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Computational modeling of human dopamine transporter structures, mechanism and its interaction with HIV-1 transactivator of transcription

This is a brief review of computational modeling studies on the detailed structures and mechanism of human dopamine transporter (hDAT), as well as its interaction with HIV-1 transactivator of transcription (Tat). Extensive molecular modeling, docking and dynamics simulations have resulted in reasonable structural models of hDAT in three typical conformational states, its dopamine uptake mechanism and its interaction with Tat. The obtained hDAT models in different conformational states and their complexes with dopamine and Tat have provided novel structural and mechanistic insights concerning how hDAT uptakes dopamine and how Tat affects the dopamine uptake by hDAT. The computational insights, that are consistent with available experimental data, should be valuable for future rational design of novel therapeutic strategies for treatment of HIV-associated neurocognitive disorders.

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Why is human dopamine transporter particularly interesting?

Human dopamine transporter (hDAT) is of particular clinical relevance because it is involved in many diseases such as schizophrenia, Parkinson's disease and drug dependence. Its native substrate, dopamine (DA), is a vital neurotransmitter for locomotor control and reward systems. According to Torres *et al.*, hDAT plays a critical role in regulating the spatial and temporal extra neuronal DA concentration [1]. It is well known that hDAT is the primary target of cocaine in the neuronal system. Cocaine is one of the most commonly abused drugs worldwide [2]. Through binding to hDAT, cocaine blocks the clearance (reuptake) of DA from CNS synapses and, thus, prolongs dopaminergic neurotransmission in brain areas associated with reward. Millions of individuals worldwide are addicted to cocaine, and there is no US FDA-approved medication specific for cocaine abuse [3,4].

In addition, hDAT also interacts with transactivator of transcription (Tat) of HIV-1. The HIV-1 Tat protein has been detected in the brain and the sera of HIV-1 patients [5–7]. Accumulating evidence [8–15] has revealed that HIV-1 Tat plays an important role in HIV-associated neurocognitive disorders (HAND) by disrupting intracellular communication [16]. Specifically, HIV-1 Tat exerts its neurotoxicity through interaction with some crucial proteins in the CNS including hDAT. Through binding with hDAT, HIV-1 Tat can block the DA uptake by hDAT. It is highly desirable to design a therapeutic agent which can block HIV-1 Tat binding with hDAT without affecting the normal function of hDAT.

Knowledge of detailed 3D structure of hDAT is essential for understanding the molecular mechanisms concerning how hDAT interacts with HIV-1 Tat and other important ligands. With the detailed 3D

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structure, one may further study the molecular mechanism of DA-transporting process in hDAT and how hDAT interacts with HIV-1 Tat, etc. These studies may eventually lead to rational design of new therapeutic agents for treatment of drug addiction and HAND. So far, there is no x-ray crystal structure reported for hDAT. Nevertheless, extensive computational modeling and simulations on the hDAT structures and mechanisms have been reported in literature, as discussed below. The most recently reported computational studies have provided a good starting point for further studies on the structures and mechanisms of hDAT and rational design of novel therapeutic agents.

A brief history of the hDAT structure modeling

Like other members of neurotransmitter sodium symporters (NSS) family [17], hDAT binds with DA released from the synaptic cleft and transports DA into pre-synaptic neurons [18–20]. This process can be modeled roughly as involving three typical conformational states of hDAT: the outward-open state (i.e., the extracellular side of binding site for the transmitter is open, while the intracellular side is closed), the outward-occluded state (i.e., both the extracellular and intracellular sides of binding site are closed such that the binding site is occluded and no longer accessible for substrate) and the inward-open state (i.e., the intracellular side of binding site for the transmitter is open, while the extracellular side is closed) [21–27], as depicted in Figure 1.

