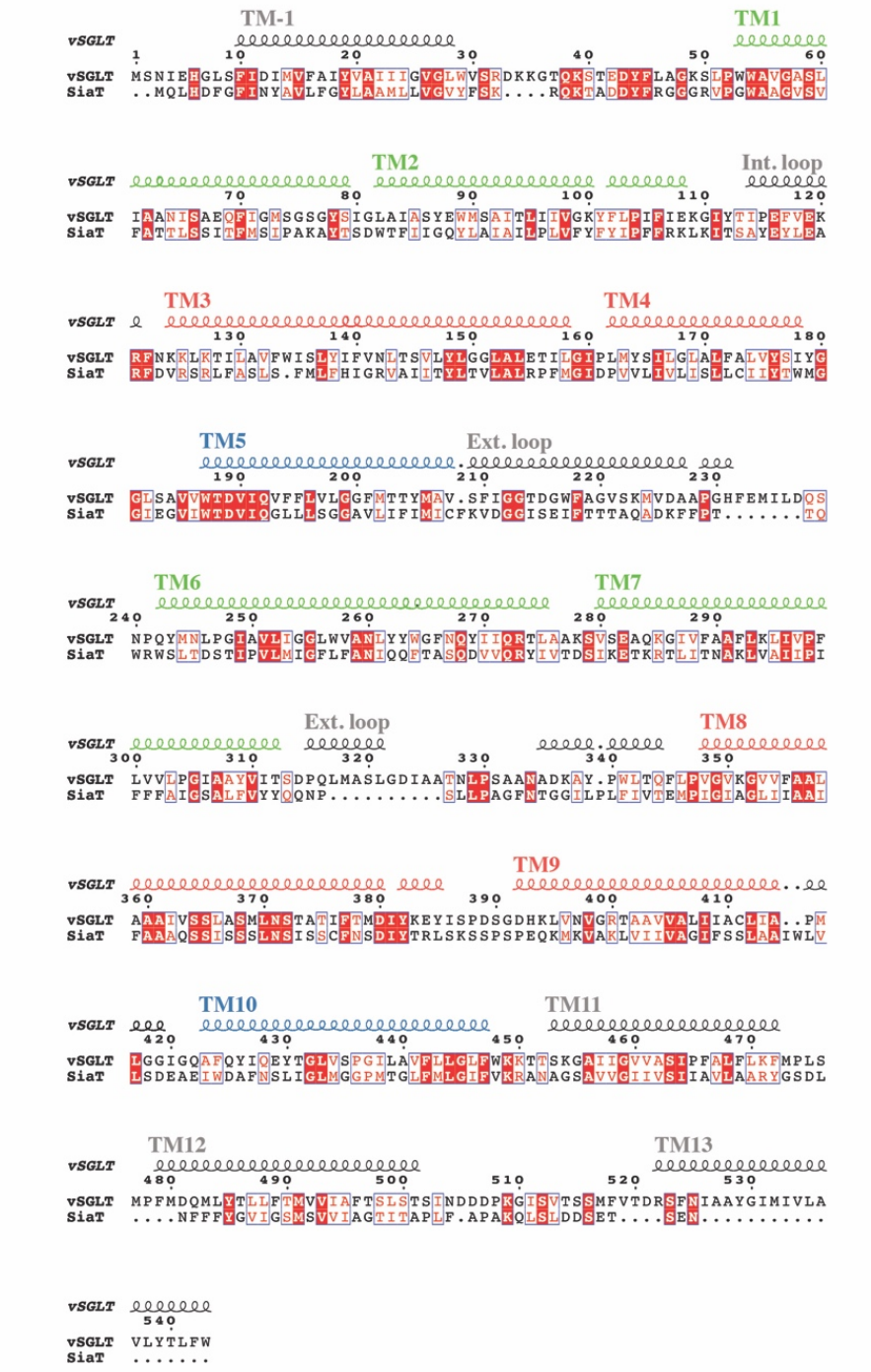
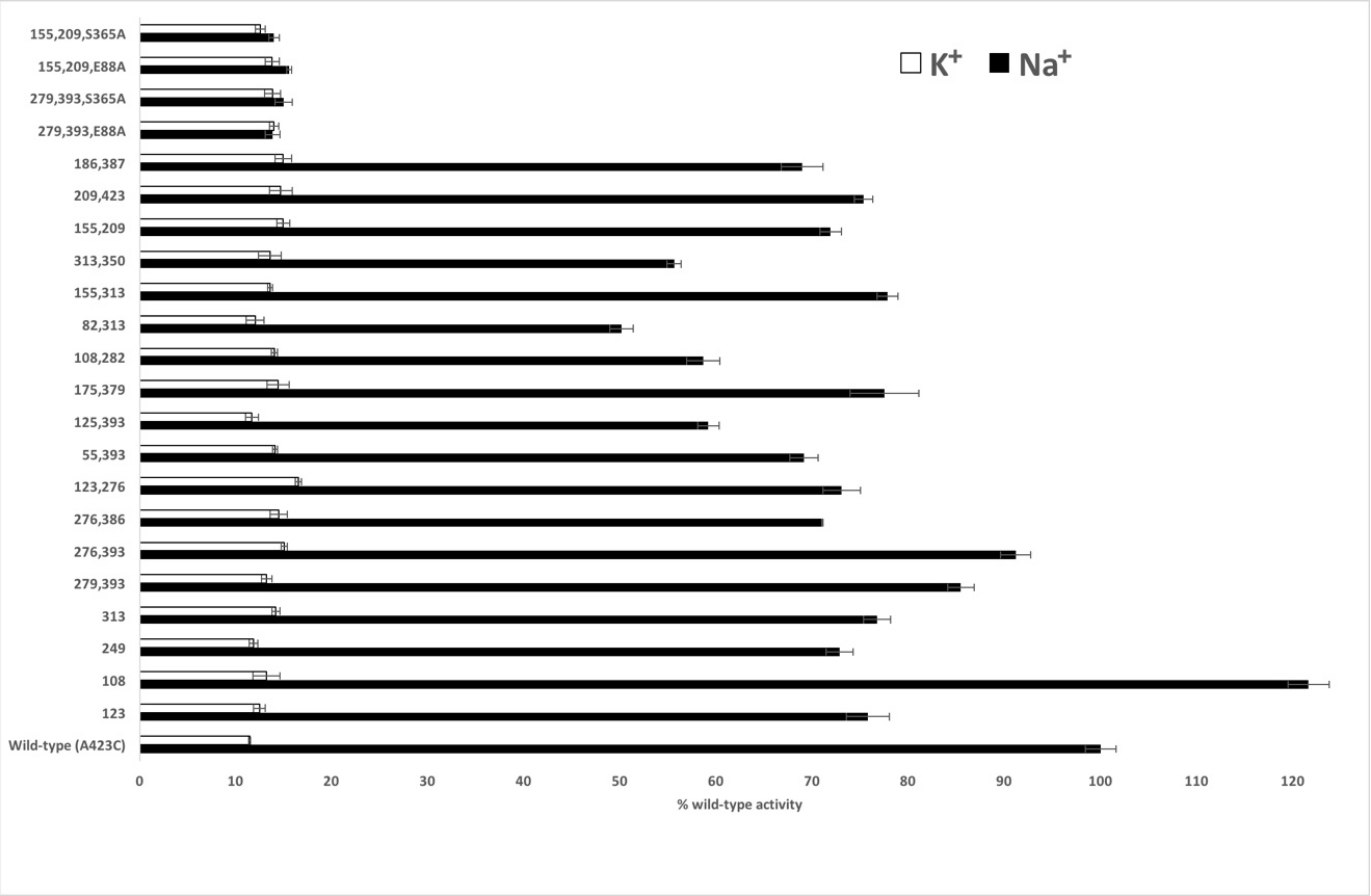
Supporting Information Appendix

