Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum (SERCA1a) is an integral membrane protein of 110K and the best characterised member of the P-type (or E1/E2-type) ion translocating ATPases. It transports 2 Ca^{2+} and counter-transports 2~3 H^+ per ATP hydrolysed. SERCA1a consists of 10 transmembrane helices, 3 cytoplasmic domains (A, actuator; N, nucleotide binding; P, phosphorylation) and small lumenal loops [1]. We have determined the crystal structures of this enzyme in 8 different states, in which the ATPase shows drastically different domain arrangements [1]. All the crystals are of type I (i.e. stacks of membraneous crystals) and required phospholipids, which form bilayers in the crystals. The crystals diffracted to fairly high resolution (better than 2.5 Å resolution for most of them) at BL41XU, SPring-8. As we use the dialysis method for crystallisation, we can accurately control important parameters, such as protein : lipid : detergent ratio. In this presentation, I will briefly describe our experience in crystallisation of SERCA1a.

[1] Toyoshima, C. *Arch. Biochem. Biophys*. in press DOI: 10.1016/j.abb.2008.04.017 (2008).

Keywords: membrane proteins, crystallisation methods, ATPases

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Crystallization of visual pigments and archaeal rhodopsins

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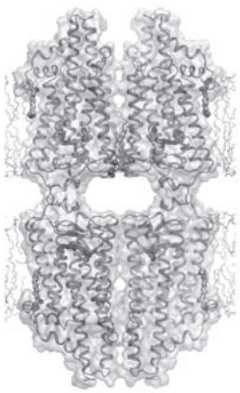
For structural investigation of bacteriorhodpsin, we previously developed the membrane fusion method, by which the trimeric bacteriorhodopsin-lipid complex was crystallized [1]. Recently, we applied this method to prepare 3D crystals of archaerhodopsin-2 without destroying the trimeric structure [2]. These crystals provided information as to the physiological roles of native lipids including bacterioruberine. For structural investigations of visual pigments, we developed a crystallization method by which the protein-lipid interactions can be maintained. Our recent study of squid rhodopsin showed that native lipids mediate the intra-membrane dimerization and that the N terminal polypeptide contributes to the inter-membrane dimerization; i.e., squid rhodopsin is able to form a tetrameric structure [3]. It is suggested that such tetramers are arranged in the apposed microvillar membranes so that the absorption dipole moments of all the retinal chromophores are aligned in parallel with the microvillar axis.



Tetramer of squid rhodopsin

1) Takeda, K., et. al. (1998) J. Mol. Biol. 283, 463. 2) Yoshimura, K. & Kouyama, T. (2008) J. Mol. Biol. 375, 1267. 3) Murakami, M. & Kouyama, T (2008) Nature, 453, 363.

Keywords: squid rhodopsin, archaerhodopsin, bacteriorhodopsin

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X-ray structure of human gap junction channel

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Inercellular signaling is one of the most essential properties exhibited in multicellular organisms. Gap junction is a structure to allow direct intercellular communication. Here, we have determined the crystal structure of human gap junction channel at 3.5Å. The crystal belonging to a monoclinic space group of *C*2 has six molecules in an asymmetric unit. The initial phases were obtained at 8.0Å resolution by the single isomorphous replacement method combined with the molecular replacement method with a hypothetical structure consisting of four helices. Phase extension was performed up to 3.5Å resolution by six-fold non-crystallographic symmetry averaging and multicrystal averaging. Anormalous dispersion signals from selenium atoms of a seleno-methionines derivative crystal and from sulfur atoms of the native crystal uniquely located amino acids and disulfide bonds in the electron density map. Consequently the trans-membrane parts a typical four－helix bundle, which was quite different from that previously proposed. The two extracellular loops in each monomer that interact with the opposing units have three intramolecular disulfide bonds. The first loop makes the wall of the channel pore and the second loop, which extends to overlay the first loop, makes subtype specific interaction. From our structure , the molecular basis of the specific junctional interaction is revealed.

Keywords: membrane protein X-ray crystal structure determina, cell adhesion, cell communication

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Diffraction-capable microfluidic crystallization chips for screening and structure determination

