Research Interests:

Membrane protein structural biology

Membrane proteins serve an astonishing range of functions from signal propagation in nerves to generation of energy through photosynthesis or oxygen respiration. Due to their central role in cell physiology, membrane proteins are of highest medical importance constituting more than half of the targets for drugs currently on the market. Despite their high importance, structural information for membrane protein is scarce with, constituting only 1-2% of the entries in the Protein Data Bank. Since structural data is central for a detailed understanding of protein function and is required for structure-based drug design, this acute shortage of membrane proteins is a major drawback in life science as well as medicine.

In our group, we primarily use X-ray crystallography combined with biochemical methods to understand the structure-function relationships of a number of different eukaryotic membrane proteins. Our main research interests are:

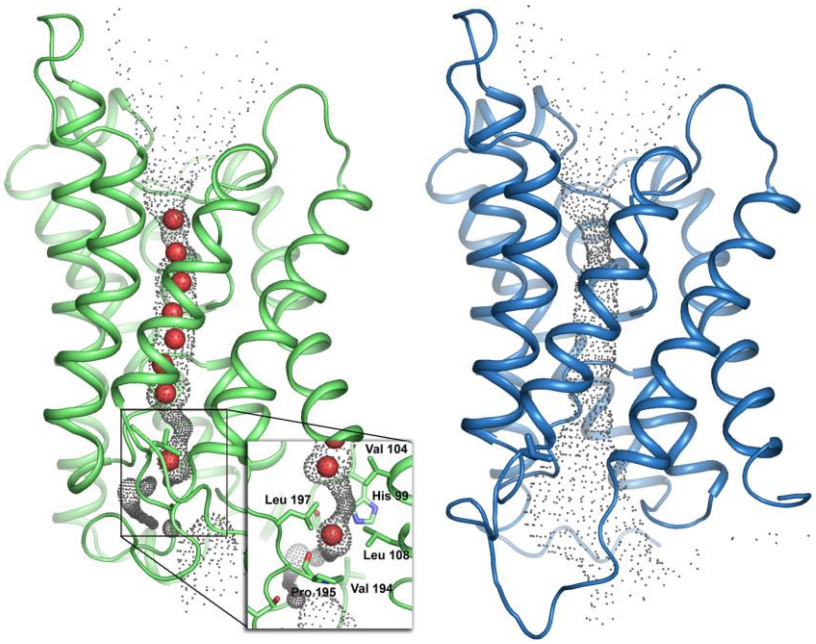
- Aquaporins
- Uncoupling proteins
- Divalent metal transporters

Aquaporins

Water transport across biological membranes requires the action of aquaporins; channel proteins that act as molecular pores through which water and sometimes other small solutes can pass along the osmotic gradients. Their importance is manifested by the fact that they are found in all kingdoms of life with remarkable structure conservation. Humans alone have 13 different isoforms that differ in the presence or absence of regulatory motifs/domains as well as their sub-cellular localisation. A number of diseases are associated with malfunctioning aquaporin systems, including skin cancer, type 2 diabetes, liver disease and brain oedema making aquaporins interesting drug targets[1,2]. In eukaryotes, aquaporins are often regulated post-translationally by gating or trafficking. Our main focus is to understand both these regulatory processes by elucidating the underlying structural mechanisms.

Plant aquaporin gating

Land living plants have evolved to cope with rapid changes in water supply by regulating all aquaporins that lie in the plasma membrane immediately inside the plant cell wall. These aquaporins are known as the plasma membrane intrinsic proteins, PIPs. During conditions of drought stress the PIPs close due to dephosphorylation of one or two highly conserved serines[3]. Interestingly, they are also closed in response to flooding, in which case the closure is attributed to the protonation of a conserved histidine[4]. We have previously solved the structure of spinach PIP2;1 (SoPIP2;1) to 2.1 Å resolution captured in a closed conformation and to 3.9 Å in an open conformation[5]. From these results, a detailed mechanism for the gating of SoPIP2;1 could be proposed. This gating mechanism successfully unifies a significant body of biochemical and genetic evidence which has identified the specific amino acid residues governing plant aquaporin gating. The aim of this project is now to further deepen our understanding of plant aquaporin gating by determining the structure of functionally relevant mutants.

