TITLE: Principles of Alternating Access in LeuT-fold Transporters: Commonalities and Divergences

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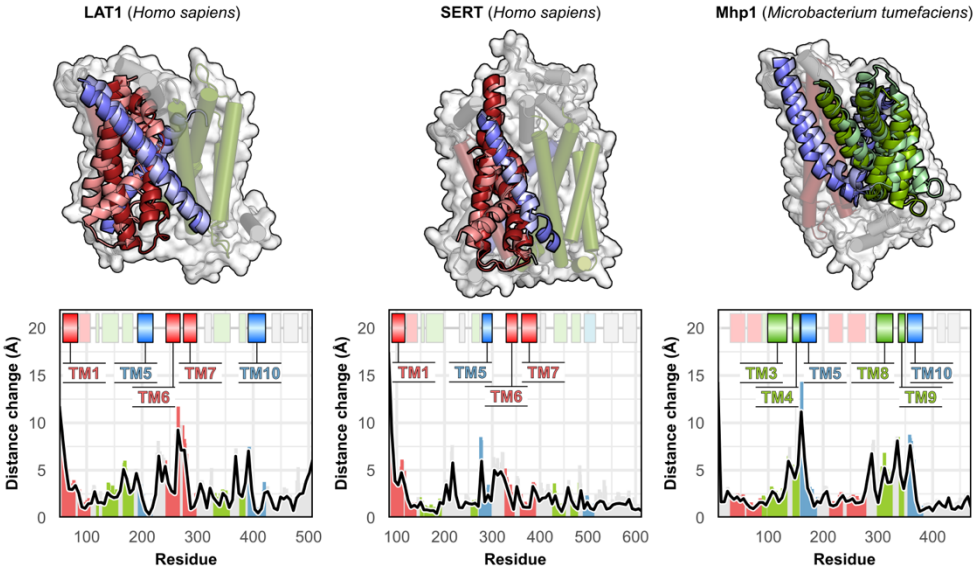
GRAPHICAL ABSTRACT:



ABSTRACT

Found in all domains of life, transporters belonging to the LeuT-fold class mediate the import and exchange of hydrophilic and charged compounds such as amino acids, metals, and sugar molecules. Nearly two decades of investigations on the eponymous bacterial transporter

LeuT have yielded a library of high-resolution snapshots of its conformational cycle linked by solution-state experimental data obtained from multiple techniques. In parallel, its topology has been observed in symporters and antiporters characterized by a spectrum of substrate specificities and coupled to gradients of distinct ions. Here we review and compare mechanistic models of transport for LeuT, its well-studied homologs as well as functionally distant members of the fold, emphasizing the commonalities and divergences in alternating access and the corresponding energy landscapes. Our integrated summary illustrates how fold conservation, a hallmark of the LeuT-fold, coincides with divergent choreographies of alternating access that nevertheless capitalize on recurrent structural motifs.

INTRODUCTION

Secondary active transporters tap the potential energy stored in electrochemical gradients to traffic nutrients and cytotoxic drugs across cell membranes[1]. Despite extensive divergence in topologies and ligands[2,3], current dogma hypothesizes that these transporters operate via alternating access, a generic term referring to the exposure of the substrate-binding site to no more than one side of the membrane at a time[4,5]. This mechanism allows transporters to couple conformational changes critical to substrate binding and/or release while minimizing the uncoupled flux or leak of ions down their electrochemical gradients. Although this basic working model of transport was devised nearly sixty years ago[4,5], describing its precise structural choreography enabling coupled ion/substrate translocation in individual transporters presents a formidable scientific challenge (Figure 1A). Until recently, capturing high-resolution snapshots of intermediates along the transport cycle was a daunting task[6,7]. To complement the static snapshots, solution-state measurements, carried out in the absence or presence of various substrates under conditions conducive to interconversion between conformations, directly report on the transporter's energy landscape and allow high-resolution snapshots to be assigned to specific intermediate states observed in the functional cycle[8–11]. However, few transporters have been exhaustively characterized to enable integration of structure, dynamics, and function[12].

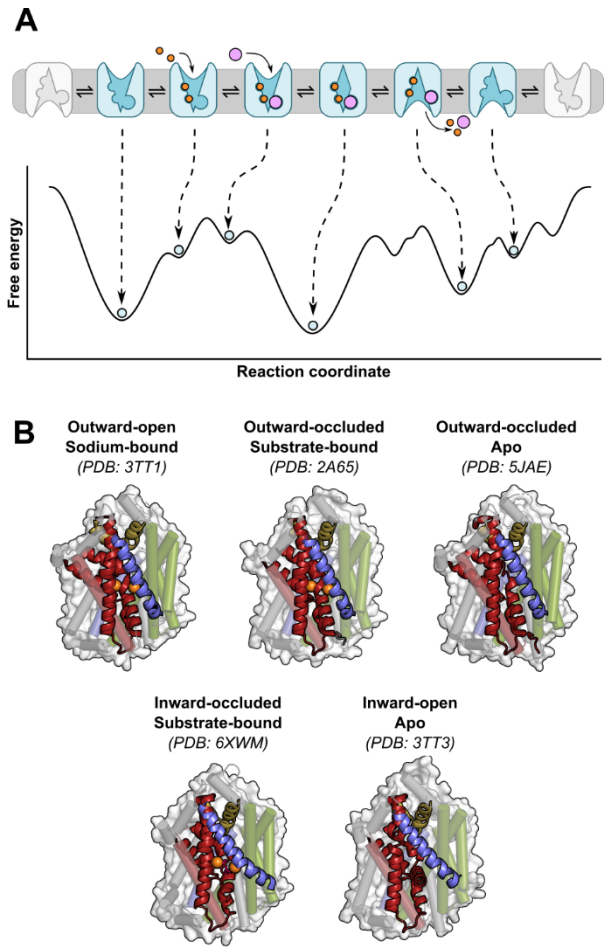


Figure 1. Transport cycle, reaction coordinate, and architecture of the bacterial amino acid transporter LeuT. (A) Cartoon depiction of a generic symporter's transport cycle as it isomerizes between outward-facing, occluded, and inward-facing conformations. Sodium ions and substrate shown as orange and purple circles, respectively. Mock reaction coordinate shown below. **(B)** Panel of experimentally resolved structures of LeuT.

The bacterial Neurotransmitter-Sodium Symporter (NSS) homolog LeuT found in the thermophilic archaea *Aquifex aeolicus* presents a rare example of a transporter that has been studied to this extent[8] (Figure 1B). LeuT couples the uptake of small aliphatic amino acids such as leucine and alanine to an inward sodium gradient and an outward proton gradient[9]. An extensive record of atomic-resolution crystal structures, complemented by solution-state experimental data, enables exquisite insight into its transport cycle although a consensus mechanism remains elusive[8,10,11,13–22]. Here, we review the LeuT body of knowledge and

explore the extent to which its structural homologs, including many transporters found in humans and whose dysfunction contributes to a range of diseases, undergo similar ligand-dependent structural rearrangements[18]. These homologs include mammalian NSSs, such as the serotonin transporter SERT and the dopamine transporter DAT[23–25], as well as a multitude of more distant transporters that have been identified across nearly a dozen families (Tables 1 and 2) belonging to the LeuT-fold. As many of these transporters have been the subject of extensive study over the course of many decades and have been the subject of numerous recent reviews[26–34], here we focus on comparing their structural dynamics and energy landscapes. Necessarily, emphasis is placed on NSSs which are the most extensively studied family of transporters with this topology.

Table 1. Curated library of LeuT-fold protein structures. Conformations are assigned to either outward-facing open (OFOp), outward-facing occluded (OFOc), fully occluded (OO), inward-facing occluded (IFOc), and inward-facing open (IFOp). Structures are marked if they were mutated (†) or bound to inhibitors (‡) or antibodies (§). Chain IDs indicated in lower-case where relevant. Redundant structures have been omitted.

Protein (*Organism*)	Conf.	PDB	Substrate	Resn.
Neurotransmitter-Sodium Symporter family				
b^0AT1 (*Homo sapiens*)	OFOc	6M17[35]	Apo	2.90 Å
DAT (*Drosophila melanogaster*)	OFOp	4XP1§[36]	2Na/Cl/Dopamine	2.89 Å
GlyT1 (*Homo sapiens*)	IFOp	6ZPL†‡§[37]	2Na/Cl/Benzoylisoindoline	3.94 Å
LeuT (*Aquifex aeolicus*)	OFOp	3TT1†§[13]	2Na	3.10 Å
		3F3A‡[15]	2Na/Trp	2.00 Å
		7DIXb[10]	Na/Leu	3.49 Å
	OFOc	2A65[8]	2Na/Leu	1.65 Å
		5JAE[38]	Apo	2.50 Å
	IFOc	6XWM†[22]	2Na/Phe	2.50 Å
	IFOp	3TT3†§[13]	Apo	3.22 Å
MhsT (*Bacillus halodurans*)	IFOc	4US3[14]	2Na/Trp	2.10 Å
SERT (*Homo sapiens*)	OFOp	5I6Z†§[24]	Apo	4.53 Å
		5I6X†‡§[24]	Paroxetine	3.14 Å
	OFOp	7LIA§[39]	Serotonin	3.10 Å
	IFOp	6DZZ‡§[40]	Ibogaine	3.60 Å
	IFOp	7LI6§[39]	Apo	3.50 Å
		7LI9§[39]	Serotonin	3.70 Å
Amino acid-Polyamine Organication Transporter family				
AdiC (*Escherichia coli*)	OFOp	3OB6[41]	Arg	3.00 Å
		7O82[42]	Apo	1.70 Å
		5J4N[43]	Agmatine	2.59 Å
	OFOc	3L1L†[44]	Arg	3.00 Å
AdiC (*Salmonella typhimurium*)	OFOp	3NCY[44]	Apo	3.20 Å
ApcT (*Methanococcus janaschii*)	IFOc	3GIA[45]	Apo	2.32 Å
$b^{(0,+)}$AT1 (*Homo sapiens*)	IFOp	6LI9[46]	Arg	2.30 Å
		6LID[46]	Apo	2.70 Å
BasC (*Carnobacterium* sp. AT7)	IFOp	6F2W§[47]	2-Aminoisobutyrate	3.40 Å
		6F2G§[47]	Apo	2.92 Å
GadC (*Escherichia coli*)	IFOp	4DJI[48]	Apo	3.19 Å
GkApcT (*Geobacillus kaustophilus*)	IFOc	5OQT[49]	Ala	2.86 Å
		6F34[49]	Arg	3.13 Å
LAT1 (*Homo sapiens*)	OFOp	7DSQ‡[50]	Diiodotyrosine	3.40 Å
	IFOp	6IRS†‡[51]	JPH203	3.30 Å
		6IRT†‡[51]	BCH	3.50 Å
		6JMQ§[52]	Apo	3.31 Å
LAT2 (*Homo sapiens*)	IFOp	7CMH[53]	Trp	3.40 Å
		7B00[54]	Apo	3.98 Å
xCT (*Homo sapiens*)	IFOp	7P9V[55]	Apo	3.40 Å
	IFOp	7P9U[55]	Glu	3.70 Å

Table 2. Curated library of LeuT-fold protein structures (continued).

Protein (*Organism*)	Conf.	PDB	Substrate	Resn.
Cation-chloride cotransporters				
KCC1 (*Homo sapiens*)	IFOp	6KKT[56]	K/2Cl	2.90 Å
		6KKR[56]	Apo	2.90 Å
KCC2 (*Homo sapiens*)	IFOp	7D8Z[57]	Apo	3.40 Å
KCC3 (*Homo sapiens*)	IFOp	6M22[‡][58]	DIOA	2.70 Å
		7D90[57]	Apo	3.60 Å
KCC4 (*Homo sapiens*)	IFOp	7D99[57]	Apo	2.90 Å
KCC4 (*Mus musculus*)		6UKN[59]	K/Cl	3.65 Å
NKCC1 (*Homo sapiens*)	IFOp	6PZT[60]	Apo	3.46 Å
NKCC1 (*Danio rerio*)	IFOp	6NPL[61]	K/2Cl	2.90 Å
Betaine/Carnitine/Choline Transporter family				
BetP (*Corynebacterium glutamicum*)	OFOp	4DOJb[†][7]	Apo	3.25 Å
	OFOc	4DOJa[†][7]	Apo	3.25 Å
	OO	4AINa[7]	Apo	3.10 Å
	IFOc	2WIT[62]	2Na/Betaine	3.35 Å
	IFOp	3P03[63]	2Na/Choline	3.35 Å
CaiT (*Escherichia coli*)	IFOp	2WSX[64]	γ-butyrobetaine	3.50 Å
		3HFX[65]	Carnitine	3.15 Å
CaiT (*Proteus mirabilis*)	IFOp	2WSW[64]	Apo	2.29 Å
Natural Resistance-Associated Macrophage Protein family				
DraNramp (*Deinococcus radiodurans*)	OFOp	6D91[†][66]	Apo	2.36 Å
		6BU5[†][66]	Mn	3.30 Å
	IFOc	6C3I[†][66]	Apo	2.40 Å
	IFOp	5KTE[†§][67]	Apo	3.94 Å
		6D9W[†§][66]	Apo	3.94 Å
EcoDMT (*Eremococcus coleocola*)	OFOp	5M8K[†][68]	Apo	3.60 Å
		5M87[†][68]	Mn	3.60 Å
EleNRMT (*Eggerthella lenta*)	IFOp	7QJI[§][69]	Apo	4.10 Å
	IFOp	7QIC[§][69]	Mg	4.10 Å
ScaDMT (*Staphylococcus capitis*)	IFOp	5M94[†§][70]	Apo	3.10 Å
		5M95[†§][70]	Mn	3.40 Å
Sodium-Solute Symporter family				
SiaT (*Proteus mirabilis*)	OFOp	5NV9[71]	2Na/Neuraminic acid	1.95 Å
	OFOp	5NVA[71]	Apo	2.26 Å
SGLT1 (*Homo sapiens*)	IFOp	7SLA[†§][72]	Apo	3.15 Å
SGLT2 (*Homo sapiens*)	OFOp	7VSI[†‡§][73]	Empagliflozin	2.95 Å
SMCT1 (*Homo sapiens*)	IFOp	7SL9[§][72]	Butyrate	3.50 Å
vSGLT (*Vibrio parahaemolyticus*)	IFOc	3DH4[74]	2Na/Galactose	2.70 Å
	IFOp	2XQ2[†][75]	Apo	2.70 Å
Potassium Uptake Permease family				
KimA (*Bacillus subtilis*)	IFOp	6S3K[76]	3K	3.70 Å
Amino acid/Auxin Permease family				
DrSLC38A9 (*Danio rerio*)	IFOp	6C08[†§][77]	Arg	3.17 Å
		7KGV[†§][78]	Apo	3.40 Å
Nucleobase-Cation Symporter-1 family				
Mhp1 (*Microbacterium tumefaciens*)	OFOp	2JLN[79]	Apo	2.85 Å
	OFOc	4D1B[80]	Na/Benzyl-hydantoin	3.80 Å
	IFOp	2X79[81]		3.80 Å
Alanine/Glycine-Cation Symporter family				
AgcS (*Methanococcus maripaludis*)	OO	6CSE[§][82]	Na/Ala	3.24 Å
		6CSF[§][82]	Na/D-Ala	3.30 Å

