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生物物理

SEIBUTSU BUTSURI

SUPPLEMENT 1-2

Vol.54

第 52回年会講演予稿集

2014.9.25（木）～ 27（土）

札幌コンベンションセンター

主催　日本生物物理学会

The Biophysical Society of Japan
一般社団法人　日本生物物理学会　http://www.biophys.jp

第 52 回日本生物物理学会年会

The 52nd Annual Meeting of the Biophysical Society of Japan

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The 52nd Annual Meeting of the Biophysical Society of Japan (BSJ2014)

第52回日本生物物理学会年会（2014年度）

札幌コンベンションセンター

会 期 ： Date：

2014年 9月25日（木）－ 27日（土）
September 25 (Thu) — 27 (Sat), 2014

会 場 ： Venue：

札幌コンベンションセンター
（札幌市白石区東札幌6条1丁目1-1）
Sapporo Convention Center
(1-1-1 Higashi-sapporo 6 jo, Shiroishi-ku, Sapporo 003-0006 Japan

年会実行委員長： Chair：

川端 和重
（北海道大学大学院先端生命科学研究院）
Kazushige Kawabata
(Hokkaido University)

HOMEPAGE http://www.aeplan.co.jp/bsj2014/

※会期後は日本生物物理学会にデータが移行されますので、学会ホームページよりご覧ください。

抄録本文（Abstract）...

オンライン講演予稿集は、こちらからダウンロードいただけます。

http://www.biophys.jp/dl/pro/52nd_proceedings.pdf
ID：ambsj52 Password ： sapporo2014

※スマートフォン・タブレット端末向けのプログラム検索・要旨閲覧アプリも現在制作中です。
（2014年 9月19日（金）公開予定）

編集・発行 ： 第52回日本生物物理学会年会実行委員会

第52回日本生物物理学会年会事務局
北海道札幌市北区北8条西5町目
北海道大学大学院先端生命科学研究院 川端教授室
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発行日：表4（裏表紙）記載

The 52nd Annual Meeting of the Biophysical Society of Japan (BSJ2014)

第52回日本生物物理学会年会（2014年度）

開催にあたって

第52回年会 実行委員長
川端 和重
（北海道大学大学院先端生命科学研究院）

一般社団法人日本生物物理学会第52回年会を2014年9月25日から27日の日程で、札幌コンベンションセンターで開催します。年会では、25 のシンポジウムと約1,000の一般発表が予定され、活気あふれた議論が展開されると期待しています。札幌で年会を開催するのは2005年以来9年ぶりです。この時期の北海道は初秋のさわやかな気候で、年会会場でも暑さに邪魔されずに快適に議論ができると期待しています。

札幌年会では、日本生物物理学会が他の学会に先駆けて行っている英語化を踏襲します。一般発表はすべてポスターとし、ポスターに英語と日本語が併記出来る環境を作ります。シンポジウムは若手研究者や男女共同参画に配慮した企画募集を基本方針としたところ、限られた枠数に多数のご応募をいただきましたことを感謝いたします。また生物物理学分野で、大型プロジェクトを組織している研究者に積極的にシンポジウムを開催していただきました。その結果、現在の生物物理学研究のトレンドに若手・男女共同参画・シニアの幅広い会員の皆様がアクセスしやすいように考えました。昨年から予稿本文の冊子体でのご提供はございませんが、参加者の皆様へ事前に予稿を検索できるpdfファイルを提供するとともに、昨年から開始したスマートフォン・タブレット用の検索アプリもご利用いただけるよう準備しました。

生物物理学は生物と物理の両方の考え方を取り入れた奥の深い学問ですが、奥の深さをいかに瀟洒に説明するかが今後の学会の発展に重要なことでしょう。札幌年会の機会を捉えて、会員の皆様が自身の研究をわかりやすくスマートに説明できる環境を作って行ければと思います。

なお、会期中、札幌大通公園で「北海道・食の祭典」さっぽろオータムフェスタが、また市内各所で札幌国際芸術祭2014が開催されております。滞在時間を有効に利用されて、観光の街・札幌を楽しまれることも期待しています。

交通のご案内／Access

■ 新千歳空港からのアクセス

◇JR新札幌駅経由の場合（所要時間約60分）

新千歳空港 → JR快速（約30分） → 新札幌駅 → 乗換 → 地下鉄新さっぽろ駅

◇JR札幌駅経由の場合（所要時間約60分）

新千歳空港 → JR快速（約40分） → 札幌駅 → 乗換 → 地下鉄さっぽろ駅 → 地下鉄南北線（約2分） → 地下鉄大通駅

◇バスご利用の場合（所要時間約60分）

新千歳空港JAL前 → 空港連絡バス（約40分） → 地下鉄大谷地駅

■ 札幌駅からのアクセス

◇地下鉄ご利用の場合（所要時間約20分）

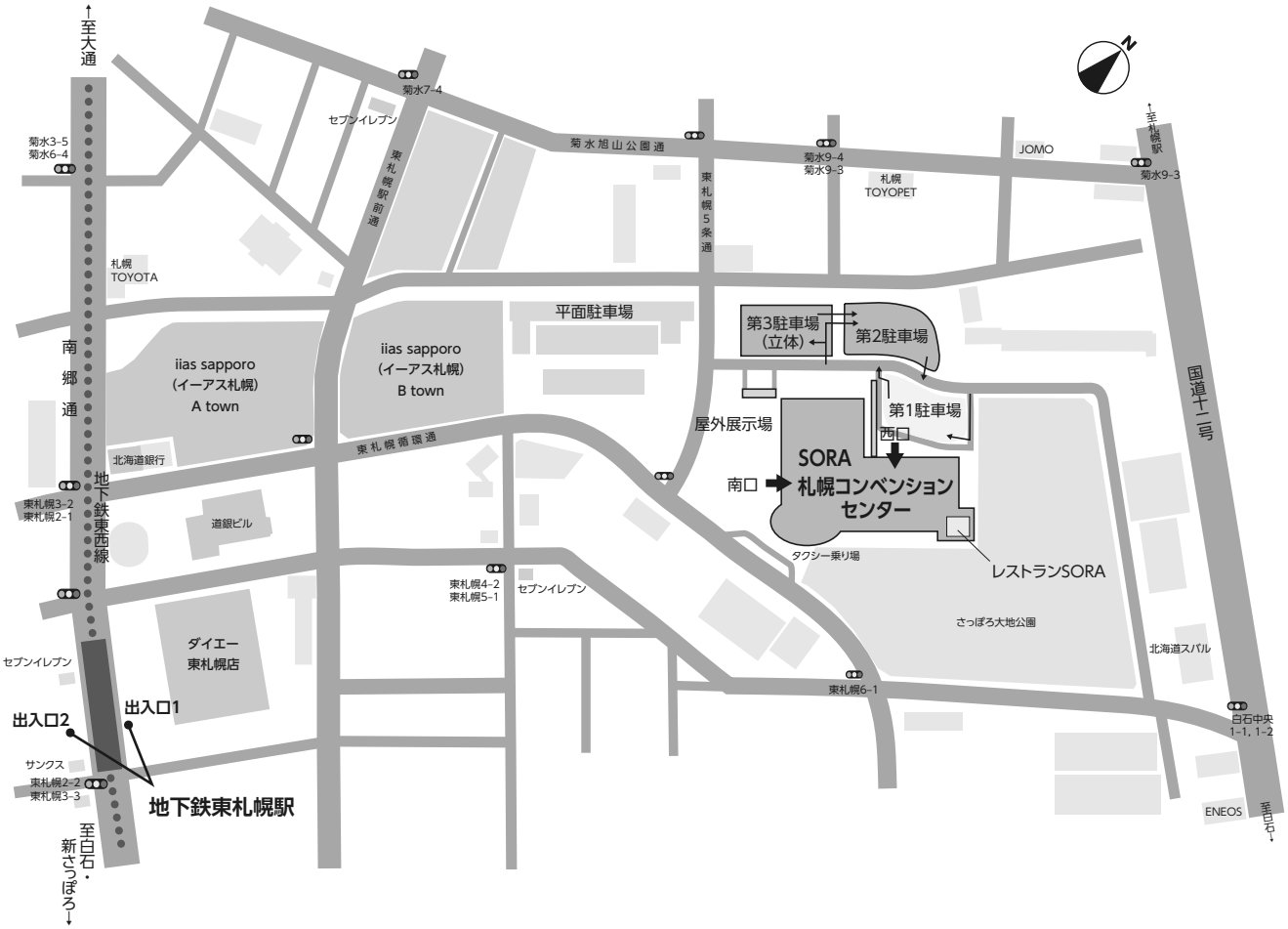
地下鉄さっぽろ駅 → 地下鉄南北線（約2分） → 地下鉄大通駅

地下鉄東西線
新さっぽろ駅より約15分
大谷地駅より約10分
大通駅より約5分

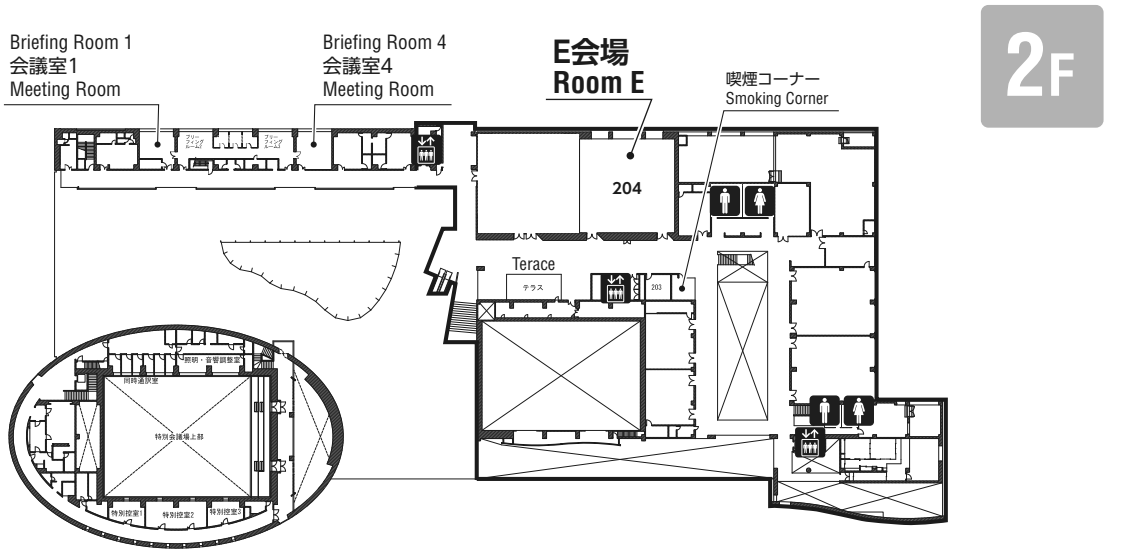
→ 札幌コンベンションセンター 東札幌駅下車 徒歩8分

周辺マップ

〒003-0006　北海道札幌市白石区東札幌6条1丁目1－1
TEL: 011-817-1010　　FAX: 011-820-4300



会場のご案内／Floor Map　〔札幌コンベンションセンター〕



9月25日（木）：年会1日目　　【Day 1】　25th Sep.

Sapporo Convention Center				8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00
札幌コンベンションセンター	1F	中ホール 1/2 Mid-sized Hall 1/2	A 会場 Room A			若手奨励賞受賞講演 Early Research in Biophusics Award			ランチョンセミナー 1LA 株式会社オプトライン OPTO-LINE, Inc.		BIOPHYSICS 論文賞受賞講演		1SAP マルチスケールに活躍する 運動超分子マシナリー Supramoleular motility machinery functioning in multi-scale scenes				
		中ホール 2/2 Mid-sized Hall 2/2	B 会場 Room B			1SBA 柔らかさが制御する生体分子系の構造形成と機能 Regulating structure formation and function of biomolecular systems with softness			ランチョンセミナー 1LB 株式会社ニコンインステック NIKON INSTECH CO.,LTD				1SBP 次世代タンパク質結晶化手法 The development of new crystallization methods for bio-macromolecular crystallography				
		107 室 Room 107	C 会場 Room C			1SCA Beyond Biophysics!: 細胞スケールにおける物理・化学・機械的な制御が生み出す新たな生物物理学 Beyond Biophysics!: Up-and-coming biophysical science achieved by physical, chemical and micromechanical control of a cell-sized space			ランチョンセミナー 1LC 株式会社菱化システム Ryoka Systems Inc.				1SCP あたかも生物のように動く 非線形化学物理系 Nonlinear physico-chemical systems moving like living organisms				
		108 室 Room 108	D 会場 Room D			1SDA 神経ダイナミクスの計測と制御 Measurement and control of neurodynamics			ランチョンセミナー 1LD DKSH ジャパン株式会社 DKSH Japan K.K.				1SDP In Cell NMR と HPC が切り開く 細胞内の蛋白質の動きと機能 Protein Dynamics and Function in Cells elucidated by in-cell NMR and High Performance Computing				
	2F	204 室 Room 204	E 会場 Room E			1SEA 脂質ラフトはどこまで分かったのか：新しい研究手法による再検証 What do we know about lipid rafts?: New landscape at the frontier							1SEP システム協同性が操る神経細胞機能 Cooperativity in shaping the nerve cell function				
	1F	大ホール Main Hall	ポスター会場 Poster Session			ポスター貼付・掲示 Poster Set-up, Viewing 9:00～13:30					ポスター討論 Poster Presentation 奇数 / Odd num. 13:30～14:30	ポスター討論 Poster Presentation 偶数 / Even num. 14:30～15:30	ポスター掲示・撤去 Poster Viewing, Removal 15:30～17:00				
			企業展示 Exhibition			機器・試薬・書籍展示 Exhibition 9:30～17:00											
		104 室 +105 室 Room 104+105	会議室 Meeting Room					第 2 回理事会 12:20～13:20			BIOPHYSICS 編集委員会 13:20～14:20		若手賞 選考委員会 16:00～17:00		臨時社員総会 18:40～19:40		
	2F	ブリーフィングルーム 4 Briefing Room 4	会議室 Meeting Room														

9月26 日（金）：年会2日目　　【Day 2】　26th Sep.

Sapporo Convention Center				8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00
札幌コンベンションセンター	1F	中ホール 1/2 Mid-sized Hall 1/2	A 会場 Room A			2SAA 膜動態から探るミトコンドリア・ネオバイオロジー Mitochondrial neo-biology explored from a membrane dynamics		分野別専門委員会 11:45～12:35	会員総会 Assembly 12:35～13:55				2SAP 少数性、数揺らぎが創出する機能のシナリオ Scenario of functions from minority and number fluctuations				
		中ホール 2/2 Mid-sized Hall 2/2	B 会場 Room B			2SBA 日本顕微鏡学会合同シンポジウム：原子レベル分解能へ向かう生物電子顕微鏡技術 Joint Symposium with the Japanese Society of Microscopy: Recent Advancement of Electron Microscopy toward Atomic Resolution from Biological Molecules							2SBP シグナル伝達機構における構造細胞生物学的新展開 New Development of Structural Cell Biology in Signal Transduction				
		107 室 Room 107	C 会場 Room C			2SCA 日本 - 中国 - 台湾　若手合同シンポジウム～超分子協同性～ Japan-China-Taiwan joint sympoium on cooperativity in supramolcular machine		ランチョンセミナー 2LC 日本エフイー・アイ株式会社 FEI Company Japan Ltd.					2SCP 構造バイオインフォマティクスによる蛋白質機能予測・解析 Prediction and analysis of protein functions from structural bioinformatics				
		108 室 Room 108	D 会場 Room D			2SDA 生命現象の基本に迫る動的クロマチン構造・機能研究の最前線 Studies of dynamic chromatin structure and function to understand fundamentals of life		ランチョンセミナー 2LD ヒューマン・フロンティア・サイエンス・プログラム機構（HFSP） The Human Frontier Science Program Organization (HFSP)					2SDP 生体分子機械の動作機構を周りの水から眺めてみる Biomolecular machinery driven by surrounding water				
	2F	204 室 Room 204	E 会場 Room E			2SEA ポンプとチャネルはどちらが偉いのか Which is important for biophysicists, pump or channel?		ランチョンセミナー 2LE 独立行政法人 理化学研究所 HPCI 計算生命科学推進プログラム RIKEN, HPCI Program for Computational Life Sciences					2SEP 生物界における光とは?：動物・植物・微生物の光科学、そしてオプトジェネティクス Light in life: photo-biology of animals, plants, microorganisms and optogenetics				
	1F	大ホール Main Hall	ポスター会場 Poster Session			ポスター貼付・掲示 Poster Set-up, Viewing 8:45～14:05					ポスター討論 Poster Presentation 奇数 / Odd num. 14:05～15:05	ポスター討論 Poster Presentation 偶数 / Even num. 15:05～16:05	ポスター掲示・撤去 Poster Viewing, Removal 16:05～17:30				
			企業展示 Exhibition			機器・試薬・書籍展示 Exhibition 9:00～17:00											
		104 室 +105 室 Room 104+105	会議室 Meeting Room					男女共同参画ミーティング 10:15～11:45	男女共同参画若手支援シンポジウム（ランチョン形式） 11:45～12:35		生物物理編集委員会 2 14:00～14:30						
	2F	ブリーフィングルーム 4 Briefing Room 4	会議室 Meeting Room											若手の会会議 17:45～18:45				

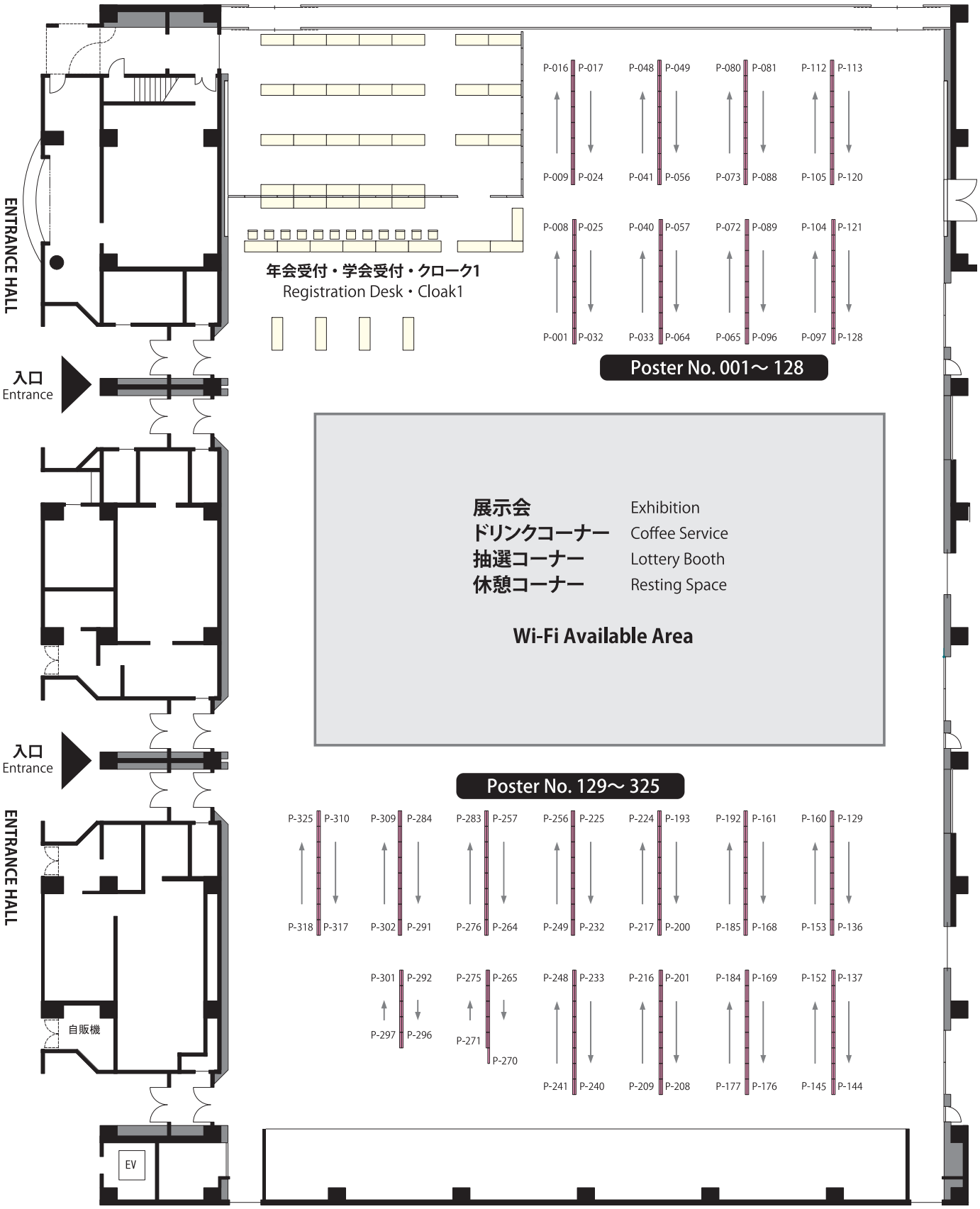
懇親会
札幌パークホテル
Banquet
Sapporo Park Hotel

9月27 日（土）：年会3日目　　【Day 3】　27th Sep.

Sapporo Convention Center				8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00
札幌コンベンションセンター	1F	中ホール 1/2 Mid-sized Hall 1/2	A 会場 Room A			3SAA 感覚と運動および知能を備えた分子ロボットの創成 Development of Molecular Robots equipped with Sensors and Intelligence											
		中ホール 2/2 Mid-sized Hall 2/2	B 会場 Room B			3SBA 分子機械デザイン Rise of molecular machines			ランチョンセミナー 3LB 日本蛋白質構造データバンク Protein Data Bank Japan								
		107 室 Room 107	C 会場 Room C			3SCA 大容量生命情報時代の新しい生物学とは? Next generation Biology at the big data era			ランチョンセミナー 3LC 公益財団法人高輝度光科学研究センター JAPAN SYNCHROTRON RADIATION RESEARCH INSTITUTE(JASRI), SPring-8								
		108 室 Room 108	D 会場 Room D			3SDA ラマン顕微分光および先端光計測が拓く生物物理の視界 Cutting-Edge Optical Imaging Approaches and Raman Micro-Spectroscopy Pioneering Bio-Physics			科研費説明会 （ランチョン形式）								
	2F	204 室 Room 204	E 会場 Room E			3SEA タンパク質物性研究の最前線：若手研究者による挑戦 Frontiers in physical properties of proteins: challenges by young scientists			ランチョンセミナー 3LE オリンパス株式会社 Olympus Corporation								
	1F	大ホール Main Hall	ポスター会場 Poster Session			ポスター貼付・掲示 Poster Set-up, Viewing 9:00～13:45				ポスター討論 Poster Presentation 奇数 / Odd num. 13:45～14:45	ポスター討論 Poster Presentation 偶数 / Even num. 14:45～15:45	撤去 Removal 15:45～16:15					
			企業展示 Exhibition			機器・試薬・書籍展示 Exhibition 9:00～16:15											
		104 室 +105 室 Room 104+105	会議室 Meeting Room				企業との意見交換会 9:30～11:00		第 3 回理事会 12:30～13:30								
	2F	ブリーフィングルーム 4 Briefing Room 4	会議室 Meeting Room														

ポスター会場のご案内 – Poster Place

札幌コンベンションセンター大ホール ー Sapporo Convention Center Main Hall



参加者へのご案内

1.　年会受付と参加登録

◇ 年会受付

場　所: 1 階大ホール内 (「会場のご案内」5 ページをご参照ください)
受付時間:　9 月 25 日(木) 9:15-17:00
9 月 26 日(金) 8:45-17:00
9 月 27 日(土) 9:00-15:00

◆事前登録

事前登録が完了された方は、日本生物物理学会会員・非会員共に参加証および領収証、プログラム集冊子が事前送付されますので、会場での受付は不要です。
当日は必ず参加証をお持ちください。

※ネームホルダーを当日配布しますので、会場内では必ず参加証をご着用ください。
配付場所: 1 階大ホール内・受付付近

注意 1)　事前登録は年会参加登録費(参加費)の振込後に完了します。振込がない場合、オンライン登録は無効となります。当日受付で当日参加費をお支払いください。

注意 2)　日本生物物理学会会員は年度会費を納めていない場合、参加証が送付されません。年度会費未納者・新規入会受付デスクにて年度会費をお支払いください。

注意 3)　参加費・年度会費ともに振込済みで、参加証が事前送付されていない場合は、総合受付デスクまでお越しください。

◆当日登録

事前登録が完了していない方は当日登録をしていただきます。
当日受付にお越しの上、参加費を現金でお支払いください。

◇ 当日年会諸費用 (一覧表)

当日参加	会員				非会員		
	正会員	シニア会員	大学院生	学部学生	一般	大学院生	学部学生
当日参加費 Registration	¥9,000	¥5,000	¥5,000	¥0	¥12,000	¥6,000	¥0
懇親会費 Banquet	¥7,000	¥5,000	¥5,000	¥3,000	¥7,000	¥5,000	¥3,000

・学部学生の参加費は無料です。当日受付で学生証を提示してください。
参加証とプログラム集冊子をお渡しします。ただし、懇親会は有料です。

・若手招待講演者、BIOPHYSICS 論文賞受賞講演者は、懇親会は招待です。
既に懇親会参加費を振り込まれている場合は、総合受付で返却します。

◇ 参加証（名札）

参加証は会場内では必ずご着用ください。参加証のない方のご入場は固くお断りいたします。
事前送付された参加証は必ず会場にお持ちください（ネームホルダーは会場内で配布いたします）。

◇ 領収書の発行

参加証とともに領収書をお渡しいたしますが、別の形式の領収書が必要な場合、お渡しした領収書と引き換えに発行いたします。

◇ プログラム集冊子/オンライン講演予稿集【8 月 20 日（水）公開予定】

プログラム集冊子（前付・プログラム・ポスター目次）は日本生物物理学会会員に事前に送付いたします（プログラム集冊子は年会受付でも当日販売（3,500 円）を行います）。なお講演予稿本文はプログラム集冊子には掲載されません。予稿本文は、オンライン講演予稿集のダウンロードシステムより閲覧していただくことになります。

オンライン講演予稿集： http://biophysjp/dl/pro/52nd_proceedings.pdf ダウンロード ID:ambsj52 パスワード：sapporo2014

プログラム（タイトル、発表者、所属）は講演予稿集発行日以降に、年会ホームページにて公開します。
年会終了後、半年ほど経て予稿本文が公開されます。日本生物物理学会ホームページの年会の記録（http://www.biophys.jp/ann/ann02.html）から CiNii（国立情報学研究所の論文情報ナビゲータ）にリンクが張られ、CiNii の生物物理のページで公開されます。
（http://ci.nii.ac.jp/organ/journal/INT1000001547_jp.html）

◇ プログラム検索（ウェブ版）【8 月 20 日（水）公開予定】

年会ホームページより「プログラム検索」を公開します。項目［演題タイトル（和文・英文）、発表者名（共著者含む）（漢字、カナ、ローマ字）、発表形式］から、演題番号、発表日、会場を検索・表示します。

◇ プログラム検索・予稿閲覧アプリ（無料）【9 月 19 日（金）公開予定】

スマートフォン(iPhone/Android)やタブレット(iPad/iPod Touch/Android)端末に対応した予稿閲覧アプリをご利用いただけます（演題検索、タイムテーブル一覧表示、ブックマーク登録等）。
App Store、Google Play（旧 Android マーケット）よりダウンロードしてください（無料）。年会ホームページにもアプリ提供サイト(App Store, Google Play)を掲載しております。

アプリケーション名：第 52 回日本生物物理学会年会 検索ワード：生物物理、日本生物物理学会、bsj2014 アプリケーションの予稿閲覧パスワード：sapporo2014

◇ 年度会費の支払いと入会の手続き

日本生物物理学会の年度会費が未納の場合は、年会受付の年度会費未納者・新規入会受付デスクでお支払いください。また、日本生物物理学会への新規入会も受け付けます。

2.　会場内のサービス・施設

◇ クローク

場　所: （第 1 クローク） 1 階大ホール内
（第 2 クローク） 1 階エントランスホール

利用時間: 9 月　25 日（木） 9:15～19:00
26 日（金） 8:45～19:15
27 日（土） 9:00～16:15

※貴重品や傘、またコンピュータなどについては、破損、紛失などの責任は負いかねますので、
各自でお持ちください。
※懇親会へ移動される際は荷物をお引き取りの上、懇親会会場にお持ちください。

◇ 昼食

ランチョンセミナー（1～3 日目）、男女共同参画・若手支援シンポジウム（2 日目）、科研費説明会（3 日目）でお弁当とお茶が無料提供されます。当日午前中に整理券を配布いたします。整理券のご利用方法は次ページ「ランチョンセミナー」をご参照ください。
この他、今回は分野別専門委員会（2 日目）でセミナー（次ページ参照）が開催されます。お弁当とお茶が無料で提供されます（整理券なし・数量に限りがあります）。積極的にご参加ください。
また会期中以下の食堂をご利用いただけます。

◇札幌コンベンションセンター1 階　レストラン「SORA」
9 月 25 日（木）～27 日（土）　11:00～17:00

◇ 呼び出し

会場内での呼び出しは、緊急の場合を除いて一切行いません。参加者間の連絡用として、年会受付に伝言板を設置しますので、ご利用ください。

◇ 駐車場

会場には会場付設の駐車場（有料）がありますが、駐車スペースに限りがありますので、できるだけ公共交通機関をご利用ください。

◇ 宿泊案内

宿泊に関しては年会ホームページ「宿泊案内」をご参照ください。

◇ インターネットならびにドリンクコーナーのご案内

・インターネット: ポスター・展示会場内休憩スペースにおいてWi-Fi (無線LAN)がご利用いただけます。
※講演会場内は利用できません。

・ドリンクコーナー: ポスター・展示会場(1階大ホール)内

◇ 託児所

年会期間中は、託児所を設置いたします。詳しくは年会ホームページをご覧ください。

3. 年会行事・プログラム

◇ 会員総会・第1回ワークショップ

一般社団法人日本生物物理学会第 1 回会員総会を年会 2 日目、9 月 26 日(金)12:35-13:55 に A 会場(中ホール 1/2)で開催しますのでご出席ください。また第1回会員総会ワークショップを開催します。詳しくは 7. 開催通知(21 ページ)をご覧ください。

◇ 若手招待講演

日本生物物理学会若手奨励賞の選考会である講演会(若手招待講演)を、年会 1 日目 9 月 25 日(木) 9:45-12:15 に A 会場(中ホール 1/2)で開催します。

◇ BIOPHYSICS 論文賞受賞講演

BIOPHYSICS 論文賞受賞の講演会を、年会 1 日目 9 月 25 日(木)15:30-16:00 に A 会場(中ホール 1/2)で開催します。

◇ 懇親会

日時: 9 月 26 日(金)19:30-21:30(年会会場から貸切バスで移動, 発車時刻の予定 18:45-19:15)
会場: 札幌パークホテル 地下 2 階『パークプラザ』(最寄り駅:地下鉄南北線・中島公園駅)
(札幌市中央区南 10 条西 3 丁目 1 番 1 号) Tel:011-511-3131
※懇親会の当日参加も受け付けいたします(受付場所:年会受付、または懇親会会場前)。

◇ 男女共同参画・若手支援シンポジウム

日時: 9 月 26 日(金)11:45-12:35
会場: 会議室(104+105 室)
昼食: お弁当とお茶が無料で提供されます(整理券を配布いたします。次ページ参照)。

◇ 分野別専門委員会(英文誌「BIOPHYSICS」セミナー)

日時: 9 月 26 日(金)11:45-12:35
会場: A 会場(中ホール 1/2)
対象: 分野別専門委員(委員以外の会員の方の参加も歓迎します)
内容: 英文誌「BIOPHYSICS」セミナーとして、波多野 薫 氏(トムソン・ロイター出版)から、
Web of Science や impact factor についての講演があります。
昼食: 委員以外の会員には、先着 20 名までお弁当とお茶が無料で提供されます(整理券なし)。

◇ 科研費説明会

日時: 9 月 27 日(土)12:30-13:20
会場: D 会場(108 室)
昼食: お弁当とお茶が無料で提供されます(整理券を配布いたします。次ページ参照)。

◇ ランチョンセミナー

昼食(お弁当とお茶、無料)をとりながらの協力企業によるセミナーにご参加ください。なお、お弁当の数に限りがあるため当日午前中に整理券を配布いたします。セミナー開始前に、会場入り口で整理券と引き換えにお弁当を受け取り、ご入場ください(整理券の発券方法は次ページ参照)。

◆整理券の発券について

ランチョンセミナー整理券は整理券配布デスクにて配布いたします(下記参照)。
時間:9月25・27日　9:00-11:00、26日　8:45-11:00
場所:ポスター・展示会場(1階大ホール内)受付付近
※整理券はランチョンセミナー共催の企業、団体よりご提供いただく昼食の引換券になります。
当日開催されるセミナー分のみ発券いたします。券は枚数が無くなり次第終了となります。

◆整理券の注意事項

整理券は各日、セミナー開始後、無効となります。
午前のプログラム終了後、ランチョンセミナー開始時間までにご来場ください。
セミナー開始までにご来場されない場合、整理券は無効となり、お弁当は整理券をお持ちでない参加者にご提供されますことをご了承ください。

◇ 機器・試薬・書籍等展示会

機器、試薬、ソフトウエア、書籍などの展示会をポスター・展示会場(1 階大ホール内)で行います。

◇ 市民講演会

テーマ:　生命を観る～世界最先端の生命科学～
日　時:　9 月 28 日(日)14:00-16:00
会　場:　北海道大学　学術交流会館(札幌市北区北 8 条西 5 丁目、JR 札幌駅・徒歩 7 分)
参　加:　無料(ポスター「一家に 1 枚　動く！タンパク質」(文科省制作・日本生物物理学会提案)提供)
お問い合わせ:　年会実行委員会サポートまでお願いします。
E-mail:　jbp2014@aeplan.co.jp

4.　禁止事項

◇ 撮影・録音

会場内でのカメラ、ビデオ、携帯電話などによる撮影や講演音声の録音などを禁止します。

◇ 喫煙・飲食

館内には所定の喫煙コーナーがございます。講演会場内での飲食はランチョンセミナー、男女共同参画・若手支援シンポジウム、科研費説明会、各種委員会など食事が提供される場合を除いて禁止します。

◇ 携帯電話

シンポジウム、ポスター発表等の講演会場内での携帯電話による通話を禁止します。講演会場内では電源をオフにするかマナーモードに設定し、呼び出し音が鳴らないようご注意ください。

5.　年会についての問い合わせ

◇ **会期中**　年会本部　(Phone number reachable during the meeting)　Tel:　070-5453-8365

◇ **会期外**　年会実行委員会　E-mail:bp_nenkai52@mail.sci.hokudai.ac.jp

参加登録・演題登録 システムサポートデスク
〒113-0033　東京都文京区本郷2-26-11　浜田ビル5F
中西印刷株式会社　東京営業部内
E-mail:　bsj2014sys-sprt@e-naf.jp

年会実行委員会サポート・展示・広告問い合わせ先

〒101-0003　東京都千代田区一ツ橋 2-4-4 岩波書店一ツ橋別館 4F
株式会社エー・イー企画　Tel:03-3230-2744(代表)　Fax:03-3230-2479
実行委員会サポート　E-mail:　jbp2014@aeplan.co.jp
広告・展示関連　E-mail:　e_jbp52@aeplan.co.jp

6.　発表者へのご案内

◇ 使用言語

シンポジウムの発表および一般発表の口頭説明は、原則として英語をお使いください。

◇ 映写機器

発表に使用できる映写機器は、液晶プロジェクターのみです。音声出力には対応しません。
会場にはパソコンを用意致しません。ご自身のノートパソコンを必ずお持ちください。

注意 1)会場スタッフがパソコンを会場に備え付けられた切り替え装置(Video Switcher)に接続致します。

注意 2)切り替え装置に繋がるパソコンの映像出力端子は、
「ミニ D-sub15 ピン端子(メス)」*(3 列あるもの)のみです。
端子の形状が異なる場合(Macintosh 等)、変換アダプターをお持ちください。
*読み:みに D さぶ 15 ぴんたんしめす

注意 3)発表に使用するパワーポイントファイルが入った USB メモリーを念のためにお持ちください。

注意 4)バッテリー切れに備え、必ず電源アダプターをお持ちください。

◇ シンポジウム、若手招待講演の座長の方へ

受付:　座長の方はシンポジウム開始 15 分前までに各会場の「座長席」までおいでください。

進行:　シンポジウムの進行と時間管理は座長に一任いたしますが、終了予定時刻を越えないようご注意ください。
会場には時間を計測するスタッフを置いています。

◇ シンポジウム、若手招待講演の講演者の方へ

受付:　講演者の方は、シンポジウム開始の 15 分前までに各会場においでください。
発表スライドをご確認頂いた後、会場スタッフがパソコンを切り替え装置(Video Switcher)に接続いたします。※スライドチェック用の試写室は設けておりません。

講演時間:　時間配分は座長に一任します。
若手招待講演の講演時間は、発表 10 分、討論 3 分、パソコンの交換に 2 分です。

若手招待講演の発表プログラムが終了後、発表会場内で若手招待講演証書が授与されます。

◇ ポスター発表の方へ

ポスターの貼付、展示、説明・討論、撤去:

		9 月 25 日(木)	9 月 26 日(金)	9 月 27 日(土)
貼付・展示		9:00-13:30／ 15:30-17:00	8:45-14:05／ 16:05-17:30	9:00-13:45
説明・討論	奇数番号	13:30-14:30	14:05-15:05	13:45-14:45
	偶数番号	14:30-15:30	15:05-16:05	14:45-15:45
撤去		17:00 までに撤去	17:30 までに撤去	16:15 までに撤去

1. ポスターは日替わりで貼り替えてください。
2. ポスターボードの大きさは、幅 90 cm、高さ 210 cm。貼付に必要な押しピンは会場に用意します。
3. 撤去時間を過ぎて残ったポスターは年会事務局にて破棄しますので、ご了承ください。

◇ ポスター発表要項

ポスターは英語で作成してください。
ただし、タイトル、所属、発表者名は、可能であれば日本語の併記もお願いいたします。
発表代表者の氏名には左肩に小さな○印を付けてください。

◇ 発表形式と演題番号(各予稿左上の番号)の見方

発表形式は、シンポジウム発表(Symposium Talk)、若手招待講演(Early Research in Biophysics Award Candidate Presentations)、ポスター発表(Poster Presentation)があります。

シンポジウム発表:(例)1SAA-03
1 文字目は発表日(1:9 月 25 日、2:9 月 26 日、3:9 月 27 日)、2 文字目は Symposium、3 文字目は会場名(A会場)、4 文字目は午前・午後(AM,PM)、最後の 2 桁の数字は発表順です。

若手招待講演:(例)1YA1045
1 文字目は発表日(1:9 月 25 日)、2 文字目は Young(Scientists)、3 文字目は会場名(A 会場)、最後の 4 桁の数字は講演開始時刻です。

ポスター発表:(例 1)1P001
1 文字目は発表日(1:9 月 25 日、2:9 月 26 日、3:9 月 27 日)、2 文字目は Poster、最後の 3 桁の数字はパネル番号を示します。

7.　一般社団法人日本生物物理学会第 1 回会員総会開催通知

日時: 9 月 26 日(金)12:35-13:55

会場: A 会場(中ホール 1/2)

一般社団法人日本生物物理学会第 1 回会員総会を開催いたします。主な議題は下記の通りです。是非ご出席ください。

議長　年会実行委員長　川端　和重

総会議題

(1)報告事項(会長　七田　芳則)

平成 25 年度および平成 26 年度第一期決算報告ならびに監査結果報告

平成 26 年度第二期会計ならびに事業の中間報告と今後の計画

平成 27･28 年度役員選挙結果の報告

次期年会について

(2)第 1 回会員総会ワークショップ:生物物理が拓く未来社会

概要:次ページ掲載

第 1 回会員総会ワークショップ：生物物理が拓く未来社会
(Biophysics paves the way for the future)

世話人：須藤 雄気（岡山大学大学院医歯薬学総合研究科）、瀧ノ上 正浩（東京工業大学大学院総合理工学研究科）、永井 健治（大阪大学産業科学研究所）、今元 泰（京都大学理学研究科）
Organizers: Yuki Sudo (Okayama Univ.), Masahiro Takinoue (Tokyo Tech.), Takeharu Nagai (Osaka Univ.) ,Yasushi Imamoto (Kyoto Univ.)

日時: 9月26日（金）12:35-13:55 会員総会中
会場: A会場（中ホール1/2）
形式: 講演会

概要:
生物物理学の主題の一つは、生命現象を解析・操作する『道具』の開発とその『利用』にある。その意味で、生物物理学は、生命科学研究の“ドラえもん”と考えることも出来る。科学技術の進展はめざましく、当初は想像の世界にすぎなかった様々な『道具』が、少しずつ現実世界でも実現しつつある。ここでは、将来作られているであろう道具やその性能・使い方について、未来創造力に優れた講演者に発表してもらい、100 年後の未来社会を想像することで、今後の生物物理学への活力としたい。

[Abstract]
One of the major topics in biophysics is to develop and utilize tools for understanding and controlling biological functions. In this sense, biophysics can be thought of as “DORAEMON” in the life science world. The remarkable progress in science and technology has made it possible to develop tools which were previously inconceivable. In this workshop, three creative scientists will present tools and/or technologies which will be developed in future. We would like to vitalize our prospects for the future of Biophysics, by imagining the future 100 years from now.

講演者・プログラム:
1. 趣旨説明

2. 浅田 稔（大阪大学工学研究科） Minoru Asada（Osaka Univ.）
人工情動に基づくロボットの共感発達
The development of robot empathy based on artificial emotion

3. 竹内 昌治（東京大学生産技術研究所） Shoji Takeuchi (The Univ. of Tokyo)
Think Hybrid. こんなことといいな、できたらいいな

4. 永井 健治（大阪大学産業科学研究所） Takeharu Nagai (Osaka Univ.)
生物発光がもたらす未来のほっこり生活
Future warm life brought about by bioluminescence

5. 会場からの提案・質問

8. 理事会、総会、各種委員会の案内

委員会等	開催日程		会場 (札幌コンベンションセンター)
ホームページ編集委員会	9 月 24 日 (水)	16:00～18:00	2階 ブリーフィングルーム 1
生物物理編集委員会 1		18:00～20:00	2階 ブリーフィングルーム 4
平成 26 年度第二期第 2 回理事会 (旧運営委員会)	9 月 25 日 (木)	12:20～13:20	1 階 104+105 室(会議室)
BIOPHYSICS 編集委員会		13:20～14:20	1 階 104+105 室(会議室)
若手賞選考委員会		16:00～17:00	1 階 104+105 室(会議室)
臨時社員総会(新旧合同委員会)		18:40～19:40	1 階 104+105 室(会議室)
男女共同参画・若手支援委員会	9 月 26 日 (金)	10:15～11:45	1 階 104+105 室(会議室)
分野別専門委員会*1		11:45～12:35	1 階 中ホール 1/2(A 会場)
会員総会(総会)*2		12:35～13:55	1 階 中ホール 1/2(A 会場)
生物物理編集委員会 2		14:00～14:30	1 階 104+105 室(会議室)
若手の会会議		17:45～18:45	2階 ブリーフィングルーム4
企業との意見交換会	9 月 27 日 (土)	9:30～11:00	1 階 104+105 室(会議室)
平成 26 年度第二期第 3 回理事会 (新運営委員会)		12:30～13:20	1 階 104+105 室(会議室)

*1; 分野別専門委員会では、英文誌「BIOPHYSICS」セミナーが開催されます(3. 年会行事・プログラム)。

*2; 会員総会では、第1回会員総会ワークショップが開催されます(7. 第 1 回会員総会開催通知)。

( )は法人化前の名称. ブリーフィングルームはエスカレーターで2階へ上がり、左側(大ホール2階)にございます。

謝 辞

本年会の開催・運営に当たり、以下の団体よりご協力・ご援助いただきました。
関係者一同より御礼申し上げます。

新学術領域研究「理論と実験の協奏による柔らかな分子系の機能の科学」
新学術領域研究「細胞シグナリング複合体によるシグナル検知・伝達・応答の構造的基礎」
新学術領域研究「少数性生物学−個と多数の狭間が織りなす生命現象の探求−」
新学術領域研究「運動超分子マシナリーが織りなす調和と多様性」
新学術領域研究「感覚と知能を備えた分子ロボットの創成」
新学術領域研究「動的クロマチン構造と機能」

第 52 回日本生物物理学会年会
実行委員長 川端 和重

9. Information for participation and presenters

◇ Registration

◆Registration Desk

Place: Sapporo Convention Center 1F Main Hall (Refer to a floor map)
Open Hours: Sep. 25(Thu) 9:15-17:00
26(Fri) 8:45-17:00
27(Sat) 9:00-15:00

◆Advance Registration

Name badge, Receipt, and Program booklet have been sent to those who have completed advance registration with a full payment of the registration fee by the due date. No necessary to stop by at the registration desk.

※Please wear your name badge during the meeting.
(Name badge holders will be provided at the meeting site.)

Note 1) Advance registration will be completed only after the payment. In case your payment cannot be confirmed by the due date, your registration is automatically cancelled. In this case, please register on-site again at the registration desk.

Note 2) A name badge has not be sent in case the payment of BSJ annual membership fee has not be completed. Please complete the payment at the BSJ desk at the meeting site.

Note 3) If both payments of the registration and BSJ annual membership fees are completed but you have not received a name badge, please visit the registration desk.

◆On-site Registration

Those who have not completed advance registration, please visit the registration desk to register on-site along with registration fees.

◇ On-site Registration fees

	BSJ Member				Non-Member		
	Regular	Senior	Student	Undergraduate student	Regular	Student	Undergraduate student
Registration fee	¥9,000	¥5,000	¥5,000	¥0	¥12,000	¥6,000	¥0
Banquet Fee	¥7,000	¥5,000	¥5,000	¥3,000	¥7,000	¥5,000	¥3,000

・Undergraduate student : Registration fee will be waived. (Please show your student ID at the registration desk to receive a name badge and the program booklet. The banquet is charged.)

・Early Research in Biophysics awardees, BIOPHYSICS paper awardees: The banquet is invited. If you have already paid the banquet fee, it will be refund at the registration desk.

◇ Name badge

Please wear your name badge during the meeting period to enter the meeting site. No admission without the badge. Make sure to bring your name badge that is sent in advance. (Name badge holders will be provided at the meeting site.)

◇ Receipt

The receipt is attached to a name badge. If you need another receipt form, it will be issued in exchange for the attached one.

◇ Program booklet / Abstracts online system【Release date: Aug. 20 (Wed)】

The abstracts are to be released only on the online system. The system is available for browsing, searching and downloading abstracts. No printed abstracts booklet has been issued. The program booklet (the parts of front matter, program, contents of poster presentations, and advertisement) has been sent to BSJ members in advance.
Program booklets are available for purchase for JPY3,500/booklet at the registration desk.

Abstracts online system: http://biophysjp/dl/pro/52nd_proceedings.pdf Download ID: ambsj52 PW: sapporo2014

Program (presentation title, presenter's name and affiliation) is to be released on this web site after the abstracts online system is open. Abstracts are to be posted a half a year later after the meeting on CiNii web site which is linked from BSJ web site. (http://ci.nii.ac.jp/organ/journal/INT1000001547_jp.html)

◇ Program search system (Web ver.)【Release date: Aug. 20 (Wed)】

Program search system is to be released on the web site.
Enter search words such as title, presenter, co-author, or presentation style, and display the result of the search (abstract number, presentation day & place).

◇ Program search system・Abstracts browsing free app【Release date: Sep. 19 (Fri)】

The app is enabled for smart phones (iPhone/Android) and tablet computers (iPad/iPod Touch/Android). Free to download it from App Store, Google Play.

App name:The 52nd Annual Meeting of the Biophysical Society of Japan Search word: bsj2014 PW for browsing abstracts:sapporo2014

◇ BSJ Membership (payment of the annual membership fee, admission procedures)

It is able to pay for the annual membership fee at the BSJ desk. Also welcome to sign up to be a member.

◇ Prohibitions

◆Photography・Recording

No photography and recording with camera, video, mobile phone and any device is allowed at the meeting site.

◆Smoking, Drinking & Eating

No smoking is allowed at the meeting site except for the separate smoking spots. No drinking & eating is allowed inside lecture rooms except for luncheon seminar, Symposium on Gender Equality, and other meetings which meals are served.

◆Mobile phone

No talking on the mobile phone in the lecture/presentation room is allowed. Please set your mobile phone on the silent mode and make sure it will not make a noise during lectures/presentations.

◇ Information for Presenters

◆Language

English

◆Presentation equipments

Please bring your own laptop and connect it to our projector for your presentation.
A sound output is not provided.
Attention 1) Our staff connects your computer to a video switcher.
Attention 2) The video output connector of presenter's laptop should be "miniD-sub15pin (female)". If the connector is not this type (for example, that of Macintosh computer), please bring an adaptor.
Attention 3) Please bring your PowerPoint file in a USB memory.
Attention 4) Please bring your AC adaptor in case the battery runs down.

◆To Symposium Chairs

Please come to the "Chair's seat" at the symposium room by 15 minutes before the starting time. The timekeeper will assist with timing by bell signal. The chairs are expected to ensure that all presentations start and finish punctually as scheduled.

◆To Symposium Speakers

Please come to the symposium room 15 minutes before the starting time. After check your presentation slides, our staff connects your computer to a video switcher.
*Please note that there is no preview room.

◆To Poster Presenters

		Day 1, Sep. 25	Day 2, Sep. 26	Day 3, Sep. 27
Set-up, Viewing		9:00-13:30／15:30-17:00	8:45-14:05／16:05-17:30	9:00-13:45
Presentation Discussion	Odd Numbers	13:30-14:30	14:05-15:05	13:45-14:45
	Even Numbers	14:30-15:30	15:05-16:05	14:45-15:45
Removal		until 17:00	until 17:30	until 16:15

If you have any questions, please contact us by email (bp_nenkai52@mail.sci.hokudai.ac.jp).

◆Instructions for Poster Presentations

Posters will be mounted at the poster room day by day.
Poster board size is 90 cm in width and 210 cm in height.

◆Presentation Types and Decoding Presentation Numbers

Presentation types are Symposium Talk, Early Research in Biophysics Award Candidate Presentations, and Poster Presentation.

Symposium Talk: (Ex.) 1SAA-03
Presentation day (1: Sep 25, 2:Sep 26, 3:Sep 27) + Symposium (S) + Session room (room A) + AM (A) / PM (P) + Order of the talk

Early Research in Biophysics Award Candidate Presentations: (Ex.) 1YA1045
Presentation day (1: Sep 25) + Young Scientists (Y) + Session room (room A) + Starting time of the talk

Poster Presentations: (Ex.) 1P001
Presentation day (1: Sep 25, 2:Sep 26, 3:Sep 27) + Poster (P) + Panel number

第52回日本生物物理学会年会 実行委員会

年会実行委員長　　川端 和重（北海道大学大学院先端生命科学研究院）

実行委員

相沢 智康　（北海道大学大学院先端生命科学研究院）
秋田谷 龍男（旭川医科大学医学部）
石森 浩一郎（北海道大学大学院理学研究院）
岩佐 達郎　（室蘭工業大学大学院工学研究科）
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内田 努　　（北海道大学大学院工学研究院）
岡嶋 孝治　（北海道大学大学院情報科学研究科）
尾瀬 農之　（北海道大学大学院薬学研究院）
神谷 昌克　（北海道大学大学院先端生命科学研究院）
菊川 峰志　（北海道大学大学院先端生命科学研究院）
北村 朗　　（北海道大学大学院先端生命科学研究院）
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小松崎 民樹（北海道大学電子科学研究所）
郷原 一寿　（北海道大学大学院工学研究院）
高井 章　　（旭川医科大学医学部）
高橋 正行　（北海道大学大学院理学研究院）
田中 良和　（北海道大学大学院先端生命科学研究院）
出村 誠　　（北海道大学大学院先端生命科学研究院）
中垣 俊之　（北海道大学電子科学研究所）
永山 昌史　（北海道教育大学教育学部 (旭川校) ）
根本 知己　（北海道大学電子科学研究所）
芳賀 永　　（北海道大学大学院先端生命科学研究院）
前仲 勝実　（北海道大学大学院薬学研究院）
眞山 博幸　（旭川医科大学医学部）
水谷 武臣　（北海道大学大学院先端生命科学研究院）
姚 閔　　（北海道大学大学院先端生命科学研究院）

※50音順。敬称略。

第 52 回日本生物物理学会年会　市民講演会
生命を観る　世界最先端の生命科学

日　時：9 月 28 日（日）14:00~16:00

会　場：北海道大学 学術交流会館 大講堂
（北海道札幌市北区北 8 条西 5 丁目）
JR 札幌駅から徒歩 7 分、北大正門そば

参加費：無料（どなたでも自由に参加できます）

主　催：第 52 回日本生物物理学会年会　実行委員会

世話人：川端 和重（第 52 回年会実行委員会委員長　北海道大学大学院先端生命科学研究院）
郷原 一寿（北海道大学大学院工学研究院）

講演プログラム

「生命を支える超分子ナノマシン」
教授　難波 啓一
（大阪大学 2012 年恩賜賞・日本学士院賞）

超分子ナノマシンとはタンパク質や核酸など生体高分子の複合体のことで、構成原子の精密な立体配置により特定の機能を発現し、あらゆる生命機能を支えるナノスケールの分子機械です。現在の工学技術をはるかにしのぐ高い精度や、桁違いに小さなエネルギーで高効率に、しかもしなやかに動作するしくみを持っています。創薬、先端医療、将来のナノマシン設計等に役立てるため、クライオ電子顕微鏡や X 線回折による立体構造解析技術を開発し、原子レベルの立体構造からその興味深いしくみの解明を進めています。

講演は日本語で行われます。

一般社団法人日本生物物理学会　第 3 回 BIOPHYSICS 論文賞受賞講演会
The 3rd Award Seminar for outstanding BIOPHYSICS paper

オーガナイザー：日本生物物理学会 BIOPHYSICS 論文賞選考委員会

Organizers: Award committee for outstanding BIOPHYSICS paper

日　時：9 月 25 日（木）15:30 ～ 16:00

場　所：A 会場（中ホール 1/2）

形　式：講演会

第 3 回 BIOPHYSICS 論文賞受賞者

金城 玲

Akira R. Kinjo

大阪大学蛋白質研究所

Institute for Protein Research, Osaka University

蛋白質リガンド結合部位の網羅的比較

Exhaustive structural comparison of interaction interfaces of proteins

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To understand the function of a protein, it is necessary to understand the interactions between the protein and other molecules. To understand the interactions, in turn, it is useful to compare the structures of interaction sites and to identify the structural patterns or motifs. It has been assumed for a long time that proteins with similar sequences share similar structures and functions. However, a wide variety of protein functions for a particular protein family forces us to recognize the importance of the diversity within the universality of protein families. Protein structure comparison is known to be a computationally hard problem so that most studies use a set of "representative" structures based on sequence similarities as well as some coarse-grained representations of structures. In order to examine the diversity of protein structures and interaction patterns, we needed to develop an extremely efficient and detailed method for structure comparison. The GIRAF method we have developed is the first to accomplish the truly exhaustive all-against-all comparison of all the interaction site structures at atomic resolution in the Protein Data Bank. Since the publication of the BIOPHYSICS paper in 2007, the method has been improved significantly and applied to exhaustive comparative studies of interaction interfaces of small ligands, proteins and nucleic acids, which have lead to the notion of the composite motif to annotate protein functions in terms of the differential combination of multiple structural motifs.

「男女共同参画・若手支援シンポジウム」
「ワークライフバランスと子育て支援の取り組み」
Work-life balance and an approach for supporting parental care

オーガナイザー：日本生物物理学会　男女共同参画・若手支援委員会

Organizers: Committee for Promoting Equal Participation of Men and Women and for Encouraging Young Researchers in the Biophysical Society of Japan

日　時：9 月 26 日（金）11:45 ～ 12:35（ランチョンセミナーの時間帯）

会　場：諸会議室（104+105 室）

昼　食：お弁当とお茶が無料で提供されます。ただし、数に限りがあります。

形　式：講演会とグループディスカッション

講演者：坂内 博子（名古屋大学）

概要：本年のシンポジウムでは、新しい試みとして講演の後にグループディスカッションの時間を設けることになりました。グループディスカッションの目的は、小さなグループに分かれて、普段思っていること感じていることについて気軽に話し合っていただき、また、同じ問題意識を持つメンバーとして交流・親睦を図ることです。この機会に、これまで個人的に会話を交わしたことのない会員とも交流を持ち、親しくなれればと思います。

講演は、1 分子イメージングの手法で脳の分子メカニズムの解明に取り組んでおられる名古屋大学の坂内博子さんにお願いし、留学経験と海外でのワークライフバランス、そして名大におけるユニークな子育て支援の取り組みについてお話しいただく予定です。男女共同参画の取り組みでは、男女ともに意識の変革が必要であることが共通の認識になってきていますが、具体的な日常のワークライフバランスは避けて通れない問題です。昨年、一昨年のシンポジウムでは「博士号を取得して多様なキャリアパスを手に入れる」をテーマとして行われました。大学で博士号を取得して研究者や専門性の高い職業につくのは今も昔も難しいことですが、日本の将来の方向性には合致しており、様々な職種についての提案がなされるようになってきました。本年はこの問題に少し別の切り口からアプローチする予定です。

講演の後、時間が許す限り講演内容も含めて様々な話題についてグループディスカッションを行いたいと考えています。話題については委員会でも用意いたします。各グループでどのような話し合いが行われたかを最後に発表していただくことを計画しています。

老若男女を問わず、ご関心のある方々の参加をお待ちしております。

科研費説明会「科研費、最近の動向」

Current Activities of the Grants-in-Aid System

世話人：後藤 祐児（大阪大学蛋白質研究所、日本学術振興会学術システム研究センター専門研究員）

Organizer: Yuji Goto (Osaka University, Institute for Protein Research, JSPS, Research Center for Science Systems, Program Officer）

日　時：9 月 27 日（土）12:30 ～ 13:20（ランチョンセミナーの時間帯）

会　場：D 会場（108 室）

昼　食：お弁当とお茶が無料で提供されます。ただし、数に限りがあります。

形　式：講演会

○「科研費」の最近の動向

講師：**日本学術振興会　研究助成第一課長　大鷲　正和**

日本学術振興会から、科研費の配分機関として制度の概要、応募から審査、決定までの流れを中心に、補助金、助成金の執行と適切な管理、不正防止に関すること、また、成果の公開、科研費の普及啓発などについても、ご説明いただきます。

○日本学術振興会の諸事業における学術システム研究センターの役割

講師：**日本学術振興会　学術システム研究センター専門調査役　樋口　和憲**

日本学術振興会には、公平・公正で透明性の高い審査・評価の実施のために、学術システム研究センターが設置され、研究機関に籍を置く第一線の研究者が、科研費等の審査委員候補者の選考、審査結果の検証、分科細目表の見直しや制度の改善など、幅広い業務に参画しています。学術的な見地から、最新の学術動向や現場の声を事業運営に反映させるための様々な活動について、ご紹介いただきます。

若手招待講演　Early Research in Biophysics Award

第 1 日目（9 月 25 日（木））／Day 1（Sep. 25 Thu.）

9:45～12:15　A 会場／Room A : Mid-sized Hall 1/2

1YA　日本生物物理学会若手奨励賞選考会
Early Research in Biophysics Award Candidate Presentations

オーガナイザー：男女共同参画・若手支援委員会
Organizer: The Committee of Promoting Gender Equality and Young Scientists

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In 2005, the Biophysical Society of Japan has established Early Research in Biophysics Award to recognize distinguished research work by young members of the BSJ. In this tenth year, we received 32 highly qualified applications. After extremely competitive first round of screening based on written application forms, the following ten applicants were selected as the "young guest speakers." For the second round of the nomination, each young speaker will be asked to make a 10-minute presentation followed by 3-minute Q&A discussion. At the end of these rounds, up to five award winners will be selected. The award winners will be announced at the banquet in the evening of Friday 26th September, and the winners will deliver a short talk. We welcome all the BSJ members to attend the oral presentations on Thursday 25th September at the Early Research in Biophysics Award Candidate Presentations and would like the members to foresee the future of biophysics in Japan through these speakers and their researches.

09:45　戎家　美紀　1P135
1YA0945　細胞間に非対称性を生み出すしくみの再構成
Reconstitution of an intercellular symmetry breaking mechanism
松田　充弘[1], 古賀　牧土[1], Woltjen　Knut[2], 西田　栄介[3], ○戎家　美紀[1]（[1]理研CDB, [2]京都大学 CiRA, [3]京都大学 生命科学研究科）
Mitsuhiro Matsuda[1], Makito Koga[1], Knut Woltjen[2], Eisuke Nishida[3], **Miki Ebisuya**[1] ([1]*RIKEN CDB*, [2]*CiRA, Kyoto Univ*, [3]*Grad Sch of Biostudies, Kyoto Univ*)

10:00　小井川　浩之　3P056
1YA1000　タンパク質の高速折り畳みダイナミクスの一分子追跡を目指したライン共焦点顕微鏡の開発
Development of the line confocal system for the single molecule tracking of fast folding dynamics of proteins
○小井川　浩之[1], 鎌形　清人[1], 新井　宗仁[2], 深澤　宏仁[3,4], 横田　浩章[4], 井出　徹[5], 高橋　聡[1]（[1]東北大 多元研, [2]東大・院総合文化, [3]浜松ホトニクス, [4]光産業創成大学院大, [5]岡山大・院自然科学）
Hiroyuki Oikawa[1], Kiyoto Kamagata[1], Munehito Arai[2], Atsuhito Fukasawa[3,4], Hiroaki Yokota[4], Toru Ide[5], Satoshi Takahashi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Arts. Sci., Univ. Tokyo*, [3]*Hamamatsu Photonics*, [4]*GPI*, [5]*Grad. Sch. Nat. Sci. and Tech., Okayama Univ*)

10:15　岡崎　圭一　1P147
1YA1015　**Multiscale analysis of functional motions in F1-ATPase: From Pi release to elasticity and friction of γ-subunit rotation**
Kei-ichi Okazaki, Gerhard Hummer (*Max Planck Institute of Biophysics*)

10:30　岡本　章玄　2P107
1YA1030　プロトン駆動力を細胞外へと捨てる微生物外膜タンパク質
Proton discarded to cell exterior via outer-membrane bound enzyme
○岡本　章玄, Kalathil　Shafeer, 徳納　吉秀, 橋本　和仁（東大院工）
Akihiro Okamoto, Shafeer Kalathil, Yoshihide Tokunou, Kazuhito Hashimoto (*Grad. Sch. Eng., Univ. of Tokyo*)

10:45　近藤　徹　1P252
1YA1045　光合成反応中心タンパク質の極低温単一分子分光
Single-molecule spectroscopic study of photosynthetic reaction center at 6 K
○近藤　徹[1], 武藤　梨沙[2], 栗栖　源嗣[2], 大岡　宏造[3], 藤芳　暁[1], 松下　道雄[1]（[1]東工大・理工, [2]阪大・蛋白研, [3]阪大・理）
Toru Kondo[1], Risa Mutoh[2], Genji Kurisu[2], Hirozo Oh-oka[3], Satoru Fujiyoshi[1], Michio Matsushita[1] ([1]*Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Institute for Protein Research, Osaka Univ.*, [3]*Grad. Sch. Sci., Osaka Univ.*)

11:00　斉藤　圭亮　1P254

1YA1100　光合成光化学系 II における MnCa クラスターの歪んだ椅子型構造の起源

Origin of the distorted-chair structure of the MnCa cluster in photosystem II

○斉藤　圭亮[1,2], 石北　央[1]（[1]東大・院工・応化, [2]JSTさきがけ）

Keisuke Saito[1,2], Hiroshi Ishikita[1] ([1]*Dep. App. Chem., Grad. Schol. Eng., Univ. Tokyo*, [2]*JST PRESTO*)

11:15　藤井　聡志　2P089

1YA1115　膜たんぱく質の進化工学手法、リポソームディスプレイ法による α-ヘモリシンの in vitro 分子進化

Directed evolution of membrane protein, alpha hemolysin, by development of liposome display method

○藤井　聡志[1], 松浦　友亮[1,2], 角南　武志[1,3], 数田　恭章[1], 四方　哲也[1,3,4]（[1]科学技術振興機構, [2]大阪大・院・工学, [3]大阪大・院・情報科学, [4]大阪大・院・生命）

Satoshi Fujii[1], Tomoaki Matsuura[1,2], Takeshi Sunami[1,3], Yasuaki Kazuta[1], Tetsuya Yomo[1,3,4] ([1]*JST*, [2]*Grad. Eng. Univ. Osaka*, [3]*Grad. Bioinfo. Univ. Osaka*, [4]*Grad. Fron. BioSci. Univ. Osaka*)

11:30　谷中　冴子　2P064

1YA1130　NMR を用いた動的構造解析により明らかとなったヒト主要組織適合複合体のペプチド認識、及び構造維持機構

The Dynamic stabilization and peptide recognition mechanism of Human Leukocyte Antigen revealed by NMR relaxation dispersion analysis

○谷中　冴子[1,2], 菅瀬　謙治[1], 上野　貴将[4], 津本　浩平[2,3]（[1](公財)サントリー生命科学財団, [2]東大・新領域, [3]東大院・工学系研究科, [4]熊大・エイズ研）

Saeko Yanaka[1,2], Kenji Sugase[1], Takamasa Ueno[4], Kouhei Tsumoto[2,3] ([1]*Sunbor*, [2]*Grad. School of Frontier Sciences, Univ. of Tokyo*, [3]*Grad. School of Engeneering, Univ. of Tokyo*, [4]*Center for AIDS Research*)

11:45　山元　淳平　2P249

1YA1145　(6-4)光回復酵素による 2 光子 DNA 修復の分子メカニズム

Molecular mechanism of the two photon DNA repair by the (6-4) photolyase

○山元　淳平[1], 清水　幸平[1], 藤原　智子[2], 藤堂　剛[2], Plaza　Pascal[3], Brettel　Klaus[4], 岩井　成憲[1]（[1]阪大院基礎工, [2]阪大院医, [3]ENS Paris, France, [4]CEA Saclay, France）

Junpei Yamamoto[1], Kohei Shimizu[1], Tomoko Fujiwara[2], Takeshi Todo[2], Pascal Plaza[3], Klaus Brettel[4], Shigenori Iwai[1] ([1]*Grad. Sch. Eng. Sci., Osaka Univ.*, [2]*Grad. Sch. Med., Osaka Univ.*, [3]*ENS Paris, France*, [4]*CEA Saclay, France*)

12:00　杉村　薫　1P134

1YA1200　組織応力の異方性が細胞の六角格子化を促進する

Anisotropic tissue stress promotes ordering in hexagonal cell packing

○杉村　薫[1], 井川　敬介[1], 石原　秀至[3]（[1]京大, [2]JST・さきがけ, [3]明治大）

Kaoru Sugimura[1], Keisuke Ikawa[1], Shuji Ishihara[3] ([1]*Kyoto Univ.*, [2]*JST PRESTO*, [3]*Meiji Univ.*)

9:45～12:15　B 会場／Room B : Mid-sized Hall 2/2

1SBA　新学術領域研究「理論と実験の協奏による柔らかな分子系の機能の科学」共催
柔らかさが制御する生体分子系の構造形成と機能
Regulating structure formation and function of biomolecular systems with softness

オーガナイザー：北尾 彰朗（東京大学），水谷 泰久（大阪大学）
Organizers: Akio Kitao (The University of Tokyo), Yasuhisa Mizutani (Osaka University)

Biomolecules and their assemblies create and control their functions by softly changing conformations. To investigate sophisticated mechanisms of biomolecular systems to function in condensed, heterogeneous, and highly noisy environments, it is necessary to employ various approaches in concerted fashion. In this symposium, we discuss the mechanisms of structure formation and functional control in soft biological molecular systems, which have been made clear by the cutting-edge theories, measurements, and molecular design.

1SBA-01　*In silico* で観察するタンパク質の柔らかで機能的な運動
Observing soft functional motion of proteins *in silico*
○北尾 彰朗（東京大学分子細胞生物学研究所）
Akio Kitao (*IMCB, Univ. Tokyo*)

1SBA-02　Continuous tracking of protein folding at microsecond resolution by a line confocal detection of single molecule fluorescence
Satoshi Takahashi (*IMRAM, Tohoku Univ.*)

1SBA-03　光応答性タンパク質の機能転換が明らかにする柔らかな構造機能相関
“Soft” structure-function relationship revealed by functional conversion of photoreceptive proteins
○神取 秀樹（名古屋工業大学）
Hideki Kandori (*Nagoya Institute of Technology*)

1SBA-04　酵素活性におけるタンパク質の柔軟性の役割
Crucial Role of Protein Flexibility in Enzymatic Catalysis
○林 重彦（京都大学大学院理学研究科化学専攻）
Shigehiko Hayashi (*Department of Chemistry, Graduate School of Science, Kyoto University*)

1SBA-05　タンパク質の機能を生み出す柔らかさの時間分解観測
Time-resolved Observation of Functionally-important Molecular Flexibility of Proteins
○水谷 泰久（大阪大学大学院理学研究科化学専攻）
Yasuhisa Mizutani (*Grad. Sch. Sci., Osaka Univ.*)

9:45～12:15　C 会場／Room C : Room 107

1SCA　Beyond Biophysics!: 細胞スケールにおける物理・化学・機械的な制御が生み出す新たな生物物理学
Beyond Biophysics!: Up-and-coming biophysical science achieved by physical, chemical and micromechanical control of a cell-sized space

オーガナイザー：瀧ノ上 正浩（東京工業大学），尾上 弘晃（慶應義塾大学），川野 竜司（東京農工大学）
Organizers: Masahiro Takinoue (Tokyo Institute of Technology), Hiroaki Onoe (Keio University), Ryuji Kawano (Tokyo University of Agriculture and Technology)

Living cells are micrometer-sized highly functional molecular systems that are hierarchically self-organized using nanometer-sized molecules. By cooperative characteristics of molecules, the living cells realize dynamic functions such as autonomous information processing, spontaneous motions, self-replication, etc. The recent progress of microtechnologies achieve control of cell-sized tiny space, molecular self-assembly, molecular reaction dynamics, mechanical properties of living cells, etc. The aim of this symposium is a further understanding of dynamical properties of life systems based on these state-of-the-art technologies, and we will discuss the current stage and the future perspectives of these novel biophysical studies.

1SCA-01　人工細胞回路を用いた DNA コンピューティングの実現
DNA computing through biological nanopore in droplet network system
○川野 竜司（東京農工大学）
Ryuji Kawano (*TUAT*)

1SCA-02　カンチレバーを用いて細胞分裂機構を探る
Examining the cell division machinery by using the cantilever system
○板橋 岳志[1], 石渡 信一[1,2]（[1]早大・理工, [2]WABIOS）
Takeshi Itabashi[1], Shin'ichi Ishiwata[1,2] ([1]*Fac. Sci. Eng., Waseda Univ.*, [2]*WABIOS*)

1SCA-03　上皮組織の発生と維持の機械・化学制御の統合的理解に向けて
Toward Understanding the Integration of Mechanical and Chemical Control of Epithelial Development and Maintenance
○杉村 薫[1,2], 梶田 美穂子[3], 藤田 恭之[3], 石原 秀至[4]（[1]京大, [2]JST・さきがけ, [3]北大, [4]明治大）
Kaoru Sugimura[1,2], Mihoko Kajita[3], Yasuyuki Fujita[3], Shuji Ishihara[4] ([1]*Kyoto Univ.*, [2]*JST PRESTO*, [3]*Hokkaido Univ.*, [4]*Meiji Univ.*)

1SCA-04　Mechanically-controlled tubular microenvironment for 3D cell culture
Hiroaki Onoe (*Dept. Mech. Eng., Keio Univ.*)

1SCA-05　MEMS technology meets scaling laws for biology
Ko Okumura (*Ochanomizu University*)

1SCA-06　生物物理学における非平衡研究のためのドロップレットマイクロ流体工学
Droplet-based microfluidics for nonequilibrium study in biophysics
○瀧ノ上 正浩[1,2]（[1]東工大・院総合理工, [2]JST・さきがけ）
Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*, [2]*PRESTO, JST*)

9:45～12:15　D 会場／Room D : Room 108

1SDA　神経ダイナミクスの計測と制御
Measurement and control of neurodynamics

オーガナイザー：伊東 大輔（北海道大学），細川 千絵（産業技術総合研究所）
Organizers: Daisuke Ito (Hokkaido University), Chie Hosokawa (National Institute of Advanced Industrial Science and Technology)

To understand higher functions of the brain, it is important to clarify spatiotemporal dynamics of nervous system at various hierarchy levels. For this purpose, novel approaches are required in the field of Biophysics. In this symposium, we invited young researchers who challenge to measure and control of neurodynamics using unique methods, such as bioimaging, nano fabrication, optical control, and multielectrode recordings. We hope that a fruitful discussion would lead to innovative studies in the future.

1SDA-01 培養神経回路網における同期バースト活動に関わる分子の探索
Analysis of the molecules involved in synchronized burst activity of cultured neuronal networks
○伊東 大輔[1], 郷原 一寿[2]（[1]北大・院先端生命, [2]北大・院工）
Daisuke Ito[1], Kazutoshi Gohara[2] ([1]*Fac. Advanced Life Sci., Hokkaido Univ.*, [2]*Fac. Engineering, Hokkaido Univ.*)

1SDA-02 培養神経細胞・神経回路操作のための表面マイクロ加工技術
Manipulating neurons and neuronal networks with micropatterned surfaces
○山本 英明[1,2], 谷井 孝至[3], 庭野 道夫[4], 平野 愛弓[2]（[1]東北大・学際研, [2]東北大・医工, [3]早大・基幹理工, [4]東北大・通研）
Hideaki Yamamoto[1,2], Takashi Tanii[3], Michio Niwano[4], Ayumi Hirano-Iwata[2] ([1]*FRIS, Tohoku Univ.*, [2]*Grad. Sch. Biomed. Eng., Tohoku. Univ.*, [3]*Sch. Fund. Sci. Eng., Waseda Univ.*, [4]*RIEC, Tohoku Univ.*)

1SDA-03 Noninvasive real-time measurement of dopamine, action potentials, and postsynaptic potentials using carbon nanotube electrodes chip
Ikuro Suzuki (*Department of Electronics, Tohoku Institute of Technology*)

1SDA-04 神経ダイナミクス解明のためのレーザー摂動技術の開発
Laser-induced perturbation into living neuronal networks: Toward understanding neurodynamics
○細川 千絵（産総研・健康工学）
Chie Hosokawa (*Health Res. Inst., AIST*)

1SDA-05 Revealing Neuronal Dynamics through Advanced Electrophysiology and Chemical Sensing using CMOS Technology
Urs Frey[1,2], Marie Engelene Obien[1], Florent Seichepine[1], Kosmas Deligkaris[1,2] ([1]*RIKEN Quantitative Biology Center*, [2]*Graduate School of Frontier Biosciences, Osaka University*)

1SDA-06 培養神経回路網における情報表現
Information presentation in cultured neuronal networks
○工藤 卓（関西学院大学 理工学部 人間システム工学科）
Suguru N. Kudoh (*Department of Human System Interaction, School of Science and Technology, Kwansei Gakuin University*)

1SDA-07 聴覚皮質における聴覚神経応答の解析及び神経ダイナミクス制御のためのマイクロデバイス開発
Analysis of auditory neural responses in the auditory cortex in vivo and development of microdevices to control neurodynamics
○西川 淳, 羽賀 健亮, 橘 唯至, 柳川 康貴, 舘野 高（北海道大学 情報科学研究科）
Jun Nishikawa, Takeaki Haga, Yuishi Tachibana, Yasutaka Yanagawa, Takashi Tateno (*Grad. Sch. of Inf. Sci. & Tech., Hokkaido Univ.*)

9:45～12:15　E 会場／Room E : Room 204

1SEA　脂質ラフトはどこまで分かったのか：新しい研究手法による再検証
What do we know about lipid rafts?: New landscape at the frontier

オーガナイザー：鈴木 健一（京都大学），森垣 憲一（神戸大学）
Organizers: Kenichi Suzuki (Kyoto University), Kenichi Morigaki (Kobe University)

The hypothesis of “lipid rafts” is now widely accepted as the basis for understanding cell membranes. However, their structures, properties, and biological functions still remain elusive. The present symposium intends to give an overview of the current understanding on the membrane heterogeneity and micro- or nano-domains in cells. We will be discussing on the most up-to-date views from recent studies using cellular membranes and model systems. By looking out on the landscape at the frontier, we seek to find new directions for future studies.

1SEA-01 GPI アンカー型タンパク質とガングリオシドの 1 分子追跡により明らかになったラフト組織化
Single-molecule tracking of GPI-anchored proteins and gangliosides revealed raft organization
○鈴木 健一（京大・iCeMS）
Kenichi G. N. Suzuki (*iCeMS, Kyoto Univ.*)

1SEA-02 細胞質分裂におけるスフィンゴミエリンラフトの役割
A role for sphingomyelin-rich lipid domains during cytokinesis
阿部 充宏, ○小林 俊秀（独立行政法人・理化学研究所）
Mitsuhiro Abe, **Toshihide Kobayashi** (*RIKEN*)

1SEA-03 Lipid Rafts and Membrane Proteins Collaborate to Organize and Shape Biological Membranes
Jeanne Stachowiak (*The University of Texas at Austin*)

1SEA-04 リポソームの膜内相分離における外場の影響
Phase separation on cell-sized liposomes in the presence of external force
○柳澤 実穂（東京農工大・工・先端物理）
Miho Yanagisawa (*Dept. Appl. Phys., Tokyo Univ. Agric. Technol.*)

1SEA-05 パターン化人工膜を用いた膜タンパク質のラフト親和性解析
Micropatterned model membrane for studying the affinity of proteins to lipid raft
○森垣 憲一, 谷本 泰士, 岡田 文子, 林 文夫（神戸大学）
Kenichi Morigaki, Yasushi Tanimoto, Fumiko Okada, Fumio Hayashi (*Kobe University*)

16:00～18:30　A 会場／Room A : Mid-sized Hall 1/2

1SAP　新学術領域研究「運動超分子マシナリーが織りなす調和と多様性」共催
マルチスケールに活躍する運動超分子マシナリー
Supramoleular motility machinery functioning in multi-scale scenes

オーガナイザー：中村 修一（東北大学），島袋 勝弥（宇部工業高等専門学校）
Organizers: Shuichi Nakamura (Tohoku Uiversity), Katsuya Shimabukuro (Ube National College of Technology)

A life system is operated by diverse supramolecular motility machineries which are finely organized functional units and allow cells intracellular material transportation and motility. In this symposium, we will invite researchers investigating the structure and operation mechanism of the motility machinery using various methodologies including structural analysis, nanophotometry, theoretical model, and molecular biological technique. We would like to present interesting and novel insights into the supramolecular motility machinery functioning in multi-scale scenes from division of genes to collective behavior.

1SAP-01 線虫精子のアメーバ運動と MSP マシナリー
Nematode sperm motility and MSP machinery
○島袋 勝弥（宇部高専・物質）
Katsuya Shimabukuro (*UNCT*)

1SAP-02 ミドリムシにおける光運動制御マシナリーの解明
Molecular machinery regulating photomovement of *Euglena*
○岩崎 憲治[1,2], 宮崎 直幸[1,2], 伊関 峰生[3], 長谷川 浩司[4], 成田 哲博[5], 松永 茂[6], 建部 益美[6], 上村 慎治[7], 渡辺 正勝[8]（[1]阪大・蛋白研, [2]生理研, [3]東邦大・薬, [4]アドバンスソフト, [5]名大・院理, [6]浜松ホトニクス（株）, [7]中大・理工, [8]光創大院）
Kenji Iwasaki[1,2], Naoyuki Miyazaki[1,2], Mineo Iseki[3], Koji Hasegawa[4], Akihiro Narita[5], Shigeru Matsunaga[6], Masumi Takebe[6], Shinji Kamimura[7], Masakatsu Watanabe[8] ([1]*Inst. for Protein Res., Osaka Univ.*, [2]*NIPS*, [3]*Pharm.Sci, Toho Univ.*, [4]*AdvanceSoft Corp.*, [5]*Struct.Biol.Res.Center, Nagoya Univ.*, [6]*Hamamatsu Photonics K.K.*, [7]*Fac.Sci.Eng., Chuo Univ.*, [8]*Grad.Sch. for the Creation of New Photonics Industry*)

1SAP-03 チューブリン様蛋白質 TubZ によるプラスミド分配の分子機構
Plasmid segregation driven by the tubulin-like GTPase TubZ
○林 郁子（横浜市立大学）
Ikuko Hayashi (*Yokohama City University*)

1SAP-04 黄色ブドウ球菌のコロニースプレッディングにおける毒素の役割
Role of toxin in *Staphylococcus aureus* colony spreading
○垣内 力, 関水 和久（東京大学 大学院薬学系研究科 微生物薬品化学）
Chikara Kaito, Kazuhisa Sekimizu (*Grad. Sch. Phar., Univ. Tokyo*)

1SAP-05 らせん細菌 *Leptospira* の遊泳力学とエネルギー論
Swimming dynamics and energetics of the spirochete *Leptospira*
○中村 修一（東北大・院工）
Shuichi Nakamura (*Grad. Sch. Eng., Tohoku Univ.*)

1SAP-06 Mechanical basis for the bacterial swimming and gliding
Hirofumi Wada (*Dep. Phys. Ritsumeikan Univ.*)

16:00～18:30　B 会場／Room B : Mid-sized Hall 2/2

1SBP　次世代タンパク質結晶化手法
The development of new crystallization methods for bio-macromolecular crystallography

オーガナイザー：田之倉 優（東京大学），姚 閔（北海道大学）
Organizers: Masaru Tanokura (The University of Tokyo), Min Yao (Hokkaido University)

Recent progress in the techniques of bio-macromolecular crystallography has made crystal structure analysis more powerful and useful method for life science. However, crystallization still remains as a major bottleneck for determining bio-macromolecular structures. Thus, further development of more advanced crystallization methods is required to increase the probability of successful crystallization. As one of the events related to International Year of Crystallography 2014, we have organized this symposium, and invited speakers who are internationally active in this field. We will discuss current hot topics and new ideas for future development.

1SBP-01 凝固したハイドロゲルを用いたタンパク質結晶の成長と特徴
Growth and characterization of protein crystals using high-strength hydrogels
○杉山 成[1,2]（[1]大阪大学大学院理学研究科化学専攻, [2]JST, ERATO脂質活性プロジェクト）
Shigeru Sugiyama[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*JST, ERATO, Lipid Active Structure Project*)

1SBP-02 微小重力下の結晶化
Protein crystallization under microgravity conditions
○田之倉 優, 中村 顕（東大・院農生科・応生化）
Masaru Tanokura, Akira Nakamura (*Dept. Appl. Biol. Chem., Grad. Sch. Agric. Life Sci., Univ. Tokyo*)

1SBP-03 結晶スポンジ法による非結晶性・極少量化合物の X 線結晶構造解析
Crystalline Sponge Method: X-ray Analysis without Crystallization on the Microgram Scale
○藤田 誠（東京大学）
Makoto Fujita (*The University of Tokyo*)

1SBP-04 膜タンパク質の結晶化法
Crystallization methods of membrane proteins
○村田 武士[1,2]（[1]千葉大・理, [2]JST・さきがけ）
Takeshi Murata[1,2] ([1]*Science/Chiba-U*, [2]*PRESTO/JST*)

1SBP-05 抗体を用いた膜蛋白質の結晶化
Crystallization of membrane proteins using antibody fragments
○岩田 想[1,2]（[1]京大院・医, [2]理研・放射光科学総合研究センター）
So Iwata[1,2] ([1]*Kyoto Univ. Grad. Sch. Med.*, [2]*RIKEN SPring8 Center*)

1SBP-06 対称性を持つタグを利用したタンパク質結晶化確率の向上
Use of symmetric tag to increase the probability of protein crystallization
○姚 閔（北海道大学大学院先端生命科学研究院）
Min Yao (*Fac. of Adv. Life Sci., Hokkaido Univ.*)

16:00～18:30　C 会場／Room C : Room 107

1SCP　あたかも生物のように動く非線形化学物理系
Nonlinear physico-chemical systems moving like living organisms

オーガナイザー：高木 清二（はこだて未来大学），住野 豊（東京理科大学），北畑 裕之（千葉大学）
Organizers: Seiji Takagi (Future University Hakodate), Yutaka Sumino (Tokyo University of Science), Hiroyuki Kitahata (Chiba University)

Recently, the motion of living organisms has been studied from the viewpoint of "active matters" in physics. To have better understanding on underlying physics, “simpler” physico-chemical systems have also been investigated rigorously. In this symposium, some examples of such “simpler” systems will be introduced, and we would like to discuss the similarities as well as differences among these physico-chemical systems and actual living systems. Through the discussion, we try to find advantages as well as critical problems in the studies on active matters based on physico-chemical systemsm, and finally hope to show a new direction of the researches.

1SCP-01　はじめに
○北畑 裕之（千葉大院理）
Hiroyuki Kitahata (*Grad. Scl. of Sci., Chiba Univ.*)

1SCP-02　なぜその材料はアクティブマターになるのか？ ～Dupeyrat システムに関する考察
A Material Discussion about the Nakache and Dupeyrat System
○松下 祥子（東京工業大学）
Sachiko Matsushita (*Tokyo Institute of Technology*)

1SCP-03　拮抗剤を介した自己推進型液滴の方向感知機能
Directional sensing of self-propelled droplets mediated by antagonists
○伴 貴彦, 中田 大樹, 谷 健太郎（阪大基礎工）
Takahiko Ban, Hiroki Nakata, Kentaro Tani (*Osaka University*)

1SCP-04　Self-propelled water droplet coupled with chemical oscillatory reaction
Nobuhiko Suematsu[1,2] ([1]*Graduate School of Advanced Mathematical Sciences, Meiji University*, [2]*Meiji Institute for Advanced Study of Mathematical Sciences (MIMS), Meiji University*)

1SCP-05　界面張力勾配に駆動される自己推進液滴のモード分岐
Mode bifurcation on a self-propelled droplet driven by interfacial tension gradient
○高畠 芙弥[1], 市川 正敏[2], 吉川 研一[3]（[1]東北大院工, [2]京大院理, [3]同志社大生命医）
Fumi Takabatake[1], Masatoshi Ichikawa[2], Kenichi Yoshikawa[3] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*Grad. Sch. Sci., Kyoto Univ.*, [3]*Grad. Sch. Life Med. Sci., Doshisha Univ.*)

1SCP-06　水路形状およびマランゴニ流構造に依存した自律運動システム
Self-propelled system depends on the structureof Marangoni flow and the shape of water chamber
○松田 唯, 中田 聡（広大院理）
Yui Matsuda, Satoshi Nakata (*Hiroshima Univ.*)

1SCP-07　界面活性剤の会合体生成に誘起される油水界面のアメーバ状運動
Amoeba like motion of the oil-water interface induced by generation of surfactant aggregate
○住野 豊（東京理科大学理学部応用物理学科）
Yutaka Sumino (*Department of Applied Physics, Faculty of Science, Tokyo University of Science*)

1SCP-08　ある巨大アメーバ生物のアメーバ運動
Amoeboid movement of a large amoeboid organism, true slime mold
○高木 清二（はこだて未来大）
Seiji Takagi (*Future Univ. Hakodate*)

16:00〜18:30　D 会場／Room D : Room 108

1SDP　In Cell NMR と HPC が切り開く細胞内の蛋白質の動きと機能
Protein Dynamics and Function in Cells elucidated by in-cell NMR and High Performance Computing

オーガナイザー：杉田 有治（理化学研究所），伊藤 隆（首都大学東京）
Organizers: Yuji Sugita (RIKEN), Yutaka Ito (Tokyo Metropolitan University)

In living cells, a variety of soluble macromolecules exist in a very crowded environment. Recent advancements in in situ observations by NMR and in large scale simulations using K computer have been contributing to investigate the various effects perturbing proteins' structures, dynamics and folding stabilities as well as the mechanisms permitting proteins to find their binding partners efficiently under the macromolecular crowding. In this symposium, we would overview the recent progresses and discuss future perspectives of the biophysical researches under intracellular environments in the field of life science.

1SDP-01　分子間五次相互作用が細胞内でのタンパク質安定性を制御する
Intermolecular Quinary Interactions Modulate Protein Stability in Living Cells
○パイラック ガリー ジェイ（ノースカロライナ大学）
Gary J. Pielak (*University of North Carolina*)

1SDP-02　分子混雑環境における蛋白質の NMR 緩和解析
NMR relaxation analysis of the protein under macromolecular crowding environment
○岡村 英保, 木川 隆則（理研・生命システム研究センター）
Hideyasu Okamura, Takanori Kigawa (*QBiC, RIKEN*)

1SDP-03　In-cell NMR 法による細胞内タンパク質の構造多様性解析
In-cell NMR analysis for protein conformational diversity in a cell
○猪股 晃介（独立行政法人 理化学研究所 生命システム研究センター）
Kohsuke Inomata (*Quantitative Biology Center (QBiC), RIKEN*)

1SDP-04　生きた細胞中の天然変性蛋白質の動態
Dynamics of intrinsically disordered proteins in living cells
○池谷 鉄兵, 井上 仁, 伊藤 隆（首都大学東京）
Teppei Ikeya, Jin Inoue, Yutaka Ito (*Tokyo Metropolitan University*)

1SDP-05　ゲノムと立体構造を結合したシミュレーション解析
Combining Structure with Genomics
○ファイグ マイケル, アスリ イードリム（ミシガン州立大学）
Michael Feig, Yildirim Asli (*Michigan State University*)

16:00〜18:30　E 会場／Room E : Room 204

1SEP　システム協同性が操る神経細胞機能
Cooperativity in shaping the nerve cell function

オーガナイザー：村越 秀治（生理学研究所），合田 裕紀子（理化学研究所）
Organizers: Hideji Murakoshi (National Institute for Physiological Sciences), Yukiko Goda (RIKEN)

The cooperative interactions between signaling molecules/cascades, subcellular compartments, or cells are integral to the flexible and plastic cellular functions underlying biological processes. This symposium addresses how cooperativity shapes cellular responses and functions by focusing on the nervous system. The speakers will discuss cooperativity involving molecular interactions in neurons, the interplay of neuronal sub-compartments such as synapses and dendritic branches and neuronal networks in supporting the unique structural and functional features of the nervous system.

1SEP-01　CaMKII によって活性化された Rho GTPase の協同的作用によるシナプス可塑性誘起
CaMKII-induced active Rho GTPases cooperatively work for the establishment of synaptic structural plasticity
○村越 秀治[1,2]（[1]生理学研究所, [2]科学技術振興機構さきがけ）
Hideji Murakoshi[1,2] ([1]*National Institute for Physiological Sciences*, [2]*JST PREST*)

1SEP-02 The basis for regulating synaptic strength heterogeneity across the dendrite
Mathieu Letellier, **Yukiko Goda** (*RIKEN Brain Science Institute*)

1SEP-03 神経細胞軸索の細胞膜にある 2 次元拡散障壁は分子選択性フィルターである
Diffusion barrier in the neuronal axon initial-segment membrane is a molecule-selective filter in the plasma membrane
○楠見 明弘[1,2], 宮原 愛美[1], 藤原 敬弘[1]（[1]京大・iCeMS, [2]京大・再生研）
Akihiro Kusumi[1,2], Manami S.H. Miyahara[1], Takahiro K. Fujiwara[1] ([1]*iCeMS, Kyoto Univ.*, [2]*Inst. for Frontier Med. Sci., Kyoto Univ.*)

1SEP-04 Activity-dependent gene expression in learning and memory
Ryang Kim[1,2], Mio Nonaka[1], Nan Yagishita-Kyo[1,2], Takashi Kawashima[1], Masatoshi Inoue[1,2], Yuichiro Ishii[1,2], Toshihiro Endo[1], Hajime Fujii[1], Sayaka Takemoto-Kimura[1], Hiroyuki Okuno[1,3], **Haruhiko Bito**[1,2] ([1]*Department of Neurochemistry, The University of Tokyo Graduate School of Medicine*, [2]*CREST-JST*, [3]*Medical Innovation Center, Kyoto University Graduate School of Medicine*)

1SEP-05 The cooperativity of neuronal molecules analyzed with imaging mass spectrometry
Mitsutoshi Setou (*Hamamatsu University School of Medicine*)

1SEP-06 Modeling the dynamical interaction of Hebbian and homeostatic plasticity
Taro Toyoizumi[1,2], Megumi Kaneko[3], Michael P. Stryker[3], Kenneth D. Miller[2] ([1]*RIKEN Brain Sci. Inst.*, [2]*Columbia Univ.*, [3]*UCSF*)

第 2 日目（9 月 26 日（金））／Day 2（Sep. 26 Fri.）

9:00～11:30　A 会場／Room A : Mid-sized Hall 1/2
2SAA　膜動態から探るミトコンドリア・ネオバイオロジー
Mitochondrial neo-biology explored from a membrane dynamics

オーガナイザー：小柴 琢己（九州大学），遠藤 斗志也（京都産業大学）
Organizers: Takumi Koshiba (Kyushu University), Toshiya Endo (Kyoto Sangyo University)

Mitochondrion, a double-membraned organelle, is the powerhouse of eukaryotic cells and is controlling essential biological processes such as generating ATP. Recent genetic studies in many model species have demonstrated that mitochondria are not just limited to respiration and apoptosis, and establishing fundamental aspects with physiological relevance. In the symposium, we invited investigators who study a broad range of species from yeast to mammals, and they will discuss current topics regarding new aspects of mitochondrial functions.

2SAA-01 ミトコンドリアと抗 RNA ウイルス自然免疫
Mitochondria and antiviral innate immunity in mammals
○小柴 琢己（九州大・院・理・生物科学）
Takumi Koshiba (*Dep. of Biol., Faculty of Sci., Kyushu Univ.*)

2SAA-02 カルシニューリンと Notch シグナリングを介してミトコンドリアの融合は心筋細胞の分化に必須である
Mitochondrial fusion controls differentiation of ESCs into cardiac cells via a novel pathway of calcineurin and Notch signaling
○笠原 敦子[1], スコラーノ ルカ[2]（[1]ジュネーブ大学, CMU, [2]パドヴァ大学）
Atsuko Kasahara[1], Luca Scorrano[2] ([1]*University of Geneva, CMU*, [2]*University of Padua, Dep. of Biology*)

2SAA-03 ミトコンドリア呼吸鎖の多様性：寄生虫からがん細胞まで
Diversity of mitochondrial respiratory chain from parasite to cancer
○北 潔（東京大学大学院医学系研究科・生物医化学教室）
Kiyoshi Kita (*Dept of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo*)

2SAA-04 オートファジーが駆動するミトコンドリア分解の仕組み
Targeting Autophagy for Mitochondrial Clearance
○岡本 浩二（阪大・院生命機能）
Koji Okamoto (*Grad. Sch. Frontier Biosci., Osaka Univ.*)

2SAA-05 酵母ミトコンドリアにおけるタンパク質と脂質の輸送機構
Mechanisms of protein and lipid transport in yeast mitochondria
○遠藤 斗志也（京産大・総合生命）
Toshiya Endo (*Fac. Life Sci., Kyoto Sangyo Univ.*)

9:00～11:30　B 会場／Room B : Mid-sized Hall 2/2

2SBA　日本顕微鏡学会合同シンポジウム：原子レベル分解能へ向かう生物電子顕微鏡技術
Joint Symposium with the Japanese Society of Microscopy: Recent Advancement of Electron Microscopy toward Atomic Resolution from Biological Molecules

オーガナイザー：宮澤 淳夫（兵庫県立大学），光岡 薫（次世代天然物研究組合）
Organizers: Atsuo Miyazawa (University of Hyogo), Kaoru Mitsuoka (JBIC)

Recently, several techniques for high-resolution electron microscopy, direct detectors, Cs corrector, phase plates, and so on, became commercially available and some of them are now applied to structural analysis of biological macromolecules and their complexes. As the result, atomic model of a membrane protein was determined using single particle analysis, for example. In this session, which is co-hosted by Japanese Society of Microscopy, these recent advancements and their application to biological samples toward atomic resolution will be discussed.

2SBA-01 電子顕微鏡法により示された線虫イネキシン 6 ギャップ結合チャネルの特徴的なサブユニット構成
Electron microscopy of C. elegans innexin-6 gap junction channels indicates a characteristic subunit organization
○大嶋 篤典[1], 松澤 朋寛[2], 村田 和義[3], 西川 幸希[1], 藤吉 好則[1]（[1]名大・CeSPI, [2]京大・院理・生物物理, [3]生理研）
Atsunori Oshima[1], Tomohiro Matsuzawa[2], Kazuyoshi Murata[3], Kouki Nishikawa[1], Yoshinori Fujiyoshi[1] ([1]*CeSPI, Nagoya Univ.*, [2]*Dept. of Biophys., Grad. Sch. Sci., Kyoto Univ.*, [3]*NIPS*)

2SBA-02 再構成系および細胞内でのアクチンフィラメント構造解析
Structural analysis of the actin filament in vitro and in vivo
○成田 哲博[1,2]（[1]名古屋大学, [2]科学技術振興機構さきがけ）
Akihiro Narita[1,2] ([1]*Nagoya Univ.*, [2]*PRESTO*)

2SBA-03 Single particle analysis of the model post-termination complex gives insights into prokaryotic ribosome recycling process
Takeshi Yokoyama (*RIKEN, CLST*)

2SBA-04 単粒子クライオ電子顕微鏡法による 8 Å 分解能サポウイルスキャプシド構造とホモロジーモデリング
Sapovirus capsid structure at 8 Å resolution by single particle cryo-electron microscopy, and homology modeling
宮崎 直幸[1], テーラー デービッド[1], ハウスマン グラント[2], 村上 耕介[2], 片山 和彦[2], ○村田 和義[1]（[1]生理学研究所, [2]国立感染症研究所）
Naoyuki Miyazaki[1], David Taylor[1], Grant Houseman[2], Kousuke Murakami[2], Kazuhiko Katayama[2], **Kazuyoshi Murata**[1] ([1]*National Institute for Physiological Sciences*, [2]*National Institute of Infectious Diseases*)

2SBA-05 Single molecular imaging and single atom spectroscopy by electron microscopy
Kazutomo Suenaga (*AIST*)

2SBA-06 低温電子顕微鏡を用いた単粒子解析による膜たんぱく質の研究
Studying integral membrane protein by single particle cryo-EM
○Cheng Yifan（カルフォルニア大学サンフランシスコ校）
Yifan Cheng (*Dep. Biochem. Biophys., UCSF*)

9:00～11:30　C 会場／Room C : Room 107

2SCA　日本-中国-台湾　若手合同シンポジウム～超分子協同性～
Japan-China-Taiwan joint sympoium on cooperativity in supramolcular machine

オーガナイザー：林 久美子（東北大学），福岡 創（東北大学）
Organizers: Kumiko Hayashi (Tohoku University), Hajime Fukuoka (Tohoku University)

Compositional units of a biological supramolecular machine such as flagellar motor and F1-ATPase exhibit the cooperative property to enhance the efficiency of their motion. We discuss the property in terms of the results obtained by using fluorescent observation, AFM observation, electron microscopic observation and MD simulation. In the symposium, young scientists from China and Taiwan also give talks as well as Japanese researchers. We believe scientific communication among young Asian scientists keep Biophysical Society of Japan more alive.

2SCA-01　Regulation of the rotational switching of bacterial flagellar motor by binding of an intracellular signaling protein CheY
Hajime Fukuoka[1], Takashi Sagawa[2], Yuichi Inoue[1], Hiroto Takahashi[1], Akihiko Ishijima[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Life Sci., Tohoku Univ.*)

2SCA-02　Visualizing stator-protein distributions of bacterial flagellar motors
Chien-Jung Lo[1,2], Tsaishun Lin[1,2] ([1]*Dept. of Phys., National Central Univ.*, [2]*Inst. Biophys., National Central Univ.*)

2SCA-03　細菌べん毛モーターの回転方向変換制御に関わる構造
Structure of the bacterial flagellar motor involved in the directional switching mechanism
○宮田 知子[1], 加藤 貴之[1], 森本 雄輔[1,2,3], 中村 修一[4], 松波 秀行[5], 難波 啓一[1,2]（[1]大阪大学大学院 生命機能研究科, [2]理研・生命システム, [3]阪大院・理, [4]東北大・工, [5]沖縄科技大・細胞膜通過輸送研究ユニット）
Tomoko Miyata[1], Takayuki Kato[1], Yusuke V. Morimoto[1,2,3], Syuichi Nakamura[4], Hideyuki Matsunami[5], Keiichi Namba[1,2] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*QBiC, RIKEN*, [3]*Grad. Sch. Sci., Osaka Univ.*, [4]*School of Engineering, Tohoku Univ.*, [5]*Trans-Membrane Trafficking Unit, OIST*)

2SCA-04　Conformational Spread as a Mechanism for Cooperativity in the Bacterial Flagellar Switch
Fan Bai (*Sch. Life Sci., Peking Univ.*)

2SCA-05　Coordination and control in the ring-shaped molecular motors
Jin Yu (*Beijing Computational Science Research Center*)

2SCA-06　高速原子間力顕微鏡によるリング状 ATPase の協同的構造変化の観察
Cooperative Conformational Change of Ring-Shape ATPase Observed by High-Speed AFM
○内橋 貴之[1,2], 飯野 亮太[3], 渡辺 洋平[4], 野地 博行[5], 安藤 敏夫[1,2]（[1]金沢大・理工, [2]金大・理工・バイオAFMセンター, [3]岡崎統合バイオ, [4]甲南大・生物, [5]東大院・工）
Takayuki Uchihashi[1,2], Ryota Iino[3], Yo-hei Watanabe[4], Hiroyuki Noji[5], Toshio Ando[1,2] ([1]*Coll.Sci. & Eng., Kanazawa Univ.*, [2]*Bio-AFM FRC, Coll.Sci. & Eng., Kanazawa Univ.*, [3]*Okazaki Inst. Integr. Biosci., NINS*, [4]*Dept. Biol., Konan Univ.*, [5]*Sch. Eng., Univ. Tokyo*)

9:00～11:30　D 会場／Room D : Room 108

2SDA　新学術領域研究「動的クロマチン構造と機能」共催

生命現象の基本に迫る動的クロマチン構造・機能研究の最前線

Studies of dynamic chromatin structure and function to understand fundamentals of life

オーガナイザー：原口 徳子（(独）情報通信研究機構 未来 ICT 研究所），徳永 万喜洋（東京工業大学）

Organizers: Tokuko Haraguchi (National Institute of Information and Communications Technology), Makio Tokunaga (Tokyo Institute of Technology)

Chromatin, which consists of DNA and proteins, plays a vital role in not only genetic activities but also biological functions. Its structure is not invariable but spatio-temporally varying in response to functions. This dynamic structural change of the chromatin is the very thing that is the basis of the biological functions and activities. Current cutting-edge researches will be presented in wide research fields, structural biology, biophysics, imaging, cell biology and so on. We discuss the basis and meaning of chromatin dynamics.

はじめに

2SDA-01　クロマチン動構造とヒストンバリアント

Structural basis of chromatin dynamics regulated by histone variants

○胡桃坂 仁志（早稲田大学理工学術院 先進理工学部）

Hitoshi Kurumizaka (*Waseda University, Faculty of Science and Engineering*)

2SDA-02　統合的イメージングアプローチによる動的クロマチン構造・機能研究

Integrated imaging approach to the study of dynamics of chromatin

○十川 久美子[1,2], 伊藤 由馬[1,2], 深川 暁弘[1], 原田 昌彦[3], 木村 宏[4], 徳永 万喜洋[1,2]（[1]東工大・院生命理工, [2]理研・統合生命医セ, [3]東北大・院農学, [4]阪大院・生命機能）

Kumiko Sakata-Sogawa[1,2], Yuma Ito[1,2], Akihiro Fukagawa[1], Masahiko Harata[3], Hiroshi Kimura[4], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*, [3]*Grad. Sch. Agr. Sci., Tohoku Univ.*, [4]*Grad. Sch. Front. Biosci. Osaka Univ.*)

2SDA-03　塩濃度変化に対する天然クロマチンファイバーの高次構造変化の直接観察

Direct observation of the higher-order structural changes of native chromatin fibers for the change of the salt concentration

○小穴 英廣[1], 西川 香里[1], 松原 央達[2], 山本 歩[2,3], 山本 孝治[4], 原口 徳子[4,5], 平岡 泰[4,5], 鷲津 正夫[1]（[1]東大院・工・機械, [2]静大院・創造科学, [3]静大・理・化学, [4]情報通信研究機構 未来ICT研, [5]阪大院・理・生物科学）

Hidehiro Oana[1], Kaori Nishikawa[1], Hirotada Matsuhara[2], Ayumu Yamamoto[2,3], Takaharu G. Yamamoto[4], Tokuko Haraguchi[4,5], Yasushi Hiraoka[4,5], Masao Washizu[1] ([1]*Dept. of Mech. Eng., The Univ. of Tokyo*, [2]*Grad. Sch. of Sci. and Tech., Shizuoka Univ.*, [3]*Dept. of Chem., Shizuoka Univ.*, [4]*Adv. ICT Res. Inst., NICT*, [5]*Dept. of Biol. Sci., Osaka Univ.*)

2SDA-04　ヒストンとＲＮＡポリメラーゼの翻訳後修飾の生細胞・生体内計測

Monitoring histone and RNA polymerase modification dynamics in living cells and organisms

○木村 宏（阪大・生命機能）

Hiroshi Kimura (*Grad Sch Frontier Biosci, Osaka Univ.*)

2SDA-05　HP1 をとおして見えてきたヘテロクロマチンの構造と機能

Elucidation of construction and function of heterochromatin through HP1 binding proteins

○小布施 力史（北海道大学 大学院先端生命科学研究院）

Chikashi Obuse (*Grad. Sch. Life, Hokudai*)

2SDA-06　相同組換え修復における損傷クロマチン動態

Nuclear topography of homologous recombinational repair

○田代 聡（広大・原医研）

Satoshi Tashiro (*RIRBM, Hiroshima Univ.*)

2SDA-07 核膜形成における核膜タンパク質とクロマチンの動的相互作用の役割
A Role of Dynamic Interaction of Nuclear Membrane Proteins with Chromatin on the Nuclear Envelope Assembly
○原口 徳子[1,2,3], 小林 昇平[1], 荒神 尚子[1], 小坂田 裕子[1], 糀谷 知子[1,4], 森 知栄[1], 平岡 泰[1,2,3]（[1]情報通信研・未来ICT研, [2]阪大・院生命機能, [3]阪大・院理学, [4]日本女子大）
Tokuko Haraguchi[1,2,3], Shouhei Kobayashi[1], Takako Koujin[1], Hiroko Osakada[1], Tomoko Kojidani[1,4], Chie Mori[1], Yasushi Hiraoka[1,2,3] ([1]*Advanced ICT Res. Inst. Kobe, NICT*, [2]*Grad. Sch. Front. Biosci., Osaka Univ.*, [3]*Grad. Sch. Sci., Osaka Univ.*, [4]*Japan Women's Univ.*)

9:00～11:30　E 会場／Room E : Room 204

2SEA　ポンプとチャネルはどちらが偉いのか
Which is important for biophysicists, pump or channel?

オーガナイザー：飯野 亮太（自然科学研究機構岡崎統合バイオサイエンスセンター），須藤 雄気（岡山大学）
Organizers: Ryota Iino (Okazaki Inst. Integ. Biosci., NINS), Yuki Sudo (Okayama University)

Membrane transporters such as pumps and channels are splendid molecular machines, and have fascinated many biophysicists for a long time because of their importance for the general understanding of the energy conversion. In this symposium, speakers will discuss the operative mechanisms of the transporters based on the results of structural analysis, single-molecule measurements, molecular simulations, creation of chimeric molecules, and cellular regulation. Particularly, each speaker will focus on their biophysical significance to gain support from the audience, and the common and individual principles of membrane transporters will be discussed together.

2SEA-01 Importance of membrane pumps and channels: an introduction
Ryota Iino[1,2,3] ([1]*Okazaki Inst. Integ. Biosci., NINS*, [2]*IMS, NINS*, [3]*Dept. of Functional Molecular Science, SOKENDAI*)

2SEA-02 VoV1 の中心回転軸における巧妙なトルク伝達機構
The ingenious structure of central rotor apparatus in VoV1; torque transmission mechanism in the central rotor of VoV1
○横山 謙（京都産業大学）
Ken Yokoyama (*Kyoto Sangyo University*)

2SEA-03 イオンポンプとの比較による、塩化物イオンチャネル CFTR の作動機構研究
Studies on the mechanism of a chloride channel CFTR in comparison with ion pumps
○政池 知子[1,2], 相馬 義郎[3]（[1]東京理科大・応用生物科学, [2]科学技術振興機構・さきがけ, [3]慶應義塾大・医学部薬理学）
Tomoko Masaike[1,2], Yoshiro Sohma[3] ([1]*Dept. Applied Biol. Science, Tokyo Univ. Science*, [2]*PRESTO, JST*, [3]*Dept. Pharmacology, School of Medicine, Keio Univ.*)

2SEA-04 多剤排出トランスポーターの薬剤取込経路の粗視化シミュレーション研究
Drug uptake pathways in multi-drug transporter studied by coarse-grained simulations
○高田 彰二（京大理 生物物理）
Shoji Takada (*Grad. Sch Sci, Kyoto Univ.*)

2SEA-05 脂質はイオンチャネルのゲート開閉をどの様に制御するのか？：新奇脂質センサーによる制御機構
How do lipids regulate the gating activity of the channel protein? : Mechanism of a novel type of the lipid sensor
○岩本 真幸, 老木 成稔（福井大・医・分子生理）
Masayuki Iwamoto, Shigetoshi Oiki (*Dept. Mol. Physiol. Biophys., Univ. Fukui Facult. Med. Sci.*)

2SEA-06 細菌多剤排出トランスポーターの制御と生理機能
Regulation and physiological function of bacterial multidrug transporters
○西野 邦彦（大阪大学産業科学研究所 感染制御学研究分野）
Kunihiko Nishino (*ISIR, Osaka Univ.*)

2SEA-07 光駆動イオンポンプから光開閉性イオンチャネルへの機能変換
Converting a light-driven ion pump into a light-gated ion channel
○須藤 雄気（岡大・院医歯薬(薬)）
Yuki Sudo (*Div. of Parm. Sci., Okayama Univ.*)

16:15〜18:45　A 会場／Room A : Mid-sized Hall 1/2

2SAP　新学術領域研究「少数性生物学―個と多数の狭間が織りなす生命現象の探求―」共催
少数性、数揺らぎが創出する機能のシナリオ
Scenario of functions from minority and number fluctuations

オーガナイザー：小松崎 民樹（北海道大学），永井 健治（大阪大学）
Organizers: Tamiki Komatsuzaki (Hokkaido University), Takeharu Nagai (Osaka University)

In intracellular environment, the number of proteins in each species is from just only a few to several thousands, which often takes a positively-skewed asymmetric distribution over single cells. Some proteins may not be described by the concept of concentration, in which discreteness in their numbers may matter, yielding individuality in molecules. Cellular individuality also exists with diverse, different numbers of proteins in each cell with same kinds of proteins. We will discuss possible roles of minorities and discreteness of numbers and, molecular and cellular individuality.

2SAP-01 分子個性と少数性
Molecular Individuality and Minority in Biology
○小松崎 民樹（北海道大学 電子科学研究所 分子生命数理研究分野）
Tamiki Komatsuzaki (*Hokkaido Univ., Res. Inst. Electronic Sci.*)

2SAP-02 触媒反応ネットワーク系における少数分子と競合
Minority molecules and competitions in a catalytic reaction network
○上村 淳, 金子 邦彦（東大総合文化）
Atsushi Kamimura, Kunihiko Kaneko (*Dept. of Basic Science, The Univ. of Tokyo*)

2SAP-03 スパインにおける確率的な Ca^{2+}の上昇はロバストでセンシティブな情報コーディングを可能にする
Stochasticity in Ca^{2+} increase in spines enables robust and sensitive information coding
○藤井 雅史[1], 上村 卓也[2], 浦久保 秀俊[1], 大橋 郁[1], 黒田 真也[1,2]（[1]東京大学大学院理学系研究科生物科学専攻, [2]東京大学理学部生物情報科学科）
Masashi Fujii[1], Takuya Koumura[2], Hidetoshi Urakubo[1], Kaoru Ohashi[1], Shinya Kuroda[1,2] ([1]*Dept. Biol. Sci., Grad. Sch. Sci, Univ. Tokyo*, [2]*Undergrad. Dept. Bioinfo. Syst, Univ. Tokyo*)

2SAP-04 定量 ATP イメージングによる単一細胞内 ATP 濃度の多様性の測定
Heterogeneity in ATP Concentrations in a Single Bacterial Cell Population Revealed by Quantitative Single-cell Imaging
○柳沼 秀幸[1,2,3], 河合 信之輔[4,5], 田端 和仁[2,6], 冨山 佳祐[3], 垣塚 彰[7], 小松崎 民樹[5], 岡田 康志[1], 野地 博行[2,3], 今村 博臣[7,8]（[1]理研・QBiC, [2]東大院・工, [3]阪大院・生命機能, [4]静大・理, [5]北大・電子研, [6]JST・さきがけ, [7]京大院・生命科学, [8]京大・白眉センター）
Hideyuki Yaginuma[1,2,3], Shinnosuke Kawai[4,5], Kazuhito Tabata[2,6], Keisuke Tomiyama[3], Akira Kakizuka[7], Tamiki Komatsuzaki[5], Yasushi Okada[1], Hiroyuki Noji[2,3], Hiromi Imamura[7,8] ([1]*QBiC, RIKEN*, [2]*Grad. Schl. Eng., Univ. Tokyo*, [3]*Grad. Schl. Frontier Biosci*, [4]*Schl. Sci, Shizuoka Univ.*, [5]*RIES, Hokkaido Univ.*, [6]*PRESTO, JST*, [7]*Grad. Schl. Biostud., Kyoto Univ.*, [8]*Hakubi Project, Kyoto Univ.*)

2SAP-05 １細胞系譜統計解析により明らかになる細胞表現型の適応度と選択圧の強さ
Single-cell lineage statistics reveals fitness and selection strength for heterogeneous phenotypic states
野添 嵩, ○若本 祐一（東大・院・総合文化）
Takashi Nozoe, **Yuichi Wakamoto** (*Univ. of Tokyo*)

2SAP-06 少数性生物学の展望
Prospect of minority biology
○永井 健治（阪大・産研）
Takeharu Nagai (*ISIR, Osaka Univ.*)

16:15〜18:45　B 会場／Room B : Mid-sized Hall 2/2

2SBP　新学術領域研究「細胞シグナリング複合体によるシグナル検知・伝達・応答の構造的基礎」共催
シグナル伝達機構における構造細胞生物学的新展開
New Development of Structural Cell Biology in Signal Transduction

オーガナイザー：前仲 勝実（北海道大学），石森 浩一郎（北海道大学）
Organizers: Katsumi Maenaka (Hokkaido University), Koichiro Ishimori (Hokkaido University)

While recent progress in structural biology has revealed fine structures of biomolecules, we have not yet understood the molecular mechanism for their functions due to lack of information on the space and time dependent interactions in the biological systems. Particularly, signal transduction processes are crucial to maintain essential biological systems, and the specific and dynamic interactions leads to the integrated regulation. In this symposium, up-and-coming young researchers will present their cutting-edge researches to discuss the contribution of structural cell biology to regulation mechanisms for signal transduction systems.

2SBP-01　構造細胞生物学とは
Structural cell biology
○箱嶋 敏雄（奈良先端科学技術大学院大学バイオサイエンス研究科）
Toshio Hakoshima (*Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol.*)

2SBP-02　C 型レクチン受容体 Mincle の糖脂質認識機構
Structural basis for glycolipid recognition mechanism by C-type lectin like receptor, Mincle
○古川 敦[1], 上敷領 淳[2], 尾瀬 農之[1], 山崎 晶[3], 前仲 勝実[1]（[1]北大・院薬, [2]福山大・薬, [3]九大・生医研）
Atsushi Furukawa[1], Jun Kamishikiryo[2], Toyoyuki Ose[1], Sho Yamasaki[3], Katsumi Maenaka[1] ([1]*Grad. Sch. of Pharm., Univ. of Hokkaido*, [2]*Sch. of Pharm., Univ. of Fukuyama*, [3]*Med. Inst. of Bioreg., Univ. of Kyushu*)

2SBP-03　コレラ菌の鉄獲得機構
Heme-iron uptake proteins from *Vibrio cholerae*
○内田 毅（北大院理）
Takeshi Uchida (*Grad. Sch. Sci., Hokkaido Univ.*)

2SBP-04　糖タンパク質品質管理システムにおける糖鎖修飾メカニズムの構造基盤
Structural basis for the glycan-processing mechanisms in glycoprotein quality control system
○佐藤 匡史[1,2], 加藤 晃一[1,3]（[1]名市大・院薬, [2]科学技術振興機構・さきがけ, [3]岡崎統合バイオ）
Tadashi Satoh[1,2], Koichi Kato[1,3] ([1]*Grad. Sch. Pharm. Sci., Nagoya City Univ.*, [2]*JST, PRESTO*, [3]*Okazaki Inst. Integ. Biosci.*)

2SBP-05　G タンパク質共役型内向き整流性カリウムイオンチャネル 1(GIRK1)の G タンパク質による機能調節の構造基盤
Structural Basis for Regulation of G Protein-activated Inwardly Rectifying Potassium Channel 1 (GIRK1) by G Proteins
○大澤 匡範[1], 間瀬 瑤子[1], 横川 真梨子[1,2], 竹内 恒[3], 嶋田 一夫[1]（[1]東大・院薬系, [2]バイオ産業情報化コンソ, [3]産総研、創薬分子プロファイリング研究セ）
Masanori Osawa[1], Yoko Mase[1], Mariko Yokoagawa[1,2], Koh Takeuchi[3], Ichio Shimada[1] ([1]*Grad. Sch. Pharm. Sci, The Univ. of Tokyo*, [2]*JBic*, [3]*Molprof, AIST*)

2SBP-06　超遠心分析と質量分析による溶液中での蛋白質間相互作用解析
In solution characterizations of protein-protein interactions by analytical ultracentrifugation and mass spectrometry
○内山 進[1,2]（[1]阪大 工 先端生命, [2]岡崎統合バイオ）
Susumu Uchiyama[1,2] ([1]*Dept.Biotech., Grad. Sch. Eng., Osaka Univ.*, [2]*Okazaki Inst. Integ. Biosci.*)

2SBP-07　毛上皮形成における S100A3-PAD3 亜鉛シグナル伝達機構の構造生物学的解明
Structural Biology for the Zinc Signal Transduction Mechanism by S100A3-PAD3 in Hair Cuticular Cells
○海野 昌喜[1,2], 木澤 謙司[3], 眞下 隆太朗[1,2], 西條 慎也[4], 清水 伸隆[4], 秋元 恵[1,2], 高原 英成[5]（[1]茨城大院理工, [2]茨城大フロンティア, [3]カネボウ化・価値創成研, [4]高エネ研・PF, [5]茨城大・農）
Masaki Unno[1,2], Kenji Kizawa[3], Ryutaro Mashimo[1,2], Shinya Saijo[4], Nobutaka Shimizu[4], Megumi Akimoto[1,2], Hidenari Takahara[5] ([1]*Grad. Sch. Sci. Eng., Ibaraki Univ.*, [2]*iFRC, Ibaraki Univ.*, [3]*Kanebo, Cosme. Inc*, [4]*KEK, PF*, [5]*Dep. Appl. Biol. Res. Sci., Ibaraki Univ.*)

16:15～18:45　C 会場／Room C : Room 107

2SCP　構造バイオインフォマティクスによる蛋白質機能予測・解析
Prediction and analysis of protein functions from structural bioinformatics

オーガナイザー：中村 春木（大阪大学），清水 謙多郎（東京大学）
Organizers: Haruki Nakamura (Osaka University), Kentaro Shimizu (The University of Tokyo)

By interpreting genome information to structural information of proteins and their interactions, estimation and deep understanding of their functions are now available with the bioinformatics techniques. In this symposium, recent methods in structural bioinformatics and their applications to clinical studies like effects by SNPs based on structures will be introduced and discussed.

2SCP-01　Toward a Computational Assessment of the Effect of Amino Acid Variation to Protein Structure and Function - Case Study on a Few Enzymes
Kei Yura[1,2] ([1]*Grad. Schl. Hum. Sci., Ochanomizu Univ.*, [2]*NIG*)

2SCP-02　From Personal Genome to Personal Proteins: Connection between the Reference sequences of genomic DNA and Proteins
Kengo Kinoshita[1,2,3] ([1]*Grad Sch Info Sci, Tohoku Univ.*, [2]*Tohoku Medical Megabank, Tohoku Univ.*, [3]*IDAC, Tohoku Univ.*)

2SCP-03　蛋白質の構造モチーフの際に着目した機能アノテーション
Composite structural motifs of binding sites for annotating functional differences
○金城 玲（阪大・蛋白研）
Akira Kinjo (*Institute for Protein Research, Osaka University*)

2SCP-04　アミノ酸残基間距離予測に基づくタンパク質立体構造モデルの評価
Model quality assessment method based on a residue-residue distance matrix prediction
○竹田ー志鷹 真由子（北里大学薬学部）
Mayuko Takeda-Shitaka (*Pharm., Kitasato Univ.*)

2SCP-05　粗視化分子動力学シミュレーションで探るタンパク質・リガンド結合過程
Protein–ligand binding processes studied by coarse-grained molecular dynamics simulations
○寺田 透, 根上 樹, 清水 謙多郎（東大・院農）
Tohru Terada, Tatsuki Negami, Kentaro Shimizu (*Grad. Sch. Agr. Life Sci., Univ. Tokyo*)

2SCP-06　超分子モデリングパイプラインの構築による相関構造解析•理論創薬支援
Supramolecular modeling pipeline for correlative structural analysis and rational drug-design
○白井 剛（長浜バイオ大学）
Tsuyoshi Shirai (*Nagahama Inst BioSci Tech*)

2SCP-07　Intrinsic disorder mediates cooperative signal transduction in STIM1
Daron M. Standley (*iFReC, Osaka University*)

16:15～18:45　D 会場／Room D : Room 108

2SDP　生体分子機械の動作機構を周りの水から眺めてみる
Biomolecular machinery driven by surrounding water

オーガナイザー：原野 雄一（姫路獨協大学），西山 雅祥（京都大学）
Organizers: Yuichi Harano (Himeji Dokkyo University), Masayoshi Nishiyama (Kyoto University)

The thermodynamic aspect of the water should be essential because the dynamical behavior of biomolecules is mostly observed at large time scales. The hydration theories are revealing the role of water in terms of the thermodynamics. At the same time, recent development of experimental technique to detect the biomolecular dynamics is quite remarkable. This session will allow theoreticians and experimentalists to get together and discuss the achievements and future directions associated with dynamical behavior of biomolecules under the influence of surrounding water.

2SDP-01 生体分子のダイナミクスに向けた水和熱力学
Hydration thermodynamics toward biomolecular dynamics
○原野 雄一（姫路獨協大学薬学部）
Yuichi Harano (*Himeji Dokkyo University*)

2SDP-02 生体分子機械を水和水であやつる
Controlling the molecular machinery by water molecules of the hydration
○西山 雅祥（京大・白眉セ）
Masayoshi Nishiyama (*The HAKUBI Center, Kyoto Univ.*)

2SDP-03 水の状態を感受する蛍光蛋白質の開発
Development of fluorescent protein to sense “state of water”
○渡邉 朋信[1,2,3]（[1](独)理研・QBiC, [2]阪大・生命機能, [3]阪大・免フロ）
Tomonobu Watanabe[1,2,3] ([1]*QBiC, RIKEN*, [2]*Grad. Sch. Front. Biosci., Osaka University*, [3]*iFRec, Osaka Univ.*)

2SDP-04 合成化学的に構築した人工分子機械によって駆動される水中での非共有結合性分子集合体の巨視的運動
Macroscopic Motion of Soft Non-covalent Molecular Assembly in Water Actuated by Chemically Synthesized Molecular Machine
○景山 義之[1,2]（[1]北海道大学大学院理学研究院, [2]ＪＳＴ・さきがけ）
Yoshiyuki Kageyama[1,2] ([1]*Fac. Sci., Hokkaido Univ.*, [2]*PRESTO, JST*)

2SDP-05 アクチンモノマーの会合と多価カチオンが媒介する同符号コロイド粒子間実効引力
Association of Actin Monomers and Effective Attraction between Like-Charged Colloidal Particles Mediated by Multivalent Cations
○秋山 良（九大 院理 化学）
Ryo Akiyama (*Dept. of Chem., Kyushu Univ.*)

2SDP-06 タンパク質の構造ゆらぎと変化に対する相互作用成分解析
Interaction-Component Analysis on Protein Structure in Explicit Solvent
○松林 伸幸（大阪大学 大学院基礎工学研究科 化学工学領域）
Nobuyuki Matubayasi (*Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University*)

16:15～18:45　E 会場／Room E : Room 204

2SEP　生物界における光とは？：動物・植物・微生物の光科学、そしてオプトジェネティクス
Light in life: photo-biology of animals, plants, microorganisms and optogenetics

オーガナイザー：井上 圭一（名古屋工業大学），中曽根 祐介（京都大学）
Organizers: Keiichi Inoue (Nagoya Institute of Technology), Yuusuke Nakasone (Kyoto University)

Light is the mother of life and it supplies huge benefits to survive. Most of organisms have evolved their unique systems to utilize light, and various photo-receptive proteins are playing central roles there. On the other hand, new techniques optically controlling living-organisms, “optogenetics”, are being rapidly developed by genetic application of those proteins. In this symposium, seven leading-researchers talk about a wide variety of photo biological- or optogenetic researches and we will discuss about future perspective of light and life from a viewpoint beyond species.

はじめに
○井上 圭一（名大・工，JSTさきがけ）
Keiichi Inoue (*Nagoya Institute of Technology*)

2SEP-01 カラフルな植物光受容体、赤、青、UV-B を見る
Colorful plant photoreceptors see red, blue and UV-B light
○徳富 哲, 吉原 静恵（阪府大院 理 生物）
Satoru Tokutomi, Shizue Yoshihara (*Osaka Prefect. Univ., Grad. School Sci., Dep. Biol. Sci.*)

2SEP-02 触覚パターンのオプトジェネティクス制御
Optogenetic patterning of touch sense
○八尾 寛[1,2,3], 横山 超一[1,2], 住吉 晃[4], 阿部 健太[1,2], 小泉 協[1,2], 江川 遼[1,2], 劉 越人[1,2], 大城 朝一[2,3], 松坂 義哉[2,3], 川島 隆太[4], 虫明 元[2,3], 石塚 徹[1,2]（[1]東北大・院生命, [2]JST-CREST, [3]東北大・院医学, [4]東北大・加齢医学研究所）
Hiromu Yawo[1,2,3], Yukiobu Yokoyama[1,2], Akira Sumiyoshi[4], Kenta Abe[1,2], Kyo Koizumi[1,2], Ryo Egawa[1,2], Yueren Liu[1,2], Tomokazu Ohshiro[2,3], Yoshiya Matsuzaka[2,3], Ryuta Kawashima[4], Hajime Mushiake[2,3], Toru Ishizuka[1,2] ([1]*Tohoku Univ. Grad. Sch. Life Sci.*, [2]*JST-CREST*, [3]*Tohoku Univ. Grad. Sch. Med.*, [4]*Tohoku Univ. IDAC*)

2SEP-03 脊椎動物クリプトクロムの多様性、機能および分子応用
Diversity, function and molecular application of vertebrate cryptochromes
○岡野 俊行（早大 先進理工 電気・情報生命）
Toshiyuki Okano (*Waseda Univ. Sch. Adv. Sci. & Eng.*)

2SEP-04 イェロープロテインを通じてみたセンサー蛋白質の作動機構
Molecular actions of the light sensor protein, Photoactive Yellow Protein, as a prototype for sensor proteins
○上久保 裕生（奈良先端大物質創成）
Hironari Kamikubo (*NAIST/MS*)

2SEP-05 BLUF タンパク質の光化学とオプトジェネティクス
Photochemistry and optogenetics with BLUF proteins
○増田 真二（東工大・バイオセンター）
Shinji Masuda (*Center for Biol. Res. & Inform., Tokyo Inst. Tech.*)

2SEP-06 脊椎動物の非視覚オプシン Opn5 の分子特性の多様性
Diversity of Molecular properties of vertebrate non-visual opsin Opn5
○山下 高廣（京大・院理・生物物理）
Takahiro Yamashita (*Grad. Sch. Sci., Kyoto Univ.*)

2SEP-07 フィトクロムは遺伝子発現の様々な段階を直接制御する
Phytochrome directly regulates various aspects of gene expression
○松下 智直[1,2]（[1]九大院・農, [2]JST さきがけ）
Tomonao Matsushita[1,2] ([1]*Fac. Agri., Kyushu Univ.*, [2]*JST PRESTO*)

おわりに
○中曽根 祐介（京大・理）
Yuusuke Nakasone (*Kyoto Univ.*)

第 3 日目（9 月 27 日（土））／Day 3（Sep. 27 Sat.）

9:45～12:15　A 会場／Room A : Mid-sized Hall 1/2

3SAA　新学術領域研究「感覚と知能を備えた分子ロボットの創成」共催
感覚と運動および知能を備えた分子ロボットの創成
Development of Molecular Robots equipped with Sensors and Intelligence

オーガナイザー：小長谷 明彦（東京工業大学），萩谷 昌己（東京大学），村田 智（東北大学），角五 彰（北海道大学）
Organizers: Akihiko Konagaya (Tokyo Institute of Technology), Masami Hagiya (The University of Tokyo), Satoshi Murata (Tohoku University), Akira Kakugo (Hokkaido University)

Recently the concept of molecular robotics, that is being motivated from the observation and understanding of highly efficient and coordinated natural biological systems, has emerged. In this symposium we are going to focus on this newly evolved field of research dealing with the molecular robotics. Comprehensive discussion will be made on various aspects of the molecular robotics that includes development and integration of robots using the combination of artificial and natural components such as synthetic polymers, poly-peptide, DNA, bio-molecular motors.

3SAA-01 分子ロボティクス―その展望と動機
Molecular Robotics — Perspectives and Motivation
○村田 智（東北大・院工学）
Satoshi Murata (*Grad. Sch. Eng., Tohoku Univ.*)

3SAA-02 Building Nanoscale Devices with DNA
Shawn Douglas (*UCSF*)

3SAA-03 核酸ナノ構造を活用した分子情報変換デバイスの設計
Designing DNA/RNA nanostructure-based information converters
○齊藤 博英[1], 遠藤 政幸[1], 瀧ノ上 正浩[2]（[1]京都大学, [2]東京工業大学）
Hirohide Saito[1], Masayuki Endo[1], Masahiro Takinoue[2] ([1]*Kyoto Univ.*, [2]*Tokyo Tech.*)

3SAA-04 分子ロボットのリアルタイムな動作を目指した試験管内での知能の実装
Implementation of *in vitro intelligence* for real-time operation of molecular robots
○小宮 健（東工大・院総理）
Ken Komiya (*Int. Grad. Sch. Sci. & Engi., Tokyo Tech.*)

3SAA-05 アメーバ型分子ロボットの課題と展望
Perspectives and objectives of amoeba-type molecular robots
○小長谷 明彦（東工大院知能システム科学）
Akihiko Konagaya (*Tokyo Institute of Technology*)

3SAA-06 ゲルに基づく分子ロボットとその計算モデル
Gel-based molecular robots and their computational models
○萩谷 昌己（東京大学）
Masami Hagiya (*The University of Tokyo*)

9:45～12:15　B 会場／Room B : Mid-sized Hall 2/2

3SBA　分子機械デザイン
Rise of molecular machines

オーガナイザー：野地 博行（東京大学），林 重彦（京都大学）
Organizers: Hiroyuki Noji (The University of Tokyo), Shigehiko Hayashi (Kyoto University)

Biomolecular machines are well investigated with respect to their conformational dynamics and reaction schemes. However, study seeking the principles of their structural design is still in its early stage. Furthermore, there are only few successful attempts of re-designing for function-gaining. To better elucidate the design principles of molecular machines, extensive redesign of natural molecular machines or de novo synthesis of completely novel molecular machines are needed. In this symposium, speakers will discuss their approaches toward synthetic re-designs.

はじめに

3SBA-01 Toward design of molecular motors
Nobuyasu Koga[1,2] ([1]*Inst. Mol. Sci. CIMoS*, [2]*JST, PRESTO*)

3SBA-02 The conformational change mechanism of the β subunit in F_1-ATPase revealed by all-atom MD simulations
Yuko Ito, Mitsunori Ikeguchi (*Grad. Sch. Med. Life Sci., Yokohama-City Univ.*)

3SBA-03 Molecular simulations of proton pumps and biomolecular motors
Qiang Cui (*Dept. of Chem., Univ. of Wisconsin, Madison*)

3SBA-04 F1 モーターの再デザインによる人工回転分子モーター開発の見通し
Prospects on artificial molecular motor by redesigning of F1-ATPase
○野地 博行（東京大学大学院工学系研究科応用化学専攻）
Hiroyuki Noji (*Applied Chem. U-Tokyo*)

3SBA-05 Is enzyme evolution reversible? Exploring fitness landscapes by laboratory evolution
Nobuhiko Tokuriki (*University of British Columbia*)

3SBA-06 Remote control of myosin and kinesin motors using light-activated gearshifting
Zev Bryant (*Stanford University*)

おわりに

9:45～12:15　C 会場／Room C : Room 107
3SCA　大容量生命情報時代の新しい生物学とは？
Next generation Biology at the big data era

オーガナイザー：諏訪 牧子（青山学院大学），有田 正規（国立遺伝学研究所）
Organizers: Makiko Suwa (Aoyama Gakuin University), Masanori Arita (National Institute of Genetics)

Biology is coming to a new turning point now. The biological "big data" of heterogeneous, many classes and dimensions is accumulated quickly every day and therefore it is just now to solve the origin questions about proncipal of biological process, which had been unsolvable before without big data. In this symposium, we would like to argue about the strategies of collecting and analyzing biological information for solving such origin questions, by exploring the point of contact of biophysics and bioinformatics.

はじめに
○諏訪 牧子（青学）
Makiko Suwa (*Aoyama Gakuin Univ.*)

3SCA-01 生物システムの理解に本質的な３つの未解決問題
Three unsolved problems for essential understanding of biological systems
○美宅 成樹（豊田理研）
Shigeki Mitaku (*Toyota Phys. Chem. Res. Inst.*)

3SCA-02 リン酸化プロテオームとメタボロームデータからのインスリン作用のグローバルネットワークの再構築
Reconstruction of global network of acute insulin action from phosphor-proteome and metabolome data
○黒田 真也, 柚木 克之（東京大学理学系研究科生物科学専攻）
Shinya Kuroda, Katsuyuki Yugi (*Biological Sciences, The University of Tokyo*)

3SCA-03 全ゲノム規模の１細胞内１分子遺伝子発現情報からのデータマイニング
Mining genome-wide datasets of single-cell gene expressions at single-molecule resolution
○谷口 雄一（理化学研究所生命システム研究センター）
Yuichi Taniguchi (*Quantitative Biology Center, RIKEN*)

3SCA-04 分子バーコーディングによる一分子の分解能をもつゲノムワイド遺伝子発現絶対定量法
Absolute genome-wide quantification of gene expression with single molecule resolution using molecular barcoding
○城口 克之（理研・統合生命医科学研究セ）
Katsuyuki Shiroguchi (*IMS RIKEN*)

3SCA-05 大容量生命情報時代の生物学のボトルネック
Fundamental bottlenecks in big-data biology
○岩崎 渉（東大・院理・生物科学）
Wataru Iwasaki (*Dept Biol Sci, Grad Sch Sci, UTokyo*)

3SCA-06 Database for Biology: which data deserve maintaining?
Masanori Arita[1,2] ([1]*National Institute of Genetics*, [2]*RIKEN CSRS*)

9:45～12:15　D 会場／Room D : Room 108

3SDA　ラマン顕微分光および先端光計測が拓く生物物理の視界
Cutting-Edge Optical Imaging Approaches and Raman Micro-Spectroscopy Pioneering Bio-Physics

オーガナイザー：盛田 伸一（東北大学），坂内 博子（名古屋大学）
Organizers: Shin-ichi Morita (Tohoku University), Hiroko Bannai (Nagoya University)

Recent progress in optical measurements for live cells to small animals provides us new and unique visions in biophysics. In this symposium, we aim to introduce cutting-edge imaging techniques that will be upcoming standards in biophysical research in near future. Young leading researchers introduce their recent studies ranging from single molecule imaging to whole-animal imaging with light-sheet microscopy, new laser sources and bio-sensors, as well as the Raman microscopy, including Raman scattering (SRS) microscope, Raman-tagged live cell imaging.

はじめに
○盛田 伸一（東北大院理）
Shin-ichi Morita (*Tohoku Univ.*)

3SDA-01　「膜分子のふるまい」を見て「細胞内シグナル」を知る
Watch the “membrane protein behavior” to know the “intracellular signaling pathway”
○坂内 博子[1,2], 丹羽 史尋[2], Triller Antoine[3], 御子柴 克彦[2]（[1]名大・院生命理学, [2]理研・BSI, [3]パリ高等師範学校生物学研究所）
Hiroko Bannai[1,2], Fumihiro Niwa[2], Antoine Triller[3], Katsuhiko Mikoshiba[2] ([1]*Nagoya Univ., Grad. Sch. Biol. Sci.*, [2]*RIKEN BSI*, [3]*IBENS*)

3SDA-02　Improvement of two-photon laser scanning microscopy for live imaging utilizing laser technology
Tomomi Nemoto[1,2,3] ([1]*RIES, Hokkaido Univ.*, [2]*JST CREST*, [3]*Grd. Sch. Info., Hokkaido Univ.*)

3SDA-03　吸収増幅顕微鏡による細胞イメージング
Cavity reflection enhanced light absorption microscopy for cellular imaging
○新井 由之[1], 山本 高之[1], 南川 丈夫[2], 高松 哲郎[2], 永井 健治[1]（[1]阪大・産研, [2]京都府立医大）
Yoshiyuki Arai[1], Takayuki Yamamoto[1], Takeo Minamikawa[2], Tetsuro Takamatsu[2], Takeharu Nagai[1] ([1]*ISIR, Osaka Univ.*, [2]*Kyoto Pref. Univ. Med.*)

3SDA-04　光シート顕微鏡による生体まるごとイメージング
Live imaging of whole organisms by light-sheet microscopy
○野中 茂紀（基生研）
Shigenori Nonaka (*NIBB*)

3SDA-05　小分子から生細胞までーラマン顕微鏡にできることー
Small Molecules and Live Cells Characterized Using Raman Microscopes
○盛田 伸一（東北大院理）
Shin-ichi Morita (*Tohoku Univ.*)

3SDA-06　近赤外吸収プローブとしての Pt(II)ジラジカル錯体の機能開発
Development of a function of Pt(II)-diradical complex as a near-infrared absorbing probe
○升谷 敦子, 田村 昂作, 星野 仁, 壹岐 伸彦（東北大院環境）
Atsuko Masuya, Kosaku Tamura, Hitoshi Hoshino, Nobuhiko Iki (*Graduate School of Environmental Studies, Tohoku University*)

3SDA-07　誘導ラマンによって何が見えるのか？
What could be visualized with stimulated Raman scattering?
○小関 泰之（東大院工）
Yasuyuki Ozeki (*UTokyo*)

9:45～12:15　E 会場／Room E : Room 204

3SEA　タンパク質物性研究の最前線：若手研究者による挑戦
Frontiers in physical properties of proteins: challenges by young scientists

オーガナイザー：新井 宗仁（東京大学），濵田 大三（三重大学）
Organizers: Munehito Arai (The University of Tokyo), Daizo Hamada (Mie University)

The aim of this symposium is to encourage discussions especially between younger generations at the earlier stages of research career particularly working on physicochemical properties of proteins. The selected speakers are PhD students, postdocs or assistant professors who are actively studying the mechanism of protein folding or methodologies for de novo protein design using variety of experimental or theoretical approaches.

3SEA-01　きわめて長い半減期をもつフォールディング中間体の解析
Analysis of an unusually stable kinetic refolding intermediate
○杉本 華幸（新潟大農）
Hayuki Sugimoto (*Fac. Agri. Univ. NIIGATA*)

3SEA-02　FRET と高速溶液混合法による SNase の凝縮過程の速度論研究
Kinetics of Chain Condensation during SNase Folding studied by FRET and ultrarapid mixing methods
○水上 琢也[1], Xu Ming[1], Cheng Hong[1], Roder Heinrich[1,2], 槇 亙介[3]（[1]フォックスチェイス癌センター, [2]ペンシルベニア大学, [3]名大・理）
Takuya Mizukami[1], Ming Xu[1], Hong Cheng[1], Heinrich Roder[1,2], Kosuke Maki[3] ([1]*FCCC*, [2]*UPenn*, [3]*Nagoya University*)

3SEA-03　様々な蛋白質間で観察される配列順序非保存な構造類似性
Non-sequential structural similarity in the protein world
○南 慎太朗[1], 太田 元規[1], 千見寺 浄慈[2]（[1]名大・情報科学, [2]名大・工）
Shintaro Minami[1], Motonori Ota[1], George Chikenji[2] ([1]*Grad. Sch. of Inf. Sci., Nagoya Univ.*, [2]*Grad. Sch. of Eng., Nagoya Univ.*)

3SEA-04　機能と構造安定性のトレードオフに対するアプローチ：アルブミン結合タンパク質を模倣したヒト型タンパク質のデザインを例として
The approach to the function-stability tradeoff: A case of the design of a humanized protein mimicking the albumin-binding protein
○大城 理志[1], 本田 真也[1,2]（[1]東大・新領域・メディカルゲノム, [2]産総研・バイオメディカル）
Satoshi Oshiro[1], Shinya Honda[1,2] ([1]*Dept. of Medical Genome Sci., Grad. Sch. of Frontier Sci., The Univ. of Tokyo*, [2]*BioMed. Research Inst., AIST*)

3SEA-05　転写因子 Sp1 と TAF4 の天然変性領域を介した相互作用
The interaction between transcription factors Sp1 and TAF4 via the intrinsically disordered regions
○日比野 絵美[1], 井上 倫太郎[2], 杉山 正明[2], 桑原 淳[3], 松崎 勝巳[1], 星野 大[1]（[1]京大院薬, [2]京大原子炉, [3]同女薬）
Emi Hibino[1], Rintaro Inoue[2], Masaaki Sugiyama[2], Jun Kuwahara[3], Katsumi Matsuzaki[1], Masaru Hoshino[1] ([1]*Grad. Pharm., Univ. Kyoto*, [2]*KURRI*, [3]*Fac. of Pharm., Doshisha WUniv.*)

3SEA-06　混雑環境下での天然変性タンパク質の振る舞い: α-synuclein のアミロイド形成に関する熱力学シミュレーション
Intrinsic disorder under crowded environment: thermodynamic simulation of α-synuclein amyloid fibril formation
○白井 伸宙（京大・院理）
Nobuhiro C. Shirai (*Grad. Sch. Sci., Kyoto Univ.*)

ポスター　Poster

第 1 日目（9 月 25 日（木））／Day 1（Sep. 25 Thu.）大ホール / Main Hall

01A. 蛋白質：構造 / 01A. Protein: Structure

1P001 **耐熱性ストマチン特異的切断プロテアーゼの構造と機能解析**
Structural and functional analysis of a thermostable stomatin-specific protease
Hideshi Yokoyama[1], Daisuke Kobayashi[1], Naoto Takizawa[1], Satoshi Fujii[1], Ikuo Matsui[2] ([1]*Sch. of Pharm. Sci., Univ. of Shizuoka*, [2]*Biomedical Res. Inst., AIST*)

1P002 **黄色ブドウ球菌由来 α-ヘモリジン単量体の結晶構造**
Crystal structure of staphylococcal α-hemolysin monomer
Takaki Sugawara[1], Daichi Yamashita[1], Yoshikazu Tanaka[1,2], Jun Kaneko[3], Yoshiyuki Kamio[3], Isao Tanaka[1,2], Min Yao[1,2] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*, [3]*Grad. Sch. Agri., Tohoku Univ.*)

1P003 ***Mycoplasma pneumoniae* 滑走メカニズムに関わる P65 の構造解析**
Structural analysis of P65 involved in *Mycoplasma pneumoniae* gliding mechanism
Masaru Yabe[1], Miki Kinoshita[1], Yukio Furukawa[2], Katsumi Imada[3], Makoto Miyata[1] ([1]*Grad. Sch. of Sci., Osaka City University*, [2]*Grad. Sch. of Frontier BioSci., Osaka University*, [3]*Grad. Sch. of Sci., Osaka University*)

1P004 **Concentration Dependence of Amyloid Peptide Assembly by Using the Molecular Dynamics Simulations**
Naohiro Nishikawa[1,2], Yoshikate Sakae[1], Yuko Okamoto[1,3,4,5] ([1]*Grad. Sch. of Sci., Nagoya Univ.*, [2]*Inst. for Mol. Sci.*, [3]*Str. Biol. Res. Cent., Grad. Sch. of Sci., Nagoya Univ.*, [4]*Cent. for Comp. Sci., Grad. Sch. of Eng., Nagoya Univ.*, [5]*Info. Tech. Cent., Nagoya Univ.*)

1P005 **Lectin-like transcript 1 (LLT1)の構造解析と LLT1-CD161 複合体のモデル作製**
Structure analysis of lectin-like transcript 1 (LLT1) and model building of LLT1-CD161 complex
Shunsuke Kita[1], Haruki Matsubara[2], Jun Kamishikiryo[3], Yuki Okabe[1], Hideo Fukuhara[1], Kimiko Kuroki[1], Katsumi Maenaka[1] ([1]*Pharm., Univ. of Hokkaido*, [2]*Grad. Univ. Advanced Studies, KEK PF SBRC, Dep. of Materials Structure Science*, [3]*Sch. of Pharm., Univ. of Fukuyama*)

1P006 **Photon Factory の BioSAXS ビームラインの現状**
Current status of BioSAXS beamlines at Photon Factory
Nobutaka Shimizu[1], Shinya Saijyo[1], Hiromasa Ota[2], Takeharu Mori[1], Yasuko Nagatani[1], Ai Kamijyo[1], Takashi Kosuge[1], Noriyuki Igarashi[1] ([1]*Photon Factory, KEK*, [2]*Mitsubishi Electric SC*)

1P007 **エネルギー表示溶液理論を用いた蛋白質複合体構造予測**
Protein-protein complex structure prediction using the solution theory in the energy representation
Kazuhiro Takemura[1], Nobuyuki Matubayasi[2], Akio Kitao[1] ([1]*IMCB, Univ. Tokyo*, [2]*Grad. Sch. Eng. Sci, Osaka Univ.*)

1P008 **B 細胞共受容体 CD72 の C 型レクチン様ドメインの X 線結晶構造解析**
Crystal structure of the C-type lectin-like domain of CD72
Kenro Shinagawa[1], Nobutaka Numoto[2], Takeshi Tsubata[2], Nobutoshi Ito[2] ([1]*Grad. Bio. Sci., Tokyo Med. and Dent. Univ.*, [2]*Med. Res. Inst., Tokyo Med. and Dent. Univ.*)

1P009 **ADP 結合型 腸球菌 A_3B_3 複合体の X 線結晶構造解析**
Crystal Structure of ADP-Bound A_3B_3 Complex of *Enterococcus hirae* V-ATPase
Kazuya Nakamoto[1], Kenji Mizutani[1,2], Kano Suzuki[1], Yoshiko Ishizuka-Katsura[3], Mikako Shirouzu[3], Shigeyuki Yokoyama[4], Ichiro Yamato[2], Takeshi Murata[1,5] ([1]*Grad. Sch. Sci., Univ. Chiba*, [2]*Dept. Bio. Sci. Tech., Tokyo Univ. of Science*, [3]*RIKEN CLST*, [4]*Structure Bio. Lab., RIKEN*, [5]*JST, PRESTO*)

1P010 **TtFbpA による新規鉄結合様式の解明**
A novel six-coordinated ferric ion binding mode of TtFbpA
Shipeng Wang, Misaki Ogata, Shoichiro Horita, Jun Ohtsuka, Koji Nagata, Masaru Tanokura (*Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo*)

1P011 **X 線 1 分子追跡法による α シヌクレイン構造揺らぎ 1 分子観察**
X-ray Single Molecule Observations of Alpha-synuclein's Structural Fluctuations by using Diffracted X-ray Tracking (DXT)
Masahiro Shimura[1], **Naruki Hara**[1], Yufuku Matsushita[1], Keigo Ikezaki[1], Hiroshi Sekiguchi[2], Naoya Fukui[3], Yasushi Kawata[3], Yuji Sasaki[1,2] ([1]*The University of Tokyo*, [2]*SPring-8/JASRI*, [3]*Tottori University*)

1P012 **分子ドッキングによるタンパク質–基質複合体の乖離構造と結合エネルギー評価**
Molecular docking study of structure and binding energy of ligand-protein complex in dissociation process
Hiroaki Saito, Kazutomo Kawaguchi, Hidemi Nagao (*Kanazawa University*)

1P013 **Some cooperative aspects of protein aggregation phenomena**
Takashi Konno (*Med., Univ. Fukui*)

1P014 **タイワンカブトムシ由来セリンプロテアーゼ阻害タンパク質オリクチンの変異体とトリプシンの複合体の構造解析**
Crystal structure of the M14R mutant of oryctin, a Kazal-type serine protease inhibitor, in complex with trypsin
Desheng Liu[1], Tatsuya Suzuki[1], Shoichiro Horita[1], Takeshi Kawai[1], Jun Ishibashi[2], Minoru Yamakawa[2], Koji Nagata[1], Masaru Tanokura[1] ([1]*Grad. Sch. of Agri. Lif Sci., Univ. Tokyo*, [2]*Nat. Ins. of Agro. Sci*)

1P015 **[NiFe]ヒドロゲナーゼ成熟化段階において Ni 挿入を担う HypAB 複合体**
Studies on intermediate HypAB complexes for Ni insertion during [NiFe] hydrogenase maturation
Takumi Kawashima[1], Satoshi Watanabe[1,2], Yuichi Nishitani[1], Tamotsu Kanai[3], Haruyuki Atomi[3], Tadayuki Imanaka[4], Kunio Miki[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*IMRAM, Tohoku Univ.*, [3]*Grad. Sch. Eng., Kyoto Univ.*, [4]*Coll. Life Sci., Ritsumeikan Univ.*)

1P016 **T4 型ファージと T2 型ファージの尾繊維先端受容体結合蛋白質の構造と機能**
Structure and function of receptor binding proteins of T4-type phages and T2-type phages
Shuji Kanamaru[1], Kazuya Uchida[1], Takahiro Momiyama[1], Kaname Nishijo[1], Fumio Arisaka[1,2] ([1]*Grad. Sch. of Biosci. & Biotech., Tokyo Inst. of Tech.*, [2]*Life Sci. Res. Center, Nihon Univ.*)

1P017 **べん毛 III 型輸送シャペロン FlgN の構造と FlhA との相互作用**
Structure of FlgN, a flagellar type III export chaperone, and its interaction with FlhA, a flagellar type III export gate protein
Yuya Ogawa[1], Yuki Nakanishi[1], Yumiko Uchida[1], Miki Kinoshita[2], Tohru Minamino[2], Katsumi Imada[1] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*Grad. Sch. Frontier BioSci., Univ. Osaka*)

1P018 **自然免疫非感受性のサルモネラ菌 FljB が形成するべん毛繊維の立体構造と FliC べん毛繊維との違い**
CryoEM structure of the flagellar filament of Salmonella FljB and implication of its difference from the FliC filament
Shoko Toma, Takayuki Kato, Keiichi Namba (*Osaka University*)

1P019 **Expression and Structural Analysis of Two Kinds of Perireceptor Proteins (PRPs)**
Xing Li[1], Durige Wen[2], Masaru Hojo[3], Mamiko Ozaki[3], Tatsuo Iwasa[1,4] ([1]*Div. Eng., Muroran Ins. of Tech.*, [2]*Div of Prod Sys Eng., Muroran Ins. of Tech.*, [3]*Dept. Biol., Grad.School Sci., Kobe Univ*, [4]*Cen. Env. Sci. Dis. Mit. Adv. Res., Muroran Ins. of Tech.*)

01B. 蛋白質：構造機能相関 / 01B. Protein: Structure & Function

1P020 **SepCysE の機能・構造解析による Cys-tRNA^{Cys} 生合成機構の解明**
The study on the structure and function of SepCysE related to Cys-tRNA^{Cys} synthesis
Yuto Nakazawa[1], Nozomi Asano[1], Akiyoshi Nakamura[2], Keisuke Komoda[3], Isao Tanaka[1,4], Min Yao[1,4] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*MB&B, Yale Univ.*, [3]*Grad. Sci. Agri. Life sci., Univ. Tokyo*, [4]*Fac. Adv. Life Sci., Hokkaido Univ.*)

1P021 **タンパク質レアイベントを抽出する効率的構造サンプリング法**
Enhanced conformational sampling methods for extracting biological rare events of proteins
Ryuhei Harada[1,3], Yu Takano[2,3], Yasuteru Shigeta[1,3] ([1]*CCS, University of Tsukuba*, [2]*IPR, Osaka University*, [3]*JST-CREST*)

1P022 **遷移温度付近のシニョリンの緩和モード解析**
Relaxation Mode Analysis of Chignolin at Transition Temperature
Ayori Mitsutake, Hiroshi Takano (*Dep. Phys, Keio Univ.*)

1P023 **真空紫外円二色性分光による α_1 酸性糖蛋白質と生体膜の相互作用機構の解明**
Interaction Mechanism of α_1-Acid Glycoprotein with Biomembrane Characterized by Vacuum-Ultraviolet Circular Dichroism Spectroscopy
Koichi Matsuo[1], Hirofumi Namatame[1], Masaki Taniguchi[1,2], Kunihiko Gekko[3] ([1]*HiSOR, Hiroshima Univ.*, [2]*Grad. Sch. Sci., Hiroshima Univ.*, [3]*ISSD, Hiroshima Univ.*)

1P024 **レチノール結合タンパク質とカルパインの立体構造上に形成されたイントロンの平面**
Planes formed with 4 introns in tertiary structures of RBP & calpain D-VI
Michiko Nosaka[1], Syunya Sunaba[2], Ryoutarou Tsuji[4], Katsuki Hitata[3] ([1]*Biol. & Mat. Eng., Sasebo College, National Inst. of Tech.*, [2]*Canon*, [3]*RHOM*, [4]*Unknown Company*)

1P025 **アカネ科由来抗腫瘍活性ペプチド RA-VII の構造解析**
Structure analysis of antitumor peptide RA-VII from Rubia Cordifolia
Yoh Noguchi[1], Hironao Yamada[1], Sakiko Mori[1], Takeshi Miyakawa[1], Ryota Morikawa[1], Satoshi Yokojima[2], Yukio Hitotsuyanagi[2], Koichi Takeya[2], Masako Takasu[2] ([1]*School of Life Sciences, Tokyo University of Pharmacy and Life Sciences*, [2]*School of Pharmacy, Tokyo University of Pharmacy and Life Sciences*)

1P026 **分子シミュレーションによるラクトースリプレッサーの転写制御機構のモデル提案**
MD and *ab initio* MO simulations on transcriptional mechanism controlled by lactose repressor protein and ligand
Yuki Matsushita, Kanako Shimamura, Masato Oishi, Tatsuya Ohyama, Noriyuki Kurita (*Toyohashi University of Technology*)

1P027 **抗体の親和性成熟に着目した抗原 - 抗体結合挙動への洞察**
Insight into the antigen binding motion of germline and affinity-matured antibodies
Yusui Sato[1], Yusuke Tanaka[1], Hiroshi Sekiguchi[2], Yuji C. Sasaki[3], Takachika Azuma[4], Masayuki Oda[1] ([1]*Grad. Sdh. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*JASRI/SPring-8*, [3]*Grad. Sch. of Fron. and Sci., Univ. of Tokyo.*, [4]*Res. Ins. for Biol. Sci., Tokyo Univ. of Sci.*)

1P028 **L-Thr と NAD^+の結合に伴う Cupriavidus necator 由来 L-スレオニン脱水素酵素の構造変化の解析**
Analysis of structural change of L-threonine dehydrogenase from Cupriavidus necator (CnThrDH) by binding of L-Thr and NAD^+
Shogo Nakano[1,3], Seiji Okazaki[1,3], Hiroaki Tokiwa[2,3], Yasuhisa Asano[1,3] ([1]*Biotech. Res. Center & Dept. Biotech., Toyama Pref. Univ.*, [2]*Dept. Chem., Rikkyo Univ*, [3]*ERATO, JST*)

1P029 **アナアオサ由来のプラストシアニンにおける弱い相互作用の役割**
The role of weak interaction in a blue copper protein, plastocyanin from Ulva pertusa
Soichiro Ikeda[1], Akiko Takashina[1], Takahide Yamaguchi[1], Risa Aoki[1], Masaki Unno[1,2], Takamitsu Kohzuma[1,2] ([1]*Grad. ins. Appl. Beam Sci., Univ. Ibaraki*, [2]*iFRC., Univ. Ibaraki*)

1P030 **ブルー銅タンパク質シュウドアズリン Met16His/His6Val 変異体の性質と構造の pH 依存性**

pH dependency of the structure and properties of a blue copper protein, Met16His/His6Val pseudoazurin mutant

Hikaru Sunagawa[1], Tsuyoshi Sakairi[1], Masaki Unno[1,2], Takamitsu Kohzuma[1,2] ([1]*Graduate School of Sci. and Eng., Ibaraki Univ.*, [2]*iFRC, Ibaraki Univ.*)

1P031 **リソスタシンの炭酸カルシウム結晶結合部位の解析**

Functional analysis of calcite-binding site of lithostathine

Maho Nara[1], Yuichi Hanada[2], Hidemasa Kondo[2,3], Sakae Tsuda[2,3] ([1]*Hokkaido College of High Technology*, [2]*Graduate School of Life Science, Hokkaido University*, [3]*Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)*)

1P032 **Production and NMR study of plant defensin-like peptides using plant cell expression system**

Yoshitaka Umetsu[1], Masashi Mori[2], Shinya Ohki[1] ([1]*JAIST*, [2]*Ishikawa Pref. Univ.*)

1P033 **FRET プローブアクチンを用いた細胞内におけるアクチンの構造多型の検出**

Polymorphism of actin in cells, detected by FRET-probed actin

Mio Okazaki[1], Saku Kijima[2,4], Yoshiaki Iwadate[3], Taro Q.P Uyeda[2,4], Taro Q.P Noguchi[1] ([1]*MNCT*, [2]*ATST*, [3]*Yamaguchi University*, [4]*University of Tsukuba*)

1P034 **HP 1 クロモドメイン/histone H3 tail 複合体の全原子構造サンプリング**

Conformational sampling of HP1 chromodomain/histone H3 tail complex

Nobuto Hashiguchi, Satoshi Omori, Kei Moritsugu, Yoshifumi Nishimura, Akinori Kidera (*Yokohama City Univ.*)

1P035 **Sox2 の DNA 結合ドメインの構造揺らぎと DNA 結合に付随するフォールディング**

Conformational Flexibility of the high-mobility group box domain of sox2 and its folding coupled with DNA binding

Erisa Harada, Tsuyoshi Konuma, Syoko Mori, Kenji Sugase (*Suntory Foundation for Life Sciences*)

1P036 **巨大ヘモグロビン結晶内での oxy 型から deoxy 型への移行**

Transition from oxy to deoxy state in crystalline giant hemoglobin

Nobutaka Numoto[1], Taro Nakagawa[2], Akiko Kita[3], Nobutoshi Ito[1], Yoshihiro Fukumori[4], Kunio Miki[5] ([1]*Med. Res. Inst., Tokyo Med. and Dent. Univ. (TMDU)*, [2]*Nagahama Inst. of Bio-Sci. and Tech.*, [3]*Research Reactor Inst., Kyoto Univ.*, [4]*College of Sci. and Eng., Kanazawa Univ.*, [5]*Grad. Sch. of Sci., Kyoto Univ.*)

1P037 **効率的な自由エネルギー計算と構造サンプリングに向けた Integrated Hamiltonian Sampling 法の開発**

Integrated Hamiltonian Sampling: towards efficient free energy calculation and conformational sampling

Toshifumi Mori[1], Qiang Cui[2] ([1]*Institute for Molecular Science*, [2]*Univ. of Wisconsin, Madison*)

1P038 **K63 および linear ユビキチン鎖の構造サンプリング**

Full-scale conformational sampling of K63 and linear polyubiquitins

Masanori Shimizu, Hafumi Nishi, Kei Moritsugu, Akinori Kidera (*Grad. Sch. of Med. Life Sci., Yokohama City University*)

1P039 **Microscopic observation of amyloid deposits associated with lipids of amyloid β-peptide**

Kenji Sasahara (*Grad. Med. Kobe Univ.*)

1P040 **微小管切断蛋白質 katanin の構造と機能に関する研究**

Structure and function of katanin, a microtubule severing protein

Naoko Iwaya[1,2], Syouta Noda[1], Natsuko Goda[1], Takeshi Tenno[1], Hidekazu Hiroaki[1] ([1]*Grad. Sch. Pharm. Sci., Univ. Nagoya*, [2]*JSPS Research Fellow*)

1P041 **リボソームストークによる翻訳伸長因子の認識の仕組み**

The study on aEF-2 recognition mechanism of ribosomal stalk

Takehito Tanzawa[1], Yuki Kumakura[1], Yoshikazu Tanaka[1,2], Toshio Uchiumi[3], Isao Tanaka[2], Min Yao[1,2] ([1]*Grad. Schl. Life. Sci., Hokkaido Univ.*, [2]*Fac. Adv. Life. Sci., Hokkaido Univ.*, [3]*Dept. Biol., Fac. Sci., Niigata Univ.*)

1P042 **HP1 クロモドメイン N 末端部分の構造探索と HP1αCD/histone H3 tail 複合体モデル構築**

Replica-exchange simulation of N-ter fragment in HP1 chromodomain and model construction of HP1αCD/histone H3 tail complex

Satoshi Omori, Nobuto Hashiguchi, Kei Moritsugu, Yoshifumi Nishimura, Akinori Kidera (*Grad. Sch. of Med. Life Sci., Yokohama City University*)

1P043 **Real-Time Observation of DNA Digestion by RecBCD with High-Speed Atomic Force Microscopy**

Weidong Zhao (*Kanazawa University*)

1P044 **Molecular basis of conformational dynamics and enzymatical maturation process of nuclear lamin A related to onset of laminopathies**

Mai Tsunoda[1], Muneyo Mio[1,2], Toshihiko Sugiki[3], Kazuhiro Mio[1,2] ([1]*AIST*, [2]*YCU*, [3]*IPR*)

1P045 **Functional Analysis of a New Type I Antifreeze Protein from Barfin plaice, *Liposetta pinnifasciata***

Sheikh Mahatabuddin[1], Kazunari Ishihara[1], Yuichi Hanada[1], Ai Miura[2], Hidemesa Kondo[1,2], Sakae Tsuda[1,2] ([1]*Graduate School of Life Science, Hokkaido University*, [2]*National Institute of Advanced Industrial Science and Technology (AIST)*)

1P046 **アクチンフィラメントの圧電特性**

Piezoelectric property of an actin filament

Jun Ohnuki[1], Takato Sato[1], Koji Umezawa[1], Taro Q.P. Uyeda[2], Mitsunori Takano[1] ([1]*Dept. of Pure & Appl. Phys., Waseda Univ.*, [2]*Biomedical Res. Inst., AIST*)

1P047 **家族性 ALS に関連した A4V、G93A 変異 SOD1 の酸化促進性獲得**

Acquisition of pro-oxidant activity by fALS-linked SOD1 mutants A4V and G93A

Ken Nishiya, Nobuhiro Fujimaki, Furi Kitamura, Takashi Miura, Takakazu Nakabayashi, Hideo Takeuchi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

1P048 **GroEL に結合した基質 BFP の一分子 FRET 計測による構造解析**
Conformation of the denatured BFP bound to GroEL by single molecule FRET measurements
Aya Yoshida[1,2], Fumihiro Motojima[3], Hiroyuki Oikawa[1], Kiyoto Kamagata[1], Hideki Taguchi[4], Masasuke Yoshida[3], Satoshi Takahashi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Sci., Tohoku Univ.*, [3]*Dept of Mol Biosci, Kyoto Sangyo Univ.*, [4]*Grad. Sci. of Biosci. Biotech., Tokyo Tech*)

1P049 **p53C 末端の負の制御ドメインに関する自由エネルギー地形**
Free-energy landscape of the C-terminal negative regulatory domain of p53
Shinji Iida[1], Haruki Nakamura[2], Junichi Higo[2] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*Institute for Protein Research*)

1P050 **タンパク質分子動力学シミュレーションの二次構造主成分分析**
Secondary Structure Principal Component Analysis of Protein Molecular Dynamics Simulations
Norifumi Yamamoto (*Chiba Inst Tech*)

1P051 **塩酸グアニジン変性における *Aspergillus niger* 由来グルコアミラーゼのデンプン結合ドメインの二つの異なる変性状態**
Two distinctive unfolded states starch binding domain of *Aspergillus niger* accumulated during GdnHCl-induced unfolding
Daizo Hamada[1], **Chiaki Ota**[2], Momoko Kitazawa[2], Hideo Miyake[1], Akiyoshi Tanaka[1] ([1]*Grad Schl Bioresource, Mie Univ*, [2]*Dept Bioresource, Mie Univ*)

1P052 **多機能性タンパク質 PHB2 のフォールディング状態に対する疎水環境の影響**
The effect of hydrophobic environment on folding states of multifunctional protein PHB2
Takeru Chigira[1], Satoru Nagatoishi[2], Toyomasa Katagiri[3], Kouhei Tsumoto[1,2,4] ([1]*Dept. of Chem. and Biol., Sch. of Eng., Univ. of Tokyo*, [2]*Dept. of Bioeng., Sch. of Eng., Univ. of Tokyo*, [3]*Inst. for Genome Res., Univ. of Tokushima*, [4]*Inst. of Med. Sci., Univ. of Tokyo*)

1P053 **AFM による酸化 LDL 粒子の物理的性質の解析**
Mechanical properties of oxidized low-density lipoprotein particles disclosed with AFM
Seiji Takeda[1], Agus Subagyo[2], Shu-Ping Hui[1], Hirotoshi Fuda[1], Kazuhisa Sueoka[2], Hitoshi Chiba[1] ([1]*Hokkaido Univ. Faculty of Health Sciences*, [2]*Hokkaido Univ. Graduate School of Information Science and Technology*)

1P054 **分子動力学シミュレーションによる抗体表面を動く水の解析**
Analysis of water dynamics at the surfaces of antibodies: Molecular dynamics study
Keiko Shinoda, Hideaki Fujitani (*RCAST, Univ. of Tokyo*)

1P055 **二次元蛍光寿命相関分光法による BdpA 折りたたみ機構解明に向けた研究:2 つの BdpA 変異体による包括的解析**
Study of BdpA folding by two-dimensional fluorescence lifetime correlation spectroscopy: Comprehensive analysis of two BdpA mutants
Takuhiro Otosu[1], Kunihiko Ishii[1], Hiroyuki Oikawa[2], Munehito Arai[3], Satoshi Takahashi[2], Tahei Tahara[1] ([1]*Mol. Spectrosc. lab., RIKEN*, [2]*IMRAM, Tohoku Univ.*, [3]*Grad. Sch. Arts. Sci., Univ. Tokyo*)

1P056 **たった 10 残基から成るタンパク質の立体構造安定性に及ぼす主鎖と側鎖の充填の効果**
Effect of Backbone and Side-chain Packing on Structural Stability of the Protein with Only Ten Residues
Satoshi Yasuda, Tomohiko Hayashi, Masahiro Kinoshita (*IAE, kyoto Univ.*)

1P057 **カルシトニンアミロイド凝集機構の解明と抑制法の考案**
Analysis of amyloid formation and inhibition mechanisms of human calcitonin
Hiroko Tanaka, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

1P058 **分子動力学シミュレーションを用いたリガンド結合による PR-Set7 の構造変化の研究**
Molecular dynamics simulations for structure changes of PR-Set7 by ligand binding
Takako Sakano, Hideaki Fujitani (*RCAST, UTokyo*)

1P059 **オクタリピート領域をもつプリオンペプチドにおける金属との競合結合性**
Competitive binding of metal ions to the octarepeat region of human prion protein
Masahiro Yagi, Kazuya Iwama, Haruto Onda, Wakako Hiraoka (*Dept. Phys., Grad. Sch. Sci. & Tech., Meiji Univ.*)

1P060 **粗視化 MD-SAXS 法の開発**
Development of Coarse-Grained MD-SAXS method
Yuichi Kokabu[1], Tomotaka Oroguchi[2], Mitsunori Ikeguchi[1] ([1]*Yokohama City Univ.*, [2]*Keio Univ.*)

1P061 **高圧分子動力学法によるヘリックス構造を持つペプチドの熱力学と構造に関する研究**
High-pressure molecular dynamics study on the thermodynamics and structures of helical peptides
Yoshiharu Mori[1], Hisashi Okumura[1,2] ([1]*IMS*, [2]*SOKENDAI*)

1P062 **トリプトファン三重項寿命測定を用いた staphylococcal nuclease の変性構造の評価**
Characterization of the denatured structure of staphylococcal nuclease by tryptophan triplet state lifetime measurements
Sadatoshi Aoyagi, Mariko Yamaguchi, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

1P063 **フェリチンのアセンブリメカニズムの研究**
A study of ferritin assembly mechanism
Daisuke Sato, Hideaki Ohtomo, Atsushi Kurobe, Ayumi Sunato, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. Bioinfo., Soka Univ.*)

1P064 **ヨウ素染色によるアミロイド線維構造の識別**
Discrimination of amyloid fibril structures by iodine staining
Takato Hiramatsu, Seongmin Ha, Yuki Masuda, Eri Chatani (*Grad.Sch.of Sci.,Univ. Kobe*)

1P065 **β-ストランドの局所配列とねじれ、曲がりの関係**
Local sequence of protein β-strands influences twist and bend angles
Kazuo Fujiwara, Shinichi Ebisawa, Yuka Watanabe, Hiromi Toda, Masamichi Ikeguchi (*Dept. of Bioinfo., Soka Univ.*)

1P066 **アミノ酸挿入によるアミロイドβペプチドの線維形成への影響**
Effects of single amino-acid insertion on amyloid β fibril formation
Kazuto Yamashita, Motonari Tsubaki, Eri Chatani (*Grad. Sch. Sci., Kobe Univ.*)

01D. 蛋白質：機能 / 01D. Protein: Function

1P067 **超好熱アーキア由来 S-layer タンパク質の糖結合特異性**
Carbohydrate binding of S-layer protein derived from hyperthermophilic archaea
Shuichiro Goda, Kenichiro Yamashita, Hideaki Unno, Tomomitsu Hatakeyama (*Grad. Sch. Eng., Nagasaki Univ.*)

1P068 **４量体型サルコシン酸化酵素の分子動力学シミュレーション: 反応物と生成物の輸送経路の解明**
Molecular dynamics simulation of heterotetrameric sarcosine oxidase: analysis of channeling of reactants and products
Daisuke Nakajima[1], Go Watanabe[2], Haruo Suzuki[2], Shigetaka Yoneda[2] ([1]*Grad. Sch. Sci., Kitasato Univ.*, [2]*Sch. Sci., Kitasato Univ.*)

1P069 **リガンド結合の動的側面：PDZ ドメインタンパク質を用いた研究**
Dynamical aspects of ligand binding: A case study for PDZ domain protein
Hiroshi Fujisaki[1], Norifumi Yamamoto[2], Kana Fuji[3], Mikito Toda[3] ([1]*Nippon Medical School*, [2]*Chiba Inst. Tech.*, [3]*Nara Women's Univ.*)

1P070 **網羅的アミノ酸置換変異によるアシル ACP 還元酵素の活性部位の解析**
Comprehensive mutagenesis reveals residues critical for aldehyde producing activity of acyl-ACP reductase
Munehito Arai[1,2], Fumitaka Yasugi[1] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

1P071 **アミロイド線維の人工設計ペプチドによる加水分解**
Hydrolysis of amyloid fibrils by artificially designed peptides
Yoshihiro Iida, Atsuo Tamura (*Grad. Sch. Sci., Univ. Kobe*)

1P072 **Large time step molecular dynamics using Torsion Angle Molecular Dynamics**
Yu Yamamori, Akio Kitao (*Tokyo Univ. ICMS*)

1P073 **鉄イオン貯蔵タンパク質に対する交流磁場の影響**
Effects of alternating magnetic fields on iron-storage protein
Yuta Yamada, Tsuyoshi Hondou, Hidetake Miyata (*Grad. Sch. Sci. , Univ. Tohoku*)

1P074 **ニトリルヒドラターゼ（NHase）によるアミド生成機構に関する理論的研究**
A QM/MM study of amide formation reaction of Nitrile Hydratase
Megumi Kayanuma[1], Mitsuo Shoji[2], Yasuteru Shigeta[2] ([1]*Grad. Sch. of Sys. and Inf. Eng., Univ. of Tsukuba*, [2]*Grad. Sch. of Pure and App. Sci., Univ. of Tsukuba*)

1P075 **Effect of C-terminal truncation of chaperonin GroEL on the yield of an in-cage folding of GFP**
So Ishino[1], Yasushi Kawata[2], Hideki Taguchi[3], Katsumi Matsuzaki[1], Masaru Hoshino[1] ([1]*Graduate School of Pharmaceutical Sciences, Kyoto Univiersity*, [2]*Department of Biotechnology, Graduate School of Engineering, Tottori University*, [3]*Department of Biomolecular Engineering Graduate School of Biosciences and Biotechnology Tokyo Institute of Technology*)

1P076 **抗体 G2 の複数の抗原を特異的に認識する機構**
Mechanism of multispecific recognition of monoclonal antibody G2
Yuji O. Kamatari[1], Masayuki Oda[2], Takahiro Maruno[3], Shohey Shimizu[2], Yuji Kobayashi[3], Naotaka Ishiguro[4] ([1]*Life Sci. Res. Ctr, Gifu Univ.*, [2]*Grad. Sch. Life Environm. Sci., Kyoto Pref. Univ.*, [3]*Grad. Sch. Engn., Osaka Univ.*, [4]*Fac. Appl. Biol. Sci., Gifu Univ.*)

1P077 **NMR およびドッキングによる抗菌ペプチドとリポ多糖の複合体構造解析**
NMR and docking structure of antimicrobial peptide complexed with lipopolysaccharide
Takahiro Kushibiki[1], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Yasuhiro Kumaki[2], Takashi Kikukawa[1], Mineyuki Mizuguchi[3], Makoto Demura[1], Syun-ichiro Kawabata[4], Keiichi Kawano[1,5] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Grad. Sch. Sci., Hokkaido Univ.*, [3]*Grad. Sch. Med. & Pharm. Univ. of Toyama*, [4]*Dept. Biol., Kyusyu Univ.*, [5]*Chitose Inst. Sci. Tech.*)

1P078 **MD シミュレーションを用いた Neuropsin - Neuregulin-1 リガンド間の相互作用に関する研究**
Molecular Dynamics Study on Interactions between Neuropsin and Neuregulin-1 Ligand
Mitsumasa Abe[1], Hideki Tamura[2], Yoshifumi Fukunishi[3], Masami Lintuluoto[1] ([1]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*L-StaR, Hoshi Univ. Sch. Pham. and Pham. Sci.*, [3]*AIST*)

1P079 **Analysis on the interaction between G3LEA proteins and other proteins by quartz crystal microbalance**
Tetsuro Yamaguchi[1], Kentaro Yamakawa[1], Takao Furuki[1], Rie Hatanaka[2] ([1]*Center for Biol. Res. & Inform., Tokyo Tech*, [2]*Natl. Inst. Agrobiol. Sci.*, [3]*Grad. Sch. of Biosci. Biotech., Tokyo Tech*)

01E. 蛋白質：計測・解析の方法論 / 01E. Protein: Measurement & Analysis

1P080 **NMR スペクトルの再構成における圧縮センシングの基底選択の影響**
Effect of basis selection in reconstructing NMR spectra using compressed sensing
Kazuya Sumikoshi[1], Teppei Ikeya[2], Yutaka Ito[2], Kentaro Shimizu[1] ([1]*Grad. Sch. Agr. Life Sci., Univ. Tokyo*, [2]*Grad. Sch. Sci. Eng., Tokyo Metropolitan Univ.*)

1P081 **べん毛 C-ring 構成要素である FliM-FliN 複合体のストイキオメトリと、べん毛輸送装置タンパク質との高次複合体**
Stoichiometry of the FliM-FliN complex, a flagellar C-ring component, and its higher order complex with flagellar export appratus proteins
Kazushi Matsushima[1], Hiroyuki Terashima[1], Miki Kinoshita[2], Tohru Minamino[2] ([1]*Grad. sch. sci. Osaka Univ.*, [2]*Grad. Sch. Front. Bio. Sci. Osaka Univ.*)

1P082 **Ｘ線１分子追跡法によるリン酸化タウタンパク質の構造動態**
X-ray Single Molecule Observations of Phosphorylated Tau protein's structural fluctuations
Masahiro Shimura[1], Yuufuku Matsushita[1], Keigo Ikezaki[1], Tomohiro Miysasaka[2], Kouhei Ichiyanagi[3], Hiroshi Sekiguchi[4], Yasuo Ihara[2], Yuji Sasaki[1,4] ([1]*Grad. School Frontier Sci., Univ. Tokyo*, [2]*Faculty of life & Medical Sci., Doshisha Univ.*, [3]*High Energy Accelerator Research Organizaiton*, [4]*Research & Utilization Div., SPring-8/JASRI*)

1P083 **ラマン分光法による高濃度タンパク質溶液の分子間相互作用の評価**
Evaluation of inter-molecular interaction of a protein in highly concentrated solution investigated by Raman spectroscopy
Sakiko Akaji[1], Chikashi Ota[1], Shintaro Noguchi[1], Kohei Tsumoto[2,3] ([1]*HORIBA, Ltd.*, [2]*Dept. of Bioeng., Grad. Sch. of Eng., Univ. of Tokyo*, [3]*Inst. Med. Sci., Univ. of Tokyo*)

1P084 **基準振動解析に基づいたタンパク質分子動的領域のモデル化**
Modeling Motion Parts of Protein Based on Normal Mode Analysis
Shinya Muraoka[1], Yutaka Ueno[1,2] ([1]*NAIST*, [2]*Health Resarch Institute, AIST*)

1P085 **プロリン異性化酵素 Pin1 のドメイン間接触頻度による機能制御**
Functional regulation of Pin1 cis-trans Pro-isomerase by the inter-domain contact frequency
Naoya Tochio[1], Ryosuke Kawasaki[2], Yu Tamari[2], Shin-ichi Tate[1,2] ([1]*RcMcD, Hiroshima Univ.*, [2]*Dept. of Math. and Life Sci., Grad Sch. of Sci. Hiroshima Univ.*)

01F. 蛋白質：蛋白質工学／進化工学 / 01F. Protein: Engineering

1P086 **アポミオグロビン折り畳み中間体に存在するノンネーティブなＨヘリックス領域構造**
Non-native H helix translocation in folding intermediate of apomyoglobin
Chiaki Nishimura[1,2], Phillip Aoto[1], Jane Dyson[1], Peter Wright[1] ([1]*Dept. Mol. Biol., Scripps Res. Inst.*, [2]*Fac. Pharm. Sci., Teikyo Heisei Univ.*)

1P087 **機能性ペプチドから創出する小型タンパク質**
Generating a small-sized protein from a functional peptide
Hideki Watanabe, Shinya Honda (*AIST BMRI*)

1P088 **ハロゲン化チロシンの多箇所への部位特異的導入によるタンパク質の安定化**
Multiple site-selective integrations of bulky halogenated tyrosines enhance protein stability
Kazumasa Ohtake[1,2], Atsushi Yamaguchi[1,2], Mitsuru Haruki[3], Kenji Yamagishi[3], Kazutaka Murayama[4], Mikako Shirouzu[1,2], Shigeyuki Yokoyama[1,5], Kensaku Sakamoto[1,2] ([1]*RIKEN Systems and Structural Biology Center*, [2]*RIKEN Center for Life Science Technologies*, [3]*Department of Chemical Biology and Applied Chemistry, College of Engineering, Nihon University*, [4]*Biomedical Engineering Research Organization, Tohoku University*, [5]*RIKEN Structural Biology Laboratory*)

1P089 **Expression and refolding of the protein from a fruits of *Richardella dulcifica***
Maria Namba, Satoko Shibuya, Naoya Hashikawa, Satoru Yamaguchi (*Okayama Univ. Sci.*)

1P090 **Introduction of negatively charged residues compensates for decreased protein solubility caused by an artificial hydrophobic surface**
Sota Yagi, Satoshi Akanuma, Akihiko Yamagishi (*Tokyo University of Pharmacy and Life Sciciences*)

1P091 **抗体精製用リガンド FPA の抗体解離メカニズムの解明と改良**
Mechanism and improvement of pH-sensitive antibody dissociation by FPA, a ligand for antibody purification
Taihei Sawada[1], Takahiro Watanabe[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

02. ヘム蛋白質 / 02. Heme proteins

1P092 **亜硝酸還元酵素と一酸化窒素還元酵素複合体の結晶構造**
Crystal structure of complex of nitrite reductase and nitric oxide reductase
Takehiko Tosha[1], Erina Terasaka[1,2], Hiroshi Sugimoto[1], Yoshitsugu Shiro[1,2] ([1]*RIKEN SPring-8*, [2]*Grad. Sch. Sci., Univ. Hyogo*)

1P093 **ハーフメト Hb M における酸素親和性と正常鎖、異常鎖の配位状態との関係**
Relationship between oxygen affinity and coordination state of normal or abnormal chain in half-met Hb Ms
Shusei Hashihara[1], Miki Okumura[1], Shigenori Nagatomo[1], Masako Nagai[2], Takashi Ogura[3], Teizo Kitagawa[3], Mafumi Hishida[1], Yasuhisa Yamamura[1], Kazuya Saito[1] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*Res. Center Micro-Nano Tech., Hosei Univ.*, [3]*Grad. Sch. Life Sci., Univ. Hyogo*)

1P094 **α 鎖あるいは β 鎖の近位ヒスチジンがグリシンに置換された変異ヘモグロビンの機能と構造**
Function and structure of mutant hemoglobins with the proximal histidine replaced by glycine in either α or β subunit
Shigenori Nagatomo[1], Yukufumi Nagai[2], Yayoi Aki[3], Hiroshi Sakurai[3], Natsumi Maruyama[4], Kiyohiro Imai[4], Naoki Mizusawa[2,4], Takashi Ogura[5], Teizo Kitagawa[5], Masako Nagai[2,3] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*Res. Center Micro-Nano Tech., Hosei Univ.*, [3]*Sch. Health Sci., Coll. Med., Pharm. and Health Sci., Kanazawa Univ.*, [4]*Dept. Frontier Biosci., Hosei Univ.*, [5]*Grad. Sch. Life Sci., Univ. Hyogo*)

1P095 **Unique reaction mechanism of MhuD, a heme-degrading enzyme from Mycobacterial tuberculosis**
Toshitaka Matsui, Syusuke Nambu, Masao Ikeda-Saito (*IMRAM, Tohoku Univ.*)

1P096 **The Caged State, the Transition State of the Regulation of Oxygen-Affinity in Hemoglobin**
Takashi Yonetani[1], Kenji Kanaori[2] ([1]*Univ. of Pennsylvania*, [2]*Kyoto Inst. of Tech.*)

1P097 異なるゾル・ゲル由来シリカゲル中ヘモグロビンのアロステリック転移の比較
Comparison of allosteric transitions in hemoglobin in different sol-gel derived silica gels
Naoya Shibayama (*Div. of Biophysics, Jichi Medical Univ.*)

1P098 シトクロム c_3 中のヘムの電子構造制御に関する計算科学的研究
Computational study of the electronic structures of hemes in cytochrome c_3
Yasuhiro Imada, Haruki Nakamura, Yu Takano (*IPR, Osaka Univ.*)

03. 膜蛋白質 / 03. Membrane proteins

1P099 分子動力学法が明らかにした ADP/ATP 透過担体の内向き開構造のモデル
A plausible model for the structurally unknown inward-facing conformation of ADP/ATP carrier: A molecular dynamics study
Koichi Tamura, Shigehiko Hayashi (*Grad. Sch. Sci., Univ. Kyoto*)

1P100 拡張アンサンブルシミュレーションを用いた繊維芽細胞増殖因子受容体(FGFR3)の膜貫通ドメインの構造予測結果と実験との比較
Comparison of the predicted structure of the FGFR3 transmembrane domain by enhanced sampling simulations with experimental results
Yumi Kashihara[1,2], Naoyuki Miyashita[1,2], Pai-Chi Li[3], Yuji Sugita[1,2,3,4] ([1]*RIKEN QBiC*, [2]*RIKEN AICS*, [3]*RIKEN TMS*, [4]*RIKEN*)

1P101 アクアポリン 3 の投影像へのホモロジーモデルの当てはめ
Fitting of a homology model to a projection map of aquaporin-3
Kaoru Mitsuoka (*Next Generation Natural Products Chemistry*)

1P102 酸素センサータンパク質 Aer のシグナル伝達機構の解明
Elucidation of signal transduction mechanism of Aer
Yoriyoshi Oka, Tatsuya Iwata, Hideki Kandori (*Nagoya Institute of Technology*)

1P103 NMR, QCM, MD シミュレーションによる κ-オピオイド受容体細胞外第２ループとダイノルフィンとの細胞膜中での相互作用解析
Interaction of ECL-II of k-opioid receptor with dynorphin in membrane environments as revealed by solid state NMR, QCM and MD simulation
Akira Naito[1], Atsushi Kira[1], Namsrai Javkalantugs[2], Takenori Miyamori[1], Yoshiyuki Sasaki[1], Masayuki Eguchi[1], Izuru Kawamura[1], Kazuyoshi Ueda[1] ([1]*Yokohama National University*, [2]*National University of Mongolia*)

1P104 分子動力学計算から明らかにされた細菌機械受容チャネル MscL のゲーティングにおける N 末端領域のメカノセンサーとしての役割
The N-terminal domain acts as a mechanosensor in the gating of the bacterial mechanosensitive channel MscL: molecular dynamics study
Yasuyuki Sawada[1], Masahiro Sokabe[2] ([1]*Dept. Physiol. Nagoya Univ. Grad. Sch. Med.*, [2]*Mechanobiology Lab. Nagoya Univ. Grad. Sch. Med.*)

1P105 改良した多リン酸力場を用いた ATP/ADP 結合状態の筋小胞体カルシウムポンプの分子動力学計算
Molecular dynamics simulations of ATP/ADP bound forms of SR Ca^{2+}-ATPase using CHARMM force field with modified polyphosphate parameters
Yasuaki Komuro[1,2,3], Suyong Re[2], Chigusa Kobayashi[3], Eiro Muneyuki[1], Yuji Sugita[2,3,4,5] ([1]*Grad. Sci and Eng., Chuo Univ.*, [2]*RIKEN*, [3]*RIKEN AICS*, [4]*RIKEN QBiC*, [5]*RIKEN iTHES*)

1P106 膜孔形成蛋白質 FraC の構造変化機構の解析：可溶性構造から膜貫通構造までの変遷
Structural analysis of the α-helical pore-forming toxin FraC; metamorphosis from a water-soluble to a transmembrane protein
Koji Tanaka[1], Jose Caaveiro[1], Kouhei Tsumoto[1,2] ([1]*Sch. of Eng., Univ. of Tokyo*, [2]*IMSUT*)

1P107 性フェロモン生合成活性化神経ペプチド受容体（PBANR）の発現、リガンド結合、会合状態の解析による結晶化の為の T4 リゾチーム置換位置の検討
Positional optimization of T4L that replaces IL3 of PBANR for crystallization by expression, ligand binding, and size exclusion analyses
Yukie Katayama[1], Tatsuya Suzuki[1], Tatsuki Ebisawa[1], Takeshi Kawai[1], Jun Ohtsuka[1], Ryo Natsume[2], Yu-Hua Lo[3], Toshiya Senda[3], Toshihiro Nagamine[4], Masaaki Kurihara[4], Jae Min Lee[4], J. Joe Hull[5], Shogo Matsumoto[4], Hiromichi Nagasawa[1], Koji Nagata[1], Masaru Tanokura[1] ([1]*UTokyo*, [2]*TDU*, [3]*KEK-PF*, [4]*RIKEN*, [5]*USDA-ARS*)

1P108 レプリカ交換アンブレラサンプリングシミュレーションでのコレステロール分子との相互作用によるアミロイド前駆体の C99 単量体の構造変化
Structural change of APP-C99 induced by interactions with cholesterol studied by Replica Exchange Umbrella Sampling (REUS) simulation
Ryo Urano[1], John E. Straub[2], Yuko Okamoto[1,3,4,5] ([1]*Dept. Phys, Grad. Sch. Sci., Nagoya Univ.*, [2]*Dept. Chem., Boston Univ.*, [3]*Cent. Comput. Sci., Grad. Sch. Eng., Nagoya Univ.*, [4]*Info. Tech. Cent., Nagoya Univ.*, [5]*Struct. Biol. Res. Cent., Sch. Sci., Nagoya Univ.*)

1P109 チャネルロドプシンの構造変化におけるカチオンの効果
Structural changes of channelrhodopsin under various cation conditions
Shota Ito[1], Hideaki Kato[2], Satomi Ohishi[3], Reiya Taniguchi[3], Tatsuya Iwata[1], Osamu Nureki[3], Hideki Kandori[1] ([1]*Nagoya Inst.Tech*, [2]*Stanford University Medical School*, [3]*Grad.Sch.of Sci., Univ. of Tokyo*)

1P110 **Effect of partial fluorination on bacteriorhodopsin reconstituted in dimerized Di-o-tetradecylphosphatidylcholine vesicle**
Naoyuki Tsuchida[1], Toshiyuki Takagi[2], Takashi Kikukawa[3], Hiroshi Takahashi[1], Toshiyuki Kanamori[2], Masashi Sonoyama[1] ([1]*Fac. Sci. & Tecnol., Gunma. Univ.*, [2]*Res. Center. Stem Cell Eng., AIST*, [3]*Grad. Sch. Sci., Hokaido. Univ.*)

1P111 Ca^{2+}-ATPase の第 2 膜貫通ヘリックス（M2）とロングレンジの共役
Second Transmembrane Helix (M2) and Long-range Coupling in Ca^{2+}-ATPase
Takashi Daiho, Kazuo Yamasaki, Stefania Danko, Hiroshi Suzuki (*Asahikawa Med. Univ.*)

1P112 **アジ化物結合型チトクロム酸化酵素の高分解能 X 線結晶構造解析によるアジ化物結合様式の解析**
High-resolution crystal structural analysis reveals that the two azide ions bind to Cytochrome c oxidase in different manner
Atsuhiro Shimada[1], Masahide Hikita[1], Hitomi Tadehara[1], Akima Yamamoto[1], Eiki Yamashita[1], Kyoko Shinzawa-Itoh[1], Tomoko Maeda[1], Tomitake Tsukihara[1,2], Shinya Yoshikawa[1] ([1]*Picobiology Inst., Univ. Hyogo*, [2]*Inst. for Prot. Res., Osaka Univ.*)

1P113 **NDQ モチーフを持つ pseudo gene の機能復元**
Functional restoration of apseudo gene of rhodopsin with NDQ motif
Yuto Suzuki[1], Rei Abe-Yoshizumi[1], Keiichi Inoue[1,2] ([1]*Nagoya Institute of Technology*, [2]*PRESTO, JST*)

1P114 **Role of a unique arginine residue on the assembly of the translocator domain in a trimeric autotransporter**
Eriko Aoki, Riki Hisata, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. of Bioinfo., Soka Univ.*)

1P115 **ヒト cytochrome b561 form 3 (hb561-3)タンパク質の機能解析**
Analyses of the physiological function of hb561-3: the cytochrome b561 form 3 protein in human
Yuma Takahashi, Takako Yamazoe, Akikazu Asada, Motonari Tsubaki (*Grad. Sch. Sci., Univ. Kobe*)

04. 核酸結合蛋白質 / 04. Nucleic acid binding proteins

1P116 **常磁性緩和促進を利用した転写因子 FMBP-1 の分子ダイナミクスの解析**
Molecular dynamics of transcription factor FMBP-1 proved by paramagnetic relaxation enhancement
Kosuke Yuhara[1], Masakatsu Kamiya[1], Yasuhiro Kumaki[1], Shigeharu Takiya[1], Takashi Kikukawa[1], Keiichi Kawano[1,2], Makoto Demura[1], Tomoyasu Aizawa[1] ([1]*Grad. Sch. Life. Sci., Hokkaido Univ.*, [2]*Chitose Inst. Sci. Tech.*)

1P117 **MBD4 蛋白質のメチル化 CpG 結合ドメインと塩基除去活性ドメインをつなぐリンカー領域の構造と機能**
Structural analysis of a linker region between methyl CpG binding and glycosylase domains in MBD4
Itaru Takeshita (*Grad. Sch. Eng., Kyoto Univ.*)

1P118 **新奇 DNA 結合ドメイン STPR を持つ転写因子 FMBP-1 の in situ コンディションにおける DNA 認識動態の FCS 解析**
FCS analysis of DNA recognition movements of transcription factor FMBP-1 contains a novel DNA binding domain STPR in situ condition
Motosuke Tsutsumi[1], Hideki Muto[1], Mai Kimoto[1], Masakatsu Kamiya[1], Takashi Kikukawa[1], Shigeharu Takiya[1], Makoto Demura[1], Keiichi Kawano[1,2], Masataka Kinjo[1], Tomoyasu Aizawa[1] ([1]*Grad. Sch. of Life Sci., Hokkaido Univ*, [2]*Chitose Inst. Sci. Tech.*)

