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Monoamine transporters: structure, intrinsic dynamics and allosteric regulation

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Abstract

Monoamine transporters (MATs) regulate neurotransmission via the reuptake of dopamine, serotonin and norepinephrine from extra-neuronal regions and thus maintain neurotransmitter homeostasis. As targets of a wide range of compounds, including antidepressants, substances of abuse and drugs for neuropsychiatric and neurodegenerative disorders, their mechanism of action and their modulation by small molecules have long been of broad interest. Recent advances in the structural characterization of dopamine and serotonin transporters have opened the way for structure-based modeling and simulations, which, together with experimental data, now provide mechanistic understanding of their transport function and interactions. Here we review recent progress in the elucidation of the structural dynamics of MATs and their conformational landscape and transitions, as well as allosteric regulation mechanisms.

Recent years have seen an explosion in the number of studies aiming at gaining a structure-based understanding of the mechanism of function of monoamine transporters (MATs). MATs belong to the solute carrier 6 (SLC6) family of human transporters, which, in turn, is a subfamily of the broader neurotransmitter:sodium symporters (NSSs) that comprise transporters from prokaryotic to human. MATs comprise three main members — the dopamine (DA) transporter (DAT)^{1–3}, serotonin transporter (SERT)^{4–6} and norepinephrine transporter (NET) — which have critical roles in regulating neurotransmission at synapses; the SLC6 family also includes the glycine transporter, GABA transporter and neutral amino acid transporter subfamilies, in addition to the MAT subfamily. Dysregulation of MATs has been linked to depression, anxiety disorder, attention-deficit-hyperactivity disorder, obsessive-compulsive disorder, substance-use disorders⁷, epilepsy, Parkinson's disease and autism-spectrum disorder^{8–11}; thus, MATs serve as pharmacological targets for several neuropsychiatric and neurodegenerative disorders.

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The surge in structure-based studies of MATs in recent years has been driven by the resolution of structures for DAT and SERT. Structural data now permit elucidation of the specific modes of actions of these transporters, beyond those originally deduced from their bacterial homologs, such as the leucine transporter LeuT. LeuT has long served as a prototype for mechanistic studies of NSS function, being the first structurally resolved member of the family^{12–14}. However, tens of structures have now been resolved for *Drosophila melanogaster* DAT (dDAT) and human SERT (hSERT) in multiple conformational states and in the presence of substrate and ions, as well as in the presence of antidepressants and/or psychostimulants. These provide an excellent starting point for delineating the functional dynamics of MATs and their modulation by ligands or drugs, as molecular dynamics (MD) simulations suggest¹⁵. The resolution of MAT structures is an extremely important milestone, as well as a solid basis for leveraging advances in computational and experimental technologies to gain a deeper understanding of their mechanisms of function and interactions.

Interactions with small molecules and ions serve a major role in mediating MAT function. First, these are, by definition, secondary transporters; i.e., the transport of their substrate (DA, serotonin, norepinephrine or other monoamines) is effectuated by co-transport of Na⁺ ions down their electrochemical gradient from the extracellular (EC) medium to the intracellular (IC) medium. Second, eukaryotic members of the SLC6 family, including MATs, are chloride dependent. Notably, even the binding events of the neurotransmitter itself, in addition to those of Na⁺ and Cl[−] ions, elicit cooperative changes in the conformation of the transmembrane (TM) domain, which facilitate its translocation. Third, some SLC6 members couple substrate influx to the reverse transport (efflux) of a K⁺ ion¹⁶. Additionally, the immediate environment has a role; for example, influx of IC water after the disruption of IC gates facilitates the dislocation of the substrate from its binding pocket and its release¹⁷, and cholesterol (CHOL) molecules affect the transporter's conformational dynamics^{18–21}. Most importantly, many small molecules, inhibitors and modulators, including drugs of abuse such as cocaine and amphetamine (AMPH), affect the structural dynamics of MATs.

In addition to interactions with small molecules, interactions with other proteins, or even self interactions to form assemblies or oligomers, enable, enhance or alter transport activity. Many studies have indicated the ability of MATs to co-exist as oligomers^{22–26} and the effect of intermolecular interactions on reversing transport^{10,11,27}. For example, DA efflux is induced by AMPH^{28,29} and is regulated by G protein β - and γ -subunits³⁰, the kinase CaMKII^{28,31}, protein kinase C³² and/or phosphatidylinositol-4,5-bisphosphate (PIP₂)^{33,34}.

Our goal is to provide here an overview of the current understanding of the molecular basis of the mechanisms by which MATs function and their interactions from the perspective of their intrinsic structural dynamics. 'Intrinsic dynamics' refers to the collective motions inherently favored ('encoded') by the three-dimensional architecture that are exploited by the protein to achieve its biological function and interactions³⁵. These comprise general properties endowed by the shared LeuT fold of MATs, as well as member-specific features that often operate at a more localized scale and allow the functional distinction of family members³⁶. In this context, we will focus on the structural dynamics of DAT, in comparison

to those of SERT, two structurally characterized types of MATs. We start with an overview of the known structural features of MATs, including their overall architecture and functional sites. We then proceed to the understanding of their intrinsic dynamics inferred from structure-based computations^{17,20,37–45}. The third section shows how structural dynamics relate to, if not determine, mechanisms of function and regulation, with an emphasis on allosteric events. Finally, the fourth section provides a glimpse into intermolecular interactions with signaling or regulatory proteins and future challenges to be addressed in the design of more-effective intervention methods for modulating the neurotransmission activities of MATs.

Overall architecture and functional sites of MATs

Overall architecture.

