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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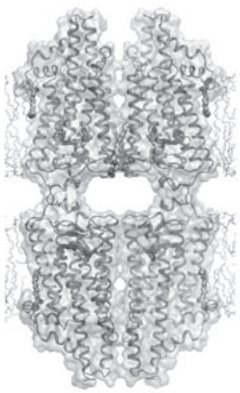
For structural investigation of bacteriorhodpsin, we previously developed the membrane fusion method, by which the trimeric bacteriorhodopsin-lipid complex was crystallized [1]. Recently, we applied this method to prepare 3D crystals of archaerhodopsin-2 without destroying the trimeric structure [2]. 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,