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Original Article

Predictive Simulation and Functional Insights of Serotonin Transporter: Ligand Interactions Explored through Database Analysis

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ABSTRACT

Through its ability to facilitate the absorption of serotonin into presynaptic neurons, the serotonin transporter, also known as SERT, an essential component in the control of neurotransmission. To discover SERT possible therapeutic application, it is essential to have a solid understanding of its dynamic behavior, ligand interactions, and functional consequences. Within the scope of this investigation, the predictive simulations is crucial to investigate the complexities of SERT to gain a fresh understanding of its operation. We use the 6AWN model to describe the sequence and simulate the behavior of SERT in silico. Within this simulation, we anticipate the conformational changes of SERT and its reaction to ligand binding with paroxetine, cholesterol, dodecyl-beta-D-maltose (DDM), and sodium hydrogen ion. We discover critical residues that are crucial in the interaction between ligands and proteins. They have paroxetine binding to I.172, I.172, Y.176, and F.341 are examples of hydrophobic interactions. Example of hydrogen bonds include A.96 and pi-stacking: F.341. The blockage of the serotonin transporter is the principal mechanism of action that paroxetine has. Cholesterol interacts with SERT W.500, W.500, W.500, W.500, L.504, and A.507, and it also interacts with the outward-facing conformation of this transporter in two different ways. In general, cholesterol interacts with SERT and ligands to stabilize their optimal activity and structure. DDM contact with SERT is also a part of this interaction. R.104, D.328, E.494, Y.495, G.498, P.499, T.503, F.556, L.557, S.559, P.561, Y.579, G.582, T.583, and F.586 are the numbers that are currently in use. Within the context of glucosyl transfer processes, DDM has been utilized as an acceptor. And the interaction of Na with SERT S.263, which causes a change in the structure of SERT. Serotonin transporters are present in the environment.

Keywords: Serotonin Transporter, Database Analysis, Functional analysis, Predictive in silico.

INTRODUCTION

Serotonin, a neurotransmitter that plays a significant role in the regulation of mood, cognition, and a variety of physiological functions, is dependent on the serotonin transporter (SERT) for its absorption into presynaptic neurons after it has been released into the synaptic cleft. The modulation of SERT activity and the change of SERT partners in the membrane trafficking route are two examples of biological mechanisms that could be responsible for the link between SERT and cognitive problem.[1] It has been discovered that increased levels of plasma serotonin (5HT) might downregulate the expression of SERT on the platelet membrane, hence restricting the uptake of SERT in platelets.[2-3] Since these mechanisms show that chronically disturbed synaptic 5-HT homeostasis might generate reversible, functional perturbations in 5-HT sensitive circuits in the brain and periphery, it is important to note that SERT may play a role in cognitive disorders.[1] Within the complex field of neurobiology, it is of the utmost importance to have a comprehensive grasp of the three-dimensional (3D) structure of important proteins to find answers to the mysteries surrounding neurotransmission SERT, which stands for serotonin transporter, is an example of a player that plays a vital role in the field of neuromodulation. Reuptake of serotonin from the synaptic cleft is the function of this membrane protein, which is responsible for the process.[1] One of the most important aspects of neurotransmission is the dynamic interaction that occurs between serotonin and serotonin. This interaction affects the duration and strength of serotonergic signaling. Having a comprehensive understanding of the complex mechanisms that govern SERT activity, particularly concerning ligand interactions, is necessary to comprehend the role that it plays in both health and sickness.

By regulating serotonin levels and exerting an influence on mood, cognition, and a variety of physiological processes, the serotonin reuptake transporter (SERT) plays a critical role. Palmitoylation is a post-translational modification that allows for the regulation of protein kinetics and trafficking.[4] SERT is a target for palmitoylation. Reducing the expression of SERT through the use of small interference RNA (SERT-siRNA) has been demonstrated to have antidepressant properties and to modify important markers of antidepressant action. These markers include the expression and function of 5-HT1A-autoreceptors, the levels of extracellular serotonin, neurogenesis, and the expression of genes related to plasticity. SERT expression on platelet membranes is downregulated when plasma serotonin levels are elevated, which limits the amount of serotonin that platelets can take in.[5] The advancements that have been made in computational biology have made it possible

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to predict the three-dimensional structure of proteins, which has provided extremely useful insights into the functions and interactions of proteins. The unraveling of the three-dimensional prediction and role of the serotonin transporter appears as an endeavor that is interesting when viewed in this light.

Through the facilitation of substrate recognition, transport, and control, the structural dynamics of SERT contribute to the role that it plays in the structure. The conformational changes and intermediate states that are involved in substrate import and binding have been uncovered through the use of Markov state models and molecular dynamics simulations.[6] As a result of this research, it has been demonstrated that the rate-limiting phase for substrate import is the transition from the occluded state to the inward-facing state and that the substrate reduces the free energy barriers to attain the inward-facing state.[7] In addition, allosteric pockets that are located outside of the substrate binding sites have been discovered, and compounds that affect the function of SERT through these pockets have been found.[8] These discoveries shed light on the molecular basis of SERT dynamics as well as its function in the transport and regulation of neurotransmitters.

Through the use of molecular simulation, the ultimate objective is to close the gap that exists between structural predictions and functional consequences. In this regard, our research endeavors to conduct an exhaustive analysis of the serotonin transporter. We make use of sophisticated predictive models to decipher the dynamic conformational changes that occur in the transporter as well as its responses to the binding of ligands. Beyond the scope of conventional structural investigations, the focus is directed toward investigating the functional implications of interactions between SERT and ligand groups. The purpose of this study is to identify trends in the interactions between ligands and SERT across a wide range of structural settings. We hope to shed light on the complicated dance that takes place between SERT and its ligands by combining predictive simulations with empirical data from the PDB. This will allow us to pinpoint critical residues that are important in ligand binding.

