THE HIGH-AFFINITY BINDING SITE FOR TRICYCLIC ANTIDEPRESSANTS RESIDES IN THE OUTER VESTIBULE OF THE SEROTONIN TRANSPORTER

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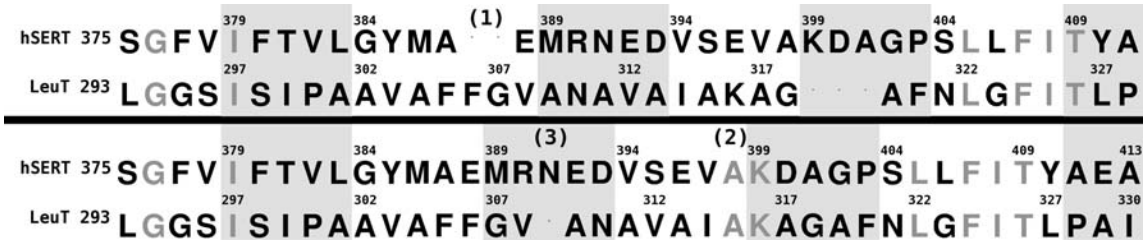
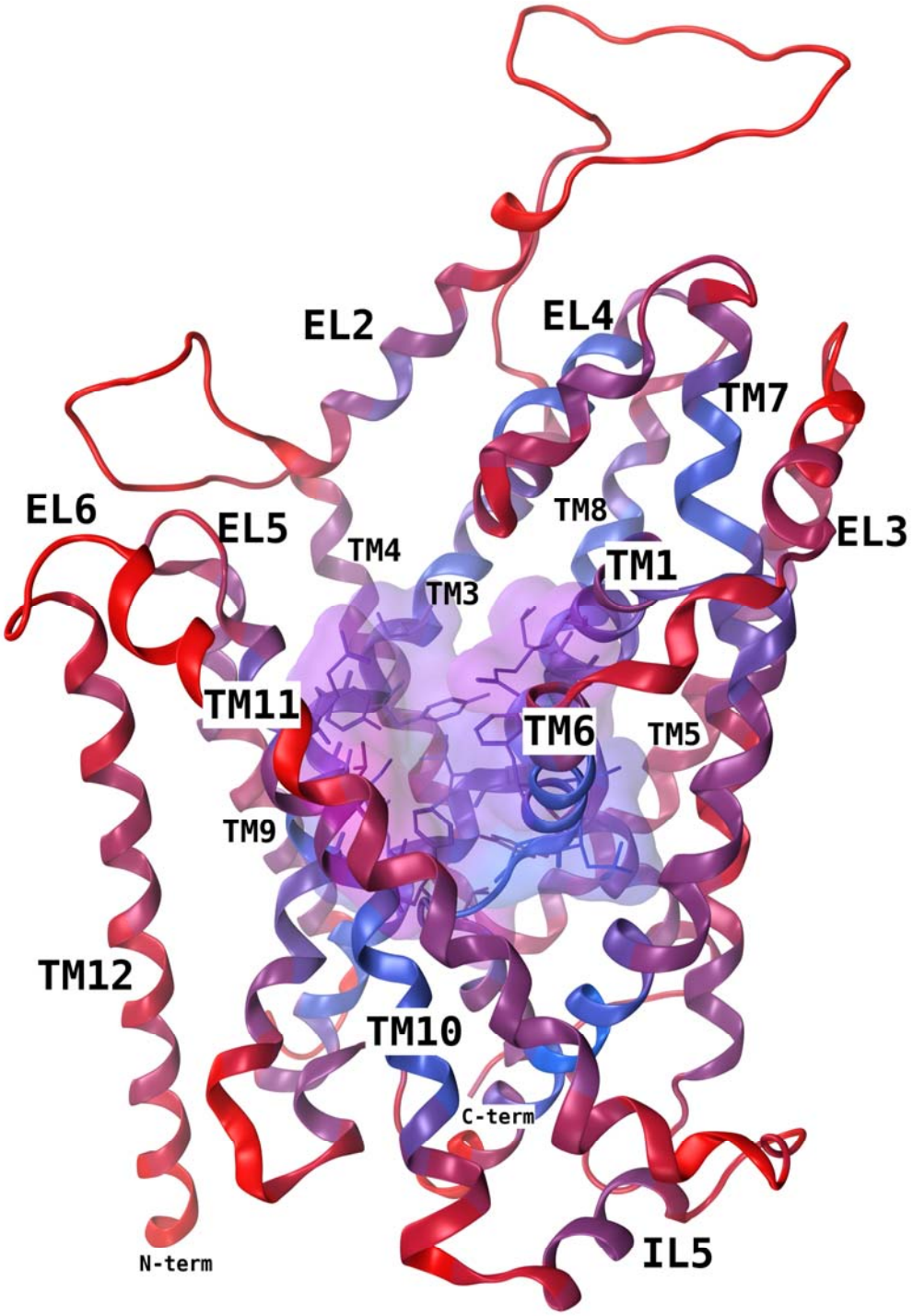
(a) Number of supplementary figures: 5

