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生物物理

SEIBUTSU BUTSURI

SUPPLEMENT 1-2

Vol.53

第 51回年会講演予稿集

2013.10.28（月）～ 30(水)

国立京都国際会館

主催　日本生物物理学会

日本生物物理学会 THE BIOPHYSICAL SOCIETY OF JAPAN http://www.biophys.jp

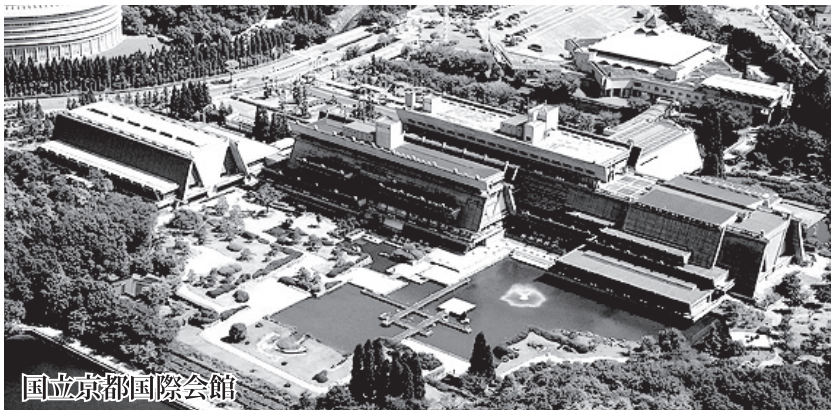
The 51st Annual Meeting of the Biophysical Society of Japan

日本生物物理学会第51回年会（2013年度）
ご案内

会 期： Date：	2013年 10月28日（月）ー 30日（水） October 28（Mon）ー 30（Wed）, 2013
会 場： Venue：	国立京都国際会館 （京都市左京区宝ヶ池） Kyoto International Conference Center （Takaragaike, Sakyo-ku, Kyoto 606-0001 Japan）
年会実行委員長： Chair：	七田 芳則 （京都大学大学院 理学研究科） Yoshinori Shichida （Kyoto University）

HOMEPAGE　http://cls.kuicr.kyoto-u.ac.jp/bsj2013/

※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。


国立京都国際会館

抄録本文は

こちらからダウンロードいただけます。

http://www.biophys.jp/dl/pro/51st_proceedings.pdf

ID：　ambsj51

パスワード：　kyoto2013

※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。
（2013年10月24日（木）公開予定）

編集・発行 ： 日本生物物理学会第51回年会実行委員会

日本生物物理学会第51回年会事務局
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日本生物物理学会第51回年会 実行委員会

年会実行委員長　　七田 芳則（京都大学大学院 理学研究科）

実行委員

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吉川　研一（同志社大学大学院 生命医科学研究科）

※50音順。敬称略。

The 51st Annual Meeting of the Biophysical Society of Japan

日本生物物理学会第51回年会（2013年度）

開催にあたって

第51回年会 実行委員長
七田 芳則
（京都大学大学院 理学研究科）

日本生物物理学会第51回年会を2013年10月28日から30日の日程で、国立京都国際会館で開催します。年会では、来年IUPABを開催するオーストラリア生物物理学会との共催を含め24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。京都で年会を開催するのは2004年以来9年ぶりであり、例年とは異なり10月末に開催するため、講義などのDutyをもっておられる方には少し不便をおかけしたかも知れません。一方、この時期の京都は気候が非常によく、ポスター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。

京都年会では、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。一方、一般発表をすべてポスターとして、ポスターに英語と日本語が併記出来る環境を作り、Abstractの字数を少なくすることも行いました。このことにより、ともすれば異なる分野間での理解度が落ちるという弊害をなくし、また、Abstractにすぐ結論が出てくるようなトピック的な記述で異なる分野の研究者にもアピールすることを期待しています。さらに、シンポジウムは会員からの応募をすべて受け付けるとともに、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会員の皆様が容易にアクセス出来るように考えました。

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。京都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、京都は町中がすべて観光地ですので、滞在時間を有効に利用されて、京都を楽しまれることも期待しています。

交通のご案内

■ 飛行機でお越しの方

関西国際空港 → JR特急「はるか」(75分) → JR京都駅
関西国際空港 → リムジンバス(95分) → JR京都駅
大阪空港 → リムジンバス(55分) → JR京都駅
JR京都駅 → 地下鉄烏丸線(20分) → 国立京都国際会館 Kyoto International Conference Center

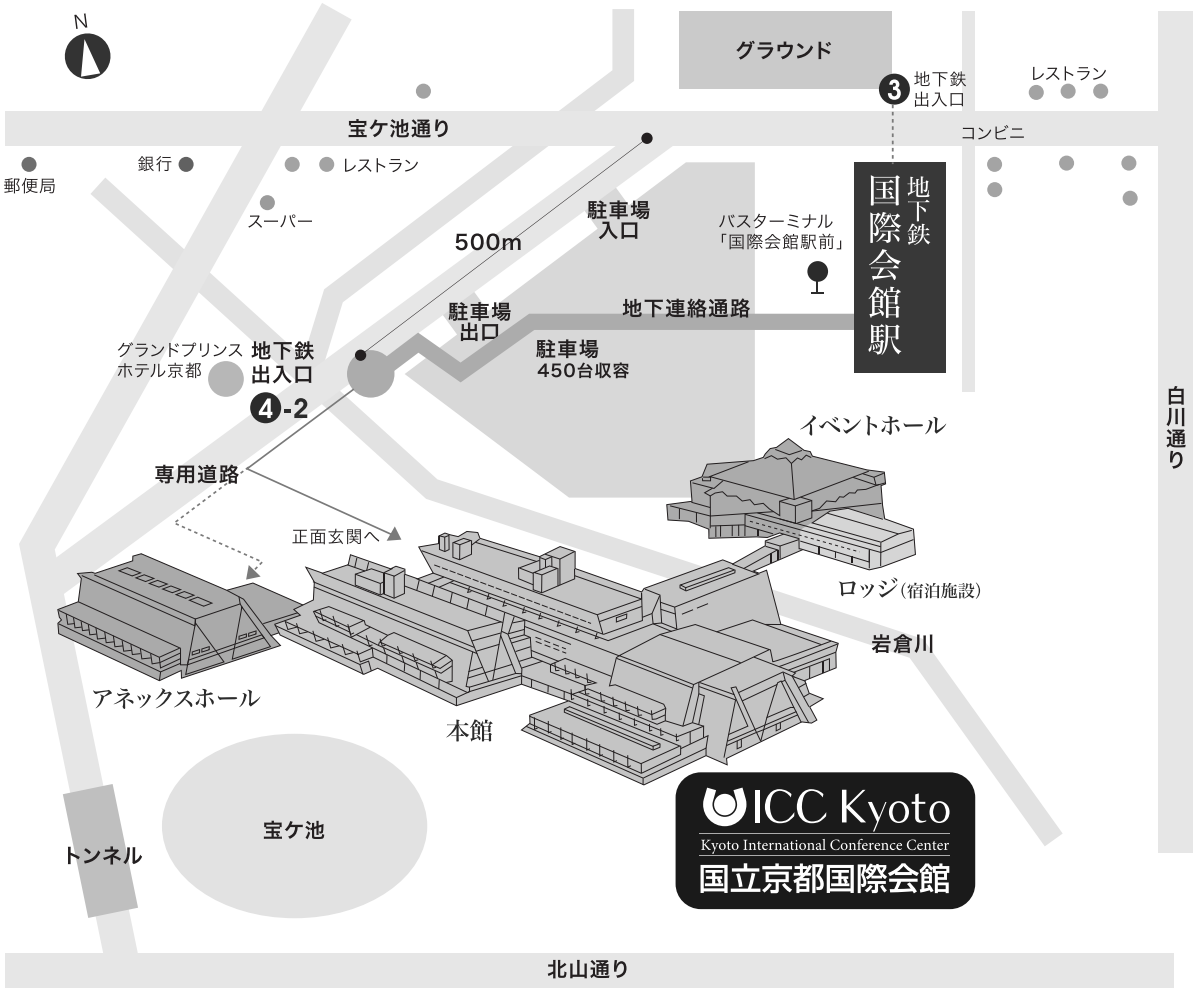
市営地下鉄烏丸線
「国際会館駅」下車

■ 電車でお越しの方

東京・中部方面より → 東海道新幹線 → JR京都駅
九州・中国方面より → 山陽新幹線 → JR京都駅
JR京都駅 → 地下鉄烏丸線(20分)（京都駅より20分・四条駅より16分） → 国立京都国際会館 Kyoto International Conference Center
大阪・神戸方面より → 阪急京都線 → 四条烏丸駅 → 地下鉄烏丸線(20分)（京都駅より20分・四条駅より16分） → 国立京都国際会館 Kyoto International Conference Center
大阪・神戸方面より → 京阪本線 → 京阪三条駅 → 地下鉄東西線・烏丸線(20分) → 国立京都国際会館 Kyoto International Conference Center

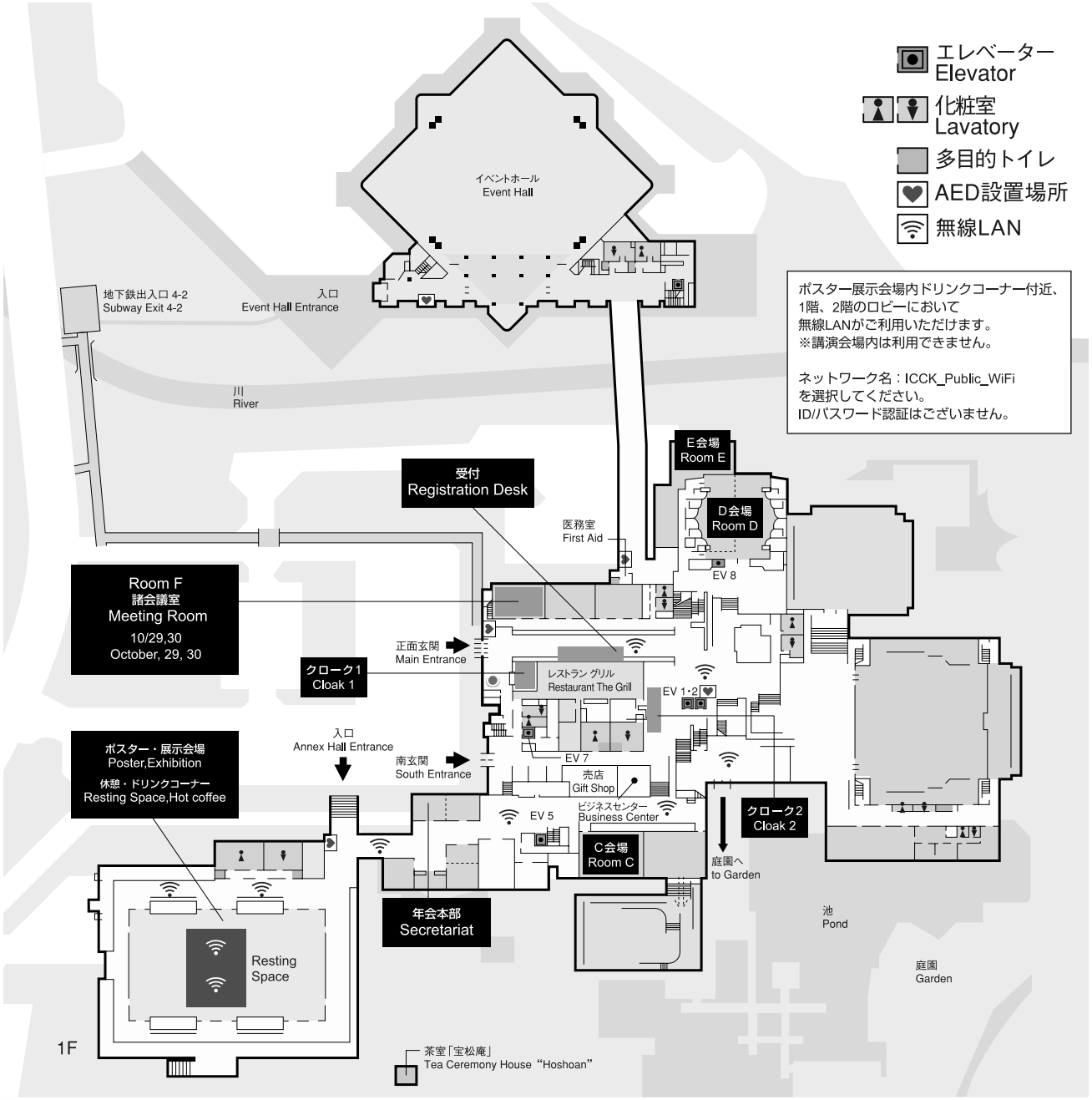
市営地下鉄烏丸線
「国際会館駅」下車

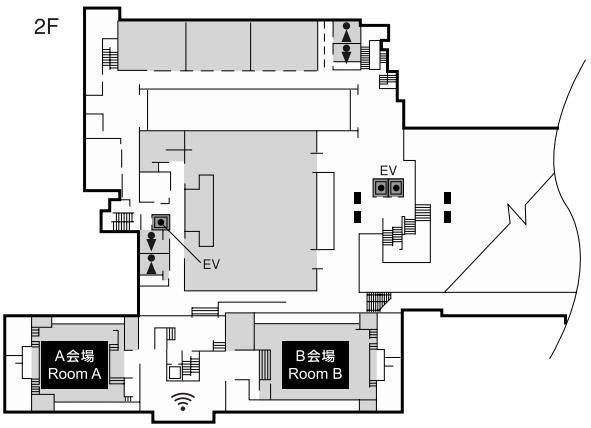
■ 周辺MAP



会場のご案内

国立京都国際会館





5F

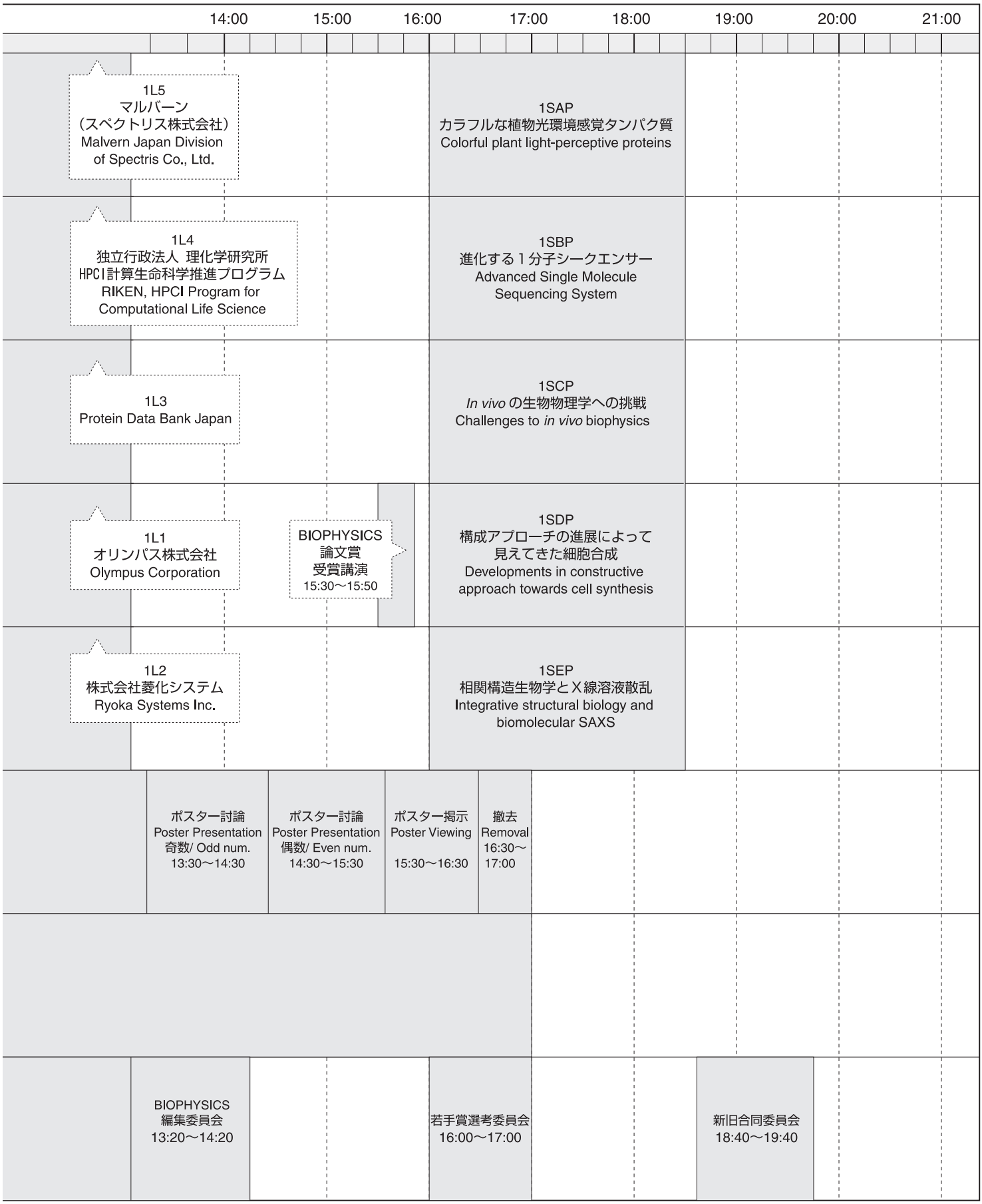
Room 553

諸会議室
Meeting Room

10/28
October, 28

▌10月28日(月) 年会1日目　【Day 1】28th Oct.

Kyoto Int'l Conf. Center			08:00	09:00	10:00	11:00	12:00	13:00
京都国際会館 2F	Room B-2	A会場 Room A			1SAA 光駆動水分解の分子機構：光化学系Ⅱと人工光合成 Molecular mechanism of light-driven water oxidation: photosystem II and artificial photosynthesis			
京都国際会館 2F	Room B-1	B会場 Room B			1SBA 生物物理学による生体分子ロボットの設計原理の探求 The Exploration of the design principle of biomolecular robots based on biophysics			
京都国際会館 1F	Room C-1	C会場 Room C			1SCA 生命システムの情報ダイナミクス Information Dynamics in Biological Systems			
京都国際会館 1F	Room D	D会場 Room D			1SDA バーグ教授記念講演と踊る運動超分子マシナリー Prof Berg's featured lecture and dancing harmonized motility machineries			
京都国際会館 1F	Room E	E会場 Room E			若手奨励賞受賞講演 Early Research in Biophysics Award			
京都国際会館 1F	アネックスホール Annex 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				
京都国際会館 1F	アネックスホール Annex Hall	企業展示 Exibition			機器・試薬・書籍展示 Exibition 9:30〜17:00			
京都国際会館 5F	Room 553	会議室 Meeting Room				第４回運営委員会 12:20〜13:20		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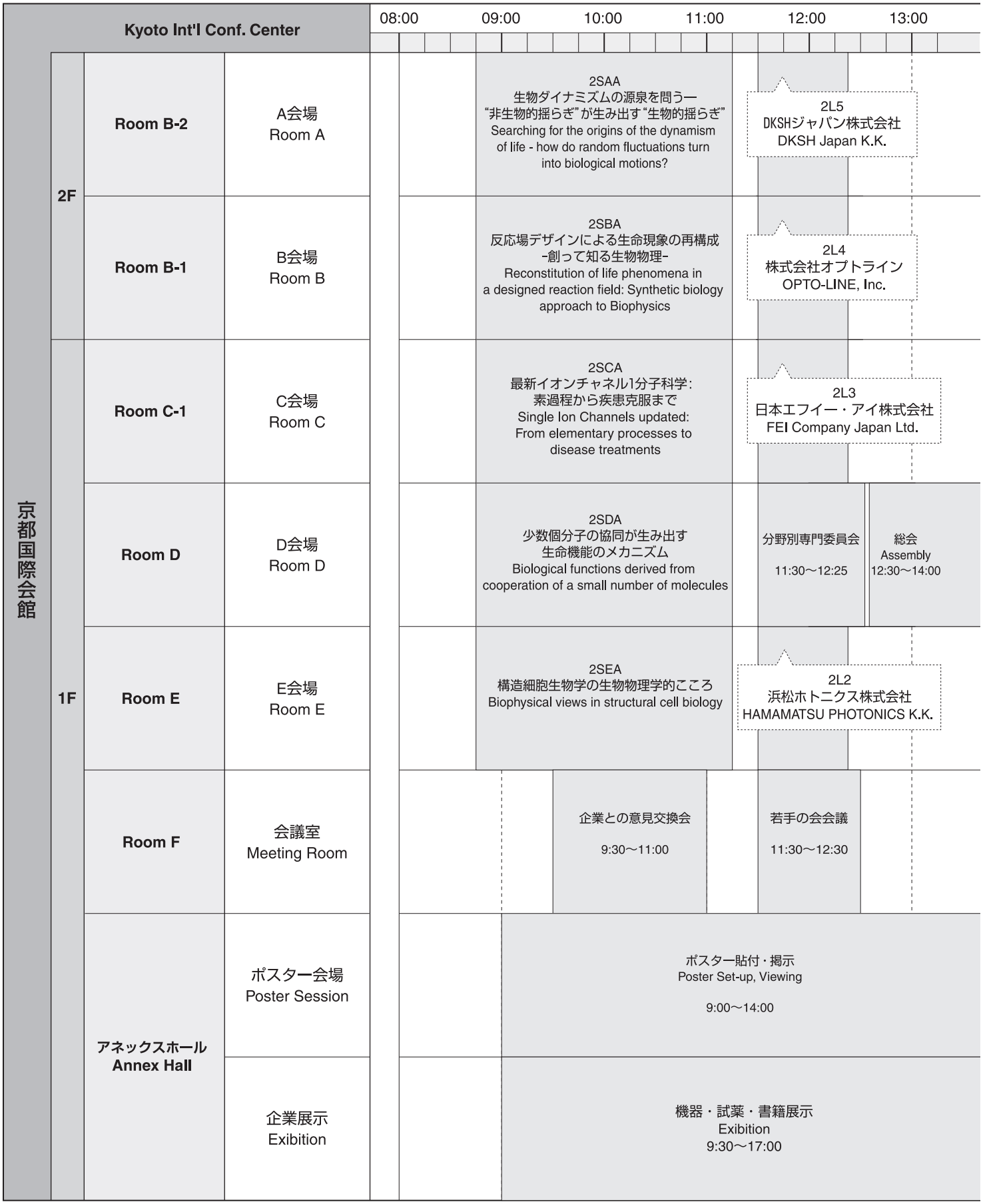
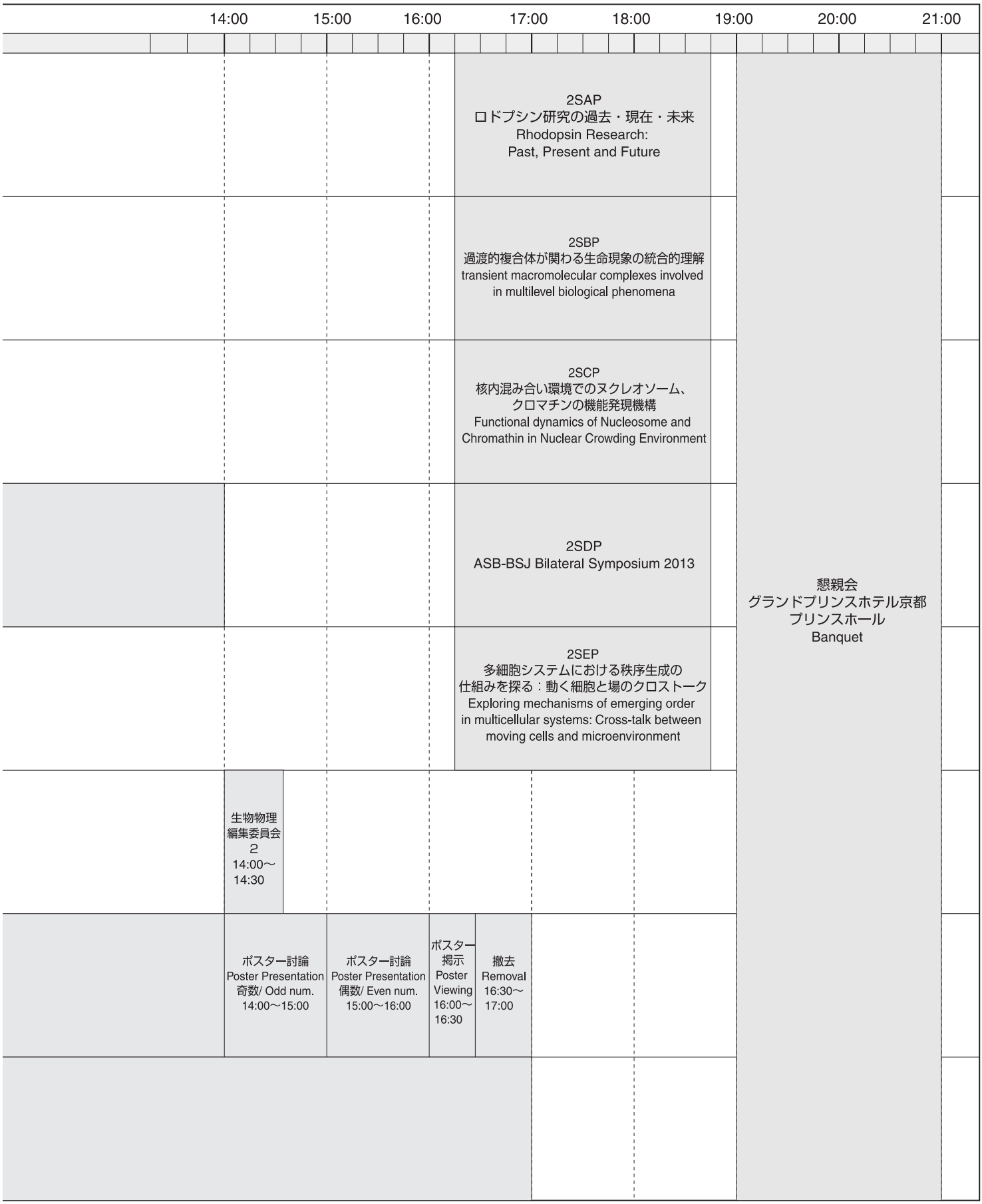
1L5
マルバーン
（スペクトリス株式会社）
Malvern Japan Division
of Spectris Co., Ltd.
1SAP
カラフルな植物光環境感覚タンパク質
Colorful plant light-perceptive proteins
1L4
独立行政法人 理化学研究所
HPCI計算生命科学推進プログラム
RIKEN, HPCI Program for
Computational Life Science
1SBP
進化する１分子シークエンサー
Advanced Single Molecule
Sequencing System
1L3
Protein Data Bank Japan
1SCP
In vivo の生物物理学への挑戦
Challenges to in vivo biophysics
1L1
オリンパス株式会社
Olympus Corporation
BIOPHYSICS
論文賞
受賞講演
15:30〜15:50
1SDP
構成アプローチの進展によって
見えてきた細胞合成
Developments in constructive
approach towards cell synthesis
1L2
株式会社菱化システム
Ryoka Systems Inc.
1SEP
相関構造生物学とX線溶液散乱
Integrative structural biology and
biomolecular SAXS
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
Poster Viewing
15:30〜16:30
撤去
Removal
16:30〜
17:00
BIOPHYSICS
編集委員会
13:20〜14:20
若手賞選考委員会
16:00〜17:00
新旧合同委員会
18:40〜19:40

10月29日(火) 年会2日目 【Day 2】29th Oct.

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2SAP
ロドプシン研究の過去・現在・未来
Rhodopsin Research:
Past, Present and Future
2SBP
過渡的複合体が関わる生命現象の統合的理解
transient macromolecular complexes involved
in multilevel biological phenomena
2SCP
核内混み合い環境でのヌクレオソーム、
クロマチンの機能発現機構
Functional dynamics of Nucleosome and
Chromathin in Nuclear Crowding Environment
2SDP
ASB-BSJ Bilateral Symposium 2013
2SEP
多細胞システムにおける秩序生成の
仕組みを探る：動く細胞と場のクロストーク
Exploring mechanisms of emerging order
in multicellular systems: Cross-talk between
moving cells and microenvironment
懇親会
グランドプリンスホテル京都
プリンスホール
Banquet
生物物理
編集委員会
2
14:00〜
14:30
ポスター討論
Poster Presentation
奇数/ Odd num.
14:00〜15:00
ポスター討論
Poster Presentation
偶数/ Even num.
15:00〜16:00
ポスター
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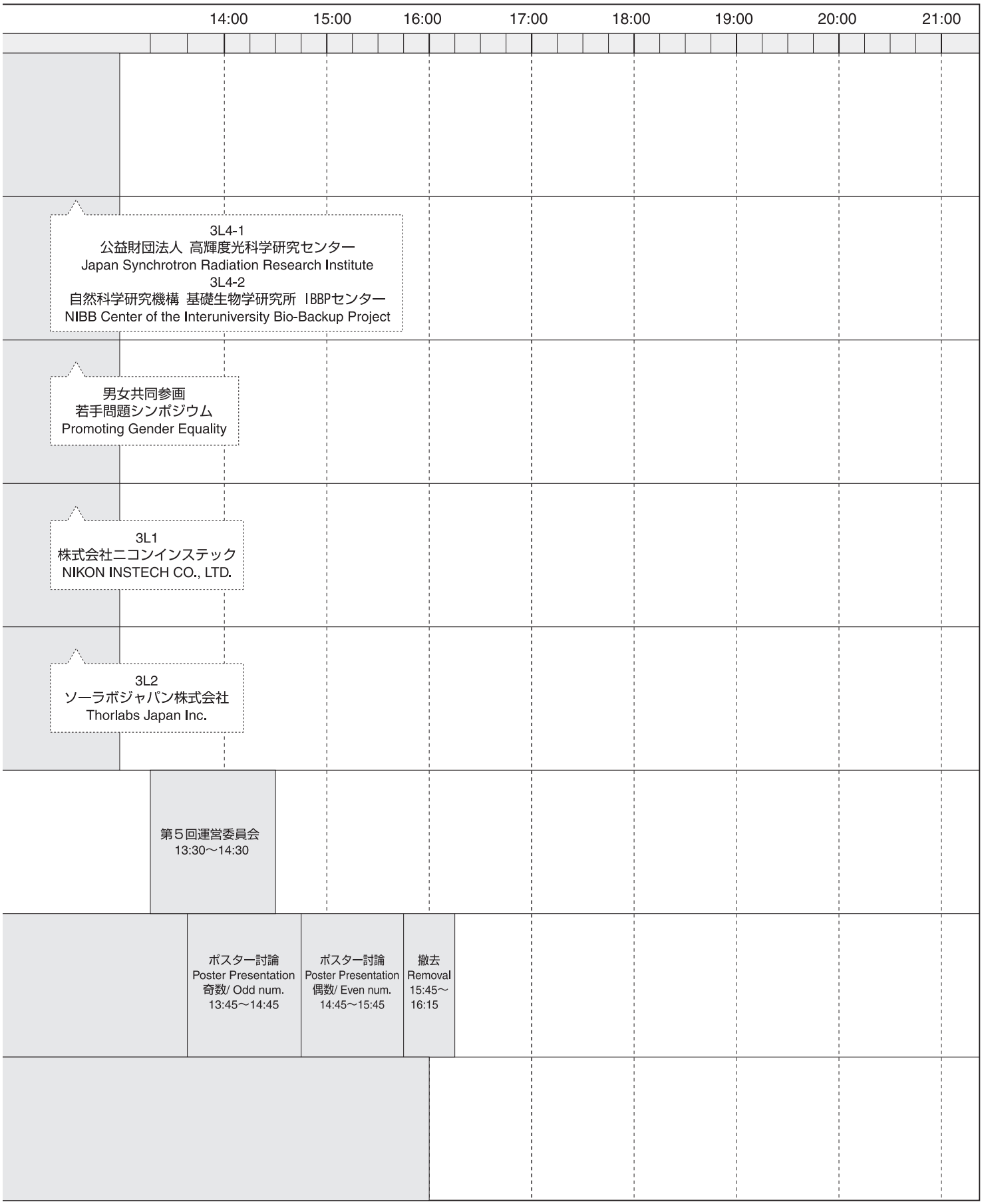
Removal
16:30〜
17:00

10月30日(水) 年会3日目 【Day 3】30th Oct.

京都国際会館 Kyoto Int'l Conf. Center				08:00	09:00	10:00	11:00	12:00	13:00
	2F	Room B-2	A会場 Room A			3SAA 生物物理学の近未来 -バイオ・ラマン研究の効きどころ- The Points in Bio-Raman Research			科研費説明会
		Room B-1	B会場 Room B			3SBA 光学イメージングによる脳神経研究の最前線 -1分子からin vivoまで- Cutting-edge optical imaging approach to neuroscience -From single molecule to in vivo-			
	1F	Room C-1	C会場 Room C			3SCA 生命現象の理解と核酸医薬を指向した機能性核酸の研究の最前線 Frontier of functional nucleic acids toward elucidation of biological events and nucleic acid medicine			
		Room D	D会場 Room D			3SDA 個体の生物物理学 -分子・細胞・個体にブリッジ- Biophysics toward *In Vivo* work			
		Room E	E会場 Room E			3SEA アミロイド線維形成における膜界面の役割 Roles of Membrane Interface in amyloidogenesis			
		Room F	会議室 Meeting Room				男女共同参画委員会 11:00～12:30		
		アネックスホール Annex 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			
			企業展示 Exibition			機器・試薬・書籍展示 Exibition 9:30～16:00			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3L4-1
公益財団法人 高輝度光科学研究センター
Japan Synchrotron Radiation Research Institute
3L4-2
自然科学研究機構 基礎生物学研究所 IBBPセンター
NIBB Center of the Interuniversity Bio-Backup Project
男女共同参画
若手問題シンポジウム
Promoting Gender Equality
3L1
株式会社ニコンインステック
NIKON INSTECH CO., LTD.
3L2
ソーラボジャパン株式会社
Thorlabs Japan Inc.
第５回運営委員会
13:30〜14:30
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
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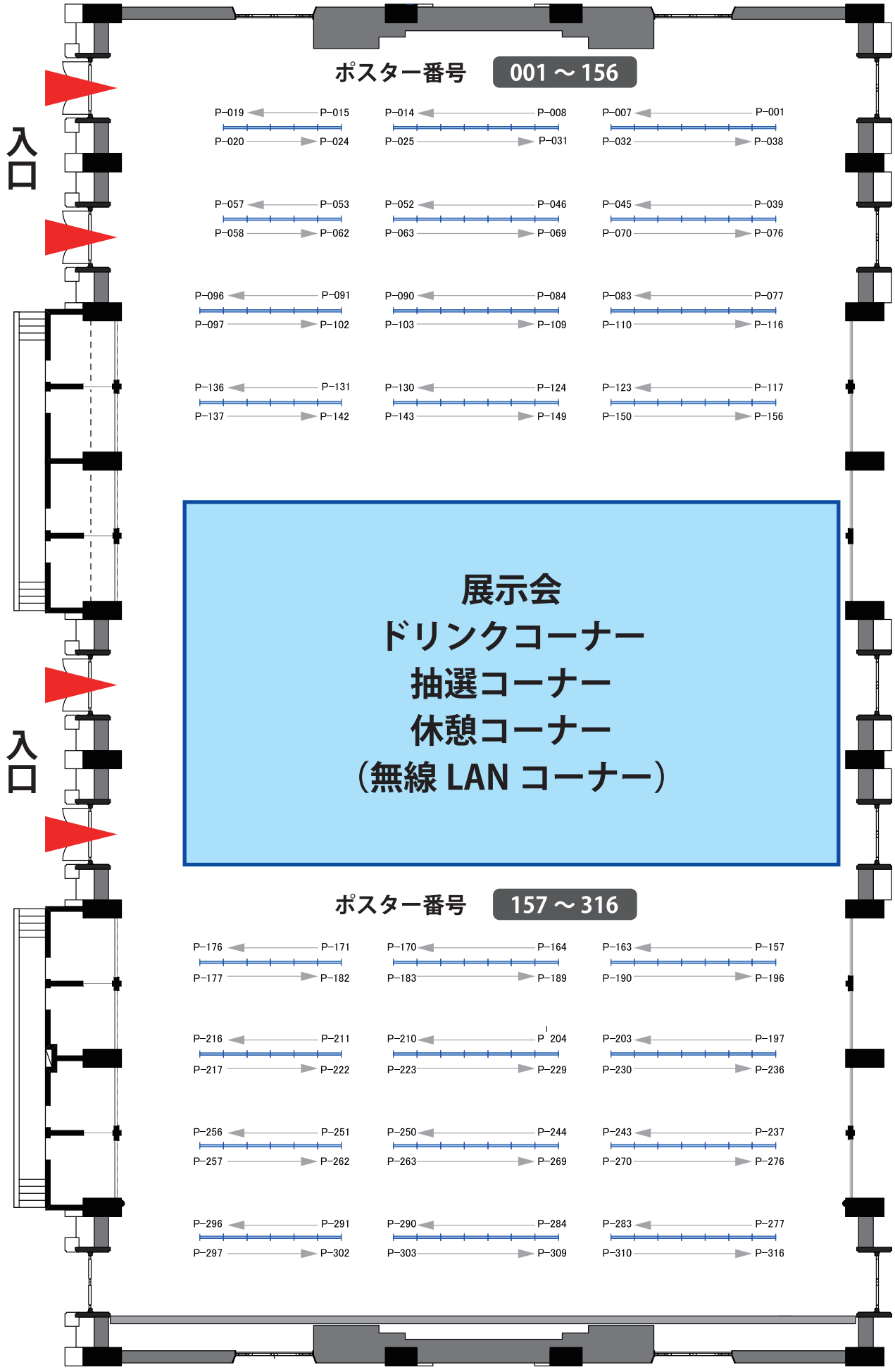
16:15

ポスター会場のご案内 ー 国立京都国際会館 アネックスホール



参加者へのご案内

1.　年会受付と参加登録

◇ 年会受付

場　　所:　国立京都国際会館　本館 1 階エントランス （フロア案内図をご参照ください）
受付時間:　10 月 28 日(月) 9:00-17:00
10 月 29 日(火) 8:00-17:00
10 月 30 日(水) 9:00-15:00

◆事前登録

日本生物物理学会 会員

事前登録が完了された方は、参加証および領収証が送付されますので、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。

非会員

事前登録が完了された方は、会期当日に受付内「非会員　事前デスク」にてプログラム集、参加証、領収証をお渡しいたします。

※ネームホルダーは当日配布しますので、会場内では必ずご着用ください。

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。
参加証と年会プログラム集をお渡しします。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/オンライン予稿集

講演予稿集は冊子体で制作いたしません。プログラム集(前付・プログラム・ポスター目次、広告を含む)は日本生物物理学会会員に事前送付いたします。抄録本文を含む講演予稿集はオンライン上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。プログラム集は年会受付でも当日販売(3,500 円)を行います。
プログラム(タイトル、発表者、所属)は予稿集発行日以後に、年会ホームページにて公開します。また年会終了後は、半年ほど経て、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(http://ci.nii.ac.jp/organ/journal/INT1000001547_jp.html)で予稿本文が公開されます。

◇ プログラム検索・要旨閲覧アプリ【2013 年 10 月 24 日(木)公開予定】

スマートフォン(iPhone/Android)やタブレット(iPad/iPod Touch/Android)端末に対応した電子抄録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)。

アプリケーション名:第 51 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、bsj2013

◇ 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: 国立京都国際会館 本館 1 階正面玄関　　(第 1 クローク)

国立京都国際会館 本館 1 階中央エレベーター前 (第 2 クローク)

利用時間: 10 月 28 日(月) 9:00～19:00
29 日(火) 8:00～19:15
30 日(水) 9:00～16:30

※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、男女共同参画・若手問題シンポジウム、科研費説明会では、お弁当とお茶が無料で提供されます。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できます。

◇国立京都国際会館　本館 1 階　レストラン「グリル」
10 月 28 日(月)～30 日(水)　10:00～17:00

◇ 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、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「宿泊について」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ドリンクコーナー付近、1階、2階のロビーにおいて
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。
ネットワーク名 : ICCK_Public_WiFi　を選択してください。
ID/パスワード認証はございません。

・ドリンクコーナー:ポスター展示会場(国立京都国際会館 アネックスホール)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

◇ 総会

日本生物物理学会第 52 回定例総会を、年会 2 日目、10 月 29 日(火)12:30-14:00 に D 会場(国立京都国際会館 Room D)で開催しますのでご出席ください。詳しくは開催通知をご覧ください。

◇ 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を、年会 1 日目、10 月 28 日(月)9:45-12:15 に E 会場(国立京都国際会館 Room E)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 10 月 28 日(月)15:30-15:50 に D 会場(国立京都国際会館 Room D)で開催します。

◇ 懇親会

日時: 2013 年 10 月 29 日(火)19:00-21:00
会場: グランドプリンスホテル京都　プリンスホール　京都市左京区岩倉幡枝町 1092-2
※懇親会の当日参加も受け付けます(会場前でも受け付けいたします)。

◇ 男女共同参画・若手問題シンポジウム

日時: 2013 年 10 月 30 日(水)12:30-13:20
会場: C 会場(国立京都国際会館 RoomC-1)
昼食: お弁当とお茶が無料で提供されます。

◇ 科研費説明会

日時: 2013 年 10 月 30 日(水)12:30-13:20
会場: A 会場(国立京都国際会館 RoomB-2)
昼食: お弁当とお茶が無料で提供されます。

◇ 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。

◆整理券の発券について

ランチョンセミナー整理券は配付デスクにて配付いたします。

時間:10月28日・30日　9:00-11:00、29日8:00-11:00

場所: 国立京都国際会館　本館1階中央エレベーター付近

※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。券は数が無くなり次第終了となります。

◆整理券の注意事項

<u>整理券は各日、セミナー開始後、無効となります。</u>

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利用いただき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国立京都国際会館 アネックスホールで行います。

◇ 市民講演会

テーマ: 生命を“診る・観る”
日時: 10 月 27 日(日) 13:00-16:00
会場: 京都大学芝蘭会館
お問い合わせ: 年会事務局までお願いします。
E-mail: ambsj2013@rh.biophys.kyoto-u.ac.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

館内は終日禁煙です。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・若手問題シンポジウム、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、口頭発表、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: 090-1664-4015

◇ 会期外

年会事務局

E-mail:ambsj2013@rh.biophys.kyoto-u.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: bsj2013sys-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

〒101-0051　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　展示会事業部
Tel: 03-3230-2744(代表)　Fax: 03-3230-2479
実行委員会サポート関連: jbp2013@aeplan.co.jp
広告関連 E-mail: adinfo@aeplan.co.jp
展示関連 E-mail: e_jbp51@aeplan.co.jp

6.　発表者へのご案内

◇ 使用言語

シンポジウムおよび一般の口頭発表は、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 ピンたんしメス

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。
会場には時間を計測するスタッフを置いています。

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。

◇ 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。

◇ ポスター発表の方へ

ポスターの貼付、展示、説明・討論、撤去:

		10 月 28 日(月)	10 月 29 日(火)	10 月 30 日(水)
貼付・展示		9:00-13:30／ 15:30-16:30	9:00-14:00／ 16:00-16:30	9:00-13:45
説明・討論	奇数番号	13:30-14:30	14:00-15:00	13:45-14:45
	偶数番号	14:30-15:30	15:00-16:00	14:45-15:45
撤去		17:00 までに撤去	17:00 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. 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。
4. 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。

◇ 発表形式と演題番号(各抄録左上の番号)の見方

発表には、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)1SAA03
1 文字目は発表日(1:10 月 28 日、2:10 月 29 日、3:10 月 30 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 桁の数字は発表順を示します。

若手招待講演: 1YEA1045
1 文字目は発表日(1:10 月 28 日)、2 文字目は Young (Scientists)、3 文字目は会場名(E 会場)、
4 文字目は午前・午後(AM,PM)、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(1:10 月 28 日、2:10 月 29 日、3:10 月 30 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。

◇ Language

English

◇ Presentation equipments

Please bring your own laptop and connect it to our projector for your presentation.
We will not provide a sound output.

Attention1) Please connect the computer to a video switcher.
Attention2) 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.
Attention3) Speaker is advised to bring his/her PowerPoint file in a USB memory.

◇ 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 min before the start of the symposium. After confirming your presentation slides, our staff will check fitness of the connection between your PC and our projector.
*Please note that there is no preview room.

◇ To Poster Presenters

		Day 1, Oct. 28	Day 2, Oct. 29	Day 3, Oct. 30
Set-up, Viewing		9:00-13:30／15:30-16:30	9:00-14:00／16:00-16:30	9:00-13:45
Presentation Discussion	Odd Numbers	13:30-14:30	14:00-15:00	13:45-14:45
	Even Numbers	14:30-15:30	15:00-16:00	14:45-15:45
Removal		until 17:00	until 17:00	until 16:15

If you have any questions, please contact us by email (ambsj2013@rh.biophys.kyoto-u.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, Young Scientists Invited Talk and Poster.

Symposium Talk: (Ex.) 1SAA03
Presentation day (**1**: Oct 28, **2**:Oct 29, **3**:Oct 30) + Symposium (S) + Session room (room A) + AM (A) /PM(P) + Order of the talk

Early Research in Biophysics Award Candidate Presentations: (Ex.) 1YEA1045
Presentation day (**1**: Oct 28) + Young Scientists(Y) + Session room (room E)+ AM(A) /PM(P) + Starting time of the talk

Poster Presentations: (Ex.) 1P001
Presentation day (**1**: Oct 28, **2**:Oct 29, **3**:Oct 30) + Poster (P) + Panel number

7. 日本生物物理学会第 52 回定例総会開催通知

日時: 2013 年 10 月 29 日(火)12:30-14:00
場所: D 会場(国立京都国際会館 Room D)
日本生物物理学会第52回定例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。
都合で出席できない方は委任状(葉書、切手貼付、本誌とじこみ)を総会開始前にご送付ください。
また、委任状は年会受付にも用意します。総会開始前に年会受付にご提出ください。

議長 年会実行委員長 七田芳則

総会議題
(1) 報告、承認事項
(会長 難波啓一)
平成 24 年度決算報告ならびに監査結果報告
平成 25 年度会計ならびに事業の中間報告、平成 25 年度の今後の計画
一般社団法人への移行について

(次期会長 七田芳則)
平成 26・27 年度役員選挙結果の報告
平成 26 年度、平成 27 年度予算案および事業計画

(2) その他

8. 運営委員会、各種委員会の案内

生物物理編集委員会 1	10 月 27 日(日)15:00ー18:00	京都大学 物質ー細胞統合システム拠点 本館 2 階セミナー室
ホームページ編集委員会	10 月 27 日(日)16:00ー18:00	京都大学 物質ー細胞統合システム拠点 本館 2 階展示室
平成 25 年度第 4 回運営委員会	10 月 28 日(月)12:20ー13:20	国立京都国際会館 5 階 Room 553
BIOPHYSICS 編集委員会	10 月 28 日(月)13:20ー14:20	国立京都国際会館 5 階 Room 553
若手賞選考委員会	10 月 28 日(月)16:00ー17:00	国立京都国際会館 5 階 Room 553
新旧合同委員会	10 月 28 日(月)18:40ー19:40	国立京都国際会館 5 階 Room 553
企業との意見交換会	10 月 29 日(火) 9:30ー11:00	国立京都国際会館 1 階 Room F
若手の会会議	10 月 29 日(火)11:30ー12:30	国立京都国際会館 1 階 Room F
分野別専門委員会	10 月 29 日(火)11:30ー12:25	国立京都国際会館 1 階 Room D
生物物理編集委員会 2	10 月 29 日(火)14:00ー14:30	国立京都国際会館 1 階 Room F
男女共同参画委員会	10 月 30 日(水)11:00ー12:30	国立京都国際会館 1 階 Room F
平成 25 年度第 5 回運営委員会	10 月 30 日(水)13:30ー14:30	国立京都国際会館 1 階 Room F

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。

京都大学芝蘭会館
新学術領域研究 「動く細胞と場のクロストークによる秩序の生成」
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 「過渡的複合体が関わる生命現象の統合的理解」
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 「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 「植物の環境感覚：刺激受容から細胞応答まで」
新学術領域研究 「人工光合成による太陽光エネルギーの物質変換：
実用化に向けての異分野融合」
新学術領域研究 「天然変性タンパク質の分子認識機構と機能発現
－生理的準安定状態を捉える新技術－」
新学術領域研究 「ナノメディシン分子科学」

日本生物物理学会第 51 回年会
実行委員長 七田 芳則

日本生物物理学会第 51 回年会　市民講演会
生命を“診る・観る”

日　時：2013 年 10 月 27 日（日）13:00~16:00

会　場：京都大学芝蘭会館
（京都市左京区吉田近衛町 京都大学医学部構内）
京都市バス 31・65・201・206 系統「京大正門前」停留所 徒歩 2 分
京阪電気鉄道「出町柳」駅 徒歩 15 分

参加費：無料（どなたでも自由に参加できます）

主　催：日本生物物理学会第 51 回年会　実行委員会

世話人：原田慶恵（京都大学物質 - 細胞統合システム拠点）
七田芳則（京都大学大学院理学研究科）

講演プログラム

「iPS 細胞の網膜疾患への応用」
高橋政代 プロジェクトリーダー
（理化学研究所 発生・再生科学総合研究センター）

「生命の不思議：1 ワットで働く脳、2 千万ワット使うスパコン」
柳田敏雄 教授
（大阪大学大学院生命機能研究科）

講演は日本語で行われます。

若手招待講演　Early Research in Biophysics Award

第 1 日目（10 月 28 日（月））／Day 1（Oct. 28 Mon.）

9:45～12:15　E 会場：Kyoto Int'l Conf. Center Room E

1YE　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・若手問題検討委員会

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 eighth year, we received 43 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 Saturday 17th September, and the winners will deliver a short talk. We welcome all the BSJ members to attend the oral presentations on Monday 28th October 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.

9:45　乙須　拓洋　3P052

1YE0945　二次元蛍光寿命相関分光法による BdpA 変性状態における構造ダイナミクスの解析

Two-dimensional fluorescence lifetime correlation spectroscopy on the conformational dynamics of the unfolded state of BdpA

○乙須　拓洋[1], 石井　邦彦[1], 小井川　浩之[2], 新井　宗仁[3], 高橋　聡[2], 田原　太平[1]（[1]理研・田原分子分光, [2]東北大・多元研, [3]東大・総合文化）

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*)

10:00　片山　耕大　1P251

1YE1000　霊長類色覚視物質の変異体に対する赤外分光研究

FTIR study of mutants of primate color pigments

○片山　耕大[1], 川田　大輝[1], 今井　啓雄[2], 和田　昭盛[3], 神取　秀樹[1]（[1]名古屋工業大学大学院工学研究科 未来材料創成工学専攻, [2]京都大学, [3]神戸薬科大学）

Kota Katayama[1], Daiki Kawata[1], Hiroo Imai[2], Akimori Wada[3], Hideki Kandori[1] ([1]*Department of Frontier Materials, Nagoya Institute of Technology*, [2]*Primate Research Institute, Kyoto University*, [3]*Organic Chemistry for Life Science, Kobe Pharmaceutical University*)

10:15　古賀　信康　1P088

1YE1015　理想タンパク質構造のデザイン原理

Principles for designing ideal protein structures

○古賀　信康[1], 古賀（巽）　理恵[1], Liu　Gaohua[2], Xiao　Rong[2], Montelione　Gaetano T.[2], Baker　David[1]（[1]Univ. Washington, Dept. of Biochemistry, [2]Rutgers Univ., Dept. Mol. Biol. and Biochem.）

Nobuyasu Koga[1], Rie Koga(Tatsumi)[1], Gaohua Liu[2], Rong Xiao[2], Gaetano T. Montelione[2], David Baker[1] ([1]*Univ. Washington, Dept. of Biochemistry*, [2]*Rutgers Univ., Dept. Mol. Biol. and Biochem.*)

10:30　古寺　哲幸　1P156

1YE1030　高速 AFM によって明らかとなったミオシン V の化学-力学変換メカニズム

Chemomechanical coupling mechanism of myosin V revealed by high-speed AFM

○古寺　哲幸[1], 内橋　貴之[1,2], 八木　健太[2], 安藤　敏夫[1,2]（[1]金沢大学理工研究域バイオAFM先端研究センター, [2]金沢大学理工研究域数物科学系）

Noriyuki Kodera[1], Takayuki Uchihashi[1,2], Kenta Yagi[2], Toshio Ando[1,2] ([1]*Bio-AFM FRC, Inst. Sci. & Eng., Kanazawa Univ.*, [2]*Sch. Math. & Phys., Col. Sci. & Eng., Kanazawa Univ.*)

10:45　小林　徹也　1P275

1YE1045　細胞システムの内因的・外因的ゆらぎに対するロバスト性に関する理論的基礎

Theoretical basis for robustness of intracellular systems against intrinsic and extrinsic fluctuation

○小林　徹也（東大、生産研）

Tetsuya Kobayashi (*IIS, Univ. Tokyo*)

11:00　斉藤　圭亮　3P255

1YE1100　光化学系 II におけるプロトン移動経路

Proton transfer pathway in photosystem II

○斉藤　圭亮[1,2], Rutherford　A. William[3], 石北　央[1,2]（[1]阪大院理・生物, [2]JSTさきがけ, [3]Dept. of Life Sci., Imperial College, London）

Keisuke Saito[1,2], A. William Rutherford[3], Hiroshi Ishikita[1,2] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*PRESTO JST*, [3]*Dept. of Life Sci., Imperial College, London*)

11:15　中根　大介　1P160

1YE1115　戦車のような仕組みで動くバクテリア

Bacterium moves like a tank

○中根　大介[1], 佐藤　啓子[2], 和田　浩史[3], McBride　Mark[4], 中山　浩次[2], 西坂　崇之[1]（[1]学習院大・理・物理, [2]長崎大・感染免疫, [3]立命館大・物理, [4]ウィスコンシン大・ミルウォーキー校・生物）

Daisuke Nakane[1], Keiko Sato[2], Hirofumi Wada[3], Mark McBride[4], Koji Nakayama[2], Takayuki Nishizaka[1] ([1]*Dept. Phys., Gakushuin Univ.*, [2]*Dept. Mol. Microbiol. Immunol., Nagasaki Univ.*, [3]*Dept. Phys., Ritsumeikan Univ.*, [4]*Dept. Biol. Sci., Univ. Wisconsin Milwaukee*)

11:30　藤原　慶　2P218

1YE1130　生細胞に極限まで近い内包物を持つ人工細胞の構築と解析

Generation of artificial cells that mimic living cells

○藤原　慶[1], 西澤　賢治[2], 栁澤　実穂[2], 野村 M.　慎一郎[1], 水野　大介[2]（[1]東北大学工学研究科, [2]九州大学 理学部）

Kei Fujiwara[1], Kenji Nishizawa[2], Miho Yanagisawa[2], Shin-ichiro M. Nomura[1], Daisuke Mizuno[2] ([1]*Tohoku university, Department of Bioengineering and Robotics*, [2]*Kyushu university, Department of Physics*)

11:45　細川　千絵　3P231

1YE1145　集光レーザービームの光摂動による神経細胞内分子動態の集合操作

Optical perturbation of intracellular molecular dynamics of single neuron in living neuronal network

○細川　千絵[1], 武田　尚子[1,2], 植田　悠介[1,2], 工藤　卓[2], 田口　隆久[1,3]（[1]産総所・健康工学, [2]関西学院大・院理工学, [3]情通研・脳情報）

Chie Hosokawa[1], Naoko Takeda[1,2], Yusuke Ueda[1,2], Suguru N. Kudoh[2], Takahisa Taguchi[1,3] ([1]*Health Res. Inst., AIST*, [2]*Grad. Sci. Eng., Kwansei Gakuin Univ.*, [3]*Cinet, NICT*)

12:00　矢野　義明　1P297

1YE1200　生細胞における膜タンパク質標識法と会合状態解析法の開発

Development of methods for labeling and oligomerization analysis of membrane proteins in live cells

○矢野　義明, 河野　健一, 大前　薫, 松崎　紗矢香, 松崎　勝巳（京大院薬）

Yoshiaki Yano, Kenichi Kawano, Kaoru Omae, Sayaka Mtsuzaki, Katsumi Matsuzaki (*Grad. Sch. Pharm. Sci., Kyoto Univ.*)

日本生物物理学会　第 2 回 BIOPHYSICS 論文賞受賞講演会
The 2nd 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：2013 年 10 月 28 日（月）15:30 ～ 15:50

場　所：D 会場（国立京都国際会館　Room D）

形　式：講演会

第 2 回 BIOPHYSICS 論文賞受賞者

目次正一 [1], 上野山敦子 [2], Jun-Adan-Kubo[2], 宮田真人 [2], 河野秀俊 [3], 由良敬 [4], 郷信広 [3]

Shoichi Metsugi[1], Atsuko Uenoyama[2], Jun-Adan-Kubo[2] Makoto Miyata[2], Hidetoshi Kono[3], Kei Yura[4], Nobuhiro Go[3]

1) 中外製薬株式会社 2) 大阪市立大学 3) 日本原子力研究開発機構 4) お茶の水女子大学

1) Chugai Pharmaceutical. Co., Ltd. 2) Osaka City University 3)Japan Atomic Energy Agency 4)Ochanomizu University

Mycoplasma mobile の運動蛋白質の配列解析による機能・構造予測

Structure and function prediction by sequence analysis for gliding proteins of *Mycoplasma mobile*

Mycoplasma mobile glides at an average velocity of about 2.0 to 4.5 μm/s, about ten times faster than the other mycoplasmas. The motile mechanism of *M. mobile* has been believed to differ from any previously identified mechanisms not only in bacteria but also in any other species. To reveal the mechanism, we carried out sequence analyses of Gli349 which is responsible for both adhesion to glass surfaces and motility, and its ortholog MYPU2110 from *Mycoplasma pulmonis*. We found that Gli349 contains 18 repeats of about 100 amino acid residues each in 3,183 residues, and MYPU2110 contains 22 in 3,216 residues. We also showed that the repeat was not homologous to any other known protein and therefore predicted three-dimensional structure [1]. The model structure of Gli349 was proposed which fit well to the images obtained by electron microscopy, assuming that the cleavage by chymotrypsin tend to occur in the regions between the repeats, and that each repeat folds into an independent structural domain [2]. Based on this model, with inhibitory antibodies and mutants, the regions directly involved in movements of *M. mobile* were suggested on Gli349 and Gli521, which are also involved in the gliding machinery [3]. A further study suggested that Gli349 should be a“leg”for the motile apparatus with Gli521 that plays a role of a“crank”for *M. mobile* [4,5]. In the presentation, such a unique motile mechanism of *M. mobile* being elucidated is introduced.

[1] Metsugi et al., Biophysics 1, 33 (2005)

[2] Adan-Kubo et al., J. Bacteriol. 188(8), 2821 (2006)

[3] Uenoyama et al., J. Bacteriol. 191(6), 1982 (2009)

[4] Nonaka et al., J. Bacteriol. 192(3), 636 (2010)

[5] Miyata M. Ann. Rev. Microbiol. 64, 519 (2010)

「男女共同参画・若手問題シンポジウム」
「博士号を取得して多様なキャリアパスを手に入れる」

Alternative Careers after PhD Course

オーガナイザー：日本生物物理学会　男女共同参画・若手問題検討委員会

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

日　時：2013 年 10 月 30 日（水）12:30 ～ 13:20（ランチョンセミナーの時間帯）

場　所：C 会場（国立京都国際会館　Room C-1）

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

講演者：3 名を予定

概要：昨年に引き続き、本年のシンポジウムも「博士号を取得して多様なキャリアパスを手に入れる」をテーマとして行うことになりました。景気は若干上向きであるとはいえ、相変わらず、大学、研究機関および企業における新規雇用が少なく、若者が社会で活躍する場が非常に少ない状態が続いています。大学で博士号を取得して研究者としてがんばっていこうと考えていたのに、思うように道を拓くことができないという話も、残念ながらよく聞くようになり、このことで、若い方々が博士号を取得する道に進まなくなってきています。博士号取得者の減少は、長い目で見ると日本の科学技術力の低下につながり、悪循環に陥ることが懸念されます。

この状況を打開するために、日本社会における博士号取得者の評価を高めることも重要ですが、大学で博士号を取得したら、次は大学や研究所で研究をするものであるという固定観念を見直す必要もありそうです。博士号を取得した後に企業で活躍されている方はかなりおられます。また、政府や大学も、博士号取得後のキャリアに関する様々な取り組みを行ってきています。

そこで、今回は生物物理学の分野で、[1] 博士の学位を取られた後、企業の研究所に勤められている方、[2] 修士修了後、公的研究機関に勤務された後に論文博士号を取得された方、[3] 修士修了後、製薬会社に勤務され、博士号取得を目指して会社から大学に派遣されている方について、企業・アカデミアでご活躍の男女若手研究者 3 名の方をお招きし、それぞれのご経歴に基づいて博士号取得の意味について話していただくことを予定しております。これらのお話を聞きながら、フロアからのご意見や質問を受け、時間が許す限りディスカッションをしていきたいと考えております。特に博士前期および後期課程学生のなまの声が聞こえてくることを期待しています。

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「科研費制度を知ろう」

Policy about the Grants-in-Aid System

世話人：豊島　陽子（東京大学大学院総合文化研究科、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: Yoko Y. Toyoshima (The Univ. of Tokyo, JSPS, Research Center for Science Systems, Program Officer）

日　時：2013 年 10 月 30 日（水）12:30~13:20（ランチョンセミナーの時間帯）
会　場：A 会場（国立京都国際会館　Room B-2）
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　松本　昌三

日本学術振興会における、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、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、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。

講演は日本語で行われます。

シンポジウム　Symposium

第 1 日目（10 月 28 日（月））／Day 1（Oct. 28 Mon.）

9:45～12:15　A 会場／Room A：Kyoto Int'l Conf. Center Room B-2

1SAA　新学術領域「人工光合成による太陽光エネルギーの物質変換：実用化に向けての異分野融合」共催
光駆動水分解の分子機構：光化学系Ⅱと人工光合成
Molecular mechanism of light-driven water oxidation: photosystem II and artificial photosynthesis

オーガナイザー：石北 央（大阪大学），野口 巧（名古屋大学）
Organizer: Hiroshi Ishikita (Osaka Univ.), Takumi Noguchi (Nagoya Univ.)

A membrane-pigment complex photosystem II (PSII) uses light to drive water oxidation and oxygen evolution processes (2H2O → O2 + 4H+ + e-), which occur at the catalytic site Mn4Ca cluster. Similar functions of light-driven water oxidation are expected for catalyst design in artificial photosynthesis systems. We will discuss molecular mechanism of light-induced water oxidation in PSII and artificial photosynthesis systems on the basis of experimental researches or theoretical chemical approaches.

1SAA-01　天然光合成における水分解・酸素発生光化学系 II
Water-splitting and oxygen-evolving photosystem II in natural photosynthesis
○神谷 信夫（大阪市立大学）
Nobuo Kamiya (*Osaka City University*)

1SAA-02　ESR 法でわかる Mn クラスターの電子構造と機能
Electronic structure and function of Mn cluster correlated with crystal structure observed by Electron Spin Resonance
○三野 広幸（名大院理）
Hiroyuki Mino (*Grad. Sch. Sci., Nagoya Univ.*)

1SAA-03　QM/MM 法による光合成酸素発生中心 S 1 状態の電子状態解析
QM/MM study on the photosystem II oxygen evolving complex at the S1 state
○庄司 光男[1], 磯部 寛[2], 山中 秀介[3], 神谷 信夫[4], 沈 建仁[2], 山口 兆[3,4]（[1]筑波大・数理物質, [2]岡山大・自然科学, [3]阪大・院理, [4]大阪市大・複合先端）
Mitsuo Shoji[1], Hiroshi Isobe[2], Shusuke Yamanaka[3], Nobuo Kamiya[4], Jian-Ren Shen[2], Kizashi Yamaguchi[3,4] ([1]*Grad. Sch. of Pure and App. Sci., Univ. Tsukuba*, [2]*Grad. Sch. Nat. Sci. & Tec., Okayama Univ.*, [3]*Grad. Sch. Sci, Osaka Univ.*, [4]*OCARINA, Osaka City Univ*)

1SAA-04　多参照波動関数理論で解く光合成系 II マンガンクラスタの電子構造
Entangled quantum electronic wavefunctions of the Mn_4CaO_5 cluster in photosystem II
○倉重 佑輝, 柳井 毅（分子科学研究所）
Yuki Kurashige, Takeshi Yanai (*Institute for Molecular Science*)

1SAA-05　OEC に関する理論的研究
Theoretical Study on OEC
○中村 振一郎（独立行政法人理化学研究所）
Shin Nakamura (*RIKEN*)

1SAA-06　人工的な遷移金属錯体を触媒とする酸素発生反応
Water Oxidation Catalyzed by Artificial Transition Metal Complexes
○正岡 重行（分子研）
Shigeyuki Masaoka (*IMS*)

1SAA-07　人工光合成の構築に向けた水の酸化触媒の開発
Development of molecular catalysts for water oxidation toward artificial photosynthesis
○八木 政行（新潟大・工）
Masayuki Yagi (*Niigata Univ.*)

9:45～12:15　B 会場／Room B：Kyoto Int'l Conf. Center Room B-1

1SBA　新学術領域「感覚と知能を備えた分子ロボットの創成」共催
生物物理学による生体分子ロボットの設計原理の探求
The Exploration of the design principle of biomolecular robots based on biophysics

オーガナイザー：瀧ノ上 正浩（東京工業大学），小宮 健（東京工業大学），齊藤 博英（京都大学）
Organizer: Masahiro Takinoue (Tokyo Inst. Tech.), Ken Komiya (Tokyo Inst. Tech.), Hirohide Saito (Kyoto Univ.)

Life systems are sophisticated "molecular robots" that are hierarchically self-organized using nanometer-sized molecular parts. Due to the cooperative behaviors of the molecular parts, the life systems achieve dynamic functions such as autonomous information processing, autonomous motions, self-replication, etc. To date, biophysics has succeeded in revealing the characteristics of individual biomolecules constituting life systems. As the next step, through the constructive approaches such as synthesis of molecular robots, the working principles of life systems would be explored more deeply. Thus, in this symposium, we introduce these novel approaches and actively discuss their prospects.

1SBA-01　動的人工細胞・分子ロボットの作製のための微小非平衡場の制御
Control of micro-sized nonequilibrium system for the construction of dynamic artificial cells and molecular robots based on microfluidics
○瀧ノ上 正浩（東工大・院総理工）
Masahiro Takinoue (*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*)

1SBA-02　分子ロボティクス指向による人工細胞構築
Molecular robotics approach for constructing an artificial cell model
○野村 M. 慎一郎[1], 藤原 慶[1, 2]（[1]東北大学　工学研究科　バイオロボティクス専攻, [2]日本学術振興会）
Shin-ichiro Nomura M.[1], Kei Fujiwara[1, 2] ([1]*Department of Bioengineering and Robotics, Division of Mechanical Engineering, TOHOKU University*, [2]*JSPS Research Fellows*)

1SBA-03　細胞サイズ液滴内での高分子混合系の相分離とゾル-ゲル転移
Aqueous phase separation and sol-gel transition of biopolymer blend in cell-sized droplets
○柳澤 実穂（九大院・理）
Miho Yanagisawa (*Grad. Sch. Sci., Kyushu Univ.*)

1SBA-04　外部環境情報をリポソーム基盤分子ロボットの内部に伝達する分子センサーの開発
A development of molecular sensor that delivers environmental information to inside of liposome-based molecular robots
○庄田 耕一郎, 陶山 明（東京大学総合文化研究科広域科学専攻生命環境科学系陶山研究室）
Koh-ichiroh Shohda, Akira Suyama (*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*)

1SBA-05　アクチン線維とミオシン、細胞サイズの膜小胞を利用した分子アメーバ構築の試み
Construction of motile artificial cell model using actomyosin and cell-sized giant liposome
○滝口 金吾, 林 真人（名古屋大学大学院理学研究科生命理学超分子機能学講座）
Kingo Takiguchi, Masahito Hayashi (*Grad. Sch. Sci., Nagoya Univ.*)

1SBA-06　人工 RNA-Protein 複合体による細胞内外で機能する分子ロボットの創出にむけて
Synthetic RNA-Protein complexes to construct molecular robot in vitro and in cells
○齊藤 博英[1,2]（[1]京都大学 iPS細胞研究所, [2]京都大学 白眉センター）
Hirohide Saito[1,2] ([1]*CiRA*, [2]*The Hakubi Center for Advanced Research*)

1SBA-07　分子ロボットを制御する試験管内知能の実装
Implementation of *in vitro* intelligence for controlling molecular robots
○小宮 健（東工大・院総理）
Ken Komiya (*Interdisci. Grad. Sch. Sci. & Engi., Tokyo Tech.*)

9:45～12:15　C 会場／Room C：Kyoto Int'l Conf. Center Room C-1

1SCA　生命システムの情報ダイナミクス
Information Dynamics in Biological Systems

オーガナイザー：小林 徹也（東京大学），黒田 真也（東京大学）
Organizer: Tetsuya J. Kobayashi (The Univ. of Tokyo), Shinya Kuroda (The Univ. of Tokyo)

Life is intrinsically dynamic and non-equilibrium phenomenon in which time and causality plays pivotal roles. This fact, on the one hand,enables us to extract parametric and non-parametric information on underlying processes from time-series and population data.
On the other hand, it suggests that biological systems are evolutionary designed so as to exploit its dynamic nature for transmitting and processing information under stochastic environment. In this symposium, we introduce cutting edge research on information dynamics and discuss its impact for understanding physical and information-theoretical principles in biological systems.

1SCA-01　遺伝子発現過程の情報ダイナミクス
Information Dynamics in Gene Expression Processes
○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*)

1SCA-02　Inferring Kinetics Objectively from Single Molecule Time Series with Full Information Content
Li Chun-Biu (*Research Institute for Electronic Science, Hokkaido University*)

1SCA-03　確率的な細胞環境感知における適応的ダイナミクスの役割
Role of Adaptive Dynamics in Stochastic Cellular Sensing Systems
○小林 徹也（東大、生産研）
Tetsuya Kobayashi (*IIS, Univ. Tokyo*)

1SCA-04　細胞内時空間データからの方程式推定
Estimating inner-cell dynamics from spatio-temporal data
○石原 秀至[1], 谷口 大相[1], 澤井 哲[1,2]（[1]東京大学大学院総合文化研究科, [2]JSTさきがけ）
Shuji Ishihara[1], Daisuke Taniguhi[1], Satoshi Sawai[1,2] ([1]*Graduate school of Arts and Sciences, The University of Tokyo*, [2]*JST PERSTO*)

1SCA-05　確率的な ERK 活性ダイナミクスと細胞増殖制御
Stochastic ERK activity pulses induced by noise and cell-to-cell propagation regulate cell density-dependent proliferation
○青木 一洋（京都大学大学院医学研究科 時空間情報イメージング拠点）
Kazuhiro Aoki (*Kyoto University, Graduate School of Medicine, Imaging Platform for Spatio-Temporal Information*)

1SCA-06　細胞内シグナル伝達経路の情報コーディング
Information coding of cellular signaling networks
○黒田 真也, 宇田 新介（東京大学理学系研究科生物化学専攻）
Shinya Kuroda, Shinsuke Uda (*Biophys. Biochem., University of Tokyo*)

9:45～12:15　D 会場／Room D：Kyoto Int'l Conf. Center Room D

1SDA　新学術領域「運動超分子マシナリーが織りなす調和と多様性」共催
バーグ教授記念講演と踊る運動超分子マシナリー
Prof Berg's featured lecture and dancing harmonized motility machineries

オーガナイザー：宮田 真人（大阪市立大学），佐藤 啓子（長崎大学）
Organizer: Makoto Miyata (Osaka City Univ.), Keiko Sato (Nagasaki Univ.)

Prof Berg at Harvard University, a pioneer of single molecule measurements has lead "biophysics", including our society for more than 40 years. He focused mainly on bacterial behaviors including motor and signal transduction, and achieved single molecule and in vivo measurements. In this symposium, he will give us special messages and also we will enjoy discussion about related several subjects.

1SDA-01 タンパク質膜透過促進因子 SecDF の構造と機能
Structure and function of SecDF, a membrane integrated protein translocation enhancing factor
○森 博幸[1], 三登 一八[1], 町田 裕紀子[1], 塚崎 智也[2,3], 伊藤 維昭[4], 秋山 芳展[1]（[1]京都大学・ウイルス研究所, [2]奈良先端科学技術大学院大学・バイオサイエンス研究科, [3]科学技術振興機構・さきがけ, [4]京都産業大学・総合生命科学部）
Hiroyuki Mori[1], Kazuya Mito[1], Yukiko Machida[1], Tomoya Tsukazaki[2,3], Koreaki Ito[4], Yoshinori Akiyama[1] ([1]*Institute for Virus Research, Kyoto University*, [2]*Grad. Sch. of Biol. Sci., NAIST*, [3]*JST, PRESTO*, [4]*Faculty of Life Sciences, Kyoto Sangyo University*)

1SDA-02 細菌べん毛ディスタルロッドの構造解析
High-resolution structure of the bacterial flagellar distal rod
○西條 由見子[1], 今田 勝巳[2], 松波 秀行[3], 藤井 高志[4], 難波 啓一[1,4]（[1]阪大・院生命機能, [2]阪大・院理・高分子, [3]沖縄科技大・細胞膜通過輸送研究ユニット, [4]理研・生命システム研究センター）
Yumiko Saijo-Hamano[1], Katsumi Imada[2], Hideyuki Matsunami[3], Takashi Fujii[4], Keiichi Namba[1,4] ([1]*FBS. Osaka Univ.*, [2]*Dept. Macromol. Sci., Grad. Sch. Sci., Osaka Univ.*, [3]*Trans-Membrane Trafficking Unit, OIST*, [4]*QBiC, RIKEN*)

1SDA-03 バチルス属細菌のべん毛モーター固定子のイオン選択性と運動性
One stator that couples to multiple different ions: flagellar stator and motility of *Bacillus* spp.
○伊藤 政博（東洋大 生命科）
Masahiro Ito (*Fac. Life Sci., Toyo Univ.*)

1SDA-04 バクテリア運動の驚異
Wonders of bacterial motility
○バーグ ハワード（ハーバード大学）
Howard C Berg (*Department of Molecular & Cellular Biology and of Physics, Harvard University*)

生体運動研究の昔と今
○大沢 文夫[1,2]（[1]大阪大学, [2]名古屋大学）
Fumio Oosawa[1,2] ([1]*Osaka Univ.*, [2]*Nagoya Univ.*)

16:00～18:30　A 会場／Room A：Kyoto Int'l Conf. Center Room B-2

1SAP　新学術領域「植物の環境感覚：刺激受容から細胞応答まで」共催
カラフルな植物光環境感覚タンパク質
Colorful plant light-perceptive proteins

オーガナイザー：徳富 哲（大阪府立大学），細川 陽一郎（奈良先端科学技術大学院大学）
Organizer: Satoru Tokutomi (Osaka Pref. Univ.), Yoichiroh Hosokawa (NAIST)

Plants sense various environmental signals for their biological responses such as seed germination, shoot growth and development, flowering and so on. Light is one of the most important environmental signals for photosynthetic plants and plants have acquired a variety of photoreceptors during the evolutional processes. The absorptions of the photoreceptors range from far-red to UV-A light. In this Symposium, the frontier research on these colorful plant photoreceptors will be introduced.

1SAP-01 UV-B photoreception by plant UVR8
John Christie (*University of Glasgow*)

1SAP-02 植物における CPD 光回復酵素と UVB 抵抗性
UVB-induced DNA damage repair enzyme “CPD photolyase” and UVB resistance in plant
○日出間 純（東北大・院・生命科学）
Jun Hidema (*Grad. Sch. Life Sci. Tohoku Univ.*)

1SAP-03 植物の青色光受容体 phototropin の全長でのシグナリング機構
Signaling mechanism in full-length phototropin, plant blue light receptor
○岡島 公司（大阪府大・理）
Koji Okajima (*Osaka Pref. Univ.*)

1SAP-04 植物における青色光に依存した気孔開口
Blue light-dependent stomatal opening in plants
○島崎 研一郎, 武宮 淳史（九大・院理・生物）
Ken-ichiro Shimazaki, Atsushi Takemiya (*Dept. of Biol., Kyushu Univ.*)

1SAP-05 フォトトロピンで誘導される葉緑体と核の運動機構
The mechanisms of chloroplast and nuclear movement mediated by blue light receptor phototropins
○和田 正三（九州大学）
Masamitsu Wada (*Kyushu University*)

1SAP-06 フィトクロム A のモジュラー構造
The modular structure of phytochrome A
○長谷 あきら[1], 岡 義人[1,2], 小野 裕也[1], 吉川 由希子[1], 小鍛治 敬生[1], 望月 伸悦[1]（[1]京大院・理, [2]理研・植物セ）
Akira Nagatani[1], Yoshito Oka[1,2], Yuya Ono[1], Yukiko Yoshikawa[1], Keio Kokaji[1], Nobuyoshi Mochizuki[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Plant Sci. Center, RIKEN*)

1SAP-07 新規光受容体群シアノバクテリオクロムの吸収波長調節メカニズム
Color tuning mechanism of novel photoreceptors cyanobacteriochrome
○成川 礼[1,2]（[1]東大・院・総合文化, [2]JST・さきがけ）
Rei Narikawa[1,2] ([1]*Univ. of Tokyo, Dept. of Life Sci.*, [2]*JST, PRESTO*)

16:00～18:30　B 会場／Room B：Kyoto Int'l Conf. Center Room B-1

1SBP　進化する 1 分子シークエンサー
Advanced Single Molecule Sequencing System

オーガナイザー：上村 想太郎（理化学研究所），谷口 正輝（大阪大学）
Organizer: Sotaro Uemura (RIKEN), Masateru Taniguchi (Osaka Univ.)

Single molecule sequencing system is one of the powerful tool for gene analysis widely. It has been greatly advancing for many varieties of applications in fact that its function is no longer just sequence but also a multianalyzer for gene analysis. However, most of technologies behind it base on the cutting edge technologies on single molecule biophysics. In this symposium, we invited frontier scientists working on single molecule sequencing system including bioinformatics for their interesting talks and discussion.

1SBP-01 1 細胞解析のための 1 分子シーケンシングシステムの開発
Development of Single Molecule Sequencing System for Single Cell Analysis
○上村 想太郎（理化学研究所ライフサイエンス技術基盤研究センター）
Sotaro Uemura (*RIKEN Center for Life Science Technologies*)

1SBP-02 REAL-TIME MONITORING OF BIOMOLECULES IN ZERO-MODE WAVEGUIDES: DNA SEQUENCING AND BEYOND
Paul Peluso (*Pacific Biosciences*)

1SBP-03 タンパク質翻訳伸長過程の実時間ダイナミクス計測
Dynamics of translation elongation in real time
○Puglisi Joseph, Tsai Albert, Chen Jin, Kornbeg Guy, Jonansson Magnus, Petrov Alexey, O'Leary Sean, Mark Capece（スタンフォード大学医学部構造生物学科）
Joseph Puglisi, Albert Tsai, Jin Chen, Guy Kornbeg, Magnus Jonansson, Alexey Petrov, Sean O'Leary, Capece Mark (*Department of Structural Biology, Stanford University School of Medicine*)

1SBP-04 Single Molecule Electrical Sequencing of DNA and microRNA
Masateru Taniguchi (*The Institute of Scientific and Industrial Research, Osaka University*)

1SBP-05 類似配列の高速な全ペア列挙に基づく NGS データの解析手法
NGS data analyses based on ultra-fast all pairs similarity search
○清水 佳奈（産業技術総合研究所 生命情報工学研究センター）
Kana Shimizu (*Computational Biology Research Center, National Institute of Advanced Industrial Science and Technology*)

16:00～18:30　C 会場／Room C：Kyoto Int'l Conf. Center Room C-1

1SCP　*In vivo* の生物物理学への挑戦
Challenges to *in vivo* biophysics

オーガナイザー：杉 拓磨（京都大学），大澤 志津江（京都大学）
Organizer: Takuma Sugi (Kyoto Univ.), Shizue Ohsawa (Kyoto Univ.)

The famous textbook "PHYSICAL BIOLOGY OF THE CELL" usually tells us the mechanism of how signaling molecules are regulated in intracellular regions based on well-accumulated *in vitro* biophysical studies. However, do we know how such mechanisms work *in vivo*? In multicellular organisms, normal development and biological functions are coordinated by inter-cellular network through cell-cell communications, although the underlying mechanisms *in vivo* remain elusive. In this symposium, we invite speakers who challenge to this problem using animal model systems and tell how cells and molecules behave *in vivo*.

1SCP-01　細胞競合を介した上皮の恒常性維持機構の分子基盤
Cell competition that regulates epithelial maintenance in Drosophila
○大澤 志津江[1], 國政 啓[1], 井垣 達吏[1,2]（[1]京都大学大学院生命科学研究科 システム機能学分野, [2]科学技術振興機構さきがけ）
Shizue Ohsawa[1], Kei Kunimasa[1], Tatsushi Igaki[1,2] ([1]*Lab.Genetics, Grad. Sch. Biostudies, Kyoto Univ.*, [2]*PRESTO, JST, Japan*)

1SCP-02　胚サイズ依存的な背腹軸パターンのスケーリング機構
Scaling of Dorsal-Ventral Patterning by Embryo Size-Dependent Degradation of Chordin
○猪股 秀彦, 柴田 達夫, 笹井 芳樹（理研・CDB）
Hidehiko Inomata, Tatsuo Shibata, Yoshiki Sasai (*CDB., RIKEN*)

1SCP-03　低温環境下の概日リズムの普遍性
Universality of circadian rhythms under low temperature conditions
○伊藤 浩史[1], 村山 依子[2], 冨田 淳[3], 近藤 孝男[4], 郡 宏[5], 八木田 和弘[6]（[1]九州大学芸術工学研究院, [2]熊本大学大学院先導機構, [3]熊本大学発生医学研究所, [4]名古屋大学, [5]お茶の水女子大学, [6]京都府立医大）
Hiroshi Ito[1], Yoriko Murayama[2], Jun Tomita[3], Takao Kondo[4], Hiroshi Kori[5], Kazuhiro Yagita[6] ([1]*Faculty of Design, Kyushu University*, [2]*Priority Organization for Innovation and Excellence, Kumamoto University*, [3]*Institute of Molecular Embryology and Genetics, Kumamoto University*, [4]*Nagoya University*, [5]*Ochanomizu Univerisity*, [6]*Kyoto Prefectural University of Medicine*)

1SCP-04　ハエトリグモの奥行き知覚における視物質の吸収特性の寄与
Contribution of a visual pigment absorption spectrum to depth perception in the jumping spider
○永田 崇（総研大・先導研）
Takashi Nagata (*Dept Evol Stud Biol Sys, Sokendai-Hayama*)

1SCP-05　Optically detected magnetic resonance spectroscopy of nitrogen-vacancy centers for subnanoscopic measurement in vivo
Ryuji Igarashi[1], Yuta Kumiya[1], Takuma Sugi[2], Fuminori Sugihara[3], Hidehito Tochio[1], Yousuke Yoshinari[2], Yoshie Harada[2], Masahiro Shirakawa[1] ([1]*Department of Molecular Engineering, Graduate School of Engineering, Kyoto University*, [2]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*, [3]*Immunology Frontier Research Center (WPI-iFReC), Osaka University*)

1SCP-06　カイメン体内で細胞及び組織が共同作業で骨片を１つ１つ組み上げて立てる建築物「骨片骨格」形成の仕組み
HOW DO SPONGE CELLS BUILD UP THE HIERARCHICAL SPICULOUS SKELETON?
○船山 典子（京都大学 大学院理学研究科 生物科学専攻 生物物理学教室 分子発生）
Noriko Funayama (*Department of Biophysics, Graduate School of Science, Kyoto Univ.*)

1SCP-07　Synergistic action of mitosis and cell shape change in epithelial invagination
Takefumi Kondo, Shigeo Hayashi (*RIKEN CDB*)

1SCP-08　生きるための細胞死～脳形態形成過程に細胞死が与える影響
Cell death for life ~ Impact of apoptosis on morphogenesis in brain development
○山口 良文（東京大学大学院薬学系研究科遺伝学教室）
Yoshifumi Yamaguchi (*Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo*)

1SCP-09 線虫 *C. elegans* の振動への馴化学習とその記憶の生物物理学的解析
Biophysical analysis of *C. elegans* mechanosensory learning and memory
○杉 拓磨[1,2]（[1]京大・iCeMS, [2]JST-PRESTO）
Takuma Sugi[1,2] ([1]*iCeMS, Kyoto Univ.*, [2]*JST-PRESTO*)

16:00～18:30　D 会場／Room D：Kyoto Int'l Conf. Center Room D

1SDP　構成アプローチの進展によって見えてきた細胞合成
Developments in constructive approach towards cell synthesis

オーガナイザー：木賀 大介（東京工業大学），野地 博行（東京大学）
Organizer: Daisuke Kiga (Tokyo Inst. Tech.), Hiroyuki Noji (The Univ. of Tokyo)

With regard to basic question that "what is cell", constructive approach to combine biomolecules has been developed, in this decade, for broad field of life science. This approach is complementary to traditional approach based on screening and analysis. At this symposium relevant to cell synthesis, we will introduce the newest research including micro fabrication technology and social impact of our field. With these lines of information, we would also like to discuss the idea of "synthesizing cell".

1SDP-01 Systems and Synthetic Biology of Biological Timings
Hiroki R. Ueda (*QBiC Riken*)

1SDP-02 無細胞合成系によるタンパク質進化技術
Protein evolution by cell-free synthesis system
○今村 千絵（豊田中央研究所）
Chie Imamura (*Toyota CRDL*)

1SDP-03 バクテリア生命システムとマイクロデバイスの融合
Hybrid system from a bacterium and a micro-device
○田端 和仁, 渡邊 力也, 野地 博行（東京大学大学院工学系研究科大学院応用化学専攻）
Kazuhito Tabata, Rikiya Watanabe, Hiroyuki Noji (*Grad. sch. eng., Univ. of Tokyo*)

1SDP-04 人工細胞の進化実験
Experimental evolution of artificial cell model
○四方 哲也[1,2]（[1]大阪大学大学院情報科学研究科, [2]科学技術振興機構 ERATOプロジェクト）
Tetsuya Yomo[1,2] ([1]*Graduate School of Information technology, Osaka University*, [2]*ERATO, JST*)

1SDP-05 合成生物学と美学
Aesthetics related with synthetic biology
○岩崎 秀雄[1,2]（[1]metaPhorest [生命美学プラットフォーム], [2]早大・理工）
Hideo Iwasaki[1,2] ([1]*metaPhorest [BioAesthetics Platform]*, [2]*Waseda Univ.*)

16:00～18:30　E 会場／Room E：Kyoto Int'l Conf. Center Room E

1SEP　新学術領域「天然変性タンパク質の分子認識機構と機能発現―生理的準安定状態を捉える新技術―」共催
相関構造生物学とX線溶液散乱
Integrative structural biology and biomolecular SAXS

オーガナイザー：佐藤 衛（横浜市立大学），清水 伸隆（高エネルギー加速器研究機構）
Organizer: Mamoru Sato (Yokohama City Univ.), Nobutaka Shimizu (KEK)

For deeper understanding of biomolecular function, it is important to analyze from many structural aspects combining several techniques in which the obtained resolution and the characteristic differ. SAXS is a method to obtain the low-resolution structure of biomolecule in solution. It is important to discuss about the structure-function relationship of biomolecules in the still larger space scale by combining a high resolution crystal structure analysis with the ab-initio low-resolution structure analysis in solution. In this symposium, not only the newest results but the current advancement of instrumentation in biomolecular SAXS will be presented.

1SEP-01 Computational Analyses of Protein Structures by Small Angle X-ray Scattering
Masaki Kojima, Yasumasa Morimoto, Takashi Nakagawa (*Tokyo University of Pharmacy and Life Sciences*)

1SEP-02 X線溶液散乱と分子動力学シミュレーションで探る蛋白質の構造揺らぎ
Protein structural fluctuations investigated by small-angle X-ray solution scattering and molecular dynamics simulations
○笠口 友隆[1], 池口 満徳[2]（[1]慶應義塾大学・理工学部・物理学科, [2]横浜市立大学・生命医科学研究科・生命医科学専攻）
Tomotaka Oroguchi[1], Mitsunori Ikeguchi[2] ([1]*Department of Physics, Faculty of Science and Technology, Keio University*, [2]*Graduate School of Medical Life Science, Yokohama City University*)

1SEP-03 カルビン回路調節複合体の SAXS と XRD による相関構造解析
Combined SAXS and XRD analysis of the Calvin cycle regulatory complex
○松村 浩由[1], 清水 伸隆[2], 井上 豪[1]（[1]大阪大学, [2]高エネルギー加速器研究機構）
Hiroyoshi Matsumura[1], Nobutaka Shimizu[2], Tsuyoshi Inoue[1] ([1]*Osaka University*, [2]*KEK*)

1SEP-04 結晶構造解析と小角散乱の併用
Applications of SAXS in structural analysis with macromolecular crystallography
○姚 閔（北海道大学大学院先端生命科学研究院）
Min Yao (*Faculty of Advanced Life Science*)

1SEP-05 フォトンファクトリーにおける小角散乱ビームラインの刷新
Refurbishment of SAXS beamlines at Photon Factory
○清水 伸隆（高エネ機構、物構研）
Nobutaka Shimizu (*IMSS, KEK*)

1SEP-06 SPring-8 理研構造生物学ビームライン I（BL45XU）の現状
Current status of RIKEN Structural Biology Beamline I (BL45XU) at SPring-8
○引間 孝明[1], 佐藤 広美[1], 河野 能顕[1], 上野 剛[1], 平田 邦生[1], 村上 博則[1], 八木 直人[1,2], 山本 雅貴[1]（[1]理研SPring-8センター, [2]JASRI/SPring-8）
Takaaki Hikima[1], Hiromi Sato[1], Yoshiaki Kawano[1], Go Ueno[1], Kunio Hirata[1], Hironori Murakami[1], Naoto Yagi[1,2], Masaki Yamamoto[1] ([1]*RIKEN SPring-8 Center*, [2]*JASRI/SPring-8*)

第 2 日目（10 月 29 日（火））／Day 2（Oct. 29 Tue.）

8:45～11:15　A 会場／Room A：Kyoto Int'l Conf. Center Room B-2

2SAA　生物ダイナミズムの源泉を問う―“非生物的揺らぎ”が生み出す“生物的揺らぎ”
Searching for the origins of the dynamism of life - how do random fluctuations turn into biological motions?

オーガナイザー：赤坂 一之（近畿大学），織田 昌幸（京都府立大学）
Organizer: Kazuyuki Akasaka (Kinki Univ.), Masayuki Oda (Kyoto Pref. Univ.)

The dynamism of all life on earth depends critically on the dynamism of bio-macromolecules themselves, most notably proteins. The origins of their conformational dynamism are actually “weak” interatomic potentials that fluctuate under physiological conditions. The fascination lies in the fact that the intrinsically random “non-biological fluctuations” of atoms and molecules are turned into specific “biological motions” to keep life going (like flowering, singing, running), by the work of life itself in the 38 billion years of evolution. Strategies for the fascinating works are beginning to be revealed through atomic, macromolecular, cellular and body levels, with “pressure” as the common variable.

2SAA-01 “非生物的揺らぎ”から“生物的揺らぎ”へ：そのからくりを問う
Turning random fluctuations into biological motions: the art work by Nature
○赤坂 一之（近畿大学先端技術総合研究所高圧力蛋白質研究センター）
Kazuyuki Akasaka (*High Pressure Protein Research Center, Institute of Adanced Technology, Kinki Univversity*)

2SAA-02 非生物的揺らぎから“生物的揺らぎ”への“水”の役割
Role of water to convert “non-biological” fluctuation to the “biological” one
○平田 文男（立命館大）
Fumio Hirata (*Univ.Ritsumeikan*)

2SAA-03 キャビティに依存した c-Myb R2R3 の構造揺らぎと DNA 結合能
Cavity-dependent conformational fluctuation and DNA-binding function of c-Myb R2R3
○織田 昌幸（京府大・院生命環境科学）
Masayuki Oda (*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*)

2SAA-04 圧力摂動やアミノ酸変異により生じる生物的揺らぎの変化：ユビキチン
Pressure and mutation enhances specific biological motion: Ubiquitin
○北原 亮（立命館大・薬）
Ryo Kitahara (*College of Pharm. Sci., Ritsumeikan Univ.*)

2SAA-05 イオンチャネルのゆらぎは細胞応答にどのように利用されているのか？
How do cells make use of single channel fluctuations for their responses?
○曽我部 正博（名古屋大・院医・メカノバイオロジー）
Masahiro Sokabe (*Mechanobiol. lab., Nagoya Univ. Grad. Sch. Med.*)

2SAA-06 ヒトで最進化した重力適応：揺らぎの階層性と分子シャペロン
Gravitational adaptation, evolved at the most in the human being: hierarchy of fluctuation and molecular chaperone
○跡見 順子[1], 藤田 恵理[1], 清水 美穂[1], 跡見 友章[2], 廣瀬 昇[2], 田中 和哉[2], 長谷川 克也[3]（[1]農工大院工・材料健康科学, [2]帝京科学大・理学療法, [3]宇宙航空研究開発機構）
Yoriko Atomi[1], Eri Fujita[1], Miho Shimizu[1], Tomoaki Atomi[2], Noboru Hirose[2], Kazuya Tanaka[2], Katsuya Hasegawa[3] ([1]*Tokyo Univ. of Agr. and Tech.*, [2]*Teikyo Univ. Sci.*, [3]*JAXA*)

8:45～11:15　B 会場／Room B：Kyoto Int'l Conf. Center Room B-1

2SBA　反応場デザインによる生命現象の再構成　-創って知る生物物理-
Reconstitution of life phenomena in a designed reaction field: Synthetic biology approach to Biophysics

オーガナイザー：多田隈 尚史（東京大学），古田 健也（情報通信研究機構），田川 美穂（名古屋大学）
Organizer: Hisashi Tadakuma (the Univ. of Tokyo), Ken'ya Furuta (NICT), Miho Tagawa (Nagoya Univ.)

Recent progresses in technology allow us to design the reaction field. In this symposium, we will focus on the motor protein / cytoskeletal function in designed reaction field to get to the heart of biological nanosystems.

2SBA-01 Designing the nano-reaction field: Introduction and application to motor protein research
Hisashi Tadakuma (*Graduate School of Frontier Science, The University of Tokyo*)

2SBA-02 Programming Nucleic Acids Self-Assembly
Peng Yin (*Wyss Institute for Biologically Inspired Engineering*)

2SBA-03 DNA セルフアセンブリによるナノ粒子超構造制御
DNA-mediated Nanoparticle Assembly
○田川 美穂[1], 磯貝 卓巳[1], 赤田 英里[1], 原田 俊太[1], 宇治原 徹[1], ヤンガー ケビン[2], ガング オレグ[2]（[1]名大・院工・マテリアル理工, [2]ブルックヘブン国研）
Miho Tagawa[1], Takumi Isogai[1], Eri Akada[1], Syunta Harada[1], Toru Ujihara[1], Kevin Yanger[2], Oleg Gang[2] ([1]*Dep. of Materials Sci. and Eng., Nagoya Univ.*, [2]*Brookhaven Nat. Lab.*)

2SBA-04 DNA ナノ構造上での分子運動の直接観察
Direct observation of molecular motions on the DNA nanostructure
○遠藤 政幸（京都大学 物質―細胞統合システム拠点）
Masayuki Endo (*Institute for Integrated Cell-Material Sciences, Kyoto University*)

2SBA-05 モータータンパク質集合体の自己組織化を操る
Controlling self-assembly of motor protein ensembles
○古田 健也（情報通研・バイオICT）
Ken'ya Furuta (*Bio ICT lab, NICT*)

2SBA-06 ミクロ閉鎖空間でアクトミオシン集合体がつくる秩序構造
Self-organized pattern formation by actomyosin mixtures in a cell-size confined space
○宮崎 牧人[1], 千葉 雅隆[1], 江口 宙輝[1], 石渡 信一[1,2]（[1]早大・物理, [2]早稲田バイオサイエンスシンガポール研究所）
Makito Miyazaki[1], Masataka Chiba[1], Hiroki Eguchi[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

2SBA-07 Directed actin self assembly and contractility
Laurent Blanchoin (*CEA Grenoble*)

8:45～11:15　C 会場／Room C：Kyoto Int'l Conf. Center Room C-1

2SCA　最新イオンチャネル 1 分子科学: 素過程から疾患克服まで
Single Ion Channels updated: From elementary processes to disease treatments

オーガナイザー：相馬 義郎（慶應義塾大学），老木 成稔（福井大学）
Organizer: Yoshiro Sohma (Keio Univ.), Shigetoshi Oiki (Univ. of Fukui)

Ion channels play pivotal roles in a number of essential physiological processes, and their dysfunctions lead to various human diseases. Ion channels are efficient targets of the pharmaceutical therapy and, historically, the single-molecule studies including the patch-clamp technique have successfully accelerated the channel-targeting drug developments. Recently the single molecule sciences *in vivo*, *in vitro* and *in silico* have been greatly advanced. This symposium is designed to mediate a closer interaction between biophysicists and physiologists for further inspiring each other.

2SCA-01 カリウムイオンチャネル KcsA のゲート開閉とリンクした脂質膜中での集合・離散ダイナミクス
Gating-related clustering-dispersion dynamics of the KcsA potassium channel on the membrane
○角野 歩[1], 山本 大輔[2], 炭竈 享司[1], 岩本 真幸[1], 出羽 毅久[3], 老木 成稔[1]（[1]福井大医, [2]福岡大理, [3]名工大院工）
Ayumi Sumino[1], Daisuke Yamamoto[2], Takashi Sumikama[1], Masayuki Iwamoto[1], Takehisa Dewa[3], Shigetoshi Oiki[1] ([1]*Fac. Med. Sci., Univ. Fukui*, [2]*Fac. Sci., Univ. Fukuoka*, [3]*Grad. Sch. Eng., Nagoya Inst. Tech.*)

2SCA-02 薬理解析により明らかになった、原核生物由来の膜電位感受性 Na チャネルにおける内腔の構造変化
The conformational rearrangement of the inner vestibule revealed by the pharmacological analysis of prokaryotic voltage-gated Na channels
○下村 拓史, 入江 克雅, 藤吉 好則（名大・細胞生理学研究センター）
Takushi Shimomura, Katsumasa Irie, Yoshinori Fujiyoshi (*CeSPI, Univ. Nagoya*)

2SCA-03 電位依存性 K+チャネルにおけるイオン透過機構に関する分子動力学的検討
Molecular Dynamics Study on Ion Conduction Mechanisms of a Voltage-sensitive Potassium Channel
○笠原 浩太[1], 城田 松之[2,3], 齊藤 俊幸[2], 近藤 寛子[2], 木下 賢吾[2,3,4]（[1]阪大蛋白研, [2]東北大院情報, [3]東北大ToMMo, [4]東北大加齢研）
Kota Kasahara[1], Matsuyuki Shirota[2,3], Toshiyuki Saito[2], Hiroko Kondo[2], Kengo Kinoshita[2,3,4] ([1]*IPR, Osaka Univ.*, [2]*Grad. Sch. Information Sci, Tohoku Univ.*, [3]*ToMMo, Tohoku Univ.*, [4]*IDAC, Tohoku Univ.*)

2SCA-04 Ligand-induced conformational changes in the cytoplasmic domain of inward rectifier potassium channels
Atsushi Inanobe (*Dept. Pharmacol., Grad. Sch. Med., Osaka Univ.*)

2SCA-05 高速 AFM によるアクアポリン 4 チャネルの直接観察
Direct observation of aquaporin-4 channels by high speed AFM
○山下 隼人, 会津 心之亮, 加藤 純悟, 阿部 陽一郎, 安井 正人, 相馬 義郎（慶應大・医・薬理学）
Hayato Yamashita, Shinnosuke Aizu, Jungo Kato, Yoichiro Abe, Masato Yasui, Yoshiro Sohma (*Pharmacol., Keio Univ. Med. Sch.*)

8:45～11:15　D 会場／Room D：Kyoto Int'l Conf. Center Room D

2SDA　新学術領域「少数性生物学―個と多数の狭間が織りなす生命現象の探究―」共催
少数個分子の協同が生み出す生命機能のメカニズム
Biological functions derived from cooperation of a small number of molecules

オーガナイザー：政池 知子（東京理科大学），広瀬 恵子（産業技術総合研究所）
Organizer: Tomoko Masaike (Tokyo Univ. of Sci.), Keiko Hirose (AIST)

Many of the biological processes utilize systems containing relatively small numbers of molecules. By cooperation, these molecules gain new functions, which cannot be explained by mere addition of the functions of individual molecules. Also, these systems cannot be readily studied by the methods we have been using for single molecules. In this symposium, we focus on recent progress in studying the mechanisms by which ensembles of a small number of molecules work.

2SDA-01　少数個分子の協働：その機構と意味解明へのアプローチ
Approaches to understand cooperative systems of small numbers of molecules
○広瀬 恵子（産総研・バイオメディカル）
Keiko Hirose (*Biomedical Res. Inst., AIST*)

2SDA-02　神経軸索への極性輸送の構造的基盤
Structural Basis for the Polarized Axonal Transport in Neuron
○岡田 康志（理研 生命システム研究センター）
Yasushi Okada (*QBiC RIKEN*)

2SDA-03　Microtubule organisation and dynamics in the anaphase spindle: properties of Cin8
Thomas Surrey (*Cancer Research UK*)

2SDA-04　生細胞内少数分子を調べるための蛋白質ラベル化技術
Protein labeling technology for investigating small number molecules in living cells
○水上 進[1,2]（[1]大阪大学大学院工学研究科生命先端工学専攻, [2]大阪大学免疫学フロンティアセンター）
Shin Mizukami[1,2] ([1]*Osaka Univ., Graduate School of Engineering*, [2]*Osaka Univ., IFReC*)

2SDA-05　１個から数個の分子が引き起こす運動と酵素反応のイメージング
Imaging of single to a few number of molecules in motion and their enzymatic reactions
○政池 知子[1,2], 池上 浩司[3], 瀬藤 光利[3], 鈴木 裕[4], 西坂 崇之[5]（[1]東京理科大・理工・応用生物科学, [2]JST さきがけ, [3]浜松医大・解剖学講座・細胞生物学分野, [4]旭川医大・生化学講座, [5]学習院大・理・物理）
Tomoko Masaike[1,2], Koji Ikegami[3], Mitsutoshi Setou[3], Hiroshi Suzuki[4], Takayuki Nishizaka[5] ([1]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*, [2]*PRESTO, JST*, [3]*Dept. Cell Biol. and Anat., Hamamatsu Univ. Sch. Med.*, [4]*Dept. Biochemistry, Asahikawa Medical Univ.*, [5]*Dept. Physics, Gakushuin Univ.*)

2SDA-06　骨格筋ミオシン分子複合体の力発生に特化したミオシン１分子の特性とダイナミクス
Molecular properties and dynamics of single skeletal myosins designed for force generations in ensemble of myosin molecules
○茅 元司, 樋口 秀男（東京大学 大学院理学系研究科 物理学専攻）
Motoshi Kaya, Hideo Higuchi (*University of Tokyo Dept of Physics*)

2SDA-07　分子イメージングから要素間の高次相互作用の定量化に向けて
Toward quantifying higher-order interactions among elements from molecular imaging
○小松崎 民樹[1,2,3]（[1]北海道大学電子科学研究所, [2]北海道大学生命科学院, [3]北海道大学数学連携推進センター）
Tamiki Komatsuzaki[1,2,3] ([1]*Research Institute for Electronic Science, Hokkaido University*, [2]*Graduate School of Life Science, Hokkaido University*, [3]*Research Center for Integrative Mathematics*)

8:45～11:15　E 会場／Room E：Kyoto Int'l Conf. Center Room E

2SEA　新学術領域「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
構造細胞生物学の生物物理学的こころ
Biophysical views in structural cell biology

オーガナイザー：箱嶋 敏雄（奈良先端科学技術大学院大学），深井 周也（東京大学）
Organizer: Toshio Hakoshima (NAIST), Shuya Fukai (The Univ. of Tokyo)

Recent advances in studies of cell-cell junctions and cell-matrix adhesions reveal that these junctions and adhesions play a role in mechano-sensing of applied forces and transduce the forces to chemical signals. Mechanotransduction by proteins comprising these contacts is now believed to control cell growth, division, motility and ultimately morphogenesis of tissues and organs. Mechanical and structural studies of these sensor proteins and mathematical description of cell mass have come on the stage center of the new era of cell biology.

はじめに
○箱嶋 敏雄（奈良先端大学院・構造生物学バイオサイエンス研究科）
Toshio Hakoshima (*Nara Institute of Science and Technology*)

2SEA-01　アドヘレンスジャンクションにおける張力感受性と上皮形態形成
Force sensitivity of the adherens junction and epithelial morphogenesis
○米村 重信（理化学研究所発生・再生科学総合研究センター電子顕微鏡解析室）
Shigenobu Yonemura (*Electron Microscope Laboratory, Riken Center for Developmental Biology*)

2SEA-02　構造細胞生物学の生物物理学的こころ
Biophysical views in structural cell biology
○箱嶋 敏雄（奈良先端大学院・構造生物学バイオサイエンス研究科）
Toshio Hakoshima (*Nara Institute of Science and Technology*)

2SEA-03　AFM を用いた接着結合分子の力学挙動解析
Mechanical Evaluation of Molecules at Adherens Junction using AFM
○韓 成雄[1], 牧 功一郎[1], 平野 良憲[2], 箱嶋 敏雄[2], 安達 泰治[1]（[1]京都大学, [2]奈良先端科学技術大学院大学）
Sung-Woong Han[1], Koichiro Maki[1], Yoshinori Hirano[2], Toshio Hakoshima[2], Taiji Adachi[1] ([1]*Kyoto University*, [2]*Nara Institute of Science and Technology*)

2SEA-04　Computational biophysics on epithelial tissue deformation: from molecular to tissue scale
Yasuhiro Inoue[1], Satoru Okuda[2], Tetsuya Fujii[3], Kohei Ohto[3], Taiji Adachi[1] ([1]*Inst. Front. Med. Sci., Kyoto Univ.*, [2]*CDB, RIKEN*, [3]*Dept. Microeng., Kyoto Univ.*)

2SEA-05　軸索伸長のためのシグナル－力変換機構
Signal-Force Transduction in Axon Outgrowth
○稲垣 直之（奈良先端大・バイオ）
Naoyuki Inagaki (*Grad. Sch. Bio., NAIST*)

2SEA-06　構造生物学からのコメント
Comments from the point of view of structural biology
○深井 周也[1,2,3]（[1]東京大学放射光連携研究機構生命科学部門, [2]東京大学分子細胞生物学研究所, [3]JST CREST）
Shuya Fukai[1,2,3] ([1]*Synchrotron Radiation Research Organization, The University of Tokyo*, [2]*Institute of Molecular and Cellular Biosciences, The University of Tokyo*, [3]*JST CREST*)

16:15～18:45　A 会場／Room A：Kyoto Int'l Conf. Center Room B-2

2SAP　ロドプシン研究の過去・現在・未来
Rhodopsin Research: Past, Present and Future

オーガナイザー：神取 秀樹（名古屋工業大学），寺北 明久（大阪市立大学）
Organizer: Hideki Kandori (Nagoya Inst.of Tech.), Akihisa Terakita (Osaka City Univ.)

Rhodopsin research is one of the main topics in biophysics (photobiology field), to which Japan has significantly contributed. Currently, activation mechanism of G protein-coupled receptors and optogenetic application have been attracting attentions for life scientists. In this symposium, we look back on the history of rhodopsin research, and discuss on the future outlook. For this aim, senior researchers give a talk and young scientists chair the presentations, by which we like to debate on what can be learned from rhodopsin research, and what is the future. Active discussion is very welcome.

2SAP-01　ロドプシン研究の新しい流れ
New trends in rhodopsin studies
○七田 芳則（京都大学大学院理学研究科生物科学専攻生物物理学教室）
Yoshinori Shichida (*Department of Biophysics, Graduate School of Science, Kyoto University*)

2SAP-02　ロドプシン群蛋白質の光誘起構造変化に関する X 線結晶解析
X-ray crystallographic studies on light-induced structural changes in rhodopsins
○神山 勉（名古屋大学理学研究科）
Tsutomu Kouyama (*Nagoya University, Graduate School of Science*)

2SAP-03　退色しないロドプシンから体色などを制御する非視覚性オプシンへ
From non-bleachable rhodopsin to non-visual opsins
○深田 吉孝（東京大学大学院理学系研究科生物化学専攻）
Yoshitaka Fukada (*Dept. Biophys. Biochem., Grad. Sch. Sci., Univ. Tokyo*)

2SAP-04　錐体 AL-OL 反応の基質特異性と活性の細胞内局在
Substrate Specificity and Localization of AL-OL Coupling Reaction in Carp Cones
佐藤 慎哉[1], 橘木 修志[1,2], 深川 貴志[2], ○河村 悟[1,2]（[1]大阪大学大学院理学研究科生物科学科, [2]大阪大学大学院生命機能研究科）
Shinya Sato[1], Shuji Tachibanaki[1,2], Takashi Fukagawa[2], **Satoru Kawamura**[1,2] ([1]*Department of Biological Sciences, Graduate School of Science, Osaka University*, [2]*Graduate School of Frontier Biosciences, Osaka University*)

16:15～18:45　B 会場／Room B：Kyoto Int'l Conf. Center Room B-1

2SBP　新学術領域「過渡的複合体が関わる生命現象の統合的理解」共催
過渡的複合体が関わる生命現象の統合的理解
Transient macromolecular complexes involved in multilevel biological phenomena

オーガナイザー：嶋田 一夫（東京大学），神田 大輔（九州大学）
Organizer: Ichio Shimada (The Univ. of Tokyo), Daisuke Kohda (Kyushu Univ.)

A view of “transient macromolecular complexes” is now widely recognized as one of the important concepts in multi level biological phenomena. Existing techniques of structural biology are not directly applicable due to the intrinsic instability and inhomogeneity of such transient complexes. We have conducted studies on new techniques for analyzing transient macromolecular complexes at atomic and molecular levels in a 5-year project for Grant-in-Aid for Scientific Research on Innovative Areas. We will discuss the potentiality of the new techniques in the filed of biophysics.

2SBP-01　メチル化 CpG 結合蛋白質 MBD4 による緩い基質 DNA 認識
Structural insight into versatile DNA recognition of methyl CpG binding protein MBD4
○有吉 眞理子[1], 大谷 淳二[2], 白川 昌宏[2]（[1]京大・iCeMS, [2]京大院・工）
Mariko Ariyoshi[1], Jyunji Otani[2], Masahiro Shirakawa[2] ([1]*iCeMS, Kyoto Univ.*, [2]*Grad. Sch. Eng., Kyoto Univ.*)

2SBP-02 タンパク質結晶中に意図的に創り出した空間を使って，タンパク質に結合した状態のリガンドの大振幅運動を解析する
Intentional creation of crystal-contact free space for monitoring large amplitude motions of ligands in protein crystals
○神田 大輔（九大・生医研）
Daisuke Kohda (*Med. Inst. Bioreg., Kyushu Univ.*)

2SBP-03 ゲル包埋型バイオリアクターを用いた生細胞内蛋白質間相互作用の NMR 観測
A gel-encapsulated bioreactor system for NMR studies of protein-protein interactions in living mammalian cells
○西田 紀貴[1], 嶋田 一夫[1,2]（[1]東大院薬系, [2]産総研・創薬分子プロファイリング研究センター）
Noritaka Nishida[1], Ichio Shimada[1,2] ([1]*Grad Sch Pharma Sci, Univ of Tokyo*, [2]*Molprof, AIST*)

2SBP-04 タンパク質解析のための生細胞でのケミカルラベリング
Chemical protein labeling in living systems for its analysis
○浜地 格（京都大学）
Itaru Hamachi (*Kyoto University*)

2SBP-05 ケモカイン受容体多量体形成による細胞動態の調節
Chemokine receptor oligomerization: a potential mechanism for regulating lymphocyte and cancer cell migration
○早坂 晴子, 小林 大地, 宮坂 昌之（大阪大学医学系研究科免疫動態学）
Haruko Hayasaka, Daichi Kobayashi, Masayuki Miyasaka (*Immunodyamics, Osaka Univ. Grad. Sch. Med.*)

2SBP-06 一回膜貫通型サイトカイン受容体 Mpl 二量体化の一分子蛍光解析
Single-molecule fluorescence analysis of the single-transmembrane cytokine receptor Mpl dimerization
坂本 明彦[1], 加藤 尚志[2], ○船津 高志[1]（[1]東大・院薬, [2]早大・教育・総合科学）
Akihiko Sakamoto[1], Takashi Kato[2], **Takashi Funatsu**[1] ([1]*Grd. Sch. Pharm. Sci., Univ. Tokyo*, [2]*Fac. Ed. and Int. Arts. Sci., Waseda Univ.*)

16:15〜18:45　C 会場／Room C：Kyoto Int'l Conf. Center Room C-1

2SCP　核内混み合い環境でのヌクレオソーム、クロマチンの機能発現機構
Functional dynamics of Nucleosome and Chromathin in Nuclear Crowding Environment

オーガナイザー：杉田 有治（理化学研究所），高橋 恒一（理化学研究所）
Organizer: Yuji Sugita (RIKEN), Koichi Takahashi (RIKEN)

Cellular nucleus is also a crowded environment where long DNA chains are packed with high densities. Recently, the highly packed DNA structures with DNA binding proteins have been investigated using X-ray crystallography, cryoelectron microscopy, and small-angle X-ray scattering (SAXS). These updated experimental information encourages multi-scale computational modeling of nucleosomes or chromatin fibers. In the symposium, both experimental and computational scientists show the latest data and discuss about the structures and dynamics of genomic DNA in chromosome or nucleus.

2SCP-01 ヌクレオソーム DNA 弛緩状態の自由エネルギープロファイル
Free Energy Profile for Nucleosomal DNA unwrapping
○河野 秀俊, 米谷 佳晃, 池部 仁善, 櫻庭 俊, 石田 恒（日本原子力研究開発機構量子ビーム応用研究部門分子シミュレーション）
Hidetoshi Kono, Yoshiteru Yonetani, Jinzen Ikebe, Shun Sakuraba, Hisashi Ishida (*Molecular Modeling and Simulation, JAEA*)

2SCP-02 クロマチン高次構造形成におけるヌクレオソーム構造多様性
Structural versatility of nucleosomes in higher order chromatin
○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*)

2SCP-03 モデルクロマチンの構造と転写因子ダイナミクスの粗視化シミュレーション研究
Structure of model chromatin and dynamics of transcription factors studied by coarse-grained simulations
○高田 彰二（京都大学理学研究科生物物理学教室）
Shoji Takada (*Department of Biophysics, Graduate School of Science, Kyoto University*)

2SCP-04 ヌクレオソームの線維は細胞内でどのように収納されているのか？
How is nucleosome fiber organized in the cell?
○前島 一博（国立遺伝学研究所）
Kazuhiro Maeshima (*National Institute of Genetics*)

2SCP-05 Diffusion-controlled reaction rate-laws in intracellular environment with molecular crowding: A single-particle-level simulation study
Kazunari Kaizu, Koichi Takahashi (*Laboratory for Biochemical Simulation, RIKEN Quantitative Biology Center (QBiC)*)

16:15～18:45　D 会場／Room D：Kyoto Int'l Conf. Center Room D

2SDP　ASB-BSJ Bilateral Symposium 2013

Organizer: Brett Hambly (University of Sydney), Jamie Vandenberg (President ASB, Victor Chang Cardiac Research Institute), Hiroyuki Noji (The University of Tokyo), Kuniaki Nagayama (National Institute for Physiological Sciences)

The Australian Society for Biophysics requests the opportunity to conduct a bilateral symposium of ~2.5 hours duration at the Biophysical Society of Japan annual conference in October 2013. The aims and purpose of the symposium are to promote the IUPAB 2014 Congress in Brisbane in 2014 to members of the BSJ. This will be achieved by 4 senior members and one early career member of ASB presenting their research results at the Symposium, to illustrate some aspects of biophysics research in Australia. Additionally, the President of ASB will briefly outline the breadth of biophysics in Australia, and the Congress Convenor will outline the structure and program of the Congress. We appreciate the opportunity that BSJ has provided us with to forge closer links between ASB and BSJ, and to promote the IUPAB 2014 Congress.

Opening remarks - Biophysics in Australia
Jawie Vandenberg (*President ASB*)

2SDP-01 High resolution imaging of malaria parasites with light, x-rays and electrons
Leann Tilley, Coralie Millet, Eric Hanssen, Matt Dixon (*Biochemistry Department, Bio21 Institute, University of Melbourne*)

2SDP-02 The dynamics of DNA origami nanostructures in Solution
Robert Hynson[1,2], Emeline Vernhes[1], Anthony Duff[3], Cy Jeffries[4], **Lawrence Lee**[1,2] ([1]*The Victor Chang Cardiac Research Institute*, [2]*The University of New South Wales*, [3]*Australian Nuclear Science and Technology Organisation*, [4]*The European Molecular Biology Organisation*)

2SDP-03 Ion channel gating and Japanese Puzzle Boxes
Jamie Vandenberg (*Victor Chang Cardiac Research Institute*)

2SDP-04 The nature of myocardial heart failure: Are hypertrophic cardiomyopathies all the same?
Amy Li[1], Dane King[1], Martijn Bos[2], Eleanor Kable[3], Peter Macdonald[4], Filip Braet[1,3], Brett Hambly[1], Shin'ichi Ishiwata[5], Michael Ackerman[2], Murat Kekic[1], Cristobal dos Remedios[1] ([1]*Bosch Institute, University of Sydney, Sydney 2006, Australia*, [2]*Mayo Medical School, Mayo Clinic, Rochester, Minnesota, 55902, USA*, [3]*Australian Centre for Microscopy & Microanalysis, University of Sydney 2006, Australia*, [4]*Heart & Lung Transplant Unit, St. Vincent's Hospital, Darlinghurst, Sydney 2010, Australia*, [5]*Department of Physics, Faculty of Science and Engineering, Waseda University, Tokyo 169-8050, Japan*)

2SDP-05 The role of matrix metalloproteinases in genetic thoracic aortic aneurysm
Yaxin Lu[1], Richmond Jeremy[2], Murat Kekic[1], Jianlin Yin[2], Donna Lai[1], **Brett Hambly**[1] ([1]*Pathology Discipline and Bosch Institute, University of Sydney*, [2]*Royal Prince Alfred Hospital, University of Sydney*)

Information on IUPAB 2014 Brisbane
Brett Hambly (*Congress convenor*)

16:15～18:45　E 会場／Room E：Kyoto Int'l Conf. Center Room E

2SEP　新学術領域「動く細胞と場のクロストークによる秩序の生成」共催

多細胞システムにおける秩序生成の仕組みを探る：動く細胞と場のクロストーク

Exploring mechanisms of emerging order in multicellular systems: Cross-talk between moving cells and microenvironment

オーガナイザー：宮田 卓樹（名古屋大学），上田 昌宏（大阪大学）

Organizer: Takaki Miyata (Nagoya Univ.), Masahiro Ueda (Osaka Univ.)

What is the principle by which moving cells with intrinsic fluctuations can develop into an ordered functional multicellular system? How can moving cells influence their environment and how can that environment restrict the randomness and freedom of cell movements, thereby alleviating strain and disorder in tissues and leading to a conducive, robust, and harmonious state? In this symposium, we introduce experimental and theoretical approaches to higher ordered phenomenon emerged through the cross-talk between individual cells and their environments in multicellular system, and discuss the principle.

2SEP-01　神経前駆細胞の集団的核移動の原理と意義

Collective nuclear migration of neural progenitors: mechanism and significance

宮田 卓樹, ○岡本 麻友美（名古屋大学大学院医学系研究科細胞生物学分野）

Takaki Miyata, **Mayumi Okamoto** (*Nagoya Univ Grad Sch Med, Anat & Cell Biol*)

2SEP-02　細胞の自発運動と確率的センシング機構との関連性

Spontaneous cell migration and its relevance to cellular stochastic sensing mechanism

○高木 拓明（奈良県立医大 医学部 物理学教室）

Hiroaki Takagi (*Department of physics, School of medicine, Nara medical University*)

2SEP-03　LFA-1/ICAM-1 によるリンパ球の‘stop and go’：ライブイメージングによるリンパ球の動態解析

Regulation of Lymphocyte “Stop and Go” via LFA-1 and ICAM-1: Lymphocyte Trafficking Analysis using Live Imaging Techniques

○木梨 達雄, 片貝 智哉, 植田 祥啓, 近藤 直幸（関西医科大学附属生命医学研究所分子遺伝学部門）

Tatsuo Kinashi, Tomoya Katakai, Yoshihiro Ueda, Naoyuki Kondo (*Dept. Molecular Genetics, Institute of Biomedical Science, Kansai Medical University*)

2SEP-04　肺の枝分れ構造の形成機構

Mechanism of lung branching morphogenesis

○三浦 岳（九州大学）

Takashi Miura (*Kyushu University*)

2SEP-05　Analysis of Tooth Germ Epithelium Morphogenesis by using Four-dimensional Cell Tracking System

Ritsuko Morita[1,2], Takashi Tsuji[2] ([1]*RIKEN CDB*, [2]*Research Inst. Sci. & Tech., Tokyo Univ. of Sci*)

2SEP-06　細胞外マトリクスとアピカル細胞膜のカップリングが気管上皮チューブの形状を決定する

Mechanical coupling of extracellular matrix with apical membrane specifies geometry of epithelial tubule

Dong Bo[1], Hannezo Edouard[2], ○林 茂生[1]（[1]理研CDB, [2]キュリー研究所）

Bo Dong[1], Edouard Hannezo[2], **Shigeo Hayashi**[1] ([1]*RIKEN CDB*, [2]*Institut Curie*)

9:45～12:15　A 会場／Room A：Kyoto Int'l Conf. Center Room B-2

3SAA　生物物理学の近未来-バイオ・ラマン研究の効きどころ-
The Points in Bio-Raman Research

オーガナイザー：盛田 伸一（理化学研究所），石垣 美歌（関西学院大学）
Organizer: Shin-ichi Morita (RIKEN), Mika Ishigaki (Kwansei Gakuin Univ.)

Recent Raman microscopy gives opportunity to measure concentrations of chemical species within individual live cells without chemical marking. (i) A single observation in bio-Raman research requires a few minutes, which allows continuous analysis of typical cellular responses; (ii) single molecular detection is possible using signal enhancing techniques; (iii) introducing Raman tags, one can visualize small bio-molecules. Through talks in the symposium, recent killer applications of the bio-Raman research will be clarified.

はじめに
○盛田 伸一（理化学研究所）
Shin-ichi Morita (*RIKEN*)

3SAA-01　表面増強ラマン散乱の機構解明と疾病関連分子や細胞表面タンパク質分子の超高感度検出への応用
Clarification of surface enhanced Raman scattering and its application to ultrasensitive detection of biomolecules
○伊藤 民武（産業技術総合研究所）
Tamitake Itoh (*AIST*)

3SAA-02　アルキンタグを用いた低分子化合物の生細胞ラマンイメージング
Alkyne-Tag Raman Imaging for Visualization of Small Molecules in Live Cells
○袖岡 幹子[1,2]（[1]理化学研究所, [2]ERATO, JST）
Mikiko Sodeoka[1,2] ([1]*RIKEN*, [2]*ERATO, JST*)

3SAA-03　ラマン散乱分光顕微鏡を用いた細胞状態を定義する「細胞指紋」の提案
Cellular fingerprints to distinguish and identify the various cellular states with Raman spectroscopy
○渡邉 朋信[1,2,3,4]（[1](独)理化学研究所生命システム研究センター, [2]大阪大学免疫学フロンティア研究センター, [3]大阪大学大学院生命機能研究科, [4](独)科学技術振興機構さきがけ）
Tomonobu Watanabe[1,2,3,4] ([1]*RIKEN, Quantitative Biology Center*, [2]*Immunology Frontier Research Center, Osaka University*, [3]*Graduate School of Frontier Bioscience, Osaka University*, [4]*PRESTO, Japan Science and Technology Agency*)

3SAA-04　ラマン分光イメージングが拓く新たな細胞周期ダイナミクス研究の可能性
In Vivo Raman Spectral Imaging of Cell Cycle Dynamics: Adding a New Dimension to Cell Cycle Research
黄 傳耿, 許 仁芳, ○重藤 真介（国立交通大・応化）
Chuan-Keng Huang, Jen-Fang Hsu, **Shinsuke Shigeto** (*Dept. Appl. Chem., National Chiao Tung Univ.*)

3SAA-05　スペクトル解析によるバイオ･ラマン研究
Spectral Analysis for Bio-Raman Research
○盛田 伸一（(独）理化学研究所 佐甲細胞情報研究室）
Shin-ichi Morita (*Cellular Informatics Laboratory, RIKEN*)

おわりに
○伊藤 民武（産業技術総合研究所）
Tamitake Itoh (*AIST*)

9:45～12:15　B 会場／Room B：Kyoto Int'l Conf. Center Room B-1

3SBA　光学イメージングによる脳神経研究の最前線―1 分子から in vivo まで―
Cutting-edge optical imaging approach to neuroscience -From single molecule to in vivo-

オーガナイザー：王 丹（京都大学），坂内 博子（名古屋大学）
Organizer: Dan Ohtan Wang (Kyoto Univ.), Hiroko Bannai (Nagoya Univ.)

Optical imaging provides a powerful approach to neuroscience - a rapidly evolving discipline filled with many fundamental unanswered questions. In this symposium, we aim to further the integration of cutting-edge optical imaging developed in the field of biophysics with brain science, one of the most important and challenging topics of this century. Seven leading neuroscience researchers will introduce their recent studies, taking advantage of various imaging techniques ranging from single molecule imaging to in vivo imaging and the development of novel technologies promoting future brain sciences.

3SBA-01　抑制性 GABA 作動性シナプス制御におけるカルシウムの驚くべき作用―1 分子イメージングで明らかになったこと―
Origin-dependent opposite effect of Ca^{2+} on the regulation of inhibitory $GABA_A$ receptor diffusion dynamics: a single molecule study
○坂内 博子[1,2], 丹羽 史尋[2], Triller Antoine[3], 御子柴 克彦[2] ([1]名大・院理・生命理学, [2]理研・脳センター, [3]IBENS, INSERM U1024, CNRS UMR8197）
Hiroko Bannai[1,2], Fumihiro Niwa[2], Antoine Triller[3], Katsuhiko Mikoshiba[2] ([1]*Nagoya Univ., Grad. Sch. Sci, Dept. Biol. Sci.*, [2]*RIKEN BSI*, [3]*IBENS, INSERM U1024, CNRS UMR8197*)

3SBA-02　シナプス内シグナル分子活性化のイメージングと操作
Imaging and controlling the activity of signaling molecules in dendritic spines of hippocampal neurons
○村越 秀治[1,2]（[1]自然科学研究機構生理学研究所, [2]科学技術振興機構さきがけ）
Hideji Murakoshi[1,2] ([1]*National Institute for Physiological Sciences*, [2]*PRESTO, JST*)

3SBA-03　Imaging with novel photochemical materials to study neuronal functions
Dan O Wang (*institute for integrated cell-material sciences*)

3SBA-04　STED imaging of synapses in living brain slices: from structure to function
U. Valentin Nägerl[1,2] ([1]*IINS, Univ. Bordeaux Segalen, France*, [2]*UMR 5297, CNRS, Bordeaux, France*)

3SBA-05　新規レーザー光技術による２光子顕微鏡の空間分解能、深部到達性の向上
Improvement of Reolution and Penetration Depth of Two-photon Microscopy with Novel Laser Techniques
○根本 知己（北海道大学電子科学研究所）
Tomomi Nemoto (*RIES, Hokkaido Univ.*)

3SBA-06　FRET sensing of transmembrane voltage
Hidekazu Tsutsui (*Osaka University*)

3SBA-07　グリアによる大脳皮質シナプス再編
Glial contribution to remodeling of cortical synapses
○鍋倉 淳一[1,2]（[1]生理研, [2]総研大）
Junichi Nabekura[1,2] ([1]*NIPS*, [2]*SOKENDAI*)

9:45～12:15　C 会場／Room C：Kyoto Int'l Conf. Center Room C-1

3SCA　生命現象の理解と核酸医薬を指向した機能性核酸の研究の最前線
Frontier of functional nucleic acids toward elucidation of biological events and nucleic acid medicine

オーガナイザー：片平 正人（京都大学），鳥越 秀峰（東京理科大学）
Organizer: Masato Katahira (Kyoto Univ.), Hidetaka Torigoe (Tokyo Univ. of Sci.)

Nucleic acids carry genetic information. This is, however, not all that nucleic acids can do. Other functions of nucleic acids have been emerging. DNA origami can be used as a molecular canvas to study various events of life science. A Microchip device with nucleic acids has been invented. Artificial base pairs have also been successfully developed. RNA aptamer can trap and inactivate a pathogenic protein. Pharmaceutical companies are pursuing to develop a next generation of drug with nucleic acids, nucleic acids medicine. Frontier of functional nucleic acids will be presented.

3SCA-01　DNA の構造と機能を制御するケミカルバイオロジー：DNA オリガミと人工遺伝子スイッチ
Chemical Biology that Controls DNA Structure and Function: DNA Origami and Artificial Genetic Switch
○杉山 弘[1,2]（[1]京都大学大学院理学研究科、, [2]物質―細胞統合システム拠点）
Hiroshi Sugiyama[1,2] ([1]*Department of Chemistry, Graduate School of Science, Kyoto University*, [2]*Institute for Integrated Cell-Material Sciences (iCeMS)*)

3SCA-02　抗プリオン活性を示すアプタマー及びカリウムイオンに感応して活性がスイッチングするインテリジェントリボザイム
Aptamer that exerts anti-prion activity and intelligent ribozyme whose activity switches in response to K^+
○片平 正人[1], 真嶋 司[1], 山置 佑大[1], 永田 崇[1], 西川 富美子[2], 西川 諭[2], 鎌足 雄司[3], 桑田 一夫[3]（[1]京都大学エネルギー理工学研究所, [2]産総研, [3]岐阜大）
Masato Katahira[1], Tsukasa Mashima[1], Yuudai Yamaoki[1], Takashi Nagata[1], Fumiko Nishikawa[2], Satoshi Nishikawa[2], Yuji Kamatari[3], Kazuo Kuwata[3] ([1]*Inst. of Adv. Energy, Kyoto Univ.*, [2]*AIST*, [3]*Gifu Univ.*)

3SCA-03　High-affinity DNA aptamer selection by a genetic alphabet expansion PCR system
Michiko Kimoto[1,2], Ken-ichiro Matsunaga[1], Rie Yamashige[1], Ichiro Hirao[1,2] ([1]*RIKEN CLST*, [2]*TagCyx Biotechnologies*)

3SCA-04　核酸医薬品への期待
The prospect for nucleic acid medicine
○坂田 恒昭[1,2]（[1]塩野義製薬（株）, [2]大阪大学大学院基礎工学研究科）
Tsuneaki Sakata[1,2] ([1]*Shionogi & Co., Ltd.*, [2]*Graduate School of Engineering Science, Osaka University*)

3SCA-05　熱力学的特性や速度論的特性に基づいた機能性核酸のデザイン戦略
Strategy to design functional nucleic acids based on their thermodynamic and kinetic properties
○鳥越 秀峰（東京理科大学理学部第一部応用化学科）
Hidetaka Torigoe (*Dep. Appl. Chem., Fac. Sci., Tokyo Univ. Sci.*)

3SCA-06　生命現象の理解に向けた超高速 DNA 分離と一分子 DNA メチル化検出のためのナノバイオデバイス
Nanobiodevices for ultrafast DNA separation and single molecular DNA methylation detection for the understanding of life phenomena
○湯川 博[1], 馬場 嘉信[1,2]（[1]名大 革新ナノバイオ研セ, [2]名大院工）
Hiroshi Yukawa[1], Yoshinobu Baba[1,2] ([1]*Res. Cent. Inno. Nanobio., Univ. Nagoya*, [2]*Grad. Sch. Eng., Univ. Nagoya*)

3SCA-07　RNA アプタマー医薬の開発動向
Development trends for RNA aptamer therapeutics
○宮川 伸（株式会社リボミック）
Shin Miyakawa (*RIBOMIC Inc.*)

9:45～12:15　D 会場／Room D：Kyoto Int'l Conf. Center Room D

3SDA　新学術領域「ナノメディシン分子科学」共催
個体の生物物理学ー分子・細胞・個体にブリッジー
Biophysics toward *In Vivo* work

オーガナイザー：樋口 秀男（東京大学），福田 紀男（慈恵会医科大学）
Organizer: Hideo Higuchi (The Univ. of Tokyo), Norio Fukuda (The Jikei Univ. Sch. of Med.)

One of the final goals of biophysics is to understand the in vivo functions of molecules including proteins, DNA and RNA. Physiological conditions in vivo are very different from those in experiments with purified proteins and in cultured cells. Therefore, in order to elucidate various processes in living systems at the molecular level, it is crucial to measure the functions of proteins in vivo. In this symposium, we will present excellent findings in mice and C. elengans eggs, and those of a heartbeat simulation study.

3SDA-01　動物の発生における PAR/aPKC 細胞極性システムの計測に基づいた数理モデル化
Measurement-based mathematical modeling of PAR/aPKC-dependent cell polarization in animal development
○荒田 幸信[1], 廣島 通夫[1,2], 白 燦基[1], 小林 徹也[3], 柴田 達夫[4], 佐甲 靖志[1]（[1]理研・佐甲細胞情報, [2]理研・生命システム・細胞シグナル動態, [3]東大・生産研・定量生物学, [4]理研・発生再生・フィジカルバイオロジー）
Yukinobu Arata[1], Michio Hiroshima[1,2], Chan-gi Pack[1], Tetsuya J. Kobayashi[3], Tatsuo Shibata[4], Yasushi Sako[1] ([1]*Cell. Info. Lab., Riken*, [2]*Lab. Cell Sig. Dyn., QBiC, Riken*, [3]*Inst. Indst. Sci., Univ. Tokyo*, [4]*Lab. Phy. Biol., CDB, Riken*)

3SDA-02　気管繊毛の運動と 3 次元構造解析
Ciliary motion and the three-dimensional structure in mouse respiratory cilia
○上野 裕則（愛教大 分子・生命）
Hironori Ueno (*Mol. func. and life sci., Aichi Univ. of Edu.*)

3SDA-03　非侵襲 in vivo 技術を用いたマウス内の好中球における高速小胞輸送解析
A non-invasive technique for the in vivo tracking of high-speed vesicle transport in mouse neutrophils
○菊島 健児, 喜多 清, 樋口 秀男（東大・院・理・物理）
Kenji Kikushima, Sayaka Kita, Hideo Higuchi (*Dept. Physics, Grad. Sch. Sci., Univ of Tokyo*)

3SDA-04　Real-time high-resolution cardiac imaging *in vivo*
Fuyu Kobirumaki-Shimozawa[1], Kotaro Oyama[2], Seine A. Shintani[2], Erisa Hirokawa[3], Togo Shimozawa[4], Takako Terui[5], Shin'ich Ishiwata[2], Norio Fukuda[1] ([1]*Dept. Cell physiol., Jieki Univ. Sch. Med.*, [2]*Dept. Physics, Waseda Univ.*, [3]*Jieki Univ. Sch. Med.*, [4]*Dept. Physics, Gakushuin Univ.*, [5]*Dept. Anesthes., Jieki Univ. Sch. Med.*)

3SDA-05　筋収縮の数理モデルとその心臓シミュレーションへの応用について
A numerical model of cross-bridge cycling and its application to a beating human heart
○鷲尾 巧[1], 米田 一徳[2], 高橋 彰仁[1], 杉浦 清了[1], 久田 俊明[1]（[1]東京大学 新領域, [2]富士通(株)）
Takumi Washio[1], Kazunori Yoneda[2], Akihito Takahashi[1], Seiryo Sugiura[1], Toshiaki Hisada[1] ([1]*Grad. Sch. of Fron. Sci., University of Tokyo,*, [2]*Fujitsu Ltd.*)

9:45～12:15　E 会場／Room E：Kyoto Int'l Conf. Center Room E

3SEA　アミロイド線維形成における膜界面の役割
Roles of Membrane Interface in amyloidogenesis

オーガナイザー：松崎 勝巳（京都大学），矢木-内海 真穂（ケンブリッジ大学）
Organizer: Katsumi Matsuzaki (Kyoto Univ.), Maho Yagi-Utsumi (Univ. of Cambridge)

Amyloidogenesis by proteins is involved in various diseases including neurodegenerative ones. Accumulating evidence suggests that membranes play a pivotal role in amyloidogenesis in vivo. Notably, membranes not only locally concentrate proteins but also lead to the formation of amyloid fibrils with different structures and toxicity from those formed in solution. This symposium will introduce recent advances in this research field and discuss roles of membrane interface in amyloidogenesis from various points of view.

3SEA-01 ガングリオシドクラスターを介したアルツハイマーアミロイド β タンパク質のフォールディングと凝集
Ganglioside Cluster-Mediated Folding and Aggregation of Alzheimer’s Amyloid beta-Protein
○松崎 勝巳（京大・院薬学）
Katsumi Matsuzaki (*Grad. Sch. Pharm. Sci., Kyoto Univ.*)

3SEA-02 ガングリオシドとの特異的な相互作用に伴うアミロイド関連タンパク質の構造転移の NMR 解析
NMR characterization of conformational transitions of amyloidogenic proteins upon their specific interactions with gangliosides
○矢木-内海 真穂（ケンブリッジ大）
Maho Yagi-Utsumi (*Univ. Cambridge*)

3SEA-03 アミロイドタンパク質の凝集過程のシミュレーション解析
Computational study on the aggregation and assemble process of amyoid beta proteins
○星野 忠次（千葉大学大学院薬学研究院）
Tyuji Hoshino (*Graduate School of Pharmaceutical Sciences, Chiba University*)

3SEA-04 脂質膜の物理的性質とアミロイド線維形成の関係
Relationship between physical properties of lipid membranes and amyloidogenesis
○三浦 隆史, 鈴木 麻紗子（東北大・院薬）
Takashi Miura, Masako Suzuki (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

3SEA-05 アミロイド形成型免疫グロブリン軽鎖可変ドメインの細胞毒性
Toward understanding the mechanism of cytotoxicity of amyloidogenic variable domain of immunoglobulin light chains
○浜田 大三（神戸大学大学院 医学研究科 生化学・分子生物学講座 構造生物学分野）
Daizo Hamada (*Division of Structural Biology, Department of Biochemistry and Molecular Biology, Graduate School of Medicine, Kobe University*)

3SEA-06 固体 NMR と TEM によるヒトカルシトニンとグルカゴンにおけるアミロイド線維形成と阻害機構の解明
Mechanisms of amyloid fibril formation and inhibition of human calcitonin and glucagon as revealed by solid-state NMR and TEM
○内藤 晶（横浜国立大学）
Akira Naito (*Yokohama National University*)

ポスター　Poster

第 1 日目（10 月 28 日（月））／Day 1（Oct. 28 Mon.）アネックスホール / Annex hall

01A. 蛋白質：構造 / 01A. Protein: Structure

1P001　高エネ機構フォトンファクトリーにおける創薬等支援基盤プラットフォーム事業による構造生物学研究の支援と高度化
Promotion of the Platform for Drug discovery, Informatics, and Structural life science (PDIS) project at Photon Factory in KEK
Ryuichi Kato[1], Naohiro Matsugaki[1], Yusuke Yamada[1], Leonard Chavas[1], Fumiaki Yumoto[1], Masato Kawasaki[1], Masahiko Hiraki[2], Toshiya Senda[1] ([1]*Photon Factory, IMSS, KEK*, [2]*Mechanical Engineering Center, ARL, KEK*)

1P002　海産無脊椎動物由来溶血性レクチン CEL-III の結晶化
Crystallization of the pore forming toxin CEL-III from marine invertebrate, Cucumaria echinata
Tomonao Nagao, Shuichiro Goda, Hideaki Unno, Tomomitu Hatakeyama (*Grad. Sch of Eng., Univ. Nagasaki*)

1P003　イェロープロテインの 150 ピコ秒時間分解能ラウエ構造解析
Time-resolved Laue crystallography of photoactive yellow protein with 150psec time resolution
Mikio Kataoka[1], Hironari Kamikubo[1], Friedrich Schotte[2], Hyun Sun Cho[2], Philip Anfinrud[2] ([1]*Grad. Sch. Mat. Sci., NAIST*, [2]*NIH*)

1P004　トマトモザイクウイルス複製タンパク質と阻害因子 Tm-1 の複合体形成機構の解明
Interaction mechanism of Tomato mosaic virus replication protein and the resistance factor Tm-1
Etsuko Katoh[1], Kazuhito Ishibashi[1], Chihoko Kobayashi[1], Hiroyoshi Matsumura[2], Masayuki Ishikawa[1] ([1]*National Institute of Agrobiological Sciences*, [2]*Osaka Univ.*)

1P005　真菌由来 TRP チャネル制御領域への Ca^{2+}イオン結合の結晶学的解析
Crystallographic analysis of the Ca^{2+}-binding sites in the regulatory-bundling region of the fungus TRP channel
Makoto Ihara[1,2], **Atsuko Yamashita**[1] ([1]*Grad. Sch. Med. Den. & Pharm. Sci., Okayama U.*, [2]*Facul. Agr., Kinki U.*)

1P006　分裂酵母由来の MAP キナーゼによりリン酸化される RNA 結合タンパク質 Nrd1 の構造解析
Structural studies of RNA-binding protein Nrd1, a fission yeast MAPK target RNA binding protein
Ayaho Kobayashi[1], Ryosuke Satoh[2], Toshinobu Fujiwara[3], Reiko Sugiura[4], Yutaka Ito[1], Masaki Mishima[1] ([1]*Grad. Sch. of Sci. & Eng., Tokyo Met. Univ.*, [2]*Lab. of Bacic Biol., Inst. of Micro. Chem.*, [3]*Grad. Sch. of Pharm. Sci., Nagoya City Univ.*, [4]*Grad. Sch. of Pharm. Sci., Kinki Univ.*)

1P007　Structural analysis of *C. elegans* innexin-6 gap junction channels by electron microscopy
Tomohiro Matsuzawa[1], Kazuyoshi Murata[2], Kouki Nishikawa[3], Yoshinori Fujiyoshi[3], Atsunori Oshima[3] ([1]*Grad. Sch. Sci., Univ. Kyoto*, [2]*NIPS*, [3]*CeSPI, Univ. Nagoya*)

1P008　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[1], Takayuki Kato[1], Keiichi Namba[1,2] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*QBiC, Riken*)

1P009　らせん対称性に基づく構造解析のための 15 プロトフィラメント微小管の調製
Preparation of seamless 15-protofilament microtubules for helical reconstruction of microtubules
Hiroko Takazaki[1], Takashi Fujii[2], Seiichi Uchimura[1], Rie Ayukawa[1], Keiichi Namba[3], Etsuko Muto[1] ([1]*BSI., Riken*, [2]*QBiC., Riken*, [3]*Grad. Sch. Frontier Biosci., Univ. Osaka*)

1P010　CD72 の構造解析に向けて
Towards the structure analysis 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.*)

1P011　多周波電子スピン共鳴によるスピンラベル変性タンパクのダイナミックス
Dynamics of Spin-labeled Denatured Protein Studied by Multi-frequency electron paramagnetic resonance
Yasunori Ohba[1], Tetsuya Itabashi[1], Munehito Arai[2], Jun Abe[3], Satoshi Takahashi[1], Seigo Yamauchi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Art and Sci, Univ. Tokyo*, [3]*IMS*)

1P012　一分子力学計測による Sup35NM の不均一構造の解明
Single Molecule Studies on the Conformational Heterogeneity of Sup35NM Structure
Yusuke Komi[1], Maillard Rodrigo[2], Carlos Bustamante[2], Motomasa Tanaka[1] ([1]*BS Inst., RIKEN*, [2]*HHMI/UC Berkeley*)

1P013　Small-angle X-ray scattering constraints and secondary-structural information can construct a coarse-grained residue-based protein model
Yasumasa Morimoto, Masaki Kojima (*Sch. Life Sci., Tokyo Univ. Pharm. & Life Sci*)

1P014　Effect of methanol on the structure of α-chymotrypsinogen A
Koichi Murayama (*Grad. Sch. Med., Gifu Univ.*)

1P015　二次構造形成に関わる分子内および分子間相互作用に関する量子化学研究
Quantum chemical study of intra- and inter-molecular interactions in secondary structures
Yu Takano, Haruki Nakamura (*Research Center for State-of-the-Art Functional Protein Analysis Institute for Protein Research, Osaka University*)

1P016　高分子複合体の密度マップ・原子モデルの混合正規分布モデルを用いた重ね合わせ計算
Superimposing density maps and atomic models of macromolecular complexes using Gaussian mixture model
Takeshi Kawabata, Hirofumi Suzuki, Akira Kinjo, Haruki Nakamura (*Institute of Protein Research, Osaka University*)

1P017 **Edge strand と central strand は異なったペアパートナー選択傾向を示す**
Edge and central strands show a different propensity for pairing partners
Hiromi Suzuki (*Sch Agri., Meiji Univ.*)

1P018 **PDB の成熟度を利用したホモロジーモデリング手法**
A new homology modeling technique that utilizes the knowledge of completeness of the PDB
Takahiro Kanemitsu[1], Shintaro Minami[2], George Chikenji[1] ([1]*Grad. Sch. of Engineering, Univ. Nagoya*, [2]*Res.Sch.of info sci, Univ. Nagoya*)

1P019 **タンパク質の構造コンプライアンス特性の計算**
Computation of the Structural Compliance Characteristics of Proteins
Keisuke Arikawa (*Fcl. Eng. , Kanagawa Inst. of Tech.*)

1P020 **NRSF/REST の競合誘起天然変性に起因する動的特性**
Dynamical Property due to Frustration Induced Intrinsic Disorder of NRSF/REST
Katsuyoshi Matsushita[1,3], Hidetoshi Sugihara[1,3], Macoto Kikuchi[1,3,4], Tomoaki Nogawa[5], Munetaka Sasaki[6] ([1]*Cybermedia Centery, Osaka University*, [2]*Institute for Protein Research, Osaka University*, [3]*Graduate School of Science, Osaka University*, [4]*Graduate School of Frontier Biosciences, Osaka University*, [5]*Faculty of Medicine, Toho University*, [6]*Department of Applied Physics, Tohoku University*)

1P021 **Adaptive lambda square dynamics シミュレーション：生体分子の効率的な構造探索法**
Adaptive lambda square dynamics simulation: an efficient conformational sampling method for biomolecules
Jinzen Ikebe, Shun Sakuraba, Hidetoshi Kono (*MMS., JAEA*)

1P022 **アラニンペプチドモデルにおける溶媒和自由エネルギーの加算性**
Analysis of additivity in the alanine peptide model of protein solvation by molecular simulations
Hironori Kokubo, B. M. Pettitt (*UTMB*)

1P023 **MD シミュレーションによる設計したタンパク質間相互作用面の評価**
Evaluation of the designed protein binding interfaces as studied by MD simulation
Masaki Fukuda, Hironao Yamada, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu, Satoshi Akanuma, Akihiko Yamagishi (*Sch. of Life Sci., Tokyo Univ. of Pharm. and Life Sci.*)

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

1P024 **赤外分光法によるカルシウム結合タンパク質並びにカルシウム結合ペプチドアナログの配位構造解析**
Coordination to divalent cations by calcium-binding proteins and calcium-binding peptide analogues studied by FTIR spectroscopy
Masayuki Nara[1], Hisayuki Morii[2], Masaru Tanokura[3] ([1]*College of Liberal Arts and Sciences, Tokyo Medical and Dental University*, [2]*National Institute of Advanced Industrial Science and Technology (AIST)*, [3]*Graduate School of Agricultural and Life Sciences, University of Tokyo*)

1P025 **蛋白質複合体の高圧放射光 X 線小角散乱データに対するグローバルフィット解析**
Global fit analysis on high pressure synchrotron small-angle x-ray scattering data of protein complexes
Tetsuro Fujisawa[1,2], Keiichi Kameyama[1], Ryo Ishiguro[1,2] ([1]*Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University*, [2]*RIKEN SPring-8 Center*)

1P026 **放射光広角散乱法によるタンパク質熱転移に対する crowding 効果の研究**
Crowding effect on thermal transition of proteins clarified by SR-WAXS
Kazuki Takeuchi, Mitsuhiro Hirai (*Graduate School of Engineering, Gunma University*)

1P027 **硬骨魚類の乳酸脱水素酵素活性の温度依存性**
Thermal stability of lactate dehydrogenase of marine teleostei: molecular adaptation of ectothermic animal to low temperature
Mizuki Nakagawa[1], Mika Yonezawa[1], Shigeyoshi Nakamura[2], Shun-Ichi Kidokoro[2], Hideki Wakui[1], **Wataru Nunomura**[1] ([1]*Life Sci., Grad. Sch. Eng. & Resource Sci., Akita Univ.*, [2]*Dept. Bioeng., Nagaoka Univ. Tech.*)

1P028 **疎水性溶媒が蛍光タンパク質の蛍光特性に及ぼす影響**
Effects of organic solvents on the properties of fluorescent proteins
Hideaki Konishi, Suguru Asai, Kunio Takeyasu, Shigehiro Yoshimura (*Kyoto university*)

1P029 **神経小胞融合過程におけるシナプトタグミンと SNARE の分子機構**
molecular mechanism of synaptotagmin and SNARE in the synaptic vesicle fusion process
Yasuhito Nagai, Tadashi Takemori (*Grad. Sch. Pure and appl sci., Univ. Tsukuba*)

1P030 **PLC-δ1 PH ドメインの分子内アロステリー**
Intramolecular allostery in the PLC-δ1 PH domain
Michikazu Tanio, Katsuyuki Nishimura (*Institute for Molecular Science*)

1P031 **分子動力学を用いた細菌機械受容チャネル MscL の脂質膜の厚みに影響される開口挙動に関する研究**
Molecular Dynamics Study on the Opening Behavior of Bacterial Mechanosensitive Channel MscL Effected by Membrane Thickness
Hiroki Katsuta[1], Yasuyuki Sawada[2], Masahiro Sokabe[2] ([1]*Sch. of Med., Nagoya Univ.*, [2]*Dept. Physiol. Nagoya Univ. Grad. Sch. Med.*)

1P032 **分子動力学シミュレーションを用いた大腸菌機械受容チャネル MscL のゲーティングに関するゆらぎ解析**
Fluctuation Analysis Study on Mechano-Gating in the E-coli Mechanosensitive Channel MscL Using Molecular Dynamics Simulations
Yuya Nakagawa, Yasuyuki Sawada, Masahiro Sokabe (*Dept. Physiol. Nagoya Univ. Grad. Sch. Med*)

1P033 **大腸菌機械受容チャネル MscL の開口過程においてメカノセンサーとゲートは密接に連動する**
Mechanosensor and gate is tightly coupled in the opening process of the bacterial mechanosensitive channel MscL
Yasuyuki Sawada[1], Takeshi Nomura[2], Masahiro Sokabe[1] ([1]*Dept. Physiol. Nagoya Univ. Grad. Sch. Med.*, [2]*Dept. Physiol. Kyoto Pref. Univ. Med.*)

1P034 **H^+/Ca^{2+}交換輸送体における対向輸送の分子基盤**

Structural Basis for the Counter-Transport Mechanism of a H^+/Ca^{2+} Exchanger

Tomohiro Nishizawa[1], Satomi Kita[2], Andres Maturana[3], Noritaka Furuya[1], Kunio Hirata[4], Go Kasuya[1], Satoshi Ogawsawara[6], Naoshi Dohmae[5], Takahiro Iwamoto[2], Ryuichiro Ishitani[1], Osamu Nureki[1] ([1]*Dept. Biophys. and Biochem., Grad. Sch. Sci., Univ. of Tokyo*, [2]*Dept. Pharmacol., Fac. Med., Fukuoka Univ.*, [3]*Dept. Bioengineering Sci., Grad. Sch. of Bioagricul. Sci.*, [4]*RIKEN SPring-8*, [5]*RIKEN Advanced Sci. Inst.*, [6]*Grad. Sch. of Med. and Faculty of Med., Kyoto Univ.*)

1P035 **RND 型薬剤排出トランスポーターの阻害活性の構造的基礎**

Structural basis for the inhibition of bacterial multidrug exporters

Keisuke Sakurai[1], Ryosuke Nakashima[1], Seiji Yamasaki[1,2], Katsuhiko Hayashi[1,2], Kunihiko Nishino[1], Akihito Yamaguchi[1] ([1]*Institute of Scientific and Industrial Research, Osaka University*, [2]*Graduate School of Pharmaceutical Sciences, Osaka University*)

1P036 **極低温電子顕微鏡を用いた電圧感受性 Na チャネルの立体構造と機能**

Two alternative conformations of a voltage-gated sodium channel

Kazutoshi Tani[1], Ching-Ju Tsai[2], Katsumasa Irie[1], Yoko Hiroaki[1], Takushi Shimomura[1], Duncan G. McMillan[3], Gregory M. Cook[3], Gebhard Schertler[2], Yoshinori Fujiyoshi[1], Xiao-Dan Li[2] ([1]*CeSPI, Nagoya Univ.*, [2]*Biomol. Res. Lab., Paul Scherrer Inst.*, [3]*Dept. Micro. Immun., Univ. Otago*)

1P037 **Roles of two coupling helices between transmembrane and cytosolic domains in ABC transporter**

Tomohiro Yamaguchi, Ryohei Jinushi, Sho Masuko, Toru Nakatsu, Hiroaki Kato (*Grad. Sch. Pharm. Sci., Kyoto Univ.*)

1P038 **分子動力学シミュレーションで探る CFTR における変異の影響**

The effects of mutations in CFTR as studied by molecular dynamics simulations

Mitsuhiko Odera[1], Tomoka Furukawa-Hagiya[1], Tadaomi Furuta[1], Yoshiro Sohma[2], Minoru Sakurai[1] ([1]*Center for Biol. Res. Info., Tokyo Tech*, [2]*Dept of Pharmacol., Sch. Med., Keio Univ.*)

1P039 **分子動力学シミュレーションで探るセルラーゼ *Tr*Cel7A の基質取り込みのメカニズム**

Mechanism of substrate uptake in cellulase *Tr*Cel7A as studied by molecular dynamics simulations

Takashi Kanazawa, Minoru Sakurai, Tadaomi Furuta (*Center for Biol. Res. Info., Tokyo Tech*)

1P040 **μs スケールの分子動力学シミュレーションによる光受容タンパク質 LOV-HTH の光応答機構の研究**

Study of the photoresponsive mechanism of LOV-HTH protein using μs scale molecular dynamics simulations

Tetsuo Kokubu, Tadaomi Furuta, Minoru Sakurai (*Center for Biol. Res. & Inform., Tokyo Tech*)

1P041 **ABC トランスポーターのヌクレオチド結合ドメイン二量体化の理論的解析—ATP と水の役割**

Theoretical analyses of the nucleotide-binding domain dimerization of ABC transporters: roles of ATP and water

Tomohiko Hayashi[1], Tomoka Furukawa-Hagiya[2], Chiba Shuntaro[2], **Tadaomi Furuta**[2], Norio Yoshida[3], Minoru Sakurai[2] ([1]*Inst. Adv. Energy, Kyoto Univ.*, [2]*Center for Biol. Res. Info., Tokyo Tech*, [3]*Dept. Chem,. Fac. Sci., Kyushu Univ.*)

1P042 **ADP/ATP 透過担体の大規模構造変化に関する理論的研究**

A theoretical study on the large conformational change of ADP/ATP carrier

Koichi Tamura, Shigehiko Hayashi (*Grad. Sch. Sci., Univ. Kyoto*)

1P043 **Computational design of short peptide inhibitors of protein-protein interactions in intracellular signaling mediated by CRK-SH2**

Junya Yamagishi[1,2], Noriaki Okimoto[2], Takuma Kasai[2], Atsushi Suenaga[3], Mariko Okada[2], Akira Imamoto[4], Makoto Taiji[1,2] ([1]*University of Tokyo*, [2]*RIKEN*, [3]*AIST*, [4]*University of Chicago*)

1P044 **Electrostatic similarities between protein and small molecules facilitate the rational design of protein-protein interaction inhibitors**

Arnout Voet, Francois Berenger, **Kam Zhang** (*Zhang Initiative Research Unit, Institute Laboratories, RIKEN*)

1P045 **レプリカ置換法による生体分子に対する効率的な構造サンプリン**

Efficient sampling for biomolecules by the replica-permutation method

Satoru Itoh[1,2], Hisashi Okumura[1,2] ([1]*IMS*, [2]*Sokendai*)

1P046 **Metadynamics: Implementation in GENESIS Software Package and Demonstration of the Efficient Computational Simulations of Biomolecules**

Raimondas Galvelis[1], Yuji Sugita[1,2,3] ([1]*RIKEN AICS*, [2]*RIKEN ASI*, [3]*RIKEN QBiC*)

1P047 **Motion Tree を利用した capping protein の動的構造解析**

Dynamical study of capping protein by Motion Tree

Motonori Ota[1], Shuichi Takeda[2], Yuichiro Maeda[2], Ryotaro Koike[1] ([1]*Info. Sci., Nagoya U.*, [2]*SBRC, Nagoya U.*)

1P048 **MSES により明らかになった蛋白質遭遇複合体構造アンサンブル**

Structural ensemble of protein encounter complex revealed by Multiscale Essential Sampling

Satoshi Omori, Kei Moritsugu, Akinori Kidera (*Grad. Sch. Med. Life Sci., Yokohama City Univ.*)

1P049 **独立成分分析 tICA を用いたタンパク質主鎖の遅い運動の解析**

Slow dynamics of protein backbone in molecular dynamics simulation revealed by time-structure based independent component analysis

Sotaro Fuchigami (*Grad. Sch. of Medical Life Science, Yokohama City Univ.*)

01C. 蛋白質：物性 / 01C. Protein: Property

1P050 **トリプリオンタンパク質に対する抗体 G2 の複数の抗原を特異的に認識する性質の特徴付け**

Characterization of multispecific monoclonal antibody G2 directed against chicken prion protein

Yuji Kamatari[1], Masayuki Oda[2], Takahiro Maruno[3], Yuji Kobayashi[3], Naotaka Ishiguro[4] ([1]*Life Sci. Res. Center, Gifu Univ.*, [2]*Grad. Sch. Life Env. Sci., Kyoto Pref. Univ.*, [3]*Graduate School of Engineering, Osaka University*, [4]*Fac. Applied Biol. Sci., Gifu Univ.*)

1P051　リソスタシンのカルサイト結合部位の同定

Identification of calcite-binding site of lithostathine

Seiya Togashi[1,2], Yuichi Hanada[1,2], Maho Nara[2,3], Sakae Tsuda[1,2] ([1]*Grad. Sch. Sci., Hokkaido Univ.*, [2]*BPRI, AIST*, [3]*Hokkaido High-Tech. Bio.*)

1P052　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イオンとの競合結合性

Competitive binding of metal ions to octarepeat region of prion protein

Masahiro Yagi, Kazuya Iwama, Haruto Onda, Wakako Hiraoka (*Graduate School of Science and Technology, Meiji University*)

1P053　QCM によるグルカゴンと酸性膜との相互作用解析

Analysis of interaction between glucagon and acidic lipid membrane by QCM

Takamichi Horie, Ayano Momose, Izumi Yamane, Hideki Fujita, Eri Yoshimoto, Izuru Kawamura, Akira Naito (*Grad. Sch. Eng., Yokohama Natl Univ.*)

1P054　ジンジパインプロテアーゼがもつ Ig-like domain の役割

Function of the Ig-like domain of gingipain proteinase

Keiko Sato[1], Hideharu Yukitake[1], Daisuke Nakane[2], Satoshi Shibata[1], Yuka Narita[1], Koji Nakayama[1] ([1]*Nagasaki Univ.*, [2]*Gakushuin Univ.*)

1P055　タンパク質の構造・安定性に及ぼす環状オリゴ糖およびポリオールの添加効果

Effects of polyol and cyclic oligosaccharide on structure and stability of protein

Takayuki Iokibe, Dai Katou, Takuya Hamada, Takayoshi Kimura (*Fac. Science, Kinki Univ.*)

1P056　タンパク質の熱安定性に及ぼすシクロデキストリンの包接効果

Inclusion effects of cyclodextrin on thermal stability of proteins

Toshiki Miki, Takayuki Iokibe, Takayoshi Kimura, Tadashi Kamiyama (*Fac. Science Kinki Univ.*)

1P057　粗視化シミュレーションを用いた STMV の自己組織化についての理論的研究

Theoretical study on the self-assembly of satellite tobacco mosaic virus using coarse grained simulation

Masato Teranishi, Micke Rusmerryani, Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Grad. Sch. Nat. Sci., Univ. Kanazawa*)

1P058　バクテリア細胞質の全原子分子動力学シミュレーション

All-Atom Molecular Dynamics Simulation of Bacterial Cytoplasm

Isseki Yu[1,2], Takaharu Mori[1], Jaewoon Jung[2], Ryuhei Harada[2], Yuji Sugita[1,2], Michael Feig[3] ([1]*RIKEN Advanced Science Institute*, [2]*RIKEN Advanced Institute for Computational Science*, [3]*Michigan State University*)

1P059　分子動力学シミュレーションによる構造エントロピー計算法の比較

Comparison of calculation methods of configurational entropy from molecular dynamics simulation trajectories

Simon Hikiri[1], Takashi Yoshidome[2], Mitsunori Ikeguchi[1,2] ([1]*Grad. Sch. of Nanobioscience, Yokohama City Univ.*, [2]*Grad. Sch. of Med. Life Sci., Yokohama City Univ.*)

1P060　分子モデリング法を用いた酸変性アポミオグロビンの構造解析

A Conformational Analysis of Acid Unfolded Apomyoglobin using a Novel Molecular Modeling Method

Yasutaka Seki[1], Takamasa Nonaka[1], Kunitsugu Soda[2] ([1]*Sch. of Pharm., Iwate Med. Univ.*, [2]*High Perform. Molec. Simula. Team, ASI, RIKEN*)

1P061　溶液中におけるタンパク質分子の配置の秩序性：小角 X 線散乱による解析

Protein's arrangement in aqueous solution before the self-assemblies: A small angle X-ray scattering study

Hiroshi Imamura[1], Takeshi Morita[1], Tomonari Sumi[2], Yasuhiro Isogai[3], Minoru Kato[4], Keiko Nishikawa[1] ([1]*Chiba Univ. Grad. Sch. Adv. Int. Sci.*, [2]*Okayama Univ. Dept. Chem*, [3]*Toyama Pref. Univ. Fac. Eng.*, [4]*Ritsumeikan Univ. Dept. Pharm.*)

1P062　チロシン/チロシネート蛍光法における圧力軸の有用性: 700 MPa を用いたニワトリオボムコイドの圧力変性研究

Utility of pressure axis on tyrosine/tyrosinate fluorescence spectroscopy: A pressure-unfolding study of chicken ovomucoid at 700 MPa

Akihiro Maeno[1,2], Hiroshi Matsuo[3], Kazuyuki Akasaka[1] ([1]*HPPRC, Kinki Univ.*, [2]*Dep. of med., Wakayama med. Univ.*, [3]*NICO*)

1P063　Staphylococcal nuclease におけるマイクロ秒スケールの主鎖の運動

Main-chain dynamics of staphylococcal nuclease in microsecond timescale

Takahiro Matsumoto, Mariko Yamaguchi, Rumi Shiba, Hironari Kamikubo, Yoichi Yamazaki, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

1P064　Staphylococcal nuclease の変性状態における局所構造の柔軟性と非局所的相互作用の関係

Local flexibility of denatured structure and its relationship to non-local interaction in staphylococcal nuclease

Toshiyuki Minemura, Mariko Yamagichi, Yoichi Yamazaki, Hironari Kamikubo, Mikio Kataoka (*Nara Institute of Science and Technology*)

1P065　FUS/TLS タンパク質の凝集が関与する筋萎縮性側索硬化症の新たな分子病理メカニズム

A new pathomechanism of amyotrophic lateral sclerosis regulated by aggregation of FUS/TLS protein

Takao Nomura[1], Shoji Watanabe[2], Kumi Kaneko[3], Koji Yamanaka[4], Nobuyuki Nukina[5], **Yoshiaki Furukawa**[1] ([1]*Dept. of Chem., Keio Univ.*, [2]*Doshisya Univ.*, [3]*RIKEN, BSI*, [4]*Nagoya Univ.*, [5]*Juntendo Univ.*)

1P066　1 分子蛍光イメージングによる脱凝集シャペロン Hsp104 の作用機構の解明

Mechanism of Hsp104 disaggregase by single-molecule imaging

Momoko Okuda, Dai Nakasaka, Tatsuya Niwa, Hideki Taguchi (*Grad. Sch. of Biosci. Biotech., Tokyo Tech*)

1P067　酵母プリオン Sup35 の細胞内 1 粒子解析

Single Particle Tracking of Yeast Prion Sup35 in Living Cells

Keita Yasaka[1], Shigeko Kawai-Noma[2], Hayashi Yamamoto[3], Hideki Taguchi[1] ([1]*Grad. Sch. of Biosci. Biotech., Tokyo Tech*, [2]*Grad. Sch. of Appl. Chem. & Biotech., Chiba Univ.*, [3]*Front. Res. Cent., Tokyo Tech*)

1P068　高圧 Native PAGE 法によるリゾチーム変異体が形成するアミロイド原繊維のかい離過程の定量的解析

Quantitative Analysis of High Pressure Native PAGE on Dissociation of Lysozyme Variant Amyloid Protofibril

Ryo Ishiguro[1,2], Hiroshi Matsuo[3], Keiichi Kameyama[1], Hideki Tachibana[4], Tetsuro Fujisawa[1,2] ([1]*Fac. Eng., Gifu Univ.*, [2]*SPring-8, RIKEN*, [3]*NICO*, [4]*Grad. Sch. Biol. Oriented Sci. Tech., Kinki Univ.*)

1P069 リゾチームジスルフィド欠損変異体の線維化反応の温度依存性

Temperature-Dependence of Fibrillation of Lysozyme Disulfide-Deficient Variant

Hideki Tachibana[1,3], Ryohei Kono[2,3] ([1]*Fac Biol-Ortd Sci Tech, Kinki Univ*, [2]*Wakayama Med Univ*, [3]*High-Pres Prot Res Center, Kinki Univ*)

1P070 SEP タグを用いたタンパク質凝集の時系列的解析

Analysis of protein aggregation kinetics using short amino acid peptide tags

Yutaka Kuroda[1], Alam Khan[1], Monirul Islam[1,2] ([1]*Dept of Biotech and Life Sci, Tokyo Univ Agr & Tech*, [2]*Dept Bioch and Mol Biol, Chittagong Univ*)

1P071 短いテトラペプチドの全原子シミュレーションによるアミノ酸の無定形な凝集性の洞察

All atom molecular dynamics simulation of short tetra-peptides shed insights into amino acid's amorphous aggregation propensities

Yuji Sato[1], Atsushi Suenaga[2], Satoshi Kosuda[1], Makoto Taiji[3], Yutaka Kuroda[1] ([1]*Department of Biotechnology and Life Sciences, Graduate School of Engineering, Tokyo University of Agriculture and Technology*, [2]*Molecular Profiling Research Center for Drug Discovery, AIST*, [3]*Quantitative Biology Center, RIKEN*)

01D. 蛋白質：機能 / 01D. Protein: Function

1P072 リポアミド脱水素酵素のフィードバック制御機構

The feedback regulation mechanism of dihydrolipoamide dehydrogenase

Tomoe Fukamichi[1], Hiromichi Nakashima[1], Etsuko Nishimoto[2] ([1]*Institute of Biophysics, Faculty of Agriculture, Graduate School of Kyushu University*, [2]*Molecular Bioscience, Bioscience and Biotechnology, Kyushu University*)

1P073 様々なアルカン産生シアノバクテリアに由来するアシル ACP 還元酵素の活性比較

Comparison of the activities of acyl-ACP reductases from various alkane producing cyanobacteria

Ryota Nawa[1], Fumitaka Yasugi[2], Yuuki Hayashi[2], Munehito Arai[1,2,3] ([1]*Dept. Basic Sci., Univ. Tokyo*, [2]*Dept. Life Sci., Univ. Tokyo*, [3]*PRESTO, JST*)

1P074 ニトリルヒドラターゼの触媒機構に関する理論的研究

Theoretical Study on Catalytic Mechanism of Nitrile Hydratase

Megumi Kayanuma[1], Kyohei Hanaoka[2], Mitsuo Shoji[2] ([1]*Grad. Sch. of Sys. and Inf. Eng., Univ. of Tsukuba*, [2]*Grad. Schl. of Pure & App. Sci., Univ. of Tsukuba*)

1P075 アデニル酸キナーゼ反応機構に関する ONIOM 法による研究

Study on the reaction mechanism of adenylate kinase with ONIOM method

Kenshu Kamiya (*Department of physics, School of science, Kitasato university*)

1P076 トレオニン合成酵素における反応制御機構の理論的解明

Theoretical elucidation on the reaction control mechanism in Threonine Synthase

Mitsuo Shoji[1,2], Kyohei Hanaoka[1], Yuzuru Ujiie[1], Wataru Tanaka[1], Megumi Kayanuma[3], Hiroaki Umeda[2], Yasuhiro Machida[4], Takeshi Murakawa[5], Hideyuki Hayashi[4] ([1]*Grad. Sch. of Pure & App. Sci., Univ. Tsukuba*, [2]*Center for Comp. Sci., Univ. Tsukuba*, [3]*Grad. Sch. of Sys. & Inf. Eng. Univ. Tsukuba*, [4]*Dep. Chem., Osaka Med. College*, [5]*Dep. of Biochem., Osaka Med. College*)

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

1P077 生体分子の分子動力学に対する時系列解析ー運動変化と構造変化の関係を探るー

Time-series analysis of molecular dynamics: Conformational change and dynamics of collective behavior

Kana Fuji[1], Masakazu Sekijima[2], Hiroshi Fujisaki[3], Mikito Toda[4] ([1]*Graduate of school Humanities and Sciences, Nara Women's Univ.*, [2]*GSIC, Tokyo Tech*, [3]*Phys., Nippon Medical School*, [4]*Sci. , Nara Women's Univ.*)

1P078 それぞれが複数の立体構造からなる複数の蛋白質構造の比較解析

Superposition of protein structures each of which is a set of multiple conformations

Takashi Amisaki, Shin-ichi Fujiwara (*Department of Biological Regulation, Faculty of Medicine, Tottori University*)

1P079 DFT によるテラヘルツ領域におけるアミノ酸とペプチドの低振動モードの帰属

DFT approach for the assignment of low-frequency vibrational modes of amino acids and peptides in the terahertz frequency region

Ohki Kambara (*RIE, Shizuoka Univ.*)

1P080 吸引式反応システムを用いたウェスタンブロッティング法によるペプチドの高感度検出

A new approach to detect small peptides clearly and sensitively by Western blotting using a vacuum-assisted detection method

Satoshi Tomisawa, Chiharu Abe, Masakatsu Kamiya, Takashi Kikukawa, Makoto Demura, Keiichi Kawano, Tomoyasu Aizawa (*Grad. Sch. Life Sci., Hokkaido Univ.*)

1P081 Grb2 結合にともなう EGFR のキネティクスとダイナミクスの１分子計測

Single-molecule measurements of kinetics and dynamics of an epidermal growth factor receptor upon Grb2-binding

Kenji Okamoto, Yasushi Sako (*RIKEN*)

1P082 ー分子力学測定によるポリプロリンヘリックスの高弾性の研究

Single molecule force spectroscopy by AFM indicates highly resilient structure of polyproline helix

Masaru Kawakami (*School of Materials Science, Japan Advanced Institute of Science and Technology*)

1P083 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 flexineri* by cryo electron microscopy

Naoko Kajimura[1,2], Martin P. Cheung[3], Takayuki Kato[1], Ariel J. Blocker[3], Keiichi Namba[1,4] ([1]*Grad. Sch. of Frontier Biosci., Osaka Univ.*, [2]*JEOL Co., Ltd.*, [3]*Sch. of Cell. & Mol. Med., Univ. of Bristol*, [4]*QBiC., RIKEN*)

1P084 **高分解能構造解析に向けた電子顕微鏡用カメラの評価**

Evaluation of cameras for high resolution structural analysis by cryoEM

Takayuki Kato[1], Tomoko Miyata[1], Keiichi Namba[1,2] ([1]*Grad. Sch. Frontier Biosci., Osaka univ.*, [2]*QBic, Riken*)

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

1P085 **Attempt of expression of the glycoprotein from *Richadella dulcifica***

Maria Namba, Naoya Hashikawa, Satoru Yamaguchi (*Okayama Univ. Sci.*)

1P086 **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*)

1P087 **An Information Theoretical Approach to Local Equilibrium State Analysis for Single-Molecule Time-Series**

J. Nick Taylor[1], C. B. Li[1], S. Kawai[1], Henning D. Mootz[2], Haw Yang[3], Tamiki Komatsuzaki[1] ([1]*Hokkaido University*, [2]*Westfalische Wilhelms-Universitat Muenster*, [3]*Princeton University*)

1P088 **理想タンパク質構造のデザイン原理**

Principles for designing ideal protein structures

Nobuyasu Koga[1], Rie Koga(Tatsumi)[1], Gaohua Liu[2], Rong Xiao[2], Gaetano T. Montelione[2], David Baker[1] ([1]*Univ. Washington, Dept. of Biochemistry*, [2]*Rutgers Univ., Dept. Mol. Biol. and Biochem.*)

1P089 **理想的な構造を持つ機能タンパク質の理論設計**

Theoretical design of functionalized proteins with ideal scaffold

Takahiro Kosugi, Nobuyasu Koga, Rie Tatsumi-Koga, David Baker (*Dept. of Biochem., Univ. Washington*)

1P090 **リポソーム内遺伝子発現を利用した進化工学による β-グルクロニダーゼの機能改変**

Directed Evolution of β-glucuronidase Using Liposome-based IVC

Takehiro Nishikawa[1], Takeshi Sunami[1,2], Tomoaki Matsuura[1,3], Tetsuya Yomo[1,2,4] ([1]*JST*, [2]*Grad. Sch. of Info. Sci. & Tech., Osaka Univ.*, [3]*Grad. Sch. of Eng., Osaka Univ.*, [4]*Grad. Sch. of Frontier Biosci., Osaka Univ.*)

1P091 **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」の構築と実践：リポソームと無細胞翻訳系による α-ヘモリシンの in vitro 分子進化**

Liposome Display: Directed evolution of membrane protein, alpha hemolysin, by using liposome and cell-free translation system

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

02. ヘム蛋白質 / 02. Heme proteins

1P092 **ニワトリクリプトクロム 1 のヘム結合モチーフ(HRM)の解析**

Characterization of HRM in Chicken Cryptochrome1

Yusuke Otsuka, Junya Kuzukawa, Keiko Okano, Toshiyuki Okano (*Dept. Eng. and Biosci., Grad. Sch. Adv. Sci. and Eng., Waseda Univ.*)

1P093 **線虫 cytochrome b561 ファミリーの生理機能解析**

Analyses on the physiological functions of the cytochrome b_{561} protein family in *C.elegans*

Yurie Hirano, Masahiro Miura, Motonari Tsubaki (*Dept. of Chem., Grad. Sch. of Sci., Kobe Univ.*)

1P094 **線虫 cytochrome b561 ホモログ Cecytb-1 の機能解析**

Analyses on the novel 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., Univ. Kobe*)

1P095 **Substrate access to slow substrate binding P450cam with mutation at the proposed gate for water egress/ingress from/to the active site**

Ayaka Kishimoto[1], Kenji Takagi[1], Tsunehiro Mizushima[1], Keisuke Sakurai[2], Katsuyoshi Harada[3], Takashi Hayashi[3], Hideo Shimada[1] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*Inst. Sci. Ind. Res., Osaka Univ.*, [3]*Grad. Sch. Eng., Osaka Univ.*)

1P096 **Heme serves as scaffold for substrate-driven active site structuring in cytochrome P450cam**

Kenji Takagi[1], Ayaka Kishimoto[1], Aya Amano[1], Keisuke Sakurai[2], Kazumasa Muramoto[1], Tsunehiro Mizushima[1], **Hideo Shimada**[1] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*Inst. Sci. Ind. Res., Osaka Univ.*)

1P097 **酵素反応の時間分解分光解析を実現するマイクロ流路フローフラッシュ法の開発**

Development of micro-channel flow-flash method for time-resolved spectroscopic study of enzymatic reactions

Tetsunari Kimura[1], Takehiko Tosha[1], Yoshitsugu Shiro[1], Minoru Kubo[1,2] ([1]*RIKEN*, [2]*PRESTO, JST*)

1P098 **C タイプヘム・銅酸素還元酵素の酸素消費活性の pH 依存性**

pH dependence of the oxygen consumption activity of the C-type heme-copper oxygen reductase

Yui Iwamoto[1], Yuriko Ando[1], Yoshitsugu Shiro[1,2], Kazumasa Muramoto[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*Harima Inst., RIKEN*)

03. 膜蛋白質 / 03. Membrane proteins

1P099 **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細胞内動態解析**

Lateral diffusion of metabotropic glutamate receptor observed in single-molecules on the living cell surface

Masataka Yanagawa[1], Michio Hiroshima[1,2], Takahiro Yamashita[3], Yoshinori Shichida[3], Yasushi Sako[1] ([1]*Cellular Informatics Laboratory, RIKEN*, [2]*Quantitative Biology Center (QBiC), RIKEN*, [3]*Department of Biophysics, Graduate School of Science, Kyoto University*)

1P100 **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], Shinsaku Maruta[1,2] ([1]*Dept. Bioinfo., Grad. Sch. Eng., Univ. Soka*, [2]*Dept. BioInfo., Fac. Eng., Univ. Soka*)

1P101 **Highly stable tubes of bovine mitochondrial F-ATP synthase suitable for electron cryo tomography**
Christoph Gerle[1], Chimari Jiko[2], Shintaro Maeda[1], Karen Davies[3], Werner Kuhlbrandt[3], Yoshinori Fujiyoshi[4], Kyoko Shinzawa-Ito[1], Shinya Yoshikawa[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*Inst. Prot. Res., Osaka Univ.*, [3]*Max Planck Biophys.*, [4]*CeSPI, Nagoya Univ.*)

1P102 **大腸菌多剤排出トランスポーター複合体 AcrAB の結合比決定**
AcrB-AcrA fusion protein indicates that multi-drug efflux transporter complex AcrAB coupling ratio is 1:1
Katsuhiko Hayashi[1,2], Ryosuke Nakashima[3], Keisuke Sakurai[3], Seiji Yamasaki[1,2], Kunihiko Nishino[4], Akihito Yamaguchi[3] ([1]*Dep. Cell Biol., Grad. Sch. Phrm. Sci., Osaka univ.*, [2]*Dep. Cell Memb. Biol., ISIR, Osaka univ.*, [3]*Lab. Cell Memb. Strac. Biol., ISIR, Osaka univ.*, [4]*Lab. Microbiol. Infec. Diseases, ISIR, Osaka univ.*)

1P103 **EGFR 細胞内領域の変異による EGF 受容体活性化**
Spontaneous activation of EGFR by mutations in its intracellular region in the absence of bound ligand
Hiraku Miyagi, Ichiro Maruyama (*OIST*)

1P104 **再構成膜におけるバクテリオロドプシンと部分フッ素化ホスファチジルコリンの低親和性**
Low affinity of bacteriorhodopsin to partially fluorinated phosphatidylcholine in reconstituted membrane
Masaru Yoshino[1], Kenji Kanayama[1], Takashi Kikukawa[2], Toshiyuki Takagi[3], Hiroshi Takahashi[1], Yasunori Yokoyama[4], Hideki Amii[1], Toshiyuki Kanamori[3], Masashi Somoyama[1] ([1]*Fac. Sch. Tech., Gunma Univ.*, [2]*Fac. Adv. Sci., Hokkaido Univ.*, [3]*R.C. Stem Cell Eng.*, [4]*Dept. Appl. Phys., Nagoya Univ.*)

1P105 **バクテリオロドプシンの色変異体に関する理論的研究**
A theoretical study on color variants of bacteriohrodopsin
Seiya Sugo[1], Motoshi Kamiya[1], Yuki Sudo[2], Shigehiko Hayashi[1] ([1]*Graduate School of Science, Kyoto Univ.*, [2]*Graduate School of Science, Nagoya Univ.*)

1P106 **計算機シミュレーションによる Hv1 プロトンチャネルの荷電性残基の影響の検討**
Evaluating the impact of charged residues in proton channel Hv1 by computer simulations
Matsuyuki Shirota[1,2], Susumu Chiba[1], Kota Kasahara[3], Hiroko Kondo[1], Kengo Kinoshita[1,2,4] ([1]*GSIS., Tohoku Univ.*, [2]*ToMMo, Tohoku Univ.*, [3]*IPR, Osaka Univ.*, [4]*IDAC, Tohoku Univ.*)

1P107 **Behavior of potassium ions around the potassium channel in relation to permeation events**
Toshiyuki Saito[1], Kota Kasahara[2], Matsuyuki Shirota[1,3], Hiroko Kondo[1], Kengo Kinoshita[1,3,4] ([1]*Grad. Sch. Information Sci, Tohoku Univ.*, [2]*IPR, Osaka Univ.*, [3]*ToMMo, Tohoku Univ.*, [4]*IDAC, Tohoku Univ.*)

1P108 **光駆動アニオンポンプハロロドプシンと発光タンパク質からなる融合タンパク質の特性**
Characteristic of fusion protein between light-driven anion pump halorhodopsin and luminescence protein
Kentaro Saito, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*School of Science and Technology, Meiji University*)

1P109 **ファラオニスハロロドプシンの高次構造及び機能に与えるカロテノイド結合の影響**
Effect of carotenoid binding to structure and function of Natronomonas pharaonis halorhodopsin
Kaede Suzuki, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*Grad. Sch. Sci. and Tech., Univ. Meiji*)

1P110 **アニオン結合に伴う膜タンパク質ハロロドプシンの三次構造変化の検出**
Detection of tertiary structural change of membrane protein halorhodopsin by anion binding
Takahiko Yokota, Noritake Katou, Yuri Mukai, Takanori Sasaki (*School of Science and Technology, Meiji University*)

1P111 **ハロロドプシン三量体が持つカロテノイド結合の特異性**
Specificity of carotenoid biding of trimer halorhodopsin
Yasuyuki Miyazaki, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*Sch. Sci. and Tech., Univ. Meiji*)

1P112 **異なるアミノ酸タグを持つ膜タンパクハロロドプシン同士での多量体形成**
Oligomer formation between membrane protein halorhodopsins with different amino acid tags
Tomokazu Wakatsuki, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*Sch. Sci. and Tech., Univ. Meiji*)

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

1P113 **PBSA 法による RecA リコンビナーゼの ssDNA 及び dsDNA との結合能の比較**
Comparison of ssDNA- and dsDNA-binding affinity of RecA recombinase using the PBSA method
Yuichi Kokabu, Mitsunori Ikeguchi (*Grad. Sch. Med. Life Sci., Yokohama city univ.*)

1P114 **DNA-binding-induced conformational changes in proteins**
Munazah Andrabi[3], Kenji Mizuguchi[1,2], **Shandar Ahmad**[1,2] ([1]*National Institute of Biomedical Innovation*, [2]*Graduate School of Frontier Bioscience, Osaka University*, [3]*Center for Developmental Biology, RIKEN*)

1P115 **Single-Molecule Studies on How Pif1 Helicases Regulate Telomerase Activity**
Hung-Wen Li[1], Jing-Ru Li[1], Jing-Jer Lin[2] ([1]*Dept. of Chemistry, National Taiwan Univ., Taiwan*, [2]*Institute of Biochemistry and Molecular Biology, National Taiwan Univ.*)

1P116 **(6–4)光回復酵素の二光子 DNA 修復反応機構**
A two photon DNA repair mechanism of the (6-4) photolyase
Junpei Yamamoto[1], Ryan Martin[2], Shigenori Iwai[1], Pascal Plaza[2], Klaus Brettel[3] ([1]*Grad.Sch.Eng.Sci., Osaka Univ.*, [2]*ENS Paris, France*, [3]*CEA Saclay, France*)

1P117 **Single Nucleosome under Tension and Torsion**
Jen-Chien Chang[1], Michel de Messieres[2], Arthur La Porta[1] ([1]*Dept. Phys., University of Maryland, USA*, [2]*National Institute of Health, Bethesda, MD, USA*)

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

1P118 **How does alcohol cause the transition of higher-order structure of DNA?**
Yuki Oda[1], Yuko Yoshikawa[3], Tadayuki Imanaka[3], Toshio Kanbe[2], Takahiro Kenmotsu[1], Kenichi Yoshikawa[1] ([1]*Faculty of Medical and Life Sciences, Doshisha University*, [2]*Nagoya University, School of Health Sciences*, [3]*Lab.Environ.Biotech, Ritsumeikann University*)

1P119 **Ultrasound-induced double-strand breaks in relation to the higher-order structure of DNA**
Rinko Kubota[1], Naoki Ogawa[1], Yukihiro Kagawa[1], Yuko Yoshikawa[2], Yoshiaki Watanabe[1], Takahiro Kenmotsu[1], Kenichi Yoshikawa[1] ([1]*Faculty of Life and Medical Sciences, Doshisha University*, [2]*Lab. Environ. Biotech., Ritsumeikan University*)

1P120 **新規抗がん性二核白金(Ⅱ)錯体による DNA の立体構造変化**
Conformational change of DNA induced by novel antitumor dinuclear Pt(II) complexes
Akira Muramatsu[1], Yuko Yoshikawa[2], Seiji Komeda[3], Wakao Fukuda[2], Tadayuki Imanaka[2], Toshio Kanbe[4], Kenichi Yoshikawa[1] ([1]*Faculty of Life and Medical Sciences, Doshisha University*, [2]*College of Life Sciences, Ritsumeikan University*, [3]*Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science*, [4]*School of Medicine, Nagoya University*)

1P121 **Mg(2+) causes shrinking on DNA but prevents spermidine(3+)-induced compaction**
Chika Tongu[1], Yuko Yoshikawa[2], Anatoly A Zinchenko[3], Ning Chen[3], Takahiro Kenmotsu[1], Kenichi Yoshikawa[1] ([1]*Faculty of Bio and Medical Sciences,Doshisha University*, [2]*Ritsumeikan University*, [3]*Nagoya University*)

1P122 **一本鎖 DNA 結合蛋白質の DNA-SWNT 複合体への選択的な吸着**
Selective adhesion of single-stranded DNA binding protein to DNA-SWNT hybrids
Daisuke Nii, Takuya Hayashida, Kazuo Umemura (*Graduate School of Science, Tokyo University of science*)

1P123 **全反射蛍光顕微鏡によるショウジョウバエ RNAi 酵素複合体形成の基本過程の解明**
Defining fundamental steps in the assembly of Drosophila RNAi enzyme complex by TIRF microscopy
Hiroshi M. Sasaki[1], Shintaro Iwasaki[1], Yuriko Sakaguchi[2], Tsutomu Suzuki[2], Hisashi Tadakuma[3], Yukihide Tomari[1,3] ([1]*IMCB, Univ. of Tokyo*, [2]*Dept. Chem. Biotech., Grad. Sch. Eng.*, [3]*Dept. Med. Genom., Grad. Sch. Front. Sci.*)

1P124 **光刺激により自律的に自己組織化する RNA 分子ロボットの構築**
Construction of an RNA molecular robot autonomously self-assembled by light stimulation
Hao Li[1,2], Hirohide Saito[3], Masahiro Talinoue[2,4] ([1]*Dep. Cont. and Sys., Engineering, Tokyo Tech.*, [2]*Interdisciplinary Grad. Sch. of Sci. and Eng., Tokyo Tech.*, [3]*The Hakubi Center, Kyoto Univ.*, [4]*PRESTO, JST*)

1P125 **蛋白質-RNA の複合体立体構造予測**
Tertiary structure prediction of Protein-RNA complexes
Tomoshi Kameda[1], Junichi Iwakiri[2], Michiaki Hamada[2], Kiyoshi Asai[1,2] ([1]*CBRC, AIST*, [2]*Grad. Sch. Frontier Sci., the Univ. of Tokyo*)

06. 電子状態 / 06. Electronic state

1P126 **緑色蛍光タンパク質の蛍光スペクトルに関する理論的研究**
A theoretical study on the fluorescent spectrum of enhanced green fluorescent protein
Yoshihiro Uchida[1], Masahiro Higashi[2], Shigehiko Hayashi[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Inst. Mol. Sci.*)

1P127 **Zero-dipole summation method for evaluating electrostatic interaction in molecular simulation of biomolecular system**
Ikuo Fukuda, Narutoshi Kamiya, Haruki Nakamura (*Institute for Protein Research, Osaka University*)

1P128 **DFTB および REUS を用いたマロンアルデヒドのプロトン移動計算**
Calculation of proton transfer in malonaldehyde using DFTB and REUS
Shingo Ito[1], Stephan Ire[2,3], Yuko Okamoto[1] ([1]*Department of Physics, Graduate School of Science, Nagoya University*, [2]*WPI-Institute of Transformative Bio-Molecules*, [3]*Department of Chemistry, Graduate School of Science, Nagoya University, Nagoya*)

1P129 **ESP 多重極子演算子を用いた QM/MM 計算法の開発**
Development of multipole electrostatic potential operator for QM/MM method
Yusuke Inoue[1], Takahiro Kosugi[2], Hiroshi Nakano[3], Takeshi Yamamoto[1], Shigehiko Hayashi[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Univ. of Washington*, [3]*Grad. Sch. Eng., Kyoto Univ.*)

1P130 **FMO 法を用いた基準振動解析によるタンパク質の赤外吸収予測**
Prediction of IR spectra by normal mode analysis based on the Fragment Molecular Orbital(FMO) method
Hiroya Nakata[1,2], Dmitri Fedorov[3], Satoshi Yokojima[4], Kazuo Kitaura[5], Shinichiro Nakamura[2] ([1]*Tokyo Institute of Technology*, [2]*RIKEN*, [3]*National Institute of Advanced Industrial Science and Technology*, [4]*Tokyo University of Pharmacy and Life Sciences*, [5]*Graduate School of System Informatics, Kobe University*)

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

1P131 **水は蛋白質を折り畳むのか？**
Does water drive a protein to fold?
Yutaka Maruyama, Yuichi Harano (*Inst. Protein Res., Osaka Univ.*)

1P132 **逆ミセル中ナノ拘束水のテラヘルツダイナミクスの温度依存性**
Temperature dependence of terahertz dynamics of nano-confined water in a reverse micelle
Hiroshi Murakami (*JAEA*)

1P133 **Terahertz dynamics of hydrated protein studied by X-ray scattering**
Koji Yoshida, Toshio Yamaguchi (*Fukuoka Univerisity*)

1P134 **テラヘルツ時間領域分光によるリゾチーム低振動ダイナミクスの温度・水和依存性の観測**
Temperature and hydration dependence of low-frequency dynamics of lysozyme studied by terahertz time-domain spectroscopy
Naoki Yamamoto[1], Atsuo Tamura[2], Keisuke Tominaga[1,2] ([1]*Molecular Photoscience Research Center, Kobe University*, [2]*Graduate School of Science, Kobe University*)

1P135 **誘電緩和分光測定によるオリゴリン酸 Na、アルキルカルボン酸 Na、アルキルスルホン酸 Na の水和特性**
Hydration properties of sodium-oligophosphates, -alkyl carboxylates and -alkyl sulfonates by dielectric relaxation spectroscopy
Kazuki Ishimori, Yangtian Wang, Norihiko Tanno, George Mogami, Tetsuichi Wazawa, Nobuyuki Morimoto, Makoto Suzuki (*Dept. Materials Processing, Tohoku Univ.*)

09. 発生・分化 / 09. Development & Differentation

1P136 **Spatiotemporal measurement of cellular and tissue elasticity in the developing brain**
Misato Iwashita[1,2], Kazunori Toida[1,2], Yoichi Kosodo[1,2] ([1]*Kawasaki Medical School*, [2]*Department of Anatomy*)

1P137 **幹細胞ミニマルモデルの *in vivo* 実装**
In vivo* realization of the minimal stem cell model in *Escherichia coli
Sumire Ono[1], Reiko Okura[1], Yuichi Wakamoto[1,2] ([1]*Grad. Sch. Arts and Sci., Univ. Tokyo*, [2]*Research Center for Complex Systems Biology, Univ. Tokyo*)

1P138 **既知遺伝子調節関係に基づいた細胞分化の力学系モデル**
Realistic dynamical system model of cell differentiation based on known gene-regulatory interactions
Tadashi Miyamoto[1], Chikara Furusawa[2], Kunihiko Kaneko[1] ([1]*Grad. Sch. Art. Sci., Univ. Tokyo*, [2]*QBiC, Riken*)

10. 筋肉 / 10. Muscle

1P139 **アクチンフィラメントの伸長メカニズムを解明するための分子シミュレーション**
A Molecular Simulation Study to Investigate Actin Filament Elongation Mechanism
Nobuhiko Wakai[1], Kazuhiro Takemura[2], Takashi Fujii[3,4], Keiichi Namba[3,4], Akio Kitao[2,5] ([1]*Grad. Sch. Frontier Sci., Univ. Tokyo*, [2]*IMCB, Univ. Tokyo*, [3]*QBiC, RIKEN*, [4]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [5]*JST, CREST*)

1P140 **F-アクチンの水和状態に及ぼすハライドイオンの効果**
Halide ion effect on hydration state of F-actin
Noriyoshi Ishida, Takahiro Watanabe, **George Mogami**, Tetsuichi Wazawa, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

1P141 **アクチン重合、ミオシン ATP 加水分解活性化に対する Tyr143 変異の効果**
Changes of polymerization and activation of myosin ATPase of Dictyostelium actin induced by mutation of Tyrosin-143
Yuki Gomibuchi[1], Taro Uyeda[2], Takeyuki Wakabayashi[1,3] ([1]*Teikyo Univ. Grad. Sciences and Engineering*, [2]*AIST*, [3]*Teikyo Univ. Dept. Judo Therapy*)

1P142 **中性子散乱による筋肉の細いフィラメントのダイナミクス変化の検出**
Changes in the dynamics of the muscle thin filaments observed by neutron scattering
Satoru Fujiwara[1], Tatsuhito Matsuo[1], Takeshi Yamada[2], Nobuaki Takahashi[3], Kazuya Kamazawa[2], Yukinobu Kawakita[3], Kaoru Shibata[3] ([1]*QuBS, JAEA*, [2]*CROSS Tokai*, [3]*J-PARC Center, JAEA*)

1P143 **SDSL-ESR による心筋トロポニン-I の N 末伸長部位の動的構造**
Structural Dynamics of N-terminal Extension of Cardiac Troponin I by Site Directed Spin Labeling-EPR
Chenchao Zhao[1], Hiroaki Yamashita[1], **Keisuke Ueda**[1,3], Shoji Ueki[2], Toshiaki Arata[1] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Tokushima-Bunri Univ.*, [3]*Inst. Prot. Res., Osaka Univ.*)

1P144 **ESR による筋肉細いフィラメントにおけるアクチンと Ca 調節タンパク質の動的構造**
Structural dynamics of actin and Ca-regulatory proteins in muscle thin filament by using ESR
Yoshiki Tsujimoto[1], Akie Yamamoto[1], Keisuke Ueda[2], Toshiaki Arata[1] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Inst. Prot. Res., Osaka Univ.*)

11. 分子モーター / 11. Molecular motor

1P145 **Effects of the KIF2C neck peptide on microtubules: lateral disintegration of microtubules and β-structure formation**
Youske Shimizu[1,2], Takashi Shimizu[2], Masayuki Nara[3], Mahito Kikumoto[1], Hiroaki Kojima[1], Hisayuki Morii[2] ([1]*NICT*, [2]*AIST*, [3]*Tokyo Medical and Dental Univ.*)

1P146 **ガラス基板上に固定した F-アクチンへのコフィリンと HMM と協同的結合**
Cooperative binding of cofilin and HMM to immobilized F-Actin on a glass surface
Yusuke Nishikawa[1], Hiroaki Ueno[1], Akira Ainai[1], Taro Ueda[2], Kiyotaka Tokuraku[1] ([1]*Grad. Sch. Appl. Sci, Muroran Inst.*, [2]*Adv. Ind. Sci. Tech, National Inst.*)

1P147 **細菌べん毛モーター固定子複合体 MotA/B チャネルのプロトン透過メカニズム**
Proton permeation mechanism through the channel of flagellar motor stator complex MotA/B
Yasutaka Nishihara, Akio Kitao (*IMCB, Univ of Tokyo*)

1P148 **高速 AFM による *Ascaris* 精子由来の MSP 線維の観察**

Observation of MSP filaments in cell-free extract from *Ascaris* sperm by high-speed atomic force microscopy

Katsuya Shimabukuro[1], Takamitsu Haruyama[2], Ryoko Chijimatsu[1], Hiroki Konnno[2] ([1]*Ube Nat. Col. Tech.*, [2]*Bio-AFM, Kanazawa Univ.*)

1P149 **The Mg^{2+} binding site of the ATP synthase ε subunit from *Bacillus subtilis* derived by Molecular Dynamics simulations**

Alexander Krah, Shoji Takada (*Theoretical Biophysics Lab, Dept. Biophysics, Kyoto University*)

1P150 **鞭毛軸糸ダイニンを駆動源として振動的屈曲運動を発生させる微小管バンドル**

A microtubule bundle that produces oscillatory bending movement with axonemal dynein

Susumu Aoyama, Yuichi Hiratsuka (*Sch. Matl. Sci., JAIST*)

1P151 **Athermal Fluctuations of Probe Particles in Active Cytoskeletal Networks**

Irwin Zaid[2], **Heev Ayade**[1], Julia Yeomans[2], Daisuke Mizuno[1] ([1]*Kyushu University*, [2]*Oxford University*)

1P152 **ポリエチレングリコールがアクチン繊維と調節繊維の運動に及ぼす影響**

Effect of polyethylene glycol on the motility of actin and regulated thin filaments on myosin molecules

Kuniyuki Hatori, Shinsuke Munakata (*Grad. Sch. Sci. Eng., Yamagata Univ.*)

1P153 **ダイニン-微小管インターフェイスの構造解析: 微小管から AAA+ ATPase ドメインにどのように情報が伝えられるか？**

Structural analysis of dynein-microtubule interface: How is a signal transmitted from microtubule to AAA+ ATPase domain?