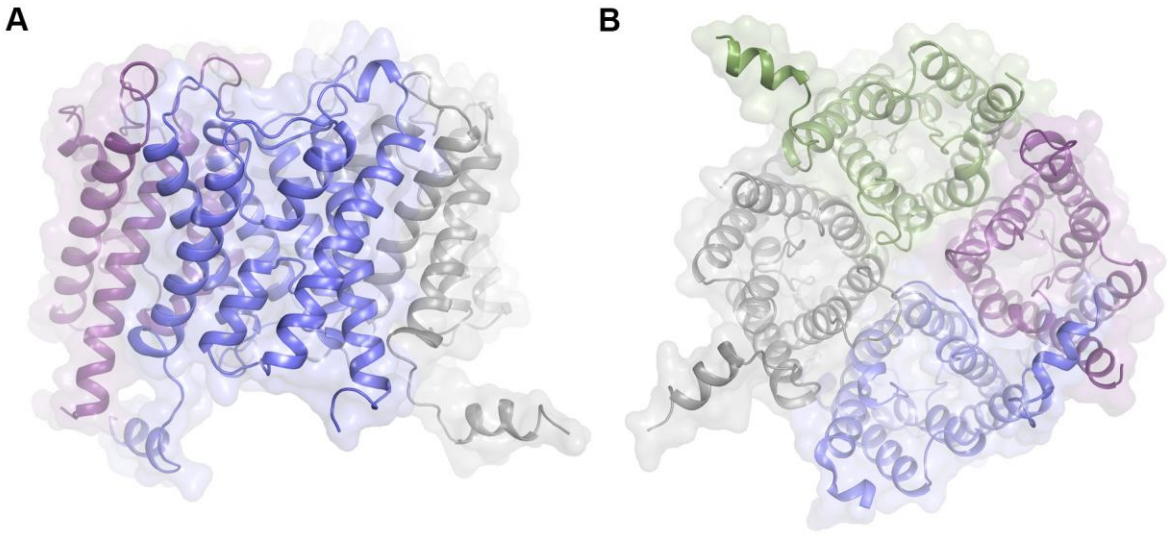


Crystal structure of SoPIP2;1 in closed (green) and open conformations (Törnroth-Horsefield et al, Nature 2006)

Trafficking of human aquaporins

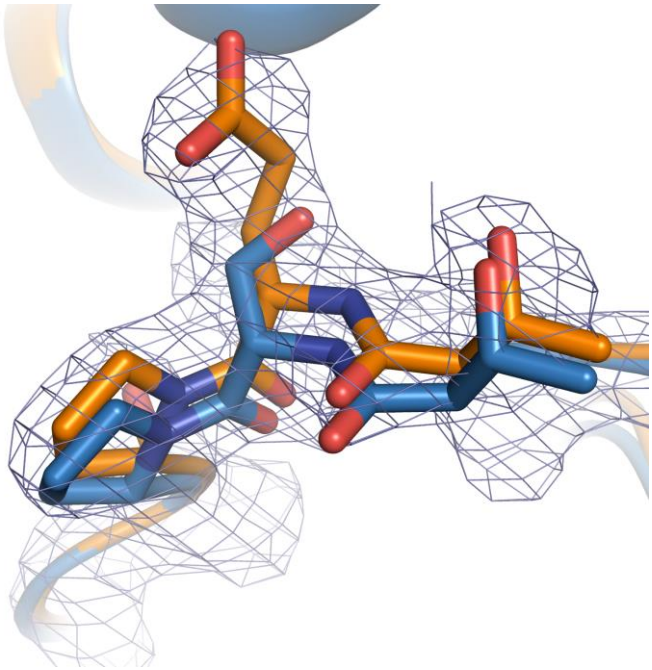
Human aquaporins are frequently regulated by trafficking, a process which involves shuttling between intracellular storage vesicles and the target membrane. The by far best characterized example is Aquaporin 2 (AQP2) present in the kidney collecting duct where its trafficking is crucial for regulating urine volume. Binding of the antidiuretic hormone vasopressin to a receptor on collecting duct cells in the kidneys triggers the movement of AQP2 from their resting state in intracellular storage vesicles to the apical membranes where they act as water channels. This process is known to involve sequential phosphorylation and protein-protein interactions[6,7].