(b) Number of supplementary tables: 3

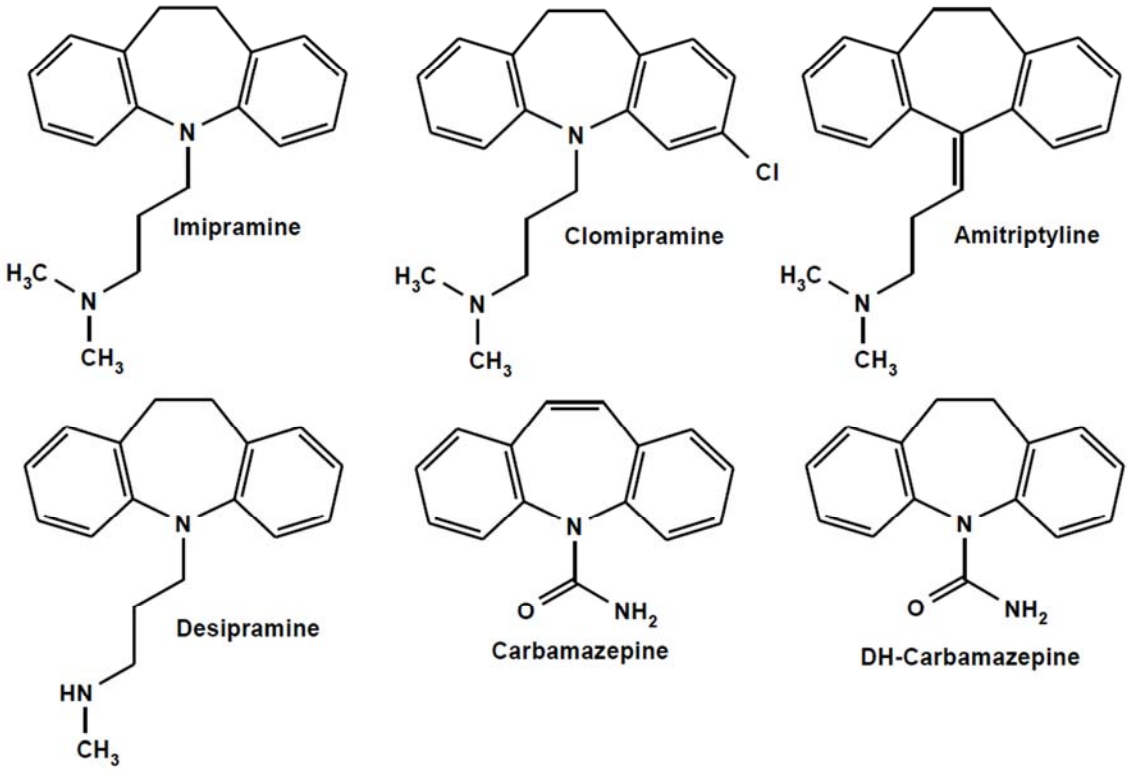
Supplementary Material



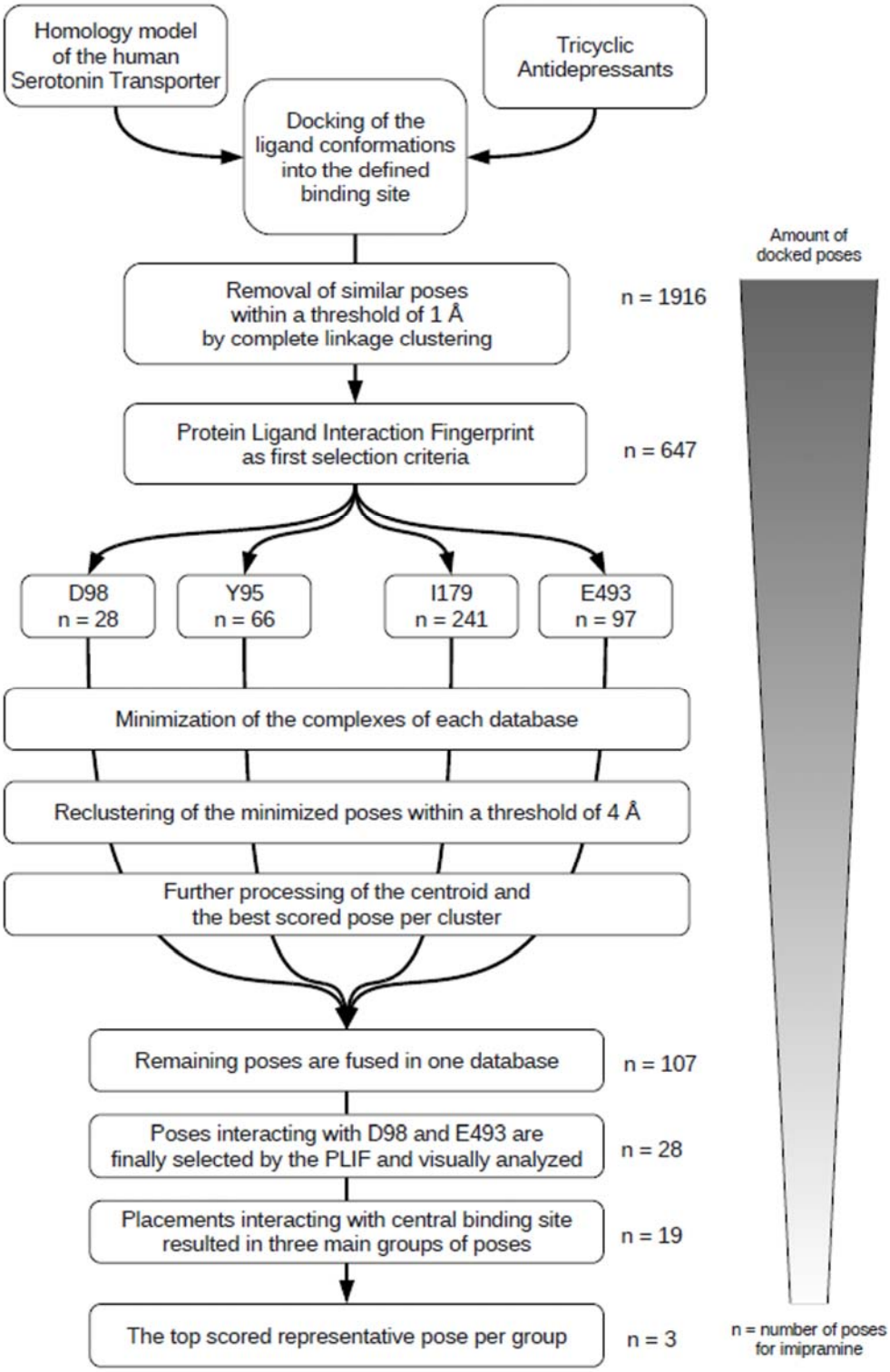
Supplementary Figure 1: Changes in the alignment compared to that published by Beuming et al. (Beuming et al. 2006). Upper row shows the original alignment. Bottom row outline the modified alignment. (1) In the query sequence a gap was removed, and (2) a match of A^{398} and K^{399} with the template was achieved. (3) N^{391} was inserted into the template at the end of TM7.



