FA1-MS08-O1

Structural Genomics of Bacterial Membrane Transport Proteins. Peter J.F. Henderson[a], Shun'ichi Suzuki[a,b], Pikyee Ma[a], Massoud Saidijam[a,c], Kim E. Bettaney[a], Gerda Szakonyi[a], Nicholas G. Rutherford[a], Simon G. Patching[a], Ryan J. Hope[a], Peter C. J. Roach[a], Tatsuro Shimamura[d], Shunsuke Yajima[d], Elisabeth P. Carpenter[d,e], Simone Weyand[d,e], Alexander D. Cameron[e], So Iwata[d,e]. [a]*Astbury Centre for Structural Molecular Biology, Institute for Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK.* [b]*Aminosciences Laboratories, Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Kanagawa 210-8681, Japan.* [c]*School of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.* [d]*Division of Molecular Biosciences, Membrane Protein Crystallography Group, Imperial College, London SW7 2AZ, UK.* [e]*Membrane Protein Laboratory, Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Oxfordshire OX11 0DE, UK.*
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Membrane transport proteins of bacteria catalyse the energized uptake of nutrients and some are multidrug (Mdr) efflux proteins that contribute to emerging antibiotic resistance. We have cloned and expressed in *Escherichia coli* over 70 genes encoding secondary active membrane transport proteins from 13 species of Gram-positive and Gram-negative bacteria. In the *E. coli* host the transport activity of the heterologous protein is established using radioisotope-labelled substrates. By incorporation of a $(His)_6$ tag at the C-terminus, purification of many of these proteins has been accomplished by NiNTA affinity and size exclusion chromatography.

The structural integrity of the purified proteins is established by biophysical measurements, including mass spectrometry, circular dichroism and FTIR spectroscopy. The preservation of transport activity and cation selectivity is confirmed by reconstitution into liposomes composed of *E. coli* lipids and measurements of counterflow of labeled substrates in different isotonic salts. The fluorescence of tryptophan residues in the protein is often altered by the binding of ligands, enabling calculation of dissociation constants for both the organic substrate and the co-cation. These assays are useful also for trials of stability and crystallisability of the purified proteins.

Crystallisation trials have yielded three proteins with diffracting crystals. The structure of one that transports indolyl methyl- and benzyl-hydantoins into *Microbacterium liquefaciens* [1] was resolved to 2.85 Å resolution by X-ray crystallography [2]. This protein, called Mhp1, belongs to the Nucleobase–Cation–Symporter-1 family of secondary active transporters, and its 12-helix structural fold revealed an unexpected similarity to LeuT of the Neurotransmitter-Sodium-Symporter family, vSGLT of the Solute-Sodium-Symport family and BetP of the Amino-Acid-Polyamine-Organocation family, hitherto thought to be unrelated. Insights into the molecular mechanism of transport have resulted.

[1] Suzuki S., Henderson P. J., *J. Bacteriol.*, 188, 3329, **2006**. [2] Weyand S, Shimamura T, Yajima S, Suzuki S, Mirza O, Krusong K, Carpenter EP, Rutherford NG, Hadden JM, O'Reilly J, Ma P, Saidijam M, Patching SG, Hope RJ, Norbertczak HT, Roach PC, Iwata S, Henderson PJ, Cameron AD, *Science*, v322, 709-713 **2008**.

Keywords: membrane transport; structural genomics; transport protein structures

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Structural Basis for Ion Conduction and Gating in Ligand Gated Ion Channels. Ricarda J.C. Hilf[a], Raimund Dutzler[a]. [a]*Department of Biochemistry, University of Zurich.*
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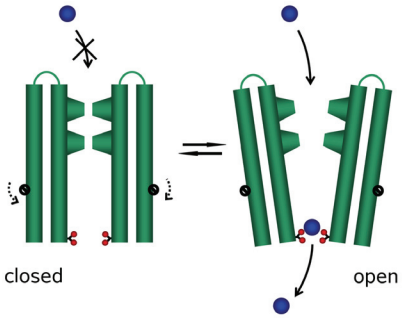
The pentameric ligand gated ion channels (pLGIC) constitute a family of selective ion channels that are key players in the control of electric signaling at chemical synapses. The family codes for a structurally conserved scaffold of channel proteins that open in response to the binding of neurotransmitter molecules. We have determined the X-ray structures of two prokaryotic family members from the bacterium *Erwinia chrysanthemi* (ELIC) at 3.3 Å resolution [1] and from the bacterium *Gloeobacter violaceus* (GLIC) at 3.1Å resolution [2]. Both proteins form cation selective channels and bear most of the structural hallmarks of the family including the N-terminal extracellular ligand binding domain and the four helices of the pore domain. Despite the overall similarity, both structures adopt distinct conformations of the ion conduction pathway:

The structure of ELIC shows a nonconductive state with rings of hydrophobic residues at the extracellular side of the pore preventing ion permeation. This hydrophobic barrier has opened in the structure of GLIC to a funnel shaped pore, where a ring of glutamate residues at the intracellular constriction of the pore creates an ion coordination site. GLIC is thus believed to represent a conducting conformation of the channel.



In combination, both structures suggest a novel gating mechanism for pentameric ligand-gated ion channels where channel opening proceeds by a change in the tilt of the pore-forming helices. Our study thus provides a first structural view at high resolution into how a pLGIC may open and selectively conduct ions.

