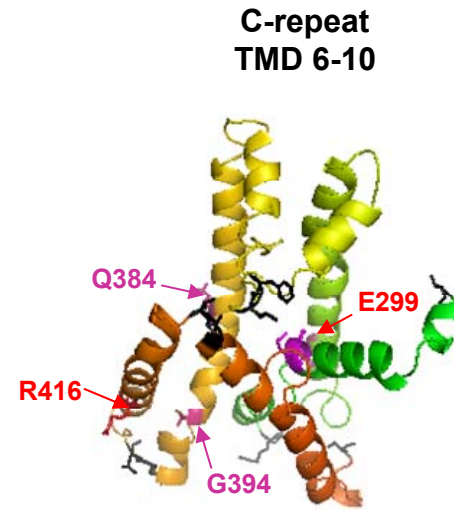
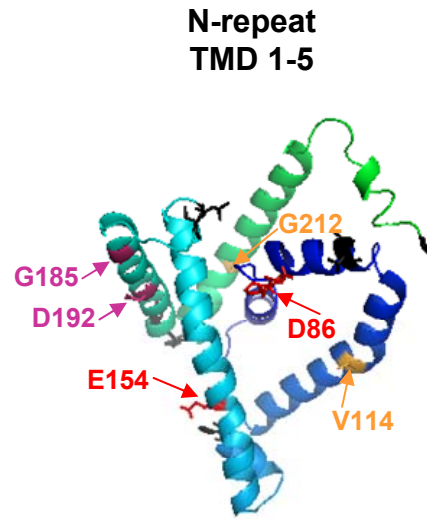


SUPPLEMENTARY FIGURE LEGENDS

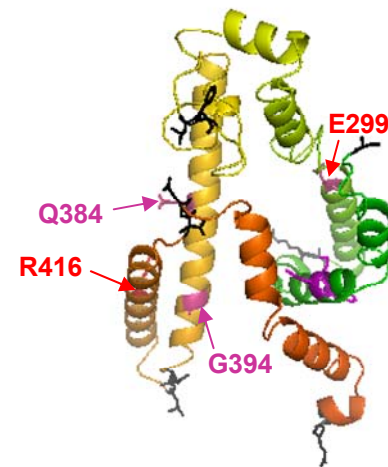
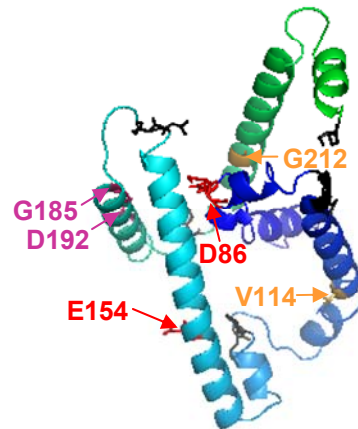
Supplementary Figure 1: *Position of functionally important amino acid residues and segments on the predicted structural model of Slc11a2.* The Slc11a2 models presented are identical to those shown in Figure 7. Positions corresponding to complete (red) or partial (pink) loss-of Slc11a2 function are shown. Mutations reported in compound heterozygote individual [17] suffering from severe anemia (but not yet functionally characterized) are shown in orange. Natural variants: TMD2 Val¹¹⁴, TMD4 Gly¹⁸⁵, TMD5 Gly²¹² and TMD9 Arg⁴¹⁶; experimental mutants: TMD1 Asp⁸⁶, TMD3 Glu¹⁵⁴, TMD4 Asp¹⁹², TMD7 Glu²⁹⁹, TMD8 Gln³⁸⁴ and Gly³⁹⁴.

Supplementary Figure 2: *Parallel view of the pseudo-symmetrical arrangement of TMDs 3-4 and 8-9 in the membrane, and position of functionally important amino acid residues.* The LeuT and vSGLT models are identical to those shown in Figure 7. The natural variants: TMD4 Gly¹⁸⁵ and TMD9 Arg⁴¹⁶, and the experimental mutants: TMD3 Glu¹⁵⁴, TMD4 Asp¹⁹², TMD8 Gln³⁸⁴ and Gly³⁹⁴ are the same as those presented in Supplementary Figure 1; another experimental loss-of-function mutant (TMD3 Gly¹⁶⁷), which was characterized using *E. coli* MntH [54] was added to show its close proximity with TMD4 Gly¹⁸⁵.