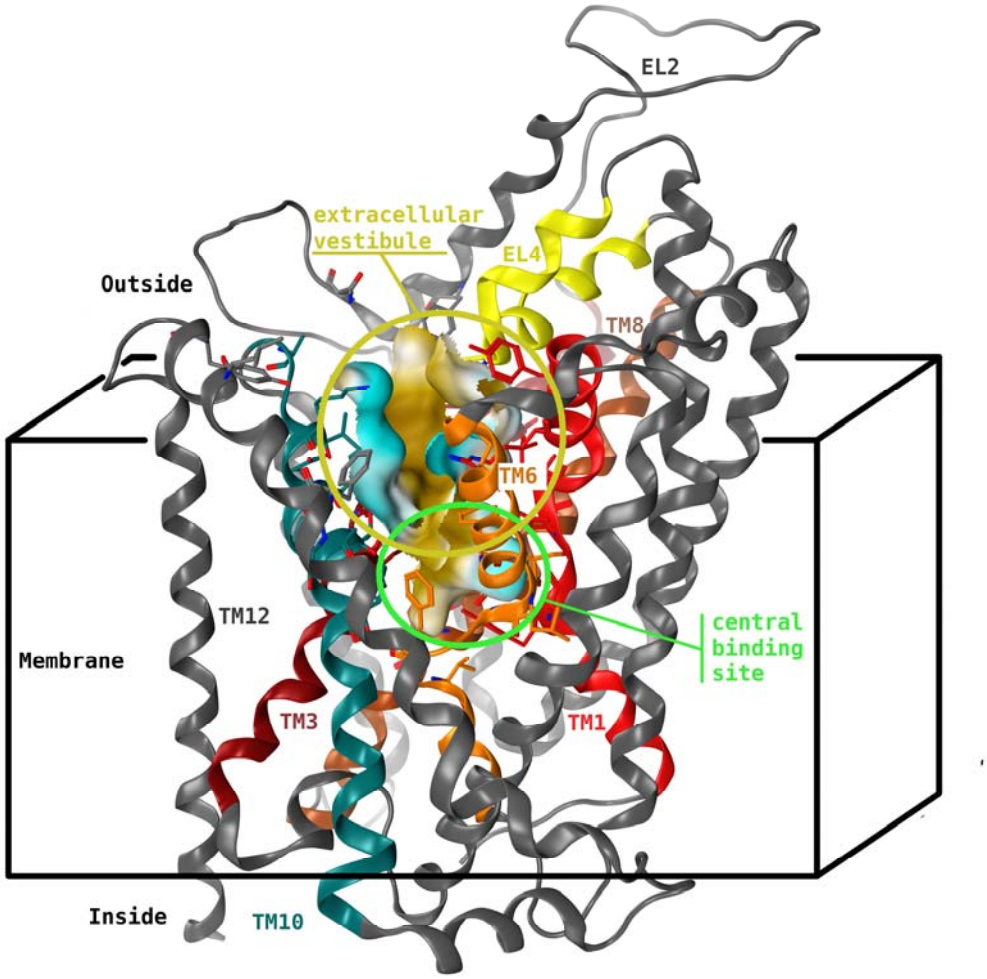
Supplementary Figure 2: Homology model of hSERT coloured according to the result of the structural assessment by the QMEAN scoring function. Regions with high accuracy (estimated error < 1 Å) are depicted in blue. The red coloured regions show an estimated error of > 3.5Å. Intermediate residues are depicted in magenta. The defined binding site is represented as transparent surface.



Supplementary Figure 3: Chemical structures of compounds used for the docking study.



Supplementary Figure 4: Protocol of the docking and evaluation procedure depicted as flow chart. The numbers on the right side correspond to the amount of poses of imipramine.



Supplementary Figure 5: The homology model of hSERT placed in a schematic membrane shows two distinct binding sites. In the 'open-to-out' conformations both sites are continuous and depicted as hydrophobicity surface (blue = hydrophilic, beige = lipophilic). Green circle: Central substrate binding site in the center of the transporter. Yellow circle: Extracellular vestibule and ligand entry path.