Early homology modeling studies on 3D structures of hDAT and other monoamine transporters were carried out by using the x-ray crystal structure of Na⁺/H⁺ antiporter or lactose permease (LacY, e.g., PDB entry of 1PV7 with a resolution of 3.6 Å) as a template. Later studies revealed that the templates used in early modeling studies are not suitable for homology modeling of any member of the NSS family [28]. Then, an x-ray crystal structure of leucine transporter from *Aquifex aeolicus* (LeuT_{Aa}) was considered as a much more reasonable template. As a bacterial homolog of NSS, LeuT_{Aa} has a structural folding and physiological features similar to other NSS family members [21,26,27]. The first homology modeling of hDAT using the LeuT_{Aa} structure as a template was reported in 2007 [28]. Based on the LeuT_{Aa} structures, various computational and experimental studies on the structures of hDAT and related transporters [12,28–37] addressed some critical questions of the NSS members including dopamine transporter (DAT). The LeuT_{Aa}-based 3D structures of hDAT have been proven valuable in predicting the hDAT–Tat interaction [38]. On the other hand, the LeuT_{Aa}-based 3D structures of hDAT are not perfect, because the sequence identity between LeuT_{Aa} and hDAT is <25%.

The most recently available x-ray crystal structures (PDB entry: 4M48, 4XP1, 4XP4, 4XP5, 4XP6, 4XP9, 4XPA, 4XPB, 4XPF, 4XPG, 4XPH, 4XPT, 4XNU and 4XNX) [39–41] of *Drosophila* DAT (dDAT) in its outward-open state have provided an extraordinary opportunity to remodel and refine the hDAT structure in the outward-open state. The first homology model of hDAT based on the dDAT structure has been reported recently [42]. The dDAT-based hDAT model in the outward-open state is essentially the same as the corresponding state of the LeuT_{Aa}-based model, but with some more reasonable details in the remodeled structure. So, the dDAT-based hDAT structure in the outward-open state may be considered as a refined 3D model of the LeuT_{Aa}-based hDAT structure.

In addition to the outward-open state, it is also important for understanding the detailed mechanism of the DA-transporting cycle in hDAT to model the 3D structures of hDAT in the other two conformational states, that is, the outward-occluded and inward-open states. However, all of the most recently available x-ray crystal structures of dDAT only provide the 3D structure of dDAT in the outward-open state. There is still no x-ray crystal structure of dDAT in the outward-occluded or inward-open state. Nevertheless, there are x-ray crystal structures of LeuT_{Aa} in all of the three conformational states (the outward-open, outward-occluded and inward-open), and it is notable that the major structural differences between the dDAT and LeuT_{Aa} structures in the outward-open state exist in the regions that are not involved in the conformational motion from the outward-open state to the outward-occluded and inward-open states. Thus, Yuan *et al.* [42] were also able to construct reasonable models of hDAT in the outward-occluded and inward-open states through a combined use of all these available x-ray crystal structures of dDAT and LeuT_{Aa}, as discussed below (the next two sections).

Modeling of the outward-open state using the dDAT structure as a template

Based on the aforementioned x-ray crystal structures of the outward-open state of dDAT, Yuan *et al.* [42] used the Modeller module (version 9v7) of Discovery Studio 2.5.5 [43] to build the structure of the outward-open state of hDAT. A complete multiple-sequence alignment was performed by using PROMALS3D server [44,45] on the amino-acid sequences of hDAT, dDAT, LeuT_{Aa}, human norepinephrine transporter and human serotonin transporter. The sequence alignment [42] indicated that hDAT is highly homologous to dDAT with a sequence similarity of 59% (identity: 46%). Moreover, the amino-acid sequence alignment between hDAT and other members of the NSS family (Figure 2) shows that 12 regions with

Figure 1. Schematic representation of the mechanisms concerning dopamine-transporting cycle of human dopamine transporter. For the sake of clear, conversion of hDAT conformation is reduced to the turning of an L-shape switch. Extracellular and intracellular lipid interfaces are represented by orange and green lines, respectively. Dopamine molecules are represented by red balls. **(A)** Holo hDAT in outward-open state. Extracellular dopamine molecules are accessible to the hDAT-binding site. **(B)** Holo hDAT in outward-occluded state. The binding site is closed and no longer accessible for dopamine. **(C)** Holo inward-open state with DA. The intracellular side of binding site is opened. **(D)** Apo inward-open state without DA. Dopamine is released to the intracellular cytoplasm. **(E)** Apo outward-occluded state without DA. **(F)** Apo outward-open state without DA. Arrows in figure indicate the transition of hDAT in DA-transporting cycle.