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We have designed and fabricated prototype microfluidic crystallization devices from which high-quality diffraction data can be collected without handling of individual crystals. We have also developed tools for straightforward handling and collection of diffraction data. These include modified synchrotron pins for collection of oscillation data from chip sections under cryogenic conditions and a X-Y stage for rapid diffraction scanning that has been integrated with the beamline control system at BL 8.3.1 at the Advanced Light Source. The prototype chips have been designed to minimize background scatter during the X-ray diffraction experiment. The prototype screening chip tests the sample against 96 crystallization reagents at two mixing ratios. The sample chamber for each of these 192 experiments holds ~ 9 nl sample. In addition to the screening chip, we have also developed a prototype chip for the growth of larger crystals that screens 24 reagents at two mixing ratios. The sample chamber for each of these 48 experiments holds ~ 90 nl sample. Using these diffraction-capable chips, we have been able to solve the structure of a seleno-methionine substituted sample using both SAD and 2-wavelength MAD methods. Data will also be presented showing the use of diffraction-scanning data,

collected using the chip and X-Y plate goniometer, for optimization of crystallization conditions for both membrane proteins and macromolecular complexes. Complete data have also been collected from crystals obtained from initial screening experiments, enabling structure determination with < 3 ul sample.

Keywords: microfluidic, *in-situ* diffraction, free-interface diffusion

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Molecular mechanisms of self-assembly and motion of the bacterial flagellum

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The bacterial flagellum is made of a rotary motor and a long helical propeller by means of which bacteria swim. The motor rotates at around 300 Hz and drives the rapid rotation of each flagellum to propel cell movements. The propeller is a relatively rigid, supercoiled filament that switches between left- and right-handed helical forms in response to reversal of the motor rotation, allowing bacteria to alternate their swimming pattern between run and tumble for taxis. A short, highly curved segment called hook connects the motor and the helical propeller, and its bending flexibility makes it work as a nano-scale universal joint. The axial structure of the flagellum is constructed by self-assembly of proteins translocated from the cytoplasm to the distal end of the growing structure through its narrow central channel, where a cap complex helps efficient self-assembly. The flagellar type III protein export apparatus, which consists of six membrane proteins that form the export gate and three cytoplasmic proteins including an ATPase, FliI, drives the export process. It had long been thought that ATP hydrolysis by FliI provides energy for the export process, but our recent finding revealed that successive unfolding and translocation of export substrate proteins is driven by proton motive force and that ATP hydrolysis is used to disassemble the FliI hexamer that binds and inserts the N-terminal chain of export substrates from the export gate and substrate proteins to facilitate the export process. We combine electron cryomicroscopy and X-ray crystallography to visualize flagellar structures at nearly atomic resolution to understand molecular mechanisms of protein export, self-assembly, and rotation.

Keywords: bacterial falgellum, self-assembly, protein export

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Mechanism of DNA packaging in bacteriophage T4

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The tailed bacteriophage T4 packages about 171 kb DNA inside the virus capsid. Its packaging motor is composed of an empty prohead and an olgomeric terminase enzyme assembled at the special dodecameric portal vertex. The T4 terminase complex consists of the 70 kDa large terminase protein, gp17 and the 18 kDa small terminase protein, gp16. The large terminase protein gp17 is the key catalytic component of the motor. It has two domains, an N-terminal ATPase domain that powers DNA translocation and a C-terminal translocation domain that pushes DNA as well as cuts it after the head is full. Mutagenesis, bioinformatics, and biochemical studies have defined various functional motifs of each domain. Single molecule studies in collaboration with Dr. Doug Smith at UCSD showed that the phage T4 packaging motor is the fastest and the most powerful reported to date. The X-ray structures of the gp17 domains as well as the full-length protein, and the cryo-EM structure of the whole packaging motor were determined in collaboration with Dr. Michael Rossmann's lab at Purdue. These studies lead to the establishment of the DNA translocation mechanism in which the gp17 large terminase protein not only supplies the ATP hydrolysis energy but also acts as a force generating motor translocating DNA into the prohead. An electrostatic inchworm translocation mechanism is proposed, which may be common to the packaging motors of tailed bacteriophages and DNA viruses.

Keywords: virus assembly, DNA packaging, molecular motor

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DNA translocation by hexameric FtsK

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FtsK is a DNA translocase that coordinates chromosome segregation and cell division in bacteria. In addition to its role as activator of XerCD site-specific recombination, FtsK can translocate double-stranded DNA rapidly and directionally, and reverse direction. We present crystal structures of the FtsK motor domain monomer, showing it has a RecA-like core, and the Ftsk hexamer, showing it is a ring with a large central annulus. Electron microscopy demonstrates the DNA-dependent existence of hexamers in solution and shows that duplex DNA passes through the middle of each ring. Comparison of FtsK monomer structures from two different crystal forms highlights a conformational change that we propose is the structural basis for a rotary inchworm mechanism of DNA translocation. We then show how the C-terminal gamma-domains recognise specific DNA sequences to reverse the direction of the motor domain's movement on DNA: three gamma domains bind to the KOPS sequence in a dircetional manner and act by loading the motor complex in one orientation only.

Keywords: DNA translocation, bacterial cell division, FtsK