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Molecular dynamics study of neurotransmitter reuptake mechanism on modeled transporter proteins

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ABSTRACT

The dopamine transporter (DAT), serotonin transporter (SERT) and norepinephrine transporter (NET) proteins are modelled based on homology modelling using Swiss model server. The modelled DAT, SERT and NET were docked with neurotransmitters dopamine, serotonin and norepinephrine respectively using Glide module of Schrodinger suite. At physiological pH, ionic interactions like hydrogen bonding interactions, cation-π interactions and π-π interactions becomes predominate between the neurotransmitter ligands and transporter proteins. It is inferred that from the docking results, phenethylamine appears to be the most important structural element accommodated by the transporter proteins. The perturbation of electronic structure at the residues by the influence of hydrogen bonding interactions, cation-π interactions and π-π interactions is noted as the significant factor in the physiological process.

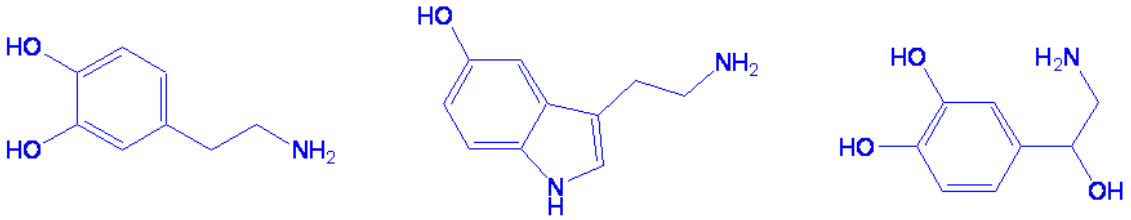
INTRODUCTION

The Dopamine transporter (DAT) has been identified from brains of homosapiens [1-5]. Among other members of the neurotransmitter transporter gene family, the mammalian DATs display highest amino-acid homology with the NET (~67%), SERT (~49%), and γ-amino butyric acid (GABA) transporter (~45%) [6]. Dopamine uptake by all the identified DATs has a similar pharmacological profile, including a highly comparable sensitivity to cocaine analogues. Dopamine a 4”-(2-aminoethyl) benzene-1, 2-diol of molecular formula C6H3 (OH) 2-CH2-CH2-NH2 is a neurotransmitter, a chemical messenger in the brain. Humans, as well as other organisms engage in behaviours that are rewarding; there are natural rewards such as food, water, sex, and nurturing, as well as artificial rewards such as drugs. The pleasurable feelings provide positive reinforcement so that the behaviour is repeated [7]. Each of these naturally rewarded behaviours is required for the survival of the species, so the reward pathway is a crucial part of the brain, as well as a key in understanding normal life and addictions. The availability dopamine at the synaptic cleft is essential for this normal physiological function of brain [8]. Dysfunction of dopaminergic neurotransmission in the CNS has been implicated in a variety of neuropsychiatric disorders, including socialphobia[9], Tourette's syndrome,[10] Parkinson's disease,[11] schizophrenia,[12], attention-deficit hyperactivity disorder (ADHD) [13]. The depletion of dopamine is mainly due to the reuptake of the dopamine by its receptor/transporter proteins [14]. Serotonin (5-hydroxy triptamine), a small monoamine molecule, responsible for the regulation of various physiological states includes cognition, mood, aggression, mating, feeding, and sleep [15-21]. An appropriate amount of serotonin at the synaptic cleft is essential for the conscientious normal behavior. Depletion of serotonin at the synaptic cleft leads to depression, which is a mental state associated with symptoms such as increased sadness and anxiety, loss of appetite, dejected mood, and loss of interest in pleasurable activities [22-24].The depletion of serotonin is mainly due to the reuptake of the serotonin by its transporter proteins [24]. The serotonin transporter protein (SERT) is a protein encoded by the SLC6A4 human gene [25]. Serotonin transporters are dependent on extracellular Na^+ and Cl^- ions. The serotonin transporters first binds a sodium ion, followed by serotonin in its protonated form (5HT+), and then binds to the Cl- ion and carry out its uptake process [26]. Norepinephrine,

