

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 processes 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 2.6 Å 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 Å Crystal Structure of a Human A_{2A} Adenosine Receptor Bound to an Antagonist *Science* 322: 1211-7.

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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 statically 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 highlights the diverse roles tyrosine residues play in various photosystems.

[1] Wöhri A.B., Wahlgren W.Y., Malmerberg E., Neutze R. & Katona, G. (2009) *Biochemistry*, 2009, 48(41), 9831. [2] Wöhri A.B., Katona G., Johansson L.C., Fritz E., Malmerberg E., Andersson M., Vincent J., Eklund M., Cammarata M., Wulff M., Davidsson J., Groenhof G. & Neutze R. *Science* 2010, 328, 630.

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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 amphipathic 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.