MS85.P05

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 phylotypic 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 membrane protein in different conformations.

Complete diffraction data covering from 200 to 3.2 Å resolution were collected at BL41XU, Spring-8, using an X-ray detector for crystals soaked in solvent of different electron density. 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 Ca²⁺-ATPase in 4 different physiological states. As phospholipids have to be added to make crystals of Ca²⁺-ATPase, it is expected that lipid bilayers are present in the crystals. Moreover, transmembrane helices of Ca²⁺-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.

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The electron density maps thus obtained clearly show that: (i) no density peaks are present underneath the amphipathic helix (M1’). Hence we conclude that the electron density peaks represent 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

MS85.P06

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 Ca²⁺-ATPase in 4 different physiological states. As phospholipids have to be added to make crystals of Ca²⁺-ATPase, it is expected that lipid bilayers are present in the crystals. Moreover, transmembrane helices of Ca²⁺-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 X-ray 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/A³ positioned at ~7 Å 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 biallyer 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 (M1’).

Keywords: membrane, contrast, protein

MS85.P07

How iodide ions inhibit the oxygen evolution of photosystem II?

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