$C_8H_{11}NO_3$, 4-[(1R)-2-amino-1-hydroxyethyl] benzene-1, 2-diol, belongs to the class of organic compounds known as catecholamines and derivatives. Norepinephrine is a catecholamine and a phenethylamine .The general function of norepinephrine is to mobilize the brain and body for action [27-29]. Norepinephrine release is lowest during sleep, rises during wakefulness, and reaches much higher levels during situations of stress or danger, in the so-called fight-or-flight response. Norepinephrine increases arousal and alertness, promotes vigilance, enhances formation and retrieval of memory, and focuses attention; it also increases restlessness and anxiety [30-35]. The delpletion of norephinephrine at the synaptic cleft creates syndrome of depression, chronic pain, irritable bowl syndrome, sleep disorders, diabatic neurophathy, agitation and insomnia and migrane prophylaxis [36]. The depletion of norepinephrine is mainly due to the reuptake of the norepinephrine by its norepinephrine transporter (NET) proteins [37]. The NET is responsible for high-affinity uptake of norepinephrine, but also mediates the transport of dopamine with higher affinity than norepinephrine itself [38, 39]. The norepinephrine transporter (NET) belongs to solute carrier family of proteins which is encoded by the SLC6A2 gene . Transporter proteins are important targets to treat psychiatric disorders [40].The natural neurotransmitters dopamine, serotonin and norepinephrine were docked with homologically modelled DAT,SERT and NET respectively .. According to their glide score their interaction at the cavity explained.

EXPERIMENTAL SECTION

The crystal structure of DAT, SERT and NET wasn't available at the PDB[41].The amino acid sequence of DAT, SERT and NET were retrieved from Uniprot [42] server. The three dimensional structures of the obtained sequences were homologically modeled using Swiss Model Server [43-46]Castp [47]server is used to find the active sites in the Modelled DAT,SERT and NET transporter proteins. The molecular dynamics of the ligands dopamine, serotonin and norepinephrine in the cavity of the DAT, SERT and NET proteins respectively is studied using Glide [48] Molecular docking module of Schrödinger suite. In glide the protein and the ligands optimised using OPLS Force field [49].



Dopamine **Serotonin** **Norepinephrine**

Fig.1. 2Dimensional structures of neurotransmitters dopamine serotonin and norepinephrine.

RESULTS AND DISCUSSION

1. Modeling of Transporter Proteins.

1.1 Modeling of DAT Proteins.

The Crystal or NMR Structure of Dopamine transporter (DAT) wasn't available in the PDB. Hence the amino acid (AA) sequence of Human DAT (hDAT) (*Gene Name: SLC6A3*) was retrieved from the Uniprot server (Unipart id: Q01959) has an AA length of 620.The fasta code for that sequence was given in Fig.2.The retrieved sequence was fed into blast [50]search tool in order to find the template protein for modeling the target hDAT sequence. The blast search tool returned with a template protein of PDB ID: 4xpa [51], having 53% of similarity with the target hDAT sequence. Using the Swiss model sever ProMod Version 3.70, the target sequence of hDAT homo logically modeled and given in Fig.2.The modeled protein validated with Z scores [52] and Rampage [53] for reliability and the results shown in Fig.3. Results show good reliability on the model. The modeled protein has been fed into the castp server to find the active sites in the 3D protein arrangement. According to castp, there are 85 pockets are identified in the modeled protein. Among the all active centres the primary one suggested by Castp was chosen, and the residues constituting the region shown in green in the Fig.4.