1P119 **転写因子はいかにして障害物を迂回するか。分子シミュレーションによるアプローチ**
How transcription factor can bypass obstacles? Molecular simulation approaches
Mami Saito[1], Tsuyoshi Terakawa[2], Shoji Takada[1] ([1]*Grad. Sch.Sci., Univ. Kyoto*, [2]*Univ. Columbia*)

05A. 核酸：構造・物性 / 05A. Nucleic acid: Structure & Property

1P120 **RecA 蛋白質の生体分子認識能力によるカーボンナノチューブ表面の DNA の生物的機能の評価**
Evaluation of biological function of DNA on the single-walled carbon nanotubes by using biomolecular recognition ability of RecA proteins
Shusuke Oura, Masahiro Ito, Yoshikazu Homma, Kazuo Umemura (*Grad. Sch. Sci., Tokyo University of Science*)

1P121 **新規プリオンアプタマーの探索とその構造研究**
Search for novel aptamer against prion protein and its structural study
Tsukasa Mashima[1], Fumiko Nishikawa[2], Yuji O. Kamatari[3], Takashi Nagata[1,4], Satoshi Nishikawa[2], Kazuo Kuwata[5], Masato Katahira[1,4] ([1]*Inst. of Adv. Energy, Kyoto Univ.*, [2]*AIST*, [3]*Life Sci. Res. Center, Gifu Univ.*, [4]*Grad. Sch. of Energy Sci., Kyoto Univ.*, [5]*Unit. Grad. Sch. of Drug Disc. and Med. Info. Sci., Gifu Univ.*)

1P122 **剪断流下における二重ラセンＤＮＡのダイナミクス**
Dynamics of double helix DNA under shear flow
Yosuke Fujita[1], Nobumasa Nakazawa[1], Takako Kato-Minoura[1], Hiroyuki Iwamoto[2], Shinji Kamimura[1] ([1]*Dept. Biol. Sci., Chuo Univ.*, [2]*SPring-8, JASRI*)

05B. 核酸：相互作用・複合体 / 05B. Nucleic acid: Interaction & Complex formation

1P123 **ヌクレオソームＤＮＡ解離の自由エネルギープロファイル**
Free Energy Profile of Nucleosomal DNA Unwrapping
Hidetoshi Kono, Shun Sakuraba, Hisashi Ishida (*Molecular Modeling and Simulation, JAEA*)

1P124 **SecM の N 末端側領域による翻訳アレストの安定化**
N-terminal region of SecM is essential for its stable translation arrest
Zhuohao Yang, Ryo Iizuka, Takashi Funatsu (*Grad. Sch. of Pharm. Sci., The Univ. of Tokyo*)

1P125 **蛍光タンパク質との融合による光二量体化モジュール（Photodimerizer）の機能評価**
Functional evaluation of the light-induced dimerizing module, Photodimerizer, fused with fluorescent proteins
Osamu Hisatomi, Yoichi Nakatani, Yuki Kai (*Grad. Sch. od Sci., Osaka Univ.*)

1P126 **大腸菌 *E.coli* ゲノム複製開始における DNA 二重らせん開裂機構の分子動力学シミュレーション研究**
DNA unwinding mechanism at DNA replication initiation of *E.coli* studied by coarse grained molecular dynamics simulation
Masahiro Shimizu[1], Tsutomu Katayama[2], Shoji Takada[1] ([1]*Dept. of Biophys., School of Sci., Kyoto Univ.*, [2]*Dept. of Mol. Biol., Kyusyu Univ.*)

1P127 **Model of reverse tRNA translocation through the ribosome analyzed by electron microscopy density maps and molecular dynamics simulations**

Hisashi Ishida, Atsushi Matsumoto (*Quantum Beam Science Center, Japan Atomic Energy Agency*)

1P128 **染色体対合形成の力学モデル**

Dynamical model of chromosome synapsis formation

Keisuke Yamamoto[1], Hiraku Nishimori[1,2], Akinori Awazu[1,2] ([1]*Dept. of Math. and Life Sci., Hiroshima Univ.*, [2]*Research Center for the Mathematics on Chromatin Live Dynamics*)

07. 水・水和／電解質 / 07. Water & Hydration & Electrolyte

1P129 **タンパク質構造に対するイオン効果のエネルギー解析**

Free-energy analysis of the effect of ions on protein structure

Yasuhito Karino[1,2], Yuji Sugita[1,2,3] ([1]*RIKEN QBiC*, [2]*RIKEN AICS*, [3]*RIKEN*)

1P130 **積分方程式理論を用いた剛体球混合系における選択的溶媒和の研究**

Preferential Solvation in Hard-Sphere Mixtures: Integral Equation Study

Yuichi Kawabata, Ryo Akiyama (*Grad. Sch. Sci., Univ. Kyushu*)

1P131 **グルタミン酸脱水素酵素のドメイン運動に協奏した水和構造変化**

Hydration structure controls domain motion of glutamate dehydrogenase

Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2] ([1]*Dept. of Phys., Keio Univ.*, [2]*Harima Inst., Riken*)

1P132 **テラピア及チョウザメコラーゲン水溶液の広帯域誘電緩和**

Broadband dielectric relaxation of the aqueous solution of tilapia and sturgeon collagen

Hiroshi Kawamata[1], Shunsuke Kuwaki[1], Masanori Mizuno[1], Kazunari Urasawa[1], Tomobumi Mishina[1], Toshiyuki Ikoma[2], Junzo Tanaka[2], Xi Zhang[3], Shinji Adachi[3], Kazuhiro Ura[3], Noriko Azuma[3], Yasuaki Takagi[3], Ryusuke Nozaki[1] ([1]*Dep. Phys., Fac. Sci., Hokkaido Univ.*, [2]*Dep. Metallurgy and Ceramics Sci., Grad. Sch. Sci. and Eng., Tokyo Inst. Tech.*, [3]*Dep. Acuaculture Life Sci., Fac. Fishers Sci., Hokkaido Univ.*)

09. 発生・分化 / 09. Development & Differentiation

1P133 **極性タンパク質 PAR-2 の非対称局在は、細胞質における拡散によって安定化される**

Stable maintenance of the cortical PAR-2 asymmetry by the cytoplasmic diffusion in the one-cell *C. elegans* embryo

Yukinobu Arata[1], Michio Hiroshima[2], Chan-gi Pack[1], Kennichi Nakazato[3], Tetsuya Kobayashi J.[4], Tatsuo Shibata[5], Yasushi Sako[1] ([1]*Cell. Info. Lab., RIKEN*, [2]*Lab. Cell Sig. Dyn., QBiC, RIKEN*, [3]*Theor. Biol. Lab., RIKEN*, [4]*Inst. Indst. Sci., Univ. Tokyo*, [5]*Lab. Phy. Biol., CDB, RIKEN*)

1P134 **組織応力の異方性が細胞の六角格子化を促進する**

Anisotropic tissue stress promotes ordering in hexagonal cell packing

Kaoru Sugimura[1], Keisuke Ikawa[1], Shuji Ishihara[3] ([1]*Kyoto Univ.*, [2]*JST PRESTO*, [3]*Meiji Univ.*)

1P135 **細胞間に非対称性を生み出すしくみの再構成**

Reconstitution of an intercellular symmetry breaking mechanism

Mitsuhiro Matsuda[1], Makito Koga[1], Knut Woltjen[2], Eisuke Nishida[3], **Miki Ebisuya**[1] ([1]*RIKEN CDB*, [2]*CiRA, Kyoto Univ*, [3]*Grad Sch of Biostudies, Kyoto Univ*)

10. 筋肉 / 10. Muscle

1P136 **低温電子顕微鏡による骨格筋の細いフィラメントの構造解析**

Structural analysis of Ca2+ regulated thin filament from skeletal muscle by electron cryomicroscopy

Yurika Yamada[1], Takashi Fujii[2], Keiichi Namba[1,2] ([1]*Grad. Sch. of Frontier Biosci., Osaka Univ.*, [2]*QBiC, RIKEN*)

1P137 **中性子非弾性散乱による F-アクチン及びミオシン S1 のダイナミクス解析**

Difference in dynamics between F-actin and myosin S1 measured by neutron scattering

Tatsuhito Matsuo[1], Toshiaki Arata[2], Toshiro Oda[3], Satoru Fujiwara[1] ([1]*Japan Atomic Energy Agency*, [2]*Osaka Univ.*, [3]*Univ. of Hyogo*)

1P138 **アクチン疎水性ヘリックスに導入した変異は細胞性粘菌の細胞運動と細胞分裂を阻害する**

Mutations introduced into the hydrophobic helix of the *Dictyostelium* actin impaired cell motility and cytokinesis

Takahiro Ohnuki[1], Yuki Gomibuchi[1], Taro Uyeda[2], Takeyuki Wakabayashi[1] ([1]*Teikyo Univ. School of Science and Engineering*, [2]*AIST*)

1P139 **心筋症特異的なトロポミオシン変異体による再構成フィラメントの光ピンセット及び熱パルスを用いた in vitro assay**

Characterization of tropomyosin mutants that cause hypertrophic cardiomyopathy (HCM): In vitro assays with optical tweezers and heat pulse

Shuya Ishii[1], Kotaro Oyama[1], Madoka Suzuki[2,3], Masataka Kawai[4], Shin'ichi Ishiwata[1,2,3] ([1]*Sch. Adv. Sci. Eng., Waseda Univ.*, [2]*WABIOS, Singapore*, [3]*Org. Univ. Res. Initiatives, Waseda Univ.*, [4]*Coll. Med., Univ. Iowa, USA*)

1P140 **ミオシンフィラメント懸濁液の ATP 存在下でのプロトン NMR 緩和経過**

Spin-spin relaxation of 1H NMR signals from myosin filaments suspension with or without ATP

Tetsuo Ohno, Maki Yamaguchi (*Dept. of Physiol., The Jikei Univ. School of Med.*)

1P141 **アクチン結合タンパク質は結合ドメイン単独でも細胞内の特定の F-アクチンに局在する**

Actin binding domains of certain actin binding proteins are sufficient to localize at specific F-actin in vivo

Keitaro Shiabta[1], Akira Nagasaki[1], Masatsune Tsujioka[2], Taro Q.P. Uyeda[1] ([1]*Biomed. Res. Inst., AIST*, [2]*Medical Res. Inst., TMDU*)

1P142 **昆虫飛翔筋線維の収縮蛋白交換実験と X 線回折**
X-ray diffraction from insect flight muscle with exchanged contractile proteins
Hiroyuki Iwamoto, Naoto Yagi (*SPring-8 JASRI*)

11. 分子モーター / 11. Molecular motor

1P143 **タキソールは、急速な微小管内チューブリン周期の伸長を誘導する**
Paclitaxel induces the quick elongation of tubulin dimer periodicity in microtubules
Shinji Kamimura[1], Megumi Kiyohara[1], Nobumasa Nakazawa[1], Yosuke Fujita[1], Yuuko Wada[1], Toshiki Yagi[3], Hiroyuki Iwamoto[2] ([1]*Dept. Biol. Sci., Chuo Univ.*, [2]*JASRI, SPring-8*, [3]*Biol. Sci., Pref. Univ. Hiroshima*)

1P144 **V1-ATPase の粗視化分子動力学シミュレーション**
Course-grained molecular dynamics simulation of V1-ATPase
Hiroki Kashimura, Yuta Isaka, Yuichi Kokabu, Mitsunori Ikeguchi (*Yokohama City Univ.*)

1P145 **フォトクロミック化合物フルギミドを結合したミオシン頭部ドメインのＸ線小角散乱**
Small-angle X-ray Scattering Study of Photochromic Fulgimide-bound Myosin
Sayaka Hayashi[1], Yasunobu Sugimoto[1,2], Nobuhisa Watanabe[1,2], Shinsaku Maruta[3] ([1]*Grad. Sch. Eng., Nagoya Univ.*, [2]*Nagoya Univ. Synchrotron radiation Center*, [3]*Fac. Eng., Univ. Soka*)

1P146 **ATP 結合で誘起されるミオシンの誘電応答**
Dielectric response of myosin induced by ATP binding
Takato Sato, Jun Ohnuki, Koji Umezawa, Mitsunori Takano (*Dept. of Pure & Appl. Phys., Waseda Univ.*)

1P147 **Multiscale analysis of functional motions in F1-ATPase: From Pi release to elasticity and friction of γ-subunit rotation**
Kei-ichi Okazaki, Gerhard Hummer (*Max Planck Institute of Biophysics*)

1P148 **Molecular mechanism of the epsilon subunit from F-type ATP synthases studied by Molecular Dynamics simulations**
Alexander Krah, Shoji Takada (*Department of Biophysics, Kyoto University*)

1P149 **ダイナクチンのアンテナ構造**
Antenna structure of Dynactin Complex
Hatsuha Kajita, Takuya Kobayashi, Kei Saito, Yoko Y. Toyoshima (*Grad. Sch. of Arts & Sci., Univ. of Tokyo*)

1P150 **Structure of dimeric axonemal dynein in cilia suggests an alternative mechanism of force generation**
Hironori Ueno[1], Bui Khanh[2,3], Takashi Ishikawa[2,3] ([1]*Mol. Func. & Life Sci., Aich Univ. Edu.*, [2]*Inst. Mol. Biol. and Biophys., ETH*, [3]*Biol. & Chem., PSI*)

1P151 **細胞質ダイニンの微小管上での運動は右方向にバイアスされている**
Cytoplasmic dynein takes a route switching randomly between protofilaments with a bias toward the right
Mitsuhiro Sugawa[1], Shin Yamaguchi[1], Keitaro Shibata[1,2], Yoko Y. Toyoshima[1], Junichiro Yajima[1] ([1]*Grad. Sch. Arts & Sciences, Univ. Tokyo*, [2]*AIST*)

1P152 **細菌べん毛モーター固定子複合体のイオン透過メカニズム**
Ion permeation mechanism through the stator complex in the flagellar motor
Yasutaka Nishihara[1], Akio Kitao[2] ([1]*Univ. of Tokyo, CMSI*, [2]*Univ. of Tokyo, IMCB*)

1P153 **V1 モーターでのトルク発生機構**
Torque generation mechanism in V1 motor
Mihori Baba[1], Shou Furuike[2], Atsuko Nakanishi[1], Jun-ichi Kishikawa[1], Nao Takeuchi[1], Ken Yokoyama[1] ([1]*Kyoto Sangyo University*, [2]*Osaka Medical College*)

1P154 **LC1 Binds to the Stalk of the Outer Arm Dynein**
Muneyoshi Ichikawa[1], Kei Saito[1], Haru-aki Yanagisawa[1], Toshiki Yagi[1,2], Ritsu Kamiya[1,3], Yasuharu Kushida[4], Kentaro Nakano[4], Osamu Numata[4], Yoko Y. Toyoshima[1] ([1]*The Univ. of Tokyo*, [2]*Pref. Univ. of Hiroshima*, [3]*Gakushuin Univ.*, [4]*Univ. of Tsukuba*)

1P155 **溶媒を陽に考慮した全原子分子動力学シミュレーションから得られたダイニンの弾性**
Elastic property of dynein motor domain obtained from all-atom molecular dynamic simulations in explicit water
Narutoshi Kamiya[1], Tadaaki Mashimo[2], Yu Takano[1,3], Takahide Kon[4,5], Genji Kurisu[1], Haruki Nakamura[1] ([1]*IPR, Osaka Univ.*, [2]*AIST*, [3]*JST, CRESTO*, [4]*Hosei Univ.*, [5]*JST, PRESTO*)

1P156 **細菌Ⅲ型分泌装置の回転運動およびエフェクター分泌に対する高粘性高分子の物理化学的影響評価**
Evaluation of physicochemical effect of viscous polymers toward rotation and effector secretion of bacterial type III secretion apparatus
Takashi Ohgita, Naoki Hayashi, Naomasa Gotoh, Kentaro Kogure (*Kyoto Pharm. Univ.*)

1P157 **The role of amino acid residues located at the catalytic site in the rotation of *Enterococcus hirae* V_1-ATPase**
Yoshihiro Minagawa[1], Hiroshi Ueno[2], Mayu Hara[1], Hiroyuki Noji[1], Takeshi Murata[3], Ryota Iino[4] ([1]*Dept. App. Chem.,Grad. Sch. Eng., The Univ. Tokyo*, [2]*Fac. Sci. & Eng., Univ. Chuo*, [3]*Grad. Sch., Univ. Chiba*, [4]*Okazaki Inst. Integ. Biosci., NINS*)

1P158 **マイコプラズマ Gli349 の構造解析**
Structural analysis of the gliding protein Gli349 from Mycoplasma mobile
Jun-ichi Inatomi[1], Yuuki Hayashi[1], Yoshito Kawakita[2], Masaru Yabe[2], Makoto Miyata[2], Munehito Arai[1,3] ([1]*Life Sci, Art and Sci, Univ.Tokyo*, [2]*Osaka City Univ*, [3]*JST.PRESTO*)

1P159 **確率的モデリングによるキネシンの化学-力学ネットワーク**

Chemomechanical network modeling of kinesin

Tomonari Sumi[1], Stefan Klumpp[2] ([1]*Dep. Chem., Okayama Univ.*, [2]*Dept. Theo. & Bio-syst., Max Planck Inst. Colloids & Interfaces*)

1P160 **F_1-ATPase の P-loop 変異体 TF_1(βG158A)に対するリン酸の阻害効果**

Inhibitory effect of Pi on F_1-ATPase P-loop mutant TF_1(βG158A)

Hitoshi Hoshina[1], Hikaru Yoshida[1], Ayumi Ito[1], Jotaro Ito[1,3], Shoichi Toyabe[2], Hiroshi Ueno[1], Eiro Muneyuki[1] ([1]*Dept. of Physics. Chuo Univ.*, [2]*Faculty of Physics. Tohoku Univ.*, [3]*School of Engineering, The University of Tokyo*)

1P161 **一定外力下での F1 の回転の観察**

Observation of the rotation of F1-ATPase under the constant external torque

Yohsuke Kikuchi[1], Hiroshi Ueno[1], Takahiro Nakayama[1], Eiro Muneyuki[1], Shouichi Toyabe[2] ([1]*Dept. Phys., Univ. Chuo*, [2]*Fac. Phys., Univ. Munchen*)

1P162 **Single-molecule fluorescent observations of the biased binding/unbinding of the tethered kinesin head**

Kouhei Matsuzaki, Michio Tomishige (*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*)

1P163 **TF1 βE190D 変異体の外部トルク依存性**

The response of TF1 βE190D mutant to the external torque

Mana Tanaka[1], Tomohiro Kawakami[1], Shoichi Toyabe[2], Hiroshi Ueno[1], Seishi Kudo[2], Eiro Muneyuki[1] ([1]*Dept. Phys., Faculty of Science and Engineering, Chuo Univ*, [2]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ*)

1P164 **軸糸外腕ダイニンによるトルク発生**

Torque generation by axonemal outer-arm dynein

Shin Yamaguchi[1], Kei Saito[1], Miki Sutoh[1], Takayuki Nishizaka[2], Yoko Y Toyoshima[1], Junichiro Yajima[1] ([1]*Department of Life sciences, Graduate School of Arts & Sciences, The University of Tokyo*, [2]*Department of Physics, Gakushuin University*)

1P165 **演題取り消し**

1P166 **偏光変調 TRIFM とデフォーカスイメージングによる単一蛍光色素の角度と回転方向の検出**

Detection of 3-D orientation and rotation of single fluorophores by combination of polarization-modulation TIRFM and defocused imaging

Shoko Fujimura, Nagisa Mikami, Tatsuro Itoh, Takayuki Nishizaka (*Dept. Phys., Gakushuin Univ.*)

12. 細胞生物的課題 / 12. Cell biology

1P167 **ビブリオ菌べん毛の MS リングを構成し大量発現で可溶性となる膜タンパク質 FliF の生化学的解析**

Biochemical analysis of the membrane protein FliF, a MS-ring component of *Vibrio* flagellar motor with being soluble when overproduced

Erika Yamaguchi, Seiji Kojima, Michio Homma (*Div. Biol. Sci., Grad. Sch. Sci., Univ. Nagoya*)

1P168 **クライオ電子線トモグラフィー法を用いたフィロポディア内構造解析**

The ultrastructure of filopodia were observed with cryo-ET

Shinji Aramaki[1], Kota Mayanagi[2], Kazuhiro Aoyama[3,4], Takuo Yasunaga[1] ([1]*Dept. of Bioscience and Bioinformatics, Kyushu Inst. of Tech.*, [2]*Medical Inst. of Bioregulation, Kyushu Univ.*, [3]*Application Lab., FEI Company Japan*, [4]*Graduate School of Frontier Bioscience, Osaka Univ.*)

1P169 **微小管結合蛋白質 MAP2、MAP4、Tau の F-アクチン-微小管束化活性の違い**

Difference of F-actin-microtubule bundling activity of microtubule-associated proteins, MAP2, MAP4, and Tau

Syouma Saito[1], Ayumu Kuramoto[1], Hikari Makihara[1], Miyuki Siga[1], Susumu Kotani[2], Kiyotaka Tokuraku[1] ([1]*Grad. Sch. Appl. sci., Muroran Inst.*, [2]*Kanagawa Univ.*)

1P170 **単離したラジアルスポークと微小管との結合**

Binding of the Isolated Radial Spokes and Microtubules

Hitoshi Sakakibara, Youské Shimizu, Hiroaki Kojima (*Bio-ICT, NICT*)

1P171 **海洋性ビブリオ菌のべん毛本数抑制に関与する FlhG の ATPase 活性の役割**

Role of ATPase activity of FlhG on the negative regulation of the flagellar number in *Vibrio alginolyticus*

Hikaru Hirata, Akari Takashima, Hiroki Ono, Michio Homma, Seiji Kojima (*Div.Bio.Sci., Grad.Sch.Sci., Univ.Nagoya*)

1P172 **細胞収縮コラーゲンゲル中における分子の拡散挙動**

Biomolecular diffusion in contracted collagen gel caused by fibroblasts

Takanori Kihara[1], Junri Ito[2], Jun Miyake[2] ([1]*Faculty Environmental Engineering, Univ. Kitakyushu*, [2]*Grad. Sch. Engineering Science, Osaka Univ.*)

1P173 **ATP および ADP 結合アクチンフィラメントに対するコフィリン結合の一分子観察**

Single molecule imaging of the binding of cofilin to ATP- and ADP-F-actin

Kimihide Hayakawa[1], Masahiro Sokabe[1], Hitoshi Tatsumi[2] ([1]*Mechanobiology Laboratory, Nagoya University*, [2]*Department of Physiology, Graduate School of Medicine, Nagoya Univerisity*)

1P174 **Na^+駆動型べん毛モーターの固定子複合体のナノディスク再構成への試み**

Attempt to reconstruct the stator complex of the bacterial Na^+-driven flagellar motor into Nanodisc

Mizuki Gohara[1], Norihiro Takekawa[1], Yohei Miyanoiri[2], Masatune Kainosho[2,3], Seiji Kojima[1], Michio Homma[1] ([1]*Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ.*, [2]*Structural Biol. Research Center, Grad. Sch. Sci., Nagoya Univ.*, [3]*Grad. Sch. Sci. Tech., Tokyo Metro. Univ.*)

1P175 **Localization and roles of F_1-ATPase subunit homologs and P42 of *Mycoplasma mobile* revealed by gene manipulation**

Tulum Isil, Masaru Yabe, Atsuko Uenoyama, Makoto Miyata (*Osaka City University*)

1P176 **原子間力顕微鏡によるマウス顎下腺組織の弾性率マッピング測定**

Mapping elastic modulus of mouse submandibular gland tissue by atomic force microscopy

Mitsuhiro Nakamura[1], Yuki Fujii[1], Hiroaki Taketa[2], Takuya Matsumoto[2], Takaharu Okajima[1] ([1]*Grad. Sch. Info. Sci. & Tech.,Univ. Hokkaido*, [2]*Grad. Sch. Med. Den. & Pham. Sci.,Univ. Okayama*)

1P177 **アクチンの Tyr143 に変異を導入した細胞性粘菌は高圧処理に対してより敏感になる**

***Dictyostelium* cells carrying the plasmids to express mutant actin (Tyr143Phe) are more susceptible to high-pressure treatment**

Yuki Gomibuchi[1], Takahiro Ohnuki[1], Taro Q.P. Uyeda[2], Masayoshi Nishiyama[3], Takeyuki Wakabayashi[1] ([1]*Teikyo Univ.*, [2]*AIST*, [3]*Kyoto Univ.*)

1P178 **粘菌管で作る懸垂線のコイル形成**

Coiling of catenaries made from *Physarum* tube

Takahiro Noguchi[1], Taito Watanabe[1], Itsuki Kunita[2], Hirofumi Wada[3], Toshiyuki Nakagaki[2], Yoshimi Tanaka[4] ([1]*Grad. Sch. Sci., Yokohama National Univ.*, [2]*RIES., Hokkaido Univ.*, [3]*Physical Sciences., Ritsumeikan Univ.*, [4]*Environment and Information Science., Yokohama National Univ.*)

1P179 **溶液中のアクチン繊維の構造のゆらぎ**

Diversity of monomers configuration within a single actin filament detected by FRET

Sakura Maesato, Kenji Kobayasi, Hajime Honda (*Department of Bioengineering, Nagaoka University of Technology*)

1P180 **蛍光顕微鏡で見た AMP-PNP アクチンの重合**

Observation of polymerization with AMP-PNP bound actin molecules

Kiwa Koike[1], Koshin Mihashi[2], Hajime Honda[1] ([1]*Deprtoment of bioengineering , Nagaoka University of Technology*, [2]*Nagoya University*)

1P181 **モータータンパク質を活用したナノバイオデバイスの開発**

Nano-Bio-Devices transporting antigens for electrical measurements

Yuto Maruko[1], Shiori Sawada[1], Shin Nanasaki[2], Kenji Moriya[2], Takashi Ishiguro[3], Shigeru Sakurazawa[4], Hajime Honda[1] ([1]*Department of bioengineering, Nagaoka University of Technology*, [2]*National Institute of Technology, Hakodate College*, [3]*Taiyo Yuden Co., Ltd.*, [4]*Future University Hakodate*)

1P182 **RAF の疾病関連変異体のコンフォメーションと機能**

Conformation and function of disease-associated RAF mutants

Kayo Hibino[1], Masahiro Ueda[1], Yasushi Sako[2] ([1]*RIKEN, QBiC*, [2]*Cellular Informatics Lab., RIKEN*)

1P183 **シロイヌナズナアクチンアイソフォームはファロイジンに結合しない**

***Arabidopsis thaliana* actin isoforms do not bind phalloidin**

Saku Kijima[1,2], Sam Geun Kong[4], Masashi Mori[3], Masamitsu Wada[4], Taro Uyeda[1,2] ([1]*Bio. Inst., AIST*, [2]*Grad. Sch. Sci., Univ. Tsukuba*, [3]*Res. Inst., Pre. Univ. Ishikawa*, [4]*Dep. Biol., Univ. Kyushu*)

1P184 **Motor activity of myosin-II is required for maintanance of the contractile ring in fission yeast**

Masak Takaine, Osamu Numata, Kentaro Nakano (*Univ. of Tsukuba*)

1P185 **コラーゲン溶液中の微粒子に働く粘性力の速度依存性**

Velocity dependence of drag force acting on a micro particle in collagen solution

Masafumi Kuroda, Yoshihiro Murayama (*Dept. of Applied Physics, Tokyo University of Agriculture and Technology*)

1P186 **Direct measurement of *Vibrio alginolyticus* polar flagellum growth rate**

Chien-Jung Lo[1,2], Meiting Chen[1,2] ([1]*Dept. of Phys., National Central Univ.*, [2]*Inst. Biophys., National Central Univ.*)

1P187 **極限環境下での超好熱始原菌の運動観察**

Motility of *Thermococcus kodakaraensis* cells at extreme environmental conditions

Masayoshi Nishiyama[1], Ryohei Tsukamoto[2], Toshiki Yagi[3,4], Masahide Kikkawa[4], Tadayuki Imanaka[5], Tamotsu Kanai[2] ([1]*The HAKUBI Center, Kyoto Univ.*, [2]*Grad. Sch. Eng., Kyoto Univ.*, [3]*Dept. Biol. Sci., Pref. Univ. Hiroshima*, [4]*Grad. Sch. Medicine, Univ. Tokyo*, [5]*Grad. Sci. Life Sci., Ritsumeikan Univ.*)

1P188 **蛍光量子ドットを用いた細胞内高速小胞輸送機構の解明**

Mechanism of high-speed vesicular transport inside cells explored by using quantum dots

Kenji Kikushima, Hideo Higuchi (*Dept. of Phys., Sch. of Sci., The Univ. of Tokyo*)

1P189 **Examining mitotic functions of bipolar kinesin Eg5 in a reconstituted minimal microtubule network**

Yuta Shimamoto[1,2,3], Scott Forth[3], Tarun Kapoor[3] ([1]*National Institute of Genetics*, [2]*JST PRESTO*, [3]*The Rockefeller University*)

1P190 **細胞サイズ液滴内でのアクトミオシンリングの自発形成と収縮**

In vitro self-assembly and contraction of actomyosin rings inside a cell-sized droplet

Makito Miyazaki[1], Masataka Chiba[1], Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

1P191 **単離マウス気管上皮繊毛が生み出す力の三次元顕微計測**

Force measurement of individual isolated mouse tracheal cilia using three-dimensional optical trapping

Takanobu Kato[1], Koji Ikegami[2], Toshihito Iwase[3], Tomoko Masaike[3,4], Mitsutoshi Setou[2], Takayuki Nishizaka[1] ([1]*Dept. Phys., Gakushuin Univ.*, [2]*Dept. Cell Biol. and Anat., Hamamatsu Univ. Sch. Med.*, [3]*Dept. Appl. Biol. Sci., Tokyo Univ. of Sci.*, [4]*PRESTO, JST*)

1P192 **微小管結合タンパク質シヌクレインは細胞質ダイニンを細胞辺縁部に運ぶ機能を持つ輸送性微小管の形成に必須である**

Synucleins are essential for the creation of transportable microtubules, which is required for anterograde transport of cytoplasmic dynein

Shiori Toba[1], Kotaro Koyasako[2], Masami Yamada[1], Takuo Yasunaga[2], Hiroaki Kojima[3], Hideki Wanibuchi[1], Shinji Hirotsune[1] ([1]*Osaka City University Graduate School of Medicine*, [2]*Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology*, [3]*Advanced ICT Research Institute, National Institute of Information and Communications Technology*)

1P193 **べん毛本数を負に制御する FlhG の ATPase モチーフは FlhF の極局在の阻害に重要である**
ATP binding motif of FlhG, a negative regulator of flagellar number, is important to prevent polar localization of FlhF
Hiroki Ono, Michio Homma, Seiji Kojima (*Grad. Sch. Sci., Univ. Nagoya*)

1P194 **大腸菌走化性シグナル伝達における CheZ 極局在の役割**
Role of the polar localization of CheZ in chemotactic signal transduction of *Escherichia coli*
Yong-Suk Che, Hajime Fukuoka, Yuichi Inoue, Hiroto Takahashi, Akihiko Ishijima (*IMRAM, Tohoku Univ.*)

1P195 **回転電場による大腸菌の強制回転が細胞内 CheY の振る舞いに与える影響**
Effect of forced-rotation of *E. coli*'s flagella motor on the behavior of intracellular CheY
Masaaki Sato, Hajime Fukuoka, Akihiko Ishijima (*IMRAM, Tohoku Univ.*)

1P196 **海洋性ビブリオ菌のべん毛モーター回転方向決定における FliG と PomB の変異の影響**
Effect of mutations in FliG and PomB on rotational direction of flagellar motor in *Vibrio alginolyticus*
Tatsuro Nishikino[1], Yasuhiro Onoue[2], Norihiro Takekawa[2], Shiwei Zhu[2], Seiji Kojima[2], Michio Homma[2] ([1]*Department of Biological Science, School of Science, Nagoya University*, [2]*Division of Biological Science, Graduate School of Science Nagoya University*)

1P197 **基質伸展刺激下でケラトサイトは伸展に垂直にも平行にも運動する**
Hybrid mechanosensing system for directional migration in fish keratocytes
Chika Okimura[1], Takafumi Mizuno[2], Yoshiaki Iwadate[1] ([1]*Fac. Sci., Yamaguchi Univ.*, [2]*AIST*)

1P198 **海洋性ビブリオ菌の c-di-GMP 結合タンパク質 PlzD における表現型および生化学的特性の解析**
Biochemical and phenotypic characterization of PlzD, a YcgR homolog of c-di-GMP binding protein in Vibrio alginolyticus
Seiji Kojima, Takuro Yoneda, Michio Homma (*Grad. Sch. Sci., Nagoya Univ.*)

1P199 **ケラトサイトとその断片の同一なかたち・細胞骨格・基質牽引力分布**
Same traction force distributions in fish keratocytes and their fragments represent the same fan-shape
Ayane Sonoda, Chika Okimura, Yoshiaki Iwadate (*Dept. Funct. Mol. Biol., Grad. Sch. Med., Yamaguchi Univ.*)

1P200 **伸縮性の表層を伝わるゾウリムシのメタクロナールウェーブ**
Ciliary metachronal wave propagation on the compliant surface of Paramecium cells
Naoki Narematsu[1], Quek Quek[2], Keng-Hwee Chiam[2], **Yoshiaki Iwadate**[1] ([1]*Fac. Sci., Yamaguchi Univ.*, [2]*A*STAR, Singapore*)

13A. 生体膜・人工膜：構造・物性 / 13A. Biological & Artificial membrane: Structure & Property

1P201 **中世膜に結合したラクトフェランピンの膜結合構造と膜親和性に基づく抗菌活性機構の解析**
Elucidation of the antimicrobial activity based on affinity and bound structure of LFampinB embedded into the neutral membrane
Masayoshi Imachi[1], Atsushi Tsutsumi[1], Atsushi Kira[2], Izuru Kawamura[1], Akira Naito[1] ([1]*Graduate School of Engineering, Yokohama National University*, [2]*Research and Development Division, ULVAC Inc*)

1P202 **diphenylhexatriene を用いる脂質膜流動性測定に与える Aβ ペプチドの影響**
Limitation of the use of diphenylhexatriene to measure the fluidity of membrane in the presence of amyloid β-peptide
Masako Suzuki, Takashi Miura, takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

1P203 **Dependence of Purple Membrane Bump Curvature on pH and Ionic Strength Analyzed by Atomic Force Microscopy Combined with Solvent Exchange**
Yasunori Yokoyama[1], Kousuke Yamada[1], Yousuke Higashi[1], Satoshi Ozaki[1], Haorang Wang[1], Naoki Koito[1], Masashi Sonoyama[1,2], Shigeki Mitaku[1,3] ([1]*Dept. Appl. Phys., Grad. Sch. Eng., Nagoya Univ.*, [2]*Div. Mol. Sci., Fac. Sci. Tech., Gunma Univ.*, [3]*Toyota Phys. Chem. Res. Inst.*)

1P204 **リン脂質/水界面における水和構造の分子動力学シミュレーション**
Structure and orientation of hydrating water molecules at phospholipid/water interface revealed by molecular dynamics simulation
Suyong Re, Wataru Nishima, Tahei Tahara, Yuji Sugita (*RIKEN,Wako*)

1P205 **膜の変形による BAR ドメインの凝集**
Assembly of BAR domains induced by membrane shape deformation
Hiroshi Noguchi (*ISSP, Univ. Tokyo*)

1P206 **コレステロールによるホスファチジルコリン膜へのクロルゾキサゾン（筋弛緩剤）の結合阻害**
Cholesterol inhibits the binding of chlorzoxazone (muscle relaxant agent) to phosphatidylcholine membranes
Ayumi Yamada, Hiroshi Takahashi (*Grad. Sch. Sci & Tech., Gunma Univ.*)

1P207 **イノシトールリン脂質が誘起する支持脂質二重膜内のドメイン構造とタンパク質反応活性との関連**
Relation between phosphatidylinositol-induced domain structure and protein reaction activity in supported lipid bilayer
Toshinori Motegi[1], Yohko Takiguchi[2], Kingo Takiguchi[2], Toshiki Itoh[3], Ryugo Tero[1,4] ([1]*Toyohashi Univ. Tech. EIIRIS*, [2]*Nagoya Univ. Dep. Sci.*, [3]*Kobe Univ. Dep. Med.*, [4]*Toyohashi Univ. Tech. Dep. Environ. Life Sci.*)

1P208 **細胞毒性を有する酸化コレステロールと酸性リン脂質（DMPG）の相互作用の構造学的研究**
Structural studies of the interaction between cytotoxic oxysterols and acidic phospholipid (DMPG) bilayer membranes
Hiroshi Takahashi[1], Takaaki Hikima[2], Masaki Takata[2], Toshihide Kobayashi[3] ([1]*Grad. Sch. Sci & Tech., Gunma Univ.*, [2]*Harima Inst., Riken*, [3]*Wako Inst., Riken*)

1P209 **脂質膜ナノチューブのダイナミクス**
Dynamics of a single nano-tube hollow of phospholipid membrane
Masatoshi Ichikawa[1], Akihisa Yamamoto[2] ([1]*Grad. Sci., Kyoto Univ.*, [2]*iCeMS, Kyoto Univ.*)

1P210 **長鎖リン脂質と短鎖リン脂質で構成される脂質多成分系の相挙動と構造変化に関する研究**
Study on the phase behavior and the structural changes of lipid multi-component system consisting of long- and short-chain phospholipids
Ryota Kobayashi, Tetsuhiko Ohba (*Dept. of Phys., Tohoku Univ.*)

13B. 生体膜・人工膜：ダイナミクス / 13B. Biological & Artificial membrane: Dynamics

1P211 **ラフト中でのアミロイド前駆体タンパク質の膜貫通部位の二量化にコレステロールが与える影響**
Cholesterols affect to the association of the transmembrane region of Amyloid Precursor Protein in the raft
Naoyuki Miyashita[1,2], Fumiko Ogushi[3], Yuji Sugita[1,2,4] ([1]*RIKEN Quantitative Biology Center*, [2]*RIKEN AICS*, [3]*Ochanomizu University*, [4]*RIKEN*)

1P212 **リポソーム内膜タンパク質合成が誘起する脂質膜の形態変化**
Morphological changes of the lipid membrane induced by in-liposome membrane protein synthesis
Kosuke Okamura, Hajime Watanabe, Tomoaki Matsuura (*Department of biotechnology, Graduate school of engineering Osaka university*)

1P213 **光応答 DNA と細胞サイズリポソームの融合による人工細胞型分子ロボット**
Artificial cell-based molecular robots by fusion of light responsive DNA and cell-sized liposomes
Masamune Morita[1], Hao Li[1], Tomonori Shibata[2], Hirohide Saito[2,3], Masahiro Takinoue[1,4] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Institute of Technology*, [2]*Center for iPS Cell Res. and App. (CiRA), Kyoto University*, [3]*The Hakubi Center for Adv. Res., Kyoto University*, [4]*PRESTO, JST*)

1P214 **チューブリン封入ジャイアントリポソームの温度・静水圧変化による可逆的形態制御**
Reversible morphological control of tubulin-encapsulated giant-liposomes induced by change of hydrostatic pressure and temperature
Masahito Hayashi[1], Masayoshi Nishiyama[2], Yuki Kazayama[3], Taro Toyota[3,4], Kingo Takiguchi[1] ([1]*Grad. Sch. Sci. Nagoya Univ.*, [2]*Kyoto Univ., iCeMS*, [3]*Grad. Sch. Arts Sci., Univ. of Tokyo*, [4]*Res. Center Complex Sys. Biol., Univ. of Tokyo*)

1P215 **粘弾性流体を内包したリポソームの膜変形**
Effects of viscoelastic cytoplasm in liposome on the shape deformation
Miho Yanagisawa[1], Kei Fujiwara[2] ([1]*Dept. Appl. Phys., Tokyo Univ. Agric. Technol.*, [2]*Dept. Biosci. Info., Keio Univ.*)

1P216 **表面張力レプリカ交換分子動力学法の開発と生体膜系への応用**
Surface-tension replica-exchange molecular dynamics simulations of biological membrane systems
Takaharu Mori, Yuji Sugita (*RIKEN*)

1P217 **一分子 FRET 測定法を用いた、膜貫通ヘリックス間相互作用への GXXXG モチーフの寄与の解明**
Contributions of GXXXG motif to transmembrane helical interactions as revealed by single molecule FRET
Kotaro Kondo, Yoshiaki Yano, Katsumi Matsuzaki (*Grad. Sch. Pha., Univ. Kyoto*)

13C. 生体膜・人工膜：興奮・チャネル / 13C. Biological & Artificial membrane: Excitation & Channels

1P218 **X 線１分子追跡法による 5 量体リガンド作動性イオンチャネル GLIC の pH 依存３D分子内運動マップ**
PH dependent 3D Motion Maps of GLIC from X-ray Single Molecule Observations
Yuji Sasaki[1,2], Hiroshi Sekiguchi[2], Yufuku Matsushita[1], Keigo Ikezaki[1], Yuri Nishino[3], Atsuo Miyazawa[3], Christele Huon[4], Jean-Pierre Changeux[4], Pierre-Jean Corringer[4] ([1]*The University of Tokyo*, [2]*SPring-8/JASRI*, [3]*University of Hyogo*, [4]*Pasteur Institute*)

1P219 **電位依存性 H^+チャネルのゲート電流**
Gating charge movement of the voltage-gated H^+ channel
Yuichiro Fujiwara, Yasushi Okamura (*Integrative Physiology, Grad Sch of Med., Osaka University*)

1P220 **再構成した電位依存性プロトンチャネルの電気生理学的計測**
The electrophysiological recording of the reconstituted voltage-gated proton channel in artificial membrane
Akira Kawanabe, Yasushi Okamura (*Grad. Sch. Med., Osaka Univ.*)

1P221 **カリウムチャネル KcsA のゲート開閉と連動した膜中集合・離散**
Gating-associated clustering-dispersion dynamics of the KcsA potassium channel in a lipid membrane environment
Ayumi Sumino[1,2], Daisuke Yamamoto[3], Masayuki Iwamoto[2], Takehisa Dewa[4], Shigetoshi Oiki[2] ([1]*JST/PRESTO*, [2]*Facult. Med. Sci., Univ. Fukui*, [3]*Facult. Sci., Univ. Fukuoka*, [4]*Grad. Sch. Eng., Nagoya Inst. Tech.*)

13D. 生体膜・人工膜：輸送 / 13D. Biological & Artificial membrane: Transport

1P222 **無細胞翻訳系による SecYEG トランスロコンの合成**
In vitro synthesis of SecYEG translocon
Hideaki Matsubayashi[1], Yutetsu Kuruma[1,2], Takuya Ueda[1] ([1]*Graduate School of Frontier Sciences, The University of Tokyo*, [2]*Earth-Life Science Institute, Tokyo Institute of Technology*)

1P223 **細菌 III 型分泌装置の in vitro 輸送再構成系の構築**
Construction of an in vitro transport assay system for the bacterial type III protein secretion
Hiroyuki Terashima[1], Akihiro Kawamoto[2], Tohru Minamino[2], Keiichi Namba[2], Katsumi Imada[1] ([1]*Grad. Sch. Sci., Osaka univ.*, [2]*Grad. Sch. Front. Biosci., Osaka Univ.*)

1P224 **人工細胞モデルを用いた RNA の脂質二分子膜透過**
Bilayer lipid membrane permeation of RNA
Kazuma Sato (*Grad. Sch. Inf Sci., Univ. Osaka*)

15. 神経・感覚 / 15. Neuroscience & Sensory systems

1P225 **カルシウムシグナルによるシナプス構造の競合的制御**
Competitive control of synaptic structure by calcium signaling
Fumihiro Niwa[1], Hiroko Bannai[2], Antoine Triller[3], Katsuhiko Mikoshiba[1] ([1]*BSI, RIKEN*, [2]*Grad. Sch. Biol. Sci., Univ. Nagoya*, [3]*IBENS*)

1P226 **背に腹は代えられぬ： モノアラガイの隠された記憶**
Necessity Knows No Law: Overwhelmed Memory in a Snail
Etsuro Ito, Miki Yamagishi (*Kagawa Sch. Pharmaceu. Sci., Tokushima Bunri Univ.*)

1P227 **Acute Modulation of long-term potentiation of Pyramidal Neurons by Hippocampal-derived Estrogen**
Hiroki Kojima, Keisuke Hotta, Yoshitaka Hasegawa, Suguru Kawato (*Department of Life Science*)

16. 神経回路・脳の情報処理 / 16. Neuronal circuit & Information processing

1P228 **昆虫の投射ニューロンにおける樹状突起 Ca^{2+}上昇はシナプス入力によって修飾される活動電位波形に依存する**
Dendritic Ca^{2+} elevation depends on spike waveform modulated by local synaptic activity in projection interneurons of insect
Hiroto Ogawa[1], Ruriko Mitani[2] ([1]*Dept Bio Sci, Fac Sci, Hokkaido Univ*, [2]*Biosystem Sci, Grad Sch Life Sci, Hokkaido Univ*)

1P229 **新規な多電極を用いた海馬の神経回路に対する神経ステロイドの作用の解析**
Analysis of neurosteroid effects on hippocampal neural circuits using novel multi-electrode probe methods
Yoshitaka Hasegawa[1], Chung Bon-chu[2], Suguru Kawato[1] ([1]*Dept. Biophysics & Life Sciences, Grad School of Arts & Sciences, Univ. of Tokyo, Tokyo, Japan*, [2]*Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan*)

17. 行動 / 17. Behavior

1P230 **滑走細菌 *Flavobacterium johnsoniae* の滑走装置**
Gliding machinery of the gliding bacterium *Flavobacterium johnsoniae*
Satoshi Shibata[1], Keiko Sato[1], Yuka Narita[1], Daisuke Nakane[2], Koji Nakayama[1] ([1]*Nagasaki Univ. Graduate Sch. of Biomedical Science*, [2]*Dept. Physics, Gakushuin Univ.*)

1P231 **トラウマストレスはエンドカンナビノイドシステムを介しオペラント条件付けによる記憶の形成を障害する**
Traumatic stress impairs learning and memory formation via an endocannabinoid system in *Lymnaea stagnalis*
Hiroshi Sunada[1], Jeremy Forest[1], Manabu Sakakibara[2], Ken Lukowiak[1] ([1]*HBI, Univ of Calgary*, [2]*Grad. Sch. Biosci., Tokai Univ*)

18A. 光生物：視覚・光受容 / 18A. Photobiology: Vision & Photoreception

1P232 **ファラオニス・ハロロドプシンの N 中間体の結晶構造解析**
Crystallographic analysis of the N intermediate of pharaonis halorhodopsin
Haruki Kawaguchi, Taichi Nakanishi, Midori Murakami, **Tsutomu Kouyama** (*Graduate School of Science, Nagoya University*)

1P233 **クラックスロドプシンの 3 量体構造と光安定性**
Photostability of the trimeric form of cruxrhodopsin
Siu Kit Chan[1], Tomomi Kitajima[1], Midori Murakami[1], Kunio Ihara[2], Tsutomu Kouyama[1] ([1]*Graduate School of Science, Nagoya University*, [2]*Center of the gene research, Nagoya University*)

1P234 **hCRBPII 及びその変異体の構造とスペクトルに関する QM/MM 理論計算**
QM/MM calculation of structure and spectral properties for human cellular retinal binding protein II (hCRBPII) and its mutants
Cheng Cheng, Motoshi Kamiya, Yoshihiro Uchida, Shigehiko Hayashi (*Grad. Sch. Sci, Kyoto U.*)

1P235 **センサリーロドプシン I-トランスデューサーの一分子 FRET 観察**
Single-molecule FRET study of the sensory rhodopsinI-transducer
Ryo Nisimura[1], Keiichi Inoue[1,2], Jin Yagasaki[3], Kenichi Kawamoto[1], Yuki Sudo[4,5,6], Hideki Kandori[1] ([1]*Nagoya Institue of Technology*, [2]*JST PREST*, [3]*Nagoya University*, [4]*Okayama University*, [5]*Institue for Molecule Science*, [6]*JST CREST*)

1P236 **海洋緑藻 *Ostreococcus tauri* 由来の光修復酵素（CPF1, CPF2）における FAD 光反応中心の分光解析**
Spectroscopic analysis of FAD photoreaction center in two photolyase (CPF1, CPF2) from a marine green alga *Ostreococcus tauri*
Shouhei Ueda[1], Kazunori Zikihara[1,2], Tomoko Ishikawa[2], Chris Bowler[3], Takeshi Todo[2], Satoru Tokutomi[1] ([1]*Grad. School Sci., Osaka Prefect. Univ*, [2]*Grad. School Med., Osaka Univ*, [3]*Ecole. Norm. Supe., Inst. Biol., Paris*)

1P237 **光修復酵素の DNA 修復能と光反応中心 FAD コンフォメーションとの相関**
Correlation of DNA repair type with FAD conformation in the photoreaction center of photolyases
Kazunori Zikihara[1,2], Shouhei Ueda[1], Takahiro Kitano[1], Kohei Kasakawa[1], Reo Fukazawa[1], Tomoko Ishikawa[2], Kristin Tessmar-Raible[3], Chris Bowler[4], Takeshi Todo[2], Satoru Tokutomi[1] ([1]*Grad. School Sci., Osaka Prefect. Univ.*, [2]*Grad. School Med., Osaka Univ.*, [3]*Max F. Perutz Labo., Univ. Vienna*, [4]*Ecole Norm. Supe., Inst. Biol., Paris*)

1P238 **チャネルロドプシンの機能理解への理論的アプローチ**
Theoretical approach toward an understanding of molecular functions of channelrhodopsin
Hiroshi C. Watanabe[1,2], Marcus Elstner[3], Minoru Sakurai[1] ([1]*Center for Biol. Res. & Inform., Tokyo Tech*, [2]*JSPS fellow*, [3]*KIT*)

1P239 **Photoactive Yellow Protein におけるアルギニン 52 のプロトン化状態**
Protonation State of Arginine 52 in Photoactive Yellow Protein
Kento Yonezawa, Hironari Kamikubo, Keito Yoshida, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

1P240 **PYP-Phytochrome Related Protein の 2 つのセンサードメインで生じる光反応の関連性**
Relationship of the photoreactions between two sensor domains in PYP-Phytochrome Related Protein
Keito Yoshida, Hironari Kamikubo, Kento Yonezawa, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

1P241 **ATP 結合におけるシロイヌナズナクリプトクロム 1 の光反応の赤外分光測定**
FTIR spectroscopy of the photoreaction of Arabidopsis Cryptochrome1 upon ATP binding
Katsuhiro Mikuni[1], Daichi Yamada[1], Tatsuya Iwata[1], Kenichi Hitomi[2], Elizabeth D. Getzoff[2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*The Scripps Res. Inst. USA*)

1P242 **光回復酵素への機能転換のためのクリプトクロム-DASH の変異導入**
Mutagenesis to convert Cyanobacterial Cryptochrome-DASH into a Photolyase
Tomohiro Suzuki[1], Tatsuya Iwata[1], I Made Mahaputra Wijaya[1], Junpei Yamamoto[2], Tomoko Ishikawa[3], Daichi Yamada[1], Elizabeth D. Getzoff[4], Takeshi Todo[3], Shigenori Iwai[2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*Grad. Sch. Eng. Sci., Osaka Univ.*, [3]*Grad. Sch. Med., Osaka Univ.*, [4]*The Scripps Res. Inst. USA*)

1P243 **培養細胞内２次メッセンジャーの発光モニタリングを利用した多様なオプシン類の分子特性解析の試み**
Analysis of molecular properties of various opsins by bioluminescence monitoring of second-messengers in cultured cells
Takashi Nagata[1], Tomohiro Sugihara[1], Mitsumasa Koyanagi[1,2], Akihisa Terakita[1] ([1]*Grad. Sch. Sci., Osaka City Univ.*, [2]*JST-PRESTO*)

1P244 **アクチノロドプシンの His-62 残基の光化学反応における役割**
Role of His-62 in the photochemistry of actinorhodopsin
Shintaro Nakamura[1], Takashi Kikukawa[1], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Martin W Hahn[2], Naoki Kamo[1], Makoto Demura[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Innsbruck Univ.*)

1P245 **QM/MM RWFE 法によるウシロドプシンの光反応中間体に関する理論研究**
A theoretical study on early intermediates of bovine rhodopsin by QM/MM RWFE method
Motoshi Kamiya, Shigehiko Hayashi (*Grad. Sch. Sci., Kyoto Univ.*)

1P246 **Accelerated MD シミュレーションを用いた AsLOV2 ドメイン光活性機構の解明**
Study of the photactivation mechanisms in AsLOV2 domain by using accelerated molecular dynamics simulations
Tomohiro Yaita, Tadaomi Furuta, Minoru Sakurai (*Cent Biol Res & Info, Tokyo Inst of Tech*)

1P247 ***Truepera radiovictrix* 由来 Na^+ポンプ型ロドプシンの機能解析**
Functional analyses of Na^+-pumping rhodopsin from *Truepera radiovictrix*
Kazuki Goto, Takashi Kikukawa, Takatoshi Hasemi, Yuta Saito, Masakatsu Kamiya, Tomoyasu Aizawa, Naoki Kamo, Makoto Demura (*Grad. Sch. Life Sci., Hokkaido Univ.*)

1P248 **光センサー蛋白質フォトトロピン 2 の光刺激による構造変化ダイナミクス**
Light induced conformational changes of a blue light receptor phototropin2: LOV2-kinase
Akira Takakado[1], Yusuke Nakasone[1], Koji Okajima[2], Satoru Tokutomi[2], Masahide Terazima[1] ([1]*Kyoto Univ. Sci.*, [2]*Osaka Prefecture Univ.*)

1P249 **DNA 光回復酵素間の機能転換**
Functional conversion of (6-4) photolyase and CPD photolyase
Daichi Yamada[1], Junpei Yamamoto[2], Tomoko Ishikawa[3], Tomohiro Suzuki[1], I Made Mahaputra Wijaya[1], Tatsuya Iwata[1], Elizabeth D. Getzoff[4], Takeshi Todo[3], Shigenori Iwai[2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*Grad. Sch. Eng. Sci., Osaka Univ.*, [3]*Grad. Sch. Med., Osaka Univ.*, [4]*The Scripps Res. Inst. USA*)

18B. 光生物：光合成 / 18B. Photobiology: Photosynthesis

1P250 **緑藻型[FeFe]ヒドロゲナーゼ成熟化機構の構造研究**
Structural studies on the maturation mechanism of [FeFe] hydrogenase maturation from green alga *Chlamydomonas reinhardtii*
Daiki Kiyota[1], Risa Mutoh[2], Chihiro Azai[3], Hirozo Oh-oka[1], Genji Kurisu[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*Institute for Protein Research, Osaka Univ.*, [3]*College of Life Sciences, Ritsumeikan Univ.*)

1P251 **光化学系 I－フェレドキシン複合体の結晶構造および NMR 解析**
X-ray structure and NMR analysis of the electron transfer complex between photosystem I and ferredoxin
Risa Mutoh[1], Hisako Kubota-Kawai[1], Marc Nowaczyk[2], Matthias Rögner[2], Hideaki Tanaka[1], Takahisa Ikegami[1], Genji Kurisu[1] ([1]*Inst. Prot. Res., Osaka Univ.*, [2]*Dept. of Plant Biochemistry, Faculty of Biology and Biotechnology, Ruhr-Univ. Bochum*)

1P252 **光合成反応中心タンパク質の極低温単一分子分光**
Single-molecule spectroscopic study of photosynthetic reaction center at 6 K
Toru Kondo[1], Risa Mutoh[2], Genji Kurisu[2], Hirozo Oh-oka[3], Satoru Fujiyoshi[1], Michio Matsushita[1] ([1]*Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Institute for Protein Research, Osaka Univ.*, [3]*Grad. Sch. Sci., Osaka Univ.*)

1P253 **同位体標識された好熱性紅色光合成細菌における膜タンパク質耐熱化の分子機構解析**
Isotope-edited ATR-FTIR analysis of the light-harvesting 1 reaction center complex from thermophilic purple photosynthetic bacteria
Yuki Yura[1], Yukihiro Kimura[1], Seiu Otomo[2], Takashi Ohno[1] ([1]*Grad. Sch. Agri. Sci., Kobe Univ.*, [2]*Fac. Sci., Ibaraki Univ.*)

1P254 **光合成光化学系 II における MnCa クラスターの歪んだ椅子型構造の起源**
Origin of the distorted-chair structure of the MnCa cluster in photosystem II
Keisuke Saito[1,2], Hiroshi Ishikita[1] ([1]*Dep. App. Chem., Grad. Schol. Eng., Univ. Tokyo*, [2]*JST PRESTO*)

1P255 **紅色硫黄細菌由来光捕集１反応中心複合体における金属―タンパク質間相互作用の熱力学的解析**
Thermodynamic analysis of metal-protein interaction in the light-harvesting 1 reaction center complex from purple sulfur bacteria
Yukihiro Kimura[1], Yusuke Hayashi[1], Seiu Otomo[2], Takashi Ohno[1] ([1]*Grad. Sch. Agri. Sci., Kobe Univ.*, [2]*Fac. Sci., Ibaraki Univ.*)

1P256 **光合成酸素発生系における表在性蛋白質と Cl^-結合部位の相互作用 ：NO_3^-置換による赤外分光解析**
Effect of the extrinsic proteins on the Cl^- binding sites of the oxygen evolving center in photosystem II: Analysis by FTIR spectroscopy
Junpei Kondo, Shin Nakamura, Ryo Nagao, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

1P257 **時間分解赤外分光法による光合成水分解反応の解析**
Proton-coupled electron transfer mechanism of photosynthetic water oxidation as revealed by time-resolved infrared spectroscopy
Hiroki Sakamoto, Ryo Nagao, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

1P258 **光化学系Ⅱにおける第二キノン電子受容体 Q_B の電子・プロトン移動機構**
Electron and proton transfer mechanism of the secondary quinone electron acceptor Q_B in photosystem II
Yukihiro Kadekawa, Yuki Kato, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

20. 生命の起源・進化 / 20. Origin of life & Evolution

1P259 **ポリメラーゼリボザイムを活性化するペプチドの試験管内創出**
In vitro selection of Polymerase Ribozyme-assisting peptides
Shigefumi Kumachi[1], Yuzuru Husimi[2], Naoto Nemoto[1] ([1]*Grad. Sci. & Tech., Saitama Univ.*, [2]*Emeritus Prof, Saitama Univ.*)

1P260 **リポソーム内 RNA 複製におけるリポソームサイズの影響**
Size effect of liposome on the inner RNA replication
Takeshi Sunami[1,2], Norikazu Ichihashi[1,2], Takehiro Nishikawa[2], Yasuaki Kazuta[2], Tetsuya Yomo[1,2,3] ([1]*Grad. Info., Osaka Univ.*, [2]*ERATO, JST*, [3]*Grad. Fron., Osaka Univ.*)

22A. 生命情報科学：構造ゲノミクス / 22A. Bioinformatics: Structural genomics

1P261 **３つ以上のドメインからなるマルチドメインタンパク質の構造予測**
Prediction of 3D structures of multidomain proteins composed of more than two domains
Masafumi Shionyu, Atsushi Hijikata, Tsuyoshi Shirai (*Fac. Bio-Sci., Nagahama Inst. Bio-Sci. Tech.*)

1P262 **細胞表面のビジュアルプロテオミクスに向けた計算技術開発：二次元電子顕微鏡画像と立体構造との照合**
Computing technology for the visual proteomics of cell surface : Collation of protein structure and electron microscopic image
Go Inoue[1], Masami Ikeda[2], Makiko Suwa[1,2] ([1]*Biol. Sci., Grad. Sci. Eng., Aoyama Gakuin Univ.*, [2]*Chem. Biol. Sci., Sci. Eng., Aoyama Gakuin Univ.*)

1P263 **Analyses of the effects of amino acid mutations on the protein folding segments by means of sequence and evolutionary analyses**
Masanari Matsuoka[1,2], Takeshi Kikuchi[1] ([1]*Ritsumeikan Univ., Col. of Life Sci., Dept. of Bioinf.*, [2]*Japan Society Promotion Science DC2*)

1P264 **Ets1 およびパートナー転写因子の協調的結合に関する分子動力学的解析**
Molecular dynamics study on cooperative binding of Ets1 and partner transcription factors on regulatory elements
Kota Kasahara, Ikuo Fukuda, Haruki Nakamura (*IPR, Osaka Univ.*)

1P265 **タンパク質相互作用データベースを利用した超分子モデリング法の開発**
Development of a method for protein-protein interaction modeling using IntAct database
Toshiyuki Tsuji, Takao Yoda, Tsuyoshi Shirai (*Nagahama Institute of Bio-Science and Technology*)

22B. 生命情報科学：機能ゲノミクス / 22B. Bioinformatics: Functional genomics

1P266 **Improved prediction of mitochondrial presequence for detecting undiscovered mitochondrial proteins**
Kenichiro Imai[1], Yoshinori Fukasawa[2], Kentaro Tomii[1,2], Paul Horton[1,2] ([1]*CBRC, AIST*, [2]*Dept. of Comp. Biol., Grad. Sch. of Frontier Sci., Univ. of Tokyo*)

22C. 生命情報科学：比較ゲノミクス / 22C. Bioinformatics: Comparative genomics

1P267 **Application of novel amino acid substitution matrix, MIQS, to the MAFFT multiple sequence aligner**
Kazunori Yamada[1], Kazutaka Katoh[1,2], Kentaro Tomii[1] ([1]*AIST*, [2]*Osaka University*)

22D. 生命情報科学：分子進化 / 22D. Bioinformatics: Molecular evolution

1P268 **Evolutionary Relationships of Clostridia Species**
Takashi Kunisawa (*Dept. Appl. Biol. Sci., Sci. Univ. Tokyo*)

23. 生態／環境 / 23. Ecology & Environment

1P269 **魚たちができること ー 実験的視点から**
The things fishes can do - From an empirical perspective
Takayuki Niizato[1], Hisashi Murakami[2], Takenori Tomaru[2], Yuta Nishiyama[3], Kohei Sonoda[4], Yukio Gunji[5] ([1]*Tsukuba University*, [2]*Kobe University*, [3]*Osaka University*, [4]*Shiga University*, [5]*Waseda University*)

24. 数理生物学 / 24. Mathematical biology

1P270 **Pattern formations of a polymer consisting of "hot" and "cold" monomers as a model of chromosome**
Akinori Awazu[1] ([1]*Dept. of Math. and Life Sciences, Hiroshima Univ.*, [2]*RcMcD, Hiroshima Univ.*)

1P271 **Bubbly vertex dynamics: 曲率をもつ細胞形状を含む上皮組織のための幾何学的動力学モデル**
Bubbly vertex dynamics: a dynamical and geometrical model for epithelial tissues with curved cell shapes
Yukitaka Ishimoto, Yoshihiro Morishita (*RIKEN CDB*)

1P272 **回転する自走粒子の渦格子**
Vortex lattice of rotating self-propelled particles
Ken Nagai (*School of Mater. Sci., JAIST*)

1P273 **細胞内環境における生体高分子の動態： ブラウン動力学法による解析**
Macromolecular dynamics in intracellular environment: Bronian dynamics simulation study
Tadashi Ando[1], Yuji Sugita[1,2,3] ([1]*QBiC, RIKEN*, [2]*RIKEN*, [3]*AICS, RIKEN*)

1P274 **受容体分子のマイクロクラスター形成はシグナル伝達におけるシグナルノイズ比を改善する**
Formation of microclusters of receptor molecules improves signal/noise ratio in cellular signal transduction
Akihiro Fukagawa[1], Kumiko Sakata-Sogawa[1,2], **Makio Tokunaga**[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

1P275 **Spatiotemporal Noisy Signal Processing in Chemotaxis**
Ryo Yokota[1,2], Tetsuya Kobayashi[1] ([1]*Inst. Ind. Sci., Univ. Tokyo*, [2]*Res. & Edu. Platf. Dyn. Liv. States*)

1P276 **神経細胞モデルの現在の発火頻度の入力およびモデルパラメータへの依存性**
The dependence of the current firing rate of a neuron model on the input and the model parameters
Takanobu Yamanobe (*Hokkaido University*)

1P277 **真性粘菌の運動に対する化学的障壁の影響**
The effect of a chemical bump on a migrating amoeba
Dai Akita[1], Itsuki Kunita[2], Shigeru Kuroda[2], Toshiyuki Nakagaki[2] ([1]*Grad. Sch. of Life Sci., Hokkaido Univ.*, [2]*RIES, Hokkaido Univ.*)

1P278 **Band-pass filtering to rhythmic input: A simple model of nonlinear response in a living cell**
Hiroshi Ueno[1], Tatsuaki Tsuruyama[2,3], Kenichi Yoshikawa[1] ([1]*Laboratory of Biological Physics, Faculty of Life and Medical Sciences, Doshisha Univ.*, [2]*Department of Pathology, Graduate School of Medicine, Kyoto University*, [3]*Department of Anatomical, Forensic Medicine, and Pathological Studies, Graduate School of Medicine, Kyoto University*)

25. 非平衡・生体リズム / 25. Nonequilibrium state & Biological rhythm

1P279 **時計タンパク質による概日リズムの同調機構**
KaiC intersubunit communication facilitates robustness of circadian rhythms in cyanobacteria
Yoko Kitayama[1], Taeko Nishiwaki-Ohkawa[1,2], Michio Homma[1], Takao Kondo[1] ([1]*Grad. Sch. Sci., Univ. Nagoya*, [2]*ITbM, Univ. Nagoya*)

1P280 **心臓組織片による同期化のメカニズム**
Synchronization of cardiac rhythms after reassembling of multiple heart fragment tissues
Tomonori Takahashi, Yuji Mitsui, Shin Arai, Kentaro Ishida, Toshiyuki Mitsui (*Dept. of Phys. & Math., Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

1P281 **酵素競合律速による動的細胞記憶の形成**
Kinetic memory based on the enzyme-limited competition
Tetsuhiro S. Hatakeyama, Kunihiko Kaneko (*Dept. of Basic Science, The Univ. of Tokyo*)

1P282 **情報熱力学による生化学シグナル伝達の情報理論**
Information thermodynamics reveals the robustness of biochemical signal transduction
Sosuke Ito[1], Takahiro Sagawa[1,2] ([1]*Department of Physics, the University of Tokyo*, [2]*Department of Basic Science, the University of Tokyo*)

1P283 **Coherence vs Dynamics on Random Boolean Networks**
Taichi Haruna (*Graduate School of Science, Kobe University*)

26. 計測 / 26. Measurements

1P284 **LN 光変調器を用いた高精度周波数領域蛍光異方性測定による蛍光色素の回転運動解析**
High-precision frequency-domain fluorescence anisotropy measurement using a waveguide LN modulator for dye rotational motion analysis
Tetsuichi Wazawa[1,2], Nobuyuki Morimoto[2], Makoto Suzuki[2] ([1]*Biomolec Sci Engin, ISIR, Osaka Univ*, [2]*Dept Mater Proc, Grad Sch Engin, Tohoku Univ*)

1P285 **近赤外蛍光ゆらぎ計測のためのナノ秒光子計数システムの構築**
A photon-timing recorder in a nano-second resolution and its application for near-infrared fluorescence fluctuation measurements
Goro Nishimura (*RIES, Hokkaido University*)

1P286 **二光子励起によるホログラフィック多点蛍光相関分光計測装置の開発**
Development of a holographic multipoint fluorescence correlation spectroscopy based on two photon excitation
Johtaro Yamamoto, Masataka Kinjo (*Faculty of Adv. Life Sci., Hokkaido Univ.*)

1P287 **Simple method for lipid bilayer formation with simultaneous incorporation of ion channels using gold electrode**
Daichi Okuno[1], Minako Hirano[2], Hiroaki Yokota[2], Yukiko Onishi[1], Toshio Yanagida[1], Toru Ide[3] ([1]*RIKEN QBiC*, [2]*GPI*, [3]*Grad. Sch. Nat. Sci. & Tec., Okayama Univ.*)

1P288 **Holliday junction DNA の自発的 branch migration 過程の FRET による解析**
FRET analysis of the spontaneous branch migration of the Holliday junction DNA
Kenji Okamoto, Yasushi Sako (*RIKEN*)

1P289 **光渦三次元トラップで生物の回転運動を操作・計測する**
The manipulation and analysis of biological rotary motions by 3D optical vortex trapping
Yu Hashimoto[1], Tomoko Otsu[2], Yuji Kimura[1], Sayaka Kazami[1], Yoshiyuki Sowa[3], Yu Takiguchi[2], Taro Ando[2], Ikuro Kawagishi[3], Hiroyasu Itoh[1] ([1]*Tsukuba Reserch Laboratory, Hamamatsu Photonics K.K.*, [2]*Central Reserch Laboratory, Hamamatsu Photonics K.K.*, [3]*Dept. Frontier Biosci., Hosei Univ.*)

1P290 **ビデオ計測による大腸菌の回転運動特性の統計解析**
Statistical analysis of rotational motion properties of tethered E. coli by video measurement
Hiroto Tanaka[1], Tadashi Matsukawa[1], Takashi Sagawa[1], Sakura Maesato[2], Yukihiro Tominari[3], Yoshiyuki Sowa[4], Ikuro Kawagishi[4], Hiroaki Kojima[1] ([1]*Bio ICT lab., NICT*, [2]*Dept. Bioeng., Nagaoka Univ. Tech.*, [3]*Nano ICT lab., NICT*, [4]*Dept. Front. Biosci., Hosei Univ.*)

1P291 **マイクロ波加熱効果：生体系に対する in situ マイクロ波照射 NMR の応用**
Microwave heating effects: Application to analyze biological system by in situ microwave irradiation NMR
Yugo Tasei[1], Fumiichi Tanigawa[1], Izuru Kawamura[1], Motoyasu Sato[2], Akira Naito[1] ([1]*Yokohama National University*, [2]*Chubu University*)

1P292 **走査型イオンコンダクタンス顕微鏡の単一細胞ナノバイオプシーへの応用と細胞内 mRNA 局在性の評価**
Single-cell nanobiopsy to investigate intracellular mRNA localization using scanning ion conductance microscopy
Yuji Nashimoto[1], Yasufumi Takahashi[2], Kosuke Ino[1], Kumi Inoue Y[1], Hitoshi Shiku[1], Tomokazu Matsue[1,2] ([1]*Enviromental studies, Tohoku Univ.*, [2]*WPI-AIMR, Tohoku Univ.*)

27. バイオイメージング / 27. Bioimaging

1P293 **高速 AFM を用いたタンパク質構造変化のライブイメージング**
Live Imaging of Protein Structural Change by High-Speed AFM
Motonori Imamura[1,2], Takayuki Uchihashi[3,4], Toshio Ando[3,4], Jonathan G. Heddle[1], Ali D. Malay[1] ([1]*Riken*, [2]*Grad. Sch. of Biosci. & Biotech., Tokyo Tech.*, [3]*Dept. Phys., Kanazawa Univ.*, [4]*Bio-AFM Frontier Research Center, Kanazawa Univ.*)

1P294 **マニフォールドの概念に基づく新規画像分類法を用いた投影イメージの解析**
Analysis of the projection images using the novel classification protocol based on the concept of manifold
Takashi Yoshidome[1], Tomotaka Oroguchi[2,3], Masayoshi Nakasako[2,3], Mitsunori Ikeguchi[1] ([1]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*, [2]*Fac. Sci., Keio. Univ.*, [3]*Harima Inst., Riken*)

1P295 **Metallothionein labeling for CLEM(Correlative Light and Electron Microscopy) method**
Ryutaro Yamanaka[1], Yuka Hirasaka[1], Mingyue Jin[1], Yanagisawa Haruaki[2], Takuo Yasunaga[1] ([1]*Kyushu Institute of Technology*, [2]*Univ. of Tokyo*)

1P296 **高速 AFM/１分子蛍光顕微鏡複合機によるタンパク質の構造動態と化学反応の同時イメージング**
Simultaneous imaging of dynamic structural and chemical events in protein by high-speed AFM combined with single-molecule TIRFM
Shingo Fukuda[1], Takayuki Uchihashi[1,2], Ryota Iino[3], Toshio Ando[1,2] ([1]*Sch. Math. & Phys., Col. Sci. & Eng., Kanazawa Univ.*, [2]*Bio-AFM FRC, Inst. Sci. & Eng., Kanazawa Univ.*, [3]*Okazaki Inst. Integr. Biosci., NINS*)

1P297 **Real time imaging of collagenase behavior by high speed atomic force microscopy**
Takahiro Nakayama, Noriyuki Kodera, Hiroki Kon'no, Toshio Ando (*Bio-AFM Frontier Research Center, Kanazawa University*)

1P298 **新規共分散 Number and Brightness 法によるグルココルチコイド受容体二量体の生細胞内空間分布解析**
Spatio-temporal distribution analysis of dimeric glucocorticoid receptor using a new Number and Brightness method based on covariance
Hideto Ishikawa[1], Johtaro Yamamoto[2], Shintaro Mikuni[2], Masataka Kinjo[2] ([1]*Grad. Life Sci., Univ.Hokkaido*, [2]*Fuc. Adv. Life Sci, Univ.Hokkaido*)

1P299 **生細胞蛍光イメージングによる ALS 関連変異体 TDP43 の構造解析**
Analysis of structural difference in ALS-linked mutant of TDP43 by fluorescence imaging in living cells
Sachiko Yuno[1], Akira Kitamura[1,2], Ai Shibasaki[1], Masataka Kinjo[1,2] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Advanced Life Sci., Hokkaido Univ.*)

1P300 **高速原子間力顕微鏡による AAA シャペロン p97 の主要 ATPase（D2）リングの構造変化の直接観察**
Direct observation of the structural changes of the major ATPase domain D2 of the AAA chaperone p97 by high-speed atomic force microscopy
Daisuke Yamamoto[1,3], Kentaro Noi[2,3], Ken-ichi Arita-Morioka[2,3], Teru Ogura[2,3] ([1]*Dept. Appl. Phys., Fac. Sci., Fukuoka Univ.*, [2]*Dept. Mol. Cell Biol., IMEG, Kumamoto Univ.*, [3]*CREST, JST*)

1P301 **マルチモーダル超高輝度化学発光タンパク質**

Multi-modal super-duper chemi-luminescent protein

Kazushi Suzuki[1], Yoshiyuki Arai[1,2], Takeharu Nagai[1,2] ([1]*Grad sch engin, Osaka Univ*, [2]*ISIR, Osaka Univ*)

1P302 **生体発光共鳴エネルギー転移（BRET）を用いたミトコンドリア外膜タンパク質 MAVS の構造基盤解析**

A structural analysis of the MAVS-regulatory mechanism using BRET

Osamu Sasaki[1], Takuma Yoshizumi[1], Misa Kuboyama[1], Takeshi Ishihara[2], Emiko Suzuki[3], Shun-ichiro Kawabata[2], Takumi Koshiba[2] ([1]*Graduate School of Systems Life Scienses, Kyushu University*, [2]*Department of Biology, Faculty of Sciences, Kyushu University*, [3]*Structural Biology Center, National Institute of Genetics and Department of Genetics*)

1P303 **ストレス顆粒内一分子 mRNA 立体構造の超解像イメージング**

Super-resolution imaging of molecular conformation of single mRNA in stress granules

Yuki Suzuki[1], Kou Sugawara[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*JST, PRESTO*)

1P304 **転写伸長因子 NELF ダイナミクスの１分子イメージング定量解析**

Single molecule imaging and quantitative analysis of dynamics of negative elongation factor NELF

Daichi Ikeda[1], Yuma Ito[1,2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

1P305 **AIP1 とコフィリン共存下でのアクチンフィラメント切断の 1 分子リアルタイムイメージング**

Real-time imaging of actin filament disassembly in the presence of cofilin and actin interacting protein 1 (AIP1)

Carina Sekiguchi[1], Kimihide Hayakawa[2], Shoichiro Ono[3], Masahiro Sokabe[2], Hitoshi Tatsumi[1] ([1]*Department of Physiology, Nagoya University Graduate School of Medicine*, [2]*Mechano-biology Laboratory, Nagoya University Graduate School of Medicine*, [3]*Department of Pathology, Emory University School of Medicine*)

1P306 **１細胞分泌実時間測定による IL-1β 非古典的分泌機序の解明**

Analysis of non-classical secretion of IL-1β using real-time single-cell secretion imaging

Yoshitaka Shirasaki[1], Ting Liu[2], Yoshifumi Yamaguchi[2], Mai Yamaguchi[1], Nobutake Suzuki[1], Kazushi Izawa[3], Jun Mizuno[4], Shuichi Shoji[4], Yoshie Harada[5], Ryuta Nishikomori[3], Toshio Heike[3], Masayuki Miura[2], Osamu Ohara[1,6] ([1]*IMS, Riken*, [2]*Grad. Sch. Pharm., Tokyo Univ.*, [3]*Grad. Sch. Med., Kyoto Univ.*, [4]*Grad. Sch. Sci. & Eng., Waseda Univ.*, [5]*iCeMS, Kyoto Univ.*, [6]*Kazusa DNA Inst.*)

1P307 **ナノスリット基板を用いたアクチンの重合の一分子観察**

Single molecule observation of actin polymerization in linear zero-mode waveguid

Masamichi Yamamoto[1], Makoto Tsunoda[1], Shun Higano[2], Kotaro Okubo[2], Takashi Tanii[2], **Takashi Funatsu**[1] ([1]*Grad. Sch. Pharm. Sci., Univ. Tokyo*, [2]*Sch. Sci. Eng., Waseda Univ.*)

1P308 **培地温度の珪藻運動に与える影響の一細胞観察による評価**

Effects of medium temperature on diatom motility studied by single cell observation

Kazuo Umemura[1], Toru Miyabayashi[1], Yoshikazu Kumashiro[2], Teruo Okano[2], Shigeki Mayama[3] ([1]*Tokyo Univ. Sci.*, [2]*Tokyo Women's Med. Univ.*, [3]*Tokyo Gakugei Univ.*)

1P309 **異物排出トランスポーター AcrD の発現は外膜チャネル TolC 遺伝子の欠失により促進される**

Expression of the xenobiotic efflux transporter AcrD is induced by the deletion of outer membrane channel gene *tolC*

Kentaro Yamamoto[1], Rei Tamai[1], Takehiko Inaba[2], Yoshiyuki Sowa[1,2,3], Ikuro Kawagishi[1,2,3] ([1]*Dept. Frontier Biosci., Grad. Sch. Sci and Eng., Hosei Univ.*, [2]*Res. Cen. Micro-Nanotech., Hosei Univ.*, [3]*Dept. Frontier Biosci., Fac. Biosci. and Appl. Chem., Hosei Univ.*)

1P310 **大腸菌異物排出トランスポーター MdtB, MdtC の膜内動態解析**

Dynamics of the xenobiotic efflux transporter components MdtB and MdtC in the cytoplasmic membrane of *Escherichia coli*

Megumi Yamazaki[1], Kentaro Yamamoto[1], Rei Tamai[1], Yoshiyuki Sowa[1,2,3], Ikuro Kawagishi[1,2,3] ([1]*Dept. Frontier Biosci., Grad. Sch. Sci and Eng., Hosei Univ.*, [2]*Res. Cen. Micro-Nanotech., Hosei Univ.*, [3]*Dept. Frontier Biosci., Fac. Biosci. and Appl. Chem., Hosei Univ.*)

28. バイオエンジニアリング / 28. Bioengineering

1P311 **電子伝達タンパク質による転写調節因子の出力変換**

Readout Conversion of Transcriptional Regulator by Electron Transfer Proteins

Hiroshi Nakajima[1], Souji Miyazaki[2], Yoshihito Watanabe[2] ([1]*Dept. Chem., Sch. Sci., Nagoya Univ.*, [2]*RCMS, Nagoya Univ.*)

1P312 **再構成無細胞系を用いた抗菌ペプチドの直接発現**

Direct expression of antimicrobial peptides in an intact form by using a reconstitued cell-free system

Satoshi Tomisawa, Masakatsu Kamiya, Takashi Kikukawa, Makoto Demura, Tomoyasu Aizawa (*Grad. Sch. Life Sci., Univ. Hokkaido*)

1P313 **カリウムイオンを感知して自らの活性をスイッチングする Tat 捕捉アプタマーおよびリボザイムの創製**

Development of Tat-binding aptamer and ribozyme which switch their activities in response to K+

Yudai Yamaoki[1,2,3], Tsukasa Mashima[1], Takashi Nagata[1,2], Masato Katahira[1,2] ([1]*Inst. Adv. Energy, Kyoto Univ.*, [2]*Grad. Sch. Energy Sci., Kyoto Univ.*, [3]*JSPS Research Fellow*)

1P314 **細胞シグナル制御を目指した機能性核酸の設計**

Design of functional nucleic acid that controls receptor signaling

Ryosuke Ueki[1], Shinsuke Sando[2] ([1]*Inamori Frontier Research Center, Kyushu University*, [2]*Grad. Sch of Eng, The University of Tokyo*)

1P315 **Fabrication of photosensiting and electron-transfer RNA-modules**

Tran Thoa Thi Thanh[1,2], Noriko Minagawa[1], Sivakumar Ponnurengam Malliappan[1], Toshiro Aigaki[2], Yoshihiro Ito[1,2], **Takanori Uzawa**[1] ([1]*RIKEN*, [2]*Tokyo Metro. Univ.*)

1P316 **Rational design of orthogonal gene transcription nano device on DNA origami**

Takeya Masubuchi[1], Hisashi Tadakuma[1], Masayuki Endo[2], Hiroshi Sugiyama[2], Yoshie Harada[2], Takuya Ueda[1] ([1]*Grad. Sch. Frontier Sci., Univ. Tokyo*, [2]*iCeMS, Univ. Kyoto*)

1P317 **微小管リング状集合体の内径を制御する方法**

How to control the size of ring-shaped microtubule assemblies

Shoki Wada[1], Masaki Ito[1], Daisuke Inoue[1], Kazuki Sada[1,2], Akira Kakugo[1,2] ([1]*Graduate School of Chemical Science and Engineering, Hokkaido University*, [2]*Faculty of Science, Hokkaido University*)

1P318 **応力場を利用した微小管集団運動の動的制御**

Dynamic Control of Collective Motion of Microtubules Propelled by Kinesin in a Stress Field

Daisuke Inoue[1], Kazuki Sada[1,2], Akira Kakugo[1,2] ([1]*Grad. Sch. of Chem. Sci. and Eng., Univ. Hokkaido*, [2]*Fac. of Sci., Univ. Hokkaido*)

1P319 **DNA の相互作用に基づいた能動的自己組織化の制御**

Control of Active Self-organization of microtubule by using DNA based interaction

Kyohei Uenishi[1], Shoki Wada[1], Daisuke Inoue[1], Kazuki Sada[1,2], Akira Kakugo[1,2] ([1]*Grad. Sch. of Chem. Sci. and Eng., Hokkaido Univ.*, [2]*Fac. of Sci., Hokkaido Univ.*)

1P320 **Cell trapping device for observation of connexin function by a single cell device**

Kosuke Inoue[1,2], Koki Kamiya[1,4], Yuta Abe[1,2], Toshihisa Osaki[1,3], Norihisa Miki[2], Shoji Takeuchi[1,3] ([1]*Kanagawa Academy of Science and Technology*, [2]*Keio University*, [3]*Institute of Industrial Science, the University of Tokyo*, [4]*PRESTO, Japan Science and Technology Agency*)

30. その他 / 30. Miscellaneous topics

1P321 **質量を変えたレプリカ交換分子動力学法**

Mass-scaling replica-exchange molecular dynamics method

Tetsuro Nagai, Takuya Takahashi (*College of Life Sciences, Ritsumeikan University*)

1P322 **分裂酵母クロマチンダイナミクスの定量的解析**

Quantitative analyses of chromatin dynamics in fission yeast

Takeshi Sugawara[1,2], Shota Masuda[3], Jun-ichi Uewaki[1,2], Akinori Awazu[1,2], Hiraku Nishimori[1,2], Masaru Ueno[1,3] ([1]*RcMcD, Hiroshima Univ.*, [2]*Department of Mathematical and Life Sciences, Faculty of Science, Hiroshima University*, [3]*Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University*)

1P323 **Dynamical heterogeneity and dynamics of cage breaking and formation in colloidal fluids**

Preetom Nag[1,2], Hiroshi Teramoto[1,2], Chun-Biu Li[2,3,4], Tamiki Komatsuzaki[1,2,4] ([1]*Graduate School of Life Science, Transdisciplinary Life Science Course, Hokkaido University*, [2]*Molecule and Life Nonlinear Sciences Laboratory, Research Institute for Electronic Science, Hokkaido University*, [3]*Graduate School of Science, Department of Mathematics, Hokkaido University*, [4]*Research Center for Integrative Mathematics, Hokkaido University*)

第 2 日目（9 月 26 日（金））／Day 2（Sep. 26 Fri.）大ホール / Main Hall

01A. 蛋白質：構造 / 01A. Protein: Structure

2P001 **イネ萎縮ウイルスの詳細な細胞侵入機構**

Detailed cell entry mechanism of Rice dwarf virus (RDV)

Naoyuki Miyazaki[1,2], Akifumi Higashiura[2], Tomoko Higashiura[2], Fusamichi Akita[3], Hiroyuki Hibino[3], Toshihiro Omura[3], Atsushi Nakagawa[2], Kenji Iwasaki[2] ([1]*NIPS*, [2]*IPR*, [3]*NARC*)

2P002 **タンパク質における埋もれた極性残基の構造と置換パターンの網羅的解析**

Comprehensive analysis on the conformation and substitution patterns of buried polar residues in protein structures

Matsuyuki Shirota[1,2,3], Kengo Kinoshita[2,3,4] ([1]*Grad. Sch. Med. Tohoku Univ.*, [2]*ToMMo, Tohoku Univ.*, [3]*GSIS, Tohoku Univ.*, [4]*IDAC, Tohoku Univ.*)

2P003 **Crowding 環境下でのタンパク質構造の熱安定性**

Thermal Structural Stability of Proteins Under Crowding Environment

Mitsuhiro Hirai[1], Shouki Sato[1], Masaaki Sugiyama[2], Noboru Ohota[3], Lionel Porcar[4], Anne Martel[4], Giuseppe Zaccai[4] ([1]*Grad. Sch.Sci. Tech., Gunma Univ.*, [2]*KURRI*, [3]*JASRI*, [4]*Institut Laue-Langevin*)

2P004 **相手に応じた様々な折り畳みを伴う一対多分子認識機構の統計熱力学：転写因子タンパク質 p53 の場合**

Statistical thermodynamics of one-to-many molecular recognition accompanied by partner-dependent folding: in the case of p53 protein

Tomohiko Hayashi, Hiraku Oshima, Satoshi Yasuda, Masahiro Kinoshita (*Institute of Advanced Energy, Kyoto University*)

2P005 **結晶環境における弾性ネットワークモデルを用いた非等方性温度因子の再現**

Anisotropic atomic fluctuations reproduced by normal modes based on an elastic-network model in the crystal environment

Shigeru Endo[1], Hiroshi Wako[2] ([1]*Dept. Phys., Sch. Science, Kitasato Univ.*, [2]*Sch. Social Sciences, Waseda Univ.*)

2P006 **レプリカ置換分子動力学法の詳細釣り合い条件の有無に対する検証と生体分子への応用**

Comparison of the replica-permutation molecular dynamics with and without detailed balance conditions and its application to biomolecules

Hiroaki Nishizawa[1], Hisashi Okumura[1,2] ([1]*IMS*, [2]*Sokendai*)

2P007 **β-sheet 中でのペア傾向は edge strand と central strand では全く異なる**
Pairing propensity in β-sheets is quite defferent between edge and central strands
Hiromi Suzuki (*School of Agriculture, Meiji Univ.*)

2P008 **最適化した力場のアミノ酸ごとのパラメータ依存性について**
Parameter dependency of an optimized force field for each amino acid
Yoshitake Sakae[1], Yuko Okamoto[1,2,3,4] ([1]*Dept. Phys., Nagoya Univ.*, [2]*Structural Biology Research Center, Nagoya Univ.*, [3]*Center for Computational Science, Nagoya Univ.*, [4]*Information Technology Center, Nagoya Univ.*)

2P009 **固体 NMR および MD シミュレーションによるヒトカルシトニン線維形成機構と構造の解析**
Fibrillation mechanism and fibril structure of human calcitonin as studied by solid-state NMR and MD simulation
Shuuhei Toyoda[1], Ganchimeg Lkhamsuren[2], Javklantugs Namsrai[1,2], Hikari Watanabe[1], Izuru Kawamura[1], Kazuyoshi Ueda[1], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Monglia Natl. Univ.*)

2P010 **４量体型サルコシン酸化酵素の分子動力学シミュレーション: 水分子の透過経路解析**
Molecular dynamics simulation of heterotetrameric sarcosine oxidase: pathways of water molecules
Go Watanabe, Akinori Hiroshima, Haruo Suzuki, Shigetaka Yoneda (*Sch. Sci., Kitasato Univ.*)

2P011 **生体高分子中性子結晶構造解析におけるフーリエマップ改善のための実践的考察**
A practical study for the improvement on the Fourier map in neutron protein crystallography(NPC)
Ichiro Tanaka[1,2], Nobuo Niimura[2] ([1]*Coll. of Eng., Ibaraki Univ.*, [2]*Frontier Center, Ibaraki Univ.*)

2P012 **分子動力学シミュレーションによる GLP-1 と Exendin-4 の構造解析**
Structure Analysis of GLP-1 and Exendin-4 by Molecular Dynamics Simulation
Sakiko Mori, Hironao Yamada, Yo Noguchi, Takeshi Miyakawa, Ryota Morikawa, Takuya Watanabe, Masako Takasu (*Sch. of Life Sci., Tokyo Univ. of Pharm. and Life Sci.*)

2P013 **分子動力学シミュレーションによる γS-WT と γS-G18V の構造変化の比較**
Structural changes of γS-WT and γS-G18V studied by molecular dynamics simulation
Ai Ozawa, Hironao Yamada, Sakiko Mori, Yo Noguchi, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu (*Tokyo University of Pharmacy and Life sciences*)

2P014 **MEGADOCK: 超並列計算環境による大規模タンパク質間相互作用予測**
MEGADOCK: a high-performance protein-protein interaction prediction tool on supercomputing environments
Masahito Ohue[1,2], Yuri Matsuzaki[3], Nobuyuki Uchikoga[4], Takashi Ishida[1], Yutaka Akiyama[1,3] ([1]*Grad. Sch. Inform. Sci. and Eng., Tokyo Tech*, [2]*JSPS Research Fellow*, [3]*ACLS, Tokyo Tech*, [4]*Dept. Phys., Chuo Univ.*)

2P015 **マイクロ流路を用いた X 線溶液散乱測定用サンプルチェンジャーの開発**
Development of a microfluidics-based auto-sample changer for solution X-ray scattering
Ryuji Okabe, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

2P016 **タンパク質の構造コンプライアンス解析手法の改良**
Improvement of a Method for Structural Compliance Analysis of Proteins
Keisuke Arikawa (*Fcl. Eng. , Kanagawa Inst. of Tech.*)

2P017 **固体 NMR 法によるタンパク質立体構造解析への常磁性緩和促進の応用**
Application of paramagnetic relaxation enhancement to solid-state NMR protein structure analysis
Hajime Tamaki[1], Ayako Egawa[2], Kouki Kido[1], Tomoshi Kameda[3], Masakatsu Kamiya[1], Takashi Kikukawa[1], Tomoyasu Aizawa[1], Toshimichi Fujiwara[2], Makoto Demura[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*IPR, Osaka Univ.*, [3]*CBRC, AIST*)

2P018 **低温電子顕微鏡による単粒子像解析法における GFP ラベル**
GFP labeling for single particle analysis with cryoEM
Takayuki Kato[1], Naoya Terahara[1], Tomoko Miyata[1], Keiichi Namba[1,2] ([1]*Grad. Sch. Front. Biosci., Osaka Univ*, [2]*QBIC, Riken*)

2P019 **赤痢菌ニードル複合体の極低温電子顕微鏡による構造解析**
Structural analysis of needle complex from *shigella flexneri* by cryo electron microscopy
Naoko Kajimura[1], Fumiaki Makino[1,2], Martin P Cheung[2], Takayuki Kato[1], Ariel J Blocker[2], Keiichi Namba[2,3] ([1]*Grad. Sch. of Frontier Biosci., Osaka Univ.*, [2]*Sch. of Cell. & Mol. Med., Univ. of Bristol*, [3]*RIKEN, QBiC*)

01B. 蛋白質：構造機能相関 / 01B. Protein: Structure & Function

2P020 **ファルネシル基の結合による hGal-1 のオリゴマー化**
Oligomerization of hGal-1 induced via the binding of farnesyl group
Kazumi Yamaguchi, Hirotsugu Hiramatsu, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ*)

2P021 **高精度自由エネルギー計算によるドラッグデザイン**
Computational Drug Design by Accurate Free Energy Calculations
Hironori Kokubo, Akihiro Yokota, Nao Morishita, Atsutoshi Okabe, Etsurou Watanabe (*Takeda Pharmaceutical*)

2P022 **変性 apo-SOD1 の Cu 結合部位における His 残基の帰属**
Assignments of His residues in the Cu^{2+}-binding sites of the denatured apo-SOD1
Nobuhiro Fujimaki, Takashi Miura, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

2P023 **Pin1 のプロリン異性化活性とタウタンパク質に対する凝集抑制活性との関係**
Relationship between Pin1's peptidyl-prolyl isomerase activity and its aggregation-inhibitory activity for tau protein
Teikichi Ikura, Nobutoshi Ito (*MRI, TMDU*)

2P024 **Hsp90 の結合ポケット内における ADP の分布に関する理論的研究**

Theoretical study of distribution of ADP in binding pocket of Hsp90

Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Institute of Science and Engineering, Kanazawa University*)

2P025 **計算と実験による黄色ブドウ球菌の Isd 蛋白質間ヘム輸送機構の解明**

Structural insight into the heme-transfer mechanism between Isd proteins in Staphylococcus aureus

Yoshitaka Moriwaki[1], Tohru Terada[1], Jose M. M. Caaveiro[2], Kouhei Tsumoto[2], Kentaro Shimizu[1] ([1]*Dept. of Biotech., Grad Sch. of Agri. Life Sci., Univ. of Tokyo*, [2]*Dept. of Bioeng., Sch. of Eng., Univ. of Tokyo*)

2P026 **滑走細菌 *Mycoplasma mobile* 由来新規シアル酸レセプターの結合活性**

Binding activity of novel sialic acid receptor from gliding bacterium, *Mycoplasma mobile*

Tasuku Hamaguchi[1], Masaru Kawakami[2], Makoto Miyata[1] ([1]*Grad. Sch. of Sci., Osaka City Univ.*, [2]*Fac. of Eng., Yamagata Univ.*)

2P027 **子嚢菌由来不凍タンパク質の機能と構造**

Function and Structure of Antifreeze Protein from Ascomycete

Daichi Fukami[1], Yuichi Hanada[1], Jing Cheng[1], Sakae Tsuda[1,2], Hidemasa Kondo[1,2] ([1]*Grad. Sch. Sci., Univ. Hokkaido*, [2]*Bioproduction Research Inst., AIST*)

2P028 **分裂酵母キネシン Cut7 の両方向運動性**

Bidirectional motility of the fission yeast kinesin-5, Cut7

Masaki Edamatsu (*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*)

2P029 **第一原理フラグメント分子軌道法による ブチリルコリンエステラーゼと阻害剤間の特異的相互作用の解析**

***Ab initio* fragment molecular orbital calculations on specific interactions between butylcholinesterase and its inhibitors**

Takeru Murakawa[1], Tomoya Suzuki[1], Tareq Khan[2], Noriyuki Kurita[1] ([1]*Toyohashi University of Technology*, [2]*University of Tromso*)

2P030 **Ab initio 分子シミュレーションによるがん細胞レセプターへのリガンド結合を阻害する新規ペプチド阻害剤の提案**

***Ab initio* molecular simulation for proposing novel peptide inhibitors blocking the ligand-binding to the receptor of cancer cell**

Tatsuro Mizushima[1], Ryushi Kadoya[1], Tomoyo Kasumi[1], Hiroshi Kobayashi[2], Noriyuki Kurita[1] ([1]*Toyohashi University of Technology*, [2]*Nara Medical University*)

2P031 **Mechanism of glycan receptor recognition for influenza virus Hemagglutinins: Comparative molecular dynamis studies**

Katumi Omagari (*Nagoya City University*)

2P032 **比較粗視化シミュレーションを用いたタンパク質-リガンド結合過程の解析**

The factors determining protein-ligand binding processes revealed by comparative coarse-grained simulations

Tatsuki Negami, Tohru Terada, Kentaro Shimizu (*Grad. Sch. of Agri. and Life Sci.*)

2P033 **DM 分子はペプチド交換時の MHC 複合体の動きを制御する**

DM defines motions of peptide/MHC complex for peptide exchange

Toshihiro Miyabe[2], Kohsuke Kasadera[1], Yufuku Matsushita[2], Yuko Kozono[1], Hiroshi Sekiguchi[3], Keigo Ikezaki[2], Yuji Sasaki[2], Haruo Kozono[1] ([1]*Res. Inst. Biomed. Sci., Tokyo Univ. of Science*, [2]*Grad. Sch. Front. Sci., Univ. Tokyo*, [3]*JASRI, JST*)

2P034 **高速 AFM による MukB の構造と機能の観察**

High-speed AFM observation of structure and function of MukB

Kenta Yagi[1], Koichi Yano[2], Noriyuki Kodera[3,4], Hironori Niki[2], Toshio Ando[1,3,5] ([1]*Div. of Math & Phys. Sci., Grad. Sch. of Nat. Sci. & Tech., Kanazawa Univ.*, [2]*Natl. Inst. of Genet.*, [3]*Bio-AFM FRC, Inst. of Sci. and Eng., Kanazawa Univ.*, [4]*PRESTO, JST*, [5]*CREST, JST*)

2P035 **アミロイドベータペプチドのオリゴマー形成機構の解析**

Analyses of the oligomerization mechanism of amyloid β peptides

Ayumi Tanaka[1], Shigeto Iwamoto[1], Takashi Saito[2], Hitomi Yamaguchi[1], Sosuke Yoshinaga[1], Toshiyuki Kohno[3], Takaomi Saido[2], Hiroaki Terasawa[1] ([1]*Fac. Life Sci., Kumamoto Univ.*, [2]*RIKEN BSI*, [3]*Kitasato Univ. Sch. Med.*)

2P036 **カメレオンモデルを用いた酸素結合に伴うヘモグロビンのアロステリック転移の研究**

A study of the allosteric transition of hemoglobin associated with oxygen binding using chameleon model

Yui Sobue, Toru Kimura, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Nagoya Univ.*)

2P037 **cAMP 結合による Catabolite Activator Protein のアロステリック構造変化のダイナミックスに関する Molecular dynamics 研究**

Molecular dynamics study on dynamics of allosteric conformational change of Catabolite activator protein induced by cAMP binding

Mayuka Ojima[1], Yoshifumi Fukunishi[2], Masami Lintuluoto[1] ([1]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*AIST*)

2P038 **カチオン分布がトロンビン-基質会合に及ぼす影響**

Influence of Cation Distribution on the Thrombin-substrate Association

Ikuo Kurisaki[1], Masayoshi Takayanagi[1,2], Masataka Nagaoka[1,2] ([1]*Grad. Sch. Info. Sci. Univ. Nagoya*, [2]*CREST, JST*)

2P039 **Ectopic A-lattice seams destabilize microtubules**

Miho Katsuki[1,2], Douglas R. Drummond[2], Robert A. Cross[2] ([1]*Fukuoka Univ., Japan*, [2]*Warwick Med. Sch., Univ. Warwick, UK*)

2P040 **cDNA ディスプレイ法による固相上のアミノ基認識ペプチドの探索とその分子認識機構の解析**

Exploring the peptide aptamer against amino group on a solid-phase by cDNA display and analysis of its molecular recognition mechanism

Yuki Mochizuki, Koichi Nishigaki, Naoto Nemoto (*Grad. Sch. of Sci. and Eng., Saitama Univ.*)

2P041 **ミトコンドリア呼吸鎖のシトクロム c-シトクロム c 酸化酵素複合体における 電子伝達反応の構造制御機構**

Conformational Gating for Electron Transfer Reaction from Cytochrome c to Cytochrome c Oxidase in Mitochondrial Respiratory Chain

Mizue Imai[1], Wataru Sato[1], Kaoru Inoue[3], Koichi Sakamoto[3], Kyoko Shinzawa[2], Takeshi Uchida[1,3], Shinya Yoshikawa[2], Koichiro Ishimori[1,3] ([1]*Grad. School of Chem. Sci. and Eng., Hokkaido Univ.*, [2]*Grad. Sch. of Life Sci., Hyogo Pred. Univ.*, [3]*Dept. of Chem., Fac. of Sci., Hokkaido Univ.*)

2P042 **MD シミュレーションを用いたマウス・線虫 ABCB1 トランスポーターの構造・ダイナミクスの解析**
Analysis of dynamics and structure of ABCB1 transporters from mouse and *C. elegans* using molecule dynamics simulations
Tatsushi Nishimoto, **Tadaomi Furuta**, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

2P043 **多剤排出トランスポーター AcrB の Motion-Tree による解析**
Motion-Tree analysis of the multidrug transporter AcrB
Tsutomu Yamane[1], Ryotaro Koike[2], Motonori Oota[2], Satoshi Murakami[3], Akinori Kidera[1], Mitsunori Ikeguchi[1] ([1]*Grad. School of Medical Life Science, Yokohama City Univ.*, [2]*Grad. School of Information Science, Nagoya Univ.*, [3]*Grad. School of Biosci. & Biotech., Tokyo Inst. Tech.*)

2P044 **Conformational states of HAMP domains interacting with SRII-membrane systems: A molecular dynamics approach**
Bikash Sahoo, Toshimichi Fujiwara (*Inst. Protein Res., Osaka Univ.*)

2P045 **高速 X 線 1 分子追跡法によるニコチン性アセチルコリン受容体の機能運動**
3D X-ray Single Molecule Tracking of nAChRs in Open, Resting, and Desensitization States
Hiroshi Sekiguchi[1], Maki Tokue[2], Yuri Nishino[3], Kouhei Ichiyanagi[4], Naoto Yagi[1], Atsuo Miyazawa[3], Tai Kubo[5], Yuji C. Sasaki[1,2] ([1]*Research & Utilization Div., JASRI/SPring-8*, [2]*Grad. School Frontier Sci., Univ. Tokyo*, [3]*Grad. School Life Sci., University of Hyogo*, [4]*Inst. Material. Struct. Sci. KEK*, [5]*Mol. Profil. Res. Ctr., AIST*)

2P046 **ドメイン運動の階層的な解析により描かれる SERCA の反応による構造変化**
Conformational changes of SERCA in response to reactions described by hierarchical domain-motion analysis
Chigusa Kobayashi[1], Ryotaro Koike[2], Motonori Ota[2], Yuji Sugita[1,3,4,5] ([1]*AICS, RIKEN*, [2]*Grad. Sch. Info. Nagoya Univ.*, [3]*TMS, RIKEN*, [4]*QBiC, RIKEN*, [5]*iTHES, RIKEN*)

2P047 **大腸菌機械受容チャネル MscL の開口における脂質膜環境に影響される脂質–タンパク質間相互作用の解析**
Analysis on Lipid-Protein Interactions Affected by Membrane Environment in Mechano-Gating of the E.coli Mechanosensitive Channel MscL
Hiroki Katsuta[1], Yasuyuki Sawada[2], Masahiro Sokabe[3] ([1]*Sch. Med., Nagoya Univ.*, [2]*Dept. Physiol. Grad. Sch. Med., Nagoya Univ.*, [3]*Mechanobiology Lab. Grad. Sch. Med., Nagoya Univ.*)

01C. 蛋白質：物性 / 01C. Protein: Property

2P048 **天然変性タンパク質と変性状態蛋白質の構造特性の比較**
A Comparison of structural properties between an intrinsically disordered protein and denatured state of proteins
Yasutaka Seki[1], Takamasa Nonaka[1], Kunitsugu Soda[2] ([1]*Sch. of Pharm., Iwate Med. Univ.*, [2]*High Perform. Molec. Simula. Team, ASI, RIKEN*)

2P049 **平衡条件下において形成される二つの天然変性蛋白質融合蛋白質のフォールディング中間状態**
Folding intermediates formed by the fusion protein of two intrisically disordered proteins under equilibrium condition
Hamada Daizo (*Dept Life Sci, Grad Schl Bioresource, Mie Univ.*)

2P050 **天然タンパク質の分子サイズに関する統計解析**
Statistical analysis on the molecular size of native proteins
Hidenobu Kawai[1], Daisuke Takahashi[2], Munehito Arai[1,2,3] ([1]*Dept. Integ Sci. Univ. Tokyo*, [2]*Dept. Life Sci., Univ. Tokyo*, [3]*PRESTO,JST*)

2P051 **アクチンフィラメントの引張，ねじり，曲げ挙動の定量評価：粗視化分子動力学法による検討**
Quantifying how actin filament is stretched, twisted and bent: A coarse grained molecular dynamics simulation study
Shinji Matsushita, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

2P052 **Calculation methods for configurational entropy from molecular dynamics simulations**
Simon Hikiri, Takashi Yoshidome, Mitsunori Ikeguchi (*Grad. Sc. of Med. Life Sci., Yokohama City Univ.*)

2P053 **ヒトカルシトニンのアミロイド様線維形成機構とその阻害効果の解析**
Analysis of amyloid fibrillation mechanism and its inhibition effects of hCT
Hikari Watanabe(Itoh)[1], Ken Takeuchi[1], Javkhlantugs Namsrai[1], Kengo Daidoji[1], Izuru Kawamura[1], Kazuyoshi Ueda[1], Hiroshi Hirota[2], Tsutomu Nakayama[3], Akira Naito[1] ([1]*Grad. Sch. Eng, Yokohama Natl. Univ.*, [2]*Wako Inst., Riken*, [3]*Faculty of Applied Life Sci., Nippon Veterinary and Life Sci. Univ.*)

2P054 **MSES 法によるリガンド結合過程の全原子構造解析**
Ligand binding process at atomistic resolution revealed by multiscale enhanced sampling
Kei Moritsugu[1], Tohru Terada[2], Akinori Kidera[1] ([1]*Grad. Sch. of Med. Life Sci., Yokohama City University*, [2]*Grad. Sch. of Agri. and Life Sci., University of Tokyo*)

2P055 **抗体を用いた抗原蛋白質の揺らぎの検出**
Detection of conformational dynamics of protein antigen by antibody
Shohey Shimizu[1], Yoshito Abe[2], Yuji O. Kamatari[3], Tadashi Ueda[2], Takachika Azuma[4], Masayuki Oda[1] ([1]*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Grad. Sch. Pharm. Sci., Kyushu Univ.*, [3]*Life Sci. Res. Ctr, Gifu Univ.*, [4]*Res. Inst. Biol. Sci., Tokyo Univ. Sci.*)

2P056 **天然変性タンパク質 HIV-1 Tat と転写コアクチベータ CBP の KIX ドメインとの相互作用**
Interaction of the intrinsically disordered HIV-1 Tat protein with the KIX domain of the transcriptional coactivator CBP
Tomoko Kunihara[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Department of Life Sciences, The University of Tokyo*, [2]*PRESTO, JST*)

2P057 **ニワトリオボムコイドにおける変性中間状態の構造と熱力学**
Structure and thermodynamics of the unfolding intermediate of hen egg ovomucoid
Akihiro Maeno[1,2], Hiroshi Matsuo[3], Sumiko Odani[4], Kazuyuki Akasaka[1] ([1]*High Pressure Protein Res. Center, Kinki Univ.*, [2]*Sch. Med., Wakayama Med. Univ.*, [3]*NICO*, [4]*Grad. Human Life Sci., Jumonji Univ.*)

2P058 **Variable temperature and pressure NMR studies on flexible conformation of c-Myb DNA-binding domain**
Satomi Inaba[1], Akihiro Maeno[2,3], Kazumasa Sakurai[3], Kazuyuki Akasaka[3], **Masayuki Oda**[1] ([1]*Grad. Sch. Life Environ. Sci., Kyoto Pref. Univ.*, [2]*Sch. Med., Wakayama Med. Univ.*, [3]*High-Pressure Protein Res. Center, Kinki Univ.*)

2P059 **Effects of chemical structure of hydrophilic tertaammonium-type ionic liquids for stability of higher-order structure of proteins**
Shigeaki Abe[1], Atsushi Hyono[1], Nobuhiro Kaneko[2], Kotaro Kaneko[2], Koji Kawai[2], Yasuhiro Yoshida[1] ([1]*Hokkaido University*, [2]*Miyoshi Oil & Fat Co., Ltd.*)

2P060 **ジスルフィド結合のシャッフリングを標的とした異常なタンパク質オリゴマー化の抑制手法**
Disulfide shuffling in Cu,Zn-superoxide dismutase is a key to develop potential drugs for neurodegeneration
Itsuki Anzai[1], Keisuke Toichi[1], Atsushi Mukaiyama[2,3], Shuji Akiyama[2,3], Yoshiaki Furukawa[1] ([1]*Dept. of Chem., Keio Univ.*, [2]*Research Center of Integrative Molecular System (CIMoS), Institute for Molecular Science*, [3]*The Graduate University for Advanced Studies (SOKENDAI)*)

2P061 **天然変性蛋白質のリン酸化に共通して見られる分子内静電相互作用の特徴**
Common intra-molecular electrostatic property of intrinsically disordered proteins for phosphorylation
Koji Umezawa[1], Jun Ohnuki[1], Yukinobu Mizuhara[1], Junichi Higo[2], Mitsunori Takano[1] ([1]*Dept. of Pure & Appl. Phys., Waseda Univ.*, [2]*IPR, Osaka Univ.*)

2P062 **金ナノ粒子の界面におけるアミロイド線維形成機構に関する研究**
Study on the mechanisms of amyloid fibrillation at the interface of gold nanoparticles
Hiroya Muta[1], Young-Ho Lee[1], Masatomo So[1], Akira Saito[2], Kazumitsu Naoe[2], Yuji Goto[1] ([1]*Inst. for Pro. Research, Osaka Univ.*, [2]*Nara National Collage of Technology*)

2P063 **Contact number diffusion model for the normal mode analysis of protein structure**
Bhaskar Dasgupta, Kota Kasahara, Narutoshi Kamiya, Haruki Nakamura, Akira Kinjo (*IPR, Osaka University*)

2P064 **NMR を用いた動的構造解析により明らかとなったヒト主要組織適合複合体のペプチド認識、及び構造維持機構**
The Dynamic stabilization and peptide recognition mechanism of Human Leukocyte Antigen revealed by NMR relaxation dispersion analysis
Saeko Yanaka[1,2], Kenji Sugase[1], Takamasa Ueno[4], Kouhei Tsumoto[2,3] ([1]*Sunbor*, [2]*Grad. School of Frontier Sciences, Univ. of Tokyo*, [3]*Grad. School of Engeneering, Univ. of Tokyo*, [4]*Center for AIDS Research*)

2P065 **Helix-turn-helix モチーフを有する蛋白質の pH 変化および DNA 結合に伴う動的構造変化の解明**
Effects of pH and DNA-binding on conformational dynamics of protein with helix-turn-helix motif
Satomi Inaba[1], Hiroshi Sekiguchi[2], Yuji C. Sasaki[3], Masayuki Oda[1] ([1]*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*JASRI/SPring-8*, [3]*Grad. Sch. Fron. and Sci., Univ. Tokyo*)

2P066 **一分子蛍光分光法による変性剤濃度ジャンプ後のユビキチンの折り畳みダイナミクスの測定**
Folding dynamics of ubiquitin after rapid mixing detected by single molecule fluorescence spectroscopy
Masataka Saito[1], Eric Chen[2], Po-Ting Chen[2], Rita P.-Y. Chen[2], Kiyoto Kamagata[1], Hiroyuki Oikawa[1], Satoshi Takahashi[1] ([1]*Institute of Multidisciplinary Research for Advanced Materials Tohoku University*, [2]*Academia Sinica*)

2P067 **タバコモザイクウイルス外被蛋白質会合体の安定性**
Stability of the Tobacco Mosaic Virus Coat Protein Assemblage
Hiroaki Fukao[1], Kazumasa Sakurai[2], Yasushige Yonezawa[2], Masao Fujisawa[1], Kazuhiro Ishibashi[3], Masayuki Ishikawa[3], Tetsuo Meshi[3], Hideki Tachibana[1,2] ([1]*Fac Biol-Ortd Sci Tech, Kinki Univ*, [2]*High-Pres Prot Res Center, Kinki Univ*, [3]*Div Plant Sci, NIAS*)

01D. 蛋白質：機能 / 01D. Protein: Function

2P068 **計算化学的手法によるアデニル酸キナーゼの反応機構の研究**
Computational Study on the reaction mechanism of adenylate kinase
Kenshu Kamiya (*Dept. Phys., Sch. Sci., Kitasato Univ.*)

2P069 **局所パッキングパターンによる GroEL 基質蛋白質の構造的特徴の記述**
Discrimination of GroEL substrate proteins using a small set of packing-patterns
Shintaro Minami[1], Tatsuya Niwa[2], Hideki Taguchi[2], Motonori Ota[1] ([1]*Grad. Sch. of Inf. Sci., Nagoya Univ.*, [2]*Grad. Sch. of Biosci. and Biotech., Tokyo Inst. of Tech.*)

2P070 **天然変性タンパク質としてのグループ３ LEA モデルペプチドの役割 -乾燥に伴うリポソームの融合を防止する効果-**
A role of group-3 LEA model peptides as IDPs. Protective effects on desiccation-induced liposome fusion
Takao Furuki, Minoru Sakurai (*Tokyo Institute of Technology*)

2P071 **An activation mechanism of human Cu,Zn-superoxide dismutase by its copper chaperone, CCS**
Carolyn T. Lim, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

2P072 **変異体を用いた分子動力学シミュレーションによる電位依存性カリウムチャネルのイオン透過機構の解析**
Analysis of the ion permeation mechanism of Kv1.2 using the molecular dynamics simulations of a single point mutant
Hiroko X. Kondo[1], Matsuyuki Shirota[1,2,3], Kota Kasahara[4], Toshiyuki Saito[1], Kengo Kinoshita[1,3,5] ([1]*GSIS, Tohoku Univ*, [2]*Grad Sch Med, Tohoku Univ*, [3]*ToMMo, Tohoku Univ*, [4]*IPR, Osaka Univ*, [5]*IDAC, Tohoku Univ*)

2P073 **コラゲナーゼによるコラーゲン原繊維分解過程の高速 AFM 観察**
High speed AFM observation for degradation process of collagen fibril by collagenase
Hayato Yamashita[1], Naoko Teramura[2], Keisuke Tanaka[2], Shunji Hattori[2], Katsumasa Iijima[2], Osamu Hayashida[2], Teru Okitsu[3], Yoshiro Sohma[1] ([1]*Pharmacol., Keio Univ. Med. Sch.*, [2]*Res. inst. Biomatrix, Nippi Inc.*, [3]*Inst. Indus. Sci., Univ. Tokyo*)

2P074 **タンパク質ジスルフィドの異常が神経変性疾患の発症に果たす役割：線虫を用いた表現型解析**
Investigating the Role of SOD1 Cysteine Residues in Neurodegeneration using C. elegans
Mariko Ogawa[1], Hisashi Shidara[2], Kotaro Oka[2], Yoshiaki Furukawa[1] ([1]*Lab. for Mechanistic Chem. of Biomolecules, Dept. of Chem., Keio Univ.*, [2]*Lab. for Biophysics and Neuroinformatics, Dept. of Biosciences and Informatics, Keio Univ.*)

2P075 **青色光センサータンパク質フォトトロピン１ LOV2 ドメインの光反応に対するクラウディング効果**
Crowding effect on the reaction dynamics of blue light sensor protein phototropin1 LOV2 domain
Tomoyuki Yoshitake[1], Yusuke Nakasone[1], Tsuguyoshi Toyooka[1], Kazunori Zikihara[2], Satoru Tokutomi[2], Masahide Terazima[1] ([1]*Kyoto Univ.*, [2]*Osaka Prefecture Univ.*)

2P076 **FTIR 測定でみたエクスパンシン活性**
Exapnsin activity observed by FTIR
Tomoya Imai[1], Masato Naruse[1], Yoshiki Horikawa[1], Katsuro Yaoi[2], Kentaro Miyazaki[2], Junji Sugiyama[1] ([1]*RISH, Kyoto Univeristy*, [2]*AIST*)

2P077 **T-, L-プラスチン EF-hand の Ca2+感受性の違い**
Different Ca2+-sensitivities between the EF-hands of T- and L-plastins
Takuya Miyakawa[1], Hiroto Shinomiya[2], Fumiaki Yumoto[1], **Yusuke Kato**[1,3], Masaru Tanokura[1] ([1]*Department of Applied Biological Chemistry, The University of Tokyo,*, [2]*Ehime Prefectural Institute of Public Health and Environmental Science*, [3]*Institute for Health Sciences, Tokushima Bunri University*)

2P078 **新規金表面親和性ペプチドの同定及びタンパク質固定化への応用**
Screening of a novel gold affinity peptide and its application on protein immobilization
Yojiro Shigemori[1], Kaori Yoshida[2], Koreyoshi Imamura[1], Yuichiro Takahashi[2], Hiroyuki Imanaka[1] ([1]*Div. of Chem. and Biotech., Grad. Sch. of Nat. Sci. & Tech., Okayama Univ.*, [2]*Div. of Biological Sci., Grad. Sch. of Nat. Sci. & Tech., Okayama Univ.*)

01E. 蛋白質：計測・解析の方法論 / 01E. Protein: Measurement & Analysis

2P079 **MMV-4SR:シングルセル生物学(SCB)基盤検出系の開発**
MMV-4SR : Development of basic detection system in Single Cell Biology (SCB)
Naoki Takeuchi[1], Tommy Nagano[2], Koichi Nishigaki[1] ([1]*Grad. Sch. of Sci. and Eng., Saitama Univ.*, [2]*DRC Co., Ltd.*)

2P080 **表面増強赤外分光法によるモデル脂質膜上におけるタンパク質フォールディングの動的挙動の解析**
Surface Enhanced IR study of Protein folding dynamics at a solid support lipid layer
Kenichi Ataka, Joachim Heberle (*Freie Universitaet Berlin, Fachbereich Physik, Experimental Molecular Biophysics*)

2P081 **Microtubule-kinesin binding assay to differentiate wild and mutant 4R tau proteins**
Subhathirai Subramaniyan Parimalam[1], Tarhan Mehmet Cagatay[2], Stan Karsten[3], Hiroyuki Fujita[2], Hirofumi Shintaku[1], Hidetoshi Kotera[1], Ryuji Yokokawa[1] ([1]*Kyoto University*, [2]*LIMMS, Institute of Industrial Science, The University of Tokyo, Japan*, [3]*NeuroInDx Inc., Signal Hill, CA, USA*)

2P082 **高速 AFM による Ascaris 精子由来の MSP 繊維の観察**
Direct observation of MSP filaments in cell-free extract from Ascaris sperm by Atomic Force Microscopy
Yutaro Yamada[1], Takamitsu Haruyama[2], Ryoko Chijimatsu[1], Hiroki Konno[2], Katsuya Shimabukuro[1] ([1]*Ube Nat. Col. Tech.*, [2]*Bio-AFM, Kanazawa Univ*)

2P083 **固体 NMR を用いた単一細胞あたりの特定タンパク質の分子数計測**
Counting of the target recombinant protein molecule in an intact Escherichia coli cell by solid-state NMR
Kazuya Yamada, Ayako Egawa, Toshimichi Fujiwara (*IPR, Osaka Univ.*)

2P084 **リポソーム内での再構成リボソームの翻訳活性**
Translation activity of a reconstructed ribosome in liposomes
Hiroki Nakanishi (*Grad. Sch. Imf., Univ. Osaka*)

01F. 蛋白質：蛋白質工学／進化工学 / 01F. Protein: Engineering

2P085 **CS 複合体形成をモデルとした高感度ペプチド―タンパク質間相互作用検出系のデザイン**
Design of highly sensitive peptide-protein interaction detection system adopting CS complex formation as the model
Runa Matsushita, Naoyuki Ishida, Koreyoshi Imamura, Hiroyuki Imanaka (*Graduate School of Natural Science and Technology, Okayama University*)

2P086 **無機基板表面を標的としたラクダ抗体から着想するスマートなバイオセンサー仕様抗体の設計**
Smart interface antibody design for biosensor
Takuma Sujino[1], Hikaru Nakazawa[1], Keiko Tawa[2], Ryutaro Asano[1], Izumi Kumagai[1], Mitsuo Umetsu[1] ([1]*Dept. Biomol. Eng., Grad. Sch. Eng., Tohoku Univ.*, [2]*HRI, AIST.*)

2P087 **タンパク質デザインの新たな方法の開発: 蛍光強度の違いを利用してファージを選別する**
Development of a new strategy of protein design: the single phage sorting based on fluorescence intensity
Rie Kiriguchi[1,2], Toshihiko Kubota[1,2], Norihisa Takahashi[1,3], Seiji Sakamoto[1,3], Hiroyuki Oikawa[1], Kiyoto Kamagata[1,2,3], Takehiko Wada[1,3], Satoshi Takahashi[1,2,3] ([1]*IMRAM., Univ Tohoku.*, [2]*Grad. Sch. Life Sci., Univ Tohoku.*, [3]*Grad, Sch. of Sci., Univ Tohoku.*)

2P088 **α/β フォールドをもつ新規ヘム蛋白質の設計と合成**
Design and syntheses of de novo heme proteins with an α/β fold
Yasuhiro Isogai[1], Kiyotaka Yamamoto[1], Hiroshi Imamura[2] ([1]*Dept. Biotech., Toyama Pref. Univ.*, [2]*Biomedical Res. Inst., AIST*)

2P089 **膜たんぱく質の進化工学手法、リポソームディスプレイ法による α-ヘモリシンの in vitro 分子進化**
Directed evolution of membrane protein, alpha hemolysin, by development of liposome display method
Satoshi Fujii[1], Tomoaki Matsuura[1,2], Takeshi Sunami[1,3], Yasuaki Kazuta[1], Tetsuya Yomo[1,3,4] ([1]*JST*, [2]*Grad. Eng. Univ. Osaka*, [3]*Grad. Bioinfo. Univ. Osaka*, [4]*Grad. Fron. BioSci. Univ. Osaka*)

2P090 **リポソームディスプレイ法によるメリチンの人工進化**
In vitro evolution of Melittin using a liposome display
Taiga Izumi[1], Takeshi Sunami[1,2], Tetsuya Yomo[1,2,3] ([1]*Grad. Sch. Info., Osaka Univ.*, [2]*ERATO,JST*, [3]*Grad. Sch. Bio., Osaka Univ.*)

02. ヘム蛋白質 / 02. Heme proteins

2P091 **チトクローム酸化酵素の反応初期過程における共役機構の解明**
Elucidation of the coupling mechanism in the initial stage of the reaction of cytochrome c oxidase
Satoru Nakashima, Minoru Kubo, Izumi Ishigami, Kyoko Itoh-Shinzawa, Shinya Yoshikawa, Takashi Ogura (*Grad. Sch. Sci., Univ. Hyogo*)

2P092 **過渡吸収分光法による一酸化窒素還元酵素の単寿命 NO 結合体の解析**
Characterization of transient NO-bound form of Nitric Oxide Reductase by Transient Absorption Spectroscopy
Tetsunari Kimura[1], Shoko Ishii[1,2], Takehiko Tosha[1], Yoshitsugu Shiro[1,2], Minoru Kubo[1,3] ([1]*RIKEN, SPring-8*, [2]*Grad. Sch. Life Sci., Univ. Hyogo*, [3]*JST, PRESTO*)

2P093 **チトクロム c 酸化酵素の酸素還元反応の時間分解赤外吸収測定を目的とした酸素肺フローシステムの開発**
Development of sample flow system with an oxygen lung for time-resolved infrared measurements of cytochrome c oxidase
Tatsuhito Nishiguchi[1], Masahide Hikita[1], Kyoko Shinzawa-Itoh[1], Shinya Yoshikawa[2], Satoru Nakashima[2], Takashi Ogura[1,2] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*Picobiology Ins., Univ. Hyogo*)

2P094 **タンパク質を基盤とした酸素濃度 FRET センサー**
Protein-based FRET sensor for oxygen concentration
Haruto Ishikawa[1], Shigetoshi Aono[2], Yasuhisa Mizutani[1] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*Okazaki Inst.*)

2P095 **細胞内の遊離ヘムの可視化に向けた融合タンパク質の開発**
Development of the FRET-based sensor protein for visualization of free heme *in vivo*
Kazuyuki Matsumoto[1], Haruto Ishikawa[1], Shigetoshi Aono[2], Yasuhisa Mizutani[1,2] ([1]*Grad.Sch.Sci.,Univ. Osaka*, [2]*Okazaki Inst.*)

2P096 **紫外共鳴ラマン分光法によるインドールアミン 2,3-ジオキシゲナーゼの三者複合体中間体モデルの研究**
Ultraviolet resonance Raman study on a ternary complex intermediate model of indoleamine 2,3-dioxygenase
Kure'e Kayama[1], Sachiko Yanagisawa[1], Hiroshi Sugimoto[2], Yoshitsugu Shiro[2], Takashi Ogura[1] ([1]*Univ. of Hyogo*, [2]*RIKEN SPring-8 center*)

2P097 **紫外共鳴ラマン分光法による酵素に結合した基質の構造解析**
Structural Analysis of the Substrate Bound to Enzyme by UV Resonance Raman Spectroscopy
Sachiko Yanagisawa[1], Masayuki Hara[1], Hiroshi Sugimoto[2], Yoshitsugu Shiro[2] ([1]*Univ. of Hyogo*, [2]*RIKEN SPring-8 center*)

03. 膜蛋白質 / 03. Membrane proteins

2P098 **マイクロ流路デバイスを用いた生体膜実時間解析システムの開発**
Development of a novel system for the real-time analysis of biological membranes by using a microfluidic device
Yuji Kimura, Sayaka Kazami, Yu Hashimoto, Hiroyasu Itoh (*Tsukuba Research Laboratory, Hamamatsu Photonics KK*)

2P099 **ブタ心臓由来ミトコンドリア調製法の確立と活性測定**
Establishment of a new process for preparation of porcine heart mitochondria, and their activity measurements
Sayaka Kazami, Yuji Kimura, Hiroyasu Itoh (*Tsukuba Research Laboratory, Hamamatsu Photonics K.K.*)

2P100 **PiericidinA によるウシ心筋 NADH-ユビキノン還元酵素の活性中心の定量**
Quantification of the active center of bovine heart NADH-ubiquinone reductase with Piericidin A
Shigefumi Uene[1], Satoru Shimada[1], Kyoko Shinzawa-Itoh[1], Tomitake Tsukihara[1,2], Shinya Yoshikawa[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*Inst. Protein Res., Osaka Univ.*)

2P101 **低分子量 G タンパク質 K-Ras のフォトクロミック分子を用いた光制御**
Photo-regulation of small G protein K-Ras using photochromic molecules
Seigo Iwata[1], Kaori Masuhara[2], Nobuhisa Umeki[3], Kazunori Kondo[2], Shinsaku Maruta[1,2] ([1]*Div. Bioinfo., Grad. Sch. Eng., SOKA Univ.*, [2]*Dept. BioInfo., Fac. Eng., SOKA Univ.*, [3]*Wako Inst., Riken*)

2P102 **ヒト癌抑制遺伝子候補 *101F6* によるカスパーゼ非依存性細胞死の機構分析**
Analyses of caspase-independent apoptosis caused by the expression of a candidate human tumor suppression gene, *101F6*
Takako Yamaoze[1], Hiroaki Okano[1], Akikazu Asada[1], Kazuo Kobayashi[2], Takahiro Kozawa[2], Motonari Tsubaki[1] ([1]*Dept. of Chemistry, Grad. Sch. Sci., Kobe Univ.*, [2]*ISIR, Osaka Univ.*)

2P103 **哺乳類ミトコンドリア呼吸鎖超複合体の精製**
Purification of the respiratory super complex from mammalian mitochondria
Kyoko Shinzawa-Itoh[1], Satoru Shimada[1,2], Ryoko Takahashi[1], Shigefumi Uene[1], Harunobu Shimomura[1], Shinya Yoshikawa[1], Tomitake Tsukihara[1,2] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*CREST, JST*)

2P104 **定常状態での GPCR シグナルは GPCR ダイマーによって誘起される**
Dimers are the key trigger for the GPCR's basic signaling without ligation
Rinshi Kasai, Akihiro Kusumi (*Inst. Front. Med. Sci., WPI-iCeMS, Kyoto Univ.*)

2P105 **1 分子イメージングによる代謝型グルタミン酸受容体の高次多量体形成と内在化の解析**
Single-molecule imaging analysis of higher-order oligomerization and internalization of metabotropic glutamate receptor
Masataka Yanagawa[1], Michio Hiroshima[1,2], Takahiro Yamashita[3], Yoshinori Shichida[3], Yasushi Sako[1] ([1]*Cell. Info. Lab., Riken*, [2]*QBiC, Riken*, [3]*Grad. Sch. Sci., Kyoto Univ.*)

2P106 **AFM Probing Opioid Signalosome on Neuroblastoma**
Lara Gay Villaruz[1], Catherine Tardin[2], Daisuke Mizuno[1] ([1]*Kyushu University*, [2]*IPBS/CNRS University of Toulouse*)

2P107 **プロトン駆動力を細胞外へと捨てる微生物外膜タンパク質**
Proton discarded to cell exterior via outer-membrane bound enzyme
Akihiro Okamoto, Shafeer Kalathil, Yoshihide Tokunou, Kazuhito Hashimoto (*Grad. Sch. Eng., Univ. of Tokyo*)

2P108 **ペリプラズム pH 追跡による細胞外電子移動酵素のプロトン移動能検討**
***In-vivo* periplasmic pH assay for studying proton export by outer membrane cytochromes in extracellular electron transport**
Yoshihide Tokunou[1], Akihiro Okamoto[2], Kazuhito Hashimoto[2] ([1]*Department of applied chemistry, Univ. Tokyo*, [2]*Department of applied chemistry, Univ. Tokyo*)

2P109 **サイズの異なるナノポアの脂質二分子膜への再構成**
Reconstitution of various-sized nanopores in lipid bilayer
Hirokazu Watanabe, Ryuji Kawano (*TUAT*)

2P110 **多剤輸送担体 EmrE の pH 依存性基質結合駆動力に対する酸性残基の役割**
Role of acidic residues of multidrug resistance transporter, EmrE for the pH dependent driving force of substrate binding
Kazumi Shimono[1,2], Toshifumi Nara[3], Tomomi Someya[2], Mikako Shirouzu[2], Shigeyuki Yokoyama[4], Seiji Miyauchi[1] ([1]*Fac. Pharm. Sci., Toho Univ.*, [2]*CLST, RIKEN*, [3]*Coll. Pharm. Sci., Matsuyama Univ.*, [4]*Struct. Biol. Lab., RIKEN*)

2P111 **Fast measurements of membrane transporter activity with attoliter-sized arrayed lipid bilayer chamber system**
Naoki Soga[1], Rikiya Watanabe[1,2], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*PRESTO, JST*)

2P112 **新世界ザルの苦味受容体 TAS2R1 および TAS2R4 のリガンド感受性の種間差と進化**
Interspecific variation of ligand sensitivity and evolution of bitter taste receptors TAS2R1 and TAS2R4 in New World monkeys
Kei Tsutsui[1], Masahiro Otoh[2], Kodama Sakurai[2], Nami Suzuki-Hashido[1], Takashi Hayakawa[1], Barbara J. Welker[3], Filippo Aureli[4,5], Colleen M. Schaffner[5], Linda M. Fedigan[6], Shoji Kawamura[2], Hiroo Imai[1] ([1]*Primate Res. Inst., Kyoto Uni.*, [2]*Dept. Integ. Biosci., Univ. Tokyo*, [3]*Dept. Anthropol., State Univ. New York Geneseo*, [4]*Res. Cent. Evol. Anthropol. Palaeoecol., Liverpool John Moores Univ.*, [5]*Inst. Neuroetol., Univ. Veracruzana*, [6]*Dept. Anthropol., Univ. Calgary*)

2P113 **新規 *Halorubrum* 属菌のもつバクテリオロドプシン類タンパク質の研究**
Study on the microbial rhodopsins from the cell membrane of *Halorubrum* sp.ejinoor
Chao Luomeng[1], Gang Dai[2], Takashi Kikukawa[3], Kunio Ihara[4], Tatsuo Iwasa[1] ([1]*Div. Eng.Composite Funct., Muroran Ins. Technol., Japan*, [2]*Coll. Chem. Environ. Sci., Inner Mongolia Normal Univ., China*, [3]*Grad. Sch. Life. Sci., Hokkaido Univ., Japan*, [4]*CGR, Nagoya Univ., Japan*)

2P114 **NpHR の三量体安定化に寄与するアミノ酸残基の特定**
Specification of amino acid residues which stabilize trimer formation of halorhodopsin
Kentaro Saito[1], Noritaka Kato[1], Takashi Kikukawa[2], Makoto Demura[2], Takanori Sasaki[1] ([1]*Sch. Sci. and Tech., Meiji Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*)

04. 核酸結合蛋白質 / 04. Nucleic acid binding proteins

2P115 **蛍光相互相関分光法を用いたグルココルチコイドレセプターと DNA 間相互作用の定量化**
Determination of the quantitative interaction between glucocorticoid receptor and DNA by fluorescence cross-correlation spectroscopy
Mari Saito[1], Shintaro Mikuni[2], Masataka Kinjo[2] ([1]*Grad. Sch. Life Sci., Univ. Hokkaido*, [2]*Grad. Sch. Advanced Life Sci., Univ. Hokkaido*)

2P116 **一分子蛍光観測法による癌抑制蛋白質 p53 の DNA 探索機構の研究**
Investigation of DNA search mechanism of tumor suppressor p53
Agato Murata[1,2], Yuji Itoh[1,2], Dwiky Rendra Graha Subekti[3], Chihiro Igarashi[1,2], Satoshi Takahashi[1], Kiyoto Kamagata[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad., Sch., Sci., Tohoku Univ.*, [3]*AMC, Fac., Sci., Tohoku Univ.*)

2P117 **二種の好熱菌由来ヌクレオチドキナーゼと変異体における高次構造変化とヌクレオチド結合の蛍光分光学的解析**
Fluorescence spectroscopic studies on conformational changes and Nucleotide binding of thermophilic nucleotide kinases and their variants
Yuto Oe[1], Momoko Abe[1], Shota Inoue[1], Shota Takahashi[1], Misaki Nakayama[1], Yurie Ohiwa[2], **Takanori Satoh**[3] ([1]*Biochem. Lab., Fac. of IAS, Tokushima Univ.*, [2]*Biochem. Lab., Grad. Sch. of SAS, Tokushima Univ.*, [3]*Biochem. Lab., Inst. of SAS, Tokushima Univ.*)

2P118 **哺乳類ヌクレオチド除去修復タンパク質 XPC の DNA 結合モードの 1 分子イメージング**
Single-molecule direct visualization of DNA binding modes of the mammalian nucleotide excision repair protein XPC
Hiroaki Yokota[1], Daisuke Tone[2], Yong-Woon Han[3], Yoshie Harada[3], Kaoru Sugasawa[2,4] ([1]*BioPhotonics Lab, GPI*, [2]*Dept. Biol., Grad. Sch. Sci., Kobe Univ.*, [3]*iCeMS, Kyoto Univ.*, [4]*Biosig. Res. Center, Kobe Univ.*)

05A. 核酸：構造・物性 / 05A. Nucleic acid: Structure & Property

2P119 **15N NMR 分光法を用いた C-Ag(I)-C 塩基対の構造解析**
Nitrogen-15 NMR spectroscopic studies of Ag (I)-mediated C-C base-pairs
Takenori Dairaku[1], Kyoko Furuita[2], Itaru Okamoto[3], Shuji Oda[1], Daichi Yamanaka[1], Yoshinori Kondo[1], Akira Ono[3], Chojiro Kojima[2], Vladimir Sychrovsky[4], Yoshiyuki Tanaka[1] ([1]*Graduate School of Pharmaceutical Sciences, Tohoku University*, [2]*Institute for Protein Research, Osaka University*, [3]*Faculty of Engineering, Kanagawa University*, [4]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic*)

2P120 **ヌクレオソーム３量体の構造ダイナミクスの粗視化分子動力学法と X 線小角散乱による研究**
Structural dynamics of tri-nucleosome studied by combination of coarse grained molecular simulation and SAXS
Yusuke Takagi[1], Yuichi Kokabu[2], Takashi Oda[2], Hiroaki Tachiwana[3], Hiroo Kenzaki[4], Hitoshi Kurumizaka[3], Mamoru Sato[2], Mitsunori Ikeguchi[2], Shoji Takada[1] ([1]*Dept. Biophys., Grad. Sch. Sci., Kyoto Univ*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ*, [3]*Faculty of Science and Engineering, Waseda Univ*, [4]*ACCC, RIKEN*)

2P121 **密度汎関数法による DNA 塩基対とラジカルの反応機構の解析**
DFT calculations on attacking mechanism of radicals to DNA base pair
Naoko Okutsu, Hideaki Tamai, Eisuke Shimizu, Noriyuki Kurita (*Toyohashi University of Technology*)

05B. 核酸：相互作用・複合体 / 05B. Nucleic acid: Interaction & Complex formation

2P122 **アルコールによって誘起される DNA の凝縮・脱凝縮二段階転移**
Condensed DNA is unfolded into elongated conformation at ethanol concentration around 80%
Yuki Oda[1], Yuko Yoshikawa[2], Tadayuki Imanaka[2], Kenichi Yoshikawa[1] ([1]*Graduate School of Life and Medical Sciences, Doshisha University*, [2]*Department of Biotechnology, College of Life Sciences, Ritsumeikan University*)

2P123 **Effect of branched polyamine from hyperthermophile on the structure of genomic DNA**
Akira Muramatsu[1], Yuko Yoshikawa[2], Naoki Umezawa[3], Shinsuke Fujiwara[4], Toshio Kanbe[5], Wakao Fukuda[2], Tadayuki Imanaka[2], Kenichi Yoshikawa[1] ([1]*Doshisha Univ.*, [2]*Ritsumeikan Univ.*, [3]*Nagoya City Univ.*, [4]*Kwansei Gakuin Univ.*, [5]*Nagoya univ.*)

2P124 **粗視化シミュレーションによる多ヌクレオソーム系の構造サンプリング**
Structural sampling of polynucleosome by coarse-grained simulations
Hiroo Kenzaki[1], Shoji Takada[2] ([1]*Advanced Center for Computer and Communications, RIKEN*, [2]*Grad. Sch. of Sci., Kyoto Univ.*)

2P125 **エステル基を有するテトラゾラト架橋白金(II)二核錯体による DNA の高次構造変化**
Action of tetrazolato-bridged dinuclear platinum(II) complexes with ester moiety on the higher order structure of DNA
Yuta Shimizu[1], Akira Muramatsu[1], Yuko Yoshikawa[2], Takahiro Tsuchiya[3], Hiroki Yoneyama[4], Shinya Harusawa[4], Seiji Komeda[3], Tadayuki Imanaka[2], Kenichi Yoshikawa[1] ([1]*Doshisha University*, [2]*Ritsumeikan University*, [3]*Suzuka University of Medical Science*, [4]*Osaka University of Pharmaceutical Sciences*)

2P126 **分子動力学計算を用いた蛋白質･RNA 複合体立体構造予測**
Three dimensional structure prediction of RNA-protein complexes by MD simulation
Kei Yura[1], Junichi Iwakiri[2], Michiaki Hamada[3], Kiyoshi Asai[2,4], **Tomoshi Kameda**[4] ([1]*Grad. School of Humanities and Sciences, Univ. of Ochanomizu*, [2]*Grad. School of Frontier Sciences, Univ. of Tokyo*, [3]*Faculty of Science and Engineering, Waseda Univ.*, [4]*CBRC, AIST*)

2P127 **光制御型 bZip モジュール Photodimerizer の二量体化分子機構**
Molecular mechanism for dimerization of the light-regulated bZip module, Photodimeizer
Yoichi Nakatani, Osamu Hisatomi (*Grad. Sch. Sci., Osaka Univ.*)

06. 電子状態 / 06. Electronic state

2P128 **FMO/3D-RISM 法の開発と応用**
Development of FMO/3D-RISM method and its applications
Norio Yoshida (*Kyushu University*)

07. 水・水和／電解質 / 07. Water & Hydration & Electrolyte

2P129 **Generalized Born モデルによる蛋白質-蛋白質間相互作用計算の重要パラメータ**
Critical parameters of the generalized Born model to simulate protein-protein interactions
Yukinobu Mizuhara, Koji Umezawa, Jun Ohnuki, Dan Parkin, Mitsunori Takano (*Dept. of Pure & Appl. Phys., Waseda Univ.*)

2P130 **シトクロム c – シトクロム c 酸化酵素間における電子伝達複合体形成機構の浸透圧を用いた解析**
Analysis of interactions in the electron transfer complex between Cytochrome c and Cytochrome c Oxidase using osmotic pressure
Wataru Sato[1], Mizue Imai[1], Takeshi Uchida[2], Kyoko Ito[3], Shinya Yoshikawa[3], Koichiro Ishimori[2] ([1]*Grad. Sch. of Chem. Sci. and Eng., Hokkaido Univ.*, [2]*Fac. of Sci., Hokkaido Univ.*, [3]*Grad. Sch. of Life Sci., Hyogo Pref. Univ.*)

2P131 **The solvent-accessible surface area of proteins is a key factor for hydration structure and dynamics in crowded environment**
Po-hung Wang[1], Isseki Yu[1], Michael Feig[2], Yuji Sugita[1,3,4] ([1]*RIKEN Theo. Mol. Sci. Lab.*, [2]*Dept. Biochem. & Mol. Biol. and Dept. Chem. MSU, USA*, [3]*RIKEN Adv. Int. Comput. Sci.*, [4]*RIKEN Quant. Biol. Center*)

2P132 **混合分布モデルにより分離されたタンパク質水和水の振る舞い：シミュレーション・データマイニングによるアプローチ**
Hydration water behavior classified by mixture model : Simulation data-mining approach
Taku Mizukami[1], Hieu Chi Dam[2], Tu Bao Ho[2], Viet Cuong Nguen[3] ([1]*JAIST, Materials Science*, [2]*JAIST, Knowledge Science*, [3]*HPC Systems, Inc*)

08. 分子遺伝・遺伝情報制御 / 08. Molecular genetics & Gene expression

2P133 **マイクロドロップアレイを用いた一分子 DNA からの無細胞タンパク質合成**
Cell free protein synthesis from single DNA in microdrop array
Hiroto Kizoe, Yi Zhang, Kazuhito Tabata, Hiroyuki Noji (*Grad. Sch. apply chem, Univ. Tokyo*)

2P134 **転写制御ダイナミクスの in vitro 1 分子計測**
In vitro single molecule assay for the dynamics of transcriptional regulation
Keisuke Fujita[1], Mitsuhiro Iwaki[1,2], Lorenzo Marcucci[1], Rika Kawaguchi[1], Toshio Yanagida[1,2] ([1]*QBiC, Riken*, [2]*Grad. Sch. of Front. Biosci., Osaka Univ.*)

09. 発生・分化 / 09. Development & Differentiation

2P135 **アポトーシスに伴う上皮恒常性維持への機械的力の寄与**
Maintenance of tissue integrity by intrinsic and extrinsic forces during apoptosis in Drosophila epithelial tissue morphogenesis
Yusuke Toyama[1,2,3] ([1]*Mechanobiology Institute, National Univ. of Singapore*, [2]*Dep. Biological Sciences, National Univ. of Singapore*, [3]*Temasek Life Sciences Lab.*)

2P136 **枯葉にそっくりな蝶の翅の模様はどのように進化してきたのか？**
Gradual and contingent evolutionary emergence of leaf wing patterns
Takao K. Suzuki, Shuichiro Tomita, Hideki Sezutsu (*NIAS, Transgenic Silkmoth Unit*)

2P137 **多細胞の形態形成における力学機構を明らかにするための三次元バーテックスモデル**
3D vertex model for revealing mechanics in multicellular morphogenesis
Satoru Okuda, Mototsugu Eiraku, Yoshiki Sasai (*RIKEN Center for Developmental Biology*)

10. 筋肉 / 10. Muscle

2P138 **細胞性粘菌由来ミオシン II の SH1 ヘリックス上の変異が運動活性に与える影響**
Mutations in SH1 helix affect the motile activity of Dictyostelium myosin II
Kotomi Shibata[1], Tsubasa Koyama[1], Sosuke Iwai[2], Shigeru Chaen[1] ([1]*College of Humanities and Sciences, Nihon University*, [2]*Faculty of Education, Hirosaki University*)

2P139 **F-アクチンの水和状態のミオシン-サブフラグメント 1 密度依存性**
Bound-Myosin Density Dependence of Hydration State of Actin-Filament
Takahiro Watanabe, Noriyoshi Ishida, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad.Sch.Eng., Tohoku Univ.*)

2P140 **ATP 結合アナログを用いたミオシンサブフラグメント 1 の水和研究**
Hydration study of myosin subflagment1 with bound ATP analogs
Hideyuki Ohsugi, Takahiro Watanabe, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

2P141 **2, 3-ブタンジオン 2-モノキシムによるミオシン II ATP 加水分解のケミカルレスキュー**
Chemical rescue of myosin II ATP hydrolysis by 2, 3-butandione 2-monoxime
Hideyuki Komatsu, Yuji Koseki, Shunsuke Aoki (*Dept. of Bioscience & Bioinformatics, Kyushu Inst. Tech.*)

2P142 **F-アクチンの水和状態に及ぼす Mg2+と Ca2+の効果**
Effect of Mg2+ and Ca2+ ions on the hydration state of F-actin
Ryotaro Chishima, Asato Imao, Takahiro Watanabe, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

2P143 **Investigating conditions for structure analysis of binding states of formin/mDia1 to the actin filament by electron microscopy**
Mizuki Matsuzaki, Akihiro Narita (*Grad. Sch. Sci., Nagoya Univ.*)

2P144 **高温の心筋細胞内でみられる高速サルコメア振動**
High-frequency sarcomeric auto-oscillations in living cardiomyocytes under hyperthermal conditions
Seine Shintani[1], Kotaro Oyama[1], Norio Fukuda[2], Shin'ichi Ishiwata[1,3] ([1]*Pure and Applied Physics, Waseda Univ.*, [2]*Dept of Cell Phy, Jikei Univ of Medicine*, [3]*Waseda Bioscience Research Institute in Singapore*)

11. 分子モーター / 11. Molecular motor

2P145 **金ナノロッドを用いた運動中キネシン 1 のモータードメイン回転の観察**
Observation of the Rotational Motion of the Motor Domain during Processive Motility of Kinesin-1 using Gold Nanorod
Yamato Niitani[1], Sawako Enoki[2], Hiroyuki Noji[2], Ryota Iino[3], Michio Tomishige[1] ([1]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*, [2]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo*, [3]*Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences*)

2P146 **X 線 1 分子計測による分子モーターの構造揺らぎ測定**
X-ray Single Molecule Observations of Structural Fluctuations in Molecular Motors
Keigo Ikezaki[1], Naruki Hara[1], Hiroshi Sekiguchi[2], Naoto Yagi[2], Yuji Sasaki[1] ([1]*Grad. Sch. Sci., Univ. Tokyo*, [2]*SPring-8/JASRI*)

2P147 **アクチンフィラメントの違いによるミオシン IXb の運動性**

The motility of Myosin IXb depend on difference of actin filaments

Masafumi D. Yamada[1], Nobuhisa Umeki[1], Mitsuo Ikebe[2], Taro Q.P. Uyeda[1] ([1]*AIST, Biomedical Research Institute*, [2]*The Univ. of Texas Health Science center at Tyler, Dept. of Cellular and Mol. Biol.*)

2P148 **全原子 MD 計算による F_o のサブユニット間水分布と相対運動の観測**

Water distribution and relative motion between subunits of F_o observed by all-atom MD simulation

Ryoichi Kiyama, Asahi Konno, Mitsunori Takano (*Dept. of Pure & Appl. Phys., Waseda Univ.*)

2P149 **タンパク質間の実行相互作用：多価カチオンを介した引力パッチ間相互作用**

Protein-Protein Effective Interaction: Interaction between Attractive Patches Mediated by Multivalent Cations

Takuto Sawayama, Ryo Akiyama (*Kyushu Univ. sci.*)

2P150 **アクチンの PEG 化は運動速度よりも運動割合に影響を与える**

Pegylation of actin affects motile fraction rather than the velocity of actin filaments on myosin molecules

Kuniyuki Hatori, Hiroki Souma (*Dept. Bio-Systems, Grad. Sch. Sci. Eng., Yamagata Univ.*)

2P151 **高速 AFM により可視化された、コフィリンによるアクチンフィラメントの協同的な構造変化の一方向的な伝播**

Cofilin-induced unidirectional cooperative conformational changes of actin filaments visualized by high speed atomic force microscopy

Kien Ngo[1], Noriyuki Kodera[2,3], Eisaku Katayama[4], Akira Nagasaki[1], Toshio Ando[2,5,6], **Taro Uyeda**[1] ([1]*Biomedical Res Inst, AIST*, [2]*Bio-AFM FRC, Inst. of Sci. and Eng., Kanazawa U.*, [3]*PRESTO, JST*, [4]*Grad. Sch. Sci., Osaka City U.*, [5]*Div. of Math & Phys. Sci., Grad. Sch. of Nat. Sci. & Tech., Kanazawa U.*, [6]*CREST, JST*)

2P152 **ミオシン・サブフラグメント 1 ネック部位の首振り運動の分子動力学シミュレーション**

Molecular dynamics simulation for the neck domain swinging motion of a myosin subfragmnet-1

Tadashi Masuda (*Fukushima Univ.*)

2P153 **ATP 非存在下におけるミオシン V のアクチンフィラメント上での歩行運動**

ATP-less walking of myosin V on actin filaments

Noriyuki Kodera[1,2], Takayuki Uchihashi[1,3,4], Toshio Ando[1,3,4] ([1]*Bio-AFM FRC, Inst. of Sci. & Eng., Kanazawa Univ.*, [2]*PRESTO, JST*, [3]*Div. of Math & Phys. Sci., Grad. Sch. of Nat. Sci. & Tech., Kanazawa Univ.*, [4]*CREST, JST*)

2P154 **直流電流を付加した導電性基盤上でのアクトミオシンの運動**

Movement of actomyosin on a conductive base under DC current

Reito Wada[1], Takao Nakamura[1], Kuniyuki Hatori[2] ([1]*Grad. Sch. Med. Sci., Yamagata Univ.*, [2]*Grad. Sch. Sci. Eng., Yamagata Univ.*)

2P155 **ミオシンフィラメント上における骨格筋ミオシン分子間の協同性**

Intermolecular cooperativity of skeletal myosins in myofilaments

Motoshi Kaya[1], Yoshiaki Tani[1], Takuya Kobayashi[2], Hideo Higuchi[1] ([1]*Graduate School of Science, University of Tokyo*, [2]*Graduate School of Arts and Sciences, University of Tokyo*)

2P156 **高速 AFM と蛍光顕微鏡観察による F-アクチンへの HMM、コフィリンの協同的結合の解析**

An analysis of cooperative binding of myosin to F-actin using high-speed atomic force microscope and fluorescence microscopy

Hiroaki Ueno[1], Yuusuke Nishikawa[1], Akira Ainai[1], Rika Hirakawa[1], Atuki Yoshino[1], Noriyuki Kodera[3], Taro Ueda[2], Kiyotaka Tokuraku[1] ([1]*Grad. Sch. Sustain. Environ. Eng., Muroran Inst.*, [2]*Biomedical Res. Inst., AIST*, [3]*Bio-AFM FRC, Inst. Sci. & Eng., Kanazawa Univ.*)

2P157 **高負荷におけるバクテリアべん毛モーターの回転ステップ解析**

Sub-14 ° steps of the bacterial flagellar motors at high load

Yuichi Inoue, Hajime Fukuoka, Hiroto Takahashi, Akihiko Ishijima (*IMRAM, Tohoku University*)

2P158 **A novel role of dynactin for dynein motility revealed by gliding assay**

Takuya Kobayashi, Hatsuha Kajita, Kei Saito, Yoko Y Toyoshima (*Grad. Sch. of Arts and Sci., Univ. of Tokyo*)

2P159 **A novel role of dynactin for dynein motility revealed by single-molecule assay**

Takuya Miyashita, Takuya Kobayashi, Hatsuha Kajita, Yoko Y. Toyoshima (*Grad. Sch. of Arts & Sci., Univ. of Tokyo*)

2P160 **ダイニン·微小管複合体の DNA 折り紙による架橋**

Cross-linking the dynein-microtubule complex by DNA origami

Keiko Hirose[1], Kangmin Yan[1], Hisashi Tadakuma[2] ([1]*Biomed. Res. Inst., AIST*, [2]*Grad. Sch. Frontier Sci., Univ. Tokyo*)

2P161 **Athermal Fluctuations of Probe Particles in Active Gel**

Heev Ayade[1], Irwin Zaid[2], Julia Yeomans[2], Daisuke Mizuno[1] ([1]*Kyushu University*, [2]*University of Oxford*)

2P162 **軸糸ダイニンが駆動する微小管が創出する in vitro での動的渦形成**

In vitro dynamic vortex formation of microtubules driven by axonemal dyneins

Naoki Kanatani[1], Hiroaki Kojima[2], Kazuhiro Oiwa[1,2,3] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*Adv. ICT Res. Inst, NICT*, [3]*JST,CREST*)

2P163 **DNA オリガミバネを用いたミオシン VI のアンカー機能の分子動態計測**

Anchoring mechanism of myosin VI revealed with a programmed DNA origami spring

Mitsuhiro Iwaki[1,2,3], Shelley Wickham[2], Keigo Ikezaki[4], Toshio Yanagida[1,3], William Shih[2] ([1]*QBiC, RIKEN*, [2]*Harvard Med. Sch.*, [3]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [4]*Dep. of Adv. Mat. Sci., Tokyo Univ.*)

2P164 **微生物の運動超分子マシナリーの単位ステップの直接観察：滑走バクテリアと遊泳古細菌について**

Direct observation of unitary steps of supermolecular motility machineries of microorganisms: gliding bacterium and swimming archaeon

Yoshiaki Kinosita[1], Daisuke Nakane[1], Makoto Miyata[2], Takayuki Nishizaka[1] ([1]*Faculty of Science, Gakushuin Univ.*, [2]*Graduate School of Science, Osaka City Univ.*)

2P165 **好熱菌 F_1 のカップリングスキーム**

Coupling scheme of thermophilic F_1

Kengo Adachi[1], Kazuhiro Oiwa[2], Masasuke Yoshida[3], Kazuhiko Kinosita, Jr.[1] ([1]*Sci. & Engin., Waseda Univ.*, [2]*Adv. ICT Res. Inst., NICT*, [3]*Dep. Mol. Biosci., Kyoto Sangyo Univ.*)

2P166 **高速 AFM によるミオシンⅥの機能の直接観察**

Direct observation of functioning myosin VI by high-speed AFM

Shiori Sano[1], Noriyuki Kodera[2,3], Daniel Safer[4], H. Lee Sweeney[4], Toshio Ando[1,2,5] ([1]*Div. of Math & Phys. Sci., Grad. Sch. of Nat. Sci.& Tech., Kanazawa Univ.*, [2]*Bio-AFM FRC, Inst. of Sci. and Eng., Kanazawa Univ.*, [3]*PRESTO, JST*, [4]*Dept. of Physiol., Univ. of Pennsylvania Sch. of Med.*, [5]*CREST, JST*)

2P167 **Investigating the Coordination of RecB and RecD Subunits within RecBCD Helicase Complex Using Cy3-labeled ATP**

Chia-Chuan Cho, Hung-Wen Li (*Dept. Chemistry, Natl. Taiwan Univ.*)

12. 細胞生物的課題 / 12. Cell biology

2P168 **海洋性細菌 *Vibrio alginolyticus* のべん毛基部体 T-ring の低温電子顕微鏡を用いた構造解析**

Structural analysis of T-ring in the flagellar basal body of *Vibrio alginolyticus* by electron cryomicroscopy

Hidemaro Hotta[1], Akihiro Kawamoto[2], Satoshi Inaba[1], Yusuke V. Morimoto[2,3], Noriko Nishioka[1], Seiji Kojima[1], Keiichi Namba[2,3], Michio Homma[1] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [3]*QBiC, RIKEN*)

2P169 **アクチニンで架橋された二次元アクチンネットワークのミオシン依存的な形態変化**

Myosin-dependent morphological changes of two-dimensional actin networks crosslinked by α-actinin

Hiroki Eguchi[1], Makito Miyazaki[1], Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

2P170 **微小管ネットワークの対称性の破れによって引き起こされる細胞質回転流動**

Cytoplasmic rotational flow induced by symmetry breaking of active microtubule networks

Kazuya Suzuki[1], Makito Miyazaki[1], Jun Takagi[2], Takeshi Itabashi[1], Shin'ichi Ishiwata[1,3] ([1]*Dept. Phys., Univ. Waseda*, [2]*Quantitative Mechanobiology Laboratory, NIG*, [3]*WABIOS*)

2P171 **二種類の固定子を持つシュードモナス･シリンゲの運動解析**

Motility analysis of Pseudomonas syringae possessing two different stator systems

Takuto Tensaka, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Tohoku. Univ.*)

2P172 **MotA に点変異を持つ細菌べん毛モーターの出力特性解析**

Rotation analysis of the bacterial flagellar motor with a point mutation in MotA

Kodai Oono[1], Shuichi Nakamura[1], Fumio Hayashi[2], Kenji Oosawa[2], Seishi Kudo[1] ([1]*Grad. Sch. Eng., Univ. Tohoku.*, [2]*Div. Mol. Sci., Fac. Sci. and Tech, Univ. Gunma.*)

2P173 **ミオシン結合タンパク質の消失は細胞が出す力に影響を与えるのか**

The examination of whether deletion of myosin binding subunit 85 would lead to alteration of cellular mechanical features

Rui Li[1], Takeomi Mizutani[2], Hisashi Haga[2], Kazushige Kawabata[2] ([1]*Graduate School of Life Science Hokkaido University*, [2]*Faculty of Advanced Life Science Hokkaido University*)

2P174 **アフリカツメガエルの卵抽出液中の方向性を持った F アクチンの流れの観察**

Observation of directional F-actin flow in Xenopus egg extracts

Masatoshi Tanabe[1], Makito Miyazaki[1], Kazuya Suzuki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

2P175 **高圧負荷によって誘導されるクラミドモナス非運動性変異株鞭毛の屈曲運動**

High pressure induces flagellar bending movements in *Chlamydomonas* paralyzed mutants

Toshiki Yagi[1,2], Masayoshi Nishiyama[3] ([1]*Dept. Biol. Sci., Pref. Univ. Hiroshima*, [2]*Grad. Sch. Med., Univ. Tokyo*, [3]*The Hakubi Center, Kyoto Univ.*)

2P176 **バクテリアの集団運動による巨大渦パターン形成**

Large-scale vortex pattern emerging from bacteria collective motion

Showko Odaka, Daisuke Nakane, Takayuki Nishizaka (*Dept. Phys., Gakushuin Univ.*)

2P177 **シグナル変換のアダプター分子 LAT は細胞膜直下の小胞膜上で働く：1 分子追跡法による解明**

Transmembrane signaling adaptor LAT works on the vesicles associated with the plasma membrane: a single-molecule tracking study

Koichiro M. Hirosawa[1], Kenta J. Yoshida[2], Taka A. Tsunoyama[1], Kenichi G.N. Suzuki[1,3], Takahiro K. Fujiwara[1], Akihiro Kusumi[1,2] ([1]*Inst. Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto Univ.*, [2]*Inst. Frontier Medical Sciences, Kyoto Univ.*, [3]*NCBS/inStem, India*)

2P178 **The bidirectional role of conserved charged residues in rotor-stator interaction in a rotary motor of bacterial flagella**

Yasuhiro Onoue, Norihiro Takekawa, Tatsuro Nishikino, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Nagoya Univ.*)

2P179 **細胞-GUV 電気融合による μm サイズ人工物の導入**

Introducing micro-meter size objects into live cells mediated by cell-GUV electrofusion

Akira C. Saito[1], Toshihiko Ogura[2], Kei Fujiwara[3], Satoshi Murata[1], Shin-ichiro M. Nomura[1] ([1]*Department of Bioengi. and Robo. Tohoku Univ.*, [2]*Depart. of Develo. of Neurobiolo. (IDAC). Tohoku. Univ.*, [3]*Department of Biosciences and Informatics Keio University*)

2P180 **量子ドットを用いた脳がん幹細胞表面の膜タンパク質の運動解析**

Membrane Protein Dynamics on Brain Tumor Stem Cell Evaluated by Using Quantum Dot

Morito Sakuma, Sayaka Kita, Hideo Higuchi (*Department of Physics, Graduate School of Science, The University of Tokyo*)

2P181 ***Xenopus* 卵抽出液を封入した脂質膜小胞中でのアクチンのダイナミクス**

Actin dynamics in *Xenopus* egg extract encapsulated in a lipid membrane

Naoki Noda, Issei Mabuchi (*Dep. Life Sci., Gakushuin Univ.*)

2P182 **神経と接着した膵島 α 細胞内顆粒動態の解析**

Analysis of granule movement in pancreatic islet α cells attached with nerves

Tadahide Furuno[1], Atsuhiro Shinohara[1], Satoru Yokawa[1], Yoshikazu Inoh[1], Naohide Hirashima[2], Mamoru Nakanishi[1] ([1]*Sch. Pharm., Aichi Gakuin Univ.*, [2]*Grad. Sch. Pharm. Sci., Nagoya City Univ.*)

2P183 **細胞極性形成におけるポジティブフィードバック機構の 1 分子イメージング解析**

PTEN Membrane Binding is Destabilized by PI(3,4,5)P_3: Positive Feedback Loop in Establishing Cellular Polarity

Satomi Matsuoka[1,2], Masahiro Ueda[1,2] ([1]*Quantitative Biology Center (QBiC), RIKEN*, [2]*Graduate School of Science, Osaka University*)

2P184 **三量体 G タンパク質と相互作用する新規タンパク質 Gip1 は走化性における応答濃度範囲を広げる働きをする**

A novel heterotrimeric G protein interacting protein (Gip1) extend chemotactic range

Yukihiro Miyanaga[1,2], Yoichiro Kamimura[2], Masahiro Ueda[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*QBiC, Riken*)

2P185 **分子の混み合いが膜上シグナル伝達過程に与える影響の分子動力学法による考察**

Analysis of Molecular Crowding effect on signal transduction process by Molecular dynamics simulation

Rei Takamoto, Hiraku Nishimori, Akinori Awazu (*Dept. Math. Life Sci, Hiroshima Univ.*)

2P186 **ウシ毛様体筋細胞においてカフェインはムスカリン受容体作動性陽イオンチャネルの活性化を誘起する**

Activation of an M_3-muscarinic receptor operated non-selective cation channel by depletion of intracellular Ca^{2+}

Motoi Miyazu, Akira Takai (*Dept. Physiol., Asahikawa Med. Univ.*)

2P187 **表皮細胞の傷の治癒には伸展によるヘミチャネルからの ATP 放出と TRPC6 を介した Ca^{2+}流入が効く**

Wound healing in keratinocyte is accelerated by mechanosensitive ATP release via hemichannels and Ca^{2+} influx through TRPC6 channels

Kishio Furuya[1,2], Hiroya Takada[2], Masahiro Sokabe[1,2] ([1]*Mechanobiology Labo, Grad Sch Med, Nagoya Univ*, [2]*Dept Physiol, Grad Sch Med, Nagoya Univ*)

2P188 **Trial for detecting the activation and inactivation of chemoreceptor array in a single *E. coli* cell**

Hajime Fukuoka[1], Tomoko Horigome[2], Yuichi Inoue[1], Hiroto Takahashi[1], Akihiko Ishijima[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Life Sci., Tohoku Univ.*)

2P189 **単離ヒドラ神経細胞の神経突起動態**

Neurite dynamics of isolated hydra nerve cells

Rui Tanaka[1], Miharu Nagaishi[2], Mio Ogawa[2], **Hiroyuki Nakagawa**[1,2] ([1]*Div. Bio., Fac. Sci., Fukuoka Univ.*, [2]*Grad. Sch. Sci., Fukuoka Univ.*)

2P190 **サルモネラ菌の遊泳行動と生物対流**

Chemotactic behavior of *Salmonella* and bioconvection

Takahiro Abe, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Tohoku Univ.*)

2P191 **Rotation analysis of the spirochete cell body by 3D dark-field microscopy**

Kyosuke Takabe, Md. Shafiqul Islam, Seishi Kudo, Shuichi Nakamura (*Grad. Sch. Engineering., Univ. Tohoku*)

2P192 **細菌の走化性に起因する リング状パターンの形成過程の解析**

Formation process of a ring-like pattern induced by bacterial chemotaxis

Tadahiko Sato, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Univ. Tohoku*)

2P193 **Theoretical and simulation study for deformation of caveolae under hypo-osmotic condition**

Masashi Tachikawa[1], Shiro Suetsugu[2] ([1]*RIKEN*, [2]*NAIST*)

2P194 **細胞性粘菌の F-アクチン波の基質依存性**

Substrate dependence of F-actin waves in Dictyostelium

Yuko Chida[1], Satoshi Sawai[1,2,3] ([1]*Graduate School of Arts and Science, University of Tokyo*, [2]*Research Center for Complex Systems Biology*, [3]*PRESTO, Japan Science and Technology Agency*)

2P195 **心筋細胞における温度依存的拍動周期の特性**

Characterization of temperature-dependent beating rate of cardiomyocytes

Tomoyuki Kaneko (*LaRC, Dept. Frontier Biosci., Hosei Univ.*)

2P196 **多電極電位計測による心筋細胞に対するエタノールの影響**

Effect of ethanol on cardiomyocytes measured by multi-electrode array system

Chiho Nihei, Tomoyuki Kaneko (*LaRC, Dept. Frontier Biosci., Hosei Univ.*)

2P197 **多電極電位計測システムを用いた薬剤の催不整脈性の検出**

Detection of arrhythmogenicity of drugs by using multi electrode array system

Shin Yoshida, Tomoyuki Kaneko (*LaRC, Grad. Sci. Eng., Hosei Univ.*)

2P198 **多電極電位計測システムを用いた薬剤試験を行うための培地条件の探索**

Exploration of the optimum medium for a toxicity testing by multi electrode array system

Yuichiro Kamei, Tomoyuki Kaneko (*LaRC, Grad. Sci. Eng., Hosei Univ*)

2P199 **Raman Micro-spectroscopy of the Dynamics of Cellular Chemical State upon Stimulation with Growth Factors**

Sota Takanezawa[1,2], Shin-ichi Morita[3], Yukihiro Ozaki[2], Yasushi Sako[1] ([1]*RIKEN. Cellular Informatics Lab.*, [2]*School. Sci. Tech., Kwansei-Gakuin Univ.*, [3]*Grad. Sch. Sci., Tohoku Univ*)

2P200 **単層上皮細胞シートの複素弾性率の空間分布：原子間力顕微鏡測定**
Spatial distribution of complex shear modulus in confluent epithelial cell sheet : Atomic Force Microscopy measurements
Yuki Fujii, Yuki Ochi, Takaharu Okajima (*Grad. Schl. Inform. Sci. and Technol., Hokkaido Univ.*)

2P201 **心筋細胞ネットーワークにおける時空間的ゆらぎ計測を用いた心毒性評価のための Quasi-*in vivo* 前臨床モデル**
Quasi-*in vivo* pre-clinical model for cardiac toxicity using spatiotemporal fluctuation measurement on human cardiomyocyte cell-network
Fumimasa Nomura[1], Tomoyuki Kaneko[2], Hideyuki Terazono[1], Kenji Yasuda[1] ([1]*IBB, Tokyo Medical & Dental Univ.*, [2]*Dept. of Frontier Bioscience, Hosei Univ.*)

13A. 生体膜・人工膜：構造・物性 / 13A. Biological & Artificial membrane: Structure & Property

2P202 **Probing the sphingomyelin clusters in pure and mix lipid bilayer by the Raman spectroscopy: A theoretical study**
Pai-Chi Li, Kiyoshi Yagi, Koichiro Shirota, Toshihide Kobayashi, Yuji Sugita (*RIKEN*)

2P203 **スズガエルの皮膚分泌物由来のペプチド Bombinin H2 および H4 により誘起される相乗的な抗菌活性および細胞膜の揺らぎ**
Synergistic antimicrobial activity and membrane disturbance induced by Bombinin H2 and H4 peptides from *Bombina variegata* skin secretion
Yuki Kitahashi, Izuru Kawamura, Akira Naito (*Grad. Sch. Eng, Yokohama Natl Univ.*)

2P204 **蛍光一分子追跡に基づいた支持脂質膜内における部分フッ素化リン脂質の熱力学的性質の評価**
Evaluation of thermodynamic property of partially fluorinated phospholipid in supported lipid bilayer based on single molecule tracking
Yoshiaki Okamoto[1], Toshinori Motegi[1], Kohei Morita[2], Toshiyuki Takagi[3], Toshiyuki Kanamori[3], Masashi Sonoyama[2], Ryugo Tero[1] ([1]*Toyohashi Univ. Tech.*, [2]*Gunma Univ.*, [3]*AIST*)

2P205 **脂質二分子膜と高分子材料を融合したハイブリッド型人工膜の創製**
Hybrid model membrane composed of phospholipid bilayer and polymeric materials
Koji Ando[1], Kenichi Morigaki[1,2] ([1]*Grad. Sch. Agri., Univ. Kobe*, [2]*Res. Cen. Env Gen., Kobe*)

2P206 **ベシクル基盤分子ロボットのための分子センサーの開発**
Development of a molecular sensor for vesicle-based molecular robots
Koh-ichiroh Shohda, Akira Suyama (*The University of Tokyo*)

2P207 **リポソームを固定した QCM センサーで脂質膜結合プローブの相互作用を測る**
Liposome fixed sensor of quartz crystal microbalance quantifies the interaction between the lipid binding probe and target membrane
Takehiko Inaba, Toshihide Kobayashi (*RIKEN (Wako)*)

2P208 **ラマン分光法によるモデル膜におけるスフィンゴミエリン会合体の研究**
Study on sphingomyelin aggregates in model membranes by Raman spectroscopy
Koichiro Shirota[1], Takehiko Inaba[1], Pai-Chi Li[2], Kiyoshi Yagi[2], Yuji Sugita[2], Toshihide Kobayashi[1] ([1]*LBL, RIKEN*, [2]*TMSL, RIKEN*)

2P209 **高速原子間力顕微鏡によるバクテリオロドプシン球殻構造体の分子構造の直接観察**
Direct observation of molecular arrangement in a bacteriorhodopsin vesicle by high-speed atomic force microscopy
Yuto Noda, Daisuke Yamamoto (*Dept. Appl. Phys., Grad. Sch. Sci., Fukuoka Univ.*)

2P210 **固液界面に形成したコレステロールとコレステロールエステルからなる自己組織化単分子膜の研究**
Self-Assembled Monolayers of Cholesterol and Cholesteryl Esters at the Liquid/Solid Interface
Masahiro Hibino[1], Hiroshi Tsuchiya[2], Junpei Abe[3] ([1]*Dept. Appl. Sci., Muroran Inst. Tech.*, [2]*Display Device Dev. Div., Sharp*, [3]*Dept. Appl. Chem., Muroran Inst. Tech.*)

2P211 **細胞膜を構成する脂質分子種の二重層間および膜平面における非対称分布の凍結割断レプリカ電顕法による解析**
Transbilayer and lateral lipid distribution in plasma membranes in nano scale
Motohide Murate[1], Mitsuhiro Abe[1], Kohji Kasahara[2], Kazuhisa Iwabuchi[3], Masato Umeda[4], Toshihide Kobayashi[1,5] ([1]*Lipid Biol. Lab., RIKEN*, [2]*Lab. Biomembrane, Tokyo Met. Inst. Med. Sci.*, [3]*Lab. Biochem., Juntendo Univ.*, [4]*Dept. Syn. Chem. Biol. Chem., Kyoto Univ.*, [5]*INSERM Unite 1060*)

13B. 生体膜・人工膜：ダイナミクス / 13B. Biological & Artificial membrane: Dynamics

2P212 **メリチンが持つ多才な膜小胞変形能力**
Multiple membrane interactions and versatile vesicle deformations elicited by melittin
Tomoyoshi Takahashi[1], Fumimasa Nomura[2], Yasunori Yokoyama[3], Yohko Tanaka-Takiguchi[1], **Kingo Takiguchi**[1] ([1]*Dev. Bio. Sci., Grad. Sch. Sci., Nagoya Univ.*, [2]*Institut Biomater. Bioengineer., Tokyo Med. Dent. Univ.*, [3]*Dept. App. Phys., Grad. Sch. Eng., Nagoya Univ.*)

2P213 **ER 膜タンパク質の膜貫通配列のリン脂質フリップフロップ誘起能の評価**
Promotion of phospholipid flip-flop by membrane-spanning sequences in the ER proteins
Hiroyuki Nakao[1], Keisuke Ikeda[2], Yasushi Ishihama[1], Minoru Nakano[2] ([1]*Grad. Sch. of Pharm. Sci., Kyoto Univ.*, [2]*Grad. Sch. of Med. and Pharm. Sci., Univ. Toyama*)

2P214 **細胞サイズリン脂質非対称膜リポソームによる膜ダイナミクスの観察**
Cell-sized asymmetric lipid vesicles for membrane dynamics observation
Koki Kamiya[1,2], Toshihisa Osaki[1,3], Kousuke Shibasaki[1], Shoji Takeuchi[1,3] ([1]*KAST*, [2]*JST PRESTO*, [3]*IIS Univ. Tokyo*)

2P215 **低い pH が誘起する DOPS/MO 膜の一枚膜リポソームからキュービック相への構造転移の初期過程**
Initial Step of Low pH-Induced Structural Transition from Unilamellar Vesicles of DOPS/MO to Inverse Bicontinuous Cubic Phase
Takahiro Saiki[1], Toshihiko Oka[1,2], Taka-aki Tsuboi[1], Masahito Yamazaki[1,2] ([1]*Grad. Sch. Sci, Shizuoka Univ.*, [2]*Res. Inst. of Electronics, Shizuoka Univ.*)

2P216 **単一 GUV 法による脂質膜に EGCg が誘起した孔構造の可視化**
Visualization of the EGCg-induced bursting of single giant unilamellar vesicles at higher time resolution
Yukihiro Tamba[1], Masahito Yamazaki[2] ([1]*Suzuka Natl Coll Tech*, [2]*Shizuoka Univ*)

2P217 **静電効果によるジャイアントリポソームの膜チューブ形成**
Formation of endocytosis-like membrane tubes in giant liposomes induced by electrostatic effect
Tamiki Umeda[1], Yohko Tanaka-Takiguchi[2], Kingo Takiguchi[3] ([1]*Grad. Sch. Maritime Sci. Kobe Univ.*, [2]*Struct. Biol. Res. Center, Nagoya Univ.*, [3]*Grad. Sch. Sci., Nagoya Univ.*)

2P218 **Self-Emergent Cell-Sized Sphere Entrapping DNA through Micro Phase-Segregation**
Naoki Nacatani[1], Kanta Tsumoto[2], Zyunya Nakamura[2], Kenichi Yoshikawa[1] ([1]*School of Life and Biomedical Sciences, Doshisha Univ.*, [2]*Graduate school of Engineering, Mie Univ.*)

13C. 生体膜・人工膜：興奮・チャネル / 13C. Biological & Artificial membrane: Excitation & Channels

2P219 **Conformational Transitions in Voltage Sensor Domains**
Morten Bertz, Kazuhiko Kinosita (*Waseda University, Dpt. of Science & Engineering*)

2P220 **破骨細胞膜に存在する新規の（?）プロトン流入経路**
A novel (?) proton influx pathway in the plasma membrane of osteoclasts
Miyuki Kuno, Guanshuai Li, Yoshiko Hino, Yoshie Moriura, Junko Kawawaki, Hiromu Sakai (*Dept Physiol, Osaka City Univ Grad Sch Med*)

2P221 **Kv1.2 でのイオン透過における透過パターンの解析**
Analysis on Ion Permeation Pattern through the Kv1.2 Channel
Takashi Sumikama[1], Shinji Saito[2], Shigetoshi Oiki[1] ([1]*Univ. of Fukui*, [2]*IMS*)

2P222 **two-pore 型カリウムチャネル TWIK-1 の特徴的なイオン選択性を生み出すメカニズムについての全反射赤外分光解析**
ATR-FTIR spectroscopic analyses of interaction modes underlying unique ion selectivity of a two-pore domain potassium channel TWIK-1
Hisao Tsukamoto[1], Koichi Nakajo[2], Yoshihiro Kubo[2], Yuji Furutani[1] ([1]*Institute for Molecular Science*, [2]*National Institute for Physiological Sciences*)

13E. 生体膜・人工膜：情報伝達 / 13E. Biological & Artificial membrane: Signal transduction

2P223 **Akt と受容体の生細胞内 1 分子追跡によるシグナル伝達機構の解明**
Signal transduction mechanism of Akt reveald by single molecule imaging of Akt and receptor molecules
Hideaki Yoshimura, Takeaki Ozawa (*Department of Chemistry, School of Science, The University of Tokyo*)

2P224 **細胞性粘菌の走化性シグナル伝達におけるグアニル酸シクラーゼ(sGC)経路の興奮性応答**
Excitability of Guanylate Cyclase (sGC) signaling pathway mediating chemotaxis in Dictyostelium cells
Yuki Tanabe[1], Masahiro Ueda[1,2] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*QBiC, Riken*)

2P225 **モデル生体膜を用いたロドプシン光受容体の脂質ラフト親和性解析**
Evaluating the raftophilicity of rhodopsin in a patterned model membrane
Yasushi Tanimoto[1], Kenichi Morigaki[1,2], Humio Hayashi[3] ([1]*Grad. Sch. Agri, Univ Kobe*, [2]*Res. Cen. Env Gen, Univ Kobe*, [3]*Grad. Sch. Scie, Univ Kobe*)

15. 神経・感覚 / 15. Neuroscience & Sensory systems

2P226 **ミミズ非連合学習における NO-cGMP シグナル経路の役割**
Role of NO-cGMP signaling in non-associative learning of the earthworm
Yoshiichiro Kitamura[1], Hitoshi Aonuma[2], Hiroto Ogawa[3], Kotaro Oka[4] ([1]*Dept Math Sci Phys, Kanto Gakuin Univ*, [2]*Res Inst Elect Sci, Hokkaido Univ*, [3]*Dept Biol Sci, Hokkaido Univ*, [4]*Dept Biosci Info, Keio Univ*)

2P227 **チャコウラナメクジ脳嗅覚中枢における匂い応答の数理解析**
Tone-Entropy analysis on odor-evoked neuronal activities in the procerebral lobe of a slug
Yoshimasa Komatsuzaki[1], Tamon Eto[1], Minoru Saito[2] ([1]*CST, Nihon Univ.*, [2]*Grad. Sch. of Int. Basic Sci., Nihon Univ.*)

2P228 **記憶学習中枢海馬の性差：海馬内ホルモン変動とシナプス変動の解析**
Sex difference in hippocampus: Fluctuation of hippocampal sex hormones and synapses
Yasushi Hojo[1,2], Asami Kato[1], Bon-chu Chung[2], Tetsuya Kimoto[1,2], Suguru Kawato[1,2] ([1]*Grad. Sch. of Arts and Sci., The Univ. of Tokyo*, [2]*JST, Japanese-Taiwanese Cooperative Programme*)

16. 神経回路・脳の情報処理 / 16. Neuronal circuit & Information processing

2P229 **マウス海馬スライスに見られる時空間活動パターンに対するゆらぎ解析**
Fluctuation analysis for spatiotemporal activity patterns in mouse hippocampal slices
Yuuta Hamasaki[1], Yoshiki Uno[2], Shodai Izumi[2], Hiromi Osanai[2], Yoshimasa Komatsuzaki[3], Minoru Saito[1,2] ([1]*The Institute of Natural Sciences, College of Humanities and Sciences, Nihon University*, [2]*Graduate School of Integrated Basic Sciences, Nihon University*, [3]*College of Science and Technology, Nihon University*)

2P230 **膜電位イメージングを用いたチャコウラナメクジ嗅覚神経回路の解析**
Characterization of the olfactory neural network of the land slug using fluorescent voltage imaging
Kohei Ishida[1], Tomoya Shimokawa[1], Yuuta Hamasaki[1], Yoshimasa Komatsuzaki[2], Satoshi Watanabe[3], Minoru Saito[1] ([1]*Graduate School of Integrated Basic Sciences, Nihon University*, [2]*College of Science and Technology, Nihon University*, [3]*Graduate School of Engineeing, Tohoku University*)

17. 行動 / 17. Behavior

2P231 **ゾウリムシにおける長期後退遊泳の膜興奮モデルによる解析**
The analyses based on a membrane excitation model for Long-term Backward Swimming in a protozoa Paramecium
Kaito Ohki[1], Itsuki Kunita[2], Shigeru Kuroda[2], Toshiyuki Nakagaki[2] ([1]*Grad. Sch. Life. Sci., Univ. Hokkaido*, [2]*RIES., Univ. Hokkaido*)

2P232 **T 細胞の自発運動の解析**
Analysis of spontaneous migration of T cell
Hiroaki Takagi[1], Tomoya Katakai[2], Tatsuo Kinashi[2] ([1]*Nara Medical University*, [2]*Kansai Medical University*)

18A. 光生物：視覚・光受容 / 18A. Photobiology: Vision & Photoreception

2P233 **高度好熱菌由来サーモフィリックロドプシンの温度依存的な不可逆構造転移**
Temperature-Dependent Irreversible Structural Transition of Thermophilic Rhodopsin
Takashi Tsukamoto[1], Makoto Demura[2], Yuki Sudo[1] ([1]*Div. Pharm. Sci., Okayama Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*)

2P234 **ナトリウムポンプロドプシンにおけるナトリウムイオンの結合の役割**
Role of Sodium Ion Binding in Sodium Pumping Rhodopsin
Keiichi Inoue[1,2], Rei Abe-Yoshizumi[1], Hideki Kandori[1] ([1]*Grad. Sch. Eng., Nagoya Inst. Tech.*, [2]*PRESTO, JST*)

2P235 **プロテオロドプシンのプロトンドナー Glu108 の変異が光化学特性に及ぼす影響について**
Effects on the photochemical properties in proteorhodopsin by the mutation of the Glu108 residue
Jun Tamogami[1], Takashi Kikukawa[2], Kimi Okubo[1], Makoto Demura[2], Toshifumi Nara[1], Naoki Kamo[2] ([1]*College Pharm. Sci., Matsuyama Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*)

2P236 ***Acetabularia* rhodopsin II (ARII)の Asp81 変異体による一過性光誘起電流**
Transient photo-induced current by Asp81 mutants of *Acetabularia* rhodopsin II (ARII) heterologously expressed in *Xenopus laevis* oocytes
Seiji Miyauchi[1], Kazumi Shimono[1], Takashi Kikukawa[2], Makoto Demura[2], Jung Kwang-Hwan[3], Naoki Kamo[2] ([1]*Toho Univ., Grad. Sch. Pharm. Sci.*, [2]*Hokkaido Univ., Grad. Sch. Life Sci.*, [3]*Sogang Univ., Inst. Biol. Interfaces*)

2P237 **Low-temperature FTIR spectroscopy of the Light-driven sodium ion pump: Krokinobacter eikastus rhodopsin 2**
Faisal Hammad Mekky Koua[1], Rei Abe-Yoshizumi[2], Hikaru Ono[2], Shota Ito[2], Yoshitaka Kato[2], Keiichi Inoue[1,2], Hideki Kandori[1,2] ([1]*OptoBioTech. Res. Cent., Nagoya Inst. Tech.*, [2]*Dept. Front. Mat., Nagoya Inst. Tech.*)

2P238 **ロドプシンの低い熱活性化頻度の分子メカニズム**
Molecular mechanism of the low thermal activation rate of rhodopsin
Keiichi Kojima[1], Masataka Yanagawa[2], Takahiro Yamashita[1], Yasushi Imamoto[1], Takesi Matsuyama Hoyos[1], Koji Nakanishi[3], Yumiko Yamano[4], Akimori Wada[4], Yasushi Sako[2], Yoshinori Shichida[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Cell. Info. Lab., Riken*, [3]*Columbia Univ.*, [4]*Kobe Pharm. Univ.*)

2P239 **ロドプシン構成的活性変異体 M257Y における構造平衡の一分子観測**
Single-molecule observation of the conformational equilibrium in constitutively active mutant of rhodopsin, M257Y
Ryo Maeda[1], Michio Hiroshima[1,3], Yasushi Imamoto[2], Takahiro Yamashita[2], Yasushi Sako[1], Yoshinori Shichida[2] ([1]*Cellular Informatics Lab., RIKEN*, [2]*Grad. Sch. Biophys., Kyoto Univ.*, [3]*QBiC, RIKEN*)

2P240 **In situ 光照射固体 NMR による光受容膜タンパク質センサリーロドプシン I の光反応過程の解析**
Photocycle of sensory rhodopsin I as revealed by in situ photo irradiation solid-state NMR
Yoshiteru Makino[1], Hiroki Yomoda[1], Yuya Tomonaga[1], Tetsurou Hidaka[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Yuki Sudo[3], Naoki Kamo[4], Akira Naito[1] ([1]*Grad. Sch. Eng, Yokohama Natl Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Grad. Sch. Pharm, Okayama Univ.*, [4]*Grad. Sch. Life Sci , Hokkaido Univ.*)

2P241 **アナベナセンサリーロドプシンの細胞質側で生じる光誘起プロトン移動反応の解析**
Light-induced proton transfer reactions at the cytoplasmic half channel of *Anabaena* sensory rhodopsin
Takatoshi Hasemi[1], Takashi Kikukawa[1], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Kwang-Hwan Jung[2], Naoki Kamo[1], Makoto Demura[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Dept. Life Sci. & Inst. Biol. Interfaces, Sogang Univ.*)

2P242 **海洋性細菌に含まれる光駆動クロライドポンプの分光研究**

Spectroscopic study of light-driven chloride pump from marine bacteria

Arisa Mori[1,2], Keiichi Inoue[2,3], Faisal Hammand Mekky Koua[2], Yoshitaka Kato[2], Rei Abe-Yoshizumi[2], Michio Homma[1], Hideki Kandori[2] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Nagoya Inst. Tech*, [3]*JST, PRESTO*)

2P243 **チャネルロドプシンの吸収波長に関する量子化学的研究**

Quantum chemical study of the absorption maximum of channelrhodopsin

Nami Yoshino[1], Tomohiko Hayashi[1], Azuma Matsuura[2], **Tadaomi Furuta**[1], Minoru Sakurai[1] ([1]*Tokyo Tech*, [2]*Fujitsu Lab*)

2P244 **固体 ^{13}C NMR によるファラオニスフォボロドプシンの機能に重要な Tyr 残基の構造解析**

Conformation of functionally important Tyr residues in *pharaonis* phoborhodopsin as studied by Solid-State ^{13}C NMR

Ryota Nishikawa[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Yuki Sudo[3], Naoki Kamo[4], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Okayama Univ.*, [4]*Grad. Sch. Life Sci, Hokkaido Univ.*)

2P245 **サル青感受性視物質の赤外分光解析**

FTIR study of monkey blue-sensitive visual pigment

Yuki Nonaka[1], Kota Katayama[1], Kei Tsutsui[2], Hiroo Imai[2], Hideki Kandori[1] ([1]*Nagoya Institute of Technology*, [2]*Primate Research Institute, Kyoto University*)

2P246 **In-situ 光照射固体 NMR によるバクテリオロドプシンの光励起過程における局所構造変化の解析**

Structural changes in the photoexcited process in retinal of Bacteriorhodopsin studied by in-situ photoirradiation solid-state NMR

Arisu Shigeta[1], Ryota Miyasa[1], Miyako Horigome[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Satoru Tuzi[3], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Univ. Hyogo*)

2P247 ***In-situ* 光照射固体 NMR によるバクテリオロドプシン Y185F 変異体に捕捉された O-中間体の評価**

Characterization of O-like intermediate trapped in Y185F mutant in Bacteriorhodopsin by *in-situ* photo-irradiation solid-state NMR

Kyosuke Oshima[1], Arisu Shigeta[1], Yoshiteru Makino[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Satoru Tuzi[3], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Univ. Hyogo*)

2P248 **光依存転写因子オーレオクロム 1 の反応ダイナミクス**

Reaction Dynamics of Light Dependent Transcription Factor Aureochrome-1

Yuki Akiyama[1], Yusuke Nakasone[1], Osamu Hisatomi[2], Yoichi Nakatani[2], Masahide Terazima[1] ([1]*Graduate School of Science, Kyoto University*, [2]*Graduate School of Science, Osaka University*)

2P249 **(6-4)光回復酵素による 2 光子 DNA 修復の分子メカニズム**

Molecular mechanism of the two photon DNA repair by the (6-4) photolyase

Junpei Yamamoto[1], Kohei Shimizu[1], Tomoko Fujiwara[2], Takeshi Todo[2], Pascal Plaza[3], Klaus Brettel[4], Shigenori Iwai[1] ([1]*Grad. Sch. Eng. Sci., Osaka Univ.*, [2]*Grad. Sch. Med., Osaka Univ.*, [3]*ENS Paris, France*, [4]*CEA Saclay, France*)

2P250 **Theoretical study of the electron transfer reaction by DNA photolyase**

Ryuma Sato[1], Hirotaka Kitoh-Nishioka[1], Tsutomu Kawatsu[2,3], Kei Yura[4], Koji Ando[5], Takahisa Yamato[1] ([1]*Grad. Sch. Sci., Univ. Nagoya*, [2]*Grad. Sch. Arts and Sci., Univ. Tokyo*, [3]*Grad. Sch. Nanobiosci., Univ. Yokohama City*, [4]*Grad. Sch. Human & Sci., Univ. Ochanomizu*, [5]*Grad. Sch. Sci., Univ. Kyoto*)

18B. 光生物：光合成 / 18B. Photobiology: Photosynthesis

2P251 **光化学系 II 複合体と層状複水酸化物からなるバイオ–無機ハイブリッド電極**

Bio-inorganic hybrid water oxidation electrodes of Photosystem II and layered double hydroxide

Masaru Kato[1], Hisako Sato[2], Miwa Sugiura[3,4] ([1]*Grad. Sch. Env. Sci., Hokkaido Univ.*, [2]*Grad. Sch. Sci., Ehime Univ.*, [3]*Proteo-Sci. Cen., Ehime Univ.*, [4]*PRESTO, JST*)

2P252 **光化学系 II における TyrZ - D1/His190 の距離と PCET の関係**

Proton-coupled electron transfer and hydrogen-bond distance of TyrZ - D1-His190 in Photosystem II

Miwa Sugiura[1], Shogo Ogami[2], Fabrice Rappaport[3], Alain Boussac[3] ([1]*PROS, Ehime Univ./JST-PRESTO*, [2]*Dep. Chem., Ehime Univ.*, [3]*IBPC*)

2P253 **光電子収量分光による非真空環境下の生体関連分子の電子構造観察：クロロフィル a 測定の試み**

Observation of the electronic structure of bio-related molecule in non-vacuum environment by using photoemission: Trial to Chlorophyll a

Yuki Takeda[1], Hiroshi Ezawa[2], Takuya Miyauchi[1], Hiroumi Kinjo[1], Yasuo Nakayama[1], Hisao Ishii[1,3] ([1]*AIS, Chiba Univ.*, [2]*Faculty of engineering, Chiba Univ.*, [3]*CFS, Chiba Univ.*)

2P254 **光捕集アンテナにおける色素の励起エネルギーの揺らぎに関する理論的研究**

Theoretical Studies on Excitation Energy Fluctuations of Pigments in a Light-Harvesting Complex

Masahiro Higashi[1], Shinji Saito[2] ([1]*Fac. Sci. Univ. Ryukyus*, [2]*IMS*)

2P255 **OEC の Kok-S2 状態の反応活性部位の分子構造に関する B3LYP 計算：Mn4 の配位水分子のプロトン化状態**

A B3LYP study on molecular structures of active site at the Kok-S2 state of OEC: protonation states of Mn4-ligated water molecules

Tomoya Ichino, Masaki Mitani, Yasunori Yoshioka (*Grad. Sch. Eng., Univ. Mie*)

2P256 **水分解 Mn_4Ca クラスター S_3 状態に関する理論的研究**

Theoretical Study of the S_3 state of the Mn_4Ca-cluster in Photosystem II: A compact chair form consisting of the short Mn-Mn pairs

Makoto Hatakeyama[1], Kouji Ogata[1], Satoshi Yokojima[2], Shinichiro Nakamura[1] ([1]*Wako Inst., Riken*, [2]*Sch. Pharm., Tokyo Univ. Pharm. Life Sci.*)

2P257 **光合成酸素発生中心(PSII-OEC)の立体構造と電荷状態変化についての理論的研究**
Theoretical investigation on the conformation-charge relationship of the photosystem II oxygen evolving complex (PSII-OEC)
Mitsuo Shoji[1], Hiroshi Isobe[2], Shusuke Yamanaka[3], Jian-Ren Shen[2], Kizashi Yamaguchi[3] ([1]*Grad. Sch.of Pure & App. Sci., Univ. Tsukuba*, [2]*Grad. Sch. Nat. Sci. & Tec., Okayama Univ.*, [3]*Grad. Sch. Sci, Osaka Univ*)

2P258 **Ca 除去と Sr 置換をした Mn クラスターの ENDOR 法による研究**
ENDOR studies on Ca depleted and Sr substituted Mn cluster in photosystem II
Hiroki Nagashima[1], Nakajima Yoshiki[2], Jian-Ren Shen[2], Hiroyuki Mino[1] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Grad. Sch. Nat. & Tech., Okayama Univ.*)

2P259 **Target analysis of the photosystem II-enriched membrane: The effect of oxidizing agent on fluorescence quenching in PSII**
Ahmed Mohamed[1], Ryo Nagao[2], Takumi Noguchi[2], Hiroshi Fukumura[1], Yutaka Shibata[1] ([1]*Grad. Sch. Sci., Univ. Tohoku*, [2]*Grad. Sch. Sci., Univ. nagoya*)