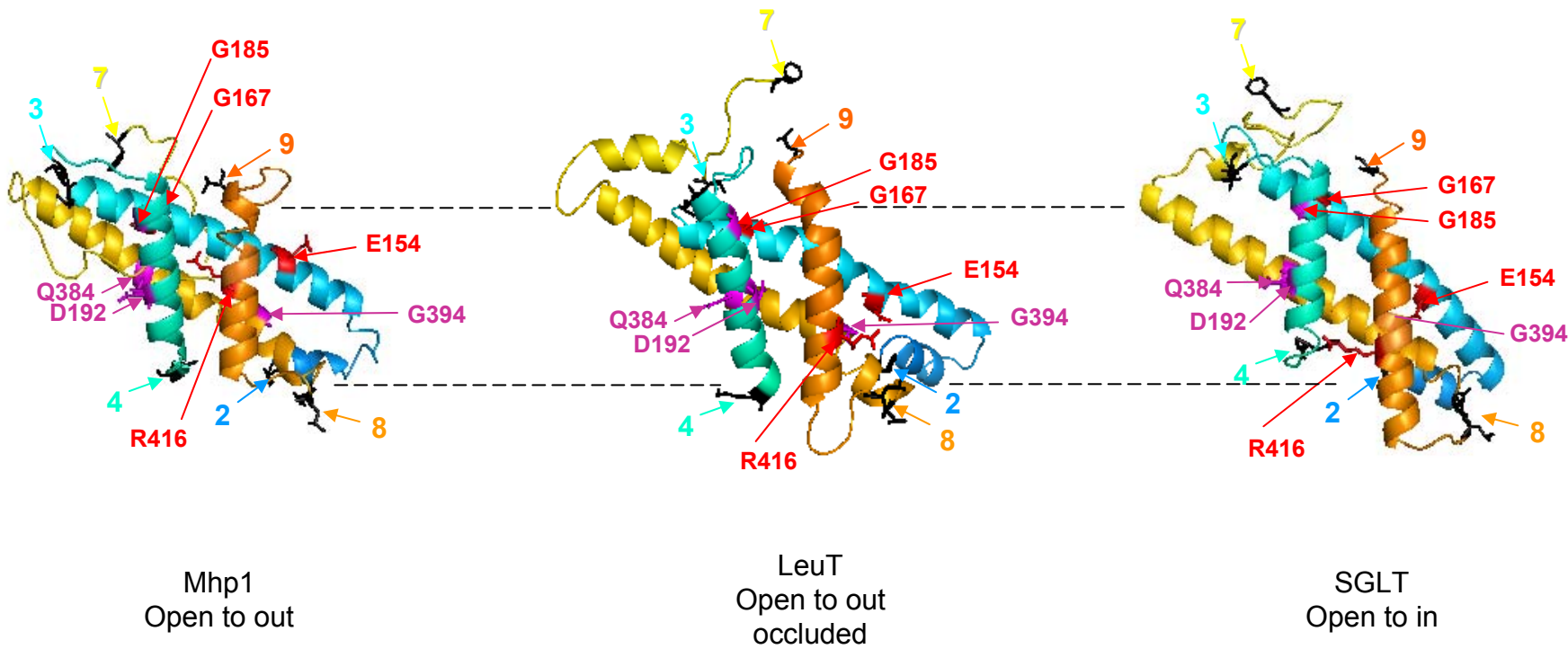
LeuT
-open to out-
(occluded)



SGLT
-open to in-



View parallel
to the membrane



Supp. Fig. 2

Supp. Table 1: Summary of mouse Slc11a2 sequence homology threading and modeling

Rank	Templates	Programs
a) Local MEta-Threading-Server (LOMETS) *		
1	vSGLT (3dh4)	HHa, HH, HHb, PPA-I, SPARKS2, SP3, PPA-II
2	Mhp1 (2jln)	PPA-I, SP3, SPARKS2, PPA-II
3	LeuT (2a65)	SPARKS2, PPA-I
4	GlpT (1pw4)	n.a.
Rank	Templates	Scores
b) Threading ASSEmbly Refinement (I-TASSER) server&		
1§	vSGLT (3dh4)	C-score=-1.74
2	Mhp1 (2jln)	C-score=-1.46
3	n.a.	C-score=-1.52
4	LeuT (2a65)	C-score=-2.24
c) MUlti-Source ThreadER (MUSTER) server%		
1	vSGLT (3dh4A)	Z-score=5.952
2	Mhp1 (2jln)	Z-score=5.480
3§	LeuT (2a65)	Z-score=5.285
4	GlpT (1pw4)	Z-score=4.997

* Templates that yielded models with scores above the respective program significance cutoff (in decreasing order)

& C-score in the range -5,2

% Z-score cutoff 5.8 ; Z-score >7.5 indicates a 'Good' template

§The two models presented in Fig. 7 and Supp. Figs. 1& 2 are indicated in bold.