Seiichi Uchimura[1], Takashi Fujii[2], Hiroko Takazaki[1], Rie Ayukawa[1], Itsushi Minoura[1], Yosuke Nishikawa[3], You Hachikubo[1], Takahide Kon[4], Genji Kurisu[3], Kazuo Sutoh[5], Keiichi Namba[6], Etsuko Muto[1] ([1]*BSI, RIKEN*, [2]*QBiC, RIKEN*, [3]*IPR, Osaka Univ.*, [4]*Fac. Biosci. Appl. Chem., Hosei Univ.*, [5]*Fac. Sci. Eng., Waseda Univ.*, [6]*Grad. Sch. Front. Biosci. Osaka Univ.*)

1P154 **キネシンの弱結合から強結合への状態変化における蝶番構造**

A mechanistic pivot-point in the weak-to-strong state transition during kinesin-microtubule interactions

Itsushi Minoura, You Hachikubo, Yoshihiko Yamakita, Hiroko Takazaki, Rie Ayukawa, Chihiro Yoshida, Seiichi Uchimura, Etsuko Muto (*RIKEN BSI*)

1P155 **高速 AFM によって明らかとなったミオシン X の歩行メカニズム**

Walking mechanism of myosin X revealed by high-speed AFM

Yusuke Sakiyama[1], Noriyuki Kodera[2], Osamu Sato[3], Mitsuo Ikebe[3], Toshio Ando[1,2] ([1]*Grad. Sch. Sci., Kanazawa Univ.*, [2]*Bio-AFM FRC, Kanazawa Univ.*, [3]*Dept. Physiol, Univ. Massachusetts Med. Sch.*)

1P156 **高速 AFM によって明らかとなったミオシン V の化学-力学変換メカニズム**

Chemomechanical coupling mechanism of myosin V revealed by high-speed AFM

Noriyuki Kodera[1], Takayuki Uchihashi[1,2], Kenta Yagi[2], Toshio Ando[1,2] ([1]*Bio-AFM FRC, Inst. Sci. & Eng., Kanazawa Univ.*, [2]*Sch. Math. & Phys., Col. Sci. & Eng., Kanazawa Univ.*)

1P157 **高速 AFM によるダイニンの機能動態の観察**

High-Speed-AFM Observation of Processive Movement of Cytoplasmic Dynein

Shuji Fujita[1], Keitaro Shibata[2], Takayuki Uchihashi[1,3], Yoko Toyoshima[2], Toshio Ando[1,3] ([1]*College of Science and Engineering, Kanazawa University*, [2]*The University of Tokyo*, [3]*Bio-AFM Frontier Research Center, College of Science and Engineering, Kanazawa University*)

1P158 **マイコプラズマモービレのあしとシアル酸の結合はヌクレオチドに依存する**

Nucleotide-dependent interaction between legs of *Mycoplasma mobile* and sialyllactose

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 University.*)

1P159 **滑走するバクテリアの戦車のような運動装置を三次元で追跡する**

Three-dimensional tracking of tank-like motility apparatus of the gliding bacterium

Showko Odaka, Daisuke Nakane, Takayuki Nishizaka (*Department of Physics, Gakushin University*)

1P160 **戦車のような仕組みで動くバクテリア**

Bacterium moves like a tank

Daisuke Nakane[1], Keiko Sato[2], Hirofumi Wada[3], Mark McBride[4], Koji Nakayama[2], Takayuki Nishizaka[1] ([1]*Dept. Phys., Gakushuin Univ.*, [2]*Dept. Mol. Microbiol. Immunol., Nagasaki Univ.*, [3]*Dept. Phys., Ritsumeikan Univ.*, [4]*Dept. Biol. Sci., Univ. Wisconsin Milwaukee*)

1P161 **方位と倒れの構造変化を１分子レベルで検出する偏光スイッチングを用いた新しい TIRFM**

Advanced TIRF microscopy to detect single-molecule conformational changes in both azimuth and axial axis using polarization switching

Nagisa Mikami[1], Tomoko Masaike[1,2], Mitsuhiro Sugawa[1], Takayuki Nishizaka[1] ([1]*Dept. phys., Gakushuin Univ.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*)

1P162 **1 分子 FRET 計測による F_1-ATPase の ATP 結合待ち構造の解析**

Analysis of the ATP-waiting form of F_1-ATPase by single-pair FRET measurement

Mitsuhiro Sugawa[1], Masaru Kobayashi[1], Takashi Matsui[1], Tomoko Masaike[2,3] ([1]*Dept. Phys., Gakushuin Univ.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. Sci.*, [3]*JST*)

1P163 **F_1-ATPase の軸とシリンダーの結合寿命の測定**

Measurement of lifetime of the bond between the shaft and the cylinder in single F_1-ATPase

Tatsuya Naito[1], Kaoru Okada[1], Tomoko Masaike[1,2], Takayuki Nishizaka[1] ([1]*Dept. phys., Gakushuin Univ*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*)

1P164 **N 末端領域変異単頭キネシンによる微小管の 3 次元コークスクリュー運動**

Three-dimensional corkscrewing motion of a microtubule driven by single-headed kinesins with mutations in the N-terminal region

Shoko Fujimura[1], Shinsuke Owada[1], Takayuki Nishizaka[1], Junichiro Yajima[2] ([1]*Dept. phys., Gakushuin Univ.*, [2]*Graduate School of Arts and Sciences, The University of Tokyo*)

1P165 **G-、F-アクチンの水和測定と偏比容測定**

Hydration and partial specific volume measurements of G- and F-actin

Asato Imao, Takahiro Watanabe, Tetsuichi Wazawa, George Mogami, Nobuyuki Morimoto, Makoto Suzuki (*Dept. Materials Processing, Tohoku Univ.*)

1P166 **アクチンに係留された色素の回転相関時間の周波数領域蛍光偏光解消法による測定**

Rotational correlation time of a fluorophore tethered to actin as studied by frequency-domain fluorescence anisotropy measurements

Tetsuichi Wazawa, Nobuyuki Morimoto, Makoto Suzuki (*Grad Sch of Engin, Tohoku Univ*)

1P167 **1,3-ジエチル尿素による骨格筋ミオシンの滑り運動の阻害と Mg-ATPase の活性化**

1,3-Diethylurea-enhanced Mg-ATPase of skeletal muscle myosin with a converse effect on the sliding motility

Tetsuichi Wazawa, Shin-ichiro Yasui, **Nobuyuki Morimoto**, Makoto Suzuki (*Grad. Sch. Engin., Tohoku Univ*)

1P168 **バクテリアべん毛モーターの高時間分解能回転ステップ計測系の開発**

Development of dark-field imaging system with high temporal resolution for angular steps by bacterial flagellar motor

Hiromichi Wakebe[1], Yuichi Inoue[2], Akihiko Ishijima[2] ([1]*Grad. Sch. Life Sci.*, [2]*IMRAM, Tohoku Univ.*)

1P169 **Analysis of angular steps of bacterial flagellar motors using an elliptic probe**

Yuichi Inoue[1], Hiromichi Wakebe[2], Takashi Sagawa[2], Hajime Fukuoka[1], Akihiko Ishijima[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad.Sch. Life Sci., Tohoku Univ.*)

1P170 **F_1-ATPase が発生するトルクの微細構造**

Microstructure of the torque generated by F_1-ATPase

Eiichiro Saita[1], Kazuhiko Kinosita[2], Masasuke Yoshida[1] ([1]*Dept. Mol. Bio., Kyoto Sangyo Univ.*, [2]*Dept. Phys., Waseda Univ.*)

1P171 **ヒト F1-ATPase の一分子解析が明らかにした、バクテリアとは異なったミトコンドリア F1 の回転スキーム**

Single molecule analyses of human F1-ATPase revealed distinct rotation scheme of mitochondrial F1 motor

Toshiharu Suzuki[1,2], Kazumi Tanaka[1], Chiaki Wakabayashi[1], Shou Furuike[3], Eiichiro Saita[1], Kazuhiko Kinosita[4], Masasuke Yoshida[1] ([1]*Dept of Mol Bioscience, Kyoto Sangyo Univ*, [2]*CRL, Tokyo Inst of Tech*, [3]*Dept of Physics, Osaka Med College*, [4]*Faculty of Science and Eng, Waseda Univ*)

1P172 **腸内連鎖球菌 V-ATPase の大腸菌発現系**

Expression of *Enterococcus hirae* V-ATPase in *E. coli* BL21 (DE3)

Shohei Matsudo[1], Suhaila Rahman[1], Shinya Saijo[1], Misaki Yamamoto[1], Yoshimi Kakinuma[2], Kenji Mizutani[1,3], Takeshi Murata[3], Ichiro Yamato[1] ([1]*Dept. Biol. Sci Tech, Tokyo Univ. Sci*, [2]*Faclty Agri, Ehime Univ*, [3]*Faculty Sci, Chiba Univ*)

1P173 **腸内連鎖球菌 V 型 ATPase の A サブユニットの精製と結晶化**

Purification and Crystallization of A subunit from *Enterococcus hirae* V-ATPase

Aki Saito[1], Yasuko Saito[1], Shinya Saijo[1], Misaki Yamamoto[1], Yoshimi Kakinuma[2], Kenji Mizutani[1,3], Takeshi Murata[3], Ichiro Yamato[1] ([1]*Dept Biol Sci Tech, Tokyo Univ Science*, [2]*Faculty Agri, Ehime Univ*, [3]*Faculty Sci, Chiba Univ*)

1P174 **Direct observation of the rotation of V_1-ATPase from *Enterococcus hirae* and its torque**

Hiroshi Ueno[1], Yoshihiro Minagawa[2], Ichiro Yamato[3], Takeshi Murata[4], Ryota Iino[2], Eiro Muneyki[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*)

1P175 **F_1-ATPase の P-loop 変異体とリン酸解離の関係**

The relationship between F_1-ATPase P-loop mutants and Pi release

Hikaru Yoshida[1], Ayumi Ito[1], Jotaro Ito[2], Tomoko Masaike[3], Takayuki Nishizaka[4], Shoichi Toyabe[5], Hiroshi Ueno[1], Eiro Muneyuki[1] ([1]*Dept, of Physics. Chuo Univ.*, [2]*School of Engineering, The university of Tokyo*, [3]*Faculty of Science and Technology, Tokyo Univercity of Science*, [4]*Dept. of Phys. Univ. Gakushuin*, [5]*Faculty of Physics, LMU Munich*)

1P176 **回転電場を用いた外力存在下での F1-ATPase の回転観察**

Observation of the rotation of F1-ATPase

Yohsuke Kikuchi[1], Takahiro Nakayama[1], Shoichi Toyabe[2], Eiroh Muneyuki[1] ([1]*Dept. Phys., Univ. Chuo*, [2]*Fac. Phys., Univ. Munchen*)

1P177 **Sopped-Flow 法を用いた β サブユニット単体と F1-ATPase へのヌクレオチド結合の比較**

Comparison of the nucleotide binding to the isolated βsubunit and the F1-ATPase using the Sopped-Flow method

Riku Nagano[1], Kiyoshi Obara[1], Tomoko Masaike[2], Hiroshi Ueno[1], Eiro Muneyuki[1] ([1]*Dept. of Physics. Chuo Univ.*, [2]*Tokyo University of science*)

12. 細胞生物的課題 / 12. Cell biology

1P178 **走化性と重力により誘起されるサルモネラ菌の生物対流**

Bioconvection of *Salmonella* induced by chemotaxis and gravity

Takahiro Abe, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Univ. Tohoku*)

1P179 **MotB ペリプラスム領域の in-flame 欠損がサルモネラ菌べん毛モーターの出力特性に及ぼす影響**

Effect of in-frame deletion in the periplasmic region of MotB on the torque-speed relationship of *Salmonella* flagellar motor

Shuichi Nakamura[1], Yusuke V. Morimoto[2], David J. Castillo[3], Yong-Suk Che[4], Nobunori Kami-ike[3], Seishi Kudo[1], Tohru Minamino[3], Keiichi Namba[2,3] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*RIKEN QBiC*, [3]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [4]*Dept. Frontier Biosci., Hosei Univ.*)

1P180 **Motility analysis of Leptospira in highly viscous environments**

Kyosuke Takabe, Md. Shafiqul Islam, Seishi Kudo, Shuichi Nakamura (*Grad.sch.engineering.,univ.tohoku*)

1P181 **Microscopic observation of chemotactic behaviors of Leptospira**

Md. Shafiqul Islam, Kyosuke Takabe, Seishi Kudo, Shuichi Nakamura (*Department of Applied Physics, Tohoku University*)

1P182 **細菌べん毛モーター蛋白質 FliG-FliM 相互作用の解析**

Interaction between FliG and FliM in the bacterial flagellar motor

Miki Kinoshita[1], Yukio Furukawa[1], Katsumi Imada[2], Keiichi Namba[1,3], Tohru Minamino[1] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*Grad. Sch. Sci., Osaka Univ.*, [3]*QBiC, RIKEN*)

1P183 **クライオ電子顕微鏡によるべん毛蛋白質輸送装置の構造と分子機構**

Molecular mechanism of the type III protein export by electron cryotomography of the flagellar basal body

Akihiro Kawamoto[1], Tomoko Miyata[1], Yusuke V. Morimoto[1,2], Tohru Minamino[1], Takayuki Kato[1], Keiichi Namba[1,2] ([1]*Grad. Sch. of frontier Biosci., Osaka Univ*, [2]*QBiC., RIKEN*)

1P184 **Mycoplasma mobile から単離した滑走装置の電子顕微鏡観察**

Electron microscopic observation of isolated gliding machinery of Mycoplasma mobile

Miyuki Nishikawa[1], Daisuke Nakane[2], Akihiro Kawamoto[3], Takayuki Katou[3], Keiichi Namba[3,4], Makoto Miyata[1] ([1]*Graduate School of Science, Osaka City University*, [2]*Department of Physics, Gakushuin University*, [3]*Graduate School of Frontier Biosciences, Osaka University*, [4]*QBiC, RIKEN*)

1P185 **FRET センサーを用いて生細胞内分子混雑を可視化する**

Visualization of the molecular-crowding effects in living cell on cellular functions using a FRET-based biosensor

Hiroaki Machiyama[1,2], Takamitsu Morikawa[3], Tomoyuki Yamaguchi[1,2], Toshio Yanagida[1,2,3], Tomonobu Watanabe[1,2,3], Hideaki Fujita[1,2] ([1]*WPI, iFReC, Osaka Univ.*, [2]*QBiC, RIKEN*, [3]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

1P186 **Positive feedback mechanism for PIP3 polarity establishment mediated by PIP3 phosphatase, PTEN**

Satomi Matsuoka[1,2], Masahiro Ueda[1,2] ([1]*QBiC, RIKEN*, [2]*Osaka University*)

1P187 **細胞内 pH 変化に伴った細胞運動**

Changes in intracellular pH mediate the cell migration

Yusuke V. Morimoto[1], Masahiro Ueda[1,2] ([1]*QBiC, RIKEN*, [2]*Grad. Sch. Sci., Osaka Univ.*)

1P188 **nonlinear stress propagation, anisotropic stiffening, and nonaffine relaxations in cytoskeletal networks**

Daisuke Mizuno[1], Lara Villaruz[1], Akiko Nakamasu[1], Emi Ikebe[1], David Head[2] ([1]*Kyushu University*, [2]*University of Leeds*)

1P189 **Roles of actin polymerization in the collective cAMP oscillations**

Fumihito Fukujin[1], Satoshi Sawai[1,2,3] ([1]*Graduate School of Arts and Science, University of Tokyo*, [2]*Research Center for Complex Systems Biology, University of Tokyo*, [3]*PRESTO, Japan Science and Technology Agency*)

1P190 **血管平滑筋細胞内の核に対する核上下のアクチンストレスファイバの力学的役割**

Actin cap fibers and basal stress fibers have different roles in mechanical regulation of nucleus in vascular smooth muscle cells

Kazuaki Nagayama, Yuki Yahiro, Mitsuhiro Ukiki, Takeo Matsumoto (*Department of Mechanical Engineering, Nagoya Institute of Technology*)

1P191 **人工設計したマイクロ構造化基質における細胞のアクチン動態**

Actin dynamics in cells cultured on engineered micro-topographical substrate

Hiromi Miyoshi[1], Takuma Kishimoto[2], Takehiko Inaba[2], Miki Nishimura[3], Michiko Sugawara[3], Jong Soo Ko[4], Taiji Adachi[1,5], Toshihide Kobayashi[2], Yutaka Yamagata[1] ([1]*RIKEN Center for Advanced Photonics*, [2]*Lipid Biology Laboratory, RIKEN*, [3]*Grad. Sch. Eng, Chiba Univ.*, [4]*Sch. Mech. Eng, Pusan National Univ.*, [5]*Inst. Front. Med. Sci. Kyoto Univ.*)

1P192 **CRP2 タンパク質によるアクチン線維のダイナミクス制御**

Smooth muscle differentiation related transcription factor CRP2 directly regulates of actin filaments dynamics

Takanori Kihara[1], Sho Shinohara[2], Satoko Shinohara[2], Yasunobu Sugimoto[3], Jun Miyake[2] ([1]*Faculty of Environmental Engineering, The University of Kitakyushu*, [2]*Graduate School of Engineering Science, Osaka University*, [3]*Nagoya University Synchrotron Radiation Research Center*)

1P193 **細胞性粘菌アクチンの疎水性ヘリックスの変異が細胞運動に与える影響**

Actin mutation introduced into the hydrophobic helix impairs cytokinesis of Dictyostelium cell

Takahiro Ohnuki[1], Yuki Gomibuchi[2], Taro Uyeda[3], Takeyuki Wakabayashi[1,2] ([1]*Teikyo Univ. Grad. Sch Medical Technology*, [2]*Teikyo Univ. Grad. Science and Engineering*, [3]*AIST*)

1P194 **クライオ電子線トモグラフィー法を用いた細胞内におけるアクチンフィラメントバンドリングメカニズム解明**

The interaction between actin filaments and fascin are observed at high resolution 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]*FEI Company Japan Ltd., Application Lab.*, [4]*Grad. School of Frontier Biosciences, Osaka Univ.*)

1P195 **非筋細胞から単離したアクチンストレスファイバーの成分について**

Molecular components of actin stress fibers isolated from nonmuscle cells

Tsubasa S. Matsui[1], Shinji Deguchi[2] ([1]*Tohoku Univ.*, [2]*Nagoya Inst. Tech.*)

1P196 **細胞性粘菌ミオシン変異株 G680V が示す骨格筋アクトミオシンの高速滑り運動**

Myosin mutant G680V accelerated sliding velocities of skeletal muscle acto-myosin

Kouhei Iwase[1], Masateru Tanaka[1], Tarou Uyeda[2], Hajime Honda[1] ([1]*Dept. Bioeng., Nagaoka Univ. Tech.*, [2]*AIST, Tsukuba*)

1P197 **鞭毛中心構造による軸糸直径調節を通じたダイニンの活性制御機構**

Flagellar central structures regulate the dynein motor activity through the change of axonemal diameter

Toshiki Yagi[1], Yosuke Fujita[2], Shinji Kamimura[2], Hiroyuki Iwamoto[3] ([1]*Grad. Sch. of Med., Univ. Tokyo*, [2]*Fac. of Sci. & Eng., Chuo Univ.*, [3]*JASRI*)

1P198 分裂酵母の細胞質分裂における単量体型 II 型ミオシンの局在と機能
Localization and function of a monomeric myosin-II during cytokinesis in fission yeast
Masak Takaine, Osamu Numata, Kentaro Nakano (*Grad. Sch. Life & Env. Sci., Univ. of Tsukuba*)

1P199 超解像光学顕微鏡による、成長円錐のアクチンの可視化解析
Acitn meshwork in the growth cone revealed with superresolution
Kaoru Katoh[1,2], Saori Mimatsu[1,2] ([1]*Biomed.Res. Inst., AIST*, [2]*Grad.Sch. of Life & Enviro.Sci., Univ Tsukuba*)

1P200 棘皮動物コラーゲン性のキャッチ結合組織を軟化させる新規タンパク質因子
A novel protein factor softening echinoderm collagenous catch connective tissues
Akira Yamada[1], Yasuhiro Takehana[2], Masaki Tamori[2], Tatsuo Motokawa[2] ([1]*Adv ICT Res Inst, NICT*, [2]*Grad Sch Biosci Biotech, Tokyo Inst Tech*)

1P201 真正粘菌の間欠的な細胞運動時にみられる細胞骨格構造の形成と破壊のダイナミクス
Formation and destruction of cytoskeletal structure during intermittent locomotion of the true slime mold, Physarum polycephalum
Seiji Takagi (*RIES, Hokkaido Univ.*)

1P202 アクチン-コフィリン相互作用の一分子解析
Analysis of Cooperative Cofilin-Actin Filament Interactions examined at the single molecule level
Kimihide Hayakawa[1], Hitoshi Tatsumi[2], Shyotaro Sakakibara[2], Masahiro Sokabe[1] ([1]*Mechanobiology Lab., Nagoya University*, [2]*Dept. of Physiology, Nagoya University Graduate School of Medicine*)

1P203 アクトミオシン収縮運動の制御機構;α-カテニンの阻害作用を中心に
Inhibition of actomyosin contractility by α-catenin, a component of adherens junction
Shuya Ishii[1], Takashi Ohki[1], Hiroaki Kubota[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),*)

1P204 アクチンフィラメントがつくる二次元ネットワーク構造とその動態
Two-dimensional network pattern of actin filaments: Structure and dynamics
Hiroki Eguchi[1], Makito Miyazaki[1], Masataka Chiba[1], Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

1P205 有糸分裂中期に観察される染色体振動の解析
Analysis of chromosome oscillation during metaphase
Keita Nakayama, Jun Takagi, Takeshi Itabashi, Shin'ichi Ishiwata (*Grad. Sch. Sci., Univ. Waseda*)

1P206 引っ張り刺激による細胞シート中のアクチンフィラメント再編成
Actin filament remodeling in cell-sheet by mechanical stretch
Madoka Suzuki[1,2], Keiko Kawauchi[3], Ee Chu Chai[1], Shota Yamauchi[3], Shin'ichi Ishiwata[1,2,4], Hideaki Fujita[5,6] ([1]*WABIOS, Waseda Univ*, [2]*Org Univ Res Initiatives, Waseda Univ*, [3]*MBI, Natl Univ Singapore*, [4]*Dept Phys, Waseda Univ*, [5]*Riken Qbic*, [6]*iFReC, Osaka Univ*)

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

1P207 パターン化モデル生体膜へのロドプシンの再構成
Reconstitution of rhodopsin into a micropatterned model biological membrane
Yasushi Tanimoto[1], Kenichi Morigaki[1], Fumio Hayashi[2] ([1]*Grad. Sch. Agr., Univ. Hyogo*, [2]*Grad. Sch. Sci., Univ. Hyogo*)

1P208 浸透圧を変化させた時の架橋脂質膜の振る舞い
Behavior of a suspended lipid membrane under varying osmotic pressure
Koji Sumitomo[1], Paul Kocher[2], Nahoko Kasai[1], Aya Tanaka[1], Yoshiaki Kashimura[1], Keiichi Torimitsu[3], John Ryan[2] ([1]*NTT Basic Research Labs.*, [2]*Oxford Univ.*, [3]*Tohoku Univ.*)

1P209 pH 転換による GUV への効率的・選択的タンパク質封入
Efficient and selective entrapment of protein into GUV by converting pH over the pI
Kanta Tsumoto (*Grad. Sch. Eng., Mie Univ.*)

1P210 中性子非鏡面散乱法による糖脂質膜の力学特性の解明
Mechanics of Glycolipid Membranes Probed by Off-Specular Neutron Scattering
Akihisa Yamamoto[1], Wasim Abuillan[2], Alexandra Burk[2], Alexander Körner[2], Daniel Werz[3], Bruno Demé[4], Motomu Tanaka[1,2] ([1]*iCeMS, Kyoto Univ.*, [2]*Phys. Chem., Univ. of Heidelberg*, [3]*Dept. Chem., Univ. Göttingen*, [4]*Inst. Laue-Langevin*)

1P211 タンパク質内包リポソームの浸透圧下における構造
Structures of liposome encapsulating proteins under the osmotic pressure
Ryota Kimura, Mitsuhiro Hirai (*Graduate School of Engineering, Gunma University*)

1P212 界面通過法で作製したジャイアントリポソームのラメラリティーの定量的解析
Measuring the lamellarity of giant liposomes prepared by inverted emulsion method
Masataka Chiba[1], Makito Miyazaki[1], Shin'ichi Ishiwata[1,2] ([1]*Dept. of Physics, Waseda Univ.*, [2]*WABIOS, Waseda Univ.*)

1P213 細胞毒性を有する酸化コレステロールのホスファチジルコリン二分子層膜内存在位置
Locations of cytotoxic oxysterols in phosphatidylcholine bilayer membranes
Tatsuya Hoshino[1], Takaaki Hikima[2], Masaki Takata[2], Toshihide Kobayashi[3], **Hiroshi Takahashi**[1] ([1]*Grad. Sch. Sci & Tech., Gunma Univ.*, [2]*Harima Inst., Riken*, [3]*Wako Inst., Riken*)

1P214 合成セラミド 2 の相挙動及びコレステロールとの相互作用
Phase Behavior of Synthetic Ceramide2((2S,3R)-2-Octadecanoylaminooctadecane-1,3-diol) and Its Interaction with Cholesterol
Kenta Takada[1], Yasuko Obata[2], Nobutaka Shimizu[3], Hiroshi Takahashi[1] ([1]*Grad.Sch.Eng.,Gunma Univ.*, [2]*Hoshi Univ.*, [3]*KEK-PF*)

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

1P215 **低い pH が誘起する DOPS/MO 膜の液晶相からキュービック相への相転移の初期過程**
Initial Step of Low pH-Induced Lamellar to Bicontinuous Cubic Phase Transition in Dioleoylphosphatidylserine/Monoolein
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.*)

1P216 **抗菌ペプチド・マガイニン２が誘起するポア形成に対する脂質膜の力学特性の効果**
Effects of Mechanical Properties of Lipid Membranes on Antimicrobial Peptide Magainin 2-Induced Pore Formation
M. A. Sayem Karal[1], Taka-aki Tsuboi[2], Md. Jahangir Alam[3], Md. Zahidul Islam[1], Masahito Yamazaki[1,2,3] ([1]*Grad. Sch. Sci. & Tech., Shizuoka Univ.*, [2]*Grad. Sch. Sci., Shizuoka Univ.*, [3]*Res. Inst. Electronics, Shizuoka Univ.*)

1P217 **張力による脂質膜のポア形成の速度定数に対する静電相互作用の効果**
Effects of Electrostatic Interactions on Rate Constants of Tension-Induced Pore Formation in Single GUVs
Taka-aki Tsuboi[1], M. A. Sayem Karal[2], Victor Levadny[2,3], Masahito Yamazaki[1,2,4] ([1]*Grad. Sch. Sci., Shizuoka Univ.*, [2]*Grad. Sch. Sci. & Tech., Shizuoka Univ.*, [3]*Rus. Acad. Sci.*, [4]*Res. Inst. Electronics, Shizuoka Univ.*)

1P218 **細胞侵入ペプチドであるトランスポータン 10 の脂質膜透過はポア形成の前に起こる**
Permeation of Cell-Penetrating Peptide Transportan 10 through Lipid Membranes before Pore Formation
Md. Zahidul Islam[1], Hirotaka Ariyama[1], Md. Jahangir Alam[2], Masahito Yamazaki[1,2] ([1]*Grad. Sch. Sci. & Tech., Shizuoka Univ.*, [2]*Res. Inst. Electronics, Shizuoka Univ.*)

1P219 **Interaction Of Warm-Sensing Chemical Capsaicin with the Biomimetic Membranes**
Neha Sharma, Pooja Gusain, Tsuyoshi Yoda, Masahiro Takagi (*Japan Advanced Institute of Science and Technology*)

1P220 **Dynamic Response of Menthol on Thermo-Induced Cell Membrane: More than Receptors**
Pooja Gusain, Neha Sharma, Tsuyoshi Yoda, Masahiro Takagi (*Japan Advanced Institute of Science and Technology*)

13C. 生体膜・人工膜：興奮・チャンネル / 13C. Biological & Artifical membrane: Excitation & Channels

1P221 **急速緩衝液交換法による時間分解全反射赤外分光法の開発**
Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode
Yuji Furutani[1,2], Tetsunari Kimura[1,2], Kido Okamoto[3] ([1]*Inst. Mol. Sci.*, [2]*SOKENDAI*, [3]*UNISOKU*)

1P222 **哺乳類 two-pore 型カリウムチャネル TWIK-1 の全反射赤外分光解析**
ATR-FTIR spectroscopic analyses on a mammalian 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*)

1P223 **有効電場中におけるグラミシジン A を含んだ脂質二重層膜の静電ポテンシャルと圧力特性**
Electrostatic potential and lateral pressure profile of lipid bilayer containing gramicidin A in effective electrostatic field
Hiroaki Saito, Kazutomo Kawaguchi, Hidemi Nagao (*Kanazawa University*)

1P224 **細菌機械受容チャネル MscS のリポソーム膜上での配向**
The orientation of MscS in liposomal membranes
Takeshi Nomura[1], Masahiro Sokabe[2], Boris Martinac[3] ([1]*Dept Mol Cell Physiol and Bio-Ionomics, Kyoto Pref Univ Med Grad Sch of Med Sci, Kyoto, Japan*, [2]*Dept Physiol, Nagoya Univ Grad Sch of Med, Nagoya, Japan*, [3]*Victor Chang Cardiac Research Institute, Mol Cardiol and Biophys Div, Sydney, Australia*)

13E. 生体膜・人工膜：情報伝達 / 13E. Biological & Artifical membrane: Signal transduction

1P225 **シグナル分子クラスターの再構成とイメージング**
Reconstitution and imaging of signaling molecule clusters
Yoshihisa Kaizuka (*National Institute for Materials Science*)

1P226 **１分子観察によるシグナル伝達分子Ａｋｔの作動機構解明**
Single-molecule imaging study of signal transduction mechanism on Akt
Hideaki Yoshimura, Takeaki Ozawa (*Dep. Chem. Sch. Sci. the Univ of Tokyo*)

14. 化学受容 / 14. Chemoreception

1P227 **海洋細菌 *Vibrio alginolyticus* 新規アミノ酸走性トランスデューサーの同定**
Identification of a novel transducer for amino acid taxis in the marine bacterium *Vibrio alginolyticus*
Yukako Tsuji[1,3], Manabu Konishi[3], Kimiko Yamamoto[1,2], So-ichiro Nishiyama[3,4], Yoshiyuki Sowa[3], Ikuro Kawagishi[1,3,4] ([1]*Dept. Frontier Biosci., Grad. Sci Eng., Hosei Univ.*, [2]*Natl. Inst. Agro-Environ. Sci.*, [3]*Dept. Frontier Biosci., Fac. Biosci. Appl. Chem., Hosei Univ.*, [4]*Res. Cen. Micro-Nano Tech., Hosei Univ.*)

1P228 **走化性レセプター発現で大腸菌の内膜に生じる形態変化の急速凍結レプリカによる観察**
A quick-freezing replica study on morphological changes in the bacterial inner membrane induced by chemoreceptor expression.
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.*)

1P229 **シグナル伝達分子間のクロストークを使った鞭毛の回転方向制御**

Control of bacterial flagellar rotation via crosstalk from a non-cognate histidine kinase to the response regulator CheY

Tohru Umemura[1], Mayumi Kobayashi[1], Chiho Hara[1], Yoshiyuki Sowa[1,2], Ikuro Kawagishi[1,2] ([1]*Department of Frontier Bioscience, Hosei University*, [2]*Research Center for Micro-Nano Technology, Hosei University*)

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

1P230 **覚醒状態の維持を担う視床下部オレキシンニューロンの同期的活動**

Synchronous activity of orexin neurons in the lateral hypothalamus

Takeshi Kanda[1], Takahiro Miyazaki[1], Ryo Ishii[1], Mari Hondo[1], Elijah Takahashi[1], Masashi Yanagisawa[1,2] ([1]*IIIS, Univ. Tsukuba*, [2]*UTSW/HHMI*)

1P231 **フェムト秒レーザー神経突起切断による神経回路網の自発活動の時空間ダイナミクス**

Spatio-temporal dynamics of spontaneous activity in living neuronal network by femtosecond laser-induced cutting of neurites

Hayato Kubo[1,2], Suguru N. Kudoh[2], Takahisa Taguchi[1,3], Chie Hosokawa[1,2] ([1]*Health Res. Inst., AIST*, [2]*Grad. Sci. Eng., Kwansei Gakuin Univ.*, [3]*Cinet, NICT*)

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

1P232 **アルツハイマー病 *in vitro* モデルを用いたアミロイド β(1-42) 伝搬毒性の評価**

Evalution of Amyloid β(1-42) toxicity propagation using Alzheimer's disease *in vitro* model

Takuma Maruyama[1], Lui Yoshida[2], Kiyoshi Kotani[2], Seiichi Suzuki[1], Yasuhiko Jimbo[2] ([1]*SEIKEI University*, [2]*The University of Tokyo*)

1P233 **前脳基底部の刺激によるラット前頭葉での応答**

Response of rat frontal neuronal activity evoked by stimulation of the basal forebrain

Kazuaki Nagasaka[1,2], Yumiko Watanabe[2], Nobuo Kunori[1,2], Riichi Kajiwara[3], Ichiro Takashima[2] ([1]*Comp. Human Sci, Univ. Tsukuba*, [2]*Human Tech Res Inst, AIST*, [3]*Biomed. Res Inst, AIST*)

1P234 **緩徐不活性化カリウムコンダクタンスが嗅周囲野 35 野の情報伝達を制御する**

Slowly inactivating potassium conductance controls transmission at area 35 of perichinal cortex: VSD imaging study

Takashi Tominaga[1], Yoko Tominaga[1], Riichi Kajiwara[2] ([1]*Inst Neurosci, Tokushima Bunri Univ.*, [2]*Biomed Res Inst, AIST*)

1P235 **Analysis of related molecules to synchronous activity of rat cultured neuronal networks**

Daisuke Ito[1], Keiko Yokoyama[2], Kazutoshi Gohara[2] ([1]*Fac. Advanced Life Sci., Hokkaido Univ.*, [2]*Fac. Engin., Hokkaido Univ.*)

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

1P236 **内モンゴルエジノル塩湖から単離された halorubrum 属菌の持つロドプシン類タンパク質遺伝子の同定**

Identification of microbial rhodopsin genes from a halorubrum species isolated from Ejinoor salt lake in Inner Mongolia of China

Luomeng Chao[1], Gang Dai[2], Tatsuo Iwasa[1] ([1]*Div. Eng.Composite Funct., Muroran Ins. Technol., Japan*, [2]*Coll. Chem. Environ. Sci., Inner Mongolia Normal Univ., China*)

1P237 **ニワトリクリプトクロム 4 の光反応特性の解析**

Spectroscopic characterization of Chicken Cryptochrome4

Hiromasa Mitsui, Toshinori Maeda, Chiaki Yamaguchi, Yusuke Tsuji, Yoko Kubo, Keiko Okano, Toshiyuki Okano (*Dept. Eng. and Biosci., Grad. Sch. Adv. Sci. and Eng., Waseda Univ.*)

1P238 **ゼブラフィッシュクリプトクロム 1a の発現・精製**

Expression and purification of zebrafish cryptochrome 1a.

Arisa Takeno, Hiromasa Mitsui, Keiko Okano, Toshiyuki Okano (*Dept. Eng. and Biosci., Grad. Sch. Adv. Sci. and Eng., Waseda Univ.*)

1P239 **近赤外ラマン円偏光二色性分光による光受容タンパク質の活性部位構造解析**

Active Site Structure of Photoactive Yellow Protein with a Locked Chromophore Analog Revealed by Near Infrared Raman Optical Activity

Takahito Shingae[1], Kensuke Kubota[1], Nicole D. Foster[2], Masato Kumauchi[2], Wouter D. Hoff[2], Masashi Unno[1] ([1]*Department of Chemistry and Applied Chemistry, Graduate School of Science and Engineering, Saga University*, [2]*Department of Microbiology and Molecular Genetics, Oklahoma State University*)

1P240 **共鳴ラマン分光法によるシアノバクテリオクローム RcaE がもつ開環テトラピロール発色団のプロトン化状態の解析**

Protonation state of the linear tetrapyrrole chromophore in cyanobacteriochrome RcaE revealed by resonance Raman spectroscopy

Shinsuke Osoegawa[1], Yuu Hirose[2], Masahiko Ikeuchi[3], Masashi Unno[1] ([1]*Grad. Sch. Sci., Univ. Saga*, [2]*EIIRIS., Univ. Toyohashi*, [3]*Sci(Bio)., Univ. Tokyo*)

1P241 **赤外分光法によるチャネルロドプシンとキメラチャネルロドプシンの比較解析**

Comparative analysis of Channelrhodopsin and its chimeras based on FTIR spectroscopy

Asumi Inaguma[1,2], Hisao Tsukamoto[1], Tetsunari Kimura[1,3], Toru Ishizuka[3,4], Hiromu Yawo[3,4], Yuji Furutani[1,2] ([1]*IMS*, [2]*PRESTO*, [3]*CREST*, [4]*Tohoku Univ.*)

1P242 **(6-4)光回復酵素における光活性化及び光修復のメカニズム**

Molecular mechanism of photoactivation and photorepair of Xenopus (6-4) photolyase

Daichi Yamada[1], Junpei Yamamoto[2], Yu Zhang[1], Tatsuya Iwata[1], Kenichi Hitomi[3], Elizabeth Getzoff[3], Shigenori Iwai[2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*Grad. Sch. Eng. Sci., Osaka Univ.*, [3]*The Scripps Res. Inst. USA*)

1P243 **大腸菌におけるチャネルロドプシン 1 の発現**

Expression of channelrhodopsin-1 in *Esherichia coli*

Arisa Mori[1], Takashi Tsukamoto[1], Zin Yagasaki[1], Michio Homma[1], Kunio Ihara[2], Yuki Sudo[1,3] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Center Gene Res., Nagoya Univ.*, [3]*JST-CREST*)

1P244 **好熱性ロドプシン：高度好熱菌から初めて発見された光駆動イオンポンプ**

Thermophilic rhodopsin: The first light-driven proton pump from an extreme thermophile

Takashi Tsukamoto[1], Yuki Sudo[1,2] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*JST, CREST*)

1P245 **レーザーフラッシュフォトリシス法によるロドプシンミミックの光化学研究**

Laser flash photolysis study on Photochemistry of Rhodopsin Mimics

Keiichi Inoue[1,2], Yuuya Ozaki[1], James H. Geiger[3], Babak Borhan[3], Hideki Kandori[1] ([1]*Grad. Sch. Eng., Nagoya Inst. Tech.*, [2]*JST PRESTO*, [3]*Dep. Chem., Michigan State Univ.*)

1P246 **酸性条件で機能する蛍光タンパク質のランダム変異によるスクリーニング**

Screening of randomly mutated fluorescent proteins that can work in acidic conditions

Tatsuya Iwata, Yukiko Ono, Masayo Iwaki, Hideki Kandori (*Grad. Sch. Eng., NITech*)

1P247 **青色光吸収型アーキロドプシン 3 変異体による内向きプロトン輸送**

Light-induced inward proton transport in a blue-shifted archaerhodopsin-3 mutant

Keiichi Inoue[1,2], Takashi Tsukamoto[3], Jin Yagasaki[3], Kazumi Shimono[4], Seiji Miyauchi[4], Shigehiko Hayashi[5], Hideki Kandori[1], **Yuki Sudo**[3,6,7] ([1]*Nagoya Institute of Technology*, [2]*JST-PRESTO*, [3]*Nagoya University*, [4]*Toho University*, [5]*Kyoto University*, [6]*Institute for Molecular Science*, [7]*JST-CREST*)

1P248 **シアノバクテリアのクリプトクロム DASH の変異体は二本鎖 CPD を修復する**

Functional conversion of cryptochrome into 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], Shigenori Iwai[2], Takeshi Todo[3], 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*)

1P249 **FTIR study of isotope-labeled CPD-Photolyase**

I M. M. Wijaya[1], Tatsuya Iwata[1], Tilo Mathes[2], Junpei Yamamoto[4], Kenichi Hitomi[3], Elizabeth D. Getzoff[3], Shigenori Iwai[4], John T. Kennis[2], Hideki Kandori[1] ([1]*Department of Frontier Materials, Nagoya Institute of Technology, Japan*, [2]*Department of Physics and Astronomy, VU University, The Netherlands*, [3]*Department of Integrative Structural and Computational Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, USA*, [4]*Graduate School of Engineering Science, Osaka University, Japan*)

1P250 **光駆動ナトリウムポンプの低温赤外分光**

Low-temperature FTIR spectroscopy of a light-driven sodium ion pump

Hikaru Ono[1], Keiichi Inoue[1,2], Rei Abe-Yoshizumi[1], Kwang-Hwan Jung[3], Hideki Kandori[1] ([1]*Nagoya Ins. Of Technol.*, [2]*JST PREST*, [3]*Sogang Univ. Korea*)

1P251 **霊長類色覚視物質の変異体に対する赤外分光研究**

FTIR study of mutants of primate color pigments

Kota Katayama[1], Daiki Kawata[1], Hiroo Imai[2], Akimori Wada[3], Hideki Kandori[1] ([1]*Department of Frontier Materials, Nagoya Institute of Technology*, [2]*Primate Research Institute, Kyoto University*, [3]*Organic Chemistry for Life Science, Kobe Pharmaceutical University*)

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

1P252 **ＯＥＣのＳ３状態の反応活性部位の分子構造と酸化状態に関する理論的研究**

Theoretical study on molecular structures and oxidation states of active site at the S3 state of OEC

Tomoya Ichino, Masaki Mitani, Yasunori Yoshioka (*Grad. Sch. Eng., Univ. Mie*)

1P253 **光化学系 II-電極による可視光照射下での水の酸化**

Visible light-driven water oxidation by Photosystem II-immobilized electrodes

Masaru Kato, Miwa Sugiura (*Proteo-Science Center, Ehime Univ.*)

1P254 **Mn 除去は光化学系 II 非ヘム鉄の酸化還元電位に影響を及ぼすか？ ーFTIR-分光電気化学計測による解析**

FTIR-Spectroelectrochemical Investigation into Whether Mn-Depletion Influences the Redox Potential of the Non-Heme Iron in Photosystem II

Yuki Kato, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

1P255 **FTIR study on the functions of the extrinsic proteins in cyanobacterial photosystem II: Evolutionary aspect of extrinsic proteins**

Ryo Nagao[1], Hanayo Ueoka-Nakanishi[1], Chihiro Uno[1], Tatsuya Tomo[2,3], Takumi Noguchi[1] ([1]*Grad. Sch. Sci., Univ. Nagoya*, [2]*Faculty of Sci., Tokyo Univ. of Sci.*, [3]*JST PREST*)

1P256 **光化学系 II における Y_Z ラジカルとヒスチジン間の高いプロトン分極を持つ水素結合：FTIR 法による検出**

FTIR evidence for the presence of a strong H-bond with high proton polarizability between the Y_Z radical and a His in photosystem II

Shin Nakamura, Ryo Nagao, Hanayo Nakanishi, Ryouta Takahashi, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

19. 放射線生物／活性酸素 / 19. Radiobiology & Active oxygen

1P257 **Role of NADPH oxidase in vitamin D_3 and PMA-induced cell differentiation**

Hiroyuki Kato[1], Asuka Kato[1], Omi Nawa[1], Masato Mutoh[2], Wakako Hiraoka[1] ([1]*Graduate School of Science and Technology, Meiji University*, [2]*Department of Materials and Human Environmental Sciences, Shonan Institute of Technology*)

1P258 **メガヘルツ超音波の抗腫瘍効果**

Antineoplastic effect of MHz ultrasound to leukemia cells

Risa Fuji, Wakako Hiraoka (*Graduate School of Science and Technology, Meiji University*)

1P259 **酸化ストレス下でのＲＯＳ検出**

ROS detection in oxidative stress

Omi Nawa, Hiroyuki Kato, Asuka Kato, Wakako Hiraoka (*Graduate School of Science and Technology, Meiji University*)

1P260 **DNA 脱塩基部位の局在性評価法の開発と放射線照射 DNA への適用**

A de novo methodology for estimating localization of apurinic (AP) sites in DNA and its application to DNA exposed to ionizing radiations

Ken Akamatsu, Naoya Shikazono (*Irradiated Cell Analysis Group, Japan Atomic Energy Agency*)

1P261 **シンクロトロン軟Ｘ線によって誘発されるバイスタンダー応答の機構**

Mechanisms of synchrotron soft X-ray-induced bystander response

Masanori Tomita[1], Munetoshi Maeda[1,2], Noriko Usami[3], Katsumi Kobayashi[3] ([1]*Radiat. Safety Res. Cent., CRIEPI*, [2]*R&D, WERC*, [3]*PF, IMSS, KEK*)

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

1P262 **海底熱水噴出孔を模擬した新型フローリアクターの製作と化学進化**

A construction of a new flow reactor simulating hydrothermal vents for chemical evolution

Eiichi Imai, Hajime Honda (*Dept. Bioengineering, Nagaoka Univ. Tech.*)

1P263 ***In vitro* selection of the preferable 3'-terminal sequences of the template for norovirus RNA replicase**

Hidenao Arai[1], Miho Suzuki[1], Naoto Nemoto[1], Koichi Nishigaki[1], Yuzuru Husimi[2] ([1]*Grad. Sch. Sci. Eng., Saitama Univ.*, [2]*Innovation Research Organization, Saitama Univ.*)

1P264 **苔に擬態した蝶の翅模様にみるノイズを利用したデザイン原理**

Noisy design of a butterfly wing pattern mimicking a lichen-covered tree bark

Takao K. Suzuki (*NIAS*)

21A. ゲノム生物学：ゲノム解析 / 21A. Genome biology: Genome analysis

1P265 **ダブルバレルカーボンプローブを用いた組織モデルからのｍＲＮＡ回収と定量評価**

Collection and quantification of messenger RNA from tissue models by double barrel carbon probe

Yuji Nashimoto[1], Yasufumi Takahashi[2], Ryosuke Takano[1], Kosuke Miyashita[1], Shukuyo Yamada[1], Kosuke Ino[1], Hitoshi Shiku[1], Tomokazu Matsue[1,2] ([1]*Enviromental studies, Tohoku Univ.*, [2]*WPI-AIMR, Tohoku Univ.*)

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

1P266 **Development of Ligand Based Virtual Screening considering protein-ligand interaction**

Koya Kato, George Chikenji (*Grad. Sch. Eng., Nagoya Univ.*)

1P267 **H-DROP:サポートベクターマシンを用いたヘリカルリンカーの予測**

H-DROP: an SVM based helical domain linker predictor trained with optimal selected features

Suzuki Ryosuke[1], Ebina Teppei[2], Yutaka Kuroda[1] ([1]*Dept of Biotech. & Life Sci., Tokyo University of Agriculture & Technology*, [2]*Brain Science Inst., RIKEN*)

1P268 **スプライシングアイソフォームの機能的有意性の評価**

Evaluation of functional significance of splicing isoforms

Masafumi Shionyu[1], Shiori Ikeda[2], Ken-ichi Takahashi[2] ([1]*Fac. Bio-Sci., Nagahama Inst. Bio-Sci. Tech.*, [2]*Grad. Sch. Bio-Sci., Nagahama Inst. Bio-Sci. Tech.*)

1P269 **タンパク質における分子トンネルの高速簡易探索法の開発ートリプトファン合成酵素への適用**

A Simple Method to Detect Molecular Tunnels in Proteins - Application to Tryptophan Synthase

Midori Yano, Kei Yura (*Grad. Sch. Hum. Sci., Univ. Ocha*)

1P270 **ｍＲＮＡ切断ポリアデニル化特異因子複合体構成サブユニットの四次構造推定**

Predicting a Quaternary Structure of mRNA Cleavage-Polyadenylation Specificity Factor Complex

Saki Aoto, Kei Yura (*Ochanomizu Univ*)

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

1P271 **ヒトリン酸化部位のデータベース解析で明らかになるシグナル伝達経路間のクロストーク**
Crosstalk between signaling pathways revealed by database analysis of human phosphorylation sites
Hafumi Nishi[1], Emek Demir[2], Anna R. Panchenko[3] ([1]*Grad. Sch. Medical Life Sci., Yokohama City Univ.*, [2]*Computational Biology Center, MSKCC*, [3]*NIH/NLM/NCBI*)

24. 数理生物学 / 24. Mathematical biology

1P272 **大自由度遺伝子発現制御モデルにおける適応応答の協同的進化**
Cooperative Adaptive Responses in Gene Regulatory Networks with Many Degrees of Freedom
Masayo Inoue[1], Kunihiko Kaneko[2] ([1]*molprof, AIST*, [2]*Univ. of Tokyo*)

1P273 **細胞の集団的意思決定の設計原理**
A Design Principle of Group-level Decision Making in Cell Populations
Koichi Fujimoto[1], Satoshi Sawai[2,3,4] ([1]*Faculty of Science, Osaka University*, [2]*Graduate School of Arts and Sciences, University of Tokyo*, [3]*Research Center for Complex Systems Biology, University of Tokyo*, [4]*PRESTO, JST*)

1P274 **Large deviation properties of population averages: An indicator of gene expression dynamics in a single cell**
Bhaswati Bhattacharyya, Ziya Kalay (*iCeMS, Kyoto University*)

1P275 **細胞システムの内因的・外因的ゆらぎに対するロバスト性に関する理論的基礎**
Theoretical basis for robustness of intracellular systems against intrinsic and extrinsic fluctuation
Tetsuya Kobayashi (*IIS, Univ. Tokyo*)

1P276 **イノシトールリン脂質代謝系が細胞の自発運動への効果の理論と実験による検証**
Theoretical and Experimental Analysis for the Effect of Phosphatidyl Inositol System on Spontaneous Cell Movement
Masato Yasui, Satomi Matsuoka, Masahiro Ueda (*Osaka University*)

1P277 **間葉ーアメーバ型遊走に関する理論モデル**
A Theoretical Model for Mesenchymal-Amoeboid Modes for Migration
Shin I. Nishimura (*Kyushu University*)

1P278 **確率的シグナル伝達経路における外因性ノイズを含む入力信号に対する応答性**
Responses of a stochastic signaling cascade to input signals with extrinsic noise
Akio Chiba[1,2], Akihiro Fukagawa[1], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

1P279 **なぜ細胞は様々なステップ数を持つシグナルカスケードを使いわけるのか？**
Why do cells use signaling cascades with a variety of the number of steps?
Akihiro Fukagawa[1], Masashi Kajita[1], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

1P280 **一分子シミュレーションによる上皮成長因子シグナル伝達経路の応答不均一性の解明**
Understanding heterogeneity in EGF pathway using simulation at the molecular resolution
Kazunari Iwamoto, Yuki Shindo, Atsushi Miyauchi, Kazunari Kaizu, Koichi Takahashi (*Laboratory for biochemical simulation, QBiC, RIKEN*)

1P281 **Diffusion-controlled reaction rate-laws in intracellular environment with molecular crowding: A single-particle-level simulation study**
Kazunari Kaizu, Koichi Takahashi (*RIKEN*)

25. 非均衡・生体リズム / 25. Equality Nonequilibrium state & Biological rhythm

1P282 **Power-law distribution derived from misunderstanding of search patterns**
Hisashi Murakami, Yukio Gunji (*Kobe University*)

1P283 **From cell-autonomous circadian clocks to tissue-level timekeeping**
Craig Jolley, Maki Ukai-Tadenuma, Dimitri Perrin, Hiroki Ueda (*RIKEN Center for Developmental Biology*)

1P284 **熱泳動現象を用いた鎖状高分子の凝集における分子構造転移の影響**
Effects of polymer chain folding for polymer aggregation in thermophoresis
Kenta Odagiri (*MIMS, Meiji Univ.*)

26. 計測 / 26. Measurements

1P285 **ベイズ統計を用いた超解像 CT アルゴリズム**
Super resolution computed tomography based on Bayesian statistics
Jun Kozuka[1], Takaki Makino[2], Haruo Mizutani[2] ([1]*QBiC, RIKEN*, [2]*Grad. Sch. Fro. Sci., Univ. Tokyo*)

1P286 **フリーズフラクチャー原子間力顕微鏡によるバクテリオロドプシンの 3 次元結晶の観察**
Observation of the crystal structure of bacteriorhodopsin by freeze fracture atomic force microscopy
Naoto Kuga, toshiaki Gotou, Tutomu Kouyama (*Nagoya Univ.*)

1P287 **楕円率変化検出ＣＤ測定法の発展とその生物系への応用**
Development of elliptically-polalization-detected CD apparatus and its application to the biological systems
Yasuyuki Araki, Yoshiyuki Hamada, Makoto Murakami, Seiji Sakamoto, Takehiko Wada (*IMRAM, Tohoku Univ.*)

1P288 **物質の非平衡加熱状態観測のための In-situ マイクロ波照射 NMR 分光法の開発**

Development of in-situ microwave irradiation NMR spectroscopy for observating non-equilibrium heating state of substances

Yugo Tasei[1], Teruaki Fujito[2], Izuru Kawamura[1], Akira Naito[1] ([1]*Graduate of Engineering, Yokohama National University*, [2]*Probe Laboratory Inc.*)

1P289 **光と磁場を用いた一分子 DNA 操作装置の開発**

A novel method for manipulation of a single DNA molecule using optical and magnetic field

Masahiro Makuta[1,2], Taishi Matsushima[1], Yoshihiro Murayama[1] ([1]*Dept. of Appl. Phys., Tokyo Univ. of Agri. and Tech.*, [2]*WPI-iCeMS, Kyoto Univ.*)

1P290 **フロー型乳酸バイオセンサを用いたマウスの脳内乳酸測定**

Measurement of lactate level in the mouse brain using a flow-type lactate biosensor

Kaoru Yamazaki, Mai Ichikawa, Ryo Shimazaki, Minoru Saito (*Graduate School of Integrated Basic Sciences, Nihon University*)

27. バイオイメージング / 27. Bioimaging

1P291 **Humidity-controlled preparation of frozen-hydrated biological samples for cryogenic coherent X-ray diffraction imaging using XFEL**

Yuki Takayama[1], Masayoshi Nakasako[1,2], Tomotaka Oroguchi[1,2], Yuki Sekiguchi[1,2], Amane Kobayashi[1,2], Masaki Yamamoto[1], Koji Yonekura[1], Takaaki Hikima[1], Saori Maki-Yonekura[1], Yukio Takahashi[1,3], Akihiro Suzuki[1,3], Sachihiro Matsunaga[4], Yayoi Inui-Tsujimoto[4], Shoichi Kato[4], Takahiko Hoshi[5] ([1]*RIKEN SPring-8 Center*, [2]*Grad. Sci. Tech., Keio Univ.*, [3]*Grad. Eng., Osaka Univ.*, [4]*Grad. Sci. Tech., Tokyo Univ. Sci.*, [5]*KOHZU PRECISION Co., Ltd*)

1P292 **ティップスキャン型高速原子間力顕微鏡による生細胞イメージング**

Live cell imaging using a tip-scan type of high-speed atomic force microscopy

Kiyohiko Tateyama[1], Akira Yagi[1], Nobuaki Sakai[1], Yoshitsugu Uekusa[1], Yuka imaoka[1], Shuichi Ito[1] ([1]*Olympus corporation*, [2]*Microtechnology R&D Division*)

1P293 **アップコンバージョンナノ蛍光体を用いた CL・蛍光イメージング**

Upconversion Nanophosphors for Correlative CL and Fluorescent Imaging

Hirohiko Niioka[1], Taichi Furukawa[1], Syoichiro Fukushima[1], Masayoshi Ichimiya[1,2], Tomohiro Nagata[3], Jun Miyake[1], Masaaki Ashida[1], Tsutomu Araki[1], Mamoru Hashimoto[1] ([1]*Grad. Sch. Eng. Sci., Osaka Univ.*, [2]*Osaka Dental Univ.*, [3]*ULVAC, inc.*)

1P294 **ファイバー共焦点レーザー蛍光顕微鏡による自由行動下マウスの神経活動の光学計測**

Fiber-optic fluorescent imaging of neural activity in freely-moving mice during sleep and wakefulness

Yasuhiro Kasagi[1], Takeshi Kanda[1], Kentaroh Honda[1], Masashi Yanagisawa[1,2] ([1]*IIIS, Univ. Tsukuba*, [2]*UTSW/HHMI*)

1P295 **生きた細胞内における内在性テロメア RNA の一分子動態解析**

Single molecule imaging of endogenous telomeric RNA in living cells

Toshimichi Yamada, Hideaki Yoshimura, Mitsuru Hattori, Takeaki Ozawa (*Grad. Sch. Sci., Univ. Tokyo*)

1P296 **Shannon エントロピーの変化でみた質量顕微鏡データ**

Analysis of the difference in Imaging Mass Spectrometry Data characterized by Shannon entropy

Noritaka Masaki, Mitsutoshi Setou (*Dept. Cell Biol. & Anatomy, Hamamatsu Univ. Sch. Med.*)

1P297 **生細胞における膜タンパク質標識法と会合状態解析法の開発**

Development of methods for labeling and oligomerization analysis of membrane proteins in live cells

Yoshiaki Yano, Kenichi Kawano, Kaoru Omae, Sayaka Mtsuzaki, Katsumi Matsuzaki (*Grad. Sch. Pharm. Sci., Kyoto Univ.*)

1P298 **走査型電気化学-イオンコンダクタンス顕微鏡を用いた神経伝達物質の放出サイトのマッピング**

Mapping of neurotransmitter releasing sites using scanning electrochemical ion conductance microscopy

Yasufumi Takahashi[1], Xiongwe Wang[2], Kosuke Ino[2], Hitoshi Shiku[2], Tomoakazu Matsue[1,2] ([1]*WPI-AIMR, Tohoku Univ.*, [2]*Enviromental studies, Tohoku Univ.*)

1P299 **光干渉法を用いた細胞-ハイドロゲル間接着の定量評価**

Quantitative evaluation of cell adhesion to hydrogels by advanced interferometric optical microscopy

Takahisa Matsuzaki[1], Gen Sazaki[2], Masami Suganuma[3], Tatsuro Watanabe[3], Takashi Yamazaki[1], Yuko Shimokawa[1], Motomu Tanaka[4], Seiichiro Nakabayashi[1], Hiroshi Yoshikawa[1] ([1]*Grad. Sch. Sci & Eng., Univ. Saitama*, [2]*Inst. Low Temp. Sci., Univ. Hokkaido*, [3]*Res. Inst. Clin. Onc., Saitama Cancer Center*, [4]*Inst. Phys. Chem., Univ. Heidelberg*)

1P300 **X-ray excited optical luminescence via bio-molecule directed metal clusters**

Yasuko Osakada[1,2], Yoshie Harada[1] ([1]*Kyoto university, iCeMS*, [2]*JST PRESTO*)

1P301 **成長円錐における単一分子レベルでのアクチン関連 mRNA の局在**

The localization of actin-related mRNAs in growth cone at a single molecule level

Hidenori Koizumi[2], Yasuko Osakada[1], Yoshie Harada[1] ([1]*iCeMS.,Univ. Kyoto*, [2]*Grad.Sch.Bio., Univ. Kyoto*)

1P302 **超音波高速 AFM の開発に向けた基礎研究 2**

Pilot study 2 for the development of high-speed ultrasonic AFM

Tomofumi Saito[1], Noriyuki Kodera[2], Toshio Ando[1,2] ([1]*Sch. Math. & Phys., Inst. Sci.*, [2]*Bio-AFM Frontier Research Center, Inst. Sci. & Eng., Kanazawa Univ.*)

1P303 **Real-time observation of amyloid fibril formation of yeast prion Sup35 by high-speed atomic force microscopy**

Liwen Zhu[1], Hiroki Konno[1], Momoko Okuda[2], Noriyuki Kodera[1], Toshio Ando[1], Hideki Taguchi[2] ([1]*Bio-AFM Frontier research center, Kanazawa University*, [2]*Department of Biomolecular Engineering, Graduate School of Biosciences and Biotechnology, Tokyo Institute of Technology*)

1P304 **高速 AFM によるバクテリアの高分解能観察**
Nanoscale investigation on bacterial cell surface using high-speed AFM
Hiroki Watanabe[1], Carriou David[1], Takayuki Uchihashi[1,2], Toshio Ando[1,2] ([1]*Dep. Phys., Col. of Sci. and Engr., Kanazawa Univ.*, [2]*Bio-AFM Frontier Res. Center*)

1P305 **高速 AFM / 光学顕微鏡複合機**
Combined system of High-speed-AFM and optical microscopy
Shingo Fukuda[1], Takayuki Uchihashi[1,2], Ryota Iino[3], Toshio Ando[1,2] ([1]*Department of Mathematics and Physics, Grad School of Natural Science and Technology, Kanazawa University*, [2]*Bio-AFM Frontier Reserch Center, College of Science and Engineering, Kanazawa University*, [3]*Department of Applied Chemistry Grad School of Engineering The University of Tokyo*)

1P306 **高速 AFM による ClpB の構造ダイナミクスの観察**
Conformations and dynamics of ClpB hexameric ring observed by high-speed AFM
Takayuki Uchihashi[1,2], Yo-hei Watanabe[3], Ryota Iino[4], Hiroki Watanabe[1], Takashi Yamasaki[3], Toshio Ando[1,2] ([1]*Dept.Phys., Kanazawa Univ.*, [2]*Bio-AFM Frontier Research Center, Kanazawa Univ.*, [3]*Dept. Biol., Konan Univ.*, [4]*Dept. Appl. Chem., Univ. of Tokyo*)

28. バイオエンジニアリング / 28. Bioengineering

1P307 **動的な DNA コンピューティングを実現するための AND ゲートモジュールの開発**
Development of AND gate module for dynamic DNA computing
Takakshi Nukada, Koh-ichiroh Shohda, Akira Suyama (*Grad. Sch. Arts and Sciences, Univ. Tokyo*)

1P308 **オンチップマルチイメージングセルソーターを用いたクラスター化細胞のリアルタイム認識と回収のための画像解析技術の研究**
Real time image analysis technology for identification and collection of clustered cells using on-chip multi-imaging cell sorter
Masao Odaka[1], Mathias Girault[1], Hyonchol Kim[1], Hideyuki Terazono[1,2], Akihiro Hattori[2], Kenji Yasuda[1,2] ([1]*KAST*, [2]*Tokyo Med. Dent. Univ.*)

1P309 **オブジェクト指向によるロボットとの認識共有**
Object-Oriented Cognition Sharing as a Method of Brain-Machine-Interface
Jun Miyake[1], Kazuyuki Hatta[1], Amalia Adiba[1], Ryuuzou Baba[2], Tadahiro Kaneda[2] ([1]*Graduate School of Engineering Science, University of Osaka*, [2]*Osaka Prefecture University College of Technology*)

1P310 **DNA Computer-Controlled Gene Expression in a Cell Model Vesicle**
Takamasa Hasegawa[1], Koh-ichiroh Shohda[2], Akira Suyama[1,2] ([1]*Univ Tokyo, Dept Phys, Grad Sch Sci*, [2]*Univ Tokyo, Dept Life Sci, Grad Sch Arts & Sci*)

1P311 **Simple and Efficient Approach for Proteomic Analysis of Subcellular Structures using Droplet-Based Microfluidics**
Haruka Okada[1], Ryo Iizuka[1], Rui Sekine[2], Dong H. Yoon[2], Tetsushi Sekiguchi[3], Shuichi Shoji[2], Takashi Funatsu[1] ([1]*Grad. Sch. of Pharm. Sci., The Univ. of Tokyo*, [2]*Major in Nanosci. and Nanoeng., Waseda Univ.*, [3]*Nanotech. Research Center, Waseda Univ.*)

1P312 **Yeast-based fluorescence assay system for detecting human G protein-coupled receptor activation in water-in-oil droplets**
Takashi Sakurai[1], Ryo Iizuka[1], Rui Sekine[2], Yoon Dong H.[2], Tetsushi Sekiguchi[3], Jun Ishii[4], Akihiko Kondo[5], Shuichi Shoji[2], Takashi Funatsu[1] ([1]*Grad. Sch. of Pharm. Sci., Univ. of Tokyo*, [2]*Major in Nanosci. and Nanoeng., Waseda Univ.*, [3]*Nanotech. Research Center, Waseda Univ.*, [4]*Org. of Advanced Sci. and Tech., Kobe Univ.*, [5]*Grad. Sch. of Sci. and Tech., Kobe Univ.*)

1P313 **Optical microdevice operated through self-organization of microtubule and kinesin: An experimental study**
Ayumu Miyata[1], Yuichi Hiratsuka[2], Takahiro Nitta[1] ([1]*Gifu University*, [2]*JAIST*)

1P314 **Optical microdevice operated through self-organization of microtubule and kinesin: A simulation study**
Takahiro Nitta[1], Yuichi Hiratsuka[2] ([1]*Gifu Univ.*, [2]*JAIST*)

1P315 **Theree-Dimensional Movements of Microtubule Diriven by Kinesin on Microfabricated Tracks Revealed with a Computer Simulation**
Yuki Ishigure, Takahiro Nitta (*Gifu University*)

1P316 **明視野/蛍光画像の同時リアルタイム解析技術を用いたオンチップ・マルチイメージング・フローサイトメーターの開発**
Development of On-chip Multi-imaging Flow Cytometer System using Real-time Bright Field/Fluorescent Dual Image Analysis High-speed Camera
Akihiro Hattori[1], Hyonchol Kim[2], Hideyuki Terazono[1], Masao Odaka[2], Mathias Girault[1], Kenji Yasuda[1,2] ([1]*Department of Biomedical Information, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University*, [2]*Kanagawa Academy of Science and Technology*)

第 2 日目（10 月 29 日（火））／Day 2（Oct. 29 Tue.）アネックスホール / Annex hall

01A. 蛋白質：構造 / 01A. Protein: Structure

2P001 **フラビン酵素 RebC 変異体の結晶構造解析とインドロカルバゾール骨格の構造多様性の創出原理の解明**
Crystal structure of a mutant flavoenzyme RebC and construction mechanism of indolocarbazole aglycone structure
Hayate Itatani[1], Eiyu Izumo[1], Saki Kageyama[2], Sayaka Kurozumi[1], Hiroyasu Onaka[3], Shumpei Asamizu[3], Tomoya Hino[1], Shingo Nagano[1] ([1]*Grad. School of Eng., Tottori Univ.*, [2]*Faculty of Eng., Tottori Univ.*, [3]*Faculty of Eng., Toyama Pref. Univ.*)

2P002 **Crystal structure of cruxrhodopsin-3 from Haloarcula vallismortis**
Siu Kit Chan[1], Tomomi Kitajima[1], Midori Murakami[1], Kunio Ihara[2], Tsutomu Kouyama[1] ([1]*Dept. Phys., Grad. Sch. Sci., Nagoya Univ.*, [2]*Center for Gene Research, Nagoya Univ.*)

2P003 **べん毛 III 型輸送装置蛋白質 FlhA の細胞質領域の構造変化**

Conformational change of a cytoplasmic fragment of FlhA, a flagellar type III protein export apparatus protein

Yuya Ogawa[1], Noritaka Hara[2], Yumiko Uchida[1], Miki Kinoshita[1,2], Tohru Minamino[2], Katsumi Imada[1] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*Grad. Sch. Frontier BioSci., Univ. Osaka*)

2P004 **コレラ菌の走化性受容体蛋白質 Mlp24 とそのリガンド複合体の構造**

Structure of a chemoreceptor protein of *Vibrio cholerae*, Mlp24, and its ligand complex

Yohei Takahashi[1], Kazuma Sumita[1], Yumiko Uchida[1], So-ichiro Nishiyama[2], Ikuro Kawagishi[2], Katsumi Imada[1] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*Dept. Front. Biosci. Sci., Univ. Hosei*)

2P005 **4-*O*-β-D-mannosyl-D-glucose phosphorylase (MGP) のＸ線結晶構造解析**

Structure of novel enzyme 4-*O*-β-D-mannosyl-D-glucose phosphorylase MGP

Setsu Nakae[1], Shigeaki Ito[2], Mariko Higa[3], Takeshi Senoura[4], Jun Wasaki[5], Atsushi Hijikata[1], Masafumi Shionyu[1], Susumu Ito[3], Tsuyoshi Shirai[1] ([1]*Dept. BioSci., Nagahama Inst. Bio-Sci. Tech.*, [2]*Central Tobacco Research Center, Japan Tobacco Inc.*, [3]*Fac. Agri.,Univ. Ryukyus*, [4]*Research Institute for Bioresources and Biotechnology, Ishikawa Pref. Univ.*, [5]*Grad. Sch. Biosphere Sci., Hiroshima Univ.*)

2P006 **組み替え human poly(ADP-ribose) polymerase 1 の精製と予備的構造解析**

Purification and preliminary structure analysis of recombinant human poly(ADP-ribose) polymerase 1

Kenichi Koyama[1], Kouta Mayanagi[2], Takayuki Eguchi[1], Hiroyuki Morita[1], Kazuo Kamemura[1], Yoshisuke Nishi[1], Masanao Miwa[1], Tuyosi Shirai[1] ([1]*Dept. BioSci., Nagahama Inst. Bio-Sci. Tech.*, [2]*Med. Inst. Bioreg. Kyusyu University.*)

2P007 **ＰＥＬＤＯＲによる時計タンパク質ＫａｉＢの構造変化の検出**

PELDOR detection of structural changes of clock protein KaiB

Ryosuke Tajika[1], Risa Mutoh[2,3], Masahiro Ishiura[1,3], Hiroyuki Mino[1] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Inst. Prot. Res., Osaka Univ.*, [3]*Center for Gene Res., Nagoya Univ.*)

2P008 **2.5 kbar におけるユビキチン高エネルギー状態の立体構造解析**

Solution structure of the "pure" high-energy state of ubiquitin: Q41N at 2.5 kbar

Ayumi Kumo[1], Soichiro Kitazawa[1], Tomoshi Kameda[2], Nicola J. Baxter[3], Michael P. Williamson[3], Ryo Kitahara[1] ([1]*College of Pharmaceutical Sciences, Ritsumeikan University*, [2]*Computational Biology Research Center, Advanced Industrial Science and Technology*, [3]*Computational Biology Research Center, Advanced Industrial Science and Technology*)

2P009 **X 線小角散乱と電子顕微鏡像を用いたハイブリッド構造解析**

Hybrid structure analysis with small-angle x-ray scattering and cryo-electron microscopic image

Shota Kaimi[1], Ryo Ishiguro[2,3], Tetsuro Fujisawa[2,3] ([1]*Grad. Sch. Eng., Gifu Univ.*, [2]*SPring-8, Riken*, [3]*Fac. Eng., Gifu Univ.*)

2P010 **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*)

2P011 **Comparative survey of image processing packages for electron computed tomography**

Nan Shen[1], Mingyue Jin[2], Takuo Yasunaga[1] ([1]*Kyushu Institute of Technology*, [2]*Osaka City University*)

2P012 **Possibility of metallothionein Labelling for CLEM method**

Ryutaro Yamanaka[1], Yuka Hirasaka[1], Mingyue Jin[1], Haruaki Yanagisawa[2], Takuo Yasunaga[1] ([1]*Kyushu Institute of Technology*, [2]*Univ. of Tokyo*)

2P013 **A new approach to build 3D atomic model from single electron microsope image**

Atsushi Matsumoto[1], Junichi Takagi[2], Kenji Iwasaki[2] ([1]*Japan Atomic Energy Agency*, [2]*Osaka University*)

2P014 **プロリンリッチなペプチドのコンホメーション特性に関する考察**

An investigation on the conformation character of proline-rich peptides

Masahito Oka (*Osaka prefecture university*)

2P015 **生体電子の流れが加速する電流生成菌の細胞外電子移動機構の発見**

Respiratory Electron Flow Enhances the Rate of Extracellular Electron Transport Processes in Current-Producing Bacteria

Akihiro Okamoto[1], Ryuhei Nakamura[2], Kenneth H. Nealson[3], Kazuhito Hashimoto[1] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Wako Inst., Riken*, [3]*Univ. South California*)

2P016 **再重法を用いたタンパク質力場パラメータの最適化**

Optimization of force-field parameters for protein systems by an energy-based reweighting approach

Yoshitake Sakae[1,2], Yuko Okamoto[1,3,4,5] ([1]*Dept. Phys., Nagoya Univ.*, [2]*IMS*, [3]*Structural Biology Research Center, Nagoya Univ.*, [4]*Center for Computational Science, Nagoya Univ.*, [5]*Information Technology Center, Nagoya Univ.*)

2P017 **ヘモグロビンの酸素結合に伴うアロステリック転移のカメレオンモデルによる研究**

A simulation study with the chameleon model: The allosteric transition of hemoglobin associated with oxygen binding

Yui Sobue, Toru Kimura, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Univ. Nagoya*)

2P018 **天然変性タンパク質の結合と共役した折りたたみ部位の相互作用解析**

Contact analysis of Protean Segments (ProSs) in intrinsically disordered proteins (IDPs)

Divya Shaji[1], Takayuki Amemiya[1], Satoshi Fukuchi[2], Motonori Ota[1] ([1]*Grad. Schl. of Info. Sci., Nagoya Univ.*, [2]*Fac. Eng. Maebashi Inst., Tech.*)

2P019 **Hras-GTP 複合体と Hras-GDP 複合体の分子動力学シミュレーションにおける水分子ネットワークの解析**
Analysis of network of water molecules in molecular dynamics simulations of Hras-GTP and GDP complexes
Takeshi Miyakawa[1], Ryota Morikawa[1], Masako Takasu[1], Kimikazu Sugimori[2], Kazutomo Kawaguchi[2], Hiroaki Saito[2], Hidemi Nagao[2] ([1]*Tokyo University of Pharmacy and Life Sciences*, [2]*Kanazawa University*)

2P020 **分子動力学シミュレーションによる GLP-1 の最適構造探索**
Optimized structure study of GLP-1 by Molecular Dynamics Simulation
Sakiko Mori, Hironao Yamada, Masaki Fukuda, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu, Takuya Watanabe (*School of Life Sciences, Tokyo University of Pharmacy and Life Sciences*)

2P021 **エネルギー表示溶液理論を用いた 分子動力学シミュレーションによる蛋白質複合体モデルの評価**
Evaluation of protein complex model using molecular dynamics simulation with the solution theory in the energy representation
Kazuhiro Takemura[1], Nobuyuki Matubayasi[2], Akio Kitao[1] ([1]*IMCB, Univ. Tokyo*, [2]*Inst. Chem. Res., Kyoto Univ.*)

2P022 **チオエステル周辺の AMBER 力場の開発および評価**
Determination and evaluation of AMBER force field parameters for thioester
Akifumi Oda[1,2], Shuichi Fukuyoshi[1], Ryoichi Nakagaki[1], 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.*)

2P023 **アミロイドβの構造探索**
Conformational Search of Amyloid β Peptide
Satoshi Yokojima (*Sch. Pharmacy, Tokyo Univ. Pharmacy and Life Sci.*)

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

2P024 **Photo synthesis of protein-based drug delivery nanoparticles for active tumor targeting**
Meng Qin (*Department of Physics, Nanjing University*)

2P025 **Single molecule force spectroscopy reveals force-enhanced binding of calcium ions by gelsolin**
Yi Cao[1], Chunmei Lv[1], Wenfei Li[1], Xiang Gao[1], Robert Robinson[2], Meng Qin[1], Leslie Burtnick[3], Wei Wang[1] ([1]*Nanjing University*, [2]*A*STAR*, [3]*University of British Columbia*)

2P026 **Direct observation of the multiple sliding modes of a tumor suppressor p53**
Agato Murata[1,2], Risa Kashima[3], Yuji Itoh[1,2], Takashi Tokino[4], Satoshi Takahashi[2], Kiyoto Kamagata[2] ([1]*IMRAM, Univ. Tohoku*, [2]*Grad. Sch. Sci., Univ. Tohoku*, [3]*CVRI, UCSF*, [4]*Research Institute for Frontier Medicine, Univ. Sapporo Medical*)

2P027 **Study of a peptidase-associated domain of an aminopeptidase from thermophilic *Aneurinibacillus* sp. AM-1**
Ryuji Tagawa[1], Hiroaki Nakano[2], Kunihiko Watanabe[1] ([1]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Dept. of Pharm., Hyogo Univ. of Health Sci.*)

2P028 **ケモカインシグナル細胞内制御因子 FROUNT とその受容体認識に関する構造生物学的研究**
Structural analyses of FROUNT, the cytosolic regulator of chemokine signaling, and its chemokine receptor recognition
Sosuke Yoshinaga[1], Tatsuichiro Tsuji[1], Akihiro Sonoda[1], Norihito Ishida[1], Yusuke Tsuchiya[1], Kaori Esaki[1], Yuya Terashima[2], Etsuko Toda[2], Takashi Saitoh[3], Daisuke Kohda[3], Ichio Shimada[4], Kouji Matsushima[2], Hiroaki Terasawa[1] ([1]*Fac. Life Sci., Kumamoto Univ.*, [2]*Grad. Sch. Med., Univ. Tokyo*, [3]*Med. Inst. Bioreg., Kyushu Univ.*, [4]*Grad. Sch. Pharm. Sci., Univ. Tokyo*)

2P029 **トウガレイ由来 I 型不凍蛋白質の構造機能解析**
Analysis of structure and function of a new type I antifreeze protein from a Japanese fish, Barfin Plaice
Kazunari Ishihara[1], Yuichi Hanada[1], Hidemasa Kondo[1,2], Ai Miura[2], Sakae Tsuda[1,2] ([1]*Graduate School of Life Science, Hokkaido University*, [2]*National Institute of Advanced Industrial Science and Technology (AIST)*)

2P030 **ジスルフィド結合が制御するバクテリア SOD1 の構造形成メカニズム**
Folding mechanism of bacterial SOD1 regulated by disulfide formation
Yasuyuki Sakurai, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

2P031 **線虫モデルを利用した神経変性疾患における病態伝播のメカニズム解明**
A worm model describing propagation of protein aggregates in neurodegenerative diseases
Mariko Ogawa[1], Hisashi Shidara[2], Kotaro Oka[2], Yoshiaki Furukawa[1] ([1]*Dept. of Chem., Keio Univ.*, [2]*Dept. of Biosci. Informatics, Keio Univ.*)

2P032 **筋萎縮性側索硬化症に関わる SOD1 タンパク質の四次構造変化を検出できるペプチドの開発**
Aberrant monomer-dimer equilibrium of mutant SOD1 in ALS: Development of peptides probing protein quaternary structures
Takao Nomura, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

2P033 **SOD1 への細胞内銅イオン輸送を制御するタンパク質ネットワーク**
A protein network regulating an intracellular copper transfer to superoxide dismutase
Kenta Nakagome, Yasushi Mitomi, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

2P034 **亜鉛イオンが制御する銅シャペロンシステムの分子認識メカニズム**
Zinc ion regulates molecular recognition in copper chaperone system
Yuma Wakahara, Kazuki Honda, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

2P035 **筋萎縮性側索硬化症に関わる変異型 SOD1 タンパク質のオリゴマー化メカニズム**
Oligomerization mechanism of mutant SOD1 proteins in a familial form of amyotrophic lateral sclerosis
Itsuki Anzai, Keisuke Toichi, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ*)

2P036 **神経変性疾患における老化の役割を検証する酵母モデルの構築**
A yeast model for testing roles of aging process in neurodegenerative diseases
Yuko Nishiura, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

2P037 **蛋白質のミスフォールド状態から生じる蛋白質の異常凝集**
Misfolding triggers a pathogenic conversion of protein conformations
Soichiro Kitazawa[1], Ryo Kitahara[1], Makoto Urushitani[2] ([1]*Pharmaceutical science, Ritsumeikan Univ.*, [2]*Moleclar Neuroscience Research Center, Shiga University of Medical Science.*)

2P038 **べん毛輸送装置構成蛋白質 FliP ペリプラズミックループの結晶化と遺伝学的解析**
Crystallization and genetic analyses of a periplasmic loop of FliP, a component of the flagellar protein export apparatus
Takuma Fukumura[1], Yumiko Saijo-Hamano[1], Yukio Furukawa[1], Tatsuya Kawaguchi[2], Katsumi Imada[2], Keiichi Namba[1], Tohru Minamino[1] ([1]*Grad. Sch, Frontier Biosci., Osaka Univ*, [2]*Grad. Sch. Sci. Osaka Univ*)

2P039 **Structural defects in fibrillin associated with Marfan syndrome**
Yaxin Lu[1], Richmond Jeremy[2], Murat Kekic[1], Jianlin Yin[2], Brett Hambly[1] ([1]*Pathology Discipline and Bosch Institute, Sydney Medical School, University of Sydney*, [2]*Central Clinical School, Sydney Medical School, University of Sydney*)

2P040 **Microtubule-associated protein 4-mediated bundle formation of microtubules and actin filaments**
Shoma Saito[1], Ayumu Kuramoto[1], Hikari Makihara[1], Tsuyoshi Yamazaki[2], Taro Q.P. Noguchi[2], Susumu Kotani[3], Kiyotaka Tokuraku[1] ([1]*Grad. Sch. Appl. sci., Muroran Inst.*, [2]*Miyakonojo Nation. Col. Tech*, [3]*Kanagawa University*)

2P041 **タンパク質の圧電効果とアロステリック制御**
Piezoelectric effect in a protein and its involvement in allosteric regulation
Jun Ohnuki[1], Takato Sato[1], Koji Umezawa[1], Taro Q.P. Uyeda[2], Mitsunori Takano[1] ([1]*Grad. Scl. of Adv. Sci. & Eng., Waseda Univ.*, [2]*Biomedical Res. Inst., AIST*)

2P042 **MD で観測された G アクチンのヌクレオチド依存構造状態と F アクチン安定性との関連**
Nucleotide-dependent structural states of G-actin observed by MD simulation and its implication for F-actin stability
Jun Ohnuki, **Mitsunori Takano** (*Dept of Phys & Appl Phys, Waseda Univ*)

2P043 **アロステリック機構の分子論的理解に向けたシグナルタンパク質 CheY の研究**
Toward a molecular level understanding of allostery in the signaling protein CheY
Toshifumi Mori, Qiang Cui (*Univ. of Wisconsin, Madison*)

2P044 **MARTINI 粗視化力場を用いたタンパク質-リガンド結合過程の比較シミュレーション**
Comparative simulations of protein-ligand binding processes using the MARTINI coarse-grained force field
Tatsuki Negami, Tohru Terada, Kentaro Shimizu (*Grad. Sch. of Agri. and Life Sci., Univ. of Tokyo*)

2P045 **粗視化モデルによる PPARγ の基質依存的な活性変化の考察**
Coarse-grained model study of ligand-dependent reaction activity of PPARγ
Tomo Matsubara, Hiraku Nishimori, Akinori Awazu (*Dept. of math and Life Sci, Hiroshima Univ*)

2P046 **構造変化を介した分子内情報伝達パターンの探索: 粗視化分子動力学計算による試み**
Screening for Mechanical Communication in Proteins by Coarse-Grained Molecular Dynamics
Yuichi Togashi (*Grad. Sch. Sys. Informat., Kobe Univ.*)

2P047 **粗視化シミュレーションによるリン酸化酵素複合体(MEK1-ERK2)のドッキングダイナミクス**
Docking dynamics of MAP kinase: MEK1-ERK2 complex system studied by coarse-grained simulation
Ryo Kanada, Shoji Takada (*Grad. Sch. Sci., Univ. Kyoto*)

2P048 **酵母 MAPK 経路における伝達制御機構の分子シミュレーション研究**
Molecular simulation study on signaling control in yeast MAPK pathway
Naoto Hori, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

2P049 **Folding coupled with binding and allosteric motions in calmodulin domains**
Wenfei LI[1], Wei WANG[1], Shoji Takada[2] ([1]*Department of Physics, Nanjing University*, [2]*Graduate School of Science, Kyoto University*)

01C. 蛋白質：物性 / 01C. Protein: Property

2P050 **FTIR 分光法を用いたユビキチンの温度-圧力変性状態の研究**
Pressure and temperature denaturation of ubiquitin by FTIR spectroscopy
Tsubasa Yamamoto[1], Minoru Kato[1,2] ([1]*Grad. Sch. of Lifescience, Ritsumeikan Univ.*, [2]*Dept. Pharma. Ritsumeikan Univ.*)

2P051 **FTIR を用いた圧力・温度可変実験による GB1(41-56)の変異体の β-hairpin 構造安定性**
Pressure and Temperature variable FTIR study on the structural stability of β-hairpin model peptides of mutants of GB1(41-56)
Keita Tsuchiya[1], Yudai Yamaoki[2], Minoru Kato[1,3] ([1]*Grad. Sch. life science, Univ. Ritsumeikan*, [2]*Institute of Advanced Energy, Univ. Kyoto*, [3]*Pharm. Univ. Ritsumeikan*)

2P052 **ペプチドにおける二次構造の圧力依存性：焼き戻し分子動力学法による研究**
Pressure dependence of the secondary structure of a peptide: A simulated tempering molecular dynamics study
Yoshiharu Mori[1], Hisashi Okumura[1,2] ([1]*Inst. Mol. Sci.*, [2]*SOKENDAI*)

2P053 **アミノ酸の物性に注目した疾患感受性遺伝子変異の判別**
Discrimination of disease-susceptibility mutations by physical properties of amino acid fragments around the mutation
Ryouta Masai[1], Shigeki Mitaku[1,2] ([1]*Dept. Applied Physics, Grad. Sch. Engineering, Nagoya Univ*, [2]*Toyoda Physical and Chemical Res. Inst.*)

2P054 シトクロム *c* 多量体の細胞膜結合

Binding of Oligomeric Cytochrome *c* to Cell Membrane

Sendy Junedi, Kazuma Yasuhara, Satoshi Nagao, Jun-ichi Kikuchi, Shun Hirota (*Grad. Sch. Mat. Sci., Nara Inst. Sci. Tech.*)

2P055 シトクロム c のドメインスワップ多量化とモルテングロビュル状態

Domain-Swapped Oligomerization and Molten Globule State of Cytochrome c

Megha Deshpande[1], Partha Parui[2], Masaru Yamanaka[1], Satoshi Nagao[1], Hironari Kamikubo[1], Mikio Kataoka[1], Hirofumi Komori[3], Yoshiki Higuchi[4], Shun Hirota[1] ([1]*Graduate School of Materials Science, Nara Institute of Science and Technology*, [2]*Department of Chemistry, Jadavpur University, Kolkata 700032, India*, [3]*Faculty of Education, Kagawa University*, [4]*Department of Life Science, Graduate School of Life Science, University of Hyogo*)

2P056 粗視化分子動力学シミュレーションによるミオグロビンのドメインスワッピング機構の研究

Domain swapping of myoglobin dimer studied by coarse-grained molecular dynamics simulations

Koji Ono, Shoji Takada (*Dept. Biophys., Grad. Sch. Sci., Kyoto Univ.*)

2P057 Wang-Landau マルチカノニカル法による Go モデル分子動力学シミュレーション

Wang-Landau Multicanonical Method for Go-model Molecular Dynamics Simulation

Mashiho Ito, Shoji Takada (*Dept. Biol., Sch. Sci., Kyoto Univ.*)

2P058 粗視化 MD を用いた SUFI のコ・トランスレーショナルフォールディングの解析

Analysis of co-translational folding of SUFI by coarse grained MD simulation

Tomohiro Tanaka, Naoto Hori, Shoji Takada (*Dept. of Biophys. Kyoto Univ.*)

2P059 粗視化 Go モデルを用いた多状態タンパクにおける遷移の回数とフォールディングコアとの関係の解析

An analysis of the relationship between the number of transitions and folding cores in multi-transition proteins by means of Go model

Masatake Sugita, Takeshi Kikuchi (*Dept. of Bioinfo., Col. of Life Sci., Ritsumeikan Univ*)

2P060 タンパク質フォールディングにおける自由エネルギーバリアと天然トポロジー間の関係

Relationships between the free energy barrier in protein folding and native topology

Koki Yamashita, Masatake Sugita, Takeshi Kikuchi (*Dept. Bioinf., Col. Life Sci., Ritsumeican Univ.*)

2P061 一分子蛍光分光法によるユビキチンの折り畳みダイナミクスの測定

Dynamics of ubiquitin folding detected by single molecule fluorescence spectroscopy

Masataka Saito[1,2], Hsin-Liang Chen[3], Rita Chen[3], Kiyoto Kamagata[1,2], Hiroyuki Oikawa[1], Satoshi Takahashi[1,2] ([1]*Tohoku University Institute of Multidisciplinary Research for Advanced Materials*, [2]*Tohoku University Department of Chemistry, Graduate School of Science*, [3]*Academia Sinica Institute of Biological Chemistry*)

2P062 プロテイン A - B ドメインの高速折り畳みダイナミクスの追跡を目指したライン共焦点顕微鏡の改良

Improvements of the line confocal system for the single molecule tracking of fast folding dynamics of the B domain of protein A

Hiroyuki Oikawa[1], Kiyoto Kamagata[1], Munehito Arai[2], Satoshi Takahashi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Arts. Sci., Univ. Tokyo*)

2P063 イソロイシンタグを付加した BPTI 変異体の熱転移における可逆的なオ リゴマー形成過程の熱力学的解析

Thermodynamic characterization of a reversible oligomerization process in the thermal transition of a BPTI variant tagged with isoleucines

Shigeyoshi Nakamura[1], Tomoka Wachi[2], Ryo Shimizu[2], Mohammad M Islam[2], Yutaka Kuroda[2], Shun-ichi Kidokoro[1] ([1]*Dept of Bioeng, Nagaoka Univ of Tech*, [2]*Dept of Biotech and Life Sci, Tokyo Univ of Agr & Tech*)

2P064 アミロイドと可溶性蛋白質の間の相互作用の幾つかの一般的側面

Some general aspects of interaction between amyloid and soluble proteins

Takashi Konno (*University of Fukui, Faculty of Medical Sciences, Molecular Physiology*)

2P065 ビーズ表面に結合した細胞外マトリクス成分は、気液界面非存在下でアルツハイマー病 β アミロイド線維の核形成を促進させる

Surface-bound basement membrane components on Sepharose beads accelerate amyloid β-peptide nucleation in air-free wells

Kazuhiro Hasegawa, Daisaku Ozawa, Tadakazu Ookoshi, Hironobu Naiki (*Div. Mol. Pathol., Dept. Pathol. Sci., Univ. Fukui*)

2P066 脂質ベシクルの疎水領域が与えるアミロイド β 線維形成への影響について

The effects of the hydrophobic area of vesicles on the fibrillation of Aβ

Mayu Suzuki, Hisashi Yagi, Yuji Goto (*Inst. Protein. Res., Osaka Univ.*)

2P067 β_2-ミクログロブリンのアミロイド形成における様々な脂肪酸の効果

Effects of various fatty acids on the amyloid fibrillation of β_2-microglobulin

Akira Ishii[1], Masatomo So[1], Hisashi Yagi[1], Hironobu Naiki[2], Yuji Goto[1] ([1]*Inst. Protein Res., Osaka Univ.*, [2]*Fac. Med. Sci., Univ. Fukui*)

2P068 β ２ミクログロブリンのアミロイド前駆状態の残余構造の特性化

The properties of the residual structure of amyloid precursor state of β2-microglobulin

Kazumasa Sakurai[1,2], Akihiro Maeno[1], Hironobu Naiki[3], Yuji Goto[2], Kazuyuki Akasaka[1] ([1]*HPPRC, Kinki Univ.*, [2]*Inst. Protein Res., Osaka Univ.*, [3]*Fac. Med. Sci., Univ. Fukui*)

2P069 超音波によるアミロイド線維形成促進のメカニズム

The mechanism of ultrasonication-induced amyloid fibril formation

Masatomo So[1], Yuichi Yoshimura[1], Hisashi Yagi[1], Hirotsugu Ogi[2], Kentaro Uesugi[2], Hironobu Naiki[3], Yuji Goto[1] ([1]*Institute for Protein Research, Osaka University*, [2]*Graduate School of Engineering Science, Osaka University*, [3]*Faculty of Medical Sciences, University of Fukui*)

2P070 **Solubility and Supersaturation-Dependent Protein Misfolding Revealed by Ultrasonication**

Yuxi Lin, Young-Ho Lee, Yuichi Yoshimura, Hisashi Yagi, Yuji Goto (*Institute for Protein research, Osaka University*)

2P071 熱測定によるアミロイド線維形成バーストに関する研究

Direct observation of burst of amyloid fibril formation by calorimetry

Tatsuya Ikenoue[1], Young-Ho Lee[1], Jozsef Kardos[2], Yuji Goto[1] ([1]*Inst. Pro. Res., Osaka Univ.*, [2]*Inst. Bio., Eotvos Lorand Univ.*)

01D. 蛋白質：機能 / 01D. Protein: Function

2P072 **Efficient Lookup Table using a Linear Function of Inverse Distance Squared**
Jaewoon Jung[1], Takaharu Mori[2,3], Yuji Sugita[1,2,3] ([1]*AICS, Riken*, [2]*Riken*, [3]*QBiC, Riken*)

2P073 **MuSTAR MD : Multi-Scale Temperature Accelerated Replica exchange Molecular Dynamics**
Yu Yamamori, Akio Kita (*Institute of Molecular and Cellular Bioscience, the University of Tokyo*)

2P074 **α-シヌクレイン繊維形成に対する分子混雑の影響**
Macromolecular crowding effect on fibril formation of α-synuclein
Nobu C. Shirai[1,2], Macoto Kikuchi[1,2,3] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*Cybermed. Cent., Osaka Univ.*, [3]*Fron. Biosci., Osaka Univ.*)

2P075 **サルモネラべん毛繊維の多型変換におけるフラジェリン Arg ４３１の役割**
The role of Arg431 of flagellin in the polymorphic transformation of Salmonella flagellar filament
Fumio Hayashi, Kenji Oosawa (*Div. Mol. Sci., Fac.Sci. and Tech, Gunma Univ.*)

2P076 **表面力測定によるシグナル伝達タンパク質間相互作用の研究**
Interactions between signal transduction proteins studied by surface forces measurement
Asuka Sakai[1], Hitomi Fujiwara[1], Masaya Fujita[3], Kazue Kurihara[1,2] ([1]*IMRAM, Tohoku Univ.*, [2]*WPI-AIMR*, [3]*Univ.Houston*)

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

2P077 **Ｘ線自由電子レーザーにより得られる低分解能データセットに対する単粒子構造解析法**
Methodology of a single biomolecular structure determination for low-resolution data set obtained by X-ray Free Electron Laser
Atsushi Tokuhisa, Osamu Miyashita, Florence Tama (*Computational Structural Biology Research Unit, AICS, RIKEN*)

2P078 **圧縮センシングを用いた NMR スペクトルの復元法**
Reconstruction of NMR spectra using compressed sensing
Kazuya Sumikoshi[1], Teppei Ikeya[2], Yutaka Ito[2], Kentaro Shimizu[1] ([1]*Grad. Sch. Agric. Life Sci., Univ. Tokyo*, [2]*Grad. Sch. Sci., Tokyo Metropolitan Univ.*)

2P079 **Intermolecular interactions and conformation of antibody dimers present in IgG1 biopharmaceuticals**
Takafumi Iura[1,2], Jun Fukuda[2], Katsuyoshi Yamazaki[2], Shuji Kanamaru[1], Fumio Arisaka[1] ([1]*Grad Sch of Biosci & Biotech, TIT*, [2]*Kyowa Hakko Kirin Co. Ltd.*)

2P080 **細胞膜上のガレクチン３もその細胞膜分子との複合体も、細胞膜上で極めて動的に振る舞う：超高速 1 分子追跡による研究**
Galectin-3 and its glyco-molecule conjugates are extremely dynamic on the cell surface: detection by ultrafast single-molecule tracking
Aiko S. Kondo[1,2], Ludger Johannes[3], Ziya Kalay[2], Ivan R. Navi[4], Manami S. H. Miyahara[1,2], Hisae Tsuboi[2], Koichiro M. Hirosawa[2], Kenta J. Yoshida[1,2], Akihiro Kusumi[1,2], Takahiro K. Fujiwara[2] ([1]*Inst. Frontier Med. Sci., Kyoto Univ.*, [2]*WPI-iCeMS, Kyoto Univ.*, [3]*Inst. Curie and CNRS*, [4]*Life Sci. Inst., Univ. of British Columbia*)

2P081 **分子動力学シミュレーションによる 1 分子 FRET のデータ同化**
Sequential data assimilation to single-molecule FRET photon-counting data by using molecular dynamics simulations
Yasuhiro Matsunaga[1], Yuji Sugita[1,2,3] ([1]*RIKEN AICS*, [2]*RIKEN ASI*, [3]*RIKEN QBiC*)

2P082 **一分子時系列から抽出されたマルコフ連鎖 定常ネットワーク における遷移確率が“最小”となる分子の“状態”の同定**
Identifying chemical states in Markov chain steady state network extracted from time series by finding “minimum” transition probability
Yutaka Nagahata[1], Hiroshi Teramoto[1,2], Chun-Biu Li[2], Tamiki Komatsuzaki[1,2] ([1]*Graduate School of Life Science, Hokkaido University*, [2]*Research Institute for Electronic Science, Hokkaido University*)

2P083 **X 線１分子追跡法による II 型シャペロニン協同的運動評価**
Cooperative Motion Analysis of group II chaperonin by X-ray Single Molecule Tracking
Hiroshi Sekiguchi[1], Yohei Yamamoto[2], Mayuno Arita[2], Naoki Ishiguro[2], Kouhei Ichiyanagi[3], Masafumi Yohda[2], Naoto Yagi[1], Yuji Sasaki[3] ([1]*Research Utilization Div., JASRI*, [2]*Dept. Biotech. Life Sci., Tokyo Univ. Agricult. Tech.*, [3]*Grad. School Frontier Sci., Univ. Tokyo*)

2P084 **X 線 1 分子計測によるタウタンパク質分子の構造揺らぎ**
Structural Fluctuations of Tau Proteins from X-ray Single　Molecule Observations
Masahiro Shimura[1], Yufuku Matsushita[1], Kouhei Ishiyanagi[1], Tomohiro Miyasaka[3], Hiroshi Sekiguchi[2], Yasuo Ihara[3], Yuji C. Sasaki[1,2] ([1]*Grad. School Frontier Sci., Univ. Tokyo*, [2]*Research & Utilization Div., SPring-8/JASRI*, [3]*Faculty of life & Medical Sci., Doshisha Univ.*)

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

2P085 **Rapid monitoring of affinity maturation process for in vitro selection by fluorescence correlation spectroscopy (FCS)**
Shigefumi Kumachi, Miho Suzuki, Koichi Nishigaki, Naoto Nemoto (*Grad. Sch. Sci. & Eng., Saitama Univ.*)

2P086 **DNA 配列相補性を用いた DNA 修飾アクチン繊維の束化制御**
Control of bundle formation of DNA-conjugated actin filaments using the complementarity of the DNA
Masahito Hayashi, Kingo Takiguchi (*Grad. Sch. Sci., Nagoya Univ.*)

2P087 **Regulation of proteasomal degradation through an unstructured initiation site of a substrate**
Kazunobu Takahashi, Tomonao Inobe (*Front. Res. Core for Life Sci., Univ. Toyama*)

2P088 **cDNA ディスプレイ法を用いた Minimum プロテアーゼの試験管内進化**
***In vitro* selection of Minimum-Protease by cDNA display**
Yuka Mashio[1,3], Shingo Ueno[2,3], Naoto Nemoto[1,3] ([1]*Grad. Sch. Sci. and Eng., Saitama Univ.*, [2]*Grad. Sch. Eng., Univ. Tokyo*, [3]*CREST, JST*)

2P089 **ナノ粒子表層セルラーゼモジュールシャッフリングによる効率的人工セルロームデザイン**
Evolutional cellulosome design from module library
Hikaru Nakazawa, Yuri Ishigaki, Eiko Kobayashi, Do-Myoung Kim, Mitsuo Umetsu (*Grad. Sch. Eng., Tohoku Univ.*)

2P090 **Green Fluorescent Protein からの機能エレメントの抽出**
Extraction of Function Elements from Green Fluorescent Protein
Toshio Morimoto, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

2P091 **人工酵素に移植した機能エレメントの役割**
Roles of functional elements transplanted into the artificial enzyme
Mai Arakawa, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

2P092 **新規ヘム蛋白質フォールドのデノボデザイン**
De novo design of new heme protein folds
Yasuhiro Isogai (*Dept. Biotech., Toyama Pref. Univ.*)

02. ヘム蛋白質 / 02. Heme proteins

2P093 **天然変性タンパクとしての Bach2 ヘム結合領域**
Heme binding region of Bach2 as intrinsically disordered protein
Kazutaka Murayama[1], Miki Matsui[2], Kazuhiko Igarashi[2] ([1]*Grad. Sch. Biomed. Eng., Tohoku Univ.*, [2]*Grad. Sch. Med., Tohoku Univ.*)

2P094 **Oxygen-affinity of hemoglobin is regulated by protein-structural dynamics**
Takashi Yonetani[1], Kenji Kanaori[2] ([1]*Biochem & Biophys., Univ. Pennsylvania*, [2]*Bioengineering, Kyoto Inst. Tech.*)

2P095 **単一結晶形中でのヘモグロビンのアロステリック転移**
Hemoglobin allosteric transition in a single crystal form
Naoya Shibayama (*Div. of Biophysics, Jichi Medical Univ.*)

2P096 **酸化型コバルトミオグロビンへの速度論的配位子結合解析**
Kinetic Analysis of Ligand Binding to Co(III) Myoglobin
Saburo Neya, Masaaki Suzuki, Tyuji Hoshino (*Chiba University, Graduate School of Pharmaceutical Sciences*)

2P097 **異なる生物種によるヘムオキシゲナーゼ反応の微調節戦略：逐次反応過程の個別制御**
Fine-tuning of heme oxygenase successive reactions: Regulation at the peculiar stages in different biological species
Norio Miyake, Atsuko Akiyama, Kouki Kimiya, **Taiko Migita** (*Fac. Agr., Dep. Biol. Chem., Yamaguchi Univ.*)

2P098 **ヘム結晶化を促進するサシガメ由来 α - グルコシダーゼのヘム結合部位の検討**
Heme binding site in *Rhodnius prolixus* α-glucosidase promoting the heme crystallization
Shotaro Kaku, Keisuke Nakatani, Haruto Ishikawa, Yasuhisa Mizutani (*Grad. Sch. Sci., Univ. Osaka*)

2P099 **時間分解共鳴ラマン分光法を用いた CO 解離に伴う CooA のタンパク質ダイナミクスの研究**
Protein dynamics of CooA upon CO dissociation studied by time-resolved resonance Raman spectroscopy
Akihiro Otomo[1], Haruto Ishikawa[1], Misao Mizuno[1], Shigetoshi Aono[2], Yasuhisa Mizutani[1] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*Okazai Inst.*)

03. 膜蛋白質 / 03. Membrane proteins

2P100 **BK チャネルの細胞質側の操作**
Manipulation of the cytoplasmic domain of BK channel
Yoshihiro Satoh, Morten Bertz, Kazuhiko Kinosita (*Waseda University*)

2P101 **アセチルコリン受容体の高速高精度３次元Ｘ線１分子内部運動計測**
3D X-ray Single Molecule Dynamics of nicotinic Acetylcholine Receptor (nAChR) with microsecond and picometre accuracy
Maki Tokue[1], Hiroshi Sekiguchi[2], Kentaro Hoshisashi[1], Kohei Ichiyanagi[1], Yuri Nishino[3], Naoto Yagi[2], Atsuo Miyazawa[3], Tai Kubo[4], Yuji Sasaki[1] ([1]*Grad. Sch. FS., Univ. Tokyo*, [2]*JASRI/SP8*, [3]*Grad. Sch. Sci., Univ. Hyogo*, [4]*ALST*)

2P102 **Computational analysis on the influence of membrane lipid composition on the structural invariance of G-protein coupled receptor**
Md. Iqbal Mahmood[1,2], Xinli Liu[1], Saburo Neya[1], Tyuji Hoshino[1] ([1]*Graduate school of pharmaceutical sciences, Chiba University*, [2]*Laboratory for system biology and medicine, RCAST, The University of Tokyo*)

2P103 **Direct monitoring of membrane protein folding process during in-vitro expression by Surface Enhanced IR spectroscopy**
Kenichi Ataka[1], Joachim Heberle[1], Axel Baumann[2], Silke Kerruth[1], Ramona Schlesinger[3], Joerg Fitter[2], Georg Bueldt[2] ([1]*Freie Universitaet Berlin, Fachbereich Physik, Experimental Molecular Biophysics*, [2]*Forschungszentrum Juelich, ICS-5*, [3]*Freie Universitaet Berlin, Fachbereich Physik, Genetic Biophysics*)

2P104 **等温滴定型熱量計による多剤輸送担体 EmrE の基質結合様式の解析**
Thermodynamics analysis of substrate binding mode of multidrug resistance transporter, EmrE by Isothermal Titration Calorimetry (ITC)
Kazumi Shimono[1,2,3,4], Yoshiro Mori[2], Toshifumi Nara[2], Tomomi Someya[3,4], Mikako Shirouzu[3,4], Shigeyuki Yokoyama[3,5], Seiji Miyauchi[1,2] ([1]*Fac. Pharm. Sci., Toho Univ.*, [2]*Coll. Pharm. Sci., Matsuyama Univ.*, [3]*SSBC, RIKEN*, [4]*CLST, RIKEN*, [5]*Struct. Biol. Lab., RIKEN*)

2P105 **多剤排出トランスポーター AcrB の Motion Tree 法による解析**
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]*Graduate School of Medical Life Science, Yokohama City University*, [2]*Graduate School of Information Science, Nagoya University*, [3]*Graduate School of Bioscience & Biotechnology, Tokyo Institute of Technology*)

2P106 **ABC トランスポーターにおける薬剤結合の影響：分子シミュレーションによる研究**

The effects of substrate binding in ABC transporter: A simulation study

Kouki Yamada[1], Hiroaki Kato[2], Akinori Kidera[1,3] ([1]*Grad. Sch. Nanobio., Yokohama City Univ.*, [2]*Grad. Sch. Pharm Sci., Kyoto Univ.*, [3]*Grad. Sch. Med Life Sci., Yokohama City Univ.*)

2P107 **紫膜表面において観測される隆起構造体の曲率に対する溶媒 pH やイオン強度の影響**

Curvature of Bump Structures on Purple Membrane Depending on pH and Ionic Strength Analyzed by Atomic Force Microscopy

Yasunori Yokoyama[1], Kosuke Yamada[1], Yosuke Higashi[1], Satoshi Ozaki[1], Haorang Wang[1], Naoki Koito[1], Masashi Sonoyama[1,2], Shigeki Mitaku[1,3] ([1]*Department of Applied Physics, Graduate School of Engineering, Nagoya University*, [2]*Division of Molecular Science, Faculty of Science and Technology, Gunma University*, [3]*Toyota Physical and Chemical Research Institute*)

2P108 **ナノディスクを用いたセンサリーロドプシン I**

Photoreaction dynamics of sensory rhodopsin I in nanodiscs

Kenichi Kawamoto[1], Keiichi Inoue[1,2], Jun Sasaki[1], jin Yagasaki[3], Yuki Sudo[3], Michio Homma[3], Hideki Kandori[1] ([1]*Nagoya Inst. Tech,*, [2]*JST PREST*, [3]*Nagoya Univ.*)

2P109 **チャネルロドプシンの活性中心における水素結合ネットワーク**

Hydrogen-bonding network in the active center of a light-gated ion channel, channelrhodopsin

Shota Ito[1], Hideaki Kato[2], Reiya Taniguchi[2], Tatsuya Iwata[1], Osamu Nureki[2], Hideki Kandori[1] ([1]*Nagoya Inst. Tech*, [2]*Grad. Sch. of Sci., Univ. of Tokyo*)

2P110 **光駆動ナトリウムポンプにおける N 末端と C 末端の役割**

Role of N- and C-terminus in a light-driven sodium ion pump

Shinya Sugita[1], Yoshitaka Kato[1], Rei Abe-Yoshizumi[1], Jun Sasaki[1], Keiichi Inoue[1,2], Kwang-Hwan Jung[3], Hideki Kandori[1] ([1]*Nagoya Inst. Tech*, [2]*JST PRESTO*, [3]*Sogang Univ. Korea*)

2P111 **プロテオロドプシンの色を決めるアミノ酸**

A Color Determining Amino Acid of Proteorhodopsin

Yuya Ozaki, Takayoshi Kawashima, Rei Abe-Yoshizumi, Hideki Kandori (*Nagoya Inst. Tech*)

2P112 **G_s および G_q の光制御に向けた新規キメラタンパク質のデザイン**

Designs of new chimeric proteins for optical activation of G_s- and G_q- 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*)

2P113 **全反射赤外分光法を用いたヒト苦味受容体の構造解析**

ATR-FTIR study of human bitter taste receptor

Tomoaki Ohashi[1], Kota Katayama[1], Masaya Iwaki[1], Kei Tsutsui[2], Hiroo Imai[2], Hideki Kandori[1] ([1]*Department of Frontier Materials, Nagoya Institute of Technology*, [2]*Primate Research Institute, Kyoto University*)

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

2P114 **Direct observation of DNA positive supercoiling by reverse gyrase**

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.*)

2P115 **TDP-43 タンパク質における複数の RNA 認識モチーフとその機能的役割**

Distinct roles of individual RNA recognition motifs in an RNA-binding protein, TDP-43

Yo Suzuki[1], Hideaki Shimizu[2], Yutaka Muto[2,3], Shigeyuki Yokoyama[2], Yoshiaki Furukawa[1] ([1]*Dept. of Chem., Keio Univ.*, [2]*RIKEN*, [3]*Dept, of Pharm. Sci., Musashino Univ.*)

2P116 **部位特異的 RNA 切断酵素 Ire1p によって認識される HAC1 mRNA の NMR 解析**

NMR analysis of HAC1 mRNA recognized by the site-specific endonuclease Ire1p

Ikumi Kawahara[1,2], Yuta Ashihara[1], Kaichiro Haruta[1], Yoshiyuki Tanaka[1], Chojiro Kojima[2] ([1]*Grad. Sch. Pharm. Sci., Tohoku Univ.*, [2]*Inst. Prot. Res., Osaka Univ.*)

2P117 **哺乳類ヌクレオチド除去修復タンパク質 XPC の 1 分子イメージング**

Single-molecule direct visualization of the mammalian nucleotide excision repair protein XPC

Hiroaki Yokota[1,2], Daisuke Tone[1], Yong-Woon Han[2], Yoshie Harada[2], Kaoru Sugasawa[1] ([1]*Biosignal Res. Center, Kobe Univ.*, [2]*iCeMS, Kyoto Univ.*)

2P118 **ナノ開口基板を用いたヘミメチル CpG 認識に関与する SRA-DNA 複合体の機能解析**

Characterization of SRA-DNA complex using Zero mode waveguides

Yong-Woon Han[1], Hiroaki Yokota[1], Mariko Ariyoshi[1,2], Yasuo Tsunaka[1,2], Takuma Iwasa[1,3], Ryuji Yokokawa[4], Ryo Hiramatsu[5], Daichi Chiba[5], Teruo Ono[5], Yoshie Harada[1] ([1]*iCeMS, Kyoto University*, [2]*PREST*, [3]*Graduate School of Biostudies, Kyoto University*, [4]*Department of Technology, Kyoto University*, [5]*Institute for Chemical Research, Kyoto University*)

2P119 **ナノ開口を用いた 1 分子イメージングによる RuvB 多量体形成機構の解明**

Single-molecule visualization of RuvB oligomer for characterizing a AAA^+ class hexameric ATPase with zero-mode waveguides

Takuma Iwasa[1], Yong-Woon Han[2], Hiroaki Yokota[2], Ryuji Yokokawa[3], Ryo Hiramatsu[4], Teruo Ono[4], Yoshie Harada[1,2] ([1]*Grad Sch. Biostudies, Kyoto Univ.*, [2]*WPI-iCeMS, Kyoto Univ.*, [3]*Grad Sch. Engineering, Kyoto Univ.*, [4]*Inst. Chem. Research, Kyoto Univ.*)

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

2P120 **Conformational Sampling of Nucleic Acids in Cellular Environments**
Asli Yildirim[1], Brad Varner[1], Monika Sharma[2], Liang Fang[2], Michael Feig[1,2] ([1]*Department of Chemistry, Michigan State University*, [2]*Department of Biochemistry and Molecular Biology, Michigan State University*)

2P121 **Local structural similarity between interphase chromatin and mitotic chromosomes 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.*)

2P122 **レドックス DNA の電子移動反応に及ぼす二本鎖内架橋の影響**
Effect of intrestrand cross-linking of redox-labeled DNA duplex on its electron transfer reaction
Yasuhiro Mie, Keiko Kowata, Yasuo Komatsu (*Bioproduction Res. Inst., AIST*)

2P123 **Bacterial ribosomal RNA as a target for sequence-specific inhibition**
Joanna Trylska[1], Sapna G. Thoduka[1], Zofia Dabrowska[1], Anna Gorska[1], Maciej Jasinski[1,2], Tomasz Witula[1] ([1]*University of Warsaw, Centre of New Technologies*, [2]*University of Warsaw, MISMAP College*)

2P124 **DNA の粗視化モデルによる Ars インスレーターの運動性と機能性の解析**
Analysis of the fluctuation and functionality of Ars-insulator by coarse -grained model of DNA
Keisuke Yamamoto, Sayuri Tatemoto, Naoaki Sakamoto, Akinori Awazu (*Department of Mathematical and Life Sciences,Hiroshima University*)

2P125 **四重鎖形成可能な相補鎖 DNA を導入することによる四重鎖リボザイムのカリウムイオン濃度依存的な活性スイッチングの高効率化**
Enhancement of a Quadruplex-ribozyme activity in response to K^+: a quadruplex-forming complementary DNA enables accurate switching
Yudai Yamaoki[1,2,3], Tsukasa Mashima[1], Takashi Nagata[1,2], Masato Katahira[1,2] ([1]*Institute of Advanced Energy, Kyoto University*, [2]*Graduate School of Energy Science, Kyoto University*, [3]*JSPS Research Fellow*)

2P126 **抗プリオンアプタマーの構造学的基盤とのその活性**
Structural basis of anti-prion aptamer and its activity
Tsukasa Mashima[1], Fumiko Nishikawa[2], Yuji O. Kamatari[3], Masayuki Saimura[1], 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.*)

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

2P127 **非水溶媒中での ATP 加水分解の熱力学的解析**
Thermodynamic analysis of ATP hydrolysis in non aqueous solvent
Hideyuki Komatsu (*Dept. Bioscience & Bioinformatics, Kyushu Inst. Tech.*)

2P128 **シミュレーション・データマイニングアプローチによる蛋白質ドッキング過程における水和水ダイナミクス**
Hydration water behavior in the protein docking process by simulating data mining approach
Taku Mizukami[1], Ayumu Sugiyama[2], Dam Hieu Chi[2], Ho Tu Bao[2] ([1]*Sch. Materials Sci., JAIST*, [2]*Sch. Knowledge Sci., JAIST*)

2P129 **蛋白質水和水の並進拡散運動と蛋白質ダイナミクスとの動的カップリング**
Translation diffusion dynamics of protein hydration water and its dynamical coupling with protein dynamics
Hiroshi Nakagawa[1], Mikio Kataoka[1,2] ([1]*Japan Atomic Energy Agency, Quantum Beam Science Directorate*, [2]*Nara Institute of Science and Technology, Graduate School of Materials Science*)

2P130 **Water behavior in buried hydration sites of human cellular prion protein and pathogenic mutation T188R**
Katsufumi Tomobe[1], Eiji Yamamoto[1], Takuma Akimoto[1], Masato Yasui[2], Kenji Yasuoka[3] ([1]*Graduate school of science and technology, Keio university*, [2]*Department of Pharmacology, School of Medicine, Keio University*, [3]*Department of mechanical engineering, Keio University*)

2P131 **Aging of water molecules on cell membrane surfaces**
Eiji Yamamoto[1], Takuma Akimoto[1], Masato Yasui[2], Kenji Yasuoka[3] ([1]*Graduate School of Science and Technology, Keio University*, [2]*Department of Pharmacology, School of Medicine, Keio University*, [3]*Department of Mechanical Engineering, Keio University*)

08. 分子遺伝・遺伝情報制御 / 08. Molecular genetics & Gene expression

2P132 **Dynamics of transcriptional apparatus in eukaryotic gene expression**
Ashwin S. S, Masaki Sasai (*Department of Computational Science and Engineering, Nagoya University*)

09. 発生・分化 / 09. Development & Differentation

2P133 **細胞性粘菌突然変異株にみられるソリトン様細胞運動**
Biological Soliton in eukaryotic multicellular movement
Hidekazu Kuwayama (*Faculty of Life and Environmental Sciences, University of Tsukuba*)

2P134 **ラミニン固定化弾性率可変ゼラチンゲルを用いた iPS 細胞のフィーダーフリー分散培養**
Feeder-free dissociated culture of iPS cells on the laminin-fixed elasticity-tunable gelatinous gels
Ayaka Utsumi[1], Tatsuya Okuda[2], Hiroshi Endo[3], Tomo Koike[3], Koji Eto[3], Satoru Kidoaki[2] ([1]*Grad. Sch. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*, [3]*CiRA, Univ. Kyoto*)

2P135 **マウス胚盤胞と桑実胚間での異なるメカニカルストレス応答**

Different responses to mechanical stimuli between mouse blastocyst and morula

Yuka Asano, Koji Matsuura, Keiji Naruse (*Grad. Sch. Med. Dent. Pharm. Sci., Okayama Univ.*)

10. 筋肉 / 10. Muscle

2P136 **SESTD1 に結合する横紋筋タンパク質の探索**

Screening of SESTD1-binding proteins in striated muscle

Akira Hanashima[1], Sumiko Kimura[2], Takashi Murayama[1] ([1]*Dept. Pharmacol., Sch. Med., Juntendo Univ.*, [2]*Dept. Biol., Grad. Sci, Chiba Univ.*)

2P137 **ギボシムシのコネクチン様タンパク質の探索**

Searching for connectin-like protein in acorn worm

Satoshi Nakayama[1], Akira Hanashima[1], Kunihumi Tagawa[2], Sumiko Kimura[1] ([1]*Department of Biology, Graduate School of Science, Chiba University*, [2]*Marine Biological Laboratory, Graduate School of Science, Hiroshima University*)

2P138 **ヤツメウナギのコネクチン様タンパク質**

Connectin-like protein of Lamprey

Mai Kanno, Yoshiharu Itoh, Akira Nishikawa, Akira Hanashima, Sumiko Kimura (*Department of Biology, Guraduate School of Science, Chiba University*)

2P139 **ウニのコネクチン様タンパク質**

Connectin-like protein of sea urchins

Tomoko Sasaki, Tetsu Matsuura, Akira Hanashima, Sumiko Kimura (*Grad. Sch. Sci., Chiba Univ.*)

2P140 **分子動力学シミュレーションを用いたトロポミオシンの柔軟性および屈曲性の解析**

Analysis of flexibility and curvature of tropomyosin by molecular dynamics simulation

Hideo Ozawa[1], Yoshihiro Ochiai[2], Koji Umezawa[1], Shin'ichi Ishiwata[1], Mitsunori Takano[1] ([1]*Dep. of Phys., Waseda Univ.*, [2]*Sch. Mar. Sci. Tec., Tokai Univ.*)

2P141 **横紋筋筋原線維 SPOC の動的特性に関するモデルシミュレーション**

Model simulation on the dynamic properties of SPOC in a striated myofibril

Koutaro Nakagome[1], Katsuhiko Sato[2], Shin'ichi Ishiwata[1,3] ([1]*Department of Physics, Faculty of Science and Engineering, Waseda University*, [2]*RIKEN Center for Developmental Biology*, [3]*Waseda Bioscience Research Institute in Singapore (WABIOS)*)

2P142 **高精度計測によるラット幼若心筋細胞内サルコメア自励振動特性の解明**

High-resolution analysis of sarcomeric auto-oscillations in rat neonatal cardiomyocytes

Seine A. Shintani[1], Kotaro Oyama[1], Shin'ichi Ishiwata[1,2], Norio Fukuda[3] ([1]*Waseda Univ., Physics, Ishiwata Lab.*, [2]*WABIOS*, [3]*Jikei Univ., Cell Phys.*)

11. 分子モーター / 11. Molecular motor

2P143 **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**

Giant Acceleration of diffusion in F_1-ATPase

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*)

2P144 **高粘性中でのキネシンによるビーズ輸送**

Transport of beads by kinesin in highly viscous environment

Naoto Sawairi[1], Takayuki Ariga[2], Michio Tomishige[2], Kumiko Hayashi[1] ([1]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*, [2]*Dept. Appl. Phys., Sch. Eng., Univ. Tokyo*)

2P145 **神経細胞の軸索輸送におけるキネシンとダイニンの数の測定：揺らぎの定理の応用**

Measuring the numbers of kinesin and dynein on neuronal cargo transport by using the fluctuation theorem

Kumiko Hayashi[1], Yasushi Okada[2] ([1]*Sch. Eng., Tohoku Univ.*, [2]*QBiC, RIKEN*)

2P146 **Observing RecBCD Translocation along Individual Chi-Containing Gapped DNA**

Cinya Chung, Hung-Wen Li (*Department of Chemistry, National Taiwan University*)

2P147 **Dynamical energy landscape theory for the force-generation process in actomyosin motor**

Qing Miao Nie[1,2,3], Masaki Sasai[1], Tomoki P. Terada[1] ([1]*Dept. of Comp. Sci. Eng., Nagoya Univ.*, [2]*Institute for Molecular Science*, [3]*Dept.of Applied Physics, Zhejiang Univ. of Tech.*)

2P148 **マイコプラズマ Gli349 タンパク質の構造ダイナミクス解析**

Structure and dynamics of the gliding protein Gli349 from *Mycoplasma mobile*

Junichi Inatomi[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

2P149 **Gene manipulation of gliding bacterium, *Mycoplasma mobile***

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

2P150 **単一糖鎖上でのマイコプラズマの滑走と結合**

Gliding and binding of mycoplasma on uniform oligosaccharide

Taioshi Kasai, Tasuku Hamaguchi, Makoto Miyata (*Grad. Sch. Sci., Univ. Osaka City*)

2P151 マイコプラズマ滑走タンパク質分子の可視化による構造解析

Structure of Proteins Involved in *Mycoplasma mobile* Gliding Revealed by Visualization

Yuhei Tahara[1], Noriyuki Kodera[2], Toshio Ando[2], Makoto Miyata[1] ([1]*Grad. Sch. Sci., Univ. Osaka City.*, [2]*Bio-AFM Frontier Research Center, Univ. Kanazawa.*)

2P152 ヒト肺炎 *Mycoplasma pneumonia* の滑走運動装置と構成タンパク質の結晶化

Crystallization of gliding machinery and component proteins of *Mycoplasma pneumoniae*

Yoshito Kawakita[1], Lisa Matsuo[1], Tsuyoshi Kenri[3], Miki Kinoshita[1], Katsumi Imada[2], Makoto Miyata[1] ([1]*Grad. Sch. Sci., Univ. Osaka City*, [2]*Grad. Sch. Sci., Univ. Osaka*, [3]*National Institute of Infectious Diseases*)

2P153 マイコプラズマ・モービレの滑走にかかわるチューブリンホモログの構造解析

Structural analysis of tubulin homolog involved in *Mycoplasma mobile* gliding

Masaru Yabe, Miki Kinoshita, Makoto Miyata (*Graduate school of science, Osaka city university*)

2P154 **Investigating stators assembly of flagellar motors in Escherichia coli**

Lin Tsai-Shun, Lo Chien-Jung (*National Central University Taiwan*)

2P155 **Tracking of bacterial flagellar motor rotation by fluorescent microscopy**

Yoshiyuki Sowa[1,2], Yong-Suk Che[1] ([1]*Dept. Frontier Bioscience, Hosei Univ.*, [2]*Reserch center for Micro-Nano Tech., Hosei Univ.*)

2P156 タンデム PomA 変異体を固定子とする Na^+駆動型キメラべん毛モーターの回転計測

Rotation Measurement of Na^+-driven Chimeric Flagellar Motor with Tandem PomA Mutants

Yong-Suk Che[1], Yoshiyuki Sowa[1,2] ([1]*Dept. Frontier Bioscience, Hosei Univ.*, [2]*Reserch center for Micro-Nano Tech., Hosei Univ.*)

2P157 **Structural study of the sheath in the magnetotactic bacterium MO-1 by electron cryomicroscopy**

Juanfang Ruan[1], Takayuki Kato[1], Claire-Lise Santini[2], Long-Fei Wu[2], Keiichi Namba[1,3] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*Lab. Chimie Bacterienne, Universite de la Mediterranee Aix-Marseille II, CNRS, France*, [3]*QBiC, RIKEN*)

2P158 細菌べん毛基部体中のスイッチ蛋白質 FliG の位置ならびに配向の同定

Identification of the location of the switch protein FliG in the flagella basal body

Tomoko Miyata[1], Takayuki Kato[1], Yusuke V Morimoto[1,2], Akihiro Kawamoto[1], Hideyuki Matsunami[3], Keiichi Namba[1,2] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*QBiC, RIKEN*, [3]*Trans-Membrane Trafficking Unit, OIST*)

2P159 **Torque-speed relationship of the flagellar motor consisting of two distinct stators**

Naoya Terahara[1], Yukina Noguchi[2], Shuichi Nakamura[1], Nobunori Kami-ike[1], Tohru Minamino[1], Masahiro Ito[2], Keiichi Namba[1] ([1]*Graduate School of Frontier Biosciences, Osaka University*, [2]*Graduate School of Lifescience, Toyo University*)

2P160 人工分子ベアリングの分子内回転の 1 分子計測

Single-Molecular Measurement of a Synthetic Molecular Bearing

Tomohio Ikeda[1], Takahiro Tsukahara[1], Masayuki Takeuchi[2], Ryota Iino[1,3], Hiroyuki Noji[1,3] ([1]*Department of Applied Chemistry, the University of Tokyo*, [2]*National Institute for Materials Science*, [3]*JST-CREST*)

2P161 F_1-ATPase の触媒活性機構の理論的解析及び新規触媒活性変異体の設計

Theoretical studies on ATP hydrolysis in F_1-ATPase and a rationally designed enzymatic reaction in its variants

Shiho Noguchi, Shigehiko Hayashi (*Grad. Sch. Sci., Univ. Kyoto*)

2P162 ATP 合成酵素の結晶化

Crystallization of ATPsynthase

Yasuo Shirakihara[1], Hiromi Tanikawa[1], Satoshi Murakami[2] ([1]*National Institute of Genetics*, [2]*Tokyo Institute of Technology*)

2P163 人工基質 RTP を用いた F_1-ATPase の回転触媒機構の解明

Base moiety of ATP is dispensable for driving the rotation of F_1-ATPase

Ayako Yukawa[1], Ryu Iwatate[2], Rikiya Watanabe[1], Mako Kamiya[2], Yasuteru Urano[2], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo.*, [2]*Grad. Sch. Med., Univ. Tokyo.*)

2P164 DNA を回転子に持つ新規回転分子モーターの創製

Creation of a hybrid F_1 motor with DNA as the rotor

Kosuke Iwamoto[1], Ryota Iino[1], Risa Yamauchi[1], Takayuki Uchihashi[2], Hiroyuki Noji[1] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Col. Sci. and Eng., Univ. Kanazawa*)

2P165 F_1-ATPase 内の DELSEED-loop のトルク伝達機構の解明

Elucidation of torque-transmission mechanism of DELSEED-loop in F_1-ATPase

Kazuma Koyasu[1], Mizue Tanigawara[2], Rikiya Watanabe[1], Hiroyuki Noji[1] ([1]*Dept. Applied Chem., Grad. Sch. Eng., Univ. Tokyo*, [2]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

2P166 高速配向イメージングによる F1-ATPase の触媒サブユニットの構造変化計測

Direct observation of domain motion of the catalytic β subunit of F1-ATPase using high-speed orientational imaging

Sawako Enoki, Ryota Iino, Hiroyuki Noji (*Grad. Eng., Univ. Tokyo*)

2P167 サポーテッド膜を用いた F_oF_1 の一分子回転計測

Single molecule observation of F_oF_1-ATP synthase in the supported lipid membrane

Yoshiki Moriizumi, Rikiya Watanabe, Kazuhito V. Tabata, Hiroyuki Noji (*Dep. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo.*)

2P168 **Basic properties of rotary dynamics of Enterococcus hirae V1-ATPAse motor protein**

Yoshihiro Minagawa[1], Hiroshi Ueno[2], Yoshiko Ishizuka-Katsura[4], Noboru Ohsawa[4], Takaho Terada[4], Mikako Shirouzu[4], Shigeyuki Yokoyama[4], Hiroyuki Noji[1], Takeshi Murata[3], Ryota Iino[1] ([1]*Dept. of App. Chem., Grad. Sch. of Eng., Univ. Tokyo*, [2]*Dept. of Phys., Fac. of Sci. and Eng., Univ. Cyuo*, [3]*Dept. of Chem., Grad. Sch. of Sci., Univ. Chiba*, [4]*RIKEN, SSBC*)

2P169 **１分子蛍光観察によるセロビオヒドロラーゼの結晶性セルロース加水分解反応素過程の解明**
Single-molecule imaging analysis of cellobiohydrolase hydrolyzing crystalline cellulose
Yusuke Shibafuji[1], Akihiko Nakamura[2], Naohisa Sugimoto[2], Kiyohiko Igarashi[2], Shingo Fukuda[3], Hiroki Watanabe[3], Takayuki Uchihashi[3], Hiroyuki Noji[1], Ryota Iino[1] ([1]*Dept. Appl. Chem. Univ. Tokyo*, [2]*Dept. Bio. Sci. Univ. Tokyo*, [3]*Dept. Phys. Kanazawa Univ*)

2P170 **負荷存在下でのキネシン頭部の運動の高時間分解能観察**
High temporal resolution observation of the stepping motion of kinesin-1 under load
Issui Akishika[1], Ryota Iino[2], Hiroyuki Noji[2], Michio Tomishige[1] ([1]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*, [2]*Dept. Appl. Chem., Grad. Sch. Eng., Univ. Tokyo*)

2P171 **ジスルフィドクロスリンクを用いたキネシン 1 の二足歩行制御機構の研究**
Strain-dependent regulation of the kinesin-1's catalytic activity as studied by disulfide-crosslinking of the neck linker
Yamato Niitani[1], Erik Jonsson[2], Ronald D.Vale[2], Michio Tomishige[1] ([1]*Dept. Appl. Phys., Grad. Sch. Eng., Univ. Tokyo*, [2]*Dept. CMP, Univ. California*)

2P172 **SDSL-ESR により検出したキネシン α-1 ヘリックスのヌクレオチド依存的な動的構造とその変位**
Nucleotide-dependent Displacement and Dynamics of α-1 Helix in Motor Protein Kinesin As Revealed by Site Directed Spin Labeling ESR
Satoshi Yasuda[1], Takanori Yanagi[1], Masafumi Yamada[2], Shinsaku Maruta[2], **Toshiaki Arata**[1] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Soka Univ.*)

2P173 **微小管上での KIF1A の選択的結合における負に荷電した C 末の役割**
The role of negatively-charged C-terminus of tubulin in selective binding of KIF1A on microtubule
Yukinobu Mizuhara, Jun Ohnuki, Koji Umezawa, Mitsunori Takano (*Dept. of Phys. & Appl. Phys, Grad. Sch. of Adv. Sci. & Eng. Waseda Univ.*)

2P174 **フォトクロミック分子を用いた有糸分裂キネシン Eg5 の光制御型阻害剤**
Photo regulted inhibitor composed of photochromic molecules for mitotic kinesin Eg5
Kanako Tohyama[1], Kumiko Ishikawa[2], Shinsaku Maruta[1,2] ([1]*Div. Bioinfo., Grad. sch. Eng., Univ. Soka*, [2]*Dep. Bioinfo., Fac. Eng., Univ. Soka*)

2P175 **有糸分裂キネシン Eg5 の機能性ループ L５へのフォトクロミック分子導入と光制御**
Incorporation of photochromic molecule into the functional loop L5 of mitotic kinesin Eg5 and its photo regulation
Kumiko Ishikawa[1], Yuki Tamura[2], Shinsaku Maruta[1] ([1]*Div. of Bioinfo., Grad. Sch. of Eng., Soka Univ.*, [2]*Dep. of Bioinfo., Fac. of Eng., Soka Univ.*)

12. 細胞生物的課題 / 12. Cell biology

2P176 **原子間力顕微鏡によるコンフルエント細胞の力学測定**
Mechanical measurements of confluent cells with an atomic force microscope
Yuki Ochi, Masahiro Tsuchiya, Yuki Saito, Takaharu Okajima (*Grad. Sch. Info. Sci. & Tech., Hokkaido Univ.*)

2P177 **ゾウリムシのメタクロナールウェーブは外液の粘性だけでなく細胞表層の弾性も使って伝播できる**
Metachronal wave travels not only in outer viscous fluid but also on elastic cell surface of *Paramecium* cells
Naoki Narematsu, **Yoshiaki Iwadate** (*Fac. Sci., Yamaguchi Univ.*)

2P178 **細胞間力学変化量の空間不均一性：原子間力顕微鏡測定**
Spatial heterogeneity of cell-to-cell mechanical variability measured by atomic force microscopy
Ryosuke Takahashi, Kaori Kuribayashi-Shigetomi, Takaharu Okajima (*Grad. Sch. Info. Sci. & Tech., Hokkaido. Univ.*)

2P179 **AFM を用いた強制剥離による細胞接着力の評価**
Evaluation of cell adhesion force by mechanical detachment using AFM
Mari Mishima[1], Ryuzo Kawamura[2], Tomoko Okada[2], Chikashi Nakamura[1,2] ([1]*Dept. Biotechnol. Life Sci., TUAT*, [2]*Biomedical Research Institute, AIST*)

2P180 **細胞内の力学環境に対する分子混み合い効果**
Molecular crowding effects on intracellular mechanical environments
Kenji Nishizawa[1], Kei Fujiwara[2], Miho Yanagisawa[1], Daisuke Mizuno[1] ([1]*Department of physics, Kyushu University*, [2]*Department of Bioengineering and Robotics, Tohoku University*)

2P181 **Molecular configurations of purified radial spoke of Chlamydomonas flagella**
Hitoshi Sakakibara, Yosuke Shimizu, Hiroaki Kojima (*Bio ICT, KARC, Nat. Inst. Info. Commn. Tech*)

2P182 **波型弾性パターニングゲル上での流れ誘導メカノタクシスに見られるがん細胞の接着スイッチング挙動**
Adhesion switching of tumor cells in shear-flow-induced mechanotaxis on wave-like elastically-patterned gels
Yuki Kubota[1], Tatsuya Okuda[2], Satoru Kidoaki[2] ([1]*Grad. Sch. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*)

2P183 **粘性流体中におけるバクテリアの Twitching 運動のシミュレーション**
Simulation study of the twitching motility of bacterium in viscous fluid
Ryota Morikawa, Masatada Tamakoshi, Takeshi Miyakawa, Masako Takasu (*School of Life Sciences, Tokyo University of Pharmacy and Life Sciences*)

2P184 **大面積弾性マイクロパターニングゲルを用いた間葉系幹細胞の分化フラストレーションの誘導と評価**
Characterization of frustrated differentiation of mesenchymal stem cells cultured on large-scale microelastically-patterned gels
Kosuke Hamano[1], Tatsuya Okuda[2], Satoru Kidoaki[2] ([1]*Grad. Sci. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*)

2P185　１分子追跡法により明らかにされた伸展中の細胞における Dystroglycan の形成中の接着斑へのリクルート

Dystroglycan recruitment to forming focal adhesions during cell spreading as observed by single-molecule tracking

Akihiro Shibata[1], Masahiro Makuta[2], Limin Chen[2], Yuuri Nemoto[2], Yuki Shirai[2], Hisae Tsuboi[2], Nao Hiramoto[2], Takaaki Tsunoyama[2], Takahiro Fujiwara[2], Akihiro Kusumi[1,2] ([1]*Institute for Frontier Medical Sciences, Kyoto University*, [2]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*)

2P186　付着型珪藻の二次元運動への培地温度の影響

Effects of variation in medium temperature on two-dimensional motion of attached diatom

Yu Imamura[1], Shunichiro Hori[1], Tomohiro Ohno[1], Shigeki Mayama[2], Kazuo Umemura[1] ([1]*Dept.Sci.univ of science.Tokyo*, [2]*Faculty of Education,Tokyo Gakugei University*)

2P187　シクロフィリン D がミトコンドリアに及ぼす影響

Effects of cyclophilin D on mitochondria

Daisuke Shinohe, Asuka Kobayashi, Hitomi Nakazato, Akiko Nagai, Yoshihiro Ohta (*Tokyo Univ. of Agric. and Tech*)

2P188　細胞分裂時におけるエネルギー状態のモニタリング

Monitoring of energy state of cells during cell division

Kotoe Hirusaki, Yoshihiro Ohta (*Tokyo University of Agriculture and Technology*)

2P189　細胞性粘菌（*Dictyostelium discoideum* Ax-2）の増殖におけるエネルギーをめぐる細胞内葛藤

Intracellular conflict on energy in the growing cellular slime mold, *Dictyostelium discoideum* Ax-2

Yatsuhisa Nagano (*Res. Ctr. Structural Thernodyn., Grad. Sch. Sci., Osaka Univ.*)

2P190　ES 細胞の分化初期段階における状態遷移

Transitions among cell states in the early stage of differentiation from embryonic stem cells

Koh Makishi, Tomoki P. Terada, Masaki Sasai (*Dept. of Comp. Sci. Eng., Univ. of Nagoya*)

2P191　巨大化大腸菌の細胞形態変化、細胞分裂の観察

Morphological change and cell division of Giant E.coli

Takao Sogo[1], Kazuhito Tabata[1,2], Hiroyuki Noji[1] ([1]*Dept. Applied Chem., Sch. Eng., Univ. Tokyo*, [2]*PREST, JST*)

2P192　細菌べん毛タンパク質輸送装置の in vitro 再構成系の構築

Construction of an in vitro assay system for the bacterial flagellar protein export

Hiroyuki Terashima[1], Tohru Minamino[2], Katsumi Imada[1] ([1]*Dep. Macromol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Grad. Sch. Fron. Biosci., Osaka Univ.*)

2P193　ケージドセリン光分解とべん毛モーター回転計測を用いた大腸菌走化性応答の高時間分解能計測

Response of flagellar motor rotation to photoreleased serine from caged-compound in an *E. coli* cell

Takashi Sagawa[1], Hajime Fukuoka[1,2], Yuichi Inoue[1,2], Hiroto Takahashi[2], Akihiko Ishijima[1,2] ([1]*Grad. Sch. Life Sci., Tohoku Univ.*, [2]*IMRAM, Tohoku Univ.*)

2P194　MS リングに変異の入ったサルモネラ菌べん毛モーターとそのシュードリバータントの構造安定性と回転特性

Structural stability and rotational characteristics of the flagellar motor of Salmonella MS-ring mutant and its psuedo-revertants

Shun Taga[1], Akira Asaumi[1], Shuichi Nakamura[1], Fumio Hayashi[2], Kenji Oosawa[2], Seishi Kudo[1] ([1]*Grad. Sch. Eng., Univ. Tohoku*, [2]*Grad. Sch. Eng, Univ. Gunma*)

2P195　電子顕微鏡によるヒト毛乳頭細胞の不動毛の構造解析

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.*)

2P196　簡単で低コストなコレラティブ顕微鏡法

Simple and cost-effective method for correlative microscopy

Teruyo Minamiyashiki, Miharu Nagaishi, **Hiryouki Nakagawa** (*Department of Earth System Science, Faculty of Science, Fukuoka University*)

2P197　顕微鏡ステージ上での微量エレクトロポレーション法

Electroporation of adherent cells with low sample volumes on a microscope stage

Harunobu Tsugiyama[1], Chika Okimura[1], Takafumi Mizuno[2], Yoshiaki Iwadate[1] ([1]*Department of Functional Molecular Biology, Graduate School of Medicine, Yamaguchi University*, [2]*Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)*)

2P198　神経ー膵島 α 細胞相互作用におけるサブスタンスＰの寄与

A neuropeptide substance P is involved in nerve-pancreatic islet α cell interaction

Tadahide Furuno, Mami Nakamura, Yoshikazu Inoh, Mamoru Nakanishi (*Sch. Pharm., Aichi Gakuin Univ.*)

2P199　RhoGAP proteins RGA-3/4 mediate spatial negative feedback of the actomyosin in C. elegans embryos

Masashi Fujita, Shuichi Onami (*RIKEN Quantitative Biology Center*)

2P200　フェムト秒レーザー誘起衝撃力による分裂酵母細胞のカルシウムイオン応答の個別解析

Individual Analysis of Changes in Calcium Concentration Induced by Femtosecond Laser Impulse in Single Fission Yeast Cells

Akinori Shigemasa[1], Yoshitaka Nakayama[2], Takanori Iino[1], Hidetoshi Iida[2], Yoichiroh Hosokawa[1] ([1]*Nara Institute of Science and Technology., Materials Science*, [2]*Univ. Tokyo gakugei., Natural Science*)

2P201　悪性高熱症関連変異をもたらしている 1 型リアノジン受容体の機能解析

Functional analysis of type 1 ryanodine receptor carrying malignant hyperthermia associated mutations

Toshiko Yamazawa[1], Takashi Murayama[2], Hedeto Oyamada[3], Junji Suzuki[4], Kazunori Kanemaru[4], Nagomi Kurebayashi[2], Masamitsu Iino[4], Shigeru Takemori[1] ([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*)

2P202 **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**
In vivo spatio-temporal distribution analysis of dimeric glucocorticoid receptor using Number and Brightness
Hideto Ishikawa[1], Johtaro Yamamoto[2], Masataka Kinjo[2] ([1]*Grad. Life Sci., Hokkaido Univ.*, [2]*Fuc. Adv. Life Sci., Hokkaido Univ.*)

2P203 **GPI アンカー型タンパク質は神経細胞膜の拡散障壁内でも高速でホップ拡散する：超高速１蛍光分子追跡による検出**
GPI-anchored proteins undergo rapid hop diffusion within the diffusion barrier in the neuronal plasma membrane
Manami Miyahara[1], Chieko Nakada[3], Takahiro Fujiwara[1], Toshiki Matsui[2], Hiroko Hijikata[1], Hiroo Iwata[2], Ziya Kalay[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*)

2P204 **免疫細胞のシグナルアダプター分子 LAT の時空間制御機構：1 分子追跡による解明**
Adaptor transmembrane protein LAT in immune signaling works in vesicles recruited to the plasma membrane: a single-molecule tracking study
Koichiro M. Hirosawa[1], Kenta J. Yoshida[1], Ankita Chadda[1], Kenichi G. N. Suzuki[1,3], Akihiro Kusumi[1,2] ([1]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS)*, [2]*Inst. Frontier Medical Sciences, Kyoto Univ.*, [3]*National Centre for Biological Science (NCBS)/Institute for Stem Cell Biology and Regenerative Medicine (inStem)*)

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

2P205 **Main phase transition of asymmetric lipid bilayers**
Antti Lamberg, Takashi Taniguchi (*Department of Chemical Engineering, Kyoto University*)

2P206 **リン脂質/コレステロール系における Lo 相形成の炭化水素鎖長依存性**
Effect of the phospholipid chain length on the cholesterol-induced liquid ordered phase formation
Tsubasa Miyoshi, Hiroshi Kitajima, Daichi Yokoi, Satoru Kato (*Grad. Sch. Sci & Tech.,Univ. Kwansei Gakuin*)

2P208 **マイクロパターン化モデル生体膜における脂質ドメインの空間的制御**
Geometrical separation of lipid domains in a micro-patterned model membrane
Fumiko Okada[1], Kenichi Morigaki[1,2] ([1]*Grad. Sch. Agri., Univ. Kobe*, [2]*Res. Cen. Env Gen., Kobe*)

2P209 **中性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の NMR と QCM による解析**
Structure and affinity analysis of bovine lactoferrampin bound to a neutral model membrane as studied by solid state NMR and QCM
Masayoshi Imachi[1], Javkhlantugs Namsrai[1], Atsushi Kira[2], Atsushi Tutsumi[1], Izuru Kawamura[1], Akira Naito[1] ([1]*Graduate School of Engineering, Yokohama National University*, [2]*Reasearch and Development Division, ULVAC Inc*)

2P210 **高圧蛍光法により明らかにされるサブゲル相中のホスファチジルコリン分子のスタッガード構造**
Staggered structure of phosphatidylcholine molecules in subgel phase revealed by high-pressure fluorometry
Masaki Goto, Nobutake Tamai, Hitoshi Matsuki (*Tokushima Univ.*)

2P211 **ジパルミトイルホスファチジルコリン二分子膜の熱的相挙動に及ぼすステロール効果**
Effects of sterols on thermotropic phase behavior of dipalmitoylphosphatidylcholine bilayer
Nobutake Tamai, Sanae Inazawa, Daiki Fujiwara, Masaki Goto, Hitoshi Matsuki (*Department of Life System, Institute of Technology and Science, The University of Tokushima*)

2P212 **グループ３ LEA タンパク質のモデルペプチドによるリポソームの乾燥誘導凝集抑制**
Anti-aggregation Effects on Liposomes during Desiccation by Model Peptides of Group-3 LEA Proteins
Takao Furuki, Takahiro Watanabe, Minoru Sakurai (*Center for biological resources and informatics, Tokyo Institute of Technology*)

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

2P213 **単一細胞膜揺らぎ計測のためのイオンコンダクタンス顕微鏡技術の開発**
Scanning ion conductance microscopy for measuring single cell membrane fluctuations
Zen Ishikura[1], Yusuke Mizutani[2], Kaori Kuribayashi-Shigetomi[1], Yuuki Fujii[1], Choi Myung-Hoon[2], Cho Sang-Joon[3], Takaharu Okajima[1] ([1]*Graduate School of Information Science and Technology, Hokkaido University*, [2]*Park Systems Inc.*, [3]*Seoul National University*)

2P214 **界面活性物質を用いた巨大細胞膜ベシクル作製方法の開発**
Development of a new method for preparation of giant plasma membrane vesicles using surfactants
Hiroaki Inuma[1], Yuta Minami[1], Toshihiko Sakurai[2], Takashi Okuno[3] ([1]*Graduate School of Science and Engineering, Yamagata University*, [2]*Depertment of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University*, [3]*Depertment of Science, Yamagata University*)

2P215 **肺サーファクタントタンパク質 SP-B によるリン脂質膜の構造変化**
Morphology Changes in Phospholipid Monolayers Induced by Lung Surfactant Protein SP-B
Masahiro Hibino[1], Hayato Suzuki[2], Takahiro Suzuki[2] ([1]*Div. Appl. Sci., Muroran Inst. Tech.*, [2]*Dept. Appl. Sci., Muroran Inst. Tech.*)

2P216 **希薄状及び飽和状なリソチーム及びナノシリカ/ダイヤが pH=7-13 のもとで起こす吸着反応の動力学**
Loose and Saturated Adsorption Reaction Dynamics of Lysozyme and Nanosilica/-diamond at pH=7-13
Victor Wei-Keh Chao[1,2] ([1]*Department of Chemical and Materials Engineering, National Kaohsiung University of Applied Sciences*, [2]*Victor Basic Research Laboratory e. V.*)

2P217 **人工物の細胞内導入：生細胞と GUV の電気融合法**
How to send artifacts into the living cell inside? -Investigating GUV-Cell electro fusion method
Akira C. Saito[1], Toshihiko Ogura[2], Shinichiro M. Nomura[1] ([1]*Department of Bioengi. and Robo. Tohoku Univ.*, [2]*Depart. of Develo. of Neurobiolo. (IDAC). Tohoku. Univ.*)

2P218 **生細胞に極限まで近い内包物を持つ人工細胞の構築と解析**

Generation of artificial cells that mimic living cells

Kei Fujiwara[1], Kenji Nishizawa[2], Miho Yanagisawa[2], Shin-ichiro M. Nomura[1], Daisuke Mizuno[2] ([1]*Tohoku university, Department of Bioengineering and Robotics*, [2]*Kyushu university, Department of Physics*)

13C. 生体膜・人工膜：興奮・チャンネル / 13C. Biological & Artifical membrane: Excitation & Channels

2P219 **不飽和脂肪酸による電位依存性プロトンチャネルへの活性増強効果**

Effects of the unsaturated fatty acids on the voltage-gated proton channel

Akira Kawanabe, Yasushi Okamura (*Grad. Sch. Med., Osaka Univ.*)

2P220 **Conformational Transitions in Voltage Sensor Domains**

Morten Bertz, Kazuhiko Kinosita (*Waseda University, Dpt. of Science & Engineering*)

2P221 **負に帯電した膜内葉表面でのアミノ末端両親媒性ヘリックスの回転が KcsA カリウムチャネルの開状態を安定化する**

Rolling of N-terminal amphipathic helix on the anionic inner membrane leaflet stabilizes the open state of the KcsA potassium channel

Masayuki Iwamoto, Shigetoshi Oiki (*Dept. Mol. Physiol. Biophys., Univ. Fukui Fac. Med. Sci.*)

2P222 **K^+チャネルの中心空洞内の水の配向は静電的相互作用を増強する**

The oriented water in the central cavity of the K^+ channel enhances the electrostatic attraction

Takashi Sumikama[1], Shinji Saito[2], Shigetoshi Oiki[1] ([1]*University of Fukui*, [2]*Institute for Molecular Science*)

13E. 生体膜・人工膜：情報伝達 / 13E. Biological & Artifical membrane: Signal transduction

2P223 **Development of a reconstituted system for localized phosphatidylinositols signaling on lipid membrane**

Kei Takahashi[1], Nao Shimada[1], Akihiko Nakajima[1], Satoshi Sawai[1,2,3], Taro Toyota[1,2] ([1]*Grad. Sch. Arts Sci.,Univ. Tokyo*, [2]*Res. Center as Complex Sys. Bio., Univ. Tokyo*, [3]*PRESTO, Japan Science and Technology Agency*)

2P224 **A Multiscale Kinetic Scheme Extracted from EGFR-Grb2 Single Molecule Reaction**

Tahmina Sultana[1], Hiroaki Takagi[2], Miki Morimatsu[3], Hiroshi Teramoto[1], Chun-Biu Li[1], Yasushi Sako[4], Tamiki Komatsuzaki[1] ([1]*Molecule and Life Nonlinear Sciences Laboratory, Research Institute for Electronic Science, Hokkaido University*, [2]*Department of Physics, Nara Medical University*, [3]*WPI-IFReC, Osaka University*, [4]*Cellular Informatics Laboratory, RIKEN, Wako*)

14. 化学受容 / 14. Chemoreception

2P225 **新世界ザルの苦味受容体 TAS2R1 および TAS2R4 の機能的多様性**

Functional diversity 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], Filippo Aureli[3], Colleen M. FedSchaffner[4], Linda M. Fedigan[5], Shoji Kawamura[2], Hiroo Imai[1] ([1]*Pri. Res. Inst., Kyoto Univ.*, [2]*Grad. Sch. Front. Sci., Univ. Tokyo*, [3]*Res. Cent. Evol. Anthropol. Palaeoecol., Liverpool John Moores Univ.*, [4]*Inst. Neuroetologia, Univ. Veracruzana*, [5]*Dept. Anthropol., Univ. Calgary*)

2P226 **コレラ菌の尿素走性と培養温度依存性**

Urea taxis of *Vibrio cholerae* and its temperature dependence

So-ichiro Nishiyama[1], Kouta Suzuki[1], Daisuke Suzuki[2], **Ikuro Kawagishi**[1] ([1]*Dept. Frontier Biosci., Hosei Univ.*, [2]*Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ.*)

2P227 **温度によるコレラ菌走化性受容体ホモログの発現制御機構の解析**

Temperature control of chemoreceptor expression in *Vibrio cholerae*

Shiori Onogi[1], Noriaki Sagoshi[1], Daisuke Suzuki[2], So-ichiro Nishiyama[3], Ikuro Kawagishi[1,3] ([1]*Frontier Biosci., Grad. Sci Eng., Hosei Univ.*, [2]*Grad. B. Engr., Nagoya Univ.*, [3]*Dept. Frontier Biosci., Fac. Biosci. Appl. Chem., Hosei Univ.*)

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

2P228 **チャコウラナメクジの脳嗅覚中枢における自発振動活動の長時間相関解析**

Long-range temporal correlations of oscillatory activities in the olfactory center in the land slug

Yuichi Tanaka[1], Tamon Eto[1], Shouhei Haga[1], Minoru Saito[2], **Yoshimasa Komatsuzaki**[1] ([1]*Nihon University, CST*, [2]*Nihon University, CHS*)

2P229 **チャコウラナメクジ嗅覚中枢における時空間神経活動パターンの膜電位イメージング**

Fluorescent Voltage Imaging of Spatiotemporal Activity Patterns in the Olfactory Center of the Land Slug

Tomoya Shimokawa[1], Kouhei Ishida[1], Yuuta Hamasaki[1], Yoshimasa Komatsuzaki[2], Minoru Saito[1] ([1]*Graduate School of Integrated Basic Sciences, Nihon University*, [2]*College of Science and Technology, Nihon University*)

2P230 **ヨーロッパモノアラガイの中枢神経系における神経活動の膜電位イメージング (II)**

Fluorescent Voltage Imaging of the Neural Activity in the Central Nervous System of the Pond Snail (II)

Yuuki Aikawa[1], Shogo Nakada[1], Makoto Hosoi[1], Yoshimasa Komatsuzaki[2], Minoru Saito[1] ([1]*Graduate School of Integrated Basic Sciences, Nihon University*, [2]*College of Science and Technology, Nihon University*)

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

2P231 **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解析法の提案**
An analysis method for spatiotemporal activity patterns in mouse hippocampal slices
Shodai Izumi[1], Yuuta Hamasaki[2], Hiromi Osanai[1], **Minoru Saito**[1,2] ([1]*College of Humanities and Sciences, Nihon University*, [2]*Graduate School of Integrated Basic Sciences, Nihon University*)

2P232 **マウス海馬スライスの CA1 領域における様々な時空間活動パターンのレーザー共焦点イメージング (II)**
Laser confocal imaging of various spatiotemporal activity patterns in the CA1 region of mouse hippocampal slices (II)
Mai Ichikawa[2], **Hiromi Osanai**[1], Yuuta Hamasaki[2], Minoru Saito[1,2] ([1]*College of Humanities and Sciences, Nihon University*, [2]*Graduate School of Integrated Basic Sciences, Nihon University*)

2P233 **視索前野の GABA 作動性神経とオレキシン神経の機能的結合について**
Functional connection between GABAergic neurons in the preoptic area and orexinergic neurons in the hypothalamus
Natsuko Kanda (Tsujino)[1], Yuki Saito[2], Manabu Abe[3], Kenji Sakimura[3], Masashi Yanagisawa[1,4], Takeshi Sakurai[1,2] ([1]*IIIS, Univ. Tsukuba*, [2]*Mol. Neurosci. Physiol., Kanazawa Univ.*, [3]*Cellular Neurobiol., Niigata Univ.*, [4]*UTSW/HHMI*)

2P234 **青斑核ノルアドレナリンニューロンへのオレキシン 2 型受容体を介した GABA 作動性の抑制性入力**
GABAergic inhibition of noradrenergic neurons through orexin type 2 receptors
Junya Fukuoka[1], Takeshi Kanda[1], Daiki Nakatsuka[1], Masashi Yanagisawa[1,2] ([1]*IIIS, Univ. Tsukuba*, [2]*UTSW/HHMI*)

17. 行動 / 17. Behavior

2P235 **アリの探索における記号創発**
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Yukio Gunji[1,2], Tomoko Sakiyama[1] ([1]*Kobe University*, [2]*University of West England*)

2P236 **群れの相互作用の多義性から自己組織化を再考する**
Rethinking about the concept of self-organization from the perspective of the interaction multiplicity in collective behavior
Takayuki Niizato (*Tsukuba University*)

2P237 **滑走細菌 *Flavobacterium johnsoniae* の菌表面構造**
Cell surface structure of the gliding bacterium *Flavobacterium johnsoniae*
Satoshi Shibata[1], Keiko Sato[1], Yuka Narita[1], Daisuke Nakane[2], Koji Nakayama[1] ([1]*Div.Microbiol./Oral infec., Grad. Sch. Bio/Med Sci., Nagasaki Univ.*, [2]*Dept. Phys.,Fac. Sci., Gakushuin Univ.*)

2P238 ***Flavobacterium johnsoniae* におけるコロニースプレッティングファクター**
Factors influencing colony spreading in *Flavobacterium johnsoniae*
Yuka Narita[1], Keiko Sato[1], Satoshi Shibata[1], Daisuke Nakane[2], Koji Nakayama[1] ([1]*Dept. Mol. Microbiol. Immunol., Grad. Sch. Biomedical Sci., Nagasaki Univ.*, [2]*Dept.Physics., Gakushuin Univ.*)

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

2P239 **自由エネルギー計算によるハロロドプシンの光駆動イオン輸送メカニズムの解析**
Study of the mechanism of the light-driven ion transport in halorhodopsin based on the free energy calculations
Hiroyuki Tamura[1], Shuntaro Chiba[1], Tadaomi Furuta[1], Shun Sakuraba[2], Nobuyuki Matsubayashi[2], **Minoru Sakurai**[1] ([1]*Tokyo Tech.*, [2]*Kyoto Univ.*)

2P240 **Aureochrome-1 の各ドメインの機能解析**
Functional analyses of each domain in Aureochrome-1
Yoichi Nakatani[1], Ken Takeuchi[1], Yosuke Izawa[1], Fumio Takahashi[2,3], Hironao Kataoka[4], Osamu Hisatomi[1] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*Grad. Sch. Life Sci., Ritsumeikan Univ.*, [3]*PRESTO, JST.*, [4]*Botanical Gardens, Tohoku Univ.*)

2P241 **Ab initio 電子状態計算における青色光受容体蛋白質の DNA 修復反応の理論的研究**
Theoretical Study of DNA Repair Mechanism of Blue Light Photoreceptors by Ab initio Electronic Structure Calculation
Ryuma Sato[1], Tsutomu Kawatsu[2,3], Takahisa Yamato[1] ([1]*Dept. of Phys., Grad. Sch. Sci., Univ. Nagoya*, [2]*CMSI*, [3]*Coll. Sci. Engn., Univ. Kanazawa*)

2P242 **In-situ 光照射固体ＮＭＲによるバクテリオロドプシン D96N 変異体の光中間体の捕捉とタンパク質構造変化の解明**
Trap of photo-intermediate and structural change of bacteriorhodopsin D96N mutant as revealed by in situ photoirradiation solid-state NMR
Akira Naito[1], Ryouta Miyasa[1], Arisu Shigeta[1], Izuru Kawamura[1], Satoru Tuzi[2], Kyosuke Oshima[1] ([1]*Yokohama National University Graduate School of Engineering*, [2]*University of Hyogo, Graduate School of Science*)

2P243 **In situ 光照射固体 NMR による 13-cis, 15-syn 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 photo excited process in 13-cis, 15-syn retinal of Bacteriorhodopsin studied by in situ photoirradiation SS-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*)

2P244 **光駆動型 Cl^-ポンプ ファラオニスハロロドプシンの Cl^-放出・取込み過程の解析**
Analysis of Cl^- release and uptake steps of light-driven Cl^- pump *Natronomonas pharaonis* halorhodopsin
Takashi Kikukawa[1], Chikara Kusakabe[1], Asami Kokubo[1], Takashi Tsukamoto[1,2], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Kunio Ihara[3], Naoki Kamo[1], Makoto Demura[1] ([1]*Grad. Sch. Life. Sci., Hokkaido Univ.*, [2]*Grad. Sch. Sci., Nagoya Univ.*, [3]*CGR, Nagoya Univ.*)

2P245 **光駆動型 Cl^-ポンプ ファラオニスハロロドプシンにおける Thr218 の役割**

Role of Thr218 in light-driven Cl^- pump mechanism of *Natronomonas pharaonis* halorhodopsin

Kousuke Shibasaki, Hiroaki Shigemura, Takashi Kikukawa, Masakatsu Kamiya, Tomoyasu Aizawa, Naoki Kamo, Makoto Demura (*Grad. Sch. Life. Sci., Hokkaido Univ.*)

2P246 **アセタブラリアロドプシン I の光化学反応**

Photochemical reaction in *Acetabularia* rhodopsin I

Jun Tamogami[1], Takashi Kikukawa[2], Kazumi Shimono[1,3,4,5], Tomomi Kimura-Someya[4,5], Mikako Shirouzu[4,5], Shigeyuki Yokoyama[4,6], Naoki Kamo[1,2] ([1]*College Pharm. Sci., Matsuyama Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*, [3]*Fac. Pharm. Sci., Toho Univ.*, [4]*RIKEN SSBC*, [5]*RIKEN Center for Life Science Technologies*, [6]*RIKEN Structural Biology Laboratory*)

2P247 ***in situ* 光照射固体 NMR による光受容膜タンパク質 *p*pR/*p*HtrII の光励起過程における transducer タンパク質膜貫通領域の構造変化の観測**

Photoactivated conformational changes of photoreceptor membrane protein *p*pR/*p*HtrII observed by *in situ* photo irradiation solid-state NMR

Yoshiteru Makino[1], Yuya Tomonaga[1], Yusuke Shibafuji[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. Sci, Nagoya Univ.*, [4]*Grad. Sch. Life Sci, Hokkaido Univ.*)

2P248 **Rhodobacter capsulatus 由来 Photoactive Yellow Protein の相互作用部位の解明**

Analysis of interaction sites on the Photoactive Yellow Protein of Rhodobacter capsulatus

Yoichi Yamazaki, Mayu Shimada, Hironari Kamikubo, Mikio Kataoka (*Graduate School of Materials science, Nara Institute of Science and Technology*)

2P249 ***Rhodobacter capsulatus* 由来 Photoactive Yellow Protein の X 線結晶構造解析**

X-ray crystal structure analysis of the Photoactive Yellow Protein of *Rhodobacter capsulatus*

Hiroshi Matsumoto, Yoichi Yamazaki, Hironari Kamikubo, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

2P250 **二種類のＰＹＰを用いたキメラタンパク質の中間体の平衡状態の解析**

Analysis of Equilibrium of intermediate states of PYP by use of chimera proteins

Yoshiaki Matsumoto, Youichi Yamazaki, Hironari Kamikubo, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

2P251 **PYP-Phytochrome Related Protein の X 線溶液散乱による研究**

X-ray Solution Scattering Studies of PYP-Phytochrome Related Protein

Keito Yoshida, Hironari Kamikubo, Kento Yonezawa, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Graduate school of Materials Science, Nara Institute of Science Technology*)

2P252 **PYP_M 中間体におけるアルギニン 52 のプロトン化状態**

Protonation state of R52 at the PYP_M intermediate state

Masayoshi Noji, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

2P253 **Excited State Proton Transfer of Fluorescent Photoactive Yellow Protein Reconstituted with Hydroxycoumarin**

Dian Novitasari, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Graduate School of Materials Science, Nara Institute of Science and Technology*)

2P254 **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, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

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

2P255 **開口数 0.9 の極低温光学顕微鏡の開発とその植物細胞内色素イメージングへの応用**

Development of a cryogenic optical microscope with NA of 0.9 and its application to studies of pigment distributions in plant cells

Yutaka Shibata[1], Keisuke Namie[1], Tomofumi Chiba[1], Mizu Kajihara[1], Wataru Kato[2], Hiroshi Fukumura[1] ([1]*Grad. Sch. Sci., Tohoku Univ.*, [2]*Grad. Sch. Sci., Nagoya Univ.*)

2P256 **ガリウム置換フェレドキシンの結晶構造と PS 1 および FNR との相互作用部位**

Crystal Structure of Ga-substituted Ferredoxin and its interaction sites for Photosystem I and Ferredoxin-$NADP^+$ reductase

Risa Mutoh, Norifumi Muraki, Hisako Kubota-Kawai, Toshiharu Hase, Takahisa Ikegami, Genji Kurisu (*Institute for Protein Research*)

2P257 **光化学系 II の Mn4 クラスター S0 状態における Mn(II)存在可能性の理論的研究**

S0-State Model of the Mn4-cluster in Photosystem II: Possibility of Mn(II)

Makoto Hatakeyama, Koji Ogata, Shinichiro Nakamura (*RIKEN*)

2P258 **フィコエリスリンを有するラン藻における励起エネルギー移動**

Excitation energy transfer in cyanobacteria containing phycoerythrin

Yuki Koge[1], Akio Murakami[1,2], Seiji Akimoto[1,3] ([1]*Graduate School of Science, Kobe University*, [2]*Kobe University Research Center for Inland Seas*, [3]*Molecular Photoscience Research Center, Kobe University*)

2P259 **ホタルルシフェラーゼとの相互作用を考慮したオキシルシフェリンの吸収スペクトルの量子化学計算**

Quantum chemical calculation of the absorption spectra of oxyluciferin interacting with firefly luciferase

Hironori Sakai[1], **Naohisa Wada**[2] ([1]*IFS. Tohoku Univ.*, [2]*Facul. of Food Sciences, Toyo Univ.*)

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

2P260 **次世代シーケンサーを用いた人工細胞モデルにおけるゲノムＲＮＡの進化プロセスの解析**
Analysis of the evolutionary process of the RNA genome in an artificial cell-like system using next generation sequencing technology
Norikazu Ichihashi[1,3], Shota Nakamura[2], Tetsuya Yomo[1,3,4] ([1]*Osaka Univ. Inf. Sci. and Tech.*, [2]*Osaka Univ. Dep. Infect. Metagenomics*, [3]*JST ERATO*, [4]*Osaka univ. Frontier Biosci.*)

2P261 **Qβ レプリケースによる RNA 複製反応中の二本鎖 RNA 形成の理解**
Double-stranded RNA formation during Qβ long RNA replication
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.*)

2P262 **人工自己複製モデルと寄生体が生み出す振動ダイナミクス**
Oscillation dynamics of Host-Parasite population in an artificial cell-like system
Yohsuke Bansho[1,2], Norikazu Ichihashi[3], Tetsuya Yomo[1,3,4] ([1]*Frontier Biosciences, Osaka University*, [2]*JSPS*, [3]*ERATO, JST*, [4]*Information Science and Technology, Osaka University*)

2P263 **Directed evolution of a self-encoding system**
Takeshi Sunami[1,2], Norikazu Ichihashi[1,2], Takehiro Nishikawa[2], Yasuaki Kazuta[2], Tomoaki Matsuura[2,3], Hiroaki Suzuki[2,4], Tetsuya Yomo[1,2,5] ([1]*Grad. Sch. Info. Sci., Osaka Univ.*, [2]*JST, ERATO*, [3]*Grad. Sch. Eng., Osaka Univ.*, [4]*Grad. Sch. Sci. Eng., Chuo Univ.*, [5]*Grad. Sch. Fro. Bio., Osaka Univ.*)

21B. ゲノム生物学：ゲノム構造 / 21B. Genome biology: Genome structure

2P264 **出芽酵母における染色体の構造変化と転写制御との関連について**
Relationship between conformational change of chromosomes and transcriptional control in budding yeast
Naoko Tokuda, Masaki Sasai (*Grad. Sch. Eng., Nagoya Univ.*)

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

2P265 **天然変性タンパク質データベース IDEAL の機能拡張 —PPI ネットワーク**
New IDEAL: availability of PPI networks involving intrinsically disordered proteins
Takayuki Amemiya[1], Shigetaka Sakamoto[2], Yukiko Nobe[1], Kazuo Hosoda[3], Yumiko Kado[1], Ryotaro Koike[1], Hidekazu Hiroaki[4], Motonori Ota[1], satoshi Fukuchi[3] ([1]*Grad. Schl of Info. Sci., Nagoya Univ.*, [2]*HOLONICS Co., Ltd.*, [3]*Fac. Engr., Maebashi Ins. Tech.*, [4]*Grad. Schl of Pharm. Sci., Nagoya Univ.*)

2P266 **Tertiary structure prediction of RNA-RNA complex structures using secondary structure information**
Satoshi Yamasaki, Kazuhiko Fukui (*molprof, AIST*)

2P267 **相互作用プロファイルを用いた Re-docking 法によるタンパク質間相互作用予測**
Re-docking scheme for prediction of protein-protein interactions using interaction fingerprints
Nobuyuki Uchikoga[1], Yuri Matsuzaki[2], Masahito Ohue[2,3], Takatsugu Hirokawa[4], Yutaka Akiyama[2,3] ([1]*Dept. Phys., Chuo Univ.*, [2]*Grad. info. sci. eng., Dept. comput. sci., Titech*, [3]*Edu. Acad. comput. life sci., Titech*, [4]*AIST, molprof*)

2P268 **Protein binding pocket and ligand shape comparison**
Chie Motono, Takatsugu Hirokawa (*Molprof, AIST*)

