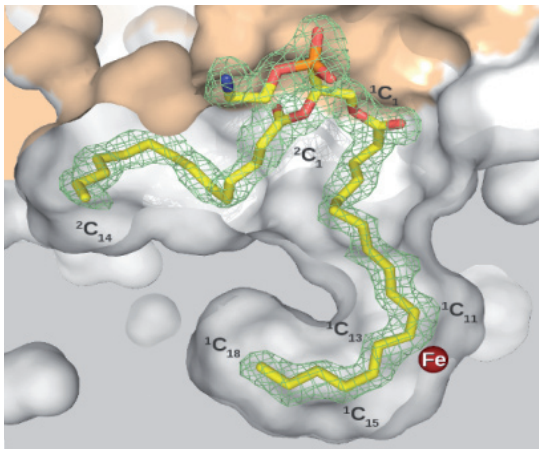

Phospholipidic cavity insight of Pa_LOX.

Keywords: lipoxygenases, membrane interaction

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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 horikoshii*, the putative operon-forming genes PH1511 and PH1510 encode stomatin and STOPP (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), and specifically cleaves the C-terminal hydrophobic region of stomatin PH1511p [1]. It is reported that stomatin forms higher-order oligomers. Stomatin is thought to act as an oligomeric scaffolding protein in lipid rafts. The cleavage of stomatin by STOPP might be involved in a certain regulatory system, but little is known about its physiological role. We determined the crystal structure of the core domain of stomatin PH1511p (residues 56-234, designated as $PhSto^{CD}$) at 3.2 Å resolution [2]. And we determined the crystal structure of the 1510-N K138A mutant at 2.3 Å resolution [3].

$PhSto^{CD}$ forms a novel homotrimeric structure. Three α/β domains form a triangle of about 50 Å on each side, and three α-helical segments about 60 Å in length extend from the apexes of the triangle. The α/β domain of $PhSto^{CD}$ is partly similar in structure to the band-7 domain of mouse flotillin-2. Flotillin is reported to be involved in endocytosis [4]. 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 structure of 1510-N, each active site around Ser97 is rich in hydrophobic residues, and is therefore suitable for 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 $Å^2$) larger than that of another dimer (1,180 $Å^2$). 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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Keywords: membrane_protein, protease, structure

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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 Gramnegative 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 the *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.

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Keywords: gram+, conjugation, protein crystallography

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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 phylogentic 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 much smaller number of binding proteins within cells (e.g. G-Proteins, Arrestins, Kinases). GPCRs have been implicated in numerous human diseases, and represent more than 50-60% of drug targets. Delivering GPCR structures is therefore of immense value. Despite the central role they play and their overrepresentation as therapeutics targets, structural information for GPCRs in general is currently minimal. These receptors are found in very small amounts in native tissues and are notoriously difficult to produce in a biologically relevant form in heterologous systems. Even when expressed, they are hard to purify with sufficient yield, as they are highly unstable when extracted from membranes and solubilized by detergents. Furthermore, GPCRs exist in a dynamic equilibrium between different functional and conformational states, a feature vital for signal trasnduction, but deleterious for crystallization.

GPCR Network has now established an efficient pipeline to solve GPCR structures and has used it in the determination of 5 new human GPCR structures (β_2-adrenergic receptor, Adenosine A_{2A} receptor, Dopamine D3, CXCR4, Histamine H1 receptor), including those of A2a bound to an antagonist and another to an agonist, providing us with glimpses of the active state conformation. Generated samples are being used to: (i) probe for novel allosteric ligand binding sites using biophysical ligand screening methodologies; (ii) understand conformational dynamics and equilibria using HDX and NMR; and (iii) determine at least 3 co-crystal structures per receptor to better define the binding sites and understand GPCR structure-function.

Collaborations are Essential in Meeting Long-Range Goals of GPCR Network: Up to 30% of targets to be studied are those nominated by the community and in addition, there are a host of questions and experiments that can be carried out on these molecules which are outside the scope of the center but for which studies can be conducted to form the basis of a more extensive collaboration. We are therefore actively recruiting new collaborators to help meet our goals. Contact Professor Stevens (stevens@scripps.edu) for details.

Keywords: membrane_protein, gpcr, psi_biology

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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 Ca^{2+}-ATPase in 4 different physiological states. As phospholipids have to be added to make crystals of Ca^{2+}-ATPase, it is expected that lipid bilayers are present in the crystals. Moreover, transmembrane helices of Ca^{2+}-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 R-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^-/A^3 positioned at ~7 A interval 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 bialyer are less than 6 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

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How iodide ions inhibit the oxygen evolution of photosystem II?

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