Supplemental Material

Mutational mapping and modeling of the (*S*)-citalopram binding site in the human serotonin transporter

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Supplemental material S1: K_i for citalopram analogs at hSERT wild-type and S438T

Experiments were performed as described in *Materials and Methods*.

Compound	K_i (nM)				Affinity change
	SERT WT		SERT S438T		$-K_i$(S438T)/K_i(WT)
	nM	*n*	*nM*	*n*	
Citalopram[a]	59 ± 7	6	10038 ± 531	6	-175
Chloro-citalopram	49 ± 11	6	7640 ± 736	3	-351
Bromo-citalopram	121 ± 13	6	5692 ± 1500	3	-55
Desfluoro-citalopram	232 ± 43	6	17581 ± 4790	3	-100
Descyano-citalopram	154 ± 16	6	8875 ± 1606	3	-62
5-Methyl-citalopram	70 ± 9	8	3310 ± 848	3	-62

[a] Data from Andersen *et al.* (2009) *J. Biol. Chem.* **284**, 10276-10284.

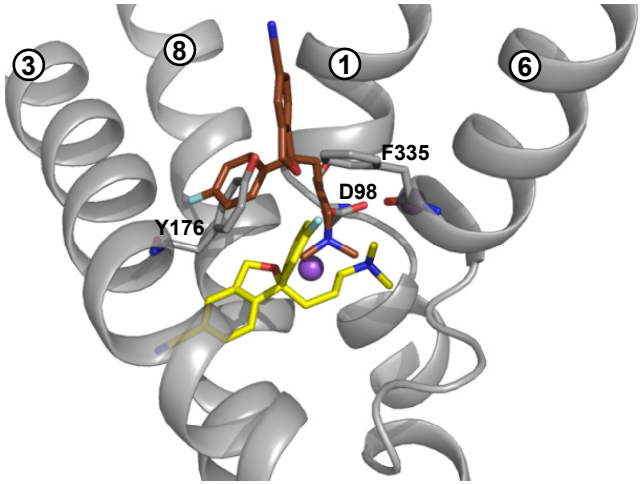
Supplemental discussion

Conclusions about (*S*)-citalopram binding to hSERT by the use of structurally related derivatives strongly relies on the assumption that the derivatives and (*S*)-citalopram have similar binding modes in hSERT. Previously, we have shown that the S438T mutation severely decreased the potency of (*S*)-citalopram, supposedly due to a steric clash between one of the methyl groups on the dimethylaminopropyl chain of (*S*)-citalopram and the introduced protein methyl group on the S438T mutant (Andersen *et al.* (2009) *J. Biol. Chem.* **284**, 10276-10284). Characterization of the five derivatives employed in the study at wild-type hSERT and the S438T mutant showed that all derivatives suffered a significant loss of potency at the S438T mutant, suggesting similar binding modes for citalopram and the analogues.

Supplemental material S2: Docking of (*S*)-citalopram into hSERT using different approaches

TMs 1, 3, 6 and 8 are shown as grey helices; the remainning TMs and intra- and extracellular loops have been removed for clarity. Key residues Asp98, Tyr176 and Phe335 are shown as stick representations and the two Na^+ ions as purple spheres. The best scoring binding mode is shown in yellow and lower ranking binding modes are shown in brown.

Model A

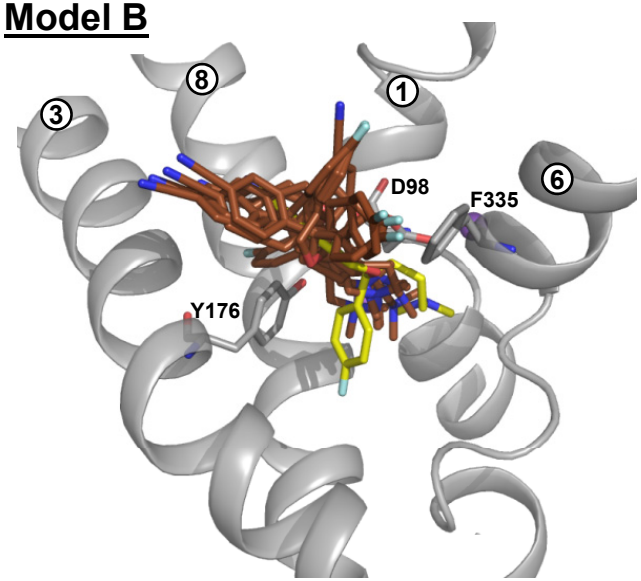


IFD of (S)-citalopram into the occluded hSERT model. Two binding modes were obtained with SP GlideScores between -9.8 and -8.4 kcal/mol.