METHODS

Using computational approaches to analyze the three-dimensional structure of the serotonin transporter (SERT). Methods that are typically utilized for the investigation of the three-dimensional structure of SERT are as follows:

The data Retrieval and Characterization of SERT details: The order of events A dependable database was consulted to retrieve the amino acid sequence of the serotonin transporter (SERT). NCBI can be found at https://www.ncbi.nlm.nih.gov/.[9] We employed the parameter protein with the protparam algorithm to characterize SERT https://web.expasy.org/protparam/.[10] In addition to predicting position with the use of Transmembrane https://services.healthtech.dtu.dk/services/TMHMM-2.0/2.[11]

Choose templates that have a high sequence resemblance to SERT, according to Alignment and Structure Prediction analyses. For the alignment sequence, we make use of a bioinformatics tool that is equipped with Clustal Omega. The SERT sequence should be aligned with the template sequences that have been chosen. in addition to a study of the gaps in the alignment and the conserved sections and template Selection to identify homologous protein structures that are known to have three-dimensional structures to represent homology using the Swiss model. To construct a three-dimensional model of SERT and its components, input the aligned sequence and template structures. The optimization of models used to alleviate steric conflicts and improve the overall geometry, the basic model should be refined through the process of energy minimization.

Verify the quality of the model and validate the model: Utilizing the Ramachandran plot, evaluate the quality of the homology model that has been developed. Confirm that the model possesses a stereochemistry that is plausible and that it falls within the parameters of acceptable structural design. SERT's Molecular Function and Another The identification and characterization of amino acid residues in proteins that are essential for the protein's biological function is the process that is referred to as functional analysis using enrichment analysis with Enrichr to determine the functional protein. proteins are involved with a variety of biological processes, molecular functions, and pathways.

An investigation into polarity and the ligands that bind. To identify the polarity of the structure of the protein It is common for polar compounds to be soluble in polar solvents, such as water, but less soluble in nonpolar solvents compared to polar solvents. It is possible to obtain qualitative information about the polarity of a molecule by observing its solubility features and identifying it with the use of https://www.creative-proteomics.com/ngpro.[12] In addition, the computational method known as "binding Ligand by Molecular Docking" is utilized to make predictions regarding the binding mechanism and affinity of a ligand to a protein using the Autodock tool to bind the ligand.

RESULTS AND DISCUSSION

Retrieval database in NCBI the term "Serotonin Transporter" or the gene symbol associated with it (e.g., SLC6A4) as Table 1.

According to Table 1, the region of SERT that is responsible for phosphorylation, transmembrane protein, Na binding site or ion binding, chemical binding, and glycosylation all have functions. On the other hand, the sodium binding sites of SERT, which are referred to as NA1 for site 1 and NA2 for site 2, are sodium selective.[13] The data shown in Table 1 shows that SERT transmembrane prediction was made by TMHMM, as shown in Fig 1 with a total probability of N-in of 0.85817.

Structure Prediction

The term "protein secondary structure" refers to the polypeptide backbone that is present in the local conformation of proteins. There are three types of secondary structure states: the α-helix (H) and the β-strand (E). Additionally, there is one type of secondary structure that is irregular, which is the coil region (C). According to hydrogen-bonding patterns, Sander devised a system for assigning secondary structure called the Dictionary of Secondary Structure of Proteins (DSSP).[14] This method automatically allocates the secondary structure into eight different states, which are H, E, B, T, S, L, G, and I. It is common practice to further simplify these eight states by distinguishing them into three states: helix, sheet, and coil. The convention that is most commonly utilized is that the helix is denoted by the letters G, H, and I, the sheet is denoted by the letters B and E, and all other states are denoted as coils. The prediction of the secondary structure of a protein is an important initial step toward the prediction of the tertiary structure of the protein. Additionally, it offers information about the activity, relationships, and functions of the protein.

Within the context of homology modeling, it is essential to have a three-dimensional (3D) structure of proteins to comprehend the characteristics and activities of proteins. A server known as SWISS-MODEL is utilized in the process of homology modeling.

The findings indicated that there were two models chosen, specifically models 1-7, which utilized the PDB code template 6vrh, 6vrl, 6vrk, 5i6z, 7txt, 6awn, and 7lwd. All of these models were selected. The evidence obtained from sequence alignment and model visualization demonstrates that models 1-7 are identical to one another. The evaluation and assessment of model 6awn on the Ramachandran Plot have a Favored area of 96.59%, a MolProbity score of 1.69, and

Table 1. Region SERT and function.

Region	Name	Function
1-630	protein hSERT	Serotonin transporter
1-59	disordered	propagated
24-64	5HT transporter N	pfam
47, 142, 276	Site type	phosphorylation
79-615	SLC6sbd_SERT	Na+ and Cl- transporter
88-112,278..297, 325..347, 361-380, 422-433,464-483, 495-516,	Site type	Transmembrane
94,97, 434, 437-438	Site type	Na binding site/ion binding
95..96,98..101,176,335..336,341,343,438,441..442	Site Type	Chemical binding
96,101,336,368	Site type	Na binding
103..104,175,179,182,403,407	Site Type	Chemical binding
208, 217	Site type	Glycosylation, N-linked (GlcNAc...) asparagine
1..630	CDS	neurotransmitter transporter

