

## Structure-guided identification of a new catalytic motif of oligosaccharyltransferase

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Asn-glycosylation is widespread not only in eukaryotes, but also in archaea and some eubacteria. Oligosaccharyltransferase (OST) catalyzes the co-translational transfer of an oligosaccharide from a lipid donor to an asparagine residue in nascent polypeptide chains. Here, we report that a thermophilic archaeon, *Pyrococcus furiosus* OST is composed of the STT3 protein alone, and catalyzes the transfer of a heptasaccharide, containing one hexouronate and two pentose residues, onto peptides in an Asn-X-Thr/Ser-motif dependent manner. We also determined the 2.7 Å-resolution crystal structure of the C-terminal soluble domain of *Pyrococcus* STT3. The structure-based multiple sequence alignment revealed a new motif, DxxK, which is adjacent to the well-conserved WWDYG motif in the tertiary structure. The mutagenesis of the DK motif residues in yeast STT3 revealed the essential role of the motif in the catalytic activity. The function of this motif may be related to the binding of the pyrophosphate group of lipid-linked oligosaccharide donors through a transiently bound cation. Our structure provides the first structural insights into the formation of the oligosaccharide-asparagine bond.

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