MS8-P1 **Conformational changes in the Calcium Pump.**
Maike Bublitz,[a,b] Johannes D. Clausen,[a,b,c] Cédric Montigny,[d,e,f] Bertrand Arnou,[a,b,c] Christine Jaxel,[d,e,f] Marc le Maire,[d,e,f] Jesper V. Møller,[a,c] and Poul Nissen[a,b] [a]*Centre for Membrane Pumps in Cells and Disease - PUMPKIN, Danish National Research Foundation, Denmark,* [b]*Department of Molecular Biology and Genetics,* [c]*Department of Physiology and Biophysics, Aarhus University, Denmark.* [d]*Institut de Biologie et Technologies de Saclay (iBiTec-S), Commissariat ŕ l'Energie Atomique (CEA),*[e]*UMR 8221 CNRS F-91191 Gif-sur-Yvette,* [f]*Université Paris-Sud, F-91405 Orsay*
E-mail: mbu@mb.au.dk

The sarco(endo)plasmic reticulum calcium pump (SERCA) is an essential membrane transport ATPase of the P-type family, moving calcium ions from the cytoplasm into the sarco- or endoplasmic reticulum, in exchange for luminal protons. During calcium transport, SERCA cycles through different conformational states, which can be divided generally into states of high (E1) or low (E2) calcium affinity. SERCA possesses two specific calcium binding sites (termed site I and II) within its membrane spanning domain, and calcium binding to site I triggers cooperative binding at site II. Mutation of the site II calcium-coordinating residue E309 leads to a loss of this cooperativity, resulting in a strongly lowered calcium affinity of site II and a loss of calcium activation of ATP hydrolysis [1]. We have solved the crystal structure of the SERCA1a mutant E309Q at 3.5 Å resolution. The protein was produced recombinantly in *S. cerevisiae* and relipidated with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) before crystallization [2]. This mutant crystallizes in a novel crystal form, under conditions where wild type SERCA adopts a different crystal form, and it represents a hitherto unknown conformational state. Two of the three cytoplasmic domains and two of the ten transmembrane helices are in new positions. The structure was solved by molecular replacement of single domains and - for one of the cytoplasmic domains - an ensemble of 30 morphing intermediates [3] between known conformational states. In the new structure, the nucleotide-binding ('N'-) domain lies in a position in between that seen in the nucleotide-free [4] and nucleotide-bound [5] E1 states, and appears to have low ATP affinity. The actuator ('A'-) domain displays a high flexibility and has moved towards the membrane surface. The structure reveals new insight into the conformational transition from the calcium-free, protonated E2 state to the calcium-bound, deprotonated E1 state of SERCA1a, which is mediated by E309 as the critical "gating" residue.

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MS8-P2 **Nearly there: The structure of human aquaporin 2** Anna Frick,[a] Urszula K Eriksson[a], Jenny vanOostrum[b], Richard Neutze[a], Wim de Grip[b], Susanna Törnroth Horsefield[a] [a]*University of Gothenburg, Sweden,* [b]*Nijmegen Center for Molecular Life Sciences, Netherlands*
E-mail: anna.frick@chem.gu.se

Aquaporins are membrane proteins which mediate the flow of water within living organisms. They occur in thirteen isoforms in the human body, tuned in different ways to preserve water homeostasis. Aquaporin 2 (AQP2) is present in the kidney where it is responsible for reabsorption of water from primary urine. Impaired function of this water channel results in nephrogenic diabetes insipidus, a disease where the patient produces tens of liters of urine every day. AQP2 also plays a part in conditions associated with water overload such as toxaemia of pregnancy and congestive heart failure. The hormone vasopressin regulates AQP2 via a signaling cascade resulting in trafficking of AQP2 from intracellular vesicles to the plasma membrane.

Aquaporins are often considered to be easy targets for membrane protein crystallization. Even so, Aquaporin 2 has resisted hard efforts, owing to low expression levels, inhomogenity, and poor reproducibility of crystals. In order to improve the results we changed the construct as well as the host. A reproducible crystallization protocol has been established, with a C-terminally truncated construct produced in Pichia pastoris. The structure has so far been possible to solve to 3.8Å, but data is twinned. We are now striving to improve the resolution to be able to gain a deeper insight into the molecular mechanism of this medically relevant aquaporin.

Keywords: membrane protein channel;macromolecular crystallography ; twinning