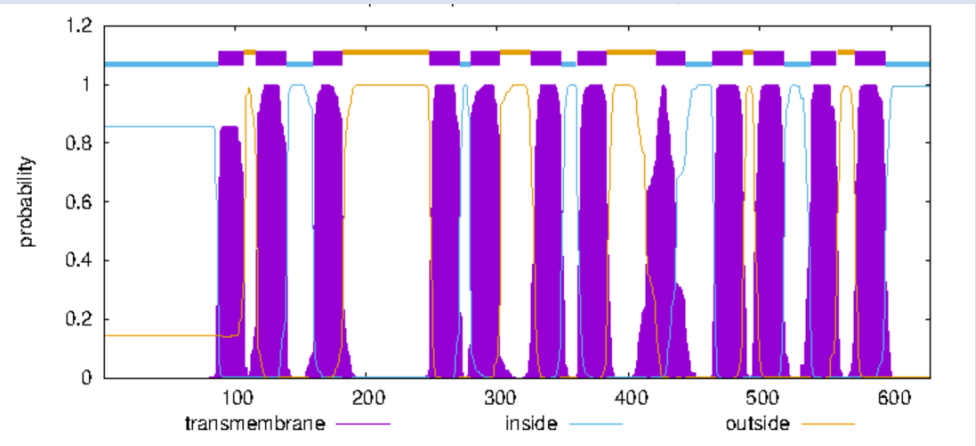

Figure 1. Prediction transmembrane of SERT using TMHMM.

a QMEAN value of 1.13. These factors indicate that the model is of high quality. A good example of a three-dimensional protein structural model is shown in Figure 3.

Structures like these Explain, using the 3D model 6awn, how the residues within the central site are responsible for the binding of a wide variety of pharmacological inhibitors and for mediating the drug selectivity of transporters based on the results of the analysis, the sequence is comparable to 6awn.1. The X-ray structure of the S439T human serotonin transporter complexed with paroxetine at the central site, as shown in Figure 3.[16]

Providing insights into the structural integrity and stereochemistry of the modeled or experimental protein structure, the Ramachandran score from MolProbity is a valuable metric for measuring the overall quality of a protein structure in terms of the backbone dihedral angles. This score may be obtained by using MolProbity. A Ramachandran score that is near one hundred percent suggests that a greater proportion of the protein residues adopt favorable backbone conformations. This is generally suggestive of a protein structure that has been refined to a high degree. There is a possibility that portions of the structure that deviate from ideal conformations are indicated by lower scores. For example, in Figure 3b, the Ramachandran score is 96.29 percent, the Rotamer score is 0.37 percent, and the poor bond score is 3/4494. According to this model, the regions that are favored reflect conformations that are both sterically permitted and energetically beneficial regarding these angles.

The molecular functional identification process involves determining the amino acid residues that play a significant role in the binding and transport of serotonin by using structural analysis. It is necessary to have an understanding of the biological processes and biochemical functions of the serotonin transporter (SERT) to undertake an analysis of its structure Subcellular Localization: - Determine the subcellular localization of SERT inside neurons, paying particular attention to its presence in presynaptic terminals. Within the context of the biological process of serotonin reuptake from the synaptic cleft into the presynaptic neuron, it is important to investigate the role that the serotonin transfer enzyme (SERT) plays. Consider researching how SERT plays a role in the regulation of neuronal signaling and neurotransmission in the central nervous system.

An Examination of the Biochemical Function: The following are some examples of binding sites and pockets, conformational change, and ligand interactions: - Through molecular docking studies, investigate the interactions that occur between SERT and serotonin, as well as other ligands or inhibitors conformational alterations for the during the process of serotonin binding and transport, investigate the conformational changes that occur in SERT. Analysis of Functional Residues Utilizing Locate the essential amino acid residues that are present in SERT and that are responsible for the biological function of the protein. Investigate the effect that mutations have on normal function. Transportation of substrate to the mechanism of serotonin transport by SERT should be investigated, including the recognition of substrates, the binding of substrates, and the translocation of serotonin across the cell membrane. According to the data shown in Table 2, the biochemical functions of SERT include transmembrane transport, transporter activity, active transmembrane transporter, specific transporter activity, ion transmembrane transporter activity, protein binding, and amine transmembrane transporter.

