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A homology model of SERT based on the $LeuT_{Aa}$ template☆

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Abstract—A human serotonin transporter (SERT) model has been constructed based on the crystal structure of the bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters from *Aquifex aeolicus* ($LeuT_{Aa}$). Amino acids in the ligand binding area predicted by ICM pocket finder included Tyr95, Ala96, Asp98, Gly100 (transmembrane helix (TMH) 1), Ala169, Ile172, Ala173, Tyr176 (TMH3), Phe335, Ser336, Gly338, Phe341, Val343 (TMH6), Thr439, Ala441, and Gly442 (TMH8). The present model is an updated working tool for experimental studies on SERT.

Structural information about the serotonin transporter (SERT) and its interactions with antidepressant drugs is important for the understanding of the mechanisms of action and for drug development. Since a direct structure determination of membrane proteins is technically difficult, molecular modelling by homology might be a step forward toward structural understanding of membrane proteins. However, homology modeling relies on experimental structures to build new 3D models. The lack of known X-ray structure of human transporter proteins makes building neurotransmitter transporters' model challenging, and a large degree of expert intervention is needed for accurate results. For the neurotransmitter transporter protein structures, like SERT, the homology modeling approach has not been possible because known protein structures close enough in sequence and function to the human neurotransmitter transporters have not been available. Instead, we have constructed models of SERT and other neurotransmitter transporter proteins from more dissimilar protein structures combined with indirect and low-resolution structural knowledge from experimental studies.[1–5]

Our first SERT[1] model was based on indirect structural knowledge of the neurotransmitter transporter proteins from ligand binding studies of cocaine to SERT and dopamine transporter (DAT) mutants.[6–16] The electron density projection map of the *Escherichia coli* sodium proton antiporter (NhaA)[17] contributed with additional information about the 3-dimensional structure of secondary transporters, and a model of NhaA was constructed[18] based on the projection map and available site-directed mutagenesis data of NhaA[19–25] and the *E. coli* lactose permease symporter (Lac Permease).[26] The NhaA model guided the construction of models of SERT, DAT, and the noradrenaline transporter (NET).[2,3] In 2003 the X-ray structure of Lac Permease was published.[27] Similar to SERT, DAT, and NET, Lac Permease is a symporter, while NhaA is an antiporter. The amino acid sequences of the neurotransmitter transporters are also closer to Lac Permease than to NhaA. In the TMHs the similarities are 11–13% with Lac Permease and 8–11% with NhaA. Thus, the models of SERT, DAT, and NET were updated[5] using the X-ray structure of Lac Permease[27] as a template.

In 2005 the crystal structure of a bacterial homologue of Na^+/Cl^- dependent neurotransmitter transporters from *Aquifex aeolicus* ($LeuT_{Aa}$), with leucine bound within the protein core, was published.[28] This is so far the X-ray crystal structure with the highest similarity to SERT, both in sequence (overall ~ 20%) and function. In the present communication, we present a SERT model based on the $LeuT_{Aa}$ crystal structure. The model is compared to previous SERT models and site-directed mutagenesis data. The relevance of using the $LeuT_{Aa}$ crystal structure as a template for homology modeling of neurotransmitter transporters is discussed.

Keywords: The serotonin transporter (SERT); Homology modeling; Template.
☆ Coordinates of the SERT model are available from the authors upon request.
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It is believed that quite accurate predictions of protein structures can be done with an amino acid sequence identity greater than 50% between the target and the template protein. However, for membrane proteins even at an overall sequence similarity less than 15%, there may be considerable structural similarities in functionally similar regions between proteins. An example is the family of G-protein coupled receptors (GPCRs), where very distantly related GPCRs share a common membrane folding of 7 TMHs.[29] The sequence identity between SERT and the template crystal structure $LeuT_{Aa}$ (~20%) is higher than between several distantly related GPCRs sharing the 7TMH membrane fold. $LeuT_{Aa}$ is a bacterial homologue of the sodium- and chloride-dependent neurotransmitter transporters, and its transport mechanism resembles the transport mechanism of SERT since they both use the electrochemical sodium gradient to provide an inward movement of substrate against a concentration gradient. The relationships between $LeuT_{Aa}$ and SERT both in function and sequence suggest that they share a common overall folding of their membrane spanning regions, and that X-ray structure of $LeuT_{Aa}$ provides an opportunity of using the traditional homology modeling approach for constructing a model of the human SERT.

