Molecular dynamics simulations of Na$^+$ and leucine transport by LeuT

Rong Chen*, Shin-Ho Chung

Research School of Biology, Australian National University, Canberra, ACT 2601, Australia

A B S T R A C T

Molecular dynamics simulations are used to gain insight into the binding of Na$^+$ and leucine substrate to the bacterial amino acid transporter LeuT, focusing on the crystal structures of LeuT in the outward-open and inward-open states. For both conformations of LeuT, a third Na$^+$ binding site involving Glu290 in addition to the two sites identified from the crystal structures is observed. Once the negative charge from Glu290 in the inward-open LeuT is removed, the ion bound to the third site is ejected from LeuT rapidly, suggesting that the protonation state of Glu290 regulates Na$^+$ binding and release. In Cl$^-$-dependent transporters where Glu290 is replaced by a neutral serine, a Cl$^-$ ion would be required to replace the role of Glu290. Thus, the simulations provide insights into understanding Na$^+$ and substrate transport as well as Cl$^-$-independence of LeuT.

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1. Introduction

Neurotransmitters released from the presynaptic terminals in the course of synaptic transmissions must be removed rapidly from the synaptic cleft by transporting them back into cells. This process involves several families of transporters, of which the solute carrier family 6 is one of the largest [1,2]. The leucine transporter (LeuT) from *Aquifex aeolicus* is a bacterial homolog of the Na$^+$-coupled neurotransmitter transporters (NSS) belonging to this family with broad specificity for small hydrophobic amino acids [3]. LeuT is considered as a prototype for studying NSS, owing to their similarities in many characteristics such as sequence, structure and function [4].

Crystal structures of LeuT in three distinct conformations (outward-open, outward-occluded, inward-open) have been reported [3,5]. These structures are consistent with the alternating access model proposed decades ago [3,5,6]. This model posits that the transporter must carry a binding site for the substrate and be able to switch between at least two different conformations, in which the binding site of the substrate is open to either the intracellular or the extracellular space. The structures of LeuT also suggest that LeuT transports two Na$^+$ ions with one substrate molecule in one cycle [3], as the crystal structure of LeuT in an outward-occluded conformation contains two Na$^+$ binding sites (Na1 and Na2) and one substrate binding site (S1) near the center of the protein [3].

The Na1 and Na2 sites are primarily formed by four (Ala22, Asn27, Thr254 and Asn286) and five (Gly20, Val23, Ala351, Thr354 and Ser355) residues, respectively. However, the precise sequence of events leading to the substrate transport remains to be elucidated [7,8].

Molecular dynamics (MD) simulations have been used extensively to understand the mechanism of substrate/ion binding and transport in LeuT [8–17], primarily focusing on the outward-occluded structure [3]. For example, a second substrate binding site (S2) not seen in the crystal structures, formed by residues Arg30 and Asp404, was predicted from MD simulations [8,9] and validated by experiment [8]. In the crystal structure of the outward-facing LeuT, a second tryptophan molecule is indeed seen to bind the S2 site [18]. It is unclear whether the substrate binds to S2 transiently before binding to S1 [19], or the presence of the S2 substrate helps trigger the required conformational changes to the transporter for subsequent substrate and ion release [20]. The outward-open and inward-open structures of LeuT reported more recently are in the apo form and less well-studied [5].

Using the outward-open and inward-open structures of LeuT, we carry out a series of unbiased MD simulations to determine where in the transporter Na$^+$ and leucine stably bind. A sodium ion in the reservoir is consistently attracted to Glu290 in both the outward-open and inward-open LeuT. Once the negative charge of Glu290 in the inward-open LeuT is removed by side chain protonation, the ion is released from LeuT rapidly. Although the importance of Glu290 in LeuT transport and Cl$^-$ dependence was proposed previously [21], the exact mechanism was not obvious

because Glu290 is involved in neither Na1 nor Na2. Our simulations provide a mechanistic understanding of how the changes in the protonation state of Glu290 may regulate the ion and substrate transport of LeuT [22].