DA: Dopamine; hDAT: Human dopamine transporter.

high homology can be assigned to 12 transmembrane (TM) helices, and the similarity in amino-acid sequence for these 12 TM helices between the hDAT and dDAT is as high as 60%. As 40% sequence identity between a template protein and a target protein is considered to be sufficient for constructing a satisfactory homology model [46,47], Yuan *et al.* [42] generated the initial structural model of hDAT with homology modeling and conformational sampling for missing residues in the template (including the first 57 residues on the N-terminal, the last 20 residues on the C-terminal and 19 residues located at extracellular loop 2 of hDAT). It should be noted that Zn^{2+} ions were added to the constructed Zn^{2+} -binding site of this hDAT model [42,48], which would contribute to the understanding for mechanism of Zn^{2+} regulation on hDAT transporting DA [49,50]. Depicted in Figure 3A & B based on the reported study [42] is a typi-

cal molecular dynamics (MD)-simulated 3D structure of the outward-open hDAT-DA-binding complex in a biological environment.

Modeling of the outward-occluded & inward-open states

Since all the available template x-ray crystal structures of dDAT represent the typical outward-open state of the DAT [39–41], such crystal structures alone are not enough for modeling the structures of the outward-occluded and inward-open states of hDAT through the routine homology modeling. In principle, there are various computational methods, such as coarse-grained simulation, normal mode analysis, enhanced sampling MD and random-expulsion MD [51], for constructing structures of different conformational states based on a starting conformation. However, using these meth-

1	hDAT	M----	SKSKCSVGL	-----	MSSVV	APAKEP	-----	NAVGP	-----	KEVEL	I	LVKEQNGVQLT	SST	LTN	PRQSPVE	-----	AQDR	ETWGGK	ID	FLLSV	I	GF	AVDL	AN	WMRFP	LY	CVYK	92									
1	dDATory	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	35										
1	dDATwt	M----	SP	TG	-----	MNP	QVQF	ENNG	-----	ADTG	PEOPLRARK	TAEL	LV	VKERNGVQ	-----	CLLA	PRDGD	-----	HI	SKSKTP	TPHNDNNS	I	S	DERET	WSGKVD	FLLSV	I	GF	AVDL	AN	WMRFP	LY	CVYK	59			
1	hNET	M----	L	LAR	-----	MET	TP	INSOKQL	SACEDGED	QENGV	LQKV	P	TPGDKV	ESGQ	ISN	-----	GYS	AVPS	PGAGD	DRHRS	IPA	TTT	TL	VAELH	-----	OGER	ETWGGK	VD	FLLSV	I	GF	AVDL	AN	WMRFP	LY	CVYK	88
1	hSERT	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	111									
1	LeuTAA	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	37									

Figure 2. Sequence alignment of human dopamine transporter, *Drosophila* dopamine transporter (dDATwt, wild-type sequence), *Drosophila* dopamine transporter (dDAT_{cr}, sequence in crystal structure), human noradrenalin transporter, human serotonin transporter and bacterial homolog of the neurotransmitter sodium symporters from *Aquifex aeolicus*, by PROMALS3D server (see facing page). Transmembrane helices are marked by black lines. Experimentally mutated residues, that is, Y88, K92 and Y470, are colored in red. Important salt bridge involving residues R86, D313 and D476 are colored in purple. Y548 and Y551 that form the 'plier' around Y470 are colored in yellow. Zn²⁺-coordinating residues are colored in cyan. Residues coordinating the internal ions (two Na⁺ and one Cl⁻) are colored in green. In addition, the proline kink in TM12 is colored in blue.

ods, some atomic details of the starting conformation could get lost in the constructed structures due to the possible bias of the conformational conversion mode.