Highest scoring binding mode: **Model A**

Template	Method	SP GlideScore	Distance (Å) Asp98(Oδ)-ligand(N)
Occluded hSERT model	IFD	-9.8 kcal/mol	3.2

Model B



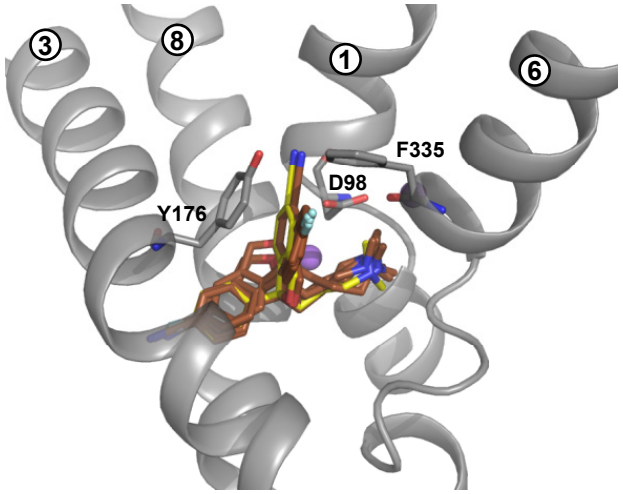
IFD of (S)-citalopram into the outward-facing hSERT model. Eight binding modes were obtained with SP GlideScores between -8.4 and -7.0 kcal/mol. A single binding mode without an interaction between the amine of the ligand and Asp98 was also obtained, but this binding mode was excluded from further analysis. Note that there is only one binding mode (the highest scoring binding mode; shown in yellow) in which one of the aromatic rings is binding below the the aromatic lid (comprised of Tyr176 and Phe335). In the remaining binding modes, both of the aromatic ringssystems are protruding into the extracellular vestibule.

Highest scoring binding mode: **Model B**

Template	Method	SP GlideScore	Distance (Å) Asp98(Oδ)-ligand(N)
Outward-facing hSERT model	IFD	-8.4 kcal/mol	3.0

Supplemental material S2 (cont'd)

Model C

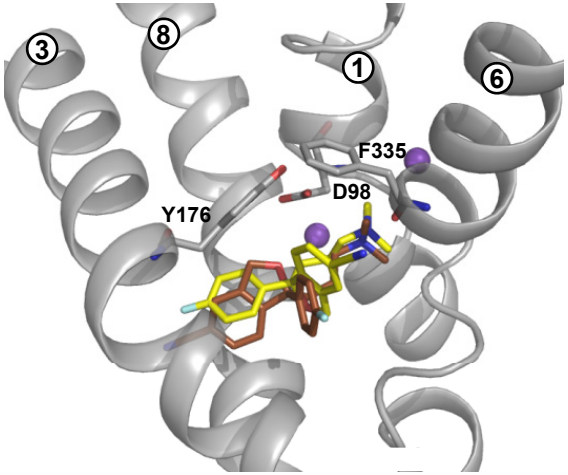


Glide docking of (*S*)-citalopram into the hSERT protein obtained from model **A**. Nine binding modes divided into two clusters were obtained with SP GlideScores between -8.6 and -7.0 kcal/mol. The binding modes in the highest scoring cluster (*cluster 1*) were identical to model A. The binding modes in the second cluster (*cluster 2*) had comparable SP GlideScores, but the aromatic ring systems of (*S*)-citalopram were flipped around in the binding site.

Highest scoring binding mode in *cluster 2*: **Model C**

Template	Method	SP GlideScore	Distance (Å) Asp98(Oδ)-ligand(N)
Occluded hSERT model	Glide	-7.2 kcal/mol	4.2

Model D



IFD of (S)-citalopram into the occluded hSERT model. Ile172 and Phe341 were mutated to alanines during the IFD procedure and added again later in the refinement of the model. Two binding modes were obtained with SP GlideScores between -10.2 and -9.0 kcal/mol.