2. Methods

2.1. Molecular dynamics simulations

The crystal structures of LeuT in the outward-open (PDB ID 3TT1 [5]) and inward-open (PDB ID 3TT3 [5]) states are considered. The crystal structures represent two mutant forms of LeuT, referred to as LeuT^K(Y108F) and LeuT^K(TSY), respectively [5]. The two mutants are back mutated to the wild type, in which the K288A background mutation is retained as it enhances substrate transport [23]. The conformation of the ten residues in the N-terminus missing in the inward-open structure is modeled using the SWISS-MODEL homology modeling server [24]. LeuT in either the outward-open or the inward-open conformation is placed in the middle of a 1-palmitoyl-2-oleoyl-*sn*-glycerol-phosphocholine bilayer. Approximately 15,800 water molecules, 60 Na$^+$ and 60 Cl$^-$ are added, corresponding to a salt concentration of 0.2 M. Two additional Cl$^-$ are added to maintain charge neutrality. The resulting system is ~90 × 90 × 90 Å^3 in size in all cases. Unless otherwise stated, the side chains of all glutamate and aspartate residues are assumed to be in the deprotonated form. Details of our simulation parameters are given in the Supplementary Material.

3. Results

3.1. Na1q in the outward-open LeuT

The crystal structure of LeuT in the outward-open state reveals two Na$^+$ binding sites, Na1 and Na2, similar to that in the outward-occluded state [3,5]. To verify if our simulations are able to reproduce the underlying physics governing the interactions between ions and LeuT, we perform two independent equilibrium simulations on the outward-open LeuT in the presence of two Na$^+$ ions at Na1 and Na2 in the starting configuration. The interactions between the two ions and their binding sites remain unchanged after 50 ns in both simulations (Fig. S1), indicating that the two ion binding sites revealed from the crystal structure are indeed stable.

Having shown that the simulations are able to reproduce some basic characteristics of the crystal structures, we carry out unbiased simulations to identify potential additional Na$^+$ binding site in the outward-open LeuT. Specifically, we perform unbiased simulations in the absence of Na1 and Na2 ions. All Na$^+$ ions are placed in the bulk and allowed to bind LeuT spontaneously.

In the two unbiased simulations of 200 ns each, two Na$^+$ ions are attracted to the central cavity of LeuT. In the outward-open LeuT, two acidic residues, Glu112 and Asp404, are located at the extracellular end of LeuT (Fig. 1A). These two residues may be involved in generating a negative electrostatic field for Na$^+$ ions. One Na$^+$ ion consistently binds to Glu290 (Fig. 1B), referred to as the Na1q site, at 165 ns and 75 ns of the two simulations, respectively. The other Na$^+$ ion binds to Ser355, which is involved in the Na2 site (Fig. 1B). This ion is also within 5 Å to the backbone oxygen atoms of Gly20 and Val23, two other residues of the Na2 site. The binding of the ion to Ser355 is stable, as the distance between the ion and Ser355 is predominantly less than 3 Å once bound (Fig. 1C). The simulations predict that the Na1q binding site is present in the outward-open LeuT.

3.2. Effect of protonation on Na$^+$ binding

The protonation state of acidic residues are not only important for generating electrostatic field for Na$^+$ ions, but also may be important for the conformational changes of LeuT [25]. Previous pKa calculations predicted that the side chain of Glu290 assumes different protonation states in the outward-open and inward-open LeuT [22]. In the outward-open LeuT the side chain of Glu290 is likely deprotonated and charged, with a predicted pKa of 5.4. However, the side chains of three residues (Glu112, Glu287 and Glu419) in the outward-occluded LeuT have been predicted to be protonated [9,21]. Our calculations employing the PROPKA program [26,27] suggest that Glu112 (pKa 7.6), Glu287 (pKa 7.6) and Glu419 (pKa 12.6) in the outward-open LeuT should also be predominantly protonated at a neutral pH. However, the pKa values predicted from those empirical methods could be overestimated, since accurate prediction of pKa is challenging even with sophisticated methods [28,29]. For example, the desolvation effect, which is considered as a major contributor to the pKa shift in PROPKA [27], is difficult to predict accurately. The protonation states of those glutamate residues thus remain to be re-examined with other methods. Here, we examine how neutralizing Glu112, which is in close proximity to Na1 and Na2 (Fig. 1A), would affect Na$^+$ binding to the outward-open LeuT.