We have solved the crystal structure of human AQP2, giving important new insights into its trafficking and role in *nephrogenic diabetes insipidus*, a disease caused by dysfunction in the vasopressin-AQP2 regulatory system, thereby causing excessive urine volumes[8]. We now aim to further advance our understanding by characterizing AQP2 in different functional states alone and in complex with known interaction partners.



Crystal structure of human AQP2. Tetramer viewed from A. Side of the membrane B. the cytoplasm (Frick et al, PNAS 2014)

We are using a similar approach to studying trafficking of AQP5, the closest human homologue of AQP2. AQP5 is mainly found in tear, saliva and sweat glands as well as in the lungs and airways[9]. Dysfunctional AQP5 trafficking leads to Sjögren's syndrome, an autoimmune disease manifesting itself as dry eyes and mouth[10]. Compared to AQP2, trafficking of AQP5 is poorly characterized. Our aim is to shed light on this process. Building on our previous crystal structure of human AQP5[9] we are now constructing and determining the structures of functionally relevant mutants, following their translocation in live cells as well as studying the interaction between AQP5 and regulatory proteins.



Crystal structure of the phosphomimicking mutant S156E of human AQP5 (orange) overlaid on the structure of wild-type human AQP5 (blue) (Kitchen et al, PLoS One 2015).

Uncoupling proteins

In the mitochondrial inner membrane, electrons derived from the oxidation of glucose and other food sources are passed along a series of integral membrane proteins collectively referred to as the electron transport chain, to the final electron acceptor oxygen. This results in a fixed number of protons being pumped across the membrane thereby establishing an electrochemical proton gradient. The energy conserved in this gradient drives the synthesis of ATP from ADP and inorganic phosphate by ATP synthase. Protons can however re-enter the mitochondrial matrix in a process that bypasses ATP synthase, thereby uncoupling the oxidative metabolism of fuels from ATP synthesis. This process is known as proton leak or uncoupled respiration and is mediated by the mitochondrial uncoupling proteins, UCP[11].

In mammals, five UCP's have been identified in various tissues (UCP1- UCP5) The best characterized of these is UCP1 which is found in brown adipose tissue where its proton leak generates heat through non-shivering thermogenesis, a process which is important for hibernating animals and infants. UCP's have been suggested to play a role in a number of disease states including obesity, cancer, cardiovascular disease and diabetes mellitus[12,13]. By determining the structures of one or several UCP's we aim to understand the underlying disease mechanisms and to open the possibility of using UCP's a drug targets.

Divalent metal-ion transporters

Metal ions participate in a wide range of cellular metabolic processes but are toxic if present in too high levels. To maintain healthy metal ion levels, cells have highly regulated mechanisms for storage, uptake and secretion[14]. A key player in this is the divalent metal-ion transporter 1 (DMT1). DMT1 is a proton-coupled metal transporter capable of transporting several divalent metal ions, including Fe^{2+}, Mn^{2+} and Cd^{2+}. It is widely expressed in our bodies and is crucial for iron uptake from the intestine as well as iron utilization in a wide range of cell types[15]. In the brain, dysfunctional regulation of DMT1 is believed to underlie the iron accumulation in substantia nigra found in patients suffering from Parkinson's disease, leading to oxidative stress that contribute to the neurodegenerative process[16]. Regulation of DMT1-levels in the membrane involves ubiquitination and subsequent degradation in the proteasome. This process has been shown to involve direct interaction with Ndfips, adaptor proteins for the Nedd4 family of ubiquitin ligases[17]. In this project, we aim to crystallize and solve the structure of mammalian DMT1 as well as study its interaction with regulatory proteins such as Ndfips. This will lead to important new insights into the mechanism of metal ion transport and its involvement in human disease.

