Structural determinants of binding of the human bile acid transporter SLC10A2 (ASBT)

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Introduction

The human apical sodium-dependent bile acid transporter (hASBT, SLC10A2) is a membrane protein that is responsible for the uptake of bile acids across the enterocytes apical membrane. hASBT is a key drug target for the treatment of hypercholesterolemia. Additionally, hASBT is an interesting target for prodrugs.
Here we describe the interactions of this transporter with its ligands using computational methods.
Our results improve our understanding on how substrate specificity is determined in hASBT, providing guiding rules for the development of new compounds targeting this pharmacologically important transporter.

Goals

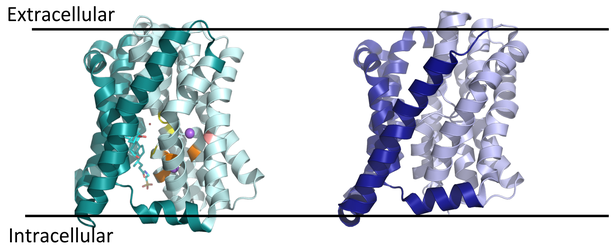
- To determine the specificity determinants of binding

=> Understanding the transporter's interaction with substrates and inhibitors at a molecular level

- Discovering new compounds

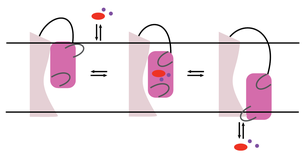
=> Used as chemical tools to understand function, or new scaffolds for the design of new drugs

Prokaryotic transporters $ASBT_{NM}$ and $ASBT_{yf}$



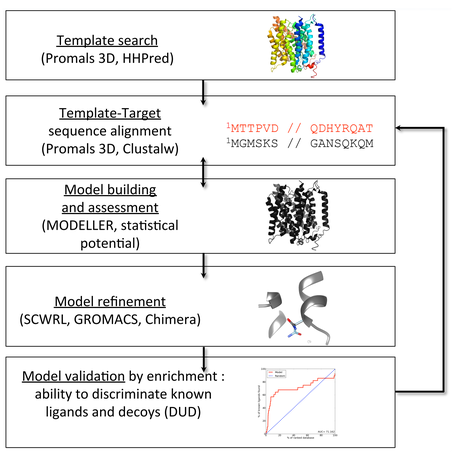
- $ASBT_{NM}$ (PDB ID 3ZUY)[1] : inward open conformation
- $ASBT_{yf}$ (PDB ID 4N7X)[2]: outward open conformation

- X-Ray structures of homologues in 2 conformations ($ASBT_{yf}$ and $ASBT_{NM}$)
- 22-26% sequence identity with human hASBT
- Elevator mechanism of transport[3]

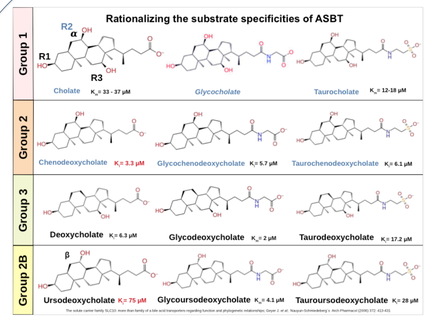


Homology modeling

- Generation of a 3D model of a protein with an unknown structure ('target') based on an experimentally determined structure of a homolog protein ('template').
- The protocol generally includes several steps (c.f. flow chart) ranging from template selection to model validation.
- The process is iterative until a suitable model is obtained.



Substrate selectivity

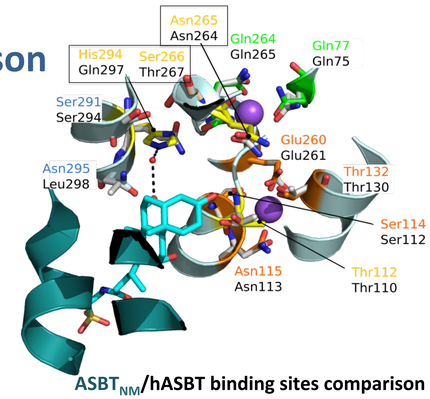


- Large discrepancy of affinities despite a similar scaffold
- Grouping of bile acids depending on their hydroxylation profile and substitutions

Binding sites comparison

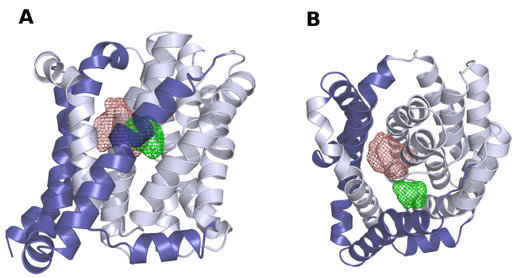
- Mutagenesis studies on the template characterized essential residues for binding
- Hydrogen bond network with a water molecule
- Residues constituting the binding sites are conserved

=> **Hypothesis :** the substrate selectivity occurs in the outward open conformation



$ASBT_{NM}$/hASBT binding sites comparison

Outward open models reveal an horizontal orientation



Binding pockets in the outward open conformation

- Homology models of the outward open conformation were generated.
- Pocket search reveal an "horizontal" orientation, as proposed for $ASBT_{yf}$.[2]
- Opportunities to design conformation-specific modulators and understand the mechanism of transport.

Future directions

Rationalize the substrate specificities of ASBT in the outward open conformation:

⇒to reveal important residues involved in binding and transport

⇒to identify conformation-specific compounds by virtual screening

Conclusions

- Our study reveals that the binding sites in the inward open conformation are conserved
- We suggest that selectivity occurs in the outward open conformation

1. Hu *et al.* Crystal structure of a bacterial homologue of the bile acid sodium symporter Asbt. Nature 478: 408 (2011)
2. Zhou *et al.* Structural basis of the alternating-access mechanism in a bile acid transporter. Nature 505: 509-573 (2013)
3. Colas *et al.* SLC Transporters: Structure, Function, and Drug Discovery MedChemComm 7(6):1069-1081 (2016)
4. Geyer *et al.* The solute carrier family SLC10: more than family of a bile acid transporters regarding function and phylogenetic relationships Arch Pharmacol 372: 413-431 (2006)