The starting configuration is generated from that shown in Fig. 1B, but with the side chain of Glu112 protonated. The system is equilibrated twice for 50 ns each. In both simulations the ions bound to Na1q and Na2 remain stable, indicating that the extent to which the protonation state of Glu112 may affect Na$^+$ binding is limited on the time scale of our simulations. The side chain of Glu112 is hydrated by several water molecules when it is deprotonated (Fig. S2). Once the side chain of this residue is protonated, the hydration number reduces to one, as the addition of a proton causes the side chain to move to an inward position and form two hydrogen bonds with Phe349 (Fig. S2). This inward motion appears to be inconsistent with the crystal structure, in which the hydrogen bonding between Glu112 and Phe349 is not evident [5]. Therefore, it is possible that Glu112 is charged, which would help generate a negative electrostatic field for the initial binding of Na$^+$ to LeuT. Similarly, we find that the side chain of Glu287 in the deprotonated form is also fully hydrated in the outward-open LeuT (Fig. S2). As the same Na1q site was predicted from simulations with Glu287 in the protonated form [25], it can be concluded that the protonation state of Glu112 and Glu287 has limited effect on Na$^+$ binding.

3.3. Binding of leucine to the outward-open LeuT

The structure of the outward-open LeuT was crystallized in the absence of a leucine substrate [5]. Our next aim is to examine the substrate binding of the outward-open LeuT using MD simulations. To save the computing resources required for the initial binding of substrate to the transporter, we place a leucine substrate inside LeuT, at a position approximately corresponding to that in the outward-occluded state [3]. The complete unbinding of leucine from LeuT observed in our simulations reveals a secondary substrate binding site at the extracellular end of the transporter, consistent with previous experimental and computational observations [8].

A leucine molecule is placed above the side chains of Phe259 and Val104, with its head group interacting with several residues in close proximity to Ser355. Two Na$^+$ ions are placed at the Na1 and Na2 sites. Three simulations each on a time scale of 200 ns are performed. In two of the simulations the position of the substrate remains unchanged, but in the third simulation a complete unbinding of the substrate from LeuT is observed. First the substrate
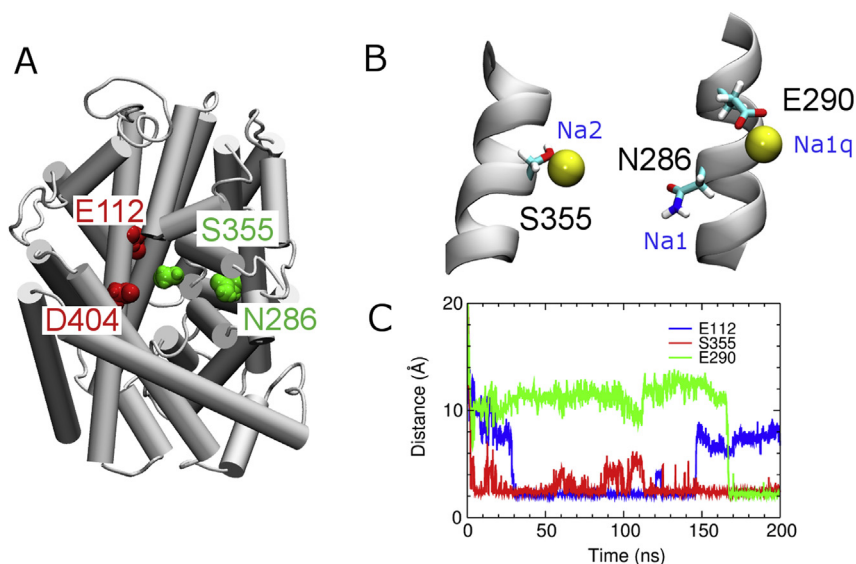
Fig. 1. Spontaneous binding of Na$^+$ ions to LeuT. In (A), four residues lining the wall of the central cavity of the outward-open LeuT are highlighted. The view is from the extracellular side approximately along the bilayer normal. In (B), the position of two Na$^+$ ions (yellow spheres) relative to three Na$^+$ binding sites (Na1, Na2, and Na1q) of LeuT predicted from MD is depicted. In (C), the time evolution of the distances between three Na$^+$ ions and three residues of LeuT is shown for one simulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

moves outward and binds to a ring of charged and hydrophobic residues near the extracellular end of LeuT. There the amine group of leucine interacts favorably with Asp404, whereas the side chain and carboxyl group of the substrate interacts intimately with Arg30, Ile111 and Leu400 (Fig. S3). The substrate remains bound to this ring over a period of 120 ns, before being completely ejected to the extracellular space. The transient binding of leucine to Arg30 and Asp404 observed here is consistent with previous simulations on the outward-occluded LeuT [8].