Both hSERT (Fig. 1a–f) and dDAT (Fig. 1g–k) contain twelve TM helices, ten of which (TM1–TM5 and TM6–TM10) are organized into two inverted-topological repeats as in the LeuT fold⁴⁵. The first helix of each repeat is broken into two segments, designated as ‘TM1a–TM1b’ and ‘TM6a–TM6b’. The TM domain harbors a primary binding site, S1, for the substrate and co-transported ions near the broken regions of TM1 and TM6^{1,6,12}.

The three-dimensional architecture of MATs has three distinct features. First, TM12 has a kink in the middle and is connected to a C-terminal latch via a broken segment. Mutations at kink-forming residues of human DAT (hDAT) TM12 alter transporter expression and activity⁴². The C-terminal latch is associated with TM1a through the IC loop IL1, suggestive of a possible role in modulating transporter activity¹. Second, MATs and their homologs GLYT1 and GAT1 have a large, sequentially variable EC loop EL2 that harbors N-linked glycosylation sites and two conserved cysteine residues that form a disulfide bond⁴⁶. Third, MATs have large N and C termini that extend into the cytoplasm. The termini are the most divergent segments in the NSSs, ranging from ~10 residues in LeuT to more than 60 (N-terminal) residues and 35 (C-terminal) residues in MATs⁴⁶. These segments have been suggested to be required for the evolutionary adaptation of MATs to diverse interactions³⁸. While there are currently no structural data on MAT termini due to their intrinsic disorder, they have been investigated by computational models and simulations for hDAT^{33,38,43} and hSERT⁴⁴, given their importance.

Primary substrate-binding site.

The primary binding site S1 for the cognate substrate and/or neurotransmitter also serves as an orthosteric binding site for common drugs and psychostimulants, such as the therapeutically active *S*-enantiomer of the antidepressant citalopram ((*S*)-citalopram) (Fig. 1f). The binding of cocaine and its analogs to the DA-binding site (Fig. 1h) was an early discovery⁴⁷ after the resolution of LeuT¹² that served as a template for DAT. Residues at site S1 and its immediate spatial vicinity, which coordinate the substrate and Na⁺ ions (Table 1), are highly conserved among MATs. As a result, MATs are not particularly selective for monoamines¹⁰; for example, DAT and NET efficiently transport both DA and norepinephrine, SERT also transports DA⁴⁸, and many drugs that bind SERT also bind DAT⁴⁹. That promiscuity is further compounded by the similarities among serotonin, DA

and norepinephrine themselves (Fig. 2a). The drugs have somewhat distinct structures (Fig. 2a), but they also bind to the S1 site in hSERT⁵ (Fig. 2b) and dDAT^{1–3} (Fig. 2c), with some structural readjustments.

Three subsites have been defined within S1^{5,50,51} (Fig. 2b,c). Subsite A, delimited by TM1b, TM6 and TM8, selectively binds the polar or amine moiety of the ligands. An aspartate therein (Asp98 in hSERT (Fig. 1f) and Asp79 or Asp46 in hDAT or dDAT, respectively (Fig. 1h)) facilitates the binding of monoamines by forming a salt bridge with the NH₃⁺ group of substrates^{2,47,50}. A glycine instead binds neutral substrates in GLY1, GAT1 and LeuT. Subsite B, lined by TM3 and TM8, sculpts the aromatic ring moieties. Subsite C, lined by TM3, TM6a and TM10, has a role in modulating inhibitor affinity and specificity⁵⁰. Two aromatic residues therein (Phe335 and Phe341 in hSERT) optimize the coordination of specific ligands by suitable isomerization.

Allosteric binding site.

The allosteric site, proposed for SERT almost three decades ago⁵³, has been confirmed in subsequent studies of SERT^{5,54} and the structural homologs LeuT^{55–59} and the NSS MhsT⁶⁰. This site (S2) is more than 10–12 Å away from S1, at the periphery of the EC vestibule, between the EC loops EL4a-EL4b, EL6 and TM1b, TM6a and TM11⁶ (Fig. 1e), in accordance with the site reported for LeuT⁵⁸. The existence of S2 for substrate or antidepressant binding, with or without simultaneous occupation of S1, is now well established among members of the NSS family⁶¹.

CHOL-binding sites.

Gouaux and co-workers have resolved three binding sites for CHOL, designated sites I and II (refs.^{1–3}) (Fig. 1j,k) and site III (refs.^{5,6}) (Fig. 1b). Coarse-grained MD simulations²⁰ have shown that all three MATs may share the sites I and II. Several CHOL-binding sites¹¹ have been predicted on the basis of consensus motifs that are yet to be validated. The effect of CHOL binding on activity may vary among members of the MAT family²⁰.

Ion-binding sites.

As mentioned above, members of the NSS family share two broken helices, TM1 and TM6, the first in each repeated pentad. The unsatisfied (otherwise hydrogen-bond-forming) groups therein provide a high-affinity medium for binding ions and polar and/or charged groups. Two Na⁺-binding sites (Na1 and Na2) and one Cl[−]-binding site are observed therein in all DAT structures, and the Na1 site is occupied in all hSERT structures. SERT bound to the selective serotonin-reuptake inhibitors (SSRIs) sertraline or fluvoxamine exhibit an electron density that represents the three ions. A serine in TM7 (Ser372 in hSERT) is conserved across all chloride-dependent eukaryotic NSSs and has a key role in chloride binding (Fig. 1d,i). In LeuT, a glutamate (Glu290) occupies the equivalent position; its replacement by serine enables Cl[−]-dependent activity, which confirms the crucial role of a serine at this position⁶². The negative charge provided by either Cl[−] or a residue side chain is presumably required for the binding and translocating of neurotransmitters⁶³.

