Phospholipidic cavity insight of Pa LOX.

**Keywords:** lipoxigenases, membrane interaction

### MS85.P03

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**Crystal structures of lipid-raft protein stomatin and its specific protease**

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Stomatin is a major integral membrane protein of human erythrocytes, the absence of which is associated with a form of hemolytic anemia known as hereditary stomatocytosis. It is reported that stomatin regulates the gating of acid-sensing ion channels in mammalian neurons. However, the function of stomatin is not fully understood. In the genomic sequence of the hyperthermophilic archaeon *Pyrococcus hortikoshii*, the putative operon-forming genes PH1511 and PH1510 encode stomatin and STOP (stomatin operon partner protein), respectively. The N-terminal region of PH1510p (1510-N) is a serine protease with a catalytic Ser-Lys dyad (Ser97 and Lys138 partner protein), respectively. The N-terminal region of PH1510p (1510-N) is degrading hydrophobic substrates. The determined structure of 1510-N K138A mutant at 2.3 Å resolution [3]. While the α/β domain is relatively rigid, the α-helical segment shows a conformational flexibility, adapting to the neighboring environment. One α-helical segment forms an anti-parallel coiled coil with another α-helical segment from a symmetry-related molecule. The α-helical segment shows a heptad repeat pattern, and mainly hydrophobic residues form a coiled-coil interface. The determined structure shows a novel trimeric fold, and the coiled-coil fold observed in the crystal probably contributes to the self-association.

1510-N preferentially degrades hydrophobic substrates. According to the gel-filtration analysis, 1510-N forms a dimer. The determined structure of 1510-N K138A shows two possible sets of dimer from the symmetry-related molecules. One stable dimer shows a contact surface area (1,498 Å²) larger than that of another dimer (1,180 Å²). In the stable dimer, the 19-residue-long loop mainly constitutes the interface of the dimer, and is significantly kinked at around Ala138, which is mutated from Lys. Thus catalytic Lys138 probably has an important role on the conformation of the loop.

In order to understand the mechanism how 1510-N protease degrades the C-terminal region of stomatin, a structural analysis of 1510-N in complex with its ligand stomatin would be needed.

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**Gram-positive bacterial conjugation: new structural insight on plasmid pIP501**


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Conjugative plasmid transfer is an important mean for horizontal gene spread (e.g. of antibiotic resistance) [1]. The model host of our Gram-positive multiple antibiotic resistance plasmid pIP501 is *Enterococcus faecalis*, which presents an important nosocomial pathogen. The plasmid conjugation process in Gram-negative bacteria has been studied in detail, whereas little information is available about the corresponding mechanisms in Gram-positive bacteria [2]. pIP501 has the broadest known host range for plasmid transfer in Gram-positive bacteria and is the first system for which also stable replication in Gram-negative bacteria was shown.

The transfer region of pIP501 is organized in an operon encoding fifteen putative transfer proteins. Three of these Tra proteins show significant sequence similarity to *Agrobacterium tumefaciens* T-DNA transfer system proteins: an ATPase (ORF5 homologue to VirB4) [3], a coupling protein (ORF10 homologue to VirD4) and a lytic transglycosylase (ORF7 homologue to VirB1) [4].

One priority of the project is to determine the structure of ORF11 and ORF14, two members of the T4SS for which no homologues exist in *Agrobacterium tumefaciens* T-DNA transfer system. 7xHis-fusion proteins of both candidates have already been successfully expressed, purified, biochemically characterized and used in crystallisation- and optimization screens.

Recently, the structure of ORF14 was solved, using selenomethionine anomalous data for phasing. The 1.4 Å structure revealed an internal dimer fold, consisting of anti-parallel beta sheets in the middle and a “helix-turn-helix” like motif on both ends. Together with previous EMSA results, these findings support the assumption that ORF14 is a DNA binding protein. To acquire detailed insight into this interaction, ORF14 has been set up with dsDNA oligos in co-crystallisation experiments.
**Poster Sessions**

**MS85.P05**

GPCR Network – Understanding Human GPCR Biology
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GPCR Network (http://gpcr.scripps.edu) is a PSI:Biology sponsored center focused on increasing our structure-function knowledge of G-Protein Coupled Receptors (GPCR). Using a protein family specific platform, the center is working closely with the community to determine the high-resolution structure and function of GPCRs distributed broadly across its phylogenetic family tree. Its primary scientific focus is the characterization of the ligand binding landscape of all GPCR families to develop a better understanding of mechanisms used in fulfilling their physiological roles, their implications to disease states, and to facilitate design of new ligands for therapeutic intervention. Plans are to solve the structures of 15-20 GPCRs by sampling sequence space and creating new templates that can be used to produce high resolution models of closely related members.

GPCRs constitute one of the largest protein families in the human genome and play essential roles in normal cell processes, most notably in cell signaling. The GPCR family contains more than 800 members and recognizes thousands of different ligands. and interact with a membrane, encompassing all types of transmembrane proteins, which play a role in many different physiological processes. GPCRs are a diverse family of proteins that are involved in various cellular processes, including signal transduction, hormone and neurotransmitter receptors, and immune system functions. Understanding the structure-function relationships of GPCRs is essential for the development of new drugs and therapies.

Keywords: gram+, conjugation, protein crystallography

**MS85.P06**

Visualization of lipid bilayers in protein crystals by contrast modulation
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A new method of X-ray solvent contrast modulation was developed to visualize lipid bilayers in crystals of membrane proteins at a high enough resolution to resolve individual phospholipids molecules (~3.5 Å). Visualization of lipid bilayer has been escaping from conventional crystallographic methods due to its extreme flexibility, and our knowledge on the behavior of lipid bilayer is still very much limited. Neutron solvent contrast modulation has been employed to visualize micelles in membrane protein crystals but so far provided only low (~12 Å) resolution information. X-ray solvent contrast modulation has also been developed but applied only to determine the envelope of soluble proteins.

Here we applied the new method of X-ray solvent contrast modulation to crystals of Ca²⁺-ATPase in 4 different physiological states. As phospholipids have to be added to make crystals of Ca²⁺-ATPase, it is expected that lipid bilayers are present in the crystals. Moreover, transmembrane helices of Ca²⁺-ATPase rearrange drastically during the reaction cycle and some of them show substantial movements perpendicular to the bilayer plane. Thus these crystals provide a rare opportunity to directly visualize phospholipids interacting with a membrane protein in different conformations.

Complete diffraction data covering from 200 to 3.2 Å resolution were collected at BL41XU, Spring-8, using an X-Axis V imaging plate detector for crystals soaked in solvent of different electron density. A new concept “solvent exchange probability”, which should be 1 in the bulk solvent, 0 inside the protein and an intermediate at interface, was introduced and used as a restraint for real space phase improvement. Anomalous signal from heavy atoms in the solvent was used for cross-validation of the phase information.

The electron density maps thus obtained clearly show that: (i) there are density peaks of about 0.4 e/A³ positioned at ~7 Å from the center surrounding the bundle of transmembrane helices, (ii) these peaks appear at the same locations with respect to the transmembrane helices in different reaction intermediates, if the movements of transmembrane helices perpendicular to the bilayer are less than ~3 Å, and that (iii) no density peaks are present underneath the amphipathic helix (M₁'). Hence we conclude that the electron density peaks represent phospholipids head groups and that phospholipids follow movements of transmembrane helices in all directions.

Keywords: membrane, contrast, protein

**MS85.P07**

How iodide ions inhibit the oxygen evolution of photosystem II?
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