3.4. Binding of Na$^+$ to the inward-open LeuT

The Na1 and Na2 sites of the outward-open LeuT are significantly perturbed in the inward-open LeuT [5], due to structural rearrangements in the core region of the transporter (TM1 and TM6). To understand ion binding of the inward-open LeuT, two unbiased simulations are performed. Na$^+$ ions corresponding to a concentration of 0.2 M are placed in the reservoir at the start of the simulation and allowed to bind LeuT spontaneously.

Two Na$^+$ binding sites, referred to as Na1q′ and Na2′, are identified from the two simulations consistently (Fig. 2A). Na1q′ is in a small cavity between the side chains of Tyr47 and Glu290. We note that Glu290 forms the Na1q site in the outward-open LeuT (Fig. 1B). When superimposed, the Na1q′ and Na2′ sites align well with the Na1q and Na2 sites of the outward-open LeuT (Fig. 2B). The binding of the ion to Na1q′ is highly stable. For example, the distance between the ion and Glu290 is almost constant at 2 Å once the ion is bound (Fig. S4). Na2′ is in a more inward position, coordinated by the side chain of Ser355, which is involved in forming Na2 in the outward-open LeuT. Na2′ is much less stable and the interactions between the ion and Ser355 break constantly (Fig. S4), suggesting that the Na2′ ion could be more readily released from LeuT during a transport cycle.

To ascertain the effect of Glu290 protonation on Na$^+$ binding to the Na1q′ site, we extend the simulation for 100 ns, but with the side chain of Glu290 protonated. The Na1q′ ion is ejected within 50 ns (Fig. 2C), indicating that the binding and release of Na$^+$ to Na1q′ is regulated by the protonation state of Glu290, in support of an earlier proposal [22].

The simulations show that the residues lining the Na$^+$ binding sites are similar between the outward-open (Na1q and Na2) and inward-open (Na1q′ and Na2′) states of LeuT (Figs. 1 and 2). Although the Na2 site is significantly disrupted in the inward-open LeuT due to TM1 and TM6 movement, the overall position of the Na2 ion relative to the protein remains largely unchanged (Fig. 2B). The minimal movement of Na1 and Na2 ions required during the conformational change of LeuT predicted from our simulations is plausible from a mechanistic point of view. A smaller motion of Na$^+$ would mean that the transport is more efficient, especially in the core of the transporter where the hydration is lower than optimal.

3.5. Binding of leucine to the inward-open LeuT

To examine the binding mode of leucine to the inward-open LeuT, we place a leucine molecule in the middle of the central cavity of LeuT, formed by four residues: Ala22, Asn21, Ser256 and Thr254. This cavity is open to the intracellular side and was shown to bind leucine tightly in previous simulations performed on the outward-occluded LeuT [9]. Two Na$^+$ ions are present at the Na1q′ and Na2′ sites, respectively, as predicted from our simulations in the absence of substrate. The system is then simulated extensively twice for 200 ns in each run.