STRUCTURAL AND FUNCTIONAL DIVERSITY WITHIN THE FOLD

The ten transmembrane helix topology

Members of the LeuT-fold class belong to the Amino Acid-Polyamine-Organocation (APC) superfamily of transporters[83] and share a core ten-helix structure consisting of two consecutive pairs of five transmembrane helices (TMHs) related by pseudo-twofold symmetry around an axis parallel to the membrane surface[84,85]. The fold encompasses symporters and antiporters engaged in ion-coupled transport as well as substrate exchange. Analysis of internal symmetry and structural similarity led to assignment of these ten helices into three domains: the bundle domain (TMHs 1, 2, 6, and 7), the hash domain (TMHs 3, 4, 8, and 9), and gating helices (TMHs 5 and 10; we use this canonical numbering scheme and ignore transmembrane helices preceding this ten-helix core in sequence). Additional N- and/or C-terminal helices flank the ten-helix core and vary across individual transporter families comprising the LeuT fold. Almost universal to members of the LeuT fold, TMH 1 and often TMH 6 contain conserved unwound regions, observed near the geometric center of the ten-helix core, and their involvement in ligand coordination is a hallmark of the fold[86]. Importantly, fold recurrence is not accompanied by sequence similarity. Few structural motifs are identifiable at the sequence level[14,18,87], which prevented these transporter families from being co-categorized into a fold prior to the determination of their structures[23,88] (Figure 2).

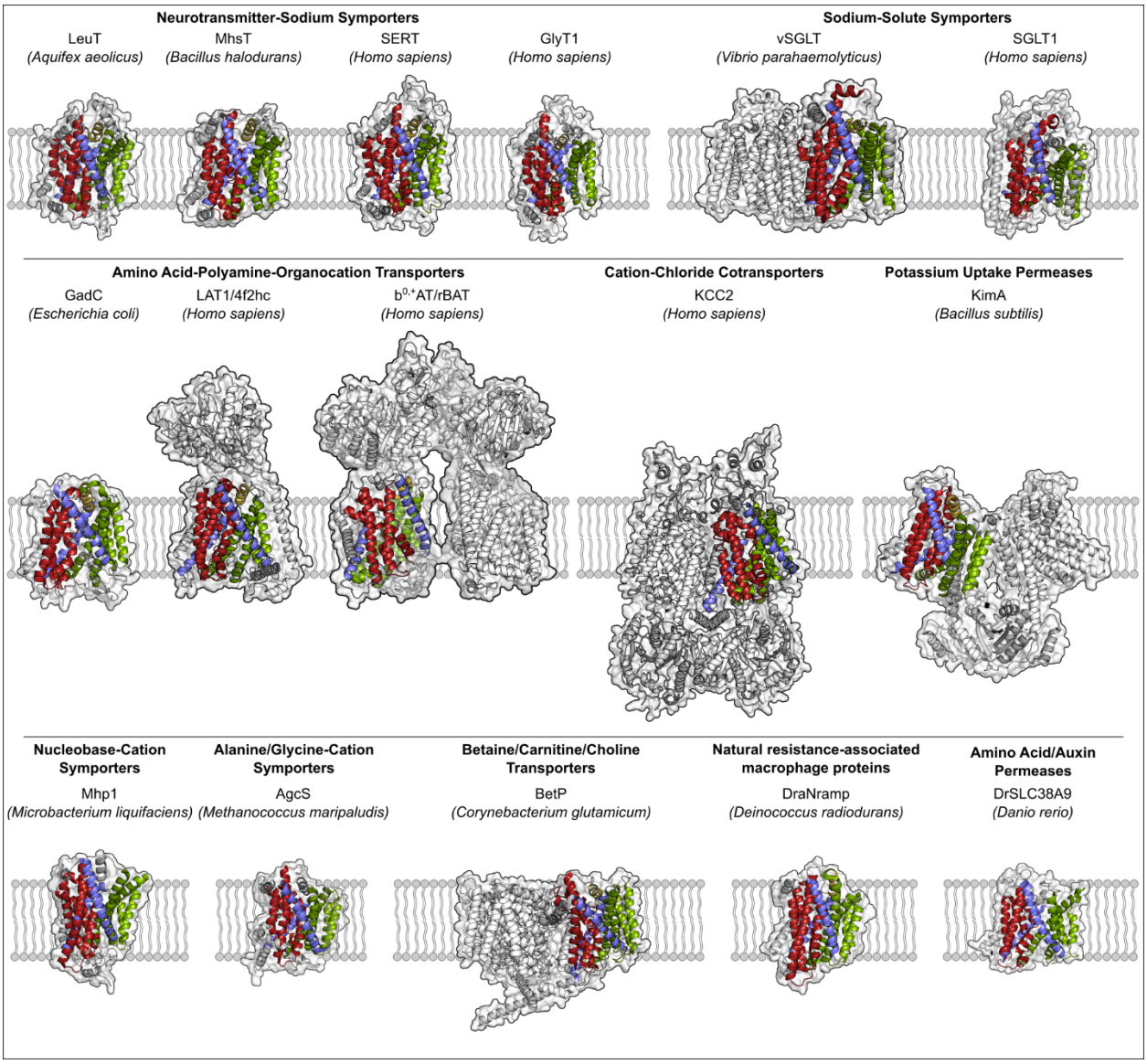


Figure 2. Functional and structural diversity within the LeuT fold. The LeuT fold is adopted by proteins found in a range of transporter families.

In addition to the NSS family[27], members of the Sodium-Solute Symporter[30] (SSS), Cation-Chloride Cotransporter[28] (CCC), Amino Acid-Polyamine-Organocation Transporter[29] (APC), Natural resistance-associated macrophage protein[26] (Nramp), Nucleobase-Cation Symporter[32] (NCS-1), and Amino Acid-Auxin Permease (AAAP) families adopt this fold. The same topology also describes transporters in protein families unique to prokaryotes, such as those in the Betaine-Carnitine-Choline Transporter[89] (BCCT), Alanine-Glycine-Cysteine Symporter (AGCS), and Potassium Uptake Permease (KUP) families. Finally, transporters in the Branched-

Chain Amino Acid-Cation Symporter (LIVCS) and the Carbon Starvation Family (CstA) families, which have no representatives in the PDB, are also predicted to adopt this fold[83].

Structural diversity outside the ten-helix core

Variations in the structural features decorating the ten-helix core further contribute to functional specialization. A remarkable example is SLC38A9, which both exports amino acids from the lysosome and activates the regulatory complex mTORC1 under nutrient-rich conditions[90,91]. An N-terminal domain elegantly couples these two functions by binding to the cytoplasmic cavity and, following displacement by the transported substrate arginine, recruiting GTPases involved in downstream signaling[78,92]. In a potential case of convergent evolution, the C-terminal domain of the pH-dependent Glu/GABA exchanger GadC arrests transport by binding to the intracellular cavity at neutral pH in a nearly identical conformation[48]. Autoinhibition by disordered terminal domains has also been visualized directly in several potassium-chloride symporters [57,58]. In other transporters, such as eukaryotic NSSs, disordered termini instead regulate transport by interacting with a range of cytoplasmic proteins and lipids[93,94]. In BetP, a cytoplasmic C-terminal helical domain regulates transport in response to osmotic stress[95,96]. These domains often go unobserved in structural studies due to truncation and/or intrinsic disorder[24,25,54,56], prompting speculation regarding their role in transport.

Further diversity is found in the range of higher-order assemblies of certain LeuT-fold transporters[97,98]. Whereas oligomeric interfaces in LeuT-fold transporters visualized by crystallography are highly compact, those of structures recently determined by cryo-EM appear weaker and more flexible. For example, several eukaryotic APC and NSS transporters associate with proteins proposed to facilitate trafficking and localization (the experimental structure of b^0AT1/ACE2 was determined as part of a larger complex that included the receptor-binding domain of the SARS-CoV-2 spike protein[35]). Additionally, protomers of the bacterial potassium transporter KimA and eukaryotic CCCs appeared to dimerize using similar domain swapping mechanisms, which may be indicative of a recurring quaternary assembly mechanism[57,59,61,76]. Notably, experimental structures and MD simulations indicate that these assemblies are highly flexible.

Conserved elements of ion/substrate binding

Retention of the ten-helix core, which encodes conserved motifs of substrate binding and ion coordination, is all the more remarkable considering the extent to which the transported ligands differ in size, structure, and polarity. The centrally located substrate-binding site, shared by symporters and antiporters, accommodates ligands ranging in size and charge from halogen ions and divalent metals to sugars and aromatic amino acids. Beyond plasticity of the substrate-binding site, coordination of the coupled ions provides rich examples of conservation and evolutionary tuning across distantly related transporters. While the identity and number of these ions tend to be family specific, the location and geometry of coordination are often recycled. A widely discussed example is the conserved sodium site, termed Na2, found in most sodium-coupled symporters[8,61,62,79,99]. The exception, the sodium-coupled amino acid symporter AgcS, coordinates its only sodium at a position equivalent to LeuT's Na1 site and leaves the Na2 site unoccupied[82]. This is despite its alanine binding site overlapping nearly perfectly with the substrate-binding sites of unrelated amino acid transporters from the NSS and APC families (Figure 3C). To our knowledge, no cations besides sodium ions have been observed in the Na2 site, and no comparable degree of structural conservation has been observed at other ligand-binding sites, such as those involved in binding potassium (transported by SERT, as well as the KUP and CCC families) or chloride (transported by NSSs and CCCs). Moreover, no such conservation is observed in the position of the other sodium binding site in symporters that bind two sodium ions such as the NSSs, SSSs, and BetP[100]. In proton-coupled symporters and exchangers, such as CaiT and members of the APC family, positively charged residues occupy the Na2 position[45,47,49,65], highlighting the malleability of ion coupling throughout the fold.

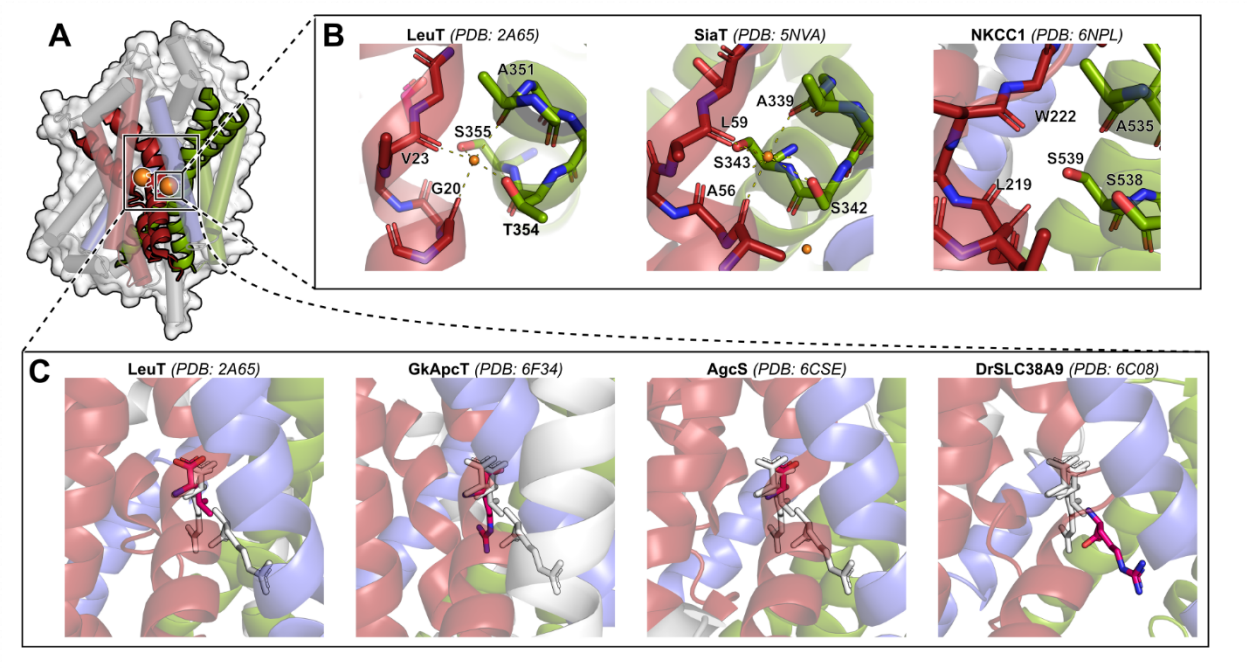


Figure 3. Examples of conserved ligand coordination. (A) Sodium ions (orange) and leucine (pink) in LeuT. **(B)** Conservation of the Na2 site. Sodium binding in NKCC1 was inferred from sequence and not directly observed. **(C)** Partial recurrence of amino acid binding modes. Native substrates are shown in purple, while substrates shown in white are shared in all four panels.