19. 放射線生物／活性酸素 / 19. Radiobiology & Active oxygen

2P260 **超音波に誘発されるケージド化合物の活性化**
Ultrasound-induced activation of caged compounds
Haruko Koura[1], Risa Fuji[1], Asuka Kato[1], Masato Mutoh[2], Wakako Hiraoka[1] ([1]*Dept. Phys., Grad. Sch. Sci. & Tech., Meiji Univ.*, [2]*Dept. Mater. & Human Env. Sci., Shonan Inst. of Tech.*)

20. 生命の起源・進化 / 20. Origin of life & Evolution

2P261 **ノロウイルス RNA 複製酵素を用いた試験管内 RNA 淘汰実験から、新奇な dsRNA 複製機構が示唆された**
A novel dsRNA replication mode was suggested from the *in vitro* RNA selection using Norovirus RNA replicase
Hidenao Arai[1], Koichi Nishigaki[2], Naoto Nemoto[1], Miho Suzuki[1], Yuzuru Husimi[2] ([1]*Grad. Sch. Sci. Eng., Saitama Univ.*, [2]*Professor Emeritus, Saitama Univ.*)

2P262 **phi29DNA 複製酵素を使った自己複製系の確立**
Establishment of a self-replication system using phi29 DNA polymerase
Yoshihiro Sakatani[1], Norikazu Ichihashi[1,2], Tetsuya Yomo[1,2,3] ([1]*Grad. Sch. Inf., Univ. Osaka*, [2]*JST, ERATO*, [3]*Grad. Sch. Bio., Univ. Osaka*)

21A. ゲノム生物学：ゲノム解析 / 21A. Genome biology: Genome analysis

2P263 **分子動力学計算を用いた β2 アドレナリン受容体と G タンパク質間の 相互作用解析**
Structural analysis of interaction between β2 adrenergic receptor and G-protein using molecular dynamics simulation
Hidenori Sakaki[1], Masami Ikeda[2], Makiko Suwa[1,2] ([1]*Biol. Sci., Grad. Sci. Eng., Aoyama Gakuin Univ.*, [2]*Chem. Biol. Sci., Sci. Eng., Aoyama Gakuin Univ.*)

2P264 **Global clustering of whole organisms enabled by the GP method**
Harshita Sharma[1], Fumihito Ohtani[1], Parmila Kumari[1], Deepti Diwan[1], Miho Suzuki[1], Naoto Nemoto[1], Takuyo Aita[2], Koichi Nishigaki[1] ([1]*Dept. of Functional Materials Science, Graduate School of Science and Engineering, Saitama University*, [2]*Graduate School of Information Science and Technology, Symbiotic Network Design Laboratory, Osaka University*)

21B. ゲノム生物学：ゲノム構造 / 21B. Genome biology: Genome structure

2P265 **Super-resolution imaging of chromatin domains in living mammalian cells**
Tadasu Nozaki[1,2], Tomomi Tani[3], Sachiko Tamura[1], Takeharu Nagai[4], Kazuhiro Maeshima[1] ([1]*Natl. Inst. Genet.*, [2]*Inst. Adv. Biosci., Keio Univ.*, [3]*Marine Biological Laboratory*, [4]*ISIR, Osaka Univ.*)

2P266 **出芽酵母の核の内側に“転写が不活発な領域”が存在する？**
Are there transcriptionally inactive regions localized in a budding yeast nucleus?
Naoko Tokuda, Shin Fujishiro, Masaki Sasai (*Grad. Sch. Engr., Univ. Nagoya*)

2P267 **Computational chromosome conformation sampling of human diploid genome**
Shin Fujishiro, Naoko Tokuda, Masaki Sasai (*Grad. Sch. Eng., Univ. Nagoya*)

22A. 生命情報科学：構造ゲノミクス / 22A. Bioinformatics: Structural genomics

2P268 **相補性に依らないタンパク質—タンパク質ドッキングポーズ予測法**
A protein-protein docking prediction method not relying on the shape complementarity
Atsushi Hijikata, Masafumi Shionyu, Tsuyoshi Shirai (*Nagahama Inst. Bio-Sci. Tech.*)

2P269 **データベース IDEAL の新機能と機能性天然変性領域の配列・構造比較**
The update of the IDEAL database, and sequence and structure comparisons of intrinsically disordered regions
Satoshi Fukuchi[1], Takayuki Mamemiya[2], Shigetaka Sakamoto[3], Yukiko Nobe[2], Yumiko Kado[2], Kazuo Hosoda[1], Ryoutaro Koike[2], Hidekazu Hiroaki[4], Motonori Ota[2] ([1]*Maebashi IT*, [2]*Nagoya Univ. SIS*, [3]*Holonics*, [4]*Nagoya Uni. BMS*)

2P270 **GGIP : GPCR-GPCR Interaction Pair Predictor**

Wataru Nemoto[1,2], Yoshihiro Yamanishi[3], Vachiranee Limviphuvadh[4], Hiroyuki Toh[2] ([1]*Grad. Sch. Sci and Eng., TDU*, [2]*CBRC., AIST*, [3]*MiB., Univ. Kyushu*, [4]*BII., A*STAR*)

2P271 **Re-docking によって正解候補構造が多く得られるタンパク質分子表面の特徴の解析**

Analysis of properties of protein-protein interaction surface areas involved in more near-native complexes by Re-docking scheme

Nobuyuki Uchikoga[1], Yuri Matsuzaki[2], Masahito Ohue[3,4], Yutaka Akiyama[2,4], Takatsugu Hirokawa[5] ([1]*Dept. Phys., Chuo Univ.*, [2]*ACLS, Tokyo Tech*, [3]*JSPS Res. Fellow*, [4]*Grad. Sch. Inform. Sci. and Eng., Tokyo Tech.*, [5]*MolProf, AIST*)

2P272 **A Ligand Based Virtual Screening method that takes into account of protein-ligand interactions**

Koya Kato, George Chikenji (*Sasai group, Department of Computational Science and Engineering, Graduate School of Engineering, Nagoya University*)

24. 数理生物学 / 24. Mathematical biology

2P273 **生命システムにおける情報の適応的価値**

Fitness Value of Information in Biological Systems

Tetsuya J. Kobayashi[1], Yuki Sughiyama[2] ([1]*IIS, Univ. Tokyo*, [2]*College of Arts and Sciences, Univ. Tokyo*)

2P274 **Detecting the selection acting on heterogeneous cell phenotypes without environmental perturbation**

Takashi Nozoe, Yuichi Wakamoto (*Univ of Tokyo*)

2P275 **完全変態昆虫の最適成長スケジュール**

Optimal growth schedule of holometabolous insects

Ken-ichi Hironaka[1,2], Yoshihiro Morishita[1] ([1]*RIKEN CDB*, [2]*JSPS Research Fellow*)

2P276 **時間依存する出生死滅過程に対する代数的アプローチ**

Algebraic approach to time-inhomogeneous birth-death processes

Jun Ohkubo (*Grad. Sch. Informatics, Kyoto Univ.*)

2P277 **多繊毛細胞における繊毛の空間配向秩序のモデル化**

Modeling of spatial distribution and orientational order of cilia mediated by multi-ciliated cells

Hironobu NOGUCHI[1], Shuji ISHIHARA[2] ([1]*Graduate School of Arts and Sciences, the University of Tokyo*, [2]*Department of Physics, School of Science and Technology, Meiji University*)

2P278 **マイクロアレイデータに基づく植物の遺伝子発現揺らぎと機能の関係**

Analysis of between gene fluctuation and function of plants based on microarray data

Kodai Hirao[1], Atsushi Nagano[2] ([1]*Dept. of Mathematical and Life Sciences, Hiroshima Univ.*, [2]*Center for Ecological Research, Kyoto Univ.*)

2P279 **化学反応における少数性効果の理論解析**

Mathematical Analysis of Small Number Effect in Biochemical Reactions

Nen Saito, Yuki Sughiyama, Kunihiko Kaneko (*Grad. Sch. Art. Sci., Univ. Tokyo*)

2P280 **人工遺伝子回路における下流レポーター遺伝子の影響**

Effects of downstream reporter genes on synthetic genetic circuits

Takefumi Moriya[1], Masayuki Yamamura[1], Daisuke Kiga[1,2] ([1]*Tokyo Institute of Technology, Department of Coputational Intelligence and Systems Science*, [2]*Tokyo Institute of Technology, Earth-Life Science Institute*)

2P281 **類似分子識別機構の数理モデル**

Mathematical modeling of molecular discrimination system

Masashi Kajita[1], Kazuyuki Aihara[1,2], Tetsuya J. Kobayashi[1,2] ([1]*Department of Mathematical Informatics, Graduate School of Information Science and Technology, The University of Tokyo*, [2]*Institute of Industrial Science, The University of Tokyo*)

25. 非平衡・生体リズム / 25. Nonequilibrium state & Biological rhythm

2P282 **油中水滴による非平衡な人工細胞システム**

Nonequilibrium artificial cell system based on water-in-oil microdroplet

Masahiro Takinoue[1,2], Haruka Sugiura[1], Hiroyuki Kitahata[3], Yoshihito Mori[4] ([1]*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*, [2]*PRESTO, JST*, [3]*Dept. Phys., Chiba Univ.*, [4]*Dept. Chem., Ochanomizu Univ.*)

2P283 **細胞濃度制御のためのマイクロ流体ケモスタット**

Microfluidic chemostat for cell density control

Manami Ito[1], Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Institute of Technology*, [2]*PRESTO, JST*)

2P284 **微小液滴を用いた非線形化学反応間の相互作用**

Interaction among nonlinear chemical reactions based on microdroplets

Tomoya Okuaki[1], Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology*, [2]*PRESTO, JST*)

2P285 **自律的に駆動する複雑形状粒子の並進、回転、円運動**

Translational, rational, circular motions of self-driven complex-shaped microparticles

Masayuki Hayakawa[1], Hiroaki Onoe[2], Ken H. Nagai[3], Masahiro Takinoue[1,4] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Dept. of Mech. Eng., Keio University*, [3]*School of Materials Science, JAIST*, [4]*PRESTO, JST*)

2P286 **The analysis of energy transfer in Chaotic Dynamical Systems 2**
Mami Kushida[1], Kana Fuji[1], Mikito Toda[2], Hiroshi Fujisaki[3] ([1]*Grad., Univ. Nara-wu.*, [2]*Univ. Nara-wu.*, [3]*NMS*)

26. 計測 / 26. Measurements

2P287 **イメージングバイオマーカーを用いた標的細胞検出のためのオンチップマルチイメージングセルソータシステムの認識アルゴリズム開発**
Development of the cell imaging biomarker identification algorism for on-chip multi imaging cell sorter system
Masao Odaka[1], Hyonchol Kim[1], Mathias Girault[1], Akihiro Hattori[1], Hideyuki Terazono[1,2], Kenji Matsuura[1], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental University*)

2P288 **標的細胞特定のためのイメージングバイオマーカー：血中循環がん細胞クラスター同定の例**
Imaging biomarkers for identification of target cells: Identification of clustered circulating tumor cells as an example
Hyonchol Kim[1], Hideyuki Terazono[1,2], Akihiro Hattori[1], Masao Odaka[1], Mathias Girault[1], Kenji Yasuda[1,2] ([1]*KAST*, [2]*Inst. Biomat. Bioeng., Tokyo Med. Dent. Univ*)

2P289 **Optimization of the cell encapsulation in the water in oil droplet using 3D printed object**
Mathias Girault[1], Akihiro Hattori[1], Hyonchol Kim[1], Kenji Matsuura[1], Masao Odaka[1], Yumi Mikami[1], Hideyuki Terazono[1,2], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental University*)

2P290 **Single particle detection of influenza virus by micro droplet array**
Shuho Kidokoro[1], Kazuhito V. Tabata[1,2], Hiroyuki Noji[1] ([1]*Department of Applied Chemistry, Graduate School of Engineering, The University of Tokyo*, [2]*PRESTO, JST*)

2P291 **水中に発生させたレーザー励起キャビテーションバブルの高速温度場イメージング**
Fast temperature measurement following single laser-induced cavitation inside a microfluidic gap
Madoka Suzuki[1,2], Pedro A. Quinto-Su[3], Claus-Dieter Ohl[4] ([1]*WABIOS, Singapore*, [2]*Org. Univ. Res. Initiatives, Waseda Univ.*, [3]*ICN, UNAM, Mexico*, [4]*SPMS, NTU, Singapore*)

2P292 **振動和周波検出赤外超解像顕微鏡法による毛髪試料の分子構造解析**
Molecular structural analysis of human hair samples by VSFG detected IR super-resolution microscopy
Makoto Sakai[1], **Yukihisa Watase**[2], Kohei Ushio[1], Haruki Ishikawa[2], Masaaki Fujii[1], Shinobu Nagase[3], Takashi Itou[3] ([1]*Tokyo Institute of Technology*, [2]*Kitasato University*, [3]*Kao Corporation*)

2P293 **筋芽細胞のインパルス応答特性とひずみエネルギ計測**
Measurements of impuls responce and strein energy for a single myoblast
Takayuki Hoshino, Yuki Miyazako, Akira Wagatsuma, Kuihiko Mabuchi (*IPC, UTokyo*)

2P294 **マイクロ電極アレイ上における非拍動性単一細胞のインピーダンスベースによる電気生理学的解析**
Non-firing impedance-based electrophysiological analysis of single cells on micro-electrode arrays
Kenji Matsuura[1], Akihiro Hattori[1], Fumimasa Nomura[2], Hideyuki Terazono[2], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental Univ.*)

2P295 **a-Si:H 光機能制御可能な生体分子固体電解質によるバイオセンサ**
Biosensor using electrochemical biomolecular element photo-controlled on hydrogenated amorphous silicon film
Yutaka Tsujiuchi[1], Hiroshi Masumoto[2], Takashi Goto[3] ([1]*Dept of Mat Sci & Eng, Akita Univ*, [2]*FRIS, Tohoku Univ*, [3]*Inst for Mat Res, Tohoku Univ*)

27. バイオイメージング / 27. Bioimaging

2P296 **時間イメージ相関分光法によるダイナミック生細胞内オルガネラ解析**
Live cell analysis of organelle dynamics using temporal image correlation spectroscopy
Yasuo Takahashi, Isao Sakane (*Olympus Corporation*)

2P297 **マウス耳介内がん細胞およびがん組織の非侵襲イメージング**
Noninvasive *in vivo* imaging of tumor cells and tissue in mouse auricles
Sayaka Kita (*Dept. of Physics, University of Tokyo*)

2P298 **炎症反応抑制タンパク質 PDLIM2 の活性化分子機構の解明**
The elucidation of the molecular mechanism of PDLIM2 activation
Satoshi Toriyama[1,2], Yuma Ito[1,2], Takashi Tanaka[2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

2P299 **超解像光学顕微鏡の動画から見積もったアクチンのダイナミクス**
Dynamics of actin and actin assoicate proteins estimated from superresolution image data
Kaoru Katoh[1,2], Saori Mimatsu[1,2], Minami Tanaka[1,2] ([1]*Biomed RI, AIST*, [2]*Grad. Sch. Life & Enviromental Sci.*)

2P300 **2 軸クライオ電子線トモグラフィーと光学顕微鏡同視野観察を用いた無傷細胞の 3D-イメージングへの挑戦**
The challenge to intact cell 3D-imaging by dual-axis Cryo-electron tomography and correlative light imaging
Ruriko Ogawa[1], Takako M. Ichinose[1], Rina Nagai[1], Kazuhiro Aoyama[2,3], **Atsuko H. Iwane**[1,2] ([1]*Cell Field Struc., QBiC, Riken*, [2]*Spec. Res. Promot. Group, Grad. Sch. Fronti. Biosci., Osaka Univ.*, [3]*Application Lab., FEI JAPAN*)

2P301 **FIB（Focused Ion Beam: 集束イオンビーム加工）-SEM による全細胞レベル、ナノスケール分解能での細胞周期の可視化**
Visualization of cell cycle by three-dimensional FIB-SEM with nanoscale resolution at whole cell level
Rina Nagai[1], Keisuke Ohota[1,2], Takako M. Ichinose[1], Akinobu Togo[2], Atsuko H. Iwane[1,3] ([1]*Cell Field Struc., QBiC, Riken*, [2]*Anatomy, Med., Kurume Univ.*, [3]*Spec. Res. Promot. Group, Grad. Sch. Fronti. Biosci., Osaka Univ.*)

2P302 **1 型リアノジン受容体 N 末領域における悪性高熱症に関わる機能的変異**
Functional mutations in N-terminal region of type 1 ryanodine receptor in malignant hyperthermia
Toshiko Yamazawa[1], Takashi Murayama[2], Hideto Oyamada[3], Junji Suzuki[4], Nagomi Kurebayashi[2], Kazunori Kanemaru[4], Maki Yamaguchi[1], Shigeru Takemori[1], Masamitsu Iino[4] ([1]*Dept Mol. Physiol., Jikei Univ. Sch. Med.*, [2]*Dept. Pharmacol., Juntendo Univ. Sch. Med.*, [3]*Dept. Pharmacol., Sch. Med., Showa Univ.*, [4]*Dept. Pharmacol., Grad. Sch. Med., The Univ.Tokyo*)

2P303 **Development of Nano Electrochemical Mircoscope for living cell imaging**
Yasufumi Takahashi[1], Sen Mustafa[2], Yoshiharu Matsumae[2], Kosuke Ino[2], Hitoshi Shiku[2], Tomokazu Matsue[1,2] ([1]*Tohoku University, WPI-AIMR*, [2]*Tohoku University, Graduate School of Environmental Studies*)

2P304 **Real-time fluorescence imaging of quantum dot-loaded single synaptic vesicles**
Masashi Ohmachi, Tomoyuki Takahashi (*OIST*)

2P305 **原子間力顕微鏡や表面力測定装置によって測定されたフォースカーブを元に溶媒和構造を計算する方法**
A method for calculating the solvation structure from force curves measured by atomic force microscopy and surface force apparatus
Ken-ichi Amano (*Grad. Sch. Eng., Kyoto Univ.*)

2P306 **細胞における緑色蛍光タンパク質のカソードルミネッセンス**
Cathodeluminescence of Green Fluorescent Protein in Cell
Kazuyoshi Murata[1], Ryusuke Ueno[2], Naoki Yamamoto[3], Hideji Murakoshi[1], Kuniaki Nagayama[4], Hiroki Minoda[2] ([1]*National Institute for Physiological Sciences*, [2]*Tokyo University of Agriculture and Technology*, [3]*Tokyo Institute of Technology*, [4]*The Graduate University for Advanced Studies (SOKENDAI)*)

2P307 **細胞内分子混雑感受性蛍光蛋白質の開発**
Glycine-inserted mutant Forster resonance energy transfer (FRET) fluorescent protein to evaluate intracellular crowding
Takamitsu Morikawa[1], Hiroaki Machiyama[2], Kazuko Okamoto[3], Keiko Yoshizawa[3], Hideaki Fujita[2,3], Taro Ichimura[3], Katsumi Imada[4], Takeharu Nagai[5], Toshio Yanagida[1,2,3], Tomonobu Watanabe[1,2,3] ([1]*Graduate School of Frontier Bioscience, Osaka University*, [2]*WPI, Immunology Frontier Research Center, Osaka University*, [3]*RIKEN Quantitative Biology Center (QBiC)*, [4]*Department of Macromolecular Science, Graduate School of Science, Osaka University*, [5]*Institute of Sciencific and Industrial Research Center, Osaka University*)

2P308 **赤外超解像イメージングによる毛髪 α-ケラチンの分子配向観察**
Orientation-sensitive IR super-resolution imaging of human hair α-keratins
Kohei Ushio[1], Yukihisa Watase[2], Haruki Ishikawa[2], Masaaki Fujii[1], Makoto Sakai[1] ([1]*Tokyo Institute of Technology*, [2]*Kitasato University*)

2P309 **オルガネラの低温コヒーレント X 線回折イメージング**
Cryogenic coherent X-ray diffraction imaging of cellular organelle particles
Yuki Sekiguchi[1,2], Amane Kobayashi[1,2], Saki Hashimoto[1,2], Tomotaka Oroguchi[1,2], **Masayoshi Nakasako**[1,2], Yuki Takayama[2], Koji Yonekura[2], Masaki Yamamoto[2], Yayoi Inui[3], Sachihiro Matsunaga[3], Yuichi Ichikawa[4], Hitoshi Kurumizaka[4], Mitsuhiro Shimizu[5] ([1]*Sci. Tech., Keio Univ.*, [2]*RIKEN SPring-8 Center*, [3]*Sci. Tech., Tokyo Univ. Sci.*, [4]*Sci. Tech., Waseda Univ.*, [5]*Sci. Tech., Meisei Univ.*)

2P310 **Real-Time Observation of Single Macromolecular Rotation Using Gold Nanorods**
Wen-Hsuan Chang, Hung-Wen Li (*Department of Chemistry, National Taiwan University*)

2P311 **生細胞における 1 分子内在性 mRNA イメージングのためのアンチセンスプローブの開発**
Development of potent antisense probes for imaging individual endogenous mRNA in live cells
Shunsuke Takeda[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad. Sch. Pharma. Sci., Univ of Tokyo*, [2]*JST, PRESTO*)

2P312 **広範囲な細胞内カルシウム濃度に対応する GECO 変異体系列**
A series of GECO mutants suitable for calcium imaging in a wide range of calcium concentration
Morio Ohki[1,2], Yuma Ito[1,2], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

2P313 **ラスター画像相関分光法の画像取得条件最適化と生細胞の定量解析への応用**
Optimization in raster image correlation spectroscopy and application of quantitative live cell measurements
Takashi Horio[1], Johtaro Yamamoto[2], Akira Sasaki[3], Masataka Kinjo[2] ([1]*Lab. Mol. Cell Dynamics, Grad. Life Sci., Hokkaido Univ.*, [2]*Lab. Mol. Cell Dynamics, Fac. Adv. Life Sci., Hokkaido Univ.*, [3]*Biomedical Research Inst., AIST.*)

28. バイオエンジニアリング / 28. Bioengineering

2P314 **リポソーム融合法を用いたハイブリッドエクソソームの構築**
Development of hybrid exosomes by liposome fusion
Yuko Sato[1,2], Kaori Umezaki[1,2], Shin-ichi Sawada[1,2], Sada-atsu Mukai[1,2], Kazunari Akiyoshi[1,2] ([1]*Grad. Sch. of Engineering, Kyoto Univ.*, [2]*JST-ERATO*)

2P315 **自律移動人工アメーバの構築に向けて**
Toward creating an autonomous mobile artificial amoeba
Yoshiaki Tanaka[1], Yuichi Hiratsuka[2], Kei Fujiwara[3], Satoshi Murata[1], Shin-ichiro M. Nomura[1] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*Sch. Matl. Sci., JAIST*, [3]*Grad. Sch. Sci. Tech., Keio Univ.*)

2P316 **96 穴 ANSI/SBS プラットフォームの 3 分間超高速 PCR と融解曲線分析に向けた温度均質性と正確な温度制御技術の開発**
A temperature-control technique with great accuracy and uniformity for a ANSI/SBS plate for 3-min PCR and a melting curve analysis
Hideyuki Terazono[1,2], Hyonchol Kim[2], Kenji Matsuura[2], Akihiro Hattori[2], Fumimasa Nomura[1], Kenji Yasuda[1,2] ([1]*Tokyo Med. Dent. Univ.*, [2]*Kanagawa Acad. Sci. Tech.*)

2P317 **Peptide-based ligand screening system for G protein-coupled receptors (GPCRs) using water-in-oil microdroplets**
Takashi Sakurai[1], Ryo Iizuka[1], Yasuyuki Nakamura[2], Jun Ishii[3], Rui Sekine[4], Yoon Dong H.[4], Tetsushi Sekiguchi[5], Akihiko Kondo[2], Shuichi Shoji[4], Takashi Funatsu[1] ([1]*Grad. Sch. of Pharm. Sci., Univ. of Tokyo*, [2]*Grad. Sch. of Sci. and Tech., Kobe Univ.*, [3]*Org. of Advanced Sci. and Tech., Kobe Univ.*, [4]*Major in Nanosci. and Nanoeng., Waseda Univ.*, [5]*Nanotech. Research Center, Waseda Univ.*)

2P318 **96 ウエル SBS フォーマットサンプルの同時実時間解析のための光学系の開発**
Investigation of wide range optical set-up for simultaneous real-time analysis of 96-well SBS formatted samples
Akihiro Hattori[1], Hideyuki Terazono[2], Kenji Matsuura[1], Hyonchol Kim[1], Masao Odaka[1], Mathias Girault[1], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental University*)

2P319 **ナノポアとナノスリットにおける DNA 通過ダイナミクス**
DNA dynamics and translocations though solid-state nanopore and nanoslit
Yuta Kato, Shohei Kawaguchi, Kensaku Shibasaki, Kentaro Ishida, Toshiyuki Mitsui (*Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

2P320 **電極付加ナノポアの DNA 通過とその挙動解析**
DNA motions near a nanopore with a voltage controlled gate embedded in dielectrics
Shohei Kawaguchi, Yuta Kato, Kensaku Shibasaki, Kentaro Ishida, Toshiyuki Mitsui (*Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

2P321 **マイクロ波でアシストされた蛋白質加水分解酵素の速度論解析**
Kinetic analysis of microwave assisted enzymatic protein digestion
Arata Shiraishi[1], Hiroya Osoegawa[1], Takeo Yoshimura[1,2], Shokichi Ohuchi[1] ([1]*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Biol. Sci. Tokyo Univ. Sci*)

2P322 **精密な温度制御下でのマイクロ波照射酵素反応**
Microwave irradiated enzyme reaction under controlled temperature
Kengo Kawachi, Fujiko Aoki, Arata Shiraishi, Shokichi Ohuchi (*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*)

2P323 **好熱菌のマイクロ波加熱培養**
Microwave heating cultivation of Thermus thermophilus
Wataru Nagayoshi[1], Ryota Nakama[1], Takeo Yoshimura[2], Makoto Kodama[3], Shokichi Ohuchi[1] ([1]*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. Sci.*, [3]*Vessel Inc.*)

第 3 日目（9 月 27 日（土））／Day 3（Sep. 27 Sat.）大ホール / Main Hall

01A. 蛋白質：構造 / 01A. Protein: Structure

3P001 **各種蛍光タンパク質が疎水的環境下において示す蛍光特性の違いに関する構造学的アプローチ**
Different sensitivity of various fluorescent proteins to hydrophobic environments
Suguru Asai, Hide A. Konishi, Kunio Takeyasu, Shige H. Yoshimura (*Grad. Schl. Biostudies., Univ. Kyoto*)

3P002 **溶液中で配向させたコラーゲンの X 線繊維回折**
X-ray diffraction study of aligned collagen fiber
Yasunobu Sugimoto[1,2], Sakurako Hayashi[3], Sayaka Hayashi[2], Nobuhisa Watanabe[1,2], Shinji Kamimura[4], Takanori Kihara[5] ([1]*Nagoya Univ. Synchrotron Radiation Research Center*, [2]*Grad. Sch. Eng., Nagoya Univ.*, [3]*Fac. Eng., Nagoya Univ.*, [4]*Fac. Sci. & Eng., Chuo Univ.*, [5]*Fac. Environmental Eng., Univ. Kitakyushu*)

3P003 **セグメンテーション＆フィッティング - 低解像度密度マップへの複数のサブユニットのあてはめ計算法 -**
Segmentation & fitting algorithm for multiple subunit fitting into a low resolution density map
Takeshi Kawabata, Hirofumi Suzuki, Haruki Nakamura (*Inst. Prot. Res., Osaka Univ.*)

3P004 **構造データベース中の 3 次元電子顕微鏡データの形状比較とフィッティング**
Shape comparison of 3D electron microscopy data using both feature-vectors and GMM-based superimpositions
Hirofumi Suzuki[1,2], Takeshi Kawabata[1], Haruki Nakamura[1,2] ([1]*IPR, Osaka-u*, [2]*PDBj*)

3P005 **スピンラベルタンパクの変性過程におけるダイナミックな電子スピン共鳴線形の解析**
An analysis of Dynamic Electron Paramagnetic Resonance Lineshape for a Denaturation Process of Spin-labeled Protein
Yasunori Ohba[1], Tetsuya Itabashi[1], Munehito Arai[2], Jun Abe[3], Toshikazu Nakamura[3], Satoshi Takahashi[1], Seigo Yamauchi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Art and Sci, Univ. Tokyo*, [3]*IMS*)

3P006 **TEM 単粒子解析と大気圧電子顕微鏡（ASEM）によるタンパク質複合体形成の観察**
TEM single particle reconstruction and atmospheric SEM of protein complex formations
Chikara Sato, Kazuhiro Mio, Nassirhadjy Memtily, Mari Sato, Tatsuhiko Ebihara, Toshihiko Ogura (*Biomed-Ri., AIST*)

3P007 **Systematic structural study of single amino acid insertion mutants of YFP**
Rumika Tanaka[1], Keiko Yoshizawa[2], Tomonobu Watanabe[2], Tatsuya Kawaguchi[1], Katsumi Imada[1] ([1]*Grad. Sch. Sci. Osaka Univ.*, [2]*QBiC, Riken.*)

3P008 **分子動力学シミュレーションを用いた Hras-GTP 複合体の溶媒水と複合体の水素結合の解析**
Analysis of hydrogen bonds between solvent water and atoms in the Hras-GTP complex by molecular dynamics simulations
Miyakawa Takeshi[1], Ryota Morikawa[1], Masako Takasu[1], Kimikazu Sugimori[2], Kazutomo Kawaguchi[2], Hiroaki Saito[2], Hidemi Nagao[2] ([1]*Tokyo Univ. of Pharmacy and Life Sci.*, [2]*Kanazawa Univ.*)

3P009 **固体 NNR を用いたヌクレオソームにおけるヒストン H2A、H4 の構造解析**
Structural analysis of histone H2A, H4 in nucleosome using by solid-state NMR
Yasuto Todokoro[1], Yoshihito Moriwaki[2], Aritaka Nagadoi[2], Hiroaki Tachiwana[3], Hitoshi Kurumizaka[3], Yoshifumi Nishimura[2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*, [3]*Sch. Adv. Sci. & Eng., Waseda Univ.*)

3P010 **細胞接着ペプチドと α2β1 インテグリン I ドメインとの結合シミュレーション**
Docking simulation of cell adhesion peptide and α2β1 integrin I domain
Hironao Yamada[1], Takeshi Miyakawa[1], Ryota Morikawa[1], Fumihiko Katagiri[2], Kentaro Hozumi[2], Yamato Kikkawa[2], Motoyoshi Nomizu[2], Masako Takasu[1] ([1]*Sch of Life Sci, Tokyo Univ of Pharm and Life Sci*, [2]*Sch of Pharm, Tokyo Univ of Pharm and Life Sci*)

3P011 **DFTB+ソフトウェアへの REUS 法の導入**
Implementation of Replica-Exchange Umbrella Sampling to the DFTB+ Simulation Package
Shingo Ito[1], Yuko Okamoto[1], **Stephan Irle**[2,3] ([1]*Dept. Phys., Grad. Sch. Sci., Univ. Nagoya*, [2]*Dept. Chem., Grad. Sch. Sci., Univ. Nagoya*, [3]*WPI-Institute of Transformative Bio-Molecules*)

3P012 **Preliminary study of voltage-gated proton channel in activated state for X-ray crystallography**
Wataru Kumano[1], Kohei Takeshita[1,2], Kohta Emura[1], Eiki Yamashita[1], Yasushi Okamura[3], Atsushi Nakagawa[1] ([1]*IPR., Osaka Univ.*, [2]*IAI., Osaka Univ.*, [3]*Grad. Sch. Med., Osaka Univ.*)

3P013 **アミロイドβの構造探索 II**
Conformational Search of Amyloid β Peptide II
Satoshi Yokojima[1,2] ([1]*Tokyo Univ. of Pharmacy and Life Sci., School of Pharmacy*, [2]*RIKEN*)

3P014 **蛋白質の二次構造形成に関わる相互作用に関する理論的研究**
Theoreical analysis of molecular interactions in secondary strucutures of proteins
Yu Takano, Ayumi Kusaka, Haruki Nakamura (*IPR, Osaka University*)

3P015 **コレラ菌走化性受容体 Mlp24,Mlp37 のリガンド認識機構**
Ligand recognition mechanism of Mlp24 and Mlp37, chemoreceptor proteins of *Vibrio cholerae*
Yohei Takhashi[1], Kazumasa Sumita[1], Yumiko Uchida[1], So-ichiro Nishiyama[2], Ikuro Kawagishi[2], Katsumi Imada[1] ([1]*Grad. Sch. Sci. Osaka Univ.*, [2]*Dept. Front. Biosci. Sci., Hosei Univ.*)

3P016 **DFG-out コンフォメーションを持つ MEK1 構造**
Structure of MEK1 in DFG-Out conformation
Setsu Nakae[1], Daishuke Fujiwara[2], Katsuya Doko[2], Tsuyoshi Shirai[1], Toshiji Tada[2] ([1]*Dept. BioSci., Nagahama Inst. Bio-Sci. Tech.*,, [2]*Grad. Sch.Sci., Osaka Pref. Univ.*)

3P017 **2D hybrid analysis: A new approach to build 3D atomic model from 2D EM image**
Atsushi Matsumoto[1], Junichi Takagi[2], Kenji Iwasaki[2] ([1]*JAEA*, [2]*Osaka University*)

3P018 **Structural analysis of the intron-encoded domain of herstatin**
Daisuke Tashiro[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dept. of Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

3P019 **分子動力学法を用いた高圧下における c-Myb R2 ドメインのキャビティ圧縮と構造変化および揺らぎとの関係**
A relationships among compression of the cavity of c-MybR2, conformational changes and fluctuation under high-pressure using MD simulation
Takuya Sogabe[1], Hisashi Yoshida[1], Kazuyuki Akasaka[2] ([1]*Graduate school of Biology-Oriented Science and Technology, Kinki University*, [2]*Department of Computational Systems Biology, Faculty of Biology-Oriented Science and Technology, Kinki University*)

01B. 蛋白質：構造機能相関 / 01B. Protein: Structure & Function

3P020 **ヒスタミン H_1 受容体の分子内情報伝達機構に関する理論的研究**
Computational study on the intramolecular signaling mechanism of histamine receptor
Yuko Ishii, Takakazu Ishikura, Takahisa Yamato (*Grad. Sch. Sci., Nagoya Univ.*)

3P021 **KcsA カリウムチャネルの開構造への構造変化前における分子揺らぎの増大**
The Enhancement of Structural Fluctuations Prior to The Opening Conformational Changes of The KcsA Potassium Channel
Hirofumi Shimizu, Masayuki Iwamoto, Yumiko Oota, Shigetoshi Oiki (*Univ.Fukui.Fac.Med.Sci*)

3P022 **Coarse-grained Generalized Born and surface area models and its application to protein docking**
Le Chang[1], Wenfei Li[2], Naoto Hori[1], Shoji Takada[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Dept. Phys., Nanjing Univ.*)

3P023 **Factor Xa に対する薬剤候補分子の結合自由エネルギー:3D-RISM 計算**
Predicting binding free energy of drug candidates to Factor Xa : 3D-RISM study
Sayaka Kohara[1], Masatake Sugita[2], Masanari Matsuoka[1], Takeshi Kikuchi[1], Fumio Hirata[1] ([1]*Dept. Bioinf., Col. Life Sci., Ritsumeikan Univ.*, [2]*Res. Org. Sci. Tech., Ritsumeikan Univ.*)

3P024 **多剤排出トランスポーター AcrB の構造回転機構に対する物理描像**
Physical Picture for Mechanism of Conformational Rotation of Multidrug Transporter AcrB
Hirokazu Mishima[1], **Hiraku Oshima**[2], Satoshi Yasuda[2], Masahiro Kinoshita[2] ([1]*Grad. Sch. Energ. Sci., Kyoto Univ.*, [2]*Inst. Adv. Energ., Kyoto Univ.*)

3P025 **Conformational motions in protein machines: elastic-network computational studies**
Holger Flechsig (*Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University*)

3P026 **野生型および変異型 CYP2B6 の柔らかさが薬物代謝に与える影響の推定**
Computational studies for the influences of protein flexibilities on enzymatic activities of the wild type and mutants of CYP2B6
Akifumi Oda[1,2], Kana Kobayashi[3], Yurie Watanabe[1], Shuichi Fukuyoshi[1], Masahiro Hiratsuka[4], Noriyuki Yamaotsu[5], Shuichi Hirono[5], Ohgi Takahashi[3] ([1]*Faculty of Pharmacy, Inst. Med. Pharm. Health Sci., Kanazawa Univ.*, [2]*Inst. Protein Res., Osaka Univ.*, [3]*Faculty of Pharm. Sci., Tohoku Pharm. Univ.*, [4]*Grad. Sch. Pharm. Sci., Tohoku Univ.*, [5]*Sch. Pharmacy, Kitasato Univ.*)

3P027 **タンパク分子内の力学的情報伝達の特徴付け―粗視化モデルによる試み**
Classification of Mechanical Communication in Proteins: A Coarse-Grained Study
Yuichi Togashi (*RCMCD, Grad. Sch. Sci., Hiroshima Univ.*)

3P028 **粗視化分子シミュレーションによる MEK1-ERK2 相互作用の調査**
Interactions of MEK1 with ERK2 in mammalian MAPK pathway studied by coarse-grained molecular simulations
Ryo Kanada, Shoji Takada (*Grad. Sch. Sci., Univ. Kyoto*)

3P029 **キャビティーが蛋白質機能を制御する**
Cavity controls protein function
Kazuyuki Akasaka (*Kinki University Institute of Advanced Technology High Pressure Protein Research Center*)

3P030 **ソーシャブルなリガンド結合部位の構造的また物理化学的特徴に関する研究**
A Study for the Structural and Physicochemical Properties of Sociable Ligand-Binding Sites in Proteins
Yoichi Murakami, Kengo Kinoshita (*Graduate School of Information Sciences, Tohoku University*)

3P031 **単一シアル酸オリゴ糖上でのマイコプラズマの滑走と結合**
Gliding and binding of mycoplasma on uniform sialylated oligosaccharide
Taishi Kasai, Tasuku Hamaguchi, Makoto Miyata (*Osaka City University, Graduate School of Science*)

3P032 **抗 HIV 因子 APOBEC3G の基質認識及びスライディング機構の実時間 NMR 解析**
Substrate Recognition and Sliding Properties of an Anti-HIV Factor APOBEC3G analyzed by Real-time NMR Monitoring Strategy
Keisuke Kamba[1,2], Takashi Nagata[1,2], Masato Katahira[1,2] ([1]*Inst. of Advanced Energy, Kyoto Univ.*, [2]*Grad. Sch. of Energy Science, Kyoto Univ.*)

3P033 **セルラーゼ TrCel7A の基質取り込み機構に関する分子シミュレーション研究**
Molecular simulation study on the mechanism of substrate uptake in cellulase TrCel7A
Takashi Kanazawa, Minoru Sakurai, Tadaomi Furuta (*Center for Biol. Res. Info., Tokyo Tech*)

3P034 **レプリカ交換 MD 及びフラグメント MO 計算によるアミロイドβダイマーの水中での安定構造の探索**
Replica exchange MD and ab initio fragment MO calculations for searching stable conformations of amyloid-b dimer in water
Hiromi Ishimura, Akisumi Okamoto, Atsushi Yano, **Noriyuki Kurita** (*Toyohashi University of Technology*)

3P035 **Elastic Network Model を用いた ABC トランスポーターの global motion の解析**
Global motion of ABC transporters using nonlinear relaxation dynamics in elastic network model
Naoki Arai, Tadaomi Furuta, Minoru Sakurai (*Center for Boil. Res. & inform., Tokyo Tech*)

3P036 **一酸化炭素型ヘモグロビンの光解離中間体のＸ線結晶構造**
X-ray crystal structures of carbonmonoxy hemoglobin photolysis intermediates
Ayana Tomita[1], Tokushi Sato[1], Hiroki Noguchi[2], Shunsuke Nozawa[1], Shin-ya Koshihara[3], Sam-Yong Park[2], Naoya Shibayama[4], Shin-ichi Adachi[1,5] ([1]*Photon Factory, KEK*, [2]*Gra. Sch. Nanobiosci., Yokohama City Univ.*, [3]*Dep. Mat. Sci., Tokyo Tech/JST-CREST*, [4]*Div. Biophys., Jichi Med. Univ.*, [5]*JST-PREST*)

3P037 **抗体デザイン手法の開発に向けた抗原認識機構の解明**
Elucidation of antigen recognition by antibodies toward the development of a method for antibody design
Yuko Tsuchiya, Kenji Mizuguchi (*NIBIO*)

3P038 **MD シミュレーションで探るマルトーストランスポーター ATP アーゼ（MalK）のダイナミクスと構造変化**
Dynamics and Structural Changes of Maltose Transporter ATPase (MalK) as studied by MD simulations
WeiLin Hsu, Tadaomi Furuta, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

3P039 **Structural analysis of the 26S proteasome by cryo-electron microscopy and Single-Particle Analysis**
Zhuo Wang[1], Yasuo Okuma[1], Daiske Kasuya[2], Kaoru Mitsuoka[3], Yasushi Saeki[4], Takuo Yasunaga[1] ([1]*Department of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology*, [2]*Biomedicinal Information Research Center, Japan Biological Information Consortium (JBIC)*, [3]*Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology*, [4]*Laboratory of Protein Metabolism, Tokyo Metropolitan Institute of Medical Science*)

3P040 **高速原子間力顕微鏡による Kai タンパク質間相互作用の観察**
Interactions between Kai Proteins observed by high-speed AFM
Shogo Sugiyama[1], Mori Tetsya[2], Takayuki Ucihashi[1,3], Carl H. Johnson[2], Toshio Ando[1,3] ([1]*Dept. of phys., Univ. Kanazawa*, [2]*Dept. of Biol. Sci., Univ. Vanderbilt*, [3]*Bio-AFM FRC., Univ. Kanazawa*)

3P041 **高速 AFM による細胞質ダイニンの動態観察**
Observation of structural dynamics of cytoplasmic dynein by High Speed AFM
Yusuke Kumagai[1], Takayuki Uchihashi[1,2], Yoko Toyoshima[3], Muneyoshi Ichikawa[3], Toshio Ando[1,2] ([1]*College of Science and Engineering, Kanazawa University*, [2]*Bio-AFM Frontier Research Center, College of Science and Engineering, Kanazawa University*, [3]*The University of Tokyo*)

3P042 **ハミルトニアンレプリカ置換分子動力学法の Aβ フラグメントへの応用**
Applications of the Hamiltonian replica-permutation molecular dynamics simulations to Aβ fragments
Satoru G. Itoh[1,2], Hisashi Okumura[1,2] ([1]*IMS*, [2]*Sokendai*)

3P043 **Mutagenesis study of an antifreeze protein isoform from a snow-mold fungus, Typhula ishikariensis**
Jing Cheng[1,2], Yuichi Hanada[1,2], Hidemasa Kondo[1,2], Sakae Tsuda[1,2] ([1]*Grad. Sch. Life. Sci., Hokkaido Univ*, [2]*Biopro. Res. Inst., AIST*)

3P044 **Thg1-like タンパク質の機能構造解析**
The functional and structural analysis of Thg1-like protein
Shoko Kimura[1], Tateki Suzuki[1], Jian Yu[2], Keisuke Komoda[3], Isao Tanaka[2], Min Yao[1,2] ([1]*Grad. Sch. Life Sci., Univ. Hokkaido*, [2]*Fac. Adv. Life Sci., Univ. Hokkaido*, [3]*Grad. Sch. Agri Life Sci., Univ. Tokyo*)

3P045 **Staphylococcal nuclease と Δ44-49 変異体の構造揺らぎの解析：酵素活性への洞察**
Analysis of the structural fluctuation in Staphylococcal nuclease and its Δ44-49 mutant: Insight into the enzymatic activity
Kana Fuji[1], Hiroshi Fujisaki[2], Tadaomi Furuta[3], Rumi Shiba[4], Mikito Toda[1] ([1]*Nara Women's Univ.*, [2]*Nippon Med. Sch.*, [3]*Tokyo Tech*, [4]*JAIST*)

3P046 **サルモネラ菌べん毛繊維の多型変換における Glu114 と Glu121 の役割**
The roles of Glu114 and Glu121 of flagellin in the polymorphic transformation of Salmonella flagellar helical filament
Atsushi Ujiie, Fumio Hayashi, Kenji Oosawa (*Div. Mol. Sci, and Tech, Gunma Univ*)

3P047 **Building an Artificial Protein Capsid**
Jonathan Heddle (*RIKEN*)

01C. 蛋白質：物性 / 01C. Protein: Property

3P048 **溶解性制御タグ（SCP タグ）によるタンパク質結晶化の解析**
Analysis of protein crystallization using short Solubility Controlling Peptide tags
Yutaka Kuroda, Mohammad Islam (*TUAT, Dept of Biotech and Life Sci*)

3P049 **Multimodal chromatography of proteins in arginine solutions**
Atsushi Hirano[1], Tsutomu Arakawa[2], Tomoshi Kameda[3] ([1]*NRI, AIST*, [2]*Alliance Protein Lab.*, [3]*CBRC, AIST*)

3P050 **フェリチン・ヘテロオリゴマーの作製**
Construction of ferritin hetero-oligomer
Atsushi Kurobe, Satsuki Takebe, Daisuke Sato, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. Bioinfo., Soka Univ.*)

3P051 **蛋白質系の静電自由エネルギーにおける有限サイズ効果**
Finite-size effect on the charging free energy for protein system
Toru Ekimoto[1], Nobuyuki Matubayasi[2], Mitsunori Ikeguchi[1] ([1]*Yokohama City Univ.*, [2]*Osaka Univ.*)

3P052 **Analysis for the structural stability of chignolin**
Yutaka Maruyama, Ayori Mitsutake (*Dep. Phys., Keio Univ.*)

3P053 **圧力効果を用いたシトクロム c の立体構造形成過程における脱水和機構の解析**
Dehydration in cytochrome c folding revealed by high pressure spectroscopy
Shohei Konno[1], Kentaro Doi[1], Takeshi Uchida[1,2], Koichiro Ishimori[1,2] ([1]*Grad. Sch. of Chem. Sci. and Eng., Hokkaido Univ.*, [2]*Fac. of Sci., Hokkaido Univ.*)

3P054 **蛍光分光法による時計タンパク質 KaiC の構造変化の解析**
Conformational transition of a cyanobacterial clock protein KaiC monitored with fluorescence spectroscopy
Atsushi Mukaiyama[1,2], Shuji Akiyama[1,2,3] ([1]*CIMoS, IMS*, [2]*Grad. Univ. for Adv. Studies*, [3]*RIKEN, SPring-8*)

3P055 **Improved Multi-Replica Metadynamics for Free Energy Calculations**
Raimondas Galvelis[1], Yuji Sugita[1,2] ([1]*RIKEN TMSL*, [2]*RIKEN AICS*)

3P056 **タンパク質の高速折り畳みダイナミクスの一分子追跡を目指したライン共焦点顕微鏡の開発**
Development of the line confocal system for the single molecule tracking of fast folding dynamics of proteins
Hiroyuki Oikawa[1], Kiyoto Kamagata[1], Munehito Arai[2], Atsuhito Fukasawa[3,4], Hiroaki Yokota[4], Toru Ide[5], Satoshi Takahashi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Arts. Sci., Univ. Tokyo*, [3]*Hamamatsu Photonics*, [4]*GPI*, [5]*Grad. Sch. Nat. Sci. and Tech., Okayama Univ*)

3P057 **アミノ酸置換による蛋白質の熱安定性変化の理論的予測**
Theoretical Prediction of Thermal-Stability Changes upon Mutations of a Protein
Shota Murakami[1], Hiraku Oshima[2], Tomohiko Hayashi[2], Masahiro Kinoshita[2] ([1]*Grad. Sch. Energ. Sci., Kyoto Univ.*, [2]*Inst. Adv. Energ., Kyoto Univ.*)

3P058 **バクテリア細胞質中の生体高分子ダイナミクスと相互作用：全原子分子動力学による研究**
Dynamics and Interactions of Macromolecules in the Bacterial Cytoplasm: All-atom Molecular Dynamics Study
Isseki Yu[1], Tadashi Ando[2], Takaharu Mori[1], Jaewoon Jung[3], Ryuhei Harada[3], Yuji Sugita[1,2,3], Michael Feig[4] ([1]*RIKEN*, [2]*RIKEN QBIC*, [3]*RIKEN AICS*, [4]*Michigan State Univ.*)

3P059 **回転および並進運動から観たタンパク質間相互作用**
Protein-protein interaction revealed by the rotational and translational motion
Akane Kato[1], Etsuko Nishimoto[2] ([1]*Institute of Biophysics, Faculty of Agriculture, Graduate School of Kyushu University*, [2]*Molecular Bioscience, Bioscience and Biotechnology, Kyushu University*)

3P060 **蛋白質のドメイン間相互作用に及ぼすホフマイスター効果**

Hofmeister effect on the domain-domain interaction of protein

Tomohiro Aoyama[1], Etsuko Nishimoto[2] ([1]*Institute of Biophysics, Faculty of Agriculture, Graduate School of Kyushu University*, [2]*Molecular Bioscience, Bioscience and Biotechnology, Kyushu university*)

3P061 **The circumventing mechanism of the folding of β-lactoglobulin**

Kazumasa Sakurai[1], Masanori Yagi[2], Chiaki Nishimura[3], Kazuyuki Akasaka[1], Yuji Goto[4] ([1]*HPPRC, Inst. Adv. Technol., Kinki Univ.*, [2]*RIMD, Osaka Univ.*, [3]*Fac. Pharm. Sci., Teikyo Heisei Univ.*, [4]*Inst. Protein Res., Osaka Univ.*)

3P062 **アポミオグロビンのドメインスワッピングとフォールディングの競合:分子シミュレーション解析**

Monomer folding versus dimer domain-swapping in apo-myoglobin studied by molecular simulations

Koji Ono[1], Mashiho Ito[1], Shun Hirota[2], Shoji Takada[1] ([1]*Dept. Biophys., Grad. Sch. Sci., Kyoto Univ.*, [2]*Nara Inst. Sci. Tech.*)

3P063 **超音波によるアミロイド線維形成と分解**

Ultrasonication dependent induction and degradation of amyloid fibrils

Sayaka Noda, Masatomo So, Masayuki Adachi, Yuji Goto (*Inst. Protein Res., Osaka Univ.*)

3P064 **中性子散乱によるヒト α-シヌクレインのダイナミクス変化の検出**

Changes in the dynamics of human α-synuclein detected by neutron scattering

Satoru Fujiwara[1], Katsuya Araki[2], Tatsuhito Matsuo[1], Hisashi Yagi[3], Takeshi Yamada[4], Kaoru Shibata[5], Hideki Mochizuki[2] ([1]*QuBS, JAEA*, [2]*Osaka Univ. Grad. Sch. Med.*, [3]*Dept. Chem. Biotech., Grad. Sch. Eng., & GSC Cntr., Tottori Univ.*, [4]*CROSS-Tokai*, [5]*J-PARC Center*)

3P065 **変異体解析を用いた緑色蛍光蛋白質の安定化機構におけるヒスチジン残基の役割に関する研究**

The role of histidine residues with abnormal pK_a values on the stability of green fluorescent protein studied by mutagenesis approach

Taichi Andou, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ*)

3P066 **ウマアポミオグロビンの pH 4 中間体と塩による中間体の速度論的性質**

Kinetic properties of pH-induced and salt-induced intermediates of horse apomyoglobin

Yukiko Abe, Takuya Mizukami, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ.*)

01D. 蛋白質：機能 / 01D. Protein: Function

3P067 **大量のアルカンを合成するシアノバクテリア変異体の構築**

Toward the construction of the cyanobacterial mutants that produce high amounts of alkanes

Hisashi Kudo[1], Mai Watabnabe[1], Masahiko Ikeuchi[1], Munehito Arai[1,2] ([1]*Department of life sciences, the university of Tokyo*, [2]*PRESTO, JST*)

3P068 **アルカンを合成するラン藻由来アルデヒド脱カルボニル化酵素のアラニンスキャン変異解析**

Alanine scanning mutagenesis of cyanobacterial aldehyde decarbonylase that synthesizes alkanes

Fumitaka Yasugi[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

3P069 **Pre-steady state kinetic studies of redox reactions between FNR from *Bacillus subtilis* and its substrates**

Daisuke Seo[1], Hidehiro Sakurai[2], Pierre Setif[3], Takeshi Sakurai[1] ([1]*Div Mat Sci, Grad Sch of Nat Sci and Tec, Kanazawa Univ.*, [2]*Res Inst Photo Hyd Prod, Kanagawa Univ.*, [3]*IBiTec-S, CEA Saclay, France*)

3P070 **一分子蛍光顕微鏡による p 53 の標的配列探索ダイナミクスの観察**

Observation of the Search Dynamics of p53 for the Target DNA Sequence by Single-molecule Fluorescence Microscopy

Yuji Itoh[1,2], Agato Murata[1,2], Seiji Sakamoto[1,2], Takehiko Wada[1,2], Satoshi Takahashi[1,2], Kiyoto Kamagata[1,2] ([1]*IMRAM, Univ. Tohoku*, [2]*Grad. Sch. Sci., Univ. Tohoku*)

3P071 **銅含有亜硝酸還元酵素の亜硝酸還元メカニズムにおける計算化学研究**

Computational study on nitirite reduction mechanism in Copper-containing nitrite reductase

Masami Lintuluoto[1], Yohta Fukuda[2,4], Tsuyoshi Inoue[2], Yoshifumi Fukunishi[3] ([1]*Grad. Sch. of Life and Env. Sci., Kyoto Pref. Univ.*, [2]*Gad. Sch. of Eng. Osaka Univ.*, [3]*AIST*, [4]*Dep. Biochem. and Mol. Biophys. , Columbia Univ.*)

3P072 **ポリアミンは α-キモトリプシンの活性化剤として機能する**

Polyamines Act as an Enzyme Activator for α-Chymotrypsin

Takaaki Kurinomaru, Kentaro Shiraki (*Grad. Sch. Pure and Appl. Sci., Univ. Tsukuba*)

3P073 **膜表面の GM1 糖鎖に対する hGal-1 結合能「増大」**

"Increased" affinity of hGal-1 to GM1 on membrane surface

Ryota Hori, Hirotsugu Hiramatsu, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

3P074 **抗菌ペプチドを用いた病原性微生物検出系のための新規スクリーニング法の開発**

Novel screening method for detection system of pathogens using antimicrobial peptides

Tatsuyuki Koshiyama[1], Satoshi Tomisawa[1], Takashi Kikukawa[1], Yasuhiro Kumaki[1], Masakatsu Kamiya[1], Keiichi Kawano[2], Makoto Demura[1] ([1]*Grad. Sch. Life Sci., Hokkaido. Univ.*, [2]*Chitose Inst. Sci. Tech.*)

3P075 **抗菌ペプチド human defensin 5 の NMR による多量体形成機構の解析**

NMR analysis of the oligomerization mechanism of antimicrobial peptide human defensin 5

Arata Hashimoto, Satoshi Tomisawa, Masakatsu Kamiya, Takashi Kikukawa, Yasuhiro Kumaki, Kiminori Nakamura, Tokiyoshi Ayabe, Tomoyasu Aizawa, Makoto Demura (*Grad. Sch. Life Sci., Hokkaido Univ.*)

3P076 トレオニン合成酵素の反応制御機構解明のための分子動力学計算：反応中間体間自由エネルギー評価
Molecular dynamics study on the reaction control mechanism of threonine synthase: evaluating the free energies of the intermediate states
Yuzuru Ujiie[1], Wataru Tanaka[1], Mitsuo Shoji[1], Megumi Kayanuma[2], Yasuteru Shigeta[1], Yasuhiro Machida[3], Takeshi Murakawa[4], Hideyuki Hayashi[3] ([1]*Grad. Sch. of Pure & Appl. Sci., Univ. of Tsukuba*, [2]*Grad. Sch. of Sys. & Inf. Eng., Univ. of Tsukuba*, [3]*Dept. Chem., Osaka Medical College*, [4]*Dept. Biochem., Osaka Medical College*)

3P077 ウリジンシチジンキナーゼの基質結合相互作用についての理論的研究
A theoretical study on the substrate bindings in uridine-cytidine kinase
Wataru Tanaka[1], Yuzuru Ujiie[1], Fumiaki Tomoike[2], Mitsuo Shoji[1], Megumi Kayanuma[3], Ryoji Masui[4], Seiki Kuramitsu[4], Yasuteru Shigeta[1] ([1]*Grad. Sch. Pure & Appl. Sci., Univ. Tsukuba*, [2]*Inst. Indus. Sci., Univ. Tokyo*, [3]*Grad. Sch. Sys. & Inf. Eng., Univ. Tsukuba*, [4]*Grad. Sch. Sci., Osaka Univ.*)

3P078 **Role of FAD N5 proximal Asn residue in CPD-Photolyase**
I M. Mahaputra Wijaya[1], Tatsuya Iwata[1], Elizabeth D. Getzoff[2], Hideki Kandori[1] ([1]*Nagoya Institute of Technology, Japan*, [2]*Scripps Research Institute, USA*)

3P079 抗菌ペプチド cecropin P1 の大腸菌発現系における発現効率に影響を与える要因の解明
Elucidation of influential factor for productivity of the antimicrobial peptide using *Escherichia coli*
Chiharu Abe[1], Taichi Nakazumi[1], Masakatsu Kamiya[1], Takashi Kikukawa[1], Keiichi Kawano[1,2], Makoto Demura[1], Tomoyasu Aizawa[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Chitose Inst. Sch. Tech.*)

01E. 蛋白質：計測・解析の方法論 / 01E. Protein: Measurement & Analysis

3P080 ベイズ推定を用いた NMR 立体構造計算法の開発
A refinement method for NMR protein structure determination based on Bayesian inference
Teppei Ikeya[1], Yutaka Ito[1], Guentert Peter[1,2] ([1]*Tokyo Metropolitan University*, [2]*Goethe University Frankfurt*)

3P081 複雑分子系の異性化反応ネットワークに埋め込まれた時間階層構造の抽出
An extraction of hierarchical organization of embedded timescales buried in complex reaction network
Yutaka Nagahata[1], Satoshi Maeda[2], Hiroshi Teramoto[1,3], Chun-Biu Li[3], Takashi Horiyama[4], Tetsuya Taketsugu[2], Tamiki Komatsuzaki[1,3] ([1]*Graduate School of Life Science, Hokkaido Univ.*, [2]*Graduate School of Science, Hokkaido Univ.*, [3]*Research Institute for Electronic Science, Hokkaido Univ.*, [4]*Information technology center, Saitama Univ.*)

3P082 RI に依存しない高感度 MGMT 活性測定法の開発と新型マイクロアレイ MMV への適応化
Development of MGMT activity assay methods of high sensitivity and being adaptabe to the novel-concept microarray
Aya Hongo, Takuto Saiki, Ran Gu, Miho Suzuki, Naoto Nemoto, Koichi Nishigaki (*Grad. Sch. of Sci. and Eng., Univ. Saitama*)

3P083 1 分子イメージングによる PI3K の活性制御機構の解析
Analysis of the Regulation Mechanism of PI3K Activity by Live-cell Single-molecule Imaging
Seiya Fukushima[1], Satomi Matsuoka[2], Masahiro Ueda[1,2] ([1]*Graduate School of Science, Osaka University*, [2]*Riken Quantitative Biology Center*)

3P084 X 線自由電子レーザーを用いたパターンマッチング法による第一原理構造モデリングの検討
Examination of ab initio structural modeling for the pattern matching method using X-ray free electron laser
Atsushi Tokuhisa, Osamu Miyashita, Florence Tama (*Advanced Institute for Computational Science, RIKEN*)

3P085 X 線 1 分子追跡法を用いたタンパク質過飽和溶液中における核形成前駆体クラスターの動態観察
Dynamical Observations of Prenucleation Clusters in Supersaturated Protein Solution from Diffracted X-ray Tracking
Yufuku Matsushita[1], Hiroshi Sekiguchi[2], Keigo Ikezaki[1], Noboru Ohta[2], Yuji Goto[3], Yuji Sasaki[1] ([1]*The University of Tokyo*, [2]*JASRI/SPring-8*, [3]*Osaka University*)

01F. 蛋白質：蛋白質工学／進化工学 / 01F. Protein: Engineering

3P086 細菌由来アルブミン結合ドメインの接触表面の模倣によるアルブミン結合ヒト型タンパク質のデザイン
Design of an Albumin-Binding Humanized Protein by Mimicking the Contact Surface of a Bacterial Albumin-Binding Domain
Satoshi Oshiro[1], Shinya Honda[1,2] ([1]*Dept. of Medical Genome Sci., Grad. Sch. of Frontier Sci., The Univ. of Tokyo*, [2]*BioMed. Research Inst., AIST*)

3P087 ファージディスプレイ法で同定した白金結合アミノ酸配列の解析
Analysis of a platinum-binding amino acid sequence identified by phage display
Asumi Kaji, Hiroya Niiro, Satoshi Akanuma, Tatsuya Uchida, Akihiko Yamagishi (*Tokyo University of Pharmacy and Life Sciences*)

3P088 エングレイルドホメオドメインを用いた新たな転写因子の設計
Designing a new artificial transcription factor based on engrailed homeodomain
Tomoko Sunami, Hidetoshi Kono (*JAEA*)

3P089 **Design of a peptide nanotube having the capability of rare metal binding**
Keisuke Ogihara, Atsuo Tamura (*Univ. Kobe*)

3P090 脂肪酸アシル-ACP 還元酵素の迅速活性評価法の開発
Development of a high-throughput method to evaluate catalytic activity of fatty acyl-ACP reductase
Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Department of Life Science, The University of Tokyo*, [2]*PRESTO*)

02. ヘム蛋白質 / 02. Heme proteins

3P091 **線虫 cytochrome b561 ホモログ Cecytb-1 の生理機能解析**
Analyses on the physiological function of Cecytb-1, a cytochrome b561 homolog in Caenorhabditis elegans
Akie Tejima, Yurie Hirano, Masahiro Miura, Motonari Tsubaki (*Dept. of Chem., Grad. Sch. Sci., Kobe Univ.*)

3P092 **一酸化窒素還元酵素における基質 NO 結合の分子機構**
Molecular mechanism for substrate NO binding to bacterial Nitric Oxide Reductase
Shoko Ishii[1,2], Tetsunari Kimura[2], Takehiko Tosha[2], Yoshitsugu Shiro[1,2], Minoru Kubo[2,3] ([1]*Grad Sch. Life Sci., Univ. of Hyogo*, [2]*RIKEN, SPring-8 Center*, [3]*JST, PRESTO*)

3P093 **チトクローム *c* とチトクローム酸化酵素複合体の X 線結晶構造解析**
X-ray structural analysis of the cytochrome *c* and cytochrome *c* oxidase
Satoru Shimada[1,2], Kyoko Shinzawa-Itoh[1], Shimpei Aoe[1], Atsuhiro Shimada[1], Jumpei Baba[1], Syuhei Takemura[1], Eiki Yamashita[3], Tomitake Tsukihara[1,2,3], Shinya Yoshikawa[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*CREST, JST*, [3]*Inst. Protein Res., Osaka Univ.*)

3P094 **亜硝酸還元酵素と一酸化窒素還元酵素の相互作用解析**
Analysis of the Interaction between Nitric Oxide Reductase and Nitrite Reductase
Kimi Matsumoto[1], Erina Terasaka[1], Takehiko Tosha[2], Yoshitsugu Shiro[1,2] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*RIKEN SPring-8 Center*)

3P095 **ナノ構造電極上でのシトクローム P450 153A13a の電気化学触媒反応**
Electrochemically-driven CYP153A13a reaction at nanostructured electrode
Yasuhiro Mie[1], Naoya Fujita[2], Toshio Cho[2], Yasuo Komatsu[1] ([1]*Bioproduction Res. Inst., AIST*, [2]*KNC Laboratories Co., Ltd.*)

3P096 **一酸化炭素よりも酸素に対して高い親和性を示すミオグロビンの創製**
Preparation of myoglobin mutants exhibiting preferential binding of oxygen over carbon monoxide
Ryu Nishimura[1], Daichi Matsumoto[1], Tomokazu Shibata[1], Sachiko Yanagisawa[2], Takashi Ogura[2], Hulin Tai[3], Takashi Matsuo[3], Shun Hirota[3], Saburo Neya[4], Akihiro Suzuki[5], **Yasuhiko Yamamoto**[1] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*Grad. Sch. Life Sci., Univ. Hyogo*, [3]*Grad. Sch. Mater. Sci., NAIST*, [4]*Grad. Sch. Pharm. Sci., Chiba Univ.*, [5]*Dept. Mater. Eng., Nagaoka Natl. Coll. Tech.*)

3P097 **ヘモグロビンの R,T,不安定 T の酸素親和力の計算：MD シミュレーションによる自由エネルギー計算**
Oxygen affinity differences of hemoglobin between the R, T, an unstable T structures: By free energy calculations based on MD simulations
Minoru Saito (*Hirosaki University*)

03. 膜蛋白質 / 03. Membrane proteins

3P098 **Engineering of Channelrhodopsin with Specific Ion Selectivity**
Monica Patti, Rieko Kamii, Toru Ishizuka, Hiromu Yawo (*Tohoku University*)

3P099 **新規キメラタンパク質による G_s タンパク質の光制御**
Optical control of G_s-protein activity by novel chimeric proteins
Kazuho Yoshida[1], Keiichi Inoue[1,2], Takahiro Yamashita[3], Rei Abe-Yoshizumi[1], Kengo Sasaki[1], Yoshinori Shichida[3], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*JST PRESTO*, [3]*Grad. Sch. Sci., Univ. Kyoto*)

3P100 **Conformation and topology of pharaonis phoborhodopsin in the lipid environment as studied by solid-state NMR**
Izuru Kawamura[1], Satoshi Nakatani[1], Yoshiteru Makino[1], Naoki Kamo[2], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Hokkaido Univ.*)

3P101 **細胞膜モデル「ナノディスク」を用いたハロロドプシンの三量体形成が持つ機能的意義**
Functional significance of homotrimer formation in the Nanodisc-embedded Halorhodopsin
Kenshiro Suzuki[1], Ayumi Yamamoto[1], Takashi Tsukamoto[2], Yoshihiro Kobashigawa[4], Takeshi Uchida[1,3], Fuyuhiko Inagaki[4], Makoto Demura[2], Koichiro Ishimori[1,3] ([1]*Grad. Sch. of Chem. Sci. and Eng. Hokkaido Univ.*, [2]*Grad. Sch. of Life Sci. Hokkaido Univ.*, [3]*Fac. of Sci. Hokkaido Univ.*, [4]*Fac. of Adv. Life. Sci. Hookaido Univ*)

3P102 **凍結割断低温原子間力顕微鏡の製作**
Fabrication of freeze fracture cryogenic atomic force microscope
Naoto Kuga, Toshiaki Gotoh, Tsutomu Kouyama (*Graduate School of science, Nagoya University*)

3P103 **緑色イオウ細菌 *Chlorobaculum tepidum* の Rieske/cyt *b* 複合体単離の試み**
Isolation of Rieske/cytochrome *b* complex from a green sulfur bacterium *Chlorobaculum tepidum*
Hirozo Oh-oka[1], Kazuya Yamamoto[1,2], Risa Mutoh[2], Chihiro Azai[3], Genji Kurisu[1,2] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Inst. for Prot. Res., Osaka Univ.*, [3]*Col. Life Sci., Ritsumeikan Univ.*)

3P104 **Significance of phospholipid composition in generating Min protein waves in vitro**
Satya N. V. Arjunan[1], Yusuke Morimoto[2], Koichi Takahashi[1] ([1]*Laboratory for Biochemical Simulation, RIKEN Quantitative Biology Center*, [2]*Laboratory for Cell Signaling Dynamics, RIKEN Quantitative Biology Center*)

3P105 **膜受容体内在化のリアルタイムモニタリング：走査型電気化学顕微鏡(SECM)による低侵襲・定量的・単一細胞レベルでの測定**
Real-time Monitoring of Membrane Receptor Internalization: Low-Invasive, Quantitative and Single-Cell Level Measurement by SECM
Yoshiharu Matsumae[1], Yasufumi Takahashi[2], Kosuke Ino[1], Hitoshi Shiku[1], Tomokazu Matsue[1,2] ([1]*Grad. Sch. Environ. Stud., Tohoku Univ.*, [2]*WPI-AIMR, Tohoku Univ.*)

3P106 **The improvement of 2D crystal quality by crystallization temperature correlated with fluidity of lipids mixture**
Shintaro Maeda[1], Kyoko Shinzawa(Itoh)[1], Atsuo Miyazawa[1], Christoph Gerle[1], Yoshinori Fujiyoshi[2], Tomitake Tsukihara[1], Shinya Yoshikawa[1] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*CeSPI.,Univ.Nagoya*)

3P107 **Molecular dynamics simulations of β2AR: the comparison of different protein-lipid force field parameters**
Md. Iqbal Mahmood, Nozomu Kamiya, Hideaki Fujitani, Yamashita Takefumi (*LSBM, RCAST, The University of Tokyo*)

3P108 **三量体ハロロドプシンの特異的なカロテノイド結合**
Specific carotenoid binding of halorhodopsin trimer
Yasuyuki Miyazaki[1], Noritaka Kato[1], Takashi Kikukawa[2], Makoto Demura[2], Takanori Sasaki[1] ([1]*Sch. Sci. and Tech., Meiji Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*)

3P109 **アルカリ条件下における三量体ハロロドプシン-バクテリオルベリン複合体の熱安定性**
Thermal stability of trimer halorhodopsin-bacterioruberin complex in alkali condition
Kaede Suzuki[1], Takashi Kikukawa[2], Makoto Demura[2], Takanori Sasaki[1] ([1]*Sch. Sci. and Tech., Meiji Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*)

3P110 **Calculation of free energy changes due to charged residues mutation from alchemical free energy calculations:Improving toxins selectivity**
Md. Harunur Rashid[1,2], Shigehiko Hayashi[1], Serdar Kuyucak[2] ([1]*Kyoto University, Japan*, [2]*University of Sydney, Australia*)

3P111 **アデノシン A2a 受容体の熱安定性を向上させるアミノ酸置換の理論的予測**
Theoretical Prediction of Mutations Improving Thermal Stability of Adenosine A2a Receptor
Yuta Kajiwara[1], Satoshi Yasuda[2], Yuki Takamuku[3], Takeshi Murata[3], Masahiro Kinoshita[3] ([1]*Graduate School of Energy Science, Kyoto University*, [2]*Institute of Advanced Energy, Kyoto University*, [3]*Graduate School of Science, Chiba University*)

3P112 **C 末端に異なるアミノ酸タグをもつハロロドプシン同士での三量体の形成**
Trimer formation between halorhodopsins with different amino acid tags at C terminus
Tomokazu Wakatsuki, Takanori Sasaki (*Sch. Sci. and Tech., Meiji Univ.*)

3P113 **ヒト由来膜タンパク質の無細胞発現と 膜局在化傾向の網羅的解析**
The comprehensive analysis of human membrane protein expression and membrane insertion in vitro
Go Takizawa (*Univ.Tokyo*)

3P114 **全反射赤外分光法を用いた苦味受容体のリガンド結合解析**
ATR-FTIR study of ligand binding in a bitter taste receptor
Tomoaki Ohashi[1], Kota Katayama[1], Masayo Iwaki[1], Kei Tsutsui[2], Hiroo Imai[2], Hideki Kandori[1] ([1]*Nagoya Institute of Technology*, [2]*Primate Reserch Institute, Kyoto University*)

3P115 **High stability of two-dimensional crystal of reconstituted bacteriorhodopsin in partially fluorinated phosphatidylcholine**
Masaru Yoshino[1], Hiroshi Takahashi[1], Kohei Morita[1], Toshiyuki Takagi[2], Hideki Amii[1], Toshiyuki Kanamori[2], Masashi Sonoyama[1] ([1]*Fac. Sci. Tech., Gunma Univ.*, [2]*R.C. Stem Cell Eng., AIST*)

04. 核酸結合蛋白質 / 04. Nucleic acid binding proteins

3P116 **Reverse gyrase likely biases thermal DNA strand passage toward overwinding**
Taisaku Ogawa[1], Katsunori Yogo[2], Shou Furuike[3], Kazuo Sutoh[1], Akihiko Kikuchi[4], Kazuhiko Kinosita[1] ([1]*Dept. Phys., Waseda Univ.*, [2]*Grad. Sch. Med. Sci., Kitazato Univ.*, [3]*Dept. Phys., Osaka Med. Coll.*, [4]*Grad. Sch. Med., Nagoya Univ.*)

3P117 **Single-molecule study of how RecA displaces SSB from single-stranded DNA**
Hung-Yi Wu, Hung-Wen Li (*Dept. of Chem., Natl. Taiwan Univ.*)

3P118 **クロマトソームの粗視化シミュレーション: H1 結合に伴うヌクレオソーム構造のコンパクト化のダイナミクス**
Coarse-grained simulation of chromatosome: H1-mediated dynamic compaction of nucleosome structure
Nobu C. Shirai, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

3P119 **Characterization of the hemi-methylated CpG methylation process using fluorescent labeled SRA**
Yubing Cui[1,2], Yong-Woon Han[2], Mariko Ariyoshi[3], Kyohei Arita[4], Isao Suetake[5], Shoji Tajima[5], Yoshie Harada[1,2] ([1]*Grad. Sch. Biostudies, Univ. Kyoto*, [2]*iCeMS Inst., Univ. Kyoto*, [3]*Grad. Sch. Tech., Univ. Kyoto*, [4]*Grad. Sch. Medical Life Sci., Univ. Yokohama City*, [5]*Protein Inst., Univ. Osaka*)

3P120 **蛍光標識ヌクレオソームを用いたクロマチン再構成複合体の機能解析**
Characterization of ATP-dependent chromatin remodeling complexes using fluorescently labeled nucleosome
Yong-Woon Han[1], Yasuo Tsunaka[1,2], Hiroaki Yokota[3], Kazuhiro Yamada[4], Mai Ohnishi[1,5], Sayaka Yamazaki[1,5], Isao Suetake[6], Shoji Tajima[6], Hisashi Tadakuma[7], Yoshie Harada[7] ([1]*iCeMS, Kyoto University*, [2]*PREST*, [3]*Bio Photonics, Grad. Sch. for the Creation of New Photonics Ind.*, [4]*Max-Planck-Inst. for Med. Res.*, [5]*Faculty of Human Life and Sci., Doshisha Woman's College of Liberal of Arts*, [6]*Inst. for Protein Res., Osaka Univ.*, [7]*Grad. Sch. of Frontier Sci., Univ. of Tokyo*)

05A. 核酸：構造・物性 / 05A. Nucleic acid: Structure & Property

3P121 **粗視化モデルを用いた Ars インスレーターの力学的特性の考察**
Analysis of dynamic characteristics of Ars-insulator by coarse-grained models
Shuhei Isami[1], Sayuri Tatemoto[1], Hiraku Nishimori[1,2], Naoaki Sakamoto[1], Akinori Awazu[1,2] ([1]*Dept. Math. and Life Sciences, Hiroshima Univ.*, [2]*Research Center for the Mathematics on Chromatin Live Dynamics*)

3P122 **等温条件で増幅可能な人工 RNA の設計原理の理解**
Design Principle of Replicable RNA under Isothermal Condition
Kimihito Usui[1], Norikazu Ichihashi[1,2], Yasuaki Kazuta[1], Tetsuya Yomo[1,2,3] ([1]*JST, ERATO, Yomo Project*, [2]*Grad. Sch. of Info. and Tech., Osaka Univ.*, [3]*Grad. Sch. of Front. Biosci., Osaka Univ.*)

05B. 核酸：相互作用・複合体 / 05B. Nucleic acid: Interaction & Complex formation

3P123 **Protective Effect of Ascorbic Acid on Double-strand Breaks of Giant DNA induced by photo- and gamma-irradiation**
Yue Ma[1], Yuko Yoshikawa[2], Toshiaki Mori[3], Tadayuki Imanaka[2], Kenichi Yoshikawa[1] ([1]*Doshisha University*, [2]*Ritsumeikan University*, [3]*Osaka Prefecture University*)

3P124 **α-hemolysin 及び T7 RNA polymerase を用いた DNA/RNA ロジックゲートの実現**
Construction of DNA computing platform using α-hemolysin and DNA/RNA with the enzyme reaction
Masayuki Ohara[1], Masahiro Takinoue[2], Ryuji Kawano[1] ([1]*TUAT*, [2]*Tokyo Tech*)

3P125 **DNA を湾曲する HMG-1/2 は塩基配列非特異的に長鎖 DNA を折り畳み**
DNA-bending protein HMG-1/2 sequence-independently folds a single giant duplex DNA chain
Hiroyuki Mayama[1], Naomi Tsumura[1], Norio Hazemoto[2], Toshio Kanbe[3], Hideaki Yamaguchi[4], Koji Kubo[5], Anatoly Zinchenko[5], Shizuaki Murata[5], Kenichi Yoshikawa[6], Tatsuo Akitaya[1] ([1]*School of Medicine, Asahikawa Med. Univ.*, [2]*Graduate School of Pharmaceutical Sciences, Nagoya City Univ.*, [3]*School of Medicine, Nagoya Univ.*, [4]*Faculty of Pharmacy, Meijo Univ.*, [5]*Graduate School of Environmental Study, Nagoya Univ.*, [6]*Faculty of Life and Medical Sciences, Doshisha Univ.*)

3P126 **転写調節タンパク質 STPR は長鎖 DNA を塩基配列非特異的に折り畳む**
Transcription modulator protein STPR induces the folding of a single giant DNA molecule in sequence-nonspecific manner
Tatsuo Akitaya[1], Naoko Makita[2], Naomi Tsumura[1], Hiroyuki Mayama[1], Norio Hazemoto[3], Toshio Kanbe[4], Hideaki Yamaguchi[5], Koji Kubo[6], Anatoly Zinchenko[6], Shizuaki Murata[6], Kenichi Yoshikawa[7], Tomoyasu Aizawa[8], Makoto Demura[8] ([1]*School of Medicine, Asahikawa Med. Univ.*, [2]*Faculty of Environmental and Information Sciences, Yokkaichi Univ.*, [3]*Graduate School of Pharmaceutical Sciences, Nagoya City Univ.*, [4]*School of Medicine, Nagoya Univ.*, [5]*Faculty of Pharmacy, Meijo Univ.*, [6]*Graduate School of Environmental Study, Nagoya Univ.*, [7]*Faculty of Life and Medical Sciences, Doshisha Univ.*, [8]*Graduate School of Life Science, Hokkaido Univ.*)

3P127 **Comparison of DNA double-strand breaks caused by ultrasound and Co60 gamma-ray with attention to the effect on its higher-order structure**
Rinko Kubota[1], Naoki Ogawa[1], Yukihiro Kagawa[1], Yuko Yoshikawa[2], Yoshiaki Watanabe[1], Takahiro Kenmotsu[1], Toshiaki Mori[3], Tadayuki Imanaka[2], Kenichi Yoshikawa[1] ([1]*Graduate School of Life and Medical Sciences, Doshisha University*, [2]*Department of Biotechnology, College of Life Sciences, Ritsumeikan University*, [3]*Radiation Research Center, Osaka Prefecture University*)

3P128 **Metal Cations(2+) Cause the Folding Transition of DNA but Inhibit Spermidine(3+)-Induced Compaction**
Chika Tongu[1], Yuko Yoshikawa[2], Zinchenko Anatoly A[3], Chen Ning[3], Takahiro Kenmotsu[1], Kenichi Yoshikawa[1] ([1]*Doshisha University*, [2]*Ritsumeikan University*, [3]*Nagoya University*)

07. 水・水和／電解質 / 07. Water & Hydration & Electrolyte

3P129 **溶質と水分子の間の LJ ポテンシャルパラメタが水和ダイナミクスに及ぼす影響**
Effects of LJ potential parameters between solute and water on the hydration dynamics
Takuya Takahashi, Tetsuro Nagai (*Coll.Life.Sc., Ritsumeikan Univ*)

3P130 **水和水が小分子のテラヘルツ振動モードに与える影響**
Effect of Hydration water on terahertz vibrational modes of small molecules
Ohki Kambara[1], Norihisa Hiromoto[2,3] ([1]*RIE, Shizuoka Univ.*, [2]*GSE, Shizuoka Univ.*, [3]*GSST, Shizuoka Univ.*)

3P131 **蛋白質間相互作用への溶媒効果を観測することの困難さ：単純なモデルでの理論研究**
Difficulty in Observing of Solvent Effect on Protein-Protein Interaction: A Theoretical Study with a Simple Model
Takumi Yamashita, Shingo Fujihara, Ryo Akiyama (*Sci., Univ. Kyushu*)

3P132 **誘電緩和分光法と分子動力学法を用いたアルカリハライドとアルカリリン酸イオンの水和ダイナミクスおよびエネルギー論**
On the Hydration Dynamics and Energetics of Alkali halide and Phosphate Ions by Dielectric Relaxation Spectroscopy and Molecular Dynamics
George Mogami[1], Kazuki Ishimori[1], Nobuyuki Matubayasi[2], Hideaki Takahashi[3], Makoto Suzuki[1] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*Grad. Sch. Eng. Sci., Osaka Univ.*, [3]*Grad. Sch. Sci., Tohoku Univ.*)

3P133 **NaCl、NaI 水溶液におけるハイパーモバイル水の熱容量及び密度の評価**
Estimation of Heat Capacity and Density of Hyper-Mobile Water in NaCl and NaI Solutions
Masayoshi Sato, George Mogami, Nobuyuki Morimoto, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

09. 発生・分化 / 09. Development & Differentiation

3P134 **低周波超音波により引き起こされたメダカ胚における卵黄球の収縮と出血**
Shrinkage of yolk sphere and bleeding on medaka embryo caused by low frequency ultrasound
Kento Yamada[1], Masato Ueda[1], Kenji Yoshida[1,2], Yasuhiro Tonoyama[3], Nobuyoshi Shimizu[3], Yoshiaki Watanabe[1] ([1]*Doshisha Univ.*, [2]*Chiba Univ.*, [3]*Keio Univ, Advanced Research Center for GSP.*)

3P135 **アフリカツメガエル卵成熟過程における卵母細胞の ATP 産生**
ATP production in *Xenopus laevis* oocytes during maturation
Takashi W. Ijiri[1], Jun-ichi Kishikawa[1], Hiromi Imamura[2], Maho Sakiie[3], Shuichi Ueno[3], Yasuhiro Iwao[3], Ken Yokoyama[1], Ken-ichi Sato[1] ([1]*Fac. Life Sci., Kyoto Sangyo Univ.*, [2]*Hakubi Center, Kyoto Univ.*, [3]*Grad. Sch. Med., Yamaguchi Univ.*)

3P136 **3 次元培養下での再生皮膚の発生における反応拡散機構による羽毛原基パターン再構築**
Reconstruction of feather bud patterning by a reaction-diffusion mechanism during bioengineered skin development in 3D culture
Kentaro Ishida, Toshiyuki Mitsui (*Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

10. 筋肉 / 10. Muscle

3P137 **電気ー機械相互作用を考慮した心筋細胞群モデル**
Model of Cardiac Muscle Cells with Reference to Electro-Mechanical Interaction
Hiroki Miyazako[1], Toshiki Murata[2], Osamu Fukayama[1,2], Kunihiko Mabuchi[1,2], Takayuki Hoshino[1,2] ([1]*Grad. Sch. IST, Univ. of Tokyo*, [2]*Sch. Eng., Univ. of Tokyo*)

3P138 **骨格筋繊維の急激な伸張に対する張力応答のシミュレーション**
Simulation of force responses to fast ramp stretches in the skeletal muscle fibres
Youjiro Tamura[1], Akira Ito[2], Andrew Cresswell[3] ([1]*SNCT*, [2]*SNCT*, [3]*UQ HMS*)

3P139 **コネクチン遺伝子の上流から転写される横紋筋タンパク質の解析**
Analysis of proteins in striated muscles that transcribed from the upstream region of connectin gene
Akira Hanashima[1], Naruki Sato[2], Sumiko Kimura[3], Takashi Murayama[1] ([1]*Dept. Pharmacol., Facl. Med., Juntendo Univ.*, [2]*Dept. Nanobiol., Grad. Adv. Int. Sci., Chiba Univ.*, [3]*Dept. Biol., Grad. Sci., Chiba Univ.*)

3P140 **“Mg-Polymer” 再考**
Revisiting “Mg-Polymer”
Mahito Kikumoto, Shuichi Takeda, Tomoharu Matsumoto, Yuichiro Maeda (*Strct. Biol. Cntr, Nagoya-univ.*)

3P141 **ヒト心筋筋原線維 SPOC の外部力学刺激に対する応答**
Response of SPOC in human cardiac myofibrils to mechanical stimuli
Toshiki Shimomura[1], Hisashi Maejima[1], Yuta Shimamoto[3], Shin’ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*, [3]*National Institute of Genetics*)

3P142 **温度変化のヒト心筋 SPOC への作用**
Effects of temperature changes on SPOC in a human myocardium
Yoshihisa Yamamura[1], Tatuya Kagemoto[1], Shin’ichi Ishiwata[1,2] ([1]*Department of Physics, Faculty of Science and Engineering, Waseda University*, [2]*Waseda Bioscience Research Institute in Singapore (WABIOS)*)

3P143 **ギボシムシのコネクチン様タンパク質のドメイン構造**
Sequential analysis of connectin-like protein in acorn worm
Shunsuke Kubota[1], Akira Hanashima[2], Hiroki Sonobe[3], Yuh Nanmoku[1], Yukihiro Seiji[1], Satoshi Nakayama[1], Mai Kanno[1], Kunifumi Tagawa[4], Sumiko Kimura[1] ([1]*Dept. Biol., Grad. Sci., Chiba Univ.*, [2]*Dept. Pharmacol., Facl. Med., Juntendo Univ.*, [3]*Dept. Nanobiol., Grad. Adv. Int. Sci., Chiba Univ.*, [4]*Marine Biol. Lab. Grad. School Sci., Hiroshima Univ.*)

11. 分子モーター / 11. Molecular motor

3P144 **高速暗視野顕微鏡による運動中のキネシン頭部の結合解離の直接観察**
Direct observation of binding and unbinding motions of kinesin motor domain during processive motility
Hiroshi Isojima[1], Ryota Iino[2], Hiroyuki Noji[3], **Michio Tomishige**[1] ([1]*Dept. Appl. Phys., Univ. Tokyo*, [2]*Okazaki Inst. Integ. Biosci., NINS*, [3]*Dept. Appl. Chem., Univ. Tokyo*)

3P145 **Control of microtubule trajectory within an electric field by altering surface charge density**
Naoto Isozaki[1], Suguru Ando[1], Hirofumi Shintaku[1], Hidetoshi Kotera[1], Edgar Meyhofer[2], Ryuji Yokokawa[1] ([1]*Kyoto University*, [2]*Michigan University*)

3P146 **キネシンとダイニンによる Tug-of-war 分子系の再構築および多分子ダイニンの発生力計測**
Tug-of-war molecular system between dynein- and kinesin-coated regions and force measurement of multiple dyneins
Fumie Oda, Hirofumi Shintaku, Hidetoshi Kotera, Ryuji Yokokawa (*Dept. Microengineering, Kyoto Univ.*)

3P147 **べん毛モーター解析のための長時間リアルタイム観察・刺激システムの構築**
Development of the real-long-time observation and local stimulation system for flagellar motor analysis
Masaru Kojima, Takahiro Motoyoshi, Mitsuhiro Horade, Kazuto Kamiyama, Yasushi Mae, Tatsuo Arai (*Grad. Sch. Eng. Sci., Osaka Univ.*)

3P148 **Visualization of functional components of the bacterial flagellar motor**
Yoshiyuki Sowa[1], Yong-Suk Che[1,2] ([1]*Dept. Frontier Biosci., Hosei Univ.*, [2]*IMRAM, Tohoku Univ.*)

3P149 **Molecular dynamics simulations of the β subunit in F_1-ATPase: Relation between the large-scale structural change and common motifs**
Yuko Ito, Mitsunori Ikeguchi (*Grad. Sch. Med. Life Sci., Yokohama-City Univ.*)

3P150 **Slow axonemal dynein e facilitates the motility of faster dynein c**
Youske Shimizu[1], Hitoshi Sakakibara[1], Hiroaki Kojima[1], Kazuhiro Oiwa[1,2] ([1]*NICT*, [2]*CREST*)

3P151 **回転電場を用いた F_1-ATPase の一分子計測による 拡散の Giant acceleration の観察 II**
Giant Acceleration of diffusion in F_1-ATPase II
Ryunosuke Hayashi[1], Shuichi Nakamura[1], Seishi Kudo[1], Kazuo Sasaki[1], Hiroyuki Noji[2], **Kumiko Hayashi**[1] ([1]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*, [2]*Dept. Appl. Chem., Sch. Eng., Univ. Tokyo*)

3P152 **$TF_1\beta$ サブユニットのヌクレオチド結合への Pi の影響**

Nucleotide binding to TF_1 β subunit in relation to the effect of Pi

Riku Nagano, Kiyoshi Obara, Hiroshi Ueno, Eiro Muneyuki (*Dept. of Physics. Chuo Univ.*)

3P153 **非天然アミノ酸導入型 F_1-ATPase を用いた１分子回転観察**

Key factors of arginine finger of F_1-ATPase clarified by an unnatural amino acid mutation

Ayako Yukawa[1], Ryota Iino[2], Rikiya Watanabe[1], Shigehiko Hayashi[3], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo.*, [2]*Okazaki Inst. Integ. Biosci., NINS*, [3]*Grad. Sch. Sci., Univ. Kyoto.*)

3P154 **Rotor-Stator Interactions in V_1 and V_o from *Enterococcus hirae* V-ATPase**

Hiroshi Ueno[1], Yoshihiro Minagawa[2], Mayu Hara[2], Ichiro Yamato[3], Hiroyuki Noji[2], Takeshi Murata[4], Ryota Iino[5], Eiro Muneyuki[1] ([1]*Fac. Sci. & Eng., Univ. Chuo*, [2]*Sch. Eng., The Univ. Tokyo*, [3]*Dept. Biol. Sci. & Tech., Tokyo Uni. Sci.*, [4]*Grad. Sch. Sci., Univ. Chiba*, [5]*Okazaki Inst. for Integr. Biosci., NINS*)

3P155 **有糸分裂キネシン Eg5 の新規 SH 基反応性フォトクロミック分子を用いた光制御**

Photo-control of mitotic kinesin Eg5 using novel SH reactive photochromic molecules

Tamura Yuki[1], Mutoh Hiroyuki[2], Tohyama Kanako[1], Kondo Kazunori[2], Maruta Shinsaku[1] ([1]*Div. Bioinfo., Grad. sch. Eng., Univ. Soka*, [2]*Dep. Bioinfo., Fac. Eng., Univ. Soka*)

3P156 **ヒト細胞質ダイニンのパワーストローク測定**

Determination of Power Stroke Distance Driven by Human Cytoplasmic Dynein

Yoshimi Kinoshita[1], Taketoshi Kambara[2], Satoshi Ikeda[1], Motoshi Kaya[1], Hideo Higuchi[1] ([1]*Graduate School of Science, The University of Tokyo*, [2]*RIKEN QBiC*)

3P157 **V-ATPase の中心回転軸におけるトルク伝達機構**

The ingenious structure of central rotor apparatus in V_oV_1; torque transmission mechanism in the central rotor of V_oV_1

Atsuko Nakanishi[1], Jun-ichi Kishikawa[1], Masatada Tamakoshi[2], Shou Furuike[3], Ken Yokoyama[1] ([1]*Dept. Mol. Biosci., Kyoto Sangyo Univ.*, [2]*Dept. Mol. Biol., Tokyo University of Pharm. & Life Sci.*, [3]*Dept. Physics, Osaka Medical College*)

3P158 **フォトクロミック分子によって誘導されるキネシン・ADP・フルオロメタル複合体の構造変化**

Conformational change of kinesin-ADP-fluorometal ternary complexes induced by photochromic molecules

Akihisa Iwata[1], Takeshi Itaba[1], Mitsuo Ohmori[2], Shinya Mitsuhashi[3], Shinsa Maruta[1,2] ([1]*Div. Bioinfo., Grad. sch. Eng., Univ. Soka*, [2]*Dep. Bioinfo., Fac. Eng., Univ. Soka*, [3]*Div. Applied Bioscience, Grad. sch. Agri., Uni. Hokkaido*)

3P159 **複数のキネシンによる協調的カーゴ輸送のメカニズムの解明**

Investigation of the mechanism of cooperative cargo transport by multiple kinesins

Naoto Sawairi[1], Takayuki Ariga[2], Mitsuhiro Iwaki[3,4], Michio Tomishige[2], Kumiko Hayashi[1] ([1]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*, [2]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*, [3]*QBiC, RIKEN*, [4]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

3P160 **MD シミュレーションによる *Enterococcus hirae.* V_1-ATPase の回転機構の解明**

Rotation mechanism of V_1-ATPase studied by MD simulation

Yuta Isaka[1], Takeshi Murata[2,3], Mitsunori Ikeguchi[1] ([1]*Grad. Schi. of Med. Life Sci., Yokohama City Univ.*, [2]*Fac. of Sci., Chiba Univ.*, [3]*JST, PRESTO*)

3P161 **リニアモータータンパク質キチナーゼの蛍光一分子観察**

Single-Molecule Fluorescence Imaging of Linear Motor Protein Chitinase

Tomoyuki Tasaki[1], Yusuke Shinafuji[1], Mayu Hara[1], Hiroyuki Noji[1], Ryota Iino[2] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Okazaki Inst. Integ. Biosci., NINS*)

3P162 **ATP synthesis by elastically coupled molecular motors**

Yasuhiro Imafuku[1], Nils Gustafsson[2], Thomas Thomas[2] ([1]*Department of Biology, Kyushu University, Japan*, [2]*School of Physics and Astronomy, University of Birmingham, UK*)

3P163 **CYK-4 による kinesin-6 の螺旋運動調節機構**

The spiraling movement of kinesin-6 regulated by CYK-4

Yohei Maruyama[1], Akihiko Sato[1], Tim Davis[2], Tetsuhiko Teshima[3], Shin Yamaguchi[1], Shoji Takeuchi[3], Masanori Mishima[2], Junichiro Yajima[1] ([1]*Dept. Life Sci., Grad. Sch. of Arts and Sci., Univ. of Tokyo*, [2]*CMCB at Warwick Med. Sch., Univ. of Warwick*, [3]*Inst. of Ind. Sci., Univ. of Tokyo*)

3P164 **Autoinhibition and cooperative activation mechanisms of cytoplasmic dynein**

Takayuki Torisawa[1,3], Ken'ya Furuta[1], Muneyoshi Ichikawa[2], Akane Furuta[1], Kei Saito[2], Kazuhiro Oiwa[1,3], Hiroaki Kojima[1], Yoko Toyoshima[2] ([1]*Bio ICT lab, NICT*, [2]*Dept. Life Sciences, Graduate School of Arts and Sciences, the Univ. of Tokyo*, [3]*CREST, JST*)

3P165 **ミオシンの協調的首振りとアクチン滑り運動のゆらぎ**

Cooperative lever-arm swings of myosins and fluctuation of actin sliding

Yota Kondo, Kazuo Sasaki (*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*)

3P166 **全反射型蛍光顕微鏡における受像偏向と偏光変調 ーF_1-ATPase の構造変化検出への応用ー**

Emitter modulation and polarization switching under TIRF illumination: Application for detection of conformational change in F_1-ATPase

Nagisa Mikami[1], Tomoko Masaike[2,3], Takayuki Nishizaka[1] ([1]*Dept. phys., Gakushuin Univ.*, [2]*PRESTO, JST*, [3]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*)

3P167 **蛍光標識 ATP アナログを用いたイネ特有のキネシン E11 の速度論的解析**

Kinetic characterization of rice plant specific kinesin E11 using fluorescent ATP analogue

Hironobu Taniguchi[1], Kouichi Miyabe[2], Nozomi Umezu-Furutani[1], Shinsaku Maruta[1] ([1]*Div.Bioinfo.,Grad.Sch.Eng.,Univ.Soka*, [2]*Dep. Bioinfo., Fac. Eng., Univ.Soka*)

3P168 **金ナノロッドを用いた高速配向イメージングシステムの開発と F1-ATPase の構造変化検出への応用**

Development of high-speed orientation imaging system for gold nanorod and application to detection of conformational change of F1-ATPase

Sawako Enoki[1], Ryota Iino[2], Yamato Niitani[3], Yoshihiro Minagawa[1], Michio Tomishige[3], Hiroyuki Noji[1] ([1]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo*, [2]*Okazaki Inst. Integ. BioSui., NINS*, [3]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*)

12. 細胞生物的課題 / 12. Cell biology

3P169 **多重周波数モジュレーション原子間力顕微鏡法：単一細胞レオロジーの高速測定**

Multi-frequency modulation atomic force microscopy for the high-speed measurement of single cell rheology

Ryosuke Takahashi, Kaori Kuribayashi-Shigetomi, Agus Subagyo, Kazuhisa Sueoka, Takaharu Okajima (*Grad. Sch. Info. Sci. & Tech., Hokkaido Univ.*)

3P170 **ケージド化合物の光分解を用いた大腸菌細胞応答の定量的計測**

Quantitative measurement of the cellular response of *Escherichia coli* using photolysis of the caged chemoattractant

Takashi Sagawa[1], Hiroto Tanaka[1], Tadashi Matsukawa[1], Yoshiyuki Sowa[2], Ikuro Kawagishi[2], Hiroaki Kojima[1] ([1]*Bio ICT Lab., NICT*, [2]*Dept. Frontier Bioscience, Hosei Univ*)

3P171 **蛍光相関分光法を用いた単一細胞由来のグルココルチコイドレセプター二量体形成と転写活性の定量**

Quantification of glucocorticoid receptor homo-dimer and transcriptional activity in single cell by fluorescence correlation spectroscopy

Sho Oasa[1], Akira Sasaki[2], Shintaro Mikuni[3], Masataka Kinjo[3] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*AIST*, [3]*Fac. Adv. Life. Sci., Hokkaido Univ.*)

3P172 **超解像顕微鏡法による上皮成長因子受容体クラスタリングの定量解析**

A Quantitative Analysis of Epidermal Growth Factor Receptor Clustering Using Super-resolution Microscopy

Michio Hiroshima[1,2], Msahiro Ueda[1], Yasushi Sako[2] ([1]*RIKEN QBiC*, [2]*RIKEN*)

3P173 **FRAP による、成長円錐のアクチンおよびアクチン関連タンパク質の動態解析**

FRAP experiments on actin and actin associate proteins in growth cones

Minami Tanaka[1,2], Takeshi Tougasaki[3], Kaoru Katoh[3] ([1]*Biomed. Res. Inst, AIST*, [2]*Grad. Sch. Life & Env. Sci., Univ. Tsukuba*, [3]*FANCL Co.*)

3P174 **神経細胞膜の分子選択的な並進拡散障壁**

Molecule-selective lateral-diffusion barrier in the neuronal axon membrane

Manami Miyahara[1], Chieko Nakada[3], Ziya Kalay[1], Toshiki Matsui[2], Hiroo Iwata[2], Takahiro Fujiwara[1], Akihiro Kusumi[1,2] ([1]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*, [2]*Institute for Frontier Medical Sciences, Kyoto University*, [3]*Instruments Company, Nikon Corporation*)

3P175 **κ オピオイド受容体の動的濃縮領域（ホットスポット）：１分子イメジングによる検出**

Single-molecule detection of hotspots for dynamic concentration of the kappa opioid receptor

Yuki Shirai[1], Peng Zhou[1], Rinshi Kasai[2], Wonhwa Cho[3], Takahiro Fujiwara[1], Akihiro Kusumi[1,2] ([1]*iCeMS, Kyoto University*, [2]*Institute for Frontier Medical Sciences, Kyoto University*, [3]*University of Illinois*)

3P176 **Noonan 症候群における SOS を介した Ras positive feedback 異常の生細胞一分子解析**

Dysregulations of SOS-mediated positive feedback on RAS activation in Noonan syndrome observed using single molecule imaging

Yuki Nakamura[1,2], Kayo Hibino[3], Yasushi Sako[1,2] ([1]*RIKEN*, [2]*Osaka Univ.*, [3]*QBiC., RIKEN*)

3P177 **二つの抗体送達システムにおける送達作用の比較**

Comparison of the delivery effects of two antibody carrier systems

Kana Kuwahara[1,2], Kazuki Harada[1,2], Takenori Yamamoto[1,2], Yasuo Shinohara[1,2] ([1]*Inst. Genome Research.*, [2]*Fac. Pharm. Sci., Univ. Tokushima*)

3P178 ***Amoeba proteus* 細胞膜の 3 次元曲率に関する研究**

Characterization of surface structures of *Amoeba proteus* in three dimensional spaces

Yukinori Nishigami[1], Atsushi Taniguchi[2], Seiji Sonobe[3], Shigenori Nonaka[2], Masatoshi Ichikawa[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Grad. Sch. Sci., Univ. Hyogo*, [3]*NIBB*)

3P179 **電子顕微鏡法によるヒト毛乳頭細胞の一次繊毛の構造解析**

Structural analysis of primary cilia in human follicle dermal papilla cells by electron microscopy

Misaki Tanaka[1], Kazuyuki Matsushima[2], Kuniyoshi Kaseda[2], Takuo Yasunaga[1] ([1]*Kyushu Institute of Technology*, [2]*Saravio Cosmetics Ltd.*)

3P180 **Intranulear particles include RPS or RPL subunits**

Saori L. Mimatsu[1,2], Maiko Kuramochi[1,2], Soyomi Uchibori[1,2], Ayako Kojima[1], Emiko Kobayashi[1], Michio Hiroshima[3,4], Yasushi Sako[3,4], Kaoru Katoh[1] ([1]*Biomed. Res. Inst., AIST*, [2]*Grad. Sch. Life & Env. Sci., Univ. Tsukuba*, [3]*REKEN ASI*, [4]*RIKEN QBiC*)

3P181 **アクチン細胞骨格と核との力学的結合が血管平滑筋細胞の分化に与える影響**

Effects of Actin-Nucleus Connections on the Vascular Smooth Muscle Cell Differentiation

Kazuaki Nagayama[1], Makoto Iwata[2], Takeo Matsumoto[2] ([1]*Department of Intelligent Systems Engineering, Ibaraki University, Japan*, [2]*Department of Mechanical Engineering, Nagoya Institute of Technology, Japan*)

3P182 **繰り返し伸展刺激によって起こるストレスファイバーの脱重合のメカニズムについて**

What is the molecular mechanism of stress fiber disassembly caused by mechanical cyclic stretch?

Wenjing Huang[2], Tsubasa Matsui[1], Masahiro Kuragano[3], Masayuki Takahashi[3], Tomohiro Kawahara[2], Masaaki Sato[4], **Shinji Deguchi**[1] ([1]*Nitech*, [2]*Kyutech*, [3]*Hokkaido Univ*, [4]*Tohoku Univ*)

3P183 **３次元コラーゲンゲル内に培養された線維芽細胞のメディウムの流れ刺激に対する反応**

Responses of fibroblasts against fluid flow stimuli in a three-dimensional collagen gel culture system

Natsumi Saito[1], Hiroaki Adachi[2], Hiroshi Tanaka[2], Satoru Nakata[2], Norifumi Kawada[1], Katsutoshi Yoshizato[1] ([1]*Dept. Hepatology, Grad. Sch. Med., Osaka City Univ.*, [2]*Nippon Menard Cosmetic Co., Ltd.*)

3P184 **細胞シート延伸における細胞核変形量の測定**

Measurements of cell nucleus deformation during stretching a cell sheet

Kota Onishi, Masahiro Tsuchiya, Takaharu Okajima (*Grad. Sch. Inform. Sci. and Techno. Hokkaido Univ.*)

3P185 **Microfluidics analyses of coordinated dynamics of F-actin and cAMP signaling in *Dictyostelium* chemotaxis**

Fumihito Fukujin[1,2], Satoshi Sawai[1,3,4] ([1]*Graduate School of Arts and Science, University of Tokyo*, [2]*Research Fellow of Japan Society for the Promotion of Science*, [3]*Research Center for Complex Systems Biology, University of Tokyo*, [4]*PRESTO, Japan Science and Technology Agency*)

3P186 **紡錘体の力学特性の遷移は染色体分配を手助けする**

Mechanical transition of the vertebrate meiotic spindle facilitates chromosome dynamics

Jun Takagi[1], Takeshi Itabashi[2], Shin'ichi Ishiwata[2,3] ([1]*Quantitative Mechanobiology Laboratory, NIG*, [2]*Fac. Sci. Engn., Waseda Univ.*, [3]*WABIOS, Waseda Univ.*)

3P187 **オリゴマイシンとロテノンはミトコンドリアの透過性遷移に相乗的な阻害作用を示す**

Synergistic inhibitory effects of oligomycin and rotenone on the mitochondrial permeability transition

Kazumasa Kotake[1,2], Yuki Inotani[1,2], Yuya Yoshimura[1,2], Kazuki Harada[1,2], Takenori Yamamoto[1,2], Yasuo Shinohara[1,2] ([1]*Inst. Genome Research., Univ. Tokushima*, [2]*Fac. Pharm. Sci., Univ. Tokushima*)

3P188 **がん細胞接着及び運動における硬さの影響の評価**

Evaluation of the Impact of Stiffness on Adhesion and Migration of Cancer Cells

Takashi Yamazaki[1], Takahisa Matsuzaki[1], Yuko Shimokawa[1], Ken Sato[1], Masami Suganuma[1,2], Motomu Tanaka[3], Seiichiro Nakabayashi[1], Hiroshi Yoshikawa[1] ([1]*Grad. Sch. Sci & Eng., Univ. Saitama*, [2]*Res. Inst. Clin. Onc., Saitama Cancer Center*, [3]*Inst. Phys. Chem., Univ. Heidelberg*)

3P189 **マイクロパターン上に培養した単一細胞の細胞核の動態**

Movement of nucleus in cells cultured on micro-patterned substrates

Xinfeng Zhu, Kaori Kuribayashi-Shigetomi, Pinggen Cai, Agus Subagyo, Kazuhisa Sueoka, Takaharu Okajima (*Graduate school of Information Science and Technology, Hokkaido University*)

3P190 **F-アクチン溶液のシアバンディング**

Shear banding in an F-actin solution

Itsuki Kunita[1], Katsuhiko Sato[2], Yoshimi Tanaka[3], Yoshinori Takikawa[4], Hiroshi Orihara[4], Toshiyuki Nakagaki[1] ([1]*RIES, Hokkaido Univ.*, [2]*RIKEN CDB*, [3]*Grad. Sch. Env. & Info. Sci., Yokohama Natl. Univ.*, [4]*Facul. Eng., Hokkaido Univ.*)

3P191 **細胞運動におけるアクチンストレスファイバと焦点接着斑の時空間ダイナミクス**

Spatio-temporal dynamics of actin stress fibers and focal adhesions during cell migration in Swiss 3T3 fibroblasts

Michiko Sugawara[1], Takuya Miura[1], Hiromi Miyoshi[2], Ken-ichi Tsubota[1], Hao Liu[1] ([1]*Grad. Sch. Eng., Chiba Univ.*, [2]*RIKEN Center for Advanced Photonics*)

3P192 **核膜孔複合体内部における疎水性分子環境の in vivo 解析**

In vivo analysis of hydrophobic molecular crowding environment in the Nuclear Pore Complex

Hide A. Konishi[1], Suguru Asai[1], Tomonobu M Watanabe[2], Shige H. Yoshimura[1] ([1]*Grad. Schl. Biostudies., Univ. Kyoto*, [2]*RIKEN, QBiC*)

3P193 **回虫精子の MSP マシナリー構成要素の探索**

Identification of protein components involved in MSP machinery in the cell-free extract of *Ascaris* sperm

Tatsuya Iida[1], Takao Kitagawa[2], Saki Uemura[1], Aya Takamori[3], Makoto Miyata[3], Katsuya Shimabukuro[1] ([1]*Ube Nat. Col. Tech.*, [2]*Grad. Sch. Med., Yamaguchi Univ.*, [3]*Grad, Sch, Sci., Osaka City Univ.*)

3P194 **Possible points of action for rectification in directional sensing model**

Akihiko Nakajima[1,2], Shuji Ishihara[3], Satoshi Sawai[1,2,4] ([1]*Grad. Sch. Arts & Sci., Univ. Tokyo*, [2]*Research Center for Complex Systems Biology, Univ. Tokyo*, [3]*Dept. Phys., Meiji Univ.*, [4]*PRESTO, JST*)

3P195 **免疫シグナルアダプター分子 SLP-76 の複合体の形成と成長：1 分子追跡による解明**

Formation and growth of the key immune signaling complex based on the adaptor protein SLP-76 revealed by single-molecule tracking

Kenta J. Yoshida[1], Koichiro M. Hirosawa[1], Takahiro K. Fujiwara[1], Akihiro Kusumi[1,2] ([1]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*, [2]*Institute for Frontier Medical Sciences, Kyoto University*)

3P196 **細胞内力学特性に対する分子混み合い効果の影響**

Crowding effects on viscoelastic properties in cell model systems

Kenji Nishizawa[1], Kei Fujiwara[2], Nobushige Nakajo[1], Miho Yanagisawa[3], Daisuke Mizuno[1] ([1]*Kyushu University*, [2]*Keio University*, [3]*Tokyo Univ. Agric. Technol.*)

3P197 **１細胞レベルにおけるバクテリア走化性のメカニズム**

Mechanism of phototaxis of bacteria at single cell level

Daisuke Nakane, Takayuki Nishizaka (*Dept. Phys., Gakushuin Univ.*)

3P198 **アクチンフィラメントの配位構造は結合タンパク質によって変化する**
Modulation of monomer configurations of actin filaments by actin binding proteins
Kouhei Monma[1], Kenji Kobayasi[1], Ryoki Isikawa[2], Hajime Honda[1] ([1]*Nagaoka University of Technology*, [2]*Gunma Prefectural College of Health Sciences*)

3P199 **Ion selectivity of the *Leptospira* flagellar motor**
Md. Shafiqul Islam[1], V. Morimoto Yusuke[2], Seishi Kudo[1], Shuichi Nakamura[1] ([1]*Tohoku University*, [2]*QBiC, RIKEN*)

3P200 **Functional and structural analysis of the flagellar protein FliL from Vibrio alginolyticus**
Ananthanarayanan Kumar, Shiwei Zhu, Seiji Kojima, Michio Homma (*Nagoya University*)

3P201 **Actin filament dynamics and organizations in liposome: A simulation study**
Takahiro Nitta (*Applied Physics Course, Gifu Univ.*)

3P202 **海洋性ビブリオ菌のべん毛形成を制御する DnaJ ファミリータンパク質 SflA の相互作用解析**
Analysis of interaction of the DnaJ family protein SflA, that is involved in regulation of flagellation in *Vibrio alginolyticus*
Satoshi Inaba, Takehiko Nishigaki, Noriko Nishioka, Seiji Kojima, Michio Homma (*Div. Biol. Sci.Grad. Sch. Sci., Nagoya Univ*)

13A. 生体膜・人工膜：構造・物性 / 13A. Biological & Artificial membrane: Structure & Property

3P203 **非対称な脂質 2 重膜の高効率な作成にむけた新規マイクロデバイスの開発**
Novel micro-device to form asymmetric lipid-bilayer membrane in a high throughput manner
Rikiya Watanabe[1,2], Naoki Soga[1], Tomoko Yamanaka[1], Hiroyuki Noji[1] ([1]*Department of Applied Chemistry, The University of Tokyo*, [2]*PRESTO, JST*)

3P204 **脂質キュービック相の単結晶化**
Single Crystallization of an Inverse Bicontinuous Cubic Phase of a Lipid
Toshihiko Oka[1,2], Hiroki Hojo[3] ([1]*Graduate School of Science, Shizuoka University*, [2]*Research Institute of Elctronics, Shizuoka University*, [3]*Faculty of Science, Shizuoka University*)

3P205 **蛍光セルソーターを用いたリポソームの融合・破壊の定量的評価**
Quantitative evaluation of GUV fusion and destruction with fluorescence activated cell sorter
Kunihiro Shimada[1], Takeshi Sunami[1,2], Tetsuya Yomo[1,2,3] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*JST, ERATO*, [3]*Grad. Sch. Sci., Univ. Osaka*)

3P206 **モデル細胞膜に対する化学物質の影響について**
Influence of chemical compounds on model cell membranes
Kazunari Yoshida, Akito Takashima, Izumi Nishio (*Coll. Sci. Eng., Aoyama Gakuin Univ.*)

3P207 **アデノウイルス由来両親媒性ペプチドの正曲率依存的な膜傷害性**
Preferential Perturbation of Positively Curved Membranes by Adenovirus-derived Amphiphilic Peptide
Tomo Murayama, Silvia Pujals, Shiroh Futaki (*Institute for Chemical Research, Kyoto Univ.*)

3P208 **動的および静的光散乱法によるリン脂質ベシクルの構造評価**
Structural evaluation of phospholipid vesicles by dynamic and static light scattering techniques
Nobutake Tamai[1], Takeshi Nobuoka[1], Masaki Goto[1,2], Hitoshi Matsuki[1] ([1]*Inst. Technol. & Sci., Univ. of Tokushima*, [2]*Lab. for Neutron Scattering, ETHZ & PSI*)

3P209 **リン脂質二重膜の圧力および化学誘起指組み構造化：形成機構の相違**
Pressure- and chemically induced interdigitation of phospholipid bilayers: difference in the formation mechanisms
Hitoshi Matsuki[1], Masaki Goto[1,2], Nobutake Tamai[1] ([1]*Inst. of Technol. & Sci., The Univ. of Tokushima*, [2]*Lab. for Neutron Scattering, ETHZ & PSI*)

3P210 **凍結超薄切片法によるテープ剥離したヒト皮膚角層構造の部位差研究**
Comparative cryo-ultrathin section study of human stratum corneum cells tape-stripped from different body regions
Keisuke Nakamura, Hiromitsu Nakazawa, Satoru Kato (*Sch. Sci&Tech. Kwansei Gakuin Univ.*)

3P211 **皮膚角層モデル膜に対する水の浸透の FTIR-ATR による解析**
FTIR-ATR analysis of water permeation into stratum corneum model membranes
Kohei Oka, Satoru Kato (*Kwansei Gakuin University*)

3P212 **時分割広角 X 線散乱によるラフトモデルリポソームとアミロイドベータタンパク質との相互作用に関する研究**
Time-resolved wide-angle X-ray scattering study of interaction between raft-model liposome and amyloid-beta protein
Shoki Sato[1], Mitsuhiro Hirai[1], Noboru Ohta[2] ([1]*Grad. Sch. Sci. Eng. Gunma Univ.*, [2]*JASRI*)

13B. 生体膜・人工膜：ダイナミクス / 13B. Biological & Artificial membrane: Dynamics

3P213 **力学的負荷下でのコレステロール含有リン脂質膜中の疎水孔形成：分子動力学シミュレーション**
Hydrophobic Pore Formation in Phospholipid/Cholesterol Bilayers under Mechanical Stretching: Molecular Dynamics Simulation
Taiki Shigematsu, Kenichiro Koshiyama, Shigeo Wada (*Grad. Sch. Eng. Sci., Osaka Univ.*)

3P214 **細胞運動における細胞内局所 pH の影響**
Effect of cytoplasmic local pH on the cell migration
Yusuke V. Morimoto[1], Masahiro Ueda[1,2] ([1]*QBiC, RIKEN*, [2]*Grad. Sch. Sci., Osaka Univ.*)

3P215 **協働的自己会合に基づく脂質―ペプチドナノ微粒子の自己複製**
Self-reproduction of lipid-peptide nanoparticles by synergistic self-assembly
Keisuke Ikeda, Minoru Nakano (*Grad. Sch. Med. Pharm. Sci., Univ. Toyama*)

3P216 **走査型イオンコンダクタンス顕微鏡を用いたコンフルエント上皮生細胞の膜揺らぎ定量化**

Membrane fluctuations of confluent epithelial cells quantified by scanning ion conductance microscopy

Zen Ishikura[1], Yusuke Mizutani[2], Myung-Hoon Choi[2], Sang-Joon Cho[2,3], Takaharu Okajima[1] ([1]*Graduate School of Information Science and Technology, Hokkaido University*, [2]*Park Systems Inc.*, [3]*Seoul National University*)

3P217 **細胞透過ペプチドであるトランスポータン 10 のベシクル内への進入と脂質膜中のポア形成に対する張力の効果**

Effects of tension on entry of cell-penetrating peptide transportan 10 into a single vesicles and its pore formation in lipid membranes

Md. Zahidul Islam[1], Mohammad Abu Sayem Karal[1], Masahito Yamazaki[1,2] ([1]*Int. Biosci., Grad. Sch. Sci. Tech., Shizuoka Univ.*, [2]*Res. Inst. Electronics, Shizuoka Univ.*)

3P218 **張力が誘起する脂質膜中のポア形成に対する静電相互作用の効果**

Effects of Electrostatic Interactions on the Rate Constant of Tension-Induced Pore Formation in Lipid Membranes

Mohammad Abu Sayem Karal[1], Taka-aki Tsuboi[2], Victor Levadny[3], Masahito Yamazaki[1,2,4] ([1]*Int. Biosci., Grad. Sch. Sci. Tech., Shizuoka Univ.*, [2]*Dept. Phys., Grad. Sch. Sci., Shizuoka Univ.*, [3]*Theo. Pro. Center Phys.-Chem. Pharm., Rus. Acad. Sci.*, [4]*Res. Inst. Electronics, Shizuoka Univ.*)

3P219 **膜の伸展により活性化される抗菌ペプチド・マガイニン２のポア形成**

Stretch-Activated Pore of the Antimicrobial peptide, Magainin 2

Md. Jahangir Alam[1], Mohammad Abu Sayem Karal[2], Tomoki Takahashi[3], Victor Levadny[4], Masahito Yamazaki[1,2,3] ([1]*Res. Inst. Electronics, Shizuoka Univ.*, [2]*Int. Biosci., Grad. Sch. Sci. Tech., Shizuoka Univ.*, [3]*Dept. Phys., Grad. Sch. Sci., Shizuoka Univ.*, [4]*Theo. Pro. Center Phys.-Chem. Pharm., Rus. Acad. Sci.*)

3P220 **張力が誘起する脂質膜中のポア形成に対する静電相互作用の効果の理論**

Theory on the electrostatic effects on tension-induced pore formation in lipid membranes

Victor Levadny[1,2], Mohammad Abu Sayem Karal[2], Taka-aki Tsuboi[3], Marina Belaya[1], Masahito Yamazaki[2,3,4] ([1]*Center Theo. Prob. Phys.-Chem.l Pharm., Rus. Acad. Sci.*, [2]*Int. Biosci., Grad. Sch. Sci. Tech., Shizuoka Univ*, [3]*Dept. Phys., Grad. Sch. Sci., Shizuoka Univ.*, [4]*Res. Inst. Electronics, Shizuoka Univ.*)

13C. 生体膜・人工膜：興奮・チャネル / 13C. Biological & Artificial membrane: Excitation & Channels

3P221 **Recording Ion-Channel Activities Based on Microfabricated Silicon Chips**

Yutaka Ishinari[1], Ayumi Hirano-Iwata[1], Yasuo Kimura[2], Michio Niwano[3] ([1]*Grad. Sch. Biomed. Eng., Univ. Tohoku*, [2]*Sch. Comp. Sci., Univ. Tech. Tokyo*, [3]*RIEC., Univ. Tohoku*)

3P222 **イオンチャネルの機能の改変**

Modifications of ion channel function

Minako Hirano[1], Daichi Okuno[2], Yukiko Onishi[2], Hiroaki Yokota[1], Toru Ide[3] ([1]*GPI*, [2]*Qbic, Riken*, [3]*Okayama Univ.*)

3P223 **チャネル内の水の水素結合鎖を介するプロトン透過の整流性**

Rectified proton permeation through the hydrogen-bonded water-chain in a channel peptide

Yuka Matsuki[1], Masayuki Iwamoto[1], Shigeki Matsunaga[2], Shigetoshi Oiki[1] ([1]*Dept. Mol. Physiol. Biophys., Univ. Fukui Fac. Med. Sci.*, [2]*Lab. Aqua. Nat. Products Chem., Grad. Sch. Agri. Life Sci., Univ. Tokyo*)

3P224 **ROS 非依存的な細胞内ミトコンドリアの一過性脱分極について**

ROS-independent transient depolarization of mitochondria in cells

Kanji Umiuchi, Yoshihiro Ohta (*Grad Sch. Engin., Tokyo Univ. Agric. & Technol.*)

14. 化学受容 / 14. Chemoreception

3P225 **大腸菌走化性レセプターの内膜における局在性解析への急速凍結レプリカ電子顕微鏡法によるアプローチ**

A quick-freezing replica electron microscopic analysis for the localization of chemoreceptors on bacterial inner membranes

Kazunori Kawasaki[1], Takehiko Inaba[2], Emiko Kobayashi[1], So-ichiro Nishiyama[3], Ikuro Kawagishi[3] ([1]*AIST*, [2]*RIKEN*, [3]*Dept. Frontier Biosci, Hosei Univ.*)

3P226 **二成分制御系間クロストークを用いた細菌べん毛の回転方向制御**

Control of the bacterial flagellar motor by cross regulation between non-cognate two-component regulatory systems

Tohru Umemura[2], Mayumi Kobayashi[2], Chiho Hara[2], Yoshiyuki Sowa[1,2], Ikuro Kawagishi[1,2] ([1]*Micro-Nano Tec. Cen., Univ. Hosei*, [2]*Dept. Frontier Bio., Univ. Hosei*)

3P227 **多刺激受容センサー Tar の温度感知領域の探索**

In search of thermosensing regions of the multimodal sensor Tar

So-ichiro Nishiyama[1,2], Masaaki Jinguji[1], Ikuro Kawagishi[1,2] ([1]*Fac. Front. Biosci., Hosei Univ.*, [2]*Res. Cen. Micro-nano Tech., Hosei Univ.*)

15. 神経・感覚 / 15. Neuroscience & Sensory systems

3P228 **線虫の単一神経細胞における Ca^{2+}時空間ダイナミクス: 数理モデルとその解析**

Spatial-temporal Ca^{2+} dynamics in a whole single neuron of C. elegans: Mathematical modeling and analysis

Yuishi Iwasaki[1,3], Sayuri Kuge[2,3], Takayuki Teramoto[2,3], Takeshi Ishihara[2,3] ([1]*Fac. Eng., Ibaraki Univ.*, [2]*Grad. Sci., Kyushu Univ.*, [3]*JST, CREST*)

3P229 **低温・Xe 加圧下における DEPC リポソーム相変化と神経細胞のラマン測定**

Raman spectra change at the phase transition of DEPC liposome and cell membrane of neuron under low temperature and xenon pressure

Tsutomu Uchida[1], Masafumi Nagayama[2], Kazutoshi Gohara[1], Amadeu K. Sum[3] ([1]*Fac. Eng., Hokkaido Univ.*, [2]*Hokkaido Univ. Edu., Asahikawa*, [3]*Colorado Sch. Mines, USA*)

3P230 **局所熱パルス法による神経細胞のカルシウム放出の誘導**

Ca2+-burst in rat hippocampal neurons induced by microscopic heat pulses

Yuki Kawamura[1], Kotaro Oyama[1], Hideki Itoh[1,2], Madoka Suzuki[3,4], Shin'ichi Ishiwata[1,3,4] ([1]*Sch. Adv. Sci. Eng., Waseda Univ., Tokyo, Japan*, [2]*Inst. Med. Biol., A*STAR, Singapore*, [3]*Org. Univ. Res. Initiatives, Waseda Univ., Tokyo, Japan.*, [4]*WABIOS, Singapore*)

16. 神経回路・脳の情報処理 / 16. Neuronal circuit & Information processing

3P231 **ヒト iPS 細胞由来ニューロンとアストロサイト共培養による電気生理学的特徴**

Electrophysiological activity of a human induced pluripotent stem cell derived neuron and astrocyte co-culture

Aoi Odawara[1,3], Ikuro Suzuki[2] ([1]*Department of Bionics, Tokyo University of Technology*, [2]*Department of Electronics and Intelligent Systems, Tohoku Institute of Technology*, [3]*Japan Society for the Promotion of Science*)

3P232 **導電性高分子含浸ファイバー電極の脳活動測定と刺激への適用**

Application of conductive polymer-coated fiber electrodes to neural recording and stimulation in vivo

Satoshi Watanabe, Hideyuki Takahashi, Keiichi Torimitsu (*Dept. Bioeng. Robotics, Grad. Sch. Eng., Tohoku Univ.*)

17. 行動 / 17. Behavior

3P233 **運動性シアノバクテリア *Pseudanabaena* sp. ILC 545 の「彗星状コロニー」の形成ダイナミクス**

Dynamics of comet-like colony formation in the filamentous cyanobacterium, *Pseudanabaena* sp. ILC 545

Yu Shoji[1], Hiroki Yamamoto[1], Yuki Fukasawa[1], Hideo Iwasaki[1,2] ([1]*Waseda University*, [2]*metaPhorest*)

3P234 **Analysis on colony formation in a filamentous cyanobacterium with an extended self-driven particle model with a cellular automaton method**

Masato Ishii[1], Yuki Fukasawa[1], Masaya Takiguchi[1], Kain Yanagi[1], Hideo Iwasaki[1,2] ([1]*Waseda University*, [2]*metaPhorest*)

18A. 光生物：視覚・光受容 / 18A. Photobiology: Vision & Photoreception

3P235 **光駆動ナトリウムポンプの pH 依存性**

The extracellular pH dependency of transport activity by light-driven sodium ion pump

Rei Abe-Yoshizumi[1], Yoshitaka Kato[1], Keiichi Inoue[1,2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*JST PRESTO*)

3P236 **ニワトリクリプトクロム 4 の光反応特性に外部環境が与える影響**

***In vitro* redox cycle of Chicken Cryptochrome4 under various ambient condition**

Hiromasa Mitsui, Toshinori Maeda, Chiaki Yamaguchi, Yusuke Tsuji, Kazuki Sakai, Keiko Okano, Toshiyuki Okano (*Dept. Eng. and Biosci., Grad. Sch. Adv. Sci. and Eng., Waseda Univ.*)

3P237 **哺乳類 NDRG1 のゼブラフィッシュ相同蛋白質の視細胞における機能解析**

Functional analysis of zebrafish orthologs of mammalian NDRG1 protein in photoreceptors

Shimpei Takita[1], Yasutaka Wada[1,2], Satoru Kawamura[1,2] ([1]*Dept. of Biol. Sci. Grad. Sch. of Sci., Osaka Univ.*, [2]*Grad. Sch. of Frontier Biosci., Osaka Univ.*)

3P238 **イエロープロテインの強い水素結合形成に関する FTIR 研究**

FTIR study on the strong hydrogen bonding formation in photoactive yellow protein

Tatsuya Iwata, Hideki Kandori (*Dept. Ftontier Mat., NITech*)

3P239 **基質の状態によらないロドプシンの脱リン酸化反応速度**

Stage-independent dephosphorylation of rhodopsin during its regeneration cycle

Hiromi Yamaoka, Shuji Tachibanaki, Satoru Kawamura (*Grad. Sch. Frontier Biosci., Osaka Univ.*)

3P240 **バクテリオロドプシン-ハロロドプシン-キメラ蛋白質の光反応サイクル**

Photoreaction cycle of a bacteriorhodopsin-halorhodopsin chimeric protein

Shinji Uyama[1], Tomomi Kitajima[1], Midori Murakami[1], Tsutomu Kouyama[1] ([1]*Graduate School of Science, Nagoya University*, [2]*Center of the gene research, Nagoya University*)

3P241 **時間分解偏光 FTIR 計測によるバクテリオロドプシンの光サイクル反応解析**

Time-resolved polarized FTIR spectroscopy on the photocyclic reaction of bacteriorhodopsin

Kuniyo Fujiwara[1], Yuji Furutani[1,2,3] ([1]*IMS*, [2]*SOKENDAI*, [3]*JST PRESTO*)

3P242 **水溶液中のオキシルシフェリン吸収スペクトルの理論的研究**

Theoretical Analysis of Absorption Spectra of Oxyluciferin in Aqueous Solutions

Miyabi Hiyama[1], Hidefumi Akiyama[1], Nobuaki Koga[2] ([1]*ISSP*, [2]*Nagoya Univ.*)

3P243 **Analysis of the photoresponse mechanism of the LOV-HTH system using accelerated molecular dynamics simulation**

Tetsuo Kokubu, Tadaomi Furuta, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

3P244 **2 種類の PYP における光誘起構造変化の違いをうむ部位の解析**
Analysis for different property of light induced structural changes between two PYPs
Yoichi Yamazaki, Yoshiaki Mathumoto, Hironari Kamikubo, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

3P245 **イエロープロテイン光反応中間体の熱平衡の解析**
Thermal equilibria between the photocycle intermediates of photoactive yellow protein
Yasushi Imamoto, Yoshinori Shichida (*Grad. Sch. Sci., Kyoto Univ.*)

3P246 **PYP の β4-5 loop 領域と発色団環境との関係性の解明**
The elucidation of the relationship between β4-5 loop region and the chromophore environment in PYPs
Atsuhiro Kawamura, Yoichi Yamazaki, Hironari Kamikubo, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

3P247 **BLUF タンパク質 PapB の FTIR 法による構造解析**
Characterization of light-induced structural changes of the BLUF protein PapB
Hiroaki Akutsu[1], Shinji Masuda[2,3] ([1]*Grad. Sch. Biosci. & Biotech., Tokyo Inst. Tech.*, [2]*Cent. Biol. Res. & Inforn., Tokyo Inst. Tech.*, [3]*ELSI, Tokyo Inst. Tech.*)

3P248 **NTQ モチーフを持つ新規微生物型ロドプシンの輸送イオン種の同定**
Ion species transported by the novel microbial rhodopsin containing NTQ motif
Naho Toyama[1], Takashi Kikukawa[1], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Kwang-Hwan Jung[2], Naoki Kamo[1], Makoto Demura[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ*, [2]*Dept. Life Sci. & Inst. Biol. Interfaces, Sogang Univ*)

3P249 **Photoactive Yellow Protein における酸誘起プロトン移動**
Acid induced proton transfer in Photoactive Yellow Protein
Masayoshi Noji, **Mai Arakawa**, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

3P250 **光駆動ナトリウムポンプのイオン取込みに関する分光研究**
Spectroscopic study on the ion uptake mechanism of the light-driven sodium ion pump
Yoshitaka Kato[1], Keiichi Inoue[1,2], Hkaru Ono[1], Rei Abe-Yoshizumi[1], Hideki Kandori[1] ([1]*Nagoya Ist. Tech.*, [2]*JST, PRESTO*)

3P251 **過渡回折格子法でみた赤色光センサー蛋白質(Cph1)の光反応ダイナミクス**
Photoreaction dynamics of the Cyanobacterial phytochrome 1 (Cph1) studied by the transient grating method
Kimitoshi Takeda, Masahide Terazima (*Graduate School of Science, Kyoto University*)

3P252 **NdR2 の D116 残基の機能的および分子的役割の解明**
Role of D116 for the Na^+ pump activity and molecular property of *Nd*R2
Shinya Sugita[1], Keiichi Inoue[1,2], Rei Abe-Yoshizumi[1], Yoshitaka Kato[1], Hideki Kandori[1] ([1]*Grad. Sch .Eng., Nagoya Inst. Tech.*, [2]*JST PRESTO*)

3P253 **New crystal forms of squid rhodopsin**
Midori Murakami, Tsutomu Kouyama (*Nagoya University*)

18B. 光生物：光合成 / 18B. Photobiology: Photosynthesis

3P254 **コンピュータシミュレーションによる光合成循環的電子伝達の非光化学消光（NPQ）の誘導における役割と寄与率の推定**
Computer simulation of photosynthetic electron transport - Prediction of contribution of the linier and the cyclic electron flow -
Ryoichi Sato[1], Hiroyuki Ohta[2], Shinji Masuda[2] ([1]*Grad. Sch. Biosci. Biotechnol., Tokyo Inst. Technol.*, [2]*Center for Biological Resources and Informatics, Tokyo Inst. Technol.*)

3P255 **光捕獲系内の励起エネルギー移動に対する分子内振動モードの役割の解析**
Analyses of intra-molecular vibrational modes affecting excitation energy transfer in light harvesting systems
Yuta Fujihashi, Akihito Ishizaki (*IMS*)

3P256 **高速原子間力顕微鏡によるホウレンソウ由来グラナ膜の観察**
Observation of grana membranes from spinach by high-speed atomic force microscopy
Ami Komata, Daisuke Yamamoto (*Dept. Appl. Phys., Grad. Sch. Sci., Fukuoka Univ.*)

3P257 **光合成蛋白質と金属ナノ粒子による水素発生人工光合成ナノデバイスの開発**
Development of an artificial light-driven water splitting nano-device using photosynthetic proteins and metal nanoparticles
Kazuki Tahara[1], Kousuke Kawahara[1], Keisuke Namie[2], Natsuko Inoue[3], Ryo Nagao[1], Yuki Kato[1], Tatsuya Tomo[4], Yutaka Shibata[2], Hiroshi Fukumura[2], Yasuhiro Kashino[3], Takumi Noguchi[1] ([1]*Div. of Mater. Sci., Grad. Sch. Sci., Nagoya Univ.*, [2]*Dept. of Chem., Grad. Sch. Sci., Tohoku Univ.*, [3]*Grad. Sch. Life Sci., Univ. of Hyogo*, [4]*Dept. of Biol., Faculty of Sci., Tokyo Univ. of Sci*)

3P258 **Detection of Transient Y_Z Radical Signals during S-State Transition in Photosystem II**
Wataru Koinuma, Hiroyuki Mino (*Grad. Sch. Sci., Nagoya Univ.*)

3P259 **QM/MM 計算による光化学系Ⅱにおける水分解 Mn4Ca クラスターのアミノ酸配位子の基準振動解析**
Vibrational Analysis of the Amino Acid Ligands to the Water Oxidizing Mn4Ca cluster in Photosystem II using QM/MM Calculations
Shin Nakamura, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

3P260 **Site-directed mutagenesis study of amino acid resides relevant to photosynthetic water oxidation in photosystem II**
Ryo Nagao, Hanayo Ueoka-Nakanishi, Takumi Noguchi (*Grad. Sch. Sci.,Univ. Nagoya*)

3P261 **Quantitative refinement of the theory of the improved variational master equation**
Yuta Fujihashi[1], **Akihiro Kimura**[2] ([1]*IMS*, [2]*Nagoya Univ.*)

3P262 **極低温顕微鏡を用いた緑化途上トウモロコシ生葉の光合成タンパク質前駆体の空間分布の測定**
Cryogenic microscope observations of photosynthetic proteins under assembly process in greening etiolated Zea mays leaves
Tomofumi Chiba, Hiroshi Fukumura, Yutaka Shibata (*Grad. Sch. Sci., Univ. Tohoku*)

3P263 **緑色硫黄細菌のタイプ 1 光合成反応中心は 2 系列のエネルギー移動系をもつ**
Two Disconnected Antenna Chlorophyll Pools in Type-1 Photosynthetic Reaction Center of Green Sulfur Bacteria
Chihiro Azai[1], Toru Kondo[2], Shigeru Itoh[3], Hirozo Oh-oka[4] ([1]*Col. Life Sci., Ritsumeikan Univ.*, [2]*Grad. Schl. Sci. & Eng., Tokyo Inst. Tech.*, [3]*Cent. Gene Res., Nagoya Univ.*, [4]*Grad. Schl. Sci., Osaka Univ.*)

20. 生命の起源・進化 / 20. Origin of life & Evolution

3P264 **復元した祖先型ヌクレオシドニリン酸キナーゼの解析**
Characterization of resurrected ancestral nucleoside diphosphate kinases
Takahiro Sasamoto, Satoshi Akanuma, Akihiko Yamagishi (*Dept. of Appl. Life Sci., Tokyo Univ. of Pharm. Life Sci.*)

3P265 **バクテリア融合チャンバーからのバクテリア再生に向けた研究**
Toward reproduction of a bacterium from hybrid chamber cells
Kazuhito Tabata, Yoshiki Moriizumi, Rikiya Watanabe, Hiroyuki Noji (*Department of Applied Chemistry, The University of Tokyo*)

3P266 **バクテリアプロトプラストとマイクロ膜チャンバーの融合反応の高感度検出**
High-sensitive detection method of bacterial protoplast fusion into a micron-sized lipid membrane chamber
Yoshiki Moriizumi[1], Kazuhito V. Tabata[1,3], Rikiya Watanabe[1,3], Hiroyuki Noji[1,2] ([1]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo.*, [2]*CREST, JST*, [3]*PRESTO, JST*)

22A. 生命情報科学：構造ゲノミクス / 22A. Bioinformatics: Structural genomics

3P267 **Structural characteristics of phosphorylation sites on disordered binding regions**
Hafumi Nishi, Akinori Kidera (*Grad. Sch. Medical Life Sci., Yokohama City Univ.*)

3P268 **De Novo protein structure modeling by rewiring old folds**
Shunsuke Nishiyama, Tatsuo Mukai, George Chikenji (*Grad. Sch. of Engineering, Nagoya Univ*)

3P269 **Template based modeling utilizing an order-made template libarary**
Kodai Takagi, Tatsuro Mukai, George Chikenji (*Grad. Sch. Eng., Nagoya Univ.*)

3P270 **Homologous protein pairs that share the same core packing but have different topology**
Takahiro Kanemitsu[1], Shintaro Minami[2], George Chikenji[1] ([1]*Grad. Sch. of Eng., Nagoya Univ.*, [2]*Grad.Sch. of Inf.Sci., Nagoya Univ.*)

3P271 **タンパク質立体構造におけるループ交差検出のためのアルゴリズム**
An algorithm for identifying loop crossing in protein structures
Tatsuo Mukai, George Chikenji (*Grad. Sch. of Eng., Dept. of Comput. Sci. and Eng., Nagoya Univ.*)

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Ziya Kalay, Yohsuke Yoshinari, Yoshie Harada (*WPI-iCeMS, Kyoto University*)

3P273 **タンパク質量バランス制御機構解明に向けた、個別タンパク質量制御ルールの分類**
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3P274 **バクテリアの Twitching 運動における線毛の伸縮規則の影響**
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Soutaro Oda[1], Chwen-Yang Shew[2], Kenichi Yoshikawa[1] ([1]*Faculty of Life and Medical Sciences, Doshisha University*, [2]*City University of New York*)

3P276 **Multicanonical Go モデル分子動力学によるタンパク質の自由エネルギーの網羅的解析**
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25. 非平衡・生体リズム / 25. Nonequilibrium state & Biological rhythm

3P280 **中和反応を駆動力とする走化性液滴:(1) ガスに対する応答**
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Hiroki Sakuta[1], Nobuyuki Magome[2], Yoshihito Mori[3], Akihisa Shioi[4], Kenichi Yoshikawa[1] ([1]*Facul. Lif. Med. Sci., Doshisha Univ.*, [2]*Chem., Dokkyo Med. Univ.*, [3]*Facul. Sci., Ochanomizu Univ.*, [4]*Facul. Sci. Eng., Doshisha Univ.*)

3P281 **中和反応を駆動力とする走化性液滴:（2）炭化水素残基効果と境界条件**
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3P282 **中和反応を駆動力とする走化性液滴：(3) 自己触媒反応による pH 変化と液滴の動き**
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Nobuyuki Magome[1], Tatsuya Okuda[1], Noriko Umezawa[1], Yoshihito Mori[2], Kenichi Yoshikawa[3] ([1]*Premedical Sci., Dokkyo Med. Univ.*, [2]*Grad. Sch. Sci., Ochanomizu Univ.*, [3]*Grad. Sch. Life and Med. Sci, Doshisha Univ.*)

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Kenju Narita, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Nagoya Univ.*)

3P284 **Epigenetic Dynamics of Cell Reprogramming**
Ashwin S.S., Masaki Sasai (*Department of Computational Sciences and Engineering, Nagoya University, Nagoya Japan.*)

26. 計測 / 26. Measurements

3P285 **アトリットル容積を持つナノセルを用いた酵素 1 分子の高速検出**
Rapid Detection of Single-Molecule Enzyme using Attoliter Well Array, Nanocell
Takao Ono[1,2], Takanori Ichki[3], Hiroyuki Noji[1,2] ([1]*Dept. Appl. Chem., Sch. Eng., Univ. Tokyo*, [2]*JST-CREST*, [3]*Dept. Bioeng., Sch. Eng., Univ. Tokyo*)

3P286 **帽子型金ナノ粒子を用いた近赤外型局在表面プラズモン共鳴バイオセンサー**
Near infrared localized surface plasmon resonance biosensing based on cap-shaped gold nanoparticles
Hiroyuki Takei[1,2], Takumi Miyashita[3], Noriyuki Bessho[3], Takayuki Okamoto[4] ([1]*Faculty of Life Sciences, Toyo University*, [2]*Bio Nano Research Centre, Toyo University*, [3]*Grad. School of Life Sciences, Toyo University*, [4]*Riken Wako*)

3P287 **フェムトリットルドロップレットアレイを用いたアルカリフォスファターゼ１分子活性の検出及び定量計測**
Detection and activity measurement of single molecule alkaline phosphatase with femtoliter droplet array
Yusuke Obayashi[1], Ryota Iino[2], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Okazaki Inst. Integ. Biosci, NINS.*)

3P288 **培養神経細胞の軸索輸送活動度の新たな評価方法**
A new quantitative method to evaluate the activity of axonal transport of cultured neurons
Takashi Katakura, Risa Isonaka, Tadashi Kawakami (*Dept. Physiol., Kitasato Univ. Sch. Med.*)

3P289 **神経細胞に対する Amyloidβ42 毒性伝搬 in vitro モデルの構築**
Development of neurotoxicity of amyloid β(1-42) proteins propagation in vitro model
Takuma Maruyama[1], Lui Yoshida[2], Kiyoshi Kotani[3], Seiichi Suzuki[1], Yasuhiko Jimbo[3] ([1]*Grad. Sch. Sci and Tech, SEIKEI Univ.*, [2]*Grad. Sch. Frontier Sci, Univ of Tokyo*, [3]*Grad. Sch. Engineering, Univ of Tokyo*)

3P290 **Automation engineering for single molecule imaging using total internal reflection fluorescence microscopy**
Jun Kozuka, Michio Hiroshima, Yasushi Sako, Masahiro Ueda (*RIKEN*)

3P291 **ナノ粒子表面へのプローブオリゴヌクレオチドの修飾密度が DNA ハイブリダイゼーション効率に与える影響**
The contribution of the density of immobilized probe oligonucleotide on nanoparticle surface for DNA hybridization efficiency
Atsushi Kira, Atsushi Suda (*Product Development Center, Japan Aviation Electronics Industry, Ltd.*)

3P292 **高速原子間力顕微鏡ピエゾドライバの広帯域駆動**
Wideband operation of high-voltage amplifier for high-speed atomic force microscopy
Hiroyuki Handa, **Daisuke Yamamoto** (*Dept. Appl. Phys., Grad. Sch. Sci., Fukuoka Univ.*)

3P293 **冷却 HPD によるサブミリ秒時間分解能の広視野蛍光 1 分子検出**
Low-background wide-field sub-millisecond single-molecule fluorescence detection by a cooled hybrid photo-detector (HPD)
Atsuhito Fukasawa[1,2], Minako Hirano[2], Toru Ide[3], Hiroaki Yokota[2] ([1]*Hamamatsu Photonics K.K.*, [2]*GPI*, [3]*Grad. Sch. Nat. Sci. Technol., Okayama Univ.*)

27. バイオイメージング / 27. Bioimaging

3P294 **単粒子解析法の高速化のための GPGPU を用いた並列化処理の実装と評価**
Implementation and evaluation of parallel processing by GPGPU for accelerating single particle analysis
Ayaka Iwasaki, Takuo Yasunaga (*Kyushu Institute of Technology*)

3P295 **生細胞内における長鎖非翻訳 RNA の一分子イメージング法の開発**
A method to visualize endogenous long non-coding RNA with single molecule sensitivity
Toshimichi Yamada, Hideaki Yoshimura, Mituru Hattori, Hiroki Segawa, Takeaki Ozawa (*Grad. Sch. Sci., Univ. Tokyo*)

3P296 **複数の光遺伝学ツールとの組み合わせが可能な化学発光膜電位センサーの開発**
Genetically-encoded chemiluminescent voltage indicator applicable in conjunction with multiple optogenetic tools
Shigenori Inagaki[1], Tomoki Matsuda[1], Yoshiyuki Arai[1], Yuka Jinno[2], hidekazu Tsutsui[2,3], Yasushi Okamura[2], Takeharu Nagai[1] ([1]*ISIR., Univ. Osaka*, [2]*Grad. Sch. Med., Univ. Osaka*, [3]*Sch. Mat. Sci., JAIST*)

3P297 **電子顕微鏡画像処理システム Eos への Web ブラウザと PIONE の統合による、ユーザビリティの向上**
Development of a user-friendly system for image processing of electron microscopy by integrating web browser and PIONE with Eos
Takafumi Tsukamoto, Takuo Yasunaga (*KIT, Creative Informatics*)

3P298 **コヒーレント X 線回折イメージングにおける回復電子密度図の多変量解析を利用した分類と評価**
Classification and assessment of reconstructed electron density maps in coherent X-ray diffraction imaging using multivariate statistics
Yuki Sekiguchi[1,2], Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2] ([1]*Grad. Sci. Tech., Keio Univ.*, [2]*RIKEN SPring-8 Center*)

3P299 **コヒーレントX線回折イメージングにおけるフリーデル対称性を拘束条件とした暗視野位相回復法の開発とシングルショット回折データ解析への応用**
Dark-field phase-retrieval method under the constraint of Friedel's symmetry for structure analyses in coherent X-ray diffraction imaging
Amane Kobayashi[1,2], Yuki Sekiguchi[1,2], Yuki Takayama[2], Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2] ([1]*Grad. Sci. Tech., Keio Univ.*, [2]*RIKEN SPring-8 Center*)

3P300 **Trafficking of membrane protein PAR-1 carried by endocytotic vesicles in cancer cells**
Seohyun Lee[1], Motoshi Kaya[1], Hideo Higuchi[1] ([1]*School of Science, the University of Tokyo*, [2]*School of Medicine, Tohoku University*)

3P301 **A method to integrate 4D images of *C. elegans* embryos expressing different fluorescent markers**
Yusuke Azuma, Shuichi Onami (*RIKEN QBiC*)

3P302 **新奇"無蛍光"蛍光タンパク質による細胞内シグナル伝達の蛍光寿命イメージング**
Imaging intracelluer signal transduction using a newly developed "non-fluorescent" fluorescent protein for FLIM-FRET
Akihiro Shibata[1], Yoshihisa Nakahata[1], Junichi Nabekura[1], Hideji Hurakoshi[1,2] ([1]*NIPS, Okazaki*, [2]*JST PREST*)

3P303 **生細胞内における microRNA の動態観測**
Observation of microRNA dynamics in living cells
Toshinari Ishikawa[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*JST-PRESTO*)

3P304 **生細胞内 mRNA のナノスケール一分子追跡**
Nanoscale Single mRNA Tracking in Living Cells
Ko Sugawara[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad. Sch. Pharm., Univ. Tokyo*, [2]*JST, PRESTO*)

3P305 **Preparation of Green-Emitting Pt Nanoclusters for Biomedical Imaging by Pre-equilibrated Pt/PAMAM (G4-OH) and Mild Reduction**
Shin-ichi Tanaka[1,2], Takashi Jin[2], Yasushi Inouye[3] ([1]*Kure National College of Technology*, [2]*RIKEN*, [3]*Osaka University*)

3P306 **高速 AFM による抗体 IgG の動的観察**
Dynamic observation of single antibody IgG using High-Speed Atomic Force Microscopy
Norito Kotani, Tomohiro Hirano, Takao Okada (*RIBM*)

3P307 **１分子イメージング計測による生細胞表面 T 細胞受容体のマイクロクラスター内外における動態変化**
T cell receptor on the surface of living cells changes in the dynamics inside microclusters revealed by single-molecule imaging analysis
Yuma Ito[1,2], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

3P308 **ミトコンドリア新規単離法の検討**
New approach to isolation of less damaged mitochondria
Takahiro Shibata[1], Rie Yamane[2], Kaoru Katoh[3], Yoshihiro Ohta[1] ([1]*Grad. Sch, Life Sci, TUAT*, [2]*Sch, Life Sci, TUAT*, [3]*AIST*)

3P309 **3D Palm Imaging at 50 Micrometers Depth in the Sample**
Audrius Jasaitis[1], Gregory Clouvel[1], Ignacio Izeddin[2], James Sillibourne[3], Mohamed El-Beheiry[3], Xavier Levecq[1], Maxime Dahan[3], Michel Bornens[3], Xavier Darzacq[2] ([1]*Imagine Optic, France*, [2]*ENS Paris, France*, [3]*Institut Curie, France*)

3P310 **Signal enhancement and Patterson-search phasing for high-spatial-resolution coherent X-ray diffraction imaging of biological objects**
Yuki Takayama[1], Saori Maki-Yonekura[1], Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2], Koji Yonekura[1] ([1]*RIKEN RSC*, [2]*Fac. Sci. Tech., Keio Univ.*)

3P311 **Investigation of intracellular temperature during stress granule formation**
Beini Shi[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad. Sch. Pha. Sci., Univ. Tokyo*, [2]*JST-PRESTO*)

3P312 **蛍光性ポリマー温度センサーを用いた生細胞内における発熱のイメージング**
Imaging of thermogenesis in living cells using fluorescent polymeric thermometer
Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad Sch Pharm Sci, Univ Tokyo*, [2]*JST, PRESTO*)

28. バイオエンジニアリング / 28. Bioengineering

3P313 **マイクロ波照射微生物における至適出力と至適温度**
Optimal microwave energy and optimal temperature on microwave irradiated microbial cultivation
Ryota Nakama[1], Wataru Nagayoshi[1], Takeo Yoshimura[2], Makoto Kodama[3], Shokichi Ohuchi[1] ([1]*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Bio. Sci. Tokyo Univ. Sci.*, [3]*Vessel inc.*)

3P314 **知能ロボットを用いたヒトとのインターフェース**
Intelligence for Robot-Human Communication
Jun Miyake, Amalia Istiqlali Adiba, Nobuyuki Tanaka (*Graduate School of Engineering Science, Osaka University*)

3P315 **マイクロ流体デバイスを用いた三次元モデルにおけるグリオーマ幹細胞と分化誘導グリオーマ細胞の浸潤形態比較**
Comparing of invasion form between glioma stem cells and the differentiated cells in a microfluidic 3D culture system
Sotaro Taki, Shingo Fujioka, Ryo Sudo (*Sci and Tech., Univ. Keio*)

3P316 **らせん構造を有する異方性マイクロゲルファイバーの作製と制御**
Control synthesis of anisotropic hydrogel microfiber with helical structure
Shoya Yasuda[1], Masayuki Hayakawa[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech., Japan*, [2]*PRESTO, JST, Japan*)

3P317 **Self-assembly of complex-shaped microgels**
Satoshi Umeyama, Masayuki Hayakawa, Masahiro Takinoue (*Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology*)

3P318 **温度感受性ゲルを用いた蛍光検出によるドロップレットソーティング技術の開発とその応用**
Development of fluorescence-activated droplet sorting system using thermoreversible gelation polymer and its application
Haruka Okada[1], Ryo Iizuka[1], Ayaka Iguchi[2], Dong H. Yoon[2], Tetsushi Sekiguchi[3], Shuichi Shoji[2], Takashi Funatsu[1] ([1]*Grad. Sch. Pharm. Sci., Univ. Tokyo*, [2]*Major in Nanosci. and Nanoeng., Waseda Univ.*, [3]*Nanotech. Research Center, Waseda Univ.*)

3P319 **Selection of RNA aptamers to develop a sensor using rhodamine as a fluorogenic probe**
Tara Bahadur KC[1,2], Hiroshi Abe[3], Yoshihiro Ito[1,2], Uzawa Takanori[1] ([1]*Emergent Bioengineering Materials Research Team, RIKEN*, [2]*Tokyo Metropolitan University*, [3]*Faculty of Pharmaceutical Sciences, Hokkaido University*)

3P320 **Control of a DNA computer-based gene-regulatory module confined in a giant unilamellar vesicle by external molecular signal**
Toru Nishikata[1,2], Takamasa Hasegawa[1,2], Yutetsu Kuruma[3], Koichiro Shoda[1,2], Akira Suyama[1,2] ([1]*Dept.Life Sci., Univ.of Tokyo*, [2]*Suyama Lab*, [3]*Earth Life.,Tokyo inst of tech*)

3P321 **進化分子工学に向けた酵素スクリニーングシステムの開発：酵素合成及び活性測定の試み**
Development of enzyme screening system for directed evolution based on enzymic activity
Yi Zhang, Hiroto Kizoe, Ryota Iino, Kazuhito Tabata, Hiroyuki Noji (*Department of Applied Chemistry, School of Engineering, The University of Tokyo*)

3P322 **シリカバイオミネラリゼーションを行う新奇人工ペプチドの設計**
The design of novel artificial peptide revealing a silica biomineralization activity
Yoshinao Murakami, Yuki Kimura, Ippei Fujiyama, Yusuke Matsuda (*Grad. Sch. Sci., Kwansei Gakuin Univ.*)

3P323 **マイクロ波照射 PCR 反応の DNA ポリメラーゼの変性**
Denaturation of DNA Polymerase on Microwave Assisted PCR
Shokichi Ohuchi[1], Takeo Yoshimura[2], Hiroya Osoekawa[1] ([1]*Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. Sci.*)

30. その他 / 30. Miscellaneous topics

3P324 **細胞体におけるミトコンドリア移動の評価法の開発**
Development of the method to evaluate mitochondrial movement in the soma
Yuki Sugimoto, Yoshihiro Ohta (*Grad. Sch, Life Sci, TUAT*)

3P325 **異種生物種由来ヘムオキシゲナーゼによるヘム分解反応の微調節：反応の pH 依存性と電子伝達速度**
Fine-tuning of the heme degradation by heme oxygenase from variable biological species: pH dependence and the electron transfer kinetics
Catharina T Migita, Norio Miyake (*Faculty of Agriculture, Yamaguchi Univ.*)

1SBA-01 *In silico* で観察するタンパク質の柔らかで機能的な運動
Observing soft functional motion of proteins *in silico*

Akio Kitao (*IMCB, Univ. Tokyo*)

Proteins exert large conformational fluctuations and changes, which are essential for their functions. This softness is a key to understand the mechanism of protein function and regulation. Molecular dynamics simulation has become a powerful tool to observe protein motion at atomic resolution normally up to microsecond order and it is still difficult to simulate slower motions by molecular dynamics without the use of special-purpose super computers. We have been developing molecular simulation methods to investigate soft protein motions efficiently. These methods utilize anisotropic nature of protein fluctuations. Relationship between soft protein motion and function elucidated by the simulation methods will be demonstrated.

1SBA-02 Continuous tracking of protein folding at microsecond resolution by a line confocal detection of single molecule fluorescence

Satoshi Takahashi (*IMRAM, Tohoku Univ.*)

We developed a line-confocal microscope combined with fast sample flow (Oikawa et al., Sci. Rep. 3, 2151 (2013)), and achieved the time resolution of 20 microsecond in obtaining single molecule FRET efficiency. We investigated the B domain of protein A (BdpA) and ubiquitin doubly labeled with donor and acceptor fluorophores. While the traces observed for BdpA can be interpreted in the framework of two-state mechanism, the native traces showed the gradual shift in the FRET efficiency, which is reminiscent of the downhill folding proteins. We also observed the folding process of ubiquitin after a rapid dilution of the denaturant. The data suggest the appearance of broad substates of the unfolded ubiquitin in the native solution condition.

1SBA-03 光応答性タンパク質の機能転換が明らかにする柔らかな構造機能相関
“Soft” structure-function relationship revealed by functional conversion of photoreceptive proteins

Hideki Kandori (*Nagoya Institute of Technology*)

Various protein functions often derive from a common protein architecture, as is seen for microbial rhodopsins (sensor, pump, channel). Researchers believe that such structure-function relationship has been optimized during evolution, but we sometimes achieved functional conversions. This suggests that structure and function are flexibly coupled with each other, and “softness” of such biomolecular systems is a key to understand successful functional conversion. I will present our experimental efforts on photoreceptive proteins such as rhodopsins and flavoproteins. We try to find new protein functions from nature, as we did for a light-driven sodium pump. We also like to attain functional conversions by mutation, as we did for light-driven proton and chloride pumps.

1SBA-04 酵素活性におけるタンパク質の柔軟性の役割
Crucial Role of Protein Flexibility in Enzymatic Catalysis

Shigehiko Hayashi (*Department of Chemistry, Graduate School of Science, Kyoto University*)

Protein functional processes involve dynamic molecular conformational changes of complex protein systems which often correlate with enzymatic chemical reactions. Hence molecular mechanism of enzymatic activities and coupling of the chemical reactions with protein molecular dynamics underlying functional processes need to be revealed for understanding of molecular nature of protein functions. In the talk, our recent molecular simulation studies on enzymatic reactions in Ras-GAP GTPase and on conformational changes of the chromophore in retinal proteins will be presented, and role of protein conformational changes and flexibility in chemical processes will be discussed.

1SBA-05 タンパク質の機能を生み出す柔らかさの時間分解観測
Time-resolved Observation of Functionally-important Molecular Flexibility of Proteins

Yasuhisa Mizutani (*Grad. Sch. Sci., Osaka Univ.*)

Dynamic structure as well as static one of proteins is needed to be elucidated because coupling of functional units in structural changes is indispensable for proteins to function. We investigate functionally-important molecular flexibility of proteins by using time-resolved resonance Raman spectroscopy, which we have developed to observe the dynamics in wide time region from picosecond to subsecond and wavelength region from visible to far ultraviolet. Site-selective observation by resonance Raman effect will provide us information on structural changes in various sites of protein. In this talk, an account will be given on protein dynamics of light-driven ion pumps and hemoglobin.

1SCA-01 人工細胞回路を用いた DNA コンピューティングの実現
DNA computing through biological nanopore in droplet network system

Ryuji Kawano (*TUAT*)

The goal of this study is to establish the calculating system using DNA and biological nanopores in artificial cell network. We are envisioning for living cells as being a replacement for the kinds of computers that we have now. As an opening gambit, we propose a binary system of NAND logic gate by using single-stranded DNA (ssDNA) and alpha-hemolysin (aHL) nanopore. Here individual ssDNA is used as inputs and outputs are obtained by monitoring electrical signal. This method is significantly different from the conventional computation using DNA in the respect that electrical signals are directly obtained. Therefore, rapid calculation in the droplet network is expected in comparison with conventional computations.

1SCA-02 カンチレバーを用いて細胞分裂機構を探る

Examining the cell division machinery by using the cantilever system

Takeshi Itabashi[1], Shin'ichi Ishiwata[1,2] ([1]*Fac. Sci. Eng., Waseda Univ.*, [2]*WABIOS*)

It has been demonstrated that the mechanical force produced inside and outside a cell plays an important role in cellular functions during the development and growth. Mitotic cells have to adapt to a variety of mechanical forces originating from cell-cell interactions or environmental fluctuations, in order to carry out the cell division properly. However, it is not known how the externally applied force affects the mechano-chemical processes in cell division. To directly address this issue, we have examined the effects of the external mechanical perturbations, which directly modulate the force balance in the mitotic cells, using a micro-fabricated cantilever system. Here we discuss the current progress in the biophysical research on the cell division machinery.

1SCA-03 上皮組織の発生と維持の機械・化学制御の統合的理解に向けて

Toward Understanding the Integration of Mechanical and Chemical Control of Epithelial Development and Maintenance

Kaoru Sugimura[1,2], Mihoko Kajita[3], Yasuyuki Fujita[3], Shuji Ishihara[4] ([1]*Kyoto Univ.*, [2]*JST PRESTO*, [3]*Hokkaido Univ.*, [4]*Meiji Univ.*)

Biological systems operate under both chemical and physical principles. I will present our recent studies on the integration of mechanical and chemical control of epithelial development and maintenance.
At the initial stage of carcinogenesis, transformation occurs in single cells within epithelia, and the transformed cells grow while being surrounded by neighboring normal epithelial cells. It has been shown that activation of signaling pathways in transformed cells leads to their exclusion from a monolayer of normal cells. Here, we identify molecular and physical mechanisms whereby neighboring normal cells actively extrude transformed cells from the epithelium.
We seek to identify mechanical principles of Drosophila wing morphogenesis by using Bayesian force inference.

1SCA-04 Mechanically-controlled tubular microenvironment for 3D cell culture

Hiroaki Onoe (*Dept. Mech. Eng., Keio Univ.*)

As well as intercellular chemical signals, mechanical stimuli are known to be an important factor for determining cell behaviors. I introduce a mechanically-controllable 3D microenvironment where cells can be cultured. The tubular microenvironment was composed of the shell of calcium alginate and the core of extracellular matrix, fabricated by using coaxial microfluidic device. Various types of cells can be cultured in the controlled microenvironment, indicating that this culture system could be an effective platform for analyzing single cellular behavior and tissue formation in 3D culture.

1SCA-05 MEMS technology meets scaling laws for biology

Ko Okumura (*Ochanomizu University*)

Scaling laws have been appreciated in many fields, starting from biology to physics, recently in particular, in the understanding of wetting or hydrodynamic phenomena. Our group has worked especially on the dynamics of fluid drops and bubbles, establishing scaling laws; One of the topics is the imbibition of textured surfaces that are fabricated by MEMS technology. This phenomenon is useful for handling of small amounts of liquids, important in various fields from biology to pharmaceutical industries. In this talk, we review our recent studies in emphasizing capability of scaling laws for the development of various devices and industrial products, and talk on our recent work that demonstrates micro-devices useful in biology on the basis of scaling laws.

1SCA-06 生物物理学における非平衡研究のためのドロップレットマイクロ流体工学

Droplet-based microfluidics for nonequilibrium study in biophysics

Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*, [2]*PRESTO, JST*)

In biophysical systems, many nonequilibrium and dynamic phenomena are found, such as autonomous motion and pattern formation of microorganisms and cells, beating hearts, neural systems, circadian rhythms, etc. Recently, artificial cells and molecular robots have attracted much attention because of their potential of elucidation of the dynamic systems and application of them. Here, I will show recent studies of control of nonequilibrium phenomena using microdroplet-based microfluidics, including construction of anisotropic complex-shaped microstructures and their autonomous motion, nonlinear chemical reactions in nonequilibrium cell-sized vesicles. Finally, I would like to discuss the future of those technologies to understand "What is Life?"

1SDA-01 培養神経回路網における同期バースト活動に関わる分子の探索

Analysis of the molecules involved in synchronized burst activity of cultured neuronal networks

Daisuke Ito[1], Kazutoshi Gohara[2] ([1]*Fac. Advanced Life Sci., Hokkaido Univ.*, [2]*Fac. Engineering, Hokkaido Univ.*)

The molecules involved in the generation and maintenance of synchronized burst activity during long-term development of neuronal networks remain unclear. To clarify molecular mechanisms underlying synchronized burst activity, we investigated the temporal relationship between the molecular expressions and the generation of synchronized burst activity of cultured neuronal networks. We cultured rat cortical neurons over 1-month. Multielectrode array-based recordings showed that spontaneous electrical activity became synchronized bursts during 1-month culture period. To investigate related molecules to synchronized burst activity, gene/protein expression analysis for 1-month culture period was performed using RT-PCR or immunofluorescence imaging of synaptic molecules.

1SDA-02 培養神経細胞・神経回路操作のための表面マイクロ加工技術
Manipulating neurons and neuronal networks with micropatterned surfaces

Hideaki Yamamoto[1,2], Takashi Tanii[3], Michio Niwano[4], Ayumi Hirano-Iwata[2] ([1]*FRIS, Tohoku Univ.*, [2]*Grad. Sch. Biomed. Eng., Tohoku. Univ.*, [3]*Sch. Fund. Sci. Eng., Waseda Univ.*, [4]*RIEC, Tohoku Univ.*)

Primary neurons cultured on a conventional coverslip grow neurites in random orientation and form a uniform network that covers the whole surface (~cm). Using micropatterned surface as a growth scaffold, direction of neurite outgrowth in single neurons or the region of network formation can be extrinsically controlled. Such micropatterned neurons and networks allow us, e.g., to study development of neurons and to investigate how structure of the network determines its function. Here we present our recent work on the application of surface nano/micro-modification techniques in manipulating neurons and their networks. (Yamamoto *et al.*, Appl. Phys. Lett. 99 (2011) 163701; Yamamoto *et al.*, J. Neurochem. 123 (2012) 904-910.)

1SDA-03 Noninvasive real-time measurement of dopamine, action potentials, and postsynaptic potentials using carbon nanotube electrodes chip

Ikuro Suzuki (*Department of Electronics, Tohoku Institute of Technology*)

Multi-electrode arrays (MEAs) can be used for noninvasive, real-time, and long-term recording of electrophysiological activity, but it is still difficult to measure presynaptic activity, such as neurotransmitter release. In this study, we describe the development of planar carbon nanotube (CNT)-MEA chips that can measure both the release of the neurotransmitter dopamine as well as electrophysiological responses. Chronoamperometric measurements using these CNT-MEA chips detected dopamine at nanomolar concentrations and synaptic dopamine release from spontaneous firings. Our CNT-MEA chips made it possible to measure presynaptic activities, postsynaptic potentials, and action potentials, which have a central role in information processing in the neuronal network.

1SDA-04 神経ダイナミクス解明のためのレーザー摂動技術の開発
Laser-induced perturbation into living neuronal networks: Toward understanding neurodynamics

Chie Hosokawa (*Health Res. Inst., AIST*)

Living neuronal networks have been widely studied to elucidate information processing in brain systems. In order to realize artificial control of spatio-temporal dynamics in neuronal activity, we propose and demonstrate laser-induced perturbation into living neurons. Optical tweezers were applied to manipulate synaptic vesicles and neural cell adhesion molecules labeled with quantum dots in neurons. The fluorescence analysis revealed that these were trapped and assembled at the focal spot, because the small assemblies were effectively trapped. Moreover, laser processing and stimulation of neurons was succeeded with a focused femtosecond laser. Our methods have potential in realizing regulation of neuronal networks without application of any drugs and genes.

1SDA-05 Revealing Neuronal Dynamics through Advanced Electrophysiology and Chemical Sensing using CMOS Technology

Urs Frey[1,2], Marie Engelene Obien[1], Florent Seichepine[1], Kosmas Deligkaris[1,2] ([1]*RIKEN Quantitative Biology Center*, [2]*Graduate School of Frontier Biosciences, Osaka University*)

Measuring neuronal activity accurately and simultaneously is key for advancing our understanding of how the brain processes information. Microelectrode arrays (MEAs) are used for highly parallel, non-invasive, and long-term measurements from large assemblies of neurons in cell cultures, brain slices and retinal preparations. The use of CMOS technology for MEAs allows increasing the sensor count and spatial resolution drastically.

We present our recent progress in advancing MEA technology and in integrating carbon nanotubes to sense the chemical microenvironment in the extracellular fluid. We discuss our research on Purkinje cell functional dynamics in acute cerebellar slices and our investigations on subcellular extracellular dynamics of neurons in in-vitro cultures.

1SDA-06 培養神経回路網における情報表現
Information presentation in cultured neuronal networks

Suguru N. Kudoh (*Department of Human System Interaction, School of Science and Technology, Kwansei Gakuin University*)

A small-scale network of dissociated neurons cultured on a multielectrodes-array-dish is one of useful models of fundamental units for brain information processing. Spatiotemporal patterns of electrical spikes evoked by external inputs were not only various but also reproducible, specific for stimulation electrodes. X-means clustering applied to the feature vectors of electrical spike activity revealed that there were numerous patterns of activity, however, highly reproducible spike patterns were not much, approximately 15 clusters in a 10-min-recording of spontaneous activity. These patterns were also modified according to the temporal pattern of input stimuli, suggesting that the cultured neuronal network possesses a short-term memory.

1SDA-07 聴覚皮質における聴覚神経応答の解析及び神経ダイナミクス制御のためのマイクロデバイス開発
Analysis of auditory neural responses in the auditory cortex in vivo and development of microdevices to control neurodynamics

Jun Nishikawa, Takeaki Haga, Yuishi Tachibana, Yasutaka Yanagawa, Takashi Tateno (*Grad. Sch. of Inf. Sci. & Tech., Hokkaido Univ.*)

Understanding sensory neural representation is important to realize bidirectional brain-machine interfaces (BMIs) to restore sensory function. In this study, we firstly analyzed neural response properties to auditory stimuli in rodent auditory cortex using spatiotemporal receptive fields (STRFs). The result showed different response properties in each cortical layer and subfield in the auditory cortex. Next, we developed a CMOS-based LSI system that is capable of both stimulation and recording of neurons at 64-ch electrode sites. We showed that various spatiotemporal patterns of stimulation can be applied and we can record neural signals from each stimulating electrode around 5 ms after stimulation. This microdevice could be a promising platform for bidirectional BMIs.

1SEA-01 GPI アンカー型タンパク質とガングリオシドの１分子追跡により明らかになったラフト組織化
Single-molecule tracking of GPI-anchored proteins and gangliosides revealed raft organization

Kenichi G. N. Suzuki (*iCeMS, Kyoto Univ.*)

Raft domains have been drawing extensive attention as a signaling platform. However, raft structure and function are still very controversial. Here, the dynamic organization of raft molecules in the plasma membrane has been investigated, using single fluorescent-molecule tracking. Virtually all of the GPI-APs (CD59, DAF, Thy-1, GFP-GPI) and gangliosides (GM1, GM2, GM3, GD1b) are mobile, and continually formed transient (100-300 ms lifetime) homodimers based on ectodomain interactions, which were stabilized by raft-lipid interactions. Furthermore, we found that GPI-AP homodimers recruited gangliosides, and ganglioside homodimers recruited GPI-APs. Our results suggest that the transient homodimers are likely one of the basic unit for raft organization.

1SEA-02 細胞質分裂におけるスフィンゴミエリンラフトの役割
A role for sphingomyelin-rich lipid domains during cytokinesis

Mitsuhiro Abe, **Toshihide Kobayashi** (*RIKEN*)

Real-time observation of cell division with a sphingomyelin (SM) specific protein, lysenin, revealed that SM is concentrated in the outer leaflet of the cleavage furrow at the time of cytokinesis. Superresolution fluorescence microscopy analysis indicates a transbilayer colocalization between the SM-rich domains in the outer leaflet and phosphatidylinositol-4,5-bisphosphate (PIP2)-rich domains in the inner leaflet of the plasma membrane. The depletion of SM disperses PIP2 and inhibits the recruitment of the small GTPase RhoA to the cleavage furrow, leading to abnormal cytokinesis. These results suggest that the formation of SM-rich domains is required for the accumulation of PIP2 to the cleavage furrow, which is a prerequisite for the proper progression of cytokinesis.

1SEA-03 Lipid Rafts and Membrane Proteins Collaborate to Organize and Shape Biological Membranes

Jeanne Stachowiak (*The University of Texas at Austin*)

By concentrating lipids and proteins, lipid rafts enable diverse cellular processes. Using model membranes and cells, our work has demonstrated two mechanisms by which proteins and rafts work together to organize membranes. First, we have precisely quantified the capacity of rafts to concentrate proteins, demonstrating that the stability of protein-lipid assemblies relies on a subtle balance between the enthalpy of membrane phase separation and the free energy of protein diffusion. Building on these findings, we have revealed that steric and electrostatic interactions among proteins confined within rafts can generate strong, highly localized membrane surface forces that drive membrane bending, helping to shape endocytic vesicles and other curved membrane structures.

1SEA-04 リポソームの膜内相分離における外場の影響
Phase separation on cell-sized liposomes in the presence of external force

Miho Yanagisawa (*Dept. Appl. Phys., Tokyo Univ. Agric. Technol.*)

Lipid domains in cell-sized liposomes upon phase separation have been extensively studied as a model of lipid rafts in cell membranes. In spite of distinct properties between liposomes and cells, comparing them should elucidate physical conditions important for lipid rafts. For example, the steric repulsive force between bulky lipids like glycolipids inhibits the size growth and stabilizes smaller domains. In this talk, we mention about the effects of external force and viscoelastic cytoplasm on phase separation of lipids. Both effects drastically vary the domain structures according to the kinetics of phase separation and the shape deformation involved. We believe that such bottom-up approach is also necessary for comprehension of complex cellular systems.

1SEA-05 パターン化人工膜を用いた膜タンパク質のラフト親和性解析
Micropatterned model membrane for studying the affinity of proteins to lipid raft

Kenichi Morigaki, Yasushi Tanimoto, Fumiko Okada, Fumio Hayashi (*Kobe University*)

Controlled association of membrane proteins to lipid rafts is believed to play important functional roles. We describe a methodology to evaluate the affinity of proteins to lipid raft by using a micropatterned model membrane composed of polymeric and fluid bilayers. The fluid bilayer has patterned regions of liquid-ordered (Lo) and liquid-disordered (Ld) bilayer domains. We observed that rhodopsin and transducin were localized in Ld domain according to their basal affinities to the disordered lipid phase. We anticipate that we can quantify the affinities of membrane proteins to lipid rafts by measuring their distributions in the micropatterned membrane. This technique should help to elucidate the functional roles of lipid rafts in the native biological membrane.

1SAP-01 線虫精子のアメーバ運動と MSP マシナリー
Nematode sperm motility and MSP machinery

Katsuya Shimabukuro (*UNCT*)

Nematode sperm lack flagellum for swimming, instead, crawls on the substrate using pseudopod like other eukaryotic cells such as fish keratocyte. Amoeboid motility is typically powered by actin machinery, nematode sperm, however, employs an unique cytoskeletal system, called MSP machinery. MSP machinery consists of a set of proteins including a filament-forming protein, major sperm protein (MSP) and other associated proteins to regulate MSP dynamics. Recent studies have shown that MSP machinery in Ascaris suum, a large parasitic roundworm found in hog intestine, can be reconstituted in vitro. In this talk an overview of nematode sperm motility and the most updated knowledge about MSP machinery will be presented.

1SAP-02 ミドリムシにおける光運動制御マシナリーの解明
Molecular machinery regulating photomovement of *Euglena*

Kenji Iwasaki[1,2], Naoyuki Miyazaki[1,2], Mineo Iseki[3], Koji Hasegawa[4], Akihiro Narita[5], Shigeru Matsunaga[6], Masumi Takebe[6], Shinji Kamimura[7], Masakatsu Watanabe[8] ([1]*Inst. for Protein Res., Osaka Univ.*, [2]*NIPS*, [3]*Pharm.Sci, Toho Univ.*, [4]*AdvanceSoft Corp.*, [5]*Struct.Biol.Res.Center, Nagoya Univ.*, [6]*Hamamatsu Photonics K.K.*, [7]*Fac.Sci.Eng., Chuo Univ.*, [8]*Grad.Sch. for the Creation of New Photonics Industry*)

Euglena tumble and change their swimming direction in response to an abrupt increase or decrease in light intensity, called step-up or step-down photophobic responses, respectively. Iseki *et al*. successfully isolated the photosensing receptor, named PAC, responsible for step-up photophobic responses from a photosensing organelle, the paraflagellar body (PFB), in Euglena gracilis and identified it to be a flavoprotein that has adenylyl cyclase activity regulated by blue light. We recently revealed how PFB is constructed from PAC using cutting edge cryo-EM techniques, such as CEMOVIS. From the view of structural biology, we draw ever closer to understanding the mechanism behind how Euglena senses light, leading to a change of swimming direction.

1SAP-03 チューブリン様蛋白質 TubZ によるプラスミド分配の分子機構
Plasmid segregation driven by the tubulin-like GTPase TubZ

Ikuko Hayashi (*Yokohama City University*)

Segregation of low-copy-number plasmids relies on partitioning systems that contain plasmid-encoded cytoskeletal proteins. Tubulin/FtsZ-like GTPase TubZ was identified as a partitioning factor of the pXO1-like plasmids in virulent Bacillus. TubZ exhibits high GTPase activity and assembles into polymers both in vivo and in vitro, and its activation is suggested to be regulated by the DNA-binding protein TubR and the centromeric DNA site. However the molecular mechanism of plasmid segregation by TubZ assembly is not well understood. Based on our recent progress in structural and biochemical studies, I would like to discuss the molecular recognition mechanism of TubR as an adaptor between the TubZ filament and DNA.

1SAP-04 黄色ブドウ球菌のコロニースプレッディングにおける毒素の役割
Role of toxin in *Staphylococcus aureus* colony spreading

Chikara Kaito, Kazuhisa Sekimizu (*Grad. Sch. Phar., Univ. Tokyo*)

S. aureus, a human pathogen, spreads on soft agar plates. We call the phenomenon “colony spreading”. High virulence *S. aureus* strains produce higher amount of toxins and exhibit higher colony-spreading abilities than low virulence strains. Deletion of a toxin encoding-gene diminishes the colony spreading. The toxin is present in both culture supernatant and cellular extract. In this study, we isolated mutants altering the toxin distribution and found that a mutant with less amount of cellular toxin decreased the colony spreading, whereas another mutant with less amount of extracellular toxin did not decrease the colony spreading ability. These findings suggest that cellular toxin has a significant role in *S. aureus* colony spreading.

1SAP-05 らせん細菌 *Leptospira* の遊泳力学とエネルギー論
Swimming dynamics and energetics of the spirochete *Leptospira*

Shuichi Nakamura (*Grad. Sch. Eng., Tohoku Univ.*)

Leptospira are spirochetes and pathogenic species cause a zoonotic disease. *Leptospira* have a right-handed and short-pitch helical cell body, which is called protoplasmic cylinder. When a cell swims in liquid, the anterior and the posterior cell ends are transformed into a left-handed and long-pitch helix (spiral), and a half circle (hook), respectively. The spiral end rotates counterclockwise and the protoplasmic cylinder rotates clockwise to generate thrust. The hook end also rotates for force balance during swimming. We have been analyzing the motion of *Leptospira* to elucidate its propulsion mechanism. In the symposium, I will present the swimming dynamics and energetics of *Leptospira* motility, which have been revealed by our recent research.

1SAP-06 Mechanical basis for the bacterial swimming and gliding

Hirofumi Wada (*Dep. Phys. Ritsumeikan Univ.*)

Flagella are primary functional components for many bacteria to propel themselves in fluids. However, there are other bacteria that are motile but do not rely on flagella or other conventional motility apparatuses. These examples provide us a unique opportunity to study novel generic mechanisms to achieve directed motions at small scales. In this talk, I will present two such examples: one is Spiroplasma, a tiny helical bacterium that can swim in fluids, and Flavobacterium johnsoniae, a rod-like bacterium that can glide fast on a solid surface. For each case, a mathematical model is developed based on recent experimental observations. In both cases, the model shows that the bacterial morphology is closely connected to its motility under a given physical environment.

1SBP-01 凝固したハイドロゲルを用いたタンパク質結晶の成長と特徴
Growth and characterization of protein crystals using high-strength hydrogels

Shigeru Sugiyama[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*JST, ERATO, Lipid Active Structure Project*)

X-ray crystallography offers an unprecedented opportunity to facilitate drug discovery. The most reliable approach is to determine the structure of the complex by soaking the ligand in apo-crystals, but many lead compounds are not readily water-soluble. Such lead compounds must be dissolved in concentrated organic solvents such as DMSO. Therefore, to date, it has been impossible to produce crystals of complexes by soaking in apo-crystals, because crystals dissolve immediately upon soaking in concentrated organic solvents. We propose an approach to avoid the damage by growing protein crystals in a hydrogel. The crystals did not dissolve for more than thirty minutes in concentrated organic solvents. Their diffraction data were suitable for structure analysis.

1SBP-02 微小重力下の結晶化
Protein crystallization under microgravity conditions

Masaru Tanokura, Akira Nakamura (*Dept. Appl. Biol. Chem., Grad. Sch. Agric. Life Sci., Univ. Tokyo*)

Microgravity conditions can improve the quality of protein crystals mainly due to the suppression of the natural convection. The space and the high magnetic fields are able to attain such microgravity environments. We have developed a protein crystallization system with strong magnetic force that enables to cancel out the gravity of a water droplet and with the *in-situ* observation device for crystal growth.
Crystallization has been carried out and a series of photographs of crystal growth have been taken. The time-lapse movie showed that protein crystals exhibited magnetic orientation while growing. X-ray diffraction experiments indicated that the quality of crystals generated in the system was more homogeneous and better than that of the control crystals.

1SBP-03 結晶スポンジ法による非結晶性・極少量化合物の X 線結晶構造解析
Crystalline Sponge Method: X-ray Analysis without Crystallization on the Microgram Scale

Makoto Fujita (*The University of Tokyo*)

X-ray single crystal diffraction (SCD) analysis has the intrinsic limitation that the target molecules must be obtained as single crystals. Recently, we report a new protocol for SCD analysis that does not require the crystallization of the sample (Nature 2013, 495, 461-466; Nat. Protoc. 2014, 9, 246-252). In our method, tiny crystals of porous complexes are soaked in the solution of a target, where the complexes can absorb the target molecules. The crystallographic analysis clearly determines the absorbed guest structures along with the host frameworks. As the SCD analysis is carried out with only one tiny crystal, the required sample amount is of the nano-to-microgram order. We demonstrate that even ~50 ng of a sample is enough to be analyzed.

1SBP-04 膜タンパク質の結晶化法
Crystallization methods of membrane proteins

Takeshi Murata[1,2] ([1]*Science/Chiba-U*, [2]*PRESTO/JST*)

Membrane proteins play crucial roles in many biological functions and are of key importance for medicine. Over 50% of commercially available drugs target membrane proteins. We need to understand membrane protein structures to provide a basic understanding of life at the molecular level and for computer aided rational design of new drugs. However, structural studies of membrane proteins have not been progressed very fast because of difficulty of the crystallization. In my talk, I would like to introduce several strategies for crystallization of membrane proteins and our recent work, and like to discuss about the future of X-ray crystallography of membrane proteins.

1SBP-05 抗体を用いた膜蛋白質の結晶化
Crystallization of membrane proteins using antibody fragments

So Iwata[1,2] ([1]*Kyoto Univ. Grad. Sch. Med.*, [2]*RIKEN SPring8 Center*)

Antibody fragments, including Fab and Fv fragments, are known to be effective to stabilise and crystallise membrane proteins. However, it has been difficult to raise monoclonal antibodies to recognise conformational epitopes of native membrane proteins using the conventional mouse hybridoma system. We have recently succeeded to raise antibodies against native membrane proteins using the system combined with improved immunization and screening methods focusing on mammalian membrane proteins. In my talk, I will present several successful examples of crystallisation of membrane proteins including human A2A adenosine receptor (A2AAR), human aninon exchanger 1 and RCE1 membrane proteinase.

1SBP-06 対称性を持つタグを利用したタンパク質結晶化確率の向上
Use of symmetric tag to increase the probability of protein crystallization

Min Yao (*Fac. of Adv. Life Sci., Hokkaido Univ.*)

Protein crystallography becomes more powerful and useful method for life science, due to astonishing progress in its techniques in the past decade. However, protein crystallization still remains as a major bottleneck. It is dependent on the accidental methods searching for special crystallization reagents and the crystal growth conditions. Therefore further development of more advanced crystallization methods is required to increase the probability of successful crystallization.

In order to increase the probability of protein crystallization, we developed a novel method by fussing target protein with crystallization tags named 2/3RS-tag. These 2/3RS-tags polymerize target proteins with 2 or 3-fold axial symmetry, and consequently accelerate formation of crystal.

1SCP-01 はじめに
Introduction

Hiroyuki Kitahata (*Grad. Scl. of Sci., Chiba Univ.*)

The motion of living organisms is one of the most interesting topics in physics as well as biophysics. In order to investigate the characteristics of such motion, some researchers do not use living materials but physico-chemical materials, such as surfactant and alcohol and so on. In this symposium, there are several speakers who are studying on physico-chemical systems in which spontaneous motion or deformation can be seen. I would like you to discuss the common and different points between living things and such physico-chemical systems, and the direction of the future study.

1SCP-02 なぜその材料はアクティブマターになるのか？ ～Dupeyrat システムに関する考察
A Material Discussion about the Nakache and Dupeyrat System

Sachiko Matsushita (*Tokyo Institute of Technology*)

From the discovery of the spontaneous interfacial motions appeared at an interface between KI + I2 nitrobenzene solution / trimethylstearylammonium chloride aqueous solution by Nakache and Dupeyrat, the spontaneous oscillation has been actively studied for the understanding of non-equilibrium systems and the construction of new chemomechanical transductions. Under these circumstances, we focused on the material point of view, i.e., “Why nitrobenzene?” and “Why iodine?”. In our presentation, we report a kind of hypothetical answers on these questions.

Reference:
Colloid. Surf. A., 429, 31-37 (2013).
Colloid. Surf. A., 395, 232-239 (2012).

1SCP-03 拮抗剤を介した自己推進型液滴の方向感知機能
Directional sensing of self-propelled droplets mediated by antagonists

Takahiko Ban, Hiroki Nakata, Kentaro Tani (*Osaka University*)

We have developed a suitable steering mechanism to maintain the orientation of self-propelled droplets in response to a chemical signal in the environmental media without external forces. Rare-earth metal ions were used as a chemical signal to control the motion of the oil droplets. The droplets show directional sensing and their motility is biased toward higher concentrations. The metal ions investigated can be arranged in decreasing order of directional sensing as Dy3+>>Nd3+>Y3+>Gd3+. We found that the suppressing effect of metal ions on the deprotonation of DEHPA is an element essential to bias the motility of self-propelled droplets. The metal ions act as antagonists that bind to a receptor and suppress the signaling processes.

1SCP-04 Self-propelled water droplet coupled with chemical oscillatory reaction

Nobuhiko Suematsu[1,2] ([1]*Graduate School of Advanced Mathematical Sciences, Meiji University*, [2]*Meiji Institute for Advanced Study of Mathematical Sciences (MIMS), Meiji University*)

Self-propelled motion and oscillatory reaction are one of the interesting behaviors associated with living systems. These phenomena have been reproduced in inanimate systems and a lot of characteristic behaviors have been found out. Here, we propose a hybrid system containing both self-propelled motion and chemical oscillatory reaction. An aqueous droplet of Belousov-Zhabotinsky (BZ) reaction was put into oil phase including surfactant. This droplet spontaneously moved and the behavior depended on the state of BZ reaction. We would like to report transition of the behavior of self-motion coupling with BZ reaction.

1SCP-05 界面張力勾配に駆動される自己推進液滴のモード分岐
Mode bifurcation on a self-propelled droplet driven by interfacial tension gradient

Fumi Takabatake[1], Masatoshi Ichikawa[2], Kenichi Yoshikawa[3] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*Grad. Sch. Sci., Kyoto Univ.*, [3]*Grad. Sch. Life Med. Sci., Doshisha Univ.*)

A chemical concentration gradient and/or thermal gradient generate the spatial variation of interfacial tension, which can cause the motion of a liquid droplet. It has been known that, by introducing internal or external asymmetry to such system, a regular motion depending on the given asymmetry is induced. However, little is known about the mode-selection under the same symmetry conditions. Here, we investigated mode bifurcations on self-propelled systems with a local energy source: the motion of a droplet attached to a solid soap and the droplet motion induced by local heating with a laser were observed. It is found that, with a change in the size of the solid or the laser power, respectively, the mode of self-motion bifurcates into translational and circular motion.

1SCP-06 水路形状およびマランゴニ流構造に依存した自律運動システム
Self-propelled system depends on the structureof Marangoni flow and the shape of water chamber

Yui Matsuda, Satoshi Nakata (*Hiroshima Univ.*)

Characteristic motions of camphor particles have been investigated as a simple self-propelled system in which the driving force is also considered to be the difference in the surface tension. As for the mechanism of motion, the dependence on the depth of the water chamber has not been thoroughly discussed since the surface tension should be independent of the water depth. However, there have been several reports on the characteristic motion of camphor particles in water chambers of varying water depth. In this presentation, we report that the dependence of speed on water depth for the camphor disk is different from that for the camphor boat. We conclude that the influence of Marangoni flow plays an important role in the motion of both the camphor disk and boat.

1SCP-07 界面活性剤の会合体生成に誘起される油水界面のアメーバ状運動
Amoeba like motion of the oil-water interface induced by generation of surfactant aggregate

Yutaka Sumino (*Department of Applied Physics, Faculty of Science, Tokyo University of Science*)

In this talk, we present a spontaneous motion of an oil-water interface induced by generation of surfactant aggregate. The aqueous phase contains cationic surfactant, stearyltrimethylammonium chloride (STAC), whereas the organic phase is tetradecane containing palmitic acid (PA). Setting the aqueous and organic phases in contact with each other, the aggregate composed of STAC and PA is generated in the aqueous phase near the oil-water interface. Accompanied with the generation of alpha-gel, the interface shows repetitive extension and retraction of spherical deformation, which resembles cellular blebbing. Here we discuss the detail of the experimental setup, as well as the underlying mechanism for the blebbing motion of the oil-water interface.

1SCP-08 ある巨大アメーバ生物のアメーバ運動
Amoeboid movement of a large amoeboid organism, true slime mold

Seiji Takagi (*Future Univ. Hakodate*)

In this symposium, motion of non-biological systems are discussed, while in this final talk, we will show amoeboid movement of a real amoeboid cell to compare the properties and mechanisms of the motion between biological and non-biological systems.
Observations of migrating plasmodia of Physarum polycephalum will be presented, e.g. non-invasive microscopic observation of the dynamic structure of cytoskeleton to visualize the correlation between motive force production and locomotion. Through this presentation we hope to bring new perspectives to the researches on physico-chemical systems.

1SDP-01 分子間五次相互作用が細胞内でのタンパク質安定性を制御する
Intermolecular Quinary Interactions Modulate Protein Stability in Living Cells

Gary J. Pielak (*University of North Carolina*)

The stabilization of protein secondary, tertiary and quaternary structure is well defined, but little is known about quinary structure, which organizes the proteome and is probably key to metabolism and signal transduction. We show that a surface mutation in the B1 domain of protein G is 10-times more destabilizing in Escherichia coli than in buffer. Using a double mutant cycle to quantify the quinary interaction, we show that interactions between the cytoplasm and the protein surface can be as important as those for the well known intramolecular contacts in the protein core. Our observation reveals a new role for surface residues in protein evolution that will aid in understanding protein chemistry under physiological conditions.

1SDP-02 分子混雑環境における蛋白質の NMR 緩和解析
NMR relaxation analysis of the protein under macromolecular crowding environment

Hideyasu Okamura, Takanori Kigawa (*QBiC, RIKEN*)

The interior of biological cells is a crowding environment. Such macromolecular crowding environment is significantly different from the experimental condition performed in diluted solutions. Therefore, it is important to obtain the information of protein dynamics under the macromolecular crowding environment. Model-free analysis for NMR spin relaxation data is a useful method for obtaining a comprehensive picture of protein dynamics. However, the application of the method has been restricted to the protein in diluted solutions. Here, we newly developed the extended model-free analysis that is applicable to the macromolecular crowding environment. The resulting data delineates the protein motion under the systems in which the adjacent proteins interfere with each other.

1SDP-03 In-cell NMR 法による細胞内タンパク質の構造多様性解析
In-cell NMR analysis for protein conformational diversity in a cell

Kohsuke Inomata (*Quantitative Biology Center (QBiC), RIKEN*)

The intracellular environment, where the most of proteins function, is highly crowded, heterogeneous and dynamic due to a high concentration of various macromolecules. Therefore, biochemical or biophysical characteristics, furthermore molecular functions, of proteins in the cellular environment can be different from those of the in vitro condition. So the methodology or technique for the direct investigation of the protein structure and dynamics in situ is strongly desired. In-cell NMR spectroscopy is a promising technique to observe structure, interactions and dynamics with an atomic level in a living cell directly. In this presentation, we will introduce to our trials for the investigation of protein conformational diversity (or dynamics) in a living mammalian cell.

1SDP-04 生きた細胞中の天然変性蛋白質の動態
Dynamics of intrinsically disordered proteins in living cells

Teppei Ikeya, Jin Inoue, Yutaka Ito (*Tokyo Metropolitan University*)

The native intracellular environment can contain more than 300 g/l of macromolecules, known as molecular crowding. The high concentration of macromolecules reaching up to 30% of a cell's volume can alter various properties of proteins by promoting specific and non-specific interactions with other molecules, the excluded volume effect, and anomalous diffusion of interior molecules as well as water. We study the dynamics of globular proteins and intrinsically disordered proteins (IDPs) in living cells and artificial crowding environments by solution NMR. Our results and several reports by other groups suggest that protein structures may be destabilized in cells. Here, we discuss the impact on the stability and dynamics of proteins in different crowding environments.

1SDP-05 ゲノムと立体構造を結合したシミュレーション解析
Combining Structure with Genomics

Michael Feig, Yildirim Asli (*Michigan State University*)

The structure of bacterial DNA has long remained elusive but driven by new data from chromosome conformation capture experiments. It is now possible to develop high-resolution nucleoid models. Using a multi-scale approach, high-resolution models consistent with the experiments were obtained. At the coarsest level the models capture the plectonemic nature of DNA in bacterial nucleoid. At an intermediate level, a multiple-nucleotide coarse-grained model is used that can be converted into fully atomistic models. The resulting nucleoid models allow an exact mapping of genes onto the nucleoid structure and provide an avenue for exploring a possible relationship between the spatial organization of genes on the chromosome and their function.

1SEP-01 CaMKII によって活性化された Rho GTPase の協同的作用によるシナプス可塑性誘起

CaMKII-induced active Rho GTPases cooperatively work for the establishment of synaptic structural plasticity

Hideji Murakoshi[1,2] ([1]*National Institute for Physiological Sciences*, [2]*JST PREST*)

Ca^{2+}/Calmodulin-dependent kinase II (CaMKII) is one of the most important signaling molecules for synaptic plasticity underlying learning and memory. Here, we developed a photo-activatable CaMKII (paCaMKII). Light-induced spine specific CaMKII activation successfully induced the structural plasticity, which suggesting that CaMKII activation is sufficient for the plasticity. In addition, we imaged the activity of Rho GTPases, RhoA and Cdc42, by using 2pFLIM and found that these molecules are activated via CaMKII. Furthermore, since the loss of function assay suggests that RhoA and Cdc42 works for triggering and maintaining the structural plasticity, respectively, these molecules may cooperatively work for establishing the spine structural plasticity.

