P 17) Considerations Concerning Homology Modeling of ABC Transporters and Neurotransmitter transporters

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Molecular models of transporters with unknown structure may be constructed by homology modeling using a transporter with a known 3D crystal structure with a sequence similarity, so called homology, to the drug target as a template. When constructing homology models of transporters, there are several pitfalls in the homology modeling procedure. Special challenges are that there are few templates available, if any, and that the resolution of these templates is generally low.
Furthermore, the homology between the target transporter and the template may also be low. An identity between the target protein and the template below 30% may be considered "borderline" of what can be considered as a basis for constructing realistic models, and structure-based drug design based on low homology models may not be as applicable as for models with identities above ~50%. But even though homology may be low, the 3D structure of homologous proteins is more conserved than sequence, so low homology models may be useful for assignment of protein fold and function.
Such models also provide tools for suggesting candidate residues for mutagenesis experiments, and active sites can be identified when combining molecular modeling and site directed mutagenesis studies. Here we present molecular models of ABC transporters and neurotransmitter transporters based on various templates. We have constructed outward-facing molecular models of ABCB1 (P-glycoprotein), ABCC4 and ABCC5 based on the *Staphylococcus aureus* ABC transporter Sav1866, which has been crystallized in an outward-facing ATP-bound state, and inward facing models of ABCB1, ABCC4 and ABCC5 based on a wide open inward-facing conformation of *Escherichia coli* MsbA. After the models were constructed, we got a unique opportunity to test our methodology when the X-ray crystal structure of the *Mus musculus* ABCB1 in a drug-bound conformation was published. Amino acids that formed a putative substrate recognition site in the ABCB1 models were confirmed by the ABCB1 X-ray crystal structure.
We have also constructed models of the dopamine transporter (DAT), the serotonin transporter (SERT), and the noradrenalin transporter (NET) based on *Aquifex aeolicus* LeuTAa crystal structures in substrate-bound and inhibitor-bound conformations. The models were also compared with site directed mutagenesis data. The transporter models are examples of how structural information and insights can be obtained even for transporter models which are based on low homology and low resolution templates. Transporters may undergo substantial conformational changes during the transport cycle, and when interpreting homology models of transporters and performing docking studies on such models, the structural flexibility of transporters should be considered. These models should be considered as working tools for generating hypotheses and designing further experimental studies related to ABC transporter and neurotransmitter structure and function, and their limitations due to uncertainties should be kept in mind.