Intrinsic dynamics of MATs

The vast majority of MAT structures are resolved in the outward-facing state.

Like all NSSs with the LeuT fold, MATs have access to two major states, outward facing (OF) and inward facing (IF), which are sampled during the respective uptake of substrate and release of substrate. In an OF conformation of DAT, sampled immediately after binding DA (Fig. 3a, purple space-filling spheres), the EC vestibule is relatively exposed to the EC region, while the cytoplasmic face is compactly packed.

All MAT structures resolved so far have been OF, except for the new cryo-EM structures of hSERT with ibogaine bound, which was resolved by Gouaux and co-workers in various states, including occluded and IF states, and now provide additional insights into inhibition and neurotransmitter-transport mechanisms⁴. Notably, molecular modeling and simulations^{17,20,38,40,42,43} and structural data for homologous NSSs have revealed conformations visited along the substrate-transport cycle. It is interesting to observe that those structural models exhibit excellent agreement with the new cryo-EM structures (Fig. 4a,e), as will be discussed below.

Conformational changes along the transport cycle revealed by molecular models and simulations.

DAT simulations have revealed a series of conformational changes and binding-unbinding events^{17,40} (Fig. 3b). The conformational changes noted were both global (Fig. 3b) and local (Fig. 3c,d)⁵⁵: the term ‘global changes’ refers to collective transitions of TM helices and loops between OF and IF states, often described as ‘alternating access’⁶⁴ or ‘rocking bundle’^{45,65}; ‘local changes’ include fluctuations between open (*o*) states and closed (*c*) states of EC and IC gates^{36,66}, side-chain rotations and ion dislocations. Table 1 lists EC- and IC-gating residues. Those and ion-coordinating residues are highly conserved among MATs, and even among bacterial transporters such as LeuT^{1,6,12,16,17}.

Global and local changes are coupled for effective intake, seclusion or release of neurotransmitters.

The initiating step of the transport cycle is the binding of a first Na⁺ ion to the NaI site⁶⁷. Many coupled local and global events follow¹⁷: Na⁺ binding promotes opening of the EC vestibule⁶⁸, which prompts the transporter to bind the substrate; subsequent substrate binding promotes the closure of the EC gates (Fig. 3c), accompanied by overall tightening of the TM helices (Fig. 3b, step 1); transition of that occluded state to the IFo[★] state is accompanied by the disruption or opening of salt bridges that serve as IC gates (Fig. 3b, step 3; the asterisk indicates that the substrate is bound). After the release of substrate and ion, back-transition to the OF state entails opening of the EC gates for the (re-)uptake of substrate and ion from the synapse (Fig. 3b, step 5). The overall transport cycle is therefore described by the following succession of highly cooperative, if not allosteric, transitions: OFo → OFc[★] → occluded → IFo[★] → IFc, and back to OFo (Fig. 3b). The occluded conformers have been resolved for MhsT (another NSS that shares the LeuT fold)⁶⁹ and very recently for hSERT⁴.

Substrate binding triggers compaction of the EC vestibule and closure of the EC gate.

What simulations have strikingly shown is that substrate binding itself drives closure of the EC gate (Fig. 3b). The closure of EC gates (the Arg85-Asp476 salt-bridge formation and the Tyr156-Phe320 association) is facilitated by the inward tilting of the EC-exposed segments TM1b and TM6a by 10–15° toward TM10, triggered by substrate binding. The substrate thus acts as an allosteric modulator of a switch to a closed state (OFc[★]) that prevents leakage and/or release back to the EC region. Numerous simulations have shown that once the substrate binds, it is committed to proceeding deeper into the binding site S1, rather than unbinding and diffusing back to the EC medium^{17,52}. Intramolecular attractions are too strong to prevent the release.

The inward tilting of TM1b and association with TM10 promote the formation of a hydrophobic cluster around a highly conserved tryptophan (Trp84) on TM1b^{17,69}, which prevents the hydration of S1. The repositioning of TM10 also alters the orientation of TM9, which induces a weakening in the associations between the IC-exposed TM helices; for example, Glu428-Arg445 and Glu446-Lys257¹⁷, which connect TM9 to TM8 and TM4 in the OF state, are disrupted (Fig. 4e). The EC thin gate (Asp476-Arg85), initially open, is observed to close down within tens of nanoseconds, and it remains closed during the next ~380 ns, while the IC-gating Arg60-Asp436 and other salt bridges at the IC vestibule break apart as the structure reconfigures into the IF state (labels above plots, Fig. 4e).

Dislocation of Na⁺ from site Na2 promotes influx of IC water, which prompts transition to the IF state.

Another interesting feature revealed by simulations^{17,43} is the driver role of the Na⁺ ion, whose dislocation from the Na2 site triggers an influx of water from the cytoplasm (Fig. 3d) and thus prompts disruption of the IC gates. Simulations⁵⁸ and biochemical and biophysical assays⁷⁰ have also shown an essential role for this Na⁺ ion in the functioning of LeuT. A similar behavior has been reported for MhsT⁶⁹, for which solvation and partial unwinding of the TM4-TM5 loop has been reported to facilitate the release of Na⁺ from site Na2. Subsequently, considerable outward tilting of TM1a breaks the salt bridges Lys66-Asp345 and Arg60-Asp436 and enables the release of ions and substrate (Fig. 4e,f). The extent of TM1a's tilting depends on the connecting N-terminal segment and may differ among members of the LeuT-fold family³⁶. Notably, the resolved cryoEM structures⁴ in the OF_o state (PDB: 5I6Z) and IF_o state (PDB: 6DZZ) have shown that the counterparts of these salt bridges in hSERT exhibit inter-residue distance changes comparable to those predicted for hDAT¹⁷.