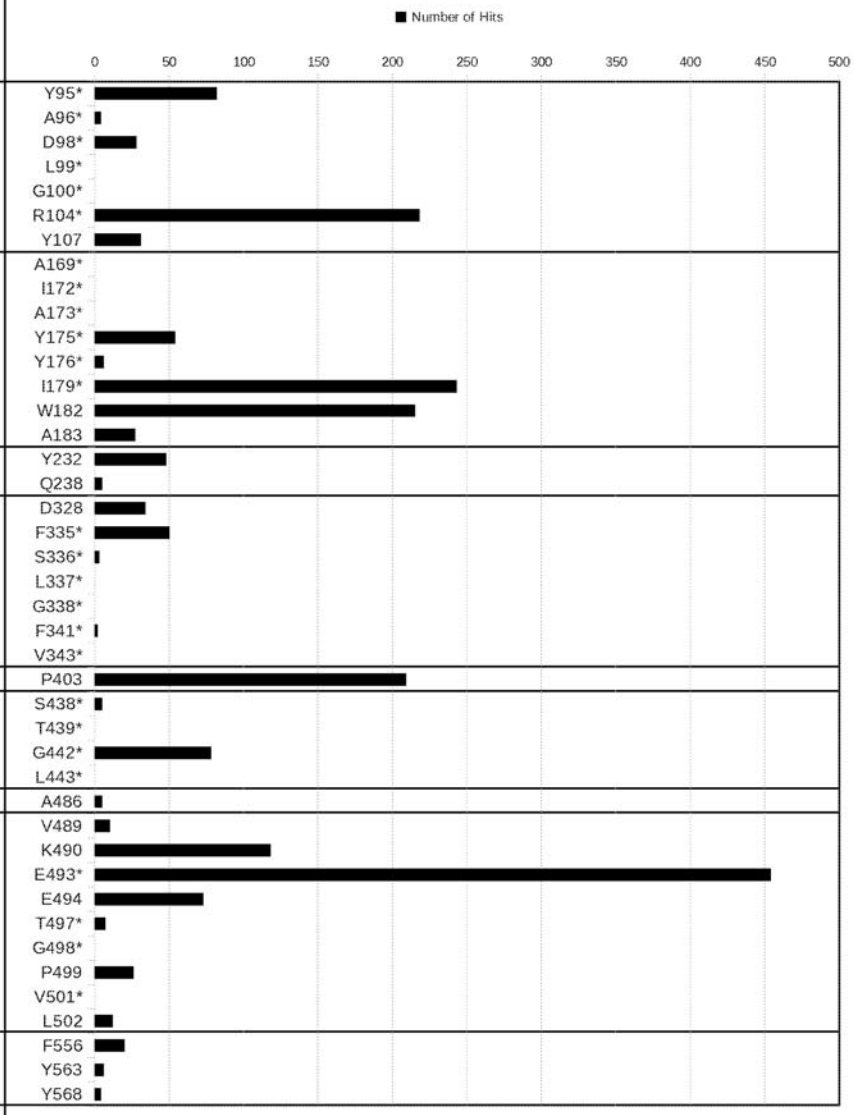
Supplementary Table 1: Mutagenesis studies used for validation of the homoloy model of hSERT