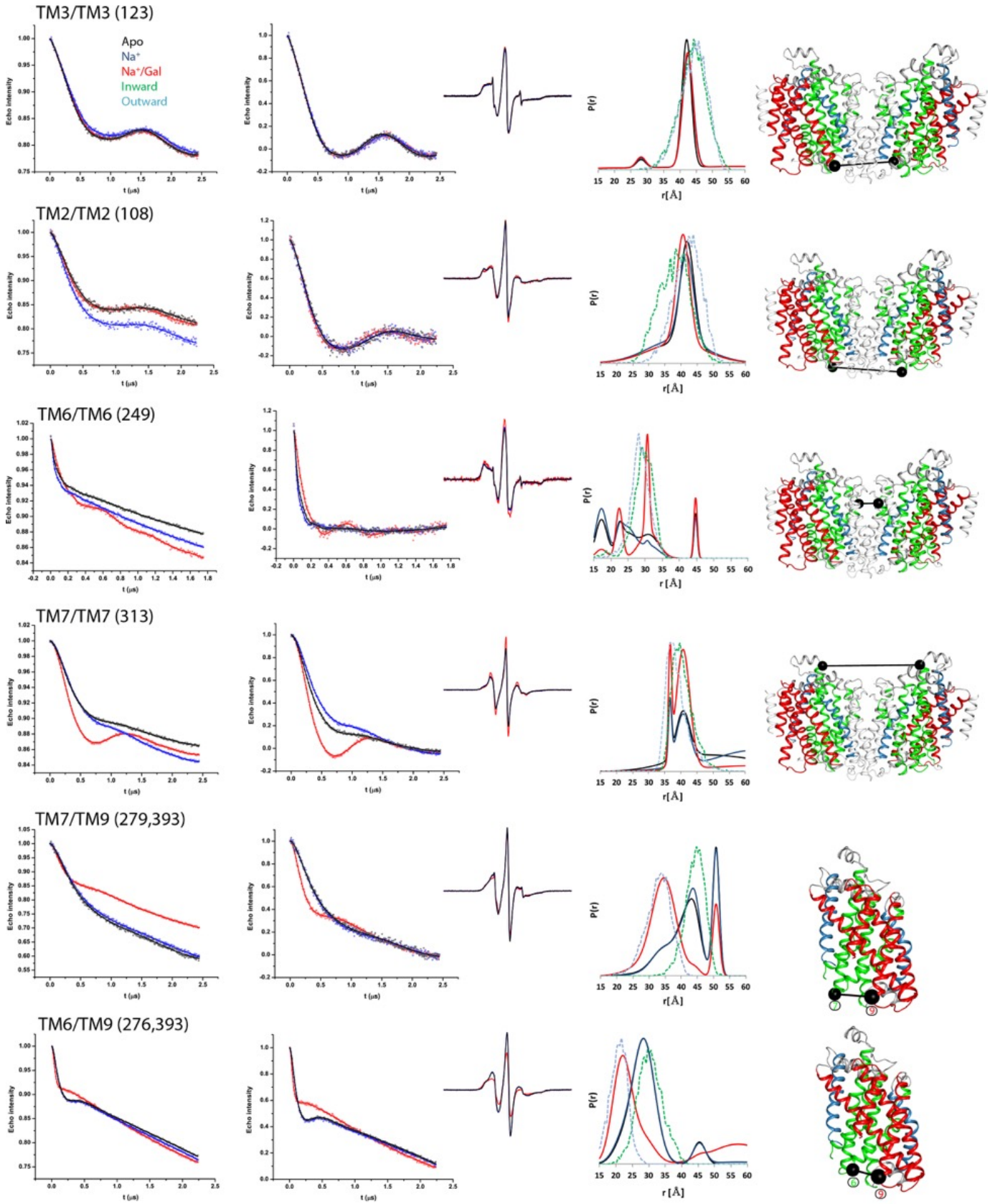
Paz et al. Conformational transitions of the sodium-dependent sugar transporter, vSGLT

