Doctoral College
Metabolic & Cardiovascular Disease

WE HAVE SEVERAL ABC EXPORTER CRYSTAL STRUCTURES: DO THEY TELL US THE RIGHT STORY?

GUEST LECTURE by

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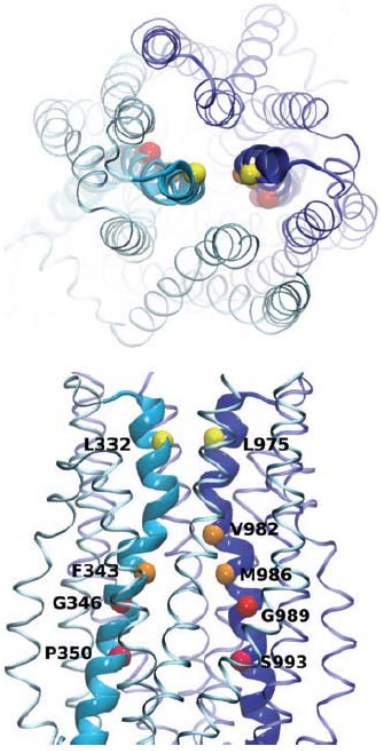
Seminar Room 07.11, Preclinics
(Harrachgasse 21, 1st floor), MUG

Abstract

The human genome contains 48 ABC protein, 44 of them are membrane transporters. We focus on the multidrug resistance transporter P-glycoprotein, which is expressed at the blood-brain-barrier, in the intestine, kidney, liver and macrophages. Sav1866 from Staphylococcus aureus was the first ABC exporter that was crystallized and showed an unexpected twisted architecture, confirmed by site-directed mutagenesis and cysteine cross-linking experiments. The same fold was observed in MsbA, mouse and C. elegans P-glycoprotein and the human mitochondrial ABCB10 transporter, suggesting that this fold is a common architecture of many ABC exporter. Although ABC exporters have now been crystallized in several conformations, uncertainty remained with respect to the physiological conformation, because these structures do not seem to be fully compatible with all biochemical evidence. The observed conformation of the ATP-bound state might be a consequence of the crystallization procedure or conditions.

It is well established that binding of ATP and subsequent hydrolysis drives the transport process. In contrary, the details of the translocation of substrate through the transporter remained elusive to a large extend. It is still unclear, how substrate enters the transporter, where it is recognized and bound to the transporter, how it signals to the nucleotide binding domain and how it is translocated to the extracellular space.

We combine modeling with experiments to address these questions. Homology modeling and MD simulations are used to determine the equilibrium conformation of the membrane inserted transporter. The aim was to test the hypothesis, if membrane inserted P-glycoprotein would be devoid of the wing-shaped conformation and found that the closed conformation is indeed energetically favorable. Side directed mutagenesis studies show the existence of two pseudo-symmetric translocation paths. Substrates seem to make use mainly of only one, with the preference depending on the nature of the ligand. Mutations in the putative substrate binding site supports this notion. Simulations indicate the importance of both hydrophobic interactions and hydrogen bonds for recognition.



Model of the cross-linked conformation of P-gp. from: Data-driven homology modelling of P-glycoprotein in the ATP-bound state indicates flexibility of the transmembrane domains.
Stockner et al. (2009) EMBO J 276:964–972