2P269 **膜タンパク質の顕微鏡画像と立体構造データとの照合用データベースの構築**
Construction of database for comparing structural data with microscopic image of transmembrane protein
Go Inoue, Masami Ikeda, Makiko Suwa (*Grad. Sch. Sci and Eng. AGU*)

2P270 **β2 アドレナリン受容体 - Gαs 間の結合要素の解析**
Structural analysis of coupling element between β2 adrenergic receptor and G-protein
Hidenori Sakaki, Masami Ikeda, Makiko Suwa (*Grad. Sch. Sci and Eng. AGU*)

23. 生態／環境 / 23. Ecology & Environment

2P271 **Flow cytometry identification of nanocyanobacteria and their limiting factors in the North Pacific Subtropical Gyre**
Mathias Girault[1,2], Hisayuki Arakawa[2], Gerald Gregori[3], Fuminori Hashihama[2], Hyonchol Kim[1], Masao Odaka[1], Kenji Yasuda[1] ([1]*KAST*, [2]*TUMSAT*, [3]*Universite de la Mediterranee*)

2P272 **実験生態系の進化、個体群、反応ダイナミクス**
Evolutionary, population, and reaction dynamics of experimental ecosystems
Kazufumi Hosoda[1], Makoto Sueyoshi[2], Itsuka Kumano[2], Masumi Habuchi[3], Kayo Yamamoto[2], Risa Takami[2], Yuhki Azuma[4], Isao Kubo[2], Shingo Suzuki[2], Tetsuya Yomo[2] ([1]*Acad Init, Osaka-u*, [2]*Info Sci, Osaka-u*, [3]*Front Bio, Osaka-u*, [4]*Eng, Osaka-u*)

24. 数理生物学 / 24. Mathematical biology

2P273 **Gain Noise Relation in Adaptation Networks**
Prabhat Shankar[1,2], Masatoshi Nishikawa[3], Tatsuo Shibata[1] ([1]*RIKEN CDB, Kobe*, [2]*Hiroshima University, HIroshima*, [3]*Max Planck Inst, Germany*)

2P274 **Adaptive random Boolean network model based on local information transfer**
Taichi Haruna, Sayaka Tanaka (*Graduate School of Science, Kobe University*)

2P275 **光合成生物との共生による利益とはーミドリゾウリムシの増殖解析**
Benefits of Acquiring Phototrophy by Hosting Algal Endosymbionts
Sosuke Iwai (*Faculty of Education, Hirosaki Univ.*)

2P276 **Diffusion in the plasma membrane with immobile molecules: significance of fluid dynamical interactions**
Ziya Kalay, Takahiro K. Fujiwara, Akihiro Kusumi (*Institute for Integrated Cell-Material Sciences, Kyoto University*)

2P277 **Competitive reaction between enzymes with normal and anomalous diffusivity**
Kenta Yashima[1], Jun Nakabayashi[2], Akira Sasaki[1] ([1]*The Graduate University for Advanced Studies*, [2]*Yokohama City University*)

2P278 **Allometries of the *Physarum* plasmodium based on the dynamics of cytoplasmic streaming**
Tomohiro Shirakawa, Hiroshi Sato (*Dept. Comp. Sci., NDA*)

2P279 **Analysis for the exploratory behavior of *Physarum* plasmodium in an unlimitedly extendable space**
Miharu Nishida, Hiroshi Satou, Tomohiro Shirakawa (*Dept. Comp. Sci., NDA*)

2P280 **Cell motility of the *Physarum* plasmodium on a non-uniform substrate**
Shinji Ishiguro, Hiroshi Sato, Tomohiro Shirakawa (*National Defense Academy of Japan*)

2P281 **過去の神経活動がどのように現在のスパイク頻度に影響を与えるのか**
How past neuronal activity affects the current firing rate
Takanobu Yamanobe (*Med. Sch., Hokkaido Univ.*)

25. 非均衡・生体リズム / 25. Equality Nonequilibrium state & Biological rhythm

2P282 **Negative feedback regulation of KaiC ATPase gives origin to the circadian periodicity of cyanobacteria**
Atsushi Mukaiyama[1,2,3], Masato Osako[4], Takaaki Hikima[3], Takao Kondo[4], Shuji Akiyama[1,2,3] ([1]*Inst. Mol. Sci.*, [2]*Grad. Univ. for Adv. Studies (SOKENDAI)*, [3]*SPring-8, RIKEN*, [4]*Nagoya Univ.*)

2P283 **マイクロドロップレットで構築された非平衡人工細胞の実験的・数理的解析**
Experimental and numerical analyses of microdroplet-based nonequilibrium artificial cells
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.*)

2P284 **膜の分子透過性へのフィードバック制御のある非平衡系人工細胞の数理解析**
Numerical analysis of non-equilibrium open artificial cell with a feedback control over molecular permeability of the cell membrane
Motosugi Murata[1], Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*, [2]*PRESTO, JST*)

2P285 **Oscillations of a genomic DNA in a cell-sized chemically open system**
Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Inst. Tech.*, [2]*PRESTO, JST*)

26. 計測 / 26. Measurements

2P286 **広帯域 X 線を用いた広角度域 X 線 1 分子追跡法の開発**
Development of wide angle Diffracted X-ray Tracking (DXT) measurement using a focusing broad band X-ray
Ichiyanagi Kouhei[1], Hiroshi Sekiguchi[2], Masato Hoshino[2], Kentaro Kajiwara[2], Kentaro Hoshisashi[1], Jae-won Chang[1], Maki Tokue[1], Yufuku Matsushita[1], Naoto Yagi[1], Yuji Sasaki[1] ([1]*Graduate School of Frontier Sciences, The University of Tokyo*, [2]*Japan Synchrotron Radiation Research Institute*)

2P287 **オンチップ画像解析システムによる形状を制御した単一心筋細胞の収縮方向の計測**
Measurement of contractile direction on single-shape-controlled cardiomyocytes by on-chip optical image analysis system
Tomoyuki Kaneko[1], Fumimasa Nomura[2], Tomoyo Hamada[2], Akihiro Hattori[2], Kenji Yasuda[2] ([1]*Dept. Frontier Bioscience, Hosei Univ.*, [2]*Dept. Biomed. Info, IBB, TMDU*)

2P288 **ビデオ解析による大腸菌回転特性の大量測定**
Large-scale measurement of rotary motion properties of tethered Escherichia coli (*E. coli*) by video analysis
Hiroto Tanaka[1], Tadashi Matsukawa[1], Yukihiro Tominari[2], Shuhei Ogawa[3], Yoshiyuki Sowa[4], Ikuro Kawagishi[4], Shukichi Tanaka[2], Kazuhiro Oiwa[1], Hiroaki Kojima[1] ([1]*Bio ICT lab., NICT*, [2]*Nano ICT lab., NICT*, [3]*Dept. Bioeng., Nagaoka Univ. Tech.*, [4]*Dept. Front. Biosci., Hosei Univ.*)

2P289 **流体力学的絞込みを用いた一分子ソーターセルの開発**
Development of hydrodynamic focusing system for single molecule sorting device
Toshihiko Kubota[1,2], Hiroyuki Oikawa[1], Kiyoto Kamagata[1,2], Satoshi Takahashi[1,2] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Life Sci., Tohoku Univ.*)

2P290 **創薬スクリーニングのための心筋細胞ネットーワークにおける空間パターンと集団サイズの重要性**
Importance of spatial arrangement and community size on cardiomyocyte network for precise and stable in vitro drug screening measurement
Fumimasa Nomura, Tomoyo Hamada, Hideyuki Terazono, Kenji Yasuda (*IBB, Tokyo Medical and Dental Univ.*)

2P291 **マニフォールドを用いた投影イメージの分類プロトコル：コヒーレント X 線イメージングによる粒子ダイナミックスの解析へ向けて**
Classification protocol of projection images by manifold: Toward analysis of dynamics of particles with coherent x-ray diffraction imaging
Takashi Yoshidome[1], Tomotaka Oroguchi[2,3], Masayoshi Nakasako[2,3], Mitunori Ikeguchi[1] ([1]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*, [2]*Dep. Phys., Keio Univ.*, [3]*Harima Inst., Riken*)

2P292 **振動和周波検出赤外超解像顕微鏡による毛髪 α-ケラチンの分子配向観察**
Observation of molecular orientation of human hair α-keratins by VSFG detected IR super-resolution microscopy
Makoto Sakai[1], Kohei Ushio[1,2], Shinobu Nagase[3], Yuuji Hirano[3], Takashi Itou[3], Haruki Ishikawa[2], Masaaki Fujii[1] ([1]*Tokyo Institute of Technology*, [2]*Kitasato University*, [3]*Kao Corporation*)

2P293 **Determination of dissociation constants of NFκB p50/p65 heterodimer using fluorescence cross-correlation spectroscopy in the living cell**
Manisha Tiwari[1], Shintaro Mikuni[2], Masataka Kinjo[2] ([1]*Graduate School of Life Science, Hokkaido University, Japan*, [2]*Faculty of Advanced Life Science, Hokkaido University, Japan*)

2P294 **蛍光・発光イメージングによる OPN5 発現細胞の Ca^{2+}応答測定**
Bioluminescent Imaging Revealed a Rapid Ca^{2+} Response in OPN5-expressing Cells
Takashi Sugiyama (*Cell-based Analysis Group, Advanced Analysis Technology R&D Dept., Olympus Corporation*)

2P295 **ライブセル超解像イメージングに向けた多重分子用アルゴリズム“Wedged Template Matching”**
Localization Algorithm of High-Density Fluorophores, “Wedged Template Matching” for Live Cell Super Resolution Imaging
Shigeo Watanabe[1], Yasushi Okada[2], Teruo Takahashi[1], Keith Bennett[3], Tomochika Takeshima[1] ([1]*Hamamatsu Photonics K.K.*, [2]*RIKEN QBiC*, [3]*Hamamatsu Corporation*)

2P296 **2 種類のシグナルノイズが PTEN の細胞内不均一性を決める**
Two types of signaling noises underlie spatiotemporal PTEN heterogeneity
Naotoshi Nakamura, Tatsuo Shibata (*Laboratory for Physical Biology, RIKEN Center for Developmental Biology*)

2P297 **2 波長同時イメージングによる PTEN の膜局在と 1 分子の同時解析**
Simultaneous Imaging of Single-molecule and Bulk Localization of PTEN
Seiya Fukushima[1], Satomi Matsuok[2], Masahiro Ueda[1,2] ([1]*Grad. Sch. Sci. Bio., Univ. Osaka*, [2]*QBiC, RIKEN*)

2P298 **Fluorescent Single Molecule Orinetation Imaging in Living Cells**
Tomomi Tani[1], Shalin Mehta[1], Rudolf Oldenbourg[1], Amy Gladfelter[2] ([1]*Marine Biologcal Laboratory*, [2]*Dartmouth College*)

2P299 **Fast positively photoswitchable fluorescent protein for superresolution nanoscopy**
Dhermendra K Tiwari, Yoshiyuki Arai, Takeharu Nagai (*Osaka University*)

2P300 **Monitoring cytosolic Mg2+ with a novel genetically encoded fluorescent indicator using a non-FRET-based ratiometric imaging approach**
Vadim Perez Koldenkova, Tomoki Matsuda, Dharmendra Tiwari, Shoji Kawakami, Takeharu Nagai (*The Institute of Scientific and Industrial Research, Osaka University*)

2P301 **GEM-GECO を用いた細胞内カルシウムのイメージング定量解析**
Quantification of calcium concentration in cells by imaging analysis using GEM-GECO
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-RCAI, RIKEN*)

2P302 **FRET による elongin B と elongin C の相互作用解析**
FRET - based analysis of interactions between elongin B and elongin C
Hirofumi Oyama[1,2], Yuma Ito[1,2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

2P303 **FRAP と 1 分子蛍光イメージングを用いた転写活性化時 Arp4β 動態の定量解析**
Quantitative analysis of molecular dynamics of Arp4β upon transcriptional activation by single-molecule fluorescence imaging and FRAP
Naomichi Inaba[1,2], Yuma Ito[1,2], Masahiko Harata[3], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*, [3]*Grad. Sch. Agr. Sci., Tohoku Univ.*)

2P304 **炎症反応抑制タンパク質 PDLIM2 の局在制御機構の解明**
The elucidation of the mechanism of PDLIM2 localization regulation
Satoshi Toriyama[1,2], Yuma Ito[1,2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

2P305 **3 色同時 1 分子イメージングによる T 細胞マイクロクラスターとシグナル膜タンパク質の相互作用解析**
Single molecule analysis of signaling membrane proteins in T cell microcluster by multicolor live cell imaging
Yuma Ito[1,2], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

2P306 **カルシウムイオン刺激による微小管伸長の動態解析**
Imaging analysis of effect of Ca^{2+} ion on micritubule polymerization
Zhihai Zheng[1], Akihiro Fukagawa[1], Yuma Ito[1,2], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech*, [2]*IMS-RACI, RIKEN*)

2P307 **T 細胞活性化における微小管形成中心の動態**
Microtubules Organizing Center (MTOC) Dynamics and Migration upon T Cell Activation
Wei Ming Lim[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-RCAI, RIKEN*)

28. バイオエンジニアリング / 28. Bioengineering

2P308 **セミインタクト細胞リシール技術による細胞内への分子導入と病態モデル細胞構築への応用**
Cell resealing technique for introducing molecules into cells and its application for establishment of disease model cells
Yoshiyuki Noguchi[1], Yuta Horiuchi[1], Daiki Nakatsu[1], Fumi Kano[1,2], Masayuki Murata[1] ([1]*Grad. Sch. of Arts and Sci., The Univ. of Tokyo*, [2]*PRESTO, JST*)

2P309 **アポフェリチン空洞内に合成した Y 化合物を母体とした Eu および Tb ナノ粒子の発光特性**
Photoluminescence Property of Eu and Tb Doped Y Based Nano-Phosphor synthesized in an apoferritin cavity
Tomoaki Harada, Hideyuki Yoshimura (*Meiji Univ.*)

2P310 **オズモシス流による FET ナノポア付近の DNA の動き制御**
Controlling the fluidic motion of DNA molecules near FET nanopores by electro-osmotic flows
Manabu Sugimoto, Yuta Kato, Kentaro Ishida, Toshiyuki Mitsui (*Grad. Sch. Sci., Aoyama. Univ.*)

2P311 **ナノ・マイクロファイバーゲルマトリックスの弾性設計による三次元細胞運動制御**
Mechanical control of 3-D cell movement in elasticity-tunable matrix of nano/micro-fiber gels
Aya Ogata[1], Satoru Kidoaki[2] ([1]*Grad. Sch. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*)

2P312 ***In vitro* selection of peptide aptamer binding to reduced ferredoxin**
Yasodha Manandhar[1,2], Takanori Uzawa[1], Toshiro Aigaki[2], Yoshihiro Ito[1,2] ([1]*RIKEN*, [2]*Tokyo Metropolitan University*)

2P313 **Selection of RNA aptamer binding to a photoredox catalyst**
Thi Thanh Thoa Tran[1,2], Toshiro Aigaki[2], Takanori Uzawa[1], Yoshihiro Ito[1,2] ([1]*RIKEN*, [2]*Tokyo Metropolitan University*)

2P314 **デザインされた DNA 高次構造体の環境安定性評価**
Stability of designed high-order DNA structures under unconventional conditions
Masahiro Endo[1], Kei Fujiwara[2], Satoshi Murata[1], Shin-ichiro Nomura[1] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*JSPS. Research Fellow. Tohoku Univ.*)

2P315 **Self-assembly and reconfiguration of multiple-sized closed structures made of DNA origami units**
Keitel Cervantes[1], Shogo Hamada[2], Shin-ichiro Nomura[1], Satoshi Murata[1] ([1]*Tohoku university*, [2]*Cornell university*)

2P316 **回転磁場による磁性粒子接着リポソームのクロール運動の観察**
Crawl movement observation of a liposome attached micro-superparamagnetic particles under a rotational magnetic field
Daiki Komatsu, Kei Fujiwara, Shin-ichiro M. Nomura (*Tohoku University*)

第 3 日目（10 月 30 日（水））／Day 3（Oct. 30 Wed.）アネックスホール / Annex hall

01A. 蛋白質：構造 / 01A. Protein: Structure

3P001 **Investigation for co-translational folding using X-ray crystallography**
Yuya Hanazono, Kazuki Takeda, Kunio Miki (*Grad. Sch. Sci., Kyoto Univ.*)

3P002 **二核フェロキシダーゼ中心をもつピロリ菌好中球活性化タンパク質の構造**
Structure of *Helicobacter pylori* neutrophil-activating protein with a di-nuclear ferroxidase center
Hideshi Yokoyama, Osamu Tsuruta, Naoya Akao, Satoshi Fujii (*Sch. of Pharm. Sci., Univ. of Shizuoka*)

3P003 **T4 ファージ gp34C 末端側半分の結晶構造から得られたファージ尾繊維に共通の構造**
The crystal structure of C-terminal half of gp34 from phage T4 reveals common architecture of phage tail fibers
Shuji Kanamaru, Mikiyoshi Namura, Fumio Arisaka (*Grad. Sch. of Biosci. & Biotech., Tokyo Institute of Technology*)

3P004 **病原性大腸菌 O-157 のタイプ 6 分泌系の VgrG1 蛋白質の C 末端断片の X 線結晶構造**
Crystal structure of the C-terminal domain of VgrG1 protein of *E.coli* O-157 Type 6 secretion system
Kazuya Uchida[1], Shuji Kanamaru[1], Petr Leiman[2], Fumio Arisaka[1] ([1]*Grad. Sch. of Biosci. & Bioeng., Tokyo Tech.*, [2]*EPFL*)

3P005 **仮性結核菌由来ヘム獲得蛋白質 HasA の結晶構造解析による新規ヘム結合様式の解明**
Crystal structure of a hemophore hasA secreted by Yersinia pseudotuberclosis shows a novel heme binding mode
Masahiro Kanadani[1], Toshiki Muroki[2], Yukie Ishimaru[2], Saki Wada[1], Takehiro Sato[3], Shin-ichi Ozaki[3], Tomoya Hino[1], Shingo Nagano[1] ([1]*Grad. Sch. Eng., Univ. Tottori*, [2]*Fac. Eng., Univ. Tottori*, [3]*Fac. Agric., Univ. Yamaguchi*)

3P006 **HLA-G2/G6 アイソフォームの単粒子構造解析**
Three dimensional reconstruction of HLA-G2/G6 isoform
Kazuhiro Mio[1], Kimiko Kuroki[2], Haruki Matsubara[2], Yoshiyuki Kasai[2], Chikara Sato[1], Katsumi Maenaka[2] ([1]*National Institute of Advanced Industrial Science and Technology, Biomedical Research Institute*, [2]*Laboratory of Biomolecular Science, Hokkaido University*)

3P007 **大気圧電子顕微鏡（ASEM）によるタンパク質微結晶と細胞内複合体の液中観察**
Direct electron microscopy of protein crystals and Mycoplasma cells in solution using the Atmospheric SEM
Tatsuhiko Ebihara[1], Masaaki Kawata[1], Hidetoshi Nishiyama[2], Miki Senda[3], Mari Sato[1], Mitsuo Suga[2], Toshiya Senda[3], **Chikara Sato**[1] ([1]*AIST*, [2]*JEOL*, [3]*KEK*)

3P008 **EM Navigator と Yorodumi による 3 次元電子顕微鏡構造データの利用**
Using 3D electron microscopy data by EM Navigator and Yorodumi
Hirofumi Suzuki[1,2], Haruki Nakamura[1,2] ([1]*IPR, Osaka univ.*, [2]*PDBj*)

3P009 **電子顕微鏡の傾斜ペアを利用した構造の異なるタンパク質単粒子画像の分類**
Separating single particle images of protein in the different conformations using tilt pair transmission electron microscopy
Yutaka Ueno, Kazunori Kawasaki, Shouhei Mine (*AIST Health Research Institute*)

3P010 **NMR タンパク質立体構造決定のための新規構造最適化法の開発**
Development of a new refinement method for NMR protein structure determination
Manato Shimazaki[1], Teppei Ikeya[1], Masaki Mishima[1], Yutaka Ito[1], Peter Guentert[1,2] ([1]*Grad. Sch. Scii., Tokyo Metropolitan Univ*, [2]*Inst. Biophys. Chem., Goethe Univ Frankfurt*)

3P011 **Structural analysis of antimicrobial peptide CP1 with LPS by NMR**
Mihwa Baek[1], Masakatsu Kamiya[1,2], Taichi Nakazumi[1], Satoshi Tomisawa[1], Yasuhiro Kumaki[3], Takashi Kikukawa[1,2], Makoto Demura[1,2], Keiichi Kawano[2], Tomoyasu Aizawa[1,2] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Fac. Adv. Life Sci., Hokkaido Univ.*, [3]*Grad. Sch. Sci., Hokkaido Univ.*)

3P012 **高圧力下で見られるべん毛繊維の動的多型性**
Dynamic polymorphism of bacterial flagellar filaments at high pressure
Masayoshi Nishiyama[1], Yoshiyuki Sowa[2] ([1]*Kyoto University*, [2]*Hosei University*)

3P013 **金属結合に伴う 3 ヘリックスバンドル形成の動的構造解析**
Dynamic structural analysis of three-helix bundle formation induced by metal-ion binding
Nobutaka Komichi[1], Hiroshi Sekiguchi[2], Yuji C. Sasaki[3], Toshiki Tanaka[4], Masayuki Oda[1] ([1]*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Jpn. Syn. Rad. Res. Inst.*, [3]*Grad. Sch. Fron. and Sci., Univ. Tokyo*, [4]*Grad. Sch. Eng., Nagoya Inst. Tech.*)

3P014 **Membrane-Induced Conformations of Proteins Characterized by Vacuum-Ultraviolet Circular-Dichroism and Flow Linear-Dichroism**
Koichi Matsuo[1], Hirofumi Namatame[1], Masaki Taniguchi[1], Kunihiko Gekko[2] ([1]*HiSOR, Hiroshima Univ.*, [2]*Inst. Sust. Sci. Devel. Hiroshima Univ.*)

3P015 **創薬等支援技術基盤プラットフォーム事業におけるタンパク質 X 線溶液散乱**
Bio-SAXS in the Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS)
Nobutaka Shimizu[1], Shinya Saijyo[1], Hiromasa Ota[2], Yasuko Nagatani[1], Ai Kamijyo[1], Takeharu Mori[1], Takashi Kosuge[1], Noriyuki Igarashi[1] ([1]*Photon Factory, KEK*, [2]*Mitsubishi Electric SC*)

3P016 **分子動力学法によるラミニン由来ペプチドの研究**
Study of peptides derived from laminin by molecular dynamics simulations
Hironao Yamada, Masaki Fukuda, Yuka Fukasawa, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu (*Tokyo University of Pharmacy and Life Sciences*)

3P017 **分子動力学法を用いたラミニン α2 由来ペプチド A2G80 の構造決定因子の同定**
Identification of structure determinant amino acid residues in the A2G80 peptide derived from laminin α2 by molecular dynamics simulation
Yuka Fukasawa[1], Jun Kumai[1], Fumihiko Katagiri[1], Yamato Kikkawa[1], Kentaro Hozumi[1], Motoyoshi Nomizu[1], Hironao Yamada[2], Masaki Fukuda[2], Takeshi Miyakawa[2], Ryota Morikawa[2], Masako Takasu[2] ([1]*School of Phamrmacy, Tokyo University of Pharmacy and Life Sciences*, [2]*School of Life Sciences, Tokyo University of Pharmacy and Life Sciences*)

3P018 **分子動力学計算による 4 量体型サルコシン酸化酵素の酵素—基質アナログ複合体の動的挙動解析**
Behavior of enzyme-substrate analogue complex of heterotetrameric sarcosine oxidase studied by molecular dynamics simulation
Go Watanabe, Akinori Hiroshima, Haruo Suzuki, Shigetaka Yoneda (*School of Science, Kitasato University*)

3P019 **The role of the flexible loop in Staphylococcal nuclease on its catalytic activity**
Rumi Shiba[1], Hironari Kamikubo[1], Yutaka Maruyama[2], Junko Yunoki[1], Keiichi Fukuyama[3], Yoichi Yamazaki[1], Mariko Yamaguchi[1], Mikio Kataoka[1] ([1]*Graduate School of Materials Science, Nara Institute of Science and Technology*, [2]*Insitute for Protein Reserch, Osaka University*, [3]*Department of Biological Science, Graduate school of Science, Osaka University*)

3P020 **触媒アスパラギン酸の電荷改変による HIV-1 プロテアーゼの分子動力学シミュレーションへの影響**
Molecular dynamics simulations of HIV-1 protease-inhibitor complex with modified charges for catalytic aspartate
Hirotaka Ode[1], Wataru Sugiura[1,2], Yoshiyuki Yokomaku[1] ([1]*Clinical Research Center, National Hospital Organization Nagoya Medical Center*, [2]*Nagoya University Graduate School of Medicine*)

3P021 **超音波によるアミロイドβオリゴマー破壊の非平衡分子動力学シミュレーション**
Non-equilibrium molecular dynamics simulation for disruption of an amyloid-β oligomer by hypersonic wave
Hisashi Okumura[1,2], Satoru Itoh[1,2] ([1]*Inst Mol Sci*, [2]*SOKENDAI*)

3P022 **Structure and Interactions in Fibrillation of Human Calcitonin Hormone**
Javkhlantugs Namsrai, Ganchimeg Lkhamsuren, Kazuyoshi Ueda, Akira Naito (*Yokohama National University*)

3P023 **結晶環境における弾性ネットワークモデルを用いた高分解能 X 線構造における温度因子の再現**
Thermal fluctuation in high-resolution crystal structures reproduced by normal modes based on an elastic-network model in the crystal
Shigeru Endo[1], Hiroshi Wako[2] ([1]*Dept. Phys., Sch. Science, Kitasato Univ.*, [2]*Sch. Social Sciences, Waseda Univ.*)

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

3P024 **カブトガニ由来抗菌ペプチド Tachyplesin I とキチン結合能に関する研究**
Analysis of chitin binding ability of an antimicrobial peptide tachyplesin I derived from horseshoe crab
Takahiro Kushibiki[1], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Yasuhiro Kumiki[2], Takashi Kikukawa[1], Makoto Demura[1], Shun-ichiro Kawabata[3], Keiichi Kawano[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Grad. Sch. of Sci., Hokkaido Univ.*, [3]*Dept. Biol., Kyusyu Univ.*)

3P025 **リジン 2,3-アミノミューターゼにおける高反応性ラジカル反応機構の解明**

Taming the Reactive 5'-Deoxyadenosyl Radical by Enforcing van der Waals Contact with Substrate in Lysine 2,3-Aminomutase

Masaki Horitani[1], Krista Shisler[2], Amanda Byer[2], Joan B. Broderick[2], Brian M. Hoffman[1] ([1]*Dept. Chem., Northwestern Univ.*, [2]*Dept. Chem. & Biochem., Montana State Univ.*)

3P026 **糖結合モジュール Trp 導入変異体の基質結合能**

Substrate binding ability of the Trp introduced mutant of carbohydrate-binding module

Tomonari Tamashiro[1], Hiromi Asada[1], Takahiro Maruno[2], Kenji Kanaori[3], Yuji Kobayashi[2], Masayuki Oda[1] ([1]*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Grad. Sch. Eng., Osaka Univ.*, [3]*Grad. Sch. Sci. and Technol., Kyoto Inst. Technol.*)

3P027 **炭酸脱水酵素のある変異体の His64 の２つの配向の間の化学交換は NMR 時間軸上十分遅い**

Chemical Exchange between Two Conformations within His64 in a Mutant of Carbonic Anhydrase Is Sufficiently Slow on the NMR Timescale

Hideto Shimahara (*JAIST CNMT*)

3P028 ***Rhodococcus rhodochrous* J1 由来ニトリラーゼの温度による構造変化の ^{1}H NMR による追跡。**

Structural changes of the J1 nitrilase from *Rhodococcus rhodochrous* upon temperatuer increase tracked by ^{1}H NMR

Kyouhei Oyama[1], Ryo Ishiguro[1,2], Teturo Fujisawa[1,2] ([1]*Department of Chemistry and Biomolecular Science,Faculty of Engineering,Gifu University*, [2]*RIKEN SPring-8 Center*)

3P029 **Analysis of unfolded structure of Staphylococcal nuclease mutants by using FRET**

Emi Ohta[1], Takuya Muto[1], Yusuke Kishi[1], Mariko Yamaguchi[1], takayoshi Watanabe[2], Yoichi Yamazaki[1], Hironari Kamikubo[1], Takahiro Hohsaka[2], mikio Kataoka[1] ([1]*Nara Institute of Science and Technology*, [2]*Japan Advanced Institute of Science and Technology*)

3P030 **高時間分解能で蛋白質の分子揺らぎと構造変化を計測するためのＸ線１分子動態計測法の開発**

The Refinement of the Diffracted X-ray Tracking Method for Recording the Single-Molecule Motions of Proteins with Higher Time Resolution

Hirofumi Shimizu, Masayuki Iwamoto, Shigetoshi Oiki (*Univ.Fukui.Fac.Med.Sci.*)

3P031 **X 線 1 分子追跡法による蛋白質安定性の解析**

Protein Stability Analysis of MHC/peptide Complex from X-ray Single Molecule Tracking

Yufuku Matsushita[1], Haruo Kozono[2], Naoki Ogawa[4,5], Kohei Ichiyanagi[1,5], Hiroshi Sekiguchi[3,5], Yuji Sasaki[1,3,5] ([1]*Grad. Sch. Sci., Univ. Tokyo*, [2]*Grad. Sch. Sci., Tokyo Univ. Sci.*, [3]*Spring-8*, [4]*Dept. Int. sci., Nippon Univ.*, [5]*CREST Sasaki team/JST*)

3P032 **表層ストレス応答を制御する膜内切断プロテアーゼ RseP のタンデム PDZ ドメインによる基質選別機構**

Substrate discrimination mechanism by a PDZ tandem in the intramembrane protease RseP that regulates extracytoplasmic stress response

Yohei Hizukuri[1], Takashi Oda[2], Sanae Tabata[3], Tamura-Kawakami Keiko[3], Mamoru Sato[2], Junichi Takagi[3], Terukazu Nogi[2], Yoshinori Akiyama[1] ([1]*Inst. Virus Res., Kyoto Univ.*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*, [3]*Inst. Prot. Res., Osaka Univ.*)

3P033 **Design of Photo-controllable Cyclic Peptides**

Shinji Kawabata, Yasuhiro Ebisu, Yuta Saeki, Masahiko Hayashi, Atsuo Tamura (*Grad. Sch. Sci., Univ. Kobe*)

3P034 **脂質ータンパク質相互作用の解明を目指した重原子標識脂肪酸の利用**

Toward an understanding of lipid-protein interactions, the use of the heavy atom labeled fatty acid analogues

Shigeru Sugiyama[1,2], Mika Hirose[1,2], Hanako Ishida[1,2], Sebastien Lethu[1,2], Hikaru Ano[1,2], Daisuke Matsuoka[1,2], Toshiaki Hara[1,2], Eiichi Mizohata[3], Tsuyoshi Inoue[3], Shigeru Matsuoka[1,2], Michio Murata[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*JST, ERATO, Lipid Active Structure Project*, [3]*Grad. Sch. Engi., Osaka Univ.*)

3P035 **嗅覚受容体モデルとしてのオプシン立体構造**

Opsin, Structural Model for Olfactory Receptors

Takefumi Morizumi[1], Jung Hee Park[2], Emil F. Pai[1], Klaus P. Hofmann[3], Hui-Woog Choe[2], Oliver P. Ernst[1] ([1]*Univ. Toronto, Dept. Biochemistry, Canada*, [2]*Chonbuk National Univ., Korea*, [3]*Charite, Univ. Med. Berlin, Germany*)

3P036 **13-cis 型が優勢となる ASR 変異体の研究**

Study of *Anabaena* Sensory Rhodopsin mutant P206D that contains the 13-cis form dominantly

Yoshitaka Kato[1], Akira Kawanabe[2], Keiichi Inoue[1], Kwang-Hwan Jung[3], Hideki Kandori[1] ([1]*Grad. Sch. Eng., Nagoya Inst. Tech.*, [2]*Grad. Sch. Med., Osaka Univ.*, [3]*Sogang Univ., Korea*)

3P037 **真菌由来エラスターゼインヒビター AFUEI と植物由来 potato I family インヒビターとの構造類似性**

Structural similarity of AFUEI, an elastase inhibitor from *Aspergillus fumigatus*, and the potato I family inhibitors from plants

Mayuko Sakuma[1], Katsumi Imada[2], Yoshiyuki Okumura[3], Kei-ichi Uchiya[3], Atsushi Hijikata[4], Tsuyoshi Shirai[4], Toshiaki Nikai[3], Michio Homma[1] ([1]*Grad. Sch. Sci., Nagoya Univ.*, [2]*Grad. Sch. Sci., Osaka Univ.*, [3]*Fac. Pharm., Meijo Univ.*, [4]*Fac. Biosci., Nagahama Inst. BioSci. Tech.*)

3P038 **酵母２６Ｓおよび２０Ｓプロテアソームの構造研究**

Structural investigation of the yeast 26S and 20S proteasome

Yuya Morita[1,2], Takuto Murakami[3], Hiroshi Yamaguchi[3], Yukio Morimoto[1,2] ([1]*Graduate School of Science, Kyoto University*, [2]*Research Reactor Institute, Kyoto University*, [3]*Graduate School of Science-Technology, Kwansei Gakuin University*)

3P039 ***Mycoplasma mobile* の滑走時に“あし”として働くシアル酸レセプターの構造解析**

Structural study of neuraminic acid receptor working as foot in *Mycoplasma mobile* gliding

Tasuku Hamaguchi, Yuhei Tahara, Makoto Miyata (*Grad. Sch. of Sci., Osaka City Univ.*)

3P040 **藍色細菌時計タンパク質 KaiA-KaiC 相互作用の ESR 解析**

Interactions between cyanobacterial clock proteins KaiA and KaiC revealed by ESR analysis

Kentaro Ishii[1], Toshiaki Arata[2], Masahiro Ishiura[1] ([1]*Center for gene research, Nagoya univ.*, [2]*Grad. Sch. Sci., Osaka Univ.*)

3P041 **黄色ブドウ球菌の Isd-NEAT ドメイン間におけるヘム輸送についての考察**
Insights into the mechanism of heme-transfer between Isd NEAT domains of Staphylococcus aureus
Yoshitaka Moriwaki[1], Tohru Terada[2], Jose M. M. Caaveiro[3], Yousuke Takaoka[4], Itaru Hamachi[4], Kouhei Tsumoto[5], Kentaro Shimizu[1] ([1]*Dept. of Biotech., Grad. Sch. of Agri. and Life Sci., Univ. of Tokyo*, [2]*Agri. Bioinfo. Res. Unit, Grad. Sch. of Agri. and Life Sci., Univ. of Tokyo*, [3]*Ins. of Med. Sci., Univ. of Tokyo*, [4]*Dept. of Synth. Chem. and Biol. Chem., Kyoto Univ.*, [5]*Dept. of Bioeng., Grad. Sch. of Eng., Univ. of Tokyo*)

3P042 **Hsp90 と ADP の解離過程における自由エネルギープロフィールと解離経路**
Free energy profile and dissociation pathway in the dissociation process of ADP from Hsp90
Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Intsitute of Science and Engineering, Kanazawa University*)

3P043 **Mechanism of glycan receptor recognition for influenza virus Hemagglutinins: Comparative molecular dynamis studies**
Katsumi Omagari (*Department of Virology, Medical School, Nagoya City University*)

3P044 **Free energy landscape of substrate passing inside proteasome - activator complex**
Hisashi Ishida (*Japan Atomic Energy Agency*)

3P045 **MD シミュレーションを用いた CD44 のヒアルロン酸結合による構造変化に関する研究**
Molecular dynamics simulation study on hyaluronan induced structural change of CD44
Saki Hongo[1], Yoshifumi Fukunishi[2], Masami Lintuluoto[1] ([1]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Natl. Instit. of Adv. Indust. Sci. and Technol.*)

3P046 **代謝型グルタミン酸受容体の活性化過程の動的モデルの構築**
Dynamical modeling of the activation process of metabotropic glutamate receptor
Kaita Fujihara, Tatsuki Negami, Tohru Terada, Kentaro Shimizu (*Dept. of Biotech., Grad Sch. of Agri. Life Sci., Univ. of Tokyo*)

3P047 **Mutation studies on the mammalian and the baterial XORs with inhibitors**
Hiroto Kikuchi[1], Hiroshi Fujisaki[1], Tadaomi Furuta[2], Ken Okamoto[3], Takeshi Nishino[4] ([1]*Dept. of Phys., Nippon Med. Sch.*, [2]*Center for Biol. Resources and Info., Tokyo Inst. Tech.*, [3]*Dept. of Biochem. and Mol. Biol., Nippon Med. Sch.*, [4]*Grad. Sch. Agri. and Life Sci.*)

3P048 **Computational studies of mutational effects on nylon degrading enzyme**
Takeshi Baba[1], Katsumasa Kamiya[2], Toru Matsui[3], Masayoshi Nakano[1], Seiji Negoro[4], Boero Mauro[5], Yasuteru Shigeta[1,6] ([1]*Grad. Sch. of Eng. Sci., Osaka Univ.*, [2]*Grad. Sch. of Pure and Applied Sci., Univ. of Tsukuba*, [3]*Adv. Ins. for Comp. Sci., RIKEN*, [4]*Grad. Sch. of Eng., Univ. Hyogo*, [5]*Univ. Strasbourg*, [6]*CREST*)

3P049 **糖鎖の構造多形予測に向けた CHARMM 力場の改良**
Revised CHARMM carbohydrate force field for improved description of conformational diversity of N-glycans
Suyong Re[1], Shigehisa Watabe[2], Wataru Nishima[1], Yuji Sugita[1] ([1]*Wako Inst., Riken*, [2]*Grad. Sch. Sci. Eng., Chuo Univ.*)

3P050 **Structural insights into enzyme-bound flavin adenine dinucleotides (FAD)**
Gopi Kuppuraj[1], Fumiko Suzuki[1], Masahiko Ikeuchi[2], Kei Yura[1] ([1]*Centre for Informational Biology, Ochanomizu University, Bunkyo, Tokyo*, [2]*Department of Life Sciences (Biology), University of Tokyo, Komaba, Meguro, Tokyo*)

01C. 蛋白質：物性 / 01C. Protein: Property

3P051 **金属結合によるヒトプリオンペプチドの配位モード**
Coordination mode in human prion peptide caused by metal binding
Kazuya Iwama, Masahiro Yagi, Haruto Onda, Wakako Hiraoka (*Graduate School and Technology, Meiji University*)

3P052 **二次元蛍光寿命相関分光法による BdpA 変性状態における構造ダイナミクスの解析**
Two-dimensional fluorescence lifetime correlation spectroscopy on the conformational dynamics of the unfolded state of BdpA
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*)

3P053 **タンパク質中のトリプトファン残基の近紫外円二色性と紫外共鳴ラマンスペクトルの特性**
Some basic properties of near-UV circular dichroism and UV resonance Raman spectra of tryptophan residues in proteins
Shigenori Nagatomo[1], Masako Nagai[2], Takashi Ogura[3], Teizo Kitagawa[3] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*Res. Center Micro-Nano Tech., Hosei Univ.*, [3]*Grad. Sch. Life. Sci., Univ. Hyogo*)

3P054 **テラヘルツ時間領域分光法によるトレハロースにコートされたタンパク質の低振動ダイナミクス**
Low-frequency dynamics of Trehalose-coated Lysozyme studied by terahertz time-domain spectroscopy
Risa Okada[1], Naoki Yamamoto[2], Atsuo Tamura[1], Keisuke Tominaga[1,2] ([1]*Grad. Sch. Sci., Univ. Kobe*, [2]*Molecular Photoscience Research Center, Univ. Kobe*)

3P055 **シクロデキストリン+タンパク質+メチルオレンジ・ヨウ素系における包接機構**
Inclusion mechanism of cyclodextrin for protein in methyl orange and iodine aqueous solution
Tomokadu Marutani, Takayoshi Kimura, Tadashi Kamiyama (*Fac.Science,Kinki Univ.*)

3P056 **タンパク質の熱変性における部分比容、断熱圧縮率、熱膨張率**
Partial specific volume, adiabatic compressibility, and thermal expansion coefficient of protein for thermal denaturation
Tetsurou Takaoka, Takuya Hamada, Takayoshi Kimura, Tadashi Kamiyama (*Fac. Science, Kinki 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 蛋白質構造安定性における溶媒エントロピー効果―蛋白質-溶媒間多体相関の重要性―

Solvent-Entropy Effect in Structural Stability of a Protein: Crucial Importance of Protein-Solvent Many-Body Correlation

Hiraku Oshima[1], Shota Murakami[2], Masahiro Kinoshita[1] ([1]*Inst. Adv. Energ., Kyoto Univ.*, [2]*Grad. Sch. Energ. Sci., Kyoto Univ.*)

3P059 天然タンパク質の鎖長と分子サイズのスケーリング関係についての包括的解析

Comprehensive analysis of the scaling relationship between the chain length and the molecular size of native proteins

Daisuke Takahashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

3P060 複雑なトポロジーを持つタンパク質のフォールディング経路ネットワーク

Network of folding pathways of topologically complex proteins

Takashi Inanami, Masaki Sasai (*Dept. of Comp. Sci. Eng., Univ. of Nagoya*)

3P061 天然変性蛋白質の立体構造特性に関わるリン酸化の静電的な制御

Phosphorylation as an electrostatic regulation of the conformational state of intrinsically disordered proteins

Koji Umezawa[1], Jun Ohnuki[1], Yukinobu Mizuhara[1], Junichi Higo[2], Mitsunori Takano[1] ([1]*Grad. Scl. of Adv. Sci. & Eng., Waseda Univ.*, [2]*IPR, Osaka Univ.*)

3P062 ウマ β ラクトグロブリン初期中間体における非天然ヘリックスのフォールディングキネティクスへの影響

Effect of non-native α-helix in the early intermediate on folding kinetics of equine β-lactoglobulin

Takahiro Okabe, Toshiaki Miyajima, Hideaki Ohtomo, Mio Ohtomo, Kanako Nakagawa, Seiichi Tsukamoto, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. of Bioinformatics, Soka Univ.*)

3P063 天然条件下における PCP 各残基アンフォールディング速度の観測ー尿素によるアンフォールディングの促進機構

Observation of unfolding rates of each residue of PCP under the native condition - Mechanism for urea to accelerate the unfolding rate

Shinya Fujii[1], Yasuo Noda[1], Katsuhide Yutani[2], Shin-ichi Segawa[1] ([1]*Sch. of Sci. and Tech., Kwansei Gakuin Univ.*, [2]*Riken SPring-8 Center, Riken Harima Institute.*)

3P064 高速溶液混合法を用いたアポミオグロビンの salt-induced 中間体のフォールディングに関する研究

Folding of salt-induced intermediate of apomyoglobin using ultrarapid mixing methods

Yukiko Abe, Takuya Mizukami, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ.*)

3P065 変異体解析を用いた緑色蛍光蛋白質のフォールディング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 in folding mechanism of green fluorescent protein studied by mutagenesis approach

Taichi Andou, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ.*)

3P066 スタフィロコッカル・ヌクレアーゼの安定性とフォールディング/アンフォールディングの研究

The Stability and Folding/Unfolding of Staphylococcal Nuclease at the Residue Level

Shun Terauchi[1], Keisuke Kamba[1], Yoshiharu Mori[2], Yoshitake Sakae[1,2], Takashi Nakamura[2,3], Yuko Okamoto[1,4,5], Kunihiro Kuwajima[2,3,6], Kosuke Maki[1] ([1]*Sch. of Sci., Nagoya Univ.*, [2]*Inst. Mol. Sci.*, [3]*Okazaki Inst. Integr. Biosci.*, [4]*Struct. Biol. Res. Center, Sch. of Sci., Nagoya Univ.*, [5]*Center for Comput. Sci., Sch. of Eng., Nagoya Univ.*, [6]*Sch. of Phys. Sci., Grad. Univ. Adv. Studies*)

3P067 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

Analyses of amyloid fibrillation mechanism and its inhibition effect of hCT as studied by ^{13}C solid-state NMR and TEM

Hikari Itoh-Watanabe[1], Miya Kamihira-Ishijima[2], Izuru Kawamura[1], Masashi Kondoh[3], Michio Sato[3], Masamichi Nakakoshi[3], Akira Naito[1] ([1]*Graduate School of Engineering, Yokohama National University, Yokohama,*, [2]*Institute of Multidisciplinary Research, Tohoku University, Sendai, Japan.*, [3]*Instrumental Analysis Center, Yokohama National University, Yokohama, Japan.*)

3P068 β_2 ミクログロブリンのアミロイド幹形成領域のスキャン探索

Scanning survey for amyloid-stem-forming region of β_2-microglobulin

Hisayuki Morii[1], Takashi Shimizu[1], Masayuki Nara[2] ([1]*National Institute of Advanced Industrial Science and Technology (AIST)*, [2]*College of Liberal Arts and Sciences, Tokyo Medical and Dental University*)

3P069 ヒトカルシトニンの酸性膜存在下でのアミロイド線維形成機構の解明

Amyloid-like fibrillization and the structure of human calcitonin in the presence of acidic lipids

Akira Asano[1], Yuki Abe[1], Ken Takeuchi[1], Miya Kamihira-Ishijima[2], Hikari Itoh-Watanabe[1], Izuru Kawamura[1], Ayyalusamy Ramamoorthy[3], Akira Naito[1] ([1]*Graduate School of Engineering, Yokohama National University*, [2]*Graduate School of Life Science, University of Hyogo*, [3]*Biophysics and Department of Chemistry, University of Michigan*)

3P070 インスリン B 鎖に見られる多様なアミロイド線維前駆中間体の観察

Observation of various types of amyloid prefibrillar intermediates of insulin B chain

Shoko Tsuhara, Eri Chatani (*Grad.Sch.of Sci.,Kobe.Univ*)

3P071 金属イオン配位によるインスリンアミロイド線維の多形誘導効果

Polymorphism of insulin amyloid fibrils induced by the coordination of metal ions

Misaki Yokoyama, Yoshito Huruie, Motonari Tubaki, Hiroshi Hori, Eri Tyatani (*Grad.Sch.of Sci.,Kobe Univ*)

3P072 **Exploring roles of water molecules on amyloid fibrillation by salt effects and Near Infrared spectroscopy**

Yuuki Masuda[1], Yutaro Tsuchisaka[2], Roumiana Tsenkova[2], Eri Chatani[1] ([1]*Graduate school of science, Kobe University*, [2]*Graduate school of Agricultural Science, Kobe University*)

01D. 蛋白質：機能 / 01D. Protein: Function

3P073 **タンパク質翻訳と共役した分子シャペロン動態の１分子蛍光イメージング**
Single-molecule fluorescence imaging of translationally-coupled chaperone action
Tatsuya Niwa[1], Hisashi Tadakuma[2], Koichi Ito[2], Takuya Ueda[2], Hideki Taguchi[1] ([1]*Grad. Sch. of Biosci&Biotech, Tokyo Institute of Technology*, [2]*Grad. Sch. of Frontier Sciences, University of Tokyo*)

3P074 **一分子蛍光法によるリポアミド脱水素酵素の作用特性の解析**
Enzymatic reaction of Dihydrolipoamide dehydrogenase revealed by single molecular fluorescence detection method
Hiromichi Nakashima[1], Tsukasa Oyakawa[1], Etsuko Nishimoto[2] ([1]*Institute of Biophysics, Faculty of Agriculture, Graduate School of Kyushu University*, [2]*Molecular Biosciences, Bioscience and Biotechnology, Kyushu University*)

3P075 **ウマ L フェリチンサブユニットへの鉄酸化活性部位の導入**
Insertion of ferroxidase center in horse L ferritin subunit
Mai Nemiti, Tomoaki Harada, Hideyuki Yoshimura (*Sch of Sci. & Tech., Meiji Univ.*)

3P076 **デザインペプチドによる脂肪滴とアミロイド線維の加水分解**
Hydrolysis of lipid droplets and amyloid fibrils by the designed peptide
Yoshihiro Iida, Atsuo Tamura (*Kobe University*)

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

3P077 **タンパク質分解酵素の速度論的安定性の熱測定による評価方法**
Calorimetric method to evaluate the kinetic stability of proteases
Shun-ichi Kidokoro, Akihiro Nagata, Keita Ochi (*Dept. Bioengineer., Nagaoka Univ. Tech.*)

3P078 **タウタンパク質に対する Pin1 のプロリン異性化活性を測定するための新しい方法**
A novel method to measure Pin1's peptidyl-prolyl isomerase activity for tau protein
Teikichi Ikura, Nobutoshi Ito (*Med. Res. Inst., Tokyo Med. Dent. Univ.*)

3P079 **New highly accurate pickup methods, MRA-StoPICK and MRMA-StoPICK methods, for single particle analysis using electron microscope**
Masaaki Kawata, Chikara Sato (*National Institute of Advanced Industrial Science and Technology*)

3P080 **積分方程式理論に基づく X 線小角散乱データを用いた蛋白質間相互作用の解析**
An integral equation approach for protein interactions using small-angle X-ray scattering data
Tomonari Sumi[1], Hiroshi Imamura[2], Keiko Nishikawa[2] ([1]*Dept. Chem., Fac. Sci., Okayama Univ.*, [2]*Grad. Sch. Adv. Integ. Sci., Chiba Univ.*)

3P081 **静的光散乱法による水溶性タンパク質の分子量の測定**
Measurements of molecular weights of soluble proteins using static light scattering
Ken Takeuchi, Youichi Nakatani, Osamu Hisatomi (*Department of Earth and Space Science, Graduate School of Science Osaka University*)

3P082 **タンパク質超高感度測定法の開発：ELISA 法と酵素サイクリング法との組み合わせの試み**
Development of super high-sensitive measurement of proteins by combination of ELISA and enzyme cycling methods
Etsuro Ito (*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University*)

3P083 **Single-molecule investigation of the force required to release SecM-mediated translation arrest**
Zhuohao Yang, Ryo Iizuka, Takashi Funatsu (*Grad. Sch. of Pharm. Sci., The Univ. of Tokyo*)

3P084 **High-Speed AFM Observation of the FliI/ FliJ Complex**
David Carriou[1], Takayuki Uchihashi[1,2], Yumiko Uchida[3], Hiroto Yanagawa[3], Tohru Minamino[4], Katsumi Imada[3], Toshio Ando[1,2] ([1]*Dept. Phys., Kanazawa Univ.*, [2]*Bio-AFM Frontier Research Center, Kanazawa Univ.*, [3]*Grad. Sch. Sci., Osaka Univ.*, [4]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

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

3P085 **穏やかな pH で抗体精製するための新規アフィニティーリガンドの開発**
Development of a novel affinity ligand for purification of antibodies at moderate pH
Taihei Sawada[1], Takaihiro Watanabe[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dep. Life Sci., Univ. Tokyo,*, [2]*PRESTO, JST*)

3P086 **アルデヒドデカルボニラーゼによるバイオアルカン生産に向けたシステイン置換体の開発**
Toward the development of cysteine-free variants of aldehyde decarbonylase for industrial bioalkane production
Yuuki Hayashi[1], Fumitaka Yasugi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

3P087 **LOV を鋳型とした酸化還元感受性タンパク質の蛍光特性**
Redox-controlled fluorescence from LOV-based proteins
Yukiko Ono, Tatsuya Iwata, Masayo Iwaki, Hideki Kandori (*Nagoya Inst. Of Technol.*)

3P088 **Addition of negatively charged residues can reverse the aggregation of a protein caused by an artificially introduced hydrophobic surface**
Sota Yagi, Satoshi Akanuma, Akihiko Yamagishi (*Tokyo university of pharmacy and life science*)

3P089 **総電荷の異なるフェリチン変異体の作製と特徴付け**
Construction and characterization of ferritin mutants having different net charges
Satsuki Takebe, Eriko Aoki, Daisuke Sato, Kazuo Fujiwara, Masamichi Ikeguchi (*Soka University*)

3P090 **人工 4 ヘリックスバンドルタンパク質上に白金結合ループを創出**
Creation of a platinum-binding loop on an artificial four-helix bundle protein
Hiroya Niiro, Satoshi Akanuma, Akihiko Yamagishi, Yuuto Akiyama, Tatuya Uchida (*Tokyo University of Pharmacy and Life Sciences*)

3P091 **アクチン発現系の確立に向けて**

Toward the establishment of an expression system for actin

Masashi Mori[1], Yoshitaka Umetsu[2], Shinya Ohki[2] ([1]*Ishikawa Prefectural University*, [2]*Japan Advanced Institute of Science and Technology*)

02. ヘム蛋白質 / 02. Heme proteins

3P092 **インドールアミン 2,3 ジオキシゲナーゼの基質トリプトファンの検出-紫外共鳴ラマン分光法**

Detection of the bound tryptophan in indoleamine 2,3-dioxygenase by UV resonance Raman spectroscopy

Sachiko Yanagisawa[1], Masayuki Hara[1], Hiroshi Sugimoto[2], Yoshitsugu Shiro[2], Takashi Ogura[1] ([1]*Univ. of Hyogo*, [2]*RIKEN Harima SPring-8 center*)

3P093 **Interaction Between Heme and Heme-Cu Binuclear Center in Cytochrome *c* Oxidase**

Miyuki Sakaguchi[1], Kyoko Shinzawa-Itoh[2], Shinya Yoshikawa[2], Takashi Ogura[1] ([1]*Department of Protein Vibrational Spectroscopy, Picobiology Institute, University of Hyogo*, [2]*Department of Protein Crystal Growth Mechanism, Picobiology Institute, University of Hyogo*)

3P094 **チトクロム *c* 酸化酵素の酸素還元反応における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 the flow system with an oxygen lung aiming at IR measurement on the oxygen reduction reaction of cytochrome *c* oxidase

Tatsuhito Nishiguchi, Masahide Hikita, Kyoko Shinzawa-Itoh, Shinya Yoshikawa, Satoru Nakashima, Takashi Ogura (*Grad. Sch. Lif. Sci., Univ. Hyogo*)

3P095 **シアン化物・アジ化物結合完全酸化型ウシ心筋チトクロム酸化酵素の構造解析**

Structural analysis of bovine heart cytochrome *c* oxidase in the cyanide- and azide-bound fully oxidized states

Kazumasa Muramoto[1], Masao Mochizuki[1], Naomine Yano[1], Tomoko Maeda[1], Kyoko Shinzawa-Itoh[1], Eiki Yamashita[2], Tomitake Tsukihara[1,2], **Shinya Yoshikawa**[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*Inst. Protein Res., Osaka Univ.*)

3P096 **Sequencing bovine/human hybrid cytochrome c oxidase genes in HeLa cells to verify mutagenesis results disapproving D-path proton pumping**

Ryohta Aminaka[1], Mai Itoh[1], Kunitoshi Shimokata[2], Yukie Katayama[1], Tomitake Tsukihara[1], Shinya Yoshikawa[1], Hideo Shimada[1] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*WORLD INTEC CO., LTD.*)

3P097 **完全酸化型チトクロム *c* 酸化酵素の酸化還元活性金属中心とアザイドの相互作用の分光学的研究**

Spectroscopic characterization of the interaction of azide with the redox-active metal sites of fully oxidized cytochrome *c* oxidase

Masahide Hikita, Akima Yamamoto, Tomoko Maeda, Kyoko Shinzawa-Itoh, Takashi Ogura, Shinya Yoshikawa (*Grad. Sch. Sci., Univ. Hyogo*)

3P098 **一酸化炭素・シアン化物結合混合原子価型ウシ心筋チトクロム酸化酵素の構造解析**

Structural analysis of bovine heart cytochrome *c* oxidase in the CO- and cyanide-bound mixed valence states

Kazumasa Muramoto[1], Masao Mochizuki[1], Maki Taniguchi[1], Naomine Yano[1], Tomoko Maeda[1], Kyoko Shinzawa-Itoh[1], Eiki Yamashita[2], Tomitake Tsukihara[1,2], Shinya Yoshikawa[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*Inst. Protein Res., Osaka Univ.*)

03. 膜蛋白質 / 03. Membrane proteins

3P099 **人工設計膜貫通ペプチドを用いたシグナル伝達モデル系の構築**

Design of transmembrane peptide for constructing a signaling model

Takato Hiramatsu, Atsuo Tamura (*Grad. Sch. Sci. chem, Univ. Kobe*)

3P100 **好熱菌 F_0F_1-ATP 合成酵素 *c* サブユニットリングの活性部位の構造**

The Active-Site Structure of Thermophilic F_0F_1-ATP Synthase *c*-Subunit Rings in Membranes

Su-Jin Kang[2], **Yasuto Todokoro**[1,5], Ikuko Yumen[1], Bo Shen[1], Iku Iwasaki[2], Toshiharu Suzuki[3,4], Atsushi Miyagi[1], Masasuke Yoshida[3,4], Toshimichi Fujiwara[1], Hideo Akutsu[1,2] ([1]*IPR, Osaka Univ.*, [2]*BpCB, Seoul Nat. Univ.*, [3]*Chem. Res. Lab., Tokyo Inst. Tech.*, [4]*Dep. Mol. Biosci., Kyoto San. Univ.*, [5]*Grad. Sch. Sci., Osaka Univ.*)

3P101 **Analysis of Structure and Function of Synaptotagmin 4**

Masayuki Goto (*Tsukuba, Material Sci,*)

3P102 **擬環状リン脂質リポソーム中のバクテリオロドプシンの構造と機能**

A Biophysical Study of Bacteriorhodopsin in Pseudocyclic Phosphatidylcholine Liposome

Masashi Sonoyama[1], So Yoshioka[1], Naoyuki Tsuchida[1], Toshiyuki Takagi[2], Hiroshi Takahashi[1], Takashi Kikukawa[3], Toshiyuki Kanamori[2] ([1]*Fac. Sci. Tech., Gunma Univ.*, [2]*R. C. Stem Cell, AIST*, [3]*Fac. Adv. Sci., Hokkaido Univ.*)

3P103 **結晶化を目指したカイコガ性フェロモン生合成活性化神経ペプチド受容体（PBANR）の細胞内第 3 ループへの T4 リゾチーム置換位置の検討**

Positional optimization of the T4 lysozyme replacing the third intracellular loop of the silkmoth PBANR for its crystallization

Yukie Katayama[1], Takeshi Kawai[1], Tatsuya Suzuki[1], Tatsuki Ebisawa[1], Jun Ohtsuka[1], Ryo Natsume[2], Yu-Hua Lo[2], Toshiya Senda[2], Toshihiro Nagamine[3], Masaaki Kurihara[3], Jae Min Lee[3], J. Joe Hull[4], Shogo Matsumoto[3], Hiromichi Nagasawa[1], Koji Nagata[1], Masaru Tanokura[1] ([1]*Univ. of Tokyo*, [2]*BRIC, AIST*, [3]*RIKEN*, [4]*USDA-ARS*)

3P104 **膜貫通ヘリックスの膜内配向決定機構の粗視化分子動力学シミュレーションによる探索**

Coarse grained molecular dynamics simulations toward the mechanism elucidation of membrane protein topogenesis

Kouya Sakuma, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

3P105 **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**
Effects of homotrimer formation on chloride pump activity in membrane mimetics, Nanodisc, embedded Halorhodopsin
Kenshiro Suzuki[1], Ayumi Yamamoto[1], Takashi Tsukamoto[2], Toshihiro 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. Hokkaido Univ.*)

3P106 **インテグリンと FAK を含む短寿命多分子複合体ラフトが GPI アンカー型受容体の IP3 シグナルを誘起するプラットフォームとなる**
Transient raft-dependent multimolecular complexes including integrin and FAK are the platforms for IP3 signaling of GPI-anchored receptors
Taka A. Tsunoyama[1], Kenichi G.N. Suzuki[1,2], Takahiro K. Fujiwara[1], Akihiro Kusumi[1,3] ([1]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*, [2]*National Centre for Biological Science/ Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India*, [3]*Institute for Frontier Medical Sciences, Kyoto University*)

3P107 **GPCR ダイマーがシグナルトリガーとしてはたらく：インバースアゴニスト効果の 1 分子イメジング解析に基づく発見**
GPCR dimers as active signal triggers: inverse agonist effects revealed by single-molecule imaging analysis
Rinshi Kasai[1], Takahiro Fujiwara[2], Akihiro Kusumi[1,2] ([1]*Inst. For Frontier Med. Sci., Kyoto Univ.*, [2]*WPI-iCeMS, Kyoto Univ.*)

3P108 **生体分子複合体を通した多剤排出の物理に関して**
On the Physics of Multidrug Efflux through a Biomolecular Complex
Hirokazu Mishima[1], Hiraku Oshima[2], Satoshi Yasuda[2], Ken-ichi Amano[3], Masahiro Kinoshita[2] ([1]*Grad. Sch. Ene., Univ. Kyoto*, [2]*Inst. Adv. Ene., Univ. Kyoto*, [3]*Pharm., Univ. Tohoku*)

3P109 **X 線結晶構造の決定に向けた膜タンパク質構造安定性の理論的向上**
Theoretical Enhancement of Structural Stability of a Membrane Protein for X-ray Crystallography
Satoshi Yasuda[1], Hiraku Oshima[1], Takeshi Murata[2], Masahiro Kinoshita[1] ([1]*Institute of Advanced Energy, Kyoto Univ.*, [2]*Department of Chemistry, Graduate School of Science, Chiba Univ.*)

3P110 **Ca^{2+}結合部位のプロトン状態変化による SERCA の構造変化**
Conformational change of SERCA upon alternating protonation states in Ca^{2+}-binding site
Chigusa Kobayashi[1], Yuji Sugita[2,3] ([1]*RIKEN, AICS*, [2]*RIKEN, QBiC*, [3]*RIKEN, TMS*)

3P111 **改良した ATP 分子力場を用いた筋小胞体カルシウムポンプの分子動力学計算**
Molecular dynamics simulations of SR Ca^{2+}-ATPase using improved ATP force field
Yasuaki Komuro[1,2], Chigusa Kobayashi[3], Suyong Re[2], Eiro Muneyuki[1], Yuji Sugita[2,3,4] ([1]*Chuo Univ., Dept. Phys.*, [2]*RIKEN*, [3]*RIKEN AICS*, [4]*RIKEN QBiC*)

3P112 **アミロイド前駆体タンパク質とコレステロールとの相互作用**
Interaction between cholesterol and transmembrane region of Amyloid Precursor Protein
Naoyuki Miyashita[1,2], Fumiko Ogushi[3], Yuji Sugita[1,2,4] ([1]*RIKEN QBiC*, [2]*RIKEN AICS*, [3]*Ochanomizu University*, [4]*RIKEN*)

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

3P113 **LRH-1 の beta-catenin による転写活性化の構造基盤**
Structural basis of transcriptional co-activation of LRH-1 by beta-catenin
Fumiaki Yumoto[1,2], Robert Fletterick[2] ([1]*KEK Structural Biology Research Center*, [2]*University of California, San Francisco*)

3P114 **RNA アプタマーとプリオン蛋白質部分ペプチドの結合の統計熱力学**
Statistical Thermodynamics for Binding of an RNA Aptamer and a Partial Peptide of a Prion Protein
Tomohiko Hayashi, Hiraku Oshima, Tsukasa Mashima, Takashi Nagata, Masato Katahira, Masahiro Kinoshita (*Institute of Advanced Energy, Kyoto Univ.*)

3P116 **The coarse grained GBSA method for simulations of biomolecular system**
Le Chang[1], Wenfei Li[2], Naoto Hori[1], Shoji Takada[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Dept. Phys., Nanjing Univ.*)

3P117 **DNA 結合蛋白質はどのようにして障害物を回避するか。分子シミュレーションによるアプローチ**
How DNA-binding proteins can bypass obstacles? Molecular simulation approaches
Mami Saito, Tsuyoshi Terakawa, Shoji Takada (*Grad.Sch.Sci, Kyoto Uni.*)

3P118 **転写因子 p53 の特異的結合部位探索・認識機構：マルチスケールシミュレーション研究**
Specific DNA sequence search and recognition mechanism of transcription factor p53: multi-scale simulation study
Tsuyoshi Terakawa[1], Junichi Higo[2], Shoji Takada[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Inst. Protein Res., Osaka Univ.*)

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

3P119 **DNA 塩基対の安定性に及ぼすコリンイオンの効果の分子動力学計算による解析**
Analysis for the effect of choline ions on the stability of DNA base pairs using molecular dynamics simulation
Miki Nakano[1], Hisae Tateishi-Karimata[1], Naoki Sugimoto[1,2] ([1]*Konan Univ. FIBER*, [2]*Konan Univ. FIRST*)

3P120 **粗視化分子動力学シミュレーションによる一本鎖 DNA 領域形成機構の駆動力の解明**
DNA unwinding mechanisms in *E. coli, oriC* region studied by coarse grained molecular dynamics simulations
Masahiro Shimizu, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

3P121 **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ダイナミクス**

Poly-nucleosome structural dynamics by coarse-grained simulations

Hiroo Kenzaki[1], Shoji Takada[2] ([1]*Advanced Center for Computer and Communications, RIKEN*, [2]*Dept. of Biophysics, Graduate School of Science, Kyoto Univ.*)

3P122 **長鎖 DNA 分子内折り畳みは高分子電解質の鎖長に依存して２つのモードを示す**

Two-mode Folding of a Single Giant Duplex DNA Chain Depneding on the Length of Cationic Polymer

Tatsuo Akitaya[1], Naomi Tsumura[1], Hiroyuki Mayama[1], Norio Hazemoto[2], Toshio Kanbe[3], Makoto Demura[4], Anatoly Zinchenko[5], Shizuaki Murata[5], Kenichi Yohikawa[6] ([1]*Dept. Chem., Asahiakwa Med. Univ.*, [2]*Grad. Sch. Pharm. Sci., Nagoya City Univ.*, [3]*Sch. Med., Nagoya Univ.*, [4]*Grad. Sch. Life Sci., Hokkaido Univ.*, [5]*Grad. Sch. Env. Study, Nagoya Univ.*, [6]*Grad. Sch. Life Med. Sci., Doshisha Univ.*)

3P123 **Fleeting secondary structure effects on hybridization kinetics**

Hiroaki Hata[1], Akira Suyama[1,2] ([1]*Grad. Sch. Sci., Univ. Tokyo*, [2]*Grad. Sch. Arts and Sci., Univ. Tokyo*)

3P124 **光応答性分子ロボット構築のための DNA マイクロカプセルの設計と作製**

Design and construction of a DNA microcapsule toward light-responsive molecular robots

Yuichi Tsuganezawa[1], Masamune Morita[1], Shogo Hamada[2], Shin-ichiro M. Nomura[3], Kenzo Fujimoto[4], Satoshi Murata[3], Masahiro Takinoue[1,5] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Kavli Inst., Cornell Univ.*, [3]*Grad. Sch. Eng., Tohoku Univ.*, [4]*Sch. Mater. Sci., JAIST*, [5]*PRESTO,JST*)

3P125 **モレキュラークラウディング環境における化学修飾を施した 2 本鎖核酸の熱力学的安定性**

Effect of molecular crowding condition on the thermodynamic stability of chemically modified duplex

Hidetaka Torigoe, Hiroshi Noguchi, Yukiko Hashizume (*Dept. Appl. Chem., Fac. Sci., Tokyo Univ. Sci.*)

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

3P126 **Development of new algolithm for calculation of the energy distribution function by GPGPU**

Takuhito Shiogama, Tsuhito Yoshida, Hiroaki Saito, Kazutomo Kawaguchi, Hidemi Nagao (*Kanazawa University*)

3P127 **浸透圧効果を利用したシトクロム c – シトクロム c 酸化酵素電子伝達複合体における相互作用の解析**

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[1,2], Kyoko Ito[3], Shinya Yoshikawa[3], Koichiro Ishimori[1,2] ([1]*Hokkaido Univ. Chem.*, [2]*Hokkaido Univ. Sci.*, [3]*Hyogo Univ. Life Sci.*)

3P128 **蛋白質およびリガンドの水和熱力学量計算に向けた形態計測法的アプローチ**

A Morphometric Approach for the Accurate Solvation Thermodynamics of Proteins and Ligands

Yuichi Harano[1], Roland Roth[2], Shuntaro Chiba[3] ([1]*IPR, Osaka Univ.*, [2]*Inst. Theo. Phys. Tuebingen Univ.*, [3]*Bio Center, TITECH*)

3P129 **溶質-溶媒間のレナードジョンズポテンシャルパラメタが溶媒和ダイナミクスに及ぼす影響**

Effects of Lennard-Jones potential parameters between the solute and solvent on the solvation dynamics

Yoshito Kondo, Tetsuro Nagai, Takuya Takahashi (*College of Life Sciences, Ritsumeikan Univ*)

3P130 **MD および QM 計算による水和水のダイナミクスと電荷計算**

MD and QM calculations of dynamics and charges of hydration water

Takuya Takahashi (*College of Life Sciences, Ritsumeikan University*)

09. 発生・分化 / 09. Development & Differentation

3P131 **カタユウレイボヤ未受精卵に一過的低張刺激を与えると、幼生での左右非対称性が乱れる**

Brief hypo-osmotic treatment on eggs disrupts the left-right asymmetry of the larvae in Ciona intestinalis

Shimpei Katsumoto, Kohei Hatta, **Masashi Nakagawa** (*Grad. Sch. Sci., Univ. Hyogo*)

3P132 **How To Achieve Sequential Local Folding of Epithelial Tube in Epididymis Development: Experimental and Mathematical Study**

Tsuyoshi Hirashima, Ryoichiro Kageyama (*Inst. for Virus Research, Kyoto Univ.*)

3P133 **アフリカツメガエル卵母細胞における全 ATP 量の測定と ATP ライブイメージング**

ATP quantification and live-imaging in *Xenopus laevis* oocyte

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.*)

10. 筋肉 / 10. Muscle

3P134 **ミオシンの金電極表面への吸着過程の粘弾性解析**

Viscoelastic analysis of myosin adsorbed to gold

Tetsuo Ohno[1], Michiru Wagatuma[2], Motoko Ichihashi[2], Atsushi Itoh[2] ([1]*Dept. Physiology, Jikie Univ. school of Med.*, [2]*Ulvac, Inc.*)

3P135 **ホッキ貝柱筋と牽引筋の天然アクトミオシン(NAM=M+A+TM1 or TM2)の「Mg-ATPase 活性の Ca 依存性」と「TM アイソフォーム TM1 と TM2 の組成比」**

Hokki clam retractor muscle NAM Mg-ATPase activities and seasonal changes of TM1 and TM2 isoform contents

Yoichi Yazawa (*Univ. Hokkaido Education*)

3P136 **中性子及び X 線散乱による F アクチン水和水の構造・ダイナミクス解析**

Characterization of the structural and dynamic properties of hydration water around F-actin detected by neutron and X-ray scattering

Tatsuhito Matsuo[1], Toshiaki Arata[2], Toshiro Oda[3], Satoru Fujiwara[1] ([1]*QuBS, JAEA*, [2]*Grad. Sch. Sci., Osaka Univ.*, [3]*Grad. Sch. Sci., Univ. Hyogo*)

3P137 **ワタリガニ骨格筋における細いフィラメントの精製および低温電子顕微鏡法による構造解析**
Isolation of native thin filament from skeletal muscle for structural analysis by cryoEM
Yurika Yamada[1], Takashi Fujii[2], Keiichi Namba[1,2] ([1]*Graduate School of Frontier Bioscience, Osaka University*, [2]*QBiC, RIKEN*)

3P138 **マルハナバチ飛翔筋トロポニン I の長い延長部の構造的役割**
The structural role of the Pro-Ala-rich extension of the troponin-I of bumblebee flight muscle
Hiroyuki Iwamoto, Naoto Yagi (*SPring-8, JASRI*)

3P139 **塩添加によるアクチン重合過程の研究**
The salt-induced polymerization of actin
Toshiro Oda[1,2], Tomoki Aihara[2], Katsuzo Wakabayashi[2,3] ([1]*Grad. Sch. Sci., Univ. Hyogo*, [2]*RIKEN, RKEN SPring-8 Center*, [3]*Grad. Sch. Eng. Sci., Osaka university*)

11. 分子モーター / 11. Molecular motor

3P140 **自己組織化生体システムの機能創発機構解明に向けたメソスケール反応場のデザインと単分子分解能計測**
Designing of self-assembled biomolecular system and the detection at the single molecule resolution
Mitsuhiro Iwaki[1,2,3], Keigo Ikezaki[1], Toshio Yanagida[1,2], William Shih[3] ([1]*RIKEN, QBiC*, [2]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [3]*Harvard Medical School*)

3P141 **ナノスリット基板を用いたアクチンの重合の観察**
Observation of actin polymerization in linear zero-mode waveguide
Masamichi Yamamoto[1], Makoto Tsunoda[1], Shun Higano[2], Kotaro Okubo[2], Takashi Tanii[2], Takashi Funatsu[1] ([1]*Grad. Pharm. Sci., Univ. Tokyo*, [2]*Sch. Sci. Eng., Waseda. Univ.*)

3P142 **中性環境における好アルカリ性 *Bacillus* 細菌が持つ Na^+駆動型べん毛モーター固定子の遊泳低下に関与するアミノ酸残基の探索**
Critical amino acid residues for motility of the Na^+ -driven flagellar motor stator in alkaliphilic *Bacillus* decrease at neutral pH
Yuka Takahashi[1,2], Yukina Noguchi[1], Masahiro Ito[1,2] ([1]*Graduate School of Life Sciences, Toyo University*, [2]*Bio-nano Electronics Research Center, Toyo University*)

3P143 **アクトミオシン複合体におけるミオシン・サブフラグメント１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 swinging lever-arm motion of a myosin subfragmnet-1 in an actomyosin complex
Tadashi Masuda (*Fukushima Univ.*)

3P144 **Nonequilibrium dissipation-free transport of F1-ATPase and the thermodynamic role of asymmetric allosterism**
Kyogo Kawaguchi[1], Shin-ichi Sasa[2], Takahiro Sagawa[3] ([1]*Dept. Phys., Univ. Tokyo*, [2]*Dept. Phys., Kyoto Univ.*, [3]*Dept. Basic Science, Univ. Tokyo*)

3P145 **エフェクター分泌機構解明を目指した細菌Ⅲ型分泌装置の回転-分泌相関の解析**
Correlation analysis between rotation and secretion of bacterial type III secretion system for elucidate of effector secretion mechanism
Takashi Ohgita, Naoki Hayashi, Susumu Hama, Naomasa Gotoh, Kentaro Kogure (*Kyoto Pharm. Univ.*)

3P146 **Simultaneous tracking of multiple motor proteins in nanoscale**
Taishi Kakizuka[1], Keigo Ikezaki[2], Hideaki Fujita[3], Taro Ichimura[2], Tomonobu Watanabe[1,2] ([1]*Grad. Sch. FBS., Univ. Osaka*, [2]*QBiC, Riken*, [3]*WPI,iFReC, Univ. Osaka*)

3P147 **クライオ電子顕微鏡法を用いたアクチンミオシン硬直複合体の高分解能密度マップ取得への試み**
Approach to obtain near-atomic resolution map of actin-myosin rigor complex by cryo-EM
Norihiro Shimizu[1], Yoshihiro Tsukada[1], Takuo Yasunaga[1,2] ([1]*Kyushu Inst. of Tech.*, [2]*JST*)

3P148 **cryo-EM と MT ラベルを用いたクラミドモナス外腕ダイニン LC4 の位置決定**
Determination of the location of chlamydomonas outer arm dynein LC4 by cryo-EM and metallothionein labelling
Reiko Chijimatsu[1], Haruaki Yanagisawa[2], Mingyue Jin[1,3,4,5], Takuo Yasunaga[1,3,4,5] ([1]*Kyushu Institute of Technology*, [2]*The University of Tokyo*, [3]*JST*, [4]*JST CREST*, [5]*JST SENTAN*)

3P149 **ヒト遺伝性難聴(DFNA20/26)γ アクチン変異体とミオシンとの相互作用**
Effects of human deafness mutations in gamma actin (DFNA20/26) on the actin/myosin interaction
Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Department of Physics, Faculty of Science and Engineering, Waseda University*, [2]*Waseda Bioscience Research Institute in Singapore*)

3P150 **ミオシン V 分子モーターの運動性に対する UV 照射の作用**
Effect of UV irradiation on myosin V motility
Seitaro Sano[1], Hiroaki Kubota[1], Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Department of Physics, Faculty of Science and Engineering, Waseda University*, [2]*Waseda Bioscience Research Institute in Singapore*)

3P151 **Ncd の運動方向性の決定機構**
The mechanism of determining the directionality of Ncd
Masahiko Yamagishi, Yoko Toyoshima, Junichiro Yajima (*Dept. Life Sciences, Grad. Sch. Arts and Sciences, Univ. Tokyo*)

3P152 **ダイニンは微小管上を短いピッチで回転しながら運動する**
Dynein moves in a short-pitch helical path around a microtubule
Shin Yamaguchi, Junichiro Yajima (*Dept Life Sciences, Graduate School of Arts and Sciences, Univ. of Tokyo*)

3P153 **破断力測定を用いた Kinesin-6 の力発生原理の研究**
Investigating the torque-generating mechanism of kinesin-6 using unbinding force measurement
Akihiko Sato[1], Tim Davis[2], Shin Yamaguchi[1], Masanori Mishima[2], Junichiro Yajima[1] ([1]*Grad. Sch. of Arts and Sciences, Univ. Tokyo*, [2]*Centre for Mechanochemical Cell Biology Warwick Medical School Univ. of Warwick*)

3P154 **細胞質ダイニンの自己阻害と協同的な活性化**

Autoinhibition and synergistic activation of cytoplasmic dynein

Takayuki Torisawa[1], Ken'ya Furuta[2], Akane Furuta[2], Muneyoshi Ichikawa[1], Yoko Toyoshima[1] ([1]*Dept. Life Sciences, Graduate School of Arts and Sciences, the Univ. of Tokyo*, [2]*Bio ICT lab, NICT*)

3P155 **Bicaudal-D2 による微小管系輸送の制御機構**

Regulatory mechanism of microtubule-based molecular motors by Bicaudal-D2

Takuya Kobayashi[1,2], Akira Hanashima[2], Yoko Y. Toyoshima[1], Takashi Murayama[2] ([1]*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo.*, [2]*Department of Pharmacology, Juntendo University School of Medicine.*)

3P156 **ダイナクチン p150 の分子構造**

Molecular architecture of dynactin p150

Kei Saito[1], Takashi Murayama[2], Tomonori Hata[1], **Yoko Y. Toyoshima**[1] ([1]*Grad. Sch. of Arts and Sci., Univ. of Tokyo*, [2]*Juntendo Univ.*)

3P157 **テトラヒメナ外腕ダイニン複合体のサブユニット構築**

Subunit structure of *Tetrahymena* outer dynein arm complex

Muneyoshi Ichikawa[1], Yasuharu Kushida[2], Kentaro Nakano[2], Osamu Numata[2], Yoko Toyoshima[1] ([1]*Department of Life Sciences, Graduate School of Arts & Sciences, The University of Tokyo*, [2]*Graduate School of Life and Environmental Sciences, University of Tsukuba*)

3P158 **ヒト細胞質ダイニンのパワーストローク測定**

Power Stroke Measurement of Human Cytoplasmic Dynein

Yoshimi Kinoshita, Taketoshi Kambara, Satoshi Ikeda, Hideo Higuchi (*Department of Physics, Graduate School of Science, The University of Tokyo*)

3P159 **細胞質ダイニンの生物物理学的・生化学的解析**

Biophysical and Biochemical characterization of human cytoplasmic dynein

Taketoshi Kambara, Yoshimi Kinoshita, Takayuki Nakayama, Hideo Higuchi (*Dept of Phys, Grad Sch of Sci, U of Tokyo*)

3P160 **骨格筋ミオシンの S1 および S2 部位と非線形弾性の関係性**

Contribution of S1 and S2 portion of myosin to nonlinear elasticity of skeletal myosin molecules

Satoshi Ikeda, Motoshi Kaya, Hideo Higuchi (*Department of Physics, Graduate School of Science, the University of Tokyo*)

3P161 **ミオシン V の前進および後退ステップ機構**

Mechanism of the forward and backward stepping motion of myosin V

Kazuo Sasaki[1], Hideo Higuchi[2] ([1]*Department of Applied Physics, Tohoku University*, [2]*Department of Physics, University of Tokyo*)

3P162 **Nucleotide turnover rates of bipolar myosin filament during actin filament sliding**

Takahiro Maruta, Shingo Miyazaki, Takahiro Kobatake, Shigeru Chaen (*department of Integrated Sciences in Physics and Biology, College of Humanities and Sciences, Nihon University*)

3P163 **細胞性粘菌ミオシン II の SH1 ヘリックス領域の変異がその運動特性に与える影響**

Effect of mutations in the SH1 helix region of Dictyosterium myosin II on the motile characteristics

Tsubasa Koyama[1], Takahiro Maruta[1], Kotomi Shibata[1], Ayaka Motiduki[1], Eri Umeki[1], Sousuke Iwai[1,2], Shigeru Chaen[1] ([1]*College of Humanities and sciences, Nihon University*, [2]*Department of Biology, Faculty of Education, Hirosaki University*)

3P164 **F1-ATPase β サブユニットの全原子溶媒和自由エネルギー解析**

All-atom hydration analysis of the β subunit in F1-ATPase

Toru Ekimoto[1], Mitsunori Ikeguchi[1], Nobuyuki Matubayasi[2] ([1]*Yokohama city Univercity*, [2]*Kyoto University*)

3P165 **腸球菌由来 V_1ATPase の軸強制回転シミュレーションによる回転機構の解明**

Rotation mechanism of V_1-ATPase studied by steered MD simulations

Yuta Isaka[1], Ichiro Yamato[2], Takeshi Murata[3,4], Mitsunori Ikeguchi[1] ([1]*Grad. Sch. of Med. Life Sci., Yokohama City Univ.*, [2]*Dept. Biol. Sci. Tech., Tokyo Univ. of Science*, [3]*Fac. of Sci., Chiba Univ.*, [4]*JST, PRESTO*)

3P166 **Free energy simulations for the conformational change of the αβ subunits in F_1-ATPase after the ATP hydrolysis**

Yuko Ito, Mitsunori Ikeguchi (*Yokohama City Univ.*)

3P167 **回転モーター F_1-ATPase の化学状態ごとのポテンシャルエネルギー**

The potential energy of the rotary motor F_1-ATPase for given chemical states

Kengo Adachi[1], Taisaku Ogawa[1], Kazuhiro Oiwa[2], Masasuke Yoshida[3], Kazuhiko Kinosita, Jr.[1] ([1]*Waseda Univ.*, [2]*Adv. ICT Res. Inst., NICT*, [3]*Kyoto Sangyo Univ.*)

3P168 **F_oF_1-ATP 合成酵素による ATP 駆動のプロトンポンプ活性の定量測定**

Quantitative assay of ATP-driven proton-pump activity of F_oF_1

Ken Tasaki[1], Yuzo Kasuya[1], Naoki Soga[1], Toshiharu Suzuki[2], Masasuke Yoshida[2], Kazuhiko Kinosita Jr[1] ([1]*Dept. Phys,. Facl. Sci. Eng., Waseda Univ.*, [2]*Dept. Mol. Bio., Facl. Life Sci., Kyoto Sangyo Univ.*)

3P169 **ドメイン交換による V_oV_1 の MgADP 阻害機構の解明**

Analysis of the MgADP-inhibition mechanism of V_oV_1 by domain swapping approach

Jun-ichi Kishikawa[1], Atsuko Nakanishi[1], Shou Furuike[2], Ken Yokoyama[1] ([1]*Life Sci., Kyoto Sangyo Univ.*, [2]*Dept. Phys., Osaka Med. College*)

3P170 **揺らぎの定理による V-ATPase のトルク測定：F サブユニットの働き**

F-subunit reinforces torque generation in V-ATPase

Junichi Kishikawa[1], **Akihiko Seino**[2], Atsuko Nakanishi[1], Naciye Esma Tirtom[3], Hiroyuki Noji[3], ken Yokoyama[1], Kumiko Hayashi[2] ([1]*Department of Biolmolecular Sciences, Kyoto Sangyo University*, [2]*Department of Applied Physics, School of Engineering, Tohoku University*, [3]*Department of Applied Chemistry, School of Engineering, University of Tokyo*)

3P171 **Binding interface between rotor subunits with low binding affinity in V_oV_1**
Atsuko Nakanishi, Jun-ichi Kishikawa, Ken Yokoyama (*Kyoto Sangyo University*)

12. 細胞生物的課題 / 12. Cell biology

3P172 **マイクロピラーアレイ上で成長したフィブロブラスト細胞の大きさと形状**
Size and Shape of Fibroblast Cells Growing on a Micro Pillar
Takuya Tsukagoshi, Uijin G. Jung, Hidetoshi Takahashi, Tetsuo Kan, Kiyoshi Matsumoto, Isao Shimoyama (*The University of Tokyo*)

3P173 **細胞および接着分子の極性の人為的制御**
Artificial control of the polarity of cells and molecular assemblies
Shinji Deguchi[1], Tsubasa Matsui[2] ([1]*Nagoya Institute of Technology*, [2]*Tohoku University*)

3P174 **Study on membrane microfluidity of living cells using Muller Matrix microscopy**
Yudai Kosaka, Tetsuhiko Ohba (*Grad. Sch. Sci., Univ. Tohoku*)

3P175 **負荷をかけた状態での単離マウス気管上皮シリアの三次元運動**
Three-dimensional motion of an isolated murine tracheal cilium under load
Takanobu Kato[1], Toshihito Iwase[2], Tomoko Masaike[2], Koji Ikegami[3], Mitsutoshi Setou[3], Takayuki Nishizaka[1] ([1]*Dept. Phys., Gakushuin Univ.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. of Sci.*, [3]*Dept. Cell Biol. and Anat., Hamamatsu Univ. Sch. Med.*)

3P176 **ケラトサイトと好中球と粘菌の遊走のための異なるメカノセンシング機構**
Mechanical responses of keratocytes, neutrophils and *Dictyostelium* cells for their optimal migrations
Chika Okimura[1], Takafumi Mizuno[2], Yoshiaki Iwadate[1] ([1]*Faculty of Science, Yamaguchi University*, [2]*Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)*)

3P177 **マイコプラズマは左回りに進むのか？**
Do Mycoplamsas glide to the left?
Hanako Morio, Taishi Kasai, Makoto Miyata (*Grad. Sch. Sci., Univ. Osaka City*)

3P178 **微小管 X 線繊維回折：チューブリンピッチの動的変化**
Dynamic changes of the axial pitch of tubulin repeat in live microtubules revealed by x-ray fiber diffraction
Shinji Kamimura[1], Yosuke Fujita[1], Yuuko Wada[1], Hiroyuki Iwamoto[2] ([1]*Dept. Biol. Sci., Chuo Univ.*, [2]*JASRI, SPring-8*)

3P179 **生細胞内における厳密な PI3K ヘテロダイマー複合体のシグナル応答**
Dynamic Signal Response of Rigorous PI3K Heterodimer in Living Cells
Chan-Gi Pack[1], Yuko Saeki[2], Mariko Okada[2], Yasushi Sako[1] ([1]*Cellular Informatics Laboratory, RIKEN*, [2]*Laboratory for Integrated Cellular Systems, RIKEN IMS-RCAI*)

3P180 **Cell signaling occurs by a specific mobility and clustering state of epidermal growth factor receptor**
Michio Hiroshima[1,2], Yasushi Sako[2] ([1]*RIKEN QBiC*, [2]*RIKEN Cellular Informatics Lab.*)

3P181 **Detection of Cellular Responses to a Differentiation Factor Using Raman Microspectroscopy**
Sota Takanezawa[1,2], Shin-ichi Morita[1], Yasushi Sako[1], Yukihiro Ozaki[2] ([1]*Cellular Informatics Lab., RIKEN*, [2]*School. Sci. Tech., Kwansei-Gakuin Univ.*)

3P182 **蛍光イメージング法による機能的べん毛モーターと走化性シグナル伝達分子 CheY の結合の直接的観察**
Direct imaging of the rotational switching of a functioning 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.*)

3P183 **細胞における核小体タンパク Nucleophosmin 1 の可視化**
Imaging a nucleolar protein, Nucleophosmin 1, in living cells
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], Kaoru Katoh[1,2] ([1]*Biomed. Res Inst, AIST*, [2]*Grad. Sch. Live & Env. Sci., Univ. Tsukuba*, [3]*RIKEN ASI*, [4]*RIKEN QBiC*)

3P184 **シグナル伝達タンパク質ＥＲＫ２の情報処理を介した細胞運命決定の定量解析**
Cell fate decisions through information processing of a signaling protein ERK2
Kazunari Mouri, Yasushi Sako (*Cellular Informatics Lab., RIKEN*)

3P185 **情報処理タンパク質 RAF の多状態性と細胞応答**
Polymorphism of a signaling protein RAF regulates cellular responses
Kayo Hibino[1], Kenji Okamoto[2], Masahiro Ueda[1], Yasushi Sako[2] ([1]*QBiC (Quantitative Biology Center), RIKEN*, [2]*Cellular Informatics Lab., RIKEN*)

3P186 **Quantitative analysis of signal transduction dynamics between Raf and ERK in living single PC12 cells**
Yuki Shindo[1,2], Kazunari Iwamoto[2], Kayo Hibino[2], Kazunari Mouri[3], Yasushi Sako[3], Koichi Takahashi[2] ([1]*Syst. Biol. Prog. Grad. Sch. Media & Governance, Keio Univ.*, [2]*RIKEN QBiC.*, [3]*Cell. Inform. Lab., RIKEN*)

3P187 **SOS を介した Ras 活性 positive feedback 調節の生細胞一分子解析**
Positive feedback regulation of SOS-mediated Ras activation detected by single-molecule analysis in living cells
Yuki Nakamura[1,2], Kayo Hibino[3], Yasushi Sako[2] ([1]*Grad, sch, FBS., okasa Univ*, [2]*wako inst., Riken*, [3]*QBIC., Riken*)

3P188 **海洋性ビブリオ菌のべん毛形成抑制に関与する DnaJ モチーフを持った SflA の細胞内局在**
The intracellular localization of SflA, the dnaJ family protein that plays a role in the suppression of flagellation in Vibrio
Takehiko Nishigaki, Noriko Nishioka, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

3P189 **Structural analysis of the flagellar basal body in intact cell 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*)

3P190 **Biochemical properties of FlhG, a negative regulator for the number of the polar flagellum in Vibrio alginolyticus**
Akari Takashima, Hiroki Ono, Michio Homma, **Seiji Kojima** (*Grad. Sch. Sci., Univ. Nagoya*)

3P191 **Stator activation requires conformational change in the periplasmic region of PomB, a Na^+-driven stator protein**
Shiwei Zhu[1], Masato Takao[2], Na Li[1], Mayuko Sakuma[1], Michio Homma[1], Seiji Kojima[1], Katsumi Imada[2] ([1]*Nagoya University*, [2]*Osaka University*)

3P192 **細菌べん毛輸送装置構成蛋白質 FlhA の変異に対するロバストネス**
Mutational robustness of FlhA, a subunit of the bacterial flagellar export apparatus
Tohru Minamino[1], Miki Kinoshita[1], Noritaka Hara[1], Satomi Koya[2], Noriko Nishioka[3], Seiji Kojima[3], Kunio Ihara[4], Michio Homma[3], Keiichi Namba[1,5] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*Dept of Food Sci. and Nutrition, Doshisha Women's College of Liberal Arts*, [3]*Grad. Sch. Sci., Nagoya Univ.*, [4]*Gene, Nagoya Univ.*, [5]*QBiC, RIKEN*)

3P193 **細菌べん毛本数を負に制御する MinD と相同性をもつ FlhG の ATPase モチーフの役割**
Role of ATP binding motif of FlhG, a MinD homolog, which regulates the number of the polar flagellum in *Vibrio alginolyticus*
Hiroki Ono, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

3P194 **Functional chimera of the flagellar stator proteins between *E. coli* MotB and *Vibrio* PomB at the periplasmic region**
Yuuki Nishino, Seiji Kojima, Michio Homma (*Div. Biol. Sci, Grad. Sch. Of Sci., Nagoya Univ.*)

3P195 **N-terminal deletion mutant of the stator protein PomA in the bacterial flagellar motor from Vibrio alginolyticus**
Yasuhiro Onoue, Rei Abe-Yoshizumi, Mizuki Gohara, Shiori Kobayashi, Noriko Nishioka, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Nagoya Univ.*)

3P196 ***Vibrio alginolyticus* 由来べん毛固定子 PomA のみによる複合体形成**
Flagellar stator protein of Vibrio PomA alone could form multimeric complex
Mizuki Gohara[1], Norihiro Takekawa[1], Yohei Miyanoiri[2], Masatune Kainosho[2,3], Seiji Kojima[1], Michio Homma[1] ([1]*Div. Bio. Sci., Grad Sch. Sci., Nagoya Univ.*, [2]*Structural Bio. Res. Cent., Grad. Sch. Sci., Nagoya Univ.*, [3]*Grad. Sch. Sci. Tech., Tokyo Metropolitan Univ.*)

3P197 **Na+ uptake activity of the plug-deleted Na+-driven stator complex from Vibrio flagellar motor using reconstituted proteoliposome**
Tetsuya Oba, Seiji Kojima, Michio Homma (*Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ.*)

3P198 **Vibrio alginolyticus の C リング付き基部体の構造解析**
Structure analysis of the basal body with C-ring components from Vibrio alginolyticus
Satoshi Inaba, Hidemaro Hotta, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

3P199 **c-di-GMP 結合タンパク質 YcgR のホモログ PlzD による *Vibrio alginolyticus* によるべん毛運動の阻害**
Flagellar motility inhibition by PlzD, a YcgR homolog of c-di-GMP binding protein, in *Vibrio alginolyticus*
Takuro Yoneda, Wakako Morimoto, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

3P200 **高度好熱菌 *Aquifex aeolicus* 由来のべん毛モーター固定子タンパク質の性質検討**
Characterization of the stator proteins of flagellar motor from extreme thermophile *Aquifex aeolicus*
Norihiro Takekawa, Mizuki Gohara, Seiji Kojima, Michio Homma (*Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ.*)

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

3P201 **アデノウイルス由来両親媒性ペプチドの曲率誘導能における配列効果**
The Sequence Effects of the Amphypathic Peptides of Adenovirus Protein VI on Their Curvature Inducing Ability
Tomo Murayama, Silvia Pujals, Shiroh Futaki (*Institute for Chemical Research, Kyoto University*)

3P202 **脂質膜の膜融合に際する水の協同性**
Water-lipid cooperativity upon lipid membrane fusion
Mafumi Hishida[1], Koichiro Tanaka[2,3], Yasuhisa Yamamura[1], Kazuya Saito[1] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*iCeMS, Kyoto Univ.*, [3]*Dept. Phys., Kyoto Univ.*)

3P203 **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に関する研究**
Study on the behavior of lipid multi-component system consisting of long- and short-chain phospholipids
Ryota Kobayashi, Tetsuhiko Ohba (*Grad.Sch.Sci., Tohoku Univ.*)

3P204 **ガラス基板上への細胞膜展開法の開発**
Development of a new method for preparation of cell membrane flat sheet on glass surface
Yuta Minami[1], Hiroaki Inuma[1], Toshihiko Sakurai[2], Takashi Okuno[3] ([1]*Graduate School of Science and Engineering, Yamagata University*, [2]*Graduate School of Engineering, Tottori University*, [3]*Department of Science, Yamagata University*)

3P205 **人工テトラエーテル型リン脂質膜と重金属イオンとの相互作用**
Interaction of heavy metal ions with artificial tetraether-type phospholipid membranes
Teruhiko Baba[1], Toshiyuki Takagi[1], Toshiyuki Kanamori[1], Tatsuya Oka[2], Hiroyuki Saito[2] ([1]*Res. Center Stem Cell Eng., AIST*, [2]*HBS, Univ. Tokushima Grad. Sch.*)

3P206 **並列化された粗視化シミュレーションを用いたベシクルの構造安定性に関する理論的研究**
Theoretical study on the structural stability of the vesicle by parallelized coarse-grained simulation
Tsuhito Yoshida, Kazuma Tamura, Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Kanazawa University*)

3P207 **Effect of cholesterol and 7-ketocholesterol on localization of Alzheimer's amyloid beta (Aβ_42) in membrane domains**
Huong Phan, Masamune Morita, Tsuyoshi Yoda, Naofumi Shimokawa, Mun'delanji Vestergaard, Masahiro Takagi (*Japan Advanced Institute of Science and Technology*)

3P208 **脂質酸化物による生体模倣膜のドメイン形成**
Effects of lipid oxidation products on domain formation of biomimetic membrane
Tsuyoshi Yoda[1,2], Wataru Inui[1], Huong Thi Than Phan[1], Naofumi Shimokawa[1], Mun'delanji C. Vestergaard[1], Tsutomu Hamada[1], Masahiro Tkagai[1] ([1]*Japan Advanced Institute of Science and Technology*, [2]*JSPS Research Fellow PD*)

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

3P209 **遠心式マイクロ流体デバイスによる細胞サイズリポソームの作製**
The synthesis of cell-sized liposomes by centrifuge-based microfluidic device
Masamune Morita[1], Miho Yanagisawa[2], Hiroaki Onoe[3], Masahiro Takinoue[1,4] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Institute of Technology*, [2]*Grad. Sch. Sci., Kyushu Univ.*, [3]*IIS, The University of Tokyo*, [4]*PRESTO, JST*)

3P210 **脂質側方拡散を増幅させる新規拡張アンサンブル法の開発と応用**
Acceleration of lipid lateral diffusion by generalized-ensemble molecular dynamics simulation
Takaharu Mori, Jaewoon Jung, Yuji Sugita (*RIKEN*)

3P211 **Time-resolved 3D Quantification and Analysis of Membrane-Lipid Signaling in Dictyostelium**
Marcel Hoerning, Tatsuo Shibata (*Physical Biology Unit, Center for Developmental Biology, RIKEN*)

3P212 **セラミド分子のフリップフロップ速度**
Transbilayer movement of sulfhydryl ceramide analogues in model membranes
Takehiko Inaba[1], Sabrina Kargoll[1], Françoise Hullin-Matsuda[1,2], Peter Greimel[1], Toshihide Kobayashi[1] ([1]*RIKEN Wako*, [2]*Inserm U1060 Universite Lyon*)

3P213 **アミロイドβタンパク質の結合に伴うラフトモデル膜のダイナミックスの変化**
Change of Dynamics of Raft-Model Membrane Induced by Amyloid-β Protein Binding
Mitsuhiro Hirai[1], Ryota Kimura[1], Kazuki Takeuchi[1], Moboru Ohta[2], Bela Farago[3], Stadler Stadler[3], Giuseppe Zaccai[3] ([1]*Grad. Eng., Gunma Univ.*, [2]*Japan Synchrotron Radiation Research Institute*, [3]*Institut Laue-Langevin*)

3P214 **抗菌ペプチドの殺菌メカニズムを探究する**
Investigating bactericidal mechanism of antimicrobial peptids
Kei Kitahara[1,2], Takeshi Sunami[1,2], Tetsuya Yomo[1,2] ([1]*Graduate School of Information Science and Technology, Osaka University*, [2]*Exploratory Research for Advanced Technology, Japan Science and Technology Agency*)

3P215 **コレステロール分子によるリン脂質二重膜の破断抑制メカニズム：分子動力学シミュレーション**
Molecular Mechanism of Inhibitory Effect of Cholesterol on Phospholipid Bilayer Rupture: Molecular Dynamics Simulation
Taiki Shigematsu, Kenichiro Koshiyama, Shigeo Wada (*Grad. Eng. Sci., Osaka Univ.*)

13C. 生体膜・人工膜：興奮・チャンネル / 13C. Biological & Artifical membrane: Excitation & Channels

3P216 **ヒト iPS 由来心筋とヒト ES 由来心筋の電気生理学性質の比較研究**
A comparative study on electrophysiological properties of human iPS- and ES-derived cardiomyocytes
Fernando Lopez-Redondo[1], Junko Kurokawa[2], Fumimasa Nomura[1], Tomoyuki Kaneko[3], Tomoyo Hamada[1], Tetsushi Furukawa[2], Kenji Yasuda[1] ([1]*Inst. Biomat. Bioeng., Tokyo Medical Dental Univ.*, [2]*Med. Res. Inst., Tokyo Med. Dental Univ.*, [3]*Grad. Sch. Sci. Eng., Hosei Univ.*)

3P217 **細胞内ミトコンドリアの一過性脱分極の観察と誘導**
Observation and induction of mitochondrial transient depolarizations in cells
Kanji Umiuchi, Yoshihiro Ohta (*Tokyo Univ. Agr. Tech.*)

3P218 **KcsA チャネルの細胞内ドメインと inactivation gate の連関**
Coordination between the cytoplasmic domain and the inactivation gate in the KcsA channel
Minako Hirano[1], Yukiko Onishi[2], Okuno Daichi[2], Toru Ide[1] ([1]*GPI*, [2]*Riken*)

3P219 **固体支持体に固定したイオンチャネルの人工平面膜への再構成**
Reconstitution of ion channel immobilized on solid support into lipid bilayer
Daichi Okuno[1], Minako Hirano[2], Yukiko Onishi[1], Toru Ide[2] ([1]*RIKEN QBiC*, [2]*The Graduate School for the Creation of New Photonics Industries*)

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

3P220 **ミトコンドリアの密集が活性に与える影響**
Effects of mitochondrial crowding on their activity
Daiki Yoshimatsu, Yoshihiro Ohta (*Tokyo Univ. of Agric. and Tech.*)

3P221 **Lipid bilayer chamber array system for massive measurement of transporter activity**
Naoki Soga, Rikiya Watanabe, Shinya Ohdate, Hiroyuki Noji (*Department of applied chemistry, School of engineering, The university of Tokyo*)

3P222 **アトリットル容積を持つナノセルを用いた膜輸送たんぱく質の 1 分子計測**
NanoCell, Attoliter Chamber Array for Single-Molecule Measurement of Membrane Transporters
Takao Ono, Rikiya Watanabe, Takanori Ichiki, Hiroyuki Noji (*Grad. Sch. Eng. Univ. Tokyo*)

3P223 **PIP2 は synaptotagmin 2 による SNARE を介した膜融合の促進に関与する**
PIP2 is involved in the enhancement of SNARE-mediated membrane fusion by synaptotagmin 2
Satoshi Tadokoro[1], Yoshikazu Inoh[2], Mamoru Nakanishi[2], Naohide Hirashima[1] ([1]*Grad. Sch. Pharm. Sci., Nagoya City Univ.*, [2]*School Of Pharmacy, Aichi Gakuin University*)

3P224 **支持体を持つ人工細胞の開発**
Development of a closed supported artificial cell
Yasuto Sasaki, Misaki Yamamoto, Ichiro Yamato (*Dept. Biol. Sci. Tech., Tokyo Univ. of Science*)

13E. 生体膜・人工膜：情報伝達 / 13E. Biological & Artifical membrane: Signal transduction

3P225 **SWAP-70 PH ドメインの脂質膜結合に対するトリプトファン残基の寄与**
Role of tryptophan residues in membrane association of the SWAP-70 PH domain
Kotono Akai[1], Michikazu Tanio[2], Katsuyuki Nishimura[2], Satoru Tuzi[1] ([1]*Grad. Sch. Life Sci., Univ. Hyogo*, [2]*Inst. Mol. Sci*)

3P226 **新規ガングリオシドプローブの 1 分子追跡によるラフト組織化と機能の解明**
Single-molecule tracking of new ganglioside probes revealed raft organization and function
Kenichi Suzuki[1], Hiromune Ando[1,2], Naoko Komura[1,2], Rahul Chadda[1], Hideharu Ishida[2], Makoto Kiso[1,2], Akihiro Kusumi[1] ([1]*iCeMS, Kyoto Univ.*, [2]*Dpt. Appl. Biol. Sci., Gifu Univ.*)

14. 化学受容 / 14. Chemoreception

3P227 **光制御水素化アモルファスシリコン薄膜上の化学反応性積層ゲルを用いたバイオセンサ**
Biosensor using electrochemical laminated gels photo-controlled on hydrogenated amorphous silicon film
Hiroki Suzuki[1], Ryohei Matsueda[1], Teruo Matsuno[1], Takahiko Sano[1], Yuta Ando[1], Hiroshi Masumoto[2], Takashi Goto[3], **Yutaka Tsujiuchi**[1] ([1]*Material Science and Engineering, Akita University*, [2]*Center for Interdisciplinary Research, Tohoku University*, [3]*Institute for Materials Research, Tohoku University*)

3P228 **金ナノ粒子キャリヤー表面に提示されたハプテンとしてのアゾベンゼン色素の免疫応答**
Immunological study with azobenzene-dye as a hapten presented on the surface of gold nanoparticle carriers
Noriyuki Ishii[1], Kaoru Tamada[2], Haruhisa Akiyama[3] ([1]*Biomedical, AIST*, [2]*IMCE, Kyushu Univ.*, [3]*Nanosystem, AIST*)

3P229 **Directional-sensing and rectified cell motion towards termporally changing gradient**
Akihiko Nakajima[1], Shuji Ishihara[1,2], Daisuke Imoto[1], Satoshi Sawai[1,2,3] ([1]*Graduate School of Arts and Sciences, University of Tokyo*, [2]*Research Center for Complex Systems Biology, University of Tokyo*, [3]*PRESTO, Japan Science and Technology Agency*)

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

3P230 **線虫においてあるモダリティーが異なるモダリティーの順応を引き起こす**
Sensory stimulation from a specific modality adapts a different modality in Caenorhabditis elegans
Hisashi Shidara, Junya Kobayashi, Ryo Tanamoto, Kohji Hotta, Kotaro Oka (*Bio and Info, Keio Univ.*)

3P231 **集光レーザービームの光摂動による神経細胞内分子動態の集合操作**
Optical perturbation of intracellular molecular dynamics of single neuron in living neuronal network
Chie Hosokawa[1], Naoko Takeda[1,2], Yusuke Ueda[1,2], Suguru N. Kudoh[2], Takahisa Taguchi[1,3] ([1]*Health Res. Inst., AIST*, [2]*Grad. Sci. Eng., Kwansei Gakuin Univ.*, [3]*Cinet, NICT*)

3P232 **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**
Sex difference in hippocampus: Fluctuation of hippocampal sex hormones and synapses
Yasushi Hojo[1,2], Asami Kato[1], Tetsuya Kimoto[1,2], Suguru Kawato[1,2] ([1]*Grad. Sch. Arts and Sci., Univ. Tokyo*, [2]*JST, Japanese-Taiwanese Cooperative Programme*)

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

3P233 **老化に伴う海馬神経シナプスの密度の減少と記憶の劣化**
Age-related decrease in synapse density of hippocampal neurons in relation to memory impairment
Suguru Kawato[1,2] ([1]*Univ of Tokyo, Grad Sch Arts and Sciences*, [2]*JST Int Collabo*)

3P234 **Acute Modulation of Synaptic Plasticity of Pyramidal Neurons by Hippocampal-derived Sex Steroids**
Yoshitaka Hasegawa[1,2], Keisuke Hotta[1], Hideo Mukai[1], Bon-chu Chung[2,3], Ooishi Yuuki[1], Hojo Yasushi[1,2], Kawato Suguru[1,2] ([1]*Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*, [2]*International collaboration program of Bioelectronics (JST)*, [3]*Institute of Molecular Biology, Taiwan*)

3P235 **脳海馬が作る男性・女性ホルモンは記憶の神経シナプスを増強する**
Hippocampus-synthesized male and female hormones increase memory-related nerve synapses
Miyuki Yoshiya, Yasushi Hojo, Suguru Kawato (*Grad. Sch. of Art and Sci.*)

3P236 **ハロロドプシンー臭素イオン複合体の N 光反応中間体の X 線結晶構造解析**
X-ray structural analysis of the N photoreaction intermediate of halorhodopsin in complex with bromide ion
Haruki Kawaguchi[1], Taichi Nakanishi[1], Hiroki Kubo[1], Kunio Ihara[2], Midori Murakami[1], Tsutomu Kouyama[1] ([1]*Graduate School of Science, Nagoya University*, [2]*Center for Gene Research, Nagoya University*)

3P237 **Trapping the photoactive form of squid rhodopsin in the P62 crytal**
Midori Murakami, Tsutomu Kouyama (*Dept. Physics, Nagoya Univ.*)

3P238 **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 orthologues of mammalian NDRG1 protein in photoreceptors
Shimpei Takita[1], Yasutaka Wada[2], Satoru Kawamura[2] ([1]*Dept. of Biol. Sci. Grad. Sch. of Sci., Osaka Univ.*, [2]*Dept. of Biol. Sci. Grad. Sch. of Sci.; Grad. Sch. of Frontier Biosci., Osaka Univ.*)

3P239 **コイ桿体と錐体とでの cGMP ホスホジエステラーゼの活性化効率の定量的理解**
Quantitative Aspects of cGMP Phosphodiesterase Activation in Carp Rods and Cones
Yuki Koshitani[1], Shuji Tachibanaki[1,2], Satoru Kawamura[1,2] ([1]*Grad. Sch. Sci., Osaka Univ.*, [2]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

3P240 **LOV タンパク質 YtvA のシグナル伝達における分子間相互作用変化の時間分解測定**
Time-resolved study on the intermolecular interaction change in the signal transduction of LOV protein YtvA
Seokwoo Choi[1], Yusuke Nakasone[1], Klaas Hellingwerf[2], Masahide Terazima[1] ([1]*Department of Chemistry, Graduate school of Science Kyoto University*, [2]*Molecular Microbial Physiology Group, Swammerdam Institute for Life Sciences, University of Amsterdam*)

3P241 **青色光センサー蛋白質 PapB の光反応ダイナミクス**
Light induced reaction dynamics of a BLUF photoreceptor PapB
Koutaro Kikukawa[1], Yusuke Nakasone[1], Shinji Masuda[2,3], Masahide Terazima[1] ([1]*Grad. Sci., Univ. Kyoto*, [2]*Center for BioRes. & Inform., Tokyo Inst. Tech.*, [3]*PRSTO, JST*)

3P242 **フォトトロピンの LOV2 ドメインからキナーゼ部位への光情報伝達ダイナミクス**
Photochemical signal transduction dynamics of the LOV2-kinase fragment of phototropin2 from *Arabidopsis*
Akira Takakado[1], Yusuke Nakasone[1], Koji Okajima[2], Satoru Tokutomi[2], Masahide Terajima[1] ([1]*Sci, Univ. Kyoto*, [2]*Sci, Univ. Osaka pref.*)

3P243 **緑藻由来の全長フォトトロピンの光反応**
Photochemistry of full-length phototropin from green algae
Yusuke Nakasone[1], Koji Okajima[2], Kenichi Hitomi[3], Yusuke Aihara[1], Akira Nagatani[1], John Christie[3], Satoru Tokutomi[2], Masahide Terazima[1] ([1]*Graduate School of Science, Kyoto Univ.*, [2]*Graduate School of Science, Osaka Prefecture Univ.*, [3]*Scripps Research Institute*)

3P244 **QM/MM RWFE 法による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.*)

3P245 **高角領域の X 線散乱によるロドプシンの構造変化の解析**
Helical rearrangement of photoactivated rhodopsin probed by high-angle X-ray scattering
Yasushi Imamoto[1], Toshihiko Oka[2], Keiichi Kojima[1], Ryo Maeda[1], Yoshinori Shichida[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Grad. Sch. Sci., Shizuoka Univ.*)

3P246 **Gタンパク質共役型受容体の構成的活性変異体に見られるGタンパク質活性化メカニズムの一分子解析**
Single-molecule analyses of the activation mechanisms of G proteins in constitutively active mutant of G protein-coupled receptor
Ryo Maeda[1], Michio Hiroshima[2,3], Yasushi Imamoto[1], Takahiro Yamashita[1], Yasushi Sako[2], Yoshinori Shichida[1] ([1]*Department of Biophysics, Graduate School of Science, Kyoto University*, [2]*Cellular Informatics Laboratory, RIKEN Advanced Science Institute*, [3]*Laboratory for Cell Signaling Dynamics, RIKEN Quantitative Biology Center*)

3P247 **光依存的なGタンパク質活性化能を失ったロドプシン類の発見とその不活性化機構の解析**
Discovery of a diffusible ligand-binding rhodopsin lacking light-dependent G protein activation ability
Keita Sato[1], Takahiro Yamashita[1], Hideyo Ohuchi[2], Sayuri Tomonari[3], Sari Fujita-Yanagibayashi[1], Kazumi Sakai[1], Atsuko Takeuchi[4], Yasushi Imamoto[1], Sumihare Noji[3], Akimori Wada[4], Yoshinori Shichida[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Grad. Sch. Med. Dent. Pharm. Sci., Okayama Univ.*, [3]*Inst. Tech. Sci., Univ. Tokushima*, [4]*Kobe Pharm. Univ.*)

3P248 **ホヤオプシン 1(Ci-opsin1)の分子特性の解析**
Analysis of molecular property of ascidian opsin, Ci-opsin1
Keiichi Kojima[1], Takahiro Yamashita[1], Yasushi Imamoto[1], Motoyuki Tsuda[2], Takehiro Kusakabe[3], Yoshinori Shichida[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Grad. Sch. Life Sci., Hyogo Univ.*, [3]*Fac. Sci. Engn., Konan Univ.*)

3P249 **脊椎動物の可視光感受性 Opn5 の分子特性解析**
Molecular properties of vertebrate visible-light sensitive Opn5
Takahiro Yamashita[1], Hideyo Ohuchi[2], Akane Yumoto[1], Keita Sato[1], Sayuri Tomonari[3], Masato Kinoshita[4], Sumihare Noji[3], Yoshinori Shichida[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Grad. Sch. Med. Dent. Pharm. Sci., Okayama Univ.*, [3]*Inst. Technol. Sci., Univ. Tokushima Grad.Sch.*, [4]*Grad. Sch. Agr., Kyoto Univ.*)

3P250 **マウスメラノプシンの分子特性**
Molecular Properties of Mouse Melanopsin
Takesi Matsuyama Hoyos, Takahiro Yamashita, Yasushi Imamoto, Yoshinori Shichida (*Kyoto University Department of Science*)

3P251 **双安定性のロドプシン類の分子特性とそれらの光遺伝学への応用の可能性**
Molecular properties of animal bistable rhodopsins and their optogenetic potential
Tomohiro Sugihara, Mitsumasa Koyanagi, Akihisa Terakita (*Grad. Sch. Sci., Osaka City Univ.*)

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

3P252 **光合成反応中心タンパク質で機能する電子移動担体の極低温１分子分光**
Cryogenic single molecule spectroscopy of the electron transfer cofactor in the photosynthetic reaction center
Toru Kondo[1], Risa Mutoh[2], Genji Kurisu[2], Hirozo Oh-oka[3], Satoru Fujiyoshi[1], Michio Matsushita[1] ([1]*Dept. Phys., Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Institute for Protein Research, Osaka Univ.*, [3]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*)

3P253 **光化学系 II における励起エネルギーと電子の輸送過程に関する階層的粗視化運動論モデル**
Hierarchical coarse-graining kinetic model for excitation energy and electron transfer processes in photosystem II
Takeshi Matsuoka[1], Shigenori Tanaka[1], Kuniyoshi Ebina[2] ([1]*Graduate School of System Informatics, Kobe University*, [2]*Graduate School of Human Development and Environment, Kobe University*)

3P254 **蛍光寿命顕微鏡による葉緑体微細構造の観察**
Fine structures of chloroplasts observed by fluorescence lifetime imaging microscopy
Ryuichi Matsuyama[1], Ryo Yamada[1], Takashi Shiina[3], Masahide Terazima[1], Shigeichi Kumazaki[1,2] ([1]*Grad. Sch. Sci., Univ. Kyoto*, [2]*PRESTO, JST*, [3]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*)

3P255 **光化学系 II におけるプロトン移動経路**
Proton transfer pathway in photosystem II
Keisuke Saito[1,2], A. William Rutherford[3], Hiroshi Ishikita[1,2] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*PRESTO JST*, [3]*Dept. of Life Sci., Imperial College, London*)

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

3P256 **実験室進化を用いた大腸菌の抗生物質耐性獲得ダイナミクスの解析**
Laboratory evolution of antibiotic resistant Escherichia coli
Shingo Suzuki, Takaaki Horinouchi, **Chikara Furusawa** (*Quantitative Biology Center, RIKEN*)

3P257 **全生物共通祖先生物の生育温度の実験による推定**
Empirical estimation of the environmental temperature of the last universal common ancestor
Satoshi Akanuma, Yoshiki Nakajima, Shin-ichi Yokobori, Akihiko Yamagishi (*Dept. of Appl. Life Sci., Tokyo Univ. of Pharm. Life Sci.*)

3P258 **On phenotypic drug tolerance based on expression noise of antibiotic resistant gene**
Takashi Nozoe[1], Reiko Okura[1], Yuichi Wakamoto[1,2] ([1]*Grad. Sch. Arts and Sci., Univ of Tokyo*, [2]*Research Center for Complex Systems Biology, Univ of Tokyo*)

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

3P259 **二次構造のパッキングの仕方は同じだがトポロジーの異なるタンパク質ペアの特徴**
Some features of protein pairs which have same SSEs packing arrangement but have different topology
Tatsuo Mukai[1], Shintaro Minami[2], George Chikenji[1] ([1]*Grad. Sch. of Engineering, Nagoya Univ.*, [2]*Grad. Sch. of Info. Sci., Nagoya Univ.*)

3P260 **デノボタンパク質立体構造予測のための新規フォールド構造生成法**
Generating novel protein folds from existing folds for de novo protein structure prediction
Yuki Nakagawa[1], George Chikenji[1], Shintaro Minami[2] ([1]*Grad. Sch. of Eng., Nagoya Univ.*, [2]*Grad. Sch. of Info. Sci., Nagoya Univ.*)

3P261 **Non-sequential structural alignment reveals fold change by segment shuffling during evolution**
Shintaro Minami[1], George Chikenji[2], Motonori Ota[1] ([1]*Dept. of Info. Sci., Nagoya Univ.*, [2]*Dept. of Eng., nagoya Univ.*)

3P262 **Motion Tree 法による蛋白質構造変化の階層的記述と網羅的分類**
Hierarchical description and extensive classification of protein structural changes by Motion Tree
Ryotaro Koike[1], Motonori Ota[1], Akinori Kidera[2] ([1]*Grad. Sch. Info. Sci., Nagoya Univ.*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*)

3P263 **beta-Trefoil タンパクのフォールディングコアの残基間平均距離統計に基づく解析**
Analyses of folding nuclei of beta-Trefoil fold proteins based on the inter-residue average distance statistics
Norihiro Kanemaru, Masanari Matsuoka, Takeshi Kikuchi (*Dept. Bioinf., Col. Life Sci., Ritsumeikan Univ.*)

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

3P264 **FCANAL（構造を基にしたタンパク質機能御予測法）の様々なタンパク質への適用**
FCANAL, structure-based protein function prediction method, applied to various types of proteins
Hiroko Sagisaka, Misaki Yamamoto, Ichiro Yamato (*Dept. Biol. Sci. Tech, Tokyo Univ. of Science*)

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

3P265 **人工プロモーター設計 Web アプリケーション PromoterCAD のためのデータベース構築**
Database Construction for Synthetic Promoter Design Web Application (PromoterCAD)
Koro Nishikata[1], Robert Cox III[1], Sayoko Shimoyama[1], Yuko Yoshida[1], Minami Matsui[2], Yuko Makita[1], Tetsuro Toyoda[1] ([1]*Integrated Database Unit, Advanced Center for Computing and Communication (ACCC), RIKEN*, [2]*Synthetic Genomics Research Team, Biomass Engineering Program Cooperation Division, Center for Sustainable Resource Science (CSRS), RIKEN*)

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

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3P267 **Lysozyme スーパーファミリーを用いた遠縁タンパク質間のフォールディング部位の頑健性についての解析**
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24. 数理生物学 / 24. Mathematical biology

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ENaC dynamics in the intracellular space: analysis of Na^+ transport in epithelial cells by mathematical model
Kouhei Sasamoto[1], Naomi Niisato[2,4], Yoshinori Marunaka[2,3,4] ([1]*Undergrad. (4th-year), Kyoto Pref. Univ. Med.*, [2]*Dept. of Mol. Cell Physiol., Kyoto Pref. Univ. Med.*, [3]*Dept. of Bio-Ionomics, Kyoto Pref. Univ. Med.*, [4]*Japan Inst. for Food Education & Health, St. Agnes' Univ.*)

3P274 **真性粘菌 *Physarum polycephalum* とそのモデルによる錯視の計算**
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3P275 **錯視を引き起こす図形パターンに対する真性粘菌変形体の反応**
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Hiroshi Kori[1,2] ([1]*Ochanomizu Univ*, [2]*CREST*)

3P277 **Dependence of cell differentiation ratio on cell-cell interaction and noise**
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25. 非均衡・生体リズム / 25. Equality Nonequilibrium state & Biological rhythm

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3P279 **KaiC タンパク質のリン酸化と ATPase 活性の概日リズムの確率シミュレーション によるモデリング**
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Eisuke Chikayama[1,2] ([1]*Niigata University of International and Information Studies*, [2]*RIKEN*)

26. 計測 / 26. Measurements

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3P283 **蛍光ダイアモンドナノ粒子を使った光検出磁気共鳴**
Optically detected magnetic resonance for fluorescent single nanodiamond in cell and c.elegance
Yohsuke Yoshinari[1], Yuta Kumiya[2], Takuma Sugi[2], Ryuji Igarashi[2], Shingo Sotoma[2], Masahiro Shirakawa[2], Yoshie Harada[1] ([1]*iCeMS, Kyoto University*, [2]*Department of Molecular Engineering, Kyoto University*)

3P284 **一分子計測と一分子粒度細胞シミュレーションの融合**
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Koichi D. Hiraoka[1], Shuichi Nakamura[2], Nobunori Kami-ike[1], Yusuke V. Morimoto[3] ([1]*Grad. Sch. of Frontier Biosci.*, [2]*Grad. Sch. of Eng., Tohoku Univ*, [3]*RIKEN, QBiC*)

3P286 **タンパク質中性子結晶構造解析におけるプロトン偏極法のための基礎的な試み**
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27. バイオイメージング / 27. Bioimaging

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Akifumi Higashiura[1], Marina Murakami[1], Kenji Iwasaki[1], Eiki Yamashita[1], Kazuki Takeda[2], Yuya Hanazono[2], Kiyofumi Takaba[2], Masahito Hibi[2], Yuriko Tomisaki[2], Kunio Miki[2], Atsushi Nakagawa[1] ([1]*Inst. for Prot. Res., Osaka Univ.*, [2]*Grad. Sch. of Sci., Kyoto Univ.*)

3P288 **hPrx2 のオリゴマー形成過程の高速 AFM 観察**
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Takamitsu Haruyama, Noriyuki Kodera, Hiroki Konno (*Bio-AFM Frontier Research Center, College of Sci. & Eng., Kanazawa Univ.*)

3P289 **細胞内蛋白質混雑感受性蛍光蛋白質の開発**
Intracellular measurement of protein-crowding condition by a gene-encoded indicator
Takamitsu Morikawa[1], Keiko Yoshizawa[2], hideaki Fujita[2,3], 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]*RIKEN Quantitative Biology Center*, [3]*WPI, Immunology Frontier Research Center, Osaka University*, [4]*Department of Macromolecular Science, Graduate School of Science, Osaka University*, [5]*Institute of Sciencific and Industrial Research Center, Osaka University*)

3P290 **マウス内がん細胞の非侵襲イメージング**
Noninvasive in vivo imaging of tumor cells in a novel xenograft model
Sayaka Kita, Hideo Higuchi (*Dep. of phys., Grad. Sch. of Sci., The Univ. of Tokyo*)

3P291 **バクテリア細胞内 ATP 濃度の一細胞計測**
Quantifying the absolute ATP concentration inside single bacteria cells
Hideyuki Yaginuma[1,2], Shinnosuke Kawai[3], Keisuke Tomiyama[2], Kazuhito V. Tabata[1,5], Tamiki Komatsuzaki[3], Hiromi Imamura[4], Hiroyuki Noji[1,2] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Grad. Sch. Front. Biosci., Osaka Univ.*, [3]*Res. Inst. Elect. Sci., Hokkaido Univ.*, [4]*Hakubi Project, Kyoto Univ.*, [5]*PRESTO, JST*)

3P292 **細胞内熱伝導率マッピング**
Mapping of thermal conductivity in single living cells
Taku Sekiguchi[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]*IMB, A*STAR, Singapore*, [3]*Org Univ Res Initiatives, Waseda Univ, Tokyo, Japan*, [4]*WABIOS, Waseda Univ, Singapore*)

3P293 **Structure and fluorescent property 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.*)

3P294 **量子ドットナノプローブを用いたアミロイドβ凝集阻害物質の新規微量ハイスループットスクリーニングシステムの開発**
Development of a novel high-throughput screening system of inhibitory substances for amyloid-β aggregation using quantum-dot nanoprobes
Toshiki Ogara, Yukako Ishigaki, Syoya Yamaguchi, Hiroyuki Tanaka, Koji Uwai, Kiyotaka Tokuraku (*Muroran Institute Of Technology*)

3P295 **Simultaneous imaging of intracellular Ca^{2+} and sarcomere length in neonatal cardiomyocytes via expression of cameleon-Nano in Z-discs**
Seiichi Tsukamoto[1], Kotaro Oyama[2], Seine Shintani[2], Fuyu Kobirumaki[1], Shin'ichi Ishiwata[2,3,4], Norio Fukuda[1] ([1]*Dept. Cell Physiol., The Jikei Univ.*, [2]*Sch. Adv. Sci. Eng., Waseda Univ.*, [3]*Org. Univ. Res. Initiatives, Waseda Univ.*, [4]*WABIOS, Waseda Univ., Singapore, Singapore*)

3P296 **超解像イメージング法により明らかとなったストレス顆粒内 mRNA の詳細分布**

Super-resolution imaging reveals nanoscale distribution of mRNA in stress granule

Ko Sugawara[1], Kohki Okabe[1,2], Akihiko Sakamoto[1], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, the University of Tokyo*, [2]*JST, PRESTO*)

3P297 **生細胞内における microRNA のイメージング**

Imaging of microRNA in living cells

Toshinari Ishikawa[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*Sakigake, JST*)

3P298 **大腸菌 RND 型異物排出トランスポーター AcrD の細胞内動態観察**

Dynamics of RND-type xenobiotic transporter AcrD in the cytoplasmic membrane of *Escherichia coli*

Rei Tamai[1], Kentaro Yamamoto[1], Takehiko Inaba[2,4], Yoshiyuki Sowa[2,3], Ikuro Kawagishi[1,2,3] ([1]*Dept. Frontier Biosci., Grad. Sch. Eng and Sci., Hosei Univ.*, [2]*Res. Cen. Micro-Nanotech., Hosei Univ.*, [3]*Dept. Frontier Biosci., Fac. Biosci. and Appl. Chem., Hosei Univ.*, [4]*RIKEN Adv. Sci. Inst.*)

3P299 **大腸菌異物排出システム AcrAB-TolC の細胞内動態解析**

Dynamics of the xenobiotic efflux system AcrAB-TolC in *Escherichia coli*

Kentaro Yamamoto[1], Rei Tamai[1], Takehiko Inaba[2,4], Yoshiyuki Sowa[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.*, [4]*RIKEN Adv. Sci. Inst.*)

3P300 **FIB（Focused Ion Beam: 集束イオンビーム加工）-SEM（Scanning Electron Microscope: 走査電子顕微鏡）による細胞まるごと三次元構造解析法の開発**

Whole cell structure reconstruction by three-dimensional Focused Ion Beam and Scanning Electron Microscopy

Rina Nagai[1], Keisuke Ohta[2], Kazuhiro Aoyama[3,4], Akinobu Togo[2], Akihiro Kawamoto[5], Atsuko H. Iwane[1,3] ([1]*Cell Field Struc., QBiC, Riken*, [2]*2Anatomy, Med., Kurume Univ.*, [3]*Spec. Res. Promot. Group, Grad. Sch. Fronti. Biosci., Osaka Univ.*, [4]*Application Lab., FEI JAPAN*, [5]*Cell Dynamics Observ., QBiC, Riken*)

3P301 **クライオ電子線トモグラフィーと STEM を用いた生細胞内オルガネラのイメージング**

Imaging of live cell organelles by Cryo-electron tomography and STEM

Ruriko Ogawa[1], Kazuhiro Aoyama[2,3], Rina Nagai[1], **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*)

3P302 **生物試料中での GFP ー CL の観察**

Observation of GFP-CL in biological specimens

Kazuyoshi Murata[1], Naoyuki Miyazaki[2], Ryusuke Ueno[2], Hiroki Minoda[2], Naoki Yamamoto[3], Kuniaki Nagayama[1] ([1]*Nat. Inst. Physiol. Sci.*, [2]*Tokyo Univ. Agricult. Tech.*, [3]*Tokyo Inst. Tech.*)

3P303 **蛍光蛋白質における光および電子発光の電子線活性化**

Electron-beam Activation of Photo- and Cathodo-luminescence in Fluorescent Proteins

Kuniaki Nagayama[1], Kazuyoshi Murata[1], Hiroki Minoda[2], Ryusuke Ueno[2], Naoki Yamamoto[3] ([1]*National Institute for Physiological Sciences*, [2]*Tokyo University of Agriculture and Technology*, [3]*Tokyo Institute of Technology*)

28. バイオエンジニアリング / 28. Bioengineering

3P304 **Genetically encoded caged Ca^{2+}**

Noritaka Fukuda[1,2], Tomoki Matsuda[1], Takeharu Nagai[1] ([1]*ISIR, Osaka Univ.*, [2]*QBiC, Riken*)

3P305 **細胞解析のためのリアルタイム化学刺激システムの構築**

Development of the real-time local chemical stimulation system for cell analysis

Masaru Kojima, Takahiro Motoyoshi, Kenichi Ohara, Mitsuhiro Horade, Yasushi Mae, Tatsuo Arai (*Grad. Sch. Eng. Sci., Osaka Univ.*)

3P306 **DNA ナノ構造体を用いた DNA-RNA ポリメラーゼ・ハイブリッドナノマシンの構築と活性評価**

Construction and functional analysis of DNA origami base DNA-RNAP hybrid nanomachine

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*)

3P307 **人工鞭毛により推進する精子型マイクロマシン**

A “sperm-like” micro-machine propelled by an artificial flagellum

Tsuyoshi Yamasaki, Susumu Aoyama, Yuichi Hiratsuka (*Japan Advanced Institute of Science and Technology*)

3P308 **インフルエンザウイルスと高い親和性を有する、Sialyllactose 修飾 3-way junction DNA**

Sialyllactose - modified Three way junction(3WJ) DNA as a inhibitor of influenza hemagglutinin

Yasuhito Ebara, Daichi Akamatsu, Naoki Hara, Anna Kono (*Grad. Sch. Hum. Dev. Env. Kobe Univ.*)

3P309 **サイズ選択的細胞回収のための超常磁性金属カップの作製**

Fabrication of Superparamagnetic Metal Cups for Size-Selective Cell Collection

Hyonchol Kim[1], Hideyuki Terazono[1,2], Hiroyuki Takei[1,3], Kenji Yasuda[1,2] ([1]*KAST*, [2]*Inst. Biomat. Bioeng., Tokyo Med. Dent. Univ.*, [3]*Fac. Life Sci., Toyo Univ.*)

3P310 **細胞表面特異的結合 DNA アプタマーの作製と心筋細胞の精製**

Non-invasive identification and purification method of target cardiomyocyte cells using cell-surface-binding ssDNA aptamers

Hideyuki Terazono[1,2], Hyonchol Kim[2], Fumimasa Nomura[1], Kenji Yasuda[1,2] ([1]*Tokyo Medical and Dental University*, [2]*Kanagawa Academy of Science and Technology*)

3P311 **DNA ナノデバイスを導入した刺激応答性ハイドロゲルの構築**
Introduction of DNA nanodevices into a hydrogel for achieving its stimuli-responsive behavior
Takashi Kitajima, Ken Komiya, Masahiro Takinoue, Masayuki Yamamura (*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*)

3P312 **遠心力を利用した複雑形状マイクロハイドロゲル粒子の高速生成**
Centrifuge-based rapid synthesis of complex-shaped microhydrogel particles
Masayuki Hayakawa[1], Hiroaki Onoe[2], Ken H. Nagai[3], Masahiro Takinoue[1,4] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*IIS, Univ. of Tokyo*, [3]*Dept. Phys, Univ. of Tokyo*, [4]*PRESTO, JST*)

30. その他 / 30. Miscellaneous topics

3P313 **Coherent dynamics in colloidal fluids in terms of Lagrangian coherent structures (LCS)**
Preetom Nag, Hiroshi Teramoto, Chun-Biu Li, Tamiki Komatsuzaki (*Research Ins. for Electronic Sci., Univ. Hokkaido*)

3P314 **レプリカ交換分子動力学計算による PA 化糖鎖の立体構造解析**
Conformational analysis of PA-glycans by replica-exchange molecular dynamics simulations
Shigehisa Watabe[1], Suyong Re[2], Eiro Muneyuki[1], Yuji Sugita[2,3,4] ([1]*Dept. Phys. Univ. Chuo*, [2]*Riken, ASI*, [3]*Riken, AICS*, [4]*Riken, QBiC*)

3P315 **Bio-inspired Connectivity Self-Healing in Wireless Mesh Networks**
Rui Teng, Ryu Miura (*The National Institute of Information and Communications Technology, Japan*)

3P316 **桿体・錐体での視物質の脱リン酸化活性の比較**
Highly effective Visual pigment Dephosphorylation in cones
Hiromi Yamaoka, Shuji Tachibanaki, Satoru Kawamura (*Grad. Sch. Frontier Biosci., Osaka Univ.*)

1SAA-01 天然光合成における水分解・酸素発生光化学系 II
Water-splitting and oxygen-evolving photosystem II in natural photosynthesis

Nobuo Kamiya (*Osaka City University*)

Photosystem II (PSII) performs light-induced water-splitting reactions, which lead to the formation of molecular oxygen in natural photosynthesis. PSII consists of twenty protein subunits, and many cofactors with a total molecular weight of 350 kDa for a monomer. The crystal structure of PSII has been reported at a resolution of 1.9 Å, in which the oxygen-evolving center (OEC) are clearly identified as a chemical composition of Mn4CaO5(H2O)4. Five oxygen atoms form oxo-bridges between the metal ions, and four water molecules directly ligated to the OEC. The significant feature of the OEC is its distorted chair form. The large distortion from a symmetric cubane is caused by the existence of Ca and O5, the fifth oxo-bridge connecting three Mn ions and one Ca ion.

1SAA-02 ESR 法でわかる Mn クラスターの電子構造と機能
Electronic structure and function of Mn cluster correlated with crystal structure observed by Electron Spin Resonance

Hiroyuki Mino (*Grad. Sch. Sci., Nagoya Univ.*)

Recently, Umena et al. have obtained an X-ray crystal structure of photosystem II (PS II) with 1.9 Å resolution and have revealed the atoms that compose the oxygen evolving complex (Mn cluster) and the coordinated amino acids. The work gives a great impact and provides a key to elucidate the secret of the oxygen evolving mechanism. Up to now, many EPR works have been performed to investigate Mn cluster, which gives good information to interpret electronic structure in intermediate states. These works are essential for elucidation oxygen evolving mechanism. In this symposium, we will discuss recent EPR works based on crystal structure.

1SAA-03 QM/MM 法による光合成酸素発生中心 S 1 状態の電子状態解析
QM/MM study on the photosystem II oxygen evolving complex at the S1 state

Mitsuo Shoji[1], Hiroshi Isobe[2], Shusuke Yamanaka[3], Nobuo Kamiya[4], Jian-Ren Shen[2], Kizashi Yamaguchi[3,4] ([1]*Grad. Sch. of Pure and App. Sci., Univ. Tsukuba*, [2]*Grad. Sch. Nat. Sci. & Tec., Okayama Univ.*, [3]*Grad. Sch. Sci, Osaka Univ.*, [4]*OCARINA, Osaka City Univ*)

Oxygen-evolving complex (OEC) is a key reaction center in photosystem II (PSII) which catalyzes the "2H2O + 4hv -> O2+ 4H+ +4e-" reaction through five redox states (Si, i = 0-4). Umena and co-workers have determined a high-resolution x-ray structure, and it was found that OEC is involved in many hydrogen-bonds (H-bonds) with waters and neighboring residues. However, it is not yet clarified for the relationship between H-bonds and OEC electronic structures. In this study, OEC electronic structures were examined at the S1 state in some different H-bond networks by using a high level QM/MM method. We used a large QM region including second coordination amino acids and waters (Total 380 atoms). Calculated ground state is singlet in consistent with experimental results.

1SAA-04 多参照波動関数理論で解く光合成系 II マンガンクラスタの電子構造
Entangled quantum electronic wavefunctions of the Mn_4CaO_5 cluster in photosystem II

Yuki Kurashige, Takeshi Yanai (*Institute for Molecular Science*)

It is a long-standing goal to understand the reaction mechanisms of catalytic metalloenzymes at an entangled many-electron level, but this is hampered by the exponential complexity of quantum mechanics. Here, by exploiting the special structure of quantum states and using the density matrix renormalization group, we compute near-exact many-electron wavefunctions of the Mn_4CaO_5 cluster of photosystem II, with more than 10^{18} quantum degrees of freedom. Our calculations support recent modifications to the X-ray crystal structure. We further identify multiple low-lying energy surfaces, highlighting multistate reactivity in the chemistry of the cluster. Mn spin-projections for current candidates were determined directly from our wavefunctions.

1SAA-05 OEC に関する理論的研究
Theoretical Study on OEC

Shin Nakamura (*RIKEN*)

The water oxidation mechanism in OEC is studied theoretically by BS-UHF method. We have obtained the most plausible form of S1 state which is consistent with various experimental data such as EXAFS, ESR and X-ray results. The possible S2 forms are also presented. Considering the experimental data of Sr replaced for Ca, we confirm our arguments on S1, S2 and partially on S3. Natural orbital analysis is used for the consistency. The role of protein environment for OEC fucnction is discussed based on the results of all atom (1,200,000) classical MD of PSII. Finally, OEC-inspired man-made electrode design-principle is to be presented.

1SAA-06 人工的な遷移金属錯体を触媒とする酸素発生反応
Water Oxidation Catalyzed by Artificial Transition Metal Complexes

Shigeyuki Masaoka (*IMS*)

Water oxidation ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$) is one of two half-reactions for water splitting, and is considered the main bottleneck for the development of energy-conversion schemes based on sunlight and/or electricity. In nature, the water oxidation is efficiently catalyzed by the oxygen evolving complex (OEC) in photosystem II (PSII). Because the replication of the OEC is extremely difficult, some easy-to-synthesize complexes have been investigated for the last decades. Recently we reported that some transition metal complexes serve as active water oxidation catalysts, the details of which will be presented in this talk.

1SAA-07 人工光合成の構築に向けた水の酸化触媒の開発

Development of molecular catalysts for water oxidation toward artificial photosynthesis

Masayuki Yagi (*Niigata Univ.*)

A photosynthetic photosystem II (PS II) model was developed by adsorbing, [(OH2)(terpy)MnIII(μ-O)2MnIV(terpy)(OH2)]3+ (1, terpy = 2,2':6',2''-terpyridine) as an oxygen evolving center and Ru(bpy)32+ (bpy = 2,2'-bipyridine) as a photoexcitation center onto mica. 1 is considered to work for photochemical water oxidation in mica adsorbate due to an efficient electron transport from deeply-intercalated 1 to S2O82- ions in a liquid phase via Ru(bpy)32+ photoexcitation near the mica adsorbate surface.

1SBA-01 動的人工細胞・分子ロボットの作製のための微小非平衡場の制御

Control of micro-sized nonequilibrium system for the construction of dynamic artificial cells and molecular robots based on microfluidics

Masahiro Takinoue (*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*)

Construction of artificial cells and molecular robots has been attracted much attention these days. To make them more complex and sophisticated, it is required to add dynamic features such as autonomous motion, autonomous information processing, etc. to them. In this presentation, I show two technologies for the construction of dynamic artificial cells and molecular robots: (i) a micro-sized nonequilibrium reaction system for an artificial cell; (ii) generation of anisotropic complex microhydrogel structures for cell-sized self-propelled matter. Finally, I would like to discuss the future of dynamic artificial cells and molecular robots.

1SBA-02 分子ロボティクス指向による人工細胞構築

Molecular robotics approach for constructing an artificial cell model

Shin-ichiro Nomura M.[1], Kei Fujiwara[1, 2] ([1]*Department of Bioengineering and Robotics, Division of Mechanical Engineering, TOHOKU University*, [2]*JSPS Research Fellows*)

Prototype artificial cell models with designed functional molecules are presented here. Artificial molecular devices based on a giant liposome were prepared to obtain specific properties that cannot be obtained from natural cells. In this context, artificial cell research is seen an extension of "molecular robotics" research. Cooperative and integrated chemical systems will be constructed from both artificial and semi-artificial molecular devices. Here, we present several aspects of the study models. : (1) gene-expressing cell model encapsulated in the liposome to simulate membrane protein synthesis, (2) multirole molecular device with a designed DNA nanostructure on the cellular membrane, and (3) designed molecules for compartmentalization. These artificial devices will be integrated to develop novel tools for constructing yet-another artificial cell.

1SBA-03 細胞サイズ液滴内での高分子混合系の相分離とゾル-ゲル転移

Aqueous phase separation and sol-gel transition of biopolymer blend in cell-sized droplets

Miho Yanagisawa (*Grad. Sch. Sci., Kyushu Univ.*)

In cells, phase separation and sol-gel transition of biopolymers plays important roles in regulating their structures, shapes and movements. To reveal the mechanism, cell-sized droplets coated by a lipid layer encapsulating polymers have been used as model cells [1]. When DNA and polyethylene glycol (PEG) bled in homogeneous phase was encapsulated in the droplets, phase separation was triggered in smaller droplets due to depletion effects among semi-flexible DNA and flexible PEG molecules. In addition, we report a phase behavior of PEG/gelatin system in droplets, where phase separation and gelation of gelatin complete with a decrease in temperature, and generate great variety of micro-gel patterns.

[1] M. Yanagisawa et al., Soft Matter, 9:5891, 2013.

1SBA-04 外部環境情報をリポソーム基盤分子ロボットの内部に伝達する分子センサーの開発

A development of molecular sensor that delivers environmental information to inside of liposome-based molecular robots

Koh-ichiroh Shohda, Akira Suyama (*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*)

In the molecular robotics project, a planned molecular robot which is based on a liposome or a gel as a body consists of molecular sensors, molecular computers, and molecular actuators. These components are communicated with single-stranded DNAs each other. We cover a development of molecular sensor which is a conjugated molecule between DNA strands and lipid. This sensor molecule located on a surface of liposome can hybridize with a complementary DNA/RNA strand which represents an environmental information. The hybridization causes a shape change of the molecular sensor, consequently a single-stranded DNA will be released into an inner water pool of liposome. The released DNA will be a trigger for molecular computers encapsulated in the liposome.

1SBA-05 アクチン線維とミオシン、細胞サイズの膜小胞を利用した分子アメーバ構築の試み

Construction of motile artificial cell model using actomyosin and cell-sized giant liposome

Kingo Takiguchi, Masahito Hayashi (*Grad. Sch. Sci., Nagoya Univ.*)

Cytoskeletal system, which consists of actin, microtubule, and their collaborating proteins and molecular motors, is a native superior micro machine. It is involved in a number of activities regarding morphogenesis, transporting, movement, and force generation, in a wide range of the biological hierarchy, for example, from swimming of unicellular organism to movements of multicellular organism that are caused by muscle contraction.

In order to utilize the performance, attempts to generate various molecular motions in vitro using purified actin and myosin and studies to develop cell-sized liposomes incorporating active actomyosin inside have been performed. Here, we introduce some of the outcomes, and describe problems to be overcome and outlook for the future.

1SBA-06 人工 RNA-Protein 複合体による細胞内外で機能する分子ロボットの創出にむけて

Synthetic RNA-Protein complexes to construct molecular robot in vitro and in cells

Hirohide Saito[1,2] ([1]*CiRA*, [2]*The Hakubi Center for Advanced Research*)

Can we construct molecular robot by leaning from nature? In naturally occurring systems, RNA-protein complexes (RNP)-mediated sophisticated nanomachines such as ribosome play important roles to control cellular functions. We aimed to design and construct synthetic RNP nanostructures that work in vitro and in human cells. Recently, we succeeded in visualizing RNA-protein interaction dynamics in single molecule resolution on the designed nanostructure. Furthermore, we could detect the specific cancer cells or control target gene expression by using the RNA nanostructures: desired functional proteins and RNAs could be attached on the scaffold. Synthetic RNA nanostructures could provide a useful tool to analyze dynamics of RNP interaction and control cellular functions.

1SBA-07 分子ロボットを制御する試験管内知能の実装

Implementation of *in vitro* intelligence for controlling molecular robots

Ken Komiya (*Interdisci. Grad. Sch. Sci. & Engi., Tokyo Tech.*)

Molecular robots are autonomous biomolecular systems, in which all components are made of synthetic biomolecular devices. Besides the development of component devices, including sensors, actuators and bodies, information processing circuits and their communication with other components should be established for achieving the sophisticated robots behaviors, just like living organisms, that are far beyond the current technologies. In the present study, we focus on the nucleic-acids-based intelligent devices to control molecular robots operation. Implementation of intelligence *in vitro* with biomolecular reaction would provide novel methodologies for the measurement, emulation and boosting of natural living organisms.

1SCA-01 遺伝子発現過程の情報ダイナミクス

Information Dynamics in Gene Expression Processes

Yuichi Taniguchi (*Quantitative Biology Center, RIKEN*)

Genetically identical cells do not have the same gene expression. It is because the cells intrinsically have diverse kinds of phenotypes that result from stochastic expressions of single proteins. This phenotypic diversity, also known as noise, can be the basis for helping cells cope and survive ever-changing environments. To reveal the system-wide architecture of the biological noise, we developed a system to quantify gene expressions in single cells with single molecule sensitivity at the proteome and transcriptome level. With this system, we discovered common rules or general mechanisms in population-level heterogeneity and time fluctuations of gene expression processes. In this presentation, we will report these findings and our recent progresses in the study.

1SCA-02 Inferring Kinetics Objectively from Single Molecule Time Series with Full Information Content

Li Chun-Biu (*Research Institute for Electronic Science, Hokkaido University*)

Statistics of the dwell times, the stationary state distributions (SSDs), are often studied to infer the underlying kinetics from single molecule finite-level time series. However, it is well known that the underlying kinetic scheme, a hidden Markov model (HMM), cannot be identified uniquely from the SSDs because some features of the underlying HMM are hidden by finite-level measurements. Here we quantify the amount of excessive information in a given HMM that is not warranted by the measured SSDs and extract the HMM with minimum excessive information as the most objective representation of the data. The method is applied to a single molecule enzymatic turnover experiment, and the origin of dynamic disorder is discussed in terms of the network properties of the HMM.

1SCA-03 確率的な細胞環境感知における適応的ダイナミクスの役割

Role of Adaptive Dynamics in Stochastic Cellular Sensing Systems

Tetsuya Kobayashi (*IIS, Univ. Tokyo*)

Sensing environment by receptors is basic cellular information processing, and acquisition of accurate information is crucial for making subsequent action. Experimental investigation revealed that our and other organism's sensory systems could detect extremely small signal even though receptor reactions are noisy. In addition, environmental signal generally contain various nuisance information that is not relevant for the decision-making. Nonetheless, biological sensory systems can effectively filter out such nuisance information.

In this talk, I demonstrate the role of adaptation for this filtering by using the theory of Bayesian information processing. Biological relevance of the result will be exemplified by using quantitative evidences for several sensory systems.

1SCA-04 細胞内時空間データからの方程式推定

Estimating inner-cell dynamics from spatio-temporal data

Shuji Ishihara[1], Daisuke Taniguhi[1], Satoshi Sawai[1,2] ([1]*Graduate school of Arts and Sciences, The University of Tokyo*, [2]*JST PERSTO*)

Accompanying with recent advances in experimental techniques, much quantitative data on the cellular processes, with finer resolution in time and space, are available. Theoretical methods should be developed that use these quantitative data as efficiently as possible, such as information contained in the fluctuation of the dynamics. Here we present a method to deduce a form of reaction-diffusion equations that describe dynamics of phophoinotitides lipids observed in *Dictyostelium* cells. Based on Bayesain procedure with smooth prior of the phase space, MCMC is implemented for the deduction. Bifurcation of the system is discussed by comparing with results from individual cells.

1SCA-05　確率的な ERK 活性ダイナミクスと細胞増殖制御

Stochastic ERK activity pulses induced by noise and cell-to-cell propagation regulate cell density-dependent proliferation

Kazuhiro Aoki (*Kyoto University, Graduate School of Medicine, Imaging Platform for Spatio-Temporal Information*)

The ERK MAP kinase plays a central role in the signaling cascades of cell growth. Here, we show that stochastic ERK activity pulses regulate cell proliferation rates in a cell density-dependent manner. A biosensor based on the principle of fluorescence resonance energy (FRET) revealed stochastic ERK activity pulses fired spontaneously or propagated from adjacent cells. Frequency, but not amplitude, of ERK activity pulses exhibited a bell-shaped response to the cell density and cell proliferation rates. Consistently, synthetic ERK activity pulses generated by a light-switchable CRaf protein accelerated cell proliferation. Taken together, these findings reveal a role of the stochastic ERK activity pulses in cell proliferation.

1SCA-06　細胞内シグナル伝達経路の情報コーディング

Information coding of cellular signaling networks

Shinya Kuroda, Shinsuke Uda (*Biophys. Biochem., University of Tokyo*)

Cellular signaling network can be regarded as a communication channel in the framework of Shannon's information theory. We can measure the distribution of phosphorylation of ERK and CREB and expression of IEGs products at a cell population level. We found that information transmission was generally more robust than averaged signal intensity despite pharmacological perturbations, and information transmission through unperturbed signaling pathways compensatorily increased in many signaling pathways. We propose that cells use information entropy as information, so that messages can be robustly transmitted despite noise and variation in molecular activities between individual cells.
Information coding will be discussed as a general property of cellular signaling.

1SDA-01　タンパク質膜透過促進因子 SecDF の構造と機能

Structure and function of SecDF, a membrane integrated protein translocation enhancing factor

Hiroyuki Mori[1], Kazuya Mito[1], Yukiko Machida[1], Tomoya Tsukazaki[2,3], Koreaki Ito[4], Yoshinori Akiyama[1] ([1]*Institute for Virus Research, Kyoto University*, [2]*Grad. Sch. of Biol. Sci., NAIST*, [3]*JST, PRESTO*, [4]*Faculty of Life Sciences, Kyoto Sangyo University*)

In bacteria, SecA, the translocation ATPase and SecYEG, the polypeptide-conducting channel, play central roles in protein translocation across the cytoplasmic membrane. Membrane proteins SecDF, conserved throughout the bacterial kingdom, form a complex with SecYEG and are required for efficient protein export. Thus, it is important to elucidate mechanisms of the SecDF enhancement of translocation. We discuss structure and function of SecDF on the basis of the crystal structure of the T. thermophilus ortholog and structure-instructed biochemical analyses of the E. coli system including site-directed in vivo photo-crosslinking. Based on the results, we propose that SecDF functions as a cation-driven molecular motor to pull a translocating polypeptide from the SecYEG.

1SDA-02　細菌べん毛ディスタルロッドの構造解析

High-resolution structure of the bacterial flagellar distal rod

Yumiko Saijo-Hamano[1], Katsumi Imada[2], Hideyuki Matsunami[3], Takashi Fujii[4], Keiichi Namba[1,4] ([1]*FBS. Osaka Univ.*, [2]*Dept. Macromol. Sci., Grad. Sch. Sci., Osaka Univ.*, [3]*Trans-Membrane Trafficking Unit, OIST*, [4]*QBiC, RIKEN*)

The flagellar rod is a competent drive shaft that transmits torque through the hook to the filament to propel the bacterial locomotion. The distal part of the rod is a helical assembly of FlgG, which shows an obvious sequence similarity with the hook protein. However, the mechanical property of the rod and the hook is quite distinct; the hook is a flexible universal joint, and the rod is a rigid drive shaft. To elucidate the structural basis of the mechanical property of the rod, we crystallized a core fragment of FlgG (FlgG47-227) and solved the structure at 2.0 Å. On the basis of the high resolution X-ray structure and the density map of the poly-rod obtained by electron cryomicroscopy, we will discuss the structure and mechanical property of the flagellar distal rod.

1SDA-03　バチルス属細菌のべん毛モーター固定子のイオン選択性と運動性

One stator that couples to multiple different ions: flagellar stator and motility of *Bacillus* spp.

Masahiro Ito (*Fac. Life Sci., Toyo Univ.*)

The bacterial flagellum acts as the propeller for cell locomotion in a variety of environments. The flagellar motor, consisting of the rotor and the stator, rotates the flagellar filament. All the bacterial flagellar motors characterized so far are energized by either transmembrane electrochemical gradients of protons or sodium ions that are coupled to motility via membrane embedded stator complexes. There are at least four groups of flagellar motors in alkaliphilic *Bacillus* species as determined by the properties of each flagellar stator. In 2012, our group identified a novel type flagellar motor which stimulates swimming speed under elevated K^+ or Rb^+ concentrations. In this symposium, we report our current progress on the novel type flagellar stator from *Bacillus*.

1SDA-04　バクテリア運動の驚異

Wonders of bacterial motility

Howard C Berg (*Department of Molecular & Cellular Biology and of Physics, Harvard University*)

Much is known about the motile behavior of *Salmonella*. and *Escherichia coli*. I will mention some history and then describe two recent vignettes, involving adaptation at the output of the sensory-transduction pathway. Receptor methylation is required for adaptation on the second time scale, which enables cells to make temporal comparisons and swim up spatial gradients of attractants. Without methylation, one still observes partial adaptation, on the minute time scale, as the motor shifts its operating point. The motor also adapts to changes in viscous load. When the load suddenly increases, additional force-generating units are added one by one; thus, the motor is a mechanosensor.

1SAP-01 UV-B photoreception by plant UVR8

John Christie (*University of Glasgow*)

The plant photoreceptor UV RESISTANCE LOCUS 8 (UVR8) triggers regulatory changes in gene expression that underpin photoresponses to UV-B. UVR8 is a 7-bladed b-propeller homodimer that monomerises in response to UV-B. Structural analyses, combined with mutagenesis and far-UV circular dichroism spectroscopy reveal that UVR8 forms a tryptophan-dominated dimer interface linked by a complex salt-bridge network. Salt-bridging arginines flank the excitonically coupled cross-dimer tryptophan “pyramid”. Photoreception disrupts these salt bridges to initiate signalling through CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1). These analyses establish how UVR8 functions as a photoreceptor without a prosthetic chromophore to promote plant survival in sunlight.

1SAP-02 植物における CPD 光回復酵素と UVB 抵抗性
UVB-induced DNA damage repair enzyme “CPD photolyase” and UVB resistance in plant

Jun Hidema (*Grad. Sch. Life Sci. Tohoku Univ.*)

UVB-induced cyclobutane pyrimidine dimers (CPDs) are one of principal cause of UVB-induced growth inhibition in plants grown under supplementary UVB radiation. The CPDs are mainly repaired by CPD photolyase, which absorbs blue/UVA light as an energy source to monomerize dimers. CPD photolyase belongs to DNA photolyase/blue light receptor family, and is widely distributed among species, ranging from bacteria to plants and mammals. CPD photolyase, which is encoded by a single-copy gene in the nuclear genome, translocates to chloroplasts, mitochondria, and nuclei and repairs CPDs, and is subjected to “triple targeting” in rice cells. These results indicate that plant may have evolved a CPD photolyase to protect cells from the harmful effects of UVB radiation.

1SAP-03 植物の青色光受容体 phototropin の全長でのシグナリング機構
Signaling mechanism in full-length phototropin, plant blue light receptor

Koji Okajima (*Osaka Pref. Univ.*)

Phototropin (phot) is a blue light (BL) sensor in plants and is involved in phototropism, chloroplast movement, stomata opening, etc. phot has two LOV (LOV1 and LOV2) as light perceiving domain and kinase as an output. Upon BL, kinase is activated through structural changes in LOV2 that is led by the formation of the adduct between FMN and Cys residue. We prepared full-length phot in green algae Chlamydomonas. Purified phot showed a photocycle and kinase activity in a light dependent manner. Small Angle X-ray Scattering indicated that conformational changes in full-length phot were induced by BL irradiation. The transduction mechanism will be discussed.

1SAP-04 植物における青色光に依存した気孔開口
Blue light-dependent stomatal opening in plants

Ken-ichiro Shimazaki, Atsushi Takemiya (*Dept. of Biol., Kyushu Univ.*)

We have demonstrated that phototropins initiate the blue light signaling and activate the plasma membrane H+-ATPase that drives stomatal opening. We also have indicated that type 1 protein phosphatase mediates the signaling between phototropins and the H+-ATPase. To identify other signaling components in this pathway, we developed a method for screening Arabidopsis plants impaired in blue light-dependent stomatal opening by infrared thermography. Using this method, we obtained a mutant that exhibited the complete loss of blue light-dependent stomatal opening. We identified the responsible gene as a novel protein kinase and named it as blue light signaling 1 (BLUS1) in Arabidopsis. We will report the functional role of BLUS1 in blue light-dependent sotmatal opening.

1SAP-05 フォトトロピンで誘導される葉緑体と核の運動機構
The mechanisms of chloroplast and nuclear movement mediated by blue light receptor phototropins

Masamitsu Wada (*Kyushu University*)

Chloroplasts and nuclei show their specific intracellular distribution patterns depending on light conditions. Under weak blue or white light chloroplasts gather at the light irradiated area, but they escape from strong blue or white light. The former accumulation response is mediated by blue light receptor phototropin1 (phot1) and phot2, but the latter avoidance response is mediated specifically by phot2. Chloroplasts use the newly found actin structure, chloroplast actin filaments (cp-actin filaments) for the movement. In this symposium, I will discuss how chloroplasts move with the cp-actin filaments. The mechanism of nuclear avoidance movement that is mediated by phot2 will also be discussed.

1SAP-06 フィトクロム A のモジュラー構造
The modular structure of phytochrome A

Akira Nagatani[1], Yoshito Oka[1,2], Yuya Ono[1], Yukiko Yoshikawa[1], Keio Kokaji[1], Nobuyoshi Mochizuki[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Plant Sci. Center, RIKEN*)

Phytochrome is a red(R)/far-red(FR)-light photoreceptor that regulates various aspects of plant development. Phytochrome A (phyA), which mediates atypical responses to continuous FR and a very low ammount of R, represents a highly specialized form of phytochromes. In this study, 16 chimeric phytochromes between phyA and phyB, a representative of canonical phytochromes, were constructed and expressed in transgenic *Arabidopsis* to identify domains that confer specific properties such as nuclear accumulation under FR, R-induced degradation and sensitization to R. Consequently, distinct structural modules were shown to be responsible for each of these properties. Hence, phyA is modularly structured to act as a highly-sensitive photoreceptor in seed plants.

1SAP-07 新規光受容体群シアノバクテリオクロムの吸収波長調節メカニズム

Color tuning mechanism of novel photoreceptors cyanobacteriochrome

Rei Narikawa[1,2] ([1]*Univ. of Tokyo, Dept. of Life Sci.*, [2]*JST, PRESTO*)

Cyanobacteriochromes (CBCRs) are cyanobacterial tetrapyrrole-binding photoreceptors that are distantly related to red/far-red light sensing phytochromes and cover the visible and near-ultraviolet regions of spectrum. CBCRs include many photoconversion types such as violet/green, blue/green, green/red and red/green reversible photoconversions. Many biochemical and spectral studies have identified that the diverse spectral properties are due to different chromophore species and different photoconversion mechanism. Recently, structures of chromophore-binding domains of red/green-type AnPixJ and blue/green-type TePixJ were determined. In this presentation, I introduce the color tuning mechanism of the diverse CBCRs based on this structural information.

1SBP-01 １細胞解析のための１分子シーケンシングシステムの開発

Development of Single Molecule Sequencing System for Single Cell Analysis

Sotaro Uemura (*RIKEN Center for Life Science Technologies*)

Recent transcriptome studies have shown that gene expression is invariably heterogeneous even in evidently similar cell types. Differences in transcriptomes may provide critical information on the composition of cell types in diseased tissues such as cancer or cancer stem cells.
Current single-cell techniques require several numbers of critical steps of preparation, such as cDNA synthesis or amplification steps. However these steps introduce multiple biases and significant sample loss that hardly reflect the original molecular counts of transcripts at single cell level. To solve these issues we are developing all in one direct single cell analysis system at single molecule detection, which contributes for minimization of number of steps and precise quantification.

1SBP-02 REAL-TIME MONITORING OF BIOMOLECULES IN ZERO-MODE WAVEGUIDES: DNA SEQUENCING AND BEYOND

Paul Peluso (*Pacific Biosciences*)

At Pacific Biosciences, we have developed a technology that observes individual biomolecules at work in real time. ZMWs are nanostructures that drastically reduce the effective optical observation volume, thereby permitting the use of higher concentrations of fluorescently tagged molecules for single-molecule studies. This technological advance provides the ability to monitor the speed, processivity, and efficiency of the enzyme to be exploited for new capabilities. The power of SMRT (Single Molecule, Real-Time) Sequencing technology - characterized by long read lengths and fast run times - is highlighted through examples such as finishing genomes, characterizing full-length transcript diversity, rapid pathogen sequencing, and the direct detection of epigenetic markers.

1SBP-03 タンパク質翻訳伸長過程の実時間ダイナミクス計測

Dynamics of translation elongation in real time

Joseph Puglisi, Albert Tsai, Jin Chen, Guy Kornbeg, Magnus Jonansson, Alexey Petrov, Sean O'Leary, Capece Mark (*Department of Structural Biology, Stanford University School of Medicine*)

Translation of proteins by the ribosome is a highly dynamic process, requiring coordination of mRNA, tRNA, protein factors and ribosomal conformation to drive the process with high speed and fidelity. We have applied a variety of single-molecule fluorescence approaches to observe conformational and compositional dynamics at each codon of an mRNA in real time. Our results have revealed the basic mechanisms of elongation and the dynamic origins of rare elongation events such as stalling, frameshifting and drug inhibition.

1SBP-04 Single Molecule Electrical Sequencing of DNA and microRNA

Masateru Taniguchi (*The Institute of Scientific and Industrial Research, Osaka University*)

Two paradigm shifts in DNA sequencing technologies, from bulk to single molecules and from optical to electrical detection, are expected to realize label-free, low-cost DNA sequencing. Here we report on single-molecule electrical sequencing of DNA and microRNA by a hybrid method of identifying single-base molecules via tunneling current and random sequencing. Our method reads short DNA and let-7 microRNA sequences. We have developed the method to control the translocation speed of single-DNA molecules passing through a nanopore, in an effort to obtain high accuracy and throughput in reading out sequences. The translocation speed can be controlled using gate voltage, in order to control electro-osmotic flow within a nanopore.

1SBP-05 類似配列の高速な全ペア列挙に基づく NGS データの解析手法

NGS data analyses based on ultra-fast all pairs similarity search

Kana Shimizu (*Computational Biology Research Center, National Institute of Advanced Industrial Science and Technology*)

Recent progress in sequencing technology calls for efficient computational methodologies for analyzing huge amount of DNA sequence fragments. In our recent study, we developed ultra-fast algorithm for evaluating sequence similarity of next generation sequencing (NGS) data. The algorithm, named SlideSort, finds all similar pairs from a set of NGS data, and serves as a building block for various important analyses including clustering analyses and identification of frequently appeared sequence patterns. In this talk, I will give a brief introduction of the algorithm and will show some application studies as well as further direction of our study.

1SCP-01 細胞競合を介した上皮の恒常性維持機構の分子基盤
Cell competition that regulates epithelial maintenance in Drosophila

Shizue Ohsawa[1], Kei Kunimasa[1], Tatsushi Igaki[1,2] ([1]*Lab.Genetics, Grad. Sch. Biostudies, Kyoto Univ.*, [2]*PRESTO, JST, Japan*)

Cell-cell interactions in multicellular organisms play crucial roles during normal development and homeostasis. 'Cell competition' is a form of cell-cell interactions in which cells with higher fitness survive and proliferate at the expense of neighboring cells with lower fitness. In *Drosophila* imaginal epithelia, clones of cells mutant for apico-basal polarity genes are eliminated by cell competition. To dissect the mechanism of this non-autonomous regulation of cell competition, we developed a "non-cell autonomous" genetic screen system. We isolated *elimination-defective* (*eld*) mutants that failed to eliminate neighboring polarity-deficient cells. The mechanism by which *eld* genes regulate the cell-elimination will be discussed.

1SCP-02 胚サイズ依存的な背腹軸パターンのスケーリング機構
Scaling of Dorsal-Ventral Patterning by Embryo Size-Dependent Degradation of Chordin

Hidehiko Inomata, Tatsuo Shibata, Yoshiki Sasai (*CDB., RIKEN*)

During early Xenopus development, a BMP gradient controls the dorsal-ventral pattern. Ventralizing signals such as BMP4 is secreted from the ventral region, while secreted BMP inhibitors such as Chordin emanate from the organizer on the dorsal side and counteract the ventralizing signals. Several studies have shown that a morphogenetic field exhibits a strong tendency of self-regulation. For instance, when a blastula embryo is bisected across the D-V axis, the dorsal half develops into a well-proportioned half-size embryo (Scaling).
Here we demonstrate that the robust formation of the graded DV pattern requires dynamic Chordin degradation linked with an axis-wide and size-sensitive feedback control involving the Chordin-proteinase inhibitor Sizzled.

1SCP-03 低温環境下の概日リズムの普遍性
Universality of circadian rhythms under low temperature conditions

Hiroshi Ito[1], Yoriko Murayama[2], Jun Tomita[3], Takao Kondo[4], Hiroshi Kori[5], Kazuhiro Yagita[6] ([1]*Faculty of Design, Kyushu University*, [2]*Priority Organization for Innovation and Excellence, Kumamoto University*, [3]*Institute of Molecular Embryology and Genetics, Kumamoto University*, [4]*Nagoya University*, [5]*Ochanomizu Univerisity*, [6]*Kyoto Prefectural University of Medicine*)

One of the key characteristics of all circadian rhythms is that the free-running period remains stable for a relatively broad range of ambient temperatures, referred to as "temperature compensation" of the period. Interestingly, outside of the range of temperature compensation, circadian clocks stop running and are arrested at a certain phase. Although essentially identical results have been found in various organisms, it remains unclear if temperature stimuli affect the circadian clock directly or indirectly through such as metabolic changes. In this presentation, we will focus on why circadian rhythms cannot be observed at low temperature conditions. We will furthermore address the conservation of type of bifurcation among species.

1SCP-04 ハエトリグモの奥行き知覚における視物質の吸収特性の寄与
Contribution of a visual pigment absorption spectrum to depth perception in the jumping spider

Takashi Nagata (*Dept Evol Stud Biol Sys, Sokendai-Hayama*)

Absorption spectra of visual pigments are adaptively tuned to optimize informational capacity in most visual systems. Our recent investigation of the visual pigments in a jumping spider revealed that the absorption spectrum of a pigment causes defocus in a part of the retina. Although defocus generally reduces visual acuity, the amount of defocus can theoretically provide a quantitative indication of the distance of an object. We will present behavioral evidence strongly suggesting a novel mechanism for depth perception in the spider based on the amount of image defocus. Our finding provides an example that a relationship of molecular properties and *in vivo* conditions are crucial for the functional role of the molecule.

1SCP-05 Optically detected magnetic resonance spectroscopy of nitrogen-vacancy centers for subnanoscopic measurement in vivo

Ryuji Igarashi[1], Yuta Kumiya[1], Takuma Sugi[2], Fuminori Sugihara[3], Hidehito Tochio[1], Yousuke Yoshinari[2], Yoshie Harada[2], Masahiro Shirakawa[1] ([1]*Department of Molecular Engineering, Graduate School of Engineering, Kyoto University*, [2]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*, [3]*Immunology Frontier Research Center (WPI-iFReC), Osaka University*)

Recent developments of fluorescence imaging techniques have enabled us to investigate the localization and dynamics of biological substances at a spatial resolution beyond the diffraction limit. However, such techniques cannot provide precise quantitative information about subnanoscopic dynamics of tissues, cells and biomolecules. Here we show a potential method for the subnano-scale resolved measurement of nitrogen-vacancy centers (NVCs) in nanodiamonds. This method is based on the property of NVCs that the fluorescence intensity sensitively depends on the ground state spin configuration which is affected by Zeeman interaction with external magnetic field. Using this method, we observed a revolving motion of a nanodiamond in an intestine of Caenorhabditis elegans.

1SCP-06 カイメン体内で細胞及び組織が共同作業で骨片を１つ１つ組み上げて立てる建築物「骨片骨格」形成の仕組み
HOW DO SPONGE CELLS BUILD UP THE HIERARCHICAL SPICULOUS SKELETON?

Noriko Funayama (*Department of Biophysics, Graduate School of Science, Kyoto Univ.*)

Spiculous skeleton construction is one of the most elegantly engineered self-organization in the organisms. It is fascinating to study how are the hierarchical spiculous skeleton is built up step by step by sponge cells which cannot see the final blueprint of the skeleton. Responses to physiological forces should be involved in these steps. By setting up a time-lapse imaging system and molecular biological tools, we found that spiculous skeleton construction is a dynamic event: mature spicules are carried by specific types of cells, one end of the spicules is raised up, fixed to the substratum, and then, additional tiers are building up by connecting additional spicules to the held-up spicules. I will talk and discuss about our current understanding of these processes.

1SCP-07 Synergistic action of mitosis and cell shape change in epithelial invagination

Takefumi Kondo, Shigeo Hayashi (*RIKEN CDB*)

Cell shape changes are induced by both internal forces generated from cytoskeletons and external forces from surrounding cells, and are critical for tissue morphogenesis. Cell division is also important for tissue shaping, but little is known how cell shape changes and mitosis are coordinated. We investigated the mechanism of epithelial invagination of the *Drosophila* tracheal placode, and found that mitosis actively facilitates invagination. This finding was unexpected because rearrangement of interphase cytoskeletons associated with mitosis has been thought to be incompatible with tissue morphogenesis, In this talk, we will present a model in which the synergistic action of mitosis and cell junction remodeling drives rapid and robust change in tissue architecture.

1SCP-08 生きるための細胞死～脳形態形成過程に細胞死が与える影響 Cell death for life ~ Impact of apoptosis on morphogenesis in brain development

Yoshifumi Yamaguchi (*Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo*)

Many cells die during development. Dysregulation of apoptosis, a major form of cell death, leads to neural tube closure (NTC) defects like exencephaly, although the mechanism is unclear. Observing apoptosis in a living context could help elucidate their origin, behavior, and influence on surrounding tissues. Using a newly developed transgenic mouse that stably expressed a genetically encoded reporter for caspase activation, we performed time-lapse imaging of apoptosis and morphogenesis in living embryos. This analysis revealed that inhibiting caspase activation perturbed and delayed the smooth progression of cranial NTC cells. We propose that cell removal by caspase-mediated apoptosis facilitates and ensues NTC completion within a limited developmental window.

1SCP-09 線虫 *C. elegans* の振動への馴化学習とその記憶の生物物理学的解析 Biophysical analysis of *C. elegans* mechanosensory learning and memory

Takuma Sugi[1,2] ([1]*iCeMS, Kyoto Univ.*, [2]*JST-PRESTO*)

Thanks to the unique futures-the body transparency and the simple neural circuit, *C. elegans* is only the model animal to noninvasively perform biophysical analysis at a single cell resolution *in vivo*. Despite such simplicity, after experiencing repetitive non-localized mechanical stimuli such as vibration, *C. elegans* can habituate to this uncomfortable stimulus and exhibit memory-based behavior. We here established the two imaging systems. First imaging system enabled us to perform high-throughput behavioral analysis and to identify the neurons responsible for memory formation. We further established the new calcium imaging system and is now quantifying the temporal activity pattern of the identified neurons in freely moving *C. elegans* during and after memory formation.

1SDP-01 Systems and Synthetic Biology of Biological Timings

Hiroki R. Ueda (*QBiC Riken*)

The logic of biological networks is difficult to elucidate without (1) comprehensive identification of network structure, (2) prediction and validation based on quantitative measurement and perturbation of network behavior, and (3) design and implementation of artificial networks of identified structure and observed dynamics.
Mammalian circadian clock system is such a complex and dynamic system consisting of complicatedly integrated regulatory loops and displaying the various dynamic behaviors. In this symposium, I will take a mammalian circadian clock as an example, and present the systems- and synthetic-biological approaches for understanding of biological timings.

1SDP-02 無細胞合成系によるタンパク質進化技術 Protein evolution by cell-free synthesis system

Chie Imamura (*Toyota CRDL*)

One of the important approaches towards cell synthesis is a protein synthesis. An *E.coli in vitro* coupled transcription/translation system is the most popular and efficient cell-free protein synthesis methods. It shows various advantages over *in vivo* protein production systems. Protein synthesis can be completed within a few hours even though in minute amounts of the reaction mixtures. Another advantage is easy modification of the composition of the reaction mixtures according to the requirements for the synthesis of each protein. According to these benefits, our novel expression system has allowed the high-throughput construction and screening of the large diversity of mutant enzymes. Here we show *in vitro* synthesis of proteins and the improvement of their functions.

1SDP-03 バクテリア生命システムとマイクロデバイスの融合 Hybrid system from a bacterium and a micro-device

Kazuhito Tabata, Rikiya Watanabe, Hiroyuki Noji (*Grad. sch. eng., Univ. of Tokyo*)

Reconstitution of a cell from molecules is highly awaited as one of the most exciting challenges in the current biology for the understanding of the principles of living molecular systems. Microfabricated reactors (microchambers) are considered as an ideal platform for cell reconstitution. To explore the feasibility of cell reconstitution with microchambers, we attempted to integrate a living bacterial cell with a microchamber. Protoplasts from E. coli cells were placed on microchambers, the top of which was sealed with lipid bilayer. A few 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.

1SDP-04　人工細胞の進化実験

Experimental evolution of artificial cell model

Tetsuya Yomo[1,2] ([1]*Graduate School of Information technology, Osaka University*, [2]*ERATO, JST*)

After the RNA world, polynucleotide, protein and lipid are supposed to self-assemble before reaching the last common ancestor of complex cells. Can we make a simple assembly of bio-molecules able to evolve to maintain and tune a life-like complex network? We have encapsulated RNA and other factors for protein synthesis into liposomes and emulsions, in which the information on RNA is translated into RNA replicase, which in turn duplicates the original RNA. With successive passage over many generations, the artificial model evolved to replicate its genome faster than any living cells by suppressing double strand RNA formation and developing parasite resistance.

1SDP-05　合成生物学と美学

Aesthetics related with synthetic biology

Hideo Iwasaki[1,2] ([1]*metaPhorest [BioAesthetics Platform]*, [2]*Waseda Univ.*)

Life has been always a matter of art. If we consider mimicking life as gestures towards inventing artificial life, then the first drawn animal/plant images may be interpreted as the origin of some aspects of biomimetics, artificial life, and synthetic biology. Recently synbio becomes attracting artists for several reasons. In 2010 NSF & ESPRC founded a program, “Synthetic Aesthetics”, where 6 pairs of scientists and artists/designers, including Oron Catts and I, have explored possible perspective of synthetic biology in terms of design or art. Our and others’ synbio-related art projects and their implications will be summarized, in relation with cultural/aesthetic notions of life.

1SEP-01　Computational Analyses of Protein Structures by Small Angle X-ray Scattering

Masaki Kojima, Yasumasa Morimoto, Takashi Nakagawa (*Tokyo University of Pharmacy and Life Sciences*)

The current bottlenecks in determining the protein structures require a new strategy using the simple design of an experiment, and SAXS is suitable for this purpose in spite of its low information content. First we demonstrated that SAXS constraints work additively to NMR-derived information in calculating structures. Next, structure calculations for nine proteins were performed using the SAXS constraints combined with the NMR-derived distance restraints for local geometry such as secondary structures or those for tertiary structure. The results show that the SAXS constraints complemented the tertiary-structural information for all the proteins. Based on these results, we were able to construct a coarse-grained protein model at amino acid residue resolution.

1SEP-02　X線溶液散乱と分子動力学シミュレーションで探る蛋白質の構造揺らぎ

Protein structural fluctuations investigated by small-angle X-ray solution scattering and molecular dynamics simulations

Tomotaka Oroguchi[1], Mitsunori Ikeguchi[2] ([1]*Department of Physics, Faculty of Science and Technology, Keio University*, [2]*Graduate School of Medical Life Science, Yokohama City University*)

Protein structural fluctuations play an important role in protein functions. Therefore, in order to understand protein functions, information on protein structural fluctuations in solution is crucial. For this purpose, we have developed the method called MD-SAXS, which uses the combination of small-angle X-ray solution scattering (SAXS) experiment and all-atom molecular dynamics (MD) simulation. In this method, the theoretical SAXS intensity of protein solution is calculated from atomic coordinates in MD trajectories with explicit treatment of the hydrated water molecules, and then compared with the experimental SAXS data. The method was applied to the studies of structural fluctuations of enzyme and intrinsically disordered proteins.

1SEP-03　カルビン回路調節複合体の SAXS と XRD による相関構造解析

Combined SAXS and XRD analysis of the Calvin cycle regulatory complex

Hiroyoshi Matsumura[1], Nobutaka Shimizu[2], Tsuyoshi Inoue[1] ([1]*Osaka University*, [2]*KEK*)

In oxygenic photosynthesis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK), the Calvin-cycle enzymes, are regulated to reversibly form the complex containing PRK, GAPDH, and the linker protein CP12, which provides light-dependent Calvin cycle regulation in a coordinated manner. CP12 acts as a linker to sequentially bind GAPDH and PRK to down-regulate both the enzymes. We previously reported the crystal structure of the GAPDH-CP12 complex; however, how association/dissociation of the PRK-GAPDH-CP12 complex coordinates the activities of PRK and GAPDH remains obscure, owing to the limited structural information. Here, we report the structural analysis of the complex, using small-angle X-ray scattering and X-ray crystallography.

1SEP-04　結晶構造解析と小角散乱の併用

Applications of SAXS in structural analysis with macromolecular crystallography

Min Yao (*Faculty of Advanced Life Science*)

X-ray crystallography is a powerful method to elucidate 3D structures of macromolecules at atomic resolution for understanding their biological function. Achieving structures of macromolecular complexes is a key challenge, since the crystallization limits its applications. In principle, SAXS is able to meet this challenge by providing information of the structural state in solution. In this presentation, two applications of SAXS with crystallography will be introduced. 1) Verify the particular tRNA-binding manner obtained from crystal structure of $tRNA^{His}$ guanylyltransferase; 2) Study on the conformation changes of translation initial factor eIF2β-NTD when interacting separately with partner protein eIF5 and eIF2Bε during translation initiation process.

1SEP-05 フォトンファクトリーにおける小角散乱ビームラインの刷新
Refurbishment of SAXS beamlines at Photon Factory

Nobutaka Shimizu (*IMSS, KEK*)

There is now two bending-magnet beamlines, BL-6A and BL-10C, for small-angle X-ray scattering (SAXS) at Photon Factory (PF), and BL-10C is especially optimized and used for Bio-SAXS experiment. A short-gap undulator beamline, new BL-15A is being constructed now, and this will be opened for users from January, 2014 after the commissioning. The automatic solution sample changer is developed, and the measurement system for high-throughput Bio-SAXS will be constructed at BL-15A in order to utilize a high brilliant beam generated by the undulator. Reconstruction of BL-10C is also planned, and optics and a diffractometer of BL-10C will be replaced and upgraded in the spring of 2014. The details of these refurbishing plans and the current status of beamlines will be presented.

1SEP-06 SPring-8 理研構造生物学ビームライン I（BL45XU）の現状
Current status of RIKEN Structural Biology Beamline I (BL45XU) at SPring-8

Takaaki Hikima[1], Hiromi Sato[1], Yoshiaki Kawano[1], Go Ueno[1], Kunio Hirata[1], Hironori Murakami[1], Naoto Yagi[1,2], Masaki Yamamoto[1] ([1]*RIKEN SPring-8 Center*, [2]*JASRI/SPring-8*)

BL45XU equipped with tandem undulators consists of two experimental station; small-angle X-ray scattering (SAXS) and small- and wide-angle X-ray scattering (SWAXS). Both equipments can be independently carried out. In the SAXS-station, time-resolved structures of non-crystalline biological materials such as protein, nucleic acid solution, membrane, muscle, and micelle system under various condition, as well as polymer materials, are studied by using SAXS and diffraction technique.

2SAA-01 “非生物的揺らぎ”から“生物的揺らぎ”へ：そのからくりを問う
Turning random fluctuations into biological motions: the art work by Nature

Kazuyuki Akasaka (*High Pressure Protein Research Center, Institute of Adanced Technology, Kinki Univversity*)

The dynamism of life must depend critically on the dynamism of bio-macromolecules themselves, notably proteins. This dynamism originates from “weak” chemical interactions or interatomic potentials that allow atoms of bio-macromolecules to fluctuate their positions under physiological conditions. The fascination lies in the fact that the basically random “non-biological fluctuations” of atoms have been “upgraded” into specific “biological motions” that couple with specific biological reactions. The upgrading has been performed by the life itself during its 3.8 billion years of evolution. Part of this miraculous work and its consequence are being disclosed throughout the atomic, molecular, cellular and body levels, with “pressure” as common variable.

2SAA-02 非生物的揺らぎから“生物的揺らぎ”への“水”の役割
Role of water to convert “non-biological” fluctuation to the “biological” one

Fumio Hirata (*Univ.Ritsumeikan*)

Two important molecular processes are working in living systems: “self-organization” and “molecular recognition.” A typical example the latter is the binding of substrates to the active site of an enzyme. Those processes do not take place spontaneously as long as protein and substrate molecules are exsisting in vacuum. The two processes are inevitably concerned with the structural fructuation of protein. So called “induced fitting” or “conformational selection” in the molecular recognition are such processes driven by the structural fluctuation, and it is “water” what makes the processes possible in native condition. Water is the material that converts the “non-biological” fluctuation of protein to the “biological” one. In this talk, I present a new theory to describe the structural fluctuation of protein in the aqueous environment.

2SAA-03 キャビティに依存した c-Myb R2R3 の構造揺らぎと DNA 結合能
Cavity-dependent conformational fluctuation and DNA-binding function of c-Myb R2R3

Masayuki Oda (*Grad. Sch. Life and Environ. Sci., Kyoto Pref. Univ.*)

The individual repeats, R2 and R3, of the minimum specific DNA-binding domain of c-Myb have similar structures. The difference between R2 and R3 is that R2 has an internal cavity, which is required for the specific DNA-binding but decreases the stability. With increasing pressure, the Trp fluorescence peaks of R2 and R2R3 showed red shifts, and the NMR signals were broadened site-specifically with changing the chemical shifts. In comparison with the cavity-filling mutant, we could detect an extensively hydrated and folded state of R2 under high pressure, which should be present under physiological conditions in equilibrium with major populated native-state. The cavity-dependent conformational flexibility gained by the hydration would facilitate the DNA-binding of R2R3.