Fortunately, it is known that there is a pseudo twofold symmetry axis between TM1-TM5 and TM6-TM10 of the NSS members, and TMs 2–5 and TMs 7–10 form a tube-like supporting frame for TM1 and TM6. Such folding pattern of the 5 (TM1-TM5) + 5 (TM6-TM10) repeat is conserved between the NSS members and the LeuT_{Aa}, and also has been shared by other non-NSS members according to available x-ray crystal structures [52]. As pointed out recently [42], available x-ray crystal structures of other NSS members, including those for different conformational states (i.e., the outward-open, outward-occluded and inward-open states) of LeuT_{Aa} [26,27], and the mechanistic studies on monoamine transporters [53,54] strongly suggested that these NSS members should share the synonymous structural architecture and features for the binding of ions, uptake of substrate and typical conformational states associated with the substrate transporting process. On the other hand, although it is acceptable to directly model the outward-occluded and inward-open states of hDAT starting from x-ray crystal structures of LeuT_{Aa} in the corresponding conformational states, the quality of these models would not be as reasonable as the outward-open hDAT model based on the x-ray crystal structure of dDAT due to the relatively lower sequence similarity between hDAT and LeuT_{Aa} as compared with the high similarity between hDAT and dDAT. Therefore, more reasonable models of hDAT in the outward-occluded and inward-open states were constructed by a combined use of the all available x-ray crystal structures of dDAT and LeuT_{Aa}, because the major structural differences between the dDAT and LeuT_{Aa} structures in the outward-open state exist in the regions that are not involved in the conformational motion from the outward-open state to the outward-occluded and inward-open states [42].

Specifically, direct homology modeling led to a total of four different homology models of hDAT using the four available x-ray crystal structures of dDAT and LeuT_{Aa}, denoted as HM1–4 (homology models 1–4) [42] for convenience. Clearly, both HM1 (dDAT-based outward-open state model of hDAT) and HM2 (LeuT_{Aa}-based outward-open state model of hDAT) are all for the outward-open state of hDAT, but HM1 should be more reliable than HM2. Then the structural dif-

ferences between HM1 and HM2 were used to correct the structures of HM3 (LeuT_{Aa}-based outward-occluded state model of hDAT) and HM4 (LeuT_{Aa}-based inward-open state model of hDAT) [42]. The reliability of this strategy was validated [42] by testing conformational conversion induced by targeted molecular dynamics simulations [42]. The targeted molecular dynamics simulations eventually led to the outward-occluded and inward-open structures that are very similar to the corresponding 'corrected' structures of HM3 and 4, suggesting that the computational strategy based on the combined use of the x-ray crystal structures of dDAT and LeuT_{Aa} is reasonable. The 'corrected' HM3 and 4 structures were used for the subsequent molecular docking (for the binding with DA) and MD simulations [42] on the outward-occluded and inward-open structures of hDAT.

Despite the similarity between dDAT and LeuT_{Aa} as noted above, the x-ray crystal structures of dDAT should be better templates than the x-ray crystal structures of LeuT_{Aa} (PDB entries as 3F3A [26], 2A65 [21] and 3TT3 [27]) for homology modeling of the hDAT structure because of some significant differences between the dDAT-based hDAT models [42,55] and the LeuT_{Aa}-based ones [28,32,38,48]. The Zn²⁺-coordinating residue H375 is located on the extracellular end of TM7 in the dDAT-based hDAT models rather than loop region in LeuT_{Aa}-based hDAT model, which is important for reasonable modeling of the Zn²⁺-binding site [42]. Besides, a short U-turn loop in EL6 was observed around the hydrophobic residues Y470, Y548, Y551 and F553 in the dDAT-based hDAT models, whereas this structural feature was missing in EL6 of the LeuT_{Aa}-based hDAT models [42]. Reasonable modeling of this U-turn loop is important for understanding the role of many key residues, such as Y470 and H547 [55]. Moreover, TM12 in the dDAT-based hDAT model is kinked significantly by residue P573 at the middle-point of the helix [42], making TM12 twisted away from the helical bundle and moved much more toward the lipid bilayer [39], whereas TM12 in the LeuT_{Aa}-based models was geometrically straight [27].

Modeling revealing critical roles of K92 & Y470 in dopamine transporting

According to computationally modeled hDAT structures [42,48], TM1 and TM6 of hDAT showed significant motion during conformational conversion in the dopamine-transporting process, with TM1a and TM6b being

significantly different in the outward-open and outward-occluded states, as shown in Figure 3C & D. Depicted in Figure 4A is the top view of the local structure around the TM1b and TM6a. Notably, both TM6a and EL4 were close to TM1b, contacting TM1b directly. Besides, R85-D476 salt bridge was reported to be a key indication for the conformational conversion from the outward-open to the outward-occluded state of hDAT [28,34,56,57], implying that TM1b (containing R85) also contacts directly with TM10 (containing D476) in the outward-occluded state. As a result, any structural changes affecting the interactions of TM1b with TM6a, EL4 and/or TM10 are expected to influence the motion of TM1b and, thus, the DA uptake by hDAT [42].