Conformational landscape of MATs.

The conformational space of macromolecules is multi-dimensional. Each conformation represents a point in the 3*N*-dimensional space whose axes represent the coordinates of the *N* atoms that compose the structure. However, for visualization purposes, we define a few metrics that provide effective discrimination of conformers of interest and examine the subspace spanned by those metrics, such as a result presented here, obtained for hDAT⁴⁰ (Fig. 4a). The TM1b-TM10 distance serves as a metric of the opening of the EC vestibule (Fig. 3c), and the TM1a-TM6b distance serves as a measure of the opening of the IC

vestibule^{17,71}. The conformational landscape generated for hDAT concurs with the diversity of structures observed for members of the NSS family. The hDAT example presented here shows the locations of the previously resolved LeuT OFc[★] structure¹², MhsT in the occluded and substrate-bound state⁶⁹ and LeuT OFo structure¹³, as well as the newly resolved hSERT cryoEM structures in the IFo and occluded states⁴ (labels and arrows, Fig. 4a), in addition to the hDAT OFo[★] and OFo homology models¹⁷ used as input in simulations (circles, Fig. 4a).

DA undergoes successive events and movements, first binding near S2, followed by stabilization near S1, further insertion and release to IC region (Fig. 4b, top), and accompanying gradual changes in interhelical distances; mainly, the TM1b-TM10 distance decreases as the DAT reconfigures from OF to IF, and it increases after return to the OF state, whereas the TM1a-TM6b distance exhibits the opposite behavior (Fig. 4b, bottom). Through the use of these two metrics, it is clearly apparent that the presence of DA favors an occluded state in which short distances are selected for both pairs of helices, which practically obstruct access to S1 from either side (Fig. 4a, left). In the absence of DA (Fig. 4a, right), on the other hand, the occluded-state peak (Fig. 4a, right, blue) is shifted to greater interhelical distances (14–17 Å), indicative of a loose channel-like packing. The passage between the endpoints OFo and IFo is therefore effectuated through a tightly packed state that effectively seals the cargo from the environment, whereas the ‘free’ transporter is more open to ‘leakage’ in both directions.

An alternative systematic method for viewing the conformational space is principal-component analysis of a representative ensemble of conformers, as in the analysis shown here for hDAT (Fig. 4c). The two principal axes PC1 and PC2 are obtained by principal-component analysis of MD trajectories, and the points represent the ‘snapshots’ projected onto this reduced space. PC1 physically refers to global (OF → IF) transitions, and PC2 refers to those between closed forms and open forms. The occluded state is neither OF nor IF, and the OF and IF states are maximally differentiated when they are both open (Fig. 4c, left). This analysis shows model structures for hDAT OFo, OFc[★] and IFo conformers (Fig. 4c, red symbols) constructed based on the LeuT OFo structure (PDB: 3TT1)¹³, the OFc[★] structure (PDB: 2A65)¹² and the IFo structure (PDB: 3TT3)¹³. The fact that the conformers evolve along diagonals (rather than horizontal or vertical directions) attests once more to the coupled global and local changes.

Detailed examination of the fluctuation profiles of residues computed using the anisotropic network model⁷² for various members (shown here for DAT (hDAT and dDAT), SERT and LeuT in OF and IF states; Fig. 4d) exposes shared patterns as well as member-specific features³⁶. Selected residues (labels (in color) above curves, Fig. 4d) have been linked to key roles (Table 1), often occupying minima (vertical dotted lines along horizontal axis, Fig. 4d). For example, the shared minimum near hDAT Asp79 is indicative of the tight interactions and spatial restrictions required for specific rearrangements at the TM1 broken helices, whereas a peak at EL2b indicates a flexibility specific to the SERT, or LeuT is distinguished by its high mobility at the TM1a segment in the IF state (but not in the OF state).

Modulation of function by small molecules

Knowledge of the key sites (Table 1) and intrinsic dynamics (Figs. 3 and 4) of MATs permits the design of methods of approach for controlling and/or modulating transport functions; i.e., identifying potential target sites and susceptible interactions that inhibitors or modulators could effectively interfere with. Studies assisted by *in silico* modeling of binding or modulatory activities of drugs, psychostimulants or lipids, and guided by knowledge of the structure-encoded dynamics of DAT and its structural homologs^{37,52,73–76}, show promise for the rational design of MAT inhibitors. Below we provide more information on specific cases of this.

Inhibition of monoamine reuptake by the binding of small molecules to S1 site.

Most inhibitors of MATs block the reuptake of monoamines by competitively binding the orthosteric site S1 (Figs. 1f and 2). Thus, they exploit the physicochemical environment that would otherwise harbor the cognate substrate, and they impair the transporter's function by stabilizing conformers that differ from the functional ones, as detected by electron paramagnetic resonance analysis⁶⁸ and hydrogen-deuterium-exchange mass spectrometry⁷⁷, obstructing transitions between the OF state and IF state or interfering with ion binding and displacements^{68,73,77}. Many inhibitors arrest the transporter in a wide-open OF conformation, presumably due to their bulky size that hinders the concerted inward tilting of EC-exposed TM helices, and impede closure of the EC gates⁵² (Fig. 5). Cocaine is a typical example of this^{47,52}.

Psychostimulants such as AMPH also prevent neurotransmitter reuptake by competing for site S1 (Fig. 5b). In this case, the binding geometry is almost identical to that of the neurotransmitter's (Fig. 5a), and the internal rearrangements that lead the translocation are not altered, but the transported molecule is AMPH, not the neurotransmitter, which results in reuptake deficiency. In contrast to the competitive binding of cocaine or AMPH, ibogaine binds noncompetitively to the MAT (for example, a SERT) in the IFo state⁷⁸.