1.2 Modeling of SERT Proteins

The Crystal or NMR Structure of Serotonin transporter (SERT) wasn't available in the PDB. Hence the amino acid (AA) sequence of Human SERT (hSERT) (*Gene Name: SLC6A3*) was retrieved from the UniProtKB/Swiss-Prot server accession number P31645. The SERT sequence contains 630 amino acids, which constitutes 12 transmembrane α-helices (TMs) connected by intra and extracellular loops.The fasta code for that sequence was given in Fig.2.The retrieved amino acid sequence was then fed into blast search tool in order to find the template protein which is vital for modeling the target hSERT sequence. The blast search tool returned with a template

protein of PDB id: 4xpa.1.A, having X-ray resolution of 3Å, and having 52.88% of structural similarity with the target hSERT sequence. Using the Swiss model sever ProMod Version 3.70, the target sequence of hSERT homologically modeled and given in Fig.2. The structural quality of homologically modeled SERT was validated with Z scores and Rampage for reliability and the results shown in Fig.3. The model was found satisfactory with the Z scores and the Ramachandran plot provided by Rampage endorsed that 91.8 percent of the residues were in the core favoured region. The modeled protein has been fed into the castp server to locate the active sites in the 3D protein arrangement. According to castp, there are 83 pockets are identified as active sites in the modeled protein. Among those active centers, the primary one suggested by castp, having volume of 3458.6$Å^3$ was chosen, and the residues constituting the region shown in green in the Fig.4.

Table 1. Glide Docking scores of dopamine, serotonin and norepinephrine at the DAT, SERT and NET proteins respectively

Docking Scores(Kcal mol^{-1})	dopamine	serotonin	norepinephrine
Glide score	-9.1	-4.1	-9.9
Dock Score	-9.1	-4.1	-9.9
Lipophilic rewards	-2.5	-0.9	-2.7
Phobic Enclosure rewards	-1.4	0.0	-1.2
Hydrogen bonding rewards	-1.8	-2.1	-2.6
Electrostatic rewards	-3.2	-1.1	-3.3
Rotational penalty	0.3	0.2	0.2

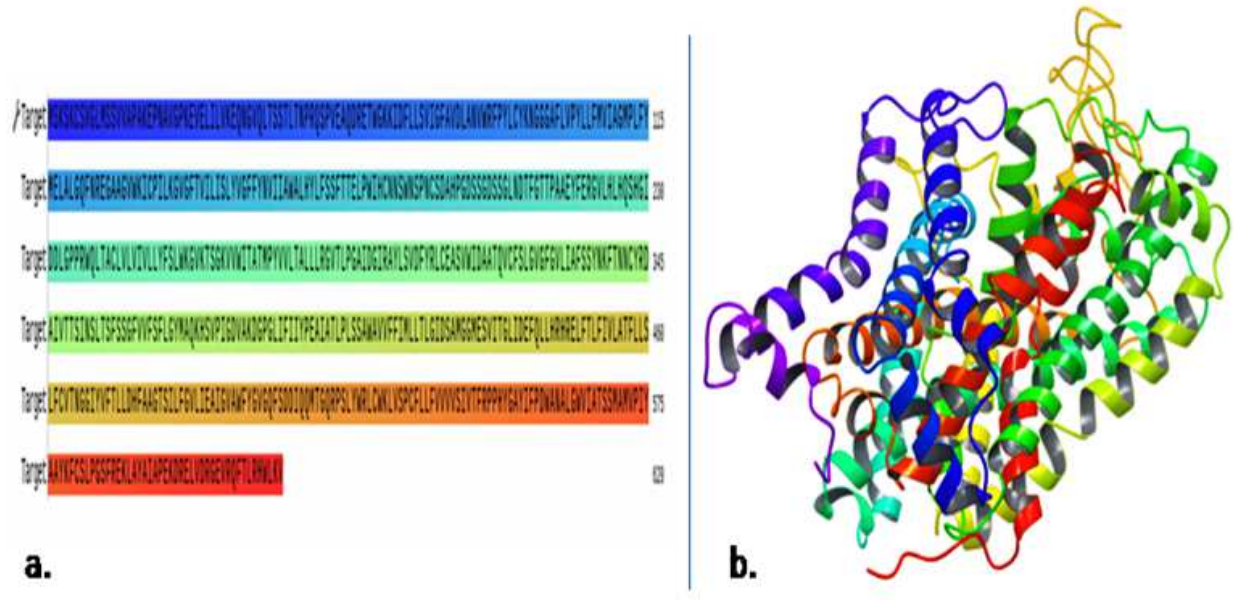


Fig.2.Fasta sequence and Modeled dopamine transporter Protein.