STRUCTURAL BASIS OF ALTERNATING ACCESS

Inferences from crystal and cryo-EM structures

Coupled transport of ions and substrates, i.e. alternating access, entails the interconversion of the transporter between conformational states. In contrast to conservation of motifs of substrate binding and ion coordination, comparison of structural elements of alternating access between LeuT-fold transporters suggest divergence is the rule, not the exception. As high-resolution snapshots of transporters in different states such as Mhp1 were determined in conjunction with comparative spectroscopic studies, this fact became apparent nearly a decade ago with the publication of structures of Mhp1[79,81], BetP[7,62], and LeuT[8,13,85]. Examination of their structural changes revealed a striking lack of consensus over the molecular details of alternating access that has since been reinforced by similar studies in SERT[24,40], DraNramp[66,67], and LAT1[50,51] (Figure 4). Additional OF-open and OF-occluded structures of AdiC[44,101], as well as IF-open and IF-occluded structures of vSGLT[75,99], further expand how these transporters grant access to the substrate-binding site. Overall, comparison of pairs of structures

from different transporters reveals fundamental differences in which helices move and which stay fixed. Below we delve into the specific elements of alternating access, with an emphasis on commonalities and divergences between different families in the fold.

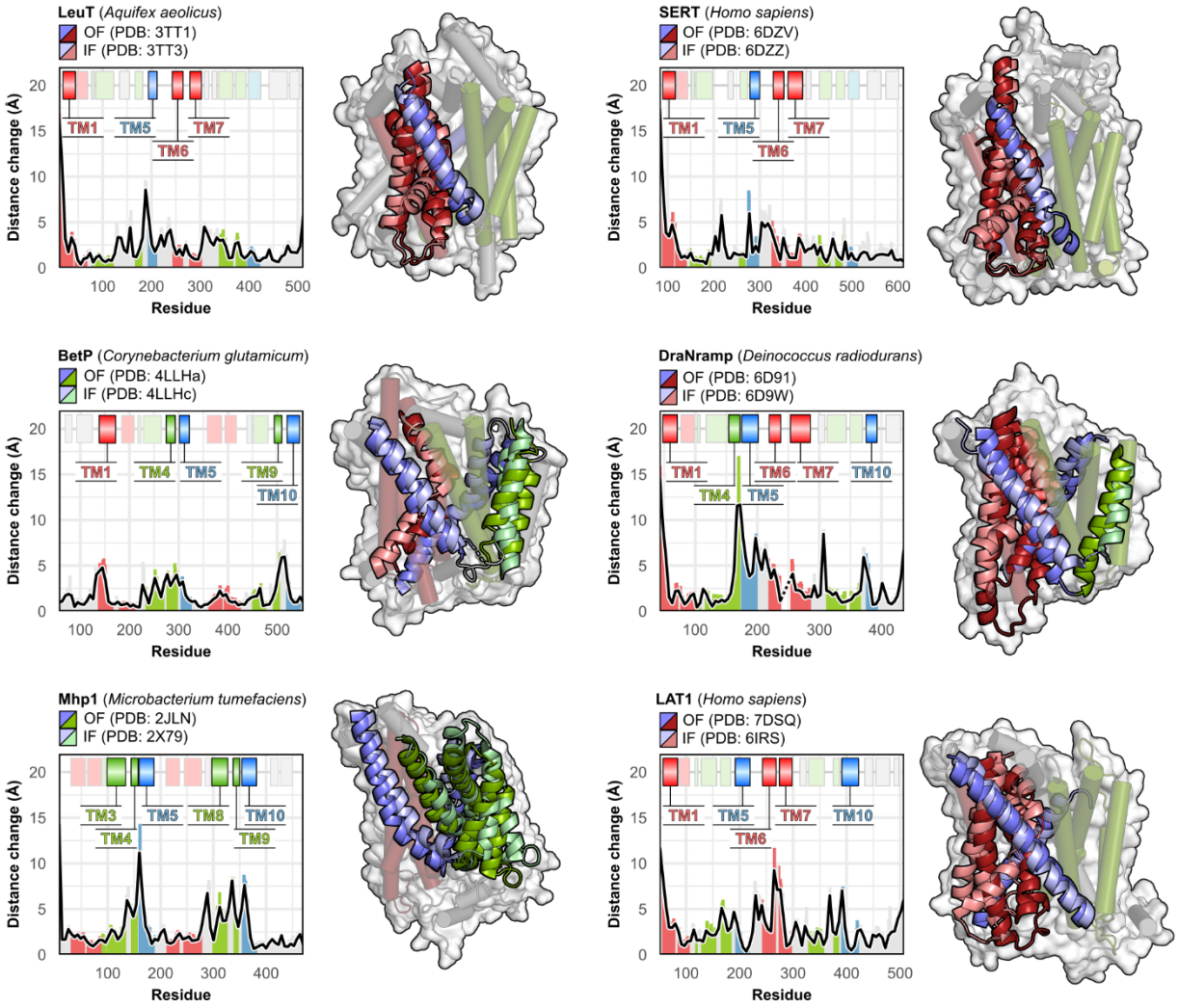


Figure 4. Variations in inferred conformational dynamics within the LeuT-fold. LeuT-fold transporters show striking differences in how alternating access between IF and OF conformations is facilitated. Dynamic and static helices are depicted as ribbons and cylinders, respectively. Charts show observed distance between alpha carbons in the indicated superimposed structures (dashed lines indicate extensive stretches of unobserved density).

The gating helix TMH5

Along with the intracellular loop preceding it, TMH5 ranks among the most consistently mobile and dynamic regions in the transporters studied so far[18,87]. In OF conformations, TMH5 nestles against the bundle domain helices TMH1a and TMH6b, forming the highly ordered intracellular "thick gate". In the IF state, by contrast, opening of the intracellular vestibule is often driven by rearrangements that vary across families and even individual transporters within families.

The contribution of TMH5 to alternating access has been most extensively studied in NSSs[18,87]. A GX_NP sequence, strictly conserved within the family and partially conserved throughout the fold, putatively mediates both bending and unfolding motions instrumental to the initiation of substrate release (Figure 5). Mutagenesis of glycine or proline with this sequence severely abrogates transport in the bacterial NSS MhsT[14], highlighting the importance of the dynamic processes facilitated by this motif. Partial unwinding of TMH5, first observed in a substrate-bound IF-occluded conformation of MhsT[14], has been corroborated in several other NSSs by hydrogen-deuterium exchange/mass spectrometry (HDXMS) studies under conditions promoting the IF conformation of each transporter[11,16,102–104]. However, although IF-open structures of LeuT, SERT, and GlyT1 show this helix protruding out from the rest of the transporter[13,22,37,40], orthogonal measurements in LeuT suggest that substrate and ion binding promote the adoption of conformations that occlude, rather than open, the intracellular cavity[20,21,105,106]. As elaborated below, however, these results in LeuT are qualified by the frequent use of leucine, which has a low transport rate and nanomolar binding affinity[15,102]. Subsequent solution-state experiments bound to different amino acids found that quenching of fluorescent probes attached to the intracellular half of TMH5 was inversely correlated with transport rate[18], suggesting that this IF-occluded conformation may be less stable, relative to IF-open, when transporting substrates with higher turnover rates such as alanine. Nevertheless, in conjunction with other findings discussed below, this points to a mechanism in which TMH5 preferentially adopts the partially unwound occluded conformation when bound to its substrates but transiently bends to facilitate their release.

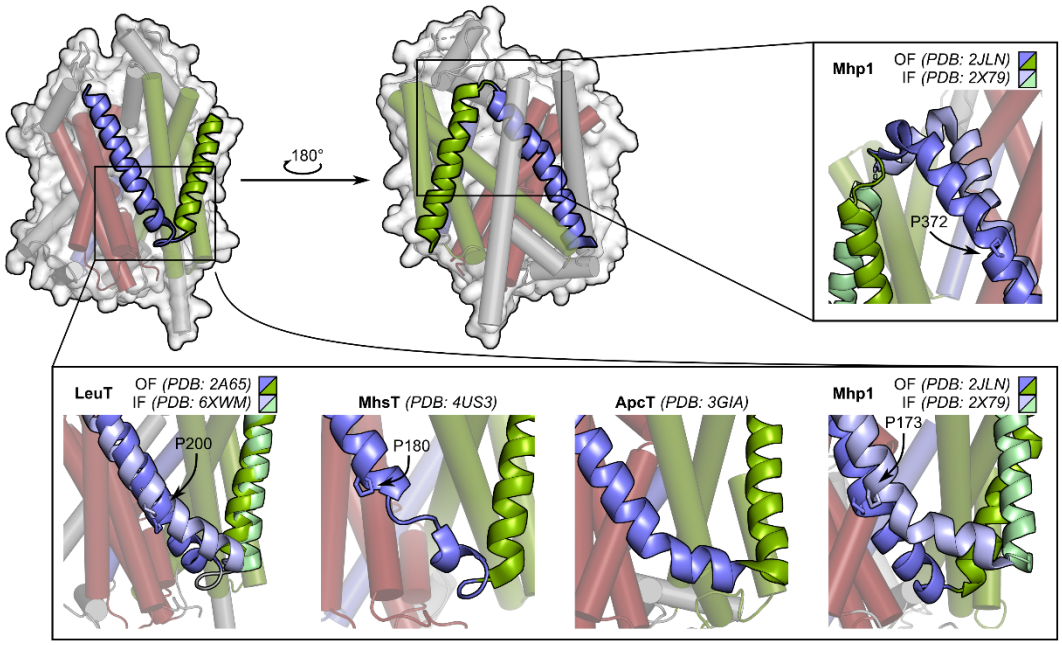


Figure 5. Bending of TMH5 and TMH10 is observed in a subset of LeuT-fold transporters. **Top left:** LeuT with TMH4/5 and TM9/10 highlighted. **Top right:** Movement of TM10 in Mhp1. **Bottom:** Movements of TMH5 observed in NSSs, ApcT, and DraNramp in the IF state. Conserved proline residues are highlighted in LeuT, MhsT and Mhp1.

It is notable that TMH5 adopts a similar, but not identical, conformation in IF-open Mhp1 which shares this GX_NP sequence[81]. Despite this agreement, electron paramagnetic resonance (EPR) spectroscopy measurements revealed disorder in TMH5 that is altogether absent from similar measurements carried out on LeuT in the presence of leucine[20,107]. Interestingly, ApcT also shares a LeuT-like bend despite lacking a proline in TMH5 at the equivalent position[45]. Since its structure has only been determined in a single conformation, and since its homologs such as GadC and BasC maintain a straight conformation of this helix[48,49], the extent to which the aforementioned NSS movements occur in ApcT and its homologs remains unclear[108]. Finally, although TMH5 is also involved in opening the intracellular cavity in DraNramp66, which also lacks the conserved mid-helical proline, it undergoes a rigid-body up-and-out translation rather than bending and unfolding. Many other IF structures, such as those observed in vSGLT and GkApcT, lack a fully resolved stretch of residues corresponding to IL2, located between TMHs 4 and 5, indicating a high degree of heterogeneity in the crystal lattice or the cryo-EM ensemble[49,75]. In summary, the crystallographic data indicate substantial conformational

variation observed across the fold in TMH5 and the loop preceding it that is corroborated by solution-state data indicative of local disorder. Ultimately, these data suggest that the protruded conformation observed in some transporters, though perhaps physiologically relevant and likely fundamental to the transport cycle, may not represent a well-defined low-energy state.

The gating helix TMH10

Movement in TMH10, despite its pseudosymmetry relation to TMH5, is less frequently observed (Figure 5). In NSSs, for example, no evidence has been advanced to show involvement in either ligand-dependent conformational dynamics or partial unwinding[11,20,104,109]. Mhp1 shows partial symmetry of TMH10 to TMH5 in both sequence and structure, with comparable increases in conformational heterogeneity detected by EPR under OF-stabilizing experimental conditions[79,81,107]. However, the movement of TMH10 inferred from crystal structures is less dramatic than that of TMH5. In both BetP and DraNramp, differences between their OF-closed and OF-open conformations in this region, though less drastic than in Mhp1, are nonetheless unmistakable; indeed, the corresponding proline in TMH10 facilitating this bend is strictly conserved in the BCCT family and partially conserved among Nramps[26,64]. Although the APC transporter AdiC both shares this specific residue and shows evidence of this structural movement, its structural similarity to the eukaryotic homolog LAT1, which instead has a cysteine at the equivalent position, indicates that this proline positioned halfway across TMH10 may be coincidental[44,50].

The bundle domain helix TMH1a

LeuT's twofold pseudosymmetry initially appeared to imply that a rigid-body rotation of the bundle domain relative to the rest of the structure mediates alternating access[84]. This elegant mechanism, elaborated under the name rocking bundle, failed to predict subsequent structural evidence in two key respects. First, the contribution of this domain to alternating access, although prominent in some transporters, is far from universal. Movement in ancillary helices and loop regions has been observed in every transporter studied thus far. Second, the bundle domain virtually never moves as a rigid body. The exception, Mhp1, locks the bundle domain into place and instead pivots the hash domain and gating helices around this scaffold[79].