1SEP-02 The basis for regulating synaptic strength heterogeneity across the dendrite

Mathieu Letellier, **Yukiko Goda** (*RIKEN Brain Science Institute*)

Dendrite is a neuronal structure specialized for receiving and processing information through its many synapses. How incoming activity modifies synaptic strength distribution across the dendrite is fundamental to understanding brain function although much remains unclear. Using a combination of electrophysiology and imaging approaches in simple circuits, we have studied the relationship between neighbouring synapses in single postsynaptic neurons. We provide evidence for a novel cellular mechanism that promotes the disparity of presynaptic strengths of convergent synaptic connections targeting the same dendritic tree.

1SEP-03 神経細胞軸索の細胞膜にある 2 次元拡散障壁は分子選択性フィルターである

Diffusion barrier in the neuronal axon initial-segment membrane is a molecule-selective filter in the plasma membrane

Akihiro Kusumi[1,2], Manami S.H. Miyahara[1], Takahiro K. Fujiwara[1] ([1]*iCeMS, Kyoto Univ.*, [2]*Inst. for Frontier Med. Sci., Kyoto Univ.*)

The plasma membrane (PM) of neuronal cells is split into two regions with distinct functions, the somatodendritic domain (neuronal signal input) and axon (output). Many PM molecules reside in either one of the two domains, which is enabled by the presence of a diffusion barrier in the PM in the initial segment (IS) region, the interface between the two domains. The diffusion barrier is formed by the cooperative assembly and binding of transmembrane proteins and scaffolding actin-binding molecules. Recently, we found that the diffusion barrier is a molecule-selective filter, allowing GPI-anchored proteins to pass through, but not transmembrane proteins and phospholipids. The mechanism for the selective passage is becoming clear, which will be discussed in this talk.

1SEP-04 Activity-dependent gene expression in learning and memory

Ryang Kim[1,2], Mio Nonaka[1], Nan Yagishita-Kyo[1,2], Takashi Kawashima[1], Masatoshi Inoue[1,2], Yuichiro Ishii[1,2], Toshihiro Endo[1], Hajime Fujii[1], Sayaka Takemoto-Kimura[1], Hiroyuki Okuno[1,3], **Haruhiko Bito**[1,2] ([1]*Department of Neurochemistry, The University of Tokyo Graduate School of Medicine*, [2]*CREST-JST*, [3]*Medical Innovation Center, Kyoto University Graduate School of Medicine*)

Long-term memory formation requires a complex coordination of signaling pathways to enable specific storage of otherwise transient information acquired as memory events happen. How does such conversion from labile into a more stable information occur? We will here present our recent findings on multiple levels of signal cooperation, at the synaptic, dendritic and nuclear compartments of a neuron, which together contribute to achieving this formidable signal processing. Recent human genome sequencing studies indicate that maladaptation of such signaling cascades may underlie several forms of neuropsychiatric disabilities.

1SEP-05 The cooperativity of neuronal molecules analyzed with imaging mass spectrometry

Mitsutoshi Setou (*Hamamatsu University School of Medicine*)

The cooperative pattern in a biological system appears when a large collection of similar molecules have concerted action to achieve a given purpose, showing either enhancing or diminishing effect, and trigger interactions with other molecules are also possible. Imaging mass spectrometry (IMS) provides information about the complex chemical composition of biological samples, together with its spatial distribution and quantification. Soft ionization techniques for IMS, such as matrix-assisted laser desorption/ionization (MALDI), allow the simultaneous detection with high spatial resolution of hundreds of intact molecules. Here we hope to present the trial to identify and analyze the cooperativity of neuronal molecules with IMS techniques.

1SEP-06 Modeling the dynamical interaction of Hebbian and homeostatic plasticity

Taro Toyoizumi[1,2], Megumi Kaneko[3], Michael P. Stryker[3], Kenneth D. Miller[2] ([1]*RIKEN Brain Sci. Inst.*, [2]*Columbia Univ.*, [3]*UCSF*)

Homeostatic synaptic plasticity, which scales synaptic strengths to restore activity toward a set-point value, is often slow relative to Hebbian plasticity. Existing models cannot robustly stabilize fast Hebbian plasticity if homeostatic plasticity is slow, as found for ocular dominance plasticity (ODP). We show that ODP dynamics are robustly captured by a model in which synaptic strength is the product of a synapse-specific Hebbian factor and a postsynaptic-cell-specific homeostatic factor. Each factor separately arrives at a stable state where it is inactive. We also experimentally confirm two key model predictions. This result highlights the importance of multiple regulatory pathways for interactions of plasticity mechanisms operating over separate timescales.

2SAA-01 ミトコンドリアと抗 RNA ウイルス自然免疫
Mitochondria and antiviral innate immunity in mammals

Takumi Koshiba (*Dep. of Biol., Faculty of Sci., Kyushu Univ.*)

Mitochondria, cellular powerhouses of eukaryotes, that undergo cycles of homotypic fusion and fission events are recently known to act as a central hub for cellular innate antiviral immunity in vertebrates, particularly mammals. Because mitochondria are believed to have evolved from organisms such as alpha-proteobacterium, their newly discovered role of branching into the host-cell defense was unexpected. In this meeting, the recent insights into the fundamental phenomenon of mitochondrial involvement in cellular innate antiviral immunity will be discussed.

2SAA-02 カルシニューリンと Notch シグナリングを介してミトコンドリアの融合は心筋細胞の分化に必須である
Mitochondrial fusion controls differentiation of ESCs into cardiac cells via a novel pathway of calcineurin and Notch signaling

Atsuko Kasahara[1], Luca Scorrano[2] ([1]*University of Geneva, CMU*, [2]*University of Padua, Dep. of Biology*)

Mitochondrial fusion-deficient mouse embryonic stem cells (ESCs) revealed altered subcellular mitochondrial distribution results in increased capacitative Ca2+ entry and calcineurin activity, and impaired cardiac differentiation. These changes caused increased nuclear NICD1 level, suggesting Notch1 activation. A pharmacological rise in intracellular Ca2+ level by ionomycin fully recapitulated the calcineurin dependent activation of Notch1, and inhibition of calcineurin normalizes Notch1 activity and rescues the cardiac differentiation in fusion-deficient ESCs. Mitochondrial shape and localization impact nuclear programs of differentiation through previously unexplored interconnected signaling pathways via Ca2+, calcineurin and canonical Notch signaling.

2SAA-03 ミトコンドリア呼吸鎖の多様性：寄生虫からがん細胞まで
Diversity of mitochondrial respiratory chain from parasite to cancer

Kiyoshi Kita (*Dept of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo*)

Starvation of glucose and hypoxia are observed in hypovascular tumors pancreatic cancer. Increased glycolysis is the principal explanation how cancer generates energy in hypoxia, but under such condition, glycolysis alone cannot explain how cancer cells maintain their required energy levels. To understand energy metabolism in tumor cells under the tumor microenvironment, we have been focusing on the mitochondrial energy metabolism, NADH-fumarate reductase system found in anaerobic organisms such as parasitic helminthes, such as Ascaris suum.
Thus, NADH-fumarate reductase system is important in maintaining mitochondrial energy metabolism of many parasites under hypoxic conditions and the tumor microenvironment, and is a novel target of anti-cancer therapy.

2SAA-04 オートファジーが駆動するミトコンドリア分解の仕組み
Targeting Autophagy for Mitochondrial Clearance

Koji Okamoto (*Grad. Sch. Frontier Biosci., Osaka Univ.*)

Mitophagy in yeast requires Atg32, a transmembrane protein anchored on the surface of mitochondria. Atg32 interacts with Atg8, a phosphatidylethanolamine (PE)-conjugated ubiquitin-like protein localized to autophagosomes. Recently, we found that mitophagy is strongly impaired in cells lacking Opi3, a phospholipid methyltransferase acting in conversion of PE to phosphatidylcholine. Strikingly, the levels of lipidated Atg8 were drastically increased in the absence of Opi3. Atg8 is conjugated to phosphatidylmonomethylethanolamine (PME) in the opi3 mutant. In vitro, Atg8-PME is not efficiently delipidated by Atg4, a cysteine protease essential for autophagy. Our data suggest that the regulation of Atg8 lipidation and recycling is critical for mitophagy.

2SAA-05 酵母ミトコンドリアにおけるタンパク質と脂質の輸送機構
Mechanisms of protein and lipid transport in yeast mitochondria

Toshiya Endo (*Fac. Life Sci., Kyoto Sangyo Univ.*)

Mitochondrial biogenesis requires synthesis and transport of its constituent proteins and lipids. Most mitochondrial proteins are synthesized outside mitochondria and subsequently imported into mitochondria. We have recently identified a new pathway for import and sorting of N-anchor mitochondrial outer membrane proteins, which unusually requires both the protein translocators in the outer and inner membranes. Mitochondrial phospholipids are synthesized by multiple phospholipid-synthetic enzymes located in different organelles including the endoplasmic reticulum (ER) and mitochondria. I will discuss our latest results on the structural basis for the possible lipid transport between ER and mitochondrial membranes.

2SBA-01 電子顕微鏡法により示された線虫イネキシン 6 ギャップ結合チャネルの特徴的なサブユニット構成
Electron microscopy of C. elegans innexin-6 gap junction channels indicates a characteristic subunit organization

Atsunori Oshima[1], Tomohiro Matsuzawa[2], Kazuyoshi Murata[3], Kouki Nishikawa[1], Yoshinori Fujiyoshi[1] ([1]*CeSPI, Nagoya Univ.*, [2]*Dept. of Biophys., Grad. Sch. Sci., Kyoto Univ.*, [3]*NIPS*)

Innexin is a molecular component of invertebrate gap junctions. The 3D structure, however, remains unknown. Here we performed an electron microscopic analysis of solubilized, reconstituted, and 2D crystallized C. elegans innexin-6 (INX-6) gap junction channels. Cryo-electron microscopy and single particle analysis of purified INX-6 channels revealed class averages with eight rotational peaks. 2D crystals of INX-6 channels were generated in a hexagonal lattice, and image processing revealed a projection map that clearly demonstrated eight separated densities around the pore. These findings suggest that the subunit number of INX-6 channels is distinct from that of vertebrate connexin channels, which contain 12 subunits in a full gap junction channel.

2SBA-02 再構成系および細胞内でのアクチンフィラメント構造解析
Structural analysis of the actin filament in vitro and in vivo

Akihiro Narita[1,2] ([1]*Nagoya Univ.*, [2]*PRESTO*)

Actin is one of most abundant proteins, which forms a double stranded filament. Actin continuously polymerizes and depolymerizes in the cell and this dynamics is crucial for many important phenomena in the cell including cytoskeleton, cell motility, cell adhesion, cell division, muscle contraction and so on. We are investigating the dynamics of the actin filament by electron microscopy. I'll review our previous work and current projects, about structural analysis of complexes of the actin filament and actin binding proteins using single particle analysis, and about structural analysis of the actin filament in the cell using electron tomography.

2SBA-03 Single particle analysis of the model post-termination complex gives insights into prokaryotic ribosome recycling process

Takeshi Yokoyama (*RIKEN, CLST*)

Single particle cryo-electron microscopy is the powerful technique to solve the three dimensional structure of biological macromolecule. By exploiting this technique, we examined one of the ribosomal complexes in ribosome recycling process, called post-termination complex (PoTC). At the end of protein synthesis, ribosome bound to mRNA on its termination codon should be recycled for the next round of protein synthesis. It is known that ribosome recycling factor and elongation factor G disassembles it into each component. The comparison of cryo-EM structures of PoTC before and after recycling reaction, reconstructed from the images of molecules observed at liquid helium temperature, clearly showed that bound tRNA and mRNA were released from the ribosome by the reaction.

2SBA-04 単粒子クライオ電子顕微鏡法による 8 Å 分解能サポウイルスキャプシド構造とホモロジーモデリング
Sapovirus capsid structure at 8 Å resolution by single particle cryo-electron microscopy, and homology modeling

Naoyuki Miyazaki[1], David Taylor[1], Grant Houseman[2], Kousuke Murakami[2], Kazuhiko Katayama[2], **Kazuyoshi Murata**[1] ([1]*National Institute for Physiological Sciences*, [2]*National Institute of Infectious Diseases*)

Sapovirus (SaV) is a member of the Caliciviridae family, which causes gastroenteritis in human. Here, we generated the SaV virus-like particles and determined the structure at 8 Å resolution by single particle cryo-electron microscopy. The capsid is composed of two functional modular domains arranged in T=3. The conserved structures among the caliciviruses allowed us to build homology models of SaV capsid. We incorporated the conserved secondary structures into the amino acid sequence alignments between the caliciviruses to build the initial homology models. The models were refined to fit well into the density map by cryo-EM. The structure revealed that the domain boundary of the capsid proteins and the possibly important residues for the capsid-related functions.

2SBA-05 Single molecular imaging and single atom spectroscopy by electron microscopy

Kazutomo Suenaga (*AIST*)

Here I show how high-resolution transmission electron microscopy (TEM) can be applied for single molecular imaging and for single atom spectroscopy. In recent developments, TEM and STEM (scanning TEM) has dramatically improved their sensitivity and spatial resolution and then become capable to detect individual carbon atoms. The application of TEM and STEM to the realistic soft matters is still largely limited. I would like to discuss the possibilities of TEM and STEM which may be usable to directly image molecules and constituent atoms.

2SBA-06 低温電子顕微鏡を用いた単粒子解析による膜たんぱく質の研究
Studying integral membrane protein by single particle cryo-EM

Yifan Cheng (*Dep. Biochem. Biophys., UCSF*)

The Transient Receptor Potential (TRP) ion channel is a large and functionally diverse superfamily, second only to potassium channels. TRPV1 is the founding member of a subfamily of thermosensitive TRP channels. Facilitated by novel single particle electron cryo-microscopy (cryo-EM) technology, we determined atomic structures of TRPV1 ion channels in three different conformations, ligand free apo state and in complexes with vanilloid agonist capsaicin, or resiniferatoxin and Double-Knot toxin. These structures revealed the atomic structure of TRPV1 ion channel, providing a structural blueprint for understanding unique aspects of TRP channel function. They also revealed the gating mechanism different from that of voltage gated ion channels.

2SCA-01 Regulation of the rotational switching of bacterial flagellar motor by binding of an intracellular signaling protein CheY

Hajime Fukuoka[1], Takashi Sagawa[2], Yuichi Inoue[1], Hiroto Takahashi[1], Akihiko Ishijima[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Life Sci., Tohoku Univ.*)

In chemotaxis system, the binding of signaling molecule, CheY-P, to a bacterial flagellar motor is believed to induce the rotational switching of a motor. In this study, by direct imaging of CheY-GFP, we directly demonstrated that the binding and dissociation of CheY-P induce CW and CCW rotation of a motor. It was found that ~ 13 CheY-P molecules bind to and dissociate from a motor within about 100 ms during switching, indicating the possibility of the cooperativity in the binding or dissociation process of CheY-P. The direct imaging of CheY-GFP also revealed that the CW motor has a higher affinity than the CCW motor for CheY-P. From these results, we would like to discuss the regulatory mechanism in the rotational switching of flagellar motor by binding of CheY-P.

2SCA-02 Visualizing stator-protein distributions of bacterial flagellar motors

Chien-Jung Lo[1,2], Tsaishun Lin[1,2] ([1]*Dept. of Phys., National Central Univ.*, [2]*Inst. Biophys., National Central Univ.*)

The bacterial flagellar motor is a natural electrical rotary molecular machine. The stator-unit consists of MotA/B proteins couples ion-flux to motor rotations. More than 10 stator-units in a motor turn over dynamically in response to the cellular energetical conditions and external loads. However, spatial features and temporal dynamics of stator-units have never been studied thoroughly. We have built a super-resolution fluorescent microscope with 10 nm resolution to study stator-units dynamics using dual color labeling on rotor- and stator- proteins. We will present protein counting, spatial distribution and dynamical properties of stator proteins.

We thank Seiji Kojima, Hajime Fukuoka, Akihiko Ishijima and Michio Homma for the bacterial strains.

2SCA-03 細菌べん毛モーターの回転方向変換制御に関わる構造
Structure of the bacterial flagellar motor involved in the directional switching mechanism

Tomoko Miyata[1], Takayuki Kato[1], Yusuke V. Morimoto[1,2,3], Syuichi Nakamura[4], Hideyuki Matsunami[5], Keiichi Namba[1,2] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*QBiC, RIKEN*, [3]*Grad. Sch. Sci., Osaka Univ.*, [4]*School of Engineering, Tohoku Univ.*, [5]*Trans-Membrane Trafficking Unit, OIST*)

Many bacteria swim by reversibly rotating flagella. The three switch proteins, FliG, FliM and FliN, form the C-ring on the cytoplasmic face of the MS ring spanning the membrane and control counterclockwise-clockwise (CCW/CW) switching of the motor rotation. CheY is a response regulator in bacterial chemotaxis, and phosphorylated CheY (CheY-P) binds to FliM and changes the rotational direction from CCW to CW. We previously reported the C ring structures locked in CCW (che deletion strain) and CW (FliG ΔPAA strain). Comparison of the two strucures showed differences in the position of the C ring and the subunit arrangement in its outer wall. In this meeting, we will report the structure of the CheY-P bound C ring and discuss the switching mecanism of flagellar rotation.

2SCA-04 Conformational Spread as a Mechanism for Cooperativity in the Bacterial Flagellar Switch

Fan Bai (*Sch. Life Sci., Peking Univ.*)

The Bacterial Flagellar Motor is a molecular machine which rotates the helical filaments that propel swimming bacteria. In our previous work, we used high-resolution optical microscopy to observe switching of single motors and uncover the stochastic multistate nature of the switch. Our observations are in quantitative agreement with a general model of allosteric cooperativity that exhibits conformational spread. On the basis of this model, we constructed a unified mathematical model describing both BFM torque generation and switching mechanism. Our model framework minimized free adjustable parameters and successfully reproduced the load-switching dynamics of the BFM reported in recent experiments and made predictions on the stator dependence in motor switching dynamics.

2SCA-05 Coordination and control in the ring-shaped molecular motors

Jin Yu (*Beijing Computational Science Research Center*)

The ring-shaped NTPase motor consists of multiple subunits that form a ring-like structure. The molecular motors use chemical free energy to move along nucleic acid or protein substrate in a directional manner. To achieve that function, inter-subunit coordination is required to ensure cooperative or sequential activities among the motor subunits. I will first show our previous modeling work on a viral DNA packaging motor, based on single molecule measurements. The focus is on how multiple subunits coordinate to achieve sequential ATP binding, hydrolysis, and ADP release around the ring, as well as on push-and-roll of DNA through the ring. Then I will introduce our preliminary work on gamma-less F_1-ATPase, focusing on biased mechanistic correlation among chemical sites.

2SCA-06 高速原子間力顕微鏡によるリング状 ATPase の協同的構造変化の観察
Cooperative Conformational Change of Ring-Shape ATPase Observed by High-Speed AFM

Takayuki Uchihashi[1,2], Ryota Iino[3], Yo-hei Watanabe[4], Hiroyuki Noji[5], Toshio Ando[1,2] ([1]*Coll.Sci. & Eng., Kanazawa Univ.*, [2]*Bio-AFM FRC, Coll.Sci. & Eng., Kanazawa Univ.*, [3]*Okazaki Inst. Integr. Biosci., NINS*, [4]*Dept. Biol., Konan Univ.*, [5]*Sch. Eng., Univ. Tokyo*)

Cooperativity among subunits in oligomeric proteins is often indispensable for their functions. Oligomeric proteins generally undergo concerted or sequential conformational transitions due to changes in intersubunit interactions upon the ligand binding/dissociation to/from proteins. Although static structures have been available for many oligomeric proteins, there have been no ways to directly observe cooperative conformational changes of subunits in the complex. Here we applied high-speed AFM, which is one of promising tools to visualize conformation of each subunit simultaneously, to ring-shaped ATPase such as F_1 -ATPase. In the presentation, we will demonstrate HS-AFM movies and discuss how the catalytic and conformational states are cooperatively regulated.

2SDA-01 クロマチン動構造とヒストンバリアント
Structural basis of chromatin dynamics regulated by histone variants

Hitoshi Kurumizaka (*Waseda University, Faculty of Science and Engineering*)

Chromatin dynamics function to regulate replication, recombination, and transcription of genomic DNA. Histones H2A, H2B, H3, and H4 are major nuclear proteins that form the core structure of the nucleosome. In many species, H2A, H2B, and H3 have isoforms (variants). Incorporation of specific histone variants into nucleosomes results in versatile structure and dynamics. These structural and physical characters of nucleosome isoforms containing histone variants play essential roles in genomic DNA regulation. In this symposium, I will discuss about the structural basis of chromatin dynamics based on the crystal structures of the nucleosomes containing histone variants.

2SDA-02 統合的イメージングアプローチによる動的クロマチン構造・機能研究

Integrated imaging approach to the study of dynamics of chromatin

Kumiko Sakata-Sogawa[1,2], Yuma Ito[1,2], Akihiro Fukagawa[1], Masahiko Harata[3], Hiroshi Kimura[4], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*, [3]*Grad. Sch. Agr. Sci., Tohoku Univ.*, [4]*Grad. Sch. Front. Biosci. Osaka Univ.*)

Chromatin remodelers are responsible to regulate gene expression of eukaryotic cells by dynamic modification of chromatin architecture. Arp4, a member of actin related protein family, is reported to serve as a regulator of chromatin remodeling possibly through its direct binding to histone. However, the detailed mechanisms are still elusive.
Aiming to clarify the dynamics of Arp4 in chromatin remodeling, we conducted quantitative imaging analysis in the nucleus. FRAP analysis revealed two components in the movements of Arp4. Single-molecule analysis by HILO microscopy discriminated the movements between Arp4 and chromatin. These results of the integrated imaging approach suggest dynamic interactions of Arp4 with either chromatin-remodeling complexes or chromatin.

2SDA-03 塩濃度変化に対する天然クロマチンファイバーの高次構造変化の直接観察

Direct observation of the higher-order structural changes of native chromatin fibers for the change of the salt concentration

Hidehiro Oana[1], Kaori Nishikawa[1], Hirotada Matsuhara[2], Ayumu Yamamoto[2,3], Takaharu G. Yamamoto[4], Tokuko Haraguchi[4,5], Yasushi Hiraoka[4,5], Masao Washizu[1] ([1]*Dept. of Mech. Eng., The Univ. of Tokyo*, [2]*Grad. Sch. of Sci. and Tech., Shizuoka Univ.*, [3]*Dept. of Chem., Shizuoka Univ.*, [4]*Adv. ICT Res. Inst., NICT*, [5]*Dept. of Biol. Sci., Osaka Univ.*)

We report a new method for the biochemical experiments of individual native chromatin fibers from single cells in a specifically designed microfluidic device using fluorescence microscopy, and dynamic changes in the higher-order structure of the chromatin fibers were demonstrated with the change of salt concentration. Chromatin fibers were obtained from fission yeast cells, and were tethered in the microfluidic device with elongated form by flow. Typical apparent contour lengths of the chromatin fibers were ca. 50-100 μm. When the concentration of NaCl in the solution was increased to 0.5 M, chromatin fibers started to elongated and typically, chromatin fibers of 200-400 μm were obtained due to dissociation of residue proteins bound to chromatin fibers.

2SDA-04 ヒストンとＲＮＡポリメラーゼの翻訳後修飾の生細胞・生体内計測

Monitoring histone and RNA polymerase modification dynamics in living cells and organisms

Hiroshi Kimura (*Grad Sch Frontier Biosci, Osaka Univ.*)

In eukaryotes, post-translational modifications on histone and RNA polymerase II (RNAPII) play an important role in gene regulation. However, how these modifications are regulated in vivo remains largely unknown. To reveal the dynamics and function of these modifications, we have developed two live-cell tracking systems by using specific monoclonal antibodies. One system uses fluorescently labeled antigen binding fragments (Fab). Another system uses genetically encoded single-chain variable fragments tagged with the fluorescent protein. This technique allows us to monitor modification dynamics in living organisms. I will present our recent data on the kinetics of histone and RNAPII modifications in response to gene activation.

2SDA-05 HP1 をとおして見えてきたヘテロクロマチンの構造と機能

Elucidation of construction and function of heterochromatin through HP1 binding proteins

Chikashi Obuse (*Grad. Sch. Life, Hokudai*)

HP1 is thought to play a role in heterochromatin formation by binding to K9 trimethylated histone H3 (H3K9me3) and its interacting proteins. By proteomic analysis, we identified 82 HP1 binding proteins (HPBPs) in human cells [Nature Cell Biol. 12: 719, 2010]. An uncharacterized HPBP was enriched in inactive X chromosomes (Xi) in association with SMCHD1, and thus we named it HBiX1. Cytological and epigenomic analyses revealed that HBiX1 and SMCHD1 mediate the compaction of Xi to form heterochromatin structure, by linking the H3K9me3 domains and the XIST/H3K27me3 domains [Nature Struct. & Mol. Biol. 20: 566, 2013]. These results enable us to discuss a molecular mecanism how epigenetic marks are translated into higher order chromatin structure.

2SDA-06 相同組換え修復における損傷クロマチン動態

Nuclear topography of homologous recombinational repair

Satoshi Tashiro (*RIRBM, Hiroshima Univ.*)

Homologous recombination repair (HR) is one of the major repair pathways of DNA double-strand breaks (DSBs). Proteins involved in HR accumulate at sites containing DNA damage. On the other hand, damaged chromatin is shown to be mobile in a manner dependent on HR factors. However, the biological significance of such dynamic rearrangements of higher order nuclear architectures in HR is still unclear. RAD51 is a key factor in the HR pathway loaded on single stranded DNA (ssDNA) processed around DSBs. The overexpressed RAD51 is accumulated within bundle-like sub-compartments in cell nucleus. ssDNA formed for HR was detected within the sub-compartments. These findings support the notion that damaged chromatins are moved into the bundle-like sub-compartment of IC for HR.

2SDA-07 核膜形成における核膜タンパク質とクロマチンの動的相互作用の役割

A Role of Dynamic Interaction of Nuclear Membrane Proteins with Chromatin on the Nuclear Envelope Assembly

Tokuko Haraguchi[1,2,3], Shouhei Kobayashi[1], Takako Koujin[1], Hiroko Osakada[1], Tomoko Kojidani[1,4], Chie Mori[1], Yasushi Hiraoka[1,2,3] ([1]*Advanced ICT Res. Inst. Kobe, NICT*, [2]*Grad. Sch. Front. Biosci., Osaka Univ.*, [3]*Grad. Sch. Sci., Osaka Univ.*, [4]*Japan Women's Univ.*)

Reformation of the normal nuclear envelope during mitosis is important to re-establish proper cellular functions. We have reported that barrier-to-autointegration factor (BAF) has a role to assemble emerin and lamin A at the NE. However, requirement of physical interaction between BAF and such NE proteins is still unclear. To obtain direct evidence, we have developed a novel experimental method using an artificial bead conjugated with a molecule of interest as an effector molecule. Using this experimental method, we found that direct interaction of BAF with emerin was required for assembly of the nuclear envelope-like membrane. We will also report results from DNA, BAF and Ran used as an effector molecule, and will discuss a role of these molecules on the NE assembly.

2SEA-01 Importance of membrane pumps and channels: an introduction

Ryota Iino[1,2,3] ([1]*Okazaki Inst. Integ. Biosci., NINS*, [2]*IMS, NINS*, [3]*Dept. of Functional Molecular Science, SOKENDAI*)

"Which is important for biophysicists, pump or channel?" You may think the title of this symposium is a bit provocative. Of course, all membrane transporters are important to support life. We used the word "important" to express our love to the membrane pumps and channels. As one of the organizers, I hope all speakers explain to audience why their target molecules are "important" for them. In my own talk, I will give brief introduction on the purpose of this symposium and the specific topics delivered by the speakers.

2SEA-02 VoV1 の中心回転軸における巧妙なトルク伝達機構
The ingenious structure of central rotor apparatus in VoV1; torque transmission mechanism in the central rotor of VoV1

Ken Yokoyama (*Kyoto Sangyo University*)

The central rotor apparatus of VoV1 of Thermus thermophilus is composed of V1-DF shaft and Vo-CL12 rotor ring. In this study, we revealed that the rod domain of V1-DF plays important roles on both reconstitution and energy coupling in VoV1 by the reconstitution and fluorescence resonance energy transfer (FRET) analysis. We propose a structural model accounting for both the detachable and sticky nature of the interaction between the central rotor apparatuses.
In addition, we discuss a twist or torsion of the central shaft in VoV1 during rotation.

2SEA-03 イオンポンプとの比較による、塩化物イオンチャネル CFTR の作動機構研究
Studies on the mechanism of a chloride channel CFTR in comparison with ion pumps

Tomoko Masaike[1,2], Yoshiro Sohma[3] ([1]*Dept. Applied Biol. Science, Tokyo Univ. Science*, [2]*PRESTO, JST*, [3]*Dept. Pharmacology, School of Medicine, Keio Univ.*)

Our task is to have a debate on which of an ion pump or a channel is "superior" to the other. We argue in favor of channels by introducing studies on Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), which conducts anions across cell membranes. Recent observation of CFTR under a high-speed AFM revealed its single-molecule structure and domain fluctuations. Attempts are made to perform single-channel measurements using new experimental setups by overcoming difficulties in detection of low ATPase activity as well as small current upon opening of the gate. The recent studies on ion pumps Ca^{2+}-ATPase and F_oF_1-ATP synthase are also introduced briefly for comparison.

2SEA-04 多剤排出トランスポーターの薬剤取込経路の粗視化シミュレーション研究
Drug uptake pathways in multi-drug transporter studied by coarse-grained simulations

Shoji Takada (*Grad. Sch Sci, Kyoto Univ.*)

Multidrug resistance has been a critical issue in current chemotherapy. In Escherichia coli, a major efflux pump responsible for the multidrug resistance contains a transporter AcrB. Crystallographic studies and mutational assays of AcrB provided much of structural and overall functional insights, which led to the functionally rotating mechanism. However, the drug uptake pathways are somewhat controversial because at least two possible pathways, the vestibule and the cleft paths, were suggested. Here, combining molecular simulations and site-directed mutagenesis experiments, we addressed the uptake echanism finding that the drug uptake pathways can be significantly different depending on the properties of drugs.

2SEA-05 脂質はイオンチャネルのゲート開閉をどの様に制御するのか？：新奇脂質センサーによる制御機構
How do lipids regulate the gating activity of the channel protein? : Mechanism of a novel type of the lipid sensor

Masayuki Iwamoto, Shigetoshi Oiki (*Dept. Mol. Physiol. Biophys., Univ. Fukui Facult. Med. Sci.*)

Membrane lipids are essential for regulation of physiological function of channel proteins. In the case of the KcsA potassium channel, it has been demonstrated that the negatively charged lipids bias the gating equilibrium to the open conformation. Here we show a novel type of the lipid sensor of the KcsA channel. The N-terminal amphipathic (M0) helix interacted with the negatively charged lipids by rolling the helix around the axis on the membrane inner surface, and stabilized the open-gate conformation. This unique type of lipid-sensing mechanism may be shared by various types of membrane proteins.

2SEA-06 細菌多剤排出トランスポーターの制御と生理機能
Regulation and physiological function of bacterial multidrug transporters

Kunihiko Nishino (*ISIR, Osaka Univ.*)

Multidrug efflux is an obstacle to the successful treatment of infectious diseases, and it is mediated by multidrug transporters that recognize and export a broad spectrum of chemically dissimilar toxic compounds. Many bacterial genome sequences have been determined, allowing us to identify drug efflux genes encoded in the bacterial genome. Here, I present an approach to identifying bacterial drug efflux genes and their regulatory networks. Multidrug transporters are often regulated by environmental signals and they are required for bacterial virulence in addition to multidrug resistance. Because multidrug transporters have roles in drug resistance and virulence, I propose that multidrug transporters have greater clinical relevance than previously considered.

2SEA-07 光駆動イオンポンプから光開閉性イオンチャネルへの機能変換

Converting a light-driven ion pump into a light-gated ion channel

Yuki Sudo (*Div. of Parm. Sci., Okayama Univ.*)

Light is the most important energy source for organisms. The photoactive retinal proteins are responsible for a variety of biological functions, such as ion pumps, ion channels and photosensors. The question arises of how these proteins can function so differently? Although ~75% of amino acid residues differ among retinal proteins, only three mutations convert a proton pump into a photosensor [1]. Recently we succeeded in conversion from a light-driven ion pump into a light-gated ion channel by three mutations [2]. The results reveal that the β-ionone ring of the chromophore is a key element for the functional difference among the active and passive ion transporters.

[1] Sudo et al., (2006) PNAS, 103, 16129, (2011) JBC, 286, 5967. [2] Inoue et al., in preparation.

2SAP-01 分子個性と少数性

Molecular Individuality and Minority in Biology

Tamiki Komatsuzaki (*Hokkaido Univ., Res. Inst. Electronic Sci.*)

Complex biological systems inherently possess hierarchies in time and space and biological functions occur across several orders of the scales. However, the question of how "individuality (=structures, dynamics) of the same kind of molecules survives in a single cell has not been addressed. We overview one of our methods, which extracts the underlying reaction scheme that enables us to address the kinetic significance of the diversity of molecular structures from time series data of single molecule experimental measurement. The resultant reaction scheme does not rely on an a priori ansatz such as detailed balance. We demonstrate the potential of our method by applying it to the analysis of, for example, a single-molecule turnover enzymatic reaction.

2SAP-02 触媒反応ネットワーク系における少数分子と競合

Minority molecules and competitions in a catalytic reaction network

Atsushi Kamimura, Kunihiko Kaneko (*Dept. of Basic Science, The Univ. of Tokyo*)

Cells integrate diverse molecules to keep their reproduction and exhibit a variety of robust functions, taking essential resources from environment. The numbers of specific components are not large, thus, competition for the few components will be relevant and commonly occurring. Understanding the effect in a general catalytic network will clarify how they contribute to the robust cellular behavior. In this talk, I will show two such examples. First results will show that a minority molecule in a catalytic reaction network results in reproductions and growth-division processes of a simple spatial structure, i.e., a protocell. Second will show depletion of and competition for a variety of resources result in reproduction of diverse compositions of protocells.

2SAP-03 スパインにおける確率的な Ca^{2+}の上昇はロバストでセンシティブな情報コーディングを可能にする

Stochasticity in Ca^{2+} increase in spines enables robust and sensitive information coding

Masashi Fujii[1], Takuya Koumura[2], Hidetoshi Urakubo[1], Kaoru Ohashi[1], Shinya Kuroda[1,2] ([1]*Dept. Biol. Sci., Grad. Sch. Sci, Univ. Tokyo*, [2]*Undergrad. Dept. Bioinfo. Syst, Univ. Tokyo*)

A dendritic spine is a very small structure (~0.1 μm^3) of a neuron that processes input timing information. Why are spines so small? Here, we provide following functional reasons obtained by the stochastic simulations of input timing-dependent Ca^{2+} increases in a cerebellar Purkinje cell's spine. Spines code input timing information by the probability of Ca^{2+} increases, rather than the amplitude, for input timing detection by utilizing the small number of molecules in a spine volume. Probability coding in a spine volume was more robust against input fluctuation and more sensitive to input numbers than amplitude coding in a cell volume. Thus, stochasticity is a strategy by which neurons robustly and sensitively code information.

2SAP-04 定量 ATP イメージングによる単一細胞内 ATP 濃度の多様性の測定

Heterogeneity in ATP Concentrations in a Single Bacterial Cell Population Revealed by Quantitative Single-cell Imaging

Hideyuki Yaginuma[1,2,3], Shinnosuke Kawai[4,5], Kazuhito Tabata[2,6], Keisuke Tomiyama[3], Akira Kakizuka[7], Tamiki Komatsuzaki[5], Yasushi Okada[1], Hiroyuki Noji[2,3], Hiromi Imamura[7,8] ([1]*QBiC, RIKEN*, [2]*Grad. Schl. Eng., Univ. Tokyo*, [3]*Grad. Schl. Frontier Biosci*, [4]*Schl. Sci, Shizuoka Univ.*, [5]*RIES, Hokkaido Univ.*, [6]*PRESTO, JST*, [7]*Grad. Schl. Biostud., Kyoto Univ.*, [8]*Hakubi Project, Kyoto Univ.*)

The fluctuation in gene expression may result in diverse concentrations of metabolites in individual cells, but the extent of heterogeneity was unknown. Here, we developed a new fluorescent indicator protein of an important metabolite, adenosine triphosphate (ATP). This indicator named "QUEEN" could quantify absolute ATP concentrations inside single Escherichia coli cells. Thus, we used QUEEN to measure the single-cell ATP concentration distribution.

We successfully visualized the ATP concentration heterogeneity. Interestingly, we found that the growth condition of the cell highly affects the shape of ATP concentration distribution. We surmise from the results that limited number of ATP synthesis pathways is active in the stationary phase of bacterial growth.

2SAP-05 １細胞系譜統計解析により明らかになる細胞表現型の適応度と選択圧の強さ

Single-cell lineage statistics reveals fitness and selection strength for heterogeneous phenotypic states

Takashi Nozoe, **Yuichi Wakamoto** (*Univ. of Tokyo*)

Different cellular phenotypic states in a clonal population are often associated with different growth rate and stress tolerance, i.e. fitness, which results in a biased representation of each state to the population. We formulated a method of single-cell lineage statistics, which gives fitness and strength of selection for heterogeneous phenotypic states quantitatively. We applied this method to the experimentally obtained single-cell lineage trees and the dynamics of protein expression levels of *Escherichia coli*, and successfully revealed fitness cost and benefit of different expression levels under several culture conditions. The results indicate the importance and utility of analyzing single-cell dynamics from the viewpoint of cell lineage (history).

2SAP-06 少数性生物学の展望
Prospect of minority biology

Takeharu Nagai (*ISIR, Osaka Univ.*)

Cooperative function/behavior in biological nanosystems consisting of small number of molecular elements is one of the most important aspects in biological phenomena. Although so many studies in terms of biochemistry and single molecule biology, which deal with Avogadro's number of molecules and single molecule, respectively, have been reported, there is almost no report showing elementary process of cooperative function/behavior among small number of molecules in living cells (from BSJ2012 Symposium abstract written by Nagai and Ishijima,). In this symposium, I will overview recent technological progress which could be applied and/or useful for investigating the small number problem, and also show several biological phenomena that should be suitable to approach it.

2SBP-01 構造細胞生物学とは
Structural cell biology

Toshio Hakoshima (*Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol.*)

At key steps involved in cell signaling pathways, multiple proteins form molecular complexes through complexed intermolecular interactions. Our primary goal of structural cell biology is to determine the three-dimensional structure of these complexes for taking a snapshot of proteins in the act of performing their functions. The obtained structural knowledge enables us to establish a scientific field that strictly defines the origins of the specificity of these interactions and to reveal the mechanisms by which proteins regulate their molecular functions.

2SBP-02 C 型レクチン受容体 Mincle の糖脂質認識機構
Structural basis for glycolipid recognition mechanism by C-type lectin like receptor, Mincle

Atsushi Furukawa[1], Jun Kamishikiryo[2], Toyoyuki Ose[1], Sho Yamasaki[3], Katsumi Maenaka[1] ([1]*Grad. Sch. of Pharm., Univ. of Hokkaido*, [2]*Sch. of Pharm., Univ. of Fukuyama*, [3]*Med. Inst. of Bioreg., Univ. of Kyushu*)

C-type lectin like receptors (CLRs) express on the surface of immune cells and recognize pathogen associated molecular patterns (PAMPs). Mincle (Macrophage inducible C-type lectin), which is one of CLRs, directly recognizes TDM (Trehalose 6,6-dimycolate), unique glycolipid on the cell surface in Mycobacterium species. TDM is well known as an adjuvant, thus the Mincle-TDM interaction plays an important role in activation of immune cells. Previous reports indicated that Mincle recognizes both sugar and lipid of TDM. However, the recognition mechanism of TDM by Mincle had not been understood. Here, we present the crystal structure of Mincle, and compare it with other C-type lectins to reveal the molecular mechanisms of substrate specificity.

2SBP-03 コレラ菌の鉄獲得機構
Heme-iron uptake proteins from *Vibrio cholerae*

Takeshi Uchida (*Grad. Sch. Sci., Hokkaido Univ.*)

Iron is an essential element for bacteria to survive. To obtain this element, bacterial pathogens utilizes heme from hemoglobin as an iron source. Recently, we found in *Vibrio cholerae* two enzymes, which liberate iron from heme in a different manner. VCA0907 (HutZ) is a heme degradation enzyme, which catalyses the O_2-dependent degradation of heme, while VC2145, whose crystal structure shows close similarity to those of a family of dye-decolorizing peroxidases, removes iron without breaking heme under anaerobic condition. The presence of two different kinds of enzymes to obtain iron from heme suggests that *V. cholerae* can adapt to different O_2-concentration environment.

2SBP-04 糖タンパク質品質管理システムにおける糖鎖修飾メカニズムの構造基盤
Structural basis for the glycan-processing mechanisms in glycoprotein quality control system

Tadashi Satoh[1,2], Koichi Kato[1,3] ([1]*Grad. Sch. Pharm. Sci., Nagoya City Univ.*, [2]*JST, PRESTO*, [3]*Okazaki Inst. Integ. Biosci.*)

Glycans act as signals for quality control of glycoproteins, ensuring their appropriate folding and trafficking in cells. Glucosidase II catalyzes two-step glucose-trimming, while folding sensor enzyme UDP-glucose glycoprotein glycosyltransferase (UGGT) transfers glucose to incompletely folded glycoproteins, thereby allowing them to access with endoplasmic reticulum (ER) chaperone complex. To unveil working mechanism of the glycoprotein-quality control system, we attempt to elucidate the 3D structures of these glycan-processing enzymes. Here, we will present our latest results including crystal structures of catalytic domain of glucosidase II and folding sensor domain of UGGT. Our findings provide structural basis for ER glycan-processing mechanisms at atomic level.

2SBP-05 G タンパク質共役型内向き整流性カリウムイオンチャネル 1(GIRK1)の G タンパク質による機能調節の構造基盤
Structural Basis for Regulation of G Protein-activated Inwardly Rectifying Potassium Channel 1 (GIRK1) by G Proteins

Masanori Osawa[1], Yoko Mase[1], Mariko Yokoagawa[1,2], Koh Takeuchi[3], Ichio Shimada[1] ([1]*Grad. Sch. Pharm. Sci, The Univ. of Tokyo*, [2]*JBic*, [3]*Molprof, AIST*)

G protein-gated inwardly rectifying potassium channel (GIRK) plays a crucial role in regulating heart rate and neuronal excitability. The gating of GIRK is regulated by the association and dissociation of G protein βγ subunits (Gβγ), which are released from pertussis toxin-sensitive G protein α subunit (Gαi/o) upon GPCR activation in vivo. Gαi/o also interacts directly with GIRK, increasing the signaling efficiency and modulating the channel activity. Here, we performed NMR analyses of the interaction of the cytoplasmic region of GIRK1 (GIRKCP) with Gβγ and Gαi3 in the GTP-bound state, respectively. Our NMR results enabled to build a docking model of Gα, Gβγ, and GIRK, providing structural basis for the regulation of the GIRK gating by G proteins.

2SBP-06 超遠心分析と質量分析による溶液中での蛋白質間相互作用解析

In solution characterizations of protein-protein interactions by analytical ultracentrifugation and mass spectrometry

Susumu Uchiyama[1,2] ([1]*Dept.Biotech., Grad. Sch. Eng., Osaka Univ.*, [2]*Okazaki Inst. Integ. Biosci.*)

Recent advancements in methods for quantitative protein-protein interaction analysis will be introduced. Analytical ultracentrifugation (AUC) is a powerful method to clarify behaviors of proteins and protein assembly in solution. Size distribution with shape information, molecular weight and interaction parameters are estimated by AUC. The sizes and shapes of each component in highly complicated solution system can be clearly identified by the combination of AUC with native mass spectrometry. Meanwhile, the detailed structural information on large protein complexes and protein aggregates can be efficiently acquired by hydrogen deuterium exchange mass spectrometry (HDX-MS). Several examples of HDX-MS will be introduced in this presentation.

2SBP-07 毛上皮形成における S100A3-PAD3 亜鉛シグナル伝達機構の構造生物学的解明

Structural Biology for the Zinc Signal Transduction Mechanism by S100A3-PAD3 in Hair Cuticular Cells

Masaki Unno[1,2], Kenji Kizawa[3], Ryutaro Mashimo[1,2], Shinya Saijo[4], Nobutaka Shimizu[4], Megumi Akimoto[1,2], Hidenari Takahara[5] ([1]*Grad. Sch. Sci. Eng., Ibaraki Univ.*, [2]*iFRC, Ibaraki Univ.*, [3]*Kanebo, Cosme. Inc*, [4]*KEK, PF*, [5]*Dep. Appl. Biol. Res. Sci., Ibaraki Univ.*)

The S100A3 homotetramer assembles upon citrullination of a specific symmetric Arg51 pair in hair cuticular cells. The binding of either Ca2+ or Zn2+ ions modulates its affinity for the other cation. A single Zn2+ ion accelerates the Ca2+-dependent tetramerization of S100A3. The Ca2+ and Zn2+ binding affinities of S100A3 were enhanced when the other cation bound in concert with the tetramerization of S100A3. The citrullination of Arg51 of S100A3 is specifically catalyzed by the peptidylarginine deiminase type III isozyme (PAD3), while its isozymes PAD1 and PAD2 can citrullinate all the arginins of S100A3 in vitro. In this conference, we will report their structures and functions.

2SCP-01 Toward a Computational Assessment of the Effect of Amino Acid Variation to Protein Structure and Function - Case Study on a Few Enzymes

Kei Yura[1,2] ([1]*Grad. Schl. Hum. Sci., Ochanomizu Univ.*, [2]*NIG*)

Genome sequencing technology has advanced to the level of identifying nucleotide sequences of individuals in a huge scale, and many differences in the genome sequences amongst human population have been reported. The differences should have effect on the phenotype of humans through differences in the expression/structure/function of RNAs and proteins. SLS-DC project, a project we launched to build a data cloud that connects databases for these pieces of information, can help to find the relationship of the variations and the phenotype through proteins. Through SLS-DC, relationship between the variations of amino acid in enzymes and the phenotype could be obtained to the extent that the phenotype could be suggested from the differences. The case study will be presented.

2SCP-02 From Personal Genome to Personal Proteins: Connection between the Reference sequences of genomic DNA and Proteins

Kengo Kinoshita[1,2,3] ([1]*Grad Sch Info Sci, Tohoku Univ.*, [2]*Tohoku Medical Megabank, Tohoku Univ.*, [3]*IDAC, Tohoku Univ.*)

The human reference genome sequence is a fundamental resources to describe a variety of data on human. In the similar way, UniProt provides the basic resources of the reference proteins with a lot of functional annotations. Literally speaking, the two “references” should be same and functional annotation in each reference should be transferred to the other reference, but they are different because the different biological resources are used. To clarify the differences and to connect the functional information in both references, we compared two references at a single base (or residue) level, and developed a method to map each genome position onto a protein residue to interpret the personal genome difference by using protein knowledge.

2SCP-03 蛋白質の構造モチーフの際に着目した機能アノテーション

Composite structural motifs of binding sites for annotating functional differences

Akira Kinjo (*Institute for Protein Research, Osaka University*)

We define composite structural motifs by classifying and integrating the results of exhaustive all-against-all comparison of binding site structures of small molecules, proteins and nucleic acids in proteins. It is shown that subtle differences in biological functions among homologous proteins can be annotated in terms of differences in composite structural motifs. We demonstrate how detailed and abundant structural information (at atomic resolution) may be usefully exploited for functional annotation by focusing on the differences of functional sites, rather than their mere similarity.

2SCP-04 アミノ酸残基間距離予測に基づくタンパク質立体構造モデルの評価

Model quality assessment method based on a residue-residue distance matrix prediction

Mayuko Takeda-Shitaka (*Pharm., Kitasato Univ.*)

Protein structure prediction methods using computers are very useful and essential in structure based drug design, function analysis and so on. At present, various prediction methods have been developed. Many of the methods are fully automated, and researchers can get protein models easily only by preparing amino acid sequences as input. When using models, the most important thing is to know their quality. Therefore, model quality assessment methods play important roles. Recently, we developed some model quality assessment methods. In this presentation, I introduce our methods and discuss about the performance of them.

2SCP-05 粗視化分子動力学シミュレーションで探るタンパク質・リガンド結合過程

Protein–ligand binding processes studied by coarse-grained molecular dynamics simulations

Tohru Terada, Tatsuki Negami, Kentaro Shimizu (*Grad. Sch. Agr. Life Sci., Univ. Tokyo*)

The process of how a small molecule binds to a protein is still unclear, despite its importance. We have explored the applicability of a coarse-grained molecular dynamics (CGMD) simulation to the study of protein–ligand binding processes. Here, we report the results of the CGMD simulations with the MARTINI force field performed on two different protein–ligand systems. We observed a number of ligand-binding and unbinding events to the ligand-binding sites. Calculated binding and unbinding rate constants and the dissociation constants agreed well with the experimental ones. Analysis of the ligand fluxes demonstrated that the CG ligand molecules entered the ligand-binding pockets through specific pathways. We will discuss the factors determining the pathways.

2SCP-06 超分子モデリングパイプラインの構築による相関構造解析•理論創薬支援

Supramolecular modeling pipeline for correlative structural analysis and rational drug-design

Tsuyoshi Shirai (*Nagahama Inst BioSci Tech*)

The structures of supramolecule are essential for rational drug-design, which is based on the mechanisms of the molecules in atomic detail. Since an elucidation of supramolecular structure is still elaborate work for a single experimental method, the correlative structural analysis, which combines several biophysical measurements, is prospected. The structural bioinformatics should play an important role in the correlative structural analysis. For this purpose, a bioinformatics resource, supramellecular modeling pipeline, is under construction. This pipeline contains the methods of knowledge-based modeling, domain/subunit docking, coarse-grained model refinement, and interface for structure mapping. Several cases of the modeling with this approach will be discussed.

2SCP-07 Intrinsic disorder mediates cooperative signal transduction in STIM1

Daron M. Standley (*iFReC, Osaka University*)

We examined the response of the STIM1 EF-SAM domain to changes in [Ca2+] using mathematical modeling based on in vitro experiments. We found that the EF-SAM domain partially unfolds and dimerizes cooperatively with respect to [Ca2+], with Hill coefficients and K1/2 very close to the values observed in vivo for STIM1 redistribution and extracellular Ca2+ influx. Our mathematical model of the dimerization reaction agreed with our AUC-based measurements as well as previously published free energies of unfolding. A simple interpretation of these results is that Ca2+ loss effectively acts as a denaturant, enabling cooperative dimerization and robust signal transduction. We present a structural model of the apo EF-SAM domain that is consistent with a wide range of evidence.

2SDP-01 生体分子のダイナミクスに向けた水和熱力学

Hydration thermodynamics toward biomolecular dynamics

Yuichi Harano (*Himeji Dokkyo University*)

Hydration is quite important in many aspects in biomolecules. In aqueous solution, a lot of interesting events, such as diffusions and chemical reactions, can only happen at the time scale of milli-seconds, seconds or even hours or days. In this study, we investigate the structural change of a biomolecule immersed in water using an all-atom molecular model. The energy function is defined for a single structure of a biomolecule as the sum of the intramolecular interaction energy of the isolated biomolecule and the hydration free energy. Here we assume local equilibrium of surrounding water is reached for a given structure of a biomolecule. With this theoretical treatment, we will argue that water can thermodynamically contribute to the structural change of a biomolecule.

2SDP-02 生体分子機械を水和水であやつる

Controlling the molecular machinery by water molecules of the hydration

Masayoshi Nishiyama (*The HAKUBI Center, Kyoto Univ.*)

In living cells, many molecular machines work in aqueous solution, interacting with many water molecules. One of the key approaches to address the mechanism of the molecular machines is to understand the role of the intermolecular interaction with surrounding water molecules by modulating the interaction with water molecules. Application of pressure is one of the powerful methods for enabling the modulation of protein hydration. We have developed a high-pressure microscope that enables us to acquire high-resolution microscopic images, regardless of applied pressures. The developed system allows us to visualize the pressure-induced changes in the structure and function of molecular machines working in living cells.

2SDP-03 水の状態を感受する蛍光蛋白質の開発

Development of fluorescent protein to sense “state of water”

Tomonobu Watanabe[1,2,3] ([1]*QBiC, RIKEN*, [2]*Grad. Sch. Front. Biosci., Osaka University*, [3]*iFRec, Osaka Univ.*)

Fluorescent proteins share a common structure composed of an eleven-stranded β-barrel containing a chromophore. The β-barrel structure prevents the chromophore from interacting with the solvent molecules, which are known to affect the absorption and emission fluorescent spectra and intensity. We here inserted amino acid before Tyr145, whose side chain phenol group is located adjacent to the chromophore in the center of the β-barrel structure, of fluorescent protein from Aequorea Victoria to enhance the solvent interaction of the chromophore. The insertion produced a dramatic structural change near the chromophore and enhanced the sensitivity to “state of water” in solution. And, we would like to introduce the application in biostudy of them.

2SDP-04 合成化学的に構築した人工分子機械によって駆動される水中での非共有結合性分子集合体の巨視的運動

Macroscopic Motion of Soft Non-covalent Molecular Assembly in Water Actuated by Chemically Synthesized Molecular Machine

Yoshiyuki Kageyama[1,2] ([1]*Fac. Sci., Hokkaido Univ.*, [2]*PRESTO, JST*)

Artificial creation of macroscopic motion triggered with structural change of small molecule is a challenging theme. In many approaches to create macroscopic motions, our salient points are in (1) using non-covalent assembly constructed in water, (2) actuation by small amount of synthesized molecular machine, (3) repeatability, and (4) spatially organized dynamics. To propagate the structural change of small molecules to macroscopic dynamics, intermolecular interaction is essential. However, simple interaction makes only simple behavior. To create highly hierarchical motion, organizations of cooperative interactions are necessary. In the symposium, mechanistic aspects of macroscopic motions of self-assemblies, in which water plays important roles, will be discussed.

2SDP-05 アクチンモノマーの会合と多価カチオンが媒介する同符号コロイド粒子間実効引力

Association of Actin Monomers and Effective Attraction between Like-Charged Colloidal Particles Mediated by Multivalent Cations

Ryo Akiyama (*Dept. of Chem., Kyushu Univ.*)

Disassembly and assembly of actin filament change a cell shape and drive the motion of amoeba-like cell. Then, the association of actin monomer regulates this function. To discuss this association mechanism we studied the attractive interaction between macroanions mediated by multivalent cations using an integral equation theory with a simple model. The calculated results indicate that the effective attraction increases as the charge of macroanion becomes larger. This result supports a hypothesis of the regulation mechanics: The association is driven by the charge of monomer and the charge is regulated by the hydrolysis of ATP. We will discuss the detail of the scenario.

2SDP-06 タンパク質の構造ゆらぎと変化に対する相互作用成分解析

Interaction-Component Analysis on Protein Structure in Explicit Solvent

Nobuyuki Matubayasi (*Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University*)

Using all-atom molecular dynamics simulation combined with a statistical-mechanical theory of solvation, the hydration effect is addressed on the structural fluctuation of cytochrome c in pure-water solvent. During the course of equilibrium fluctuation, the variation of the protein intramolecular energy is shown to be induced and compensated by the solvent water through the exchange of the electrostatic interaction energy. The urea effect on protein structure is also analyzed in terms of the transfer free energy from pure-water solvent to the urea-water mixed solvent. It is found that the transfer free energy correlates strongly with the change in the van der Waals interaction upon transfer and not with the change in the electrostatic interaction.

2SEP-01 カラフルな植物光受容体、赤、青、UV-B を見る

Colorful plant photoreceptors see red, blue and UV-B light

Satoru Tokutomi, Shizue Yoshihara (*Osaka Prefect. Univ., Grad. School Sci., Dep. Biol. Sci.*)

Immovable plants have to see the light condition precisely to adapt the environment. For this purpose, plants have acquired a variety of photoreceptors in a spectral range from far-red to UV-B. Phytochrome, a red- and far-red reversible receptor, regulates photomorphogenetic responses including flowering. Cryptochrom, a blue light receptor, acts for the regulation of similar physiological responses to those of phytochrome. Phototropin, a member of LOV blue light receptor families, serve to maximize photosynthetic activities by regulating phototropic responses, chloroplast movements, stomata opening, etc. Furthermore, UVR8 senses UV-B light. Molecular bases for the light-signal reception of these photoreceptors will be reviewed.

2SEP-02 触覚パターンのオプトジェネティクス制御

Optogenetic patterning of touch sense

Hiromu Yawo[1,2,3], Yukiobu Yokoyama[1,2], Akira Sumiyoshi[4], Kenta Abe[1,2], Kyo Koizumi[1,2], Ryo Egawa[1,2], Yueren Liu[1,2], Tomokazu Ohshiro[2,3], Yoshiya Matsuzaka[2,3], Ryuta Kawashima[4], Hajime Mushiake[2,3], Toru Ishizuka[1,2] ([1]*Tohoku Univ. Grad. Sch. Life Sci.*, [2]*JST-CREST*, [3]*Tohoku Univ. Grad. Sch. Med.*, [4]*Tohoku Univ. IDAC*)

The rodent whisker-barrel system has been an ideal model for studying somatosensory representations in the cortex. However, it remains a challenge to experimentally stimulate whiskers with a given pattern under spatiotemporal precision. Here we identified the selective expression of channelrhodopsin-2 (ChR2) in the large mechanoreceptive neurons in the trigeminal ganglion (TG) as well as their peripheral nerve endings innervating the whisker follicles of a transgenic rat. The spatiotemporal pattern of whisker irradiation thus produced a barrel-cortical response with a specific spatiotemporal. Our methods of generating an optogenetic tactile pattern (OTP) should open a new avenue for investigating complex senses such as shape, size and movement.

2SEP-03 脊椎動物クリプトクロムの多様性、機能および分子応用

Diversity, function and molecular application of vertebrate cryptochromes

Toshiyuki Okano (*Waseda Univ. Sch. Adv. Sci. & Eng.*)

Cryptochromes (CRY) are blue-light photoreceptors highly related to photolyases. Cry genes have been diverged into several groups shared by a wide range of living organisms including plants, animals and fungi. In vertebrates, CRY1 and CRY2 are established circadian clock proteins, playing a pivotal role for the oscillatory transcription loops as negative regulators. Non-mammalian vertebrates have additional Cry genes, Cry3 and Cry4, and interestingly, these CRYs could be photoreceptors and/or magnetoreceptors. For future optogenetical application as well as functional characterization, we have been studying these CRYs by a wide variety of molecular approaches such as immunohistochemistry, biochemical analyses of recombinant proteins, and spectroscopic analyses.

2SEP-04 イェロープロテインを通じてみたセンサー蛋白質の作動機構
Molecular actions of the light sensor protein, Photoactive Yellow Protein, as a prototype for sensor proteins

Hironari Kamikubo (*NAIST/MS*)

Photoactive Yellow Protein (PYP) is a light sensor protein that serves as a prototype for studying the molecular mechanisms of light sensor proteins. Chromophore isomerization, local chemical reactions such as proton transfer, and tertiary structural changes occur during the photoreaction of PYP; elucidating the relationship between these fundamental molecular processes can provide insights into the mechanism of sensor proteins. To this end, a high-resolution structure containing hydrogen atoms in the dark state and intermediate structures have been determined. In this symposium, recent advances in the investigation of the molecular actions of the light sensor protein are discussed.

2SEP-05 BLUF タンパク質の光化学とオプトジェネティクス
Photochemistry and optogenetics with BLUF proteins

Shinji Masuda (*Center for Biol. Res. & Inform., Tokyo Inst. Tech.*)

BLUF is a small (~15 kDa) flavin-binding domain that functions as a blue light-sensing module. BLUF controls a wide variety of light-regulated physiological activities including photosystem synthesis, biofilm formation, and the photoavoidance response. The photo-activation process of BLUF is unique in that only small structural changes of the flavin are involved rather than an isomerization or covalent bond formation. Recent spectroscopic, biochemical, and structural studies have begun to elucidate how BLUF domains transmit the light-induced signal and identify related, subsequent changes in the domain structures. This information has been applied for optogenetics to artificially control physiology by light.

2SEP-06 脊椎動物の非視覚オプシン Opn5 の分子特性の多様性
Diversity of Molecular properties of vertebrate non-visual opsin Opn5

Takahiro Yamashita (*Grad. Sch. Sci., Kyoto Univ.*)

Opsins are the universal photoreceptive molecules for visual and non-visual photoreceptions in animals and are classified into several distinct groups based on their amino acid sequences. Opn5 forms an independent group whose members in vertebrates are diversified into four subgroups. The comprehensive analysis of the molecular properties showed that Opn5 group shares G protein coupling property (Gi activation) and is diversified based on their spectral sensitivities (visible light- or UV light-sensitivity). In addition, preference for retinal isomers (binding of 11-cis and/or all-trans retinals) is also different among four subgroups. I would like to discuss the physiological importance of the diversity of the molecular properties in Opn5 group.

2SEP-07 フィトクロムは遺伝子発現の様々な段階を直接制御する
Phytochrome directly regulates various aspects of gene expression

Tomonao Matsushita[1,2] ([1]*Fac. Agri., Kyushu Univ.*, [2]*JST PRESTO*)

Plants adapt to their environment by monitoring ambient light conditions through several photoreceptors such as phytochrome. It is widely believed that, upon absorbing red light, phytochrome induces light responses by regulating the transcription of numerous target genes. Here we provide clear evidence that phytochrome controls not only transcription, but also alternative splicing in Arabidopsis. We reveal that 6.9% of the annotated genes in the Arabidopsis genome undergo rapid changes in their alternative splicing patterns in a red light- and phytochrome-dependent manner. Our results demonstrate that phytochrome simultaneously regulates two different aspects of gene expression and reveal that alternative splicing plays an important role in light signaling in plants.

3SAA-01 分子ロボティクス―その展望と動機
Molecular Robotics — Perspectives and Motivation

Satoshi Murata (*Grad. Sch. Eng., Tohoku Univ.*)

Molecular Robotics is a new research field emerging at intersection of Biology, Chemistry and Systems Scicence. I this talk I would like to introduce perspectives and motivation of Molecular Robotics.

3SAA-02 Building Nanoscale Devices with DNA

Shawn Douglas (*UCSF*)

The programmability of DNA makes it an attractive material for constructing intricate nanoscale shapes. One method for creating these structures is DNA origami, in which a multiple kilobase single-stranded scaffold is folded into a custom nanoscale shape by interacting with hundreds of short oligonucleotide staple strands. I will talk about our efforts to realize demand-meeting applications with this method, including our recent development of nanoscale devices to mimic cell signaling stimulation carried out by our own immune systems.

3SAA-03 核酸ナノ構造を活用した分子情報変換デバイスの設計
Designing DNA/RNA nanostructure-based information converters

Hirohide Saito[1], Masayuki Endo[1], Masahiro Takinoue[2] ([1]*Kyoto Univ.*, [2]*Tokyo Tech.*)

The purpose of the molecular sensor team is conducting research in two directions, (1) sensing a variety of signals by employing bionanostructures and (2) transmitting the information to the signals which can be used by computers and actuators of molecular robots. Recently, studies in the emerging field of DNA/RNA nanotechnology revealed that a variety of functional nanostructures can be constructed by employing a set of DNA/RNA motifs. Indeed, DNA/RNA-based nanostructures or gene regulatory devices can sense several trigger factors and control their structures and functions dependent on the environment. In this presentation, we introduce our recent progress and scientific achievement on the molecular robotics sensor project.

3SAA-04 分子ロボットのリアルタイムな動作を目指した試験管内での知能の実装
Implementation of *in vitro intelligence* for real-time operation of molecular robots

Ken Komiya (*Int. Grad. Sch. Sci. & Engi., Tokyo Tech.*)

Nucleic acid strands that can autonomously hybridize based on Watson-Crick complementarity are promising materials for construction of chemical reaction systems to perform computation. In the Molecular Robotics project, the Molecular Intelligence team aims to design and implement chemical reaction circuits with nucleic acid strands, which serve as central components for controlling molecular robots operation. In this presentation, we will discuss key criteria for evaluating the performance of a reaction circuit, including time efficiency, time responsiveness, and so on. We also report the current effort for speeding up the computation and construction of interfaces to achieve integration of sensing, computation and actuation operation of an intelligent molecular robot.

3SAA-05 アメーバ型分子ロボットの課題と展望
Perspectives and objectives of amoeba-type molecular robots

Akihiko Konagaya (*Tokyo Institute of Technology*)

The Amoeba Robot team aims at developing an amoeba-like molecular robot with molecular sensors, a molecular controller and molecular actuators enveloped in a molecular container. The amoeba robots are considered the first generation in the scenario of molecular robot evolution. The main challenge for the construction of amoeba-type molecular robots is system integration of molecular components in a molecular container. Giant liposome is one of the most promising molecular containers for amoeba-type molecular robots. Integration of advanced molecular sensing systems and molecular control systems is a remaining issue in our future work.

3SAA-06 ゲルに基づく分子ロボットとその計算モデル
Gel-based molecular robots and their computational models

Masami Hagiya (*The University of Tokyo*)

The Slime Mold Team of the Molecular Robotics Project aims at constructing molecular robots with gels as actuators and bodies. Such molecular robots are called slime mold robots. They are expected to process and amplify molecular and other kinds of signals to drive gel actuators, which make conformational changes to robot bodies and eventually result in motions such as locomotion. After explaining the concept of slime mold robots, I briefly introduce research contributions the team has made so far, including some pieces of work on DNA-based hydrogels. I then talk about mathematical models for designing slime mold robots and analyze their computational power, including the model called gellular automata, in which solutions are separated into cells by walls made of gels.

3SBA-01 Toward design of molecular motors

Nobuyasu Koga[1,2] ([1]*Inst. Mol. Sci. CIMoS*, [2]*JST, PRESTO*)

One of the grand challenges for protein design in biophysics is to create protein molecular motors completely from scratch. The molecular motors perform unidirectional motion using external energies such as the energy released by ATP hydrolysis. How do we rationally design such protein molecules from scratch? Having this goal in mind provides us opportunities to develop technologies for precisely designing protein's basic properties, folding, oligomerization, conformational changes and etc., and to explore their fundamental understandings. We have developed the computational methods for designing amino-acid sequences that can fold into targeted structures. In this talk, we will present the achievements, and discuss how we go beyond the achievements toward the goal.

3SBA-02 The conformational change mechanism of the β subunit in F_1-ATPase revealed by all-atom MD simulations

Yuko Ito, Mitsunori Ikeguchi (*Grad. Sch. Med. Life Sci., Yokohama-City Univ.*)

The rotation of F_1-ATPase is induced by the conformational changes of the catalytically active β subunits. In short, the β structural change is *'the main engine'* of this molecular motor. The conformational change of the β subunit have two driving forces; one is the ATP binding and the other is the ATP hydrolysis. Our all-atom MD simulations elucidate both their mechanisms, where strongly conserved residues among the ATPase protein family; such as the P-loop and Walker A/B, play important roles. Furthermore, based on our predictions, single molecule experiments are being conducted in an experimental laboratory to confirm the mechanism underlying the structural change.

3SBA-03 Molecular simulations of proton pumps and biomolecular motors

Qiang Cui (*Dept. of Chem., Univ. of Wisconsin, Madison*)

I'll discuss the application of molecular simulations to proton pumps and biomolecular motors. The applications highlight that calibrated QM/MM methods are valuable because they provide not only energetic/kinetic information for the relevant chemical driving force (e.g., ATP hydrolysis) but also spectroscopic observables that can be compared to experiments. A feature emerged from these studies is that changing hydration level of protein cavities may play an important role in modulating the reactivity of key groups and thus the timing of chemical events. Thus, connections between conformational transitions, hydration changes and chemical activities form the basis of "mechanochemical coupling" in biomolecular machines.

3SBA-04 F1 モーターの再デザインによる人工回転分子モーター開発の見通し
Prospects on artificial molecular motor by redesigning of F1-ATPase

Hiroyuki Noji (*Applied Chem. U-Tokyo*)

F1-ATPase, soluble and catalytic domain of ATP synthase is the rotary motor protein in which the catalytic stator ring rotates the inner rotary shaft upon ATP hydrolysis. Chemomechanical coupling mechanism of F1 has been well studied and F1 became one of the best-characterized molecular motors. Recent studies revealed that the rotation mechanism of F1 is unexpectedly robust against global mutagenic perturbations. Thus, the basis has been set for the rational or semi-rational designing of F1. Such a synthetic approach will test the working hypotheses and give important insights on the design principle of F1. After reviewing on recent studies on the design principle of F1, I will introduce the current status of ongoing F1 design project.

3SBA-05 Is enzyme evolution reversible? Exploring fitness landscapes by laboratory evolution

Nobuhiko Tokuriki (*University of British Columbia*)

The extent to which mutations interact each other or epistasis dictates how protein evolves to new functions. Although importance of epistasis has been well recognized in protein as well as organismal evolution, molecular basis underpinning epistasis is poorly studied.
Here I present experimental evolution to explore fitness landscape. I discuss our findings of highly restricted the fitness landscape of phosphotriesterase (PTE) activity by performing laboratory evolution between PTE and arylesterase. I present molecular constraints underlying extensive epistasis between mutations, leading genetic irreversibility and incompatibility. Our findings indicate that understanding constraints in evolution will help us to understand and predict protein evolution and design.

3SBA-06 Remote control of myosin and kinesin motors using light-activated gearshifting

Zev Bryant (*Stanford University*)

Engineering biomolecular motors provides direct tests of structure-function relationships and potential tools for controlling cellular processes or harnessing molecular transport. I will describe the creation of a panel of cytoskeletal motors that reversibly change gears — speed up, slow down, or switch directions — when exposed to blue light. Our genetically encoded structural designs incorporate a photoactive protein domain to enable light-dependent conformational changes in an engineered lever arm. Using in vitro motility assays, we have confirmed robust spatiotemporal control over motor function and characterized the kinetics of optical gearshifting. Our modular approach has yielded controllable motors for both actin-based and microtubule-based transport.

3SCA-01 生物システムの理解に本質的な３つの未解決問題
Three unsolved problems for essential understanding of biological systems

Shigeki Mitaku (*Toyota Phys. Chem. Res. Inst.*)

I discuss the relationship among three certain unsolved problems for understanding biological systems: (1) Is there any simple principle of the protein structure formation? (2) How an enormously large number of cellular processes are harmonized in biological systems? (3) How the integration of many random mutations can design the robustness of living things? Such problem seems unsolvable, if we do not take the close relationship among the three problems into account. However, suppose that any cellular system for regulating mutations has been developed in the evolutionary process, we will have an obvious solution of all the problems. I will show several evidences for the relationship among the unsolved problems and discuss on the future direction of the bioinformatics.

3SCA-02 リン酸化プロテオームとメタボロームデータからのインスリン作用のグローバルネットワークの再構築
Reconstruction of global network of acute insulin action from phosphor-proteome and metabolome data

Shinya Kuroda, Katsuyuki Yugi (*Biological Sciences, The University of Tokyo*)

Cellular responses are composed of dynamic molecular interactions between multiple layers including protein phosphorylation, and metabolites. To reveal an unbiased whole picture, simultaneous quantitative and global measurements in these layers, rather than pin-point analysis of some selected molecules, is needed. Here, we simultaneously performed metabolomic and phospho-proteomic analysis in insulin-stimulated Fao hepatoma cells in collaboration with Prof. Soga (Keio Univ), and Dr. Matsumoto and Prof. Nakayama (Kyushu Univ), respectively, and developed an reconstruction method of insulin-dependent metabolic control pathway directly from trans-OMICS data.
In this symposium, reconstruction of global molecular network directly from trans-OMICS data is discussed.

3SCA-03 全ゲノム規模の１細胞内１分子遺伝子発現情報からのデータマイニング

Mining genome-wide datasets of single-cell gene expressions at single-molecule resolution

Yuichi Taniguchi (*Quantitative Biology Center, RIKEN*)

In modern biological science, a common issue that researchers face is handling and mining a large amount of datasets. Here I argue on this issue by showing our large-scale data analyses as an example. The data to be discussed is datasets from quantitative genome-wide analyses of protein and mRNA expression in individual living Escherichia coli cells with single-molecule sensitivity. With statistical analyses and physical model predictions, we found genome-wide rules of gene expression noise properties, such as protein copy number distributions and protein expression networks. I will further discuss with our recent data on gene expression analyses in single eukaryote cells, and will discuss on its perspective for large-scale data mining.

3SCA-04 分子バーコーディングによる一分子の分解能をもつゲノムワイド遺伝子発現絶対定量法

Absolute genome-wide quantification of gene expression with single molecule resolution using molecular barcoding

Katsuyuki Shiroguchi (*IMS RIKEN*)

Big data, generated by system-wide measurements, is used to further understand biological systems or link different biological layers. In such measurements, accurate and absolute molecular counting (e.g. copy per cell) is important as it enables integration of multiple omics analyses and quantitative mathematical modeling, which relies on quality of raw data. I will present our newly developed method, which enables the digital counting of the absolute copy number of RNA molecules genome-wide from 1-100 cells by using molecular barcoding. This highly accurate quantification allows us to analyze even low-copy cellular RNAs, which are important since, for example biologically, the expression level of some transcription factors that play crucial roles are known to be low.

3SCA-05 大容量生命情報時代の生物学のボトルネック

Fundamental bottlenecks in big-data biology

Wataru Iwasaki (*Dept Biol Sci, Grad Sch Sci, UTokyo*)

We are welcoming the "big data era" of biology. Under this background, the importance of computational approaches is repeatedly pointed out; however, there seem fundamental bottlenecks. In this presentation, I review some of those bottlenecks and discuss how biophysics and bioinformatics can eliminate them.

3SCA-06 Database for Biology: which data deserve maintaining?

Masanori Arita[1,2] ([1]*National Institute of Genetics*, [2]*RIKEN CSRS*)

The digital age enabled us to catalog large scale information, not only original but secondary ones. What has been overlooked is the maintenance cost for such information, including open access journal articles. Availability of larger information can accelerate biological discovery but not all of them are essential. Recently several important databases start to charge development cost on users, and researchers are forced to seriously evaluate their data values. In this talk, I would like to introduce history of biological databases and their outlook from the perspective of data maintenance. The research project to develop a shared metabolome repository will also be introduced.

3SDA-01 「膜分子のふるまい」を見て「細胞内シグナル」を知る

Watch the “membrane protein behavior” to know the “intracellular signaling pathway”

Hiroko Bannai[1,2], Fumihiro Niwa[2], Antoine Triller[3], Katsuhiko Mikoshiba[2] ([1]*Nagoya Univ., Grad. Sch. Biol. Sci.*, [2]*RIKEN BSI*, [3]*IBENS*)

Plasma membrane of the cell has functional microdomains that are essential for cell-cell signal transduction. Detailed analysis of diffusive behavior of membrane protein often helps us to understand the molecular mechanism underlying the formation and the maintenance of signal microdomains. In this talk, we introduce the application of Quantum-dot single particle tracking (QD-SPT), a powerful tool to analyze the membrane protein behavior, into the field of neuroscience. Using QD-SPT, we uncovered novel signaling pathway responsible for the stabilization of the GABAergic synapse, a primary inhibitory synapse in mammalian brain. We also found that two independent Ca^{2+} signal pathways have opposite effect on GABAergic synapse stability.

3SDA-02 Improvement of two-photon laser scanning microscopy for live imaging utilizing laser technology

Tomomi Nemoto[1,2,3] ([1]*RIES, Hokkaido Univ.*, [2]*JST CREST*, [3]*Grd. Sch. Info., Hokkaido Univ.*)

To elucidate the molecular mechanism underlying neural activities, visualizing fine structures at a high resolution within deeper layers of brain tissue, while keeping neural circuits nearly intact, is required. Fluorescence imaging by two-photon microscopy is widely used for this purpose. However, the tissue penetration depth, and the spatial and the temporal resolutions of laser scanning microscopy are limited by several physical factors. In this presentation, I will show our several approaches to break these limitations by utilizing novel laser technology including a semiconductor-laser-base 1030-nm light pulse source, vector beams generated by transparent liquid crystal devices, or multi-point beam scanning of a high-power NIR ultra-short pulse laser.

3SDA-03 吸収増幅顕微鏡による細胞イメージング
Cavity reflection enhanced light absorption microscopy for cellular imaging

Yoshiyuki Arai[1], Takayuki Yamamoto[1], Takeo Minamikawa[2], Tetsuro Takamatsu[2], Takeharu Nagai[1] ([1]*ISIR, Osaka Univ.*, [2]*Kyoto Pref. Univ. Med.*)

Absorption spectrum has been known as “molecular fingerprint”. However, because of the short optical path length of cells, it is hard to measure absorption spectrum for individual cells. Here, we developed optical cavity enhanced light absorption spectroscopic microscopy for two-dimensional light absorption imaging. This microscopy enabled to detect absorption spectrum with only 10 μ m optical path length samples at subcellular spatial resolution, and image various cell types that showed the cellular diversity among not only different species but also identical cell types without any staining. Thus, our microscopy allows to measure the absorption spectra of biological samples, thereby detecting individuality of cells.

3SDA-04 光シート顕微鏡による生体まるごとイメージング
Live imaging of whole organisms by light-sheet microscopy

Shigenori Nonaka (*NIBB*)

Light-sheet microscopy is a recently developed technology that uses thin sheet-shaped excitation light to illuminate the focal plane of a detection objective. This method is characterized by low bleaching and phototoxicity, deep penetration length, and high-speed image acquisition. These features are extremely suitable for live imaging of whole organisms of submillimeter scale.
Here I show two examples that are first enabled by this microscopy: One is gastrulating mouse embryos, by which we revealed migration pattern of invaginated mesodermal cells. The other is high-speed 4D imaging of moving *Amoeba proteus*, that is enabled by moving the light-sheet and the detection objective instead of the specimen.

3SDA-05 小分子から生細胞までーラマン顕微鏡にできることー
Small Molecules and Live Cells Characterized Using Raman Microscopes

Shin-ichi Morita (*Tohoku Univ.*)

It is now possible to measure the Raman spectrum of a single live cell. Proteins, nucleic acids, lipids, etc. are vibrated with different wavenumbers. These oscillators are interacted with the light, eventually detected as Raman scattering. Using Raman signals, it is capable to analyze the distribution of biomolecules. It is however difficult to distinguish Raman bands of similar molecules. Raman analysis is therefore ambiguous. To clarify this, we are interested in developing Raman probes such as alkyne tags for small molecules. Also, we have been interested in direct measurements of Raman spectra of live cells, because using Raman microscopes differentiation of live cells was distinguished. Recent results were discussed in the talk.

3SDA-06 近赤外吸収プローブとしての Pt(II)ジラジカル錯体の機能開発
Development of a function of Pt(II)-diradical complex as a near-infrared absorbing probe

Atsuko Masuya, Kosaku Tamura, Hitoshi Hoshino, Nobuhiko Iki (*Graduate School of Environmental Studies, Tohoku University*)

Near-infrared (NIR) light of 700-900 nm effectively penetrates into tissues, thus imaging technique based on NIR absorption such as photoacoustic imaging has been received much attention to visualize deep structures. In this technique, an NIR-absorbing dye plays an important role as a probe improving the S/N ratio. However, there are less investigations on NIR-absorbing probes contrary to the case of NIR-fluorescent probes. As canditdates of the NIR-absorbing probes, we have investigated a Pt(II) complex with o-diiminobenzosemiquinonate radical which shows intense NIR absorption. Herein, we present the NIR absorption change induced by recognition of chemical environments such as pH and hydrophobic cavity to be suitable for monitoring micro-environment in vivo.

3SDA-07 誘導ラマンによって何が見えるのか？
What could be visualized with stimulated Raman scattering?

Yasuyuki Ozeki (*UTokyo*)

Recent advance in stimulated Raman scattering (SRS) microscopy has been proving a variety of attractive features of SRS such as the capability of real-time imaging and the accessibility to the spectral information. Indeed, we have recently developed SRS spectral microscopy, which allows for rapid, multi-color imaging of tissue. Nevertheless, it is still unclear and not fully explored: what kind of insights can SRS give us? The talk will introduce imaging examples with SRS, compare SRS with other Raman modality, and discuss the future perspective.

3SEA-01 きわめて長い半減期をもつフォールディング中間体の解析
Analysis of an unusually stable kinetic refolding intermediate

Hayuki Sugimoto (*Fac. Agri. Univ. NIIGATA*)

Refolding of a thermally unfolded disulfide-deficient mutant of the starch-binding domain of glucoamylase was investigated using DSC, ITC, CD, and NMR. When the sample was rapidly cooled from a higher temperature, a kinetic intermediate was formed during refolding. This intermediate was unusually stable with a half-life of 11 h (pH 7.0, 5°C). It was shown that this intermediate contained substantial secondary structure and tertiary packing and had the same ligand binding ability as the native state. These characteristics differ from those of partially folded intermediates. It was suggested that in the intermediate, the aromatic cluster at the surface is structurally less organised, whereas the interior of the protein has relatively rigid, native-like side-chain packing.

3SEA-02 FRET と高速溶液混合法による SNase の凝縮過程の速度論研究

Kinetics of Chain Condensation during SNase Folding studied by FRET and ultrarapid mixing methods

Takuya Mizukami[1], Ming Xu[1], Hong Cheng[1], Heinrich Roder[1,2], Kosuke Maki[3] ([1]*FCCC*, [2]*UPenn*, [3]*Nagoya University*)

Continuous-flow FRET is a powerful technique to obtain site-specific information on tertiary structure formation during early stages of protein folding. We studied the chain condensation within the beta-barrel domain and between beta-barrel and alpha-helical domains during SNase folding. Variants with a single FRET pair in the beta-barrel domain showed an increase in the FRET efficiency on the sub-millisecond time scale. In contrast, the variant with a donor in the alpha-helical domain and an acceptor in the beta-barrel domain showed an increase in the FRET efficiency only after 100 ms of folding. These results indicate that the beta-barrel domain in SNase adopts a compact structure early in folding while the alpha-helical domain forms only during the final stages.

3SEA-03 様々な蛋白質間で観察される配列順序非保存な構造類似性

Non-sequential structural similarity in the protein world

Shintaro Minami[1], Motonori Ota[1], George Chikenji[2] ([1]*Grad. Sch. of Inf. Sci., Nagoya Univ.*, [2]*Grad. Sch. of Eng., Nagoya Univ.*)

In some proteins, secondary structure elements are arranged spatially in the same manner, but they are connected in the alternative ways. Analysis on such non-sequential structural similarity in proteins is important because it provides a deeper understanding of the structural geometry of protein. This can be also observed even in the homologous proteins, indicating the non-sequential structural similarity is significant in the protein evolution. However, the non-sequential structural similarity in proteins is less investigated. We performed comprehensive non-sequential structural comparison among homologous and non-homologous proteins by using MICAN program, which we have developed. Based on the result, protein structural geometry and evolution will be discussed.

3SEA-04 機能と構造安定性のトレードオフに対するアプローチ：アルブミン結合タンパク質を模倣したヒト型タンパク質のデザインを例として

The approach to the function-stability tradeoff: A case of the design of a humanized protein mimicking the albumin-binding protein

Satoshi Oshiro[1], Shinya Honda[1,2] ([1]*Dept. of Medical Genome Sci., Grad. Sch. of Frontier Sci., The Univ. of Tokyo*, [2]*BioMed. Research Inst., AIST*)

Protein design is an effective method to gain the artificial proteins that have superior functions, and various artificial proteins have been designed. However, the gain of function with the sacrifice of structural stability, so-called “function-stability tradeoff”, still complicates the success of protein design. In this lecture, we will talk about the approach to the problem of function-stability tradeoff in the protein design, based on the design of a humanized artificial protein mimicking the bacterial albumin-binding protein.

3SEA-05 転写因子 Sp1 と TAF4 の天然変性領域を介した相互作用

The interaction between transcription factors Sp1 and TAF4 via the intrinsically disordered regions

Emi Hibino[1], Rintaro Inoue[2], Masaaki Sugiyama[2], Jun Kuwahara[3], Katsumi Matsuzaki[1], Masaru Hoshino[1] ([1]*Grad. Pharm., Univ. Kyoto*, [2]*KURRI*, [3]*Fac. of Pharm., Doshisha WUniv.*)

Many transcription factors contain Glutamine-rich domains (Q-domains) which are considered to be important for molecular recognition. The transcription factors Sp1 and TAF4 are proposed to interact via their Q-domains. However, structural characterization of these Q-domains has not been performed, and the molecular mechanism of the interaction is still unclear. Here, we measured NMR, CD, SPR, SAXS and SANS of Q-domain fragments of Sp1 and TAF4. The results indicated that all these Q-domains were kind of IDPs and that Sp1 formed homo oligomers weakly by themselves and hetero oligomers with TAF4 site-specifically. Interestingly, no significant conformational change was observed upon oligomer formations. This may be a novel binding mode of IDPs.

3SEA-06 混雑環境下での天然変性タンパク質の振る舞い: α-synuclein のアミロイド形成に関する熱力学シミュレーション

Intrinsic disorder under crowded environment: thermodynamic simulation of α-synuclein amyloid fibril formation

Nobuhiro C. Shirai (*Grad. Sch. Sci., Kyoto Univ.*)

Physicochemical properties of intrinsically disordered proteins (IDPs) differ from those of folded proteins because of their structural flexibility; IDPs have a larger radius of gyration; and binding of IDPs to their biological targets significantly reduces the conformational entropy of IDPs. α-synuclein (α-syn), which is known as an IDP, forms amyloid fibrils, and this fibrillation process is accelerated by molecular crowding.
In order to investigate the effect of crowding on the amyloid formation of IDPs, we developed a lattice gas model of α-syn and analyzed an equilibrium state of a system including with crowding agents.
As a result of the analysis, we found that fibrillation of IDPs is more sensitive to macromolecular crowding than fibrillation of folded proteins.

1P001 耐熱性ストマチン特異的切断プロテアーゼの構造と機能解析
Structural and functional analysis of a thermostable stomatin-specific protease

Hideshi Yokoyama[1], Daisuke Kobayashi[1], Naoto Takizawa[1], Satoshi Fujii[1], Ikuo Matsui[2] ([1]*Sch. of Pharm. Sci., Univ. of Shizuoka*, [2]*Biomedical Res. Inst., AIST*)

The N-terminal domain of PH1510p (1510-N) from the hyperthermophilic archaeon *Pyrococcus horikoshii* is a thermostable serine protease, and specifically cleaves the stomatin PH1511p. Stomatin is thought to act as an oligomeric scaffolding protein in lipid rafts. 1510-N stomatin-peptide complex was crystallized after heat-treatment, and the structure was determined to understand the function of 1510-N. The comparison between the structures of the heat-treatment and no heat-treatment previously determined indicates that the N-terminal half of the peptide binds to 1510-N more tightly than the C-terminal half of the peptide. After the protease reaction of 1510-N mixed with stomatin substrates, two degraded products were produced via acyl-enzyme intermediates.

1P002 黄色ブドウ球菌由来 α-ヘモリジン単量体の結晶構造
Crystal structure of staphylococcal α-hemolysin monomer

Takaki Sugawara[1], Daichi Yamashita[1], Yoshikazu Tanaka[1,2], Jun Kaneko[3], Yoshiyuki Kamio[3], Isao Tanaka[1,2], Min Yao[1,2] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*, [3]*Grad. Sch. Agri., Tohoku Univ.*)

Staphylococcal α-hemolysin (αHL) is a β-barrel pore-forming toxin expressed by *Staphylococcus aureus*. αHL is secreted as a water-soluble monomeric protein, which forms membrane-inserted heptameric pores on the target cell. Although the crystal structures of pore and monomer bound with antibody have been determined, that of monomeric αHL without binder has not yet been elucidated. To obtain crystals of αHL monomer, we focused on H35A mutant, which showed marked decrease in oligomerization activity in the previous mutation studies. As expected, the revealed structure was monomeric αHL, in which the stem region was held by cap-domain, and N-terminal amino latch located the side of cap-domain. Based on the revealed structure, we discuss the pore formation mechanism of αHL.

1P003 *Mycoplasma pneumoniae* 滑走メカニズムに関わる P65 の構造解析
Structural analysis of P65 involved in *Mycoplasma pneumoniae* gliding mechanism

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M pneumoniae, a human pathogen, glides to one direction with a unique mechanism. The gliding machinery localizing at a cell pole contains an internal structure composed of the terminal button, the paired plates, and the bowl complex from the front end. In the present study, we focus on P65 (MPN309 protein), an essential component for gliding localizing at the terminal button. We expressed a recombinant P65 in *Escherichia coli* and purified it. The rotary-shadowing electron microscopy showed that the P65 forms a multimer composed of a central globule 30 nm in diameter and many filaments about 10 nm sticking out. We obtained rod-shaped crystals of a N-terminal 1-209 fragment with diffraction up to 2.5 Å resolution. A structural analysis is underway.

1P004 Concentration Dependence of Amyloid Peptide Assembly by Using the Molecular Dynamics Simulations

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Alzheimer's disease, which is our research target, is a kind of the folding diseases. It is well-known that Alzheimer's disease is caused by the misfolding of the amyloid-beta peptides. According to some hypothesis, the cause of Alzheimer's disease is the misfolding into the beta-sheet structures of amyloid-beta peptides, and to form the insoluble fibrous proteins is called "amyloid fibrils".
Recently, an interesting report about the mechanisms of amyloid fibril formations has published by the experimental group. They suggested that the concentration is important for the self-assembly of amyloid fibrils. In order to examine the concentration dependence of the amyloid peptide aggregation theoretically, we have performed the molecular dynamics simulations.

1P005 Lectin-like transcript 1 (LLT1)の構造解析と LLT1-CD161 複合体のモデル作製
Structure analysis of lectin-like transcript 1 (LLT1) and model building of LLT1-CD161 complex

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NK cell activity is regulated by signals through numerous cell surface receptors. CD161 (also known as NKRP1A) is one of these receptors, which binds to the ligand molecule, lectin-like transcript 1 (LLT1). Both LLT1 and CD161 belong to C-type lectin family and extracellular regions have responsible for ligand binding. Here we determine the crystal structure of extracellular region of LLT1.
LLT1 was refolded by dilution method. Crystals of LLT1 were obtained by vapor diffusion method and X-ray diffraction data were collected at NW12A PF in Tsukuba. The structure of LLT1 was solved by molecular replacement method.
LLT1 adopts C-type lectin-like folds and forms dimer related by two-fold axis. Using LLT1 dimer structure, LLT1-CD161 binding model was constructed.

1P006 Photon Factory の BioSAXS ビームラインの現状
Current status of BioSAXS beamlines at Photon Factory

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The biological small-angle X-ray solution scattering (BioSAXS) is useful technique to analyze solution structure of biological molecules in low resolution. The hybrid method approach will help to discuss about the structure and the function of the molecules by combining a high-resolution structure analyzed by the crystallography and the NMR with a molecular shape obtained by the ab-initio analysis of the BioSAXS. We, Photon Factory promote a national platform project, PDIS (http://pford.jp/) for the structural life science, and not only provide the BioSAXS beamtime but support the beginner user as collaborative work in PDIS. We will present the current status of BioSAXS beamlines and PDIS in the BioSAXS field of PF.

1P007 エネルギー表示溶液理論を用いた蛋白質複合体構造予測
Protein-protein complex structure prediction using the solution theory in the energy representation

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As a protein-protein complex structure prediction method, we have developed a procedure to combine recently developed two approaches. The first one is a clustering and reranking method named CyClus which performs a fast clustering using a cylindrical approximation of interface and improves results of rigid-body docking. The second one is free energy evaluation through molecular dynamics simulation using the solution theory in the energy representation. Using this method we can calculate binding free energy differences of generated complex models. The clustering using CyClus efficiently reduces the number of candidates to be evaluated and the free energy analysis serves to accurate evaluation.

1P008 B 細胞共受容体 CD72 の C 型レクチン様ドメインの X 線結晶構造解析
Crystal structure of the C-type lectin-like domain of CD72

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CD72 is a 45 kDa type II membrane protein expressed mainly in B lymphocytes and plays a regulatory role in B cell activation. CD72 contains an immunoreceptor tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic region, and negatively regulates B-cell receptor (BCR) signaling. CD72 contains a C-type lectin-like domain （CTLD） in the extracellular region, but its lectin activity is not clear. The CD72 CTLD was expressed in Escherichia coli, refolded and purified. NMR measurements indicated the correct folding of the refolded protein and possibilities of interaction with ligands. The crystals of the CD72 CTLD were obtained by the hanging-drop method. We have solved the crystal structure of the CD72 CTLD in ligand free form. Structural details will be discussed.

1P009 ADP 結合型 腸球菌 A_3B_3 複合体の X 線結晶構造解析
Crystal Structure of ADP-Bound A_3B_3 Complex of *Enterococcus hirae* V-ATPase

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V-ATPase functions as ATP-dependent proton pump. The A_3B_3, which is the catalytic domain of V-ATPase, forms a hexagonal ring by the three catalytic A subunits and the three non-catalytic B subunits. We previously reported the asymmetric structures of the nucleotide-free (2.8 Å) and AMP-PNP-bound (3.4 Å) A_3B_3 complex from *Enterococcus hirae*. Two AMP-PNP molecules were found at the nucleotide-binding pockets in two AB pairs. Here we report the crystal structure of ADP-bound A_3B_3 complex at 2.7 Å resolution. Three ADP molecules are found at the nucleotide-binding pockets in all AB pairs. In my poster, I would like to discuss the intermediate structure of A_3B_3 complex.

1P010 TtFbpA による新規鉄結合様式の解明
A novel six-coordinated ferric ion binding mode of TtFbpA

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TtFbpA is a ferric ion-binding protein from Thermus thermophilus HB8. Here we report the crystal structures of the apo and ferric ion-bound form of TtFbpA at 1.8-Å and 1.7-Å resolutions, respectively. The latter crystal structure shows the ferric ion forms a novel six-coordinated complex with three tyrosine residues, two bicarbonates and a water molecule. A five-coordinated ferric ion-binding mode formed by three tyrosine residues and a carbonate bound in bidentate manner was reported earlier. The difference would probably result from the different pHs used for crystallization: pH 5.5 (six-coordinated) vs. pH 7.5 (five-coordinated). We propose TtFbpA can act as a ferric ion-binding protein over the wide pH range by taking at least two different coordination manners.

1P011 X 線 1 分子追跡法による α シヌクレイン構造揺らぎ 1 分子観察
X-ray Single Molecule Observations of Alpha-synuclein's Structural Fluctuations by using Diffracted X-ray Tracking (DXT)

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The structure of alpha-synuclein (α-syn) in solution resembles that of a random coil. But, alpha-synuclein is of great interest to Parkinson's researchers because it is a major constituent of Lewy bodies. Here, we try to observe the structural fluctuations of alpha-synuclein and its mutants (WT, A53T, and E46K) by using Diffracted X-ray Tracking (DXT) as x-ray single molecule observations. In DXT, we observed Brownian motions of his-tagged alpha-synucleins, which are adsorbed on the substrate's surface. DXT experiments used the energy of quasi-white x-rays (energy peak-width of 2% using undulator radiations, 10-20 KeV, BL40XU, SPring-8). From DXT data, we discovered that these alpha-synuclein's dynamic states (structural fluctuation=0.1-0.2 nm) had a clear difference.

1P012 分子ドッキングによるタンパク質–基質複合体の乖離構造と結合エネルギー評価
Molecular docking study of structure and binding energy of ligand-protein complex in dissociation process

Hiroaki Saito, Kazutomo Kawaguchi, Hidemi Nagao (*Kanazawa University*)

Molecular docking explores the binding modes of two interacting molecules. The technique is increasingly popular for studying protein-ligand interactions and for drug design. A fundamental problem with molecular docking is that orientation space is very large and grows combinatorially with the number of degrees of freedom of the interacting molecules. Here, we describe and evaluate algorithms that improve the efficiency and accuracy of a shape-based docking method. We adopt the molecular organization and sampling techniques to remove the exponential time dependence on molecular size in docking calculations. The possible structures and binding energies in the dissociation process are discussed by using the developed docking program.

1P013 Some cooperative aspects of protein aggregation phenomena

Takashi Konno (*Med., Univ. Fukui*)

We previously repoted that amyloid formation of Ab(25-35) peptide is strongly modified by soluble proteins with electrostatic interactions. An extension of this study was done for providing further insights into interactions between amyloidgenic and other soluble proteins, partly motivated by the question whether the interaction is observed for more pathologically important cases. The results gave some general aspects of the interaction by demonstrating several different modes of the interactions. It was, for example, indicated that distinction between specific and non-specific modes of the interactions are rather ambiguous, and some more important suggestions were also obtained.

1P014 タイワンカブトムシ由来セリンプロテアーゼ阻害タンパク質オリクチンの変異体とトリプシンの複合体の構造解析
Crystal structure of the M14R mutant of oryctin, a Kazal-type serine protease inhibitor, in complex with trypsin

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Oryctin is a 66-amino-acid protein purified from the larval haemolymph of the coconut rhinoceros beetle Oryctes rhinoceros, which shows no sequence similarity to any other proteins known but has a similar backbone fold to the turkey ovomucoid domain 3, OMTKY3, a Kazal-type serine protease inhibitor. Oryctin inhibits some serine proteases such as α-chymotrypsin, leukocyte elastase, endopeptidase K and subtilisin Carlsberg, but cannot inhibit trypsin at all. In this study, we found that M14R mutant of oryctin can inhibit trypsin and solved the crystal structure M14R-oryctin-trypsin complex at 2.0-Å resolution. We are going to present how this oryctin mutant binds and inhibits trypsin.

1P015 [NiFe]ヒドロゲナーゼ成熟化段階において Ni 挿入を担う HypAB 複合体
Studies on intermediate HypAB complexes for Ni insertion during [NiFe] hydrogenase maturation

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[NiFe] hydrogenases, which catalyze reversible H_2 production, contain a $NiFe(CN)_2CO$ cluster in the active site. This cluster is not formed spontaneously but synthesized with the aid of six maturation proteins, HypABCDEF. HypCDEF proteins biosynthesize the $Fe(CN)_2CO$ moiety and insert it into immature hydrogenases, while HypA and B carry out the Ni insertion. However, the mechanism of this maturation process has been only partially revealed.
We have focused on HypB, which has ATPase activity. SEC analyses have shown that only the ATP-bound form of HypB interacts with HypA, forming a 2:2 complex. To determine the HypAB structure and elucidate the Ni transfer mechanism, we now perform crystallography of the HypAB complex.

1P016 T4 型ファージと T2 型ファージの尾繊維先端受容体結合蛋白質の構造と機能
Structure and function of receptor binding proteins of T4-type phages and T2-type phages

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T4 and T2 phages recognize the receptor of their host E. coli cell with the distal end of long tail fibers (LTFs). It is known that T4 and T2 are very similar each other but only several features are obviously different. One of the differences is the receptor binding protein (RBP) of LTFs. In T4 phage, gp37 is utilized to recognize the receptor of the host E. coli. In phage T2, gp38 is attached at the C-terminus of gp37 and acts as adhesin. We compared the T4 gp37-type LTFs and T2 gp37-gp38-type LTFs to the sequences in the database. We found T4-type LTFs have a unique HXH iron binding motif at the tip of the gp37. On the other hand, gp37 of T2-type LTFs undergoes C-terminal processing. The removed C-terminus might be an intramolecular chaperone for the folding of gp37.

1P017 べん毛 III 型輸送シャペロン FlgN の構造と FlhA との相互作用
Structure of FlgN, a flagellar type III export chaperone, and its interaction with FlhA, a flagellar type III export gate protein

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Flagellar export chaperones are multifunctional proteins. They bind their cognates to prevent premature aggregation in cytoplasm, promote their export, and control the flagellar gene expression to fine-tune the flagellar biogenesis. FlgN is the export chaperone for the junction proteins, FlgK and FlgL. Recently, the interaction of FlgN with FlhA, a flagellar type III export gate protein, is found to be required for efficient export of the junction proteins. To understand the molecular mechanism of the flagellar protein export assisted by the export chaperone, we solved the structure of FlgN at 2.3 Å and analyzed the interaction of FlgN with FlhA. We will show the conformational change of FlgN is essential for the interaction with its cognates and the gate protein.

1P018 自然免疫非感受性のサルモネラ菌 FljB が形成するべん毛繊維の立体構造と FliC べん毛繊維との違い
CryoEM structure of the flagellar filament of Salmonella FljB and implication of its difference from the FliC filament

Shoko Toma, Takayuki Kato, Keiichi Namba (*Osaka University*)

The flagellar filament of Salmonella Typhimurium is constructed from a single protein, flagellin. It has been postulated that atomic structures of the flagellar filaments are very similar among all homologous bacteria. Through three-dimensional cryo-EM reconstruction of flagellar filaments composed of FljB flagellin, we have recently found flagellar filaments composed of FljB flagellin have a different D3 domain orientation. Implications of this finding will be discussed.

1P019 **Expression and Structural Analysis of Two Kinds of Perireceptor Proteins (PRPs)**

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Around olfactory dendrites there are soluble proteins in the high concentration, suggesting an important role in the odour or pheromone perception. In insects two classes of soluble proteins have been so far identified, odorant-binding proteins (OBPs) and chemosensory proteins (CSPs). However, we found only OBPs from common Japanese newt. We call them perireceptor protein (PRP) and study to elucidate the role in chemosensation. We have cloned, expressed and analyzed two kinds of OBPs (Cp-Lip1, -Lip2) of *Cynops pyrrhogaster*, and CSP7 found in the aunt chemosensoryorgan. In the present study, the expression and purification of Cp-Lip1 and CSP7 and the fluorescent assay and CD measurements are reported.

1P020 **SepCysE の機能・構造解析による Cys-tRNA^{Cys} 生合成機構の解明**

The study on the structure and function of SepCysE related to Cys-tRNA^{Cys} synthesis

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In the synthesis process of aa-tRNA, amino acid is normally attached to tRNA directly by aminoacyl-tRNA synthetase (aaRS). However, methanogenic archaea require two enzymes to form Cys-tRNA^{Cys} synthesis in indirectly pathway, which tRNA^{Cys} is acylated with phosphoserine (Sep) to form Sep-tRNA^{Cys} by phosphoseryl-tRNA synthetase (SepRS), and then Sep is converted to Cys by Sep-tRNA:Cys-tRNA synthetase (SepCysS).
Here we revealed a crystal structure of SepCysS in complex with SepCysE that is recently discovered as an essential factor for Cys-tRNA^{Cys} synthesis. Moreover, we carried out binding assay with SepRS, SepCysS and SepCysE. The results indicated that these three proteins form a stable complex as a functional unit.

1P021 **タンパク質レアイベントを抽出する効率的構造サンプリング法**

Enhanced conformational sampling methods for extracting biological rare events of proteins

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Biological rare events are related to functions of proteins. In the most of biological processes, the rare events are observed as structural transitions related to the biological functions. In this study, we propose two enhanced conformational sampling methods by repeating (i) Selections of seeds and (ii) Conformational re-sampling for the seeds. One is Fluctuation Flooding Method (FFM), where largely fluctuating structures projected onto low frequency modes are selected as the seeds. Another is Outlier FLOODing Method (OFLOOD), where sparse distributions of states of proteins (outliers) in conformational spaces are selected as the seeds. We show these simple protocols might drastically promote structural transitions like a conformational flooding from the seeds.

1P022 **遷移温度付近のシニョリンの緩和モード解析**

Relaxation Mode Analysis of Chignolin at Transition Temperature

Ayori Mitsutake, Hiroshi Takano (*Dep. Phys, Keio Univ.*)

Relaxation mode analysis (RMA) developed to investigate "dynamic" properties of polymer, homo-polymer, systems. In the method, slow relaxation modes are extracted from a molecular dynamics simulation. In RMA, the time correlation matrices of structural fluctuations for two different times are calculated. Then, by solving a generalized eigenvalue problem for these matrices, the relaxation rates and modes are estimated from the eigenvalues and eigenvectors, respectively. RMA has been applied to protein systems to investigate dynamic properties of structural fluctuations. Here, we present the results of RMA for a simulation of chignolin at a transition temperature in which many transitions between the global energy minimum state and metastable states are observed.

1P023 **真空紫外円二色性分光による α_1 酸性糖蛋白質と生体膜の相互作用機構の解明**

Interaction Mechanism of α_1-Acid Glycoprotein with Biomembrane Characterized by Vacuum-Ultraviolet Circular Dichroism Spectroscopy

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α_1-Acid glycoprotein (AGP) interacts with biomembrane to change the conformation ($\alpha \rightarrow \beta$ transition) and decrease the drug (hormone)-binding capacity. To clarify the interaction mechanism, the vacuum-ultraviolet circular dichroism spectra of AGP were measured in the presence or absence of phosphatidylcholine (PC), phosphatidic acid (PA), and phosphatidylglycerol (PG) liposomes. It was found that the helical regions of AGP increased upon interacting with the PA and PG liposomes, the latter more strongly inducing the helices. However, there was no secondary-structure change of AGP in the PC liposome. These results suggest that the polar head groups of phospholipids play an important role and their negative net charge is essential for the AGP biomembrane interaction.

1P024 **レチノール結合タンパク質とカルパインの立体構造上に形成されたイントロンの平面**

Planes formed with 4 introns in tertiary structures of RBP & calpain D-VI

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We report a novel relationship between introns and the tertiary structures of retinol binding protein and calpain domain VI. We identified “intron-positions” on the residues on which or just after which introns are found in their corresponding nucleotide sequences, and then found that 4 intron-positions form a plane. We also found some relationships between the planes and the ligands. To evaluate the statistical significance of the planarity, we calculated the mean distance of each intron-position from the plane defined by the other three intron-positions, and simulated to confirm that their probabilities are significantly smaller than those calculated from randomly generated locations.

1P025 アカネ科由来抗腫瘍活性ペプチド RA-VII の構造解析
Structure analysis of antitumor peptide RA-VII from Rubia Cordifolia

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RA-VII is antitumor cyclic hexapeptide, which is isolated from Rubia cordifolia and Rubia akane. This peptide is comprised of alanines and modified tyrosines. The peptide has an 18-membered cyclohexapeptide ring and a 14-membered cycloisodityrosine ring in the structure. It is suggested that 14-membered ring and Tyr-3 of 18-membered ring are indispensable for the antitumor activity, and the active conformation of 14-membered ring is maintained by the presence of the 18-membered ring. We study the conformers of RA-VII and its analogs by quantum chemical calculation to explore the relationship between the structure of RA-VII and the antitumor activity.

1P026 分子シミュレーションによるラクトースリプレッサーの転写制御機構のモデル提案
MD and *ab initio* MO simulations on transcriptional mechanism controlled by lactose repressor protein and ligand

Yuki Matsushita, Kanako Shimamura, Masato Oishi, Tatsuya Ohyama, Noriyuki Kurita (*Toyohashi University of Technology*)

Lactose repressor protein (LacR) controls the transcriptional mechanism of gene information from DNA to mRNA in a ligand-dependent manner. Although the ligand-binding to LacR was found to change the mechanism drastically, the effect of ligand-binding on the conformation of LacR+DNA complex has not been clarified at atomic and electronic levels. We here investigated the change in conformation of LacR-dimer+DNA complex induced by the ligand-binding, using molecular dynamics simulations and ab initio fragment molecular orbital calculation. The results elucidate that the binding of an inducer to LacR significantly changes the LacR structure to cause strong interactions between LacR monomers, resulting in weakening the interactions between LacR-dimer and DNA.

1P027 抗体の親和性成熟に着目した抗原 - 抗体結合挙動への洞察
Insight into the antigen binding motion of germline and affinity-matured antibodies

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The main purpose of this study is to elucidate the change of structural dynamics of antibodies during affinity maturation and their antigen binding mechanisms. We expressed single-chain Fvs (scFvs) of anti-(4-hydroxy-3-nitrophenyl)acetyl antibodies in *E. coli*, and purified them. Analysis of antigen binding kinetics using Biacore showed that affinity-matured C6 scFv formed more stable complex than germline N1G9 scFv and mutants, mainly due to the dissociation rates. Analysis of structural dynamics at the single-molecule level using diffracted X-ray tracking showed that the fluctuations of scFvs were decreased upon the antigen binding. In comparison with N1G9, C6 with antigen had less fluctuated motion, which might be correlated with the more stable complex.

1P028 L-Thr と NAD^+の結合に伴う Cupriavidus necator 由来 L-スレオニン脱水素酵素の構造変化の解析
Analysis of structural change of L-threonine dehydrogenase from Cupriavidus necator (CnThrDH) by binding of L-Thr and NAD^+

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We determined and analyzed the structural changes of CnThrDH: apo (2.25Å), NAD^+-binding (2.5Å) and L-Thr and NAD^+ binding (2.5Å) forms. Differences of these three structures indicated that switching of “open” and “closed” states occurs at the regions of 80-87 and 180-186 during the dehydrogenation reaction. MD simulation and kinetic analysis also supported the switching of the states: the closed state changed to open state during the simulation, and the kcat/Km values of variants which are difficult to form the closed state are much lower than that of wild type. We concluded that the switching is essential to exhibit the high reactivity of CnThrDH [1].

[1] Nakano et al., J. Biol. Chem. 2014, 289: 10445-10454

1P029 アナアオサ由来のプラストシアニンにおける弱い相互作用の役割
The role of weak interaction in a blue copper protein, plastocyanin from Ulva pertusa

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Plastocyanin from Ulva pertusa (UPc) is a blue copper protein, which performs as an electron carrier between cytochrome b6f complex and P700. The active site copper atom of UPc is coordinated by His37, His87, Cys84, and Met92 with a distorted tetrahedral structure.

Several weak interaction in the protein has been considered to express the unique structure and properties of UPc. The X-ray crystallographic structure analyses of UPc were reinvestigated to know the more details of the correlation between the weak interaction and properties. The EXAFS studies of UPc was also performed to obtain the precise active site structural information. The crystallographic structural data and the bond distances evaluated from EXAFS of UPc demonstrated different structural parameters.

1P030 ブルー銅タンパク質シュウドアズリン Met16His/His6Val 変異体の性質と構造の pH 依存性
pH dependency of the structure and properties of a blue copper protein, Met16His/His6Val pseudoazurin mutant

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The active site of pseudoazurin (PAz) is coordinated by two nitrogen atoms (His40, His81), one thioether S (Met86), and thiol S (Cys78) with distorted tetrahedral structure. Met16 is located at the vicinity of His81. The weak interaction between Met16 and His81 has been explained to be important for regulation of electronic structure of PAz. Met16His/His6Val double mutant was constructed to know the effect of His closed to active site.

The electronic absorption spectra of the Met16His/His6Val mutant protein in the acidic pH range demonstrated the increasing of the axial component, and a pKa of newly introduced His16 was estimated to be 4.62, which is relatively lower pKa value of the normal His imidazole group by the positive charge effect of the active site copper ion.

1P031 リソスタシンの炭酸カルシウム結晶結合部位の解析
Functional analysis of calcite-binding site of lithostathine

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Lithostathine (LIT) binds to calcite ($CaCO_3$), which is a main component of pancreatic stone, to modify its morphology. Although LIT can be utilized to control the crystal growth of calcite, its molecular mechanism has been unclear. In the present study, we prepared LIT mutants introduced to acidic residues clustered on its molecular surface and evaluate their binding activities to calcite. Ala mutant exhibited less binding efficiency to calcite and less modification of the calcite morphology compared with wild type LIT. In contrast, Asn/Gln mutant retained binding efficiency and fully modified the calcite morphology. These results suggest that the electric charge and the side chain structure of the mutated region are responsible for the calcite binding.

1P032 Production and NMR study of plant defensin-like peptides using plant cell expression system

Yoshitaka Umetsu[1], Masashi Mori[2], Shinya Ohki[1] ([1]*JAIST*, [2]*Ishikawa Pref. Univ.*)

We studied structure-activity relationship of plant defensin-like peptides, stomagen and embryo surrounding factor 1.3 (ESF 1.3). Stomagen increases the stomatal density of plants, and ESF1.3 regulates early embryo patterning in flowering plants. For NMR analysis, we frequently choose *E. coli* expression system for producing isotope-labeled proteins. However, these peptides were not expressed as functional form. Therefore, we established a new system using suspension-cultured plant BY-2 cells with an inducible virus vector. In this method, the peptides are expressed and folded correctly with proper disulfide bonds. From our elucidated structures, we revealed that the loop region of stomagen and two Trp residues of ESF 1.3 play an important role for their activity.

1P033 FRET プローブアクチンを用いた細胞内におけるアクチンの構造多型の検出
Polymorphism of actin in cells, detected by FRET-probed actin

Mio Okazaki[1], Saku Kijima[2,4], Yoshiaki Iwadate[3], Taro Q.P Uyeda[2,4], Taro Q.P Noguchi[1] ([1]*MNCT*, [2]*ATST*, [3]*Yamaguchi University*, [4]*University of Tsukuba*)

Actin subunits in filaments take multiple structural states in vitro, and this polymorphism affects affinities for actin-binding proteins. To examine if actin in cells also take multiple structures, we used actin with two fluorophores for intramolecular FRET. The donor and acceptor dyes were attached at the tips of large and small domains, and we previously reported that this FRET-probed actin detects myosin-induced structural changes in vitro. Now, the FRET-probed actin was introduced into HeLa and Dictyostelium cells by electroporation. Microscopic FRET measurements showed that FRET index differs locally, demonstrating polymorphism of actin, in cells. We are analyzing the relationship between the polymorphism and function of actin structures.

1P034 HP 1 クロモドメイン/histone H3 tail 複合体の全原子構造サンプリング
Conformational sampling of HP1 chromodomain/histone H3 tail complex

Nobuto Hashiguchi, Satoshi Omori, Kei Moritsugu, Yoshifumi Nishimura, Akinori Kidera (*Yokohama City Univ.*)

Heterochromatin formation is regulated by the binding of heterochromatin protein 1α chromodomain (HP1αCD) to histone H3 lysine 9 methylation (H3K9me). It is known that phosphorylation at four consecutive serine residues in the N-terminal disordered loop of HP1αCD enhances the associated binding affinity. Conformational samplings of the HP1αCD/ histone H3 tail complex with and without serine phosphorylation help us to understand the molecular mechanism interaction in relation to serine phosphorylation. Here, we used replica-exchange molecular dynamics simulation for this purpose. The structural model of the complex was constructed by Omori et al. The structural ensembles derived demonstrated the potential to regulate the association process via phosphorylation.

1P035 Sox2 の DNA 結合ドメインの構造揺らぎと DNA 結合に付随するフォールディング
Conformational Flexibility of the high-mobility group box domain of sox2 and its folding coupled with DNA binding

Erisa Harada, Tsuyoshi Konuma, Syoko Mori, Kenji Sugase (*Suntory Foundation for Life Sciences*)

Transcriptional factor protein sox2 possess a high-mobility group box (HMG) domain, which specifically binds to DNA to exert its function. However, DNA recognition mechanism of sox2 is not fully understood. To elucidate how sox2 recognizes the target sequence, we investigated dynamics of the HMG domain upon DNA binding using NMR. Although the HMG domain is known to form three helices in the DNA complex, we found that those helices were almost unfolded in the free state. Interestingly, structural change in the helical region was also observed when the HMG domain nonspecifically binds to random DNA sequences. These data suggest that the HMG domain adjusts its structure on DNA binding and finally forms the stable complex with the target sequence.

1P036 巨大ヘモグロビン結晶内での oxy 型から deoxy 型への移行
Transition from oxy to deoxy state in crystalline giant hemoglobin

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Mechanisms of allosteric oxygen-binding of hemoglobin (Hb) have been widely discussed but the structure of intermediate states between the oxy and deoxy forms without any artificial modification of the Hb molecule is still unclear. Our previous studies of the extracellular giant Hb (V2Hb; 400 kDa) of a tubeworm, *Lamellibrachia satsuma*, demonstrated that the oxy crystals can transform to the deoxy state in keeping a crystalline structure by the soaking methods. We tested various soaking times and the obtained 'intermediate' structures reveal that the electron densities at some oxygen-binding sites were either very weak or disappeared. The quaternary rearrangement of V2Hb might arise just before a complete dissociation of all the oxygen molecules from all the subunits.

1P037 効率的な自由エネルギー計算と構造サンプリングに向けた Integrated Hamiltonian Sampling 法の開発
Integrated Hamiltonian Sampling: towards efficient free energy calculation and conformational sampling

Toshifumi Mori[1], Qiang Cui[2] ([1]*Institute for Molecular Science*, [2]*Univ. of Wisconsin, Madison*)

Adequate sampling in conformational space is essential for understanding protein functions and evaluating free energy differences. To this end, novel methods (e.g. Hamiltonian repilca exchange method and its variants) have been developed and applied successfully (especially to small protein folding simulations). Here we show an alternative approach, Integrated Hamiltonian Sampling (IHS) method [1], which builds a reference Hamiltonian on the fly, and can enhance the sampling in conformational space and estimate free energy differences from a single simulation. The method is applied to estimate pKa shifts of amino acids in solution and conformational sampling of poly-peptides in solution and in solid/liquid interface.

[1] T. Mori et al., J. Phys. Chem. B (in press)

1P038 K63 および linear ユビキチン鎖の構造サンプリング
Full-scale conformational sampling of K63 and linear polyubiquitins

Masanori Shimizu, Hafumi Nishi, Kei Moritsugu, Akinori Kidera (*Grad. Sch. of Med. Life Sci., Yokohama City University*)

Ubiquitin is a small regulatory protein which participates in disparate biological processes. The variations are considered to be attained by different linkages of polyubiquitin chains, namely how ubiquitins are conjugated through seven lysines and the first methionine (linear). Here, as the first attempt, both K63 and linear di-ubiquitin chains, which have similar static structures, were studied to understand how binding target molecules of polyubiquitins are specifically selected by use of different linkages. We performed conformational samplings of the two di-ubiquitin chains by replica-exchange molecular dynamics simulation. The conformational spaces and energy landscapes were analyzed in terms of dynamic binding processes to target molecules.

1P039 Microscopic observation of amyloid deposits associated with lipids of amyloid β-peptide

Kenji Sasahara (*Grad. Med. Kobe Univ.*)

Deposition of amyloid β-peptide (Aβ) in the brain is one of the pathological features of Alzheimer disease. Recent reports have shown that the association of Aβ with membranes plays an pivotal role in cell dysfunction and death. However, the detailed processes of amyloid deposition of Aβ under membrane environment are not fully understood. In this study, vesicle fusion method was introduced in the process of Aβ aggregation to microscopically observe the amyloid deposits formed under membrane environment. The experimental system allowed us to image Aβ deposits associated with vesicle components such as ganglioside GM1. The results suggest that the application of vesicle fusion method is a useful approach for characterizing the membrane-mediated deposition of Aβ.

1P040 微小管切断蛋白質 katanin の構造と機能に関する研究
Structure and function of katanin, a microtubule severing protein

Naoko Iwaya[1,2], Syouta Noda[1], Natsuko Goda[1], Takeshi Tenno[1], Hidekazu Hiroaki[1] ([1]*Grad. Sch. Pharm. Sci., Univ. Nagoya*, [2]*JSPS Research Fellow*)

Katanin is a microtubule severing protein, that consists of the p60 catalytic subunit and the p80 regulatory subunit. We determined the structure of the N-terminal domain of katanin p60, that showed a striking similarity against the N-terminal MIT domain of Vps4, an ESCRT-III disassembling enzyme. Both the two enzymes harbor an AAA domain. We further found each Ca^{2+}-binding site in the N-terminal domains of katanin p60 and Vps4 by NMR. Despite their structural similarity, the effects of Ca^{2+} on function of katanin p60 and Vps4 are different and unique. Finally, we will report the artificial peptides that bind N-terminal domain of katanin p60. The peptides were discovered by phage display experiment and evaluated their interaction to katanin p60 by NMR.

1P041 リボソームストークによる翻訳伸長因子の認識の仕組み
The study on aEF-2 recognition mechanism of ribosomal stalk

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The energy for translation process on ribosome is provided by GTP hydrolysis of GTP-bound translation factors (GTPase) at ribosomal "GTPase-associated center". In this center, a protein complex that is highly flexible protuberance called "ribosomal stalk" exists, which is conserved in all domains of life and responsible for the recruitment of GTPase to ribosomal large subunit.

Archaeal stalk is composed of P0 protein and multi copies of P1 dimer, and the C-termini of P0/P1 (P0/P1-CTD) involve in the interaction with GTPase.

In this study, in order to elucidate GTPase recognition mechanism of P1-CTD, we solved crystal structure of archaeal elongation factor 2 complexed with P1-CTD. Moreover, based on the structural information, binding assay of mutants was performed.

1P042 HP1 クロモドメイン N 末端部分の構造探索と HP1αCD/histone H3 tail 複合体モデル構築
Replica-exchange simulation of N-ter fragment in HP1 chromodomain and model construction of HP1αCD/histone H3 tail complex

Satoshi Omori, Nobuto Hashiguchi, Kei Moritsugu, Yoshifumi Nishimura, Akinori Kidera (*Grad. Sch. of Med. Life Sci., Yokohama City University*)

Binding of heterochromatin protein 1α chromodomain (HP1αCD) to histone H3 lysine 9 methylation yields heterochromatin formation which represses gene expressions. Recent studies revealed the increase in binding affinity by phosphorylation of four consecutive serine residues in the HP1αCD N-ter disordered loop. To clarify the associated molecular mechanism, firstly, conformational samplings of the N-ter loop with/without serine phosphorylation were performed by replica-exchange molecular dynamics simulation. The phosphorylation was found to cause large structural and dynamic changes, which may enhance the binding affinity. Then, structural models containing both the full-length HP1αCD/H3 tail peptide, constructed based on the NMR data, were examined using MD simulations.

1P043 Real-Time Observation of DNA Digestion by RecBCD with High-Speed Atomic Force Microscopy

Weidong Zhao (*Kanazawa University*)

RecBCD, which contains two helicases and a nuclease, plays important roles in DNA digestion and repair. We directly traced the enzyme functional process at high spatial (a few nanometers) and temporal (~0.3 s) resolution with high-speed atomic force microscopy. At the beginning, RecBCD bound to the end of dsDNA, while the other end labeled with biotin was blocked by streptavidin. When ATP was added, RecBCD translocated and digested dsDNA. After the reaction stopped at Chi which is a regulatory eight-nucleotide DNA sequence for ~10 s, RecBCD restarted to translocate and generated ssDNA. The movement of RecBCD was much less vigorous than before the pause. Thus, the functional processes of RecBCD, including Chi sequence recognition have been successfully visualized.

1P044 Molecular basis of conformational dynamics and enzymatical maturation process of nuclear lamin A related to onset of laminopathies

Mai Tsunoda[1], Muneyo Mio[1,2], Toshihiko Sugiki[3], Kazuhiro Mio[1,2] ([1]*AIST*, [2]*YCU*, [3]*IPR*)

The nuclear lamins are type V intermediate filament proteins constituting lamina, critically important for the structural properties of the nucleus. Lamin A is expressed as prelamin and suffers several steps of enzymatic processing to generate matured lamin. The mutations in counterpart enzyme cause the laminopathies, however the molecular basis of these diseases is not completely understood. To understand the mechanisms of laminopathies onset, we analyze the structures of lamin A. Electron microscopy clearly shows the reversible transition among the dimer, filaments, and paracrystal conformation, which are also modulated by the cell division kinases. The enzymtic cleavage of prelamin A by the membrane integrated peptidase zmpste24 is also studied.

1P045 Functional Analysis of a New Type I Antifreeze Protein from Barfin plaice, *Liposetta pinnifasciata*

Sheikh Mahatabuddin[1], Kazunari Ishihara[1], Yuichi Hanada[1], Ai Miura[2], Hidemesa Kondo[1,2], Sakae Tsuda[1,2] ([1]*Graduate School of Life Science, Hokkaido University*, [2]*National Institute of Advanced Industrial Science and Technology (AIST)*)

Antifreeze proteins (AFPs) have been identified in a variety of organisms living in cold environment. AFP type I has been known as a 37-residue monomeric α-helical peptide identified from a flat-fish named Winter flounder (WfAFP). The authors recently found a new 40-residue type I AFP from Barfin Plaice, *Liposetta pinnifasciata* (BpAFP), which exhibited 80% sequence identity with that of WfAFP. Significantly, BpAFP showed high thermal hysteresis (TH), solubility and thermal stability. It also appeared that its TH activity is not affected by pH change between 3 and 11. Therefore, BpAFP is very different from any other species of known AFPs. To understand these unique properties of BpAFP in molecular level we will discuss its structure-function relationship.

1P046 アクチンフィラメントの圧電特性
Piezoelectric property of an actin filament

Jun Ohnuki[1], Takato Sato[1], Koji Umezawa[1], Taro Q.P. Uyeda[2], Mitsunori Takano[1] ([1]*Dept. of Pure & Appl. Phys., Waseda Univ.*, [2]*Biomedical Res. Inst., AIST*)

Protein molecules seem to utilize mechanical stress to fulfill functions. Actin is likely to regulate the affinity for actin-binding proteins (ABPs) via applied mechanical stress. However, the molecular mechanism of such a mechanical response of actin is unclear. By molecular dynamics (MD) simulation, we previously reported that myosin possesses piezoelectricity: strain applied to the lever-arm region of myosin induces electric charge displacement in the motor domain. Here, to investigate whether piezoelectric property is inherent in actin filaments, we conducted MD simulation of actin filament to which external forces are applied. We focus on electrostatic responses of actin subunits in the filament and discuss possible effects on interaction with ABPs.

1P047 家族性 ALS に関連した A4V、G93A 変異 SOD1 の酸化促進性獲得
Acquisition of pro-oxidant activity by fALS-linked SOD1 mutants A4V and G93A

Ken Nishiya, Nobuhiro Fujimaki, Furi Kitamura, Takashi Miura, Takakazu Nakabayashi, Hideo Takeuchi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

More than 100 types of mutations have been identified for Cu,Zn-superoxide dismutase (SOD1) in familial ALS (fALS) patients. Previously, we found that the metal-depleted apo form of a SOD1 mutant, His43Arg (H43R), denatured under physiological conditions and then acquired pro-oxidant activity upon Cu^{2+} binding. In this study, we have extended our study to two other representative mutants, Ala4Val (A4V) and Gly93Ala (G93A), to verify whether pro-oxidant activity is common to fALS-linked SOD1 mutants. It is found that both the mutants also denatures under physiological conditions and then acquires pro-oxidant activity. Their Cu^{2+} binding modes are analogous to those of H43R, implying a common mechanism of pro-oxidant activity, at least, for these fALS-linked mutants.

1P048 GroEL に結合した基質 BFP の一分子 FRET 計測による構造解析
Conformation of the denatured BFP bound to GroEL by single molecule FRET measurements

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It remains unclear how chaperonin GroEL assists the folding of the substrate. We measured single-molecule FRET efficiencies of the double-labeled BFP in the equilibrium unfolding process in the presence and absence of GroEL by using line-confocal microscopy. The data obtained in the absence of GroEL suggest the presence of several folding intermediates. The FRET efficiency of BFP-GroEL complex is similar to that of the folding intermediate of BFP. In constant to the previous reports describing broad FRET distributions for the GroEL substrate complexes, the current results showed a relatively narrow distribution. We will examine BFP conformation change induced by the binding of ADP and ATP analogs to GroEL by using single-molecule FRET technique.

1P049 p53C 末端の負の制御ドメインに関する自由エネルギー地形
Free-energy landscape of the C-terminal negative regulatory domain of p53

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Intrinsically disordered protein (IDP) has unique molecular recognition mechanism, coupled folding and binding. In addition, IDP has a hub feature that can be explained with the coupled folding and binding mechanism.
To understand the hub feature and the binding mechanism of IDP, we studied a well-known IDP, the C-terminal regulatory domain of p53, using an all-atom virtual-system coupled multicanonical molecular dynamics simulation with explicit solvent.
First, we calculated potential mean force for the p53 C-terminal domain in the single state, and produced free energy landscapes. These results have shown that the p53 C-terminal domain adopts various conformations. We discuss the recognition mechanism of this domain binding to a partner molecule, the p53-s100bb.

1P050 タンパク質分子動力学シミュレーションの二次構造主成分分析
Secondary Structure Principal Component Analysis of Protein Molecular Dynamics Simulations

Norifumi Yamamoto (*Chiba Inst Tech*)

We propose a straightforward method to extract the essential information regarding secondary structure interconversions from molecular dynamics simulations. Based on this method, named secondary structure principal component analysis (SSPCA), we have been studying about structural basis of intrinsic disorder proteins, structurally ambivalent peptides, and chameleon sequences, which lack a strong intrinsic secondary structure, thus promoting α-helix/β-sheet conformational interconversions. Recently, we applied the SSPCA to the prion protein and the C-terminal fragment of protein G. We found that the SSPCA could systematically construct a free-energy landscape by mapping protein structural data into a reduced space according to their characteristic secondary structures.

1P051 塩酸グアニジン変性における *Aspergillus niger* 由来グルコアミラーゼのデンプン結合ドメインの二つの異なる変性状態
Two distinctive unfolded states starch binding domain of *Aspergillus niger* accumulated during GdnHCl-induced unfolding

Daizo Hamada[1], **Chiaki Ota**[2], Momoko Kitazawa[2], Hideo Miyake[1], Akiyoshi Tanaka[1] ([1]*Grad Schl Bioresource, Mie Univ*, [2]*Dept Bioresource, Mie Univ*)

Starch binding domain of glucoamylase (SBD) from *Aspergillus niger* is a small globular protein which could be one of the model proteins for the analysis on the mechanism of protein folding. We here analysed guanidium hydrochloride (GdnHCl)-unfolding process of SBD using various spectroscopic approach. SBD cooperatively unfolded into U1 state at 4-6 M GdnHCl but further addition of GdnHCl induced the formation of another unfolded state, U2 at ~7M GdnHCl. Both U1 and U2 indicated circular dichroism spectra typical of fully unfolded state. Thus, the presence of residual aromatic clusters in U1 was speculated whereas they are disrupted in U2. We are currently characterising the properties of U1 and U2 to clarify the role of the residual structures on the folding by SBD.

1P052 多機能性タンパク質 PHB2 のフォールディング状態に対する疎水環境の影響
The effect of hydrophobic environment on folding states of multifunctional protein PHB2

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Prohibitin-2 (PHB2) plays many important roles in diverse cellular activities, including cell signaling, aging and tumorigenesis. Because of its multifunctionality, PHB2 is an attractive target for some therapeutic applications. To characterize the structure and folding state of PHB2, recombinant PHB2 was analyzed using circular dichroism spectrometry (CD) and differential scanning calorimetry (DSC). In DSC measurements, PHB2 showed weak peak under buffer A condition (10 mM HEPES, pH 7.5, 150 mM NaCl). On the other hand, distinct endothermic peaks and concentration dependent increasing in T_m of PHB2 were observed when measured in the buffer A with DMSO or Tween 20. It implies hydrophobicity of solvent induces the folding of PHB2.

1P053 AFM による酸化 LDL 粒子の物理的性質の解析
Mechanical properties of oxidized low-density lipoprotein particles disclosed with AFM

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Evaluation of oxidized low-density lipoproteins (LDLs) is a possible positive risk factor for the development of cardiovascular disease. Although many chemical and immunological evaluation were done for the oxidized LDLs, change in the physical properties of LDL particles due to oxidation has not been well investigated. In this study, using an atomic force microscopy, we measured the topographies of LDL particles immobilized on an Au-coated mica surface before and after oxidation. This results showed that AFM might serve as a new tool for evaluation of oxidation in LDL particles and extend our knowledge of the role for oxidized LDLs in the development of cardiovascular disease.

1P054 分子動力学シミュレーションによる抗体表面を動く水の解析
Analysis of water dynamics at the surfaces of antibodies: Molecular dynamics study

Keiko Shinoda, Hideaki Fujitani (*RCAST, Univ. of Tokyo*)

In biological processes such as protein folding, hydrophobic hydration is considered to have an important role. We examine dynamics of water molecules at the water-protein interface of an antibody that has hydrophobic patch at the bottom of frame and a mutant that is not have the patch at the region by molecular dynamics simulations. In this meeting, we discuss the difference of water dynamics at the two surfaces, hydrophobic and non-hydrophobic surfaces of antibodies.

1P055 二次元蛍光寿命相関分光法による BdpA 折りたたみ機構解明に向けた研究:2 つの BdpA 変異体による包括的解析

Study of BdpA folding by two-dimensional fluorescence lifetime correlation spectroscopy: Comprehensive analysis of two BdpA mutants

Takuhiro Otosu[1], Kunihiko Ishii[1], Hiroyuki Oikawa[2], Munehito Arai[3], Satoshi Takahashi[2], Tahei Tahara[1] ([1]*Mol. Spectrosc. lab., RIKEN*, [2]*IMRAM, Tohoku Univ.*, [3]*Grad. Sch. Arts. Sci., Univ. Tokyo*)

Elucidation of the folding process of proteins is indispensable for understanding how proteins acquire a unique conformation. To this goal, we recently developed two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS). In the Biophysical society meeting last year, we reported a 2D FLCS study on the conformational transition dynamics of B domain of protein A (BdpA). The obtained results suggested that the conformational dynamics of BdpA occur within 10 microseconds. In this study, 2D FLCS was performed for two mutants of BdpA in which FRET pairs are attached on different positions. The results strongly suggested that the folding process of BdpA does not fit the simple two-state model. In the presentation, we discuss the folding mechanism of BdpA.

1P056 たった 10 残基から成るタンパク質の立体構造安定性に及ぼす主鎖と側鎖の充填の効果

Effect of Backbone and Side-chain Packing on Structural Stability of the Protein with Only Ten Residues

Satoshi Yasuda, Tomohiko Hayashi, Masahiro Kinoshita (*IAE, kyoto Univ.*)

The small protein with only 10 residues, CLN025, folds into the β-hairpin structure. We show that the enthalpy increase arising from the break of hydrogen bonds with water molecules is compensated by the enthalpy decrease due to intramolecular hydrogen bonding. The water-entropy (WE) gain arising from close packing of the backbone and side chains including those with the four large aromatic residues is powerful enough to surpass the conformational-entropy (CE) loss. In its design template (GPM12) with only two aromatic residues, however, the WE gain yields to the CE loss though there is no enthalpy change, and the unfolded state is stabilized. The above elucidation is made possible by decomposing our free-energy function into physically insightful constituents.

1P057 カルシトニンアミロイド凝集機構の解明と抑制法の考案

Analysis of amyloid formation and inhibition mechanisms of human calcitonin

Hiroko Tanaka, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Human calcitonin (hCT) is a peptide hormone used as a medicine. It easily forms amyloid fibrils to reduce the efficiency. Salmon calcitonin (sCT) is known to inhibit amyloid formation of hCT. We revealed that the chimera peptides derived from sCT and hCT suppresses the amyloid formation. In order to develop an inhibitor of hCT amyloid formation, we aimed to clarify the inhibition mechanism. We measured the amyloid fibrillation process by thioflavin T fluorescence. The fluorescence intensity decreases with increasing the molar ratio of the chimera CT. The amyloid formation was completely suppressed by the equivalent amount of chimeric CT, indicating that hetero-dimer comprising hCT and the chimera CT forms at the initial process and inhibits the further fibrillation.

1P058 分子動力学シミュレーションを用いたリガンド結合による PR-Set7 の構造変化の研究

Molecular dynamics simulations for structure changes of PR-Set7 by ligand binding

Takako Sakano, Hideaki Fujitani (*RCAST, UTokyo*)

X-ray crystal structure of a target protein is used to find new active molecules, Virtual Screening, or their docking pose prediction. However, some X-ray crystal structures are not suitable for such purposes, because some proteins significantly change their structure by allosteric effect of ligand binding.
We obtained apo structure of histone methyltransferase PR-Set7 by molecular dynamic simulations started from the X-ray crystal structure (PDB:1ZKK) without histone H4 peptide and other cofactor.
We predicted docking poses of known ligands to the apo-PR-Set7 with some tools, and performed MD simulations of them.
We present the results: how the structures differ and how they effect ligand docking.

1P059 オクタリピート領域をもつプリオンペプチドにおける金属との競合結合性

Competitive binding of metal ions to the octarepeat region of human prion protein

Masahiro Yagi, Kazuya Iwama, Haruto Onda, Wakako Hiraoka (*Dept. Phys., Grad. Sch. Sci. & Tech., Meiji Univ.*)

The misfolded form of prion protein (PrP) cause neurodegenerative diseases. It is thought that the binding of PrP to Cu^{2+} may result in the misfolding of PrP. To clarify the process of misfolding and subsequent aggregation, we investigated the structure of the octarepeat peptide (PHGGGWGQ) of human PrP that binds to Cu^{2+} and the stability of this bound structure. ESR spectra indicated two kinds of coordination modes for the binding of peptide -Cu^{2+}. Competitive binding of other divalent metal ions, such as Co^{2+}, Ni^{2+}, Zn^{2+}, and Mn^{2+}, to peptide-Cu^{2+} revealed that these metal ions were partially substitute for Cu^{2+}. These results suggest that the stability of PrP-Cu^{2+} is possibly affected by the presence of other metal ions.

1P060 粗視化 MD-SAXS 法の開発

Development of Coarse-Grained MD-SAXS method

Yuichi Kokabu[1], Tomotaka Oroguchi[2], Mitsunori Ikeguchi[1] ([1]*Yokohama City Univ.*, [2]*Keio Univ.*)

The structural fluctuations of proteins are important for protein functions. Small angle x-ray scattering (SAXS) is a powerful tool to characterize the dynamically fluctuating solution structure. However, the ab-initio modeling of flexible molecules from SAXS data is difficult due to low resolution. Combination of molecular dynamics (MD) simulation and SAXS is a promising method to model flexible molecules such as multi-domain proteins. In particular, coarse-grained (CG) MD is capable of wide sampling even for muti-domain proteins. Therefore, we developed a CG-MD-SAXS method that computes SAXS profiles from the structural ensemble obtained by CG-MD. SAXS profiles calculated from CG-models were in agreement with experimental data.

1P061 高圧分子動力学法によるヘリックス構造を持つペプチドの熱力学と構造に関する研究

High-pressure molecular dynamics study on the thermodynamics and structures of helical peptides

Yoshiharu Mori[1], Hisashi Okumura[1,2] ([1]*IMS*, [2]*SOKENDAI*)

Structural changes of proteins and peptides induced by high pressure have recently been studied theoretically and experimentally. Some peptides are more stable in the folded state than in the random coil state at high pressure. We studied high-pressure effect on helical peptides which are stable at high pressure by using molecular dynamics simulations with simulated tempering. We found that the population of the helical structures of the peptides increases with pressure. We calculated several thermodynamic parameters, and these results were consistent with experimental results. Structural properties such as the radius of gyration and interatomic distances were also calculated, and these indicate that the peptides are squeezed under high-pressure conditions.

1P062 トリプトファン三重項寿命測定を用いた staphylococcal nuclease の変性構造の評価

Characterization of the denatured structure of staphylococcal nuclease by tryptophan triplet state lifetime measurements

Sadatoshi Aoyagi, Mariko Yamaguchi, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

The elucidation of non-local interactions in denatured structure of a protein is essential for the deep understanding of protein folding. We revealed that non-local interactions around W140 form in the early stage of the folding of Staphylococcal nuclease. In this study, we investigate the collision frequency between W140 and other residues in the denatured state by tryptophan triplet quenching. We prepared several Cys-substituted mutants to observe the quenching efficiency. The triplet lifetimes of W140 with/ without Cys were measured to estimate the contact rate. The contact rates were not necessarily related to the distance between W140 and C on the amino acid sequence, indicating that the non-local interactions around W140 are not random in denatured structure.

1P063 フェリチンのアセンブリメカニズムの研究

A study of ferritin assembly mechanism

Daisuke Sato, Hideaki Ohtomo, Atsushi Kurobe, Ayumi Sunato, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. Bioinfo., Soka Univ.*)

Ferritin (Ftn) is a spherical shell-shaped 24-mer protein. It dissociates into 2-mers at acidic pH and can reassemble into 24-mer when pH is increased. To clarify Ftn assembly mechanism, we monitored the reassembly kinetics by time-resolved small-angle X-ray scattering. The initial rate was proportional to the square of initial Ftn concentration, suggesting that a second order reaction is rate-limiting. By native polyacrylamide gel electrophoresis, only 2-mer and 24-mer bands were observed during the reassembly reaction. From these observations, we propose the assembly scheme, in which the 4-mer formation from two 2-mers is rate-limiting, and then subsequent addition of 2-mer to the pre-formed oligomer is much faster than the 4-mer formation.

1P064 ヨウ素染色によるアミロイド線維構造の識別

Discrimination of amyloid fibril structures by iodine staining

Takato Hiramatsu, Seongmin Ha, Yuki Masuda, Eri Chatani (*Grad.Sch.of Sci.,Univ. Kobe*)

Amyloid fibrils are implicated in many diseases such as Alzheimer's disease. Iodine staining of amyloid fibrils was first described more than 150 years ago, but its mechanism has not been well understood. In this study, we tried to elucidate the mechanism by using insulin amyloid fibrils. The fibrils were prepared by seeding reaction with seeds generated by spontaneous nucleation in the presence of several different salts. As a result, it certainly showed that the fibrils obtained were stained with iodine, and interestingly, they represented different colors with distinct absorption spectra in visible region. The spectral shape was conserved even after repeated self-seeding, suggesting that iodine successfully discriminate various structures of amyloid fibrils.

1P065 β-ストランドの局所配列とねじれ、曲がりの関係

Local sequence of protein β-strands influences twist and bend angles

Kazuo Fujiwara, Shinichi Ebisawa, Yuka Watanabe, Hiromi Toda, Masamichi Ikeguchi (*Dept. of Bioinfo., Soka Univ.*)

β-Sheet twisting is thought to be mainly determined by interstrand hydrogen bonds with little contribution from side chains, but some proteins have large, flat β-sheets, suggesting that side chains influence β-structures. We therefore investigated the relationship between amino acid composition and twists or bends of β-strands. We calculated and analyzed the twist and bend angles of short frames of β-strands in known protein structures. The most frequent twist angles were strongly negatively correlated with the proportion of serine, threonine, and asparagine residues. Furthermore, the majority of serine, threonine, and asparagine side-chains in β-strands made contacts with nitrogen atoms of the main chain, suggesting that these residues suppress b-strand twisting.

1P066 アミノ酸挿入によるアミロイドβペプチドの線維形成への影響

Effects of single amino-acid insertion on amyloid β fibril formation

Kazuto Yamashita, Motonari Tsubaki, Eri Chatani (*Grad. Sch. Sci., Kobe Univ.*)

Amyloid β (Aβ) is a representative amyloidogenic protein, the deposition of which is a key pathogenic event in Alzheimer's disease. In this study, we investigated the effects of single amino-acid insertion at different positions of Aβ1-40 on their amyloid fibril formation. Wild-type and mutant Aβ1-40 peptides were prepared by expressing glutathione S-transferase (GST) tagged fusion proteins. After purification, the GST tag was removed by cleaving with a protease. Effects of mutation were then evaluated by analyzing seeding reaction of the mutant peptides with the wild-type seeds. As a result, an inhibitory effect was observed for a certain mutant, and along with the results for other mutants, amino-acid region(s) important for the fibril elongation will be discussed.

1P067 超好熱アーキア由来 S-layer タンパク質の糖結合特異性
Carbohydrate binding of S-layer protein derived from hyperthermophilic archaea

Shuichiro Goda, Kenichiro Yamashita, Hideaki Unno, Tomomitsu Hatakeyama (*Grad. Sch. Eng., Nagasaki Univ.*)

Archaeal S-layer protein is associated with the outside of the cytoplasmic membrane and directly exposed to the extreme environment. We purified PH1395.1 protein from the crude extract of *Pyrococcus horikoshii* by using glucose affinity column chromatography. The function of PH1395.1 is predicted as S-layer protein. PH1395.1 gene was expressed in *Eschelichia coli*. Carbohydrate binding specificity of PH1395.1 was measured by isothermal titration calorimetry. PH1395.1 showed heat change by binding to GlcNAc. Coaggregation assay was carried out by mixing PH1395.1 with Micrococcus lysodeikticus. Molecular weight of the PH1395.1 was determined by small-angle x-ray scattering. This results showed that the PH1395.1 forms oligomer and this cause coaggregation activity.

1P068 ４量体型サルコシン酸化酵素の分子動力学シミュレーション: 反応物と生成物の輸送経路の解明
Molecular dynamics simulation of heterotetrameric sarcosine oxidase: analysis of channeling of reactants and products

Daisuke Nakajima[1], Go Watanabe[2], Haruo Suzuki[2], Shigetaka Yoneda[2] ([1]*Grad. Sch. Sci., Kitasato Univ.*, [2]*Sch. Sci., Kitasato Univ.*)

Heterotetrameric sarcosine oxidase (SO) catalyzes the oxidative demethylation of sarcosine. The structural and biochemical studies for the SO-dimethylglycine (DMG) complex have suggested channeling of reactants and products through several tunnels. In the previous study, we have performed molecular dynamics simulations for the SO-DMG complex (pdb: 1X31) with the Amber force field and have analyzed pathways of surrounding water molecules. These pathways corresponded to the tunnels proposed by the experiments. In order to understand the mechanism of the channeling related to the catalysis of SO, we analyze the motion of hydrogen peroxide from precise molecular dynamics simulations for the SO-methylthioacetate complex (pdb: 3AD7).

1P069 リガンド結合の動的側面：PDZ ドメインタンパク質を用いた研究
Dynamical aspects of ligand binding: A case study for PDZ domain protein

Hiroshi Fujisaki[1], Norifumi Yamamoto[2], Kana Fuji[3], Mikito Toda[3] ([1]*Nippon Medical School*, [2]*Chiba Inst. Tech.*, [3]*Nara Women's Univ.*)

Ligand binding is one of the key functions of biomolecules, initiating enzymatic catalysis or conformational change, and leading to signal transduction in a cell. Hence it is computationally important to characterize the interaction between a ligand and a protein (or DNA) and the binding free energy is often calculated by employing different levels of methods. In this presentation, we investigate the dynamical aspects of ligand binding, that is, the ligand binding kinetics and pathways using the transition path sampling (TPS) method, which is a general and powerful method to characterize nonequilibrium dynamics of (bio)molecular systems. Our target system is a PDZ domain protein and its interaction with the ligand peptide is the main concern.

1P070 網羅的アミノ酸置換変異によるアシル ACP 還元酵素の活性部位の解析
Comprehensive mutagenesis reveals residues critical for aldehyde producing activity of acyl-ACP reductase

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Biosynthesis of alkanes is an attractive way of producing renewable substitutes for gasoline, diesel, and jet fuel. A key enzyme in cyanobacterial alkane synthesis is an acyl-(acyl carrier protein (ACP)) reductase (AAR) that catalyzes reduction of fatty acyl-ACP to aldehydes, which is then converted into alkanes or alkenes by aldehyde decarbonylase. However, it is still unknown which residues are critical for aldehyde production by AAR. Here, we performed alanine-scanning mutagenesis on AAR (341 residues) and revealed a catalytic cysteine residue and other residues important for its activity. Our ongoing studies to improve the AAR activity by comprehensive mutagenesis will also be presented.

1P071 アミロイド線維の人工設計ペプチドによる加水分解
Hydrolysis of amyloid fibrils by artificially designed peptides

Yoshihiro Iida, Atsuo Tamura (*Grad. Sch. Sci., Univ. Kobe*)

Amyloidosis has been a serious problem in the aging society. To remedy amyloidosis, the most effective way is to hydrolyze amyloid fibrils. We thus tried to design short peptides having hydrolysis activity against amyloid fibrils. As a strategy for the design, we made the peptide to have the catalytic triad composed of histidine, aspartic acid and serine. Based on the strategy, we synthesized 9 peptides named me1-9. It has been shown that me5 takes the alpha-helical conformation and can hydrolyze amyloid fibrils. We tried to hydrolyze variety of amyloids: beta lactoglobulin, insulin, amyloid beta40, and amyloid beta42. It is concluded that the peptide me5 can be regarded as a hydrolase which is capable of hydrolyzing the amyloid fibrils.

1P072 Large time step molecular dynamics using Torsion Angle Molecular Dynamics

Yu Yamamori, Akio Kitao (*Tokyo Univ. ICMS*)

Molecular dynamics (MD) of proteins in torsion angle space can enlarge the MD time step without significant loss of information because protein conformation is mainly determined by torsion angles, and the torsion angle motion does not strongly couple with bond and angle motions. However, solving complex equations of motions in torsion angle space directly requires inverse matrix calculation of a large matrix. We adopted the Articulated-Body Algorithm to significantly reduce the cost of inverse matrix calculation. We also investigated the effect of fixing torsion angles of terminal side chains and tested the performance for peptides. The results show that 8 fs time step can be adopted if the terminal chemical groups are fixed.

1P073 鉄イオン貯蔵タンパク質に対する交流磁場の影響

Effects of alternating magnetic fields on iron-storage protein

Yuta Yamada, Tsuyoshi Hondou, Hidetake Miyata (*Grad. Sch. Sci. , Univ. Tohoku*)

Epidemiological studies have suggested that alternating magnetic fields affect human body, but mechanism of influences on cell or human body has not established in the biological experiment yet. In order to reveal primary site of action by alternating magnetic fields, we focus on ferritin, iron-storage protein, because ferritin is source of iron ions which produce reactive oxygen species. We exposed ferritin solution to alternating magnetic fields (50Hz~500kHz for 3 h) and measured the concentration of iron ions outside ferritin. The results show that concentration of iron ions is decreased after the exposure. We are currently performing the experiment under wider conditions and will report the detailed analysis of this phenomenon.

1P074 ニトリルヒドラターゼ（NHase）によるアミド生成機構に関する理論的研究

A QM/MM study of amide formation reaction of Nitrile Hydratase

Megumi Kayanuma[1], Mitsuo Shoji[2], Yasuteru Shigeta[2] ([1]*Grad. Sch. of Sys. and Inf. Eng., Univ. of Tsukuba*, [2]*Grad. Sch. of Pure and App. Sci., Univ. of Tsukuba*)

Nitrile Hydratase (NHase) catalyzes hydration of nitriles to the corresponding amides and has been used for industrial productions of various amides. The active site of NHase consists of an Fe(III) or a Co(III) ion coordinated with two carboxamido nitrogens, one cysteine-sulfer (Cys-S), one cysteine-sulfenic (Cys-SO) and one cysteine-sulfinic (Cys-SO2) moieties. Several reaction paths have been proposed for the catalytic mechanism of NHase, and recently quantum chemical calculations using active-site models were reported (Hopmann et al., 2007, 2008, and 2014). In the present study, we have examined four reaction mechanisms by using Quantum Mechanics/Molecular Mechanics (QM/MM) method to reveal the effects of surrounding protein environment on the reaction process.

1P075 Effect of C-terminal truncation of chaperonin GroEL on the yield of an in-cage folding of GFP

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The chaperonin GroE encapsulates a substrate in its inner cavity, assisting the folding in an ATP-dependent manner. Although the function of GroEL has been well characterized, the role of its C-terminal region remains elusive.
Here, we investigated the effect of C-terminal truncation on the GroE-mediated folding of GFP. We found that the yield of an in-cage folding mediated by a single-ring GroEL was significantly decreased by truncation. In contrast, the yield of an in-cage folding mediated by an ATPase-deficient double-ring GroEL, which formed a stable football shaped complex, was not affected by truncation. These results suggest that the C-terminal region of GroEL functions as a barrier between rings, preventing the leakage of GFP through the bottom space of the cage.

1P076 抗体 G2 の複数の抗原を特異的に認識する機構

Mechanism of multispecific recognition of monoclonal antibody G2

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We generated a monoclonal antibody (mAb), G2, by immunizing mice with residues 174-247 of the chicken prion protein and found that G2 possessed an extremely unusual characteristic for a mAb. It can react with two completely different amino acid sequences (Pep18mer and Pep8) specifically. ELISA, SPR, and ITC experiments indicated that these two peptides have similar binding affinity for G2. However, we observed that these two peptides substantially differed in several binding characteristics from SPR and ITC experiments. Antibody inhibition test using each peptide indicated that the binding sites of the two different peptides overlapped each other. Therefore, an antigen-recognition site of G2 may adopt multiple binding conformations through different binding pathways.

1P077 NMR およびドッキングによる抗菌ペプチドとリポ多糖の複合体構造解析

NMR and docking structure of antimicrobial peptide complexed with lipopolysaccharide

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Antimicrobial peptides (AMPs) are ubiquitously found in living organisms and has a broad antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi. Some AMPs can bind to lipopolysaccharide (LPS) that is a major component of the outer membrane of Gram-negative bacteria and cause fatal septic shock. Therefore, AMPs that interact with LPS are expected to be novel antibiotics for sepsis. Tachyplesin I (TP I) is one of the AMPs that binds to LPS, but complex structure between TP I and LPS and binding mechanism are still unknown. We investigated the NMR structure of TP I in the presence of LPS and determined the complex structure using docking program. It was indicated that cationic and aromatic residues of TP I are important to bind to LPS.

1P078 MD シミュレーションを用いた Neuropsin - Neuregulin-1 リガンド間の相互作用に関する研究

Molecular Dynamics Study on Interactions between Neuropsin and Neuregulin-1 Ligand

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Neuropsin is a serine protease that is expressed locally in the limbic system. Neuropsin is involved with memory formation and psychiatric disorder. However, the signaling mechanism underlying neuropsin is not completely understood. Recently, neuregulin-1 (NGR-1) has been suggested as an important substrate for neuropsin. In this Molecular Dynamics (MD) study, we use the peptide models for NGR-1 in order to investigate the specific signaling substrates, the cleavage active sites, and the various initial neuropsin-NGR-1 ligand complexes. The aim of this work is to evaluate neuropsin's structural and dynamical behavior and relative stability on the neuropsin-ligand complexes, and to obtain new knowledge to reveal the signaling mechanism.

1P079 Analysis on the interaction between G3LEA proteins and other proteins by quartz crystal microbalance

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We have developed a model peptide (PvLEA-22) which consists of two tandem repeat of 11 -mer motifs of G3LEA proteins in *P. vanderplanki*. And we have indicated that PvLEA-22 maintained the structural and thermodynamic properties of the original G3LEA. In particular, PvLEA-22 suppresses desiccation-induced aggregation of various proteins, including lysozyme and HybD, To elucidate the functional mechanism of PvLEA22, we here determined the kinetic constants between PvLEA22 and its partner proteins using quartz crystal microbalance (QCM). Similar experiments were performed for a native LEA protein (AavLEA). These results demonstrated that PvLEA-22 tends to associate its partner in aqueous solution, leading to shielding the surface of the partner protein.

1P080 NMR スペクトルの再構成における圧縮センシングの基底選択の影響

Effect of basis selection in reconstructing NMR spectra using compressed sensing

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Multidimensional NMR spectroscopy often requires long measurement time, especially when handling large molecules. However, this requirement is prohibitive when the targets change their state quickly, as those in a living cell. We have therefore developed a method using compressed sensing (CS) theory, which can exploit the sparseness of NMR spectra and infer one from much less sampling points, i.e., with much shorter time, than conventionally required. In applying CS, the level of sparseness affects the performance of signal reconstruction; meaning that the selection of basis functions for representing signals is important for achieving better performance. In this report, we present the differences in reconstruction performance between several basis functions.

1P081 べん毛 C-ring 構成要素である FliM-FliN 複合体のストイキオメトリと、べん毛輸送装置タンパク質との高次複合体

Stoichiometry of the FliM-FliN complex, a flagellar C-ring component, and its higher order complex with flagellar export appratus proteins

Kazushi Matsushima[1], Hiroyuki Terashima[1], Miki Kinoshita[2], Tohru Minamino[2] ([1]*Grad. sch. sci. Osaka Univ.*, [2]*Grad. Sch. Front. Bio. Sci. Osaka Univ.*)

FliG, FliM and FliN are flagellar basal body component proteins, and form the C-ring beneath the MS-ring. The C-ring is responsible for switching of the direction of rotation, torque generation, and flagellar protein export. The C-ring contains 26 copies of FliG and 34 copies of FliM, however, the copy number of FliN is still obscure. FliM and FliN interact with the FliH-FliI complex, which are components of the flagellar protein export apparatus, but it is unknown how many C-ring subunits are involved in the interaction with the FliH-FliI complex. Here we purified the FliM-FliN and the FliM-FliN-FliH-FliI complex, and determined their stoichiometry. On the basis of the result, we will discuss the structure of the C-ring and the flagellar protein export mechanism.

1P082 Ｘ線１分子追跡法によるリン酸化タウタンパク質の構造動態

X-ray Single Molecule Observations of Phosphorylated Tau protein's structural fluctuations

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Alzheimer Disease is a neurodegenerative pathology, which is caused by hyperphosphorylated tau. Although it is necessary for understanding the protein's functions to find out its structure, tau proteins structure hasn't identified because Tau protein is one of the intrinsically disordered proteins.

Here we phosphorylated wild-type and mutated recombinant tau proteins with GSK–3β. The diffraction X–ray tracking method was used to observe their structural fluctuations at the beam line SPring–8 BL40XU. In consequence, we found tau protein molecules were fluctuating between 0.3 – 1 nm. Additionally, their fluctuations were changed after phosphorylation. Finally, we specified the phosphorylation sites that affect structural fluctuations of tau.

1P083 ラマン分光法による高濃度タンパク質溶液の分子間相互作用の評価

Evaluation of inter-molecular interaction of a protein in highly concentrated solution investigated by Raman spectroscopy

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Recently, how we can formulate the highly concentrated solution of protein is critical from the view point of biomedicine. To investigate the molecular structure and the interaction of protein in highly concentrated solution, Raman spectroscopic measurements of lysozyme solution as model system over a wide range of concentrations (2.5 mg/ml - 300 mg/ml) were carried out. In this study, we focused on the specific Raman bands (Amide I, Tyr and Trp) to analyze. The results revealed that the molecular interaction changes in the stepwise processes as an order of magnitude of the distance between molecules changes. These results suggest that the practically effective maker bands are capable of evaluating appropriate formulation of the highly concentrated solution of protein.

1P084 基準振動解析に基づいたタンパク質分子動的領域のモデル化

Modeling Motion Parts of Protein Based on Normal Mode Analysis

Shinya Muraoka[1], Yutaka Ueno[1,2] ([1]*NAIST*, [2]*Health Resarch Institute, AIST*)

The molecular animation is an approach to visualize molecular reactions in the cell with comprehensive understanding of the whole molecular system for both beginners and expert researchers. So far molecular models are made by atomic coordinates of proteins, it is difficult to add motions to model by an imaginative hypotheses derived from experimental results. Based on the normal mode analysis of protein movements, we propose a method with coarse grained motion parts for the movement of atomic model. The regions of the atomic model that move mostly together in the major normal mode motions are segmented. Our method applied in making a molecular movie is discussed toward a closer look at the actual in vivo molecular activities.

1P085 プロリン異性化酵素 Pin1 のドメイン間接触頻度による機能制御

Functional regulation of Pin1 cis-trans Pro-isomerase by the inter-domain contact frequency

Naoya Tochio[1], Ryosuke Kawasaki[2], Yu Tamari[2], Shin-ichi Tate[1,2] ([1]*RcMcD, Hiroshima Univ.*, [2]*Dept. of Math. and Life Sci., Grad Sch. of Sci. Hiroshima Univ.*)

Pin1 isomerizes specific pSer/pThr-Pro motif and regulates diverse cellular processes. Pin1 has two domains; WW phosphor-peptide binding domain and catalytic PPIase domain, connected by unstructured linker. The domains transiently contact at specific surfaces to modulate activity. Many Pin1 targets possess multiple Pin1 binding sites. To elucidate role of interdomain contact to multisite Pin1-ligand binding, we performed NMR titration experiments using a series of Pin1 mutants having various linker lengths, which are supposed to have differently frequent contacts. The affinities of two domains for the mutants showed a correlation with the contact frequency, which implicates transiently interdomain contact could affect Pin1 activity via changing each domain's affinity.

1P086 アポミオグロビン折り畳み中間体に存在するノンネーティブなHヘリックス領域構造

Non-native H helix translocation in folding intermediate of apomyoglobin

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Apomyoglobin folding from the unfolded state to the native state is somewhat slower (within a few seconds) than those of the other proteins. The kinetic intermediate was analyzed using the pH-pulse labeling hydrogen-deuterium exchange, and it was shown to be composed of ABGH helices. In this study, the fluorescence energy transfer as well as the cysteine quench was employed using the Cys-mutants for the analyses of the distance between Trp14 and either AEDANS or Cys during the apomyoglobin folding. The non-native interaction between G and H helices in intermediate was observed as a helix turn shifted. Furthermore, the disulfide mutant between S108C (G-helix) - L135C (H-helix) adopted the native-like intermediate.

1P087 機能性ペプチドから創出する小型タンパク質

Generating a small-sized protein from a functional peptide

Hideki Watanabe, Shinya Honda (*AIST BMRI*)

We have previously revealed that the use of a 10-residue β-hairpin chignolin facilitated structural and functional evolution of a polypeptide. Here we applied this concept to generating a small-sized protein from a given functional peptide. Segment-based combinatorial libraries were generated where each molecule consisted of a functional segment, a chignolin-derived bending segment, and an adjusting segment composed of a randomized sequence to stabilize the functional segment. Phage display selection resulted in 40,600-fold affinity-enhancement of the functional segment, generating a 54-residue protein termed AF.p17. This method can take advantage of given functional peptides by providing them with a tailor-made protein scaffold that supports their active conformation.

1P088 ハロゲン化チロシンの多箇所への部位特異的導入によるタンパク質の安定化

Multiple site-selective integrations of bulky halogenated tyrosines enhance protein stability

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Genetic codes with artificial assignments of the UAG codon combine the natural and non-natural diversity of amino acids for synthesizing protein variants. In this study, we show that protein stability can be enhanced by incorporating UAG-encoded halogenated tyrosines at multiple selected sites, with the bulky halogen moieties filling inter-residue spaces to achieve a tightly packed protein interior. This new mechanism for protein stabilization illustrated that the artificial codes expand engineering strategies.

1P089 Expression and refolding of the protein from a fruits of *Richardella dulcifica*

Maria Namba, Satoko Shibuya, Naoya Hashikawa, Satoru Yamaguchi (*Okayama Univ. Sci.*)

We tried to express protein contained in the fruits of *Richardella dulcifica*. The functional structure of this protein has dimer structure in solution. As we reported in the previous meeting, this water-soluble protein was expressed as inclusion body by using vector pET16b and *Escherichia coli* BL21. In this meeting, we report a result of the protein expression used various strains and vectors with chaperone plasmid. We also present a result of refolding of it to soluble dimer.

1P090 Introduction of negatively charged residues compensates for decreased protein solubility caused by an artificial hydrophobic surface

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A hydrophobic region on a protein surface often interacts with other molecules. However, a hydrophobic surface artificially introduced into the soluble protein may decrease its solubility. We first produced 6L, a mutant of an acidic protein sulerythrin, by creating a hydrophobic surface consisted of six leucines. After expressed in Escerichia coli, 6L was found in the insoluble fraction. To reverse this lowered solubility, we introduced charged residues around the hydrophobic surface. Introduction of negatively charged residues, but not positively charged residues, surrounding the hydrophobic surface on the acidic protein can most efficiently counteract the effects on solubility induced by the hydrophobic surface.

1P091 抗体精製用リガンド FPA の抗体解離メカニズムの解明と改良

Mechanism and improvement of pH-sensitive antibody dissociation by FPA, a ligand for antibody purification

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Protein A is frequently used as an affinity ligand for antibody purification through affinity chromatography. However, acidic pH is required to dissociate antibodies from protein A, which has risks of acid denaturation and aggregation of antibodies. We have previously developed an affinity ligand, FPAF, which dissociates antibodies at pH ~5. FPAF is composed of the B-domain of protein A connected with two leucine zipper regions of c-Fos. Here, we designed FPA, a truncated form of FPAF, and elucidated the mechanism of pH-sensitive antibody dissociation by FPAF. Besides, to dissociate antibodies at more neutral pH, we have created His mutants of FPA, in which homodimeric coiled-coil formation of FPA is controlled by the positive charges of His residues.

1P092 亜硝酸還元酵素と一酸化窒素還元酵素複合体の結晶構造

Crystal structure of complex of nitrite reductase and nitric oxide reductase

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Nitric oxide (NO) is produced as an intermediate product in nitrate respiration called denitrification. Cytotoxic NO must be degraded by nitric oxide reductase (NOR) immediately after the production by nitrite reductase (NiR) in denitrification. This raises the possibility that NOR and NiR form a complex to effectively decompose NO. On the basis of this idea, here, a formation of NOR-NiR complex was examined by X-ray structural analysis. The structure of the NOR-NiR complex was determined at a resolution of 3.2 Å. While there was no obvious NO channel between NOR and NiR, the complex structure implies that NO could be produced by NiR at the close proximity to NOR by the complex formation, thereby facilitating rapid NO reduction by the NiR/NOR system in denitrification.

1P093 ハーフメト Hb M における酸素親和性と正常鎖、異常鎖の配位状態との関係

Relationship between oxygen affinity and coordination state of normal or abnormal chain in half-met Hb Ms

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Hemoglobin Ms (Hb M), in which proximal or distal histidine was replaced by tyrosine, or the valine (βValE11) was replaced by glutamic acid (βGluE11), were investigated to elucidate a regulation mechanism of oxygen affinity (OA) of α or β normal chain. Tetramer of half met Hb M enables us to measure OA of α or β normal chain since it is steadily present as valence-hybrid ($Fe^{2+}Fe^{3+}$). Hb M Iwate (αH87Y) whose OA is also known to be scarcely changed between pH 6 and 9, gave the Fe-His band at 218 cm^{-1} in β normal chain without pH dependence. The Fe-His frequency of α normal chain in Hb M Milwaukee (βV67E) also did not show pH dependence in spite of showing pH dependence of OA. We discuss a relationship between OA of normal chain and coordination state of abnormal chain.

1P094 α 鎖あるいは β 鎖の近位ヒスチジンがグリシンに置換された変異ヘモグロビンの機能と構造

Function and structure of mutant hemoglobins with the proximal histidine replaced by glycine in either α or β subunit

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Mutant hemoglobins (Hbs) in which the proximal histidine (HisF8) was replaced by glycine (GlyF8) in the presence of imidazole (Im) were investigated. Mutant Hbs have the Fe-Im bond but Gly(F8) is detached from Fe of heme. A movement of F-helix concomitant with ligand binding to heme does not occur in the subunit in which HisF8 was replaced by GlyF8. Recombinant Hb αH87G and Hb βH92G were investigated by resonance Raman, 1H NMR, and near-UV CD. Hb αH87G showed distinct biphasic oxygen equilibrium curves (OEC), while Hb βH92G showed OECs of high affinity without cooperativity. It is suggested that the absence of the Fe-His bond in α subunits keeps oxygen affinity of β subunit low, while the absence of the bond in β subunits enhances oxygen affinity in α subunits.

1P095 Unique reaction mechanism of MhuD, a heme-degrading enzyme from Mycobacterial tuberculosis

Toshitaka Matsui, Syusuke Nambu, Masao Ikeda-Saito (*IMRAM, Tohoku Univ.*)

MhuD, a heme degrading enzyme from Mycobacterial tuberculosis, is characterized by unusual non-planarity of heme bound to the enzyme. We have recently shown that MhuD affords a unique heme catabolite, mycobilin, whose structure is indicative of mechanistic discrepancy of MhuD from canonical heme oxygenases. In this study, we have performed detailed reaction analysis on MhuD to explore its unique mechanism. An observed intermediate during the reductive O_2-activation was successfully identified as a ferrous-oxy heme of MhuD. The H_2O_2-dependent mycobilin formation by MhuD suggests formation of an iron-hydroperoxy (FeOOH) intermedaite. We also have examined possible involvement of hydroxyheme in the MhuD reaction as a key intermediate.

1P096 The Caged State, the Transition State of the Regulation of Oxygen-Affinity in Hemoglobin

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The “caged” state of hemoglobin (Hb), in which the heme-oxygen bond is broken and the unbonded oxygen is trapped within the heme pocket, is the transition state in the regulation of the oxygen-affinity of Hb. The oxygen-affinity of Hb is regulated by heterotropic effectors with detectable changes in neither static molecular structures of the protein nor the coordination/ electronic structures of the hemes. The rate of dissociation of oxygen from the “caged” state is enhanced by effector-linked enhanced thermal fluctuations, which simultaneously reduce the rate of geminate-recombination of oxygen to hemes, resulted in the reduction of the apparent oxygen-affinity of Hb. Thus, the oxygen-affinity of Hb is regulated by protein dynamics, rather than static structures.

1P097 異なるゾル・ゲル由来シリカゲル中ヘモグロビンのアロステリック転移の比較

Comparison of allosteric transitions in hemoglobin in different sol-gel derived silica gels

Naoya Shibayama (*Div. of Biophysics, Jichi Medical Univ.*)

Encapsulation of human hemoglobin in wet silica gels slows protein motion, allowing investigation of allosteric transition intermediates that are short-lived in solution. Recently, however, several research groups have performed distinctive gel experiments, giving different conclusions to different groups. To solve this inconsistency, we have empirically compared our sol-gel methods and others by monitoring both directions of the hemoglobin allosteric transition (R-to-T and T-to-R) by use of optical absorption and near-UV CD spectroscopy. The results reveal method-dependent variations not only in slow-down factor, but also in kinetic resolution between tertiary and quaternary structural changes, resolving some inconsistency between the studies.

1P098 シトクロム c_3 中のヘムの電子構造制御に関する計算科学的研究

Computational study of the electronic structures of hemes in cytochrome c_3

Yasuhiro Imada, Haruki Nakamura, Yu Takano (*IPR, Osaka Univ.*)

Cytochrome c_3 (cyt c_3) is a periplasmic electron transport protein found in sulfate-reducing bacteria. Cyt c_3 possesses four c-type hemes in a compact configuration and exhibits extremely low reduction potentials. NMR experiments show that point mutations on F20 lead to remarkable changes in redox potential, however, their 3D structures are not largely changed. The X-ray structures are equivalent to each other within 0.3 Å backbone RMSD.

In this study, quantum mechanics (QM) calculations were performed to investigate the relationship between the mutations and the redox potentials. We will discuss how the mutations influence π-π interactions, porphyrin distortion, and electrostatic interactions of the protein, leading to the difference in the redox potentials.

1P099 分子動力学法が明らかにした ADP/ATP 透過担体の内向き開構造のモデル

A plausible model for the structurally unknown inward-facing conformation of ADP/ATP carrier: A molecular dynamics study

Koichi Tamura, Shigehiko Hayashi (*Grad. Sch. Sci., Univ. Kyoto*)

ADP/ATP carrier (AAC) is a mitochondrial integral membrane protein. As the name implies, its functional role is to exchange ADP with ATP, with a 1:1 stoichiometry. AAC can be trapped in two distinct conformational substates, outward-facing and inward-facing state, by specific inhibitors. Currently, three-dimensional structures for the outward-facing state are available, but that of the inward-facing state is yet to be found. Recently, we have developed a molecular dynamics method, linear response path following (LRPF), that enables one to explore the conformational transition pathway of proteins upon ligand bindings. The method was applied to elucidate the experimentally unknown inward-facing structure. Currently a model for the inward-facing state was obtained.

1P100 拡張アンサンブルシミュレーションを用いた繊維芽細胞増殖因子受容体(FGFR3)の膜貫通ドメインの構造予測結果と実験との比較

Comparison of the predicted structure of the FGFR3 transmembrane domain by enhanced sampling simulations with experimental results

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FGFR3 is a kind of the receptor tyrosine kinase related to the regulation of skeletal growth and development. The transmembrane (TM) domain of the FGFR3 forms the monomer and dimer. The dimer has two conformations, active and inactive. The difference between active and inactive forms is linked with the signal control. The active structure of FGFR3 TM dimer in the micelle has been solved by NMR spectroscopy. However, the inactive structure and the structures in the lipid bilayer system have not been determined yet. To predict the dimer and monomer structures in the lipid bilayer, we performed multi-dimensional umbrella sampling and replica-exchange MD simulations with the implicit/solvent membrane. We'll show the difference between predicted structures and NMR results.

1P101 アクアポリン 3 の投影像へのホモロジーモデルの当てはめ

Fitting of a homology model to a projection map of aquaporin-3

Kaoru Mitsuoka (*Next Generation Natural Products Chemistry*)

Aquaporins are a family of water channels that facilitate efficient transport of water and small solutes across plasma membranes. They are divided into two subfamilies on the basis of their permeability, the aquaporin and the aquaglyceroporin subfamilies. Members of the former are highly specific for water, but members of the latter are permeated by water and small molecules such as glycerol and urea. AQP3, one of aquaglyceroporins, conducts glycerol as well as water. AQP3 is abundantly expressed in the skin, and plays a key role in the mammalian skin hydration, elasticity, and barrier function. Here we present fitting of an homology model to a projection map by cryo-electron crystallography and discuss about the implications between the structure and the functions.

1P102 酸素センサータンパク質 Aer のシグナル伝達機構の解明

Elucidation of signal transduction mechanism of Aer

Yoriyoshi Oka, Tatsuya Iwata, Hideki Kandori (*Nagoya Institute of Technology*)

Aer is a membrane protein for aerotaxis in E.coli, which is a kind of taxis receptors. Aer has FAD-binding PAS domain as a sensor domain; redox reaction of the FAD causes the structural changes of PAS, followed by the activation of signaling domain. However, little is known about the signal transduction mechanism.

In order to reveal the aerotaxis mechanism, we investigated its spectroscopic properties such as UV-vis and FTIR. First, we constructed expression system of Aer in E.coli and purification conditions. Next, to change redox states of FAD, we searched for reducing reagents to reduce FAD in Aer and sodium dithionite can reduce the FAD. The signal transduction mechanism will be discussed on these spectroscopic properties.

1P103 NMR, QCM, MD シミュレーションによる κ-オピオイド受容体細胞外第２ループとダイノルフィンとの細胞膜中での相互作用解析

Interaction of ECL-II of k-opioid receptor with dynorphin in membrane environments as revealed by solid state NMR, QCM and MD simulation

Akira Naito[1], Atsushi Kira[1], Namsrai Javkalantugs[2], Takenori Miyamori[1], Yoshiyuki Sasaki[1], Masayuki Eguchi[1], Izuru Kawamura[1], Kazuyoshi Ueda[1] ([1]*Yokohama National University*, [2]*National University of Mongolia*)

k-receptor is a member of the opioid receptor family and selectively interacts with dynorphin (Dyn). Interaction of extracellular loop II (ECL-II) of the k-receptor and N-terminal a-helix of Dyn was investigated. 13C MAS NMR spectra were recorded and the 13C chemical shift perturbation clearly indicated that Dyn interacts with ECL-II at the location of Val10-Ala15. QCM measurements were performed to determine the binding constant of ECL-II with Dyn and indicated that the binding constant between Dyn and ECL-II was 72 times larger than that between Dyn and lipids. The result of the MD simulation indicates that the C-terminus of Dyn interacts with the amino acid residues of the region between Val10-Gln14 of ECL-II.

1P104 分子動力学計算から明らかにされた細菌機械受容チャネル MscL のゲーティングにおける N 末端領域のメカノセンサーとしての役割

The N-terminal domain acts as a mechanosensor in the gating of the bacterial mechanosensitive channel MscL: molecular dynamics study

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E-coli mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane (TM) helices, and its 3D structure of the closed state has been resolved. The major issue is to understand the gating mechanism driven by tension in the membrane. We performed MD simulations for the opening of new MscL model with the N-terminal (S1) helices running parallel to the cytoplasmic membrane. Upon membrane stretch, the TM helices are dragged and tilted by lipids at Phe78 at the periplasmic side, leading to the gate expansion. Furthermore, it is found that the cytoplasmic side of the TM helices is expanded more widely than that of the previous MscL model with the bundled S1 helices, suggesting that the S1s of the new model MscL play a role of a mechsnosensor.

1P105 改良した多リン酸力場を用いた ATP/ADP 結合状態の筋小胞体カルシウムポンプの分子動力学計算

Molecular dynamics simulations of ATP/ADP bound forms of SR Ca^{2+}-ATPase using CHARMM force field with modified polyphosphate parameters

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Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase transports two Ca^{2+} from the cytoplasm into the SR lumen utilizing ATP hydrolysis. It is fundamental to know how ATP/ADP can bind stably in the cytoplasmic domain of the ATPase before/after the reaction. We performed all-atom molecular dynamics (MD) simulations of the ATPase in the ATP/ADP bound forms with explicit solvent and a DOPC lipid bilayer. We observed the phosphates structure in the bound ATP changed rapidly into the extended form using the original CHARMM force field. After some modifications of the force field, we could simulate the protein without such changes. We examine the essential interactions stabilizing bound ATP with the ATPase and discuss the relationship between the simulation results and experimental data.

1P106 膜孔形成蛋白質 FraC の構造変化機構の解析：可溶性構造から膜貫通構造までの変遷

Structural analysis of the α-helical pore-forming toxin FraC; metamorphosis from a water-soluble to a transmembrane protein

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The pore forming toxins (PFTs) are cytolytic proteins that bind to cellular membranes, where spontaneously metamorphose from a water-soluble form to transmembrane pores. Fragaceatoxin C (FraC) is an α-PFT secreted by the sea anemone *Actinia fragacea*.
In this study we have investigated the structural transition of FraC by determining its crystal structures in the water-soluble and lipid-bound monomeric forms and that of an assembly intermediate. We are also working on the preparation of crystals of the transmembrane pore of FraC.
Our results demonstrate multivalent protein-lipid and protein-protein interactions in the context of the membrane trigger the spontaneous conformational change and assembly of a soluble protein into a multitopic membrane protein.

1P107 性フェロモン生合成活性化神経ペプチド受容体（PBANR）の発現、リガンド結合、会合状態の解析による結晶化の為の T4 リゾチーム置換位置の検討

Positional optimization of T4L that replaces IL3 of PBANR for crystallization by expression, ligand binding, and size exclusion analyses

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The silkmoth pheromone biosynthesis-activating neuropeptide receptor (PBANR), a class-A GPCR, activates the biosynthesis of sex pheromone by binding PBAN. We aim to solve the crystal structure of the PBAN-PBANR complex. For crystallization, we designed several PBANR constructs with truncated N- and C-termini and the third intracellular loop (IL3) mostly replaced by T4 lysozyme (T4L). The best PBAN-PBANR microcrystal so far diffracted X-rays to 12-Å resolution. To obtain better diffracting crystals of the complex, we have optimized the insertion position of T4L out of 21 PBANR constructs by comparing their expression levels, ligand binding ability and elution patterns in FSEC. Based on these data, we have chosen two as the constructs suitable for crystallization.

1P108 レプリカ交換アンブレラサンプリングシミュレーションでのコレステロール分子との相互作用によるアミロイド前駆体の C99 単量体の構造変化

Structural change of APP-C99 induced by interactions with cholesterol studied by Replica Exchange Umbrella Sampling (REUS) simulation

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The Aβ peptide, critical to Alzheimer's Disease (AD), is produced by cleavage of the transmembrane of Amyloid-Precursor-Protein-C99 (C99) by γ-secretase. It has been observed that increased levels of cholesterol are correlated with early onset of AD. It has also been proposed that cholesterol binds specifically to C99 and that interaction with cholesterol induces a structural change in C99, possibly affecting the recognition of C99 by γ-secretase. This research investigated the C99-cholesterol complex structures, including the structural change of C99 (686-726) monomer, by all-atom REUS simulations in an implicit membrane model. Results of REUS simulations were compared with NMR experiments to gain insight into the impact of cholesterol on C99 structure and processing.

1P109 チャネルロドプシンの構造変化におけるカチオンの効果
Structural changes of channelrhodopsin under various cation conditions

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Optogenetics has revolutionized neurosciences, where ion-transporting microbial rhodopsins are utilized as tools for light-induced neuronal excitation and suppression. Channelrhodopsin (ChR), a light-gated cation channel, is used to excite neurons by light. Recently, several chimeric proteins have been designed for better optogenetics application.
Here we applied low-temperature spectroscopy to the chimeric ChR, ReaChR (Red-activatable ChR). We found accumulation of photointermediates at low temperatures by UV-vis spectroscopy. Light-induced difference FTIR spectra were measured between dark state and photointermediates in the presence of various cations. Structural changes of ReaChR and its cation effects will be discussed.

1P110 Effect of partial fluorination on bacteriorhodopsin reconstituted in dimerized Di-o-tetradecylphosphatidylcholine vesicle

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A comparative study of bacteriorhodopsin (bR) reconstituted in vesicles composed of a pseudocyclic Di-o-tetradecylphosphatidylcholine(PC-DTPC), which corresponds to a homologous dimeric molecule through a linkage of a single alkyl chain between two DTPC molecules, and of its partially fluorinated analog (PC-F4DTPC) has been performed by using several biophysical techniques. Upon partial fluorination of PC-DTPC, absorption maximum of reconstituted bR shifted from 575 nm to 565 nm and bR trimeric structure was highly stabilized against heat up to ~70°C. Laser flush photolysis experiments showed that after photo-excitation, bR in PC-F4DTPC recovers to the original ground state faster than bR in PC-DTPC. More details of fluorination effect on bR will be discussed.

1P111 Ca^{2+}-ATPase の第 2 膜貫通ヘリックス（M2）とロングレンジの共役
Second Transmembrane Helix (M2) and Long-range Coupling in Ca^{2+}-ATPase

Takashi Daiho, Kazuo Yamasaki, Stefania Danko, Hiroshi Suzuki (*Asahikawa Med. Univ.*)

The Actuator (A) domain of sarcoplasmic reticulum Ca^{2+}-ATPase undergoes large rotational movements that influence the distant transport sites through connections with transmembrane helices M1 and M2. Here we explore the importance of M2 and its junction with the A domain in coupling between Ca^{2+}-transport and ATP hydrolysis, E2P hydrolysis, and dephosphorylated enzyme transition by a series of mutations. Results are most clear with five-glycine insertions. The results pinpoint which parts of M2 control cytoplasm gating and which are critical for luminal gating at each stage, and suggest that proper gate function requires appropriate interactions, tension and/or rigidity in the M2 region at appropriate times for coupling with A-domain movements and catalysis.

1P112 アジ化物結合型チトクロム酸化酵素の高分解能 X 線結晶構造解析によるアジ化物結合様式の解析
High-resolution crystal structural analysis reveals that the two azide ions bind to Cytochrome c oxidase in different manner

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Cytochrome c oxidase (CcO) pumps protons coupled with O2 reduction. The azide-bound oxidized resting fast CcO has been studied to probe the function of the O2 reduction site. The reported structure of azide-CcO (2.9 Å) shows that one azide ion bridges between CuB and Fea3 in the O2 reduction site. In this study, effect of azide concentration between 2 to 20 mM was examined on the X-ray structure of the O2 reduction site at high resolution (1.9 to 1.7 Å) and the azide binding to CcO was confirmed by absorption spectrum. These results reveal that previously determined electron density assigned as the azide ion is due to that of a peroxide ion. Namely, the peroxide in the O2 reduction site was replaced completely by azide-binding to both CuB and Fea3 at 20 mM azide.

1P113 NDQ モチーフを持つ pseudo gene の機能復元
Functional restoration of apseudo gene of rhodopsin with NDQ motif

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Pseudo gene is the gene which is not expressed in vivo. However, many pseudo genes have similar sequences to the functional genes. We focused on pseudo genes with the characteristic NDQ motif of the sodium pump rhodopsins. These rhodopsin genes encoded in the locus of pseudo gene must not be expressed because codon-frame shift occurs by the loss of base(s) and mutation(s). We fixed the frame shift of putative pseudo genes of rhodopsins with NDQ motif by introducing identical sequence as sodium pump rhodopsin KR2. As the result, we successfully expressed the rhodopsins. They showed sodium pump functions. However, their expression levels were low and pump activities were weaker than KR2. We discuss the molecular mechanism of the two proteins.

1P114 Role of a unique arginine residue on the assembly of the translocator domain in a trimeric autotransporter

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Haemophilus influenzae adhesin (Hia) belongs to a trimeric autotransporter family and consists of a passenger domain and a translocator domain. The crystal structure of Hia translocator domain (HiaT) has shown that HiaT forms a transmembrane β-barrel of 12 β-strands, four of which are provided from each subunit. This protein has a unique arginine residue at 1077. Arg1077 side chains from three subunits protrude toward the center of the b-barrel and are close to each other. To investigate role of this residue on the trimer assembly and stability, we replaced this arginine with the neutral amino acid, methionine, and the properties of the mutant were investigated. Although the mutation accelerated the reassembly, it seems to compromise the integrity of the trimer.

1P115 ヒト cytochrome b561 form 3 (hb561-3)タンパク質の機能解析
Analyses of the physiological function of hb561-3: the cytochrome b561 form 3 protein in human

Yuma Takahashi, Takako Yamazoe, Akikazu Asada, Motonari Tsubaki (*Grad. Sch. Sci., Univ. Kobe*)

Cytochrome b561 is a membrane protein having a role in regeneration of vesicular ascorbate (AsA) by transmembrane electron transfer. However, the physiological function of hb561-3 is not clarified. In the present study, we investigated the intercellular localization of hb561-3 protein in cultured human A549 cells and its interaction in the purified state with AsA. We found that hb561-3 protein is expressed in endoplasmic reticulum (ER), as found previously for its very close homolog, 101F6 protein. Upon interaction with AsA, oxidized heme of hb561-3 protein was reduced, but with a much slower rate than that of 101F6 protein. These results suggested that hb561-3 protein might interact with a distinct substrate other than AsA as the physiological electron donor.

1P116 常磁性緩和促進を利用した転写因子 FMBP-1 の分子ダイナミクスの解析
Molecular dynamics of transcription factor FMBP-1 proved by paramagnetic relaxation enhancement

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FMBP-1 is a transcription factor of silk protein, fibroin H chain. FMBP-1 has a STPR domain, which is a novel DNA binding motif found in wide spectrum of eukaryotes. CD and other spectroscopic experiments showed that STPR domain is partially folded and has a flexible structure in the DNA-free state and DNA binding induces a conformational change into a more rigid structure. However, the detail of its binding process remains almost unknown due to the lack of information at the atomic-level about structure and dynamics of STPR domain. Here, we used paramagnetic relaxation enhancement (PRE) to characterize the dynamical aspect of DNA binding of FMBP-1. We measured the intramolecular contacts of STPR domain from PRE experiments and estimated the conformational dynamics.

1P117 MBD4 蛋白質のメチル化 CpG 結合ドメインと塩基除去活性ドメインをつなぐリンカー領域の構造と機能
Structural analysis of a linker region between methyl CpG binding and glycosylase domains in MBD4

Itaru Takeshita (*Grad. Sch. Eng., Kyoto Univ.*)

Methyl CpG Binding Domain Protein 4 (MBD4) is a DNA repair enzyme that recognizes and excises a mismatch thymine base in methylated CpG sequence. MBD4 has two functional domains, the N-terminal methyl CpG binding domain (MBD) and the C-terminal glycosylase domain (GD), separated by a 265 amino acid linker. The crystal structures of these domains have been already solved. However, it remains unclear how these two domains act together in MBD4 function. In this study, structural properties of full length MBD4 were analyzed using SAXS combined with biochemical techniques. Our data suggest that MBD and GD are spatially coordinated relatively close to each other despite the long linker region. The linker region is implied to facilitate coordinated action of MBD and GD.

1P118 新奇 DNA 結合ドメイン STPR を持つ転写因子 FMBP-1 の in situ コンディションにおける DNA 認識動態の FCS 解析
FCS analysis of DNA recognition movements of transcription factor FMBP-1 contains a novel DNA binding domain STPR in situ condition

Motosuke Tsutsumi[1], Hideki Muto[1], Mai Kimoto[1], Masakatsu Kamiya[1], Takashi Kikukawa[1], Shigeharu Takiya[1], Makoto Demura[1], Keiichi Kawano[1,2], Masataka Kinjo[1], Tomoyasu Aizawa[1] ([1]*Grad. Sch. of Life Sci., Hokkaido Univ*, [2]*Chitose Inst. Sci. Tech.*)

Fibroin modulator binding protein-1 (FMBP-1) is a transcription factor of the fibroin H chain gene in the silkworm *Bombyx mori*. FMBP-1 has a unique tandem repeat DNA binding domain “the one score and three amino acid peptide repeat (STPR)”. However, the functional details of STPR and molecular dynamics in live cell remain unknown. Here, we report an analysis of diffusion properties of FMBP-1 in posterior silk gland cells of *Bombyx mori* by fluorescence correlation spectroscopy (FCS). In our observation, EGFP fusion of FMBP-1 showed clear nuclear localization and the existence of movement with four distinct diffusion properties in a single measurement. This result might indicate co-existence of "facilitated diffusion" with specific binding on chromosomal DNA.

1P119 転写因子はいかにして障害物を迂回するか。分子シミュレーションによるアプローチ
How transcription factor can bypass obstacles? Molecular simulation approaches

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According to single molecular experiments, transcription factors search their target sites with sliding on DNA and 3D diffusion. These experiments mainly were under the conditions of liner DNA without obstacles or with fixed obstacles by antibodies. But in cells the obstacles are other transcription factors that can detach from DNA or histones wounded by DNA. Although the searching mechanism on molecular level in such conditions is still unclear, the transcription factor behavior in such condition is difficult to investigate with experiments. So we tried to reveal the searching mechanism with coarse-grained molecular dynamics software, Cafemol.

1P120 RecA 蛋白質の生体分子認識能力によるカーボンナノチューブ表面の DNA の生物的機能の評価
Evaluation of biological function of DNA on the single-walled carbon nanotubes by using biomolecular recognition ability of RecA proteins

Shusuke Oura, Masahiro Ito, Yoshikazu Homma, Kazuo Umemura (*Grad. Sch. Sci., Tokyo University of Science*)

We examined the biological function of single-stranded DNA (ssDNA) molecules on the single-walled carbon nanotubes (SWNTs) by using biomolecular recognition ability of RecA proteins. In atomic force microscopy, the height distribution of observed rod-like hybrids showed that the ssDNA-SWNTs with RecA had larger height than the ssDNA-SWNTs. In agarose gel electrophoresis, the migration speed of the ssDNA-SWNTs was obviously decreased after adding RecA. For comparison, we performed similar tests for carboxymethylcellulose-SWNTs (CMC-SWNTs). Unlike the case of ssDNA-SWNTs, RecA didn’t bind to CMC-SWNTs. Our results revealed that ssDNA molecules on the SWNT surfaces maintain their native function because RecA proteins recognized ssDNA on the SWNT surfaces precisely.

1P121 新規プリオンアプタマーの探索とその構造研究
Search for novel aptamer against prion protein and its structural study

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We have elucidated the structural basis of anti-prion activity of aptamer against prion protein, r(GGAGGAGGAGGA) (R12). R12 folds into unique quadruplex and its structure is responsible for high affinity to prion protein and anti-prion activity. Here, we have performed screening of the anti-prion activity for the nucleic acids that fold into various kinds of quadruplexes. Then, we have obtained a nucleic acid that reduced the amount of an abnormal prion protein down to 10% of that in the control. We estimated the 50% inhibitory concentration of the newly selected nucleic acid and it was around 100 nM. Analyses of the structure and mechanism by which the newly selected nucleic acid exerts higher anti-prion activity are now in progress.

1P122 剪断流下における二重ラセンＤＮＡのダイナミクス
Dynamics of double helix DNA under shear flow

Yosuke Fujita[1], Nobumasa Nakazawa[1], Takako Kato-Minoura[1], Hiroyuki Iwamoto[2], Shinji Kamimura[1] ([1]*Dept. Biol. Sci., Chuo Univ.*, [2]*SPring-8, JASRI*)

The DNA double helix of Watson-Crick model, which is called B-DNA, is composed of base pairs arranged with an approximately 3.4 nm helical pitch. The structure has been believed to be corresponding primarily to that of DNA inside live cells. However, direct evidence to show the structure of native DNA strands in solution has not been enough to get more detailed insights. In the present study, we used a modified rotary rheometer device, which has been successfully applied to align microtubules in solution for the analysis of X-ray fiber diffractions. We observed X-ray diffraction of salmon sperm DNA in solution at BL45XU in SPring-8. We found dynamic and reversible pitch conversions of DNA between from 3.4 nm to 2.9 nm under shear flow conditions.

1P123 ヌクレオソームＤＮＡ解離の自由エネルギープロファイル
Free Energy Profile of Nucleosomal DNA Unwrapping

Hidetoshi Kono, Shun Sakuraba, Hisashi Ishida (*Molecular Modeling and Simulation, JAEA*)

Eukaryotic genome is compactly stored into a tiny nucleus of cell in the form of protein-DNA complex. This protein-DNA complex is called as nucleosome which is composed of a histone octamer formed by two copies each of the four core histones H3, H4, H2A and H2B and about 150bp of DNA wrapping almost twice around the octamer. This compact form, however, has to be unwrapped in transcription, DNA duplication and DNA repair processes. To study the detailed molecular mechanism, free energy profiles for unwrapping nucleosomal DNA on several nucleosomes were calculated. So far, we estimated that a cost for unwrapping the outer DNA is 0.1 to 0.4 kcal/mol/1bp, consistent with a value experimentally obtained. In the meeting, we will report the cost for unwrapping the inner DNA.

1P124 SecM の N 末端側領域による翻訳アレストの安定化
N-terminal region of SecM is essential for its stable translation arrest

Zhuohao Yang, Ryo Iizuka, Takashi Funatsu (*Grad. Sch. of Pharm. Sci., The Univ. of Tokyo*)

SecM, a bacterial secretary protein, contains a specific amino acid sequence at the C-terminus, which interacts with the ribosomal tunnel and arrests its translation. It is accepted that the sequence, called arrest sequence, is sufficient and necessary to induce SecM-mediated translation arrest. However, in cell-free translation assays, we have found that the arrest sequence is necessary but not sufficient to induce translation arrest, when fused to the C-terminus of HaloTag. We have also found that the stability of the translation arrest depends on the protein structure outside the ribosome and the presence of the N-terminal region of SecM increases the stability. We then concluded that the N-terminal region of SecM is essential for its stable translation arrest.