Publication	Residue	Criterion	fulfilled; comment
Andersen et al.	I179	in extracellular vestibule; part of the entrance	yes
	D400	in EL4; no effect or slight effect on inhibitor binding	yes; orientated towards extracellular medium
	S438	Important for recognition of substrate and inhibitors; near D98	yes
	L486	in extracellular vestibule; no effect or slight effect on inhibitor binding	yes
	V489	in extracellular vestibule; no effect or slight effect on inhibitor binding	yes
	K490	in extracellular vestibule; no effect or slight effect on inhibitor binding	no; group 3 shows pi-cation interaction
Barker et al.	D98	important for transporter function; is placed central in binding site 1	yes; coordinates one Na+ and Ligands
Chen et al.	I172	MTS-accessible from both sites; protected by substrates and inhibitors	yes; located in central binding site
	I179	protected only by substrates; may be buried in hydrophobic environment	partly
Field et al.	T323	MTSEA-Biotin accessible	yes
	G324	MTSEA-Biotin accessible	yes
	I327	MTSEA-Biotin accessible	yes
	A331	MTSEA-Biotin accessible	yes
	I333	MTSEA-Biotin accessible, may distinguish between 5HT and MDMA	no; orientated towards TM2
	F334	MTSEA-Biotin accessible	yes
	F335	MTSEA-Biotin accessible	yes
	S336	MTSEA-Biotin accessible, coordination of Na, Cl, 5HT; opposed to N101	yes
	G338	domain movement - no function when mutated	located in unwound region
	G342	domain movement - no function when mutated	located in unwound region
	F347	MTSEA-Biotin accessible	no; below central binding site
Forrest et al.	Y95	accessible from both sides of the membrane	not in our 'open-to-out' model
	G442	not accessible; part of the central binding site	yes
	L443	not accessible; part of the central binding site	yes
Henry et al. 2003	D98	accessible, protected by 5HT	yes
	G100	accessible, protected by 5HT and Cocaine	yes
	N101	accessible, protected by 5HT and Cocaine	partly
	W103	not well accessible	yes
	Y107	accessible, protected by 5HT	partly, on the extracellular end of the helix
	I108	accessible	yes
Henry et al. 2006	Y95C/I172C	no Cd++ binding site; too distant	yes
	N101C/I179C	Cd++ binding site; close enough	no, too distant
	I172	located in the central binding site; involved in ligand binding	yes
	I179	indirectly involved in ligand binding	yes
White et al.	V102C/D98	Zn++ binding site	partly; both residues are in proximity
	V102C/M180C	inhibited by MTS-3-MTS; suggesting a distance of 5 to 6.5 A	orientation matches; distance larger
	V102C/A183C	disulphide bond	orientation matches; distance larger
	W103H/M180C	sensitive for Zn++ inhibition; not W103C/M180C (too distant)	orientation matches; distance larger
	Distances (in Å) between the mentioned residues in the model of White et al.		distances measured from Cβ in our model
	V102-I179	8,5	13,3
	V102-M180	8,6	13,6
	V102-A181	n.d. - opposite side of the helix	opposite side of the helix
	V102-W182	9,9	14,1
	V102-V183	7,5	11,7
	V102-L184	n.d. - opposite side of the helix	opposite side of the helix

The homology model of hSERT was validated with mutagenesis studies. Concerning the location and orientation of residues important for ligand binding, most of the criteria are fulfilled.

Supplementary Table 2: Interactions of the docking poses of imipramine with residues of hSERT

Domain	Interacting Residue	Number of Hits	Type of Main Interaction
TM1	Y95*	82	contact
	A96*	4	backbone donor
	D98*	28	ionic
	L99*	0	
	G100*	0	
	R104*	218	contact
	Y107	31	contact
TM3	A169*	0	
	I172*	0	
	A173*	0	
	Y175*	54	sidechain donor
	Y176*	6	sidechain donor
	I179*	243	contact
	W182	215	contact
	A183	27	contact
EL2	Y232	48	contact
	Q238	5	contact
TM6	D328	34	contact
	F335*	50	contact
	S336*	3	backbone donor
	L337*	0	
	G338*	0	
	F341*	2	backbone donor
	V343*	0	
EL4	P403	209	contact
TM8	S438*	5	backbone donor
	T439*	0	
	G442*	78	contact
	L443*	0	
EL5	A486	5	contact
TM10	V489	10	contact
	K490	118	contact
	E493*	454	contact
	E494	73	ionic
	T497*	7	sidechain donor
	G498*	0	
	P499	26	contact
	V501*	0	
	L502	12	contact
TM11	F556	20	contact
	Y563	6	contact
	Y568	4	contact
	Na^+_1*	0	



Amount and the type of the most frequent populated interactions of imipramine with residues of hSERT. The docking algorithm prefers placements in the extracellular vestibule near E493 and I179. Residues marked with an asterisk have been part of the defined binding site for docking.

Supplementary Table 3: Interactions of poses in group 3

	Residue					
Pose	D98	Y175	Y176	F335	K490	E493
1	ionic		π-cation			
2*	ionic		π-cation	π-cation	π-cation	
3	ionic					
4		H-bond				ionic
5	ionic				π-cation	
6	ionic			π-cation	π-cation	
7	ionic		π-cation			

The type of interactions of the docking poses in group 3 with residues of the homology model of hSERT were obtained by the function 'Ligand Interactions' implemented in MOE. (*)Docking pose 2 as the top scored one of this group was finally selected as representative.