MS8-02 **Membrane transporters and bilayers studied by X-ray crystallography**
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Active transport is driven by an input of energy, such as ATP hydrolysis or electrochemical gradients. Typically, transporters follow either rotary or "alternating access" mechanisms, in both cases controlling the uptake and release of the transported substrates through half-channels, separeted in the functional cycle by occluded states. Unlike channels, transporters therefore typically undergo large conformational changes during the functional cycle while at the same time forming a tight seal to prevent any passive flow across the membrane. Using X-ray crystallography we have revealed some of the structural rationales of these mechanism, both from structure determination of several states relating to the functional cycle (e.g. the calcium pump and sodium-dependent transporters), or by direct visualization at low resolution of bilayer features interacting with transporters.

Similarly we study the regulatory or inhibitory mechanisms of ligands, autoregulatory domains and protein-protein interactions such as the inhibition of Na+,K+-ATPase by cardiotonic steroids and the activation of the plasma-membrane Ca2+-ATPase by calmodulin.

Recently we have obtained new information on the function of the heavy-metal transporters of the P1B-ATPase family. Using a combination of X-ray crystallography, Molecular Dynamics simulations, and mutational studies we have analysed the structure-function relationships of the copper release mechanism of the *Legionalla pneumophila* Cu+-ATPase LpCopA.

MS8-03 **Structure of the human Histamine H1 receptor**
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Allergies are widespread amongst human beings. The allergic reaction occurs to usually harmless environmental substances such as dust, pollen, animal hair or food. Treatments for allergies include the use of . Over the years the pharmaceutical industry developed a wide range of different drugs. A common problem of such drugs are strong side effects, which are mainly due to the low selectivity of the receptor and the penetration across the blood brain barrier. Improved drugs significantly reduce brain permeability, although residual CNS effects are still reported. We determined the high resolution structure of the human Histamine H1 receptor in complex with a 1st generation antihistamine bound to it. The receptor protein was crystallized in the Lipidic Cubic Phase, crystals were optimized and flash frozen in liquid nitrogen and and screened at the Diamond Light Source microfocus beamline I24. Each loop with these very small microcrystals (in average 10 x 10 x 3 microns) was gridscreened with a total of more than 700 loops in order to locate and center the protein crystals. We then collected data with a microbeam (10 x 10) and combined several wedges with typical 12 – 15 degrees oscillation each. The structure shows the human Histamine H1 receptor with the 1st generation antihistamine Doxepin and phosphate ion bound in the active site. This protein belongs to the family of GPCRs and shares the common feature of 7 helices spanning the membrane. The well-conserved pocket with mostly hydrophobic nature contributes to low selectivity of the first-generation drugs. It is associated with an anion-binding region occupied by the phosphate ion and docking of various second-generation H1R-antagonists shows that the unique carboxyl-group present in this class of compounds interacts with two lysines, both of which form part of the anion-binding region. This region is not conserved in other aminergic receptors defining how minor differences in receptor lead to pronounced selectivity differences with small molecules. The detailed analysis of the structure gives in depth insights into the protein-drug relationship of this protein for the first time and enables a much more rational drug design.

[1] Shimamura T, Shiroishi M, Weyand S, Tsujimoto H, Winter G, Katritch V, Abagyan R, Cherezov V, Liu W, Han GW, Kobayashi T, Stevens RC, Iwata S. (2011) *Nature*. 22;475(7354):65-70.

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