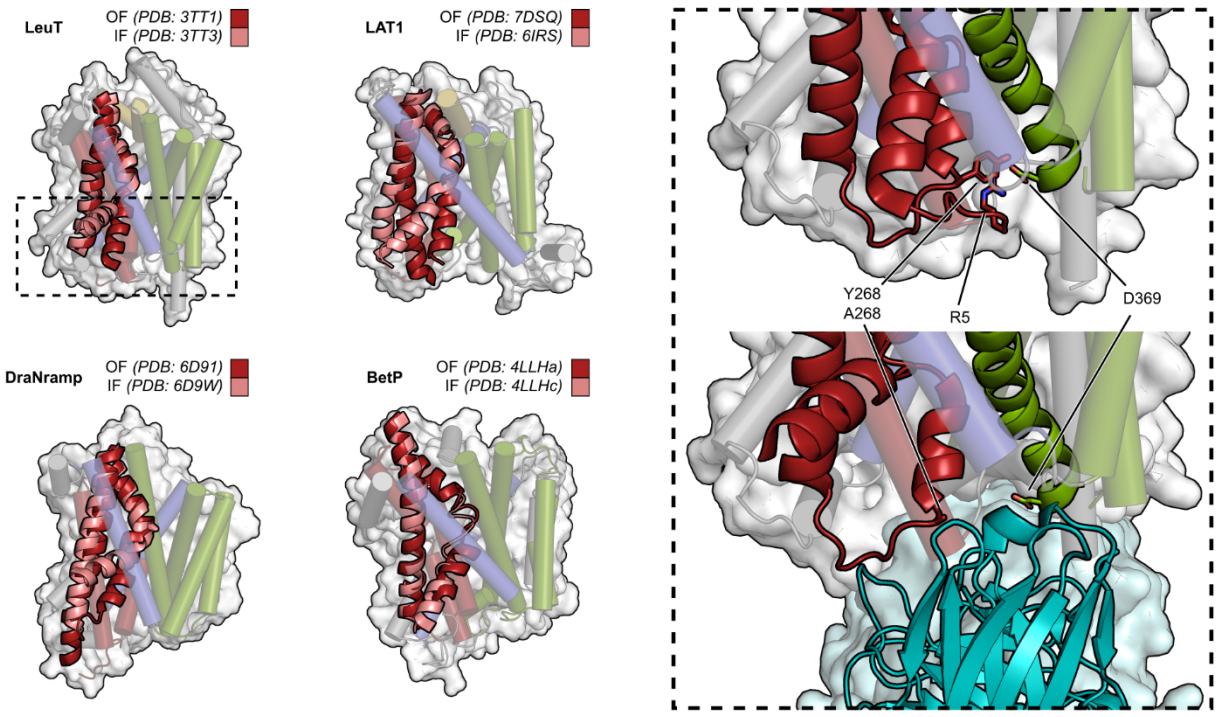


Figure 6. Movement of TMH1 and TMH7 across the LeuT fold. Left: Four representative LeuT-fold transporters with experimental structures determined in both OF and IF conformations. **Right:** Comparison of the LeuT's intracellular side in an outward-facing occluded conformation (top, PDB: 2A65) and an inward-open conformation (bottom, PDB: 3TT3). Stabilization of the IF conformation required disruption of an intracellular hydrogen bond network by mutagenesis of a conserved tyrosine (Y268) and the introduction of a high-affinity antibody, shown in teal. Electron density corresponding to the N-terminus, which contains R5, was not resolved in the inward-facing conformation.

Movements of TMH1a relative to the rest of the intracellular vestibule, in the context of alternating access, appear to be the most variable across this superfamily. Considered critical for substrate release, these movements were observed in X-ray and cryo-EM structures of NSSs and Nramps[13,40,66] and were corroborated in solution (both families, it should be noted, lack N-terminal helices and oligomeric interfaces capable of restricting the dynamics of TMH1a). Particular controversy surrounds the relevance of the signature 45° pivot observed in LeuT, which has been attributed to the use of short-chain detergents commonly used in membrane protein crystallography[103,110], alanine mutagenesis of a conserved tyrosine residue essential for function[13,111], and a conformationally selective high-affinity antibody (Figure 6). Molecular

dynamics simulations of LeuT's IF-open crystal structure in a lipid bilayer later revealed the steep energetic cost of this movement in a more physiological membrane environment[110]. Although this brought attention to the contribution of the membrane mimetic in stabilizing such an extreme conformer, these findings, alongside experimental measurements obtained using both luminescence resonance energy transfer[110] and HDXMS[16] in lipid environments, nonetheless corroborated the more general hypothesis that TMH1a becomes conformationally disordered in the IF-open state. Because these experiments were executed on similar tyrosine-to-alanine mutants, they do not address the extent to which this IF-open conformation is sampled by the wildtype protein in solution. For example, EPR measurements on equivalent tyrosine-to-alanine mutants indicate comparable disorder in TMH1a; by contrast, no such movement was detected without this mutation[11,20]. Experiments in SERT painted a similar picture to LeuT of large amplitude movements of TMH1a[40,104], and recent cryo-EM structures reinforce the distribution of conformations adopted by this helix[39].

This movement is also a hallmark of alternating access in Nramps[66]. In fact, deletion of residues in THM1a was necessary to determine the first atomic-resolution structure of an Nramp, suggestive of disorder comparable to LeuT and SERT[70]. By contrast, transporters in the BCCT, SSS, and other families show marginal movement, which may be in part due to steric hindrance by N- and C-terminal helices[7,72,73,75,99] (Figure 6). Instead, opening of the cytoplasmic pathway is mediated by structural changes elsewhere. Overall, the data suggest that transient detachment of TMH1a from the rest of the bundle domain exposes the substrate-binding site to the cytoplasm in a subset of LeuT-fold transporters.

The rest of the bundle domain

A similar pattern of increased disorder under conditions promoting opening of the cytoplasmic cavity has also been observed on the intracellular side of TMH7. EPR and HDX/MS experiments in LeuT suggest that TMH7 partially unfolds and/or translates under such conditions[11,20], contrasting with its lack of movement inferred from the crystal structures. Similarly, in DraNramp's IF-open conformation, but not its IF-occluded conformation, the eight N-terminal residues of TMH7 could not be assigned to electron density, suggesting increases in disorder correlated with substrate release[66]. The most pronounced motion of TMH7 is likely found in LAT1, which swings over 15 Å to close its intracellular cavity[50]. Notably, this

movement does not coincide with comparable movements on the extracellular side, which contrasts with observations in its bacterial homologs[44,47,48,101].

TMH1b and TMH6a, located on the extracellular sides of the LeuT topology, consistently undergo smaller scale but nonetheless significant movements [15,19]. These helices appear to open the extracellular vestibule by moving in concert with the conserved helix extracellular loop 4 (EL4), located between TMH7 and TMH8, in NSSs[8,13,24,40], APC transporters[50,51], and Nramps[66–68,70]. However, EPR spectroscopy of LeuT indicates that the crystal structures may understate the helices' movement[19,20], a finding attributed to detergent molecules wedged in the extracellular vestibule[112]. Interestingly, on the intracellular side, no equivalent coupling between TMH1a, TMH6b, and intracellular loop 1 (IL1) has been detected to our knowledge. Indeed, unlike EL4, IL1 appears to be firmly stapled to the hash domain. Finally, TM2 was not shown to undergo any significant movement among the panel of transporters shown in Figure 4.

The hash domain

Relative to movements outlined above, independent helical movements within the hash domain are relatively rare. Mhp1 stands out in rigidly "rocking" this domain, alongside bending in TMH5 and TMH10, to mediate alternating access[79,81,107]. Similar movements were reported in vSGLT using EPR[113], although these coincided with additional movements distributed throughout the rest of the structure. In contrast, inferred hash domain movements in other transporters are limited to bending of TMH4 on the intracellular side and TMH9 on the extracellular side to complement bending of TMH5 and TMH10, respectively. The contribution of ancillary helices and/or oligomeric interfaces, which are frequently found adjacent to the hash domain, to this phenomenon is unclear. In an interesting twist, a preprint publication describing the IF-to-OF transition in KCC1 proposes that alternating access is purely mediated by movement of TMH3 and TMH8, while TMH4 and TMH9 remain fixed[56,114].

Experimental conditions, mutations, and antibodies confound interpretation of alternating access

Many LeuT-fold transporters, with Mhp1 and BetP being noteworthy exceptions, could only be coaxed into specific conformations using drastic mutations, conformationally selective antibodies, or high-affinity transport inhibitors (see Tables 1 and 2). In addition to the controversial

use of a transport-abrogating tyrosine-to-alanine mutation in LeuT discussed above[13], stabilization of a purported IF-occluded state was also achieved by mutating a conserved tryptophan similarly found to be essential for function[22]. Crystallographic snapshots of DraNramp in OF and IF conformations required glycine-to-arginine mutations in TMH1a and glycine-to-tryptophan mutations in TMH6a, respectively, that prevented isomerization by obstructing closure of the appropriate vestibule[66,67]. As noted in the release of these structures, the equivalent missense mutation in TMH1a in human Nramps is correlated with severely reduced iron uptake *in vivo*[115], highlighting the extent to which transport function is impaired. Similarly, crystallization of IF-open vSGLT required a lysine-to-alanine mutation that prevented ligand binding and showed no transport activity[75]. Capture of the OF-occluded conformation of AdiC, achieved using an aspartate-to-alanine mutation, may have played a role in stabilizing a ligand pose distinct from those observed in subsequent ligand-bound crystal structures of the wildtype protein[116]. Equivalent studies of the eukaryotic transporters SERT and LAT1 have employed potent inhibitors that preferentially bind to specific conformations[40,50,114]. Whereas apo SERT readily crystallized in OF conformations, capture of IF state required the small molecule ibogaine. Similarly, LAT1 was structurally characterized in an IF conformation in the absence of ligands[51,52] but could only be described in OF conformations using inhibitors[50]. The introduction of small molecules and/or inactivating mutations arrested both transporters by stabilizing conformers that may be off-path with respect to the protein's functional cycle.

CONNECTING THE DOTS: FROM STRUCTURES TO LANDSCAPES

Because secondary active transporters lack a molecular motor such as an ATPase, they must rely on the energy input of ion and ligand gradients to navigate their energy landscapes[117]. Mechanistic models of transport must, therefore, also map the conditions under which specific conformations are populated. The outstanding structural record of LeuT-fold transporters underrepresents conformations that are not amenable to structural characterization[118,119]. Although the experimentally determined structures can be foundational to mechanistic models of transport, such as for instance the glide-symmetry symport mechanisms in NSSs[120] and CCCs[121], these structures do not represent a direct test to these models. An example that will not be discussed further is the possible existence of an allosteric binding site in LeuT and other

prokaryotic NSSs, which remains controversial despite nearly two decades of investigations[9,122,123].

Rigorous evaluation of energy landscapes requires solution-state techniques reporting on protein kinetics and thermodynamics during the transport cycle[85]. Although several LeuT-fold transporters have been studied using these techniques, it remains difficult to ascertain the extent to which these properties are conserved both within and between families. Below, we summarize the current data on NSSs and SSSs, the only LeuT-fold transporter families with data that permit these fundamental questions to be tackled.

Conformational dynamics of NSSs

The energy landscapes of NSSs have been extensively characterized by ensemble and single molecule biophysical studies carried out in solution. Among the conserved themes emerging from these studies: sodium binding stabilizes OF conformations, substrates tend to stabilize occluded conformations, and absence of either promotes interconversion between IF-open, occluded, and OF-open[11,20,124–126] (Figure 7). These data provide additional context for the experimental structures by reporting on how ligand-binding events bias the conformational ensemble. Concomitantly, the data can reveal steps unanticipated by canonical symport and/or antiport mechanisms. For example, recent data suggesting that potassium stabilizes LeuT into an IF-open conformation indicate a step in which intracellular potassium ions indirectly participate in the transport cycle by competing with sodium and accelerating its release from the central binding site[11,17] Facilitation of substrate release by allosterically bound ions has since been reported in several CCCs[56,59,127].

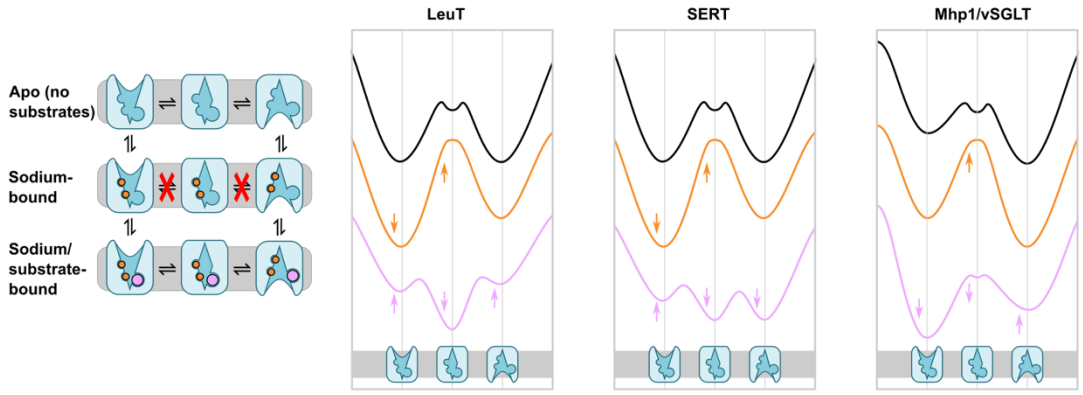


Figure 7. Ligand dependence of conformations for selected LeuT-fold transporters. Left: A canonical sodium-coupled symport cycle. Although NSSs, SSSs, and NCS1 transporters all rely on sodium gradients to import cargo, the ligand-dependence of their conformational dynamics deviate from one another. Right: Free energy diagram capturing the conformational dynamics of sodium-coupled symporters.

