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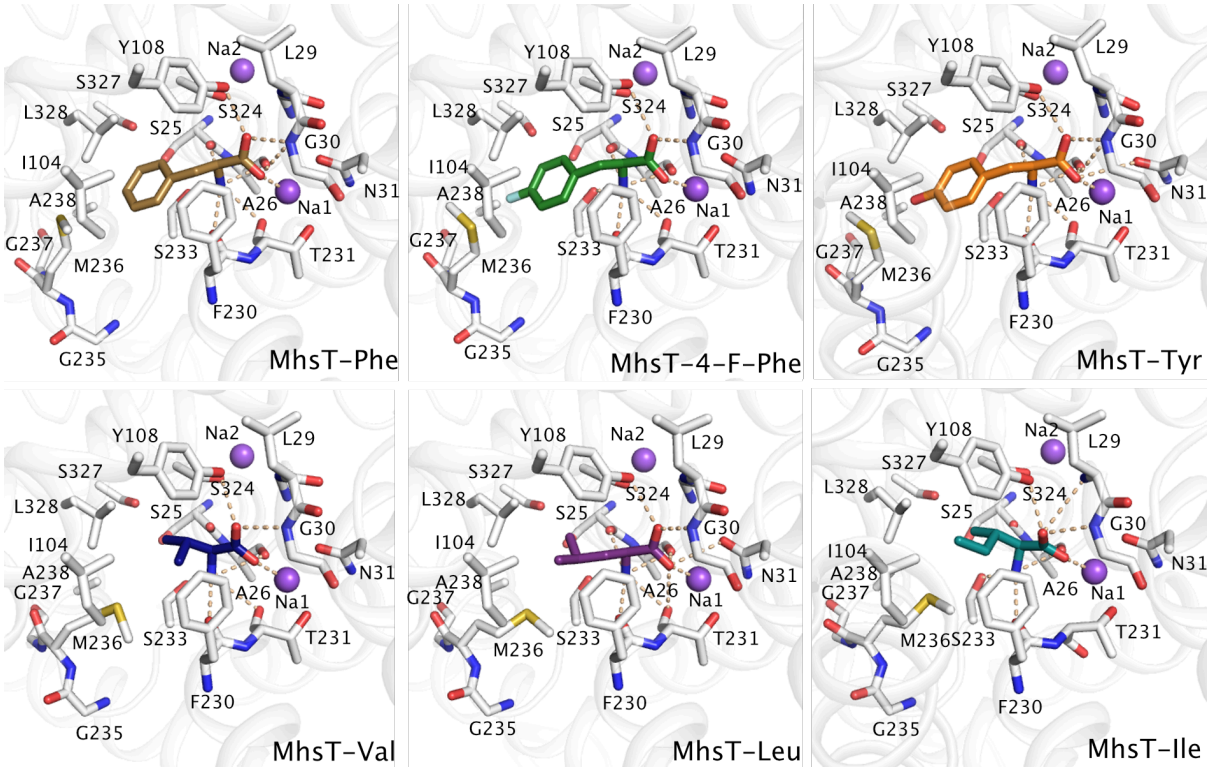
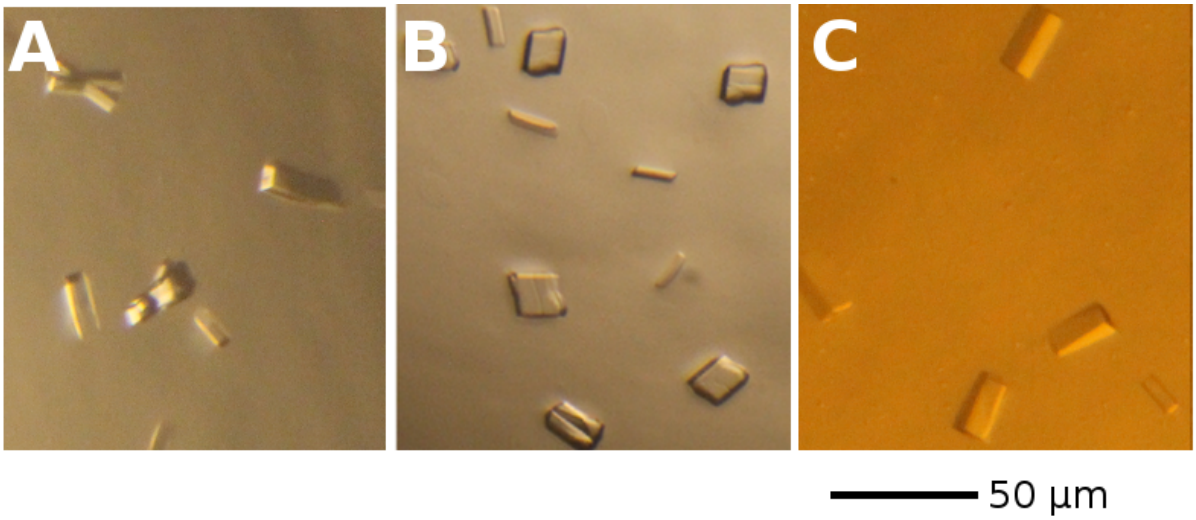
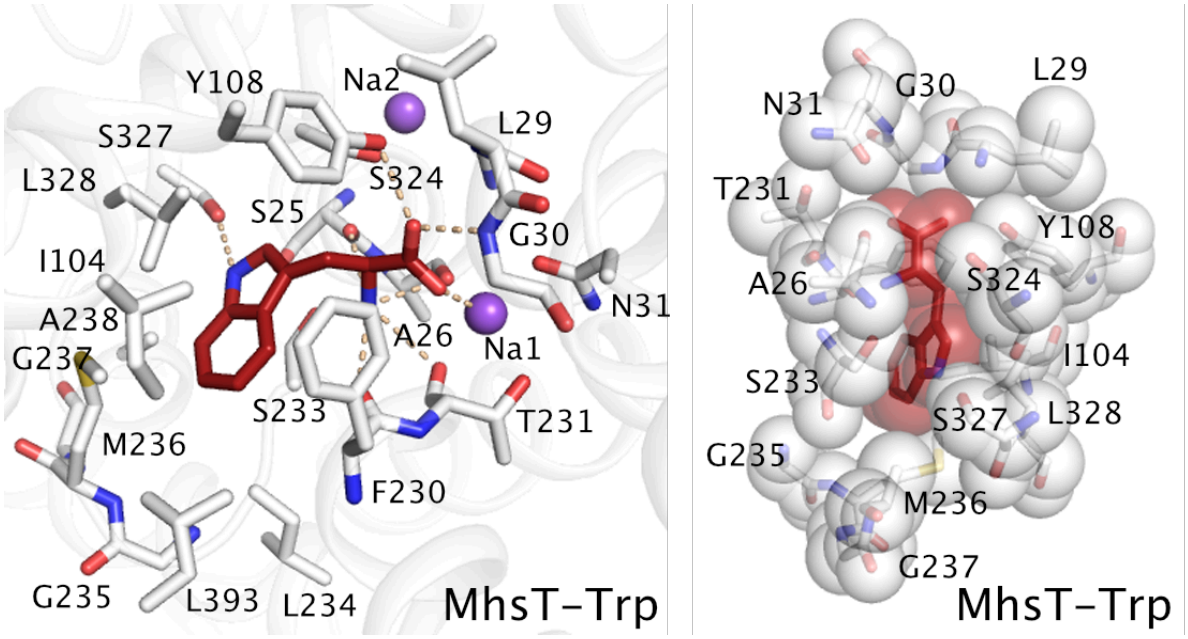