Reported MD simulations [42,48] revealed a favorable salt bridge between K92(TM1b) and D313(TM6a) side chains in all of three typical conformational states of hDAT. As the motions of both TM1b and TM6a are necessary for the conformational conversion, elimi-

nation of K92-D313 salt bridge by K92M mutation is expected to isolate TM1b with TM6a, which may impair the DA uptake process [48]. In addition, TM1b also plays an important role in inducing the conformational conversion of hDAT. Previous studies [28,34,56,57,34] revealed that the formation of a stable D476-R85 salt bridge is an essential structural feature for the conformational conversion of hDAT from the outward-open state to the other states during the dopamine-transporting process. The positively charged R85(TM1b) side chain only forms direct intramolecular interaction with the negatively charged D476(TM10) side chain in the outward-occluded and inward-open state rather than outward-open state hDAT, which indicates that disturbing the D476-R85 salt bridge would impair the TM1b and TM10 motion-related conformational conversion of hDAT [55].

The computational insight was confirmed by experimental tests. Based on the site-directed mutagenesis and

Figure 3. Overview of molecular dynamics-simulated human dopamine transporter dopamine-binding structure.

(A) The MD-simulated outward-open hDAT-DA-binding structure in the lipid bilayer. The hDAT-DA structure is represented as rainbow ribbons. The side chains of lipid molecules are shown in semitransparent stick-and-ball style, whereas the head group atoms as semitransparent molecular surface. The DA and cholesterol molecules are represented in stick-and-ball style and colored in red. The Zn²⁺ ion is shown as a gray ball, the Na⁺ ions as green balls and Cl⁻ as a purple ball. (B) Local view of the extracellular side of hDAT. The lipid molecules are wiped off for clarity. (C) Superimposition of the outward-open hDAT-DA structure (gray color) with the outward-occluded hDAT-DA structure (green color) and the inward-open hDAT-DA structure (purple color). All the hDAT-DA structures are represented as cylinders. (D) The other side view of the superimposed structures. DA: Dopamine; hDAT: Human dopamine transporter.

This figure is based on energy minimized structure from previous work [42,48].

Figure 4. Local view of molecular dynamics-simulated human dopamine transporter dopamine-binding structure. (A) Top view over the extracellular side of the outward-occluded hDAT-DA-binding structure with the focus on the local structures around TM1b, TM6a, EL4, EL6 and TM10 near its extracellular end. The hDAT-DA structure is represented in gray ribbon, with TM1b and TM6a in cyan, EL4 colored in green, EL6 colored in purple and part of TM10 in blue. Residues R85, Y88, K92, D313, Y470 and D476 are shown in stick style and colored in orange. The R85-D476 salt bridge is represented as dashed lines, and the Zn²⁺ ion as an orange ball. (B) The local hydrophobic and/or aromatic residues around the Y470 in the outward-open hDAT-DA structure. The Y470 is shown in orange stick, while the other residues are in stick style with cyan color. DA: Dopamine; hDAT: Human dopamine transporter. This figure is based on energy minimized structure from previous work [42,48].

in vitro pharmacological activity assays performed by Midde *et al.* [48], K92M mutation indeed decreased the $V_{\max}$ for DA uptake by approximately threefold [48], which is consistent with the computational insight. Furthermore, the D313N mutation reported by Chen *et al.* [58] had an approximately fourfold decrease in the $V_{\max}$ for DA uptake, which further supports the interaction between K92 and D313. In addition, K92 is not involved in DA binding based on the computation model [42], which is consistent with the observation that the K92M mutation did not significantly change the K_m [48].

