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

Keywords: membrane protein; ion channel; protein X-ray crystallography

FA1-MS08-O3

Photosystem II at 2.9 Å Resolution - Quinones, Lipids, Channels and Chloride Ion.
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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.

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FA1-MS08-O4

Structure and Molecular Mechanism of a Nucleobase -Cation-Symport-1 Family Transporter.
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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].

[1] Jardetzky O., Nature, 211, 969, **1966**. [2] Suzuki S., Henderson P. J., J. Bacteriol., 188, 3329, **2006**. [3] 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; membrane protein structures; membrane protein X-ray structure determination

FA1-MS08-O5

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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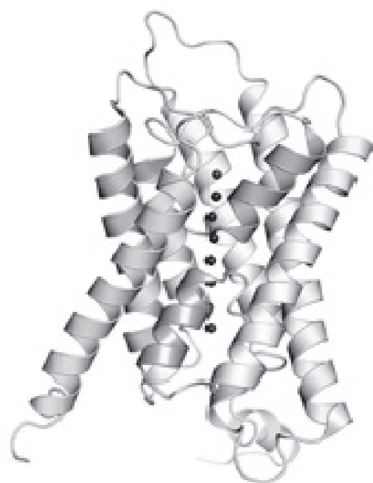

Aquaporins are water – but interestingly not proton– transporting proteins, located in both plasma and internal membranes of cells throughout all kingdoms of life.
The sole aquaporin Aqy1 of the yeast Pichia pastoris has been crystallized and its three-dimensional structure has been solved to 1.15 Å 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.

FA1-MS08-O6

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.

[1] J. C. Skou., Biochim Biophys Acta., 2, **1957**. [2] Morth et al., Crystal structure of the sodium-potassium pump. Nature. 450, **2007**.

Keywords: ATPase; membrane channel transport; membrane protein