2SAA-04 圧力摂動やアミノ酸変異により生じる生物的揺らぎの変化：ユビキチン
Pressure and mutation enhances specific biological motion: Ubiquitin

Ryo Kitahara (*College of Pharm. Sci., Ritsumeikan Univ.*)

High pressure NMR spectroscopy revealed that ubiquitin and ubiquitin like proteins, NEDD8 and SUMO-2, have closely similar high-Gibbs energy states. These results indicate that high-energy states are conserved in molecular evolution. Here, we demonstrate that rational mutation based on the structure and dynamics characteristics of ubiquitin obtained from high-pressure NMR experiments is a new strategy for amplifying particular fluctuations in the protein. Turning the hydrogen-bond between I36-Q41 down by mutation substantially increases a population of the high-energy state N2. The N2 state produced by high pressure and mutation is closely similar in structure and dynamics. The N1-N2 fluctuation matches changes seen on binding to the E2 conjugation enzyme.

2SAA-05 イオンチャネルのゆらぎは細胞応答にどのように利用されているのか？
How do cells make use of single channel fluctuations for their responses?

Masahiro Sokabe (*Mechanobiol. lab., Nagoya Univ. Grad. Sch. Med.*)

Channel proteins fluctuate between open and closed states driven by thermal fluctuations. Proper stimuli (S) alter free energy levels of the two states to control the open probability that shows a sigmoid-curve with respect to S. As the cell response (R) is the sum of single channel activities, R also follows a sigmoid-curve with a threshold and saturation. The maximum sensitivity occurs at the inflection point where fluctuations are largest, indicating that cells can increase sensitivity by keeping a moderate spontaneous activity. Another property characterizing R is the response speed that is governed by the open to/from closed transition rates. Sub-molecular mechanisms how open-close fluctuations are regulated will be discussed based on channel protein structures.

2SAA-06 ヒトで最進化した重力適応：揺らぎの階層性と分子シャペロン
Gravitational adaptation, evolved at the most in the human being: hierarchy of fluctuation and molecular chaperone

Yoriko Atomi[1], Eri Fujita[1], Miho Shimizu[1], Tomoaki Atomi[2], Noboru Hirose[2], Kazuya Tanaka[2], Katsuya Hasegawa[3] ([1]*Tokyo Univ. of Agr. and Tech.*, [2]*Teikyo Univ. Sci.*, [3]*JAXA*)

Cells in the multicellular organism are dynamically regulated by cytoskeletons, which provide tension, shape, and respond to the mechanical stimuli. Among them tubulin/microtubule system shows intrinsic property of “dynamic instability (DI)”. Bipedal human beings well develop anti-gravitational muscles, so as they can stand, move, and maintain body weight and posture without fatigue. Long-lasting “tonic” and rhythmic contractions increase the molecular chaperone, αB-crystallin (αB), which localizes at areas where receiving tension in the cell. Time-laps imaging of GFP-αB in beating heart cell shows striated patterns. Use reagents impeding DI and FRA analysis shows αB’s rapid association with tubulin, which links hierarchy of biological fluctuation to human health.

2SBA-01 Designing the nano-reaction field: Introduction and application to motor protein research

Hisashi Tadakuma (*Graduate School of Frontier Science, The University of Tokyo*)

Recent progresses in technology allow us to design the reaction field. I will first introduce the two approaches of designing reaction field: bottom-up and top-down approach. Especially, I will focus the recent bottom-up approach using DNA nano-technology. The three dimensional structure of DNA nano-structure could be predicted using computer aided design software, and many kinds of material (e.g. protein, metal etc) could be attached to the specific position within DNA nano-structure on-demand, meaning one can design the reaction field in nano- to micro-meter scale. These characters may open up new research area in Biophysics. In the latter part of the talk, I will also introduce our recent achievements.

2SBA-02 Programming Nucleic Acids Self-Assembly

Peng Yin (*Wyss Institute for Biologically Inspired Engineering*)

I will discuss my lab’s (http://molsys.net) research on engineering synthetic, nucleic acid-based nanostructures and applications. We have recently invented a general framework for programming the self-assembly of short synthetic nucleic acid strands into prescribed target shapes or demonstrating their prescribed dynamic behavior, e.g. 2D (Nature 2012) and 3D (Science 2012) structures on the 100-nanometer scale with nanometer precision, or dynamic behavior of DNA hairpins such as catalytic circuits and autonomous locomotion (Nature 2008). By interfacing these synthetic, nucleic acid nanostructures with functional molecules, we are developing a diverse range of applications. I’ll also discuss our ongoing work to move the nanostructures from test tubes to living cells.

2SBA-03 DNA セルフアセンブリによるナノ粒子超構造制御
DNA-mediated Nanoparticle Assembly

Miho Tagawa[1], Takumi Isogai[1], Eri Akada[1], Syunta Harada[1], Toru Ujihara[1], Kevin Yanger[2], Oleg Gang[2] ([1]*Dep. of Materials Sci. and Eng., Nagoya Univ.*, [2]*Brookhaven Nat. Lab.*)

We demonstrate the formation control of lattice using DNA-functionalized gold nanoparticles (DNA-AuNPs) linked by designed DNA nanostructures. Using SAXS analysis, we revealed the assembly of DNA-AuNPs, linked by DNA tetrahedral constructs, into a diamond structure. The successful formation of this crystal structure demonstrates a power of the proposed approach for assembly of complex lattices using designed DNA nanostructures. We will also present the recent progress on two-dimensional programmable assembly of nanoparticles on supported lipid bilayers (SLBs). Owing the high mobility of lipid molecules, two-dimensional diffusion of DNA-AuNPs on SLB was achieved and DNA-AuNPs assemble into a densely-packed single-layered two-dimensional lattice through DNA hybridization.

2SBA-04 DNA ナノ構造上での分子運動の直接観察
Direct observation of molecular motions on the DNA nanostructure

Masayuki Endo (*Institute for Integrated Cell-Material Sciences, Kyoto University*)

Direct imaging of the molecular motions is one of the fundamental issues for investigating the mechanical behavior of the molecules during the reactions. We designed various DNA nanostructures using DNA origami method for the preparation of single-molecule observation scaffolds. Using the designed DNA scaffold and high-speed atomic force microscopy (AFM), the behaviors of the enzymes were directly observed in the target dsDNAs placed in the DNA nanostructure. DNA structural changes were also visualized. Single-molecule motions of a DNA motor were investigated on the DNA origami surface. The combination of the designed DNA scaffold modified with target DNA strands and high-speed AFM is valuable for visualizing and analyzing the biological reaction events.

2SBA-05 モータータンパク質集合体の自己組織化を操る
Controlling self-assembly of motor protein ensembles

Ken'ya Furuta (*Bio ICT lab, NICT*)

Intracellular transport is thought to be achieved by teams of motor proteins bound to a cargo. However, the coordination within a team remains poorly understood as a result of the experimental difficulty in controlling the number and composition of motors. Here, I used experimentally defined assemblies in vitro to investigate how the functions of motor proteins depend on these factors. I linked multiple molecules of different types of kinesin motors, processive kinesin-1 or nonprocessive kinesin-14, or cytoplasmic dynein motors. TIRF microscopy and optical trapping experiment showed that the efficient cooperation among motors depends inversely on the individual motor processivity, and revealed the unexpected feedback mechanisms among dyneins in collective transport.

2SBA-06 ミクロ閉鎖空間でアクトミオシン集合体がつくる秩序構造
Self-organized pattern formation by actomyosin mixtures in a cell-size confined space

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

Eukaryotic cells possess various kinds of cytoskeletal structures composed of actin filaments and myosin motors. To date, signaling cascades that regulate assembly of actin cytoskeleton have been clarified, however, the self-assembly mechanisms still remain unclear. To elucidate the basic features of cytoskeletal assembly, we encapsulated purified proteins inside cell-size confined spaces (water-in-oil emulsions or liposomes). By controlling the concentrations and the molar ratios of actin, myosin, and the regulatory factors (e.g., F-actin bundling factors), we found that ordered patterns reminiscent of actin cortex and contractile rings were self-organized. We will present dynamics of these patterns and discuss the assembly mechanisms in a confined microscopic space.

2SBA-07 Directed actin self assembly and contractility

Laurent Blanchoin (*CEA Grenoble*)

The organization of actin filaments into higher-ordered structures governs eukaryotic cell shape and movement. We have developed a micropatterning method that enables the spatial control of actin nucleation sites for in vitro assays. These actin templates were used to study the response of oriented actin structures to myosin-induced contractility. We determine that myosins selectively contract and disassemble anti-parallel actin structures while parallel bundles remain unaffected. This "orientation selection" mechanism reveals how the dynamics of the cellular actin cytoskeleton is spatially controlled by contractility. Further application of this method will be presented in particular data on the reconstitution of a cellular lamellipodium or 3D electrical connections.

2SCA-01 カリウムイオンチャネル KcsA のゲート開閉とリンクした脂質膜中での集合・離散ダイナミクス
Gating-related clustering-dispersion dynamics of the KcsA potassium channel on the membrane

Ayumi Sumino[1], Daisuke Yamamoto[2], Takashi Sumikama[1], Masayuki Iwamoto[1], Takehisa Dewa[3], Shigetoshi Oiki[1] ([1]*Fac. Med. Sci., Univ. Fukui*, [2]*Fac. Sci., Univ. Fukuoka*, [3]*Grad. Sch. Eng., Nagoya Inst. Tech.*)

KcsA is a pH-dependent potassium channel, and with the available crystal structure the gating mechanism has been studied extensively. We have elucidated the open gate structure of the KcsA channel in the membrane-embedded condition using atomic force microscopy (AFM). Here, we found the reversible clustering-dispersion dynamics of the channel. At neutral pH, the closed channels form nano-clusters. When pH was changed to acidic, the channels in the clusters slightly shortened their longitudinal length, but still remained closed, and then dispersed until the channels were isolated separately with their gate open. These results suggest that the gating conformational changes are associated with the inter-molecular interaction, leading to the nano-cluster formation.

2SCA-02 薬理解析により明らかになった、原核生物由来の膜電位感受性 Na チャネルにおける内腔の構造変化
The conformational rearrangement of the inner vestibule revealed by the pharmacological analysis of prokaryotic voltage-gated Na channels

Takushi Shimomura, Katsumasa Irie, Yoshinori Fujiyoshi (*CeSPI, Univ. Nagoya*)

We investigated the inhibition mechanism of prokaryotic voltage-gated Na channels (NavBacs) by quaternary ammoniums (QAs) and local anesthetics, which are the inhibitors for K and Na channels, respectively. Mutagenesis study showed that a key residue in the inner vestibule is important for both local anesthetic and QA recognition. The QAs are not accessible to the inner vestibule unless the channels are activated, as generally observed in K channels. In contrast to this similarity, the QA action to NavBacs was different in that the bound QA is robustly trapped in the open state, but released in the inactivated state. These results imply that the inner vestibule of Na channels, the inhibitor occupying site, substantially rearranges its conformation after channel opening.

2SCA-03 電位依存性 K+チャネルにおけるイオン透過機構に関する分子動力学的検討
Molecular Dynamics Study on Ion Conduction Mechanisms of a Voltage-sensitive Potassium Channel

Kota Kasahara[1], Matsuyuki Shirota[2,3], Toshiyuki Saito[2], Hiroko Kondo[2], Kengo Kinoshita[2,3,4] ([1]*IPR, Osaka Univ.*, [2]*Grad. Sch. Information Sci, Tohoku Univ.*, [3]*ToMMo, Tohoku Univ.*, [4]*IDAC, Tohoku Univ.*)

Voltage-sensitive potassium channels conduct essential functions for life system by permeating potassium ions across plasma membranes. Molecular mechanisms of their ion conduction processes are still largely unclear because direct observation of atomic motions in such a complex system is not straightforward by experimental techniques. Here, we present a molecular dynamics study of a Kv1.2 potassium channel under several conditions in ion concentration and membrane voltage. As a result, ion conductions went along in two distinct mechanisms, and preference in these mechanisms depends on both of the ion concentration and membrane voltage. High concentrations and low voltages tended to retain a larger number of potassium ions in the selectivity filter.

2SCA-04 Ligand-induced conformational changes in the cytoplasmic domain of inward rectifier potassium channels

Atsushi Inanobe (*Dept. Pharmacol., Grad. Sch. Med., Osaka Univ.*)

Mammalian inward rectifier K^+ channels (Kir) participate in the formation of deep resting membrane potentials, the regulation of cell excitability, and the transport of K^+ ions. Four subunits of Kirs assemble to form transmembrane and cytoplasmic domains: the former primarily functions as a gate and the latter receives various stimuli to control the gate. Thus, ligand-induced structural alteration within the cytoplasmic domain governs the channel activity. Electrophysiological and structural analyses for the cytoplasmic domain of Kir subunit Kir3.2 revealed that the pore expansion at the center of the domain is an intrinsic conformational change for ion conduction and the change is supported by the rearrangement of two pore-facing structural elements within the domain.

2SCA-05 高速 AFM によるアクアポリン 4 チャネルの直接観察 Direct observation of aquaporin-4 channels by high speed AFM

Hayato Yamashita, Shinnosuke Aizu, Jungo Kato, Yoichiro Abe, Masato Yasui, Yoshiro Sohma (*Pharmacol., Keio Univ. Med. Sch.*)

Aquaporin-4 (AQP4) is the predominant water channel in the human brain and involved in cranial nerve diseases such as brain edema and neuromyelitis optica (NMO). It is known that AQP4 assembles as regular square arrays in astrocyte endfoot. However the structure-function relationship of array formation by AQP4 still remains unrevealed. We prepared proteoliposome of purified human AQP4 and observed them by high speed atomic force microscopy (HS-AFM). This microscopy showed AQP4 M23 isoform assembled in large orthogonal arrays composed of AQP4 tetramer whereas M1 isoform did not. Furthermore, we visualized the binding process of autoantibodies to AQP4s which causes NMO. These direct observations will help us to understand the mechanism of AQP4-related diseases.

2SDA-01 少数個分子の協働：その機構と意味解明へのアプローチ Approaches to understand cooperative systems of small numbers of molecules

Keiko Hirose (*Biomedical Res. Inst., AIST*)

In cells, there are systems in which a small number of molecules work cooperatively by means of specific interactions or other factors. For example, in flagellar axonemes, each outer arm dynein molecule has direct interactions with just two neighboring dynein molecules out of thousands of dynein molecules present in the axoneme. Individual dynein molecules also communicate with other dyneins via the force they produce. By making these cooperative systems, the molecules would gain functions different from either those of single or multiple molecules. In this talk, different approaches to study the systems composed of small numbers of molecules and recent findings will be discussed.

2SDA-02 神経軸索への極性輸送の構造的基盤 Structural Basis for the Polarized Axonal Transport in Neuron

Yasushi Okada (*QBiC RIKEN*)

Newly sysntesized axonal plasma membrane proteins are transported from TGN directly into axons. This polarized axonal transport is mainly driven by kinesin-1 motors, and we have previously shown that the motor domain of kinesin-1 preferentially runs along axonal microtubules than dendritic microtubules. As the basis for this preference, we have found that axonal microtubules, especially those at the initial segment of axons, are enriched with GTP-tubulin. In vitro binding assay revealed approximately threefold stronger binding of kinesin-1 motor domain to GTP-form microtubules than to GDP-form microtubules. We will discuss its mechanism by combining the structural analyses and the computer simulation.

2SDA-03 Microtubule organisation and dynamics in the anaphase spindle: properties of Cin8

Thomas Surrey (*Cancer Research UK*)

During anaphase of mitosis, various proteins in the midzone bundle and slide antiparallel microtubules. It is an open question of how these proteins cooperate. Kinesin-5 Cin8, the major motor that drives spindle elongation in budding yeast, has an unexpected property: It switches directionality depending on motor number (Roostalu et al, Science, 2011). When many motors crosslink antiparallel microtubules, they move towards the microtubule plus-end, like in cells during spindle elongation. However, when individual motors interact with single microtubules, they move into the minus-end direction. I will present recent experiments addressing the molecular mechanism underlying this directional switch and discuss its relevance for the in vivo situation.

2SDA-04 生細胞内少数分子を調べるための蛋白質ラベル化技術 Protein labeling technology for investigating small number molecules in living cells

Shin Mizukami[1,2] ([1]*Osaka Univ., Graduate School of Engineering*, [2]*Osaka Univ., IFReC*)

Protein labeling technology holds promise to overcome various limitations in fluorescent protein-based live cell imaging. Hence this technology has attracted an increasing attention in life sciences. We recently developed a novel protein labeling system using a mutant β-lactamase and synthesized β-lactam probes. The appropriate molecular design enabled fluorogenic protein labeling or intracellular protein labeling. Now, we are trying the artificial regulation of cellular protein dynamics or functions by exploiting protein labeling technologies. Through this approach, we are trying to construct a new methodology to clarify the significance of small number molecules in cells.

2SDA-05 １個から数個の分子が引き起こす運動と酵素反応のイメージング

Imaging of single to a few number of molecules in motion and their enzymatic reactions

Tomoko Masaike[1,2], Koji Ikegami[3], Mitsutoshi Setou[3], Hiroshi Suzuki[4], Takayuki Nishizaka[5] ([1]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*, [2]*PRESTO, JST*, [3]*Dept. Cell Biol. and Anat., Hamamatsu Univ. Sch. Med.*, [4]*Dept. Biochemistry, Asahikawa Medical Univ.*, [5]*Dept. Physics, Gakushuin Univ.*)

The aim of our research is to visualise how motions and functions of proteins are coupled when they are in small numbers. We detected dynamic motions of F1-ATPase and a cilium through fairly large polystyrene or magnetic beads under optical microscopes. Moreover, we monitored local conformational changes of proteins by detecting orientation of fluorophores under TIRF microscopy. Furthermore, we are developing a system to detect inorganic phosphate released from enzymes by encapsulating phosphate-binding protein in microchambers. This system accomplishes micromolar concentrations by small numbers of molecules. These observation systems will be integrated and thus contribute to understanding of biological systems.

2SDA-06 骨格筋ミオシン分子複合体の力発生に特化したミオシン 1 分子の特性とダイナミクス

Molecular properties and dynamics of single skeletal myosins designed for force generations in ensemble of myosin molecules

Motoshi Kaya, Hideo Higuchi (*University of Tokyo Dept of Physics*)

In our previous study, we suggested that the nonlinear elasticity of skeletal myosin might be advantageous feature for force generations of myosin ensemble. Moreover, our recent experiments showed cooperative force generations between myosins particularly against high loads. However, these experimental interpretations were made based on observations of a single actin filament interacted with myosins rather than direct observations of myosin molecules. Therefore, our next challenge is to directly observe single myosin molecules during force generations by labeling the regulatory light chain with gold nanoparticles, while force outputs of myofilament are monitored by optical tweezers. From this approach, molecular dynamics of myosin force generation will be discussed.

2SDA-07 分子イメージングから要素間の高次相互作用の定量化に向けて

Toward quantifying higher-order interactions among elements from molecular imaging

Tamiki Komatsuzaki[1,2,3] ([1]*Research Institute for Electronic Science, Hokkaido University*, [2]*Graduate School of Life Science, Hokkaido University*, [3]*Research Center for Integrative Mathematics*)

How does each element communicate with each other in complex systems and in what order of interactions? How can one quantify such hierarchical interactions solely from a set of data? In this presentation, we would like to overview and generalize the recently-developed information-theoretic quantity, connected information (Schneidman et al., PRL 91, 238701 (2003); Nature 440, 1007(2006)) to quantify the hierarchical interactions buried in complex systems. While the connected information is formulated globally to include all contributions from the elements, we derive decomposition rules for the connected information to capture local interactions. A possible application to molecular imaging is discussed.

2SEA-01 アドヘレンスジャンクションにおける張力感受性と上皮形態形成

Force sensitivity of the adherens junction and epithelial morphogenesis

Shigenobu Yonemura (*Electron Microscope Laboratory, Riken Center for Developmental Biology*)

Recent progress has shown that the adherens junction (AJ) is not only a devise for cell-cell recognition and adhesion but also a platform for mechanotransduction. Several years ago, we found that the AJ is a force-sensitive junction that can develop in terms of vinculin accumulation and ultrastructure when forces are applied so that it can resist and transmit the strong forces. We further elucidate that the force-sensitivity resides in alpha-catenin molecule, an essential component of the cadherin-catenin complex. In this talk, AJ structure and the basic function of alpha-catenin are reviewed first. Then, our recent progress on the understanding of the involvement of the force-sensitivity in epithelial morphogenesis especially in the 3-D culture system will be shown.

2SEA-02 構造細胞生物学の生物物理学的こころ

Biophysical views in structural cell biology

Toshio Hakoshima (*Nara Institute of Science and Technology*)

Recent advances in studies of cell-cell junctions and cell-matrix adhesions reveal that these junctions and adhesions play a role in mechano-sensing of applied forces and transduce the forces to chemical signals. Mechanotransduction by proteins comprising these contacts is now believed to control cell growth, division, motility and ultimately morphogenesis of tissues and organs. Mechanical and structural studies of these sensor proteins and mathematical description of cell mass have come on the stage center of the new era of cell biology.

2SEA-03 AFM を用いた接着結合分子の力学挙動解析

Mechanical Evaluation of Molecules at Adherens Junction using AFM

Sung-Woong Han[1], Koichiro Maki[1], Yoshinori Hirano[2], Toshio Hakoshima[2], Taiji Adachi[1] ([1]*Kyoto University*, [2]*Nara Institute of Science and Technology*)

Adherens junctions (AJs) are essential for the maintenance and morphogenesis of epithelial cell sheets. AJ is composed of several transmembrane adhesion proteins; E-cadherin, β-catenin, α-catenin, and vinculin, are directly or indirectly anchored to actin filaments. α-catenin is known as a linker protein between β-catenin and the actin cytoskeleton for AJ formation. It has been proposed that that α-catenin senses tension from the adjacent cell and changes the conformation, thus it results in the vinculin recruitment. However the molecular mechanism of α-catenin conformational change by tension is still unclear. Here we introduce the probing the mechanical evaluation of the AJ molecules behavior using an AFM single molecule force spectroscopy.

2SEA-04 Computational biophysics on epithelial tissue deformation: from molecular to tissue scale

Yasuhiro Inoue[1], Satoru Okuda[2], Tetsuya Fujii[3], Kohei Ohto[3], Taiji Adachi[1] ([1]*Inst. Front. Med. Sci., Kyoto Univ.*, [2]*CDB, RIKEN*, [3]*Dept. Microeng., Kyoto Univ.*)

Tissue shape emerges from multicellular dynamics with the balance of mechanical forces, which are sensed by molecules altering cell activities to regulate morphogenesis. In this study, we introduce a multiscale computational approach to reveal dynamics ranging from molecular to tissue scales. At the tissue scale, we suggest a vertex-based model to analyze multicellular dynamics and demonstrate dynamic epithelial sheet deformations driven by cell proliferation in silico. To reveal a mechanosensing mechanism at the molecular scale, we conduct molecular dynamics simulations of an intracellular protein. Through this study, we provide future perspectives for evaluating the manner in which multicellular and molecular dynamics can be coupled to regulate morphogenesis.

2SEA-05 軸索伸長のためのシグナルー力変換機構
Signal-Force Transduction in Axon Outgrowth

Naoyuki Inagaki (*Grad. Sch. Bio., NAIST*)

Motile cells receive external signals and achieve properly controlled cellular protrusion and migration. However, how cell signaling controls forces for cellular motility remains unclear. Shootin1 functions as a linker molecule that couples F-actin retrograde flow and the substrate at neuronal growth cones. We found that a chemoattractant, netrin-1, positively regulates traction forces at axonal growth cones via Pak1-mediated shootin1 phosphorylation. This phosphorylation enhanced the interaction between shootin1 and F-actin flow, thereby promoting F-actin-substrate coupling, force generation, and concomitant filopodium extension and axon outgrowth. Thus, shootin1 is located at a critical interface, transducing a chemical signal into traction forces for axon outgrowth.

2SEA-06 構造生物学からのコメント
Comments from the point of view of structural biology

Shuya Fukai[1,2,3] ([1]*Synchrotron Radiation Research Organization, The University of Tokyo*, [2]*Institute of Molecular and Cellular Biosciences, The University of Tokyo*, [3]*JST CREST*)

Comments will be presented from the point of view of structural biology.

2SAP-01 ロドプシン研究の新しい流れ
New trends in rhodopsin studies

Yoshinori Shichida (*Department of Biophysics, Graduate School of Science, Kyoto University*)

The term “rhodopsin” was originally used for the photoreceptive protein involved in the rod photoreceptor cells in our eyes. Nowadays, however, this term is used for photoreceptive proteins that contain retinal as a chromophore. Rhodopsins are classified into two types; Type I and Type II rhodopsins. Recent developments in genome projects have identified several thousands of rhodopsin genes. In addition to their well-known role as G protein-coupled receptors, rhodopsins have been known to function as pumps, channels, and even as regulators of gene expression. In the present talk, I will introduce the new research trends, including studies on functional analysis of rhodopsins by use of evolutionary trace and those toward optogenetics applications.

2SAP-02 ロドプシン群蛋白質の光誘起構造変化に関する X 線結晶解析
X-ray crystallographic studies on light-induced structural changes in rhodopsins

Tsutomu Kouyama (*Nagoya University, Graduate School of Science*)

In our group, we have performed X-ray crystallographic studies on five light-driven proton pumps (bacteriorhodopsin (BR); archaerhodopsin-1 & -2, cruxrhodopsin and deltarhodopsin), a light-driven chloride pump (pharaonis halorhodopsin (pHR)) and a visual pigment (squid rhodopsin). Comparison of these structures provides us a clue to elucidate the common structural motif that is utilized to stabilize their tertiary structures as well as unique architectures that are relevant to specific functions. From our recent studies on light-induced structural changes in BR and pHR, we proposed a novel hypothesis that BR functions as a water/proton antiporter, whereas pHR functions as an HCl/proton antiporter.

2SAP-03 退色しないロドプシンから体色などを制御する非視覚性オプシンへ
From non-bleachable rhodopsin to non-visual opsins

Yoshitaka Fukada (*Dept. Biophys. Biochem., Grad. Sch. Sci., Univ. Tokyo*)

Early studies on rhodopsin employed reconstitution of opsin with isomers of 11-*cis*-retinal, such as 9-*cis*- and 7-*cis*-retinal, which yield ‘rhodopsin isomers’. Simultaneously, contribution of organic chemists developed a variety of ‘rhodopsin analogs’ by reconstitution of opsin with 11-*cis*-retinal-related compounds. I started my research on rhodopsin analog that does not bleach upon light absorption due to the chemically 11-*cis*-locked structure with a cyclopentene ring. Later on, we found pinopsin in 1994 that represnts the first example of non-visual opsins. This study triggered a huge wave of researches of ‘non-visual’ opsins. At present, my group’s endeavors are directed toward elucidation of physiological roles of these NEW opsins with unknown function.

2SAP-04 錐体 AL-OL 反応の基質特異性と活性の細胞内局在
Substrate Specificity and Localization of AL-OL Coupling Reaction in Carp Cones

Shinya Sato[1], Shuji Tachibanaki[1,2], Takashi Fukagawa[2], **Satoru Kawamura**[1,2] ([1]*Department of Biological Sciences, Graduate School of Science, Osaka University*, [2]*Graduate School of Frontier Biosciences, Osaka University*)

Cones regenerate visual pigments from 11-cis retinol. Previous study showed that carp cones specifically have an enzyme activity to oxidize 11-cis retinol (alcohol; OL) to 11-cis retinal (aldehyde; AL) with concomitant reduction of all-trans retinal (AL) to all-trans retinol (OL) (AL-OL coupling reaction, Miyazono et al., 2008). In this reaction, NAD(H) or NADP(H) usually required for retinoid oxidation or reduction was not added. In the present study, we examined the substrate specificity and subcellular localization of this AL-OL coupling reaction. Oxidation of alcohols was specific to 11-cis and 9-cis retinol while reduction was not specific: many retinal isomers and also benzaldehyde were reduced. The activity was found in the inner segment in cones.

2SBP-01 メチル化 CpG 結合蛋白質 MBD4 による緩い基質 DNA 認識
Structural insight into versatile DNA recognition of methyl CpG binding protein MBD4

Mariko Ariyoshi[1], Jyunji Otani[2], Masahiro Shirakawa[2] ([1]*iCeMS, Kyoto Univ.*, [2]*Grad. Sch. Eng., Kyoto Univ.*)

The methyl-CpG binding domain (MBD) protein MBD4 is a DNA glycosylase that excises mismatched bases generated in methylated CpG sequences. Combined with biochemical data, the crystal structrues of the methyl-CpG binding domain of MBD4 (MBDmbd4) in complex with DNA clearly demonstrate that MBDmbd4 recognizes a wide range of 5-methylcytosine modifications via an extensive water network. Conformational adaptability at the DNA interface of MBD4 is a key aspect to broad base recognition that is unique to MBDmbd4. Such a versatile base recognition ability of MBDmbd4 implies multi-functional roles for MBD4 in the regulation of dynamic DNA methylation patterns coupled with deamination and/or oxidation of 5-methylcytosine.

2SBP-02 タンパク質結晶中に意図的に創り出した空間を使って，タンパク質に結合した状態のリガンドの大振幅運動を解析する
Intentional creation of crystal-contact free space for monitoring large amplitude motions of ligands in protein crystals

Daisuke Kohda (*Med. Inst. Bioreg., Kyushu Univ.*)

Contacts between molecules in protein crystals often restrict internal motions of proteins. The crystal-contact effect provides crystallographic snapshots of working proteins in crystal lattice. It is possible to collect many snapshots under different crystallization conditions, but the snapshots may not represent the true distribution because of non-random sampling. To analyze the amplitude of motions, we propose intentional creation of space in protein crystal lattice, and place a target region in the space created. The key technique is the rigid connection to fix the relative orientation of the protein of interest and a tag protein. We applied this technique to analyze the dynamics of a mitochondrial presequence peptide as bound to the mitochondrial receptor Tom20.

2SBP-03 ゲル包埋型バイオリアクターを用いた生細胞内蛋白質間相互作用の NMR 観測
A gel-encapsulated bioreactor system for NMR studies of protein-protein interactions in living mammalian cells

Noritaka Nishida[1], Ichio Shimada[1,2] ([1]*Grad Sch Pharma Sci, Univ of Tokyo*, [2]*Molprof, AIST*)

In-cell NMR is a useful approach for obtaining structural information within living cells, but the rapid increase of the dead cells has limited the usage of various NMR experiments that require long-term measurements. To overcome this problem, we developed a novel bioreactor system, in which fresh culture medium is supplied to cells encapsulated in thermoreversible Mebiol gel inside the NMR tube, thereby maintaining intracellular ATP level and reducing the dead cell population. Using the bioreactor established above, we successfully identified the binding site of the externally introduced protein, CG1 (the microtubule-binding domain of CLIP-170), for the endogenous microtubule based on the in-cell transferred cross saturation (in-cell TCS) experiments.

2SBP-04 タンパク質解析のための生細胞でのケミカルラベリング
Chemical protein labeling in living systems for its analysis

Itaru Hamachi (*Kyoto University*)

Here I describe our recent results for the development of chemistry-based methods for specific protein labeling. A tosylate reactive group that tethers a ligand module for selective binding to a target protein and a probe module to be labeled to the protein surface was designed. This chemical reagent was incubated with a protein in cell, cell lysate, as well as in a pure sample of test tubes, which allow us to conduct the endogenous protein selective and site selective labeling. This type of ligand-directed chemistry is now expanded to useful methods applicable to selective labeling on surface or inside of live cells where target proteins was endogenously expressed.

2SBP-05 ケモカイン受容体多量体形成による細胞動態の調節
Chemokine receptor oligomerization: a potential mechanism for regulating lymphocyte and cancer cell migration

Haruko Hayasaka, Daichi Kobayashi, Masayuki Miyasaka (*Immunodyamics, Osaka Univ. Grad. Sch. Med.*)

Recent studies indicate that transient protein-protein interactions are involved in a wide range of biological processes. Chemokines and their G-protein coupled receptors are such molecules undergoing transient interactions, regulating cell adhesion and motility. We have found that multiple chemokines can synergistically act on lymphocytes and cancer cells to enhance their migration and that cognate chemokine receptors transiently accumulate in the migration front, where they undergo homo- and hetero-oligomerization during receptor activation. Here we discuss the possibility that such receptor oligomerization may determine the level of chemokine receptor activation, providing a potential mechanism for cell migration under physiological and pathological conditions.

2SBP-06 一回膜貫通型サイトカイン受容体 Mpl 二量体化の一分子蛍光解析

Single-molecule fluorescence analysis of the single-transmembrane cytokine receptor Mpl dimerization

Akihiko Sakamoto[1], Takashi Kato[2], **Takashi Funatsu**[1] ([1]*Grd. Sch. Pharm. Sci., Univ. Tokyo*, [2]*Fac. Ed. and Int. Arts. Sci., Waseda Univ.*)

Mpl is a single-transmembrane cytokine receptor that regulates platelet production and early hematopoiesis. In the previous meeting, we proposed a positive feedback regulation of Mpl phosphorylation by the stabilization of the Mpl dimer. In this model, Mpl dimers are stabilized by their phosphorylation, and the stabilized Mpl dimers in turn promote their phosphorylation, which should contribute to the effective stimulation of Mpl-expressing cells. Although this model is based on our understanding of Mpl dimerization, the single-molecule analysis of the Mpl dimer has been difficult because of the diffraction limit. Here, we solve this problem by single-molecule super-resolution microscopy using compressed sensing and the à trous wavelet transform.

2SCP-01 ヌクレオソーム DNA 弛緩状態の自由エネルギープロファイル

Free Energy Profile for Nucleosomal DNA unwrapping

Hidetoshi Kono, Yoshiteru Yonetani, Jinzen Ikebe, Shun Sakuraba, Hisashi Ishida (*Molecular Modeling and Simulation, JAEA*)

Eukaryotic Genome is stored in a nucleus in the form of chromatin. Along with DNA binding proteins, this chromatin structure plays a key role in gene regulation. The chromatin has the fundamental structural unit called nucleosome which is composed of two copies of histones H2A, H2B, H3 and H4 and DNA wrapped around the histone proteins almost twice. Transcription does not occur until nucleosome is unwrapped to expose DNA, which can then be recognized by regulatory proteins. To understand this unwrapping process, MD simulations were performed on two nucleosomes: the canonical H3 and the H3 variant called CENP-A, essential for the kinetochore assembly. In the talk, how these nucleosomes are different in free energy profiles and what causes the differences are discussed.

2SCP-02 クロマチン高次構造形成におけるヌクレオソーム構造多様性

Structural versatility of nucleosomes in higher order chromatin

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

In eukaryotes, genomic DNA is highly compacted and packaged into nucleus as chromatin, in which nucleosome is the basic repeating unit. Nucleosome accomodates about 145-147 base pairs of DNA, which are wrapped around a histone octamer composed of two of each histones H2A, H2B, H3, and H4. It has recently been revealed that nucleosomes are not structurally homogeneous, and the nucleosome versatilities in structure and dynamics play essential roles in gene regulation in eukaryotes. The nucleosome structures and dynamics are regulated by histone variants and post-translational modifications. In this symposium, I will discuss about structural basis of regulation of higher-order chromatin architecture and dynamics by histone variants and modifications.

2SCP-03 モデルクロマチンの構造と転写因子ダイナミクスの粗視化シミュレーション研究

Structure of model chromatin and dynamics of transcription factors studied by coarse-grained simulations

Shoji Takada (*Department of Biophysics, Graduate School of Science, Kyoto University*)

In eukaryotic cells, genome is stored in highly condensed form of chromatin. How nucleosomes are arranged and how DNA-binding proteins seek cognate sequences in condensed chromatin is unclear. Using multiscale methods, we have been simulating structure and dynamics of mono- and poly-nucleosome model chromatin systems and dynamics of transcription factors on them. Some of these recent works are presented.

2SCP-04 ヌクレオソームの線維は細胞内でどのように収納されているのか？

How is nucleosome fiber organized in the cell?

Kazuhiro Maeshima (*National Institute of Genetics*)

DNA is wrapped around core histones and forms a nucleosome fiber. This nucleosome fiber has long been assumed to be folded into a 30-nm chromatin fiber, and a further helically folded larger fiber. However, when we observed human mitotic cells using cryoelectron microscopy, no higher-order structures including 30-nm chromatin fibers were found. Furthermore, our small-angle x-ray scattering analysis, which can detect periodic structures in noncrystalline materials in solution, demonstrated no structural feature larger than 11 nm. We also found a similar scattering pattern in interphase nuclei of human cells. Our findings suggest a common structural feature in interphase and mitotic chromatin: compact and irregular folding of nucleosome fibers.

2SCP-05 Diffusion-controlled reaction rate-laws in intracellular environment with molecular crowding: A single-particle-level simulation study

Kazunari Kaizu, Koichi Takahashi (*Laboratory for Biochemical Simulation, RIKEN Quantitative Biology Center (QBiC)*)

Although intracellular environment is significantly different from the ideal conditions expected in conventional biochemical simulations, the effect is poorly understood. In particular, there is no general quantitative theory for the reaction kinetics with molecular crowding. To derive the practical theory, we quantified the effect of molecular crowding on a rate-law in various conditions by using a single-particle-level simulation. In contrast to the common theory for diffusion-controlled reactions like Collins-Kimball's, the recombination probability was evaluated as a kinetic parameter. The rate-law revised on the basis of these calculations allowed simulations of intracellular signaling pathways and revealed the impact of crowding on cellular responses.

2SDP-01 High resolution imaging of malaria parasites with light, x-rays and electrons

Leann Tilley, Coralie Millet, Eric Hanssen, Matt Dixon (*Biochemistry Department, Bio21 Institute, University of Melbourne*)

New microscopy techniques are providing amazing views of the cellular landscape. We have used 3-D structured illumination microscopy (SIM), cryo X-ray microscopy and 3D-Electron Tomography to explore the sub-cellular topography of the malaria parasite, Plasmodium falciparum.
We have probed membrane systems in the cytoplasm of its host cell that are used to traffic virulence proteins to the erythrocyte surface. We have explored changes in the cellular structure of the parasite as it prepares for transmission to a mosquito and sexual reproduction. We have also probed the parasite's digestive processes and show that endocytosis and haemoglobin digestion occur via an orchestrated process.

2SDP-02 The dynamics of DNA origami nanostructures in Solution

Robert Hynson[1,2], Emeline Vernhes[1], Anthony Duff[3], Cy Jeffries[4], **Lawrence Lee**[1,2] ([1]*The Victor Chang Cardiac Research Institute*, [2]*The University of New South Wales*, [3]*Australian Nuclear Science and Technology Organisation*, [4]*The European Molecular Biology Organisation*)

DNA self-assembly is an emerging technology whose potential applications are only just beginning to be explored. It allows programmed construction of structures on a molecular scale with near atomic precision including the synthesis of nanoscale maps of the world and DNA robots that ‘walk’ along DNA mats. These spectacular advances clearly indicate the maturity of the method to reliably make atomically precise nanoscale objects for technological advance.
Here, we explore the potential to use DNA nanostructures to template the artificial synthesis of large protein assemblies. This includes an experimental characterization of the dynamic properties of the DNA origami tile in solution with X-ray scattering and the templated assembly of oligomeric protein complexes.

2SDP-03 Ion channel gating and Japanese Puzzle Boxes

Jamie Vandenberg (*Victor Chang Cardiac Research Institute*)

The choreographed opening and closing of voltage gated ion channels underlies the rapid and extremely sensitive electrical signaling which is critical for processes such as information transfer in the nervous system and the regulation of muscle contraction. In this presentation we will discuss the application of rate energy free energy relationship (REFER) analysis to interrogate the dynamics of gating in the human ether-a-go-go-related gene (hERG) K+ channel, a key regulator of the rhythm of the heartbeat. These studies reveal that a complex spatial and temporal sequence of widespread domain motions connects the open and inactivated states of the hERG K+ channel, analogous to the opening and closing of a Japanese Puzzle Box.

2SDP-04 The nature of myocardial heart failure: Are hypertrophic cardiomyopathies all the same?

Amy Li[1], Dane King[1], Martijn Bos[2], Eleanor Kable[3], Peter Macdonald[4], Filip Braet[1,3], Brett Hambly[1], Shin’ichi Ishiwata[5], Michael Ackerman[2], Murat Kekic[1], Cristobal dos Remedios[1] ([1]*Bosch Institute, University of Sydney, Sydney 2006, Australia*, [2]*Mayo Medical School, Mayo Clinic, Rochester, Minnesota, 55902, USA*, [3]*Australian Centre for Microscopy & Microanalysis, University of Sydney 2006, Australia*, [4]*Heart & Lung Transplant Unit, St. Vincent’s Hospital, Darlinghurst, Sydney 2010, Australia*, [5]*Department of Physics, Faculty of Science and Engineering, Waseda University, Tokyo 169-8050, Japan*)

Hypertrophic cardiomyopathy (HCM) has an incidence of 1:500 with at least 40% of this population directly caused by sarcomeric gene mutations. In this study we examine two of the most frequently mutated contractile proteins in HCM patients; MYBPC3 and the troponin family (Tn). SPontaneous Oscillatory Contractions (SPOCs) were induced in myectomy samples to identify functional characteristics of these mutations in the contraction-relaxation cycles that are intrinsic to the sarcomeres. PCA demonstrated distinctive clustering of the two HCM mutant myectomy populations and donors. However, we found no differences between the types of mutations and their pathological variants. This information will help us to understand the underlying mechanics of the failing HCM phenotype.

2SDP-05 The role of matrix metalloproteinases in genetic thoracic aortic aneurysm

Yaxin Lu[1], Richmond Jeremy[2], Murat Kekic[1], Jianlin Yin[2], Donna Lai[1], **Brett Hambly**[1] ([1]*Pathology Discipline and Bosch Institute, University of Sydney*, [2]*Royal Prince Alfred Hospital, University of Sydney*)

Genetic thoracic aortic aneurysm is more severe in males than females. MMPs participate in extra-cellular matrix remodelling in aneurysm disease. Plasma MMP-3 (stromelysin-1) was measured by ELISA in controls and Marfan Syndrome (MFS) patients. MMP-3 levels were higher in control males (12 ng/ml) versus control females (6 ng/ml), and were also higher in control males with larger Body Surface Area (BSA). Paradoxically, MMP-3 levels were found to be significantly decreased in MFS patients. These data contrast with atherosclerotic thoracic aneurysms, where plasma MMP-3 levels have been shown to rise. The higher levels of MMP-3 in males may partially explain the increased severity of genetic thoracic aortopathy in males.

2SEP-01 神経前駆細胞の集団的核移動の原理と意義
Collective nuclear migration of neural progenitors: mechanism and significance

Takaki Miyata, **Mayumi Okamoto** (*Nagoya Univ Grad Sch Med, Anat & Cell Biol*)

Neural progenitor cells exhibit nuclear migration along the apicobasal axis in a cell cycle-dependent manner (called interkinetic nuclear migration, INM). How INM occurs collectively (i.e. how directional and random movements are coordinated) is unclear. Our live imaging and MSD analysis demonstrated that basally-elongated cells move their nuclei directionally, while nuclei of non-elongated cells move less directionally. Coexistence of these two different nucleokinesis mechanisms based on morphological prioritization streamlines INM in a way analogous to “staggered commuting”. Contractility of the apical surface and physiological crowding involving horizontally-dividing M-phase cells may induce passive nucleokinesis away from the surface.

2SEP-02 細胞の自発運動と確率的センシング機構との関連性
Spontaneous cell migration and its relevance to cellular stochastic sensing mechanism

Hiroaki Takagi (*Department of physics, School of medicine, Nara medical University*)

We organized collaborative researches between theory and experiment to answer function of cellular spontaneous activity by adopting Dictyostelium discoideum. We focused on spontaneous cell migration, applied statistical analysis based on the theory of Brownian movement to cellular trajectories, and identified a model that can reproduce the revealed statistical nature. Through the numerical simulations and theoretical analysis of this model, we predicted functional significance of fluctuation in cell migration dynamics. Then, we experimentally verified a relationship between random and directional cell migration under DCEF (electrotaxis). Now we apply corresponding analysis to spontaneous migration of immune T cell, in order to examine the generality of the view.

2SEP-03 LFA-1/ICAM-1 によるリンパ球の'stop and go' : ライブイメージングによるリンパ球の動態解析
Regulation of Lymphocyte "Stop and Go" via LFA-1 and ICAM-1: Lymphocyte Trafficking Analysis using Live Imaging Techniques

Tatsuo Kinashi, Tomoya Katakai, Yoshihiro Ueda, Naoyuki Kondo (*Dept. Molecular Genetics, Institute of Biomedical Science, Kansai Medical University*)

Regulation of T-cell trafficking plays important roles in its development, antigen responses and homing. We established live two-photon microscopy using tissue slices of the lymph nodes and thymus, which revealed that LFA-1 and ICAM-1 mediate the dynamic migration and antigen recognition of T cells and thymocytes. The mammalian hippo homolog Mst1 mediates Rap1 signaling and regulates LFA-1 activity. We found that Mst1 was required for three dimensional high-velocity movement as well as rapid deceleration upon encounter of on antigen-presenting dendritic cells and thymic epithelial cells, leading to immunological synapse formation. These results demonstrate that Mst1 plays a key role in regulating antigen scanning through LFA-1 and ICAM-1 in the periphery and thymus.

2SEP-04 肺の枝分れ構造の形成機構
Mechanism of lung branching morphogenesis

Takashi Miura (*Kyushu University*)

Vertebrate lung has tree-like structure. This pattern is generated via complex epithelial-mesenchymal interaction. Various molecules are known to be involved in this process, but how the interactions result in branching morphogenesis is not well understood. At first we modeled mesenchyme-free lung epithelium culture system. with two species reaction-diffusion model, and reproduce the experimentally observed pattern.
Next, we try to model epithelial-mesenchymal interaction in vivo. By concentrating on expression regulation of FGF10, we could simplify the complex interaction to the rule "epithelium downregulates FGF10 in surrounding mesenchyme". We implemented this rule with interface equation and convolution, and reproduced pattern formation in vivo.

2SEP-05 Analysis of Tooth Germ Epithelium Morphogenesis by using Four-dimensional Cell Tracking System

Ritsuko Morita[1,2], Takashi Tsuji[2] ([1]*RIKEN CDB*, [2]*Research Inst. Sci. & Tech., Tokyo Univ. of Sci*)

Organ shapes are formed by accumulation of diverse cell behaviors such as cell growth, movement and differentiation. In this study, we developed a four-dimensional (4-D) cell tracking system as a quantitative kinetic analysis and analyzed tooth germ epithelium morphogenesis as an organ model. We found that deformation pattern of tooth germ epithelium correlated well with the cell proliferation pattern. We also found that cell proliferation and motility are orchestrated during epithelial morphogenesis. Our 4-D cell kinetic analysis enable bridging cellular event and morphological change of tissue and may shed new light on how multicellular events in tooth morphogenesis are controlled across space and time.

2SEP-06 細胞外マトリクスとアピカル細胞膜のカップリングが気管上皮チューブの形状を決定する
Mechanical coupling of extracellular matrix with apical membrane specifies geometry of epithelial tubule

Bo Dong[1], Edouard Hannezo[2], **Shigeo Hayashi**[1] ([1]*RIKEN CDB*, [2]*Institut Curie*)

In biological tubular network, ECMs are transiently secreted from epithelial or endothelial cells and accumulates in the lumen contacting with apical membrane. We present that the apical ECM of the embryonic tracheal tube of Drosophila is a complex visco-elastic material coupled to apical membrane. Based on a study of mutations altering tracheal tube geometry and theoretical modeling, we propose a model in which aECM elasticity, apical-membrane growth, and their association are three vital parameters determining the stability of biological tubes. Our findings suggest that the interaction of the apical membrane and elastic aECM determines the stability of biological tubes, independent of cell shape.

3SAA-01 表面増強ラマン散乱の機構解明と疾病関連分子や細胞表面タンパク質分子の超高感度検出への応用
Clarification of surface enhanced Raman scattering and its application to ultrasensitive detection of biomolecules

Tamitake Itoh (*AIST*)

We review the fundamental study of SERS with emphasis on experiments that attempted to identify the enhancement mechanism using single Ag nanoparticle (NP) dimers attached by dye molecules. The results are quantitatively discussed in the framework of electromagnetic (EM) mechanism. We also review recent our results in basic SERS applications for biological sensing regarding detections of cell surface molecules and distinction of disease biomarker molecules under single cell and single molecule levels using four types of biological targets; yeast, helicobacter pylori, E. coli, and Hemoglobin A1c (HbA1c) to explore the real application of SERS.

3SAA-02 アルキンタグを用いた低分子化合物の生細胞ラマンイメージング

Alkyne-Tag Raman Imaging for Visualization of Small Molecules in Live Cells

Mikiko Sodeoka[1,2] ([1]*RIKEN*, [2]*ERATO, JST*)

Alkyne has a unique Raman band that does not overlap with Raman scattering from any endogenous molecule in live cells. Therefore, alkyne-tag Raman imaging (ATRI) is a promising approach for visualizing small molecules in live cells. An examination of structure-Raman shift/intensity relationships revealed that alkynes conjugated to an aromatic ring and/or to a second alkyne (conjugated diynes) have strong Raman signals in the cellular silent region and can be excellent tags. As a proof of concept, imaging of 5-ethynyl-2'-deoxyuridine (EdU) in living HeLa cells has been demonstrated. Simultaneous imaging of two small molecules, EdU and a CoQ analogue, with distinct Raman tags was also demonstrated.

3SAA-03 ラマン散乱分光顕微鏡を用いた細胞状態を定義する「細胞指紋」の提案

Cellular fingerprints to distinguish and identify the various cellular states with Raman spectroscopy

Tomonobu Watanabe[1,2,3,4] ([1]*RIKEN, Quantitative Biology Center*, [2]*Immunology Frontier Research Center, Osaka University*, [3]*Graduate School of Frontier Bioscience, Osaka University*, [4]*PRESTO, Japan Science and Technology Agency*)

Self renewal and differentiation capability of ES cells are maintained as a result of complex physico-chemical interactions between various elements, such as DNA, protein and lipids, within a nano-scale volume. Microscopy is a powerful tool to observe biological events because of the applicability for observation of living specimens, however, diffraction limit of light restrict the spatial resolution of the microscope to few hundred nano-meters. To overcome the problem, we have been challenging to detect the nano-scale events with light that includes the information in nano-scale. Raman scattering is one of the scattered lights, and inheres all the vibration mode of the molecule that scattered. In this meeting, we would like to show our challenge to describe potential landscape of ES cell differentiation by Raman's "cellular fingerprints".

3SAA-04 ラマン分光イメージングが拓く新たな細胞周期ダイナミクス研究の可能性

***In Vivo* Raman Spectral Imaging of Cell Cycle Dynamics: Adding a New Dimension to Cell Cycle Research**

Chuan-Keng Huang, Jen-Fang Hsu, **Shinsuke Shigeto** (*Dept. Appl. Chem., National Chiao Tung Univ.*)

The cell cycle plays a pivotal role in reproduction of all living organisms. Dysregulation of the cell cycle components may lead to tumor formation. Detailed molecular-level study of the cell cycle dynamics will not only deepen our understanding of life, but it will also open up new possibilities of diagnosis/prognosis of cancer cells. In this work, we use a hybrid of time-lapse Raman imaging and multivariate curve resolution to disentangle complicated spatiotemporal behaviors of the major intracellular components during the cell cycle of a single fission yeast cell. We have detected a protein component associated with tyrosine phosphorylation, which cannot be seen with the univariate approach. Further we extend our work to the cell cycle of colon cancer cells.

3SAA-05 スペクトル解析によるバイオ･ラマン研究

Spectral Analysis for Bio-Raman Research

Shin-ichi Morita (*Cellular Informatics Laboratory, RIKEN*)

Spectral analysis is essential to bio-Raman research to disentangle Raman data since they are complicated and fluctuated multivariable. This talk, we sought to predict cell fates, including cellular differentiation of multicellular system. Two types of cellular systems were used, that is, filamentous cyanobacteria and human breast cancer cell line. For cyanobacteria, Raman technique yielded marker vibrations characteristic to differentiation. The multivariable analysis suggested that the diverse states of undifferentiated cells were converged into a specific state through differentiation.

3SBA-01 抑制性 GABA 作動性シナプス制御におけるカルシウムの驚くべき作用―1 分子イメージングで明らかになったこと―

Origin-dependent opposite effect of Ca^{2+} on the regulation of inhibitory $GABA_A$ receptor diffusion dynamics: a single molecule study

Hiroko Bannai[1,2], Fumihiro Niwa[2], Antoine Triller[3], Katsuhiko Mikoshiba[2] ([1]*Nagoya Univ., Grad. Sch. Sci, Dept. Biol. Sci.*, [2]*RIKEN BSI*, [3]*IBENS, INSERM U1024, CNRS UMR8197*)

By analyzing the diffusion properties of type-A GABA receptors ($GABA_AR$) on the cell surface using single molecule imaging technique with quantum dots, we found that Ca^{2+} influx evoked by neuronal excitation increased $GABA_AR$ diffusion dynamics and contributed to rapid and plastic reduction in GABAergic synaptic transmission. Conversely, another intracellular Ca^{2+} signaling pathway, i.e. Ca^{2+} release from the intracellular Ca^{2+} stores reduced the surface $GABA_AR$ mobility and had an effect to stabilize the GABAergic synapses. These results indicate that Ca^{2+} from different sources could have the opposite effect on the regulation of GABAergic synapses.

3SBA-02 シナプス内シグナル分子活性化のイメージングと操作

Imaging and controlling the activity of signaling molecules in dendritic spines of hippocampal neurons

Hideji Murakoshi[1,2] ([1]*National Institute for Physiological Sciences*, [2]*PRESTO, JST*)

Ca2+/Calmodulin-dependent kinase II (CaMKII) is one of the most important signaling molecules for long-term potentiation and associated spine enlargement underlying learning and memory. Here, to understand the function of CaMKII for synaptic plasticity, we developed genetically encoded light-inducible CaMKII inhibitor and photo-activatable CaMKII by using LOV2 derived from phototropin. We applied these newly developed optogenetic tools for the study of structural plasticity of single dendritic spines by using 2-photon fluorescence microscope and 2-photon glutamate uncaging, and found that 1) ~60 s of CaMKII activation is sufficient for inducing transient and sustained spine enlargement, 2) CaMKII activation alone is sufficient for triggering structural plasticity.

3SBA-03 Imaging with novel photochemical materials to study neuronal functions

Dan O Wang (*institute for integrated cell-material sciences*)

Novel photochemical materials potentiate new approaches to study brain functions. We are developing imaging methods to study two fundamental signaling pathways in neurons: RNA dynamics and gas molecules. We demonstrate that in vivo electroporation of an exciton-controlled hybridization-sensitive oligonucleotide probe allows monitoring the behavior of endogenous RNA species in multiple living cellular contexts at a single-cell resolution without noticeable cytotoxicity. To study gas signalling, we target nitric oxide (NO), a crucial signalling molecule with highly site-specific and concentration-dependant activity. The spatiotemporal control of NO releasing is realized by a synthetic platform based on photoactive porous materials.

3SBA-04 STED imaging of synapses in living brain slices: from structure to function

U. Valentin Nägerl[1,2] ([1]*IINS, Univ. Bordeaux Segalen, France*, [2]*UMR 5297, CNRS, Bordeaux, France*)

Neuronal synapses are complex (and tiny) structures ensheated by glia processes, forming elementary functional units for fast and flexible communication in the brain. Understanding how synapses are built during development and modified by experience is a central challenge for neuroscience.
I will review our progress in developing STED microscopy for live-cell nanoscale imaging of neural structures in living brain slices, including: 1) nanoscale imaging up to 100 µm below tissue surface in acute brain slices by a novel combination of two-photon and STED microscopy; 2) spine structure - function analysis based on a combination of STED imaging and FRAP experiments; 3) nanoscale alterations of dendritic spine morphology in a mouse model of fragile X.

3SBA-05 新規レーザー光技術による２光子顕微鏡の空間分解能、深部到達性の向上
Improvement of Reolution and Penetration Depth of Two-photon Microscopy with Novel Laser Techniques

Tomomi Nemoto (*RIES, Hokkaido Univ.*)

"In vivo" two-photon microscopy has revealed vital information on neural activity for brain function, even in light of its limitation in imaging events at depths greater than a several hundred micrometers from the brain surface. To break the limit of this penetration depth, we introduced a novel light source based on a semiconductor laser. The light source successfully visualized not only cortex layer V pyramidal neurons at a superior S/N ratio, but also hippocampal CA1 neurons in young adult mice. Furthermore, we developed liquid crystal devices to convert linearly polarized beams to vector beams, that enabled us to identify individual φ170nm fluorescent beads; smaller than classical PSF width. We will here discuss these improvements on the basis of our recent data.

3SBA-06 FRET sensing of transmembrane voltage

Hidekazu Tsutsui (*Osaka University*)

Sensitive detection of spatiotemporal electrical activities in a complex network of excitable cells is one of the long-lasting challenges in the field of physiology. We have been interested in engineering of protein-based voltage probes by coupling the voltage-sensor domain derived from a voltage-sensing phosphatase to fluorescence reporters. In this presentation, I would like to talk on our recent advances regarding the functional evaluations of test probes, knowledge on the motion of the voltage sensor domain, as well as the development of an improved voltage probe.

3SBA-07 グリアによる大脳皮質シナプス再編
Glial contribution to remodeling of cortical synapses

Junichi Nabekura[1,2] ([1]*NIPS*, [2]*SOKENDAI*)

Recent advance in brain maging techniques, e.g. MRI, allows us to visualize the morphological change in physiological and pathological conditions. However, with their spatial/temporal limitation, it is difficult it to elucidate their underlying mechanisms at neuronal circuits' level in vivo. Here, we introduce two evidences of dynamics of neuronal structures in pathologic brains and the contribution of glia to their synapse remodeling with taking an advantage of two photon microscopy. 1) an observation of microglial surveillance of synapses in intact and ischemic brain with real-time in vivo imaging. 2) phase-specific increase synapse turnover driven by astrocyte-releasing thrombospondin in chronic pain condition observed with a long term time lapse imaging.

3SCA-01 DNA の構造と機能を制御するケミカルバイオロジー：DNA オリガミと人工遺伝子スイッチ
Chemical Biology that Controls DNA Structure and Function: DNA Origami and Artificial Genetic Switch

Hiroshi Sugiyama[1,2] ([1]*Department of Chemistry, Graduate School of Science, Kyoto University*, [2]*Institute for Integrated Cell-Material Sciences (iCeMS)*)

We have been undertaking original research on the molecular recognition of DNA by antitumor antibiotics, and the analysis of atom-specific chemical reaction toward DNA with these agents. By reconstituting such knowledge, various functionalized sequence-specific DNA binders were synthesized as an artificial genetic switch. We have successfully developed synthetic genetic switches that could trigger cellular reprogramming by switching "ON" the transcriptional machinery conferring to pluripotency. Unlike other small molecules that can control the fate of stem cells, PIP conjugates can be programmed to bind with predetermined DNA sequences and could perturb the architecture of the packed chromatin. Therefore, strategies to expand our tunable SAHA-PIPs could create an epoch-making approach in cellular reprogramming and regenerative medicine to modulate the desired genes. We recently reported the design of "DNA frame" using the DNA origami method to examine enzymatic action. We newly developed the tension-controlled dsDNA substrates in the DNA frame and showed the importance of DNA strand relaxation in allowing double helix bending during enzymatic reaction. In addition, the DNA frame is valuable for analyzing the motion of the enzyme because of the defined coordinated space. The exact location and displacement of the enzyme in the reaction on the dsDNA can be monitored and analyzed. Therefore, the time-resolved reaction coordination between the enzymes and substrate can be estimated at meso-scale spatial resolution. Recent progress of regulation of the epigenetic gene expression using designed molecules, and elucidation the mechanism using single molecular imaging technique will be discussed.

3SCA-02 抗プリオン活性を示すアプタマー及びカリウムイオンに感応して活性がスイッチングするインテリジェントリボザイム

Aptamer that exerts anti-prion activity and intelligent ribozyme whose activity switches in response to K^+

Masato Katahira[1], Tsukasa Mashima[1], Yuudai Yamaoki[1], Takashi Nagata[1], Fumiko Nishikawa[2], Satoshi Nishikawa[2], Yuji Kamatari[3], Kazuo Kuwata[3] ([1]*Inst. of Adv. Energy, Kyoto Univ.*, [2]*AIST*, [3]*Gifu Univ.*)

On the basis of structural analysis by NMR, we elucidated the way how RNA aptamer against prion protein, (GGAGGAGGAGGA), tightly binds to its target. It was found that simultaneous double interactions between aptamer and protein cause tight binding. The assay experiment with neural cells revealed that the aptamer exerts the activity to repress the formation of an abnormal form of prion protein. We also succeeded to develop an intelligent ribozyme whose activity switches in response to K^+. In response to K^+, quadruplex is induced in this ribozyme, which results in the formation of the active catalytic core of the ribozyme and thus the enhancement of the activity. The same method is supposed to be applicable to switch the activity of various aptamers.

3SCA-03 High-affinity DNA aptamer selection by a genetic alphabet expansion PCR system

Michiko Kimoto[1,2], Ken-ichiro Matsunaga[1], Rie Yamashige[1], Ichiro Hirao[1,2] ([1]*RIKEN CLST*, [2]*TagCyx Biotechnologies*)

We developed a new method for generating high-affinity DNA aptamers containing five different bases. Nucleic acid aptamers are DNA or RNA fragments that specifically bind targets and obtained by evolutionary engineering (SELEX) using nucleic acid libraries with random base sequences. However, nucleic acid aptamers consisting of only the four natural bases often show low affinities to target proteins. To improve the affinity, we added the fifth hydrophobic bases into DNA libraries. In PCR amplification of the library, we used an artificial third base pair between the fifth and its pairing partner sixth bases. This genetic alphabet expansion SELEX method would be useful for producing aptamers with greatly augmented affinity toward diagnostic and therapeutic applications.

3SCA-04 核酸医薬品への期待

The prospect for nucleic acid medicine

Tsuneaki Sakata[1,2] ([1]*Shionogi & Co., Ltd.*, [2]*Graduate School of Engineering Science, Osaka University*)

I would like to talk about how a pharmaceutical company in Japan views nucleic acid medicines. Nucleic acid drugs, as a class of molecular-targeted drugs, can be defined as molecular-targeted drugs that suppress the onset and progression of an illness through regulating the gene expression and function of disease-related molecules. However, only 2 nucleic acid drugs have been marketed to date. The desires or trends regarding nucleic acid drugs as a new class of drug will be discussed.

3SCA-05 熱力学的特性や速度論的特性に基づいた機能性核酸のデザイン戦略

Strategy to design functional nucleic acids based on their thermodynamic and kinetic properties

Hidetaka Torigoe (*Dep. Appl. Chem., Fac. Sci., Tokyo Univ. Sci.*)

Artificial regulation of target gene expression by functional nucleic acids, such as antisense and antigene technologies, is important not only for basic study to analyze unknown biological functions of target genes but also for therapeutic applications to repress expression of undesirable target genes. Serious difficulties, such as poor ability of oligonucleotides added from outside to bind to target gene or mRNA, may limit practical applications of these technologies *in vivo*. Many chemical modifications of oligonucleotides have been developed to overcome the difficulties. Thermodynamic and kinetic properties are important to design chemical modification. Here, strategy to design functional nucleic acids based on thermodynamic and kinetic properties will be discussed.

3SCA-06 生命現象の理解に向けた超高速 DNA 分離と一分子 DNA メチル化検出のためのナノバイオデバイス

Nanobiodevices for ultrafast DNA separation and single molecular DNA methylation detection for the understanding of life phenomena

Hiroshi Yukawa[1], Yoshinobu Baba[1,2] ([1]*Res. Cent. Inno. Nanobio., Univ. Nagoya*, [2]*Grad. Sch. Eng., Univ. Nagoya*)

Our group has performed the preparation of nanobiodevices mainly for ultrafast DNA separation and single molecular DNA methylation detection for the understanding of life phenomena. Using the nanopillar array chip, we found that the mixture of lambda DNA (48.5 kbp) and T4 DNA (165.6 kbp) molecules could be separated by a 380 mm long nanopillar region within only 10s. A nanowall array structure realized 30s separation for a mixture of DNA fragments (48.5 and 1 kbp fragments) by applying an electric voltage. Moreover, we could perform the methylation mapping of DNA molecules specifically in nanoslit devices by using the quantum dots (QDs) combined with methyl-CpG-binding domain protein (MBDp) at a single molecule level. I would like to introduce these two technologies.

3SCA-07 RNA アプタマー医薬の開発動向

Development trends for RNA aptamer therapeutics

Shin Miyakawa (*RIBOMIC Inc.*)

RNA aptamer is a single-stranded short RNA that binds to targeted proteins with high affinity and specificity. The anti-VEGF aptamer, Macugen, was approved by FDA in 2004 for treating wet age-related macular degeneration. Other ten programs including anti-coagulant and anti-inflammatory aptamers are now in clinical studies. In this presentation, current topics of RNA aptamer developments will be presented. Toxicology, pharmacokinetics and chemistry manufacturing control specifically relating to aptamers will be also presented.

3SDA-01 動物の発生における PAR/aPKC 細胞極性システムの計測に基づいた数理モデル化

Measurement-based mathematical modeling of PAR/aPKC-dependent cell polarization in animal development

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

Asymmetric protein localization functions as a source of positional information in cells and embryos during animal development. However, quantitative aspects-how molecular reactions control protein dynamics for pattern formation remains largely unexplored. We succeeded in detecting single molecules of polarity protein PAR-2 in living *C. elegans* embryos. The dissociation and association rate constants of PAR-2 proteins with the cell cortex were changed along the anterior-posterior axis. A mathematical model, in which all parameter values were determined by *in vivo* measurements, reproduced the asymmetric localization. In this study, we demonstrated that bi-stable polarity system is maintained by phosphorylation and oligomerization of PAR-2 proteins in *C. elegans* embryos.

3SDA-02 気管繊毛の運動と 3 次元構造解析

Ciliary motion and the three-dimensional structure in mouse respiratory cilia

Hironori Ueno (*Mol. func. and life sci., Aichi Univ. of Edu.*)

Mucociliary clearance of the tracheal lumen is an important component of lung defense. However, the axonemal structure that achieves effective ciliary motion and the mechanisms by which cilia generate directional flow are unknown. In this study, we examined individual ciliary motion with 7-9 nm spatial precision using quantum dots. Moreover, we analyzed the conformational change in each dynein head of outer dynein arm 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 are dissociated and alternatively moves in different ways in the presence of nucleotide. It probably contributes force generation of ciliary dynein in axoneme during fast ciliary motion.

3SDA-03 非侵襲 in vivo 技術を用いたマウス内の好中球における高速小胞輸送解析

A non-invasive technique for the in vivo tracking of high-speed vesicle transport in mouse neutrophils

Kenji Kikushima, Sayaka Kita, Hideo Higuchi (*Dept. Physics, Grad. Sch. Sci., Univ of Tokyo*)

Neutrophils play an essential role in the innate immune response. We developed a new non-invasive technique for the in vivo imaging of neutrophils labeled with quantum dots, up to 100 um below the skin surface of mice. The quantum dots were endocytosed into vesicles in the neutrophils, allowing us to track the vesicles at 12.5 msec/frame with 15-24 nm accuracy. Most intriguingly, the vesicles containing quantum dots were transported at higher speed than the in vitro velocity of a molecular motor such as kinesin or dynein. This is the first report in which non-invasive techniques have been used to visualize the internal dynamics of neutrophils. In this symposium, I'll report recent progress in the molecular mechanism of the vesicle transport in the neutrophil.

3SDA-04 Real-time high-resolution cardiac imaging *in vivo*

Fuyu Kobirumaki-Shimozawa[1], Kotaro Oyama[2], Seine A. Shintani[2], Erisa Hirokawa[3], Togo Shimozawa[4], Takako Terui[5], Shin'ich Ishiwata[2], Norio Fukuda[1] ([1]*Dept. Cell physiol., Jieki Univ. Sch. Med.*, [2]*Dept. Physics, Waseda Univ.*, [3]*Jieki Univ. Sch. Med.*, [4]*Dept. Physics, Gakushuin Univ.*, [5]*Dept. Anesthes., Jieki Univ. Sch. Med.*)

To explore the molecular mechanisms of cardiac muscle contraction under physiologic conditions, we directly imaged the motions of single sarcomeres in the beating mouse heart *in vivo* at high spatial and temporal resolution. The Z-lines were labeled with α-actinin-GFP (as in rat neonatal cardiomyocytes) via infection of adenoviruses, and sarcomere lengths were measured by using a spinning disk confocal microscope at ~100 fps (resolution in the XY plane, ~20 nm). We successfully imaged sarcomeric motions, simultaneously with electrocardiogram and left ventricular pressure. Likewise, we imaged Ca^{2+} waves / transients in the cardiomyocytes of the isolated mouse heart. At the meeting, we will discuss how cardiac muscle contraction is organized at the molecular level *in vivo*.

3SDA-05 筋収縮の数理モデルとその心臓シミュレーションへの応用について

A numerical model of cross-bridge cycling and its application to a beating human heart

Takumi Washio[1], Kazunori Yoneda[2], Akihito Takahashi[1], Seiryo Sugiura[1], Toshiaki Hisada[1] ([1]*Grad. Sch. of Fron. Sci., University of Tokyo,*, [2]*Fujitsu Ltd.*)

Numerical modeling of cross-bridge cycling is an important issue to utilize numerous experimental molecular scale findings for understanding organ scale functionalities of muscle. In this talk, we propose a simple state-transition model, which includes a cooperative property with its neighboring units and the two stage head-rotation mechanism proposed by Huxley and Simons (1971 model). First, we show that typical experimental results in the myofibril level, such as the pCa-force relationship, the tension responses for quick release and stretch, and for oscillatory length change, can be reproduced with this model. Second, we examine how the features in the cross-bridge model are related with the performance of pumping human heart by multiscale numerical simulations.

3SEA-01 ガングリオシドクラスターを介したアルツハイマーアミロイドβタンパク質のフォールディングと凝集

Ganglioside Cluster-Mediated Folding and Aggregation of Alzheimer's Amyloid beta-Protein

Katsumi Matsuzaki (*Grad. Sch. Pharm. Sci., Kyoto Univ.*)

The aggregation of Abeta is considered to be the key step in the development of Alzheimer's disease. We propose the following mechanism of Abeta aggregation in membranes. Abeta specifically binds to a GM1 cluster, changing its conformation from a random coil to an α-helix-rich structure. At Abeta/GM1 ratios of between ~0.013 and ~0.044, the helical species and aggregated beta-sheets (~15 mer) coexist. However, the beta-structure is stable and does not form larger aggregates. At Abeta/GM1 values above ~0.044, the beta-structure is converted to a second, seed-prone beta-structure. Amyloid fibrils formed in solution are almost nontoxic, whereas fibrils formed in lipid rafts are cytotoxic and have different morphologies and secondary structures.

3SEA-02 ガングリオシドとの特異的な相互作用に伴うアミロイド関連タンパク質の構造転移の NMR 解析

NMR characterization of conformational transitions of amyloidogenic proteins upon their specific interactions with gangliosides

Maho Yagi-Utsumi (*Univ. Cambridge*)

Lipid membranes provide active platform for dynamic interactions of a variety of biomolecules on cell surfaces, where glycolipids are involved in physiological and pathological molecular recognition events. Growing evidences have demonstrated that gangliosides on neuronal cell membranes can be targets for various amyloidogenic proteins that are associated with neurodegenerative disorders (e.g. α-synuclein in Parkinson's disease and amyloid β in Alzheimer's disease). Our NMR spectroscopic data have indicated that size and curvature of ganglioside clusters along with ganglioside density are the determining factors for the membrane-interactions and the consequent conformational transition of the amyloidogenic proteins.

3SEA-03 アミロイドタンパク質の凝集過程のシミュレーション解析

Computational study on the aggregation and assemble process of amyoid beta proteins

Tyuji Hoshino (*Graduate School of Pharmaceutical Sciences, Chiba University*)

The neurodegenerative disorder is characterized by the deposition of amyloid plaques in a human brain. The complex of amyloid beta (Aβ) and GM1-ganglioside is considered to act as a seed for the amyloid aggregation. In spite of much computational studies, there is no report clarifying the embryonic stage of Aβ fibrils under the physiological condition like that in the human brain cell. In this study, we performed huge computational analysis using the calculation model including Aβ peptides, the GM1-ganglioside clustering mixed lipid membrane, and the water layer. Multiple simulations demonstrated the binding of Aβ peptide to the membrane and the formation of Aβ complex. The motions of Aβs to evolve into a bundle of Aβ peptides were monitored through computer simulation.

3SEA-04 脂質膜の物理的性質とアミロイド線維形成の関係

Relationship between physical properties of lipid membranes and amyloidogenesis

Takashi Miura, Masako Suzuki (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

Self-association of amyloid β-peptide (Aβ) is considered to be an initial step in the development of Alzheimer's disease and is known to be promoted by lipid membranes. We have recently shown that physical properties of lipid membranes, such as ordering or packing of lipid hydrocarbon chains, could be factors influencing not only the affinity of Aβ to the membrane but also the self-association of the peptide. In order to further understand roles of cellular membranes in the formation of fibrils and the cytotoxic action by Aβ, we have investigated the effects of Aβ binding on physical properties of lipid membranes, such as rigidifying or fluidifying effect of Aβ, by using fluorescence, circular dichroism, and Raman spectroscopy.

3SEA-05 アミロイド形成型免疫グロブリン軽鎖可変ドメインの細胞毒性

Toward understanding the mechanism of cytotoxicity of amyloidogenic variable domain of immunoglobulin light chains

Daizo Hamada (*Division of Structural Biology, Department of Biochemistry and Molecular Biology, Graduate School of Medicine, Kobe University*)

Accumulation of amyloid fibrils by immunoglobulin light chain (LC) at various organs is associated with AL amyloidosis. Interactions of amyloidogenic LCs to biological membranes induce the cell death. However, only little is known about the mechanism of this phenomena. In order to clarify this problem, the effects of an amyloidogenic mutants of variable domain (VL) from REI LC on the properties of lipid membranes were analysed by various model systems. The results obtained here suggest that the amyloidogenic VL binds to various types of cellular membrane and is converted to different conformational states to reduce the membrane fluidity that may be one of the triggers to induce the cell death.

3SEA-06 固体 NMR と TEM によるヒトカルシトニンとグルカゴンにおけるアミロイド線維形成と阻害機構の解明

Mechanisms of amyloid fibril formation and inhibition of human calcitonin and glucagon as revealed by solid-state NMR and TEM

Akira Naito (*Yokohama National University*)

Human calcitonin (hCT) is a peptide hormon that forms amyloid fibril. The kinetics of F16L-, F19L, TL-hCT were examined using solid-state NMR, indicating that the fibril elongation rate (k_2) was turned out to be significantly inhibited. The fibrillation process in HEPES buffer was examined using solid-state NMR and transmission electron microscopy. Spherical intermediates were observed during the early stage of fibril formation in HEPES buffer.

Glucagon ia a 29 amino acid peptide hormone and forms amyloid fibrils. It turned out that the N-terminal α-helix did not change to β-sheet in the presence of bicelles. Rate constant of nucleation process (k_1) is faster and elongation process (k_2) is slower than the case in the absence of bicelles.

1P001 高エネ機構フォトンファクトリーにおける創薬等支援基盤プラットフォーム事業による構造生物学研究の支援と高度化

Promotion of the Platform for Drug discovery, Informatics, and Structural life science (PDIS) project at Photon Factory in KEK

Ryuichi Kato[1], Naohiro Matsugaki[1], Yusuke Yamada[1], Leonard Chavas[1], Fumiaki Yumoto[1], Masato Kawasaki[1], Masahiko Hiraki[2], Toshiya Senda[1] ([1]*Photon Factory, IMSS, KEK*, [2]*Mechanical Engineering Center, ARL, KEK*)

Platform for Drug discovery, Informatics, and Structural life science (PDIS) is a five-year national project started FY2012. KEK has been selected as one of the institutes / universities participating in the project. In the project, KEK supplies protein crystallization, synchrotron X-ray beamtime, diffraction data collection and structure determination activities. PDIS will accept numerous applications of interest in the fields of basic science, medical, and pharmaceutical researches. All Japanese scientists can apply to use resources open for the PDIS project. In addition to the support, we promote technology development in the project.

1P002 海産無脊椎動物由来溶血性レクチン CEL-III の結晶化

Crystallization of the pore forming toxin CEL-III from marine invertebrate, Cucumaria echinata

Tomonao Nagao, Shuichiro Goda, Hideaki Unno, Tomomitu Hatakeyama (*Grad. Sch of Eng., Univ. Nagasaki*)

CEL-III is a hemolytic lectin isolated from the sea cucumber Cucumaria echinata. This lectin binds to the cell surface carbohydrate chains and associates to form transmembrane pores. We prepared the pore-forming oligomer using rabbit erythrocytes. The oligomer was solubilized with n-dodecyl-β-D-maltoside (DDM) from rabbit erythrocytes and purified by lactose-fixed affinity column chromatography. The purified oligomer was homogeneous on SDS-PAGE and used to crystallization. The crystals of pore-forming oligomer were obtained by vapor diffusion method.

1P003 イェロープロテインの 150 ピコ秒時間分解能ラウエ構造解析

Time-resolved Laue crystallography of photoactive yellow protein with 150psec time resolution

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In order to understand the molecular mechanism of signal transduction in photoactive yellow protein (PYP), we investigated its structural dynamics with time-resolved Laue crystallography. We acquired 1.65Å; diffraction data over 10 decades of time with ~150 psec time resolution, the highest time resolution achieved thus far. The time-resolved diffraction data could be decomposed into 4 intermediates (pR0, pR1, pR2, pB0) plus the unphotolyzed ground state (pG). The chromophore in pR0 is highly contorted, with its carbonyl oriented perpendicular to the phenolate plane. This strained cis state is short-lived (~600 ps lifetime), and transitions reversibly to pR1, a planar cis state. The structural changes observed as PYP advances through its photocycle will be described.

1P004 トマトモザイクウイルス複製タンパク質と阻害因子 Tm-1 の複合体形成機構の解明

Interaction mechanism of Tomato mosaic virus replication protein and the resistance factor Tm-1

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Tm-1 gene is a resistance gene of tomato against Tomato mosaic virus (ToMV). Tm-1 binds the helicase domain of ToMV replication proteins (ToMV-Hel) and inhibits its RNA replication. However, little is yet known about the interaction mechanism of ToMV-Hel and Tm-1. In this study, we determined the crystal structures of ToMV-Hel and N-terminal domain of Tm-1 complex. Furthermore, the binding energies of Tm-1 with ToMV-Hel were measured by isothermal titration calorimetry under various conditions. The asymmetric unit of the crystal contains a tetrameric complex in a 2:2 stoichiometry, comprising of a Tm-1 dimer and two monomeric ToMV-Hel. The complex formation is enthalpically driven and requires an ATP analog or ADP.

1P005 真菌由来 TRP チャネル制御領域への Ca^{2+}イオン結合の結晶学的解析

Crystallographic analysis of the Ca^{2+}-binding sites in the regulatory-bundling region of the fungus TRP channel

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TRPGz is a fungus TRP channel responding to osmotic upshock. Previously, we identified the crucial region for the osmotic responses in the cytosolic region of TRPGz, and revealed that the interprotomer helix-bundle formation by the region is critical for the response. In this study, we analyzed the divalent-cation binding to this region. Crystallographic analyses revealed the binding of Ca^{2+} and other cations with the similar ionic radii of Ca^{2+} at the interprotomer interfaces, while no significant binding or binding at a different position was observed in the case for other cations with different ionic radii from Ca^{2+}. The results suggested the existence of the Ca^{2+}-specific binding sites in the region and their regulatory roles for the osmotic responses.

1P006 分裂酵母由来の MAP キナーゼによりリン酸化される RNA 結合タンパク質 Nrd1 の構造解析

Structural studies of RNA-binding protein Nrd1, a fission yeast MAPK target RNA binding protein

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Negative regulator of differentiation 1 (Nrd1) is known as a negative regulator of sexual differentiation in fission yeast. Further, Nrd1 binds and stabilizes the Cdc4 mRNA which encodes a myosin II light chain, and thereby suppressing the cytokinesis. Pmk1 (yeast MAPK) phosphorylates Nrd1 resulting in markedly reduced RNA binding activity. The mechanism by which Pmk1 regulates the RNA binding activity of Nrd1 is unknown. In an effort to delineate the relationship between Nrd1 structure and function, we prepared each RRM (RNA recognition motif) of Nrd1. The structure of the second RRM of Nrd1 has been determined (Kobayashi et al., *BBRC*, in press). Furthermore, we will discuss usage of split intein and paramagnetic relaxation enhancement for the full-length Nrd1.

1P007 Structural analysis of *C. elegans* innexin-6 gap junction channels by electron microscopy

Tomohiro Matsuzawa[1], Kazuyoshi Murata[2], Kouki Nishikawa[3], Yoshinori Fujiyoshi[3], Atsunori Oshima[3] ([1]*Grad. Sch. Sci., Univ. Kyoto*, [2]*NIPS*, [3]*CeSPI, Univ. Nagoya*)

Innexin is a structural component of invertebrate gap junctions. The molecular dimensions of *Caenorhabditis elegans* innexin-6 (INX-6) gap junction channels were characterized by negative-staining electron microscopy. Cryo-electron microscopy and single particle analysis of purified INX-6 channels showed class averages with eight rotational peaks that would be related to the INX-6 subunits. Two-dimensional crystallization of INX-6 is now in progress. Electron tomography demonstrated that the INX-6 channels in the reconstituted membranes form hemichannels rather than junction channels. Single particle analysis of the reconstituted INX-6 hemichannels suggests that the oligomeric number of the INX-6 channel is distinct from that of the dodecameric connexin channel.

1P008 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[1], Takayuki Kato[1], Keiichi Namba[1,2] ([1]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [2]*QBiC, Riken*)

Salmonella enterica serovar Typhimurium expresses two flagellins, FliC and FljB. The *fli*C^{+}/*flj*B^{-} strain is a robust activator of the innate immune system whereas the *fli*C^{-}/*flj*B^{+} strain is a very poor activator (1). It has been postulated that the atomic structures of the flagellar filaments are very similar among many bacteria. Three-dimensional cryoEM image reconstruction of the flagellar filament composed of FljB has shown that the orientation of the outer domain (D3) of flagellin is markedly different from that of the FliC filament. Implications of this finding will be discussed.

1. Simon, R., & Samuel, CE. Activation of NF-kappaB-dependent gene expression by Salmonella flagellins FliC and FljB. Biochem Biophys Res Commun. 2007 Mar 30;355(1):280-5.

1P009 らせん対称性に基づく構造解析のための 15 プロトフィラメント微小管の調製
Preparation of seamless 15-protofilament microtubules for helical reconstruction of microtubules

Hiroko Takazaki[1], Takashi Fujii[2], Seiichi Uchimura[1], Rie Ayukawa[1], Keiichi Namba[3], Etsuko Muto[1] ([1]*BSI., Riken*, [2]*QBiC., Riken*, [3]*Grad. Sch. Frontier Biosci., Univ. Osaka*)

Electron cryomicroscopy and helical reconstruction are powerful tools for structural analyses of helical protein polymers. However, the helical reconstruction algorithm cannot be simply applied to microtubules (MTs), because MTs in vitro consist of a variable number of protofilaments (pfs), most with a 'seam' in their lattice. Here we expressed and purified MEC-12 (α-tubulin) and MEC-7 (β-tubulin), which are responsible for the formation of seamless 15-pf MTs in *C. elegans* neurons. About 70 % of the purified MEC MTs was composed of 15-pfs, and all become 15-pf MTs, when K40 of MEC-12 is acetylated. By using this material, we succeeded in reconstructing 3D maps of MTs at a sub-nanometer resolution. At the meeting, we will discuss our structural model of the MT.

1P010 CD72 の構造解析に向けて
Towards the structure analysis of CD72

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CD72 is a 45 kDa type II membrane protein expressed mainly in B lymphocytes. It contains a C-type lectin-like domain （CTLD） in the extracellular region, but its lectin activity is not clear. Recently, some candidates for ligands of CD72 were found by glycan array assays. To study the molecular basis for CD72-mediated ligand recognition, we have expressed recombinant CD72 for structure analysis by NMR and X-ray crystallography. Overexpression of CD72 in bacteria leads to the formation of inclusion bodies. Therefore, we tried protein refolding or expression as a fusion protein to soluble tags. NMR measurements indicated the correct folding of the refolded protein and possibilities of interaction with ligands.

1P011 多周波電子スピン共鳴によるスピンラベル変性タンパクのダイナミックス
Dynamics of Spin-labeled Denatured Protein Studied by Multi-frequency electron paramagnetic resonance

Yasunori Ohba[1], Tetsuya Itabashi[1], Munehito Arai[2], Jun Abe[3], Satoshi Takahashi[1], Seigo Yamauchi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Art and Sci, Univ. Tokyo*, [3]*IMS*)

Dynamic behavior of protein during denaturation process is studied by using the SDSL (site directed spin labeling) method with multi-frequency electron paramagnetic resonance (EPR) approach. The B domain of protein A (BDPA) is labeled at 22 (on the helix H2) and 55 (H3) th residue by nitroxide radical (MTSL) and denaturation is caused by addition of guanidinium chloride (GdmCl) up to 5 M. We observed significant line width changes of nitroxide X-band EPR (9.5 GHz)(1). In the present paper, we reports W-band EPR (95 GHz) linewidth analyses of spin labeled BDPA for the denaturation process to discriminate several types of dynamics exist in the protein.

1P012 一分子力学計測による Sup35NM の不均一構造の解明
Single Molecule Studies on the Conformational Heterogeneity of Sup35NM Structure

Yusuke Komi[1], Maillard Rodrigo[2], Carlos Bustamante[2], Motomasa Tanaka[1] ([1]*BS Inst., RIKEN*, [2]*HHMI/UC Berkeley*)

Sup35NM is the aggregation domain of a yeast prion protein Sup35. The N-domain in Sup35NM has Q/N-rich regions that are required for prion formation. The role of the Middle domain remains unknown. Our studies showed that the variable amyloid properties of Sup35NM are linked to different aggregated states of the protein. However, it is not known how the monomer conformation leads to the formation of distinct oligomeric states and amyloid properties. Here we use optical tweezers to study the conformational heterogeneity of single Sup35NM monomers. We found that the folding pathway of Sup35NM displays multiple, interconverting conformational states. The first 150aa organize in three conformations whereas the last 100aa arranges in two states.

1P013 Small-angle X-ray scattering constraints and secondary-structural information can construct a coarse-grained residue-based protein model

Yasumasa Morimoto, Masaki Kojima (*Sch. Life Sci., Tokyo Univ. Pharm. & Life Sci*)

We have developed a new methodology that determines protein structures using Small-angle X-ray scattering (SAXS) data. The current bottlenecks in determining the protein structures require a new strategy using the simple design of an experiment, and SAXS is suitable for this purpose in spite of its low information content. For nine proteins taking different folds, we performed structure calculations using the SAXS constraints combined with the various type of structural information from NMR. The results show that the SAXS constraints complemented the tertiary-structural information for all the proteins, and furthermore, a coarse-grained protein model at amino acid residue resolution can be constructed only from SAXS constraints and secondary-structural information.

1P014 Effect of methanol on the structure of α-chymotrypsinogen A

Koichi Murayama (*Grad. Sch. Med., Gifu Univ.*)

The structural characterization of protein on native/denatured state is of primary importance for understanding the mechanism of protein stability and folding. Herein, to explore structural characteristics of protein on alcohol-induced denaturation, far/near-UV CD, Trp fluorescence and FT-IR spectra of protein in the methanol solutions were measured. α-Chymotrypsinogen A (aCTgn), which is one of the major component of pancreatic secretions, was used as a sample. The following results were obtained; (1) A formation of helical structures by helix-inducing ability of methanol to aCTgn was observed. (2) Local environment of Trp residues in aCTgn was changed. (3) Methanol concentration range for helical structure formation was different from that in local environment of Trp.

1P015 二次構造形成に関わる分子内および分子間相互作用に関する量子化学研究

Quantum chemical study of intra- and inter-molecular interactions in secondary structures

Yu Takano, Haruki Nakamura (*Research Center for State-of-the-Art Functional Protein Analysis Institute for Protein Research, Osaka University*)

The three-dimensional structure of a protein determines its functions and chemical properties. The second structures such as α-helices and β-sheets are important components for the protein architecture. The intra- and inter-molecular interactions, in particular hydrogen bonding, play significant roles in the formation of the secondary structures. Quantitative estimate of these interactions is required to understand the principle of the formation of the three-dimensional protein structure. In the present study, we have investigated the intra- and inter-molecular interactions in the α-helices and β-sheets, using quantum chemical methods and molecular mechanics. The characteristic interactions essential for forming the secondary structures are discussed quantitatively.

1P016 高分子複合体の密度マップ・原子モデルの混合正規分布モデルを用いた重ね合わせ計算

Superimposing density maps and atomic models of macromolecular complexes using Gaussian mixture model

Takeshi Kawabata, Hirofumi Suzuki, Akira Kinjo, Haruki Nakamura (*Institute of Protein Research, Osaka University*)

More than 1,800 of 3D density maps are now registered in EMDB; their comparisons can lead us to new biological findings. We introduce the program gmfit for superimposing macromolecular complexes, which employs a Gaussian mixture model, composed of Gaussian distribution functions, to approximate shapes of 3D density map and atomic models. To find the optimal configuration, initial configurations are randomly generated, and sequentially improved by the steepest descent method. The gmfit can perform not only one-vs-one, but also many-vs-one superimpositions. We have invented a new approach “segmentation fitting”, for many-vs-one superimposition. A WEB service for one-vs-one superimposition of the density maps in EMDB will appear in EM navigator.

1P017 Edge strand と central strand は異なったペアパートナー選択傾向を示す

Edge and central strands show a different propensity for pairing partners

Hiromi Suzuki (*Sch Agri., Meiji Univ.*)

Formation of β-sheets is one of the key steps for the tertiary structure of proteins. Considering the difference of physicochemical properties of edge and central strands into account, we analyzed residue pairing patterns in the PDB. For hydrogen bond pairs, residues on a strand tended to avoid inconvenient residues (negative selection) rather than to select convenient residues (positive selection), and this propensity was not changed whether edge and central strands were distinguished or not. For non-hydrogen bond pairs, however, the propensity was quite different between edge and central strands; negative and positive selections were preferred for central and edge strands, respectively. These results imply importance of non-hydrogen bond pairs for β-sheet formation.

1P018 PDB の成熟度を利用したホモロジーモデリング手法

A new homology modeling technique that utilizes the knowledge of completeness of the PDB

Takahiro Kanemitsu[1], Shintaro Minami[2], George Chikenji[1] ([1]*Grad. Sch. of Engineering, Univ. Nagoya*, [2]*Res.Sch.of info sci, Univ. Nagoya*)

Homology modeling technique is currently the most reliable method for predicting protein structures. However, if there is a large insertion in the amino acid sequence of the target protein relative to the template, the predicted structure for such a region is likely to be unreliable.
Here, we propose a new homology modeling method that can effectively generate reasonable structure candidates of such unreliable regions. The idea of the method is based on the knowledge of completeness of secondary structure packing in PDB. The detailed description of the method and the results of benchmark tests will be presented in the presentation.

1P019 タンパク質の構造コンプライアンス特性の計算
Computation of the Structural Compliance Characteristics of Proteins

Keisuke Arikawa (*Fcl. Eng. , Kanagawa Inst. of Tech.*)

We developed a method for calculating the structural compliance of proteins using PDB data. The protein model used in this method consists of main chains, springs between the alpha carbons, and constraints of distances between the specific alpha carbons. The method is formulated not just for a single protein molecule model but for a system containing multiple molecule models, with the prospect of efficient computation including parallel and incremental computation. We confirmed that it was possible to calculate the structural compliance of a system containing more than eight thousand amino acid residues. The calculated results provide information regarding the motion of molecules in the system as well as the structural compliance characteristics.

1P020 NRSF/REST の競合誘起天然変性に起因する動的特性
Dynamical Property due to Frustration Induced Intrinsic Disorder of NRSF/REST

Katsuyoshi Matsushita[1,3], Hidetoshi Sugihara[1,3], Macoto Kikuchi[1,3,4], Tomoaki Nogawa[5], Munetaka Sasaki[6] ([1]*Cybermedia Centery, Osaka University*, [2]*Institute for Protein Research, Osaka University*, [3]*Graduate School of Science, Osaka University*, [4]*Graduate School of Frontier Biosciences, Osaka University*, [5]*Faculty of Medicine, Toho University*, [6]*Department of Applied Physics, Tohoku University*)

An intrinsically disordered region in NRSF/REST exhibits a coupled binding and folding [1]. Recently, we theoretically investigated this coupled folding and binding, and proposed the hypothesis that the cooperativity between the folding and binding is induced by the frustrating interaction in this region [2]. The frustrating interaction is expected to exert effects on the kinetics of the coupled folding and binding. The effects on the kinetics are still unclear.
In the present work, we develop an analytical method of folding kinetics. We apply this method to a model of NRSF/REST and describe the frustration effects on the coupled folding and binding.
[1] M. Nomura et al., J. Mol. Bio. 354 (2005) 903.
[2] K. Matsushita and M. Kikuchi, J. Chem. Phys. 138 (2013) 105101.

1P021 Adaptive lambda square dynamics シミュレーション：生体分子の効率的な構造探索法
Adaptive lambda square dynamics simulation: an efficient conformational sampling method for biomolecules

Jinzen Ikebe, Shun Sakuraba, Hidetoshi Kono (*MMS., JAEA*)

A novel, efficient sampling method for biomolecules is proposed. The partial multicanonical molecular dynamics (partial McMD) was recently developed as a generalized ensemble (GE) method to focus sampling only on a part of a system (GEPS); however, it was not tested well. We found that partial McMD did not work well for poly-lysine decapeptide and gave significantly worse sampling efficiency than a conventional GE. Herein, we elucidate the fundamental reason for this and propose a novel GEPS, adaptive lambda square dynamics (ALSD), which can resolve the problem faced when using partial McMD. We demonstrate that ALSD greatly increases the sampling efficiency over a conventional GE.

1P022 アラニンペプチドモデルにおける溶媒和自由エネルギーの加算性
Analysis of additivity in the alanine peptide model of protein solvation by molecular simulations

Hironori Kokubo, B. M. Pettitt (*UTMB*)

The protein solvation model implying additivity of the alanine peptide free energy of solvation is computationally tested by solvation free energy simulations. We found that both the fixed extended conformations and the flexible ones showed the ideally additive solvation free energy as the chain length increased. The slope of the total solvation free energy of the extended conformations was negatively larger than that of the flexible ones as expected. However, we found that the vdW solvation free energy decreased as the chain length increased for the extended peptides, on the other hand, it increased as the chain length increased for the flexible peptides. We discuss these trends and their implications in protein solvation.

1P023 MD シミュレーションによる設計したタンパク質間相互作用面の評価
Evaluation of the designed protein binding interfaces as studied by MD simulation

Masaki Fukuda, Hironao Yamada, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu, Satoshi Akanuma, Akihiko Yamagishi (*Sch. of Life Sci., Tokyo Univ. of Pharm. and Life Sci.*)

The purpose of our study is to design protein surfaces which induce a protein-protein interaction. We mimicked naturally occurring 4-helix bundle that are found at interfaces between two proteins. To strengthen the protein-protein interaction, we introduced hydrophobic residues on the surface and charged residues on the side of interface *in silico*. We evaluated the binding of two protein molecules with the designed interface by molecular dynamics simulation. Then, we analyzed the distance between the interfaces, solvent-accessible surface area, root mean square fluctuation, and short-range (1nm) part of Coulomb potential energy in the system. We eventually found an interface that seemed to be suitable for inducing the protein-protein interaction.

1P024 赤外分光法によるカルシウム結合タンパク質並びにカルシウム結合ペプチドアナログの配位構造解析
Coordination to divalent cations by calcium-binding proteins and calcium-binding peptide analogues studied by FTIR spectroscopy

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EF-hand motif is a Ca^{2+} binding domain in common between many intracellular Ca^{2+}-binding proteins such as calmodulin and troponin C. FTIR spectroscopy has been applied to study Ca^{2+}-binding proteins and the synthetic peptide analogues corresponding to the EF-hand motif. The COO^- stretching vibration modes can be used to identify the coordination modes of COO^- groups of Ca^{2+}-binding proteins to metal ions: bidentate, unidentate, and pseudo-bridging. As a result, the downshift of the COO^- antisymmetric stretching mode from 1565 cm^{-1} to 1555 - 1540 cm^{-1} upon Ca^{2+} binding was a commonly observed feature of FTIR spectra for EF-hand proteins. The implication of COO^- stretches was discussed on the basis of the FTIR spectra of calcium-binding peptide analogues.

1P025 蛋白質複合体の高圧放射光 X 線小角散乱データに対するグローバルフィット解析

Global fit analysis on high pressure synchrotron small-angle x-ray scattering data of protein complexes

Tetsuro Fujisawa[1,2], Keiichi Kameyama[1], Ryo Ishiguro[1,2] ([1]*Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University*, [2]*RIKEN SPring-8 Center*)

Small-angle scattering (SAS) from protein solutions now retrieves distinct structures in solution as an ensemble by rigid body ensemble fitting. Together with other structural methods, these SAS models appears to be "real" in solution. However, it is true that the fitting is susceptible to the quality of data and modeling method. Alternative approaches are required to reinforce this methodology. The combinational use of high hydrostatic pressure (HP) below 400MPa with SAS seems promising, since it enables that the free energy contribution on protein stability and equilibrium can be finely tuned without breaking covalent bonds. We discuss the theoretical foundations of global fitting and its application on high-pressure SAS data.

1P026 放射光広角散乱法によるタンパク質熱転移に対する crowding 効果の研究

Crowding effect on thermal transition of proteins clarified by SR-WAXS

Kazuki Takeuchi, Mitsuhiro Hirai (*Graduate School of Engineering, Gunma University*)

Synchrotron radiation wide-angle X-ray scattering (SR-WAXS) method is useful to detect the structural characteristics of proteins depending on different hierarchal structure levels. On the other hand, crowding environment in cells is assumed to affect structure and function of proteins through the change of chemical potential of water. By using SR-WAXS, we have been studying the thermal stability of proteins under crowding condition generated by the presence high- and/or low-molecular weight neutral molecules as co-solutes. The co-solutes used were polyvinylpyrrolidone (Mw. 40,000) and glycerol. SR-WAXS measurements were performed at SPring-8 and PF. We will discuss about the difference of crowding effect on the thermal transition feature depending on co-solutes.

1P027 硬骨魚類の乳酸脱水素酵素活性の温度依存性

Thermal stability of lactate dehydrogenase of marine teleostei: molecular adaptation of ectothermic animal to low temperature

Mizuki Nakagawa[1], Mika Yonezawa[1], Shigeyoshi Nakamura[2], Shun-Ichi Kidokoro[2], Hideki Wakui[1], **Wataru Nunomura**[1] ([1]*Life Sci., Grad. Sch. Eng. & Resource Sci., Akita Univ.*, [2]*Dept. Bioeng., Nagaoka Univ. Tech.*)

To understand adaptation of exothermal animals to low temperature, we highly purified lactate dehydrogenase (LD) from muscle of slime flounder (*Microstomus achne*), a bottom-dwelling fish living in 10°C sea. The slime flounder muscle contained homogenous single isoform LD at the position of M_2H_2 LD isozyme of bovine muscle. The activity of LD was lost at 62°C (thermostability). Although the maximum activity of LD was measured at 30°C, the values of K_m and V_{max} were increased dependent with temperature between 5°C and 40°C. Differential scanning calorimetry (DSC) measuring revealed that the each subunit of LD simultaneously denatured by heat. We discuss about the relationship between enzyme activity and evolution of ectothermy to endothermy based on the present study.

1P028 疎水性溶媒が蛍光タンパク質の蛍光特性に及ぼす影響

Effects of organic solvents on the properties of fluorescent proteins

Hideaki Konishi, Suguru Asai, Kunio Takeyasu, Shigehiro Yoshimura (*Kyoto university*)

Fluorescent properties of fluorophores are known to be affected by the environment (e.g. pH and permittivity of solvent). The properties of fluorescent proteins (FPs) are also affected in a complicated manner due to their unique chromophore structures. Here, the fluorescent properties of various FPs were elucidated in different hydrophobic environments. The fluorescence intensities of ECFP and EGFP, but not mRFP and mCherry, were decreased in 2,2,2-trifluoroethanol (TFE)-containing solution, whereas lower alcohols had almost no effect. Molecular dynamics revealed that the addition of TFE induced a significant conformational change in the chromophore structure of ECFP.

1P029 神経小胞融合過程におけるシナプトタグミンと SNARE の分子機構

molecular mechanism of synaptotagmin and SNARE in the synaptic vesicle fusion process

Yasuhito Nagai, Tadashi Takemori (*Grad. Sch. Pure and appl sci., Univ. Tsukuba*)

Synaptotagmin (Syt) family proteins play crucial roles in synaptic vesicle fusion. They have a peculiarly intriguing universal structure of tandem C2 domains with a flexible linker of a highly conserved length in between, but its role is still unresolved. Points of contact between Syt-I and SNAP25 have been revealed, but the strength of molecular binding seems modest at those binding sites. We shall report molecular dynamics simulation of the interaction between Syt-I and SNARE complexes, to examine the mode of binding between various parts, and to figure out how they can work in concert to assist SNARE formation on the synaptic cell membrane. We shall present a model of the sequence of events leading up to SNARE formation prior to vesicle fusion.

1P030 PLC-δ1 PH ドメインの分子内アロステリー

Intramolecular allostery in the PLC-δ1 PH domain

Michikazu Tanio, Katsuyuki Nishimura (*Institute for Molecular Science*)

Protein activities are generally regulated by intramolecular allosteric interactions, by which spatially separated sites in a protein molecule communicate. Molecular mechanisms of intramolecular allosteric interactions in the phospholipase C (PLC)-δ1 pleckstrin homology (PH) domain were investigated. Mutational analyses using native PAGE and solution NMR methods provide evidence for intramolecular interactions in the PLC-δ1 PH domain, the function of which could be allosterically regulated by modifications at sites spatially separated from the ligand-binding site through the intramolecular interaction network.

1P031 分子動力学を用いた細菌機械受容チャネル MscL の脂質膜の厚みに影響される開口挙動に関する研究

Molecular Dynamics Study on the Opening Behavior of Bacterial Mechanosensitive Channel MscL Effected by Membrane Thickness

Hiroki Katsuta[1], Yasuyuki Sawada[2], Masahiro Sokabe[2] ([1]*Sch. of Med., Nagoya Univ.*, [2]*Dept. Physiol. Nagoya Univ. Grad. Sch. Med.*)

One of mechanosensitive channels MscL is homopentamer with two transmembrane inner (TM1) and outer (TM2) helices and activates by sensing membrane tension. The major issue of MscL is to solve its gating mechanism. Previous studies revealed that MscL embedded in a thinner lipid bilayer opens more easily. However, it remains unclear why the channel opening depends on the bilayer thickness. Thus we performed MD simulations of MscL embedded in three types of the bilayer to explain the dependence of the opening behavior in atomic detail. As a result, MscL in a thinner membrane actually expanded widely. Also it was found that a tilt angle of the tranemembrane helices increased as the thickness decreased, leading to smaller interaction energy between MscL and the membrane.

1P032 分子動力学シミュレーションを用いた大腸菌機械受容チャネル MscL のゲーティングに関するゆらぎ解析

Fluctuation Analysis Study on Mechano-Gating in the E-coli Mechanosensitive Channel MscL Using Molecular Dynamics Simulations

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One of mechanosensitive channels, MscL, is homopentamer of a subunit with transmembrane inner and outer helices. The major issue of MscL is to understand the gating mechanism driven by membrane tension. To address this question, MD simulations have been performed, however, it remains unclear the relationship between tension sensing and the gate opening. Thus, we performed opening simulations of the channel and analyzed thermal fluctuations using principal component analysis and get insight into the coupling between a mechanosensor and the gate. We modeled wild type, F78N and G22N mutant MscLs, and performed simulations for 40 ns to sample 5000 coordinate data sets. As a result, it was found that a fluctuation coupling of between 22nd and 78th amino acids is important.

1P033 大腸菌機械受容チャネル MscL の開口過程においてメカノセンサーとゲートは密接に連動する

Mechanosensor and gate is tightly coupled in the opening process of the bacterial mechanosensitive channel MscL

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The bacterial mechanosensitive channel MscL is known to gate by membrane tension and we previously found that F78 acts as a tension sensor. In this study we performed MD simulations of several MscL mutants to get insights into the relationship between the tension sensor F78 and the gate. The GOF mutant G22N is easier to open, while the LOF mutant F78N cannot be opened upon strong membrane stretch. To test whether the behavior of G22N is independent of the tension sensing at F78, we performed simulations of the double mutant G22N/F78N and found that G22N/F78N MscL did not open the gate, suggesting that the tension sensor and the gate of MscL is tightly connected and that the interaction between the tension sensor and lipids is essentially important for the MscL opening.

1P034 H^+/Ca^{2+}交換輸送体における対向輸送の分子基盤

Structural Basis for the Counter-Transport Mechanism of a H^+/Ca^{2+} Exchanger

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Ca^{2+}/cation antiporters catalyze the exchange of Ca^{2+} with various cations across biological membranes to regulate cytosolic calcium levels.
Here, we report the crystal structure of a H^+/Ca^{2+} exchanger from Archaeoglobus fulgidus (CAX_Af) in the two representatives of the inward-facing conformation at 2.3 Å; resolution. The structures suggested Ca^{2+} or H^+ binds to the cation-binding site mutually exclusively. Structural comparison of CAX_Af with previously a reported CaCA protein revealed that the first and sixth transmembrane helices alternately create hydrophilic cavities on the intra- and extracellular sides. The structures and functional analyses provide insight into the mechanism of how the inward- to outward-facing state transition is triggered by the Ca^{2+} and H^+ binding.

1P035 RND 型薬剤排出トランスポーターの阻害活性の構造的基礎

Structural basis for the inhibition of bacterial multidrug exporters

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The multidrug efflux transporter AcrB and its homologs are important in the multidrug resistance of Gram-negative pathogens. ABI-PP is AcrB and MexB-specific inhibitor that does not inhibit MexY; MexB and Y are principal multidrug exporters in Pseudomonas aeruginosa. We have determined the first inhibitor-bound structures of AcrB and MexB. ABI-PP tightly binds to a narrow pit composed of a phenylalanine cluster located in the binding site and sterically hinders the functional rotation. We found that the difference of affinity for the inhibitor between AcrB/MexB and MexY is the volume of the residues of the end of the pit. The structure of the hydrophobic trap described in this study will contribute to the development of universal inhibitors of MexB and MexY.

1P036 極低温電子顕微鏡を用いた電圧感受性 Na チャネルの立体構造と機能

Two alternative conformations of a voltage-gated sodium channel

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We present two conformations of a voltage-gated sodium channel (Nav) from C. thermarum reconstituted into lipid bilayers in one crystal at 9Å resolution based on electron crystallography. Despite a voltage sensor arrangement identical to that in the activated form, we observed two distinct pore domain structures of a prominent form with a relatively open inner gate and a closed inner gate conformation similar to the first prokaryotic Nav structure. Our analyses together with mutational and electrophysiological experiments indicated that widening of the inner gate was dependent on interactions among the S4-S5 linker, the N-terminal part of S5 and its adjoining part in S6, and on inter-helical repulsion by a negatively charged C-terminal region subsequent to S6.

1P037 Roles of two coupling helices between transmembrane and cytosolic domains in ABC transporter

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ATP Binding Cassette transporters are membrane proteins moving substrates energized by ATP hydrolysis. A eukaryotic ABC transporter consists of two domains: nucleotide-binding domains hydrolyzing ATP and transmembrane domains facilitating substrate-transport. These domains contact via two helices called coupling helices, CH1 and CH2, from the transmembrane domain. To understand the roles of CH1 and CH2 in the ATP-driven transport mechanism, we performed functional study by site-directed mutagenesis based on the crystal structure of a new eukaryote ABC exporter, we recently determined at high resolution. This study revealed some residues on CH1 and CH2 play important functions such as maintaining the structure of the helices or mediating interaction of the two domains.

1P038 分子動力学シミュレーションで探る CFTR における変異の影響

The effects of mutations in CFTR as studied by molecular dynamics simulations

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CFTR is a member of ABC transporters, and is a unique chloride channel. The channel opening is triggered by the ATP-driven NBD dimerization. There are several mutations in the CFTR gene, which lead to the genetic disease cystic fibrosis (CF). In spite of intense investigation, the effects of mutations have not been understood in detail. In this study, we focus on the conformational changes of CFTR wild type and mutants. We conducted the comparison between dynamics of wild type and those of several mutants by molecular dynamics simulations, where simulations were started from inward-facing homology models. We will discuss the structure-function relationships in CFTR, and also the role of ATP on NBD dimerization.

1P039 分子動力学シミュレーションで探るセルラーゼ *Tr*Cel7A の基質取り込みのメカニズム

Mechanism of substrate uptake in cellulase *Tr*Cel7A as studied by molecular dynamics simulations

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 with aromatic residues, which is important for substrate uptake. Here, we conducted molecular dynamics (MD) simulations and free energy calculations to clarify the roles of tryptophan residues in *Trichoderma reesei* cellobiohydrolase Cel7A. The MD results show that the cellulose chain entered into the tunnel from subsite -6 to -3 in W40A mutant (in which the entrance Trp40 is mutated to alanine), whereas no apparent entering was observed in wild type. Moreover, from the free energy calculation, we found that the tryptophan residues lining up along the tunnel have a significant effect on substrate binding in *Tr*Cel7A.

1P040 μs スケールの分子動力学シミュレーションによる光受容タンパク質 LOV-HTH の光応答機構の研究

Study of the photoresponsive mechanism of LOV-HTH protein using μs scale molecular dynamics simulations

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 μs scale MD 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). The present simulations indicated that formation of the cysteinyl-FMN (LOV chromophore) adduct causes significant dynamics changes in the HTH domain and does the H-bond network among residues in the vicinity of FMN in the light state. Based on these results, we propose the light signal path ways through Q136-S137-R215 and via Jα-helix.

1P041 ABC トランスポーターのヌクレオチド結合ドメイン二量体化の理論的解析—ATP と水の役割

Theoretical analyses of the nucleotide-binding domain dimerization of ABC transporters: roles of ATP and water

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ATP-binding into the nucleotide-binding domains (NBDs) of ABC transporter induces NBD dimerization, which triggers the transportation of substrates across membranes. However the energetics of these processes has not been well understood. Here, we evaluated the free energy difference (ΔG_{bind}) of NBD dimerization in MalK and CFTR cases by the MM/3D-RISM method. In the case of MalK, the ΔG_{bind} of ATP-bound NBDs is -6.34 kcal/mol, which is in good agreement with the experimental value. We also found that the processes are driven by a large entropy gain of water, and that the subsequent Pi releases destabilize the NBD dimer with a significant decrease of entropy gain of water. Similarly an entropy-driven NBD dimerization was also observed in the case of CFTR.

1P042 ADP/ATP 透過担体の大規模構造変化に関する理論的研究

A theoretical study on the large conformational change of ADP/ATP carrier

Koichi Tamura, Shigehiko Hayashi (*Grad. Sch. Sci., Univ. Kyoto*)

ADP/ATP carrier(AAC) is a membrane protein embedded in the inner membrane of mitochondria. Its fundamental function is to exchange an ADP or ATP in one side of membrane to an ADP(ATP) in the other side. The exchange is thought to be accompanied with protein's large conformational change and the strctural analysis of AAC will explain the mechanism of the 1:1 exchange. The only available structure (PDBid: 1okc) is opened toward the inter membrane space and the other structure is still not available. LRPF method, which enables us to predict protein's conformational transition pathway, was applied to the problem and revealed a channel-like intermediate structure.

1P043 Computational design of short peptide inhibitors of protein-protein interactions in intracellular signaling mediated by CRK-SH2

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CRK is a signal transducing adaptor protein, which mediates protein-protein interactions in signaling pathways. CRK has several protein-binding modules named SH2 and SH3 domains. Our goal is the design of high-affinity peptides binding to CRK SH2.
First, we developed original GPU docking program using MM potential energy and the generalized born (GB) solvent as scoring functions. After pose predictions, MM-PBSA rescoring were conducted. In rescoring, we compared several radii sets which determines the boundary between solute and solvent. Furthermore, we performed additional conformational search of peptides in the unbound states to take account of ligand's reorganization effects. Our methods showed high performances in discrimination of known binding sequences.

1P044 Electrostatic similarities between protein and small molecules facilitate the rational design of protein-protein interaction inhibitors

Arnout Voet, Francois Berenger, **Kam Zhang** (*Zhang Initiative Research Unit, Institute Laboratories, RIKEN*)

We have developed a method (Elekit) that measures the similarity of the electrostatic fields for the discovery of protein-protein interaction inhibitors. The electrostatic values are mapped onto a 3D grid surrounding the molecules. A bitmask is created such that only the grid points, representing the electrostatic field towards the receptor protein, are taken into account. The Spearman rank correlation coefficient between the SMPPII ligand and the known protein ligand is computed. Analysis of all available SMPPII structures indicates that SMPPII have similar electrostatic properties as the ligand proteins of the same receptor. Elekit can be used as a post-processing filter for docking and/or pharmacophore based SMPPII virtual screening experiments.

1P045 レプリカ置換法による生体分子に対する効率的な構造サンプリン
Efficient sampling for biomolecules by the replica-permutation method

Satoru Itoh[1,2], Hisashi Okumura[1,2] ([1]*IMS*, [2]*Sokendai*)

Efficient sampling in the conformational space is necessary to predict the native structures of proteins. The replica-exchange method (REM) is one of the most well-known methods among the generalized-ensemble algorithms which realize efficient sampling in the conformational space. We had recently proposed a better alternative to the REM, the replica-permutation method (RPM) [1], in which temperatures are permutated among more than two replicas. Furthermore, the Suwa-Todo algorithm is employed in RPM instead of the Metropolis algorithm.
We will show the results of RPM in our presentation. These results will be compared with those of REM to see sampling efficiency of RPM.
References
[1] S. G. Itoh and H. Okumura, J. Chem. Theory Comput. 9, 570 (2013).

1P046 Metadynamics: Implementation in GENESIS Software Package and Demonstration of the Efficient Computational Simulations of Biomolecules

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Metadynamics (MTD) is an accelerated sampling algorithm aiming to maximize the output of molecular dynamics (MD) simulations. MTD, by design, is capable of the efficient simulation of biosystems with large energetic barriers and rough energy landscape within the limited time scale of MD simulations, and it is applicable to arbitrary large systems and complex phenomena (i.e. folding, binding, chemical reactions, etc.). Our implementation of MTD in GENESIS is focused toward scalability to utilize massively-parallel computers (i.e. "K computer"). The accuracy and efficiency is demonstrated with several simulations of biomolecules and compared with an another established accelerated sampling method, replica-exchange MD (REMD).

1P047 Motion Tree を利用した capping protein の動的構造解析
Dynamical study of capping protein by Motion Tree

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Capping protein (CP) binds to the barbed end of an actin filament and inhibits the further polymerization. V-1 and CARMIL inhibit CP to bind the barbed end, but their inhibition mechanisms are quite different: V-1 sterically inhibits the CP binding to actin filament, and allosterically does CARMIL. In addition, CARMIL can uncap CP from actin filament or V-1.
To elucidate the regulation mechanism of CARMIL, we conducted molecular dynamic simulation for the structures of free CP, CP/CARMIL and CP/V-1 complexes, and investigate the dynamic properties of CP. For the snapshot ensemble of CP, Motion Tree was applied and the distribution of rigid bodies was examined. We found the CARMIL binding suppresses large domain motions of CP.

1P048 MSES により明らかになった蛋白質遭遇複合体構造アンサンブル
Structural ensemble of protein encounter complex revealed by Multiscale Essential Sampling

Satoshi Omori, Kei Moritsugu, Akinori Kidera (*Grad. Sch. Med. Life Sci., Yokohama City Univ.*)

Many proteins perform their functions by specific complex formation. Paramagnetic relaxation enhancement (PRE) experiments indicate the existence of non-specific encounter complex preceding the formation of the specific complex. However, structural details of the encounter complex still remain unclear even after many experimental and computational studies. Here, we simulated the process of the complex formation between N-terminal domain of enzyme 1 (EIN) + HPr (PDB ID: 3EZB) by Multiscale Essential Sampling (MSES), which allows an enhanced sampling of solvated all-atom structures. The structural ensemble including the encounter complexes successfully reproduced the PRE data. Free energy landscape of the complex formation revealed the role of the encounter complexes.

1P049 独立成分分析 tICA を用いたタンパク質主鎖の遅い運動の解析

Slow dynamics of protein backbone in molecular dynamics simulation revealed by time-structure based independent component analysis

Sotaro Fuchigami (*Grad. Sch. of Medical Life Science, Yokohama City Univ.*)

Protein motions occur over a wide range of time scales, but not all are important for protein functions, which time scales are generally longer. Thus, it would be reasonable to consider that slower motions of proteins are more relevant to their functions. To identify such slow protein dynamics from simulation results, we have proposed the time-structure based independent component analysis (tICA). In the present study, we selected lysine-, arginine-, ornithine-binding protein (LAO) as a target protein, and performed one-microsecond molecular dynamics simulation in explicit water. By applying the tICA to the simulation results, several motions in the LAO were identified as slow modes, and were confirmed with additional analyses to be actually occurred.

1P050 トリプリオンタンパク質に対する抗体 G2 の複数の抗原を特異的に認識する性質の特徴付け

Characterization of multispecific monoclonal antibody G2 directed against chicken prion protein

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We generated a monoclonal antibody, designated G2, with residues 174-247 of the chicken prion protein (ChPrPC) and found that G2 could react with at least three proteins other than ChPrPC. Surprisingly, there were no regions of amino acid sequence similarity between ChPrPC and those proteins. The amino acid sequence of the G2 epitope within one of the protein was not related to the G2 epitope within ChPrPC. ELISA, SPR, and ITC experiments indicated that these two epitope peptides have similar binding affinity for G2. However, we found that these two peptides differed substantially in several binding characteristics in SPR and ITC experiments. These differences may be explained by G2 adopting different binding conformations and undergoing different binding pathways.

1P051 リソスタシンのカルサイト結合部位の同定

Identification of calcite-binding site of lithostathine

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Lithostathine (LIT) binds to surfaces of calcite ($CaCO_3$) crystal to modify its morphology. LIT is proteolyzed into two fragments (LIT133 and LIT11) by trypsin. To identify the calcite-binding site of LIT, we compared binding efficiency of intact LIT, LIT133 and LIT11 by examining their ability to modify the calcite morphology. Intact LIT modified calcite into a prismatic shape, implying that the protein binds to the prism planes ((110) and (1-10)). LIT133 modified the prism planes less efficiently than intact LIT. No morphological change of calcite was detected for LIT11 even at high concentrations. These results suggest that calcite-binding sites are located in LIT133 portion, where acidic residues aligned in its molecular surface.

1P052 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イオンとの競合結合性

Competitive binding of metal ions to octarepeat region of prion protein

Masahiro Yagi, Kazuya Iwama, Haruto Onda, Wakako Hiraoka (*Graduate School of Science and Technology, Meiji University*)

Metal ions binding to prion protein (PrP) is related to cause and progress prion disease. Cu^{2+} binding to octarepeat region in PrP is considered as an important process for misfolding of PrP. In this report, competitive binding of metal ions (Cu^{2+}, Co^{2+}, Ni^{2+}, etc) to the octarepeat peptide (PHGGGWGQ) was investigated with spectroscopic studies. Visible circular dichroism (CD) and visible absorption spectra showed the binding of each metal ion to the peptide. UV-CD spectra indicated the conformational change of secondary structure in the metal binding peptide. To determine the affinity of each metal ion to the peptide. the metal binding peptide was titrated with competitive metal ion, using visible CD and visible absorption spectroscopy.

1P053 QCM によるグルカゴンと酸性膜との相互作用解析

Analysis of interaction between glucagon and acidic lipid membrane by QCM

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Glucagon is a peptide hormone consisting of 29 amino acid residues, and forms amyloid-like fibrils in aqueous solutions. Previously, kinetics of fibrillation in the presence of neutral (DMPC) and acidic (DMPC + DMPG) lipid membrane was investigated under acetic acid solution by solid state NMR. It suggested that fibril nucleation rate in the presence of acidic lipids was faster than that in the neutral lipids. In this study, association constants (Ka) between glucagon and lipids were determined by QCM. As a result, Ka values significantly rose upon increasing acidity of lipids. Membrane disruption was also observed in the lipids including 25% of DMPG. It turned out that glucagon-acidic lipids interaction accelerates the fibril nucleation rate.

1P054 ジンジパインプロテアーゼがもつ Ig-like domain の役割

Function of the Ig-like domain of gingipain proteinase

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Porphyromonas gingivalis possesses cysteine proteinases, named gingipains, which are important virulence factors of the periodontal pathogen. A proprotein of Lys-gingipain (Kgp) consists of signal peptide, propeptide, proteinase domain, Ig-like domain, adhesin domains and C-terminal domain. Ig-like domains, which are found in a variety of proteins, are the widespread structural motif that has a sandwich-like structure formed by two sheets of antiparallel beta strands. Kgp lacking the Ig-like domain was degraded by the HtrA protease that plays a role in protein quality control in the periplasm of Gram-negative bacteria, suggesting that the Ig-like domain functions as an intra-molecular chaperone.

1P055 タンパク質の構造・安定性に及ぼす環状オリゴ糖およびポリオールの添加効果

Effects of polyol and cyclic oligosaccharide on structure and stability of protein

Takayuki Iokibe, Dai Katou, Takuya Hamada, Takayoshi Kimura (*Fac. Science, Kinki Univ.*)

Polyol and cyclic oligosaccharide (cyclodextrin, CD) influence structure and stability of protein through hydrophobic interaction. We have determined thermodynamic properties of molten globule (MG) state of cytochrome c induced by polyol and unfolded state by temperature with CD. In this study, to reveal the thermodynamic properties of MG state of myoglobin, thermal denaturation of myoglobin in aqueous sorbitol solution were measured by circular dichroism and DSC. Sorbitol induced the MG state from acid unfolded state at pH2, 290 K, and over 3 M of sorbitol, and the MG state was unfolded by increasing temperature with low cooperativity. The kinetic properties of MG state was determined by stopped flow measurement.

1P056 タンパク質の熱安定性に及ぼすシクロデキストリンの包接効果

Inclusion effects of cyclodextrin on thermal stability of proteins

Toshiki Miki, Takayuki Iokibe, Takayoshi Kimura, Tadashi Kamiyama (*Fac. Science Kinki Univ.*)

The protein structure is maintained by a significant small stability as a compensated result of hydrophobic interaction, hydrogen bond, electrostatic interaction, structural entropy, and other enthalpic and entropic factors. Cyclodextrins (CDs) can influence the conformation and stability of protein via their inclusion ability. In this study, thermal denaturations of several globular proteins in aqueous CD solutions were measured by DSC. CD concentration dependence of midpoint temperature and change in enthalpy for thermal denaturation of proteins indicate the CD destabilized the folded state of proteins by stabilizing the unfolded state due to inclusion. The effect was dependent on the property of proteins.

1P057 粗視化シミュレーションを用いた STMV の自己組織化についての理論的研究

Theoretical study on the self-assembly of satellite tobacco mosaic virus using coarse grained simulation

Masato Teranishi, Micke Rusmerryani, Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Grad. Sch. Nat. Sci., Univ. Kanazawa*)

Satellite tobacco mosaic virus (STMV) is composed of 60 identical copies of a single protein with an icosahedral structure. In this work, we investigate the assembly mechanism of the STMV particle by simulations with Go like model, which is a kind of coarse grained model. For the purpose of understanding the assembly mechanism of a virus particle, it is significant to study interactions both intra- and inter-subunits of capsid proteins because the interactions highly contribute to the structural stability of a whole virus particle. Electrostatic, Van der Waals, hydrophobic and hydrogen bonding interactions are applied to protein-protein interactions. We then discuss the stability and which interaction is dominant for completing the assembly of the STMV particle.

1P058 バクテリア細胞質の全原子分子動力学シミュレーション

All-Atom Molecular Dynamics Simulation of Bacterial Cytoplasm

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Computer simulation of the realistic cellular environment is one of the major challenges for biophysical scientists. It is indispensable in obtaining the molecular-level dynamic picture of the biochemical reaction networks. For this purpose, we constructed all-atom model of cytoplasm in the Mycoplasma genetalium, the smallest bacteria. The size of the system is 50x50x50 nm, which is greatly exceeds that of typical MD simulations, covering about 1% of the volume of an entire cell. Using the trajectories generated by highly parallelized MD program GENESIS on K computer, the dynamics of the macromolecules/metabolites are analyzed. The correlation between MD-derived spatiotemporal data with experimentally known biochemical reaction networks is also discussed.

1P059 分子動力学シミュレーションによる構造エントロピー計算法の比較

Comparison of calculation methods of configurational entropy from molecular dynamics simulation trajectories

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Configurational entropy (S^c) is one of important properties in many biomolecular processes such as molecular binding. So far, many methods to calculate S^c from trajectories of the molecular dynamics simulations have been proposed. Recently, Harpole and Sharp proposed the Clausius method which enables us to exactly calculate the temperature difference in S^c without any approximations such as quasi-harmonic distributions.[1]
In this study, we compared results of the Clausius method with those of several methods for entropy calculation, including quasi-harmonic methods based on principal component analysis using Cartesian coordinates.
[1] K. W. Harpole and K. A. Sharp, *J. Phys. Chem. B*, **115**, 9461(2011).

1P060 分子モデリング法を用いた酸変性アポミオグロビンの構造解析

A Conformational Analysis of Acid Unfolded Apomyoglobin using a Novel Molecular Modeling Method

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The unfolded protein in solution consists of an ensemble with a great number of conformations. It is necessary to integrate diverse experimental data for elucidating its structural properties. We developed a new computational method for generating conformations of unfolded protein (Seki, Y. et al., *JCTC*, **2011**). Using our method, we have tried to find an ensemble which best reproduces experimental data obtained from both residual dipolar couplings of NMR and solution X-ray scattering. As a result, it was clarified that structural properties of the acid-unfolded (AU) apomyoglobin. In the annual meeting, we will discuss differences in structural properties between urea unfolded state and AU state.

1P061 溶液中におけるタンパク質分子の配置の秩序性：小角 X 線散乱による解析

Protein's arrangement in aqueous solution before the self-assemblies: A small angle X-ray scattering study

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In protein solution, protein's number density in water, i.e. the concentration, is not uniform because the intermolecular attractive and/or repulsive interactions modulate the distance between the protein molecules. From protein's interparticle interference in a small-angle X-ray scattering (SAXS), we can capture the protein's arrangement in aqueous solution. In addition, intermolecular potential of a protein can be estimated. We demonstrate that change in the SAXS profile by removal of the heme of myoglobin could be explained by an increase in intermolecular attractive interaction potential at short range from the protein molecule. The holo-form of myoglobin can escape from the aggregation due to the attractive potential comparable to thermal energy at short range.

1P062 チロシン/チロシネート蛍光法における圧力軸の有用性: 700 MPa を用いたニワトリオボムコイドの圧力変性研究

Utility of pressure axis on tyrosine/tyrosinate fluorescence spectroscopy: A pressure-unfolding study of chicken ovomucoid at 700 MPa

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The utility of tyrosine/tyrosinate fluorescence for pressure-unfolding studies of Trp-lacking proteins has been explored for the first time, with chicken ovomucoid (OVM) as target. A newly developed fluorescence spectrometer working in the range 0.1-700 MPa is employed for this purpose. At 25^{o} C at pH 12, all six Tyr residues give tyrosine emission at 306 nm, implying that all five Tyr residues are well buried in the folded OVM, except one giving "half-tyrosinate" emission at 325 nm. Upon increasing pressure, however, a distinct intermediate state, in which domains 1 and 2 are selectively unfolded, appears and increases up to 700 MPa. This intermediate state was characterized with the obtained thermodynamic parameters, ΔG^0 and ΔV^0.

1P063 Staphylococcal nuclease におけるマイクロ秒スケールの主鎖の運動

Main-chain dynamics of staphylococcal nuclease in microsecond timescale

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Staphylococcal nuclease reduces the catalytic activity greatly by deleting residues from T44 to K49. Since the deleted mutant takes the native structure, it is suggested that the deletion affects the main-chain structure dynamically to reduce the activity. We measured the lifetime of tryptophan (Trp) triplet state in microsecond timescale to explore the main-chain dynamics. In order to provide information on the dynamics, a cysteine (Cys) was substituted as a quencher for appropriate residues, which are apart from the introduced Trp in the native structure. The lifetime should decrease if the main chain has microsecond motions so that Trp and Cys encounter within the lifetime. We discuss the difference between wild type and the mutants based on the main-chain dynamics.

1P064 Staphylococcal nuclease の変性状態における局所構造の柔軟性と非局所的相互作用の関係

Local flexibility of denatured structure and its relationship to non-local interaction in staphylococcal nuclease

Toshiyuki Minemura, Mariko Yamagichi, Yoichi Yamazaki, Hironari Kamikubo, Mikio Kataoka (*Nara Institute of Science and Technology*)

Staphylococcal nuclease (SNase) has a hydrophobic cluster around W140 at the C terminus (W cluster) important for the formation of the tertiary structure, which is stabilized by the non-local interactions between the N-terminal and C-terminal domains. Alanine insertion into the W-cluster region significantly changes the local properties and leads to denaturation of SNase. We studied the influence of alanine insertion on the local properties of the W-cluster region by measuring the contact rates between W140 and a substituted cysteine for K134. The contact between W140 and K134 observed for the insertion mutants, indicating the increase of local flexibility. The origin on the instability of the insertion mutants will be discussed based on the local flexibility.

1P065 FUS/TLS タンパク質の凝集が関与する筋萎縮性側索硬化症の新たな分子病理メカニズム

A new pathomechanism of amyotrophic lateral sclerosis regulated by aggregation of FUS/TLS protein

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Dominant mutations in FUS/TLS cause a familial form of amyotrophic lateral sclerosis (fALS). Many of pathogenic mutations have been shown to deteriorate the nuclear localization signal in FUS and thereby facilitate cytoplasmic mislocalization of mutant proteins. Here, we show that a pathogenic mutation, G156E, did not disturb the nuclear localization of FUS but significantly increased the propensities for aggregation of FUS in vitro and in vivo. Notably, aggregates of mutant FUS functioned as efficient seeds to trigger the aggregation of wild-type protein and exerted cytotoxicity in rat primary neurons. Intranuclear aggregation of FUS triggered by a subset of pathogenic mutations is hence an alternative pathomechanism of FUS-related fALS diseases.

1P066 1 分子蛍光イメージングによる脱凝集シャペロン Hsp104 の作用機構の解明

Mechanism of Hsp104 disaggregase by single-molecule imaging

Momoko Okuda, Dai Nakasaka, Tatsuya Niwa, Hideki Taguchi (*Grad. Sch. of Biosci. Biotech., Tokyo Tech*)

AAA+ chaperone Hsp104 can solubilize the aggregates in cooperation with Hsp70/40 system. Hsp104 is also required for the stable propagation of yeast prions by dividing the amyloid aggregates into small one. We attempt to investigate the function of Hsp104 by a single-molecule approach. Using a total internal reflection fluorescence microscopy, the association and dissociation of Hsp104 with the Sup35 fibrils were observed in the presence of ATP. Regarding the solubilization of amorphous aggregates by Hsp104 system, we have constructed a method to watch the aggregates using highly inclined thin illumination. Addition of both Hsp104 and Hsp70/40 system decreased the size of firefly luciferase aggregates. Further statistical analyses are in progress and will be presented.

1P067 酵母プリオン Sup35 の細胞内１粒子解析
Single Particle Tracking of Yeast Prion Sup35 in Living Cells

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Yeast prion Sup35 forms self-propagating fibrillar aggregates. The mechanism of yeast prion propagation consists of fibril growth, division, and transmission to daughter cells. To unravel how individual prion particles propagate and transmit to the daughter cell, here we have conducted single particle tracking of Sup35-GFP aggregates in living yeast cells using highly inclined thin light. Since the prion particles were abundant in a cell, we had to reduce the number of the foci. Treated with guanidine hydrochloride, which specifically inhibits Hsp104, we succeeded in tracking the distinct particles in the cytoplasm, and the mean square displacement analysis showed that most prion particles underwent Brownian motion.

1P068 高圧 Native PAGE 法によるリゾチーム変異体が形成するアミロイド原繊維のかい離過程の定量的解析
Quantitative Analysis of High Pressure Native PAGE on Dissociation of Lysozyme Variant Amyloid Protofibril

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A disulfide-deficient variant of hen lysozyme, 0SS, is known to form an amyloid protofibril spontaneously, and to dissociate into monomer under high hydrostatic pressure. We carried out native PAGE at various pressures and temperatures, to characterize the dissociation equilibrium of 0SS amyloid protofibrils. The obtained electrophoresis patterns were composed of the leading band corresponding to the monomeric 0SS and the protofibrils stacked on the top of gel media. The amount of monomeric 0SS increased cooperatively with pressure, and none of the intermediates emerged. These observations were quantified by densitometry, and analyzed based on the theoretical model, to reveal the volumetric parameters of amyloid protofibril.

1P069 リゾチームジスルフィド欠損変異体の線維化反応の温度依存性
Temperature-Dependence of Fibrillation of Lysozyme Disulfide-Deficient Variant

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Hen lysozyme disulfide-deficient variant 0SS, which is intrinsically unfolded, spontaneously forms amyloid-like fibrils in a strongly salt-concentration dependent manner. Here we studied temperature-dependence of the 0SS fibrillation by monitoring the progress of polymer formation primarily with size-exclusion chromatography. The fibrillation rate generally became lower with increasing temperature indicating that some reaction step with equilibrium of negative temperature-dependence is involved. The observation that the elution profile of the fibrils formed at lower temperatures showed a higher fraction of smaller fibrils, and therefore a higher number concentration of polymers, suggests that the nucleation of fibrils has the negative equilibrium temperature-dependence.

1P070 SEP タグを用いたタンパク質凝集の時系列的解析
Analysis of protein aggregation kinetics using short amino acid peptide tags

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We report the effects of 10 representative amino acids on the aggregation kinetics of proteins measured as the solubility of a simplified BPTI variant, to which short artificial tags containing the amino acid of interest were added at its C-terminus. We determined the solubility of the tagged variants as a function of equilibration time (20 minutes to 48 hours) and total protein concentration (0.10 mg/ml to 25.0 mg/ml). We rationalized our observations by identifying three different solubility values: A “Transient Solubility (TS)”, an “Aggregation Initiation Concentration (AIC)” and a “Long-term Solubility (LS)”. TS was dependent on the measurement conditions, but AIC and LS can be considered as amino acid intrinsic properties.

1P071 短いテトラペプチドの全原子シミュレーションによるアミノ酸の無定形な凝集性の洞察
All atom molecular dynamics simulation of short tetra-peptides shed insights into amino acid’s amorphous aggregation propensities

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In this study, we aim at relating experimentally measured amino acid aggregation propensities to values estimated from 100ns all atom molecular dynamics (MD) simulations. The experimental amino acids aggregation propensities were determined as the soluble fraction upon centrifugation of our model protein (BPTI-19A) to which a 5-residue tag made from a single amino acid type was fused. The simulation was carried out using AMBER 8 on a MD Grape special purpose computer for a 104Å cubic box filled with >30,000 water molecules and containing 27 peptides made of a single amino acid type. We performed the analysis for 14 types of representative amino acids. Overall, our simulation was able to reproduce the relative solubilities of all residues.

1P072 リポアミド脱水素酵素のフィードバック制御機構
The feedback regulation mechanism of dihydrolipoamide dehydrogenase

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The feedback regulation mechanism displayed by Dihydrolipoamide dehydrogenase (E_3) was confirmed through the steady state and time-resolved fluorescence analysis of FAD included in E_3. The enzymatic activity analysis suggested that K_m for dihydrolipoamide (DHLip), one substrate of E_3 was reduced by NAD^+ which was the other substrate. When the fluorescence of E_3 was measured in the presence of DHLip, it was rather suppressed by NAD^+ to suggest that the conversion of FAD to $FADH_2$ by DHLip would be accelerated by the binding of NAD^+ with E_3. The time-resolved fluorescence of FAD and Trp in E_3 demonstrated that the space around FAD would be so extended by the binding of NAD^+ .The conformational change that DHLip could approach more easily to FAD is induced by NAD^+ binding.

1P073 様々なアルカン産生シアノバクテリアに由来するアシル ACP 還元酵素の活性比較

Comparison of the activities of acyl-ACP reductases from various alkane producing cyanobacteria

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Some kinds of cyanobacteria generates alkanes and alkenes with acyl-ACP reductase (AAR) and aldehyde decarbonylase (AD). AAR reduces fatty acyl-ACP into aldehyde, which is then transformed into alkane by AD. It is experimentally shown that activities of AD vary between species of cyanobacteria. However, differences in activities of AAR from various alkane producing cyanobacteria has not been investigated. To reveal them, we have synthesized AAR genes of various cyanobacteria including *Synechococcus elongatus* PCC 7942, *Nostoc punctiforme* PCC 73102, and *Synechocystis sp.* PCC 6803. Each AAR protein is overexpressed in *E. coli*, and the amount of aldehydes produced in *E. coli* is measured by GC-MS. Detailed results will be presented in the poster.

1P074 ニトリルヒドラターゼの触媒機構に関する理論的研究

Theoretical Study on Catalytic Mechanism of Nitrile Hydratase

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Nitrile hydratase (NHase) catalyses hydration of nitrile to the corresponding amide and has been used as a biocatalyst in chemical industry. However, the detailed catalytic mechanism of this enzyme has not been elucidated. In the present study, we analyze several proposed reaction pathways of NHases comprising Fe(III) at the active site by using Quantum Mechanics / Molecular Mechanics (QM/MM) method. First we analyze three reaction mechanisms which have been suggested in previous theoretical studies using active-site models (Hopmann et al., 2007&2008) to reveal the effects of surrounding protein environment on the reaction process.

1P075 アデニル酸キナーゼ反応機構に関する ONIOM 法による研究

Study on the reaction mechanism of adenylate kinase with ONIOM method

Kenshu Kamiya (*Department of physics, School of science, Kitasato university*)

In order to elucidate the nature of the reaction of adenylate kinase,
which catalyzes the reaction: ATP + AMP + Mg2+ -> ADP + ADP + Mg2+ ,we have been studying the theoretical model using molecular mechanics and quantum chemistry.
We constructed the model of complex structure of enzyme and substrates, ATP and AMP withMg 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.
In these calculations, small barrier shown in the low level model vanishes with the higher level, and the present highest model gives the reaction barrier of about 19 kcal/mol, and 10 kcal/mol exothermicity.

1P076 トレオニン合成酵素における反応制御機構の理論的解明

Theoretical elucidation on the reaction control mechanism in Threonine Synthase

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Threonine synthase (TS) catalyzes the last step of L-Thr biosynthesis and its reaction is the most complex among the PLP enzymes. To elucidate the detailed mechanism, we performed comparative Quantum Mechanics/ Molecular Mechanics calculations with an exhaustive search for the reaction pathways in the reaction-specificity-determining-process. Satisfactory agreements with the experimental data were obtained. Contrary to the earlier proposal, the base that abstracts a proton from the attacking water was the Lys61 amino group rather than the phosphate ion. We also determined that phosphate ion forms a stable H-bond with the L-Thr moiety, which is critical for the reaction specificity. Additionally, a new mechanism was proposed for the transaldimination process.

1P077 生体分子の分子動力学に対する時系列解析ー運動変化と構造変化の関係を探るー

Time-series analysis of molecular dynamics: Conformational change and dynamics of collective behavior

Kana Fuji[1], Masakazu Sekijima[2], Hiroshi Fujisaki[3], Mikito Toda[4] ([1]*Graduate of school Humanities and Sciences, Nara Women's Univ.*, [2]*GSIC, Tokyo Tech*, [3]*Phys., Nippon Medical School*, [4]*Sci. , Nara Women's Univ.*)

Dynamics of proteins have harmonic and unharmonic motion. In recent years, the wavelet transformation as a method to analyze unharmonic motion has been proposed.
We aim to develop analysis for MD simulations, focusing in particular on the relationship between conformational change and motion of proteins.In this study, we analyze a trajectory data of chignolin (consisting of 10 amino acid residues) from all atom MD simulation. Our method is "Wavelet PCA", which combines the wavelet transformation and the principal component analysis (PCA). Wavelet PCA can be applied to characterize frequency of each of degrees of freedom of proteins.
We find that conformational change from folded to misfolded states results in shift to a lower frequency side.

1P078 それぞれが複数の立体構造からなる複数の蛋白質構造の比較解析

Superposition of protein structures each of which is a set of multiple conformations

Takashi Amisaki, Shin-ichi Fujiwara (*Department of Biological Regulation, Faculty of Medicine, Tottori University*)

The mathematics for superposition of two protein conformations are well known. Methods for superimposing more than two conformations have been reported in recent years. Stepping further, we propose a method for superimposing multiple proteins simultaneously each having multiple conformations. The method is based on mixed-effects models and uses maximum a posteriori estimation, i.e., the deviations of individual proteins are estimated by borrowing information from overall conformations. The portions that have deviations are narrowed by means of L1-regularization to clarify the characteristic deviation of each protein molecule. We will illustrate the applications of the method for analyzing molecular dynamics trajectories.

1P079 DFT によるテラヘルツ領域におけるアミノ酸とペプチドの低振動モードの帰属

DFT approach for the assignment of low-frequency vibrational modes of amino acids and peptides in the terahertz frequency region

Ohki Kambara (*RIE, Shizuoka Univ.*)

To understand the low-frequency vibrational modes observed in the terahertz (THz) spectroscopic region, not only the experimental study but also the theoretical works are needed. In this paper, the frequency calculation of amino acid including glycine and L-alanine and these short peptides are performed with density functional theory (DFT) package, CRYSTAL09, where the periodic boundary condition (PBC) is implemented. The number of theoretically obtained normal modes in the THz region increase with increasing chain length. This trend continues to the bigger polypeptides, proteins, whose THz spectra are uniformly smoothed and featureless. On the other hand, temperature dependence of calculated outcomes is discussed at the same time.

1P080 吸引式反応システムを用いたウェスタンブロッティング法によるペプチドの高感度検出

A new approach to detect small peptides clearly and sensitively by Western blotting using a vacuum-assisted detection method

Satoshi Tomisawa, Chiharu Abe, Masakatsu Kamiya, Takashi Kikukawa, Makoto Demura, Keiichi Kawano, Tomoyasu Aizawa (*Grad. Sch. Life Sci., Hokkaido Univ.*)

Western blotting is a widely used technique for the detection and quantification of proteins and peptides. However, it is difficult to detect small peptides efficiently by the conventional Western blotting method with shaking. In this study, we show that the previously developed vacuum-assisted detection method greatly improves the detection of small peptides without additional protocol modification. The vacuum-assisted method was developed to shorten the time required for all immunodetection steps, and all the Western blotting solutions penetrated the membrane quickly and efficiently by this method. By using this vacuum method, we succeeded in detecting small peptides that were completely undetectable by the conventional Western blotting method.

1P081 Grb2 結合にともなう EGFR のキネティクスとダイナミクスの１分子計測

Single-molecule measurements of kinetics and dynamics of an epidermal growth factor receptor upon Grb2-binding

Kenji Okamoto, Yasushi Sako (*RIKEN*)

Epidermal growth factor receptor (EGFR) is one of the membrane proteins which trigger cellular signal transduction pathways. EGFR has multiple Grb2-binding sites on its intracellular C-tail domain, which is intrinsically disordered (ID). Years ago, Grb2-binding kinetics was found to be nonlinear and it has been suggested that structural dynamics of this ID domain plays an important role for the kinetics. We have prepared the C-tail fragment molecule and investigated the Grb2-binding kinetics and the structural dynamics on it by *in vitro* single-molecule imaging and single-molecule fluorescence resonance energy transfer (FRET) measurement. The results suggest the existence of multiple conformational states induced by Grb2 binding and dynamical transitions between them.

1P082 一分子力学測定によるポリプロリンヘリックスの高弾性の研究

Single molecule force spectroscopy by AFM indicates highly resilient structure of polyproline helix

Masaru Kawakami (*School of Materials Science, Japan Advanced Institute of Science and Technology*)

The polyproline helix II (PPII) plays an important role in protein-protein interaction for intercellular cohesion or enzyme activation. The PPII structure has also known as a “rigid rod”, and has been used as “spacer” or “molecular ruler” in the fluorescence spectroscopy. We performed single molecule force spectroscopy by AFM to measure the mechanical property of PPII. PPII showed a completely linear response to tensile force, which is quite different from other polypeptide chains. This is indicating that PPII behaves as a rigid spring. Force curve analysis provided a quantitative estimate of the molecular stiffness of PPII, and we found that the stiffness of the PPII and PPI are comparable to those of globular proteins, or much higher than those of helical proteins.

1P083 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 flexineri* by cryo electron microscopy

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Many gram negative pathogenic bacteria possess a Type III Secretion System (T3SS) which plays a key role in injecting virulence proteins into host cells. This huge molecular machinery is believed to be composed of more than 20 different proteins and divided into three domains: an extracellular needle, a transmembranous basal body and cytoplasmic domain. Although, a part of T3SS components have been characterized by structural, biochemical and genetic approach, many questions concerning the arrangement of components and of the regulation of virulence effector protein secretion are remained. We will report the results of structural analysis and characterization of T3SS complex purified from *shigella* using single particle image analysis.

1P084 高分解能構造解析に向けた電子顕微鏡用カメラの評価

Evaluation of cameras for high resolution structural analysis by cryoEM

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The CCD and CMOS cameras have been developed as a new recording media substituting for the film, and they are now widely accepted by those in the electron microscopy field. However, the point spread function (PSF) of the CCD/CMOS cameras is worse than that of the film, so nowadays the film is still used for high resolution structural analysis of biological macromolecules. A state of the art camera, direct electron detector, can detect electrons without converting to photons by scintillators and therefore, a PSF as good as that of the film can be achieved. Here, we will compare the PSF and the 3D structure of biological macromolecules reconstructed from the data recorded on commercially available standard cameras and direct electron detecting cameras.

1P085 Attempt of expression of the glycoprotein from *Richadella dulcifica*

Maria Namba, Naoya Hashikawa, Satoru Yamaguchi (*Okayama Univ. Sci.*)

A plant *Richadella dulcifica* has a glycoprotein; miraculin. This protein makes homodimer structure by forming a disulfide bond at the position of Cys138. In addition, this protein has three disulfide bonds in one subunit. Generally, when it carried out overexoression of protein using *E.coli*, the various problems, such as the formation of inclusion bodies, can occur. In this study, we constructed an overexpression system using *E.coli* BL21 with plasmid pET-16b in order to obtain the active form of the protein. As a result of expression, the protein was confirmed but it was isolated insoluble fraction for the inclusion body. We examined the various plasmid, species, strain and culture conditions. Further, the purification procedure was also examined.

1P086 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 have been carrying out alanine-scanning mutagenesis of AD from *Nostoc punctiforme*. So far, 184 sites among 231 residues of AD (79%) are substituted into Ala one at a time. The amount of hydrocarbons produced in *E. coli*, in which mutant AD is overexpressed, is measured by GC-MS. We find that some residues are essential for the activity of AD, because their substitutions into Ala greatly reduced the activity. On the other hand, changes at some other sites enhance the activity of AD. These mutations are useful in improving the alkane production of AD.

1P087 An Information Theoretical Approach to Local Equilibrium State Analysis for Single-Molecule Time-Series

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Extraction of sub-ensemble molecular properties and behavior is now a well-known capability of single-molecule experiments. Recently, local equilibrium state (LES) analysis was developed as a means of extracting 'local equilibrium states' from a scalar time series. LES analysis is comprised of three components: (1) constructing short-time distributions, (2) computing distortion among these distributions, and (3) clustering the distributions into local equilibrium states. Here we apply the methods of rate-distortion theory to LES analysis and demonstrate its ability to identify conformational states within both simulated and experimental single-molecule FRET data without a priori knowledge of the number of states that underlie the empirical data.

1P088 理想タンパク質構造のデザイン原理
若手招待講演
Principles for designing ideal protein structures

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We describe an approach for designing ideal protein structures stabilized by completely consistent local and non-local interactions. The approach is based on a set of rules relating local structures to non-local structures, which was identified using folding simulations and analyses of naturally occurring proteins. Building backbone structures according to the rules, and placing sidechains stabilizing the backbones, we can readily design the proteins which have funnel-shaped energy landscapes. Using the approach, we designed ideal protein structures consisting of α-helices, β-strands and minimal loops with the Rosetta program. Designs were found to be monomeric and very stable and to adopt structures in solution nearly identical to the computational models.

1P089 理想的な構造を持つ機能タンパク質の理論設計
Theoretical design of functionalized proteins with ideal scaffold

Takahiro Kosugi, Nobuyasu Koga, Rie Tatsumi-Koga, David Baker (*Dept. of Biochem., Univ. Washington*)

Many artificial functionalized proteins, for example enzymes and small molecule binding proteins, have been reported, because they are interesting and important for both academic research and industry. Some proteins of them have higher affinity than that of the native proteins. However, these designed proteins have almost the same structure with a native protein because they are not designed from scratch and the functions are improved based on a native structure. Recently, a theoretical approach to make an arbitrary ideal protein scaffold from scratch has been developed and some stable proteins were designed successfully. In this study, we improved this approach and made some functionalized proteins from scratch without using the structure of native protein.

1P090 リポソーム内遺伝子発現を利用した進化工学による β-グルクロニダーゼの機能改変
Directed Evolution of β-glucuronidase Using Liposome-based IVC

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Liposome-based in vitro compartmentalization (IVC) is one of the selection methods for the directed evolution of proteins. This method is experimentally performed using cell-sized liposomes for in vitro protein synthesis and fluorescence activated cell sorter (FACS) for high-throughput screening of liposomes encapsulating the gene of our interest. In this report, we focus on the role of liposome size for screening β-glucuronidase (GUS). Liposomes exhibiting catalytic activity were sorted by following the criteria for fluorescence intensity of reaction product and liposome sizes. Iterative rounds of gene screen experiment using 80fL-sized liposomes enriched active variants of GUS and finally identified GUS variants with capability of faster assembly of tetramer.

1P091 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」の構築と実践：リポソームと無細胞翻訳系による α-ヘモリシンの in vitro 分子進化

Liposome Display: Directed evolution of membrane protein, alpha hemolysin, by using liposome and cell-free translation system

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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.

1P092 ニワトリクリプトクロム 1 のヘム結合モチーフ(HRM)の解析

Characterization of HRM in Chicken Cryptochrome1

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Molecular properties of a group of heme-binding proteins are regulated by binding of heme to the heme-regulatory motif (HRM) such as CP motif. Although the heme-binding is not shown, HRM is retained commonly in vertebrate cryptochromes (CRYs), which are known to play a pivotal role in the vertebrate circadian clockwork by closing a transcription-translation-based negative feedback loop as a transcriptional repressor. CRYs are also implied to function as a blue-light photoreceptor or light-driven magnetoreceptor. In order to examine the binding and explore the possible regulatory role of HRM in CRYs, we prepared a partial recombinant protein of chicken CRY1 and its point mutants to examine its heme-binding ability by UV-visible spectroscopy.

1P093 線虫 cytochrome b561 ファミリーの生理機能解析

Analyses on the physiological functions of the cytochrome b_{561} protein family in *C.elegans*

Yurie Hirano, Masahiro Miura, Motonari Tsubaki (*Dept. of Chem., Grad. Sch. of Sci., Kobe Univ.*)

Cytochrome b_{561} (b561) is a heme protein residing in neuroendocrine vesicles and regenerates intravesicular ascorbate (AsA) for the biosynthesis of neurotransmitters by transferring electrons from cytosolic AsA to intravesicular monodehydroascrobate radical. In higher animals, there are homologs belonging to the b561 family, but their physiological functions are not known in detail. In this study, we focused on Cecytb-5 protein, one of seven b561 homologs in *C.elegans*, and found that it has two isoforms, which are different in the length of cytosolic loop. Heterologously expressed Cecytb-5 proteins using *Pichia pastoris* system showed slightly different visible absorption spectra. To reveal the functions of them, we conducted *in situ* hybridization and RNAi on *C.elegans*.

1P094 線虫 cytochrome b561 ホモログ Cecytb-1 の機能解析

Analyses on the novel function of Cecytb-1, a cytochrome b561 homolog in Caenorhabditis elegans

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Cytochrome b561 is a membrane protein and has a role in regeneration of vesicular ascorbate (AsA) by a transmembrane electron transfer. Recently, b561 family proteins were found to distribute in eukaryotes. There are seven homologs in *C.elegans*. However, their physiological functions remain unclear. We attempted to clarify the function of Cecytb-1, the closest homolog to neuroendocrine b561, and showed previously its distribution in muscle tissues. Analysis of the microsomal fraction obtained from *Pichia pastoris* containing the heterologously expressed Cecytb-1 protein showed a peak at 561 nm in AsA-reduced spectrum. In the present study, we continued to clarify the nature by solubilization of Cecytb-1 protein with n-octyl-β-D-glucoside.

1P095 Substrate access to slow substrate binding P450cam with mutation at the proposed gate for water egress/ingress from/to the active site

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P450cam, heme-bearing D-camphor (Cam) monooxygease, contains in the catalytic center waters that are removed by Cam. We have proposed the water egresses via the gate formed by Asp297: Its mutation to Leu drastically slows Cam binding. Here we report x-ray structure of the Cam-free and -partially bound Leu297-mutant. The former exhibits open substrate channel; its top narrower than that of the WT. Analysis by Caver 3.0 program revealed the mutation renders no change in Cam access to heme. The latter retains open channel; Cam anchors to heme by hydrophobic interaction displacing liganded water. Crystallization with DTE closed the channel of the Cam-bound form, indicating normal channel. The present results suggest the mutation does not block the Cam access.

1P096 Heme serves as scaffold for substrate-driven active site structuring in cytochrome P450cam

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P450cam containing heme *b* in the catalytic center catalyzes absolute regio- and stereo-specific hydroxylation of D-camphor (Cam). Exclusive binding of properly oriented Cam to the Cam site achieves the specificity. Cam-free protein exhibiting open substrate channel (open form) changes upon interaction with Cam to the closed channel form (closed form), structuring the active site, although this process, essential to the catalysis, is unknown. Here we report x-ray structure of P450cam in a Cam bound open form, a possible encounter complex. Cam anchors to heme by hydrophobic interaction, displacing liganded water yielding a high-spin state. A loop near the Cam is disordered. The present results suggest heme serves as scaffold for Cam driven transition to the closed form.

1P097 酵素反応の時間分解分光解析を実現するマイクロ流路フローフラッシュ法の開発

Development of micro-channel flow-flash method for time-resolved spectroscopic study of enzymatic reactions

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Time-resolved spectroscopy is powerful to clarify the molecular mechanism of enzymes. Although the solution-mixing is a general technique to trigger the reaction, high sample consumption and limited time-resolution have prevented its extensive application for enzymatic reactions. To improve the time-resolution, we choose the flow-flash method with caged-compounds, where the flash can quickly release substrates. In addition, based on the approach reported by Nakashima et al. at this Society meeting (3I1034 in 2012), we are developing a micro-channel flow-cell connected with pulse-synchronized syringe-pump. Only 1 nl sample volume is required to obtain a time-resolved spectrum. The latest developments of the devices will be reported in this presentation.

1P098 C タイプヘム・銅酸素還元酵素の酸素消費活性の pH 依存性

pH dependence of the oxygen consumption activity of the C-type heme-copper oxygen reductase

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In the aerobic respiratory chain, the heme-copper oxygen reductase (HCOR) families catalyze O_2 reduction to H_2O coupling to proton pump. To understand energy transduction mechanism, we performed structural and functional analyses of C-type HCOR, one of subfamilies. C-type shows high O_2 affinity and transfers both substrate and pumped protons by single pathway.
In this study, we analyzed O_2 consumption activity of C-type purified from *Vibrio cholerae* O395-N1 cells. The activity was measured by using ascorbate as electron donor and TMPD as mediator. The maximal activity was observed at pH 7.8 suggesting that proton and/or electron transfer was affected by pH. To examine the activity under physiological condition, we currently construct ascorbate/cytochrome c_4 system.

1P099 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細胞内動態解析

Lateral diffusion of metabotropic glutamate receptor observed in single-molecules on the living cell surface

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G protein-coupled receptors (GPCRs) constitute the largest superfamily of membrane proteins in the human genome. For about two decades, over 140 different GPCR dimers or oligomers have been reported and attracted much attention as potential drug targets. However, the size and dynamics of GPCR oligomers under physiological condition are yet to be cleared. Here we show the dynamics of lateral diffusion of metabotropic glutamate receptor (mGluR) molecules on the living cell surface, which are well known to function as a constitutive homo-dimer, by using single-molecule imaging technique. We will discuss the states of mGluR having different diffusion constants and their relationship with the size of oligomer or with the activation upon agonist stimulation.

1P100 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-proteins known as a molecular switch mediating cellular signalling. In this study, we performed basic study to control the function of Ras reversibly using photochromic molecules, 4-phenylazophenyl maleimide (PAM) and monoiodoacetic spiropyran (IASP) upon visible (VIS) light and ultra-violet (UV) light irradiation. We have prepared the three kinds of Ras mutants Y32C, I36C, and Y64C. The mutants were modified with PAM and IASP stoichiometrically. And the GTPase activity of Ras was monitored by the quantitative analysis of GTP and GDP using HPLC with reverse phase column. It was suggested that the GTPase activities of Ras mutants modified with these photochromic molecules were reversibly altered upon VIS and UV light irradiations.

1P101 Highly stable tubes of bovine mitochondrial F-ATP synthase suitable for electron cryo tomography

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The structure of the mitochondrial F-ATP synthase has been characterized by X-ray crystallography of subcomplexes and electron cryo microscopy of the detergent solubilized intact enzyme.
Thus far, none of the methods used to study the structure of the F-ATP synthase has the potential to give insights into its conformational state during ATP synthesis under a proton motive force.
Here, we present tubes of tightly packed, membrane reconstituted bovine mitochondrial F-ATP synthases, which are suitable for structural studies by electron cryo tomography.
The enclosed nature of the tubes make them ideal candidates to elucidate the structure of mitochondrial F-ATP synthase under proton motive force in synthesis mode.

1P102 大腸菌多剤排出トランスポーター複合体 AcrAB の結合比決定

AcrB-AcrA fusion protein indicates that multi-drug efflux transporter complex AcrAB coupling ratio is 1:1

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RND-type multi-drug efflux transporters are the major cause of multi-drug resistance of Gram-negative bacteria. They act as a tripartite complex of an exporter, membrane-fusion protein and outer membrane channel, however, the stoichiometry in the active complex has not been known. We constructed the fusion protein gene of an exporter AcrB and a membrane-fusion protein AcrA connected with glycine-serine repeat linker. When this AcrB-AcrA fusion protein was expressed in acrAB deletion strain of Escherichia coli, the resultant strain showed multi-drug resistance, indicating that the AcrB/AcrA stoichiometry is 1:1.

1P103 EGFR 細胞内領域の変異による EGF 受容体活性化
Spontaneous activation of EGFR by mutations in its intracellular region in the absence of bound ligand

Hiraku Miyagi, Ichiro Maruyama (*OIST*)

EGFR is a single-path transmembrane protein with a tyrosine kinase in its intracellular domain. Aberrant activation of EGFR causes various cancers. The 'dimerization model', in which ligand binding to monomers induces dimers, has been used to explain the activation of EGFR. However, we have shown that EGFR exists as preformed, yet inactive, dimers prior to ligand binding, and have proposed an alternative 'twist model'. To test our model, we examined which residues are essential for the dimer formation by introducing various mutations into the intracellular domains. Deletion of four charged residues spontaneously activated EGFR without bound ligand, suggesting that consistently with our model, the receptor dimer is stabilized through ionic interaction of the region.

1P104 再構成膜におけるバクテリオロドプシンと部分フッ素化ホスファチジルコリンの低親和性
Low affinity of bacteriorhodopsin to partially fluorinated phosphatidylcholine in reconstituted membrane

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Partially fluorinated lipids are expected to be very useful for biophysical and biochemical studies of membrane proteins. The reconstituted bacteriorhodopsin (bR) in diF4H10-PC retained the trimeric structure even after the chain melting phase transition and showed a photocycle similar to native purple membrane, which is in a striking contrast with bR reconstituted in DMPC. In order to investigate the factors for retaining the trimeric structure of bR molecules in diF4H10-PC liposome in the liquid crystalline phase, we have examined the distribution behavior of bR in binary immiscible membrane of diF4H10-PC and DMPC. We found that bR molecules have a tendency to be selectively distributed into DMPC-rich domain, suggesting that bR is essentially immiscible to diF4H10-PC.

1P105 バクテリオロドプシンの色変異体に関する理論的研究
A theoretical study on color variants of bacteriohrodopsin

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Bacteriorhodopsin(bR) is a transmembrane protein which absorbs light around 570 nm and functions as a light-driven proton pump. We attempted a theoretical design of its colar variants, which exhibits a large spectral blue-shift (>50 nm), by chaging several residues around the retinal chromophore. We propose several mutations that stabilize conformational deformation of the chromophore and in turn leads to the spectral shifts. Structural models and spectral properties are examined by QM/MM RWFE-SCF method.

1P106 計算機シミュレーションによる Hv1 プロトンチャネルの荷電性残基の影響の検討
Evaluating the impact of charged residues in proton channel Hv1 by computer simulations

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Hv1 is a human voltage-gated proton channel. Since its crystal structure is unknown, the proton permeation mechanism through this channel is unclear. In this study we used homology modeling and molecular dynamics simulation to explore the influence of the charged residues in proton permeation. We observed that Asp 112 and Arg211, which are reportedly critical to proton selectivity, are included in the intra-molecular salt bridge network and interact with water molecules in the narrowest part of the channel. Mutations of these residues to Ala significantly distorted the structure and impaired hydrogen bond network. These results indicate the importance of stable interactions between protein and water molecules in the predicted proton conduction pathway.

1P107 Behavior of potassium ions around the potassium channel in relation to permeation events

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Voltage-dependent K+ channels play a critical role in the movement of K+ ions across cell membrane under influence of membrane potential. Transition of K+ ions through the pore of the channel has been intensively studied by using molecular dynamics (MD) simulations but how the ions attach to and detach from the pore is not well understood. Here we present the analysis of ion movement around Kv1.2-2.1 chimera channel embedded in lipid bilayer by using MD trajectories. The ions frequently occupied the extracellular side of the pore in comparison with the bulk water and the ion density of this position decreased as the ion concentration or the membrane voltage increased. Our results imply that the conformation outside the pore can influence ion permeation events.

1P108 光駆動アニオンポンプハロロドプシンと発光タンパク質からなる融合タンパク質の特性
Characteristic of fusion protein between light-driven anion pump halorhodopsin and luminescence protein

Kentaro Saito, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*School of Science and Technology, Meiji University*)

Seven-transmembrane helix protein, halorhodopsin (NpHR), is an inward light-driven anion pump which exists in biomembrane of N. pharaonis. To construct the anion pump system utilizing the emission light by bioluminescent protein, we established the E.coli expression system for fusion protein between the NpHR and luciferase from click beetle (ELuc). NpHR fused ELuc at the C-terminal was successfully expressed on the biomembrane, and bioluminescence from ELuc by reaction with D-luciferine was observed. Furthermore, separation between the NpHR and ELuc by the poly-glycine linker contributed to the increase of the luminescence intensity. The capability of the liminescence energy transfer from the Eluc to the retinal chromophore in the NpHR is discussed.

1P109 ファラオニスハロロドプシンの高次構造及び機能に与えるカロテノイド結合の影響

Effect of carotenoid binding to structure and function of Natronomonas pharaonis halorhodopsin

Kaede Suzuki, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*Grad. Sch. Sci. and Tech., Univ. Meiji*)

Light driven anion pump halorhodopsin (NpHR), which exists on the biomembrnae of N. Pharaonis, forms trimer and binds a carotenoid of bacterioruberin (BR). In this study, the effect to the structure and function of NpHR by binding of the BR were investigated. NpHR obtained from an E.coli expression system had ability to bind the BR tightly and the binding led to the remarkable increase of the thermal stability of the tertiary and quaternary structure of NpHR. These results indicate that the BR binding has the effect to reduce the thermal fluctuation of the NpHR following to the increase of the structural stability. The effect of the BR binding to the anion pump activity of the NpHR is also discussed based on the Patch-Clamp experiment.

1P110 アニオン結合に伴う膜タンパク質ハロロドプシンの三次構造変化の検出

Detection of tertiary structural change of membrane protein halorhodopsin by anion binding

Takahiko Yokota, Noritake Katou, Yuri Mukai, Takanori Sasaki (*School of Science and Technology, Meiji University*)

Halorhodopsin (NpHR) from Natronomonas pharaonis is a retinal protein with a seven-transmembrane helix and acts as an inward light-driven anion pump. In this study, tertiary structural change of NpHR in the ground state accompanied by anion binding was investigated. It is known that NpHR in the desalted condition is easily bleached by thermal treatment. This bleached NpHR has bound the retinal again by addition of anion, suggesting that the anion binding to the specific site in the apoprotein induces its tertiary structural change following to the retinal binding. Tertiary structural changes of the ground state NpHR by addition of anion were also confirmed as the remarkable stabilization of its trimer structure.

1P111 ハロロドプシン三量体が持つカロテノイド結合の特異性

Specificity of carotenoid biding of trimer halorhodopsin

Yasuyuki Miyazaki, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*Sch. Sci. and Tech., Univ. Meiji*)

A light driven anion pump, halorhodopsin (NpHR) exists on the biomembrane of N. pharaonis, and binds a kind of carotenoid, bacterioruberin (BR). In this study, we investigated the carotenoid recognition mechanism of the NpHR obtained from a heterologous over expression system in E. coli. Although solubilized trimer NpHR in detergent has bound the BR very tightly, monomer NpHR has lost the binding ability. There results indicate that the BR binds only to the intermolecular interface formed by NpHRs. The trimer NpHRs mutated on the amino acid residues located in the interface has not changed the binding ability of the BR. These results suggest that trimer NpHR recognizes the BR by the entire hydrophobic region in the interface but not by the local amino acid residues.

1P112 異なるアミノ酸タグを持つ膜タンパクハロロドプシン同士での多量体形成

Oligomer formation between membrane protein halorhodopsins with different amino acid tags

Tomokazu Wakatsuki, Noritaka Kato, Yuri Mukai, Takanori Sasaki (*Sch. Sci. and Tech., Univ. Meiji*)

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 in the E.coli membrane. In this study, we examined whether the His-tagged NpHR can form an oligomer with the Strep-tagged NpHR. At first, each trimer NpHR with His-tag and Strep-tag was monomerized by thermal treatment. After that, NpHRs with each amino acid tag were mixed and reconstituted on the artificial lipid. Finally, NpHRs re-formed trimer were selected by affinity chromatography. As a result, the ratio of the trimer composed of both NpHRs with His-tag and Strep-tag was only about 10%, suggesting that the amino acid residues at the C-terminal region affect the trimer formation.

1P113 PBSA 法による RecA リコンビナーゼの ssDNA 及び dsDNA との結合能の比較

Comparison of ssDNA- and dsDNA-binding affinity of RecA recombinase using the PBSA method

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In the strand exchange reaction, the RecA recombinase binds to the single-stranded DNA (ssDNA), and then the strand exchange occurs between the RecA-bound ssDNA and the homologous strand of the double-stranded DNA (dsDNA). RecA preferentially binds to ssDNA over dsDNA. The difference of the affinities is important for the strand exchange reaction.
In this study, we calculated the binding free energy of RecA-ssDNA and RecA-dsDNA using the PBSA method. The calculated binding free energy of RecA and ssDNA was largely more negative than that of RecA and dsDNA, and key residues of the enhanced affinity were identified.

1P114 DNA-binding-induced conformational changes in proteins

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Differences between the DNA-bound and their unbound counterparts complicate efforts to design drugs targeting these interactions, even if the structure of free protein is known. The mechanism of conformational changes upon complex formation is not well understood. We present a comprehensive analysis of conformational changes observed in known protein-DNA complexes. Compiling a detailed database of pairs of bound and unbound proteins, we classified conformational changes into six geometric categories and explored their relationship with electrostatic properties, intrinsic flexibility, target specificity and stability. Our results provide a global picture of the mechanism and consequences of conformational changes between DNA-bound and unbound structures of proteins.

1P115 Single-Molecule Studies on How Pif1 Helicases Regulate Telomerase Activity

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The repetitive TG-rich DNA sequence at the chromosome end, telomere, protects cells from the end-replication problem in eukaryotic cells. S. cerevisiae telomerase (Est2) is the reverse transcriptase responsible for extending telomeres in yeast. Pif1 helicase has been implicated in regulating the telomerase activity. We used single-molecule experiments to investigate how Pif1 helicases regulate telomerase activity. We found that Est2 telomerase stayed bound to telomere end after extension, but Pif1 helicases remove telomerase from the telomere. In the presence of Pif1 helicases, multiple runs of the telomerase-mediated telomere lengthening were observed. This suggests a model that Pif1 helicases remove telomerase from the telomere ends, allowing telomerase recycling.

1P116 (6–4)光回復酵素の二光子 DNA 修復反応機構 A two photon DNA repair mechanism of the (6-4) photolyase

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Ultraviolet (UV) irradiation of DNA induces two harmful cross-links between adjacent pyrimidine bases: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts ((6-4)PPs). These lesions can be repaired by DNA photolyases -flavoenzymes using blue to near UV light for their catalytic action. For CPD repair, it has been established that the transfer of an electron from the excited fully reduced flavin cofactor to the lesion is required. In contrast, repair of the (6-4)PPs still remains obscure, and numbers of photons required for the (6-4)PP repair has not been determined experimentally. Here, we studied repair of the (6-4)PP-containing substrate by (6-4) photolyase under photon-regulated conditions, revealing a novel two photon mechanism.

1P118 How does alcohol cause the transition of higher-order structure of DNA?

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For the purpose to extent DNA from cells, ethanol precipitation is commonly used. It is also well known that B-A transition of double-helix DNA is induced by high concentrations of ethanol. However, the effect of alcohol on the higher-order structure of DNA has not been fully understood yet. Here, we investigated the higher-order structural changes of individual long DNA molecules in the presence of various concentrations of ethanol or 2-propanol using fluorescence microscopy together with CD spectroscopy and electron microscopy. Interestingly, it was found that ethanol causes dual reentrant transition, fold-unfold-fold states, accompanied by the increase of the alcohol concentration.

1P119 Ultrasound-induced double-strand breaks in relation to the higher-order structure of DNA

Rinko Kubota[1], Naoki Ogawa[1], Yukihiro Kagawa[1], Yuko Yoshikawa[2], Yoshiaki Watanabe[1], Takahiro Kenmotsu[1], Kenichi Yoshikawa[1] ([1]*Faculty of Life and Medical Sciences, Doshisha University*, [2]*Lab. Environ. Biotech., Ritsumeikan University*)

We have been currently performing the study on double-strand breaks by use of single DNA observation with fluorescence microscopy. It was found that ultrasound causes the double-strand breaks only above a threshold power and, above the threshold, the damage increases almost linearly with the power. Here, we studied how the frequency of double-strand breaks caused by ultrasound depends on the higher order structure of DNA. In order to examine the effect of the DNA conformation, we prepared tightly compact DNA molecules by the addition of spermidine (3+). After the ultrasound irradiation against the compact DNA, we added salt to obtain elongated DNA molecules. We then performed the direct observation on the fragmented DNA molecules by use of fluorescence microscopy.

1P120 新規抗がん性二核白金(Ⅱ)錯体による DNA の立体構造変化 Conformational change of DNA induced by novel antitumor dinuclear Pt(II) complexes

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Cisplatin is the most widely used platinum-based anticancer drug. However, its clinical use is limited due to severe side effects. Therefore, much attention has focused on designing new platinum compounds with improved pharmacological properties. Recently, Komeda et al. developed a series of antitumor-active tetrazolato-bridged dinuclear platinum (II) complexes with alkyl chain lengths ranging from C2 to C9. In this study, we examined the effect of these platinum complexes on the higher-order structure of single DNA molecules under fluorescence microscope. It was found that the efficacy of these complexes in inducing DNA compaction changed in an alkyl chain length-dependent manner. We discussed these results together with changes of DNA secondary structure.

1P121 Mg(2+) causes shrinking on DNA but prevents spermidine(3+)-induced compaction

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It is known that, Mg(2+) plays an important role in the structure and function of nucleic acid. Here we study the influence of Mg(2+) on the higher-order structure of DNA. By use of single DNA observation by fluorescence microscopy, we confirmed the experimental condition to cause the compaction of giant DNA, T4 phage DNA (166 kbp, 57 μm). With the addition of Mg(2+) to the solution of DNA compacted with the spermidine(3+), we found that DNA molecules unfold into an elongated coil state. On the contrary, in the absence of spermidine, Mg(2+) induces DNA shrinking. Thus, it becomes clear that Mg(2+) exhibits opposite effect on the DNA conformation either in the absence or presence of spermidine(3+).

1P122 一本鎖 DNA 結合蛋白質の DNA-SWNT 複合体への選択的な吸着

Selective adhesion of single-stranded DNA binding protein to DNA-SWNT hybrids

Daisuke Nii, Takuya Hayashida, Kazuo Umemura (*Graduate School of Science, Tokyo University of science*)

In this research, we investigated selective adhesion of single-stranded DNA binding (SSB) protein onto SWNT surfaces that were wrapped with single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA). DNA-SWNT hybrids were prepared in an aqueous solution prior to the injection of SSB molecules. Atomic force microscopy and electrophoresis revealed that SSB protein adsorbed only to the ssDNA-SWNT hybrids. The results clearly showed that molecular recognition function of SSB protein molecules is available even for the DNA molecules attached on the SWNT surfaces. Further, the data suggested that dsDNA molecules retained their double-stranded structures on the SWNT surfaces although the molecules were sonicated during the sample preparation.

1P123 全反射蛍光顕微鏡によるショウジョウバエ RNAi 酵素複合体形成の基本過程の解明

Defining fundamental steps in the assembly of Drosophila RNAi enzyme complex by TIRF microscopy

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siRNAs and Argonaute (Ago) proteins form RNA-induced silencing complexes (RISCs) that silence expression of target mRNAs. Although *Drosophila* RISC assembly requires the Dicer-2/R2D2 heterodimer and the Hsc70/Hsp90 chaperone machinery, the details remain unclear. Here, by following the assembly of single RISCs, we find that an siRNA bound to Dicer-2/R2D2 associates with Ago2 only transiently. The chaperone machinery extends the dwell time of Dicer-2/R2D2/siRNA on Ago2, in a manner dependent on the 5' phosphate recognition on the siRNA guide strand. We propose that the chaperone machinery acts to support a productive conformation of Ago2, allowing it to load authentic siRNA duplexes. Our results define the molecular basis for the chaperone-assisted assembly of RISC.

1P124 光刺激により自律的に自己組織化する RNA 分子ロボットの構築

Construction of an RNA molecular robot autonomously self-assembled by light stimulation

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Molecular computers and molecular self-assembled structures have recently attracted attention as sophisticated bio-inspired systems. Although many nanostructures and autonomous molecular computers based on DNA/RNA molecules have been reported up to now, molecular robots integrating the nanostructures and molecular computers have never been developed yet. Here, we propose an autonomously self-assembled RNA molecular robot as an integrated system of molecular computers and nanostructures. In this system, RNA transcription starts by light stimulation as input information, and the RNA molecules autonomously self-assembles into an RNA molecular robot body. We believe that this molecular robot will be applied to molecular robots with more complex functions in the future.

1P125 蛋白質-RNA の複合体立体構造予測

Tertiary structure prediction of Protein-RNA complexes

Tomoshi Kameda[1], Junichi Iwakiri[2], Michiaki Hamada[2], Kiyoshi Asai[1,2] ([1]*CBRC, AIST*, [2]*Grad. Sch. Frontier Sci., the Univ. of Tokyo*)

In general, it is difficult to solve the three dimensional (3D) structure of biomolecule complex compared to a monomeric protein. So, the computational 3D structure prediction of complex (often called “docking problem”) has been studied. Although 3D structure prediction of protein-protein complex and protein-compound complex has been investigated by many researchers during decades, there are few studies about protein-nucleic acid complex.
Now, we introduce the method to predict 3D structure of protein-RNA complex. Our method is applied to 72 complex structures, its success rate is ~29%, which may be world record in this research area. Moreover, it usually requires within only an hour to acquire result by using a general desktop computer.

1P126 緑色蛍光タンパク質の蛍光スペクトルに関する理論的研究

A theoretical study on the fluorescent spectrum of enhanced green fluorescent protein

Yoshihiro Uchida[1], Masahiro Higashi[2], Shigehiko Hayashi[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Inst. Mol. Sci.*)

Fluorescent proteins have played a crucial role in biological imaging and analysis. The fluorescent spectrum is one of the important photophysical properties of fluorescent protein. Here, we talk about a research on the molecular simulation of the spectral shape of fluorescent protein. First, we introduce a method to calculate fluorescent spectra of protein. For this purpose, it is needed to evaluate accurately both excited states of the chromophore and structural fluctuations of the protein. Next, by applying the method to enhanced green fluorescent protein (EGFP), the origin of the spectral shape is discussed. This new method for calculating fluorescent spectrum is useful to develop the functions of fluorescent proteins such as color variant and ion sensor.

1P127 Zero-dipole summation method for evaluating electrostatic interaction in molecular simulation of biomolecular system

Ikuo Fukuda, Narutoshi Kamiya, Haruki Nakamura (*Institute for Protein Research, Osaka University*)

Appropriate treatment of electrostatic interaction of charged particles is critical for computational study of biomolecular system. We introduced a novel idea, zero-dipole summation[1], which is based on cut-off approach to calculate the electrostatic interactions, but prevents electrically non-neutral states artificially generated by straight truncation. The resulting formula is very simple and does not necessarily need periodic boundary condition, which is often problematic. We discuss the theory, timing, and results of molecular dynamics simulations applied to a membrane protein system and a DNA system. [1] I. Fukuda, et al. J. Chem. Phys. 134, 164107 (2011); 137, 054314 (2012); Biophys. Rev. 4, 161 (2012); N. Kamiya, et al. Chem. Phys. Lett. 568, 26 (2013) .

1P128 DFTB および REUS を用いたマロンアルデヒドのプロトン移動計算

Calculation of proton transfer in malonaldehyde using DFTB and REUS

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We have investigated the free energy profile of the intramolecular proton transfer of malonaldehyde by molecular simulations. The computational methods combine the self-consistent-charge Density Functional based Tight Binding (DFTB) method for fast calculations of quantum effects and the Replica-Exchange Umbrella Sampling (REUS) for enhanced conformational sampling. The reaction coordinate for proton transfer which defines the umbrella potential was taken to be the difference of two distances between the hydrogen atom and the two oxygen atoms in malonaldehyde. We will explain the details of this new method and present the results of free energy calculations. We want to carry out similar calculations of a larger system based on the same method.

1P129 ESP 多重極子演算子を用いた QM/MM 計算法の開発

Development of multipole electrostatic potential operator for QM/MM method

Yusuke Inoue[1], Takahiro Kosugi[2], Hiroshi Nakano[3], Takeshi Yamamoto[1], Shigehiko Hayashi[1] ([1]*Grad. Sch. Sci., Kyoto Univ.*, [2]*Univ. of Washington*, [3]*Grad. Sch. Eng., Kyoto Univ.*)

Combined QM/MM method is a useful simulation technique to model molecular systems including biomacromolecules. Although one can obtain QM/MM free energy through a MD simulation, the computation is very demanding due to a large number of coulombic integrals required to be computed in the simulation. To overcome this problem, a point charge approximation is widely used. However, the approximation cannot describe properly anisotropy and screening of electron density. In the present study, we solved this problem by introducing higer order terms of multipoles in addition to point charges. We incorporated the multipole operators into QM/MM reweighting free energy (RWFE) SCF method, which is an efficient method for calculating QM/MM free energy.

1P130 FMO 法を用いた基準振動解析によるタンパク質の赤外吸収予測

Prediction of IR spectra by normal mode analysis based on the Fragment Molecular Orbital(FMO) method

Hiroya Nakata[1,2], Dmitri Fedorov[3], Satoshi Yokojima[4], Kazuo Kitaura[5], Shinichiro Nakamura[2] ([1]*Tokyo Institute of Technology*, [2]*RIKEN*, [3]*National Institute of Advanced Industrial Science and Technology*, [4]*Tokyo University of Pharmacy and Life Sciences*, [5]*Graduate School of System Informatics, Kobe University*)

IR spectroscopy is a powerful method to understand the reaction mechanism of enzymes. Isotopic substitution or site-directed mutagenesis can be used to assign the amino acid sequence based on IR spectra. Normal mode analyses have been performed for a variety of biological systems providing free energy and structural information. We proposed a method to do normal mode analysis using the analytic second-order derivative (Hessian) of the energy for FMO, in which ab initio calculations of fragments are performed and then the properties of the total system are evaluated. The efficiency and accuracy of the FMO Hessians are demonstrated, and we present the results for several polypeptide isomers, and discuss the effect of the secondary structure on IR peak positions.

1P131 水は蛋白質を折り畳むのか？

Does water drive a protein to fold?

Yutaka Maruyama, Yuichi Harano (*Inst. Protein Res., Osaka Univ.*)

We investigate the thermodynamic stability of a protein in water by using all-atom molecular model. The free energy change upon the protein folding is calculated by the way of a combined approach based on molecular dynamics simulation and integral-equation theory of molecular liquids. Through this approach, we can analyze components of the free energy such as the protein intramolecular energy, the solvation energy, and the salvation entropy. Based on the analysis for the free energy change upon protein folding, we show how water can contribute to the structural stability of a protein in terms of thermodynamic quantities. As a role of water, the hydration entropy is thus crucial for protein folding.

1P132 逆ミセル中ナノ拘束水のテラヘルツダイナミクスの温度依存性

Temperature dependence of terahertz dynamics of nano-confined water in a reverse micelle

Hiroshi Murakami (*JAEA*)

A reverse micelle is a nanometer-scale cage filled with water. We have made THz spectroscopy of water in AOT/isooctane reverse micelles at temperatures from ~ 273 K to room temperature, and found that for the reverse micelle with its aqueous cavity radius of ~5 nm, the relaxation time of the order of 10 ps increases rapidly in the temperature range examined. Further, the Stokes radius of the reverse micelle derived from a dynamic light scattering measurement increases with temperature dependence similar to that of the relaxation time. This is proposed to be due to formation of hydrogen-bond network of water at lower temperatures. In the presentation, we will discuss the micelle-size dependence of temperature behavior of the relaxation time and Stokes radius.

1P133 Terahertz dynamics of hydrated protein studied by X-ray scattering

Koji Yoshida, Toshio Yamaguchi (*Fukuoka Univerisity*)

Terahertz spectroscopy of biological samples has recently attracted much attention for chemical biologists since the dynamics of proteins in terahertz domain is concerned with their functions. Since X-ray scattering is coherent and the wavelength is comparable with an atomic distances, inelastic X-ray scattering (IXS) of hydrated proteins would reveal the collective dynamics of the protein and hydration water as a function of wavevector. In the present study, IXS of hydrated proteins (β-lactoglobulin, antifreeze protein, and filamentous actin) was measured at 180-298 K. The difference between the collective dynamics and a single particle dynamics investigated by neutron scattering will be discussed.