In addition, according to the reported computational models [42,55], residue Y470 of TM10 stayed at the center of the local hydrophobic region, which was formed by hydrophobic and aromatic residues including L224, I230, I469, Y548, Y551 and F553 (Figure 4B). In this local hydrophobic region, residues Y548 (on EL6) and Y551 (on EL6) packed the aromatic side chain of Y470 in a way like a plier [55]. Therefore, the tight interactions between Y470, Y548 and Y551 are expected to hold the position of Y470, and then stabilize TM10, which is important for the hDAT activity [55]. Notably, the stable Y548-Y470-Y551 interaction unit (denoted as YYY motif) exists in all of the three typical conformational states of hDAT, suggesting that the stability of the YYY motif is important for dopamine-transporting process [55]. Thus, hDAT mutation such as Y470H or Y470A is expected to disrupt the hydrophobic interactions among the three residues in the YYY motif, which would decrease the efficiency of dopamine transporting [55]. Experimental tests performed

by Midde *et al.* [48] confirmed that Y470H or Y470A mutation only significantly decreased the $V_{\max}$ without a significant change on the K_m , which is consistent with the computational model.

Considering the overall consistency between the computational predictions and experimental data [38,42,48,55] on the effects of mutations Y470 and K92, the computational model based on the dDAT template has provided further clues in understanding the mechanism of DA transporting and regulation of the hDAT activity.

Modeling of hDAT interacting with HIV-1 Tat

It is particularly interesting to understand how hDAT interacts with HIV-1 Tat at molecular level, especially the detailed hDAT-Tat-binding mode. It would be a grand challenge to determine an x-ray crystal structure of hDAT-Tat-binding complex in the physiological membrane environment. There is also no x-ray crystal structure available even for hDAT itself. On the other hand, state-of-the-art molecular modeling techniques provide useful tools to model the possible hDAT-Tat interaction. The computational models [28,32,42] of hDAT can be used to investigate how hDAT interacts with Tat. In the first computational modeling study (reported in 2013 [38]) on the hDAT-Tat interaction, the LeuT_{Aa}-based hDAT model [28,32] was used. The modeling provided novel mechanistic insights concerning how HIV-1 Tat interacts with hDAT and inhibits dopamine uptake by hDAT. According to the computational study using the LeuT_{Aa}-based hDAT model, the hDAT-Tat binding does not block hDAT-DA binding, but prevents the confor-

mational conversion from the outward-open state to the other states (outward-occluded and inward-open states) and, thus, inhibits the DA uptake by hDAT. In addition, according to the computational modeling using the LeuT_{As}-based hDAT model, Y470 of hDAT was predicted to be key residue involved in the interaction between hDAT and Tat, and the Y470H mutation would significantly attenuate the hDAT–Tat binding. These computational predictions were supported by subsequent experimental validation, showing that the Y470H mutation indeed significantly attenuated the inhibitory effect of Tat on DA uptake by hDAT [38].

Further, based on the newly available dDAT-based hDAT models, further computational studies have been performed to re-examine how hDAT binds with Tat with more detailed structural information, leading to qualitatively the same hDAT–Tat-binding mode obtained from the use of the LeuT_{As}-based hDAT model. According to the computational modeling using the dDAT-based hDAT model, Tat binds most favorably with the outward-open state of hDAT (see Figure 5). Further analysis of the MD-simulated hDAT–Tat-binding structure has revealed that Y88, K92 and Y470 of hDAT are key residues involved in the interaction between hDAT and Tat (Figure 6). The roles of these hDAT residues in the interaction with Tat have been validated by experimental tests through site-directed mutagenesis and DA uptake assays. The agreement between the computational and experimental data suggests that the computationally predicted hDAT–Tat-binding mode and mechanistic insights are reasonable and provide a new starting point to design further pharmacological studies on the molecular mechanism of HAND.

Possible gene therapy for HAND in light of H547A mutation of hDAT

Many mutations on hDAT such as Y470H, K92M and Y88F reported previously not only attenuate the hDAT–Tat binding, they also significantly decrease the dopamine uptake activity of hDAT. However, in a most recently reported study [55], a combined computational experimental study based on the aforementioned hDAT–Tat model has demonstrated that residue H547 of hDAT plays a crucial role in the hDAT–Tat binding and dopamine uptake by hDAT, and that the H547A mutation can not only considerably attenuate Tat-induced inhibition of dopamine uptake, but also significantly increase the V_{max} of hDAT for dopamine uptake. The unusual outcomes, along with the new structural and mechanistic insights, obtained from the study [55] may help to develop novel concepts for future therapeutic treatment of the HAND. For example, it has been known that the HAND-related