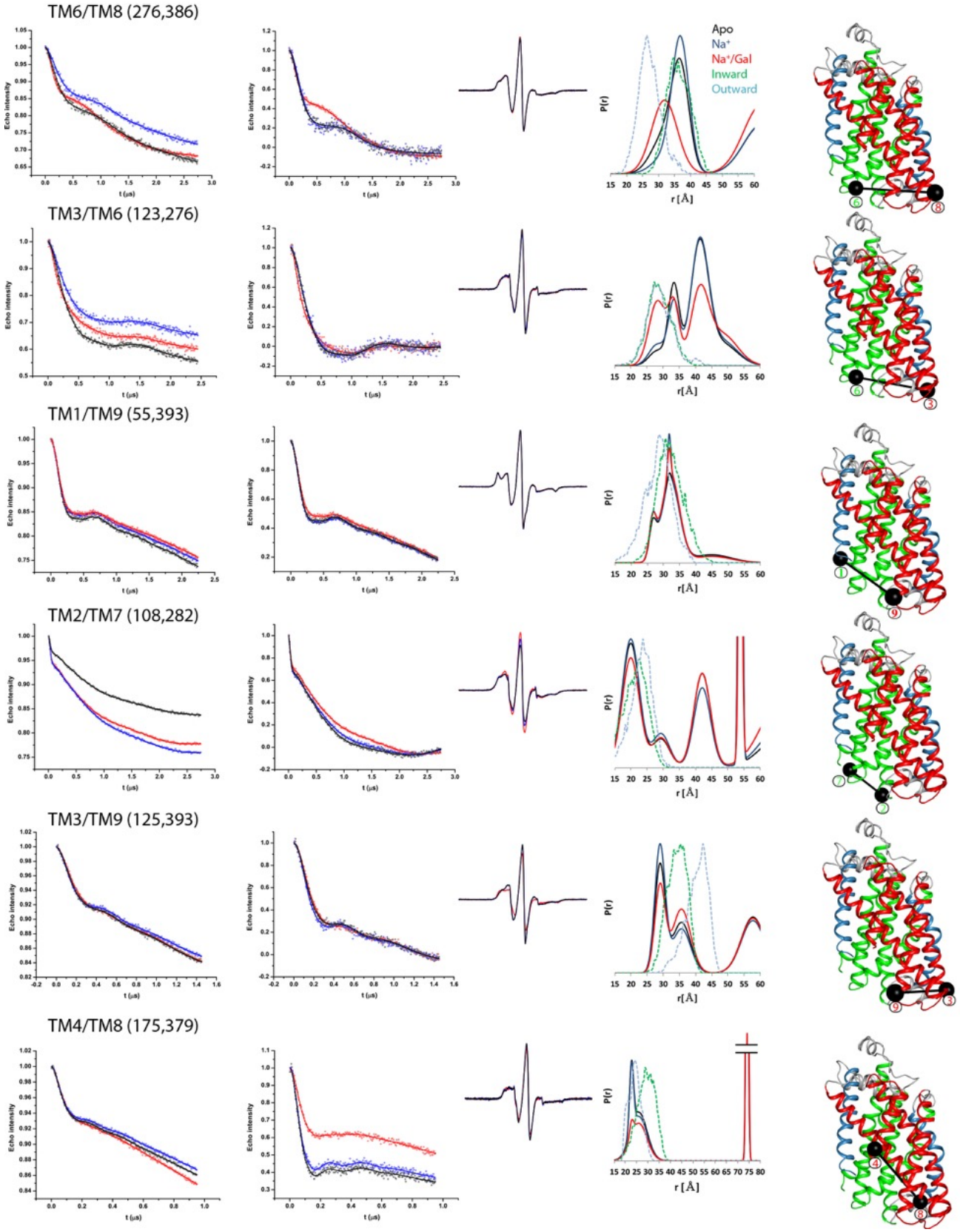


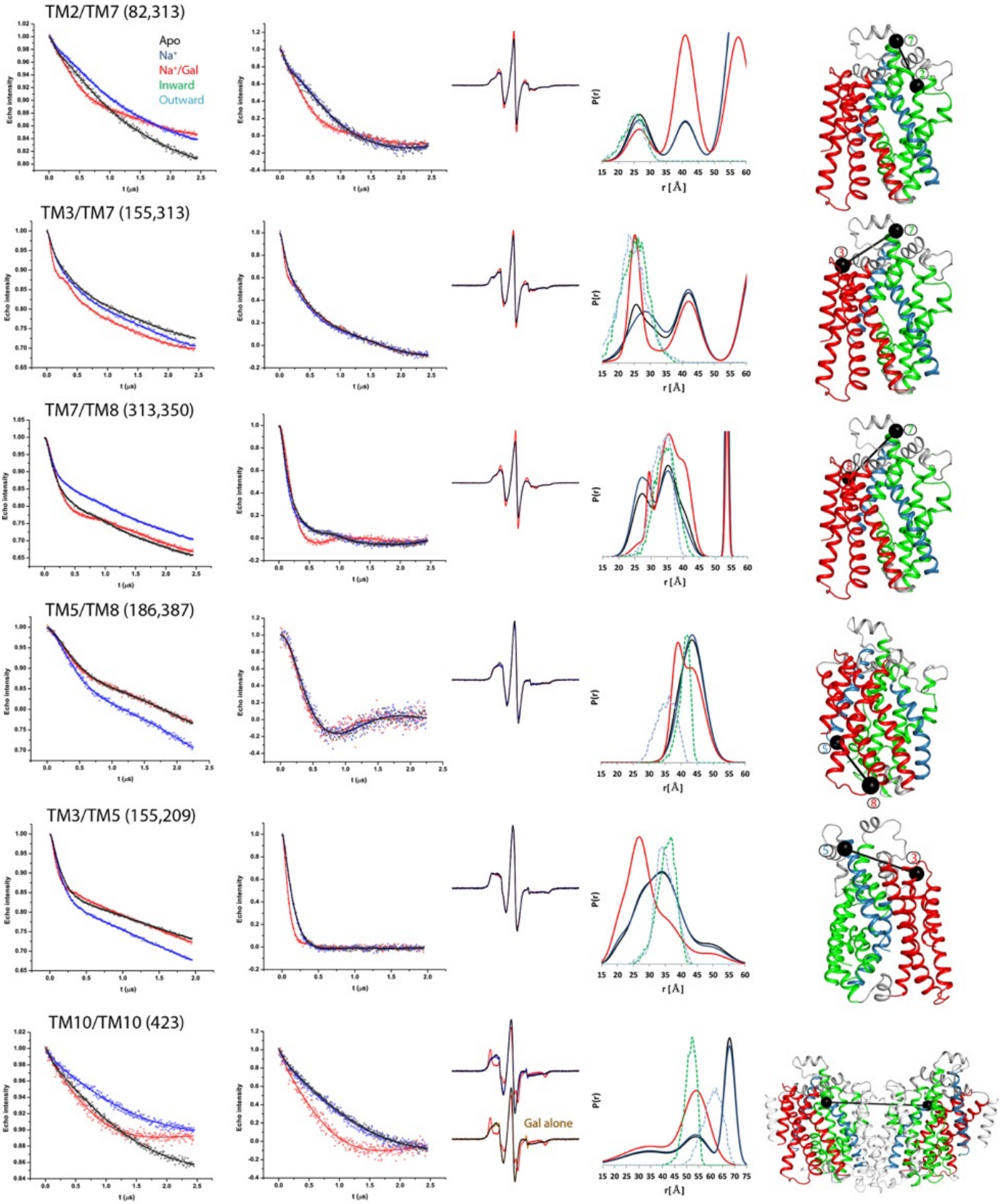
Supplementary Figure S1 A sequence alignment of vSGLT and *Proteus Mirabilis* SiaT that served as the template for modeling the outward-facing conformation of vSGLT. The alignment was generated by Clustal Omega(1, 2) and ESPript 3.0(3) was used for generating this figure. The TMs of vSGLT are noted using the same color scheme as in Fig. 1.