Despite their structural similarity, LeuT, SERT, and DAT were observed to have distinct steps underlying their conformational dynamics. Whereas LeuT adopts an occluded conformation when bound to leucine[11,20], serotonin-bound SERT fluctuates between IF-occluded and IF-open[104]. More intriguingly, the helical unwinding in IL2 and TMH5 initially proposed by the IF-occluded structure of MhsT[14], although plausible in LeuT and SERT, was altogether inconsistent with HDXMS data collected in DAT suggesting a lack of cooperative movement in this region[109]. In contrast, the results pointed to movement in IL4, a nearby region previously found to be critical to IF-opening in other eukaryotic NSSs but static in LeuT[20,128] (lack of coverage in HDXMS studies of SERT prevented this region from being studied[104]). The presence of lipids and cholesterol in DAT samples presents a confounding factor when attempting to directly compare these results to those collected in SERT, which was probed in detergent micelles. Previous studies reported modulation of conformational dynamics in both eukaryotic NSSs and other active transporters by detergent and lipids[40,104,129–134]. Therefore, while these results highlight the extent to which conformational steps are conserved, they also reveal key deviations in how evolution has fine-tuned these transporters to traffic substrates.

Single-molecule visualization of conformational changes using fluorescent resonance energy transfer (FRET) has added a layer of detail entirely missed by ensemble-level measurements[135,136]. A recent study employing this technique showed how LeuT appears to undergo uncoupled movements on the intracellular and extracellular sides of the membrane in the absence of substrate, including sampling a channel-like conformation simultaneously open to both sides[124]. Although canonical symport mechanisms of alternating access forbid population of such intermediates[137], LeuT appears to avoid uncoupled sodium flux by sealing the intracellular cavity in response to sodium binding. Additionally, this study corroborated previous experimental and computational studies on LeuT and other NSSs suggesting that substrate dissociation, and specifically substrate-dependent sodium dissociation from the Na2 site, is the rate-limiting step in

transport[18,138,139]. However, not all NSSs appear to follow this pattern. A study on wildtype MhsT, in which soluble amino acid-binding proteins labeled with pairs of complementary fluorescent probes were introduced to the interior of MhsT-containing proteoliposomes, determined that the substrate-free IF-to-OF transition was instead rate-limiting[123]. Electrophysiology studies in human NSSs led to similar conclusions[140], suggesting that divergences outlined above in the conformational dynamics of NSSs extend to their landscapes as well.

Conformational dynamics in SSSs

The differences in conformational dynamics among NSSs, although not trivial, are dwarfed by those distinguishing them from SSSs such as the eukaryotic sodium/glucose symporter SGLT1, the prokaryotic sodium/galactose symporter vSGLT, and the prokaryotic sodium/proline symporter PutP (Figure 7). While not as extensively studied, these proteins traverse an energy landscape that is fundamentally distinct from those of NSSs. Moreover, the outstanding data highlight the challenges inherent to the interpretation of solution-state dynamics data. EPR measurements of vSGLT[113] and PutP[141] as well as fluorescent labeling and cysteine accessibility measurements in SGLT1[142–144] suggest that ligand-dependent conformational dynamics are effectively inverted relative to NSSs, with apo and/or sodium-rich conditions favoring IF conformations and substrate binding stabilizing the OF conformation. As with NSSs discussed above, key differences exist between these SSS transporters: whereas sodium was not observed to engender large structural changes in vSGLT[113], sodium binding to PutP led to closing of EL4 and increased accessibility of residues lining the intracellular vestibule[141,145,146]. Importantly, similar sodium-invariant conformational dynamics were independently reported in the unrelated bacterial transporter Mhp1 using both EPR and cysteine accessibility measurements[107,147,148], and sodium-driven stabilization of an IF state was also suggested by EPR data in BetP[149].

These data prompted the conclusion that symport in SSSs may be driven by ion electrochemical gradients and/or membrane potentials absent in these *in vitro* studies[113]. Consistent with this hypothesis, accessibility measurements and fluorescent labeling data collected in human SGLT1 in cells that actively maintain a sodium gradient showed a conformational landscape nearly identical to NSSs: unrestricted isomerization between OF and IF in the apo

state[144] and stabilization of the OF conformation in the presence of sodium and absence of glucose[142,150]. Critically, the OF-promoting effect of sodium diminished when the electrochemical gradient was decreased[142]. However, while these data support the hypothesis that the gradient may play a similar role in prokaryotic SSSs, a critical difference is that SGLT1 is electrogenic, with a 2:1 sodium-to-glucose stoichiometry that contrasts with the 1:1 stoichiometry of the prokaryotic model systems discussed above.

CONCLUDING REMARKS: DETERMINANTS OF SUBSTRATE TRANSLOCATION

Single particle cryo-EM has resolved dozens of structures of LeuT-fold transporters that have reinforced questions about the extent to which distinct conformational steps are conserved across transporters with this fold. Some facets of this question can be answered with confidence, such as the evolutionary persistence of the Na2 site or the ligand-binding modes of transporters within the same family. For example, point mutations in residues involved in substrate binding in GAT-1[151], dDAT[152], and BetP[153] were sufficient to modulate cargo preferences. This indicates that, even without access to structures in multiple conformational states, initial crystallographic data could answer key questions about how evolution fine-tunes the substrate-binding site within individual families.

In sharp contrast, the recent surge of structural data has failed to unmask deeper relationships surrounding the sequence and structural determinants of alternating access. The source of variations in the alternating access mechanisms and the corresponding energy landscapes between NSSs and SSSs remains to be unlocked. Indeed, it is unclear why this variation evolved at all, or if they are relevant to other understudied families such as APC and/or Nramp transporters. In part, this knowledge gap stems from the recent prioritization of the acquisition of high-resolution structures, rather than a deeper understanding of dynamics. Recent HDX-MS studies in the NSSs LeuT, SERT, and DAT have shed light on how the kinetics and structural movements of even closely related transporters can differ[11,104,109]. Moreover, spectroscopic and cysteine accessibility in studies in the SSSs SGLT1, vSGLT, and PutP highlight variations in their response to sodium binding, suggesting that these intra-family differences extend to energy landscapes as well. Thus, the apparent variation between LeuT-fold transporters in different families may reflect accumulated changes that occur at the family level which are only now coming to light.

Although formidable, the task of determining alternating access mechanisms of LeuT-fold transporters will almost certainly benefit from the recent development of high accuracy *de novo* structure prediction tools, particularly for families lacking representatives in the Protein Data Bank[83,154,155]. Further advancements and modifications that facilitate prediction of conformational dynamics may reveal the extent to which mechanisms of alternating access are conserved both within and across the LeuT-fold[156]. However, despite these advancements, only experiments can provide a rich understanding of transport dynamics of individual proteins, such as their energy minima and the rate-limiting steps of transport.

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REFERENCES

[1] P.D. Bosshart, D. Fotiadis, Secondary Active Transporters, Subcellular Biochemistry. 92 (2019) 275–299. https://doi.org/10.1007/978-3-030-18768-2_9.

[2] D. Drew, O. Boudker, Shared Molecular Mechanisms of Membrane Transporters, Annual Review of Biochemistry. 85 (2016) 543–572. https://doi.org/10.1146/annurev-biochem-060815-014520.

[3] Y. Shi, Common folds and transport mechanisms of secondary active transporters, Annu Rev Biophys. 42 (2013) 51–72. https://doi.org/10.1146/annurev-biophys-083012-130429.

[4] O. Jardetzky, Simple allosteric model for membrane pumps [27], Nature. 211 (1966) 969–970. https://doi.org/10.1038/211969a0.

[5] P. Mitchell, A general theory of membrane transport from studies of bacteria, Nature. 180 (1957) 134–136. https://doi.org/10.1038/180134a0.

[6] S. Hofmann, D. Januliene, A.R. Mehdipour, C. Thomas, E. Stefan, S. Brüchert, B.T. Kuhn, E.R. Geertsma, G. Hummer, R. Tampé, A. Moeller, Conformation space of a heterodimeric ABC exporter under turnover conditions, Nature. 571 (2019) 580–583. https://doi.org/10.1038/s41586-019-1391-0.

[7] C. Perez, C. Koshy, Ö. Yildiz, C. Ziegler, Alternating-access mechanism in conformationally asymmetric trimers of the betaine transporter BetP, Nature. 490 (2012) 126–130. https://doi.org/10.1038/nature11403.

[8] 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. https://doi.org/10.1038/nature03978.
[9] Y. Zhao, D. Terry, L. Shi, H. Weinstein, S.C. Blanchard, J.A. Javitch, Single-molecule dynamics of gating in a neurotransmitter transporter homologue, Nature. 465 (2010) 188–193. https://doi.org/10.1038/nature09057.
[10] J. Fan, Y. Xiao, M. Quick, Y. Yang, Z. Sun, J.A. Javitch, X. Zhou, Crystal structures of LeuT reveal conformational dynamics in the outward-facing states, Journal of Biological Chemistry. 296 (2021) 100609. https://doi.org/10.1016/j.jbc.2021.100609.
[11] P.S. Merkle, K. Gotfryd, M.A. Cuendet, K.Z. Leth-Espensen, U. Gether, C.J. Loland, K.D. Rand, Substrate-modulated unwinding of transmembrane helices in the NSS transporter LeuT, Science Advances. 4 (2018). https://doi.org/10.1126/sciadv.aar6179.
[12] D. Ciftci, C. Martens, V.G. Ghani, S.C. Blanchard, A. Politis, G.H.M. Huysmans, O. Boudker, Linking function to global and local dynamics in an elevator-type transporter, Proceedings to the National Academy of Sciences of the United States of America. 118 (2021) 1–9. https://doi.org/10.1073/pnas.2025520118/-/DCSupplemental.
[13] H. Krishnamurthy, E. Gouaux, X-ray structures of LeuT in substrate-free outward-open and apo inward-open states, Nature. 481 (2012) 469–474. https://doi.org/10.1038/nature10737.
[14] 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, Nature Structural and Molecular Biology. 21 (2014) 1006–1012. https://doi.org/10.1038/nsmb.2894.
[15] S.K. Singh, C.L. Piscitelli, A. Yamashita, E. Gouaux, A competitive inhibitor traps LeuT in an open-to-out conformation, Science (1979). 322 (2008) 1655–1661. https://doi.org/10.1126/science.1166777.
[16] S. Adhikary, D.J. Deredge, A. Nagarajan, L.R. Forrest, P.L. Wintrode, S.K. Singh, Conformational dynamics of a neurotransmitter: Sodium symporter in a lipid bilayer, Proc Natl Acad Sci U S A. 114 (2017) E1786--E1795. https://doi.org/10.1073/pnas.1613293114.
[17] C.B. Billesbølle, J.S. Mortensen, A. Sohail, S.G. Schmidt, L. Shi, H.H. Sitte, U. Gether, C.J. Loland, Transition metal ion FRET uncovers K+ regulation of a neurotransmitter/sodium symporter, Nature Communications. 7 (2016). https://doi.org/10.1038/ncomms12755.
[18] C.B. Billesbølle, M.B. Krüger, L. Shi, M. Quick, Z. Li, S. Stolzenberg, J. Kniazeff, K. Gotfryd, J.S. Mortensen, J.A. Javitch, H. Weinstein, C.J. Loland, U. Gether, Substrate-induced unlocking of the inner gate determines the catalytic efficiency of a neurotransmitter: Sodium symporter, Journal of Biological Chemistry. 290 (2015) 26725–26738. https://doi.org/10.1074/jbc.M115.677658.
[19] D.P. Claxton, M. Quick, L. Shi, F.D. de Carvalho, H. Weinstein, J.A. Javitch, H.S. McHaourab, Ion/substrate-dependent conformational dynamics of a bacterial homolog of neurotransmitter:sodium symporters, Nature Structural and Molecular Biology. 17 (2010) 822–829. https://doi.org/10.1038/nsmb.1854.
[20] 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,

Nature Structural and Molecular Biology. 21 (2014) 472–479. https://doi.org/10.1038/nsmb.2816.
[21] Y. Zhao, D.S. Terry, L. Shi, M. Quick, H. Weinstein, S.C. Blanchard, J.A. Javitch, Substrate-modulated gating dynamics in a Na+-coupled neurotransmitter transporter homologue, Nature. 474 (2011) 109–113. https://doi.org/10.1038/nature09971.
[22] K. Gotfryd, T. Boesen, J.S. Mortensen, G. Khelashvili, M. Quick, D.S. Terry, J.W. Missel, M. v LeVine, P. Gourdon, S.C. Blanchard, J.A. Javitch, H. Weinstein, C.J. Loland, P. Nissen, U. Gether, X-ray structure of LeuT in an inward-facing occluded conformation reveals mechanism of substrate release, Nature Communications. 11 (2020). https://doi.org/10.1038/s41467-020-14735-w.
[23] T. Beuming, L. Shi, J.A. Javitch, H. Weinstein, A comprehensive structure-based alignment of prokaryotic and eukaryotic neurotransmitter/Na+ symporters (NSS) aids in the use of the LeuT structure to probe NSS structure and function, Molecular Pharmacology. 70 (2006) 1630–1642. https://doi.org/10.1124/mol.106.026120.
[24] J.A. Coleman, E.M. Green, E. Gouaux, X-ray structures and mechanism of the human serotonin transporter, Nature. 532 (2016) 334–339. https://doi.org/10.1038/nature17629.
[25] A. Penmatsa, K.H. Wang, E. Gouaux, X-ray structure of dopamine transporter elucidates antidepressant mechanism, Nature. 503 (2013) 85–90. https://doi.org/10.1038/nature12533.
[26] A.T. Bozzi, R. Gaudet, Molecular Mechanism of Nramp-Family Transition Metal Transport, Journal of Molecular Biology. (2021) 166991. https://doi.org/10.1016/j.jmb.2021.166991.
[27] M.H. Cheng, I. Bahar, Monoamine transporters: structure, intrinsic dynamics and allosteric regulation, Nature Structural and Molecular Biology. 26 (2019) 545–556. https://doi.org/10.1038/s41594-019-0253-7.
[28] T.A. Chew, J. Zhang, L. Feng, High-resolution views and transport mechanisms of the NKCC1 and KCC transporters, Journal of Molecular Biology. 433 (2021) 167056. https://doi.org/10.1016/j.jmb.2021.167056.
[29] A. Nicolàs-Aragó, J. Fort, M. Palacín, E. Errasti-Murugarren, Rush hour of LATs towards their transport cycle, Membranes (Basel). 11 (2021). https://doi.org/10.3390/membranes11080602.
[30] T. Henriquez, L. Wirtz, D. Su, H. Jung, Prokaryotic solute/sodium symporters: Versatile functions and mechanisms of a transporter family †, International Journal of Molecular Sciences. 22 (2021) 1–21. https://doi.org/10.3390/ijms22041880.
[31] E.M. Krammer, M. Prévost, Function and Regulation of Acid Resistance Antiporters, Journal of Membrane Biology. 252 (2019) 465–481. https://doi.org/10.1007/s00232-019-00073-6.
[32] S.G. Patching, Recent developments in nucleobase cation symporter-1 (NCS1) family transport proteins from bacteria, archaea, fungi and plants, Journal of Biosciences. 43 (2018) 797–815. https://doi.org/10.1007/s12038-018-9780-3.
[33] G. Rudnick, W. Sandtner, Serotonin transport in the 21st century, Journal of General Physiology. 151 (2019) 1248–1264. https://doi.org/10.1085/JGP.201812066.
[34] N. Singh, G.F. Ecker, Insights into the structure, function, and ligand discovery of the large neutral amino acid transporter 1, lat1, International Journal of Molecular Sciences. 19 (2018) 1–32. https://doi.org/10.3390/ijms19051278.