LeuT is highly rigid in the absence of any restraint, as the maximum RMSD of the backbone with reference to the crystal structure is less than 2 Å over the course of both simulations. In one simulation the substrate changes to a deeper binding mode, and its amine and carboxyl groups now interact favorably with the Na2′ ion and LeuT (Fig. 3). The side chain of the substrate is orientated toward the intracellular side, consistent with the binding mode in the outward-open LeuT. In the other simulation a complete release of the Na2′ ion from LeuT is observed at 120 ns (Fig. S5). The release of the ion is decoupled from that of the substrate, as the substrate remains bound to the transporter at the end of the simulation (Fig. S5), suggesting that the Na2′ ion is released before the substrate. The release of the Na2′ ion observed here is consistent with previous experiment [30] and simulations [17,31], which suggest that the Na2/Na2′ site is water-accessible in both the outward-facing and inward-facing conformations.
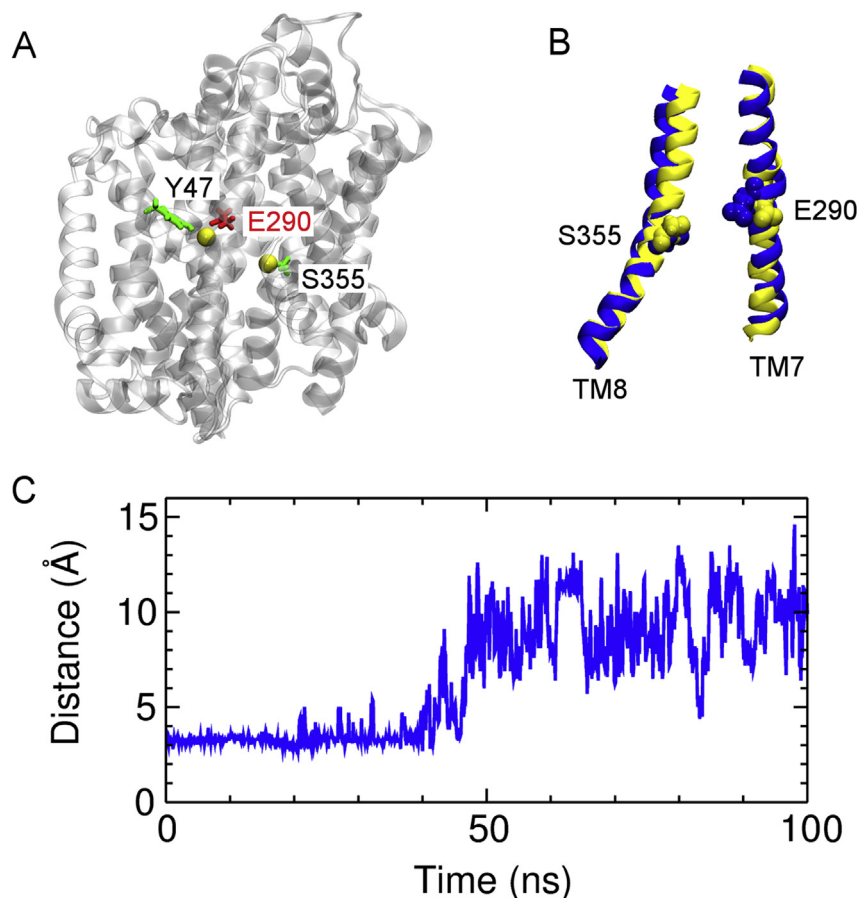
Fig. 2. Binding of Na$^+$ to the inward-open LeuT in the absence of a leucine substrate. (A) The position of two Na$^+$ ions (yellow spheres) relative to Na1′q (Tyr47 and Glu290) and Na2′ (Ser355) predicted from simulations. (B) Superimposition of the TM7 and TM8 helices of the outward-open (yellow) and inward-open (blue) LeuT. (C) The distance between the Na$^+$ ion and the side chain oxygen atoms of Glu290 as a function of simulation time after the side chain of Glu290 is protonated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
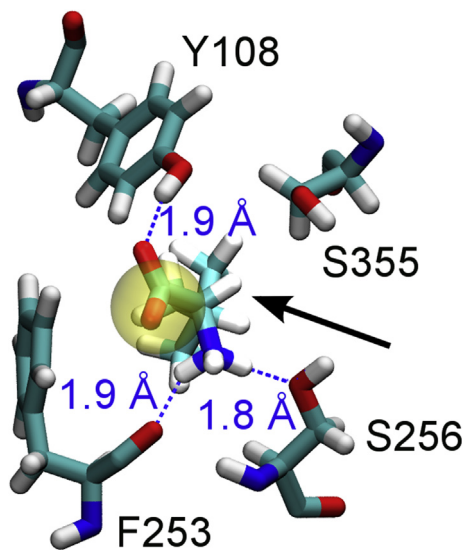

Fig. 3. The position of a leucine substrate (arrow) relative to four residues of the inward-open LeuT and the Na2′ ion (transparent yellow sphere) predicted from MD simulations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

MD simulations are used to understand the binding of Na$^+$ and leucine substrate to the recently solved structures of LeuT in the outward-open and inward-open states. A third Na$^+$ binding site involving Glu290, referred to as Na1q for the outward-open and Na1q′ for the inward-open LeuT, is identified. The protonation of Glu290 causes inward release of Na$^+$, indicating that Glu290 is important for the transport mechanism of LeuT.