1P134 テラヘルツ時間領域分光によるリゾチーム低振動ダイナミクスの温度・水和依存性の観測

Temperature and hydration dependence of low-frequency dynamics of lysozyme studied by terahertz time-domain spectroscopy

Naoki Yamamoto[1], Atsuo Tamura[2], Keisuke Tominaga[1,2] ([1]*Molecular Photoscience Research Center, Kobe University*, [2]*Graduate School of Science, Kobe University*)

Theoretical calculations have indicated protein low-frequency motions exist in a terahertz (THz; 1 THz ~ 33 cm^{-1}) region. These motions are functionally-related movements such as open-close motions of active cites. Since protein structure is always thermally fluctuating surrounded by solvent, it is essential to study temperature and hydration dependence of protein low-frequency dynamics. We obtained THz spectra depending on these parameters by THz time-domain spectroscopy from 83 to 293 K. In a dehydrated state absorption spectra linearly increased with a rise in temperature. In contrast to this, at a hydrated state increasing rates of the spectra became more intense at around 190 K. We discuss spectral components in the hydrated state by fitting with model functions.

1P135 誘電緩和分光測定によるオリゴリン酸 Na、アルキルカルボン酸 Na、アルキルスルホン酸 Na の水和特性

Hydration properties of sodium-oligophosphates, -alkyl carboxylates and -alkyl sulfonates by dielectric relaxation spectroscopy

Kazuki Ishimori, Yangtian Wang, Norihiko Tanno, George Mogami, Tetsuichi Wazawa, Nobuyuki Morimoto, Makoto Suzuki (*Dept. Materials Processing, Tohoku Univ.*)

Our previous studies revealed that hyper-mobile water (HMW) with a higher dielectric relaxation (DR) frequency than that of bulk water (fc = 17GHz at 20°C) existed in sodium halide aqueous solutions. In this study, we have investigated the effect of monovalent anion group, $-RO_x^-$ (R=C, S, P; x=2, 3, 4), on HMW formation, using high-resolution DR spectroscopy for sodium-oligophosphates, -alkyl carboxylates and -alkyl sulfonates aqueous solution.
We found that 1) with increasing the number of valence, HMW (fc = 17.5-21.0GHz) increased 2) with increasing the length of hydrophobic alkyl group, constrained water (fc = 4.6-11.2GHz) increased. These results indicate that the number of valence seems to govern the HMW formation.

1P136 Spatiotemporal measurement of cellular and tissue elasticity in the developing brain

Misato Iwashita[1,2], Kazunori Toida[1,2], Yoichi Kosodo[1,2] ([1]*Kawasaki Medical School*, [2]*Department of Anatomy*)

Previous studies showed that substrate elasticity can direct stem cell fate *in vitro*. These results lead a possibility that physical stimulations from its environment act as the extrinsic factor for fate determination of neural stem cells. However, no clue is currently available if there is spatiotemporal transition of tissue elasticity *in vivo*.
We are addressing to the point by focusing on the mammalian cerebral cortex, since the tissue shows dramatic changes of its cytoarchitecture during development. We established a method to measure the elasticity of living cortical tissue by using Atomic Force Microscope. We found that layers of cortical tissue show significant changes in their elasticity during brain development.

1P137 幹細胞ミニマルモデルの *in vivo* 実装

In vivo* realization of the minimal stem cell model in *Escherichia coli

Sumire Ono[1], Reiko Okura[1], Yuichi Wakamoto[1,2] ([1]*Grad. Sch. Arts and Sci., Univ. Tokyo*, [2]*Research Center for Complex Systems Biology, Univ. Tokyo*)

Stem cells can differentiate spontaneously and irreversibly. To investigate fundamental gene regulatory network (GRN) of stem cell differentiation, we have constructed an artificial GRN, whose regulation is equivalent to the minimal stem cell model [1].
The network regulation of the model is composed of two regulatory factors, one of which must diffuse intercellularly. We constructed an equivalent network using three regulatory factors, in which the lux operon of *V. Fischeri* achieves intercellular communication via diffusible factor.
We have carried out time-lapse microscopy of the constructed *E. coli* strains, and monitored the network activities in live cells by fluorescent markers. Further results will be discussed.
[1] Goto & Kaneko (2013) arXiv:1303.7319

1P138 既知遺伝子調節関係に基づいた細胞分化の力学系モデル

Realistic dynamical system model of cell differentiation based on known gene-regulatory interactions

Tadashi Miyamoto[1], Chikara Furusawa[2], Kunihiko Kaneko[1] ([1]*Grad. Sch. Art. Sci., Univ. Tokyo*, [2]*QBiC, Riken*)

Pluripotent genes such as Oct4 and Nanog interact with differentiation marker genes as Gata6, which is associated with pluripotency and differentiation. The interactions of these genes are known as gene regulatory network (GRN) of self-renewal and differentiation. Differentiation is interpreted as changing structure of GRN, but the mechanism to trigger cellular state transition is coarse and fragmented. We then constructed small GRN related to pluripotency and differentiation. Modeling differential equations based on the GRN, we conducted a simulation. As a result, we found that our model could generate three differentiated states from one stem cell state. In addition, we are planning to evaluate the consistency between our model and RNA-seq data.

1P139 アクチンフィラメントの伸長メカニズムを解明するための分子シミュレーション

A Molecular Simulation Study to Investigate Actin Filament Elongation Mechanism

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One of the main components of muscle fiber is actin, which plays many important roles in cellular functions. Although the atomic structure of filamentous actin has recently been determined by electron cryomicroscopy, the filament elongation mechanism is not yet fully understood at molecular level. We performed molecular dynamics simulations of monomeric and filamentous actins to investigate the elongation mechanism. The results show that change in salt bridges around actin hinge region is key to control the difference of the propeller angle (dihedral angle defined by four subdomains) between ATP- and ADP-actins.

1P140 F-アクチンの水和状態に及ぼすハライドイオンの効果
Halide ion effect on hydration state of F-actin

Noriyoshi Ishida, Takahiro Watanabe, **George Mogami**, Tetsuichi Wazawa, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ.*)

Water should play a key role in muscle contraction (Suzuki et al., 2004; Amano et al., 2010). Large halide ions induce high protein solubility as known to be the Hofmeister effect. In the previous study, water with higher dielectric relaxation frequency than bulk water was found around F-actin (FA) (Kabir et al., 2003). This water is referred to as hyper-mobile water (HMW). Halide ions of KX (X: F, Cl, Br or I) are known to affect ATPase activity of actomyosin. In this study the effects of halide ions (X: Cl^-, Br^-, or I^-) on the hydration states of FA were investigated using dielectric relaxation spectroscopy and density measurements at 10 and 20 °C. As a result, constrained water, HMW, and partial specific volume of FA were found to decrease in the order: Cl^-, Br^-, I^-.

1P141 アクチン重合、ミオシン ATP 加水分解活性化に対する Tyr143 変異の効果
Changes of polymerization and activation of myosin ATPase of Dictyostelium actin induced by mutation of Tyrosin-143

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When actin polymerizes, the hydrophobic cleft opens and the Tyr143 becomes more exposed (1). This region has been known as one of the important myosin-binding sites. We have studied the influence of mutations introduced to this residue to Dictyostelium actin. Polymerization assay shows that the polymerizability of the Tyr143Phe-actin is poorer compared with the wild-type actin. However, when phalloidin was added, mutant actins polymerize normally. The Lineweaver-Burk plot of the HMM ATPase activated by Tyr143Trp-actin shows the decrease of Km, indicating that this mutant associates more tightly with HMM-ADP-Pi. This indicates the importance of Tyr143 of actin for the polymerization and for the weak binding to myosin.

(1) Murakami et al. (2010) Cell, 143, 275-287

1P142 中性子散乱による筋肉の細いフィラメントのダイナミクス変化の検出
Changes in the dynamics of the muscle thin filaments observed by neutron scattering

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In order to investigate the role of protein dynamics in the regulatory mechanism of muscle contraction, we carried out neutron inelastic scattering experiments on the native thin filaments (NTF) in the presence and absence of Ca^{2+} and F-actin using the dynamics analysis spectrometer DNA at J-PARC. The mean square displacements estimated from the elastic incoherent neutron scattering measurements showed that NTF in the $-Ca^{2+}$ state is more dynamically disordered than in the $+Ca^{2+}$ state. Comparison with F-actin suggests that the difference arises from the regulatory proteins. The dynamic change in the regulatory proteins suggests that the dynamics of these proteins plays an important role in the regulatory mechanism of muscle contraction.

1P143 SDSL-ESR による心筋トロポニン-I の N 末伸長部位の動的構造
Structural Dynamics of N-terminal Extension of Cardiac Troponin I by Site Directed Spin Labeling-EPR

Chenchao Zhao[1], Hiroaki Yamashita[1], **Keisuke Ueda**[1,3], Shoji Ueki[2], Toshiaki Arata[1] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Tokushima-Bunri Univ.*, [3]*Inst. Prot. Res., Osaka Univ.*)

Troponin I in cardiac muscle (cTnI) has a unique flexible N-terminal extension (50 residues), that is firmly essential for modulating Ca sensitivity and force by phosphorylation of itself. We previously showed that N-lobe of cTnC opened to less extent and became weakly bound to TnI regulatory domain by phosphorylation. Here, we determined the secondary structure of the N-extension by measuring the distance distribution between two spin labels attached on i and i+4 residues. Only 23-27 or 43-47 region which has PKA or PKC phosphorylation sites, transmitted from flexible (wide 2nm distribution width) in monomer to relatively stable or helical conformation (narrow 0.8-1.1 nm) in cTnI-cTnC complex. These data suggest dynamic interaction of the N-extension with cTnC.

1P144 ESR による筋肉細いフィラメントにおけるアクチンと Ca 調節タンパク質の動的構造
Structural dynamics of actin and Ca-regulatory proteins in muscle thin filament by using ESR

Yoshiki Tsujimoto[1], Akie Yamamoto[1], Keisuke Ueda[2], Toshiaki Arata[1] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*Inst. Prot. Res., Osaka Univ.*)

It is a central problem of muscle contraction how conformational changes in the thin filament in response to myosin and Ca^{2+} binding. Average interspin distances between Mn^{2+} at nucleotide binding site and a spin label at the amino acid residue Lys61 within actin monomer were estimated to be ~2.0 nm. The distance between Cys374 and Mn^{2+} was ~2.0 nm as consistent with the crystal structure, and decreased by ~0.3 nm upon polymerization. The distance increased by ~0.3 nm at 30-60% occupancy by myosin S1 and returned nearly to the original level at 100% occupancy, suggesting cooperative conformation change. The distance also increased by ~0.3 nm with tropomyosin-troponin. Now we are trying to measure the distance between tropomyosin and actin on the thin filament.

1P145 Effects of the KIF2C neck peptide on microtubules: lateral disintegration of microtubules and β-structure formation

Youske Shimizu[1,2], Takashi Shimizu[2], Masayuki Nara[3], Mahito Kikumoto[1], Hiroaki Kojima[1], Hisayuki Morii[2] ([1]*NICT*, [2]*AIST*, [3]*Tokyo Medical and Dental Univ.*)

KIF2C kinesin depolymerizes MTs. The “neck” region should be important in the MT depolymerization. Peptides from the neck region induced an increase in the turbidity of a MT suspension. For the increase, it was suggested that electrostatic interactions should be crucial and proper secondary structure formation would be of significance. EM observations revealed ring structures surrounding the MTs and free thin straight filaments. Therefore, the neck region, even without the catalytic domain, should induce lateral disintegration of MTs into protofilaments. The neck region of KIF2C was considered to be flexible, but our IR analysis suggested formation of β-structure in the presence of MTs. Our results provide important clues to the depolymerization steps by intact KIF2C.

1P146 ガラス基板上に固定した F-アクチンへのコフィリンと HMM と協同的結合

Cooperative binding of cofilin and HMM to immobilized F-Actin on a glass surface

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In amoeboid (crawling) cells, cofilin and myosinII are localized in the anterior and posterior regions, respectively. In the last meeting, we reported the exclusive binding of cofilin and HMM to F-Actin at the cooperative binding condition, and concluded that this exclusive binding is correlated with their differential localization. However, quantitative analysis in that experiment was difficult because of the bundling of F-Actin. In this study, therefore, cooperative and exclusive binding of cofilin and HMM was analyzed for immobilized F-actin on a glass surface. The results showed that cooperative binding of cofilin and HMM were also observed on immobilized F-Actin. Now we are analyzing the exclusive binding of cofilin and HMM to immobilized F-actin.

1P147 細菌べん毛モーター固定子複合体 MotA/B チャネルのプロトン透過メカニズム

Proton permeation mechanism through the channel of flagellar motor stator complex MotA/B

Yasutaka Nishihara, Akio Kitao (*IMCB, Univ of Tokyo*)

Bacterial flagellar motors are powered by ions (proton in *Escherichia coli* and sodium ion in *Vibrio alginolyticus*) but the molecular mechanism is still unclear. The motor consists of a rotor and stators, and the latter that comprises MotA and MotB proteins in *E. coli* acts as a torque-generating unit.

To examine the structural changes coupled with ion permeation through the stator channel, we performed steered molecular dynamics calculations using the model structures which we had modeled with the experimental data. Our results showed that the channel gating was controlled by Leu46 on MotB and that helix movements were induced by ion permeation. We will also discuss the motions of the cytoplasmic domain in MotA induced by these structural changes.

1P148 高速 AFM による *Ascaris* 精子由来の MSP 線維の観察

Observation of MSP filaments in cell-free extract from *Ascaris* sperm by high-speed atomic force microscopy

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Crawling movement in eukaryotic cells requires dynamic assembly and disassembly of cytoskeleton. In Ascaris sperm, motility is powered by an unique cytoskeletal protein, Major Sperm Protein (MSP), instead of actin which is commonly used for amoeboid motility. To understand the MSP dynamics at single filament level, we have observed MSP filaments attached to the aminosilane-coated mica by high-speed AFM. Despite of high protein concentration (~ 1 - 2 mg/ml) in cell-free extract from *Ascaris* sperm, filaments with 200-nm length and 9-nm diameter on average were clearly visible. Portions of filaments that were not attached to the surface fluctuated substantially, indicating that MSP filaments are highly flexible unlike actin filaments.

1P149 The Mg^{2+} binding site of the ATP synthase ε subunit from *Bacillus subtilis* derived by Molecular Dynamics simulations

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ATP synthases are the main producer of ATP, the universal energy source, in all living cells, from microbes to humans. The mechanism of ATP hydrolysis inhibition differs in bacteria and mitochondria, driven by either the [ATP] dependent subunit ε in bacteria or the pH dependent inhibitor protein IF_1 in mitochondria. The ATPase inhibitory extended state in bacteria is found at low [ATP], while the ATP binding non-inhibitory down state is observed at high [ATP]. In addition the conformational change from the extended to the down state of subunit ε from *Bacillus subtilis* has been shown to be [Mg^{2+}] dependent along with its [ATP] dependency. In this work we predict the Mg^{2+} binding site of the ε subunit from *Bacillus subtilis*, using Molecular Dynamics simulations.

1P150 鞭毛軸糸ダイニンを駆動源として振動的屈曲運動を発生させる微小管バンドル

A microtubule bundle that produces oscillatory bending movement with axonemal dynein

Susumu Aoyama, Yuichi Hiratsuka (*Sch. Matl. Sci., JAIST*)

The beating of the flagellar axoneme is produced by regulated active sliding of aligned microtubules, which is driven by dynein motor protein. How the sliding movement is converted to the oscillatory bending is not clearly elucidated. To solve the mystery, we tried to assemble oscillatory units composed of microtubules, dynein, and some additional components. We constructed polarity-arranged microtubule arrays and added axonemal dynein and microtubule-bundling protein to the array, which made bundles of microtubules. When ATP was added, the microtubule bundles sometimes displayed oscillatory bending movement. This result suggests that axonemal dynein (outer arm dynein) has some mechanical sensors and is regulated by itself in loosely linked microtubules.

1P151 Athermal Fluctuations of Probe Particles in Active Cytoskeletal Networks

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A reconstituted active cytoskeletal networks consisting of an actin filament network coupled to myosins have been shown rich in dynamical and mechanical behaviors that is often in contrast to passive, equilibrium system. The athermal fluctuations observed in the network are linked to the active force generation process by motor proteins which give more relevant information including the interaction with the surrounding materials. In this study, we investigated the nonequilibrium statistics and dynamics of the active network by analyzing the athermal fluctuations of probe particles embedded in the active system. The model developed here is based on truncated Levy statistics which is generally observed for the force generators whose impact decays as 1/r2.

1P152 ポリエチレングリコールがアクチン繊維と調節繊維の運動に及ぼす影響

Effect of polyethylene glycol on the motility of actin and regulated thin filaments on myosin molecules

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Increased concentrations and molecular weights of polyethylene glycols (PEG1000—20000) decreased the velocities of actin and regulated thin filaments (at pCa 4). Further, in the absence of Ca^{2+}, regulated thin filaments were moderately motile in the presence of PEG. The excluded volume change (ΔV) during their interactions was estimated by determining the relationship between osmotic pressure exerted by PEG and the decreased ratio of the velocities in the presence and absence of PEG. ΔV for regulated thin filaments was approximately 2-fold higher than that of actin filaments. This result suggests that the geometric configuration on filaments influences the excluded volume during interactions of actomyosin and modulates the motility.

1P153 ダイニン-微小管インターフェイスの構造解析: 微小管から AAA+ ATPase ドメインにどのように情報が伝えられるか？

Structural analysis of dynein-microtubule interface: How is a signal transmitted from microtubule to AAA+ ATPase domain?

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We have previously identified two residues in α-tubulin critical for directional movement and ATPase activation of cytoplasmic dynein. To clarify the structural basis for this observation, we here analyzed the structure of the dynein-microtubule-binding domain (MTBD) bound to microtubules (MTs) using electron cryomicroscopy. The result revealed a key structure at the interface of the MTBD, possibly transmitting the MT binding signal to the ATPase domain: two critical residues in tubulin interact with the charged residues in MTBD, linking the distal end of each helix in the stalk coiled-coil (CC1 and CC2, respectively) to the MT. Based on this observation, we propose a model addressing how MTBD-MT interaction is coupled to ATPase activation and directional movement.

1P154 キネシンの弱結合から強結合への状態変化における蝶番構造

A mechanistic pivot-point in the weak-to-strong state transition during kinesin-microtubule interactions

Itsushi Minoura, You Hachikubo, Yoshihiko Yamakita, Hiroko Takazaki, Rie Ayukawa, Chihiro Yoshida, Seiichi Uchimura, Etsuko Muto (*RIKEN BSI*)

The molecular mechanisms underlying the microtubule-dependent activation of kinesin ATPase remains unclear. To elucidate the role of the microtubule in the kinesin-ATPase cycle, we previously identified 6 acidic tubulin residues that are critical for ATPase activation; while 4 α-tubulin mutants showed a reduced k_{cat} of kinesin ATPase, the K_{mMT} of 2 β-tubulin mutants showed an increase. In this study, we identified a basic residue (R262) in β-tubulin, which, when mutated, lowers k_{cat} and increases K_{mMT}. Biochemical and biophysical analyses revealed that this residue interacts with loop 12 in kinesin in both the weak- and strong-binding states and serves as a mechanistic pivot-point in the weak-to-strong state transition during kinesin-microtubule interactions.

1P155 高速 AFM によって明らかとなったミオシン X の歩行メカニズム

Walking mechanism of myosin X revealed by high-speed AFM

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We succeeded in direct visualization of walking behavior of myosin X (M10) by high-speed AFM (HS-AFM). At low ATP concentration, M10 mostly stepped forward but sometimes stepped backward. Interestingly, we noticed that the leading head (LH) of M10 showed structural changes between a straight neck (ST) and a sharply bent (BN). In addition, LH tended to take ST before M10 showed large forward step, and LH tended to take BN before LH dissociated from actin. Based on our myosin V (M5) observations, it is indicated that LH of M10 with BN holds no nucleotide and thus can bind to new ATP and results in the head dissociation, which well explains frequently seen irregular movements of M10. These observations suggest that M10 has a poor gating mechanism compared with M5.

1P156 高速 AFM によって明らかとなったミオシン V の化学-力学変換メカニズム

若手招待講演

Chemomechanical coupling mechanism of myosin V revealed by high-speed AFM

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Our previous high-speed AFM (HS-AFM) observations of myosin V (M5) suggest that M5 does not need the ADP.Pi state to rebind to actin and that the powerstroke performed by the leading head spontaneously occurs and dose not need the Pi release process, which is challenging the widely accepted chemomechanical coupling of myosin motor. Here, we performed an experiment to further evidence our suggestion, in which in the presence of only ADP we applied a force to only the trailing head to dissociate this head from actin using a newly developed scanning mode of HS-AFM. Then, we successfully observed walking behaviors of M5, very similar to that seen in the presence of ATP. This result supports our suggestion and requires reconsideration of the chemomechanical coupling model.

1P157 高速 AFM によるダイニンの機能動態の観察

High-Speed-AFM Observation of Processive Movement of Cytoplasmic Dynein

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Cytoplasmic dynein is a microtubule-based motor protein which transports cargos toward the minus end of microtubules. So far, several studies based on structural analysis and single molecule observations have been reported to elucidate the working mechanisms. However, no studies have been performed to directly observe the conformational dynamics of dynein in action due to the lack of technique. On the other hand, we have recently succeeded in directly visualizing hand-over-hand behavior of myosin V. Here, we applied high-speed AFM to observing cytoplasmic dynein moving along microtubules. In this presentation, we will discuss what information about the dynein's mechanisms can be gained with high-speed AFM.

1P158 マイコプラズマモービレのあしとシアル酸の結合はヌクレオチドに依存する

Nucleotide-dependent interaction between legs of *Mycoplasma mobile* and sialyllactose

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 University.*)

Mycoplasma mobile has hundreds of legs approximately 100 nm long and glides on the substrate at the speed of 2.5 μm/sec. It is hypothesized that a cycle of attachment to and detachment from surface-immobilized sialyllactose (SL) drives the force of the gliding. To reveal the mechanism of directed movement as the sum of the force in each leg, we quantified the interaction between legs and SL by using optical tweezers in the presence of different nucleotides or analogues: AMP-PNP ; ADP; ADP+Vi ; nucleotide-free condition. We found that unbinding force depends on chemical states, and also, loading directions in terms of a bacterium gliding polarity. These nucleotide-dependent asymmetric variations could contribute to decipher the gliding mechanism at the molecular level.

1P159 滑走するバクテリアの戦車のような運動装置を三次元で追跡する

Three-dimensional tracking of tank-like motility apparatus of the gliding bacterium

Showko Odaka, Daisuke Nakane, Takayuki Nishizaka (*Department of Physics, Gakushin University*)

Flavobacterium johnsoniae exhibits rapid gliding motility over surfaces. The cell does not have flagella or pili, and instead, rely on a novel motility apparatus, the cell-surface adhesion, by a unique mechanism for efficient gliding. The latest contribution showed that the adhesions flow travels along a closed loop on a cell surface like a caterpillar in a tank (Nakane *et al*., *PNAS*, *in press*). Here we extend this approach and apply the three-dimensional tracking method to the flow by nanometer-scale localization of 0.1 μm fluorescent beads to visualize the motion of adhesions on gliding cells. This method reveals the accurate flow pathway and its directionality: the left-handed helical closed loop with the helix of 3 μm in the rotational pitch and of 0.4 μm in radius.

1P160 戦車のような仕組みで動くバクテリア

若手招待講演

Bacterium moves like a tank

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Flavobacterium johnsoniae exhibits rapid gliding motility over surfaces by a unique mechanism. These cells do not have flagella or pili; instead, they rely on a novel motility apparatus. SprB, a 669 kDa cell-surface adhesin, is required for efficient gliding. Here, we showed dynamic movements of SprB were observed by fluorescent microscopy. SprB moved at a constant speed of 2 μm/s on the cell surface along a left-handed helical closed loop, appears that the cell have a moving conveyer belt. Attachment of SprB to the substratum was associated with cell movement, suggesting a model for gliding, in which adhesins are propelled along a helical track, generating rotation and translation of the cell.

1P161 方位と倒れの構造変化を１分子レベルで検出する偏光スイッチングを用いた新しい TIRFM

Advanced TIRF microscopy to detect single-molecule conformational changes in both azimuth and axial axis using polarization switching

Nagisa Mikami[1], Tomoko Masaike[1,2], Mitsuhiro Sugawa[1], Takayuki Nishizaka[1] ([1]*Dept. phys., Gakushuin Univ.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*)

To elucidate domain motions that correlate with protein functions at the single molecular level, we have developed objective-type TIRF microscopes with polarization modulation. We here extend this technique to detect not only conformational changes in azimuth but also tilting motions in axial angle, i.e., the component parallel to the optical axis, by switching the laser polarization. A pair of images under *s*- and *p*-polarized illuminations is continuously captured while spots from single fluorophores are rotated on the camera plate in synchronously. By fitting the intensity profile of each image, orientation of surface-immobilized catalytic subunit of F_1-ATPase was detected with an angular resolution of ～1° under the exposure of 800 millisecond.

1P162 1 分子 FRET 計測による F_1-ATPase の ATP 結合待ち構造の解析

Analysis of the ATP-waiting form of F_1-ATPase by single-pair FRET measurement

Mitsuhiro Sugawa[1], Masaru Kobayashi[1], Takashi Matsui[1], Tomoko Masaike[2,3] ([1]*Dept. Phys., Gakushuin Univ.*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. Sci.*, [3]*JST*)

To uncover conformational changes in F_1-ATPase, we performed single-pair FRET measurement to detect distance changes between subunits having a FRET pair (Cy3 and Cy5), using the mutant F1(βE190D/L398C). FRET signal showed at least two-state transition between high (~0.8) and low (~0.5) FRET efficiencies. The high FRET state represents the two β subunits taking a closed form. We also performed a simultaneous measurement of FRET and rotational steps. The high FRET state occurred at one of the three catalytic dwells. In other dwells, FRET efficiency remained lower. These results suggest that in the ATP-waiting dwell two of three β subunits should not take the closed form as in the catalytic dwell.

1P163 F_1-ATPase の軸とシリンダーの結合寿命の測定

Measurement of lifetime of the bond between the shaft and the cylinder in single F_1-ATPase

Tatsuya Naito[1], Kaoru Okada[1], Tomoko Masaike[1,2], Takayuki Nishizaka[1] ([1]*Dept. phys., Gakushuin Univ*, [2]*Dept. Appl. Biol. Sci., Tokyo Univ. of Science*)

F_1-ATPase is a rotary motor protein in which the central γ subunit rotates inside the cylinder consisting of $\alpha_3\beta_3$ subunits. To investigate interactions between the γ shaft and the cylinder at the molecular scale, three-dimensional single-particle tracking using a wedge prism has been combined with optical tweezers to unbind γ in the direction along which the shaft penetrates the cylinder. Lifetime under various constant loads was measured using high-speed camera with a time resolution of 2 ms, which enabled estimation of the interaction distance (Δd) and the lifetime with no load (τ_0) to be 1 nm and 50 s, respectively.

1P164 N 末端領域変異単頭キネシンによる微小管の 3 次元コークスクリュー運動

Three-dimensional corkscrewing motion of a microtubule driven by single-headed kinesins with mutations in the N-terminal region

Shoko Fujimura[1], Shinsuke Owada[1], Takayuki Nishizaka[1], Junichiro Yajima[2] ([1]*Dept. phys., Gakushuin Univ.*, [2]*Graduate School of Arts and Sciences, The University of Tokyo*)

Kinesins are microtubule (MT) -based motor proteins that power intracellular transport. By using the 3-D tracking microscopy we develped, it was shown that MT rotates in a left-handed manner with the pitch of ～0.3 μm when it slides on the lawns of single-headed kinesins. We here introduced mutations at the N-terminal region which was proposed to interact with the necklinker as a cover strand (CS) form to build a β-sheet. By tracking quantum dot attached to MT, the corkscrewing motion of MT driven by mutants were directly and quantitatively visualized in 3-D. The pitch depends on the type of mutations, indicating that one of the structural bases that causes torque is CS.

1P165 G-、F-アクチンの水和測定と偏比容測定

Hydration and partial specific volume measurements of G- and F-actin

Asato Imao, Takahiro Watanabe, Tetsuichi Wazawa, George Mogami, Nobuyuki Morimoto, Makoto Suzuki (*Dept. Materials Processing, Tohoku Univ.*)

The role of hydration water is a key issue in actin polymerization. We studied hydration properties and partial specific volume of actin by dielectric relaxation spectroscopy (DRS) and density measurements (DM). By DRS, constrained water (fc = 5.1 GHz at 10 °C) and hyper mobile water (HMW) (fc = 20 GHz) were detected around G- and F-actin. The HMW content was higher for F-actin than for G-actin. DMs of actin solutions showed that partial specific volumes of G- and F-actin were 0.715 ± 0.006 and 0.726 ± 0.003 ml/g at 10 °C, respectively. The thermal expansibilities were $6.8 \times 10^{-4} \pm 0.4 \times 10^{-4}$ for G and $4.7 \times 10^{-4} \pm 0.4 \times 10^{-4}$ (K^{-1}) for F. Thus, changes in hydration state and partial specific volume have been revealed upon actin polymerization.

1P166 アクチンに係留された色素の回転相関時間の周波数領域蛍光偏光解消法による測定

Rotational correlation time of a fluorophore tethered to actin as studied by frequency-domain fluorescence anisotropy measurements

Tetsuichi Wazawa, Nobuyuki Morimoto, Makoto Suzuki (*Grad Sch of Engin, Tohoku Univ*)

Hydration water is vital to molecular processes involving proteins and has altered properties such as modulated local viscosity. Since rotational motion of a fluorophore reflects its surrounding viscosity, we have investigated fluorescence anisotropy of a fluorophore tethered to a protein.
A frequency-domain fluorometer was in-house constructed to measure time resolved fluorescence anisotropy. Rhodamine 6G (R6G) was tethered to C374 of actin through oligo(ethylene glycol).
Two components were found in the anisotropy decay of R6G and the rotational correlation times were 0.5 ns and 25 ns at 20°C, which would correspond to rotation of R6G and tumbling of actin, respectively. The former fast component is likely to be a measure of the hydration water viscosity around actin.

1P167 1,3-ジエチル尿素による骨格筋ミオシンの滑り運動の阻害と Mg-ATPase の活性化

1,3-Diethylurea-enhanced Mg-ATPase of skeletal muscle myosin with a converse effect on the sliding motility

Tetsuichi Wazawa, Shin-ichiro Yasui, **Nobuyuki Morimoto**, Makoto Suzuki (*Grad. Sch. Engin., Tohoku Univ*)

Alkylureas modulate protein properties and enzymatic activities. Cosolvent- and drug-induced changes in actomyosin have been exploited to better understand its mechanism of force generation. In this study, we have investigated effects of alkylureas on actomyosin.
Mg-ATPase of myosin subfragment-1 in the presence of urea, 1,3-dimethylurea, 1,3-diethylurea (DEU), or thiourea below 1 M showed that DEU enhanced the turnover rate by up to 7-fold whereas the others had a lesser effect on it. Interestingly, the maximal rate V_{max} of actin-activated heavy meromyosin ATPase was almost unchanged with 0.3 M DEU, whereas the sliding speed of myosin-driven actin filaments was reduced to 1/16 of control. Thus, DEU effectively uncouples the sliding motility from actomyosin ATPase.

1P168 バクテリアべん毛モーターの高時間分解能回転ステップ計測系の開発

Development of dark-field imaging system with high temporal resolution for angular steps by bacterial flagellar motor

Hiromichi Wakebe[1], Yuichi Inoue[2], Akihiko Ishijima[2] ([1]*Grad. Sch. Life Sci.*, [2]*IMRAM, Tohoku Univ.*)

The rotational angular steps of flagellar motor have been studied under very low ion motive force conditions in which rotation speed is unusually slow and unstable. To understand the mechanism of the angular steps, it is required to detect the steps more clearly at normal rotational speed with high temporal resolution.
Here, we develop a new optics of laser dark-field illumination for the fast tracking. Two separated incident laser illuminating the sample obliquely from opposite sides was used to image a nanoparticle attached to the flagellum with low background scattering came from the cell. Using this technique, we achieved the high S/N over 100 to track the rotation as 1 kHz. The high S/N will enable us to track the steps more faster than the previous reports.

1P169 Analysis of angular steps of bacterial flagellar motors using an elliptic probe

Yuichi Inoue[1], Hiromichi Wakebe[2], Takashi Sagawa[2], Hajime Fukuoka[1], Akihiko Ishijima[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad.Sch. Life Sci., Tohoku Univ.*)

Bacterial flagellar motor is a rotary motor with ~26 steps/revolution at low load (Sowa et al., 2005). The step analysis suffered from low frequency noise that limited resolution as several degrees. Here we developed a simple method to understand the mechanism of the motor rotation.
A rotating E. coli cell tethered through a flagellum on a glass was imaged by high speed camera as an elliptic probe. The images were processed to binary images to calculate the angles by the linear fitting. During the slow rotation of ~0.5 Hz driven by sodium-driven chimeric motor under low concentration of sodium ion, the steps of ~14° were detected at a fast frame rate of 1-2 kHz with angular resolution of 0.83°, indicating that motor steps even with a high load.

1P170 F_1-ATPase が発生するトルクの微細構造

Microstructure of the torque generated by F_1-ATPase

Eiichiro Saita[1], Kazuhiko Kinosita[2], Masasuke Yoshida[1] ([1]*Dept. Mol. Bio., Kyoto Sangyo Univ.*, [2]*Dept. Phys., Waseda Univ.*)

F_1-ATPase (F_1) is an ATP-driven rotary motor. Average output of the torque during ATP hydrolysis reaction is reported to be 40-50 pNnm. However, the detailed torque profile as a function of rotor angle remains unknown. To know it, we have measured the torque by using magnetic tweezers.
One of primary findings is that F_1 torque has a "microstructure", that is, a gradual decrease/increase followed by a steep jump/fall in torque repeats at least three times per 120 degree rotation during ATP hydrolysis/synthesis, respectively. In addition, the efficiency of mechanochemical energy conversion is almost 100% in both ATP hydrolysis/synthesis reactions. Finally, the torque profiles provide a simple model that explains how F_1 attains 100% energy conversion and how F_1 rotates.

1P171 ヒト F1-ATPase の一分子解析が明らかにした、バクテリアとは異なったミトコンドリア F1 の回転スキーム

Single molecule analyses of human F1-ATPase revealed distinct rotation scheme of mitochondrial F1 motor

Toshiharu Suzuki[1,2], Kazumi Tanaka[1], Chiaki Wakabayashi[1], Shou Furuike[3], Eiichiro Saita[1], Kazuhiko Kinosita[4], Masasuke Yoshida[1] ([1]*Dept of Mol Bioscience, Kyoto Sangyo Univ*, [2]*CRL, Tokyo Inst of Tech*, [3]*Dept of Physics, Osaka Med College*, [4]*Faculty of Science and Eng, Waseda Univ*)

A rotary motor enzyme F1-ATPase (F1) is a catalytic domain of FoF1-ATP synthase that produces the majority of ATP in respiring cells. Here, we visualize rotation of human mitochondrial F1, and present how each catalytic event drives rotation. Starting from rotor angle 0° of the γ-subunit in the stator α3β3 ring, ATP binding to one β-subunit in the ring drives a 0°>65° rotation, the Pi release from another β drives the next 65°>90° rotation, ATP hydrolysis on the third β occurs at 90°, and gamma turns to 120° to complete one cycle reaction. Rotation is often interrupted by persistent ADP binding and is stalled by azide or IF1. Based on this rotation scheme, the previous crystal structures of mitochondrial F1s are successfully assigned to each of the rotational isoforms.

1P172 腸内連鎖球菌 V-ATPase の大腸菌発現系

Expression of *Enterococcus hirae* V-ATPase in *E. coli* BL21 (DE3)

Shohei Matsudo[1], Suhaila Rahman[1], Shinya Saijo[1], Misaki Yamamoto[1], Yoshimi Kakinuma[2], Kenji Mizutani[1,3], Takeshi Murata[3], Ichiro Yamato[1] ([1]*Dept. Biol. Sci Tech, Tokyo Univ. Sci*, [2]*Faclty Agri, Ehime Univ*, [3]*Faculty Sci, Chiba Univ*)

There are several ion-pumping ATPases in cell membranes, working to maintain the homeostasis of a cell. V-ATPases function as ATP-dependent proton pumps in the membranes of acidic organelles of eukaryotic cells. V-ATPase is also expressed in a prokaryote, *E. hirae*, which transports Na^+.
We have already established expression and purification of V-ATPase in *E. hirae*. We are now trying to express this heterologous genes of V-ATPase whole complex in *E. coli*. In this poster, we report expression and purification of V-ATPase in *E. coli* along with its activity measurement.

1P173 腸内連鎖球菌 V 型 ATPase の A サブユニットの精製と結晶化

Purification and Crystallization of A subunit from *Enterococcus hirae* V-ATPase

Aki Saito[1], Yasuko Saito[1], Shinya Saijo[1], Misaki Yamamoto[1], Yoshimi Kakinuma[2], Kenji Mizutani[1,3], Takeshi Murata[3], Ichiro Yamato[1] ([1]*Dept Biol Sci Tech, Tokyo Univ Science*, [2]*Faculty Agri, Ehime Univ*, [3]*Faculty Sci, Chiba Univ*)

The vacuolar ATPases (V-ATPases) function as ATP-dependent proton pumps in the membranes of acidic organelles of eukaryotic cells. The V_1 domain is composed of A_3B_3DF complex which hydrolyzes ATP. To understand details of the assembly mechanism of V_1 domain, it is necessary to obtain the structures of every subunit and partial complex. We have already reported structures of A_3B_3 and A_3B_3DF.
A subunit was expressed in *E. coli* with His tag, purified with Ni^{2+} affinity and gel filtration chromatography. In this poster, we will report the results of crystallization of purified A subunit with and without nucleotides.

1P174 Direct observation of the rotation of V_1-ATPase from *Enterococcus hirae* and its torque

Hiroshi Ueno[1], Yoshihiro Minagawa[2], Ichiro Yamato[3], Takeshi Murata[4], Ryota Iino[2], Eiro Muneyki[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*)

We constructed an experimental setup to observe the rotation of *Enterococcus hirae* V_1-ATPase (EhV_1). Proteins were prepared by Cell-free or *E. coli* expression systems that we developed recently. Rotation mutant was reconstituted from His_6-A_3B_3 and biotinylated-DF complexes, because mutation of endogenous cysteine residues in A-subunit decreased the ATPase activity. We have succeeded in observing the rotation of EhV_1 and the basic properties were determined by using a 40-nm gold colloid as a load-free probe (Minagawa, Y. *et al.*, this meeting). Furthermore, we estimated the torque of EhV_1 from continuous rotation of large beads at 4 mM ATP using fluctuation theorem (Hayashi, K. *et al.*, PRL 2010). Interestingly, torque of EhV_1 was ~3 times lower than that of F_1-ATPase.

1P175 F_1-ATPase の P-loop 変異体とリン酸解離の関係

The relationship between F_1-ATPase P-loop mutants and Pi release

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We studied P-loop mutants (βG158S and βG158C) of F_1-ATPase which may release Pi slowly based on previous studies (Sanjay et al. Biochemistry 2002, 41.14421-14429). In the case of F_1 (βG158S), 1 mM and 5 mM Pi decreased ATPase activity by about 20% and 40% respectively at 1 mM ATP. Rotation assay of this mutant revealed long pauses at the catalytic positions more frequently in the presence of 5 mM Pi compared with the wild type. These long pauses may be caused by decrease in the ATPase activity. In the case of βG158C, it showed very low ATPase activity (1.4 /s) in the range of 5 mM to 50 μM ATP. Because Ser has similar characteristics with Cys, these two mutants may have common inhibitory mechanism by Pi.

1P176 回転電場を用いた外力存在下での F1-ATPase の回転観察
Observation of the rotation of F1-ATPase

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F_1-ATPase is a rotary molecular motor which consists of the $\alpha_3\beta_3$ ring and the rotor subunit (γ-shaft). While F_1 hydrolyzing ATP to ADP and phosphate, γ-shaft rotates to counter clockwise. On the other hand, while F_1-motor synthesizes ATP from ADP and phosphate, γ-shaft rotates to clockwise powered by an enough strong external torque. In order to study this energy transduction, we observed the rotation of F_1, from thermophillic Bacilus PS3 (TF_1), under the external torque and estimated maximum work from of the torque when the probe particle showed the bidirectional stepwise fluctuations with 120° steps. To apply a constant external torque of precisely controlled magnitude, we use the electrorotaion method.

1P177 Sopped-Flow 法を用いた β サブユニット単体と F1-ATPase へのヌクレオチド結合の比較
Comparison of the nucleotide binding to the isolated βsubunit and the F1-ATPase using the Sopped-Flow method

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Nucleotide binding is important for the rotation of F1-ATPase. In order to investigate the effect of the complex formation on the nucleotide binding to the β subunit, we compared nucleotide binding rate constant (kon) to the isolated β subunit and the α3β3γ subcomplex containing βY341W mutation that shows fluorescence intensity decreases upon nucleotide binding. kon ATP=1.3×10^7 M-1 s-1, kon ADP=1.1×10^7 M-1 s-1 of the isolated βsubunit and kon ADP=1.0×10^7 M-1 s-1 of the α3β3γ subcomplex have been obtained at 286.5 K with a stopped-flow apparatus. Furthermore we found that Pi did not interfere with nucleotide binding of the isolated β subunit. We will report also the kon and Pi effect for the α3β3γ subcomplex at room temperature.

1P178 走化性と重力により誘起されるサルモネラ菌の生物対流
Bioconvection of *Salmonella* induced by chemotaxis and gravity

Takahiro Abe, Shuichi Nakamura, Seishi Kudo (*Grad. Sch. Eng., Univ. Tohoku*)

When cell density is low, the chemotactic response has been known as a biased random walk. However, it still remains unknown how increase of cell density influences the chemotaxis. In this study, 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. As a result, we found that there were at least three phases when bacteria show the chemotactic response, which were significantly affected by cell density. Analysis of the flow by PIV (Particle Image Velocimetry) showed that large-scale convection flow was generated when bacteria accumulated to attractant and the direction of convection was affected by the direction of gravitational force.

1P179 MotB ペリプラスム領域の in-flame 欠損がサルモネラ菌べん毛モーターの出力特性に及ぼす影響
Effect of in-frame deletion in the periplasmic region of MotB on the torque-speed relationship of *Salmonella* flagellar motor

Shuichi Nakamura[1], Yusuke V. Morimoto[2], David J. Castillo[3], Yong-Suk Che[4], Nobunori Kami-ike[3], Seishi Kudo[1], Tohru Minamino[3], Keiichi Namba[2,3] ([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*RIKEN QBiC*, [3]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [4]*Dept. Frontier Biosci., Hosei Univ.*)

MotA and MotB form the stator of the bacterial flagellar motor. Because the deletion of residues 52-71 of MotB causes excessive proton leakage, this region is thought to act as a plug to suppress the undesirable proton influx. In this study, we examined the torque-speed relationships of the Salmonella motors driven by the Δplug stator to understand how the periplasmic region of MotB is involved in torque generation. Rotation assays revealed that torque produced by the Δplug motor was wild-type level at high loads but markedly reduced as the load decreased. However, the Δplug motor could spin as fast as the wild-type one near zero load. These suggest that the loss of the plug causes proton leakage but does not affect the coupling of proton influx with torque generation.

1P180 Motility analysis of Leptospira in highly viscous environments

Kyosuke Takabe, Md. Shafiqul Islam, Seishi Kudo, Shuichi Nakamura (*Grad.sch.engineering.,univ.tohoku*)

Leptospira is a member of spirochete. The motility of Leptospira is believed to be an important factor for the infection. It has been known that Leptospira cells positively respond to a gradient of viscosity. In this study, to understand mechanism of the taxis-like behavior called viscotaxis, the motility and morphology of individual Leptospira cells were examined. When the Leptospira cell moves, transformation of the cell body between symmetric and asymmetric shapes occurs. The asymmetric body is required for translation. Microscopic analysis showed that the frequency of morphological switching and the duration of the asymmetric shape increased with viscosity. The time when cells efficiently translate was elongated at high viscosity, which would induce viscotaxis.

1P181 Microscopic observation of chemotactic behaviors of Leptospira

Md. Shafiqul Islam, Kyosuke Takabe, Seishi Kudo, Shuichi Nakamura (*Department of Applied Physics, Tohoku University*)

Leptospira is a member of spirochete. Recently, several chemoattractants for Leptospira cells were identified but chamotactic behaviors of individual cells are still elusive. In this study, we analyzed motility of individual Leptospira cells attracted to sugars. Leptospira cells swim with spiral (S) anterior end and hooked (H) posterior end, and change swimming direction with rapid transformation of cell ends. Observation using a dark-field microscope showed that the switching frequency of swimming direction was suppressed in the presence of sugars. Also, the fraction of cells swimming with S-H shape increased in concentration gradient of sugars. These suggest that chemotaxis signals allow cells to retain asymmetric morphology which is proper for efficient translation.

1P182 細菌べん毛モーター蛋白質 FliG-FliM 相互作用の解析
Interaction between FliG and FliM in the bacterial flagellar motor

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The flagellar motor of Salmonella rotates in both counterclockwise (CCW) and clockwise (CW) directions. FliG, FliM and FliN are involved in switching the direction of flagellar motor rotation. The binding of a signaling protein to FliM and FliN induces conformational changes in FliG, thereby switching the motor to spin in the CW direction. A middle domain of FliM binds to the middle and C-terminal domains of FliG. A three-amino-acid deletion at positions 169-171 of Salmonella FliG results in an extreme CW-biased rotation. In this study, we analyzed the effect of this in-frame deletion on the FliG-FliM interaction. We show that the deletion significantly reduces the binding affinity of FliG for FliM. We will discuss the switching mechanism of the flagellar motor.

1P183 クライオ電子顕微鏡によるべん毛蛋白質輸送装置の構造と分子機構
Molecular mechanism of the type III protein export by electron cryotomography of the flagellar basal body

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The flagellar type III protein export apparatus is a nanomachine spanning two bacterial membranes to export flagellar axial proteins from the cytoplasm for flagellar assembly. The molecular mechanism remains unclear due to the lack of detailed structural information. Recently, two cytoplasmic structures within and below the MS-C ring have been visualized *in situ* by electron cryo-tomography (ECT) and putatively identified as part of the apparatus. However, since they look isolated from the MS-C ring, it is not clear how they are stably attached and what they are. To identify them for more detailed understanding of the export mechanism, we analyzed the basal body structures of some mutant strains by ECT. We will discuss the current model of the molecular mechanisms.

1P184 Mycoplasma mobile から単離した滑走装置の電子顕微鏡観察
Electron microscopic observation of isolated gliding machinery of Mycoplasma mobile

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Mycoplasma mobile, a fish pathogen, glides on solid surfaces in the direction of a membrane protrusion at a cell pole by a unique mechanism. Previously, we succeeded in isolating the gliding machinery from M. mobile, named “gliding head”. Although this fraction should be useful to study the gliding mechanism, it contains only 30% gliding head in various membrane pieces. In the present study, we improved the isolation process and achieved 50% gliding head, visualized “leg” structure, cell membrane, cytoskeletal “jellyfish structure”, and plasmalemmal undercoat by electron microscopy. Cryo-electron microscopy is undertaken to get the three dimensional images of these structures.

1P185 FRET センサーを用いて生細胞内分子混雑を可視化する
Visualization of the molecular-crowding effects in living cell on cellular functions using a FRET-based biosensor

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Cells are densely packed with many molecules (DNA, proteins, etc.). Molecular crowding has been shown to modify the biochemical reactions in vitro. However, it is unclear how molecular crowding in living cells effects on the various cellular processes. In this study, we developed a FRET-based biosensor for the spatio-temporally resolved observation of molecular crowding and confirmed that FRET ratio of the biosensor was changed in dependent on the concentration of surrounding proteins in vitro. To examine whether molecular crowding can be a regulatory factor of cellular functions such as migration and cell division, the biosensor was fused to cytoskeleton, focal adhesion proteins and nucleosomes and expressed in fibroblasts. We will discuss the results in the meeting.

1P186 Positive feedback mechanism for PIP3 polarity establishment mediated by PIP3 phosphatase, PTEN

Satomi Matsuoka[1,2], Masahiro Ueda[1,2] ([1]*QBiC, RIKEN*, [2]*Osaka University*)

Cell motility involves establishing anterior-posterior polarity with a definite localization of signaling molecules such as PIP3 at the anterior membrane. The confined localization may be achieved via a positive feedback, which works so that PIP3 leads to further accumulation of PIP3 on the nearby membrane. However, it has not been confirmed yet. By analyzing the behavior of PIP3 phosphatase, PTEN, in living *Dictyostelium* cells, we showed that PIP3 inhibits PTEN membrane localization resulting in the inhibition of PIP3 dephosphorylation. Single-molecule imaging revealed that PTEN membrane dissociation is enhanced after PIP3 dephosphorylation. Therefore, PIP3 polarity is likely to be established via the positive feedback mediated by the enzymatic activity of PTEN.

1P187 細胞内 pH 変化に伴った細胞運動
Changes in intracellular pH mediate 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 observed intracellular pH changes and cell dynamics simultaneously by fluorescence microscopy. We will discuss the role of intracellular pH in signal transduction.

1P188 nonlinear stress propagation, anisotropic stiffening, and nonaffine relaxations in cytoskeletal networks

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The mechanics of cells or cytoskeletons have been understood either as a network of semi-flexible polymers or a glass. Here we employ optical-trap based microrheology to apply forces to cytoskeleton and measure the high-bandwidth response at an anterior point. Simulating the highly nonlinear and anisotropic stress-strain propagation assuming affinity, we found that theoretical predictions for the quasi-static response are only realized at high frequencies inaccessible to conventional rheometers. We give a theoretical basis for determining the critical frequency when both affinity and quasi-staticity are valid, and discuss with experimental evidence that the relaxations at lower frequencies can be characterized by the experimentally obtained non-affinity parameter.

1P189 Roles of actin polymerization in the collective cAMP oscillations

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In eukaryotic chemotaxis, actin polymerization appears to be part of a positive feedback circuit that amplifies receptor-mediated intracellular signaling. In *Dictyostelium*, there is a significant overlap between pathways responsible for chemotaxis and cell-cell signaling. Here, we report on the relationship between cAMP-induced cAMP relay response and the amount of F-actin. Using microfluidics and live-cell imaging, we show that the amplitudes and duration of the response at the single cell level diminished when actin polymerization was pharmacologically inhibited. The collective oscillations of cAMP in the population of cells were also suppressed as expected from the single cell experiments. We will also address the contribution of cell motility to cell-cell signaling.

1P190 血管平滑筋細胞内の核に対する核上下のアクチンストレスファイバの力学的役割
Actin cap fibers and basal stress fibers have different roles in mechanical regulation of nucleus in vascular smooth muscle cells

Kazuaki Nagayama, Yuki Yahiro, Mitsuhiro Ukiki, Takeo Matsumoto (*Department of Mechanical Engineering, Nagoya Institute of Technology*)

Actin stress fibers (SFs), bundles of actin-myosin contractile cytoskeletal structures, play important roles in cellular mechanotransduction. In this study, we cut the apical SFs running across the top surface of the nucleus (actin cap fibers, ACFs) or the basal SFs (BSFs) by the laser ablation and observed the mechanical responses of SFs and nucleus. Shortening of the dissected fibers was significantly greater in ACFs than in BSFs. Nuclei moved in the direction of SF retraction, and local deformation of nucleus and redistribution of DNA were more remarkable after the dissection of ACFs. These indicate that ACFs and BSFs play different roles in mechanical regulation of nucleus, and that intracellular tension is transmitted to nucleus more efficiently by ACFs.

1P191 人工設計したマイクロ構造化基質における細胞のアクチン動態
Actin dynamics in cells cultured on engineered micro-topographical substrate

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Extracellular environmental topographical feature is an important determinant for various cell functions, such as cell motility, differentiation and proliferation. The aim of this study is to clarify the mechanism how cells detect the topographical features and integrate these inputs to express various functions. We developed an experimental system for high resolution image analysis of actin cytoskeleton and related signaling molecules in cells cultured on engineered micro-grooved substrates. The signaling molecule and actin dynamics changed largely depending on the groove width. Our findings will be a basis of deductive design of cell culture substrates to manipulate cellular function with the topographical feature.

1P192 CRP2 タンパク質によるアクチン線維のダイナミクス制御
Smooth muscle differentiation related transcription factor CRP2 directly regulates of actin filaments dynamics

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Actin filaments form many types of structures and dynamically regulate the cell morphology and movement, and furthermore, they play a mechanosensory role for extracellular stimuli. In this study, we clarified that smooth muscle related transcription factor, CRP2 (cysteine-rich protein 2), directly bound to actin filaments and regulated their dynamics. CRP2 blocked the association of cofilin and actin filaments and then inhibited the actin filaments depolymerization. Furthermore, smooth muscle cells with active CRP2 expression showed higher mechanical stiffness. Thus, that actin-bound CRP2 plays roles in the stabilization of actin filament formation in smooth muscle cells.

1P193 細胞性粘菌アクチンの疎水性ヘリックスの変異が細胞運動に与える影響
Actin mutation introduced into the hydrophobic helix impairs cytokinesis of Dictyostelium cell

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To reveal the function of the hydrophobic helix of actin, mutations were introduced to this helix of Dictyostelium actin. When Dictyostelium cells are starved, they aggregate each other. The cells with the expression vector of the mutant actin showed the abnormal behavior in cytokinesis, even though they still express the wild-type actin together with the mutant actin, as detected with SDS-gel electrophoresis. They formed smaller mounds. The number of the mounds was larger. When cultured with E. coli B/r, they showed lower mobility, with the spreading velocity being about one half. When cells were cultured on the plastic surface, the average number of nuclei per cell increased. In suspension culture, there was no significant change in the growth rate of the cells.

1P194 クライオ電子線トモグラフィー法を用いた細胞内におけるアクチンフィラメントバンドリングメカニズム解明

The interaction between actin filaments and fascin are observed at high resolution with cryo-ET

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Actin filaments have very important roles in the cell. In a half decade their detailed structure was revealed by EM. However their interaction with their accessary proteins in the cell is unclear, so it is important to know how to interact with each other in biological conditions.
Here we combined cryo-ET and image processing with computer. Tilt series from EM have very low S/N ratio, which restricts resolution, so we overcome the problem with that.
We obtained high resolution structure of "*in vivo*" actin filaments in filopodia by cryo-ET. Moreover, the interactions between actin and fascin were revealed. Hence our techniques have potential to reveal interaction between actin and various interaction proteins such as motor protein at subnanometer resolution.

1P195 非筋細胞から単離したアクチンストレスファイバーの成分について

Molecular components of actin stress fibers isolated from nonmuscle cells

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Contractile actin stress fibers play important roles in various physiological processes, such as migration, survival, and differentiation. However, their biophysical characteristics and structural components are largely unknown. To identify the critical constituents required for the contraction, we have tried to develop an in-vitro contractile system of actin stress fibers isolated from nonmuscle cells. We demonstrate that the phosphorylation level of myosin regulatory light chains is maintained in our isolation procedure, and the stress fibers are highly contractile even without Rho kinases. This system enables us to measure the contractile force of single stress fibers, which has never been reported.

1P196 細胞性粘菌ミオシン変異株 G680V が示す骨格筋アクトミオシンの高速滑り運動

Myosin mutant G680V accelerated sliding velocities of skeletal muscle acto-myosin

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Myosin mutant G680V from *Dictyostelium discoideum* cannot move actin filament *in vitro*. Previously, we have reported that the sliding velocity of an actin filament was remarkably accelerated in a intermittent manner by mixing of the slight amount of the mutant into skeletal HMM. In order to examine the capability of the mutant in solution, G680V-HMM was added to assay in solution. The actin filament in the condition also demonstrated two-times faster velocities than those without mutants. Furthermore, the movement was not intermittent but continuous. Neither intact nor NEM-treated HMM demonstrate any acceleration. These results suggest that the attaching of G680V myosin molecules to an actin filament might alter the sliding capacity of the filament.

1P197 鞭毛中心構造による軸糸直径調節を通じたダイニンの活性制御機構

Flagellar central structures regulate the dynein motor activity through the change of axonemal diameter

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Eukaryotic flagellar movements are based on the sliding between microtubules (MTs) powered by dynein. Dynein is thought to be regulated by a central pair and radial spokes, but the mechanism is not understood. *Chlamydomonas* mutants lacking the flagellar central structures are non-motile, but the mutant axonemes display beating in the presence of ATP and various kinds of salts. Here we performed X-ray fiber diffraction studies to examine the structural base for the motility. We found that the diameters in mutants were smaller than that in wild type (~185 nm) by 2-3 nm, and that the diameters increased by 1-2 nm under the motility recovery conditions. This suggests that the central structures regulate the axoneme diameter to induce the effective dynein-MT interactions.

1P198 分裂酵母の細胞質分裂における単量体型 II 型ミオシンの局在と機能

Localization and function of a monomeric myosin-II during cytokinesis in fission yeast

Masak Takaine, Osamu Numata, Kentaro Nakano (*Grad. Sch. Life & Env. Sci., Univ. of Tsukuba*)

During cytokinesis in many eukaryotic cells, myosin-II concentrates at the equatorial cortex with F-actin and is supposed to generate forces to divide the cell into two, which is called the contractile ring (CR) hypothesis. However, molecular details of the actin-myosin-II interaction are still unknown. To address this issue, we used fission yeast *S. pombe* as a model system and investigated the localization and function of a monomeric myosin-II, Myo3. Myo3 localized only to the CR depending on F-actin at late anaphase. Modification of the Myo3's motor activity altered its accumulation kinetics and local concentration in addition to its function. These results suggest that motor activity itself regulates myosin-II's localization during cytokinesis.

1P199 超解像光学顕微鏡による、成長円錐のアクチンの可視化解析

Acitn meshwork in the growth cone revealed with superresolution

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Super-resolution microscopes (STED, SIM, PALM, STORM etc.) reveal fine structures smaller than resolution limits of conventional optical microscopy. We, therefore, applied STED and SIM to observe actin meshwork in the growth cones.
A growth cone is an enlargement of thin cytoplasm at the tip of growing neurite. The growth cone crawls about and finds the path of neuronal elongation. Their movements depend on the dynamics of actin filaments (actin meshwork) which is too small to observe with conventional optical microscope.
STED and SIM revealed actin meshwork in the growth cones with resolution of 40nm in STED and of 100nm in SIM. Moreover, we recorded the actin dynamics as movies. Present hypothesis of growth cone advance will be directly examined in the presentation.

1P200 棘皮動物コラーゲン性のキャッチ結合組織を軟化させる新規タンパク質因子

A novel protein factor softening echinoderm collagenous catch connective tissues

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Catch connective tissues shown in echinoderm animals such as sea urchins, sea stars, and sea cucumbers can extensively and reversibly change their stiffness in a few minutes under nervous control. There are some granule-containing cells in the tissues, which are assumed to secret some factors under the nervous control causing changes in the tissue stiffness. The tissues contain a large amount of the extracellular matrix mainly consisting of collagen fibrils. It seems that cross-linking between the fibrils are formed or broken during the change of the stiffness of the tissues. Here, we purified a novel protein factor from an extract of sea cucumber body wall dermis, one of the known catch connective tissues, which softens the detergent-treated cell-free dermal pieces.

1P201 真正粘菌の間欠的な細胞運動時にみられる細胞骨格構造の形成と破壊のダイナミクス

Formation and destruction of cytoskeletal structure during intermittent locomotion of the true slime mold, Physarum polycephalum

Seiji Takagi (*RIES, Hokkaido Univ.*)

Plasmodium of the true slime mold, Physarum polycephalum, is a large amoeboid unicellular organism, and is known to perform intelligent behaviors such as maze solving and optimal transportation network design. These behaviors are achieved based on the amoeboid movement. Although such a cell motility is generated by self-assembled contractile proteins, long time observation of the cytoskeletal structure in a migrating plasmodium has not been performed yet. In this study, using polarized light microscope (LC-Pol scope) which can analyze all orientations of the birefringence axis, we observed the cytoskeletal structure. The formation and destruction of the structures accompanied by the intermittent locomotion will be reported.

1P202 アクチン-コフィリン相互作用の一分子解析

Analysis of Cooperative Cofilin-Actin Filament Interactions examined at the single molecule level

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ADF/cofilin proteins bind to F-actin in a cooperative manner, and modulate actin dynamics by severing the F-actin. We have directly evaluated the thermodynamic parameters (on-rate, off-rate, and cooperative parameter ω) associated with cooperative cofilin binding to F-actin by single molecule imaging techniques. The initial non-contiguous cofilin bindings facilitated additional cofilin bindings in the vicinity (300 nm) of an initial cofilin binding site, indicating that the cooperative binding of cofilin to F-actin was directly observed. Gausian fitting of the fluorescence intensity centroid revealed that the additional cofilin binding was facilitated in the vicinity (ca. 60 nm) of the initial binding site.

1P203 アクトミオシン収縮運動の制御機構;α-カテニンの阻害作用を中心に

Inhibition of actomyosin contractility by α-catenin, a component of adherens junction

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α-Catenin forms a complex with β-catenin and E-cadherin in cell-cell adherens junctions and plays an essential role as an anchor, directly or indirectly, of E-cadherin-mediated adherens junctions and the cytoskeletal F-actin networks. Biochemical studies suggested that the dimer of α-catenin was is formed by the dissociation from the complex,bound binds to F-actin with micromolar affinity, and bundles it. In the present study, we report that α-catenin inhibits actomyosin contraction in the dose-dependent manner,which was characterized by an in vitro actin-filament motility assay and the measurement of ATPase activity with myosin II HMM.Herein, we will discuss how α-catenin inhibits actomyosin contraction and the implications of that in for maintaining adherence junctions.

1P204 アクチンフィラメントがつくる二次元ネットワーク構造とその動態

Two-dimensional network pattern of actin filaments: Structure and dynamics

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Stress fibers are cytoskeletal actin bundles essential for cell motility and morphology. Stress fibers are mainly composed of actin filaments, myosin motors, and the F-actin bundling protein α-actinin, but the self-assembly mechanisms are poorly understood. Here we restricted the movement of F-actin on the glass surface by polyethylene glycol and observed spontaneous formation of actin bundles by the addition of α-actinin. We found that the network pattern drastically changed depending on the surface density of F-actin and the bulk concentration of α-actinin. The network pattern was disassembled by myosin in the concentration-dependent manner. We show the dynamic structure of actin network patterns and discuss the assembly mechanisms of stress fibers.

1P205 有糸分裂中期に観察される染色体振動の解析

Analysis of chromosome oscillation during metaphase

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Accurate chromosome segregation during mitosis is critical to prevent severe developmental defects. There is a process that chromosomes are aligned at spindle equator. In this process, chromosomes move back and forth at spindle midzone. This phenomenon is called chromosome oscillation. There are remaining questions, such as: “What determines the oscillatory property?” The dynamics of chromosome oscillation is thought to be generated by balance of polar ejection force and poleward force. To examine mechanisms of chromosome oscillation, we externally applied physical forces to mitotic Retinal Pigment Epithelial cells and perturbed the balance of forces in the mitotic spindle. We discuss the current progress in the research on the mechanism of chromosome oscillation.

1P206 引っ張り刺激による細胞シート中のアクチンフィラメント再編成
Actin filament remodeling in cell-sheet by mechanical stretch

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Focal adhesions and stress fibers have been the targets of intensive studies in mechano-sensing. These structures, however, are not observed in cells cultured on soft substrates. Here we report a cell-sheet based assay. The cells in the cell-sheet are sustained by cell-cell junctions, allowing us to examine the mechano-sensing with minimal influence from the substrate. We observed cell-sheets of fibroblasts expressing RFP-actin and GFP-tagged proteins under a real-time confocal microscope. Mechanical stretch is applied, while the cells are not attached to the substrate. This method enables us to study the responses to mechanical stretch mediated through adherens junctions in conditions that mimic the cellular environment inside soft tissues *in vivo*.

1P207 パターン化モデル生体膜へのロドプシンの再構成
Reconstitution of rhodopsin into a micropatterned model biological 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 Rh*-Gt complex has a higher affinity toward lipid rafts (raftophilicity) compared with Rh (*Hayashi et al*. J. Biol. Chem (2001) 276, 20813-16). We are currently developing a methodology to quantitatively evaluate the raftophilicity of Rh by using a micropatterned model membrane with controlled phase separation of lipid ordered (*l*o) and lipid disordered (*l*d) domains. We incorporated Rh into the micropatterned membrane by rapidly diluting solubilized Rh. We observed that incorporated Rh molecules were mobile. We discuss on the reconstitution process and localization of Rh and Rh*-Gt complex in *l*o and *l*d domains to elucidate their raftophilicity.

1P208 浸透圧を変化させた時の架橋脂質膜の振る舞い
Behavior of a suspended lipid membrane under varying osmotic pressure

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We are developing nanobiodevices that consist of artificial lipid membranes containing functional membrane proteins: the membrane is suspended over a microcavity which contains cytoplasmic fluid. Membrane stress is one of the most important factors controlling the function of certain membrane proteins. Here, we describe the behavior of a suspended lipid bilayer under conditions of varying osmotic pressure. Upon diluting the external solution, a phenomenon akin to blebbing in apoptosing cells was observed: a bud formed in the bilayer as a result of the increased osmotic pressure inside the cavity. In some cases budding-off from the bilayer was observed, forming a small vesicle containing the internal solution from the cavity, in a process akin to endocytosis in cells.

1P209 pH 転換による GUV への効率的・選択的タンパク質封入
Efficient and selective entrapment of protein into GUV by converting pH over the pI

Kanta Tsumoto (*Grad. Sch. Eng., Mie Univ.*)

Incorporation of proteins into GUVs with the functions active is an important step for studies of artificial cell systems. Here, I show a simple method with which proteins could be efficiently entrapped with charged GUVs generated by gentle hydration; hydration of the lipid films at the pH below the pI of the target proteins (BSA, MG, LZM; positive) can enhance associations between DOPC/DOPG (negative) membranes and the proteins, and then by converting the pH to be above the pI the protein can be dissociated from the membranes leading to at most 10-fold efficient (and selective) entrapment. The efficiencies depended on lipid composition, salt concentration and temperature, implying that association and closure processes may be determinant.

1P210 中性子非鏡面散乱法による糖脂質膜の力学特性の解明
Mechanics of Glycolipid Membranes Probed by Off-Specular Neutron Scattering

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We investigated the effect of glycolipid Gb3 on the mechanics of the phospholipid bilayer by using the technique of specular/off-specular neutron scattering. Multi-stacked planar membrane of Gb3 molecules with deutrated alkyl chains is formed on the Silicon substrate. The reflectivity is measured with respect to scattering vector components parallel and perpendicular to the substrate. This profile is calculated by taking membrane displacement correlation function into account under Born approximation [N. Lei, et al., J. Phys. (2003); E. Schneck, et al., Phys. Rev. E (2008)], which yields the bending rigidity and the compression modulus of the membrane. In the presentation, we discuss the effect of the molecular structure on the mechanical property of the membrane.

1P211 タンパク質内包リポソームの浸透圧下における構造
Structures of liposome encapsulating proteins under the osmotic pressure

Ryota Kimura, Mitsuhiro Hirai (*Graduate School of Engineering, Gunma University*)

By using synchrotron radiation small-angle and wide-angle X-ray scattering, we studied the structural characteristics of liposomes entrapping proteins. The aim of this study is to clarify structure of liposomes entrapping proteins under osmotic pressure. LUVs were prepared as follows. The lipid mixtures dissolved in protein solutions in 10 mM HEPES buffer at pH 7.5 were subjected to natural swelling, ultra-sonic dispersion, freeze-thaw, extrusion molding, and spin-filtration, successively. The protein occluded in liposomes was myoglobin. The mixture containing glycosphingolipid shows the structural transition from a uni-lamellar vesicle to a double-layered vesicle by the rise of osmotic pressure. However, Structure of the liposome encapsulating protein did not change.

1P212 界面通過法で作製したジャイアントリポソームのラメラリティーの定量的解析

Measuring the lamellarity of giant liposomes prepared by inverted emulsion method

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The inverted emulsion method of liposome formation is pushing water-in-oil droplets through the water/oil interface. This method is efficient for encapsulating proteins and is thus widely used for modeling cells. However, the lamellarity of liposomes prepared by this method has not been evaluated quantitatively (e.g., dependency on lipid composition). Here we prepared fluorescently labeled liposomes and analyzed the fluorescence intensity of the membrane of individual liposomes under the microscope. By comparing the intensities of the membrane before and after quenching of the fluorescent dye on the outermost monolayer and monitoring the permeability of the inner and the outer buffers in the presence of hemolysin, we concluded that >90% of liposomes were unilamellar.

1P213 細胞毒性を有する酸化コレステロールのホスファチジルコリン二分子層膜内存在位置

Locations of cytotoxic oxysterols in phosphatidylcholine bilayer membranes

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Some types of oxysterols are cytotoxic. For example, 7β-hydroxycholesterol (7β-OH) and 25-hydroxycholesterol (25-OH) have been reported to be associated with neurodegenerative diseases. In this study, we have explored the locations of these oxysterols in in phosphatidylcholine (POPC) bilayers. It was found that 25-OH is located more near the center of the POPC bilayers in comparison with normal cholesterol. On the other hand, 7β-OH is located more near the polar POPC headgroup regions. We estimated these locations from the combination of the X-ray diffraction data and the molecular volume data obtained by the D_2O/H_2O flotation method. We will compare these results with those of a recent MD simulation study (*JACS*, 131 (2009) 4854).

1P214 合成セラミド 2 の相挙動及びコレステロールとの相互作用

Phase Behavior of Synthetic Ceramide2((2S,3R)-2-Octadecanoylaminooctadecane-1,3-diol) and Its Interaction with Cholesterol

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Stratum corneum plays an important role in the barrier and moisture retention function of the skin. The synthetic ceramide2 (Cer2) has a similar chemical structure to ceramide2 that is the main ceramide component of stratum corneum. In this study, to elucidate the role of ceramide in the stratum corneum, we investigated the structural changes and phase behavior of Cer2. We found that Cer2 converts to a stable phase from a metastable phase by incubating just below of melting temperature of Cer2. Such stable phase of Cer2 has not been taken into consideration in previous studies on Cer2 system. Thermal behavior of Cer2/cholesterol now is under investigation, paying attention to the existence of stable phase of Cer2.

1P215 低い pH が誘起する DOPS/MO 膜の液晶相からキュービック相への相転移の初期過程

Initial Step of Low pH-Induced Lamellar to Bicontinuous Cubic Phase Transition in Dioleoylphosphatidylserine/Monoolein

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We investigated the initial step of low pH-induced L_α to Q_{II}^D phase transition in mixed lipid membrane of dioleoylphosphatidylserine(DOPS)/monoolein using small angle X-ray scattering with a stopped flow apparatus. We observed that the L_α phase of MLV converted 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 depended on final pH and DOPS concentrations. We also found the difference in the L_α- H_{II} transition between non-filtered MLV and filtered one through 1μm pores. The results indicate L_α phase of large MLV converted into H_{II} phase fast, while that of small MLV did slow. The rate of the small MLV might be controlled by the size growth of lipid particles.

1P216 抗菌ペプチド・マガイニン２が誘起するポア形成に対する脂質膜の力学特性の効果

Effects of Mechanical Properties of Lipid Membranes on Antimicrobial Peptide Magainin 2-Induced Pore Formation

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Antimicrobial peptide magainin 2(mag) forms pores in biomembranes to induce leakage in cells, which is a main cause of its bactericidal activity. Here we investigated the effect of mechanical properties of lipid membranes on the rate constant (k_p) of mag-induced pore formation using the single GUV method. Lower tension induced pore formation in 40%diC12:0-phosphatidylglycerol(DLPG)/60%diC13:0-phosphatidylcholine(DTPC) than in 40%DOPG/60%DOPC membranes. On the other hand, k_p values in 40%DLPG/60%DTPC-GUVs were much larger than those of 40%DOPG/60%DOPC-GUVs (e.g., 4.8×10^{-2} and 1.6×10^{-2} s^{-1} for 30 μM mag, respectively). In contrast, presence of high concentration of cholesterol inhibited the mag-induced pore formation. We discuss the mechanism of these results.

1P217 張力による脂質膜のポア形成の速度定数に対する静電相互作用の効果

Effects of Electrostatic Interactions on Rate Constants of Tension-Induced Pore Formation in Single GUVs

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When external forces are applied to cells, tension is induced in biomembranes. We investigated the rate constant (k_p) of tension-induced pore formation in lipid membranes of GUVs, which were analyzed using the mean first passage time [1]. Here we investigated effects of the electrostatic interactions. k_p values of 40%dioleoylphosphatidylglycerol (DOPG)/60%dioleoylphosphatidylcholine(DOPC)-GUVs were larger than those of DOPC-GUVs (e.g., 1.9×10^{-2} and 2.9×10^{-3} s^{-1} at tension 7.0 mN/m). The decrease in salt concentration increased k_p values of 40%DOPG/60%DOPC-GUVs (e.g., at 5.0 mN/m, 1.9×10^{-2} and 1.7×10^{-3} s^{-1} in 0 mM and 150 mM NaCl). These data indicate that k_p increases as the electrostatic interactions increase.

[1] *Langmuir*, 29, 3848, 2013

1P218 細胞侵入ペプチドであるトランスポータン 10 の脂質膜透過はポア形成の前に起こる
Permeation of Cell-Penetrating Peptide Transportan 10 through Lipid Membranes before Pore Formation

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The elementary process and the mechanism of permeation of cell-penetrating peptide transportan 10 (TP10) through lipid membranes are not well known. Here we investigated the permeation of carboxyfluorescein(CF)-labeled TP10 through lipid membranes and the leakage of water-soluble fluorescein probe Alexafluor (AF) in 20%DOPG/ 80%DOPC-GUVs simultaneously using the single GUV method with confocal microscopy. We found that CF-TP10 entered into the inside of GUV from the outside before starting leakage of AF, i.e., the pore formation. The fraction of entry of CF-TP10 among all the observed GUVs increased with an increase in CF-TP10 concentration. Through the TP10-induced pores, larger molecules such as dextran 10K and lipid vesicles passed through the membranes.

1P219 Interaction Of Warm-Sensing Chemical Capsaicin with the Biomimetic Membranes

Neha Sharma, Pooja Gusain, Tsuyoshi Yoda, Masahiro Takagi (*Japan Advanced Institute of Science and Technology*)

Capsaicin, one of main components of chilli peppers produces warming sensation. It is used in pain relievers, topical ointments. It specifically interacts with TRPV1 membrane receptor and depletes a substance which is transmitter of pain and heat. However, the non-specific interaction of Capsaicin and lipid bilayers are not negligible.
Here, cell-sized liposome was used to investigate thermosensitive response of Capsaicin-containing membranes. It was shown that capsaicin-containing membranes are more thermosensitve than control at high temperature range. This could be that the hydrophobic interaction between the non-polar tail groups of lipid membrane and Capsaicin. These findings are useful to understand mechanism behind the effect of capsaicin on biological membranes.

1P220 Dynamic Response of Menthol on Thermo-Induced Cell Membrane: More than Receptors

Pooja Gusain, Neha Sharma, Tsuyoshi Yoda, Masahiro Takagi (*Japan Advanced Institute of Science and Technology*)

It is important to understand the physicochemical mechanism that is responsible for the morphological changes in the cell membrane in the presence of various stimuli and sensory compounds. Menthol, popularly known for its cooling effect, activates TRPM8- a cold activated thermoTRP ion channel. We are interested in direct interaction of menthol with bio-membrane.
In this study, we demonstrate how menthol affects the dynamical movement of thermo-induced membrane and reveal uncleared mechanism behind cooling sensation of menthol. Hence we used cell-sized liposomes to study the cell dynamics. We found that menthol containing liposomes are more thermo-sensitive. This can be explained by hydrophilic interaction between menthol with head group of lipid molecules.

1P221 急速緩衝液交換法による時間分解全反射赤外分光法の開発
Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode

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Attenuated total reflectance (ATR)-FTIR spectroscopy has been widely used to probe protein structural changes under various stimuli. Here we developed a novel, rapid buffer-exchange system for time-resolved ATR-FTIR spectroscopy to monitor the ligand- or ion-binding reaction of a protein. By using the step-scan mode (time resolution; 2.5 ms), we confirmed the completion of the buffer-exchange reaction within ~25 ms; the process was monitored by the infrared absorption change of a nitrate band at 1350 cm^{-1}. We also demonstrated the anion-binding reaction of a membrane protein, *Natronomonas pharaonis* halorhodopsin (*p*HR). The formation of chloride- or nitrate-bound *p*HR was confirmed by an increase of the retinal absorption band at 1528 cm^{-1}.

1P222 哺乳類 two-pore 型カリウムチャネル TWIK-1 の全反射赤外分光解析
ATR-FTIR spectroscopic analyses on a mammalian two-pore domain potassium channel, TWIK-1

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A mammalian two-pore domain potassium channel TWIK-1 (or KCNK1, K2P1) contributes to generate a "leak" K^+ conductance that is essential for the resting membrane potential. TWIK-1 and other two-pore domain channels possess two selectivity filter (pore) domains per subunit and function as a dimer, although conventional potassium channels have one pore domain per subunit and function as a tetramer. Interestingly, TWIK-1 can permeate Na^+ under some conditions whereas other mammalian potassium channels are highly K^+-selective. The mechanism underlying the unique property is unclear whereas crystal structure of TWIK-1 was solved. In this study, we carried out ATR-FTIR spectroscopic analyses on TWIK-1 with an aim to reveal how the channel accomplishes the unique function.

1P223 有効電場中におけるグラミシジン A を含んだ脂質二重層膜の静電ポテンシャルと圧力特性
Electrostatic potential and lateral pressure profile of lipid bilayer containing gramicidin A in effective electrostatic field

Hiroaki Saito, Kazutomo Kawaguchi, Hidemi Nagao (*Kanazawa University*)

The linear peptide gramicidin A (GA) forms an ion channel specific for monovalent cation and has been extensively used to study the organization, dynamics and function of GA channels. On the other hand, the electric field and lateral pressure of the membrane influences the permeability of polar small molecules and ions across the membrane. In this study, we carry out a molecular dynamics (MD) simulation of a GA in the DMPC lipid bilayers to investigate the structure of GA/DMPC membrane and the ion selectivity of the GA channel. We provide the electrostatic potential maps of the GA/DMPC membrane in the effective electrostatic field. The lateral pressure profile across the membrane is estimated, and the mechanism of ion permeation of the GA in the membrane is discussed.

1P224 細菌機械受容チャネル MscS のリポソーム膜上での配向
The orientation of MscS in liposomal membranes

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The mechanosensitive channel of small conductance (MscS) plays a crucial role in the protection of cells against hypoosmotic challenge. Despite many studies of MscS reconstituted into liposomes the channel orientation in liposome patches still remains unknown. Here we examined the orientation of MscS in liposomes using its previously determined electrophysiological and pharmacological properties and found that in liposomes MscS retains the right-side-out orientation as in giant spheroplasts based on the following evidence: 1) I-V curves exhibited rectification, 2) MscS showed a voltage-dependent hysteresis, and 3) trifluoroethanol caused inactivation. These findings indicate that the cytoplasmic (CP) domain of MscS faces the CP side in liposomes as in native membranes.

1P225 シグナル分子クラスターの再構成とイメージング
Reconstitution and imaging of signaling molecule clusters

Yoshihisa Kaizuka (*National Institute for Materials Science*)

In signal transduction processes, many signal proteins form complexes in cells, ranging from multimeric oligomers to submicron or micron sized clusters. To investigate how signaling is regulated in such clusters, we reconstitute clusters of signal proteins in vitro and analyze them by direct imaging of single clusters. As a model system, we study T cell receptor proximal signaling that involves many kinds of molecular clusters in cell membranes and that can be reconstituted biochemically in artificial lipid bilayers. By imaging reconstituted clusters and reactions of receptor-kinase-phosphatase network of T cell on planar lipid bilayers, we can analyze the receptor phosphorylation states regulated by differentially clustered proteins.

1P226 １分子観察によるシグナル伝達分子Ａｋｔの作動機構解明
Single-molecule imaging study of signal transduction mechanism on Akt

Hideaki Yoshimura, Takeaki Ozawa (*Dep. Chem. Sch. Sci. the Univ of Tokyo*)

Akt is a hub molecule of signal transduction in mammalian cells. Upon cells receive external signal through a receptor and generate PIP3 on the plasma membrane, Akt is recruited to the plasma membrane through interacting with PIP3. Then Akt is activated and phosphorylate downstream molecules. Though Akt transmits various kinds of signals, the transmission mechanism without signal crossing is unknown. The dynamics of Akt on plasma membrane should be important to understand the mechanism because the whole signaling events from receiving external signals to phosphorylation of downstream molecules take place on the plasma membrane. In this presentation, we show single-molecule imaging of Akt in living cells and will discuss the mechanism of Akt signal transduction.

1P227 海洋細菌 *Vibrio alginolyticus* 新規アミノ酸走性トランスデューサーの同定
Identification of a novel transducer for amino acid taxis in the marine bacterium *Vibrio alginolyticus*

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Vibrio alginolyticus, a Gram-negative marine bacterium, has two types of flagella, the polar flagellum (Pof) and the lateral flagella (Laf). In liquid, a rod-shaped swimmer cell is propelled by Pof, whereas on a surface, the cell differentiates into an elongated swarmer cell with numerous Laf. These cells respond differently to cysteine: the former is attracted well but the latter is not. Among homologs of methyl-accepting chemotaxis protein (MCP), which we name MCP-like protein (MLPs), Vamlp9 was found to mediate attractant responses to amino acids, including cysteine. The expression of Vamlp9 in swarmer cells did not enhance their cysteine responses, suggesting the involvement of additional factor(s), such as a periplasmic binding protein, in cysteine taxis.

1P228 走化性レセプター発現で大腸菌の内膜に生じる形態変化の急速凍結レプリカによる観察
A quick-freezing replica study on morphological changes in the bacterial inner membrane induced by chemoreceptor expression.

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) are receptor proteins that span bacterial inner membrane with two transmembrane domains per subunit. MCP dimers assemble into large clusters consisting of thousands of copies. For understanding the molecular organization of the MCP cluster within the inner membrane, an *Escherichia coli* strain HCB436 that lacks all MCPs was transformed to express a single chemoreceptor (Tar) and the resulting cells were examined with quick-freezing replica electron microscopy. On fractured cytoplasmic faces (P-faces) of inner membranes were observed densely studded intramembrane particles (IMPs). Changes in the distribution of IMPs, such as quasi-crystalline arrays of IMPs and IMP-free domains, were examined in the observed images.

1P229 シグナル伝達分子間のクロストークを使った鞭毛の回転方向制御
Control of bacterial flagellar rotation via crosstalk from a non-cognate histidine kinase to the response regulator CheY

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Escherichia coli has various two-component regulatory systems, consisting of a histidine kinase (HK) and a response regulator (RR). In chemotaxis, CheA (HK) and CheY (RR) play a central role in the control of flagellar rotation: binding of phospho-CheY to the flagellar motor induces its clockwise (CW) rotation. An in vitro study showed HKs transfer their phosphoryl groups to non-cognate RRs (Yamamoto et al., 2005). Here we examined in vivo whether the activity of CheY can be regulated by non-cognate HKs. A nonchemotacitc strain co-expressing DcuS (HK) and CheY, but not CheA, rotated its flagella with higher CW biases than the strain expressing CheY alone. Thus, crosstalk from DcuS to CheY appears to occur in vivo, providing a novel system to regulate flagellar rotation.

1P230 覚醒状態の維持を担う視床下部オレキシンニューロンの同期的活動

Synchronous activity of orexin neurons in the lateral hypothalamus

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How does the brain sustain wakefulness? Orexin neurons, located in the lateral hypothalamus (LH), are thought to be essential for the maintenance of wakefulness. Although their projections to other regions are systematically investigated, the functional connections within the LH are poorly understood. To visualize selectively orexin neuron activity, we generated cre-inducible viral vector for the Ca^{2+} indicator GCaMP7 and injected it into the LH of orexin-cre mice. Orexin neuron-specific Ca^{2+} imaging revealed that orexin neurons fired synchronously with each other and had a response to orexin in a dose-dependent manner. Our results suggest that there are excitatory connections between orexin neurons, thereby contributing to the maintenance of wakefulness.

1P231 フェムト秒レーザー神経突起切断による神経回路網の自発活動の時空間ダイナミクス

Spatio-temporal dynamics of spontaneous activity in living neuronal network by femtosecond laser-induced cutting of neurites

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Understanding functional connectivity in neuronal network is essential to elucidate information processing in brain system. We evaluated spatio-temporal dynamics of spontaneous activity in living neuronal network cultured on multi-electrode arrays (MEAs) by femtosecond laser-induced cutting of neurites. When a femtosecond laser was focused on neurites of hippocampal neurons, they were cut at the focal point. Calcium imaging of fluo-4 in neurons showed rapid increase of fluorescence intensity after laser cutting. After neurons cultured on MEAs were taken off along laser scanning line, the synchronization of spontaneous activity in neurons drastically decreased over the divided area along the line, indicating diminution of functional connectivity in neuronal network.

1P232 アルツハイマー病 *in vitro* モデルを用いたアミロイドβ(1-42)伝搬毒性の評価

Evalution of Amyloid β(1-42) toxicity propagation using Alzheimer's disease *in vitro* model

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The Alzheimer's disease(AD) is the most famous cause of dementia in people of advanced age. AD is disorder which induces reduction of cognitive function of infected individual with time and deteriorates the quality of life(QOL). The exerting harmful effect on central nervous system is not well understood so far. And so in this study, we aimed at evaluation of pathogenic effect of infected neuronal cell to co-cultured normal neuronal cell. We developed co-culture device in which we can culture different types of neuronal cells separately.
The neurits of Neuronal cells were observed by immunostaining. Feasibility of the device was evaluated by immunostaining and measurement of extracellular potential was demonstrated.

1P233 前脳基底部の刺激によるラット前頭葉での応答

Response of rat frontal neuronal activity evoked by stimulation of the basal forebrain

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The projection from the basal forebrain to the frontal cortex has been implicated in a variety of cognitive functions such as memory and attention. This projection is thought to arise largely from cholinergic neurons in the basal forebrain. However, the spatiotemporal dynamics of neuronal ensemble activity in the frontal cortex after stimulation of the basal forebrain are not well understood. Therefore, we visualized the spatiotemporal dynamics of frontal activity evoked by electrical stimulation of the basal forebrain by using neurophysiological technique and voltage-sensitive dye imaging technique in anaesthetized rats. As a result, even single-pulse stimulation of the basal forebrain elicited a widespread response in the frontal cortex.

1P234 緩徐不活性化カリウムコンダクタンスが嗅周囲野 35 野の情報伝達を制御する

Slowly inactivating potassium conductance controls transmission at area 35 of perichinal cortex: VSD imaging study

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The perirhinal cortex (PC) and entorhinal cortex (EC) act as the link between the neocortex (NC) and hippocampus (HP). Area 35 of the PC is thought to act as a gate of the neuronal signal. Here, we examined the neuronal mechanism of the gate by visualizing the neural signals with voltage-sensitive dye (VSD) imaging methods. Upon a 40Hz stimulation, area 35 showed transient depolarization with subsequent inactivation of 300ms. We hypothesized that the inactivation is controlling the gate and is mediated by slowly inactivating potassium conductance (ID). Hence, we used 4-aminopyridine (4-AP) to block ID. In the presence of 4-AP, area 35 showed prolonged depolarization. We propose that the ID of area 35 neurons contributes to the gate function of the PC.

1P235 Analysis of related molecules to synchronous activity of rat cultured neuronal networks

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The molecules involved in the generation and maintenance of synchronous activity during long-term development of cultured neuronal networks remain unclear. In this study, we focused on neuron-specific gene expression and investigated the generation of synchronized bursts over a 35 day culture of rat cortical neurons. Multielectrode array-based recordings showed that synchronized bursts were observed starting at 14 days in vitro (DIV). Gene expression analysis revealed that Arc gene expression was not observed at 1-7 DIV, but the gene was expressed from 14 up to 35 DIV. Furthermore, the first expression of the Arc gene occurred at the same culture DIV as the generation of synchronized bursts under this culture condition.

1P236 内モンゴルエジノル塩湖から単離された halorubrum 属菌の持つロドプシン類タンパク質遺伝子の同定

Identification of microbial rhodopsin genes from a halorubrum species isolated from Ejinoor salt lake in Inner Mongolia of China

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Microbial rhodopsins are photoactive proteins that use retinal molecule as the photoactive center and have been studied intensively as an excellent model system for structural biology. We have identified the new microbial rhodopsin genes from halorubrum species isolated from Ejinoor salt lake in Inner Mongolia. Pairs of PCR primers targeted bacteriorhodopsin (bR) gene or phoborhodopsin (pR) gene were made. We got the full-length sequence of bR-like gene by genome walking. The deduced amino acid sequence has 87% of identity with that of H.s. All the amino acids important for the proton pumping are conserved. The cloned pR homolog showed 60% of identity with that of H.s. in deduced amino acids sequence.

1P237 ニワトリクリプトクロム 4 の光反応特性の解析

Spectroscopic characterization of Chicken Cryptochrome4

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Cryptochromes (CRYs) are flavoproteins suspected to function as blue-light photoreceptors, and they have been found in bacteria, plants, and animals. The phototransduction pathway and physiological function of vertebrate CRYs are almost unknown, although circadian repressor function is extensively studied. In this study, we investigated the photic reaction of recombinant chicken CRY4 (cCRY4) by UV-visible spectroscopy. Blue-light irradiation changed dark-adapted state of cCRY4 probably having the oxidized FAD chromophore to semi-reduced or further fully-reduced photointermediate that are reoxidized to the oxidized state in the dark. The dark reaction and effect of a reducing agent dithiothreitol was investigated to characterize the photointermediates.

1P238 ゼブラフィッシュクリプトクロム 1a の発現・精製

Expression and purification of zebrafish cryptochrome 1a.

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Cryptochromes (CRYs) function as blue light photoreceptors in bacteria, plants, invertebrates, and non-mammalian vertebrates. In order to understand molecular properties of CRYs in lower vertebrates, we focused on zebrafish cryptochromes, the physiological function of which remains elusive. We expressed a HAT-tagged zebrafish CRY1a (zCRY1a) in *Saccharomyces cerevisiae* exogeneously and zCRY1a was purified by using immobilized metal ion affinity chromatography. The purified zCRY1a protein did not show characteristic spectral feature indicating flavin binding, but it bound with flavin adenine dinucleotide (FAD) when FAD was added. This novel system will enable us to investigate photic reaction of zCRY1a by UV-visible spectroscopy.

1P239 近赤外ラマン円偏光二色性分光による光受容タンパク質の活性部位構造解析

Active Site Structure of Photoactive Yellow Protein with a Locked Chromophore Analog Revealed by Near Infrared Raman Optical Activity

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Raman optical activity (ROA) is expected to yield a wealth of stereo-chemical information about conformational details of molecules. We recently applied the near-infrared ROA (NIR-ROA) to photoactive yellow protein (PYP), which contains a p-coumaric acid (pCA) chromophore. Here, we applied the NIR-ROA to a PYP analog, which is reconstituted with a locked-pCA chromophore. Furthermore, we have performed quantum mechanical/molecular mechanical (QM/MM) calculations to analyze the observed spectra. The use of the NIR-ROA combined with QM/MM calculations is a novel and generally applicable spectroscopic tool to study the chromophore distortions within a protein environment.

1P240 共鳴ラマン分光法によるシアノバクテリオクローム RcaE がもつ開環テトラピロール発色団のプロトン化状態の解析

Protonation state of the linear tetrapyrrole chromophore in cyanobacteriochrome RcaE revealed by resonance Raman spectroscopy

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Cyanobacteriochromes (CBCRs) are cyanobacterial members of the phytochrome superfamily of photosensors and contain a linear tetrapyrrole (bilin) chromophore. RcaE from *Fremyella diplosiphon* has been shown to undergo a reversible photoconversion between a green-absorbing Pg state and a red-absorbing Pr state. A previous pH titration experiment suggested that the bilin chromophore is deprotonated in Pg but protonated in Pr (1). Here we have measured the resonance Raman spectra of RcaE in Pg and Pr states and provide spectroscopic evidence that the Pg/Pr photoconversion is associated with a protonation/deprotonation of the bilin chromophore.

(1) Y. Hirose et al. (2013) Proc. Natl. Acad. Sci. USA 110, 4-74-4979.

1P241 赤外分光法によるチャネルロドプシンとキメラチャネルロドプシンの比較解析

Comparative analysis of Channelrhodopsin and its chimeras based on FTIR spectroscopy

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Channelrhodopsin (ChR) is a light-gated cation channel, widely used to evoke the nerve and muscle action potential with the stimulation of light. We analyzed the conformational changes between the dark state and the photostationary state of ChR-2 and chimeric ChRs, using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. We have found that the FTIR difference spectra of chimeric ChRs, ChR5/2 (TM1-5: ChR1, TM6-7: ChR2) and ChR2/5 (TM1-2: ChR1, TM3-7: ChR2), are very similar, but different from that of ChR2, suggesting that the first two transmembrane is crucial to show the characteristic change of ChR1 and ChR2.

1P242 (6-4)光回復酵素における光活性化及び光修復のメカニズム

Molecular mechanism of photoactivation and photorepair of Xenopus (6-4) photolyase

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(6-4) photolyase ((6-4)PHR) is a flavoprotein that specifically revert UV-induced (6-4) photoproduct into normal bases. Recently, we reported structural changes upon photoactivation and photorepair of Xenopus (6-4)PHR using difference Fourier-transform infrared (FTIR) spectroscopy [1].
Here we study detailed molecular mechanism of photoactivation and photorepair by combination of low-temperature FTIR spectroscopy, isotope labeling and site-directed mutagenesis. Three intermediate states are detected during the repair process of (6-4) photoproduct, and the molecular mechanisms will be discussed based on the present vibrational identifications.
[1] Zhang et al. Biochemistry 50, 3591 (2011); J. Phys. Chem. Lett. 2, 2774 (2012); Yamada et al. Biochemistry 51, 5774 (2012).

1P243 大腸菌におけるチャネルロドプシン 1 の発現

Expression of channelrhodopsin-1 in *Esherichia coli*

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Channelrhodopsins (ChRs) are light-gated ion channels from green algae. Although they are utilized as optogenetic tools to induce membrane depolarization and action potentials in the cells by light, the molecular mechanism is still unclear. In this study, we tried to express several portions of ChRs with various lengths in *E. coli*. As a result, a ChR1 mutant lacking N- and C-terminal portions was successfully expressed in *E. coli* membrane and showed the absorption maximum at around 453 nm. The induction of the truncated ChR1 gene caused severe growth repression both in the culture medium and on the agar plate, suggesting the constitutive activity. Using this heterologous expression system, the ion transport mechanism in ChR1 would be investigated.

1P244 好熱性ロドプシン：高度好熱菌から初めて発見された光駆動イオンポンプ

Thermophilic rhodopsin: The first light-driven proton pump from an extreme thermophile

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So far, retinal proteins have not been discovered in thermophilic organisms. In this study, we characterized a rhodopsin derived from an extreme thermophilic bacterium, *Thermus thermophilus* JL-18, which lives in a hot spring at ~75°C. The rhodopsin named thermophilic rhodopsin (TR) was successfully expressed in *E. coli* cells and showed an absorption maximum at 530 nm. Of note, TR maintained its absorption at 75°C for more than 4 hours [1]. Additionally, TR shows the temperature-induced absorption change with a transition at around 68°C, indicating the existence of two states. Structural and photochemical properties on these states are investigated.
[1] Tsukamoto, T., Inoue, K., Kandori, H., Sudo, Y. (2013) *J. Biol. Chem.*, in press.

1P245 レーザーフラッシュフォトリシス法によるロドプシンミミックの光化学研究

Laser flash photolysis study on Photochemistry of Rhodopsin Mimics

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Rhodopsin mimics (Rho-mimics) are the artificial soluble retinal binding proteins designed from human cellular retinol binding protein II and the colors can be regulated in the range more than 200 nm (425-644 nm). Here, we studied the photochemistry of three Rho-mimics, M1, M4 and M9 whose λmaxs are at 425, 508 and 613 nm, respectively, by laser flash photolysis. In the case of M4, no transient absorption change was observed. This suggests the isomerization of retinal is strongly suppressed in M4. In contrast, M1 was unstable for light illumination and bleaching process was observed. M9 was stable for light as M4. However, it was thermally bleached at room temperature. The origin of the difference among them will be discussed in the presentation.

1P246 酸性条件で機能する蛍光タンパク質のランダム変異によるスクリーニング

Screening of randomly mutated fluorescent proteins that can work in acidic conditions

Tatsuya Iwata, Yukiko Ono, Masayo Iwaki, Hideki Kandori (*Grad. Sch. Eng., NITech*)

Green fluorescent protein (GFP) and its mutants are indispensable tools for non-destructive observation of living cells and organisms in the life science field. Recently, functional fluorescent proteins have been reported based on not only GFP but also LOV domain, which flavin-binding blue light receptor domain. Both GFP and LOV cannot function in acidic condition because the lost of fluorescence and dissociation of flavin, respectively.
Here we propose screening method of GFP and LOV mutants which are functional in the acidic condition using *E.coli* in vivo. Randomly mutated fluorescent protein candidates are expressed in the periplasmic space of *E.coli*, where light-induced proton pump, microbial rhodopsin is expressed at the inner membrane.

1P247 青色光吸収型アーキロドプシン 3 変異体による内向きプロトン輸送

Light-induced inward proton transport in a blue-shifted archaerhodopsin-3 mutant

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Archaerhodopsin-3 (AR3) functions as a light-driven “outward” proton pump to produce electrochemical gradient across the membrane. AR3 absorbs green/red light (~552 nm) and is utilized as an optogenetic tool for neural silencing. Recently we succeeded in production of a blue-shifted mutant whilst preserving the robust pumping activity and illumination of the C. elegans with blue light lead to the effective locomotory paralysis [1]. In this study, we tried to make a further blue-shifted variant. As a result, introduction of G132V lead to large spectral blue-shift (~450 nm). Unexpectedly the mutant showed light-driven “inward” proton transport activity. The characteristic feature will be confirmed and investigated.
[1] Sudo et al., (2013) J. Biol. Chem., in press.

1P248 シアノバクテリアのクリプトクロム DASH の変異体は二本鎖 CPD を修復する

Functional conversion of cryptochrome into photolyase

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Cryptochrome (CRY) and photolyase (PHR) are flavoproteins whose structures are similar, but functions are entirely different. PHR repairs UV-induced DNA lesions, while CRY is involved in photomorphogenesis, biological clock and magnetoreception. Cryptochrome-DASH (CRY-DASH) is a new type of CRY originally found from cyanobacteria. In vitro experiment showed that CRY-DASH repairs CPD photoproduct in a single-strand DNA (ssCPD), but not in a double-strand DNA (dsCPD). In this study, we introduced mutation to CRY-DASH, and a mutant protein successfully repaired dsDNA, which was monitored by light-induced difference FTIR spectroscopy. Molecular mechanism of functional conversion of CRY into PHR will be discussed.

1P249 FTIR study of isotope-labeled CPD-Photolyase

I M. M. Wijaya[1], Tatsuya Iwata[1], Tilo Mathes[2], Junpei Yamamoto[4], Kenichi Hitomi[3], Elizabeth D. Getzoff[3], Shigenori Iwai[4], John T. Kennis[2], Hideki Kandori[1] ([1]*Department of Frontier Materials, Nagoya Institute of Technology, Japan*, [2]*Department of Physics and Astronomy, VU University, The Netherlands*, [3]*Department of Integrative Structural and Computational Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, USA*, [4]*Graduate School of Engineering Science, Osaka University, Japan*)

CPD-photolyases (PHRs) are flavoproteins that repair CPD-type UV-induced damaged DNA by use of near UV/blue light. We recently reported light-induced difference FTIR spectra of light-induced activation and DNA repair of E. coli CPD-PHR[1], whose signals originate from the chromophore (FAD), apo-protein or substrate. To identify vibrational signals, we prepared 13C- and 15N-labeled CPD-PHR enzymes whose chromophore is similarly labeled or unlabeled. Molecular mechanism of activation and DNA repair of CPD-PHR will be discussed on the basis of the FTIR spectral comparison of these systems.

[1] I M. M.Wijaya et al. Biochemistry 52, 1019 (2013).

1P250 光駆動ナトリウムポンプの低温赤外分光

Low-temperature FTIR spectroscopy of a light-driven sodium ion pump

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Light-driven outward H^+ pump bacteriorhodopsin (BR) and inward Cl^- pump halorhodopsin (HR) were discovered from Halophilic archaea 35-40 years ago. While HR can pump not only Cl^-, but also other monovalent cations such as Br^-, I^- and NO_3^-, microbial rhodopsins can pump only H^+, no other cations. It may be reasonable because the chromophore (protonated Schiff base of all-trans retinal) is positively charged, so that cations cannot stay in the Schiff base region except for the covalently attached H^+. However, recent discovery of light-driven outward Na^+ pump (Inoue et al. Nature Commun. 2013) challenges such common understanding in the field. Here we report low-temperature FTIR spectroscopy of a sodium ion pump, from which molecular mechanism will be discussed.

1P251 霊長類色覚視物質の変異体に対する赤外分光研究

若手招待講演

FTIR study of mutants of primate color pigments

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We human distinguish various colors using three proteins that contain a common chromophore, 11-cis-retinal, whereas structural information of color tuning by protein is little known. We have recently reported structural analysis of monkey red (MR) and green (MG) sensitive proteins by low-temperature FTIR spectroscopy. In this study, we prepared mutant proteins (S180A, Y277F and T285A MR, and N318Q MG), and the FTIR spectra were compared to those of MR and MG. We conclude that primate red-green discrimination primarily originates from dipolar interaction of specific amino acids, rather than structural difference. In contrast, we observed water-containing hydrogen-bonding network extended to the cytoplasmic domain, which differs between MR and MG.

1P252 ＯＥＣのＳ３状態の反応活性部位の分子構造と酸化状態に関する理論的研究

Theoretical study on molecular structures and oxidation states of active site at the S3 state of OEC

Tomoya Ichino, Masaki Mitani, Yasunori Yoshioka (*Grad. Sch. Eng., Univ. Mie*)

During the catalysis of water oxidation in nature, the oxygen evolving complex (OEC) passes through five oxidation states (S0 ~ S4). At the S3 state, the oxidation states of four Mn atoms were experimentally assigned as [4 Mn(IV) ions] or [Mn(III) and 3 Mn(IV) ions]. There are also proposals that substrate instead of Mn atom is oxidized to yield an oxygen radical species. In this study, the molecular structures and oxidation states of the active site at the S3 state of OEC have been investigated using B3LYP method. It is found that the most stable state in the triplet state is not the [4 Mn(IV) ions] but the [Mn(III) and 3 Mn(IV) ions] with the outer Mn4(IV)-oxyl radical bond. The oxidation states with the Mn4(IV)-OH radical or Ca(II)-OH radical bond are unstable.

1P253 光化学系 II-電極による可視光照射下での水の酸化

Visible light-driven water oxidation by Photosystem II-immobilized electrodes

Masaru Kato, Miwa Sugiura (*Proteo-Science Center, Ehime Univ.*)

Solar light-driven water splitting into molecular hydrogen and oxygen provides a sustainable route for the production of hydrogen, which is a potentially alternative fuel. The water oxidation reaction to molecular oxygen is known as the bottleneck in the development of water splitting devices, and many water oxidation catalysts including semiconductors and coordination compounds have been developed all over the world.

A photosynthetic protein Photosystem II is the natural water oxidation enzyme and sets the benchmark in terms of oxygen evolution rate under ambient conditions for the development of water oxidation catalysts. We report our progress on the development of metal oxide-based electrodes with PSII for visible light-driven water oxidation.

1P254 **Mn 除去は光化学系 II 非ヘム鉄の酸化還元電位に影響を及ぼすか？ ーFTIR-分光電気化学計測による解析**
FTIR-Spectroelectrochemical Investigation into Whether Mn-Depletion Influences the Redox Potential of the Non-Heme Iron in Photosystem II

Yuki Kato, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

Photosystem II (PSII) drives photo-induced transmembrane electron transfer from the Mn_4Ca cluster to plastoquinone. It was previously reported that removal of the Mn ions from PSII shifts by ca. +150 mV the redox potential E_m of the primary quinone electron acceptor Q_A. However, Q_A is over 40Å distant from the Mn_4Ca cluster, and hence the structural rational behind such a long range interaction is still unknown. In this work, we applied an FTIR-spectroelectrochemical technique to study the influence of Mn-depletion on E_m of the non-heme iron, located near Q_A, to probe the long range interaction in more detail. The result showed that the E_m value shifted by +9 mV at most. A possible explanation for the long range phenomena is discussed.

1P255 **FTIR study on the functions of the extrinsic proteins in cyanobacterial photosystem II: Evolutionary aspect of extrinsic proteins**

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Extrinsic proteins in PSII regulate the reactions in the water-oxidizing center (WOC), although its molecular mechanism is unknown. We investigated the effects of the extrinsic proteins on the WOC structure in cyanobacterial PSII using FTIR spectroscopy. S2/S1 FTIR difference spectra exhibited significant changes upon removal of all the extrinsic proteins (PsbO, V, U), whereas spectral features were mostly recovered by rebinding of PsbO. The amide I bands further recovered by rebinding of PsbV, and completely recovered by PsbU. Thus, the three extrinsic proteins affect the structure of WOC in different manners. With the results of a higher plant (PsbO, P, Q) and a red alga (PsbO, V, U, Q'), evolutionary aspects of the functions of extrinsic proteins are discussed.

1P256 **光化学系 II における Y_Z ラジカルとヒスチジン間の高いプロトン分極を持つ水素結合：FTIR 法による検出**
FTIR evidence for the presence of a strong H-bond with high proton polarizability between the Y_Z radical and a His in photosystem II

Shin Nakamura, Ryo Nagao, Hanayo Nakanishi, Ryouta Takahashi, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

The redox-active tyrosine Y_Z, which is an immediate electron acceptor of the water oxidizing center (WOC) in PSII, is involved in a H-bond network from WOC to the lumen. To clarify the role of Y_Z in proton release in the water oxidation mechanism, we investigated the H-bonded structure of Y_Z using FTIR spectroscopy. A $Y_Z^{\cdot}/Y_Z$ FTIR difference spectrum showed a broad positive band around 2800 cm^{-1}, which was absent in a $Y_D^{\cdot}/Y_D$ spectrum. DFT calculation on the model complexes of Tyr and His reproduced this vibration as the NH stretch of $HisH^+$ H-bonded with a Tyr radical. It was thus concluded that Y_Z oxidation produces a strong H-bond with high proton polarizability between $Y_Z^{\cdot}$ and protonated D1-His190, which may play a crucial role in proton release during water oxidation.

1P257 **Role of NADPH oxidase in vitamin D_3 and PMA-induced cell differentiation**

Hiroyuki Kato[1], Asuka Kato[1], Omi Nawa[1], Masato Mutoh[2], Wakako Hiraoka[1] ([1]*Graduate School of Science and Technology, Meiji University*, [2]*Department of Materials and Human Environmental Sciences, Shonan Institute of Technology*)

We have studied the role of reactive oxygen species (ROS) from NADPH oxidase in the process of monocytic differentiation of myeloid leukemia PLB-985 cells. Cells were exposed to bioactive 1alpha, 25-dihydroxyvitamin D_3 (vitamin D_3) and incubated for 3 days for comparison of monocyte differentiation between normal cells and $gp91^{phox}$(-) cells (NADPH oxidase knockout). Microscopic observation showed that normal cells differentiated to monocyte. Although knockout cells were not. Expression of Mac-1alpha, one of the monocytic antigen markers, was higher in normal cells than that of in knockout cells. These results suggested that monocyte differentiation induced by vitamin D_3 required ROS generation derived from NADPH oxidase.

1P258 **メガヘルツ超音波の抗腫瘍効果**
Antineoplastic effect of MHz ultrasound to leukemia cells

Risa Fuji, Wakako Hiraoka (*Graduate School of Science and Technology, Meiji University*)

The relationship between cavitation-induced OH radical and cell killing after ultrasound irradiation was investigated in order to properly evaluate MHz ultrasound for therapeutic effects and safety. Irradiation was performed for 10 s at 1.6, 2.4, 5.4 and 7.9 MHz, respectively. Considering cell damage in normal tissue, we examined the proliferation rate of U937 cells. In addition, we examined the induction of apoptosis after irradiation by phosphatidylserine externalization. As a result, cell death was induced by direct burst and necrosis at the frequency and intensity where OH radicals were generated. On the other hand, selective apoptosis was induced in the proper condition that OH radicals were not generated.

1P259 **酸化ストレス下でのＲＯＳ検出**
ROS detection in oxidative stress

Omi Nawa, Hiroyuki Kato, Asuka Kato, Wakako Hiraoka (*Graduate School of Science and Technology, Meiji University*)

Qualitative and quantitative analysis of reactive oxygen species (ROS) lead to clarify the signal transduction of oxidative stress, which plays an important part in lots of diseases. In this study, we evaluated the method of ROS detection among the chemiluminescence using methyl-6-p-methoxyphenyl-ethynyl-imidazopyrazinone (MPEC), ESR-spin trapping using 5-(2,2-dimethyl-1,3-propoxy cyclophosphoryl)-5-methyl-1- pyrroline N-oxide and aminophenyl fluorescein as a ROS detector. We also reported the efficiency of chemiluminesce in the reactions of MPEC with superoxide, hydroxyl radical, hydrogen peroxide and nitroxide in vitro. Furthermore, each ROS detecting system was compared in PLB-985 cells treated with phorbol ester.

1P260 DNA 脱塩基部位の局在性評価法の開発と放射線照射 DNA への適用

A de novo methodology for estimating localization of apurinic (AP) sites in DNA and its application to DNA exposed to ionizing radiations

Ken Akamatsu, Naoya Shikazono (*Irradiated Cell Analysis Group, Japan Atomic Energy Agency*)

It is well-known that DNA lesions induced by ionizing radiation and chemicals can cause mutation and carcinogenesis. In particular, 'clustered damage' site, that is a DNA region with multiple lesions within a few helical turns, is believed to hardly be repaired. However, chemical and spatial details of them are not known. We have developed a methodology for estimating localization of AP sites using Förster resonance energy transfer (FRET). We have recently found that experimentally-obtained FRET efficiencies for the heat-treated AP-DNA correspond to theoretical ones calculated on the basis of a Poisson process. Now we are applying the FRET methodology to a plasmid irradiated with ^{60}Co γ-rays and $^{4}He^{2+}$ ion beam. The results and the prospective will be discussed.

1P261 シンクロトロン軟Ｘ線によって誘発されるバイスタンダー応答の機構

Mechanisms of synchrotron soft X-ray-induced bystander response

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A radiation-induced bystander response, which is generally defined as an intercellular response that is induced in non-irradiated cell that received bystander signals from directly irradiated cells within an irradiated cell population. We studied mechanisms of bystander response using soft X-rays of the resonance peak and off peak of the K-shell photoabsorption of phosphorus. When 0.4% of normal human fibroblasts were irradiated in the population, the bystander cell killing was observed at the doses of higher than 0.4 Gy, and cell survival decreased to about 85% that was not enhanced by the K-shell photoabsorption of phosphorus. Additionally, we found that bystander cell killing was mainly initiated/mediated by nitric oxide signalling pathway.

1P262 海底熱水噴出孔を模擬した新型フローリアクターの製作と化学進化

A construction of a new flow reactor simulating hydrothermal vents for chemical evolution

Eiichi Imai, Hajime Honda (*Dept. Bioengineering, Nagaoka Univ. Tech.*)

We have been focusing on the environments of hydrothermal vents in the primitive ocean for the proscenium of the chemical evolutions for a life. Recently we have proposed a newly designed flow reactor intended for simulating submarine hydrothermal vents in more faithful geometry. In the new flow reactor, a high-temperature high-pressure chamber was located at the bottom of the apparatus. The fluid at 200-350 degrees, 24 MPa was ejected upward from the bottom of the low-temperature chamber. The amount of di-glycine was maximized when the temparature of fluid was around 300 degrees which was found to be higher than previous studies. Furthermore, the reaction rate was accelerated by the presence of Basalt particles in the low-temperature chamber.

1P263 *In vitro* selection of the preferable 3'-terminal sequences of the template for norovirus RNA replicase

Hidenao Arai[1], Miho Suzuki[1], Naoto Nemoto[1], Koichi Nishigaki[1], Yuzuru Husimi[2] ([1]*Grad. Sch. Sci. Eng., Saitama Univ.*, [2]*Innovation Research Organization, Saitama Univ.*)

We have been characterizing the *in vitro* activities of norovirus RNA replicase (NV3D^{pol}) *in vitro* for the construction of an autonomous *in vitro* evolution system. NV3D^{pol} synthesizes the complementary strand from the 3'-terminus of RNA template in primer-independent manner and amplified a double-stranded RNA *in vitro*. We have found that C-stretch sequence at the 3'-terminus of RNA template was one of the preferable sequences, and in a case of ---CCCC-3', NV3D^{pol} amplifies the RNA strand about 54-fold. In this work, we attempted to identify the 3'-terminal sequences of RNA template for the most efficient initiation reaction of *in vitro* RNA replication by NV3D^{pol} using an *in vitro* selection method, excluding the self-priming sequences.

1P264 苔に擬態した蝶の翅模様にみるノイズを利用したデザイン原理

Noisy design of a butterfly wing pattern mimicking a lichen-covered tree bark

Takao K. Suzuki (*NIAS*)

Butterfly wings often display colored patterns, which play defensive roles for escape from their enemies. For an effective escape, it is hypothesized these patterns have 'good' designs to deceive enemies. Previously, we revealed a leaf mimicry pattern of a moth implements a modular structure to stabilize a fluctuation of its leaf venation pattern. However, other design principles of patterns have been poorly understood. Here, we show a design principle of a pattern mimicking lichen. First, we quantified variation of pattern elements and found its lower than that in the leaf mimicry pattern. In addition, we calculated the finely subdivided modules in the lichen pattern. Our results may suggest that these characteristics likely improve the visual trick for crypsis.

1P265 ダブルバレルカーボンプローブを用いた組織モデルからのｍＲＮＡ回収と定量評価

Collection and quantification of messenger RNA from tissue models by double barrel carbon probe

Yuji Nashimoto[1], Yasufumi Takahashi[2], Ryosuke Takano[1], Kosuke Miyashita[1], Shukuyo Yamada[1], Kosuke Ino[1], Hitoshi Shiku[1], Tomokazu Matsue[1,2] ([1]*Enviromental studies, Tohoku Univ.*, [2]*WPI-AIMR, Tohoku Univ.*)

We introduce the double-barrel carbon probe (DBCP) which enables the analysis of single cells independent of cellular positioning. The target cells were punctured by an electric pulse between the two electrodes in DBCP. The mRNA in cellular lysates were collected and quantified by qPCR. The histograms of single-cell relative gene expression were fit to a theoretical distribution. In the angiogenesis model, we evaluated multiple gene expression analysis using the mRNA collected by DBCP. Finally, we applied DBCP for the embryonic stem (ES) cell-derived cardiomyocytes to substantiate the capability of collecting cells, even from high-volume samples. This method achieves high sensitivity for mRNA at the single-cell level and is applicable in the various biological samples.

1P266 Development of Ligand Based Virtual Screening considering protein-ligand interaction

Koya Kato, George Chikenji (*Grad. Sch. Eng., Nagoya Univ.*)

Virtual screening plays an important role in the early drug discovery stage. There are two broad categories of screening techniques: ligand-based and structure-based. Both techniques have their own advantages and weaknesses and there is still much room of improvement for both techniques. Here, we developed a new virtual screening method that combines the both techniques. The method is basically based on the ligand based techniques; A candidate ligand is compared with one or more active ligand structures by the 3D geometric hashing method. Besides, it also takes into account of ligand-protein interactions. The performance of the method was evaluated against the DUD set. We will report the detailed description of the method and the results.

1P267 H-DROP:サポートベクターマシンを用いたヘリカルリンカーの予測

H-DROP: an SVM based helical domain linker predictor trained with optimal selected features

Suzuki Ryosuke[1], Ebina Teppei[2], Yutaka Kuroda[1] ([1]*Dept of Biotech. & Life Sci., Tokyo University of Agriculture & Technology*, [2]*Brain Science Inst., RIKEN*)

Domain linker prediction is the focus of interest as it can help identify novel domains suitable for high throughput analysis. Yet, most domain linker predictors focus on non-helical linkers, and there are no performant predictor for helical linker. Here, we present H-DROP, an SVM-based helical linker predictor trained with a dataset consisting of helical boundary regions between two structural domains. Optimal features were selected by combining a random forest and a stepwise feature selection protocols. The prediction sensitivity and precision of H-DROP were 35.2% and 38.8%. These values are over 10.7% and 23.0% higher than the respective values of other predictors and random prediction. H-DROP is the only efficient helical linker predictor that is currently reported.

1P268 スプライシングアイソフォームの機能的有意性の評価

Evaluation of functional significance of splicing isoforms

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Although many genes in higher eukaryotes undergo alternative splicing, functions of the majority of Splicing isoforms are not analyzed experimentally. We developed a pipeline to evaluate functional significance of splicing isoforms using three criteria: expression in the protein level, positional conservation among different species and regulation of expression in the mRNA level. About 80% of known functional splicing isoforms were judged as functionally significant, showing the validity of the pipeline. We, then, evaluated splicing isoforms losing their parts of hydrophobic core, which accounted for a large fraction of splicing isoforms in human. As a result, about 20% of them were judged as functionally significant.

1P269 タンパク質における分子トンネルの高速簡易探索法の開発ートリプトファン合成酵素への適用

A Simple Method to Detect Molecular Tunnels in Proteins - Application to Tryptophan Synthase

Midori Yano, Kei Yura (*Grad. Sch. Hum. Sci., Univ. Ocha*)

The active sites of tryptophan synthase α- and β- subunits are connected with a long molecular tunnel and the ligand is transferred through it, avoiding accidental reactions with other molecules. This tunnel was first discovered by visual inspection of the protein structure. Enormous number of known protein structures, however, prevents us from finding tunnels by visual inspection, and an automatic method to detect a tunnel is required. We, therefore, developed a new and fast method to search the whole proteins in PDB for molecular tunnels. The method is applied to different entries of tryptophan synthases in PDB. We found that the structures of the tunnel were diverse and the differences may relate to the regulation of the ligand transport between the subunits.

1P270 ｍＲＮＡ切断ポリアデニル化特異因子複合体構成サブユニットの四次構造推定

Predicting a Quaternary Structure of mRNA Cleavage-Polyadenylation Specificity Factor Complex

Saki Aoto, Kei Yura (*Ochanomizu Univ*)

A messenger RNA (mRNA) undergoes a polyadenylation process after being transcribed from DNA. The process is regulated by quite a number of proteins associated with the mRNAs, but the three-dimensional (3D) structure of the proteins and the mechanisms of the regulation remain to be elucidated. Here, we predicted a 3D structure of cleavage and polyadenylation specificity factor (CPSF), a hetero-tetramer molecule and one of the components for regulating polyadenylation of mRNA. The 3D structures of the subunits were built by comparative modeling method. We then docked the four subunits utilizing information obtained from docking software and amino acid sequence conservation. Based on the obtained reasonable quaternary structure, we predicted the mRNA interfaces of CPSF.

1P271 ヒトリン酸化部位のデータベース解析で明らかになるシグナル伝達経路間のクロストーク

Crosstalk between signaling pathways revealed by database analysis of human phosphorylation sites

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Cellular fate depends on the spatio-temporal separation and integration of signaling processes which in turn can be provided by phosphorylation events. We address this topic at both the systems and molecular levels by linking distinct phosphorylation events with their functional outcomes and signaling pathways. We find that multiple phosphosites are clustered in sequence and space, and have a coherent effect on protein stability if they share regulatory functions and regulate similar pathways. In addition we observe a strong association of tyrosine phosphorylation with activating signaling events, and activating phosphosites more extensively participate in switching the signals from different pathways than inhibitory ones.

1P272 大自由度遺伝子発現制御モデルにおける適応応答の協同的進化

Cooperative Adaptive Responses in Gene Regulatory Networks with Many Degrees of Freedom

Masayo Inoue[1], Kunihiko Kaneko[2] ([1]*molprof, AIST*, [2]*Univ. of Tokyo*)

Cells generally adapt to environmental changes to achieve homeostasis. Although simple mechanisms for adaptation through network motifs consisting of a few genes are well understood, how regulatory networks involving many genes that activate or repress each other can generate adaptive behaviors is unclear. Here, by numerically evolving gene regulatory networks, we obtained a class of genes whose expression dynamics showed adaptation over almost all genes, albeit sometimes partial adaptation, from which we revealed the general logic underlying such adaptive dynamics with many degrees of freedom, which was not reducible to common motifs with a few genes. These results have implications in the significance to the biological homeostasis of systems with many components.

1P273 細胞の集団的意思決定の設計原理

A Design Principle of Group-level Decision Making in Cell Populations

Koichi Fujimoto[1], Satoshi Sawai[2,3,4] ([1]*Faculty of Science, Osaka University*, [2]*Graduate School of Arts and Sciences, University of Tokyo*, [3]*Research Center for Complex Systems Biology, University of Tokyo*, [4]*PRESTO, JST*)

Although the gene circuits that underlie the switches are well understood at the level of single cells, the ways in which such circuits work in concert among many cells to support group-level switches are not fully explored. Here, we theoretically show two distinct forms of bistability are uniquely determined by a dimensionless parameter, which compares the synthesis and the transport of the inducing molecules. The parameter in bacterial quorum-sensing circuits appears to be tuned so that the cells can use either the cell-level or the group-level switch. Furthermore, in negative feedback circuits, the same parameter determines the group-level transitions. The design principle identified here serves as the basis for the control of cellular collective decision making.

1P274 Large deviation properties of population averages: An indicator of gene expression dynamics in a single cell

Bhaswati Bhattacharyya, Ziya Kalay (*iCeMS, Kyoto University*)

Population of genetically identical cells are heterogenous in their phenotypes. Consider a model of gene expression network where activated genes produce mRNA and protein in a sequential manner. The output data of a collection of cells give us the mean signal which contains only partial information. Instead, we calculate the sample mean for a set of sub-population of cells, obtaining a distribution whose variance and large deviation properties are characterised by the underlying gene expression network. To perform this calculation, we obtain the probability generating function for chemical master equations for three simple regulatory networks and also exactly calculate the first and second moments in the steady state.

1P275 細胞システムの内因的・外因的ゆらぎに対するロバスト性に関する理論的基礎

若手招待講演

Theoretical basis for robustness of intracellular systems against intrinsic and extrinsic fluctuation

Tetsuya Kobayashi (*IIS, Univ. Tokyo*)

Intracellular systems, even with intrinsic stochasticity in intracellular reactions, can robustly and dynamically sustain their homeostatic state by flexibly adapting to unpredictably changing environment. The underlying mechanisms how this robustness and flexibility emerge from stochastic components have yet to be revealed. In this work, I demonstrate that Bayesian and information theories can be applied for unveiling the mechanisms by reframing the problem of the robustness as efficient information transfer in noise and uncertainty. I also clarify that the network that transfer information effectively behaves as if it utilizes noise and fluctuation. This result may serve as a theoretical basis for the origin of spontaneous activity in living cells.

1P276 イノシトールリン脂質代謝系が細胞の自発運動への効果の理論と実験による検証

Theoretical and Experimental Analysis for the Effect of Phosphatidyl Inositol System on Spontaneous Cell Movement

Masato Yasui, Satomi Matsuoka, Masahiro Ueda (*Osaka University*)

We have analyzed the effect of phosphatidyl inositol system on spontaneous cell movement from single molecule to a cell level, experimentally and theoretically. In our experiment, PTEN mutants were used to modulate phosphatidyl inositol system. Then, the on and off rate of PTEN mutants were measured by using single molecule imaging to understand the mutation effect on membrane localization. Next, the localization of PTEN mutants, and spontaneous cell movement were measured. Finally, we have constructed a mathematical model, and we compared the model and experimental data (on and off rate, localization, and cell movement). As a result, our model explained experimental results. We believe our model becomes fundamental model to connect molecule and cell level.

1P277 間葉ーアメーバ型遊走に関する理論モデル

A Theoretical Model for Mesenchymal-Amoeboid Modes for Migration

Shin I. Nishimura (*Kyushu University*)

Tumor cells exhibit two distinct modes for migration, mesenchymal and amoeboid modes, in three-dimensional environment. The Mesenchymal tumor type is similar to fibroblasts that synthesize the extracellular matrix. In contrast, the amoeboid type is rather similar to leukocytes that eliminate external microbes. Certain types of tumor cells can transform one mode to the other. To understand why the tumor cells exhibit two distinct modes, a theoretical model is introduced. The simulated cell on a flat substrate forms two distinct, amoeboid and keratocyte-like shapes by changing model parameters. In three-dimensional substrates, the amoeboid type cell again forms three-dimensional amoeboid shape but the keratocyte type transforms its body into fibroblast-like shape.

1P278 確率的シグナル伝達経路における外因性ノイズを含む入力信号に対する応答性

Responses of a stochastic signaling cascade to input signals with extrinsic noise

Akio Chiba[1,2], Akihiro Fukagawa[1], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

Intracellular signaling cascades such as the MAPK pathway play an important role in amplification of input signals, notably faint signals. However, statistical fluctuations or noise in input signals are inevitable in vital signals. The signaling cascades amply not only signals but also noise, which often deteriorates the quality of the received signal. The properties of the deterioration in signaling cascades remain unclear. In this study, we performed the numerical simulations with a stochastic mathematical model of signaling cascade. We analyzed the effects of the extrinsic noise on the signal deterioration as a function of the parameters of input signals and cascades. We will discuss how living cells treat the extrinsic noise optimizing signaling cascades.

1P279 なぜ細胞は様々なステップ数を持つシグナルカスケードを使いわけるのか？

Why do cells use signaling cascades with a variety of the number of steps?

Akihiro Fukagawa[1], Masashi Kajita[1], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

Cellular signal transduction involves multiple steps, which are thought to be essential for signal intensity amplification. However, each reaction step adds an undesirable intrinsic noise to output signal. Why do living cells use such multistep processes? To answer this, we performed the numerical simulations based on a stochastic cascade model. We found signaling cascades maximize the signal-to-noise ratio at an optimal number of steps. The number is logarithmically dependent on the input intensity, and is decreased with increasing the number of molecules at steps. This result indicates that cells select a signaling cascade system with an optimal number of steps and molecules according to the input signal intensity.

1P280 一分子シミュレーションによる上皮成長因子シグナル伝達経路の応答不均一性の解明

Understanding heterogeneity in EGF pathway using simulation at the molecular resolution

Kazunari Iwamoto, Yuki Shindo, Atsushi Miyauchi, Kazunari Kaizu, Koichi Takahashi (*Laboratory for biochemical simulation, QBiC, RIKEN*)

Cellular growth, proliferation and differentiation are regulated by epidermal growth factor (EGF) signaling pathway, and mutation of several proteins in EGF signaling pathway caused malignant transformation. In this pathway, ERK protein was finally activated and translocated into nucleus, which showed different dynamics between individual cells (called heterogeneity). Since heterogeneity affected the malignant transformation and drug resistance of cells, it is essential to elucidate the mechanism of heterogeneity. In this study, we simulate EGF signaling pathway at the molecular resolution and analyze the simulation results to understand mechanism of heterogeneity. Our simulation results showed one of mechanisms that can generate heterogeneity in EGF pathway.

1P281 Diffusion-controlled reaction rate-laws in intracellular environment with molecular crowding: A single-particle-level simulation study

Kazunari Kaizu, Koichi Takahashi (*RIKEN*)

Although intracellular environment is significantly different from the ideal conditions expected in conventional biochemical simulations, the effect is poorly understood. In particular, there is no general quantitative theory for the reaction kinetics with molecular crowding. To derive the practical theory, we quantified the effect of molecular crowding on a rate-law in various conditions by using a single-particle-level simulation. In contrast to the common theory for diffusion-controlled reactions like Collins-Kimball's, the recombination probability was evaluated as a kinetic parameter. The rate-law revised on the basis of these calculations allowed simulations of intracellular signaling pathways and revealed the impact of crowding on cellular responses.

1P282 Power-law distribution derived from misunderstanding of search patterns

Hisashi Murakami, Yukio Gunji (*Kobe University*)

In the study of random search, though the Levy strategy is consistent with the searching behavior of real animals, some researchers claim that the Levy-like distributions exhibited by animals are not necessarily produced by a Levy process. Here, we propose an intermittent two-phase search model that does not include a Levy process, in which agent is basically a correlated random walker, but it memorizes its trajectory and counts the number of crossovers in a trajectory. If the number exceeds a threshold, the agent makes ballistic movement in the direction uncorrelated to the past. We show this model can optimize the trade-off. Finally, we demonstrate another model that misunderstand the rule to switch the two phases can show a Levy-like distribution of time intervals.

1P283 From cell-autonomous circadian clocks to tissue-level timekeeping

Craig Jolley, Maki Ukai-Tadenuma, Dimitri Perrin, Hiroki Ueda (*RIKEN Center for Developmental Biology*)

In mammals, the suprachiasmatic nucleus (SCN) is the central pacemaker of the brain. Each cell has an autonomous rhythm of gene expression, and ~20,000 neurons are coupled to produce a stable rhythmic output. We have developed a single-cell model of clock gene expression and parameterized it to experimental mRNA/protein expression data. We can predict the response of individual cells to perturbations, and we hope that these predictions will aid us in understanding intercellular coupling in the SCN. Currently, we are interested in how spatiotemporal “waves” of circadian gene expression arise at the tissue level. More coarse-grained models of the SCN will incorporate experimental data on intercellular coupling and help us understand organism-level behavioral outputs.

1P284 熱泳動現象を用いた鎖状高分子の凝集における分子構造転移の影響

Effects of polymer chain folding for polymer aggregation in thermophoresis

Kenta Odagiri (*MIMS, Meiji Univ.*)

We theoretically investigate aggregation of flexible polymer chains caused by competition between thermophoresis and entropic effect, inspired by the recent experiments on finding the polymer structure dependence of DNA accumulation in a PEG solution under temperature gradient. The experiment showed that the coil-globule transition for DNA is observed for a large PEG volume fraction and the transition causes the wide range of ring-like localization. To explain the experimental results, we construct a new model which takes into account effects of
the polymer structural transition caused by PEG volume fraction explicitly. The phase transition for the PEG distribution and the change of the size of ring-like distribution for long polymer will be discussed.

1P285 ベイズ統計を用いた超解像 CT アルゴリズム

Super resolution computed tomography based on Bayesian statistics

Jun Kozuka[1], Takaki Makino[2], Haruo Mizutani[2] ([1]*QBiC, RIKEN*, [2]*Grad. Sch. Fro. Sci., Univ. Tokyo*)

The reconstruction of a high-quality three-dimensional image from a low-resolution sinogram, which is a visual representation of the two-dimensional projection data obtained from computed axial tomography, is an important problem which arises in fields such as microscope and medical imaging. It is known that several artifacts originated from the inverse Radon transformation arise during typical reconstruction approaches. We have developed a Bayesian treatment of the super-resolution computed tomography problem. This approach is rendered tractable through the introduction of Gaussian processes. Results indicate a significant improvement over techniques based on the filtered back projection.

1P286 フリーズフラクチャー原子間力顕微鏡によるバクテリオロドプシンの 3 次元結晶の観察

Observation of the crystal structure of bacteriorhodopsin by freeze fracture atomic force microscopy

Naoto Kuga, toshiaki Gotou, Tutomu Kouyama (*Nagoya Univ.*)

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 intracellular structures by electron micrography, the freeze-fracture method has been utilized. In this study, we have improved a cryogenic AFM and freeze fracture apparatus to obtaine topological images of a fractured surface of a frozen crystal of bR. Firstly, a 3D crystal of bR was dipped in cold ethanol at -100℃, and fractured using a knife. Then the fractured sample was mounted on a cryogenic stage of AFM, by which the fractured surface was observed in cold ethanol. In the P622 crystal of bR that was prepared by the membrane fusion method, a periodic arrangement on BR trimers was observed.

1P287 楕円率変化検出ＣＤ測定法の発展とその生物系への応用

Development of elliptically-polalization-detected CD apparatus and its application to the biological systems

Yasuyuki Araki, Yoshiyuki Hamada, Makoto Murakami, Seiji Sakamoto, Takehiko Wada (*IMRAM, Tohoku Univ.*)

Circular Dichroism (CD) spectral study is one of the most powerful and versatile methods for detection of interaction and binding of biomolecules. Though, it is hard to measure CD spectra with good to excellent S/N, as a result of small differences in extinction coefficients of asymmetric left and right circularly polarized light. In order to improve the sensitivity disadvantage, a new control method of ellipticity of pseudo-linearly elliptically polarized light by the azimuth of retarder to get high sensitivity in CD detection has been proposed in this work. We have also confirmed that this technique has been suitable for the time-resolved CD spectroscopy. Some results of time-resolved CD study of the biological systems have also been demonstrated in this poster.

1P288 物質の非平衡加熱状態観測のための In-situ マイクロ波照射 NMR 分光法の開発

Development of in-situ microwave irradiation NMR spectroscopy for observating non-equilibrium heating state of substances

Yugo Tasei[1], Teruaki Fujito[2], Izuru Kawamura[1], Akira Naito[1] ([1]*Graduate of Engineering, Yokohama National University*, [2]*Probe Laboratory Inc.*)

Microwave(MW) is widely used in the acceleration of organic reaction. However, detailed molecular mechanism of MW heating effect on the chemical reaction has not well understood yet. We investigated the MW heating effect by using newly developed *in-situ* microwave irradiation NMR spectroscopy. ^{1}H NMR spectra of liquid crystalline samples were observed under MW irradiation. The results indicate significant change of the chemical shifts. Surprisingly, the chemical shift value of isotropic phase under MW irradiation was corresponding to 200 degree higher than that of thermally equilibrated isotropic phase. *In-situ* microwave irradiation NMR spectroscopy can be applicable to analyze MW heating effects in biological systems under MW irradiation.

1P289 光と磁場を用いた一分子 DNA 操作装置の開発

A novel method for manipulation of a single DNA molecule using optical and magnetic field

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A nucleosome, a unit structure of folded DNA, is formed by DNA and proteins called histones, and is responsible for the condensation of DNA in the cell nucleus as well as for regulating gene expression. For understanding the mechanism by which the nucleosome is formed, the knowledge on the mechanical characteristics and electrostatic interaction of DNA with histone is critically important. The relationships between tension and length of DNA have been characterized by optical tweezers or AFM. However, the knowledge on torsional characteristics of DNA is very limited because it is difficult to operate the torsion with controlling DNA tension. Here, we developed a method to observe the responses of DNA to tension and torsion using optical and magnetic fields, respectively.

1P290 フロー型乳酸バイオセンサを用いたマウスの脳内乳酸測定

Measurement of lactate level in the mouse brain using a flow-type lactate biosensor

Kaoru Yamazaki, Mai Ichikawa, Ryo Shimazaki, Minoru Saito (*Graduate School of Integrated Basic Sciences, Nihon University*)

Lactate biosensors which enable to measure lactate concentration in blood and other body fluids are applicable to some medical fields such as sports medicine. We fabricated a lactate biosensor by a flow-type sensing system, in which the injected sample flows into an immobilized enzyme (lactate oxidase) column. The generated H_2O_2 or consumed O_2 in the column was detected by the H_2O_2 electrode or O_2 electrode. The fabricated biosensor could measure lactate concentration up to 10 mM. By using it, we measured lactate levels of the mouse brain. The samples were prepared by homogenizing the brain slices in ACSF. The results showed the differences of lactate levels among each part of the brain and among each age of the mouse.

1P291 Humidity-controlled preparation of frozen-hydrated biological samples for cryogenic coherent X-ray diffraction imaging using XFEL

Yuki Takayama[1], Masayoshi Nakasako[1,2], Tomotaka Oroguchi[1,2], Yuki Sekiguchi[1,2], Amane Kobayashi[1,2], Masaki Yamamoto[1], Koji Yonekura[1], Takaaki Hikima[1], Saori Maki-Yonekura[1], Yukio Takahashi[1,3], Akihiro Suzuki[1,3], Sachihiro Matsunaga[4], Yayoi Inui-Tsujimoto[4], Shoichi Kato[4], Takahiko Hoshi[5] ([1]*RIKEN SPring-8 Center*, [2]*Grad. Sci. Tech., Keio Univ.*, [3]*Grad. Eng., Osaka Univ.*, [4]*Grad. Sci. Tech., Tokyo Univ. Sci.*, [5]*KOHZU PRECISION Co., Ltd*)

Coherent X-ray diffraction imaging (CXDI) has potential to reveal internal structures of whole cells and organelles with sizes of μm to sub-μm at high spatial resolutions without sectioning of the samples. With X-ray free electron laser (XFEL), we can obtain diffraction patterns from such non-crystalline samples before they are destroyed by single exposure. To make the best use of XFEL pulses coming at a high repetition rate, we have developed a humidity-controlled sample preparation system [1] to produce sample particles at a high number density in thin vitreous ice. Here we report details of the cryogenic CXDI and first results obtained by the Japanese XFEL facility, SACLA.

[1] Takayama and Nakasako (2012) Rev. Sci. Instrum. 83, 054301 (6 pages).

1P292 ティップスキャン型高速原子間力顕微鏡による生細胞イメージング

Live cell imaging using a tip-scan type of high-speed atomic force microscopy

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High-speed AFM is a means which can observe the molecular activity on living cell surface. However there is one drawback. It can not identify what is observed, because the acquired images indicate only the shape. We developed a tip-scanning type of high-speed AFM which make it possible to simultaneously observe the shape and fluorescence of living cells surface. Using this device, it can be expected to obtain useful information on the life sciences by direct observation of cell surface molecules with fluorescent Information. We introduce the example observation of living cell surface and the device.

1P293 アップコンバージョンナノ蛍光体を用いた CL・蛍光イメージング

Upconversion Nanophosphors for Correlative CL and Fluorescent Imaging

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We propose a new correlative imaging method using upconversion (UC) nanophosphors that emit light via both near-infrared (NIR) light and electron-beam excitation, where UC is a process in which lower energy, longer wavelength, excitation light is transduced to higher energy, shorter wavelength, emission light and the light emission induced by an electron beam is called cathodoluminescence (CL). Due to the electron beam excitation, the spatial resolution of CL microscopy matches to 10 nm order. NIR light allow us to image deep tissue region because NIR light is insusceptible to absorption, scattering, and autofluorescence. We investigated the UC and CL spectra of Y2O3:Tm, Yb and Y2O3:Ho, Yb nanophosphors and imaged the phosphors inside HeLa cells with UC and CL.

1P294 ファイバー共焦点レーザー蛍光顕微鏡による自由行動下マウスの神経活動の光学計測

Fiber-optic fluorescent imaging of neural activity in freely-moving mice during sleep and wakefulness

Yasuhiro Kasagi[1], Takeshi Kanda[1], Kentaroh Honda[1], Masashi Yanagisawa[1,2] ([1]*IIIS, Univ. Tsukuba*, [2]*UTSW/HHMI*)

Cortical neurons exhibit distinct firing patterns, depending on the brain state. Their activity is thought to be synchronized during sleep, and desynchronized during wakefulness. However it is technically difficult to record firing of an individual neuron during the natural sleep-wake cycle, because sleep is often disturbed by the stressful circumstance such as the head-restrained condition. To overcome the technical problems and clarify the cortical ensemble activity, it is required to monitor the discharge of a large number of neurons in the animals under the head-unrestrained condition. Using fibered confocal microscopy and Ca^{2+} indicators, we optically observed cortical neuron activity with a cellular resolution in freely moving mice during sleep and wakefulness.

1P295 生きた細胞内における内在性テロメア RNA の一分子動態解析

Single molecule imaging of endogenous telomeric RNA in living cells

Toshimichi Yamada, Hideaki Yoshimura, Mitsuru Hattori, Takeaki Ozawa (*Grad. Sch. Sci., Univ. Tokyo*)

Telomeric RNA is a long-noncoding RNA from telomere and its function is likely essential for telomere maintenance. To reveal the RNA function, we developed a method for single telomeric RNA imaging in live cell. An RNA-binding protein, PUM-HD, was modified by amino acid mutations to recognize the target RNA. Each end of the mutated PUM-HD was connected with N- and C-terminal fragments of EGFP so that the probes emit fluorescence by EGFP reconstitution upon binding to telomeric RNA. Using total internal reflection fluorescence microscopy, fluorescence of the probe was detected in single molecule level. We revealed that telomeric RNAs were confined around telomere regions and diffused on nucleoplasm by analyzing mean square displacement and diffusion coefficient.

1P296 Shannon エントロピーの変化でみた質量顕微鏡データ
Analysis of the difference in Imaging Mass Spectrometry Data characterized by Shannon entropy

Noritaka Masaki, Mitsutoshi Setou (*Dept. Cell Biol. & Anatomy, Hamamatsu Univ. Sch. Med.*)

Through studies using Imaging Mass Spectrometry (IMS), we could successfully reveal key molecules and their distributions in various biological phenomena and diseases. On the other hand, we also found that these molecules usually behave not independently but relating to other molecules. To characterize the difference of IMS data taking account such interactions, we introduced Shannon entropy to overview mass spectra varied from position-to-position. In our previous presentation in this biophysical society, we discussed characteristic spatial distribution of Shannon entropy in mouse brain irrespective to anatomical representation. In this study, we will present which types of signals are dominant in Shannon entropy estimation and how they contribute to its variation.

1P297 生細胞における膜タンパク質標識法と会合状態解析法の開発
若手招待講演
Development of methods for labeling and oligomerization analysis of membrane proteins in live cells

Yoshiaki Yano, Kenichi Kawano, Kaoru Omae, Sayaka Mtsuzaki, Katsumi Matsuzaki (*Grad. Sch. Pharm. Sci., Kyoto Univ.*)

We have developed a specific labeling method for live-cell fluorescence imaging of membrane proteins using a high-affinity coiled-coil peptide pair to overcome limitations of conventional methods. Taking advantages of cell-surface-specific labeling and easiness in multicolor labeling, the oligomeric states of membrane proteins were precisely determined by spectral imaging FRET. We found that the β2 adrenoceptor does not form constitutive homooligomers, and homooligomerizaiton is not necessary for their function. Furthermore, the influenza A M2 protein, which is proposed to form a tetrameric proton channel, was found to form a functional dimer at neutral pH. Thus, this cell-based method is powerful for analysis of oligomerization of various membrane proteins.

1P298 走査型電気化学-イオンコンダクタンス顕微鏡を用いた神経伝達物質の放出サイトのマッピング
Mapping of neurotransmitter releasing sites using scanning electrochemical ion conductance microscopy

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Neurotransmitters are the chemical medium through which the signals move from one neuron to the next at the synapses. One technique with the potential to map chemically specific fluxes on the nanoscale is scanning electrochemical microscopy (SECM), but a lack of reliable distance control and difficulties in fabricating small-scale electrodes have largely restricted the technique to the microscale. We introduce an extremely quick and simple process with a high success rate for making double-barrel carbon nanoprobes for use in SECM-scanning ion conductance microscopy (SICM). The overall probe radius is controllable on the nano- to microscale, and the probes can be used for simultaneous chemical and topographical imaging.

1P299 光干渉法を用いた細胞-ハイドロゲル間接着の定量評価
Quantitative evaluation of cell adhesion to hydrogels by advanced interferometric optical microscopy

Takahisa Matsuzaki[1], Gen Sazaki[2], Masami Suganuma[3], Tatsuro Watanabe[3], Takashi Yamazaki[1], Yuko Shimokawa[1], Motomu Tanaka[4], Seiichiro Nakabayashi[1], Hiroshi Yoshikawa[1] ([1]*Grad. Sch. Sci & Eng., Univ. Saitama*, [2]*Inst. Low Temp. Sci., Univ. Hokkaido*, [3]*Res. Inst. Clin. Onc., Saitama Cancer Center*, [4]*Inst. Phys. Chem., Univ. Heidelberg*)

Hydrogels are important tissue engineering scaffolds and ideal materials to direct cell function by serving key functionalities (e.g., mechanics) of native extracellular matrix. However, the imaging of physical contacts between cells and hydrogels with a nanometer resolution along the optical axis remain challenging because of low reflectivity of cell/hydrogel interfaces. In this work, to visualize cell/hydrogel interfaces, we have developed an interferometric optical microscopy combined with high-throughput optics, a confocal system, and a new class of monochromatic light source. By using the microscopy, we succeeded in evaluating the dependence of cancer cell spreading on hydrogel stiffness in a quantitative manner.

1P300 X-ray excited optical luminescence via bio-molecule directed metal clusters

Yasuko Osakada[1,2], Yoshie Harada[1] ([1]*Kyoto university, iCeMS*, [2]*JST PRESTO*)

Understanding the nature of X-ray excited optical luminescence is not only of fundamental interest but would also be meaningful for the development of novel computed tomography (CT) imaging. In this study, we, for the first time, investigated the X-ray excited optical luminescence from bio-molecule directed metal clusters. Interestingly, the emission was only observed via metal clusters directed with bovine serum albumin, whereas neither lysozyme protein metal cluster nor DNA-directed cluster does not induce significant emission upon X-ray irradiation. These results provide new insight of metal clusters into the development of novel CT imaging probes.

1P301 成長円錐における単一分子レベルでのアクチン関連 mRNA の局在
The localization of actin-related mRNAs in growth cone at a single molecule level

Hidenori Koizumi[2], Yasuko Osakada[1], Yoshie Harada[1] ([1]*iCeMS.,Univ. Kyoto*, [2]*Grad.Sch.Bio., Univ. Kyoto*)

In neurons, axon terminal is called “growth cone” which is ameba-like motile structure. At growth cone, the polymerization of actin-filaments is one of the most important factors for nerve growth and maintenance. However, how actin-related mRNAs function at axon terminal is not fully understood.
Therefore, we are investigating the dynamics and localization of actin-related mRNAs at growth cone. In this study, we designed new visualization system to image actin-related mRNAs at single molecule level. Here, we plan to talk about our preliminary results observed at the growth cone of PC12 cells.

1P302 超音波高速 AFM の開発に向けた基礎研究 2
Pilot study 2 for the development of high-speed ultrasonic AFM

Tomofumi Saito[1], Noriyuki Kodera[2], Toshio Ando[1,2] ([1]*Sch. Math. & Phys., Inst. Sci.*, [2]*Bio-AFM Frontier Research Center, Inst. Sci. & Eng., Kanazawa Univ.*)

We have developed high-speed AFM (HS-AFM) that enables us to directly visualize dynamic structural changes of protein molecules at high spatiotemporal resolution. So far, various biological processes have been directly filmed. However, the observations by HS-AFM are generally limited only to phenomena occurring on relatively hard surfaces, meaning that we can observe neither objects placed on a soft surface nor objects placed under a surface. Ultrasonic techniques must be useful in breaking this limitation because they have been widely used for non-invasiveness imaging of objects lying on/under a surface. Thus, we are currently attempting to find a way to combine our HS-AFM with ultrasonic techniques. In the presentation, we will report our pilot study on this subject.

1P303 Real-time observation of amyloid fibril formation of yeast prion Sup35 by high-speed atomic force microscopy

Liwen Zhu[1], Hiroki Konno[1], Momoko Okuda[2], Noriyuki Kodera[1], Toshio Ando[1], Hideki Taguchi[2] ([1]*Bio-AFM Frontier research center, Kanazawa University*, [2]*Department of Biomolecular Engineering, Graduate School of Biosciences and Biotechnology, Tokyo Institute of Technology*)

Yeast prion [PSI+] is caused by ordered protein aggregates, called amyloids, of Sup35 protein. To investigate the mechanism of how the amyloids elongate and then propagate is critical to unravel the amyloid biology. Although there have been some studies of real-time amyloid formation of Sup35, especially using fluorescence probes, the detailed mechanism of the amyloid formation still remains unclear. We used the high-speed atomic force microscope (HS-AFM) to investigate the dynamic behaviors of the Sup35 fibrils. We have developed procedures to watch real-time fibril formation of the recombinant N-terminal and medium domains of Sup35 in liquid at a high spatial resolution.

1P304 高速 AFM によるバクテリアの高分解能観察
Nanoscale investigation on bacterial cell surface using high-speed AFM

Hiroki Watanabe[1], Carriou David[1], Takayuki Uchihashi[1,2], Toshio Ando[1,2] ([1]*Dep. Phys., Col. of Sci. and Engr., Kanazawa Univ.*, [2]*Bio-AFM Frontier Res. Center*)

A bacterial cell has variety of proteins and structures on the surface, such as membrane proteins, peptidoglycan layers, a flagellum and secretion apparatus. Cryo-electron microscopy can visualize their static structures and also optical microscopy can observe localization of labeled target molecules. However, there have been no techniques which can directly observe the structure of cell surface. Recently it was demonstrated that high-speed atomic force microscopy (HS-AFM) can directly visualize dynamics of protein molecules on a bacterial cell surface. Here, we applied HS-AFM to visualize the dynamics and structures on gram-negative bacteria. We will show high-resolution images of the outer membrane covered by a net structure and a lysis process induced by a detergent.

1P305 高速 AFM / 光学顕微鏡複合機
Combined system of High-speed-AFM and optical microscopy

Shingo Fukuda[1], Takayuki Uchihashi[1,2], Ryota Iino[3], Toshio Ando[1,2] ([1]*Department of Mathematics and Physics, Grad School of Natural Science and Technology, Kanazawa University*, [2]*Bio-AFM Frontier Reserch Center, College of Science and Engineering, Kanazawa University*, [3]*Department of Applied Chemistry Grad School of Engineering The University of Tokyo*)

Combined system of high-speed atomic force microscopy (HS-AFM) and total internal reflection fluorescence microscopy (TIRFM) has been developed. This novel instrument can provide a way to correlate conformational change of protein to ligand-binding events, specify the tip position on a large object such as a living cell, and identify target molecules even in a complex system. Also unlike conventional combination of AFM and optical microscopy, our system can simultaneously capture AFM and TIRFM images at single molecular level. The capability of this combined microscope is demonstrated by simultaneous HS-AFM/TIRFM imaging of myosin V walking on an actin filament.

1P306 高速 AFM による ClpB の構造ダイナミクスの観察
Conformations and dynamics of ClpB hexameric ring observed by high-speed AFM

Takayuki Uchihashi[1,2], Yo-hei Watanabe[3], Ryota Iino[4], Hiroki Watanabe[1], Takashi Yamasaki[3], Toshio Ando[1,2] ([1]*Dept.Phys., Kanazawa Univ.*, [2]*Bio-AFM Frontier Research Center, Kanazawa Univ.*, [3]*Dept. Biol., Konan Univ.*, [4]*Dept. Appl. Chem., Univ. of Tokyo*)

The molecular chaperon ClpB is a ring-forming AAA+ machine that rescues aggregated proteins. So far, conformations of ClpB in a hexameric ring depending on nucleotides have been investigated by electron microscopic single-particle analysis and biochemical analysis. Here we applied high-speed atomic force microscopy (HS-AFM) to directly visualize conformations and dynamics of the ClpB ring. The HS-AFM images revealed that the ring shows deformed, 2-fold rotational symmetry without nucleotide, and one of the interfaces between subunits at axis of symmetry repeats reversible opening and closing. On the other hand, the ring showed 6-fold rotational symmetry in high ATP. These dynamic conformational changes in the ClpB ring would play roles for protein disaggregation.

1P307 動的な DNA コンピューティングを実現するための AND ゲートモジュールの開発
Development of AND gate module for dynamic DNA computing

Takakshi Nukada, Koh-ichiroh Shohda, Akira Suyama (*Grad. Sch. Arts and Sciences, Univ. Tokyo*)

RTRACS (Reverse-transcription and TRanscription-based Autonomous Computing System) is a modular biomolecular computing system composed of DNA, RNA and enzymes, whose mechanism is based on retroviral replication. The modules of RTRACS communicate with each other through RNA for the sophisticated computational operations. Here, we report AND gate module for RTRACS that produces output RNA after receiving input RNA and changes output RNA dynamically depending on input RNA. The module allows RTRACS to perform a time-dependent computation. It is expected that other RTRACS modules dynamically worked are also capable of being developed in the same way of the AND gate module.

1P308 オンチップマルチイメージングセルソーターを用いたクラスター化細胞のリアルタイム認識と回収のための画像解析技術の研究

Real time image analysis technology for identification and collection of clustered cells using on-chip multi-imaging cell sorter

Masao Odaka[1], Mathias Girault[1], Hyonchol Kim[1], Hideyuki Terazono[1,2], Akihiro Hattori[2], Kenji Yasuda[1,2] ([1]*KAST*, [2]*Tokyo Med. Dent. Univ.*)

We report the newly-developed real time image analysis technology to identify and sort the clustered cells using an on-chip multi-imaging cell sorter, which was combined with a multi-color imaging unit, an ultra-high speed camera, and a microfluidic cell separation unit. The bright-field and fluorescence images of cells were captured simultaneously, and their morphological parameters were analyzed to identify clustered cells. The target clustered cells were collected from a cell suspension successfully by applying electrical pulses in microfluidic flow. The results showed the potential of the morphological index of cells to identify and collect the target cells such as clustered cells in the cell suspension.

1P309 オブジェクト指向によるロボットとの認識共有

Object-Oriented Cognition Sharing as a Method of Brain-Machine-Interface

Jun Miyake[1], Kazuyuki Hatta[1], Amalia Adiba[1], Ryuuzou Baba[2], Tadahiro Kaneda[2] ([1]*Graduate School of Engineering Science, University of Osaka*, [2]*Osaka Prefecture University College of Technology*)

Brain-Machine Interface is useful for disabled people on daily life support. Difficulties are the control and the weight. We propose a separate-type arm/hand on a mobile robot controlled with neural network. The robot has a certain intellectual capability to understand the order and execute the demand. Object-oriented interactive system is the method to share the cognition: 1) pointing the object by eye-tracker or laser beam, 2) the robot extracts the meaning of the object by its shape. The robot moves the arm to serve for the person. No complicated order is required to function, i.e., the object extracts the verb to function, reducing the order. The cognition sharing method provides a simple control of a robot. The capability depends on the power of cognition.

1P310 DNA Computer-Controlled Gene Expression in a Cell Model Vesicle

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The ability to directly interface with biological systems, which none of the other computers could possess, is useful for biological and medical applications of DNA computers. Here, we report a result of experiments carried out to aim for biomedical application of RTRACS, which is a modular DNA computing system. An RTRACS module receives input RNA for two inputs and produces output mRNA coding GFP as a consequence of the AND operation. This module was encapsulated into a Giant Unilamellar Vesicle (GUV). Expression of GFP gene controlled by input RNA strands was observed by GFP translated from the output mRNA in a GUV. The present result would be the first small but important step toward the GUV-based synthetic biology and highly-intelligent drug delivery system.

1P311 Simple and Efficient Approach for Proteomic Analysis of Subcellular Structures using Droplet-Based Microfluidics

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We have developed a simple and efficient method for isolating target subcelluar structures using droplet-based microfluidics. Using this method, we demonstrated the quick and efficient isolation of structurally intact mitochondria from crude cell homogenate. Mitochondria were fluorescently labeled in living HeLa cells. The cell homogenate was encapsulated into water-in-oil droplets using microfluidics, and the droplets containing fluorescent mitochondria were collected by a glass capillary. PCR resulted in the successful amplification of the cytochrome b gene fragment of mitochondrial DNA from a single droplet containing a single mitochondrion. Now, we are trying to perform mass spectrometry-based proteomic analysis of the mitochondria isolated using droplets.

1P312 Yeast-based fluorescence assay system for detecting human G protein-coupled receptor activation in water-in-oil droplets

Takashi Sakurai[1], Ryo Iizuka[1], Rui Sekine[2], Yoon Dong H.[2], Tetsushi Sekiguchi[3], Jun Ishii[4], Akihiko Kondo[5], Shuichi Shoji[2], Takashi Funatsu[1] ([1]*Grad. Sch. of Pharm. Sci., Univ. of Tokyo*, [2]*Major in Nanosci. and Nanoeng., Waseda Univ.*, [3]*Nanotech. Research Center, Waseda Univ.*, [4]*Org. of Advanced Sci. and Tech., Kobe Univ.*, [5]*Grad. Sch. of Sci. and Tech., Kobe Univ.*)

We have established a yeast-based fluorescence assay system to detect G protein-coupled receptor (GPCR) activation in water-in-oil droplets. Microfluidics is used to encapsulate template DNA encoding peptides, *in vitro* transcription-translation system, and yeast cells expressing GPCR into droplets. The yeast cells are genetically engineered to express GFP via their intracellular signaling pathway in response to ligand stimulation of GPCR. Thus, yeast cells would be fluorescent when synthesized peptides stimulate the GPCRs in droplets. We successfully demonstrated that the synthesized peptide ligand (somatostatin, SST) activated the cognate human GPCR (SSTR5) in droplets. The system will be useful for designing novel peptide ligands for both liganded and orphan GPCRs.

1P313 Optical microdevice operated through self-organization of microtubule and kinesin: An experimental study

Ayumu Miyata[1], Yuichi Hiratsuka[2], Takahiro Nitta[1] ([1]*Gifu University*, [2]*JAIST*)

Inspired by fish melanocytes, we have envisioned an optical microdevice operated through self-organizations of microtubule (MT) and kinesin. Formation and disassembly of MT asters lead to accumulation and dispersion of the kinesin-streptavidin complexes, corresponding to bright and dark states of fish melanocytes, respectively. By selectively activating microchambers in an array, images can be drawn. In order to realize it, we confined MTs and kinesin-streptavidin complexes into hexagonal microchambers, and found that MT asters were formed in the chambers. At the center of the MT aster, the kinesin-streptavidin complexes were accumulated. We will discuss the condition at which the MT aster formations in the microscale chambers.

1P314 Optical microdevice operated through self-organization of microtubule and kinesin: A simulation study

Takahiro Nitta[1], Yuichi Hiratsuka[2] ([1]*Gifu Univ.*, [2]*JAIST*)

Fish melanocytes change their appearances through aggregations and dispersions of melanosomes, corresponding to bright and dark, respectively. Here, we have envisioned an optical microdevice which changes its color through self-organizations of microtubules and kinesins. Formations and disassemblies of microtubule asters lead to aggregations and dispersions of kinesin-streptavidin complexes, which are "melanosomes" in the device. We investigated the feasibility of the device with a computer simulation. The simulation showed that the kinesin-streptavidin complexes initially distributed all over the chamber could be accumulated at the center of the aster. With the computer simulation, we will show guidelines for the design of the envisioned optical device.

1P315 Theree-Dimensional Movements of Microtubule Diriven by Kinesin on Microfabricated Tracks Revealed with a Computer Simulation

Yuki Ishigure, Takahiro Nitta (*Gifu University*)

Motility assay of kinesin and microtubule (MT) has been utilized to power Lab-on-a-Chip devices, which are miniaturized chemical analysis systems. On the devices, driven by kinesin motors on microfabricated tracks, MTs carrying cargo are delivered to their destinations. When a MT comes to a boundary of the tracks, outcomes are either MT continuing to move along the boundary or MT dissociation. Although, predictions of the outcomes in given conditions are important in designing microfabricated tracks, such predictions are hampered by limited information obtained from optical microscopes on details of MT movements. Here, we performed a computer simulation, and revealed the details of MT movements at the boundaries of microfabricated tracks.

1P316 明視野/蛍光画像の同時リアルタイム解析技術を用いたオンチップ・マルチイメージング・フローサイトメーターの開発 Development of On-chip Multi-imaging Flow Cytometer System using Real-time Bright Field/Fluorescent Dual Image Analysis High-speed Camera

Akihiro Hattori[1], Hyonchol Kim[2], Hideyuki Terazono[1], Masao Odaka[2], Mathias Girault[1], Kenji Yasuda[1,2] ([1]*Department of Biomedical Information, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University*, [2]*Kanagawa Academy of Science and Technology*)

We have developed an on-chip multi-imaging flow cytometer system for a real-time bright field/fluorescent dual-image analysis. The system consists of (1) a disposable microfluidic chip, (2) a bright field/fluorescent dual-image microscopic optical system, and (3) a real-time high-speed digital camera with image-processing function. For the high-speed image acquisition, we adopted single-band width LED light sauce, synchronized with camera shutter intervals, and FPGA circuit was directly connected to the camera part. Using this system, we analyzed not only shapes of cell, but also nuclei formation with faster than 1/200 s. In this meeting, we introduce the potential and possibilities of this system and the new index of cell identification, 'imaging biomarkers'.

1P117 Single Nucleosome under Tension and Torsion

Jen-Chien Chang[1], Michel de Messieres[2], Arthur La Porta[1] ([1]*Dept. Phys., University of Maryland, USA*, [2]*National Institute of Health, Bethesda, MD, USA*)

The fundamental chromatin packing unit in eukaryotes is the nucleosome. Prior single-molecule experiments have exerted linear tension to stretch both chromatin fibers and mononucleosome, which have given information on the nature of the free-energy barrier for a particular disruption pathway. We develop a theoretical model including torsional constraints, which suggests that the disruption pathway may be sensitive to the torsional loading of the nucleosome. Experimentally we apply force and torque simultaneously to disrupt a mononucleosome using an optical torque wrench. Positive supercoiling is found to destabilize the nucleosome while negative supercoiling has little effect, which is consistent with our model.

2P001 フラビン酵素 RebC 変異体の結晶構造解析とインドロカルバゾール骨格の構造多様性の創出原理の解明

Crystal structure of a mutant flavoenzyme RebC and construction mechanism of indolocarbazole aglycone structure

Hayate Itatani[1], Eiyu Izumo[1], Saki Kageyama[2], Sayaka Kurozumi[1], Hiroyasu Onaka[3], Shumpei Asamizu[3], Tomoya Hino[1], Shingo Nagano[1] ([1]*Grad. School of Eng., Tottori Univ.*, [2]*Faculty of Eng., Tottori Univ.*, [3]*Faculty of Eng., Toyama Pref. Univ.*)

Flavoenzymes RebC and StaC are responsible for selective production of arcyriaflavin A and K252c in indolocarbazole biosynthesis, respectively. In this study, we have determined X-ray crystallographic structure of a mutant RebC F216V/R239N that has StaC-type activity at 2.4-Å resolution with reduced flavin. Compared to the putative substrate-bound WT RebC, the structural changes are confined to the active site. The side chain of Arg230, which are in between the two mutation sites, moves farther away from substrate binding site. As a result, the distance between substrate and FAD is longer than that in WT, which may not appropriate for flavin dependent monooxygenation in the mutant.

2P002 Crystal structure of cruxrhodopsin-3 from Haloarcula vallismortis

Siu Kit Chan[1], Tomomi Kitajima[1], Midori Murakami[1], Kunio Ihara[2], Tsutomu Kouyama[1] ([1]*Dept. Phys., Grad. Sch. Sci., Nagoya Univ.*, [2]*Center for Gene Research, Nagoya Univ.*)

Cruxrhodopsin-3 (cR3), a retinylidene protein found in Haloarcula vallismortis, functions as a light-driven proton pump. cR3 was crystallized into a trigonal crystal (P321, a=b=106.2 Å, c=55.4-60.2 Å), and its structure was determined at 2.1 Å resolution. The trimeric structure of cR3 is very similar to that observed in archaerhodopsin-2. Diffraction data collected at pH 4.0, 5.0 and 7.0 showed that two glutamates (E198 and E208) in the proton release pathway form a paired structure that is stabilized by a low-barrier hydrogen bond. The C-terminal region forms a short helix, which fills the space between the AB and EF loops at the cytoplasmic side.

2P003 べん毛 III 型輸送装置蛋白質 FlhA の細胞質領域の構造変化

Conformational change of a cytoplasmic fragment of FlhA, a flagellar type III protein export apparatus protein

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FlhA is the largest component protein of the flagellar type III protein export apparatus. It consists of the N-terminal transmembrane region ($FlhA_{TM}$) and the C-terminal cytoplasmic region ($FlhA_C$). $FlhA_C$ is an assembly platform for the export substrate and the soluble components of the apparatus. $FlhA_C$ is conposed of four sub-domains and sub-domain motion is thought to be required for protein secretion. To elucidate the molecular mechanism of the flagellar protein secretion, we crystallized and solved the structures of $FlhA_C$ and its mutants. The structure of the new crystal form of wild-type $FlhA_C$ adopts different conformation from the previous one. We will discuss the sub-domain motion of $FlhA_C$ and its role on protein export based on the structures.

2P004 コレラ菌の走化性受容体蛋白質 Mlp24 とそのリガンド複合体の構造

Structure of a chemoreceptor protein of *Vibrio cholerae*, Mlp24, and its ligand complex

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Toxigenic *Vibrio cholerae*, the causative agent of cholera, is a Gram-negative bacterium with a single polar flagellum. *V.cholerae* has at least 45 genes for methyl-accepting chemotaxis protein-like proteins (MLPs). Among them, Mlp24 is required for production of cholera toxin upon mouse infection. Mlp24 is known to bind multiple amino asids, such as Ser, Asn, Arg, and Pro. Thus, sensing of these amino acids by Mlp24 is thought to trigger the expression of virulence factors. We have solved the structures of a periplasmic fragment of Mlp24 and its complex with arginine. Mlp24 has two PAS domains and arginine is bound to the distal PAS domain. The structures indicated that arginine binding induces a large conformational change of the PAS domain.

2P005 4-*O*-β-D-mannosyl-D-glucose phosphorylase (MGP) の X 線結晶構造解析

Structure of novel enzyme 4-*O*-β-D-mannosyl-D-glucose phosphorylase MGP

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A novel component of the mannan biodegradation system, 4-*O*-β-D-mannosyl-D-glucose phosphorylase (MGP), crucially converts 4-*O*-β-D-mannosyl-D-glucose to yield D-mannose-1-phosphate and D-glucose in an inverting manner. The structure of MGP has been determined with an X-ray crystallography to a 1.68 Å resolution. The crystal structure revealed a unique homohexameric quaternary structure of MGP, which was formed by using two helices attached to the N- and C-terminals as a tab for sticking between subunits. The MGP complex structures with substrate and product molecules were also determined. The substrate/product complex structures implied a novel catalytic mechanism, since the predicted general acid/base Asp131 did not exist close to the nucleophilic substrate atom.

2P006 組み替え human poly(ADP-ribose) polymerase 1 の精製と予備的構造解析

Purification and preliminary structure analysis of recombinant human poly(ADP-ribose) polymerase 1

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Human poly(ADP-ribose) polymerase 1 (h-PARP-1) performs a posttranslational modification called poly ADP-ribosylation. Although the crystal structure of h-PARP-1 without several domains has been reported recently, the tertiary structure of an intact complex is not yet known. The overall tertiary structure is required to develop h-PARP-1 specific inhibitor as an effective anticancer drug. We have developed the high yield recombinant h-PARP-1 production system in E. coli, and established a purification scheme through affinity and cation exchange chromatographies, in order to analyze the overall structure of h-PARP-1. In this presentation, the results of h-PARP-1 purification and preliminary structure analysis with single-particle electron microscopy will be discussed.

2P007 ＰＥＬＤＯＲによる時計タンパク質ＫａｉＢの構造変化の検出

PELDOR detection of structural changes of clock protein KaiB

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The circadian clock is an endogenous biological mechanism that generates autonomous daily cycles of physiological activity. The cyanobacterial circadian clock, which consists of three clock proteins, KaiA, KaiB, and KaiC, works as a clock oscillator by forming the KaiABC complex.
We examined the structural changes of KaiB during incubation with both KaiA and KaiC by PELDOR method. The PELDOR is a well-established method to determine the distance between radicals with high accuracy. PELDOR results show that the inter-distance between the spin labels in KaiB was initially about 33.0 Å;, and changed after incubation for several hours. These results indicate structural changes of KaiB in the formation of KaiABC complex.

2P008 2.5 kbar におけるユビキチン高エネルギー状態の立体構造解析

Solution structure of the "pure" high-energy state of ubiquitin: Q41N at 2.5 kbar

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By using high-pressure NMR spectroscopy, solution structure of the alternatively folded state N2 of ubiquitin was determined. Comparing solution structures between N1 and N2, we designed ubiquitin mutants stabilizing N2 state of the protein. In the case of the Q41N mutant, in which hydrogen bond between I36 carboxyl group and Q41 side chain amide group is weakened, the N2 state is 71% populated even at 1 bar. Here, we demonstrate structural determination of Q41N at 2500 bar by high-pressure NMR spectroscopy, where the N2 state is about 98% populated. This allows us to characterize the structure of the “pure” N2 state. The combination of high-pressure NMR spectroscopy and amino acid substitution is powerful tool to characterize important intermediates of the protein.

2P009 X 線小角散乱と電子顕微鏡像を用いたハイブリッド構造解析

Hybrid structure analysis with small-angle x-ray scattering and cryo-electron microscopic image

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Recently, small-angle x-ray scattering (SAXS) analysis on protein complexes by integrating other data such as cryo-electron microscopic (EM) images has been reported to be useful for improving the accuracy of the modeling. We attempted a hybrid structural analysis by use of two data of SAXS and EM image database (EMDB1313) on active oligomeric nitrilase complex in solution, the association of which is closely related to its activity. First, the EM data was used for homology modeling of the basic building block, a dimer. Second, the helical structure of active nitrilase complex was determined by SAXS. We report the result of the structure analysis and discuss about the effect of EM data in structure refinement process.

2P010 Structural analysis of the 26S proteasome by cryo-electron microscopy and Single-Particle Analysis

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The 26S proteasome (26S) undertakes the controlled degradation of proteins. The 2.5-MDa molecular machine comprises two types of subcomplexes: the barrel 20S core particle (CP) and the two 19S regulatory particles, which associate with the CP and prepare substracts for degradation. Since the weak assembly of the 26S makes structural analysis more difficult, we added 10% glycerol in the specimen for stabilization. But the low-contrast which made ‘pick up’ more difficult has been found in the 26S photograms. Thus we used the Gradient Fixation method to purify and stabilize the 26S. Then, CTF compensation using Wiener filter has been used to improve the contrast. As a result, particles could be visible and easy to be picked up. The refined 3D maps will be reported here.

2P011 Comparative survey of image processing packages for electron computed tomography

Nan Shen[1], Mingyue Jin[2], Takuo Yasunaga[1] ([1]*Kyushu Institute of Technology*, [2]*Osaka City University*)

Electron tomography (ET) is one of widely applicable methods to obtain the three-dimensional structure of organelles or cell components. Using tilt series of phantom data and axonemes by cryo-transmission electron microscopy, We compared the 3D reconstruction by three ET software packages (IMOD, Inspect3D and TEMography) and Eos, respectively. IMOD can automatically determine tilt axis by using fiducial marker, while Inspect3D needs to manually determine tilt axis but gave us more precise alignment than that of IMOD in case of no markers, judging from reconstructed 3D.We also compared them systematically to make our developed Eos more convenient for higher resolution analysis. More comparison between them will here be reported and discussed.

2P012 Possibility of metallothionein Labelling for CLEM method

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CLEM (Correlative light electron microscopy) is one of the powerful techniques to elucidate the localization and structure of the target proteins or their complexes in cell. Our previous study, metallothionein with cadmium ions functions as a label for electron microscopy [Nishino et al., 2007] whereas, in the presence of cadmium ions and selenium ions, it forms Qdot-like nano-particles, which generate fluorescence as reported by Park et al., (2010). We have also been challenging to use it as a fluorescent and heavy metal label for CLEM. We here report the possibility of metallothionein as a CLEM label and the trial to detect localizations and stuctures of two proteins, FAP20 and PACRG, in the axoneme of Chlamydomonas reinhardtii.

2P013 **A new approach to build 3D atomic model from single electron microsope image**

Atsushi Matsumoto[1], Junichi Takagi[2], Kenji Iwasaki[2] ([1]*Japan Atomic Energy Agency*, [2]*Osaka University*)

We have developed a new approach to build a 3D atomic model from a single electron microscope (EM) image of a bio-molecule. In this approach, the initial structure (X-ray crystal structure or modeled structure) of the molecule is deformed by computational techniques and many atomic models with different conformations are generated. The deformation is performed in such a way that the internal energy of the molecule does not increase so much. For this purpose, the lowest frequency normal modes are used in the deformation. Then, each atomic model is projected to many different directions, and each projection is fitted to the EM image and the fitting-score is calculated. The atomic models with high fitting-scores are the candidates that reproduce the EM image.

2P014 **プロリンリッチなペプチドのコンホメーション特性に関する考察**

An investigation on the conformation character of proline-rich peptides

Masahito Oka (*Osaka prefecture university*)

It has been considered that proline-rich peptides would generally form the polyproline-II structure in warter by the speculation on the CD spectral pattern of proline-rich peptides. In this work, theoretical investigation on the conformation character of proline-rich peptides in water was carried out by molecular dynamic simulations using gromacs algorism. It was shown that all examined peptides dynamically fluctuate among many stable conformations. The stability of straight-rod-like conformations decreases with a decrease in proline content and the stability of compactly folded conformations increases with an increase in the content of the residue having large backbone flexibility. These results almost corresponded to the pattern of CD spectra for peptides in water.

2P015 **生体電子の流れが加速する電流生成菌の細胞外電子移動機構の発見**

Respiratory Electron Flow Enhances the Rate of Extracellular Electron Transport Processes in Current-Producing Bacteria

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Vectorial electron transfer (ET) sustained by positive feedbacks from structural fluctuation in photosynthesis and respiration has been predicted by theoretical studies, yet not been experimentally confirmed in molecular level. Recently we developed a 3-electrode system with electrogenic bacteria Shewanella to directly monitor the respiratory ET process via outer-membrane-bound deca-heme c-type cytochromes (OMDCs) in-vivo, and electrochemically identified the active state of OMDCs, which increases the ET rate 1000-fold compared with non-active state. Here we observed that respiratory electron flow itself is required to sustain OMDCs in its active state to speed up ET, suggesting OMDCs mediate the vectorial ET via its static active state under non-equilibrium condition.

2P016 **再重法を用いたタンパク質力場パラメータの最適化**

Optimization of force-field parameters for protein systems by an energy-based reweighting approach

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A force field is widely used in the field of molecular simulations for biomolecular systems. In this study, we propose a new method for refining force-filed parameters, which modifies the force-field parameters to minimize the root-mean-square deviation of backbone dihedral angles in various protein structures. Here, we compare the experimental data obtained from NMR with the trajectory data obtained from molecular dynamics simulations. In order to decrease the simulation cost, we also apply an energy-based reweighting approach to a molecular dynamics trajectory to efficiently screen a large number of trial force fields. By using this approach, we can refine the backbone-torsion-angle parameters for each amino acid at once.

2P017 **ヘモグロビンの酸素結合に伴うアロステリック転移のカメレオンモデルによる研究**

A simulation study with the chameleon model: The allosteric transition of hemoglobin associated with oxygen binding

Yui Sobue, Toru Kimura, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Univ. Nagoya*)

To elucidate the mechanism of the allosteric transition of hemoglobin, we have performed molecular dynamics simulation using a newly developed potential model, ‘the chameleon model’. In this model, the most stable position of locally defined potential is switched according to whether the local structure around it is similar to that in the T or R state. This gives rise to the global structural transition which is sensitively controlled by the subtle change of distance between histidine and Fe atom. Using this model system, we will discuss the molecular details of transition state in the allosteric transition process dependent on oxygen binding by characterizing the average structure of the transition state ensemble and the correlation of the movement of each residue.

2P018 **天然変性タンパク質の結合と共役した折りたたみ部位の相互作用解析**

Contact analysis of Protean Segments (ProSs) in intrinsically disordered proteins (IDPs)

Divya Shaji[1], Takayuki Amemiya[1], Satoshi Fukuchi[2], Motonori Ota[1] ([1]*Grad. Schl. of Info. Sci., Nagoya Univ.*, [2]*Fac. Eng. Maebashi Inst., Tech.*)

Intrinsically disordered proteins (IDPs) lack a stable, tertiary structure. Many regions of disordered proteins can undergo disorder to order transitions upon binding to their partners, known as coupled folding and binding mechanism. We call these regions Protean segments (ProSs). A dataset of ProSs was extracted from IDEAL database. The ProSs structures were classified into surface, interior and interface (core, rim and support). We calculated the average number of internal and external contacts of ProSs, their interaction partners and heterodimers. The results indicated that ProSs have less number of internal contacts and high number of external contacts than interaction partners and heterodimers. We will also discuss the binding efficiency of ProSs.

2P019 Hras-GTP 複合体と Hras-GDP 複合体の分子動力学シミュレーションにおける水分子ネットワークの解析
Analysis of network of water molecules in molecular dynamics simulations of Hras-GTP and GDP complexes

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In order to understand the mechanism of hydrolysis of GTP in the Hras-GTP complex, we study the structures of Hras-GTP and GDP complexes in water solvent by molecular dynamics (MD) simulations.
We evaluated the potential parameters and the atomic charges 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 angular distribution of water around GTP is different from the distribution around GDP. We proposed that this difference of causes the difference of hydrolyzability between GTP and GDP.
In this study, we analyze the network of water molecules using the site-dipole introduced by Higo et al.

2P020 分子動力学シミュレーションによる GLP-1 の最適構造探索
Optimized structure study of GLP-1 by Molecular Dynamics Simulation

Sakiko Mori, Hironao Yamada, Masaki Fukuda, Takeshi Miyakawa, Ryota Morikawa, Masako Takasu, Takuya Watanabe (*School of Life Sciences, Tokyo University of Pharmacy and Life Sciences*)

We have performed simulated annealing of glucagon-like peptide-1-(7-36)-amide (GLP-1) with molecular dynamics approach using GROMACS software package. GLP-1, a 30 amino acid peptide with the sequence: HAEGTFTSDVSSTLSGQAAKEFIAWLVKGR [Protein Data Bank at RCSB code: 1D0R], has the function to increase insulin secretion.
Simulated annealing is a method of optimizing the protein structure. We analyze Coulomb energy of the system without the interaction between water molecules because the energy between water molecules is much larger than the energy concerning proteins, and we observe the secondary structures.

2P021 エネルギー表示溶液理論を用いた 分子動力学シミュレーションによる蛋白質複合体モデルの評価
Evaluation of protein complex model using molecular dynamics simulation with the solution theory in the energy representation

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In protein-protein complex prediction, $10^3 \sim 10^4$ complex structure models are typically generated and evaluated. Accurate evaluation of generated complex models is a key to predict correct complex model. We recently developed a method to evaluate binding free energy differences of generated complex models through molecular dynamics simulation using the solution theory in the energy representation. Using this method, we can select “near-native” models, which are similar to crystal structure, as low energy structures. To consider a larger number of complex models, we are developing a procedure to combine the free energy evaluation with a method in which $\sim 10^6$ complex models can be clustered and reranked.

2P022 チオエステル周辺の AMBER 力場の開発および評価
Determination and evaluation of AMBER force field parameters for thioester

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Sulfur atoms frequently play important roles in biomolecules. Several enzymes include acetylcysteine or acetyl CoA in the active center, and these molecules contain a thioester sulfur. In this study, the AMBER force field parameters around the sulfur atom of the thioester moiety were determined by high-accuracy quantum chemical calculations. Because the atomic charges are mostly indispensable to classical force field calculations, the atomic charges of acetylcysteine were also calculated. To evaluate the newly determined parameters, quantum chemical and molecular mechanical calculations of short peptides including acetylcysteine were carried out, and the results were compared to evaluate the new parameters.

2P023 アミロイドβの構造探索
Conformational Search of Amyloid β Peptide

Satoshi Yokojima (*Sch. Pharmacy, Tokyo Univ. Pharmacy and Life Sci.*)

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 carry out MD simulations to explore the conformational space of an amyloid β peptide to understand the possible structures and the build-up mechanism.

2P024 Photo synthesis of protein-based drug delivery nanoparticles for active tumor targeting

Meng Qin (*Department of Physics, Nanjing University*)

In this work, we present a novel method to photo synthesize protein based drug delivery system with active targeting function to tumor site. The disulfide bonds in protein bovine α-lactalbumin (BLA) can be ruptured by controlled UV illumination, which triggers the formation of nano-sized protein aggregates and releases free thiol groups for the modification of active targeting ligand of circular RGD. The synthesis approach is very convenient and cost-effective and can be accomplished in physiological condition. Both in vitro and in vivo experiments validate that this DDS system possesses much greater drug delivery efficiency to the tumor sites and better inhibition capability to tumor growth than the unmodified counterparts.

2P025 Single molecule force spectroscopy reveals force-enhanced binding of calcium ions by gelsolin

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Force is increasingly recognized as an important element in controlling biological processes. Forces are able to deform native protein conformations leading to protein-specific effects. Here we demonstrate that the calcium-binding affinity of the actin-binding protein gelsolin domain G6 is enhanced by mechanical force. Using a recently developed single molecule-binding assay based on atomic force microscopy, we establish that the calcium-binding affinity of G6 increases exponentially with the applied force, up to the point of G6 unfolding. This implies that gelsolin will be activated at lower calcium ion levels when subjected to tensile forces and suggests a basis for enhanced cooperativity during multi-cation induced activation.

2P026 Direct observation of the multiple sliding modes of a tumor suppressor p53

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1D sliding of a tumor suppressor p53 along DNA is an essential dynamics for its effective search of the target sequence. The 1D sliding of p53 has been assumed to be described as a single diffusive movement. However, it is conceivable that p53 has multiple sliding modes depending on the quaternary structures of the p53-DNA complex. In this study, the sliding of p53 along DNA was measured by a single-molecule fluorescence microscopy. The observed trajectories of p53 were analyzed by the change point analysis, which can detect boundaries of trajectories with different diffusion constants. Our results showed that p53 possesses at least three different diffusion modes, and further suggests that the quaternary structures of the p53-DNA complex modulate the sliding modes.

2P027 Study of a peptidase-associated domain of an aminopeptidase from thermophilic *Aneurinibacillus* sp. AM-1

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A thermophilic bacterium *Aneurinibacillus* sp. AM-1 produces an aminopeptidase (AM1AP) belonging to M28 family. The enzyme shows strong prolyl aminopeptidase activity toward prolyl-*p*-nitroanilide. X-ray crystal structure of AM1AP revealed that the enzyme is composed of a peptidase domain (PD, 31 kDa) and a peptidase-associated domain (PAD, 14 kDa). PD, typical for M28 family enzymes, contains an active site with two zinc atoms, whereas PAD widely occurs in peptidases/proteases beyond this family. To date, the roles of PAD is undefined yet. To elucidate its roles, PD and PAD was separately expressed in *E. coli*, and purified, and then their molecular properties (specific activity, gel filtration, CD in the presence and absence of zinc ion, etc) were investigated.

2P028 ケモカインシグナル細胞内制御因子 FROUNT とその受容体認識に関する構造生物学的研究
Structural analyses of FROUNT, the cytosolic regulator of chemokine signaling, and its chemokine receptor recognition

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Chemokine-dependent migration of the leukocytes to sites of inflammation is essential for immune defense, but also causes autoimmune inflammatory diseases. We identified a cytosolic regulator of the chemokine signaling, FROUNT, which specifically binds to the C-terminal regions (Pro-Cs) of chemokine receptors CCR2 and CCR5. The aim of this study is to elucidate the Pro-C recognition mechanism of FROUNT. We revealed the three-dimensional structure of the chemokine receptor-binding domain of FROUNT (FNT-C) and its Pro-C binding site by NMR. We built a docking model of FNT-C and Pro-C, using the Pro-C structure we previously determined. Current studies of the FROUNT-CCR2/5 interaction provide a structural basis to develop anti-inflammatory agents with high specificity.

2P029 トウガレイ由来 I 型不凍蛋白質の構造機能解析
Analysis of structure and function of a new type I antifreeze protein from a Japanese fish, Barfin Plaice

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Antifreeze proteins (AFP) bind onto an ice crystal to arrest its crystal growth within a temperature range, which was called thermal hysteresis (TH). An alpha-helical type 1 AFP from winter flounder (denoted WfAFP) exhibited 1 degC of TH at its solubility limit. In contrast, hyperactive AFPs from insects and bacteria exhibited higher TH values (ex. 3 - 5 degC). Here we found that a 40-residue AFP from Barfin Plaice (BpAFP) exhibited approximately 3 degC of TH. It has 80 % of sequence identity with WfAFP, while the mechanism of such high activity is unknown. Here we present biochemical properties of BpAFP, for which multinuclear multidimensional NMR experiments were performed for a 13C and 15N-labeled protein of BpAFP.