[35] R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Guo, Q. Zhou, Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2, Science (1979). 367 (2020) 1444–1448. https://doi.org/10.1126/science.abb2762.
[36] K.H. Wang, A. Penmatsa, E. Gouaux, Neurotransmitter and psychostimulant recognition by the dopamine transporter, Nature. 521 (2015) 322–327. https://doi.org/10.1038/nature14431.
[37] A. Shahsavar, P. Stohler, G. Bourenkov, I. Zimmermann, M. Siegrist, W. Guba, E. Pinard, S. Sinning, M.A. Seeger, T.R. Schneider, R.J.P. Dawson, P. Nissen, Structural insights into the inhibition of glycine reuptake, Nature. 591 (2021) 677–681. https://doi.org/10.1038/s41586-021-03274-z.
[38] L. Malinauskaite, S. Said, C. Sahin, J. Grouleff, A. Shahsavar, H. Bjerregaard, P. Noer, K. Severinsen, T. Boesen, B. Schiøtt, S. Sinning, P. Nissen, A conserved leucine occupies the empty substrate site of LeuT in the Na+-free return state, Nature Communications. 7 (2016) 1–11. https://doi.org/10.1038/ncomms11673.
[39] D. Yang, E. Gouaux, Illumination of serotonin transporter mechanism and role of the allosteric site, 2021. https://www.science.org.
[40] J.A. Coleman, D. Yang, Z. Zhao, P.C. Wen, C. Yoshioka, E. Tajkhorshid, E. Gouaux, Serotonin transporter–ibogaine complexes illuminate mechanisms of inhibition and transport, Nature. 569 (2019) 141–145. https://doi.org/10.1038/s41586-019-1135-1.
[41] L. Kowalczyk, M. Ratera, A. Paladino, P. Bartoccioni, E. Errasti-Murugarren, E. Valencia, G. Portella, S. Bial, A. Zorzano, I. Fita, M. Orozco, X. Carpena, J.L. Vázquez-Ibar, M. Palacín, Molecular basis of substrate-induced permeation by an amino acid antiporter, Proc Natl Acad Sci U S A. 108 (2011) 3935–3940. https://doi.org/10.1073/pnas.1018081108.
[42] H. Ilgü, J.M. Jeckelmann, D. Kalbermatter, Z. Ucurum, T. Lemmin, D. Fotiadis, High-resolution structure of the amino acid transporter AdiC reveals insights into the role of water molecules and networks in oligomerization and substrate binding, BMC Biology. 19 (2021). https://doi.org/10.1186/s12915-021-01102-4.
[43] H. Ilgü, J.M. Jeckelmanna, V. Gapsys, Z. Ucurum, B.L. de Grootc, D. Fotiadis, Insights into the molecular basis for substrate binding and specificity of the wild-type L-arginine/agmatine antiporter AdiC, Proc Natl Acad Sci U S A. 113 (2016) 10358–10363. https://doi.org/10.1073/pnas.1605442113.
[44] Y. Fang, H. Jayaram, T. Shane, L. Kolmakova-Partensky, F. Wu, C. Williams, Y. Xiong, C. Miller, Structure of a prokaryotic virtual proton pump at 3.2 Åresolution, Nature. 460 (2009) 1040–1043. https://doi.org/10.1038/nature08201.
[45] P.L. Shaffer, A. Goehring, A. Shankaranarayanan, E. Gouaux, Structure and mechanism of a Na+-independent amino acid transporter, Science (1979). 325 (2009) 1010–1014. https://doi.org/10.1126/science.1176088.
[46] R. Yan, Y. Li, Y. Shi, J. Zhou, J. Lei, J. Huang, Q. Zhou, Cryo-EM structure of the human heteromeric amino acid transporter b0,+AT-rBAT, Science Advances. 6 (2020). https://doi.org/10.1126/sciadv.aay6379.
[47] E. Errasti-Murugarren, J. Fort, P. Bartoccioni, L. Díaz, E. Pardon, X. Carpena, M. Espino-Guarch, A. Zorzano, C. Ziegler, J. Steyaert, J. Fernández-Recio, I. Fita, M. Palacín, L amino acid transporter structure and molecular bases for the asymmetry of substrate interaction, Nature Communications. 10 (2019). https://doi.org/10.1038/s41467-019-09837-z.

[48] D. Ma, P. Lu, C. Yan, C. Fan, P. Yin, J. Wang, Y. Shi, Structure and mechanism of a glutamate-GABA antiporter, Nature. 483 (2012) 632–636. https://doi.org/10.1038/nature10917.
[49] K.E.J. Jungnickel, J.L. Parker, S. Newstead, Structural basis for amino acid transport by the CAT family of SLC7 transporters, Nature Communications. 9 (2018) 1–12. https://doi.org/10.1038/s41467-018-03066-6.
[50] R. Yan, Y. Li, J. Müller, Y. Zhang, S. Singer, L. Xia, X. Zhong, J. Gertsch, K.H. Altmann, Q. Zhou, Mechanism of substrate transport and inhibition of the human LAT1-4F2hc amino acid transporter, Cell Discovery. 7 (2021). https://doi.org/10.1038/s41421-021-00247-4.
[51] R. Yan, X. Zhao, J. Lei, Q. Zhou, Structure of the human LAT1–4F2hc heteromeric amino acid transporter complex, Nature. 568 (2019) 127–130. https://doi.org/10.1038/s41586-019-1011-z.
[52] Y. Lee, P. Wiriyasermkul, C. Jin, L. Quan, R. Ohgaki, S. Okuda, T. Kusakizako, T. Nishizawa, K. Oda, R. Ishitani, T. Yokoyama, T. Nakane, M. Shirouzu, H. Endou, S. Nagamori, Y. Kanai, O. Nureki, Cryo-EM structure of the human L-type amino acid transporter 1 in complex with glycoprotein CD98hc, Nature Structural and Molecular Biology. 26 (2019) 510–517. https://doi.org/10.1038/s41594-019-0237-7.
[53] R. Yan, J. Zhou, Y. Li, J. Lei, Q. Zhou, Structural insight into the substrate recognition and transport mechanism of the human LAT2–4F2hc complex, Cell Discovery. 6 (2020) 20–23. https://doi.org/10.1038/s41421-020-00207-4.
[54] C.F. Rodriguez, P. Escudero-Bravo, L. Díaz, P. Bartoccioni, C. García-Martín, J.G. Gilabert, J. Boskovic, V. Guallar, E. Errasti-Murugarren, O. Llorca, M. Palacín, Structural basis for substrate specificity of heteromeric transporters of neutral amino acids, Proceedings of the National Academy of Sciences. 118 (2021) e2113573118. https://doi.org/10.1073/pnas.2113573118.
[55] J.L. Parker, J.C. Deme, D. Kolokouris, G. Kuteyi, P.C. Biggin, S.M. Lea, S. Newstead, Molecular basis for redox control by the human cystine/glutamate antiporter system xc−, Nature Communications. 12 (2021). https://doi.org/10.1038/s41467-021-27414-1.
[56] S. Liu, S. Chang, B. Han, L. Xu, M. Zhang, C. Zhao, W. Yang, F. Wang, J. Li, E. Delpire, S. Ye, X.C. Bai, J. Guo, Cryo-EM structures of the human cation-chloride cotransporter KCC1, Science (1979). 366 (2019) 505–508. https://doi.org/10.1126/science.aay3129.
[57] Y. Xie, S. Chang, C. Zhao, F. Wang, S. Liu, J. Wang, E. Delpire, S. Ye, J. Guo, Structures and an activation mechanism of human potassium-chloride cotransporters, Science Advances. 6 (2020). https://doi.org/10.1126/sciadv.abc5883.
[58] G. Chi, R. Ebenhoch, H. Man, H. Tang, L.E. Tremblay, G. Reggiano, X. Qiu, T. Bohstedt, I. Liko, F.G. Almeida, A.P. Garneau, D. Wang, G. McKinley, C.P. Moreau, K.D. Bountra, P. Abrusci, S.M.M. Mukhopadhyay, A. Fernandez-Cid, S. Slimani, J.L. Lavoie, N.A. Burgess-Brown, B. Tehan, F. DiMaio, A. Jazayeri, P. Isenring, C. v Robinson, K.L. Dürr, Phospho-regulation, nucleotide binding and ion access control in potassium-chloride cotransporters, The EMBO Journal. (2021) 1–20. https://doi.org/10.15252/embj.2020107294.
[59] M.S. Reid, D.M. Kern, S.G. Brohawn, Cryo-EM structure of the potassium-chloride cotransporter KCC4 in lipid nanodiscs, Elife. 9 (2020). https://doi.org/10.7554/eLife.52505.

[60] X. Yang, Q. Wang, E. Cao, Structure of the human cation–chloride cotransporter NKCC1 determined by single-particle electron cryo-microscopy, Nature Communications. 11 (2020). https://doi.org/10.1038/s41467-020-14790-3.
[61] T.A. Chew, B.J. Orlando, J. Zhang, N.R. Latorraca, A. Wang, S.A. Hollingsworth, D.H. Chen, R.O. Dror, M. Liao, L. Feng, Structure and mechanism of the cation–chloride cotransporter NKCC1, Nature. 572 (2019) 488–492. https://doi.org/10.1038/s41586-019-1438-2.
[62] S. Ressl, A.C. Terwisscha Van Scheltinga, C. Vonrhein, V. Ott, C. Ziegler, Molecular basis of transport and regulation in the Na+/betaine symporter BetP, Nature. 458 (2009) 47–52. https://doi.org/10.1038/nature07819.
[63] C. Perez, K. Khafizov, L.R. Forrest, R. Krämer, C. Ziegler, The role of trimerization in the osmoregulated betaine transporter BetP, EMBO Reports. 12 (2011) 804–810. https://doi.org/10.1038/embor.2011.102.
[64] S. Schulze, S. Köster, U. Geldmacher, A.C. Terwisscha Van Scheltinga, W. Kühlbrandt, Structural basis of Na+-independent and cooperative substrate/product antiport in CaiT, Nature. 467 (2010) 233–236. https://doi.org/10.1038/nature09310.
[65] L. Tang, L. Bai, W.H. Wang, T. Jiang, Crystal structure of the carnitine transporter and insights into the antiport mechanism, Nature Structural and Molecular Biology. 17 (2010) 492–496. https://doi.org/10.1038/nsmb.1788.
[66] A.T. Bozzi, C.M. Zimanyi, J.M. Nicoludis, B.K. Lee, C.H. Zhang, R. Gaudet, Structures in multiple conformations reveal distinct transition metal and proton pathways in an nramp transporter, Elife. 8 (2019) 1–63. https://doi.org/10.7554/eLife.41124.
[67] A.T. Bozzi, L.B. Bane, W.A. Weihofen, A. Singharoy, E.R. Guillen, H.L. Ploegh, K. Schulten, R. Gaudet, Crystal Structure and Conformational Change Mechanism of a Bacterial Nramp-Family Divalent Metal Transporter, Structure. 24 (2016) 2102–2114. https://doi.org/10.1016/j.str.2016.09.017.
[68] I.A. Ehrnstorfer, C. Manatschal, F.M. Arnold, J. Laederach, R. Dutzler, Structural and mechanistic basis of proton-coupled metal ion transport in the SLC11/NRAMP family, Nature Communications. 8 (2017) 1–11. https://doi.org/10.1038/ncomms14033.
[69] K. Ramanadane, M.S. Straub, R. Dutzler, C. Manatschal, Structural and functional properties of a magnesium transporter of the SLC11/NRAMP family, Elife. 11 (2022). https://doi.org/10.7554/elife.74589.
[70] I.A. Ehrnstorfer, E.R. Geertsma, E. Pardon, J. Steyaert, R. Dutzler, Crystal structure of a SLC11 (NRAMP) transporter reveals the basis for transition-metal ion transport, Nature Structural and Molecular Biology. 21 (2014) 990–996. https://doi.org/10.1038/nsmb.2904.
[71] W.Y. Wahlgren, E. Dunevall, R.A. North, A. Paz, M. Scalise, P. Bisignano, J. Bengtsson-Palme, P. Goyal, E. Claesson, R. Caing-Carlsson, R. Andersson, K. Beis, U.J. Nilsson, A. Farewell, L. Pochini, C. Indiveri, M. Grabe, R.C.J. Dobson, J. Abramson, S. Ramaswamy, R. Friemann, Substrate-bound outward-open structure of a Na+-coupled sialic acid symporter reveals a new Na+ site, Nature Communications. 9 (2018) 1–14. https://doi.org/10.1038/s41467-018-04045-7.
[72] L. Han, Q. Qu, D. Aydin, O. Panova, M.J. Robertson, Y. Xu, R.O. Dror, G. Skiniotis, L. Feng, Structure and mechanism of the SGLT family of glucose transporters, Nature. 601 (2022) 274–279. https://doi.org/10.1038/s41586-021-04211-w.