abnormal neurocognitive function is associated with dysfunctions in dopamine neurotransmission [12,14,31]. It might be possible to develop a gene therapy for this patient population through effective delivery of a gene for an engineered hDAT mutant (such as one including the H547A mutation) with a significantly improved dopamine uptake activity without binding with HIV-1 Tat. For development of an effective gene therapy for the HAND treatment, it is essential to have an hDAT mutant, which does not significantly bind with Tat so that Tat will not significantly affect the dopamine uptake activity of the hDAT mutant. In addition, the higher the dopamine uptake activity of the hDAT mutant, the more effective the gene therapy would be. Alternatively, one may develop a possible small-molecule drug, which can bind with hDAT to affect the hDAT functions in a way similar to the effects of the H547A mutation and, thus, effectively block the hDAT binding with Tat and enhance/keep the normal dopamine uptake activity of native hDAT [55].

Dopamine reuptake inhibitors as potential therapies for drug abuse

Dopamine transporter is known as a typical target of psychostimulants such as cocaine, and it became preclinical focus in the early stage of drug abuse therapy development. In particular, selective dopamine reuptake inhibitors that bind with hDAT have been developed as possible therapies for cocaine abuse [59–63]. This therapeutic strategy is based on the hypothesis that a dopamine reuptake inhibitor may partially substitute for cocaine, thus decreasing cocaine self-administration and minimizing the craving for cocaine [64–66]. It is also possible to develop potent allosteric hDAT modulators that can partially block DA uptake by hDAT without affecting the hDAT–DA binding [67]. Notably, previously developed LeuT-based hDAT models and x-ray crystal structures of dDAT have been used to study how the known inhibitors bind with the transporter [19,25,28,32,68–77]. The most recently developed hDAT models and obtained mechanistic insights discussed above may be used to rationally design more potent dopamine reuptake inhibitors. In particular, a potent dopamine reuptake inhibitor may bind with hDAT in any of the three conformational states discussed previously in this review.

Conclusion

In the absence of an x-ray crystal structure of human dopamine transporter (hDAT), recently performed extensive molecular modeling, docking, and dynamics simulations have provided reasonable

3D structural models of hDAT in its three typical conformational states, its DA uptake mechanism, and its interaction with HIV-1 Tat. In particular, computational studies have successfully predicted the first-ever hDAT–Tat interaction model, and the predicted model has been supported by all of the experimental validation tests so far. The modeled 3D structures of hDAT in different conformational states and the complexes with DA and HIV-1 Tat have provided detailed structural and mechanistic insights concerning how hDAT uptakes DA and how

HIV-1 Tat influences the normal function of hDAT. The general features of the modeled 3D structures of hDAT and its interactions with DA and Tat are consistent with available experimental data in literature, and have been further supported by the subsequent experimental validation tests. The detailed structural and mechanistic insights obtained from the computational studies on hDAT and hDAT–Tat interactions should be valuable for future rational design of novel therapeutic strategies for treatment of HIV-associated neurocognitive disorders (HAND).

Figure 5. Schematic representation of the mechanism concerning how HIV-1 transactivator of transcription blocks the dopamine transporting cycle. All of the structures were generated with the PyMol software (DeLano Scientific LLC, NY, USA) from the energy-minimized structures. All of the initial structures used in the energy minimization were obtained from our previous studies [32,38,42,48]. Protein hDAT is represented by white semitransparent cylinder. The channel for substrate entry or leaving in hDAT is represented by semitransparent surface in cyan. DA is shown as mesh surface and colored in red. Tat is shown as cartoon and colored in orange. (A) S_0 indicates the apo hDAT in the outward-open state. The substrate-binding site is opened to extracellular side of the neuro cell for DA uptake. (B) S_1 indicates the holo hDAT in outward-open state. (C) S_2 indicates the holo hDAT in the outward-occluded state. The substrate-binding site is occluded and no longer accessible for DA entry. (D) S_3 indicates the apo hDAT in the inward-open state. The substrate-binding site is opened to intracellular cytoplasm and dopamine could leave. The change from S_0 to S_3 represents the process of hDAT-transporting dopamine from the extracellular side to the intracellular side. (E) Tat could bind to the S_0 state of apo hDAT without occupying the dopamine-binding site. So this S_0^* state is still available for dopamine binding. (F) Tat could also bind to the S_1 state of holo hDAT without occupying the dopamine-binding site, which stabilizes the S_1 state (S_1^*). The noncompetitive inhibition effect of Tat blocks the $S_1 \rightarrow S_2$ transition step of hDAT transporting. As a result, Tat is expected to block DA transporting by hDAT.