2P030 ジスルフィド結合が制御するバクテリア SOD1 の構造形成メカニズム
Folding mechanism of bacterial SOD1 regulated by disulfide formation

Yasuyuki Sakurai, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) is an enzyme that scavenges superoxide radicals, and is conserved among aerobic organisms. To gain catalytic activity, SOD1 requires two post-translational modifications: binding of a copper ion and formation of a disulfide bond. In eukaryotes, these modifications are carried out simultaneously by a copper chaperone, CCS. In bacteria, however, no CCS homologue is present, and the activation mechanism of bacterial SOD1 remains unknown. Here we show that, in *E. coli* SOD1, formation of the disulfide bond precedes binding of metal ions and is crucial for attaining a structure that is resistant to degradation *in vivo*. In bacteria, a pool of apo-SOD1 with disulfide would thus exist waiting for a copper ion to become enzymatically active.

2P031 線虫モデルを利用した神経変性疾患における病態伝播のメカニズム解明

A worm model describing propagation of protein aggregates in neurodegenerative diseases

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Fibrils caused by protein misfolding have the tendency to propagate inter/intracellularly, a mechanism known as "seeding". Here, we investigated the seeding mechanism of Cu,Zn-superoxide dismutase (SOD1), which is known to trigger amyotrophic lateral sclerosis (ALS) when mutation occurs in the gene. An ALS-causing mutant, SOD1L126X, was used to test whether it forms aggregates in vitro with the addition of seeds. We confirmed that adding insoluble seeds to its soluble counterpart accelerates aggregation process in vitro. Also, we are currently using C. elegans to further understand the seeding reaction of SOD1 in a living organism. Worms expressing mutant SOD1 will become a new model of seeded aggregation in vivo.

2P032 筋萎縮性側索硬化症に関わる SOD1 タンパク質の四次構造変化を検出できるペプチドの開発

Aberrant monomer-dimer equilibrium of mutant SOD1 in ALS: Development of peptides probing protein quaternary structures

Takao Nomura, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) protects aerobic organisms from superoxide by catalyzing its disproportionation but gains toxic properties by mutations identified in a familial form of amyotrophic lateral sclerosis. Pathogenic mutations have been considered to facilitate the monomerization of SOD1, which usually forms a tight homo-dimer. It is, however, difficult to clarify effects of mutations on the quaternary structure of SOD1 *in vivo/ex vivo*. Here, we have developed peptides discriminating monomeric and dimeric SOD1 by using a phage display method. The peptides were found to bind exclusively to either a monomeric or dimeric state of SOD1 with a sub-micromolar dissociation constant, enabling a pull-down assay to evaluate the SOD1 quaternary structures.

2P033 SOD1 への細胞内銅イオン輸送を制御するタンパク質ネットワーク

A protein network regulating an intracellular copper transfer to superoxide dismutase

Kenta Nakagome, Yasushi Mitomi, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) binds a catalytic copper ion for its antioxidant activity, which is delivered by a copper chaperone for SOD1 (CCS) *in vivo*. CCS is composed of three domains, among which central and C-terminal domains play critical roles in recognition and activation of apo-SOD1, respectively. In contrast, physiological roles of the N-terminal domain (CCS^{dI}) remain obscure. Given that CCS^{dI} possesses a ferredoxin-like fold with a CxxC motif for binding a cuprous ion, we suspected if CCS^{dI} functions as a receptor site for a copper ion and thus involves in the activation of SOD1. Here we have found a certain ferredoxin-like protein that appears to exchange a copper ion with CCS^{dI}, suggesting a new intracellular pathway for copper supply to SOD1.

2P034 亜鉛イオンが制御する銅シャペロンシステムの分子認識メカニズム

Zinc ion regulates molecular recognition in copper chaperone system

Yuma Wakahara, Kazuki Honda, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) catalyzes the disproportionation of superoxide radicals at its bound copper ion, which is supplied by a copper chaperone for SOD1 (CCS) *in vivo*. CCS is composed of three domains, among which the central domain (CCS^{dII}) plays critical roles in recognizing the substrate, apo-SOD1. CCS^{dII} assumes a similar structural fold to that of SOD1 and, more interestingly, possesses a binding site for a zinc ion. Here we show that CCS^{dII} is required to bind a zinc ion for specific recognition of apo-SOD1. Zinc binding exerts a conformational change in CCS^{dII}, which enables to form a tight complex with apo-SOD1. It is hence possible that *in vivo* transfer of a copper ion from CCS to SOD1 is regulated by a heterogeneous metal ion, *i.e.* zinc ion.

2P035 筋萎縮性側索硬化症に関わる変異型 SOD1 タンパク質のオリゴマー化メカニズム

Oligomerization mechanism of mutant SOD1 proteins in a familial form of amyotrophic lateral sclerosis

Itsuki Anzai, Keisuke Toichi, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ*)

Dominant mutations in Cu,Zn-superoxide dismutase (SOD1) cause a familial form of amyotrophic lateral sclerosis (fALS). Wild-type SOD1 forms an intramolecular disulfide, while pathological inclusions contain mutant SOD1 oligomers cross-linked via disulfides. Recently, we have shown that isomerization from an "intra"- to an "inter"-molecular disulfide bond is facilitated by destabilization of SOD1 by mutations. Based upon our proposing mechanism, we attempted to develop anti-oligomerization drugs effective to mutant SOD1, which may be a cure for SOD1-fALS. Several drugs screened from a drug library have been found to inhibit SOD1 oligomerization by structural stabilization of SOD1. Detailed examination on interactions of those drugs with SOD1 is now underway.

2P036 神経変性疾患における老化の役割を検証する酵母モデルの構築

A yeast model for testing roles of aging process in neurodegenerative diseases

Yuko Nishiura, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Most of neurodegenerative diseases are characterized by its late onset and specific deterioration of a nervous system. Damages would accumulate in non-proliferating neurons with aging and then exceed a certain threshold to cause neurodegeneration. Pathological roles of aging in neurodegenerative diseases, however, remain obscure partly due to its time-consuming aspect. Here, we have attempted to reveal effects of aging on pathologies of amyotrophic lateral sclerosis by using the budding yeast, S.cerevisiae, as a model organism. The chronological aging of yeast as a survival of its non-dividing state can be a convenient model for the aging process in post-mitotic cells. Biochemical changes of pathogenic proteins by aging will be discussed.

2P037 蛋白質のミスフォールド状態から生じる蛋白質の異常凝集
Misfolding triggers a pathogenic conversion of protein conformations

Soichiro Kitazawa[1], Ryo Kitahara[1], Makoto Urushitani[2] ([1]*Pharmaceutical science, Ritsumeikan Univ.*, [2]*Moleclar Neuroscience Research Center, Shiga University of Medical Science.*)

Although conformational fluctuations of a protein among different states have been recognized to be important for protein function, such fluctuations may trigger a pathogenic conversion of protein conformations. Aggregation of a nuclear protein TDP-43 in abnormal cytoplasmic inclusions in motor neurons is a pathological signature of sporadic ALS. Here, we demonstrate that pressure perturbation accelerates an irreversible misfolding of RNA recognition motif-1 (RRM1) of TDP-43, which triggers aggregation of TDP-43. NMR spectroscopy revealed structure and dynamic characteristics of the misfolded conformation of RRM1.

2P038 べん毛輸送装置構成蛋白質 FliP ペリプラズミックループの結晶化と遺伝学的解析
Crystallization and genetic analyses of a periplasmic loop of FliP, a component of the flagellar protein export apparatus

Takuma Fukumura[1], Yumiko Saijo-Hamano[1], Yukio Furukawa[1], Tatsuya Kawaguchi[2], Katsumi Imada[2], Keiichi Namba[1], Tohru Minamino[1] ([1]*Grad. Sch, Frontier Biosci., Osaka Univ*, [2]*Grad. Sch. Sci. Osaka Univ*)

Most of flagellar proteins are transported by a specific export apparatus from the cytoplasm to the growing distal end of the flagellum where they self-assemble. The export apparatus consists of a water-soluble ATPase complex and a proton-driven export gate made of six membrane proteins. FliP is one of the component of the gate complex. FliP is predicted to have four transmembrane helices (TM) and has a relatively large periplasmic loop (FliPp) between TM-2 and TM-3. Genetic analysis showed that the C-terminal region of FliPp, especially Glu-178, is important for the export function. We overexpressed and purified Thermotoga maritime FliPp and found that FliPp forms homo tetramer. We also succeeded in crystallizing FliPp, and the crystal diffracted to 2.2Å resolution.

2P039 Structural defects in fibrillin associated with Marfan syndrome

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Fibrillin (FBN) is a 350kDa glycoprotein vital for formation of elastic and non-elastic fibres in connective tissue. A FBN protein complex also controls TGF-β bioavailability. FBN mutations cause Marfan syndrome (MFS), leading to both defective microfibrils and dysregulation of TGF-β signalling. FBN1 consists of repeating epidermal growth factor (EGF) and TGF-β-binding protein (TB) domains. Paired EGF domains bind Ca2+ for structural rigidity. 600 MFS mutations in FBN have been classified in terms of their structural and functional consequences. A large majority of missense mutations alter EGF tandem repeat structural rigidity, by either altering disulphide formation, Ca2+ binding or domain interaction. Some mutations in TB domains alter storage of latent TGF-β.

2P040 Microtubule-associated protein 4-mediated bundle formation of microtubules and actin filaments

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Microtubule-associated protein (MAP) 4 is a non-neural MAP that plays important roles in the intracellular organization of microtubules. Recent study revealed that MAP4 also binds to actin filaments at the C-terminal part of the Proline-rich region in the microtubule-binding domain (Matsushima et al., J. Biochem., 2012). In this study, we examined MAP4 functions when both actin filaments and microtubules coexisted in a sample as well as inside cells by fluorescence microscopic observation and coprecipitation assay. The results showed that MAP4 bound to microtubules lost actin filament-binding ability. On the other hand, MAP4 bound to actin filaments retained microtubule-binding and microtubule assembly-promoting abilities.

2P041 タンパク質の圧電効果とアロステリック制御
Piezoelectric effect in a protein and its involvement in allosteric regulation

Jun Ohnuki[1], Takato Sato[1], Koji Umezawa[1], Taro Q.P. Uyeda[2], Mitsunori Takano[1] ([1]*Grad. Scl. of Adv. Sci. & Eng., Waseda Univ.*, [2]*Biomedical Res. Inst., AIST*)

Intermolecular interaction of proteins is often regulated by mechanical strain. For instance, the affinity between myosin and actin filament is affected by the internal strain of myosin due to lever-arm swing or by the strain imposed on the actin filament. However, its molecular mechanism is not well-established. By conducting molecular dynamics simulation, we first investigated the response within the myosin molecule that accompanies forced lever-arm swing, and discovered an electric charge response in the actin-binding region via large-scale hydrogen-bond rearrangements: myosin behaves like a piezoelectric actuator. We will examine if this piezoelectricity actually affects the myosin-actin interactions, and if it also occurs in actin filaments.

2P042 MD で観測された G アクチンのヌクレオチド依存構造状態と F アクチン安定性との関連
Nucleotide-dependent structural states of G-actin observed by MD simulation and its implication for F-actin stability

Jun Ohnuki, **Mitsunori Takano** (*Dept of Phys & Appl Phys, Waseda Univ*)

Actin treadmill is fundamental to cellular motility, which is based on ATP-consuming transformation between monomeric (G) and filamentous (F) states. It is obvious that the ATP energy drives treadmill, but how it is used is unclear, even though the structural change upon ATP hydrolysis, which is accelerated in F-state, is likely to hold the key. We thus conducted MD simulation of G-actin to investigate intrinsic structural difference between the ATP and ADP-bound states. Extensive MD simulation allowed us to obtain statistically-definite results: ADP-bound actin is more rigid and less conforming to F-state, indicating how F-actin is destabilized upon ATP hydrolysis. By analyzing hydrogen-bond network we further clarify how this difference arises.

2P043 アロステリック機構の分子論的理解に向けたシグナルタンパク質 CheY の研究

Toward a molecular level understanding of allostery in the signaling protein CheY

Toshifumi Mori, Qiang Cui (*Univ. of Wisconsin, Madison*)

Allostery is one of the most important regulatory mechanisms of cellular processes, but the detailed dynamic events that underlie allosteric conformational transitions remain largely unknown. CheY is a response regulator protein that exhibits allosteric transitions upon phosphorylation, and recent NMR experiments suggested that it does not follow the conventional two-state switching mechanism. In this work, using μs-MD simulations and various recently developed analysis techniques, we have identified the structural coupling network responsible for allosteric communication. We will further discuss the μs~ms switching mechanism of the leading residues by characterizing the relevant free energy landscape and comparing the results to state-of-the-art NMR studies.

2P044 MARTINI 粗視化力場を用いたタンパク質-リガンド結合過程の比較シミュレーション

Comparative simulations of protein-ligand binding processes using the MARTINI coarse-grained force field

Tatsuki Negami, Tohru Terada, Kentaro Shimizu (*Grad. Sch. of Agri. and Life Sci., Univ. of Tokyo*)

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 have shown that the ligand-binding processes can be reproduced in coarse-grained (CG) simulations with MARTINI. In this study, we classified protein-ligand complex structures in PDB into groups according to the physicochemical and geometric properties of the ligands and the ligand-binding pockets. Then, the CG simulations were performed for a representative protein-ligand pair from each group. We will discuss the effects of these properties on the ligand-binding processes.

2P045 粗視化モデルによる PPARγ の基質依存的な活性変化の考察

Coarse-grained model study of ligand-dependent reaction activity of PPARγ

Tomo Matsubara, Hiraku Nishimori, Akinori Awazu (*Dept. of math and Life Sci, Hiroshima Univ*)

PPARγ is a nuclear receptor of transcription factors controlling genes implicated in Antidiabetic effect and Bone metabolism. Recent experimental studies reported that PPARγ binds with some ligands and its activity is influenced by the ligand properties. However, the recent X-ray crystal structure analysis implies the structure of PPARγ is almost independent of ligand types. This fact indicates the ligand modifies not the structure but the dynamics of PPARγ, and such dynamics is important for its activity.

Then, we construct and analyze a coarse-grained model of PPARγ. By the normal mode analysis and the molecular dynamics simulation, we identify the ligand-dependent effective intra-molecular interaction network and its contribution to the molecular function.

2P046 構造変化を介した分子内情報伝達パターンの探索: 粗視化分子動力学計算による試み

Screening for Mechanical Communication in Proteins by Coarse-Grained Molecular Dynamics

Yuichi Togashi (*Grad. Sch. Sys. Informat., Kobe Univ.*)

Molecular machines such as motors operate through their conformational changes. Some of them can respond to external stimuli (e.g. forces) and change their properties. As they are typically a huge molecule or complex with multiple subunits, communication inside the molecule or complex is essential for their concerted operation. To probe mechanical communication, we employed steered molecular dynamics simulations using coarse-grained elastic network models. We applied static forces in many randomly-chosen directions to each residue, to show transmission patterns of mechanical perturbations. As an application of this method to a variety of structural data, here we present screening for proteins with a certain mode of mechanical communication.

2P047 粗視化シミュレーションによるリン酸化酵素複合体(MEK1-ERK2)のドッキングダイナミクス

Docking dynamics of MAP kinase: MEK1-ERK2 complex system studied by coarse-grained simulation

Ryo Kanada, Shoji Takada (*Grad. Sch. Sci., Univ. Kyoto*)

MAP kinases are central transducers of extracellular signals from hormones, growth factors, cytokines, and environmental stresses. Experimentally, the most studied mammalian MAP kinase pathway is the Raf/MEK/ERK pathway. However the molecular (detailed) mechanism for activation (phosphorylation) of ERK2 by MEK1 is still unknown, because the complex structure for MEK1-ERK2 system is not available. So, in this study, to investigate the stable complex structure of the MEK-ERK system and their docking dynamics which is required for their efficient signal transmission, we apply the atomistic interaction based coarse-grained (AICG) model in which the parameters of interaction are physico-chemically well tuned depend on amino-acid sequence (and secondary structures).

2P048 酵母 MAPK 経路における伝達制御機構の分子シミュレーション研究

Molecular simulation study on signaling control in yeast MAPK pathway

Naoto Hori, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

Signal transmission is important and highly regulated in the cell. In yeast, pheromone response is transmitted through a MAPK pathway, a cascade of phosphorylation of signaling proteins; Ste11, Ste7, and Fus3. Ste7 is commonly used in another pathway and thus its activity and target are precisely controlled to prevent crosstalk. Experimental studies have suggested that a scaffold protein, Ste5, has crucial roles in that regulation. To reveal the entire mechanism at the molecular level, we performed a series of coarse-grained MD simulations. Particularly we focused dynamics of a long tail at the N-terminus of Ste7, which contains Fus3-binding motifs. Our simulations show how the phosphorylation state and/or the scaffold protein affect the accessibility of substrates.

2P049 Folding coupled with binding and allosteric motions in calmodulin domains

Wenfei LI[1], Wei WANG[1], Shoji Takada[2] ([1]*Department of Physics, Nanjing University*, [2]*Graduate School of Science, Kyoto University*)

Most proteins need to fold for functioning. Previously, the folding studies mostly focus on the proteins with unique native structure. However, there are a large number of functionally important allosteric proteins which can have two functional structures, depending on the binding state of cofactors. For these proteins, co-factor binding, conformational changes and folding can be tightly coupled, but the coupling mechanism is still unclear. In this work, by using the atomic interaction based coarse grained (AICG) model with a multiple-basin Hamiltonian, we study the folding coupled with binding and allosteric motions in calmodulin domains. In this meeting, we will present the details of this work.

2P050 FTIR 分光法を用いたユビキチンの温度-圧力変性状態の研究
Pressure and temperature denaturation of ubiquitin by FTIR spectroscopy

Tsubasa Yamamoto[1], Minoru Kato[1,2] ([1]*Grad. Sch. of Lifescience, Ritsumeikan Univ.*, [2]*Dept. Pharma. Ritsumeikan Univ.*)

The secondary structure of ubiquitin is known to be constant around various pH, however its thermodynamic stability and FTIR spectrum drastically change on the boundary at pH 4. This fact indicate that minor conformation and hydrogen bond pattern of ubiquitin change by pH, and these difference may affect to thermodynamic properties.
We observed pressure and temperature denaturation of ubiquitin by FTIR spectroscopy at pD (alternate of pH in heavy water) 2 and pD 5. Ubiquitin at higher pD was more stable than at lower pD. Moreover, the pressure denaturation process at higher pD showed at least three-state transition, while the denaturation at lower pD showed two-state transition.

2P051 FTIR を用いた圧力・温度可変実験による GB1(41-56)の変異体の β-hairpin 構造安定性
Pressure and Temperature variable FTIR study on the structural stability of β-hairpin model peptides of mutants of GB1(41-56)

Keita Tsuchiya[1], Yudai Yamaoki[2], Minoru Kato[1,3] ([1]*Grad. Sch. life science, Univ. Ritsumeikan*, [2]*Institute of Advanced Energy, Univ. Kyoto*, [3]*Pharm. Univ. Ritsumeikan*)

In general, protein folds into a unique structure and the structure unfolds by perturbing temperature and pressure. In the present study, we focus on the β-haripin structure, which is the simplest β-structure. Previously, we have reported that Trpzip4 (W43W45W52W54) which is a triple mutant of GB1 segment peptide (W43Y45F52V54) refolded by pressure. In the present work, we have expanded the research project to the other kinds of mutants, W43W45F52W54 (WWFW) and W43W45Y52W54 (WWYW). We observed the temperature and pressure effect on these peptides using FT-IR and CD spectroscopy. We compare the results and discuss the substitutional effects on the structural stability of the β-hairpin structure.

2P052 ペプチドにおける二次構造の圧力依存性：焼き戻し分子動力学法による研究
Pressure dependence of the secondary structure of a peptide: A simulated tempering molecular dynamics study

Yoshiharu Mori[1], Hisashi Okumura[1,2] ([1]*Inst. Mol. Sci.*, [2]*SOKENDAI*)

Pressure effect on the structure of proteins and peptides has 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 pressure effect on a peptide that is stable at high pressure by using molecular dynamics simulations.
We performed simulated tempering molecular dynamics simulations for the system of an AK16 peptide in explicit water molecules. We found that the population of the secondary structure of the peptide increases with pressure and that the peptide shrinks under high pressure conditions. We also calculated the partial molar volume change, and the calculated results were consistent with experimental results.

2P053 アミノ酸の物性に注目した疾患感受性遺伝子変異の判別
Discrimination of disease-susceptibility mutations by physical properties of amino acid fragments around the mutation

Ryouta Masai[1], Shigeki Mitaku[1,2] ([1]*Dept. Applied Physics, Grad. Sch. Engineering, Nagoya Univ*, [2]*Toyoda Physical and Chemical Res. Inst.*)

There are double conditions for the disease-susceptibility of mutations: the change in the activity of protein function, and the change in the phenotype. We studied the correlation between the disease-susceptibility and the physical properties of amino acid fragments around the mutations, using the data for secretory proteins. The parameter, AUF, which we previously developed for indexing the molecular recognition of proteins, showed good correlation with the disease-susceptibility. Several other properties such as hydrophobicity, electric charge density and the density of aromatic amino acids showed minor but significant correlation. The results suggested that the physical properties of amino acid fragments are responsible for the disease susceptibility.

2P054 シトクロム *c* 多量体の細胞膜結合
Binding of Oligomeric Cytochrome *c* to Cell Membrane

Sendy Junedi, Kazuma Yasuhara, Satoshi Nagao, Jun-ichi Kikuchi, Shun Hirota (*Grad. Sch. Mat. Sci., Nara Inst. Sci. Tech.*)

Domain-swapped oligomeric cytochrome *c* (cyt *c*) possesses many positively charged lysine residues at its protein surface. Although the biological properties of monomeric cyt *c* have been investigated in detail, the properties of oligomeric cyt *c* have not been elucidated. We found that oligomeric horse cyt *c* binds to the plasma membrane of HeLa cells stronger compared to monomeric cyt *c*. Binding of cyt *c* oligomers to the negatively charged liposome (dipalmitoylphosphatidylglycerol/ dimyristoylphosphatidyl choline) induced phase separation in the membrane, and binding of oligomers to HeLa cells caused cell morphological changes, whereas the monomer did not. We conclude that cyt *c* oligomers bind to the negatively charged lipids in the cell membrane and disrupt the membrane.

2P055 シトクロム c のドメインスワップ多量化とモルテングロビュル状態

Domain-Swapped Oligomerization and Molten Globule State of Cytochrome c

Megha Deshpande[1], Partha Parui[2], Masaru Yamanaka[1], Satoshi Nagao[1], Hironari Kamikubo[1], Mikio Kataoka[1], Hirofumi Komori[3], Yoshiki Higuchi[4], Shun Hirota[1] ([1]*Graduate School of Materials Science, Nara Institute of Science and Technology*, [2]*Department of Chemistry, Jadavpur University, Kolkata 700032, India*, [3]*Faculty of Education, Kagawa University*, [4]*Department of Life Science, Graduate School of Life Science, University of Hyogo*)

We have previously shown that horse cytochrome c (cyt c) forms oligomers by treatment with ethanol. The C-terminal α-helix was swapped in the cyt c dimer. In the present study, we found that domain-swapped oligomeric cyt c forms after refolding cyt c from its molten globule state. More cyt c oligomers were obtained by increasing the protein concentration at the molten globule state. More oligomers were also obtained from the molten globule state with higher concentration of salt, such as NaCl and $NaClO_4$. The formation of oligomeric cyt c from the molten globule state was more sensitive to $NaClO_4$ compared to NaCl. These results show that domain-swapped oligomeric cyt c forms from its molten globule state, at which ions play an important role.

2P056 粗視化分子動力学シミュレーションによるミオグロビンのドメインスワッピング機構の研究

Domain swapping of myoglobin dimer studied by coarse-grained molecular dynamics simulations

Koji Ono, Shoji Takada (*Dept. Biophys., Grad. Sch. Sci., Kyoto Univ.*)

Self-organization of proteins are related to cellular functions as well as diseases, such as Alzheimer's disease. Some of those proteins form oligomers, such as dimer and trimer, exchanging their identical domains, which are called the domain swapping. Hirota group solved a crystal structure of the domain swapped myoglobin dimer, that was formed in certain solvent conditions. Here, using coase-grained molecular dynamics (CGMD) simulation which treat an amino acid as a bead, we study folding and self-organization to the domain-swapped form of apo-myoglobin dimer.
In CGMD, based on the AICG2+ interaction model which includes atomic-interaction based bias Go-like terms, amino acid. Iwe extended it to included inter-chain interactions in a symmetric form.

2P057 Wang-Landau マルチカノニカル法による Go モデル分子動力学シミュレーション

Wang-Landau Multicanonical Method for Go-model Molecular Dynamics Simulation

Mashiho Ito, Shoji Takada (*Dept. Biol., Sch. Sci., Kyoto Univ.*)

Molecular dynamics (MD) simulation by Go model can accelerate calculation of folding/unfolding process of proteins. However, for large proteins, it is difficult to sample entire folding pathways reversibly. Here we introduced the Wang-Landau multicanonical method for Go model MD simulations. By this method, we can obtain free energy surface of protein folding for larger proteins than before. Now we are working on comprehensive analysis of the data set from the Protein Data Bank (PDB).

2P058 粗視化 MD を用いた SUFI のコ・トランスレーショナルフォールディングの解析

Analysis of co-translational folding of SUFI by coarse grained MD simulation

Tomohiro Tanaka, Naoto Hori, Shoji Takada (*Dept. of Biophys. Kyoto Univ.*)

Proteins are synthesized gradually from N-terminus and extruded from ribosome. Nascent proteins start folding during synthesis, which is termed co-translational folding. Interestingly, the rate of translation is not uniform, but is affected by tRNAs concentration and others. It has been found that the correct folding of SUFI, a three-domain protein, is maintained by particular use of low-abundance tRNAs.
Here, using a coarse grained model we simulated 1) post-translational, 2) co-translational(uniform rate), and 3) co-translational(non-uniform rate) folding of SUFI.
The success ratio of folding in co-translational conditions was much higher than that in post-translational condition. We addressed the differences in folding mechanisms among these conditions.

2P059 粗視化 Go モデルを用いた多状態タンパクにおける遷移の回数とフォールディングコアとの関係の解析

An analysis of the relationship between the number of transitions and folding cores in multi-transition proteins by means of Go model

Masatake Sugita, Takeshi Kikuchi (*Dept. of Bioinfo., Col. of Life Sci., Ritsumeikan Univ*)

Understanding how each transition occurs has been an important issue to elucidate the common view of folding mechanisms and there are many models trying it. In our previous work, we showed that the number of transition in the Ferredoxin-like fold proteins can be explained by the number of core regions which fold cooperatively by means of coarse-grained Go model. In this work, we apply the same method with previous work to other proteins which are suggested to fold into the native structures through multiple transitions and affirm whether we can reproduce multi-state transition or not. We also investigate that the characteristics of the regions to contribute to barrier constructions.

2P060 タンパク質フォールディングにおける自由エネルギーバリアと天然トポロジー間の関係

Relationships between the free energy barrier in protein folding and native topology

Koki Yamashita, Masatake Sugita, Takeshi Kikuchi (*Dept. Bioinf., Col. Life Sci., Ritsumeican Univ.*)

The free energy landscape for protein folding is not fully understood yet although many studies on folding mechanisms have been performed by both experiments and computations. In this decade, it has been revealed that a native topological index such as RCO of a protein correlates with its folding rate. However, the relationships of the number of free energy barriers with native topology do not clarify. In this study, we investigate whether the native topology of a protein governs the height of the folding free energy barrier based on our original coarse grained Go model. We also investigate the relationship between number of free energy barriers and native topology. The results show the correlation between the height of a barrier and RCO as it has been observed so far.

2P061 一分子蛍光分光法によるユビキチンの折り畳みダイナミクスの測定

Dynamics of ubiquitin folding detected by single molecule fluorescence spectroscopy

Masataka Saito[1,2], Hsin-Liang Chen[3], Rita Chen[3], Kiyoto Kamagata[1,2], Hiroyuki Oikawa[1], Satoshi Takahashi[1,2] ([1]*Tohoku University Institute of Multidisciplinary Research for Advanced Materials*, [2]*Tohoku University Department of Chemistry, Graduate School of Science*, [3]*Academia Sinica Institute of Biological Chemistry*)

The presence of the intermediate in the folding of ubiquitin is still under debate. Two distinct folding models have been proposed: two-state model and non-two state model involving the intermediates. To reveal each state hidden by the ensemble averaging in a conventional method, we investigated the folding dynamics of ubiquitin at a single molecule level by using a line confocal microscope coupled with a microfluidic tip. We used the mutant of ubiquitin labeled with two different dyes. More than 100 time series of the FRET efficiency were obtained at several GdnHCl concentrations. Our data clearly indicate that the folding intermediate whose FRET efficiency is located between the native and denatured states populates significantly at a moderate GdnHCl concentration.

2P062 プロテイン A - B ドメインの高速折り畳みダイナミクスの追跡を目指したライン共焦点顕微鏡の改良

Improvements of the line confocal system for the single molecule tracking of fast folding dynamics of the B domain of protein A

Hiroyuki Oikawa[1], Kiyoto Kamagata[1], Munehito Arai[2], Satoshi Takahashi[1] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Arts. Sci., Univ. Tokyo*)

Single-molecule fluorescence measurements with the microsecond resolution are expected to reveal the detailed process of protein folding. We recently developed a two-color line-confocal system, which enabled us to follow a time evolution of FRET efficiency from single molecules with the time resolution of ~20 μs. The time series of FRET efficiency from the B domain of protein A (BdpA) labeled with fluorophores suggest conformational heterogeneity and dynamic fluctuations in the unfolded state. For further improvement of the time resolution, we optimized the optical system, the flow speed, and the fluorophore-labeling sites of BdpA. The improvements are expected to enable the tracking the fast dynamics of folding transitions.

2P063 イソロイシンタグを付加した BPTI 変異体の熱転移における可逆的なオ リゴマー形成過程の熱力学的解析

Thermodynamic characterization of a reversible oligomerization process in the thermal transition of a BPTI variant tagged with isoleucines

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We previously developed short 3 to 9 residues tags that can control protein solubility over >100 fold concentration range. Here, we analyzed the thermal transition curve of a simplified BPTI variant (BPTI-21) tagged with 1, 3 and 5 isoleucines by DSC at concentrations of 0.3-5mg/ml and pH 4.7. All of the thermal transitions were fully reversible, and the transition temperature of BPTI-21 with 1 or 3 isoleucines did not depend on the concentration. That with 5 ones clearly decreased when the concentration increased. The inverse dependence is frequently reported for oligomer proteins. Precise global fitting analysis suggested that BPTI-21 with 5 isoleucines forms a reversible tetramer at high temperature while it is monomeric in the native state.

2P064 アミロイドと可溶性蛋白質の間の相互作用の幾つかの一般的側面

Some general aspects of interaction between amyloid and soluble proteins

Takashi Konno (*University of Fukui, Faculty of Medical Sciences, Molecular Physiology*)

Amyloid-type protein aggregates and its on- and off-pathway intermediates are formed in complex biological media, and have chances to interact with a variety of biological molecules, among which soluble proteins constitute one of the most conspicuous molecular groups. The present study focuses on this type of amyloid-protein interaction that will substantially modify appearance of the amyloid-related molecular events. Conforming diverse amyloid species and soluble proteins to simple in vitro systems, the interaction and its consequence were systematically studied by several biophysical methods. The results could elucidate some general origins of the strong interaction.

2P065 ビーズ表面に結合した細胞外マトリクス成分は、気液界面非存在下でアルツハイマー病 β アミロイド線維の核形成を促進させる

Surface-bound basement membrane components on Sepharose beads accelerate amyloid β-peptide nucleation in air-free wells

Kazuhiro Hasegawa, Daisaku Ozawa, Tadakazu Ookoshi, Hironobu Naiki (*Div. Mol. Pathol., Dept. Pathol. Sci., Univ. Fukui*)

We constructed an in vitro system to examine the hypothesis that the vascular basement membrane may act as a scaffold of amyloid β-peptide (Aβ) carried by perivascular drainage flow and accelerate its amyloid fibril formation in vivo. The basement membranes in the media of cerebral arteries were reproduced by conjugating Matrigel and other proteins on the surface of Sepharose beads. The perivascular drainage flow was reproduced by using these beads as stirrers in air-free wells containing 5 μM Aβ solution. We found that several basement membrane components accelerate the initiation of Aβ fibril growth. Our data support the essential role of proteins in vascular basement membranes in the development of cerebral amyloid angiopathy.

2P066 脂質ベシクルの疎水領域が与えるアミロイド β 線維形成への影響について

The effects of the hydrophobic area of vesicles on the fibrillation of Aβ

Mayu Suzuki, Hisashi Yagi, Yuji Goto (*Inst. Protein. Res., Osaka Univ.*)

When amyloid β peptides (Aβ) form amyloid fibrils, they interact with lipid membranes, especially lipid rafts. Many researchers investigated the interaction using the vesicles of lipids. However there are few studies considering the size and concentration of vesicles. In order to investigate their effects, we prepared the small sized vesicles with methanol and ultrasonication. A small amount of methanol didn't affect the fibrillation but stabilized the vesicles of small size. This results showed that although the low concentration of vesicles promoted the nucleation, the high concentration inhibited the fibrillation of Aβ. We also discuss the role of the hydrophobic area of the vesicles in the conformational change.

2P067 β_2-ミクログロブリンのアミロイド形成における様々な脂肪酸の効果

Effects of various fatty acids on the amyloid fibrillation of β_2-microglobulin

Akira Ishii[1], Masatomo So[1], Hisashi Yagi[1], Hironobu Naiki[2], Yuji Goto[1] ([1]*Inst. Protein Res., Osaka Univ.*, [2]*Fac. Med. Sci., Univ. Fukui*)

Amyloid fibrillation is dominated by the environmental factors. Although various additives, such as SDS, phospholipids and RNA aptamers, are known to accelerate and/or inhibit fibrillation, little is known about the mechanism how these additives effect fibrillation. Clarifying the mechanisms of acceleration and inhibition of fibrillation by additives is useful not only to treat amyloid-related diseases but also to understand physical properties of amyloid fibrils. In this study, we investigated the dependence of fibrillation on the concentration of various fatty acids and found that there is an optimal concentration for each compound to accelerate fibrillation and that fibrillation was inhibited under the high concentrations.

2P068 β２ミクログロブリンのアミロイド前駆状態の残余構造の特性化

The properties of the residual structure of amyloid precursor state of β2-microglobulin

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β2-Microglobulin (β2m), associated with dialysis-related amyloidosis, is known to form amyloid fibrils at low pH via a partially structured state. Based on the results of the various NMR experiments, we previously suggested the partially structured state to be “activated” to become extensively unfolded, in which state the hydrophobic residues are exposed and associate with the seed. In addition, under high pressure conditions, increase in HSQC signal intensity over the sequence and decrease in transverse relaxation rate on the 60’s aromatic-rich region were observed, suggesting that β2m is experiencing inter- and intramolecular association processes simultaneously. We will further discuss the thermodynamic nature of the interactions.

2P069 超音波によるアミロイド線維形成促進のメカニズム

The mechanism of ultrasonication-induced amyloid fibril formation

Masatomo So[1], Yuichi Yoshimura[1], Hisashi Yagi[1], Hirotsugu Ogi[2], Kentaro Uesugi[2], Hironobu Naiki[3], Yuji Goto[1] ([1]*Institute for Protein Research, Osaka University*, [2]*Graduate School of Engineering Science, Osaka University*, [3]*Faculty of Medical Sciences, University of Fukui*)

Amyloid fibril formation takes a long time because of a high free-energy barrier of nucleation. Although ultrasonication has been used for breaking the mature fibrils, it found to be one of the most powerful methods for accelerating spontaneous fibril formation. However, the mechanism is still unclear. To understand the effects of ultrasonication on fibril formation, we measured ultrasonic wave using a piezoelectric probe. The lag time of fibril formation was significantly correlated with ultrasonic amplitude. The results indicate that ultrasonic amplitude is a dominant factor determining the fibril formation. We will discuss the effects of ultrasonication on fibril formation and fibril morphologies.

2P070 Solubility and Supersaturation-Dependent Protein Misfolding Revealed by Ultrasonication

Yuxi Lin, Young-Ho Lee, Yuichi Yoshimura, Hisashi Yagi, Yuji Goto (*Institute for Protein research, Osaka University*)

We studied the alcohol-induced fibrillation of lysozyme at various concentrations of ethanol, trifluoroethanol (TFE) and hexafluoropropanol (HFIP). Under the conditions where the alcohol-denatured lysozyme retained metastability, ultrasonication effectively triggered fibrillation. The optimal alcohol concentration depended on the alcohol species. HFIP showed a maximum at 12-16%. For TFE, a broad maximum at 40-80% was observed. Ethanol showed an increase of fibrillation above 60%. These profiles were opposite to the equilibrium solubility of lysozyme in water/alcohol mixtures. The results indicate that, although fibrillation is determined by solubility, supersaturation prevents conformational transition and that ultrasonication is effective in breaking supersaturation.

2P071 熱測定によるアミロイド線維形成バーストに関する研究

Direct observation of burst of amyloid fibril formation by calorimetry

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Although amyloid fibrils are related to various pathologies, energetic natures of fibrillation remain largely unclear. We show bursting formation of amyloid fibrils of β2-microglobuin (β2m) using isothermic titration calorimetry. β2m amyloid burst occurred with emitting large heat after a lag phase for nucleation. Enthalpy changes (ΔH) of β2m misfolding were smaller than β2m folding, and displayed a negative heat capacity change (ΔCp) related to buried area. By contrast, amorphous aggregation was followed by smaller ΔH than amyloid burst without a lag phase. Interestingly, (mis)folding and aggregation of acid-denatured β2m showed similar ΔCp values, implying similar buried area following structural conversions with distinct level of ordered molecular contacts.

2P072 Efficient Lookup Table using a Linear Function of Inverse Distance Squared

Jaewoon Jung[1], Takaharu Mori[2,3], Yuji Sugita[1,2,3] ([1]*AICS, Riken*, [2]*Riken*, [3]*QBiC, Riken*)

The major bottleneck in molecular dynamics (MD) simulations of biomolecules exists in the calculation of short-range non-bonded interactions. Non-bonded interaction includes time-consuming inverse square roots and complementary error functions in particle mesh Ewald (PME). To avoid such time-consuming operations while keeping accuracy, we propose a new lookup table for short-range interaction by defining energy and gradient as a linear function of inverse distance squared. In our lookup table approach, densities of table points are inversely proportional to squared pair distances, enabling accurate evaluation of energy and gradient at small pair distances. The new lookup table scheme allows fast pairwise non-bonded calculations owing to efficient usage of cache memory.

2P073 MuSTAR MD : Multi-Scale Temperature Accelerated Replica exchange Molecular Dynamics

Yu Yamamori, Akio Kita (*Institute of Molecular and Cellular Bioscience, the University of Tokyo*)

We developed a new sampling method, MuSTAR MD which is an extension of temperature accelerated MD and can also be considered as a variation of replica-exchange MD. In the MuSTAR MD, each replica contains an all-atom model, at least one coarse-grained model, and a collective variable model that interacts with the other models through coupling terms. The coarse-grained model is introduced to drive efficient sampling of large conformational space and the all-atom model can serve to conduct accurate conformational sampling. The coupling strengths are exchanged between neighboring replicas in some interval obeying the Metropolis method. MuSTAR MD was applied to Ala-dipeptide and met-enkephalin. Comparison with existing methods shows the efficiency and accuracy of MuSTAR MD.

2P074 α-シヌクレイン繊維形成に対する分子混雑の影響
Macromolecular crowding effect on fibril formation of α-synuclein

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An aggregated form of α-synucleins are found in the Lewy body, which is the pathological hallmark of Parkinson's disease. The native state of α-synucleins are in a stable α-helical tetrameric state, while their monomeric state is disordered state. An *in vitro* experiment showed that fibril formation of α-synucleins occurs in their disordered state. These fibrils are considered as seeds for the aggregated form. In order to investigate the mechanism of transition from stable tetrameric state to monomeric disordered state, we constructed a simple lattice gas model considering the effects of macromolecular crowding. We found that decrease of macromolecular density causes tetramer-monomer transition and leads to subsequent fibril formation.

2P075 サルモネラべん毛繊維の多型変換におけるフラジェリン Arg４３１の役割
The role of Arg431 of flagellin in the polymorphic transformation of Salmonella flagellar filament

Fumio Hayashi, Kenji Oosawa (*Div. Mol. Sci., Fac.Sci. and Tech, Gunma Univ.*)

Salmonella flagellar filament is a μm-length scale and helical structure composed of ~30,000 molecules of a single protein flagellin. The filament transforms among several helical structures, which are different in curvature and twist, and the morphological transformation is called polymorphic transformation. Elucidating the atomic mechanism of the transformation observed at μm scale provides a new insight into the actuating mechanism of large protein machines. Previously, we proposed that Arg431 of flagellin is one of the key residues for the polymorphic transformation by *fliC*-intragenic suppressor analysis. In the present study, we created mutants carrying mutations at Arg431 and investigated their filament shape and polymorphic transformation activity.

2P076 表面力測定によるシグナル伝達タンパク質間相互作用の研究
Interactions between signal transduction proteins studied by surface forces measurement

Asuka Sakai[1], Hitomi Fujiwara[1], Masaya Fujita[3], Kazue Kurihara[1,2] ([1]*IMRAM, Tohoku Univ.*, [2]*WPI-AIMR*, [3]*Univ.Houston*)

Spoluation in Bacillus subtilis is controlled by phosphorelay signal transduction. In this series of phosphorylation reactions, KinA binds ATP and autophosphorylates at a histidine residue (KinA-P). The phosphoryl moiety on KinA is transferred to Spo0F and then to Spo0B. We have succeeded in measuring the specific interactions between KinA and Spo0F only in the presence of ATP by using colloid probe AFM. In this study, we focused on the second step of the series of phosphorylation reactions. Proteins were immobilized on a glass substrate using Langmuir Blodget films of a chelate amphiphile. The conditions of absorbing proteins were determined using quartz crystal microbalance. We measured the interactions between Spo0F and Spo0B in buffer solution with/without KinA-P.

2P077 Ｘ線自由電子レーザーにより得られる低分解能データセットに対する単粒子構造解析法
Methodology of a single biomolecular structure determination for low-resolution data set obtained by X-ray Free Electron Laser

Atsushi Tokuhisa, Osamu Miyashita, Florence Tama (*Computational Structural Biology Research Unit, AICS, RIKEN*)

X-ray Free Electron Laser offer new possibility of the single biomolecular structure determination. In experiment, bright coherent X-ray pulse is irradiated onto a single biomolecule with unknown orientation, and diffraction patterns are recorded repeatedly for 3D structure. However, in the current status, we can obtain only low resolution data set because of the absence of efficient sample delivery system. Therefore, we are developing a methodology for structure determination using currently attainable data set. To compensate low resolution data set, we assume structure models from simulation and perform diffraction pattern matting by using a similarity detection algorithm (Tokuhisa et al., 2012, Acta Cryst A. 68. 336-381). We report current status of our effort.

2P078 圧縮センシングを用いた NMR スペクトルの復元法
Reconstruction of NMR spectra using compressed sensing

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NMR spectroscopy is a non-destructive technique used to reveal structural information of molecules, including biomolecules in solution. Since the invention of multidimensional NMR spectroscopy, the area of its application in structural biology has been growing, and the current interest reached as far as using it to inspect proteins in a living cell. But the largest obstacle for its realization is the severe requirement for measurement time; it has to be much shorter than those of usual cases due to the deterioration of a cell.

To solve the problem, we attempted using compressed sensing theory, a cutting-edge theory for reconstructing data from thinned-out sampling points, to achieve shorter measurement time by measuring with much less sampling points.

2P079 Intermolecular interactions and conformation of antibody dimers present in IgG1 biopharmaceuticals

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Intermolecular interactions and conformation in dimer species in Palivizumab, a monoclonal antibody (IgG1), were investigated to elucidate the physical and chemical properties of the dimerized antibody. Palivizumab solution contains approximately 1 % of dimer species with 99 % monomer. The dimer species was isolated by size exclusion chromatography (SEC) and analyzed by a number of methods. The results indicated that approximately half of the dimer species was non-covalently associated, whereas the other half was dimerized by covalent bond including disulfide and di-tyrosine bonds. The dimer species were formed between Fab and Fc, or Fab and Fab. The higher-order structure and thermal stability were very similar between the dimer and monomer.

2P080 細胞膜上のガレクチン３もその細胞膜分子との複合体も、細胞膜上で極めて動的に振る舞う：超高速 1 分子追跡による研究

Galectin-3 and its glyco-molecule conjugates are extremely dynamic on the cell surface: detection by ultrafast single-molecule tracking

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Galectin-3 (Gal) is an N-glycan-binding lectin, whose pentamers could generate "lattices" by multiply crosslinking glyco-molecules on the plasma membrane (PM) surface, thus modulating their locations and functions. We performed ultrafast single-molecule tracking of Cy3-Gal (0.1-ms resol.), and found that each Gal molecule stays on the PM for 110 ms, and alternatingly binds and unbinds N-glycans (for 5.1 and 0.87 ms, with diffusion coefficients of 1.1 and 30 μm2/s, respectively). The latter rate is the fastest ever observed in/on the PM, suggesting levitated diffusion. Since Gal-bound glyco-molecules still undergo diffusion (1.1 μm2/s) and Gal oligomers last <10 ms, Gal pentamers and lattices are likely dynamic entities, forming and dispersing continually on a ms scale.

2P081 分子動力学シミュレーションによる 1 分子 FRET のデータ同化

Sequential data assimilation to single-molecule FRET photon-counting data by using molecular dynamics simulations

Yasuhiro Matsunaga[1], Yuji Sugita[1,2,3] ([1]*RIKEN AICS*, [2]*RIKEN ASI*, [3]*RIKEN QBiC*)

Single molecule FRET (smFRET) measurement provides valuable insights into the dynamical heterogeneities of biomolecules. Here we propose a method, based on the particle filter, to sample hidden conformation states from smFRET data by using molecular dynamics simulations. In the method, a set of coarse-grained models are concurrently simulated and also filtered/resampled during the simulation like the bootstrap methods according to the likelihood of the smFRET photon-counting data in each short time bin. For emulated smFRET data of polyproline and other biomolecules, we demonstrate the performance of the method by using ten thousands of coarse-grained model simulations on K computer.

2P082 一分子時系列から抽出されたマルコフ連鎖 定常ネットワークにおける遷移確率が“最小”となる分子の“状態”の同定

Identifying chemical states in Markov chain steady state network extracted from time series by finding “minimum” transition probability

Yutaka Nagahata[1], Hiroshi Teramoto[1,2], Chun-Biu Li[2], Tamiki Komatsuzaki[1,2] ([1]*Graduate School of Life Science, Hokkaido University*, [2]*Research Institute for Electronic Science, Hokkaido University*)

Protein dynamics is considered as having multiple time scale for execute their functions such as folding/unfolding, allosteric regulations, and signal transaction. Such property was visualized by disconnectivity graph based on potential energy profile. The approach was recently extended into disconnectivity graph based on free energy profile in equilibrium call transition discconectivity graph. In this talk we introduce novel method to construct disconnectivity graph. Our approach is starting from Markov chain steady state network that can be extracted from time series of single molecule measurement. Then extract hierarchical property of transition from the network based on transition probability between clusters of network.

2P083 X 線 1 分子追跡法による II 型シャペロニン協同的運動評価

Cooperative Motion Analysis of group II chaperonin by X-ray Single Molecule Tracking

Hiroshi Sekiguchi[1], Yohei Yamamoto[2], Mayuno Arita[2], Naoki Ishiguro[2], Kouhei Ichiyanagi[3], Masafumi Yohda[2], Naoto Yagi[1], Yuji Sasaki[3] ([1]*Research Utilization Div., JASRI*, [2]*Dept. Biotech. Life Sci., Tokyo Univ. Agricult. Tech.*, [3]*Grad. School Frontier Sci., Univ. Tokyo*)

Group II chaperonin, found in archaea and in the eukaryotic cytosol, is an indispensable protein that captures a nonnative protein and refolds it to the correct conformation in an ATP dependent manner. ATP-induced structural changes are essential for chaperonin activity. We had reported that the diffracted X-ray tracking (DXT) could trace ATP induced conformational change of group II chaperonin at single molecule level (Sekiguchi et al., PLoS ONE 2013). In DXT, nanocrystal immobilized on one side of chaperonin-ring is used as tracer for structural change of chaperonin. In this study, we analyzed how ATPase deficient mutant modulate dynamic motion of chaperonin and will discuss intra- and inter-ring cooperativity of group II chaperonin from motion analysis view.

2P084 X 線 1 分子計測によるタウタンパク質分子の構造揺らぎ

Structural Fluctuations of Tau Proteins from X-ray Single Molecule Observations

Masahiro Shimura[1], Yufuku Matsushita[1], Kouhei Ishiyanagi[1], Tomohiro Miyasaka[3], Hiroshi Sekiguchi[2], Yasuo Ihara[3], Yuji C. Sasaki[1,2] ([1]*Grad. School Frontier Sci., Univ. Tokyo*, [2]*Research & Utilization Div., SPring-8/JASRI*, [3]*Faculty of life & Medical Sci., Doshisha Univ.*)

The structure of tau proteins in solution resembles that of a random coil. But, tau proteins in Alzheimer paired helical filaments-like fibers have very little secondary structure. Here, we tried to observe the structural fluctuations of tau proteins using Diffracted X-ray Tracking (DXT) as x-ray single molecule observation method. In DXT, we observed Brownian motions of recombinant tau proteins and his-tagged tau proteins, which are adsorbed on the substrate’s surface. These adsorbed tau proteins were reacted to many antibodies and were phosphorylated by several kinases. From DXT data, the tau protein combined with the antibody was confirmed that structure fluctuation had become slightly small compared with that which is not combined.

2P085 **Rapid monitoring of affinity maturation process for in vitro selection by fluorescence correlation spectroscopy (FCS)**

Shigefumi Kumachi, Miho Suzuki, Koichi Nishigaki, Naoto Nemoto (*Grad. Sch. Sci. & Eng., Saitama Univ.*)

In vitro selection (one of evolutionary molecular engineering to generate functional biomolecules) has required rapid analysis of each selection process to screen peptides and proteins with a high affinity. This study showed that affinities of the whole selected molecules in the library were measured by fluorescence correlation spectroscopy (FCS) rapidly. We synthesized a fluorescent labeled peptide library by using both a puromycin linker and a cell-free translation system. Then, we could evaluate the affinity maturation process of the whole library by FCS analysis of molecular interaction between the target molecule and each labeled library. As a model selection, in vitro selection against Anti-FLAG antibody with a peptide library was performed by cDNA display.

2P086 **DNA 配列相補性を用いた DNA 修飾アクチン繊維の束化制御**
Control of bundle formation of DNA-conjugated actin filaments using the complementarity of the DNA

Masahito Hayashi, Kingo Takiguchi (*Grad. Sch. Sci., Nagoya Univ.*)

We are trying to construct amoeba-like molecular robots based on a giant liposome that encapsulates DNA computers and protein actuators. To make an informational linkage between the computers and actuators, we developed DNA-conjugated actin (DNA-actin) using heterobifunctional cross linkers. The DNA-actin kept its filament structure when the coupling ratio was up to 50%. In the presence of HMM, ATP and metylcellurose, DNA-actin filaments formed aster-like structure, suggesting that DNA-actin filaments keep their basic function as active cytoskeleton. When DNA-actin filaments with sense and antisense ssDNA were mixed, they immediately formed dense bundles, indicating that we can control their bundle formation using the complementarity of the conjugated DNAs.

2P087 **Regulation of proteasomal degradation through an unstructured initiation site of a substrate**

Kazunobu Takahashi, Tomonao Inobe (*Front. Res. Core for Life Sci., Univ. Toyama*)

Efficient proteasome-mediated degradation requires an unstructured initiation site in addition to the ubiqutin modification. We investigated whether the proteolysis by the proteasome could be inhibited by a small molecule that modifies the property of the unstructured initiation site of the substrate using model substrates. As a result, in the presence of the small molecules, the model substrates could escape from the degradation by the purified proteasome and the endogenous proteasome in the HEK293T cells. Our experiments suggest that similar mechanism exist for some of the proteasome substrates *in vivo* and provide a useful strategy to selectively control the cellular concentration of specific proteins.

2P088 **cDNA ディスプレイ法を用いた Minimum プロテアーゼの試験管内進化**
***In vitro* selection of Minimum-Protease by cDNA display**

Yuka Mashio[1,3], Shingo Ueno[2,3], Naoto Nemoto[1,3] ([1]*Grad. Sch. Sci. and Eng., Saitama Univ.*, [2]*Grad. Sch. Eng., Univ. Tokyo*, [3]*CREST, JST*)

Development of more useful enzymes has been expected in various industrial fields. *In vitro* display technologies such as ribosome display, mRNA display and cDNA display enable an efficient artificial protein evolution by using a huge mutant library. However, a few artificial evolution experiments of proteins by *in vitro* display technologies have been succeeded until now.
Recently, we have started the challenge to perform the evolution of a minimum-metalloprotease by cDNA display. The system was designed to select the cDNA coding an active protease by releasing it from the solid-phase when the displayed protease could cleave the peptide substrate in the puromycin-linker. In this presentation, we will show the selection system in detail and discuss about some results.

2P089 **ナノ粒子表層セルラーゼモジュールシャッフリングによる効率的人工セルロームデザイン**
Evolutional cellulosome design from module library

Hikaru Nakazawa, Yuri Ishigaki, Eiko Kobayashi, Do-Myoung Kim, Mitsuo Umetsu (*Grad. Sch. Eng., Tohoku Univ.*)

Recently, we propose a new design of artificial cellulosome to enhance cellulase activity: biotinylated catalytic domain(CD) and carbohydrate binding module(CBM) was clustered on streptavidin-modified inorganic nanomaterials via biotin-avidin interaction, and the design of CBM valency and synergistic effect of CD showed drastic enhancement of catalytic activity. Here, we constracted a biotinylated CD and CBM library with a kind of 65CDs and 21CBMs selected from cellulases reported in PDB, Pfam and Cazy database, and the appropriate cluster format attempted to be identified by analyzing the phosphate acid swollen cellulose degradation. Consequently, we succeeded in conveniently identifying the optimal format of the cellulosome and optimal combination of CD and CBM.

2P090 **Green Fluorescent Protein からの機能エレメントの抽出**
Extraction of Function Elements from Green Fluorescent Protein

Toshio Morimoto, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Function element is defined as a region responsible for function. The chromophore of GFP is reconstituted from own amino acid residues (SYG) involved in the amino acid sequence. We attempted to extract function elements related to the spontaneous reconstruction of the chromophore in GFP. For the purpose, we prepared all possible alanine insertion mutants of GFP ranging from the 50th to the 102nd amino acid residue containing the SYG sequence. The fluorescence of each insertion mutant was examined with the mutant-containing colony on a cultivation plate and supernatant of cell extraction under blue light. From the results, we concluded that the function element responsible for the fluorescence is the region from the 53rd to the 76th residues.

2P091 人工酵素に移植した機能エレメントの役割
Roles of functional elements transplanted into the artificial enzyme

Mai Arakawa, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

We succeeded to obtain an artificial enzyme, TSN-nuclease, by transplanting a complete set of the function elements extracted from staphylococcal nuclease (SNase) into a TSN domain of human transcription factor, p100. We applied the alanine insertion analysis to TSN-nuclease to clarify the role of the transplanted function elements. We prepared the mutants in which an alanine is inserted into a transplanted function element as well as the mutants in which alanine into an originally TSN region, and examined their activities. The activity was reduced by the insertion into an element, while the activity remained by the insertion into the other regions, indicating that the transplanted function element acts as a function element of TSN-nuclease.

2P092 新規ヘム蛋白質フォールドのデノボデザイン
De novo design of new heme protein folds

Yasuhiro Isogai (*Dept. Biotech., Toyama Pref. Univ.*)

Designing proteins *de novo* is a productive challenge to elucidate principles of the protein structure and function. Here we have designed tertiary structures and amino acid sequences of globular heme proteins with an α/β fold that does not occur in nature. The protein strcutures were modeled to accommodate heme between secondary structure elements by considering basic rules relating local structures to tertiary motifs (Koga et al., Nature 491, 222-229, 2012). The amino acid sequences to fold into a modeled structure were designed with Rosetta. The heme binding site is constructed by positioning two His residues at the sites for coordination of the heme iron and restricting the amino acid composition around the bound heme to leave space within the protein core.

2P093 天然変性タンパクとしての Bach2 ヘム結合領域
Heme binding region of Bach2 as intrinsically disordered protein

Kazutaka Murayama[1], Miki Matsui[2], Kazuhiko Igarashi[2] ([1]*Grad. Sch. Biomed. Eng., Tohoku Univ.*, [2]*Grad. Sch. Med., Tohoku Univ.*)

Bach2 is a transcriptional repressor, which is involved in B-cell development and plays critical roles in the process of plasma cell differentiation. Heme participates in these functional regulations of Bach2. Bach2 includes the BTB domain at the N-terminal and the b-Zip motif in the C-terminal region. As a heme acceptor, Bach2 has five Cys-Pro motifs. UV-Vis spectroscopic analyses suggested that the central region (331-520) of Bach2 shows similar heme binding manner with the full-length protein. However, no special secondly structural element was assigned in this region. Here, we focused on this central heme binding region and characterized it based on bioinformatical analyses. The results indicated that the central heme binding region is not fully random structure.

2P094 Oxygen-affinity of hemoglobin is regulated by protein-structural dynamics

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The O2-affinity of Hb is regulated by the interaction of Hb with heterotropic effectors without changes in either static molecular structures of the protein or the coordination structure of the hemes. The very-high-frequency (>1GHz) thermal fluctuations of oxy-Hb are entropically enhanced upon deoxygenation and/or by binding of heterotropic effectors, in parallel to the decreases in the O2-affinity. The rate of dissociation of O2 from oxy-Hb is enhanced by effector-linked enhanced thermal fluctuations, which simultaneously reduce the rate of geminate-recombination of O2, resulted in the reduction of the apparent O2-affinity of Hb. Therefore, the heterotropic effectors are the pivotal signaling molecule to generate diverse functionality of physiological relevance in Hb.

2P095 単一結晶形中でのヘモグロビンのアロステリック転移
Hemoglobin allosteric transition in a single crystal form

Naoya Shibayama (*Div. of Biophysics, Jichi Medical Univ.*)

Nine distinct allosteric equilibrium conformers of human hemoglobin in the half-liganded and fully-liganded states have been characterized, within a novel crystal form, by X-ray structural analysis and direct oxygen equilibrium measurements on three isomorphous crystals, each capturing three different conformations. The observed nine conformations cover the complete conformational space of hemoglobin, spanning from T to R2 (second relaxed state) through R, with various relaxed intermediate forms between R and R2. Moreover, we found a novel intermediate form with an intermediate oxygen affinity between T and R, which may be the missing link between hemoglobin structure and function for over several decades. Details of these results will be presented.

2P096 酸化型コバルトミオグロビンへの速度論的配位子結合解析
Kinetic Analysis of Ligand Binding to Co(III) Myoglobin

Saburo Neya, Masaaki Suzuki, Tyuji Hoshino (*Chiba University, Graduate School of Pharmaceutical Sciences*)

Myoglobin reconstituted with oxidized Co(III) deuteroheme was found to exhibit relatively large affinities to cyanide, azide, pyridine, and imidazole contrary to the early proposal. The relaxation kinetic analysis revealed that the ligand association rates were small and that the dissociation rates were still much smaller. The relatively large ligand affinities in Co(III) myoglobin were found due to the compensation of small association rates with fairly smaller dissociation rates. The rationale for the characteristic ligand-binding behavior of Co(III) myoglobin was provided on the basis of the properties of Co(III) which has an additional negative charge and forms stronger metal-ligand bonds than Fe(III).

2P097 異なる生物種によるヘムオキシゲナーゼ反応の微調節戦略：逐次反応過程の個別制御

Fine-tuning of heme oxygenase successive reactions: Regulation at the peculiar stages in different biological species

Norio Miyake, Atsuko Akiyama, Kouki Kimiya, **Taiko Migita** (*Fac. Agr., Dep. Biol. Chem., Yamaguchi Univ.*)

Heme oxygenase (HO) catabolizes heme into biliverdin, iron, and carbon monoxide by consuming electrons and molecular oxygen and participates in several physiological processes in a variety of species. To clarify the strategy employed by different organism for HO tuning, the HO activity from different organism are compared. EPR of the respective HO complexes has revealed similar but distinctive heme axial coordination structures. Oxygen consumption rate monitored by micro-sensor, stopped-flow kinetics analyses, and the kinetics of reactant, intermediate, and product degradation/formation observed by ESI-MS, revealed that rat, fish, soybean, and cyanobacteria HO reactions are uniquely and finely tuned at different steps of the successive reactions.

2P098 ヘム結晶化を促進するサシガメ由来 α - グルコシダーゼのヘム結合部位の検討

Heme binding site in *Rhodnius prolixus* α-glucosidase promoting the heme crystallization

Shotaro Kaku, Keisuke Nakatani, Haruto Ishikawa, Yasuhisa Mizutani (*Grad. Sch. Sci., Univ. Osaka*)

A crystal of heme called hemozoin (Hz) was found in *Rhodnius prolixus* that is a blood feeding insect. Although it is suggested that the heme crystallization is promoted by α-glucosidase which is a membrane protein, the detailed mechanism has not been revealed [1]. To reveal the mechanism, we established the recombinant expression system of the non-tagged α-glucosidase and the purification method using a gel filtration chromatography for the first time. The purified α-glucosidase showed Hz formation activity and electronic absorption spectra typical for heme proteins, suggesting that α-glucosidase can bind heme. Moreover, we carried out the further spectroscopic study to identify the heme binding site.

Reference [1] Mury, F.B. *et al.* (2009) PLoS ONE 4: e6966.

2P099 時間分解共鳴ラマン分光法を用いた CO 解離に伴う CooA のタンパク質ダイナミクスの研究

Protein dynamics of CooA upon CO dissociation studied by time-resolved resonance Raman spectroscopy

Akihiro Otomo[1], Haruto Ishikawa[1], Misao Mizuno[1], Shigetoshi Aono[2], Yasuhisa Mizutani[1] ([1]*Grad. Sch. Sci., Univ. Osaka*, [2]*Okazai Inst.*)

CooA is a CO sensor protein that controls the transcription of enzymes involved in the oxidation of CO. Association/dissociation of CO from the heme induces a sequence of conformational changes, which is transmitted to the DNA binding region. To elucidate the CO sensing mechanism of CooA, we studied structural changes upon CO dissociation by time-resolved resonance Raman (RR) spectroscopy. Time-resolved visible RR spectra of the CO-dissociated form indicated the structure of the heme and heme pocket do not change in picosecond to submillisecond regime. In addition, based on ultraviolet RR spectra, we will discuss structural change of the C-helix and heme pocket.

2P100 BK チャネルの細胞質側の操作

Manipulation of the cytoplasmic domain of BK channel

Yoshihiro Satoh, Morten Bertz, Kazuhiko Kinosita (*Waseda University*)

Ion channels play an important role in signal transduction. They change between an open and closed state by reacting to signals such as changes in membrane potential, ligand binding, and so on. These signals trigger conformational changes in the ion channel protein, but the nature of these conformational changes is not yet clear. Here, we are using BK, a potassium channel that is activated by both changes in membrane potential and intracellular calcium. The crystal structure suggests how calcium binds to the cytoplasmic domain and opens the transmembrane pore. To confirm this conformational change, we are trying to manipulate the channel by applying force through a probe attached to the cytoplasmic domain while observing open/close transitions by patch clamp.

2P101 アセチルコリン受容体の高速高精度３次元Ｘ線１分子内部運動計測

3D X-ray Single Molecule Dynamics of nicotinic Acetylcholine Receptor (nAChR) with microsecond and picometre accuracy

Maki Tokue[1], Hiroshi Sekiguchi[2], Kentaro Hoshisashi[1], Kohei Ichiyanagi[1], Yuri Nishino[3], Naoto Yagi[2], Atsuo Miyazawa[3], Tai Kubo[4], Yuji Sasaki[1] ([1]*Grad. Sch. FS., Univ. Tokyo*, [2]*JASRI/SP8*, [3]*Grad. Sch. Sci., Univ. Hyogo*, [4]*ALST*)

Nicotinic acetylcholine receptor (nAChR) is ligand gating ion channel protein compounded of 5 subunits in bilayer of lipids. The construction of nAChR is already found out, but it's fixed in crystal condition and we need measuring intermolecular motion of nAChR to understand the essential motion mechanism. In recent research, α subunit presses β subunit in χ direction and β subunit moves in θ direction when nAChR opens. I observed diffraction spots from α subunits which bind Au nano crystals by use of Diffracted X-ray tracking (DXT) with microsecond and picometre accuracy to reveal opening, closed and desensitized state. I injected some types of ligands into the cell taking out of torpedo which is almost composed of nAChR to compare the the nAChR motion.

2P102 Computational analysis on the influence of membrane lipid composition on the structural invariance of G-protein coupled receptor

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β2 adrenergic receptor is a member of G-protein coupled receptors, which transduce a wide range of extracellular signals across the membrane of cell. GPCRs are most important drug targets in pharmaceutical field. We performing molecular dynamics simulations of three states: apo and agonist or antagonist bound β2AR and embedded in four types of membranes: (i) POPC (ii) POPC/CHL (iii) POPC/CHL/GM1 (iv) POPC/POPE/CHL/SM. β2ARs in POPC/CHL bilayer are unstable in which the ionic lock is not formed between R131 and E268. A strong hydrophobic interaction between CHL and β2AR was observed at helix5 in POPC/CHL/GM1 and POPC/POPE/CHL/SM. These results suggest that lipid composition affects the conformation of GPCR and essentially concerns for activation.

2P103 Direct monitoring of membrane protein folding process during in-vitro expression by Surface Enhanced IR spectroscopy

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Surface Enhance Infrared Absorption (SEIRAS) have unique properties that enhances signals at vicinity (in an order of several nm's) of a substrate metal. This property is useful to determine solely the surface chemical process distinguished from that occurred in bulk phase. Therefore when a biological 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 a folding process of membrane protein (bacteriorhodopsin) during cell free expression on the nano-disc artificial lipid bilayer.

2P104 等温滴定型熱量計による多剤輸送担体 EmrE の基質結合様式の解析

Thermodynamics analysis of substrate binding mode of multidrug resistance transporter, EmrE by Isothermal Titration Calorimetry (ITC)

Kazumi Shimono[1,2,3,4], Yoshiro Mori[2], Toshifumi Nara[2], Tomomi Someya[3,4], Mikako Shirouzu[3,4], Shigeyuki Yokoyama[3,5], Seiji Miyauchi[1,2] ([1]*Fac. Pharm. Sci., Toho Univ.*, [2]*Coll. Pharm. Sci., Matsuyama Univ.*, [3]*SSBC, RIKEN*, [4]*CLST, RIKEN*, [5]*Struct. Biol. Lab., RIKEN*)

EmrE is a substrate/proton antiporter, which extrudes a wide range of lipophilic cations. Here, we had demonstrated with ITC how tetraphenyl phosphonium ion (TPP^+) binds to the wild-type and mutants (E14Q, D, E25Q). As pH increase, the driving force of EmrE/TPP^+ binding shifted from enthalpy-driven to entropy-driven and the binding affinity was more potent, inferring that EmrE has a more open conformation at acidic pH, and a closed conformation at neutral and alkaline pH. Especially, Glu25 might be one of determinant residues to drive the substrate binding, because in E25Q, at pH 7.4, the contribution of enthalpy to interaction energy remained a little, but significantly. The mechanism of proton coupled substrate transport will be discussed here.

2P105 多剤排出トランスポーター AcrB の Motion Tree 法による解析

Motion Tree analysis of the multidrug transporter AcrB

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The multidrug transporter AcrB actively exports a wide variety of noxious compounds using the proton-motive force as an energy source in Gram-negative bacteria. AcrB adopts an asymmetric structure comprising three protomers with different conformations that are sequentially converted during drug export. These cyclic conformational changes for drug export are called the functional rotation. The Motion Tree is a new method to describe the structural change between two states of proteins in a hierarchical manner. This method can detect the rigid body motions and clarify the relations among them. In the present study, we applied the Motion Tree analysis to the cyclic conformational changes of AcrB. As a result, the key motions for the functional rotation were observed.

2P106 ABC トランスポーターにおける薬剤結合の影響：分子シミュレーションによる研究

The effects of substrate binding in ABC transporter: A simulation study

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ATP-binding cassette (ABC) transporters constitute a large family of membrane proteins that transport diverse substrate across the cell membrane with the help of ATP hydrolysis. It is known in some ABC transporters that substrate binding promotes the ATPase activity. The effect of substrate binding is thus an important clue to understanding the functional mechanism of the ABC transporter. In present study, we performed all-atom molecular dynamics simulations of the system comprising ABC transporter of the inward-facing form, lipid bilayer, and water, both in the substrate bound state and in the apo state. The substrate binding effect was studied by comparing the simulation results in the substrate bound state with those in the apo state.

2P107 紫膜表面において観測される隆起構造体の曲率に対する溶媒 pH やイオン強度の影響

Curvature of Bump Structures on Purple Membrane Depending on pH and Ionic Strength Analyzed by Atomic Force Microscopy

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Purple membrane (PM) is a membrane patch which is formed by self-assembly of membrane protein bacteriorhodopsin with archaeal lipids. Atomic force microscopy (AFM) studies for PM supported onto mica substrate shows dome-like "bump" structures on the surface. Since PM must have a curvature to form the bump structure, bump formations will be related to a mechanism for structural formations via self-assembly. To elucidate effects of asymmetric distribution of charged residues between two aqueous domains on bump curvature, AFM observations were carried out toward identical PM sheets with varying solvent pH and ionic strength. The bump curvature studies indicated large influence of electrostatic interaction. The model for the bump structure will be discussed.

2P108 ナノディスクを用いたセンサリーロドプシン I

Photoreaction dynamics of sensory rhodopsin I in nanodiscs

Kenichi Kawamoto[1], Keiichi Inoue[1,2], Jun Sasaki[1], jin Yagasaki[3], Yuki Sudo[3], Michio Homma[3], Hideki Kandori[1] ([1]*Nagoya Inst. Tech.*, [2]*JST PREST*, [3]*Nagoya Univ.*)

Sensory rhodopsin I (SRI) is a member of microbial rhodopsins, functioning as a phototaxis sensor by interacting with a cognate transducer protein. In this study, we have tried to study photoactivation dynamics of SRI by spectroscopic methods, but the measurements in lipid bilayer, the native environment, was difficult, particularly for transient grating method. Here we reconstitute SRI from Haloarcula vallismortis (HvSRI) into nanodiscs, and photoreaction dynamics are studied by both transient grating and transient absorption methods. Low scattering of the sample into nanodiscs, allowed us to obtain signals, from which we reveal that the dynamics of HvSRI significantly differ between membrane and detergent.

2P109 チャネルロドプシンの活性中心における水素結合ネットワーク

Hydrogen-bonding network in the active center of a light-gated ion channel, channelrhodopsin

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Optogenetics has revolutionized neurosciences, where ion-transporting microbial rhodopsins are utilized as the tools for light-induced neuronal excitation and suppression. Channelrhodopsin (ChR), a light-gated ion channel, is used to excite neurons by light, and the X-ray structure of a chimeric protein of ChR was determined recently (Kato et al. Nature 2012).
Here we applied low-temperature FTIR spectroscopy to this protein and the mutants of the active center. The FTIR data suggest that protein-bound water molecules form specific hydrogen-bonding network in the active site of ChR. Functional role of protein-bound water molecules in ChR will be discussed on the basis of the X-ray structure and the present FTIR results.

2P110 光駆動ナトリウムポンプにおける N 末端と C 末端の役割

Role of N- and C-terminus in a light-driven sodium ion pump

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Recent finding of a light-driven sodium ion pump from marine bacteria showed that nature creates and utilizes sodium transport for light-energy conversion in marine environment [1]. In the new rhodopsin from Krokinobacter eikastus (KR2), a sodium binds near the extracellular surface, but interestingly, the binding is not required for sodium ion pump [1]. KR2 can also pump lithium ions, but converts to a proton pump when presented with potassium chloride or salts of larger cations. Nonlabens dokdonensis possesses a homologous protein, and here we studied molecular mechanism of the protein. We particularly focused on the functional role of N- and C-terminus, which possibly affect the binding and transport of sodium ions.
[1] Inoue et al. *Nature Commun.4, 1678* (**2013**).

2P111 プロテオロドプシンの色を決めるアミノ酸

A Color Determining Amino Acid of Proteorhodopsin

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Proteorhodopsin (PR) is a light-driven proton pump found in marine bacteria, and thousands of PRs are classified into blue-absorbing PR (BPR) (λ_{max}~490 nm) or green-absorbing PR (GPR) (λ_{max}~520 nm). The amino acid at position 105 is known to be color determinant, where Gln and Leu are characteristic of BPR and GPR, respectively. However, mechanism of color tuning has been little understood.
In this study, we replaced Leu105 of GPR into 19 different amino acids, and measured absorption spectra and pKa of the Schiff base counter ion (Asp97). All mutants exhibited spectral shift and pKa changes specifically, suggesting the critical role of position 105. We will discuss how nature optimized color tuning in marine bacterial rhodopsins on the basis of the present results.

2P112 G_s および G_q の光制御に向けた新規キメラタンパク質のデザイン

Designs of new chimeric proteins for optical activation of G_s- and G_q- 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) transmit various stimuli to G-proteins. In order to control the signal transduction by light in cell, we are developing chimeras between GPCRs and photo receptive microbial rhodopsins.
In this study, new chimeras between GR, a proton pumping microbial rhodopsin, and β_2-adrenergic receptor (GR/β_2AR) or squid rhodopsin (GR/SqRho) were designed for G_s- and G_q-activations, respectively. Furthermore, we constructed chimeras with various lengths of the cytoplasmic domains of GPCR and introduced E132Q mutation which makes the photocycle slower. GR/β_2AR chimeras showed light-dependent G_s-activation and the activities were determined by G protein activation assay. We will discuss the activation mechanism in this poster.

2P113 全反射赤外分光法を用いたヒト苦味受容体の構造解析

ATR-FTIR study of human bitter taste receptor

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Humans recognize the bitterness mediated by the bitter taste receptor (TAS2Rs), which is one 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 specific tuning range, bitter compounds. Despite of the progress on understanding 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.

2P114 Direct observation of DNA positive supercoiling by reverse gyrase

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Reverse gyrase is a type I topoisomerase that can introduce positive supercoils into DNA only at high temperature (>70°C). We have investigated the activity of purified reverse gyrase under an optical microscope: a magnetic bead attached to the free end of a double-stranded DNA tethered to a glass surface rotated in one direction to relax the positive superhelical turns introduced by the enzyme. Inclusion of a single-stranded bubble in the DNA greatly enhanced the activity. Apparently a single reverse gyrase molecule stayed at the bubble to continuously rotate the DNA for >100 revolutions, indicating a high processivity. When we prohibited the bead rotation, the bead sank as the DNA was supercoiled, showing that the enzyme can work against torsional stress.

2P115 TDP-43 タンパク質における複数の RNA 認識モチーフとその機能的役割

Distinct roles of individual RNA recognition motifs in an RNA-binding protein, TDP-43

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RNA recognition motif (RRM), composed of a βαββαβ fold, is the most abundant RNA-binding unit in higher vertebrates, which describes about 1 % of human genome. Each of RRMs recognizes its own target nucleotide sequence with distinct specificity, and interestingly, many of RRM-containing proteins are equipped with multiple copies of RRMs. Despite this, functional significance of such “RRM multiplicity” remains obscure. Here we have noted TDP-43 as a model protein containing two RRMs in tandem (RRM1 and RRM2) and developed a new assay system to evaluate the binding of each RRM with various nucleotide sequences. RRM1 but not RRM2 was found to bind nucleotides, and structural analysis describing such distinct binding capabilities of RRM1/2 is now underway.

2P116 部位特異的 RNA 切断酵素 Ire1p によって認識される HAC1 mRNA の NMR 解析

NMR analysis of HAC1 mRNA recognized by the site-specific endonuclease Ire1p

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The consensus sequence of HAC1, 'CNGNNGN', is cleaved at the single specific site by yeast endonuclease Ire1p. Interestingly, HAC1 mRNA has ten CNGNNGN sequences, but Ire1p cleaves two intron-exon junctions of HAC1 mRNA correctly. Here, we attempted to uncover the molecular mechanism how the specific processing sites of this HAC1 mRNA are selected. To probe the roles of the conserved sequences of HAC1 mRNA, we recorded the NMR spectra of HAC1 mRNA with the conserved residues ^{15}N-labeled. Based on our NMR data, we found that the sequence around this consensus sequence took a stem-loop structure where the consensus sequence is included in the loop region. This finding helps to explain the specific interaction between Ire1p and HAC1 mRNA.

2P117 哺乳類ヌクレオチド除去修復タンパク質 XPC の 1 分子イメージング

Single-molecule direct visualization of the mammalian nucleotide excision repair protein XPC

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Although XPC protein complexed with the RAD23B protein is known to be responsible for damage recognition in mammalian nucleotide excision repair, it is unclear how the protein complex discriminates base lesions from a vast excess of normal bases. To address this issue, we labeled XPC with a Qdot and developed a PEG-based single-molecule imaging platform. We found that the XPC protein complex preferably bound to DNA lesions on λDNA created by UV-irradiation. We also found that the protein complex performed one-dimensional free diffusion on undamaged λDNA. The obtained diffusion coefficient indicates that the protein complex moves on DNA by hopping as well as scanning, suggesting that the protein complex uses these modes for efficient search of DNA lesions.

2P118 ナノ開口基板を用いたヘミメチル CpG 認識に関与する SRA-DNA 複合体の機能解析

Characterization of SRA-DNA complex using Zero mode waveguides

Yong-Woon Han[1], Hiroaki Yokota[1], Mariko Ariyoshi[1,2], Yasuo Tsunaka[1,2], Takuma Iwasa[1,3], Ryuji Yokokawa[4], Ryo Hiramatsu[5], Daichi Chiba[5], Teruo Ono[5], Yoshie Harada[1] ([1]*iCeMS, Kyoto University*, [2]*PREST*, [3]*Graduate School of Biostudies, Kyoto University*, [4]*Department of Technology, Kyoto University*, [5]*Institute for Chemical Research, Kyoto University*)

DNA modification such as CpG methylation regulates chromatin structures and eukaryotic gene expression. Many eukaryotic genes contain highly GC-rich sequences and methylation of cytosine at CpG dinucleotides is essential for silencing of parasitic DNA, genomic imprinting and embryogenesis. DNA methyltransferase 1 (Dnmt1) is the enzyme to methylate hemi-methylated CpG regions. Uhrf1 is methylated CpG binding protein and interacts with Dnmt1, followed by recruitment of Dnmt1 to hemi-methylated CpG regions. SRA domain of Uhrf1 is responsible for hemi-methylated CpG binding activity. We characterize the process of hemi-methylated CpG recognition by SRA domain using Single-Molecule technique, and in this meeting, we will show our present data.

2P119 ナノ開口を用いた 1 分子イメージングによる RuvB 多量体形成機構の解明

Single-molecule visualization of RuvB oligomer for characterizing a AAA$^+$ class hexameric ATPase with zero-mode waveguides

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E. coli RuvB, which is classified AAA$^+$ proteins family, promotes branch migration of Holliday junction DNA together with RuvA in homologous recombination. However, despite of the importance of RuvB oligomer ring formation for its activity, the formation mechanism is poorly understood. To clarify the reaction mechanism of Holliday junction DNA branch migration by RuvA-RuvB, we prepared Cy5-RuvB and zero-mode waveguides (ZMWs) consisting of a nano-hole array. We observed interaction between Cy5-RuvB and Holliday junction, and determined the number of RuvBs under various nucleotide conditions in ZMWs. Our data demonstrated that RuvBs form higher oligomers in the presence of ATPγS and ADP, compared with those in the presence of ATPγS or ADP.

2P120 Conformational Sampling of Nucleic Acids in Cellular Environments

Asli Yildirim[1], Brad Varner[1], Monika Sharma[2], Liang Fang[2], Michael Feig[1,2] ([1]*Department of Chemistry, Michigan State University*, [2]*Department of Biochemistry and Molecular Biology, Michigan State University*)

Macromolecular crowding has significant effects on structure and dynamics of biomolecules and on their biological roles in cells. Since the role of these crowders on biomolecules is controlled by electrostatic interactions one possibility of modeling cellular environments involves reduced dielectric constants. Herein, the effect of cellular environment on the conformational sampling of A-DNA, B-DNA and RNA structures is modeled with reduced dielectric constants of 20, 40, and 80. This is realized with molecular dynamics simulations using implicit solvent based on the Generalized Born (GB) methodology. The results for the nucleic acids suggest that reduced dielectric environments as found in cellular environments may lead to a conformational shift towards A-DNA forms.

2P121 Local structural similarity between interphase chromatin and mitotic chromosomes in living mammalian cells

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The long string of genomic DNA must be organized three dimensionally in the cell to utilize genome information. Recently we demonstrated that the nucleosome fiber (10-nm fiber), in which DNA is wrapped around core histones, is irregularly folded in the cell without a 30-nm chromatin fiber. Our super-resolution nucleosome 2-D mapping identified numerous compact chromatin domains in both interphase chromatin and mitotic chromosomes in living cells. This finding provides us a novel view on the mitotic chromosome assembly: in the interphase nuclei, numerous compact chromatin domains are already formed. During mitosis, these chromatin domains are assembled together, presumably by condensins and other protein factors to create a rod-like chromosomal shape.

2P122 レドックス DNA の電子移動反応に及ぼす二本鎖内架橋の影響

Effect of intrestrand cross-linking of redox-labeled DNA duplex on its electron transfer reaction

Yasuhiro Mie, Keiko Kowata, Yasuo Komatsu (*Bioproduction Res. Inst., AIST*)

The electrochemical investigation of interstrand cross-linked DNA (ICL-DNA) would help us to understand the biological DNA electron transfer and to construct efficient bioelectronics as well. In this study, the first insight into this matter is presented. We prepared 17-mer DNA duplexes incorporating Nile blue (NB) at one end as a redox marker and a disulfide tether at the other end for immobilization onto an electrode. The duplexes were covalently cross-linked by bifunctional cross-linkers that utilize either a propyl or naphthalene residue for replacing a base pair. Interestingly, the ICL-DNA with naphthalene residues exhibited DNA-mediated electron transfer, while the standard DNA and ICL-DNA with propyl-linker showed direct electron transfer pathway.

2P123 Bacterial ribosomal RNA as a target for sequence-specific inhibition

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Many antibiotics block protein synthesis in pathogenic bacteria by interacting with bacterial ribosomal RNA (rRNA) and inhibiting the ribosome. However, bacteria have developed resistance against known antibiotics so there is a need to look for new antibacterials. We design short modified oligonucleotides to target in a sequence-specific manner rRNA. We test different DNA analogues such as 2'O-methyl-RNA, peptide nucleic acid (PNA), and locked nucleic acid (LNA) which hybridize well with RNA and are resistant to cellular enzymes. PNA is also known to have good strand invasion properties. Next, we check the ability of these oligonucleotides to inhibit protein synthesis in vitro in a cell-free transcription/translation system.

2P124 DNA の粗視化モデルによる Ars インスレーターの運動性と機能性の解析

Analysis of the fluctuation and functionality of Ars-insulator by coarse -grained model of DNA

Keisuke Yamamoto, Sayuri Tatemoto, Naoaki Sakamoto, Akinori Awazu (*Department of Mathematical and Life Sciences,Hiroshima University*)

Appropriate gene expressions are achieved by proper actions of enhancers. Since transcriptional activation by enhancer is independent of orientation and distance from promoter, interference of regulatory actions between neighboring genes may cause inappropriate gene expressions. In order to block inappropriate enhancer-promoter communications, insulator sequences are inserted between such inappropriate pair of the enhancer promoter. In this study, we focus on the structure and dynamics of Ars-insulator in sea urchin to uncover the mechanism of such insulator activity. For this purpose, we construct a coarse-grained model of DNA involving Ars-insulator region and some mutants and perform the normal mode analysis to compare the molecular fluctuation and activity.

2P125 四重鎖形成可能な相補鎖 DNA を導入することによる四重鎖リボザイムのカリウムイオン濃度依存的な活性スイッチングの高効率化

Enhancement of a Quadruplex-ribozyme activity in response to K^+: a quadruplex-forming complementary DNA enables accurate switching

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An RNA R11, $r(GGA)_3GG$, forms a quadruplex under high K^+ conditions. We replaced a part of a hammerhead ribozyme by R11. Although an activity of a designed ribozyme was enhanced in response to K^+ via quadruplex formation in R11, our ribozyme showed non-negligible residual activity even in the absence of K^+. In this study, in order to repress this residual activity, we introduced a complementary DNA that also can form a quadruplex in the presence of K^+, to our ribozyme. It drastically repressed the residual activity. As a result, an activity ratio with/without K^+ has reached 16-fold. Because K^+ concentration is low outside the cell and high inside the cell our ribozyme may have a potential as a therapeutic drug and as an advanced drug delivery system.

2P126 抗プリオンアプタマーの構造学的基盤とのその活性

Structural basis of anti-prion aptamer and its activity

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We have obtained an RNA aptamer as to prion protein (PrP), r(GGAGGAGGAGGA) (R12). R12 tightly binds to PrP with the dissociation constant of 10^{-8} M. We identified two separated lysine rich clusters, designated as P1 and P16, within bovine PrP as binding sites for R12. Here, we have revealed that in the R12:PrP complex, one protomer of the R12 dimer interacts with P1 and the other with P16, simultaneously. This simultaneous double binding renders the high affinity to R12 against PrP.

We also demonstrate the anti-prion activity of R12 using mouse neuronal cells persistently infected with a human TSE agent, which constitutively expressed PrP^{Sc}. After incubation with R12, the accumulation of PrPSc in the cell was explicitly reduced.

2P127 非水溶媒中での ATP 加水分解の熱力学的解析
Thermodynamic analysis of ATP hydrolysis in non aqueous solvent

Hideyuki Komatsu (*Dept. Bioscience & Bioinformatics, Kyushu Inst. Tech.*)

The effect of hydration on ATP hydrolysis energy has been evaluated using a model system, where ATP is hydrolyzed via the transfer of ATP from water to organic solvent, the hydrolysis in the organic solvent, and the transfer of ADP and P_i from the organic solvent to water. In this model, alkyl amine-containing octanol was employed as a solvent, in which ATP, ADP and P_i are highly soluble. Because the hydrolysis is not detectable in the organic solvent, the partitioning of ATP, ADP and P_i between water and organic solvent was thermodynamically analyzed. Although the Gibbs energy of hydrolysis in the organic solvent is similar with that in water, ATP hydrolysis in the organic solvent is entropy-driven, as opposed to that the hydrolysis in water is enthalpy-driven.

2P128 シミュレーション・データマイニングアプローチによる蛋白質ドッキング過程における水和水ダイナミクス
Hydration water behavior in the protein docking process by simulating data mining approach

Taku Mizukami[1], Ayumu Sugiyama[2], Dam Hieu Chi[2], Ho Tu Bao[2] ([1]*Sch. Materials Sci., JAIST*, [2]*Sch. Knowledge Sci., JAIST*)

One of the central interest of hydration is the huge water contribution to the dynamics of protein. The identification of the hydration water, however, has been a quite difficult task, because the physicochemical water parameters disperse widely. To overcome these difficulties, we employed "the simulated data mining approach".
In this study, under the motivation to investigate the solvation effect on the protein docking process, we focused the water behavior in the vicinity of Barnase-barstar complex. The MD trajectory of the individual water molecule was projected into the feature-space, and classification of a new category hydration water was succeeded. In the presentation, we will discuss the large scale water structure of the protein complex system.

2P129 蛋白質水和水の並進拡散運動と蛋白質ダイナミクスとの動的カップリング
Translation diffusion dynamics of protein hydration water and its dynamical coupling with protein dynamics

Hiroshi Nakagawa[1], Mikio Kataoka[1,2] ([1]*Japan Atomic Energy Agency, Quantum Beam Science Directorate*, [2]*Nara Institute of Science and Technology, Graduate School of Materials Science*)

Protein dynamics is coupled with hydration water dynamics. Here, we examined the translational diffusion dynamics of protein hydration water by MD simulation and incoherent quasi-elastic neutron scattering at various hydration levels. The results of MD simulation show that the diffusion constants of hydration water are significantly suppressed below the hydration level at the percolation transition of hydration water. The diffusion constants are consistent with those estimated by neutron scattering experiments. The experimental data show that the hydration water dynamics can be described by a jump-diffusion model. The residence time in this model is correlated with the hydrogen bond kinetics between water molecules.

2P130 Water behavior in buried hydration sites of human cellular prion protein and pathogenic mutation T188R

Katsufumi Tomobe[1], Eiji Yamamoto[1], Takuma Akimoto[1], Masato Yasui[2], Kenji Yasuoka[3] ([1]*Graduate school of science and technology, Keio university*, [2]*Department of Pharmacology, School of Medicine, Keio University*, [3]*Department of mechanical engineering, Keio University*)

The secondary structural conversion of cellular prion protein (PrPc) to scrapie prion protein is a key process for prion disease. However this process remains unclear. We performed all-atom molecular dynamics simulations of human PrPc and pathogenic point mutant T188R to investigate conformational and hydration changes. Conformational fluctuation increases in a wide range by point mutation. Analyzing the mean residence time, we found four buried hydration sites in PrPc, while there are no buried hydration sites in point mutant T188R. These results suggest that the increase of fluctuation and the disruption of buried hydration sites may be important for secondary structural changes.

2P131 Aging of water molecules on cell membrane surfaces

Eiji Yamamoto[1], Takuma Akimoto[1], Masato Yasui[2], Kenji Yasuoka[3] ([1]*Graduate School of Science and Technology, Keio University*, [2]*Department of Pharmacology, School of Medicine, Keio University*, [3]*Department of Mechanical Engineering, Keio University*)

Water molecules near membrane surfaces form bridges that connect lipid molecules and stabilize cell membranes. An unsolved problem is clarifying how water molecules on the membrane surface diffuse. Using all-atom molecular dynamics simulations of lipid membranes, we analyze trajectories of water molecules on lipid membrane surfaces. Here, translational as well as rotational diffusion of water molecules on lipid membrane surfaces is found to show anomalous diffusion. Moreover, our approach, including the aging and the mean maximal excursion analyses, provides clear evidence that both divergent mean trapping time (continuous-time random walk) and long-correlated noise (fractional Brownian motion) contribute to this subdiffusion.

2P132 Dynamics of transcriptional apparatus in eukaryotic gene expression

Ashwin S. S, Masaki Sasai (*Department of Computational Science and Engineering, Nagoya University*)

Unlike simple bacterial gene switches, gene expression in eukaryotes is orchestrated by formation and resolution of a molecular complex, transcription apparatus (TA), at gene locus. TA is an assembly of regulating proteins such as transcription factors, mediators and RNA polymerase around the looped DNA region near the gene locus. Since formation of TA is a cooperative process including DNA looping and remodeling of nucleosomes, the time scale of formation/resolution of TA could be longer than the time scale of binding/unbinding of regulating proteins to/from DNA. We present a stochastic model to elucidate the effects of coexistence of multiple time scales on gene expression dynamics. Using mean field and simulation, we report a novel phase arising due to TA dynamics.

2P133 細胞性粘菌突然変異株にみられるソリトン様細胞運動

Biological Soliton in eukaryotic multicellular movement

Hidekazu Kuwayama (*Faculty of Life and Environmental Sciences, University of Tsukuba*)

Solitons have been observed in various physical phenomena. In this meeting, we show that the distinct characteristics of solitons are present in the mass cell movement of non-chemotactic mutants of the cellular slime mould, Dictyostelium discoideum. During starvation, D. discoideum forms multicellular structures that differentiate into spore or stalk cells and, eventually, a fruiting body. Non-chemotactic mutant cells do not form multicellular structures; however, they do undergo mass cell movement in the form of a pulsatile soliton-like structure (SLS). We also found that SLS induction is likely to be mediated by adhesive cell-cell interactions. These observations provide novel insights into the significant role of solitons in multicellular morphogenesis.

2P134 ラミニン固定化弾性率可変ゼラチンゲルを用いた iPS 細胞のフィーダーフリー分散培養

Feeder-free dissociated culture of iPS cells on the laminin-fixed elasticity-tunable gelatinous gels

Ayaka Utsumi[1], Tatsuya Okuda[2], Hiroshi Endo[3], Tomo Koike[3], Koji Eto[3], Satoru Kidoaki[2] ([1]*Grad. Sch. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*, [3]*CiRA, Univ. Kyoto*)

In recent years, fate control of mesenchymal stem cell depending on the mechanical conditions of extracellular matrix has attracted the strong attentions in the biomedical and tissue engineering fields. Relating to such stem cell mechanobiology, how about the mechanical manipulation for iPS cells? In this study, we designed laminin-fixed elasticity-tunable gelatinous gels, and investigated the conditions for feeder-free dissociated culture of iPS cells. As the result, we achieved to increase the cell proliferation rate compared to conventional matrigel culture by optimization of substrate elasticity and density of surface-fixed laminin. Cultured cells were confirmed to maintain undifferentiated state by immunostaining the marker proteins.

2P135 マウス胚盤胞と桑実胚間での異なるメカニカルストレス応答

Different responses to mechanical stimuli between mouse blastocyst and morula

Yuka Asano, Koji Matsuura, Keiji Naruse (*Grad. Sch. Med. Dent. Pharm. Sci., Okayama Univ.*)

The mammalian embryo in vivo is exposed to fluid shear stress (SS) when it traverses the fallopian tube. To find the difference of the response to SS between embryonic stages, we recorded time-resolved confocal microscopy images of mouse morula and blastocyst in the polydimethylsiloxane microfluidic channels. The embryos were hold using holding pipette in the microfluidic channel with continuous flow using a syringe pump. The shape of the blastocyst was not changed, when the applied SS was 2 dyne/cm^2. However blastomares of the molura were enlarged after the SS application. Based on the observation and our experimental results, we consider that mouse embryos at the morula stage are likely to be more sensitive to physical and/or chemical stimuli than the blastocysts.

2P136 SESTD1 に結合する横紋筋タンパク質の探索

Screening of SESTD1-binding proteins in striated muscle

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SESTD1 is a novel protein that consists of SEC14 domain, three spectrin repeats and unique sequences and exists in various tissues. One research suggests that SESTD1 works in the planar cell polarity pathway during mammalian embryonic development and another research suggests that SESTD1 regulates some transient receptor potential channels in smooth muscle. However, it remains unclear how SESTD1 functions in striated muscles. In this research, we searched the SESTD1-binding proteins from skeletal and heart muscle cDNA libraries using yeast two-hybrid methods. Alpha-actin was identified as a binding candidate of SESTD1 N-terminal region in skeletal muscle. We are currently investigating the role of SESTD1 in actin filament assembly.

2P137 ギボシムシのコネクチン様タンパク質の探索

Searching for connectin-like protein in acorn worm

Satoshi Nakayama[1], Akira Hanashima[1], Kunihumi Tagawa[2], Sumiko Kimura[1] ([1]*Department of Biology, Graduate School of Science, Chiba University*, [2]*Marine Biological Laboratory, Graduate School of Science, Hiroshima University*)

Connectin is a high molecular elastic protein in vertebrate striated muscle. On the other hand, invertebrate striated muscles also have connectin-like proteins (eg: I-connectin, projectin). To investigate evolutionary roots of connectin and connectin-like proteins, we searched for gene that encodes connectin-like protein in the genome of acorn worm, *Ptychodera flava*, using BLAST search program. Subsequently, we identified some domains including in connectin and connectin-like proteins, such as immunoglobulin (Ig), fibronectin type 3 (Fn3) and kinase domains, using SMART program. The results of analyses revealed that acorn worm connectin-like protein has Ig, Fn3 and kinase domains. Based on the tentative sequence, we performed RT-PCR and determined its partial sequence.

2P138 ヤツメウナギのコネクチン様タンパク質

Connectin-like protein of Lamprey

Mai Kanno, Yoshiharu Itoh, Akira Nishikawa, Akira Hanashima, Sumiko Kimura (*Department of Biology, Guraduate School of Science, Chiba University*)

Connectin is an elastic muscle protein in vertebrate striated muscle. We searched for a gene that encodes connectin using sea lamprey (*Petromyzon marinus*) information. Next, we identified characteristic domains present in connectin, such as immunoglobulin (Ig), fibronectin type 3 (Fn3) and kinase domains, in the lamprey using the SMART program and manual validation. Furthermore, the existence of these domains and the unique sequences between each domain were confirmed by RT-PCR using cDNA from lamprey skeletal muscle. The domain structure of lamprey connectin was similar to that of human connectin in that it possessed tandem Ig domains, PEVK region (rich in proline, glutamic acid, valine and lysine), Ig-Fn3 super repeats and a kinase domain at the C-terminus.

2P139 ウニのコネクチン様タンパク質

Connectin-like protein of sea urchins

Tomoko Sasaki, Tetsu Matsuura, Akira Hanashima, Sumiko Kimura (*Grad. Sch. Sci., Chiba Univ.*)

We performed SDS-PAGE to search for a connectin-like proteinin echinoderm sea urchins. We found a band that had high molecular weight, and predicted that it might be a connectin-like protein. In addition, we performed RT-PCR based on the sea urchin genome sequence, and determined the sequence of a connectin-like protein. Its size was about 80 kbp, and its molecular weight was estimated to be about 3 million. We noticed that this protein contains both connectin-like and characteristic structures from tandem immunoglobulin domains, a PEVK region, and a tandem fibronectin type 3 domain. Additionally, we prepared an antibody based on its sequence, and performed western blotting. We found that this antibody showed a reaction to the high molecular weight band.

2P140 分子動力学シミュレーションを用いたトロポミオシンの柔軟性および屈曲性の解析

Analysis of flexibility and curvature of tropomyosin by molecular dynamics simulation

Hideo Ozawa[1], Yoshihiro Ochiai[2], Koji Umezawa[1], Shin'ichi Ishiwata[1], Mitsunori Takano[1] ([1]*Dep. of Phys., Waseda Univ.*, [2]*Sch. Mar. Sci. Tec., Tokai Univ.*)

Tropomyosin (TM) is a dimeric parallel-coiled-coil protein, which binds the actin filament and participates in the regulation of muscle contraction. In addition, TM plays a role in the actin assembly in non-muscle cells. It is likely that the flexibility and curvature of TM affect these functions, through the interaction with actin. In order to evaluate these mechanical properties in detail, we performed molecular dynamics simulation with implicit solvent (by amber 10) for rabbit full-length TM. The crystal structure of pig TM (PDB ID 1C1G) was used as an initial structure, and the amino acids were replaced by rabbit TM sequence. In this study, the highly flexible and curved residues were determined and the relevance to the actin binding will be discussed.

2P141 横紋筋筋原線維 SPOC の動的特性に関するモデルシミュレーション

Model simulation on the dynamic properties of SPOC in a striated myofibril

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SPOC (Spontaneous Oscillatory Contraction) is the phenomenon observed in striated muscle under the intermediate activation conditions between contraction and relaxation. Recently, we constructed a unit model to describe the auto-oscillatory properties of sarcomeres, a structural and functional unit of striated muscle, and obtained a phase diagram consisting of contraction, SPOC and relaxation regions (Sato, K. et al., 2011), and extended it to a connected model by visco-elastically connecting a unit model in series. This model could explain the dynamic properties of SPOC such as SPOC wave (Sato, K. et al., submitted). In the present study, we examined various oscillatory patterns, the response to external mechanical perturbations and so on, based on the connected model.

2P142 高精度計測によるラット幼若心筋細胞内サルコメア自励振動特性の解明

High-resolution analysis of sarcomeric auto-oscillations in rat neonatal cardiomyocytes

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We developed a novel experimental system for simultaneous nanoscale analysis of single sarcomere dynamics and intracellular Ca^{2+} changes in rat neonatal cardiomyocytes via expression of AcGFP in Z-discs. First, our analysis revealed that during autonomous beating, the averaging of sarcomere lengths caused marked underestimation of lengthening speed due to superposition of different timings for lengthening between sequentially connected sarcomeres. The waveform was similar to that occurred at partial Ca^{2+} activation with blockage of sarcoplasmic reticulum (Cell-SPOC). Likewise, the waveform properties were qualitatively explained by our mathematical SPOC model. Thus, this system provides a powerful tool for unveiling single sarcomere dynamics in cardiomyocytes.

2P143 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

Giant Acceleration of diffusion in F_1-ATPase

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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.

2P144 高粘性中でのキネシンによるビーズ輸送

Transport of beads by kinesin in highly viscous environment

Naoto Sawairi[1], Takayuki Ariga[2], Michio Tomishige[2], Kumiko Hayashi[1] ([1]*Dept. Appl. Phys., Sch. Eng., Tohoku Univ.*, [2]*Dept. Appl. Phys., Sch. Eng., Univ. Tokyo*)

Kinesin-1 is a motor protein which transports organelles along microtubules in living cells.

Because intracellular viscosity is about ten times larger than water's, we used highly viscous buffer to construct a similar environment to the intracellular one.

Then we performed an *in vitro* single-molecule experiment on kinesin-1 in which a 1μm-sized bead was attached to it.

In a previous study, physical quantities such as velocity and drag force have been measured in a highly viscous environment using motility assay of kinesin-1 [A. J. Hunt *et al*., Biophys. J. 1994].

In our study, we aim to measure the drag force exerted by kinesin-1 by using the fluctuation theorem and to compare our result with the result obtained in the previous study.

2P145 神経細胞の軸索輸送におけるキネシンとダイニンの数の測定：揺らぎの定理の応用

Measuring the numbers of kinesin and dynein on neuronal cargo transport by using the fluctuation theorem

Kumiko Hayashi[1], Yasushi Okada[2] ([1]*Sch. Eng., Tohoku Univ.*, [2]*QBiC, RIKEN*)

We observed organelles, which are transported by kinesin and dynein along microtubules in neurons, by using fluorescence microscopy. We investigated non-equilibrium fluctuation in the constant drag motion of the organelle's center position. We estimated the drag force acting on the organelle exerted by the motors using the fluctuation theorem, a theorem for entropy production that has been applied to measure forces of bio-motors. We found that the distribution of the drag force, which was calculated from the drag force of each organelle, had several peaks. The values at these peaks may correspond to forces exerted cooperatively by multiple motors. The number of motors attached to an organelle is discussed based on the drag force distributions.

2P146 Observing RecBCD Translocation along Individual Chi-Containing Gapped DNA

Cinya Chung, Hung-Wen Li (*Department of Chemistry, National Taiwan University*)

The E. coli RecBCD initiates the repair of double-strand DNA breaks (DSB). Translocating and recognizing chi site (crossover hotspot instigator, 5'-GCTGGTGG-3') induces a conformation change that enables the enzyme to preserve the 3'-to-5' single-stranded DNA for later RecA assembly. RecBCD is composed of three subunits, RecB (3'-to-5' helicase), RecD (5'-to-3' helicase), and RecC, which is believed to be responsible for chi recognition. Here we use a single-molecule tethered particle motion technique to directly monitor the translocation of RecBCD along chi-contained DNA. Using bead-labeled enzymes, we monitored the RecBCD translocation along DNA by measuring the gradual decrease in the bead Brownian motion as the enzyme moves along the DNA towards the surface.

2P147 Dynamical energy landscape theory for the force-generation process in actomyosin motor

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The mechanism of the myosin II motor is still under debate. To resolve the controversy between the lever-arm model and the biased Brownian motion model, we investigate the roles of conformational and nucleotide state changes by using a coarse-grained model of actomyosin. The free energy landscape is calculated for different conformational and nucleotide states in the force generation process, and kinetic transitions among states are simulated by stochastic jumps among landscapes. We found that the free energy landscape is much affected by both the conformational change of myosin and change in electrostatic interactions between actin and myosin, and the jumps among these landscapes explain how the lever-arm and the biased Brownian motion contribute to force generation.

2P148 マイコプラズマ Gli349 タンパク質の構造ダイナミクス解析

Structure and dynamics of the gliding protein Gli349 from *Mycoplasma mobile*

Junichi Inatomi[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

Mycoplasma mobile clings to a cell surface and glides over it using a plausible leg protein Gli349 through its large conformational changes. Previous studies suggest that Gli349 is composed of 18 repeats of ~100 residues. However, detailed structure and dynamics of the protein remain unclear. One approach to solve this problem is to study the structure and dynamics of separate fragments corresponding to the repeats of Gli349 and then reconstruct its overall structure. To this end, we have synthesized the genes corresponding to the repeats J, K, and L and a hinge h2 separately. These regions are considered to be essential for the conformational changes of Gli349. Structural characterization of these regions will be presented at the meeting.

2P149 Gene manipulation of gliding bacterium, *Mycoplasma mobile*

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

Mycoplasma mobile, a pathogenic bacterium, glides on solid surfaces with a unique mechanism, not related to known bacterial motility systems or conventional motor proteins. The gliding machinery is composed of at least three huge surface proteins and an ATPase, and potentially more than ten cytoskeletal proteins. To elucidate this mechanism, first we constructed a transformation system for *M. mobile*, by optimizing parameters such as electroporation conditions, recovery time and reduction of film-spot. We will present localization of at least two gliding proteins by EYFP tagging, replacement of gliding proteins by modified ones, and the discussion about the updated working model for gliding mechanism.

2P150 単一糖鎖上でのマイコプラズマの滑走と結合

Gliding and binding of mycoplasma on uniform oligosaccharide

Taioshi Kasai, Tasuku Hamaguchi, Makoto Miyata (*Grad. Sch. Sci., Univ. Osaka City*)

Mycoplasmas, pathogenic bacteria bind to solid surface and glide to one direction. Previously, we showed that mycoplasma cells catch sialylated oligosaccharides in gliding, but mycoplasma gliding on uniform oligosaccharide has never been observed. In the present study, we analyzed the gliding and binding of mycoplasma to 53 uniform oligosaccharides fixed to a solid surface, and found that the gliding speed relate inversely to the affinity of mycoplasma for sialylated oligosaccharide. The recognition of mycoplasma foot for sialylated oligosaccharide also has been suggested as follows. (i) The reducing terminal Neu5Ac is tightly recognized in an occluded position. (ii) The second and third sugars are loosely recognized. (iii) The third sugar is recognized from one side.

2P151 マイコプラズマ滑走タンパク質分子の可視化による構造解析
Structure of Proteins Involved in *Mycoplasma mobile* Gliding Revealed by Visualization

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Mycoplasma mobile, a fish pathogen, glides on solid surfaces with a unique mechanism. In this mechanism, Gli521 (521kDa) transmits movements from motor to leg as "crank", and Gli349 (349kDa) attaches to solid surface and pull cell body as "leg". Here, we analyzed molecular shapes of these proteins by negative-staining electron microscopy and high speed AFM. Gli521 molecule is 120 nm long and shaped "ω", featured with a central hinge, at which the molecule bends only to one direction. Gli349, known as 100 nm " 8th note ", shaped molecule by rotary shadowing TEM was divided into small and large domains connected by a thin, elastic filament. We will discuss the movements of molecules in the gliding mechanism.

2P152 ヒト肺炎 *Mycoplasma pneumonia* の滑走運動装置と構成タンパク質の結晶化
Crystallization of gliding machinery and component proteins of *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae, a human pathogenic bacterium glides with repeated catch and release of sialylated oligosaccharides on host cell surfaces through a membrane protrusion at a pole. The rod structure on the attachment organelle can be divided into three parts, Terminal button, Paired plates, and Wheel complex from the tip. To study the functions and structures, we expressed and purified four Wheel complex proteins coded by MPN311, MPN312, MPN387 and MPN526, a Terminal button protein coded by MPN309, and P1 adhesin coded by MPN141. To date, MPN311 and MPN387, responsible for the connection of tip to cell body and motility, respectively, are successful for crystallization after modifying some protein constructs.

2P153 マイコプラズマ・モービレの滑走にかかわるチューブリンホモログの構造解析
Structural analysis of tubulin homolog involved in *Mycoplasma mobile* gliding

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Mycoplasma mobile glides with a unique mechanism. We focus on P42 protein composed of 356 amino acids, a component of the gliding machinery. Its amino acid sequence showed 23% identity in C-terminal 86 amino acids residues with a bacterial tubulin, FtsZ of Thermus sp. RL. N-terminal 235 amino acids, however, did not show similarity with FtsZ, suggesting that P42 evolved from tubulin and achieved a role specific for *Mycoplasma* gliding. To study its functions and structure, we expressed P42 and purified. As the full length protein easily formed clumps, we removed a P42 specific coil-coil near the C terminus. The protein did not show GTPase activity, consistent with the sequence analysis.

2P154 Investigating stators assembly of flagellar motors in Escherichia coli

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Stators of bacterial flagella motor are the drivers to prompt motor rotation for swimming at low Reynolds number environment. Motor basal part is about 45 nm in diameter and surrounded by ~11 stators which work independently. The whole motor can rotate at few hundred hertz. Here we develop a super-resolution microscopy to study the stator binding position in the motor. We use photo-active green fluorescent protein (PAGFP) fused stator protein (PomA) in Escherichia coli. Under low expression level, motor rotate at low speed (~ few Hz) with few stators only. By measuring the relative position of these stators, we investigate the number of stators and the assembly process of these stators in these nanometer powerful molecular machines.

2P155 Tracking of bacterial flagellar motor rotation by fluorescent microscopy

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The bacterial flagellar motor is a reversible rotary nano-machine, about 45 nm in diameter, embedded in the bacterial cell envelope. It is powered by the flux of coupling ions across the cytoplasmic membrane driven by an electrochemical gradient. The torque is thought to be generated at the cytoplasmic side of the motor components, FliG, FliM, FliN, MotA and MotB. To understand how the motor works, we have developed the assay to track functional motor components labeled with fluorophores by total-internal reflection fluorescent microscopy. Single fluorescent molecules in *E. coli* cells were detectable using our system. We have been trying to observe behaviors of each component during motor rotation at single molecule level.

2P156 タンデム PomA 変異体を固定子とする Na^+駆動型キメラべん毛モーターの回転計測
Rotation Measurement of Na^+-driven Chimeric Flagellar Motor with Tandem PomA Mutants

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Bacterial flagellar motor with chimeric stators (PomA/PotB) works as a Na^+-driven motor in *E. coli*, which has H^+-driven one in nature. The motor is driven by independent stator complexes consist of two sets of A_2B sub-complexes (A_4B_2 in total). To study the functional roles of individual A subunits, we carried out rotation measurements of single flagellar motors with tandem fused PomA dimmer, which expressed as a single peptide. The motor with tandem PomA can rotate smoothly, but its speed was ~30% lower than that with monomeric PomA. Furthermore, single mutation of crucial charged residue in either side of the tandem PomA still allowed motor rotation, indicating that four A subunits do not work equivalently in the stators for torque generation.

2P157 Structural study of the sheath in the magnetotactic bacterium MO-1 by electron cryomicroscopy

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MO-1 is a magnetotactic bacterium capable of swimming as fast as 300 micrometer per second. Recently, we reported the three-dimensional structure of its flagellar apparatus. It is a complex organelle consisting of 7 flagella and 24 fibrils that form a tight bundle enveloped by a sheath. Their basal bodies are arranged in an intertwined hexagonal array similar to the thick and thin filaments of vertebrate skeletal muscles.
The sheath plays an essential role in thrust production driven by flagellar rotation. It was assembled from a big glycoprotein denoted as sheath-associated protein in a calcium ion-dependent manner, but the detailed structure remains unknown. Here, we show the structural study of the sheath obtained by electron cryomicroscopy.

2P158 細菌べん毛基部体中のスイッチ蛋白質 FliG の位置ならびに配向の同定
Identification of the location of the switch protein FliG in the flagella basal body

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Many bacteria swim by a reversiblely rotating flagella. The switch complex consists of three switch proteins, FliG, FliM and FliN which control counterclockwise-clockwise (CCW/CW) switching of the motor rotation, form the C-ring on the cytoplasmic face of the MS ring. FliG is most directly involved in torque generation and motor switching.
In the previous meeting, we reported the structure of the CCW and CW type C-ring. FliM and FliN were well fitted into the middle and lower parts of the outer region of the C-ring, but the location of FliG remains uncertain. To determine the location of FliG, we performed the FliG labelling experiments by electron cryomicroscopy, we report the psudo atomic model of the C ring and discuss the switching mecanism of flagellar rotation.

2P159 Torque-speed relationship of the flagellar motor consisting of two distinct stators

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The bacterial flagellar motor is powered by electrochemical potential difference of ions across the cytoplasmic membrane. Rotational energy is mediated by inward ion flux through the channel of the stator complexes. *Bacillus subtilis* has not only H^+-dependent stator MotAB, but also Na^+-dependent stator MotPS. We have shown that both MotAB and MotPS contribute in motility, but there has been no direct evidence to date that these two stator complexes coexist in the flagellar motor. Here, we measured the rotation speed of single motors by bead assay to see whether these two stators actually work together in a motor. The torque-speed curve showed that the motor of *Bacillus subtilis* is driven by both MotAB and MotPS in a motor.

2P160 人工分子ベアリングの分子内回転の 1 分子計測
Single-Molecular Measurement of a Synthetic Molecular Bearing

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Single-molecule measurements using large probes have revealed mechanisms of biomolecular motors such as steps, pauses, velocities and forces. Here we applied single-molecule technique to double-decker porphyrin (DD) known as a synthetic molecular bearing (~1 nm in size). By using a magnetic bead (~200 nm) as a probe, we successfully visualized non-biased rotary diffusion with 90-deg. steps, consistent with the 4-fold rotational symmetry of the DD. Distribution of duration times of the pause was reproduced by single-exponential decay function with time constant of 0.36 s, corresponding to the barrier height of 3 kBT. Our approach will open a way studying the fundamental properties of synthetic molecular machines which cannot be resolved by ensemble-molecule measurement.

2P161 F_1-ATPase の触媒活性機構の理論的解析及び新規触媒活性変異体の設計
Theoretical studies on ATP hydrolysis in F_1-ATPase and a rationally designed enzymatic reaction in its variants

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We study catalytic reaction of F_1-ATPase by hybrid QM/MM methodology. First, reaction profile of ATP hydrolysis was investigated by means of QM/MM RWFE method which enables one to take into account protein thermal fluctuation. Second, we attempted to rationally design mutants of F_1-ATPase furnished with catalytic activity for an unnatural chemical reaction, Kemp elimination. The ATP binding site is modified to accommodate and chemically activate a substrate of the reaction, 5-Nitrobenzisoxazole, which is attached to ribose instead of the adenine ring of the native substrate, ATP. The mutants that possibly enhance the reaction rate will be discussed based on reaction profiles obtained by QM/MM calculations.

2P162 ATP 合成酵素の結晶化
Crystallization of ATPsynthase

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ATP synthase is responsible for ATP production in living cells, and is a membrane protein. We crystallized ATP synthase from a thermophilic bacterium PS3, but crystals were not good enough. In the course of improving those crystals, we suddenly got unable to reproduce them.
Subsequent efforts to improve PS3 synthase preparation were unfruitful. Also synthase preparations from other 27 bacterial strains, which we then isolated, were not good enough. We are now back to PS3.
After lots of points, possibly leading to good preparations, have been examined so far, our current view is that good bacterial stocks, among many currently available, should be used in combination of a use of a fermenter (not flasks). Data will be presented on why we think so.

2P163 人工基質 RTP を用いた F_1-ATPase の回転触媒機構の解明

Base moiety of ATP is dispensable for driving the rotation of F_1-ATPase

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F_1-ATPase (F_1) is a rotary motor protein driven by ATP hydrolysis. We have previously examined the role of base moiety of ATP on the rotation of F_1 by using base-substituted analogs; GTP and ITP. These analogues supported the rotation of F_1. Based on these findings, it was hypothesized that the base moiety is dispensable for the rotation of F_1. To verify this, we synthesized an artificial substrate "ribose-triphosphate" (RTP), lacking the base moiety, and examined its competency to drive the rotation of F_1. As a result, we found that RTP can also drive F_1 rotation, although its binding affinity was 10^6 times lower than ATP. Thus, we confirmed that the base moiety of ATP is important for its binding to F_1, but is dispensable for driving the rotation.

2P164 DNA を回転子に持つ新規回転分子モーターの創製

Creation of a hybrid F_1 motor with DNA as the rotor

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F_1-ATPase (F_1) is a molecular motor that rotates the rotor γ-subunit against the stator $\alpha_3\beta_3$ ring by hydrolyzing ATP. The three β-subunits generate a torque for the unidirectional rotation. They show the well-coordinated power-stroke motion in the isolated $\alpha_3\beta_3$ ring (Uchihashi, Iino et al Science 2011). This result raised a hypothesis that the $\alpha_3\beta_3$ can rotate the objects other than the γ-subunit. To verify this hypothesis, we have created a hybrid motor that consists of the $\alpha_3\beta_3$ as the stator and DNA as the rotor. Double-stranded DNA was successfully incorporated into the $\alpha_3\beta_3$ ring. When observed using gold nanorod as a probe, some molecules showed three pausing positions, although unidirectional rotation has not been observed yet.

2P165 F_1-ATPase 内の DELSEED-loop のトルク伝達機構の解明

Elucidation of torque-transmission mechanism of DELSEED-loop in F_1-ATPase

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F_1-ATPase (F_1) is an ATP-driven rotary motor whose torque is generated at the interface between the catalytic β subunit and the rotary γ subunit. Previously, we reported that the mutant F_1 of which residues in DELSEED-loop (D-loop) of β *C*-terminus domain were substituted with glycine to disrupt the interaction with γ (Gly^{loop}) halved the torque, suggesting the crucial role of D-loop in torque-transmission. Here, we investigated how the glycine substitution of D-loop affects the angular dependencies of the equilibrium constants of ATP binding and ATP hydrolysis. Gly^{loop} showed evidently lower angular dependency of ATP binding step while that of ATP hydrolysis step was not affected by the mutation, suggesting that D-loop transmits the torque in ATP binding process.

2P166 高速配向イメージングによる F1-ATPase の触媒サブユニットの構造変化計測

Direct observation of domain motion of the catalytic β subunit of F1-ATPase using high-speed orientational imaging

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F_1-ATPase is an ATP-driven rotary molecular motor. Rotation of the rotor subunit γ is driven by coordinated conformational change of three catalytic subunits β in the stator ring. To elucidate the mechanism how each catalytic subunit β rotates the γ subunit, direct observation of the conformational change of individual subunit β at high temporal and spatial resolutions is requisite. Here, we attached a gold nanorod to the subunit β, and observed defocused scattering images of gold nanorod with time resolution of 3 μs. As results, we could resolve rapid and reversible changes of the catalytic subunit β between open and closed states driven by ATP hydrolysis reaction. We will discuss the observed conformational transition process and the rotation mechanism of F_1-ATPase.

2P167 サポーテッド膜を用いた F_oF_1 の一分子回転計測

Single molecule observation of F_oF_1-ATP synthase in the supported lipid membrane

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F_oF_1-ATP synthase is the rotary motor, in which the mechanical rotation mediates the energy conversion between ATP hydrolysis and the proton translocation. F_oF_1 from Thermophilic *Bacillus* PS3 (TF_oF_1) is the best-characterized F_oF_1 in single-molecular studies. While 3-step rotation of F_1-part, reflecting the 3-fold symmetry of TF_1 structure, has been well studied, 10-step rotation of TF_oF_1 corresponding to the structural 10-fold symmetry of F_o has not been observed. In this study, we developed the novel experimental setup in which TF_oF_1 was reconstituted to the supported lipid membrane. Using this setup, we observed the ATPase rotation of TF_oF_1, some of which showed 10-step rotations and we will report the kinetic analysis of the stepping behavior of TF_oF_1.

2P168 Basic properties of rotary dynamics of Enterococcus hirae V1-ATPAse motor protein

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we observed rotation of *Enterococcus hirae* V_1-ATPase (EhV_1) by using a 40-nm gold colloid as a load-free probe. EhV_1 rotated unidirectionally with maximum rotation speed (V_{max}) of 107 rps and the Michaelis constant (K_m) of 154 μM. Second-order binding rate constant for ATP (k_{on}^{ATP}) determined by $3\times V_{max}/K_m$ was 2.1×10^6 $M^{-1}s^{-1}$. At saturating ATP concentration, EhV_1 showed three catalytic pauses separated by 120° each other. The distribution of duration times of the catalytic pause showed convex-shape and was reproduced by consecutive reaction with two time constants of 2.5 ms and 0.5 ms. No substeps were resolved in the rotation observed around K_m. The k_{on}^{ATP} determined from duration times at 10 μM ATP was 2.3×10^6 $M^{-1}s^{-1}$, consistent with the value of $3\times V_{max}/K_m$.

2P169 １分子蛍光観察によるセロビオヒドロラーゼの結晶性セルロース加水分解反応素過程の解明

Single-molecule imaging analysis of cellobiohydrolase hydrolyzing crystalline cellulose

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Cellobiohydrolase is a new type of linear molecular motor that hydrolyzes crystalline cellulose into water-soluble disaccharide at solid/liquid interface. Here, with single-molecule fluorescence microscopy and high-speed AFM, we revealed the adsorption-desorption dynamics and linear translocation of *Trichoderma reesei* cellobiohydrolase (*Tr*Cel7A) hydrolyzing Type I_α crystalline celluloses. *Tr*Cel7A showed binding rate constant of 9.7×10^6 M^{-1} μm^{-2} s^{-1}, including productive and non-productive binding that lasted 8.6 s and 1.2 s respectively. The velocity of movement on cellulose in the productive binding was 5.0 nm/s (5 s^{-1} in turnover rate). Our results shed light on the enzyme reaction mechanism at solid/liquid interface at the single-molecule level for the first time.

2P170 負荷存在下でのキネシン頭部の運動の高時間分解能観察

High temporal resolution observation of the stepping motion of kinesin-1 under load

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Kinesin-1 is a motor protein that walks along microtubules to transport cargoes. The velocity of the movement decreases with increasing load, however it is still unclear which conformational transition is the load-dependent. At the last meeting, Isojima in our lab demonstrated that we could distinguish bound and unbound states of the motor head using dark-field microscopy with 50-μs temporal resolution. In this study, we constrained the kinesin movement by fusing a mutant head that cannot detach from the microtubule to the stalk of wild-type dimer. Then we observed the movement of a wild-type head, which showed discrete 16 nm steps and then stalled after several steps. We will discuss the effect of load on the duration of bound and unbound states of a head.

2P171 ジスルフィドクロスリンクを用いたキネシン 1 の二足歩行制御機構の研究

Strain-dependent regulation of the kinesin-1's catalytic activity as studied by disulfide-crosslinking of the neck linker

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Kinesin-1 walks along microtubules by alternately hydrolyzing ATP and moving two motor domains, although the mechanism of the alternate catalysis remains unknown. Here we focused on the neck linker that connects two motor domains and investigated the effect of the neck linker tension on the motor activity by constraining the neck linker in the forward or backward extended conformation using disulfide-crosslinking. Stopped flow and single molecule measurements showed that the forward-constraint of the neck linker reduced ADP release rate although the backward-constraint suppresses either ATP hydrolysis or Pi release rate. These results suggest that ATP hydrolysis cycle can be differently regulated depending on the direction of the neck linker tension.

2P172 SDSL-ESR により検出したキネシン α-1 ヘリックスのヌクレオチド依存的な動的構造とその変位

Nucleotide-dependent Displacement and Dynamics of α-1 Helix in Motor Protein Kinesin As Revealed by Site Directed Spin Labeling ESR

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We show the nucleotide-dependent displacement of the α-1 helix of kinesin on microtubule by ESR spectroscopy. Kinesin monomer was doubly spin-labeled at α-1 and α-2. The inter-helix distance distribution was determined by spectral broadening and showed that 40% of spins had a peak at 1.4-1.7 nm, which was close to that from crystal structure, but 60% beyond sensitivity (>2.5 nm). The fraction of 1.4-1.7 nm was 20 and 25% in the presence of AMPPNP and ADP, respectively. These nucleotide-induced decreases in the fraction of 1.4-1.7 nm were reversely related to those in the docking fraction of neck-linker on motor core, suggesting that shift of spatial equilibrium of α-1 helix from 1.4-1.7 nm toward >2.5 nm makes its C-terminal end to be exposed and bind neck-linker.

2P173 微小管上での KIF1A の選択的結合における負に荷電した C 末の役割

The role of negatively-charged C-terminus of tubulin in selective binding of KIF1A on microtubule

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KIF1A, a well-studied motor protein, functions as a monomer, where the force-generating biased binding to the microtubule (MT) has been observed during the weak-to-strong binding transition. While it is obvious that the weak binding is maintained by the electrostatic interaction between the lysine-rich K-loop of KIF1A and the glutamate-rich C-terminus of tubulin (E-hook), it is less clear why KIF1A selectively binds on the α-β tubulin junction within the tubulin dimer rather than the β-α junction between the tubulin dimers. In this study, by calculating the binding energy between KIF1A and MT, we investigated the molecular mechanism of the selective binding of KIF1A, which also has close relevance to the biased binding, and show that E-hook plays the key role.

2P174 フォトクロミック分子を用いた有糸分裂キネシン Eg5 の光制御型阻害剤

Photo regulted inhibitor composed of photochromic molecules for mitotic kinesin Eg5

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S-trityl-L-cysteine (STLC) is a well known specific inhibitor for mitotic kinesin Eg5. Trityl group of STLC is a key moiety to inhibit Eg5 ATPase potently. In this study, we designed and synthesized the photo regulated inhibitors to control Eg5 activity. The photochromic inhibitor 4,4'-bis(N-(2-(triphenylmethylamino)acetyl)amino)azobenzene (BTAB) was synthesized. by the coupling reaction of 4,4'-diaminoazobenzene with tritylglycine. And other related inhibitors were also synthesized. BTAB showed cis-trans isomerization upon 380 nm and 480 nm light irradiations. BTAB inhibited reversibly Eg5 ATPase activity in the absence of microtubules by light irradiations. We also examined which step along ATPase kinetic pathway is inhibited by BTAB using stopped flow apparatus.

2P175 有糸分裂キネシン Eg5 の機能性ループ L 5 へのフォトクロミック分子導入と光制御

Incorporation of photochromic molecule into the functional loop L5 of mitotic kinesin Eg5 and its photo regulation

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It is believed that the loop L5 of kinesin is important region for motor function. Interestingly mitotic kinesin Eg5 has a several times longer L5 in comparing with other kinesins. It has been demonstrated that the L5 of Eg5 performed as a stabilizer for the Eg5-specific inhibitors (STLC, monastrol) complexes. In this study, we prepared 8 mutants of Eg5 which have a single cysteine in L5 in order to incorporate photochromic molecules. We also synthesized thiol reactive spiropyran derivative monoiodoacetyl-spiropyran (IASP). IASP was incorporated into the mutants stoichiometrically. The Eg5 mutant E118C modified with IASP showed reversible alteration of microtubule dependent ATPase activity upon UV and visible light irradiations. The other mutants were also examined.

2P176 原子間力顕微鏡によるコンフルエント細胞の力学測定

Mechanical measurements of confluent cells with an atomic force microscope

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Cells have mechanical interactions with surrounding cells. Thus, it is crucial to identify mechanical properties of cells in a large scale from intercellular regions. Recent studies revealed the traction forces of confluent cells during cell migration, but the stiffness of confluent cells has not fully understood. To measure such a mechanical property, we developed a home-made atomic force microscope (AFM), with a wide-range scanner, equipped with an upright optical microscope. In the AFM, a liquid-immersion objective lens was employed to focus and collect the laser light for optical lever. The AFM allowed us to map the height and the Young's modulus of cells in a range of about 300 mm × 300 mm. The spatial correlation of confluent cells' mechanics will be discussed.

2P177 ゾウリムシのメタクロナールウェーブは外液の粘性だけでなく細胞表層の弾性も使って伝播できる

Metachronal wave travels not only in outer viscous fluid but also on elastic cell surface of *Paramecium* cells

Naoki Narematsu, **Yoshiaki Iwadate** (*Fac. Sci., Yamaguchi Univ.*)

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. To confirm this, we planed to breakdown the hydrodynamic coupling of ciliary movements at a restricted portion of a *Paramecium* cell and observe whether metachronal coordination collapses or not. Metachronal waves passed over the portion where the hydrodynamic coupling was broken. To clarify the other mediator of the wave, we applied cyclic stretching of cell body. The frequency of metachronal wave became equal to that of the cyclic stretching.

2P178 細胞間力学変化量の空間不均一性：原子間力顕微鏡測定

Spatial heterogeneity of cell-to-cell mechanical variability measured by atomic force microscopy

Ryosuke Takahashi, Kaori Kuribayashi-Shigetomi, Takaharu Okajima (*Grad. Sch. Info. Sci. & Tech., Hokkaido. Univ.*)

Cell mechanics is crucial not only for understanding the mechanism of cell functions but also for diagnosing cell disease. Previous studies revealed that the averaged cell mechanical properties largely changed in intracellular positions. However, little is known how the cell-cell variability of cell mechanics depends on the cell positions. Using atomic force microscopy, we investigated the complex shear modulus, which exhibits single power-law behavior [1], of single cells cultured on micro-patterned substrates. We found that the variation of the cell modulus decreased toward the cell center. We will show the detail relationship between the variability and the cell cytoskeleton. [1] Fabry et al. Phys. Rev. E (2003)

2P179 AFM を用いた強制剥離による細胞接着力の評価

Evaluation of cell adhesion force by mechanical detachment using AFM

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We developed a method to measure cell adhesion force by mechanical detachment from substrate. An AFM probe was fabricated to a needle shape with a hook-shaped tip to penetrate cellular membrane and pull up cells vertically. The adhesion forces defined as peak forces in detaching process were successfully measured for seven cell lines. We supposed that the force volume might be correlated with number of focal adhesions. Since the cell adhesion has close relations with the cell motility, we measured the average velocity of the cells. As a result, the migration velocity showed negative correlation with the adhesion forces among the measured cell lines. Concerning murine breast cancer cell lines, the adhesion force tends to be smaller in the more metastatic cells.

2P180 細胞内の力学環境に対する分子混み合い効果

Molecular crowding effects on intracellular mechanical environments

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We investigate crowding effects on cell mechanics by high-bandwidth microrheology. For the model systems, viscoelasticity of BSA (family of globular proteins) and extracts taken from Escherichia coli were measured by changing their concentrations.
The concentration dependence of viscosity fits well to empirical function $\eta=\eta 0\ \exp\{A\varphi/(\varphi 0-\varphi)\}$ which is known to describe the characteristic behavior near glass transition. The concentration $\varphi 0$ at which the viscosity divergies, however, seems largely different between BSA solutions and extracts from cells.
We found that the viscosity in cells is much lower than that in cell extracts. The uncaging in model systems are driven purely thermally while the spontaneous athermal forces can facilitate flows in cells or active systems.

2P181 Molecular configurations of purified radial spoke of Chlamydomonas flagella

Hitoshi Sakakibara, Yosuke Shimizu, Hiroaki Kojima (*Bio ICT, KARC, Nat. Inst. Info. Commn. Tech*)

Radial spokes of eukaryotic flagella are large protein complexes (~2 M) which connect 9 peripheral microtubules and the central-pair apparatus. They are thought to play important roles for generating well-ordered flagellar waveforms. In order to examine properties and molecular configurations of radial spoke in vitro, we purified radial spoke from Chlamydomonas. For purification, we used the strain (pf14::PF14-12His). Radial spoke was purified from the 0.6M-NaBr extract of axonemes successfully by combining the His-tag purification method and sucrose-density gradient centrifugation. By negative-staining electron microscopy, we observed structures consisting of a base, a shaft, and a heart shaped head in the sample in the same way as radial spokes in flagellar axonemes.

2P182 波型弾性パターニングゲル上での流れ誘導メカノタクシスに見られるがん細胞の接着スイッチング挙動
Adhesion switching of tumor cells in shear-flow-induced mechanotaxis on wave-like elastically-patterned gels

Yuki Kubota[1], Tatsuya Okuda[2], Satoru Kidoaki[2] ([1]*Grad. Sch. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*)

Epithelial tumor cells migrate into mesenchyme, invade into blood vessel, and spread to other organs in their metastasis process. In this process, motility of tumor cells is affected by the mechanical conditions of matrix-stiffness and shear stress. In this study, to systematically address the effect of those conditions on the motility regulation of tumor cells, we newly developed flow-chamber system with the microelastically-patterned gels, and analyzed the migration of breast cancer cell line MDA-MB-231 in the system. Interestingly, MDA-MB-231 cells were found to exhibit significant adhesion switching especially on wave-like elastically-patterned gels under the shear-induced mechanotaxis, while normal mammary epithelial cells did not show such switching phenomenon.

2P183 粘性流体中におけるバクテリアの Twitching 運動のシミュレーション
Simulation study of the twitching motility of bacterium in viscous fluid

Ryota Morikawa, Masatada Tamakoshi, Takeshi Miyakawa, Masako Takasu (*School of Life Sciences, Tokyo University of Pharmacy and Life Sciences*)

We present a dynamical model of a bacterium, which crawls over solid surfaces by extending and retracting their multiple pili. This motion is called “twitching motility” which has been experimentally investigated on P. aeruginosa and T. thermophilus. In the model, the drag force of the bacterial body in the viscous fluid balances with the other force caused by retracting movement of the pili. We investigate dynamical properties of our bacterial model by computer simulation. The peculiar motion found in experiments, in which the bacterial body turns in a short time like oversteering, is reproduced in our simulation. We suggest that the bacteria skillfully locomote against a viscous fluid by means of twitching on the solid surfaces.

2P184 大面積弾性マイクロパターニングゲルを用いた間葉系幹細胞の分化フラストレーションの誘導と評価
Characterization of frustrated differentiation of mesenchymal stem cells cultured on large-scale microelastically-patterned gels

Kosuke Hamano[1], Tatsuya Okuda[2], Satoru Kidoaki[2] ([1]*Grad. Sci. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*)

In the previous study, we found the emergence of suppressed differentiation of mesenchymal stem cells (MSCs) into multiple lineages when cultured on microelatically-patterned gelatinous gels having alternating arrays of hard and soft bands. On the gels MSCs migrate among the different region of elasticity, receive a kind of oscillating mechanosignals from substrate during culture, and are blocked to differentiate into the elasticity-specific lineages. We named such phenomenon as “frustrated differentiation”. In the present study, to fully characterize stemness of MSCs in the frustrated differentiation, we investigated multi-lineage differentiation ability of the MSCs using newly-developed large-scale elastically-patterned gels to prepare enough numbers of MSCs.

2P185 １分子追跡法により明らかにされた伸展中の細胞における Dystroglycan の形成中の接着斑へのリクルート
Dystroglycan recruitment to forming focal adhesions during cell spreading as observed by single-molecule tracking

Akihiro Shibata[1], Masahiro Makuta[2], Limin Chen[2], Yuuri Nemoto[2], Yuki Shirai[2], Hisae Tsuboi[2], Nao Hiramoto[2], Takaaki Tsunoyama[2], Takahiro Fujiwara[2], Akihiro Kusumi[1,2] ([1]*Institute for Frontier Medical Sciences, Kyoto University*, [2]*Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University*)

Dystroglycan (DG) is a transmembrane protein, considered to be responsible for mechanically linking the plasma membrane (PM) to the actin cytoskeleton (ASK) via dystrophin (DP). Another key mechanical linkage between PM and ASK is done by the focal adhesion (FA). Here, we found that DG was recruited to forming FAs during cell spreading, but not mature FAs in steady-state cells. Within the FA, DG underwent diffusion with intermittent, transient binding to, perhaps, the FA-protein islands in the archipelago architecture of the FA reported previously (Shibata et al. 2012 and 2013, both published in Cytoskeleton). Since the DG-DP complex and the FA had been considered to work totally independently, the crosstalk between the FA and the DG-DP complex was unexpected.

2P186 付着型珪藻の二次元運動への培地温度の影響
Effects of variation in medium temperature on two-dimensional motion of attached diatom

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Some types of diatoms are known to glide on a substrate. In this research, we observed the variation in the velocity of a gliding diatom cell by varying the temperature of a medium to 15°C, 18°C, and 25°C. We selected samples of freshwater pennate diatoms, Nitzschia palea, and used glass, polystyrene, and PDMS substrates. Our results show that with an increase in the medium temperature, the ratio of the stationary cells to the total number of cells decreased and the average velocities of the cells increased. These velocity changes were observed for all the used substrates. These results indicate that a change in temperature is an important factor affecting the velocity of cell motion.

2P187 シクロフィリン D がミトコンドリアに及ぼす影響
Effects of cyclophilin D on mitochondria

Daisuke Shinohe, Asuka Kobayashi, Hitomi Nakazato, Akiko Nagai, Yoshihiro Ohta (*Tokyo Univ. of Agric. and Tech*)

Cyclophilin D (CypD) is a peptidyl-prolyl cis-trans isomerase (PPIase) in the mitochondrial matrix.
Many studies have shown that CypD is involved in necrosis and various diseases. However, the physiological role of CypD is unknown. To investigate the physiological role of CypD, we prepared C6 glioma cell line with overexpressed wild-type or PPIase-deficient mutants (R97A) of cyclophilin D and control cells transfected with the corresponding empty vecter.
In the present study, we investigated energy metabolism, quantity of mitochondrial protein, and uptake of the fluorescent dye to mitochondria among these cells. As a result, we found that CypD significantly affected pyruvate metabolism and translocation of calcein-AM into mitochondria.

2P188 細胞分裂時におけるエネルギー状態のモニタリング
Monitoring of energy state of cells during cell division

Kotoe Hirusaki, Yoshihiro Ohta (*Tokyo University of Agriculture and Technology*)

Cell division is a dynamic process, and consists of many ATP-dependent reactions. In the present study, to examine the energy state of cells during cell division, we monitored changes in mitochondrial membrane potential and ATP concentration in C6 glioma cell. Mitochondrial membrane potential was examined with TMRE, a membrane potential sensitive dye, and cytosolic ATP concentration was measured with Go-ATeam. Go-ATeam is a fluorescence resonance energy transfer (FRET) based ATP indicator. We found that TMRE intensity was once decreased in the beginning of M-phase and increased again in the end of M-phase. By contrast, TMRE intensity was constant in interphase. Unlike TMRE fluorescence, ATP concentration at the cytosol was constant in both phases.

2P189 細胞性粘菌（*Dictyostelium discoideum* Ax-2）の増殖におけるエネルギーをめぐる細胞内葛藤
Intracellular conflict on energy in the growing cellular slime mold, *Dictyostelium discoideum* Ax-2

Yatsuhisa Nagano (*Res. Ctr. Structural Thernodyn., Grad. Sch. Sci., Osaka Univ.*)

Thermogenesis of *D. discoideum Ax-2* in growing phase was measured at various temperatures by using high-precision isothermal calorimetry. Total energy consumption of a cell during doubling time shows a minimum in the vicinity of the optimal temperature, T = 295 K. The magnitude of thermogenesis indicates the activity of mitochondria. Temperature jump from the optimal temperature induces significant changes of magnitude and growth rate of thermogenesis, which can be attributed to temperature adaptation and conflict of mitochondria against the other organelles. Calorimetry provides a prominent non-invasive method to evaluate the state of mitochondria in living cells.

2P190 ES 細胞の分化初期段階における状態遷移
Transitions among cell states in the early stage of differentiation from embryonic stem cells

Koh Makishi, Tomoki P. Terada, Masaki Sasai (*Dept. of Comp. Sci. Eng., Univ. of Nagoya*)

Embryonic stem (ES) cells can differentiate into all three germ layers. This capability is called pluripotency, and it has been shown that Sox2, Oct4, and Nanog (SON) are the important genes for pluripotency. We built a model of the network of core genes, and performed the stochastic simulation. The model takes into account of processes to regulate the gene expression in the model; binding/unbinding of transcription factors (TF), formation/dissolution of transcription apparatus (TA), and modification of histone code. The results show that the slow formation/dissolution of TA at Nanog locus explains the observed phenotypic heterogeneity of ES cells. By using the epigenetic landscape picture, we will discuss the role of the heterogeneity in the differentiation process.

2P191 巨大化大腸菌の細胞形態変化、細胞分裂の観察
Morphological change and cell division of Giant E.coli

Takao Sogo[1], Kazuhito Tabata[1,2], Hiroyuki Noji[1] ([1]*Dept. Applied Chem., Sch. Eng., Univ. Tokyo*, [2]*PREST, JST*)

Large spherical E.coli cells (giant E.coli) with 5 to 10 μm in diameter that were prepared under inhibitory condition for cell wall synthesis are expected as a model cell system to be integrated with artificial micro reactors. In this study, we report morphological change of giant E.coli cells after depletion of the cell-wall synthesis inhibitor. While many cells grew keeping spherical shape until cell lysis, some cells changed their shape and others showed protrusion from the cell and a few cells showed node-formation at end of the protrusion like cell division. We will also report the recovery process of cell wall and the intracellular distribution of FtsZ.

2P192 細菌べん毛タンパク質輸送装置の in vitro 再構成系の構築
Construction of an in vitro assay system for the bacterial flagellar protein export

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The bacterial flagellum is a long filamentous organelle to locomote in a wet environment. Most of its component proteins are translocated across the cell membrane through the flagellar export apparatus. In spite of many in vivo studies, the molecular mechanism of secretion is still obscure, due to complex intra-cellar regulatory networks that affect the protein secretion. Thus, an in vitro assay system is required for further understanding of the protein export. Here we report an inverted-membrane-vesicle based export assay system. We examined that the system exports flagellar proteins depending on proton motive force. This system will allow us to precise and accurate measurements of the protein export under various well-controlled conditions.

2P193 ケージドセリン光分解とべん毛モーター回転計測を用いた大腸菌走化性応答の高時間分解能計測

Response of flagellar motor rotation to photoreleased serine from caged-compound in an *E. coli* cell

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E. coli cell controls rotational direction of flagellar motor by chemotaxis system. To understand the signaling process in high-temporal resolution, a cellular response time and duration of response to photoreleased serine from caged-compound were measured via the rotational direction of a motor. From the response time and the duration of response, the same values of threshold were estimated in the increase and decrease of serine-concentration, indicating a cell senses absolute value of serine-concentration. Moreover, we found out the response time depends on the distance between receptor and motor. This dependency in response time informed us that diffusion-based propagation of signaling molecules and the 240 ms of enzymatic reaction involved in the signaling process.

2P194 MS リングに変異の入ったサルモネラ菌べん毛モーターとそのシュードリバータントの構造安定性と回転特性

Structural stability and rotational characteristics of the flagellar motor of Salmonella MS-ring mutant and its psuedo-revertants

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The bacterial flagellar motor is a rotary nanomachine fueled by proton motive force.It has been known that one of Salmonella FliF mutants has fragile flagella as compared with those of a wild type strain. In this study, we quantitatively measured the mechanical strength and torques of the wild-type and FliF mutant flagella. As a result, the strength of the FliF mutant decreased to a half of the wild type. Several pseudo-revertants of the FliF mutant suppressed fragility of the flagella. In contrast, rotation assays revealed that motor torques of the FliF mutant and pseudo-revertants were almost the same with those of the wild-type at high loads. These suggest that the mutation in FliF decreases structural stability of the motor but does not affect torque generation.

2P195 電子顕微鏡によるヒト毛乳頭細胞の不動毛の構造解析

Structural analysis of primary cilia in human follicle dermal papilla cells by electron microscopy

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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 in cell. Dermal papilla cells (DP cells) with primary cilia (Matsushima, 2009) in the skin can induce hair growth. Their ultrastructure is, however, unclear. Here we observed the structure of primary cilia of DP cells by confocal laser microscopy and scanning electron microscopy. As a result, we, for the first time, observed the 80 % primary cilia of the cells around the outside of the nucleus, which is ~2.9um in length and 200nm in diameter. We believe that these results help to reveal the function of primary cilia of DP cells.

2P196 簡単で低コストなコレラティブ顕微鏡法

Simple and cost-effective method for correlative microscopy

Teruyo Minamiyashiki, Miharu Nagaishi, **Hiryouki Nakagawa** (*Department of Earth System Science, Faculty of Science, Fukuoka University*)

Recently it has been growing a demand to observe cytoskeleton in a locomoting cell at molecular level. The methods combining a live cell imaging to an electron-microscopic observation have been reported as correlative microscopy requiring hi-end equipments. Here we show a simple and cost-effective method for the correlative microscopy. BG2-c6 cells, Drosophila neural cell line, were cultured on formvar film attached on the finder grid. After fixation and extraction of cells with the glutaraldehyde and detergent mixture at the end of live imaging, the finder grid was picked up to negative-stain cells. Under a transmission electron microscope, cells of interest were located by the specific pattern of the grid to observe the fine structure at high magnification.

2P197 顕微鏡ステージ上での微量エレクトロポレーション法

Electroporation of adherent cells with low sample volumes on a microscope stage

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Electroporation is a powerful technique to label specific molecules in living cells for investigating intracellular molecular dynamics. However, the loading of samples into “adherent” cells with normal electroporators is difficult. Here, we developed a new electroporator with four special characteristics: (1) Electric pulses are applied to the adherent cells directly, without removing them from the substratum. (2) Samples can be loaded into the adherent cells while observing them on the stage of an inverted microscope. (3) Only 2 microliter of sample solution is sufficient. Samples could be loaded into keratocytes, neutrophil-like HL-60 cells and *Dictyostelium* cells. The new device should be useful for a wide range of adherent cells.

2P198 神経ー膵島 α 細胞相互作用におけるサブスタンスＰの寄与

A neuropeptide substance P is involved in nerve-pancreatic islet α cell interaction

Tadahide Furuno, Mami Nakamura, Yoshikazu Inoh, Mamoru Nakanishi (*Sch. Pharm., Aichi Gakuin Univ.*)

Autonomic neurons innervate pancreatic islets of Langerhans and maintain blood glucose homeostasis by regulating hormonal levels. Using in vitro coculture of αTC6 cells, a murine islet α cell line, with superior cervical ganglia (SCG), we observed the oscillation of intracellular Ca^{2+} concentration in αTC6 cells after SCG activation specifically with scorpion venom. In addition, we found that the neurokinin (NK)-1 receptor, a substance P receptor, was expressed in αTC6 cells, and that pretreatment with CP99,994, an NK1 receptor antagonist, suppressed the responding rate of αTC6 cells. These results suggested that substance P released from stimulated neurites functioned as a mediator to activate α cells.

2P199 RhoGAP proteins RGA-3/4 mediate spatial negative feedback of the actomyosin in C. elegans embryos

Masashi Fujita, Shuichi Onami (*RIKEN Quantitative Biology Center*)

In one-cell embryos of C. elegans, contraction of an actomyosin network drives flows of cell cortex from posterior to anterior. These flows anteriorly transport the small GTPase Rho, which is a positive regulator of contractility. This raises a question how a positive feedback loop, between flow-driven concentration of Rho and Rho-regulated actomyosin contraction, is prevented. We found that RhoGAP proteins RGA-3/4 colocalizes with actomyosin. Expression of a fusion of the RGA-3 GAP domain and an F-actin binding domain MOE rescued lethality of rga-3/4 RNAi. These results suggest that the positive feedback between Rho and actomyosin is counterbalanced by a negative feedback between actomyosin and RhoGAP.

2P200 フェムト秒レーザー誘起衝撃力による分裂酵母細胞のカルシウムイオン応答の個別解析

Individual Analysis of Changes in Calcium Concentration Induced by Femtosecond Laser Impulse in Single Fission Yeast Cells

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When an infrared femtosecond laser pulse is focused into culture medium through an objective lens, an impulsive force due to local explosion of water is generated at the laser focal point. We can selectively stimulate a single cultured cell near the laser focal point by the impulse. In this work, an impulsive machano-response in single fission yeast cells (S. pombe) was investigated by monitoring the cytoplasmic Ca2+ concentration that was measured by a FRET-based Ca2+ sensor protein, Yellow Cameleon, containing a Ca2+-binding pocket of calmodulin between CFP and YFP. We found that fluorescence intensities of CFP and YFP were changed when the impulse was loaded on the single cells. This suggests that the impulse generates a mechano-response at the single-cell level.

2P201 悪性高熱症関連変異をもたらしている 1 型リアノジン受容体の機能解析

Functional analysis of type 1 ryanodine receptor carrying malignant hyperthermia associated mutations

Toshiko Yamazawa[1], Takashi Murayama[2], Hedeto Oyamada[3], Junji Suzuki[4], Kazunori Kanemaru[4], Nagomi Kurebayashi[2], Masamitsu Iino[4], Shigeru Takemori[1] ([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*)

Malignant hyperthermia (MH) is a disorder of Ca^{2+}-induced Ca^{2+} release (CICR) via type 1 ryanodine receptor (RyR1) in skeletal muscles. More than 200 mutations in the RyR1 gene have been reported in MH patients. However, there were only a few experimental results confirming those mutations being responsible for the increment of the CICR sensitivities. We characterized the functional mutations on RyR1 in HEK293 cells. It was found that disease-associated mutations of the RyR1 resulted in enhanced Ca^{2+} release activity, therefore these mutations would be responsible for the MH incidence. These results suggest that exploration of the functional mutations of RyR1 is probably effective in preventive diagnosis of patients associated with MH disease.

2P202 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

In vivo spatio-temporal distribution analysis of dimeric glucocorticoid receptor using Number and Brightness

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Glucocorticoid receptor (GR) belongs to the nuclear receptor super family. When GR binds to a steroid hormone such as the dexamethasone (Dex), it forms a homodimer and works as a transcription factor for various genes. Most of GR exists in cytoplasm in the absence of ligand and translocates to nuclear in the presence of 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 optimized the imaging of the GR distribution in living cell. Finally, we succeeded in the visualization of EGFP-GR dimers distribution in living cell in the presence of Dex.

2P203 GPI アンカー型タンパク質は神経細胞膜の拡散障壁内でも高速でホップ拡散する：超高速 1 蛍光分子追跡による検出

GPI-anchored proteins undergo rapid hop diffusion within the diffusion barrier in the neuronal plasma membrane

Manami Miyahara[1], Chieko Nakada[3], Takahiro Fujiwara[1], Toshiki Matsui[2], Hiroko Hijikata[1], Hiroo Iwata[2], Ziya Kalay[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*)

The initial segment (IS) domain in the neuron is located between the axon and the somatodendritic domain, separating them by generating a diffusion barrier in the plasma membrane (PM), which blocks the diffusion of even phospholipids, very basic membrane molecules. The diffusion barrier consists of the actin mesh and its associated transmembrane proteins (pickets). Here, by using ultraspeed single-molecule tracking of GPI-anchored proteins, we found that the compartment size in the IS-PM was 60 nm and that they undergo rapid hop diffusion in the IS-PM. Besides, higher hydrophilicity of the protein domain was responsible for the enhanced hops, suggesting that it shifted the molecular location toward the membrane surface, enhancing hops across the compartment boundaries.

2P204 免疫細胞のシグナルアダプター分子 LAT の時空間制御機構：1 分子追跡による解明

Adaptor transmembrane protein LAT in immune signaling works in vesicles recruited to the plasma membrane: a single-molecule tracking study

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In immune cells, a transmembrane protein called Linker for Activation of T cells (LAT) exists, which plays a key role in transferring the receptor signal to cytoplasmic signaling molecules in the plasma membrane (PM). However, how LAT works is unknown. Using rat basophilic leukemia (RBL) cells, which are largely responsible for allergic reactions, we found that LAT exists in cytoplasmic vesicles as well as in the PM, and that, upon antigen stimulation, these vesicles are recruited to/near the PM cytoplasmic surface with a lifetime of ~ 5.0 s, and under these conditions, 40% of LAT in-near the PM existed in the recruited vesicles. Furthermore, we found that the LAT molecules on the vesicles, rather than those in the PM are responsible for the downstream signaling.

2P205 Main phase transition of asymmetric lipid bilayers

Antti Lamberg, Takashi Taniguchi (*Department of Chemical Engineering, Kyoto University*)

Physical properties of lipid membranes are often deduced from measurements on supported bilayers. The support can act to cause asymmetry in the lipid composition. Asymmetric compositions also naturally occur in living cells. By means of a simple theory and accompanying coarse grained molecular dynamics simulations, we study how the stress in these systems varies as a function of temperature as the membrane undergoes a phase transition. Based on our findings, we propose a physical mechanism, mediated by membrane freezing, for the gating of some thermosensitive ion channels.

2P206 リン脂質/コレステロール系における Lo 相形成の炭化水素鎖長依存性

Effect of the phospholipid chain length on the cholesterol-induced liquid ordered phase formation

Tsubasa Miyoshi, Hiroshi Kitajima, Daichi Yokoi, Satoru Kato (*Grad. Sch. Sci & Tech.,Univ. Kwansei Gakuin*)

Cholesterol, a versatile modulator of biomembrane properties, induces the relative rigid liquid ordered (Lo) phase when added to phospholipid bilayers in the liquid-disordered (Ld) phase. We have already reported on cholesterol effect on the partial molecular volume of DPPC in the binary bilayers, studied by buoyant density method. In this study, we employed DMPC instead of DPPC to get insight into the influence of hydrocarbon chain length on the cholesterol-induced Lo phase formation in the temperatures higher than the main transition temperature. On the basis of the comparison between the partial molecular volumes in the DMPC/ cholesterol and DPPC/cholesterol systems, we will discuss the molecular mechanism of cholesterol-induced Lo phase formation.

2P208 マイクロパターン化モデル生体膜における脂質ドメインの空間的制御

Geometrical separation of lipid domains in a micro-patterned model membrane

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We developed a micropatterned model membrane that can spatially control the separation of liquid-ordered (*lo*) and liquid-disordered (*ld*) phases. The micro-patterned membrane comprised polymeric and fluid lipid bilayers, in which the fluid bilayer spontaneously segregated into *lo* and *ld* domains due to the affinity of *ld* domains to the boundaries of polymeric bilayer domains. In the present study, we demonstrate that the distribution of *lo* and *ld* domains can be controlled both by the area fraction and geometry of polymerized bilayer regions. Controlled separation of *lo*/ *ld* domains allows us to construct a lipid-raft model with pre-defined geometries, and provides a means to sort membrane-bound proteins according to their affinities to membrane domains.

2P209 中性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の NMR と QCM による解析

Structure and affinity analysis of bovine lactoferrampin bound to a neutral model membrane as studied by solid state NMR and QCM

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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 LFampinB formed α-helix in the N-terminal region and the α-helical axis rotated rapidly about the bilayer normal with the tilt angle of 40 degree to the axis. 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.

2P210 高圧蛍光法により明らかにされるサブゲル相中のホスファチジルコリン分子のスタッガード構造

Staggered structure of phosphatidylcholine molecules in subgel phase revealed by high-pressure fluorometry

Masaki Goto, Nobutake Tamai, Hitoshi Matsuki (*Tokushima Univ.*)

The phosphatidylcholine (PC) bilayers form the most stable phase called the subgel (L_c) phase but the exact mechanism and the structure of the L_c phase are still unknown. We performed the thermotropic and barotropic measurements in reference to the temperature-pressure phase diagram of the myristoylpalmitoyl-PC (MPPC) bilayer by fluorescence spectroscopy using a polarity-sensitive fluorescent probe Prodan. On the basis of a second-derivative analysis of the fluorescence data, the line shape for the L_c phase was characterized by a broad peak with a minimum wavelength of ca. 460 nm under high pressure. From the fluorescence behavior of Prodan for the L_c phase, we concluded that the L_c phase takes highly probably a staggered structure depending on pressure.

2P211 ジパルミトイルホスファチジルコリン二分子膜の熱的相挙動に及ぼすステロール効果

Effects of sterols on thermotropic phase behavior of dipalmitoylphosphatidylcholine bilayer

Nobutake Tamai, Sanae Inazawa, Daiki Fujiwara, Masaki Goto, Hitoshi Matsuki (*Department of Life System, Institute of Technology and Science, The University of Tokushima*)

Stigmasterol is one of phytosterols and ergosterol is found in yeast and fungal cell membranes. These sterols are considered to have similar functions to those of cholesterol in animal cell membranes. We performed differential scanning calorimetric and fluorescence spectroscopic measurements for dipalmitoylphosphatidylcholine (DPPC)-stigmasterol and DPPC-ergosterol binary bilayer membranes to construct temperature-composition phase diagrams. By comparing these phase diagrams with that of the DPPC-cholesterol binary bilayer membrane, we discussed how the sterol effects on the bilayer phase behavior of DPPC are affected by the slight variation in the chemical structure of these kinds of sterols.

2P212 グループ３ LEA タンパク質のモデルペプチドによるリポソームの乾燥誘導凝集抑制

Anti-aggregation Effects on Liposomes during Desiccation by Model Peptides of Group-3 LEA Proteins

Takao Furuki, Takahiro Watanabe, Minoru Sakurai (*Center for biological resources and informatics, Tokyo Institute of Technology*)

We tested the anti-aggregation effects of the following four peptides on 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 PvLEA22, although its sequence is scrambled. 3) Poly-L-glutamic acid, and 4) Poly-L-lysine. Based up on the results of the turbidity measurements, Fourier transform infrared (FT-IR) spectroscopic measurements, and the molecular dynamics simulations for the POPC membrane-PvLEA22 system, we discuss the underlying mechanism for the anti-aggregation effects of the LEA model peptides.

2P213 単一細胞膜揺らぎ計測のためのイオンコンダクタンス顕微鏡技術の開発

Scanning ion conductance microscopy for measuring single cell membrane fluctuations

Zen Ishikura[1], Yusuke Mizutani[2], Kaori Kuribayashi-Shigetomi[1], Yuuki Fujii[1], Choi Myung-Hoon[2], Cho Sang-Joon[3], Takaharu Okajima[1] ([1]*Graduate School of Information Science and Technology, Hokkaido University*, [2]*Park Systems Inc.*, [3]*Seoul National University*)

Scanning ion conductance microscopy (SICM) is a powerful tool for imaging topography of soft and fragile sample surfaces such as living cells. Our recent study [1] reported that SICM could measure membrane fluctuations of cells by analyzing the ion current curves, which are usually used as a feedback signal to control the tip-surface distance. However, due to limitations of SICM used, the cell membrane fluctuation at the single cell level could not be measured. In this study, we developed a SICM technique, which allowed us to map the ion current curves with a commercial SICM system and investigated the spatial dependence of membrane fluctuations of cells cultured on micropattened surfaces, which will be shown in detail. [1] Mizutani et al. Appl. Phys. Lett. (2013).

2P214 界面活性物質を用いた巨大細胞膜ベシクル作製方法の開発

Development of a new method for preparation of giant plasma membrane vesicles using surfactants

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Giant plasma membrane vesicles (GPMVs) isolated from cultured cells by chemical (formaldehyde: FA) were induced by plasma membrane vesiculation or blebbing. An irreversible denaturation of membrane proteins by FA is problem for using GPMVs as model plasma membrane. Analysis by GFP-fusion protein was quite difficult, because GFP fluorescent was immediately quenched by FA treatment. Development of a new method for GPMVs preparation that is avoidable or reductive of the denaturation of membrane proteins is required. We found that moderate sodium lauroyl sarcosinate (SLS) and some detergents were good chemicals for inducing blebbing from HeLa cells. We found that SLS reduced fluorescent quenching rate of GFP on cell membrane and obtained GPMVs labeled with GFP.

2P215 肺サーファクタントタンパク質 SP-B によるリン脂質膜の構造変化

Morphology Changes in Phospholipid Monolayers Induced by Lung Surfactant Protein SP-B

Masahiro Hibino[1], Hayato Suzuki[2], Takahiro Suzuki[2] ([1]*Div. Appl. Sci., Muroran Inst. Tech.*, [2]*Dept. Appl. Sci., Muroran Inst. Tech.*)

The influence of synthetic peptides based on the surfactant-specific protein SP-B on the morphology and function of phospholipid monolayers, a model system of pulmonary surfactant assembly, was studied using Langmuir isotherms, fluorescence microscopy, and atomic force microscopy. The monolayers at the air-water interface appeared to be phase separated under compression and retained the continuous LE phase network surrounding islands of C phase. When the two-dimensional monolayers were compressed beyond the equilibrium surface pressure, they collapsed and assumed a three-dimensional formation. The amino-terminal peptides of SP-B inhibited the formation of condensed phases in monolayersand and induced a reversible folding transition at monolayer collapse upon expansion.

2P216 希薄状及び飽和状なリソチーム及びナノシリカ/ダイヤが pH=7-13 のもとで起こす吸着反応の動力学

Loose and Saturated Adsorption Reaction Dynamics of Lysozyme and Nanosilica/-diamond at pH=7-13

Victor Wei-Keh Chao[1,2] ([1]*Department of Chemical and Materials Engineering, National Kaohsiung University of Applied Sciences*, [2]*Victor Basic Research Laboratory e. V.*)

Adsorption dynamics of lysozyme and nanosilica/-diamond with diameter 100 nm and 0.25 μg/μL, lysozyme in 0-1000 nM of 7 mM PPBS at pH=7, 9, 11, and 13 have been investigated by Fluorescence spectroscopy. The highest adsorp. capabilities and conformational efficiencies at pH=13 have been obtained. Lysozyme can be prepared, adsorbed and carried with optimal activity and helicity, with 10 and 2 mg/m^2 on nanosurface, 150 and 130 mg/g in g of nanoparticle, within the linear coverages at 150-250 nM and four pH values for nanosilica and nanodiamond, respectively. They can be prepared in the tightest packed form, with 55 and 20 mg/m^2, 580-1100 and 810-1680 mg/g at adsorp. thresholds and four pH for nanosilica and nanodiamond, respectively. **Ref.**Chin.J.Chem.Phys. **26**, 295 (2013).

2P217 人工物の細胞内導入：生細胞と GUV の電気融合法

How to send artifacts into the living cell inside? -Investigating GUV-Cell electro fusion method

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Our group has been investigating biochemical reactions in GUV (Giant Unilamellar Vesicle) as an artificial cell-model. In this research, we aim to evaluate the quasi-living dynamics of artificial systems constructed by using GUV-cell electrofusion method. The cell electrofusion method has been shown to be more effective than the other conventional methods, such as PEG or Sendai virus. The feasibility of this method has already been reported by Shirakashi's group (cell-GUV) and also Yomo's group (GUV-GUV). By using this technique, one might expect that designed artifacts could be introduced into living cells. Here, we show that we successfully introduced DNA into cultured cells by the GUV-fusion method. Based on our results, we'll discuss a new direction of our research.

2P218 生細胞に極限まで近い内包物を持つ人工細胞の構築と解析

若手招待講演

Generation of artificial cells that mimic living cells

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To fill in gaps between living cells and materials, mimicking intracellular conditions in vitro is an essential process. Here we developed a method to condense macromolecules in liposomes, and generated artificial cells containing macromolecules at physiological intracellular concentrations of *Escherichia coli*. The artificial cells showed many tubulation around their core sphere, and resembled irregular-shaped giant spheroplast cells of *E. coli*. Fluorescence recovery after photobleaching revealed macromolecule diffusions in the artificial cells are much slower than those in living cells. Viscosity under various concentrations of cell extracts estimated by micro-rheology supported the result, raising a new insight into molecular crowding studies.

2P219 不飽和脂肪酸による電位依存性プロトンチャネルへの活性増強効果

Effects of the unsaturated fatty acids on the voltage-gated proton channel

Akira Kawanabe, Yasushi Okamura (*Grad. Sch. Med., Osaka Univ.*)

The unsaturated fatty acids are important component of the phospholipids of biological membranes and mediators of cellular signaling. It is well-known that the unsaturated fatty acids, particularly arachidonic acid (AA, C20:4), modulate functions of various ion channels in a distinct manner; some ion channels are activated whereas others are inhibited. The molecular mechanisms of these actions have been unclear. The voltage-gated proton channel (VSOP/Hv1), one of smallest channels and can control the proton permeation by membrane voltage and pH. The proton currents in native cells have been reported to be activated by AA. Here we report the effects of AA on the proton currents through VSOP/Hv1 heterologously expressed in HEK293T cells by patch clamp technique.

2P220 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 on 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 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, with both small translations and large-scale movements reported. Here, we attempt to shed light on this transition using a combination of engineered probes, mutagenesis, and manipulation.

2P221 負に帯電した膜内葉表面でのアミノ末端両親媒性ヘリックスの回転が KcsA カリウムチャネルの開状態を安定化する

Rolling of N-terminal amphipathic helix on the anionic inner membrane leaflet stabilizes the open state of the KcsA potassium channel

Masayuki Iwamoto, Shigetoshi Oiki (*Dept. Mol. Physiol. Biophys., Univ. Fukui Fac. Med. Sci.*)

Presence of anionic phospholipids such as phosphatidylglycerol in the membrane is prerequisite for the activity of the KcsA potassium channel. In this study we demonstrated that the N-terminal amphipathic helix (M0) of the KcsA channel mediates the lipid effect on channel gating. Fluorescence measurement showed that the M0 revolves around the axis of the helix on the inner membrane surface upon gating, rendering the positively charged residues on the M0 interact with the negative head groups of the lipids. Single-channel current analysis revealed that the rolling of the M0 stabilizes the open state of the channel. This novel type of lipid-sensing mechanism may be shared by various types of membrane proteins.

2P222 K^+チャネルの中心空洞内の水の配向は静電的相互作用を増強する

The oriented water in the central cavity of the K^+ channel enhances the electrostatic attraction

Takashi Sumikama[1], Shinji Saito[2], Shigetoshi Oiki[1] ([1]*University of Fukui*, [2]*Institute for Molecular Science*)

The K^+ channel serves a reservoir space, named the central cavity (CV), for permeating ions, where hydrated ions subsequently dehydrate and enter into the selectivity filter. The surface of the CV is lined with the hydrophobic residues, the role of which remained elusive. We performed the molecular dynamics simulation on the ion conduction through the K^+ channel and found that the water molecules in the CV were mostly oriented such that their dipoles directed towards the selectivity filter due to the interaction with the hydrophobic surface. This orientation of water molecules decreases the dielectric constant in the CV, and ions located around the channel entrance are attracted to the CV by enhancing the electrostatic attraction. The CV facilitates the ion conductance.

2P223 Development of a reconstituted system for localized phosphatidylinositols signaling on lipid membrane

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Pseudopod formation and contraction during amoeba motion of a cell are governed by localized patches of phosphatidylinositols signaling on the plasma membrane. It is not clear how these patches are realized. We developed “cytosol-membrane dynamic system” - a reconstituted system consisted of cytosolic extract and a supported lipid bilayer. Using cytosol from *Dictyostelium discoideum*, localized patches can be observed and studied under a confocal laser scanning fluorescence microscope. We found that change in composition of various lipids and proteins related to signaling have a significant effect on the appearance of localized patches. We will discuss how the current our system can be employed to address a partial machinery of amoeba motion.

2P224 A Multiscale Kinetic Scheme Extracted from EGFR-Grb2 Single Molecule Reaction

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We apply a recently developed model-free time series analysis method to single molecule time series of dissociation and association kinetics between epidermal growth factor receptor (EGFR) and Grb2 to elucidate the underlying multiscale dynamics by extracting multiscale state space network (SSN). To deal with nonstationarity in time series, we proposed a simple scheme to construct a series of multiple SSN from a set of resampled data sequences. We found that the underlying SSNs change their topographical structure as a function of the timescale: while the corresponding SSN is simple at the short timescale, the SSN at the longer timescales becomes rather complicated in order to capture multiscale nonstationary kinetics emerging at longer timescales.

2P225 新世界ザルの苦味受容体 TAS2R1 および TAS2R4 の機能的多様性

Functional diversity of bitter taste receptors TAS2R1 and TAS2R4 in New World monkeys

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Bitter taste perception is mediated by G-protein-coupled bitter taste receptors (TAS2Rs). In recent years, human TAS2Rs have been functionally characterized. However, functional diversity of TAS2Rs among different species, which is thought to be related to adaptations to species-specific environments, is largely unknown. In this study, we focused on TAS2Rs of New World monkeys, which have variable amino acid sequences among different species. By calcium imaging in HEK293T cells, we found that TAS2R1 and TAS2R4 orthologues of marmoset, capuchin monkey, owl monkey and spider monkey exhibit different sensitivity for some bitter tastants. Furthermore, we performed site-directed mutagenesis to identify the amino acid residues responsible for the functional differences.

2P226 コレラ菌の尿素走性と培養温度依存性

Urea taxis of *Vibrio cholerae* and its temperature dependence

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Vibrio cholerae, an etiological agent of cholera, showed taxis toward various amino acids for their survival and virulence. We found that *V. cholerae* is attracted by urea, a common and essential organic compound in mammals, such as humans, used for nitrogen metabolisms. Interestingly, both tactic responses to urea and serine were significantly enhanced when cells were cultured at 37°C compared to those of cells cultured at 30°C, implying the relevance of taxis to urea and serine upon infection. Deletions of the *mlp24* and *mlp37* genes, encoding major chemoreceptors for various amino acids, significantly attenuated the urea taxis, suggesting that these chemoreceptors are also involved in urea taxis.

2P227 温度によるコレラ菌走化性受容体ホモログの発現制御機構の解析

Temperature control of chemoreceptor expression in *Vibrio cholerae*

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Vibrio cholerae, the etiological agent of cholera, shows chemotaxis that plays a role in pathogenicity. The bacterium has more than 40 homologs of methyl-accepting chemotaxis protein (MCP), which we name MCP-like protein (MLP). In this study, we examined whether *V. cholerae* modulates the MLP expression in response to changes in temperature. The response of wild-type cells to serine was stronger when cultured at 37℃ than when cultured at 23℃ or 30℃. Northern blotting revealed that cultivation at 37℃ increases the cellular amout of mRNA of *mlp37*, encoding one of the two major amino acid chemoreceptors. These results suggest that expression of at least some *mlp* genes is modulated by culture temperature at the transcriptional level.

2P228 チャコウラナメクジの脳嗅覚中枢における自発振動活動の長時間相関解析

Long-range temporal correlations of oscillatory activities in the olfactory center in the land slug

Yuichi Tanaka[1], Tamon Eto[1], Shouhei Haga[1], Minoru Saito[2], **Yoshimasa Komatsuzaki**[1] ([1]*Nihon University, CST*, [2]*Nihon University, CHS*)

Spontaneous oscillation of activity in olfactory system is important for odor-information processing. We investigated the statistical property of spontaneous oscillation in the procerebrum (PC), an olfactory center of the land slug. We performed a detrended fluctuation analysis (DFA), which is a scaling analysis technique used to provide a quantitative parameter (scaling exponent, α). The fluctuation of spike timing of the PC possessed a long-term correlation at timescales larger than 10^2 spikes (α~1.21). The application of cychloheximide, an inhibitor of protein synthesis, induced a decrease in the scaling exponent α at the larger time window. This result indicates that the fluctuations of spike timings are characterized by a dynamics of mRNA translation in the PC.

2P229 チャコウラナメクジ嗅覚中枢における時空間神経活動パターンの膜電位イメージング

Fluorescent Voltage Imaging of Spatiotemporal Activity Patterns in the Olfactory Center of the Land Slug

Tomoya Shimokawa[1], Kouhei Ishida[1], Yuuta Hamasaki[1], Yoshimasa Komatsuzaki[2], Minoru Saito[1] ([1]*Graduate School of Integrated Basic Sciences, Nihon University*, [2]*College of Science and Technology, Nihon University*)

In the olfactory center (procerebrum; PC) of *the land slug Limax valentianus*, the local field potential (LFP) of the PC shows an oscillation of about 1 Hz, and the oscillatory activity was changed by various odor stimuli to the tentacle. In the present study, we examined spatiotemporal activity patterns in the PC by fluorescence voltage imaging technique. As a result, an oscillation of fluorescence intensity was observed in the PC, and the oscillation had a phase delay along the distal-proximal axis. After aversive odor stimuli, the phase delay disappeared, which shows coherent activities of neurons in the PC. By this technique, we also examined the function of the superior and inferior tentacles on the olfactory processing.

2P230 ヨーロッパモノアラガイの中枢神経系における神経活動の膜電位イメージング (II)

Fluorescent Voltage Imaging of the Neural Activity in the Central Nervous System of the Pond Snail (II)

Yuuki Aikawa[1], Shogo Nakada[1], Makoto Hosoi[1], Yoshimasa Komatsuzaki[2], Minoru Saito[1] ([1]*Graduate School of Integrated Basic Sciences, Nihon University*, [2]*College of Science and Technology, Nihon University*)

Some neurons spontaneously fire and often exhibit complex activities. We have found that a regulatory neuron (cerebral giant cell; CGC), which is involved in feeding responses, in the cerebral ganglion of the pond snail *Lymnaea stagnalis* shows regular beating, regular bursting and irregular bursting discharges depending on the d.c. current through the cell membrane. In the present study, we examined how the activities of CGC affect the neural activities of the buccal ganglion (BuG) by using fluorescence voltage imaging technique. The activities of CGC were measured simultaneously by intracellular recording using a glass electrode. The fluorescence images showed that the firing patterns of some neurons in the BuG were affected by the discharge of CGC.

2P231 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解析法の提案

An analysis method for spatiotemporal activity patterns in mouse hippocampal slices

Shodai Izumi[1], Yuuta Hamasaki[2], Hiromi Osanai[1], **Minoru Saito**[1,2] ([1]*College of Humanities and Sciences, Nihon University*, [2]*Graduate School of Integrated Basic Sciences, Nihon University*)

Recently, functional multineuron calcium imaging (fMCI), which enables us to access brain function with single-neuron resolution, has been developed. We have observed various spatiotemporal activity patterns in the CA1 region of mouse hippocampal slices by fMCI. For example, some dozens of neurons fired incoherently in some slices, while they exhibited more coherent activity patterns in other slices. In the present study, we propose an analysis method to evaluate the coherency of the neural activity patterns and the similarity among each neural activity pattern. As a result, the analysis showed the neural activity under a higher K^+ concentration and the existence of bicuculline became more frequent but incoherent than the normal condition.

2P232 マウス海馬スライスの CA1 領域における様々な時空間活動パターンのレーザー共焦点イメージング (II)

Laser confocal imaging of various spatiotemporal activity patterns in the CA1 region of mouse hippocampal slices (II)

Mai Ichikawa[2], **Hiromi Osanai**[1], Yuuta Hamasaki[2], Minoru Saito[1,2] ([1]*College of Humanities and Sciences, Nihon University*, [2]*Graduate School of Integrated Basic Sciences, Nihon University*)

Recently, functional multineuron calcium imaging (fMCI), which enables us to access brain function with single-neuron resolution, has been developed. In the present study, we observed spatiotemporal activity patterns in the CA1 region of mouse hippocampal slices by fMCI. The slices (350 μm) were prepared from 1-week-old male ddY mouse. The slice preparation was stained with a Ca^{2+}-sensitive dye, oregon green. The stained slice was illuminated by an Ar laser (488 nm; 532-BS-A04, Melles Griot), and the 520 nm fluorescence images were acquired through a Nipkow confocal unit (CSU-10, Yokogawa) and a CCD camera (iXon X3 897, Andor). As a result, some dozens of neurons fired incoherently in some slices, while they exhibited more coherent activity patterns in other slices.

2P233 視索前野の GABA 作動性神経とオレキシン神経の機能的結合について

Functional connection between GABAergic neurons in the preoptic area and orexinergic neurons in the hypothalamus

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The preoptic area (POA) GABAergic neurons are thought to be important for driving and maintaining sleep, whereas orexinergic neurons in the lateral hypothalamus maintain wakefulness. Our research team examined the functional connectivity between the GABAergic and the orexinergic neurons using channelrhodopsin 2 (ChR2). ChR2 was expressed selectively in the POA GABAergic neurons using AAV-mediated gene transfer. We found that ChR2-expressing neurons projected to the lateral hypothalamus and the fibers were located adjacent to orexin neurons. Optogenetic stimulations of GABAergic fibers or cell bodies resulted in a strong inhibition of activity of orexin neurons both in vitro and in vivo. We revealed that the POA GABAergic neurons functionally connect to orexin neurons.

2P234 青斑核ノルアドレナリンニューロンへのオレキシン 2 型受容体を介した GABA 作動性の抑制性入力

GABAergic inhibition of noradrenergic neurons through orexin type 2 receptors

Junya Fukuoka[1], Takeshi Kanda[1], Daiki Nakatsuka[1], Masashi Yanagisawa[1,2] ([1]*IIIS, Univ. Tsukuba*, [2]*UTSW/HHMI*)

The locus coeruleus (LC), the noradrenergic (NA) nucleus in the pons, plays pivotal roles in generating and sustaining the state of wakefulness and REM sleep. Orexin neurons are also involved in the maintenance of wakefulness. Orexin neurons excite NA neurons in the LC through orexin type-1 receptors (OX1Rs). Recently, it is found that orexin type-2 receptors (OX2Rs) are expressed in GABAergic neurons in and around the LC. Using patch-clamp recordings in the mouse LC slices, we investigated the functions of OX2Rs on the LC GABAergic neurons. Activation of OX2Rs reduced spontaneous firing of NA neurons. The effect was not observed after blockade of $GABA_A$Rs. These results suggest that orexin neurons could inhibit NA neurons through OX2R-mediated GABAergic transmission.

2P235 アリの探索における記号創発

Emergence of symbol in ant navigatioin

Yukio Gunji[1,2], Tomoko Sakiyama[1] ([1]*Kobe University*, [2]*University of West England*)

Ant visual navigation is well studied with respect to taxon-like navigation or map-like navigation. Little evidence has been obtained in support of map-like navigation. We here generalize the idea of landmark navigation by navigation based on visual cues and their paired accessibility, which is expressed by a topological system, and predict that an ant can learn the relationship between a collection of visual cues and a collection of accessible sites. Here, we demonstrate that the garden ant C. japonicus can perform AND and XOR logical operations for the accessibility of visual cues, and also that ants can generate emergent symbol of which many cues combined by OR is united as one cue.

2P236 群れの相互作用の多義性から自己組織化を再考する

Rethinking about the concept of self-organization from the perspective of the interaction multiplicity in collective behavior

Takayuki Niizato (*Tsukuba University*)

The concept of the self-organization is widely known for the emergent phenomena from local simple interactions. Almost of the self-organization's models only consider one aspect of phenomena. However, this attitude may miss the problem of the functional differentiation from a local interaction. Thus, we propose multiplicity of interaction in a simple model constructed from three factors: asynchronous updating, learning site patterns, and agent anticipation. We found that the first two contribute to an efficient searching strategy, and that adding agent anticipation enables sign making in heterogeneous environments. Our model suggests that searching strategies and territorial behavior such as boundary marking emerge from two aspects of our simple interaction rule.

2P237 滑走細菌 *Flavobacterium johnsoniae* の菌表面構造

Cell surface structure of the gliding bacterium *Flavobacterium johnsoniae*

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Cells of *Flavobacterium johnsoniae* move over surfaces, which is called gliding motility. Gliding motility is unrelated to well-studied motility mechanisms using flagella and type IV pili. In our recent study (Nakane et al., 2013) we proposed a "helical loop track model" for gliding motility, where the SprB molecule is propelled along left-handed helical loop on the cell surface.

Using transmission electron microscopy to characterize the gliding machinery, "Fettuccini pasta-like structures" with 22.1 ± 0.8 nm in width and 8.2 ± 0.2 nm in thickness were observed on the cell surface, and also similar structures were able to be isolated from the cell surface. The structure may play an role in gliding motility. We are in the process of identifying its function.

2P238 *Flavobacterium johnsoniae* におけるコロニースプレッティングファクター

Factors influencing colony spreading in *Flavobacterium johnsoniae*

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Flavobacterium johnsoniae is an aerobic Gram-negative bacterium, which glides rapidly over solid surfaces at about 2 μm/s. *F. johnsoniae* shows colony spreading on a poor medium such as PY2 agar, whereas it forms non-spreading colonies on an enriched medium. Size of colony in PY2 agar was decreased by presence of NaCl and glucose in a dose-dependent manner. A number of mutants that showed colony spreading on PY2 agar containing 150 mM NaCl and 10 mM glucose were isolated from a transposon (Tn) mutant library of *F. johnsoniae* ATCC17061. Tn insertion sites in the mutants were determined, which suggested that amino acid biosynthesis, transporter and stress response pathways influence NaCl- and glucose-mediated suppression of colony spreading of *F. johnsoniae*.

2P239 自由エネルギー計算によるハロロドプシンの光駆動イオン輸送メカニズムの解析

Study of the mechanism of the light-driven ion transport in halorhodopsin based on the free energy calculations

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Halorhodopsin (hR) uses the light energy to transport chloride ions from the extracellular side to the cytoplasmic side. The process is thought to be divided into at least the three steps: (i) a chloride ion passively enters the binding site near retinal Schiff base linkage in the protein, (ii) the chloride ion is moved to the cytoplasmic side after the photoisomerization of retinal, and iii) the chloride ion is released to the cytoplasmic medium. The purpose of this work is to reveal the free energy profiles of these processes. The free energies were calculated using the method of energy representation combined with molecular dynamics simulations. Based on these results, we discuss the mechanism of the light-driven ion transport in hR.

2P240 Aureochrome-1 の各ドメインの機能解析

Functional analyses of each domain in Aureochrome-1

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Aureochrome-1 (AUREO1) found in *Vaucheria frigida* consists of a basic leucine zipper domain (bZip) and a light-oxygen-voltage-sensing (LOV) domain, and is believed to be a blue light-regulated transcription factor[1]. We prepared recombinant AUREO1 proteins, containing full coding sequence (FL), bZIP and LOV domains (ZL), and LOV domain. In addition, we made a deletion mutant (dZL) lacking the basic region of ZL. The dZL protein showed a similar spectral change to ZL upon illumination with blue light, but lost the affinity to DNA. Our results suggested that each domain in AUREO1 works as a functional unit and is available to create a new molecular tool.

1. Takahashi, F., et al. *Proc. Natl. Acid. Sci. USA* 104, 19625-19630 (2007)

2P241 Ab initio 電子状態計算における青色光受容体蛋白質の DNA 修復反応の理論的研究

Theoretical Study of DNA Repair Mechanism of Blue Light Photoreceptors by Ab initio Electronic Structure Calculation

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Molecular origin of efficient electron transfer (ET) in DNA photolyase (PHR) was illustrated by the analysis of electron tunneling pathways from FADH- to CPD.

Due to non-local nature and structural sensitivity of the active site electronic states, it is important to select the necessary and sufficient range of the QM region. Therefore, we evaluated the sensitivity of the tunneling matrix element to the perturbation of the Hamiltonian at each amino acid site, and measured the contribution of each site to ET.

We will discuss the role of each amino acid residues in the active site of PHR with respect to (1) explicit contribution to ET and (2) stabilization of the active site structure.

2P242 In-situ 光照射固体ＮＭＲによるバクテリオロドプシン D96N 変異体の光中間体の捕捉とタンパク質構造変化の解明

Trap of photo-intermediate and structural change of bacteriorhodopsin D96N mutant as revealed by in situ photoirradiation solid-state NMR

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Bacteriorhodopsin (bR) is an integrated membrane protein that functions light-driven proton pump. Upon photoirradiation, all-trans retinal start photocycle through K-, L-, M-, N-, O-intermediates. During the photocycle, proton is transported from cytoplasmic to extracelluler side. When the M-intermediate generates, proton moves from retinal to Asp96 accompanied by a large dynamical changes, which may play an important role for proton pump. Because D96N-bR shows a longer half life of the M-intermediate than the WT, we could trap the M-intermediate using in situ photoirradiation solid-state NMR by observing the ^{15}N NMR signal of [^{15}N-ε-Lys]-D96N-bR at -60 ^{o}C. It was observed that mobility of F-helix and C-terminus in protein was increased in the M-intermediate.