References

1 Agre, P. & Kozono, D. Aquaporin water channels: molecular mechanisms for human diseases. *FEBS Lett* **555**, 72-78 (2003).

2 King, L. S., Kozono, D. & Agre, P. From structure to disease: the evolving tale of aquaporin biology. *Nat Rev Mol Cell Biol* **5**, 687-698 (2004).

3 Johansson, I., Larsson, C., Ek, B. & Kjellbom, P. The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca2+ and apoplastic water potential. *Plant Cell* **8**, 1181-1191 (1996).

4 Tournaire-Roux, C. *et al.* Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**, 393-397 (2003).

5 Tornroth-Horsefield, S. *et al.* Structural mechanism of plant aquaporin gating. *Nature* **439**, 688-694, doi:10.1038/nature04316 (2006).

6 van Balkom, B. W. *et al.* The role of putative phosphorylation sites in the targeting and shuttling of the aquaporin-2 water channel. *J Biol Chem* **277**, 41473-41479, doi:10.1074/jbc.M207525200 (2002).

7 Zwang, N. A. *et al.* Identification of phosphorylation-dependent binding partners of aquaporin-2 using protein mass spectrometry. *J Proteome Res* **8**, 1540-1554, doi:10.1021/pr800894p (2009).

8 Frick, A. *et al.* X-ray structure of human aquaporin 2 and its implications for nephrogenic diabetes insipidus and trafficking. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 6305-6310, doi:10.1073/pnas.1321406111 (2014).

9 Horsefield, R. *et al.* High-resolution x-ray structure of human aquaporin 5. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 13327-13332, doi:10.1073/pnas.0801466105 (2008).

10 Tsubota, K., Hirai, S., King, L. S., Agre, P. & Ishida, N. Defective cellular trafficking of lacrimal gland aquaporin-5 in Sjogren's syndrome. *Lancet* **357**, 688-689, doi:S0140-6736(00)04140-4 [pii]
10.1016/S0140-6736(00)04140-4 (2001).
11 Krauss, S., Zhang, C. Y. & Lowell, B. B. The mitochondrial uncoupling-protein homologues. *Nat Rev Mol Cell Biol* **6**, 248-261 (2005).
12 Harper, M. E. & Gerrits, M. F. Mitochondrial uncoupling proteins as potential targets for pharmacological agents. *Curr Opin Pharmacol* **4**, 603-607 (2004).
13 Lowell, B. B. & Shulman, G. I. Mitochondrial dysfunction and type 2 diabetes. *Science* **307**, 384-387 (2005).
14 Nevo, Y. & Nelson, N. The NRAMP family of metal-ion transporters. *Biochim Biophys Acta* **1763**, 609-620, doi:10.1016/j.bbamcr.2006.05.007 (2006).
15 Mackenzie, B., Takanaga, H., Hubert, N., Rolfs, A. & Hediger, M. A. Functional properties of multiple isoforms of human divalent metal-ion transporter 1 (DMT1). *Biochem J* **403**, 59-69, doi:10.1042/BJ20061290 (2007).
16 Salazar, J. *et al.* Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 18578-18583, doi:10.1073/pnas.0804373105 (2008).
17 Foot, N. J. *et al.* Regulation of the divalent metal ion transporter DMT1 and iron homeostasis by a ubiquitin-dependent mechanism involving Ndfips and WWP2. *Blood* **112**, 4268-4275, doi:10.1182/blood-2008-04-150953 (2008).