1P125 蛍光タンパク質との融合による光二量体化モジュール（Photodimerizer）の機能評価
Functional evaluation of the light-induced dimerizing module, Photodimerizer, fused with fluorescent proteins

Osamu Hisatomi, Yoichi Nakatani, Yuki Kai (*Grad. Sch. od Sci., Osaka Univ.*)

A synthetic gene encoding the light-induced dimerizing module, Photodimerizer (Pd), was prepared and introduced in *E-coli* cells. Pd protein showed the same properties with a N-terminally truncated mutant (ZLC_2S) of aureochrome-1 [1]. To evaluate the performance of Pd, we prepared YFP-Pd and mCherry-Pd fusion proteins and investigted their oligomeric structures and DNA-bindings. Similar to Pd, both fusion proteins are monomeric in the dark state, and form dimers and increase their affinity for the target DNA sequence in the light state. Our results suggest that Pd is a useful molecular tool for the light-dependent dimerization and DNA-binding of the fusion proteins.

[1] Hisatomi *et al.* (2014) J. Biol. Chem. *in press* (published online)

1P126 大腸菌 *E.coli* ゲノム複製開始における DNA 二重らせん開裂機構の分子動力学シミュレーション研究
DNA unwinding mechanism at DNA replication initiation of *E.coli* studied by coarse grained molecular dynamics simulation

Masahiro Shimizu[1], Tsutomu Katayama[2], Shoji Takada[1] ([1]*Dept. of Biophys., School of Sci., Kyoto Univ.*, [2]*Dept. of Mol. Biol., Kyusyu Univ.*)

The DNA replication initiation complex is formed on *oriC* region in *E.coli* genome. This complex unwinds the DNA duplex of A/T rich region in *oriC*. But structure of the DNA replication initiation complex, which contains about ten proteins and DNA, is unknown. So the mechanism how this complex unwinds DNA remains elusive. In order to reveal the unwinding mechanism, we conducted coarse-grained molecular dynamics simulation. First, we elucidated the structure of replication initiation complex of *E.coli* including *oriC* left-half region, IHF protein and domain III-IV of DnaA protein. Second we investigated interaction between DnaA protein and A/T rich region in *oriC*. Based on our simulation, we propose the DNA unwinding mechanism in DNA replication initiation complex.

1P127 Model of reverse tRNA translocation through the ribosome analyzed by electron microscopy density maps and molecular dynamics simulations

Hisashi Ishida, Atsushi Matsumoto (*Quantum Beam Science Center, Japan Atomic Energy Agency*)

To understand the mechanism of reverse tRNA translocation, all-atom molecular dynamics simulations of the ribosome-tRNAs-mRNA-EFG complex were performed. The complex at the post-translocational state was directed towards the pre-translocational states by fitting the complex into cryo-EM density maps. Multistep structural changes, such as a ratchet-like motion and rotation of the head of the small subunit were observed. The results indicate that the coupled motion of the head rotation and tRNA translocation plays an important role in opening and closing of the P/E-gatet. Conformational change of EF-G was interpreted as the result of the combination of the external motion by L12 around an axis passing near the sarcin-ricin loop, and internal hinge-bending motion.

1P128 染色体対合形成の力学モデル
Dynamical model of chromosome synapsis formation

Keisuke Yamamoto[1], Hiraku Nishimori[1,2], Akinori Awazu[1,2] ([1]*Dept. of Math. and Life Sci., Hiroshima Univ.*, [2]*Research Center for the Mathematics on Chromatin Live Dynamics*)

Several eukaryote exhibit “homologous recombination” during meiosis to keep their genetic diversity. Homologous recombination requires juxtaposition and synapsis formation of homologous loci in maternal and paternal chromosomes all along their length. In recent studies of budding yeast, C. elegans and fission yeast, the dramatic dynamical motions of nucleus and chromosomes are observed during meiotic prophase. Although such motion might play important rolls for the pairing of homologous loci, the contributions of it remains unclear. Then, in this study, we construct a coarse-grained model of chromosomes at meiotic prophase. By the simulation of this model, we investigate the influences of nucleus and chromosome motions on the synapsis formations of homologous loci.

1P129 タンパク質構造に対するイオン効果のエネルギー解析
Free-energy analysis of the effect of ions on protein structure

Yasuhito Karino[1,2], Yuji Sugita[1,2,3] ([1]*RIKEN QBiC*, [2]*RIKEN AICS*, [3]*RIKEN*)

Recently, several studies for a crowded cellular environment using the molecular dynamics simulation have been performed, and the crowded environment stabilizes/destabilizes the folded states of proteins. The influence of ions near the protein is still not negligible to the protein stability even in such environment. The solvation free energy of the system is one of the most important physical quantities but it is difficult to be obtained from the conventional free-energy calculations. To understand the effect of water and ions on protein stability, we calculated the solvation free energy of a protein in solution including ions by using the method of energy representation. In the future, we will try to calculate the ion effect in the crowded environment.

1P130 積分方程式理論を用いた剛体球混合系における選択的溶媒和の研究
Preferential Solvation in Hard-Sphere Mixtures: Integral Equation Study

Yuichi Kawabata, Ryo Akiyama (*Grad. Sch. Sci., Univ. Kyushu*)

Crowders affect the stability of a protein in aqueous solution through preferential solvation. To elucidate the effect of molecular crowding, we studied solvation free energy (SFE) in hard-sphere mixture system. We solved the HNC-OZ equation for the system which contains solvent (small-sized sphere), crowders (medium-sized sphere), and a solute (large-sized sphere), varying the composition of the mixture at constant pressure. To evaluate the SFE of the solute, we adopted two methods; one was based on Morita-Hiroike equation, and the other was Kirkwood-Buff theory. We obtained opposite tendencies for two methods when the size of crowder molecule was large.

1P131 グルタミン酸脱水素酵素のドメイン運動に協奏した水和構造変化
Hydration structure controls domain motion of glutamate dehydrogenase

Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2] ([1]*Dept. of Phys., Keio Univ.*, [2]*Harima Inst., Riken*)

Protein folds and functions only in aqueous solution, and therefore water should play a crucial role in the functionality of biomolecules. In this study, we investigate how functional motions of glutamate dehydrogenase (GDH) couple with its hydration structure by using 200-ns MD simulation. The structure of GDH is homohexamer, and each subunit consists of two domains. The analyses of cryogenic X-ray crystal structure and the MD trajectory indicate that the major dynamics of GDH in solution is a open-close motion between these two domains. From the detailed analyses of water molecules in the trajectory, we have found that the domain motion concerts with the cooperative changes of the hydration structures in the hydrophobic cleft between the two domains.

1P132 テラピア及チョウザメコラーゲン水溶液の広帯域誘電緩和
Broadband dielectric relaxation of the aqueous solution of tilapia and sturgeon collagen

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We measured broadband dielectric response of the collagen aqueous solutions with several concentrations in both microwave and terahertz regions. The collagen structure depends on the species and site, which is grouped as type I and II for our sample (tilapia and sturgeon). The dominant Debye relaxation at 20GHz and its succeeding tiny one at 0.5 THz remains on the subtle addition of the collagen type I to pure water. This result suggests that the microscopic structure of water hydrogen-bonding network is unaffected by huge collagen molecules (length 150 nm, molecular weight 300,000), while macroscopically the dissolved collagen to pure water increases the solution viscosity drastically. The difference with concentration, spices, and site will be discussed.

1P133 極性タンパク質 PAR-2 の非対称局在は、細胞質における拡散によって安定化される

Stable maintenance of the cortical PAR-2 asymmetry by the cytoplasmic diffusion in the one-cell *C. elegans* embryo

Yukinobu Arata[1], Michio Hiroshima[2], Chan-gi Pack[1], Kennichi Nakazato[3], Tetsuya Kobayashi J.[4], Tatsuo Shibata[5], Yasushi Sako[1] ([1]*Cell. Info. Lab., RIKEN*, [2]*Lab. Cell Sig. Dyn., QBiC, RIKEN*, [3]*Theor. Biol. Lab., RIKEN*, [4]*Inst. Indst. Sci., Univ. Tokyo*, [5]*Lab. Phy. Biol., CDB, RIKEN*)

Asymmetric localizations of polarity proteins are maintained by feedback controls, but how the dynamics are balanced *in vivo* remains unexplored. Using single-molecule detection technologies, we measured dynamics of *C. elegans* polarity protein PAR-2 comprehensively. We identified the novel cortical fractions that dissociated in a fast rate and were laterally immobile on the cortex. Single-molecule imaging revealed that the novel fractions contain less-oligomerized and highly-phosphorylated PAR-2. Our mathematical model showed that dynamics measured *in vivo* measurements were sufficient for the cortical asymmetry by bi-stability, but unexpectedly emphasized a role of the components for the stability of asymmetry. A similar mechanism may function in other polarity systems.

1P134 組織応力の異方性が細胞の六角格子化を促進する

若手招待講演

Anisotropic tissue stress promotes ordering in hexagonal cell packing

Kaoru Sugimura[1], Keisuke Ikawa[1], Shuji Ishihara[3] ([1]*Kyoto Univ.*, [2]*JST PRESTO*, [3]*Meiji Univ.*)

Many epithelial tissues pack cells into a honeycomb pattern to support their structural and functional integrity. Developmental changes in cell packing geometry have been shown to be regulated by mechanical and biochemical interactions between cells; however, it is largely unknown how molecular and cell-level dynamics and tissue mechanics are orchestrated to realize the correct and robust development of hexagonal cell packing. By combining mechanical and genetic perturbations along with live imaging and Bayesian force inference, we identified the mechanism through which the mechanical anisotropy in a tissue promotes ordering in cell packing geometry. We are currently investigating a regulatory mechanism of force sensation and generation of F-actin during this process.

1P135 細胞間に非対称性を生み出すしくみの再構成

若手招待講演

Reconstitution of an intercellular symmetry breaking mechanism

Mitsuhiro Matsuda[1], Makito Koga[1], Knut Woltjen[2], Eisuke Nishida[3], **Miki Ebisuya**[1] ([1]*RIKEN CDB*, [2]*CiRA, Kyoto Univ*, [3]*Grad Sch of Biostudies, Kyoto Univ*)

Cells diversify into different cell-types during development. We have recently reconstituted a mechanism for creating a stable difference among cells, which is mediated by Delta-Notch signaling. In our synthetic gene circuit, production of a membrane protein, Delta, in a cell inhibits production of Delta in the adjacent cells. Such intercellular mutual inhibition functions as a (double-negative) positive feedback loop, which amplifies a small initial difference between adjacent cells. In fact, mammalian cultured cells engineered with our gene circuit spontaneously split into two distinct cell-types: Delta-positive and -negative populations. Interestingly, the ratio of two cell-types was robust against perturbation and also adjustable by the architecture of gene circuit.

1P136 低温電子顕微鏡による骨格筋の細いフィラメントの構造解析

Structural analysis of Ca2+ regulated thin filament from skeletal muscle by electron cryomicroscopy

Yurika Yamada[1], Takashi Fujii[2], Keiichi Namba[1,2] ([1]*Grad. Sch. of Frontier Biosci., Osaka Univ.*, [2]*QBiC, RIKEN*)

Muscle contraction is caused by sliding between the thick and thin filaments. The thin filament is composed of actin filament, tropomyosin (Tm) and troponin (TnC, TnI, TnT). It is thought that Ca2+ released from sarcoplasmic reticulum binds to TnC, leading to a conformational change of Tm on the actin filament to allow actin-myosin interaction. To understand this regulatory mechanism by electron cryomicroscopy, we developed a method to isolate skeletal muscle thin filament from a crab, Portunus trituberculatus. We treated the thin filament by glutaraldehyde to crosslink Tm and Tn with actin filament and obtained a density map of the filament fully decorated with Tm. Interestingly, the cross-linked thin filament retained the ability of Ca2+ regulation.

1P137 中性子非弾性散乱による F-アクチン及びミオシン S1 のダイナミクス解析

Difference in dynamics between F-actin and myosin S1 measured by neutron scattering

Tatsuhito Matsuo[1], Toshiaki Arata[2], Toshiro Oda[3], Satoru Fujiwara[1] ([1]*Japan Atomic Energy Agency*, [2]*Osaka Univ.*, [3]*Univ. of Hyogo*)

The dynamics of F-actin and myosin S1 were studied by quasi-elastic neutron scattering (QENS). The QENS measurements were conducted on D_2O solution samples of F-actin and S1 at 300 K using the cold-neutron disk chopper spectrometer AMATERAS in J-PARC. In the current analysis, it was found that while the correlation time (τ) of atomic motions of S1 was similar to that of other proteins such as hemoglobin, the τ value of F-actin was shorter than S1. Furthermore, F-actin had a population of the atoms undergoing diffusive motions with larger amplitudes than S1. These results suggest that F-actin is more flexible than other proteins including S1. The results of a more detailed analysis will be given in the presentation.

1P138 アクチン疎水性ヘリックスに導入した変異は細胞性粘菌の細胞運動と細胞分裂を阻害する

Mutations introduced into the hydrophobic helix of the *Dictyostelium* actin impaired cell motility and cytokinesis

Takahiro Ohnuki[1], Yuki Gomibuchi[1], Taro Uyeda[2], Takeyuki Wakabayashi[1] ([1]*Teikyo Univ. School of Science and Engineering*, [2]*AIST*)

The hydrophobic helix of actin is a part of myosin-binding site(s). *Dictyostelium* cells carrying plasmids expressing the mutant actin (Tyr337Cys/Leu349Cys) showed dominant negative phenotypes. The size of starvation-induced mounds was smaller. The number of mounds was increased 10 times. When co-cultured with *E. coli* B/r, they migrated slower than wild-type cells. When cultured in suspension, the number of nuclei per cell increased by a factor of two, indicating impaired cytokinesis. The results are consistent with the reports showing the hydrophobic helix is important for the interaction with myosin. Surprisingly, ultracentrifugation analysis of the purified mutant actin showed the significantly higher polymerizability than the expressed wild-type actin.

1P139 **心筋症特異的なトロポミオシン変異体による再構成フィラメントの光ピンセット及び熱パルスを用いた in vitro assay**
Characterization of tropomyosin mutants that cause hypertrophic cardiomyopathy (HCM): In vitro assays with optical tweezers and heat pulse

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HCM is characterized by thickening of the left ventricular wall and the interventricular septum, and abnormality is suspected in contractile functions. Here, we studied the role of a human α-tropomyosin (Tm) mutant V95A in Ca2+ regulation of contraction. Thin filaments reconstituted with actin, troponin and Tm V95A were examined in in vitro motility assays, where the sliding velocity was measured between R.T. and ~40℃ and the sliding force with optical tweezers at R.T. We found that the thermal sensitivity of thin filaments reconstituted with V95A decreased compared to WT. This can be attributed to the lower binding affinity of V95A to actin, which may initiate maladaptive HCM. Similar results have been obtained in D175N, which is also known to cause HCM in humans.

1P140 **ミオシンフィラメント懸濁液の ATP 存在下でのプロトン NMR 緩和経過**
Spin-spin relaxation of 1H NMR signals from myosin filaments suspension with or without ATP

Tetsuo Ohno, Maki Yamaguchi (*Dept. of Physiol., The Jikei Univ. School of Med.*)

The dynamic changes of water molecules structure surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of 1H-NMR signals from suspension of myosin filaments prepared from rabbit could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T2). The slowest two components (T2 around 0.4s and 0.15s) dominated over faster relaxation components. This may suggest that the potential of the water molecules existing around myosin filaments is high.

1P141 **アクチン結合タンパク質は結合ドメイン単独でも細胞内の特定の F-アクチンに局在する**
Actin binding domains of certain actin binding proteins are sufficient to localize at specific F-actin in vivo

Keitaro Shiabta[1], Akira Nagasaki[1], Masatsune Tsujioka[2], Taro Q.P. Uyeda[1] ([1]*Biomed. Res. Inst., AIST*, [2]*Medical Res. Inst., TMDU*)

F-actin plays important roles in various cellular activities through interaction with actin binding proteins (ABPs). The ABPs specify function and intracellular localization of F-actin. However, how each ABP recognizes specific actin filaments within a cell is largely unknown. Here, we identified ABPs whose actin binding domains (ABDs) can localize at specific actin filaments within *Dictyostelium* cells when expressed as GFP-fusions. Since the ABDs are not expected to interact with any molecules except F-actin, they probably distinguish the structural difference of F-actin. Our findings suggest that the interaction between F-actin and an ABP cooperatively changes the F-actin structure, increasing or decreasing the affinity of the F-actin for various ABPs.

1P142 **昆虫飛翔筋線維の収縮蛋白交換実験と X 線回折**
X-ray diffraction from insect flight muscle with exchanged contractile proteins

Hiroyuki Iwamoto, Naoto Yagi (*SPring-8 JASRI*)

The function of stretch activation is essential for the asynchronous action of insect flight muscle (IFM), and the molecular mechanism for sensing of mechanical stretch has been unknown. We have shown evidence that the stretch-sensing mechanism is built into myosin heads themselves, and have proposed a mechanism in which pre-force attached myosin heads are converted to a force producing form when they are distorted in a specific manner by stretch. To further test this proposal, we are currently trying to exchange contractile proteins of IFM fibers with those from different sources, such as vertebrate skeletal muscle. Fluorescently labeled rabbit myosin is incorporated into IFM fibers, and X-ray diffraction patterns indicate the presence of myosin filaments.

1P143 **タキソールは、急速な微小管内チューブリン周期の伸長を誘導する**
Paclitaxel induces the quick elongation of tubulin dimer periodicity in microtubules

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Microtubules (MT) are key components of the cytoskeleton in eukaryotic cells. One of the most fundamental questions is how MT dynamics is associated with the molecular conformation of tubulin dimers within MTs. To address the issues, we applied our new technique for the rapid shear-flow alignment of biological filaments, which enabled us to acquire X-ray fiber diffraction data in seconds from native MTs under various physiological conditions. We found that the longitudinal repeat of tubulin dimers in MTs were elongated from 3.88 to 4.03 nm within 1 min after adding paclitaxel. Diameter changes appeared to be occurring in a slow time course (10-20 min). It is suggested paclitaxel induces quick elongation of tubulin dimers in MTs.

1P144 **V1-ATPase の粗視化分子動力学シミュレーション**
Course-grained molecular dynamics simulation of V1-ATPase

Hiroki Kashimura, Yuta Isaka, Yuichi Kokabu, Mitsunori Ikeguchi (*Yokohama City Univ.*)

V-ATPase is a molecular motor acting as ion pump. The soluble domain of V-ATPase (V1) is composed of the central stalk (DF) and hexamer ring (A3B3): three catalytic A-subunits and three B-subunits are alternatively arranged around DF. Coupled with an ATP hydrolysis, conformational changes of A3B3 induce a 120-degree rotation of DF. To elucidate the relationship between the conformational changes and the rotation, we conducted a coarse-grained MD simulation using Go-model. By switching Go-potentials of A3B3, we forced to change the A3B3 structure, and prepared two states before and after the 120-degree rotation of DF. We then analyzed the dynamics of DF in response to the conformational changes of A3B3.

1P145 フォトクロミック化合物フルギミドを結合したミオシン頭部ドメインのX線小角散乱

Small-angle X-ray Scattering Study of Photochromic Fulgimide-bound Myosin

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To clarify the structure of myosin head domain that bounds the photochromic fulgimide ATP analog, we have carried out the small-angle X-ray scattering experiments.
Fulgimide compound synthesized de novo by Maruta et al is a photochromic molecule that changes color and ring structure by light irradiation. It is possible to have an ATP similar structure to tri-phosphorylates fulgimide which binds to the ATP binding site of S1. We have examined the global structural changes of S1 by binding fulgimide ATP analog and light irradiation, using small-angle X-ray scattering. The S1 bound fulgimide ATP analog showed the shape change such as the radius of gyration increased when UV light was irradiated.

1P146 ATP 結合で誘起されるミオシンの誘電応答

Dielectric response of myosin induced by ATP binding

Takato Sato, Jun Ohnuki, Koji Umezawa, Mitsunori Takano (*Dept. of Pure & Appl. Phys., Waseda Univ.*)

Binding of a ligand induces allosteric response in a protein. The allosteric response is often understood by a sort of the principle of leverage. However, the ligand, with specific distribution of electric charge, should induce a large-scale response of protein through the rearrangements of charges and dipoles in the protein. Myosin, a well-studied molecular motor, is a typical allosteric protein, the ligands of which are a nucleotide and a divalent cation. When Mg-ATP binds to myosin, myosin dissociates from actin, the mechanism of which is still unclear. By conducting extensive all-atom model molecular dynamics simulation, we investigated the dielectric response of myosin upon ATP binding, and discuss the role of this dielectric response in the motor function.

1P147 Multiscale analysis of functional motions in F1-ATPase: From Pi release to elasticity and friction of γ-subunit rotation

若手招待講演

Kei-ichi Okazaki, Gerhard Hummer (*Max Planck Institute of Biophysics*)

F1-ATPase, the catalytic domain of ATP synthase, synthesizes most of the ATP in living organisms. Running in reverse powered by ATP hydrolysis, it creates torque on its central γ-subunit. First, we use molecular dynamics (MD) simulations to study the timing of Pi release coupled to the rotation. On the basis of metadynamics simulations and rate calculations, we clarify the timing and pathway of Pi release. Second, from the MD trajectories we construct a simple model to deduce elasticity and friction of γ-rotation. The deduced elastic properties are consistent with experiments. According to our analysis, the work performed in the torque-driven rotation is mostly stored as elastic energy with remarkably little dissipation, which explains high efficiency of the motor.

1P148 Molecular mechanism of the epsilon subunit from F-type ATP synthases studied by Molecular Dynamics simulations

Alexander Krah, Shoji Takada (*Department of Biophysics, Kyoto University*)

ATP synthases are the main producer of ATP, the universal energy source in all living cells. For ATP synthesis, they use a transmembrane electrochemical gradient. Vice versa they can generate an electrochemical gradient, hydrolysing ATP. While in mammals a pH dependent inhibitory protein IF1 regulates the ATP hydrolysis activity, bacteria avoid ATP hydrolysis by the [ATP] dependent subunit epsilon, which carries out a conformational change from the contracted to the inhibitory extended state if [ATP] gets too low. Understanding the ATP hydrolysis prevention mechanisms in bacteria, might help to develop new antibiotics. Using MD simulations and taking previous experiments into account, we propose novel features for the bacterial epsilon subunit of F-type ATP synthases.

1P149 ダイナクチンのアンテナ構造

Antenna structure of Dynactin Complex

Hatsuha Kajita, Takuya Kobayashi, Kei Saito, Yoko Y. Toyoshima (*Grad. Sch. of Arts & Sci., Univ. of Tokyo*)

Dynactin is a multi-molecular protein complex which regulates dynein activity. Previously we have shown that the coiled coil 1 domain (CC1) of p150 extrudes from the dynactin head and forms an antenna structure. Since CC1 directly binds dynein and regulates dynein activity, we purified CC1 fragments with different lengths and restricted the dynein binding region. By estimating molecular weight, each of the CC1 fragments appeared as a mixture of dimer and tetramer. We further purified the first half and the second half fragments of the CC1 and found that they did not bind each other. These results suggest the folding model that CC1 makes a parallel dimer first and then folds back at some middle points.

1P150 Structure of dimeric axonemal dynein in cilia suggests an alternative mechanism of force generation

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The ciliary outer arm dynein produces force during ciliary beating. However, it is still unknown how the ciliary dynein translocate the microtubule using the two different types of heads. Here, we analyzed the conformational change and its distribution in each dynein head of mouse respiratory cilia by cryo-electron tomography and image processing. Most of two heads were in the same form and tightly packed in the non-nucleotide condition, whereas they were dissociated and alternatively moves in the presence of nucleotide. In a significant number of dyneins in the presence of ADP/Vi, two heads overlap each other in the proximal shifting form, indicating that ciliary heterodimeric dynein translocates a microtubule by moving with short steps.

1P151 細胞質ダイニンの微小管上での運動は右方向にバイアスされている

Cytoplasmic dynein takes a route switching randomly between protofilaments with a bias toward the right

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Yeast cytoplasmic dynein (CD) motor proteins walk processively along a microtubule (MT). What path does CD move along a MT? To address this question, we developed three-dimensional tracking assay which allowed the CD-coated beads to move freely along and around the MT anchored on an etched glass as a suspension bridge, unhindered by glass surface. Observing movement of the CD-coated beads among all surfaces of the suspended MT, we found that the CD-coated beads moved laterally toward both the right and the left, when it moves toward the minus end of the MT. Our analysis showed that CD does not follow the MT's protofilament (Pf) axis, but that CD takes a route switching randomly between Pfs with a bias toward the right, inducing the mainly right-handed corkscrew path.

1P152 細菌べん毛モーター固定子複合体のイオン透過メカニズム

Ion permeation mechanism through the stator complex in the flagellar motor

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Bacterial flagellar motors are powered by ions (protons in *Escherichia coli* and sodium ions in *Vibrio alginolyticus*). The motor consists of a rotor and stators. The stator acts as a torque generation unit and comprises MotA and MotB protein in *E. coli* or PomA and PomB protein in *V. alginolyticus*. However the molecular mechanism for torque generation is still unclear.
To investigate the structural changes coupled with ion permeation through the stator, we performed molecular dynamics calculations with our model structures. Our results showed that the movements of helices in MotA and MotB were induced by ion permeation. We will also discuss the difference between the helix movements of MotA/MotB and PomA/PomB.

1P153 V1 モーターでのトルク発生機構

Torque generation mechanism in V1 motor

Mihori Baba[1], Shou Furuike[2], Atsuko Nakanishi[1], Jun-ichi Kishikawa[1], Nao Takeuchi[1], Ken Yokoyama[1] ([1]*Kyoto Sangyo University*, [2]*Osaka Medical College*)

The V1 rotary ATPases contain a rotor that rotates against a catalytic A3B3 stator. The rotor V1-DF is composed of both anti-parallel coiled coil and globular-loop parts. Single molecular analysis indicates that an anti-parallel coiled-coil domain of V1-D functions as a rotor in A3B3. Contrary, FliJ protein from flagellar, also composed of an anti-parallel coiled-coil, did not show the unidirectional rotation.
In this study, we report the essential region of D subunit for unidirectional rotation by a domain swapping approach.

1P154 LC1 Binds to the Stalk of the Outer Arm Dynein

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Outer arm dynein (OAD) complex drives beating of flagella/cilia. OAD complex is composed of three heavy chains (α, β, γ HCs), intermediate chains (ICs), and light chains (LCs), but the exact subunit architecture has not been elucidated.
LC1 is a 22-kDa light chain and widely preserved among many species. To directly label the LC1 in ODA complex, we expressed recombinant His-tagged LC1 in *Tetrahymena* and *Chlamydomonas*. By electron microscopy aided by Ni-NTA-gold labeling, LC1 was found at the γ-stalk (*Chlamydomonas*), unlike the previous assumption that LC1 bound at the γ-head. Pull-down assay revealed that LC1 preferentially binds to γ-stalk at 1:1 molar ratio. These results raise the possibility that LC1 regulates OAD activity by changing its affinity to microtubules.

1P155 溶媒を陽に考慮した全原子分子動力学シミュレーションから得られたダイニンの弾性

Elastic property of dynein motor domain obtained from all-atom molecular dynamic simulations in explicit water

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Dyneins are large microtubule motor proteins. ADP-bound high-resolution structures have revealed the organization of the dynein motor domain which comprises the AAA+ ring, the linker, stalk/strut and C sequence. However, a high-resolution ATP-bound dynein conformer remains unclear. We modeled the ATP-bound form, and carried out a 200-ns molecular dynamics simulation for both ADP- and ATP-bound forms using our psygene-G program to investigate the effect of ATP on the structure and dynamics. The stalk of the ATP-bound form was more flexible than that of the ADP-bound form. Two additional 50-ns simulations starting from the ADP-bound form with ATP ligand reproduced this flexibility. The rigidness of both obtained trajectories qualitatively agrees with experimental results.

1P156 細菌Ⅲ型分泌装置の回転運動およびエフェクター分泌に対する高粘性高分子の物理化学的影響評価

Evaluation of physicochemical effect of viscous polymers toward rotation and effector secretion of bacterial type III secretion apparatus

Takashi Ohgita, Naoki Hayashi, Naomasa Gotoh, Kentaro Kogure (*Kyoto Pharm. Univ.*)

Like drug-injection, bacteria inject effector proteins into host cells via needle-like type III secretion apparatus (T3SA). However, the mechanism of effector transport through T3SA is unclear. T3SA shows structural similarities with flagellum. Effector secretion via T3SA requires proton-motive force (PMF) like flagellar rotation. Based on the facts, we hypothesized that T3SA would secrete effectors by PMF-dependent rotation. Previously, we succeeded in observing PMF-dependent T3SA rotation. In this study, we examined whether the rotation correlates with the secretion. The effector secretion was suppressed, when T3SA rotation was physicochemically inhibited by viscous polymers. Consequently, it was suggested that effector secretion is induced by T3SA rotation.

1P157 The role of amino acid residues located at the catalytic site in the rotation of *Enterococcus hirae* V_1-ATPase

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We investigated the role of amino acid residues loated at the catalytic site in the rotation of *Enterococcus hirae* V_1-ATPase. We observed the rotation of the mutants A-F506E, A-F425E (both residues interact wit hadenine ring of ATP), and B-R350K (the arginie finger) by using a 40 or 50 nm Au colloid as a load free probe. All mutatns rotated unidirectionally and the rotation rates obeyed the Michaelis-Menten kinetics. A-F506E and A-F425E reduced the second-order binding rate constant for ATP (k_{on}^{ATP}) by 40 and 4000 times. Additionaly, A-F425E reduced not only k_{on}^{ATP} but also the maximal rotation rate by 20 times. B-R350K only reduced the maximal rotation rate by 300 times. Our results revealed the different roles of these amino acid residues.

1P158 マイコプラズマ Gli349 の構造解析
Structural analysis of the gliding protein Gli349 from Mycoplasma mobile

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Mycoplasma mobile clings to a cell surface and glides over it using a leg protein Gli349 through its large conformational changes. Previous studies suggest that Gli349 is composed of 18 repeats of ~100 residues, which are named A to V. However, detailed structure of the protein remains unclear. Here, we constructed 15 fragments of the Gli349 protein and found that only the KLM fragment, which overlaps the regions of repeats K, L, and M, is soluble. We carried out small-angle X-ray scattering measurements of the KLM fragment and revealed that the fragment is composed of three globular domains connected sequentially. The results suggest that full-length Gli349 has a tandem repeat structure of globular domains.

1P159 確率的モデリングによるキネシンの化学-力学ネットワーク
Chemomechanical network modeling of kinesin

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We present a chemomechanical network model for kinesin based on the general network theory of Liepelt and Lipowsky for cytoplasmic molecular motors with two heads. It had been pointed out that the internal strain between two heads induces asymmetries between the chemical transition rates on the leading and trailing heads. In this study, we systematically constructed an 8-state network model for kinesin by taking into account the symmetry/asymmetry between state transitions in the network. We found that the main chemomechanical cycle strongly depends on the ATP concentration and the main cycles at high and low ATP concentrations are very different from the straightforward regular cycle that is obtained from several reduced-state models.

1P160 F_1-ATPase の P-loop 変異体 TF_1(βG158A)に対するリン酸の阻害効果
Inhibitory effect of Pi on F_1-ATPase P-loop mutant TF_1(βG158A)

Hitoshi Hoshina[1], Hikaru Yoshida[1], Ayumi Ito[1], Jotaro Ito[1,3], Shoichi Toyabe[2], Hiroshi Ueno[1], Eiro Muneyuki[1] ([1]*Dept. of Physics. Chuo Univ.*, [2]*Faculty of Physics. Tohoku Univ.*, [3]*School of Engineering, The University of Tokyo*)

The reaction scheme of F_1-ATPase has mostly been established. But the timing of Pi release remains to be determined. In 2010 Watanabe et al gave an answer to this question that F_1 released Pi after ADP (Nat Chem Biol. 2010 Nov; 6 (11)794-5). To challenge this issue by another clue we paid our attention to p-loop structure in β subunit. The structure accommodates γPi of the ATP so this part seems to be related with the Pi release. We investigated the properties of TF_1 p-loop mutant (βG158A). By bulk assay this mutant was less active, difficult to entrap in the ADP inhibition and easy to be affected by Pi. By single molecule observation we found that Pi prolonged the catalytic dwell like the ADP inhibition. The scheme of F_1 rotation is under investigation.

1P161 一定外力下での F1 の回転の観察
Observation of the rotation of F1-ATPase under the constant external torque

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F_1-ATPase is a rotary molecular motor which consists of the $\alpha_3\beta_3$ ring and a rotor subunit (γ-shaft). When F_1 hydrolyze ATP, g-shaft rotates to counter clockwise. On the other hand, when an external torque force γ-shaft to rotate to clockwise, F_1 synthesizes ATP from ADP and Pi. The detailed kinetics and reaction scheme of ATP hydrolysis have been studied intensively. But knowledge of ATP synthesizes is less than one of ATP hydrolysis, due to the difficulty in observation of the F_1 rotation under the constant external torque. In this study, we observed the rotation of F_1 and discuss the kinetics under the constant external torque. To apply a constant external torque of precisely controlled magnitude, we use the electrorotaion method.

1P162 Single-molecule fluorescent observations of the biased binding/unbinding of the tethered kinesin head

Kouhei Matsuzaki, Michio Tomishige (*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*)

Kinesin-1 is a motor protein that moves processively along microtubules by alternately hydrolyzing ATP. To move in a hand-over-hand manner, the trailing head should detach from microtubules prior to the leading head, although the underlying mechanism remains unknown. In this study we anchored a kinesin head to microtubule via a long flexible linker that allows the head to diffuse and access to the ±16 nm forward/backward binding sites and observed the binding and unbinding motions of the head using FIONA. The observation showed that the dwell time at the forward binding site was three times longer than that at the backward binding site, suggesting that the backward tension posed to the neck linker decreases the detachment rate from microtubule.

1P163 TF1 βE190D 変異体の外部トルク依存性

The response of TF1 βE190D mutant to the external torque

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F1-ATPase is a motor protein driven by ATP hydrolysis. It converts chemical free energy change into the kinetic energy of the rotation. Toyabe examined the external torque dependency of the rotation rate of wild type F1-ATPase (PANS 2011). He found the maximum work estimated from stall torque is nearly equal to the free energy change of ATP hydrolysis, reached conclusion of high energy efficiency. Thus, interest issue is to find out the mechanism of this high efficiency. In the previous annual meeting, we reported that βE190D mutant exhibited response to external torque different from WT. Furthermore, we found that the stall torque was smaller than that of WT. Here, we discuss origin of the characteristic response of βE190D mutant to the external torque.

1P164 軸糸外腕ダイニンによるトルク発生

Torque generation by axonemal outer-arm dynein

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Outer-arm dynein is the main engine providing the motive force in cilia. *Tetrahymena* outer-arm dynein consists of three different heavy chains, termed α, β and γ, and is decomposed into two-headed βγ subparticle and one-headed α subparticle by chymotoryptic digestion. Using three-dimensional tracking microscopy, we found that contrary to previous reports the outer-arm dynein and its subparticles rotated sliding microtubules around their longitudinal axis in an *in vitro* microtubule gliding assay. Furthermore microtubule corkscrewing motion driven by the dynein and its subparticles showed different ATP concentration dependencies. Our results suggest that the three-headed outer-arm dynein integrates three dynein motor domains with distinct mechanical properties.

1P165 演題取り消し

1P166 偏光変調 TRIFM とデフォーカスイメージングによる単一蛍光色素の角度と回転方向の検出

Detection of 3-D orientation and rotation of single fluorophores by combination of polarization-modulation TIRFM and defocused imaging

Shoko Fujimura, Nagisa Mikami, Tatsuro Itoh, Takayuki Nishizaka (*Dept. Phys., Gakushuin Univ.*)

Determination of rotating conformational changes in a protein at the single molecular level is crucial to uncover mechanochemical characteristics of working enzymes. Under TIRF illumination with *p*-polarized beam, the intensity of the fluorophore becomes maximal when its dipole directs perpendicular to the sample plane. Sequential defocused images of sparsely labelled microtubules, which slid on the lawns of single-headed kinesin-1, were captured under the illumination, and applied for the verification of our system. The left-handed rotation was detected as in 3-D tracking method (Yajima, Mizutani & Nishizaka, 2008), and the dipole angle against the microtubule axis was successfully estimated as ˜30°.

1P167 ビブリオ菌べん毛の MS リングを構成し大量発現で可溶性となる膜タンパク質 FliF の生化学的解析

Biochemical analysis of the membrane protein FliF, a MS-ring component of *Vibrio* flagellar motor with being soluble when overproduced

Erika Yamaguchi, Seiji Kojima, Michio Homma (*Div. Biol. Sci., Grad. Sch. Sci., Univ. Nagoya*)

The MS-ring is a part of the bacterial flagellar basal structure embedded in the inner membrane and composed of membrane protein FliF. It has two transmembrane segments and a large periplasmic region. When *Vibrio alginolyticus fliF* is overexpressed in *Escherichia coli*, FliF is equally detected from soluble and membrane fractions. Size exclusion chromatography showed that soluble FliF behaves as an oligomer.
Here we examined several conditions for *Vibrio* FliF purification and found that it was eluted at smaller molecular size from a gel filtration chromatography, when solubilized by detergent at an alkaline condition (pH 11). We will optimize the purification procedures to purify FliF as a monomer and crystallize.

1P168 クライオ電子線トモグラフィー法を用いたフィロポディア内構造解析

The ultrastructure of filopodia were observed with cryo-ET

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In the last decade, the *in vivo* biophysical research techniques have been developed. However most methods for structural biology have been limited to the *in vitro* level. Hence, we revealed the structure of proteins in cells with cryo-ET.
We focus on the intracellular structure of filopodia, and we successfully obtained the 3D volumes of filopodia with cryo-ET. In filopodia, actin filaments were bundled tightly and parallelly. They were bundled by actin bundling protein, presumably assigned as fascia. It bound to actin filaments in 36 nm period, which is equal to the half pitch of actin filaments.
In the next step, we are introducing 3D subtomogram averaging techniques to improve the resolution of intracellular protein structures.

1P169 微小管結合蛋白質 MAP2、MAP4、Tau の F-アクチン-微小管束化活性の違い

Difference of F-actin-microtubule bundling activity of microtubule-associated proteins, MAP2, MAP4, and Tau

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The structural microtubule-associated proteins, MAP2, MAP4 and tau,play an important role in the intracellular organization of microtubules by binding to microtubule. Recently, we revealed that MAP4 also could bind to actin filaments as MAP2, and the main actin-binding site was the C-terminal part of the Pro-rich region in microtubule-binding domain. In this study, we examined behavior of these structural MAPs when both actin filaments and microtubules coexisted in a sample. The results showed that MAP4 and MAP2 but not tau bound to actin filament retained microtubule binding abilities, and consequently induced actin filament-microtubule bundles. On the other hand, all MAPs bound to microtubules could not bind to actin filaments.

1P170 単離したラジアルスポークと微小管との結合

Binding of the Isolated Radial Spokes and Microtubules

Hitoshi Sakakibara, Youské Shimizu, Hiroaki Kojima (*Bio-ICT, NICT*)

Radial spokes of eukaryotic flagella are large protein complexes (~2 M), which connect 9 peripheral microtubules and the central-pair apparatus. Two or three radial spokes are aligned in a 96 nm structural period on the A subfiber of the peripheral microtubules. Using purified radial spokes of *Chlamydomonas* and microtubules of porcine brain tubulin, we examined the binding of the radial spokes and microtubules. When the sample of radial spokes was incubated with microtubules and followed by centrifugation, they were co-sedimented. By negative-stain electron microscopy, we observed that the structures of similar configurations to the radial spokes were attached to the microtubules. These suggest that the purified radial spoke contains microtubule binding subunits.

1P171 海洋性ビブリオ菌のべん毛本数抑制に関与する FlhG の ATPase 活性の役割

Role of ATPase activity of FlhG on the negative regulation of the flagellar number in *Vibrio alginolyticus*

Hikaru Hirata, Akari Takashima, Hiroki Ono, Michio Homma, Seiji Kojima (*Div.Bio.Sci., Grad.Sch.Sci., Univ.Nagoya*)

Marine bacteria *Vibrio alginolyticus* has a single polar flagellum whose the number is regulated positively by FlhF and negatively by FlhG. FlhG is a homolog of MinD, a cell division inhibitor of Escherichia coli, which has an ATPase motif. Mutational analysis revealed that the ATPase motif is necessary for proper function of FlhG. Here we overproduced and purified FlhG, and measured its ATPase activity. We found that FlhG actually hydrolyzed ATP, and mutation D171A enhanced its activity although the corresponding mutation in MinD abolished ATPase activation by MinE. We expect that ATPase activity is related to the function of FlhG and that FlhG behaves in different way from MinD. We will discuss a mechanism of regulating the number of flagella in the meeting.

1P172 細胞収縮コラーゲンゲル中における分子の拡散挙動

Biomolecular diffusion in contracted collagen gel caused by fibroblasts

Takanori Kihara[1], Junri Ito[2], Jun Miyake[2] ([1]*Faculty Environmental Engineering, Univ. Kitakyushu*, [2]*Grad. Sch. Engineering Science, Osaka Univ.*)

Many biomolecules secreted from cells diffuse throughout the ECM. Therefore, investigation of the diffusive behaviors of biomolecules in the extracellular environment is critical. In this study, we investigated the diffusion coefficients of biomolecules of various sizes by fluorescence correlation spectroscopy in contracted collagen gel caused by fibroblasts. In collagen gels populated with fibroblasts, the diffusion coefficient at the cell vicinity clearly decreased in the first 24 h of culture. Furthermore, molecular diffusion was greatly restricted, with a central focus on the populated cells. Thus, biomolecular diffusion is restricted in the vicinity of the cells where collagen fibers are highly condensed.

1P173 ATP および ADP 結合アクチンフィラメントに対するコフィリン結合の一分子観察

Single molecule imaging of the binding of cofilin to ATP- and ADP-F-actin

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Biding of cofilin to actin filaments modulates the actin dynamics by severing actin filaments, and is thought to be crucial for cell growth, survival, and cell motility. Cofilin binds to actin filaments with positive cooperativity, and the binding accelerates the conversion of ATP-actin to ADP-actin. Binding of cofilin to ADP- and ATP-F-actin was imaged at the single molecule level, which showed that the on-rate of cofilin binding to ATP-F-actin was lower than that of to the ADP-F-actin. The cooperativity factor ω of cofilin binding to the ATP-F-actin was ca. 3 times higher than that of to the ADP-F-actin, suggesting that ATP-actin is locally converted to ADP-actin, and the on-rate of cofilin binding is elevated there.

1P174 Na^+駆動型べん毛モーターの固定子複合体のナノディスク再構成への試み

Attempt to reconstruct the stator complex of the bacterial Na^+-driven flagellar motor into Nanodisc

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The stator complex in the bacterial flagellar motor forms the specific ion-conducting pathway. The ion flux of stator generates a torque by interacting with FliG. The stator of the Na^+-driven motor of *Vibrio alginolyticus* consists of 4 PomA and 2 PomB molecules. Toward the determination of the whole structure of the stator complex or the observation of function and interaction, we have tried to purify some variations of the stator complex and reconstruct them into liposome or Nanodisc. In this poster, we show the successful result of Nanodisc reconstruction by using *E. coli* polar lipid (EPL). From these results, now we are planning to reveal the physical or the structural properties by using NMR spectroscopy.

1P175 Localization and roles of F_1-ATPase subunit homologs and P42 of *Mycoplasma mobile* revealed by gene manipulation

Tulum Isil, Masaru Yabe, Atsuko Uenoyama, Makoto Miyata (*Osaka City University*)

M. mobile, the fastest mycoplasma species, glides on solid surfaces. In this study, we elucidated the subcellular localization of two proteins plausibly involved in the gliding mechanism: P42 and a homolog of the F_1-ATPase α-subunit , by using the EYFP. The F_1-ATPase α and β subunit homologs form the tentacles of the "jellyfish structure", the intracellular part of gliding machinery and suggest that the movements for gliding are generated by ATP hydrolysis at this structure. The sequence analysis of P42 suggested that it might evolve from a common ancestor with FtsZ, and could play a role in supporting or bridging part in the machinery. To clarify the roles of these two proteins, we will replace them by dominant negative alleles.

1P176 原子間力顕微鏡によるマウス顎下腺組織の弾性率マッピング測定

Mapping elastic modulus of mouse submandibular gland tissue by atomic force microscopy

Mitsuhiro Nakamura[1], Yuki Fujii[1], Hiroaki Taketa[2], Takuya Matsumoto[2], Takaharu Okajima[1] ([1]*Grad. Sch. Info. Sci. & Tech.,Univ. Hokkaido*, [2]*Grad. Sch. Med. Den. & Pham. Sci.,Univ. Okayama*)

It is recognized that biological functions of cells and tissue change depending their mechanical microenvironment during morphogenesis. Recent study [1] revealed that the stiffness of substrate affects the morphogenesis of tissue. To elucidate how tissue feels the surrounding external forces, the mechanical properties of cells and tissue during morphogenesis is crucial, but the less direct mechanical measurement of tissue has been examined. Here, we reported the local elastic modulus E of mouse submandibular gland tissue (SMG) measured by atomic force microscopy. The results showed that the E of the SMG was highly correlated with the height. The detailed relationship between E and the internal structures will be presented.

[1] Miyajima et al. Biomaterials 32 (2011) 6754

1P177 アクチンの Tyr143 に変異を導入した細胞性粘菌は高圧処理に対してより敏感になる

Dictyostelium cells carrying the plasmids to express mutant actin (Tyr143Phe) are more susceptible to high-pressure treatment

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Actin is important for cytoplasmic processes including motility, cytokinesis, cell adhesion, cellular signaling. *Dictyostelium* cells carrying the plasmid to express Tyr143Phe-actin migrated slowly, with the spreading velocity being ~1/2. The effects of high-pressure on such *Dictyostelium* cells were examined using a high-pressure microscope. The shape of the cells became spherical. The recovery phase after the treatment (60 MPa, 2 min) was observed using a usual microscope. Cells tended to show thin filopodia-like protrusions. The cells carrying plasmids to express Tyr143Phe-actin showed more protrusions than those carrying plasmids to express the wild-type actin or the plasmid-free wild-type cells that appeared most robust against high pressure.

1P178 粘菌管で作る懸垂線のコイル形成

Coiling of catenaries made from *Physarum* tube

Takahiro Noguchi[1], Taito Watanabe[1], Itsuki Kunita[2], Hirofumi Wada[3], Toshiyuki Nakagaki[2], Yoshimi Tanaka[4] ([1]*Grad. Sch. Sci., Yokohama National Univ.*, [2]*RIES., Hokkaido Univ.*, [3]*Physical Sciences., Ritsumeikan Univ.*, [4]*Environment and Information Science., Yokohama National Univ.*)

Plasmodia of *Physarum polycephalum* consist of anterior migrating parts and posterior tubular parts. In our experiment, we made catenaries from tube parts of plasmodia and observed active movements of the 'living' catenaries. The plasmodia catenaries fluctuated in the earlier stage, and then took left-handed coiling shapes. We discuss the self-coiling phenomena from physiological and mechanical points of view.

1P179 溶液中のアクチン繊維の構造のゆらぎ

Diversity of monomers configuration within a single actin filament detected by FRET

Sakura Maesato, Kenji Kobayasi, Hajime Honda (*Department of Bioengineering, Nagaoka University of Technology*)

The conformations of actin monomer in various conditions have been discussed recent years. Signals from FRET-pair located on the same monomer demonstrate the existence of at least two different conformational states (2007). We have focused on the inter-monomer configurations within a filament in contrast to intra-monomer ones. Either donor or acceptor was labeled to G-actin. Donor-labeled, acceptor-labeled and non-labeled monomers were mixed to form filaments. Fluorescent signal from FRET occurred within a filament was measured. Fluctuation of the time-developed signal indicated at least four different monomer configurations. In addition, the configuration spread about one micron along the filament and changing about several seconds interval.

1P180 蛍光顕微鏡で見た AMP-PNP アクチンの重合

Observation of polymerization with AMP-PNP bound actin molecules

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Bound nucleotides of actin monomers are structural stability but not to be essential for their polymerization. However, hydrolysis of ATP to ADP at the near end of each filament is certainly playing a central role for the dynamic property of the filaments in cells. Then, how many monomers are binding ATP at the end of the filament? In order to answer the question, we have tried to reveal the localization of ATP within a single filament. We have prepared AMP-PNP bound actin monomers. The monomers were found to polymerize into short filament about 0.1~0.2 micrometers long. On the other hand, ATP-bound ones formed filament of about 10 micrometers. Nucleotide exchange and hydrolysis within a filament would be discussed.

1P181 モータータンパク質を活用したナノバイオデバイスの開発
Nano-Bio-Devices transporting antigens for electrical measurements

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Motor proteins have been studied intensively but not devoted for engineering applications yet. Recently, we have developed a motor protein-equipped electronic device detecting various antigens related to human diseases. Actin and myosin molecules are used to catch, transport and concentrate the target antigen molecules. The target molecules were put down on the set of electrode where the impedance of the solution was measured. As the accretion of the molecule proceeds, the changes in impedance can be continuously measured. This device provides superior cost-effectiveness as it could operate only 10 nL of specimen without additional solvents. This device should be the first products based on the motor-protein research outcomes.

1P182 RAF の疾病関連変異体のコンフォメーションと機能
Conformation and function of disease-associated RAF mutants

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RAF is an important intracellular signaling molecule involved in cell-proliferation and differentiation. It has been reported that some activating mutations in RAF relate to the serious developmental disorder Noonan syndrome, however, the pathogenic mechanisms of these mutations are unknown in detail. Here, using FRET based probes of RAF, we investigated conformations of RAF and its mutants simultaneously with the cellular responses upon stimulation with epidermal growth factor, which causes RAF activation, in individual cells. Additionally, we investigated the activation level of MEK, a downstream component of RAF. Based on the results, we will discuss the mechanism of hyper activation found in the disease-associated mutants of RAF.

1P183 シロイヌナズナアクチンアイソフォームはファロイジンに結合しない
Arabidopsis thaliana actin isoforms do not bind phalloidin

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We expressed two major vegetative actin isoforms in *Arabidopsis thaliana*, AtACT2 and AtACT7, as fusion proteins with thymosin (Noguchi et al., 2007), and purified intact actins individually. Both plant actins co-polymerized with skeletal muscle (sk) actin. Also the two actins were similar to sk actin in persistence length. But phalloidin and rhodamine-phalloidin bound weakly to AtACT7 and almost not at all to AtACT2, despite the conserved sequence of the phalloidin binding site. Additionally the rates of polymerization of plant actins were two-fold faster than sk or *Dictyostelium* actin. To understand unique functional requirements of plant actin, we are performing additional biochemical analyses to reveal similarities and differences among plant, sk and amoeba actins.

1P184 Motor activity of myosin-II is required for maintanance of the contractile ring in fission yeast

Masak Takaine, Osamu Numata, Kentaro Nakano (*Univ. of Tsukuba*)

In many eukaryotic cells, actin and myosin-II coassemble surrounding the equator after metaphase. The actomyosin ring, which is called the cytokinetic contractile ring (CR), is supposed to mechanically divide the cell into two. The molecular details of the actin-myosin-II interaction remain unclear. We address this issue by using a fission yeast model. In this study, we investigated the localization and function of a monomeric myosin-II Myo3. Modification of the Myo3's motor activity altered its accumulation kinetics and local concentration. Moreover, in the absence of functional myosin-II, the CR normally formed but frequently collapsed during late anaphase, suggesting that the myosin-II's motor activity is primarily vital for maintenance of the CR.

1P185 コラーゲン溶液中の微粒子に働く粘性力の速度依存性
Velocity dependence of drag force acting on a micro particle in collagen solution

Masafumi Kuroda, Yoshihiro Murayama (*Dept. of Applied Physics, Tokyo University of Agriculture and Technology*)

Using a loosely trapping optical tweezers and three dimensional position tracking of thermally fluctuating microspheres, we can observe local structures of surrounding polymer solution. In contrast to the normal homogeneous fluid, in a collagen solution the motion of probe particle shows extraordinary distorted distribution, which indicates polymer networks deform with relatively slow dynamics. We demonstrated the velocity dependence of drag force acting on a micro particles traveling in polymer solution which reflects the inhomogeneous structure and corresponding variation in effective viscosity of the environment.

1P186 Direct measurement of *Vibrio alginolyticus* polar flagellum growth rate

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Bacterial flagella are self-assembled external tubular filaments. Flagellins, flagellum monomers (FliC), are pumped out from the basal body associated with a nanometer transportation system. As FliC reaches the end of flagellar filament, it becomes the new extending part of filaments. By using a fast flagella protein binding assay and laser scanning confocal microscope, we were able to observe short flagella and measure the growth rate directly. We found that the filament growth rate decrease exponentially with filament length. We also found the growth rate increase as temperature increase indicating the enhancement of transporting rate. We will discuss the implication of physical mechanisms.

We thank Seiji Kojima and Michio Homma for bacterial strains.

1P187 極限環境下での超好熱始原菌の運動観察
Motility of *Thermococcus kodakaraensis* cells at extreme environmental conditions

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Thermococcus kodakarensis is a species of thermophilic archaea. Unlike other types of bacteria, thermophiles can survive at much hotter temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures. Here, we studied the motility machinery of *Thermococcus kodakaraensis* strain KOD1. Electron microscopy revealed that the cells have several dozen polar flagella. We directly observed the swimming motility of KOD1 cells at high temperature conditions. Half of the cells swam smoothly with a speed of 110 μm s^{-1} at optimum growth temperature of 85 °C. A rotating tethered cell assay showed the cell body rotated with a speed of 4 Hz. We will discuss the detailed mechanism of the motility machinery of KOD1 cells in the meeting.

1P188 蛍光量子ドットを用いた細胞内高速小胞輸送機構の解明
Mechanism of high-speed vesicular transport inside cells explored by using quantum dots

Kenji Kikushima, Hideo Higuchi (*Dept. of Phys., Sch. of Sci., The Univ. of Tokyo*)

Previously, we have succeeded in non-invasive in vivo imaging of vesicle movements inside the neutrophil in mouse by using quantum dots. Surprisingly, we found that the velocity of these vesicles occasionally reached almost 4 um/s, which is about 4 times faster than that of molecular motors observed in vitro. In order to elucidate molecular mechanisms of the high-speed vesicular transport, here we tested the effects of some cytoskeletal agents to the vesicular transport inside the purified neutrophil or several cell lines. We revealed that the high-speed vesicular transport is result from the coordination of microtubule- and actin-based molecular motors.

1P189 Examining mitotic functions of bipolar kinesin Eg5 in a reconstituted minimal microtubule network

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The proper assembly of the mitotic spindle requires a balance of forces whose magnitudes must somehow be regulated within its dynamic microtubule network. However, it has been unclear if such a regulation can be achieved by any microtubule-associated protein. Here we show, using an optical trap- and TIRF-based reconstitution assay, that ensembles of mitotic kinesin Eg5 crosslink two adjacent microtubules while generating pushing and resisting forces, of which magnitude and direction depend on the microtubule overlap length and the relative polymer polarity. Based on these findings we discuss how simple geometric features in cytoskeletal networks can regulate mechanical outputs of motor proteins to assemble functional structures required for error-free cell division.

1P190 細胞サイズ液滴内でのアクトミオシンリングの自発形成と収縮
In vitro self-assembly and contraction of actomyosin rings inside a cell-sized droplet

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During cytokinesis, animal cells assemble a contractile ring composed of actin filaments and myosin motors to separate the cell body into two. Here, we constructed a simple model system from purified actomyosin, actin bundling factor, and cell-sized droplets that captured basic features of the entire process of cytokinetic ring dynamics. Actin polymerization inside the droplets induced spontaneous formation of ring-shaped actin bundles at the equatorial plane. Myosin assisted ring formation by dynamic remodeling of actin networks. A spontaneous increase in motor density on the actin ring triggered complete contraction, followed by the ring disassembly. This in vitro system models simple design principles and minimal essential elements of cytokinetic rings in cells.

1P191 単離マウス気管上皮繊毛が生み出す力の三次元顕微計測
Force measurement of individual isolated mouse tracheal cilia using three-dimensional optical trapping

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Beating of airway epithelial cilia generates fluid flow, composed of clearly distinguished effective and recovery strokes. We herein aim to understand the correlation between the cilium-generated forces and the asymmetric beating by combining optical tweezers with 3-D tracking microscopy. A fluorescent bead was attached to the tip of the single cilium, and was trapped at various points apart from the position of the post-effective stroke. The maximum force increased from 10 to 80 pN as the trap center was displaced from that position. We also measured the stiffness of a cilium; the spring constant of cilium was approximately 6 pN/μm in ADP·Vi state. Our results suggest that the increase in the force arises partially from the bending deformation of the ciliary axoneme.

1P192 微小管結合タンパク質シヌクレインは細胞質ダイニンを細胞辺縁部に運ぶ機能を持つ輸送性微小管の形成に必須である
Synucleins are essential for the creation of transportable microtubules, which is required for anterograde transport of cytoplasmic dynein

Shiori Toba[1], Kotaro Koyasako[2], Masami Yamada[1], Takuo Yasunaga[2], Hiroaki Kojima[3], Hideki Wanibuchi[1], Shinji Hirotsune[1] ([1]*Osaka City University Graduate School of Medicine*, [2]*Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology*, [3]*Advanced ICT Research Institute, National Institute of Information and Communications Technology*)

Synuclein is a neuronal protein that is linked genetically and neuropathologically to Parkinson's disease. Physiological role of synuclein is remaining largely unknown. Here, we show that synuclein families are an essential component to create transportable microtubules, which functions as a freighter for anterograde transport of cytoplasmic dynein.

Mutated synuclein shows different effect on microtubules from normal protein. Depletion of synuclein dramatically inhibited an anterograde and retrograde transport of cytoplasmic dynein.

Our findings uncovered the surprising functional relevance of synuclein proteins, microtubules and cytoplasmic dynein for anterograde transport, which will shed light on the novel pathogenesis of Parkinson disease.

1P193 べん毛本数を負に制御する FlhG の ATPase モチーフは FlhF の極局在の阻害に重要である

ATP binding motif of FlhG, a negative regulator of flagellar number, is important to prevent polar localization of FlhF

Hiroki Ono, Michio Homma, Seiji Kojima (*Grad. Sch. Sci., Univ. Nagoya*)

Marine bacterium *Vibrio alginolyticus* has a single polar flagellum whose number is regulated positively by FlhF and negatively by FlhG. FlhG is an ATPase: ATP binding motif of FlhG is important for flagellation, motility and subcellular localization of FlhG. Here we investigated the effect of FlhG mutations on subcellular localization of FlhF. We found strong FlhF polar localization in the ATP binding motif mutant FlhG(K36Q), which could not negatively regulate flagellation. The flagellar number may be determined by the number of FlhF molecules at pole, which is regulated by FlhG with ATPase motif. The effect of FlhG(D171A), which exhibits higher ATPase activity and severely inhibits flagellation, to subcellular localization of FlhF will be investigated.

1P194 大腸菌走化性シグナル伝達における CheZ 極局在の役割

Role of the polar localization of CheZ in chemotactic signal transduction of *Escherichia coli*

Yong-Suk Che, Hajime Fukuoka, Yuichi Inoue, Hiroto Takahashi, Akihiko Ishijima (*IMRAM, Tohoku Univ.*)

Binding of phosphorylated CheY (CheY-P) to the flagellar motor switches the rotational direction from CCW to CW. We proposed a transient increase and decrease in the CheY-P concentration are propagated from the receptor patch at cell pole to motors with a delay time. Here, we investigated the switching delay of the CCW-to-CW and CW-to-CCW between two different motors on the same cell. In wild-type cell, the switching delay clearly correlated with each motor's relative distance from the receptor patch. However in the localization-less mutant of CheZ (phosphatase for CheY-P), the distant dependency of switching delay was not observed in CW-to-CCW switching. The polar localization of CheZ is critical for the directed propagation of the decrease in CheY-P concentration.

1P195 回転電場による大腸菌の強制回転が細胞内 CheY の振る舞いに与える影響

Effect of forced-rotation of *E. coli*'s flagella motor on the behavior of intracellular CheY

Masaaki Sato, Hajime Fukuoka, Akihiko Ishijima (*IMRAM, Tohoku Univ.*)

E. coli senses chemotactic signals and regulates the rotational direction of its flagella motors by phospholyrated-CheY (CheY-P) binding to a motor complex. To investigate if external load to a flagella motor affects the CheY-P binding through conformational change of the motor, we applied the eternal load to a tethered cell by electro-rotation generated with 4 tungsten microelectrodes. When the cell was compulsorily rotated in clockwise direction, the fluorescent intensity from CheY-GFP at a motor was increased, but not in counter-clockwise direction. As for FliM-GFP, the fluorescent intensity at a motor was not changed by the electro-rotation to both directions. These results imply that the external load to flagella motors regulates the CheY-P binding.

1P196 海洋性ビブリオ菌のべん毛モーター回転方向決定における FliG と PomB の変異の影響

Effect of mutations in FliG and PomB on rotational direction of flagellar motor in *Vibrio alginolyticus*

Tatsuro Nishikino[1], Yasuhiro Onoue[2], Norihiro Takekawa[2], Shiwei Zhu[2], Seiji Kojima[2], Michio Homma[2] ([1]*Department of Biological Science, School of Science, Nagoya University*, [2]*Division of Biological Science, Graduate School of Science Nagoya University*)

Most of the bacteria can swim towards a favorable condition by rotating their flagella and changing their rotational direction. FliG is a component of the rotor complex and interacts with the stator complex that is composed of PomA and PomB. In this study, we investigated the role of FliG on rotational switching using a mutational approach. We observed that in the wild type, counterclockwise/clockwise rotational bias was 7 to 3, but some mutants showed more biased rotation (9 to 1 and 1 to 9). We also found a PomB mutant that can swim only in the presence of attractant. Combining these mutations of FliG and PomB, we are trying to understand the relation between stator-rotor interaction and rotational direction of flagellar motor.

1P197 基質伸展刺激下でケラトサイトは伸展に垂直にも平行にも運動する

Hybrid mechanosensing system for directional migration in fish keratocytes

Chika Okimura[1], Takafumi Mizuno[2], Yoshiaki Iwadate[1] ([1]*Fac. Sci., Yamaguchi Univ.*, [2]*AIST*)

The mechanical interaction between the cells and the substrata regulates the cell polarity for migration. Cyclic stretching-recovery of substratum (CSS) is one of the most appropriate techniques to estimate the relationship between the force from the substratum and the cell function. Fish epidermal keratocytes are known as fast migrating cells containing stress fibers in the cell body. It is well known that stress fibers of keratocytes depolymerize under low concentration of blebbistatin or some kinds of protein kinases. In response to CSS, "normal" keratocytes migrated parallel to the direction of CSS. Whereas, "stress fiber-less" keratocytes migrated perpendicular to that. We will discuss the possible mechanism of the bidirectional migration.

1P198 海洋性ビブリオ菌の c-di-GMP 結合タンパク質 PlzD における表現型および生化学的特性の解析

Biochemical and phenotypic characterization of PlzD, a YcgR homolog of c-di-GMP binding protein in Vibrio alginolyticus

Seiji Kojima, Takuro Yoneda, Michio Homma (*Grad. Sch. Sci., Nagoya Univ.*)

A small molecule, cyclic diguanylate (c-di-GMP) has been emerging as a second messenger that is involved in diverse bacterial signal transduction pathways. YcgR, a c-di-GMP binding protein in Escherichia coli, is known to inhibit flagellar motility by binding to the motor. Vibrio alginolyticus has a YcgR homolog, PlzD, and overproduction of PlzD reduced its polar flagellar motility on soft agar plate. Here, we overproduced and purified the recombinant PlzD expressed in E. coli. PlzD is easy to aggregate and so hampered further biochemical analyses. Immunoblot showed that PlzD could be detected at early stationary phase, but not in overnight culture. This suggests that PlzD may function in growth phase-dependent control of swimming, as found for YcgR in E. coli.

1P199 ケラトサイトとその断片の同一なかたち・細胞骨格・基質牽引力分布

Same traction force distributions in fish keratocytes and their fragments represent the same fan-shape

Ayane Sonoda, Chika Okimura, Yoshiaki Iwadate (*Dept. Funct. Mol. Biol., Grad. Sch. Med., Yamaguchi Univ.*)

Fish epidermal keratocytes show crawling migration keeping their fan-shape. Application of staurosporine, a kind of protein kinase inhibitor, induces fragmentation of the cells. After the fragmentation, two kinds of fragments, one is the fragment with cytoplasm and a nucleus and the other without them, continue locomotion keeping fan-shape, same as original keratocytes, for more than 1 hour. We observed actin stress fibers, filamentous myosin II and vinculin, a kind of focal adhesion protein, in original keratocytes and two kinds of fragments, and measured the traction forces of them. Comparing the distribution of the cytoskeletal proteins and traction forces among them, we will discuss the relationships between traction forces and the cell shape determination.

1P200 伸縮性の表層を伝わるゾウリムシのメタクロナールウェーブ

Ciliary metachronal wave propagation on the compliant surface of Paramecium cells

Naoki Narematsu[1], Quek Quek[2], Keng-Hwee Chiam[2], **Yoshiaki Iwadate**[1] ([1]*Fac. Sci., Yamaguchi Univ.*, [2]*A*STAR, Singapore*)

Ciliary movements in protozoa show metachronal coordination so as to maintain a constant phase difference between adjacent cilia. This coordination is called as “metachronal wave”.
It is now generally thought that metachronal waves arise from hydrodynamic coupling between adjacent cilia at extracellular fluid. However, in *Paramecium* cells, metachronal waves passed over the portion where the hydrodynamic coupling was broken.
Here, we propose that the compliant cell surface can also serve as a mediator of metachronal waves by combining computational modeling and experiments.

1P201 中世膜に結合したラクトフェランピンの膜結合構造と膜親和性に基づく抗菌活性機構の解析

Elucidation of the antimicrobial activity based on affinity and bound structure of LFampinB embedded into the neutral membrane

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Bovine lactoferrampin(LFampinB) is an antimicrobial peptide found in the N1-domain of bovine lactoferrin(268-284). The structure of LFampinB bound to the neutral membrane(DMPC:DMPG=5:1) was determined by analyzing the chemical shift anisotropies of carbonyl carbons of Leu3, Leu4, Ala7, Gln8, Phe11, Gly12. These results indicated that N-terminal region of LFampinB formed α-helix, and inserted into the bilayer and rotated rapidly about the bilayer normal with the tilt angle of 39 degree. The association constant (Ka) of LFampinB with the neutral lipid was 300 times smaller than that with the acidic membrane determined by QCM. The difference of the Ka value explains that LFampinB selectively interacts with the acidic bacterial membrane.

1P202 diphenylhexatriene を用いる脂質膜流動性測定に与える Aβ ペプチドの影響

Limitation of the use of diphenylhexatriene to measure the fluidity of membrane in the presence of amyloid β-peptide

Masako Suzuki, Takashi Miura, takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

The effect of amyloid β-peptide (Aβ) on the membrane fluidity has long been a subject of controversy, because both rigidifying and fluidizing effects have been reported. To reveal the reason for this discrepancy, we have re-examined the use of 1,6-diphenyl-1,3,5-hexatriene (DPH) as a fluorescent probe to measure the membrane fluidity.
We found that positive CD signals of DPH appeared regardless of D/L-chirality of DPPC when Aβ was added to DPH-labeled DPPC membranes. A chiral environment of Aβ is most likely to be the origin of the induced CD of DPH. These findings provide evidence that DPH molecules at least partly translocate from the membrane to Aβ. The result indicates a limitation of the use of DPH as a probe of the membrane fluidity in the presence of Aβ.

1P203 Dependence of Purple Membrane Bump Curvature on pH and Ionic Strength Analyzed by Atomic Force Microscopy Combined with Solvent Exchange

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Purple membrane (PM), which is self-assembly of membrane protein bacteriorhodopsin, is a good study subject for supramolecular formation mechanism of membrane proteins. Atomic force microscopy (AFM) studies for PM on mica substrate showed dome-like structures (bump) on the surface for unexplained reasons. The cause of bump appearance will be related to mechanism for spontaneous supramolecular formation because PM must have a curvature to form the bump. To study effects of electrostatic interaction on the bump curvature, AFM topography of identical PM sheets was collected varying solvent ionic strength and pH. The bump curvature was significantly influenced by salt concentration and pH, which suggests a significance of electrostatic repulsive force on the bump formation.

1P204 リン脂質/水界面における水和構造の分子動力学シミュレーション

Structure and orientation of hydrating water molecules at phospholipid/water interface revealed by molecular dynamics simulation

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Structure and dynamics of lipid/water interface are vital for the functions of membranes and membrane proteins. We have performed all-atom molecular dynamics simulation of zwitterionic phospholipid (POPC) with explicit water solvent and analyzed the lipid/water interactions at the interface. The results show that interfacial water forms a patch-like hydration structure near the interface. The water molecules heads one of their H atoms toward the phosphate group, while those around the choline group tend to have opposite orientation. The result is consistent with the recent experimental study (Mondal et al. (2012) J. Am. Chem. Soc., 134, 7842). Distinct structures of hydrating water at lipid/water interface are relevant for membrane-mediated biological functions.

1P205 膜の変形による BAR ドメインの凝集

Assembly of BAR domains induced by membrane shape deformation

Hiroshi Noguchi (*ISSP, Univ. Tokyo*)

It is known that BAR family proteins modify local curvature of biomembranes and often assembles into a tubular structure on the membrane. However, the assembly mechanism is not well understood. We have studied membrane-curvature mediated interactions between BAR domains using coarse-grained meshless membrane simulations. The BAR domain is assumed permanently adsorbed on the membrane and modeled as a rod curved in the normal direction of the membrane. We found that membrane shape deformation induces a two-step and three-step assemblies of the rods on a tubular membrane and vesicle, respectively, as the rod curvature is increased.

1P206 コレステロールによるホスファチジルコリン膜へのクロルゾキサゾン（筋弛緩剤）の結合阻害

Cholesterol inhibits the binding of chlorzoxazone (muscle relaxant agent) to phosphatidylcholine membranes

Ayumi Yamada, Hiroshi Takahashi (*Grad. Sch. Sci & Tech., Gunma Univ.*)

Many sorts of drugs are metabolized by cytochrome P450 (CYP) family in endoplasmic reticulum(ER) membranes. In this process, the following things have been proposed; before binding to CYP, drugs bind to the lipid membrane regions. However, if the drugs bind all types of biomembranes including containing no CYP, the metabolism would not work smoothly. Thus, we assume that the bonds of drugs are controlled by the content of cholesterol, from the fact that the cholesterol content of ER significantly lower than the other types of biomembranes. In this study, we examined the interaction to model membranes of chlorzoxazone using various techniques. As a result, we have found that the presence of cholesterol inhibits the penetration into phosphatidylcholine of the drug.

1P207 イノシトールリン脂質が誘起する支持脂質二重膜内のドメイン構造とタンパク質反応活性との関連

Relation between phosphatidylinositol-induced domain structure and protein reaction activity in supported lipid bilayer

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In plasma membranes, phosphatidylinositol (PI) is an anchorage for membrane-protein reactions relating to cellular signals, but the distribution of PI and the details on the reactions remain to be elucidated. Supported lipid bilayers (SLBs) are valuable system for the molecular-level approach with atomic force microscopy and single molecule tracking. The association between the structure of PI-containing SLB and the reaction activity of the membrane tublation of BAR domain proteins (FBP-17) was investigated. The observation of the PI-SLB on mica reveled that sub-micron depletion domains were formed and worked as the barrier for molecular diffusion at sub-second regime. We revealed that the domains worked as the nucleating site for the protein assembly on the PI-SLB.

1P208 細胞毒性を有する酸化コレステロールと酸性リン脂質（DMPG）の相互作用の構造学的研究

Structural studies of the interaction between cytotoxic oxysterols and acidic phospholipid (DMPG) bilayer membranes

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Many kinds of oxysterols are cytotoxic. For example, 7β-hydroxycholesterol (7β-OH) and 25-hydroxycholesterol (25-OH) have been reported to be associated with some diseases. At the last meeting, we reported the possible locations of these oxysterols in neutral phosphatidylcholine (POPC) bilayers, on the basis of the X-ray diffraction data. In this study, small angle X-ray scattering method was used to investigate the effects of these oxysterols on the structure of acidic phospholipid (DMPG) bilayers. The preliminary analysis suggested that 25-OH exhibits a similar thickness increasing effect of normal cholesterol, but 7β-OH does not. The detailed analysis on the locations of these sterols in the DMPG bilayers is now under progress.

1P209 脂質膜ナノチューブのダイナミクス

Dynamics of a single nano-tube hollow of phospholipid membrane

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We investigated the dynamics of a single soft nanotube hollow of phospholipids to extract nanoscale information such as the size of the tube hollow, which were several tens to hundreds of nanometers thick. The dynamic properties of the tubes obtained from direct observation by fluorescent microscopy, such as their persistence length, enable us to access the nanoscale characteristics through a simple elastic model of the membrane. The present methodology should be applicable to the nanosized membrane structure in living cells.

1P210 長鎖リン脂質と短鎖リン脂質で構成される脂質多成分系の相挙動と構造変化に関する研究

Study on the phase behavior and the structural changes of lipid multi-component system consisting of long- and short-chain phospholipids

Ryota Kobayashi, Tetsuhiko Ohba (*Dept. of Phys., Tohoku Univ.*)

The binary mixtures of long- and short-chain phospholipids show a complicated phase behavior depending on the lipid composition, concentration in water and temperature. For example, DMPC/DHPC mixtures are transparent or turbid, and have high viscosity at intermediate temperatures. The structure at this range is not yet clear although some models such as worm-like or disk-like aggregates had been proposed.
In this study, we observed the mixtures using a high-sensitivity different scanning calorimetry to elucidate the mechanism of structural changes in terms of thermodynamics.
The results show that there are complicated peaks caused by the melting of the hydrocarbon depending on the concentration and a peak caused by any change other than the melting at high temperature.

1P211 ラフト中でのアミロイド前駆体タンパク質の膜貫通部位の二量化にコレステロールが与える影響

Cholesterols affect to the association of the transmembrane region of Amyloid Precursor Protein in the raft

Naoyuki Miyashita[1,2], Fumiko Ogushi[3], Yuji Sugita[1,2,4] ([1]*RIKEN Quantitative Biology Center*, [2]*RIKEN AICS*, [3]*Ochanomizu University*, [4]*RIKEN*)

The bio-membrane often forms nano-domains, rafts, and provides a special environment to a membrane protein. Amyloid precursor protein (APP) produces an Amyloid beta peptide, which is the source of senile plaque in a brain. The APP forms dimer or monomer conformations in the bio-membrane. Recent experiments have suggested that the cholesterols attach to the Gly-xxx-Gly motif in the raft-like environments. However, the Gly-xxx-Gly motif is the important interface of the dimerization of APP too. We investigated how the cholesterols compete with the dimerization of APP, using Martini Coarse Grained (CG) model simulations. The cholesterols, of course, favored the dimer interface. Our results suggested that the cholesterols assist to the association of APP too.

1P212 リポソーム内膜タンパク質合成が誘起する脂質膜の形態変化

Morphological changes of the lipid membrane induced by in-liposome membrane protein synthesis

Kosuke Okamura, Hajime Watanabe, Tomoaki Matsuura (*Department of biotechnology, Graduate school of engineering Osaka university*)

Letm1, one of the mitochondrial inner membrane proteins, is assumed to be involved in the morphological changes of mitochondria. In vivo, it has been found that Letm1 dysfunction induced by knockdown or knockout experiments resulting morphological abnormalities of mitochondria. However, the exact role of Letm1 on the membrane morphology remains unclear. We, thus, aimed to elucidate it by the bottom-up approach. We synthesized Letm1 using a reconstituted in vitro transcription-translation system (the PURE system) inside a cell-size liposome, thereby investigating the role of the membrane protein on the dynamics of the cellular membrane. We observed clear morphological changes of the lipid membrane by synthesizing Letm1 inside the liposome.

1P213 光応答 DNA と細胞サイズリポソームの融合による人工細胞型分子ロボット

Artificial cell-based molecular robots by fusion of light responsive DNA and cell-sized liposomes

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Molecular robotics has attracted much attention as a novel engineering concept of self-assembled biological systems. Although nano-sized molecular robots based on the DNA/RNA molecules have been reported, micro-sized molecular robots have never been developed yet. Here, we proposed an artificial cell-based molecular robots as an integrated system of designed DNA/RNA molecules and cell-sized liposomes. In this system, we designed that RNA transcription starts by light stimulation, and the RNA and proteins autonomously self-assembles into a RNA-protein complex. We will report the more complex functions within interaction between RNA-protein complex and liposome.

1P214 チューブリン封入ジャイアントリポソームの温度・静水圧変化による可逆的形態制御

Reversible morphological control of tubulin-encapsulated giant-liposomes induced by change of hydrostatic pressure and temperature

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We examined the effect of hydrostatic pressure and temperature on the tubulin-encapsulated giant-liposomes. Many of the liposomes kept a bipolar shape with a central sphere and two tubular protrusions that aligned in a straight line at room temperature and ambient pressure as reported previously (Kaneko et.al., 1998). Immediately after the application of high pressure (80 MPa) or low temperature (4°C), the protrusions shrunk within several tens of seconds. This process was reversible; after the pressure was released or the temperature increased to 25°C, the protrusions regenerated within several minutes. Our results demonstrate that the shape of giant liposomes is controllable using the polymerization and depolymerization process of microtubules.

1P215 粘弾性流体を内包したリポソームの膜変形

Effects of viscoelastic cytoplasm in liposome on the shape deformation

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Liposomes have been used as a model of living cells. Under a hypotonic condition, the liposomes deform their shapes to minimize the bending energy. We investigated how the existence of macromolecules inside affects the liposomal deformation. We prepared liposomes containing 80 mg/ml macromolecules of BSA or a protein mixture extracted from *Escherichia coli*. The macromolecules were concentrated up to the physiological concentration, i.e. 300 mg/ml. Consequently, the condensation process and the shape deformation differed between BSA- and Cell Eextract-containing liposomes. These results strongly suggested that the crowded environment in cells is different from that found in typical single-component systems.

[1] K. Fujiwara and M. Yanagisawa, ACS Synth. Biol., in press.

1P216 表面張力レプリカ交換分子動力学法の開発と生体膜系への応用

Surface-tension replica-exchange molecular dynamics simulations of biological membrane systems

Takaharu Mori, Yuji Sugita (*RIKEN*)

Conformational sampling is fundamentally important for simulating complex bio-molecular systems. Here, we propose a new enhanced simulation method for membrane systems, which we call the surface-tension replica-exchange molecular dynamics method. We tested the method on two biological membrane systems: DPPC lipid bilayers and WALP23-POPC membranes. During these simulations, large-scale lateral deformation of the membranes took place, and there was accelerated lateral diffusion of DPPC lipid molecules compared with conventional MD simulation. We succeeded to predict the optimal orientation of WALP23 based on the free-energy calculation. Our method could be applicable to a wide variety of biological membrane systems.

1P217 一分子 FRET 測定法を用いた、膜貫通ヘリックス間相互作用への GXXXG モチーフの寄与の解明

Contributions of GXXXG motif to transmembrane helical interactions as revealed by single molecule FRET

Kotaro Kondo, Yoshiaki Yano, Katsumi Matsuzaki (*Grad. Sch. Pha., Univ. Kyoto*)

Transmembrane helix-helix interactions are important for folding and conformational changes of α-helical membrane proteins. These interactions are dependent on amino acid sequences, lipid compositions, and helical orientations, however, their mechanism is not fully understood. In this study, we focused on the transmembrane dimerization GXXXG motif to measure the association-dissociation dynamics of GXXXG-inserted transmembrane helices in lipid bilayers by single molecule FRET. We revealed that the insertion of the GXXXG motif significantly promoted the parallel association of helices compared with the control helices. We also found that not only amino acid sequences but also lipid compositions strongly regulated the transmembrane helix-helix interactions.

1P218 X 線１分子追跡法による 5 量体リガンド作動性イオンチャネル GLIC の pH 依存３Ｄ分子内運動マップ

PH dependent 3D Motion Maps of GLIC from X-ray Single Molecule Observations

Yuji Sasaki[1,2], Hiroshi Sekiguchi[2], Yufuku Matsushita[1], Keigo Ikezaki[1], Yuri Nishino[3], Atsuo Miyazawa[3], Christele Huon[4], Jean-Pierre Changeux[4], Pierre-Jean Corringer[4] ([1]*The University of Tokyo*, [2]*SPring-8/JASRI*, [3]*University of Hyogo*, [4]*Pasteur Institute*)

GLIC is a proton-gated bacterial ion channel from Gloeobacter violaceus and is member of pentameric ligand-gated ion channels. Recently structural information of GLIC in activated state (acidic pH) and closed state (neutral pH) are available and the gating mechanism is discussed based on these information. However the dynamic information of each state in physical point of view was not available. Here we used Diffracted X-ray Tracking (DXT) method to detect rotational motion of extracellular or transmembrane domain of GLIC from neutral to acidic pH. We found that tilting motion of transmembrane domain of GLIC and the both tilting and twisting motions of extracellular domain of GLIC were enhanced in acidic pH. The detailed dynamic information will be discussed.

1P219 電位依存性 H^+チャネルのゲート電流

Gating charge movement of the voltage-gated H^+ channel

Yuichiro Fujiwara, Yasushi Okamura (*Integrative Physiology, Grad Sch of Med., Osaka University*)

Voltage-gated channels are responsible for sensing membrane potential and generating electrical impulses in many organs. In the voltage sensing process, positively charged residues in the voltage sensor domain are known to sense membrane potential and move across the membrane electric filed, generating a transient 'gating current' ahead of an ionic current. However, it is mysterious that the voltage-gated H^+ channel (Hv) does not show the gating current. Here we report that we succeeded in recording the gating current, and it was affected by changing the pHo/i gradient and Zn^{2+}o. This suggests that the voltage sensing and the H^+ permeation can be considered separately in Hv, and the physiological modifiers affect the voltage sensing process.

1P220 再構成した電位依存性プロトンチャネルの電気生理学的計測

The electrophysiological recording of the reconstituted voltage-gated proton channel in artificial membrane

Akira Kawanabe, Yasushi Okamura (*Grad. Sch. Med., Osaka Univ.*)

The voltage-gated proton channel protein (VSOP/Hv1) is known to be controlled by membrane voltage and pH. A number of fundamental questions remain unanswered. These include mechanisms of voltage gating, sensing the pH difference across membrane and proton selective permeation. The reconstitution system is ideally suited for answering these questions. We therefore construct the reconstitution system of the expressed VSOP into the artificial membrane.
Mouse-VSOP/Hv1 protein heterologously expressed in E.coli was purified and reconstituted into the artificial membrane liposome. The activity of VSOP/Hv1 was confirmed upon liposomal pH change. We repot here our trial of electrophysiological recording of VSOP/Hv1 in the reconstituted proteoliposome.

1P221 カリウムチャネル KcsA のゲート開閉と連動した膜中集合・離散

Gating-associated clustering-dispersion dynamics of the KcsA potassium channel in a lipid membrane environment

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The KcsA potassium channel is a pH-dependent channel, and the activation gate opens at acidic pH. Using atomic force microscopy (AFM), we revealed pH-dependent clustering-dispersion behavior of the KcsA channels on the membrane, which is associated with the gating. At neutral pH, the closed channels formed self-assembled nanoclusters. At acidic pH, the open-gated channels were dispersed as singly-isolated channels. High-speed AFM revealed that the clustering-dispersion dynamics were completed within several minutes. The interplay between the gating conformational change of individual channels and the collective behavior of the clustering-dispersion provides insight into understanding membrane-mediated protein-protein interactions and functional cooperativity.

1P222 無細胞翻訳系による SecYEG トランスロコンの合成

In vitro synthesis of SecYEG translocon

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SecYEG is the bacterial protein conducting channel which facilitates the transport of membrane proteins and secretory proteins. In contrast to the structural and functional analysis, its biogenesis is still unknown. In order to study how SecYEG subunits assemble into complex, we aimed to reconstruct SecYEG on lipid vesicles by synthesizing its subunit proteins in the PURE system: the reconstituted cell-free protein synthesis system. Synthesized subunit SecY, SecE, and SecG proteins spontaneously formed SecYEG complexes on lipid membrane and showed the protein translocation activity. The results suggested that SecYEG protein could form an active complex in self-assembly process.

1P223 細菌 III 型分泌装置の in vitro 輸送再構成系の構築
Construction of an in vitro transport assay system for the bacterial type III protein secretion

Hiroyuki Terashima[1], Akihiro Kawamoto[2], Tohru Minamino[2], Keiichi Namba[2], Katsumi Imada[1] ([1]*Grad. Sch. Sci., Osaka univ.*, [2]*Grad. Sch. Front. Biosci., Osaka Univ.*)

The bacterial flagellum is a long filamentous organelle to swim in water. Most of components are translocated across the cytoplasmic membrane through the type III secretion complex. In spite of many genetic and biochemical studies, the molecular mechanism of protein transport is still unclear because regulatory feedbacks affect the flagellar construction. Thus, an in vitro transport assay system that enables precise control of the measurement conditions is required for further understanding of the protein transport. Here we show an inverted-membrane-vesicle based transport assay system. We succeeded in transporting flagellar proteins depending on proton motive force and hydrolysis energy of ATP. This system will allow us to precise measurement of the protein transport.

1P224 人工細胞モデルを用いた RNA の脂質二分子膜透過
Bilayer lipid membrane permeation of RNA

Kazuma Sato (*Grad. Sch. Inf Sci., Univ. Osaka*)

It has been thought that lipid bilayer is impermeable to charged molecules. But the possibility that some amino acids and NTP which have charge pass through the lipid bilayer was shown. So, we thought charged molecule such as RNA may also permeate the lipid bilayer and tried to demonstrate the RNA permeation. As an artificial cell model, we used giant liposome encapsulating cell-free protein synthesis system and added RNA encoding GFP gene to outside of liposome. If RNA pass through the lipid bilayer, GFP will be synthesized in liposome. So we detected the green fluorescence using fluorescence activated cell sorter and confocal laser scanning microscope. As a result, GFP synthesis in some liposomes was confirmed and the possibility of RNA permeation was suggested.

1P225 カルシウムシグナルによるシナプス構造の競合的制御
Competitive control of synaptic structure by calcium signaling

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We uncovered a novel homeostatic signaling pathway that promotes the stabilization of inhibitory GABAergic synaptic structure and transmission, using quantum dot single molecule imaging technique. IP_3 receptor-dependent calcium release evoked by metabotropic glutamate receptor signaling activated protein kinase C to promote GABAergic synaptic clustering and efficacy, through the regulation of $GABA_A$ receptor ($GABA_AR$) lateral diffusion. This process prevented $GABA_AR$ lateral dispersion by counteracting NMDAR-dependent influx of calcium and calcineurin activation. These finding show that distinct glutamate receptor signaling pathways trigger competing calcium signals to dynamically control the stability and efficacy of inhibitory synapses.

1P226 背に腹は代えられぬ： モノアラガイの隠された記憶
Necessity Knows No Law: Overwhelmed Memory in a Snail

Etsuro Ito, Miki Yamagishi (*Kagawa Sch. Pharmaceu. Sci., Tokushima Bunri Univ.*)

The pond snail *Lymnaea stagnalis* can be conditioned to avoid food and to consolidate the learning into a long-term memory (LTM). This is referred to as conditioned taste aversion (CTA). One-day food deprivation results in the best CTA-LTM; whereas 5-day food deprivation before training results in neither learning nor LTM. Here we asked if snails are food-deprived for 5 days and then subjected to taste-aversion training do in fact learn and form memory according to ‘necessity knows no law’. Thus, we trained 5-day food-deprived snails with 20 paired presentations of a sucrose solution and an electric shock. We found that memory was overwhelmed by snails being in a stressed-traumatic state due to severe food deprivation, and also found that CTA-LTM was context dependent.

1P227 Acute Modulation of long-term potentiation of Pyramidal Neurons by Hippocampal-derived Estrogen

Hiroki Kojima, Keisuke Hotta, Yoshitaka Hasegawa, Suguru Kawato (*Department of Life Science*)

Hippocampal-derived sex hormones are neuromodulators in the hippocampus. We investigated effects of Estradiol (E2). Adult male hippocampus synthesized 8nM E2, and synaptic estrogen receptors (ERα) exist in spines of hippocampal neurons. Localization of ERα in spines was demonstrated by immunogold electron microscopy. We demonstrated that E2 induced the long-term potentiation (LTP) of the hippocampal neurons by weak theta burst stimulation (sub-threshold stimulation) in hippocampal CA1 pyramidal neurons. E2 rapidly drives PKA, PKC, and MAPK through ERα and ERβ in hippocampal synapses. These kinases may phosphorylate NR2B subunit of NMDA receptor, leading to the increase in calcium influx and CaMKII activation, resulting in induction of LTP.

1P228 昆虫の投射ニューロンにおける樹状突起 Ca^{2+}上昇はシナプス入力によって修飾される活動電位波形に依存する
Dendritic Ca^{2+} elevation depends on spike waveform modulated by local synaptic activity in projection interneurons of insect

Hiroto Ogawa[1], Ruriko Mitani[2] ([1]*Dept Bio Sci, Fac Sci, Hokkaido Univ*, [2]*Biosystem Sci, Grad Sch Life Sci, Hokkaido Univ*)

Ca^{2+} imaging of mammalian cortical neurons have demonstrated that action potential (AP) evoked by synaptic stimulation back-propagates into the dendritic arbor and causes global Ca^{2+} elevation throughout whole dendrites. However, we found that selective stimulation of presynaptic afferents made difference in spatial pattern of dendritic Ca^{2+} rise even if either stimulation evoked single AP in the insect interneurons. Comparing the AP recorded at various locations in dendrites, the stimulation of ipsilateral afferents to recording site evoked larger dendritic spike than the contralateral stimulus. It is possible that spike-initiation site is shifted by local synaptic activity, resulting in input site-dependency in dendritic Ca^{2+} elevation via spike-waveform modulation.

1P229 新規な多電極を用いた海馬の神経回路に対する神経ステロイドの作用の解析

Analysis of neurosteroid effects on hippocampal neural circuits using novel multi-electrode probe methods

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By using custom multielectrode probes, suitable for hippocampal neural circuits, we have investigated rapid regulation of synaptic plasticity by neurosteroids in rat hippocampal slices. The long-term potentiation (LTP) is essential in memory encoding processes. Neurosteroids synthesized within the hippocampus. The mechanism of neurosteroids action is still not clear. We blocked the synthesis of pregnenolone, which is a kind of neurosteroids by AG (aminoglutethimide), inhibitor of cytochrome P450scc. AG perfusion also rapidly decreased EPSP (excitatory postsynaptic potentiation). We can rescue the suppression of LTP and EPSP by supplementation of exogenous pregnenolone. These results show the effect of pregnenolone on rapid regulation of neuronal synaptic plasticity.

1P230 滑走細菌 *Flavobacterium johnsoniae* の滑走装置

Gliding machinery of the gliding bacterium *Flavobacterium johnsoniae*

Satoshi Shibata[1], Keiko Sato[1], Yuka Narita[1], Daisuke Nakane[2], Koji Nakayama[1] ([1]*Nagasaki Univ. Graduate Sch. of Biomedical Science*, [2]*Dept. Physics, Gakushuin Univ.*)

Cells of *F. johnsoniae* move over surfaces, which is called gliding motility. This motility is unrelated to other well-sutudy motility mechanisms. In our recent study we proposed a "Helical loop track model", where the SprB adhesion is propelled along left-handed helical loop on the cell surface. Microscopic analysis with fluorescent labeling of non-SprB surface proteins, which are located at fixed position between poles when the cell body moves forward, verified that cells glide on surfaces with rotating CCW. In addition, tracking of SprB signals revealed that an SprB can overtake another SprB, change lanes and move on a different route during pole-pole oscillation. These results suggest the presence of multiple helical lanes for SprB movement on the cell surface.

1P231 トラウマストレスはエンドカンナビノイドシステムを介しオペラント条件付けによる記憶の形成を障害する

Traumatic stress impairs learning and memory formation via an endocannabinoid system in *Lymnaea stagnalis*

Hiroshi Sunada[1], Jeremy Forest[1], Manabu Sakakibara[2], Ken Lukowiak[1] ([1]*HBI, Univ of Calgary*, [2]*Grad. Sch. Biosci., Tokai Univ*)

Intense stress can cause impairment of learning and memory. This impairment may be due to activation of the endocannabinoid system. Here we determined in *Lymnaea stagnalis* whether traumatic stress altered both learning and long-term memory (LTM) formation. Snails received traumatic stress and we tested the ability to form LTM 2, 4, and 7 days later. Learning and memory were impaired on day 2 and 4, and was fully recovered by day 7. Next, we examined if the actions of endocannabinoid receptor 1 (CB1R) mediate this deficit. Injection of WIN55, an agonist for CB1R, in the absence of stress, mimicked the stress-induced memory impairment. Conversely, administration of AM251, a CB1R antagonist, prior to the intense stressor significantly mitigated LTM impairment.

1P232 ファラオニス・ハロロドプシンの N 中間体の結晶構造解析

Crystallographic analysis of the N intermediate of pharaonis halorhodopsin

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Halorhodopsin utilizes light energy to transport chloride ions from the extracellular side to the cytoplasmic side. In this study, we investigated light-induced structural changes in pharaonis halorhodopsin (pHR) using the C2 crystal. When the bromide-ion-bound purple form of pHR was exposed to red light, the N intermediate with a bromide ion in the cytoplasmic vicinity of the retinal Schiff base was generated efficiently in one of the three subunits in the asymmetric unit. This subunit has the EF loop facing a free space, so that a profound outward movement of the cytoplasmic half of helix F was induced upon formation of the N state. This structural change was accompanied by a large swing of Phe259 in helix G, creating a water channel in the cytoplasmic half.

1P233 クラックスロドプシンの 3 量体構造と光安定性

Photostability of the trimeric form of cruxrhodopsin

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Cruxrhodopsin-3 (cR3) is a light-driven proton pump found in the cell membrane of Haloarcula vallismortis. Our previous crystallographic analysis has shown that cR3 forms a trimeric assembly which is stabilized by direct protein-protein interactions. In this study, we investigated how the photostability of cR3 is dependent strongly on the protein-lipid and/or protein-protein interactions. We will discuss structural factors that affect the stability of the trimeric assembly.

1P234 hCRBPII 及びその変異体の構造とスペクトルに関する QM/MM 理論計算

QM/MM calculation of structure and spectral properties for human cellular retinal binding protein II (hCRBPII) and its mutants

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Structures and spectral properties of water soluble retinal binding proteins, human cellular retinal binding protein II(hCRBPII) and its mutants, were recently reported by Borhan's and co-workers. In their study, molecular engineering created a series of mutants which exhibit photoabsorption with absorption maxima widely distributed from 425 nm to 644 nm. However, the underlying color tuning mechanism remains unclear. In the present study, we calculated the absorption maxima with QM/MM reweighting free energy SCF (RWFE-SCF) method to elucidate how the mutations of several essential residues change the structure of protein and thus alter the spectral proterties of the chromophore.

1P235 センサリーロドプシン I-トランスデューサーの一分子 FRET 観察

Single-molecule FRET study of the sensory rhodopsinI-transducer

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Sensory rhodopsin I, SRI, is a dual photo-receptor membrane protein regulating both positive- and negative phototaxis in collaboration with its cognate transducer protein, HtrI. Here we used single-molecule fluorescence-resonance-energy-transfer (FRET) method to observe conformational changes and structural fluctuations in the complex.
We designed the fusion complex of SRI and HtrI. The protein was labeled with the dyes, which are expected to work as FRET-donor and the acceptor. We obtained the fluorescence signals from the labeled SRI-HtrI by illuminating with evanescent wave produced by the total internal reflection of the laser beam. As the result, single-molecular FRET between SRI and HtrI was successfully observed by this system. Its implications will be discussed.

1P236 海洋緑藻 *Ostreococcus tauri* 由来の光修復酵素（CPF1, CPF2）における FAD 光反応中心の分光解析

Spectroscopic analysis of FAD photoreaction center in two photolyase (CPF1, CPF2) from a marine green alga *Ostreococcus tauri*

Shouhei Ueda[1], Kazunori Zikihara[1,2], Tomoko Ishikawa[2], Chris Bowler[3], Takeshi Todo[2], Satoru Tokutomi[1] ([1]*Grad. School Sci., Osaka Prefect. Univ*, [2]*Grad. School Med., Osaka Univ*, [3]*Ecole. Norm. Supe., Inst. Biol., Paris*)

Cryptochrome/Photolyase-Family (CPF) is widely distributed from bacteria to plant and animal kingdoms. Generally, photolyase binds two chromophores, a flavin adenine dinucleotide (FAD) and a N5,N10-methenyl-5,6,7,8-tetrahydrofolate (MTHF) non-covalently. Recently, five CPF genes were identified in marine green alga *Ostreococcus tauri* (*Ot*). It was reported that *Ot*_CPF1 and *Ot*_CPF2 have (6-4)-photolyase activity cyclobutane pyrimidine dimer (CPD)-photolyase activity, respectively. We measured photochemistry and structural change of the chromophores using UV-Vis absorption and circular dichroism (CD) spectroscopies, respectively. In comparison of these results, the difference in MTHF binding and FAD conformation between *Ot*_CPF1 and *Ot*_CPF2 will be discussed.

1P237 光修復酵素の DNA 修復能と光反応中心 FAD コンフォメーションとの相関

Correlation of DNA repair type with FAD conformation in the photoreaction center of photolyases

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Far-UV light produces two major DNA photoproducts, cyclobutane pyrimidine dimer (CPD) and pyrimidine-pyrimidone (6-4) photoproduct. Most organisms have two photolyases to repair these photoproducts using near UV and blue light. They cannot repair different types of photoproduct each other. Both binds a flavin adenine dinucleotide (FAD) non-covalently in the photoreaction center. We studied the FAD conformations of a variety of photolyases with circular dichroism (CD) spectroscopy and found that CPD- and (6-4)-photrolyases show a negative and a positive CD signal of FAD in its fully reduced form, respectively.

1P238 チャネルロドプシンの機能理解への理論的アプローチ

Theoretical approach toward an understanding of molecular functions of channelrhodopsin

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Channelrhodopsins (ChRs), light-gated caion channels, were originally found in green algae where they serve as sensory photoreceptors for photophobic response. Recent establishment of ChR expression in mammalian neurons enables optical control of nerve impulse on intact brain. We succeeded in structural modeling for unknown structures of ChRs with theoretical computations. Then, we attempted to describe their functional mechanism to extend optogenetic toolbox in combination of the computational model and a later published x-ray structure at the atomic level. On Basis of the computational analysis, we present several structural characteristics that are highly involved in the channel function including ion selectivity, photocycle kinetics and color-tuning mechanism.

1P239 Photoactive Yellow Protein におけるアルギニン 52 のプロトン化状態

Protonation State of Arginine 52 in Photoactive Yellow Protein

Kento Yonezawa, Hironari Kamikubo, Keito Yoshida, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

We have revealed the deprotonated R52 and low barrier hydrogen bond (LBHB) near the chromophore in photoactive yellow protein. We also revealed that the R52 of the LBHB lacked E46Q is protonated. Based on the results, we consider that the formation of LBHB is coupled to the deprotonation of R52. In this study, the effect of the mutation on the IR bands is observed to examine the protonation state of R52, in order to further confirm our hypothesis. The difference spectra between ^{15}N labeled R52 and unlabeled PYP's showed substantial differences between WT and E46Q. The difference comes from their dark states, suggesting that the protonation state of R52 at the dark state is different between WT and E46Q. The assignment of the bands will be discussed.

1P240 PYP-Phytochrome Related Protein の 2 つのセンサードメインで生じる光反応の関連性

Relationship of the photoreactions between two sensor domains in PYP-Phytochrome Related Protein

Keito Yoshida, Hironari Kamikubo, Kento Yonezawa, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

PYP-Phytochrome Related Protein (Ppr) is comprised of two light sensor domains, PYP and Bph, and a His-kinase domain. The PYP and Bph domains show similar photoreactions to Hh PYP and other bacterial phytochromes, respectively. In order to reveal the physiological roles of two photosensor domains, it is essential to understand the relationship of their photoreactions. We examined the blue-light induced photoreaction of holo-holo Ppr with and without red-light pre-irradiation. While an M-like intermediate was accumulated in PYP without pre-irradiation, an L-like intermediate was observed with pre-irradiation. Solution structural changes are also different. We conclude that the photoreaction of Bph controls the photo-induced domain rearrangement of Ppr.

1P241 ATP 結合におけるシロイヌナズナクリプトクロム 1 の光反応の赤外分光測定

FTIR spectroscopy of the photoreaction of Arabidopsis Cryptochrome1 upon ATP binding

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Cryptochrome1 from Arabidopsis (AtCRY1) is a blue-light receptor for photomorphogenesis. Previous studies have shown that AtCRY1 can bind ATP, which promotes a photoreaction (reduction of FAD) of AtCRY1 in vitro.
In order to investigate how ATP influences structural changes in AtCRY1 during the photoreaction, we compared light-induced difference FTIR spectra in the absence and presence of ATP. Differences were observed in the amide I region, which reflects the secondary structure of peptide backbone. A signal showing deprotonation of a carboxylic acid was observed in the presence of ATP. Relationship among the structural changes caused by ATP binding, the structural changes upon photoreaction and the efficiency of the photoreaction will be discussed.

1P242 光回復酵素への機能転換のためのクリプトクロム-DASH の変異導入

Mutagenesis to convert Cyanobacterial Cryptochrome-DASH into a Photolyase

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Cryptochrome (CRY) and photolyase (PHR) are flavoproteins with similar structures but different functions. PHR repairs UV-induced DNA lesions, while CRY is involved in photomorphogenesis in plants and circadian rhythms in animals. CRY-DASH is a new type of CRY, which was first found from cyanobacteria. In vitro, CRY-DASH repairs CPD in single-strand DNA but does not repair CPD in double-strand DNA (dsCPD).
We tried functional conversion of CRY-DASH into PHR by mutagenesis, then monitored enzymatic activity with FTIR spectroscopy. We measured repair of dsCPD in CRY-DASH mutants including the Y398W mutation. However, the mutants did not function as PHR by in vivo survival experiments. Further mutation studies of functional design of CRY-DASH are in progress.

1P243 培養細胞内２次メッセンジャーの発光モニタリングを利用した多様なオプシン類の分子特性解析の試み

Analysis of molecular properties of various opsins by bioluminescence monitoring of second-messengers in cultured cells

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Previous biochemical and biophysical studies have suggested that diversified properties of opsin-based pigments contribute to functional variety of photoreceptive systems in animals. However, in the case of no small number of opsins, it has been difficult to obtain a large amount of functional proteins sufficient for biochemical and biophysical assays by expressing them in cultured cell. In this study, we aimed to develop an experimental system that allows us to investigate molecular properties of opsins that exhibit low-level expression in cultured cells. We monitored intracellular second messenger levels in living cultured cells by using bioluminescent protein probe and succeeded in quantitative evaluation of spectral sensitivity of various opsin-based pigments.

1P244 アクチノロドプシンの His-62 残基の光化学反応における役割

Role of His-62 in the photochemistry of actinorhodopsin

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Actinobacteria are common inhabitants in freshwater environments and encode predicted H^+-pumping rhodopsins named actinorhodopsins (ActRs), which are similar to xanthorhodopsin but form the distinct cluster. We examined the photochemistry of ActR from strain MWH-Dar1. Illumination to the native cell suspension caused pH decrease due to the H^+-pumping activity of ActR. This activity was further confirmed in the ActR-expressing *E. coli* cells. Next, we analyzed the photocycle using the purified ActR. Below pH 7, ActR showed a similar behavior with proteorhodopsin (PR). However, above pH 8, unanticipated H^+ release occurred during the M decay. This probably reflects the H^+ release from His62 (corresponding to His75 of PR) just after the reprotonation of the Schiff base.

1P245 QM/MM RWFE 法によるウシロドプシンの光反応中間体に関する理論研究

A theoretical study on early intermediates of bovine rhodopsin by QM/MM RWFE method

Motoshi Kamiya, Shigehiko Hayashi (*Grad. Sch. Sci., Kyoto Univ.*)

Visual rhodopsins undergo a series of structural changes upon the photoisomerization of their chromophore, 11-cis retinal. Although the structural changes of bovine rhodopsin are very well investigated, there are still controversial issues such as the relaxation pathway of the isomerized retinal. In this study, we investigated the structural relaxation of the retinal and opsin after the photoabsorption by using the classical Molecular Dynamics and QM/MM RWFE calculations. The mechanism of the relaxation and the intermediate structures (BSI, Lumi, and briefly Meta I) will be discussed by using the calculated structures, vibrational frequencies, and absorption energies.

1P246 Accelerated MD シミュレーションを用いた AsLOV2 ドメイン光活性機構の解明

Study of the photactivation mechanisms in AsLOV2 domain by using accelerated molecular dynamics simulations

Tomohiro Yaita, Tadaomi Furuta, Minoru Sakurai (*Cent Biol Res & Info, Tokyo Inst of Tech*)

LOV domains are blue light photoreceptors that play a role in controlling the activity of phototropins. Upon illumination unfolding of the Jα helix occurs. By using accelerated MD (aMD), we investigated the dynamics and structural changes of Aslov2 domain (PDB ID: 2V1A, 2V1B) both in the dark and light states. And, to investigate the interaction between the Jα helix and the A'α helix, aMD was also performed for ΔA'α. The simulations for the wild type suggest that the signal is transmitted to the β sheet from FMN, leading to unstabilization of the N-terminal side of the Jα helix. The simulations for ΔA'α revealed how the C-terminal side of the Jα helix unfolds: the hydrophobic interactions present around the A'α helix disappear in ΔA'α.

1P247 ***Truepera radiovictrix* 由来 Na^+ポンプ型ロドプシンの機能解析**

Functional analyses of Na^+-pumping rhodopsin from *Truepera radiovictrix*

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Truepera radiovictrix, a thermophilic bacterium isolated from the hot spring in the Azores, encodes two predicted Na^+-pumping rhodopsins (NaR), TR1 and TR2. NaR homologues in other organisms share high amino acid sequence identities (~81%), while TR1 and TR2 have relatively low identities to other NaR homologues (TR1, ~58%; TR2, ~40%). Here we report the functional analyses on TR1. Similar to NaR in *K. eikastus* (KR2), TR1 pumps Na^+ and Li^+, but converts to H^+ pump in the absence of transportable cations. However, unlike KR2, Na^+-binding to TR1 causes a slight absorption shift. Moreover, M intermediate is not detected in the Na^+-pumping photocycle, implying that the deprotonation of the Schiff base is not essential for this ion-pumping function.

1P248 **光センサー蛋白質フォトトロピン 2 の光刺激による構造変化ダイナミクス**

Light induced conformational changes of a blue light receptor phototropin2: LOV2-kinase

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Phototropin2, a blue light receptor, consists of two light sensing domains (LOV1 and LOV2) and a kinase domain. When the LOV2 domain is stimulated by blue light, interaction between the LOV2 domain and the kinase domain changes to activate the kinase domain. In this study, to uncover the signal transduction mechanism at the molecular level, we investigated the reaction dynamics of LOV2-kinase constructs by the time-resolved transient grating technique. We have detected several steps of the diffusion coefficient change upon photoexcitation, which represents the light information is transmitted to induce global changes in the protein part. Comparing the reaction with that of the LOV2 construct, we will discuss how the kinase domain is activated.

1P249 **DNA 光回復酵素間の機能転換**

Functional conversion of (6-4) photolyase and CPD photolyase

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Photolyases (PHRs) are DNA repair enzymes that revert UV-induced photoproducts into normal bases. Two types of PHRs have been reported: CPD-PHR repairs cyclobutane pyrimidine dimers (CPDs), while (6-4)PHR repairs (6-4) photoproducts. However, the features that distinguish how CPD-PHR and (6-4)PHR recognize and repair their respective damaged DNA are not fully understood.

Here, we aim to redesign CPD-PHR from (6-4)PHR by site-directed mutagenesis. We successfully obtained the photorepair signal of CPD for a (6-4)PHR triple mutant by FTIR spectroscopy. We also try to redesign (6-4)PHR from CPD-PHR by reversing the mutations in the conversion of (6-4)PHR into CPD-PHR. The repair mechanisms for both CPD and (6-4) PHRs will be discussed based on the present results.

1P250 **緑藻型[FeFe]ヒドロゲナーゼ成熟化機構の構造研究**

Structural studies on the maturation mechanism of [FeFe] hydrogenase maturation from green alga *Chlamydomonas reinhardtii*

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[FeFe] hydrogenase (HydA1) from green alga *Chlamydomonas reinhardtii* is a ferredoxin dependent enzyme that catalyzes hydrogen production. HydA1 has a binuclear FeS cluster, consisting of a [4Fe4S] cluster and a [2Fe] subcluster. In the cells, three maturation genes are required for subcluster assembly. Therefore, expression of HydA1 in *Escherichia coli* led to non-active form lacking the subcluster.

In this study, HydA1 was heterologously expressed in a green sulfur bacterium *Chlorobaculum tedium*, and was obtained with high purity. Crystal structure at 2 Å resolution revealed the presence of an immature [2Fe] subcluster in addition to the [4Fe-4S] cluster, implying the nonspecific maturation process of [FeFe] hydrogenases without specific maturation genes.

1P251 **光化学系 I－フェレドキシン複合体の結晶構造および NMR 解析**

X-ray structure and NMR analysis of the electron transfer complex between photosystem I and ferredoxin

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Ferredoxin (Fd) is an electron transfer protein with a [2Fe-2S] cluster, mediating one-electron from photosystem I (PSI) to several Fd-dependent enzymes. Intrinsically the electron transfer complex is formed transiently and dissociated easily upon redox. To obtain a stable complex of PSI and Fd for structural study, Ga-substituted Fd (GaFd) was used as a replacement of native Fd. Crystal structure of PSI-GaFd complex was solved at 4.2 Å resolution, and amino acid residues important for complex formation were assigned.

In parallel to X-ray analysis, we measured NMR spectra of ^{15}N-labelled wild-type and mutants of GaFd with and without PSI, and observed different NMR chemical shift perturbation. These results were consistent with the crystal structure.

1P252 **光合成反応中心タンパク質の極低温単一分子分光**

若手招待講演

Single-molecule spectroscopic study of photosynthetic reaction center at 6 K

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Efficiency of electron transfer as well as that of energy transfer in photosynthetic reaction center (RC) is surprisingly high. To elucidate the mechanism, we have applied single-molecule spectroscopy to heliobacterial RC (hRC). The hRC includes two molecules of chlorophyll *a* (A_0) as an electron/energy carrier. Fluorescence excitation spectrum of hRC was measured at 6 K using a newly-built light source and reflecting microscope. One-step sudden change of the fluorescence intensity suggests that the signal is from a single hRC. Fluctuations of the peak position and line shape were also observed.

1P253 同位体標識された好熱性紅色光合成細菌における膜タンパク質耐熱化の分子機構解析

Isotope-edited ATR-FTIR analysis of the light-harvesting 1 reaction center complex from thermophilic purple photosynthetic bacteria

Yuki Yura[1], Yukihiro Kimura[1], Seiu Otomo[2], Takashi Ohno[1] ([1]*Grad. Sch. Agri. Sci., Kobe Univ.*, [2]*Fac. Sci., Ibaraki Univ.*)

An attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectroscopy was applied to uniformly 15N- or 13C-labeled Light-harvesting 1 reaction center (LH1-RC) complexes from Thermochromatium (Tch.) tepidum to investigate metal-sensitive structural changes involved in the enhanced thermal stability of this complex. The characteristic ATR-FTIR difference bands of unlabeled LH1-RC complexes were induced by the metal exchange from Ca2+ to other divalent cations. The detected bands exhibited clear isotopic shifts upon the 15N- or 13C-labeling. The molecular mechanism enhancing the thermal stability is discussed based on the present results and the recent X-ray crystallographic structure of the Tch. tepidum LH1-RC complex.

1P254 光合成光化学系 II における MnCa クラスターの歪んだ椅子型構造の起源

若手招待講演

Origin of the distorted-chair structure of the MnCa cluster in photosystem II

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The Mn_4CaO_5 cluster is a catalytic site of the water-splitting reaction in Photosystem II (PSII), where the bonds between Mn and O atoms are elongated causing it to have a distorted-chair structure. The distortion plays an important role in catalytic activity in water splitting. So far, the idea was accepted that the presence of Ca distorted the structure. By employing quantum mechanics/molecular mechanics (QM/MM) calculation, we have clarified that the direct cause of the distortion was not Ca located in the seat of the chair-like structure, but Mn located in the back of the chair-like structure. The Ca atom may play a key role in keeping hydrogen-bond networks around Mn_4CaO_5 that is important to control the proton transfer involved in the water-splitting reaction.

1P255 紅色硫黄細菌由来光捕集 1 反応中心複合体における金属—タンパク質間相互作用の熱力学的解析

Thermodynamic analysis of metal-protein interaction in the light-harvesting 1 reaction center complex from purple sulfur bacteria

Yukihiro Kimura[1], Yusuke Hayashi[1], Seiu Otomo[2], Takashi Ohno[1] ([1]*Grad. Sch. Agri. Sci., Kobe Univ.*, [2]*Fac. Sci., Ibaraki Univ.*)

The enhanced thermal stability of the light-harvesting 1 reaction center (LH1-RC) from purple sulfur bacterium Thermochromatium (Tch.) tepidum is achieved by the binding of Ca2+ to the LH1. In this study, we analyzed metal-protein interactions in the wild-type and biosynthetically Sr2+-substituted Tch. tepidum LH1-RCs by means of isothermal titration calorimetry. The number of the metal binding site was estimated to be 16 in both wild-type and Sr2+-substituted LH1-RCs although the binding constant was significantly reduced in the latter. Furthermore, the Ca2+-binding to the wild-type or Sr2+-substituted LH1-RC was favorable enthalpically or entropically. These differences may be important for understanding the enhanced thermal stability of the Tch. tepidum LH1-RC.

1P256 光合成酸素発生系における表在性蛋白質と Cl^-結合部位の相互作用 ：NO_3^-置換による赤外分光解析

Effect of the extrinsic proteins on the Cl^- binding sites of the oxygen evolving center in photosystem II: Analysis by FTIR spectroscopy

Junpei Kondo, Shin Nakamura, Ryo Nagao, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

Extrinsic proteins, PsbP and PsbQ, in photosystem II (PSII) of higher plants regulate the O_2 evolving reaction by controlling the binding of Ca_2^+ and Cl^- ions in the O_2 evolving center (WOC). To understand the molecular mechanism of this regulation, we investigated the effect of PsbP,Q on the Cl^- binding sites using FTIR spectroscopy. Light-induced S_2/S_1 FTIR difference spectra were recorded with PSII membranes in which Cl^- ions were replaced with infrared-active NO_3^- ions. Upon removal of PsbP,Q, the frequencies of the $^{14}NO_3^-/^{15}NO_3^-$ bands were slightly shifted, suggesting the changes in the NO_3^- interactions. This result provides evidence that binding of PsbP,Q to PSII perturbs the Cl^- sites in WOC, causing the changes in the dissociation constants of Cl^- ions.

1P257 時間分解赤外分光法による光合成水分解反応の解析

Proton-coupled electron transfer mechanism of photosynthetic water oxidation as revealed by time-resolved infrared spectroscopy

Hiroki Sakamoto, Ryo Nagao, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

Photosynthetic water oxidation takes place at the Mn cluster in photosystem II through five intermediates called S states (S_0-S_4). In this study, the mechanism of proton-coupled electron transfer in the S_2-to-S_3 transition was investigated using time-resolved infrared spectroscopy. The time course at 1400 and 2500 cm^{-1}, which reveals the changes in carboxylate groups and polarizable protons in H-bond networks, respectively, showed two phases with fast (40-100 μs) and slow (200-1800 μs) time constants at pH 4-7, with slower values at lower pHs. This pH dependence together with the H/D effect suggested that proton release occurs before electron transfer in the S_2-to-S_3 transition to remove an excess positive charge accumulated on the Mn cluster at the S_2 state.

1P258 光化学系Ⅱにおける第二キノン電子受容体 Q_B の電子・プロトン移動機構

Electron and proton transfer mechanism of the secondary quinone electron acceptor Q_B in photosystem II

Yukihiro Kadekawa, Yuki Kato, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

In photosystem II (PSII), electrons abstracted from water is transferred to the secondary quinone electron acceptor Q_B, which is converted to plastoquinol (PQH_2) after two turnovers. It is generally thought that PQH_2 is released from the Q_B pocket and is replaced with a new PQ. However, there is another model that only the Q_B head flips to the outside to transfer electrons and protons to another PQ. In this study, we investigated which model is correct using FTIR spectroscopy. PSII membranes supplemented with either ^{12}C-PQ or ^{13}C-PQ were illuminated with a train of flashes, and then flash-induced $S_2Q_B^-/S_1Q^B$ difference spectra were measured. The CO band of Q_B^- at 1480 cm^{-1} was unchanged between ^{12}C-PQ and ^{13}C-PQ, strongly supporting the latter flip-flop model.

1P259 ポリメラーゼリボザイムを活性化するペプチドの試験管内創出

In vitro selection of Polymerase Ribozyme-assisting peptides

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In the origin of the life, it is thought that a primitive functional protein gradually arose in "RNA world" by an appearance early translation system, thereby "RNP world" was emerged. Then, what kind of functional protein could appear in the early stage of translation system? In RNA world, it can be thought that the RNA dependent RNA polymerase ribozyme existed for self replication and reproduction of genome RNA. If a peptide enhances the activity of this ribozymes which have hyper cycle-like property, they could coevolve with the translation system. Therefore we examined the possibility whether such ribozyme-activating peptide can be selected by modified cDNA display. We will show the selected peptide which enhances the polymerase ribozyme activity.

1P260 リポソーム内 RNA 複製におけるリポソームサイズの影響

Size effect of liposome on the inner RNA replication

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Cell membrane is important as a basic compartment which can inhibit the diffusion of internal materials, and the compartment size may strongly affect on the inner biochemical reaction. We demonstrate the size effect using giant liposome in which RNA is replicated by Qbeta replicase encoded on the RNA. A reporter RNA was introduced to detect the RNA replication as green fluorescence, and a fluorescence-activated cell sorter was used for analysis of in-liposome reactions and collection of liposomes for further RT-qPCR analysis. RNA replication hardly progressed in larger (230 fL) liposome, but greatly progressed in smaller (7.7 fL) liposome. Our experimental results showed that smaller reaction chamber can strongly enhance the inner biochemical reaction in some cases.

1P261 ３つ以上のドメインからなるマルチドメインタンパク質の構造予測

Prediction of 3D structures of multidomain proteins composed of more than two domains

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In proteomes, many proteins are composed of several structural domains. To elucidate the details of molecular function of such multidomain proteins, it is important to know the possible spatial arrangement between each domain. In many cases, the domains of multidomain proteins are attached to each other by flexible linkers. Therefore, 3D structure determination of multidomain proteins by experimental methods is often difficult. We have developed a structure prediction method for two-domain proteins based on domain-domain docking, named DINE. The evaluation shows that DINE is more accurate than the other methods. Using the domain poses obtained by DINE, we are developing a prediction method for 3D structures of multidomain proteins composed of more than two domains.

1P262 細胞表面のビジュアルプロテオミクスに向けた計算技術開発：二次元電子顕微鏡画像と立体構造との照合

Computing technology for the visual proteomics of cell surface : Collation of protein structure and electron microscopic image

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It is important to visualize the interaction of the transmembrane proteins (TMPs), to understand their functions. We have been studying to identify kind of TMPs on the cell by comparing their electron microscopic (EM) images and tomographic images of their tertiary structure. We have constructed a database including 859 entries (2,216 chains) of TMPs, with their tomographic images and shape parameters. The performance of collation between these tomographic images and EM images in EMDB is enhanced by considering accessible surface area (ASA) around tomographic images. We optimized probe radius of ASA that shows the best structural similarity and this value shows strong correlation (Pearson's correlation coefficient of r=0.7515, p<0.05) to the circularity of EM image.

1P263 Analyses of the effects of amino acid mutations on the protein folding segments by means of sequence and evolutionary analyses

Masanari Matsuoka[1,2], Takeshi Kikuchi[1] ([1]*Ritsumeikan Univ., Col. of Life Sci., Dept. of Bioinf.*, [2]*Japan Society Promotion Science DC2*)

Protein folding is a very significant process for a protein. If it fails, not only functional disorders of proteins, but also some lethal folding diseases could be induced. However, the relationship between sequence and folding is not fully understood yet. Therefore, we study how robust the folding of a protein is among its homologues using Average Distance Map analysis. In order to clarify the significant factors for protein folding, we make the same analyses for artificially generated homologues and the results are compared with the previous data. According to the current result, in addition to the mutation matrices of amino acids, the intrinsic properties of an original sequence seems to be a factor to prevent misfolding via mutations.

1P264 Ets1 およびパートナー転写因子の協調的結合に関する分子動力学的解析

Molecular dynamics study on cooperative binding of Ets1 and partner transcription factors on regulatory elements

Kota Kasahara, Ikuo Fukuda, Haruki Nakamura (*IPR, Osaka Univ.*)

Transcription factor (TF) Ets1 recognizes specific regulatory elements by cooperative binding with other TFs such as Runx1, CBFbeta, and Pax5. In spite of biological importance of this recognition process, mechanistic details are still largely unclear in particular at the atomistic level. We performed molecular dynamics simulations for several TFs-DNA complexes including Ets1 and analyzed the correlation network by using our original method named multi-modal dynamic cross correlation, which can analyze correlation of motions between atoms with considering of transiently formed interactions and multi-modality of motions. Comparing correlation networks reveled conserved correlations regardless of types of the partner TF.

1P265 タンパク質相互作用データベースを利用した超分子モデリング法の開発
Development of a method for protein-protein interaction modeling using IntAct database

Toshiyuki Tsuji, Takao Yoda, Tsuyoshi Shirai (*Nagahama Institute of Bio-Science and Technology*)

Most of the biomolecules such as proteins and nucleic acids are assembled into large complexes to perform complicated functions in the cell. The interaction networks of biomolecules are essential for living body. However, experimental determination of the structures of supramoelcule or interaction network is still challenging.
We developed a method to modeling network structure by combining PDB and IntAct. If dimeric protein structures of A-B and A-C are determined, and protein A interacts with proteins B and C at different interfaces, B-A-C trimer would be assembled by superimposing the dimers on protein A. This process can be continued as far as template is available. We have predicted several undetermind or altenative protein-protein interfaces by constructing models.

1P266 Improved prediction of mitochondrial presequence for detecting undiscovered mitochondrial proteins

Kenichiro Imai[1], Yoshinori Fukasawa[2], Kentaro Tomii[1,2], Paul Horton[1,2] ([1]*CBRC, AIST*, [2]*Dept. of Comp. Biol., Grad. Sch. of Frontier Sci., Univ. of Tokyo*)

Despite intense study, the complete list of mitochondrial proteins is not known. Some bioinformatics based prediction tools are widely used but their prediction is imperfect and little progress has been made in the last decade. We report MitoFates, a novel prediction method for cleavable N-terminal mitochondrial targeting signal (presequence) incorporating novel features such as amphiphilic sequence motifs, refined amphiphilic helical moment score and explicit modeling of presequence cleavage by the secondary proteases Oct1 and Icp55. On independent test data, MitoFates predicts those cleavage sites with 19-31% fewer errors than previous methods and achieved a higher 73% (14-32% improvement) sensitivity at false positive rate of 1.7% in presequence prediction.

1P267 Application of novel amino acid substitution matrix, MIQS, to the MAFFT multiple sequence aligner

Kazunori Yamada[1], Kazutaka Katoh[1,2], Kentaro Tomii[1] ([1]*AIST*, [2]*Osaka University*)

Multiple sequence alignment is an essential step in comparative sequence analysis. Currently, more and more biological sequences are becoming available and the importance of aligning sequences quickly and accurately is growing than ever. MAFFT, one of the most popular multiple sequence aligners, is applicable to large data. In the meantime, previously we developed a novel amino acid substitution matrix, MIQS, which was of fine homology detection performance and alignment quality in a pairwise alignment standard. In this study, we combined MAFFT and MIQS to examine whether MIQS could improve the performance of MAFTT or not, using large-scaled database, HomFam. As a result, MIQS had a positive effect on MAFFT, especially for aligning extremely large dataset.

1P268 Evolutionary Relationships of Clostridia Species

Takashi Kunisawa (*Dept. Appl. Biol. Sci., Sci. Univ. Tokyo*)

The evolutionary relationships of the class Clostridia in the phylum Firmicutes are not well established. Tree re-constructions of 21 concatenated ribosomal protein sequences with the Bayesian and maximum likelihood methods, PhyloBayes and RAxML, respectively, were carried out. The tree topology was examined in the light of the other analysis of gene order comparison. These analyses suggest that Coprothermobacter proteolyticus and Thermodesulfobium narugense, both of which belong to the family Thermodesulfobiaceae and are members of the order Thermoanaerobacteriales in the class Clostridia according to the Bergey's Mannual of Systematic Bacteriology should be placed outside the Firmicutes.

1P269 魚たちができること ー 実験的視点から
The things fishes can do - From an empirical perspective

Takayuki Niizato[1], Hisashi Murakami[2], Takenori Tomaru[2], Yuta Nishiyama[3], Kohei Sonoda[4], Yukio Gunji[5] ([1]*Tsukuba University*, [2]*Kobe University*, [3]*Osaka University*, [4]*Shiga University*, [5]*Waseda University*)

What the things can do for fishes? Rrecent empirical studies pose the question against previous assumption such as "velocity matching".The empirical study would propose new interaction principle for the collective behavior. In this study, we examine real collective behavior of fish schools. Plecoglossus altivelis, which we called them "ayu" in Japanese, We found that the patterns of collective behavior are mainly divided into two, which are the parallel movement and the torus (making circles) movement. Furthermore, we also found out power law distribution between the duration times and its frequency. The slopes of figures are around -1.0. In other words, the collective behavior would obey Zip's law. This finding would have a relation to scale-free correlation.

1P270 Pattern formations of a polymer consisting of "hot" and "cold" monomers as a model of chromosome

Akinori Awazu[1] ([1]*Dept. of Math. and Life Sciences, Hiroshima Univ.*, [2]*RcMcD, Hiroshima Univ.*)

We consider the dynamics and pattern formations of a nonequilibrium polymer involving of "hot" and "cold" monomer regions confined in a spherical container. This polymer is considered as a simple model of a chromosome in nucleus. Here, the regions containing "hot" monomers corresponds to the transcriptional active regions on DNA because several proteins such as chromatin remodeling factors and transcription factors often access such DNA regions and produce several mechanical perturbations through the ATP hydrolysis energy consumption. By focusing on the container size dependent behaviors of this polymer, we consider the mechanisms of the cell type-dependent chromatin positioning and mechanical contributions to transcriptional controls.

1P271 Bubbly vertex dynamics: 曲率をもつ細胞形状を含む上皮組織のための幾何学的動力学モデル

Bubbly vertex dynamics: a dynamical and geometrical model for epithelial tissues with curved cell shapes

Yukitaka Ishimoto, Yoshihiro Morishita (*RIKEN CDB*)

In order to describe two-dimensionally packed cells in epithelial tissues theoretically, there have been developed several sorts of geometrical models. So far, in any attempt, pressures have not neatly been dealt with and curvatures of the cell boundaries have been even omitted through their approximations. Focusing on these quantities, we formulate a model with curvatures and vertices, and its algorithm is given for simulation. We demonstrate, by simulation, a typical time course of the bubbly dynamics, the mitotic cell rounding, and the two-vertex cell which could be important for sensory organs. The retinal epithelium of fruit fly and some examples of cell sorting will also be given. We will discuss its possible extensions and applications in the closing part.

1P272 回転する自走粒子の渦格子

Vortex lattice of rotating self-propelled particles

Ken Nagai (*School of Mater. Sci., JAIST*)

Recently, it has been reported that microtubules driven by the dyneins grafted to a glass plate form the lattice of vortex (Y. Sumino, et al., Nature (2012).). The key factor of this swarming is the long correlation time of the rotating speed of particles. To clarify the physical mechanism of this swarming, we calculated the continuous description for the swarming of the rotating self-propelled particles. We found that there is the effective potential between particles, which is short-range attractive and long-range repulsive. The effective potential is significant to form the vortex lattice of rotating self-propelled particles. Using our continuous description, the transition point to the vortex lattice of microtubules is predicted.

1P273 細胞内環境における生体高分子の動態： ブラウン動力学法による解析

Macromolecular dynamics in intracellular environment: Bronian dynamics simulation study

Tadashi Ando[1], Yuji Sugita[1,2,3] ([1]*QBiC, RIKEN*, [2]*RIKEN*, [3]*AICS, RIKEN*)

One of the most characteristic features of the cellular interior is crowding. How does the crowding alter dynamics of macromolecules? By employing Brownian dynamics (BD) simulations of a coarse-grained (CG) model, we try to elucidate the principles of intermolecular dynamics in the crowded environment of cells. We first developed a parallelized BD algorithm that can rigorously handle hydrodynamic effects between particles. Next, we built a CG model of a Mycoplasma genitalium cytoplasm, where macromolecules are represented by spherical objects with their Stokes radii. Using the CG system and algorithm, long-time BD simulations were performed. In the meeting, we discuss these simulation results.

1P274 受容体分子のマイクロクラスター形成はシグナル伝達におけるシグナルノイズ比を改善する

Formation of microclusters of receptor molecules improves signal/noise ratio in cellular signal transduction

Akihiro Fukagawa[1], Kumiko Sakata-Sogawa[1,2], **Makio Tokunaga**[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

We reported that T cell activation is initiated by formation of microcluseters of TCR (T cell receptor), which contains tens or hundreds of TCR molecules. Microclustering is a distinct and key feature in the immune system. However, the meaning of the MC formation are still unclear. To answer this, we performed numerical simulations based on a stochastic cascade model, where microclustering of the receptor molecules in the first step is incorporated. We found that the signal/noise ratio of transduced downstream signals was increased non-linearly with increasing the number of microclustered receptor molecules. This result gives a comprehensive account of experimental results of single molecule imaging and analysis.

1P275 Spatiotemporal Noisy Signal Processing in Chemotaxis

Ryo Yokota[1,2], Tetsuya Kobayashi[1] ([1]*Inst. Ind. Sci., Univ. Tokyo*, [2]*Res. & Edu. Platf. Dyn. Liv. States*)

Some types of cells such as bacteria and immune cells decide their behavior by using chemical gradient of environment. However, under noisy and dynamic circumstances, it remains unclear how to process the signals and to make the decision. The previous studies simulated how cellular system processes only one of either temporal or spatial information of gradient sensing for decision making. In this study, we modeled the mechanism of spatiotemporal information processing, and evaluated the difference between this combinational model and previous ones.

1P276 神経細胞モデルの現在の発火頻度の入力およびモデルパラメータへの依存性

The dependence of the current firing rate of a neuron model on the input and the model parameters

Takanobu Yamanobe (*Hokkaido University*)

One of the candidates of the information carrier in nervous systems is firing rate. It has been used spike density method that uses the convolution integral to calculate the firing rate. In this method, it is assumed a kernel function to estimate the firing rate, or spike density, of given spike data. The kernel function determines the contribution of the past firing to the current one. One question is "what is the appropriate function to calculate the spike density?" In this study, we evaluate the contribution of the past firing activity to the current one using a stochastic neuronal oscillator, and we analyze the dependence of the contribution on the relaxation rate, the noise strength and the parameters of impulsive input.

1P277 真性粘菌の運動に対する化学的障壁の影響
The effect of a chemical bump on a migrating amoeba

Dai Akita[1], Itsuki Kunita[2], Shigeru Kuroda[2], Toshiyuki Nakagaki[2] ([1]*Grad. Sch. of Life Sci., Hokkaido Univ.*, [2]*RIES, Hokkaido Univ.*)

An amoeboid organism Physarum plasmodium, true slime mold, migrating in an agar lane has diversity of behavioral options that develops when it encounters the repellent quinine. To understand the mechanism of this diversity, a mathematical model has been already proposed based on Gray-Scott model. However, behavior of a slime mold facing other types of chemical bumps has not been considered. In this study, to realize the general behavior of a plasmodium encountering a chemical bump, we conduct experiments with other chemical substances or a different chemical pattern in an agar lane and compare the results to the model behavior by examining the correspondence of model parameters. Then, we discuss behavioral diversity from the effect of chemical bumps.

1P278 Band-pass filtering to rhythmic input: A simple model of nonlinear response in a living cell

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All of the living organisms maintain their lives under period stimulus, including daily and annual cycles. Here, we study the effect of time-periodic stimuli by considering a simple model of cell signaling pathway. As for the kinetics of the signaling, we adapt a usual allosteric model under the choice of the Hill coefficient of 2-3; with constant strength of the stimulus at different frequencies, the amplitude of the sinusoidal input is taken as a constant. With such kind of framework of the modeling, we find that this signaling pathway plays the role as an 'on-off' type band-pass filter, i.e., simuli with suitable range of frequency leads the response to 'on' state. We will discuss such kind of unique characteristic of the response in relation to MAPK Cascade.

1P279 時計タンパク質による概日リズムの同調機構
KaiC intersubunit communication facilitates robustness of circadian rhythms in cyanobacteria

Yoko Kitayama[1], Taeko Nishiwaki-Ohkawa[1,2], Michio Homma[1], Takao Kondo[1] ([1]*Grad. Sch. Sci., Univ. Nagoya*, [2]*ITbM, Univ. Nagoya*)

The cyanobacterial circadian clock is the only model to have been reconstituted in vitro. The central clock protein, KaiC, is a homohexameric ATPase with autokinase and autophosphatase activities. We analyze the molecular mechanism underlying the regulation of KaiC's activity, in the context of its hexameric structure. We identify two types of regulatory mechanisms with distinct functions. First, local interactions between adjacent phosphorylation sites regulate KaiC's activities, coupling the nucleotide-binding states. Second, the phosphorylation states of the protomers affect the overall activity of KaiC hexamers via intersubunit communication. Our findings indicate that intra-hexameric interactions play an important role in sustaining robust circadian rhythmicity.

1P280 心臓組織片による同期化のメカニズム
Synchronization of cardiac rhythms after reassembling of multiple heart fragment tissues

Tomonori Takahashi, Yuji Mitsui, Shin Arai, Kentaro Ishida, Toshiyuki Mitsui (*Dept. of Phys. & Math., Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

Synchronization process of two oscillating systems is important to study dynamical biological phenomena at the molecular level with the aid of numerical simulations. We have been experimentally analyzing the physiological cardiac rhythms by attaching a pair of heart tissue fragments from chick embryos. Initially, these tissues show independent contraction and relaxation cycles in their inter-beat intervals (IBI) and phases. However, these tissues synchronize the cycle with 24 h. Interestingly, the tissues tend to lead the cycle between the pair as an internal clock were the one exhibiting the faster IBI than the others or removed from atrium. These results will be of interest for clinical applications, especially for regenerative medical techniques.

1P281 酵素競合律速による動的細胞記憶の形成
Kinetic memory based on the enzyme-limited competition

Tetsuhiro S. Hatakeyama, Kunihiko Kaneko (*Dept. of Basic Science, The Univ. of Tokyo*)

Cellular memory, which allows cells to retain information from their environment, is important for a variety of cellular functions. While posttranslational modifications have received much attention as a source of cellular memory, mechanisms directing such alterations have not been fully uncovered. We propose "kinetic memory" for cellular memory, in which memory is stored as a slow-relaxation process far from a stable fixed-state. We study models in which multimeric proteins undergo catalytic modifications, and find that a slow relaxation process of the modification state, logarithmic in time, appears when the concentrations of an enzyme are lower than that of the substrates. This kinetic memory provides a novel insight to a broad class of cellular memory and functions.

1P282 情報熱力学による生化学シグナル伝達の情報理論
Information thermodynamics reveals the robustness of biochemical signal transduction

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The information transfer and feedback play significant roles to achieve the robust signal transduction in thermally-fluctuating environment of cells. In a rather different context, thermodynamics of information processing has been developed, which establishes a unified theory of thermodynamics and information theory in small fluctuating world. In this presentation, we show the fundamental link between these two distint world, the biological signal transduction and thermodynamics of information. The second law of thermodynamics with information reveals the fundamental limit of the robustness of the signal transduction against the environmental fluctuation.

1P283 Coherence vs Dynamics on Random Boolean Networks

Taichi Haruna (*Graduate School of Science, Kobe University*)

Coherence of a network means that it is made up of functions of entities glued together. In our previous work [Haruna, T., 2013. BioSystems 114, 125-148], we showed that coherence of directed networks can be captured by the lateral path which is dual to the usual directed path. Here, we study propagation of coherence along lateral paths associated with dynamics on random Boolean networks (RBNs). We show that the critical condition for coherence propagation is generally different from that for dynamical criticality by a mean-field theory. An RBN can be dynamically subcritical in parallel with being supercritical for coherence propagation, and vice versa. We discuss the criticality hypothesis of biological networks in terms of our result.

1P284 LN 光変調器を用いた高精度周波数領域蛍光異方性測定による蛍光色素の回転運動解析
High-precision frequency-domain fluorescence anisotropy measurement using a waveguide LN modulator for dye rotational motion analysis

Tetsuichi Wazawa[1,2], Nobuyuki Morimoto[2], Makoto Suzuki[2] ([1]*Biomolec Sci Engin, ISIR, Osaka Univ*, [2]*Dept Mater Proc, Grad Sch Engin, Tohoku Univ*)

Time-resolved fluorescence anisotropy provides a measure of rotational motion of dyes. Since fluorophores at room temperature often undergo rapid rotational motion, it is necessary to precisely measure anisotropy including low values near to zero, which is difficult to be measured by time-correlated single photon counting. To solve this, we have developed a novel frequency-domain fluorometry method.
Our technique used a waveguide LN modulator, which could generate single-frequency amplitude-modulated excitation light up to as high as 1 GHz. As an evaluation, a modulation anisotropy of free rhodamine 6G in water was measured to be 0.023±0.004 (SD) at 1 MHz. The present technique is useful to probe the local viscosity around dye and dynamics of proteins that bind to dye.

1P285 近赤外蛍光ゆらぎ計測のためのナノ秒光子計数システムの構築
A photon-timing recorder in a nano-second resolution and its application for near-infrared fluorescence fluctuation measurements

Goro Nishimura (*RIES, Hokkaido University*)

A field reprogrammable gate array (FPGA) based photon-timing recorder has been constructed and demonstrated in fluorescence fluctuation measurements to characterize near-infrared fluorescence dye labeled proteins and micelles for *in vivo* imaging. The new device has a capability to record time-series of photon with a 960MHz sampling time at a maximum (timing-resolution is about 1.0ns with 2-channels) with the cost less than $1,000.
The device was used to construct a fluorescence fluctuation measurement system in solution phase, actually an inverted confocal fluorescence microscope with a near-infrared laser diode or a femto-second Ti:Sapphire laser. The performance in the measurements with near-infrared fluorescent dye labeled serum albumin and micelles will be discussed.

1P286 二光子励起によるホログラフィック多点蛍光相関分光計測装置の開発
Development of a holographic multipoint fluorescence correlation spectroscopy based on two photon excitation

Johtaro Yamamoto, Masataka Kinjo (*Faculty of Adv. Life Sci., Hokkaido Univ.*)

We previously developed a holographic multipoint fluorescence correlation spectroscopy (H-MP-FCS) system based on the spatial light modulator. In the system, multiple focused laser beam pattern were simultaneously generated in the sample and recorded the fluorescence intensity fluctuation at each laser beam spot. There were, however, a problem of photo-toxicity caused by multiple focused laser beam in the biological specimen.
In this report, a new H-MP-FCS system based on two photon excitation were developed. We succeeded in FCS measurements of fluorescent dye and protein in vitro, and fluorescent protein in living cell using the system. This system is expected as new biological tool to analyze protein dynamics inside cells in biological tissues.

1P287 Simple method for lipid bilayer formation with simultaneous incorporation of ion channels using gold electrode

Daichi Okuno[1], Minako Hirano[2], Hiroaki Yokota[2], Yukiko Onishi[1], Toshio Yanagida[1], Toru Ide[3] ([1]*RIKEN QBiC*, [2]*GPI*, [3]*Grad. Sch. Nat. Sci. & Tec., Okayama Univ.*)

Although single channel recording techniques enable us to examine the detailed pharmacological properties of various types of ion channel protein, there are weaknesses such as membrane fragility and a low channel reconstitution rate. To overcome these problems, we have developed a novel single channel recording technique, in which the lipid bilayers are formed and channel proteins are incorporated into the membranes at the same time by contacting a gold electrode with a lipid-solution interface. Using this technique, we succeeded to measure single channel currents of some channels. This simplicity allows to overcome the weaknesses and promises the application of highly-efficient measurement system such as high-throughput screening devices.

1P288 Holliday junction DNA の自発的 branch migration 過程の FRET による解析
FRET analysis of the spontaneous branch migration of the Holliday junction DNA

Kenji Okamoto, Yasushi Sako (*RIKEN*)

The Holliday junction (HJ) is a DNA four-way junction structure and its crossover point migrates by rearrangement of base-pairs around the junction. The spontaneous branch migration of HJ has been investigated by the single-molecule FRET (smFRET) measurement and subsequently analyzed by the variational Bayes-hidden Markov model (VB-HMM) method to reproduce the state transition trajectories (STTs). The FRET distributions and the state transition networks are reconstructed from those STTs for mobile and immobile control HJs. Based on the results, we discuss the dependence of HJ migration dynamics on, such as the nucleotide sequence around the junction or the concentration of divalent cation, which is thought to lower the transition rates.

1P289 光渦三次元トラップで生物の回転運動を操作・計測する
The manipulation and analysis of biological rotary motions by 3D optical vortex trapping

Yu Hashimoto[1], Tomoko Otsu[2], Yuji Kimura[1], Sayaka Kazami[1], Yoshiyuki Sowa[3], Yu Takiguchi[2], Taro Ando[2], Ikuro Kawagishi[3], Hiroyasu Itoh[1] ([1]*Tsukuba Reserch Laboratory, Hamamatsu Photonics K.K.*, [2]*Central Reserch Laboratory, Hamamatsu Photonics K.K.*, [3]*Dept. Frontier Biosci., Hosei Univ.*)

Optical vortex (OV) is light twisted around the propagation axis. Optical trap by OV exerts constant torque on a dielectric object in the same direction of angular momentum at any moment because OV has an orbital angular momentum. Recently, we have succeeded in manipulating beads three dimensionally using OV (Otsu *et.al, Sci. Rep.*, 2014).
In this study, we tried to demonstrate that OV trapping can be used for an analysis tool for biological rotary motors. As a result, DNA and F_1-ATPase was manipulated by OV *via* beads in a desired direction and speed. *Escherichia coli* cells tethered on a coverslip by a single flagellum was accelerated and decelerated by OV. These results show that OV has a potential to reveal the torque generation mechanism of molecular motors.

1P290 ビデオ計測による大腸菌の回転運動特性の統計解析
Statistical analysis of rotational motion properties of tethered E. coli by video measurement

Hiroto Tanaka[1], Tadashi Matsukawa[1], Takashi Sagawa[1], Sakura Maesato[2], Yukihiro Tominari[3], Yoshiyuki Sowa[4], Ikuro Kawagishi[4], Hiroaki Kojima[1] ([1]*Bio ICT lab., NICT*, [2]*Dept. Bioeng., Nagaoka Univ. Tech.*, [3]*Nano ICT lab., NICT*, [4]*Dept. Front. Biosci., Hosei Univ.*)

Most responses of organism to inputs are stochastic, and statistical analysis is important to discuss for biological behaviors (functions). Although it would be the best approach to perform large number of measurements with high accuracy, such approach is often not practical because of consumption of much time. When focused on statistical analysis, increasing the number of measurements even at low measurement precision is often effective in extracting the required information of target functions. Here, using video measurement, we quantitatively estimate rotational movement characteristics of tethered E. coli, as a model measuring samples. Under low special resolution conditions, we statistically extract information such as direction, speed, center, torque of rotation.

1P291 マイクロ波加熱効果：生体系に対する in situ マイクロ波照射 NMR の応用
Microwave heating effects: Application to analyze biological system by in situ microwave irradiation NMR

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Microwave heating is widely used in the acceleration of organic reaction as well as activity enhancement of enzymes. However, detailed molecular mechanism of microwave heating effect has not well understood yet. It is especially important to understand microwave induced denaturation of lipid membrane and protein. We therefore newly developed in situ microwave irradiation NMR spectroscopy. ^{1}H NMR spectra of liquid crystalline samples were observed under microwave irradiation as a model of biological system. Chemical shift and line width was indicated that non-equilibrium local heating caused the liquid crystalline to isotropic phase transition. Moreover, we successfully raise the temperature of samples around 200 degree in a short time within the NMR probe.

1P292 走査型イオンコンダクタンス顕微鏡の単一細胞ナノバイオプシーへの応用と細胞内 mRNA 局在性の評価
Single-cell nanobiopsy to investigate intracellular mRNA localization using scanning ion conductance microscopy

Yuji Nashimoto[1], Yasufumi Takahashi[2], Kosuke Ino[1], Kumi Inoue Y[1], Hitoshi Shiku[1], Tomokazu Matsue[1,2] ([1]*Enviromental studies, Tohoku Univ.*, [2]*WPI-AIMR, Tohoku Univ.*)

The cellular function is controlled by temporal and spatial changes in gene expression. Recent advances in qPCR technology make it possible to obtain information on single-cellular transcriptome, but the methods to quantify mRNA localization in single cell are still limited. Here we introduce the single-cell nanobiopsy using scanning ion conductance microscopy (SICM) technique for analysis of intracellular mRNA localization. For nanobiopsy platform, electrowetting in glass nano-pipette which enable the fL-pL extraction was used. By adopting the SICM technology, the positioning of nano-pipette was precisely controlled and the information of cellular topography could be obtained. We'll introduce the result of quantitativity of this system and application of living cell.

1P293 高速 AFM を用いたタンパク質構造変化のライブイメージング
Live Imaging of Protein Structural Change by High-Speed AFM

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The *trp*-RNA binding attenuation protein (TRAP), from *Bacillus stearothermophilus*, is a ring shaped protein composed of 11 subunits. A lysine residue, located at the outer surface of the ring, was mutagenized to cysteine forming TRAP-K35C. After adding 1.4 nm diameter gold nanoparticles (GNPs) to TRAP-K35C, the thermostable ring structure transformed into a larger hollow sphere. Of significance is the addition of reducing agents which cause the hollow sphere to revert back to the original ring structure. In this study, high-speed atomic force microscopy (HS-AFM) was used to observe the dynamics of sphere disassembly. Insights into the sphere structure, mechanism of assembly/disassembly, and structural intermediates will be discussed.

1P294 マニフォールドの概念に基づく新規画像分類法を用いた投影イメージの解析
Analysis of the projection images using the novel classification protocol based on the concept of manifold

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We have recently proposed a novel classification protocol of projection images toward applications to the data obtained through coherent X-ray diffraction imaging (CXDI) experiments. The protocol is based on the concept of manifold, and can be applied to projection images involving those generated from the particle conformations with a subtle difference as well as those with a large difference. Such projections would be obtained through CXDI experiments. In the present study, we apply the classification protocol to the projection images generated on a computer. We also analyze the classified data in terms of the projection direction (the Euler angle). We are going to discuss usefulness of the protocol through the application and the analysis.

1P295 Metallothionein labeling for CLEM(Correlative Light and Electron Microscopy) method

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CLEM is one the efficient techniques to elucidate the localization and the structure of target protein. CLEM needs a label, which functions in both fluorescence microscopy and electron microscopy. The candidate of the labels is metallothionein, which is heavy metal binding protein. Our previous studies showed that metallothionein with cadmium ions functions as a label for EM [Nishino et al., 2007]. The other groups showed that, in the presence of cadmium and selenium ions, metallothionein formed nanoparticles like Q-dot, which generates fluorescence [Park et al., 2010]. In fluorescence microscopy, we also observed fluorescence of metallothionein to form nanoparticle. We are now trying to observe larger electron density from metallothionein with cadmium for CLEM.

1P296 高速 AFM/1 分子蛍光顕微鏡複合機によるタンパク質の構造動態と化学反応の同時イメージング
Simultaneous imaging of dynamic structural and chemical events in protein by high-speed AFM combined with single-molecule TIRFM

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To elucidate the functional mechanism of protein nanomachines driven by chemical reactions, it is crucial to know how chemical events give rise to mechanical events (and vice versa). To this end, fluorescence microscopy has thus far been employed to study chemomechanical coupling mechanism of protein. However it has been difficult to directly visualize the protein's conformational dynamics. Here we develop simultaneous observation system of HS-AFM and single-molecule fluorescence microscopy which enable us to observe conformational dynamics of a molecule and binding/dissociation events of fluorescent ligands. We apply this system to rotor-less F1-ATPase hydrolyzing Cy3-ATP to observe both chemical and structural events that are synchronously occurring therein.

1P297 Real time imaging of collagenase behavior by high speed atomic force microscopy

Takahiro Nakayama, Noriyuki Kodera, Hiroki Kon'no, Toshio Ando (*Bio-AFM Frontier Research Center, Kanazawa University*)

Collagenase G (ColG) is a representative bacterial collagenase which is suggested to be processively moving on collagen microfibril and unwinding collagen chains during collagenolysis. The behavior of ColG on collagen fibrils, however, have been still unclarified. To revealing ColG motion on collagen simultaneous observation of substrate collagen structure and collagenase dynamics is beneficial. Here, we applied the high-speed atomic force microscopy to simultaneously observe collagen structure and collagenase dynamics during collagenolysis. In the conference, we show the movies of ColG behavior on collagen degradation and discuss collagen-collagenase dynamics.

1P298 新規共分散 Number and Brightness 法によるグルココルチコイド受容体二量体の生細胞内空間分布解析
Spatio-temporal distribution analysis of dimeric glucocorticoid receptor using a new Number and Brightness method based on covariance

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Glucocorticoid receptor (GR) belongs to the nuclear receptor super family. When GR binds to synthetic steroid hormone such as the dexamethasone (Dex), it forms a homodimer and works as a transcription factor for various genes. Most GR exists in cytoplasm in the absence of ligand and translocates to nuclear in the presence of the ligand. However, where GR forms dimer is unclear yet. In this study, the Number and Brightness (N&B) analysis which is a statistical imaging analysis was adapted to visualize the distribution of dimeric GR. For this purpose, a software for the N&B analysis was developed and a shot noise free N&B method using covariance was established. Finally, we performed N&B analysis of EGFP-GR dimers distribution in living cell in the presence of Dex.

1P299 生細胞蛍光イメージングによる ALS 関連変異体 TDP43 の構造解析
Analysis of structural difference in ALS-linked mutant of TDP43 by fluorescence imaging in living cells

Sachiko Yuno[1], Akira Kitamura[1,2], Ai Shibasaki[1], Masataka Kinjo[1,2] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Advanced Life Sci., Hokkaido Univ.*)

The amyotrophic lateral sclerosis (ALS)-linked mutations of TAR RNA/DNA-binding protein 43kDa (TDP43) have been identified. However it remains unclear how ALS-linked mutation in TDP43 affects the intact folding state. To investigate structural difference of ALS-linked mutant of TDP43, we performed time-lapse imaging and FRET analysis of TDP43 tagged with fluorescent proteins. As the results, rapid translocation of ALS-linked mutant TDP43 (Q331K) from the nucleus into the cytoplasm was observed during caspase3 activation, suggesting Q331K may form caspase3-sensitive structure. FRET analysis suggests that distance between N and C terminus of TDP43 is more than 10 nm. Further analysis is needed, ALS-linked mutation in TDP43 may potentially involve in protein stability.

1P300 高速原子間力顕微鏡による AAA シャペロン p97 の主要 ATPase（D2）リングの構造変化の直接観察
Direct observation of the structural changes of the major ATPase domain D2 of the AAA chaperone p97 by high-speed atomic force microscopy

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p97 is an AAA chaperone, which plays crucial roles in a variety of cellular processes. p97 comprises three domains, N-terminal domain and two ATPase domains (D1 and D2), and forms a homo-hexameric ring. Our previous high-speed AFM observation has revealed that the N-D1 ring rotates relative to the D2 ring upon binding to ATP at the major ATPase domain D2. However, the conformational changes of D2 during the cycle of ATP hydrolysis remains to be elucidated. Here, we have succeeded to observe the p97 D2 ring by high-speed AFM. For this purpose, we used N-terminally his-tagged p97 and NTA-conjugated streptavidin. On this substrate, the hexameric ring structure of p97 D2 ring was clearly observed. We will discuss the structural changes of the D2 ring in the presence of ATP.

1P301 マルチモーダル超高輝度化学発光タンパク質

Multi-modal super-duper chemi-luminescent protein

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Chemi-luminescence imaging has shed light on bioscience research field in situations when fluorescence cannot be used, for example non-invasive deep tissue imaging of whole organisms and light-induced biological processes. Despite of their potential utility, the universal applications are precluded by low brightness and restricted color hue. To overcome these, we had developed a brighter luminescent protein, Nano-lantern and several functional indicators(Nat. Commn. 3, 1262, 2012). Here we report further development of a super-duper chemi-luminescent protein and its color variant as well as Ca2+ indicator obtained by protein engineering, thereby we visualized multiple kind of tumor tissues in freely locomoting mouse, and also multifunctional imaging at single cell level

1P302 生体発光共鳴エネルギー転移（BRET）を用いたミトコンドリア外膜タンパク質 MAVS の構造基盤解析

A structural analysis of the MAVS-regulatory mechanism using BRET

Osamu Sasaki[1], Takuma Yoshizumi[1], Misa Kuboyama[1], Takeshi Ishihara[2], Emiko Suzuki[3], Shun-ichiro Kawabata[2], Takumi Koshiba[2] ([1]*Graduate School of Systems Life Sciences, Kyushu University*, [2]*Department of Biology, Faculty of Sciences, Kyushu University*, [3]*Structural Biology Center, National Institute of Genetics and Department of Genetics*)

In mammals, mitochondria act as a platform for antiviral innate immunity. Mitochondrial-mediated antiviral immunity depends on the participation of mitochondrial antiviral signaling (MAVS), which is localized on the mitochondrial outer membrane. After RNA virus infection, MAVS activates and undergoes a conformational change that is essential for downstream signaling on the mitochondrial outer membrane, although its structural features are poorly understood. Here we examined the MAVS-regulatory mechanism on the mitochondrial outer membrane using bioluminescence resonance energy transfer (BRET) in live cells. Our results reveal structural features underlying the precise regulation of MAVS signaling on the mitochondrial outer membrane.

1P303 ストレス顆粒内一分子 mRNA 立体構造の超解像イメージング

Super-resolution imaging of molecular conformation of single mRNA in stress granules

Yuki Suzuki[1], Kou Sugawara[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*JST, PRESTO*)

During stress, cytoplasmic mRNAs aggregate and form stress granules (SGs), where they are remodeled for repression of translation. It is suggested that circularization of mRNA is important for translation regulation. However, the coordination of translational repression in SGs and circularization of mRNAs has been unknown because a detailed distribution and a conformation of mRNAs in SGs remain unclear. We combined multicolor labeling of single mRNA and super-resolution microscopy to investigate molecular conformation of single mRNA. We observed two spots in close proximity which were most likely two points on single mRNA in vitro and in SGs in cells. Analysis of a distance between two spots to confirm whether a circularization occurs in SGs is under investigation.

1P304 転写伸長因子 NELF ダイナミクスの１分子イメージング定量解析

Single molecule imaging and quantitative analysis of dynamics of negative elongation factor NELF

Daichi Ikeda[1], Yuma Ito[1,2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

NELF contributes to the regulation of transcription elongation by promoter-proximal pausing, which can be released by phosphorylation of the CTD of RNA polymerase II by a kinase P-TEFb. Despite of the important role in transcription regulation, detailed dynamics of NELF is still unclear.
Aiming to elucidate the dynamic properties of NELF in the living cells, we performed single molecule imaging and quantitative analysis. We constructed SNAP-tag fusion protein of NELF and established a cell-line harboring single copy of SNAP-NELF. By controlling the concentration of fluorescent ligand to SNAP, we visualized the movements of single molecules of NELF in the nucleus. Increase of binding NELF by the inhibitor of P-TEFb supported the single molecule imaging of the molecule.

1P305 AIP1 とコフィリン共存下でのアクチンフィラメント切断の 1 分子リアルタイムイメージング

Real-time imaging of actin filament disassembly in the presence of cofilin and actin interacting protein 1 (AIP1)

Carina Sekiguchi[1], Kimihide Hayakawa[2], Shoichiro Ono[3], Masahiro Sokabe[2], Hitoshi Tatsumi[1] ([1]*Department of Physiology, Nagoya University Graduate School of Medicine*, [2]*Mechano-biology Laboratory, Nagoya University Graduate School of Medicine*, [3]*Department of Pathology, Emory University School of Medicine*)

Severing of actin filaments by cofilin is thought to be essential for cell survival, growth, and motility, whereas the rate of severing by cofilin is very low *in vitro* (less than 2·10^-3 event ·s^-1 ·μm^-1). AIP1 enhances the disassembly of actin filaments by cofilin *in vitro* and *in vivo*. However, the molecular process of the enhancement is largely unknown. We directly imaged the binding of Alexa-labeled-AIP1 to actin filaments by total internal reflection fluorescence microscopy, and analyzed the process of the disassembly, which showed that actin filaments were severed within a second at or in the vicinity (<150 nm) of AIP1 binding in most cases. This suggests that AIP enhances the severing activity of cofilin at or near its binding site.

1P306 １細胞分泌実時間測定による IL-1β 非古典的分泌機序の解明

Analysis of non-classical secretion of IL-1β using real-time single-cell secretion imaging

Yoshitaka Shirasaki[1], Ting Liu[2], Yoshifumi Yamaguchi[2], Mai Yamaguchi[1], Nobutake Suzuki[1], Kazushi Izawa[3], Jun Mizuno[4], Shuichi Shoji[4], Yoshie Harada[5], Ryuta Nishikomori[3], Toshio Heike[3], Masayuki Miura[2], Osamu Ohara[1,6] ([1]*IMS, Riken*, [2]*Grad. Sch. Pharm., Tokyo Univ.*, [3]*Grad. Sch. Med., Kyoto Univ.*, [4]*Grad. Sch. Sci. & Eng., Waseda Univ.*, [5]*iCeMS, Kyoto Univ.*, [6]*Kazusa DNA Inst.*)

Interleukin (IL) 1β is an important cytokine for inflammatory responses. It does not have a signal peptide for ER/Golgi pathway, and is thought to be secreted via non-classical pathway. Some research suggested that IL-1β secretion is associated with caspase-1 mediated programmed cell death. We figured out this cell-death associated IL-1β secretion by our real-time single cell secretion imaging. We found that individual cells digitally secreted massive IL-1β with inflammasome activating stimuli. We also found that caspase-1 activation, membrane pore formation and IL-1β bursting occurred sequentially within a few minutes. This result indicates the importance of single cell measurement for research of biological secretion activity.

1P307 ナノスリット基板を用いたアクチンの重合の一分子観察
Single molecule observation of actin polymerization in linear zero-mode waveguid

Masamichi Yamamoto[1], Makoto Tsunoda[1], Shun Higano[2], Kotaro Okubo[2], Takashi Tanii[2], **Takashi Funatsu**[1] ([1]*Grad. Sch. Pharm. Sci., Univ. Tokyo*, [2]*Sch. Sci. Eng., Waseda Univ.*)

Actin dynamics contribute to many cellular processes, while the detail mechanism of actin polymerization is unknown. Thus, a single molecule imaging of actin polymerization should be performed. As critical concentration of actin is >100 nM, we applied a linear zero-mode waveguide (ZMW), which has a slit structure with 50 nm in width. Short Cy3-labelld F-actin was immobilized in a linear ZMW. BODIPY-FL-labelled actin elongated from the end of immobilized actin at the rate similar to that on a cover glass. Actin polymerization was observed at elongation phase from G-actin or at steady state of polymerization. Monomer actin predominantly attached to the end of actin filament at elongation phase, while both monomer and oligomer actin attached at steady state.

1P308 培地温度の珪藻運動に与える影響の一細胞観察による評価
Effects of medium temperature on diatom motility studied by single cell observation

Kazuo Umemura[1], Toru Miyabayashi[1], Yoshikazu Kumashiro[2], Teruo Okano[2], Shigeki Mayama[3] ([1]*Tokyo Univ. Sci.*, [2]*Tokyo Women's Med. Univ.*, [3]*Tokyo Gakugei Univ.*)

Some types of diatom cells actively glide on solid surfaces in culture medium. In this work, we studied the effects of temperature of the medium on the gliding of Navicula pavillardii cells. In order to realize detailed analysis, single cell observation using a microchamber was demonstrated. After confining a few diatom cells in the microchamber, the sample was incubated for one or two nights at 17 degrees under 4000 lux irradiation in order to stabilize the cell condition. Then, single cell movements was continuously observed at 17, 25, and 30 degrees using an optical microscope system. As a result, cells were activated at 25 degrees, and damaged at 30 degrees. This is the first report of temperature dependence of the diatom motility studied by single cell analysis.

1P309 異物排出トランスポーター AcrD の発現は外膜チャネル TolC 遺伝子の欠失により促進される
Expression of the xenobiotic efflux transporter AcrD is induced by the deletion of outer membrane channel gene *tolC*

Kentaro Yamamoto[1], Rei Tamai[1], Takehiko Inaba[2], Yoshiyuki Sowa[1,2,3], Ikuro Kawagishi[1,2,3] ([1]*Dept. Frontier Biosci., Grad. Sch. Sci and Eng., Hosei Univ.*, [2]*Res. Cen. Micro-Nanotech., Hosei Univ.*, [3]*Dept. Frontier Biosci., Fac. Biosci. and Appl. Chem., Hosei Univ.*)

The RND-type xenobiotic efflux transporter AcrD forms a tripartite complex with the membrane fusion protein AcrA and the outer membrane channel TolC. The expression of *acrD* is induced by external stimuli such as indole, as was visualized by constructing the *acrD-gfp* gene with the native promoter. When induced, most fluorescent foci of AcrD-GFP hardly showed lateral movements, whereas they were moving incessantly in the absence of TolC *in vivo* with TIRFM. In the latter background, AcrD-GFP was expressed without external stimuli, which was complemented by expressing TolC from a plasmid-borne gene. This *acrD-gfp* induction was found to involve the two-component regulatory systems, BaeSR and CpxSA, which are responsible for a variety of environmental responses.

1P310 大腸菌異物排出トランスポーター MdtB, MdtC の膜内動態解析
Dynamics of the xenobiotic efflux transporter components MdtB and MdtC in the cytoplasmic membrane of *Escherichia coli*

Megumi Yamazaki[1], Kentaro Yamamoto[1], Rei Tamai[1], Yoshiyuki Sowa[1,2,3], Ikuro Kawagishi[1,2,3] ([1]*Dept. Frontier Biosci., Grad. Sch. Sci and Eng., Hosei Univ.*, [2]*Res. Cen. Micro-Nanotech., Hosei Univ.*, [3]*Dept. Frontier Biosci., Fac. Biosci. and Appl. Chem., Hosei Univ.*)

The inner membrane transporters MdtB and MdtC of *Escherichia coli* are unique in that they can form heterotrimers to assemble into an RND-type xenobiotic efflux complex with the membrane fusion protein MdtA and the outer membrane channel TolC. Among all possible trimers, the most potent is the heterotrimer of two MdtB protomers and one MdtC protomer. Taking advantage of this property, we examined how the efflux complex is assembled. When observed with TIRFM, most fluorescent MdtB-GFP foci were fixed, whereas MdtC-GFP foci were moving. Further analyses detected monomers, dimers and trimers of MdtB-GFP, but MdtC-GFP was found to be almost exclusively monomeric. These results suggest that even a monomer of MdtB, but not that of MdtC, can associate with TolC and MdtA.

1P311 電子伝達タンパク質による転写調節因子の出力変換
Readout Conversion of Transcriptional Regulator by Electron Transfer Proteins

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In a biological system, various transcriptional regulator proteins are evolved to detect environmental factors and retain the homeostasis of living cells at the transcriptional level. The high, specific sensitivity of these proteins is attractive in light of a novel bio-based sensor. However, facile conversion of the biological readout from the protein to a readily detectable signal is a major issue to be solved before application.
In this study, we show a facile conversion of readout from a transcriptional regulator to electronic signal by using a couple of electron transfer proteins which serve as a junction of electron transfer pathway switched on/off by the transcriptional regulator. We'll also discuss possible application of the present system to a biosensor.

1P312 再構成無細胞系を用いた抗菌ペプチドの直接発現
Direct expression of antimicrobial peptides in an intact form by using a reconstitued cell-free system

Satoshi Tomisawa, Masakatsu Kamiya, Takashi Kikukawa, Makoto Demura, Tomoyasu Aizawa (*Grad. Sch. Life Sci., Univ. Hokkaido*)

Recombinant production of antimicrobial peptides is difficult due to their susceptibility to host proteases and toxicity to the host cells. The use of cell-free expression system is thought to be useful in producing antimicrobial peptides. In a cell-free system, antimicrobial peptides were usually expressed as a fusion protein to improve the productivity. However, in fusion protein systems, enzymatic cleavage is inevitable to remove the fusion protein tag. Therefore, in this study, we investigated to express peptides in an intact form by using a reconstitued cell-free system derived from *Escherichia coli*. Some antimicrobial peptides showed a single band on SDS-PAGE but the others demonstrated plural bands that indicated degradation even in the cell-free system.

1P313 カリウムイオンを感知して自らの活性をスイッチングする Tat 捕捉アプタマーおよびリボザイムの創製
Development of Tat-binding aptamer and ribozyme which switch their activities in response to K+

Yudai Yamaoki[1,2,3], Tsukasa Mashima[1], Takashi Nagata[1,2], Masato Katahira[1,2] ([1]*Inst. Adv. Energy, Kyoto Univ.*, [2]*Grad. Sch. Energy Sci., Kyoto Univ.*, [3]*JSPS Research Fellow*)

An RNA R11, r(GGA)$_3$GG, forms a quadruplex under high K^+ concentration. We inserted R11 into the middle of the binding site of Tat-binding RNA aptamer and the active core of hammerhead ribozyme. Thus, both of the newly constructed molecules comprise 5′-subunit, R11, and 3′-subunit. In the absence of K^+, they showed no activity because the two subunits located far apart. However, upon addition of K^+, these aptamer and ribozyme exhibited Tat-binding and target RNA cleavage activity, respectively. We have also found that they do not respond to Na^+, thus K^+ is the specific switching stimulator. Since, the concentration of K^+ is low outside the cell but high inside the cell, we expect that they are silent outside the cell, but turn on their activity upon entering the cell.

1P314 細胞シグナル制御を目指した機能性核酸の設計
Design of functional nucleic acid that controls receptor signaling

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Our effort toward controlling receptor signaling using fuctional nucleic acid will be discussed in this presentation. The receptor tyrosine kinases (RTKs) are known to plays a key role such as cell survival and proliferation. Therefore, the signaling triggered by RTKs has been an important target in broad subject such as tissue repair and cancer therapy. We focused on aptamer, oligonucleotide sequence that binds specific target molecule, as a ligand for RTKs. We will present the molecular strategy to control the activity of RTK molecules using DNA aptamer.

1P315 Fabrication of photosensiting and electron-transfer RNA-modules

Tran Thoa Thi Thanh[1,2], Noriko Minagawa[1], Sivakumar Ponnurengam Malliappan[1], Toshiro Aigaki[2], Yoshihiro Ito[1,2], **Takanori Uzawa**[1] ([1]*RIKEN*, [2]*Tokyo Metro. Univ.*)

We aim for fabricating a desired enzyme by assembling aptamers that are selected through in vitro molecular evolution. Specifically aiming for creating an enzyme that harnesses sun light as its energy source, we selected two RNA aptamers that bind for the respective target: photosensitizer ($Ru(bpy)_3^{2+}$) and an electron acceptor (methyl viologen). Both aptamers strongly bind to the respective target (K_d on the order of 10nM), but do not disrupt electron transfer between $Ru(bpy)_3^{2+}$ and methyl viologen. For the fabrication of a photo-driven enzyme we will use these aptamers as photosensitizer and electron-transfer modules.

1P316 Rational design of orthogonal gene transcription nano device on DNA origami

Takeya Masubuchi[1], Hisashi Tadakuma[1], Masayuki Endo[2], Hiroshi Sugiyama[2], Yoshie Harada[2], Takuya Ueda[1] ([1]*Grad. Sch. Frontier Sci., Univ. Tokyo*, [2]*iCeMS, Univ. Kyoto*)

In the cell, gene expression is highly controlled. To create biologically inspired nanoscale device enabling the control of gene expression, we made hybrid nanomachine (T7-tile) using DNA origami tile as the skeletal structure and T7 RNA polymerase (T7-RNAP) as the functional module. T7-tile hybrid allowed us to evaluate the effects of intermolecular distance of enzyme (T7-RNAP) and substrate (target gene containing T7 promoter). We will show our recent achievements.

1P317 微小管リング状集合体の内径を制御する方法
How to control the size of ring-shaped microtubule assemblies

Shoki Wada[1], Masaki Ito[1], Daisuke Inoue[1], Kazuki Sada[1,2], Akira Kakugo[1,2] ([1]*Graduate School of Chemical Science and Engineering, Hokkaido University*, [2]*Faculty of Science, Hokkaido University*)

Biomolecular motor systems such as microtubule-kinesin are regarded as smallest natural machine which can perform work with a high efficiency. Recently we have developed a method termed as active self-organization to integrate microtubules (MT) into assembled structures on a kinesin-coated surface by using a specific biotin-streptavidin interaction. Active self-organization of MT produces ring-shaped MT structures which can produce rotational motion. In this work, we aim at controlling the size of MT rings by regulating MT length, rigidity and density. In future, size-controlled MT rings could be used as a highly efficiency rotational nanodevice.

1P318 応力場を利用した微小管集団運動の動的制御
Dynamic Control of Collective Motion of Microtubules Propelled by Kinesin in a Stress Field

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Biomolecular motor kinesin immobilized on a substrate can propel microtubules in the presence of ATP. Over high densities, microtubules start to move in large groups which is manifested by collective motion that spontaneously form ordered network on a large scale over time. Controlling the movement of such large scale microtubule network may provide us means to establish a large scale and cooperative molecular transportation system. In this work we employed uni-axial stretching stimuli on microtubules showing collective motion on kinesin coated elastomer substrate to control direction of movement of microtubules. We evaluated the response of microtubules to the change of stretching strain, frequency and mode of stretching.

1P319 DNA の相互作用に基づいた能動的自己組織化の制御
Control of Active Self-organization of microtubule by using DNA based interaction

Kyohei Uenishi[1], Shoki Wada[1], Daisuke Inoue[1], Kazuki Sada[1,2], Akira Kakugo[1,2] ([1]*Grad. Sch. of Chem. Sci. and Eng., Hokkaido Univ.*, [2]*Fac. of Sci., Hokkaido Univ.*)

Biomolecular motor such as kinesin converts chemical energy into mechanical work with a high efficiency and moves along the microtubule (MT). MTs, introducing biotin-streptavidin interaction, were integrated into assembled structures on a kinesin-coated surface and MT assemblies provide specific motion. However, reversible control of the MT association / dissociation was not achieved. Hence, we prepared DNA-modified MT and control the self-organization of MTs by using DNA interaction. DNA-modified MTs can reversibly associate and dissociate depending on the DNA sequence used. It will offer means to control the assembly of MT and thereby may widen the application of biomolecular motor based nanodevice.

1P320 Cell trapping device for observation of connexin function by a single cell device

Kosuke Inoue[1,2], Koki Kamiya[1,4], Yuta Abe[1,2], Toshihisa Osaki[1,3], Norihisa Miki[2], Shoji Takeuchi[1,3] ([1]*Kanagawa Academy of Science and Technology*, [2]*Keio University*, [3]*Institute of Industrial Science, the University of Tokyo*, [4]*PRESTO, Japan Science and Technology Agency*)

Membrane proteins play an important role in several biological functions including single transduction, energy production and cellular communication. Understanding the characteristic of membrane proteins is important for drug screening. Accordingly, the cells should be trapped for the analysis of membrane proteins' functions. In this study, we first arranged three cells in the order of donor-acceptor-acceptor cells using the parylene slide device in which the number and position of trapping cells was controlled by sliding parylene rails (5μm×54μm) on comb-shaped blocker. Next, we investigated the permeability of two fluorescent molecular (calcein and cytored) in Cx channel from the donor cell to the acceptor cells on the parylene rail.

1P321 質量を変えたレプリカ交換分子動力学法
Mass-scaling replica-exchange molecular dynamics method

Tetsuro Nagai, Takuya Takahashi (*College of Life Sciences, Ritsumeikan University*)

We present a new method of replica-exchange molecular dynamics (REMD) simulation, mass-scaling REMD (MSREMD) method, which improves trajectory accuracy at high temperatures and thereby contributes to numerical stability. In addition, the MSREMD method can simplify a replica-exchange routine by eliminating velocity scaling. As a pilot system, a Lennard-Jones fluid is simulated. The results show that the MSREMD method improves the trajectory accuracy at high temperatures over the conventional REMD method. We compare the computational costs of the REMD and MSREMD simulations, concluding that the MSREMD method optimizes the computational resources with simpler algorithm under the constant trajectory accuracy at all temperatures.

1P322 分裂酵母クロマチンダイナミクスの定量的解析
Quantitative analyses of chromatin dynamics in fission yeast

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Based on genome-wide chromatin interactions obtained from 3C experiments, 3D models of chromosomes have been constructed for some organisms. However, such models are constructed from population-based data on fixed cells and thus do not contain information about dynamic aspects of chromosomes. We are interested in how chromatin dynamically changes its structure during cell cycle, then we visualized gene loci in *S. pombe* and tracked the 3D positions of the loci. We statistically analyzed the positions and dynamics of the loci, and then revealed quantitative features of chromatin dynamics. In this poster presentation, we will report recent results and finally discuss our perspectives toward understanding possible roles that chromatin dynamics play within the cell nucleus.

1P323 Dynamical heterogeneity and dynamics of cage breaking and formation in colloidal fluids

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We study on colloidal fluids to understand system's mechanical response under a microscopic perturbation arises from a single optically trapped particle. Particles that undergo distortions of their packing configuration by the time scale of perturbation, resulting in a cage breaking and formation dynamics, are investigated by the power in Wavelet transform. We compare such a cage breaking and fomation dynamics with the underlying dynamical structure identified as finite-time Lyapunov exponent (FTLE) based Lagrangian Coherent Structures (LCSs) which are mobile separatrices that separate the flow into regions of different dynamical behavior. We have established a relationship between LCSs and cage breaking and formation dynamics.

2P001 イネ萎縮ウイルスの詳細な細胞侵入機構
Detailed cell entry mechanism of Rice dwarf virus (RDV)

Naoyuki Miyazaki[1,2], Akifumi Higashiura[2], Tomoko Higashiura[2], Fusamichi Akita[3], Hiroyuki Hibino[3], Toshihiro Omura[3], Atsushi Nakagawa[2], Kenji Iwasaki[2] ([1]*NIPS*, [2]*IPR*, [3]*NARC*)

Viral host cell recognition is required for a successful virus infection and replication cycle. The minor outer capsid protein P2 of RDV is essential for the viral host cell recognition. Here, we clarified the structure of P2 and the interactions between the P2 protein and the host cell. Negative stain EM single-particle image processing showed flexible structure of P2. In addition, 3D structure of the RDV virion by cryo-EM revealed the location and the partial structure of P2 in the capsid. Furthermore, we investigated the actual interactions between RDV and the host cell by electron tomography. By combining the in vitro and in vivo structural information, we could get new insights into the detailed mechanism about the viral cell attachment and entry of RDV.

2P002 タンパク質における埋もれた極性残基の構造と置換パターンの網羅的解析
Comprehensive analysis on the conformation and substitution patterns of buried polar residues in protein structures

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Although the stability of protein structure is mainly attributed to burying hydrophobic residues in the protein core, protein structure in nature sometimes include polar residues buried in the protein internal. These buried polar residues are suggested to be important in achieving marginal stability in aqueous condition and in energetically distinguishing the native structure relative to misfolded ones, but the understanding of their role is limited due to their small occurrence. In this study, we searched the Protein Data Bank for the conformations of buried polar residues and their substitutions with hydrophobic residues between homologous proteins. We discuss the patterns of burying polar residues and their roles in protein structures.

2P003 Crowding 環境下でのタンパク質構造の熱安定性
Thermal Structural Stability of Proteins Under Crowding Environment

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Hydration of proteins is the key determinant for isothermal, concentration-dependent effects on protein equilibria, such as folding. As shown in the studies using such as inelastic neutron scattering and molecular-dynamics simulations, the dynamics of proteins is coupled with water molecules surrounding proteins. Although there is no doubt that crowding changes protein equilibria, interpretations of the changes remain controversial since structural studies of protein folding and stability were conducted in dilute solutions in many cases. Therefore, we have studied the thermal structural transition of proteins under molecular crowding environment by using complementally wide-angle X-ray scattering and inverse-contrast variation method of neutron scattering.

2P004 相手に応じた様々な折り畳みを伴う一対多分子認識機構の統計熱力学：転写因子タンパク質 p53 の場合
Statistical thermodynamics of one-to-many molecular recognition accompanied by partner-dependent folding: in the case of p53 protein

Tomohiko Hayashi, Hiraku Oshima, Satoshi Yasuda, Masahiro Kinoshita (*Institute of Advanced Energy, Kyoto University*)

A transcription factor protein, p53, recognizes over 100 biomolecules. Here we investigate the mechanism of its molecular recognition accompanied by partner-dependent folding of its partial peptide. The C-terminal residues (374-388) of p53, which lacks rigid 3D structure without a partner, form a helix in binding to S100ββ, a sheet in binding to sirtuin, and a coil with two distinct backbone conformations in binding to CBP or cyclin A2. We calculate changes in thermodynamic quantities upon these bindings using a statistical-mechanical approach combined with molecular models for water. We discuss a common mechanism underlying the one-to-many molecular recognition by p53 from the viewpoint of hydration thermodynamics.

2P005 結晶環境における弾性ネットワークモデルを用いた非等方性温度因子の再現
Anisotropic atomic fluctuations reproduced by normal modes based on an elastic-network model in the crystal environment

Shigeru Endo[1], Hiroshi Wako[2] ([1]*Dept. Phys., Sch. Science, Kitasato Univ.*, [2]*Sch. Social Sciences, Waseda Univ.*)

We have developed a computer program that performs normal mode analysis (NMA) based on an elastic network model which includes intermolecular atom pairs between a target molecule and surrounding molecules in a crystalline environment. Taking advantage of the relatively small number of degrees of freedom required to describe a molecular structure in dihedral angle space, we aimed to develop the program applicable to a full-atom system of any molecule in the Protein Data Bank (PDB). Here, we show that the atomic fluctuations calculated by NMA reproduce anisotropic displacement parameters as well as isotropic temperature factors in high-resolution crystal structures in the PDB.

2P006 レプリカ置換分子動力学法の詳細釣り合い条件の有無に対する検証と生体分子への応用
Comparison of the replica-permutation molecular dynamics with and without detailed balance conditions and its application to biomolecules

Hiroaki Nishizawa[1], Hisashi Okumura[1,2] ([1]*IMS*, [2]*Sokendai*)

The replica-permutation molecular dynamics that adopts a way to be able to minimize the rejection ratio, namely Suwa-Todo algorithm, is successful in efficient sampling in comparison with the traditional replica-exchange molecular dynamics. The first Suwa-Todo algorithm is suggested as a method that does not fulfill the detailed balance conditions. Recently, Suwa and Todo proposed a new algorithm which satisfies the detailed balance conditions. However, the sampling efficiency of the algorithm is yet to be assessed in detail.
In this presentation, we apply two methods, i.e. replica-permutation molecular dynamics with and without detailed balance conditions, to a biomolecular system, and assess the sampling efficiency.

2P007 β-sheet 中でのペア傾向は edge strand と central strand では全く異なる

Pairing propensity in β-sheets is quite defferent between edge and central strands

Hiromi Suzuki (*School of Agriculture, Meiji Univ.*)

We analyzed residue pairing patterns in β-sheets to understand the mechanism of β-sheet formation which is one of the key steps for forming the tertiary structure of proteins. Only 12 pairs showed different pairing propensity between hydrogen-bonding (HB) and non-bonding (nHB) pairs in anti-parallel sheets when pairs including Pro were neglected. When edge and central strand pairs were distinguished, however, 37 and 39 pairs showed different pairing propensity for edge and central strand pairs, respectively, and only 7 of these pairs showed similar propensity between edge and central strand pairs. These results indicated that edge and central pairs possessed quite different propensity each other and this difference might be one of criteria to select edge strand pairs.

2P008 最適化した力場のアミノ酸ごとのパラメータ依存性について

Parameter dependency of an optimized force field for each amino acid

Yoshitake Sakae[1], Yuko Okamoto[1,2,3,4] ([1]*Dept. Phys., Nagoya Univ.*, [2]*Structural Biology Research Center, Nagoya Univ.*, [3]*Center for Computational Science, Nagoya Univ.*, [4]*Information Technology Center, Nagoya Univ.*)

A force field is widely used in the field of molecular simulations for biomolecular systems. Recently, we proposed a new method for refining force-filed parameters, which modifies the force-field parameters for each amino acid to minimize the root-mean-square deviation of backbone dihedral angles in various protein structures. Here, we compared the experimental data obtained from NMR with the trajectory data obtained from molecular dynamics simulations. In addition, we also applied an energy-based reweighting approach to a molecular dynamics trajectory to efficiently screen a large number of trial force fields in order to decrease the simulation cost. In this study, we examined the parameter dependency of optimized force filed for each amino acid.

2P009 固体 NMR および MD シミュレーションによるヒトカルシトニン線維形成機構と構造の解析

Fibrillation mechanism and fibril structure of human calcitonin as studied by solid-state NMR and MD simulation

Shuuhei Toyoda[1], Ganchimeg Lkhamsuren[2], Javklantugs Namsrai[1,2], Hikari Watanabe[1], Izuru Kawamura[1], Kazuyoshi Ueda[1], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Monglia Natl. Univ.*)

Human calcitonin (hCT) is known to form amyloid fibrils by taking two step autocatalytic reaction mechanisms. In the initial stage of fibrillation of hCT in HEPES solution, spherical stage of intermediates were observed by TEM experiment. This result gain insight into the fibrillation mechanism of hCT. To determine the fibril structure of hCT in phosphate buffer at pH7.5, 13C-15N internuclear distance were evaluated using [1-13C]Phe16, [15N]Phe19-hCT by means of REDOR experiment in solid-state NMR. REDOR results indicated that Phe19 and Phe16 located one residue away registry in the antiparallel β-sheet structure. MD simulation results indicate that the fibril structure is stabilized by interactions between inter-strand aromatic interchain.

2P010 ４量体型サルコシン酸化酵素の分子動力学シミュレーション: 水分子の透過経路解析

Molecular dynamics simulation of heterotetrameric sarcosine oxidase: pathways of water molecules

Go Watanabe, Akinori Hiroshima, Haruo Suzuki, Shigetaka Yoneda (*Sch. Sci., Kitasato Univ.*)

Heterotetrameric sarcosine oxidase (SO) containing FAD and FMN catalyzes the oxidative demethylation of sarcosine. The structural and biochemical analyses have shown that the SO-dimethylglycine (DMG) complex contains a large cavity near the catalytic site and have suggested channeling of reactants and products through several tunnels. Toward realistic simulations of channeling, we have carried out molecular dynamics simulations for the SO-DMG complex using the GROMACS program with the Amber force field. As a result, the structure simulated was in good agreement with the X-ray data. In our present study, the trajectories of water molecules were analyzed accurately to understand their pathways and obtain new insights into the channeling related to the catalysis of SO.

2P011 生体高分子中性子結晶構造解析におけるフーリエマップ改善のための実践的考察

A practical study for the improvement on the Fourier map in neutron protein crystallography(NPC)

Ichiro Tanaka[1,2], Nobuo Niimura[2] ([1]*Coll. of Eng., Ibaraki Univ.*, [2]*Frontier Center, Ibaraki Univ.*)

Neutron scattering length (b [cm]) is independent of sinθ/λ unlike X-ray atomic form factor (f [cm]). However, the resolution or a set of reflections of NPC has been determined as observed minimum d-spacing by considering R-merge etc, like in case of X-ray's. In order to find new criteria in NPC, we drew several Fourier maps by including a set of calculated Fc(hkl) from neutron PDB data, which consists of only larger |Fc|s than the mean value at the higher resolution range, and found that the quality of the map became better than one at the conventional resolution. This suggests that the practical improvement on the map will be possible if we use reflections ignored by the conventional method.

2P012 分子動力学シミュレーションによる GLP-1 と Exendin-4 の構造解析

Structure Analysis of GLP-1 and Exendin-4 by Molecular Dynamics Simulation

Sakiko Mori, Hironao Yamada, Yo Noguchi, Takeshi Miyakawa, Ryota Morikawa, Takuya Watanabe, Masako Takasu (*Sch. of Life Sci., Tokyo Univ. of Pharm. and Life Sci.*)

We analyzed the structure of glucagon-like peptide-1 (GLP-1) and exendin-4 by molecular dynamics (MD) simulation. GLP-1, a peptide of 30 amino acids, enhances insulin secretion when it binds to GLP-1 receptor (GLP-1R) in pancreas. This function is beneficial for treatment of type II diabetes. However, GLP-1 is decomposed by dipeptidyl peptidase-4 (DPP-4), and the activity is turned off. On the other hand, exendin-4 is a GLP-1R agonist and it has the resistance to DPP-4. We have performed MD simulation and compared the conformation of GLP-1 and exendin-4. In our simulations, we studied the effects on the conformations of several conditions of environment, for example, with 0.9 % NaCl in water solvent system.

2P013 分子動力学シミュレーションによるγS-WTとγS-G18Vの構造変化の比較

Structural changes of γS-WT and γS-G18V studied by molecular dynamics simulation

Ai Ozawa, Hironao Yamada, Sakiko Mori, Yo Noguchi, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu (*Tokyo University of Pharmacy and Life sciences*)

γS-crystallin is a protein which exists in crystalline lens. γS-crystallin maintains transparency of the crystalline lens and increases the refraction index. The aggregation of this protein enhances the development of cataract. In addition, frequency of aggregation of mutant γS-crystallin is higher than that of wild-type γS-crystallin (γS-WT). We focus on γS-G18V which is a mutant γS-crystallin where 18th glycine is replaced by valine. This protein is related to childhood-onset cortical cataract.
In the present study, we calculated the root mean square deviation (RMSD), radius of gyration (Rg), and obtained Ramachandran plot of the protein structure and compared the structures of γS-WT and γS-G18V by performing molecular dynamics simulation.

2P014 MEGADOCK: 超並列計算環境による大規模タンパク質間相互作用予測

MEGADOCK: a high-performance protein-protein interaction prediction tool on supercomputing environments

Masahito Ohue[1,2], Yuri Matsuzaki[3], Nobuyuki Uchikoga[4], Takashi Ishida[1], Yutaka Akiyama[1,3] ([1]*Grad. Sch. Inform. Sci. and Eng., Tokyo Tech*, [2]*JSPS Research Fellow*, [3]*ACLS, Tokyo Tech*, [4]*Dept. Phys., Chuo Univ.*)

Protein-protein interaction (PPI) plays a core role in cellular functions. In recent years, PPI prediction methods based on protein docking have been developed and have been applied for large-scale PPI network prediction based on tertiary structures. However, such network prediction requires much computing resources, and a faster PPI prediction method is eagerly demanded.
We have developed a high throughput PPI prediction system based on rigid-body protein docking, "MEGADOCK". MEGADOCK can perform faster docking based on its original scoring function and make extensive use of recent supercomputing environments such as K computer and TSUBAME. We have analyzed PPI networks such as bacterial chemotaxis, human apoptosis pathway, and EGFR pathway by using MEGADOCK.

2P015 マイクロ流路を用いたX線溶液散乱測定用サンプルチェンジャーの開発

Development of a microfluidics-based auto-sample changer for solution X-ray scattering

Ryuji Okabe, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

In order to obtain an ideal solution scattering profile from a protein, we need to collect a series of scattering profiles at various protein concentrations. Sample change is the most time-consuming step in the scattering experiments. We developed a microfluidics-based auto-sample exchanger to overcome the problem. Both protein solution and buffer are injected to inlet channels simultaneously by syringe pumps. A long path from the injected point to the measurement chamber enables to mix two solutions by diffusion to the expected concentration. We confirmed the designed performance of the sample exchanger, which makes a series of solution scattering at desired concentrations possible within 30min. Some examples of the application will be discussed.

2P016 タンパク質の構造コンプライアンス解析手法の改良

Improvement of a Method for Structural Compliance Analysis of Proteins

Keisuke Arikawa (*Fcl. Eng. , Kanagawa Inst. of Tech.*)

We have shown that it is possible to obtain information regarding the internal motion of proteins by analyzing the structural compliance of their elastic network models. We improved the method for more practical application. By separating the protein parts where deformations are observed from those where forces are applied, it becomes possible to analyze the motion interaction between different parts or to calculate the effective forces that cause the deformation in other specified parts. Moreover, by identifying and fixing the conformation variables (dihedral angles on the main chains) that do not affect compliance properties, we can reduce the computational cost. Through the analysis of PDB data of some real proteins, we verified the effect of these improvements.

2P017 固体NMR法によるタンパク質立体構造解析への常磁性緩和促進の応用

Application of paramagnetic relaxation enhancement to solid-state NMR protein structure analysis

Hajime Tamaki[1], Ayako Egawa[2], Kouki Kido[1], Tomoshi Kameda[3], Masakatsu Kamiya[1], Takashi Kikukawa[1], Tomoyasu Aizawa[1], Toshimichi Fujiwara[2], Makoto Demura[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*IPR, Osaka Univ.*, [3]*CBRC, AIST*)

Solid-state NMR has become a powerful method for structural analysis of insoluble proteins. However, it is still challenging to analyze them. One of the biggest obstacles is obtaining long-range distance restraints for structural calculation because of peak line width broadening caused by molecular immobility. To overcome this problem, we have developed a new approach for structural analysis using paramagnetic relaxation enhancement (PRE). In this study, we acquired 2D-DARR spectra for Mn^{2+} doped proteinG B1 domain (GB1) and obtained PRE data. PRE data showed good correlations between signal intensities and nucleus-electron distances. In addition, we demonstrated spectral simulation is useful for structure determination by solid-state NMR.

2P018 低温電子顕微鏡による単粒子像解析法におけるGFPラベル

GFP labeling for single particle analysis with cryoEM

Takayuki Kato[1], Naoya Terahara[1], Tomoko Miyata[1], Keiichi Namba[1,2] ([1]*Grad. Sch. Front. Biosci., Osaka Univ*, [2]*QBIC, Riken*)

The single particle image analysis by cryoEM is very powerful tool for the structural analysis. Recently, some structures have been analyzed beyond 4 angstrom resolution. But in the case of a flexible or a heterogeneous molecule, the resolution is lower than ~15 angstrom. Such a intermediate resolution structure, the orientation of the molecule or boundary of the each molecule in the molecular complex cannot be even decided.
On the other hands, GFP is widely used for various biological phenomena analysis under the optic microscopy. Florescence from GFP can not be detected by electron microscopy, but extra mass can be visualize. I will discuss about possibility of the GFP labeling to decide the molecular orientation and position for single particle analysis.

2P019 赤痢菌ニードル複合体の極低温電子顕微鏡による構造解析
Structural analysis of needle complex from *shigella flexneri* by cryo electron microscopy

Naoko Kajimura[1], Fumiaki Makino[1,2], Martin P Cheung[2], Takayuki Kato[1], Ariel J Blocker[2], Keiichi Namba[2,3] ([1]*Grad. Sch. of Frontier Biosci., Osaka Univ.*, [2]*Sch. of Cell. & Mol. Med., Univ. of Bristol*, [3]*RIKEN, QBiC*)

The needle complex (NC) is a huge membrane-embedded complex and is a central component of the Type III Secretion System of pathogenic bacteria. Single particle image analysis (SPA) has shown that NC has a syringe-like structure with three domains: an extracellular needle, a transmembranous basal body and core inner membrane export apparatus. While the advancement in the technology of SPA, determining the high-resolution structure of intact NC is challenging due to the structural heterogeneities, which includes the stoichiometry of component proteins, the symmetry of the major ring in basal body, and so on.
We report the result of structural analysis and characterization of T3SS complex purified from shigella using SPA.

2P020 ファルネシル基の結合による hGal-1 のオリゴマー化
Oligomerization of hGal-1 induced via the binding of farnesyl group

Kazumi Yamaguchi, Hirotsugu Hiramatsu, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ*)

Human galectin-1 (hGal-1) binds to the farnesyl group of H-Ras and assembles the H-Ras clusters. We have studied the role of hGal-1 in the clustering of H-Ras using farnesyl thiosalicylic acid (FTS), a model compound having the farnesyl group. The generated oligomers of hGal-1 disappear with the addition of lactose, indicating that the oligomerization occurs around the sugar binding pocket of hGal-1. The oligomerization is not owing to the lectin activity because the activity of FTS-bound hGal-1 is lower than that of hGal-1. With the addition of FTS in solution, the twisted β-sheet of hGal-1 becomes loose and that Trp68 in the pocket becomes exposed to water. These result indicate that the oligomerization occurs with the interaction among the side chain of Trp68.

2P021 高精度自由エネルギー計算によるドラッグデザイン
Computational Drug Design by Accurate Free Energy Calculations

Hironori Kokubo, Akihiro Yokota, Nao Morishita, Atsutoshi Okabe, Etsurou Watanabe (*Takeda Pharmaceutical*)

We have tackled two issues in order to realize drug design based on free energy calculations. First, we performed molecular dynamics simulations of proteins in various cosolvent solutions in order to predict small candidate molecules with high affinity and those binding modes. Our simulations successfully identified experimentally-known ligand partial structures. A protein structural change associated with the ligand binding was also observed properly, and we expect that this method can be a large-scale virtual screening method based on accurate free energy calculations. Second, we performed binding free energy calculations of protein-ligand complexes. Predicted binding free energies were in reasonable agreements in some cases, but over/under-estimated in other cases.