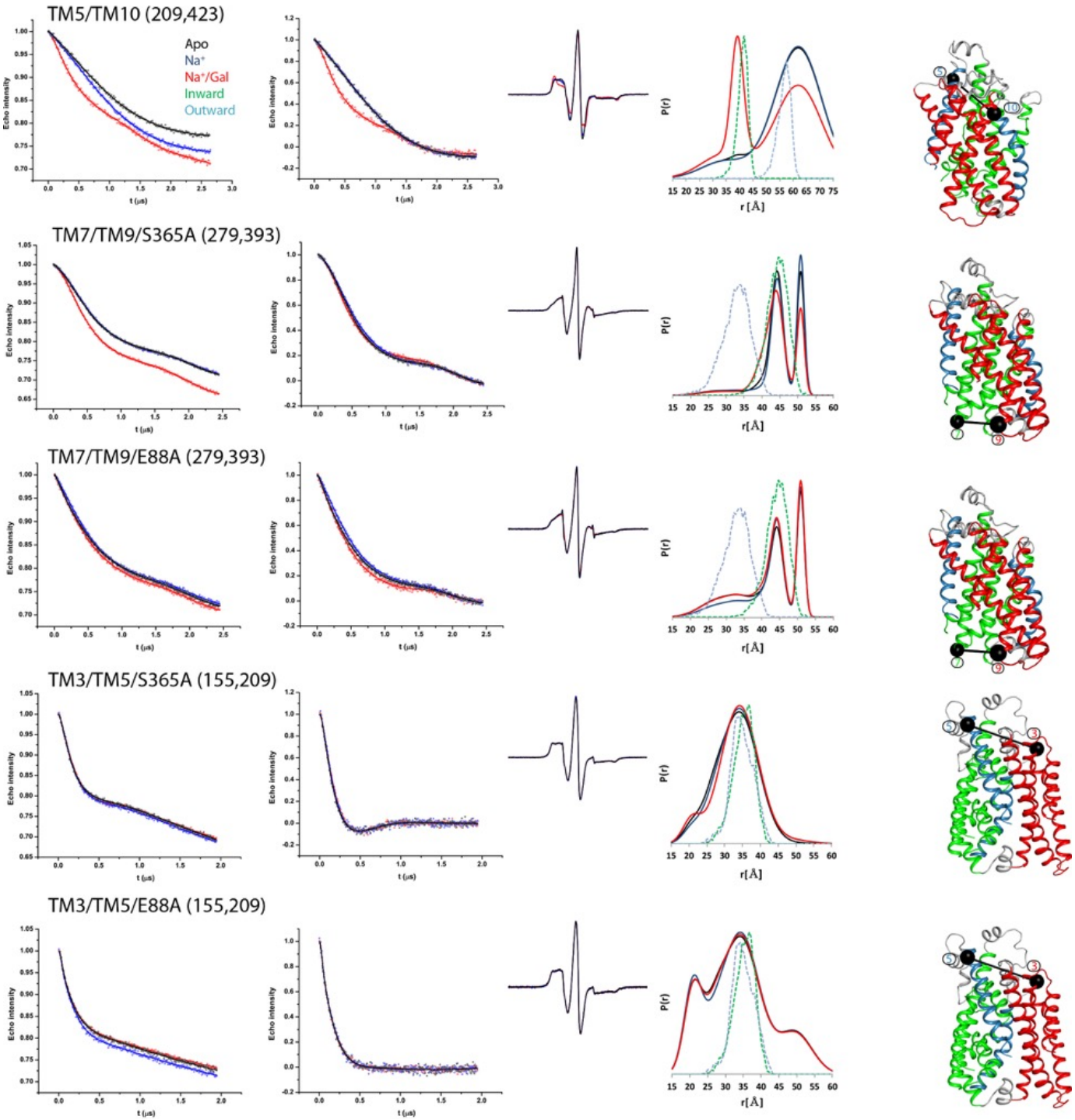


Supplementary Figure S2 D-Galactose uptake for the constructs used in this study. Results are expressed in percentage of uptake compared to the wild-type vSGLT, either in 100mM NaCl (black bars) or in 100mM KCl (white bars) with error bars representing the standard deviation from the mean.

