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

FA1-MS08-O2

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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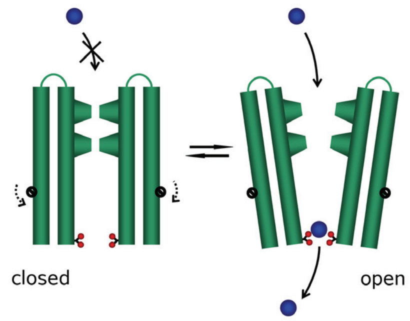
The pentameric ligand gated ion channels (pLGIC) constitute a family of selective ion channels that are key players in the control of electric signaling at chemical synapses. The family codes for a structurally conserved scaffold of channel proteins that open in response to the binding of neurotransmitter 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.