[73] Y. Niu, R. Liu, C. Guan, Y. Zhang, Z. Chen, S. Hoerer, H. Nar, L. Chen, Structural basis of inhibition of the human SGLT2–MAP17 glucose transporter, Nature. 601 (2022) 280–284. https://doi.org/10.1038/s41586-021-04212-9.
[74] S. Faham, A. Watanabe, G.M. Besserer, D. Cascio, A. Specht, B.A. Hirayama, E.M. Wright, J. Abramson, The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport, Science (1979). 321 (2008) 810–814. https://doi.org/10.1126/science.1160406.
[75] A. Watanabe, S. Choe, V. Chaptal, J.M. Rosenberg, E.M. Wright, M. Grabe, J. Abramson, The mechanism of sodium and substrate release from the binding pocket of vSGLT, Nature. 468 (2010) 988–991. https://doi.org/10.1038/nature09580.
[76] I. Tascón, J.S. Sousa, R.A. Corey, D.J. Mills, D. Griwatz, N. Aumüller, V. Mikusevic, P.J. Stansfeld, J. Vonck, I. Hänelt, Structural basis of proton-coupled potassium transport in the KUP family, Nature Communications. 11 (2020). https://doi.org/10.1038/s41467-020-14441-7.
[77] H.T. Lei, J. Ma, S. Sanchez Martinez, T. Gonen, Crystal structure of arginine-bound lysosomal transporter SLC38A9 in the cytosol-open state, Nature Structural and Molecular Biology. 25 (2018) 522–527. https://doi.org/10.1038/s41594-018-0072-2.
[78] H.T. Lei, X. Mu, J. Hattne, T. Gonen, A conformational change in the N terminus of SLC38A9 signals mTORC1 activation, Structure. 29 (2021) 426-432.e8. https://doi.org/10.1016/j.str.2020.11.014.
[79] S. Weyand, T. Shimamura, S. Yajima, S.N.I. Suzuki, O. Mirza, K. Krusong, E.P. Carpenter, N.G. Rutherford, J.M. Hadden, J. O'Reilly, P. Ma, M. Saidijam, S.G. Patching, R.J. Hope, H.T. Norbertczak, P.C.J. Roach, S. Iwata, P.J.F. Henderson, A.D. Cameron, Structure and molecular mechanism of a nucleobase-cation-symport-1 family transporter, Science (1979). 322 (2008) 709–713. https://doi.org/10.1126/science.1164440.
[80] K.J. Simmons, S.M. Jackson, F. Brueckner, S.G. Patching, O. Beckstein, E. Ivanova, T. Geng, S. Weyand, D. Drew, J. Lanigan, D.J. Sharples, M.S. Sansom, S. Iwata, C.W. Fishwick, A.P. Johnson, A.D. Cameron, P.J. Henderson, Molecular mechanism of ligand recognition by membrane transport protein, Mhp1, The EMBO Journal. 33 (2014) 1831–1844. https://doi.org/10.15252/embj.201387557.
[81] T. Shimamura, S. Weyand, O. Beckstein, N.G. Rutherford, J.M. Hadden, D. Sharpies, M.S.P. Sansom, S. Iwata, P.J.F. Henderson, A.D. Cameron, Molecular basis of alternating access membrane transport by the sodium-hydantoin transporter Mhp1, Science (1979). 328 (2010) 470–473. https://doi.org/10.1126/science.1186303.
[82] J. Ma, H.T. Lei, F.E. Reyes, S. Sanchez-Martinez, M.F. Sarhan, J. Hattne, T. Gonen, Structural basis for substrate binding and specificity of a sodium-alanine symporter AgcS, Proc Natl Acad Sci U S A. 116 (2019) 2086–2090. https://doi.org/10.1073/pnas.1806206116.
[83] A. Vastermark, S. Wollwage, M.E. Houle, R. Rio, M.H. Saier, Expansion of the APC superfamily of secondary carriers, Proteins: Structure, Function and Bioinformatics. 82 (2014) 2797–2811. https://doi.org/10.1002/prot.24643.
[84] L.R. Forrest, Y.W. Zhang, M.T. Jacobs, J. Gesmonde, L. Xie, B.H. Honig, G. Rudnick, Mechanism for alternating access in neurotransmitter transporters, Proc Natl Acad Sci U S A. 105 (2008) 10338–10343. https://doi.org/10.1073/pnas.0804659105.

[85] K. Kazmier, D.P. Claxton, H.S. Mchaourab, Alternating access mechanisms of LeuT-fold transporters: trailblazing towards the promised energy landscapes, Current Opinion in Structural Biology. 45 (2017) 100–108. https://doi.org/10.1016/j.sbi.2016.12.006.
[86] E. Screpanti, C. Hunte, Discontinuous membrane helices in transport proteins and their correlation with function, Journal of Structural Biology. 159 (2007) 261–267. https://doi.org/10.1016/j.jsb.2007.01.011.
[87] S. Stolzenberg, Z. Li, M. Quick, L. Malinauskaite, P. Nissen, H. Weinstein, J.A. Javitch, L. Shi, The role of transmembrane segment 5 (TM5) in Na2 release and the conformational transition of neurotransmitter:sodium symporters toward the inward-open state, Journal of Biological Chemistry. 292 (2017) 7372–7384. https://doi.org/10.1074/jbc.M116.757153.
[88] J. Abramson, E.M. Wright, Structure and function of Na+-symporters with inverted repeats, Current Opinion in Structural Biology. 19 (2009) 425–432. https://doi.org/10.1016/j.sbi.2009.06.002.
[89] C. Ziegler, E. Bremer, R. Krämer, The BCCT family of carriers: From physiology to crystal structure, Molecular Microbiology. 78 (2010) 13–34. https://doi.org/10.1111/j.1365-2958.2010.07332.x.
[90] M. Rebsamen, L. Pochini, T. Stasyk, M.E.G. de Araújo, M. Galluccio, R.K. Kandasamy, B. Snijder, A. Fauster, E.L. Rudashevskaya, M. Bruckner, S. Scorzoni, P.A. Filipek, K.V.M. Huber, J.W. Bigenzahn, L.X. Heinz, C. Kraft, K.L. Bennett, C. Indiveri, L.A. Huber, G. Superti-Furga, SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1, Nature. 519 (2015) 477–481. https://doi.org/10.1038/nature14107.
[91] S. Wang, Z.Y. Tsun, R.L. Wolfson, K. Shen, G.A. Wyant, M.E. Plovanich, E.D. Yuan, T.D. Jones, L. Chantranupong, W. Comb, T. Wang, L. Bar-Peled, R. Zoncu, C. Straub, C. Kim, J. Park, B.L. Sabatini, D.M. Sabatini, Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1, Science (1979). 347 (2015) 188–194. https://doi.org/10.1126/science.1257132.
[92] S.A. Fromm, R.E. Lawrence, J.H. Hurley, Structural mechanism for amino acid-dependent Rag GTPase nucleotide state switching by SLC38A9, Nature Structural and Molecular Biology. 27 (2020) 1017–1023. https://doi.org/10.1038/s41594-020-0490-9.
[93] A. Cooper, D. Woulfe, F. Kilic, Post-translational modifications of serotonin transporter, Pharmacological Research. 140 (2019) 7–13. https://doi.org/10.1016/j.phrs.2018.10.018.
[94] C.S. Karam, J.A. Javitch, Phosphorylation of the Amino Terminus of the Dopamine Transporter: Regulatory Mechanisms and Implications for Amphetamine Action, Advances in Pharmacology. 82 (2018) 205–234. https://doi.org/10.1016/bs.apha.2017.09.002.
[95] G. Güler, R.M. Gärtner, C. Ziegler, W. Mäntele, Lipid-protein interactions in the regulated betaine symporter BetP probed by infrared spectroscopy, Journal of Biological Chemistry. 291 (2016) 4295–4307. https://doi.org/10.1074/jbc.M114.621979.
[96] R. Krämer, C. Ziegler, Regulative interactions of the osmosensing C-terminal domain in the trimeric glycine betaine transporter BetP from Corynebacterium glutamicum, Biological Chemistry. 390 (2009) 685–691. https://doi.org/10.1515/BC.2009.068.
[97] M. Gur, M.H. Cheng, E. Zomot, I. Bahar, Effect of Dimerization on the Dynamics of Neurotransmitter:Sodium Symporters, Journal of Physical Chemistry B. 121 (2017) 3657–3666. https://doi.org/10.1021/acs.jpcb.6b09876.

[98] L. Ponzoni, S. Zhang, M.H. Cheng, I. Bahar, Shared dynamics of LeuT superfamily members and allosteric differentiation by structural irregularities and multimerization, Philosophical Transactions of the Royal Society B: Biological Sciences. 373 (2018). https://doi.org/10.1098/rstb.2017.0177.
[99] S. Faham, A. Watanabe, G.M. Besserer, D. Cascio, A. Specht, B.A. Hirayama, E.M. Wright, J. Abramson, The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport, Science (1979). 321 (2008) 810–814. https://doi.org/10.1126/science.1160406.
[100] K. Khafizov, C. Perez, C. Koshy, M. Quick, K. Fendler, C. Ziegler, L.R. Forrest, Investigation of the sodium-binding sites in the sodium-coupled betaine transporter BetP, Proc Natl Acad Sci U S A. 109 (2012). https://doi.org/10.1073/pnas.1209039109.
[101] X. Gao, F. Lu, L. Zhou, S. Dang, L. Sun, X. Li, J. Wang, Y. Shi, Structure and mechanism of an Amino Acid Antiporter, Science (1979). 324 (2009) 1565–1568. https://doi.org/10.1126/science.1176088.
[102] D. Calugareanu, I.R. Möller, S.G. Schmidt, C.J. Loland, K.D. Rand, Probing the Impact of Temperature and Substrates on the Conformational Dynamics of the Neurotransmitter:Sodium symporter LeuT, Journal of Molecular Biology. 434 (2022) 167356. https://doi.org/10.1016/j.jmb.2021.167356.
[103] I.R. Möller, P.S. Merkle, D. Calugareanu, G. Comamala, S.G. Schmidt, C.J. Loland, K.D. Rand, Probing the conformational impact of detergents on the integral membrane protein LeuT by global HDX-MS, Journal of Proteomics. 225 (2020) 103845. https://doi.org/10.1016/j.jprot.2020.103845.
[104] I.R. Möller, M. Slivacka, A.K. Nielsen, S.G.F. Rasmussen, U. Gether, C.J. Loland, K.D. Rand, Conformational dynamics of the human serotonin transporter during substrate and drug binding, Nature Communications. 10 (2019). https://doi.org/10.1038/s41467-019-09675-z.
[105] L. Shi, M. Quick, Y. 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, Molecular Cell. 30 (2008) 667–677. https://doi.org/10.1016/j.molcel.2008.05.008.
[106] Y. Zhao, D. Terry, L. Shi, H. Weinstein, S.C. Blanchard, J.A. Javitch, Single-molecule dynamics of gating in a neurotransmitter transporter homologue, Nature. 465 (2010) 188–193. https://doi.org/10.1038/nature09057.
[107] K. Kazmier, S. Sharma, S.M. Islam, B. Roux, H.S. Mchaourab, E.M. Wright, Conformational cycle and ion-coupling mechanism of the Na+/hydantoin transporter Mhp1, Proc Natl Acad Sci U S A. 111 (2014) 14752–14757. https://doi.org/10.1073/pnas.1410431111.
[108] L. Shi, W. Harel, Conformational rearrangements to the intracellular open states of the LeuT and ApcT transporters are modulated by common mechanisms, Biophysical Journal. 99 (2010) L103–L105. https://doi.org/10.1016/j.bpj.2010.10.003.
[109] A.K. Nielsen, I.R. Möller, Y. Wang, S.G.F. Rasmussen, K. Lindorff-Larsen, K.D. Rand, C.J. Loland, Substrate-induced conformational dynamics of the dopamine transporter, Nature Communications. 10 (2019) 1–14. https://doi.org/10.1038/s41467-019-10449-w.
[110] A. Sohail, K. Jayaraman, S. Venkatesan, K. Gotfryd, M. Daerr, U. Gether, C.J. Loland, K.T. Wanner, M. Freissmuth, H.H. Sitte, W. Sandtner, T. Stockner, The Environment