The ICM software version 3.4–4[30] was used to construct the SERT model. A Psi-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) amino acid sequence alignment of $LeuT_{Aa}$, the human glycine transporter (GlyT1b), the human GABA transporter (GAT1), the human dopamine transporter (DAT), and the human SERT[28] was used as input for the construction of a SERT model from the amino acid sequence (Swiss-Prot accession code P31645) using the $LeuT_{Aa}$ (PDB id 2a65) as a template. Homology modeling by ICM is performed by constructing the model from a few core sections defined by the average of C_α atom positions in the conserved regions, and loops are searched for by matching them in regard to sequence similarity and sterical interactions with the surroundings of the model, within several thousand high quality structures in the PDB databank.[31] Maps around are calculated and the loops are scored based on their relative energies, selecting the best fitting one. The RefineModel macro of ICM, which globally optimizes side-chain positions and anneals the backbone, was used for energy refinement of the SERT model. This macro performs (1) a side-chain conformational sampling using 'Montecarlo fast',[32] (2) iterative annealing with tethers provided, and (3) a second side-chain sampling. In step 1, the program module 'Montecarlo fast' samples conformational space of a molecule with the ICM global optimization procedure. The iterations of this procedure consist of a random move followed by a local energy minimization, and the complete energy is then calculated. The iteration is accepted or rejected based on the energy and the temperature. The tethers included in the annealing of the backbone are harmonic restraints pulling an atom in the model to a static point in space represented by a corresponding atom in the template. The ECEPP3 charges[33] were used for the amino acids, and a surface based implicit solvation model[30] was included in the calculations. Membrane molecules were not included. The ICM pocket finder was used to explore possible binding pockets in the model, using a tolerance level of 3.0.

The stereochemical quality of the SERT model was checked using the Savs Metaserver for analyzing and validating protein structures (http://nihserver.mbi.ucla.edu/SAVS/). Programs run were Procheck, What_check, Verify_3D, Errat, and Prove. The overall quality factor of the SERT model was 82.6. According to the Ramachandran plot, 95.4% of the residues of the SERT model were in the most favored regions, 4.3% were in additional allowed regions, 0.3% were in generously allowed regions, and 0.0% were in disallowed regions.

The SERT model is shown in Figure 1. The protein featured 12 TMHs, with TMHs 1–5 and 6–10 arranged as in the template,[28] with a pseudo-twofold axis in the membrane plane. TMH6 is kinked near the ligand binding site. The template $LeuT_{Aa}$ has the substrate leucine bound at the active site indicating that the SERT model also corresponds to the substrate-bound SERT conformation, being closed at both sides of the membrane. Based on knowledge from other membrane spanning proteins it is reasonable to believe that SERT needs to be structurally flexible, and that the process of substrate recognition, transporter–substrate complexation, and substrate translocation induces large conformational changes. The conformation of SERT recognized by the substrate serotonin is therefore not necessarily completely similar to that of the present SERT model. Accurate predictions of SERT-ligand binding affinities require quite accurate structural models of the target, and have so far been impossible. The present model provides a possibility for more accurate docking than the previous models. However, the structural flexibility of the transporter is a challenge for accurate docking and predictions, and the docking should be performed using several low-energy conformations of the model.

The availability of site-directed mutagenesis data is very important for the validation of structural models with low homology between the template and the target. However, to interpret if an amino acid is directly involved in ligand binding, or structurally important by maintaining a correct structure of the binding pocket is not straightforward. The ICM pocket finder reported a possible binding pocket of the SERT model in an area corresponding to the substrate binding pocket of leucine in the $LeuT_{Aa}$ crystal structure.[28] Amino acids in the predicted ligand binding area included Tyr95, Ala96, Asp98, Gly100 (TMH1), Ala169, Ile172, Ala173, Tyr176 (TMH3), Phe335, Ser336, Gly338, Phe341, Val343 (TMH6), Thr439, Ala441, and Gly442 (TMH8). Site-directed mutagenesis data on SERT, DAT, and NET confirm the involvement of amino acids in TMH1,[6,34,35,7,36] TMH3,[34,9,37–39,36] TMH6,[40] and TMH8[40,41] in the ligand binding area. Both our first SERT model based on indirect structural knowledge[1] and the second model based on the NhaA model[18] included TMH1 and TMH3 in the binding pocket, but failed to predict a direct involvement of amino acids in