2P022 変性 apo-SOD1 の Cu 結合部位における His 残基の帰属
Assignments of His residues in the Cu^{2+}-binding sites of the denatured apo-SOD1

Nobuhiro Fujimaki, Takashi Miura, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

The metal-depleted apo form of H43R mutant of SOD1 misfolds into a denatured structure and acquires pro-oxidant activity upon Cu^{2+} binding at the two sites composed of His residues. In this study, to reveal the origin of the pro-oxidant activity, we have assigned His residues in the two Cu-binding sites of the denatured apo-H43R. We measured visible absorption spectra of the denatured apo-H43R bound by 1 equivalent Co^{2+}, exhibiting the strong absorption of the d-d transition. The d-d absorption of Co^{2+} was drastically diminished when any of His63, 71, and 80, which constitute the Zn-binding site of the native SOD1, was replaced with Ala. This indicates that these three His residues still construct one of the two Cu^{2+}-binding sites of the denatured apo-H43R.

2P023 Pin1 のプロリン異性化活性とタウタンパク質に対する凝集抑制活性との関係
Relationship between Pin1's peptidyl-prolyl isomerase activity and its aggregation-inhibitory activity for tau protein

Teikichi Ikura, Nobutoshi Ito (*MRI, TMDU*)

The Alzheimer's disease-related protein, tau, aggregates into neurofibrillary tangles when it is hyperphosphorylated. A peptidyl-prolyl isomerase (PPIase), Pin1, restores the function of tau by presumably catalyzing isomerization of a specific pS/T-P motif. The function of Pin1 for tau, however, is still unclear. Recently we developed a novel method to measure Pin1's PPIase activity for tau, and reported it last year. In the present study, we produced various kinds of mutant Pin1 and measured their activities to rescue a tau peptide from aggregating. The PPIase activity of the mutants varied and the relationship between Pin1's peptidyl-prolyl isomerase activity and its aggregation-inhibitory activity for tau protein will be discussed on the basis of these results.

2P024 Hsp90 の結合ポケット内における ADP の分布に関する理論的研究
Theoretical study of distribution of ADP in binding pocket of Hsp90

Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Institute of Science and Engineering, Kanazawa University*)

Heat Shock Protein 90 (Hsp90) is one of a group of molecular chaperones required for protein folding. The functional cycle of Hsp90 is driven by ATP binding, hydrolysis of ATP to ADP and ADP dissociation. These nucleotides are located in the binding pocket with a length of about 1.5 nm in the binding state. In our previous study, we calculated the free energy profile for ADP dissociation and found effective attraction [K. Kawaguchi, et al., Chem. Phys. Lett., 2013]. In this study, to elucidate the dissociation mechanism from such a deep binding pocket, we performed molecular dynamics simulations and calculated the spherical distribution at various binding distances during the ADP dissociation. We will discuss the influence of amino acid residues on ADP dissociation.

2P025 計算と実験による黄色ブドウ球菌の Isd 蛋白質間ヘム輸送機構の解明

Structural insight into the heme-transfer mechanism between Isd proteins in Staphylococcus aureus

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Although the tertiary structures of the IsdH-N3, IsdA-N, and IsdC-N domains of Staphylococcus aureus have been determined by X-ray crystallography, the mechanism of the rapid and unidirectional transfer of heme between these domains has remained unclear. We performed MD simulations on these domains and identified two distinct regions that may cause the difference in the affinity for heme. This prediction was validated by *in vitro* experiments. The rapid transfer of heme implies the existence of the heme-transfer intermediate complexes. Here, we propose novel structural models for them. We found that the complex structures were stable during 200-ns MD simulations. We will discuss how heme is transferred in each complex based on the results of QM/MM calculations.

2P026 滑走細菌 *Mycoplasma mobile* 由来新規シアル酸レセプターの結合活性

Binding activity of novel sialic acid receptor from gliding bacterium, *Mycoplasma mobile*

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M. mobile binds to sialylated oligosaccharides and glides on the host cell. Gli349 composed of 3,183 amino acids acts as “leg” and has a sialic acid receptor domain at a C-terminal 463 amino acids region (foot). The binding activity of this receptor is critical for *Mycoplasma* gliding.
We prepared a soluble recombinant foot (rfoot) with high refolding efficiency and measured the binding force to sialylated glycoprotein by atomic force microscopy (AFM). The rfoot showed binding activity with the average force 64.5±34.0 pN, which was similar to that of Gli349 isolated from *M. mobile*. Crystallization of rfoot is undertaken for determination of three dimensional structure.

2P027 子嚢菌由来不凍タンパク質の機能と構造

Function and Structure of Antifreeze Protein from Ascomycete

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Antifreeze proteins (AFPs) bind specifically to ice. A novel AFP (AnpAFP) from ascomycete, Antarctomyces psychrotrophicus, has been found in Antarctica. AnpAFP shows a high homology to that from a fungus Typhula ishikariensis (TisAFP) at the amino acid level. The thermal hysteresis (TH) activity of AnpAFP was measured to be 0.8℃. In contrast, TisAFP is known to have a TH of 0.5℃. Fluorescence-based ice plane affinity analysis showed that AnpAFP bound to the prism plane of ice, whereas TisAFP binds to both the prism and basal planes. The ice-binding site of AnpAFP was deduced from the homology model obtained by using the crystal structure of TisAFP. These results would lead to understanding of the structural basis of AnpAFP to exhibit ice-binding function.

2P028 分裂酵母キネシン Cut7 の両方向運動性

Bidirectional motility of the fission yeast kinesin-5, Cut7

Masaki Edamatsu (*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*)

Kinesin-5 is a homotetrameric motor with its motor domain at the N-terminus. Kinesin-5 crosslinks microtubules and functions in separating spindle poles during mitosis. In this study, the motile properties of Cut7, fission yeast kinesin-5, were examined for the first time. In in vitro motility assays, full-length Cut7 moved toward minus-end of microtubules, but the N-terminal half of Cut7 moved toward the opposite direction. Furthermore, additional truncated constructs lacking the N-terminal or C-terminal regions, but still contained the motor domain, did not switch the motile direction. These indicated that Cut7 was a bidirectional motor, and microtubule binding regions at the N-terminus and C-terminus were not involved in its directionality.

2P029 第一原理フラグメント分子軌道法による ブチリルコリンエステラーゼと阻害剤間の特異的相互作用の解析

***Ab initio* fragment molecular orbital calculations on specific interactions between butylcholinesterase and its inhibitors**

Takeru Murakawa[1], Tomoya Suzuki[1], Tareq Khan[2], Noriyuki Kurita[1] ([1]*Toyohashi University of Technology*, [2]*University of Tromso*)

Butyrylcholinesterase (BChE) exists mainly at hippocampus and hydrolyses neurotransmitter acetylcholine. Many types of ligands have been produced to inhibit the activity of BChE. We here investigate the specific interactions between BChE and several types of ligands Kx, using ab initio fragment molecular orbital (FMO) calculations. The binding energies between BChE and Kx evaluated by FMO are well correlated with the IC50 obtained by the previous experiments. In addition, the FMO calculations highlight the amino acid residues of BChE important for the binding between BChE and Kx. Based on the results, we propose some novel ligands and elucidate that one of the ligands can bind more strongly to BChE than Kx.

2P030 Ab initio 分子シミュレーションによるがん細胞レセプターへのリガンド結合を阻害する新規ペプチド阻害剤の提案

***Ab initio* molecular simulation for proposing novel peptide inhibitors blocking the ligand-binding to the receptor of cancer cell**

Tatsuro Mizushima[1], Ryushi Kadoya[1], Tomoyo Kasumi[1], Hiroshi Kobayashi[2], Noriyuki Kurita[1] ([1]*Toyohashi University of Technology*, [2]*Nara Medical University*)

Recent biochemical experiments have revealed that many proteases play important roles in cancer invasion and metastasis. Among these proteases, the binding of urokinase-type plasminogen activator (uPA) and uPA receptor (uPAR) existing on the surface of a cancer cell is a trigger for cancer invasion. Therefore, the blocking of the binding is expected to inhibit cancer invasion. In the present study, we created a various types of peptides for blocking the ligand-binding pocket of uPAR. The binding energies between uPAR and the peptides were evaluated by ab initio fragment molecular orbital calculations, and the peptide with the largest binding energy was proposed as a potent inhibitor to cancer invasion.

2P031 Mechanism of glycan receptor recognition for influenza virus Hemagglutinins: Comparative molecular dynamis studies

Katumi Omagari (*Nagoya City University*)

The heamagglutinin (HA) of influenza viruses mediate receptor binding, the initial event in virus infection. The differences in receptor-binding specificity of human and avian viruses are determined by the amino acid residues in the HA receptor-binding pocket. Asp at position 190 and 225 of H1 HAs confer binding to human-type receptors, whereas E190 and G225 confer binding to avian-type receptors. However some isolated viruses have E190 or G225, and D190E/D225G substituted virus does not prefer avian-receptor always. To clarify the detail effects of changes on binding for different HAs, molecular dynamics simulations were performed for the H1HA-glycan receptor complexes which comprise wild type and one point amino acid substituted HAs at positions 190 or 225.

2P032 比較粗視化シミュレーションを用いたタンパク質-リガンド結合過程の解析
The factors determining protein-ligand binding processes revealed by comparative coarse-grained simulations

Tatsuki Negami, Tohru Terada, Kentaro Shimizu (*Grad. Sch. of Agri. and Life Sci.*)

Clarifying the mechanism of protein-ligand interactions is one of the most important research subjects in the field of biophysics. However, most of the research efforts have been devoted to predicting docked structures. The process of the ligand binding remains to be clarified. Previously, we analyzed ligand binding processes of several protein ligand pairs that differ in physicochemical and geometric properties of the ligands and the ligand-binding pockets using the coarse-grained simulation. The results suggested that the ligand binding process depends on these properties. In this study, we applied this method to various additional protein-ligand systems. We will discuss the generality of the tendencies of the ligand binding processes.

2P033 DM 分子はペプチド交換時の MHC 複合体の動きを制御する
DM defines motions of peptide/MHC complex for peptide exchange

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DM catalyze peptide exchange reaction of MHC II. Crystal structures suggest that DM bound form of MHC II generate novel threshold for peptide binding by creating a hydrogen bond inside the binding groove, that form half bound intermediate peptides complexs. To examine the hypothesis, we used fluorescein labeled peptides to check the degree of quenching that results in a remarkable difference; fluorescein peptides give twice a signal with DM, which suggests peptides floating half way from MHC II. DXT analyses with gold nano-crystal at the same position of the peptide revealed that peptide/MHC complex has two ground states without DM, and the two states converged to a single state by addition of DM. We will discuss the mechanism of DM action based on these observations.

2P034 高速 AFM による MukB の構造と機能の観察
High-speed AFM observation of structure and function of MukB

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MukB is a structural maintenance of chromosomes (SMC) protein that performs chromosome segregation and condensation in E. coli. Structurally, MukB forms homodimer at a central globular dimerization domain each of which is followed by a long coiled-coil and an ATPase head domain. Although it is important to understand how MukB interacts with DNA and maintains chromosomal structure, these reaction mechanisms at the single molecular level remain poorly understood. Here, we apply high-speed AFM to directly observe structure and function of MukB. At the moment, structural details of single MukB and binding manners between MukB and plasmid DNA were visualized at nanometer spatial resolution. In the presentation, we will report the obtained results.

2P035 アミロイドベータペプチドのオリゴマー形成機構の解析
Analyses of the oligomerization mechanism of amyloid β peptides

Ayumi Tanaka[1], Shigeto Iwamoto[1], Takashi Saito[2], Hitomi Yamaguchi[1], Sosuke Yoshinaga[1], Toshiyuki Kohno[3], Takaomi Saido[2], Hiroaki Terasawa[1] ([1]*Fac. Life Sci., Kumamoto Univ.*, [2]*RIKEN BSI*, [3]*Kitasato Univ. Sch. Med.*)

The deposition of senile plaques is observed in the brains of Alzheimer's disease (AD) patients. Amyloid β peptide (Aβ) oligomers, formed in the process of senile plaque production, appear to be neurotoxic in AD. The Aβ(1–40), Aβ(1–42) and Aβ(1–43) species, which have different C–terminal lengths, were previously identified. Aβ(1–42) and Aβ(1–43) aggregate more easily than Aβ(1–40). Additionally, N–terminally modified Aβ species, pyroglutamate Aβs, are also more prone to aggregation than unmodified Aβs. To elucidate the roles of Aβ species on the Aβ oligomer formation, we utilized solution NMR, PICUP (Photo–Induced Cross–linking of Unmodified Proteins) and ESI–TOF MS. Our results indicated that multiple regions of Aβ contribute to the oligomer formation.

2P036 カメレオンモデルを用いた酸素結合に伴うヘモグロビンのアロステリック転移の研究
A study of the allosteric transition of hemoglobin associated with oxygen binding using chameleon model

Yui Sobue, Toru Kimura, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Nagoya Univ.*)

We investigate the mechanism of the allosteric transition of hemoglobin by Langevin dynamics simulation using a new coarse-grained potential model, "the chameleon model". In this model, the energy minimum of local potential is switched between two positions depending on the local structure around the interaction site. We characterized the allosteric transition by the average structure of the transition state ensemble as well as the correlation of the movement of each residue. We found the positive correlation between the structural change around Fe atom and the global structural changes, which has been considered as sequential events in the Perutz model. Based on these results, we discuss the molecular mechanism underlying the cooperativity of allosteric transition.

2P037 cAMP 結合による Catabolite Activator Protein のアロステリック構造変化のダイナミックスに関する Molecular dynamics 研究

Molecular dynamics study on dynamics of allosteric conformational change of Catabolite activator protein induced by cAMP binding

Mayuka Ojima[1], Yoshifumi Fukunishi[2], Masami Lintuluoto[1] ([1]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*AIST*)

The Catabolite activator protein (CAP) is a bacterial DNA binding protein. CAP is a homodimer, and each subunit consists of two domains which are a cyclic adenosine monophosphate (cAMP) binding domain (CBD) and a DNA binding domain (DBD). The binding of cAMP to the CBD of CAP induces allosteric conformation change of the DBD into the DNA binding form. We perform a molecular dynamics (MD) study to investigate the dynamics of allosteric conformation change of CAP induced by cAMP binding. Our results for the non-ligand CAP show the flexibility in both of the CBD and the DBD, while cAMP binding to the CAP makes the CBD more rigid compared to the DBD. From our thermodynamics results, the first cAMP binding step is supposed to be the rate-determining step.

2P038 カチオン分布がトロンビン-基質会合に及ぼす影響

Influence of Cation Distribution on the Thrombin-substrate Association

Ikuo Kurisaki[1], Masayoshi Takayanagi[1,2], Masataka Nagaoka[1,2] ([1]*Grad. Sch. Info. Sci. Univ. Nagoya*, [2]*CREST, JST*)

Thrombin is activated by Na^+, and the mechanism has been explained by site-directed interaction of Na^+. However, the recent challenge of redesigning K^+-activated thrombin suggested that site-directed interaction does not simply result in the enzymatic activation. To explain this inconsistency, we performed molecular dynamics (MD) simulations of thrombin in three aqueous solutions of NaCl, LiCl and CsCl. We analyzed cation's distributions around thrombin, and found that the number of cations changes in inverse proportion to their ionic radius. Both surplus and deficit of cations around thrombin should prevent substrates from binding to thrombin. This observation suggests that thrombin-substrate association is optimized under moderate distribution of Na^+.

2P039 Ectopic A-lattice seams destabilize microtubules

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Microtubules (MTs) typically include one A-lattice seam within a B-lattice tube. It is currently unclear how A-lattice seams influence MT dynamics. Here we find that including extra A-lattice in GMPCPP MTs destabilizes them, enhancing their shrinkage rate. Dynamic MTs nucleated by seeds containing extra A-lattice seams have growth rates similar to MTs nucleated by B-lattice seeds, yet have increased catastrophe frequencies at both ends. Furthermore, binding B-lattice GDP MTs to a rigor kinesin surface stabilizes them against shrinkage, whereas MTs with extra A-lattice seams are stabilized only slightly. On this basis, we propose that the single A-lattice seam of natural B-lattice MTs may act as a trigger point, and potentially a regulation point, for catastrophe.

2P040 cDNA ディスプレイ法による固相上のアミノ基認識ペプチドの探索とその分子認識機構の解析

Exploring the peptide aptamer against amino group on a solid-phase by cDNA display and analysis of its molecular recognition mechanism

Yuki Mochizuki, Koichi Nishigaki, Naoto Nemoto (*Grad. Sch. of Sci. and Eng., Saitama Univ.*)

Disulphide-rich peptides are an attractive non-antibody affinity reagent because of their extreme specificity and affinity towards a target protein. However, molecular recognition ability of disulphide-rich peptides against non-protein target molecules including small molecules has not been investigated yet. We have screened peptide aptamers that specifically bind to the amino group on a solid-phase by in vitro selection using the cDNA display method [1]. We revealed the identified peptides have a unique structure containing two cyclic loops with disulphide bonds and a linkage region, which were indispensable for molecular recognition.

[1] Y. Mochizuki, K. Nishigaki, and N. Nemoto, *Chem. Commun.*, **50**, 5608-5610 (2014).

2P041 ミトコンドリア呼吸鎖のシトクロム c-シトクロム c 酸化酵素複合体における 電子伝達反応の構造制御機構

Conformational Gating for Electron Transfer Reaction from Cytochrome c to Cytochrome c Oxidase in Mitochondrial Respiratory Chain

Mizue Imai[1], Wataru Sato[1], Kaoru Inoue[3], Koichi Sakamoto[3], Kyoko Shinzawa[2], Takeshi Uchida[1,3], Shinya Yoshikawa[2], Koichiro Ishimori[1,3] ([1]*Grad. School of Chem. Sci. and Eng., Hokkaido Univ.*, [2]*Grad. Sch. of Life Sci., Hyogo Pred. Univ.*, [3]*Dept. of Chem., Fac. of Sci., Hokkaido Univ.*)

Cytochrome c (Cyt c) mediates single electron transfer (ET) from cytochrome bc1 complex to cytochrome c oxidase (Cyt c) in the mitochondrial respiratory chain, which promotes reduction of molecular oxygen to two water molecules. Based on the NMR measurements, we proposed the "gating ET mechanism", where the disruption of hydrogen bond between Lys13 and Glu90 in Cyt c regulates the ET reaction with CcO. Here, we mutated Lys13 to disrupt the hydrogen bond and measured the NMR spectra in the presence and absence of CcO.The NMR spectra revealed that the area of the interaction site with CcO in the mutant is reduced and the interactions with CcO are perturbed, showing that the disruption of the hydrogen bond would play the primary role in the ET reaction from Cyt c to CcO.

2P042 MD シミュレーションを用いたマウス・線虫 ABCB1 トランスポーターの構造・ダイナミクスの解析

Analysis of dynamics and structure of ABCB1 transporters from mouse and *C. elegans* using molecule dynamics simulations

Tatsushi Nishimoto, **Tadaomi Furuta**, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

P-glycoprotein (P-gp) is a multidrug transporter that catalyzes the transport of several substrates. In order to reveal the underlying mechanism of this type of substrate transport, we performed molecular dynamics (MD) simulations using the X-ray crystal structures of mouse and *C. elegans* P-gps, which have inward-facing conformations. Our simulations showed that the dimerization of the NBDs is driven by the binding of ATP to the NBDs and/or the binding of the substrate (verapamil) to a cavity in the TMDs, and that the so-called tetrahelix bundle might mediate communication between the NBDs and TMDs. In contrast, only partial contacts between the NBDs were observed in the ATP-free (Apo) state during the 100 ns MD simulations.

2P043 多剤排出トランスポーター AcrB の Motion-Tree による解析
Motion-Tree analysis of the multidrug transporter AcrB

Tsutomu Yamane[1], Ryotaro Koike[2], Motonori Oota[2], Satoshi Murakami[3], Akinori Kidera[1], Mitsunori Ikeguchi[1] ([1]*Grad. School of Medical Life Science, Yokohama City Univ.*, [2]*Grad. School of Information Science, Nagoya Univ.*, [3]*Grad. School of Biosci. & Biotech., Tokyo Inst. Tech.*)

AcrB is one of the proton-driven multidrug transporters from the Gram-negative bacteria E. coli., and forms homo trimer with different conformations that are sequentially converted during drug export. These cyclic conformational changes are called functionally rotating mechanism. We have studied about the structural changes through functionally rotating mechanism using the Motion-Tree analysis, which is a new method to describe the structural change as rigid body motions, and some of the key motions were observed. In the present study, we added the analysis of rigid body motions by using other conventional methods, and obtained the supportive information for the results of Motion-Tree analysis. We will show the detail of the results in the poster presentation.

2P044 Conformational states of HAMP domains interacting with SRII-membrane systems: A molecular dynamics approach

Bikash Sahoo, Toshimichi Fujiwara (*Inst. Protein Res., Osaka Univ.*)

Signal transduction in sensory rhodopsin-transducer (SRII-HtrII) complex is still elusive due to structural inadequacy in N. pharaonis. We studied SRII-HtrII complex by molecular dynamics simulation in various force fields, lipid bilayers, and environments. Results showed, the HtrII having two four-helix bundles (HAMP1 and 2) was stable at 323K and 1.15M NaCl concentration. MM/PBSA computed ΔG of -355.4 (ground), -229.1 (intermediate) state, and -156.6, -92.6 and -83.7 kJ/mol for different HtrII dimer fragments. Mutation at T189 and N74 (SRII), and E43 and S62 (HtrII) minified the ΔG. The HAMP conformation and stability were highly influenced by the complex state (monomer/1:1/2:2). The helix rotations depicted a new insights into the signaling mechanism.

2P045 高速 X 線 1 分子追跡法によるニコチン性アセチルコリン受容体の機能運動
3D X-ray Single Molecule Tracking of nAChRs in Open, Resting, and Desensitization States

Hiroshi Sekiguchi[1], Maki Tokue[2], Yuri Nishino[3], Kouhei Ichiyanagi[4], Naoto Yagi[1], Atsuo Miyazawa[3], Tai Kubo[5], Yuji C. Sasaki[1,2] ([1]*Research & Utilization Div., JASRI/SPring-8*, [2]*Grad. School Frontier Sci., Univ. Tokyo*, [3]*Grad. School Life Sci., University of Hyogo*, [4]*Inst. Material. Struct. Sci. KEK*, [5]*Mol. Profil. Res. Ctr., AIST*)

The nicotinic acetylcholine receptor (nAChR) is well-studied neurotransmitter receptors and there are several conformational states of nAChRs, such as open, resting, desensitized states. However the structural information of each state and mechanism of changing states remains incompletely understood. We addressed to elucidate dynamic information of nAChRs in different states by single molecule technique with diffracted X-ray tracking. We found that the combined tilting and twisting motions of α-subunit nAChR were enhanced with ACh, those motions were inhibited with toxins, and only tilting motion was inhibited with excess nicotine in the solution. The finding is consistent with recent electron crystallography study and the detailed dynamic information will be discussed.

2P046 ドメイン運動の階層的な解析により描かれる SERCA の反応による構造変化
Conformational changes of SERCA in response to reactions described by hierarchical domain-motion analysis

Chigusa Kobayashi[1], Ryotaro Koike[2], Motonori Ota[2], Yuji Sugita[1,3,4,5] ([1]*AICS, RIKEN*, [2]*Grad. Sch. Info. Nagoya Univ.*, [3]*TMS, RIKEN*, [4]*QBiC, RIKEN*, [5]*iTHES, RIKEN*)

Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps Ca^{2+} across membranes against a large concentration gradient. The X-ray structures reveal rearrangement of transmembrane helices coupled with large-scale cytoplasmic domain motions. To characterize the conformational changes, we illustrate 'Motion Tree (MT)' based on crystal structures of SERCA. MT elucidates coupling between the motions of transmembrane helices and the cytoplasmic domain motions in individual reaction step. By gathering information from all MTs, rigid structural units of SERCA throughout the whole enzymatic cycle are identified. We discuss conformational changes of SERCA in response to individual reactions by characterizing its intrinsic conformational rigidity and flexibility.

2P047 大腸菌機械受容チャネル MscL の開口における脂質膜環境に影響される脂質–タンパク質間相互作用の解析
Analysis on Lipid-Protein Interactions Affected by Membrane Environment in Mechano-Gating of the E.coli Mechanosensitive Channel MscL

Hiroki Katsuta[1], Yasuyuki Sawada[2], Masahiro Sokabe[3] ([1]*Sch. Med., Nagoya Univ.*, [2]*Dept. Physiol. Grad. Sch. Med., Nagoya Univ.*, [3]*Mechanobiology Lab. Grad. Sch. Med., Nagoya Univ.*)

One of mechanosensitive channels MscL is homopentamer with two transmembrane inner and outer helices and opened by sensing membrane tension. It is known that MscL in thinner bilayer can open easily, however, its atomic detail remains unclear. So in this study, we prepared four types of membrane with different thickness and performed MD simulations for the opening process of MscL. As a result, MscL in thinner membrane model actually opened more widely. Furthermore, a helical structure of the periplasmic side of the outer helices, corresponding to be a major tension sensor region, was slightly broken. From these results, it is suggested that the breaking may be important for stable interaction with lipids and energetically favorable in the opening process of MscL.

2P048 天然変性タンパク質と変性状態蛋白質の構造特性の比較
A Comparison of structural properties between an intrinsically disordered protein and denatured state of proteins

Yasutaka Seki[1], Takamasa Nonaka[1], Kunitsugu Soda[2] ([1]*Sch. of Pharm., Iwate Med. Univ.*, [2]*High Perform. Molec. Simula. Team, ASI, RIKEN*)

The unfolded protein in solution consists of an ensemble with a great number of conformations. As an experiment gives only a piece of structural information of the unfolded protein, it is necessary to integrate diverse experimental data for elucidating its structural properties. We developed a computational method for generating conformations of unfolded protein. It enables us to generate a large number of conformations very rapidly. Using our method, we have tried to find an ensemble which best reproduces experimental residual dipolar couplings data. As a result, the structural properties of α-synuclein was clarified. In the annual meeting, we will discuss differences in structural properties between α-synuclein and some other denatured state of proteins.

2P049 平衡条件下において形成される二つの天然変性蛋白質融合蛋白質のフォールディング中間状態

Folding intermediates formed by the fusion protein of two intrisically disordered proteins under equilibrium condition

Hamada Daizo (*Dept Life Sci, Grad Schl Bioresource, Mie Univ.*)

GTPase binding domain of WASP (GBD) assumes partially folded state in isolated form but readily folded into well-ordered structure by binding to either Rho GTPase, VCA domain or R33 of EspFU. However, the less much is known about this folding process.
Here, the equilibrium unfolding processes of a fusion protein of GBD C-terminally connected to R33 (GBD-R33) were analysed by circular dichroism and fluorescence spectroscopy under various conditions. GBD-R33 assumes stable native-like folded state at pH 7.0, whereas it unfolded into the intermediate state at acidic pH. The data suggested that this intermediate contains partially folded GBD and highly unfolded R33. Thus, GBD-R33 could be a useful model to analyse the mechanism of coupled folding and binding by GBD and R33.

2P050 天然タンパク質の分子サイズに関する統計解析

Statistical analysis on the molecular size of native proteins

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A radius of gyration, R_g, is an essential parameter that represents an outline of protein structure, especially a size of a protein molecule. It has been theoretically shown that an R_g of a polymer scales with the number of residues, N, according to the equation: $R_g = R_0N^{\nu}$, where ν is a scaling exponent. To comprehensively examine whether the scaling relationship holds for native proteins, we calculated R_g of 167,546 protein domains classified in SCOPe database. The results show that the scaling relationship holds to a large extent for native proteins. Moreover, there appears to be the lowest limit of R_g at each chain length. Structural and functional characteristics of the maximally compact native proteins will be presented at the meeting.

2P051 アクチンフィラメントの引張，ねじり，曲げ挙動の定量評価：粗視化分子動力学法による検討

Quantifying how actin filament is stretched, twisted and bent: A coarse grained molecular dynamics simulation study

Shinji Matsushita, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

Actin filaments are double helix-shaped polymers that display conformational stretching, twisting and bending motions. Although modulation of these motions is crucial for regulatory actin-binding protein functions in many mechanical cellular activities, couplings between these dynamics, stretch-twist, twist-bend and bend-stretch couplings, have not been evaluated. In the present study, we quantitatively evaluate the stretching, twisting and bending dynamics of the filament by using coarse grained molecular dynamics simulations. We analyzed stretching, twisting and bending Brownian motions of the filament consisting of 56 actin subunits, then evaluated the coupling between these motions.

2P052 Calculation methods for configurational entropy from molecular dynamics simulations

Simon Hikiri, Takashi Yoshidome, Mitsunori Ikeguchi (*Grad. Sc. of Med. Life Sci., Yokohama City Univ.*)

Configurational entropy is an important factor of free energies in biomolecular systems. Here, we evaluate seven calculation methods for configurational entropy from molecular dynamics (MD) simulation. The Clausius method is used to assess other methods, because the Clausius method is capable of accurately estimating entropy changes with temperature. The other methods are based on the quasi-harmonic approximation with Cartesian or internal coordinate system. For convergence of configurational entropy, replica exchange MD simulations were used to generate trajectories for small molecules. Our results indicate that the internal coordinate system is more suited to calculate configurational entropy.

2P053 ヒトカルシトニンのアミロイド様線維形成機構とその阻害効果の解析

Analysis of amyloid fibrillation mechanism and its inhibition effects of hCT

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Human calcitonin (hCT) is known to form amyloid fibril and has been studied as a model peptide to clarify the mechanism of amyloid fibril formation. TOCSY NMR experiments were performed on the fibrillations of hCT in the addition of Curcumin (Cur) at pH 7.4. It was noted that peaks of Phe16, 19 and His20 did not disappear in the spectrum, indicating that those residues may interact with Cur. By the turbidity measurements, rate constants, k_1s of hCT were determined to be significantly slower than those of hCT in the presence of Cur. These results indicate that the core part of hCT is inhibited in the first step of a two-step autocatalytic reaction mechanism. Besides, the results of MD simulation support that Cur interacts with hCT at the sites around Phe16, 19 and His20.

2P054 MSES 法によるリガンド結合過程の全原子構造解析

Ligand binding process at atomistic resolution revealed by multiscale enhanced sampling

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Ligand binding to protein molecules plays a fundamental role for biological processes such as signal transduction. Since atom interaction and desolvation are involved, in this study, we aim at simulating the ligand binding process at atomistic resolution and including explicit solvent using multiscale enhanced sampling (MSES).
MSES allows an enhanced sampling of the all-atom structure in explicit solvent, by coupling with the accelerated dynamics of the coarse-grained model (CG). Here, this extension using multiple CGs has been applied to glutamine binding protein which has been studied as a simplest ligand-binding model. Free energy landscape calculated from the sampled conformations has revealed the coupling between ligand interaction and protein structural change.

2P055 抗体を用いた抗原蛋白質の揺らぎの検出
Detection of conformational dynamics of protein antigen by antibody

Shohey Shimizu[1], Yoshito Abe[2], Yuji O. Kamatari[3], Tadashi Ueda[2], Takachika Azuma[4], Masayuki Oda[1] ([1]*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Grad. Sch. Pharm. Sci., Kyushu Univ.*, [3]*Life Sci. Res. Ctr, Gifu Univ.*, [4]*Res. Inst. Biol. Sci., Tokyo Univ. Sci.*)

We showed that an anti-hen egg lysozyme (HEL) monoclonal antibody could distinguish Cys6-Cys127 alkylated lysozyme ($CM^{6,127}$-HEL) from native HEL whose crystal structures are similar. The decreased binding affinity to $CM^{6,127}$-HEL was mainly due to the decreased association rate constant (k_{on}), indicating that k_{on} is an indicator of proportion of the native format determinant in equilibrium. It should also be noted that the antibody could recognize the difference of conformational dynamics. In order to analyze the dynamic property of HEL, especially the effects of the reduction, in detail, we expressed HEL and C6A/C127A mutant in *E. coli*, and purified them. Based on the analysis using Biacore and NMR, we discuss the conformational dynamics of HEL detected by antibody.

2P056 天然変性タンパク質 HIV-1 Tat と転写コアクチベータ CBP の KIX ドメインとの相互作用
Interaction of the intrinsically disordered HIV-1 Tat protein with the KIX domain of the transcriptional coactivator CBP

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HIV-1 transactivator of transcription (Tat) is an intrinsically disordered protein, which interacts with multiple target proteins during HIV-1 gene expression. One of the targets of Tat is the KIX domain of the transcriptional coactivator CREB-binding protein (CBP). However, the structure and mechanism of the Tat-KIX interaction is poorly understood. To investigate the structure of the bound form and the mechanism of coupled folding and binding of Tat, we constructed the expression systems of the full-length Tat (86 residues) and KIX, and purified them with the Ni-affinity chromatography and gel filtration. We have measured CD spectra of the free and bound forms of Tat and KIX. NMR measurements of the Tat-KIX interactions will be presented at the meeting.

2P057 ニワトリオボムコイドにおける変性中間状態の構造と熱力学
Structure and thermodynamics of the unfolding intermediate of hen egg ovomucoid

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Chicken ovomucoid (OVM) is a potent allergen from egg white, the thermodynamic characterization of which is an unsolved, but important issue in reducing its allergenicity. We studied conformational stability of OVM, at pH 8.0, in the wide pressure (3~700 MPa) and temperature (5~50°C) range using tyrosine/tyrosinate fluorescence and 1H-NMR spectroscopy. We found no full unfolding is possible for OVM, but a stable intermediate is present under certain conditions of P and T, allowing us to draw a P-T phase diagram between the folded state (N) and the intermediate state (I). 1H-NMR at 0.1 MPa, and the proteolysis reaction under pressure, suggest that in state I only the 3rd-domain is folded. Such a stable I state is likely to be the major cause of the allergenicity.

2P058 Variable temperature and pressure NMR studies on flexible conformation of c-Myb DNA-binding domain

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The main purpose of this study is to find details of the dynamic conformational state of the c-Myb DNA-binding domain, R2R3, under the physiological conditions. Both 1D ^{1}H and 2D $^{15}N/^{1}H$ HSQC NMR spectra of R2R3 showed that, with increasing temperature in the range below the thermal denaturation, most peaks lost their intensities rather heterogeneously over residues, and nearly diminished at the physiological temperature. The extensive conformational fluctuations are rather unusual for proteins like enzymes, but would be rather normal for DNA binding proteins, as conformational disorder is often advantageous in its function. We also show the results obtained using high-pressure NMR experiments, and discuss the flexible property in correlation with the function.

2P059 Effects of chemical structure of hydrophilic tertaammonium-type ionic liquids for stability of higher-order structure of proteins

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Ionic liquid (ILs) is an organic salt that is liquid at room temperature. Their unique characteristics have been recieved much attention in wide fields. For example, some researchers have reported that ILs can be applied for a solvent of enzyme reactions. But the mechanism has not be enough clear. For understanding them, we synthesized several hydrophilic tetraammonium-type ionic liquids, and investigated relationships between their chemical structure and their higher-order structures in IL solution. Compared with conventional buffer solution or ionic liquids such imidazolium-type ILs, our ILs indicated excellent solubility and stability on proteins including some enzymes. This property can be applied them as a novel stock system for functional proteins such as enzyme.

2P060 ジスルフィド結合のシャッフリングを標的とした異常なタンパク質オリゴマー化の抑制手法
Disulfide shuffling in Cu,Zn-superoxide dismutase is a key to develop potential drugs for neurodegeneration

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Dominant mutations in Cu,Zn-superoxide dismutase (SOD1) cause a familial form of amyotrophic lateral sclerosis, of which a pathological hallmark is the formation of abnormal SOD1 oligomers in affected spinal cords. Our group has recently revealed that mutations facilitate the disulfide shuffling within and among SOD1 proteins and thereby form the disulfide-crosslinked oligomers. Here, we have identified several drugs that can effectively and selectively inhibit the formation of SOD1 oligomers and found that those drugs act on the disulfide-shuffling process between specific cysteine residues without affecting structural stability of mutant SOD1 proteins. Further investigation on roles of cysteine residues in the SOD1 oligomerization is now in progress.

2P061 天然変性蛋白質のリン酸化に共通して見られる分子内静電相互作用の特徴

Common intra-molecular electrostatic property of intrinsically disordered proteins for phosphorylation

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The intrinsically disordered protein (IDP) has no unique structure, which is a corollary of high content of charged residues. The IDP often undergoes phosphorylation to fulfill biological functions. Phosphorylation should largely affect the conformation of IDP due to the electrostatic interaction between the added phosphate group and the charged residues. To investigate the electrostatic impact of phosphorylation on IDP, we have conducted an enhanced sampling with a side-chain-coarse-grained model to obtain nonphosphorylated and phosphorylated conformational ensembles for 4 IDPs: kinase-inducible domain of CREB, stathmin, α-synuclein and tau protein. The results indicate a common electrostatic property which makes the conformation of IDP susceptible to phosphorylation.

2P062 金ナノ粒子の界面におけるアミロイド線維形成機構に関する研究

Study on the mechanisms of amyloid fibrillation at the interface of gold nanoparticles

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Amyloid fibrils form in supersaturated solutions via nucleation and growth mechanisms. Interfaces have shown effects on aggregation of absorbed proteins by inducing structural changes and/or increasing local concentrations. However, the mechanisms of surface-induced amyloid fibrillations and amorphous aggregations are still unclear. Here, we show the effects of gold nanoparticles (AuNPs) which mimic interfaces of membranes on aggregation behaviors of proteins. Based on ThT, CD, AFM analyses, AuNPs promoted lysozyme fibrillation and delayed insulin fibrillation. Further increasing in the AuNPs concentrations inhibited lysozyme fibrillation and induced amorphous aggregation. These suggested distinct effects of AuNPs on protein aggregation by adjusting initial nucleation.

2P063 Contact number diffusion model for the normal mode analysis of protein structure

Bhaskar Dasgupta, Kota Kasahara, Narutoshi Kamiya, Haruki Nakamura, Akira Kinjo (*IPR, Osaka University*)

The dynamics of a protein depends on its sequence. A popular method to investigate native protein dynamics is Elastic network model (ENM) which requires all pairwise-specific distance information. However, how the protein sequence can encode this amount of information is not trivial. Here we introduce Contact Number Diffusion model (CND) that uses O(N) restraints to capture native dynamics (where, N is sequence length). In CND we treat sequentially non-local interactions in terms of contact numbers. The sequentially local interactions are treated to be pairwise-specific. We observed the native dynamics of CND is comparable or sometimes better to that obtained from ENM. This tells us that specific non-local interactions are not fully necessary to capture native dynamics.

2P064 NMR を用いた動的構造解析により明らかとなったヒト主要組織適合複合体のペプチド認識、及び構造維持機構

若手招待講演

The Dynamic stabilization and peptide recognition mechanism of Human Leukocyte Antigen revealed by NMR relaxation dispersion analysis

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Human leukocyte antigen (HLA) presents various antigenic peptides to cytotoxic T lymphocyte (CTL) to kill infected cells. The stability of the peptide-HLA complex (pHLA), important for CTL activity, highly depends on bound peptides. However, how pHLA stabilizes and recognises various peptides remains elusive. Here we examined the mechanism of pHLA peptides recognition and stabilization by elucidating its conformational dynamics using relaxation dispersion NMR spectroscopy. Experiments showed that the peptide binding-domain of pHLA fluctuates in solution, and forms a well-packed minor state. We revealed that pHLA loosely recognizes various peptides with the major conformation, and transiently forms a well-packed state, resulting in circumvention of pHLA disintegration.

2P065 Helix-turn-helix モチーフを有する蛋白質の pH 変化および DNA 結合に伴う動的構造変化の解明

Effects of pH and DNA-binding on conformational dynamics of protein with helix-turn-helix motif

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The minimum unit for specific DNA-binding of a transcriptional factor c-Myb, R2R3, has a large conformational fluctuation under the physiological conditions. In order to elucidate the effects of pH and DNA-binding, we analyzed the physical properties of R2R3 under the different solvent conditions. The analysis using circular dichroism showed that the apparent α-helical contents and thermal stability of R2R3 were increased upon the DNA-binding, and the secondary and ternary structures were largely perturbed by pH. The single-molecule analysis using diffracted X-ray tracking showed that the conformational fluctuation of R2R3 was restricted by DNA-binding. Taken together with the NMR analysis, we discuss the conformational dynamics, different from the averaged structure.

2P066 一分子蛍光分光法による変性剤濃度ジャンプ後のユビキチンの折り畳みダイナミクスの測定

Folding dynamics of ubiquitin after rapid mixing detected by single molecule fluorescence spectroscopy

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Two distinct models have been proposed to explain the folding of ubiquitin: the two-state model and the non-two state model involving intermediates. We investigated the folding dynamics of ubiquitin at the single molecule(sm) level by using the line confocal microscope. We first obtained the time series of sm-FRET efficiency from the ubiquitin labeled with two different dyes at the equilibrium conditions. Two distributions were detected in the FRET efficiency histograms at different urea concentrations, suggesting the two-state model. We next obtained the time series of sm-FRET efficiency after the urea concentration jump. The data showed the presence of metastable intermediates among the folding pathway, which is rather consistent with the non-two state model.

2P067 タバコモザイクウイルス外被蛋白質会合体の安定性
Stability of the Tobacco Mosaic Virus Coat Protein Assemblage

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Tobacco mosaic virus coat protein (TMVCP) is known to form self-assemblages such as single-helical rods or disks. We have produced TMVCP in E.coli by using an auto-inducing expression system with a yield of more than a hundred mg of pure protein per three liter of culture. In 20 mM TrisCl, pH 7.4, the dissociation of a disk-like assemblage of the produced TMVCP occurred in the urea-concentration range of 0 to 2 M, and the unfolding of the secondary structure in the range of 2 to 4 M. Thermodynamic analyses based on a simple all-at-once association scheme showed that a decrease in the dissociation free energy change by only several kJ per mol of monomeric protein unit caused the assembly to be dissociated.

2P068 計算化学的手法によるアデニル酸キナーゼの反応機構の研究
Computational Study on the reaction mechanism of adenylate kinase

Kenshu Kamiya (*Dept. Phys., Sch. Sci., Kitasato Univ.*)

Adenylate kinase catalyzes the reaction: ATP + AMP + Mg2+ -> ADP + ADP + Mg2+. We have been studying the theoretical model of the reaction using MM/QM method. We constructed the model of complex structure of enzyme and substrates, ATP and AMP with Mg ion with some water molecules surronding the active center using MM or MD calculation with AMBER99 force field. The truncated models were used for the calculations with ONIOM method, and the reactant, product, transition structures were optimized. The highest level of the theory (B3LYP/ 6-31+G(d):Amber(embed)) with 853 atoms (89 atoms in QM) gives the reaction barrier of about 19 kcal/mol. The details about the model size, the conformational differences, or the free energy profiles, will be discussed.

2P069 局所パッキングパターンによる GroEL 基質蛋白質の構造的特徴の記述
Discrimination of GroEL substrate proteins using a small set of packing-patterns

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Chaperonin GroEL plays an essential role in preventing protein misfolding by assisting the folding reaction. Recent proteome-wide experiments indicate a strong correlation between GroEL substrate proteins and their structure, e.g. α/β class folds are enriched in substrates. This suggests that folded structure can be related to the specific substrate recognition by GroEL. Here, we analyzed GroEL substrates by focusing on the local packing-patterns of secondary structures shared among them, applying a non-sequential structure comparison method MICAN. As a result, a decision tree was derived to discriminate the substrates from others with more than 95% sensitivity. The packing-patterns used in the decision tree will be discussed in terms of the GroEL-substrate recognition.

2P070 天然変性タンパク質としてのグループ３ LEA モデルペプチドの役割 -乾燥に伴うリポソームの融合を防止する効果-
A role of group-3 LEA model peptides as IDPs. Protective effects on desiccation-induced liposome fusion

Takao Furuki, Minoru Sakurai (*Tokyo Institute of Technology*)

The following four peptides were tested as candidates of anti-fusion reagents for dried liposomes prepared with POPC: 1) PvLEA-22, which consists of two tandem repeats of the 11-mer motif characteristic to LEA proteins from an African sleeping chironomide, 2) its control, i.e. the peptide with the amino acid composition identical with that of PvLEA-22, although its sequence is scrambled. 3) Poly-L-glutamic acid, and 4) Poly-L-lysine. Based upon the results of the size distribution measurements and the leakage tests of a fluorescent marker inside liposomes, we argue that the protective efficiency of the peptides depends not only on the amino acid composition but also on their sequence.

2P071 An activation mechanism of human Cu,Zn-superoxide dismutase by its copper chaperone, CCS

Carolyn T. Lim, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

A catalytic copper ion in human Cu,Zn-superoxide dismutase (SOD1) is supplied by the copper chaperone for SOD1 (CCS). CCS is composed of three domains, among which domain I and III have Cu(I)-binding Cys-x-x-Cys (CxxC) and Cys-x-Cys (CxC) motifs, respectively. While both motifs have been known to tightly bind to cuprous ion, it remains controversial as to which motif is important for donating a copper ion to SOD1. Here, we show that the CxC motif in domain III is essential for human SOD1 activation; and in contrast, the CxxC motif in domain I was not necessary. Investigation on the roles of CCS domain I in SOD1 activation are currently in progress using our yeast model expressing human SOD1 and human CCS proteins.

2P072 変異体を用いた分子動力学シミュレーションによる電位依存性カリウムチャネルのイオン透過機構の解析
Analysis of the ion permeation mechanism of Kv1.2 using the molecular dynamics simulations of a single point mutant

Hiroko X. Kondo[1], Matsuyuki Shirota[1,2,3], Kota Kasahara[4], Toshiyuki Saito[1], Kengo Kinoshita[1,3,5] ([1]*GSIS, Tohoku Univ*, [2]*Grad Sch Med, Tohoku Univ*, [3]*ToMMo, Tohoku Univ*, [4]*IPR, Osaka Univ*, [5]*IDAC, Tohoku Univ*)

Voltage-gated potassium channels play a crucial role in recovering membrane potentials from its depolarized state. Potassium ions permeate outward through the selectivity filter (SF) in single-file, which requires the entering ions to be dehydrated in the central cavity. Here we analyzed the mechanism of ion permeation by performing the molecular dynamics simulations of recently reported single point mutant of Kv-channel with high conductance under several conditions. In the ion permeation process through this mutant channel entering of ions into the cavity is not a rate-limiting step. These results suggested that the water molecules in the central cavity having orientation tend to push ions toward SF though the external field is required for the permeation through SF.

2P073 コラゲナーゼによるコラーゲン原繊維分解過程の高速 AFM 観察

High speed AFM observation for degradation process of collagen fibril by collagenase

Hayato Yamashita[1], Naoko Teramura[2], Keisuke Tanaka[2], Shunji Hattori[2], Katsumasa Iijima[2], Osamu Hayashida[2], Teru Okitsu[3], Yoshiro Sohma[1] ([1]*Pharmacol., Keio Univ. Med. Sch.*, [2]*Res. inst. Biomatrix, Nippi Inc.*, [3]*Inst. Indus. Sci., Univ. Tokyo*)

Collagen is a major protein (~30%) in mammalian body and resistant to common proteinases, while digested by collagenases. The degradation of collagen fibril is very important in biological function and its biomaterial application. However, the molecular mechanism of digesting collagen by collagenase remains unclear whereas it has been intensively investigated by indirect methods. In this study, we applied high speed AFM and succeeded to directly observe the collagen fibrils showing characteristic ~67 nm periodic banding pattern and the degradation process of the fibrils by bacterial collagenase. These direct observations will provide new insights into understanding the mechanisms of collagenase activity at single molecule level.

2P074 タンパク質ジスルフィドの異常が神経変性疾患の発症に果たす役割：線虫を用いた表現型解析

Investigating the Role of SOD1 Cysteine Residues in Neurodegeneration using C. elegans

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Mutations in Cu,Zn-superoxide dismutase (SOD1) is known to cause amyotrophic lateral sclerosis (ALS), and formation of insoluble SOD1 aggregates in affected motorneurons is a pathological hallmark. Cysteine residues of SOD1 have been proposed to play a key role in aggregation by forming abnormal disulfide cross-links; however, roles of such abnormal disulfides in the development of this disease remain obscure. Here, we investigate pathological effects of cysteine residues in mutant SOD1 on ALS by constructing C. elegans models. Worms that express ALS-mutant SOD1 lacking cysteine residues in neurons have been examined to clarify effects of cysteine residues on disease-related phenotypes.

2P075 青色光センサータンパク質フォトトロピン１ LOV2 ドメインの光反応に対するクラウディング効果

Crowding effect on the reaction dynamics of blue light sensor protein phototropin1 LOV2 domain

Tomoyuki Yoshitake[1], Yusuke Nakasone[1], Tsuguyoshi Toyooka[1], Kazunori Zikihara[2], Satoru Tokutomi[2], Masahide Terazima[1] ([1]*Kyoto Univ.*, [2]*Osaka Prefecture Univ.*)

Intracellular environment in which proteins play their roles is highly condense with a lot of macromolecules such as proteins, RNAs. Such crowding environment has several effects on protein's structure, oligomeric state and reaction. In this study, we have investigated the crowding effect on reaction dynamics of blue light sensor protein phototropin1 LOV2 domain. Photoexcitation of FMN leads to a dissociation and subsequent unfolding of C terminal α helix of LOV2 domain in dilute buffer conditions. In the presence of Ficoll 70, the crowding effect was observed as decrease of reaction rates of these steps and increase of the diffusion coefficient of the product molecule, indicating the conformation of protein tends to be compact due to the exclusion volume effect.

2P076 FTIR 測定でみたエクスパンシン活性

Exapnsin activity observed by FTIR

Tomoya Imai[1], Masato Naruse[1], Yoshiki Horikawa[1], Katsuro Yaoi[2], Kentaro Miyazaki[2], Junji Sugiyama[1] ([1]*RISH, Kyoto Univeristy*, [2]*AIST*)

Expansin is one of the plant cell wall proteins, and plays an important role for the cell expansion. The direct activity of expansin is believed to be the cleavage of hydrogen bond between polysaccharides in the cell wall, which allows the wall to be loosen and remodeled for the cell expansion. This hypothesis is generally accepted, while it is difficult to provide the evidence. We challenged this hypothesis with FTIR analysis of intracrystalline deuterated cellulose. The hydroxyl groups in the cellulose crystallite usually do not accept H/D exchange because of hydrogen bonding in and between molecules. We however show that expansin actively allows H/D exchange in the crystallite, supporting that expansin disturbs hydrogen bonding in cellulose crystallite.

2P077 T-, L-プラスチン EF-hand の Ca2+感受性の違い

Different Ca2+-sensitivities between the EF-hands of T- and L-plastins

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Plastins are Ca2+-regulated actin-bundling proteins, and essential for developing and stabilizing actin cytoskeletons. T-plastin is expressed in epithelial and mesenchymal cells of solid tissues, whereas L-plastin is expressed in mobile cells such as hemopoietic cell lineages and cancer cells. Using various spectroscopic methods, gel-filtration chromatography, and isothermal titration calorimetry, we here demonstrate that the EF-hand motifs of both T- and L-plastin change their structures in response to Ca2+, but the sensitivity to Ca2+ is lower in T-plastin than in L-plastin. These results suggest that T-plastin is suitable for maintaining static cytoskeletons, whereas L-plastin is suitable for dynamic rearrangement of cytoskeletons.

2P078 新規金表面親和性ペプチドの同定及びタンパク質固定化への応用

Screening of a novel gold affinity peptide and its application on protein immobilization

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In the field of biotechnology, gold has been used as a useful substrate for biosensor as it has excellent properties such as corrosion resistivity, high electrical conductivity, and so on. In that case, immobilization of proteins with maintaining their conformation and controlling their orientation are very important factors for higher interaction detection sensitivity. In this research, we screened novel 6 a.a. gold affinity peptides (Au-tags) from T7 phage random peptide library, and their characteristics were investigated using various Au-tag conjugated esterases. Examination of remaining activities after immobilization and analysis of adsorption characteristics by SPR suggested that rational design of Au-tag conjugation pattern would be important.

2P079 MMV-4SR:シングルセル生物学(SCB)基盤検出系の開発
MMV-4SR : Development of basic detection system in Single Cell Biology (SCB)

Naoki Takeuchi[1], Tommy Nagano[2], Koichi Nishigaki[1] ([1]*Grad. Sch. of Sci. and Eng., Saitama Univ.*, [2]*DRC Co., Ltd.*)

Manipulating a single cell with the conventional system is not easy due to the loss occurring in operations. Since the scale of operation is inevitably minute in SCB, any special handling tools are necessary to deal with such a minute quantity. At the same time, novel detection systems need to be developed besides the conventional microscopic one. Considering these, we have developed the operation system at the micro-scale, that is, MMV (Microarray with Manageable Volume), which does not require pipette operations. In addition, we devised the 4SR (Stacked Slice-gel System for Separation and Reactions) which can detect DNA/RNA derived from a single cell owing to the PCR-amplification and ELISA mechanism equipped with the MMV. Here, we report this novel 4SR system.

2P080 表面増強赤外分光法によるモデル脂質膜上におけるタンパク質フォールディングの動的挙動の解析
Surface Enhanced IR study of Protein folding dynamics at a solid support lipid layer

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Surface Enhance Infrared Absorption (SEIRAS) have unique properties that enhances signals at vicinity of a substrate metal. This property is useful to determine solely the surface chemical process distinguished from that occurred in bulk phase. When a sample of interest is confined to such surface, one can selectively monitor a chemical reaction of the target regardless of the complex ensemble biological process in the bulk phase. We present an application of SEIRAS to in-situ investigation for following folding process of protein on the artificial lipid bilayer, a) aggregation and pore formation of Melittin, b) pH induced pore formation of Anthrax Protective Antigen, c)folding of bacteriorhodopsin during cell-free expression

2P081 Microtubule-kinesin binding assay to differentiate wild and mutant 4R tau proteins

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We have extended the application of kinesin-microtubule molecular set-up for differentiating wild and mutant 4R tau proteins. Microtubule-associated protein tau is a biomarker for neurodegenerative conditions related to tauopathies. We target the excessive need of non-immunological tau detection; by assaying tau-decorated TAMRA-labeled MTs in a kinesin-coated micro device comprising of MT reservoir, channel and collector region. Wild tau intervenes in MT-kinesin binding, eventually reducing the landing rate, density and velocity of MTs, which is reflected by the number of MTs collected at the collectors. By measuring the fluorescent intensity at the collector regions, we were able to distinguish wild and mutant taus.

2P082 高速 AFM による Ascaris 精子由来の MSP 繊維の観察
Direct observation of MSP filaments in cell-free extract from Ascaris sperm by Atomic Force Microscopy

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Crawling movement in eukaryotic cells require dynamic assembly and disassembly of cytoskeleton. In Ascaris sperm, motility is powered by an unique cytoskeletal protein, called Major Sperm Protein (MSP), instead of actin. To understand the property of MSP filament, we have observed them attached to the aminosilane-coated mica surface by AFM. Despite of high protein concentration (~1-2 mg/ml) in cell-free extract from Ascaris sperm, filamentous structures were clearly visible. Analysis of 245 filaments showed that their average length and diameter are 205 nm and 9.2 nm, respectively. Further analysis of filaments attached partially to the surface also revealed that MSP filaments are highly flexible unlike actin filaments.

2P083 固体 NMR を用いた単一細胞あたりの特定タンパク質の分子数計測
Counting of the target recombinant protein molecule in an intact Escherichia coli cell by solid-state NMR

Kazuya Yamada, Ayako Egawa, Toshimichi Fujiwara (*IPR, Osaka Univ.*)

The structural biology which clarifies the structure and function of proteins is essential to understand lives. A lot amount of target proteins is usually obtained by an expression system of *Escherichia coli*. However the quantification of the protein synthesis ability of *E. coli* was not reported. Here, we report the measurement of the number of recombinant protein molecules in living *E. coli* cells by using quantitative solid-state NMR. First, the relation between the integral value of ^{31}P 1D spectra and the number of cells was obtained by using cell counting. Next, the amount of the protein were evaluated from the signal intensity of the high-resolution ^{13}C NMR. From these results, we calculated the number of the protein molecules in an *E. coli* cell.

2P084 リポソーム内での再構成リボソームの翻訳活性
Translation activity of a reconstructed ribosome in liposomes

Hiroki Nakanishi (*Grad. Sch. Imf., Univ. Osaka*)

In vitro ribosome reconstruction is a critical step towards the directed evolution of ribosome. Here, as an initial step towards this goal, we report a method to reconstruct E. coli ribosomes in a cell-free translation system (PURE system) and the measurement of the translation activity in liposomes. We reconstituted E.coli ribosome in PURE system in a co-transcriptional manner from purified 16S rRNA, 30S proteins, and 50S subunit. The ribosomes were encapsulated into liposomes, and the translation activities were measured by flow cytometer. This method enables highly sensitive detection of translation activity of the reconstituted ribosome.

2P085 CS 複合体形成をモデルとした高感度ペプチド―タンパク質間相互作用検出系のデザイン

Design of highly sensitive peptide-protein interaction detection system adopting CS complex formation as the model

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Biomolecules such as protein, peptide and nucleic acids interact with each other in the cell controlling its life activities. Therefore, techniques to reproduce biomolecular interactions correctly on the solid substrate would be a good versatile way to clarify the biological phenomena. In this research, we investigated the influence of protein orientation and density on peptide-protein interaction (adopting cysteine synthase (CS) complex formation as the model) detection with employing site specific protein immobilization technique. As the results, consideration of the location of functional site with avoiding the steric hindrance by adjusting the protein density and the degree of freedom of the peptide would be important to design higher interaction detection system.

2P086 無機基板表面を標的としたラクダ抗体から着想するスマートなバイオセンサー仕様抗体の設計

Smart interface antibody design for biosensor

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Some biosensors have a sensor chip bearing antibody for detecting a specific target molecule and antibody's capturing action is transduced to physical signals. The density and orientation of immobilized antibody are critical for detection sensitivity of sensors, but several complicated processes should be considered for designing the surface of sensor chip. Recently, we devised a method for creating a camel antibody with high affinity for a specific inorganic material, and the material-binding antibody can spontaneously and high-densely bind to the material surface. In this study, we tried to design the bispecific interface molecules from material-binding antibody and antigen-binding antibody fragments, which can immobilize antigen-binding probes on sensor chip.

2P087 タンパク質デザインの新たな方法の開発: 蛍光強度の違いを利用してファージを選別する

Development of a new strategy of protein design: the single phage sorting based on fluorescence intensity

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We aim to develop a new strategy of protein design based on the phage display and single-molecule sorting. In the first step of the method, a library of phages expressing a target protein with randomized sequence is prepared. In the second step, the expressed proteins are labeled by fluorophore(s) and are sorted based on the fluorescence signals from single phages. To realize the strategy, we prepared a phage expressing C-terminal fragment (214-230) of GFP. The addition of the N terminal fragment (1-214) of GFP, purified separately, to the phage caused the reconstitution of GFP and the appearance of the fluorescence signal. We are currently conducting the observation and sorting of the phages having the reconstituted GFP on the surface.

2P088 α/β フォールドをもつ新規ヘム蛋白質の設計と合成

Design and syntheses of de novo heme proteins with an α/β fold

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De novo protein design is a constructive approach to elucidate principles of the protein structure and function. We have computationally designed tertiary structures and amino acid sequences of globular heme proteins with an α/β fold that does not occur in nature. The protein 3D architectures were modeled to accommodate heme between secondary structure elements. The amino acid sequences to fold into the modeled structures were designed with Rosetta. The heme binding site is constructed by positioning two His residues at the sites for coordination of the heme iron. In the present study, $\beta_2\alpha\beta\alpha\beta_2$ folds with the heme binding site were designed and synthesized. The synthesized proteins exhibited the secondary structure contents and heme-binding activity as designed.

2P089 膜たんぱく質の進化工学手法、リポソームディスプレイ法による α-ヘモリシンの in vitro 分子進化

若手招待講演

Directed evolution of membrane protein, alpha hemolysin, by development of liposome display method

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In vitro methods have enabled the rapid and efficient evolution of proteins and successful generation of novel and highly functional proteins. However, the available methods deal only with globular proteins (e.g., antibodies and enzymes), and not membrane proteins. Here, we report the development of a method named liposome display that can evolve the properties of membrane proteins entirely in vitro. This method, which involves in vitro protein synthesis inside liposomes was applied to the pore-forming activity of alpha-hemolysin (AH), a membrane protein derived from Staphylococcus aureus. The obtained AH mutant possessed only two point mutations but exhibited a 30-fold increase in its pore-forming activity compared with the wild-type.

2P090 リポソームディスプレイ法によるメリチンの人工進化

In vitro evolution of Melittin using a liposome display

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Recently, a new method was developed, called "liposome display", which can evolve the properties of membrane proteins in vitro. So, using this method, we tried to evolve the pore-forming activity of melittin, an antimicrobial peptide derived from bee venom. Melittin consists of 26 amino acids and exhibits antimicrobial activity by lysing cell membrane. Starting from a random mutagenized DNA library of melittin, we obtained a mutant which exhibited higher pore-forming activity and induced higher molecular weight-dependent permeability, compared with the wild type after several rounds of selection using a liposome display. This analogue of melittin which we obtained can be expected to be applied widely, for example drug delivery vesicles.

2P091 チトクローム酸化酵素の反応初期過程における共役機構の解明

Elucidation of the coupling mechanism in the initial stage of the reaction of cytochrome c oxidase

Satoru Nakashima, Minoru Kubo, Izumi Ishigami, Kyoko Itoh-Shinzawa, Shinya Yoshikawa, Takashi Ogura (*Grad. Sch. Sci., Univ. Hyogo*)

CO photolysis of Cytochrome c oxidase has been studied by using time-resolved IR and resonance Raman spectroscopy. It was observed that multi parts of the protein cooperate each other to attain its proton pumping function. Especially, for time-resolved IR spectroscopy, we developed a new IR spectrometer, which makes it possible to pursue the protein reaction in water solution under physiological conditions. From the observed results, the ligand attached to CuB site just after the photodissociation, and escaped toward bulk solution within 2 μsec. Accompanied with this ligand behavior, the iron-His bond became weak, and α-helix structure along proton pump pathway, changed its structure to close the gate of the water channel, which protects the back leak of protons.

2P092 過渡吸収分光法による一酸化窒素還元酵素の単寿命 NO 結合体の解析

Characterization of transient NO-bound form of Nitric Oxide Reductase by Transient Absorption Spectroscopy

Tetsunari Kimura[1], Shoko Ishii[1,2], Takehiko Tosha[1], Yoshitsugu Shiro[1,2], Minoru Kubo[1,3] ([1]*RIKEN, SPring-8*, [2]*Grad. Sch. Life Sci., Univ. Hyogo*, [3]*JST, PRESTO*)

Nitric oxide reductase (NOR) is a membrane protein, which reduces NO to N_2O at the catalytic center consisting of heme *b* and non-heme Fe_B. Although the clarification of the transient NO-bound form is critical to understand the molecular mechanism of NO reduction, capturing this early intermediate is difficult because of the fast NO reduction (μs to ms). To observe the NO-bound form in real-time manner, we developed the time-resolved visible (TR-vis) and infrared (TR-IR) spectroscopic measurement systems, in which caged-NO is used to trigger the reaction. TR-vis spectroscopy showed that the NO-bound intermediate formed within 4 μs. The further characterization of NO-bound form by TR-IR, which monitors the N-O stretching vibrational mode, is now underway.

2P093 チトクロム c 酸化酵素の酸素還元反応の時間分解赤外吸収測定を目的とした酸素肺フローシステムの開発

Development of sample flow system with an oxygen lung for time-resolved infrared measurements of cytochrome c oxidase

Tatsuhito Nishiguchi[1], Masahide Hikita[1], Kyoko Shinzawa-Itoh[1], Shinya Yoshikawa[2], Satoru Nakashima[2], Takashi Ogura[1,2] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*Picobiology Ins., Univ. Hyogo*)

A new flow system aimed at measuring time-resolved (TR) IR spectra of the oxygen reduction reaction by cytochrome c oxidase (CcO) in aqueous solution was developed. Oxygen is supplied by the inserted artificial oxygen lung. The reaction is triggered by CO photolysis of CO-bound CcO. To measure the IR spectra for aqueous solution, the path length of the flow cell must be made extremely thin (50 μm) to reduce high absorbance background of water and, in this case, smooth flowing of the solution should be warranted. To evaluate the system, TR visible absorption spectra were observed. Since the reported intermediates and their dynamics were well reproduced, proper functioning of the system was confirmed. Currently, we are trying to measure TR IR spectra with this system.

2P094 タンパク質を基盤とした酸素濃度 FRET センサー

Protein-based FRET sensor for oxygen concentration

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The sea lamprey hemoglobin (slHb) is primarily monomeric in oxy form, while slHb self-associates to dimers or tetramers upon deoxygenation. The oxygen dependent monomer-dimer transition of slHb is useful to apply to the FRET approach. The single cysteine resiudes in slHb were labeled by Alexa Fluor 488 or 594. Purified slHb with Alexa Fluor dyes was mixed and measured by the fluorescence spectroscopy. There was no FRET in our FRET system when the oxygen concentration is high. On the other hand, the labeled slHb exhibited FRET signal under low oxygen concentration. Since slHb self-associates upon deoxygenation, slHb would bring the Alexa Fluor dye in close enough proximity to allow for FRET. Our FRET system could be useful to detect the oxygen concentration in the cell.

2P095 細胞内の遊離ヘムの可視化に向けた融合タンパク質の開発

Development of the FRET-based sensor protein for visualization of free heme *in vivo*

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Free heme, not taken in hemeproteins, is cytotoxic. The concentration of free heme regulates a variety of gene expression. However, the cellular labile heme pool has not been well characterized. Therefore, we developed the FRET-based sensor proteins. The heme-binding protein HrtR regulates the expression of the heme-efflux system in *Lactococcus lactis*. Thus, we fused HrtR mutant and fluorescent protein and measured FRET. The measurements of the fusion proteins demonstrated the decrease of FRET efficiency with the increase of the heme concentration *in vitro*. This result can be applied to develop the sensitive probe for the concentration of cellular free heme.

2P096 紫外共鳴ラマン分光法によるインドールアミン 2,3-ジオキシゲナーゼの三者複合体中間体モデルの研究

Ultraviolet resonance Raman study on a ternary complex intermediate model of indoleamine 2,3-dioxygenase

Kure'e Kayama[1], Sachiko Yanagisawa[1], Hiroshi Sugimoto[2], Yoshitsugu Shiro[2], Takashi Ogura[1] ([1]*Univ. of Hyogo*, [2]*RIKEN SPring-8 center*)

Indoleamine 2,3-dioxygenase (IDO) is a heme-enzyme and catalyzes insertion of two oxygen atoms into L-tryptophan (Trp), yielding N-formylkynurenine. Two oxygen atoms had been proposed to be inserted into Trp without cleaving the O-O bond. However, detection of a Fe=O species during reaction indicated that the O-O bond cleaved before insertion of the second oxygen atom.

The unusual reaction might be enabled by distinct heme-pocket environment including conformation of the IDO-bound substrate. In this study, we measured UV resonance Raman spectra of CN^--ligated and Trp-bound IDO as a model of IDO-O_2-Trp ternary complex, which is a key reaction intermediate. By utilizing UV resonance enhancement, we can selectively obtain vibrational spectra of the bound-substrate.

2P097 紫外共鳴ラマン分光法による酵素に結合した基質の構造解析
Structural Analysis of the Substrate Bound to Enzyme by UV Resonance Raman Spectroscopy

Sachiko Yanagisawa[1], Masayuki Hara[1], Hiroshi Sugimoto[2], Yoshitsugu Shiro[2] ([1]*Univ. of Hyogo*, [2]*RIKEN SPring-8 center*)

We have developed a new method to analyze structure of substrate, which is bound to enzyme, by detecting its resonance Raman (RR) spectra. We have applied the new methods to indoleamine 2,3-dioxygenase (IDO) which catalyses direct incorporation of two oxygen atoms into tryptophan (Trp) and whose reaction mechanism is not fully understood due to the lack of structural information. We have successfully obtained UVRR spectra of the bound-substrate in IDO and found some spectral change in Trp bands by binding to IDO; 10 cm^{-1} down-shift of W3 mode and 10 cm^{-1} up-shift of W18 mode. These results clearly show that the bound Trp to IDO as substrate has a specific conformation as compared to Trp in solution.

2P098 マイクロ流路デバイスを用いた生体膜実時間解析システムの開発
Development of a novel system for the real-time analysis of biological membranes by using a microfluidic device

Yuji Kimura, Sayaka Kazami, Yu Hashimoto, Hiroyasu Itoh (*Tsukuba Research Laboratory, Hamamatsu Photonics KK*)

We developed a real-time analysis system for biological membranes in the whole-cell recording configuration: two micro-fabricated flow channels connected with a micro aperture (2 μm) where a large-sized liposome is captured. To validate the system, we assessed the proton pump activity of complex I (CI) of the respiratory chain as follows. First, a large-sized protoplast from *E. coli* (10 μm) was trapped and perforated at the aperture with an electrical pulse. Then, one of the channels was infused with NADH, a substrate for CI. As NADH diffused into the *E. coli* via the aperture, pH fluorescent indicator started to become bright at another channel. It suggests proton was transferred across the membrane by CI, as observed in bulk biochemical assays.

2P099 ブタ心臓由来ミトコンドリア調製法の確立と活性測定
Establishment of a new process for preparation of porcine heart mitochondria, and their activity measurements

Sayaka Kazami, Yuji Kimura, Hiroyasu Itoh (*Tsukuba Research Laboratory, Hamamatsu Photonics K.K.*)

We have been developing a novel real time monitoring device for membrane protein activities. As a membrane sample, we chose the mitochondrial inner membrane (IM) prepared from porcine hearts. Porcine heart, which is not required any assessment of BSE, is extremely fresher than bovine heart, because the hearts can be obtained immediately after the animals are slaughtered.
We confirmed the proton pumping activity of the IM proteins in bulk experiment by using a fluorescent pH indicator. The respiratory complexes were selectively activated depending on various substrates, which is consistent with bovine IM assay. By fusing the IM, we also successfully prepared a giant vesicle (>10 μm), which will allow us to image membrane protein activities using our device.

2P100 PiericidinA によるウシ心筋 NADH-ユビキノン還元酵素の活性中心の定量
Quantification of the active center of bovine heart NADH-ubiquinone reductase with Piericidin A

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NADH-ubiquinone reductase (Complex I) pumps protons coupled with electron transfer from NADH to ubiquinone. For crystallization of this large membrane protein, the method for quantitative evaluation of the integrity of the purified enzyme sample was searched for improvement of the purification method by extensive examinations of the effect of temperature on binding rate of a specific inhibitor, Piericidin A (PA), to Complex I. Under the maximum binding conditions (10 min preincubation of Complex I preparation at 10 °C), the content of the integral Complex I and the PA-binding affinity in each preparation can be quantified reproducibly. Correlation between PA sensitivity and the integral enzyme content has been examined for elucidation of the PA inhibition mechanism.

2P101 低分子量 G タンパク質 K-Ras のフォトクロミック分子を用いた光制御
Photo-regulation of small G protein K-Ras using photochromic molecules

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Ras is one of small G-protein known as a molecular switch mediating cellular signalling. We performed basic study to control the function of Ras reversibly using photochromic molecules, 4-phenylazophenyl maleimide (PAM) upon visible (VIS) light and ultra-violet (UV) light irradiation. We have prepared the Ras mutants which have a single cysteine at functional sites and modified with PAM stoichiometrically. The GTPase activities of PAM-Ras were reversibly altered upon VIS and UV light irradiations. In order to monitor the effect on GTPase kinetic pathway by the photoisomerization of PAM, We synthesized a novel fluorescent GTP analogue, NBD-GTP. The Kinetic studies suggested that initial binding step of NBD-GTP to Ras mutant Y32C is regulated with PAM.

2P102 ヒト癌抑制遺伝子候補 *101F6* によるカスパーゼ非依存性細胞死の機構分析
Analyses of caspase-independent apoptosis caused by the expression of a candidate human tumor suppression gene, *101F6*

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Since the tumor suppression activity was enhanced by ascorbate(AsA), there is a possibility that 101F6 protein, which belongs to cytochrome b561 family, utilizes redox reactions using AsA to induce apoptosis. Objective of our research is clarification of the molecular mechanism of caspase-independent apoptosis caused by 101F6 protein. Analyses of intracellular localization of the 101F6 gene product showed that the protein was expressed in endoplasmic reticulum. Pathway analyses of the apoptosis signaling using cultured A549 cells derived from human lung adenocarcinoma suggested that 101F6 was involved in the Keap1-Nrf2 system. Moreover, mutation analyses on the putative AsA-binding site showed that 101F6 might have a distinct electron-transferring mechanism.

2P103 哺乳類ミトコンドリア呼吸鎖超複合体の精製

Purification of the respiratory super complex from mammalian mitochondria

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The mitochondrial respiratory chain consists of 5 large multi-subunit complexes, complex I - V. Complexes I, III and IV catalyze the transfer of electrons from NADH to molecular oxygen and use the energy released in electron transfer to pump protons across the inner membrane. Though the individual complexes have been studied, little is known about how these complexes interact in the membrane to perform their tasks. These complexes assemble into higher level of organization, respiratory supercomplexes, comfirmed by BN-PAGE analysis.
Here, we report the purification of respiratory supercomplexes from mammalian mitochondria. The purified complexes stabilized with amphipol show the KCN-sensitve NADH oxidation activity. The absorption spectra of the complexes were obtained.

2P104 定常状態での GPCR シグナルは GPCR ダイマーによって誘起される

Dimers are the key trigger for the GPCR's basic signaling without ligation

Rinshi Kasai, Akihiro Kusumi (*Inst. Front. Med. Sci., WPI-iCeMS, Kyoto Univ.*)

Monomers and dimers of G-protein coupled receptors (GPCRs) are in dynamic equilibrium, with dimer lifetimes of ~100 ms. Here, we found that trimeric G proteins were recruited to both GPCR monomers and dimers in the steady state, but the addition of the inverse agonist, which blocks the GPCR basal activity, a unique feature of GPCRs, decreased the G-proteins' recruitment to GPCR dimers, using β2 adrenergic receptor (b2AR) as a paradigm, suggesting that GPCR dimers are responsible for the basic b2AR signaling. A b2AR mutant with lower basal activity formed dimers with a shorter lifetime, comparable to the lifetime for the wild type b2AR bound by the inverse agonist. These results together indicate that GPCR dimers are the key trigger for the GPCR's basic signaling.

2P105 1 分子イメージングによる代謝型グルタミン酸受容体の高次多量体形成と内在化の解析

Single-molecule imaging analysis of higher-order oligomerization and internalization of metabotropic glutamate receptor

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Metabotropic glutamate receptor (mGluR) is a G protein-coupled receptor involved in the synaptic transmission. It is well-known that mGluR functions as a constitutive homodimer. However, the higher-order oligomerization of mGluR under physiological condition is yet to be cleared. Here we show that deceleration of the diffusion followed by the internalization occurs accompanied with the oligomerization upon mGluR activation. The diffusion and oligomerization of fluorescence-tagged mGluR on HEK293 cell surface were monitored by total internal reflection fluorescence microscopy and analyzed by variational Bayesian hidden Markov model. We will discuss the dynamics of the clathrin-dependent internalization of mGluR from the dual-color single-molecule imaging analysis.

2P106 AFM Probing Opioid Signalosome on Neuroblastoma

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Opiate drugs exert their analgesic actions by binding to mu opioid receptors. However, opioid use is limited by tolerance development. MOR activation results in receptor desensitization and internalization. In this study, dynamic force spectroscopy AFM is used to measure affinity and distribution of MOR on live and fixed SHSY5Y cell treated with agonist by coating the AFM tips with antibody. The agonist-induced internalization is measured in terms of the relative change in the number of binding sites observed during force volume mapping. This approach in receptor localization will facilitate further studies on the correlation of proper receptor function with the dynamic physical properties of the MOR signalosome.

2P107 プロトン駆動力を細胞外へと捨てる微生物外膜タンパク質

若手招待講演

Proton discarded to cell exterior via outer-membrane bound enzyme

Akihiro Okamoto, Shafeer Kalathil, Yoshihide Tokunou, Kazuhito Hashimoto (*Grad. Sch. Eng., Univ. of Tokyo*)

Certain microbes can produce energy for sustaining life by transporting electrons from cell interior respiratory electron chain to insoluble electron acceptors located outside the cell, a process referred to as extracellular electron transport (EET). This EET process was recently found to occur via semiquinone active species bound to outer-membrane c-type cytochromes. Here, our in-vivo electrochemical analysis shows that the bound flavin cofactor transports not only electrons but also protons to cell exterior from periplasm, implying ATP is produced by substrate-level phosphorylation. Discarding proton motive force would be a unique strategy to slow down microbial growth in a solid surface environment with extremely high cell density.

2P108 ペリプラズム pH 追跡による細胞外電子移動酵素のプロトン移動能検討

***In-vivo* periplasmic pH assay for studying proton export by outer membrane cytochromes in extracellular electron transport**

Yoshihide Tokunou[1], Akihiro Okamoto[2], Kazuhito Hashimoto[2] ([1]*Department of applied chemistry, Univ. Tokyo*, [2]*Department of applied chemistry, Univ. Tokyo*)

Microbial extracellular electron transport (EET) is a process to transport electrons to insoluble substrates located outside cell wall. We recently reported that a cell-secreted flavin binds to outer-membrane *c*-type cytochromes to enhance the rate of EET despite its unfavorable redox potential. Since the redox reaction of flavin couples proton transport, it is, therefore, assumable that the unfavorable flavin potential comes from need for EET to export proton from periplasm to cell exterior. Here, we constructed a periplasmic pH (pH_p) assay based on the membrane permeability of flavin during EET. Our *in-vivo* pH_p assay showed that EET does not accumulate proton in periplasm, suggesting that proton and electron are simultaneously exported to cell exterior by flavin.

2P109 サイズの異なるナノポアの脂質二分子膜への再構成
Reconstitution of various-sized nanopores in lipid bilayer

Hirokazu Watanabe, Ryuji Kawano (*TUAT*)

It is well known that nanopore sensing is strong tool in the field of high sensitive biosensing. To date, α-hemolysin (1.4 nm in diameter) pore has been used in the most of the nanopore sensing. This size of pore can detect a single-stranded DNA (1 nm in diameter) molecule because of the great size-matching. The size of the nanopores often used is nearly 1 nm scale in diameter. However, much larger nanopore is required for detecting proteins, secondly-structured DNA/RNA, and antigen, a few examples have been reported previously. In this study, we show two different proteins, magainin (2 nm in diameter) and human perforin (~10 nm in diameter).We believe that these nanopores can detect the molecules of which size is too large to be detected by α-hemolysin pore.

2P110 多剤輸送担体 EmrE の pH 依存性基質結合駆動力に対する酸性残基の役割
Role of acidic residues of multidrug resistance transporter, EmrE for the pH dependent driving force of substrate binding

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EmrE is H^+/lipophilic cations antiporter, which has three acidic residues (Glu14, Glu25 and Asp84). We reported previously the pH dependent driving force of substrate binding to EmrE. Here, we had demonstrated with isothermal titration calorimetry (ITC) how substrate binds to the EmrE mutants (E14D, E25Q and D84N). Asp84 is a mainly key residue to drive the substrate binding, because in D84N, the driving forces of substrate binding were enthalpy at pH 6.5 and 7.4, not shifted. These results indicate that Asp84 might become the protonated form at the intermediate state between inward-facing (entropy-driven binding) and outward-facing (enthalpy-driven binding) conformation. The thermodynamics-based mechanism of H^+/substrate transport in EmrE will be discussed.

2P111 Fast measurements of membrane transporter activity with attoliter-sized arrayed lipid bilayer chamber system

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Transporters play pivotal roles by transporting molecules across bio-membranes. We recently developed the femto-liter sized arrayed chambers sealed by lipid bilayers (ALBiC), which enable the fluorescent detection of single transporter activity. However, it still had some drawbacks, i.e. slow detection (>1 h). To address this issue, we in this study developed the attoliter-sized ALBiC (aL-ALBiC). aL-ALBiC demonstrated the fast detection of passive transport activity by a-hemolysin within hundreds seconds, which was 10-fold faster than that of conventional ALBiC. This fast detection of single transporter molecules would reduce possible artifacts such as photobleach. Thus, aL-ALBiC would be the platform for single-molecule analysis of the various membrane proteins.

2P112 新世界ザルの苦味受容体 TAS2R1 および TAS2R4 のリガンド感受性の種間差と進化
Interspecific variation of ligand sensitivity and evolution of bitter taste receptors TAS2R1 and TAS2R4 in New World monkeys

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Bitter taste is mediated by bitter taste receptors (TAS2Rs). In recent years, sensitivity of human TAS2Rs to various bitter compounds has been elucidated. However, it remains largely unknown how far this knowledge is applicable to other primates and how variable the sensitivity is among species. In this study we focused on TAS2R1 and TAS2R4 of New World monkeys with dietary and sensory (visional) diversity. By calcium imaging in HEK293T cells, we demonstrated interspecific variation of sensitivity to some bitter tastants in both receptors. Furthermore, we applied the functional assay to ancestral receptors reconstructed based on the maximum likelihood phylogenetic inference and identified amino acid substitutions relevant to the evolutionary shift of ligand sensitivity.

2P113 新規 *Halorubrum* 属菌のもつバクテリオロドプシン類タンパク質の研究
Study on the microbial rhodopsins from the cell membrane of *Halorubrum* sp.ejinoor

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We have identified the new bR-like and pR-like genes from a halorubrum species (*H.*sp.ejinoor ; (*H.e.*) isolated from Ejinoor salt lake in Inner Mongolia and reported last year. The brown membrane was isolated from *H.e.* by a step sucrose gradient centrifugation like purple membrane. The photoreaction and ion-pump activities were measured on the brown membrane. The photoreaction cycle with M, O-intermediate absorbance was observed, and kinetics were close to that of Xanthorhodopsin. Measurement of ion transportation suggests the existence of proton pump and chloride pump activities in the membrane vesicles of *H.e.*. The results suggest that the brown membrane contains not only bR and pR-like pigments, but also chloride pump, hR-like pigment.

2P114 NpHR の三量体安定化に寄与するアミノ酸残基の特定
Specification of amino acid residues which stabilize trimer formation of halorhodopsin

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Halorhodopsin (NpHR) from *N. pharaonis* is a retinal protein and forms trimer as a unit. At room temperature, the trimer of NpHR is retained both in the presence and absence of anion although the former is more stable. In this study, we aimed to specify the amino acid residue which stabilizes the NpHR trimer. Mutation of N145 or T147 located in the hydrophilic region of the molecular interface to A led to dramatic destabilization of the NpHR trimer in the absence of anion, compared to the case of the wild type. On the other hand, the trimer structures of those mutants were stable similarly to that of the wild type in the presence of anion. These results suggest that N145 and T147 have important role to keep the packing between NpHRs especially in the absence of anion.

2P115 蛍光相互相関分光法を用いたグルココルチコイドレセプターと DNA 間相互作用の定量化

Determination of the quantitative interaction between glucocorticoid receptor and DNA by fluorescence cross-correlation spectroscopy

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Glucocorticoid receptor (GR) belongs the nuclear receptor superfamily. Upon binding to the ligand, GR regulates the transcription of target genes through direct binding to glucocorticoid response element (GRE) as a dimer. Recently, it was reported that a monomeric GR could regulate the genes which is different from that controlled by dimeric GR. Our purpose is determination of dissociation constant of monomeric and dimeric GR toward GRE to reveal the mechanism as initiation of transcription. For this purpose, human wild type GR and dimerization deficient mutant were purified as fusion protein with EGFP from insect cells. We quantified the interaction between purified EGFP-GR and Alexa647-labeled GRE in vitro by fluorescent cross-correlation spectroscopy.

2P116 一分子蛍光観測法による癌抑制蛋白質 p53 の DNA 探索機構の研究

Investigation of DNA search mechanism of tumor suppressor p53

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Tumor suppressor protein p53 is considered to perform 1D sliding, 3D search and intersegmental transfer to find the target sequence on DNA genome. In this study, we aim to investigate the heterogeneity in the 1D sliding of p53 and the search mechanism of p53 on the collapsed DNA. Firstly, we measured the sliding of p53 along the stretched DNA using a single-molecule fluorescence microscopy, and revealed that the 1D sliding of p53, previously considered as the simple diffusive movement, has multiple sliding modes with different diffusion constants. Secondly, we constructed the novel experimental system based on the rapid cycling of stretch and collapse of DNA, and examined the frequency of 3D search or intersegmental transfer of p53 on the collapsed DNA.

2P117 二種の好熱菌由来ヌクレオチドキナーゼと変異体における高次構造変化とヌクレオチド結合の蛍光分光学的解析

Fluorescence spectroscopic studies on conformational changes and Nucleotide binding of thermophilic nucleotide kinases and their variants

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In previous studies, we reported the application of fluorescence spectroscopic methods for the conformational changes by ANS, and also the binding of aromatic molecule by fluorescence quenching.
In this study, we investigated the conformational changes of two thermophilic nucleotide kinases and their variants by using intrinsic Tyr residues and ANS as fluorescence probe, and their nucleotide binding properties by quenching of Tyr-excited fluorescence. As results, it was elucidated that evaluations for the differences in the conformational changes after heating, and nucleotide binding properties might be possible even in cases of thermophilic nucleotide kinases and their variants by these fluorescence spectroscopic applications.

2P118 哺乳類ヌクレオチド除去修復タンパク質 XPC の DNA 結合モードの 1 分子イメージング

Single-molecule direct visualization of DNA binding modes of the mammalian nucleotide excision repair protein XPC

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XPC-RAD23B protein complex is known to be responsible for damage recognition in mammalian nucleotide excision repair. To address the damage recognition mechanism, we have visualized single-molecule Qdot-labeled XPC-RAD23B protein complexes along λDNA tethered by our developed PEG-based single-molecule imaging platform. On damaged λDNA created by UV-irradiation, we visualized association of XPC-RAD23B with a lesion most of which remained bound at least several hours. On undamaged λDNA, in contrast, XPC-RAD23B dissociated from DNA in several seconds on average and some performed one-dimensional bidirectional diffusion with a variety of diffusion coefficients. We will present the data and discuss relationship between the observed binding modes and damage recognition.

2P119 15N NMR 分光法を用いた C-Ag(I)-C 塩基対の構造解析

Nitrogen-15 NMR spectroscopic studies of Ag (I)-mediated C-C base-pairs

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In DNA duplex, a Ag (I) ion is selectively captured by a cytosine-cytosine mismatch and formed Ag (I)-mediated cytosine-cytosine base pair (C-Ag(I)-C). However, the structure of C-Ag(I)-C in DNA duplex was not fully understood. To determine the chemical structure of C-Ag(I)-C, uniformly 15N-labeled DNA duplex was enzymatically synthesized. In the 1D 15N NMR spectra of the 15N-labeled DNA duplex, the 15N signals of cytosine residues of C-Ag(I)-C base pair were observed. We will discuss determined chemical structure of C-Ag(I)-C base pair.

2P120 ヌクレオソーム３量体の構造ダイナミクスの粗視化分子動力学法と X 線小角散乱による研究

Structural dynamics of tri-nucleosome studied by combination of coarse grained molecular simulation and SAXS

Yusuke Takagi[1], Yuichi Kokabu[2], Takashi Oda[2], Hiroaki Tachiwana[3], Hiroo Kenzaki[4], Hitoshi Kurumizaka[3], Mamoru Sato[2], Mitsunori Ikeguchi[2], Shoji Takada[1] ([1]*Dept. Biophys., Grad. Sch. Sci., Kyoto Univ*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ*, [3]*Faculty of Science and Engineering, Waseda Univ*, [4]*ACCC, RIKEN*)

The dynamic change of chromatin structure is the basis of transcriptional activity control. Thus it is important to know structures of nucleosome-array in a thermal equilibrium state. Since the angle made of contiguous three nucleosomes is especially critical to understand the packing of chromatin, tri-nucleosome can be a nice and minimal system to address folding of chromatin. In this research, the structure dynamics of the tri-nucleosome was investigated by the coarse graining molecular simulation. Using structural ensemble of various simulations, we computed SAXS profiles, which were compared with the experimental SAXS data.

2P121 密度汎関数法による DNA 塩基対とラジカルの反応機構の解析

DFT calculations on attacking mechanism of radicals to DNA base pair

Naoko Okutsu, Hideaki Tamai, Eisuke Shimizu, Noriyuki Kurita (*Toyohashi University of Technology*)

Recently, the influence of radiation on human body has been recognized as a serious problem. In particular, highly-reactive radicals produced by the radiation react with DNA, resulting in damage on its structure and electronic properties. It is thus important to investigate the reaction mechanism of radicals to DNA for elucidating the initial damage in DNA induced by the radiation. In the present study, we search for the transition states of the attacking mechanism between base-pair (G-C or A-T) and radical (OH and H radicals) in vacuum and in water, using the density functional theory calculations. The results elucidate that OH radical can cause mutation in A-T base pair, while H radical affects significantly on G-C base pair.

2P122 アルコールによって誘起される DNA の凝縮・脱凝縮二段階転移

Condensed DNA is unfolded into elongated conformation at ethanol concentration around 80%

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We report that condensed DNA caused in ethanol solution becomes solvable at ethanol concentration around 80%. Through the observation of individual giant DNA molecules by use of fluorescence and bright microscopies, it becomes clear that DNA undergoes a reentrant coil-to-globule-to-coil transition with the increase of ethanol concentration. CD measurements indicate its secondary structure exhibits the step-wise change, B-C-A-form, corresponding to the observed reentrant transition. We will discuss the mechanism of the unfolding transition at ethanol concentrations around 80%, in relation to the possible occurrence of micro-phase segregation of the ethanol solution in the presence of DNA as a polyelectrolyte.

2P123 Effect of branched polyamine from hyperthermophile on the structure of genomic DNA

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Naturally occurring polyamines such as spermidine and spermine are present in both prokaryotic and eukaryotic cells and known to play important roles in various cellular processes. Interestingly, long- and/or branched-chain polyamines are found in thermophilic archaea and bacteria. These unique polyamines are considered to support the growth of thermophilic microorganisms under high temperature conditions. We studied the effect of these polyamines on the higher order structure of DNA by fluorescence microscopy together with atomic force microscopy. It is found that such specific polyamines exhibit the effect to bridge between the segments of genomic DNA molecule. We may discuss the role of such a bridging effect in relation to the thermoduric properties.

2P124 粗視化シミュレーションによる多ヌクレオソーム系の構造サンプリング

Structural sampling of polynucleosome by coarse-grained simulations

Hiroo Kenzaki[1], Shoji Takada[2] ([1]*Advanced Center for Computer and Communications, RIKEN*, [2]*Grad. Sch. of Sci., Kyoto Univ.*)

In eucaryotes, the nucleosome is the fundamental repeating unit of chromatin, and the strings of nucleosomes take hierarchical structure. The choromatin structure may be essential for the searching mechanism of transcription factors to achieve the target sites on DNA duplex. Thus we performed structural sampling of polynucleosome by coarse-grained protein and DNA model to investigate local structural and dynamical features of choromatin.

2P125 エステル基を有するテトラゾラト架橋白金(II)二核錯体による DNA の高次構造変化

Action of tetrazolato-bridged dinuclear platinum(II) complexes with ester moiety on the higher order structure of DNA

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Platinum-based complexes such as cisplatin and carboplatin are widely used in cancer chemotherapy. Unfortunately, their usage has been limited due to severe side effects. To minimize the side effect, various derivatives of platinum compound are actively under development. In this study, we investigated the effect of tetrazolato-bridged dinuclear platinum(II) complexes having ester group on the higher order structure of DNA by fluorescence microscopy. Single-molecule observation revealed that the potency for inducing DNA compaction was in the order propyl > ethyl > methyl ester derivatives. We will discuss the effect of DNA compaction by these platinum compounds in relation to cytotoxicity.

2P126 分子動力学計算を用いた蛋白質･RNA 複合体立体構造予測

Three dimensional structure prediction of RNA-protein complexes by MD simulation

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RNA-protein interactions play fundamental roles. To understand these interactions, it is necessary to know the three-dimensional structures of RNA-protein complexes. However, determining the tertiary structure of these complexes is often difficult, suggesting that an accurate prediction method for RNA-protein tertiary structures is needed. Previously, we propose a novel method based on docking for predicting three-dimensional structures of RNA-protein complexes, which had higher success rates than other methods. However, our method requires the apo form structure of both protein and RNA. Comparing with protein, quite few structures of RNA are determined. Thus, we developed a novel method based on MD simulation from protein structure and RNA sequence.

2P127 光制御型 bZip モジュール Photodimerizer の二量体化分子機構

Molecular mechanism for dimerization of the light-regulated bZip module, Photodimeizer

Yoichi Nakatani, Osamu Hisatomi (*Grad. Sch. Sci., Osaka Univ.*)

Photodimerizer (Pd) is a blue light-regulated dimerizing module consisting of a basic leucine zipper (bZip) and a light-oxygen-voltage-sensing (LOV) domains. To understand the molecular mechanism for dimerization, we investigated the concentration dependencies of Pd and N-terminally-truncated Pds, composed of Zip-LOV, linker-LOV and only LOV domain. The dynamic light scattering and size exclusion chromatography measurements indicated that blue light induces the dimerization of all Pds. In the dark state, monomeric forms of bZip-LOV and Zip-LOV are more stable than those of linker-LOV and LOV, probably due to the synergistic interactions between Zip and LOV domains. Consequently, blue light drives conformational switching of monomeric Pd into the dimeric form.

2P128 FMO/3D-RISM 法の開発と応用

Development of FMO/3D-RISM method and its applications

Norio Yoshida (*Kyushu University*)

An efficient implementation of the three-dimensional reference interaction site model (3D-RISM) theory to the fragment molecular orbital (FMO) method was proposed. The method allows us to treat an electronic structure of whole part of macromolecules, such as protein, as well as a solvent distribution around the solute macromolecules. In this study, we propose a procedure to save the computational cost for calculating the electrostatic potential in the framework of FMO method. The results are compared with those from the other methods.

2P129 Generalized Born モデルによる蛋白質-蛋白質間相互作用計算の重要パラメータ

Critical parameters of the generalized Born model to simulate protein-protein interactions

Yukinobu Mizuhara, Koji Umezawa, Jun Ohnuki, Dan Parkin, Mitsunori Takano (*Dept. of Pure & Appl. Phys., Waseda Univ.*)

The generalized Born (GB) model is a widely-used implicit solvent model to simulate biopolymer electrostatics. The GB model has several critical parameters that determine the burial of charges which is accompanied by large energy change and hence largely affect intermolecular interaction. These parameters are to estimate the Born radius, to which much effort has been devoted. However, some potentially important parameters have been less well studied. Here we show how much these parameters affect binding free-energy between two small helices with a single salt-bridge pair, comparing to the result obtained from the simulation with explicit solvents. We then interpret the binding behavior between kinesin and tubulin observed in the simulation using the GB model.

2P130 シトクロム c – シトクロム c 酸化酵素間における電子伝達複合体形成機構の浸透圧を用いた解析

Analysis of interactions in the electron transfer complex between Cytochrome c and Cytochrome c Oxidase using osmotic pressure

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In the mitochondrial respiratory chain, Cytochrome c oxidase (CcO) accepts electrons from Cytochrome c (Cyt c) to reduce molecular oxygen. The complex formation of Cyt c with CcO is characterized by the positive entropy change, suggesting the dehydration from hydrophobic residues, but no evidence of such dehydration has been reported. Here, we examined the osmotic pressure dependence of the dissociation constant for the complex formation and estimated the number of dehydrated water molecules. We successfully identified that about 17 water molecules are dehydrated in the complex formation and found that the dehydration is a primary factor to increase the entropy for the complexation. Based on the results, we will discuss contribution of dehydration to protein folding.

2P131 The solvent-accessible surface area of proteins is a key factor for hydration structure and dynamics in crowded environment

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In this study, using molecular dynamics simulations we found that the solvent-accessible surface area (SASA) of proteins in a crowded environment affects the structure and dynamics of water, even at the same protein volume fraction. A series of protein G and bovine serum albumin (BSA) systems were built and simulated for 50 ns. Hydration structure and water dynamics were analyzed as a function of total SASA. The results show that the water structure and dynamics were altered differently. The diffusion of water slowed down and showed a linear trend as a function of total SASA. The change in the water dynamics can be related to the ratio of water in the bulk region. The results suggest a novel view on the protein crowding effect on hydration.

2P132 混合分布モデルにより分離されたタンパク質水和水の振る舞い：シミュレーション・データマイニングによるアプローチ

Hydration water behavior classified by mixture model : Simulation data-mining approach

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Water plays important roles in bio-molecular dynamics. The identification of the hydration water has been a difficult task, because the physicochemical parameters disperse widely. We employed “the simulation data mining approach” to overcome these difficulties.
In this study, under the motivation to investigate the water dynamics surrounding protein, we focused the water behavior in the vicinity of soluble proteins. The MD trajectory of the individual water molecule was mapped into the feature-space by means of mixture model. The classification on the feature-space of new categories of hydration water was succeeded. One of the classes of water behavior is consistent with the first hydration water. A long-range water structure originated by protein was detected.

2P133 マイクロドロップアレイを用いた一分子 DNA からの無細胞タンパク質合成

Cell free protein synthesis from single DNA in microdrop array

Hiroto Kizoe, Yi Zhang, Kazuhito Tabata, Hiroyuki Noji (*Grad. Sch. apply chem, Univ. Tokyo*)

Protein plays a very important role in cells. It is known that number of proteins in each cell is heterogeneous because regulation of gene expression takes place at a single DNA locus within a cell. On the other hand, cell-free protein synthesis system was developed. However, it is unknown whether heterogeneity of protein expression in vivo and that of proteins synthesized with reconstituted protein synthesis system are same or not. Then it is necessary to examine fluctuation of synthetic quantity of proteins from single DNA with reconstituted protein synthesis system such as PURE system. In this study, precise quantification of protein synthesis from single DNA is implemented with microdroplet array. At present, kinetic analysis of protein synthesis is attempted.

2P134 転写制御ダイナミクスの in vitro 1 分子計測

In vitro single molecule assay for the dynamics of transcriptional regulation

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Stochastic gene expression is generally explained by a two-state model of gene regulation, where a gene stochastically fluctuates between on and off states. The on state is explained by a molecular mechanism where RNA polymerase binds to the promoter, initiates transcription, and mRNA is produced at a constant rate. Comparatively little is known, however, about the off state.
In this study, we reconstructed prokaryote transcription and transcriptional regulation in vitro, and visualized mRNA production at the single molecule level by using fastFISH. Our data suggest that our experimental system is capable of clarifying the transcription kinetics, especially the transcription initiation step. We are applying this method to investigate the off state.

2P135 アポトーシスに伴う上皮恒常性維持への機械的力の寄与

Maintenance of tissue integrity by intrinsic and extrinsic forces during apoptosis in Drosophila epithelial tissue morphogenesis

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How tissue integrity is preserved during apoptosis in epithelia is largely unknown. We studied tissue replacement process know as histoblast expansion during Drosophila metamorphosis and revealed 1) the apoptotic cell is squeezed out from the tissue by the contraction of actomyosin cables form within the dying cell and within the neighboring non-apoptotic cells formed upon apoptosis and 2) the cell adhesion between dying and non-dying cells are well regulated, for instance, E-Cadherin is dissociated from the plasma membrane during the apical constriction. Our results provide new mechanical and molecular insights of the maintenance of tissue integrity during apoptosis and the mechanical contribution of apoptosis in epithelial tissue morphogenesis.

2P136 枯葉にそっくりな蝶の翅の模様はどのように進化してきたのか？

Gradual and contingent evolutionary emergence of leaf wing patterns

Takao K. Suzuki, Shuichiro Tomita, Hideki Sezutsu (*NIAS, Transgenic Silkmoth Unit*)

How leaf mimicry evolved has fascinated many biologists, but it remains unclear. Here we show evolutionary origin and process of leaf wing patterns of butterflies (*Kallima*). First, comparative morphological analyses indicated that *Kallima* leaf patterns are decomposed into the same pattern elements as 31 closely related species share, suggesting that the pattern elements have been inherited across species. Next, we used Bayesian phylogenetic methods to estimate the past wing patterns, and revealed that the leaf pattern has evolved through several intermediate patterns from a non-mimetic ancestor. Finally, we estimated the temporal order of state evolution in the pattern elements. In summary, our study provides the first evidence for gradual evolution of leaf mimicry.

2P137 多細胞の形態形成における力学機構を明らかにするための三次元バーテックスモデル

3D vertex model for revealing mechanics in multicellular morphogenesis

Satoru Okuda, Mototsugu Eiraku, Yoshiki Sasai (*RIKEN Center for Developmental Biology*)

In biological development, global deformations of multicellular tissues emerge from dynamics of subcellular structures such as cytoskeletons. One fundamental challenge is to understand how subcellular mechanical behaviors can be orchestrated into global tissue deformations on the regulatory background. To elucidate such physical phenomena in multi-component systems, in this study, we have developed a novel 3D vertex model. The proposed vertex model has been succeeded expressing changes in cell configurations, cell divisions, and 3D dynamic deformation processes. In this presentation, we would like to demonstrate the applicability of the proposed model for revealing mechanics in tissue morphogenesis.

2P138 細胞性粘菌由来ミオシン II の SH1 ヘリックス上の変異が運動活性に与える影響

Mutations in SH1 helix affect the motile activity of Dictyostelium myosin II

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Mutations at the SH1 helix region of the myosin II motor domain have been reported to link to some autosomal-dominant diseases. The SH1 helix region acts as a linker for transmitting the structural changes of ATP-binding site in the catalysis domain to the lever arm. To investigate the effects on the motile activity, we introduced each of three mutations (E683K and R686C) into the SH1 helix in Dictyostelium myosin II. The mutation resulted in a decrease in the actin-myosin sliding velocity and the thermal stability, and the thermal aggregation of the myosin, which might be implicated in the disease process.

2P139 F-アクチンの水和状態のミオシン-サブフラグメント 1 密度依存性

Bound-Myosin Density Dependence of Hydration State of Actin-Filament

Takahiro Watanabe, Noriyoshi Ishida, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad.Sch.Eng., Tohoku Univ.*)

Hydration state change of actin filament(F-actin) with bound myosin S1 after ATP hydrolysis were measured by precision microwave dielectric spectroscopy(DRS) at different molar ratio from 0:13 to 3:13 of S1:actin. In the previous study (BBRC 322(2004) 340-346), the hydration state of F-actin with bound S1 exhibited a higher level of hypermobile water(HMW) number per actin than that of F-actin in spite of the absence of HMW around S1. Inthis study, with increasing the number of bound S1 on F-actin, amount of HMW was found to increase non linearly, exhibiting a cooperative structural change in actin filament.

2P140 ATP 結合アナログを用いたミオシンサブフラグメント１の水和研究

Hydration study of myosin subflagment1 with bound ATP analogs

Hideyuki Ohsugi, Takahiro Watanabe, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

Hydration properties of myosin subfragment-1 (S1) with bound ADP.Pi analogs were measured by precision microwave dielectric spectroscopy (DRS). In the previous study (BJ, 72 (1997) 18-23), the intermediate state of S1 during ATP hydrolysis reaction, the mixed state of S1.ATP and S1.ADP.Pi, exhibited a lower level of hydration number, which was consistent with the thermodynamic parameter changes in entropy and heat capacity given by Kodama et al (Physiol. Rev. 65 (1985)467-551). However, in the present study, the hydration levels of S1.ADP.AlF4 and S1.ADP.Vi were higher than that of S1.ADP. It suggests that the S1 states with bound ADP.AlF4 and ADP.Vi are different from that in the dynamic ATP-hydrolysis cycle.

2P141 2, 3-ブタンジオン 2-モノキシムによるミオシン II ATP 加水分解のケミカルレスキュー

Chemical rescue of myosin II ATP hydrolysis by 2, 3-butandione 2-monoxime

Hideyuki Komatsu, Yuji Koseki, Shunsuke Aoki (*Dept. of Bioscience & Bioinformatics, Kyushu Inst. Tech.*)

BDM (2, 3-butandione 2-monoxime) is well characterized as an inhibitor of myosin motor. However, its inhibitory mechanism is not fully understood. We herein demonstrated that BDM elevates skeletal myosin II K^+, EDTA-ATPase activity. In addition, BDM reactivates the ATPase activity of partially unfolded myosin. Furthermore, computational molecular modeling of docking of BDM on *Dictyostelium*myosin II-nucleotide complexes suggests two putative BDM-binding sites located around the the ATP site. Analogously to oxime compounds such as pralidoxime, which reactivate organophosphate-inactivated acetylcholinesterase, the oxime group of BDM may rescue the ATP hydrolysis of myosin. Based on these results, the mechanism of the inhibition of myosin motor will be discussed.

2P142 F-アクチンの水和状態に及ぼす Mg2+と Ca2+の効果

Effect of Mg2+ and Ca2+ ions on the hydration state of F-actin

Ryotaro Chishima, Asato Imao, Takahiro Watanabe, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

F-actin was reported to have dual hydration shell containing restrained water and hypermobile water (HMW) (BJ 85 (2003)3154-3161) and to exhibit marked increase in HMW upon binding mysion S1 by the previous study (BBRC 322(2004)340-346). In this study, hydration property of F-actin was measured in two buffer solutions at 10C: (1) 2mM MgCl2, 50mM KCl, 10mM HEPES,1mM DTT, 0.1mM ATP at pH 7.8, (2) 2mM CaCl2, 50mM KCl, 10mM HEPES, 1mM DTT, 0.1mM ATP at pH 7.8. As a result, the HMW signal (fc-fw)δ was markedly stronger in Ca-F-actin solution than in Mg-F-actin solution, indicating a structural change of F-actin.

2P143 Investigating conditions for structure analysis of binding states of formin/mDia1 to the actin filament by electron microscopy

Mizuki Matsuzaki, Akihiro Narita (*Grad. Sch. Sci., Nagoya Univ.*)

We investigated suitable conditions for structure analysis of binding states of formin/mDia1 to the actin filament by electron microscopy. We found the mDia1 gradually aggregated and its polymerization activity decreased as time passed after the purification. To solute the problems, we changed several conditions including shortening purification experiments and we achieved a better set of conditions without aggregation and with high activity. We also developed a new device to quantify binding activity to the actin filaments (and other filaments) at a very low concentration, to evaluate formin/mDia1 binding activity.

2P144 高温の心筋細胞内でみられる高速サルコメア振動

High-frequency sarcomeric auto-oscillations in living cardiomyocytes under hyperthermal conditions

Seine Shintani[1], Kotaro Oyama[1], Norio Fukuda[2], Shin'ichi Ishiwata[1,3] ([1]*Pure and Applied Physics, Waseda Univ.*, [2]*Dept of Cell Phy, Jikei Univ of Medicine*, [3]*Waseda Bioscience Research Institute in Singapore*)

We have developed an experimental system for nano-scale analysis of sarcomere dynamics and Ca^{2+} changes via expression of AcGFP in Z-discs (Shintani et al., J. Gen. Physiol., 2014). By using this system, we found that an increase in temperature by IR laser irradiation generated sarcomeric auto-oscillations with frequencies faster than those of normal Ca^{2+} -dependent beating in living cardiomyocytes. The sarcomeric auto-oscillations, termed Hyperthermal Sarcomeric Oscillations (HSOs), were induced independent of Ca transients and exhibited stable oscillation properties (e.g., frequency and amplitude). However, under conditions where normal cardiac beat was blocked, the HSOs were gradually organized. Therefore, the normal cardiac beating may need to induce stable HSOs.

2P145 金ナノロッドを用いた運動中キネシン 1 のモータードメイン回転の観察

Observation of the Rotational Motion of the Motor Domain during Processive Motility of Kinesin-1 using Gold Nanorod

Yamato Niitani[1], Sawako Enoki[2], Hiroyuki Noji[2], Ryota Iino[3], Michio Tomishige[1] ([1]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*, [2]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo*, [3]*Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences*)

Kinesin-1 is a motor protein that moves along microtubules in a hand-over-hand manner. Recent crystal structure solved in our lab showed that kinesin head undergoes ~25 degree rotational motion of a subdomain upon ATP binding. In this study, we directly observed ATP-dependent rotational motion of the subdomain during processive motility using gold nanorod attached to one of the head. The centroid position and the angle of the nanorod were determined by fitting the focused dark-field image recorded at 100 μs temporal resolution to simulated PSF, and demonstrated that the angle of the nanorod showed two state transition while the labeled head binds to microtubule. We will also show the results of kinetics analysis and discuss the implication for the gating mechanism.

2P146 X 線 1 分子計測による分子モーターの構造揺らぎ測定

X-ray Single Molecule Observations of Structural Fluctuations in Molecular Motors

Keigo Ikezaki[1], Naruki Hara[1], Hiroshi Sekiguchi[2], Naoto Yagi[2], Yuji Sasaki[1] ([1]*Grad. Sch. Sci., Univ. Tokyo*, [2]*SPring-8/JASRI*)

Molecular motors alter their molecular structures and exert mechanical force using the chemical energy of ATP hydrolysis. So for, many researchers revealed structural changes of molecular motors during ATP hydrolysis by using optical microscopy and atomic force microscopy. However, no one succeeds to clarify inter molecular fluctuations during ATP hydrolysis because of the resolution limit of their methods.
Recently, we are trying to observe inter molecular fluctuations of myosin molecular motors using DXT measurement (described below). At the meeting, we will report the latest progress. Please look forward to it!
Note: Diffracted X-ray Tracking
DXT allows us to know the orientations of gold nano-crystals attached to protein surfaces.
(SPring-8,BL40XU)

2P147 アクチンフィラメントの違いによるミオシン IXb の運動性

The motility of Myosin IXb depend on difference of actin filaments

Masafumi D. Yamada[1], Nobuhisa Umeki[1], Mitsuo Ikebe[2], Taro Q.P. Uyeda[1] ([1]*AIST, Biomedical Research Institute*, [2]*The Univ. of Texas Health Science center at Tyler, Dept. of Cellular and Mol. Biol.*)

Myosin IXb is a single-headed processive motor. Recent studies from two groups reported that the motility direction of myosin IXb was to either barbed end or point end direction. This discrepancy may arise from different sources of myosin or methods of actin preparation. Therefore, we hypothesized that the motility of myosin IXb is highly sensitive to subtle differences in actin structure. In this study, we first established a reproducible baculovirus-dependent system to express motile human myosin IXb. Movement of this myosin on Cy3-actin was to the barbed end, but interestingly, phalloidin was slightly inhibitory (0.66-fold slower). We will report the motile properties of several other types of actin filaments on this myosin.

2P148 全原子 MD 計算による F_o のサブユニット間水分布と相対運動の観測

Water distribution and relative motion between subunits of F_o observed by all-atom MD simulation

Ryoichi Kiyama, Asahi Konno, Mitsunori Takano (*Dept. of Pure & Appl. Phys., Waseda Univ.*)

F_oF_1-ATP synthase is an energy-transducing molecular motor. Compared to F_1, membrane-embedded F_o portion is less well studied. F_o is composed of the *a* and *b*-subunits and the *c*-ring. H^+ current across membrane down the electrochemical potential gradient is thought to drive the rotary motion of *c*-ring relative to *a*-subunit. As a molecular mechanism of the rotary motion, the half-channel model is widely accepted, where the two different H^+ channels at the interface of *a*-subunit and *c*-ring are assumed to play a key role. To investigate this model, we conducted all-atom molecular dynamics simulation, and analyzed water distribution in the membrane-embedded region. We further studied the inter-subunit motion between *c*-ring and *a*-subunit.

2P149 タンパク質間の実行相互作用：多価カチオンを介した引力パッチ間相互作用

Protein-Protein Effective Interaction: Interaction between Attractive Patches Mediated by Multivalent Cations

Takuto Sawayama, Ryo Akiyama (*Kyushu Univ. sci.*)

Acidic proteins strongly attract each other only in the multivalent cation solution whose concentration is about 10-3M, although they repel each other in more dilute or dense electrolyte solution. We are studying the effective attraction between like-charged macroions immersed in an electrolyte solution based on an HNC-OZ theory with a charged hard sphere model. The previous results showed above reentrant behavior. In the present study, we took account of the protein's structure.We regarded an attractive patch as a carboxylate oxygen in an acidic residue. To discuss the effective attraction between the patches, we calculated the effective interaction between oxygen sized ions.We will discuss the protein association based on the idea of attractive patches.

2P150 アクチンの PEG 化は運動速度よりも運動割合に影響を与える

Pegylation of actin affects motile fraction rather than the velocity of actin filaments on myosin molecules

Kuniyuki Hatori, Hiroki Souma (*Dept. Bio-Systems, Grad. Sch. Sci. Eng., Yamagata Univ.*)

The motility of actomyosin was examined when actin was covalently bound with poly(ethylene glycol) methyl ether maleimide (molecular weight of 2k, 5k, and 10k). PEG-maleimide could bind to actin monomer at a ratio of 1:1. PEG2k-bound actin filaments still exhibited the motility, whereas PEG5k- and PEG10k-bound actin did not. When copolymer filaments were prepared from a mixture of PEG2k- or PEG5k-actin and intact actin, it was found that both motile and unmoved filaments co-existed in the same preparation. The velocity of motile filaments was decreased by only 10% with the increase in the ratio of PEG-actin within the filaments in the range of 0-60%. The fraction of unmoved filaments significantly increased as the ratio of PEG-actin and size of PEG were increased.

2P151 高速 AFM により可視化された、コフィリンによるアクチンフィラメントの協同的な構造変化の一方向的な伝播

Cofilin-induced unidirectional cooperative conformational changes of actin filaments visualized by high speed atomic force microscopy

Kien Ngo[1], Noriyuki Kodera[2,3], Eisaku Katayama[4], Akira Nagasaki[1], Toshio Ando[2,5,6], **Taro Uyeda**[1] ([1]*Biomedical Res Inst, AIST*, [2]*Bio-AFM FRC, Inst. of Sci. and Eng., Kanazawa U.*, [3]*PRESTO, JST*, [4]*Grad. Sch. Sci., Osaka City U.*, [5]*Div. of Math & Phys. Sci., Grad. Sch. of Nat. Sci. & Tech., Kanazawa U.*, [6]*CREST, JST*)

It was previously shown that cofilin forms clusters along actin filaments and induces supertwisting of helix and severing of the filaments. Here, we used high speed AFM to live-image cofilin binding, cluster growth, supertwisting, and severing of actin filaments on positively charged lipid membrane at pH 6.8. The supertwisted filament structure in cofilin clusters was cooperatively propagated to the adjacent bare zone only on the P-end side of the cluster. The growth direction of cofilin clusters was also to the P-end, and this was independent on the bound nucleotide of actin. Severing was frequently observed at or near the boundaries between the bare zone and the cofilin cluster, as well as within the cofilin clusters.

2P152 ミオシン・サブフラグメント１ネック部位の首振り運動の分子動力学シミュレーション

Molecular dynamics simulation for the neck domain swinging motion of a myosin subfragmnet-1

Tadashi Masuda (*Fukushima Univ.*)

Molecular dynamics (MD) simulation was conducted for a myosin subfragment-1. The myosin residues assumed to be engaged in the docking with an actin filament were fixed to the space. External force of 17 pN was applied to the neck end in the direction opposite to the power stroke. After the neck domain was bent and took the pre-power stroke configuration, the applied force was released to see whether the neck returned to the original position.
Ten times of MD calculations were conducted over 80 ns, but the myosin neck did not show any tendencies to return to the original position except for one case. Much longer time may be required for the recovery of the neck, or the pull force applied to the myosin was too large and broke the internal structure of the molecule.

2P153 ATP 非存在下におけるミオシン V のアクチンフィラメント上での歩行運動

ATP-less walking of myosin V on actin filaments

Noriyuki Kodera[1,2], Takayuki Uchihashi[1,3,4], Toshio Ando[1,3,4] ([1]*Bio-AFM FRC, Inst. of Sci. & Eng., Kanazawa Univ.*, [2]*PRESTO, JST*, [3]*Div. of Math & Phys. Sci., Grad. Sch. of Nat. Sci. & Tech., Kanazawa Univ.*, [4]*CREST, JST*)

We have recently developed a new technique for high-speed AFM, called the “interactive imaging mode”. In this mode, one can apply a controlled strong tapping-force onto a targeted locus of the sample during imaging. Here we use this technique to detach from actin either head of a two-headed bound myosin V (M5) molecule under nucleotide-free conditions as well as in the presence of ADP. When the trailing head is detached in this mechanical way, M5 almost always steps forwards by ~36 nm, whereas it moves neither backwards nor forwards when the leading head is detached. Thus, the ATP hydrolysis energy is not required for the generation of intramolecular tension and hence for the execution of a lever-arm swing.

2P154 直流電流を付加した導電性基盤上でのアクトミオシンの運動

Movement of actomyosin on a conductive base under DC current

Reito Wada[1], Takao Nakamura[1], Kuniyuki Hatori[2] ([1]*Grad. Sch. Med. Sci., Yamagata Univ.*, [2]*Grad. Sch. Sci. Eng., Yamagata Univ.*)

Because Indium Tin Oxide (ITO) slide glass has conductivity and transparency, direct observation of fluorescently labeled actin filaments by microscopy can be performed on ITO slide glass under DC current. We investigated the response of the movement of actin filaments interacting with myosin molecules to the current applied on ITO slide glass. When voltage was applied up to 8 V (0.24 A), the temperature of the ITO slide glass surface increased with time and reached 65°C. Sliding velocity of actin filaments was increased with the increase in temperature, according to the Arrhenius equation. At present, we are investigating a relationship between the movement direction and the current direction.

2P155 ミオシンフィラメント上における骨格筋ミオシン分子間の協同性

Intermolecular cooperativity of skeletal myosins in myofilaments

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Muscle contraction is powered by the cyclic interaction of skeletal myosin molecules with actin filaments. Recent experiments suggest cooperative actions between myosin molecules, when part of an ensemble. In order to elucidate the mechanism of intermolecular cooperativity of myosin in a myosin ensemble, displacements of actin driven by ~20 interacting myosins embedded in myofilament were measured by optical tweezers. Results showed stepwise displacements of actin under high loads of ~30 pN, implying that the individual steps may be generated by synchronous actions of several myosin motors. Potential mechanisms of synchronous steps between myosin motors in myofilaments can be elucidated by developing the simulation model implemented with two force-generating states.

2P156 高速 AFM と蛍光顕微鏡観察による F-アクチンへの HMM、コフィリンの協同的結合の解析

An analysis of cooperative binding of myosin to F-actin using high-speed atomic force microscope and fluorescence microscopy

Hiroaki Ueno[1], Yuusuke Nishikawa[1], Akira Ainai[1], Rika Hirakawa[1], Atuki Yoshino[1], Noriyuki Kodera[3], Taro Ueda[2], Kiyotaka Tokuraku[1] ([1]*Grad. Sch. Sustain. Environ. Eng., Muroran Inst.*, [2]*Biomedical Res. Inst., AIST*, [3]*Bio-AFM FRC, Inst. Sci. & Eng., Kanazawa Univ.*)

Heavy Meromyosin (HMM) binds cooperatively to F-actin and form clusters on the filaments. Although we believe that the cooperative binding is achieved through conformational changes of actin subunits, which is evoked by binding of HMM, the molecular mechanism remains unknown. To analyze the detailed cooperative binding, we tried to immobilize F-actin on a glass surface using biotin-avidin system and lipid membrane, and observed the binding of HMM-GFP by fluorescence microscopy. The results showed that cooperative binding of HMM-GFP to immobilized F-actin on lipid membrane was observed, but biotin-avidin system was not appropriate to observe it. The cooperative binding of HMM-GFP to F-actin on lipid membrane could be also observed by high-speed atomic force microscope.

2P157 高負荷におけるバクテリアべん毛モーターの回転ステップ解析

Sub-14 ° steps of the bacterial flagellar motors at high load

Yuichi Inoue, Hajime Fukuoka, Hiroto Takahashi, Akihiko Ishijima (*IMRAM, Tohoku University*)

Bacterial flagellar motor is a rotary motor with unitary steps of ~14 ° at low load (Sowa et al., 2005). We developed a simple method to detect the motor steps at high load using an elliptic probe (Annual meeting, 2013).
To understand the mechanism of the motor rotation, here we analyzed the steps at high load. With decreasing concentration of external sodium ion, rotational speed of the sodium-driven motor reduced until the motor showed unstable angular change, due to angular diffusion in the absence of stator complex. Histograms of the angular change showed the ~7° steps as well as the reported steps of ~14°, suggesting that the motor rotation is based on the stator-independent steps of < 14°.

2P158 A novel role of dynactin for dynein motility revealed by gliding assay

Takuya Kobayashi, Hatsuha Kajita, Kei Saito, Yoko Y Toyoshima (*Grad. Sch. of Arts and Sci., Univ. of Tokyo*)

Dynactin is multi molecular protein complex and mediates dynein and the transporting cargo and regulates dynein motility. Although the coiled-coil 1 (CC1) region of dynactin p150 is known to bind dynein, the details of p150 binding to dynein motility has not been elucidated.
Because the CC1 region is extruded from the dynactin head, we produced the CC1 fragment and characterized. We performed in vitro motility assay in the presence of CC1 fragment and found that microtubule dissociation was increased by CC1 fragment. The remaining microtubules moved faster than the condition without CC1 fragment. Although it has been thought that dynactin is a simple activator, these results suggest that dynactin is a complicated regulator for dynein motility.

2P159 A novel role of dynactin for dynein motility revealed by single-molecule assay

Takuya Miyashita, Takuya Kobayashi, Hatsuha Kajita, Yoko Y. Toyoshima (*Grad. Sch. of Arts & Sci., Univ. of Tokyo*)

Dynactin is thought to interact with dynein motor protein by binding to it directly. Single-molecule assay of dynactin complex by total internal reflection fluorescence (TIRF) microscopy revealed that exon 5-7 present in the basic domain of dynactin p150 is necessary for binding and interaction between microtubules and dynactin. We found that coiled-coil domain (CC1) of p150 N-terminal, which binds to the N-terminal region of dynein intermediate chain, caused dissociation of dynein from microtubules by TIRF W-view imaging of these proteins. Dynactin complex also dissociated dynein from microtubules as with CC1, indicating that CC1 dissociate dynein from microtubules. This study demonstrates CC1 is intrinsically important to regulation of dynein motility by dynactin.

2P160 ダイニン･微小管複合体の DNA 折り紙による架橋

Cross-linking the dynein-microtubule complex by DNA origami

Keiko Hirose[1], Kangmin Yan[1], Hisashi Tadakuma[2] ([1]*Biomed. Res. Inst., AIST*, [2]*Grad. Sch. Frontier Sci., Univ. Tokyo*)

To understand how dynein produces force and moves along a microtubule, it is necessary to observe the structural changes of dynein molecules during force production. However, the dynein-microtubule complex, which has been used for electron microscopic observation of dynein in microtubule-bound states, tends to disassemble when ATP is added to induce movement. To prevent disassembly, we cross-linked the microtubules using DNA origami structures. DNA origami tube structures were biotinylated at both ends, and bound to biotinylated microtubules via streptavidin. Cryo-electron microscopic images showed bundles of microtubules with bound dynein and DNA origami both in the presence and absence of ATP.

2P161 Athermal Fluctuations of Probe Particles in Active Gel

Heev Ayade[1], Irwin Zaid[2], Julia Yeomans[2], Daisuke Mizuno[1] ([1]*Kyushu University*, [2]*University of Oxford*)

In a reconstituted active gel, a single myosin which acts as a contractile force generator drives athermally the probe particles located at a separation distance r away from the myosin. The probe particles sense a displacement field proportional to 1/r2 coming from the myosin which is in continuum, elastic actin network. The non-equilibrium statistics and dynamics of active gel are investigated by analyzing the non-Gauss athermal fluctuations of probe particles. We found out that the full positional probability density distribution of probe particles follows the truncated Levy distribution with a characteristic exponent of 1.5.

2P162 軸糸ダイニンが駆動する微小管が創出する in vitro での動的渦形成

In vitro dynamic vortex formation of microtubules driven by axonemal dyneins

Naoki Kanatani[1], Hiroaki Kojima[2], Kazuhiro Oiwa[1,2,3] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*Adv. ICT Res. Inst, NICT*, [3]*JST,CREST*)

We found in vitro self-organized pattern formation of fluorescently-labeled microtubules driven by inner-arm dynein subspecies c and g of Chlamydomonas. These dyneins attached on a glass surface at densities higher than 1000 molecules/um2, are capable of moving microtubules on glass surface at velocities of 6-12 um/sec in the presence of 1 mM Mg-ATP at 23 C. Microtubules moving on the surface often collide with each other and are gradually aligned and form streams. The behavior of microtubules in the collision is nematic. These microtubule streams grow into vortices with the diameter of 200-500 um and finally the vortices form arrays covering the surface of a whole flow cell. We have investigated biological factors which determine parameters of this vortex formation.

2P163 DNA オリガミバネを用いたミオシン VI のアンカー機能の分子動態計測

Anchoring mechanism of myosin VI revealed with a programmed DNA origami spring

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Myosin VI physiologically functions as transporter and anchor in a cell. Recent single molecule high accuracy nano imaging (Nishikawa et al., 2010) revealed myosin VI has inchworm-like steps and hand-over-hand steps. These diverse stepping mode may relate to the switch between transporter and anchor, however, there's no evidence for that.
Here, we examined the relashionship by a direct observation of stepping dynamics under tension, because tension triggers the switching between transporter and anchor. We achieved FIONA under tension by constructing nano sized spring using DNA nanotechnology (DNA origami). We found, during anchoring, myosin VI does not only prolong dwell time but repeats inchworm-like step and back steps to keep the anchoring state.

2P164 微生物の運動超分子マシナリーの単位ステップの直接観察：滑走バクテリアと遊泳古細菌について

Direct observation of unitary steps of supermolecular motility machineries of microorganisms: gliding bacterium and swimming archaeon

Yoshiaki Kinosita[1], Daisuke Nakane[1], Makoto Miyata[2], Takayuki Nishizaka[1] ([1]*Faculty of Science, Gakushuin Univ.*, [2]*Graduate School of Science, Osaka City Univ.*)

Mycoplasma mobile has a huge gliding machinery and glides on the substrate at the speed of 2.5 μm/sec. We proposed the "centipede model" in which leg protein portion of the machinery attaches to and detaches from sialylated oligosaccharides with a force production driven by ATP. Recently, we've detected this motion and reported *M. mobile* took 70 nm steps.
Haloferax volcanii swims with a speed of 2 μm/sec by rotating archaeal flagella. It was functionally similar to a bacterial flagella though structurally resembled a bacterial typeIV pili. To clarify the force generation mechanism of flagella, we constructed a tethered cell assay. Notably, 6-9 steps per revolution were detected, which were roughly consistent with the periodicity of the active ATPase, the FlaI protein.

2P165 好熱菌 F_1 のカップリングスキーム

Coupling scheme of thermophilic F_1

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F_1 is an ATP-driven rotary molecular motor in which three catalytic sites, primarily hosted by a β subunit, hydrolyze ATP sequentially to power the rotation of γ subunit. The rotation occurs in steps of 120° per ATP, and the 120° step is further resolved into 80-90° and 40-30° substeps. In the basic coupling scheme, ATP binding starts rotation at 0°, and the ATP is hydrolyzed at 200°, and the ADP is released around 240° after a third ATP is bound. The timing of Pi release is yet unsettled: either at 200° or 320°. The timing of ATP hydrolysis, too, is not yet unequivocal. To complete the scheme, we observed the rotation driven by fluorescently (Cy3) labeled ATPγS and AMPPNP with single fluorophore imaging and by ATPγS with high speed imaging.

2P166 高速 AFM によるミオシンⅥの機能の直接観察

Direct observation of functioning myosin VI by high-speed AFM

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Myosin VI, the only class of myosin that moves towards the minus end of actin filaments, performs various cellular functions including intracellular transports and structural maintenance of Golgi apparatus and stereocilla. Previous single molecule studies have demonstrated that myosin VI dimer moves processively with a step size (~36 nm) larger than that expected from its canonical lever-arm length. To gain an insight into the motile mechanism, we here use high-speed AFM to directly observe moving myosin VI. The AFM movies successfully visualize myosin VI molecules moving wiggly towards the minus end of actin filaments with large and small strides. We will report the observation results in detail.

2P167 Investigating the Coordination of RecB and RecD Subunits within RecBCD Helicase Complex Using Cy3-labeled ATP

Chia-Chuan Cho, Hung-Wen Li (*Dept. Chemistry, Natl. Taiwan Univ.*)

RecBCD is the essential hetero-trimeric enzyme that initiates homologous recombination repair in E. coli. RecBCD is a DNA helicase containing two ATPases with single-stranded DNA (ssDNA) translocase activities with opposite polarity: RecB (3'-to-5') and RecD (5'-to-3'). How these two translocases communicate is not known. Here, we used total internal reflection fluorescence (TIRF) microscopy to image the binding of individual Cy3-ATP by RecBCD. By analyzing the dwell time and binding frequency, we aim to define the communication between RecB and RecD subunits.

2P168 海洋性細菌 *Vibrio alginolyticus* のべん毛基部体 T-ring の低温電子顕微鏡を用いた構造解析

Structural analysis of T-ring in the flagellar basal body of *Vibrio alginolyticus* by electron cryomicroscopy

Hidemaro Hotta[1], Akihiro Kawamoto[2], Satoshi Inaba[1], Yusuke V. Morimoto[2,3], Noriko Nishioka[1], Seiji Kojima[1], Keiichi Namba[2,3], Michio Homma[1] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [3]*QBiC, RIKEN*)

Marine bacterium *Vibrio alginolyticus* has a motility organelle called flagellum at the cell pole. The rotation is driven by the motor located at the base of the flagellum called the flagellar basal body and is composed of the rotor and the stator. To understand the mechanisms of motor rotation and torque generation, we observed the basal body structure isolated from the cell by transmission electron microscopy and cryotomography. We also observed it in the mini cell to clarify the details by merging information from these two structures. In this report, we focus on the T-ring structure that is a specific for the sodium driven *Vibrio* motor and composed of MotX and MotY. We want also to discuss the features in comparison with the proton driven motor of *Salmonella*.

2P169 アクチニンで架橋された二次元アクチンネットワークのミオシン依存的な形態変化

Myosin-dependent morphological changes of two-dimensional actin networks crosslinked by α-actinin

Hiroki Eguchi[1], Makito Miyazaki[1], Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

The stress fiber composed of actin filaments, myosin motors, and α-actinin is essential to maintain a cell shape. To investigate the self-assembly mechanism of the stress fiber, we constructed 2D actin networks crosslinked by α-actinin in vitro, and observed the network contraction by the addition of myosin with ATP. At high [myosin], the network collapsed, forming large clusters. At the intermediate [myosin], we found that the thin actin bundles were fused and straightened, resulting in the formation of thick bundle networks. We confirmed that the successful thick bundle network formation was determined by the myosin vs. actinin molar ratio. Our results suggest that the local density ratio of α-actinin and active myosin in cytoplasm regulates the stress fiber assembly.

2P170 微小管ネットワークの対称性の破れによって引き起こされる細胞質回転流動

Cytoplasmic rotational flow induced by symmetry breaking of active microtubule networks

Kazuya Suzuki[1], Makito Miyazaki[1], Jun Takagi[2], Takeshi Itabashi[1], Shin'ichi Ishiwata[1,3] ([1]*Dept. Phys., Univ. Waseda*, [2]*Quantitative Mechanobiology Laboratory, NIG*, [3]*WABIOS*)

Cytoskeletal networks are essential for cellular functions. While properties of actin networks are well understood, it remains unclear how microtubule (MT) networks behave in cytoplasm. Here, we employed *Xenopus* egg extracts encapsulated in droplets as a model system to examine the behavior of cytoplasmic MT networks. After the encapsulation, the networks spontaneously contracted, accumulating cytoplasmic materials to the droplet center. In contrast, when dynein was inactive, extensile MT bundles pushed the droplet boundary, resulting in a rotational vortex. This vortex induced cytoplasmic rotational flow, which seems useful for dispersing organelles. Our results suggest that cellular MT networks have two different modes, and cells utilize dynein activity as a switch.

2P171 二種類の固定子を持つシュードモナス･シリンゲの運動解析

Motility analysis of Pseudomonas syringae possessing two different stator systems

Takuto Tensaka, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Tohoku. Univ.*)

The bacterial flagellar motor consists of a rotor and a stator, which convert the flow of ions, such as proton and sodium ion, to rotation. Pseudomonas syringae is known to possess two sets of stators, MotA/B and MotC/D. In this study, to elucidate how the dual stator system functions in the P. syringae flagellar motor, we tested the motilities of P. syringae mutants lacking motA/B or motC/D at various viscous conditions. Both ΔmotA/B and ΔmotC/D cells showed motility in a low viscous medium. However, in a high viscous medium containing 20% Ficoll, the ΔmotA/B cells swam as fast as the wild-type cell, while the ΔmotC/D cells were non-motile. These suggest that the MotC/D complex rather than the MotA/B one would play a major role in highly viscous environments.

2P172 MotA に点変異を持つ細菌べん毛モーターの出力特性解析

Rotation analysis of the bacterial flagellar motor with a point mutation in MotA

Kodai Oono[1], Shuichi Nakamura[1], Fumio Hayashi[2], Kenji Oosawa[2], Seishi Kudo[1] ([1]*Grad. Sch. Eng., Univ. Tohoku.*, [2]*Div. Mol. Sci., Fac. Sci. and Tech, Univ. Gunma.*)

The bacterial flagellar motor converts the energy of proton flow through the stator into the mechanical work. The stator complex consists of MotA and MotB. Though it has been proposed that the electrostatic interaction between MotA and a rotor protein FliG generates the torque, the mechanism of torque generation is not fully elucidated. In this study, we investigated the rotation characteristics of flagellar motors of several Salmonella strains possessing point mutations in MotA. Interestingly, some mutants showed different behaviors between their swimming speeds and flagellar rotation rates over the load range examined. We will present differences in the torque-speed relationships among motors from the MotA mutants and the wild-type.

2P173 ミオシン結合タンパク質の消失は細胞が出す力に影響を与えるのか

The examination of whether deletion of myosin binding subunit 85 would lead to alteration of cellular mechanical features

Rui Li[1], Takeomi Mizutani[2], Hisashi Haga[2], Kazushige Kawabata[2] ([1]*Graduate School of Life Science Hokkaido University*, [2]*Faculty of Advanced Life Science Hokkaido University*)

Gene therapy requires the permanent integration of transgenes into genomic locus of cells. AAVS1 is considered to be one of such genomic sites. Yet, AAVS1 is the locus where myosin binding subunit 85 (MBS85) located. MBS85 is a member of myosin II phosphatase located downstream of the RhoA-ROCK pathway. At this point it is unclear whether deletion of MBS85 would lead to alteration of cellular myosin-dependent mechanical features. Our primitive data shows that alteration of myosin II phosphatase will change cellular elasticity. In this annual meeting, we will present our findings on the alteration in cellular mechanical features of MBS85-knockout cells.

2P174 アフリカツメガエルの卵抽出液中の方向性を持った F アクチンの流れの観察

Observation of directional F-actin flow in Xenopus egg extracts

Masatoshi Tanabe[1], Makito Miyazaki[1], Kazuya Suzuki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

Unidirectional F-actin flow from the leading edge to the rear end (retrograde flow) is important for cell migration. To elucidate the polarization mechanism of F-actin flow, we used droplets of Xenopus egg extracts surrounded by oil as a cell model system, and observed F-actin flow. Our preliminary experiments showed that F-actin flow emerged from the droplet periphery. This flow carried cytoplasmic materials including vesicles inward, which led to form a large aggregate at the droplet center. In the case of small droplets, this aggregate tended to position near the droplet periphery and asymmetric F-actin flow toward the aggregate was observed. On the basis of the droplet size dependency, we will discuss how the retrograde flow is generated in motile cells.

2P175 高圧負荷によって誘導されるクラミドモナス非運動性変異株鞭毛の屈曲運動

High pressure induces flagellar bending movements in *Chlamydomonas* paralyzed mutants

Toshiki Yagi[1,2], Masayoshi Nishiyama[3] ([1]*Dept. Biol. Sci., Pref. Univ. Hiroshima*, [2]*Grad. Sch. Med., Univ. Tokyo*, [3]*The Hakubi Center, Kyoto Univ.*)

Chlamydomonas mutants lacking flagellar central structures are paralyzed. Recently we found that the mutants display beating under high pressure (20-80 MPa). To further examine the effect of pressure, we analyzed pressure-induced beating in *Chlamydomonas* mutants by high-speed video microscopy. Previous studies showed that flagellar waveform changes from asymmetric to symmetric types over 10^{-5}M Ca. Interestingly, pressure application increased the number of cells displaying symmetric waveform. In addition, similar waveform change was also observed in demembranated and reactivated flagella in the absence of Ca^{2+}, indicating that pressure application induced symmetric beating without Ca^{2+}. We suggest that pressure and Ca^{2+}have similar effects on flagellar structure.

2P176 バクテリアの集団運動による巨大渦パターン形成

Large-scale vortex pattern emerging from bacteria collective motion

Showko Odaka, Daisuke Nakane, Takayuki Nishizaka (*Dept. Phys., Gakushuin Univ.*)

Swarming bacteria is an ideal example of collective motion because of their limited cognition between individuals and the experimental reproducibility. We found *Flavobacterium johnsoniae*, gliding bacteria with the rod shape of 5 μm in length and 0.5 μm in width, exhibits the large-scale vortex pattern during spreading on the non-nutrient agar plate. The size of the single vortex grew up to 3 mm in a 24-hour period, and the number of cells including the vortex was estimated to be 10^7. Notably, all vortices rotated in a counter-clockwise manner at a speed of 0.4 μm/s, which is presumably attributable to the left-side biased movement of single cell revealed by the tracking method with a high accuracy.

2P177 シグナル変換のアダプター分子 LAT は細胞膜直下の小胞膜上で働く：1 分子追跡法による解明

Transmembrane signaling adaptor LAT works on the vesicles associated with the plasma membrane: a single-molecule tracking study

Koichiro M. Hirosawa[1], Kenta J. Yoshida[2], Taka A. Tsunoyama[1], Kenichi G.N. Suzuki[1,3], Takahiro K. Fujiwara[1], Akihiro Kusumi[1,2] ([1]*Inst. Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto Univ.*, [2]*Inst. Frontier Medical Sciences, Kyoto Univ.*, [3]*NCBS/inStem, India*)

Recently, Linker for Activation of T cells (LAT), a transmembrane adaptor protein vital for signaling in immune cells, is drawing extensive attention: it was reported that LAT constitutively forms clusters in the plasma membrane (PM) and that the LAT clusters are the key for signal transduction. However, our single-molecule tracking of LAT in mast cells revealed that ~10% of LAT exists in cytoplasmic vesicles located near the PM, and that, upon antigen stimulation, downstream signaling molecules are recruited to the LAT molecules on the vesicles rather than LAT monomers or clusters in the PM. These results suggest that LAT-containing vesicles serve as a signaling platform in immune cells. These vesicles were enriched in particular phosphoinositides and their kinases.

2P178 The bidirectional role of conserved charged residues in rotor-stator interaction in a rotary motor of bacterial flagella

Yasuhiro Onoue, Norihiro Takekawa, Tatsuro Nishikino, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Nagoya Univ.*)

Bacterial flagellum is bidirectionally rotated by the flagellar motor located in the inner membrane. It is known that some conserved charged residues are crucial for rotor-stator interaction but this evidence is based on previous studies focused on the rotation in forward (counterclockwise) direction only. Here, using tethered-cell assay, we verified the function of the residues in the interaction even in the reverse (clockwise) direction. In WT and charge-neutralizing mutations of the stator, rotational speeds in both directions were almost the same. But in the case of some mutations in the rotor, they differed totally. These results support a current model that the rotor changes its conformation depending on rotational direction.

2P179 細胞-GUV 電気融合による μm サイズ人工物の導入

Introducing micro-meter size objects into live cells mediated by cell-GUV electrofusion

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Here, we report a method for introducing large objects, up to micrometer level, into living cells by electrofusion using an artificial giant unilamellar vesicle (GUV or Liposome). The mixture dispersion of GUVs and HeLa cells were exposed to an AC field to induce cell-GUV alignment and were loaded DC pulse to cause puncture of the contact area. We demonstrated that a plasmids, designed DNA nanostructure (origami), and magnetic beads could be introduced into live cells by using this approach. The treated cells were cultured to confluence without significant damage. By using external magnetic fields, we also demonstrated rotating HeLa cells that introduced magnetic beads. These bioengineered hybrid cells will be useful for elucidation of cell mechanisms in the future.

2P180 量子ドットを用いた脳がん幹細胞表面の膜タンパク質の運動解析

Membrane Protein Dynamics on Brain Tumor Stem Cell Evaluated by Using Quantum Dot

Morito Sakuma, Sayaka Kita, Hideo Higuchi (*Department of Physics, Graduate School of Science, The University of Tokyo*)

Brain tumor stem cells (BTSCs) populate in a small portion of tumor and are responsible for the recurrence of the tumor after treatment. BTSCs have also been known to have unique characteristics, such as high resistance to radiotherapy and chemotherapy, invasion, metastasis, multilineage and self-renewal potential. Despite such well-known properties, the difference in membrane functions between BTSCs and non-BTSCs remains unclear. A potential factor is dynamics of the BTSC membrane protein marker, CD133. Thus, we evaluate dynamics of CD133 labeled with quantum dots using a confocal microscope. BTSCs showed higher motility and expression level of CD133 than that of non-BTSCs, suggesting that dynamics of CD133 might be a key property in membrane functions of BTSCs.

2P181 *Xenopus* 卵抽出液を封入した脂質膜小胞中でのアクチンのダイナミクス

Actin dynamics in *Xenopus* egg extract encapsulated in a lipid membrane

Naoki Noda, Issei Mabuchi (*Dep. Life Sci., Gakushuin Univ.*)

Actin dynamics in *Xenopus* egg extract confined in oil as a droplet was investigated. To visualize the actin dynamics, we added fluorescently labeled actin monomers to the extract. In the droplet, a spherical aggregate of cytoplasmic materials, which we call *X* body, was formed and centripetal actin flow occurred. The flow seemed to initiate at the oil-extract interface and terminated at the *X* body. It lasted for about an hour. Although the centripetal actin flow converged at the *X* body, the fluorescence intensity at the *X* body did not change over time. The speed of the actin flow was decreased by addition of a myosin II ATPase inhibitor, blebbistatin, in a dose dependent manner.

2P182 神経と接着した膵島 α 細胞内顆粒動態の解析

Analysis of granule movement in pancreatic islet α cells attached with nerves

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Autonomic neurons innervate pancreatic islets of Langerhans and maintain blood glucose homeostasis by regulating hormone levels. Because the islet α cells release glucagon by exocytotic pathway, we have here analyzed the intracellular movement of granules in αTC6 cells, an α cell line, attached with superior cervical ganglia neurites. Granules were observed to move rapidly in αTC6 cells without neurites in resting condition. However, granules in αTC6 cells densely attached with neurites hardly moved. The population of spontaneous Ca^{2+}-oscillating αTC6 cells was also less in the attached group. These results suggested that dense innervation to α cells in the pancreatic islet negatively regulated their glucagon secretion activity.

2P183 細胞極性形成におけるポジティブフィードバック機構の 1 分子イメージング解析

PTEN Membrane Binding is Destabilized by PI(3,4,5)P_3: Positive Feedback Loop in Establishing Cellular Polarity

Satomi Matsuoka[1,2], Masahiro Ueda[1,2] ([1]*Quantitative Biology Center (QBiC), RIKEN*, [2]*Graduate School of Science, Osaka University*)

Cell migration requires mutual antagonism of PIP_3 that locally accumulates on anterior cell membranes and the phosphatase, PTEN, due to self organization. Here, we revealed a molecular mechanism of positive feedback amplifying PIP_3 through PIP_3-induced membrane dissociation of PTEN. Single-molecule imaging revealed that PIP_3 dephophorylation destabilizes PTEN membrane binding in *Dictyostelium* cells. A mathematical model explaining the molecular behaviors predicts the PIP_3-induced exclusion from the membrane. The model was shared by human PTEN that rescues defective migration of *pten*- cells. Therefore, PIP_3/PTEN polarity formation through the catalysis-mediated positive feedback seems to be evolutionally conserved and essential for cell motility.

2P184 三量体 G タンパク質と相互作用する新規タンパク質 Gip1 は走化性における応答濃度範囲を広げる働きをする

A novel heterotrimeric G protein interacting protein (Gip1) extend chemotactic range

Yukihiro Miyanaga[1,2], Yoichiro Kamimura[2], Masahiro Ueda[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*QBiC, Riken*)

Chemotactic responses are mediated by GPCRs in various eukaryotic cells. GPCRs transduce signals to diverse downstream molecules via trimeric G proteins. Although G protein activation is saturated by a chemoattractant stimulation of around ten nM in Dictyostelium chemotaxis, these cells can sense chemical gradient even in several hundred nM of attractant. There would be some factor to expand response range. We found a novel G protein interacting protein named Gip1. Biochemical analysis has revealed that complex formation of trimeric G was reduced in gip1- cells. Moreover gip1- cells had defect in chemotaxis at higher chemoattractant concentration. These data suggest that Gip1 would regulate the interaction of G protein subunits and extend chemotactic range.

2P185 分子の混み合いが膜上シグナル伝達過程に与える影響の分子動力学法による考察

Analysis of Molecular Crowding effect on signal transduction process by Molecular dynamics simulation

Rei Takamoto, Hiraku Nishimori, Akinori Awazu (*Dept. Math. Life Sci, Hiroshima Univ.*)

Several organisms can sense the environmental variation by the signal transduction processes. Such processes start with the activation of receptors and signaling protein on the cell membrane. Recent studies suggest that various macromolecules are crowded in the cell, and the excluded volumes of molecules often affect their biochemical processes. Such effects are expected to influence more seriously on molecular motions on 2-dimensional field like cell membranes.
Thus, we investigate the influences of the excluded volume of molecules using a model of signal transduction process on the cell membrane of retina cell. We perform the coarse-grained molecular dynamics simulations and analyze the receptor density dependencies of signal transduction velocity of retina cell.

2P186 ウシ毛様体筋細胞においてカフェインはムスカリン受容体作動性陽イオンチャネルの活性化を誘起する

Activation of an M_3-muscarinic receptor operated non-selective cation channel by depletion of intracellular Ca^{2+}

Motoi Miyazu, Akira Takai (*Dept. Physiol., Asahikawa Med. Univ.*)

In primary cells of bovine ciliary muscle, we studied the effects of caffeine on the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ as well as on the activity of the non-selective cation channels (NSCCs) with unitary conductances of 35 pS and 100 fS which are known to be opened by M_3-muscarinic stimulation. In the ciliary myocytes under whole-cell voltage clamp at -50 mV, caffeine (5-20 mM) induced opening of 100 fS-NSCC without concomitant activation of 35 pS-NSCC. Caffeine also caused an elevation of the $[Ca^{2+}]_i$ monitored using Fluo-4 as the indicator. Thapsigargin (300 nM) or ryanodine (10 μM) made these caffeine-induced responses irreversible. These results suggest involvement of a mechanism related to depletion of intracellular Ca^{2+} store in activation of 100 fS-NSCC.

2P187 表皮細胞の傷の治癒には伸展によるヘミチャネルからの ATP 放出と TRPC6 を介した Ca^{2+}流入が効く

Wound healing in keratinocyte is accelerated by mechanosensitive ATP release via hemichannels and Ca^{2+} influx through TRPC6 channels

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The cutaneous wound healing was accelerated by stretch and impaired in TRPC6-KO mice. HaCaT keratinocyte treated with hyperforin, a traditional herbal medicine and also TRPC6 activator, were cultured in an elastic chamber. At 3 h after making scratching, ATP release and intracellular Ca^{2+} response by stretch were determined by live-imaging. ATP release was observed only from foremost cells and it caused spreading Ca^{2+} waves. The Ca^{2+} response and wound healing were inhibited by apyrase, suramin, CBX and diC8-PIP_2. In addition, hemichannel permeable dye calcein only entered ATP-releasing cells. These results suggested that stretch-accelerated wound closure was due to ATP release via hemichannels from the foremost cells and subsequent Ca^{2+} waves mediated by TRPC6.

2P188 Trial for detecting the activation and inactivation of chemoreceptor array in a single *E. coli* cell

Hajime Fukuoka[1], Tomoko Horigome[2], Yuichi Inoue[1], Hiroto Takahashi[1], Akihiko Ishijima[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Life Sci., Tohoku Univ.*)

In chemotaxis system, the intracellular signal protein, CheY, is phosphorylated and dephosphorylated at the chemoreceptor array localized at cellular pole. Recently, we demonstrated multiple flagellar motors coordinately switch the rotational direction and such rotational switching was induced by binding and dissociation of CheY-P. Therefore, we speculated that the receptor array might produce pulses of CheY-P production by spontaneous activation of receptor array in steady-state of an *E. coli*. To investigate this possibility, we tried to simultaneously detect the activity of receptor array from the fluorescently labeled chemotaxis proteins and the rotational switching of a motor in a single *E. coli* cell. We would like to discuss our results in the annual meeting.

2P189 単離ヒドラ神経細胞の神経突起動態

Neurite dynamics of isolated hydra nerve cells

Rui Tanaka[1], Miharu Nagaishi[2], Mio Ogawa[2], **Hiroyuki Nakagawa**[1,2] ([1]*Div. Bio., Fac. Sci., Fukuoka Univ.*, [2]*Grad. Sch. Sci., Fukuoka Univ.*)

Hydra, or cnidaria in general, is one of the first organisms with nerve cells evolving into the brain of higher bilaterians, such as insect and vertebrate. It is expected to be retained basic nature of nerve cell common to all nervous systems in them. However a few studies have been reported about their cellular characters as nerve cell, such as neurite dynamics. We, thus, isolated nerve cells from fresh water hydra, *Hydra magnipapillata*, and observed the dynamics of their neurites. Most of nerve cells well-attached to glass surface were observed as bipolar or multi-polar shape. During observation, neurites containing actin filament and microtubule were repeated the cycle of elongation and retraction at the rate of about 2 micrometer/sec.

2P190 サルモネラ菌の遊泳行動と生物対流

Chemotactic behavior of *Salmonella* and bioconvection

Takahiro Abe, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Tohoku Univ.*)

When cell density is low, chemotactic response of bacteria has been known as a biased-random walk. However, that at high cell density has not been fully understood. We analyzed chemotactic responses of *Salmonella* by a capillary assay under an optical microscope with changing cell density to characterize the chemotaxis in high cell density. Analysis by PIV (Particle Image Velocimetry) showed that large-scale convection flow was generated when bacteria accumulated to attractant. The direction and stability of convection were affected by the direction and effective magnitude of gravitational force. Random swimming of individual cells was observed even during the convection.

2P191 Rotation analysis of the spirochete cell body by 3D dark-field microscopy

Kyosuke Takabe, Md. Shafiqul Islam, Seishi Kudo, Shuichi Nakamura (*Grad. Sch. Engineering., Univ. Tohoku*)

Leptospira are spirochetes and have flagellar motors at both cell ends. When Leptospira swim, the anterior end is spiral-shaped (S) and the posterior end is hook-shaped (H). The S-end is known to rotate counterclockwise (CCW) to propel the cell. In contrast, the H-end has been believed to rotate CCW. Based on a theoretical analysis, we recently proposed a hypothesis that the H-end rotates clockwise (CW). However, direct observations of the H-end rotation to determine its rotational direction have not been performed. In this study, we examined the rotational direction of the H-end by three-dimensional dark-field microscopy. We found that most cells showed the CW rotations in the H-ends, which gives an evidence for the counterrotation of the H-end against the S-end.

2P192 細菌の走化性に起因する リング状パターンの形成過程の解析

Formation process of a ring-like pattern induced by bacterial chemotaxis

Tadahiko Sato, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Univ. Tohoku*)

Motile bacteria respond to the concentration gradient of chemicals and migrate toward the place more suitable for growth, which is called chemotaxis. It is known that, when an agar drop including an attractant is placed in a bacterial suspension, a ring-like pattern is formed around the drop. Although the ring formation is caused by positive chemotaxis, the formation mechanism is still unclear. In this study, we quantitatively analyzed the formation process of the ring. We found that the ring-formation speed and the ring size after reaching the steady state were increased with the concentration of attractants. We are now trying to observe the behavior of individual cells during the ring formation.

2P193 Theoretical and simulation study for deformation of caveolae under hypo-osmotic condition

Masashi Tachikawa[1], Shiro Suetsugu[2] ([1]*RIKEN*, [2]*NAIST*)

Caveolae are invagination structures in plasma membrane, size of about 100 nm. The main function of caveolae is still under debate. Recently it is proposed that the caveolae become flattened as buffers of plasma membrane at hypo-osmotic shock. We performed theoretical analysis and computer simulation to test this hypothesis. In the theoretical analysis, we calculated force acted on the caveolae and found that, the direction of force depends on the shape of caveolae: the expanding force and constriction force are both expected. The constriction force may give the opposite result; constricted caveolae. Then we performed coarse-grained membrane (polygon membrane) simulation and confirmed the existence of constricted caveolae, which is a novel membrane structures.

2P194 細胞性粘菌の F-アクチン波の基質依存性 Substrate dependence of F-actin waves in Dictyostelium

Yuko Chida[1], Satoshi Sawai[1,2,3] ([1]*Graduate School of Arts and Science, University of Tokyo*, [2]*Research Center for Complex Systems Biology*, [3]*PRESTO, Japan Science and Technology Agency*)

F-actin waves that propagate at the membrane cortex are known in a variety of moving cells. In Dictyostelium, waves of PIP3/F-actin are enriched in PI3Kinase and Arp2/3 complex thus appear related to leading edge structure of migratory cells. However, the exact conditions supporting waves and their link to cell migration remains elusive. Here, by combining confocal and TIRF microscopy, we demonstrate that wave nucleation in Dictyostelium is substrate-dependent. The PIP3/F-actin waves are suppressed on a poly-lysine coated coverglass or PDMS. The suppression is negated by treating PDMS with air plasma, which renders the substrate negatively charged. We will discuss the relation between strength of cell-substrate attachment, its spatio-temporal change and actin-waves.

2P195 心筋細胞における温度依存的拍動周期の特性 Characterization of temperature-dependent beating rate of cardiomyocytes

Tomoyuki Kaneko (*LaRC, Dept. Frontier Biosci., Hosei Univ.*)

Cardiomyocytes are known to change the beating rate depending on temperature. To characterize the beating rate of cardiomyocytes with changing temperature, we measured the inter-spike interval (ISI) of cardiomyocytes derived from chick embryo (E13) by multi-electrode array (MEA) system. The beating rate was not increased immediately after starting the temperature rises and was increased sigmoidal as temperature rises from 15°C to 32°C after lag time of several minutes. The stationary phase of the beating rate was constant at repeated procedure of three times. These results suggested that cardiomyocytes could possess an inherent beating rate corresponding to surrounding environments.

2P196 多電極電位計測による心筋細胞に対するエタノールの影響 Effect of ethanol on cardiomyocytes measured by multi-electrode array system

Chiho Nihei, Tomoyuki Kaneko (*LaRC, Dept. Frontier Biosci., Hosei Univ.*)

Ethanol is contained in liquor and had various influence to human body. To examine the effect of ethanol to heart, we applied ethanol to cardiomyocytes derived from chicken embryo (E13). We measured inter spike interval (ISI) and field potential duration (FPD) of the cells by multi-electrode array system. A low concentration of ethanol (0.1%) had no apparent effect on both ISI and FPD. As shortening of ISI and FPD occurred at the concentration of more than 0.5% of ethanol, it was shown that ethanol made beating of cardiomyocytes faster. At the waveform of extracellular potential, the peak values of Na^+ and K^+ were decreased in a concentration-dependent manner. Therefore, it was suggested that ethanol might simultaneously affect several ion channels on cardiomyocytes.

2P197 多電極電位計測システムを用いた薬剤の催不整脈性の検出 Detection of arrhythmogenicity of drugs by using multi electrode array system

Shin Yoshida, Tomoyuki Kaneko (*LaRC, Grad. Sci. Eng., Hosei Univ.*)

An electrocardiographic QT interval often extends just before fatal arrhythmia such as torsades de pointes caused by the dosage of drugs. Recently, multi electrode array (MEA) system has been used to measure the field potential duration (FPD) equivalent of QT interval. To examine the availability of MEA system with cardiomyocytes derived from chick embryo (E13) for detect arrhythmogenicity of drugs, we applied K^+ blocker E-4031 to this system, at a final concentration 10 nM, FPD was extended to 1.2 times compared with control. This result suggested that it is possible to use this system for detection of arrhythmogenicity of drugs.

2P198 多電極電位計測システムを用いた薬剤試験を行うための培地条件の探索 Exploration of the optimum medium for a toxicity testing by multi electrode array system

Yuichiro Kamei, Tomoyuki Kaneko (*LaRC, Grad. Sci. Eng., Hosei Univ*)

Recently, multi electrode array (MEA) system has been noted as a toxicity testing tools. However, its optimum protocol for toxicity testing is not established. To explore the optimum medium, we measured inter spike interval (ISI) and field potential duration (FPD) by MEA system with cardiomyocytes derived from chick embryo (E13). In standard DMEM, ISI and FPD largely varied and were unstable. To stable the pH in the medium, DMEM including HEPES buffer and CO_2 independent medium were used. In both medium, ISI and FPD hardly varied and were stable at least for 2 hours. As a result, we found that pH changes had a negative influence on cardiomyocytes. Therefore it was suggested that the medium suppressing pH changes could be used for a toxicity testing during several hours.

2P199 Raman Micro-spectroscopy of the Dynamics of Cellular Chemical State upon Stimulation with Growth Factors

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Cell fate changes stimulated by external factors, often show large cell-to-cell variation in a clonal population. Such variation should reflect differences in the internal chemical states of cells. By defining and tracking the chemical state of individual cells, it would be possible to find a pathway of cell fate determination. Raman microspectroscopy is a powerful method to obtain information about the comprehensive cellular states. In this study, we tracked the cellular chemical states in the principal components plane defined from the time-course Raman spectra of the single living cells upon stimulation with growth factors. Results of the analysis indicated that differentiation process is affected by the initial chemical states. While proliferation looked stochastic.

2P200 単層上皮細胞シートの複素弾性率の空間分布：原子間力顕微鏡測定
Spatial distribution of complex shear modulus in confluent epithelial cell sheet : Atomic Force Microscopy measurements

Yuki Fujii, Yuki Ochi, Takaharu Okajima (*Grad. Schl. Inform. Sci. and Technol., Hokkaido Univ.*)

It is commonly recognized that mechanical properties of cell strongly influence various biological functions. To elucidate detailed relationship between mechanical properties and functions in soft cell systems, we investigate the viscoelastic behaviors of cell population. To do this, we developed a newly designed atomic force microscopy (AFM), which allows us to measure the local complex shear modulus of cell monolayer in a wide spatial range. The measurements of epithelial Madin-Darby canine kidney (MDCK) cells showed a longer spatial correlation in both the storage and loss moduli. We found that the spatial correlation was significantly affected by cytoskeletal structure as well as cell-cell contacts. The detailed results about those mentioned will be reported.

2P201 心筋細胞ネットーワークにおける時空間的ゆらぎ計測を用いた心毒性評価のための Quasi-*in vivo* 前臨床モデル
Quasi-*in vivo* pre-clinical model for cardiac toxicity using spatiotemporal fluctuation measurement on human cardiomyocyte cell-network

Fumimasa Nomura[1], Tomoyuki Kaneko[2], Hideyuki Terazono[1], Kenji Yasuda[1] ([1]*IBB, Tokyo Medical & Dental Univ.*, [2]*Dept. of Frontier Bioscience, Hosei Univ.*)

To overcome the limitations of conventional prediction of arrhythmic cardiotoxicity, we have developed a quasi-*in vivo* pre-clinical model employing the two step measurement of spatiotemporal fluctuation (short-term variability; STV) of (1) cell's repolarization and (2) cell-to-cell conduction, representing two origins of lethal arrhythmia. Temporal STV of field potential duration showed a potential to predict the risks of lethal arrhythmia originated from repolarization dispersion, even for false negative compounds. Spatial STV of conduction time also unveiled the proarrhythmic risk of asynchronous propagation in cell networks. The results indicate the importance of the two spatiotemporal fluctuation viewpoint of *in vitro* cell networks to reach clinical assessment.

2P202 Probing the sphingomyelin clusters in pure and mix lipid bilayer by the Raman spectroscopy: A theoretical study

Pai-Chi Li, Kiyoshi Yagi, Koichiro Shirota, Toshihide Kobayashi, Yuji Sugita (*RIKEN*)

Recent experimental evidence have suggested that sphigomyelin (SM) forms clusters in SM/DOPC liposomes to which GFP-lysenin proteins can bind specifically, while these phenomenons are not observed in the SM/DPPC liposomes. Moreover, the Raman spectra of SM, SM/DOPC and SM/DPPC liposomes show significant difference in the intensity of the amide I band, indicating that the environment of the amide group of SM is different in these liposomes. However, the structure of the SM in these liposomes remains elusive. Here, we propose a simulation procedure to predict the Raman spectrum (amide I band) for these three bilayers. This study provides insight into the changes in Raman intensity of the amide I band caused by the distinct SM cluster structure in the three liposomes.

2P203 スズガエルの皮膚分泌物由来のペプチド Bombinin H2 および H4 により誘起される相乗的な抗菌活性および細胞膜の揺らぎ
Synergistic antimicrobial activity and membrane disturbance induced by Bombinin H2 and H4 peptides from *Bombina variegata* skin secretion

Yuki Kitahashi, Izuru Kawamura, Akira Naito (*Grad. Sch. Eng, Yokohama Natl Univ.*)

Bombinin H2 and H4 are antimicrobial peptides varying at the 2nd position with L-Ile in H2 and D-allo-Ile in H4. The previous study showed H4 has stronger antimicrobial and hemolytic activities than H2. In antimicrobial activity measurements, we found the mixture of H2 and H4 was stronger than each peptide components. CD results indicate the mixture mainly formed α-helix in DMPC. Line width lipid bilayers determined by ^{31}P static NMR showed the strong interaction of mixed peptides with lipid head groups and the reduction of the order of lipids compared with each peptide in DMPC. To summarize, the mixture synergistically enhanced antimicrobial activity and disturbed the membrane order. The structural difference of N-terminus may be crucial to exhibit strong activity.

2P204 蛍光一分子追跡に基づいた支持脂質膜内における部分フッ素化リン脂質の熱力学的性質の評価
Evaluation of thermodynamic property of partially fluorinated phospholipid in supported lipid bilayer based on single molecule tracking

Yoshiaki Okamoto[1], Toshinori Motegi[1], Kohei Morita[2], Toshiyuki Takagi[3], Toshiyuki Kanamori[3], Masashi Sonoyama[2], Ryugo Tero[1] ([1]*Toyohashi Univ. Tech.*, [2]*Gunma Univ.*, [3]*AIST*)

Fluorinated amphiphiles are expensively studied in biotechnological and biomedical fields because of the biological inertness of fluorocarbons by weakly intermolecular interaction of carbon-fluorine bonds. Partially fluorinated phospholipid (F-PL) substituted with fluorocarbon on its hydrophobic chain is expected to the application for drug delivery system, protein extraction, and protein crystallization. We performed the single molecule tracking of the F-PL bilayer on a SiO_2/Si substrate, and evaluated the diffusion coefficient calculated from the mean square displacement analysis. Based on the thermal dependence of molecular diffusion, we evaluated the thermodynamic property of the F-PL bilayer.

2P205 脂質二分子膜と高分子材料を融合したハイブリッド型人工膜の創製

Hybrid model membrane composed of phospholipid bilayer and polymeric materials

Koji Ando[1], Kenichi Morigaki[1,2] ([1]*Grad. Sch. Agri., Univ. Kobe*, [2]*Res. Cen. Env Gen., Kobe*)

We developed a hybrid model membrane by combining natural and polymeric phospholipid bilayers, and silicone elastomer (polydimetylsiloxan; PDMS). A micropatterned membrane composed of polymeric and fluid bilayers was generated on a glass substrate. The polymeric bilayer was made of a diacetylene phospholipid, and had a reactive amine moiety on the surface. We bonded PDMS microstructures onto the polymeric bilayer by attaching biotin onto the surface and using the biotin-streptavidin linkage. We also employed lipid vesicles as a bonding agent between polymeric bilayer and PDMS. The model membrane achieved a unique 3D structure for confining and transporting molecules in a small space between the membrane and PDMS, which can be used as a novel biosensing platform.

2P206 ベシクル基盤分子ロボットのための分子センサーの開発

Development of a molecular sensor for vesicle-based molecular robots

Koh-ichiroh Shohda, Akira Suyama (*The University of Tokyo*)

A molecular robot based on a vesicle or a gel as a body is composed of molecular sensors, molecular computers, and molecular actuators in the molecular robotics project. We develop a molecular sensor that is a conjugated molecule of DNA strands and a hydrophobic molecule. The sensor molecule located into a membrane of giant unilamellar vesicle can hybridize with a complementary DNA/RNA strand that represents an environmental signal. The hybridization approximates two sensor molecules, consequently a single-stranded DNA will be released into an inner water pool of vesicle. The released DNA will be a trigger for molecular computers encapsulated in GUV-based molecular robots.

2P207 リポソームを固定した QCM センサーで脂質膜結合プローブの相互作用を測る

Liposome fixed sensor of quartz crystal microbalance quantifies the interaction between the lipid binding probe and target membrane

Takehiko Inaba, Toshihide Kobayashi (*RIKEN (Wako)*)

Cells contain thousands of lipid varieties. To know the lipid roles, each lipid behavior is important. Lipid binding probes have been used to visualize their target lipids. To characterize the interaction between lipids and probes, we try to construct the system based on quartz crystal microbalance (QCM). Although the simple method for the fixation of lipid membrane on sensor is vesicle fusion, the method is not suited for the complex composition of lipids. Therefore, we applied the linker based liposome fixation. In this method, the sensor was covered with planar lipid bilayers containing linkers and target liposomes were fixed with linkers. We are able to characterize the binding kinetics of lipid probe in the flexible liposome condition (composition, size, density).

2P208 ラマン分光法によるモデル膜におけるスフィンゴミエリン会合体の研究

Study on sphingomyelin aggregates in model membranes by Raman spectroscopy

Koichiro Shirota[1], Takehiko Inaba[1], Pai-Chi Li[2], Kiyoshi Yagi[2], Yuji Sugita[2], Toshihide Kobayashi[1] ([1]*LBL, RIKEN*, [2]*TMSL, RIKEN*)

Sphingomyelin (SM), which shows a gel phase at room temperature (RT), is miscible with dipalmitoylphosphatidylcholine (DPPC) that also takes a gel phase at RT. In contrast, SM is immiscible with dioleoylphosphatidylcholine (DOPC) that has a liquid crystal phase at RT, and thus forms clusters. Here, we have observed aggregates of SM molecules and those between SM and phospholipid molecules in model membranes by Raman spectroscopy to elucidate the mechanism of lipid domain formation. The spectra of SM mixtures with DPPC and DOPC differ in C=O stretching vibrations of the amide group due to hydrogen bonding.

2P209 高速原子間力顕微鏡によるバクテリオロドプシン球殻構造体の分子構造の直接観察

Direct observation of molecular arrangement in a bacteriorhodopsin vesicle by high-speed atomic force microscopy

Yuto Noda, Daisuke Yamamoto (*Dept. Appl. Phys., Grad. Sch. Sci., Fukuoka Univ.*)

Bacteriorhodopsin (bR) is a light-driven proton pump that forms two-dimensional crystal, called purple membrane (PM). While PM is a flat membrane in a native environment, PM spontaneously converts its membrane structure into a uniformly-sized vesicle (50 nm in diameter) in the presence of a detergent. So far, the molecular arrangement in the bR-vesicle is undissolved. Here, we applied high-speed atomic force microscopy to directly visualize the molecular arrangement of bR in the bR-vesicle. On mica surface, the bR-vesicle collapsed to form a flat membrane with a thickness of 8 nm. In high-magnification images, honeycomb structure was observed in the bR-vesicle. This molecular arrangement of bR is similar to that in a crystal that is grown from the bR-vesicle.

2P210 固液界面に形成したコレステロールとコレステロールエステルからなる自己組織化単分子膜の研究

Self-Assembled Monolayers of Cholesterol and Cholesteryl Esters at the Liquid/Solid Interface

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The molecular arrangements of self-assembled monolayers (SAM) of cholesterol, cholesteryl laurate, and cholesteryl stearate adsorbed on a graphite surface were studied using scanning tunneling microscopy (STM) at the liquid/solid interface. The STM images of the SAMs showed two-dimensional periodic arrays of bright regions that corresponded to the sterol rings. The STM images of cholesteryl ester monolayers had molecular resolution and showed pairs of cholesteryl ester molecules oriented in an antiparallel manner, with their fatty acid chains located in the central regions. These results show that the self-assembly at the liquid/solid interface is controlled by the interactions between sterol rings, between alkyl chains, and between alkyl chains and the substrate.

2P211 細胞膜を構成する脂質分子種の二重層間および膜平面における非対称分布の凍結割断レプリカ電顕法による解析

Transbilayer and lateral lipid distribution in plasma membranes in nano scale

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Methods to examine transbilayer lipid distribution in biomembranes are limited. Commonly used biochemical characterization requires highly purified membrane and re-organization of lipids during treatment cannot be excluded. We employed a freeze-fracture replica immunoelectron microscopy in combination with different lipid-specific probes to examine both transbilayer and lateral distribution of various phospholipids. Our results indicated that phospholipids were exclusively distributed either the outer or the inner leaflet of red blood cell membrane, inconsistent with previous studies. The data also revealed the characteristic inner leaflet domains of sphingomyelin in nucleated cells and the selective budding from specific lipid domains in the stimulated platelets.

2P212 メリチンが持つ多才な膜小胞変形能力

Multiple membrane interactions and versatile vesicle deformations elicited by melittin

Tomoyoshi Takahashi[1], Fumimasa Nomura[2], Yasunori Yokoyama[3], Yohko Tanaka-Takiguchi[1], **Kingo Takiguchi**[1] ([1]*Dev. Bio. Sci., Grad. Sch. Sci., Nagoya Univ.*, [2]*Institut Biomater. Bioengineer., Tokyo Med. Dent. Univ.*, [3]*Dept. App. Phys., Grad. Sch. Eng., Nagoya Univ.*)

Melittin induces various reactions in membranes and has been widely studied as a model for membrane-interacting peptide; however, the mechanism whereby melittin elicits its effects remains unclear. Here, we observed melittin-induced changes in individual giant liposomes using direct real-time imaging by dark-field optical microscopy, and the mechanisms involved were correlated with results obtained using CD, cosedimentation, fluorescence quenching of Trp residues, and EM. The results obtained indicate that the various effects of melittin result from its ability to adopt various structures and membrane-binding states depending on the conditions.

2P213 ER 膜タンパク質の膜貫通配列のリン脂質フリップフロップ誘起能の評価

Promotion of phospholipid flip-flop by membrane-spanning sequences in the ER proteins

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Phospholipid flip-flop is very rapid in the endoplasmic reticulum (ER) in order to maintain the membrane integrity. However, the mechanism to promote the flip-flop in the ER remains to be elucidated. We have previously reported that model peptides which have a hydrophilic residue in the center of transmembrane sequence facilitate the flip-flop of phospholipids. We have also shown that there exist human ER membrane proteins which have hydrophilic residues in the center of the transmembrane domain. In this study, under the hypothesis that these transmembrane regions of the proteins may promote the phospholipid flip-flop in the ER, we synthesized peptides with native membrane-spanning sequences of the ER proteins and investigated their flip-flop promotion ability.

2P214 細胞サイズリン脂質非対称膜リポソームによる膜ダイナミクスの観察

Cell-sized asymmetric lipid vesicles for membrane dynamics observation

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Cell membranes are composed of asymmetric phospholipid layers. In the cell membranes of eukaryotic cells, phosphatidylserine (PS) and phosphatidylethanolamine (PE) are located on the intracellular leaflet, while phosphatidylcholine (PC) is located on the extracellular leaflet. Membrane asymmetry plays a role in the biological reactions in living cells. To investigate lipid dynamics in asymmetric membranes that emulate the components of cell membranes, we prepared cell-sized asymmetric lipid vesicles from a planar asymmetric lipid bilayer by using jet flow. The lipid dynamics (flip-flop) on the asymmetric vesicles were observed using a confocal laser scanning microscopy.

2P215 低い pH が誘起する DOPS/MO 膜の一枚膜リポソームからキュービック相への構造転移の初期過程

Initial Step of Low pH-Induced Structural Transition from Unilamellar Vesicles of DOPS/MO to Inverse Bicontinuous Cubic Phase

Takahiro Saiki[1], Toshihiko Oka[1,2], Taka-aki Tsuboi[1], Masahito Yamazaki[1,2] ([1]*Grad. Sch. Sci, Shizuoka Univ.*, [2]*Res. Inst. of Electronics, Shizuoka Univ.*)

We investigated the initial step of low pH-induced structural transition from large unilamellar vesicles (LUVs) of dioleoylphosphatidylserine (DOPS)/monoolein (MO) to bicontinuous cubic (Q_{II}^{D}) phase using small angle X-ray scattering with a stopped flow apparatus. After the pH-jump from pH 6.7 to pH 2.6-2.8, the LUVs transformed into the H_{II} phase, and then the H_{II} phase converted into the Q_{II}^{D} phase. The rate constant of the initial step decreased with increasing pH, and much smaller than that of multilamellar vesicles (MLVs) of DOPS/MO. The DLS data indicate that the LUVs associated and thus increased in size with time after the pH-jump to the low pH. The difference in the rate constant of between LUVs and MLVs can be explained by the classical nucleation theory.

2P216 単一 GUV 法による脂質膜に EGCg が誘起した孔構造の可視化

Visualization of the EGCg-induced bursting of single giant unilamellar vesicles at higher time resolution

Yukihiro Tamba[1], Masahito Yamazaki[2] ([1]*Suzuka Natl Coll Tech*, [2]*Shizuoka Univ*)

Tea catechins such as epigallocatechin gallate (EGCg) have antibacterial activity. Using the single GUV method, we found that EGCg induced a large hole in PC-membrane resulting in bursting of GUVs. Here, we visualized the process of the EGCg-induced bursting with a time-resolution of 5 ms. In the beginning of bursting, a region of lipid defect suddenly appeared in the membrane of a GUV, and at the same time dense small particles appeared in the membrane. At 10 ms after the appearance, the defect clearly became a large hole. And, at edge of the hole, other dense lipid particles appeared. With growing of these dense particles, the diameter of the GUV decreased. Based on these results, we discuss the mechanism of the EGCg-induced bursting of GUVs.

2P217 静電効果によるジャイアントリポソームの膜チューブ形成
Formation of endocytosis-like membrane tubes in giant liposomes induced by electrostatic effect

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We observed giant liposomes composed of negatively charged lipids (PC/PI) and containing a sugar solution, and found that when they were exposed to a hypertonic KCl solution, very thin membrane tubes protruded into the interior of the spherical liposomes. To investigate the mechanism of this phenomenon, we numerically calculated the free energy of curved membranes, which is determined by the membrane elasticity, the surface charge density and the distribution of electrolytes in the aqueous solution. The result showed that unless the lipid molecules have bulky heads, a cylindrical charged membrane has lower energy than a flat one. This fact suggests that the thin tubes observed in the experiment may be induced by the electrostatic effect of the membrane charge.

2P218 Self-Emergent Cell-Sized Sphere Entrapping DNA through Micro Phase-Segregation

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Environments inside living cells are crowded with macromolecular substances including DNAs, proteins, etc. Recently, microcompartments have been actively studied as model cellular system, by focusing on stabilities and functions of lipid vesicle. Generally, the “aqueous two-phase system (ATPS)” such as the dextran/PEG mixture is applied to separation of biochemical macromolecules. In the present study, we examined an emulsified dextran/PEG solution containing DNAs, and found that, under suitable experimental condition, DNAs were exclusively located inside cell-sized dextran-rich droplets by avoiding the fusion among the droplets. Emergence of such kind of cell-like structure is discussed in relation to the mechanism of origin of life.

2P219 Conformational Transitions in Voltage Sensor Domains

Morten Bertz, Kazuhiko Kinosita (*Waseda University, Dpt. of Science & Engineering*)

Voltage gating - the opening and closing of ion channels in response to changes in membrane potential - is fundamental to signal transduction in living organisms. Voltage gating is achieved by four voltage sensing domains (VSDs) that surround the ion-conducting pore of the channel protein. In these VSDs, conserved positively charged residues located in the S4 transmembrane helix move according to the transmembrane electrical field, and the resulting conformational change is transmitted to the channel pore. The extent and direction of this movement, however, remain controversial. Here, we attempt to shed light on this transition using a combination of engineered probes, mutagenesis, observation, and manipulation.

2P220 破骨細胞膜に存在する新規の（?）プロトン流入経路
A novel (?) proton influx pathway in the plasma membrane of osteoclasts

Miyuki Kuno, Guanshuai Li, Yoshiko Hino, Yoshie Moriura, Junko Kawawaki, Hiromu Sakai (*Dept Physiol, Osaka City Univ Grad Sch Med*)

Osteoclasts, multinuclear bone-resorbing cells, face to highly acidic extracellular environments (~pH 4) in the resorption pit formed on the bone surface. Resultant pH gradient across the plasma membrane could generate a large driving force for protons entering into the cells, but the proton flux mechanism is unknown. In murine osteoclasts, we identified proton influx currents activated by extracellular acidification (< pH 5.5). The currents were featured by proton-selectivity, inward rectification and insensitivity to blockers for acid-sensitive cation channels, like ASIC and TRP channels. These results suggested that osteoclalsts may possess an electrogenic proton-selective pathway which could mediate proton influx upon severe extracellular acidification.

2P221 Kv1.2 でのイオン透過における透過パターンの解析
Analysis on Ion Permeation Pattern through the Kv1.2 Channel

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States in ion permeation through the K^+ channel can be defined by the queue of water and ions in the selectivity filter. The cyclic paths formed by the transition among states are called the permeation patterns here. Ion permeation is now described by the transitions among the permeation patterns. We analyzed the rates of the patterns used in permeation through the Kv1.2 channel at several concentrations using the molecular dynamics simulation. The transport ratios of water molecules over ions, which can be estimated by the measurement of the streaming potential, are also evaluated, yielding to a similar concentration dependency of them to the experiment. It is found that a slight difference in the ratio reflects a large difference in the patterns used in permeation.

2P222 two-pore 型カリウムチャネル TWIK-1 の特徴的なイオン選択性を生み出すメカニズムについての全反射赤外分光解析
ATR-FTIR spectroscopic analyses of interaction modes underlying unique ion selectivity of a two-pore domain potassium channel TWIK-1

Hisao Tsukamoto[1], Koichi Nakajo[2], Yoshihiro Kubo[2], Yuji Furutani[1] ([1]*Institute for Molecular Science*, [2]*National Institute for Physiological Sciences*)

Most of K^+ channels are highly K^+ selective and impermeable to Na^+, but a mammalian two-pore domain K^+ channel TWIK-1 (or K2P1, KCNK1) shows a "loose" ion selectivity. In this study, we applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to TWIK-1 in order to elucidate the molecular mechanism underling the unique ion selectivity. The difference spectra upon ion exchange from K^+ to other monovalent cations were recorded in TWIK-1 WT and a mutant having a high K^+ selectivity. We compared the spectra between WT and the mutant, and found specific absorption bands that reflect different interaction modes of the channels with cations. Based on the IR spectra, we propose a model explaining how TWIK-1 achieves the unique ion selectivity.

2P223 Akt と受容体の生細胞内 1 分子追跡によるシグナル伝達機構の解明

Signal transduction mechanism of Akt reveald by single molecule imaging of Akt and receptor molecules

Hideaki Yoshimura, Takeaki Ozawa (*Department of Chemistry, School of Science, The University of Tokyo*)

This study aims at revealing the mechanism of Akt to produce specific signaling output patterns depending on the input stimuli. Akt in cytosol is recruited to the plasma membrane upon the cell receives external stimuli such as growth factors. The Akt on the plasma membrane is activated and mediates the external signal to downstream molecules to generate specific signal output patterns. The mechanism of specific signal transduction is unknown. The dynamics of Akt on plasma membrane is important to understand the mechanism because the whole signaling events on Akt occur on the plasma membrane. We here show a study of single-molecule imaging of Akt in living cells and will discuss the mechanism of Akt signal transduction.

2P224 細胞性粘菌の走化性シグナル伝達におけるグアニル酸シクラーゼ(sGC)経路の興奮性応答

Excitability of Guanylate Cyclase (sGC) signaling pathway mediating chemotaxis in Dictyostelium cells

Yuki Tanabe[1], Masahiro Ueda[1,2] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*QBiC, Riken*)

Chemotaxis, the directed migration in chemical gradients, is a vital function in many biological processes such as immune response and synapse formation. Chemotaxis is mediated by some parallel signaling pathways, e.g. PI3K-path, sGC-path and so on. It has been considered that the excitable response to chemoattractant is important to amplify the signal and the previous work showed that PI3K-path response is an excitable system. However, what kind of property sGC-path has is unknown.
By using the pulsed chemoattractant stimulation, we found that sGC response follows all-or-none law and has the refractory period. These features are characteristics of excitable system like PI3K-path. We discuss the chemotactic signaling network consists of multiple excitable pathways.

2P225 モデル生体膜を用いたロドプシン光受容体の脂質ラフト親和性解析

Evaluating the raftophilicity of rhodopsin in a patterned model membrane

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Rhodopsin (Rh) is a photoreceptor that forms a complex with transducin (Gt) upon activation by light. It has been reported that the affinity of Rh to lipid raft (raftophilicity) increases after photoactivation and complexation with Gt (Hayashi et al. ARVO meeting (2011)). To elucidate the roles of the raftophilicity, we are developing a methodology to evaluate the raftophilicity of Rh in a model membrane that has micro-patterned liquid ordered (Lo) (raft model) and liquid disordered (Ld) (non-raft model) domains. We observed that reconstituted Rh and Gt before photoactivation were localized in Ld domains, suggesting their affinity to non-raft bilayers. We discuss on the observed distributions of Rh, Gt and Rh*-Gt complex in the patterned membrane.

2P226 ミミズ非連合学習における NO-cGMP シグナル経路の役割

Role of NO-cGMP signaling in non-associative learning of the earthworm

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Extracellular recording from the ventral nerve cord of the earthworm revealed number of action potentials in the median giant fiber decreased after repeated tactile stimulus to the body wall. NO donor and cGMP analogue also decreased the number of action potentials by single tactile stimulus. Not only cGMP analogue but also guanylate cyclase inhibitor decreased the number of action potentials. These results indicate that cGMP has an excitatory effect on action potential generation, though excess of cGMP reduced the responsibility to tactile stimulus. In conclusion, cGMP in earthworm has a bi-directional effect on neural activity, and the non-associative learning such as habituation in earthworm is assumedly formed due to cGMP accumulation.

2P227 チャコウラナメクジ脳嗅覚中枢における匂い応答の数理解析

Tone-Entropy analysis on odor-evoked neuronal activities in the procerebral lobe of a slug

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We investigate how information is included in the oscillatory activity of procerebrum (PC), an olfactory center of Limax. Here, we analyzed conditioned odor-evoked responses of the PC by using the tone-entropy (T-E) method. The T-E method calculates two indexes: tone which represents a balance between acceleration and inhibition of the oscillatory activity, and entropy which indicates the total activity of physiological mediators. After the administration of conditioned odor, the mean entropy value significantly increased in both naïve and conditioned groups, whereas the mean tone value increased in conditioned group alone. The results suggest that the balance of excitatory and inhibitory synapses in PC is altered with aversive conditioning.

2P228 記憶学習中枢海馬の性差：海馬内ホルモン変動とシナプス変動の解析

Sex difference in hippocampus: Fluctuation of hippocampal sex hormones and synapses

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The hippocampus, a center for learning and memory, does not have sex difference at the anatomical level including its volume and the number of neurons. Nevertheless, the significant sex difference in the performance of hippocampus-dependent task.
We hypothesized that the sex difference in the hippocampal structure exists at more subtle level, that is, synaptic level. A novel software, Spiso-3D, which we developed, allowed us to reveal the sex difference in the density of spines and the fluctuation of synapses in female with a period of 4 days (estrous cycle).
The estrous cycle-dependent fluctuation of the spine density in female rat hippocampus had a good correlation with the cyclic fluctuation of hippocampal levels of female hormones (estradiol and progesterone).

2P229 マウス海馬スライスに見られる時空間活動パターンに対するゆらぎ解析

Fluctuation analysis for spatiotemporal activity patterns in mouse hippocampal slices

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We have observed various spatiotemporal activity patterns in mouse hippocampal slices by functional multineuron calcium imaging (fCMI), which enables us to access brain function with single-neuron resolution. For example, some dozens of neurons exhibited incoherent activity patterns in the CA1 region under the normal condition, while they exhibited more coherent ones under a higher K^+ concentration and the existence of bicuculline. In the present study, we analyzed the spatiotemporal activity patterns using detrended fluctuation analysis (DFA), which is a scaling analysis used to provide a quantitative parameter (scaling exponent, α). As a result, 1/f fluctuations (α=1) were seen for the neural activity patterns in the normal slices.

2P230 膜電位イメージングを用いたチャコウラナメクジ嗅覚神経回路の解析

Characterization of the olfactory neural network of the land slug using fluorescent voltage imaging

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In the olfactory center (procerebrum; PC) of the land slug *Limax valentianus*, the local field potential (LFP) shows an oscillation of about 1 Hz. We have found using fluorescent voltage imaging that the oscillatory activity has a phase delay along the distal-proximal axis of the PC. However, how the output signals from the PC affect the neural activities of other regions in the cerebral ganglion is not elucidated. In the present study, we measured the neural activity of the metacerebrum using fluorescent voltage imaging together with the LFP oscillation in the PC. As a result, when the superior tentacle nerve was electrically stimulated, the LFP oscillation pattern was changed, and burst-like activities appeared in some neurons in the metacerebrum.

2P231 ゾウリムシにおける長期後退遊泳の膜興奮モデルによる解析

The analyses based on a membrane excitation model for Long-term Backward Swimming in a protozoa Paramecium

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We observed how a Paramecium attempts to retreat from the dead-end of capillary that is too narrow for turning. When the forward swimming Paramecium bumped against the end of capillary, the specimen exhibited short-term backward swimming (SBS). After that, the specimen exhibited long-term backward swimming (LBS) that lasted three to four times longer than SBS. LBS is a novel behavior for retreat from a long narrow space. Since the mechanism of backward swimming in Paramecium can be described by the Hodgkin-Huxley (H-H) type equations, we propose a mathematical model based on the H-H model for understanding a possible mechanism for LBS. The physiological implications and physical mechanism of the development of LBS are discussed.

2P232 T 細胞の自発運動の解析

Analysis of spontaneous migration of T cell

Hiroaki Takagi[1], Tomoya Katakai[2], Tatsuo Kinashi[2] ([1]*Nara Medical University*, [2]*Kansai Medical University*)

Cell migration is a fundamental process associated with many physiological phenomena. We have confirmed that spontaneous cell migration shows anomalous super diffusion with velocity memory, multiplicative fluctuation, and different timescale dynamics in Dictyostelium cells. Also, we have verified functional links between spontaneous cell migration and cellular response against external signals. Then, we tried to verify the generality of this view by analyzing different cell species, especially spontaneous migration of T cells. We extensively performed single T cell trajectory analysis in 2D and 3D cases. In this presentation, we discuss the common aspects and differences in these migration dynamics by introducing the techniques of statistical physics on Brownian motion.

2P233 高度好熱菌由来サーモフィリックロドプシンの温度依存的な不可逆構造転移

Temperature-Dependent Irreversible Structural Transition of Thermophilic Rhodopsin

Takashi Tsukamoto[1], Makoto Demura[2], Yuki Sudo[1] ([1]*Div. Pharm. Sci., Okayama Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*)

Several forms of oligomers have been found in microbial ion-pumping rhodopsins. The assembly is reversibly associated and dissociated at a certain time interval and is related to physiological properties, such as stabilizing protein structure and regulating photoreaction dynamics. Here, we investigated the oligomeric assembly of a H^+-pump thermophilic rhodopsin (TR) derived from *Thermus thermopiles* JL-18 living at 75°C. TR formed a trimer in a detergent solution at room temperature, but the trimer is irreversibly dissociated into monomers at 68°C, where the visible absorption maximum was also shifted. The irreversible transition allowed us to investigate photoreaction rates of the both forms. Physiological significance of the irreversible transition will be discussed.

2P234 ナトリウムポンプロドプシンにおけるナトリウムイオンの結合の役割

Role of Sodium Ion Binding in Sodium Pumping Rhodopsin

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Sodium ion-pumping rhodopsin (NaR) is a member of microbial rhodopsins and it outwardly pumps Na^+ by energy of light. Previously, we revealed that NaR binds Na^+ in the extracellular side. However, the mutants which lost the Na^+-binding activity still showed effective Na+-transport as wildtype. This means Na^+-binding is not required for Na^+-transport and its physiological role has been unrevealed. In this study, we investigated the effect of Na^+-binding to the thermal stability of NaR. We found that the stability was lowered by the removal of Na+ from the binding site. This indicates that Na^+-binding is physiologically important to maintain the higher stability of NaR and the molecular mechanism will be discussed.

2P235 プロテオロドプシンのプロトンドナー Glu108 の変異が光化学特性に及ぼす影響について

Effects on the photochemical properties in proteorhodopsin by the mutation of the Glu108 residue

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Proteorhodopsin (PR) is one of the microbial rhodopsins, which acts as a light-driven proton pump. It has been considered that Glu108 in PR (corresponding to Asp96 in bacteriorhodopsin) functions as an internal proton donor of the Schiff base during its cyclic photochemical reaction (called photocycle). On the other hand, the corresponding residue in a novel PR-like protein found in *Exiguobacterium sibiricum* (ESR) was a lysine, however, ESR had the proton pumping activity over a wide pH range and fast turnover photocycle. This indicates that the existence of a carboxylic residue in the cytoplasmic domain is not necessary for proton pumping. In this study, we investigated the effects of the mutation of Glu108 on the photocycle and accompanying proton transfer in PR.

2P236 *Acetabularia* rhodopsin II (ARII)の Asp81 変異体による一過性光誘起電流

Transient photo-induced current by Asp81 mutants of *Acetabularia* rhodopsin II (ARII) heterologously expressed in *Xenopus laevis* oocytes

Seiji Miyauchi[1], Kazumi Shimono[1], Takashi Kikukawa[2], Makoto Demura[2], Jung Kwang-Hwan[3], Naoki Kamo[2] ([1]*Toho Univ., Grad. Sch. Pharm. Sci.*, [2]*Hokkaido Univ., Grad. Sch. Life Sci.*, [3]*Sogang Univ., Inst. Biol. Interfaces*)

ARII is a light-driven proton pump rhodopsin. Asp81 in ARII corresponding to Asp85 in baceriorhodopsin is considered to function as a proton acceptor from the protonated Schiff base.
We here determined how this proton acceptor coordinates with the protonated Schiff base and other amino acid residues to pump out a proton. The photo-induced current by D81S mutant exhibited transient; the light illumination induced the transient inward-current, whereas the turn-off of the light illumination induced the outward transient current. These transient currents remained unchanged irrespective of extracullular ions, Na^+ and Cl^-, implying that the transient currents might be due to an intracellular circulation of a certain ion.

2P237 Low-temperature FTIR spectroscopy of the Light-driven sodium ion pump: Krokinobacter eikastus rhodopsin 2

Faisal Hammad Mekky Koua[1], Rei Abe-Yoshizumi[2], Hikaru Ono[2], Shota Ito[2], Yoshitaka Kato[2], Keiichi Inoue[1,2], Hideki Kandori[1,2] ([1]*OptoBioTech. Res. Cent., Nagoya Inst. Tech.*, [2]*Dept. Front. Mat., Nagoya Inst. Tech.*)

Krokinobacter eikastus rhodopsin 2 (KR2) is a newly discovered light-driven compatible sodium ion-proton pump[1]. KR2 pumps Na+ and Li+, while turning into proton pump in the presence of K+ or larger cations. The Na+ ion pumping activity has been shown to be completely suppressed at acidic pH and for D116N, the retinal Schiff base counterion mutant. To understand the mechanism behind this, we applied low-temperature light-induced difference FTIR[2] and UV-vis spectroscopy at a range of pH for the wild type KR2 and the D116N mutant. We discuss the possible mechanisms of the light-driven Na+ ion pumping based on the analysis for these proteins and other mutants.
[1] Inoue, K et al. *Nat. Commun.* 4, 1678-1687 (2013).
[2] Ono, H et al. *J. Phys. Chem. B* 118, 4784-4792 (2014).

2P238 ロドプシンの低い熱活性化頻度の分子メカニズム

Molecular mechanism of the low thermal activation rate of rhodopsin

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Most vertebrate retinas contain two types of photoreceptor cells, rods and cones, which are responsible for scotopic and photopic vision, respectively. Because rods show a low threshold of photon detection and high sensitivity, the low thermal activation rate of rhodopsin, rod visual pigment, is essential for the function of rods. Phylogenetic analysis indicates that rhodopsin has evolved out of cone visual pigment, but it remains unknown how rhodopsin acquired the low thermal activation rate in the molecular evolution. We estimated the thermal activation rates of rhodopsin, cone visual pigments and their mutants by biochemical and spectroscopic measurements. Based on our results, we discuss the molecular mechanism of the low thermal activation rate of rhodopsin.

2P239 ロドプシン構成的活性変異体 M257Y における構造平衡の一分子観測

Single-molecule observation of the conformational equilibrium in constitutively active mutant of rhodopsin, M257Y

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Rhodopsin, a G-protein coupled receptor, has retinal as a ligand and the ligand-free state opsin scarcely activates G-protein. Previous studies have shown that opsin state of constitutively active mutant (CAM) activates G-protein much effectively than that of wild type but the mechanism has been unclear. We labeled M257Y (a typical CAM) with Alexa594 fluorophore and monitored the conformational changes directly by single-molecule measurements using TIRFM. We compared conformational dynamics in the dark, photoactivated, and opsin states of M257Y and wild type rhodopsin, and found that conformational changes occurred more frequently in M257Y opsin than wild type opsin. The frequent generation of active state would account for the constitutive activity of opsin.