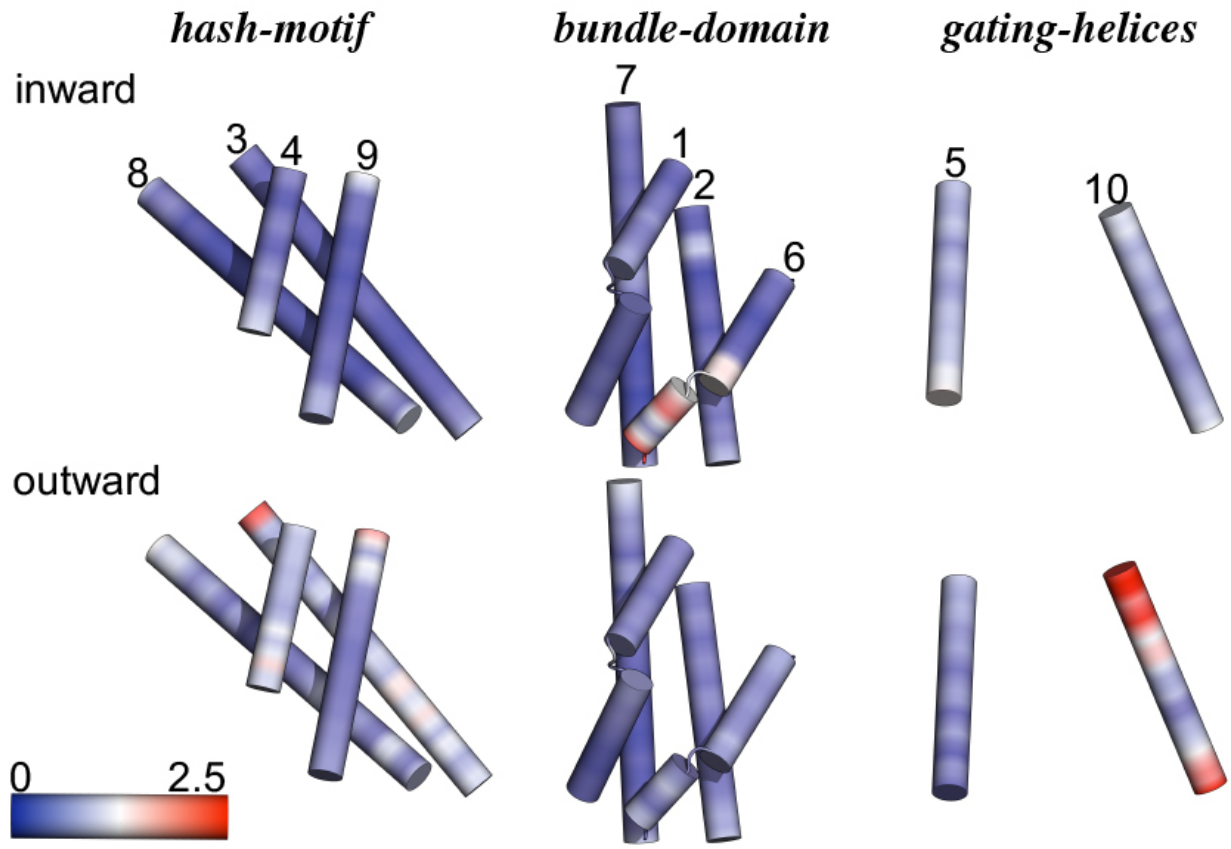

TM3/TM3 (123)
Apo
Na+
Na+/Gal
Inward
Outward
TM2/TM2 (108)
TM6/TM6 (249)
TM7/TM7 (313)
TM7/TM9 (279,393)
TM6/TM9 (276,393)
Echo intensity
t (μs)
P(r)
r[Å]


TM6/TM8 (276,386)
TM3/TM6 (123,276)
TM1/TM9 (55,393)
TM2/TM7 (108,282)
TM3/TM9 (125,393)
TM4/TM8 (175,379)
Apo
Na+
Na+/Gal
Inward
Outward
Echo intensity
t (μs)
P(r)
r [Å]