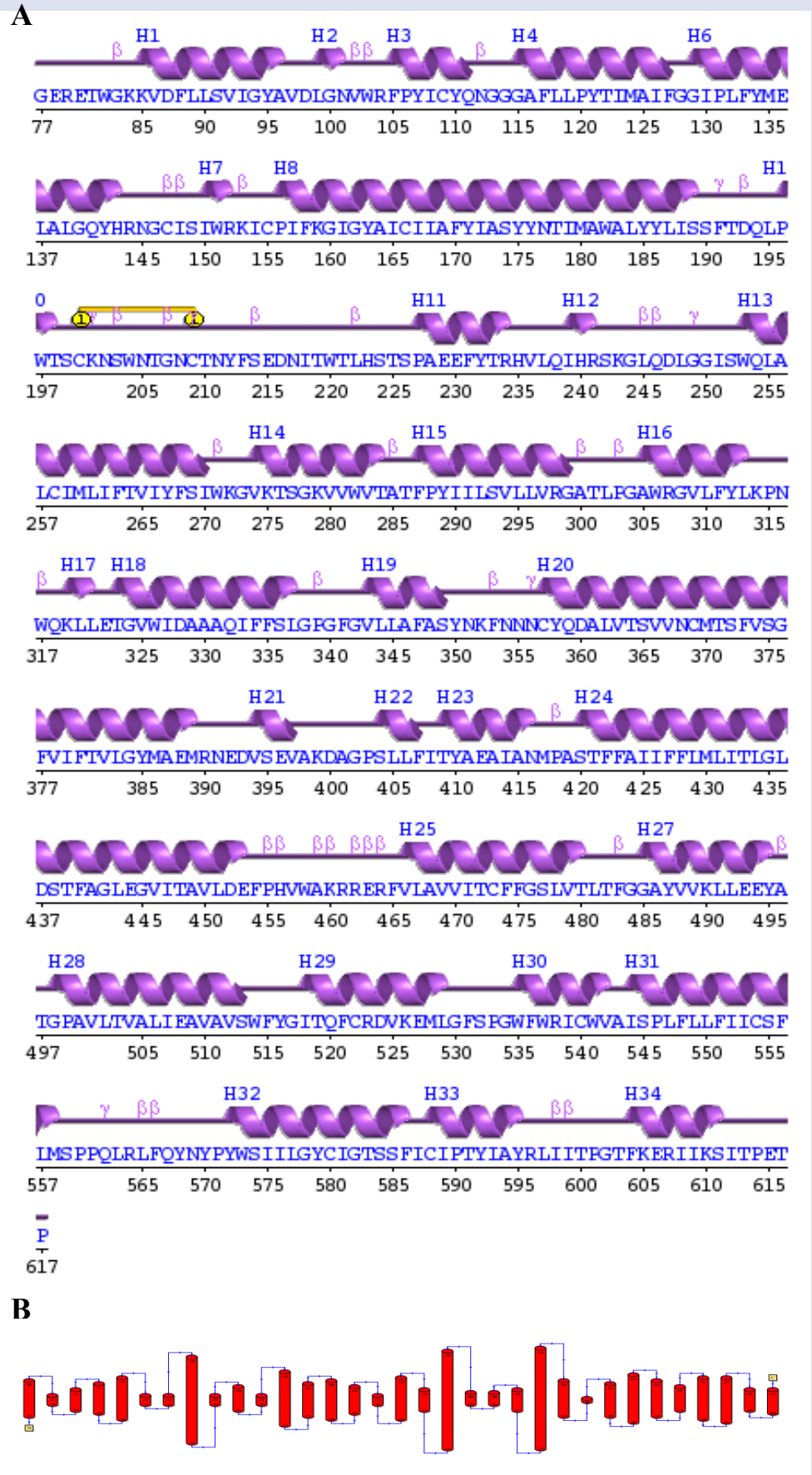

Figure 2. Prediction structure of SERT. A)Secondary structure of SERT, B) modeling loop for 3D of SERT[15]

Table 2. Molecular function identification using FuncPro.

Type	GO no.	Description	Score	BLAST	IPR	SUP	PDB	SSM	ENZ	DNA	LIG	REV
Cellular component												
	0016021	integral to membrane	22.48	-	0.04 (0)	-	19.09 (0)	-	-	-	0.99 (0)	2.36 (0)
	0005623	cell	22.48	-	0.04 (0)	-	19.09 (0)	-	-	-	0.99 (0)	2.36 (0)
	0044464	cell part	22.48	-	0.04 (0)	-	19.09 (0)	-	-	-	0.99 (0)	2.36 (0)
	0016020	membrane	22.48	-	0.04 (0)	-	19.09 (0)	-	-	-	0.99 (0)	2.36 (0)
	0044425	membrane part	22.48	-	0.04 (0)	-	19.09 (0)	-	-	-	0.99 (0)	2.36 (0)
	0031224	intrinsic to membrane	22.48	-	0.04 (0)	-	19.09 (0)	-	-	-	0.99 (0)	2.36 (0)
	0005886	plasma membrane	17.62	-	-	-	14.36 (0)	-	-	-	1.04 (0)	2.22 (0)
	0005887	integral to plasma membrane	14.01	-	-	-	10.95 (0)	-	-	-	1.03 (0)	2.03 (0)
	0044459	plasma membrane part	14.01	-	-	-	10.95 (0)	-	-	-	1.03 (0)	2.03 (0)
	0031226	intrinsic to plasma membrane	14.01	-	-	-	10.95 (0)	-	-	-	1.03 (0)	2.03 (0)
Biological process												
	0051234	establishment of localization	10.87	-	-	-	9.09 (0)	-	-	-	0.88 (0)	0.90 (0)
	0006810	transport	10.87	-	-	-	9.09 (0)	-	-	-	0.88 (0)	0.90 (0)
	0051179	localization	10.87	-	-	-	9.09 (0)	-	-	-	0.88 (0)	0.90 (0)
	0009987	cellular process	8.45	-	-	-	7.17 (0)	-	-	-	0.85 (0)	0.43 (0)
	0065007	biological regulation	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0050789	regulation of biological process	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0050794	regulation of cellular process	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0032879	regulation of localization	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0051049	regulation of transport	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0055085	transmembrane transport	4.69	-	-	-	4.69 (0)	-	-	-	-	-
	0050896	response to stimulus	4.44	-	-	-	3.07 (0)	-	-	-	0.63 (0)	0.74 (0)
	0042221	response to chemical stimulus	4.44	-	-	-	3.07 (0)	-	-	-	0.63 (0)	0.74 (0)
	0015837	amine transport	4.44	-	-	-	3.07 (0)	-	-	-	0.63 (0)	0.74 (0)
Biochemical function												
	0022857	transmembrane transporter activity	23.62	-	-	-	20.09 (0)	-	-	-	1.04 (0)	2.49 (0)
	0005215	transporter activity	23.62	-	-	-	20.09 (0)	-	-	-	1.04 (0)	2.49 (0)
	0015293	symporter activity	20.16	-	-	-	16.80 (0)	-	-	-	1.03 (0)	2.32 (0)
	0022804	active transmembrane transporter activ	20.16	-	-	-	16.80 (0)	-	-	-	1.03 (0)	2.32 (0)
	0015291	secondary active transmembrane trans	20.16	-	-	-	16.80 (0)	-	-	-	1.03 (0)	2.32 (0)
	0022892	substrate\-specific transporter activity	6.99	-	-	-	5.37 (0)	-	-	-	0.78 (0)	0.84 (0)
	0022891	substrate\-specific transmembrane trar	6.99	-	-	-	5.37 (0)	-	-	-	0.78 (0)	0.84 (0)
	0015075	ion transmembrane transporter activity	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0008324	cation transmembrane transporter activ	4.71	-	-	-	3.68 (0)	-	-	-	0.68 (0)	0.35 (0)
	0005515	protein binding	4.44	-	-	-	3.07 (0)	-	-	-	0.63 (0)	0.74 (0)
	0005488	binding	4.44	-	-	-	3.07 (0)	-	-	-	0.63 (0)	0.74 (0)
	0005275	amine transmembrane transporter activ	4.44	-	-	-	3.07 (0)	-	-	-	0.63 (0)	0.74 (0)