2P240 In situ 光照射固体 NMR による光受容膜タンパク質センサリーロドプシン I の光反応過程の解析

Photocycle of sensory rhodopsin I as revealed by in situ photo irradiation solid-state NMR

Yoshiteru Makino[1], Hiroki Yomoda[1], Yuya Tomonaga[1], Tetsurou Hidaka[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Yuki Sudo[3], Naoki Kamo[4], Akira Naito[1] ([1]*Grad. Sch. Eng, Yokohama Natl Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Grad. Sch. Pharm, Okayama Univ.*, [4]*Grad. Sch. Life Sci , Hokkaido Univ.*)

Salinibactor ruber Sensory rhodopsin I (*Sr*SRI) is a photoreceptor membrane protein with a retinal chromophore, and has a dual function as positive and negative phototaxis. To characterize photo-intermediates in the photocycle of *Sr*SRI, configuration change of [20-^{13}C] retinal was distinctly observed by *in situ* photo irradiation solid state NMR [1]. During green and light irradiation, the retinal changed from all-*trans* to 13-*cis*, indicating that M-intermediate (attractant) was stationary trapped. M-intermediate changed to P-intermediate (repellent) with protonated Schiff base by illumination with UV-light. In conclution, the dual function in *Sr*SRI is induced by the color-discriminating isomerization of retinal. [1]Y. Makino et al.(2014) *Angew. Chem. Int. Ed. in press*

2P241 アナベナセンサリーロドプシンの細胞質側で生じる光誘起プロトン移動反応の解析

Light-induced proton transfer reactions at the cytoplasmic half channel of *Anabaena* sensory rhodopsin

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The photocycle of *Anabaena* sensory rhodopsin (ASR), which is the photoreceptor for the cyanobacterial chromatic adaptation, was examined by focusing on the H^+ transfer reactions. Photoinduced deprotonation of the Schiff base induces the formation of M intermediate. However, the M formation of ASR was accompanied with the H^+ uptake from the medium. This indicates the contributions of two residues: a H^+-accepting residue from the Schiff base and a H^+-capturing residue from the medium. The mutation experiments suggested that these residues are assigned to Asp217 and Glu36, respectively, and their protonation states influence each other. We will discuss the relationship between the H^+ transfer reactions and the conformational change of ASR.

2P242 海洋性細菌に含まれる光駆動クロライドポンプの分光研究

Spectroscopic study of light-driven chloride pump from marine bacteria

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Thousands of microbial rhodopsins have been recently found from marine bacteria. Most of them are light-driven proton pumps, possessing the DTE motif at the corresponding positions of D85, T89 and D96 in bacteriorhodopsin. In contrast, some rhodopsins contain the NDQ or NTQ motif, which functions as light-driven sodium or chloride pump, respectively. The latter is evolutionally far different from halorhodopsin, well-known chloride pump in Halophilic Archaea. Here we present spectroscopic study of the new chloride pump. The results are compared with those of halorhodopsin, and mutant proteins are also used in this study. Molecular mechanism of uni-directional chloride transport will be discussed based on the present results.

2P243 チャネルロドプシンの吸収波長に関する量子化学的研究

Quantum chemical study of the absorption maximum of channelrhodopsin

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Herein, the absorption maximum of channelrhodopsin (ChRh) is calculated using our recently developed method in which the whole protein can be treated quantum mechanically at the level of INDO/S-CIS. This calculation successfully reproduced the so-called opsin shift of ChRh. We also applied the same calculation for different mutants, each of which was constructed by replacing any one of the amino acid residues of the wild-type ChRh with Gly. This substitution made it possible to elucidate the extent to which each amino acid contributes to the opsin shift and to estimate the inter-residue synergistic effect. Based on these data, we discuss the spectral tuning mechanism in ChRh.

2P244 固体 ^{13}C NMR によるファラオニスフォボロドプシンの機能に重要な Tyr 残基の構造解析

Conformation of functionally important Tyr residues in *pharaonis* phoborhodopsin as studied by Solid-State ^{13}C NMR

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Pharaonis phoborhodopsin(*p*pR) has 7 transmembrane α-helices with a retinal. Tyr174 and Tyr199 residues in *p*pR are important for the signal transduction from *p*pR to *p*HtrII [1]. Here we present a Solid-State ^{13}C NMR spectra of [15,20-^{13}C]retinal, [ζ-^{13}C]Tyr and [ε-^{13}C]Lys in *p*pR, T204A and D75N incorporated in the EggPC lipid bilayer. We observed each of the correlated peaks of Cζ-Tyr174 with retinal by proton-driven spin diffusion 2D NMR experiments. Besides, we successfully assigned ^{13}C chemical shift of Cζ-Tyr199. These site-specific chemical shifts have revealed interesting information on the Tyr conformation. We will discuss about retinal-protein and *p*pR-*p*HtrII interaction by comparing the structure with its mutant. [1]Y. Sudo & J.L. Spudich(2006) *PNAS*. 103, 16129.

2P245 サル青感受性視物質の赤外分光解析

FTIR study of monkey blue-sensitive visual pigment

Yuki Nonaka[1], Kota Katayama[1], Kei Tsutsui[2], Hiroo Imai[2], Hideki Kandori[1] ([1]*Nagoya Institute of Technology*, [2]*Primate Research Institute, Kyoto University*)

Primates including human have three types of color visual pigments; blue, green, and red. Although these pigments contain the same chromophore molecule, 11-cis-retinal, different chromophore-protein interactions allow absorption of different colors. In color tuning, three factors are generally considered; (i) chromophore distortion, (ii) interaction between the protonated Schiff base and the counterion, (iii) polarity around the chromophore.

In this study, structural basis of color tuning in monkey blue-sensitive visual pigment was examined by light-induced FTIR spectroscopy. The color tuning mechanism of blue pigment will be discussed based on the FTIR spectral comparison with the other visual pigments.

2P246 In-situ 光照射固体 NMR によるバクテリオロドプシンの光励起過程における局所構造変化の解析

Structural changes in the photoexcited process in retinal of Bacteriorhodopsin studied by in-situ photoirradiation solid-state NMR

Arisu Shigeta[1], Ryota Miyasa[1], Miyako Horigome[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Satoru Tuzi[3], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Univ. Hyogo*)

Bactriorhodopsin (BR) is an seven-helical membrane protein that functions as a light-driven proton pump and has retinal chromophore which forms two different configurations of all-trans (AT) and 13-*cis*, 15-*syn* (CS) with 1:1 ratio in the dark state, and changes to ~100% AT under photoirradiation at 20°C. Using in-situ photoirradiation SS-NMR and [1-^{13}C]Tyr-, [20-^{13}C]Retinal-BR, CS-like intermediate and N intermediate were trapped under green light illumination at -20°C. Large structural changes between dark and light state were observed not only in retinal configuration but also in protein structure. These results gain insight into an essential role of CS state in proton pump function. At the presentation, trapping yield of N intermediate will be displayed.

2P247 *In-situ* 光照射固体 NMR によるバクテリオロドプシン Y185F 変異体に捕捉された O-中間体の評価

Characterization of O-like intermediate trapped in Y185F mutant in Bacteriorhodopsin by *in-situ* photo-irradiation solid-state NMR

Kyosuke Oshima[1], Arisu Shigeta[1], Yoshiteru Makino[1], Izuru Kawamura[1], Takashi Okitsu[2], Akimori Wada[2], Satoru Tuzi[3], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Kobe Pharm. Univ.*, [3]*Univ. Hyogo*)

Bacteriorhodopsin (bR) in a purple membrane of *H. salinarum* shows a light-driven proton pump activity under photo-irradiation. In the photocycle, it is difficult to trap O intermediate and hence its detailed structure has not revealed yet. Here, we demonstrate that the O-like intermediate in Y185F mutant can be stationary trapped by *in-situ* photo-irradiation solid-state NMR. In the dark adapted state, ^{13}C NMR spectrum of [15, 20-^{13}C]Ret-[1-^{13}C]Tyr-Y185F-bR indicates that the ratio of 13-cis and All-trans retinals is about 4:1. Under photo-irradiation with green light, both N and O intermediates were distinctly observed in the ^{13}C NMR spectrum. Subsequently, the N-intermediate transformed to the O-intermediate under the dark condition.

2P248 光依存転写因子オーレオクロム 1 の反応ダイナミクス

Reaction Dynamics of Light Dependent Transcription Factor Aureochrome-1

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Aureo1 is a blue light dependent transcriptional factor which has two domains; photoreceptive LOV domain and DNA binding bZIP domain. Aureo1 exists as dimer in vitro by forming disulfide-linkages and binds to DNA both in the light and dark states. In vivo, however, it has recently been supposed that the Aureo1 can exist as monomer and light dependent dimerization should be relevant for the DNA binding. In order to clarify the reaction of monomeric Aureo1, we constructed C162S/C182S mutant and studied its reaction dynamics by the transient grating method. Upon photoexcitation, diffusion coefficient (D) decreased significantly by the dimerization at the rate constant k= 2.5 s-1. In the presence of DNA, light dependent DNA binding was observed as a further decrease of D.

2P249 (6-4)光回復酵素による 2 光子 DNA 修復の分子メカニズム

若手招待講演

Molecular mechanism of the two photon DNA repair by the (6-4) photolyase

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UV in sunlight causes formation of crosslinks between two adjacent pyrimidine bases in DNA, namely cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts ((6-4)PPs). Since these lesions lead to mutagenesis and cell death, organisms have developed UV-protecting systems that can remove the UV lesions and restore intact nucleobases. The (6-4) photolyase is a unique flavoenzyme that can repair the (6-4)PP by utilizing blue light. We have recently reported that the repair of the (6-4)PP by the (6-4) photolyase requires two photons. In this study, we investigated the role of the amino acid residues located proximal to the lesion and performed biochemical and spectroscopic studies. Their molecular role in the two-photon DNA repair will be discussed.

2P250 Theoretical study of the electron transfer reaction by DNA photolyase

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The DNA photolyase repairs thymine dimers found in UV-induced DNA lesions by electron transfer reaction. In this work, the molecular mechanism involved in the efficient electron transfer of DNA photolyase was investigated by the analysis of electron tunneling pathways from FADH- to CPD using ab initio and fragment molecular orbital calculations. In particular, we focused on the roles of amino acid residues and crystallographic water molecules in the active site.
According to previous studies, the calculation of electron transfer pathways, considering excited state of FADH- and three amino acids (Gln283, Asn349 and Met353), have not been performed. We computed the electron transfer pathways from FADH- to CPD using ab initio and fragment molecular orbital method.

2P251 光化学系 II 複合体と層状複水酸化物からなるバイオ–無機ハイブリッド電極

Bio-inorganic hybrid water oxidation electrodes of Photosystem II and layered double hydroxide

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We report bio-inorganic hybrid electrodes consisting of Photosystem II (PSII) and layered double hydroxides (LDHs) for visible light-driven water oxidation. PSII is a photosynthetic protein, which catalyzes the oxidation of water to molecular oxygen under visible light irradiation. LDHs are composed of cationic double hydroxide nanosheets and anions placed in the interlayers of the cationic nanosheets. We synthesized PSII-LDH electrodes and recorded their photocurrent in a buffered aqueous solution at pH 6.5 under visible light irradiation. In our system efficient interfacial electron transfer occurred from PSII to LDH, allowing us to experimentally determine turnover numbers of PSII in vitro for the first time.

2P252 光化学系 II における TyrZ - D1/His190 の距離と PCET の関係

Proton-coupled electron transfer and hydrogen-bond distance of TyrZ - D1-His190 in Photosystem II

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Here we show that the geometry of the TyrZ phenol and its environment, likely the Tyr-O---H---Nε-His bonding, are modified in PsbA2-PSII when compared to PsbA(1/3)-PSII of *Thermosynechococcus elongatus*. These results point to the dynamics of the proton coupled electron transfer processes associated with the oxidation of TyrZ being affected. From sequence comparison we propose that the Cys144Pro and Pro173Met substitutions in PsbA2-PSII versus PsbA(1/3)-PSII, respectively located upstream of the α-helix bearing TyrZ and between the 2 α-helices bearing TyrZ and its hydrogen bonded partner, His190, are responsible for these changes.

2P253 光電子収量分光による非真空環境下の生体関連分子の電子構造観察：クロロフィル a 測定の試み

Observation of the electronic structure of bio-related molecule in non-vacuum environment by using photoemission: Trial to Chlorophyll a

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To elucidate various bio-related processes, the electronic structures of key molecules are indispensable. For non-living materials, Photoelectron Spectroscopy (PES) is a powerful technique and widely used to examine their electronic structures. However, PES needs vacuum environment and its application to bio system has been much limited. In this study, we tried to observe the electronic structure of bio-related molecule in non-vacuum environment by using Photoelectron Yield Spectroscopy (PYS) because PYS can be carried out not only in vacuum but also in atmospheric condition. The observed electronic structure of chlorophyll a solution, which is a key molecule in photosynthesis, will be reported.

2P254 光捕集アンテナにおける色素の励起エネルギーの揺らぎに関する理論的研究

Theoretical Studies on Excitation Energy Fluctuations of Pigments in a Light-Harvesting Complex

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Excitation energy fluctuations of pigments in light-harvesting complexes play an important role in the excitation energy transfer dynamics. However, the detailed mechanism is still unknown. The high computational cost of reliable electronic structure calculations for excited states prevents us from carrying out a large number of sampling needed to evaluate the excitation energy fluctuations. To overcome this difficulty, we develop a new method called molecular mechanics with Shepard interpolation corrections (MMSIC), which enable us to generate potential energy surfaces for pigments in light-harvesting complexes efficiently. We illustrate the new method by application to bacteriochlorophyll a pigments in the Fenna-Matthews-Olson complex.

2P255 OEC の Kok-S2 状態の反応活性部位の分子構造に関する B3LYP 計算：Mn4 の配位水分子のプロトン化状態

A B3LYP study on molecular structures of active site at the Kok-S2 state of OEC: protonation states of Mn4-ligated water molecules

Tomoya Ichino, Masaki Mitani, Yasunori Yoshioka (*Grad. Sch. Eng., Univ. Mie*)

The Kok cycle shows that during the catalysis of photosynthetic water oxidation, the oxygen evolving complex passes through five oxidation states (S0 to S4). Using B3LYP method, we determined the molecular structure at the S2 state in doublet, where the two water ligands on Mn4 are H2O molecule (W1) and OH anion (W2). In this work, we found that a Grotthuss-proton transfer (PT) mechanism along a hydrogen bonding network from W1 to Asp61 would thermodynamically proceed from the previous S2 structure. The product, where both of W1 and W2 are OH anions, is energetically lower than the reactant. The oxidation states of (Mn1, Mn2, Mn3, Mn4) = (III, IV, IV, IV) do not change before and after the PT reaction. We will suggest that the product is the Kok-S2 state.

2P256 水分解 Mn_4Ca クラスター S_3 状態に関する理論的研究

Theoretical Study of the S_3 state of the Mn_4Ca-cluster in Photosystem II: A compact chair form consisting of the short Mn-Mn pairs

Makoto Hatakeyama[1], Kouji Ogata[1], Satoshi Yokojima[2], Shinichiro Nakamura[1] ([1]*Wako Inst., Riken*, [2]*Sch. Pharm., Tokyo Univ. Pharm. Life Sci.*)

Mn_4Ca-cluster in photosystem II protein accumulates the oxidizing equivalents and catalyzes the water-splitting reaction of photosynthesis. The cluster shows the structural change when it transits from the S_2 state to the S_3 state in the Kok-cycle. For example, the number of the long (R>3 Å) Mn-Mn or Mn-Ca pairs decreases in the Mn-EXAFS spectra. While, the short (2.7-2.8 Å) Mn-Mn pairs have been found in the spectra. Thus, the cluster consisting of 2.7-2.8 Å Mn-Mn pairs has been expected for S_3 state. We investigated the possible S_3 state cluster by using DFT calculation based on the chair form shown in the crystal structure. We will report the ligand environment of the cluster and the dependence on the deprotonated water (OH^-) site.

2P257 光合成酸素発生中心(PSII-OEC)の立体構造と電荷状態変化についての理論的研究

Theoretical investigation on the conformation-charge relationship of the photosystem II oxygen evolving complex (PSII-OEC)

Mitsuo Shoji[1], Hiroshi Isobe[2], Shusuke Yamanaka[3], Jian-Ren Shen[2], Kizashi Yamaguchi[3] ([1]*Grad. Sch.of Pure & App. Sci., Univ. Tsukuba*, [2]*Grad. Sch. Nat. Sci. & Tec., Okayama Univ.*, [3]*Grad. Sch. Sci, Osaka Univ*)

Photosystem II oxygen evolving complex (OEC) catalyzes a four-electron-oxidation of two water molecules. This reaction is important in photosystem and is one of the most important systems in life. Reaction mechanisms of OEC have attracted considerable attentions, however, the knowledge is still limited for many difficulties. Especially for (1) relationships between the OEC core structures and the Mn charge states and (2) interactions from surrounding amino acid residues, water molecules and protons. In this study, accurate QM/MM calculations were performed by taking a large QM region at the S0, S1 and S2 states.

2P258 Ca 除去と Sr 置換をした Mn クラスターの ENDOR 法による研究

ENDOR studies on Ca depleted and Sr substituted Mn cluster in photosystem II

Hiroki Nagashima[1], Nakajima Yoshiki[2], Jian-Ren Shen[2], Hiroyuki Mino[1] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Grad. Sch. Nat. & Tech., Okayama Univ.*)

Electron nuclear magnetic resonance (ENDOR) was applied to the S_2 state Mn cluster to reveal locations of protons which are not detected in the X-ray crystal structure analysis. Similar ENDOR spectra of the S_2 state spinach membranes and *thermosynechococcus elongatus* were observed and indicating there are no structural difference in two species. ENDOR studies also revealed that the positions of protons were modified by the Ca-depletions and recovered by Sr-substitutions. These results indicates Ca is essential for the hydrogen bonding network.

2P259 Target analysis of the photosystem II-enriched membrane: The effect of oxidizing agent on fluorescence quenching in PSII

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We studied picosecond time-resolved fluorescence spectra of photosystem II (PSII)-enriched membrane at 77 K by a streak-camera system to reveal its light-harvesting dynamics. We globally fitted data by using the target analysis to build up a new compartment model for PSII, which is consistent with a microscopic model based on the structure [Shibata, Y., et al. (2013), JACS]. Excitation was made at 430 nm and 460 nm with different powers to populate different antenna components.
Though there were several studies on the fluorescence quenching in PSII induced by oxidizing chlorophyll Z (ChlZ+) or carotenoids, its mechanism has not been well clarified so far. We will use our compartment model to shed light on the mechanism of the oxidant-induced quenching in PSII.

2P260 超音波に誘発されるケージド化合物の活性化
Ultrasound-induced activation of caged compounds

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Caged compounds are widely used as tracers and activators of biological reactions and their activities can be temporarily masked using a photosensitive moiety. However, photoirradiation is not always successful in clinical applications. Therefore, we attempted to activate caged compounds using ultrasound rather than light. To determine the mechanism of ultrasound-induced activation of CMNB-caged fluorescein, an irradiated product was analyzed by fluorospectrometry and HPLC. As a result, fluorescent unmasked molecules were detected in an ultrasound-irradiated caged compound solution. However, the fluorospectrometric and HPLC profiles of its degradation products were not identical to photoirradiated caged fluorescein.

2P261 ノロウイルス RNA 複製酵素を用いた試験管内 RNA 淘汰実験から、新奇な dsRNA 複製機構が示唆された
A novel dsRNA replication mode was suggested from the *in vitro* RNA selection using Norovirus RNA replicase

Hidenao Arai[1], Koichi Nishigaki[2], Naoto Nemoto[1], Miho Suzuki[1], Yuzuru Husimi[2] ([1]*Grad. Sch. Sci. Eng., Saitama Univ.*, [2]*Professor Emeritus, Saitama Univ.*)

We had confirmed that Norovirus RNA replicase ($NV3D^{pol}$) amplified dsRNA isothermally. In order to identify the optimal 3'-terminal sequence of the RNA template for $NV3D^{pol}$, an *in vitro* selection using the serial transfer was performed for a random library (3'-terminal 4 nts). The population landscape on the 4-dimensional sequence space of the 17^{th} round of transfer gave a main peak around the CAAC. In the batch amplification, the C-stretch was much more effective than the CAAC, but in the serial transfer condition in which the CAAC was sustained stably, the C-stretch was washed out. Based on these results we proposed the existence of the "shuttle mode" replication of dsRNA. We also proposed the optimal terminal sequences of RNA for *in vitro* evolution with $NV3D^{pol}$.

2P262 phi29DNA 複製酵素を使った自己複製系の確立
Establishment of a self-replication system using phi29 DNA polymerase

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Living organisms have replication systems of their genetic information, in which genomic DNA is replicated by its encoded proteins. This self-replication of the genetic information is essential for living system to evolve. To understand how life can evolve, we are attempting to construct a self-replication system in which a DNA encoded polymerase is replicated through translation of the protein. This system was assembled by a circular DNA encoding phi29 DNA polymerase and a cell-free translation system reconstituted from purified proteins. We found phi29 DNA polymerase is translated from the genomic DNA and replicate it. If we add a recursivity to this system, it would be possible to observe the evolution of DNA self-replication system.

2P263 分子動力学計算を用いた β2 アドレナリン受容体と G タンパク質間の 相互作用解析
Structural analysis of interaction between β2 adrenergic receptor and G-protein using molecular dynamics simulation

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The study of the interaction between G-protein coupled receptor (GPCR) and G-protein (Gα) is important to understand the feature of Gα binding selectivity, which cause different downstream phenomenon of GPCR.
Based on the 3D structure (PDB ID: 3sn6) of β_2 adrenergic receptor (β2AR) with G_s, we made several mutated complexes (β2AR with $G_{i/o}$, $G_{q/11}$ or $G_{12/13}$), by comparative modeling, and evaluated behaviors of native or mutated complexes, by using molecular dynamics simulations in vacuum or in implicit water solvent with AMBER99 force field.
It was suggested that the native β2AR - G_s complex is the most stable through 12 ns simulation. This stability was caused by attractive interaction of key residues on C terminal of Gα, on DRY motif of TM3 and on ICL2 of β2AR.

2P264 Global clustering of whole organisms enabled by the GP method

Harshita Sharma[1], Fumihito Ohtani[1], Parmila Kumari[1], Deepti Diwan[1], Miho Suzuki[1], Naoto Nemoto[1], Takuyo Aita[2], Koichi Nishigaki[1] ([1]*Dept. of Functional Materials Science, Graduate School of Science and Engineering, Saitama University*, [2]*Graduate School of Information Science and Technology, Symbiotic Network Design Laboratory, Osaka University*)

In this study, we demonstrate genome profiling (GP)-based global clustering by taking an example of a case with 48 samples from five classes of animalia. The resultant cluster showed well-discriminated clustering of five classes of kingdom animalia. In addition, this cluster showed correspondence to the phenotype-based classification. Furthermore, we constructed a genome sequence space where these 48 samples were separated by their corresponding genome distance with high correlation to Euclidean distance. The results support the effectiveness and universality of genome distance-based approach which does not require a priori knowledge of sequence for analysis and can be ubiquitously applied. To our knowledge, this is the first trial and success of this kind.

2P265 Super-resolution imaging of chromatin domains in living mammalian cells

Tadasu Nozaki[1,2], Tomomi Tani[3], Sachiko Tamura[1], Takeharu Nagai[4], Kazuhiro Maeshima[1] ([1]*Natl. Inst. Genet.*, [2]*Inst. Adv. Biosci., Keio Univ.*, [3]*Marine Biological Laboratory*, [4]*ISIR, Osaka Univ.*)

Genomic DNA must be organized three dimensionally in the cell to utilize genome information. Our recent studies demonstrated the nucleosome fiber is irregularly folded in the cell without the 30-nm chromatin fiber. Using newly developed single nucleosome imaging, we identified numerous compact nucleosome clusters (chromatin domains) in both interphase chromatin and mitotic chromosomes in living mammalian cells. Subsequently, we also observed that nucleosomes dynamically moved around in the chromatin domains. Furthermore, we detected that some chemical perturbations could change the chromatin domains and nucleosome dynamics. These findings provide a novel insight into the genome organization and dynamics in living mammalian cells.

2P266 出芽酵母の核の内側に“転写が不活発な領域”が存在する？ Are there transcriptionally inactive regions localized in a budding yeast nucleus?

Naoko Tokuda, Shin Fujishiro, Masaki Sasai (*Grad. Sch. Engr., Univ. Nagoya*)

3-dimensional structures of genomes have been inferred from Hi-C methods with which the chromosome-conformation-capture method and the next-generation DNA sequencing have been combined (Tanizawa et al., 2012). However, several biases have been reported in the Hi-C data(Yaffe & Tanay, 2011), and the definite way for estimating these biases has not yet been established. We used “HiCNorm”(Hu et al., 2012) to estimate the biases in a Hi-C data of budding yeast, which was measured by Duan et al.(2010). We will discuss whether HiCNorm is an effective method and to what degree the biases affect the genome structure simulation. By using the corrected Hi-C data, we will check whether there are transcriptionally inactive regions as we reported at the last BSJ meeting.

2P267 Computational chromosome conformation sampling of human diploid genome

Shin Fujishiro, Naoko Tokuda, Masaki Sasai (*Grad. Sch. Eng., Univ. Nagoya*)

The chromosome conformation capture techniques offer pairwise contact frequencies of DNA fragments in nucleus, which have been enabled researchers to investigate three-dimensional (3D) structures of chromosomes in interphase nucleus. However, the current experimental methods have a flaw when applied to a diploid genome: 2×2 = 4 distinct contact frequencies for diploid alleles are merged into a single value, which has to be decomposed into 2×2 frequencies to construct a 3D model of genome. In this presentation we propose a computational method which explicitly infers 2×2 contact probabilities for inter-chromosomal contacts and generates a conformation ensemble of human 46 chromosomes, and discuss the possible application of this method.

2P268 相補性に依らないタンパク質—タンパク質ドッキングポーズ予測法 A protein-protein docking prediction method not relying on the shape complementarity

Atsushi Hijikata, Masafumi Shionyu, Tsuyoshi Shirai (*Nagahama Inst. Bio-Sci. Tech.*)

Many proteins in living things function as high-order complexes, thus the 3D structure of the complex at atomic resolution is crucial for fully understanding of its functions. Current protein-protein docking algorithms predominantly rely on the shape complementarity between proteins to make contacts, and it may be problematic if a case forming a complex followed by conformation changes. To tackle this issue, we propose a new method to predict a docking poses of protein pairs, which is not necessarily relying on the shape complementarity, employing a vector-match algorithm of an empirical dataset of amino acid residue pairs in the interaction interface from the Protein Data Bank. We discuss the performance and accuracy of the method with a conventional docking method.

2P269 データベース IDEAL の新機能と機能性天然変性領域の配列・構造比較 The update of the IDEAL database, and sequence and structure comparisons of intrinsically disordered regions

Satoshi Fukuchi[1], Takayuki Mamemiya[2], Shigetaka Sakamoto[3], Yukiko Nobe[2], Yumiko Kado[2], Kazuo Hosoda[1], Ryoutaro Koike[2], Hidekazu Hiroaki[4], Motonori Ota[2] ([1]*Maebashi IT*, [2]*Nagoya Univ. SIS*, [3]*Holonics*, [4]*Nagoya Uni. BMS*)

IDEAL (http://www.ideal.force.cs.is.nagoya-u.ac.jp/IDEAL/) is a collection of intrinsically disordered proteins (IDPs) that cannot take stable globular structures in the physiological conditions. The new IDEAL incorporates the interaction of IDPs and their binding partners more explicitly, and illustrates the protein-protein interaction (PPI) networks and the structures of protein complexes. Redundant experimental data are arranged based on the clustering of Protein Data Bank entries, where similar sequences in a same binding mode are grouped. In this session, I will introduce the new IDEAL functions and some results of sequence/ structure comparisons of IDPs that adopt 3D structures when they bind to their binding partners.

2P270 GGIP : GPCR-GPCR Interaction Pair Predictor

Wataru Nemoto[1,2], Yoshihiro Yamanishi[3], Vachiranee Limviphuvadh[4], Hiroyuki Toh[2] ([1]*Grad. Sch. Sci and Eng., TDU*, [2]*CBRC., AIST*, [3]*MiB., Univ. Kyushu*, [4]*BII., A*STAR*)

Many GPCRs function through mutual interactions. The association of such GPCR pairs with various diseases has been discovered. However, our understandings on the GPCR interactions are far from the whole image due to the lack of the systematic and/or large scale analyses. A tool to accurately predict an interacting pair of GPCRs could accelerate various studies related to the GPCR-GPCR interactions. We have developed a high performance method to predict interacting hetero GPCR pairs by using structures and sequences. Then, we found two predicted GPCR pairs, follicle stimulating hormone receptor-neuromedin B receptor, and gonadotropin releasing hormone receptor-Kiss1 receptor, may be involved in ovarian cancer and idiopathic hypogonadotropic hypogonadism, respectively.

2P271 Re-docking によって正解候補構造が多く得られるタンパク質分子表面の特徴の解析

Analysis of properties of protein-protein interaction surface areas involved in more near-native complexes by Re-docking scheme

Nobuyuki Uchikoga[1], Yuri Matsuzaki[2], Masahito Ohue[3,4], Yutaka Akiyama[2,4], Takatsugu Hirokawa[5] ([1]*Dept. Phys., Chuo Univ.*, [2]*ACLS, Tokyo Tech*, [3]*JSPS Res. Fellow*, [4]*Grad. Sch. Inform. Sci. and Eng., Tokyo Tech.*, [5]*MolProf, AIST*)

To understand mechanisms of protein-protein interactions, we have used rigid-body docking algorithm generating many protein complexes, called decoys. This docking method is popular and useful but with some serious cases of no near-native decoys (NN-decoys). We developed Re-docking scheme, iterating rigid-body docking for generating NN-decoys using Interaction FingerPrints (IFPs). By this scheme, after re-docking process in each interfaces, we could obtain more NN-decoys.
To predict interaction surfaces with NN-decoys, we investigated several parameters of interaction surfaces. In this work, we analyzed interaction surfaces of decoys by IFPs, and will discuss interaction surfaces generating more near-native decoys by re-docking process.

2P272 A Ligand Based Virtual Screening method that takes into account of protein-ligand interactions

Koya Kato, George Chikenji (*Sasai group, Department of Computational Science and Engineering, Graduate School of Engineering, Nagoya University*)

Recently, Virtual screening (VS) are increasingly playing an important role in the early drug discovery stage. There are two main VS approaches; the ligand-based and the structure-based. It is known that both approaches have their own advanteges and weaknesses. Here, we developed a new VS method that combines the both approaches to improve active hit rates. The method is based on the ligand-based technique, in which a candidate compound is compared with one or more active ligand structures using the 3D geometric hashing method. Beside, this method takes into protein-ligand interaction effect. We will report the performance of its method against the DUD as well as the IPAB contest.

2P273 生命システムにおける情報の適応的価値

Fitness Value of Information in Biological Systems

Tetsuya J. Kobayashi[1], Yuki Sughiyama[2] ([1]*IIS, Univ. Tokyo*, [2]*College of Arts and Sciences, Univ. Tokyo*)

Most of cells are equipped with sensory systems that can obtain environmental information, and they change their phenotype depending on the information. The amount of information obtained, therefore, may be linked quantitatively to the gain of evolutionary advantage (fitness), but such quantitative relation has not yet been clarified both experimentally and theoretically.
In this work, by combining information theory and population dynamics, we show a relation between information and fitness. This theoretical result will be a fundamental basis to understand what is relevant information for biological systems and how we can quantify the information.

2P274 Detecting the selection acting on heterogeneous cell phenotypes without environmental perturbation

Takashi Nozoe, Yuichi Wakamoto (*Univ of Tokyo*)

Although the fluctuation of gene expression level in a clonal cell population can be measured at single-cell level, the relation between gene expression level and cellular fitness has still been evaluated essentially by measuring changes of population parameters. Here, we present a practical method to reveal the quantitative relation between historical reproductive rate and “coarse-grained” historical phenotypes from single-cell lineage trees. Then, we quantify the contributions of phenotypic variations to the selection acting on individual histories from those datasets. Such approach would be beneficial because we can evaluate the strength of selection acting on arbitrary measurable phenotypic quantities without any environmental perturbations.

2P275 完全変態昆虫の最適成長スケジュール

Optimal growth schedule of holometabolous insects

Ken-ichi Hironaka[1,2], Yoshihiro Morishita[1] ([1]*RIKEN CDB*, [2]*JSPS Research Fellow*)

Once holometabolous insect larvae pass the critical size, juvenile hormone (JH) titers decline and imaginal discs begin to grow rapidly. The subsequent rise in ecdysteroid titers causes the larva to stop feeding and wander away from its food to find a place to metamorphose. The period between attainment of the critical size and ecdysteroid secretion is called the terminal growth period (TGP). Although all holometabolous insects are thought to have the critical size for metamorphosis, the mechanisms for nutritional regulation of the critical size and the TGP differ between species. To study the ecological causes of this diversity, we develop a resource allocation model and analyze it with the Pontryagin's maximum principle.

2P276 時間依存する出生死滅過程に対する代数的アプローチ

Algebraic approach to time-inhomogeneous birth-death processes

Jun Ohkubo (*Grad. Sch. Informatics, Kyoto Univ.*)

Some analytical solutions for simple time-inhomogeneous birth-death processes are investigated based on an algebraic approach. The algebraic approach is called the Doi-Peliti formalism, and its mathematical structure is similar to quantum mechanics. Although non-commutative treatments are needed, the framework gives us useful theoretical tools and results. Combining the Wei-Norman method, I will mainly discuss analytical results for transition probabilities. Although further investigations are necessary, it would be expected that these algebraic approaches become useful tools for studies of small systems in biological and ecological issues.

2P277 多繊毛細胞における繊毛の空間配向秩序のモデル化

Modeling of spatial distribution and orientational order of cilia mediated by multi-ciliated cells

Hironobu NOGUCHI[1], Shuji ISHIHARA[2] ([1]*Graduate School of Arts and Sciences, the University of Tokyo*, [2]*Department of Physics, School of Science and Technology, Meiji University*)

Multi-ciliated cells make a regulated flow by cooperatively moving their cilia. Recent study have reported that the spatially and orientationally well-arranged distribution of cilium in a cell is crucial to the correct motions and that microtubules connected to the basal foot of each cilia may help to organize this distribution.
To verify this hypothesis, we performed a numerical study, modeling the collective motions of the basal bodies which generate microtubules from their basal feet as those of simple particles acting as anchors binding two microtubules of the same length with a fixed angle. The MD like simulations showed that four characteristic spatio-temporal patterns emerged depending on the density of the basal bodies and the angular parameter.

2P278 マイクロアレイデータに基づく植物の遺伝子発現揺らぎと機能の関係

Analysis of between gene fluctuation and function of plants based on microarray data

Kodai Hirao[1], Atsushi Nagano[2] ([1]*Dept. of Mathematical and Life Sciences, Hiroshima Univ.*, [2]*Center for Ecological Research, Kyoto Univ.*)

Fluctuations of gene expression levels among individuals (phenotype fluctuation) are often observed in several organisms even if they have completely the same genome and live in the same environmental conditions.
Although some potential roles of such fluctuations are suggested, the meanings of the fluctuations of gene expressions have not yet been clarified. In this presentation, we analyze the microarray data of some plants to clarify the meanings of the fluctuation of gene expressions. We found the magnitude of the phenotype fluctuations depend on the function of genes where stress related genes tend to exhibit large fluctuations. We also found the high positive correlations between such fluctuation and response against the environmental variations.

2P279 化学反応における少数性効果の理論解析

Mathematical Analysis of Small Number Effect in Biochemical Reactions

Nen Saito, Yuki Sughiyama, Kunihiko Kaneko (*Grad. Sch. Art. Sci., Univ. Tokyo*)

Biochemical reactions are typically described by a set of deterministic ordinary differential equations under implicit assumptions that concentration of molecules is continuous and fluctuations in concentration are negligible. However, stochasticity in the reaction due to fluctuation in the number of reactants is non-negligible, especially when the number of molecules is small. Such stochasticity can introduce a qualitative change in the behavior of the reaction dynamics, which is referred to as "small number effect".
In this talk, I discuss what kind of “motif” of chemical reaction network can give rise to the small number effects, and present a mathematical description of this phenomenon. Biological relevance of the small number effect is also discussed.

2P280 人工遺伝子回路における下流レポーター遺伝子の影響

Effects of downstream reporter genes on synthetic genetic circuits

Takefumi Moriya[1], Masayuki Yamamura[1], Daisuke Kiga[1,2] ([1]*Tokyo Institute of Technology, Department of Coputational Intelligence and Systems Science*, [2]*Tokyo Institute of Technology, Earth-Life Science Institute*)

In order to understand and regulate complex genetic networks in living cells, it is important to build simple and well-defined genetic circuits. We designed such circuits using a synthetic biology approach that included mathematical modeling, simulation, and microscopic experiments with a focus on the effects by which downstream reporter genes are involved in the regulation of synthetic genetic circuits.
Our findings regarding the effects of downstream genes on regulatory genes and the role of impedance in driving large-scale and complex genetic circuits may facilitate the design of more accurate genetic circuits. This design will have wide applications in future studies of systems and synthetic biology.

2P281 類似分子識別機構の数理モデル

Mathematical modeling of molecular discrimination system

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Discrimination of molecules is a necessary function of all kinds of intracellular phenomena. Among others DNA replication, protein synthesis and T cell antigen discrimination can detect and amplify small difference between right and wrong molecules. Hopfield and Ninio introduced kinetic proofreading mechanism to show that accurate discrimination can be achieved by the non-equilibrium systems. From this point of view, we propose a minimum model for molecular discrimination using a non-equilibrium reaction network. This model, focusing on T cells antigen discrimination, can reproduce several features of T cell behavior. We investigate the underlying mechanism of discrimination of our model.

2P282 油中水滴による非平衡な人工細胞システム

Nonequilibrium artificial cell system based on water-in-oil microdroplet

Masahiro Takinoue[1,2], Haruka Sugiura[1], Hiroyuki Kitahata[3], Yoshihito Mori[4] ([1]*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*, [2]*PRESTO, JST*, [3]*Dept. Phys., Chiba Univ.*, [4]*Dept. Chem., Ochanomizu Univ.*)

In recent years, artificial cells as physical models of living cells have been proposed to study essential dynamic mechanisms of life systems. However, sustained dynamic nonequilibrium phenomena such as nonlinear chemical oscillations have not been achieved because of closed nature of the artificial cells. In this study, we propose an artificial cell with chemically open nonequilibrium condition. The nonequilibrium is achieved by the control of water-in-oil microdroplets, and is kept by the controlled influx and dissipation of chemicals. In this presentation, we report theoretical analyses and experimental demonstrations. We believe that this system will help the study of cell-sized dynamic chemical systems in future.

2P283 細胞濃度制御のためのマイクロ流体ケモスタット
Microfluidic chemostat for cell density control

Manami Ito[1], Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Institute of Technology*, [2]*PRESTO, JST*)

Recently, synthetic biology based on constructive approaches, such as genetically engineering of living cells, has attracted attention. For genetic engineering, culturing living cells in a steady condition is required, and chemostats based on inflow and outflow of culture media are often used. However, it is difficult to observe a cell while culturing because chemostats require large volume of media (>0.1 L-) . In this study, to conquer this problem, we propose a microfluidic chemostat based on water-in-oil microdroplets. The inflow and outflow of media are controlled by droplet fusion and fission. We formulated a numerical model and investigated it by simulations. We believe that this chemostat can be applied to single-cell observation and promote synthetic biology.

2P284 微小液滴を用いた非線形化学反応間の相互作用
Interaction among nonlinear chemical reactions based on microdroplets

Tomoya Okuaki[1], Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology*, [2]*PRESTO, JST*)

The interaction among cells plays an important role in collective behavior of cells. Especially, interaction between cells processing information like neural cells is asymmetric, and the asymmetry of their interaction is considered to be essential. Although the interactions between cells have been theoretically and experimentally studied, the effects of asymmetric interaction have not been revealed yet. Here, to study asymmetric interactions, we propose a system based on microdroplets that contain a nonlinear chemical reaction. In this system, the interaction between droplets is controlled by fusion and fission of droplets. We believe that our system will help to understand the complex behavior exhibited by cells processing information.

2P285 自律的に駆動する複雑形状粒子の並進、回転、円運動
Translational, rational, circular motions of self-driven complex-shaped microparticles

Masayuki Hayakawa[1], Hiroaki Onoe[2], Ken H. Nagai[3], Masahiro Takinoue[1,4] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Dept. of Mech. Eng., Keio University*, [3]*School of Materials Science, JAIST*, [4]*PRESTO, JST*)

Microorganisms produce their autonomous motions based on chemical-mechanical energy conversion with their motor proteins. Recently, micrometer-sized self-driven particles using chemical energy have received attention because they provide interesting physical insights about dynamical structures of microorganisms such as bioconvection, colony formation, etc. However, existing self-driven particles show only translational motion due to their simple shapes (ex. sphere, rod, etc.). Here, we report anisotropic complex-shaped self-driven particles exhibiting translational, rotational, and circular motions. We hope our particles will provide many insights for understanding of autonomous motion and micron-sized dynamical structures based on chemical-mechanical energy conversion.

2P286 The analysis of energy transfer in Chaotic Dynamical Systems 2

Mami Kushida[1], Kana Fuji[1], Mikito Toda[2], Hiroshi Fujisaki[3] ([1]*Grad., Univ. Nara-wu.*, [2]*Univ. Nara-wu.*, [3]*NMS*)

We propose a new method for analysis of the mechanism of the energy transfer in systems of large degrees of freedom including proteins.
It is a combination of the wavelet transformation and PCA and is called the wavelet PCA.
We have applied the wavelet PCA to the Fermi-Pasta-Ulam（FPU）coupled oscillator system.
In addition, we study a coarse graining model of biomolecules called the elastic network model using our method.
We will also think of another method for the analysis of the energy transfer mechanism in these sysrems.

2P287 イメージングバイオマーカーを用いた標的細胞検出のためのオンチップマルチイメージングセルソータシステムの認識アルゴリズム開発
Development of the cell imaging biomarker identification algorism for on-chip multi imaging cell sorter system

Masao Odaka[1], Hyonchol Kim[1], Mathias Girault[1], Akihiro Hattori[1], Hideyuki Terazono[1,2], Kenji Matsuura[1], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental University*)

We have developed an on-chip multi imaging cell sorter system to identify and purify target cells based on their morphological characteristics, which we call “imaging biomarker”. In this study, we adopted an algorism for typical parameters such as cell area, perimeter, and nucleus area were evaluated to identify characteristic target cells, which express "imaging biomarkers". In result, target cells can be successfully identified and purified using those imaging biomarkers, and by using the system, clustered circulating tumor cells were purified from a sample blood as an example. These results indicate the developed algorism is useful for identification of target cells having characteristic morphologies depending on imaging biomarkers.

2P288 標的細胞特定のためのイメージングバイオマーカー：血中循環がん細胞クラスター同定の例
Imaging biomarkers for identification of target cells: Identification of clustered circulating tumor cells as an example

Hyonchol Kim[1], Hideyuki Terazono[1,2], Akihiro Hattori[1], Masao Odaka[1], Mathias Girault[1], Kenji Yasuda[1,2] ([1]*KAST*, [2]*Inst. Biomat. Bioeng., Tokyo Med. Dent. Univ*)

An on-chip multi-imaging cell sorter was developed to purify target cells using "imaging biomarkers", which are indexes for image information of cell morphology including cell clustering and the cluster size. One advantage for using imaging biomarkers is overcoming undesired cell contamination by removing clustered cells. Another is achievement of the collection of clustered samples without any dye staining. The formation of cell clusters is important information for diseases, because blood immune system responds to antigens and forms their clusters. We applied it for the identification of circulating tumor cells (CTCs) without any labeling, and successfully identified them from model rat bloods. These results indicated the usefulness of the imaging biomarkers.

2P289 Optimization of the cell encapsulation in the water in oil droplet using 3D printed object

Mathias Girault[1], Akihiro Hattori[1], Hyonchol Kim[1], Kenji Matsuura[1], Masao Odaka[1], Yumi Mikami[1], Hideyuki Terazono[1,2], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental University*)

Maintain a constant single cell encapsulation is a key factor to perform reliable experiments for single cell level without any contamination of other heterogeneous phenotypes in the results. Although the water droplet formation exploiting sedimentation mechanism is one of the most powerful methods to create an isolated single cell-handling environment, it can significantly decrease the efficiency of cell encapsulation. To improve the efficiency, we applied a passive method using a three-dimensional (3D) microfluidic structure. This method maintains the single cell encapsulation during 80 min at a nearly constant rate without any modification of the sample composition. Moreover, 3D microstructure had no significant effect on the growth rate of cells.

2P290 Single particle detection of influenza virus by micro droplet array

Shuho Kidokoro[1], Kazuhito V. Tabata[1,2], Hiroyuki Noji[1] ([1]*Department of Applied Chemistry, Graduate School of Engineering, The University of Tokyo*, [2]*PRESTO, JST*)

Detecting influenza infection in early stage is important for early medical treatment.
However, current immunochromatography (IC) method, which is widely used in clinic, could detect only after getting fever. In order to detect, over 10^3 - 10^5 pfu virus (virus per mL) is needed.
In order to improve sensitivity, we attempted to develop detection method of influenza at single particle level.
Single virus particle detection is achieved by combination of fluorescent bio-assay and micro droplet array.
The limit of detection (LOD) of this method reaches several 10^2 pfu. This value is 10 to 1000 times more sensitive than that of conventional IC method.
The detail of result will be reported in the annual meeting.

2P291 水中に発生させたレーザー励起キャビテーションバブルの高速温度場イメージング
Fast temperature measurement following single laser-induced cavitation inside a microfluidic gap

Madoka Suzuki[1,2], Pedro A. Quinto-Su[3], Claus-Dieter Ohl[4] ([1]*WABIOS, Singapore*, [2]*Org. Univ. Res. Initiatives, Waseda Univ.*, [3]*ICN, UNAM, Mexico*, [4]*SPMS, NTU, Singapore*)

Laser-induced transient microbubbles are routinely utilized in biomedical applications like selective destruction and membrane permeabilization of cells. Here we study the residual heating following the collapse of a single laser-induced microbubble (maximum radius ~ 10 - 35 μm) using non-invasive fluorescence thermometry combined with high speed imaging at up to 90,000 frames per second. We measure the evolution of the spatial temperature profile and find moderate (< 12.8°C), localized (< 15 μm) and short lived (< 1.3 ms) temperature rises. Extracted thermal diffusivities are explained with 3 dimensional temperature distributions affected by jetting and bubble migration that depend on the geometry of the fluid environment.

2P292 振動和周波検出赤外超解像顕微鏡法による毛髪試料の分子構造解析
Molecular structural analysis of human hair samples by VSFG detected IR super-resolution microscopy

Makoto Sakai[1], **Yukihisa Watase**[2], Kohei Ushio[1], Haruki Ishikawa[2], Masaaki Fujii[1], Shinobu Nagase[3], Takashi Itou[3] ([1]*Tokyo Institute of Technology*, [2]*Kitasato University*, [3]*Kao Corporation*)

We have developed a vibrational sum-frequency generation (VSFG) detected IR super-resolution microscope with a sub-micrometer spatial resolution. In this study, we applied this microscope to human hair samples. Human hair is well-known to compose of medulla, cortex, and cuticle regions from inside to outside. At the cortex area, the α-helical keratin protein forms a line in the shape of fiber along the longitudinal direction of human hair, and controls physical properties such as flexibility, strength, softness and curliness. To understand the relationship between physical properties and the internal nanostructure, we attempted IR super-resolution imaging in the 6-9 μm region. In the presentation, the internal nanostructure of human hair will be discussed in detail.

2P293 筋芽細胞のインパルス応答特性とひずみエネルギ計測
Measurements of impuls responce and strein energy for a single myoblast

Takayuki Hoshino, Yuki Miyazako, Akira Wagatsuma, Kuihiko Mabuchi (*IPC, UTokyo*)

Mechanical responce of myoblast C2C12 was evaluated using inverted electron beam lithgraphy. The mechanical strein energy of the myoblast was measured from an impuls responce of which input was applied by 120-nm focused EB spot on focal adhesion.

2P294 マイクロ電極アレイ上における非拍動性単一細胞のインピーダンスベースによる電気生理学的解析
Non-firing impedance-based electrophysiological analysis of single cells on micro-electrode arrays

Kenji Matsuura[1], Akihiro Hattori[1], Fumimasa Nomura[2], Hideyuki Terazono[2], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental Univ.*)

In recent years, studies on electrophysiological analysis of cells have been progressed by the development of non-invasive multi-microelectrode array (MEAs) measurement technologies. In particular, experiment of effect of drugs to the cells have been promoted vigorously. However, the conventional electrophysiological measurement is based on the firing-analysis based measurement, and especially, non-firing cells does not suite for the conventional electrophysiological measurements. Hence, to overcome this limitation, we examined the effectiveness of the impedance-based measurement of cells on the multi-microelectrode arrays. In the meeting, we introduce the set-up of the impedance measurement system and also results of the measurements of cells.

2P295 a-Si:H 光機能制御可能な生体分子固体電解質によるバイオセンサ

Biosensor using electrochemical biomolecular element photo-controlled on hydrogenated amorphous silicon film

Yutaka Tsujiuchi[1], Hiroshi Masumoto[2], Takashi Goto[3] ([1]*Dept of Mat Sci & Eng, Akita Univ*, [2]*FRIS, Tohoku Univ*, [3]*Inst for Mat Res, Tohoku Univ*)

For creation of elements coated with multi-layer functional thin-films using a combination of different technologies, such as the rectification property of ionic conduction, photo-induction control, and the use of wavelength conversion materials, ion conductive behavior of neutral, acidic, or basic amino acid dispersed in gel are able to be controlled by thickness of hydrogenated amorphous silicon (a-Si:H) film and hydrogen concentration in preparation of the film. We have conducted an advanced attempt of reproduction of sensor performance by supplying small amount of water into 99% removed element which is capable of storing for long period.

2P296 時間イメージ相関分光法によるダイナミック生細胞内オルガネラ解析

Live cell analysis of organelle dynamics using temporal image correlation spectroscopy

Yasuo Takahashi, Isao Sakane (*Olympus Corporation*)

Temporal image correlation spectroscopy (TICS) is an imaging variant of FCS that measures the dynamics of fluorescently labeled molecules by extracting the temporal fluctuations from microscopic images. There have been several studies that employed TICS to analyze the dynamics of subcellular structures such as endosomes and mitochondria.
In this report, we discuss the capability of TICS to measure large subcellular structures such as mitochondria using experimental images of mitochondria acquired from live cells of different origins and cells that are treated with nocodazole. Analysis results of experimental images will be discussed with regard to the analysis of computer-simulated images.

2P297 マウス耳介内がん細胞およびがん組織の非侵襲イメージング

Noninvasive *in vivo* imaging of tumor cells and tissue in mouse auricles

Sayaka Kita (*Dept. of Physics, University of Tokyo*)

In the previous studies, we performed *in vivo* imaging of tumor surgically exposed by a dissection of skin and epithelium, resulting in lack of oxygen and nutrient supply. In order to avoid such deficient factors, we developed new noninvasive imaging methods.
We injected five kinds of human cancer cell lines into the mouse ear auricle, which is very thin (~150-200μm) and accessible to visualize cells without dissection. Tumor was successfully formed using each cell lines. Also, we could successfully perform a real-time observation of the breast cancer cells and tissue with GFP fluorescence in noninvasive condition using confocal microscope.
We developed methods of preparing xenograft model and imaging tumor cells noninvasively in auricles of SCID mice.

2P298 炎症反応抑制タンパク質 PDLIM2 の活性化分子機構の解明

The elucidation of the molecular mechanism of PDLIM2 activation

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PDLIM2 is an E3 ubiquitin ligase which promotes NF-κB degradation in the nucleus to suppress the excessive inflammation. However, the mechanism how PDLIM2 is activated remains unclear. Previously, we observed translocation of GFP fusion proteins of PDLIM2 from the cytoplasm to the nucleus upon stimulation. In this study, aiming to elucidate the activation mechanism of PDLIM2, we analyzed the localization and the function of serine phosphorylation mutants of PDLIM2. The results of a reporter assay suggest an important role of serine 72 phosphorylation in PDLIM2 activation. In addition, we narrowed down the candidates of the kinase of PDLIM2 by analyzing the effect of phosphorylation inhibitors.

2P299 超解像光学顕微鏡の動画から見積もったアクチンのダイナミクス

Dynamics of actin and actin assoicate proteins estimated from superresolution image data

Kaoru Katoh[1,2], Saori Mimatsu[1,2], Minami Tanaka[1,2] ([1]*Biomed RI, AIST*, [2]*Grad. Sch. Life & Enviromental Sci.*)

Super-resolution microscopes reveal fine structures smaller than resolution limits of conventional optical microscopes. We used super-resolution to observe fine structures of growth cone.
Growth cone is an enlargement of thin cytoplasm at the tip of growing neurite. The growth cone crawls about and finds path of neuronal elongation. Their movements depend on the dynamics of actin filaments (actin meshwork) which are too small to observe with conventional optical microscope.
Here, we recorded dynamics of actin meshwork in the growth cones as superresolution movies. Polymerization rate and other parameters of actin meshwork were estimated from the movie and were compared with previous ones. We will also discuss about the dynamics of actin associate proteins.

2P300 2 軸クライオ電子線トモグラフィーと光学顕微鏡同視野観察を用いた無傷細胞の 3D-イメージングへの挑戦

The challenge to intact cell 3D-imaging by dual-axis Cryo-electron tomography and correlative light imaging

Ruriko Ogawa[1], Takako M. Ichinose[1], Rina Nagai[1], Kazuhiro Aoyama[2,3], **Atsuko H. Iwane**[1,2] ([1]*Cell Field Struc., QBiC, Riken*, [2]*Spec. Res. Promot. Group, Grad. Sch. Fronti. Biosci., Osaka Univ.*, [3]*Application Lab., FEI JAPAN*)

Cryo-EM tomography of intact cells can be expected to reveal the spatial arrangements of key proteins and complexes during intracellular signaling and mechanical events. In this meeting, we describe the 3D-imaging of intact organelles by Cryo-EM tomography using the Titan Krios EM and HAADEF detector. Living cells were grown directly on Quantifoil and labeled by organelle specific fluorescent dye and then were done vitrification. To obtain a clearer structural model cells were dual-axially imaged over an angular range from -65 to 65 degrees. Fluorescent-labeled organelles in the cell were correlatively observed using light microscopy at just before and after vitrification. At future, our system can provide any new information about many kinds of organelles.

2P301 FIB（Focused Ion Beam: 集束イオンビーム加工）-SEM による全細胞レベル、ナノスケール分解能での細胞周期の可視化
Visualization of cell cycle by three-dimensional FIB-SEM with nanoscale resolution at whole cell level

Rina Nagai[1], Keisuke Ohota[1,2], Takako M. Ichinose[1], Akinobu Togo[2], Atsuko H. Iwane[1,3] ([1]*Cell Field Struc., QBiC, Riken*, [2]*Anatomy, Med., Kurume Univ.*, [3]*Spec. Res. Promot. Group, Grad. Sch. Fronti. Biosci., Osaka Univ.*)

For whole single cell 3D-structure analysis, we are using some technologies including (1) Cryo-Tomography, (2) FIB-SEM, etc. A new biological application of FIB-SEM for the 3D reconstruction of an entire cell at a nanoscale resolution that lies between those of EM tomography and X-ray tomography.
Last annual meeting, we presented the base 3D model of *C. merolae* , which is thinking as the primitive unicellular red algae using FIB-SEM. Our system could image simple individual double or single membrane organelles like nucleus, chloroplasts, mitochondria, ER and Golgi inside *C. merolae* of 2-5 μm in length. Using synchronizing cells, we would like to present the typical 3D-structural models against each cell division stage at whole cell level in this meeting.

2P302 1 型リアノジン受容体 N 末領域における悪性高熱症に関わる機能的変異
Functional mutations in N-terminal region of type 1 ryanodine receptor in malignant hyperthermia

Toshiko Yamazawa[1], Takashi Murayama[2], Hideto Oyamada[3], Junji Suzuki[4], Nagomi Kurebayashi[2], Kazunori Kanemaru[4], Maki Yamaguchi[1], Shigeru Takemori[1], Masamitsu Iino[4] ([1]*Dept Mol. Physiol., Jikei Univ. Sch. Med.*, [2]*Dept. Pharmacol., Juntendo Univ. Sch. Med.*, [3]*Dept. Pharmacol., Sch. Med., Showa Univ.*, [4]*Dept. Pharmacol., Grad. Sch. Med., The Univ.Tokyo*)

Ryanodine receptors (RyRs), located in the sarcoplasmic/endoplasmic reticulum (SR/ER) membrane, are required for intracellular Ca^{2+} release. Mutations in RyR1 can lead to severe genetic conditions that affect skeletal muscle, e.g., malignant hyperthermia (MH) and central core disease. We investigated properties of the RyR1 channels carrying disease-associated mutations at the N-terminal region. HEK293 cells expressing the mutant RyR1 channels exhibited alterations in Ca^{2+} homeostasis, i.e., enhanced caffeine sensitivity, decrease of ER Ca^{2+} contents, increase in resting cytoplasmic Ca^{2+} concentration. These results suggest that exploration of the functional mutations of RyR1 is probably effective in preventive diagnosis of patients associated with MH disease.

2P303 Development of Nano Electrochemical Mircoscope for living cell imaging

Yasufumi Takahashi[1], Sen Mustafa[2], Yoshiharu Matsumae[2], Kosuke Ino[2], Hitoshi Shiku[2], Tomokazu Matsue[1,2] ([1]*Tohoku University, WPI-AIMR*, [2]*Tohoku University, Graduate School of Environmental Studies*)

Scanning electrochemical microscopy (SECM) uses a microelectrode tip for detecting electroactive chemical species and is an effective tool for the investigation of the localized chemical properties of sample surfaces. But, the probe is often micrometer scale and the probe vertical position is kept at a constant-height during probe scanning. Here, we report a nano electrochemical microscope(nanoSECM) developed to achieve constant-distance mode measurements and the possibility of (electro) chemical flux measurements at the same location, in order to improve the resolution of SECM imaging. The nanoSECM yields highly resolved topographical structures and electrochemical features of a cell surface, for instance microvilli and lamellipodium.

2P304 Real-time fluorescence imaging of quantum dot-loaded single synaptic vesicles

Masashi Ohmachi, Tomoyuki Takahashi (*OIST*)

The dynamic movement of synaptic vesicles is thought to be a key feature of synaptic transmission. However, the nature of synaptic vesicle mobility at nerve terminals still remains unclear. Here, we investigated synaptic vesicle mobility at the cultured calyx of Held, a giant brainstem presynaptic terminal, using real-time imaging of quantum dot-loaded single vesicles. This approach allows us to examine the movement of single vesicles in the presence and absence of presynaptic activity. Our results indicated that mobility of individual vesicles near the release site tended to increase in response to presynaptic activity. Thus, single vesicle dynamics can be investigated in real time imaging at nanometer scale resolution at mammalian central presynaptic terminals.

2P305 原子間力顕微鏡や表面力測定装置によって測定されたフォースカーブを元に溶媒和構造を計算する方法
A method for calculating the solvation structure from force curves measured by atomic force microscopy and surface force apparatus

Ken-ichi Amano (*Grad. Sch. Eng., Kyoto Univ.*)

Atomic Force Microscopy (AFM) and Surface Force Apparatus (SFA) in liquid can measure the force acting between the probe and the solid plate. The force is mainly composed of the solvation force and the probe-solid plate interaction force. In the presentation, a method for transforming the solvation force into the solvation structure is explained and its verification results are shown. The transform method is derived based on the statistical mechanics of simple liquids. It is found that the transform method works well when the number density of the liquid is lower. When the number density is higher, the contact number density and the layer spacing are not well reproduced.

2P306 細胞における緑色蛍光タンパク質のカソードルミネッセンス
Cathodeluminescence of Green Fluorescent Protein in Cell

Kazuyoshi Murata[1], Ryusuke Ueno[2], Naoki Yamamoto[3], Hideji Murakoshi[1], Kuniaki Nagayama[4], Hiroki Minoda[2] ([1]*National Institute for Physiological Sciences*, [2]*Tokyo University of Agriculture and Technology*, [3]*Tokyo Institute of Technology*, [4]*The Graduate University for Advanced Studies (SOKENDAI)*)

Cathodeluminescence (CL) is an optical phenomenon in which electrons hitting on a luminescent materials cause the emission of photons. A lyophilized powder of green fluorescent protein (GFP) has been reported to show the CL in scanning electron microscope (Fischer et al., 2008). Here, bacteria expressing the GFP gene were observed in transmission and scanning electron microscopes. The CL of GFP was successfully identified in the cell. The special resolution of fluorescent was better than the regular photoluminescence, in addition to the small specimen damage. The analysis of CL spectrum showed it has two emission peaks of 500 and 580 nm. The CL of GFP has a potential to be a new site-specific labels in biological electron microscopy.

2P307 細胞内分子混雑感受性蛍光蛋白質の開発
Glycine-inserted mutant Forster resonance energy transfer (FRET) fluorescent protein to evaluate intracellular crowding

Takamitsu Morikawa[1], Hiroaki Machiyama[2], Kazuko Okamoto[3], Keiko Yoshizawa[3], Hideaki Fujita[2,3], Taro Ichimura[3], Katsumi Imada[4], Takeharu Nagai[5], Toshio Yanagida[1,2,3], Tomonobu Watanabe[1,2,3] ([1]*Graduate School of Frontier Bioscience, Osaka University*, [2]*WPI, Immunology Frontier Research Center, Osaka University*, [3]*RIKEN Quantitative Biology Center (QBiC)*, [4]*Department of Macromolecular Science, Graduate School of Science, Osaka University*, [5]*Institute of Sciencific and Industrial Research Center, Osaka University*)

The Intracellular environment is very crowded, containing various molecules, proteins and nucleotides. This crowded condition is an indispensable factor for cellular functions of proteins. In the past, the diffusion coefficient of a chemical probe has been used as an evaluation index of the intracellular crowded condition. However, crowding depends not only on the mobility, but also the density of the crowding agents. To measure the density of the crowding agents, we developed a sensor for crowding density via hydrophobicity based on fluorescent protein engineering. Combined the sensor with measurement of diffusion coefficient, we revealed the diffusion coefficient alone is insufficient for defining crowding.

2P308 赤外超解像イメージングによる毛髪 α-ケラチンの分子配向観察
Orientation-sensitive IR super-resolution imaging of human hair α-keratins

Kohei Ushio[1], Yukihisa Watase[2], Haruki Ishikawa[2], Masaaki Fujii[1], Makoto Sakai[1] ([1]*Tokyo Institute of Technology*, [2]*Kitasato University*)

Vibrational sum-frequency generation (VSFG) detected IR super-resolution microscopy has a possibility to measure the orientation-sensitive IR image with sub-micrometer scale spatial resolution. In this study, we applied this microscopy to human hair samples and succeeded in the orientation-sensitive IR super-resolution imaging of human hair α-keratins. In the case of the amide III band at 1250 cm-1, the human hair gave strong VSFG signals in the cortex area. This enabled us to observe the distribution of α-keratins. On the other hand, VSFG signals disappeared completely when the amide I band at 1650 cm-1 was monitored. From the polarization dependence of VSFG signals, it is concluded that α-keratins are well oriented along the longitudinal direction of the human hair.

2P309 オルガネラの低温コヒーレント X 線回折イメージング
Cryogenic coherent X-ray diffraction imaging of cellular organelle particles

Yuki Sekiguchi[1,2], Amane Kobayashi[1,2], Saki Hashimoto[1,2], Tomotaka Oroguchi[1,2], **Masayoshi Nakasako**[1,2], Yuki Takayama[2], Koji Yonekura[2], Masaki Yamamoto[2], Yayoi Inui[3], Sachihiro Matsunaga[3], Yuichi Ichikawa[4], Hitoshi Kurumizaka[4], Mitsuhiro Shimizu[5] ([1]*Sci. Tech., Keio Univ.*, [2]*RIKEN SPring-8 Center*, [3]*Sci. Tech., Tokyo Univ. Sci.*, [4]*Sci. Tech., Waseda Univ.*, [5]*Sci. Tech., Meisei Univ.*)

Coherent X-ray diffraction imaging (CXDI) bridges a serious gap between optical and electron microscopy regarding the spatial resolution and thickness of sample particles. In CXDI experiment, spatially isolated sample particle is irradiated by coherent X-rays, and the electron density map projected along the direction of X-ray is retrieved from diffraction pattern using the phase-retrieval method. We have developed a suite of X-ray diffraction devices suitable for cryogenic CXDI experiment of organelle particles at a resolution of better than 50 nm using X-ray free electron laser pulses. Here we report our experimental devices, protocols and analysis method for diffraction patterns from chloroplast of schyzon and nucleus of yeast in a period of the cell cycle.

2P310 Real-Time Observation of Single Macromolecular Rotation Using Gold Nanorods

Wen-Hsuan Chang, Hung-Wen Li (*Department of Chemistry, National Taiwan University*)

Gold nanoparticles have higher absorption and scattering cross section. They don't photobleach as seen in fluorescent dyes. These properties make gold nanoparticles suitable for long-time and fast optical imaging. In particular, the scattering light of gold nanorods is highly polarized along the long axis, allowing the anisotropic detection. Using dark-field microscopy and a birefringent prism with a cross-polarization imaging system, we directly visualized the single-molecule anisotropy change of individual gold nanorods indicative of the rotation motion of the attached macromolecule in real-time. We expect that this method can be used to monitor the RecA-family protein assembly process on DNA in high-resolution.

2P311 生細胞における 1 分子内在性 mRNA イメージングのためのアンチセンスプローブの開発
Development of potent antisense probes for imaging individual endogenous mRNA in live cells

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Investigating gene-specific and dynamic behaviors of mRNA are important in understanding cell functions. To reveal those mRNA behaviors, we aimed individual endogenous mRNA imaging using antisense probes. Because unbound probes cannot be washed out in live cells, the probes with high binding rate are needed to detect individual mRNA. Here, we investigated relation between probe competence in live cells and the probe sequence. As a result, we suggest a general strategy for designing potent probes toward given mRNA targets. By complying with the strategy, we were able to obtain potent probes effectively, which enabled to observe bright spots derived from individual endogenous mRNA. These results highlighted the advantage of our approach in mRNA study.

2P312 広範囲な細胞内カルシウム濃度に対応する GECO 変異体系列
A series of GECO mutants suitable for calcium imaging in a wide range of calcium concentration

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Ca^{2+} ions have important roles as a second messenger by changing their concentration. To understand mechanisms of the signal transduction systems, it is crucial to image and quantify [Ca^{2+}] changes inside living cells. In this research, we tried to capture [Ca^{2+}] changes in cells by using GECO, one of the latest cpFP based Ca^{2+} sensors, which gives greater responses (fluorescence change) toward Ca^{2+} compared to previous indicators. However, it has narrow available range of [Ca^{2+}] to use in cells where [Ca^{2+}] differs drastically depending on compartments. To overcome this problem, we mutated GECO into several variants of different affinities for Ca^{2+} to observe [Ca^{2+}] changes in large variety of [Ca^{2+}] environments.

2P313 ラスター画像相関分光法の画像取得条件最適化と生細胞の定量解析への応用

Optimization in raster image correlation spectroscopy and application of quantitative live cell measurements

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Raster image correlation spectroscopy (RICS) is an advanced analysis method for molecular dynamics in living cells. RICS derives both distributions of diffusion coefficient and concentration from image of laser scanning microscopy using 2D autocorrelation function analysis. However, speed of laser scanning beam affects results of RICS analysis, especially the diffusion coefficient. This drawback makes difficult to quantify the diffusion coefficient of indeterminate samples. In this work, global fitting method was introduced to RICS analysis to solve this serious problem. By using this method, the range of the diffusion coefficient quantified accurately was expanded. We applied this method to in cellulo analysis.

2P314 リポソーム融合法を用いたハイブリッドエクソソームの構築

Development of hybrid exosomes by liposome fusion

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Exosomes are vesicles with the diameter ~150 nm and derived from endosome. Exosomes contain RNA, DNA and proteins; they play a key role in intracellular communication. Exosomes are utilized as delivery carriers of biological molecules for their targeting ability to specific cells. However, it is difficult to control exosomes inclusions and membrane proteins. We propose new strategy for bionanotechnological applications of exosomes using liposome fusion methods. Lipid mixing and nanoparticle tracking analysis revealed that exosomes are fused with small unilamellar vesicles by some conventional methods. Next, we observed the interaction between exosomes and Giant Vesicles by CLSM. As a result, GVs were coated with exosomes, and then, partly fusion occurred on GV surface.

2P315 自律移動人工アメーバの構築に向けて

Toward creating an autonomous mobile artificial amoeba

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We are aiming at creating molecular robots with controllable mobility.
Here, we report our attempt to build an autonomous mobile artificial amoeba-like structure consists of molecular-motors (kinesin/microtubules) and giant liposomes.
It is known that liposomal membrane can be transformed by the combination of kinesin and microtubules. By adding ATP to the mixture of kinesin-coated liposomes and microtubules, an aster-like structure was organized. The bundled microtubules were observed by phase contrast microscopy. After formation of the structures, then they started translational motion to random direction and fusion each other.
We are planning to utilize the aster as a movable frame or a controllable skeleton of an amoeba-like molecular robot.

2P316 96 穴 ANSI/SBS プラットフォームの 3 分間超高速 PCR と融解曲線分析に向けた温度均質性と正確な温度制御技術の開発

A temperature-control technique with great accuracy and uniformity for a ANSI/SBS plate for 3-min PCR and a melting curve analysis

Hideyuki Terazono[1,2], Hyonchol Kim[2], Kenji Matsuura[2], Akihiro Hattori[2], Fumimasa Nomura[1], Kenji Yasuda[1,2] ([1]*Tokyo Med. Dent. Univ.*, [2]*Kanagawa Acad. Sci. Tech.*)

Polymerase chain reaction (PCR) is an essential technique for all life science by amplifying the few target DNA copies. Melting curve analysis is also essential for detecting differences of PCR amplicons such as single nucleotide polymorphism. However, as the conventional PCR technologies lack the uniformity of temperature on the wider area of SBS format with fast and accurate temperature control, the potential of these methods has not been expanded to high-throughput screening (HTS) effectively. Hence, we expanded our 3-min rapid real-time qPCR and melting-curve-analysis device in SBS format for HTS application. With this device, PCR was accomplished within 3 min and melting curve can be analyzed rapidly while the temperature uniformity is maintained.

2P317 Peptide-based ligand screening system for G protein-coupled receptors (GPCRs) using water-in-oil microdroplets

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We are developing a peptide-based ligand screening system for GPCRs, combining *in vitro* compartmentalization with yeast-based GPCR assay. In droplets, yeast cells express fluorescent protein when synthesized peptides stimulate GPCRs, enabling easy identification of ligand candidates. In this study, to increase eventual fluorescent protein expression, a number of the DNA molecules encoding a peptide were immobilized onto a bead, and encapsulated into a droplet with necessary components for ligand assay. As a result, yeast cells expressing the target GPCR in droplets with the beads showed about 10-fold brighter than yeast cells in droplets without the beads. Our system will be a powerful tool for identifying novel peptide ligands both for liganded and orphan GPCRs.

2P318 96 ウエル SBS フォーマットサンプルの同時実時間解析のための光学系の開発

Investigation of wide range optical set-up for simultaneous real-time analysis of 96-well SBS formatted samples

Akihiro Hattori[1], Hideyuki Terazono[2], Kenji Matsuura[1], Hyonchol Kim[1], Masao Odaka[1], Mathias Girault[1], Kenji Yasuda[1,2] ([1]*Kanagawa Academy of Science and Technology*, [2]*Tokyo Medical and Dental University*)

The Society for Biomolecular Sciences (SBS) microtiter plate is one of the most commonly used sample cultivation and analysis format for high-throughput screening (HTS). Especially 96-well format is the powerful platform for cultivation of plurality of cells simultaneously. However, because of its wider size and format, the conventional microscopy optical system cannot cover such a large whole area of SBS format for simultaneous observation, and adopted the time consuming scanning procedures. To overcome the limitation of the conventional optical microscopy, we newly designed and developed an optical set-up for the SBS microtiter platform in which whole image of 96-well reactions can be observed at a time both in bright-field and multi-fluorescence images.

2P319 ナノポアとナノスリットにおける DNA 通過ダイナミクス
DNA dynamics and translocations though solid-state nanopore and nanoslit

Yuta Kato, Shohei Kawaguchi, Kensaku Shibasaki, Kentaro Ishida, Toshiyuki Mitsui (*Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

Solid-state nanopores have revealed the capability of single polymer molecule sensing by measuring the ionic current through the nanopore. Although the ionic current profile has successfully provided the information of the length and configurations of the molecules, this nanopore based detection method has shown serious issues i.e. DNA clogging of the pore. To elucidate the clogging mechanism, we have observed the interactions between a pore and single DNA molecules before their translocations by optical microscopy. We have also fabricated nanoslit to observe the pore shape dependence of the interactions. In this presentation, we will discuss the ideal shape of pore for the reliable DNA detection devices.

2P320 電極付加ナノポアの DNA 通過とその挙動解析
DNA motions near a nanopore with a voltage controlled gate embedded in dielectrics

Shohei Kawaguchi, Yuta Kato, Kensaku Shibasaki, Kentaro Ishida, Toshiyuki Mitsui (*Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

Sensing devices using solid-state nanopores have been developed in order to detect single DNA molecules. Resent trends in the nanopore device technology are adding electrodes to nanopores toward single DNA sequencing as an ultimate goal. However, the electric potential differences between the electrodes and electrolyte would generate liquid flows near the electrode surface called electroosmosis. We have developed experimental devices that allow us to directly observe the motions and translocations of DNA molecules in the vicinity of a nanopore, which contains a voltage controlled Au gate as an electrode. We will discuss the magnitude of the electroosmosis and the possibility to control the DNA motions by the gate voltages.

2P321 マイクロ波でアシストされた蛋白質加水分解酵素の速度論解析
Kinetic analysis of microwave assisted enzymatic protein digestion

Arata Shiraishi[1], Hiroya Osoegawa[1], Takeo Yoshimura[1,2], Shokichi Ohuchi[1] ([1]*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Biol. Sci. Tokyo Univ. Sci*)

We have been demonstrated the enzymatic hydrolysis reaction of protein under microwave irradiation. For example, trypsin digestion was accelerated 100 times with microwave condition. Additionally, when hydrolyzed with a protease protein such as the trypsin, usually only the recognized amide sequence of protein surface was broken. However, under microwave condition could break the almost amide bonds including the protein inside amide bonds. In this study, we will discuss the kinetic analysis of microwave assisted enzymatic protein digestion under temperature-controlled condition.

2P322 精密な温度制御下でのマイクロ波照射酵素反応
Microwave irradiated enzyme reaction under controlled temperature

Kengo Kawachi, Fujiko Aoki, Arata Shiraishi, Shokichi Ohuchi (*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*)

We have applied the reaction accelerative effect by a microwave irradiation to enzyme reactions, such as a hydrolysis reaction and a gene amplification reaction. And we found that the effect had given by a microwave irradiation with various enzymes. In this research, we analyzed about the hydrolase of lipase and the oxidoreductase using the microwave described previously according to making reaction temperature into low temperature from the usual method. In the result, it turned out that the progress condition of a reaction changes with reaction temperature.

2P323 好熱菌のマイクロ波加熱培養
Microwave heating cultivation of Thermus thermophilus

Wataru Nagayoshi[1], Ryota Nakama[1], Takeo Yoshimura[2], Makoto Kodama[3], Shokichi Ohuchi[1] ([1]*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. Sci.*, [3]*Vessel Inc.*)

We have studied on the effect of microwave for bacteria. In this research, we investigated about microwave irradiated cultivation of Thermus thermophilus. The microwave cultivation with some thermophilus was carried out on various condition of temperature, culture medium and microwave power. It was found that the microwave power gave useful effects and the threshold of life or death of the microwave power for some bacterium under microwave irradiation was observed.

3P001 各種蛍光タンパク質が疎水的環境下において示す蛍光特性の違いに関する構造学的アプローチ

Different sensitivity of various fluorescent proteins to hydrophobic environments

Suguru Asai, Hide A. Konishi, Kunio Takeyasu, Shige H. Yoshimura (*Grad. Schl. Biostudies., Univ. Kyoto*)

Hydrophobic interaction is one of the key factors in protein folding, structure and function. Here we examined the structural stability of various fluorescent proteins originated either GFP or mRFP in different hydrophobic environments. Fluorescence of GFP variants, but not mRFPs, were well quenched in 2,2,2-trifluoroethanol (TFE). Circular dichroism spectra analysis indicated that the β-barrel of GFP variants was significantly collapsed by TFE, whereas that of mRFP was not affected. Molecular dynamics simulation revealed that the disruption of the β-barrel occurred the β-sheets between 9-4, 10-11, and 7-8, where GFP variants have more hydrophobic residues than mRFPs, indicating that these hydrophobic residues are important for the structural stability.

3P002 溶液中で配向させたコラーゲンの X 線繊維回折

X-ray diffraction study of aligned collagen fiber

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A filamentous polymer structure is one of the most typical features in biological materials. Commonly seen protein molecules, such as microtuble, fibronectin, and collagen have filament structures that relate to their biological functions. These filamentous polymers have difficulty to solve their structures by the X-ray crystallography because of their lengths. Sugiyama et al. introduced an alignment method and X-ray fiber diffraction to investigate filamentous polymer structures under physiological condition.
We have investigated the type II collagen structure using small-angle X-ray scattering and alignment methods as well as disorder protein solution.

3P003 セグメンテーション＆フィッティング - 低解像度密度マップへの複数のサブユニットのあてはめ計算法 -

Segmentation & fitting algorithm for multiple subunit fitting into a low resolution density map

Takeshi Kawabata, Hirofumi Suzuki, Haruki Nakamura (*Inst. Prot. Res., Osaka Univ.*)

Single particle CryoEM typically produces 3D density maps the macromolecular complex with low resolutions. For understanding these maps, it is essential to fit atomic structure of individual subunits into the map of the complex. However, huge computation costs are often required to search the configurations of large number of subunits. We invented a new fitting algorithm “segmentation & fitting” based on our program gmfit using Gaussian mixture model. The algorithm repeats two steps. The “segmentation” step puts labels of subunits onto parts of the map. The “fitting” step superimposes each subunit onto the labeled part of the map. This algorithm successfully rebuilds the 21 subunits of GroEL/ES with the symmetric constraints with a short computation time.

3P004 構造データベース中の 3 次元電子顕微鏡データの形状比較とフィッティング

Shape comparison of 3D electron microscopy data using both feature-vectors and GMM-based superimpositions

Hirofumi Suzuki[1,2], Takeshi Kawabata[1], Haruki Nakamura[1,2] ([1]*IPR, Osaka-u*, [2]*PDBj*)

3D electron microscopy have generated more than 3000 structures (density maps and models), they have been stored in EMDB and PDB. The shape comparison is necessary to make a full use of these data. We have developed a system to search similar molecular shape using both feature-vector and superposition comparisons. First, the feature-vector comparison roughly extracts the similar data from the database in a short time. The distance distributions of representative points were employed as the feature vectors. Next, the detected data are examined in detail by the superimposition in 3D space using Gaussian mixture model (GMM), it clearly shows similar and different parts of two maps or models. A part of our comparison method is now available in the EM Navigator website.

3P005 スピンラベルタンパクの変性過程におけるダイナミックな電子スピン共鳴線形の解析

An analysis of Dynamic Electron Paramagnetic Resonance Lineshape for a Denaturation Process of Spin-labeled Protein

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Dynamic behavior of BDPA protein (the B domain of protein A) in a denaturation process is studied by electron paramagnetic resonance (EPR) by the site-directed spin-labeling method. BDPA is doubly labeled at helix H2 and H3 by nitroxide radical (MTSL) to study inter-helices motion during denaturation which is caused by addition of guanidinium chloride (GdmCl) up to 5 M. We observed linewidth changes of nitroxide radical. In addition, difference between the linewidths of singly and doubly labeled BDPA indicate significant effects of spin-spin interaction on the linewidth in doubly labeled BDPA. In this paper, we discuss detailed analysis of linewidth of doubly labeled protein which is essential to extract inter-helix motion.

3P006 TEM 単粒子解析と大気圧電子顕微鏡（ASEM）によるタンパク質複合体形成の観察

TEM single particle reconstruction and atmospheric SEM of protein complex formations

Chikara Sato, Kazuhiro Mio, Nassirhadjy Memtily, Mari Sato, Tatsuhiko Ebihara, Toshihiko Ogura (*Biomed-Ri., AIST*)

Among structure determination methods of protein using TEM, single particle analysis (SPA) has minimum requirements for samples, i.e., not protein crystals but just 50 - 500 ng-purified proteins are needed. Using SPA, the three-dimensional structure of a protein is reconstructed from its EM images. We have determined the structure of signal peptide peptidase (SPP), which works in the clearance of signal peptides and misfolded membrane proteins. SPP has a significant role in the maturation of several pathogens including the hepatitis C virus. The new atmospheric scanning electron microscope (ASEM) observes samples in solution under an open atmosphere. Using this system, immuno-ASEM successfully visualized axonal segmentation and platelet formation from megakaryocyte.

3P007 Systematic structural study of single amino acid insertion mutants of YFP

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YFP is a mutant derivative of green fluorescent protein, and is widely used as a fluorescent indicator in molecular biology. Recently we found that YFP mutant variants with single amino acid insertion in β7 showed various fluorescence responses to environmental change. The fluorescence responses depend on the nature of the inserted amino acid residue. To elucidate the molecular mechanism how the insertion affects the fluorescent property, we prepared twenty single amino acid insertion variants of YFP and solved the structures. Here we show the structures of eighteen mutant YFPs. They show unexpected conformational variation in β7 dependent on the inserted residue. We will discuss the relationship between the mutant structure and the fluorescent property.

3P008 分子動力学シミュレーションを用いた Hras-GTP 複合体の溶媒水と複合体の水素結合の解析
Analysis of hydrogen bonds between solvent water and atoms in the Hras-GTP complex by molecular dynamics simulations

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In order to understand the mechanism of hydrolysis of GTP in the Hras-GTP complex, we study the structures of the Hras-GTP and GDP complexes in water solvent by molecular dynamics (MD) simulations.
We evaluated the potential parameters around Mg2+ in Hras-GTP and GDP complexes by quantum chemical calculations. We performed MD simulations of the Hras-GTP and GDP complexes in water solvent using parameters of AMBER03 and our parameters around Mg2+. We found that the site dipole fields around GTP are different from those around GDP. We proposed that this difference causes the difference of hydrolyzability between GTP and GDP.
In this study, we analyze the individual hydrogen bonds between solvent water and atoms in the Hras-GTP complex.

3P009 固体 NNR を用いたヌクレオソームにおけるヒストン H2A、H4 の構造解析
Structural analysis of histone H2A, H4 in nucleosome using by solid-state NMR

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In eukaryotic cells the fundamental structural unit of chromatin is a nucleosome core, consisting of two copies of four histones and DNA, whose crystalline structures have been determined. However in the crystals the N- and C-terminal histone tails, which are responsible for gene regulations by their chemical modifications could not be well identified. Here, we have examined the structures of N- and C-terminal histone tails of H2A and H4 in a model of chromatin by solid-state NMR and MD simulations. These suggested that both histone tails in chromatin hold flexible random coil structures, which might play significant roles in gene regulations.

3P010 細胞接着ペプチドと α2β1 インテグリン I ドメインとの結合シミュレーション
Docking simulation of cell adhesion peptide and α2β1 integrin I domain

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Laminins, which are glycoprotein in basement membrane, have diverse biological activities. EF1 peptide (DYATLQLQEGRLHFMFDLG) derived from α1 chain of laminin has cell adhesion mediated by α2β1 integrin. Suzuki et al. analyzed active core sequences to investigate minimal sequences of EF1 and found that cell adhesion of EF1 is conformation-dependent by cyclization of minimal sequences of EF1. Previously, we showed that EF1 has hairpin structure by simulated annealing, and we studied dynamical behavior of EF1 in water solution (300K, 1bar). We are also interested in relationship of structure between EF1 and receptor. In this study, we perform docking simulation of EF1 and α2β1 integrin. We use structure of α2β1 integrin I domain determined by X-ray diffraction.

3P011 DFTB+ソフトウェアへの REUS 法の導入
Implementation of Replica-Exchange Umbrella Sampling to the DFTB+ Simulation Package

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We have investigated the computational methods which combined the self-consistent-charge Density Functional based Tight Binding (DFTB) method[1] for fast calculations of quantum effects and the Replica-Exchange Umbrella Sampling (REUS)[2] for enhanced conformational sampling. One of the excellent QM-MD simulation package named DFTB+ does not have REUS method incorporated. We thus modified DFTB+ to include the REUS method. We will compare the results of DFTB+ calculations with those by another simulation package and present the results in small molecules.
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3P012 Preliminary study of voltage-gated proton channel in activated state for X-ray crystallography

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Voltage-gated proton channel (VSOP) has a voltage sensor domain that is similar to the voltage sensors of canonical voltage-gated ion channels, but it lack of ion pore domain. VSOP is related to superoxide production in phagocytes and human sperm motility. We recently determined the crystal structure of VSOP, which showed first resting-state structure as voltage sensor, and it showed that the proton pathway was closed. We are trying to determine the crystal structure in activated-state, which is expected to understand the gating mechanism of VSOP. In our poster, we will discuss the procedure to prepare the activated-state of VSOP using gel filtration chromatography with anti-VSOP Fab fragment labeled fluorescence.

3P013 アミロイドβの構造探索 II
Conformational Search of Amyloid β Peptide II

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The cause for Alzheimer's disease is considered to be due to the accumulation of amyloid β peptides in the brain. The excess amount of amyloid β peptides leads to the formation of aggregates which are neurotoxic. Therefore, it is important to obtain the conformation of the aggregate of amyloid β peptides. Recently, we have examined the growth process of the aggregates of the amyloid β peptides by FCS and obtained the size of the aggregates. However, we could not identify the conformation of the aggregates. Here, we continue to work on the topic and carry out MD simulations to explore the conformational space of an amyloid β peptide to understand the possible structures and the build-up mechanism.

3P014 蛋白質の二次構造形成に関わる相互作用に関する理論的研究
Theoreical analysis of molecular interactions in secondary strucutures of proteins

Yu Takano, Ayumi Kusaka, Haruki Nakamura (*IPR, Osaka University*)

The second structures such as α-helices and β-sheets are important components for the protein architecture, which dominates its functions and chemical properties. Molecular interactions, in particular hydrogen bonding, play significant roles in the formation of the secondary structures. In the present study, we have investigated the local and nonlocal molecular interactions in the α-helices and parallel and antiparallel β-sheets composed of alanine residues, using quantum chemical methods (QM) (B97D/ 6-31+G(d)) and molecular mechanics (MM) (AMBER99-SB). The MM force field overestimates the interaction energies of the α-helix, compared to the QM calculation, We will discuss the origin of the deviation between the MM force field and the QM calculation.

3P015 コレラ菌走化性受容体 Mlp24,Mlp37 のリガンド認識機構
Ligand recognition mechanism of Mlp24 and Mlp37, chemoreceptor proteins of *Vibrio cholerae*

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Vibrio cholerae is the causative agent of cholera. At least 45 genes for methyl-accepting chemotaxis protein-like proteins (MLPs) have been identified in *V. cholerae*. Unlike other well-studied chemoreceptors, such as Tar and Tsr, some of the MLPs are expected to have PAS-like domains. Among them, Mlp24 is involved in the production of cholera toxin and recognizes multiple amino acids. Thus, sensing of amino acids is thought to trigger the expression of virulence factors. We have determined the structures of periplasmic fragments of Mlp24 and Mlp37, the closest homolog of Mlp24, and their substrate complexes. They have two PAS-like domains, but only the distal domain binds the ligand. We will discuss the ligand recognition mechanism of this class of chemoreceptors.

3P016 DFG-out コンフォメーションを持つ MEK1 構造
Structure of MEK1 in DFG-Out conformation

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MEK1/2 (MAPK/ERK kinases) are dual-specificity kinases and activated via the phosphorylation at two Ser residues in the activation segment by their upstream kinases. Only MEK1/2 can activate ERK1/2 by phosphorylating regulatory Thr and Tyr residues. Protein kinases show two characteristic conformations: DFG-in and DFG-out, but the stabilization of each conformation remains unclear. The crystal structure of unphopshorylated MEK1 (38-381 amino acids) in complex with ATP-gamma-S has been determined at 2.2 Å resolution. Contrary to previous reports, MEK1 had the DFG-out conformation of the active segment. The activation segment had a highly extended conformation. ATP-gamma-S exhibited a novel binding mode.

3P017 2D hybrid analysis: A new approach to build 3D atomic model from 2D EM image

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We have been developing a new computational approach to build a 3D atomic model from an electron microscope (EM) image of a biological molecule. In the usual approach, a 3D-EM model is constructed from many EM images. Then, a 3D atomic model is built mainly by deforming the X-ray crystal structure so that it fits into the 3D-EM model better. Our approach, on the other hand, uses only a single EM image (or an averaged image) and an X-ray crystal structure (or a modeled structure), and has the advantage of being applicable even to flexible molecules, for which it is difficult to construct 3D-EM models. We will report the results about the application to integrins, transmembrane proteins that involved in cell-cell and cell-extracellular matrix adhesions.

3P018 Structural analysis of the intron-encoded domain of herstatin

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A receptor tyrosine kinase HER2 forms a dimer to promote cell proliferation, but aberrant expression of HER2 causes cancers. Therefore, a HER2-binding protein that blocks its dimerization is expected as an antitumor drug. A HER2 alternative splicing product, herstatin, is an autoinhibitor of HER2, because the C-terminal, intron 8-encoded domain of herstatin (Int8) binds HER family receptors. Interestingly, Int8 is predicted to be an intrinsically disordered protein. To investigate the structure and dynamics of Int8, we constructed its E. coli expression system. We will perform NMR measurements of Int8, and the results will be presented at the meeting.

3P019 分子動力学法を用いた高圧下における c-Myb R2 ドメインのキャビティ圧縮と構造変化および揺らぎとの関係

A relationships among compression of the cavity of c-MybR2, conformational changes and fluctuation under high-pressure using MD simulation

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c-Myb protein is a transcription factor connected with proliferation and differentiation of hematopoietic cells. In this study, wild-type (WT) c-Myb with a cavity and a mutant-type (V103L) with a vanishingly small cavity were studied by molecular dynamics simulation at 1 and 3k bar for 200 ns in order to establish the relationship among the cavity, the compression, and conformational changes. We calculated the root mean square deviation (RMSD), the protein volume (probe size 1.1Å), cavity volume, and a difference map (DM) of c-Myb under pressure at 1 and 3k bar from the trajectories. From these results, we conclude that there must be a relationship between the cavity and characteristic conformational changes.

3P020 ヒスタミン H_1 受容体の分子内情報伝達機構に関する理論的研究

Computational study on the intramolecular signaling mechanism of histamine receptor

Yuko Ishii, Takakazu Ishikura, Takahisa Yamato (*Grad. Sch. Sci., Nagoya Univ.*)

The histamine H_1 receptor (H_1R), a member of GPCR, plays a key role in allergic and inflammatory reactions. In this work, the characteristic pattern of the inter-residue interactions of the wild-type H_1R was represented as an energy exchange network (EEN) using the current calculation for protein (CURP) program[1,2]. The allosteric mechanism of H_1R was then investigated by comparing the EEN of H_1R with those of rhodopsin and β2 adrenergic receptor.

[1] T. Ishikura, T. Hatano and T. Yamato, ***CPL*** 539, 144-150, (2012).
[2] T. Ishikura and T. Yamato, ***CPL*** 432, 533-537, (2006).

3P021 KcsA カリウムチャネルの開構造への構造変化前における分子揺らぎの増大

The Enhancement of Structural Fluctuations Prior to The Opening Conformational Changes of The KcsA Potassium Channel

Hirofumi Shimizu, Masayuki Iwamoto, Yumiko Oota, Shigetoshi Oiki (*Univ.Fukui.Fac.Med.Sci*)

We have refined the Diffracted X-ray Tracking (DXT) method to reveal the conformational changes of the KcsA channels upon gating. To trace the gating conformational change in the single molecule level, the solution pH was jumped from neutral to acidic by using the caged proton, and the opening of the activation gate was examined. The structural fluctuation was enhanced immediately after the pH jump, and the twisting conformational change was initiated after a delay. This enhanced fluctuation was also observed at equilibrium acidic conditions. These findings will lead us to unveil the mechanism of gating.

3P022 Coarse-grained Generalized Born and surface area models and its application to protein docking

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The electrostatic and hydrophobic interactions are two major driving force of protein-protein docking. We developed the coarse-grained (CG) Generalized Born (GB) and surface area (SA) models in CafeMol software to achieve better accuracies in protein interactions. This work is divided into three parts. First, the fitting of CG GBSA parameters to all-atom GBSA energies. Second, optimizing the GBSA scaling factors by matching to all-atom GBSA forces. Finally, we test the optimized parameters in CG simulations.

3P023 Factor Xa に対する薬剤候補分子の結合自由エネルギー:3D-RISM 計算

Predicting binding free energy of drug candidates to Factor Xa : 3D-RISM study

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It is of essential significance to evaluate the binding free energy for searching a candidate of active drug in the in silico pharmaceutical design. To estimate the binding free energy, it is important to evaluate not only the interaction energy between protein and drug candidates but also the desolvation free energy. But, it is not an easy task for the MD simulation because of the difficulty in sampling of water configuration, especially, at the active site. The “3D-RISM theory” has been successful in evaluating the solvation thermodynamics of complicated solutes. In this study, we calculate the binding free energy between Factor Xa and the ligands using 3D-RISM theory, and compare the theoretical results for the relative binding free energy with experimental values.

3P024 多剤排出トランスポーター AcrB の構造回転機構に対する物理描像

Physical Picture for Mechanism of Conformational Rotation of Multidrug Transporter AcrB

Hirokazu Mishima[1], **Hiraku Oshima**[2], Satoshi Yasuda[2], Masahiro Kinoshita[2] ([1]*Grad. Sch. Energ. Sci., Kyoto Univ.*, [2]*Inst. Adv. Energ., Kyoto Univ.*)

The multidrug transporter AcrB, which exports a variety of drugs in Gram-negative bacteria, comprises three protomers with different conformations. The conformations change in a cyclic way by the proton-motive force, leading to an apparent, conformational rotation of AcrB which induces the drug exportation. Here we investigate the packing structure of AcrB in terms of the entropic effect originating from the translational displacement of water molecules or hydrocarbon groups constituting nonpolar chains of lipid molecules. We find that the packing in AcrB is highly asymmetric. We construct a physical picture for the mechanism of conformational rotation elucidating how each protomer achieves such a drastic conformational change using only a small free energy.

3P025 Conformational motions in protein machines: elastic-network computational studies

Holger Flechsig (*Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University*)

Protein machines and motors are involved in virtually all processes in biological cells. The function of these nanoscale engines relies on cyclic changes of their conformation, powered by binding and hydrolysis of ATP molecules. In computational studies the relevant motions cannot be traced with the highest possible resolution since they are too slow to be followed by all-atom molecular-dynamics methods. To explore functional aspects of these proteins, coarse-grained models such as elastic-network descriptions have been shown to be remarkably successful. I will show how elastic networks can be used to understand ATP-induced functional dynamics in protein motors and machines. Recent applications will be presented and discussed.

3P026 野生型および変異型 CYP2B6 の柔らかさが薬物代謝に与える影響の推定

Computational studies for the influences of protein flexibilities on enzymatic activities of the wild type and mutants of CYP2B6

Akifumi Oda[1,2], Kana Kobayashi[3], Yurie Watanabe[1], Shuichi Fukuyoshi[1], Masahiro Hiratsuka[4], Noriyuki Yamaotsu[5], Shuichi Hirono[5], Ohgi Takahashi[3] ([1]*Faculty of Pharmacy, Inst. Med. Pharm. Health Sci., Kanazawa Univ.*, [2]*Inst. Protein Res., Osaka Univ.*, [3]*Faculty of Pharm. Sci., Tohoku Pharm. Univ.*, [4]*Grad. Sch. Pharm. Sci., Tohoku Univ.*, [5]*Sch. Pharmacy, Kitasato Univ.*)

The genetic polymorphisms of drug metabolizing enzymes play important roles in individual differences of the drug efficacies and adverse effects. In CYP2B6, several mutants whose enzymatic activities were lower than the wild type were reported. In this study, three dimensional structures of the wild type and mutants of CYP2B6 were predicted and the dynamical properties of these proteins were investigated by using molecular dynamics simulations. The root mean square fluctuations (RMSFs) were calculated for the wild type and the mutants, and the cavities of the proteins were detected. The flexibilities of some mutants were largely different even though only one residues were mutated, and the change of flexibilities may influence the enzymatic activities of CYP2B6 mutants.

3P027 タンパク分子内の力学的情報伝達の特徴付け―粗視化モデルによる試み

Classification of Mechanical Communication in Proteins: A Coarse-Grained Study

Yuichi Togashi (*RCMCD, Grad. Sch. Sci., Hiroshima Univ.*)

Protein molecules sometimes form a complex, and each molecule consists of subdomains. Their function is often regulated by external stimuli, such as forces or binding of ligands. Generally, for the whole complex to work properly, communication between the parts (molecules or subunits) is required. However, the regulatory parts (sensor) and functional parts (actuator) are not always close to each other. We adopted steered molecular dynamic simulations using coarse-grained elastic networks, to predict mechanical communication pathways within a complex. Using a variety of structural data, we demonstrate how proteins can be classified according to their mechanical communication profiles.

3P028 粗視化分子シミュレーションによる MEK1-ERK2 相互作用の調査

Interactions of MEK1 with ERK2 in mammalian MAPK pathway studied by coarse-grained molecular simulations

Ryo Kanada, Shoji Takada (*Grad. Sch. Sci., Univ. Kyoto*)

Cell growth and response to extracellular signals could be realized by a system of intracellular reaction network: MAPK cascade. Since the collapse of MAPK cascade causes the serious disease such as cancer, the comprehension of this system is medically important. However the molecular mechanism for activation of ERK2 by MEK1, which is one of the representative players in mammalian MAPK pathway, remains to be elucidated, because the complex structure for MEK1-ERK2 is not available. So, we investigated MEK1-ERK2 association dynamics by coarse-grained molecular simulation with well-tuned inter-chain interaction including hydrophobic and electrostatic ones. Based on the sampled structures, we will investigate the important interaction for association of MEK1-ERK2 system.

3P029 キャビティーが蛋白質機能を制御する

Cavity controls protein function

Kazuyuki Akasaka (*Kinki University Institute of Advanced Technology High Pressure Protein Research Center*)

The crucial part of the protein dynamism lies in its “excited state” paradigm, which is manifested in the non-linear part of pressure response of 1HN and 15NH chemical shifts in high-pressure NMR spectra [1]. The non-linear response is a manifestation of “excited states” that merge into the ground state within the NMR time scale.

3P030 ソーシャブルなリガンド結合部位の構造的また物理化学的特徴に関する研究

A Study for the Structural and Physicochemical Properties of Sociable Ligand-Binding Sites in Proteins

Yoichi Murakami, Kengo Kinoshita (*Graduate School of Information Sciences, Tohoku University*)

Comparison of protein structures available in the Protein Data Bank (PDB) has so far enabled us to elucidate the natural mechanisms of molecular interactions. In this study, we have compared a number of ligand-binding sites shared with different ligands, called as sociable ligand-binding sites (SLBS), with those accommodating only one single ligand or its analogues, called as un-sociable ligand-binding sites (USLBS). In order to more understand the difference between SLBS and USLBS, we have statistically analyzed structural and physicochemical properties of both SLBS and USLBS, such as amino acid composition, hydropathy, secondary structure and accessible surface area. We will present observation results and discuss their distinctive differences.

3P031 単一シアル酸オリゴ糖上でのマイコプラズマの滑走と結合
Gliding and binding of mycoplasma on uniform sialylated oligosaccharide

Taishi Kasai, Tasuku Hamaguchi, Makoto Miyata (*Osaka City University, Graduate School of Science*)

Mycoplasmas glide in the direction of the membrane protrusion at a pole. Generally, mycoplasmas glide on mixed sialylated oligosaccharides (SOs), and the gliding motility on uniform SO has never been observed.
In the present study, we analyzed binding and gliding of M. mobile on a plastic plate covered by 53 uniform oligosaccharides on isolated spots. The gliding speed related inversely to the affinity of mycoplasma to the SO, suggesting that the mycoplasma legs generate drag force in gliding. Mycoplasma cells glided faster, more constantly, with acceralated pivoting on uniform SO than on mixed SO, suggesting that they can glide efficiently on uniform SO and processively on mixed SO.

3P032 抗 HIV 因子 APOBEC3G の基質認識及びスライディング機構の実時間 NMR 解析
Substrate Recognition and Sliding Properties of an Anti-HIV Factor APOBEC3G analyzed by Real-time NMR Monitoring Strategy

Keisuke Kamba[1,2], Takashi Nagata[1,2], Masato Katahira[1,2] ([1]*Inst. of Advanced Energy, Kyoto Univ.*, [2]*Grad. Sch. of Energy Science, Kyoto Univ.*)

Human APOBEC3G protein (A3G) destroys the HIV genomic information by converting cytidines into uridines in the minus DNA strand synthesized from RNA genome of HIV. This deamination activity of A3G is only targeted towards DNA. A3G effectively deaminates cytidines that are located close to the 5' end. The mechanism of the DNA recognition by A3G has not been clarified so far. In this study, we have applied the real-time NMR monitoring strategy to the systematically designed oligonucleotides: DNAs that are partially substituted with RNA or abasic nucleotides. We will discuss the identified DNA determinants for the recognition by A3G and A3G's DNA sliding property.

3P033 セルラーゼ TrCel7A の基質取り込み機構に関する分子シミュレーション研究
Molecular simulation study on the mechanism of substrate uptake in cellulase TrCel7A

Takashi Kanazawa, Minoru Sakurai, Tadaomi Furuta (*Center for Biol. Res. Info., Tokyo Tech*)

Cellobiohydrolases is an enzyme that hydrolyzes glycosidic linkages in cellulose, and its catalytic domain has a tunnel for substrate to pass through. Here, we conducted MD simulations of the catalytic domain and free energy calculations to examine the contribution of four tryptophan residues (W38, W40, W367, W376), which are lined up along the tunnel, for initial threading of a cellulose chain into the catalytic tunnel of the Family 7 cellobiohydrolase from Trichoderma reesei (TrCel7A). Simulations for W40A and W38A mutants revealed the role of these tryptophan residues in initial uptake. Moreover, we would discuss the dynamics of entire TrCel7A bound to crystalline cellulose toward further understanding of cellulose decrystallization.

3P034 レプリカ交換 MD 及びフラグメント MO 計算によるアミロイドβダイマーの水中での安定構造の探索
Replica exchange MD and ab initio fragment MO calculations for searching stable conformations of amyloid-b dimer in water

Hiromi Ishimura, Akisumi Okamoto, Atsushi Yano, **Noriyuki Kurita** (*Toyohashi University of Technology*)

The aggregation of amyloid-B peptides (ABs) is involved in the pathogenesis of Alzheimer's disease, and the conformations of AB aggregates have been investigated widely. In the present study, we performed replica exchange molecular dynamics simulations to obtain various conformations of AB(1-42) dimer in explicit water molecules and determined the most stable conformation of the solvated AB dimer by ab initio fragment molecular orbital calculations. In addition, the specific interactions between AB monomers were investigated to elucidate which residues of AB contribute to the AB dimerization in water.

3P035 Elastic Network Model を用いた ABC トランスポーターの global motion の解析
Global motion of ABC transporters using nonlinear relaxation dynamics in elastic network model

Naoki Arai, Tadaomi Furuta, Minoru Sakurai (*Center for Boil. Res. & inform., Tokyo Tech*)

ABC transporters have two TMDs and two NBDs. TMD and NBD are connected with ICL. Although several X-ray structures have been reported, their dynamic properties are not well understood. This study investigated the structural transition and communication between TMDs and NBDs by applying nonlinear relaxation dynamics in elastic network model (ENM) (Togashi et al. PNAS 2007) to MsbA. At first, ENM was constructed from its X-ray structure. Then, we added a force of bringing the two NBDs approaching each other as a simulation of ATP binding, but the TMDs never opened outward. Next, a force of rotating the NBDs was added. Then, the TMD opening was observed. The detailed analysis suggested that the motion of TMDs opening results from power transmission through ICL.

3P036 一酸化炭素型ヘモグロビンの光解離中間体の X 線結晶構造
X-ray crystal structures of carbonmonoxy hemoglobin photolysis intermediates

Ayana Tomita[1], Tokushi Sato[1], Hiroki Noguchi[2], Shunsuke Nozawa[1], Shin-ya Koshihara[3], Sam-Yong Park[2], Naoya Shibayama[4], Shin-ichi Adachi[1,5] ([1]*Photon Factory, KEK*, [2]*Gra. Sch. Nanobiosci., Yokohama City Univ.*, [3]*Dep. Mat. Sci., Tokyo Tech/JST-CREST*, [4]*Div. Biophys., Jichi Med. Univ.*, [5]*JST-PREST*)

Hemoglobin (Hb) is an $\alpha_2\beta_2$ tetrameric oxygen transport protein that binds gaseous ligands such as O_2 and CO cooperatively at the four heme irons. Although Hb was one of the first protein structures ever to be solved by X-ray crystallography, its static structures do not tell us much about the gas migration pathway from the outside of the protein to the deeply buried heme. We report a series of X-ray crystal structures of the photolysis intermediates of HbCO at 140 K, visualizing the irradiation time-dependent position of CO in the α and β subunits. Photo-dissociated CO moves toward the protein internal cavities with shift of amino acid residues around the cavities. We assign the individual ligand migration pathways in both subunits of Hb.

3P037 抗体デザイン手法の開発に向けた抗原認識機構の解明
Elucidation of antigen recognition by antibodies toward the development of a method for antibody design

Yuko Tsuchiya, Kenji Mizuguchi (*NIBIO*)

Understanding antigen recognition by antibody is essential for the design of a novel antibody. Among the six CDR loops, CDR-H3 is the most important to antigen recognitions, because of its sequence diversity. In addition, the loop conformations of CDR-H3 are very diverse, particularly in long CDR-H3 loops, which may affect the mechanisms of antigen recognition.
In this study, we focus on the relationship between CDR-H3 conformations and antigen-recognition patterns, particularly in antibodies with long CDR-H3 loops. We constructed a new method for describing and characterizing CDR loop conformations. Using this method, we aim to relate CDR-H3 conformations to their sequences and interaction patterns.

3P038 MD シミュレーションで探るマルトーストランスポーター ATP アーゼ（MalK）のダイナミクスと構造変化
Dynamics and Structural Changes of Maltose Transporter ATPase (MalK) as studied by MD simulations

WeiLin Hsu, Tadaomi Furuta, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

ABC transporters translocate many substrates across cellular membrane by coupling with ATP hydrolysis. Currently, the mechanism for the substrates translocation still remains elusive. Here we focus on MalK, the subunit of the maltose transporter from E. coli. To elucidate the dimerization mechanism of MalK, we performed the cMD and aMD. Our present results indicate that the H-bond network formation between D-loop and walkerA located near the NBD interface may be a critical factor to facilitate the dimerization of MalK. We also observed the entering of water in ABP sites may further stabilize the H-bond network. On the other hands, the results of apo systems show the opening of NBDs. The phenomenon may be explained by the unfastened amino acids due to the absence of ATP.

3P039 Structural analysis of the 26S proteasome by cryo-electron microscopy and Single-Particle Analysis

Zhuo Wang[1], Yasuo Okuma[1], Daiske Kasuya[2], Kaoru Mitsuoka[3], Yasushi Saeki[4], Takuo Yasunaga[1] ([1]*Department of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology*, [2]*Biomedicinal Information Research Center, Japan Biological Information Consortium (JBIC)*, [3]*Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology*, [4]*Laboratory of Protein Metabolism, Tokyo Metropolitan Institute of Medical Science*)

The 2.5-MDa 26S proteasome is composed of a 20S core particle together with one or two 19S regulatory particles. Since the weak assembly of the 26S makes structural analysis more difficult, the GraFix (Gradient Fixation) method was used to purify and stabilize holoenzyme 26S proteasome. After using GraFix, not only could the glycerol which was contributed to the low contrast of EM images be easy removed, but also the mount of particles could be increased. But even the glycerol remove, the EM images are still lacking in visibility. So the Wiener filter which can remove the additive noise was used in here. As a result, we got higher contrast images to pick up. And the single particles were used to obtain the 3D structures. Finally, we defined the Rpn10 position.

3P040 高速原子間力顕微鏡による Kai タンパク質間相互作用の観察
Interactions between Kai Proteins observed by high-speed AFM

Shogo Sugiyama[1], Mori Tetsya[2], Takayuki Ucihashi[1,3], Carl H. Johnson[2], Toshio Ando[1,3] ([1]*Dept. of phys., Univ. Kanazawa*, [2]*Dept. of Biol. Sci., Univ. Vanderbilt*, [3]*Bio-AFM FRC., Univ. Kanazawa*)

The circadian rhythm in cyanobacteria is generated by an oscillator that comprises three Kai proteins (KaiA, KaiB and KaiC). The interactions between Kai proteins change the phospholylation state of the hexameric ring of KaiC, which determines the phase of circadian rhythms. Mutational and structural analyses have suggested that both KaiA and KaiB bind to the C-terminal side of the KaiC ring (1), whereas a possibility of KaiB binding to the N-terminal side has also been proposed (2). Here we observe the interactions between KaiC and KaiA/B proteins using high-speed AFM to investigate the interaction dynamics.
1) Vakonakis, I. & LiWang, A.C. Proc. Natl. Acad. Sci. USA 101, 10925 (2004)
2) Chang, Y.G. et al., Proc. Natl. Acad. Sci. USA 109, 16847 (2012)

3P041 高速 AFM による細胞質ダイニンの動態観察
Observation of structural dynamics of cytoplasmic dynein by High Speed AFM

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Cytoplasmic dynein is one of cytoskeletal motor proteins and processively moves along a minus-end-directed microtubule due to ATP hydrolysis. So far, several studies based on X-ray crystallography and single-molecule fluorescence microscopy have been performed to elucidate the molecular mechanism of dynein motility. However, no studies have been performed to directly observe the conformational dynamics of dynein in action due to the lack of technique. Here, we applied high-speed AFM to observing conformational dynamics of yeast and human cytoplasmic dyneins in action. In the presentation, we will show the recent progress of this observation.

3P042 ハミルトニアンレプリカ置換分子動力学法の Aβ フラグメントへの応用
Applications of the Hamiltonian replica-permutation molecular dynamics simulations to Aβ fragments

Satoru G. Itoh[1,2], Hisashi Okumura[1,2] ([1]*IMS*, [2]*Sokendai*)

We have recently proposed the Hamiltonian replica-permutation method (HRPM). HRPM combines the advantages of RPM and the Hamiltonian replica-exchange method (HREM). RPM is a better alternative to REM. In RPM not only temperature exchanges between two replicas but also temperature permutations among more than two replicas are performed with the Suwa-Todo algorithm. In HREM, by exchanging the parameters that are related only to limited degrees of freedom, the number of replicas can be decreased in comparison with REM. HRPM realizes efficient sampling more than HREM and is able to reduce the number of replicas in comparison with RPM. We will introduce HRPM in our presentation. The results of HRPM simulations with Aβ fragments will also be shown.

3P043 Mutagenesis study of an antifreeze protein isoform from a snow-mold fungus, Typhula ishikariensis

Jing Cheng[1,2], Yuichi Hanada[1,2], Hidemasa Kondo[1,2], Sakae Tsuda[1,2] ([1]*Grad. Sch. Life. Sci., Hokkaido Univ*, [2]*Biopro. Res. Inst., AIST*)

Antifreeze proteins (AFPs) facilitate cold-survival of the organisms living under freezing environment by inhibiting ice crystal growth. In this study, we focused on a fungal AFP denoted TisAFP secreted from a psychrophilic snow-mold fungus, Typhula ishikariensis. It reported seven isoforms of TisAFP that share a high sequence identity. Significantly, fluorescence-based ice plane affinity (FIPA) analysis showed that the ice binding specificity of TisAFP8 is quite different from the others. To clarify what determines the uniqueness of TisAFP8, we performed mutagenesis experiments on TisAFP8, especially on the residues constructing its putative ice-binding site. The data suggested an importance of inward pointing residues not just outward pointing residues of TisAFP8.

3P044 Thg1-like タンパク質の機能構造解析
The functional and structural analysis of Thg1-like protein

Shoko Kimura[1], Tateki Suzuki[1], Jian Yu[2], Keisuke Komoda[3], Isao Tanaka[2], Min Yao[1,2] ([1]*Grad. Sch. Life Sci., Univ. Hokkaido*, [2]*Fac. Adv. Life Sci., Univ. Hokkaido*, [3]*Grad. Sch. Agri Life Sci., Univ. Tokyo*)

A guanylyl base (G_{-1}) of histydyl tRNA ($tRNA^{His}$) is an important element for tRNA recognition of HisRS. In eukaryotes, G_{-1} is added by $tRNA^{His}$ guanylyltransferase (Thg1) which functions as a reverse polymerase. Interestingly, some archaea and bacteria possess Thg1-like-protein (TLP) although G_{-1} of $tRNA^{His}$ is genomically encoded. Previous studies show that archaeal TLPs add nucleotides to 5'-truncated tRNAs, suggesting that TLPs may work as a repair enzyme of tRNAs. Moreover, there are differences in tRNA recognition and cofactor affinity between TLPs and Thg1. Here, we determined the structures of archaeal TLP (aTLP), aTLP-ATP, and aTLP-GTP. The structural differences between TLPs and Thg1 provide important information for understanding the reaction mechanism of TLP.

3P045 Staphylococcal nuclease と Δ44-49 変異体の構造揺らぎの解析：酵素活性への洞察
Analysis of the structural fluctuation in Staphylococcal nuclease and its Δ44-49 mutant: Insight into the enzymatic activity

Kana Fuji[1], Hiroshi Fujisaki[2], Tadaomi Furuta[3], Rumi Shiba[4], Mikito Toda[1] ([1]*Nara Women's Univ.*, [2]*Nippon Med. Sch.*, [3]*Tokyo Tech*, [4]*JAIST*)

Staphylococcal nuclease (SNase) is one of the proteins whose structural fluctuations play important roles in enzymatic activities. Omega-loop deletion mutant (Δ44-49) of SNase shows reduced enzymatic activity, while its structure and ligand-binding activity are similar to those of the wild type (Wt). However the relationship between the enzymatic activity and fluctuations still remains unclear. To investigate the structural fluctuations of SNase related to the enzymatic activity, MD simulations of Wt and Δ44-49 were conducted in three ligand-binding states: a ligand-free, Ca^{2+}-bound, and prAp & Ca^{2+}-bound states. We will show the results of the simulations and discuss the role of the structural fluctuation on the enzymatic activity.

3P046 サルモネラ菌べん毛繊維の多型変換における Glu114 と Glu121 の役割
The roles of Glu114 and Glu121 of flagellin in the polymorphic transformation of Salmonella flagellar helical filament

Atsushi Ujiie, Fumio Hayashi, Kenji Oosawa (*Div. Mol. Sci, and Tech, Gunma Univ*)

The salmonella flagellar filament is a biological nanomachine acting as a screw to move the cell. The filament transforms among several helical structures, which are different in curvature and twist, and such morphological transformation is called polymorphic transformation. From suppressor mutant assays, we suggested that seven amino acid residues play important roles on transformation. Previously, we supposed that Arg431, one of the key residues, interact with the side chain of Gln117. From mutation assays at the sites, there were no apparent result. In this study, we suppose that Arg431 forms salt bridges with both Glu114 and Glu 121, and report filament shapes with mutations at twise sites.

3P047 Building an Artificial Protein Capsid

Jonathan Heddle (*RIKEN*)

A stable ring-shaped protein can be transformed into a regular hollow sphere structure - similar to a viral capsid simply by the addition of gold nanoparticles. The mechanism whereby this occurs is not clear. We have tried to understand the driving forces behind this assembly by using a mixture of microscopy and biochemical techniques and the results sugget a highly unusual process and unique protein structure. The work sheds new light on the special catalytic properties of gold particles in relation to biological molecules as well as introducing some new features of protein complex self assembly that may be useful in bionanoscience and drug delivery.

Malay, A. D. et al. Gold Nanoparticle-Induced Formation of Artificial Protein Capsids. Nano Lett. 12, 2056-2059 (2012).

3P048 溶解性制御タグ（SCP タグ）によるタンパク質結晶化の解析
Analysis of protein crystallization using short Solubility Controlling Peptide tags

Yutaka Kuroda, Mohammad Islam (*TUAT, Dept of Biotech and Life Sci*)

Short peptide tags attached to recombinant proteins are emerging as important tools for biochemical research. Here, we report the effects of ten Solubility Controlling Peptide tags on the crystallization behavior of a Bovine Pancreatic Trypsin Inhibitor (BPTI) variant. The tags did not affect the structure, stability, and activity of BPTI. Moreover, eight of the tagged variants crystallized under the same condition and six of them diffracted at high resolution. All variants with long-term solubility (LS) between 1-6mg/mL produced good crystals, while variants with LS>6mg/mL crystallized poorly. SPC tags introduce a mere 3-5 residues elongation and could thus provide a useful technology for tuning protein solubility without affecting its other properties.

3P049 Multimodal chromatography of proteins in arginine solutions

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Arginine is effective in elution of proteins from chromatography columns. In this study, effects of arginine on the elution from multimodal chromatography columns, the resins of which have multiple functional groups, were examined using bovine serum albumin and a monoclonal antibody against interleukin-8. The resins used here were Capto MMC and Capto adhere, which are multimodal cation and anion exchangers, respectively. As expected, arginine effectively eluted the proteins from the columns. Mechanism of the elution was examined by molecular dynamics simulations. The results showed that the affinity of arginine was primarily associated with electrostatic interaction for Capto MMC and with hydrophobic and π-π interactions as well as hydrogen bonding for Capto adhere.

3P050 フェリチン・ヘテロオリゴマーの作製
Construction of ferritin hetero-oligomer

Atsushi Kurobe, Satsuki Takebe, Daisuke Sato, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. Bioinfo., Soka Univ.*)

Ferritin (Ftn) is a spherical shell-shaped 24-mer protein. It dissociates to dimers at acidic pH, and is able to reassemble to 24-mer at neutral pH. Utilizing this property, a hetero 24-mer may be designable from different mutant Ftns. To test this possibility, we constructed several mutants that have different net charges. After dissociation at acid pH, different mutants were mixed, and then reassembled. Resultant hetero 24-mers were analyzed by analytical gel filtration, circular dichroism, and native polyacrylamide gel electrophoresis (PAGE). In the native PAGE, the hetero 24-mer originated from a mutant and wild-type (WT) Ftns showed a single band with the mobility between those of the mutant and WT, indicating that a hetero 24-mer has limited compositions.

3P051 蛋白質系の静電自由エネルギーにおける有限サイズ効果
Finite-size effect on the charging free energy for protein system

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The electrostatic interaction is a key for determining the protein stability in solvent. The solvent effect on the stability is governed by the free energy of solvation and its electrostatic component is quantified by the charging free energy. The finite-size effect refers to the cell-size dependence of the charging free energy at different cell sizes. This effect is one of the difficulties of MD simulations, and its correction is needed to compare a computed energetic quantity with the experimental. We examine this effect on protein with explicit solvent over a variety of the charged states and cell sizes. We show the extent of this effect on protein, assess the performance of the self-energy correction proposed by Hummer et al., and propose an additional correction.

3P052 Analysis for the structural stability of chignolin

Yutaka Maruyama, Ayori Mitsutake (*Dep. Phys., Keio Univ.*)

Chignolin, which consist of ten amino acid residues, is used for verification of various techniques. We investigated how the water solvent would affect the structural stability of chignon in this study. We generated various structures by the molecular dynamics simulation and calculated the hydration free energy using the 3D-RISM theory. The hydration free energy of the denatured state is lower than that of the native state. Thus the water around chignolin acts so as not to contribute to the folding, but it smooths the total free energy surface.

3P053 圧力効果を用いたシトクロム c の立体構造形成過程における脱水和機構の解析
Dehydration in cytochrome c folding revealed by high pressure spectroscopy

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Dehydration is a key step of protein folding. While the dehydration from peptide chains was supposed to be the primary driving force of the protein folding, the molecular mechanism is still unclear. Since the dehydration is reflected in the partial molar volume change (ΔV), we estimated ΔV for the cytochrome c folding by using high pressure spectroscopy. The pressure dependence of the absorption spectra in the presence of various concentrations of the denaturant clearly showed negative ΔV, which is characteristic of the dehydration from hydrophobic groups. Interestingly, ΔV for the urea denaturation was more negative than that for the guanidine hydrochloride denaturation. Based on the results, we will discuss molecular mechanisms of the dehydration on protein folding.

3P054 蛍光分光法による時計タンパク質 KaiC の構造変化の解析
Conformational transition of a cyanobacterial clock protein KaiC monitored with fluorescence spectroscopy

Atsushi Mukaiyama[1,2], Shuji Akiyama[1,2,3] ([1]*CIMoS, IMS*, [2]*Grad. Univ. for Adv. Studies*, [3]*RIKEN, SPring-8*)

Cyanobacterial circadian clock is composed of the three proteins, KaiA, KaiB, and KaiC. *In vitro* incubation of the three Kai proteins with ATP generates the phosphorylation cycle of KaiC with a period of approximately 24h.
Previous study has revealed that hexameric KaiC expands and contracts its C-terminal ring during circadian oscillation in order to assemble/ disassemble with KaiA and KaiB, while the conformational transition of its N-terminal ring remains to be well analyzed.
To examine the conformational transition of the N-terminal ring of KaiC, we constructed a mutant having a fluorescent probe at the N-terminal ring and tracked the conformational transition of this mutant using fluorescence spectroscopy. In this meeting, we are going to discuss the details.

3P055 Improved Multi-Replica Metadynamics for Free Energy Calculations

Raimondas Galvelis[1], Yuji Sugita[1,2] ([1]*RIKEN TMSL*, [2]*RIKEN AICS*)

Metadynamics (MTD) is an enhanced sampling algorithm applicable for the computational studies of biosystems with high energy barriers and slow modes of motion. It based on a history-dependent bias potential in a collective variable (CV) space. In particular, we are focused on multi-replica approaches such as the bias-exchange MTD (BE-MTD), where the CV space is divided into lower dimensionality subspaces for efficiency and scalability. We demonstrate that BE-MTD can be improved (shorter simulation times are required for the same accuracy) by replacing Metropolis-Hastings algorithm with advanced replica exchange schemes. Benchmarks on alanine polypeptides and a small protein are shown.

3P056 タンパク質の高速折り畳みダイナミクスの一分子追跡を目指したライン共焦点顕微鏡の開発

若手招待講演

Development of the line confocal system for the single molecule tracking of fast folding dynamics of proteins

Hiroyuki Oikawa[1], Kiyoto Kamagata[1], Munehito Arai[2], Atsuhito Fukasawa[3,4], Hiroaki Yokota[4], Toru Ide[5], Satoshi Takahashi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Arts. Sci., Univ. Tokyo*, [3]*Hamamatsu Photonics*, [4]*GPI*, [5]*Grad. Sch. Nat. Sci. and Tech., Okayama Univ*)

Single-molecule measurements are expected to reveal the detailed process of protein folding. By using the line confocal system, we could trace the single-molecule time evolution of FRET efficiency from the B domain of protein A (BdpA) with the time resolution of ~100 μs. The time series from the BdpA suggest conformational heterogeneity in the unfolded state. In the case for our existing system, however, there is a trade-off between the time resolution and the observation time for single-molecule tracking. To obtain the better time resolution, we built a new system based on hybrid photo detectors. We could obtain time series with the time resolution of ~10 μs and the observation time of ~ 10 ms. We will discuss the time series obtained by the improved system.

3P057 アミノ酸置換による蛋白質の熱安定性変化の理論的予測

Theoretical Prediction of Thermal-Stability Changes upon Mutations of a Protein

Shota Murakami[1], Hiraku Oshima[2], Tomohiko Hayashi[2], Masahiro Kinoshita[2] ([1]*Grad. Sch. Energ. Sci., Kyoto Univ.*, [2]*Inst. Adv. Energ., Kyoto Univ.*)

We have recently proposed a measure of the thermal stability of a protein, Σ: the water-entropy gain upon folding at 298 K normalized by the number of residues. It is calculated using a molecular model for water. In the present study, we apply Σ to the prediction of thermal-stability changes upon mutations of a protein. The structure of the mutant is assumed to be unknown. We find that the performance becomes considerably higher when an enthalpic component is incorporated in the measure: The resultant measure is denoted by Σ_{New}. Let ΔX be "X of the mutant" minus "X of the wild type". We plot ΔT_m (T_m is the denaturation temperature) against $\Delta\Sigma_{\mathrm{New}}$ for significantly many proteins and find that the two quantities exhibit fairly high correlation.

3P058 バクテリア細胞質中の生体高分子ダイナミクスと相互作用：全原子分子動力学による研究

Dynamics and Interactions of Macromolecules in the Bacterial Cytoplasm: All-atom Molecular Dynamics Study

Isseki Yu[1], Tadashi Ando[2], Takaharu Mori[1], Jaewoon Jung[3], Ryuhei Harada[3], Yuji Sugita[1,2,3], Michael Feig[4] ([1]*RIKEN*, [2]*RIKEN QBIC*, [3]*RIKEN AICS*, [4]*Michigan State Univ.*)

Discovering the molecular-level dynamics and interactions of macromolecules in realistic cellular environment is one of the major challenges for biophysical scientists. For this purpose, we constructed all-atom model of cytoplasm in the Mycoplasma Genetalium, the smallest known bacteria. The size of the system is 100 nm x 100 nm x 100 nm, which is greatly exceeds that of typical molecular dynamics (MD) simulations, covering about 10% of the volume of an entire bacterium cell. Using the atomic trajectories generated by highly parallelized MD program GENESIS on K computer, translational and rotational diffusion of macromolecules are analyzed. The influence of crowding environment and protein-protein interactions on the dynamics of individual macromolecules are discussed.

3P059 回転および並進運動から観たタンパク質間相互作用

Protein-protein interaction revealed by the rotational and translational motion

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Precise description for weak protein-protein interaction has not been established. In the present studies, we extracted the experimental parameters concerning with the interaction from the diffusion of protein. The fluorescence anisotropy and correlation were employed for the interaction between lysozymes. The interaction parameter, k_{rot} estimated from the rotational diffusion, showed that the attractive interaction would be induced by monovalent cations. Since k_{rot} could discriminate the effect of cations, it is concluded that k_{rot} is an appropriate parameter for the weak protein interaction. The other parameter, k_{trans} was more effective for weaker interaction. Based on the results of k_{rot} and k_{trans}, protein-protein interaction induced by cations is discussed.

3P060 蛋白質のドメイン間相互作用に及ぼすホフマイスター効果

Hofmeister effect on the domain-domain interaction of protein

Tomohiro Aoyama[1], Etsuko Nishimoto[2] ([1]*Institute of Biophysics, Faculty of Agriculture, Graduate School of Kyushu University*, [2]*Molecular Bioscience, Bioscience and Biotechnology, Kyushu university*)

Hofmeister effect is generally known as the cation or anion effect on the protein solubility and stability. Because of concerning with various biological phenomena, vigorous theoretical and experimental approaches have been examined. But the details are remained unclear. In the present investigation, monovalent cation effects on the domain-domain interaction of GFP-GST fusion protein. Using FRET between GFP chromophore and Cy3 conjugated with the terminal residue of GST, the change in the FRET distance were evaluated to be LiCl>NH_4Cl>NaCl>KCl>CsCl. This result suggests the domain-domain interaction would respond to the usual Hofmeister series. But it should be also interesting to note that LiCl and NH_4Cl shrunk FRET distance while other cations prolonged.

3P061 The circumventing mechanism of the folding of β-lactoglobulin

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Bovine β-lactoglobulin (βLG) has a folding intermediate with a non-native α-helical structure. Our previous study indicated that the moderate α-helical propensity of the wild-type sequence likely contributes to circumventing non-productive intermediates. In the present study, we analyzed the dynamics of the denatured βLG and performed high-pressure NMR measurements and H/D exchange pulse labeling experiments to obtain structural information of the intermediates. The results suggested that the three portions of the sequence, the C-terminal, the middle, and the N-terminal regions, sequentially attain individual native structures. Probably, the order of folding of these regions is programed in the βLG sequence to avoid non-native aggregations.

3P062 アポミオグロビンのドメインスワッピングとフォールディングの競合:分子シミュレーション解析

Monomer folding versus dimer domain-swapping in apo-myoglobin studied by molecular simulations

Koji Ono[1], Mashiho Ito[1], Shun Hirota[2], Shoji Takada[1] ([1]*Dept. Biophys., Grad. Sch. Sci., Kyoto Univ.*, [2]*Nara Inst. Sci. Tech.*)

Recent experiments showed that myoglobin can form a domain-swapped dimer in a certain solvent condition. Here, using a coarse-grained symmetrized Go model, we performed a series of folding simulations of two apo-myoglobin molecules restrained in a high density condition, addressing competition of formation of a domain-swapped dimer with folding to two monomer structures. In addition to the domain-swapped dimer found in the X-ray crystallography, we also found some other forms of domain swapping. Folding pathway analysis clarified that separation between monomer folding and domain-swapping arose at a relatively early state, where inter-chain contacts between helices AB of one chain and helices GH of another chain tend to result in the domain swapping.

3P063 超音波によるアミロイド線維形成と分解

Ultrasonication dependent induction and degradation of amyloid fibrils

Sayaka Noda, Masatomo So, Masayuki Adachi, Yuji Goto (*Inst. Protein Res., Osaka Univ.*)

Amyloid fibrils, associated with more than 20 degenerative diseases including Alzheimer's disease, has been thought to be highly stable aggregates consisting of cross β structure. Recently, our group reported that ultrasonication is one of the powerful methods of agitation for accelerating spontaneous fibrillation. More recently, it has been found that high amplitude of ultrasonication also induces the decrease of Thioflavin T fluorescence intensity, suggesting that fibrils convert to amorphous aggregates. Here, we compared the effects of ultrasonication on the fibrils and native monomers using human insulin and β_2-microglobulin. We discuss the stability of fibrils and monomers against surface denaturation at the cavitation bubbles induced by ultrasonication.

3P064 中性子散乱によるヒトα-シヌクレインのダイナミクス変化の検出

Changes in the dynamics of human α-synuclein detected by neutron scattering

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Amyloid fibrils of α-synuclein (α-Syn) (and/or its intermediate structures toward the mature fibrils) is involved with pathogenesis of Parkinson's disease. Elucidation of the mechanism of amyloid fibril formation of α-Syn is thus important. Here we compared the dynamic behavior of α-Syn in the monomeric and the fibril states by quasielastic neutron scattering experiments using the backscattering spectrometer BL02 (DNA) at MLF/J-PARC. It was shown that in the fibril state, amplitudes of the local motions such as side-chain motions increase in fibril state. Since the increase in the amplitudes of the local motions implies wider distributions of conformational substates, this result suggests that the fibril state is entropically favorable.

3P065 変異体解析を用いた緑色蛍光蛋白質の安定化機構におけるヒスチジン残基の役割に関する研究

The role of histidine residues with abnormal pK_a values on the stability of green fluorescent protein studied by mutagenesis approach

Taichi Andou, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ*)

Green fluorescent protein (GFP) accumulates a native-like equilibrium intermediate (N') at pH~5. N' emits reduced green fluorescence compared with that of the native state. The accumulation of N' is suggested to arise from abnormal pK_a values of histidine residues of GFP.
For the purpose of understanding the role of the histidine residues on the stability of GFP, here we constructed a series of variants by replacing the histidines with other amino acids and measured the acid-induced equilibrium unfolding transition curves by monitoring the tryptophan and chromophore fluorescence. The results indicated that buried histidine residues are responsible for the stability of GFP. The contribution of each histidine residue to the pH-dependent stability of N' will be discussed.

3P066 ウマアポミオグロビンの pH 4 中間体と塩による中間体の速度論的性質

Kinetic properties of pH-induced and salt-induced intermediates of horse apomyoglobin

Yukiko Abe, Takuya Mizukami, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ.*)

Apomyoglobin accumulates not only kinetic intermediates during the folding, but also equilibrium intermediates under various denaturing conditions such as at pH ~ 4 ($I_{pH\,4}$)) or at pH 2 and high salt concentrations (I_{salt}). $I_{pH\,4}$ is considered to be a counterpart of kinetic intermediates. In this study, we measured kinetic folding reactions of these intermediates and compared their kinetic properties. Urea concentration dependence of refolding from acid unfolded state to these intermediates were observed by monitoring tryptophan fluorescence. From these results, $I_{pH\,4}$ and I_{salt} have similar kinetic property; additional intermediate, I', is accumulated before the formation of $I_{pH\,4}$ or I_{salt} for both of them.

3P067 大量のアルカンを合成するシアノバクテリア変異体の構築
Toward the construction of the cyanobacterial mutants that produce high amounts of alkanes

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Cyanobacteria synthesize a small amount of hydrocarbons accessible as biofuels. This reaction is catalyzed by acyl-(acyl carrier protein) reductase (AAR) and aldehyde decarbonylase (AD). Because both enzymes have low activities, mutational studies for improving their activities are ongoing. It is expected that cyanobacteria expressing highly active mutants of AAR and AD produce high amounts of alkanes. To ensure this, here we carried out transformation of the cyanobacteria Synechocystis sp. PCC6803 with the plasmid containing the wild-type AAR and AD genes. We found that these cyanobacterial mutants produce higher amounts of heptadecane than the control strain, suggesting that expression of highly active mutants of AAR and AD will further improve alkane biosynthesis.

3P068 アルカンを合成するラン藻由来アルデヒド脱カルボニル化酵素のアラニンスキャン変異解析
Alanine scanning mutagenesis of cyanobacterial aldehyde decarbonylase that synthesizes alkanes

Fumitaka Yasugi[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

Cyanobacteria synthesize a small amount of alkanes from fatty acyl-ACP using aldehyde decarbonylase (AD). However, little is known about its function. To clarify which residues are responsible for its catalytic activity, we carried out alanine-scanning mutagenesis of AD from *Nostoc punctiforme* PCC73102 (232 residues). The amounts of hydrocarbons produced in *E. coli*, in which mutant AD is overexpressed, were measured by GC-MS. We find that some residues are essential for the activity of AD, while changes at other sites enhanced the activity. These mutations are useful in improving the alkane production of AD.

3P069 Pre-steady state kinetic studies of redox reactions between FNR from *Bacillus subtilis* and its substrates

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Ferredoxin-NADPH oxidoreductases (FNRs) from *Bacillus subtilis* (BsFNR) are a unique homo-dimeric flavoprotein with a significant structural homology to the bacterial NADPH-thioredoxin reductase (TrxR). We report the pre-steady state kinetic studies of redox reactions of BsFNR by a stopped-flow spectrophotometry. Reaction with NADP/H yielded a rapid formation of two kinds of charge transfer complexes followed by a rate determining hydride transfer. The rate for the reduction by NADPH was much faster than that for the oxidation by NADP. Mixing reduced BsFd with oxidized BsFNR demonstrated a rapid formation of one-electron reduced neutral semiquinone radical followed by a slow reduction to the hydroquinone form.

3P070 一分子蛍光顕微鏡による p 53 の標的配列探索ダイナミクスの観察
Observation of the Search Dynamics of p53 for the Target DNA Sequence by Single-molecule Fluorescence Microscopy

Yuji Itoh[1,2], Agato Murata[1,2], Seiji Sakamoto[1,2], Takehiko Wada[1,2], Satoshi Takahashi[1,2], Kiyoto Kamagata[1,2] ([1]*IMRAM, Univ. Tohoku*, [2]*Grad. Sch. Sci., Univ. Tohoku*)

The tumor suppressor p53 binds to the target sequences of DNA and induces cell cycle arrest, apoptosis, or DNA repair. However, the detailed search mechanism of p53 for the target sequences remains unclear. In this study, we observed the 1D sliding movement of p53 along DNA containing the target sequence by using TIRF microscopy. p53 was labeled with atto532. The DNA containing the target sequence in its center was attached on a flow cell. The analysis of the trajectories of p53 sliding along the DNA revealed that the averaged diffusion coefficients are 0.110 ± 0.028 and 0.085 ± 0.011 $\mu m^2/s$ with and without a flow pressure, respectively. We observed that p53 passed over the target sequence a few times, suggesting that the binding efficiency of p53 is not 100%.

3P071 銅含有亜硝酸還元酵素の亜硝酸還元メカニズムにおける計算化学研究
Computational study on nitirite reduction mechanism in Copper-containing nitrite reductase

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Copper-containing nitrite (CuNiR) reductase catalyze the reduction of nitrite to nitric oxide. The CuNiR reduction mechanism is supposed to have two steps: H-addition to nitrite at T2Cu site, and electron transfer from T1- to T2Cu. However, the exact order of these two steps, and the details of H-addition mechanism are still under debate. In this study, we use computational methods to investigate the binding form of nitrite at T2Cu and the following catalytic reduction mechanism of CuNiR. Our results show that the binding form and the stability of nitrite depend on the protonation states of amino acid residues and the oxidation states of T2Cu. H-bonding network around reaction site is also found to have a very important role on H addition to nitrite.

3P072 ポリアミンは α-キモトリプシンの活性化剤として機能する
Polyamines Act as an Enzyme Activator for α-Chymotrypsin

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Polyamines are multifunctional metabolites in living cells. It is known that polyamines bind to biomolecules through electrostatic interaction, resulting in stabilization and modulation of the biological entity. Here, we demonstrate that polyamines act as a nonessential enzyme activator for α-chymotrypsin (ChT) that catalyzed to anionic substrates. The catalytic rates of ChT increased 2.1-, 4.6-, and 5.6-fold in the presence of 50 mM putrescine, spermidine, and spermine, respectively, at pH 7.5. Enzyme kinetic study revealed that polyamines increased the catalytic constants (k_{cat}), whereas slightly decreased the Michaelis constants (K_M). It was suggested that enhancement of the catalytic cycles of ChT were contributed with a binding of polyamines to ChT.

3P073 膜表面の GM1 糖鎖に対する hGal-1 結合能「増大」
"Increased" affinity of hGal-1 to GM1 on membrane surface

Ryota Hori, Hirotsugu Hiramatsu, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

Human galectin-1 (hGal-1), a soluble lectin, exists in homodimer under physiological conditions. hGal-1 plays important roles in cell adhesion and signaling by cross-linking multiple ligands, which may gather to form microdomains on membrane surfaces. We, therefore, studied differences in the binding of hGal-1 to the ligands on the membrane surfaces and in solution. We prepared 1 mM liposome using neutral lipid (POPC) and glycolipid (ganglioside GM1), and measured fluorescence spectra of Trp68 in hGal-1 for different fraction of GM1 to POPC. Both the binding constant and the cooperativity of hGal-1 to GM1 became larger on the membrane than those in solution, suggesting that the clustering of GM1 on the membrane surface increase in the affinity of GM1 to hGal-1.

3P074 抗菌ペプチドを用いた病原性微生物検出系のための新規スクリーニング法の開発
Novel screening method for detection system of pathogens using antimicrobial peptides

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Antibody-based assays such as an enzyme-linked immunosorbent assay (ELISA) are important techniques for rapid and efficient detection and identification of pathogens. However, in the case of some target pathogens, it is very difficult to develop antibodies with both high sensitivity and high specificity and practical pathogen detection systems. To solve this problem, we previously developed a colorimetric sandwich assay for detection of pathogens by using antimicrobial peptides (Amps) as detection probes.
To improve the performance, we developed a rapid and simple method to screen a large number of Amps using thioredoxin fusion expression system. Moreover, this method was also able to be used as screening for Amps with potential antimicrobial activity.

3P075 抗菌ペプチド human defensin 5 の NMR による多量体形成機構の解析
NMR analysis of the oligomerization mechanism of antimicrobial peptide human defensin 5

Arata Hashimoto, Satoshi Tomisawa, Masakatsu Kamiya, Takashi Kikukawa, Yasuhiro Kumaki, Kiminori Nakamura, Tokiyoshi Ayabe, Tomoyasu Aizawa, Makoto Demura (*Grad. Sch. Life Sci., Hokkaido Univ.*)

Human defensin 5 (HD5), one of the human alpha-defensins is key player in the innate immune response in the gastrointestinal and serve to prevent colonization by invading pathogenic microbes. Recent research has revealed HD5 forms dimer and tetramer in aqueous solution and the oligomerization state is condition-dependent. Although the oligomerization is considered to be important for antimicrobial activity, the oligomerization mechanism is only partially understood. To gain additional insights into the oligomerization mechanism, we tried to determine the structure of HD5 tetramer. We established a new high expression system of HD5 and used NMR spectroscopy to determine the interfaces of the tetramer.

3P076 トレオニン合成酵素の反応制御機構解明のための分子動力学計算：反応中間体間自由エネルギー評価
Molecular dynamics study on the reaction control mechanism of threonine synthase: evaluating the free energies of the intermediate states

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Threonine Synthase (ThrS) catalyzes a formation of L-threonine from O-phospho-L-homoserine. A series of reactions catalized by ThrS is complicated and full of regiospecific and stereospecific steps. Therefore, the mechanisms of the reaction controls (product-assisted catalysis) are not yet elucidated.
In this study, molecular dynamics simulations of ThrS were performed with the thermodynamics integration approach, and accurate free energy differences between different intermediates were evaluated by changing the phosphate ion, which is one of the products of the enzyme reaction, to a sulfate ion. The molecular mechanism of the product-assisted catalysis was examined.

3P077 ウリジンシチジンキナーゼの基質結合相互作用についての理論的研究
A theoretical study on the substrate bindings in uridine-cytidine kinase

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Uridine-cytidine kinase (UCK) catalyzes the phosphorylation of uridine and cytidine. UCK from *Thermus thermopiles* HB8 (ttCK) has a substrate specificity toward only cytidine. It was revealed experimentally that Y93H mutant of ttCK has a phosphorylation activity toward both uridine and cytidine, but the detailed mechanism in a molecular level has not been elucidated.
In this study, we investigated the substrate specificities of ttCK with MD simulations and the MM-PBSA method. By calculating the binding free energy between ttCK and substrates, we have reproduced that ttCK binds uridine less stably than cytidine. Uridine in the keto form causes steric hindrance, thus we also investgated the binding stabilities between ttCK and uridine in other protonation states.

3P078 Role of FAD N5 proximal Asn residue in CPD-Photolyase

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Cyclobutane Pyrimidine Dimer-Photolyase (CPD-PHR) is a DNA repair enzyme that specifically repairs CPD-type UV-induced DNA damage. CPD-PHR possesses FAD as a chromophore, and asparagine residue (Asn341) near the N5 position of FAD. Using FTIR spectroscopy, we aim to elucidate the roles of Asn341 for stabilization of FADH°.
From the large downshifted signals at 1800-1600 cm^{-1} of the uniformly ^{13}C-labeled enzyme, signals from Asn upon FAD activation could be observed by reverse labeling of Asn (unlabeled Asn in the ^{13}C-labeled enzyme). We found hydrogen bonding changes in the carbonyl of Asn with the N5-H group of FAD upon photoactivation. New insights into the stabilization mechanisms for the FADH° state in CPD-PHR will be discussed on the basis of the FTIR results.

3P079 抗菌ペプチド cecropin P1 の大腸菌発現系における発現効率に影響を与える要因の解明

Elucidation of influential factor for productivity of the antimicrobial peptide using *Escherichia coli*

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Cecropin P1 (CP1), an antimicrobial peptide found in nematodes from the stomachs of pigs, is 31 amino acids in length. In a membrane-like environment, CP1 forms an α-helical structure and believed to damage microbial membranes. We designed a soluble fusion protein that contains thioredoxin on the N-terminal side of CP1. This fusion construct still showed the cytotoxicity upon the expression in the *Escherichia coli* cells. However, this toxic behavior was improved when we tried the expression of the derivatives lacking a few C-terminal residues. In this study, we focused on the C-terminal region of CP1 and investigated the contribution of this region to the inhibition of *E. coli* growth and to the interaction with *E. coli* membranes using some fluorescent dyes.

3P080 ベイズ推定を用いた NMR 立体構造計算法の開発

A refinement method for NMR protein structure determination based on Bayesian inference

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Conventional NMR structure determination requires to determine a weight factor for balancing experimental data and a physical forcefield, and to estimate calibration constants that convert NOE data into three-dimensional structure information. Thus, it is necessary in the conventional method to empirically fix those parameters despite of different attributes of the experimental data derived from noise and ambiguities. We developed a refinement method for NMR protein structure determination based on Bayesian inference.
The new structure calculation method shows that it can provide sufficiently accurate structures even with less NOE peaks. Moreover, it dramatically improves protein structures of the proteins in living E.coli cells.

3P081 複雑分子系の異性化反応ネットワークに埋め込まれた時間階層構造の抽出

An extraction of hierarchical organization of embedded timescales buried in complex reaction network

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Reactions involved in complex molecular systems such as proteins have a variety of timescales from fs to seconds or much longer even in a molecule. To provide theoretical framework for multiple timescales systems, we develop a novel method for constructing a timescale hierarchal diagram of a Markov chain (or “energy landscape”). Since one can derive a Markov chain from a 1st order chemical reaction, our method can capture the hierarchical organization of embedded timescales buried in complex reaction network. As an illustrative example, we apply our method to a reaction network of Claisen rearrangement of allyl vinyl ether, computed quantum-chemically in terms of the global reaction route mapping method and compare the results with the experimental rate constant.

3P082 RI に依存しない高感度 MGMT 活性測定法の開発と新型マイクロアレイ MMV への適応化

Development of MGMT activity assay methods of high sensitivity and being adaptabe to the novel-concept microarray

Aya Hongo, Takuto Saiki, Ran Gu, Miho Suzuki, Naoto Nemoto, Koichi Nishigaki (*Grad. Sch. of Sci. and Eng., Univ. Saitama*)

For the treatment of a brain cancer gliobrastoma, MGMT behaves as a suppressor of anti-cancer drug TMZ (temozolomide) due to the cancellation effect of TMZ-derived methylation. We have devised a novel *in vitro* selection system based on MMV (well type novel concept microarray), which enables function-based screening of target molecules. For this screening system, RI-nondependent high sensitivity assay system of MGMT was prerequisite and thus we have devised and developed it originally with utilizing fluorescence and PCR technologies and adapting them to the MGMT system. These RI-nondependent approaches were estimated to be the highest sensitivity in measuring the MGMT activity, especially PCR-based one, constructing the function-based *in vitro* selection system.

3P083 1 分子イメージングによる PI3K の活性制御機構の解析

Analysis of the Regulation Mechanism of PI3K Activity by Live-cell Single-molecule Imaging

Seiya Fukushima[1], Satomi Matsuoka[2], Masahiro Ueda[1,2] ([1]*Graduate School of Science, Osaka University*, [2]*Riken Quantitative Biology Center*)

Cell migration depends on pseudopod formation, which is mediated by PIP3 that induces actin polymerization and is produced by PI3K. Previous studies proposed that an efficient migration requires a positive feedback loop that activates PI3K at the leading edge via F-actin-dependent membrane localization, although the mechanism is remained unclear. In this study, by single-molecule imaging of PI3K in living Dictyostelium cells, we found that membrane-binding lifetime was prolonged and lateral diffusion became immobile in the presence of functional F-actin. This indicates that F-actin stabilizes membrane binding of PI3K. By including an analysis of activation of PI3K by Ras-binding, we will discuss the essential molecular processes of the positive feedback.

3P084 X 線自由電子レーザーを用いたパターンマッチング法による第一原理構造モデリングの検討

Examination of ab initio structural modeling for the pattern matching method using X-ray free electron laser

Atsushi Tokuhisa, Osamu Miyashita, Florence Tama (*Advanced Institute for Computational Science, RIKEN*)

Coherent diffraction patterns observed by X-ray free electron laser provide information on the structure and dynamics of biomolecues. New computational approaches are necessary to annotate experimental data. We are exploring new algorithms to model biomolecular structures from a limited amount of experimental data. Such algortihms would rely on conformational sampling methods and similarity detection to experimental data. In our preliminary study, an exhaustive search approach is used, to generate the positions of gold colloids. Simulated diffraction patterns from modeled assemblies of gold particles are compared with the reference diffraction pattern to determine the actual structure of the colloids.

3P085 X 線 1 分子追跡法を用いたタンパク質過飽和溶液中における核形成前駆体クラスターの動態観察

Dynamical Observations of Prenucleation Clusters in Supersaturated Protein Solution from Diffracted X-ray Tracking

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Recently, comprehension of supersaturated phenomena in protein solution is one of the most important subject. For example, elucidation of amyloid fibril formation process which is known as a main causative agent causing Alzheimer disease. In our study, we utilized Diffracted X-ray Tracking (DXT) as a high time resolution (100us) and high positional accuracy (-pm) detection system for the time resolved dynamical interactions between a single gold nanocrystal and localized prenucleation clusters in supersaturated solution (at SPring-8,BL40XU). At this presentation, We explain the DXT method and obtained physical parameters of clusters as a dynamic, size and occupied spaces by DXT analysis. In finally, We present a comprehensive model of protein supersaturated solutions

3P086 細菌由来アルブミン結合ドメインの接触表面の模倣によるアルブミン結合ヒト型タンパク質のデザイン

Design of an Albumin-Binding Humanized Protein by Mimicking the Contact Surface of a Bacterial Albumin-Binding Domain

Satoshi Oshiro[1], Shinya Honda[1,2] ([1]*Dept. of Medical Genome Sci., Grad. Sch. of Frontier Sci., The Univ. of Tokyo*, [2]*BioMed. Research Inst., AIST*)

Attachment of a bacterial albumin-binding protein module is an attractive strategy to prolong the plasma residence time of protein therapeutics. However, the induction of an unfavorable immune reaction by bacterial albumin binding module-fused protein or peptide is concerned.
To address this issue, we designed an alternative albumin-binding protein by transferring key residues of a bacterial albumin-module, onto human derived 6 helix-bundle proteins. We utilized structural information to avoid the deleterious mutation in the design.
As a result, one of the designed proteins specifically binds to human serum albumin (HSA). Competitive binding assay and thermodynamics analysis showed that the best binder have similar HSA-binding site of bacterial module.

3P087 ファージディスプレイ法で同定した白金結合アミノ酸配列の解析

Analysis of a platinum-binding amino acid sequence identified by phage display

Asumi Kaji, Hiroya Niiro, Satoshi Akanuma, Tatsuya Uchida, Akihiko Yamagishi (*Tokyo University of Pharmacy and Life Sciences*)

Creation of a metal-binding site on a desired position of a protein may contribute to develop a highly sensitive biosensor. Previously, we created a platinum-binding loop on an artificial four-helix bundle protein, LARFH. In that study, we constructed a LARFH library that contained random amino acid sequences within a loop and then selected a LARFH variant interacting with platinum by using the T7 phage display system. The selected LARFH variant contained a 6-residue YKRGYK sequence at the loop. In this study, we analyzed the affinity of the LARFH variant to platinum by means of Quartz Crystal Microbalance analysis. As the result, we found that the YKRGYK sequence really enhances the affinity to platinum.

3P088 エングレイルドホメオドメインを用いた新たな転写因子の設計

Designing a new artificial transcription factor based on engrailed homeodomain

Tomoko Sunami, Hidetoshi Kono (*JAEA*)

Recently, zinc finger nucleases which are artificially fused a zinc finger transcription factor and a nuclease have been widely used for genome editing. However, the applications are limited because they only recognize GC-rich sequences. To make such artificial proteins recognize broader sequences other than GC-rich ones, we are rationally designing novel proteins based on an engrailed homeodomain. Engrailed homeodomain proteins recognize six bases. To recognize longer DNA sequences, we connected two homeodomains with a short linker. We will report that the designed molecule show a strong affinity and high specificity for the target sequence. We will also report a DNA sequence profile of the protein obtained by B1H assay.

3P089 Design of a peptide nanotube having the capability of rare metal binding

Keisuke Ogihara, Atsuo Tamura (*Univ. Kobe*)

There has been compelling need for recovery of precious rare metals from the urban mines these days. We focused on the peptide nanotube (PNT), which does not give a burden on the environment because it is biodegradable and water-soluble. It also leads to energy saving because it can be used at normal temperature and pressure. Therefore, PNT is valuable as a new material for the metal recovery technology. So far we have designed PNT by self-assembly of cyclic peptides. However, it can only bind to base metals. Here we try to transform the PNT to have the ability to bind the rare metals. As a result, it has been confirmed that the newly designed PNT can bind to palladium and silver, indicating that the PNT can be conferred the capability of rare metal binding.

3P090 脂肪酸アシル-ACP 還元酵素の迅速活性評価法の開発

Development of a high-throughput method to evaluate catalytic activity of fatty acyl-ACP reductase

Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Department of Life Science, The University of Tokyo*, [2]*PRESTO*)

Alkanes produced in microorganisms are focused on as renewable alternatives of biofuels. Cyanobacterial fatty acyl-ACP reductase (AAR) catalyzes the reduction of fatty acyl-ACP to fatty aldehyde, which is an initial step in alkane biosynthesis. However, due to low catalytic activity of AAR, it is required to improve its activity for industrial use. Directed protein evolution is a powerful method to improve an enzymatic activity, but it requires a rapid and convenient method to evaluate the activity of a library of mutants. Here, we developed a high-throughput method to evaluate the catalytic activity of AAR. This enables high-throughput screening of E. coli colonies expressing active AAR from random mutant libraries generated by error-prone PCR.

3P091 線虫 cytochrome b561 ホモログ Cecytb-1 の生理機能解析

Analyses on the physiological function of Cecytb-1, a cytochrome b561 homolog in Caenorhabditis elegans

Akie Tejima, Yurie Hirano, Masahiro Miura, Motonari Tsubaki (*Dept. of Chem., Grad. Sch. Sci., Kobe Univ.*)

Cytochrome b561 in neuroendocrine vesicles is known to have a role in regeneration of vesicular ascorbate (AsA) by a transmembrane electron transfer. Many b561 homologs were found to distribute in eukaryotes. There are 7 homologs in *C. elegans*. However, their physiological functions remain unclear. We attempted to clarify the function of Cecytb-1, the closest homolog to neural b561. Analysis of the microsomal fraction prepared from *P. pastoris* expressing the recombinant Cecytb-1 showed a characteristic peak in AsA-reduced UV-visible spectra. In the present study, we analyzed the localization of Cecytb-1 expression by *in situ* hybridization and immunostaining. Both results suggested that Cecytb-1 was expressed in dorsal region of adult worms.

3P092 一酸化窒素還元酵素における基質 NO 結合の分子機構

Molecular mechanism for substrate NO binding to bacterial Nitric Oxide Reductase

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Membrane-bound nitric oxide reductase (NOR) reduces NO to N_2O in microbial denitrification. Towards understanding the NO reduction mechanism in NOR, we focused on the NO binding to NOR, an initial process for the catalytic reaction. To identify NO channel(s), we utilized Xe whose properties, such as the solubility in water and the size, are similar to NO as a structural probe, and Xe binding sites were determined by X-ray structural analysis. Xe atoms tracked a Y-shaped channel from the protein surface to the heme/non-heme Fe active center, suggesting that the Y-shaped channel could be a potential NO access channel in NOR. We will also present the kinetic analysis of the NO binding and following reduction by flow-flash time-resolved visible absorption spectroscopy.

3P093 チトクロム *c* とチトクロム酸化酵素複合体の X 線結晶構造解析

X-ray structural analysis of the cytochrome *c* and cytochrome *c* oxidase

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Cytochrome *c* oxidase (CcO) reduces molecular oxygen, coupled with proton pumping using electrons from cytochrome *c* (Cyt.c). The electron transfer from Cyt.c to CcO is one of the critical processes in CcO. Although X-ray structures of mammalian CcO and Cyt.c have been reported at high resolution, no Cyt.c-CcO complex has been determined. For elucidation of the mechanism of electron transfer from Cyt.c to CcO, it is desirable to determine the crystal structure of the Cyt.c-CcO complex at high resolution.

We succeeded in obtaining the complex crystals reproducibly from mammalian purified sample (Cyt.c/CcO = 1.2). These crystals diffracted X-ray up to 1.8 Å resolution. The calculated density map revealed the presence of one cyt.c molecule bound to CcO monomer.

3P094 亜硝酸還元酵素と一酸化窒素還元酵素の相互作用解析

Analysis of the Interaction between Nitric Oxide Reductase and Nitrite Reductase

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Denitrification is a form of microbial anaerobic respiration, in which NO_3^- is converted to N_2 through four stepwise reduction processes. In this process, while cytotoxic NO is generated as an intermediate product by nitrite reductase (NiR), NO is rapidly degraded by nitric oxide reductase (NOR) to prevent NO from diffusing into cellular environment. We, therefore, hypothesize that NOR forms a complex with NiR for effective elimination of NO. To test this idea, interaction between NOR and NiR was examined by surface plasmon resonance (SPR). The addition of NiR to NOR immobilized onto the sensor chip gave an increase in SPR signal, demonstrating the interaction between NiR and NOR. Functional significance of the formation of NiR-NOR complex will be discussed.

3P095 ナノ構造電極上でのシトクロム P450 153A13a の電気化学触媒反応

Electrochemically-driven CYP153A13a reaction at nanostructured electrode

Yasuhiro Mie[1], Naoya Fujita[2], Toshio Cho[2], Yasuo Komatsu[1] ([1]*Bioproduction Res. Inst., AIST*, [2]*KNC Laboratories Co., Ltd.*)

Cytochrome P450 enzymes (CYPs) catalyze a vast array of oxidative biotransformations that are potentially useful for industrial and pharmaceutical synthesis. Constructing an electrochemically-driven CYP system, which does not require NADPH and additional redox protein(s) to activate the enzyme, is a promising way to develop the efficient screening system for the substrates of the enzymes. In this study, we prepared CYP153A13a and immobilized on the nanostructured electrode to investigate the electrocatalytic reaction of the enzyme. The direct electron transfer between the electrode and CYP was detected with a relatively larger rate constant compared to the conventional methods. We also observed the electrocatalytic currents corresponding to the substrate conversion.

3P096 一酸化炭素よりも酸素に対して高い親和性を示すミオグロビンの創製

Preparation of myoglobin mutants exhibiting preferential binding of oxygen over carbon monoxide

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We analyzed the oxygen (O_2) and carbon monoxide (CO) binding properties of some myoglobin mutants reconstituted with chemically-modified heme cofactors possessing a heme Fe atom with various electron densities. The study provided valuable information about the effects of amino acid replacements and changes in the electron density of the heme Fe atom (ρ_{Fe}) on the O_2 versus CO discrimination. We found that the preferential binding of O_2 over CO by the L29F mutant, where the Leu29 residue being replaced by Phe, was achieved through increasing ρ_{Fe}. We also found that the O_2 versus CO discrimination of the protein could be regulated over a range of ~10^5 through combined use of the amino acid replacements and the chemical modification of heme cofactors.

3P097 **ヘモグロビンの R,T,不安定 T の酸素親和力の計算：MD シミュレーションによる自由エネルギー計算**

Oxygen affinity differences of hemoglobin between the R, T, an unstable T structures: By free energy calculations based on MD simulations

Minoru Saito (*Hirosaki University*)

Oxygen affinities of hemoglobin were calculated for the R, T, and an unstable T structures by free energy perturbation method based on MD simulations. Differences in the affinity between the structures were close to experimental values. The free energy calculations and MD simulations were carried out for all atoms of hemoglobin in water with all degrees of freedom and without any constraints or any fitting parameters. All calculations were performed by using the software developed by myself, COSMOS90, PERTURB, and FENE.

3P098 **Engineering of Channelrhodopsin with Specific Ion Selectivity**

Monica Patti, Rieko Kamii, Toru Ishizuka, Hiromu Yawo (*Tohoku University*)

Channelrhodopsins (ChRs) are members of the microbial-type rhodopsin family; they are characterized by a seven-pass transmembrane structure with a covalently bound retinal. Light absorption induces conformational changes of the molecule, which allow the channel structure to become permeable to cations. This enables very rapid generation of a photocurrent in cell membranes expressing ChRs. Despite extensive applications of ChRs in the field of optogenetics, researchers have yet to describe how the ions flow through the molecule. Here we report that a single point mutation of ChRWR, which is one of chimerae between ChR1 and 2, generates peculiar ions selectivity. Therefore, this residue is suggested to be critical for the ion selectivity determination.

3P099 **新規キメラタンパク質による G_s タンパク質の光制御**

Optical control of G_s-protein activity by novel chimeric proteins

Kazuho Yoshida[1], Keiichi Inoue[1,2], Takahiro Yamashita[3], Rei Abe-Yoshizumi[1], Kengo Sasaki[1], Yoshinori Shichida[3], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*JST PRESTO*, [3]*Grad. Sch. Sci., Univ. Kyoto*)

G-protein-coupled receptors (GPCRs) are heptahelical transmembrane receptors, which transduce signals through specific G-proteins to intracellular signaling cascades. We design new chimeras between photo receptive microbial rhodopsins and GPCRs for the optical control of G-protein signaling by light.

In this study, we focused on various ion-pumping microbial rhodopsins, *Gloeobacter* rhodopsin (GR), *Natronomonous pharaonis* halorhodopsin (*Np*HR), and *Indibacter* rhodopsin 2 (*Indi*R2), and designed their chimeras with cytoplasmic loops of β_2-adrenergic receptor (β_2AR) for G_s-activation. Among them, only GR chimeras showed light-dependent G_s-activation. In the poster, we will discuss the molecular properties based on the results of spectroscopic measurements.

3P100 **Conformation and topology of pharaonis phoborhodopsin in the lipid environment as studied by solid-state NMR**

Izuru Kawamura[1], Satoshi Nakatani[1], Yoshiteru Makino[1], Naoki Kamo[2], Akira Naito[1] ([1]*Grad. Sch. Eng., Yokohama Natl. Univ.*, [2]*Hokkaido Univ.*)

Solid-state NMR spectroscopy can provide detailed information regarding the conformation, orientation and dynamics of membrane protein in the lipid environment. Here we present magic angle spinning (MAS) NMR study of a retinal-binding seven-helical photoreceptor, *pharaonis* phoborhodopsin (ppR). We prepared uniformly ^{13}C, ^{15}N-labeled *p*pR reconstituted into lipid bilayers and performed correlated NMR experiments. We will discuss the conformation and topology of *p*pR in the lipid environment w/o its cognate transducer protein.

3P101 **細胞膜モデル「ナノディスク」を用いたハロロドプシンの三量体形成が持つ機能的意義**

Functional significance of homotrimer formation in the Nanodisc-embedded Halorhodopsin

Kenshiro Suzuki[1], Ayumi Yamamoto[1], Takashi Tsukamoto[2], Yoshihiro Kobashigawa[4], Takeshi Uchida[1,3], Fuyuhiko Inagaki[4], Makoto Demura[2], Koichiro Ishimori[1,3] ([1]*Grad. Sch. of Chem. Sci. and Eng. Hokkaido Univ.*, [2]*Grad. Sch. of Life Sci. Hokkaido Univ.*, [3]*Fac. of Sci. Hokkaido Univ.*, [4]*Fac. of Adv. Life. Sci. Hookaido Univ*)

Halorhodopsin (HR), a light-driven chloride pump, forms a homotrimer in the cytoplasmic membranes. Although the trimeric structure of HR is supposed to be essential for its chloride transport, the detailed analyses have been reported only in the detergent-solubilized system. To discuss the effect of the trimeric formation on chloride transport in the membrane, we reconstruct trimeric and monomeric HR into Nanodisc, a membrane mimetic system. The UV and CD spectra characterized the structures and the chloride titration determined the dissociation constant of the nanodisc-embedded trimeric HR for chloride. The monomeric HR also embedded into Nanodisc, to examine the chloride affinity, and together with these data, significance of the trimeric structure will be discussed.

3P102 **凍結割断低温原子間力顕微鏡の製作**

Fabrication of freeze fracture cryogenic atomic force microscope

Naoto Kuga, Toshiaki Gotoh, Tsutomu Kouyama (*Graduate School of science, Nagoya University*)

Atomic force microscopy (AFM) has been established as an important tool to analyze the surface topology of a biological sample at nanometer resolution. For observation of intercellular structures by AFM, freeze-fracture method was invented. In this study, we fabricated a cryogenic AFM to analyze a surface topology of a freeze-fractured surace of a biological membrane dipped in cold ethanol at -100℃.

3P103 緑色イオウ細菌 *Chlorobaculum tepidum* の Rieske/cyt *b* 複合体単離の試み

Isolation of Rieske/cytochrome *b* complex from a green sulfur bacterium *Chlorobaculum tepidum*

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Cytochrome (Cyt) *bc* complexes are crucial energy conserving enzymes, which couple electron transport with proton transfer across membranes, resulting in the production of chemiosmotic potential essential for ATP synthesis. As a transcription unit that encodes the complex in green sulfur bacteria lacks a region encoding cyt c_1, it has been supposed to form the most primitive structure composed of only two subunits, Rieske iron-sulfur protein (ISP) and cyt *b*. In order to elucidate its structural organization, we constructed the expression system of the His-tagged Rieske ISP or cyt *b* at their amino (N)- or carboxy (C)-termini in *Chlorobaculum tepidum*. At present, we could obtain a crude fraction containing two major components of Rieske ISP and cyt *b*.

3P104 Significance of phospholipid composition in generating Min protein waves in vitro

Satya N. V. Arjunan[1], Yusuke Morimoto[2], Koichi Takahashi[1] ([1]*Laboratory for Biochemical Simulation, RIKEN Quantitative Biology Center*, [2]*Laboratory for Cell Signaling Dynamics, RIKEN Quantitative Biology Center*)

In Escherichia coli, Min proteins direct the cytokinetic ring to the midcell by oscillating between the cell poles. MinD is an ATPase that binds to the inner membrane and is activated by MinE. Together, MinDE generates ATP-driven surface waves in vitro on supported bilayers of E. coli lipid extract, made up of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin. Here, we report the effects of varying compositions of the phospholipids in the bilayer on the MinDE surface waves. Significantly, our results indicate that a single phospholipid type is sufficient to generate the waves. We have also developed a detailed particle simulation model that recapitulates the in vitro surface waves.

3P105 膜受容体内在化のリアルタイムモニタリング：走査型電気化学顕微鏡(SECM)による低侵襲・定量的・単一細胞レベルでの測定

Real-time Monitoring of Membrane Receptor Internalization: Low-Invasive, Quantitative and Single-Cell Level Measurement by SECM

Yoshiharu Matsumae[1], Yasufumi Takahashi[2], Kosuke Ino[1], Hitoshi Shiku[1], Tomokazu Matsue[1,2] ([1]*Grad. Sch. Environ. Stud., Tohoku Univ.*, [2]*WPI-AIMR, Tohoku Univ.*)

We developed a scanning electrochemical microscopy (SECM)-based quantitative receptor-internalization measurement method. The epidermal growth factor receptor (EGFR) is one of the key membrane proteins associated with cancer. C225 is a monoclonal antibody that binds to the EGFR and inhibits growth of cancer cells. C225 internalizes the EGFR, however, this mechanism is not completely clear. To measure the internalization process by SECM, EGFR was labeled with glucose oxidase (GOx)-modified C225. Microelectrode of SECM system was positioned near the cells and set at 0.7 V vs. Ag/AgCl to detect H_2O_2 produced by GOx. High currents were detected near the cells, and it decreased rapidly within 2 hours because of the internalization of EGFR-GOx complex.

3P106 The improvement of 2D crystal quality by crystallization temperature correlated with fluidity of lipids mixture

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Mitochondrial F-ATP synthase produces the majority of ATP for cellular functions requiring free energy. The structural basis for proton motive force driven rotational catalysis of ATP formation in the holoenzyme remains to be determined.

We have succeeded in purification and 2D crystallization of bovine enzyme in active oligomycin-sensitive form. 2D crystals were grown using dimyristoyl phosphocholine as lipid at 27°C. For data collections, the qualities of 2D crystal have to be improved. Though the enzyme is stabilized at low temperature, 2D crystallization has to be done at high temperature than transition temperature of lipids. The choice of crystallization temperature and the length of acyl chain in lipid molecule were critical for quality of 2D crystals.

3P107 Molecular dynamics simulations of β2AR: the comparison of different protein-lipid force field parameters

Md. Iqbal Mahmood, Nozomu Kamiya, Hideaki Fujitani, Yamashita Takefumi (*LSBM, RCAST, The University of Tokyo*)

To predict physical properties accurately based on MD simulations, good force fields are necessary for all molecules in a system. This work is to compare the different force fields on the structural behavior of β2AR. We perform MD simulations of β2AR-POPC bilayer with FUJI+Slipids, FUJI+GAFFlipid, C27 and C36 force fields and discuss the choice of force field affects of β2AR. FUJI+GAFFlipid model, β2AR forms an ionic lock between R131 and E268 whereas the ionic lock is broken in FUJI+Slipids. C27 and C36, the ionic lock forms transiently. The distance between K267 and D331 in FUJI+GAFFlipid is shorter than others. These data suggest that the force field influences the structural changes and lead us to develop a more accurate force field for protein-lipid simulations.

3P108 三量体ハロロドプシンの特異的なカロテノイド結合

Specific carotenoid binding of halorhodopsin trimer

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A light driven anion pump halorhodopsin (NpHR) exists on the biomembrane of *N. pharaonis*. Its trimer binds a kind of carotenoid bacterioruberin (BR), at the intermolecular interface.

In this study, we investigated the carotenoid-binding specificity of the trimer NpHR obtained from a heterologous over-expression system in *E. coli*.

Although some kinds of carotenoid (β-carotene, astaxanthin and lycopene) were mixed to the trimer NpHR solution, these did not bind to the NpHR.

In addition, acetylated BR at its hydroxyl groups also led to a decrease in the affinity to the NpHR.

These results indicate that the binding specificity of the NpHR to the BR is very high.

3P109 アルカリ条件下における三量体ハロロドプシン-バクテリオルベリン複合体の熱安定性

Thermal stability of trimer halorhodopsin-bacterioruberin complex in alkali condition

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A light driven anion pump halorhodopsin (NpHR), which exists on the membrane of *N. Pharaonis*, forms trimer and binds a carotenoid of bacterioruberin (BR). Trimer NpHR obtained from *E.coli* over-expression system also can bind the BR in the detergent system. In this study, we examined the effect of the BR binding for thermal stability of the NpHR in the pH range of 7.0-8.5. By thermal treatment at 40 ℃ for 30 min, bleaching ratio of NpHR unbound BR was about 64 % at pH 8.5, 12 % higher than that at pH 7.0. Contrary to this, bleaching ratio of the NpHR-BR complex was about 37 % at pH 8.5, and only 6 % higher than that at pH 7.0. These results suggest that the BR binding contributes to the stabilization of the NpHR not only at neutral pH but also at alkali condition.

3P110 Calculation of free energy changes due to charged residues mutation from alchemical free energy calculations:Improving toxins selectivity

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How a mutation affects the binding free energy of a ligand is a fundamental problem in molecular biology/biochemistry with many applications in pharmacology and biotechnology, e.g., design of drugs and enzymes. Free energy change due to a mutation can be determined most accurately by performing alchemical free energy calculations in molecular dynamics simulations. Here we discuss the necessary conditions for success of free energy calculations using toxin peptides ShK and HsTx1 that bind to the voltage gated potassium channels Kv1.1 and Kv1.3 as examples. We show that preservation of the binding mode is an essential requirement for the success of the FEP calculations. The proposed method will be useful in improving the affinity/selectivity of drug leads and enzymes.

3P111 アデノシン A2a 受容体の熱安定性を向上させるアミノ酸置換の理論的予測

Theoretical Prediction of Mutations Improving Thermal Stability of Adenosine A2a Receptor

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G protein-coupled receptors (GPCRs) form the most important target for drug design. However, their mass production and experimental structure determination are quite difficult to achieve due to the low thermal stability. In the present study, through mutations for the antagonist-binding structure of the adenosine A2a receptor, we investigate how to predict the mutants which lead to enhanced thermal stability using our free-energy function recently developed for membrane proteins. Such mutations are theoretically proposed with the emphasis on the entropic effect arising from the translational displacement of hydrocarbon groups which constitute nonpolar chains of lipid molecules, and they are experimentally examined. Currently, the success rate is about 1/2.

3P112 C 末端に異なるアミノ酸タグをもつハロロドプシン同士での三量体の形成

Trimer formation between halorhodopsins with different amino acid tags at C terminus

Tomokazu Wakatsuki, Takanori Sasaki (*Sch. Sci. and Tech., Meiji Univ.*)

A light-driven anion pump, halorhodopsin (NpHR), forms homotrimer on the membrane of N. pharaonis. It is known that the NpHR with amino acid tag at its C terminus also can be expressed on the E.coli membrane. In this study, we examined whether the His-tagged NpHR can form a hetero-trimer with the Strep-tagged NpHR. Solubilized NpHR monomers with each amino acid tag in dodecyl-maltoside were mixed and reconstituted on the artificial lipid. As a result, the ratio of the hetero-trimer was only about 10%, suggesting that the amino acid residues at the C-terminal region affect the trimer formation. Furthermore, we constructed His-tag/Strep-tag NpHR coexpression system in E.coli, for convenient detection of hetero-trimer.

3P113 ヒト由来膜タンパク質の無細胞発現と 膜局在化傾向の網羅的解析

The comprehensive analysis of human membrane protein expression and membrane insertion in vitro

Go Takizawa (*Univ.Tokyo*)

Cell-free protein synthesis system has recently been applied to express membrane proteins in vitro in combination with para-biomembrane. However, it is still difficult to predict what type of membrane protein can be successfully synthesized and integrated in lipid bilayer in the cell-free system.
This study aims to analyze the relationship between the insertion efficiency of various membrane proteins and their amino acid sequences by expressing in the cell-free system. The results indicated that the membrane proteins with the shorter hydrophilic N-terminus have the higher insertion efficiency, suggesting the long hydrophilic N-terminus region can prevent the insertion process.

3P114 全反射赤外分光法を用いた苦味受容体のリガンド結合解析

ATR-FTIR study of ligand binding in a bitter taste receptor

Tomoaki Ohashi[1], Kota Katayama[1], Masayo Iwaki[1], Kei Tsutsui[2], Hiroo Imai[2], Hideki Kandori[1] ([1]*Nagoya Institute of Technology*, [2]*Primate Reserch Institute, Kyoto University*)

Bitter taste is mediated by the bitter taste receptors (TAS2Rs), which make a family of the G protein-coupled receptors expressed in taste cells of the tongue. There are 25 putatively functional receptors in human and each receptor has different and specific tuning range for bitter compounds. Despite of the progress in understanding on the functions of TAS2Rs, no structural analysis has been so far achieved.
In the present study, we attempted to elucidate the binding mechanism of human TAS2Rs with bitter substances. For this aim, we apply ATR (Attenuated Total Reflection) FTIR spectroscopy, which monitors structural changes of TAS2Rs upon ligand binding. Structural analysis is now in progress, which will be presented in the poster.

3P115 High stability of two-dimensional crystal of reconstituted bacteriorhodopsin in partially fluorinated phosphatidylcholine

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We have reported successful reconstitution of bacteriorhodopsin (bR) into novel partially fluorinated phosphatidylcholine (diF4H10-PC) vesicles, in that the reconstituted bR has native-like higher order structure and photocycle. The present study on structural stability of the reconstituted bR demonstrated that 2D crystals as well as trimeric structure of bR molecules are maintained and no light-induced denaturation is observed up to ~40 °C, which is much higher than the gel-to-liquid crystalline phase transition temperature (5 °C) of pure diF4H10-PC bilayer. The high stability of the reconstituted bR in diF4H10-PC is in stark contrast with bR in DMPC showing phase transition-induced disassembly of bR molecules and remarkable denaturation by visible light.

3P116 Reverse gyrase likely biases thermal DNA strand passage toward overwinding

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Reverse gyrase is a type I topoisomerase that can introduce positive supercoils into DNA only at >70°C. Here we have imaged the overwinding reaction at 71°C under a microscope and found that a single reverse gyrase molecule processively winds DNA for >100 turns. The rate is nearly two orders of magnitude higher than previous biochemical bulk results. The enzyme ceases to work when the torsional stress in DNA rises to mere ~4 pN·nm, as expected if its role is to keep DNA slightly overwound for protection against thermal melting. The activity is also highly sensitive to tension in DNA. All our results are consistent with the mechanism where strand passage relying on thermal motions, as in topoisomerase IA, is actively, but loosely, biased toward overwinding.

3P117 Single-molecule study of how RecA displaces SSB from single-stranded DNA

Hung-Yi Wu, Hung-Wen Li (*Dept. of Chem., Natl. Taiwan Univ.*)

RecA competes with SSB (single-stranded DNA binding proteins) for single-stranded DNA (ssDNA) substrates to form RecA nucleoprotein filaments in E coli, essential for DNA homologous recombinational repair. In this study, we monitored individual RecA filament formation by tethered particle motion (TPM) technique in real-time. Binding of RecA on the SSB wrapped-ssDNA extends the DNA substrate, visible by the increase in bead Brownian motion in TPM imaging. By varying the ssDNA gap sizes, we prepared the SSB-ssDNA complex with different ssDNA lengths. Interestingly, we found a single amino acid mutation of RecA (RecA E38K) accelerates the SSB removal rate compared to wild-type RecA. Our data suggests the presence of direct RecA-SSB interaction on ssDNA.

3P118 クロマトソームの粗視化シミュレーション: H1 結合に伴うヌクレオソーム構造のコンパクト化のダイナミクス
Coarse-grained simulation of chromatosome: H1-mediated dynamic compaction of nucleosome structure

Nobu C. Shirai, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

The linker histone H1 has two disordered regions separated by a globular domain, and it stabilizes compact chromatin structure by binding to nucleosome and forming chromatosome.
Formation and dissociation of chromatosome dramatically change the accessibility of regulatory proteins, and thus the binding process of H1 is important to understand the indirect regulation of transcription and replication.
In this study, we performed molecular dynamics simulations using a coarse-grained model of chromatosome to analyze dynamic compaction processes.
As a result of the simulations, we observed that the positively charged C-terminal disordered domain of H1 induces compact structures of multi-nucleosome by placing itself in the middle of the negatively charged linker DNAs.

3P119 Characterization of the hemi-methylated CpG methylation process using fluorescent labeled SRA

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DNA methylation plays a central role in the epigenetic regulation of genes expression and cell differentiation. Dnmt1 (DNA methyltransferase) is the enzyme responsible for the maintenance of cytosine methylation at CpG dinucleotide in mammalian genome. It is suggested that the SRA domain of Uhrf1 recognizes the hemi-methylated CpG sites, and Dnmt1 interacts with SRA to facilitate accession of the catalytic center to hemi-methylated DNA, resulting in the maintenance of DNA methylation pattern. In this study, we prepared fluorescent labeled SRAs and characterized SRA-DNA complex formation kinetics with biochemical analysis.

3P120 蛍光標識ヌクレオソームを用いたクロマチン再構成複合体の機能解析
Characterization of ATP-dependent chromatin remodeling complexes using fluorescently labeled nucleosome

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Eukaryotic gene expression is regulated by chromatin structures and/or DNA modification such as CpG methylation. The basic unit of eukaryotic chromatin structure is a nucleosome consisting of approximately 150 bp DNA wrapped in 1.7 superhelical turns around a histone octamer. Posttranslational histone modifications such as acetylation, methylation, phosphorylation and ubiquitylation regulate chromatin structure, resulting in activation or repression of gene expression. ATP-dependent chromatin remodeling complexes also regulate gene expression by changing chromatin structure. In this study, we construct fluorescently labeled mono-nucleosomes and characterize ATP-dependent chromatin remodeling complexes, LSH and CHD.

3P121 粗視化モデルを用いた Ars インスレーターの力学的特性の考察

Analysis of dynamic characteristics of Ars-insulator by coarse-grained models

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Insulator is one of the transcription control elements on DNA. This sequence works as the boundary among DNA domains to archive appropriate gene expressions by blocking the communications between the promoter and inappropriate enhancers. Recently, some insulator sequences are identified in some model organisms. However, the molecular mechanisms of their activities are not clearly understood, yet. In this study, we focus on the physical aspects of Ars-insulator identified in sea urchin to elucidate the mechanism of insulator activities. We construct the coarse-grained DNA models of Ars-insulator sequence and other typical sequences. We perform the simulations of these models and compare their sequence dependent motions and functional activities.

3P122 等温条件で増幅可能な人工 RNA の設計原理の理解

Design Principle of Replicable RNA under Isothermal Condition

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The exponential amplification of nucleic acids is a core technology in life science and diagnostics. The development of isothermal nucleic acids-amplification system is important. Qβ replicase (RNA-dependent RNA polymerase of coli phage Qβ) can amplify some target RNAs exponentially under isothermal condition. In this study, we tried to reveal design principle of replicable RNAs by Qβ replicase. We analyzed secondary structure of two RNAs, which are point mutants and show different template activities, by Selective 2'-Hydroxyl Acylation and Primer Extension (SHAPE). We revealed effects of secondary structure to replicability of template RNA and succeeded in construction of replicable RNA by manipulating the secondary structure of non-replicable RNA.

3P123 Protective Effect of Ascorbic Acid on Double-strand Breaks of Giant DNA induced by photo- and gamma-irradiation

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In order to evaluate the protective effect of ascorbic acid (AsA) on DNA molecules, we studied the double-strand breaks (DSBs) of DNA caused by visible light and γ-rays through a single-molecule observation using fluorescence microscopy. For induction of DSBs by visible light, a fluorescent cyanine dye, YOYO-1, was adapted as a generator of reactive oxygen species under light illumination at 450-490nm. We studied double-strand breakage reaction under focused illumination by real-time observation. As a result, it is shown that the DNA damage is inhibited by AsA. With regard to DSBs caused by γ-rays, we counted the number of DSBs, n, with respect to the γ-ray dose, D. From the slope of linear relationship; n~D, we have evaluated the degree of protection by AsA.

3P124 α-hemolysin 及び T7 RNA polymerase を用いた DNA/RNA ロジックゲートの実現

Construction of DNA computing platform using α-hemolysin and DNA/RNA with the enzyme reaction

Masayuki Ohara[1], Masahiro Takinoue[2], Ryuji Kawano[1] ([1]*TUAT*, [2]*Tokyo Tech*)

Construction of the operation element which combined DNA and channel protein is attractive as a new algorithm of a DNA computer. We have tried to apply nanopore method which can be detected single strand DNA and RNA electrically to DNA computing. Here we proposed a logic gate operation by using enzyme reaction. We used RNA synthesis in order to increase the concentration of the output molecules. As the result, we are able to constrict an AND logic gate using this concept. In this system, the concentration of output molecules can be around 100 times higher than that of the input molecules. This method will be controlled the concentration of output molecules, apply to paralleled DNA computing, and apply the element to the combined like cells network.

3P125 DNA を湾曲する HMG-1/2 は塩基配列非特異的に長鎖 DNA を折り畳み

DNA-bending protein HMG-1/2 sequence-independently folds a single giant duplex DNA chain

Hiroyuki Mayama[1], Naomi Tsumura[1], Norio Hazemoto[2], Toshio Kanbe[3], Hideaki Yamaguchi[4], Koji Kubo[5], Anatoly Zinchenko[5], Shizuaki Murata[5], Kenichi Yoshikawa[6], Tatsuo Akitaya[1] ([1]*School of Medicine, Asahikawa Med. Univ.*, [2]*Graduate School of Pharmaceutical Sciences, Nagoya City Univ.*, [3]*School of Medicine, Nagoya Univ.*, [4]*Faculty of Pharmacy, Meijo Univ.*, [5]*Graduate School of Environmental Study, Nagoya Univ.*, [6]*Faculty of Life and Medical Sciences, Doshisha Univ.*)

Chromatin structure and gene regulation are performed by interactions between proteins and nucleic acids that sometimes reads morphological change of local structure of DNA such as bending in sequence-independent manner. High Mobility Group (HMG) -1 and close relative HMG-2 proteins take part in general modulation of chromatin structure and gene activity in vertebrate. HMG-1/2 bend DNA upon binding and stabilize bent and supercoiled DNA but the effects on higher-order structure of DNA are still unclear. We found that HMG-1/2 induced the folding of a single giant duplex DNA chain in steep manner. Coexisting metal cations enhanced DNA-HMG-1/2 complex formation whereas HMG-1/2 involved long acidic (anionic) C-terminal region. Mechanisms of the folding will be discussed.

3P126 転写調節タンパク質 STPR は長鎖 DNA を塩基配列非特異的に折り畳む

Transcription modulator protein STPR induces the folding of a single giant DNA molecule in sequence-nonspecific manner

Tatsuo Akitaya[1], Naoko Makita[2], Naomi Tsumura[1], Hiroyuki Mayama[1], Norio Hazemoto[3], Toshio Kanbe[4], Hideaki Yamaguchi[5], Koji Kubo[6], Anatoly Zinchenko[6], Shizuaki Murata[6], Kenichi Yoshikawa[7], Tomoyasu Aizawa[8], Makoto Demura[8] ([1]*School of Medicine, Asahikawa Med. Univ.*, [2]*Faculty of Environmental and Information Sciences, Yokkaichi Univ.*, [3]*Graduate School of Pharmaceutical Sciences, Nagoya City Univ.*, [4]*School of Medicine, Nagoya Univ.*, [5]*Faculty of Pharmacy, Meijo Univ.*, [6]*Graduate School of Environmental Study, Nagoya Univ.*, [7]*Faculty of Life and Medical Sciences, Doshisha Univ.*, [8]*Graduate School of Life Science, Hokkaido Univ.*)

The STPR domain is a novel DNA-binding domain composed of repeats of 23 amino acid peptide found in the fibroin-modulator binding protein (FMBP-1) of the silkworm Bombyx mori. We found that sequence-specific DNA-binding protein STPR induced sequence-independent folding of a single giant T4 genomic DNA (166 kbp) in a 'mild-and-continuous folding' mode at similar order of the STPR concentration in transcription modulation. The salt-bridge of STPR was independent from the folding whereas it was significant to transcription modulation. Sequence-independent folding of T4 DNA was suppressed dependent on the concentration of salt in the solution. Attainable molecular mechanisms of dual-mode modulation through sequence-independent folding of DNA by STPR will be discussed.

3P127 Comparison of DNA double-strand breaks caused by ultrasound and Co60 gamma-ray with attention to the effect on its higher-order structure

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The effect of ultrasound and gamma-ray on double-strand breaks is reported by use of single DNA observation with fluorescence microscopy. It is shown that ultrasound causes the double-strand breaks only above a threshold power and that, above the threshold, the damage increases linearly with the power. Whereas, gamma-ray causes the double-strand breaks almost linearly with the power, where there exists no apparent threshold on the breaking efficiency. We have evaluated how the probability of the double-strand breaks depends on the higher-order structure of DNA both for the damages by gamma-ray and ultrasound. It will be reported that folded compact DNA molecules are significantly stable against these damages compared to unfolded elongated DNA.

3P128 Metal Cations(2+) Cause the Folding Transition of DNA but Inhibit Spermidine(3+)-Induced Compaction

Chika Tongu[1], Yuko Yoshikawa[2], Zinchenko Anatoly A[3], Chen Ning[3], Takahiro Kenmotsu[1], Kenichi Yoshikawa[1] ([1]*Doshisha University*, [2]*Ritsumeikan University*, [3]*Nagoya University*)

We report the results of our observation on the higher order structure of a giant DNA by use of fluorescence microscopy. It is shown that divalent metal cations, Mg(2+) and Ca(2+), cause shrinking on DNA conformation. Sperimidine(3+) also induces folding transition from an elongated coil state with higher potency than divalent cations. Interestingly, it is found that divalent metal cations have the inhibiting effect on the folding transition induced by spermidine(3+). In other words, it becomes clear that spermidine(3+) and divalent cations exhibit an antagonistic effect each other on the higher order structure of DNA. The mechanism of such antagonistic effect is discussed by considering the physic-chemical contribution of the translational entropy of counter ions.

3P129 溶質と水分子の間の LJ ポテンシャルパラメタが水和ダイナミクスに及ぼす影響
Effects of LJ potential parameters between solute and water on the hydration dynamics

Takuya Takahashi, Tetsuro Nagai (*Coll.Life.Sc., Ritsumeikan Univ*)

Previous experiments and simulations suggest that mobility of water molecules around structure-breaker solutes increase. They have larger translational self-diffusion coefficients than a water molecule in bulk has. Here, a set of suitable Lennard-Jones potential parameters of interactions between TIP5P water and solutes to reproduce the dynamical behavior of water molecules. The effect of the potential parameter on the hydration dynamics was not so simple as expected. In addition, we analyze the number of hydrogen bonds and the radial distribution functions with a number of combinations of Lennard-Jones parameter values. We then investigate their correlations to the dynamical behavior to study how the water dynamical behavior is governed.

3P130 水和水が小分子のテラヘルツ振動モードに与える影響
Effect of Hydration water on terahertz vibrational modes of small molecules

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Organic molecules in vivo including biomolecules or chemicals usually function only in the presence of water. To study hydration process of the molecule, vibrational spectra of small organic molecules in the low frequency region are measured by terahertz time domain spectroscopy (THz-TDS). In the case of ampicillin, THz spectrum of trihydrate sample shows large absorption band at around 60-90 cm^{-1}, which is not observed for that of anhydrous sample. The effects of hydration water molecules are compared and interpreted with the results of periodic boundary condition (PBC) implemented DFT calculations by CRYSTAL09. Low-frequency spectra of L-asparagine anhydrous and its monohydrate samples will be also discussed.

3P131 蛋白質間相互作用への溶媒効果を観測することの困難さ：単純なモデルでの理論研究
Difficulty in Observing of Solvent Effect on Protein-Protein Interaction: A Theoretical Study with a Simple Model

Takumi Yamashita, Shingo Fujihara, Ryo Akiyama (*Sci., Univ. Kyushu*)

Understanding of effective interactions between proteins in a solution is important in biology.
We calculated effective interaction between proteins w(r) (= -kTln[g(r)]) and the structure factor S(k) by using an integral equation theory with a simple model. Explicit solvent model and implicit solvent model were examined. Although the g(r) of the explicit solvent model was completely different from that of the implicit model, these structure factors S(k) were nearly the same each other. To reproduce the significant peak arising from the translational motion of solvent molecules in g(r) the experimental S(k) is not sufficiently long. These results indicate that the usual small angle scattering analysis cannot deny the existence of solvent effect.

3P132 誘電緩和分光法と分子動力学法を用いたアルカリハライドとアルカリリン酸イオンの水和ダイナミクスおよびエネルギー論
On the Hydration Dynamics and Energetics of Alkali halide and Phosphate Ions by Dielectric Relaxation Spectroscopy and Molecular Dynamics

George Mogami[1], Kazuki Ishimori[1], Nobuyuki Matubayasi[2], Hideaki Takahashi[3], Makoto Suzuki[1] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*Grad. Sch. Eng. Sci., Osaka Univ.*, [3]*Grad. Sch. Sci., Tohoku Univ.*)

Alkali halide and phosphate ions were found to have constrained water and hypermobile water (HMW) which has higher dielectric relaxation (DR) frequency than bulk water in the hydration region of ions. Marked progress in the understanding of HMW has been achieved by theoretical (M.Kinoshita et al.,2009; Y.Kubota et al.,2012) and Raman spectroscopic (Y.Okazaki et al.,2014) studies. In this study, we focused on dynamic and energetic property of HMW and carried out hydration measurements of sodium oligo-phosphates and sodium halides by DRS and thermodynamics calculations of ionic solvation by molecular dynamics (MD) simulation using energy representation (ER) method. As a result it was indicated that HMW was mainly induced by many-body interactions.

3P133 NaCl、NaI 水溶液におけるハイパーモバイル水の熱容量及び密度の評価

Estimation of Heat Capacity and Density of Hyper-Mobile Water in NaCl and NaI Solutions

Masayoshi Sato, George Mogami, Nobuyuki Morimoto, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

Hydration shells of ions in NaCl and NaI solutions were previously reported to consist of hypermobile water (HMW) which has higher dielectric relaxation frequency than bulk water as given by precision microwave dielectric spectroscopy (DRS).(J. Phys. Chem. A 117, (2013) 4851-4862) In this study, apparent density and heat capacity of HMW was estimated based on difference scanning calorimetry (DSC) and solution density measurements. Using the numbers of HMW per alkali-halide by DRS, both heat capacities of HMW around ions in NaI and NaCl solutions were estimated to be about 10% lower than that of bulk water. Density of HMW was estimated to be lower than that of bulk water even if there was bound water on Na+ ions having higher density.

3P134 低周波超音波により引き起こされたメダカ胚における卵黄球の収縮と出血

Shrinkage of yolk sphere and bleeding on medaka embryo caused by low frequency ultrasound

Kento Yamada[1], Masato Ueda[1], Kenji Yoshida[1,2], Yasuhiro Tonoyama[3], Nobuyoshi Shimizu[3], Yoshiaki Watanabe[1] ([1]*Doshisha Univ.*, [2]*Chiba Univ.*, [3]*Keio Univ, Advanced Research Center for GSP.*)

This study focuses on the biological effect of acoustic cavitation on living tissue. A medaka embryo was exposed to continuous low-frequency and high intensity ultrasound (30 kHz, 20-150 kPa). The standing wave was formed in the experimental bath filled with degas water. The medaka embryo in a tube was located in the pressure antinode. Based on the stage of embryonic development, the samples was categorized into 3 types, within 1) 12 hours, 2) 5 days, 3) 10 days. After exposure, the effect was investigated in the microscope. The biological effect was confirmed as the shrinkage of yolk sphere in case of 1), the bleeding from blood vessels around the yolk in case of 3). High-speed imaging during exposure suggested that acoustic cavitaion related to these damages.