Table 2. Molecular interactions observed at Dopamine-DAT, Serotonin-SERT and Norepinephrine-NET complexes

Observed Molecular Interactions	Residues on Ligands	Residues on Transporter Proteins
Dopamine-DAT complex		
Hydrogen bonding interactions	NH_3^+	Phe 320, Ala 77, Ser 321, Asp 79
cation-π Interactions	NH_3^+	Phe 320
π-π Interactions	aromatic ring of dopamine	Phe326,Phe76
Hydrophobic Interactions	CH_2,CH_2-CH_2 on aromatic ring of dopamine	Ser 422,val 38, Phe 326, Phe 76, Tyr 156
Serotonin-SERT complex		
Hydrogen bonding Interactions	NH_3^+	Glu493
	OH	Gln332
Norepinephrine-NET complex		
HB Interactions	NH_3^+	Ala73,Asp75,phe317
	OH	Phe317
π-π Interactions	aromatic ring of norepinephrine	Tyr152,Phe72,Phe323
Cation –pi interacions	NH_3^+	Phe317

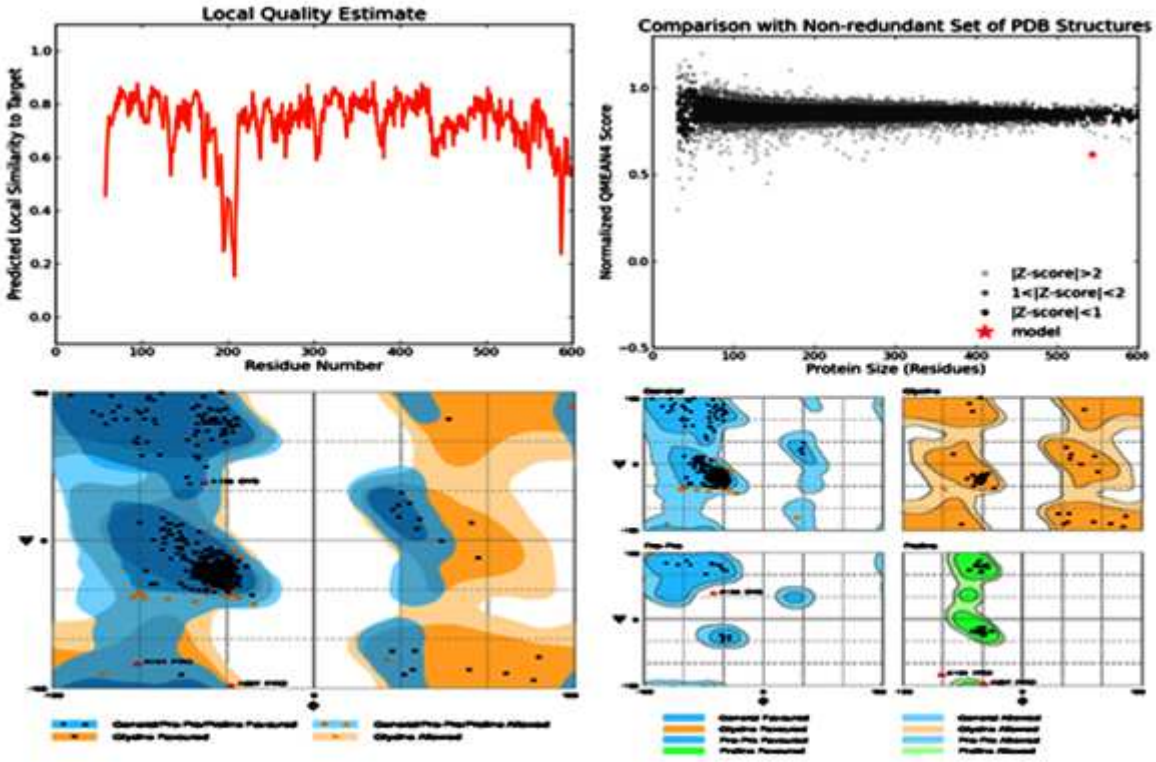


Fig. 3 Z-Score and Rampage Validation of Modeled DAT Protein

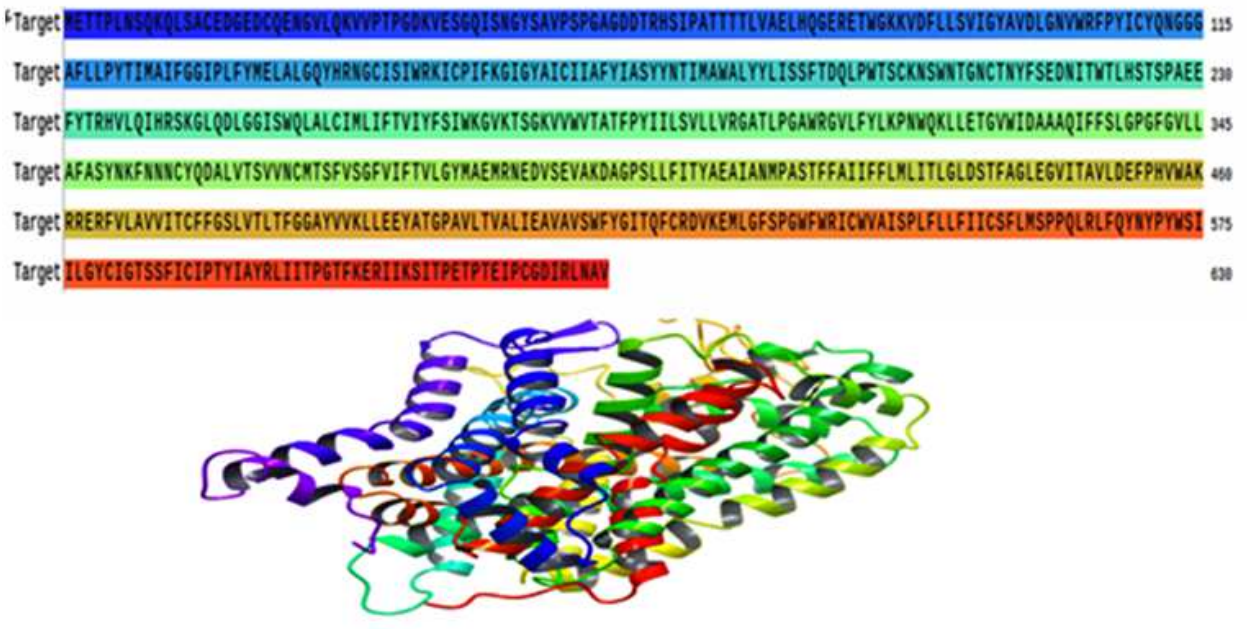


Fig. 4. Fasta sequence and Modeled serotonin (SERT) transporter Protein.

1.3 Modeling of NET Proteins

The Crystal or NMR Structure of Norepinephrine transporter (NET) wasn't available in the PDB. Hence the amino acid (AA) sequence of human NET (hNET) (*Gene Name: SLC6A2*) was retrieved from the UniProtKB/Swiss-Prot server accession number P23975.NET contains 617 amino acids that forms 12 transmembrane α-helices (TMs) connected by intra- and extracellular loops.The fasta code for that sequence was given in Fig.2.The retrieved sequence was fed into blast search tool in order to find the template protein for modeling the target hNET sequence. The blast search tool returned with a template protein of PDB Id: 4xpa.1.A , X-ray resolution of 2.95Å, having 59.07% of similarity with the target hNET sequence. Using the Swiss model sever ProMod Version 3.70, the target

sequence of hNET homologically modeled and given in Fig.2. The structural quality of the modeled NET was validated with QMEAN scores and Rampage for reliability and the results shown in Fig.3. The model was found satisfactory with the QMEAN score of -6.33 achieved for global and per-residue model quality assessment and the Ramachandran plot provided by Rampage showed that 95.9 percent of the residues were in the favoured core region and 3.1 percent of the residues were in the allowed region.The modeled protein has been fed into the castp server to find the active sites in the 3D protein arrangement. According to castp, there are 84 pockets are identified as active sites in the modeled protein. Among the all active centres the primary one suggested by castp was chosen, and the residues constituting the region shown in green in the Fig.4.