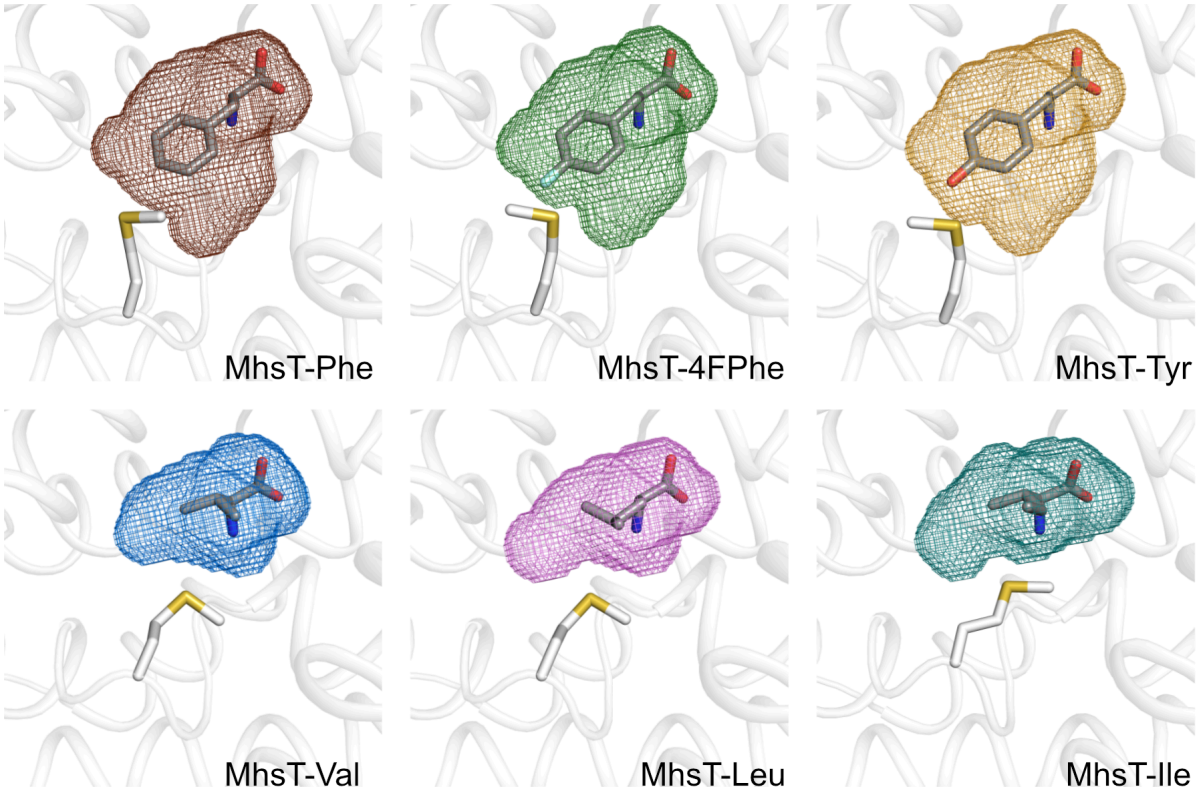
Appendix Figure S1: Comparison of the ligand binding sites of MhsT in complex with the various substrates: L-Phe, L-4F-Phe (Tyr analogue), L-Tyr, L-Val, L-Leu and L-Ile. Protein is shown as white ribbon with the binding site defined with white sticks. Substrates are visualised as coloured sticks and the sodium ions as purple spheres. Hydrogen bonds and electrostatic interactions are identified by dashed lines.