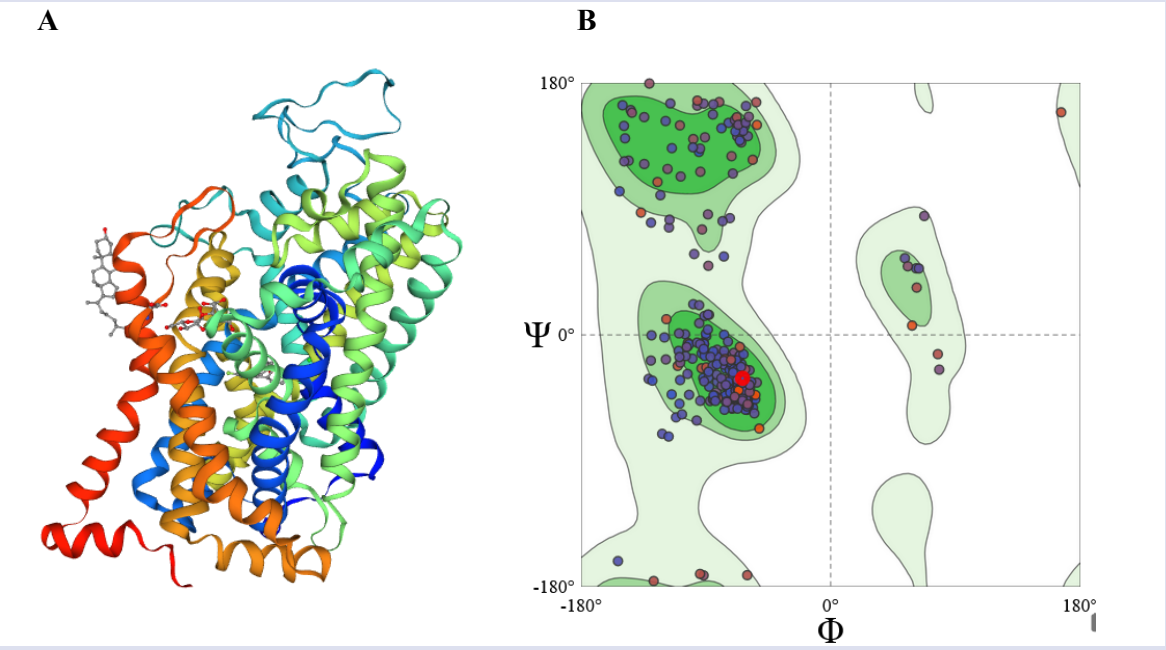

Figure 3. Structure of SERT. A) 3D structure have similar with 6awn.1 and B) Ramachandran plot of SERT

The polarity of SERT, understanding the structure-function relationship of SERT, its interactions with its environment, and the dynamics involved in serotonin transport are all dependent on having a solid understanding of the polarity of SERT. The serotonin transporter, also known as SERT, is a membrane protein that is accountable for the reuptake of serotonin (5-HT) from the synaptic cleft and into the presynaptic neuron. In terms of polarity, there are a few elements of SERT that can be considered to be associated with the location of membrane proteins. Figure 4 of the polarity SERT is a transmembrane protein, which means that it can traverse the lipid bilayer that is present in the cell membrane. This transmembrane nature of the protein suggests that it contains both hydrophobic and hydrophilic regions within its structure. Within the lipid bilayer, the transmembrane segments of SERT are normally hydrophobic, giving them a grey appearance. These segments are also deeply entrenched. There is a possibility that specific loops, as well as extracellular and intracellular sections, may contain hydrophilic residues (blue), which facilitate interactions with the watery environment that surrounds the biological system. Both Binding and Transporting, There is a possibility that hydrophilic areas of SERT are involved in the interaction with serotonin and other ligands that are present in the extracellular environment during the processes of binding and transport. By interacting with the lipid bilayer, the hydrophobic regions of SERT contribute to the stability of the protein and help it to be anchored in the membrane.

Aliphatic chemicals are organic molecules that are made up of carbon atoms arranged in carbon chains that are either straight or branched. Several amino acids are included in the structure of the serotonin transporter, which is a transmembrane protein. These amino acids provide the transporter with its overall activity. Aliphatic amino acids, which are distinguished by the presence of aliphatic side chains, are fundamental amino acids that are found in proteins. These aliphatic amino acids, along with many others, make a cumulative contribution to the structure and function of the serotonin transporter as a whole. A particular factor that plays a significant role in the integration of the protein into the lipid bilayer of the cell membrane is the hydrophobic nature of aliphatic residues, which are represented by the color blue. Figure 4b demonstrates that the distribution of aliphatic amino acids within the serotonin transporter and the role that they play is essential for acquiring an understanding of the structural stability of the serotonin transporter, as well as its interactions with other molecules and its overall activity, particularly in the context of neurotransmitter transport.