3P135 アフリカツメガエル卵成熟過程における卵母細胞の ATP 産生

ATP production in *Xenopus laevis* oocytes during maturation

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To understand ATP metabolism during oocyte maturation, we developed an ATP imaging system in *Xenopus* oocytes with FRET-based ATP indicator, ATeam. Now, we are using albino oocytes to observe more accurate ATP changing. ATP imaging in albino oocytes is under investigation with 2-deoxyglucose and oligomycin A as inhibitors of glycolysis and oxidative phosphorylation, respectively. These inhibitors are also injected into wild-type *Xenopus* oocytes to study the effect(s) on ATP production during oocyte maturation. White spot is the hallmark of maturation in *Xenopus* oocytes; therefore, the timing of the appearance of white spot was compared between oocytes with or without inhibitor(s) during progesterone induced maturation.

3P136 3 次元培養下での再生皮膚の発生における反応拡散機構による羽毛原基パターン再構築

Reconstruction of feather bud patterning by a reaction-diffusion mechanism during bioengineered skin development in 3D culture

Kentaro Ishida, Toshiyuki Mitsui (*Coll. of Sci. & Eng., Aoyama Gakuin Univ.*)

Feather bud development requires epithelial-mesenchymal interactions that result in the periodic pattern formation of the feather buds during skin development. To explain this pattern formation, Turing's reaction-diffusion model for the activator-inhibitor type has been adopted. We have developed a bioengineered chick skin and feather buds that exhibited normal skin characteristics, including the size of the feather buds, the inter-bud distance and several gene expressions. Inhibition of FGF signaling by treating with inhibitors during the feather bud formation led to fusion of the feather buds, which in turn can be described by the reaction-diffusion model. Thus, we have developed a 3D culture system to regenerate embryonic chick skin as a feather bud-forming field.

3P137 電気ー機械相互作用を考慮した心筋細胞群モデル

Model of Cardiac Muscle Cells with Reference to Electro-Mechanical Interaction

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Cardiac stretch-activated ion channels have an important role in interaction between ion current and stretch activation in heart muscle. Though it is important to evaluate the effect of interaction quantitatively, there are few models for such electro-mechanical interaction. In this study, we propose a simple network model of cardiac muscle cells with reference to electro-mechanical interaction in order to analyze propagation of cardiac excitation-contraction coupling theoretically and numerically. In spite of the simplicity of the proposed model, it realized normal and abnormal behavior of cardiac impulse propagation among cells by changing the strength of interaction between electronic and mechanical dynamics.

3P138 骨格筋繊維の急激な伸張に対する張力応答のシミュレーション

Simulation of force responses to fast ramp stretches in the skeletal muscle fibres

Youjiro Tamura[1], Akira Ito[2], Andrew Cresswell[3] ([1]*SNCT*, [2]*SNCT*, [3]*UQ HMS*)

We have proposed a muscle model based on a viscous fluid between actin and myosin molecules (1992). Macroscopically, this fluid model makes a muscle model that consists of Maxwell elements (2005). The muscle model is essentially different from Huxley model, because the force responses due to a sudden length changes come from the viscous elements. Bagni et al. (1998) measured the force responses to fast ramp stretches in frog skeletal muscle fibres and recorded the velocity changes during the stretches. We test the muscle model and conclude that the initial fast phase comes from a viscous element in parallel. Since the viscosity is centesimal of that of the Maxwell element, we think that the viscosity originates in the parallel components like titin and/or collagen.

3P139 コネクチン遺伝子の上流から転写される横紋筋タンパク質の解析

Analysis of proteins in striated muscles that transcribed from the upstream region of connectin gene

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Connectin is the largest elastic protein that connects between Z-line and M-line of sarcomere and functions as a molecular spring of vertebrate striated muscles. In the upstream region of connectin gene, there are two genes for proteins which domain structures are closely related with that of connectin. Although RT-PCR experiments revealed that these two genes are expressed in striated muscle, their function remains unknown. In this study, we investigated the domain structures, isoforms, localizations and binding partners of these proteins in striated muscles. To know their physiological roles, we examined the effects of overexpression or knock-down of these proteins using cultured skeletal muscle cells. In this presentation, we introduce the result of our experiments.

3P140 “Mg-Polymer”再考

Revisiting “Mg-Polymer”

Mahito Kikumoto, Shuichi Takeda, Tomoharu Matsumoto, Yuichiro Maeda (*Strct. Biol. Cntr, Nagoya-univ.*)

We search for another state of F-actin to clarify the relationship between polymorphism of actin and its state. We reinvestigate the “Mg-Polymer” (Totsuka and Hatano, BBA, 1970; Kamiya, Maruyama et a., BBA, 1972). We polymerized Mg-ATP-actin with 2mM MgCl2 in the presence of CP(CapZ) and in the absence of KCl. This actin showed extremely low viscosity and high ATPase, in contrast to those polymerized in the absence of CP or in the presence of KCl. We have found effective molar ratio of CP(CapZ) to actin for half change of viscosity and ATPase as ca. 1:500 and 1:3000, respectively. This implies the influence of CP(CapZ) reaches beyond 1μm along the actin filament. We are identifying mechanism of this state of actin filament.

3P141 ヒト心筋筋原線維 SPOC の外部力学刺激に対する応答

Response of SPOC in human cardiac myofibrils to mechanical stimuli

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The self-oscillation phenomenon called SPOC (Spontaneous Oscillatory Contraction) in striated muscle occurs under an intermediate activation conditions between contraction and relaxation (pCa, 6). We previously reported that the SPOC frequency and phase in rabbit glycerinated skeletal myofibrils are synchronized with cyclic mechanical stimuli. Recently, we reported that the transition rate of sarcomeres induced by mechanical stretch during SPOC is related with loading rate and the amplitude. (41th Ann. Meeting Biophys. Soc. Jap.) In the present study, we examined the response of SPOC patterns in human cardiac myofibrils to the mechanical impulses (stretch and release). We will discuss the response of single myofibrils and individual sarcomeres to the mechanical stimuli.

3P142 温度変化のヒト心筋 SPOC への作用

Effects of temperature changes on SPOC in a human myocardium

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Skinned myofibrils show auto-oscillation of sarcomeres called SPOC under partial activation conditions. We previously reported the SPOC properties of human cardiac myofibrils; that is, the SPOC period of Dilated cardiomyopathy (DCM) was longer than that of non-failing myofibrils (50th Ann. Meeting of Biophys. Soc. Japan: 1PS016: 90th Ann. Meeting of Physiol. Soc. Japan: 1PK-155). In the present study, we examined the temperature dependence of the SPOC properties (a period, an amplitude and SPOC patterns) in human non-failing myofibrils and found that the SPOC period became shorter on increasing temperature, mainly because of higher shortening velocity of sarcomeres at higher temperatures. This research has been approved by Human Ethics Committee at Waseda University.

3P143 ギボシムシのコネクチン様タンパク質のドメイン構造

Sequential analysis of connectin-like protein in acorn worm

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Connnectin is an elastic muscle protein that connects between Z-line and M-line of the sarcomere in vertebrate striated muscle. The domain structure of connectin divided into N-terminus Z-line, I-band, A-band and C-terminus M-line regions. Connectin-like proteins are also found in invertebrate muscles but the structure is different from vertebrate connectin and diverse among animals. To investigate evolutional roots of connectin and connectin-like proteins, we searched for a connectin-like protein using the genome information of Ptychodera flava. Based on this tentative sequence, we performed RT-PCR and determined the partial sequence. As a result, the connectin-like protein in the acorn worm (*Ptychodera flava*) has immunoglobulin, fibronectin and kinase domains.

3P144 高速暗視野顕微鏡による運動中のキネシン頭部の結合解離の直接観察

Direct observation of binding and unbinding motions of kinesin motor domain during processive motility

Hiroshi Isojima[1], Ryota Iino[2], Hiroyuki Noji[3], **Michio Tomishige**[1] ([1]*Dept. Appl. Phys., Univ. Tokyo*, [2]*Okazaki Inst. Integ. Biosci., NINS*, [3]*Dept. Appl. Chem., Univ. Tokyo*)

Kinesin-1 is a dimeric motor protein that moves along microtubule by alternately moving two motor domains in a hand-over-hand manner. The 8-nm stepping motion has been extensively studied, although it is still technically challenging to dissect the detailed process within the step. Here we observed the movement of one of kinesin heads by labeling with gold probe and observing it using total internal reflection dark-field microscopy with 55 μs temporal resolution. Using this technique we distinguished between bound and unbound state of the head and identified the structure and kinetics of the intermediate states between steps. These observations provide detailed information on the head motion and also serve to understand how kinesin coordinates two motor domains.

3P145 Control of microtubule trajectory within an electric field by altering surface charge density

Naoto Isozaki[1], Suguru Ando[1], Hirofumi Shintaku[1], Hidetoshi Kotera[1], Edgar Meyhofer[2], Ryuji Yokokawa[1] ([1]*Kyoto University*, [2]*Michigan University*)

A challenge for using microtubules (MTs) driven by kinesin motors in functional nanosystems is to control their direction of movement. A method was developed to guide kinesin-propelled MTs in multiple directions under an electric field by designing a charged surface of MT minus ends labeled with dsDNA via a streptavidin-biotin interaction. Experimental trajectories were in good agreement with values predicted from measured electrophoretic mobilities. As the effective charge of labeled DNA molecules matches to that of freely dispersed DNA molecules, MT trajectory can be estimated even by selecting labeling molecules with known charges. Our molecular design and prediction methodology demonstrate the feasibility of using molecular sorters driven by motor proteins.

3P146 キネシンとダイニンによる Tug-of-war 分子系の再構築および多分子ダイニンの発生力計測
Tug-of-war molecular system between dynein- and kinesin-coated regions and force measurement of multiple dyneins

Fumie Oda, Hirofumi Shintaku, Hidetoshi Kotera, Ryuji Yokokawa (*Dept. Microengineering, Kyoto Univ.*)

A large number of microtubule-based motor proteins, such as kinesin and dynein, are involved in mitosis. It is believed that chromosome oscillations in mitosis arise from tug-of-war between two opposite-polarity motors. However, the behavior of multiple motors as a team is poorly understood. Here, to contribute for understanding of multiple motor behavior in mitosis, we adopted microtubules motility assay on dynein- and kinesin-coated substrate. First, we measured force generated by multiple dyneins using optical tweezers. Dyneins exerted ~60 pN against optical trap to a bead attached to the tail of a microtubule. Moreover, we designed a tug-of-war molecular system between dynein and kinesin by photolithographically pattering motors in two different regions.

3P147 べん毛モーター解析のための長時間リアルタイム観察・刺激システムの構築
Development of the real-long-time observation and local stimulation system for flagellar motor analysis

Masaru Kojima, Takahiro Motoyoshi, Mitsuhiro Horade, Kazuto Kamiyama, Yasushi Mae, Tatsuo Arai (*Grad. Sch. Eng. Sci., Osaka Univ.*)

Recently, in cell analysis, local environment control technology for single cell analysis is being established. In this study, we try to develop a real-long-time observation and cultivation system that can changing and keeping local environment condition stably and apply this system for analyze the bacteria flagella motor. We have implemented “fast response measurement” and “solution spouting by micropipette” for this system. And then we confirm the performance of this system by measuring the rotational speed of bacteria flagellar motor for long-time with stable condition and controlling the rotational speed of flagellar motor diametrically.

3P148 Visualization of functional components of the bacterial flagellar motor

Yoshiyuki Sowa[1], Yong-Suk Che[1,2] ([1]*Dept. Frontier Biosci., Hosei Univ.*, [2]*IMRAM, Tohoku Univ.*)

The bacterial flagellar motor is a reversible rotary nano-machine embedded in the cell envelope. The motor consists of a rotor ~45 nm in diameter surrounded by ~10 independent stator units functioning as ion-channels and the torque is generated by interaction between the rotor and stator. To understand how the motor works, direct observation of motor movements at the site of torque generation is required. For this purpose, we have been developing the assay to detect motor dynamics by fluorescent microscopy at single-molecule level. In this study, we tried to track the movement of stator units MotB labeled with tetramethylrhodamine of rotating tethered cell.

3P149 Molecular dynamics simulations of the β subunit in F_1-ATPase: Relation between the large-scale structural change and common motifs

Yuko Ito, Mitsunori Ikeguchi (*Grad. Sch. Med. Life Sci., Yokohama-City Univ.*)

The F_1-ATPase is rotated by the structural changes of the catalytically active β subunits which have two driving forces; the ATP binding and ATP hydrolysis. The former is elucidated in our previous MD simulations (Y. Ito, *et al.* JACS, 2011). Therefore, in this study, we simulated the latter, which contains important issues; 1) the timing of the Pi release, 2) the unsolved half-closed form, and 3) the ADP releasing mechanism. The simulation solved all these issues. Also, we found that conserved residues among the ATPase family, induced the large (open-closed) structural change. This leverage like relationship was also observed in our previous study (the structural change by the ATP binding), suggesting that the obtained mechanism may be shared with other ATPase proteins.

3P150 Slow axonemal dynein e facilitates the motility of faster dynein c

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We highly purified the Chlamydomonas flagellar inner-arm dynein "e" and "c." EM observations and single particle analysis showed that the head domains of these two dyneins were morphologically similar. The ATPase activities of dynein e were lower than those of dynein c. Dynein e was expected to be a processive motor judged from microtubule landing rate assays. From in vitro motility assays, microtubule translocation by dynein e was found to be slow (V = 1.2 ± 0.1 μm/s), whereas that by dynein c was very fast (Vmax = 15.8 ± 1.5 μm/s). The microtubule translocation of the "fast" dynein c became even faster in the presence of the "slow" dynein e. In flagella, dynein e would act as a "facilitator" by holding adjacent microtubules to aid dynein c’s power stroke.

3P151 回転電場を用いた F_1-ATPase の一分子計測による 拡散の Giant acceleration の観察 II

Giant Acceleration of diffusion in F_1-ATPase II

Ryunosuke Hayashi[1], Shuichi Nakamura[1], Seishi Kudo[1], Kazuo Sasaki[1], Hiroyuki Noji[2], **Kumiko Hayashi**[1] ([1]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*, [2]*Dept. Appl. Chem., Sch. Eng., Univ. Tokyo*)

Diffusion coefficients are often measured in small biological systems to characterize their fluctuation. Giant acceleration of diffusion is one of the theories on diffusion in the field of non-equilibrium statistical mechanics. We apply this theory to F_1-ATPase which is an ATP-driven rotary motor protein. According to the theory, when we apply a constant torque to F_1 by using an electric rotating field, the diffusion coefficient of a rotary probe attached to F_1 as a function of the applied torque exhibits a resonance peak. The resonance peak corresponds to the torque value near the critical tilt of the rotary potential of F_1.

3P152 $TF_1\beta$ サブユニットのヌクレオチド結合への Pi の影響

Nucleotide binding to TF_1 β subunit in relation to the effect of Pi

Riku Nagano, Kiyoshi Obara, Hiroshi Ueno, Eiro Muneyuki (*Dept. of Physics. Chuo Univ.*)

Nucleotide binding is important for the rotation of F_1-ATPase. Here, we examined nucleotide binding to the isolated β subunit and $\alpha_3\beta_3\gamma$ subcomplex containing βY341W mutation in relation to the effect of Pi. We found that $k_{on\ ATP}$ and $k_{on\ ADP}$ for the isolated β subunit were insensitive to 100 mM Pi. Next we examined the effect of Pi on nucleotide binding to $\alpha_3\beta_3\gamma$ subcomplex. In order to prevent ATP hydrolysis during experiment, we first used β(E190Q/Y341W) mutant for nucleotide binding to subcomplex. One hundred mM Pi partially released pre-loaded ADP but not bound ATP from $\alpha_3\beta(E190Q/Y341W)_3\gamma$ subcomplex. Next, we repeated the same experiment using $\alpha_3\beta(Y341W)_3\gamma$. In this case, 100mM Pi partially released the bound nucleotide irrespective of ATP or ADP was pre-loaded.

3P153 非天然アミノ酸導入型 F_1-ATPase を用いた１分子回転観察

Key factors of arginine finger of F_1-ATPase clarified by an unnatural amino acid mutation

Ayako Yukawa[1], Ryota Iino[2], Rikiya Watanabe[1], Shigehiko Hayashi[3], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo.*, [2]*Okazaki Inst. Integ. Biosci., NINS*, [3]*Grad. Sch. Sci., Univ. Kyoto.*)

A catalytically important arginine, called Arg finger (Arg), is employed in many enzymes to regulate their functions. F_1-ATPase (F_1), a rotary motor protein, possesses Arg, which contributes to catalyze ATP efficiently. In this study, to identify chemical determinants of the Arg catalysis, we mutated Arg into an unnatural amino acid Lyk, a lysine analogue with an alkyl chain elongated by one CH_2 unit. Single molecule observations showed that terminal guanidium group in Arg is indispensable for catalyzing ATP and that geometric chain length matching prevented the inhibited-state of F_1. We showed that utilization of unnatural amino acids extends biochemical approaches for elucidation of molecular mechanism of protein functions at a high chemical resolution.

3P154 Rotor-Stator Interactions in V_1 and V_o from *Enterococcus hirae* V-ATPase

Hiroshi Ueno[1], Yoshihiro Minagawa[2], Mayu Hara[2], Ichiro Yamato[3], Hiroyuki Noji[2], Takeshi Murata[4], Ryota Iino[5], Eiro Muneyuki[1] ([1]*Fac. Sci. & Eng., Univ. Chuo*, [2]*Sch. Eng., The Univ. Tokyo*, [3]*Dept. Biol. Sci. & Tech., Tokyo Uni. Sci.*, [4]*Grad. Sch. Sci., Univ. Chiba*, [5]*Okazaki Inst. for Integr. Biosci., NINS*)

We developed an *E. coli* expression system for *E. hirae* V-ATPase (EhV_oV_1) which functions as a Na^+ pump and observed the rotation of EhV_oV_1. Even at high Na^+ concentration, with a load-free probe, EhV_oV_1 rotated slower than EhV_1 without clear three pauses separated by 120°, suggesting that EhV_o limits the rotation of EhV_1. The torque of EhV_1 estimated from the continuous rotation was nearly half of that of EhV_oV_1 although the stepping torque of EhV_1 was comparable to the torque of EhV_oV_1, suggesting that EhV_1 has unstable state where EhV_1 generates low torque whereas rotor-stator interactions in EhV_oV_1 are stabilized by two peripheral stalks. We found that the torque of EhV_oV_1 was lower than those of other V_1 and F_1, indicating low energy conversion efficiency in EhV_oV_1.

3P155 有糸分裂キネシン Eg5 の新規 SH 基反応性フォトクロミック分子を用いた光制御

Photo-control of mitotic kinesin Eg5 using novel SH reactive photochromic molecules

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Kinesin Eg5 is essential for bipolar spindle formation during eukaryotic cell division. Previously, we prepared mutants of mitotic kinesin Eg5, which have a single cysteine in the functional region and modified with photochromic molecules, iodoacetyl-spiropyran and 4-phenylazomaleinanil. ATPase activities of the modified Eg5 mutants W127C and D130C were photo-reversibly regulated by light irradiations. I this study, we synthesized novel SH group crosslinkable photochromic molecules, iodo-fulgide (IAFG) and iodo-trityl azobenzene (IATAB) and tried to control function of Eg5. IAFI and IATAB were incorporated into the Eg5 mutants, E116C, E118C, Y125C, W127C and D130C. Photo-control of ATPase and motility activities of the modified Eg5 were investigated.

3P156 ヒト細胞質ダイニンのパワーストローク測定

Determination of Power Stroke Distance Driven by Human Cytoplasmic Dynein

Yoshimi Kinoshita[1], Taketoshi Kambara[2], Satoshi Ikeda[1], Motoshi Kaya[1], Hideo Higuchi[1] ([1]*Graduate School of Science, The University of Tokyo*, [2]*RIKEN QBiC*)

Cytoplasmic dynein is a motor protein moving along microtubules toward the minus-end dominantly with 8.2nm step, and plays an important role in cellular processes. Dynein's conformational change, called "power stroke", is assumed to generate driving forces moving along the microtubule. However, it has not been clarified the mechanism of how the power stroke contributes to individual steps. Thus, we measured the power stroke distance of single-headed dynein using optical tweezers. Results showed that the power stroke distance is less than 8.2nm, implying the following scenario; the attached head goes on power stroke, while the other head detaches, undergoes diffusive search and rebinds to the next site on microtubule.

3P157 V-ATPase の中心回転軸におけるトルク伝達機構
The ingenious structure of central rotor apparatus in V_oV_1; torque transmission mechanism in the central rotor of V_oV_1

Atsuko Nakanishi[1], Jun-ichi Kishikawa[1], Masatada Tamakoshi[2], Shou Furuike[3], Ken Yokoyama[1] ([1]*Dept. Mol. Biosci., Kyoto Sangyo Univ.*, [2]*Dept. Mol. Biol., Tokyo University of Pharm. & Life Sci.*, [3]*Dept. Physics, Osaka Medical College*)

The central rotor apparatus of V_oV_1 of *Thermus thermophilus* is composed of V_1-DF shaft and V_o-CL_{12} rotor ring. In this study, we revealed that the rod domain of V_1-DF plays important roles on both reconstitution and energy coupling in V_oV_1 by the reconstitution and fluorescence resonance energy transfer (FRET) analysis. We propose a structural model accounting for both the detachable and sticky nature of the interaction between the central rotor apparatuses.
In addition, we discuss a twist or torsion of V_1-DF shaft in V_oV_1 during rotation.

3P158 フォトクロミック分子によって誘導されるキネシン・ADP・フルオロメタル複合体の構造変化
Conformational change of kinesin-ADP-fluorometal ternary complexes induced by photochromic molecules

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In the presence of ADP, kinesin forms stable ternry complexes with phosphate analogues, BeFn, AlF4- and Vi, each of which mimic different transient state along ATPase cycle. In this study, we tried to induce transition between the different transient states of the ternary complexes in ATPase by photoisomerization incorporated into the functional region of kinesin. To monitor the conformational change of the ternary complexes by 19F-NMR, we synthesized fluorine labelled ADP analogue, 4-Fluoro-benzoic-ADP (4-FB-ADP). In the presence of BeFn, 4-FB-ADP was trapped within the ATPase site of kinesin resulted in formation of Photochromic-kinesin/4-FB-ADP/BeFn ternary complexes. The conformational change of the complex induced by light irradiation was monitored by 19F-NMR.

3P159 複数のキネシンによる協調的カーゴ輸送のメカニズムの解明
Investigation of the mechanism of cooperative cargo transport by multiple kinesins

Naoto Sawairi[1], Takayuki Ariga[2], Mitsuhiro Iwaki[3,4], Michio Tomishige[2], Kumiko Hayashi[1] ([1]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*, [2]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*, [3]*QBiC, RIKEN*, [4]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

In cells, organelles are considered to be transported by multiple motor proteins such as kinesin and dynein along microtubules. It is important to elucidate the transport mechanism. In the previous study[1], the drag force was measured using fluctuation theorem in Neuron, and the number of motors was estimated from the distribution of the drag force. Then we performed an *in vitro* single-molecule experiment on kinesin-1 with controlling the number of kinesins using DNA-origami. Multiple kinesins are connected with a 1um-sized bead via DNA-origami. We aim to reproduce the result[1] in this *in vitro* single molecule experiments.
[1] K. Hayashi, Y. Okada, 2p145, 51th annual meeting of BSJ (2013)

3P160 MD シミュレーションによる *Enterococcus hirae.* V_1-ATPase の回転機構の解明
Rotation mechanism of V_1-ATPase studied by MD simulation

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V-ATPase is a molecular motor acting as a proton pump in cell membranes, which includes a proton-translocation domain (V_O) and a rotary ATP-hydrolytic domain (V_1). Recently, Arai et al. have determined a high-resolution crystal structure of V_1, composed of the central stalk (DF) and the asymmetrically-packed hexamer ring (A_3B_3). To elucidate the relationship between rotation dynamics of DF and conformation changes of A_3B_3, we conducted a 100-ns MD simulation using an all-atom model of V_1 with explicit solvent, and analyzed dynamical correlation among the subunits. We found that several residues exhibit highly correlated motions during MD, suggesting that the correlated motions play key roles on the rotation dynamics of V_1.

3P161 リニアモータータンパク質キチナーゼの蛍光一分子観察
Single-Molecule Fluorescence Imaging of Linear Motor Protein Chitinase

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Chitinase A (ChiA) is a linear motor protein witch processively hydrolyzes and moves on crystalline chitin. Turnover of ChiA measured in biochemical assay is about one-seventeenth lower than that calculated from the result of single-molecule observation with high-speed AFM (Uchiyama et al. JBC, 276 (2001), 41343-41349, Igarashi, Uchihashi et al. Nature Commun, in press.). This inconsistency can be explained if we assume the two different binding modes of ChiA to chitin, productive and non-productive bindings which accompany hydrolysis and movement or not, respectively. With single-molecule florescence observation, we determined fraction and velocity of productively binding ChiA. As a result, we found that most ChiA molecules bind to chitin non-productively.

3P162 ATP synthesis by elastically coupled molecular motors

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We have studied a simplified analogue of ATP synthase, consisting of two elastically coupled molecular motors pulling against each other in a molecular 'tug of war'. When one of the molecular motors is driven strongly enough by an external free-energy source, it pulls the other ATP-driven molecular motors backwards so that it synthesizes ATP.
Monte-Carlo simulations of the molecular tug of war allow us to study the dependence of the ATP synthesis rate on the free-energy drive ΔG and on the stiffness λ of the spring coupling the two motors. The model reproduces the experimentally observed dependence of ATP synthesis rate on free energy at two crucial points, the maximum ATPase velocity and the thermodynamic equilibrium where the velocity of the ATPase is zero.

3P163 CYK-4 による kinesin-6 の螺旋運動調節機構
The spiraling movement of kinesin-6 regulated by CYK-4

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Centralspindlin, kinesin-6 (motor)/CYK-4(Rho-GAP) complex is essential for assembly of the central spindle. While the motility of kinesin-6 bound to CYK-4 was examined in a microtubule sliding assay, it is not clear how binding of CYK-4 to kinesin-6 regulates the motor activity. In this work, using 3-D tracking microscopy, three dimensional movements of both kinesin-6 and centralspindlin along a suspended microtubule were quantified. We found that both kinesin-6 and centralspindlin displayed a left-handed spiraling motion around the microtubule. The velocity of centralspindlin was slower than that of kinesin-6 and the rotational pitch of centralspindlin was longer than that of kinesin-6, indicating that CYK-4 modulates the motor activity of kinesin-6.

3P164 Autoinhibition and cooperative activation mechanisms of cytoplasmic dynein

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Cytoplasmic dynein is a microtubule-based motor responsible for diverse intracellular movements. Here, we show single dynein molecules are in an autoinhibited state, in which the two motor heads are stacked together. In this state, dynein moves diffusively along a microtubule with a small bias toward the minus end of the microtubule. When the two heads were physically separated, the movement of dynein molecules became directed and processive. Furthermore, assembling of multiple dynein molecules on a single cargo enabled directed movement and cooperative force production. We propose a mechanism of autonomous on-off switching of cargo transport, in which single dynein molecules in the cell is autoinhibited and become active when assembled as a team on a cargo.

3P165 ミオシンの協調的首振りとアクチン滑り運動のゆらぎ
Cooperative lever-arm swings of myosins and fluctuation of actin sliding

Yota Kondo, Kazuo Sasaki (*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*)

Myosin II is a motor protein which swings a lever arm and generates in a group the sliding movement of an actin filament. It is customarily thought that the actions of individual myosin motors are independent when an actin filament slides. If this is the case, the effective diffusion coefficient of actin will be inversely proportional to the number of myosins (K. Sekimoto and K. Tawada, Biophys. Chem., 2001). However, a measurement of actin sliding *in vitro* showed that the effective diffusion coefficient is almost independent of the number of myosins (N. Noda *et al*., Biophysics, 2005), which implies that lever-arm swings of myosins are not independent. We construct a theoretical model that can explain the experiment and analyze cooperative lever-arm swings of myosins.

3P166 全反射型蛍光顕微鏡における受像偏向と偏光変調 ーF_1-ATPase の構造変化検出への応用ー
Emitter modulation and polarization switching under TIRF illumination: Application for detection of conformational change in F_1-ATPase

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To elucidate domain motions of the catalytic β subunits of a molecular motor F_1-ATPase at the single molecular level, we have developed TIRFM under polarization modulation. A pair of images under *s*- and *p*-polarized illuminations is continuously captured by fast switching between two beams. Single emitting images of spots were rotated at the camera plate synchronously with the rotation of the polarization orientation under *s*-polarized light, and resultant circular images were fitted with an approximated equation. Feasibility of the analyses was validated by reconstructed images to estimate the changes in tilting and azimuthal angles of the C-terminal helix of β.

3P167 蛍光標識 ATP アナログを用いたイネ特有のキネシン E11 の速度論的解析
Kinetic characterization of rice plant specific kinesin E11 using fluorescent ATP analogue

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We have previously demonstrated that some kinesins derived from rice plant have unique biochemical characteristic properties and structures. In this study, we focused on rice kinesin E11 that belongs to the plant specific At1 subfamily in kinesin-7 family. E11 motor domain was expressed in E.coli BL21(DE3) and purified by Co-chelate column in order to characterize biochemical and ATPase kinetic properties. We employed two fluorescent ATP analogues, Mant-ATP and NBD-ATP for the kinetic characterization. we have successfully observed significant FRET between Mant-ATP and intrinsic tryptophane (Trp23) in E11. The rates of initial binding of Mant-ATP and release of Mant-ADP from E11 were analyzed by monitoring the FRET using stopped flow apparatus.

3P168 金ナノロッドを用いた高速配向イメージングシステムの開発と F1-ATPase の構造変化検出への応用
Development of high-speed orientation imaging system for gold nanorod and application to detection of conformational change of F1-ATPase

Sawako Enoki[1], Ryota Iino[2], Yamato Niitani[3], Yoshihiro Minagawa[1], Michio Tomishige[3], Hiroyuki Noji[1] ([1]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo*, [2]*Okazaki Inst. Integ. BioSui., NINS*, [3]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*)

We developed two kind of high-speed orientational imaging systems for single gold nanorod (AuNR) by using objective-type backscattering dark-field microscopy with microsecond temporal resolution. These two systems are based on (i) intensity analysis of focused images of AuNR split to two orthogonally polarized components, and (ii) analysis of defocused image of AuNR. We compared angle precision and accuracy of the systems and found that orientations resolved by analysis of defocused image had better angular accuracy of 5 degree with 10 μs temporal resolution. Using analysis of defocused image, rotation of the rotor γ subunit and domain motion of catalytic β subunit of rotary molecular motor F_1-ATPase were monitored with time resolution of 3 μs.

3P169 多重周波数モジュレーション原子間力顕微鏡法：単一細胞レオロジーの高速測定

Multi-frequency modulation atomic force microscopy for the high-speed measurement of single cell rheology

Ryosuke Takahashi, Kaori Kuribayashi-Shigetomi, Agus Subagyo, Kazuhisa Sueoka, Takaharu Okajima (*Grad. Sch. Info. Sci. & Tech., Hokkaido Univ.*)

Measurement of cell mechanical property is crucial for both understanding various cell functions and diagnosing cell disease. Previous atomic force microscopy (AFM), in which a stepwise change in frequency was employed, revealed that the cell complex shear modulus, G^* exhibited a large cell-cell variation depending on frequency [1]. However the AFM method required a measurement time longer than the conventional force curve measurements. Here, we proposed a multi-frequency AFM for obtaining simultaneously G^* of cell in a wide frequency range and demonstrated that this AFM technique allowed us to measure the power-law frequency dependence of G^* [2] within several seconds.

[1] Cai et al. Biophys. J. 105,1093-1102 (2013) [2] Fabry et al. Phys. Rev. Lett. 87,14 (2001)

3P170 ケージド化合物の光分解を用いた大腸菌細胞応答の定量的計測

Quantitative measurement of the cellular response of *Escherichia coli* using photolysis of the caged chemoattractant

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Using chemotaxis signal processing system, *Escherichia coli* (*E. coli*) regulates rotational direction of flagellar motor. When *E. coli* cell recognizes chemoattractant by transmembrane receptor, rotational direction of the flagellar motor changes to counterclockwise direction. In order to investigate kinetic property of the signal processing induced by chemoattractant, we statistically measured time course of directional biases of rotationing tethered cells. By using caged chemoattractants and high-temporal resolution measurement system (250 Hz), we traced precisely the directional biases triggered by photolysis of caged attractants. We would like to discuss about detail of the kinetic property in the signal processing induced by chemoattractant in this annual meeting.

3P171 蛍光相関分光法を用いた単一細胞由来のグルココルチコイドレセプター二量体形成と転写活性の定量

Quantification of glucocorticoid receptor homo-dimer and transcriptional activity in single cell by fluorescence correlation spectroscopy

Sho Oasa[1], Akira Sasaki[2], Shintaro Mikuni[3], Masataka Kinjo[3] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*AIST*, [3]*Fac. Adv. Life. Sci., Hokkaido Univ.*)

The relationship between the amounts of homo-dimeric glucocorticoid receptor (GR) and transcriptional activity is studied using single-cell method combined with Fluorescence Correlation Spectroscopy (FCS) and microwell chip (FCS-microwell system). It is well-known that GR translocates to nucleus after ligand binding and then, homo-dimeric GR binds to genome and activates its functions as transcriptional factor. However, the relationship between the amounts of homo-dimeric GR and its transcriptional activity remains unclear, yet. In this study, FCS-microwell system was established, namely the amounts of homo-dimeric GR and transcriptional activity were determined at single-cell lysate in microwell simultaneously by FCS and fluorescent reporter assay.

3P172 超解像顕微鏡法による上皮成長因子受容体クラスタリングの定量解析

A Quantitative Analysis of Epidermal Growth Factor Receptor Clustering Using Super-resolution Microscopy

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Activation of epidermal growth factor receptor (EGFR), which evokes various cellular signaling, has been suggested to be regulated through diffusion-driven receptor clustering. For a precise analysis of the EGFR clustering, photo-activation localization microscopy (PALM) was employed to distinguish adjacent oligomers with a distance of shorter than the conventional optical resolution. The distribution of oligomer size quantified by a hierarchical clustering algorithm on the PALM data was distinct from that obtained by the previous methods. The localization accuracy has been improved for enabling investigation of the spatial receptor arrangement in oligomers. The clustering dependent mechanism of signaling regulation unveiled by the novel method will be discussed.

3P173 FRAP による、成長円錐のアクチンおよびアクチン関連タンパク質の動態解析

FRAP experiments on actin and actin associate proteins in growth cones

Minami Tanaka[1,2], Takeshi Tougasaki[3], Kaoru Katoh[3] ([1]*Biomed. Res. Inst, AIST*, [2]*Grad. Sch. Life & Env. Sci., Univ. Tsukuba*, [3]*FANCL Co.*)

Growth cones are enlargement of cytoplasm which appear at the tip of growing dendrites or axons. The growth cone movement plays an important roles in path finding and neuronal navigation. The movement is controlled by the balance between rate of polymerization of g-actin and of retrograde flow of f-actin. Growth cone advances when rate of polymerization is faster than that of retrograde flow. The actin dynamics is regulated by actin associate proteins. We, therefore, performed FRAP experiments on actin, arp2/3 and other actin associate proteins. We observed not only retrograde flow of actin but also anterograde movement. Mechanism of anterograde movement of actin and future experiments will also be discussed.

3P174 神経細胞膜の分子選択的な並進拡散障壁

Molecule-selective lateral-diffusion barrier in the neuronal axon membrane

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The presence of a diffusion barrier in the plasma membrane (PM) in the neuronal initial segment (IS) region has been established by us and others. Here, we found that GPI-anchored proteins (GPI-APs) diffuse rapidly in the IS-PM (only ~2x slower than in other PM regions), but not phospholipids, showing that the diffusion barrier is a molecule-selective filter. Ultrahigh-speed single-molecule tracking revealed that the IS-PM is partitioned into ~60-nm compartments, and that GPI-APs hop to an adjacent compartment an average of once every ~2.0 ms, but phospholipids do so ~300x less frequently. With an increase in hydrophilicity in the headgroup moiety, the hop frequency was increased, suggesting that the molecules' vertical location in the PM affects the hop frequency.

3P175 κオピオイド受容体の動的濃縮領域（ホットスポット）：1分子イメジングによる検出

Single-molecule detection of hotspots for dynamic concentration of the kappa opioid receptor

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The kappa opioid receptor (kOR), a G-protein-coupled receptor, was expressed in the plasma membrane (PM) of cultured cells and observed at the single-molecule level. The kOR molecules underwent thermal diffusion, but dynamically concentrated in many submicron areas: many molecules enter and exit from the area continually, but the average molecular density remained high in the area (hotspot). kOR's scaffolding protein EBP50, which could link kOR to the actin filament via ERM proteins, is dynamically present in the hotspots, but at variance with the general view of the kOR-EBP50 interaction, their interactions are transient, with a colocalization lifetime of ~0.3 s. These results indicate a possibility that kOR signaling occurs in transient kOR oligomers including EBP50.

3P176 Noonan 症候群における SOS を介した Ras positive feedback 異常の生細胞一分子解析

Dysregulations of SOS-mediated positive feedback on RAS activation in Noonan syndrome observed using single molecule imaging

Yuki Nakamura[1,2], Kayo Hibino[3], Yasushi Sako[1,2] ([1]*RIKEN*, [2]*Osaka Univ.*, [3]*QBiC., RIKEN*)

Noonan syndrome (NS) is a congenital disorder with cardiac disease. The 10 % of NS patients has mutation on SOS, an activator (guanine-nucleotide exchange factor, GEF) of a small GTPase RAS, a crucial factor of cell proliferation. In this study, we observed fluorescently labelled SOS molecules on the plasma membrane using single-molecule imaging and analyzed the dissociation kinetics reflecting GEF activity. It is known that GEF activity of SOS is stimulated by interaction with the active form of RAS (positive feedback), which is crucial for RAS activation in living cells. Our observation indicated that roles of the positive feedback were different among types of SOS mutants in NS. Mutants that become gain of function might exist independent of the positive-feedback.

3P177 二つの抗体送達システムにおける送達作用の比較

Comparison of the delivery effects of two antibody carrier systems

Kana Kuwahara[1,2], Kazuki Harada[1,2], Takenori Yamamoto[1,2], Yasuo Shinohara[1,2] ([1]*Inst. Genome Research.*, [2]*Fac. Pharm. Sci., Univ. Tokushima*)

Experimental systems, which can deliver antibodies into living cells, are useful to develop antibody therapies. In these systems, the delivery strategy using cell-penetrating peptide has some advantages such as low toxicity. We here compared two kinds of the peptide-based antibody delivery system: the system using protein A module and that using a peptide with hydrophobic tryptophan-rich motif. We delivered the fluorescent labeled antibodies to HeLa cells using each delivery system. As a result, both systems enabled to deliver the antibodies into the cells. However, the system using a hydrophobic tryptophan-rich motif caused the red-shift of the fluorescent spectrum. The possible reasons for the shift will be discussed.

3P178 *Amoeba proteus* 細胞膜の 3 次元曲率に関する研究

Characterization of surface structures of *Amoeba proteus* in three dimensional spaces

Yukinori Nishigami[1], Atsushi Taniguchi[2], Seiji Sonobe[3], Shigenori Nonaka[2], Masatoshi Ichikawa[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Grad. Sch. Sci., Univ. Hyogo*, [3]*NIBB*)

Amoeba proteus migrates actively and during the locomotion the shape of cell surface is dynamically deformed. In this study, we observed the surface structure of *A. proteus* in three dimensional spaces using a modified digital scanned light-sheet microscope. Interestingly, pseudopods were actively elongated to various directions in three dimensional spaces, which have never seen in two dimensional observations. To characterize the surface structure of the cell, we calculated three dimensional curvatures (Gaussian curvature and Mean curvature) of the structure of cell surface and compared the curvatures with direction of cell locomotion. As a result, cell surface curvatures were unevenly distributed in the cell depending on polarity of cell migration.

3P179 電子顕微鏡法によるヒト毛乳頭細胞の一次繊毛の構造解析

Structural analysis of primary cilia in human follicle dermal papilla cells by electron microscopy

Misaki Tanaka[1], Kazuyuki Matsushima[2], Kuniyoshi Kaseda[2], Takuo Yasunaga[1] ([1]*Kyushu Institute of Technology*, [2]*Saravio Cosmetics Ltd.*)

Most cells of multicellular organisms have primary cilia, which are single, non-motile, and sensory cilia. They have been reported to detect and transform mechanical stimulation to intracellular, but the mechanisms are not still well. Dermal papilla cells (DPCs) with primary cilia (Matsushima, 2013) in the skin can induce hair growth. Here we observed the structure of DPCs (2D and clumps) by scanning electron microscopy (SEM) for microstructural analysis. In SEM we prepared the specimens under near-living conditions with ionic liquid and so. Moreover we challenged CLEM, which can observe the cilia by SEM after identifying them by fluorescence microscopy. Here we report the recent progress to get the ultrastructure of cells and primary cilia under near-living conditions.

3P180 Intranulear particles include RPS or RPL subunits

Saori L. Mimatsu[1,2], Maiko Kuramochi[1,2], Soyomi Uchibori[1,2], Ayako Kojima[1], Emiko Kobayashi[1], Michio Hiroshima[3,4], Yasushi Sako[3,4], Kaoru Katoh[1] ([1]*Biomed. Res. Inst., AIST*, [2]*Grad. Sch. Life & Env. Sci., Univ. Tsukuba*, [3]*REKEN ASI*, [4]*RIKEN QBiC*)

Apodized phase contrast microscope (APC) reduces halo artifacts with spacial filtering.and reveals fine structures without any staining. To identify structures in the APC images with fluorescent marker, our external APC system have simultaneous imaging system of APC (546nm) and fluorescence image (594nm-700nm).

Here, we re-examined and identified intracellular structures observed with APC in simultaneous image records of APC and fluorescence. APC directly revealed 1) ER 2) mitochondria, 3) PML body, and 4) intranuclear granules. The granules included, ribosomal protein of small (RPS) or large (RPL) subunit, but RPS and RPL were not co-localized in the granules. Farther image data will be discussed in the presentation.

3P181 アクチン細胞骨格と核との力学的結合が血管平滑筋細胞の分化に与える影響

Effects of Actin-Nucleus Connections on the Vascular Smooth Muscle Cell Differentiation

Kazuaki Nagayama[1], Makoto Iwata[2], Takeo Matsumoto[2] ([1]*Department of Intelligent Systems Engineering, Ibaraki University, Japan*, [2]*Department of Mechanical Engineering, Nagoya Institute of Technology, Japan*)

Vascular smooth muscle cells (VSMCs) dedifferentiate from contractile to synthetic phenotype under pathological conditions. To understand smooth muscle pathophysiology, it is important to understand the mechanism of their differentiation and dedifferentiation. We recently found that the actin stress fibers (SFs) have a mechanical interaction with the nucleus, and the internal forces of SFs were transmitted directly to the nucleus. Thus, it is possible that the mechanical interaction between SFs and nuclei could be associated with gene transcription and cell differentiation. Here we investigated the alterations in the mechanical interaction between actin cytoskeleton and the nucleus during the differentiation and dedifferentiation processes of VSMCs.

3P182 繰り返し伸展刺激によって起こるストレスファイバーの脱重合のメカニズムについて

What is the molecular mechanism of stress fiber disassembly caused by mechanical cyclic stretch?

Wenjing Huang[2], Tsubasa Matsui[1], Masahiro Kuragano[3], Masayuki Takahashi[3], Tomohiro Kawahara[2], Masaaki Sato[4], **Shinji Deguchi**[1] ([1]*Nitech*, [2]*Kyutech*, [3]*Hokkaido Univ*, [4]*Tohoku Univ*)

The mechanism underlying selective disassembly of stress fibers (SFs) oriented in the direction of cyclic stretch remains unclear. Here, we show that fast shortening of cells that exceeds the intrinsic contraction speed of SFs causes disassembly of SFs. SFs that contained myosin light chain mutants whose actin-myosin-II interactions were restricted were more prone to disassembly upon fast cell shortening. We overexpressed active LIM-kinase mutants to inactivate cofilin, and found that disassembly of SFs still occurred in a manner similar to that of controls. We suggest that the disassembly is caused by the unbundling of constituent actin filaments in a manner critically dependent on myosin II but independent of the severing activity of cofilin.

3P183 ３次元コラーゲンゲル内に培養された線維芽細胞のメディウムの流れ刺激に対する反応

Responses of fibroblasts against fluid flow stimuli in a three-dimensional collagen gel culture system

Natsumi Saito[1], Hiroaki Adachi[2], Hiroshi Tanaka[2], Satoru Nakata[2], Norifumi Kawada[1], Katsutoshi Yoshizato[1] ([1]*Dept. Hepatology, Grad. Sch. Med., Osaka City Univ.*, [2]*Nippon Menard Cosmetic Co., Ltd.*)

Cells *in vivo* are bathed in the interstitial fluid and it has been said that the fluid flow-derived stimulus is one of the basic constraints that determine cells' basic phenotypes *in vivo*. Cells in the three-dimensional (3D) collagen gel culture are known to recapitulate their *in vivo* phenotypes. The effects of medium flow-stimulus on human fibroblasts were studies using this culture method. The cells were supplied with medium through a pump-driven syringe at a regulated velocity (0.012 μm/s to 1.5 μm/s) and characterized for their various phenotypes. The medium flow-stimulus activated the cell growth in a velocity-dependent manner, the expression of hyaluronan synthetase 2 gene, and markedly increased the gel's wet volume.

3P184 細胞シート延伸における細胞核変形量の測定

Measurements of cell nucleus deformation during stretching a cell sheet

Kota Onishi, Masahiro Tsuchiya, Takaharu Okajima (*Grad. Sch. Inform. Sci. and Techno. Hokkaido Univ.*)

Cells have an ability to activate their cell functions in response to external forces. Mechanical properties of cells have been widely studied in single cells, but less in cell population. Here, we investigated how cell nucleus deforms in response to uniaxial external forces, using cell sheet of COS-Fucci (RIKEN) [1] by monitoring the fluorescence of nucleus depending on cell phase. We found that the deformation of nucleus was proportional to that of the whole cell sheet, but the former value was much less than the latter indicating a heterogeneous deformation of intracellular structures. Detailed results for the cell cycle dependence will be presented. We thank Dr. Atsushi Miyawaki for COS-Fucci and NMuMG-Fucci cells. [1]A. Sakaue-Sawano.et.al.,Cell 132, 487 (2008)

3P185 Microfluidics analyses of coordinated dynamics of F-actin and cAMP signaling in *Dictyostelium* chemotaxis

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In migrating *Dictyostelium*, chemoattractant field is dynamic, self-generated and amplified by the moving cells themselves. How the cell movement and the amplification of guidance cue are coordinated in space and time remains elusive. Here we study the role of actin polymerization on the chemoattractant cAMP-induced elevation of cAMP. We show by combining FRET-based live-cell imaging of cytosolic cAMP and microfluidics that the amplitude and the timescale of the response are altered when actin polymerization is pharmacologically inhibited. The population-level oscillations of cAMP also diminished under latrunculin treatment, suggesting that self-generation of chemoattractant cAMP field is linked strictly to cell movement.

3P186 紡錘体の力学特性の遷移は染色体分配を手助けする

Mechanical transition of the vertebrate meiotic spindle facilitates chromosome dynamics

Jun Takagi[1], Takeshi Itabashi[2], Shin'ichi Ishiwata[2,3] ([1]*Quantitative Mechanobiology Laboratory, NIG*, [2]*Fac. Sci. Engn., Waseda Univ.*, [3]*WABIOS, Waseda Univ.*)

Activities of molecules such as molecular motors and microtubules are well regulated depending on cell cycle phases to organize a mitotic/meiotic spindle. Currently, we do not know whether the mechanical properties of the spindle differ in stages of mitotic/meiotic phase, and whether this difference contributes to mitotic stage-dependent mechanical works such as congression and segregation of chromosomes. Here, we measured the mechanical properties of the metaphase and anaphase spindles assembled in *Xenopus* egg extracts using a pair of force-calibrated micro-needles. We found that anaphase spindles are less elastic than metaphase spindles against the stretching force, of which direction is the same as that of spindle elongation at anaphase.

3P187 オリゴマイシンとロテノンはミトコンドリアの透過性遷移に相乗的な阻害作用を示す

Synergistic inhibitory effects of oligomycin and rotenone on the mitochondrial permeability transition

Kazumasa Kotake[1,2], Yuki Inotani[1,2], Yuya Yoshimura[1,2], Kazuki Harada[1,2], Takenori Yamamoto[1,2], Yasuo Shinohara[1,2] ([1]*Inst. Genome Research., Univ. Tokushima*, [2]*Fac. Pharm. Sci., Univ. Tokushima*)

Mitochondrial inner membrane shows high resistance against permeation of molecules or ions. However, under certain conditions such as in the presence of Ca^{2+} and inorganic phosphate, the membrane becomes permeable to various solutes up to 1500 Da. This phenomenon is referred to as mitochondrial permeability transition (PT). The physiological meanings of PT induction have established as a part of the processes regulating cellular apoptosis. However, the mechanisms of PT induction have been unclear. We here investigated the effects of oligomycin, F_oF_1-ATPase inhibitor, on the mitochondrial PT. As a Result, oligomycin inhibited the PT induction. Moreover, the inhibitory effects of oligomycin were enhanced in the presence of rotenone, respiratory chain complex I inhibitor.

3P188 がん細胞接着及び運動における硬さの影響の評価

Evaluation of the Impact of Stiffness on Adhesion and Migration of Cancer Cells

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Recently, a number of studies have provided substantial evidence that highly metastatic cancer cells are softer than normal cells and lowly metastatic cancer cells. Thus stiffness of cells now draws much attention as a new physical measure for malignancy of cancer cells. Alternatively, stiffness of cellular microenvironments has also been increasingly recognized as a physical cue that can determine various cell functions (e.g., differentiation). However, how the interplay of stiffness of cancer cells and their microenvironments is involved in cancer progression remains much unknown. In this work, we evaluated adhesion and migration of cancer cells with different metastatic ability on hydrogel with tunable mechanical properties.

3P189 マイクロパターン上に培養した単一細胞の細胞核の動態

Movement of nucleus in cells cultured on micro-patterned substrates

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Physical quantities such as the shape and the displacement of the cell nucleus are crucial parameters for understanding how cell organelle is mechanically regulated to express various cell functions. Here, we measured the nucleus movement of cells placed on micro-patterned substrates, which allow us to confine the cells in a restricted region and thus to quantify the dynamic behaviors of the cell nucleus. Time-lapse images of stained nucleus were obtained, and the mean square displacement (MSD) was analyzed in different micro-patterns. It was found that cell nucleus of square-shaped cells spatially fluctuates in a random manner. The detailed results of cells with cytoskeleton modifications as well as of disease cells will be presented.

3P190 F-アクチン溶液のシアバンディング

Shear banding in an F-actin solution

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It has been reported that the shear stress of F-actin solution was constant with respect to upward changes in a wide range of applied shear rate (Maruyama et al., BBA, 1974). To understand the mechanism, we examined the relation between the rheological property and flow field in an F-actin solution (Kunita et al., PRL, 2012). The F-actin solution showed 'shear banding', which is characterized by the spontaneous separation of homogeneous shear flow into two macroscopic domains of different definite shear rates. In our presentation, we discuss the dynamic aspect and biological implications of shear banding in an F-actin solution.

3P191 細胞運動におけるアクチンストレスファイバと焦点接着斑の時空間ダイナミクス

Spatio-temporal dynamics of actin stress fibers and focal adhesions during cell migration in Swiss 3T3 fibroblasts

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Actin stress fibers (SFs) and focal adhesions (FAs) play a central role in cell migration that is a combination of protrusive and contractile events. The aim of this study is to clarify the mechanism how cells turn during migration. We observed migration of Swiss 3T3 fibroblasts expressing GFP-actin and RFP-zyxin, and spatio-temporal dynamics of SFs and FAs were analyzed by kymograph studies and quantitative measurements. As a result, at the side of the cell, there were gradually stabilizing FAs attached to the end of the dorsal SFs. They connected to the arc and subsequently formed a SF with FAs at the both ends, possibly becoming a contractile ventral SF at the cell rear during the next cycle of cell migration. This process might cause turn in cell migration.

3P192 核膜孔複合体内部における疎水性分子環境の in vivo 解析

In vivo analysis of hydrophobic molecular crowding environment in the Nuclear Pore Complex

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Nuclear Pore Complexes (NPCs) is composed of more than 30 different kinds of subunits. The central channel of the pore is thought to have hydrophobic crowding environment, due to high contents of Phe-Gly (FG) motifs in pore-forming subunits. Here, we analyzed spatiotemporal formation of hydrophobic pore within NPCs by utilizing fluorescent protein probe (ECFP-YFP-G1), which is affected by high concentration of proteins. The pore forming subunits fused with ECFP-YFP-G1 were localized in the nuclear envelope of HeLa cells. Quantitative analysis of FRET signal revealed that subunits showed different reduction of YFP-G1 signal, indicating that the hydrophobic microenvironment isn't uniform. We further analyzed the process of post-mitotic reassembly of the NPC subunits.

3P193 回虫精子の MSP マシナリー構成要素の探索
Identification of protein components involved in MSP machinery in the cell-free extract of *Ascaris* sperm

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Nematode sperm crawls on the substrate in the same fashion as other eukaryotic cells, however, it utilizes an unique cytoskeleton system, called MSP (Major Sperm Protein) machinery instead of actin machinery. MSP, the most abundant protein in sperm, forms filaments like actin, but biochemical studies have shown that, in addition to MSP, other MSP associate proteins are critical to power MSP machinery. To identify those proteins, we performed 2D electrophoresis and found almost 100 spots in the cell free-extract from *Ascaris* sperm. Now we have been trying to identify those proteins by mass spectrometry and hoping that we are able to make a list of proteins associated with MSP machinery.

3P194 Possible points of action for rectification in directional sensing model

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How cells integrate spatial and temporal information from the surrounding environment to determine the direction of movement is not clear. We have previously reported that directional migration in *Dictyostelium* is associated with asymmetric change in the activity of the small GTPase Ras depending on the time derivative of the chemoattractant concentrations. Here by taking theoretical approach we analyzed how a modified local-excitation global-inhibition (LEGI) model of gradient sensing is able to explain the Ras dynamics in the spatiotemporal gradients of the chemoattractant concentration. We show that the feed-forward type gradient sensing circuit works as, in addition to a well-understood differentiator aspect, a “rectifier” for spatio-temporally changing gradients.

3P195 免疫シグナルアダプター分子 SLP-76 の複合体の形成と成長：1 分子追跡による解明
Formation and growth of the key immune signaling complex based on the adaptor protein SLP-76 revealed by single-molecule tracking

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The cytoplasmic adaptor protein, SH2-domain-containing leukocyte phosphoprotein of 76 kDa (SLP-76), is critical for antigen-induced cytoplasmic responses in mast cells and other immune cells. Here, using two-color single-molecule tracking, we found, upon engagement of the IgE receptor, SLP-76 forms small complexes with other signaling molecules, Gads and PLCγ1 within a minute, but that, at variance with the general concept of cytoplasmic responses of immune cells, these complexes were not colocalized with the IgE receptor or LAT, a transmembrane adaptor molecule considered to be a key molecule to relay the activated receptor signal to downstream signaling molecules. Furthermore, the SLP-76-based signaling complex grew in size in the course of several 10s of minutes.

3P196 細胞内力学特性に対する分子混み合い効果の影響
Crowding effects on viscoelastic properties in cell model systems

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We investigate the effects of molecular crowding on cellular mechanics, using a globular protein solution (BSA) and cell extracts (E. coli and HeLa cells) as the model cytoplasm which lacks cytoskeletons. Viscosity of these solutions rapidly increases as the solid contents become higher. It diverges at ~0.7 g/ml for BSA solution and at the physiological ~0.3g/ml for cell extracts. These results suggest that inert cytoplasmic extracts can experience glass transition at physiological concentration. Microrheology experiments in the cultured living cells (HeLa cells), however, confirmed finite fluidity. This implies that the cytoplasm in metabolically active cells is fluidized due to the spontaneous agitation.

3P197 １細胞レベルにおけるバクテリア走化性のメカニズム
Mechanism of phototaxis of bacteria at single cell level

Daisuke Nakane, Takayuki Nishizaka (*Dept. Phys., Gakushuin Univ.*)

Synechocystis sp. PCC 6803 is a cyanobacterium with the size of 1 μm and shows phototaxis as demonstrated in colony spreading on agar. At the single cell level, the motility is hypothesized to be powered by the repetition of extension and retraction of multiple filaments called type IV pili. To uncover the mechanism of its signal transduction process of the light-sensing motility apparatus, we here directly visualized the motility of single cells as the response to various wavelengths of light under an optical microscope. When a lateral light turned on, over 90‰ of cells started to show directional movement at a speed of 0.2 μm/sec in 20 sec. This response was depend on the light direction rather than intensity, and reversibly switched by 535-and 470-nm light.

3P198 アクチンフィラメントの配位構造は結合タンパク質によって変化する
Modulation of monomer configurations of actin filaments by actin binding proteins

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Several actin-binding proteins (ABPs) were reported to alter the conformation or configuration of actin filaments revealed by the electron microscopic studies. In addition, binding of these ABPs on a single filament have been examined vigorously under fluorescence microscope. We have being trying to detect the conformational changes on the side of actin filament in solution. For this purpose, fluctuations of inter-monomer FRET signal originated from monomer-configurations were measured. Distribution of fluctuating signal from intact actin filaments indicated more than four stable configurations. And these stable configurations varied with the addition of myosin heads, tropomyosin and drebrin molecules at physiological conditions.

3P199 Ion selectivity of the *Leptospira* flagellar motor

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Leptospira have intracellular flagella that rotate within the periplasmic space. Each flagellar filament is linked to a flagellar motor. Although the bacterial flagellar motor is powered by the electrochemical potential gradient of specific ions across the cytoplasmic membrane, coupling ions driving the *Leptospira* motor has not been investigated. We tested effects of a sodium channel inhibitor, EIPA, and a protonophore, CCCP, on the motility of saprophytic *Leptospira biflexa*, showing that the motility is completely inhibited by EIPA but not by CCCP. We also found that the motility is significantly enhanced with concentrations of NaCl added to media. These results suggest that the flagellar motor of *Leptospira* can utilize Na^+ as a coupling ion for torque generation.

3P200 Functional and structural analysis of the flagellar protein FliL from Vibrio alginolyticus

Ananthanarayanan Kumar, Shiwei Zhu, Seiji Kojima, Michio Homma (*Nagoya University*)

The bacterial flagellum consists of many different proteins. Although the functions of most of these proteins have been characterized, there are a few which remains to be studied in detail. FliL is one such enigmatic integral membrane protein. To clarify the function of FliL, we deleted fliL gene from chromosome of Vibrio alginolyticus. Pilot experiments showed that motility phenotype of the mutant was not different from that of the wild type. Recombinant full length FliL and its periplasmic region were over-expressed in Escherichia coli, purified and attempts are being undertaken to crystallize the purified proteins in order to solve its structure. Studies are also being carried out to understand its localization and possible interaction with other flagellar proteins.

3P201 Actin filament dynamics and organizations in liposome: A simulation study

Takahiro Nitta (*Applied Physics Course, Gifu Univ.*)

Dynamics and organizations of actin filaments play essential roles in cell morphology and motility. The dynamics and organizations were known to be affected by associated proteins and surrounding boundaries (cell membranes). However, complication in cellular environments has hampered quantitative and systematic investigations on what are essential ingredients for phenomena of interests. Reconstructed systems, actin filaments and associated proteins in liposomes, has enabled to investigate the dynamics and organizations with well-defined components, enabling direct comparison with simulations. Here, with a computer simulation, we will present a systematic investigation on dynamics and organizations of actin filaments in liposomes.

3P202 海洋性ビブリオ菌のべん毛形成を制御する DnaJ ファミリータンパク質 SflA の相互作用解析

Analysis of interaction of the DnaJ family protein SflA, that is involved in regulation of flagellation in *Vibrio alginolyticus*

Satoshi Inaba, Takehiko Nishigaki, Noriko Nishioka, Seiji Kojima, Michio Homma (*Div. Biol. Sci.Grad. Sch. Sci., Nagoya Univ*)

Marine bacterium *Vibrio alginolyticus* usually has a single polar flagellum, and its number and location are regulated by FlhF and FlhG. Mutation in *sflA* restores motility of the severely reduced Δ*flhFG* mutant, and the single transmembrane protein SflA was found to be located at the cell pole. We speculated that SflA interacts with FlhF, FlhG and other proteins, and suppress the flagellation. Function of the large N-terminal periplasmic region of SflA is not well understood, and its cytoplasmic region may interact with FlhF and FlhG. In order to test our hypothesis, we investigate binding protein(s) of SflA by the pull down assay using N- or C-terminal fragment of SflA. Our recent results about crystallization will be also presented.

3P203 非対称な脂質 2 重膜の高効率な作成にむけた新規マイクロデバイスの開発

Novel micro-device to form asymmetric lipid-bilayer membrane in a high throughput manner

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Most biological membranes possess an asymmetric transbilayer distribution of phospholipids. In particular, a plasma membrane of eukaryotes maintain a high asymmetry, controlling various cellular functions. To understand them, extensive studies has been performed to develop artificial lipid-bilayer systems; however, it remains difficult to form asymmetric lipid-bilayers in a high-throughput manner. In this study, we developed a novel micro-device to form 10,000 asymmetric lipid-bilayers at a time with high efficiency of 97 %. This micro-device extends the versatility of artificial lipid-bilayer system, which had been in general limited to symmetric-bilayer formation, and will contribute to the understanding of the role of lipid compositional asymmetry on cell physiology.

3P204 脂質キュービック相の単結晶化

Single Crystallization of an Inverse Bicontinuous Cubic Phase of a Lipid

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We report a simple method to produce a single crystal region of an inverse bicontinuous cubic (Q_{II}) phase of a lipid, 1-monoolein. By starting with the lipid of the sponge (L_3) phase in the presence of 1,4-butanediol, we can obtain a single crystal region of the double-diamond Q_{II} phase in one week by controlled dilution of 1,4-butanediol. The length of the single crystal region in a 0.5 mm diameter capillary was on the order of mm. X-ray diffraction images changed rotation angle dependently. We could assign Miller indices to all of the distinguishable diffraction spots from the region. This method would bring benefits to the basic and application researches of the Q phases.

3P205 蛍光セルソーターを用いたリポソームの融合・破壊の定量的評価

Quantitative evaluation of GUV fusion and destruction with fluorescence activated cell sorter

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We have focused on fusion and division of cell and revealed the mechanism of spontaneous division triggered by macromolecules using giant unilamellar vesicle (GUV) composed of phospholipid bilayer. In this study, we aimed to reveal correlation between various parameters such as lipid compositions of GUV and GUV fusion and destruction ratio with fluorescence activated cell sorter (FACS). FACS can quantify the properties of many GUVs in a short time. So we can get each ratio using FACS more easily and correctly than using microscope. As a result, we constructed the detection system of GUV fusion and destruction using FACS. Then, we revealed that charged lipid contained in GUV and AC pulse applied during fusion process affected on GUV fusion and destruction.

3P206 モデル細胞膜に対する化学物質の影響について

Influence of chemical compounds on model cell membranes

Kazunari Yoshida, Akito Takashima, Izumi Nishio (*Coll. Sci. Eng., Aoyama Gakuin Univ.*)

Interaction between local anesthetics such as lidocaine and cell membranes that have ion channels has been considered to play an important role in the anesthetic function. Therefore, the effect of local anesthetic molecules on cell membranes has been studied for several years. However, detailed mechanism of functional expression is still unclear. In this study, we investigated the effect of anesthetic molecules on lateral phase separation in liposomes which are model systems of cell membranes. We measured the miscibility transition temperature of the liposomes composed of dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and cholesterol with and without the anesthetic molecules using a fluorescence microscope. The result of the measurement will be discussed.

3P207 アデノウイルス由来両親媒性ペプチドの正曲率依存的な膜傷害性

Preferential Perturbation of Positively Curved Membranes by Adenovirus-derived Amphiphilic Peptide

Tomo Murayama, Silvia Pujals, Shiroh Futaki (*Institute for Chemical Research, Kyoto Univ.*)

The N-terminus segment of Adenovirus internal protein VI (AdVpVI) is known as a potentially amphipathic helical structure and is essential for the membrane interaction of AdVpVI. The peptide corresponding to AdVpVI (positions 33-55, ori) and derivatives were prepared by Fmoc-solid-phase synthesis. The studies on their modes of interaction differential calorimetry suggested that ori increased the fluidity of membrane hydrophobic core. The membrane perturbation activities of peptides were assessed by the dye-leakage from the different sized large unilamellar vesicles possessing different degree of curvature. We report the peptide-membrane binding to increase membrane fluidity should be critical for the curvature sensitivity of the membrane perturbation by AdVpVI (33-55).

3P208 動的および静的光散乱法によるリン脂質ベシクルの構造評価

Structural evaluation of phospholipid vesicles by dynamic and static light scattering techniques

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A large number of structural studies on various phospholipid aggregates have so far been extensively carried out by means of the wide- and small-angle X-ray scattering techniques to establish their detailed microscopic structure (e.g., bilayer thickness and molecular packing state). On the other hand, structural features of phospholipid vesicles at a mesoscopic scale (e.g., the shape of the vesicle and the distribution of the vesicle size) have been less focused despite the fact that they are more directly relevant to the bulk properties of the phospholipid vesicle system. In this study, we attempted to evaluate structural features of the phospholipid vesicle, including the lamellarity, by exploiting the general dynamic and static light scattering techniques.

3P209 リン脂質二重膜の圧力および化学誘起指組み構造化：形成機構の相違

Pressure- and chemically induced interdigitation of phospholipid bilayers: difference in the formation mechanisms

Hitoshi Matsuki[1], Masaki Goto[1,2], Nobutake Tamai[1] ([1]*Inst. of Technol. & Sci., The Univ. of Tokushima*, [2]*Lab. for Neutron Scattering, ETHZ & PSI*)

Some phospholipids form nonbilayers depending on experimental conditions. Interdigitated structure is one of such nonbilayers. So far, it has been reported that small amphiphiles like short-chain alcohols induce bilayer interdigitation of phosphatidylcholines (PCs) such as dipalmitoyl-PC (DPPC). On the other hand, the bilayer interdigitation is also induced by applying pressure. Since pressure decreases a total volume of the system and strengthens the molecular interaction among lipid molecules, the mechanism of pressure-induced interdigitation cannot be explained by the same mechanism as that of the chemically induced interdigitation. Here, we explain the difference in the formation mechanisms based on our recent data of PC bilayers.

3P210 凍結超薄切片法によるテープ剥離したヒト皮膚角層構造の部位差研究

Comparative cryo-ultrathin section study of human stratum corneum cells tape-stripped from different body regions

Keisuke Nakamura, Hiromitsu Nakazawa, Satoru Kato (*Sch. Sci&Tech. Kwansei Gakuin Univ.*)

Stratum corneum (SC) structures and barrier properties vary depending on body region, season, subject and so on. In order to investigate human SC structures under a wide spectrum of conditions, it is required to develop a simple observation technique in combination with noninvasive sampling. In this study we examined dependence of SC structures on body region by using a modified cryo-ultrathin section technique, where SC cells almost noninvasively stripped from the skin were sandwiched between two adhesive tapes and sectioned with a cryo-ultramicrotome. We found that morphological characteristics of SC cells from various sampling regions are well preserved and can be distinguished. Thus, our method is suitable for evaluating SC structures under various conditions.

3P211 皮膚角層モデル膜に対する水の浸透の FTIR-ATR による解析

FTIR-ATR analysis of water permeation into stratum corneum model membranes

Kohei Oka, Satoru Kato (*Kwansei Gakuin University*)

We examined permeation of D2O through SC model membranes by FTIR-ATR. The model membranes were composed of only one kind of ceramide, cholesterol and free fatty acid with hydrocarbon chain length of 16~24. The lipid mixture in chloroform/methanol was directly sprayed onto the ATR prism to form a homogeneous film. After removal of the solvent, a drop of D2O was mounted on the film. An increasing signal around 2500 kayser appears as the D2O molecules reach into the region where their FTIR signal is detectable. The time course analysis of the FTIR signal revealed that the longer is the hydrocarbon chain length, the lower is the permeation rate. Based on the results, we'll discuss the relationship between the molecular packing in the membrane and the water permeability.

3P212 時分割広角 X 線散乱によるラフトモデルリポソームとアミロイドベータタンパク質との相互作用に関する研究

Time-resolved wide-angle X-ray scattering study of interaction between raft-model liposome and amyloid-beta protein

Shoki Sato[1], Mitsuhiro Hirai[1], Noboru Ohta[2] ([1]*Grad. Sch. Sci. Eng. Gunma Univ.*, [2]*JASRI*)

Recently, the significance of studies of proteins and membranes in cell environment has been pointed out. On the other hand, in-vitro spectroscopic studies of amyloid-beta (Ab) proteins have been done in dilute solutions in many cases. Therefore, the mechanism of amyloid formation under crowding environment still remains controversial and ambiguous.
By using time-resolved wide-angle X-ray scattering (WAXS), we have studied the effect of the interaction with Ab protein on the structure of raft-model membrane liposomes (ternary mixture: glycosphingolipid / cholesterol / phospholipid) under macromolecular crowding environment. The time-course of WAXS data suggests that the presence of Ab tends to induce multi-lamellar formation under crowding.

3P213 力学的負荷下でのコレステロール含有リン脂質膜中の疎水孔形成：分子動力学シミュレーション

Hydrophobic Pore Formation in Phospholipid/Cholesterol Bilayers under Mechanical Stretching: Molecular Dynamics Simulation

Taiki Shigematsu, Kenichiro Koshiyama, Shigeo Wada (*Grad. Sch. Eng. Sci., Osaka Univ.*)

Pore structure penetrating lipid bilayers plays an important role in membrane permeabilization under mechanical stretching. We perform molecular dynamics simulations of stretched DPPC/Cholesterol bilayers, expressed by applying various constant areal strains. When the areal strain exceeds a critical value, a hydrophobic pore (HoP) forms in the DPPC/Cholesterol bilayer, unlike the hydrophilic pore (HiP), whose edge is lined with hydrophilic headgroups of lipids, in pure bilayers. Furthermore, we analyzed the free energy difference between HoP and HiP in the DPPC/Cholesterol bilayer and found that the energy for HoP is lower than that for HiP, which rationalize the formation HoP in the DPPC/Cholesterol bilayers under mechanical stretching.

3P214 細胞運動における細胞内局所 pH の影響

Effect of cytoplasmic local pH on the cell migration

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Intracellular pH plays important roles in signal transduction in the cellular slime mould *Dictyostelium discoideum*, which is a model organism for studies on cell motility, chemotaxis and differentiation. Chemotactic stimulation by cAMP was reported to elicit an efflux of protons and increase the speed of moving cells. However it remains unknown how the intracellular pH change is transduced in moving cells. To investigate the transduction mechanism of intracellular pH, we constructed *Dictyostelium* strains expressing pH-sensitive fluorescent proteins and then observed cytoplasmic local pH changes and cell dynamics simultaneously by fluorescence microscopy. We will discuss the role of cytoplasmic local pH in signal transduction.

3P215 協働的自己会合に基づく脂質―ペプチドナノ微粒子の自己複製

Self-reproduction of lipid-peptide nanoparticles by synergistic self-assembly

Keisuke Ikeda, Minoru Nakano (*Grad. Sch. Med. Pharm. Sci., Univ. Toyama*)

Self-replication is one of the fundamental functions of living organism. We describe a self-reproduction of lipid-peptide nanoparticles (nanodiscs) formed by chemical ligation of precursors and self-assembly of the building-blocks. The ligation reaction was accelerated on lipid bilayer surfaces and the products spontaneously assembled into the nanoparticles with lipid molecules. When the nanodiscs were formed, a rapid proliferation occurred not by a template- or complex-enhanced ligation of precursors but by the spatial rearrangements of the molecules between the pre-existed nanodiscs and the unreacted materials. Our study suggests that the synergistic self-assembly possibly underlie the principles of heterogeneous multimolecular self-replicating systems.

3P216 走査型イオンコンダクタンス顕微鏡を用いたコンフルエント上皮生細胞の膜揺らぎ定量化

Membrane fluctuations of confluent epithelial cells quantified by scanning ion conductance microscopy

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Membrane of cells fluctuates thermally and actively owing to the intracellular dynamics and external cell-to-cell interactions. Recent our study [1] using scanning ion conductance microscopy (SICM) revealed that membrane fluctuations of adherent cells exhibited a spatial dependence, but the origin of the spatial dependence is still unknown. To elucidate the mechanism of the fluctuation, we used a modified SICM to map membrane fluctuations of cells. It is found that spatial distribution of cell membrane fluctuations significantly varies among cells. We will show in detail how the membrane fluctuations are affected by the dynamic behaviors of underlying cytoskeleton and discuss the mechanism.
[1] Mizutani et al. Appl. Phys. Lett. 102, 173703 (2013).

3P217 細胞透過ペプチドであるトランスポータン 10 のベシクル内への進入と脂質膜中のポア形成に対する張力の効果

Effects of tension on entry of cell-penetrating peptide transportan 10 into a single vesicles and its pore formation in lipid membranes

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Transportan 10 (TP10) can enter a GUV from its outside by translocating across the lipid membrane before TP10-induced pore formation (1). Here we investigated the effects of tension on pore formation and entry of TP10 into a 20%DOPG/80%DOPC-GUVs. During the interaction of TP10 with a single GUV with a constant tension the fractional change in the area of the GUV membrane increased with time, then suddenly pore formation occurred. The rate constant of pore formation in the presence of same concentration of TP10 increased with an increase in tension. Tension also enhanced the fraction of the entry of TP10 into the inside of GUV before pore formation. We discussed the mechanism of the effect of tension on these phenomena. (1)Biochemistry 53, 386, 2014.

3P218 張力が誘起する脂質膜中のポア形成に対する静電相互作用の効果

Effects of Electrostatic Interactions on the Rate Constant of Tension-Induced Pore Formation in Lipid Membranes

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Using the method developed in our previous paper (1), we investigated the effects of electrostatic interactions on the rate constant (k_p) of constant tension-induced pore formation in lipid membranes. The increase in surface charge density increased k_p of dioleoylphosphatidylglycerol(DOPG) /dioleoylphosphatidylcholine(DOPG)-giant unilamellar vesicle (GUVs) at 150 mM NaCl. The decrease in salt concentration increased k_p in 40%DOPG/ 60%DOPC-GUVs. These results indicate that k_p values increases with an increase in the electrostatic interactions. Bending modulus of DOPG/DOPC-GUVs decreased with surface charged density. It may change the line tension of a prepore. We discussed the mechanism for the effects of electrostatic interactions on k_p.

(1) Langmuir, 29, 3848, 2013.

3P219 膜の伸展により活性化される抗菌ペプチド・マガイニン２のポア形成

Stretch-Activated Pore of the Antimicrobial peptide, Magainin 2

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The pore formation induced by antimicrobial peptide magainin 2 (mag) was investigated using single giant unilamellar vesicles (GUVs). The binding of mag to the membrane of GUVs increased the fractional change in the area of the membrane, δ, which increased the rate of pore formation. The tension of a membrane following aspiration of a GUV activated mag-induced pore formation. These indicate that the mag-induced pore is a stretch-activated pore. Simultaneous measurements of the leakage of a fluorescent probe, the location of carboxyfluorescein (CF)-mag, and mag-induced change in δ indicate that mag cannot translocate from the outer to the inner monolayer until just before pore formation. Based on these results, we discuss the mechanism for mag-induced pore formation.

3P220 張力が誘起する脂質膜中のポア形成に対する静電相互作用の効果の理論

Theory on the electrostatic effects on tension-induced pore formation in lipid membranes

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Recently, we found that the rate constant of tension-induced pore formation increased as the electrostatic interactions due to the membrane surface charges increased (1, 2). Here we developed a theory for this result. The decrease in the energy barrier of the potential energy of a prepore due to the electrostatic repulsive interaction among the charged prepore surface is one of the main factors for the increase of rate constant of tension-induced pore formation. Our theory is based on the Poisson-Boltzmann equation and takes into account the electrostatic repulsion between the pore walls. The result of the theory can explain the experimental results reasonably.

(1)Langmuir, 29, 3848, 2013, (2) Karal et al., the presentation in this conference.

3P221 Recording Ion-Channel Activities Based on Microfabricated Silicon Chips

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Ion-channel proteins are membrane proteins that are of crucial physiological importance and are major targets for drug design. Among them, the human ether-a-go-go-related gene (hERG) channel has been drawing considerable attention, because it has been found to be related to serious arrhythmic side effects following drug treatment. In the present study, we report on the reconstitution of the hERG channels in artificially formed bilayer lipid membranes (BLMs) formed in the micropores which were fabricated in silicon chips. Ion currents of hERG channels were investigated in terms of single-channel conductance, voltage dependence, sensitivity to typical drugs and potassium concentration dependence.

3P222 イオンチャネルの機能の改変

Modifications of ion channel function

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Increasing structural information of ion channels has clarified the structure-function relationship, which is now being used to design ion channels that respond to specific stimuli and select specific ions. Using this knowledge, we describe a series of engineered ion channels based on modification of the KcsA channel whose structural-functional relationship has been predicted by ours and other groups. Some mutations changed the selectivity from K^+ to Ca^{2+}. Furthermore, inserting the site of the KcsA channel responsible for pH sensitivity into another channel conferred similar pH sensitivity. We will discuss how these engineered channels can be used to regulate cell function by changing the ion permeability.

3P223 チャネル内の水の水素結合鎖を介するプロトン透過の整流性
Rectified proton permeation through the hydrogen-bonded water-chain in a channel peptide

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We examined the proton conduction of a channel peptide, polytheonamide B (pTB), by use of the planar lipid bilayer technique. pTB forms a pore with a uniform diameter of 4 Å. pTB is highly conductive to proton, and shows weak inward-rectification in the *I–V* curve. The mechanism underlying the rectified proton permeation was addressed. The single channel recording in the presence of high concentration buffer revealed that the conductance of pTB is diffusion-limited across the access region, and the rectification is yielded inside the pore. The proton permeation through pTB was modeled with the discrete-state Markov model, involving contribution of the diffusion-limitation. We discussed the mechanism of rectified proton permeation inside the pore of the pTB channel.

3P224 ROS 非依存的な細胞内ミトコンドリアの一過性脱分極について
ROS-independent transient depolarization of mitochondria in cells

Kanji Umiuchi, Yoshihiro Ohta (*Grad Sch. Engin., Tokyo Univ. Agric. & Technol.*)

Reactive Oxygen Species (ROS) are mainly produced in mitochondria and induce cell injuries and cell death. Previously, we found that the transient depolarization of mitochondria suppressed ROS production and was induced by the shortage of protons in mitochondrial matrix which would induce ROS production. However, it is not unclear that ROS induce the transient depolarizations. In this research, we investigated the effect of ROS on the transient depolarizations. The presence of the antioxidant MnTMPyP did not affect the frequency of the transient depolarizations, both with or without oligomycin, an inhibitor of FoF1-ATP synthetase. These results indicate that the transient depolarizations induced by the shortage of protons in the matrix occur independently of ROS.

3P225 大腸菌走化性レセプターの内膜における局在性解析への急速凍結レプリカ電子顕微鏡法によるアプローチ
A quick-freezing replica electron microscopic analysis for the localization of chemoreceptors on bacterial inner membranes

Kazunori Kawasaki[1], Takehiko Inaba[2], Emiko Kobayashi[1], So-ichiro Nishiyama[3], Ikuro Kawagishi[3] ([1]*AIST*, [2]*RIKEN*, [3]*Dept. Frontier Biosci, Hosei Univ.*)

Methyl-accepting chemotaxis proteins (MCPs), receptors in the bacterial inner membrane, form large clusters observed as bright patches at cell poles by fluorescence microscopic imaging. Here we aimed at a higher-resolution analysis on the subcellular localization of MCPs. An *Escherichia coli* strain that lacks all MCPs was transformed to express a single chemoreceptor (Tar) fused to green fluorescent protein (GFP). The resulting strain was examined with quick-freezing replica electron microscopy with two kinds of staining for detection of Tar-GFP: 3,3'-diaminobenzidine staining based on photoconversion by fluorescence from GFP, and immune-replica method using anti-GFP antibody. Details of the current protocols and the results will be presented.

3P226 二成分制御系間クロストークを用いた細菌べん毛の回転方向制御
Control of the bacterial flagellar motor by cross regulation between non-cognate two-component regulatory systems

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Escherichia coli has various two-component regulatory systems, typically consisting of a histidine kinase (HK) and a response regulator (RR). Among them, CheA (HK) and CheY (RR) are responsible for controlling the rotational sense of the flagellar motor: phospho-CheY induces the clockwise (CW) rotation. Here we tried to establish a system to control the CheY activity by non-cognate HKs. Cells co-expressing DcuS (HK) and CheY showed significantly higher CW rotation bias than cells expressing CheY only. However, the CW bias of the latter strain decreased when the *ackA* gene, encoding acetate kinase, was deleted, suggesting that CheY can also be phosphorylated by acetyl-CoA. We therefore plan to examine crosstalk between DcuS and CheY in the *ackA*-deletion background.

3P227 多刺激受容センサー Tar の温度感知領域の探索
In search of thermosensing regions of the multimodal sensor Tar

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The aspartate chemoreceptor Tar of *Escherichia coli* can sense thermal stimuli to mediate its thermotactic behavior. Tar is a homodimeric integral membrane protein, each subunit with two transmembrane (TM) helices. Here we ask whether there is a specific region for thermosensing. First, we examined a mutant Tar protein lacking its periplasmic domain. At least two of its derivatives showed abilities to mediate thermoresponses, suggesting that the periplasmic domain is dispensable for thermosensing. Next, we examined the cytoplasmic fragments of Tar. Any constructs tested had no thermosensing ability, while they retained sufficient protein levels. Thermosensing is therefore suggested to involve transmembrane segments, membrane anchoring and/or clustering of the receptor.

3P228 線虫の単一神経細胞における Ca^{2+}時空間ダイナミクス: 数理モデルとその解析
Spatial-temporal Ca^{2+} dynamics in a whole single neuron of C. elegans: Mathematical modeling and analysis

Yuishi Iwasaki[1,3], Sayuri Kuge[2,3], Takayuki Teramoto[2,3], Takeshi Ishihara[2,3] ([1]*Fac. Eng., Ibaraki Univ.*, [2]*Grad. Sci., Kyushu Univ.*, [3]*JST, CREST*)

In an odorsensory neuron of C. elegans, Ca^{2+} response to a stimulus was slightly different at dendrite, soma and axon. To understand the observed spatial-temporal Ca^{2+} dynamics, we construct an electrodiffusion model in a whole single neuron. Ca^{2+} and K^{+} concentrations are determined by the Nernst-Planck equation with chemical reactions. Membrane potential, whose value is determined by the law of charge conservation, governs the opening of ion channels. Concentration of fluorescent protein is introduced to provide a quantitative correspondence between "fluorescence intensity" in our model and Ca^{2+} imaging data. From a correspondence between our model and the cable equation under certain assumptions, we estimate electrical conductivity of the neurite.

3P229 低温・Xe 加圧下における DEPC リポソーム相変化と神経細胞のラマン測定

Raman spectra change at the phase transition of DEPC liposome and cell membrane of neuron under low temperature and xenon pressure

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The change of spectra of DEPC liposome with temperature and xenon pressure was observed by microscopic Raman spectroscopy to identify the phase transition of liposome. At atmospheric pressure, DEPC liposome transforms from gel to liquid crystal at approximately 12oC and shifts to lower temperature at xenon pressure. The Raman spectra indicated the slight change of C-H stretching modes at the phase transition temperature under xenon pressure. These findings support the previously obtained results by MD simulations, which showed the evidence of xenon dissolved in the lipid bilayer. Based on these observations, the Raman spectra of neurons under low temperature and xenon pressure were observed.

3P230 局所熱パルス法による神経細胞のカルシウム放出の誘導

Ca2+-burst in rat hippocampal neurons induced by microscopic heat pulses

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Temperature is an essential parameter determining physical and chemical processes in cells. We previously reported highly thermosensitive Ca2+ dynamics in HeLa cells (V. Tseeb et al, 2009). In a search of novel methods controlling neural activities, we studied the thermoresponsive Ca2+ dynamics in neurons. We found that single rat hippocampal neurons responded to microscopic heat pulses induced by a infra-red laser beam focused nearby. The Ca2+ change in neurite was larger than that in soma. In addition, we achieved to build a molecular model for the thermoresponsive Ca2+ signaling, which is different from HeLa cells in function of ion channels. Our results demonstrate that microscopic heat pulses can potentially be useful for exciting neurons locally and noninvasively.

3P231 ヒト iPS 細胞由来ニューロンとアストロサイト共培養による電気生理学的特徴

Electrophysiological activity of a human induced pluripotent stem cell derived neuron and astrocyte co-culture

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Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for drug discovery and cell-based therapy. However, the immaturity of cultured human iPSC-derived neurons and the lack of established functional evaluation methods are problematic. We here used a multi-electrode array (MEA) system to investigate the effects of the co-culture of rat astrocytes with hiPSC-derived neurons on the long-term culture, spontaneous firing activity, and drug responsiveness effects. The co-culture facilitated the long-term culture of hiPSC-derived neurons for >3 months and long-term spontaneous firing activity was also observed. As a result, The co-culturing with rat astrocytes allowed hiPSC-derived neurons to maintain long time spontaneous activity.

3P232 導電性高分子含浸ファイバー電極の脳活動測定と刺激への適用

Application of conductive polymer-coated fiber electrodes to neural recording and stimulation in vivo

Satoshi Watanabe, Hideyuki Takahashi, Keiichi Torimitsu (*Dept. Bioeng. Robotics, Grad. Sch. Eng., Tohoku Univ.*)

Electrodes for chronic implantation are desired to be made of soft material that induces minimal stress, and should have low impedance and biocompatibility for stable long-term functioning. We fabricated a fiber-shaped electrode by coating silk fibers with the conductive polymer, poly(3,4-ethylenedioxythiophene) p-toluenesulfonate (PEDOT-pTS), by chemical polymerization. Using this electrode, we recorded field potentials from embryonic chick brain with a signal-to-noise ratio comparable to conventional metal electrodes. The fiber electrode is expected to apply for clinical purposes such as deep brain stimulation in neuropathological diseases.

3P233 運動性シアノバクテリア *Pseudanabaena* sp. ILC 545 の「彗星状コロニー」の形成ダイナミクス

Dynamics of comet-like colony formation in the filamentous cyanobacterium, *Pseudanabaena* sp. ILC 545

Yu Shoji[1], Hiroki Yamamoto[1], Yuki Fukasawa[1], Hideo Iwasaki[1,2] ([1]*Waseda University*, [2]*metaPhorest*)

We have analyzed colony-pattern formations in the multicellular (filamentous) cyanobacterium, *Pseudanabaena* sp. ILC 545, which we isolated previously. ILC 545 strain glides their major axis. As the bacterial density increases, we observed various colony patterns, due to collisions and entanglement. These colony patterns can be classified into three groups: "bundle" colonies with nematic alignment, "comet-like" colonies with fast population movement, and "disk" colonies forming vortices.
We focused on the comet-like colony formation. Concretely we analyzed the relationship between colony size and speed, and movement of one filament in the colony. Here, we repot a model on the morphogenesis of comet-like colony formation, and some related quantitative analyses.

3P234 Analysis on colony formation in a filamentous cyanobacterium with an extended self-driven particle model with a cellular automaton method

Masato Ishii[1], Yuki Fukasawa[1], Masaya Takiguchi[1], Kain Yanagi[1], Hideo Iwasaki[1,2] ([1]*Waseda University*, [2]*metaPhorest*)

Cyanobacteria, a group of eubacteria performing oxygenic photosynthesis, show a variety of patterns depending on environmental conditions such as light and nutrients. The motile cyanobacterium, *Geitlerinema* sp. ILC 546, forms a unique colony pattern, which is characterized by multiple rings on an agar medium with robust trails. We performed some quantitative analysis on the movement of gliding filaments, and employed a cellular automaton method to modify the self-driven particle (Vicsek) model for population dynamics of filamentous units. Some critical conditions which seem important for the robust-trail ring pattern formation will be reported.

3P235 光駆動ナトリウムポンプの pH 依存性

The extracellular pH dependency of transport activity by light-driven sodium ion pump

Rei Abe-Yoshizumi[1], Yoshitaka Kato[1], Keiichi Inoue[1,2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*JST PRESTO*)

Nature created two kinds of light-driven ion pumps, bacteriorhodopsin (BR) and halorhodopsin (HR) functioning as outward proton and inward chloride pumps, respectively. Recently, we found a light-driven sodium ion pump, KR2, from marine bacterium. KR2 can pump not only Na^+ but also H^+ in KCl solution. In this study, we examined the effect of pH on the ion transport activity of another sodium pump rhodopsin (NaR).
We expressed a new sodium ion pump GlR2 from *Gillisia limnaea* R-8282 in *E. coli* and measured light induced pH change. GlR2 transport Na^+ in NaCl and H^+ in KCl, and the ion transport showed pH-dependency. We will discuss about the molecular mechanism of the pH dependency on the ion transport.

3P236 ニワトリクリプトクロム 4 の光反応特性に外部環境が与える影響

***In vitro* redox cycle of Chicken Cryptochrome4 under various ambient condition**

Hiromasa Mitsui, Toshinori Maeda, Chiaki Yamaguchi, Yusuke Tsuji, Kazuki Sakai, Keiko Okano, Toshiyuki Okano (*Dept. Eng. and Biosci., Grad. Sch. Adv. Sci. and Eng., Waseda Univ.*)

Cryptochromes (CRYs) are flavoproteins found in many living species. In addition to a repressive role in circadian oscillator, CRYs are considered to work as photoreceptors or light-driven magnetoreceptors. These functions still remain elusive in vertebrates, and therefore we have been focused our study on uncharacterized vertebrate CRYs. We previously reported light-dependent conformational changes of chicken CRY4 and revealed its photocycle, in which the redox state of the FAD chromophore dynamically changes. In this study, its photocycle is further characterized in detail under various experimental conditions including redox and temperature environments. Our present report would provide novel insights to elucidate the physiological function of vertebrate CRY.

3P237 哺乳類 NDRG1 のゼブラフィッシュ相同蛋白質の視細胞における機能解析

Functional analysis of zebrafish orthologs of mammalian NDRG1 protein in photoreceptors

Shimpei Takita[1], Yasutaka Wada[1,2], Satoru Kawamura[1,2] ([1]*Dept. of Biol. Sci. Grad. Sch. of Sci., Osaka Univ.*, [2]*Grad. Sch. of Frontier Biosci., Osaka Univ.*)

A carp ortholog of mammalian *ndrg1*, *ndrg1b*, is a gene with unknown function expressed in cones but not in rods. The purpose of this study is to understand the role of this protein in cones in zebrafish, akin to carp. NDRG1b protein was apparently localized throughout the plasma membrane in cones. Newly isolated its paralog NDRG1a-v1 showed similar subcellular localization in cones, and also in rods except that this protein was not present in the outer segment (OS) of a rod. Knockdown of *ndrg1b* or *ndrg1a-v1* at larval stage impaired proper cone and retina formation. Forced-expression of NDRG1b or NDRG1a-v1 in rods altered the shape and the length of their OS. NDRG1b and NDRG1a-v1 proteins seem to be important for cone OS morphology and therefore, retinal development.

3P238 イエロープロテインの強い水素結合形成に関する FTIR 研究

FTIR study on the strong hydrogen bonding formation in photoactive yellow protein

Tatsuya Iwata, Hideki Kandori (*Dept. Ftontier Mat., NITech*)

Photoactive yellow protein (PYP) is a blue light sensor in purple bacteria. X-ray and neutron diffraction results of PYP crystals show that the chromophore, *p*-coumaric acid, and Glu46 forms short hydrogen bond or low-barrier hydrogen bond (LBHB). It is debated whether LBHB is really formed or not. We detected a broad negative band with some peaks at 2600-2400 cm^{-1} in the difference FTIR spectrum of PYP. This shows the presence of very strong hydrogen bonds of the X-H (X=O, N) group in the unphotolyzed state, which is the proof of abnormal hydrogen bonding environment as is the case for BLUF [1]. To identify the origin of the signal, mutants and isotope-labeled PYP were measured by FTIR spectroscopy.
[1] Iwata et al. (2011) ***J. Phys. Chem. Lett. 2***, 1015.

3P239 基質の状態によらないロドプシンの脱リン酸化反応速度

Stage-independent dephosphorylation of rhodopsin during its regeneration cycle

Hiromi Yamaoka, Shuji Tachibanaki, Satoru Kawamura (*Grad. Sch. Frontier Biosci., Osaka Univ.*)

In vertebrate photoreceptors, light-activated rhodopsin is inactivated by phosphorylation and decomposed into free opsin plus all-trans retinal. Rhodopsin is then regenerated by binding of 11-cis retinal to opsin, but for the full regeneration, the phosphates bound to opsin should be removed during its regeneration cycle. In this study, using a rod soluble protein fraction, we measured the rate of this dephosphorylation at various stages of rhodopsin in the cycle, including bleaching intermediates, retinal-free opsin and phosphorylated rhodopsin regenerated by addition of 11-cis retinal. The results showed that the dephosphorylation rates are similar in these substrates, which suggests that the dephosphorylation takes place constantly throughout the regeneration cycle.

3P240 バクテリオロドプシン-ハロロドプシン-キメラ蛋白質の光反応サイクル

Photoreaction cycle of a bacteriorhodopsin-halorhodopsin chimeric protein

Shinji Uyama[1], Tomomi Kitajima[1], Midori Murakami[1], Tsutomu Kouyama[1] ([1]*Graduate School of Science, Nagoya University*, [2]*Center of the gene research, Nagoya University*)

Bacteriorhodopin (bR) is a light-driven proton pump, whereas halorhodopsin is a light-driven chloride ion pump. Recent crystallographic studies of pharaonis halorhodopsin (pHR) using the C2 crystal have shown that it is possible to observe a large structural change in the cytoplasmic half of helix F that occurs upon formation of the N state. With the aim at investigating whether a similar structural change occurs during the proton pumping cycle of bR, we have designed, expressed and purified a chimeric light-driven proton pump with a structural property similar to that of pHR. Its spectroscopic properties will be discussed

3P241 時間分解偏光 FTIR 計測によるバクテリオロドプシンの光サイクル反応解析

Time-resolved polarized FTIR spectroscopy on the photocyclic reaction of bacteriorhodopsin

Kuniyo Fujiwara[1], Yuji Furutani[1,2,3] ([1]*IMS*, [2]*SOKENDAI*, [3]*JST PRESTO*)

Bacteriorhodopsin (BR) is a heptahelical transmembrane protein and functions as a light-driven proton pump. BR is the most well-known membrane protein from the point of view of biophysics. BR molecules form 2D crystals in purple membrane in nature, which enables us to prepare a highly ordered protein sample on an IR window, whose transmembrane helices are oriented to the normal of the window, just by drying the suspension. By analyzing the angle dependence of time-resolved FTIR spectra collected during the BR photocycle, we can know more information about the angles of vibrational modes which change during the proton transportation. It would become a new method for the analysis of structural dynamics of protein, if we can get highly ordered protein samples.

3P242 水溶液中のオキシルシフェリン吸収スペクトルの理論的研究

Theoretical Analysis of Absorption Spectra of Oxyluciferin in Aqueous Solutions

Miyabi Hiyama[1], Hidefumi Akiyama[1], Nobuaki Koga[2] ([1]*ISSP*, [2]*Nagoya Univ.*)

To elucidate the assignment of absorption spectra of oxyluciferin, the excitation and emission energies of oxyluciferin are calculated by the TDDFT method and the absorption spectra in the aqueous solutions with various pH values are assigned using the relative absorption intensity estimated from the theoretical pK_a values. We estimated the relative concentrations of the species in solutions with various pH values, to reasonably analyze the absorption spectra of oxyluciferin. The obtained molar fractions for these chemical species are in good agreement with the experimental ones, in which the concentrations were determined by the absorption spectra for chemically modified oxyluciferin derivatives.

3P243 Analysis of the photoresponse mechanism of the LOV-HTH system using accelerated molecular dynamics simulation

Tetsuo Kokubu, Tadaomi Furuta, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

LOV (light-oxygen-voltage) domains are blue light-activated signaling modules integral to a wide range of photosensory proteins. To reveal their photoresponsive mechanism, we performed accelerated MD (aMD) simulations for a light-regulated DNA-binding protein construct LOV-HTH (helix-turn-helix) in both the dark (before light absorption) and light states (after light absorption). We also performed aMD for L120K mutant in the dark state. The present simulations indicated that formation of the cysteinyl-FMN (LOV chromophore) adduct causes significant unfolding in the Jα-helix in the light state and the mutation of L120K darastically changes interactions between LOV and HTH. Based on these results, we propose the light signal path ways from LOV to HTH via Jα-helix.

3P244 2 種類の PYP における光誘起構造変化の違いをうむ部位の解析

Analysis for different property of light induced structural changes between two PYPs

Yoichi Yamazaki, Yoshiaki Mathumoto, Hironari Kamikubo, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Halorhodospira halophila PYP (Hh-PYP) and Rhodobacter capsulatus PYP (Rc-PYP) share key residues near the chromophore. However, both PYPs show different intermediate states in each photocycle. To clarify which part in their sequence is the origin of the differences, we created chimera PYPs to compare their spectroscopic properties with that of Hh-PYP. Spectroscopic measurements of chimera PYPs revealed that the replacement of the second block (31-57) showed different intermediate states compared with that of the other chimera PYPs and Hh-PYP. Replacement of non-conserved residues in the 2nd block, Q41K and A44K on Hh-PYP showed also different intermediate states. Thus these two residues are responsible for the difference of intermediate state between two PYPs.

3P245 イエロープロテイン光反応中間体の熱平衡の解析

Thermal equilibria between the photocycle intermediates of photoactive yellow protein

Yasushi Imamoto, Yoshinori Shichida (*Grad. Sch. Sci., Kyoto Univ.*)

Photoactive yellow protein (PYP) undergoes the photocycle after absorption of a blue photon. It has been established that the spectral changes in the microsecond-millisecond time scale is explained by the existence of three intermediates, L (pR), M^{acid} (pB'), and M^{alkali} (pB). They appear in this order, but there are substantial equilibria between them. We kinetically and spectroscopically analyzed the photocycle of PYP, in which the equilibria are taken into consideration. Our results demonstrated that L and M^{acid} are in almost 1:1 equilibrium in microsecond time scale, and L-like intermediate in the equilibrium with M^{alkali} is energetically distinct state from that of L. Based on these findings, the photocycle of PYP is revisited.

3P246 PYP の β4-5 loop 領域と発色団環境との関係性の解明

The elucidation of the relationship between β4-5 loop region and the chromophore environment in PYPs

Atsuhiro Kawamura, Yoichi Yamazaki, Hironari Kamikubo, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Photoactive Yellow Protein (PYP) is a soluble light receptor protein containing *p*-coumaric acid as a chromophore. The properties of PYP from *Rhodobacter capsulatus* (Rc-PYP) are substantially different from the intensively studied PYP of *Halorhodospira halophila* PYP (Hh-PYP). M100 is one of the key residues to determine the properties of Hh-PYP, which is converted to G99 in Rc-PYP. We exchanged some residues in β4-5 loop region of Rc-PYP to elucidate the origin of the differences. Spectroscopic properties of either G99M or N96D of Rc-PYP were identical to that of Rc-PYP, while. G99M/N96D of Rc-PYP showed similar spectroscopic features to Hh-PYP, indicating that the β4-5 loop region affects the chromophore environment and the concerted effect of M100 and D97 is essential.

3P247 BLUF タンパク質 PapB の FTIR 法による構造解析
Characterization of light-induced structural changes of the BLUF protein PapB

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BLUF domain proteins have a flavin chromophore to sense blue light. PapB contains a BLUF domain and controls the enzymatic activity of a phophodiesterase PapA for modulating the biofilm formation of the purple photosynthetic bacterium *Rhodopseudomonas palustris*. We have applied FTIR (Fourier Transform Infrared) spectroscopy, coupled with isotopic labeling techniques, to identify necessitated structural changes of PapB for activating PapA. We recently succeeded to establish reconstitution condition of PapB that allows us to characterize PapB samples containing non-labeled flavin and isotopic labeled apo-protein. The obtained spectra are compared with those of non-labeled samples to identify specific light-induced structural changes of PapB that is sensed by PapA.

3P248 NTQ モチーフを持つ新規微生物型ロドプシンの輸送イオン種の同定
Ion species transported by the novel microbial rhodopsin containing NTQ motif

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Novel type of microbial rhodopsins, containing NTQ motif, was found to act as inward Cl^- pump (Inoue *et al.*, ICRP 2012; Yoshizawa *et al.*, PNAS 2014). We examined ion selectivity of a NTQ rhodopsin from *Fulvimarina pelagi* (FR) by detecting the light-induced pH changes of the suspensions of FR-expressing *E. coli* cells. In the solvents containing NaCl, KCl and choline Cl, the illumination caused the pH increases, which were not depressed by CCCP. These reflect the Cl^- transport by FR. However, the pH increases were also observed in Na_2SO_4, Rb_2SO_4, $MgSO_4$ and in the additional presence of CCCP. Thus, FR might pump cations or SO_4^{2-} when Cl^- is absent. We will discuss the ion species transported by FR basing on other experimental results.

3P249 Photoactive Yellow Protein における酸誘起プロトン移動
Acid induced proton transfer in Photoactive Yellow Protein

Masayoshi Noji, **Mai Arakawa**, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Photoactive yellow protein (PYP) is a photoreceptor protein with *p*CA as a chromophore. Its signaling state, PYP_M, is blue-shifted from the dark state, indicating that a proton is covalently attached to the chromophore. Similar blue shift is also observed only by decreasing pH. We investigated the proton transfer mechanism by FTIR measurements for WT and R52Q at an acidic pH. An obvious band at $1771cm^{-1}$ attributed to a protonated E46 observed for R52Q indicates the protonation of both *p*CA and E46. On the contrary, the corresponding band cannot be seen in WT, suggesting that *p*CA is protonated but E46 is deprotonated in WT. Based on the results, we concluded that the acid-induced "proton transfer" from E46 to *p*CA occurs just decreasing the pH in WT.

3P250 光駆動ナトリウムポンプのイオン取込みに関する分光研究
Spectroscopic study on the ion uptake mechanism of the light-driven sodium ion pump

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Light-driven Na^+ pumping rhodopsin (NaR) actively transports Na^+ in NaCl solution, but it converts to H^+ pump in the presence of larger cations. Na^+ uptake occurs upon formation of O intermediate, while photocycles largely differ between Na^+ and H^+ pumps [1].
In this study, we investigated molecular mechanism of NaR by applying flash photolysis measurement under several conditions. The measurements for different pH revealed that formation of the O intermediate is accelerated at low pH. This result suggests that H^+ uptake occurs upon O formation similarly to Na^+ pump. We will discuss the relation between the function and photorcycle dynamics.
[1] Inoue et al. *Nat. Commun.*, 2013, 4, 1678

3P251 過渡回折格子法でみた赤色光センサー蛋白質(Cph1)の光反応ダイナミクス
Photoreaction dynamics of the Cyanobacterial phytochrome 1 (Cph1) studied by the transient grating method

Kimitoshi Takeda, Masahide Terazima (*Graduate School of Science, Kyoto University*)

Cyanobacterial phytochrome 1 (Cph1) is a red and far red light sensor protein. The primary structure of the Cph1 is very similar to the phytochrome derived from the higher plants. In the photoreaction of Cph1, interconversion between the inactive Pr state and active Pfr state is induced by the red and far-red light absorption. In this study, we measured the photoreaction dynamics associated with the conversion from Pr to Pfr state by using the time-resolved transient grating (TG) method. The TG signal of Cph1 showed significant diffusion coefficient (D) change and volume change, which indicates that the structural change and interdomain interaction change occurred in the protein part. We will discuss the mechanism of signal transduction based on the results.

3P252 NdR2 の D116 残基の機能的および分子的役割の解明
Role of D116 for the Na^+ pump activity and molecular property of *Nd*R2

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Nonlabens dokdonensis possesses microbial rhodopsin (*Nd*R2) which functions as a light-driven outward Na^+ pump. *Nd*R2 has the unique NDQ motif in helix-C conserved among Na^+ pumps [1]. D116 is the counterion of the retinal Schiff base. In this study, we constructed various variants of D116. We measured pumping functions and UV-vis spectra in various pH for these variants to investigate the effect of mutation of D116. Based on these results, we will discuss about the role of D116 for Na^+ pump function and molecular property.
[1] Inoue et al. Nat. Commun, 2013, 4, 1678

3P253 New crystal forms of squid rhodopsin

Midori Murakami, Tsutomu Kouyama (*Nagoya University*)

Rhodopsin is a photo-receptor protein in vertebrate and inveretebrate eyes. To investigate the activation mechanism of rhodopsin, we have performed crystallographic studies of the early photo-reaction intermediates of squid rhodopsin using the P62 crystal. In this study, we obtained new crystal forms of squid rhodopsin for analysis of the late intermediates. When microvillar membranes were treated with hydroxylamine, rhodopsin was completely bleached within 48 hours. The opsin was crystallized into a hexagonal form. The other crystal was obtained using full-length rhodopsin with long C-terminal loop. When the membrane was partially delipidated and then mixed with octyl-thioglucoside, full-length rhodopsin was crystallized into a hexagonal form.

3P254 コンピュータシミュレーションによる光合成循環的電子伝達の非光化学消光（NPQ）の誘導における役割と寄与率の推定

Computer simulation of photosynthetic electron transport - Prediction of contribution of the linier and the cyclic electron flow -

Ryoichi Sato[1], Hiroyuki Ohta[2], Shinji Masuda[2] ([1]*Grad. Sch. Biosci. Biotechnol., Tokyo Inst. Technol.*, [2]*Center for Biological Resources and Informatics, Tokyo Inst. Technol.*)

On the Earth, sunlight is changeable in its intensity and spectral quality during the day, and plants have to perform photosynthesis with this fluctuating light. To adapt the fluctuating conditions, plant have mechanisms called Non-photochemical quenching (NPQ) which is regulated by the proton gradient across the thylakoid membrane (ΔpH) in chloroplast. The ΔpH is generated by two electron flows: the linear electron flow (LEF) and the cyclic electron flow (CEF). However, the respective significance of LEF and CEF for NPQ induction is largely unknown. Here we applied computer simulation analysis to estimate the respective contribution of LEF and CEF to the dynamics of NPQ induction. The simulation also suggested a novel function of the PGR5-dependent CEF.

3P255 光捕獲系内の励起エネルギー移動に対する分子内振動モードの役割の解析

Analyses of intra-molecular vibrational modes affecting excitation energy transfer in light harvesting systems

Yuta Fujihashi, Akihito Ishizaki (*IMS*)

In the last decade the technique of 2D electronic spectroscopy has been applied to explore light harvesting proteins. Recent analyses of the 2D spectra suggest that quantum mechanically mixed electronic and vibrational states (vibronic states) in pigments might play a important role in understanding excitation energy transfer (EET) dynamics. However, such quantum mixtures are thought to be fragile against protein-induced fluctuations. In order to unveil roles of vibrational or vibronic states in EET, we investigated EET dynamics and 2D spectra in model systems with vibrational modes by means of a numerically accurate quantum dynamic theory. In this poster, we demonstrate to what extent vibrational modes assist EET under the influence of protein-induced fluctuations.

3P256 高速原子間力顕微鏡によるホウレンソウ由来グラナ膜の観察

Observation of grana membranes from spinach by high-speed atomic force microscopy

Ami Komata, Daisuke Yamamoto (*Dept. Appl. Phys., Grad. Sch. Sci., Fukuoka Univ.*)

Grana membrane is a membrane system that converts energy from light and optimizes itself under varying environmental conditions. Here, we observed grana membranes from spinach by high-speed atomic force microscopy (HS-AFM) to reveal the molecular arrangement and its dynamics within the membrane. On mica surface, the observed height of the grana membranes was about 20 nm on average and the diameter was several hundred nanometers, which agree with those expected. On the grana membranes, many particles protruding from the membrane surface were discerned, probably reflecting Photosystem II (PSII). A significant lateral diffusion of PSII was unidentified by the HS-AFM observations, suggesting that the intramembranous structure of the grana membrane is relatively rigid.

3P257 光合成蛋白質と金属ナノ粒子による水素発生人工光合成ナノデバイスの開発

Development of an artificial light-driven water splitting nano-device using photosynthetic proteins and metal nanoparticles

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Development of efficient artificial photosynthetic systems is crucial to solve the energy and environmental crises. In this study, we developed a nano-device using photosynthetic proteins and metal nanoparticles aiming at light-driven H_2 production by water splitting. A conjugate of cyanobacterial PSI and PSII with a gold nanoparticle (PSI-GNP-PSII) was prepared and binding of both PSI and PSII on a GNP was confirmed by single-particle fluorescence measurement at a cryogenic temperature. The relaxation of photooxidized P700 indicated the transfer of an electron from PSII to PSI through DCPIP. Furthermore, H_2 production was detected using a conjugate of PSI with a platinum nanoparticle (PSI-PtNP) in the presence of ascorbate and TMPD as electron donors.

3P258 Detection of Transient Y_Z Radical Signals during S-State Transition in Photosystem II

Wataru Koinuma, Hiroyuki Mino (*Grad. Sch. Sci., Nagoya Univ.*)

The photosystem II (PS II) protein complex catalyzes water oxidation reaction. Y_Z residue works as an intermediate redox-active component for electron transfer between P680 and Mn-cluster, in PS II. Y_Z might work not only as an electron transfer intermediate but also as a proton transfer intermediate. But conclusive evidences related to proton transfer are not found. In order to clarify the role of Y_Z, time-resolved EPR measurement was performed. Up to now, $Y_Z^{\cdot}$ transient EPR signal has been not resolved well because of short lifetime. We have improved time resolution of the EPR by using high frequency Lock-in amplifier. We applied the new system to the measurement of $Y_Z^{\cdot}$ and obtained four kinds of $Y_Z^{\cdot}$ decay components, depending on S state transitions.

3P259 QM/MM 計算による光化学系Ⅱにおける水分解 Mn4Ca クラスターのアミノ酸配位子の基準振動解析

Vibrational Analysis of the Amino Acid Ligands to the Water Oxidizing Mn4Ca cluster in Photosystem II using QM/MM Calculations

Shin Nakamura, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

Photosynthetic water oxidation is catalyzed at the Mn_4Ca cluster in photosystem II. Six carboxyl groups and one imidazole group function as ligands to the Mn_4Ca cluster. To clarify electronic and vibrational structure of the Mn_4Ca cluster, we have performed the normal mode analysis of the Mn_4Ca cluster including the amino acid ligands using QM/MM calculations. The calculated S_2/S_1 difference spectra satisfactorily reproduced the symmetric COO^- region of the experimental FTIR spectrum. In addition, the $^{12}C/^{13}C$ double difference spectrum of D1–A344 (C–terminus) was well reproduced. These data provide the information of the oxidation states of the individual Mn ions in the S_1 and S_2 states as well as the assignments of the FTIR bands of amino acid ligands.

3P260 Site-directed mutagenesis study of amino acid resides relevant to photosynthetic water oxidation in photosystem II

Ryo Nagao, Hanayo Ueoka-Nakanishi, Takumi Noguchi (*Grad. Sch. Sci.,Univ. Nagoya*)

The high-resolution X-ray structure of photosystem II shows the ligand structure of the Mn4Ca cluster and postulated proton pathways. However, the roles of amino acid residues involved in the O2 evolution mechanism remain to be clarified. In this study, we constructed the site-directed mutants of amino acid residues on the D1 and D2 subunits responsible for the ligands to the Mn4Ca cluster and putative proton pathways using the cyanobacterium Synechocystis sp. PCC 6803 and investigated the effects of mutation on the growth and O2-evolving reaction. The mutants of the residues outside the first shell grew photoautotrophically, whereas those of the direct ligands to the Mn4Ca cluster did not. Spectroscopic characterization of the photoautotrophic mutants is underway.

3P261 Quantitative refinement of the theory of the improved variational master equation

Yuta Fujihashi[1], **Akihiro Kimura**[2] ([1]*IMS*, [2]*Nagoya Univ.*)

The excitation energy transfer (EET) mechanism depends on the degrees of EET interaction V and reorganization energy in the reaction system. In the photosynthetic antenna system, because some values of V are in the intermediate coupling cases, it is difficult to analyze the EET dynamics under simple perturbation theory.

Recently, we qualitatively improved the variational master equation by McCutcheon and Nazir[1]. There however exist the quantitative differences between the results by our theory and those by the hierarchy equation of motion. We studied the refinement of the disagreements by analyzing the local minima states in the free energy and the damping effect by the higher order perturbation terms.

[1] Y. Fujihashi and A. Kimura, J. Phys. Soc. Jpn. 83 014801 (2014).

3P262 極低温顕微鏡を用いた緑化途上トウモロコシ生葉の光合成タンパク質前駆体の空間分布の測定

Cryogenic microscope observations of photosynthetic proteins under assembly process in greening etiolated Zea mays leaves

Tomofumi Chiba, Hiroshi Fukumura, Yutaka Shibata (*Grad. Sch. Sci., Univ. Tohoku*)

The purpose of this study is to directly observe the spatial distributions of the precursors to photosynthetic pigment-binding proteins in vivo in greening etioplasts of Zea mays leaves. We froze thin sections of greening leaves to stop further development of greening and then observed them using a novel cryogenic microscope with an NA of 0.9. The improved NA of the microscope led to a drastic enhancement of the spatial resolution. This study revealed spatial distributions of chlorophyll precursors (protochlorophyllide, etc.) in non-irradiated and 10-min irradiated etioplasts. This gives novel information about the collapse process of the prolamellar body in the earliest stage of greening. We will also present experimental results about the later stage of the greening.

3P263 緑色硫黄細菌のタイプ 1 光合成反応中心は 2 系列のエネルギー移動系をもつ

Two Disconnected Antenna Chlorophyll Pools in Type-1 Photosynthetic Reaction Center of Green Sulfur Bacteria

Chihiro Azai[1], Toru Kondo[2], Shigeru Itoh[3], Hirozo Oh-oka[4] ([1]*Col. Life Sci., Ritsumeikan Univ.*, [2]*Grad. Schl. Sci. & Eng., Tokyo Inst. Tech.*, [3]*Cent. Gene Res., Nagoya Univ.*, [4]*Grad. Schl. Sci., Osaka Univ.*)

Photosynthetic reaction center (RC) is a pigment-associated membrane protein supercomplex that drives light-induced electron transfer reactions. RCs can be classified into two groups, type 1 and 2, based on their significant structural and functional similarities. In this study, organization of antenna chlorophylls in the type-1 RC of green sulfur bacteria (gRC) was investigated by leucine substitutions of universally conserved histidine residues, which are expected to be axial ligands of chlorophylls. Fluorescence lifetime analysis showed gRC has two groups of antenna bacteriochlorophylls *a* that are energetically disconnected at 77 K. This is different from the situation in another type-1 RC, photosystem 1, where antenna chlorophylls *a* are homogeneously organized.

3P264 復元した祖先型ヌクレオシドニリン酸キナーゼの解析

Characterization of resurrected ancestral nucleoside diphosphate kinases

Takahiro Sasamoto, Satoshi Akanuma, Akihiko Yamagishi (*Dept. of Appl. Life Sci., Tokyo Univ. of Pharm. Life Sci.*)

Using phylogenetic trees built from a large number of extant nucleoside diphosphate kinase (NDK) sequences, we previously resurrected several NDKs that might be hosted by the last common ancestors of archaea and of bacteria. The resurrected NDKs were highly thermally stable and showed the highest catalytic activity at 80°C, suggesting that both the common ancestors of archaea and of bacteria were (hyper)thermophilic. In the current study, we also analyzed the pH dependences for catalytic activity and for thermal stability of the resurrected NDKs. We found that all of the resurrected NDKs analyzed displayed the highest activities around pH 10 and the maximum stability at a neutral pH. Therefore, the ancestral NDKs might function optimally at a neutral or alkaline pH.

3P265 バクテリア融合チャンバーからのバクテリア再生に向けた研究

Toward reproduction of a bacterium from hybrid chamber cells

Kazuhito Tabata, Yoshiki Moriizumi, Rikiya Watanabe, Hiroyuki Noji (*Department of Applied Chemistry, The University of Tokyo*)

Hybrid chamber cells are devices of femtoliter (fL) size that house membrane and cellular components of the cell and allow for the fusion of animate and inanimate materials. It is considered as an ideal platform for cell reproduction and reconstitution. To explore the feasibility of cell reconstitution with fL-chambers, we attempted to integrate a living bacterial cell with fL-chamber. Protoplasts from E. coli cells were placed on fL-chambers, the top of which was sealed with lipid bilayer. Some of protoplasts showed membrane fusion that released the intracellular components into a chamber and the membrane components into the artificial bilayer membrane. We are currently conducting viability assessments of the hybrid system.

3P266 バクテリアプロトプラストとマイクロ膜チャンバーの融合反応の高感度検出

High-sensitive detection method of bacterial protoplast fusion into a micron-sized lipid membrane chamber

Yoshiki Moriizumi[1], Kazuhito V. Tabata[1,3], Rikiya Watanabe[1,3], Hiroyuki Noji[1,2] ([1]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo.*, [2]*CREST, JST*, [3]*PRESTO, JST*)

We have been trying to develop 'cyber-cell system' by fusing a protoplast of *E. coli* with a micron-scaled reactor sealed with lipid bilayer. Although we observed fusion events and viability of the cyber-cell chamber in some cases, the yield of the cyber-cell chamber formation is still low. To optimize the protocol, quantitative measurement of the fusion process is required. In this study, we are developing an complementation-based method to detect the fusion; the α-peptide of β-galactosidase (β-gal) is introduced in the chamber prior to the fusion of protoplast *E. coli* expressing ω-peptide. Upon the fusion, the formed β-gal will give clear fluorescent signal, hydrolyzing a fluorogenic substrate. This assay will facilitate optimization of the fusion process.

3P267 Structural characteristics of phosphorylation sites on disordered binding regions

Hafumi Nishi, Akinori Kidera (*Grad. Sch. Medical Life Sci., Yokohama City Univ.*)

Phosphorylation is one of the most common post-translational modifications in eukaryotic cells. Several comprehensive studies have indicated phosphorylation sites are generally abundant on disordered regions and may serve as a switch of disorder-order transition upon binding; however, structural details of coupling between phosphosites and disorder are not fully understood. In this study, we aimed to describe the relation at atomic level using experimentally validated data. We obtained 142 phosphorylation sites on disordered binding regions (disordered regions which become ordered upon binding) from PhosphoSitePlus, Phospho.ELM and IDEAL databases, and classified them based on their structural properties and functions.

3P268 De Novo protein structure modeling by rewiring old folds

Shunsuke Nishiyama, Tatsuo Mukai, George Chikenji (*Grad. Sch. of Engineering, Nagoya Univ*)

The primary obstacle to de novo protein structure prediction is conformational sampling: the native state generally has lower free energy than nonnative structures but is exceedingly difficult to reach in folding simulations. Here, we develop a new method of generating protein structures for de novo prediction.
The method generates novel structures by permutating the secondary structure elements and reversing the N- to C-term chain direction of old folds. This technique allows us to effectively produce novel fold structures which have a stable and well packed hydrophobic core. In the presentation, we will report the detailed description of the method and the results of the benchmark test.

3P269 Template based modeling utilizing an order-made template libarary

Kodai Takagi, Tatsuro Mukai, George Chikenji (*Grad. Sch. Eng., Nagoya Univ.*)

Template based modeling is currently the most reliable method for predicting protein structures. However, there is still much room for improvement in predicting hard targets, for which only distantly related homologous or analogous protein is available as templates. For these targets, the key is appropriately exploiting structure information. Here, we propose a new method of template based modeling. The characteristic feature of our method is that, unlike many of the other method, templated structures are modified so that template structures be compatible with predicted stucture features of the target.In the presentation, the detailed description of the method and the results of benchmark tests will be presented.

3P270 Homologous protein pairs that share the same core packing but have different topology

Takahiro Kanemitsu[1], Shintaro Minami[2], George Chikenji[1] ([1]*Grad. Sch. of Eng., Nagoya Univ.*, [2]*Grad.Sch. of Inf.Sci., Nagoya Univ.*)

Some homologous proteins sharing the same spatial arrangement of secondary structure elements show different topology. Investigation of such homologous proteins will lead us to deeper understanding of mechanism of protein evolution. We performed comprehensive protein structure comparison against the SCOP database, using a non-sequential protein structure alignment program MICAN, and identified all non-sequentially similar homologous protein pairs. We found that some of them show complex structure relationship beyond circular-permutation or other known structure relationships, and obtained some sugggestion of possible evolutionary events that change protein topology.

3P271 タンパク質立体構造におけるループ交差検出のためのアルゴリズム
An algorithm for identifying loop crossing in protein structures

Tatsuo Mukai, George Chikenji (*Grad. Sch. of Eng., Dept. of Comput. Sci. and Eng., Nagoya Univ.*)

It is widely accepted that the total number of stable protein folds is limited by physicochemical rules. Among them, prohibiting loop crossing has been recognized as one of the most important rules, because it has been thought that loop crossing is rarely observed in nature. However, it has not been estimated how frequently loop crossing occurs. That is because the algorithm that can detect loop crossing in protein structures has not been developed so far.
Here, we develop an algorithm that identifies loop crossing. In the algorithm, loop structures is represented by triangles and a decision whether loop crossing occurs is made based on a triangle-triangle intersection. We will report the detailed description of the algorithm and the results of the benchmark test.

3P272 Observing the rotational diffusion of nanodiamonds with arbitrary nitrogen vacancy center configurations

Ziya Kalay, Yohsuke Yoshinari, Yoshie Harada (*WPI-iCeMS, Kyoto University*)

Diamond nanoparticles containing nitrogen vacancy centers (NVCs) are promising probes for cell biology as they can report both rotational and translational motion and do not blink or bleach. We present theoretical results on the relation between the rotational diffusion coefficient of a nanodiamond undergoing Brownian motion and the configuration of NVCs in the particle. Via exact calculations, we obtain the fluorescence intensity autocorrelation function measured in optically detected magnetic resonance experiments at single particle level, relate it to the rotational diffusion coefficient and discuss the influence of different NVC configurations on the outcomes. Our results can be useful in interpreting observations on nanodiamonds that contain multiple NVCs.

3P273 タンパク質量バランス制御機構解明に向けた、個別タンパク質量制御ルールの分類
Production and degradation balancing mechanism of each protein controls the whole protein balance

Masayo Inoue, Katsuhisa Horimoto (*molprof, AIST*)

The balance of each protein is essential for maintaining healthy state on the whole. Each protein amount is determined through its production and degradation balance. Studying its regulatory mechanism, i.e., whether fitting the production rate or the degradation rate according to conditions is important for understanding the whole balance control. We report how we can distinguish the two regulatory mechanisms from the transcription-factor (TF) and its regulating gene relation. In case of the production-fitting, TF adjusts the necessary production rate. On the contrary, in case of the degradation-fitting, the excessive amount is generated and later decreased to the required level. We studied exhaustive classification and found some systematic classification rules.

3P274 バクテリアの Twitching 運動における線毛の伸縮規則の影響
Effect of the extending and retracting rule of the bacterial pili in twitching motility

Ryota Morikawa, Masatada Tamakoshi, Takeshi Miyakawa, Masako Takasu (*Sch. of Life Sci., Tokyo Univ. of Pham. and Life Sci.*)

Studying characteristic individual motion of the bacteria and their social behavior in the environment of the low Reynolds number leads to suggesting the difference between life and non-life from the physical viewpoint. Our dynamical model of a twitching bacterium, which crawls over solid surfaces by extending and retracting their multiple type IV pili, indicates that a dimensionless retraction velocity of the pilus controls the moving direction of the twitching bacterium. In this presentation, we report how the bacterial motion is affected by the difference of the extending and retracting frequency of the pili.

3P275 細胞の動的構造・機能とモデリング
Modeling of crowded environment under micro-confinement: Detachment of a large object from the surface

Soutaro Oda[1], Chwen-Yang Shew[2], Kenichi Yoshikawa[1] ([1]*Faculty of Life and Medical Sciences, Doshisha University*, [2]*City University of New York*)

Living cells maintain their activities within closed membrane, encapsulating macromolecules in a highly crowded condition with 30-40% volume occupied. Here, Monte Carlo simulation is applied to elucidate the spatial distribution of a large sphere mixing with smaller crowders in a spherical confinement as a greatly simplified model to abstract the essence of the nuclear of a eukaryotic cell constituting giant genomes under molecular crowding with smaller proteins. From the simulation, we find the conditions that the large sphere is localized either around the center or on the surface in crowded capsules. We compare such a specific localization of the confined large sphere with smaller crowders via a simple real-world experiment agitated by mechanical vibration.

3P276 Multicanonical Go モデル分子動力学によるタンパク質の自由エネルギーの網羅的解析
Comprehensive analysis of protein folding energy landscape by multicanonical Go-model molecular dynamics simulation

Mashiho Ito, Shoji Takada (*Dept. Biophysics, Sch. Sci., Kyoto Univ.*)

In protein folding, coarse-grained Go model simulations have been widely used to simulate the folding reactions as well as to sample conformaitons. However, even with Go model, it is difficult to sample proteins longer than one hundred residues currently. Here we introduced Wang-Landau multicanonical method to Go model simulation and enabled to sample proteins larger than 300 residues. We did sampling over than 50 proteins and characterized folding barrier heights, structures of transition state and transition path lengths.

3P277 多変数多項式による動的恒常維持パターンの解析へ向けて
Toward analysis of multicell-turnover patterns by using multivariable polynomials

Hirosi Yosida (*Faculty of Math. Kyushu Univ.*)

Campbell, who performed many experiments in Hydra, noted: “The cells and tissues of hydra are in continuous flux. The polyp undergoes perpetual growth and tissue loss, coupled by balanced cell renewal patterns involving all cell types” [1]. I studied a bio-inspired model of patterns that regenerate in flux, namely regenerate through turnover in [2,3], where I parameterized the manner of the redistribution of *Dachsous* and *Fat*, and then derived equations to make turnover feasible. I here propose a method for analyzing multicell-turnover patterns by using multivariable polynomials.

[1] R.D. Campbell *Am. Zool.* 1974.
[2] Yoshida, H. *Biosystems*, 2012.
[3] Yoshida, H. et al. *Scientific Reports*, 2014.

3P278 Role of intronic delay in oscillatory gene expression

Bhaswati Bhattacharyya, Ziya Kalay (*Institute for Integrated Cell-Material Sciences, Kyoto University*)

Growing evidence in favor of multi-stage maturation of mRNA from immature RNA (pre-mRNA) strongly suggests the role of delay in maturation process. We propose to consider a gene switch model, where in each stage an intron is removed from a pre-mRNA or other modifications occurs. We consider the final gene product, which is a protein, offers negative feedback on itself. To find the conditions for stable sustained oscillations, we explore two features of this network (i) the role of delay and (ii) the effect of cooperativity gene switching rate. We calculate autocorrelation function, by using simulations and proteomic field theory to compare them. We aim to construct a phase diagram to identify the parameters and number of introns necessary for sustained oscillation.

3P279 不均質環境下における個体群動態の解析
Analysis of population dynamics in heterogeneous environment

Kenta Yashima[1], Sayaki Suzuki[2], Akira Sasaki[1] ([1]*The Graduate University for Advanced Studies*, [2]*National Agricultural Research Center*)

In order to deal with the heterogeneous nature of the environment, we have utilized a theoretical framework based on Fractal reaction kinetics which is efficient in dealing with the chemical reactions in heterogeneous medium. This theory is effective when the movements of the reactants exhibit a peculiar behavior termed anomalous diffusion. It has been found recently that the movements of some walking insects show an anomalous diffusion. Given these results, we propose a new theoretical framework in mathematical ecology; application of Fractal reaction theory to population dynamics in heterogeneous environment. We have applied the theory to a plant pest disease model and derived an analytic form of basic reproduction ratio R0.

3P280 中和反応を駆動力とする走化性液滴:(1) ガスに対する応答
Chemotactic behavior of a liquid droplet:(1) Smelling and running

Hiroki Sakuta[1], Nobuyuki Magome[2], Yoshihito Mori[3], Akihisa Shioi[4], Kenichi Yoshikawa[1] ([1]*Facul. Lif. Med. Sci., Doshisha Univ.*, [2]*Chem., Dokkyo Med. Univ.*, [3]*Facul. Sci., Ochanomizu Univ.*, [4]*Facul. Sci. Eng., Doshisha Univ.*)

We report the physical realization of chemotaxis with an artificial material, which self-propels in response to a stimulant gas. We will show experimental verification on the formation of self-moving object with the ability of chemotactic response, by adapting floating oil droplets on a water surface. For example, it is reported that a droplet of oleic acid exhibits negative chemotaxis against the source of ammonium vapor. Similarly, a droplet of oleylamine exhitis the motion by repelling the vapor of hydrochloric acid. The mechanism such chemotactic behavior will be discussed in terms of the spontaneous generation of the interfacial gradient caused by the stimulant gas.

3P281 中和反応を駆動力とする走化性液滴:（2）炭化水素残基効果と境界条件
Chemotactic behavior of a liquid droplet: (2) Hydrocarbon residue effects and boundary conditions

Lui Mihara[1], Aya Fujii[1], Kyoka Shiraki[1], Miku Shimada[1], **Yoshihito Mori**[1], Kenichi Yoshikawa[2] ([1]*Ochanomizu Univ. Sci*, [2]*Doshisha Univ. Life & Med. Sci.*)

We designed a chemotacitc liquid droplet based on an acid - base reaction and demonstrated an oleic-acid droplet reacting ammonia gas and an oleylamine droplet reacting hydrogen chloride gas in a vessel. In this work the prototype was developed by replacing oleylamine by decylamine or aniline to modulate the movement. The movement can be generated by fluid flow around a droplet interface and the fluid flow is supposed to depend on hydrocarbon residue of the amine at the interface. Moreover we discuss various types of vessels to bring the effects of the boundary conditions on the movement.

3P282 中和反応を駆動力とする走化性液滴：(3) 自己触媒反応による pH 変化と液滴の動き
Chemotactic behavior of a liquid droplet: (3) pH change by autocatalytic reaction and liquid droplet motion

Nobuyuki Magome[1], Tatsuya Okuda[1], Noriko Umezawa[1], Yoshihito Mori[2], Kenichi Yoshikawa[3] ([1]*Premedical Sci., Dokkyo Med. Univ.*, [2]*Grad. Sch. Sci., Ochanomizu Univ.*, [3]*Grad. Sch. Life and Med. Sci, Doshisha Univ.*)

We present autonomous liquid motion induced by chemical wave of pH change. Liquid droplet motion depends on a chemical composition of aqueous phase. The pH of the aqueous phase can be changed in a spatiotemporal manner by an autocatalytic reaction. The autocatalytic reaction, which is important process in nonlinear reactions, exhibits sudden shift of pH from acidic state to basic state. This reaction causes neutralization of monocarboxylic fatty acid on the reacting aqueous phase, then Marangoni convection due to local change of interfacial tension is induced.

3P283 Interplay between the ATPase activity and the structural change of KaiC protein studied by stochastic simulation

Kenju Narita, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Nagoya Univ.*)

KaiA, KaiB, KaiC proteins and ATP produce the temperature-compensated rhythm in the phosphorylation level of KaiC with the period of about 24 hours in vitro. In addition, ATPase activity of KaiC also shows the temperature-compensated circadian rhythm, the period of which is correlated with the phosphorylation level oscillation. Thus we here consider the computational model of a single hexamer of KaiC comprised by monomers, each of which can take two structures and four different states of ligands. We assume that structural changes are at equilibrium in the time scale of the ATPase activity and monitor the stochastic change of bound ligands. By using this model, the mechanism of ATPase activity oscillation and the temperature compensation will be discussed.

3P284 Epigenetic Dynamics of Cell Reprogramming

Ashwin S.S., Masaki Sasai (*Department of Computational Sciences and Engineering, Nagoya University, Nagoya Japan.*)

Reprogramming is a mechanism by which one reverses a differentiated cell to iPS cells by inducing specific factors in the cell. Reprogramming involves collaboration at levels separated by orders magnitude in timescale, namely the epigenetic change and transcription/translation. We propose a model integrating these temporally separated mechanisms and show that stable states on the epigenetic landscape should be viewed as a superposition of subset of basin minima. Our model elucidates how barrier crossing during reprogramming is aided by valleys created due to epigenetic dynamics. We additionally show the similarity of latency profiles in our model with experiments and resolve a puzzle raised by a recent model on the stability of pluripotent and differentiated cells.

3P285 アトリットル容積を持つナノセルを用いた酵素 1 分子の高速検出
Rapid Detection of Single-Molecule Enzyme using Attoliter Well Array, Nanocell

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Microwell array allows detecting single enzyme molecule by encapsulating enzymatic products inside the well. However, even in μm-scale, fL-volume wells, the concentration of products rises so slowly that the application of the detection is severely limited. In this study, nanofabricated device "Nanocell", which confines well volume to 8~200 aL, was developed for high-speed detection of single-molecule enzyme. Several minutes after randomly encapsulated in Nanocell, β-galactosidase molecules were individually detected by their fluorescent products, while the fluorescent detection takes several hours in fL-volume wells. Nanocell has the potential to expand the application range of single-molecule assay.

3P286 帽子型金ナノ粒子を用いた近赤外型局在表面プラズモン共鳴バイオセンサー
Near infrared localized surface plasmon resonance biosensing based on cap-shaped gold nanoparticles

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We describe a non-labeled biosensing method based on localized surface plasmon resonance, LSPR, of surface-adsorbed cap-shaped gold nanoparticles. Gold nanoparticles are well known to exhibit an extinction peak whose peak wavelength depends on the refractive index of the surrounding. Typically, the peak appears in the visible regime, but an additional peak appears in the 900-1400 nm regime due to dimer structures. This peak shows greater sensitivity toward the surrounding refractive index than the visible peak. The figure of merit, expressed in terms of nm/refractive index unit, RIU, improves from 120 nm/RIU to over 500 nm/RIU. It is hoped that the improved sensitivity, combined with simplicity of an LSPR system, will lead to wider acceptance of this method.

3P287 フェムトリットルドロップレットアレイを用いたアルカリフォスファターゼ１分子活性の検出及び定量計測
Detection and activity measurement of single molecule alkaline phosphatase with femtoliter droplet array

Yusuke Obayashi[1], Ryota Iino[2], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Okazaki Inst. Integ. Biosci, NINS.*)

Digital enzyme-linked immunosorbent assay (dELISA) enables the detection of antigen molecules at the single-molecule level. To realize multiplex dELISA, it is highly awaited to expand the color variation of the detection enzyme. In the present study, we conducted single-molecule fluorogenic assay of alkaline phosphatase (ALP) that is widely used in conventional ELISA. When individual ALP molecules were encapsulated with fluorogenic substrate in femtoliter droplet array, each droplet showed clear fluorescent signal. The determined turnover rate of ALP was 891s-1, well consistent with bulk measurement. Simultaneous single-molecule fluorogenic assay of ALP and β-gal was also achieved, supporting the feasibility of multiplex dELISA.

3P288 培養神経細胞の軸索輸送活動度の新たな評価方法
A new quantitative method to evaluate the activity of axonal transport of cultured neurons

Takashi Katakura, Risa Isonaka, Tadashi Kawakami (*Dept. Physiol., Kitasato Univ. Sch. Med.*)

We have developed a new method to quantify the axonal transport of cultured neurons. Enhanced video images of axons transporting organelles were captured, cropped and converted into video-stacks. We analysed the video-stacks using KBI-Flow plugin on ImageJ. The velocity vectors and the number of moving organelles were calculated. We define the activity of axonal transport as the sum of particles whose moving velocity is greater than 0.1 μm/s within a fixed area per certain unit of time. We estimate the velocity of moving organelles is directly proportional to the activity of axonal transport. The activity decrease of axonal transport caused by Neurotropin calculated by KBI-Flow plugin is well coincided with our previous data measured by the traditional method.

3P289 神経細胞に対する Amyloidβ42 毒性伝搬 in vitro モデルの構築

Development of neurotoxicity of amyloid β(1-42) proteins propagation in vitro model

Takuma Maruyama[1], Lui Yoshida[2], Kiyoshi Kotani[3], Seiichi Suzuki[1], Yasuhiko Jimbo[3] ([1]*Grad. Sch. Sci and Tech, SEIKEI Univ.*, [2]*Grad. Sch. Frontier Sci, Univ of Tokyo*, [3]*Grad. Sch. Engineering, Univ of Tokyo*)

A co-culture device was fabricated by use of photolithographically which is one of the micromachining.The MEA has 64 microelectrodes which is capable of measuring extracellular potential.The chambes, which can culture neuronal cells, are connected by microfluidic channels.The chambar was made of PDMS , and MEA was made of Indium Tin Oxide base material. We culture separately hippocampal neuronal cells in the different culture compartments of the co-culture chambers. Neurites of hippocampal neuronal cells structure nerve connections between the different groups of neuronal cells pass through the microfluidic channels.In this research, we evaluated toxicity propagation from neuronal cells treated with Amyloid β42 to normal neuronal cells by use of the co-culture device.

3P290 Automation engineering for single molecule imaging using total internal reflection fluorescence microscopy

Jun Kozuka, Michio Hiroshima, Yasushi Sako, Masahiro Ueda (*RIKEN*)

Automation engineering for single molecule imaging using total internal reflection fluorescence microscopy (TIRFM) technique which typically involves very thin (~500 nm) optical sectioning, which makes automatic observation of cells adhering to the glass surface difficult. High-throughput systems, such as multi-well plates, further require constant feeding of oil to the objective lens. We have developed 1) an image processing algorithm for single cell detection that uses the coefficient of variation of the pixel intensity of an image, 2) an auto-focusing system that uses the contrast of the aperture, which is located conjugate to the glass surface, and 3) an objective lens adapter that feeds immersion oil to the objective lens via an oil inlet and outlets.

3P291 ナノ粒子表面へのプローブオリゴヌクレオチドの修飾密度が DNA ハイブリダイゼーション効率に与える影響

The contribution of the density of immobilized probe oligonucleotide on nanoparticle surface for DNA hybridization efficiency

Atsushi Kira, Atsushi Suda (*Product Development Center, Japan Aviation Electronics Industry, Ltd.*)

The biomolecular sensing methods using nanotechnology have been known to be a powerful tool to understand how biological systems work. Amoung these methods, nanomaterials including nanoparticles (NPs) are used for label or signal enhancement. The affinity between probe and target contributes directly to the amount of target molecules trapped by probe. Thus the regulation of probe consisting of probe molecular immobilized nanomaterials is one of the key for the quantitative and reliable analysis.
We will report about the investigation in the change of DNA hybridization efficiency of thiolated single stranded DNA (ssDNA) immobilized on Au-NPs with respect to the immobilization density of ssDNA and consideration about the contribution of interaction in nanomaterials.

3P292 高速原子間力顕微鏡ピエゾドライバの広帯域駆動

Wideband operation of high-voltage amplifier for high-speed atomic force microscopy

Hiroyuki Handa, **Daisuke Yamamoto** (*Dept. Appl. Phys., Grad. Sch. Sci., Fukuoka Univ.*)

We have developed a method to operate the high-voltage amplifier (HVA) of high-speed atomic force microscopy (HS-AFM) with a wide bandwidth. The frame rate of HS-AFM is considered to mainly be restricted by the delay of cantilever, amplitude measurement instrument, and z-piezo in the feedback loop during the scanning. Here, we found that the capacitive load, the value of which is comparable to that of the z-piezo, causes a significant delay in the response of the HVA used to drive the z-piezo. We compensated this delay using the circuit that simulates the inverse transfer function of HVA. As a result, the frequency response of HVA was improved to about 1 MHz, even with a capacitive load. We will discuss the effect of our method on the image acquisition speed of HS-AFM.

3P293 冷却 HPD によるサブミリ秒時間分解能の広視野蛍光 1 分子検出

Low-background wide-field sub-millisecond single-molecule fluorescence detection by a cooled hybrid photo-detector (HPD)

Atsuhito Fukasawa[1,2], Minako Hirano[2], Toru Ide[3], Hiroaki Yokota[2] ([1]*Hamamatsu Photonics K.K.*, [2]*GPI*, [3]*Grad. Sch. Nat. Sci. Technol., Okayama Univ.*)

Although EM-CCDs, single-photon counting avalanche photodiodes and photomultiplier tubes have been widely used for single-molecule fluorescence detection, none of them are capable of low-background wide-field single-molecule fluorescence detection with sub-millisecond time resolution. Here, we demonstrate such detection using a cooled hybrid photo-detector (HPD) (Hamamatsu Photonics) consisting of a photocathode and an avalanche photodiode. We will present time courses of single-molecule fluorescence intensity of fluorophores such as a Qdot (0.1 ms resolution) obtained by a cooled HPD whose fluorescence images were simultaneously monitored by an EM-CCD. The high performance of HPD allows us to elucidate sub-millisecond dynamics of mobile single biomolecules.

3P294 単粒子解析法の高速化のための GPGPU を用いた並列化処理の実装と評価

Implementation and evaluation of parallel processing by GPGPU for accelerating single particle analysis

Ayaka Iwasaki, Takuo Yasunaga (*Kyushu Institute of Technology*)

Single particle analysis (SPA) is one of the most powerful methods to analyze structure of proteins. The method can obtain 3D reconstruction by back projection of identical particles with various projection directions. In our lab., the image processing application called Eos has been developed for SPA. Using the application, it takes from 1 week to 1 month to obtain 3D reconstruction. Thus the purpose of this study is to accelerate SPA by implementation of parallel processing with GPGPU. Actually we implemented programs of Eos that is part of programs related to the SPA such as the addition of images, rotation, projection and so on. These programs were 20-50 times faster than those using only CPU. I will implement more complex SPA-related programs with GPGPU.

3P295 生細胞内における長鎖非翻訳 RNA の一分子イメージング法の開発

A method to visualize endogenous long non-coding RNA with single molecule sensitivity

Toshimichi Yamada, Hideaki Yoshimura, Mituru Hattori, Hiroki Segawa, Takeaki Ozawa (*Grad. Sch. Sci., Univ. Tokyo*)

Increasing knowledge about long non-coding RNAs (lncRNAs) from in vitro experiments highlights a need to study lncRNAs in live cells. We here present a method for imaging repeat regions of lncRNA in living cells. A mutant PUM-HD labels tandem repeats in a RNA sequence. Each split fragment of EGFP is connected to N and C terminal of mutant PUM-HD. When two probe molecules come close to each other on the repeat regions, split fragments of EGFP between the probes reconstitute an EGFP. Single particle tracking of TERRA revealed heterogeneities of the dynamics and its transient localization to telomere. Single molecule imaging of endogenous lncRNA provides quantitative verification of in vitro results and moreover, leads to the hypothesis by investigating unseen phenomenon.

3P296 複数の光遺伝学ツールとの組み合わせが可能な化学発光膜電位センサーの開発

Genetically-encoded chemiluminescent voltage indicator applicable in conjunction with multiple optogenetic tools

Shigenori Inagaki[1], Tomoki Matsuda[1], Yoshiyuki Arai[1], Yuka Jinno[2], hidekazu Tsutsui[2,3], Yasushi Okamura[2], Takeharu Nagai[1] ([1]*ISIR., Univ. Osaka*, [2]*Grad. Sch. Med., Univ. Osaka*, [3]*Sch. Mat. Sci., JAIST*)

Genetically encoded voltage indicators (GEVIs) are powerful tools to extract information from large number of neurons and combination with optogenetic tools must provide insights into the new function of neural circuit. However, excitation light irradiating GEVIs often restrict optogenetic tools we can use due to spectral overlap. In the conference, we will introduce a chemiluminescent GEVI. Since this novel GEVI emits very bright chemiluminescence without excitation light, it enables 333 frames / sec imaging. Besides, our GEVI can visualize the voltage change triggered by ChR2 and HR. Our results demonstrate the chemiluminescent GEVI are very useful for particularly long-term imaging with multiple optogenetic tools to understand the mechanism of neural circuit.

3P297 電子顕微鏡画像処理システム Eos への Web ブラウザと PIONE の統合による、ユーザビリティの向上

Development of a user-friendly system for image processing of electron microscopy by integrating web browser and PIONE with Eos

Takafumi Tsukamoto, Takuo Yasunaga (*KIT, Creative Informatics*)

Eos (Extensible Object-oriented System) is one of the applications for image processing of electron microscopy. In usual cases, Eos works with only character user interfaces (CUI) under the operating systems (OS) such as OS-X or Linux, not user-friendly. Thus we extended Eos to a web system independent of OS with graphical user interfaces (GUI). The part of system implemented properly and produced image analysis, but remained a problem of workspace for analysis: We need to provide common workspace for analysis because the client is physically separated from server. To solve workspace problems, we have developed the system employing PIONE, our developing platform that works under cloud environments.

3P298 コヒーレント X 線回折イメージングにおける回復電子密度図の多変量解析を利用した分類と評価

Classification and assessment of reconstructed electron density maps in coherent X-ray diffraction imaging using multivariate statistics

Yuki Sekiguchi[1,2], Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2] ([1]*Grad. Sci. Tech., Keio Univ.*, [2]*RIKEN SPring-8 Center*)

Coherent X-ray diffraction imaging (CXDI) is expected to bridge a serious gap between optical and electron microscopy in terms of spatial resolution and sample thickness. However, CXDI is still premature for structure analyses even fifteen years after its experimental demonstration. This is mainly attributed to difficulty in reconstruction of correct electron density maps from recorded diffraction patterns. Although many reconstruction algorithms have been proposed, there are still few criteria to evaluate obtained maps adequately. In this presentation, we introduce a way to classify and assess reconstructed electron density maps in multi-dimensional image space for finding global solutions applying multivariate statistics.

3P299 コヒーレントＸ線回折イメージングにおけるフリーデル対称性を拘束条件とした暗視野位相回復法の開発とシングルショット回折データ解析への応用

Dark-field phase-retrieval method under the constraint of Friedel's symmetry for structure analyses in coherent X-ray diffraction imaging

Amane Kobayashi[1,2], Yuki Sekiguchi[1,2], Yuki Takayama[2], Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2] ([1]*Grad. Sci. Tech., Keio Univ.*, [2]*RIKEN SPring-8 Center*)

In coherent X-ray diffraction imaging, electron density maps of non-crystalline particles are reconstructed by applying the phase-retrieval method to the Fraunhofer diffraction patterns. In practice, the lack of diffraction patterns in very small-angle by beam-stopper and detector saturation results in the loss of information on the overall shapes and total electrons of particles, and makes the reconstruction difficult. To overcome this problem, we have developed the dark field phase retrieval method under the constraint of Friedel's symmetry in small-angle diffraction. Here we report the theory, a set of simulation to assess the method and the application to structure analyses for the single-shot diffraction patterns collected using X-ray free electron laser.

3P300 Trafficking of membrane protein PAR-1 carried by endocytotic vesicles in cancer cells

Seohyun Lee[1], Motoshi Kaya[1], Hideo Higuchi[1] ([1]*School of Science, the University of Tokyo*, [2]*School of Medicine, Tohoku University*)

Protease activated receptor 1 (PAR-1), has been known to be one of the most essential membrane protein that mediates intracellular signals promoting cell motility, which is closely related to cancer metastasis. Since the machinery of vesicles carrying this PAR-1 proteins plays a key role in signal transfer, we imaged the trafficking of PAR-1 carrying vesicles, mainly focused on the moment of endocytosis, to analyze the movement of activated PAR-1 after internalization. Our triple-view method consisting of dual-focus fluorescence and phase contrast optics, enabled us to track endocytotic vesicles in 3-dimension. Also, using confocal microscopy, we were able to diagnose the characteristic movements of PAR-1 proteins with respect to their relative position in a cell.

3P301 A method to integrate 4D images of *C. elegans* embryos expressing different fluorescent markers

Yusuke Azuma, Shuichi Onami (*RIKEN QBiC*)

To understand cellular dynamics, we need to track a variety of proteins and visualize their relationships. However, multi-color imaging is limited to displaying only three to four proteins. Here, we solved this problem by developing a method to integrate 4D images of *C. elegans* embryos expressing different fluorescent markers. It takes advantage of the invariant embryogenesis of *C. elegans* and allows integrating unlimited number of proteins. We evaluated performance of the method by applying it to images of pairs of embryos whose nucleus and plasma membrane were labeled by different fluorescent proteins. The overlap volume ratio was calculated by segmenting the membrane regions and was about 85% for early stages. We plan to introduce this method to heterochronic genes.

3P302 新奇“無蛍光”蛍光タンパク質による細胞内シグナル伝達の蛍光寿命イメージング
Imaging intracelluer signal transduction using a newly developed “non-fluorescent” fluorescent protein for FLIM-FRET

Akihiro Shibata[1], Yoshihisa Nakahata[1], Junichi Nabekura[1], Hideji Hurakoshi[1,2] ([1]*NIPS, Okazaki*, [2]*JST PREST*)

Monitoring fluorescence resonance energy transfer using fluorescence lifetime imaging microscopy (FLIM-FRET) is a powerful technique that enables the imaging of biochemical reactions. Here, we developed a nonfluorescent green fluorescent protein named ShadowG for FLIM-FRET acceptor by using random mutagenesis method. ShadowG has similar absorption spectrum and extinction coefficient to those of EGFP, but has 150-fold smaller quantum yield. Since ShadowG has remarkable folding efficiency and darkness which excludes an artifact due to spectral overlap, we applied it for the previously reported FRET sensors and found that ShadowG-based sensors show improved sensitivity and reproducibility in HeLa cells. Thus, we believe ShadowG is currently the best FLIM-FRET acceptor.

3P303 生細胞内における microRNA の動態観測
Observation of microRNA dynamics in living cells

Toshinari Ishikawa[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*JST-PRESTO*)

MicroRNA (miRNA) takes part in translational regulations by targeting mRNA to trigger either cleavage or translational repression. Although the importance of miRNA activity is recognized, dynamic characteristics of miRNA such as biogenesis in living cells are still unknown due to the lack of method to capture them in real time. Here, we show a new approach to chase the intracellular dynamics of miRNA by introducing fluorescent precursors of miRNA into living cells. Intracellular amount, intracellular distribution, and function of the introduced fluorescent miRNA were examined by fluorescence imaging, and dynamics of miRNA biogenesis was analyzed by F(C)CS. This indicated its applicability to investigate functionality of miRNA in living cells.

3P304 生細胞内 mRNA のナノスケール一分子追跡
Nanoscale Single mRNA Tracking in Living Cells

Ko Sugawara[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad. Sch. Pharm., Univ. Tokyo*, [2]*JST, PRESTO*)

Revealing the dynamic behavior of intracellular mRNA is important in understanding cell functions. Here, we demonstrate the tracking of endogenous single mRNA in living cells by using the photoswitching attributes of fluorescent dyes. Stochastic activation of fluorophores attached to endogenous mRNA via linear antisense 2'-*O*-methyl RNA probes provided low background and spatially resolved molecular information, which enabled nanoscale tracking of single mRNA. Owing to stochastic property of this method, we could track single mRNA even in high-density regions. We performed the tracking of endogenous GAPDH mRNA and revealed that its motility was different inside of and outside of cytoplasmic mRNA granules. This powerful approach will make a breakthrough in mRNA studies.

3P305 Preparation of Green-Emitting Pt Nanoclusters for Biomedical Imaging by Pre-equilibrated Pt/PAMAM (G4-OH) and Mild Reduction

Shin-ichi Tanaka[1,2], Takashi Jin[2], Yasushi Inouye[3] ([1]*Kure National College of Technology*, [2]*RIKEN*, [3]*Osaka University*)

Noble-metal (Au, Ag and Pt) nanoclusters have been gaining attention as next-generation bioimaging probes owing to their sub-nanometer size, low cytotoxicity and size-dependent photoluminescent wavelength. Green-emitting Pt nanoclusters (em: 520 nm, ex: 460 nm) were synthesized by pre-equilibrating a Pt ion/PAMAM (G4-OH) solution in the dark for 24 h at 4°C and using a mild reduction reaction. The structural characteristics of the resulting Pt nanoclusters, Pt8L8 (L=C2H2O2S), were determined by ESI mass spectroscopy. These nanoclusters possess a 28% quantum yield and are brighter than either Au or Ag nanoclusters. In addition, since Pt nanoclusters have considerably low cytotoxicity, our green-emitting Pt nanoclusters offer great promise for biomedical imaging.

3P306 高速 AFM による抗体 IgG の動的観察
Dynamic observation of single antibody IgG using High-Speed Atomic Force Microscopy

Norito Kotani, Tomohiro Hirano, Takao Okada (*RIBM*)

Antibody IgG molecule is a “Y” shape protein. It has two Fab regions, which bind to antigens, and one Fc region. IgG is composed two heavy chains and two light chains. A hinge region is a center of heavy chain and connects between Fab and Fc region.
High-Speed Atomic Force Microscopy (HS-AFM) can observe dynamic behaviors as movie in liquid condition. IgG molecule was distinguished as “Y” shape with HS-AFM. The Y shape of IgG was not “rigid”, shook variable angles as like swinging arms. The swinging Fab behavior depends on the flexible hinge region. We estimated the flexibility of the IgG hinge in liquid quantitatively by using HS-AFM. Those flexible hinges behave effectively to bind to antigen. HS-AFM can reveal antibody details.

3P307 １分子イメージング計測による生細胞表面 T 細胞受容体のマイクロクラスター内外における動態変化

T cell receptor on the surface of living cells changes in the dynamics inside microclusters revealed by single-molecule imaging analysis

Yuma Ito[1,2], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS, RIKEN*)

Formation of microclusters (MC) of T cell receptor (TCR) plays a crucial role in the initiation of adaptive immune response. However, spatio-temporal changes of TCR molecules by MC formation are still unclear. We have devised a simple method to observe molecular dynamics and interactions on the surface of living T cells with stimulatory planar lipid bilayers. We performed simultaneous imaging of individual MC's and single TCR molecules, using GFP-tagged TCR and quantum dot-tagged TCR, respectively. By classifying the trajectories and kinetics of single TCR molecules inside and outside of MC, we found two diffusion modes of TCR in the MC. This suggests that the structure of MC is not uniform and MC serves multiple functions through distinct interactions.

3P308 ミトコンドリア新規単離法の検討

New approach to isolation of less damaged mitochondria

Takahiro Shibata[1], Rie Yamane[2], Kaoru Katoh[3], Yoshihiro Ohta[1] ([1]*Grad. Sch, Life Sci, TUAT*, [2]*Sch, Life Sci, TUAT*, [3]*AIST*)

The aim of this study is to isolate less damaged mitochondria from cells. Mitochondria are subcellular organelle that play important roles for intracellular energy production and cell death. Isolated mitochondria have an advantage over intracellular mitochondria, because we can modify the medium surrounding mitochondria. However, when we isolate mitochondria from cells in the conventional procedure, tubular mitochondria will be broken off during homogenization of cells. As a result, a lot of damaged mitochondria will be contained in the mitochondria preparation. In this study, we isolated mitochondria from cells without homogenization and characterized those mitochondria in terms of structure and activity.

3P309 3D Palm Imaging at 50 Micrometers Depth in the Sample

Audrius Jasaitis[1], Gregory Clouvel[1], Ignacio Izeddin[2], James Sillibourne[3], Mohamed El-Beheiry[3], Xavier Levecq[1], Maxime Dahan[3], Michel Bornens[3], Xavier Darzacq[2] ([1]*Imagine Optic, France*, [2]*ENS Paris, France*, [3]*Institut Curie, France*)

Photoactivation localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) enable us to locate fluorescent molecules with nanometric resolution. Unfortunately in current implementations these techniques are efficient only in first few micrometers from the coverslip. Imaging deeper is perturbed by sample-induced aberrations. In this work we corrected these aberrations using adaptive optics device MicAO 3DSR and for 3D imaging we used astigmatism induced by the deformable mirror. As a result, at different depths we obtained axial localization precision comparable to the surface. We performed 3D PALM imaging of centrosomal protein centrin-1 and showed that 3D PALM imaging can be successfully carried out at 50 micrometers depth.

3P310 Signal enhancement and Patterson-search phasing for high-spatial-resolution coherent X-ray diffraction imaging of biological objects

Yuki Takayama[1], Saori Maki-Yonekura[1], Tomotaka Oroguchi[1,2], Masayoshi Nakasako[1,2], Koji Yonekura[1] ([1]*RIKEN RSC*, [2]*Fac. Sci. Tech., Keio Univ.*)

Coherent X-ray diffraction imaging has revealed internal structures of whole biological cells and organelles. However, attainable spatial resolution is limited to several tens of nanometers so far mainly due to poor scattering power of biological samples. We propose a method to extend spatial resolutions by imaging biological targets together with colloidal gold particles. Weak signals from the biological objects are enhanced to a detectable level by interference with strong diffraction waves from the colloidal gold. The positions of gold particles determined by the Patterson analysis can serve as reliable initial phase for iterative phase retrieval. Our calculation based on current experiments clearly demonstrates improvement of the resolution by a factor of two.

3P311 Investigation of intracellular temperature during stress granule formation

Beini Shi[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad. Sch. Pha. Sci., Univ. Tokyo*, [2]*JST-PRESTO*)

Temperature governs a wide variety of cellular processes. However, the detail and significance of intracellular temperature are still unclear. In this study, we focused on the intracellular temperature during stress. In eukaryotic cells, cytoplasmic mRNAs assemble and form stress granule (SG), responsible for translation regulation. Given the results that arsenite stress, SG inducer, targeted mitochondria, we hypothesized that the temperature change concerning mitochondria in the cell under stress might involve in the stress response signaling. Here we demonstrated that SG formation had intrinsic relationship with local temperature change, which may be a novel mechanism of this phenomenon.

3P312 蛍光性ポリマー温度センサーを用いた生細胞内における発熱のイメージング

Imaging of thermogenesis in living cells using fluorescent polymeric thermometer

Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Grad Sch Pharm Sci, Univ Tokyo*, [2]*JST, PRESTO*)

Intracellular temperature distribution reflects cellular thermodynamics and function. We previously demonstrated monitoring and imaging of intracellular temperature based on a fluorescent polymeric thermometer and quantitative fluorescence imaging techniques, showing non-homogeneous temperature distribution in living cells. Here, we have investigated intracellular thermogenesis to understand the detailed property of temperature change. The local temperature change provoked by both endogenous heat production from mitochondria and external heating using infrared laser was examined. The results showed the local temperature variation inside living cells, suggesting an important contribution to cell functions.

3P313 マイクロ波照射微生物における至適出力と至適温度
Optimal microwave energy and optimal temperature on microwave irradiated microbial cultivation

Ryota Nakama[1], Wataru Nagayoshi[1], Takeo Yoshimura[2], Makoto Kodama[3], Shokichi Ohuchi[1] ([1]*Dept. Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Bio. Sci. Tokyo Univ. Sci.*, [3]*Vessel inc.*)

We have utilized microwave heating for cultivating microorganisms. Increase in the number of viable cells and the growth rate was observed by cultivation of Escherichia coli under microwave heating. By scanning electron microscopy, the microwave energy sensitivity different from the real temperature had been observed from the shape of the cells. In this study, cultured in a microwave heating Bacillus subtilis and E. coli, it is compared with normal culture was investigated various temperature and microwave output energy under microwave irradiation condition. As a result, we have confirmed the presence of the optimal microwave output energy for cell cultivation.

3P314 知能ロボットを用いたヒトとのインターフェース
Intelligence for Robot-Human Communication

Jun Miyake, Amalia Istiqlali Adiba, Nobuyuki Tanaka (*Graduate School of Engineering Science, Osaka University*)

The intelligence of robot is a key factor in the communication with human being. We study a separate-type arm/hand for disabled persons, with the method to share the cognition: 1) pointing the object by eye-tracker etc, 2) the robot extracts the meaning of the object by its shape by neural network. The robot has a certain intellectual capability to understand the object and execute the demand given by its interpretation. The result indicated that the robot communicates with human by sharing the cognition. The human-human communication could be a model. This study is useful for understanding the nature of communication and modeling the intellectual behavior. The role of the intelligence leads us a reconsideration of the difference between human and robot.

3P315 マイクロ流体デバイスを用いた三次元モデルにおけるグリオーマ幹細胞と分化誘導グリオーマ細胞の浸潤形態比較
Comparing of invasion form between glioma stem cells and the differentiated cells in a microfluidic 3D culture system

Sotaro Taki, Shingo Fujioka, Ryo Sudo (*Sci and Tech., Univ. Keio*)

Aims: To investigate the invasion process of glioma, we demonstrated the difference of glioma invasion form and invasiveness between the glioma stem cells and the differentiated cells.
Methods: Microfluidic devices were made by using a soft lithography technique. A mixed-gel solution was injected into a center flow channel. Glioma cells were injected into upper side channel. This invasion process was compared between the stem cells and differentiated cells.
Results and Discussion: We found that stem cells had high invasiveness with single cell invasion form while differentiated cells had low invasiveness with collective cell invasion form. This result suggested that glioma stem cells have a potential to play a role of the leader cell in the process of invasion.

3P316 らせん構造を有する異方性マイクロゲルファイバーの作製と制御
Control synthesis of anisotropic hydrogel microfiber with helical structure

Shoya Yasuda[1], Masayuki Hayakawa[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech., Japan*, [2]*PRESTO, JST, Japan*)

Hydrogel microfibers have been attracting much attention as soft biocompatible material. The formation method of homogeneous and Janus microfibers has been reported; however, fibers with helical structure cannot be formed by conventional method like microchannels. Here we report a method for the synthesis of anisotropic helical hydrogel microfiber. We used a centrifuge-based fiber extruding device and a centrifugal mixer that generates axial spin and orbital revolution. We constructed a method of controlling diameter and helical pitch of fibers. Furthermore, multiple-helical fibers were also formed successfully. We hope our helical fiber will achieve higher-order functions characteristic of its three-dimensional structure: twisting, expanding and contracting, etc.

3P317 Self-assembly of complex-shaped microgels

Satoshi Umeyama, Masayuki Hayakawa, Masahiro Takinoue (*Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology*)

Recently, magnetically functionalized microparticles attract much attention due to their unique properties including self-assembly, and remote controllability with an external magnetic field. Using complex-shaped microparticles, those properties are expected to be extended to self-assembled smart materials, drug delivery, and bioassay, etc. However, only self-assembly using simple-shaped particles (ex. sphere, ellipse, etc.) has been studied up to date. Here, we propose self-assembly of complex-shaped magnetic microparticles (ex. two-thirds of sphere, propeller, etc.) using a magnetic field. We believe that our study will contribute to understanding of dynamic self-assembly of microparticles.

3P318 温度感受性ゲルを用いた蛍光検出によるドロップレットソーティング技術の開発とその応用
Development of fluorescence-activated droplet sorting system using thermoreversible gelation polymer and its application

Haruka Okada[1], Ryo Iizuka[1], Ayaka Iguchi[2], Dong H. Yoon[2], Tetsushi Sekiguchi[3], Shuichi Shoji[2], Takashi Funatsu[1] ([1]*Grad. Sch. Pharm. Sci., Univ. Tokyo*, [2]*Major in Nanosci. and Nanoeng., Waseda Univ.*, [3]*Nanotech. Research Center, Waseda Univ.*)

We have developed a fluorescence-activated microfluidic droplet sorting system using thermoreversible gelation polymer (TGP) as a switching valve. In this system, water-in-oil droplets are converted into water-in-oil-in-water droplets, hydrodynamically focused by two buffer solutions containing TGP, and actively sorted based on their fluorescence. Using the sorter, fluorescent droplets were separated from non-fluorescent droplets with accuracy and purity better than 90%. As an application of the system, we have demonstrated the isolation of the small subcellular structure (human artificial chromosome; HAC) from crude cell homogenate. Now, we are trying to understand the structure of HAC by mass spectrometry-based proteomic analysis from HAC in collected droplets.

3P319 Selection of RNA aptamers to develop a sensor using rhodamine as a fluorogenic probe

Tara Bahadur KC[1,2], Hiroshi Abe[3], Yoshihiro Ito[1,2], Uzawa Takanori[1] ([1]*Emergent Bioengineering Materials Research Team, RIKEN*, [2]*Tokyo Metropolitan University*, [3]*Faculty of Pharmaceutical Sciences, Hokkaido University*)

Fluorescence signal generation and amplification is a powerful tool to sense a target molecule in bacteria and eukaryotic cells. As a fluorogenic probe we have used rhodamine 110 derivatives; reduction of rhodamine azide (Rh Az) to rhodamine amine (Rh Am) generates fluorescence. Aiming for achieving fluorescence amplification of the rhodamine system, here we propose a system in which Rh Az is captured but, after its reduction, fluorescent Rh Am is released. To realize this system, we selected RNA aptamers that specifically bind to Rh Az but not to Rh Am. After six rounds of selection, 48 different RNA sequences were obtained in which the highest repetition of eight was seen in two sequences. Binding affinity and specificity of those aptamer candidates will be evaluated.

3P320 Control of a DNA computer-based gene-regulatory module confined in a giant unilamellar vesicle by external molecular signal

Toru Nishikata[1,2], Takamasa Hasegawa[1,2], Yutetsu Kuruma[3], Koichiro Shoda[1,2], Akira Suyama[1,2] ([1]*Dept.Life Sci., Univ.of Tokyo*, [2]*Suyama Lab*, [3]*Earth Life.,Tokyo inst of tech*)

We have constructed a DNA computer-based gene regulatory module using RTRACS, which is a DNA computing system composed of enzymatic reaction modules communicating with each other through RNA strands. The constructed module was to regulate gene expression using two input RNA sequences. Only when both input RNA sequences are received, the module can transcribe mRNA strands. We confined this module in a giant unilamellar vesicle (GUV) with a cell-free protein synthesis system, and demonstrated that the gene expression was regulated with the two input RNA added to the GUV in advance. Here, we report a result of our next-step experiment on control of the module in a GUV by external molecular signal. This experiment is an important step toward the GUV-based synthetic biology.

3P321 進化分子工学に向けた酵素スクリニーングシステムの開発：酵素合成及び活性測定の試み
Development of enzyme screening system for directed evolution based on enzymic activity

Yi Zhang, Hiroto Kizoe, Ryota Iino, Kazuhito Tabata, Hiroyuki Noji (*Department of Applied Chemistry, School of Engineering, The University of Tokyo*)

There is an urgent need to establish a screening system that combines protein synthesis and activity measurement for directed evolution of enzymes. Previous studies based on w/o emulations were always limited by either flexibility or throughput of the system, and such limitations could be overcome by using droplet-based system. We proposed an approach to fabricate a chamber array device consisting of million chambers with uniform diameter and depth of several microns in which single molecules can be encapsulated. We applied this device to protein synthesis by introducing cell-free expression system. We synthesized β-galactosidase and monitored the activity from single droplet. Our system holds great promise for the creation of new enzymes with improved activities.

3P322 シリカバイオミネラリゼーションを行う新奇人工ペプチドの設計
The design of novel artificial peptide revealing a silica biomineralization activity

Yoshinao Murakami, Yuki Kimura, Ippei Fujiyama, Yusuke Matsuda (*Grad. Sch. Sci., Kwansei Gakuin Univ.*)

Silaffin in diatom shell have clustered positive charges, suggesting that the positively charged macromolecules are polymerize silicate. Given this hypothesis, we designed artificial silica forming peptides based on a partial secondary structure of a diatom chloroplastic carbonic anhydrase, PtCA1. Lysine or arginine were introduced in the C-terminal helix of PtCA1. This peptide polymerized silicate and formed several hundred nm of spherical silica. Solvent polarity changed by the addition of alcohol effect the formation of plate-formed or clumping biosilica. The reaction at the boundary layer of different polarity solvent also formed sheet shaped silica. Tandemly linked peptides produced bigger spherical silica although any change in the biosilica shape was observed.

3P323 マイクロ波照射 PCR 反応の DNA ポリメラーゼの変性
Denaturation of DNA Polymerase on Microwave Assisted PCR

Shokichi Ohuchi[1], Takeo Yoshimura[2], Hiroya Osoekawa[1] ([1]*Biosci. & Bioinform., Kyushu Inst. Tech.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. Sci.*)

We had already demonstrated the microwave heating for the processes of the thermal denaturation and the enzymatic elongation reaction. For example, ExTaq polymerase was decreased the fluorescence intensity by the microwave irradiation. On the other hand, Vent polymerase was shown same intensity of fluorescence between the microwave irradiation and the conventional condition. The ExTaq polymerase showed the different phenomena between the microwave irradiation and the conventional heating in spite of same temperature. This fact might mean a new state of protein denaturation, what we would call “Microwave Denaturation”. In this study, microwave energy and the real temperature will be discussed.

3P324 細胞体におけるミトコンドリア移動の評価法の開発
Development of the method to evaluate mitochondrial movement in the soma

Yuki Sugimoto, Yoshihiro Ohta (*Grad. Sch, Life Sci, TUAT*)

Mitochondrial movement is thought to play an important role for cellular activities. However, since it has been measured mainly in the neurite, the role of the movement has not been clarified in many types of cells. In present study, we aimed at developing the method to evaluate the mitochondrial movement in the soma by time-lapse imaging. To observe the movement, C6 glioma cells were stained with Mito-Tracker Red and were observed with fluorescence microscopy at intervals of 3 s. The movements were significantly dependent on the cells, but they did not change during the observation for 1 min. The movements at 37℃ were more active than those at 25℃. Additionally we would like to report the effect of mitochondrial inhibitors on the movements.

3P325 **異種生物種由来ヘムオキシゲナーゼによるヘム分解反応の微調節：反応の pH 依存性と電子伝達速度**

Fine-tuning of the heme degradation by heme oxygenase from variable biological species: pH dependence and the electron transfer kinetics

Catharina T Migita, Norio Miyake (*Faculty of Agriculture, Yamaguchi Univ.*)

Heme oxygenase (HO) catabolizes regiospecific heme degradation by use of molecular oxygen and electrons in a variety of organisms. The HO reaction is related with many physiological processes: not only iron recycling but also releasing a signaling molecule, producing biliverdin, antioxidant and the starting material of photoreceptive molecules. To clarify the strategy for HO fine tuning employed by different biological species, we have examined several factors affecting HO activity. Here, effects of pH and of electron transfer efficiency on the HO reaction were examined. The apparent heme degradation rate varied depending on pH between 6 and 9, uniquely, by HOs with different origins of mammal, fish, plant, and cyanobacteria.

Name Index (索引)

名字（Family Name）のアルファベット順にソートしています。すべて、オンラインで入力されたデータのまま、表示しています。

Name Index

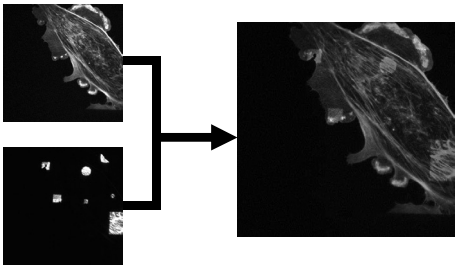
第52回日本生物物理学会年会

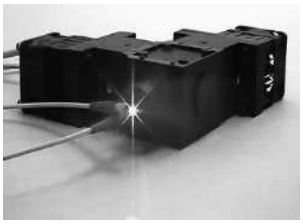
オプトライン ランチョンセミナー

ハイパワーLEDが拓く
マルチモダリティ オプトジェネティクスシステム

日　時　9月25日（木）12：30～ 13：20

会　場　A会場（札幌コンベンションセンター 中ホールA）





演　者

永井　健治　先生　　大阪大学産業科学研究所　　教授

マルチカラーオプトジェネティクスによる細胞機能の多元操作と
化学発光膜電位センサーによる高速計測

演　者

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日本生物物理学会第 52 回年会　共催ランチョンセミナー

自発的ブリンキング機能を有する超解像イメージングプローブの開発

東京大学医学系研究科 生体物理医学専攻医用生体工学講座 生体情報学

神谷 真子 先生 、 浦野 泰照 先生

日時 2014 年 9 月 25 日（木）12:30～13:20

会場 札幌コンベンションセンター 中ホールB

超解像イメージング法の一種である PALM/STORM は、蛍光分子を確率的に光らせ、その位置を決定することで、数 10 nm の空間分解能の画像を取得する手法である。しかし、市販の蛍光色素を用いる場合、チオール（還元剤）や GLOX（酸素除去剤）、高強度のレーザー照射が必要である場合が多く、生細胞イメージングには適していない。そこで我々は、光化学的な観点から蛍光色素の特性を最適化することで、汎用性の高い超解像イメージングプローブの開発を行った。具体的には、分子内求核基を有するローダミン誘導体が pH に応じて無色・無蛍光の閉環体と吸収・蛍光を示す開環体の構造を取ることに着目し（分子内スピロ環化平衡）、平衡定数及び熱的な閉環速度を最適化することで、自発的なブリンキング機能を有する蛍光プローブを開発した。開発したブリンキングプローブの特性を蛍光顕微鏡下で評価した結果、添加物やレーザー強度に依らずブリンキングすることが明らかになった。さらに、開発したプローブを用いて、プラスミド DNA 上の RecA フィラメントや固定／生細胞における微小管の超解像イメージングを達成した。

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第５２回日本生物物理学会年会

株式会社菱化システム ランチョンセミナー

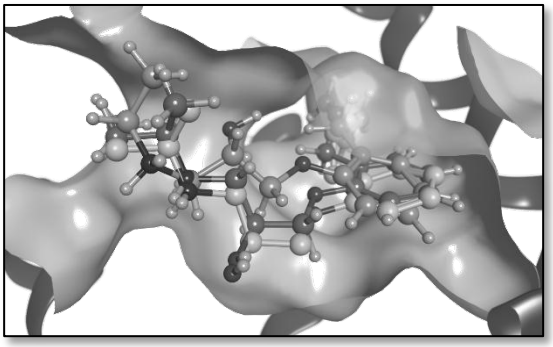
統合計算化学システム MOE によるタンパク質ーリガンド相互作用解析

日時： ９月 25 日（木） 12:30～13:20
場所： C 会場（107 会議室）

MOE を活用したドッキング計算支援

独立行政法人産業技術総合研究所　創薬分子プロファイリング研究センター　広川 貴次

タンパク質立体構造に対する化合物のドッキング計算において、プレ・ポストドッキング解析は、ドッキング計算精度を向上させる重要な手続きである。例えば、アポ構造に対するドッキング計算では、化合物の結合部位の同定が困難なケースが多く、プレドッキング解析による結合部位予測が重要となる。また、ポストドッキング解析においては、分子動力学計算による計算結果を解析するために相互作用フィンガープリントなどが活用されている。本発表では、MOE の SiteFinder や Protein Ligand Interaction Fingerprint 機能を用いた実施例を紹介する。



ヒト β２アドレナリン受容体（PDB:3NYA）に対するアンタゴニスト（alprenolo）のドッキングシミュレーション例

MOE のタンパク質ーリガンド結合状態の特徴づけ機能の紹介

株式会社菱化システム 科学技術システム事業部 木村 嘉朗

MOE では、タンパク質とリガンドの結合状態の様々な特徴付けを行うことができます。例えば、リガンドが結合している部分のタンパク質表面を描画し、静電ポテンシャル、水素結合性などに基づいて色付けできます。また、溶媒原子の確率密度や溶媒和自由エネルギーなどの分布を可視化することで、溶媒分子の局在する領域を予測できます。本セミナーでは、これらの特徴づけ機能とともに、溶媒原子の確率密度から水分子の位置を予測する最新機能を紹介します。

Ryoka Systems Inc.
株式会社菱化システム
科学技術システム事業部
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DYNAMIC BIOSENSORS

DKSH

第52回日本生物物理学会年会

DKSHジャパン株式会社 ランチョンセミナー

日時：9月25日（木）12:30 – 13:20　会場：D会場(108会議室)

DNA ナノレバーを用いた新規バイオセンサーテクノロジー SwitchSENSE 法による分子の形状変化と相互作用解析

Analysis of molecular interactions and biophysical properties with switchSENSE - a chip based platform with electroswitchable nanolevers

Dr. Ralf Strasser　Dynamic Biosensors GmbH, Munich, Germany

Measurements in stationary or mobile phases are fundamental principles in protein analysis. Although the immobilization of moleculeson solid supports allows for the parallel analysis of interactions, properties like size or shape are usually inferred from the molecular mobility under the influence of external forces. However, as these principles are mutually exclusive, a comprehensive characterization of proteins usually involves a tedious multi-step workflow. Here we show how these measurement modalities can be reconciled by tethering proteins to a surface via dynamically actuated nanolevers. Short DNA strands, which are switched by alternating electric fields, are employed as capture probes to bind target proteins. By swaying the proteins over nanometer amplitudes and comparing their motional dynamics to a theoretical model, the protein diameter can be quantified with Angstrom accuracy. Alterations in the tertiary protein structure (folding) and conformational changes are readily detected, and even post-translational modifications are revealed by time-resolved molecular dynamics measurements [1]. Moreover, we demonstrate the analysis of protein interactions with exceptional sensitivity, i.e. the quantification of dissociation constants in the femtomolar concentration regime. Real-time measurements yield association and dissociation rate constants, and we discuss the artefact free characterization of high-affinity interactions like the binding of monoclonal antibodies to antigens. Since the capture probe surface density can be adjusted through an electrical desorption process, the interaction of multivalent analytes with the sensor surface can be can be evaluated which directly discloses the occurrence of avidity effects [2]. The thermal stability (denaturation) of proteins in the p resence or absence of co-factors can be monitored in parallel and melting temperatures are quantified.
Finally, we discuss the possibilities and limitations of utilizing electro-switchable DNA nanolevers in the format of a 24x parallel microelectrode chip for the high-content analysis of molecular interactions.

[1] Nature Communications 4:2099 (2013)
[2] J. Am. Chem. Soc. 134, 15225 (2012).

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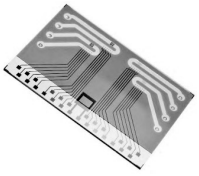
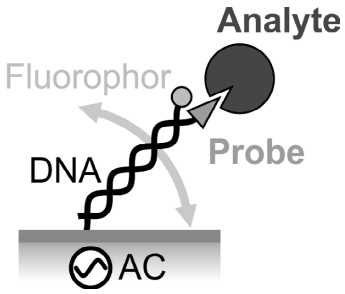


4×6 Chip

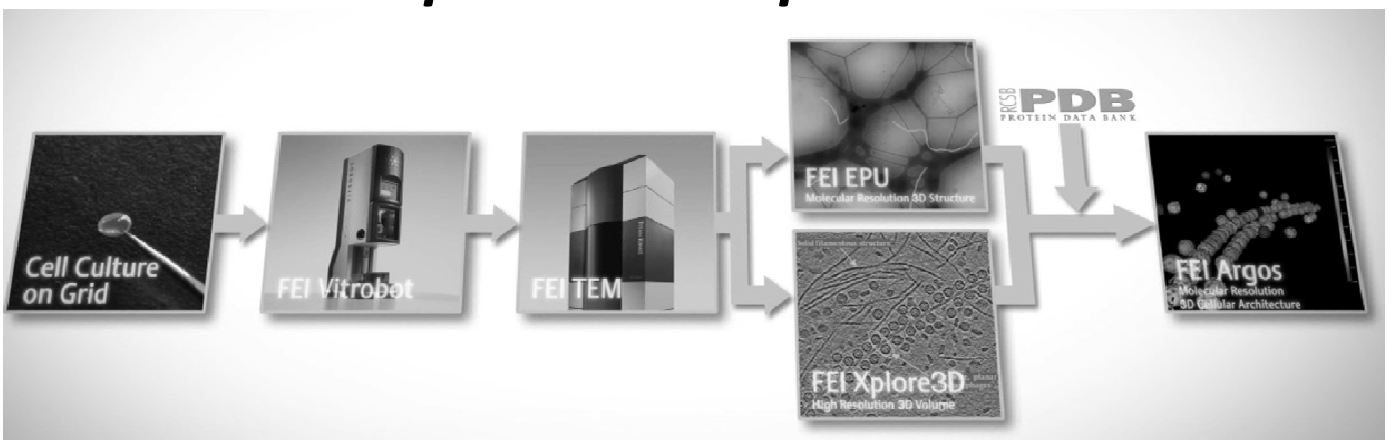


Switch SENSE Principle

DKSHジャパン株式会社
テクノロジー事業部門 科学機器部
Tel : 03-3767-4510　Fax :03-3767-4569
e-mail: tp.labtyo@dksh.com

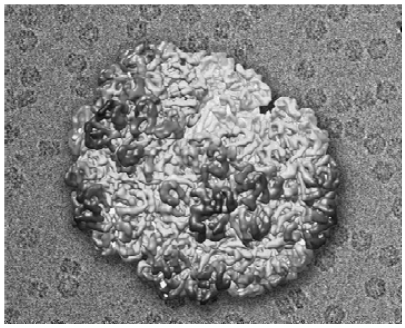
第52回日本生物物理学会年会ランチョンセミナー

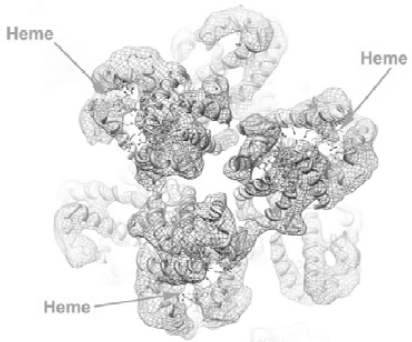
Breakthrough of cryo-TEM for high resolution structural analysis of protein complexes

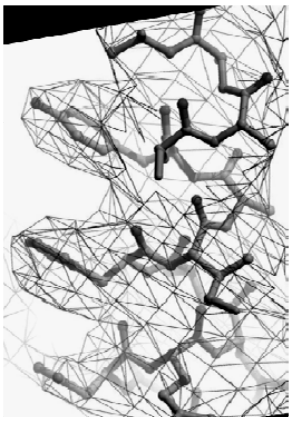


- 開催日時: 2014年9月26日 (金) 11:45 – 12:35
- 会場: C会場 (107会議室)
- 演者: Dr. 葦原 雅道 (Product Marketing Engineer, Life Science Market, FEI)
- 要旨:

クライオ電子顕微鏡法は近年、急速な進展を遂げた。ウィルスやリボソーム等分子量の大きい超分子複合体は電子顕微鏡の密度マップのみから原子モデル構築が可能な分解能で構造解析が可能である。さらに、従来解析が困難であった分子量数100 kDa程度の複合体や膜タンパク質もサブナノメートル分解能で構造解析がルーティンに行えるようになった。
クライオ電子顕微鏡法の高分解能化により、タンパク質複合体の構造解析手法としてX線結晶構造解析法やNMR法と相補的に用いる相関構造生物学が主流となりつつある。
本セミナーでは、クライオ電子顕微鏡法の実験の流れから技術的革新まで構造解析例とともに紹介する。







L. terrestris haemoglobin Courtesy of S. De Carlo and M. van Heel, NeCEN, Netherlands

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日時：9 月 26 日（金）　11：45ー12：35　　Time and Date : Sep 26th (Fri) 11:45-12:35
会場：Ｄ会場　　Venue : Room D

ヒューマン・フロンティア・サイエンス・プログラム機構（フランス・ストラスブール）
グラント部門部長　ジェフ・リチャード

ＨＦＳＰ（ヒューマン・フロンティア・サイエンス・プログラム）は、ライフサイエンス分野における革新的な国際共同研究を推進するため、1989 年に創設された国際プロジェクトです。本プログラムでは、基礎的な生物機能を解明するため、広く異なった分野の専門知識を融合することに重点をおいています。研究対象とする生物機能としては、細胞構造における詳細な分子状態から、神経システム科学における複雑な相互作用にまで及びます。ＨＦＳＰがサポートするフロンティアサイエンス分野には、情報生命科学者や生物物理学者らの参画も次第に一定規模を占めるようになっている中で、物理学分野からの参画もまた多く見られます。

今回の講演の中では、ＨＦＳＰの共同研究グラントプログラムについて、応募申請時におこしがちな失敗や、採択されやすい事例についての説明を交えながら意見交換をします。課題解決のための様々な専門領域や科学文化による「新規性」や革新的発想を備えた研究チームが、好結果を挙げています。

Geoff Richards　Director of Research Grants
The Human Frontier Science Program Organization, Strasbourg, France

HFSP (the Human Frontier Science Program) was established in 1989 so as to encourage innovative international collaboration in the life sciences. An emphasis is made on combining widely different expertise to understand fundamental biological mechanisms. These mechanisms can range from the finest molecular details of cellular structure to complex interactions in systems neuroscience. In the frontier science supported by HFSP, the biology is increasingly quantitative with contributions from computational biologists and biophysicists although other contributions from the physical sciences are also common.

In this talk we will discuss the HFSP collaborative grant programs, explaining the common errors in applications and the kind of project that is likely to win support. Successful teams take a ‘new look’ and bring novel ideas from different disciplines and scientific cultures together to solve a problem.

The International Human Frontier Science Program Organization (HFSPO)
12, Quai Saint-Jean - B.P.10034　67080 Strasbourg-cedex, FRANCE
http://www.hfsp.org　　Specific enquiries to grant@hfsp.org

文部科学省高性能汎用計算機高度利用事業

HPCI 戦略プログラム　分野1「予測する生命科学・医療および創薬基盤」

スーパーコンピューティング
- 計算科学と情報科学の接点 -

日　時：2014 年 9 月 26 日（金） 11:45 – 12:35
会　場：E 会場（204 会議室）

生命は細胞に始まります。しかし、その 10^{-15} から 10^{-11} リットルという極めて小さな細胞ですら、その振る舞いを再現することは極めて難しいです。そのため従来の計算科学では、タンパク質という分子に限定するか、細胞の詳細を見ずに連続体として扱うことでシミュレーションを行ってきました。しかし、それだけでは、生物学の本質的問題の解決にはなかなかつながりません。生物学の世界ではどうしても、恐ろしく複雑で不均質な細胞を見ていく必要があります。近年、生物学の世界では、ゲノム情報に加えて、計測技術の飛躍的進歩により、個体レベルから一細胞レベルまで、日常的に莫大な実験データが蓄積されてきています。今情報科学では、それらビッグデータから効果的に知識を取り出す必要にせまられています。これら2つの必要性に応えていく一つの手段としてスーパーコンピューティングの役割を考えます。ここではスーパーコンピューティング、計算科学、情報科学の接点における最新の研究成果と将来の方向性について紹介します。

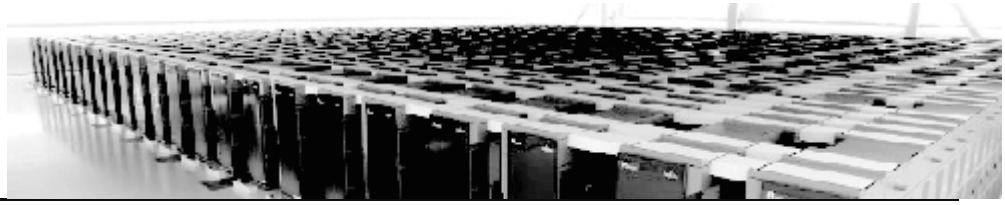
演者 1：「京」コンピュータは生命科学の何をどこまで解決できるのか

木寺 詔紀（理化学研究所 HPCI 計算生命科学推進プログラム・副プログラムディレクター／横浜市立大学生命医科学研究科・教授）

演者 2：データ駆動型生命情報科学と計算科学との接点を探る

木下 賢吾（東北大学 大学院情報科学研究科・教授／東北メディカル・メガバンク機構 ゲノム解析部門・副部門長）

司　会：江口 至洋（理化学研究所 HPCI 計算生命科学推進プログラム・副プログラムディレクター）



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PDBj：Protein Data Bank Japan
（日本蛋白質構造データバンク）

Modifications to the Protein Data Bank: A new PDB format, Data Deposition, and Validation Report

Genji KURISU, Institute for Protein Research, Osaka University

The Protein Data Bank Japan (PDBj, http://pdbj.org) is a member of the worldwide Protein Data Bank (wwPDB, http://wwpdbj.org/) and accepts and processes the deposited data of experimentally determined macromolecular structures. While maintaining the archive in collaboration with other wwPDB partners, PDBj also provides a wide range of services and tools for analyzing structures and functions of proteins, which are summarized in this article. The wwPDB has recently decided to change its traditional “PDB format” in a plain text style to the “PDBx/mmCIF format, because of very many limitations in the “PDB format”, such as limited chain and atom numbers, no bond order or chirality for ligands. We will discuss what are the differences, and how we are going to move to use the new format and its derived formats, PDBML and PDB/RDF, which are considered to be useful for data integration. Other important news from wwPDB is an opening of new annotation system for data deposition. This new annotation system enables workload balancing of data centers, increased productivity and better quality assurance of ligand chemistry and polymer sequences. We will also discuss about the validation report that summarizes quality of the deposited X-ray data and overview of the residue-based structural quality for every polymer. It will be used for reviewing papers describing macromolecular structures.

Browsing, searching, and comparing 3D electron microscopy data.

Hirofumi SUZUKI, Institute for Protein Research, Osaka University

Structure data derived by 3D electron microscopy (3DEM) deposited on PDB and EM Data Bank (EMDB) are increasing. We have been provided services for the 3DEM data. *EM Navigator* is a data explorer 3DEM data in both databanks. *Yorodumi* is interactive 3D structure viewer running on PCs and some mobile devices. While it is designed to see complex structures such as 3DEM data, all the PDB data and chemical component data also can be viewed. Recently, we start a new service, Omokage search, which is low-resolution shape similarity search system. Users can find structure data having similar shapes to users query structure from both of EMDB and PDB. Atomic models, 3D density maps, and SAXS bead models can be applied as a search query. Found 3DEM structure pair sharing shapes can be fitted by *gmfit*, and viewed interactively within web browsers. In the seminar, recent changes of PDB/EMDB format related to 3DEM in addition to our services will be introduced.

Tools for highly automated NMR analysis and applications using database

Naohiro KOBAYASHI, Institute for Protein Research, Osaka University

Owing to the development of NMR analysis, highly automated methods have been available for NMR signal assignments and structure determination. The database for structure and experimental data has been growing so fast significantly raising the possibility to find homologues for the target macromolecule in the database. Nowadays we can apply homology modeling to NMR analysis.

We have recently developed a stand-alone system to expedite the tasks of NMR data analysis. The system is composed of a number of modules including graphical user interface (GUI) modules integrated by MagRO-Core and external programs such as a program for fully automated NOE assignments and structure determination (CYANA) and fully automated NMR signal assignments (FLYA). The modules for setting up FLYA and CYANA calculations are including fully automated peak picking, noise filtering and conversion of file format. Using the modules, the user can easily prepare peak lists for a wide variety of NMR spectra. For the calculations of FLYA and CYANA, the modules for importing the calculation results into the MagRO system support user to readily correct and assign remaining NMR signals. In the lecture, the procedure for analysis of the samples showing very broad signals will be shown, demonstrating that successful NMR signal assignments and structure determination can be performed using the structure generated by homology modeling.

PDBj: Protein Data Bank Japan
http://pdbj.org

Institute for Protein Research, Osaka University,
3-2 Yamadaoka, Suita, Osaka 5650871, JAPAN
TEL: +81-6-6879-4311

第52回　日本生物物理学会年会

SPring-8／JASRI　ランチョンセミナー

日時：9月27日（土）12:30～12:55

場所：C会場／107会議室

講演者：SPring-8/JASRI　熊坂　崇、関口　博史

大型放射光施設SPring-8は、世界最高性能の放射光を利用することができる大型の実験施設であり、国内外の研究者等に広く開かれた共同利用施設として、幅広い分野の研究に用いられています。生物物理学の研究分野では、タンパク質の結晶解析をはじめとして、溶液中での構造変化を探るためのＸ線小角散乱解析、筋肉やアミロイド繊維などを対象とする繊維回折実験、組織を非破壊的にイメージングするＸ線コンピュータ断層撮影（Ｘ線CT）、微結晶を利用した１分子計測などに利用されています。

特に、タンパク質の結晶回折測定には、BL41XU（アンジュレーター）、BL38B1（偏向電磁石）の2本の共用ビームラインの他、BL32XU（アンジュレーター）、BL26B1/BL26B2（共に偏向電磁石）の3本の理研ビームラインのビームタイムを提供し、多様な試料や測定手法に対応しています。

ご利用に際しては、原則として春秋年2回行う課題募集にて、実験内容を申請していただきます。ただし、タンパク質結晶回折測定用途では、毎月応募可能なビームタイム枠も設けています。また、測定に慣れた方向けには、SPring-8に来なくても測定のできる遠隔測定システムも用意されています。

今回、 SPring-8の運転・維持管理及び利用支援を行っている公益財団法人高輝度光科学研究センター(JASRI)では、生物物理学の研究にさらに利用していただくため、施設の取り組み・研究内容・利用方法などをご紹介するランチョンセミナーを開催します。奮ってご参加ください。

Japan Synchrotron Radiation Research Institute

（公財）高輝度光科学研究センター

http://www.spring8.or.jp/ja

第52回日本生物物理学会年会（BSJ2014）

オリンパス株式会社 ランチョンセミナー 3LE

日 時

9月27日［土］ 12:30～13:20

会 場

札幌コンベンションセンター　204会議室（E会場）

演 者

茅 元司 先生　　東京大学大学院理学系研究科 物理学専攻生物物理

筋の収縮動態を生体レベルから分子レベルというマルチスケールで研究に取り組む東京大学大学院理学系研究科 茅 元司助教。
本セミナーでは、2010年に米国科学誌「Science」で報告した内容をはじめ、近年取り組んでいる顕微鏡を使った生体レベルでのアクチンとミオシンの相互作用について紹介いただく予定です。

※　演題等、セミナー詳細については、学会年会にてご案内します。

※ オリンパス㈱ 顕微鏡技術開発者の企業プレゼンテーションも予定しています。

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   E-mail bpsjp@biophys.jp
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6. 日本生物物理学会の www ホームページ
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本誌記事の動物実験における実験動物の扱いは,
所属機関のルールに従っています.

生物物理 SEIBUTSU BUTSURI
日本生物物理学会 THE BIOPHYSICAL SOCIETY OF JAPAN

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