Allosteric effect of the binding of substrate or inhibitor to site S2.

Another mechanism of inhibition is the binding of drugs to the allosteric site S2 (Fig. 1e), which may help in the synthesis of high-affinity and high-selectivity inhibitors of SERT⁵⁴. Occupancy of S2 by a substrate not only sterically hinders unbinding of the substrate from S1⁶ but also facilitates its cytoplasmic release^{58,79,80} by triggering cooperative movements that engage the two sites.

Such couplings have been observed in LeuT simulations in the presence of two alanine residues (also a substrate for LeuT), Ala1 and Ala2, bound to the respective sites S1 and S2⁵⁵ (Fig. 5d–f). The isomeric rotation of an EL4b residue, Phe320, has been shown to elicit an allosteric conformational switch that concertedly displaces both alanine residues, concurrent with closure of the thin gate (Arg30-Asp404, equivalent to Arg85-Asp476 in hDAT) that sequesters Ala2 from the EC environment. In this case, the occupancy of S2 by Ala2 prompts the insertion of Ala1 deeper into the binding pocket below S1, which completely seals it from the EC region after closure of the second EC gate (Tyr108-Phe253,

equivalent to Tyr156-Phe320 in hDAT). On the other hand, occupancy of S2 by a bulky drug would have the opposite effect. Antidepressants bound to S2 indeed interfere with EC gating and block the access and/or passage of synaptic neurotransmitters to the S1 site⁶⁰.

In a systematic study of the role of surrounding residues in mediating the allosteric activity of antidepressants bound to the SERT S2 site⁸¹, it was found that the most pronounced decrease in allosteric potency occurred after substitution of the EL4a-EL4b linker residue Gly402 by histidine. This substitution resulted in complete elimination of the allosteric action of (*S*)-citalopram. Strikingly, this residue is sequentially adjacent to LeuT residue Phe320, which has been noted to trigger allosteric responses in LeuT⁵⁵. These two completely independent studies, one a computational analysis of LeuT⁵⁵ and the other an experimental study of a SERT⁸¹, have thus identified a critical residue in the same region. Notably, all MATs have a conserved glycine at this position, indicative of their functional importance.

Closer examination shows that the S2 pocket can accommodate substrates or drugs in slightly different 'poses' (Fig. 5h), depending on the transporter and the drug, but the coordination by the flexible (or rotatable) EL4a-EL4b linker residue Gly402 (or Phe320) appears to be essential for allosteric action. Notably, the mutation L406E in the immediate vicinity of Gly402 induces a remarkable gain of potency for a range of SERT inhibitors, in further support of the proposal of a role for EL4 in allosterically modulating inhibitor actions⁸². Finally, R104K is another SERT mutation found to cause a drastic decrease in potency⁸¹. The critical role of this residue that otherwise controls the thin EC gate again attests to the impact of interfering with intrinsic dynamics.

Modulation of MAT function by lipids.

CHOL regulates the kinetics of DAT⁸³ and SERT⁸⁴, among many other membrane proteins. Depletion of CHOL results in a decrease in MAT activity^{18,21}. This effect has been attributed to the prevention of TM5 reorientation, shown in published simulations²⁰, and/or to steric constraints exerted on TM1a¹ (Fig. 5g). TM1a, TM5 and TM7 line a pocket with critical rearrangements that promote progress to the occluded state (Fig. 3b,d). The effect of CHOL on transport activity presumably stems from the restrictions on the pre-existing susceptibility to undergo functional motions.

PIP₂ is another lipid that modulates or even alters MAT function^{33,34}. While an essential role of PIP₂ is to control the gating of ion channels, studies suggest a similar regulatory role for PIP₂ in the context of MATs that promotes the efflux of neurotransmitters^{33,34}. Combined experimental and computational studies indicate that interactions of PIP₂ with the DAT N terminus facilitate AMPH-induced efflux³³ and may even be a prerequisite for AMPH's actions, as observed for a SERT³⁴, without affecting inward transport. Computations have shown that the PIP₂ lipid component redistributes the IC-gating pairs on IC loop IL4 (for example, Glu428-Arg445 in hDAT; Fig. 4e) to expose the IC vestibule and release the Na⁺ ion originally bound to site Na2^{38,43}. This is another example of exploitation of the pre-existing potential of Na2 to dislocate. It is triggered in this case by a phospholipid, PIP₂, for which microdomains or rafts are often enriched.

Multimerization promoted by small molecules.

There has been an upsurge in exploring the ability of MATs to form oligomers (aggregates), a property suggested in earlier studies^{26,85}. Whereas DAT^{1–3} and SERT^{5,6} have been resolved as monomers, several structurally homologous members of the LeuT-fold family have been resolved as dimers^{12,69} or trimers^{86,87}. Published work has shown that trimerization of hDAT is promoted by the binding of a heterocyclic drug (furoprimidine AIM-100)²⁵. DAT forms stable dimers in a PIP₂- and CHOL-independent manner²², whereas for SERT, the formation of oligomers is promoted by the binding of PIP₂ (ref.²⁴). Computational studies of the multimerization properties of members of the LeuT-fold family have revealed that (i) trimerization exploits the intrinsic ability of the LeuT architecture undergo a conformational change that pre-disposes the EL3 loop (LeuT) or H7 helix (the betaine transporter BetP) to make intermonomer contacts³⁶, as observed for the BetP trimer⁸⁷; (ii) a variety of dimerization geometries that involve the scaffold domain are accessible to DAT⁴¹, and preferred oligomerization interfaces depend on the specific TM sequences and geometries³⁶, such as the interfacial contacts of TM9 and TM12 in LeuT^{12,13}, or TM12 in a SERT³⁹; (iii) the ability to oligomerize depends on the conformational state of the transporter²⁵, which also affects its aggregation in selected localizations⁸⁸; and (iv) dimerization may facilitate the intrinsically accessible IF-OF transition³⁶ while promoting allostery⁴². Overall, these studies point to the possibility of stimulating the assembly of MATs into multimers or aggregates, the functional impact of which is yet to be established.