Paroxetine is classified as a selective serotonin reuptake inhibitor (SSRI), and its principal function is to inhibit the serotonin transporter (SERT). The absorption of serotonin (5-HT) from the synaptic cleft into the presynaptic neuron is the responsibility of a protein known as the serotonin transporter. This reuptake process is disrupted by selective serotonin reuptake inhibitors (SSRIs) like paroxetine, which results in an elevated concentration of serotonin in the synaptic cleft. According to Figure 5A, paroxetine inhibits the reuptake of serotonin by interacting with the SERT amino acid Y.95, A.96, D.98, A.169, I.172, A.173, Y.176, F.335, S.336, G.338, F.341, S.438, T.439, G.442, L.443, T.497, and V.501. binding by contact The hydrophobic interactions of peroxetine are present. There are hydrogen bonds A.96 and pi-stacking: F.341, as well as I.172, A: I.172, A: Y.176, and F.341. The blockage of the serotonin transporter is the principal mechanism of action that paroxetine has. The ability of the serotonin transporter to internalize serotonin from the synaptic cleft into the presynaptic neuron is inhibited by the binding of paroxetine to the serotonin transporter pathway.

When it comes to modifying the functional characteristics of the serotonin transporter (SERT), cholesterol is a factor that cannot be ignored. A decrease in SERT activity is caused by the depletion of membrane cholesterol, which results in a loss of affinity for substrate and ligand binding as well as a drop in the maximal transport rate.[17] On the other hand, the distribution of SERT to the plasma membrane is not affected by the depletion of cholesterol.[18] Cholesterol has a significant role in the upkeep of the outward-facing conformation of the dopamine transporter (DAT) as well as the typical rates of structural interconversions.[19] It was demonstrated in Figure 5B that cholesterol interacts with SERT W.500, W.500, W.500, W.500, L.504, and A.507 and that these interactions involve two parts and are characterized by an outward-facing conformation of this transporter. The overall effect of cholesterol's interactions with SERT and ligand is to stabilize the optimal activity and structure of these molecules.[20]

DDM, also known as dodecyl-beta-D-maltose, is a non-ionic detergent that can interact with the serotonin transporter mechanism. It has been demonstrated that it is capable of occupying anesthetic binding sites in model proteins, such as human serum albumin (HSA) and horse spleen apoferritin, hence preventing the binding of general anesthetics like propofol and isoflurane.[21] In addition, DDM has been utilized in molecular dynamics simulations to investigate the impact that surfactant conformation has on micelle properties to investigate.

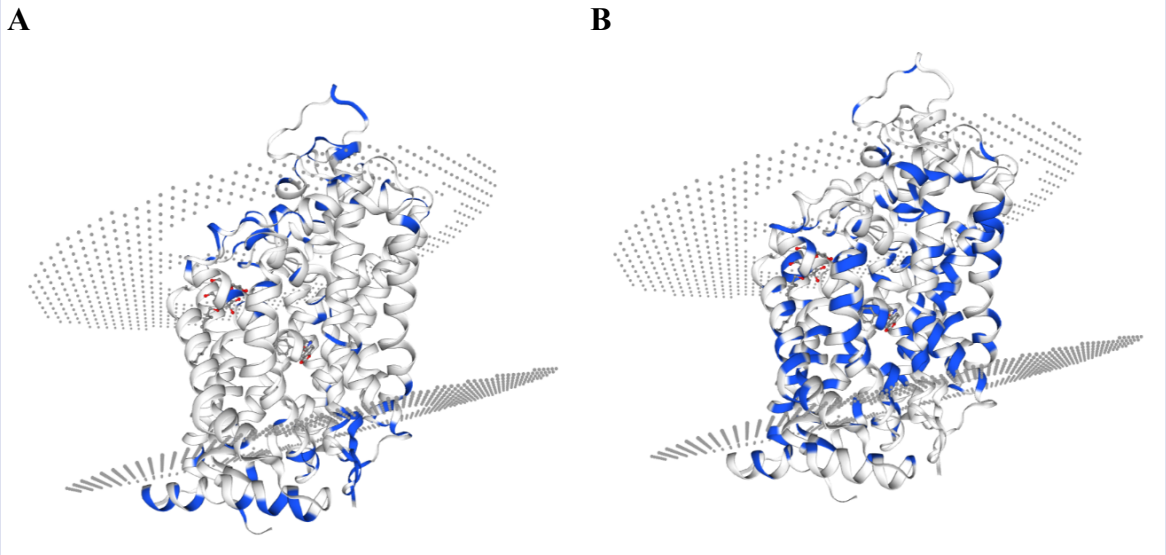

Figure 4. Characteristics of SERT A) Polarity region of SERT with transmembrane, B) Aliphatic R group of amino acid SERT

