Supporting Information

for

The role of local hydration and hydrogen-bonding dynamics in ion and solute release from ion-coupled secondary transporters

Method S1, GCMC Simulations

Following the equilibrium MD simulations in Step 2.1, a generalized solvent boundary potential (GSBP) system was generated for the occluded LeuT with atoms within 20 Å of the leucine substrate explicitly represented while all other atoms in the system were included implicitly using a GSBP representation (*1*). After the GSBP maps were generated, the reduced systems were minimized and equilibrated for 500 ps. GCMC simulations were then run to stably insert water molecules inside the models (*2*). The area of interest is within 15 Å distance of the leucine substrate. Within the GCMC framework, water molecules are randomly inserted into or deleted from this interested area following a Monte-Carlo algorithm. The new conformation will be either accepted or rejected based on the change in net free energy following insertion or deletion of a water molecule. Each cycle of GCMC protocol consists of 20000 Monte-Carlo steps combined with 20 ps of the Langevin MD simulations to ensure proper relaxation of the side-chains at the position of the insertion/deletion. The convergence of simulations was monitored by the counting of total number of water molecules in the system. 100 cycles of GCMC/MD simulations were performed to ensure saturation with water molecules.

Method S2, Free Energy Perturbation of uncharging of the Na^+ in Na2

The uncharging free energy of Na^+ in Na2 is an approximation of the binding free energy of Na^+ to Na2. The value of Na^+ uncharging from Na2 we got from FEP computation is -97.8 kcal/mol. To obtain the (approximate) binding free energy, the uncharging free energy of Na^+ in water molecules (-91.6 kcal/mol) was subtracted. This results in an approximate binding affinity of -6.2 kcal/mol. which is within the error to the value, ~-5.2 kcal/mol, in ref. (*3*).

The computational protocol for the computation of the uncharging binding free energy is: Following the equilibrium MD simulations in Step 2.1, FEP for Na2 Na^+ uncharging was carried out using the CHARMM (*4*) PERTurb command. Each FEP experiment was run in windowed mode with 50 windows and 100 ps per window, with the thermodynamic coupling parameter λ varying between 0.0 and 1.0 by increments of 0.02. The integration timestep used was 1 fs. The ion is constrained to within 3.8 Å of the 5 coordinating oxygen's using the NOE module in CHARMM with a force constant of 5 kcal/(mol·A^2). This constraint effectively prevents the ion escaping from the site yet shall have only minor impact on the obtained uncharging free energy. The weighted histogram analysis method (*5*) was used to post-process the FEP calculation data. To obtain the uncharging free energy of Na^+ in water, Similar FEP computation was performed for Na^+ solvated in 1125 water molecules.

Fig. S1, The full simulation cell of LeuT membrane transporter embedded in DPPC lipid membrane.
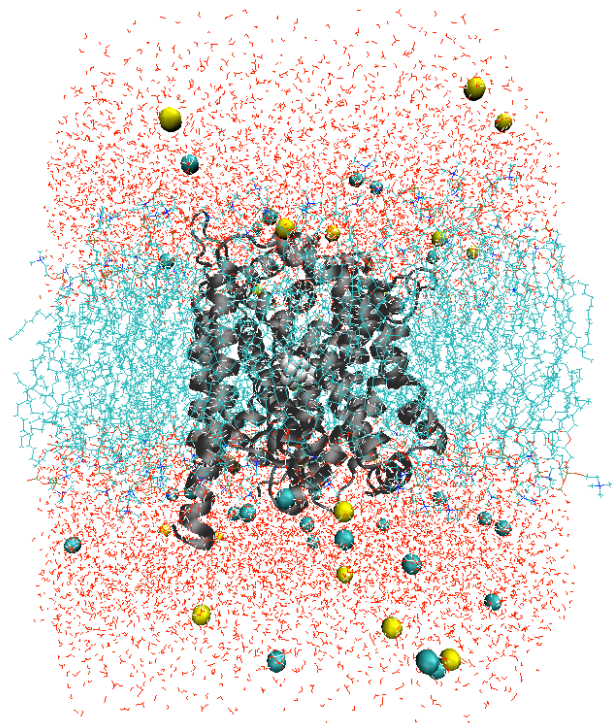

Molecular graphics view of the MD simulation setup of the leucine transporter LeuT. The protein is shown in grey ribbon mode. Ions, including the two Na^+ bound in the protein, and the leucine substrate, are shown in spacefill mode. Water molecules are shown in red lines and DPPC lipid molecules are shown in blue lines. The full simulation box is hexagonal in shape and includes more than 60,000 atoms.