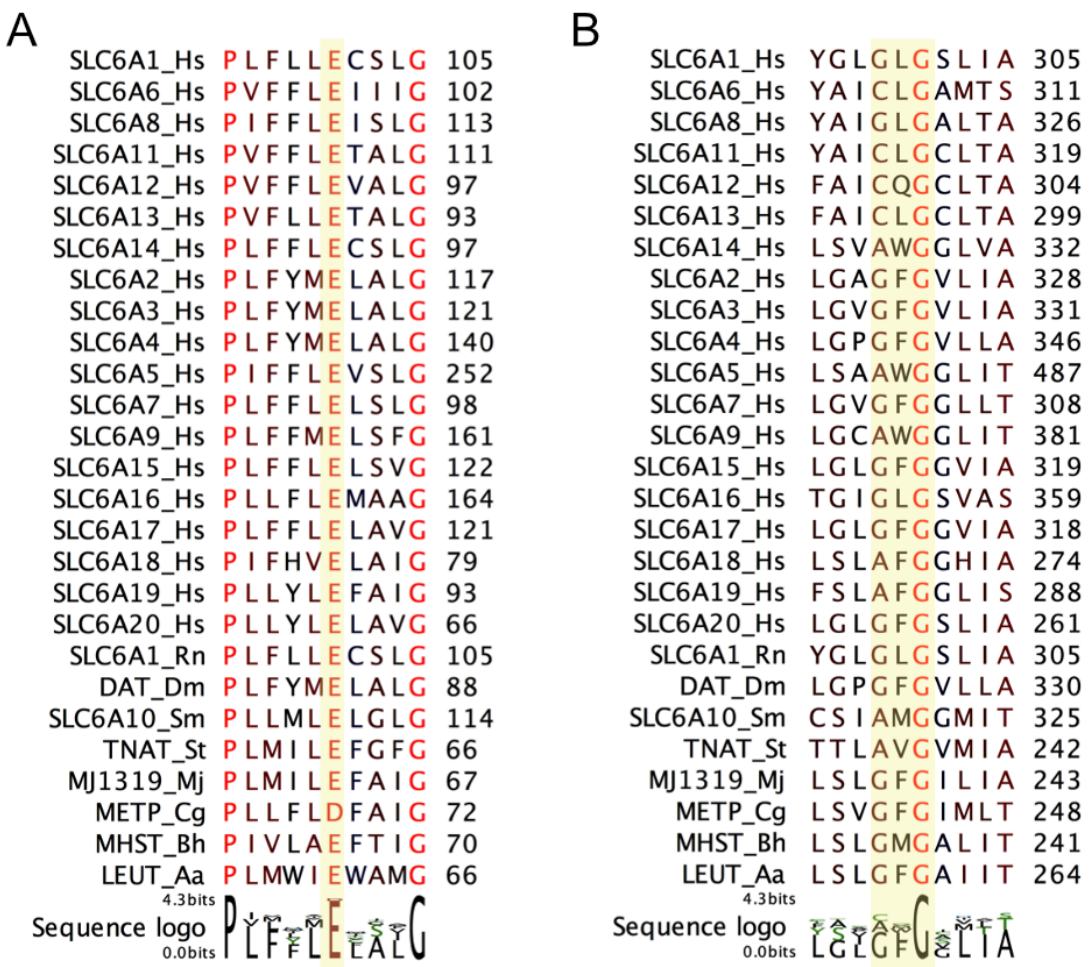
Appendix Figure S2: Examples of MhsT crystals obtained in complexes with different substrates. A) MhsT+Leu complex obtained using HiLiDe in hanging drop plates, 0.5 mM L-Leu, 0.1 M HEPES-NaOH pH 7.0, 16 % PEG 400. 0.4 M NaCl, 2x cmc OTG, 10 % glycerol; B) MhsT+4-F-Phe complex obtained using HiLiDe in hanging drop plates, 0.5 mM L-Phe, 0.1 M Tris-HCl pH 7.0, 14 % PEG 400, 0.4 M NaCl, 5 % TMANO, 2x cmc OTG, 5 % glycerol; C) MhsT+Val complex crystals obtained in hanging drop plates with protein:DOPC ration of 3:0.8 (w/w), 2x cmc NG; 5 % glycerol, 12 % PEG400, 0.1M HEPES-NaOH pH 7.0, 0.3 M NaCl, 5 % TMANO.