Our simulations together with available theoretical and experimental data suggest a complete transport cycle of LeuT (Fig. 4). Initially two Na$^+$ ions bind to the Na1q and Na2 sites (Fig. 1B) and one leucine substrate binds to the S2 site, primarily formed by Asp404 and Arg30 in the outward-open LeuT. Then the transporter transforms to an outward-occluded state, preventing the ions and substrate from moving back to the extracellular space. During this transformation the Na1q ion moves to the Na1 site (PDB ID 2A65 [3]) and the substrate to its inner primary binding site. The transporter then switches to an inward-occluded state with the position of the two ions unchanged (PDB ID 4US3 [30]). Subsequently the transporter transforms to an inward-open state and the Na1 ion moves to the Na1q′ site (Fig. 2A). The Na2′ ion interacts with the inward-open LeuT rather weakly, and thus is released to the intracellular space, followed by the release of the substrate. The Na1q′ ion is finally released once the side chain of Glu290 becomes protonated and neutral. The transport cycle is now complete and LeuT switches to an outward-open state to start a new cycle. Our
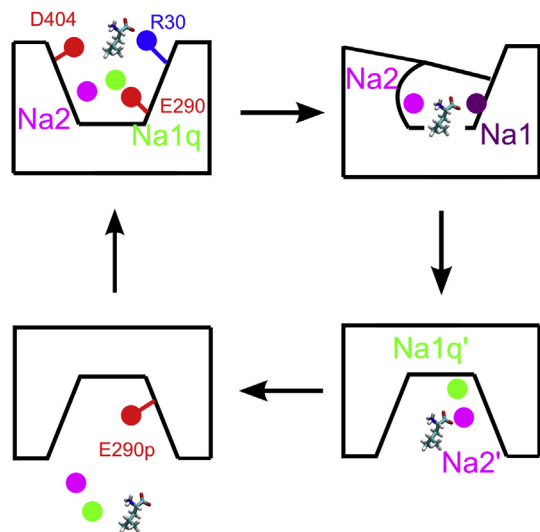
Fig. 4. Schematic of the transport cycle of LeuT.

model does not take into account the possibility that a second substrate is involved in transport [20], but the key role of Glu290 in Na^+ binding and release revealed from our simulations is likely to be valid.

The transport of Na^+ and substrate by LeuT is independent of Cl^- [3]. In contrast, some other NSS isoforms, such as the GABA transporter GAT-1 [32], are Cl^--dependent. Glu290 is replaced by a neutral serine in GAT-1, and was found to be important for Cl^--independence of LeuT from mutagenesis experiments [21]. Our simulations suggest that in Cl^--dependent transporters Cl^- would be required to replace the negative charge carried by Glu290 in the outward-facing conformation necessary for the formation of the Na1q site. The protonation of Glu290 in the inward-open LeuT would be equivalent to the release of Cl^- in GAT-1 to the intracellular space. Thus, the simulations provide structural basis for understanding Na^+ and substrate transport as well as Cl^- dependence/independence in LeuT, GAT-1 and some other NSS isoforms.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.143.

Transparency document

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References

[1] S. Bröer, U. Gether, The solute carrier 6 family of transporters, Br. J. Pharmacol. 167 (2012) 256–278.

[2] P.J. Focke, X. Wang, H.P. Larsson, Neurotransmitter transporters: structure meets function, Structure 21 (2013) 694–705.

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

[4] B.I. Kanner, E. Zomot, Sodium-coupled neurotransmitter transporters, Chem. Rev. 108 (2008) 1654–1668.

[5] H. Krishnamurthy, E. Gouaux, X-ray structures of LeuT in substrate-free outward-open and apo inward-open states, Nature 481 (2012) 469–474.

[6] O. Jardetzky, Simple allosteric model for membrane pumps, Nature 211 (1966) 969–970.

[7] K. Kazmier, S. Sharma, M. Quick, S.M. Islam, B. Roux, H. Weinstein, J.A. Javitch, H.S. McHaourab, Conformational dynamics of ligand-dependent alternating access in LeuT, Nat. Struct. Mol. Biol. 21 (2014) 472–479.

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

[9] L. Celik, B. Schiott, E. Tajkhorshid, Substrate binding and formation of an occluded state in the leucine transporter, Biophys. J. 94 (2008) 1600–1612.

[10] S.Y. Noskov, B. Roux, Control of ion selectivity in LeuT: two Na^+ binding sites with two different mechanisms, J. Mol. Biol. 377 (2008) 804–818.

[11] C. Zhao, S.Y. Noskov, The molecular mechanism of ion-dependent gating in secondary transporters, PLoS Comput. Biol. 9 (2013) e1003296.

[12] M.H. Cheng, I. Bahar, Complete mapping of substrate translocation highlights the role of LeuT N-terminal segment in regulating transport cycle, PLoS Comput. Biol. 10 (2014) e1003879.