Fig. S2. Water interacting with residues involving in Na1 and substrate binding.

(A)

(B)

(C)

(D)
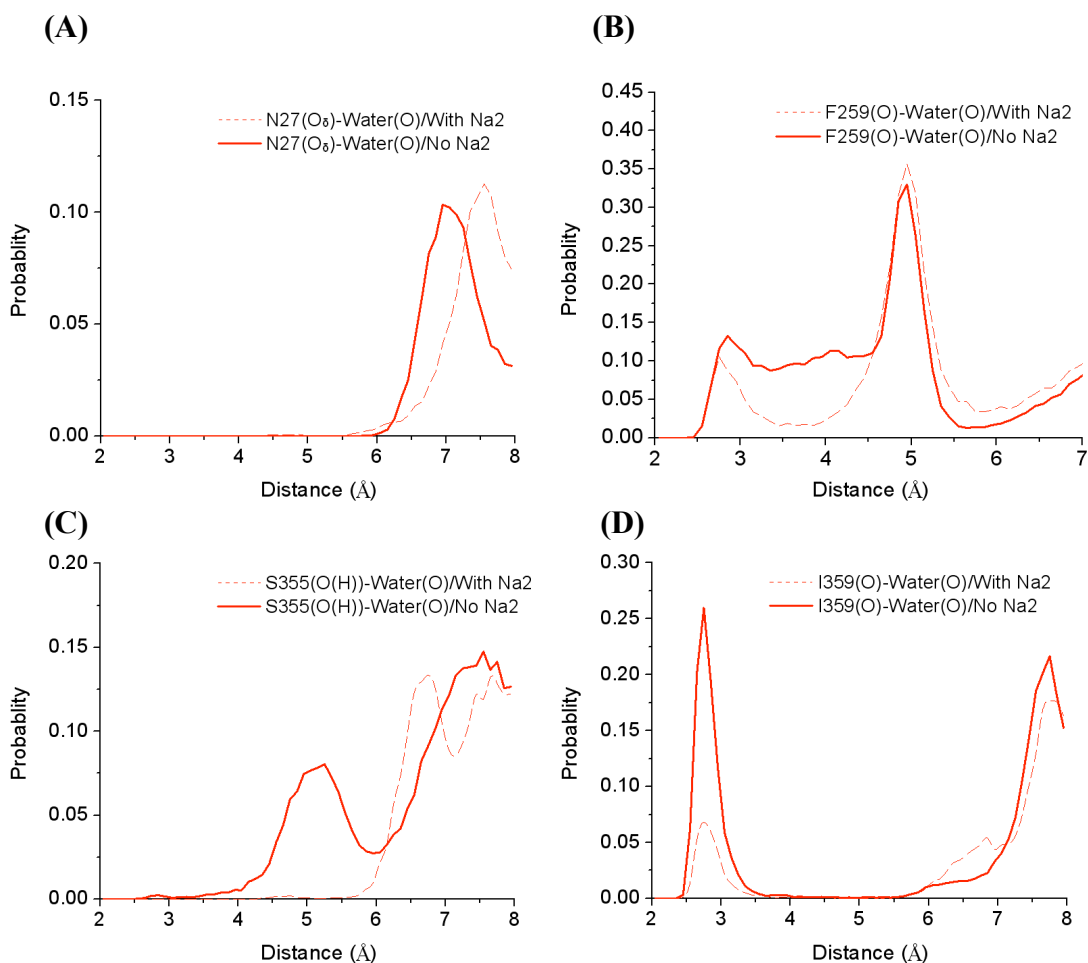
The probability distributions of the distance between the N27 delta oxygen, F359 oxygen, S355 hydroxyl oxygen, I359 oxygen, with oxygen from nearby water molecules are shown for the Na2 bound (dashed) and Na2 free (solid) LeuT in (A), (B), (C), and (D) respectively.