Importance of structure-encoded dynamics

We have presented here an overview of what has been learned in the past decade about the molecular bases and dynamics of the transport function and interactions of MATs, after the resolution of the first NSS structure, LeuT, in 2005. Since then, a wealth of studies based on structural data, including those on DAT and SERT in the presence of different ligands and drugs (Figs. 1 and 2) have improved the understanding of MAT machinery and its modulation by small molecules. Notably, early insights provided by LeuT and its structural homologs proved tenable, indicating the importance of the evolutionarily conserved LeuT fold as a determinant of functional dynamics. As shown for enzymes⁸⁹ and many allosteric proteins, the intrinsic dynamics encoded by the fold offer pre-existing accessible paths on the conformational space⁹⁰, and movements along them, triggered by the binding of a substrate, ion or small molecule, cooperatively enable or disable functional events. The same holds true for MATs. Neurotransmitter translocation and release involves coupled local and global rearrangements (Figs. 3 and 4) encoded by the LeuT fold and sharpened by sequence and structure features unique to MATs.

Such advances suggest new strategies for controlling MAT function via either orthosteric inhibition or allosteric modulation (Fig. 5). For example, orthosteric inhibitors of DAT do not show specific association with TM10, in contrast to SSRIs (Fig. 2), and the design of small molecules that promote the association with TM10 might be explored to enhance binding affinity. Likewise, MATs have structurally and sequentially distinctive TM11 segments near site S2, which might be exploited for the design of selective allosteric inhibitors (Fig. 5h). However, there is a great deal of knowledge still missing. DAT and

SERT are similar in terms of sequence and structure^{2,6} and, not surprisingly, SERT also transport DA⁴⁸, many drugs that bind SERT also bind DAT⁴⁹, and there is a close correlation between the actions of DAT and those of SERT, as well as those of NET^{91,92}. While SSRIs are successful in selectively targeting SERT over DAT and NET⁴⁸, and atypical DAT inhibitors have proven useful against cocaine-abuse disorder^{74,93–95}, future challenges must still be addressed, some of which are briefly described below.

Future directions

The tightly regulated structural dynamics of MATs do not solely enable the translocation of substrates and ions. They mediate the assembly, complexation or oligomerization of MATs, their sub-cellular localization, and selective activation of downstream signals^{8,16,46,85,96}. Post-translational modifications of MATs have major roles in maintaining subcellular localization and interactions with regulatory proteins^{8–11,46,85}. Such events are tightly dependent on conformational state and dynamics; for example, cGMP-dependent phosphorylation of SERT is diminished by Na⁺ and cocaine, which stabilize the OFo state, and is increased by agents such as ibogaine that stabilize the IFo[★] state⁹⁷. Overall, there is an interdependence among the conformation, post-translational modification and reuptake and/or efflux activity of MATs^{8,97}. Given the effects on cellular networks, dysfunction and diseases (such as the effect of mitophagy on neurons^{98,99}, membrane trafficking¹⁰⁰, dysfunctional peripheral immunity and Parkinson's disease¹⁰¹, just to name a few), an increased focus on characterizing the structure and dynamics of the cytoplasmic interactions of MATs is anticipated in the near future.

The N and C termini of MATs serve as regulatory hubs.

The N and C termini of MATs remain largely unknown in terms of their structure and dynamics, due to their intrinsically disordered nature. These regions serve as regulatory hubs¹⁰ to modulate reuptake, psychostimulant action, endocytosis, and export from the endoplasmic reticulum for axonal targeting and/or cytoplasmic interactions^{9–11,46}. The N and C termini are the most divergent structural units of the NSS family³⁸. Insertions and increases in the length of eukaryotic neurotransmitter transporters' termini may have resulted from evolutionary adaptation³⁸ to myriad interactions^{10,11}, including those that promote neurotransmitter efflux or mediate intracellular signaling¹⁰².

Oligomerization.

The functional and pharmacological roles of MAT oligomerization remain elusive. Early studies suggested that oligomerization assists in efficient export from the endoplasmic reticulum, surface expression and endocytosis^{26,41}. An oligomer-based counter-transport model was proposed to describe the action of AMPH (reviewed in ref.¹⁰). Cooperativity between protomers has been reported for DAT dimers and oligomers¹⁰³. Interestingly, DAT oligomer complexes have been identified as a novel therapeutic target for cocaine addiction¹⁰⁴. There is compelling need to learn more about the biological importance of MAT oligomerization.

Missense mutations.

Several disease-linked missense mutants are listed in Table 1. These mutations have been shown to alter ion binding¹⁰⁵ and transporter conformation^{106,107}, disrupt post-translational modifications and microdomain distributions¹⁰⁸, stimulate anomalous efflux of neurotransmitters and leakage of ions^{34,105}, and disrupt intermolecular interactions¹⁰⁹. With the advances in structural determination of MATs^{1–3,5,6} and modeling of their functional dynamics, as well as advances in assessing the pathogenicity of missense mutations by taking account of their effects on structural dynamics¹¹⁰, researchers are now in a better position to rationalize the effects of these mutations and design more-effective intervention strategies.