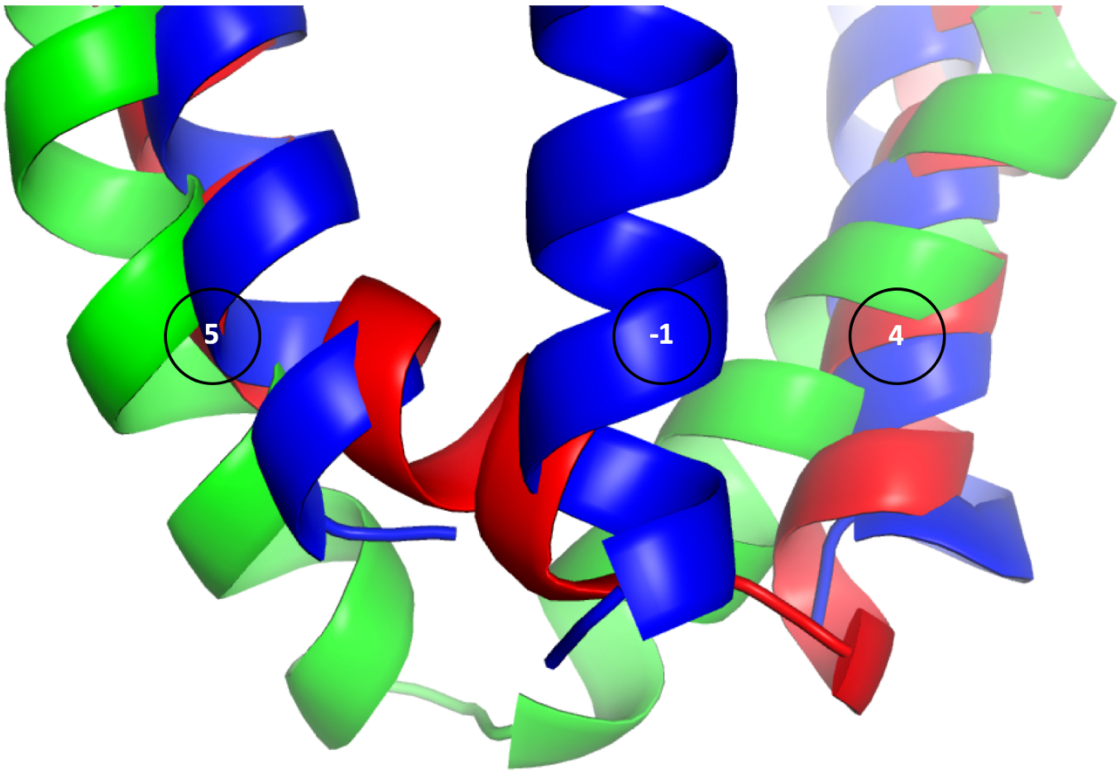

TM2/TM7 (82,313)
Apo
Na+
Na+/Gal
Inward
Outward
TM3/TM7 (155,313)
TM7/TM8 (313,350)
TM5/TM8 (186,387)
TM3/TM5 (155,209)
TM10/TM10 (423)
Gal alone
Echo intensity
t (μs)
P(r)
r [Å]



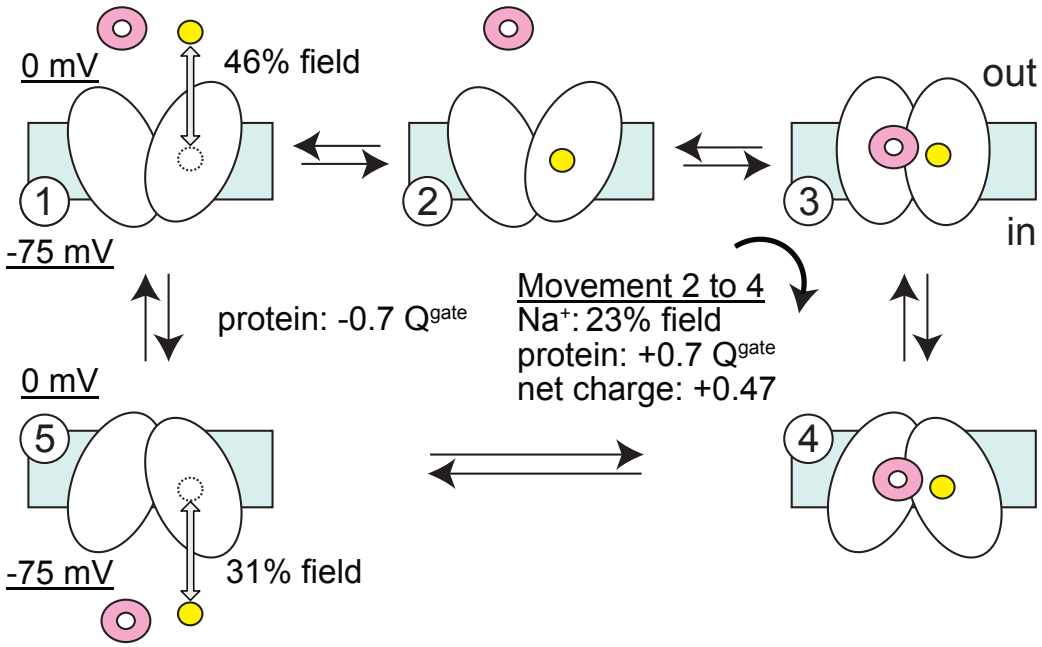
Supplementary Figure S3 DEER raw-data and fits, normalized background-corrected data and fits, CW-EPR lineshapes, distance distributions and a cartoon depiction of the labeling site(s) for each construct.