**Fig. S3.** Water interacting with Na2 ion
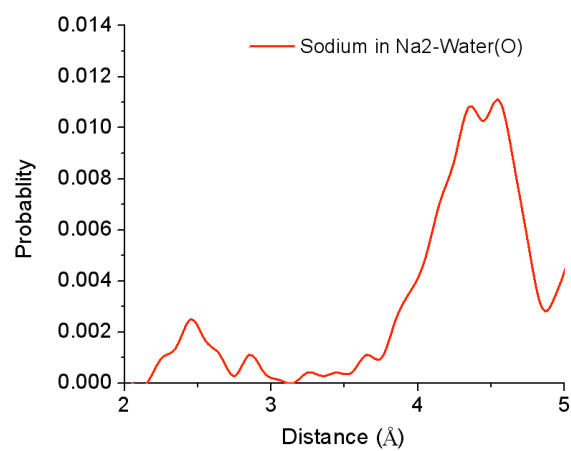
Pair distribution function of the distance between the Na2 ion and oxygen atom from nearby water molecules are plotted for the Na2 bound LeuT.

**Fig. S4.** Reorientation of the N-C$_\alpha$-C$_\beta$-O$_\gamma$ dihedral of T354 of the vacant Na2 binding site.
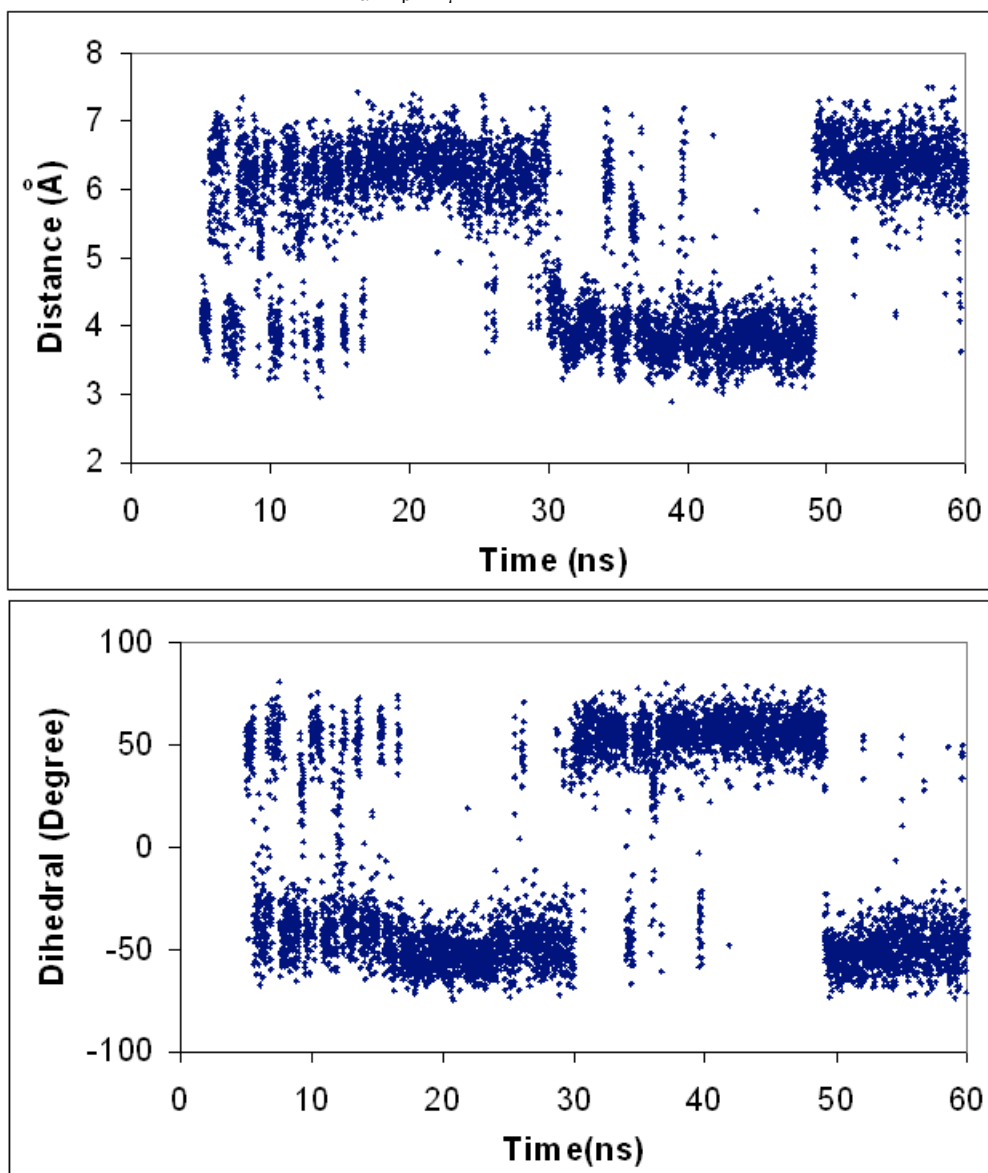
The distance between G20(O) – T354 (hydroxyl O) and the dihedral N-C$_\alpha$-C$_\beta$-O$_\gamma$ of T354 is plotted as a function of simulation time in the top and the bottom panel respectively. Please note that the open (corresponding to a distance around 6 Å in the top panel) and close (corresponding to a distance around 4 Å in the top panel) are correlated with the original (corresponding to a dihedral around 50 degree in the bottom panel) and reoriented (corresponding to a dihedral around -50 degree in the bottom panel) dihedral N-C$_\alpha$-C$_\beta$-O$_\gamma$ of T354.