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Na⁺ ions (yellow spheres; Na1 and Na2) (**i**), and the two CHOL molecules (CHOL-I and CHOL-II; stick representation) bound to dDAT in the crystal structure (**j,k**). Helix colors in **a.g**: orange, TM1–TM5; blue, inverted counterpart TM6–TM10. The TM11–EL6–TM12 C-terminal segment (red ribbons) distinguishes MATs from their bacterial counterpart LeuT.

Fig. 2 |. The primary site S1 for substrate binding serves as an orthosteric drug-binding site in hSERT and dDAT.

a, Chemical structures of substrates (blue shading), SSRIs (green shading) and DAT ligands (orange shading) resolved in SERT and DAT^{2,3,5}. **b**, Alignment of the SSRIs paroxetine (blue; Prozac), (S)-citalopram (orange), Br-citalopram (green), sertraline (purple) and fluvoxamine (cyan) bound to hSERT^{5,6}. The same drugs in different structures are colored slightly differently; residues that coordinate paroxetine are indicated by thick red lines, and others are indicated by thin lines with the same color as the ligand. **c**, Alignment of antidepressant nortriptyline (blue), the norepinephrine-reuptake inhibitors nisoxetine (magenta) and reboxetine (yellow), the substrates DA (violet), methamphetamine (tan), D-amphetamine (cyan) and DCP (2-(3,4-dichlorophenyl) ethanamine) (ochre), and the DAT inhibitors cocaine (lime), cocaine analog RTI55 (pink) and β -CFT (mauve), all bound to dDAT¹⁻³. Residues that interact with DA are indicated by thick red lines; others are indicated by thin lines with the same color as the ligand. Interacting residues are defined as those making atom-atom contacts closer than 3.5 Å with the ligand. A salt bridge (dashed line) forms between DA and Asp46; binding of DCP induces isomerization of Phe319 toward a partially closed OF conformation (orange arrow). The circles indicate the subsites A-C (labeled).

Fig. 3 |. Neurotransmitter transport cycle of hDAT.

a, ‘Snapshot’ of hDAT in the OF state (orange), embedded in membrane bilayer (yellow), from MD simulations. A few residues distinguished by their key roles are labeled in the diagram, including Asp79, which stably coordinates the bound DA; Asp476 and Arg85, which serve as a thin EC gate after salt-bridge formation; and Asp436 and Arg60, which serve as an IC gate, forming a salt bridge in the OF state of DAT. **b**, Global changes in TM helices 1a–1b, 5, 6a–6b and 10 during the transport cycle as the structure, originally in a substrate- or ion-free state, binds the substrate and ions (step 1) to reconfigure into a closed form (OFc^{*}), further rearrangement of which, together with Na2 dislocation, leads to an occluded state (step 2), which promotes an influx of water and transition to conformer IFo^{*} (step 3). Release of the substrate and ions (step 4) leads to the long-lived conformer IFc, before opening of the EC vestibule (step 5) and resumption of a new cycle. **c,d**, Local events in step 1 (**c**) and step 2 (**d**); mainly, substrate binding prompts the closure of two EC gates (Asp476–Arg85 and Tyr156–Phe320) (**c**), and dislocation of Na2 preceded by disruption of the close association of Asn82, Asn353 and Na⁺ and Cl[−] ions stimulates an influx of water (small red and white spheres) (**d**). More details in ref.¹⁷.

Fig. 4 |. Conformational space and structural dynamics of hDAT, and signature dynamics of MATs.

a, Comparison of the conformational space accessible to DAT in the presence (left) or absence (right) of DA (adapted from ref. 40). The probability distribution of conformers observed in simulations (from highest (blue) to lowest (dark red)) are presented as a function of interhelical distances TM1b–TM10 and TM1a–TM6b, representative of EC vestibule openings and IC vestibule openings, respectively⁴⁰. Several known NSS structures (labels in plots) lie in the space sampled by hDAT, as shown. occl, occluded state. **b**, Diffusion of DA from the EC medium to the DAT interior (top) and the accompanying change in TM1b–TM10 and TM1a–TM6b distances (bottom) as the structure undergoes the global changes OF → IF → OF^{17,40}. **c**, The hDAT trajectory from OFo to occluded to IFo resulting from eight MD trajectories (each in a different color), projected onto the subspace spanned by PC1 and PC2¹⁷. **d**, Shared signature dynamics of MATs and LeuT in the OF state (solid curves) or IF state (dashed curves). Curves are vertically shifted for visual clarity. Residue

numbers refer to hDAT. **e**, Time dependency of interhelical distances TM1b–TM10 and TM6a–TM10 (EC) and TM1a–TM6b (IC) as the structure evolves from the OFo[★] state to an occluded state to the IFo[★] state (top), with concurrent opening of TM1a (middle) and the accompanying changes in salt-bridge-forming pairs at the EC vestibule (Asp476–Arg85) and IC vestibule (Asp436–Arg60, Asp345–Lys66 and Glu428–Arg445)¹⁷. Arrows (bottom) indicate the distances for the equivalent salt-bridge-forming pairs Asp493–Arg104 (blue), Asp452–Arg79 (red), Asp360–Lys85 (pink) and Glu444–Arg462 (green) in the cryo-EM structures resolved for hSERT in the IFo state (right)⁴ and the OFo state (left). **f**, Location of the residues in **e** (bottom plot) and the repositioning of TM1a, TM8 and TM6b as the structure evolves from an occluded state (cyan) to the IFo[★] state (green). **g**, Pathways and trace of DA and ions being released from a DAT¹⁷. The spheres show the successive positions of DA (purple), Na2 (orange), Na1 (yellow) and a Cl[−] ion (cyan)¹⁷. TM5 and TM8 line the pore.