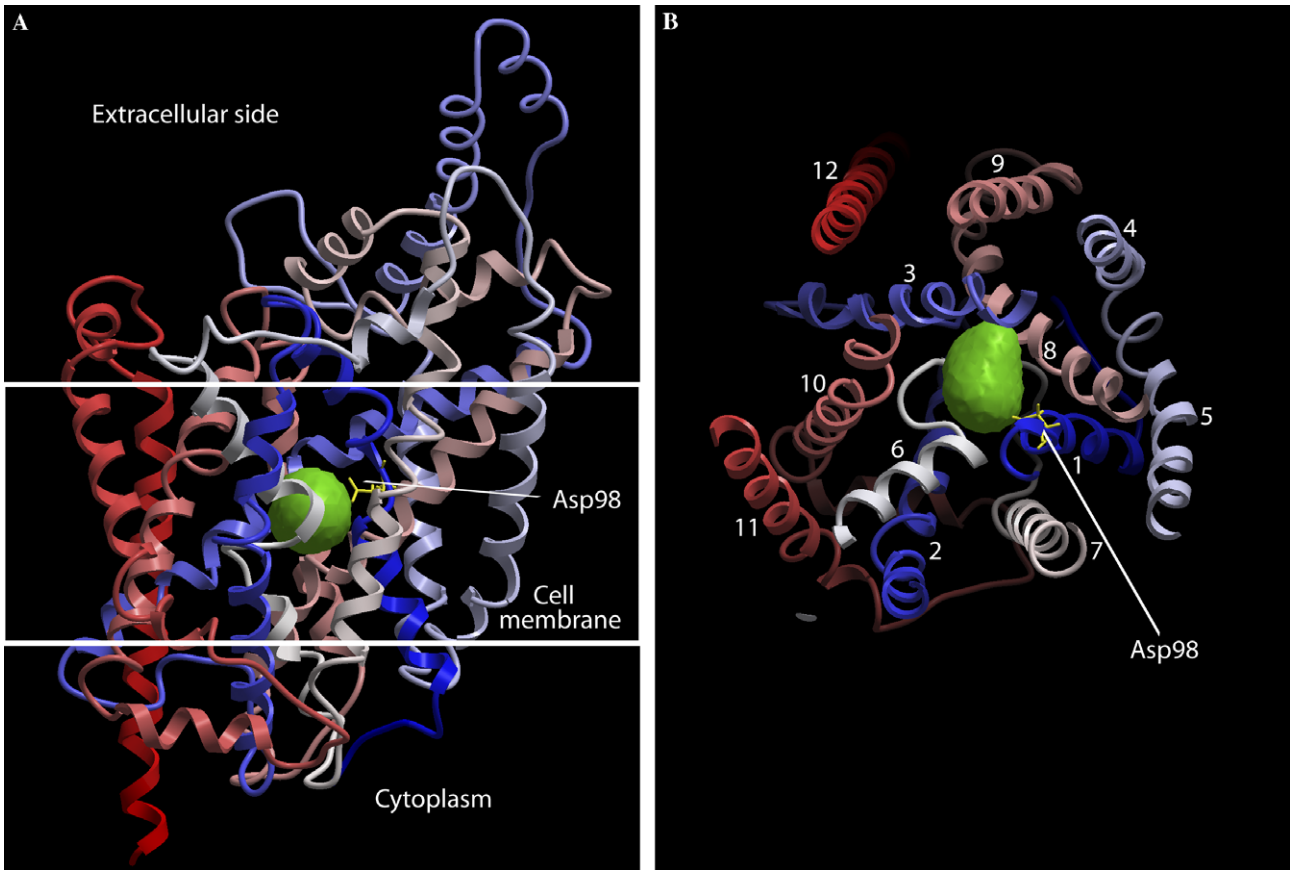

Figure 1. C_α traces of the SERT model viewed in the membrane plane (A), and from the extracellular side (B). Color code of the SERT model is blue via white to red from N-terminal to C-terminal. Asp98 is shown in yellow. The putative substrate binding pocket identified by the ICM pocket finder is shown in green.

TMH6 and TMH8 in substrate binding. The third model[5] suggested that TMHs 1, 2, 4, 5, 7, 8, 10, and 11 are in close proximity to the substrate translocation area, and in contrast to the present model failed to predict the involvement of TMH3 and TMH6. One study also suggests that TMH4 and TMH5 could be involved in ligand binding,[10] which was also predicted by our previous SERT model.[5] The data reported on TMH4 and TMH5 emphasize the importance of how to interpret site-directed mutagenesis studies. However, TMH5 is close to TMH1, and as studies on Lac Permease have indicated,[26] widespread co-operative conformational changes including sliding and tilting motions of the TMHs may occur during ion and substrate transport, and the 12 TMHs may be loosely packed in different conformations associated with transport of substrate molecules. SERT may therefore involve several conformational changes during its transport cycle, both in TMHs and in loop segments. Thus, TMH5 may be closer to a substrate recognition site than the substrate binding site observed in the present SERT model. Other TMHs in close proximity to the substrate translocation area are TMH7 and TMH10.

Based on the fact that the homology between SERT and $LeuT_{Aa}$ is two times higher than between SERT and Lac Permease, and that site-directed mutagenesis data are confirming our predictions,[6,7,9,34–41] it is reasonable to believe that the present SERT model represents a step toward structural understanding of the SERT protein. Molecular modeling and site-directed mutagenesis studies are complementary to each other in an iterating process towards a better understanding of the structure and function of transporter proteins. The SERT model can be used for docking of ligand molecules into the putative binding site and to identify amino acids in the model interacting with the ligand, which will aid the selection of amino acids for further site-directed mutagenesis studies. Furthermore, the interactions seen in drug–target complexes can lead to understanding of the intermolecular forces determining the specificity and the potency of the drug. This information can aid the process of structure-aided drug design, where new drugs can be designed based on information from 3-dimensional models of drug target proteins.

The amino acid residues located in the putative binding pocket are in correspondence with several site-directed mutagenesis studies on SERT, DAT, and NET,[6,7,9,34–41] and partly by our previous modeling studies.[1,3] Together with the functional and sequential similarities between SERT and $LeuT_{Aa}$ this suggests that $LeuT_{Aa}$ is a suitable template for homology modeling of SERT. Compared with our previous models, the present SERT model must be considered as an improved working tool for generating hypotheses and

designing further experimental studies, and more precise predictions of function and drug binding can be performed using the present SERT model than our previous SERT models.

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