Fig. S5, Fluctuation of the Na1 site upon removal of Na$^+$ in Na2.
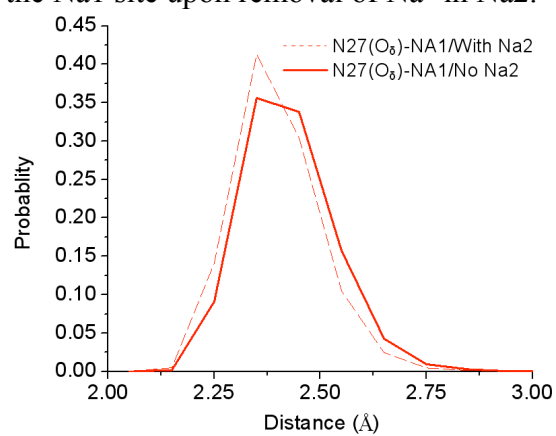
The probability distributions of the distance between N27(O$_\delta$) and Na1 are plotted for Na2 bound (dashed) and Na2 free (solid) LeuT.

Fig. S6, Illustration of the major conformational states of the proposed ion-coupled secondary membrane transporter transporting cycle.
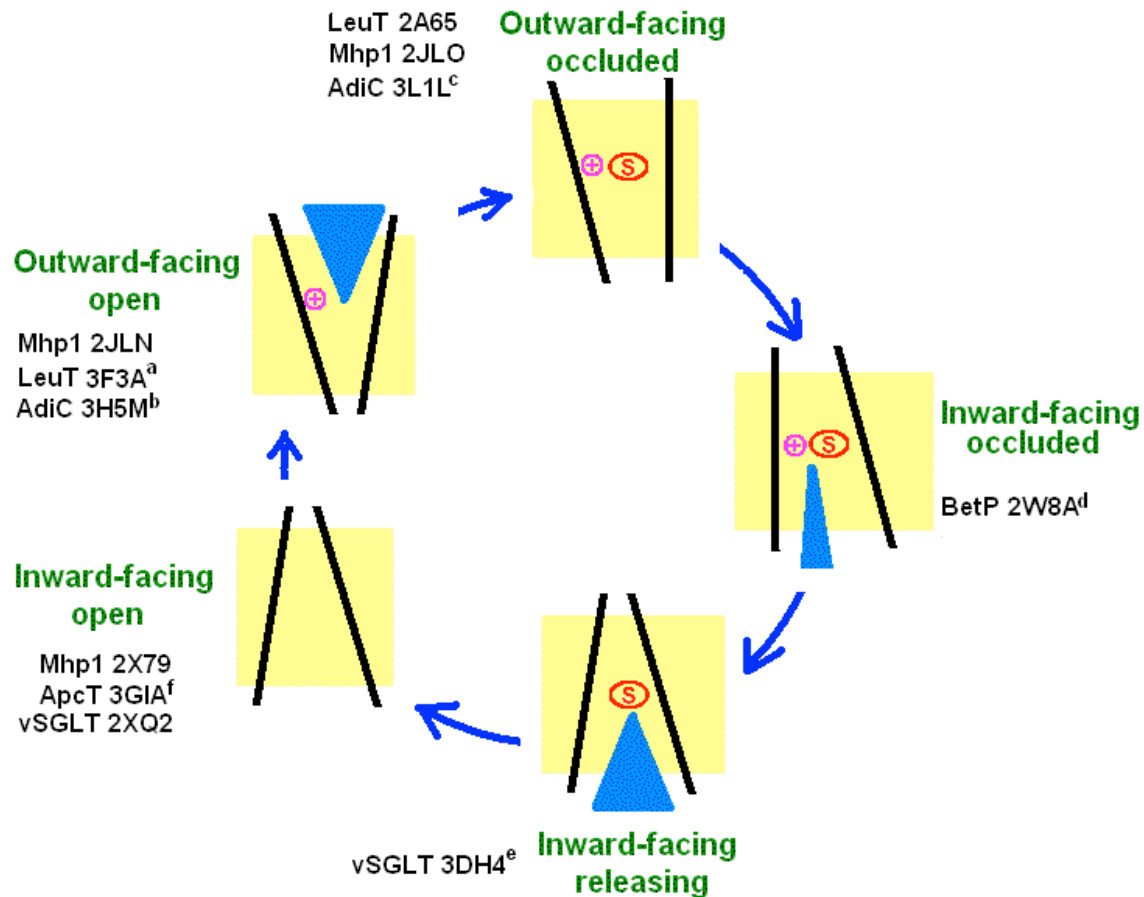
The transporters are simplistically represented by two black sticks. The coupled ion is represented by the magenta cross in a circle while the substrate is represented by an S character in an oval. The current collection of crystal structures (transporter name followed by pdb database entry symbol) that belong to transporters with similar topologies to LeuT are assigned to outward-facing open, outward-facing occluded, inward-facing occluded, inward-facing releasing, and inward-facing open based on their conformational states (*6-15*).

a: The leuT 3F3A structure is locked in an putative outward-facing open state by the binding of an competitive inhibitor tryptophan (*13*).

b,c,f: The AdiC (*10*), and ApcT structures are proton-dependent instead of sodium-dependant. The ApcT 3GIA structure is believed to be in a state of inward-facing without the proton and a substrate and the authors described it as an inward-facing occluded apo structure.

d: The BetP 2W8A is in an occluded state with 2 sodium ions and a betaine substrate bound. Its conformation is relatively inward-facing and is believed to be in between the states of LeuT 2A65 and vSGLT 3DH4 (*9*).

e: The vSGLT 3DH4 structure is inward-facing and has a substrate bound (*14*). The sodium ion might be either being released or released (*6, 16*).

References

1. Im, W., Berneche, S., and Roux, B. (2001) Generalized solvent boundary potential for computer simulations, *Journal of Chemical Physics 114*, 2924-2937.

2. Woo, H. J., Dinner, A. R., and Roux, B. (2004) Grand canonical Monte Carlo simulations of water in protein environments, *J Chem Phys 121*, 6392-6400.

3. Shi, L., Quick, M., Zhao, Y. F., Weinstein, H., and Javitch, J. A. (2008) The mechanism of a neurotransmitter : sodium symporter - Inward release of Na+ and substrate is triggered by substrate in a second binding site, *Molecular Cell 30*, 667-677.

