Department of Structural Biology

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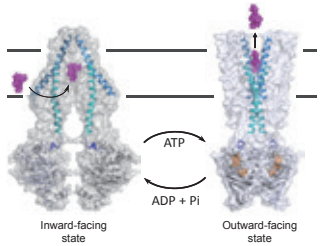
Research Projects:

Three-dimensional structure determination of biological macromolecules is the most powerful way to elucidate the molecular mechanisms underlying biological phenomena. However, owing to the distinct structural changes of the molecules during their action, static views of those structures are not enough to explain their mechanisms. General objectives of this department are to understand the mechanisms of protein molecules based on dynamic motion involved in their functions. We aim to capture the structural changes of molecular machinery at atomic resolution by X-ray crystallography and to develop new crystallographic techniques that acquire time-resolved dynamic structural changes of proteins in action. Our current research subjects are listed below.

1) Structural-basis for pharmacologic behavior of ATP binding Cassette transporters: ATP Binding Cassette (ABC) transporters comprise a family of structurally related membrane proteins sharing well-conserved nucleotide binding domains. They commonly use ATP hydrolysis as an energy source for transport of the substrate across the lipid bilayer membrane. Membrane protein transporters generally function by the alternating access model of transport in which the substrate binding site alternately faces either side of the membrane through conformational changes between an inward- and outward-facing state. One of the most famous and pharmacologically important ABC transporter is P-glycoprotein, also called ABCB1 or MDR1. It is a multi-drug transporter that not only plays essential roles in normal physiology by protecting tissues from various toxic xenobiotics and endogenous metabolites but also contributes to multidrug resistance (MDR) in tumors, a major obstacle to effective chemotherapeutic treatment. Understanding the mechanism of the multidrug transport is crucial for designing drugs of good bioavailability and efficient cancer chemotherapy. Because of low thermal stability and low crystallizability of human P-glycoprotein (hP-gp), we searched for ABC transporters closely resembling hP-gp in the genome of *Cyanidioschyzon merolae*, a thermophilic unicellular eukaryote and found CmABCB1 whose amino acid sequence, multidrug specificity, and kinetics of ATP hydrolysis are the most similar to those of hP-gp. We have determined a high-resolution crystal structure of CmABCB1 at an inward-facing conformation and elucidated its gating mechanism during the substrate transport. In addition, we have discovered a novel inhibitor, which disables the diverging outward motions of the trans-membrane helices by clamping them from the outside of the transporter, and the mode of action of the inhibitor supports our proposed gating mechanism. Recently, we have succeeded in determining both inward- and outward-facing structures of the same molecule of P-gp molecule for the first time. These structures will allow us to explain the pharmacologic behavior of ABC transporters, especially to solve the ambivalent issue in controlling multi-drug transporters. We aim to determine the dynamic structures between the inward- and outward-facing conformations by fluorescence and NMR spectroscopies and kinetic (time-resolved) X-ray crystallographic techniques.

2) Development of new techniques for Structural analysis using X-ray free electron laser: The biggest issues in X-ray crystallography are necessity of large and good quality crystals, and radiation damage that hampers accurate structure determination. Thus, their overcome is the most important research subject for the current crystallographers. To overcome these issues, a new light source has emerged in the form of the X-ray free electron laser (XFEL), with improvements upon many of the properties of synchrotron radiation sources. The SPring-8 Angstrom Compact free electron LAser (SACLA) in Japan generates X-rays a billion times brighter than a third generation synchrotron, SPring-8. The extremely bright XFEL pulses enable data collection using micrometer-sized microcrystals without radiation damage at room temperature through the diffraction-before-destruction principle. We have been involved in the development of serial femtosecond crystallography (SFX) measurement systems using XFEL at the SACLA. We have succeeded in experimental phasing that enables *de novo* structure determination by SFX. We are planning to advance SFX development that can revolutionize the way in which we study matter at the atomic and molecular level. We are expecting to capture atomic resolution snapshots on the ultrafast timescale associated with the intrinsic atomic motions of proteins in action.



Inward-facing (left) and outward-facing (right) structures of CmABCB1.
The outward-facing structure is complexed with ATP analog, AMP-PNP (orange). The trans-membrane helix, TM1s (cyan) and TM3s (blue) are drawn with ribbons. The ATP binding is proposed to connect two cytosolic nucleotide binding domains (NBDs) and its structural change of NBDs drives conformational changes of the transmembrane helices. These conformational changes reduce the volume of an inner-chamber where the substrate binds at the inward-facing state, thus squeezes out the substrate from the inner chamber into the extracellular space at the outward-facing state.

Recent publications

- Kodan *et al.* Inward- and outward-facing X-ray crystal structures of homodimeric P-glycoprotein CmABCB1. *Nat Commun*, **10**(1), 88, 2019.
- Yamashita *et al.* Experimental phase determination with selenomethionine or mercury- derivatization in serial femtosecond crystallography. *IUCrJ*, **4**, 639, 2017.
- Yamashita *et al.* An isomorphous replacement method for efficient de novo phasing for serial femtosecond crystallography. *Sci Rep*, **5**, 14017, 2015.
- Kodan *et al.* Structural basis for gating mechanisms of a eukaryotic P-glycoprotein homolog. *Proc Natl Acad Sci USA*, **111**, 4049, 2014.