[13] M.H. Cheng, I. Bahar, Coupled global and local changes direct substrate translocation by neurotransmitter-sodium symporter ortholog LeuT, Biophys. J. 105 (2013) 630–639.

[14] S.A. Shaikh, J. Li, G. Enkavi, P.C. Wen, Z. Huang, E. Tajkhorshid, Visualizing functional motions of membrane transporters with molecular dynamics simulations, Biochemistry 52 (2013) 569–587.

[15] J. Li, S.A. Shaikh, G. Enkavi, P.C. Wen, Z. Huang, E. Tajkhorshid, Transient formation of water-conducting states in membrane transporters, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 7696–7701.

[16] J. Shan, J.A. Javitch, L. Shi, H. Weinstein, The substrate-driven transition to an inward-facing conformation in the functional mechanism of the dopamine transporter, PLoS One 6 (2011) e16350.

[17] J. Grouleff, S. Søndergaard, H. Koldsø, B. Schiøtt, Properties of an inward-facing state of LeuT: Conformational stability and substrate release, Biophys. J. 108 (2015) 1390–1399.

[18] S.K. Singh, C.L. Piscitelli, A. Yamashita, E. Gouaux, A competitive inhibitor traps LeuT in an open-to-out conformation, Science 322 (2008) 1655–1661.

[19] H. Krishnamurthy, C.L. Piscitelli, E. Gouaux, Unlocking the molecular secrets of sodium-coupled transporters, Nature 459 (2009) 347–355.

[20] M. Quick, A.M. Winther, L. Shi, P. Nissen, H. Weinstein, J.A. Javitch, Binding of an octylglucoside detergent molecule in the second substrate (S2) site of LeuT establishes an inhibitor-bound conformation, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5563–5568.

[21] L.R. Forrest, S. Tavoulari, Y.W. Zhang, G. Rudnick, B. Honig, Identification of a chloride ion binding site in Na+/Cl -dependent transporters, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12761–12766.

[22] Y. Zhao, M. Quick, L. Shi, E.L. Mehler, H. Weinstein, J.A. Javitch, Substrate-dependent proton antiport in neurotransmitter:sodium symporters, Nat. Chem. Biol. 6 (2010) 109–116.

[23] C.L. Piscitelli, H. Krishnamurthy, E. Gouaux, Neurotransmitter/sodium symporter orthologue LeuT has a single high-affinity substrate site, Nature 468 (2010) 1129–1132.

[24] K. Arnold, L. Bordoli, J. Kopp, T. Schwede, The SWISS-MODEL workspace: a web-based environment for protein structure homology modeling, Bioinformatics 22 (2006) 195–201.

[25] C. Zhao, S. Stolzenberg, L. Gracia, H. Weinstein, S. Noskov, L. Shi, Ion-controlled conformational dynamics in the outward-open transition from an occluded state of LeuT, Biophys. J. 103 (2012) 878–888.

[26] M.H.M. Olsson, C.R. Søndergard, M. Rostkowski, J.H. Jensen, PROPKA3: consistent treatment of internal and surface residues in empirical pKa predictions, J. Chem. Theory Comput. 7 (2011) 525–537.

[27] H. Li, A.D. Robertson, J.H. Jensen, Very fast empirical prediction and rationalization of protein pKa values, Proteins 61 (2005) 704–721.

[28] J. Ho, M.L. Coote, pKa calculation of some biologically important carbon acids – an assessment of contemporary theoretical procedures, J. Chem. Theory Comput. 5 (2009) 295–306.

[29] J. Ho, M.L. Coote, A universal approach for continuum solvent pKa calculations: are we there yet? J. Chem. Theory Comput. 125 (2010) 3–21.

[30] L. Malinauskaite, M. Quick, L. Reinhard, J.A. Lyons, H. Yano, J.A. Javitch, P. Nissen, A mechanism for intracellular release of Na^+ by neurotransmitter/sodium symporters, Nat. Struct. Mol. Biol. 21 (2014) 1006–1012.

[31] C. Zhao, S.Y. Noskov, The role of local hydration and hydrogen-bonding dynamics in ion and solute release from ion-coupled secondary transporters, Biochemistry 50 (2011) 1848–1856.

[32] E. Zomot, A. Bendahan, M. Quick, Y. Zhao, J.A. Javitch, B.I. Kanner, Mechanism of chloride interaction with neurotransmitter:sodium symporters, Nature 449 (2007) 726–730.