Shapes the Inner Vestibule of LeuT, PLoS Computational Biology. 12 (2016). https://doi.org/10.1371/journal.pcbi.1005197.
[111] C.J. Loland, L. Norregaard, T. Litman, U. Gether, Generation of an activating Zn2+ switch in the dopamine transporter: Mutation of an intracellular tyrosine constitutively alters the conformational equilibrium of the transport cycle, Proc Natl Acad Sci U S A. 99 (2002) 1683–1688. https://doi.org/10.1073/pnas.032386299.
[112] M. Quick, A.M.L. 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. https://doi.org/10.1073/pnas.0811322106.
[113] A. Paz, D.P. Claxton, J.P. Kumar, K. Kazmier, P. Bisignano, S. Sharma, S.A. Nolte, T.M. Liwag, V. Nayak, E.M. Wright, M. Grabe, H.S. McHaourab, J. Abramson, Conformational transitions of the sodium-dependent sugar transporter, vSGLT, Proc Natl Acad Sci U S A. 115 (2018) E2742–E2751. https://doi.org/10.1073/pnas.1718451115.
[114] Y. Zhao, J. Shen, Q. Wang, M. Zhou, E. Cao, Inhibitory and Transport Mechanisms of the Human Cation-Chloride Cotransport KCC1, BioRxiv. (2020) 1–9. https://doi.org/10.1101/2020.07.26.221770.
[115] M. Barrios, M.-I. Moreno-Carralero, N. Cuadrado-Grande, M. Baro, J.-L. Vivanco, M.-J. Morán-Jiménez, The homozygous mutation G75R in the human SLC11A2 gene leads to microcytic anaemia and iron overload, British Journal of Haematology. 157 (2012) 510–514. https://doi.org/10.1111/j.1365-2141.2012.09042.x.
[116] X. Gao, L. Zhou, X. Jiao, F. Lu, C. Yan, X. Zeng, J. Wang, Y. Shi, Mechanism of substrate recognition and transport by an amino acid antiporter, Nature. 463 (2010) 828–832. https://doi.org/10.1038/nature08741.
[117] O. Boudker, G. Verdon, Structural perspectives on secondary active transporters, Trends in Pharmacological Sciences. 31 (2010) 418–426. https://doi.org/10.1016/j.tips.2010.06.004.
[118] A. Mullen, J. Hall, J. Diegel, I. Hassan, A. Fey, F. MacMillan, Membrane transporters studied by EPR spectroscopy: Structure determination and elucidation of functional dynamics, Biochemical Society Transactions. 44 (2016) 905–915. https://doi.org/10.1042/BST20160024.
[119] C.J. Tsai, C. Ziegler, Coupling electron cryomicroscopy and X-ray crystallography to understand secondary active transport, Current Opinion in Structural Biology. 20 (2010) 448–455. https://doi.org/10.1016/j.sbi.2010.06.005.
[120] G. Rudnick, Serotonin transporters - Structure and function, Journal of Membrane Biology. 213 (2006) 101–110. https://doi.org/10.1007/s00232-006-0878-4.
[121] J.M. Russell, Sodium-Potassium-Chloride Cotransport, Physiology Reviews. (2000) 211–276. http://www3.
[122] C.L. Piscitelli, H. Krishnamurthy, E. Gouaux, Neurotransmitter/sodium symporter orthologue LeuT has a single high-affinity substrate site, Nature. 468 (2010) 1129–1133. https://doi.org/10.1038/nature09581.
[123] G.A. Fitzgerald, D.S. Terry, A.L. Warren, M. Quick, J.A. Javitch, S.C. Blanchard, Quantifying secondary transport at single-molecule resolution, Nature. 575 (2019) 528–534. https://doi.org/10.1038/s41586-019-1747-5.
[124] D.S. Terry, R.A. Kolster, M. Quick, M. v. LeVine, G. Khelashvili, Z. Zhou, H. Weinstein, J.A. Javitch, S.C. Blanchard, A partially-open inward-facing intermediate conformation of

LeuT is associated with Na+ release and substrate transport, Nature Communications. 9 (2018). https://doi.org/10.1038/s41467-017-02202-y.
[125] Y.W. Zhang, S. Tavoulari, S. Sinning, A.A. Aleksandrova, L.R. Forrest, G. Rudnick, Structural elements required for coupling ion and substrate transport in the neurotransmitter transporter homolog LeuT, Proc Natl Acad Sci U S A. 115 (2018) E8854--E8862. https://doi.org/10.1073/pnas.1716870115.
[126] Y.W. Zhang, S. Uchendu, V. Leone, R.T. Bradshaw, N. Sangwa, L.R. Forrest, G. Rudnick, Chloride-dependent conformational changes in the GlyT1 glycine transporter, Proc Natl Acad Sci U S A. 118 (2021) 1–10. https://doi.org/10.1073/pnas.2017431118.
[127] S. Zhang, J. Zhou, Y. Zhang, T. Liu, P. Friedel, W. Zhuo, S. Somasekharan, K. Roy, L. Zhang, Y. Liu, X. Meng, H. Deng, W. Zeng, G. Li, B. Forbush, M. Yang, The structural basis of function and regulation of neuronal cotransporters NKCC1 and KCC2, Communications Biology. 4 (2021) 1–15. https://doi.org/10.1038/s42003-021-01750-w.
[128] N. Hansra, S. Arya, M.W. Quick, Intracellular Domains of a Rat Brain GABA Transporter that Govern Transport, Journal of Neuroscience. 24 (2004) 4082–4087. https://doi.org/10.1523/JNEUROSCI.0664-04.2004.
[129] K.L. Jagessar, D.P. Claxton, R.A. Stein, H.S. Mchaourab, Sequence and structural determinants of ligand-dependent alternating access of a MATE transporter., Proc Natl Acad Sci U S A. 117 (2020) 4732–4740. https://doi.org/10.1073/pnas.1917139117.
[130] S. Zakrzewska, A.R. Mehdipour, V.N. Malviya, T. Nonaka, J. Koepke, C. Muenke, W. Hausner, G. Hummer, S. Safarian, H. Michel, Inward-facing conformation of a multidrug resistance MATE family transporter, Proc Natl Acad Sci U S A. 116 (2019) 12275–12284. https://doi.org/10.1073/pnas.1904210116.
[131] C. Martens, R.A. Stein, M. Masureel, A. Roth, S. Mishra, R. Dawaliby, A. Konijnenberg, F. Sobott, C. Govaerts, H.S. McHaourab, Lipids modulate the conformational dynamics of a secondary multidrug transporter, Nature Structural and Molecular Biology. 23 (2016) 744–751. https://doi.org/10.1038/nsmb.3262.
[132] A. Penmatsa, K.H. Wang, E. Gouaux, X-ray structure of dopamine transporter elucidates antidepressant mechanism, Nature. 503 (2013) 85–90. https://doi.org/10.1038/nature12533.
[133] K. Immadisetty, J. Hettige, M. Moradi, Lipid-Dependent Alternating Access Mechanism of a Bacterial Multidrug ABC Exporter, ACS Central Science. 5 (2019) 43–56. https://doi.org/10.1021/acscentsci.8b00480.
[134] C. Martens, M. Shekhar, A.J. Borysik, A.M. Lau, E. Reading, E. Tajkhorshid, P.J. Booth, A. Politis, Direct protein-lipid interactions shape the conformational landscape of secondary transporters, Nature Communications. 9 (2018) 1–12. https://doi.org/10.1038/s41467-018-06704-1.
[135] M.F. Juette, D.S. Terry, M.R. Wasserman, Z. Zhou, R.B. Altman, Q. Zheng, S.C. Blanchard, The bright future of single-molecule fluorescence imaging, Current Opinion in Chemical Biology. 20 (2014) 103–111. https://doi.org/10.1016/j.cbpa.2014.05.010.
[136] B. Schuler, Single-molecule FRET of protein structure and dynamics - a primer., J Nanobiotechnology. 11 Suppl 1 (2013). https://doi.org/10.1186/1477-3155-11-S1-S2.
[137] L.R. Forrest, G. Rudnick, The rocking bundle: A mechanism for ion-coupled solute flux by symmetrical transporters, Physiology. 24 (2009) 377–386. https://doi.org/10.1152/physiol.00030.2009.

[138] M.C. Chan, B. Selvam, H.J. Young, E. Procko, D. Shukla, The substrate import mechanism of the human serotonin transporter, Biophysical Journal. (2022). https://doi.org/10.1016/j.bpj.2022.01.024.
[139] A.M. Razavi, G. Khelashvili, H. Weinstein, How structural elements evolving from bacterial to human SLC6 transporters enabled new functional properties, BMC Biology. 16 (2018) 1–14. https://doi.org/10.1186/s12915-018-0495-6.
[140] S. Bhat, M. Niello, K. Schicker, C. Pifl, H.H. Sitte, M. Freissmuth, W. Sandtner, Handling of intracellular k+ determines voltage dependence of plasmalemmal monoamine transporter function, Elife. 10 (2021) 1–23. https://doi.org/10.7554/eLife.67996.
[141] M. Raba, S. Dunkel, D. Hilger, K. Lipiszko, Y. Polyhach, G. Jeschke, S. Bracher, J.P. Klare, M. Quick, H. Jung, H.J. Steinhoff, Extracellular loop 4 of the proline transporter PutP controls the periplasmic entrance to ligand binding sites, Structure. 22 (2014) 769–780. https://doi.org/10.1016/j.str.2014.03.011.
[142] D.D.F. Loo, B.A. Hirayama, E.M. Gallardo, J.T. Lam, E. Turk, E.M. Wright, Conformational changes couple Na+ and glucose transport, Proc Natl Acad Sci U S A. 95 (1998) 7789–7794. https://doi.org/10.1073/pnas.95.13.7789.
[143] D.D.F. Loo, B.A. Hirayama, M.H. Karakossian, A.K. Meinild, E.M. Wright, Conformational dynamics of hSGLT1 during Na +/glucose cotransport, Journal of General Physiology. 128 (2006) 701–720. https://doi.org/10.1085/jgp.200609643.
[144] M. Sala-Rabanal, B.A. Hirayama, D.D.F. Loo, V. Chaptal, J. Abramson, E.M. Wright, Bridging the gap between structure and kinetics of human SGLT1, American Journal of Physiology - Cell Physiology. 302 (2012) 1293–1305. https://doi.org/10.1152/ajpcell.00397.2011.
[145] G. Jeschke, C. Wegener, M. Nietschke, H. Jung, H.J. Steinhoff, Interresidual Distance Determination by Four-Pulse Double Electron-Electron Resonance in an Integral Membrane Protein: The Na +/Proline Transporter PutP of Escherichia coli, Biophysical Journal. 86 (2004) 2551–2557. https://doi.org/10.1016/S0006-3495(04)74310-6.
[146] C. Wegener, S. Tebbe, H.J. Steinhoff, H. Jung, Spin labeling analysis of structure and dynamics of the Na+/proline transporter of Escherichia coli, Biochemistry. 39 (2000) 4831–4837. https://doi.org/10.1021/bi992442x.
[147] A.N. Calabrese, S.M. Jackson, L.N. Jones, O. Beckstein, F. Heinkel, J. Gsponer, D. Sharples, M. Sans, M. Kokkinidou, A.R. Pearson, S.E. Radford, A.E. Ashcroft, P.J.F. Henderson, Topological Dissection of the Membrane Transport Protein Mhp1 Derived from Cysteine Accessibility and Mass Spectrometry, Analytical Chemistry. 89 (2017) 8844–8852. https://doi.org/10.1021/acs.analchem.7b01310.
[148] S. Weyand, T. Shimamura, O. Beckstein, M.S.P. Sansom, S. Iwata, P.J.F. Henderson, A.D. Cameron, The alternating access mechanism of transport as observed in the sodium-hydantoin transporter Mhp1, Journal of Synchrotron Radiation. 18 (2011) 20–23. https://doi.org/10.1107/S0909049510032449.
[149] V. Leone, I. Waclawska, K. Kossmann, C. Koshy, M. Sharma, T.F. Prisner, C. Ziegler, B. Endeward, L.R. Forrest, Interpretation of spectroscopic data using molecular simulations for the secondary active transporter BetP, Journal of General Physiology. 151 (2019) 381–394. https://doi.org/10.1085/jgp.201812111.
[150] A.K. Meinild, B.A. Hirayama, E.M. Wright, D.D.F. Loo, Fluorescence studies of ligand-induced conformational changes of the Na+/glucose cotransporter, Biochemistry. 41 (2002) 1250–1258. https://doi.org/10.1021/bi011661r.

[151] 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. https://doi.org/10.1038/nature06133.
[152] S. Pidathala, A.K. Mallela, D. Joseph, A. Penmatsa, Structural basis of norepinephrine recognition and transport inhibition in neurotransmitter transporters, Nature Communications. 12 (2021). https://doi.org/10.1038/s41467-021-22385-9.
[153] C. Perez, B. Faust, A.R. Mehdipour, K.A. Francesconi, L.R. Forrest, C. Ziegler, Substrate-bound outward-open state of the betaine transporter BetP provides insights into Na+ coupling, Nature Communications. 5 (2014). https://doi.org/10.1038/ncomms5231.
[154] K. Tunyasuvunakool, J. Adler, Z. Wu, T. Green, M. Zielinski, A. Žídek, A. Bridgland, A. Cowie, C. Meyer, A. Laydon, S. Velankar, G.J. Kleywegt, A. Bateman, R. Evans, A. Pritzel, M. Figurnov, O. Ronneberger, R. Bates, S.A.A. Kohl, A. Potapenko, A.J. Ballard, B. Romera-Paredes, S. Nikolov, R. Jain, E. Clancy, D. Reiman, S. Petersen, A.W. Senior, K. Kavukcuoglu, E. Birney, P. Kohli, J. Jumper, D. Hassabis, Highly accurate protein structure prediction for the human proteome., Nature. (2021). https://doi.org/10.1038/s41586-021-03828-1.
[155] M. Baek, F. Dimaio, I. Anishchenko, J. Dauparas, S. Ovchinnikov, G.R. Lee, J. Wang, Q. Cong, L.N. Kinch, R.D. Schaeffer, C. Millán, H. Park, C. Adams, C.R. Glassman, A. Degiovanni, J.H. Pereira, A. v Rodrigues, A.A. van Dijk, A.C. Ebrecht, D.J. Opperman, T. Sagmeister, C. Buhlheller, T. Pavkov-Keller, M.K. Rathinaswamy, U. Dalwadi, C.K. Yip, J.E. Burke, K.C. Garcia, N. v Grishin, P.D. Adams, R.J. Read, D. Baker, Accurate prediction of protein structures and interactions using a 3-track network, Science (1979). 373 (2021) 871–876. https://doi.org/10.1126/science.abj8754.
[156] D. del Alamo, D. Sala, H.S. Mchaourab, J. Meiler, Sampling alternative conformational states of transporters and receptors with AlphaFold2, Elife. (2022). https://doi.org/10.1101/2021.11.22.469536.
[157] Y. Zhang, J. Skolnick, TM-align: A protein structure alignment algorithm based on the TM-score, Nucleic Acids Research. 33 (2005) 2302–2309. https://doi.org/10.1093/nar/gki524.