4. Brooks, B. R., Brooks, C. L., Mackerell, A. D., Nilsson, L., Petrella, R. J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A. R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R. W., Post, C. B., Pu, J. Z., Schaefer, M., Tidor, B., Venable, R. M., Woodcock, H. L., Wu, X., Yang, W., York, D. M., and Karplus, M. (2009) CHARMM: The Biomolecular Simulation Program, *Journal of Computational Chemistry 30*, 1545-1614.

5. Kumar, S., Bouzida, D., Swendsen, R. H., Kollman, P. A., and Rosenberg, J. M. (1992) The Weighted Histogram Analysis Method for Free-Energy Calculations on Biomolecules .1. The Method, *Journal of Computational Chemistry 13*, 1011-1021.

6. Watanabe, A., Choe, S., Chaptal, V., Rosenberg, J. M., Wright, E. M., Grabe, M., and Abramson, J. (2010) The mechanism of sodium and substrate release from the binding pocket of vSGLT, *Nature 468*, 988-U162.

7. Shimamura, T., Weyand, S., Beckstein, O., Rutherford, N. G., Hadden, J. M., Sharples, D., Sansom, M. S. P., Iwata, S., Henderson, P. J. F., and Cameron, A. D. (2010) Molecular Basis of Alternating Access Membrane Transport by the Sodium-Hydantoin Transporter Mhp1, *Science 328*, 470-473.

8. Claxton, D. P., Quick, M., Shi, L., de Carvalho, F. D., Weinstein, H., Javitch, J. A., and Mchaourab, H. S. (2010) Ion/substrate-dependent conformational dynamics of a bacterial homolog of neurotransmitter: sodium symporters, *Nature Structural & Molecular Biology 17*, 822-U868.

9. Ressl, S., van Scheltinga, A. C. T., Vonrhein, C., Ott, V., and Ziegler, C. (2009) Molecular basis of transport and regulation in the Na+/betaine symporter BetP, *Nature 458*, 47-U41.

10. Gao, X., Lu, F. R., Zhou, L. J., Dang, S. Y., Sun, L. F., Li, X. C., Wang, J. W., and Shi, Y. G. (2009) Structure and Mechanism of an Amino Acid Antiporter, *Science 324*, 1565-1568.

11. Fang, Y. L., Jayaram, H., Shane, T., Kolmakova-Partensky, L., Wu, F., Williams, C., Xiong, Y., and Miller, C. (2009) Structure of a prokaryotic virtual proton pump at 3.2 angstrom resolution, *Nature 460*, 1040-1043.

12. Weyand, S., Shimamura, T., Yajima, S., Suzuki, S., Mirza, O., Krusong, K., Carpenter, E. P., Rutherford, N. G., Hadden, J. M., O'Reilly, J., Ma, P., Saidijam, M., Patching, S. G., Hope, R. J., Norbertczak, H. T., Roach, P. C. J., Iwata, S.,

Henderson, P. J. F., and Cameron, A. D. (2008) Structure and Molecular Mechanism of a Nucleobase-Cation-Symport-1 Family Transporter, *Science 322*, 709-713.

13. Singh, S. K., Piscitelli, C. L., Yamashita, A., and Gouaux, E. (2008) A Competitive Inhibitor Traps LeuT in an Open-to-Out Conformation, *Science 322*, 1655-1661.

14. Faham, S., Watanabe, A., Besserer, G. M., Cascio, D., Specht, A., Hirayama, B. A., Wright, E. M., and Abramson, J. (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport, *Science 321*, 810-814.

15. Yamashita, A., Singh, S. K., Kawate, T., Jin, Y., and Gouaux, E. (2005) Crystal structure of a bacterial homologue of Na+/Cl--dependent neurotransmitter transporters, *Nature 437*, 215-223.

16. Li, J., and Tajkhorshid, E. (2009) Ion-Releasing State of a Secondary Membrane Transporter, *Biophysical Journal 97*, L29-L31.