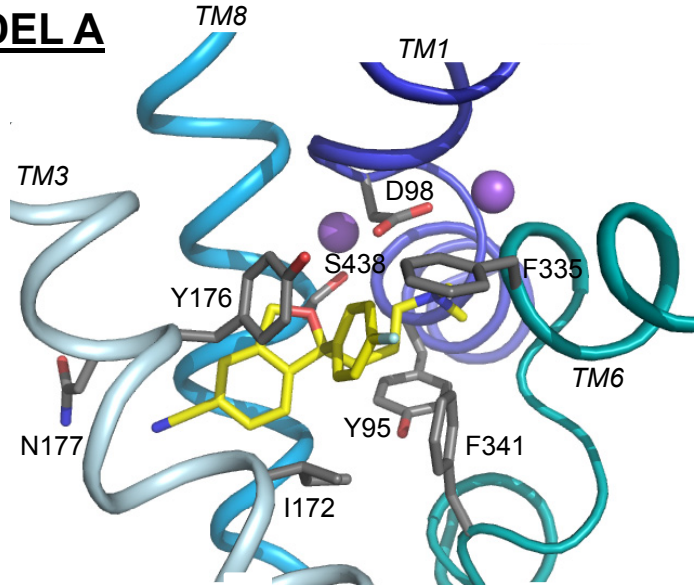
Highest scoring binding mode: **Model D**

Template	Method	SP GlideScore	Distance (Å) Asp98(Oδ)-ligand(N)
Occluded hSERT model	IFD	-10.2 kcal/mol	3.6

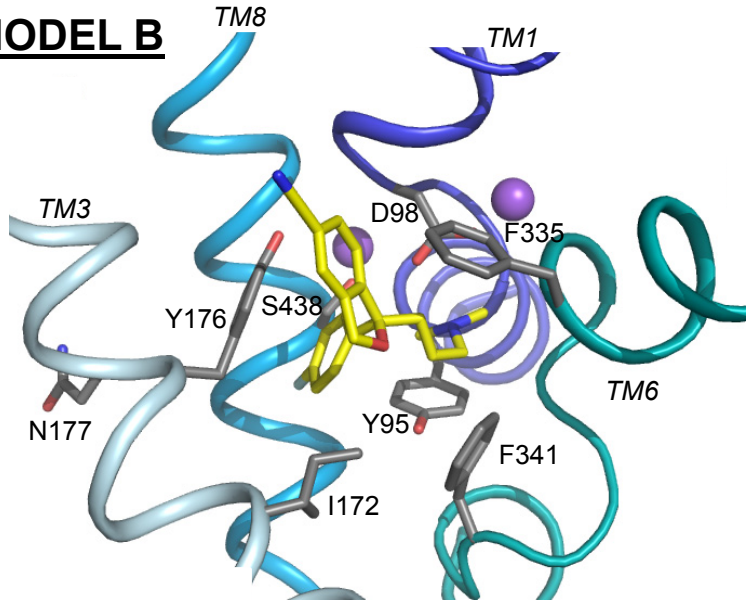
Supplemental material S3: Molecular details of model A-D

TMs 1, 3, 6 and 8 are shown in various shades of blue; the remaining TMs and loops have been removed for clarity. (*S*)-citalopram is shown in yellow and the six key residues (Tyr95, Asp98, Ile172, Asn177, Phe341 and Ser438) in addition to Tyr176 and Phe335 are shown in grey. The two Na^+ ions are shown as purple spheres.

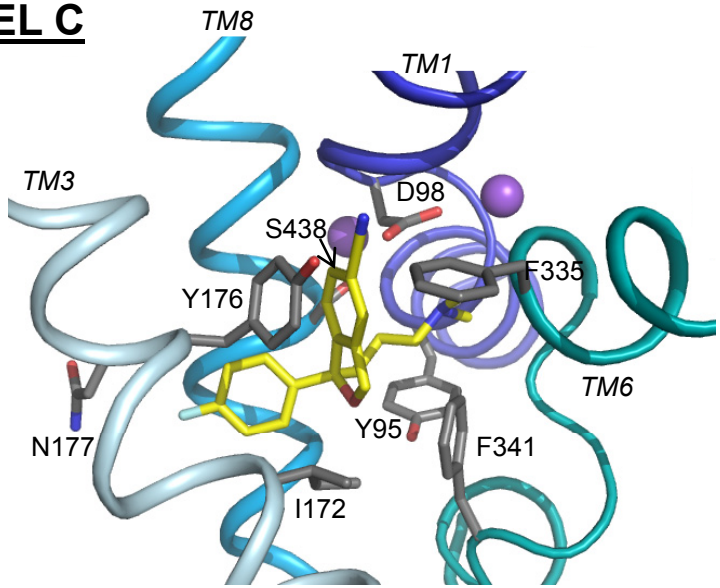
MODEL A



MODEL B



MODEL C



MODEL D