Fig. 5 |. Effect of small molecules bound to orthosteric (S1) sites and allosteric (S2) sites on intrinsic dynamics.

a–c, Comparison of the binding ‘poses’ of DA (**a**), AMPH (**b**) and the anti-Parkinson drug orphenadrine⁵² (**c**) with hDAT. Binding of DA or AMPH leads to transport. In contrast, orphenadrine locks hDAT in the OFo state. TM1, TM6, and TM10 are shown in the OFo state at the initiation of simulation (orange) and after ~100 ns of relaxation after binding of the substrate or drug (yellow). The white ribbon and side chains (sticks with labels) indicate the relaxed, stabilized conformation. The IC gate (Arg60–Asp436) remains closed throughout this process. **d–f**, Successive conformations observed⁵⁵ in MD simulations of LeuT in the presence of two alanine residues (Ala1 and Ala2) originally placed in the EC medium. Phe320 undergoes a rotational isomerization (**d** versus **e**; outlined with a gray circle) that pushes both substrates (magenta space-filling spheres) deeper into the TM region, which stabilizes them in the respective S1 and S2 sites. **g**, CHOL binds to an interfacial groove in hDAT composed of TM1a, TM5 and TM7 that stabilizes the OF conformation (orange tubes). Superposition of the IFo* conformer (cyan tubes; bottom) in the absence of CHOL shows how the opening of TM1a would cause a steric clash (ref.¹). **h**, Close-up view of S2 showing the superposition of two allosteric drugs: a tricyclic antidepressant (TCA) (dark yellow) bound to LeuT (PDB: 2Q6H), and the SSRI (S)-citalopram ((S)-CIT) (green) bound to a SERT. TM11 is shown for both transporters. Side chains in red and blue indicate those observed to coordinate (S)-citalopram in simulations⁸¹ and a crystal structure (PDB: 5I73; Fig. 1e). Gly402 on the EL4 loop is critical for enabling allosteric potency⁸¹.

Table 1 |
Functional residues in human MAT s and their role in enabling or regulating neurotransmitter transport

Roles or regulation effects	Domain	hDAT	hSERT	hNET	Refs.
Substrate-binding site S1 in the OF ^a , facilitation of the OF-to-IF transition; inhibitor binding, stabilization of the OF conformation	TM1	F76, D79	Y95, D98	F72, D75	1,3,6,111
	TM3	S149, V152, G153, Y156	A169, I172, A173, Y176	A145, V148, G149, Y152	
	TM6	F320, F326	F335, F341	F317, F323	
Na1 sodium-binding site ^a , stabilization of OF form for substrate binding	TM8	S422, G426	S438, G442	S419, G423	
	TM1	A77, N82	A96, N101	A73, N78	
	TM6	S321	S336	S318	
Na2 sodium-binding site ^a , triggering of the IF transition	TM7	N353	N368	N350	
	TM1	G75, V78	G94, V97	G71, V74	
	TM8	L418, D421, S422	L434, D437, S438	L415, D418, S419	
CF-binding site ^a , co-transport for balancing charges	TM2	Y102	Y121	Y98	
	TM6	Q317, S321	Q332, S336	Q314, S318	
	TM7	S357	N368, S372	S354	
EC gates involved in opening and closure of the EC vestibule	TM1, TM10	R85-D476	R104-D493/E494	R81-D473	1,6,17,43,112
	TM3, TM6	Y156-F320	Y176-F339	Y152-F317	
	TM1, TM8	R60-D436	R79-D452	R56-D433	
IC gating pairs that facilitating opening and closure of the IC vestibule	TM1, TM7	K66-D345	K85-D360	K62-D342	
	TM1, TM6	W63-Y335	W82-Y350	W59-Y332	
	EL4	R85, G386, I390	R104, G402, L406	R81, G383, V387	81,82
Allosteric regulation sites near S2 ^b CHOL site I ^a , stabilization of OF and modulation of transporter kinetics	TM1	I67, L70, L71, I74	V86, L89, L90, I93	I63, L66, L67, V70	1,20,84
	TM5	T271, Y274, L277, T278	T286, Y289, L292, S293	T268, Y271, L274, F275	
	TM7	V348, I352, L355, S359	V363, V367, M370, V374	L345, I349, I352, V356	
PIP ₂ -regulated dynamics, efflux or oligomerization	N terminus or TMs	K3, K5	R144, K352, K460		33,34,43
	N terminus	S2, S4, S7, S12, S13, T48, S53	S13, Y47, T81	T30	8,113
	TMs	T62	Y142, S149, T276, S277	T258, S259	
Phosphorylation ^c , regulation of transporter activities, internalization, and trafficking	C terminus	S582, T613	T603, S611, T613, T616		

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Roles or regulation effects	Domain	hDAT	hSERT	hNET	Refs.
N-linked glycosylation ^c	EL2	N181, N188, N205	N208, N217	N184, N192, N198	46
Disulfide bond ^c	EL2	C180, C189	C200, C209	C176, C185	6,46,114
Missense mutations ^c : loss or gain of functions, alterations of cell expressing, conformation		R51W, V55A, R237Q AN336, T356M, L368Q V382A, P395L, D421N A559V, E602G, R615C	G56A, I425L, I425V F465L, L550V, K605N K201N	A457P, F528C, R121Q T283M, V245I	9,46,105– 107,109

^aSubstrate, sodium, chloride and CHOL site I for MATs are based on the equivalent positions in the crystal structures resolved for dDAT and hSERT, and computational and mutational studies¹¹¹.

^bReported for SERT only^{81,82} and computationally predicted for LeuT⁵⁵.

^cExperimentally validated, compiled from previous reviews or publications.