Supp. Table 2: Compilation of mouse Slc11a2 sequence homology modeling using the 9 component meta-server LOMETS

n.a.me	Programs		Models		
	Approach	Z-score cutoff	Rank (Z-score)	Z-score	% seq ID w/ template
Template: Mhp1 (2jln.a.)					
MUSTER	[1]	5.8	2	5.507	11.5
HHsearch v1.0	[2]	13	n.a.		
HHsearch v1.5a	[2]	9.5	n.a.		
HHsearch v1.5b	[2]	11.5	n.a.		
SPARKS2	[3]	7	1	9.774	13.2
SP3	[4]	7	1	9.892	14.7
PROSPECT2	[5]	3.2	n.a.		
PPA-I	[6]	9.3	2	12.409	11.4
FUGUE	[7]	7	n.a.		
Template: LeuT (2a65A)					
MUSTER	[1]	5.8	3	5.311	12.7
HHsearch v1.0	[2]	13	2	3.959	11.8
HHsearch v1.5a	[2]	9.5	2	2.187	13.3
HHsearch v1.5b	[2]	11.5	n.a.		
SPARKS2	[3]	7	3	7.603	11.7
SP3	[4]	7	7	4.522	12.9
PROSPECT2	[5]	3.2	n.a.		
PPA-I	[6]	9.3	7	8.043	11.8
FUGUE	[7]	7	n.a.		
Template: vSGLT (3dh4A)					
MUSTER	[1]	5.8	1	5.982	11.8
HHsearch v1.0	[2]	13	1	18.245	13
HHsearch v1.5a	[2]	9.5	1	15.105	13
HHsearch v1.5b	[2]	11.5	1	15.98	12.8
SPARKS2	[3]	7	2	8.022	12.3
SP3	[4]	7	2	7.553	11.2
PROSPECT2	[5]	3.2	n.a.		
PPA-I	[6]	9.3	1	13.533	10.3
FUGUE	[7]	7	n.a.		
Template: GlpT (1pw4A)					
MUSTER	[1]	5.8	4	5.021	10.1
HHsearch v1.0	[2]	13	3	3.867	7.3
HHsearch v1.5a	[2]	9.5	n.a.		
HHsearch v1.5b	[2]	11.5	4	5.038	11.4
SPARKS2	[3]	7	n.a.		
SP3	[4]	7	n.a.		
PROSPECT2	[5]	3.2	n.a.		
PPA-I	[6]	9.3	4	8.435	9.8
FUGUE	[7]	7	n.a.		

Supp. Table 3: Assessment of Slc11a2 modeling accuracy.

Templates	Nramp2 MUST ER Models							
	Resolution (Å)	Conformation	Homology to Rmsd (Å) ^	BetP* % sed ID	Homology to template & Rmsd (Å) ^	% sed ID	Homology to Rmsd (Å) ^	BetP* % sed ID
Mhp1	2.85	open-to-out	4.9 +/- 0.5	7.6 +/- 1.2	1.4 +/- 0.5	10.9 +/- 1.0	5.0 +/- 0.8	9.4 +/- 1.5
LeuT	1.65	open to out occluded	4.3 +/- 0.2	11 +/- 1.5	1.1 +/- 0.1	12.1 +/- 1.2	4.3 +/- 0.3	6.4 +/- 1.6
vSGLT	2.7	open-to-in	5.0 +/- 0.7	9.3 +/- 1.2	1.9 +/- 0.1	10.3 +/- 0.6	4.8 +/- 0.8	7.5 +/- 1.3
GlpT	3.3	open-to-out	n.t.	n.t.	1.8 +/- 0.1	9.2 +/- 1	5.9 +/- 2.9	6.8 +/- 3.6

*BetP structure represents a translocation state where the bound substrate is occluded from both sides of the membrane by aromatic residues; This structure is perceived as a conformational intermediate between the structures of LeuT (open to out, lightly occluded) and vSGLT (open to in) (Ressl S et al., 2009 Nature, 458: 47-53).

& rmsd >3 Å is typically expected for sequence identity <20 % (Zhang Y, 2009, Curr Opin Struct Biol, 19:145–155, Forrest LR et al., 2006, Biophys J 91: 508–517).

^ Pairwise structural alignments were obtained using different algorithms (Dalilite v. 3, Holm L et al., 2008, Bioinformatics, 24:2780-1, Softberry Inc's 3DMap, Matras, Kawabata T, 2003, Nucleic Acids Res. 31:3367-9); the results are expressed in average +/- SEM.