FA1-MS03-T01

The Crystallographic Model of a Human A_{2A} Adenosine Receptor. Veli-Pekka Jaakola. *Biocenter Oulu and Department of Biochemistry, University of Oulu, Oulu, Finland.*
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The important role that extracellular adenosine plays in many physiological process is mediated by the adenosine class of G protein-coupled receptors, a class of receptors that also responds to the antagonist caffeine, the most widely used pharmacological agent in the world. The crystallographic model of the human adenosine A_{2A} receptor was recently solved to 2.6Å in complex with the antagonist ZM241385 [1], which is also referred to as "*super-caffeine*" because of its strong antagonistic effect on adenosine receptors. The crystallographic model revealed some unexpected and unusual features of the adenosine A_{2A} receptor structure that have led to new studies on the receptor and the re-examination of pre-existing data. Compared to other known GPCR structures, the adenosine A_{2A} receptor has a unique ligand binding pocket that is nearly perpendicular to the membrane plane. The ligand binding site highlights the integral role of the helical core together with the extracellular loops and the four disulfide bridges in the extracellular domain, in ligand recognition by the adenosine class of GPCRs. Here we describe the strategy employed to obtain the crystal structure of adenosine A_{2A} receptor and main structural features of adenosine A_{2A} receptor.

[1] Jaakola, V.P., Griffith, M.T., Hanson, M.A., Cherezov, V., Chien, E.Y., Lane, J.R., Ijzerman, A.P. and Stevens, R.C. (2008) The 2.6 Angstrom Crystal Structure of a Human A2A Adenosine Receptor Bound to an Antagonist *Science 322: 1211-7.*

Keywords: G protein-coupled receptors, Adenosine A_{2A} receptor, Xray crystallography

FA1-MS03-T02

Lipidic sponge phase crystallization of photosynthetic reaction centres. Gergely Katona[a], Annemarie Wöhri[b], Weixiao Y. Wahlgren[a], Linda C. Johansson[a], Emelie Fritz[b], Erik Malmerberg[a], Magnus Andersson[b], Jonathan Vincent[c], Mattias Eklund[c], Marco Cammarata[d], Michael Wulff[d], Jan Davidsson[c], Gerrit Groenhof[e], Richard Neutze[a]. *[a]Department of Chemistry, Biochemistry and Biophysics, University of Gothenburg, Box 462, SE-40530 Göteborg, Sweden. [b]Department of Chemical and Biological Engineering, Chalmers University of Technology, Box 462, SE-40530 Göteborg, Sweden. [c]Department of Photochemistry and Molecular Science,Uppsala University, Box 523, SE-75120 Uppsala, Sweden. [d]European Synchrotron Radiation Facility, BP 220, Grenoble Cedex 38043, France. [e]Computational Biomolecular Chemistry Group, Department of Theoretical and Computational Biophysics, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany.*
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Bacterial photosynthetic reaction centres from *Blastochloris viridis* convert visible light into chemical energy with remarkable efficiency. This membrane protein contains a special bacteriochlorophyll dimer, two accessory bacteriochlorophylls, two bacteriopheophytins and a staticly bound menaquinone. On the cytoplasmic side of the membrane there is a binding site for a mobile ubiquinone molecule, which is the terminal electron acceptor of the light induced charge separation.
Recently we studied the crystal structure of *Bl. viridis* reaction center grown from lipidic sponge phase. [1] The liquid sponge phase was formed upon mixing monoolein and Jeffamine-M600 and the protein was allowed to diffuse freely into the crystallization matrix. The 1.85 Å resolution structure revealed the precise location of cofactors and revealed lipid molecules bound on the surface of the protein. The mobile ubiquinone binding site appears to be occupied by a monoolein molecule from the crystallization environment. The crystals retained low mosaicity even after repeated cycles of illumination by intense optical laser pulses. This property was essential later for studying a light induced structural change by Laue diffraction techniques. [2] We observed that Tyr-L162 residue moves closer to the special pair upon illumination which attributed to the electrostatic attraction between the oxidized special pair and the deprotonated tyrosine residue. This subtle conformational change highlight the diverse roles tyrosine residues play in various photosystems.