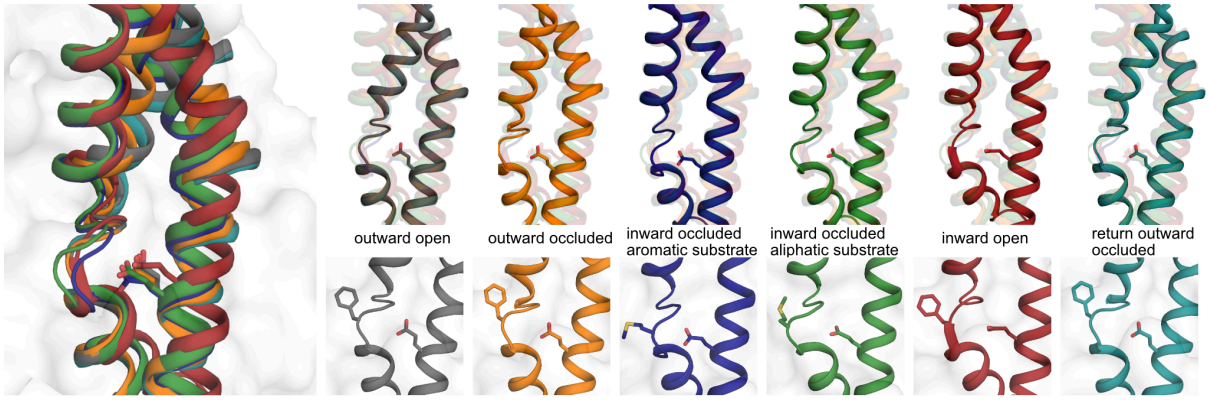
Appendix Figure S3: Binding of Trp (PDB ID: 4US3). Ligand binding site of MhsT in complex with L-Trp and volume of the binding pockets visualised by means of the van der Waals representation of the ligand and coordinating residues of MhsT in complex with L-Trp.