Supplementary Figure S4 Simulation reveals the state-dependent flexibility of vSGLT domains. MD simulations of the inward-facing structure and the outward-facing model of vSGLT were performed as described in the Methods, and root mean square fluctuation of the Cα atoms are shown as a heat-map projected onto the structure of vSGLT. In both states, the *bundle-domain* is more rigid than the *hash-motif* and *gating-helices*. TM10 displays the highest degree of flexibility for the outward-facing conformation.



Supplementary Figure S5 TM-1 in vSGLT could limit the conformational space available for the internal gate (TM5). A cartoon representation of TM -1, TM4 and TM5 of the inward-open conformation of vSGLT (PDB ID: 2XQ2 in blue) is superimposed on TM4 and TM5 of the inward-open structure of Mhp1 (PDB ID: 2X79, in red) and on TM4 and TM5 of the outward-open structure of Mhp1 (PDB ID: 2JLN, in green). Mhp1 is a 10 TM protein whereas vSGLT is composed of 14 TM. The functional dynamics of the internal gate in vSGLT is limited when compared to the dynamics observed for the same helix in Mhp1. During gating in Mhp1, TM5 opens up towards a position that is not sterically available in vSGLT due to the presence of TM (-1).



Supplementary Figure S6 Kinetic states for vSGLT with charge transfer values indicated.

Supplementary Table 1. 5-state hSGLT1 model.

k_{12} = 50,000 $M^{-2}{\cdot}s^{-1}$	k_{21} = 300 s^{-1}	ε_{12} = 0.3	ε_{21} = -0.3
k_{15} = 600 s^{-1}	k_{51} = 25 s^{-1}	ε_{15} = -0.7	ε_{51} = 0.7
k_{23} = 45,000 $M^{-1}{\cdot}s^{-1}$	k_{32} = 20 s^{-1}	ε_{23} = 0.0	ε_{32} = 0.0
k_{34} = 50 s^{-1}	k_{43} = 50 s^{-1}	ε_{34} = 0.0	ε_{43} = 0.0
k_{45} = 10 s^{-1}	k_{54} = 156,250 $M^{-3}{\cdot}s^{-1}$	ε_{45} = 0.0	ε_{54} = 0.0

Supplementary Table 2. 5-state vSGLT model.

k_{12} = 5,000 $M^{-1}{\cdot}s^{-1}$	k_{21} = 300 s^{-1}	ε_{12} = 0.23	ε_{21} = -0.23
k_{15} = 600 s^{-1}	k_{51} = 25 s^{-1}	ε_{15} = -0.35	ε_{51} = 0.35
k_{23} = 45,000 $M^{-1}{\cdot}s^{-1}$	k_{32} = 20 s^{-1}	ε_{23} = -0.1175	ε_{32} = 0.1175
k_{34} = 50 s^{-1}	k_{43} = 50 s^{-1}	ε_{34} = -0.1175	ε_{43} = 0.1175
k_{45} = 10 s^{-1}	k_{54} = 15,625 $M^{-2}{\cdot}s^{-1}$	ε_{45} = 0.155	ε_{54} = -0.155

Supplementary Table 3. Probability of being in the inward or outward facing states.

state	100 mM Na^+ [out]	100 mM Na^+ [out]	145 mM Na^+ [out]	145 mM Na^+ [out]
	100 mM Na^+ [in]	100 mM Na^+ [in]	10 mM Na^+ [in]	10 mM Na^+ [in]
	V = 0 mV	V = -75 mV	V = 0 mV	V = -75 mV
In (hSGLT1)	90%	4%	84%	2%
Out (hSGLT1)	10%	96%	16%	98%
In (vSGLT1)	90%	30%	88%	23%
Out (vSGLT)	10%	70%	12%	77%

References

1. Goujon M, et al. (2010) A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res* 38(Web Server):W695–W699.
2. Sievers F, et al. (2014) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7(1):539–539.
3. Robert X, Gouet P (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res* 42(W1):W320–W324.