[1] A. Malik, E.-K. Çelik, C. Bohn, U. Böckelmann, K. Knobel, E. Grohmann, *FEMS Microbiol Lett* **2008,** *279,* 207–216. [2] E. Grohmann, G. Muth, M. Espinosa, *Microbiol. Mol. Biol.* **2003,** *67*, 277-301. [3] M.Y. Abajy, J. Kopeć, K. Schiwon, M. Burzynski, M. Döring, C. Bohn, E. Grohmann, *J. Bacteriol* **2007,** *189*, 2487-2496. [4] E.-K. Çelik, et al. *The pIP501 type IV secretion-like system encodes a specialised lytic transglycosylase: purification and functional characterisation of the enzyme* (manuscript in preparation).

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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Oxygen-evolving photosystem II (PSII) is the site of light-induced water-splitting and provides us with molecular oxygen indispensible for life on the earth. The components of PSII include 17 membrane-spanning subunits, 3 hydrophilic, peripheral subunits, and over 70 cofactors, including chlorophylls, carotenoids, Mn, Ca, Fe, Cl, and plastoquinones, which give rise to a total molecular mass of 350 kDa for a monomer. Chloride ion (Cl^-) is an essential cofactor for oxygen evolution of PSII, and is closely associated with the Mn_4CaO_5-cluster. Two Cl^--binding sites have been identified in the vicinity of the Mn_4CaO_5-cluster by substitution of Cl^- with bromide ion (Br^-) or iodide ion (I^-). Substitution of Cl^- with I^- completely inhibited oxygen evolution of PSII, whereas substitution of Cl^- with Br^- did not inhibit oxygen evolution [1].

Recently, we succeeded in solving the crystal structure of PSII at 1.9 Å resolution, and confirmed the two chloride-binding sites in native PSII [2]. However, the cause for the inhibition of oxygen evolution by I^- has not been clarified. In order to elucidate the inhibition mechanism of oxygen evolution by I^-, we crystallized Br^- and I^- substituted PSII crystals, and analyzed these crystal structures at resolutions of 2.06 and 2.03 Å, respectively. The diffraction data of PSII crystals was collected at beamline BL41XU of SPring-8 in JAPAN and processed with MOSFLM. Each initial phase angles of the reflections were determined by the molecular replacement method with MOLREP in the CCP4 program suit using the previously determined 1.9 Å resolution structure of PSII (PDB code: 3ARC) as a search model. Model improvement and structural refinement were carried out using COOT and REFMAC5, respectively. The results showed that two I^- ions (I-1, I-2) indeed replaced the two Cl^--binding sites in the vicinity of the Mn_4CaO_5-cluster; however, the positions of the two I^--binding sites were slightly different from those of the two Cl^--binding sites, whereas two Br^- ions bound to the same positions of Cl^-. Moreover, fine structural changes were found in the residues surrounding the I-2 site, especially in the C-terminal residue of D1 subunit, Ala344, the carboxylate group of which is directly coordinated to Ca and Mn2 of the Mn_4CaO_5-cluster. These results suggest that the inhibition of oxygen evolution by I^- can be ascribed to the structural changes caused by substitution of Cl^- with I^-.

[1] K. Kawakami, Y. Umena, N. Kamiya, J.R. Shen, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8567-8572. [2] Y. Umena, K. Kawakami, J.R. Shen, N. Kamiya, *Nature* **2011**, in press.

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Production of membrane histidine kinases from mycobacterium tuberculosis

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Bacterial pathogens frequently use Two-Components Systems (TCS) to recognize and respond to the changing environmental conditions within the host, and they normally do this by means of a phosphotransfer reaction between a membrane-localizated histidine kinase sensor protein (HK) and a cytoplasmic response regulator (RR), usually a transcription factor. Mycobacterium tuberculosis contains few TCS compared to many other bacteria [1], even so, these TCS appear to play important roles in early intracellular survival of the pathogen as well as in aspects of virulence [2], what makes them potential targets for the development of new chemotherapeutic agents.

We have selected all the integral membrane HKs present in the Mycobacteria tuberculosis genome for its production to initiate structural studies. Here we describe our medium-throughput strategy including ligation independent cloning in two T7 based expression vectors, auto-induction protein expression in Escherichia coli, and a membrane solubilization screening with the most successful detergents used in structural analysis.

After optimization of protein expression and detergent-mediated solubilization, we achieved large-scale purification of 70% of the membrane HK cloned, yielding enough quantities for biochemical, biophysical and structural analysis.

[1] Cole *et al*., *Nature* **1998,** *393*, 537-544. [2] T. Parish, D.A. Smith, S. Kendall, N. Casali, G.J. Bancroft, N.G. Stoker, *Infect. Immun.* **2003,** *71*, 1134-1140.

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Exploring the Na^+/K^+-ATPase : src kinase complex

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The Na^+/K^+-ATPase is a plasma membrane protein of fundamental physiological significance. During one reaction cycle, 3 Na^+ ions are exported and 2 K^+ are imported under the consumption of one ATP molecule. The ion gradients being formed are important for maintenance of resting potentials and regulation of cell volume, and are exploited by ion channels as well as secondary transporters that facilitate the transport of e.g. ions, nutrients or neurotransmitters across biological membranes.

Research over the last few years suggests that the Na^+/K^+-ATPase is implicated in interactions with several other proteins including the Src kinase, where it serves as a signal transducer [1]. The Src kinase is inhibited while it is bound to the Na^+/K^+-ATPase. Upon binding of cardiotonic steroids (e.g. ouabain) to the extracellular side of the Na^+/K^+-ATPase, the kinase domain of Src is supposedly released and activated. Subsequent phosphorylation of downstream proteins by Src activates for instance MAPK signal cascades and production of mitochondrial reactive oxygen species.

To expand the availability of Na^+/K^+-ATPase from different animal sources, the purification of the Na^+/K^+-ATPase from bovine kidneys was established based on the purification of pig Na^+/K^+-ATPase [2]. The individual domains of the Src kinase are produced recombinantly in *E. coli.* Interactions between the Src kinase domains and the Na^+/K^+-ATPase are analyzed using sucrose cushion assays and microscale thermophoresis [3]. The complexes are furthermore applied in screenings for crystallization conditions.

[1] J. Tian, T. Cai, Z. Yuan, H. Wang, L. Liu, M. Haas, E. Maksimova, X.Y. Huang, Z.J. Xie *Mol Biol Cell* **2006**, *17*, 317-326. [2] I. Klodos, M. Esmann, R.L. Post *Kidney Int* **2002**, *62*, 2097-2100. [3] C.J. Wienken, P. Baaske, U. Rothbauer, D. Brain, S. Duhr *Nat Commun* **2010**, *1*, 100.

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