DA: Dopamine; hDAT: Human dopamine transporter; Tat: Transactivator of transcription.

Figure 6. The key residues involved in the binding between HIV-1 transactivator of transcription and human dopamine transporter in the outward-open state. (A) Typical hDAT-Tat-binding structure. Tat is represented as gold ribbons. Protein hDAT is represented by semitransparent cyan surface. Substrate dopamine, cholesterol, sodium ions, chloride ion and zinc ion are represented by the sphere style and colored in red, purple, blue, green and yellow, respectively. The contact interface of hDAT between Tat and hDAT is colored in pink. **(B)** Atomic interactions on the binding interface of the typical hDAT-Tat-binding structure. HIV-1 Tat protein is shown as ribbon and colored in gold, and hDAT is shown as cyan ribbon. Residues T-M1, T-P18 and T-K19 of HIV-1 Tat are shown in ball-stick style and colored in yellow. Residues D-Y470, D-Y88 and D-K92 are shown in ball-stick style and colored in green. Dashed lines represent intermolecular hydrogen bonds with labeled distances. For D-Y470, the red point indicates the center of its aromatic ring, and the dashed line pointing to the red ball represents the cation- π interaction with labeled distance.

hDAT: Human dopamine transporter; Tat: Transactivator of transcription.

This figure is based on energy minimized structure from previous work [42].

Future perspective

The new structural and mechanistic insights obtained from the computational studies may lead to development of new concepts concerning hDAT-related therapeutic design. For example, since HIV-1 Tat and DA do not compete in their binding with hDAT, it might be possible to design a novel type of therapeutic agent, which can prevent Tat from binding with hDAT without blocking the DA uptake by hDAT. In fact, our research team is currently exploring this possibility in light of the new mechanistic insights.

The similar computational strategies and approaches used to study hDAT structures and mechanism may be employed to determine the detailed structures and mechanisms of other monoamine transporters, including norepinephrine and serotonin transporters, and other members of the NSS family. Understanding the detailed interactions of Tat with all possibly relevant transporters and receptors could help to better understand all of the possible physiological effects of HIV-1 Tat in the brain, to better understand the molecular mechanisms of the HAND and to design more effective therapeutics.

In general, the success of the aforementioned computational studies on the hDAT structures, mechanism and its interaction with HIV-1 Tat indicates that state-of-the-art computational techniques are truly valuable tools that can be used to reliably predict the complex protein structures, mechanisms and their interactions with other proteins or drugs. For this reason, one may expect to see more and more computational studies on other proteins for their structures, mechanisms and protein-protein interactions in the future.

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Executive summary

- Human dopamine transporter (hDAT) is a common target for a variety of drug design and discovery efforts, but there is no x-ray crystal structure available for hDAT.
- Extensive molecular modeling, docking and dynamics simulations have provided reasonable 3D structural models of hDAT in its three typical conformational states.
- The recent 3D models of hDAT have been used to study how hDAT uptakes dopamine and how HIV-1 transactivator of transcription (Tat) blocks dopamine uptake by hDAT.
- Computational modeling led to development of the first-ever detailed 3D structural model of hDAT–Tat binding.
- According to the computational modeling of hDAT–Tat interaction, HIV-1 Tat binds most favorably with the outward-open state of hDAT.
- Computational modeling has also revealed key residues affecting the hDAT mechanisms for its normal function and interaction with Tat.
- The computational insights are consistent with available experimental data and supported by further experimental validation.
- The detailed structural and mechanistic insights obtained from recent computational studies on hDAT and hDAT–Tat interaction should be valuable for future rational design of novel therapeutic strategies for treatment of HIV-associated neurocognitive disorders.

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