2P243 In situ 光照射固体 NMR による 13-cis, 15-syn 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 photo excited process in 13-cis, 15-syn retinal of Bacteriorhodopsin studied by in situ photoirradiation SS-NMR

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Bactriorhodopsin (BR) is an integral membrane protein that functions 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 (dark-adapted state (DA)), and changes to ~100% AT under photoirradiation (light-adapted state (LA)). In this study, retinal configuration change in BR was observed using [1-^{13}C]Tyr-, [20-^{13}C]Retinal-BR by means of *in situ* photoirradiation SS-NMR. At 20°C, change from DA to LA is seen; however, at -20°C, an CS-like intermediate (CS*) is observed stationary under green light illumination. Large structural change was also observed in protein side in CS*. These results suggest a new photocycle, in which CS may play an essential role in proton pump function.

2P244 光駆動型 Cl$^-$ポンプ ファラオニスハロロドプシンの Cl$^-$放出・取込み過程の解析

Analysis of Cl$^-$ release and uptake steps of light-driven Cl$^-$ pump *Natronomonas pharaonis* halorhodopsin

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KM-1 is a mutant of *Natronomonas pharaonis* expressing a large amount of NpHR-bacterioruberin (Brub) complex. At the formation of O-intermediate, flash-photolysis results of the NpHR-Brub complex showed wavy absorbance changes (Wac) that were originated from the potential-modulated Brub. On the other hand, the opposite-directed changes of Wac were observed when Cl$^-$ was added to the free NpHR. These indicated that at O, Cl$^-$ is released to the cytoplasmic (CP) space. On the other hand, imposition of the interior negative membrane potential of envelope membrane vesicles decreased the O-accumulation and slowed down the O-decay. These indicated that Cl$^-$ release and uptake occur during the formation and decay of O, respectively, as proposed by previous works.

2P245 光駆動型 Cl$^-$ポンプ ファラオニスハロロドプシンにおける Thr218 の役割

Role of Thr218 in light-driven Cl$^-$ pump mechanism of *Natronomonas pharaonis* halorhodopsin

Kousuke Shibasaki, Hiroaki Shigemura, Takashi Kikukawa, Masakatsu Kamiya, Tomoyasu Aizawa, Naoki Kamo, Makoto Demura (*Grad. Sch. Life. Sci., Hokkaido Univ.*)

Thr218 is considered to be functionally important, because this hydrophilic residue is located near the retinal of hydrophobic cytoplasmic (CP) channel. We analyzed the photocycle of various T218 mutants. Wild-type and T218S show 1) the fastest L2 to N transition and 2) the largest dissociation constant of Cl$^-$ (K_d) to CP. Thus, Thr or Ser at this position is suitable for the Cl$^-$ movement which involves the transfer from the binding site in the extracellular channel followed by the release to the CP space. Pressure dependence of K_d yielded the volume differences between N and O (ΔV). Results suggested the water entry at O, which may increase K_d. The relationship between K_d and ΔV may propose a special coordination of the water molecules with OH group of Thr or Ser.

2P246 アセタブラリアロドプシン I の光化学反応

Photochemical reaction in *Acetabularia* rhodopsin I

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Acetabularia rhodopsin (AR) is a light-driven proton pump found in marine alga, *Acetabularia acetabulum*. Recently, Jung *et al*. cloned two homologous AR opsins, and named them ARI and ARII. Previously we reported details of photochemical properties of ARII prepared by the cell-free synthesis. In this study, we focused on the photochemistry of another homologous one, ARI. The photocycle and photo-induced proton transfer of ARI was investigated by the flash photolysis and photoelectrochemical measurement with indium tin-oxide (ITO) electrodes, respectively. On the basis of these results, the expected photocycle and proton transfer scheme of ARI were suggested, and finally discussed compared with ARII or well-known bacteriorhodopsin.

2P247 *in situ* 光照射固体 NMR による光受容膜タンパク質 *p*pR/*p*HtrII の光励起過程における transducer タンパク質膜貫通領域の構造変化の観測

Photoactivated conformational changes of photoreceptor membrane protein *p*pR/*p*HtrII observed by *in situ* photo irradiation solid-state NMR

Yoshiteru Makino[1], Yuya Tomonaga[1], Yusuke Shibafuji[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. Sci, Nagoya Univ.*, [4]*Grad. Sch. Life Sci, Hokkaido Univ.*)

*p*pR/*p*HtrII is a complex of photo receptor and signal transducer membrane proteins and functions as negative phototaxis. [15,20-^{13}C] retinal *p*pR and [1-^{13}C]Val,[2-^{13}C]Gly,[3-^{13}C]Ala *p*HtrII was expressed respectively and 2:2 complex was prepared. Conformational changes of *p*pR/*p*HtrII complex in a membrane (Egg-PC) were observed using *in situ* photo irradiation NMR. The experimental result demonstrated that transmembrane region of *p*HtrII changed from α-helix to random coil, while retinal of *p*pR from all-*trans*(grand states) to 13-*cis* (M-intermediates). In addition, we observed the presence of at least 3 types of M-intermediates. These results can provide a clue to clarify the mechanisms of signal transduction of photo-sensor proteins.

2P248 Rhodobacter capsulatus 由来 Photoactive Yellow Protein の相互作用部位の解明

Analysis of interaction sites on the Photoactive Yellow Protein of Rhodobacter capsulatus

Yoichi Yamazaki, Mayu Shimada, Hironari Kamikubo, Mikio Kataoka (*Graduate School of Materials science, Nara Institute of Science and Technology*)

Photoactive Yellow Protein (PYP) is a photoreceptor protein that absorbs blue light with p-coumaric acid as a chromophore. We identified the light dependent interaction protein of PYP named PBP from Rhodobacter capsulatus (Rc). This is the only target protein of PYP homologues revealed so far. In order to clarify the mechanism of light dependent interaction of PBP with Rc-PYP, we aimed to identify the interaction sites on Rc-PYP by using chimeric mutants of Rc-PYP and Hh-PYP and Ala mutations on Rc-PYP. As a result of verification of binding ability in each mutant, Lysine 72 is assigned as an interaction key role residue. K72A mutant decreased its binding affinity to PBP, but K72Q was not. This indicates that the side chain length is important to interaction with PBP.

2P249 *Rhodobacter capsulatus* 由来 Photoactive Yellow Protein の X 線結晶構造解析

X-ray crystal structure analysis of the Photoactive Yellow Protein of *Rhodobacter capsulatus*

Hiroshi Matsumoto, Yoichi Yamazaki, Hironari Kamikubo, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Photoactive Yellow Protein (PYP) is a water-soluble photoreceptor protein that absorbs blue light with *p*-coumaric acid as a chromophore. *Rhodobacter capsulatus* PYP (Rc-PYP) has several different properties in its absorption spectrum and photocycle from that of well- known PYP from *Halorhodopsira halophila*. Especially, a target protein of PYP has been identified only for Rc-PYP. So far, crystal structure of Rc-PYP was not solved. In order to clarify the origin of the difference of the spectroscopic properties and the molecular mechanism of light signal transduction, we attempted the crystal structure analysis of Rc-PYP. We succeeded to crystalize Rc-PYP, which gives diffraction to 3Å resolution. Preliminary analysis is under way.

2P250 二種類のＰＹＰを用いたキメラタンパク質の中間体の平衡状態の解析

Analysis of Equilibrium of intermediate states of PYP by use of chimera proteins

Yoshiaki Matsumoto, Youichi Yamazaki, Hironari Kamikubo, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Despite that the key residues around the chromophore are common for *Halorhodospira halophila*PYP (Hh-PYP) and *Rhodobacter capsulatus* PYP (Rc-PYP), they show different intermediate states in their photocycles. To clarify the molecular basis of the origin of the differences, we created chimera proteins between Hh-PYP and Rc-PYP. The PYP amino acid sequence was divided into four blocks and one of four Hh-PYP blocks was replaced with the corresponding block of Rc-PYP. The chimera with the second block of Rc-PYP caused different spectral properties of intermediate from the other chimera PYPs and Hh-PYP, suggesting the shift of equilibrium between two intermediates. We assume that this block is a determinant of equilibrium of intermediate states.

2P251 PYP-Phytochrome Related Protein の X 線溶液散乱による研究

X-ray Solution Scattering Studies of PYP-Phytochrome Related Protein

Keito Yoshida, Hironari Kamikubo, Kento Yonezawa, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Graduate school of Materials Science, Nara Institute of Science Technology*)

PYP - Phytochrome Related Protein (Ppr) is a blue/red light sensor protein isolated from *Rhodospirillum centenum*. Ppr is a multi-domain protein comprised of PYP, Bph, and His-kinase domains. The former two act as sensor domains containing a *p*-coumaric acid and biliverdin as chromophores, respectively. To understand the regulation mechanism of photo-signal transduction we carried out the time-resolved solution X-ray scattering experiments under illumination using red and/or blue light and after turning off the light. It was found that domain rearrangement of Ppr occurs only when both of the PYP and Bph domains are activated, indicating that the two signals of red and blue light are integrated to promote the following biological response.

2P252 PYP_M 中間体におけるアルギニン 52 のプロトン化状態

Protonation state of R52 at the PYP_M intermediate state

Masayoshi Noji, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Our previous neutron study revealed that R52 of Photoactive Yellow Protein (PYP) is deprotonated under the dark state. In order to verify the protonation state of R52 in a solution condition, the pH titration experiments for WT and R52Q were performed. While the UV-Vis spectra of WT and R52Q are identical under neutral pH, those at the acidic state were different from each other, suggesting the protonation of R52 at the acidic condition. The comparison of UV-Vis spectra of PYP_M between WT and R52Q showed that spectral difference is similar to that observed at the acidic pH. We concluded that R52 in WT takes neutral form in the dark state under the solution condition, and R52 is expected to be protonated during the photo-reaction.

2P253 Excited State Proton Transfer of Fluorescent Photoactive Yellow Protein Reconstituted with Hydroxycoumarin

Dian Novitasari, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (*Graduate School of Materials Science, Nara Institute of Science and Technology*)

Photoactive yellow protein (PYP) shows high efficient photoisomerization of chromophore upon light absorption, resulting in little emission of fluorescence. We succeeded to reconstitute fluorescent PYP by introducing a “trans-locked” chromophore, 7-hydroxycoumarin-3-carboxylic acid, called as PYP-coumarin. Because its pKa value is almost identical to that of the intact PYP, their hydrogen bonding network is similar. It strongly emits fluorescence with λmax of 466nm at neutral pH. At acidic pH, it shows bimodal fluorescence spectrum, indicating the existence of both protonated and unprotonated fluorophore, whereas free coumarin shows a single peak. Based on these results, we assume that the excited state proton transfer (ESPT) of hydroxycoumarin occurs in PYP-coumarin.

2P254 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, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Our neutron structural analysis of Photoactive Yellow Protein (PYP) revealed an unusual deprotonated arginine (R52) with a low barrier hydrogen bond (LBHB) between the chromophore and E46. To examine the protonation state of R52 in solution, we performed FTIR spectroscopy. The comparison of PYP_L/PYP_{Dark} difference spectra between WT and R52Q confirmed the substantial differences in the region from 1500 cm^{-1} to 1600 cm^{-1}, which is assigned to a C-N stretch vibration of the deprotonated guanidino group. The observation indicates that the deprotonated R52 of WT is slightly perturbed during the reaction. The signals show clear isotope effect with ^{15}N labeled Arg. We conclude that R52 in PYP is surely deprotonated in solution.

2P255 開口数 0.9 の極低温光学顕微鏡の開発とその植物細胞内色素イメージングへの応用

Development of a cryogenic optical microscope with NA of 0.9 and its application to studies of pigment distributions in plant cells

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Sharpening of spectral bands and reduced photo-damage of samples upon lowering temperature enable us to get detailed information about the functional states and spatial distributions of chlorophyll (Chl)-protein complexes within a plant cell. To realize fluorescence microspectroscopy observations of photosynthetic components at cryogenic temperatures, we developed a laser-scanning cryogenic microscope, in which the objective lens is set in the adiabatic vacuum achieving drastic shortening of the distance between the lens and a sample. Pigment distributions in etiolated *Zea mays* leaves were studied. We could dissolve the distributions of protochlorophyllide, the precursor to Chl, in different spectral forms with a much improved spatial resolution from previous studies.

2P256 ガリウム置換フェレドキシンの結晶構造と PS 1 および FNR との相互作用部位

Crystal Structure of Ga-substituted Ferredoxin and its interaction sites for Photosystem I and Ferredoxin-$NADP^+$ reductase

Risa Mutoh, Norifumi Muraki, Hisako Kubota-Kawai, Toshiharu Hase, Takahisa Ikegami, Genji Kurisu (*Institute for Protein Research*)

Plant-type ferredoxin (Fd) is an electron transfer protein with a [2Fe-2S] cluster, mediating one-electron from photosystem I (PSI) to several Fd-dependent enzymes such as Ferredoxin-$NADP^+$ reductase (FNR). For the interaction study between Fd and Fd-dependent enzymes, NMR spectroscopy has used Ga-substituted Fd (Ga-Fd) due to the paramagnetic properties of [2Fe-2S] cluster. To establish how much Ga-Fd can be used as a replacement of native Fd, we solved the crystal structure of Ga-Fd at 1.4-Å resolution, and confirmed the comparable binding affinities of native-Fd and Ga-Fd using an FNR immobilized column. NMR chemical shift perturbation experiments of both Fds also exhibit no significant structural differences in the interaction sites for PSI.

2P257 光化学系 II の Mn4 クラスター S0 状態における Mn(II)存在可能性の理論的研究

S0-State Model of the Mn4-cluster in Photosystem II: Possibility of Mn(II)

Makoto Hatakeyama, Koji Ogata, Shinichiro Nakamura (*RIKEN*)

Mn4-cluster in photosystem II protein accumulates oxidizing equivalents generated by the photoreaction-center and catalyzes the water-splitting of photosynthesis. Then, depending on the number of oxidizing equivalents, Mn4-cluster shows different configuration of possible Mn(II)/Mn(III)/Mn(IV) ions. For the Mn4-cluster having no oxidizing equivalent (S0 state), spectroscopic studies have proposed two possible configurations; Mn4(III,III,III,IV) or Mn4(II,III,IV,IV). To elucidate a more possible configuration at S0, we focus on the two factors; (a) the stable Mn4-structure on each possible valence-configuration and (b) Mn-Mn distances shown in EXAFS. We will report QM/MM optimization results for each configuration and the agreements with EXAFS.

2P258 フィコエリスリンを有するラン藻における励起エネルギー移動

Excitation energy transfer in cyanobacteria containing phycoerythrin

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Cyanobacteria are the most primitive oxygenic photosynthetic organisms, in which phycobilisome (PBS) works as major light-harvesting pigments. Cyanobacteria, *Synechococcus* sp. CCMP1334 (*Synechococcus*) and *Phormidium* sp. NIBB1081 (*Phormidium*), show red color, because these two species contain large amount of phycoerythrin (PE) in PBS. We examine excitation energy transfer in *Synechococcus* and *Phormidium* by means of steady-state and time-resolved fluorescence spectroscopies. In steady-state excitation spectra, *Synechococcus* showed R-PE-type spectra, whereas *Phormidium*, C-PE-type. Results of the time-resolved measurements indicated that energy transfer within PE occurred about two times faster in *Synechococcus*.

2P259 ホタルルシフェラーゼとの相互作用を考慮したオキシルシフェリンの吸収スペクトルの量子化学計算

Quantum chemical calculation of the absorption spectra of oxyluciferin interacting with firefly luciferase

Hironori Sakai[1], **Naohisa Wada**[2] ([1]*IFS. Tohoku Univ.*, [2]*Facul. of Food Sciences, Toyo Univ.*)

Firefly is most investigated among the bioluminescent species. Its reaction mechanism is oxygenation of substrate luciferin by firefly luciferase (E) under the existence of Mg-ATP and oxygen. Visible light is emitted when final product of this reaction, electronically excited oxyluciferin (OxyLn*), goes down to its ground state. By X-ray diffraction study, tertiary structure of E with OxyLn was already reported (PDB; 2D1R). In this study, we attempted to predict the absorption spectra of OxyLn interacting with E including all water molecules. After correction of missing residues and atoms, QM/MM geometry optimization was performed using Discovery studio (Accelrys). At last, INDO/S calculation was performed for optimized structure of 2D1R by MO-S (Fujitsu).

2P260 次世代シーケンサーを用いた人工細胞モデルにおけるゲノムＲＮＡの進化プロセスの解析

Analysis of the evolutionary process of the RNA genome in an artificial cell-like system using next generation sequencing technology

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Evolution is a key function of living things that produce present-day living world. However, the detailed process of evolution is still obscure due to the lack of sufficiently simple experimental model. Recently, we have constructed a simple cell-like system which contains a single-stranded RNA genome and has the ability of evolution through the continuous fusion-division cycle of cell-like compartment. Here, we investigated the evolutionary process in detail by using the next-generation sequencing technology. The analysis revealed the existence of clonal interference and positive epistasis throughout the evolutionary process. The method used in this study and the obtained result would contribute to the deeper understanding of evolutionary process.

2P261 Qβ レプリケースによる RNA 複製反応中の二本鎖 RNA 形成の理解

Double-stranded RNA formation during Qβ long RNA replication

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Qβ replicase is an RNA-dependent RNA polymerase of coliphage Qβ, which synthesizes the complementary RNA using a single-stranded RNA as a template. The formation of non-replicable double-stranded RNA (dsRNA) by hybridization between newly synthesized RNA and the template RNA hinders the broader application of Qβ replicase. Here, we developed a kinetic model of Qβ RNA replication consisting of two reaction pathways of dsRNA formation, which quantitatively explains the dynamics of dsRNA formation of three template RNAs. We also found that part of the Qβ phage genomic RNA sequence including the central hairpin loop significantly decreases the rate of dsRNA formation and revealed that the secondary structure of RNA affects the dsRNA formation during Qβ RNA replication.

2P262 人工自己複製モデルと寄生体が生み出す振動ダイナミクス

Oscillation dynamics of Host-Parasite population in an artificial cell-like system

Yohsuke Bansho[1,2], Norikazu Ichihashi[3], Tetsuya Yomo[1,3,4] ([1]*Frontier Biosciences, Osaka University*, [2]*JSPS*, [3]*ERATO, JST*, [4]*Information Science and Technology, Osaka University*)

The appearance of parasite is one of the biggest hurdles for primitive self-replicators in prebiotic evolution. It casts a question: how has the primitive self-replication been established under threats of parasites? To understand this question, we are attempted to construct the artificial host-parasite interacting self-replication system derived from RNA phage Q-beta.
We found that this artificial host-parasite system long-term lasting in water-in-oil emulsion with the populations of the host and the parasite oscillating. Moreover, the host genomic RNA acquired frequent changes of the genetic sequence during the oscillation. It might suggest that this parasite-driven oscillation dynamics facilitates the evolution of the host RNA.

2P263 Directed evolution of a self-encoding system

Takeshi Sunami[1,2], Norikazu Ichihashi[1,2], Takehiro Nishikawa[2], Yasuaki Kazuta[2], Tomoaki Matsuura[2,3], Hiroaki Suzuki[2,4], Tetsuya Yomo[1,2,5] ([1]*Grad. Sch. Info. Sci., Osaka Univ.*, [2]*JST, ERATO*, [3]*Grad. Sch. Eng., Osaka Univ.*, [4]*Grad. Sch. Sci. Eng., Chuo Univ.*, [5]*Grad. Sch. Fro. Bio., Osaka Univ.*)

We have constructed a simplified system, in which the genetic information is replicated by self-encoded replicase in liposomes. Here we demonstrate the directed evolution of the self-encoding system. Cell-sized giant liposome was used as a micro reactor, and fluorescence-activated cell sorter (FACS) was used to analyze and sort the liposomes. The antisense of the beta-galactosidase gene was used as a reporter gene. Fluorescent liposomes with higher active replicase gene were sorted by FACS. The DNA templates for next selection round were amplified from the selected RNA in a test tube. After 69 rounds of selection, the self-replication activity of the selected mutants was evaluated in liposomes, and the mutants were higher active than wild-type replicase.

2P264 出芽酵母における染色体の構造変化と転写制御との関連について

Relationship between conformational change of chromosomes and transcriptional control in budding yeast

Naoko Tokuda, Masaki Sasai (*Grad. Sch. Eng., Nagoya Univ.*)

Recently, 3-dimensional eukaryotic genome structures have been inferred from next-generation sequencing combined with chromosome conformation capture (Tanizawa et al., 2012). However, it has not been clarified how the 3-dimensional genome structure affects transcriptional regulation. For example, Taddei et al., Genome Res. (2009) showed that the expression pattern of many genes in budding yeast is changed when mutation is introduced to prevent telomeres from being anchored to the nuclear periphery, but the mechanism of this misregulation is elusive. In this talk, we will discuss the reason why genes are misregulated in the mutant by using a dynamical structural model of chromosomes (Tokuda et al., 2012) and microarray gene-expression data.

2P265 天然変性タンパク質データベース IDEAL の機能拡張 —PPI ネットワーク

New IDEAL: availability of PPI networks involving intrinsically disordered proteins

Takayuki Amemiya[1], Shigetaka Sakamoto[2], Yukiko Nobe[1], Kazuo Hosoda[3], Yumiko Kado[1], Ryotaro Koike[1], Hidekazu Hiroaki[4], Motonori Ota[1], satoshi Fukuchi[3] ([1]*Grad. Schl of Info. Sci., Nagoya Univ.*, [2]*HOLONICS Co., Ltd.*, [3]*Fac. Engr., Maebashi Ins. Tech.*, [4]*Grad. Schl of Pharm. Sci., Nagoya Univ.*)

The IDEAL database (http://www.ideal.force.cs.is.nagoya-u.ac.jp/IDEAL/) has been constructed to collect the knowledge of intrinsically disordered proteins (IDPs). Because it is quite important to understand IDPs in the context of a biological system and/or a protein-protein interaction (PPI) network, IDEAL has been improved to show the PPI networks. We regarded a protein in IDEAL as a NODE, and an interaction of two proteins as an EDGE, preparing NODE pages as well as EDGE pages. Users can easily walk around a PPI network by clicking the link bottoms which connect the NODE and the EDGE pages each other. The birds-eye view of the PPI network is also provided. We will demonstrate the features of new IDEAL with some examples.

2P266 Tertiary structure prediction of RNA-RNA complex structures using secondary structure information

Satoshi Yamasaki, Kazuhiko Fukui (*molprof, AIST*)

Recent progress in molecular biology and genome science leads to finding many functional RNAs which control gene expression and replication. These functions are achieved through the protein-RNA and RNA-RNA interactions. Deriving the tertiary structures of these complex is very helpful to clarify the mechanism of those functions in detail. We developed the new method based on fragment assembly algorithm to predict RNA-RNA complex structures from nucleotide sequence and secondary structure information. We applied our method to predict several kinds of RNA-RNA complex structures which include kissing-loops, hammerhead ribozymes and other difficult targets, and derived successful results especially in the prediction of kissing-loop targets.

2P267 相互作用プロファイルを用いた Re-docking 法によるタンパク質間相互作用予測
Re-docking scheme for prediction of protein-protein interactions using interaction fingerprints

Nobuyuki Uchikoga[1], Yuri Matsuzaki[2], Masahito Ohue[2,3], Takatsugu Hirokawa[4], Yutaka Akiyama[2,3] ([1]*Dept. Phys., Chuo Univ.*, [2]*Grad. info. sci. eng., Dept. comput. sci., Titech*, [3]*Edu. Acad. comput. life sci., Titech*, [4]*AIST, molprof*)

We approach to problems of protein-protein Interaction network using rigid-body docking algorithm, generating many decoys including false positives. Although this docking method is popular and useful, there are some serious cases with no near-native decoys. Then, we developed iterated method for generating more near-native decoys using Interaction FingerPrints (IFPs). We applied this method to obtaining region including native interacting residue pairs for re-docking process.
We examined re-docking process using IFPs after an initial-docking process after investigating docking surfaces of proteins. As results, we could obtain a set of decoys with higher similarities than that of decoys generated in the initial docking process.

2P268 Protein binding pocket and ligand shape comparison

Chie Motono, Takatsugu Hirokawa (*Molprof, AIST*)

The aim of the study is to understand molecular recognition for drug discovery and design. We analyzed binding pocket and corresponding ligand with respect to their shapes using selected data from DUD (directory of useful decoys). The respective ligand and pocket shape overlap was calculated. In addition, we addressed the effect of protein molecular dynamics on shape similarity between a protein pocket and its ligand. MD simulations were used to explore the structural change of a target protein, especially of its active site. We also assessed if a comparison of shape and size of protein pockets and ligands could be helpful in virtual screening context. It is discussed if the comparison method can filter out compounds prior to other docking methods.

2P269 膜タンパク質の顕微鏡画像と立体構造データとの照合用データベースの構築
Construction of database for comparing structural data with microscopic image of transmembrane protein

Go Inoue, Masami Ikeda, Makiko Suwa (*Grad. Sch. Sci and Eng. AGU*)

It is important to visualize the interaction between the transmembrane proteins (TMPs) which are difficult to determine structure by X-ray crystallography and NMR, when we understand these functions. Recent methodology “visual proteomics” is expected to annotate comprehensively the name of whole proteins in the cell by comparing 3D volume with cryo-electron tomography images. We have been studying to identify localization of TMPs, projected on membrane surface by comparing their electron microscopic images with tomographic images of 3D structure of TMPs. We have constructed a database including 734 entries of TMPs (2,021 chains), with their calculated features such as the tomographic image, the perimeter, the image area, and the circularity for each structure.

2P270 β2 アドレナリン受容体 - Gαs 間の結合要素の解析
Structural analysis of coupling element between β2 adrenergic receptor and G-protein

Hidenori Sakaki, Masami Ikeda, Makiko Suwa (*Grad. Sch. Sci and Eng. AGU*)

G protein-coupled receptors (GPCRs) transduce signals from extracellular ligands to intracellular G-proteins. The study of the GPCR-G protein interface is important to understand the functional mechanism of GPCRs. Based on the 3D structure of β2 adrenergic receptor (βAR) with Gs type G-protein complex, we made several mutated structures (βAR with non-Gs proteins), by comparative modeling, and optimized the side chain structures of native / mutated complex, by using AMBER99 force field. Comparing interactions energy between these modeled structures, it was suggested that the native βAR structure, binding with Gs, is most stable with interaction energy several tens or more kcal/mol less than that of other mutants.

2P271 Flow cytometry identification of nanocyanobacteria and their limiting factors in the North Pacific Subtropical Gyre

Mathias Girault[1,2], Hisayuki Arakawa[2], Gerald Gregori[3], Fuminori Hashihama[2], Hyonchol Kim[1], Masao Odaka[1], Kenji Yasuda[1] ([1]*KAST*, [2]*TUMSAT*, [3]*Universite de la Mediterranee*)

The distribution of ultraphytoplankton was investigated in the western North Pacific Subtropical Gyre (NPSG) during La Nina event. Among the organisms sorted by flow cytometry, we focussed on the distribution of nanocyanobacteria. In the NPSG nanocyanobacteria are usually associated with the low nitrate and phosphate concentrations and are expected to play a key role in the nutrient cycle. Our results suggested that contrasting to the cyanobacteria Prochlorococcus and Synechococcus, nanocyanobacteria are not mainly controlled by the nutrient concentrations but rather by the frontal system observed at the 22.83N. Principal component analysis and partial redundancy analysis performed on the data set tend to confirm the major role of temperature and salinity in the NPSG.

2P272 実験生態系の進化、個体群、反応ダイナミクス
Evolutionary, population, and reaction dynamics of experimental ecosystems

Kazufumi Hosoda[1], Makoto Sueyoshi[2], Itsuka Kumano[2], Masumi Habuchi[3], Kayo Yamamoto[2], Risa Takami[2], Yuhki Azuma[4], Isao Kubo[2], Shingo Suzuki[2], Tetsuya Yomo[2] ([1]*Acad Init, Osaka-u*, [2]*Info Sci, Osaka-u*, [3]*Front Bio, Osaka-u*, [4]*Eng, Osaka-u*)

Organisms rarely live alone in nature. All functions (or phenotypes) of organisms, achieved by numerous numbers of biochemical reactions, work in ecosystems including interactions among individual organisms and among different populations. Those functions finally provide advantages to the organisms for propagation or survival in the evolutionary competition. Thus, it is important to connect the mechanisms of the phenotypes, which is based on biochemical reactions, to the population and evolutionary dynamics in an ecosystem. Experimental reconstruction of simple ecosystems is an efficient strategy to comprehend those dynamics. Here, we summarize the results obtained from multiple experimental ecosystems that were composed of different microbial populations.

2P273 Gain Noise Relation in Adaptation Networks

Prabhat Shankar[1,2], Masatoshi Nishikawa[3], Tatsuo Shibata[1] ([1]*RIKEN CDB, Kobe*, [2]*Hiroshima University, HIroshima*, [3]*Max Planck Inst, Germany*)

Perfect adaptation is shown by many noisy biochemical systems. The relation between the response and intrinsic noise is not clearly understood for them. It was shown recently that in bacterial chemotaxis, response time is proportional to the noise. Here, we study network topologies which show perfect adaptation, and find that: a) Gain is limited by intrinsic noise b) An optimal gain-noise performance can be reached for some parameters. c) Intrinsic noise dominates the extrinsic noise. We also derive gain in terms of the intrinsic noise using the fluctuation-dissipation theorem. We hope this study will help to understand the role of network topologies in achieving perfect adaptation in noisy enviornments, and will help in determining their parameters experimentally.

2P274 Adaptive random Boolean network model based on local information transfer

Taichi Haruna, Sayaka Tanaka (*Graduate School of Science, Kobe University*)

It has been suggested that gene regulation networks of living systems are working close to criticality. Lizier et al. (2008) showed that random Boolean networks close to criticality balance coherent information transfer and information storage. Here, information is quantified globally from the outside of the system. However, information usage within a system could be relevant to evolution towards criticality. In this presentation, we propose a new adaptive random Boolean network model taking account of how information is utilized locally within a system. We show that the proposed model can spontaneously evolve towards a critical state by both computer simulations and theoretical analysis based on an annealed approximation.

2P275 光合成生物との共生による利益とはーミドリゾウリムシの増殖解析
Benefits of Acquiring Phototrophy by Hosting Algal Endosymbionts

Sosuke Iwai (*Faculty of Education, Hirosaki Univ.*)

Many species of protists and metazoans have acquired phototrophy by hosting algal endosymbionts. These species benefit from enhanced growth due to access to photosynthetic products, while the precise contributions of the endosymbiosis remain elusive. To reveal the benefits of acquiring phototrophy, here we analyze growth of *Paramecium bursaria*, a ciliate that hosts chlorella-like algae as endosymbionts. To quantitatively analyze the growth, *P. bursaria* cells were grown in a simple, bacteria-free monoxenic culture system using yeast as the sole food. Comparison of the growth between wild-type and aposymbiotic (chlorella-free) cells shows that the benefits of acquiring phototrophy for *P. bursaria* are gains of both maintenance energy and a carbon source for cell growth.

2P276 Diffusion in the plasma membrane with immobile molecules: significance of fluid dynamical interactions

Ziya Kalay, Takahiro K. Fujiwara, Akihiro Kusumi (*Institute for Integrated Cell-Material Sciences, Kyoto University*)

Due to the coupling between the plasma membrane and the actin cytoskeleton, membrane molecules such as receptor proteins can become immobilized by binding to static structures. We investigate the effect of immobile membrane molecules on the diffusion of mobile ones. By modeling the membrane as a 2-d fluid composed of hard-particles and performing event driven molecular dynamics simulations, we show that the diffusion coefficient sharply decreases with increasing immobile fraction. We argue that the fluid dynamical interactions play a major role in this slowdown. We discuss the relevance of our findings to single molecule observations of membrane lipids and to theoretical findings on the influence of fluid dynamics on molecular transport in a 2-d fluid.

2P277 Competitive reaction between enzymes with normal and anomalous diffusivity

Kenta Yashima[1], Jun Nakabayashi[2], Akira Sasaki[1] ([1]*The Graduate University for Advanced Studies*, [2]*Yokohama City University*)

Molecules within heterogeneous medium may diffuse anomalously. Hindered by the obstacles for reactant molecules, classical mass action law fails, requiring the application of the fractal reaction theory. In this study we analyze the system where two enzymes with different diffusivity, one with normal diffusivity and the other with anomalous diffusivity compete for the same substrate. Analytical formula derived from the fractal reaction theory shows the production rate from anomalously diffusing enzyme is reduced several orders of magnitudes compared with that from normally diffusing one. These results are confirmed by extensive Monte Carlo simulations on lattice structured media. We will discuss possible physiological implications of this effect.

2P278 Allometries of the *Physarum* plasmodium based on the dynamics of cytoplasmic streaming

Tomohiro Shirakawa, Hiroshi Sato (*Dept. Comp. Sci., NDA*)

The plasmodium of *Physarum polycephalum* is a unicellular and multinuclear giant amoeba. In the previous studies, we found some allometric scaling laws in the cell motility and exploratory behavior of the plasmodium. For example, the area of plasmodial body is proportional to the cell weight to the power of three fourths, and the velocity of cell motility is proportional to the length of the plasmodium. In this study, we tried to investigate how such allometries emerge, and found that the allometric laws can be explained in terms of the dynamics of cytoplasmic streaming.

2P279 Analysis for the exploratory behavior of *Physarum* plasmodium in an unlimitedly extendable space

Miharu Nishida, Hiroshi Satou, Tomohiro Shirakawa (*Dept. Comp. Sci., NDA*)

The plasmodium of *Physarum polycephalum* is a unicellular giant amoeba. Recent studies clarified that the plasmodium has an ability to perform computation such as maze-solving and optimization of a network. These studies targeted the behavior of the plasmodium in a closed space and thus there is no study on the long-term exploratory behavior of the organism in an open and wide space. In this study, we developed an experimental system that provides unlimitedly extendable 2-dimensional space. The setup consists of 9 square plates with wet paper towel substrate on each of them. By rearranging the plates according to the motility of the plasmodium, we can extend the 2-dimensional space arbitrarily. As a result, we succeeded to observe long-term motility of the plasmodium.

2P280 Cell motility of the *Physarum* plasmodium on a non-uniform substrate

Shinji Ishiguro, Hiroshi Sato, Tomohiro Shirakawa (*National Defense Academy of Japan*)

The plasmodium of true slime mold *Physarum polycephalum* is a unicellular and multinuclear giant amoeba. Since the plasmodium has amorphous body, the cell motility of the plasmodium is continuous and omnidirectional. This nature makes the motility of the plasmodium complex and gives rise to some difficulty in observation. Therefore we developed an experimental system that is able to limit the motility of the plasmodium. In our experiment, we used a non-uniform substrate such that the hydrophilic surfaces with circular shape are arranged in a 2-dimensional lattice and surrounded by hydrophobic plastic surface. By using this substrate, we succeeded to limit the motility of the plasmodium to 4-directional neighbors, and to stepwise one.

2P281 過去の神経活動がどのように現在のスパイク頻度に影響を与えるのか

How past neuronal activity affects the current firing rate

Takanobu Yamanobe (*Med. Sch., Hokkaido Univ.*)

Spikes need to be generated independently of the past activity of the neuron for encoding information into spike pattern. The duration of the past neuronal activity is a key to understand information coding in nervous systems. We evaluated how the past neuronal activity affects the current firing rate of a neuron theoretically and experimentally. We examined the response of squid giant axons to time-varying current pulses to investigate the dependence on the past neuronal activity. We introduced a Markov operator that describes the density evolution of a neuronal oscillator. Specifically, we analyzed the dynamics of the number of spikes per unit time based on the invariant and transient properties of the Markov operator.

2P282 Negative feedback regulation of KaiC ATPase gives origin to the circadian periodicity of cyanobacteria

Atsushi Mukaiyama[1,2,3], Masato Osako[4], Takaaki Hikima[3], Takao Kondo[4], Shuji Akiyama[1,2,3] ([1]*Inst. Mol. Sci.*, [2]*Grad. Univ. for Adv. Studies (SOKENDAI)*, [3]*SPring-8, RIKEN*, [4]*Nagoya Univ.*)

Circadian clocks are endogenous timing devices to accommodate the biochemical and physiological processes to dairy alterations in external environments. In cyanobacterium *Synechococcus elongatus* PCC7942, circadian clocks consist 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.
The frequency of the phosphorylation cycle is linearly correlated with the ATPase activity of KaiC alone. This means that KaiC ATPase determines the period. Additionally, KaiC ATPase is extremely low, and temperature-compensated, suggesting that it seems to be under negative feedback regulation. In this meeting, we provide experimental evidences to support it.

2P283 マイクロドロップレットで構築された非平衡人工細胞の実験的・数理的解析

Experimental and numerical analyses of microdroplet-based nonequilibrium artificial cells

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.*)

Recently, construction of artificial cells as simplified models of living cells has been proposed to study essential dynamic mechanisms of life systems. However, most of them have limitations in transporting exterior/interior molecules through their interface, preventing implementation of sustained dynamic nonequilibrium reactions such as nonlinear oscillations. Here, we demonstrate an artificial cell construction based on water-in-oil microdroplets. In this system, the artificial cell was kept nonequilibrium by the controlled influx/efflux of molecules in a microfluidic channel; sustained nonlinear chemical oscillations were successfully achieved. We believe that this system will promote the study of dynamic chemical systems in artificial cells in future.

2P284 膜の分子透過性へのフィードバック制御のある非平衡系人工細胞の数理解析

Numerical analysis of non-equilibrium open artificial cell with a feedback control over molecular permeability of the cell membrane

Motosugi Murata[1], Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. & Eng., Tokyo Tech.*, [2]*PRESTO, JST*)

Recently, artificial cells have been attracted much attention as a model of living cells. The living cells are non-equilibrium chemically open systems and transport molecules in and out through their cell membrane in a controlled manner. However, artificial cells with a chemically open feature and a controlled transport of molecules have never been achieved yet. Here, we propose a chemically open artificial cell with a feedback control over molecular permeability of the cell membrane. To implement this feedback control system, diffusion coefficient inside the artificial cell is changed according to the concentration of inside chemicals. We believe that this numerical analysis will promote the understanding of dynamic chemical systems in artificial cells.

2P285 Oscillations of a genomic DNA in a cell-sized chemically open system

Haruka Sugiura[1], Masahiro Takinoue[1,2] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Inst. Tech.*, [2]*PRESTO, JST*)

Temporal self-organization such as biochemical oscillation is universally observed in non-equilibrium open systems including living cells; it is utilized to trigger and regulate time-dependent behaviors of gene expressions etc. However, temporal self-organization has still not been realized in artificial cells that are models to study dynamical aspects of living cells. Thus, generating an intrinsic oscillation is strongly desired in the study of artificial cells. Here we present two models of DNA structural oscillations in a cell-sized chemically open system. Our numerical analyses derive that DNA exhibits synchronized and self-excited oscillations. In the future, our study will promote the construction and regulation of temporal dynamics in artificial cells.

2P286 広帯域 X 線を用いた広角度域 X 線 1 分子追跡法の開発

Development of wide angle Diffracted X-ray Tracking (DXT) measurement using a focusing broad band X-ray

Ichiyanagi Kouhei[1], Hiroshi Sekiguchi[2], Masato Hoshino[2], Kentaro Kajiwara[2], Kentaro Hoshisashi[1], Jae-won Chang[1], Maki Tokue[1], Yufuku Matsushita[1], Naoto Yagi[1], Yuji Sasaki[1] ([1]*Graduate School of Frontier Sciences, The University of Tokyo*, [2]*Japan Synchrotron Radiation Research Institute*)

Diffracted X-ray tracking (DXT) enables the tilting and twisting motions of single protein molecules to be monitored with micorradian resolution using a highly brilliant and broad band X-ray source. We have developed a new technique to measure in-situ single molecule motion combined with a single X-ray toroidal mirror at the BL28B2 to SPring-8 in an energy range of 10 to 20 keV. In this study, we present the specification of wide angle DXT measurement system. The intramolecular motions of a human serum albumin and its complex with 2-anthracencarboxly acid were investigated using wide angle DXT. The each random tilting and twisting intramolecular motions was shown to be directly linked.

2P287 オンチップ画像解析システムによる形状を制御した単一心筋細胞の収縮方向の計測

Measurement of contractile direction on single-shape-controlled cardiomyocytes by on-chip optical image analysis system

Tomoyuki Kaneko[1], Fumimasa Nomura[2], Tomoyo Hamada[2], Akihiro Hattori[2], Kenji Yasuda[2] ([1]*Dept. Frontier Bioscience, Hosei Univ.*, [2]*Dept. Biomed. Info, IBB, TMDU*)

Evaluation of mechanophysiological responses of cardiomyocytes has become more important for precise prediction of cardiotoxicity. For the accurate detection of cardiomyocyte contraction, we have developed an on-chip optical image analysis system that records the contractile motions of cardiomyocytes with noninvasive/nondestructive measurement. Using this system, we measured the displacement and direction of contraction of single circular and rectangular cardiomyocytes. The results indicated that the rectangular cardiomyocytes tend to contract along the longitudinal direction as in a real heart. This system enables the accurate measurement of cardiomyocyte contraction, and is expected to be applicable to the precise detection of irregular motions such as arrhythmia.

2P288 ビデオ解析による大腸菌回転特性の大量測定

Large-scale measurement of rotary motion properties of tethered Escherichia coli (*E. coli*) by video analysis

Hiroto Tanaka[1], Tadashi Matsukawa[1], Yukihiro Tominari[2], Shuhei Ogawa[3], Yoshiyuki Sowa[4], Ikuro Kawagishi[4], Shukichi Tanaka[2], Kazuhiro Oiwa[1], Hiroaki Kojima[1] ([1]*Bio ICT lab., NICT*, [2]*Nano ICT lab., NICT*, [3]*Dept. Bioeng., Nagaoka Univ. Tech.*, [4]*Dept. Front. Biosci., Hosei Univ.*)

E. coli modifies the way in which it swims, by switching directions of reversible rotary motors, which drive flagellum, in response to chemical compounds (attractants, repellents). Namely, *E. coli* functions as a chemical bio-sensor (input : chemical compounds, output : ratio of direction of reversible rotary motor). Focusing on the sensing functions, we have tried developing bio-sensor system with bacteria as an application of bio-materials. As a fundamental technology for developments of bio-sensor, we report construction of automated detection system for rotary motion of tethered *E. coli* under an optical microscope. With X20 objective, high speed camera (> 500 fps) and image analysis techniques, we accomplish analysis of ~500 cells in a screen automatically.

2P289 流体力学的絞込みを用いた一分子ソーターセルの開発

Development of hydrodynamic focusing system for single molecule sorting device

Toshihiko Kubota[1,2], Hiroyuki Oikawa[1], Kiyoto Kamagata[1,2], Satoshi Takahashi[1,2] ([1]*IMRAM, Tohoku Univ.*, [2]*Grad. Sch. Life Sci., Tohoku Univ.*)

Sorting of micro particles is widely used in life sciences; however, the sorting at the single molecule level is still difficult. We constructed the fluorescence detection and flow systems for the sorting of single proteins. To achieve single molecule sensitivity, the detection system was designed based on confocal alignment and avalanche photodiode. The flow system was constructed using mechanical valves and a microfluidic cell. The cell possesses a dam structure for the three-dimensional focusing of sample flow. We confirmed that the detection system can discriminate fluoresence signals from single fluorophores from background. We further verified that the hydrodynamic focusing enough for the detection of all the flowing samples at the single molecule level.

2P290 創薬スクリーニングのための心筋細胞ネットーワークにおける空間パターンと集団サイズの重要性

Importance of spatial arrangement and community size on cardiomyocyte network for precise and stable in vitro drug screening measurement

Fumimasa Nomura, Tomoyo Hamada, Hideyuki Terazono, Kenji Yasuda (*IBB, Tokyo Medical and Dental Univ.*)

We have investigated the community size and spatial arrangement dependence of cardiomyocyte network response against pharmaceutical compounds. Typical four types of cell networks, small cluster, small closed loop, two-dimensional sheet, and a large closed loop, were formed in those fabricated agarose microchambers on a multielectrode array chip and comparison of their responses against Quinidine. The beating rhythm of the two-dimensional sheet was most durable and stable against administration of Qunidine even 100 μM, whereas the large closed loop network was most sensitive and showed lethal arrhythmia from 1 μM. The results indicate that the cell community for drug screening should be tuned to the proper community size and spatial arrangement pattern.

2P291 マニフォールドを用いた投影イメージの分類プロトコル：コヒーレントX線イメージングによる粒子ダイナミックスの解析へ向けて

Classification protocol of projection images by manifold: Toward analysis of dynamics of particles with coherent x-ray diffraction imaging

Takashi Yoshidome[1], Tomotaka Oroguchi[2,3], Masayoshi Nakasako[2,3], Mitunori Ikeguchi[1] ([1]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*, [2]*Dep. Phys., Keio Univ.*, [3]*Harima Inst., Riken*)

Coherent x-ray diffraction imaging enables us to collect huge amounts of diffraction images of a single particle for a short time. Since images represent projections of snapshots of a particle, they reflect its dynamics. Thus, dynamics can be analyzed using the images. One of the requirements for the analysis is a classification of images. Usual classification methods, however, would generate a great deal of classes because there are images arising from snapshots with a subtle difference as well as those with a large difference. This makes the analysis difficult. Here we propose a classification protocol of projection images using manifold, by which the issue above can be solved. We demonstrate the usefulness of the protocol using the images constructed in a computer.

2P292 振動和周波検出赤外超解像顕微鏡による毛髪 α-ケラチンの分子配向観察

Observation of molecular orientation of human hair α-keratins by VSFG detected IR super-resolution microscopy

Makoto Sakai[1], Kohei Ushio[1,2], Shinobu Nagase[3], Yuuji Hirano[3], Takashi Itou[3], Haruki Ishikawa[2], Masaaki Fujii[1] ([1]*Tokyo Institute of Technology*, [2]*Kitasato University*, [3]*Kao Corporation*)

IR super-resolution images of cross-sections of human hairs were measured by using a vibrational sum-frequency generation (VSFG) detected IR microscope with a sub-micrometer spatial resolution. For the amide III band, the cross-section sample gave clear strong VSFG signals at the cortex area. This enabled us to measure the distribution of an α-helix structure of keratin proteins (α-keratins) in the hair. On the other hand, the VSFG signal disappeared completely when the amide I band was monitored by the same polarization of incident light. From the polarization dependence of VSFG, it is concluded that the α-keratins are well aligned along the axial direction in human hair. In the presentation, the results of the oblique-sections will be also reported in detail.

2P293 Determination of dissociation constants of NFκB p50/p65 heterodimer using fluorescence cross-correlation spectroscopy in the living cell

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The p50/p65 heterodimer plays various roles in gene regulation. The quantitative value of affinity, namely the Kd, for the heterodimer in living cells is not known yet. We used fluorescence cross-correlation spectroscopy to quantify the heterodimerization of p50/p65 in the living cell. We determined the Kd of the transiently expressed mCherry tandem dimer (mCherry2) and green fluorescent protein (EGFP) protein fused with IPT domains of p50 and p65, respectively, in living cells. The Kd values of mCherry2-p50 and EGFP-p65 were determined to be 0.46 μM in the cytoplasm and 1.06 μM in the nucleus. These results suggest the different binding affinities of the p50/p65 heterodimer in the cytoplasm and nucleus of the living cell.

2P294 蛍光・発光イメージングによる OPN5 発現細胞の Ca^{2+}応答測定

Bioluminescent Imaging Revealed a Rapid Ca^{2+} Response in OPN5-expressing Cells

Takashi Sugiyama (*Cell-based Analysis Group, Advanced Analysis Technology R&D Dept., Olympus Corporation*)

Fluorescence imaging is a powerful tool for investigating the intracellular signaling, but it may be difficult to apply to photo-receptive or photo-sensitive cells. In this report, we demonstrated that a light-induced Ca^{2+} response was different between fluorescence imaging and bioluminescence imaging in human opsin5 (OPN5)-expressing cells. In addition, the irradiation induced the phosphorylation of MAPK when OPN5 was stimulated under dark condition, but this phosphorylation was altered when OPN5 was irradiated under the bright condition. These findings suggested that comparison of the responses in these imaging provide a new technique to investigate the differences of physiological characteristics between light-adapted and dark-adapted photoreceptors.

2P295 ライブセル超解像イメージングに向けた多重分子用アルゴリズム“Wedged Template Matching”

Localization Algorithm of High-Density Fluorophores, “Wedged Template Matching” for Live Cell Super Resolution Imaging

Shigeo Watanabe[1], Yasushi Okada[2], Teruo Takahashi[1], Keith Bennett[3], Tomochika Takeshima[1] ([1]*Hamamatsu Photonics K.K.*, [2]*RIKEN QBiC*, [3]*Hamamatsu Corporation*)

Localization microscopy excites a statistical fraction of molecules over time, allowing the sub-diffraction location of each molecule to be mathematically determined. A limitation of this method is the need for many (>5,000) raw frames to produce meaningful reconstructions. To address this issue we developed a localization algorithm of high density fluorophores, “Wedged Template Matching (WTM)”, which can localize simultaneously overlapping molecules. On simulated data, WTM can localize overlapping molecules at greater than 20 molecules/μm^2 density with localization precision (FWHM) of 24 nm. We demonstrate WTM for live cell super resolution imaging.

2P296 2 種類のシグナルノイズが PTEN の細胞内不均一性を決める
Two types of signaling noises underlie spatiotemporal PTEN heterogeneity

Naotoshi Nakamura, Tatsuo Shibata (*Laboratory for Physical Biology, RIKEN Center for Developmental Biology*)

Cells employ various intracellular signaling molecules to determine their front/back polarity. PtdIns(3,4,5)P3 phosphatase PTEN is known to be excluded from the front and is necessary for correct pseudopod formation. However, the role of noise in the formation of PTEN polarity has not been elucidated. Here we studied spatiotemporal PTEN heterogeneity in individual cells by imaging PTEN molecules conjugated with green or red fluorescent probes. Spatial and temporal correlation functions of the fluorescence intensities revealed the kinetics of PTEN and its binding sites. The results suggest that two types of noises, one from PTEN itself and the other from its binding sites, contribute to PTEN heterogeneity.

2P297 2 波長同時イメージングによる PTEN の膜局在と 1 分子の同時解析
Simultaneous Imaging of Single-molecule and Bulk Localization of PTEN

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PTEN, an enzyme that catalyzes dephosphorylation of PIP3 to PIP2, plays an important role in dynamic self-organizing distributions of PIP2 and PIP3. In a previous study, we demonstrated self-organizing patterns by analysing PIP3 and PTEN distributions at the membrane. In addition, we measured the single-molecule level kinetics of PTEN. In order to understand the self-organization mechanism at the level of individual molecules, it is necessary to couple these results. To achieve this, we analyse single-molecule by bulk PTEN density level, and find that PTEN kinetics vary as a function of bulk PTEN localization and fluctuation patterns. To identify the mechanisms, we are now performing this analysis with other molecules involved in the self-organizing molecular network.

2P298 Fluorescent Single Molecule Orinetation Imaging in Living Cells

Tomomi Tani[1], Shalin Mehta[1], Rudolf Oldenbourg[1], Amy Gladfelter[2] ([1]*Marine Biologcal Laboratory*, [2]*Dartmouth College*)

We are proposing a light microscopy to detect changes in intra-molecular structure or inter-molecular organization based on orientation imaging of fluorescent single molecules. We have developed robust instrumentation for polarized fluorescence imaging exhibiting the speed and sensitivity required to monitor 3D angular changes of individual fluorophores that are rigidly connected to proteins of interest. While developing the optical arrangement and required acquisition and processing algorithms, we use the system to monitor the organization of cytoskeletal molecules in a filamentous fungus, Ashbya gossypii, and in budding yeast. In this presentation we describe our single-molecule approaches which include instrumentation, image acquisition and processing algorithms.

2P299 Fast positively photoswitchable fluorescent protein for superresolution nanoscopy

Dhermendra K Tiwari, Yoshiyuki Arai, Takeharu Nagai (*Osaka University*)

Reversibly photoswitchable fluorescent proteins (RSFPs) have been used for superresolution nanoscopy such as PALM. Among the available RSFPs, most of them categorized in negatively switchable RSFP (NS-RSFP) share the light for switching-off with that for fluorescence excitation. The switching-off property during fluorescence imaging hampers fast image acquisition with higher signal-to-noise ratio. Although positively switching RSFP (PS-RSFP) can be switching-on by a light for fluorescence excitation, the slow switching speed have restricted their application to nanoscopy. To overcome this, we developed the fastest PS-RSFP which shows much faster photoswitching properties than those of conventional PS-RSFP.

2P300 Monitoring cytosolic Mg2+ with a novel genetically encoded fluorescent indicator using a non-FRET-based ratiometric imaging approach

Vadim Perez Koldenkova, Tomoki Matsuda, Dharmendra Tiwari, Shoji Kawakami, Takeharu Nagai (*The Institute of Scientific and Industrial Research, Osaka University*)

Although lots of genetically encoded Ca2+ indicators including cameleons, G-CaMPs and GECOs are available today, an indicator for Mg2+, the major cytosolic divalent cation required for many physiological events, is not available at all. Here we describe a functionalized variant of the fluorescent protein Venus, which fluorescence intensity increases upon Mg2+ binding. The dissociation constant for Mg2+ is 5.1 mM allowing Mg2+ concentration to be measured in the sub-millimolar to millimolar range. Fusing the Mg2+-sensitive Venus variant with the Mg2+-insensitive fluorescent protein mCherry, used as a reference, we obtained a ratiometric Mg2+ indicator. With this novel indicator, we succeeded visualization of Mg2+ dynamics in living cells under different conditions.

2P301 GEM-GECO を用いた細胞内カルシウムのイメージング定量解析
Quantification of calcium concentration in cells by imaging analysis using GEM-GECO

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Ca^{2+} ions have important roles as second messenger by changing their concentration via ER or plasma membrane. 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 used GEM-GECO to image [Ca^{2+}] changes using fluorescence microscopy. It has two emission wavelengths which show inverse proportional changes depending on [Ca^{2+}]. Time-change of [Ca^{2+}] caused by stimulant was imaged and analyzed. Furthermore, GEM-GECO was mutated into several variants of different affinities for Ca^{2+}. We used these mutants with localizing tags to observe [Ca^{2+}] changes in organelles such as ER and mitochondria where [Ca^{2+}] is much higher than cytosol.

2P302 FRET による elongin B と elongin C の相互作用解析
FRET - based analysis of interactions between elongin B and elongin C

Hirofumi Oyama[1,2], Yuma Ito[1,2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

Elongin B and elongin C are found together with elongin A in the elongin (SIII) complex that increases the efficiency of elongation by RNA polymerase II via suppressing transient transcriptional pausing. Elongin B and elongin C bind stably to each other in the complex. The interactions of two proteins were investigated using FRET-FLIM. However, the mechanism of supression of pausing by the elongin complex is not known in details. We focused on conformational change of elongin C induced by binding of elongin B. We measured intramolecular FRET using a protein of elongin C linked to fluorescent proteins at N- and C-terminus, respectively. We will discuss suppression of pausing of Pol II by using this probe.

2P303 FRAP と 1 分子蛍光イメージングを用いた転写活性化時 Arp4β 動態の定量解析
Quantitative analysis of molecular dynamics of Arp4β upon transcriptional activation by single-molecule fluorescence imaging and FRAP

Naomichi Inaba[1,2], Yuma Ito[1,2], Masahiko Harata[3], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*, [3]*Grad. Sch. Agr. Sci., Tohoku Univ.*)

Actin-related protein 4β (Arp4β) is a component of chromatin remodeling complex. It is thought to control transcription through binding to histone directly. However, the detailed mechanism remains elusive. Aiming to clarify the dynamics of Arp4β in transcriptional control, we performed quantitative imaging analysis of Arp4β in the nucleus. Both single molecule imaging and FRAP analysis revealed dynamic movements of Arp4β in the nucleus. After stimulation with PMA, the residence time of Arp4β became shorter. These results suggest dynamic interaction of Arp4β with either chromatin remodeling complex or chromatin. We will further discuss the dynamics of Arp4β.

2P304 炎症反応抑制タンパク質 PDLIM2 の局在制御機構の解明
The elucidation of the mechanism of PDLIM2 localization regulation

Satoshi Toriyama[1,2], Yuma Ito[1,2], Makio Tokunaga[1,2], Kumiko Sakata-Sogawa[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, [2]*IMS-RCAI, RIKEN*)

NF-κB plays an important role in the immune responses through activation of inflammatory gene transcription. As excessive inflammatory responses cause massive damage to host cells, activation of NF-κB should be tightly regulated. Recently PDLIM2 was found to target NF-κB and terminates NF-κB activation in the nucleus. We observed translocation of GFP fusion proteins of PDLIM2 from the cytoplasm to the nucleus upon stimulation. In this study, aiming to elucidate localization regulation mechanism of PDLIM2, we analyzed the localization of phosphorylation mutants of PDLIM2. We found that 72Ser phosphorylation may have an important role of PDLIM2 localization regulation.

2P305 3 色同時 1 分子イメージングによる T 細胞マイクロクラスターとシグナル膜タンパク質の相互作用解析
Single molecule analysis of signaling membrane proteins in T cell microcluster by multicolor live cell imaging

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T cell receptor (TCR) microcluster is important to initiate and sustain T cell activation. Although various signaling molecules were found enriched in microcluster, the mechanism of microcluster formation remains unclear. To visualize the dynamics of membrane proteins related to microcluster formation, we performed single molecule imaging of TCR and phosphatase CD45 (excluded from microcluster) with GFP labeled microcluster simultaneously. On the activated T cell surface, TCR molecules showed slower diffusion and longer residence time in microclusters than CD45. It suggests the dynamic interaction of these molecules with microcluster. Based on the quantitative imaging analysis, we will discuss the mechanisms of microcluster formation in T cell activation.

2P306 カルシウムイオン刺激による微小管伸長の動態解析
Imaging analysis of effect of Ca^{2+} ion on micritubule polymerization

Zhihai Zheng[1], Akihiro Fukagawa[1], Yuma Ito[1,2], Kumiko Sakata-Sogawa[1,2], Makio Tokunaga[1,2] ([1]*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech*, [2]*IMS-RACI, RIKEN*)

EB1 (end-binding protein1) is one of the microtubule binding proteins and it dynamically tracks microtubule plus ends. EB1 is also known to associate with an ER resident Ca^{2+} sensor protein, STIM1 (stromal interaction molecule 1). In spite of the important role in microtubule dynamics, the molecular dynamics of EB1 especially in relation with STIM1 remains unclear. In this study we imaged movements of EB1 in HeLa cells and measured the polymerization speed of microtubules. We will discuss the effect of Ca^{2+} ion in microtubule dynamics.

2P307 T 細胞活性化における微小管形成中心の動態
Microtubules Organizing Center (MTOC) Dynamics and Migration upon T Cell Activation

Wei Ming Lim[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-RCAI, RIKEN*)

Cellular polarity and division is related to microtubule (MT) dynamics. As MT performs major roles in cellular activities notably cell division and morphological structuring, a comprehensive insight of MT and MTOC mechanism is crucial. The phenomena of MTOC migration during T cell activation has been well established, suggesting MT interaction with polymerized actin as the main influence. However, recent studies showed kinase protein exhibits a crucial role in manipulating MT dynamics and critical potential initiating MTOC migration. Our data on instability of MTOC structure enables further deduction of ER transfer along MTOC migration. We will further discuss the dynamics of MTOC using quantitative molecular imaging analysis.

2P308 セミインタクト細胞リシール技術による細胞内への分子導入と病態モデル細胞構築への応用

Cell resealing technique for introducing molecules into cells and its application for establishment of disease model cells

Yoshiyuki Noguchi[1], Yuta Horiuchi[1], Daiki Nakatsu[1], Fumi Kano[1,2], Masayuki Murata[1] ([1]*Grad. Sch. of Arts and Sci., The Univ. of Tokyo*, [2]*PRESTO, JST*)

We demonstrate the novel method for introducing various molecules into cells by the cell resealing technique. First, the cells were permeabilized with streptococcal pore-forming toxin, streptolysin O (SLO). The permeabilization of plasma membrane allowed various molecules such as proteins, siRNAs, plasmids, and membrane-impermeable chemical compounds to enter into cells by diffusion. Then, the lesion of plasma membrane was repaired by addition of Ca2+, making the permeabilized cells become intact again. We call these cells “resealed cells”. In this study, we show the several examples of introducing proteins in resealed cells and the establishment of disease model cells by exchanging the normal cytosol to the pathogenic one as its application.

2P309 アポフェリチン空洞内に合成した Y 化合物を母体とした Eu および Tb ナノ粒子の発光特性

Photoluminescence Property of Eu and Tb Doped Y Based Nano-Phosphor synthesized in an apoferritin cavity

Tomoaki Harada, Hideyuki Yoshimura (*Meiji Univ.*)

Eu and Tb doped Y (Y:Eu and Y:Tb) nanoparticles are synthesized in an apoferritin cavity of 7 nm diameter. Electron diffraction pattern of Y nanoparticles corresponds to the Y_2O_3 crystal spacing. Y:Eu nanoparticles exhibit red photoluminescence (emission peaks: 590 and 614 nm), while Y:Tb nanoparticles exhibit green photoluminescence (emission peaks: 488, 544, 582 and 618 nm). The main excitation peak of Y:Eu nanoparticles is detected as 252 nm, which is assigned to be Eu-O charge transfer transition. The intense excitation peak of Y:Tb nanoparticles is observed at 237 nm, which is attributed $4f^8$-$4f^75d^1$ transition of Tb^{3+} ion. The most optimal dopant content for luminescence of Y:Eu and Y:Tb nanoparticles in apoferritin cavity are about 60% and 40%, respectively.

2P310 オズモシス流による FET ナノポア付近の DNA の動き制御

Controlling the fluidic motion of DNA molecules near FET nanopores by electro-osmotic flows

Manabu Sugimoto, Yuta Kato, Kentaro Ishida, Toshiyuki Mitsui (*Grad. Sch. Sci., Aoyama. Univ.*)

Recent trends of nanopore related researches are the translocation studies of DNA through nanopores fabricated on conductive membranes called FET nanopores as the membranes for gate electrodes. The gate potentials can be varied relative to the biased potentials applied to trans/cis fluidic solutions. As a result, the polarity and the concentrations of ions near nanopore can be controlled and this induces electro-osmotic flows through the nanopores. We will present the observation of the fluorescent tagged DNA’s motions in the flows near nanopores. We have carefully estimated the flow velocities of ionic solutions. Finally, we will discuss the possibility of removing heavily clogged DNA’s in pore holes using the electro-osmotic flows.

2P311 ナノ・マイクロファイバーゲルマトリックスの弾性設計による三次元細胞運動制御

Mechanical control of 3-D cell movement in elasticity-tunable matrix of nano/micro-fiber gels

Aya Ogata[1], Satoru Kidoaki[2] ([1]*Grad. Sch. Eng., Univ. Kyushu*, [2]*IMCE, Univ. Kyushu*)

The cell movements are critically affected by the stiffness of extracellular surroundings. To scrutinize the mechanobiology of the cell motility, systematic design of the extracellular mechanical surroundings is quite essential. In this study, we developped elasticity-tunable matrix of nano/micro-fiber gels which consists of nonwoven mesh of swollen fiberous gels, in order to clarify the effect of matrix stiffness on the 3-D cell movement. By regulating the fabrication conditions, elastic modulus of fiber gel sheet was well-tuned. Assessment of movement of 3T3 fibroblasts on the fiber-gel sheets with different stiffness showed that cells tend to get into only harder sheet. This result suggested that 3-D cell mechanotaxis can be driven depending on matrix-stiffness.

2P312 *In vitro* selection of peptide aptamer binding to reduced ferredoxin

Yasodha Manandhar[1,2], Takanori Uzawa[1], Toshiro Aigaki[2], Yoshihiro Ito[1,2] ([1]*RIKEN*, [2]*Tokyo Metropolitan University*)

Photosynthesis is the only known biological process that can convert solar energy into organic substances. The photo-induced electrons produced by the photosystems are transferred to a small redox protein named ferredoxin. If we could use the reduced ferredoxin for our purposes including the productions of solar cell and an artificial substance, we would open up new possibility to harness solar energy. Thus motivates we used ribosome display technique to select the peptide aptamers that binds to the reduced ferredoxin. After the eight rounds of ribosome-display selection, we obtained eleven candidate peptides. We will discuss about those binding assays to the reduced ferredoxin.

2P313 Selection of RNA aptamer binding to a photoredox catalyst

Thi Thanh Thoa Tran[1,2], Toshiro Aigaki[2], Takanori Uzawa[1], Yoshihiro Ito[1,2] ([1]*RIKEN*, [2]*Tokyo Metropolitan University*)

Aiming for the application of $Ru(bpy)_3^{2+}$ (Ru) as a redox enzyme, we selected its RNA aptamer. We selected 8 RNA sequences after 12 rounds of SELEX. Truncation experiments of one of the RNA sequence identified a minimum functional sequence that possesses high binding affinity, 183nM of Kd. The phosphorescence intensity of Ru increases 3.3 folds upon binding of the RNA; in the same line the phosphorescence lifetime is elongated 4.2 folds. We found that the Ru-bound RNA exhibits a cooperative melting curve, whereas the free RNA does not. Those observations suggest that RNA tightly interlocks the Ru inside and thus the phosphorescence intensity increases due to reduction of the quenching efficiency by dioxygen and energy dissipation to water molecules.

2P314 デザインされた DNA 高次構造体の環境安定性評価
Stability of designed high-order DNA structures under unconventional conditions

Masahiro Endo[1], Kei Fujiwara[2], Satoshi Murata[1], Shin-ichiro Nomura[1]
([1]*Grad. Sch. Eng., Tohoku Univ.*, [2]*JSPS. Research Fellow. Tohoku Univ.*)

Recently, rapid progress of DNA nanotechnology enables us to produce various types of molecular devices, such as nanostructures, logic gates and memories made of DNA. Especially, regarding to the two-dimensional structures, any shapes we desire can be constructed. Most of the reported artificial DNA structures, however, have been observed in buffer solution at room temperature and 1 atm. Assessing and extending the stability of DNA structures are essentially important to the future applications, for instance, an artificial "panspermia" in extremely vacuum and dry condition. Here we report our result of the stability of designed DNA structures under unconventional extreme conditions.

2P315 Self-assembly and reconfiguration of multiple-sized closed structures made of DNA origami units

Keitel Cervantes[1], Shogo Hamada[2], Shin-ichiro Nomura[1], Satoshi Murata[1]
([1]*Tohoku university*, [2]*Cornell university*)

Nanoscale units able to dynamically change their shape are one route to realize environment-responsive materials. However, the self-assembly of dynamic units has only been proposed at the computational level. Here, we show a dynamic unit that self-assemble simultaneously into closed structures of multiple sizes. This unit made of DNA origami has bilateral symmetry in which a nick and two tunable ssDNA connect both parts, giving flexibility to the unit. The bond specificity of the unit is coded using pi-pi stacking of DNA blunt-ends as in the seminal work of Woo et al. (Nature Chemistry, 2011). The assembly and reconfiguration of the closed structures is examined in response to the environment, in this case by addition of salt concentration in the buffer.

2P316 回転磁場による磁性粒子接着リポソームのクロール運動の観察
Crawl movement observation of a liposome attached micro-superparamagnetic particles under a rotational magnetic field

Daiki Komatsu, Kei Fujiwara, Shin-ichiro M. Nomura (*Tohoku University*)

We aimed to construct a "micrometer-sized supramolecular crawler" which moves while interfacing with tissue surfaces in biological environments. Since this structure actively makes contact with external environments, it will be possible to salvage important molecules from contact surfaces. To prepare this crawler, streptavidin superparamagnetic particles (about 1 μm in diameter) were attached to liposomes (10 - 60 μm in diameter). Crawling of the liposome was observed under rotating magnetic field generated by a ball-type neodymium magnet. We will also discuss about the crawling on various surfaces (glass, polymer, and living cells).

3P001 Investigation for co-translational folding using X-ray crystallography

Yuya Hanazono, Kazuki Takeda, Kunio Miki (*Grad. Sch. Sci., Kyoto Univ.*)

Nascent polypeptide chains are synthesized on the ribosomes. The newly synthesized polypeptide chains *in vivo* fold co-translationally, and each intermediate takes consistently most stable conformation. However, both experimental and theoretical investigations of folding have been mostly performed for full-length peptides.
We revealed a series of the hPin1 WW domain N-terminal fragment of increasing amino acid length. Imitating the ribosome, the maltose-binding protein was fused just behind the WW domain N-terminal fragment. From X-ray crystallographic study, though the full-length fragment is composed of two beta-hairpins between the three-stranded beta-sheet, the intermediate-length fragments are mainly composed of the alpha helix.

3P002 二核フェロキシダーゼ中心をもつピロリ菌好中球活性化タンパク質の構造
Structure of *Helicobacter pylori* neutrophil-activating protein with a di-nuclear ferroxidase center

Hideshi Yokoyama, Osamu Tsuruta, Naoya Akao, Satoshi Fujii (*Sch. of Pharm. Sci., Univ. of Shizuoka*)

H. pylori neutrophil-activating protein (HP-NAP) is a Dps-like iron storage protein forming a dodecameric shell, and promotes adhesion of neutrophils to endothelial cells. The crystal structure of HP-NAP in a Zn^{2+} or Cd^{2+}-bound form reveals the binding of two zinc or two cadmium ions and their bridged water molecule at the ferroxidase center (FOC). The two zinc ions are coordinated in a tetrahedral manner to the conserved residues among HP-NAP and Dps proteins. The two cadmium ions are coordinated in a trigonal-bipyramidal and distorted octahedral manner. In both structures, the second ion is more weakly coordinated than the first. Another zinc ion is found inside of the negatively-charged three-fold-related pore, which is suitable for metal ions to pass through.

3P003 T4 ファージ gp34C 末端側半分の結晶構造から得られたファージ尾繊維に共通の構造
The crystal structure of C-terminal half of gp34 from phage T4 reveals common architecture of phage tail fibers

Shuji Kanamaru, Mikiyoshi Namura, Fumio Arisaka (*Grad. Sch. of Biosci. & Biotech., Tokyo Institute of Technology*)

At the beginning of phage T4 infection, gp37 trimer, tip of long tail fiber (LTF) recognizes an *E. coli* outer surface. The signal of the recognition transmitted to phage baseplate through gp34 trimer which is proximal half of LTF. Here we report the crystal structure of the C-terminal 500 residues of gp34 (gp34C). Overall structure is trimeric fibrous rod-shaped structure with 300 Å; long and 50 Å; wide (narrower shaft 20 Å;). There are two major structural motifs which were reported in short tail fiber (gp12) structure, 3-stranded β-helix and α - β - long loop - β, are found in the gp34C. We are also able to fit the gp34C structure into the density of LTF of EM 3D reconstruction data of T4 phage particle.

3P004 病原性大腸菌 O-157 のタイプ 6 分泌系の VgrG1 蛋白質の C 末端断片の X 線結晶構造
Crystal structure of the C-terminal domain of VgrG1 protein of *E.coli* O-157 Type 6 secretion system

Kazuya Uchida[1], Shuji Kanamaru[1], Petr Leiman[2], Fumio Arisaka[1] ([1]*Grad. Sch. of Biosci. & Bioeng., Tokyo Tech.*, [2]*EPFL*)

Bacterial Type 6 Secretion System (T6SS) is a molecular machine which translocates toxins or effector molecules to target cells. The gene cluster of T6SS contains several proteins which are homologous to the proteins forming the tail of contractile bacteriophages. VgrG protein is one of these proteins and it is a homolog of gp27-gp5 cell puncturing complex of phage T4.
To elucidate the structural relationship between T6SS and the bacteriophage, we focused on the VgrG1 of *E.coli* O-157 and determined the structure of its C-terminal 73 residues by X-ray crystallography. It forms a triangular prism-shaped trimer which mainly consists of antiparallel β-sheets. Although the polypeptide topology is different, overall structure is similar to the β-helix found in gp5 of phage T4.

3P005 仮性結核菌由来ヘム獲得蛋白質 HasA の結晶構造解析による新規ヘム結合様式の解明
Crystal structure of a hemophore hasA secreted by Yersinia pseudotuberclosis shows a novel heme binding mode

Masahiro Kanadani[1], Toshiki Muroki[2], Yukie Ishimaru[2], Saki Wada[1], Takehiro Sato[3], Shin-ichi Ozaki[3], Tomoya Hino[1], Shingo Nagano[1] ([1]*Grad. Sch. Eng., Univ. Tottori*, [2]*Fac. Eng., Univ. Tottori*, [3]*Fac. Agric., Univ. Yamaguchi*)

In the bacterial heme acquisition system (Has), HasA picks up a heme and then shuttles it to a membrane receptor, HasR. In this study, we solved the crystal structure of HasA from Yersinia pseudotuberuculosis (YpsHasA). Unlike other extensively studied HasAs, the heme iron of YpsHasA lacks His ligand and is coordinated by Tyr75. Around the 6th ligation site, two Arg side chains make hydrogen bonds with heme propionate, which likely compensate the missing axial His ligand. Around Tyr75 there are a number of hydrophobic interactions with heme. One of the Arg residue that makes hydrogen bond with heme is conserved for HasA having 5-coordinated heme and most of hydrophobic residues around Tyr75 are highly conserved for both HasA having 5- and 6-coordinated heme.

3P006 HLA-G2/G6 アイソフォームの単粒子構造解析
Three dimensional reconstruction of HLA-G2/G6 isoform

Kazuhiro Mio[1], Kimiko Kuroki[2], Haruki Matsubara[2], Yoshiyuki Kasai[2], Chikara Sato[1], Katsumi Maenaka[2] ([1]*National Institute of Advanced Industrial Science and Technology, Biomedical Research Institute*, [2]*Laboratory of Biomolecular Science, Hokkaido University*)

The extracellular domain of HLA-G6 (identical to HLA-G2) protein was expressed in E.coli, which was confirmed as a homodimer. We applied electron microscopy to the negatively stained HLA-G molecules. Particle images were picked up from the recorded films, digitized, and reconstructed to generate its 3D structure by the single particle analysis. The final structure has an pai-shaped (one plate with two legs) with slightly twisted. The structure from the EM analysis fit well with the predicted atomic coordinate, which is obtained from a crystal structure of the closely related molecule. Current data will provide a structural basis for understanding the molecular mechanisms of beta2m-free form of HLA-G dimer.

3P007 大気圧電子顕微鏡（ASEM）によるタンパク質微結晶と細胞内複合体の液中観察

Direct electron microscopy of protein crystals and Mycoplasma cells in solution using the Atmospheric SEM

Tatsuhiko Ebihara[1], Masaaki Kawata[1], Hidetoshi Nishiyama[2], Miki Senda[3], Mari Sato[1], Mitsuo Suga[2], Toshiya Senda[3], **Chikara Sato**[1] ([1]*AIST*, [2]*JEOL*, [3]*KEK*)

The new Atmospheric Scanning Electron Microscope (ASEM) observes samples in solution under an open atmosphere. It is, at the same time, a Correlative Light-Electron Microscope (CLEM), in which the optical microscope (OM) and SEM quasi-simultaneously observes samples. In the system, an inverted SEM observes the wet sample from beneath an open dish while an OM observes it from above. The film of ASEM dish can be coated variously like glass, which allows various cells to be cultured including neurons.
Immuno-ASEM successfully visualized molecular complexes in neuron primary culture and STIM1 in T cells in solution [1]. The ASEM not only visualized cells, but protein micro-crystals [2].
[1] J Struct Biol 180, (2012) 259-270. [2] Int. J. Mol. Sci. 13, (2012) 10553-10567.

3P008 EM Navigator と Yorodumi による 3 次元電子顕微鏡構造データの利用

Using 3D electron microscopy data by EM Navigator and Yorodumi

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More than 2300 structure data analyzed by 3D electron microscopy (3DEM) are deposited in EM Data Bank (EMDB) and Protein Data Bank (PDB). Compared to representative structure data, it is more difficult to utilize the 3DEM data. The structures are large and complex, and EMDB data are in 3D map format, not atomic models. To make it easy even for 3DEM non-specialist, we have been developing and managing two web sites. EM Navigator (http://pdbj.org/emnavi/) is a 3DEM data explorer for EMDB and 3DEM data in PDB. Yorodumi (http://pdbj.org/yorodumi/) is a 3D structure viewer especially for complex data in both databanks. In the presentation, we will describe the recent progress of these services.

3P009 電子顕微鏡の傾斜ペアを利用した構造の異なるタンパク質単粒子画像の分類

Separating single particle images of protein in the different conformations using tilt pair transmission electron microscopy

Yutaka Ueno, Kazunori Kawasaki, Shouhei Mine (*AIST Health Research Institute*)

In single particle analysis of protein and biological macromolecules, tilt pair images have been utilized to build the first sturcutral models. With negatively stained specimens, particle images are sometimes stochastically distorted, although it could also happen in the native environment. We tried to segment single particle images in the different conformation using tilt pair observation. Based on a simple flattening model, a tilt angle correction was examined to yield correct volume reconstruction for the target molecule. The segmentation was considered as a typical graph-cut problem and images from different conformations were segmented. Our preliminary results in application for a structural study of glucosaminidase purified from archea will be discussed.

3P010 NMR タンパク質立体構造決定のための新規構造最適化法の開発

Development of a new refinement method for NMR protein structure determination

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NMR protein structure calculation adopts severe simplifications of the nonbonded interactions to rapidly obtain global structures. It is thus necessary to perform the subsequent refinement of the structures with a physical forcefield. While various refinement approaches are proposed using MD simulations, there are a few examples to optimize the NOESY assignment and calibration of the distances from NOE peak intensities based on the refined conformations. Recently, we implemented into the program CYANA a new structure refinement method that recursively improves the structures and NOE assignments, and addresses the calibration based on Bayesian inference. Here, we show the results applying it to several test data and its feasibility for the protein structure refinement.

3P011 Structural analysis of antimicrobial peptide CP1 with LPS by NMR

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Cecropin P1 (CP1) is 31 amino acids peptide and exhibits antimicrobial activity against gram-negative bacteria. In this study, we investigated the interaction with LPS and $CP1_{1-29}$ because AMPs that target Gram-negative bacteria bind to the negatively charged LPS. We have applied tr-NOESY to determine the high-resolution structure of $CP1_{1-29}$ in LPS. NOE peaks were observed at the C-terminal region in LPS, but, were not detected in aqueous solution. CD measurement was also carried out using CP1 and analogues of CP1 ($CP1_{1-29}$ and $CP1_{1-20}$, the lacks of C-terminal amino acids). In LPS, α-helical content of $CP1_{1-20}$ is lower than CP1 WT or $CP1_{1-29}$'s one. We suggest that $CP1_{1-29}$ accepts α-helical structure with LPS at C-terminal region.

3P012 高圧力下で見られるべん毛繊維の動的多型性

Dynamic polymorphism of bacterial flagellar filaments at high pressure

Masayoshi Nishiyama[1], Yoshiyuki Sowa[2] ([1]*Kyoto University*, [2]*Hosei University*)

Escherichia coli cells smoothly swim in solution by rotating their flagellar filaments, each of which is composed of a single protein, flagellin. In response to mechanical torque and solvent conditions, the supercoiled structure is dynamically changed from a left-handed form (normal) to the others including right-handed forms, and vice versa. Here, we show that application of pressure can change the supercoiled structure of flagellar filaments from normal to coiled or curly I form. The application of pressure is thought to enhance the structural fluctuation and/or association of water molecules with the exposed regions of flagellin molecules, and results in switching the helical shape from normal to coiled or curly I form.

3P013 金属結合に伴う 3 ヘリックスバンドル形成の動的構造解析
Dynamic structural analysis of three-helix bundle formation induced by metal-ion binding

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We designed a 72-residue protein based on a *de novo* designed three-helix bundle protein, α_3D, reported previously. In order to evaluate the conformational change from random to three-helix bundle structure only in the presence of metal-ion, three residues in the hydrophobic core of α_3D were replaced to His, and additional several residues were replaced to Ala. We analyzed the metal-ion dependent helix formation of overexpressed and purified α_3D-His using circular dichroism spectrometry. We further analyzed the Laue diffraction spots from gold nanocrystal attached to the introduced Met of α_3D-His in the presence or absence of metal-ion using the single-molecule detection system called diffracted X-ray tracking. The dynamic property of α_3D-His was evaluated.

3P014 Membrane-Induced Conformations of Proteins Characterized by Vacuum-Ultraviolet Circular-Dichroism and Flow Linear-Dichroism

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Vacuum-ultraviolet (VUV) circular-dichroism (CD) and flow linear-dichroism (FLD) spectra of α-lactalbumin, β-lactoglobulin, and thioredoxin were measured down to 170 nm in the presence (pH 4.5) and absence (pH 7.5) of phosphoglyceride liposome in order to clarify the conformations of these proteins interacting with membrane. The VUVCD analysis showed that the α-helix regions on the secondary-structure sequence largely increase due to liposome binding in all proteins. The FLD spectra indicated that the membrane-induced helix regions interact with the surface of liposome. These results suggest that a VUVCD coupled with a FLD spectroscopy can give new insight into the conformations of proteins interacting with membrane.

3P015 創薬等支援技術基盤プラットフォーム事業におけるタンパク質 X 線溶液散乱
Bio-SAXS in the Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS)

Nobutaka Shimizu[1], Shinya Saijyo[1], Hiromasa Ota[2], Yasuko Nagatani[1], Ai Kamijyo[1], Takeharu Mori[1], Takashi Kosuge[1], Noriyuki Igarashi[1] ([1]*Photon Factory, KEK*, [2]*Mitsubishi Electric SC*)

PDIS is a national platform project started from FY2012 to promote the structural life science. KEK is one of groups to support the structural analysis studied by protein crystallography and protein solution scattering, supplying the beamtime of Photon Factory. Biomolecule small-angle X-ray scattering (Bio-SAXS) is a powerful technique for analyzing not only the protein structure in the solution but the molecular arrangement of complexes by ab-initio method. The hybrid analysis using PX and SAXS would lead to the further understanding about a structure state of biomolecules. We now support measurement and analysis of beginners of Bio-SAXS and improve the core technologies in the Bio-SAXS experiment.

3P016 分子動力学法によるラミニン由来ペプチドの研究
Study of peptides derived from laminin by molecular dynamics simulations

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We are analyzing the EF1 (DYATLQLQEGRLHFMFDLG, mouse α1 chain 2762-2780 residues) and the EF2 (DFATVQLRNGFPYFSYDLG, mouse α2 chain residues 2808-2826) peptides derived from the laminin. Laminin, a glycoprotein which is a component of basement membrane, has diverse biological activities. In previous research (EF1 and EF2), relation of structure to biological activity was indicated. Thus, we study the differences of dynamical properties in EF1 and EF2. In our previous research, we showed that hairpin-structure is found in EF1 (but not in EF2) by simulated annealing. In this study, we investigate the behavior of EF1 and EF2 peptides at 300K and 1bar. We calculate RMSD and RMSF. Furthermore, we will analyze our results by using PCA.

3P017 分子動力学法を用いたラミニン α2 由来ペプチド A2G80 の構造決定因子の同定
Identification of structure determinant amino acid residues in the A2G80 peptide derived from laminin α2 by molecular dynamics simulation

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Laminins are glycoproteins composed of α,β, and γchains. Laminins have diverse biological functions, such as cell adhesion, migration and angiogenesis. Laminin α2 chain is expressed in muscle and nerve tissues, and the interaction between laminin α2 chain and α-dystroglycan (αDG) is essential for proper muscle function.
The A2G80 peptide derived from laminin α2 chain binds to αDG specifically. We performed molecular dynamics simulation of A2G80 to compare with crystal structure obtained by X-rays. Furthermore, to identify structure determinants, alanine-substituted peptides were also simulated, and compared with their circular dichroic spectra.

3P018 分子動力学計算による 4 量体型サルコシン酸化酵素の酵素—基質アナログ複合体の動的挙動解析
Behavior of enzyme-substrate analogue complex of heterotetrameric sarcosine oxidase studied by molecular dynamics simulation

Go Watanabe, Akinori Hiroshima, Haruo Suzuki, Shigetaka Yoneda (*School of Science, Kitasato University*)

Heterotetrameric sarcosine oxidase (SO) catalyzes oxidative demethylation of sarcosine to generate glycine and hydrogen peroxide. SO is composed of four non-identical subunits, and FAD, FMN, NAD^+, and Zn^{2+}. The structural and biochemical analyses have shown that the SO-dimethylglycine (DMG) complex contains a large cavity and suggested channeling of oxygen, sarcosine, and products through the cavity. Toward realistic simulations of channeling, we have carried out molecular dynamics simulations for the SO-DMG complex using the GROMACS program with the GROMOS force field. In the present study, we are performing more accurate simulations by using the AMBER force filed than the previous one. As a result, the structure simulated was in good agreement with X-ray data.

3P019 The role of the flexible loop in Staphylococcal nuclease on its catalytic activity

Rumi Shiba[1], Hironari Kamikubo[1], Yutaka Maruyama[2], Junko Yunoki[1], Keiichi Fukuyama[3], Yoichi Yamazaki[1], Mariko Yamaguchi[1], Mikio Kataoka[1] ([1]*Graduate School of Materials Science, Nara Institute of Science and Technology*, [2]*Insitute for Protein Reserch, Osaka University*, [3]*Department of Biological Science, Graduate school of Science, Osaka University*)

Deleting an internal loop of SNase substantially reduces its hydrolytic activity. In order to clarify the role of the loop, we performed crystal structure and 3D-RISM analysis on the Δ44-49 and WT. While their static structures are almost identical, but dynamical properties especially on loops were found to be different from each other. The crystal structures also showed that a water molecule involved in the catalytic reaction locates at the same position between WT and the mutant. The 3D-RISM analysis, however, revealed that the coordination of the water to the ligand is altered upon the deletion. From these results, the loops influence the dynamical properties, and in addition, the local reaction coordinate, which might be indispensable for catalytic reactions.

3P020 触媒アスパラギン酸の電荷改変による HIV-1 プロテアーゼの分子動力学シミュレーションへの影響
Molecular dynamics simulations of HIV-1 protease-inhibitor complex with modified charges for catalytic aspartate

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HIV-1 protease (PR) exists as a homo-dimer. Either of two catalytic aspartates (D25/D25') seems to be protonated in PR with its inhibitors (PIs). Therefore, for molecular dynamics (MD) simulations of PR-PI, distinct protonation states and charges are generally set between D25/D25'. However, since PR has structure with C2-symmetry, we hypothesized that symmetrical charges for D25/D25' can improve stability of structure of PR-PI like those in the crystal structures, during their MD simulations. Indeed, the hydrogen bond network around D25/D25' as seen in the crystal structures were stabilized during MD simulations with AMBER9 and with the modified charges, compared with the simulations by the default charges. The modification would be useful to develop novel PIs.

3P021 超音波によるアミロイドβオリゴマー破壊の非平衡分子動力学シミュレーション
Non-equilibrium molecular dynamics simulation for disruption of an amyloid-β oligomer by hypersonic wave

Hisashi Okumura[1,2], Satoru Itoh[1,2] ([1]*Inst Mol Sci*, [2]*SOKENDAI*)

We performed non-equilibrium MD simulations of an amyloid-β oligomer in explicit water to study cavitation around the oligomer and the oligomer destruction by the bubbles. The simulation was started from the experimentally-known amyloid oligomer structure in the amyloid fibril. To express supersonic wave, sinusoidal pressure was applied between -100 MPa and 300 MPa. When the pressure was decreased to a negative value of -100 MPa from a room pressure, a bubble formation was observed around the C-terminal region, the amino acid residues of which were hydrophobic. Even after the bubble size increased, the secondary structures of the oligomer were maintained. When the pressure was increased to a positive value, the bubble shrank, and the oligomer was disrupted.

3P022 Structure and Interactions in Fibrillation of Human Calcitonin Hormone

Javkhlantugs Namsrai, Ganchimeg Lkhamsuren, Kazuyoshi Ueda, Akira Naito (*Yokohama National University*)

The human calcitonin (hCT) is a thyroid polypeptide hormone that has a 32 amino acid residues. The hCT peptide aggregates to form insoluble fibril toward the diseases of osteoporosis. In the fibril formation of hCT, the local conformation changes from an alpha-helix in a monomeric hCT to a beta-sheet structure in a fibril. The beta sheet conformation was determined as mixed antiparallel structure using by REDOR-NMR experiments. The role of the amino acid residues is unclear, therefore, we simulated to determine the interaction energies between amino acid residues for stability of fibril using molecular dynamics simulation.

3P023 結晶環境における弾性ネットワークモデルを用いた高分解能X線構造における温度因子の再現
Thermal fluctuation in high-resolution crystal structures reproduced by normal modes based on an elastic-network model in the crystal

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 crystalline state. 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 illustrate the performance of the program with proteins, nucleic acids, and their complexes, and show that the atomic fluctuations calculated by NMA reproduce the temperature factor data of these molecules in high-resolution crystal structures in the PDB.

3P024 カブトガニ由来抗菌ペプチド Tachyplesin I とキチン結合能に関する研究
Analysis of chitin binding ability of an antimicrobial peptide tachyplesin I derived from horseshoe crab

Takahiro Kushibiki[1], Masakatsu Kamiya[1], Tomoyasu Aizawa[1], Yasuhiro Kumiki[2], Takashi Kikukawa[1], Makoto Demura[1], Shun-ichiro Kawabata[3], Keiichi Kawano[1] ([1]*Grad. Sch. Life Sci., Hokkaido Univ.*, [2]*Grad. Sch. of Sci., Hokkaido Univ.*, [3]*Dept. Biol., Kyusyu Univ.*)

Tachyplesin I (TP I), an antimicrobial peptide found in hemocytes of horseshoe crab, has a broad antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi. Although it has been already known that TP I can bind to chitin which is a component of cell wall of fungi, there are no structural information about the interaction between them. In order to clarify the key structural factor for chitin-binding of TP I, we investigated the binding of TP I for chitin. From the NMR studies, F4, R9, Y13 and R17 residues of TP I were suggested to be involved in binding to chitin. Then we performed chitin binding assay of TP I mutant whose F4, R9, Y13 and R17 were replaced with Ala. The results showed that these residues are essential for binding to chitin.

3P025 リジン 2,3-アミノミューターゼにおける高反応性ラジカル反応機構の解明

Taming the Reactive 5'-Deoxyadenosyl Radical by Enforcing van der Waals Contact with Substrate in Lysine 2,3-Aminomutase

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Lysine 2,3-aminomutase (LAM) utilizes a [4Fe-4S] cluster, *S*-adenosyl-*L*-methionine (SAM) and pyridoxal 5'-phosphate to isomerize L-*α*-Lys to L-*β*-Lys. LAM is a member of the radical-SAM enzyme superfamily in which a $[4Fe\text{-}4S]^{+}$ cluster reductively cleaves SAM to produce the 5'-deoxyadenosyl radical (5'-dAdo*), which abstracts an H-atom from Lys. 5'-dAdo* is so reactive that it has never been observed, thus this makes characterization of this step difficult. We utilize multinuclear ENDOR to characterize this radical mechanism in LAM by using SAM surrogate. We conclude that the active-site facilitates hydrogen atom transfer by enforcing van der Waals contact between radical and Lys. This constraint enables the enzyme to minimize and even eliminate side reactions.

3P026 糖結合モジュール Trp 導入変異体の基質結合能

Substrate binding ability of the Trp introduced mutant of carbohydrate-binding module

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The carbohydrate binding module attached to endo-1,3-β-glucanase from *Cellulosimicrobium cellulans* DK-1, CBM-DK, have putative imperfect tandem α-, β-, and γ-repeats. Among the three repeats, we recently showed that the α-repeat mainly contributes to the carbohydrate binding, in which Asp270 and Trp273 have the critical role [1]. While the residues corresponding to Asp 270 are conserved in both β- and γ-repeats, the residues corresponding to Trp273 are Asp314 and Gly358, respectively. In this study, we generated the Trp introduced mutants, D314W and G358W, and analyzed the interactions with laminarin and laminarioligosaccharides, using surface plasmon resonance biosensor and isothermal titration calorimetry.

[1] Tamashiro *et al*., Glycoconj. J. 29, 77-85, 2012.

3P027 炭酸脱水酵素のある変異体の His64 の２つの配向の間の化学交換は NMR 時間軸上十分遅い

Chemical Exchange between Two Conformations within His64 in a Mutant of Carbonic Anhydrase Is Sufficiently Slow on the NMR Timescale

Hideto Shimahara (*JAIST CNMT*)

Human carbonic anhydrase II (hCAII) has been extensively studied as a model system for investigating the finely tuned movement of protons in an efficient histidine-regulated hydrogen bond relay during enzymatic catalysis. Here, we report structural information of the histidine (His64), using site-directed mutagenesis and 2D $^{15}N/^{1}H$ NMR experiments for determining the tautomeric constant of histidine residues. We found that the NMR resonance of ^{15}N nucleus in the imidzole of His64 splits into two signals in a mutant enzyme. This shows that 1) His64 has two conformations and 2) the chemical exchange is sufficiently slow on the NMR timescale. This feature strongly supports that the proton transfer process does not require a change in orientation of His64 during catalysis.

3P028 *Rhodococcus rhodochrous* J1 由来ニトリラーゼの温度による構造変化の ^{1}H NMR による追跡。

Structural changes of the J1 nitrilase from *Rhodococcus rhodochrous* upon temperatuer increase tracked by ^{1}H NMR

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Nitrilase is an industrial enzyme that hydrolyzes nitrile compounds into ammonia and carboxylic acids. The nitrilase from *Rhodococcus rhodochrous* (J1-NTase) associates from an inactive dimer to an active oligomer upon heating. The C-terminus of J1-Ntase is known to be easily degraded by proteases and its loss induces a helical complex with higher activity.

We, therefore, examined structural change of J1-Ntase upon heating by ^{1}H NMR. Despite of its large molecular weight (80kDa in dimer), the methyl signal peak of J1-Ntase was clearly resolved at low temperatures and gradually decreased with temperature increase. We are going to interpret these sepctral changes in comparison with small-angle scattering data.

3P029 Analysis of unfolded structure of Staphylococcal nuclease mutants by using FRET

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Staphylococcal nuclease mutants, 33A34 and W140A, exhibit induced folding but their mechanisms have been shown to be different from each other. To understand the difference, we focused on the unfolded structures of these mutants under physiological conditions. We assume that the initial unfolded structure determines the induced-folding mechanism. The unfolded structures were characterized by the fluorescence resonance energy transfer (FRET) between a donor at D146 and an acceptor at K5. The FRET efficiency of W140A was higher than that of 33A34, suggesting that W140A contains more residual structures than 33A34. The results suggest that the induced folding depends on the local stiffness and flexibility.

3P030 高時間分解能で蛋白質の分子揺らぎと構造変化を計測するためのＸ線１分子動態計測法の開発

The Refinement of the Diffracted X-ray Tracking Method for Recording the Single-Molecule Motions of Proteins with Higher Time Resolution

Hirofumi Shimizu, Masayuki Iwamoto, Shigetoshi Oiki (*Univ.Fukui.Fac.Med.Sci.*)

In the Diffracted X-ray Tracking method, synchrotron white x-rays are irradiated to samples to track the motions of the Laue diffraction spots from a gold nanocrystal attached to a protein molecule as a probe. Random fluctuations and global conformational changes of a single-molecule protein are readily measured with the high spatial resolution. In the unfocused white x-ray beamline, BL28B2, in SPring8, we have newly introduced the 1 m of toroidal mirror designed for focusing the beam effectively and the x-ray spectrum measurement system. The set of equipment enabled us to optimize the spectrum for tracking the protein motions with minimal radiation damages of proteins, leading to the recordings in sub-millisecond time resolutions.

3P031 X 線 1 分子追跡法による蛋白質安定性の解析
Protein Stability Analysis of MHC/peptide Complex from X-ray Single Molecule Tracking

Yufuku Matsushita[1], Haruo Kozono[2], Naoki Ogawa[4,5], Kohei Ichiyanagi[1,5], Hiroshi Sekiguchi[3,5], Yuji Sasaki[1,3,5] ([1]*Grad. Sch. Sci., Univ. Tokyo*, [2]*Grad. Sch. Sci., Tokyo Univ. Sci.*, [3]*Spring-8*, [4]*Dept. Int. sci., Nippon Univ.*, [5]*CREST Sasaki team/JST*)

MHC (Major HistoCompatibility) is a dimer protein which is known as antigen peptide presentation in the immune system. However, perspectives on the antigen peptide presentation mechanism have not yet been unified. Therefore, we focused on the single molecule motion and chose six different peptides binding MHC/peptide complex for the comparison. We measured multi-molecule analysis to complement molecular dynamics data from single-molecule measurement.
We succeeded in confirming the relationship between single-molecule dynamics from Diffracted X-ray Tracking (DXT) and dimer stability from SDS-PAGE. DXT will be observed the in vivo single molecule stability without separation of dimer proteins.

3P032 表層ストレス応答を制御する膜内切断プロテアーゼ RseP のタンデム PDZ ドメインによる基質選別機構
Substrate discrimination mechanism by a PDZ tandem in the intramembrane protease RseP that regulates extracytoplasmic stress response

Yohei Hizukuri[1], Takashi Oda[2], Sanae Tabata[3], Tamura-Kawakami Keiko[3], Mamoru Sato[2], Junichi Takagi[3], Terukazu Nogi[2], Yoshinori Akiyama[1] ([1]*Inst. Virus Res., Kyoto Univ.*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*, [3]*Inst. Prot. Res., Osaka Univ.*)

During the σ^E pathway of the extracytoplasmic stress response in *Escherichia coli*, intramembrane protease RseP cleaves the anti-σ^E protein RseA only after membrane-anchored protease DegS truncates the periplasmic part of RseA. Here we analyzed the 3D structure of the two tandemly-arranged PDZ domains (PDZ tandem) of an RseP orthologue and revealed that the two putative ligand-binding grooves constitute a single pocket-like structure. Complete removal of the PDZ tandem from *E. coli* RseP led to the deregulated cleavage of RseA without prior truncation by DegS. From these and other results we propose that the PDZ tandem serves as a size-exclusion filter to discriminate between the intact and truncated forms of RseA.

3P033 Design of Photo-controllable Cyclic Peptides

Shinji Kawabata, Yasuhiro Ebisu, Yuta Saeki, Masahiko Hayashi, Atsuo Tamura (*Grad. Sch. Sci., Univ. Kobe*)

β-sheet-like Cyclic Peptide Nanotubes (CPNs) offer a variety of applications in chemistry, biology and micro electronics. CPNs are produced by stacking of cyclic peptides (CPs) containing alternated D-L-α amino acids. CPs can adopt a flat, closed-ring shape and stack through intermolecular hydrogen bonding oriented perpendicular to the plane of the ring structure. However, it is difficult to control self-assemblies of CPs without drastically changing pH or temperature. Photoregulation using chromophores, such as azobenzene, is a powerful approach to control a function and conformation of biomolecules. Herein we report design, synthesis, and characterization of novel azobenzen-CPs complex in which the β-sheet content can be reversibly photo-controlled.

3P034 脂質ータンパク質相互作用の解明を目指した重原子標識脂肪酸の利用
Toward an understanding of lipid-protein interactions, the use of the heavy atom labeled fatty acid analogues

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Fatty acid binding protein (FABP) is small cytosolic protein that is involved in fatty acid (FA) processing. FABP are also widely distributed in all tissues and exhibits high-affinity binding to a single long-chain FA. In order to understand the functions of lipids and the role of lipid-protein interactions, we performed the crystal structure analysis of FABP in complex with FA. However, the electron density of alkyl chain of the FA molecule was initially very poor. This result suggests that the FA may be highly flexible molecule or possess some different conformations. To verify this, we used the FA analogues with Se or Br atoms instead of C atoms of FA. We report the crystal structures of FABP in complex with FA analogues.

3P035 嗅覚受容体モデルとしてのオプシン立体構造
Opsin, Structural Model for Olfactory Receptors

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Among the large family of G protein-coupled receptors, about half of them are olfactory receptors (ORs), for which high-resolution structures remain elusive. Rhodopsin is the photoreceptor in vision and its ligand, retinal, shares high hydrophobicity with OR ligands. Here we report a new crystal structure of the opsin apoprotein. A molecule of octylglucoside was identified in the ligand-binding pocket and stabilizing the active receptor conformation. As prototypic mimics of odorants, several detergents were tested for their capability to enter opsin's ligand-binding pocket. The study provides an explanation on how hydrophobic odorants can enter OR, supports odorant-receptor hydrogen bonding for OR activation and establishes opsin as a new basis for OR homology modeling.

3P036 13-cis 型が優勢となる ASR 変異体の研究
Study of *Anabaena* Sensory Rhodopsin mutant P206D that contains the 13-cis form dominantly

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Anabaena Sensory Rhodopsin (ASR) is a microbial rhodopsin found in *Anabaena* (*Notstoc*) sp. PCC7120, a freshwater cyanobacterium. ASR regulates transcription by sensing light. We found that the photoreaction of ASR is completely photochromic; the stable photoproduct of the all-trans form is 100 % 13-cis, and that of the 13-cis form is 100 % all-trans [1].
In present study, we focus on an interesting mutant, ASR P206D. Pro206 is characteristic for ASR, because the corresponding amino acid is highly conserved Asp in microbial rhodopsins (Asp212 in BR). HPLC analysis shows that ASR P206D contains 13-cis retinal dominantly both in dark and light conditions. We will discuss its photoreaction and structual change.
[1] Kawanabe et al., *J. Am. Chem. Soc.* 129, 8644 (2007).

3P037 真菌由来エラスターゼインヒビター AFUEI と植物由来 potato I family インヒビターとの構造類似性

Structural similarity of AFUEI, an elastase inhibitor from *Aspergillus fumigatus*, and the potato I family inhibitors from plants

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AFUEI is an *Aspergillus fumigatus* elastase inhibitor that has a high inhibitory activity against elastases of *A. fumigatus*, *A. flavus* and human neutrophil elastase (HNE). Elastase from Aspergillus sp. is a major pathogenic factor for aspergillosis, therefore, AFUEI is one of the strong drug candidates of acute lung injury (ALI). We have determined the crystal structure of AFUEI and it shows remarkable similarity to serine protease inhibitors of the potato inhibitor I family, although their amino acid sequences are not similar. This similarity allowed us to build a complex model of AFUEI with HNE and suggests that AFUEI inhibits its cognate proteases through the same mechanism as the potato I family inhibitors.

3P038 酵母２６Ｓおよび２０Ｓプロテアソームの構造研究

Structural investigation of the yeast 26S and 20S proteasome

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The 26S Proteasome is a large multiprotein complex involved in a regulated degradation of ubiquitinated protein in the cell. It has not been the way itself how the 26S proteasome degrades ubiquitinated proteins. We have experimented that degradation process and/or particle forming by a small angle X-ray scattering. Scattering intensities were collected by use of a BioSAXS-1000 installed FR-E X-ray generator with a protein concentration of 1mg/mL. We have calculated a liberal structure of the 26S and 20S component without any constraints on shapes of components.
We have purified another 20S particle whose fusion site was exchanged, and compared with a single cap 26S or a 20S core particle. In the session, we will discuss our strongly and weakly bound 26S characters.

3P039 *Mycoplasma mobile* の滑走時に“あし”として働くシアル酸レセプターの構造解析

Structural study of neuraminic acid receptor working as foot in *Mycoplasma mobile* gliding

Tasuku Hamaguchi, Yuhei Tahara, Makoto Miyata (*Grad. Sch. of Sci., Osaka City Univ.*)

M. mobile glides on solid surface by a unique gliding mechanism. The "Leg" protein, Gli349 composed of 3183 amino acids has been suggested to have a neuraminic acid (Neu5Ac) receptor domain at its C-terminal part (foot). However, no similarity of amino acid sequence has been found to any other proteins. We predicted the foot structure based on the alignment of β-strands and found that the predicted structure is consistent with our current image from electron microscopy and has high similarity with toll-like receptor 4, involved in natural immune system. To elucidate the structure and functions of foot, we expressed and purified the foot composed of 465 amino acids using a synthesized gene and *E. coli* system.

3P040 藍色細菌時計タンパク質 KaiA-KaiC 相互作用の ESR 解析

Interactions between cyanobacterial clock proteins KaiA and KaiC revealed by ESR analysis

Kentaro Ishii[1], Toshiaki Arata[2], Masahiro Ishiura[1] ([1]*Center for gene research, Nagoya univ.*, [2]*Grad. Sch. Sci., Osaka Univ.*)

The cyanobacterial circadian clock can be reconstituted *in vitro* from three clock proteins, KaiA, KaiB, and KaiC, in the presence of ATP. Here, we investigated interactions between KaiA and KaiC by the site directed spin labeling-electron spin resonance method, which can provide information on the location and environment of an individual residue within a protein. We labeled each cystein residue of Cystein-substituted mutants of KaiC and KaiA derived from the thermophilic cyanobacterium *Thermosynechococcus elongatus* with spin labels, performed ESR analyses, and identified specific residues involved in KaiA-KaiC interactions.

3P041 黄色ブドウ球菌の Isd-NEAT ドメイン間におけるヘム輸送についての考察

Insights into the mechanism of heme-transfer between Isd NEAT domains of Staphylococcus aureus

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IsdH-N3, IsdA-N, and IsdC-N are near transporter (NEAT) domains of iron-regulated surface determinant (Isd) proteins of *Staphylococcus aureus*. These domains bind heme and transfer it from IsdH-N3 to IsdC-N via IsdA-N in an affinity-driven manner. However, as the structures of the NEAT domains are quite similar to each other, it was difficult to explain the physicochemical basis of the difference in affinity.
To solve this problem, we performed MD simulations for these domains. They revealed important residues that have large contribution to the affinity. Then, we made mutant proteins with substitutions of these residues and measured their affinities for heme with ITC. We found that the mutations reduced the affinity for heme, as predicted by the simulations.

3P042 Hsp90 と ADP の解離過程における自由エネルギープロフィールと解離経路

Free energy profile and dissociation pathway in the dissociation process of ADP from Hsp90

Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Intsitute of Science and Engineering, Kanazawa University*)

Hsp90 (Heat Shock Protein 90) 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. The processes of ATP binding and ADP dissociation of are not revealed at atomic resolution. In this study, we performed all-atom molecular dynamics (MD) simulations of Hsp90 with ADP to calculate the free energy profile for ADP dissociation and to reveal the dissociation pathway of ADP. Thermodynamic integration method was applied to calculate the free energy profile for ADP dissociation. We will report the results of the free energy calculation and the possible pathway of ADP dissociation.

3P043 Mechanism of glycan receptor recognition for influenza virus Hemagglutinins: Comparative molecular dynamis studies

Katsumi Omagari (*Department of Virology, Medical School, Nagoya City University*)

The heamagglutinins (HAs) 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.

3P044 Free energy landscape of substrate passing inside proteasome - activator complex

Hisashi Ishida (*Japan Atomic Energy Agency*)

Proteasome is involved in the degradation of the majority of cellular proteins. Proteasome activators bind to 20S proteasome core particle (CP) and facilitate opening a gate on the end of the CP. Then, the CP allows protein substrates to move into its interior through a channel between the gate and the α-annulus in order to hydrolyze the substrates to small fragments, and releases the products outside. To understand the gating mechanism of the CP, molecular dynamics simulations of the CP complexed with a PAN (analog of ATP-dependent 19S activator)-like activator, and of the CP complexed with an ATP-independent PA26 activator. The free-energies of the translocation of a poly-peptide substrate through the channel of these complexes were estimated.

3P045 MD シミュレーションを用いた CD44 のヒアルロン酸結合による構造変化に関する研究
Molecular dynamics simulation study on hyaluronan induced structural change of CD44

Saki Hongo[1], Yoshifumi Fukunishi[2], Masami Lintuluoto[1] ([1]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*, [2]*Natl. Instit. of Adv. Indust. Sci. and Technol.*)

CD44 contains hyaluronan binding domain (HABD) in the extracellular region. The 3D structures of the HABD in HA-bound and -free states were revealed by NMR and X-ray studies. The structures found by both studies in the HA-free state were similar, and two modes of the HA binding to the HABD were suggested. Moreover, the NMR structure in the HA-bound state revealed that the C-terminal segment of the HABD enhanced the flexibility compared to the HA-free state. Our molecular dynamics study showed that the NMR structure in the HA-free state is different from that of the X-ray on the HA binding surface and has lower free energy compared to its crystal structure. We will report the difference and the dynamics between the structures of the HA-bound and -free states.

3P046 代謝型グルタミン酸受容体の活性化過程の動的モデルの構築
Dynamical modeling of the activation process of metabotropic glutamate receptor

Kaita Fujihara, Tatsuki Negami, Tohru Terada, Kentaro Shimizu (*Dept. of Biotech., Grad Sch. of Agri. Life Sci., Univ. of Tokyo*)

The metabotropic glutamate receptor (mGluR) is one of the G-protein coupled receptors. It has a large, ligand-binding extracellular (EC) region. It exists as homo dimer where two mGluRs interact with each other at the EC region. Based on the comparison of the crystal structures of the ligand-free and the ligand-bound forms of the EC region, it is proposed that the relative orientation between the two EC regions changes upon ligand binding, which brings the two transmembrane (TM) regions close to each other and leads to the activation of the receptor. To construct a dynamical model of this activation process, we performed coarse-grained MD simulations with MARTINI. We will discuss how the conformational change in the EC regions causes the change in the TM regions.

3P047 Mutation studies on the mammalian and the baterial XORs with inhibitors

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Xanthine oxidoreductase (XOR), which is distributed widely in various species, is a target of drug for gout. Febuxostat is a commercially available drug that inhibits strongly the mammalian XORs but not the bacterial XOR although the substrate-binding pockets of mammalian and bacterial XOR are well-conserved. We have been experimentally and computationally studying the detailed mechanism of this difference. In this study, we further investigate this problem, comparing new experimental and computational results, including MD simulations with several mutations, binding free energy calculations via MM-PBSA(PBGA) method, and the interaction between XOR and different inhibitors.

3P048 Computational studies of mutational effects on nylon degrading enzyme

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Nylon-6 degrading enzyme (NylB) breaks the amide bond of the unnatural compound. On the basis of the several mutants, important residues at the catalytic site are unraveled as Ser112, Lys115, Tyr170, and Tyr 215. However, reaction mechanisms are still unknown. A major difference between NylB and other proteases is existence of Tyr170, which belongs to a loop region and binds to the substrate. In order to clarify the role of Tyr170, we inspected mutational effects (Tyr170 to Phe170) on the reaction mechanism of hydrolysis undergoing in this enzyme by using first-principles molecular dynamics with metadynamics approach for free energy analyses. We proposed detailed reaction mechanisms and identified two possible reasons for deactivation due to the mutation.

3P049 糖鎖の構造多形予測に向けた CHARMM 力場の改良
Revised CHARMM carbohydrate force field for improved description of conformational diversity of N-glycans

Suyong Re[1], Shigehisa Watabe[2], Wataru Nishima[1], Yuji Sugita[1] ([1]*Wako Inst., Riken*, [2]*Grad. Sch. Sci. Eng., Chuo Univ.*)

Glycans-protein interaction is essential for a variety of biological functions and diseases. However, the inherent flexibility of the glycan molecules makes conventional X-ray or NMR structural analysis quite difficult. Here, we employ molecular dynamics (MD) simulations to reveal the glycan-protein complex structures in atomic details. To this end, we revised the current CHARMM force field for carbohydrates by refitting the parameters so as to increase the accuracy in the potential for the α1-6 glycosidic bond. Replica-exchange MD simulations, with extensive sampling of N-glycan structures in solution, show that our revised force filed parameters better reproduce the experimental NMR data and more accurately represent glycan rotameric conformations.

3P050 Structural insights into enzyme-bound flavin adenine dinucleotides (FAD)

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The coenzyme FAD is an absolute requirement for certain enzymes to carry out metabolic reactions and to repair DNA damaged by UV radiation. Hence, understanding the conformations adopted by FAD bound to different enzymes is essential. Here, we sampled 927 enzyme-bound FAD conformations in the Protein data Bank (PDB). The results show that FAD exists in a variety of conformations from compact to “open” structures. FAD dihedral angle distributions indicate that structures are locally conserved to recognize different chemical groups within the coenzyme. The conclusions provided herein may prove valuable in the development of rotamer libraries and fragment-based approaches for docking/drug targets in the aforementioned FAD-bound enzymes.

3P051 金属結合によるヒトプリオンペプチドの配位モード
Coordination mode in human prion peptide caused by metal binding

Kazuya Iwama, Masahiro Yagi, Haruto Onda, Wakako Hiraoka (*Graduate School and Technology, Meiji University*)

In this report we investigated the conformational change in human prion protein (huPrP) which have flexible regions caused by metal binding, such as Cu^{2+}, $Ni^{2+}$and Co^{2+}.
Metal binding to peptide fragments containing His96 and/or His 111 was examined using visible absorption spectra and Circular Dichroism (CD). Secondary structure of peptide fragments was observed using Far-UV CD. Vis absorption and Vis CD indicated the characteristic spectra from metal binding, which were derived from d-d transition of metal ion. Far-UV CD spectra showed coordination modes depended on pH value, metal and chain length of huPrP.
We will also investigate metal binding mode of other huPrP peptide fragments to more understand mechanism of prion diseases.

3P052 二次元蛍光寿命相関分光法による BdpA 変性状態における構造ダイナミクスの解析
若手招待講演

Two-dimensional fluorescence lifetime correlation spectroscopy on the conformational dynamics of the unfolded state of BdpA

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*)

Characterization of the unfolded state of protein is highly required to understand the initial stage of protein folding. This state does not possess a well-defined structure but is described as an ensemble of conformers. However, the sensitivity, time resolution and quantitativity provided by conventional analytical methods are not sufficient to fully explore it. We have recently developed two-dimensional fluorescence lifetime correlation spectroscopy and applied it to examine the unfolded ensemble of B domain of protein A (BdpA). The results showed the presence of two distinct unfolded ensembles (extended and compact). It was also revealed that the compact unfolded ensemble is highly heterogeneous and the dynamics in the ensemble occur within 10 microseconds.

3P053 タンパク質中のトリプトファン残基の近紫外円二色性と紫外共鳴ラマンスペクトルの特性
Some basic properties of near-UV circular dichroism and UV resonance Raman spectra of tryptophan residues in proteins

Shigenori Nagatomo[1], Masako Nagai[2], Takashi Ogura[3], Teizo Kitagawa[3] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*Res. Center Micro-Nano Tech., Hosei Univ.*, [3]*Grad. Sch. Life. Sci., Univ. Hyogo*)

Near-UV circular dichroism (CD) and UV resonance Raman (UVRR) spectra of L-Trp residues and model compounds were investigated to utilize Trp signals of proteins as a structural marker. All the indole-C_3 derivatives except for L-Trp gave no CD bands (L_a and L_b), indicating that the asymmetric carbon (C_α) is essential to appearance of CD. The 229-nm excited UVRR spectra were almost the same between L-Trp and indole-C_3 derivatives, suggesting that Raman intensity comes from resonance with B_b but not with L_a or L_b. Both UVRR and CD spectra of L-Trp were influenced by protonation of amino- and/or carboxyl-groups, suggesting that these protonations are communicated to indole through σ-bonds containing C_α and influence both chirality and the electronic state.

3P054 テラヘルツ時間領域分光法によるトレハロースにコートされたタンパク質の低振動ダイナミクス
Low-frequency dynamics of Trehalose-coated Lysozyme studied by terahertz time-domain spectroscopy

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It is known that trehalose preserves proteins from damage such as denaturation due to dehydration or extreme change in temperature. This function of trehalose is related to inhibition of protein collective motions, which correspond to low-frequency motions in several wavenumber regions. In this study, we measured low-frequency dynamics of trehalose-coated lysozyme by terahertz time-domain spectroscopy to understand effect of coating by trehalose from 83 K to 293 K. Terahertz absorption coefficients increase linearly as the temperature increasing. In contrast to the case of hydrated lysozyme, “dynamical transition”, in which increasing rate of absorption coefficients become more intense, is never observed in this temperature region.

3P055 シクロデキストリン+タンパク質+メチルオレンジ・ヨウ素系における包接機構

Inclusion mechanism of cyclodextrin for protein in methyl orange and iodine aqueous solution

Tomokadu Marutani, Takayoshi Kimura, Tadashi Kamiyama (*Fac.Science,Kinki Univ.*)

Cyclodextrin (CD) can destabilize folded state of proteins by stabilizing the unfolded state by inclusion of hydrophobic part into the hydrophobic interior of CD. We have estimated the change in number of bound CD between folded and unfolded state by CD concentration dependence of midpoint temperature for thermal denaturation, although the number of bound CD at each state had not been determined yet. In this study, to determine the number of bound CD, the interaction between CD with proteins were relatively determined in methyl orange (MO) or iodine aqueous solution by ITC and spectrophotmetry. The binding constant of CD with MO decreased with increasing protein concentration indicating that CD can interact with protein competitively.

3P056 タンパク質の熱変性における部分比容、断熱圧縮率、熱膨張率

Partial specific volume, adiabatic compressibility, and thermal expansion coefficient of protein for thermal denaturation

Tetsurou Takaoka, Takuya Hamada, Takayoshi Kimura, Tadashi Kamiyama (*Fac. Science, Kinki Univ.*)

Partial specific volume of protein can reflect conformational packing and hydration of protein, and the volume fluctuation and entropy-volume cross fluctuation is thermodynamically related to the isothermal compressibility and thermal expansion coefficient, respectively. In this study, to determine the thermodynamic properties of protein at folded and unfolded state, density and sound velocity of aqueous protein solutions were measured at various temperatures. The adiabatic compressibility of solution had a minimum against temperature and shifted to lower temperature by adding solute. Thermal expansion coefficient of protein at unfolded state was larger than the folded state due to increase in the hydration and conformational freedom by the unfolding.

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 proposed a measure of the thermal stability of a protein: the water-entropy gain upon folding at 298 K normalized by the number of residues, Σ. It has been shown for homologous proteins that the order of the measure is in accord with that of the thermal denaturation temperature T_m. In this study, we apply this measure to the prediction of thermal-stability changes upon mutations. Let ΔX be "X of the mutant" minus "X of the wild-type". We plot ΔT_m against $\Delta\Sigma$ for significantly many proteins and find that the two quantities exhibit a fairly high correlation. The structure of the mutant is assumed to be unknown. When the structure and T_m of the wild-type are known, the quantitative prediction of the change in T_m upon a mutation is often successful.

3P058 蛋白質構造安定性における溶媒エントロピー効果―蛋白質-溶媒間多体相関の重要性―

Solvent-Entropy Effect in Structural Stability of a Protein: Crucial Importance of Protein-Solvent Many-Body Correlation

Hiraku Oshima[1], Shota Murakami[2], Masahiro Kinoshita[1] ([1]*Inst. Adv. Energ., Kyoto Univ.*, [2]*Grad. Sch. Energ. Sci., Kyoto Univ.*)

Using a hybrid method of the integral equation theory and the morphometric approach, we have shown that the solvent-entropy effect originating from the protein-solvent many-body correlation plays crucially important roles in the following subjects: the protein-folding mechanism, sugar-induced enhancement of the thermal stability, and pressure and cold denaturating of a protein. Here we investigate the differences in the thermal stability among homologous proteins obtained from thermophiles and mesophiles. The thermal-stability change upon mutation is also considered. On the basis of the results obtained, we argue the essential roles of the protein-solvent many-body correlation in a unified manner within the same theoretical framework.

3P059 天然タンパク質の鎖長と分子サイズのスケーリング関係についての包括的解析

Comprehensive analysis of the scaling relationship between the chain length and the molecular size of native proteins

Daisuke Takahashi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

It has been theoretically shown that the radius of gyration of a polymer, R_g, scales with the number of its residues, N, according to the equation $R_g = R_0 N^{\nu}$, where R_0 is a constant. The scaling exponent, ν, equals 1/3, when a polymer collapses into a compact globular structure in a poor solvent. To examine whether the scaling relationship holds for native proteins, we calculate R_g and N of ~110,000 protein domains from the SCOP database. We reveal that there appears to be the lowest limit of R_g at each chain length and that the ensemble of the maximally compact native structures obeys the scaling law with $\nu \sim 1/3$. This suggests that the structural property of densely-folded native proteins is similar to that of polymers collapsed in a poor solvent.

3P060 複雑なトポロジーを持つタンパク質のフォールディング経路ネットワーク

Network of folding pathways of topologically complex proteins

Takashi Inanami, Masaki Sasai (*Dept. of Comp. Sci. Eng., Univ. of Nagoya*)

The funnel model has explained folding of single-domain proteins, but folding mechanism of multi-domain proteins still remains elusive. Especially, the multi-domain proteins with N and C termini belonging to the same domain are topologically complex, requiring the new method of analysis. We extend the Wako-Saito-Munoz-Eaton model to analyze the tendency of the synchronous formation of N and C terminal structures in this type of proteins, and apply it to dihydrofolate reductase (DHFR) and other proteins. By analyzing the multi-dimensional free energy landscape, we found that many folding pathways and intermediates coexist and that the kinetic flow through the network of pathways explains the observed features (M. Arai et al. J Mol Biol 2011) of folding of DHFR.

3P061 天然変性蛋白質の立体構造特性に関わるリン酸化の静電的な制御

Phosphorylation as an electrostatic regulation of the conformational state of intrinsically disordered proteins

Koji Umezawa[1], Jun Ohnuki[1], Yukinobu Mizuhara[1], Junichi Higo[2], Mitsunori Takano[1] ([1]*Grad. Scl. of Adv. Sci. & Eng., Waseda Univ.*, [2]*IPR, Osaka Univ.*)

An intrinsically disordered protein (IDP) often exhibits folding when binding to its partner, which is frequently regulated by phosphorylation; e.g., KID (kinase inducible domain) binds to its partner protein upon phosphorylation and adopts an alpha-helix. One of the effects of phosphorylation should be the electrostatic perturbation on the intra-molecular interaction which affects the conformational state of IDP; in fact, phosphorylated KID (pKID) exhibits higher helix content than KID. We here studied the conformational change of IDPs upon phosphorylation using a side-chain-coarse-grained model that can reproduce the increase of helical content for pKID, and suggest the switching mechanism between inter and intra-molecular electrostatic interactions.

3P062 ウマβラクトグロブリン初期中間体における非天然ヘリックスのフォールディングキネティクスへの影響

Effect of non-native α-helix in the early intermediate on folding kinetics of equine β-lactoglobulin

Takahiro Okabe, Toshiaki Miyajima, Hideaki Ohtomo, Mio Ohtomo, Kanako Nakagawa, Seiichi Tsukamoto, Kazuo Fujiwara, Masamichi Ikeguchi (*Dept. of Bioinformatics, Soka Univ.*)

Equine β-lactoglobulin is a small globular protein (162 residues) which is monomeric at any pH. Although ELG adopts a predominantly β-sheet structure consisting of nine anti-parallel β-strands (A-I) and one major α-helix in the native state, it has a markedly high helical propensity throughout the sequence. Previously, we have shown that a non-native α-helical intermediate accumulates in the early folding stage of ELG and that the region corresponding to the H strand assumes a non-native α-helix. To investigate the contribution of non-native α-helix on refolding kinetic of ELG, we construct a mutant in which helical propensity at the H strand region is decreased and then the refolding kinetics were investigated by the stopped-flow CD and fluorescence.

3P063 天然条件下における PCP 各残基アンフォールディング速度の観測－尿素によるアンフォールディングの促進機構

Observation of unfolding rates of each residue of PCP under the native condition - Mechanism for urea to accelerate the unfolding rate

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To study the unfolding process under the native condition, H/D exchange reactions were measured every residue. H/D exchange reactions with the slowest rate followed the EX1 mechanism, reflecting the unfolding rate of individual residues. The structural fluctuations in the native state are extremely restricted, and water penetration into the interior of protein was prohibited. The influence of urea was examined on the structural fluctuations in the native state. Urea had no influence on the regions easily exposed to water, but had strong influence on the interior of protein into which water penetration is prohibited. We will discuss the mechanism for urea to accelerate the unfolding rate.

3P064 高速溶液混合法を用いたアポミオグロビンの salt-induced 中間体のフォールディングに関する研究

Folding of salt-induced intermediate of apomyoglobin using ultrarapid mixing methods

Yukiko Abe, Takuya Mizukami, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ.*)

Proteins consisted of more than 100 amino acid residues accumulate folding intermediate during the folding process. Some proteins accumulate equilibrium intermediate under moderately denaturing conditions, which have similar properties to the folding intermediate. An additional intermediate is formed under acid denaturing conditions in the presence of high concentration of salt (salt-induced intermediate). In this study, folding mechanisms of salt-induced and equilibrium intermediate of horse apomyoglobin are compared in terms of the equilibrium and kinetic properties of these intermediates by means of fluorescence measurement using ultrarapid mixing methods.

3P065 変異体解析を用いた緑色蛍光蛋白質のフォールディング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 in folding mechanism of green fluorescent protein studied by mutagenesis approach

Taichi Andou, Kosuke Maki (*Grad. Sch. Sci., Nagoya Univ.*)

Green fluorescent protein (GFP) accumulates several intermediates under moderately acidic conditions. A previous study suggested that GFP accumulated a native-like equilibrium intermediate (N') with reduced green fluorescence at pH 5, which arose from histidine residues with abnormal pK_a values.
In this study, to examine the role of hidtidine residues in unfolding of GFP, a series of variants was constructed by replacing histidines with other amino acids and the acid-induced equilibrium unfolding measurements were carried out by monitoring the chromophore fluorescence. From these results, the contribution of each histidine residues on the pH-dependent stability of GFP will be discussed.

3P066 スタフィロコッカル・ヌクレアーゼの安定性とフォールディング/アンフォールディングの研究

The Stability and Folding/Unfolding of Staphylococcal Nuclease at the Residue Level

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we used native-state hydrogen-deuterium (H/D) exchange NMR to estimate the stability of staphylococcal nuclease at the residue level, and replica exchange molecular dynamics simulations to monitor the secondary structure formation/unfolding on temperature-induced unfolding. These results suggest that the more stable regions (β-barrel domain) under the native conditions unfold at the higher temperatures. In addition, the pattern of the stability obtained the native-state H/D exchange NMR experiments is associated with that of the protection factors at 100 ms after the initiation of the folding obtained by the previous H/D exchange pulse-labeled NMR study. Our results indicate the relationship between proteins' stability and conformation change in protein folding.

3P067 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

Analyses of amyloid fibrillation mechanism and its inhibition effect of hCT as studied by ^{13}C solid-state NMR and TEM

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Human calcitonin (hCT) known as peptide thyroid hormone, plays a central role in calcium-phosphorus metabolism. HCT forms amyloid fibril in concentrated aqueous solution. We have shown that the fibrillation mechanism of hCT can be analyzed using a two-step autocatalytic reaction mechanism. In this study, we particularly investigated the early process in the fibril formation in HEPES solution. The morphology of the early process was examined by means of TEM, and turned out that the fibril formation in HEPES is much slower than that in acetic acid solution. It was nated that intermediate was seen in the early process. This observation clearly supports that hCT fibril takes a two-step autocatalytic reaction mechanism.

3P068 β_2 ミクログロブリンのアミロイド幹形成領域のスキャン探索

Scanning survey for amyloid-stem-forming region of β_2-microglobulin

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Amyloid formation of β_2-microglobulin is responsible for dialysis-related amyloidosis. The most relevant region to amyloidogenicity is considered to be (20-41). Structural models of the amyloid have been reported using isotope-labeling IR and solid-state NMR for K3-peptide that corresponds to (20-41). Herein, we developed 2-dimensional scanning method in order to identify a pair of β-strands that form the amyloid-stem, a cross-β structure in fibrils. The survey was carried out with the peptides consisting of systematically selected two 6-residue sequences and a designed turn-inducing linker, Asp-($_D$-Ala). The specific residue-residue interactions between thus-identified two β-strands in K3-peptide region were found to have an important role for amyloidogenicity.

3P069 ヒトカルシトニンの酸性膜存在下でのアミロイド線維形成機構の解明

Amyloid-like fibrillization and the structure of human calcitonin in the presence of acidic lipids

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Human calcitonin (hCT) is a 32-residue peptide hormone, involves in bone calcium metabolism and known as amyloid peptide. However, the detailed fibrillization mechanism is not well understood. In this study, the structure and fibrillation kinetics of hCT in solution containing neutral and acidic phospholipids (bicelles and micelles) were observed using NMR and UV-Vis spectroscopies. The fibrillation kinetics was revealed using a two-step autocatalytic reaction model composed of fibril nucleation (k_1) and fibril elongation reaction (k_2). In the presence of lipids, the first reaction was accelerated but the second reaction was decelerated. The surface condensation effect and the electrostatic interaction between hCT and lipid may play a role in fibril formation.

3P070 インスリン B 鎖に見られる多様なアミロイド線維前駆中間体の観察

Observation of various types of amyloid prefibrillar intermediates of insulin B chain

Shoko Tsuhara, Eri Chatani (*Grad.Sch.of Sci.,Kobe.Univ*)

Amyloid fibrils are protein aggregates associated with many diseases. To elucidate detailed mechanism of amyloid fibril formation, we have investigated the fibrillation process of insulin B chain. Immediately after dissolving this peptide in buffer solution, significant ThT fluorescence intensity was observed, and the needle-like mature fibrils were formed subsequently without any lag phase, suggesting the formation of a prefibrillar intermediate at the initial stage. Interestingly, different types of prefibrillar intermediates in terms of cross-β content and morphology were observed dependent of pH, and these species further developed to form different mature fibrils, implying that these intermediates play a role as nuclei determining final amyloid structures.

3P071 金属イオン配位によるインスリンアミロイド線維の多形誘導効果

Polymorphism of insulin amyloid fibrils induced by the coordination of metal ions

Misaki Yokoyama, Yoshito Huruie, Motonari Tubaki, Hiroshi Hori, Eri Tyatani (*Grad.Sch.of Sci.,Kobe Univ*)

Amyloid fibrils are protein assemblies with a potential application as a new material in the field of nanotechnology. To control physicochemical properties of amyloid fibrils, polymorphism, a feature with which a variety of fibril structures are formed even from one protein sequence, may be useful. In this study, we have attempted to induce polymorphic fibrils of insulin by altering initial association state with zinc ions. As a result, we could obtain fibrils both in the presence and absence of zinc ions, and the amyloid fibrils formed with zinc ions had stronger tolerance for pH-induced dissociation than those formed without zinc. This result indicates that metal ion is an effective factor to manifest polymorphism of insulin fibrils.

3P072 Exploring roles of water molecules on amyloid fibrillation by salt effects and Near Infrared spectroscopy

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To clarify mechanisms of the formation of amyloid fibrils, we have performed systematic analysis of salt effects and near infrared (NIR) spectroscopic measurements for the fibrillation of insulin. When nucleation and extension rates were measured in the presence of various types and concentrations of salts, both of acceleration and deceleration were observed in a concentration-dependent manner for all salts, and especially, the deceleration observed at high concentrations followed Hofmeister series. Furthermore, we could find some characteristic changes in water structures during the fibrillation by time-resolved NIR spectroscopy. From these results, it has been suggested that specific states of hydrating and bulk water contribute to efficient fibril formation.

3P073 タンパク質翻訳と共役した分子シャペロン動態の１分子蛍光イメージング

Single-molecule fluorescence imaging of translationally-coupled chaperone action

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Molecular chaperones assist the folding of newly synthesized proteins during and after the translation in cells. In bacteria, the prevailing view is that Trigger Factor and DnaK/DnaJ chaperones interact with nascent polypeptide co-translationally and GroEL/ES chaperone act mainly after the translation termination. To elucidate the mechanism of these chaperones on the folding of nascent polypeptides, we try to observe the dynamic behavior of chaperones on the translating ribosome at the single-molecule level. By using TIRF microscopy and reconstituted cell-free translation system (PURE system), we watched the interaction between fluorescence-labeled Trigger Factor and a immobilized translating ribosome.

3P074 一分子蛍光法によるリポアミド脱水素酵素の作用特性の解析

Enzymatic reaction of Dihydrolipoamide dehydrogenase revealed by single molecular fluorescence detection method

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Dihydrolipoamide dehydrogenase (E3) subtracts hydrogen from dihydrolipoamide to reduce NAD to NADH. FAD as an active center of E3 mediates this enzymatic reaction by the periodic redox. Since FAD is fluorescent and the reduced form is non-fluorescent, the fluorescence from single molecule of E3 repeats the periodic transition between the bright and dark states. The duration time and fluorescence intensity of the bright and dark state responding to the enzymatic turn over were analyzed by the home built single molecular fluorescence detection system using a CW laser, fluorescence microscope and MCS. The fluctuation and the cross correlation of two FADs of the enzymatic activity of E3 are reported in detail.

3P075 ウマ L フェリチンサブユニットへの鉄酸化活性部位の導入

Insertion of ferroxidase center in horse L ferritin subunit

Mai Nemiti, Tomoaki Harada, Hideyuki Yoshimura (*Sch of Sci. & Tech., Meiji Univ.*)

Ferritin is a ubiquitous cage-like protein and has a capability to store iron. It is co-polymers of two different subunits (H and L). H subunit has ferroxidase activity, instead of this, L subunit consists of nucleation site of oxidized product. Recombinant L ferritin, fer0, is well studied because it is extremely stable and has potential to synthesize nanoparticles (NPs) of various metal compounds. Although recombinant H ferritin has higher ability for oxidation, it has not been utilized for NPs synthesis, because it is easy to coagulate. To improve NPs synthesis efficiency, we have designed the L-mutant (Y24E) that is one of the essential residues for ferroxidase activity in H subunit. We will report the ability of NPs synthesis in fer0 and the mutant.

3P076 デザインペプチドによる脂肪滴とアミロイド線維の加水分解

Hydrolysis of lipid droplets and amyloid fibrils by the designed peptide

Yoshihiro Iida, Atsuo Tamura (*Kobe University*)

Obesity has become a huge problem in modern life. To avoid obesity, we should hydrolyze triglyceride in lipocyte. As a candidate for an anti-obesity drug, we tried to design peptides having enzyme activity like the lipase. As a strategy for the design, we made the peptide to have the catalytic triad composed of histidine, asparagine acid and serine. Based on the strategy, we synthesized 5 peptides named me1-5. It was shown that me5 takes the alpha-helical conformation and can hydrolyze triglyceride. We also tried to hydrolyze amyloid fibrils by using me5, and it was confirmed. We conclude that the peptide me5 can be regarded as a hydrolase which is capable of hydrolyzing the lipid droplets and amyloid fibrils.

3P077 タンパク質分解酵素の速度論的安定性の熱測定による評価方法

Calorimetric method to evaluate the kinetic stability of proteases

Shun-ichi Kidokoro, Akihiro Nagata, Keita Ochi (*Dept. Bioengineer., Nagaoka Univ. Tech.*)

The thermal denaturation of protease is known to be irreversible because of self-digestion. As differential scanning calorimetry (DSC) was a dynamic method in nature, several papers have reported that DSC was useful to evaluate the kinetic stability of proteases. While only the first-order reaction was used in the analysis traditionally, the second-order reaction, self-digestion, becomes apparent in many cases and is introduced in the new model of this study. The concentration dependence of the irreversible thermal denaturation observed by DSC was well explained by the new model, and the kinetic parameters from the model were found to agree well with those determined by other method, analyzing the time course of the remaining enzymatic activity.

3P078 タウタンパク質に対する Pin1 のプロリン異性化活性を測定するための新しい方法

A novel method to measure Pin1's peptidyl-prolyl isomerase activity for tau protein

Teikichi Ikura, Nobutoshi Ito (*Med. Res. Inst., Tokyo Med. Dent. Univ.*)

The Alzheimer's disease-related protein, tau, aggregates into neurofibrillary tangles when it is hyperphosphorylated. A peptidyl-prolyl isomerase (PPIase), Pin1, restored the function of tau by presumably catalyzing isomerization of a specific pSer/Thr-Pro motif. The function of Pin1 for tau, however, is still unsolved due to the lack of methods to measure Pin1's PPIase activity for tau. Here we developed a novel method for this purpose. In this method, catalytic reaction by Pin1 is coupled with dephosphorylation by a trans-isomer-specific phosphatase PP2A. Then the PPIase activity is detected as degree of dephosphorylation by ELISA. In the present study, we demonstrated availability of this method for a synthetic peptide including the pThr231-Pro232 motif of tau.

3P079 New highly accurate pickup methods, MRA-StoPICK and MRMA-StoPICK methods, for single particle analysis using electron microscope

Masaaki Kawata, Chikara Sato (*National Institute of Advanced Industrial Science and Technology*)

For highly accurate reconstruction of three-dimensional (3D) structure of biological macromolecules using electron microscope, we have developed new pickup methods based on probability distribution of particles on micrograph, in which the multi-reference alignment (MRA) or multi-reference multiple alignment (MRMA) is used to evaluate the probability and we refer to them as MRA-StoPICK and MRMA-StoPCIK methods. Both methods demonstrated a superior performance to the previous methods. Accurate particle image libraries generated by those methods have enabled higher resolution 3D reconstruction using cryo-electron micrographs. Those methods are key algorithms for fully automated data processing for high resolution single particle analysis.

3P080 積分方程式理論に基づく X 線小角散乱データを用いた蛋白質間相互作用の解析
An integral equation approach for protein interactions using small-angle X-ray scattering data

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A model-potential-free analysis of protein interactions using small-angle X-ray scattering data is presented. This method gave the better description of experimental structure factor for protein solutions than DLVO model potential. On the bases of results obtained from the model-potential-free approach, we introduced a solvent-induced model potential to improve the DLVO description of protein interactions. The DLVO plus solvent-induced potential provided a quite good agreement with the experimental structure factors. This analysis revealed that solvent effects on protein interactions stabilize contact-pair conformations and simultaneously raise an activation barrier against approaching each other.

3P081 静的光散乱法による水溶性タンパク質の分子量の測定
Measurements of molecular weights of soluble proteins using static light scattering

Ken Takeuchi, Youichi Nakatani, Osamu Hisatomi (*Department of Earth and Space Science, Graduate School of Science Osaka University*)

Light scattering is a convenient technique to investigate the size of the molecules, and has been used for investigation of polymers and macromolecules. However, the applications to biomolecules, such as proteins were limited. We investigated the molecular weights (M_W) of globular proteins by static light scattering (SLS), and compared them to those deduced from dynamic light scattering (DLS) and size exclusion chromatography (SEC). The apparent M_W showed smaller differences from those calculated from amino acid sequences, in comparison with the M_W estimated from DLS and SEC. Our results suggested that static light scattering is a useful tool for investigation of M_W of globular proteins.

3P082 タンパク質超高感度測定法の開発：ELISA 法と酵素サイクリング法との組み合わせの試み
Development of super high-sensitive measurement of proteins by combination of ELISA and enzyme cycling methods

Etsuro Ito (*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University*)

To develop a super high-sensitive measurement for determination of a small amount of protein, we noted that an enzyme cycling method could amplify the signal that is released from ELISA. We adopted the thio-NAD cycling method as one of the enzyme cycling methods following ELISA. We employed alkaline phosphatase as an enzyme labeled with an antibody and andorosterone phosphate as a substrate for the alkaline phosphatase in ELISA. We then applied a dehydrogenase with a coenzyme, thio-NAD, to the thio-NAD cycling method. The signal was measured as the absorbance of thio-NADH. Our measurement system showed that the detection limit of alkaline phosphatase reached 10^{-20} mol and that the detection limit of a given protein was 10^{-19} mol.

3P083 Single-molecule investigation of the force required to release SecM-mediated translation arrest

Zhuohao Yang, Ryo Iizuka, Takashi Funatsu (*Grad. Sch. of Pharm. Sci., The Univ. of Tokyo*)

SecM contains an arrest sequence that interacts with the ribosomal tunnel to induce a translation arrest. The arrest is considered to be released as the N-terminus of SecM is pulled by Sec apparatus. To clarify the molecular mechanism, we are trying to examine the force required to release the translation arrest. First, we tried to examine the rupture force for the interaction between ribosome and SecM arrest sequence by using optical tweezers. Unexpectedly, the interaction was very weak, and the rupture force was unmeasurable. Then, we are examining the force required to release the translation arrest using microfluidic single-molecule force spectroscopy, in which translation-arrested ribosome complexes are pulled through the fluid by a constant force (< 2 pN).

3P084 High-Speed AFM Observation of the FliI/ FliJ Complex

David Carriou[1], Takayuki Uchihashi[1,2], Yumiko Uchida[3], Hiroto Yanagawa[3], Tohru Minamino[4], Katsumi Imada[3], Toshio Ando[1,2] ([1]*Dept. Phys., Kanazawa Univ.*, [2]*Bio-AFM Frontier Research Center, Kanazawa Univ.*, [3]*Grad. Sch. Sci., Osaka Univ.*, [4]*Grad. Sch. Frontier Biosci., Osaka Univ.*)

FliI and FliJ are soluble component proteins of the flagellar-specific protein export apparatus. Strong structural similarity between FliI/J and F1-ATPase has been revealed by X-ray crystallography and electron microscopy. FliI is an ATPase and assembles into a hexameric ring, whose formation is promoted by binding of FliJ to the center of the ring. The working mechanism of the FliI/J complex is supposed to be similar to that of F1-ATPase. Here we applied HS-AFM to directly observe the structure of the FliI/J complex and its structural dynamics. The HS-AFM images showed that the FliI hexamer is not symmetric ring but a ring formed by three dimers. We will discuss how FliJ binds to the FliI hexamer and the dynamics of the FliI/J complex.

3P085 穏やかな pH で抗体精製するための新規アフィニティーリガンドの開発

Development of a novel affinity ligand for purification of antibodies at moderate pH

Taihei Sawada[1], Takaihiro Watanabe[1], Yuuki Hayashi[1], Munehito Arai[1,2] ([1]*Dep. Life Sci., Univ. Tokyo,*, [2]*PRESTO, JST*)

Protein A is frequently used as an affinity ligand for purification of antibodies through affinity chromatography. However, acidic pH is required to dissociate antibodies from protein A, which has risks of acid denaturation and aggregation of antibodies. To dissociate antibodies at more neutral pH, we develop a novel affinity ligand, FPAF. FPAF is composed of the B-domain of protein A (BDPA) connected with the leucine zipper region of c-Fos. A rationale for the design is given by mutually exclusive folding, in which homodimeric coiled-coil formation of c-Fos below pH 6 perturbs the interactions between BDPA and an antibody. As expected, FPAF dissociates antibodies at more neutral pH than BDPA. Therefore, FPAF is potentially useful for purifying antibody medicines.

3P086 アルデヒドデカルボニラーゼによるバイオアルカン生産に向けたシステイン置換体の開発

Toward the development of cysteine-free variants of aldehyde decarbonylase for industrial bioalkane production

Yuuki Hayashi[1], Fumitaka Yasugi[1], Munehito Arai[1,2] ([1]*Dept. Life Sci., Univ. Tokyo*, [2]*PRESTO, JST*)

In 2010, acyl carrier protein reductase (AAR) and aldehyde decarbonylase (AD), which synthesize alkanes from fatty acid metabolites, were identified from cyanobacteria. AD catalyzes decarbonylation of aldehydes generated by AAR to produce alkanes. It has three free cysteines at residues 71, 107, and 117. For industrial use of AD as a catalyst of bioalkane production, it is desirable to remove all the free cysteines to avoid a risk of oxidative denaturation, without any loss of activity. Here, we explore the structural property and activity of the wild-type AD and its cysteine-deficient variants by circular dichroism, size-exclusion chromatography, and GC-MS, and evaluate their potential as an industrial catalyst. Detailed results will be presented at the meeting.

3P087 LOV を鋳型とした酸化還元感受性タンパク質の蛍光特性

Redox-controlled fluorescence from LOV-based proteins

Yukiko Ono, Tatsuya Iwata, Masayo Iwaki, Hideki Kandori (*Nagoya Inst. Of Technol.*)

The LOV domain, which was first identified in the plant phototropin, functions as a blue-light sensor. Upon blue light absorption, a covalent bond is formed between the FMN chromophore and a nearby cysteine residue. The Cys-to-Ala (C/A) mutant was known to exhibit photoreduction of fluorescent FMN to non-fluorescent semiquinone in the presence of external reductants. This suggests that novel redox-sensitive and light-switchable fluorescent proteins can be designed by genetic modification based on the C/A mutant. In this paper, the highly functional recombinant LOV protein was screened in *E. coli*, and its physicochemical properties were examined by UV/vis spectroscopy combined with electrochemistry. The *in vivo* application will be also demonstrated.

3P088 Addition of negatively charged residues can reverse the aggregation of a protein caused by an artificially introduced hydrophobic surface

Sota Yagi, Satoshi Akanuma, Akihiko Yamagishi (*Tokyo university of pharmacy and life science*)

A hydrophobic patch on the surface of a protein can induce aggregation. Conversely, a hydrophobic patch may be required so that the protein can bind other molecules. The mutant 6L of the dimeric, α-helical protein sulerythrin, which contains an additional six Leu arranged to form a hydrophobic patch on its surface forms insoluble aggregation. To reverse this aggregation, we mutated 6L so that it contained an additional three to six negative charged residues (Glu or Asp) that would surround the hydrophobic Leu patch. The mutants with six Glu or six Asp appeared to exist mostly as a dimer. These results demonstrate that negative charged residues surrounding a hydrophobic patch can reverse the aggregation of a protein caused by a hydrophobic patch.

3P089 総電荷の異なるフェリチン変異体の作製と特徴付け

Construction and characterization of ferritin mutants having different net charges

Satsuki Takebe, Eriko Aoki, Daisuke Sato, Kazuo Fujiwara, Masamichi Ikeguchi (*Soka University*)

Non-heme ferritin from E.coli forms a spherical shell-shaped 24mer with 4/3/2 symmetry. It dissociates into 12 dimers at acidic pH, and it is able to reassemble into the native 24mer when pH is returned to a neutral one. Utilizing this property, we aimed to engineer hetero-oligomer from subunits having different net charges. The candidates for mutation sites are positively or negatively charged residues far from the subunit interface and forming no salt bridge with other residues, that is, Glu5, Glu8, Glu12, Glu85, Glu89 and Arg56. Mutants prepared have multiple Glu->Gln subsitutions, multiple Glu->Lys subsitutions, and Arg->Glu substitution. Some of them were able to form native-like 24mer as judged by gel filtration chromatography and circular dichroism spectroscopy.

3P090 人工 4 ヘリックスバンドルタンパク質上に白金結合ループを創出

Creation of a platinum-binding loop on an artificial four-helix bundle protein

Hiroya Niiro, Satoshi Akanuma, Akihiko Yamagishi, Yuuto Akiyama, Tatuya Uchida (*Tokyo University of Pharmacy and Life Sciences*)

Proteins and peptides that bind to metals are important components of biosensor. For example, glucose oxidase is immobilized on a platinum electrode of a glucose sensor. Although a number of artificial metal binding peptides have been constructed, no example where a metal-binding site was created on a protein's surface has been reported. In this study, we created a platinum-binding site within a loop of an artificial four-helix bundle protein, LARFH. We constructed a LARFH library that contains random amino acid sequences within a loop and then selected a LARFH variant interacting with platinum by using the T7 phage display system. The purified LARFH variant showed platinum-binding ability as judged by AFM and QCM analyses.

3P091 アクチン発現系の確立に向けて
Toward the establishment of an expression system for actin

Masashi Mori[1], Yoshitaka Umetsu[2], Shinya Ohki[2] ([1]*Ishikawa Prefectural University*, [2]*Japan Advanced Institute of Science and Technology*)

Protein sample preparation is exactly the first step in every biophysical study. Curious proteins, however, are often difficult to be expressed by the popular systems including E. coli and Pichia. Actin has been recognized as one of such challenging proteins. So far, employment of various analytical methods requiring recombinant proteins is impeded for studying actin. To overcome this issue, we have been developing a new protein expression protocol with an inducible viral vector infection system in suspension-cultured plants cells. In the presentation, we will report overview of our original protein expression system and the preliminary results for actin.

3P092 インドールアミン 2,3 ジオキシゲナーゼの基質トリプトファンの検出-紫外共鳴ラマン分光法
Detection of the bound tryptophan in indoleamine 2,3-dioxygenase by UV resonance Raman spectroscopy

Sachiko Yanagisawa[1], Masayuki Hara[1], Hiroshi Sugimoto[2], Yoshitsugu Shiro[2], Takashi Ogura[1] ([1]*Univ. of Hyogo*, [2]*RIKEN Harima SPring-8 center*)

A heme containing enzyme, indoleamine 2,3-dioxygenase (IDO) catalyses direct incorporation of two oxygen atoms into tryptophan (Trp) and its reaction mechanism is not fully understood due to the lack of structural information. Structural information of the bound substrate and intermediate products during the reaction is essential to understand the reaction mechanism. UV resonance Raman (RR) spectroscopy has been applied to selectively detect Trp in this study. We have successfully obtained RR spectra of the bound-substrate in IDO by the aid of its isotopomers. Some of the Raman bands exhibited intensity and/or frequency shift relative to those of free Trp. Here, we will present the UVRR spectra of the bound-Trp and discuss its specific structure.

3P093 Interaction Between Heme and Heme-Cu Binuclear Center in Cytochrome *c* Oxidase

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The heme a_3-Cu_B site and heme *a* in cytochrome *c* oxidase function as a O_2 reduction site and the driving element of H^+-pump. Axial ligands of hemes *a* and a_3 are closely adjacent each other and they are on one α-helix, helix X. This structural feature is important for the coupling mechanism of the O_2 reduction reaction and the H^+-pump.
In the resonance Raman spectra, the vibrational modes, which originate from the formyl substituent and the Fe-ligand bond of heme a_3, showed frequency shifts upon the oxidation state change of heme *a*. This heme-heme interaction is most likely propagated through helix X. However, the frequency shifts were restored upon reduction of Cu_B. It suggests that the interaction could be regulated by the oxidation state of Cu_B.

3P094 チトクローム *c* 酸化酵素の酸素還元反応における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 the flow system with an oxygen lung aiming at IR measurement on the oxygen reduction reaction of cytochrome *c* oxidase

Tatsuhito Nishiguchi, Masahide Hikita, Kyoko Shinzawa-Itoh, Shinya Yoshikawa, Satoru Nakashima, Takashi Ogura (*Grad. Sch. Lif. Sci., Univ. Hyogo*)

Although proton pump mechanisms of cytochrome *c* oxidase (CcO) have been studied vigorously, the detail is still unrevealed. Since IR spectra gives the information about the hydrogen bond related structure, it is extraordinary important to measure IR spectra during the reaction. Here we have developed a new flow system that enables to measure the time-resolved IR spectra on the oxygen reduction reaction of CcO in aqueous solution. The inserted artificial oxygen lung supplies oxygen, which is necessary for the reaction. To measure the IR spectra in aqueous solution, it is necessary to supply enough amount of flow rate in the extremely thick (50 μm) water line. We could develop the flow system that could be used for time-resolved IR measurements.

3P095 シアン化物・アジ化物結合完全酸化型ウシ心筋チトクロム酸化酵素の構造解析
Structural analysis of bovine heart cytochrome *c* oxidase in the cyanide- and azide-bound fully oxidized states

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Cytochrome *c* oxidase (CcO) in the mitochondrial respiratory chain catalyzes the O_2 reduction coupled to proton pump. In the catalytic intermediate states, some of oxygen spices are bound to the Fe_{a3}-Cu_B site. Cyanide (CN^-) and azide (N_3^-) ions, known as inhibitors, are bound to the Fe_{a3}-Cu_B site in the Fe^{3+} and Cu^{2+} state, which mimics oxidized form (O-form) intermediate.
In this study, we analyzed X-ray crystal structures of bovine heart CcO in the CN^-- and N_3^--bound fully oxidized states. The refined structures indicate that CN^- bridges between Fe and Cu atoms and N_3^- shows two conformers, Fe- or Cu-bound form. Conformations of the Fe_{a3}-Cu_B site and proton transfer pathways are similar to those in the resting oxidized state, in which peroxide (O_2^{2-}) ion is bound.

3P096 Sequencing bovine/human hybrid cytochrome c oxidase genes in HeLa cells to verify mutagenesis results disapproving D-path proton pumping

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D-path proton pumping has been proposed from mutational studies on bacterial cytochrome c oxidase (CcO). Its structural similarity and amino acid conservation with bovine D-path imply D-path pumping in bovine CcO, contrary to our H-path proposal for bovine CcO. A bacterial D-path mutation abolishes proton pumping retaining O_2 reduction activity, supporting D-path model. However, as reported at previous meeting, the corresponding bovine mutation employing HeLa cell bovine/human hybrid CcO expression system does not change the activity, in contrast to the bacterial results. Here, for examination of the possible occurrence of back mutation of CcO in HeLa cells, we sequenced the CcO subunit genes or their cDNAs. No mutation other than the original one was identified.

3P097 完全酸化型チトクロム *c* 酸化酵素の酸化還元活性金属中心とアザイドの相互作用の分光学的研究

Spectroscopic characterization of the interaction of azide with the redox-active metal sites of fully oxidized cytochrome *c* oxidase

Masahide Hikita, Akima Yamamoto, Tomoko Maeda, Kyoko Shinzawa-Itoh, Takashi Ogura, Shinya Yoshikawa (*Grad. Sch. Sci., Univ. Hyogo*)

Cytochrome *c* oxidase (CcO) is the terminal oxidase in the mitochondrial respiratory chain. It catalyzes the reduction reaction of the molecular oxygen, which is coupled with the proton-pumping. Azide is a potent inhibitor of CcO, and considered to bind to heme a_3 in the oxidized state. Thus far, there is no resonance Raman spectrum of azide-bound CcO.
Here we report spectroscopic characterization of the interaction of azide with fully oxidized CcO. Addition of azide to CcO shifts the Soret peak and changes the intensity of the band, showing two azide-binding sites with K_d values of 59 μM and 44 mM at pH 7.4. Resonance Raman analysis using 441.6 nm as the exciting laser shows very small spectral changes. The results suggest the absence of direct azide binding to heme a_3.

3P098 一酸化炭素・シアン化物結合混合原子価型ウシ心筋チトクロム酸化酵素の構造解析

Structural analysis of bovine heart cytochrome *c* oxidase in the CO- and cyanide-bound mixed valence states

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Cytochrome *c* oxidase (CcO) catalyzes the O_2 reduction coupled to proton pump. CcO contains four redox active metal sites, Cu_A, Fe_a, Fe_{a3} and Cu_B. Carbon monoxide (CO) and cyanide ion (CN^-) are bound to the Fe_{a3}-Cu_B site and stabilize oxidized (Fe^{3+}-Cu^{2+}) and reduced (Fe^{2+}-Cu^{1+}) states, respectively. Previously, structures in the CN^--bound fully oxidized and CO-bound fully reduced states were analyzed.
To investigate redox dependent effect of Cu_A and Fe_a on the proton transfer pathways, we analyzed X-ray crystal structures of bovine heart CcO in the CO- and CN^--bound mixed valence states. The refined structures indicate that conformations in H-pathway, a proposed proton pump pathway, are regulated by redox states of these metal sites.

3P099 人工設計膜貫通ペプチドを用いたシグナル伝達モデル系の構築

Design of transmembrane peptide for constructing a signaling model

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Transmembrane(TM) signaling proteins such as receptor tyrosine kinases and T-cell receptors control many fundamental cellular processes. Signaling of TM proteins is frequently activated by ligand-mediated dimerization of TM domains in membrane. The interaction between TM domains is very important for control of signaling. However, due to the large molecular weight and hydrophobicity of TM proteins, it is quite difficult to analyze the interaction in aqueous solution. We thus tried to constitute a simple and small model of the signaling by using designed peptides based on the TM domain,EphA1. It was shown that the peptides associated in membrane-mimetic environment and its association state could be controlled by external stimulus.

3P100 好熱菌 F_oF_1-ATP 合成酵素 *c* サブユニットリングの活性部位の構造

The Active-Site Structure of Thermophilic F_oF_1-ATP Synthase *c*-Subunit Rings in Membranes

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In F_oF_1-ATP synthase, the electrochemical potential across membranes or ATP hydrolysis is used to rotate the F_oc-subunit ring. To facilitate elucidation of the chemical mechanism, we have developed a solid-state NMR method for analysis of a focused structure of the c-subunits from thermophilic Bacillus (TF_oc) in membranes. Structural information on its active site was obtained, using stereo-array-isotope-labeling (SAIL) with a cell-free system. The membrane-reconstituted TF_oc-oligomer was confirmed by the H^+-translocation activity and high-speed atomic force microscopic images. The Cδ chemical shift of Glu56 essential for H^+-translocation revealed that its carboxyl group is protonated in membranes, forming the H^+-locked conformation with Asn23.

3P101 Analysis of Structure and Function of Synaptotagmin 4

Masayuki Goto (*Tsukuba, Material Sci,*)

Synaptotagmin-4 (Syt4) regulates exocytosis of brain-derived neurotrophic factor (BDNF). Despite sharing the universal molecular design with other members of Syt family, having tandem C2 domains with a flexible linker of conserved length in between, mammalian Syt4 appears not to function as a Ca ion sensor. A preliminary molecular dynamics simulation also indicates that Syt4 does not bind Ca^{2+} ions as well as Synaptotagmin-1(Syt1) in the presence of anionic phospholipid such as Phosphatidylserine (PS) or phosphatidylinositol 4,5-bisphosphate (PIP_2). We shall report the MD results to discuss the molecular mechanism behind the different behavior as a result of minor structural differences. We shall also speculate on the role of Syt4 in LTP and BDNF regulation.

3P102 擬環状リン脂質リポソーム中のバクテリオロドプシンの構造と機能

A Biophysical Study of Bacteriorhodopsin in Pseudocyclic Phosphatidylcholine Liposome

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It is well known that in Archaea, macrocyclic tetraether phospholipids are a major component of biomembrane and contribute to its high stability against extreme environments. In the present study, a novel pseudo-cyclic phosphatidylcholine (PC), an acyclic analog of cyclic phospholipids bearing the two PC groups as the head groups, was synthesized and the reconstitution of bacteriorhodopsin (bR) was performed. Laser flash photolysis experiments on successfully reconstituted bR demonstrated that bR in the pseudo-cyclic PC has a photocycle very similar to native purple membrane, which is significantly different from that of bR in DMPC. Effects of pseudo-cyclization of the phospholipid on structure and function of bR and physical properties of membrane will be discussed.

3P103 結晶化を目指したカイコガ性フェロモン生合成活性化神経ペプチド受容体（PBANR）の細胞内第3ループへのT4リゾチーム置換位置の検討

Positional optimization of the T4 lysozyme replacing the third intracellular loop of the silkmoth PBANR for its crystallization

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The silkmoth pheromone biosynthesis-activating neuropeptide receptor (PBANR), a member of class-A GPCRs, regulates the biosynthesis of sex pheromone by interacting with PBAN. We obtained low resolution X-ray diffraction image (ca. 10 Å resolution) for the PBAN-PBANR complex, where the third intercellular loop of PBANR was mostly replaced by T4 lysozyme (T4L) for stabilization. To obtain better diffracting crystals of PBANR, we have optimized the insertion position of T4L. Twenty-one PBANR constructs were expressed with C-terminally fused EGFP in Sf9 cells, and their (1) expression level, (2) subcellular localization, and (3) binding to fluorescent-labeled PBAN were compared. We have obtained several promising constructs, which are being subjected to further screenings.

3P104 膜貫通ヘリックスの膜内配向決定機構の粗視化分子動力学シミュレーションによる探索

Coarse grained molecular dynamics simulations toward the mechanism elucidation of membrane protein topogenesis

Kouya Sakuma, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

How membrane proteins fold remains largely unclear. It has at least two distinct stages; membrane integration and tertiary structure formation. Our present study focuses on the former stage and simultaneously occurring topogenesis of nascent chains mediated by the Sec translocon, a highly conserved protein-conducting channel.
Aiming to reproduce and rationalize the famous positive-inside rule and other sub-rules for topogenesis, we performed molecular dynamics simulations. We modeled geometric and electrostatic features of phospholipid bilayer and utilized coarse grained models for proteins to simulate the long-timescale process of interest. Results indicated some requirements for strings of transmembrane helices to be integrated neatly into membrane environment.

3P105 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

Effects of homotrimer formation on chloride pump activity in membrane mimetics, Nanodisc, embedded Halorhodopsin

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Halorhodopsin (HR), a light-driven chloride anion pump, forms a homotrimeric structure 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, not in the reconstituted membrane. To discuss the functional significance of the trimeric formation in the membrane, we tried to reconstruct trimeric and monomeric HR into Nanodisc, a membrane mimetic. The UV and CD spectra indicate that the Nanodisc-embedded trimeric HR is active and the dissociation constant for chloride was determined. Together with the spectroscopic data from monomeric HR, the functional significance of the trimeric formation in the membrane will be discussed.

3P106 インテグリンとFAKを含む短寿命多分子複合体ラフトがGPIアンカー型受容体のIP3シグナルを誘起するプラットフォームとなる

Transient raft-dependent multimolecular complexes including integrin and FAK are the platforms for IP3 signaling of GPI-anchored receptors

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GPI-anchored receptor (GPI-AR) signaling is hardly understood. Using single-molecule tracking (SMT), we previously found that a GPI-AR CD59 forms tetramer rafts upon engagement, and becomes a platform for IP3 (and thus Ca2+) signaling by recruiting PLC. Surprisingly, the platform was able to recruit PLC for only ~0.4 s (lifetime) when it is bound to f-actin and immobilized (called STALL): the engaged CD59 tetramer raft undergoing STALL is the KEY SITE for signal transfer from outside to inside the cell. Here, we found that, using SMT, integrin and/or FAK recruited to the tetramer raft, where they are activated by recruited Lyn, which in turn is activated by recruited trimeric G-protein and CD47, mediated the raft binding to f-actin, inducing STALL and PLC recruitment.

3P107 GPCRダイマーがシグナルトリガーとしてはたらく：インバースアゴニスト効果の1分子イメジング解析に基づく発見

GPCR dimers as active signal triggers: inverse agonist effects revealed by single-molecule imaging analysis

Rinshi Kasai[1], Takahiro Fujiwara[2], Akihiro Kusumi[1,2] ([1]*Inst. For Frontier Med. Sci., Kyoto Univ.*, [2]*WPI-iCeMS, Kyoto Univ.*)

A unique feature of the G-protein-coupled receptor (GPCR) signaling is that low levels of constitutive signals always exist. Consistently, we found, even without stimulation, trimeric G-protein (G) transiently but continually bound a prototypical GPCR adrenergic receptor (AR) monomers and dimers, which are in dynamic equilibrium, for ~100 ms for each G biding event. Inverse agonists (IAs) block constitutive signals, and are becoming important in basic and clinical medicine. The IA addition abolished the G binding to AR dimers, whereas it increased the duration of G binding to AR monomers by ~2 fold, from 95 to 185 ms. These results together with others suggest that AR dimers are active AR species, whereas lower monomer activity is reduced by slowed G protein turnover.

3P108 生体分子複合体を通した多剤排出の物理に関して

On the Physics of Multidrug Efflux through a Biomolecular Complex

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We consider “multidrug efflux” where solutes (drug molecules) with diverse properties can be inserted and released into and from a vessel comprising biopolymers. We have shown that the solute-vessel potential of mean force (PMF) induced by the solvent plays critical roles in the insertion/release process. When the vessel inner surface is neither solvophilic nor solvophobic, the entropic component (EC) of the PMF, which is insensitive to the solute properties, dominates. In our view, the multidrug efflux is realized when the process is achieved by the EC. We show that the entropically inserted solutes with various sizes can be released by a continuous variation of the vessel geometry forming a time-dependent entropic force accelerating the solute motion to the exit.

3P109 X 線結晶構造の決定に向けた膜タンパク質構造安定性の理論的向上

Theoretical Enhancement of Structural Stability of a Membrane Protein for X-ray Crystallography

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Structure determination of integral membrane proteins (IMPs) by the X-ray crystallography is very difficult due to their low thermal stability in detergents. Though the stability can be enhanced by introducing mutations into IMPs, a random search accompanying a heavy experimental burden is currently employed to obtain mutations leading to sufficient enhancement. In this study, we show that the entropic effect originating from the translational displacement of hydrocarbon groups constituting nonpolar chains of the lipid bilayer plays crucially important roles in the structural stability of IMPs. A theoretical method for finding mutations which present considerably higher stability is proposed, and its reliability is discussed on the basis of some test calculations.

3P110 Ca^{2+}結合部位のプロトン状態変化による SERCA の構造変化

Conformational change of SERCA upon alternating protonation states in Ca^{2+}-binding site

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Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps Ca^{2+} from muscle cell to lumen by alternating Ca^{2+} affinity of Ca^{2+}-binding site. Proton counter-transport plays a quite important role in the transport against a large concentration gradient. Experimental studies revealed the conformational change as changing Ca^{2+} affinity at the higher pH. They also postulated that change of protonation states in the binding site brings the conformational change. To understand how SERCA utilize the counter-transport, we performed MD simulations of SERCA with different protonation states by using all-atom model. We show that proton transfer from the binding site induces the conformational change of important acidic residues and opens of water channels as pathway of Ca^{2+}.

3P111 改良した ATP 分子力場を用いた筋小胞体カルシウムポンプの分子動力学計算

Molecular dynamics simulations of SR Ca^{2+}-ATPase using improved ATP force field

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Ca^{2+}-ATPase transports two Ca^{2+} across the SR membrane utilizing chemical energy released in ATP hydrolysis. We focus on the ATP bound state and perform MD simulation in solution with DOPC lipid bilayer to clarify the mechanism of the ion transport induced by structural changes. Since the conformation of ATP immediately changed from the X-ray structure in the original CHARMM27 force field (ff), we improved angle/dihedral parameters of ATP following the CHARMM protocol. Reliability of the new ff was validated based on statistical information by performing replica-exchange MD of ATP as well as quantum chemical calculation. In the improved ff the ATP bound state reproduced the X-ray structure stably. The details of the improvement of ATP ff will be reported at the meeting.

3P112 アミロイド前駆体タンパク質とコレステロールとの相互作用

Interaction between cholesterol and transmembrane region of Amyloid Precursor Protein

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Bio-membrane consists of several types of lipids and membrane proteins. Such mixed membrane often forms micro-domains, which are called Raft. The activity of Amyloid Precursor Protein (APP), which is one of membrane proteins, is affected by Raft. This protein plays a very important role in the early stage of Alzheimer's disease. Recently, P.J Barrette et al has suggested that the cholesterol binding sites of the APP are mainly located in the upper layer region. We have investigated the relation between cholesterol and a small membrane protein, using Martini Coarse Grained model simulations. We'll show that the interactions between cholesterol and APP are stronger than the interaction between cholesterol and a normal model membrane protein.

3P113 LRH-1 の beta-catenin による転写活性化の構造基盤

Structural basis of transcriptional co-activation of LRH-1 by beta-catenin

Fumiaki Yumoto[1,2], Robert Fletterick[2] ([1]*KEK Structural Biology Research Center*, [2]*University of California, San Francisco*)

LRH-1 (NR5A2) is a member of the nuclear hormone receptor family of transcription factors with essential roles in development, metabolism, and cancer. It is essential for pregnancy and critical for ES cells pluripotency. LRH-1 binds beta-catenin, which accumulates in the nucleus on Wnt activation. We reported the structure of a beta-catenin in complex with the LRH-1 ligand binding domain (Yumoto et al., PNAS, 2012). Targeted mutagenesis of amino acids forming both sides of the LRH-1/beta-catenin interface reveals that they are essential for stable interactions between these proteins in solution. Based on the results, we are studying full-length LRH-1 with multiple biophysical methods toward structure determination of intact LRH-1 in complex with DNA and beta-catenin.

3P114 RNA アプタマーとプリオン蛋白質部分ペプチドの結合の統計熱力学

Statistical Thermodynamics for Binding of an RNA Aptamer and a Partial Peptide of a Prion Protein

Tomohiko Hayashi, Hiraku Oshima, Tsukasa Mashima, Takashi Nagata, Masato Katahira, Masahiro Kinoshita (*Institute of Advanced Energy, Kyoto Univ.*)

It has been reported that a novel RNA aptamer with 12 residues (R12) binds to a partial peptide of a prion protein (P16). This binding is expected to prevent prion diseases, but its driving force remains rather unclear. Here we calculate the free-energy change upon the binding of R12 to P16 using molecular mechanics, the three-dimensional reference interaction site model theory, and the hybrid method in which the angle-dependent integral equation theory applied to a multipolar water model is combined with the morphometric approach. We show that the large decrease in the intermolecular energy upon the binding is almost cancelled out by a correspondingly large increase in the hydration energy. The binding is driven by a large gain in the translational entropy of water.

3P116 The coarse grained GBSA method for simulations of biomolecular system

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The Generalized Born with solvent accessible surface area (GBSA) method has been widely used as a quick and reasonably accurate implicit solvation free energy model in all-atom (AA) molecular dynamics (MD) simulations of biomolecules. However, it has never been used in coarse grained (CG) MD simulations. Here, using a one-bead-per-residue CG representation, we provide the protocols to apply GBSA method in CGMD simulations. The CG GB term is fitted against AA Poisson-Boltzmann solvation energy. The CG SA is fitted against AA SA. Together, the CG GBSA method will give better accuracy for electrostatic and hydrophobic interactions than traditional CG force fields.

3P117 DNA 結合蛋白質はどのようにして障害物を回避するか。分子シミュレーションによるアプローチ
How DNA-binding proteins can bypass obstacles? Molecular simulation approaches

Mami Saito, Tsuyoshi Terakawa, Shoji Takada (*Grad.Sch.Sci, Kyoto Uni.*)

Recent researches suggest that DNA-binding proteins(DBPs) search target sites by 1D-sliding, hopping and 3D-diffusion because the theoretical search time with only 3D-diffusion is much longer than the time from experimental results. But it is unclear how DBPs bypass the obstacles consisted of other DBPs and nucleosome on DNA.
This research aims to reveal how DBPs bypass the obstacles by using coarse-grained molecular dynamics simulation software, CafeMol.

3P118 転写因子 p53 の特異的結合部位探索・認識機構：マルチスケールシミュレーション研究
Specific DNA sequence search and recognition mechanism of transcription factor p53: multi-scale simulation study

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A transcription factor p53 is composed of five domains, of which three are intrinsically disordered regions, which prevent to elucidate the functional mechanism based on static structural analyses. In order to elucidate the role of functional cross-talks among these domains from a dynamic structural analysis, we constructed coarse-grained model for p53 based on the result of all-atom simulation. The result of multi-scale MD simulation using the constructed model revealed the specific DNA sequence search and recognition mechanism of p53 and the effect of post-translational modification on it, suggesting efficacy of multi-scale approach to elucidation of functional mechanism of the highly flexible proteins.

3P119 DNA 塩基対の安定性に及ぼすコリンイオンの効果の分子動力学計算による解析
Analysis for the effect of choline ions on the stability of DNA base pairs using molecular dynamics simulation

Miki Nakano[1], Hisae Tateishi-Karimata[1], Naoki Sugimoto[1,2] ([1]*Konan Univ. FIBER*, [2]*Konan Univ. FIRST*)

In physiological-condition, G-C base pairs are more stable than A-T base pairs. However, A-T base pairs are stabilized in the solution containing choline ion while G-C base pairs are destabilized. In this study, we clarified unique binding style of choline ion with DNA atoms using molecular dynamics simulation. The binding style of sodium ion to DNA is tight but fragile; in contrast, the style of choline ion is relatively loose but stable through multiple-hydrogen-bond network. Choline ions prevent the formation of hydrogen bonds between G-C base pairs by binding to atoms between base pairs, while choline ions stabilize by fitting their narrow minor groove. We will discuss about the effects of choline ions on the stability of DNA base pairs in the presentation.

3P120 粗視化分子動力学シミュレーションによる一本鎖 DNA 領域形成機構の駆動力の解明
DNA unwinding mechanisms in *E. coli, oriC* region studied by coarse grained molecular dynamics simulations

Masahiro Shimizu, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

In the initiation of DNA replication of *E.coli* , unwinding DNA duplex at *oriC* region is a critical step. When DnaA proteins bind DNA to form DnaA-*oriC* complex, *oriC* region is bent to be positive supercoil structure and then DNA duplex is unwound. But, mechanisms of how this region is unwound are poorly understood. We assumed that *oriC* is a characteristic region which is easy to be ssDNA by a kind of driving force. In this study we first addressed thermodynamic stability of duplex in the *oriC* region, especially in AT-rich region, using coarse grained molecular dynamics simulation. We then investigated what kind of force play crucial role for DNA unwinding by reproducing the DnaA-*oriC* complex forming.

3P121 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ダイナミクス
Poly-nucleosome structural dynamics by coarse-grained simulations

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Nucleosome is a basic unit of chromatin structure in which DNA duplex wraps about 1 and 3/4 times around a histone octamer. Poly-nucleosome takes higher order chromatin structure to store the very long genome into a compact form. Density fluctuation of chromatin structure may be essential for the searching mechanism of transcription factors to achieve the target sites on DNA duplex. Previously, we simulated mono-nucleosome dynamics by using coarse-grained DNA-protein model, in which one amino acid is represented by one bead and one nucleotide is represented by three beads corresponding to phosphate, sugar, and base. In this study, we try to reproduce poly-nucleosome conformations at various densities of nucleosomes.

3P122 長鎖 DNA 分子内折り畳みは高分子電解質の鎖長に依存して２つのモードを示す

Two-mode Folding of a Single Giant Duplex DNA Chain Depneding on the Length of Cationic Polymer

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The effects of coexisting salts were observed on the folding of T4 DNA induced with tetramer and 100-mer poly-L-lysine (PLL), concerning with an ON/OFF manner of folding and precise structure of condensed DNA. Coexisting salt inhibited the ON/OFF folding of T4 DNA chain by tetramer PLL, but enhanced continuous folding by 100-mer PLL. The large-scale structure of T4 DNA in aqueous solution was directly observed with fluorescence microscopy. Precise structure of folded structure was analyzed with TEM and ζ-potential. Folding analysis were also demonstrated for nonspecific DNA-binding protein HMG-1,2 and specific protein STPR. Mechanisms and biological behavior of discrete (ON/OFF) / continuous change in large-scale structure of genomic DNA will be discussed.

3P123 Fleeting secondary structure effects on hybridization kinetics

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We report that fleeting secondary structures surprisingly suppress the hybridization kinetics. The fleeting single-stranded DNA secondary structures that are less stable than unstructured, or random coil, have been regarded as ignorable for hybridization kinetics. We measured the hybridization kinetics for an enormous number (over a hundred) of designed 23mer probe-target pairs having a uniform T_m but varying degrees of secondary structure stabilities by fluorescence spectroscopy with a stopped-flow apparatus and microarrays in solution and on the surface, respectively. Kinetic rate constants derived from the resultant data obviously showed a correlation with the stability of fleeting secondary structures in both solution and surface environments.

3P124 光応答性分子ロボット構築のための DNA マイクロカプセルの設計と作製

Design and construction of a DNA microcapsule toward light-responsive molecular robots

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In recent years, molecular robots constructed with biomolecules such as DNA and proteins have attracted much attention seeking for application such as artificial cell models, drug delivery systems, etc. At present, it is desired to construct molecular robots with environmental responsiveness. In this study, we propose a light responsive DNA microcapsule for the construction of micrometer-sized molecular robots. The DNA microcapsule is self-assembled on an inner surface of a water-in-oil (W/O) microdroplet. Here, a cyanovinylcarbazole nucleotide (cnvK) is used to bond DNA strands, which can result in light-responsive collapse of DNA microcapsules by UV irradiation. We believe that light-responsive molecular robots can be constructed based on these technologies.

3P125 モレキュラークラウディング環境における化学修飾を施した 2 本鎖核酸の熱力学的安定性

Effect of molecular crowding condition on the thermodynamic stability of chemically modified duplex

Hidetaka Torigoe, Hiroshi Noguchi, Yukiko Hashizume (*Dept. Appl. Chem., Fac. Sci., Tokyo Univ. Sci.*)

To achieve efficient artificial regulation of target gene expression by antisense strategy, the high stability of the duplex composed of the target mRNA and the antisense oligonucleotide added from outside is quite necessary. Chemical modifications of the antisense oligonucleotides have been used to stabilize the duplex. However, their stabilizing effect has been analyzed only under dilute condition. It has not been examined under molecular crowding condition similar to intracellular environment condensing many biological molecules. Here, we examined the thermodynamic stability of the duplex composed of the target mRNA and the chemically modified antisense oligonucleotide under the molecular crowding condition containing 10-40% polyethylene glycol 200.

3P126 Development of new algolithm for calculation of the energy distribution function by GPGPU

Takuhito Shiogama, Tsuhito Yoshida, Hiroaki Saito, Kazutomo Kawaguchi, Hidemi Nagao (*Kanazawa University*)

Because the free energy calculation generally requires extensive molecular interaction calculations, the acceleration and effective parallelization of the computation such as MPI and GPGPU should be important. In this study, we develop the new algorithm for the free energy calculation in the energy representation method by GPGPU acceleration. In the energy representation method, the energy distribution function rho_0 should be evaluated by extensive insertions of the solute molecule to the solvent system. In our new algorithm, these molecular insertions are parallelized by GPGPU calculation. We compare this algorithm with conventional MPI parallelization and discuss the efficiency of our approach.

3P127 浸透圧効果を利用したシトクロム c – シトクロム c 酸化酵素電子伝達複合体における相互作用の解析

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[1,2], Kyoko Ito[3], Shinya Yoshikawa[3], Koichiro Ishimori[1,2] ([1]*Hokkaido Univ. Chem.*, [2]*Hokkaido Univ. Sci.*, [3]*Hyogo Univ. Life Sci.*)

In the mitochondrial respiratory chain, Cytochrome c oxidase (CcO) accepts electrons from Cytochrome c (Cyt c) to reduce molecular oxygen. While one of the crucial steps in the electron transfer reaction, the complex formation of Cyt c with CcO, is characterized by the positive entropy change, suggesting the dehydration from hydrophobic residues, no experimental evidence has been reported. Here, we measured the dissociation constant for the complex formation under various osmotic pressures and showed the dissociation constant was decreased with the osmotic pressure, which corresponds to the dehydration from hydrophobic residues. The dehydration from hydrophobic residues, therefore, promotes the complex formation to facilitate the electron transfer from Cyt c to CcO.

3P128 蛋白質およびリガンドの水和熱力学量計算に向けた形態計測法的アプローチ

A Morphometric Approach for the Accurate Solvation Thermodynamics of Proteins and Ligands

Yuichi Harano[1], Roland Roth[2], Shuntaro Chiba[3] ([1]*IPR, Osaka Univ.*, [2]*Inst. Theo. Phys. Tuebingen Univ.*, [3]*Bio Center, TITECH*)

We have developed a versatile method for calculating solvation thermodynamic quantities for molecules, starting from their atomic coordinates. The contribution of each atom to the thermodynamic quantities is estimated as a linear combination of four fundamental geometric measures of the atomic species, which are defined by Hadwiger's theorem, and the coefficients reflecting their solvation properties. This treatment enables us to calculate the solvation free energy with high accuracy despite of the limited computational load. The method can readily be applied to macromolecules in an all-atom molecular model, allowing the stability of these molecules' structures in solution to be evaluated.

3P129 溶質-溶媒間のレナードジョンズポテンシャルパラメタが溶媒和ダイナミクスに及ぼす影響

Effects of Lennard-Jones potential parameters between the solute and solvent on the solvation dynamics

Yoshito Kondo, Tetsuro Nagai, Takuya Takahashi (*College of Life Sciences, Ritsumeikan Univ*)

It has been reported that mobility-increased water molecules exist around structure-breaker solutes experimentally. They have larger translational self-diffusion coefficients than a water molecule in bulk has.
In this study, we seek for a set of suitable Lennard-Jones potential parameters of interactions between solvent and solute based on TIP5P water model to more quantitatively reproduce the dynamical behavior of water molecules. In addition, we analyze the radial distribution functions and the number of hydrogen bonds 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 MD および QM 計算による水和水のダイナミクスと電荷計算

MD and QM calculations of dynamics and charges of hydration water

Takuya Takahashi (*College of Life Sciences, Ritsumeikan University*)

Classical MD simulations of water molecules around several types of solutes (poly-phosphates, AMP, and monovalent ions) were done with modified TIP5P water model. The hydration water molecules around mono-phosphate and di-phosphate molecules with a moderate negative charge showed increased mobility compared with bulk water and the results were partly consistent with the experimental results. Then, QM calculations were done with Gaussian software. In case of the tri-phosphate molecules, the calculated absolute charge of water oxygen atom was smaller as the solute charge became negative and the tendency was not changed in several calculation conditions such as different system size, basis function and chemical model.

3P131 カタユウレイボヤ未受精卵に一過的低張刺激を与えると、幼生での左右非対称性が乱れる

Brief hypo-osmotic treatment on eggs disrupts the left-right asymmetry of the larvae in Ciona intestinalis

Shimpei Katsumoto, Kohei Hatta, **Masashi Nakagawa** (*Grad. Sch. Sci., Univ. Hyogo*)

Ascidian Ciona intestinalis tadpole larvae exhibit left-right asymmetry. Neurula embryos transiently rotate within the chorion. In tailbud embryos, the Ci-pitx gene is expressed in the left-side epidermis. The photoreceptors are situated only on the right side. We found that a brief hypo-osmotic treatment before, but not after, the neurula stage resulted in bilateral expression of Ci-pitx in tailbud embryos and the randomization of the location of photoreceptors in larvae. This treatment impaired the transient counterclockwise rotation within the chorion at the neurula stage. These results suggest that dead test cells blocked the neural rotation and impaired left-right asymmetry.

3P132 How To Achieve Sequential Local Folding of Epithelial Tube in Epididymis Development: Experimental and Mathematical Study

Tsuyoshi Hirashima, Ryoichiro Kageyama (*Inst. for Virus Research, Kyoto Univ.*)

In the murine epididymis development an epithelial tube of epididymis shows sequential folding in its head region locally although it remains unclear what is required for the morphogenesis of epididymal tube in the cellular level. Our mathematical model, in which the tube is represented by a bead-spring chain, predicts two sufficient conditions for the sequential local folding: local mitosis in the head region and high viscosity of a tissue surrounding in the tube. We confirmed experimentally that there are more mitotic cells in the head region than in the tail region and myofibroblasts that can produce the viscosity surround in a periphery of the tube. Furthermore, some perturbation experiments such as a transfection or an inhibitor assay verify the model prediction.

3P133 アフリカツメガエル卵母細胞における全 ATP 量の測定と ATP ライブイメージング

ATP quantification and live-imaging in *Xenopus laevis* oocyte

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ATP plays various physiological roles; however, ATP metabolism in *Xenopus* oocyte has not been studied fully yet. First, we quantified global ATP in an oocyte using luciferase reaction-based reagent. The result did not show significant difference between immature and mature oocytes. Next, we set up an ATP live-imaging system using FRET-based indicator, ATeam. To observe its fluorescence, we prepared translucent immature oocytes that were injected with ATeam protein. Under fluorescence microscopy, the oocytes displayed strong FRET without background. Importantly, the FRET consistently increased in response to the addition of ATP, suggesting that ATeam works in the translucent oocytes. Local ATP distribution during oocyte maturation is currently under investigation.

3P134 ミオシンの金電極表面への吸着過程の粘弾性解析
Viscoelastic analysis of myosin adsorbed to gold

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We observed the adsorption process of myosin to the gold surface by QCM (quartz crystal microbalance). Viscoelastic property of the myosin adsorbed to the surface of the gold electrode and its surrounding solution as a whole was studied using the AFFINIXQN Pro (Initium, Tokyo),
When myosin adsorbed more sparsely than 0.2 ug/cm^2, viscoelastic change accompanied with myosin adsorption was almost the same as to a solid globular protein. On the other hand, when myosin adsorbed at higher density, large viscoelastic change has been obserbed. Viscoelastic analysis indicates that myosin plays as a protein having viscoelasticity, and that binding ATP or ADP to myosin head changes the viscoelasticity of the protein.