Appendix Figure S4: Binding site volumes calculated by use of VOIDOO for MhsT in complex with Phe, 4FPhe, Tyr, Val, Leu and Ile.



Appendix Figure S5: Conservation of the A) Glu66^{MhsT} and B) GMG motif within the unwound part of TM6 across the human SLC6 family membranes and their homologues from other organisms. Accession numbers: SLC6A1_Hs - P30531; SLC6A2_Hs - P23975; SLC6A3_Hs - Q01959; SLC6A4_Hs - P31645; SLC6A5_Hs - Q9Y345; SLC6A6_Hs - P31641; SLC6A7_Hs - Q99884; SLC6A8_Hs - P48029; SLC6A9_Hs - P48067; SLC6A11_Hs - P48066; SLC6A12_Hs - P48065; SLC6A13 - Q9NSD5; SLC6A14_Hs - Q9UN76; SLC6A15_Hs - Q9H2J7; SLC6A16_Hs - Q9GZN6; SLC6A17_Hs - Q9H1V8; SLC6A18_Hs - Q96N87; SLC6A19_Hs - Q695T7; SLC6A20_Hs - Q9NP91; SLC6A1_Rn - P23978; DAT_Dm - Q7K4Y6; SLC6A10_Sm - A0A0H3YEX6; TNAT_St - O50649; MJ1319_Mj - Q58715; METP_Cg - A0A0D6FTY1; MhsT - Q9KDT3; LeuT - O67854.



Appendix Figure S6: Movement of TM6, TM2 and Glu66 during the transport cycle. Structures are aligned based on scaffold TMs 3-4, and TMs 8-9.LeuT outward open conformation (PDB ID: 3TT1): gray color; LeuT outward-occluded conformation (PDB ID: 2A65) – orange color; Mhst-Val inward-occluded conformation with a small substrate – green color; MhsT- 4FPhe inward-occluded conformation with an aromatic substrate- blue color; LeuT inward-open conformation (PDB ID: 3TT3) – red color; LeuT return-occluded conformation (PDB ID: 5JAE)– cyan color.

Appendix Table S1: r.m.s.d. values of the different complexes when compared to MhsT-Trp (PDB ID: 4US3) and comparison of the binding site cavity volumes for the substrate bound MhsT structures.

Complex	r.m.s.d. value	Cavity volume on plot grid (Å^3)*
MhsT-Trp	n.a.	230
MhsT-Tyr	0.185	224
MhsT-4FPhe	0.165	215
MhsT-Phe	0.162	210
MhsT-Ile	Chain A : 0.351 Chain B : 0.340	159
MhsT-Leu	Chain A : 0.292 Chain B : 0.287	158
MhsT-Val	Chain A: 0.274 Chain B: 0.267	144

**Calculated with VOIDOO software [65]*

Appendix Table S2: Comparison of the binding site residues of MhsT, SLC6A18 and SLC6A19.

MhsT	LeuT	SLC6A18	SLC6A19	dDAT	SERT
Ala26	Ala	Ala	Cys	Ala	Ala
Leu29	Leu	Leu	Leu	Leu	Leu
Gly30	Gly	Gly	Gly	Ala	Gly
Asn31	Asn	Asn	Asn	Asn	Asn
Ile104	Val	Ile	Val	Ile	Ile
Tyr108	Tyr	Tyr	Tyr	Tyr	Tyr
Phe230	Phe	Phe	Phe	Phe	Phe
Thr231	The	Ser	Ser	Ser	Ser
Ser233	Ser	Ser	Ser	Gly	Gly
Met236	Phe	Phe	Phe	Phe	Phe
Ala238	Ala	Gly	Gly	Val	Val
Ser324	Ser	Thr	Ser	Ser	Ser
Ser327	Ala	Gly	Gly	Ala	Gly
Leu328	Ile	Thr	Asn	Gly	Gly
Val331	Pro	Ala	Gly	Gly	Ala