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

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Yeast Aquaporin Gating - A Membrane Protein At 1.15Å Resolution. Gerhard Fischer[a], Urszula Kosinska-Eriksson[a], Madelene Palmgren[b], Kristina Hedfalk[a], Stefan Hohmann[b], Richard Neutze[a], Karin Lindkvist-Petersson[b]. [a]*Dept. of Chemistry/ Biochemistry, Univ. of Gothenburg.* [b]*Dept of Cell- and Molecular Biology, Univ. of Gothenburg.*
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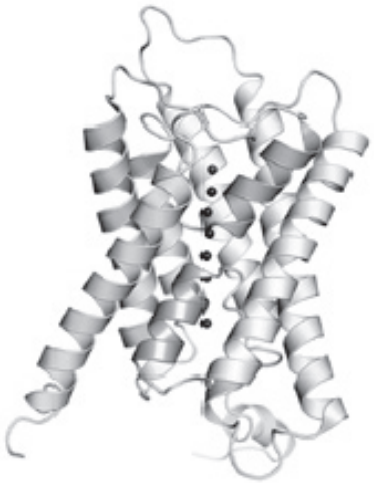

Aquaporins are water – but interestingly not proton–transporting proteins, located in both plasma and internal membranes of cells throughout all kingdoms of life.
The sole aquaporin Aqy1 of the yeast Pichia pastoris has been crystallized and its three-dimensional structure has been solved to 1.15 Å resolution. This unprecedented resolution for membrane proteins provides insights in the properties of the water pore with unequalled precision and will serve as a model system for further characterization of its human homologues.
The structure confirms the well-known tetrameric formation, where each monomer folds according to the "hour-glass"-model, i.e. forms 6 transmembrane helices and two half-helices which are formed by loops B and E. The water file through the channel can be clearly observed, as well as the ar/R constriction region, which serves as a size filter, and the so-called NPA-region, which is thought to prevent proton conductance.
The main difference to most known aquaporins is an extended N-terminus on the cytosolic side which bundles up with its counterparts from the neighboring monomers, thus stabilizing the tetramer. The structure shows that this N-terminus also leads to a closure of the channel by plugging it with the residue Tyrosine 31. Functional studies have been performed, confirming the channel being able to open. In order to do this, a full-length and a truncated version – lacking the N-terminus and thus not being able to close – of the protein were cloned and assayed using a spheroplast and proteoliposome assay. An additional mutational study confirms gating via phosphorylation at Serine 107 – as was suggested by molecular dynamics simulations.
Thus, we present the structure of Aqy1 with new insights on the water exclusion mechanism. We also suggest a novel gating mechanism for aquaporins, where the N-terminus of Aqy1 prevents water flux by capping the pore, which can be regulated by phosphorylation.

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X-Ray Crystallographic Studies of the Pig Renal Na+,K+-ATPase. Thomas LM Sorensen[c], J. Preben Morth[a], Bjoern P. Pedersen[a], Hanne Poulsen[a], Mads S. Toustrup-Jensen[b], Janne Pedersen[b], Jens Peter Andersen[b], Bente Vilsen[b], Poul Nissen[a]. [a]*Department of Molecular Biology, Aarhus, University, Denmark.* [b]*Department of Physiology and Biophysics, Aarhus University, Denmark.* [c]*Diamond Light Source, UK.*
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The Na+,K+-ATPase, the sodium-potassium pump, was first described in 1957 by Jens C. Skou [1] - a discovery for which he was awarded the Nobel prize in Chemistry in 1997. The Na+,K+-ATPase belongs to the P-type ATPase family, and via formation and break-down of phosphoenzyme intermediates it derives the energy from ATP hydrolysis to pump Na+ out of the cell and K+ into the cell. This energises the plasma membrane with steep electrochemical gradients for these key cations and enables e.g. electrical signalling.
The crystal structure was recently published [2]. A complete native dataset was obtained at 3.5 Å resolution on the X06SA beam line at the Swiss Light Source (SLS). The brilliant light source present at SLS was used to obtain useful data from these very weakly diffracting crystals. The crystal form has 75% solvent and contains two-fold NCS. Careful density modification with NCS and inter-crystal averaging was applied and extended the MIRAS phases to 3.5 Å resolution thus allowing for model building and refinement of the structure. Structural comparison between Na+,K+-ATPase and Ca2+-ATPase as well as between the human isoforms alpha1-3 will be discussed.

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Keywords: ATPase; membrane channel transport; membrane protein