[1] Hilf, RJC., Dutzler, R., *Nature* 452, 375-379, **2008**. [2] Hilf, RJC., Dutzler, R., *Nature* 457, 115-118, **2009**.,

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Photosystem II at 2.9 Å Resolution - Quinones, Lipids, Channels and Chloride Ion. Wolfram Saenger[a], Albert Guskov[a], Azat Gabdulkhakov[a], Matthias Broser[b], Jan Kern[b], Athina Zouni[b]. [a]*Freie Universitaet Berlin, Institute for Chemistry and Biochemistry/Crystallography, Berlin, Germany.* [b]*Technische Universitaet Berlin, Max Volmer Laboratory for Biophysical Chemistry, Berlin, Germany.*
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Photosystem II (PSII) is a large homodimeric protein-cofactor complex that acts as light-driven water:plastoquinone oxidoreductase and is located in the photosynthetic thylakoid membrane of plants, green algae and cyanobacteria. The principal function of PSII is to oxidize two water molecules at the unique Mn_4Ca cluster to molecular (atmospheric) oxygen, 4 protons and 4 electrons. The protons serve to drive ATP synthetase and the electrons reduce plastoquinone (Q_B) to plastoquinol (Q_BH_2) that is exported and delivers the electrons (through the cytochrome b_6f complex) to photosystem I. Here the electrons gain a high reducing potential and serve at NADP reductase to generate NADPH that together with ATP reduces CO_2 to carbohydrates in the Calvin cycle.
The crystal structure of PSII from *Thermosynechococcus elongatus* at 2.9-Å resolution [1] allowed the unambiguous assignment of all 20 protein subunits and complete modeling of all 35 chlorophyll *a*, 2 pheophytin, 2 cytochrome, 2 plastoquinone, and 12 carotenoid molecules, 25 integral lipids, 1 chloride ion and the Mn_4Ca cluster per PSII monomer. The presence of a third plastoquinone Q_C and a second plastoquinone-transfer channel, which were not observed before, suggest mechanisms for plastoquinol-plastoquinone exchange, and we calculated other possible water or dioxygen and proton channels. Putative oxygen positions obtained from Xenon derivative crystals indicate a role for lipids in oxygen diffusion to the cytoplasmic side of PSII. The chloride position suggests a role in proton-transfer reactions because it is bound through a putative water molecule to the Mn_4Ca cluster at a distance of 6.5 Å and is close to two possible proton transfer channels.

[1] Guskov A., Gabdulkhakov A., Broser M., Kern, J., Zouni A. Nat. *Struct. Mol. Biol*. **2009**, 16, 334.

Keywords: photosynthesis; membrane protein; cofactors

FA1-MS08-O4

Structure and Molecular Mechanism of a Nucleobase -Cation-Symport-1 Family Transporter. Simone Weyand[a,b,c], Tatsuro Shimamura[b,c,d], Shunsuke Yajima[b,c], Shun'ichi Suzuki[e], Osman Mirza[b], Kuakarun Krusong[b], Elisabeth P. Carpenter[a,b], Nicholas G. Rutherford[e], Jonathan M. Hadden[e], John O'Reilly[e], Pikyee Ma[e], Massoud Saidijam[e], Simon G. Patching[e], Ryan J. Hope[e], Halina T. Norbertczak[e], Peter C. J. Roach[e], So Iwata[a,b,c,d,f], Peter J. F. Henderson[e], Alexander D. Cameron[a,b,c]. [a]*Membrane Protein Laboratory, Diamond Light Source, Harwell Science and Innovation Campus, Chilton, Didcot, Oxfordshire OX11 0DE, UK.* [b]*Division of Molecular Biosciences, Membrane Protein Crystallography Group, Imperial College, London SW7 2AZ, UK.* [c]*Human Receptor Crystallography Project, ERATO, Japan Science and Technology Agency, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606- 8501, Japan.* [d]*Department of Cell Biology, Graduate School of Medicine, Kyoto University, Yoshida-Konoe, Sakyo-Ku, Kyoto 606-8501, Japan.* [e]*Astbury Centre for Structural Molecular Biology, Institute for Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK.* [f]*Systems and Structural Biology Center, RIKEN, 1-7-22 Suehiro-cho Tsurumi-ku, Yokohama 230-0045 Japan.*
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Membrane transport proteins are usually classified into three groups: the primary active transporters, the secondary active transporters and those using diffusion without energy. The 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 enabling the uptake of indolyl methyl- and benzyl-hydantoins into M. liquefaciens. This is part of a metabolic salvage pathway for their conversion to amino acids [2].
Mhp1 has been cloned, heterologously expressed in E.coli, purified and crystallized. The strucure was solved by MIRAS and refined at 2.85 Å resolution to R=24% and R free=28.1% [3]. A second structure with the substrate bound was solved by molecular replacement.
The overall architecture of the protein shows a monomer with 12 transmembrane helices. The helices are arranged in two repeating units (1-5 and 6-10), showing an opposite topology with respect to the membrane and are related to each other by a rotation of 168° around an axis in the center of the membrane and parallel to its plane. The substratesand cation-binding sites are all located in between a central four-helix bundle and the surrounding helix coat.
The outward-facing open and outward-facing occluded structures of this protein give detailed insights in the closing mechanism of the substrate binding site. A comparison to proteins with similar fold, LeuT Aa and vSGLT, discloses the symmetrically inverted arrangement of the cavities in the outward and inward facing conformations. The reciprocal opening and closing of these cavities is synchronized by the inverted repeat helices 3 and 8.
These results give for the first time structural insight in the molecular mechanism of the alternate access model [3].