3P135 ホッキ貝柱筋と牽引筋の天然アクトミオシン (NAM=M+A+TM1 or TM2)の「Mg-ATPase 活性の Ca 依存性」と「TM アイソフォーム TM1 と TM2 の組成比」
Hokki clam retractor muscle NAM Mg-ATPase activities and seasonal changes of TM1 and TM2 isoform contents

Yoichi Yazawa (*Univ. Hokkaido Education*)

We have studied about protein components actomyosin(NAM)of retractor and adductor muscles of Hokki clam,*Spisula sacchalinensis*.
In autumn (September~November), Ca sensitivities of NAM were (15~50%). M.W. of TM2 was 43.000. Them of NAM prepared in other three seasons were higher (88~97%). M.W. of TM1 was 32,000.
TM2 were found in this report for the first time.

3P136 中性子及び X 線散乱による F アクチン水和水の構造・ダイナミクス解析
Characterization of the structural and dynamic properties of hydration water around F-actin detected by neutron and X-ray scattering

Tatsuhito Matsuo[1], Toshiaki Arata[2], Toshiro Oda[3], Satoru Fujiwara[1] ([1]*QuBS, JAEA*, [2]*Grad. Sch. Sci., Osaka Univ.*, [3]*Grad. Sch. Sci., Univ. Hyogo*)

The structural and dynamic properties of hydration water around F-actin and myosin S1 were investigated using small-angle neutron/X-ray scattering and quasi-elastic neutron scattering. S1 was shown to have typical hydration water, which has 10-15% higher average density with lower mobility than bulk water. On the other hand, F-actin was shown to have hydration water with unusual properties: the average density of the hydration water is at least 19% higher than that of bulk water and mobility is close to that of bulk water. These results indicate the diversity of hydration shell around proteins in terms of both structural and dynamic properties. The unusual hydration water around F-actin may be related to the suggested existence of “hyper-mobile water” around F-actin.

3P137 ワタリガニ骨格筋における細いフィラメントの精製および低温電子顕微鏡法による構造解析
Isolation of native thin filament from skeletal muscle for structural analysis by cryoEM

Yurika Yamada[1], Takashi Fujii[2], Keiichi Namba[1,2] ([1]*Graduate School of Frontier Bioscience, Osaka University*, [2]*QBiC, RIKEN*)

Muscle contraction is caused by sliding between the thick and thin filaments by repeated association and dissociation of myosin head and actin and is regulated by intracellular Ca2+ concentration. The thin filament is composed of actin filament, tropomyosin (Tm), and troponin (Tn) complex (TnC, TnI, TnT). Ca2+ released from sarcoplasmic reticulum binds to TnC, leading to a conformational change of Tm on the actin filament. To understand the regulatory mechanism of muscle contraction by electron cryomicroscopy, we developed a method to isolate skeletal muscle thin filament from a crab, Portunus trituberculatus. The result of image analysis showed that only 25% of the isolated thin filaments were fully decorated with Tm and Tn.

3P138 マルハナバチ飛翔筋トロポニン I の長い延長部の構造的役割
The structural role of the Pro-Ala-rich extension of the troponin-I of bumblebee flight muscle

Hiroyuki Iwamoto, Naoto Yagi (*SPring-8, JASRI*)

The structural role of the unusually long extension of insect flight muscle (IFM)-specific isoform of troponin-I was examined by X-ray diffraction and electron microscopy. Electron microscopy revealed that, after enzymatic removal of the extension from bumblebee IFM, a substantial fraction of the thin filaments were at the trigonal position between three thick filaments, like in vertebrate skeletal muscle, while the rest of the thin filaments remained in their original position (midway between two neighboring thick filaments). This result explains the dramatic change in X-ray diffraction patterns after removal of the extension (from insect-type to vertebrate-type), and suggests that its role is to keep the filament lattice in the correct configuration for IFM.

3P139 塩添加によるアクチン重合過程の研究
The salt-induced polymerization of actin

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Actin is one of the most important proteins in eukaryotic cells. Actin has two states, G-actin and F-actin. We are interested in the transformation of G- to F-actin. We recorded the time-resolved small angle scattering intensities during actin polymerization at SPring-8 BL45XU. The actin polymerization was initiated by mixing of salt to G-actin prepared in the low salt solution. The time courses of SAXS intensity profiles were analyzed by using the sequential polymerization model. We concluded that actin formed dimer immediately after mixing of salt, and growth of filament was initiated when pentamer was formed by chance.

3P140 自己組織化生体システムの機能創発機構解明に向けたメソスケール反応場のデザインと単分子分解能計測

Designing of self-assembled biomolecular system and the detection at the single molecule resolution

Mitsuhiro Iwaki[1,2,3], Keigo Ikezaki[1], Toshio Yanagida[1,2], William Shih[3] ([1]*RIKEN, QBiC*, [2]*Grad. Sch. Frontier Biosci., Osaka Univ.*, [3]*Harvard Medical School*)

Biological system is unique in that component biomolecules are self-organized and emerge adaptable, stable and energy-saving functions. To understand the essential condition for reproducing the properties and constructing stable system from unstable flexible components, we are designing artificial muscle using myosin and DNA nanostructure. System size, spatial positioning, degree of intrinsic noise and the mechanical communication in the synthesized muscle can be modulated and we'll observe the internal dynamics and the system behavior at the single molecule resolution. We have developed several novel tools to modulate the system and monitor the internal dynamics with high precision.

3P141 ナノスリット基板を用いたアクチンの重合の観察

Observation of actin polymerization in linear zero-mode waveguide

Masamichi Yamamoto[1], Makoto Tsunoda[1], Shun Higano[2], Kotaro Okubo[2], Takashi Tanii[2], Takashi Funatsu[1] ([1]*Grad. 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, it is not possible to observe actin polymerization at a single molecule level by TIRFM. Hence, we applied a linear zero-mode waveguide (ZMW), which has a 100-nm wide slit structure, allowing single molecule imaging at higher concentrations. We found that single BODIPY-FL-labelled actin was observed in a linear ZMW at 100 nM. F-actin was immobilized in a linear ZMW. Actin elongated in a linear ZMW at a similar elongation rate on a cover glass. Actin polymerization at a single molecule level in a linear ZMW is now under investigation.

3P142 中性環境における好アルカリ性 *Bacillus* 細菌が持つ Na^+駆動型べん毛モーター固定子の遊泳低下に関与するアミノ酸残基の探索

Critical amino acid residues for motility of the Na^+ -driven flagellar motor stator in alkaliphilic *Bacillus* decrease at neutral pH

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Alkaliphilic *Bacillus pseudofirmus* OF4 has a MotPS complex as a stator and the flagellum is driven by a Na^+-motive force. Previous studies showed that strain OF4 swimming is dependent on Na^+ at pH 8-10, but exhibited poor motility at neutral pH even in the presence of Na^+. It was hypothesized there could be competitive inhibition by H^+ of the Na^+translocation by the stator-force generator MotPS. In contrast, *B. subtilis* has a MotPS complex similar to strain OF4, but it was reported that *B. subtilis* can swim dependent on Na^+ concentrations at neutral pH. We investigated important amino acid residues for the motility decrease at neutral pH of the alkaliphilic *Bacillus*. Critical amino acid residues for reduced motility at neutral pH were identified in the MotP subunit.

3P143 アクトミオシン複合体におけるミオシン・サブフラグメント１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 swinging lever-arm motion of a myosin subfragmnet-1 in an actomyosin complex

Tadashi Masuda (*Fukushima Univ.*)

Molecular dynamics simulation was conducted for an actomyosin complex consisting of a myosin subfragment-1 and seven actin monomers solved in water. External force was applied to the end of the neck domain in the direction opposite to the power stroke by using the "pull code" in the GROMACS software.

The myosin neck domain showed a swinging lever-arm motion from the post-power stroke position to the pre-power stroke position, while the myosin head did not detach from the actin filament. This motion was in accordance with the myosin power stroke mechanism named "Driven by Detachment (DbD)" theory, which assumes that the power stroke is caused by the elasticity at the joint between the head and the neck domains and is not directly related to ATP hydrolysis.

3P144 Nonequilibrium dissipation-free transport of F1-ATPase and the thermodynamic role of asymmetric allosterism

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Recent experiments on F1-ATPase have clarified that the dissipative heat inside the motor is very small, irrespective of the velocity of rotation and energy transport. In this presentation, we focus on the problem that the amount of internal dissipation is not simply determined by the sequence of equilibrium pictures, but also relies on the truely nonequilibrium aspect. We show that the totally asymmetric affinity model, where ATP binding to F1 is assumed to have low dependence on the angle of the rotating shaft, produces results that are most consistent with experiment. The principle adopted in the model is simple enough to be considered generic in molecular motors, and may help providing a blueprint for artificial nano-machines.

3P145 エフェクター分泌機構解明を目指した細菌Ⅲ型分泌装置の回転-分泌相関の解析

Correlation analysis between rotation and secretion of bacterial type III secretion system for elucidate of effector secretion mechanism

Takashi Ohgita, Naoki Hayashi, Susumu Hama, Naomasa Gotoh, Kentaro Kogure (*Kyoto Pharm. Univ.*)

Bacterial type III secretion apparatus (T3SA) is a secretion-machinery for effector proteins, which involved in infection or toxicity. The secretion mechanism is still unknown. Based on the high similarities between T3SA and flagellum, we hypothesized that T3SA would secrete effectors via the proton-motive force-dependent rotation of T3SA needle like flagellum. Thus, we attempted to observe T3SA rotation, and evaluate the relationship between rotation and effector secretion. As the result, we succeeded in observation of T3SA rotation in situ on Pseudomonas aeruginosa, and found out its proton motive force dependence and its correlation with effector secretion. These results suggested that T3SA secretes effectors via the proton-motive force-dependent rotation of T3SA.

3P146 Simultaneous tracking of multiple motor proteins in nanoscale

Taishi Kakizuka[1], Keigo Ikezaki[2], Hideaki Fujita[3], Taro Ichimura[2], Tomonobu Watanabe[1,2] ([1]*Grad. Sch. FBS., Univ. Osaka*, [2]*QBiC, Riken*, [3]*WPI,iFReC, Univ. Osaka*)

Recent advances in single molecule tracking technique using optical microscopy have provided us valuable information on linear motor proteins. However, these techniques cannot achieve simultaneous observation of multiple molecules working in nanoscale because of limitation of the spatial resolution. In this study, we have developed a high-speed multiple-molecule tracking technique especially for linear motor protein study, which uses multi-color labeling and polychromatic detection. We succeeded in simultaneous tracking individual myosin molecules walking on an actin filament with nanometric precision and 10-millisecond temporal resolution. This technique could become a helpful tool to reveal the cooperativity of multiple motor proteins such as muscle myosins.

3P147 クライオ電子顕微鏡法を用いたアクチンミオシン硬直複合体の高分解能密度マップ取得への試み
Approach to obtain near-atomic resolution map of actin-myosin rigor complex by cryo-EM

Norihiro Shimizu[1], Yoshihiro Tsukada[1], Takuo Yasunaga[1,2] ([1]*Kyushu Inst. of Tech.*, [2]*JST*)

Actin-myosin interaction is involved in muscle contraction and intercellular transport, but the interaction mechanism in detail hasn't been figured out yet. Other researchers have previously reported their 8~13 Å resolution density maps, but the resolutions weren't high enough to discuss the actin-myosin interaction at the atomic level. We also have been challenging to obtain high resolution maps beyond 4 Å by cryo-EM and helical particle analysis. We also processed 85 filaments (3000 segments) to estimate the dependency of the segment number on the resolution. We therefore found to need about 20000 segments with HITACHI EF2000 EM for ~4 Å. We once reported the 3D from 5500 segments. Thus we are trying to obtain more images to improve the resolution of 3D.

3P148 cryo-EM と MT ラベルを用いたクラミドモナス外腕ダイニン LC4 の位置決定
Determination of the location of chlamydomonas outer arm dynein LC4 by cryo-EM and metallothionein labelling

Reiko Chijimatsu[1], Haruaki Yanagisawa[2], Mingyue Jin[1,3,4,5], Takuo Yasunaga[1,3,4,5] ([1]*Kyushu Institute of Technology*, [2]*The University of Tokyo*, [3]*JST*, [4]*JST CREST*, [5]*JST SENTAN*)

Chlamydomonas outer arm dynein has three heavy chain (α,β,γHCs). Previous works reported that γHC contains calmodulin-like LC4, but its detailed location are unknown. Thus we used cryo-EM and metallothionein (MT) labels to identify LC4's detailed location. We here used the strains whose LC4 is fused to MT. Crude dynein was prepared from the cells and fractionated by HPLC to be isolated into γHC and αβγHC, which were then incubated on ice with 10 μM Cd^{2+} overnight. Finally, isolated dynein under two types of ionic strength (600mM KCl, 50mM K-acetate) were observed by cryo-EM. As a result, we observed higher density that is due to MT binding Cd^{2+} and identified the position as LC4. Note the filamentous protein with 24nm repeat was observed under low ion strength.

3P149 ヒト遺伝性難聴(DFNA20/26)γ アクチン変異体とミオシンとの相互作用
Effects of human deafness mutations in gamma actin (DFNA20/26) on the actin/myosin interaction

Takashi Ohki[1], Shin'ichi Ishiwata[1,2] ([1]*Department of Physics, Faculty of Science and Engineering, Waseda University*, [2]*Waseda Bioscience Research Institute in Singapore*)

Mutations in non-muscle gamma-actin at the DFNA20/26 locus cause autosomal dominant non-syndromic sensorineural progressive hearing loss (ADNSHL), indicating that these mutations in some way disrupt actin function. However, the molecular basis underlying this actin-dependent hearing loss is unknown. To address this problem, we expressed the gamma-actin mutants, T89I, K118M, I122V, P264L, T278I, P332A, and V370A, in a baculovirus expression system. We focused on their interaction with myosin isoforms (classes II, V, and VI) that are expressed in hair cells and cochlea. At this meeting, we report their biochemical and biophysical properties in vitro.

3P150 ミオシン V 分子モーターの運動性に対する UV 照射の作用
Effect of UV irradiation on myosin V motility

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The question, how the molecular motors work, has not been elucidated based on the 3-D structures. The Relay helix, Relay loop and Switch-2 have been reported to be essential domains for force-generation and stepwise movement. Power stroke and recovery stroke are caused by conformational changes of these domains. And, some aromatic amino-acids existing in these domains are considered to play an important role. These aromatic rings absorb UV light because of its conjugated system. In this experiment, myosin V in the *in vitro processivity assay* under TIRFM was irradiated with UV light which is absorbed by tryptophan and tyrosine. We examined how the motility of myosin V, a processive molecular motor, was affected by UV irradiation.

3P151 Ncd の運動方向性の決定機構
The mechanism of determining the directionality of Ncd

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Kinesins are the unidirectional motor proteins. Among the kinesin superfamily, kinesin-1 moves toward the plus-end of the microtubule while kinesin-14 is the minus-end directed motor. A series of experiments using chimera motors, whose components are exchanged between kinsin-1 and Ncd, demonstrated that the neck region, adjacent to the motor core, was crucial for determination of the directionality. We here succeeded in changing the directionality of Ncd without any mutation but by linking the motor core to the glass surface via its C-terminus. We also engineered some directionality-altered motors. These results indicate that kinesins motor core has a default plus-end directionality and both the N-terminal neck and the C-terminus of Ncd make Ncd the minus-ended motor.

3P152 ダイニンは微小管上を短いピッチで回転しながら運動する
Dynein moves in a short-pitch helical path around a microtubule

Shin Yamaguchi, Junichiro Yajima (*Dept Life Sciences, Graduate School of Arts and Sciences, Univ. of Tokyo*)

Dynein is a microtubule (MT)-based motor protein. *Tetrahymena* axonemal outer arm dynein (OAD) rotated sliding MTs around their axis in an *in vitro* MT gliding assay, indicating that OAD generate torque. However, the MT gliding assay may not accurately quantify the torque generation of OAD, because a lot of OADs simultaneously act on a MT and rotational marker attached to MTs may be a steric hindrance to rotation. To overcome them, we tracked a moving bead coated with OADs along a MT anchored on an etched glass as a suspension bridge to investigate movement of a small number of OADs without hindrance to rotation. We find that the beads moved in a right-handed helical path, and the pitch is 0.8 μm, which is shorter than MT supertwist pitches.

3P153 破断力測定を用いた Kinesin-6 の力発生原理の研究
Investigating the torque-generating mechanism of kinesin-6 using unbinding force measurement

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Kinesin-6 (ZEN-4) is a microtubule (MT)-based motor protein which localizes at central spindle in metaphase cells. We previously found that ZEN-4 rotates sliding MTs in an *in vitro* 3-D gliding assay. This means that ZEN-4 generates forces not only along the longitudinal axis of MT but also along the rotational direction. To generate a unidirectional MT rotation, ZEN-4 is considered to tend to unbind from MT after a step against the direction of rotation and not to unbind after a step toward the direction of rotation. In this study, we focused on the unbinding force of ZEN-4-MT complexes and measured the unbinding force imposing external load in every direction with optical tweezers in order to reveal the mechanism of the generation of rotational force of ZEN-4.

3P154 細胞質ダイニンの自己阻害と協同的な活性化
Autoinhibition and synergistic activation of cytoplasmic dynein

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Cytoplasmic dynein is involved in a wide range of cellular activities. To perform diverse functions, dyneins are required to up- and down-regulated. However, single-molecule motility of mammalian cytoplasmic dynein has been controversial among research groups, which makes it unclear how dynein's activity is regulated and tuned for a specific function. Here, we show that single dyneins are autoinhibited, in which two motor heads are stacked together, whereas multiple dyneins can be activated when clustered on a cargo. Optical trapping suggested that this activation is mediated by dynein's unconventional force response, which leads to mutual activation among multiple dyneins. We propose that this synergistic property enables self-regulation depending on cellular context.

3P155 Bicaudal-D2 による微小管系輸送の制御機構
Regulatory mechanism of microtubule-based molecular motors by Bicaudal-D2

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Bicaudal-D2 (BICD2) is a dynein adaptor which plays an important role in intracellular transport. It has been suggested that BICD2 interacts with two microtubule-based molecular motors: kinesin and dynein. However, it remains unclear how BICD2 regulates these motors. To address the question, we here investigated interaction between BICD2 and the molecular motors in vivo and in vitro. In live-cell imaging, full-length BICD2 was observed as foci moving along microtubules. A series of deletion mutants of each domain showed that the mutants are localized toward cell edges or cell center, suggesting that the corresponding domains may be involved in interaction between kinesin or dynein motor. In vitro interaction experiments are now in progress.

3P156 ダイナクチン p150 の分子構造
Molecular architecture of dynactin p150

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Dynactin is known to be a regulator of cytoplasmic dynein and play a part in intracellular transport. Dynactin is a large complex composed of multiple subunits including p150, p50 and Arp1, and has a characteristic architecture. It has been shown that p150 forms a dimer and appears two-headed structure and thin rod which extends from the Arp1 rod as a side arm. Here, we investigated the molecular architecture of dynactin p150 by electron microscopy using recombinant human proteins expressed in HEK cells, and found that the dynein binding region of p150 forms a new protrusion from the head of p150. Our results provide a new insight into the dynein-dynactin interaction.

3P157 テトラヒメナ外腕ダイニン複合体のサブユニット構築
Subunit structure of *Tetrahymena* outer dynein arm complex

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Outer dynein arm (ODA) complex is a major component in cilia and drives its beating. *Tetrahymena* ODA complex is composed of three heavy chains (α, β, γHCs), two intermediate chains (ICs) and several light chains (LCs), but the exact subunit arrangement has not been determined.
We engineered a codon-optimized hEGFP-tag (EGFP inserted His-tag) and introduced hEGFP-tag at C-termini of ODA components by homologous recombination. Western blot and live cell imaging verified that hEGFP-tagged ODA component was incorporated into cilia. PCR analysis confirmed complete replacement of the target locus with the hEGFP-tagged sequence. By Ni-NTA-gold labeling and electron microscopy of the His-tagged ODA complex, we will elucidate the subunit architecture of *Tetrahymena* ODA complex.

3P158 ヒト細胞質ダイニンのパワーストローク測定
Power Stroke Measurement of Human Cytoplasmic Dynein

Yoshimi Kinoshita, Taketoshi Kambara, Satoshi Ikeda, Hideo Higuchi (*Department of Physics, Graduate School of Science, The University of Tokyo*)

Cytoplasmic dynein is a motor protein moving along microtubules toward the minus-end, and plays an important role in cellular processes. Previously we reported that dynein moves continuously along microtubule, and takes dominantly 8 nm step. The mechanism of dynein movement remains unknown. Here, we used dumbbell dual-trap optical tweezers instrument to measure the size of power stroke driven by conformational changes of single-headed dynein. We expressed the motor domain of human cytoplasmic dynein in Sf9 cells and purified by Flag-tag affinity. Mutant kinesin, T93N, was used for adhesion between microtubule and bead. We finished setting up the dumbbell instrument and started to measure the power stroke distance.

3P159 細胞質ダイニンの生物物理学的・生化学的解析
Biophysical and Biochemical characterization of human cytoplasmic dynein

Taketoshi Kambara, Yoshimi Kinoshita, Takayuki Nakayama, Hideo Higuchi (*Dept of Phys, Grad Sch of Sci, U of Tokyo*)

Dynein is a molecular motor that moves toward the minus-end of microtubules, and responsible for transporting various cargos, positioning the Golgi complex and mitotic spindles in cells. The motor properties of dynein are relatively well understood for yeast dynein, while those of human dynein are still obscure. Here, we performed biophysical and biochemical characterization of human cytoplasmic dynein. The maximum microtubule gliding velocity of the motor domain of dynein was 976 um/s with Km of 49uM for ATP. ADP was competitive inhibitor with Ki=135uM. When nucleotide was depleted from dynein, dynein was unable to bind to microtubules in the absence of ATP. Furthermore, dynein movement was rescued when ATP was added to nucleotide-depleted dynein, unlike kinesin.

3P160 骨格筋ミオシンの S1 および S2 部位と非線形弾性の関係性
Contribution of S1 and S2 portion of myosin to nonlinear elasticity of skeletal myosin molecules

Satoshi Ikeda, Motoshi Kaya, Hideo Higuchi (*Department of Physics, Graduate School of Science, the University of Tokyo*)

We had revealed the nonlinear elasticity of single myosins. Stiffness is greater when they are stretched, while it substantially decreases when they are compressed. In particular, it was suggested that the difference in elasticity between S1 and S2 portion of myosin may contributes to the nonlinearity of elasticity. However, the question of which part of myosin contributes to nonlinear elasticity remains unresolved. Hence, in order to clarify this question, we measured displacements of S1. Myosin heads are labeled by attaching gold nanoparticles to biotinylated regulatory light chain. Forces exerted on myosin are measured by optical tweezers. We will discuss the contribution of S1 and S2 elasticity to nonlinear elasticity of myosin.

3P161 ミオシン V の前進および後退ステップ機構
Mechanism of the forward and backward stepping motion of myosin V

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Myosin V is a two-headed molecular motor and moves processively along an actin filament toward its plus end in a hand-over-hand fashion. Single-molecule experiments have accumulated evidence that supports the idea that myosin V undergoes a lever-arm swing and a Brownian search to achieve its forward stepping motion. Myosin V takes also backward steps when large load forces are applied. The mechanism of how backward steps occur has not been clarified yet. We propose a simple theoretical model that can quantitatively explain experimental data not only on forward steps but also on backward steps. The model takes into account the lever-arm swing, the Brownian search, and the elasticity of lever arm.

3P162 Nucleotide turnover rates of bipolar myosin filament during actin filament sliding

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In in vitro motility assay, actin filaments moved at a fast speed towards the bare zone and at a slower speed away from the bare zone. Myosin heads in the reverse sliding might rotate 180 degrees so as to face the actin in the right configuration, and might be constrained so that the detachment of the rotated heads from actin would occur at slower rate. Currently, we have shown that ADP release step depends on the sliding direction of actin filament by measuring the displacement of fluorescent nucleotide bound to myosin heads using flash photolysis of caged-ATP. Furthermore, we try to observe whether ADP release rate changes in accord with the changes in the speed of actin sliding when its leading end begins to slide along the proper direction of myosin filament.

3P163 細胞性粘菌ミオシン II の SH1 ヘリックス領域の変異がその運動特性に与える影響
Effect of mutations in the SH1 helix region of Dictyosterium myosin II on the motile characteristics

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Point mutations in fast myosin heavy chain gene are known to cause a human myopathy and autosomal dominant diseases. In the present study, we examined the effect of mutations, glutamic acid to lysine at the 706th, arginine to cysteine at the 702nd and arginine to threonine at the 703rd, on the motile activities. These mutations are located at SH1 helix region, which has been thought to act as a linker for transmitting the structural changes of ATP-binding site in the catalysis domain to the lever arm. We have introduced the corresponding mutations into the SH1 helix of Dictyosterium myosin II (E683K, R686C and R689T), and measured the actin-myosin sliding velocity, thermal stability and the thermal aggregation of the myosin.

3P164 F1-ATPase β サブユニットの全原子溶媒和自由エネルギー解析

All-atom hydration analysis of the β subunit in F1-ATPase

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F1-ATPase is a protein complex to hydrolyze adenosine tri-phosphate (ATP). Within the complex, the part called β subunit carries the catalytic activity. In this study, we focus on the ATP binding of the β subunit and treat the β subunit isolated in solvent water. To separately treat the water and ATP effects on the binding, we conceptually decompose the binding process into two steps. The first step is the conformational change of the β subunit from the open to closed states. The second is the ATP binding to the closed state. The effect of water is taken into account at all-atom level using the molecular dynamics simulation coupled with the method of energy representation. We will show that the solvent water promotes the first step and inhibits the second.

3P165 腸球菌由来 V_1ATPase の軸強制回転シミュレーションによる回転機構の解明

Rotation mechanism of V_1-ATPase studied by steered MD simulations

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V-ATPase is a rotary molecular motor functioning as a proton pump in cell membranes. A soluble part of V-ATPase is called V_1, mainly composed of the A3B3 hexamer ring and the central stalk, D and F subunits. Recently, the high-resolution X-ray crystal structure of Entercoccus hirae V_1 ATPase was determined. Because the crystal structure is a snapshot of the dynamic processes in its functions, molecular dynamics (MD) simulations were required for elucidating the rotation mechanism. In this study, we performed the steered MD simulations, in which the central stalk was forced to be rotated. Conformational changes in the A3B3 hexamer ring in response to the stalk rotation were observed during the MD simulations.

3P166 Free energy simulations for the conformational change of the αβ subunits in F_1-ATPase after the ATP hydrolysis

Yuko Ito, Mitsunori Ikeguchi (*Yokohama City Univ.*)

F_1-ATPase is a rotary motor enzyme. A single molecule experiment has identified that after the ATP hydrolysis, one of the β subunits in the F_1-ATPase changes the conformation from the closed form to the half closed form. We performed ensemble sampling simulations to elucidate this structural change mechanism. The simulations were carried out with different nucleotide states. The obtained energy profiles agree well with the facts observed in single molecule experiments. Also, in the structural change from the open to closed form, we could obtain the half closed form (β_{HC}) in a minimum of the free energy surface.

3P167 回転モーター F_1-ATPase の化学状態ごとのポテンシャルエネルギー

The potential energy of the rotary motor F_1-ATPase for given chemical states

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F_1-ATPase is an ATP-driven rotary molecular motor that synthesizes ATP when forcibly rotated in reverse. The remaining challenge is to determine the potential energy that governs the mechanical conformational change in the motor. To measure the potential energy for rotation for given chemical states in the three catalytic sites, we controlled the rotation with magnetic tweezers by attaching a magnetic bead(s) to the central rotor, and simultaneously imaged the binding of fluorescently (Cy3) labeled ADPs and ATPs with TIRF microscopy. The torque was measured by the bead(s) displacement against magnets for the binding events of Cy3-nucleotides. We will report the individual potential which is estimated from integrating the torque for each chemical state.

3P168 F_oF_1-ATP 合成酵素による ATP 駆動のプロトンポンプ活性の定量測定

Quantitative assay of ATP-driven proton-pump activity of F_oF_1

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The F_oF_1-ATP synthase (F_oF_1) synthesizes ATP when proton motive force drives protons through F_o. As a reverse reaction, F_oF_1 can pump protons in the opposite direction by ATP-hydrolysis.Although ATPase/synthesis functions have been deeply investigated, the proton-pump activity has been poorly characterized, qualitative even in bulk assays. Here, we report quantitative estimation of the proton-pump activity of F_oF_1 of thermophilic origin reconstituted into liposomes. The activity was monitored with a pH sensitive fluorescent probe pH-rodo conjugated to phospholipid, and the buffering capacity of the system was carefully estimated in several conditions. We also measured the ATP-hydrolysis activity under identical conditions.

3P169 ドメイン交換による V_oV_1 の MgADP 阻害機構の解明

Analysis of the MgADP-inhibition mechanism of V_oV_1 by domain swapping approach

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V-type ATPase (V_oV_1) of *Thermus thermophilus* shows sensitivity to MgADP-inhibition, favorable for ATP synthesis reaction. Contrary, V_oV_1 from *Enterococcus hirae* and eukaryotes show insensitivity to MgADP-inhibition, favorable for continuous ATP hydrolysis. To clarify how these V_oV_1 show the different sensitivity to MgADP-inhibition, we produced domain swapping V_1 consisting of both *T. thermophilus* and *E. hirae* enzymes. Our results indicate that the domain-domain interaction in catalytic A subunit defines the binding affinity of V_1 for phosphate, which is critical for the sensitivity to MgADP-inhibition in V_1. Based on recent reports of crystal structures of both V_1, we discuss the molecular basis of MgADP-inhibition mechanism of V_oV_1.

3P170 揺らぎの定理による V-ATPase のトルク測定：F サブユニットの働き

F-subunit reinforces torque generation in V-ATPase

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V_1, a water soluble domain of V-ATPase (rotary ATPase) is a rotary motor protein and the minimal rotary unit of V_1 is A_3B_3D. The rotor D-subunit is likely considered to be a structural analog of γ-subunit of F_1 although it lacks a globular domain unlike γ-subunit. To investigate how the lack of a globular domain affects on the rotary motion of V_1, we performed a single molecule experiment and measured the torque of A_3B_3D. We found that A_3B_3D produced significantly low torque compared with V_1, and recovered the high torque value only when globular shaped F-subunit was fused with D-subunit. The torque was measured precisely by using the fluctuation theorem that is the recently proposed method in the field of non-equilibrium statistical mechanics.

3P171 Binding interface between rotor subunits with low binding affinity in V_oV_1

Atsuko Nakanishi, Jun-ichi Kishikawa, Ken Yokoyama (*Kyoto Sangyo University*)

The central rotor complex of V_oV_1 of *Thermus thermophilus* is composed of V_1-DF shaft and V_o-CL_{12} rotor ring. ATP synthesis/hydrolysis in V_1 is coupled with proton translocation in V_o by rotation of central rotor complex relative to a surrounding stator apparatus.
In this study, we directly demonstrate that low binding affinity of V_1-DF for V_o-CL_{12} by both FRET and reconstitution experiments. Our results conflict with an idea that the binding interface between DF and C subunit should be tight sufficient for energy coupling between V_1 and V_o. A unique mode for the binding interface between rotor subunits should be necessary to explain the torque transmission mechanism in V_oV_1.

3P172 マイクロピラーアレイ上で成長したフィブロブラスト細胞の大きさと形状

Size and Shape of Fibroblast Cells Growing on a Micro Pillar

Takuya Tsukagoshi, Uijin G. Jung, Hidetoshi Takahashi, Tetsuo Kan, Kiyoshi Matsumoto, Isao Shimoyama (*The University of Tokyo*)

We investigated human fibroblast cells (ATCC CRL-2097) growing on silicon micro pillars to study mechanical properties of the cells, such as locomotion, adhesion, and elasticity. Using a silicon-on-insulator wafer, we fabricated micro pillars (20 μm in width), which were approximately equal in size to a fibroblast cell before its adhering to the substrate. The cells were able to bridge geological gaps until 15 μm between the pillars. For larger gaps, the cells sank down to the bottom of the gap. Once the cell clings to the top of the pillar, it could grow over a gap onto the next pillar. The height of the pillars (35 μm) enabled us to know the vertical location of the cells. Our results will contribute to ways to know mechanical properties of cells on microstructures.

3P173 細胞および接着分子の極性の人為的制御

Artificial control of the polarity of cells and molecular assemblies

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The subcellular localization of individual focal adhesions, a molecular assembly necessary for cell adhesion, determines the cell morphology; however, the molecular and biophysical mechanisms remain unclear regarding how the localization is determined. Here we observed that cells exhibited marked Rac1-dependnet membrane ruffles when the polarity of focal adhesions was artificially controlled, using our novel micro-patterning technique, not to be consistent with that of the cells. Thus, our results suggested that focal adhesions align preferentially along the cellular long axis because of the high efficiency for inactivating Rac1, which in turn activates RhoA and then promotes cell adhesion maturation.

3P174 Study on membrane microfluidity of living cells using Muller Matrix microscopy

Yudai Kosaka, Tetsuhiko Ohba (*Grad. Sch. Sci., Univ. Tohoku*)

It is widely accepted that membranes of living cells haveinhomogeneous fluidity but direct measurements on such heterogeneityof membrane fluidity are scarce. Recently we developped a MuellerMatrix imaging system which can measure any polarization changes bythe sample at optical microscopic resolution. It operates two measurement modes; one is the transmission mode which can visualize optical anisotropy, e.g. retardance and its direction, and the other is the epi-fluorescence mode which can visualize local orientation and rotational diffusion rate of fluorophores, We applied this apparatus to Swiss 3T3 fibroblast cells. The results will be discussed on poster presentation.

3P175 負荷をかけた状態での単離マウス気管上皮シリアの三次元運動

Three-dimensional motion of an isolated murine tracheal cilium under load

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Beating of airway epithelial cilia generates fluid flow to protect the mammalian respiratory system from harmful inhaled materials. Thus their response to the external force is crucial to understanding of the mechanism of regulated strokes. To address this point, we here combined 3-D tracking microscopy with optical tweezers. A fluorescent bead was attached to the tip of the isolated tracheal cilium, and trapped with various power of the infrared laser in the presence of ATP. The bead escaped from the trap when the low laser power, < 20 mW, was applied, whereas it showed irregular oscillation within the range of 350 nm under the higher power of laser. The apparent maximum force is estimated as 15pN, and the periodic beating was randomized under that load.

3P176 ケラトサイトと好中球と粘菌の遊走のための異なるメカノセンシング機構

Mechanical responses of keratocytes, neutrophils and *Dictyostelium* cells for their optimal migrations

Chika Okimura[1], Takafumi Mizuno[2], Yoshiaki Iwadate[1] ([1]*Faculty of Science, Yamaguchi University*, [2]*Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)*)

Crawling migration is achieved even under no external attractants. How do cells generate their migrating polarity? Cells adhering to substrata must receive and respond to mechanical stimuli from the substrata. The mechanical force is a potent candidate which regulates migrating polarity.
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 keratocytes, neutrophil-like HL-60 cells and *Dictyostelium* cells are commonly used as materials for investigation of fast crawling cell migration. In response to the CSS, they showed different migrations. We will discuss the difference dependent on cell types and molecular dynamics in each cell type.

3P177 マイコプラズマは左回りに進むのか？

Do Mycoplamsas glide to the left?

Hanako Morio, Taishi Kasai, Makoto Miyata (*Grad. Sch. Sci., Univ. Osaka City*)

Mycoplasma mobile, a fish pathogenic bacterium, forms a membrane protrusion at one pole and glides in its direction. The gliding does not occur along a straight line. We checked the gliding direction and found that 77% cells glide with curving to the left. The units of gliding machinery may align helically around the cell neck and generate the propelling force biased from the cell axis. The gliding machinery pulls the other part of cell, and generates the drag force at other cell parts, resulting in the bias in gliding direction. We traced a 200 nm bead artificially bound to the cell, and found that the cell does not rotate around the axis. This may be explained by assuming that the legs always pull the cell body rather than row.

3P178 微小管 X 線繊維回折：チューブリンピッチの動的変化

Dynamic changes of the axial pitch of tubulin repeat in live microtubules revealed by x-ray fiber diffraction

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Microtubule (MT) is one of the key components of eukaryote cytoskeleton. Our question is how MT assembly/disassembly dynamics is correlated with the conformation of tubulin dimers inside MT. The technique we developed for the quick shear-flow alignment of biological filaments enabled us to analyze the x-ray fiber diffractions of MTs under various physiological conditions. We found that MTs were classified into 3 groups of distinct structural properties, which varied depending on GTP-hydrolysis and taxol content. Interestingly, the axial pitch of tubulin repeat of GMPCPP-MTs became longer by lowering temperatures. It is strongly suggested that even in assembled MTs, both GTP- and GDP-tubulin dimers can undergo dynamic conversion between short- and long- configurations.

3P179 生細胞内における厳密な PI3K ヘテロダイマー複合体のシグナル応答

Dynamic Signal Response of Rigorous PI3K Heterodimer in Living Cells

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In mammals, class IA phosphoinositide 3-kinases (PI3Ks) consist of a p110 catalytic subunit (p110a or p110b) bound to any of five regulatory subunits including p85a and p85b. It has been suggested previously that free p85a negatively regulates PI3K signaling by competition with p85/p110 heterodimers for recruitment to phosphotyrosine residues of ErbB. In contrast, it was recently shown by quantitative mass spectroscopy that p85 and p110 subunits are present in equimolar amount in mammalian cell lines suggesting the nonredundant complex formation between PI3K subunits. However, compositions of PI3K subunits have never been studied in vivo conditions. Here, we for the first time detect spatio-temporal dynamics of the interactions between PI3K subunits in living cells.

3P180 Cell signaling occurs by a specific mobility and clustering state of epidermal growth factor receptor

Michio Hiroshima[1,2], Yasushi Sako[2] ([1]*RIKEN QBiC*, [2]*RIKEN Cellular Informatics Lab.*)

An information-theory based analysis combining with fluorescence single-molecule imaging showed that epidermal growth factor receptor (EGFR) moves across the plasma membrane with rapid transitions among several mobility and clustering states. EGF stimulation induced an equilibrium shift to the state of slowest diffusion and larger cluster where interactions between EGFR and Grb2 adaptor protein occurred significantly more frequent compared to other states. Receptor molecules in the state undergo confined diffusion and the diffusion characteristics were affected by membrane cholesterol depletion. Therefore, EGF signaling arises through dynamic interactions between the receptor molecules in a specific state susceptible to membrane components.

3P181 Detection of Cellular Responses to a Differentiation Factor Using Raman Microspectroscopy

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Raman microspectroscopy is one of powerful methods to obtain comprehensive information about molecular compositions in living cells. We measured Raman spectra in the cytoplasm and nucleus of human breast cancer derived MCF-7 cells at 0-2 hours of heregulin (HRG) stimulation. HRG induces differentiation of MCF-7 into mammalian grand-like cells. Average and standard deviation of the spectra from HRG-stimulated cells were compared with those from control cells. In the HRG-stimulated cells, decreases of lipid and protein fractions were observed with an increase of water fraction. This result suggests that cell morphological changes were induced, and/or concentration of intracellular molecules was diluted by HRG stimulation.

3P182 蛍光イメージング法による機能的べん毛モーターと走化性シグナル伝達分子 CheY の結合の直接的観察

Direct imaging of the rotational switching of a functioning 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 signaling system, the binding of signaling molecule, CheY-P, to a bacterial flagellar motor is believed to induce the rotational switching of a motor. However, the rotational switching by binding of CheY-P has not been directly showed in a functioning motor. In this study, by direct imaging of CheY-GFP, we demonstrated that the binding and dissociation of CheY-P induce CW and CCW rotation of a motor, respectively. It was found that ~10 CheY-P molecules bind to and dissociate from a motor within about 100 ms during switching. Thus we succeed in measuring a molecular process of signal transduction occurred on subsecond and clarified CheY-P that is produced by receptor-kinase cluster directly regulates the switching of motor via the binding of CheY-P.

3P183 細胞における核小体タンパク Nucleophosmin 1 の可視化

Imaging a nucleolar protein, Nucleophosmin 1, in living cells

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], Kaoru Katoh[1,2] ([1]*Biomed. Res Inst, AIST*, [2]*Grad. Sch. Live & Env. Sci., Univ. Tsukuba*, [3]*RIKEN ASI*, [4]*RIKEN QBiC*)

Nucleophosmin 1(NPM1) plays important roles in ribosome biogenesis, nucleocytoplasmic transport, transcriptional regulation, and DNA duplication. Increase in mutation of NPM1 is also observed in leukemia. Thus, many researchers focused their attention to dynamics of NPM1 and observed them with fluorescence microscopy and by immunoelectron microscopy, although detail of moving NPM1 complex remains still unknown.
Here, we will present moving NPM1 complexes can be observed with pupil projection apodized phase contrast(PPAPC) microscopy without any staining. Interactive observation of electron and optical microscopy will reveal fine structure of moving NPM1 complexes. Molecules involved in the NPM1 complexes will also be demonstrated in this presentation.

3P184 シグナル伝達タンパク質ＥＲＫ２の情報処理を介した細胞運命決定の定量解析

Cell fate decisions through information processing of a signaling protein ERK2

Kazunari Mouri, Yasushi Sako (*Cellular Informatics Lab., RIKEN*)

The heterogeneity that arises from stochastic fate decisions has been reported for several clonal cells. Focusing on the PC12 cell fates, we have found that phenotypic heterogeneity in a population of cell started from the proliferative state increased with time under constant serum and growth factor conditions, suggesting stochasticity in single cells. To clarify origins of this heterogeneity, we tracked the dynamics of nuclear translocation of ERK2-GFP, which is a key protein in cell fate decision, for several days after activation by a growth factor NGF under a fluorescence microscope. We applied statistical analyses to evaluate how much each cell fate is determined by ERK2 activity and whether fluctuations in ERK2 activity cause heterogeneous cell fate decisions.

3P185 情報処理タンパク質 RAF の多状態性と細胞応答

Polymorphism of a signaling protein RAF regulates cellular responses

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

Conformational polymorphism is important for the regulation of protein functions and cell-signaling. The intracellular signaling molecule RAF is a polymorphic protein involved in cell-proliferation, differentiation, and carcinogenesis. From single-molecule imaging and the spectrum analysis of RAF-FRET probes in single cells, we found that RAF adopts various conformational states that relate to multiple phosphorylations. Interestingly, based on the ensemble average of RAF conformations, cells could be categorized into one of five states, each of which showing different responses of RAF activation to stimulation. We conclude that RAF has cell-memory which determines the RAF conformation and therefore has the potential to predict the cellular response prior to stimulation.

3P186 Quantitative analysis of signal transduction dynamics between Raf and ERK in living single PC12 cells

Yuki Shindo[1,2], Kazunari Iwamoto[2], Kayo Hibino[2], Kazunari Mouri[3], Yasushi Sako[3], Koichi Takahashi[2] ([1]*Syst. Biol. Prog. Grad. Sch. Media & Governance, Keio Univ.*, [2]*RIKEN QBiC.*, [3]*Cell. Inform. Lab., RIKEN*)

ERK is one of the most major MAPKs and its dynamical behavior regulates cell fate decisions such as growth and differentiation in PC12 cells. By using live cell fluorescence imaging analysis, we previously found that ERK activation which was characterized as nuclear translocation in response to growth factor stimulation varied extensively among individual cells. To investigate the mechanisms that induce such heterogeneity, we focused on membrane translocation dynamics of Raf that is the MAPKKK in the ERK MAPK cascade. By using the hybrid system of confocal laser scan microscopy and TIRF microscopy, dynamics of Raf and ERK were observed simultaneously in living single cells, unveiling tight associations between dynamics of Raf and resulting ERK responses.

3P187 SOS を介した Ras 活性 positive feedback 調節の生細胞一分子解析

Positive feedback regulation of SOS-mediated Ras activation detected by single-molecule analysis in living cells

Yuki Nakamura[1,2], Kayo Hibino[3], Yasushi Sako[2] ([1]*Grad, sch, FBS., okasa Univ*, [2]*wako inst., Riken*, [3]*QBIC., Riken*)

A small GTPase Ras is a crucial transducer of proliferation and differentiation signals. The inactive form of Ras binding GDP is converted into the active form binding GTP by a guanine nucleotide exchange factor, Son of Sevenless (SOS). *In vitro* studies have suggested that an interaction between SOS and Ras-GTP generates a positive feedback loop for Ras activation. However it is unclear whether the positive feedback functions or not in living cells and, if so, how it affects SOS dynamics. In this study, we observed SOS molecules on the plasma membrane by single-molecule imaging. The positive feedback functioned to prolong both the dwell time of individual SOS molecules on the membrane and ensemble molecule activity of SOS in living cells after EGF stimulation.

3P188 海洋性ビブリオ菌のべん毛形成抑制に関与する DnaJ モチーフを持った SflA の細胞内局在

The intracellular localization of SflA, the dnaJ family protein that plays a role in the suppression of flagellation in Vibrio

Takehiko Nishigaki, Noriko Nishioka, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

Bacterial flagella are formed in various numbers and locations in different kinds of bacteria. A Vibrio alginolyticus strain VIO5 that lacks lateral flagella, has a single polar flagellum whose number is regulated positively by FlhF and negatively by FlhG. The ΔflhFG strain, derived from VIO5, has mostly no flagellum, but the ΔflhFGΔsflA triple deletion mutant had peritricous flagella, so SflA suppressed formation of polar flagellum in lateral positions. Here, we showed the localization of SflA. In ΔsflA strain, which was the same phenotype as VIO5, GFP-SflA was localized at the cell pole, while in ΔflhFGΔsflA strain, GFP-SflA was localized on the surface of cells. This may suggest that SflA interact with FlhF, FlhG, or some other proteins at the flagellar basal body.

3P189 Structural analysis of the flagellar basal body in intact cell 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 and swims by its rotation as a screw propeller. 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 by electron cryotomography in the mini cell produced by FtsZ overproduction. We are also trying to look at the structure of the basal body isolated from the cell to clarify the details by merging information from these two structures. We will discuss structural features of the sodium driven *Vibrio* motor in comparison with the proton driven motor of *Salmonella*.

3P190 Biochemical properties of FlhG, a negative regulator for the number of the polar flagellum in Vibrio alginolyticus

Akari Takashima, 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, a MinD homolog and putative ATPase, interacts with FlhF to prevent FlhF from localization at cell pole. We recently found that mutations in the ATPase motif of FlhG affect its function (see, poster for Ono et al). To investigate the relationship between ATPase activity and FlhG function, we overproduced and purified recombinant FlhG in E. coli. Purified FlhG tends to aggregate, and stable protein requires high concentration of NaCl. Size exclusion chromatography showed that purified FlhG behaves as a monomer in solution. ATPase activity will be investigated and discussed in the meeting.

3P191 Stator activation requires conformational change in the periplasmic region of PomB, a Na^+-driven stator protein

Shiwei Zhu[1], Masato Takao[2], Na Li[1], Mayuko Sakuma[1], Michio Homma[1], Seiji Kojima[1], Katsumi Imada[2] ([1]*Nagoya University*, [2]*Osaka University*)

The energy for the flagellar motor, which is a H^+ and/or Na^+ ion flux, is provided only when a protein complex called stator is incorporated into the motor. In order to understand the activation mechanism for the Na^+-driven stator, we determined the crystal structure of a periplasmic region of PomB. The structure suggests that the stator activation requires a conformational change of this region. Here we tested the hypothesis by engineered disulfide-bridge formation. Results showed that the disulfide bridges reversibly inhibit stator function without affecting the assembly of stators around the rotor. Thus, we conclude that the conformational change in the periplasmic region of PomB required for stator activation occurs after the stator incorporation into the motor.

3P192 細菌べん毛輸送装置構成蛋白質 FlhA の変異に対するロバストネス

Mutational robustness of FlhA, a subunit of the bacterial flagellar export apparatus

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For assembly of the bacterial flagellum, most flagellar proteins are exported to the distal end of the growing structure by a specific export apparatus composed of an export gate and an ATPase complex. FlhA is a subunit of the export gate and plays a role in the energy coupling mechanism along with the ATPase complex. It has been shown that mutations of highly conserved charged residues of FlhA are tolerated, but result in loss-of-function when FliH and FliI are missing. In this study, we carried out cross-complementation analysis of FlhA. We show that Vibrio alginolyticus FlhA fully complements a Salmonella flhA mutant in the presence of FliH and FliI but not at all in their absence. We will discuss the molecular basis of the mutational robustness of FlhA.

3P193 細菌べん毛本数を負に制御する MinD と相同性をもつ FlhG の ATPase モチーフの役割

Role of ATP binding motif of FlhG, a MinD homolog, which regulates the number of the polar flagellum in *Vibrio alginolyticus*

Hiroki Ono, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

Bacterial flagella are generated in wide variety of locations and numbers. Marine bacterium *Vibrio alginolyticus* has a single polar flagellum whose number is regulated positively by FlhF and negatively by FlhG. FlhG interacts with FlhF to prevent FlhF from localization at cell pole. FlhG is the homolog of bacterial cell division inhibitor (MinD) and putative ATPase. To know the role of FlhG in molecular level, we mutated its putative ATPase motif. FlhG mutants affected motility, flagellation and subcellular localization. From the results, ATPase motif of is important for FlhG function, especially for its localization at the cell pole that affects negative regulation of flagellar number. FlhF localization and interaction with FlhG mutants will be investigated.

3P194 Functional chimera of the flagellar stator proteins between *E. coli* MotB and *Vibrio* PomB at the periplasmic region

Yuuki Nishino, Seiji Kojima, Michio Homma (*Div. Biol. Sci, Grad. Sch. Of Sci., Nagoya Univ.*)

We have determined the crystal structure of $PomB_{C5}$, the periplasmic region fragment of PomB, which is the stator protein of the flagellar motor of *Vibrio*. Based on the structural information, we constructed three chimeric proteins between PomB and MotB, named PotB91, PotB129 and PotB138, with various chimeric junctions in addition to PotB. When these chimeric proteins were produced with PomA in the *ΔmotAB* strain of *E. coli* or the *ΔpomAB* and *ΔpomABΔmotX* strains of *Vibrio*, their motility was examined. All the chimeras are functional in either *E. coli* or *Vibrio* and either with or without MotX that are specific motor proteins for *Vibrio* though the motilities were very weak in *E. coli*. We try to find out what caused chimeras to give the different abilities.

3P195 N-terminal deletion mutant of the stator protein PomA in the bacterial flagellar motor from Vibrio alginolyticus

Yasuhiro Onoue, Rei Abe-Yoshizumi, Mizuki Gohara, Shiori Kobayashi, Noriko Nishioka, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Nagoya Univ.*)

PomA is a membrane protein essential for torque generation in the bacterial flagellar motor from Vibrio alginolyticus. Previously, we succeeded in expression of the cytoplasm Loop domain (Q54-D148) of PomA using cold shock vector and GB1 tag at N-terminus (Biophysics 2013 Abe-Yoshizumi et al.). However, this mutant has a loose tertiary structure and shows slight dominant negative effect on motility in E. coli. To further characterize the property of the whole cytoplasmic domain, we constructed N-terminal deficient PomA (Q54-E253) with GB1 tag. This construct was expressed in both cytoplasmic and membrane fraction and showed strong dominant negative effect on motility in E. coli. We are currently studying the detailed molecular mechanism of this effect.

3P196 *Vibrio alginolyticus* 由来べん毛固定子 PomA のみによる複合体形成
Flagellar stator protein of Vibrio PomA alone could form multimeric complex

Mizuki Gohara[1], Norihiro Takekawa[1], Yohei Miyanoiri[2], Masatune Kainosho[2,3], Seiji Kojima[1], Michio Homma[1] ([1]*Div. Bio. Sci., Grad Sch. Sci., Nagoya Univ.*, [2]*Structural Bio. Res. Cent., Grad. Sch. Sci., Nagoya Univ.*, [3]*Grad. Sch. Sci. Tech., Tokyo Metropolitan Univ.*)

The stator complex in the bacterial flagellar motor forms the specific ion-conducting pathway. Ion flux through the stator through this pathway couples to the interaction between the cytoplasmic region of the stator and the rotor to generate torque. The stator of the Na+-driven motor of *Vibrio alginolyticus* consists of 4 PomA and 2 PomB molecules. Only the structure of periplasmic region of PomB has been determined. Toward the determination of the whole structure of the stator, we first tried to purify PomA alone. However, against our prediction, purified PomA behaved as the multimer as judged by the size-exclusion chromatography. Currently we are examining the stoichiometry, interaction site and functional meaning of this PomA complex.

3P197 Na+ uptake activity of the plug-deleted Na+-driven stator complex from Vibrio flagellar motor using reconstituted proteoliposome

Tetsuya Oba, Seiji Kojima, Michio Homma (*Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ.*)

The PomA/PomB stator complex couples Na+ influx to torque generation. We have been tried to quantify Na+ influx through the PomA/B complex by using not wild type but plug-deleted stator to facilitate detection of Na+ uptake. Last year, we reported a pilot experiment to detect Na+ uptake by reconstituted PomABΔL complex, which lacks plug segment. Since then, we tried to establish this experiment, but we faced difficulties, such as low yield of proteoliposome. So we tested different expression system and improved overexpression and purification protocol of PomA/BΔL complex. And, to confirm the Na+ influx, we reconstitute mutant stator, whose Na+ binding site (Asp24) is mutated. From these experiments, we will discuss the details of Na+ influx through the stator.

3P198 Vibrio alginolyticus の C リング付き基部体の構造解析
Structure analysis of the basal body with C-ring components from Vibrio alginolyticus

Satoshi Inaba, Hidemaro Hotta, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

The basal body is one of the rotor components of the flagella motor in many bacteria. The C-ring is attached to the cytoplasmic side of the basal body. The polar flagellum of Vibrio alginolyticus has Na+ driven motor. We attempt to isolate the intact polar flagellar basal body with C-ring components from Vibrio. We have isolated the basal body with only FliG which is one of the C-ring components (FliG,FliM,FliN). FliM and FliN of Vibrio are easier to dissociate from the basal body than those of Salmonella. We would like to isolate the basal body without acid treatment to eliminate filaments. We attempt to isolate filamentless multi polar flagella mutants in Vibrio, and to purify the intact basal body with C-ring.

3P199 c-di-GMP 結合タンパク質 YcgR のホモログ PlzD による *Vibrio alginolyticus* によるべん毛運動の阻害
Flagellar motility inhibition by PlzD, a YcgR homolog of c-di-GMP binding protein, in *Vibrio alginolyticus*

Takuro Yoneda, Wakako Morimoto, Seiji Kojima, Michio Homma (*Grad. Sch. Sci., Univ. Nagoya*)

Bacterial flagellar torque is considered to be generated by interaction between the rotor, a component of flagellar basal body, and the stator, which skirts around the basal body. Marine bacterium *Vibrio alginolyticus* has two types of flagella, one is polar flagellum to swim in the liquid and the other is lateral flagellum to swarm on the surface. Recently it was found that YcgR inhibits motility in the presence of cyclic dimeric GMP(c-di-GMP).

We found that PlzD in *V. alginolyticus*, a homolog of YcgR, inhibits swimming and that PlzD-GFP localizes at the flagellated pole in the nutrient-poor condition. Also PlzD represses swarming on the plate. These results may indicate that PlzD functions as brake for both polar and lateral flagellum.

3P200 高度好熱菌 *Aquifex aeolicus* 由来のべん毛モーター固定子タンパク質の性質検討

Characterization of the stator proteins of flagellar motor from extreme thermophile *Aquifex aeolicus*

Norihiro Takekawa, Mizuki Gohara, Seiji Kojima, Michio Homma (*Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ.*)

For the function of the bacterial flagellar motor, ion influx through the stator and interaction between the stator and the rotor are essential. Stator is composed of four MotA and two MotB molecules. Periplasmic region of MotB is anchored to the peptidoglycan layer, and cytoplasmic region of MotA interact with rotor to generate torque. Despite stator is most important part for motor, little is known for structure associated with its function. In this study, we established the method for overproduction and purification of recombinant MotA and MotB of extreme thermophile *A. aeolicus*. The production of MotA of *A. aeolicus* was higher compared to those from the other species. We are doing the functional and structural analysis of these proteins.

3P201 アデノウイルス由来両親媒性ペプチドの曲率誘導能における配列効果

The Sequence Effects of the Amphypathic Peptides of Adenovirus Protein VI on Their Curvature Inducing Ability

Tomo Murayama, Silvia Pujals, Shiroh Futaki (*Institute for Chemical Research, Kyoto University*)

Membrane curvature plays a crucial role in cellular functions. Amphipathic peptides induce or sense membrane curvature by their insertion into lipid bilayers, but the contribution of each amino acid is still obscure. The infomation would be helpful for the design of membrane interacting peptides with novel functions. We synthesized a peptide corresponding to N-terminal amphipathic helical segment of Adenovirus internal protein VI and the several derivatives, and their modes of interaction with bilayers were studied using large unilamellar vesicles. Their abilities to induce membrane curvature were also examined using differencial scanning calorimetry and confocal microscope observation of giant vesicles.

3P202 脂質膜の膜融合に際する水の協同性

Water-lipid cooperativity upon lipid membrane fusion

Mafumi Hishida[1], Koichiro Tanaka[2,3], Yasuhisa Yamamura[1], Kazuya Saito[1] ([1]*Dept. Chem., Univ. Tsukuba*, [2]*iCeMS, Kyoto Univ.*, [3]*Dept. Phys., Kyoto Univ.*)

It is not fully understood whether water play an important role for bimolecular self-assembly or it is just a background medium. To approach this issue, here we show a cooperativity between water and lipid membranes during an intermediate process of membrane fusion by use of small-angle X-ray scattering, grazing-incident small-angle X-ray scattering measurements and THz spectroscopy; recent progressed methodology for precisely detecting hydration state. With comparing two species of lipids, the results indicate that the structural change of the lipid membranes depend on the water behaviors in the hydration layer. The different behaviors of water at each membrane surfaces are caused by the different behavior between the hydrophilic and hydrophobic hydration waters.

3P203 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に関する研究

Study on the behavior of lipid multi-component system consisting of long- and short-chain phospholipids

Ryota Kobayashi, Tetsuhiko Ohba (*Grad.Sch.Sci., Tohoku Univ.*)

The binary mixtures of long- and short-chain phospholipids show a complicated phase behavior depending on the lipid composition, concentration 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.
Therefore, we observed the mixtures using Mueller matrix microscope developed in our laboratory to elucidate the mechanism of structural changes in the mixtures.
As a result, we obtained the results that the mixtures form elongated structures and its structures intertwine in a circle at intermediate temperature. These results suggest that this aggregates cause high viscosity.

3P204 ガラス基板上への細胞膜展開法の開発

Development of a new method for preparation of cell membrane flat sheet on glass surface

Yuta Minami[1], Hiroaki Inuma[1], Toshihiko Sakurai[2], Takashi Okuno[3] ([1]*Graduate School of Science and Engineering, Yamagata University*, [2]*Graduate School of Engineering, Tottori University*, [3]*Department of Science, Yamagata University*)

Cytoplasmic side of cell membrane is critical interface for substance transporting and signal transduction. We developed new method for preparation of cell membrane flat sheet (CMFS), on which detail analysis can be performed from cytoplasmic side. Giant plasma membrane vesicles (GPMVs) consisting of cell membrane derived from HeLa cells were prepared. Due to adsorption of the GPMVs on glass, the spherical form changed to dome shape. CMFS was obtained by removing a dome part of the GPMVs adsorbed on glass. Cytoplasmic side of CMFS was faced up, because GFP protein was confirmed on the CMFS prepared from cell membrane of which cytoplasmic side was modified with palmitoyl-GFP. To avoid physical fixation, CMFS on micro-plate patterning small holes was prepared.

3P205 人工テトラエーテル型リン脂質膜と重金属イオンとの相互作用

Interaction of heavy metal ions with artificial tetraether-type phospholipid membranes

Teruhiko Baba[1], Toshiyuki Takagi[1], Toshiyuki Kanamori[1], Tatsuya Oka[2], Hiroyuki Saito[2] ([1]*Res. Center Stem Cell Eng., AIST*, [2]*HBS, Univ. Tokushima Grad. Sch.*)

Archaeal branched-chain ether-type lipid (diether, tetraether) membranes are expected as useful reconstitution matrices for ion transporters owing to the high membrane stability and the high membrane barrier property to ionic solutes. Tetraether analog with phosphocholine groups (PTEPC) was prepared to examine its membrane barrier property to metal ions (Co^{2+}, Cu^{2+}, Hg^{2+}) in comparison with those of bilayer-forming PC membranes. The partition of metal ions into PTEPC membrane phase was evaluated by fluorescence quenching and lifetime measurements with fluorescent probes such as dansyl-PE. The lower partition of ions for PTEPC compared with bilayer-forming PCs was discussed in terms of the difference in the dielectric property of glycerol backbone region in membranes.

3P206 並列化された粗視化シミュレーションを用いたベシクルの構造安定性に関する理論的研究

Theoretical study on the structural stability of the vesicle by parallelized coarse-grained simulation

Tsuhito Yoshida, Kazuma Tamura, Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Kanazawa University*)

Biomembrane has lipid bilayer structure and the function as protein transporting. Lipid bilayer has a property to bend. Lipid bilayer on large scale is transformed from plane membrane to spherical membrane which is called vesicle. In this study, we perform molecular dynamics (MD) simulation of the vesicle to study structural stability by using different parameters of the interaction between two lipid molecules. We use coarse-grained model in which the lipid molecules are represented as 3 elastic chains of beads and develop MD program to perform parallel calculation for large scale and long time simulation. We observe membrane properties such as curvature. We will report the stability of the vesicle and the acceleration of MD simulation by parallel calculation.

3P207 Effect of cholesterol and 7-ketocholesterol on localization of Alzheimer's amyloid beta (Aβ_42) in membrane domains

Huong Phan, Masamune Morita, Tsuyoshi Yoda, Naofumi Shimokawa, Mun'delanji Vestergaard, Masahiro Takagi (*Japan Advanced Institute of Science and Technology*)

Lipid raft domains are considered as sites for amyloid beta (Aβ) binding in cell membranes. Cholesterol (Chol) and its oxidized derivative, 7-ketocholesterol (7keto), have been reported to affect Aβ/membranes interaction. However, very little is known about their effects on Aβ localization in membrane domains.
We have shown that Chol controlled the association of Aβ with Lo domains of model membranes. 7keto did not significantly increase Aβ localization in Lo domains but facilitated the peptide to insert into Ld phase. The effect of Chol and 7keto on the interaction of Aβ with membrane domains has been discussed by means of their ability to change membrane fluidity. These results are useful for understanding the role of Chol and its oxidation in Aβ-induced cytotoxicity.

3P208 脂質酸化物による生体模倣膜のドメイン形成

Effects of lipid oxidation products on domain formation of biomimetic membrane

Tsuyoshi Yoda[1,2], Wataru Inui[1], Huong Thi Than Phan[1], Naofumi Shimokawa[1], Mun'delanji C. Vestergaard[1], Tsutomu Hamada[1], Masahiro Tkagai[1] ([1]*Japan Advanced Institute of Science and Technology*, [2]*JSPS Research Fellow PD*)

Lipid rafts, physiologically important domains in plasma membranes, may be formed by mainly saturated lipids and cholesterol. Lipid oxidation has been reported to change dynamics of membranes1. However, the influence of lipid oxidation on domain structure and stability of membranes has not been well understood.
We made phase diagrams of membranes containing oxidized cholesterols, such as 7-ketocholesterol and 7β-hydroxycholesterol. We found that nonanal, an oxidized product of dioleoylphosphatidylcholine, significantly affects domain structure. Further, we characterized miscibility temperatures of oxidized membranes. Finally, the mechanism behind domain formation and stability of oxidized lipid-containing membranes was discussed.
[1] T. Yoda, et al., Chem. Lett. (2010)

3P209 遠心式マイクロ流体デバイスによる細胞サイズリポソームの作製

The synthesis of cell-sized liposomes by centrifuge-based microfluidic device

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Cell-sized liposomes are widely used to mimic the bio-membrane in biophysics and biochemistry. To efficiently use liposome for biological application, the production methods are required to control unilamellarity, encapsulation efficiency, and size. In recently, microfluidics method for producing a cell-sized liposomes have been proposed. However, the utility of this technology is low because to make microchannel requires for a microfabrication process. Here, we constructed simple production method of cell-sized liposomes as control unilamellarity, encapsulation efficiency, and size by centrifuge-based droplet shooting device (Maeda et al., Adv. Mater. 2012). We hope that this method will allow to make cell-sized liposomes for a given biological application.

3P210 脂質側方拡散を増幅させる新規拡張アンサンブル法の開発と応用

Acceleration of lipid lateral diffusion by generalized-ensemble molecular dynamics simulation

Takaharu Mori, Jaewoon Jung, Yuji Sugita (*RIKEN*)

Conformational sampling is important for simulating complex bio-molecular systems. In this study, we propose a new generalized-ensemble simulation algorithm for membrane systems, which we call the surface-tension replica-exchange molecular dynamics method. In the method, each replica is simulated in the NPγT ensemble, where surface tensions in a pair of replicas are exchanged at certain intervals during the simulation. We tested the method on a fully hydrated DPPC lipid bilayer system. We observe that large-scale lateral deformation of DPPC membranes takes place in all of the replicas. There is accelerated lateral diffusion of lipid molecules compared with conventional MD simulation. Our method could be applicable to a wide variety of biological membrane systems.

3P211 Time-resolved 3D Quantification and Analysis of Membrane-Lipid Signaling in Dictyostelium

Marcel Hoerning, Tatsuo Shibata (*Physical Biology Unit, Center for Developmental Biology, RIKEN*)

On the basis of 3D time-resolved observations on actin-polymerization-inhibited Dictyostelium cells using ultra-fast spin confocal microscopy, we systematically analyze and map the dynamics and patterns of membrane-bounded PIP3 molecules. We show that a whole zoo of pattern formation is possible, even exotic ones such as periodic target waves, bouncing waves and standing waves. Quantitative analysis of a large ensemble of cells lead to unique dependencies that are found to be bounded by the geometrical constrain of the cell membrane. We additionally performed realistic 3D stochastic simulations and compare the results with our experimental findings.
This study is a unique (may be first) attempt to analyze and understand pattern formation in chemotactic cells in 3D.

3P212 セラミド分子のフリップフロップ速度
Transbilayer movement of sulfhydryl ceramide analogues in model membranes

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Cell membrane and organelle have many kinds of lipids. The distribution is changed depending on the function. To study the lipid dynamics, the behavior of lipid molecules is important. There are two lipid movements, lateral diffusion within a leaflet and flip-flop between leaflets. In contrast to the fast lateral diffusion, flip-flop is rather slow because of the energy barrier to leap the polar head group over the hydrophobic core in membrane. Ceramide, cell-signaling messenger lipid, has small head and this might promotes flip-flop. Therefore, we synthesized sulfhydryl ceramide analogues to quantify the ceramide flip-flop in model membrane. This system clarifies the ceramide flip-flop is affected by lipids composition, such as fluidity and lipid backbone structure.

3P213 アミロイドβタンパク質の結合に伴うラフトモデル膜のダイナミックスの変化
Change of Dynamics of Raft-Model Membrane Induced by Amyloid-β Protein Binding

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'Lipid rafts' have been considered to have a function as platforms for signaling and sorting. Recent spectroscopic studies show that the interaction between monosialoganglioside and amyloid beta (Aβ) protein promotes the transition of Aβ to amyloid aggregates. However, there is few evidence on the dynamics of 'lipid rafts' membranes. By using neutron spin-echo and small-angle X-ray scattering, we have found that the interaction between the Aβ proteins and the model membrane significantly suppresses a bending-diffusion motion with a minor effect on the membrane structure. The present results suggest non-receptor-mediated disorder through a change of membrane dynamics by Aβ binding.

[References]
M. Hirai, et al., Eur. Phys. J. E (2013) 36, in press.

3P214 抗菌ペプチドの殺菌メカニズムを探究する
Investigating bactericidal mechanism of antimicrobial peptids

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Antimicrobial peptides (AMPs), which interfere with bacterial membrane, are promising candidates for new therapeutic antibiotics. Using giant unilamellar vesicles (GUVs), we performed quad-color flow-cytometric analysis to investigate how melittin leaks GUV's internal contents, revealing that melittin stochastically acts as either pore-former or detergent-like destabilizer of the membrane. By carefully comparing behaviors of GUVs and *E. coli* to melittin, we found that destabilization of bacterial cytoplasmic membrane was critical for sterilization. On the other hand, formation of (troidal) pores on cytoplasmic membrane did not cause cell death. It is anticipated that these results provide a basis for detailed understanding on the bactericidal mechanism of AMPs.

3P215 コレステロール分子によるリン脂質二重膜の破断抑制メカニズム：分子動力学シミュレーション
Molecular Mechanism of Inhibitory Effect of Cholesterol on Phospholipid Bilayer Rupture: Molecular Dynamics Simulation

Taiki Shigematsu, Kenichiro Koshiyama, Shigeo Wada (*Grad. Eng. Sci., Osaka Univ.*)

We perform molecular dynamics simulations of phospholipid (DPPC)/ cholesterol bilayers under various areal strains. The critical areal strain of the DPPC/cholesterol bilayers, where the rupture occurs, is larger than that of the pure DPPC bilayer, in agreement with previous experimental observations. With increasing areal strain, DPPC and cholesterol molecules once become disordered, and then, just before the rupture, the tilt angle of cholesterol recovers and this rectifies the order of DPPC and cholesterol molecules. From these results, we speculate that the rectification of DPPC and cholesterol molecules increases the number of the hydrogen bonds between DPPC and cholesterol, resulted in inhibiting the rupture.

3P216 ヒト iPS 由来心筋とヒト ES 由来心筋の電気生理学性質の比較研究
A comparative study on electrophysiological properties of human iPS- and ES-derived cardiomyocytes

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Special attention is directed to the potential application of human induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) as a potential *in vitro* human panel screening source for predictive cardiotoxicity. However, variations of electrophysiological properties in iPS-CMs confine precise drug safety evaluation. To describe the variations objectively, action potential (AP) parameters recorded from perforated patch-clamped iPS-CMs were systematically compared with those from human ES-derived cardiomyocytes (hES-CMs). Although we found statistical differences in the mean values of APD_{50}, upstroke velocity and beating rate, detailed analysis of the variation with Gaussian fitting revealed marked differences in frequency distributions between iPS- and hES-CMs.

3P217 細胞内ミトコンドリアの一過性脱分極の観察と誘導
Observation and induction of mitochondrial transient depolarizations in cells

Kanji Umiuchi, Yoshihiro Ohta (*Tokyo Univ. Agr. Tech.*)

Reactive Oxygen Species (ROS) are mainly produced in mitochondria and induce cell injuries and cell death. Previously, we found with isolated mitochondria that the shortage of protons in mitochondrial matrix causes transient depolarizations, and that the depolarizations suppress ROS production. However, it is not unclear that the phenomena occur in cells. In this research, we investigated the frequency of transient depolarizations of mitochondria in h9c2. The presence of oligomycin, a inhibitor of FoF1-ATP synthetase, increased the frequency of the transient depolarizations. This increase was ceased by the addition of the protonophore CCCP. These results indicate that the shortage of protons in the matrix induces the transient depolarizations of mitochondria in cells.

3P218 KcsA チャネルの細胞内ドメインと inactivation gate の連関
Coordination between the cytoplasmic domain and the inactivation gate in the KcsA channel

Minako Hirano[1], Yukiko Onishi[2], Okuno Daichi[2], Toru Ide[1] ([1]*GPI*, [2]*Riken*)

The KcsA channel is a representative potassium channel that is activated by protons. Recently, we found that the cytoplasmic domain (CPD) regulates opening and closing of the KcsA channel. The inactivation gate which is in a selectivity filter region is also known to regulate the gating. However, it is not clear how the CPD and the inactivation gate coordinate to regulate the gating. In this study, to clarify the relationship between these two sites, an effect of the CPD or the inactivation gate on the other gate was investigated. Changes in the conformation of the inactivation gate did not influence on the conformation of the CPD. In contrast, the conformation of the CPD influenced on the function of the inactivation gate.

3P219 固体支持体に固定したイオンチャネルの人工平面膜への再構成
Reconstitution of ion channel immobilized on solid support into lipid bilayer

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To elucidate the molecular mechanism of ion channels to regulate the ion flow, it is necessary to investigate the relationship between the conformation and function and interaction with some factors. To aim this task, Ion channel have to be kept suppressing lateral diffusion in the membrane to measure conformational change, ligand binding/unbinding and current, simultaneously. In this study, we successfully reconstituted ion channels (KcsA) into a lipid bilayer using glass needle. This means that the use of the near-field illumination from the tip of optical fiber probe has a potential to facilitate building the simultaneous measurement system of optical and electrical recording for single ion channel.

3P220 ミトコンドリアの密集が活性に与える影響
Effects of mitochondrial crowding on their activity

Daiki Yoshimatsu, Yoshihiro Ohta (*Tokyo Univ. of Agric. and Tech.*)

Mitochondria are organelles which produce ATP and are considered to gather at the regions where ATP is highly required. In the present study, we examined the effects of mitochondrial crowding on their activity. Mitochondria were isolated from porcine hearts. When we adsorbed mitochondria at the higher density, each mitochondrion produced ATP at a higher rate. In addition, mitochondria were more polarized by their crowding. Since mitochondria are the main ROS source in mammalian cells, we also examined the effect of mitochondria crowding on their ROS production. The mechanism by which mitochondrial crowding affects their activity on cover slips will be discussed.

3P221 Lipid bilayer chamber array system for massive measurement of transporter activity

Naoki Soga, Rikiya Watanabe, Shinya Ohdate, Hiroyuki Noji (*Department of applied chemistry, School of engineering, The university of Tokyo*)

Transporters carry out physiological function of transmembrane transport coupling with the conformational change. To investigate the coupling mechanism of conformational change and transporting activity, it has been required to detect the conformational change simultaneous with the transport activities at a single molecule level, although it has not been accomplished yet. In this study, we develop the chamber system closed by the lipid membrane, which enables us to detect the transporting activity with high sensitivity. Using this system, we at a single molecule level observed the passive transport of fluorescent dye by α-hemolysin, and moreover, have been trying to measure the active transport by transporters, such as Ca2+-ATPase or FoF1-ATP synthase.

3P222 アトリットル容積を持つナノセルを用いた膜輸送たんぱく質の 1 分子計測
NanoCell, Attoliter Chamber Array for Single-Molecule Measurement of Membrane Transporters

Takao Ono, Rikiya Watanabe, Takanori Ichiki, Hiroyuki Noji (*Grad. Sch. Eng. Univ. Tokyo*)

Membrane transporters carry out various physiological functions by transporting small molecules across bio-membranes. To measure their transport activities, some artificial lipid membrane systems were developed, however, they had too low sensitivity to quantitatively detect the activities. To solve this problem, we developed the novel experimental setup to form a lipid bilayer membrane on nano-fabricated device “NanoCell (NC)” which confined the device volume to attoliter scale. Using this setup, the passive transport activity by α-Hemolysin was rapidly detected as release of 200-μM dye from NC within a second, which was mainly due to the small volume of NC. This setup is basically applicable to the other membrane proteins such as ion pumps or active transporters.

3P223 PIP2 は synaptotagmin 2 による SNARE を介した膜融合の促進に関与する
PIP2 is involved in the enhancement of SNARE-mediated membrane fusion by synaptotagmin 2

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Mast cells are involved in allergic responses. Antigen stimulation causes intracellular Ca2+ concentration, which induces exocytotic release of inflammatory mediators. Recent researches revealed that SNARE proteins are involved in this exocytotic membrane fusion process. PIP2 is a acidic phospholipid and localizes on the inner leaflet of the plasma membrane. It was reported that synaptotagmin 1, a Ca2+ sensor for exocytosis at nerve terminal, interacted with PIP2 in a Ca2+ dependent manner. However, the role of PIP2 in mast cell exocytosis is not clear. In this study, we investigated the role of PIP2 in mast cell exocytosis using liposome-based membrane fusion assay. We found that PIP2 is involved in the enhancement of SNARE-mediated membrane fusion by synaptotagmin 2.

3P224 支持体を持つ人工細胞の開発

Development of a closed supported artificial cell

Yasuto Sasaki, Misaki Yamamoto, Ichiro Yamato (*Dept. Biol. Sci. Tech., Tokyo Univ. of Science*)

Liposomes, a kind of artificial cells, have been used for studies of biomembranes and membrane proteins. However, liposomes without support are weak physically. Therefore, Supported Lipid Bilayer (SLB) was developed, mostly on planar supports. In this respect, we are trying to develop a closed supported lipid bilayer.
We used Sephadex as the support, having glutathione linker, and PutP (*E.coli* Na^+/proline symporter)-GST fusion protein as a membrane protein. After reconstitution with *E.coli* phospholipid, the Sephadex beads seemed to be closed and showed Uptake activity of proline, suggesting that we obtained a closed supported artificial cell.

3P225 SWAP-70 PH ドメインの脂質膜結合に対するトリプトファン残基の寄与

Role of tryptophan residues in membrane association of the SWAP-70 PH domain

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SWAP-70 is involved in the PI3K-dependent signaling pathway at the plasma membrane and contribute to the cytoskeletal reorganization. We investigate the SWAP-70 PH domain-membrane interaction by using Trp residues as probes of local environment. Two of the three Trp residues in the PH domain were individually replaced by Phe (W227F, W231F) and the effects of the mutations were estimated by fluorescence spectroscopy and vesicle coprecipitation assay. Membrane-binding affinity of W227F was 10-fold weaker than WT, suggesting that Trp227 contributes to the binding mechanism. Fluorescence of Trp231 was indifferent to the membrane association. Together with WT-like membrane-binding affinity of W231F, Trp231 is suggested to be excluded from the membrane-binding mechanism.

3P226 新規ガングリオシドプローブの 1 分子追跡によるラフト組織化と機能の解明

Single-molecule tracking of new ganglioside probes revealed raft organization and function

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Gangliosides are considered to be strongly associated with rafts in cellular plasma membranes. However, how they interact with rafts and function in rafts have been unknown due to the lack of their fluorescent analogues that behave like the parent molecules. Here, we succeeded in synthesizing several fluorescent ganglioside probes. Surprisingly, single-molecule observation revealed that all the fluorescent ganglioside probes (GM1, GM2, GM3, GD1b) transiently formed homodimers which were induced by glyco-chain interactions and stabilized by raft-lipid interactions. Furthermore, we found that the homodimers inhibited dimerization of EGF receptors. Our results suggest that the transient homodimers are likely one of the basic unit for raft organization and function.

3P227 光制御水素化アモルファスシリコン薄膜上の化学反応性積層ゲルを用いたバイオセンサ

Biosensor using electrochemical laminated gels photo-controlled on hydrogenated amorphous silicon film

Hiroki Suzuki[1], Ryohei Matsueda[1], Teruo Matsuno[1], Takahiko Sano[1], Yuta Ando[1], Hiroshi Masumoto[2], Takashi Goto[3], **Yutaka Tsujiuchi**[1] ([1]*Material Science and Engineering, Akita University*, [2]*Center for Interdisciplinary Research, Tohoku University*, [3]*Institute for Materials Research, Tohoku University*)

On an attempt for fabrication of biosensor using photo-controlled film system, we have been researching, by using ionic conduction in laminated gels on hydrogenated amorphous silicon film. Ionic conduction in solution as well as in intermediate states between a liquid and a solid, such as gels, is a key phenomenon to inter-conversion between light energy, chemical energy, and electric energy, are able to be achieved using amino acids that is the elementally element of bio molecule and has potential of diversity to electro chemical device. In this conference, we report an attempt of introducing some chemical reaction on the targeting molecules and large bio-molecules.

3P228 金ナノ粒子キャリヤー表面に提示されたハプテンとしてのアゾベンゼン色素の免疫応答

Immunological study with azobenzene-dye as a hapten presented on the surface of gold nanoparticle carriers

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The azobenzene moiety which is well-known not only for its reversible *cis*-to-*trans* photoisomerization but also as a hapten did not induce antibody production on its own in rabbits, but we confirmed an *in vivo* response against azobenzene dye presented on the entire surface of gold nanoparticles (azo-nanoparticles), where the gold nanoparticles appeared to play a role as a carrier for the hapten. A high yield of immunoglobulin G (IgG) against the azobenzene derivative took place in rabbits injected with azo-nanoparticles, whereas no increase in IgG was recognized in other rabbits treated solely with chemically equivalent azobenzene dye instead of azo-nanoparticles. Investigation of the immune mechanism at molecular level using cultured cell lines is under progress.

3P229 Directional-sensing and rectified cell motion towards termporally changing gradient

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Dictyostelium aggregation is mediated by chemotaxis towards traveling waves of cAMP. It remains unclear why the cells do not fall into futile back and forth movements despite the gradient reversal. By employing a microfluidic system, we analyzed chemotaxis towards well-defined cAMP traveling pulses and alternating gradients. We found that, (1) cells move towards the direction of in-coming pulse for waves with appropriate timescale, (2) Pseudopod formation correlates with Ras activation, and (3) Localized activation of Ras disappears even in the presence of spatial gradient when the cells are exposed to cAMP that is decreasing in time. These results suggest that the directional sensing implements a half-wave rectifier to filter out response to the back of the cAMP wave.

3P230 線虫においてあるモダリティーが異なるモダリティーの順応を引き起こす

Sensory stimulation from a specific modality adapts a different modality in Caenorhabditis elegans

Hisashi Shidara, Junya Kobayashi, Ryo Tanamoto, Kohji Hotta, Kotaro Oka (*Bio and Info, Keio Univ.*)

In study on C. elegans, the neural functions and circuits have been revealed at just single sensory modality, but it is unknown how a specific modality influences others. Here, we showed that chemotaxis to specific volatile odorants decreased by 5 min pre-exposure to gustatory cue (Mg2+) by behavior sassy, suggesting that the cross modality adaptation is observed in C. elegans. A specific neuron ablation with a phototaxis protein, KillerRed, revealed that AIY interneuron, which is projected by olfactory and gustatory sensory neurons, required for this adaptation. The function of AIY was also confirmed by intracellular Ca2+ measurement. The Ca2+ response to odor stimulation was diminished by the adaptation, also indicating that AIY is necessary for the adaptation.

3P231 集光レーザービームの光摂動による神経細胞内分子動態の集合操作

若手招待講演

Optical perturbation of intracellular molecular dynamics of single neuron in living neuronal network

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Intracellular molecular dynamics at synaptic terminals in neurons are essential for synaptic plasticity and subsequent modulation of neuronal network. For aiming artificial control of synaptic activity, we demonstrate optical perturbation of synaptic vesicles and neural cell adhesion molecules (NCAMs) at plasma membrane in living neurons with optical tweezers. Synaptic vesicle dynamics and NCAMs labeled with Q-dots in an optical trap obtained from fluorescence correlation spectroscopy were revealed that the particle motion was constrained at the focus due to optical trapping force. Optical manipulation suppressed releasing synaptic vesicles after high K^+ stimulation, suggesting that our method has a potential to manipulate synaptic transmission at single synapse level.

3P232 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

Sex difference in hippocampus: Fluctuation of hippocampal sex hormones and synapses

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The hippocampus, a center for learning and memory, is not sex different at the anatomical level such as its number of neurons. Nevertheless, the significant sex difference exists in the performance of hippocampus-dependent task such as spatial memory. To date, hippocampal sex difference had been attributed to the blood hormone level.
However, we revealed that the hippocampal level of sex hormones is much higher than the blood level. Moreover, hippocampal levels of female sex hormones (estradiol and progesterone) were sex different and those in female fluctuated across a 4 day-period (estrous cycle), with a high correlation with spine density (postsynapse).
Hippocampal sex difference at synaptic level may be due to the difference in hippocampal hormonal level.

3P233 老化に伴う海馬神経シナプスの密度の減少と記憶の劣化

Age-related decrease in synapse density of hippocampal neurons in relation to memory impairment

Suguru Kawato[1,2] ([1]*Univ of Tokyo, Grad Sch Arts and Sciences*, [2]*JST Int Collabo*)

We investigated the spine (postsynapse) density in hippocampus center for learning and memory. We found the age-related decrease in the spine density and change in morphology, indicating a reduction of memory performance.
The density of spines of 3 months rats (young) and 24 months rats (aged) was analyzed with Spiso-3D mathematical software. In aged rats, spine density (1.71 spines/μm) eas decreased from young rat (2.29 spines/μm). The density of large-head spine and middle-head spine was significantly lower in aged rats. W found the considerable decrease in testosterone to 1/100 in aged rat hippocampus, by using LC-MS/MS. The testosterone replacement therapy rescued the spine density. Note that BDNF was not at all decreased by aging.

3P234 Acute Modulation of Synaptic Plasticity of Pyramidal Neurons by Hippocampal-derived Sex Steroids

Yoshitaka Hasegawa[1,2], Keisuke Hotta[1], Hideo Mukai[1], Bon-chu Chung[2,3], Ooishi Yuuki[1], Hojo Yasushi[1,2], Kawato Suguru[1,2] ([1]*Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*, [2]*International collaboration program of Bioelectronics (JST)*, [3]*Institute of Molecular Biology, Taiwan*)

Hippocampal-derived sex hormones are neuromodulators in the hippocampus. We investigated effects of Estradiol(E2), Testosterone(T) and Dihydrotestosterone(DHT). Adult hippocampus synthesized 8nM E2. Synaptic estrogen receptors(ERα) are in spines of hippocampal neurons. Localization of ERαin 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. E2 also increased the density of spines in CA1 pyramidal neurons in hippocampal slices. Effects of E2 are induced by mitogen-activated protein kinase (MAPK) in hippocampal neurons.

3P235 脳海馬が作る男性・女性ホルモンは記憶の神経シナプスを増強する

Hippocampus-synthesized male and female hormones increase memory-related nerve synapses

Miyuki Yoshiya, Yasushi Hojo, Suguru Kawato (*Grad. Sch. of Art and Sci.*)

Dendritic spines are postsynapses which store the memory in the hippocampus. We already demonstrated that sex hormones are synthesized locally in the hippocampus. We focus on the rapid effect of male and female hormones on the synaptic plasticity. Following a 2 h treatment, the density of spines was significantly increased. Mathematic analysis by Spiso-3D software distinguished kinase signaling induced by these sex hormones. The signaling of andorogen and estrogen involve synaptic estrogen and andorogen receptors, which drive PKC, PKA and MAPK, resulting in actin polymerization and new spine formation.

3P236 ハロロドプシン－臭素イオン複合体の N 光反応中間体の X 線結晶構造解析

X-ray structural analysis of the N photoreaction intermediate of halorhodopsin in complex with bromide ion

Haruki Kawaguchi[1], Taichi Nakanishi[1], Hiroki Kubo[1], Kunio Ihara[2], Midori Murakami[1], Tsutomu Kouyama[1] ([1]*Graduate School of Science, Nagoya University*, [2]*Center for Gene Research, Nagoya University*)

Halorhodopsin from N. pharaonis (pHR) is a retinylidene protein that functions as a light-driven chloride ion pump.
In this study, we investigated the structure of a reaction intermediate of pHR-bromide ion complex that was accumulated under illumination at 240K.
The structure analysis showed that the three subunits in the asymmetric unit underwent different structural changes.
In the subunit with the EF loop facing a free space, a profound outward movement of the cytoplasmic half of helix F took place, while the middle moiety of helix C moved inward. In this reaction state (possibly the N state), the bromide ion that initially existed in the active site moved across the Schiff base and occupied a site at the cytoplasmic vicinity of the Schiff base.

3P237 Trapping the photoactive form of squid rhodopsin in the P62 crytal

Midori Murakami, Tsutomu Kouyama (*Dept. Physics, Nagoya Univ.*)

Upon absorption of light, rhodopsin undergoes a photocycle and, via several intermediates, forms a final photo-product which stimulates the intracellular signaling pathway to excite the visual cell. We have performed crystallographic analyses of squid rhodopsin. In this study, we developed a method to trap the late intermediate of squid rhodopsin. When the P62 crystal was kept above 293K, rhodopsin molecules in the crystal were bleached, suggesting the formation of opsin. When this crystal was soaked with all-trans retinal, the color turned to yellow-orange and red-orange in alkali and acidic conditions, respectively, indicating that a large fraction of opsin was converted into the photo-active state.

3P238 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 orthologues of mammalian NDRG1 protein in photoreceptors

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A carp orthologue of mammalian *ndrg1* (*n-myc downstream-regulated gene 1*), *ndrg1b*, is a cone-specific gene with unknown function. The purpose of this study is to understand the function of this protein in cones using zebrafish, akin to carp. NDRG1b protein was localized to the plasma membrane (PM) of the inner segment (IS) and the outer segment (OS) in cones. Its newly isolated paralogue NDRG1a-v1 showed similar subcellular localization in cones, but in rods, this protein was present only in the IS PM. Knockdown of *ndrg1b* at larval stage impaired proper cone maturation. Forced-expression of NDRG1b or NDRG1a-v1 in rods altered the length and the shape of their OS. It was found that NDRG1b is important for cone maturation, and both proteins for photoreceptor OS morphology.

3P239 コイ桿体と錐体とでの cGMP ホスホジエステラーゼの活性化効率の定量的理解

Quantitative Aspects of cGMP Phosphodiesterase Activation in Carp Rods and Cones

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Light sensitivity is lower in cones than in rods. We previously showed that activation of cGMP phosphodiesterase (PDE), a hydrolyzing enzyme of the 2nd messenger cGMP in rods and cones, requires >200 times more light in cones than in rods. This lower PDE activation efficiency in cones is partly due to 5 times lower activation rate of transducin by activated visual pigment (R*) in cones than in rods. However, this difference does not explain the >200 times difference in the PDE activation between rods and cones. In the present study, therefore, to understand the underlying mechanism of the remaining >40 times difference, we compared in rods and cones (1) the efficiency of PDE activation by activated transducin (Tr*), and (2) the contribution of lifetimes of R* and Tr*.

3P240 LOV タンパク質 YtvA のシグナル伝達における分子間相互作用変化の時間分解測定

Time-resolved study on the intermolecular interaction change in the signal transduction of LOV protein YtvA

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YtvA is a blue light sensor protein composed of the N-terminal LOV domain, linker domain, and the C-terminal STAS domain. Physiological experiments have shown that the YtvA acts as a positive regulator for environmental stress responses (light and salt stresses) regulated by the σB factor. However, the molecular mechanism of its signal transduction has not been revealed yet. For understanding this mechanism, we have studied the photoreaction dynamics of full-length protein and its truncated constructs. In addition, we are investigating the intermolecular interaction change with RsbRA, which is a partner protein of YtvA in the living cell. We will discuss the signaling process in more detail at the conference.

3P241 青色光センサー蛋白質 PapB の光反応ダイナミクス

Light induced reaction dynamics of a BLUF photoreceptor PapB

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The BLUF (Blue-Light Using Flavin adenine dinuculeotide) protein PapB is a blue light receptor which controls the biofilm formation of the purple photosynthetic bacterium Rhodopseudomonas palustris by enhancing the phosphodiesterase activity of the EAL domain protein PapA, which hydrolyzes the second messenger cyclic dimeric AMP. In order to elucidate the signaling mechanism, we investigated the light-induced reaction of PapB in vitro by Transient Grating method. Analyzing the TG signals, we detected light-induced volumetric change commonly observed in BLUF proteins. This dynamics was followed by significant change of diffusion coefficient. These reactions were spectrally silent processes and the details of photochemistry are presented at the symposium.

3P242 フォトトロピンの LOV2 ドメインからキナーゼ部位への光情報伝達ダイナミクス

Photochemical signal transduction dynamics of the LOV2-kinase fragment of phototropin2 from *Arabidopsis*

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Phototropin, a blue-light receptor protein, consists of two light sensing domains (LOV1 and LOV2) and a kinase domain whose activity is regulated by the LOV2 domain mainly. However, the interaction between the kinase domain and the LOV2 domain has been remained unclear so far. In this study, to understand the signal transduction mechanism of the kinase activation, we investigated the reaction dynamics of LOV2-kinase construct by Transient Grating and Transient Lens techniques. We have detected the large changes in the diffusion coefficient upon photoexcitation, which represents the global reaction was induced in the protein part. Comparing the reaction with that of the LOV2 construct, we will discuss how the kinase domain is activated.

3P243 緑藻由来の全長フォトトロピンの光反応

Photochemistry of full-length phototropin from green algae

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Phototropins are blue-light dependent kinases in plants and algae. So far, biophysical studies of phototropin has been restricted to shorter fragments because of the difficulty of purification of the full-length protein. In this study, however, we obtained full-length phototropins from *Chlamydomonas* and *Ostreococcus*. In order to understand the mechanism of the kinase activation, we have investigated the reactions of these proteins using Transient Grating method. We have detected conformational changes of protein moiety in milliseconds time scale as a decrease of diffusion coefficient. CD measurements also showed that the secondary structure was partially unfolded. We will discuss the photochemistry of intact proteins on the basis of these findings.

3P244 QM/MM RWFE 法による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.*)

Rhodopsin is a photoreceptor for twilight vision. The photoisomerization of its chromophore, 11-cis retinal, initiates a series of structural changes. The purpose of this study is to elucidate the structure of early intermediates and the molecular mechanism of the relaxation process by using classical MD and QM/MM calculations. We analyzed the structure and vibrational frequencies of rhodopsins and the intermediates by using QM/MM RWFE method (Kosugi and Hayashi, JCTC 8, 322 (2012)), recently developed in our laboratory. The mechanism of Schiff base frequency upshift in lumirhodopsin will also be discussed.

3P245 高角領域の X 線散乱によるロドプシンの構造変化の解析

Helical rearrangement of photoactivated rhodopsin probed by high-angle X-ray scattering

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G-protein coupled receptors have common structural motif composed of seven transmembrane helices. In response to stimuli, transmembrane helices are rearranged and G proteins are activated. However, the technique to directly observe the helical movement in the physiological environment has been limited. Here light-induced helical rearrangement of bovine rhodopsin was monitored by high angle X-ray scattering. Rhodopsin was incorporated into nanodiscs, and the X-ray scattering ranging from Q=0.1 to 1.5 $Å^{-1}$ was measured in the dark and after photoexcitation. Characteristic intensity changes were observed at Q=0.2 and 0.6 $Å^{-1}$, which are consistent with the crystal structure of metarhodopsin II.

3P246 Gタンパク質共役型受容体の構成的活性変異体に見られるGタンパク質活性化メカニズムの一分子解析

Single-molecule analyses of the activation mechanisms of G proteins in constitutively active mutant of G protein-coupled receptor

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Rhodopsin, a member of GPCRs, activates G protein in agonist-bound form (Meta-II), but scarcely activates it in ligand free form (opsin). Opsin state of constitutively active mutant (CAM) of rhodopsin activates G-protein much effectively than that of wild type but the mechanisms have been unclear. In order to elucidate the mechanisms, we labeled M257Y (a typical CAM) with Alexa594 fluorophore and monitored conformational changes in opsin state by florescence changes. Bulk measurements showed that fluorescence intensity of Alexa594 bound to M257Y opsin was greater than that to wild type opsin. The conformational changes of M257Y opsin in single-molecule level were monitored by using TIRFM and the conformational dynamics were analyzed.

3P247 光依存的なGタンパク質活性化能を失ったロドプシン類の発見とその不活性化機構の解析

Discovery of a diffusible ligand-binding rhodopsin lacking light-dependent G protein activation ability

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In general, animal photoreceptor opsin covalently binds a ligand 11-cis-retinal at the conserved lysine in the inactive state, and light isomerizes the retinal to all-trans-retinal (ATR), which induces the formation of the active state to couple with G protein. Here we show that Opn5L1 found in non-mammalian vertebrates, whose physiological function remains unknown, exclusively bound an agonist ATR to activate G protein, and was inactivated by the formation of covalent bond between retinal and the neighbor cysteine, that is, lysyl-retinal-cysteinyl adduct is a ligand in the inactive state of Opn5L1. We also show that the inactive state thermally reverted to the ATR bound active state. We will discuss unique molecular mechanism to regulate Opn5L1 activity.

3P248 ホヤオプシン 1(Ci-opsin1)の分子特性の解析

Analysis of molecular property of ascidian opsin, Ci-opsin1

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Opsins are photoreceptive proteins in animals which belong to G protein-coupled receptors (GPCRs). Ci-opsin1, ascidian (Ciona intestinalis) opsin, was found to be expressed in the ocellus of ascidian larvae to regulate the swimming behavior. The phylogenetic analysis indicated that Ci-opsin1 was more closely related to vertebrate visual opsins than invertebrate ones. However, the detailed molecular properties of Ci-opsin1 remain unknown. Our spectroscopic analysis indicated that Ci-opsin1 showed intermediate photoreaction and G protein activation efficiency between those of vertebrate and invertebrate visual opsins. Based on our results, we discuss the molecular properties of Ci-opsin1 in the linkage of vertebrate and invertebrate visual opsins.

3P249 脊椎動物の可視光感受性 Opn5 の分子特性解析

Molecular properties of vertebrate visible-light sensitive Opn5

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Opsins are the universal photoreceptive molecules for visual and non-visual photoreceptions in animals and are classified into seven distinct groups based on their amino acid sequences. Opn5 forms an independent group whose members in vertebrates are diversified into four subgroups. Previously we showed that two subgroups (Opn5m and Opn5L2) are Gi-coupled UV-sensitive bistable pigments. Here we show that Opn5n subgroup found in non-mammalian vertebrates was reconstituted with 11-*cis* retinal to form a visible light-sensitive pigment and activated Gi after *cis-trans* photoisomerization of the retinal. These results suggest that Opn5 group shares G protein coupling property and is diversified based on their spectral sensitivities.

3P250 マウスメラノプシンの分子特性

Molecular Properties of Mouse Melanopsin

Takesi Matsuyama Hoyos, Takahiro Yamashita, Yasushi Imamoto, Yoshinori Shichida (*Kyoto University Department of Science*)

Melanopsin is the photosensitive pigment of ipRGCs (intrinsically photosensitive Retinal Ganglion Cells), which mediate irradiance detection functions such as pupillary light reflex and photo-entrainment of the circadian rhythm. Although melanopsin's physiological relevance is well established now, its molecular properties remain largely unexplored. In order to address this gap in our understanding, we have characterized melanopsin's molecular properties, using recombinant mouse melanopsin exogenously expressed in culture cells. We have conducted a comprehensive analysis of spectroscopic properties of mouse melanopsin and of its Gq activation properties. Based on these results, we discuss the possible consequences of such molecular properties on irradiance detection.

3P251 双安定性のロドプシン類の分子特性とそれらの光遺伝学への応用の可能性

Molecular properties of animal bistable rhodopsins and their optogenetic potential

Tomohiro Sugihara, Mitsumasa Koyanagi, Akihisa Terakita (*Grad. Sch. Sci., Osaka City Univ.*)

We previously revealed that most non-visual opsin-based pigments of animals exhibited a bistable nature, photoregeneration ability of the stable photoproduct, unlike vertebrate visual opsin-based pigments. We also showed that some of non-visual opsins bound to retinal isomers ubiquitously present in animal bodies. These properties might contribute to enable non-visual opsins to function as photosensitive pigments in tissues containing not enough 11-cis retinal and also suggest optogenetic potential of non-visual opsins. In this study, in order to investigate their optogenetic potential, we tested responsiveness of cultured cells expressing non-visual opsins to light. Obtained results suggested some non-visual opsins might be useful for optogenetic applications.

3P252 光合成反応中心タンパク質で機能する電子移動担体の極低温１分子分光

Cryogenic single molecule spectroscopy of the electron transfer cofactor in the photosynthetic reaction center

Toru Kondo[1], Risa Mutoh[2], Genji Kurisu[2], Hirozo Oh-oka[3], Satoru Fujiyoshi[1], Michio Matsushita[1] ([1]*Dept. Phys., Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*Institute for Protein Research, Osaka Univ.*, [3]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*)

Nature achieved almost 100% electron transfer efficiency in the photosynthetic reaction center (RC). One of the mechanisms to realize the high efficiency is considered to be a local structural change following the light-induced charge separation, which leads to the stabilization of the radical pair. The structural change may affect optical spectra of an electron transfer cofactor. In order to measure optical spectra free from thermal fluctuation and ensemble averaging, we apply cryogenic single molecule spectroscopy to the RC of *Heliobacterium modesticaldum*. Here we present development of microscope setup and a home-built light source, tunable from 500 to 800 nm. The cryogenic fluorescence excitation spectrum of a single Chl a, serving as a cofactor A0, was obtained.

3P253 光化学系 II における励起エネルギーと電子の輸送過程に関する階層的粗視化運動論モデル

Hierarchical coarse-graining kinetic model for excitation energy and electron transfer processes in photosystem II

Takeshi Matsuoka[1], Shigenori Tanaka[1], Kuniyoshi Ebina[2] ([1]*Graduate School of System Informatics, Kobe University*, [2]*Graduate School of Human Development and Environment, Kobe University*)

Photosynthesis is characterized as a multi-scale reaction system. Chlorophyll a fluorescence induction (FI) shows such a property of photosynthesis. To understand this phenomenon, a number of studies based on various mathematical models have been attempted. However, there are problems : (1) understanding of phenomenon based on very complex model is difficult and (2) high computation cost is required for integration over multi-time-scale. Here, a reduction method of coarse-graining in time which resolves these problems is applied to PSII model for exciton and electron transfer kinetics. Analysis of FI based on coarse-grained model of PSII indicates that light-intensity dependent branching off of reaction path occurs.

3P254 蛍光寿命顕微鏡による葉緑体微細構造の観察

Fine structures of chloroplasts observed by fluorescence lifetime imaging microscopy

Ryuichi Matsuyama[1], Ryo Yamada[1], Takashi Shiina[3], Masahide Terazima[1], Shigeichi Kumazaki[1,2] ([1]*Grad. Sch. Sci., Univ. Kyoto*, [2]*PRESTO, JST*, [3]*Grad. Sch. of Life and Environ. Sci., Kyoto Pref. Univ.*)

Chloroplasts in plants and algae show autofluorescence of chlorophyll a mainly from photosystem II, its light-harvesting complexes, and to a less extent from photosystem I and its antenna. The lifetime of fluorescence reflects photochemical reaction in chloroplasts. We have applied fluorescence lifetime imaging microscopy (FLIM) to chloroplasts in a green algae, in order to study fine structures of chloroplast in situ and their changes. We have also developed software to automatically analyze the results of FLIM data to extract fluorescence lifetime from grana thylakoid and stroma-exposed thylakoid separately. Three dimensional structures inside the chloroplasts and the dependence of the fluorescence lifetime on excitation laser intensity will be described.

3P255 光化学系 II におけるプロトン移動経路

若手招待講演

Proton transfer pathway in photosystem II

Keisuke Saito[1,2], A. William Rutherford[3], Hiroshi Ishikita[1,2] ([1]*Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ.*, [2]*PRESTO JST*, [3]*Dept. of Life Sci., Imperial College, London*)

PS II possesses the redox active tyrosine TyrD in the electron-transfer inactive subunit D2. When TyrD is oxidized, the H+ of TyrD is released forming a very stable neutral radical TyrD-O^dot. The PT energetics analysis shows that the H+ released on oxidation of TyrD is transferred to a water molecule near the phenolic O of TyrD, triggering a concerted PT along a pathway involving D2-Arg180 and a series of waters, through which the proton reaches the aqueous phase at D2-His61. From the symmetry between the D2 and the electron-transfer active subunit D1, we deduced a PT pathway from the tyrosine Z to D1-Asp61 through one of the Cl- sites in the D1.

3P256 実験室進化を用いた大腸菌の抗生物質耐性獲得ダイナミクスの解析

Laboratory evolution of antibiotic resistant Escherichia coli

Shingo Suzuki, Takaaki Horinouchi, **Chikara Furusawa** (*Quantitative Biology Center, RIKEN*)

Laboratory evolution experiments provide valuable information about the nature of adaptive evolution. Here, we performed evolution experiments of Escherichia coli under each of 33 antibiotics, and obtained antibiotic resistant strains. Then, we performed gene expression analysis by microarrays and resequencing analysis by next-gen sequencer. Furthermore, for each resistant strain, cross-resistance/sensitivity to other antibiotics were quantified. As results, we found although the patterns of cross-resistance/sensitivity and expression profiles were similar among the resistant strains for same antibiotics, the fixed genetic mutations were often diverse. This suggested that, the phenotypic convergence is based, as least in part, on diverse genetic/epigenetic mechanisms.

3P257 全生物共通祖先生物の生育温度の実験による推定

Empirical estimation of the environmental temperature of the last universal common ancestor

Satoshi Akanuma, Yoshiki Nakajima, Shin-ichi Yokobori, Akihiko Yamagishi (*Dept. of Appl. Life Sci., Tokyo Univ. of Pharm. Life Sci.*)

We experimentally address the environmental temperature of the universal common ancestor of life (Commonote). Our approach involved phylogenetic tree building to infer the amino acid sequences of archaeal and bacterial ancestral nucleoside diphosphate kinases (NDK) and characterizing their unfolding temperatures. The unfolding temperature of an NDK correlates well with the environmental temperature of its host. The ancestral NDKs are very thermally stable proteins. Because the amino acids conserved among the ancestral sequences probably include those of the Commonote and because the amino acids that varied in a given position did not substantially affect the thermal stability, the Commonote probably possessed a thermostable NDK, i.e., it was a thermophile.

3P258 On phenotypic drug tolerance based on expression noise of antibiotic resistant gene

Takashi Nozoe[1], Reiko Okura[1], Yuichi Wakamoto[1,2] ([1]*Grad. Sch. Arts and Sci., Univ of Tokyo*, [2]*Research Center for Complex Systems Biology, Univ of Tokyo*)

To investigate general principles of bacterial phenotypic tolerance for lethal stress, we constructed *E.coli* strain expressing from an inducible promoter an antibiotic resistant gene fused with fluorescent protein and observed recovery of its population fitness in the antibiotic exposure. Moreover, we constructed some variants among which production rate and degradation rate of the fusion protein were different; those variants enabled us to study effects of gene expression noise on the fitness recovery. Thus, by conducting timelapse experiments, we evaluated population dynamics of those constructed variants in different growth conditions. We discuss relation between phenotypic tolerance and noise property of the antibiotic resistant gene expression.

3P259 二次構造のパッキングの仕方は同じだがトポロジーの異なるタンパク質ペアの特徴

Some features of protein pairs which have same SSEs packing arrangement but have different topology

Tatsuo Mukai[1], Shintaro Minami[2], George Chikenji[1] ([1]*Grad. Sch. of Engineering, Nagoya Univ.*, [2]*Grad. Sch. of Info. Sci., Nagoya Univ.*)

Many studies have been reported that there is a limited repertoire of secondary structure packing arrangemnets and current PDB is sufficiently complete.
These observations suggest that new fold protein structures can always be modeled by rewiring a currently exsiting fold.
In order to develop a new prediction method for new fold targets with the rewiring technique, we need to understand features of the same secondary structure packing arrangemnets.
Fot this purpose, we performed large scale non-sequential structure alignment between protein pairs deposited in PDB and searched for the characteristic feature of the protein pairs that have the same core packing but have different topology. We will report detailed description of the method and the results.

3P260 デノボタンパク質立体構造予測のための新規フォールド構造生成法

Generating novel protein folds from existing folds for de novo protein structure prediction

Yuki Nakagawa[1], George Chikenji[1], Shintaro Minami[2] ([1]*Grad. Sch. of Eng., Nagoya Univ.*, [2]*Grad. Sch. of Info. Sci., Nagoya Univ.*)

The primary obstacle to de novo protein structure prediction is conformational sampling: the native state generally has lower free energy than non-native 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 builds novel structures by rewiring the secondary structure elements of a existing fold. This technique allows us to effectivly 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 large scale benchmark test of de novo protein structure prediction with the new method.

3P261 Non-sequential structural alignment reveals fold change by segment shuffling during evolution

Shintaro Minami[1], George Chikenji[2], Motonori Ota[1] ([1]*Dept. of Info. Sci., Nagoya Univ.*, [2]*Dept. of Eng., nagoya Univ.*)

It has been known that homologous proteins sharing the same architecture could take different topology. Further investigation of such homologous proteins will lead us to deep understanding of protein evolution. We performed comprehensive database search employing non-sequential structural alignment. We calculated structural similarity and profile correlation for all protein pairs in the PDB regardless of their sequence-order. As a result, we found a number of candidates of homologs that adopt similar architecture and different topology. Some of them can not be explained as circular-permuted or segment-swapped proteins. In the presentation, we'll show detailed results and discuss some examples.

3P262 Motion Tree 法による蛋白質構造変化の階層的記述と網羅的分類

Hierarchical description and extensive classification of protein structural changes by Motion Tree

Ryotaro Koike[1], Motonori Ota[1], Akinori Kidera[2] ([1]*Grad. Sch. Info. Sci., Nagoya Univ.*, [2]*Grad. Sch. Med. Life Sci., Yokohama City Univ.*)

The crystal structures of the same protein, determined under different conditions, provide clues toward understanding the role of structural changes in the protein's function. To describe protein structural changes, we propose a method based on hierarchical clustering. This method enables the illustration of a wide range of protein motions in a single tree diagram, named the "Motion Tree". We applied the method to 432 proteins exhibiting large structural changes, and classified their Motion Trees in terms of the characteristic indices of the trees. This classification of the Motion Trees revealed clear relationships to their protein functions. Especially, complex structural changes are significantly correlated with multi-step protein functions.

3P263 beta-Trefoil タンパクのフォールディングコアの残基間平均距離統計に基づく解析

Analyses of folding nuclei of beta-Trefoil fold proteins based on the inter-residue average distance statistics

Norihiro Kanemaru, Masanari Matsuoka, Takeshi Kikuchi (*Dept. Bioinf., Col. Life Sci., Ritsumeikan Univ.*)

The beta-Trefoil structure is a trilateral symmetry proteins consisting of beta-sheets. In this study, we aim to predict the regions of the folding nuclei of a beta-Trefoil protein by means of a predicted contact map (ADM) and the inter-residue contact frequency (F-value) calculated from the inter-residue potential based on the inter-residue average distance statistics. The results indicate that the location of folding nuclei and the high F-value regions correspond well to the highly protected regions in a HD exchange experiment, which are in the central part of the protein. There were also some conserved residues in the Cytokine Superfamily around the F-value peaks. We consider that these regions would become the folding nuclei of the beta-Trefoil protein.

3P264 FCANAL（構造を基にしたタンパク質機能御予測法）の様々なタンパク質への適用

FCANAL, structure-based protein function prediction method, applied to various types of proteins

Hiroko Sagisaka, Misaki Yamamoto, Ichiro Yamato (*Dept. Biol. Sci. Tech, Tokyo Univ. of Science*)

Although sequence-based function prediction methods have been used extensively, structure-based prediction is expended to provide higher specificity and sensitivity because functions are closely related to the three-dimensional structures of functional sites.
We have developed FCANAL(Fast Calculable protein function ANALyzer), a method to predict functions using score matrices obtained from the distances between C^α atoms and frequencies of amino acid appearance around functional sites of proteins with known functions. FCANAL can predict many protein functions of many proteins; binding proteins of low molecular weight substrates, suger binding proteins, DNA binding proteins, enzymes, etc.
Here, I report FCANAL applied to predict functions of G-protein-coupled receptors.

3P265 人工プロモーター設計 Web アプリケーション PromoterCAD のためのデータベース構築

Database Construction for Synthetic Promoter Design Web Application (PromoterCAD)

Koro Nishikata[1], Robert Cox III[1], Sayoko Shimoyama[1], Yuko Yoshida[1], Minami Matsui[2], Yuko Makita[1], Tetsuro Toyoda[1] ([1]*Integrated Database Unit, Advanced Center for Computing and Communication (ACCC), RIKEN*, [2]*Synthetic Genomics Research Team, Biomass Engineering Program Cooperation Division, Center for Sustainable Resource Science (CSRS), RIKEN*)

Synthetic promoters can control the timing, location and amount of gene expression for any organism. PromoterCAD is a web application for designing synthetic promoters with altered transcriptional regulation. We constructed 4 types of expression-motif databases for PromoterCAD by combining 2 types of expression data (AtGenExpress, Diurnal) and 2 types of regulatory sequence motif (ATTED-II, PPDB). These include information on 21,000 genes from Arabidopsis and 1,410,000 microarray data measurements in 20 growth conditions and 79 tissue organs and developmental stages. We demonstrate data mining tools for finding motifs related to circadian oscillations and tissue-specific expression patterns. The application provides user interface, so users can design promoters easily.

3P266 保存された連続反応を用いた代謝系のモジュール構造の同定とその進化に関する考察

Identification of metabolic pathway modules by conserved reaction sequences and its application to evolutionary analysis

Ai Muto, Masaaki Kotera, Toshiaki Tokimatsu, Yuki Moriya, Zenichi Nakagawa, Minoru Kanehisa, **Susumu Goto** (*Inst. Chem. Res., Kyoto Univ.*)

Enzymatic reactions are key components in the metabolic pathway and have been classified in several ways. We have also developed a classification system for the enzymatic reactions based on chemical transformation mechanisms. Using this classification system, we extracted and annotated conserved sequences of reactions from the KEGG PATHWAY database, successfully defined 33 functionally well explained sequences, and termed them as reaction modules. These modules often correspond to gene clusters on bacterial genomes even though they are defined only based on chemical reactions. We also found that some modules are repeatedly used and/or combined with specific partner modules, indicating an evolutionary aspect of the metabolic pathway.

3P267 Lysozyme スーパーファミリーを用いた遠縁タンパク質間のフォールディング部位の頑健性についての解析

The analysis of the robust folding units among highly diverse proteins in the lysozyme superfamily

Michirou Kabata[1], Yousuke Kawai[2], Takeshi Kikuchi[1] ([1]*Dept. Bioinf., Col. Life Sci., Ritsumeikan Univ.*, [2]*Dept. Bioinf., Fac. Eng., Maebashi Ins. Tech.*)

The relationships between the evolution of protein and the protein folding mechanisms are not completely elucidated. In the lysozyme superfamily the structural similarity of each type of lysozyme suggests they have a common ancestor despite their low sequence similarity. In this study, we examine the robustness of folding nuclei of the lysozyme superfamily. That is, location of the possible folding nuclei of these proteins is predicted only from their amino acid sequences based on the inter-residue average distance statistics. The phylogenetic trees are built using the multiple sequence alignment to investigate the evolutionary change of the possible folding nuclei. The results suggest the robustness of folding nuclei during the evolution of the lysozyme superfamily.

3P268 天然変性タンパク質における自然淘汰の dN/dS 比に関する解析

Estimating the strength of natural selection on intrinsically disordered proteins in terms of dN/dS ratio

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A significant fraction of eukaryotic proteins includes intrinsically disordered regions (IDR) that do not exhibit compact rigid structure under physiological conditions. Typically, functional fragments in IDRs switch to more ordered states upon binding to target molecules, a phenomenon known as coupled folding and binding (CFB). We conducted an analysis to compare the degree of selective pressure observed between ordered regions, IDRs, and functional IDRs. dN/dS, that represents the vulnerability of amino acid substitutions relative to the neutral nucleotide mutation rate, of the functional IDRs suggests that the regions have been subjected to stronger negative selection than IDRs. Higher dN/dS of IDRs may be accounted for by the abundance of neutrally evolved codons.

3P269 間期染色体の３ D モデル：ゲノム構造と機能の理解に向けて

3D model of interphase chromosomes: toward understanding of genome structure and function

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Recent progress of the chromosome conformation capture technology such as 3C and Hi-C enabled studies of the spatial organization of genome-wide chromosome structures. Yet, how the 3D structure of the genome affects gene function (ex. transcription, replication, etc.) remains elusive. Using published data and computational approaches, we examined the gene positioning and other properties for several organisms, compared them with the chromatin organization, and then revealed some functional role of the spatial organization of the genome.

3P270 Krylov 部分空間法による相関したブラウンノイズの計算

Krylov subspace methods for computing correlated Brownian noise vectors in Brownian dynamics simulations with hydrodynamic interactions

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Brownian dynamics is a molecular simulation algorithm that can incorporate hydrodynamic interactions between particles. In the simulations, a Cholesky factorization is commonly used to compute correlated Brownian noise vectors. However, this factorization is an $O(N^3)$ operation for an N-particle system, which is generally the bottleneck in the Brownian dynamics. Here, we study methods based on Krylov subspace approximations for computing Brownian noise vectors to overcome this difficulty. We show that the computational time of Krylov subspace methods scales very nearly as $O(N^2)$ without any loss of simulation accuracy. Thus, Krylov subspace methods are recommended for performing large-scale Brownian dynamics simulations with hydrodynamic interactions.

3P271 3P271 は 2P183 に移動しました.

3P272 力を介して細胞の増殖速度の差を感知する仕組み

Interface mechanics between two clonal cell populations with different growth rates --- A theoretical study of cell competition

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When two types of clonal cell populations competitively grow in epithelial tissues, only at the boundary between the two clones, apoptosis selectively occurs in the cells with lower division rate. Although genetic studies have suggested that F-actin dependent mechanical force is a major regulator of cell competition, how the difference of division rates affects the mechanical force is little investigated. Using the cell vertex model, we numerically found that the boundary of the clones was more smoothly rounded only as the difference of division rates increases. This boundary shape is consistent with experiments in *Drosophila*. Moreover, the slower dividing cells exhibited boundary-specific abnormalities not only in cell shape, but also in tensile force.

3P273 ENaC 細胞内動態の数理モデル構築による上皮 Na^+輸送制御解析

ENaC dynamics in the intracellular space: analysis of Na^+ transport in epithelial cells by mathematical model

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Epithelial Na^+ channels (ENaC) are translocated to the apical cell membrane from the intracellular store site. Experimental observations on ENaC-mediated Na^+ transport indicate that at least following 4 steps are involved in ENaC translocation: i.e. 1) insertion, 2) endocytosis, 3) recycling, and 4) degradation.
To understand more details about regulation of Na^+ transport in epithelial cells and which state is the rate-limiting one, we applied a mathematical model including 4 steps for electrophysiological parameter of Na^+ transport, suggesting that the amount of recycled ENaCs depends on quality control of ENaC in the intracellular store site. This means that this model leads us to novel understandings on ENaC-mediated Na^+ transport.

3P274 真性粘菌 *Physarum polycephalum* とそのモデルによる錯視の計算

Computing visual illusion by *Physarum* plasmodium and the model

Iori Tani, Masaki Yamachiyo, Pegio-Yukio Gunji (*Department of Earth and Planetary Sciences, Graduate School of Science, Kobe University*)

True slime mold *Physarum polycephalum* plasmodium is a huge unicellular amebic organism. It is widely used in biocomputing studies.
However, the most of these studies used plasmodium as optimization calculator.
By contrast, we propose *Physarum* biosensor element that duplicate human KANSEI/feeling information.
We show *Physarum* plasmodium and our asynchronous cellular automata model are able to "calculate" visual illusions such as subjective contour of Kanizsa's triangle and Muller-Lyer illusion.

3P275 錯視を引き起こす図形パターンに対する真性粘菌変形体の反応

Behavior of the physarum plasmodium to the graphical pattern that provide the optical illusion

Masaki Yamachiyo, Iori Tani, Pegio-Yukio Gunji (*Department of Earth and Planetary Sciences, Graduate School of Science, Kobe University*)

The plasmodium of Physarum polycephalum is a giant acellular organism that crawls on planar surfaces foraging food sources and forms a network of protoplasmic tubes connecting the masses of protoplasm at the food sources. In this study, we investigated the behavior of the plasmodium to the graphical food sources arranged in the pattern that provide the optical illusion Kanizsa triangle. We found that such graphical patterns have an effect on the plasmodium same as optical illusion in terms of connecting tube network between food sources. Moreover, in this experiments, we introduced the epochal material of food source made from nutrient rich agar, which is easily-shaped into various figures and sizes.

3P276 概日中枢時計のウエーブパターンとその機能について

Wave-like structure and its function in the circadian master clock

Hiroshi Kori[1,2] ([1]*Ochanomizu Univ*, [2]*CREST*)

Modeling and Analysis of circadian rhythm
In this presentation, I will present our recent mathematical studies, in collaboration with experimentalists, on the wave-like structure and its function in the circadian master clock.

3P277 Dependence of cell differentiation ratio on cell-cell interaction and noise

Fumiko Ogushi, Hiroshi Kori (*Ochanomize University*)

Differentiation is one of the most fundamental ability of biological cells and cell differentiation is considered to be controlled by the expression level of certain genes. Recent experimental studies indicate that cell-cell interaction plays an important role in cell-fate decision. To understand the control mechanisms of cell differentiation, we introduce a simple multiple-cell model. In this presentation, we report the effect of interaction and noise on cell differentiation ratio.

3P278 クラミドモナスの鞭毛波形変異体における生物対流現象
Bioconvection in waveform mutants of *Chlamydomonas reinhardtii*

Azusa Kage, Yoshihiro Mogami (*Graduate School of Humanities & Sciences, Ochanomizu Univ.*)

Many motile microorganisms show bioconvection behavior, in which density variation of the microorganism develops into two-dimensional macroscopic patterns. We have previously reported a peculiar spontaneous pattern transition in the bioconvection of *Chlamydomonas*, and also suggested that this response is triggered by the changes of the gyrotaxis of the cell, a tendency of entrainment into the downward flow. As gyrotaxis is caused by the vorticity of the advective fluid flow, it is likely that the changes in the total geometry of the cell including the beating wave configuration of the flagella affect gyrotaxis. Here we investigated bioconvection of the waveform mutants of *Chlamydomonas reinhardtii* in relation to the pattern transition response.

3P279 KaiC タンパク質のリン酸化と ATPase 活性の概日リズムの確率シミュレーション によるモデリング
A modeling study of the circadian rhythm of phosphorylation and ATPase activity of KaiC protein by stochastic simulation

Kenju Narita, Masaki Sasai, Tomoki P. Terada (*Grad. Sch. Eng., Nagoya Univ.*)

Cyanobacterial proteins KaiA, KaiB, KaiC and ATP produce the temperature-compensated rhythm of phosphorylation level of KaiC with the period of about 24 hours in vitro. In addition, ATPase activity of KaiC also shows circadian rhythm, which is temperature-compensated and affected by the mutation in a manner correlated with the phosphorylation level oscillation. Although some mechanisms have been proposed only for the phosphorylation level oscillation, no model has describe oscillations of the phosphorylation level and the ATP hydrolysis activity at the same time. We focus on a single hexamer of KaiC to examine the correlated dynamics of both two oscillations by using the stochastic simulation of chemical reactions to address the underlying mechanism.

3P280 The analysis of energy transfer in Chaotic Dynamical Systems

Mami Kushida (*Grad., Univ. Narajoshi*)

One of the fundamental laws of the classical statistical mechanics is equipartition of energy. However, a celebrated work of Fermi, Pasta and Ulam revealed that equipartition of energy does not necessarily hold for a model of coupled nonlinear oscillators. This finding raises a question of how energy transfer takes place in nonlinearly coupled systems of large degrees of freedom. The same question applies to biomolecules such as proteins. In this study, we propose a new method for understanding the mechanism of the energy transfer. Our method is "Wavelet PCA", which combines the wavelet transformation and the principal component analysis. We will report results of our analysis for the energy transfer of the FPU coupled oscillator system.

3P281 非線形関数のステップ関数表示の公式
Step Function Representation of Nonlinear Function

Eisuke Chikayama[1,2] ([1]*Niigata University of International and Information Studies*, [2]*RIKEN*)

Nonlinear dynamical system is a biophysical, theoretical biological, and bioinformatic tool for analysing various temporal patterns in biological systems. For the question how biological systems, physico-chemically, can behave in complex and functional manners, we have concluded that any complicated behaviours can synthesized by mixing chemical catalysts of which the reactions are expressed with only Hill equations, simple cooperative reactions. We have not, however, open the complete formula of step function representation for a multidimensional nonlinear function, which will be shown, that is the base of the conclusion.

3P282 DNA マイクロアレイを基盤とした無標識 miRNA の定量法の開発
Label-free quantification of miRNA using Ligase-Assisted Sandwich-Hybridization based on DNA microarray

Taro Ueno, Takashi Funatsu (*The University of Tokyo*)

Micro RNAs (miRNAs), which are small RNAs of ~22 nucleotides, have been one of the potential biomarkers of cancers for several years. We constructed a platform to quantify miRNAs without the need of pre-labeling using ligase-assisted sandwich hybridization (LASH). T4 DNA ligase was used to compensate for the low affinity between miRNAs and two short complementary DNA probes, and it improved the hybridization yield ~50,000 times. LASH assay allowed us to determine the amount of endogenous miR-143 exported from HEK293 cells as well as to distinguish homologous miRNAs by a multi-color detection. Furthermore, this labeling-free quantification system was applicable in a microfluidic chip, which device is well-qualified candidate for a full-automatic diagnosis system.

3P283 蛍光ダイアモンドナノ粒子を使った光検出磁気共鳴
Optically detected magnetic resonance for fluorescent single nanodiamond in cell and c.elegance

Yohsuke Yoshinari[1], Yuta Kumiya[2], Takuma Sugi[2], Ryuji Igarashi[2], Shingo Sotoma[2], Masahiro Shirakawa[2], Yoshie Harada[1] ([1]*iCeMS, Kyoto University*, [2]*Department of Molecular Engineering, Kyoto University*)

We developed new spectroscopy for measuring local dynamics in living biological samples. The method is based on optically detected magnetic resonance (ODMR) for a fluorescent impurity, nitrogen vacancy centers (NVCs) in diamond nanoparticle. The well-established magnetic resonance technique and analysis are applied even for single nanodiamond present in a living biological samples. We will present a few experimental results such as, selective imaging of nanodiamonds, creation of NVC by ion irradiation with post-anneal and monitoring a rotational dynamics of the nano particle in the intestine of living C.elegance.

3P284 一分子計測と一分子粒度細胞シミュレーションの融合
Development of Fluorescence Microscopy/Spectroscopy Monte Carlo Simulation

Masaki Watabe, **Satya Arjunan**, Koichi Takahashi (*RIKEN*)

Quantitative modelling has become an essential procedure toward understanding the cell at the systems level. Insights into the inner workings of biological networks can be obtained from mathematical analysis of biochemical models that might not be possible from direct experimentation alone. E-Cell System is one of the cell simulation platforms that allow us model cellular biochemical reaction networks in single-molecule resolution. For further quantitative understanding of intracellular molecular dynamics and kinetics, we have developed fluorescence microscopy/ spectroscopy Monte Carlo simulation to have direct comparison of the simulated data to single molecule experimental data.

3P285 細菌べん毛モーターへの CheY-P の結合は回転方向だけでなく速度にも影響する。
CheY-P binding to the bacterial flagellar motor affects not only the direction but also the speed of rotation

Koichi D. Hiraoka[1], Shuichi Nakamura[2], Nobunori Kami-ike[1], Yusuke V. Morimoto[3] ([1]*Grad. Sch. of Frontier Biosci.*, [2]*Grad. Sch. of Eng., Tohoku Univ*, [3]*RIKEN, QBiC*)

Many bacteria swim in liquid environments by rotating helical flagella by an ion-driven rotary motor. The motor rotates in the counter-clockwise (CCW) direction constantly and in the clockwise (CW) direction occasionally for smooth swimming and tumbling, respectively. The CW rotation occurs by the binding of phosphorylated CheY (CheY-P) to the motor as the signal molecule. The behavior and specification of the motor have been well studied in the CCW rotation but little is known about the CW rotation. We carried out bead-rotation assay on a FliG mutant motor that rotates only in the CW direction without CheY-P and found that the motor function is symmetrical in the CCW and CW rotation. We also found that CheY-P binding to the motor disturbs the motor function.

3P286 タンパク質中性子結晶構造解析におけるプロトン偏極法のための基礎的な試み
Fundamental trials for proton polarization technique in neutron protein crystallography

Ichiro Tanaka[1,2], Katsuhiro Kusaka[2], Toshiyuki Chatake[3], Nobuo Niimura[2] ([1]*Coll. of Eng., Ibaraki Univ.*, [2]*Frontier, Ibaraki Univ.*, [3]*RRI, Kyoto Univ.*)

Isotope effect in the conventional neutron protein crystallography (NPC) can be eliminated by proton polarization technique (ppt). Furthermore, the ppt can make hydrogen visibility about 8 times larger than that in the conventional NPC, etc. On the other hand, several technical difficulties should be overcome in order to perform the ppt. In this presentation, several fundamental studies to realize ppt will be presented; the high pressure flash cooling was found a promising technique to make bulk water amorphous, and an ESR measurement will be presented in order to detect the spin distribution in a protein single crystal which is important to polarize protons in a protein.

3P287 X線自由電子レーザーを利用した球状生体超分子複合体のコヒーレントX線イメージングへの取り組み
Approaches to cohherent X-ray diffraction imaging of single virus particle using X-ray free-electron laser

Akifumi Higashiura[1], Marina Murakami[1], Kenji Iwasaki[1], Eiki Yamashita[1], Kazuki Takeda[2], Yuya Hanazono[2], Kiyofumi Takaba[2], Masahito Hibi[2], Yuriko Tomisaki[2], Kunio Miki[2], Atsushi Nakagawa[1] ([1]*Inst. for Prot. Res., Osaka Univ.*, [2]*Grad. Sch. of Sci., Kyoto Univ.*)

The advent of X-ray free-electron leasers (X-FEL) creates new capabilities for coherent x-ray diffraction imaging (CXDI) of a single macromolecule. The first image reconstruction of a biological molecule using CXDI techniques was performed by Seibert et al. (2011). Low-resolution images of mimi-virus were calculated from the single diffraction pattern using X-FEL from the Linac Coherent Light Source (LCLS) at Stanford. In Japan, an X-FEL facility, SACLA (SPring-8 Angstrom Compact Free Electron Laser) has been developed, which produces high brilliance and short X-ray pulses at Angstrom wavelength. The use of the facility began with the start of user operation in March, 2012. We obtained some diffraction images from virus particles in the beamline BL3 at SACLA.

3P288 hPrx2 のオリゴマー形成過程の高速 AFM 観察
Investigation of hPrx2 oligomerization process by high-speed AFM

Takamitsu Haruyama, Noriyuki Kodera, Hiroki Konno (*Bio-AFM Frontier Research Center, College of Sci. & Eng., Kanazawa Univ.*)

Peroxiredoxin (Prx) catalyzes the decomposition of reactive oxygen species (ROS) like peroxide, organic hydroperoxide, and peroxynitrite. Interestingly, several Prxs act not as peroxidases but as molecular chaperones in high H_2O_2 stress. The functional change is related to a structural change from low molecular weight oligomers, including dimers and decamers, to high molecular weight complexes in high H_2O_2 conditions.
To investigate the dynamics of the structural change in detail, we observed the oligomerization process of human peroxiredoxin 2 (hPrx2) by using high-speed AFM, as well as the dissociation process of the high molecular weight complexes by sulfiredoxin (Srx). The details of the oligomerization mechanism will be discussed.

3P289 細胞内蛋白質混雑感受性蛍光蛋白質の開発
Intracellular measurement of protein-crowding condition by a gene-encoded indicator

Takamitsu Morikawa[1], Keiko Yoshizawa[2], hideaki Fujita[2,3], 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]*RIKEN Quantitative Biology Center*, [3]*WPI, Immunology Frontier Research Center, Osaka University*, [4]*Department of Macromolecular Science, Graduate School of Science, Osaka University*, [5]*Institute of Sciencific and Industrial Research Center, Osaka University*)

Intracellular environment is overcrowded with enormous number of various macromolecules such as proteins. This high protein concentration causes molecular crowding effects on protein folding and enzyme activity, which can have a significant effect on protein function. Despite its significance, no techniques can directly measure protein concentration in living cells. Instead, protein concentration is estimated from protein diffusion, which is measured by techniques like FRAP or FCS. To directly measure protein concentration, we here describe a protein-concentration sensor based on fluorescent protein engineering and show that it can reveal molecular crowding effects not realized when measuring only protein diffusion.

3P290 マウス内がん細胞の非侵襲イメージング
Noninvasive in vivo imaging of tumor cells in a novel xenograft model

Sayaka Kita, Hideo Higuchi (*Dep. of phys., Grad. Sch. of Sci., The Univ. of Tokyo*)

We developed new noninvasive imaging methods to visualize molecules. We focused on the auricle of mouse for observation of tumor cells because it is very thin and limited hypodermal tissue. We developed a novel xenograft model of the auricle with cancer cells in order to observe them noninvasively. We injected four kinds of human cancer cell lines into the auricle of mice. Tumor was successfully formed using each cell lines. To image molecules, specific antibodies to recognize these cells were labeled with fluorescence quantum dots and then injected to tail vein after the formation of tumor. We could perform noninvasive real time observation of quantum dots within cancer cells and on its membrane.

3P291 バクテリア細胞内 ATP 濃度の一細胞計測
Quantifying the absolute ATP concentration inside single bacteria cells

Hideyuki Yaginuma[1,2], Shinnosuke Kawai[3], Keisuke Tomiyama[2], Kazuhito V. Tabata[1,5], Tamiki Komatsuzaki[3], Hiromi Imamura[4], Hiroyuki Noji[1,2] ([1]*Grad. Sch. Eng., Univ. Tokyo*, [2]*Grad. Sch. Front. Biosci., Osaka Univ.*, [3]*Res. Inst. Elect. Sci., Hokkaido Univ.*, [4]*Hakubi Project, Kyoto Univ.*, [5]*PRESTO, JST*)

Adenosine triphosphate (ATP) is necessary for the progress of many intracellular reactions. Our previous ATP biosensor could report ATP concentration change in single cells, but the absolute value was difficult to measure. Here, we developed a biosensor called BQueen, which can be used to determine the absolute intracellular ATP concentration. Using BQueen, we found that the ATP concentration in *Escherichia coli* cells varies from cell to cell. The ATP concentration was distributed in a positively-skewed shape around 1-8 mM. Interestingly, the cell division rate was the fastest in the moderate ATP concentrations. From the distribution width of ATP concentration in different bacteria growth conditions, we will discuss the change in the metabolic pathways inside the cell.

3P292 細胞内熱伝導率マッピング
Mapping of thermal conductivity in single living cells

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Advanced fluorescence thermometry has enabled us to measure the temperature changes in living cells. The details of heat transfer in cells are still not clear due to the lack of information on the thermal properties such as thermal conductivity and heat capacity. In the present study, we mapped two-dimensional distribution of the thermal conductivity in living cells. We applied fluorescent dyes as fluorescent thermometers which uniformly distribute in both nucleus and cytoplasm. The dye is sensitive to temperature changes but resistant to environmental changes such as pH and ionic strength. We locally heated either nucleus or cytoplasm with a focused infra-red laser beam. The temperature distribution indicated heterogeneous thermal conductivity in living cells.

3P293 Structure and fluorescent property 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.*)

Green fluorescent protein based indicators are widely used to detect intracellular environment. We recently found that single amino acid insertion in β7 of YFP dramatically affects its fluorescent property in response to environmental change. The fluorescent property highly depends on nature of the inserted amino acid. This finding has opened the door to designing novel fluorescent base biosensors, however, the molecular mechanism of the mutational effect is unknown. To understand the structural basis of the mutational effect, we prepared a series of amino acid insertion mutant YFPs and solved the structures. Here we show the structures of mutant YFPs, (YFP-1E, F, G, L, M, R, Y) and discuss the relationship between the mutant structure and the fluorescent property.

3P294 量子ドットナノプローブを用いたアミロイドβ凝集阻害物質の新規微量ハイスループットスクリーニングシステムの開発
Development of a novel high-throughput screening system of inhibitory substances for amyloid-β aggregation using quantum-dot nanoprobes

Toshiki Ogara, Yukako Ishigaki, Syoya Yamaguchi, Hiroyuki Tanaka, Koji Uwai, Kiyotaka Tokuraku (*Muroran Institute Of Technology*)

Aggregation of amyloid β (Aβ) protein causes Alzheimer's disease (AD), suggesting that the screening of inhibitory compounds for Aβ aggregation can be the first important step to develop preventive and/or therapeutic drugs for AD. In this study, we successfully developed the microliter-scale and high-throughput screening system for Aβ aggregation inhibitors. First, we evaluated Aβ aggregation inhibitory activities of ethanolic extracts from 52 spices using this system, and found that mint family showed significantly higher activity. Next, we tried to isolate the main inhibitory compound from summer savory, and revealed it was rosmarinic acid. Inhibitory activity of rosmarinic acid has reported, demonstrating that this system could be applied to the actual screening.

3P295 Simultaneous imaging of intracellular Ca^{2+} and sarcomere length in neonatal cardiomyocytes via expression of cameleon-Nano in Z-discs

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To understand the cardiac E-C coupling at the single sarcomere level, we have developed a novel experimental system for simultaneous measurement of intracellular Ca^{2+} and sarcomere length (SL) via expression of cameleon-Nano, a FRET-based Ca^{2+} indicator, in Z-discs (α-actinin) in primary-cultured rat neonatal cardiomyocytes. Under dual-view microscopy, we measured Ca^{2+} changes by imaging the fluorescence emission ratio (i.e., YFP/CFP) and analyzed SL by measuring the fluorescence profiles. As a result, we found that SL changed in response to a change in the YFP/CFP ratio. The present experimental system is useful for simultaneous imaging of local Ca^{2+} and single sarcomeres at high spatial and temporal resolution in living cardiomyocytes in health and disease.

3P296 超解像イメージング法により明らかとなったストレス顆粒内 mRNA の詳細分布

Super-resolution imaging reveals nanoscale distribution of mRNA in stress granule

Ko Sugawara[1], Kohki Okabe[1,2], Akihiko Sakamoto[1], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, the University of Tokyo*, [2]*JST, PRESTO*)

Under cellular stress, cytoplasmic mRNAs assemble and form stress granules (SGs). However, the fine structure of SGs has been unclear. We investigated the issue by stochastic optical reconstruction microscopy (STORM), which provided us super-resolution images with spatial resolution of several tens of nanometers. STORM imaging showed that endogenous mRNAs located in spherical compartments with a diameter of ~200 nm. Furthermore, some SGs components showed distinct spatial patterns in SGs. These results suggested that STORM is a powerful tool to investigate the fine structure of granular architecture in cells and that SGs have highly organized composition that would be responsible for the physiological functions in cellular stress responses.

3P297 生細胞内における microRNA のイメージング

Imaging of microRNA in living cells

Toshinari Ishikawa[1], Kohki Okabe[1,2], Takashi Funatsu[1] ([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*Sakigake, JST*)

MicroRNA (miRNA), a small noncoding RNA, takes part in translational regulations by targeting mRNA to trigger either cleavage or translational repression. It is known that miRNAs are closely related to certain disease; for instance, let-7 miRNA is depleted in cells of human lung cancer. Although the importance of miRNA activity is recognized, dynamics of miRNA in living cells are still unknown because no method has been available to capture them. Here, we show a new approach to chase the cellular dynamics of miRNA by introducing fluorescent precursors of miRNA into living cells followed by fluorescence imaging. This method allowed us to quantitatively image miRNA in living cells, which would be applicable to investigate functionality of miRNA in living cells.

3P298 大腸菌 RND 型異物排出トランスポーター AcrD の細胞内動態観察

Dynamics of RND-type xenobiotic transporter AcrD in the cytoplasmic membrane of *Escherichia coli*

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AcrD is an inner membrane transporter of the resistance-nodulation-cell division (RND) family for xenobiotic efflux. Upon induction by indole, AcrD forms a tripartite complex with the outer membrane channel TolC and the membrane fusion protein AcrA. In this study, we asked how newly expressed AcrD forms a tripartite complex in the cytoplasmic membrane and whether AcrA affects dynamics of AcrD as the effect of AcrA on AcrB dynamics has not been explored. When observed using TIRFM, most fluorescent foci of chromosome-encoded AcrD-GFP showed little lateral displacements, whereas they were moving incessantly in the *tolC* or *acrA*-deletion background. These results suggest that AcrA plays a critical role for assembly or stabilizaton of the tripartite complex.

3P299 大腸菌異物排出システム AcrAB-TolC の細胞内動態解析

Dynamics of the xenobiotic efflux system AcrAB-TolC in *Escherichia coli*

Kentaro Yamamoto[1], Rei Tamai[1], Takehiko Inaba[2,4], Yoshiyuki Sowa[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.*, [4]*RIKEN Adv. Sci. Inst.*)

Among the five RND-type xenobiotic efflux systems of *Escherichia coli*, only AcrB, which forms a complex with the membrane fusion protein AcrA and the outer membrane channel TolC, is constitutively expressed. In this study, we aim at visualization of the construction processes of the efflux protein complex. When observed with TIRFM, most fluorescent foci of chromosome-encoded AcrB-GFP hardly showed lateral movements, whereas they were moving incessantly in the absence of TolC. The fraction of moving AcrB-GFP foci increased with increasing levels of the closely related transporter AcrD, which forms a ternary complex with AcrA and TolC. We therefore propose that AcrD can replace AcrB in the complex with AcrA and TolC.

3P300 FIB（Focused Ion Beam: 集束イオンビーム加工）-SEM（Scanning Electron Microscope: 走査電子顕微鏡）による細胞まるごと三次元構造解析法の開発

Whole cell structure reconstruction by three-dimensional Focused Ion Beam and Scanning Electron Microscopy

Rina Nagai[1], Keisuke Ohta[2], Kazuhiro Aoyama[3,4], Akinobu Togo[2], Akihiro Kawamoto[5], Atsuko H. Iwane[1,3] ([1]*Cell Field Struc., QBiC, Riken*, [2]*2Anatomy, Med., Kurume Univ.*, [3]*Spec. Res. Promot. Group, Grad. Sch. Fronti. Biosci., Osaka Univ.*, [4]*Application Lab., FEI JAPAN*, [5]*Cell Dynamics Observ., QBiC, Riken*)

We describe a new biological application of FIB-SEM, which is normally used to visualize metals and ceramics, for the 3D reconstruction of an entire cell at a nanoscale resolution that lies between those of Electron Microscopy tomography and X-ray tomography.
In this meeting, we used FIB-SEM to visualize the 3D architecture of C. merolae, which is thinking as the primitive unicellular red algae. Our system could image simple individual double-membrane organelles like nucleus, chloroplasts, and mitochondria, and single-membrane organelles like the ER and Golgi inside C. merolae of 2-5 μm in length. By synchronizing cells to a 6-h light/18-h dark cycle, we describe the imaging of these organelles during cell division.

3P301 クライオ電子線トモグラフィーと STEM を用いた生細胞内オルガネラのイメージング

Imaging of live cell organelles by Cryo-electron tomography and STEM

Ruriko Ogawa[1], Kazuhiro Aoyama[2,3], Rina Nagai[1], **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 is an emerging technology that compliments crystallography, NMR and SM imaging techniques. Its strength is in that it reveals the spatial arrangements of key proteins and complexes during intracellular signaling and mechanical events. In this meeting, we describe the imaging of live organelles by Cryo-EM tomography using the Titan Krios EM and HAADEF detector. Observed living cells were grown directly on Quantifoil and were done vitrification instead of the usual chemical fixation for keeping soluble materials. Cells were imaged over an angular range from -70 to 70 degrees at 2 degrees x cosΘ and analyzed with Inspect 3D and Amira software.
At future, our system can provide any new information about many kinds of cells and organelles.

3P302 生物試料中での GFP ー CL の観察
Observation of GFP-CL in biological specimens

Kazuyoshi Murata[1], Naoyuki Miyazaki[2], Ryusuke Ueno[2], Hiroki Minoda[2], Naoki Yamamoto[3], Kuniaki Nagayama[1] ([1]*Nat. Inst. Physiol. Sci.*, [2]*Tokyo Univ. Agricult. Tech.*, [3]*Tokyo Inst. Tech.*)

GFP-CL is a phenomenon that fluorescent proteins like GFP generate fluorescent light by electron irradiation (CL: cathodeluminescence). To develop a new technique of correlative light and electron microscopy (CLEM), we employed GFP-CL and identified GFP-labeled proteins in biological specimens using electron microscope. GFP was expressed in yeast cells, and the CL was observed in the cells using the hybrid light-electron microscope (200kV) developed by Nagayama and coworkers (Iijima et al., submitted). The GFP-CL showed a good matching with the photoluminescence (PL). The spectra of GFP-CL were distributed between 500 to 900 nm. An extreme electron irradiation sometimes enhanced the PL and CL intensities. GFP-CL was also observed in other GFP-transfected cells.

3P303 蛍光蛋白質における光および電子発光の電子線活性化
Electron-beam Activation of Photo- and Cathodo-luminescence in Fluorescent Proteins

Kuniaki Nagayama[1], Kazuyoshi Murata[1], Hiroki Minoda[2], Ryusuke Ueno[2], Naoki Yamamoto[3] ([1]*National Institute for Physiological Sciences*, [2]*Tokyo University of Agriculture and Technology*, [3]*Tokyo Institute of Technology*)

Cathodoluminescence(CL) is a kind of fluorescence(FL) generated by not photon but electron irradiation. Recently we have found that some classes of fluorescent proteins (FPs) act as stable and bright CL substances with an associated enhancement of FL itself, which is quite contradictory to our general comprehension to organic materials prone to be damaged by electron bombardment. To clarify this novel finding, the electron-beam activated luminescence, we have made experiments by focusing on following points; i)generality among FPs, ii)FL and CL spectral properties before and after electron irradiation and iii) efficiency of light emission in CL and FL. Possible causes of the novel luminescent event induced with the electron beam is also to be discussed.

3P304 Genetically encoded caged Ca^{2+}
Noritaka Fukuda[1,2], Tomoki Matsuda[1], Takeharu Nagai[1] ([1]*ISIR, Osaka Univ.*, [2]*QBiC, Riken*)

In living organism, Ca^{2+} is one of the most important second messenger to mediates between stimuli and biological responses. To decipher biological events, many Ca^{2+} indicators are developed, although development of Ca^{2+} regulators were insufficient.
Here, we report genetically encoded caged Ca^{2+} that termed PhotoActivatable Ca^{2+} Releaser (PACR). This protein is consist of Ca^{2+} binding protein and light-sensitive domain. Upon photoirradiation, decrease PACR affinity for Ca^{2+} release Ca^{2+}. Its direct Ca^{2+} perturbation ability, localizability and inheritance enabled cells and organelle specific Ca2+ pertubation and control freely moving organisms. This tool will be useful for studying the role of Ca2+ dynamics in complex biological events.

3P305 細胞解析のためのリアルタイム化学刺激システムの構築
Development of the real-time local chemical stimulation system for cell analysis

Masaru Kojima, Takahiro Motoyoshi, Kenichi Ohara, Mitsuhiro Horade, Yasushi Mae, Tatsuo Arai (*Grad. Sch. Eng. Sci., Osaka Univ.*)

The local environment control technology for single cell analysis is being established. In this study, we try to develop a real-time local chemical stimulation system that cans dynamically changing local environment condition and apply this system for analyze and control of bacteria flagella motor. As a first step, 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 and controlling the rotational speed of bacteria flagellar motor with local ion concentration control.

3P306 DNA ナノ構造体を用いた DNA-RNA ポリメラーゼ・ハイブリッドナノマシンの構築と活性評価
Construction and functional analysis of DNA origami base DNA-RNAP hybrid nanomachine

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.

3P307 人工鞭毛により推進する精子型マイクロマシン
A “sperm-like” micro-machine propelled by an artificial flagellum

Tsuyoshi Yamasaki, Susumu Aoyama, Yuichi Hiratsuka (*Japan Advanced Institute of Science and Technology*)

Motor proteins form a molecular complex with other proteins by self-assembly, and therefore generate various sophisticated functions, such as contraction of the sarcomere and beating of the flagellum. In a field of mechanical engineering, a device incorporating such functions is expected as a next-generation micro-machine. In this study, we focused on an oscillatory motion of flagellar axoneme among the biological motion driven by motor proteins and we aim to create novel bio-hybrid micro device which is propelled by the oscillatory motion. Currently, we attempt to construct a “sperm-like” micro-machine which is composed of a photo-lithographically fabricated head part and artificial flagellum synthesized by self-assembly of tubulin and dynein.

3P308 インフルエンザウイルスと高い親和性を有する、Sialyllactose 修飾 3-way junction DNA

Sialyllactose - modified Three way junction(3WJ) DNA as a inhibitor of influenza hemagglutinin

Yasuhito Ebara, Daichi Akamatsu, Naoki Hara, Anna Kono (*Grad. Sch. Hum. Dev. Env. Kobe Univ.*)

Sialyllactose(SL) is present on cellular surface and plays a important roles in the infection on influenza virus. The SL is recognized by hemagglutinin(HA) protein on the virus. A compound that inhibits the interaction between SL on the cell and hemagglutinin has been thought to prevent infection of influenza virus, because the amino acids sequence of sialic acid binding site in HAs are highly conserved among influenza virus strains. In this study, SL-modified dUTP was synthesized as a substrate of DNA polymerase, and these SLs were incorporated on 3-way junction DNAs. These DNAs have high affinity (10 nM) to the hemagglutinin on influenza virus A/PR/8/34(H1N1). These DNAs are expected to be used as influenza virus sensing or inhibitors of influenza infection

3P309 サイズ選択的細胞回収のための超常磁性金属カップの作製

Fabrication of Superparamagnetic Metal Cups for Size-Selective Cell Collection

Hyonchol Kim[1], Hideyuki Terazono[1,2], Hiroyuki Takei[1,3], Kenji Yasuda[1,2] ([1]*KAST*, [2]*Inst. Biomat. Bioeng., Tokyo Med. Dent. Univ.*, [3]*Fac. Life Sci., Toyo Univ.*)

We propose a method for the fabrication of cup-shaped superparamagnetic hemispheres and its application for size-selective cell collection without serious damages. Polystyrene spheres were used as templates, coated with magnetic materials by thermal evaporation, and burned to remove the sphere templates. Cup-shaped hemispheres composed of the evaporated magnetic materials were then obtained. We succeeded to fabricate superparamagnetic cups by controlling the metal thicknesses strictly at the thermal evaporation. By using inner void of the fabricated cups, target cells were size-selectively captured and collected with an application of the magnetic field. The collected cells can be re-cultivated, indicating an ability of the cup for size-selective cell collections.

3P310 細胞表面特異的結合 DNA アプタマーの作製と心筋細胞の精製

Non-invasive identification and purification method of target cardiomyocyte cells using cell-surface-binding ssDNA aptamers

Hideyuki Terazono[1,2], Hyonchol Kim[2], Fumimasa Nomura[1], Kenji Yasuda[1,2] ([1]*Tokyo Medical and Dental University*, [2]*Kanagawa Academy of Science and Technology*)

For the re-construction of cell network assay, the development of non-invasive purification procedure of phenotypically identified target cells from mixture of differentiated cells is essential. Hence, we have developed a new non-invasive cell collection technique to acquire target cells using ssDNA aptamers specifically bound to particular targets on the cardiomyocyte cell surfaces. For choosing the specific aptamer, we adopted a modified conventional SELEX method combining with a next generation sequencing method, and we have successfully acquired the target cardiomyocytes from the mixture of the cells. They suggest that this method may facilitate cell preparation assay and transplantation of ES- or iPS-derived cardiomyocytes for regenerative medicine.

3P311 DNA ナノデバイスを導入した刺激応答性ハイドロゲルの構築

Introduction of DNA nanodevices into a hydrogel for achieving its stimuli-responsive behavior

Takashi Kitajima, Ken Komiya, Masahiro Takinoue, Masayuki Yamamura (*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*)

DNA is a promising component for the construction of nanostructures and functional nanodevices by the sequence-specific hybridization between complementary DNA strands. The programmable binding between complementary DNA strands was utilized for crosslinking in hydrogel materials. The response of such DNA-crosslinked hydrogel materials at a macroscopic scale can be controlled by DNA under a mild solution condition. In this study, we constructed a micrometer-sized stimuli-responsive hydrogel by taking advantage of DNA nanodeveices, with the use of a centrifuge-based droplet shooting device. For emulating a cellular microenvironment, construction and analysis of a micrometer-sized hydrogel would be important. We report the stimuli-response of the present microhydrogel.

3P312 遠心力を利用した複雑形状マイクロハイドロゲル粒子の高速生成

Centrifuge-based rapid synthesis of complex-shaped microhydrogel particles

Masayuki Hayakawa[1], Hiroaki Onoe[2], Ken H. Nagai[3], Masahiro Takinoue[1,4] ([1]*Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech.*, [2]*IIS, Univ. of Tokyo*, [3]*Dept. Phys, Univ. of Tokyo*, [4]*PRESTO, JST*)

Complex-shaped microparticles are becoming important because such particles can offer unique applications including translational/rotational motion dynamics, self-assembly with higher-order structures like living systems. Therefore, today, various synthesizing methods for complex microparticles are reported. However, those methods have common problems: it takes much time and cost to synthesize complex microparticles. Here, we propose a rapid synthesizing method of complex-shaped microhydrogel particles. Our method needs a tabletop mini centrifuge and common experimental supplies such as microtubes, glass capillaries, etc. and requires only 20 minutes for the whole process. We hope our method will occupy crucial position in the field of microparticles synthesis.

3P313 Coherent dynamics in colloidal fluids in terms of Lagrangian coherent structures (LCS)

Preetom Nag, Hiroshi Teramoto, Chun-Biu Li, Tamiki Komatsuzaki (*Research Ins. for Electronic Sci., Univ. Hokkaido*)

Studies on LCS have been received a significant attention to understand transport phenomena in a non-autonomous dynamical system. Roughly, LCS serves as a robust transport barrier between regions in which particles have different dynamical behavior. The simplest diagnostic tool for identifying LCS is to identify the maximum ridges of finite-time Lyapunov exponent(FTLE) field, where FTLE measures the maximum separation rate between nearby set of trajectories over a finite time interval. Here we present our LCS analysis and compare our results with string-like cooperative motion, so-called Donati string, in colloid fluids system. Our results suggest that the ridges of FTLE field should play a significant role to differentiate persistent and fragile strings in this system.

3P314 レプリカ交換分子動力学計算による PA 化糖鎖の立体構造解析

Conformational analysis of PA-glycans by replica-exchange molecular dynamics simulations

Shigehisa Watabe[1], Suyong Re[2], Eiro Muneyuki[1], Yuji Sugita[2,3,4] ([1]*Dept. Phys. Univ. Chuo*, [2]*Riken, ASI*, [3]*Riken, AICS*, [4]*Riken, QBiC*)

Structural diversity of glycans is essential for a variety of biological functions, such as immune response. However, the accurate and rapid identification of glycan structures is difficult due to the presence of complex isomeric forms. In this study, we performed replica-exchange molecular dynamics (REMD) simulations of a set of isomeric pyridylaminated (PA) glycans. We newly developed force field parameters of PA-glycans for the simulations. We calculated the distribution of collision cross-sections for each isomer, which is directly comparable to the ion mobility mass spectrometry (IM-MS) experiments, by using a set of conformers in trajectories. Our REMD results provide a structural basis of the observed IM-MS data for isomeric PA-glycans.

3P315 Bio-inspired Connectivity Self-Healing in Wireless Mesh Networks

Rui Teng, Ryu Miura (*The National Institute of Information and Communications Technology, Japan*)

Self-healing is a phenomenon widely found in the biology systems. For example, in the wound healing, cells around the wound area are "polarized" with a directional movement, accompanying with a collective behavior of cells.
This paper studies the self-healing mechanism of wireless mesh network in case there are disconnections among mesh base stations. The target is to enable the discovery and recovery of disconnected mesh base station by the adjustment of the communication patterns of mesh base stations. We employ the directional antennas to adjust the communication connections among mesh base stations. By utilizing a collection operation of directional pairs, the network can be self-repaired and keep the high connectivity among network nodes.

3P316 桿体・錐体での視物質の脱リン酸化活性の比較

Highly effective Visual pigment Dephosphorylation in cones

Hiromi Yamaoka, Shuji Tachibanaki, Satoru Kawamura (*Grad. Sch. Frontier Biosci., Osaka Univ.*)

In the vertebrate retina, rods mediate night vision, and cones daylight vision. In both cells, visual pigments are once activated by light to trigger phototransduction cascade, and then phosphorylated and inactivated. Visual pigments are regenerated so that the phosphates incorporated into opsin have to be removed. Cones function in daylight where quick pigment regeneration is required. For this, we expected that the removal of the phosphates (dephosphorylation) could be faster in cones. To examine it, we compared dephosphorylation activities between carp rods and cones. The activity was 4-fold higher in cone membranes than in rod membranes. In both rods and cones, the activity was increased by 60-fold by addition of the cytoplasmic fraction prepared from rods or cones.

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。

1L1
オリンパス株式会社

倒立型顕微鏡を用いたリアルタイム1分子イメージング：蛍光法と暗視野法

日　時
10月28日 [月]　**12:30〜13:20**

場　所
京都国際会館 D会場(Room D)

演　者
飯野 亮太 先生
東京大学大学院 工学系研究科 応用化学専攻

倒立顕微鏡をベースにした対物レンズ型全反射蛍光顕微鏡（エバネッセント顕微鏡）はリアルタイム1分子イメージングに汎用されている。我々はこの手法を改変した対物レンズ型全反射暗視野顕微鏡を開発し、金コロイド微粒子の高速イメージングに適用した。この手法は、蛍光顕微鏡のダイクロイックミラーとバリアフィルターを中央に穴のあいた開口ミラーに交換するだけで構築でき簡便である。さらに我々は対物レンズを用いた後方散乱型暗視野顕微鏡も開発し、デフォーカスイメージングによる金ナノロッドの高速配向イメージングに適用した。こちらの手法も中央部のみがミラーになっているスポットミラーに交換するだけで構築でき簡便である。本ランチョンセミナーでは、これらの手法を用いた分子モーターの1分子計測例を紹介する。

Ryoka Systems Inc.

第 51 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

分子設計　～低分子から生体高分子まで～

日時：　10 月 28 日（月）　12:30～13:20

場所：　E 会場（Room E）

計算化学的手法を利用した抗ウイルス薬の探索研究

大阪大学大学院薬学研究科　川下　理日人

主として熱帯地域で流行しており、年に 5000 万人～１億人の感染がみられるデングウイルス感染症は、しばしば死亡率の高いデング出血熱を発症する。この感染症はネッタイシマカなどの蚊によって媒介されているが、近年の地球温暖化に伴って蚊の生息範囲が徐々に北上する傾向にあるため、将来的にはこの感染症の日本への流入が危惧されている。その一方で、この感染症に対するワクチンや治療薬は現在のところ臨床利用されておらず、デングウイルス感染症の治療にはこれらの開発が重要な位置を占める。本セミナーではそのような背景下、MOE を用いたデングウイルス NS3 蛋白質に対する阻害剤探索を中心に紹介したい。また、時間が許せば他の感染症における阻害剤探索にも触れる予定である。

MOE によるタンパク質デザイン／タンパク質工学

株式会社菱化システム 科学技術システム事業部 東田 欣也

MOE のタンパク質デザイン機能は、目的に応じて網羅的な変異体モデルを自動的に構築すると同時に、タンパク質立体構造に関連する様々な物性を予測します。アラニンスキャンによるタンパク質の熱力学的な安定性のホットスポット検出や、Cys 置換によりジスルフィド結合が形成可能な位置の探索、1 塩基置換により起こり得る残基置換による変異体性スキャンなどを行うことができます。また、これらの変異体に対する熱安定性や等電点、リガンドや抗原との相互作用エネルギー変化の予測を行います。セミナーでは、これらのタンパク質デザイン機能について紹介します。

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PDBj
Protein Data Bank Japan

http://pdbj.org/

PDBj：Protein Data Bank Japan
（日本蛋白質構造データバンク）

Activities of PDBj and wwPDB: A new PDB format, Data Deposition, Validation, and Data Integration

Haruki Nakamura
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”. 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 latest facilities of data deposition and validation will also be introduced.

The new PDBj web Interface: Customizable, Modern and User-friendly

Gert-Jan Bekker
Institute for Protein Research, Osaka University

The new PDBj web interface was launched in May 2013 and now several months have passed. The new web interface offers a powerful search function, tightly integrated services, a customizable, modern and unified design, which also provides better responsiveness. All features and services familiar to users in the previous website (legacy) are still present in the new interface, but they have been redesigned and integrated into a single unified design. During the past couple of months we have made several modifications and improvements to the web interface. We have also developed a new version of the Mine RDB (Relational Data Base), which we will also introduce. During this talk we will introduce the new features and will give a demonstration on how the PDBj web interface can be used. As time passes, more functionality will be added. Upon user's requests, new features can also be added. Please contact us to suggest a feature to be included: http://pdbj.org/contact.

PDBj-BMRB: Publishing NMR Spectroscopy Derived Data of BioMagResBank in Structured Data Formats, XML and RDF

Masashi Yokochi
Institute for Protein Research, Osaka University

The PDBj-BMRB (bmrbdep.protein.osaka-u.ac.jp) at Osaka University operates a BioMagResBank (BMRB) site, which accepts data depositions and carries out data processing and annotation in close collaboration with the BMRB at the University of Wisconsin-Madison (www.bmrb.wisc.edu).

The BMRB is a repository for experimental and derived data gathered from nuclear magnetic resonance (NMR) spectroscopic studies of biological molecules. The NMR-STAR data format has been used in the ADIT-NMR deposition system, entry validation systems and the BMRB relational database for 15 years.

To enhance the interoperability of the BMRB archival data in addition to the NMR-STAR format, the PDBj-BMRB began to provide the data in standard structured data formats, which are an Extensible Markup Language (XML) and a Resource Description Framework (RDF). Collected data files of the formats are named BMRB/XML and BMRB/RDF, respectively.

We will discuss differences between the formats and introduce new publishing services (bmrbpub.protein.osaka-u.ac.jp) for the BMRB/XML and BMRB/RDF with short examples.

PDBj: Protein Data Bank Japan
http://pdbj.org

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TEL: +81-6-6879-4311

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」

日　時：10 月 28 日（月）　12：30 – 13：20
会　場：B 会場（Room B-1）

生命科学と計算科学がみる未来

演者 1：大規模計算って意外に重要かも?
柳田敏雄（理化学研究所 HPCI 計算生命科学推進プログラム / 大阪大学生命機能研究科）

「10 ペタフロップスのスパコンを使って、力づくで計算して生物を研究するなんて、芸がないことやろ。頭を使って生物の原理を探求するところがおもろいところやないか。」と思っていたのだけど、生物は超複雑だし頭と計算機の両方を使わんと研究が進まんな、と最近思ってきた。

演者 2：計算生命科学における大規模計算の重要性
木寺詔紀（理化学研究所 HPCI 計算生命科学推進プログラム / 横浜市立大学生命医科学研究科）

生命科学の対象は、シミュレーションであれ、情報解析であれ、常に自由度間の相関の大きさに起因する大規模複雑系という困難さがあり、また系の著しい多様性による個別論として扱わざるを得ない。個別論とは、系における詳細にいたる特殊性が機能発現に与える影響を見ようというものであり、そこに考慮すべきモデルの自由度が増大するひとつの理由、即ち大規模計算の重要性がある。

演者 3：スーパーコンピュータ「京」と HPCI へのご勧誘
江口至洋（理化学研究所 HPCI 計算生命科学推進プログラム）

文部科学省が進めている HPCI は、スーパーコンピュータ「京」や 9 大学の計算機環境を高速ネットワークで統合したものである。HPCI は大学等研究機関や民間企業の研究機関に対して開かれている。「戦略プログラム　分野1」では多くの生命科学研究者がその HPCI を活用し、計算生命科学の研究開発の進展に寄与してもらうために、スーパーコンピュータ「京」と互換性を有する SCLS 計算機システムを開放している。是非、みなさんがこれら計算機環境を活用されることを願う。

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
http://www.kobe.riken.jp/stpr1-life/

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マルバーン ランチョンセミナー

Malvern

題目：

共振式質量・粒子径計測装置「アルキメデス」による サブミクロン粒子の定量測定

Quantitative analysis of sub-micron particles, using “Archimedes” resonant mass measurement system

日時　2013 年 10 月 28 日（月） 12:30～13:20
第 51 回日本生物物理学会年会 ランチョンセミナー

会場　A 会場（Room B-2）

講演者　スペクトリス株式会社 マルバーン事業部
プロダクトスペシャリスト 池田英幸
Hideyuki Ikeda
Product Specialist, Malvern Instruments, A division of Spectris Co., Ltd.

発表内容：

「アルキメデス」は、マイクロカンチレバー内の流路を通過する粒子により、カンチレバーの固有振動数がわずかに変化することを利用した、新しい原理の粒子径・粒子数測定装置である。SEC では測定出来ないほど大きく、光学顕微鏡では測定出来ないほど小さい、いわゆる「サブビジブル粒子」と呼ばれる、100nm 程度から数μm の粒子については、これまで再現性良く、かつ定量的に測定出来る有効な手法が存在しなかった。DLS は、短時間で粒子径分布を把握するのに有効であるが、個々の粒子を個別にカウントすることは出来ない。また電子顕微鏡は、サンプルの前処理および測定に時間がかかり、統計的に精度のある数の粒子数を測定することが難しい。アルキメデスでは、適切な濃度であれば、複雑な前処理を行なうことなく、短時間で多くの数の粒子を精度および再現性良く測定することが出来る。また、アルキメデスは、その名の通り、液体よりも密度の小さい「浮く」粒子と、密度の大きい「沈む」粒子を区別して測定することが出来る。このため、従来の方法では不可能だった、注射器薬中のタンパク質凝集体とシリコンオイル油滴それぞれの定量測定や、有機物とエアバブルの判別などが可能になる。本セミナーでは、アルキメデスの動作原理と既存のアプリケーション、将来の応用の可能性について説明を行なう。

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第51回 日本生物物理学会年会

浜松ホトニクス株式会社
ランチョンセミナー

◇ **発表番号:2L2**

◇ **日時:2013年10月29日(火) 11:30 ～ 12:20**

◇ **会場:E会場 (Room E)**

演題1

受容体のモノマー・ダイマー動的平衡と機能:
生細胞での1蛍光分子高速追跡

笠井 倫志 先生
(京都大学 再生医科学研究所 楠見研究室 助教)

【 要旨 】

Gタンパク質共役型受容体(G-protein-coupled receptor = GPCR)は、約800種類からなる大きなスーパーファミリーを形成しており、認知・情動・感覚・代謝・内分泌・循環・炎症・免疫などの多様な生理的機能に関与している。現在、全世界で用いられている薬のおよそ半分が、GPCRをターゲットにしていると言われている。

我々は、生細胞中で、時間分解能4ミリ秒での1蛍光分子高速追跡を行い、調べた全てのクラスAのGPCRでは、モノマーとダイマーの間で変換を繰り返していること、生理的条件で、1秒間に数回から数十回くらい、相手を変えながら、次々と2分子対を形成することを見出した。この頻度は、アゴニストなどの結合によって変化した。

一方、GPCRによって活性化される3量体Gタンパク質は、アゴニスト結合前から、GPCRのモノマーにもダイマーにも、0.1秒程度の寿命で結合して解離することを繰り返していた。また、シグナル変換は、主にダイマーが担っているらしいこともわかってきた。実験は、主に浜松ホトニクス社のイメージインテンシファイアを同社のsCMOSカメラにつなげて行い、その高速性、高S/N、広視野、多波長画像同時取得によって、大きく進展した。

演題2

浜松ホトニクスの最新イメージング技術

伊東 克秀
(浜松ホトニクス株式会社 システム事業部 第1設計部)

第51回日本生物物理学会年会ランチョンセミナー

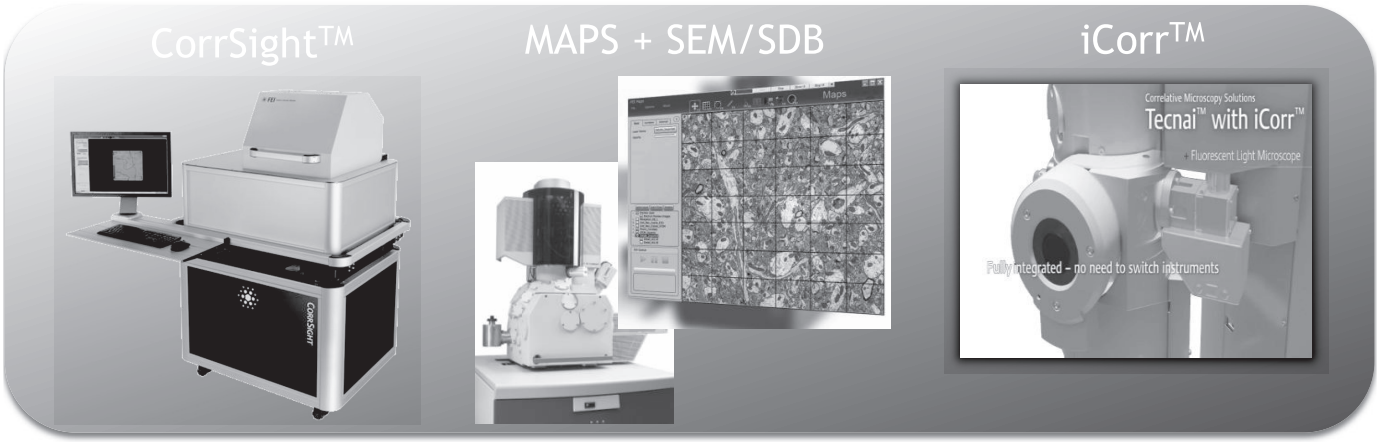
Correlative Microscopy Workflow

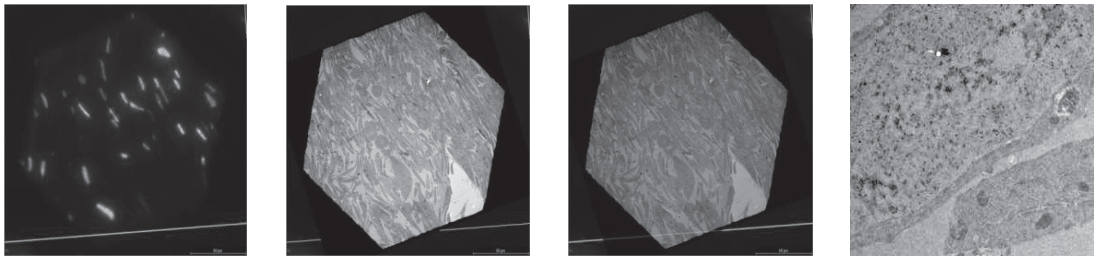
光学顕微鏡と電子顕微鏡をつなぐ新たなアプリケーション

- 開催日時: 2013年10月29日 (火) 11:30 – 12:20
- 会場: C会場（Room C-1）
- 演題: Correlative Microscopy Workflow
- 講演者: 葦原　雅道　(Product Marketing Engineer, FEI Company)
- 要旨:

光学顕微鏡と電子顕微鏡双方の特長を相補的に用いることが利点であるCorrelative Microscopyにより，超微形態像の中で特定の標識タンパク質の局在，さらには分子間の相互作用様式を細胞内で議論することが可能となる。

本セミナーではFEI Company が提供するCorrelativeワークフローを紹介する。





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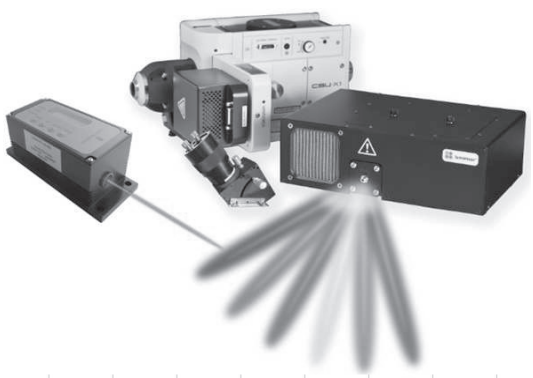
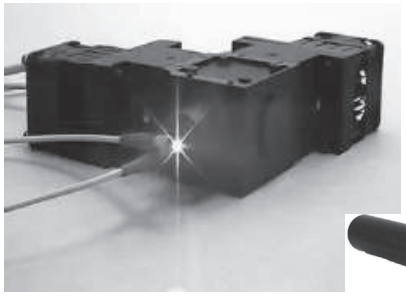
第５１回 日本生物物理学会年会
オプトライン ランチョンセミナー

～ ハイパワーLED光源を生命科学研究に活かす ～

生命科学研究において、ハイパワーLED光源の利用価値はまだまだ成長過程にあります。

レーザー併用マルチカラー共焦点システム『 MESSIA-α 』
多点・パターン刺激 LED照明システム『 Leopard 』





日　時　10月29日（火）11：30 ～ 12：20

会　場　B会場（京都国際会館 Room B-1）

演　者　**永井　健治**　大阪大学産業科学研究所
生体分子機能科学研究分野 教授

ハイパワーLED光源の特徴を活かした、生命科学研究へのアプローチ例 とその可能性

演　者　**岩井　亮一**　株式会社オプトライン

ハイパワーLED光源をベースとした2つのシステム紹介
- レーザー併用マルチカラー共焦点システム『MESSIA-α』
- 多点・パターン刺激LED照明システム『Leopard』

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第51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

構造生物学研究向け装置とアプリケーション紹介

日時：10 月 29 日（火）　11：30〜12：20
会場：A 会場（Room B-2）

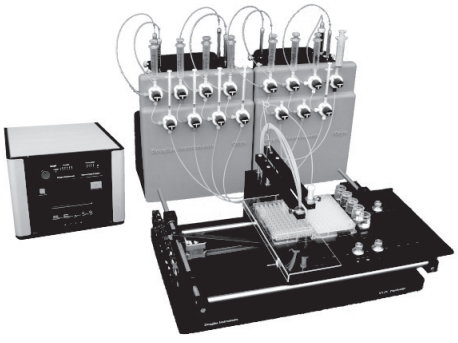
1. Avacta Analytical 社　ハイスループット蛋白質安定性評価装置 Optim®2 のご紹介

Avacta Analytica 社の Optim®2 はタンパク質の安定性評価を微量で迅速に解析することを目的に開発された新しいタンパク質安定性評価装置です。1サンプルわずか 9ul で、48 サンプルのタンパク質の変性温度（T_m）と凝集開始温度（T_{agg}）を同時に測定することができます。

主に抗体医薬品開発における開発候補スクリーニングや製剤処方条件検討に活用されていますが、タンパク質の安定性研究にも幅広く活用可能なシステムです。今回のランチョンセミナーでは、装置原理の概要と、最近のアプリケーション例であるタンパク質リフォールディングの評価例やワクチンの安定性評価例などをご紹介いたします。

2. 効率よく結晶を得るための random Microseed Matrix Screening と Douglas Instruments 社 結晶化ロボット Oryx のご紹介

結晶化ドロップに種結晶（シード）を添加するマイクロシーディングは結晶成長を促進させる手法として最適化実験でしばしば用いられますが、このシーディングの手法をランダムスクリーニングで活用する結晶化のアプローチ方法がランダム・マイクロシード・マトリックス・スクリーニング（rMMS）です。この結晶化スクリーニングを用いて結晶が得られた例と、合わせて Douglas Instruments 社結晶化ロボット Oryx シリーズの概要をご紹介します。



DKSH ジャパン株式会社
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日本生物物理学会第 51 回年会　共催ランチョンセミナー

細胞内シグナル伝達系の多階層イメージング解析

上田　昌宏　先生

大阪大学大学院・理学研究科
理化学研究所・生命システム研究センター

日時	2013年10月30日（水）12:30～13:20
会場	京都国際会館　D会場（Room D）

細胞スケールの小さな空間では，分子反応や分子運動の確率性に起因する「分子数ゆらぎ（分子数ノイズ，molecular noise）」がしばしば無視できない大きさとなり，シグナル伝達や遺伝子発現，細胞運動などの機能に影響することが知られている．細胞の複雑な動態の理解と操作を実現するには，分子レベルの変化が細胞レベルの動態に影響する仕組みを多階層にわたって明らかにし，生物階層システムが持つ確率論的ダイナミクスをも含めて複雑な細胞動態を定量的に予測する方法論の構築が必要となる．この実現のためには，細胞動態を定量的に捉えるライブイメージング解析は今後ますます重要な基盤技術となるだろう．本セミナーでは，走化性シグナル伝達系を例にして，分子の確率性・少数性に着目した１分子レベルからの多階層イメージング解析と，そこで得られる不規則時系列データの統計解析について紹介したい．走化性細胞においては，分子の確率性を起源として細胞の振る舞いに確率的要素を加えることで，変動する環境へ柔軟に適応するシステムを作り上げているようだ．

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ソーラボジャパン株式会社 ランチョンセミナー

セミナータイトル

個体レベル研究における 多光子顕微鏡のこれから

Advances in whole animal 2 photon microscope systems

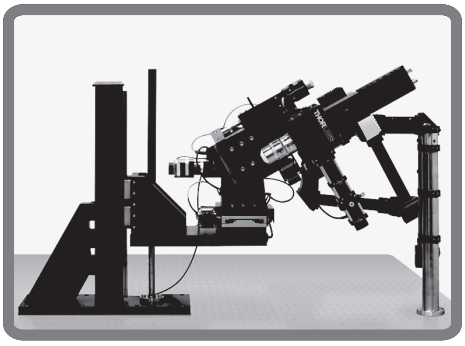
■ 日　時：2013年10月30日（水）12：30～13：20
■ 会　場：E会場（Room E）
■ 座　長：熊崎 茂一 先生　京都大学大学院 理学研究科
■ 講演者：Enrique Chang　Imaging Division, Thorlabs Inc.
ソーラボグループ イメージング部門

■ 要　旨：

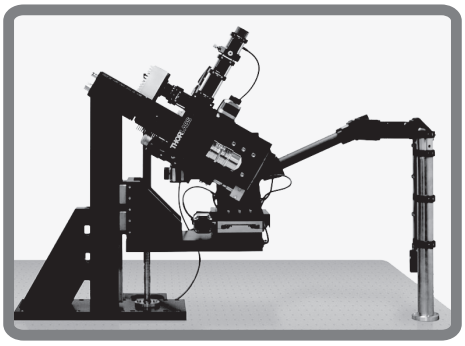
神経科学や生物物理学の分野では、脳機能のさらなる研究と解明のために革新的な実験ツールを必要としています。その実験ツールの1つが2光子顕微鏡ですが、従来の2光子顕微鏡では個体実験においてその機能に限界があります。2光子顕微鏡に、試料の周りで顕微鏡を機械的に動かす機能を追加することにより、実験中の試料への刺激導入を軽減することができます。生体脳のより深部の観察を目指し、ソーラボ社は科学者により科学者のために考案された実験用ツールを日々開発しています。

The neuroscience and biophysics community is in need of innovative tools in order to further study and map the brain. One of the many tools available is the two photon microscope. The traditional two photon system has its limitations in the whole animal study arena. By providing a mechanized moveable two photon microscope in which the microscope moves around the specimen, it mitigate the introduction of tactical stimulation during an experimental run. With the emphasis is peeking deeper into a live brain, Thorlabs is regularly developing tools developed by scientist for scientist.

多光子励起レーザ走査顕微鏡 B-SCOPE



右に最大限回転させたB-Scope



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第 51 回 日本生物物理学会年会

JASRI/SPring-8 ランチョンセミナー

日時　　：10 月 30 日 (水) 12:30 ～ 12:52
会場　　：B 会場 (Room B-1)
講演者　：JASRI/SPring-8 関口博史

大型放射光施設 SPring-8 は国内外の研究者等に広く開かれた施設として多くの放射光利用研究が行われています。
生物物理学研究の分野では、タンパク質の X 線結晶構造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。
今回、生物物理・研究分野の研究者の方々への利用を促進させるためにランチョンセミナーを開催し、SPring-8 における研究の内容、取り組み , 利用方法などについてご紹介します。

萌芽的研究支援 (大学院生対象)

SPring-8 では、放射光研究を担う人材の育成を目的に、大学院生が研究代表者として研究を行う萌芽的研究支援課題を募集し、若手学生を支援しております。
この課題は、学生でも無理なく課題を実施できるように、旅費 (滞在費込) 及び、消耗品実費負担費等の支援を行っています。

http://www.spring8.or.jp/ja/students/　　SPring-8 萌芽的研究　検索

平成 26 年 1 月～ 3 月は熱源設備の更新が予定されている為、運転停止を予定しています。
新規に放射光利用をお考えの方にとっては、研究テーマの相談や施設内見学の良い機会です。お気軽にご相談ください

http://www.spring8.or.jp/ja/users/　　SPring-8 利用　検索

Japan Synchrotron Radiation Research Institute
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JASRI　SPring-8

大学連携バイオバックアッププロジェクト（IBBP）
―誰もが利用できる生物遺伝資源のバックアップ拠点形成―

成瀬　清（基礎生物学研究所 IBBP センター）
naruse@nibb.ac.jp

大学連携バイオバックアッププロジェクト（IBBP）とは

平成 23 年 3 月 11 日に発生した東日本大震災では、東北地方を中心に多くの大学・研究所が被災いたしました。震災による直接的な被害とともに長期間の停電によって、恒温室やフリーザーの維持が不可能になり、実験研究に用いる変異体や遺伝子導入個体など長年の努力によって作成してきた貴重な系統、cDNA/ゲノムクローンのような研究になくてはならない実験材料など多くの生物遺伝資源が失われました。その結果、研究の遅滞や研究方向の転換を余儀なくされた研究者も多くおいでになります。規模の違いはあれ、そのような災害や事故は今後も起きる可能性があると考えられます。

予期しない生物遺伝資源の喪失を防ぎ、我が国の生命科学研究の災害対応能力を高めることを目的として平成 24 年 6 月より開始された国家プロジェクトが IBBP（http://www.nibb.ac.jp/ibbp/）です。このプロジェクトでは生物遺伝資源バックアップの中核施設である大学連携バイオバックアップセンター（IBBP センター）を基礎生物学研究所に設置し、連携する 7 大学（北海道大学、東北大学、東京大学、名古屋大学、京都大学、大阪大学、九州大学）とともに、研究途上にある生物遺伝資源を中心にバックアップ保管を行い、災害や事故により研究者が保持する生物遺伝資源が失われた際には速やかにバックアップ保管している生物遺伝資源をお返しすることで、研究の迅速な再開を支援します。また IBBP センターに設置した分注用ロボット、レプリカ作成装置、コロニーピッカー、キャピラリーシークエンサー、クリーンベンチ、プログラムフリーザー等の機器（http://www.nibb.ac.jp/ibbp/ibbp-center/facilities.html）を用いてバックアップ用のサンプル調整、ライブラリーのコピーや中規模な塩基配列決定、ライブラリースクリーニングシステムの構築などを支援いたします。このような支援によって研究者がもつ生物遺伝資源の付加価値を向上させることもプロジェクトの重要な活動の一つとなっています。

新規長期保存技術開発を目指した共同利用研究の実施

研究に利用される様々な生物遺伝資源は、必ずしも安定した長期保存技術が確立されているとは限りません。長期保存技術が確立されていない生物遺伝資源は、現状では IBBP センターでのバックアップ保管が困難です。そこで平成 25 年度よりそのような生物遺伝資源を対象に、新規長期保存技術開発を目的として共同利用研究を IBBP として実施することとなりました。この共同利用研究では生物遺伝資源のバックアップ保管を推進するため新規保存技術の確立および基礎的な低温生物学的研究 IBBP センターおよび IBBP 大学サテライト拠点の教員（教授。准教授・助教）と共同で行う研究です。一件あたり年間 700 万円程度を上限に研究費（旅費、滞在費及び消耗品費）を補助することができます。詳細は IBBP センターホームページ（http://www.nibb.ac.jp/ibbp/collabo/）をご参照ください。

IBBP は国内の全ての研究者が利用可能な生物遺伝資源のバックアップ拠点形成を目指した日本初のプロジェクトです。利用しやすいプロジェクト運営を心がけることで、より多くの研究者に利用されるように、このプロジェクトを育て、日本の生命科学研究の災害対応能力を高め、その安定した発展をサポートいたします。

本学会の連絡先は下記の通りです.

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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.

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