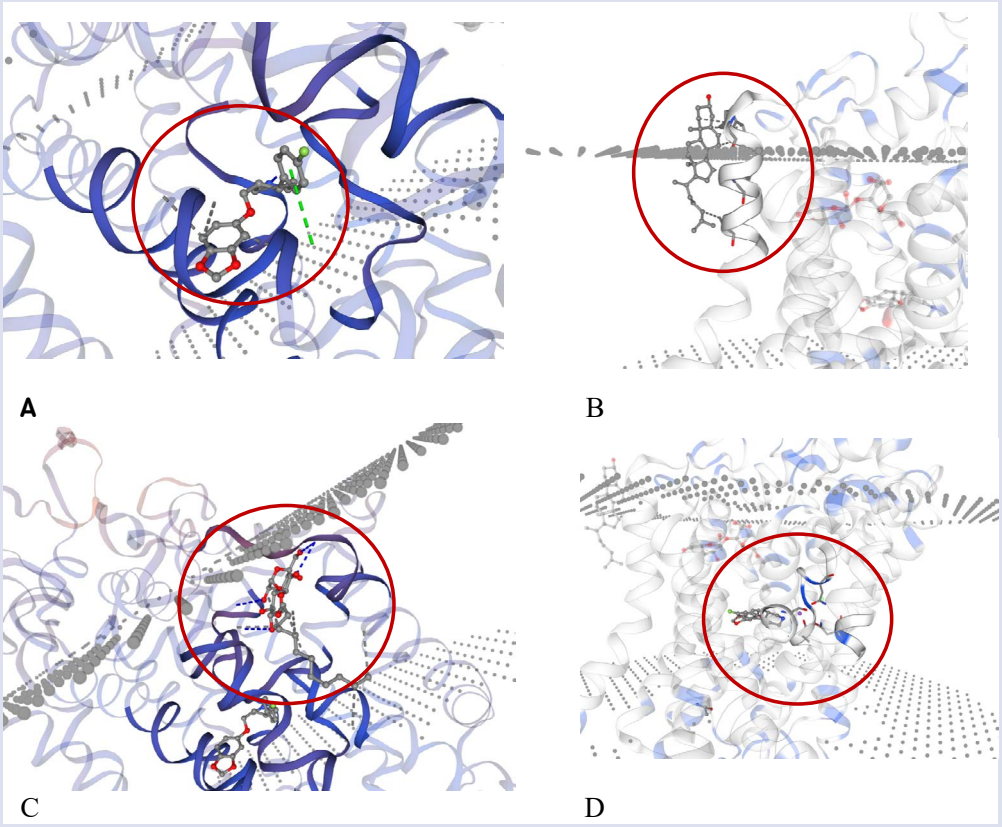

Figure 5. Binding interaction of SERT with ligand. A) binding interaction of Paroxetine, B) binding interaction of Cholesterol, C) binding interaction of Dodecyl beta D Maltose, and D) binding interaction of Na+ (red circles)

The results of these simulations have demonstrated that the geometry of DDM micelles is somewhat ellipsoidal and that the dynamics of interfacial water are slower than those of bulk water, regardless of the conformation of the headgroup.[22] Figure 5C demonstrated that the DDM interaction with SERT R.104, D.328, E.494, Y.495, G.498, P.499, T.503, F.556, L.557, S.559, P.561, Y.579, G.582, T.583, and F.586 was observed during the experiment. In glucosyl transfer processes, DDM has been employed as an acceptor, which suggests that it may play a role in the production of glycogen.[23] Further applications of DDM include the extraction and purification of membrane proteins, as well as the solubilization of membrane proteins to conduct experimental research on the structure, dynamics, and ligand binding of membrane proteins.[24]

The serotonin transporter is responsible for facilitating the uptake of serotonin into nerve cells, and sodium plays a role in this process. The neurotransmitter transporters (NTs) that are dependent on sodium and chloride are a family of homologous membrane proteins that are accountable for the reuptake of neurotransmitters from the synaptic cleft. These neurotransmitters include dopamine, serotonin (5-HT), norepinephrine, γ-aminobutyric acid (GABA), and glycine, in addition to other small molecules like proline, creatine, betaine, and taurine. These transporters are responsible for coordinating the flow of sodium along its electrochemical gradient with the transportation of substrate across the plasma membrane. To transport their substrate neurotransmitters, these transporters make use of transmembrane ion gradients of sodium, chloride, and potassium.[25-26] During the process of transporting serotonin into the cell, sodium ions are also brought in, while potassium ions are transported out of the cell. Numerous psychostimulants and essential medicinal medicines are aimed at this particular target.

The SERT enzyme is responsible for catalyzing the influx of 5-HT, together with Na+ and Cl–, in a stoichiometry of 1:1:1. one K+ ion can undergo coupled efflux within the same catalytic cycle. When it comes to transporting amino acids and amines, SERT is a member of a wide family of transporters that are thought to use processes of transport that are comparable to one another.[27] This process contributes to the regulation of extracellular serotonin levels and the termination of its action at receptor sites during the process. According to research carried out by Friederich and colleagues in 1997, presynaptic Na+-coupled neurotransmitter transporters are an indispensable component of neurotransmission and play a position that is of utmost significance in the treatment of mental diseases and substance abuse. Our objective is to solve the three-dimensional structure of one of these transporters so that we may better understand the role that it plays. Our understanding of the molecular mechanisms underlying the operation and control of transporters would be substantially accelerated as a result of this, and these mechanisms could potentially open up new avenues for the creation of pharmaceuticals and treatments.[28] It was demonstrated in Figure 5D that the interaction between Na and SERT S.263 and the change in conformation of SERT. Additionally, the presence of serotonin transporters enables other types of cells to concentrate and store serotonin for eventual secretion.[29]

CONCLUSION

Structures of SERT indicate that NA1 and NA2 play different roles, with NA1 being primarily responsible for substrate binding and the ion in NA2 being critical for stabilizing the outward-open state. NA1 is primarily responsible for binding to substrates. The simulations that were conducted to study the influence of bound ions began with bound ions or investigated the situation in which there were no bound ions.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