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Keywords: lipidic sponge phase, membrane protein, time-resolved crystallography

FA1-MS03-T03

Passing the expression hurdle for membrane proteins. Eric R. Geertsma, Raimund Dutzler. *Biochemistry department, University of Zurich, Switzerland.*
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Membrane proteins are notoriously difficult to overproduce and crystallize due to their amphipatic nature. Predicting whether a given membrane protein is amenable to overproduction and ultimately, to crystallization is currently not possible.
Given these conditions, our lab employs a broad screening approach that typically involves 50-100 homologs of the target protein. We aim to rapidly identify proteins within this set that are stable and well expressed and consequently can be submitted to crystallization screening. We initially screen a limited set of conditions, but gradually increase the complexity if required. Parameters varied include the expression hosts, promoter strength, types and location of tags/fusion proteins and detergents.
Here, we discuss our experiences with technologies that allow one to rapidly identify well-produced, stable target proteins ready for crystallization screening. Amongst others, this involves high-throughput cloning and screening for overexpression and stability using GFP.

Keywords: membrane proteins, high-throughput, green fluorescent protein

FA1-MS03-T04

A systematic approach to membrane protein crystallization in bilayers. Xiang-Yu Liu[a], Pontus Gourdon[a], Jacob Lauwring Andersen[a], Maike Bublitz[a], Bjørn P. Pedersen, Laure Yatime, Maria Nyblom,Claus Olesen, Jesper V. Møller, Poul Nissen, J. Preben Morth.
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Methods to get high quality membrane protein crystals are in high demand and in a constant development. A preferred method of choice is to maintain low levels of detergent, near the Critical Micelle Concentration (CMC), to shield the hydrophobic regions throughout the purification and crystallization process. This approach typically results in 'Type II' crystals that are built up through interactions between the hydrophilic surfaces of the molecules [1]. In contrast, 'Type I' crystals (often described as stacked 2D crystals) [2, 3] are characterized by continuous bilayers, formed by a lipid/detergent saturated environment, in which the proteins are packed. We present an improved method to induce growth of bilayer membrane protein crystals in high concentrations of lipids and detergent. The straightforward procedure includes a systematic screening approach for lipidation and crystallization, and subsequent improvement of diffraction properties of membrane protein crystals by optimization of added amounts of detergents and lipids.

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Keywords: Membrane proteins, crystallization, bilayers, type I crystals

FA1-MS03-T05

Access Membrane Transport by the Sodium-Hydantoin Transporter Mhp1. Simone Weyand[a,b,d*], Tatsuro Shimamura[a,b,c*] , Oliver Beckstein[e*] , Nicholas G. Rutherford[f] , Jonathan M. Hadden[f] , David Sharples[f], Mark S. P. Sansom[d], So Iwata,[a,b,c,d,g], Peter J. F. Henderson[f], Alexander D. Cameron[a,b,d]. [a]*Division of Molecular Biosciences, Membrane Protein Crystallography Group, Imperial College, London SW7 2AZ, UK.* [b]*Japan Science and Technology Agency, Exploratory Research for Advanced Technology, Human Receptor Crystallography Project, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan.* [c]*Department of Cell Biology, Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-Ku, Kyoto 606-8501, Japan.* [d]*Membrane Protein Laboratory, Diamond Light Source, Harwell Science and Innovation Campus, Chilton, Didcot, Oxfordshire OX11 0DE, UK.* [e]*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK.* [f]*Astbury Centre for Structural Molecular Biology, Institute for Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK.* [g]*Systems and Structural Biology Center, RIKEN, 1-7-22 Suehiro-cho Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan.*
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Membrane transport proteins are classified into different groups. However, the common molecular mechanism of all of them is based on the alternating access model [1]. Mhp1 belongs to the nucleobase–cation–symport-1 family of secondary active transporters with indolyl methyl- and benzyl-hydantoins as substrates in *M. liquefaciens*. Two crystal structures of this protein were already solved and present the outward facing and occluded forms [2]. Recently, a crystal structure in a third conformation, inward facing, was solved and revealed detailed insights into the alternate access model. The strucure was first solved by molecular replacement and later on by SAD and refined at 3.8 Å resolution to R=27.3% and R_{free}=31.3.1% [3].
Mhp1 comprises a five-helix inverted repeat, a common motif among secondary transporters. This new crystal structure is complementing its previously described structures in outward-facing and occluded states. From analyses of the three structures and molecular dynamics simulations, a mechanism for the transport cycle in Mhp1 could be proposed. The switch from the outward- to the inward-facing state, to effect the inward release of sodium and benzylhydantoin, is primarily achieved by a rigid body movement of transmembrane helices 3, 4, 8, and 9 relative to the rest of the protein. This forms the basis of an alternating access mechanism applicable to many transporters of this emerging superfamily.

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Keywords: membrane transport; membrane protein structure; membrane protein X-ray structure determination