REFERENCES

1. Antonio, Verdejo-García. (2016). Cognitive training for substance use disorders: Neuroscientific mechanisms. Neuroscience & Biobehavioral Reviews, 68:270-281. doi: 10.1016/J.NEUBIOREV.2016.05.018
2. Seenae, Eum., S., Kristian, Hill., Jeffrey, R., Bishop. (2022). Considering medication exposure in genomic association studies of cognition in psychotic disorders. Pharmacogenomics, 23(14):791-806. doi: 10.2217/pgs-2022-0070
3. Charles, P., Mercado., Fusun, Kilic. (2010). Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. Molecular Interventions, 10(4):231-241. doi: 10.1124/MI.10.4.6
4. James, D., Foster. (2023). Palmitoylation regulates human serotonin transporter activity, trafficking, and expression and is modulated by escitalopram. doi: 10.1101/2023.05.09.540092
5. Albert, Ferrés-Coy., Fencible, Pilar-Cuéllar., Rebeca, Vidal., Verónica, Paz., Mercè, Masana., Mercè, Masana., et al. (2013). RNAi-mediated serotonin transporter suppression rapidly increases serotonergic neurotransmission and hippocampal neurogenesis. Translational Psychiatry, 3(1) doi: 10.1038/TP.2012.135
6. Ara, M., Abramyan., Rachel, D., Slack., Sitaram, Meena., et al. (2019). Computation-guided analysis of paroxetine binding to hSERT reveals functionally important structural elements and dynamics. Neuropharmacology, 161:107411-107411. doi: 10.1016/J.NEUROPHARM.2018.10.040
7. Matthew, Chan., Erik, Procko., Diwakar, Shukla. (2021). Structural rearrangement of the intracellular gate of the serotonin transporter induced by Thr276 phosphorylation. bioRxiv, doi: 10.1101/2021.10.13.464332
8. Yuji, Tokunaga., Thibault, Viennet., Haribabu, Arthanari., Koh, Takeuchi. (2020). Spotlight on the Ballet of Proteins: The Structural Dynamic Properties of Proteins Illuminated by Solution NMR. International Journal of Molecular Sciences, 21(5):1829-. doi: 10.3390/IJMS21051829
9. https://www.ncbi.nlm.nih.gov/.
10. Gasteiger E, Hoogland C, Gattiker A, Duvaud SE, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. Humana press; 2005.
11. Krogh A, Larsson B, von Heijne G, Sonnhammer EL: Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001, 305(3):567–580.
12. https://www.creative-proteomics.com/ngpro.
13. Kabsch, W. & Sander, C. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* 22, 2577–2637 (1983).
14. Felts B., Pramod A. B., Sandtner W., Burbach N., Bulling S., Sitte H. H., et al.. (2014). The two Na+ sites in the human serotonin transporter play distinct roles in the ion coupling and electrogenicity of transport. J. Biol. Chem. 289, 1825–1840. 10.1074/jbc.M113.504654
15. Wang J et al. (2023), *"The conserved domain database in 2023"*, Nucleic Acids Res.51(D)384-8.
16. Coleman, J.A. et al., Structural basis for recognition of diverse antidepressants by the human serotonin transporter. Nat. Struct. Mol. Biol. (2018)
17. M., Scanlon., D., C., Williams., Patrick, Schloss. (2001). Membrane cholesterol modulates serotonin transporter activity. Biochemistry, 40(35):10507-10513. doi: 10.1021/BI010730Z
18. Carmen, M., Deveau., Eric, A., Rodriguez., Allen, Schroering., Bryan, K., Yamamoto. (2021). Serotonin transporter regulation by cholesterol-independent lipid signaling. Biochemical Pharmacology, 183:114349-114349. doi: 10.1016/J.BCP.2020.114349
19. Kymry, T., Jones., Juan, Zhen., Maarten, E., A., Reith. (2012). Importance of cholesterol in dopamine transporter function. Journal of Neurochemistry, 123(5):700-715. doi: 10.1111/JNC.12007
20. Peter, Fischer., Gruenblatt, E., Pietschmann, P., Tragl, Kh. (2006). Serotonin transporter polymorphism and LDL-cholesterol. Molecular Psychiatry, 11(8):707-709. doi: 10.1038/SJ.MP.4001837
21. Stéphane, Abel., François, Yves, Dupradeau., E., Prabhu, Rahman., Alexander, D., MacKerell., Marchi, Massimo. (2010). Molecular Simulations of Dodecyl-D-Maltose Micelles in Water: Influence of the Headgroup Conformation and the Force Field Parameters. Biophysical Journal, 98(3):5-7. doi: 10.1016/J.BPJ.2009.12.3074
22. Yvonne, Dempsie., Margaret, R., MacLean. (2008). Role of the serotonin transporter in pulmonary arterial hypertension. Expert Review of Clinical Pharmacology, 1(6):749-757. doi: 10.1586/17512433.1.6.749
23. Longhe, Xu., Felipe, Matsunaga., Jin, Xi., Min, Li., Jingyuan, Ma., Renyu, Liu. (2014). n-Dodecyl β-D-maltose specifically competes with general anesthetics for anesthetic binding sites. Journal of Biomolecular Structure & Dynamics, 32(11):1833-1840. doi: 10.1080/07391102.2013.838699
24. Stephen, M., Manzella., Lennart, Rodén., Elias, Meezan. (1995). Dodecyl-β-D-maltose as a substrate for glucosyl and xylosyl transfer by glycogenin. Glycobiology, 5(2):263-271. doi: 10.1093/GLYCOB/5.2.263
25. Naomi, R., Goldberg., Thijs, Beuming., Harel, Weinstein., Jonathan, A., Javitch. (2004). A Structural Context for Studying Neurotransmitter Transporter Function. 213-234. doi: 10.1007/978-1-59259-672-0_12
26. D., Graham., S.Z., Langer. (1992). Structure and function of the serotonin transporter. European Neuropsychopharmacology, 2(3):271-272. doi: 10.1016/0924-977X(92)90105-H
27. Gary, Rudnick. (2006). Structure/function relationships in serotonin transporter: new insights from the structure of a bacterial transporter. Handbook of experimental pharmacology, 175(175):59-73. doi: 10.1007/3-540-29784-7_3
28. Friedrich, M., Titgemeyer., Patrick, Schloss., Cara, R., Baker., D., Clive, Williams., et al. (1997). Towards Overexpression of the Serotonin Transporter. 773-780. doi: 10.1007/978-1-4615-5405-9_128
29. Coleman J. A., Yang D., Zhao Z., Wen P.-C., Yoshioka C., Tajkhorshid E., et al.. (2019). Serotonin transporter-ibogaine complexes illuminate mechanisms of inhibition and transport. Nature 569, 141–145. 10.1038/s41586-019-1135-1

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