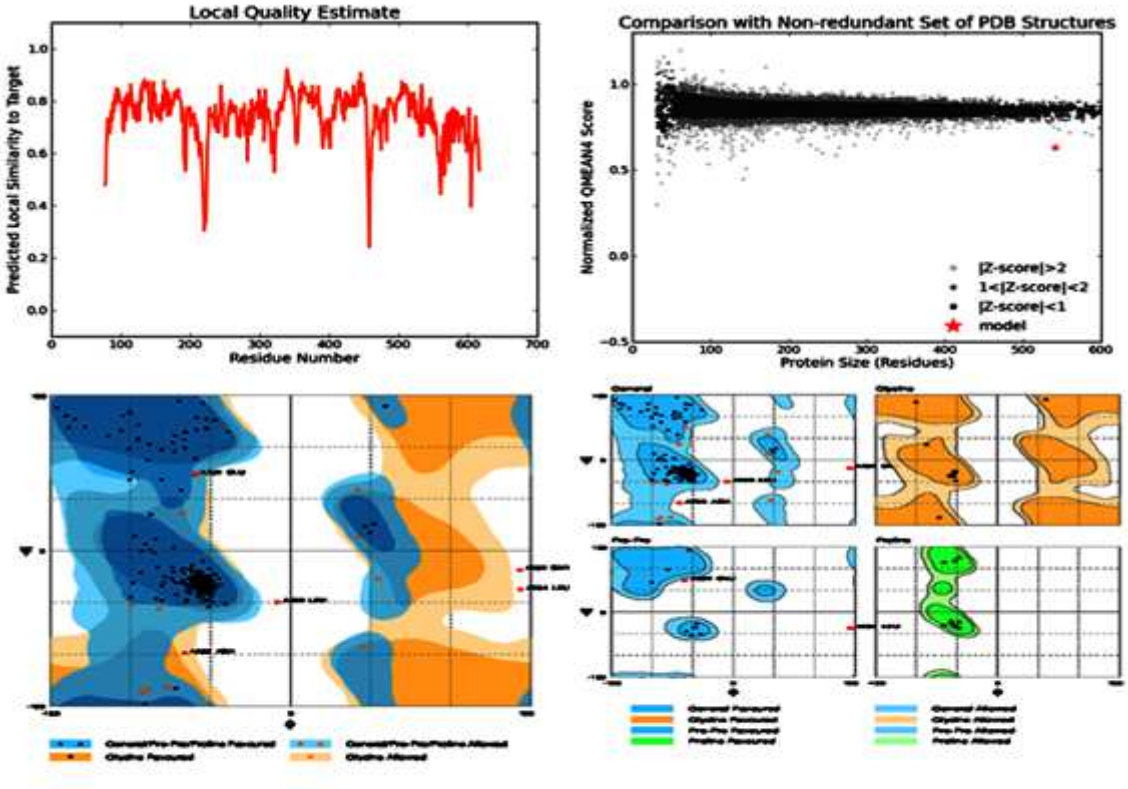


Fig.5 Z-Score and Rampage Validation of Modeled SERT Protein

2. Docking of Neurotransmitters into Transporter Protein.

2.1 Docking of Dopamine into Dopamine Transporter Protein.

The Molecular Dynamics study done using the Glide Module of Schrodinger Suite reveals the interaction of the dopamine with its transporter. The numerical values, which describe the magnitude of interaction given in Table 1.The mode of interaction is presented in the Table.2 and illustrated in Fig.5. Glide score is an empirical scoring function that approximates the ligand binding free energy. The glide score for Dopamine transporter-Dopamine docking is -9.1 kcal mol^{-1}, shows the effective binding between the neurotransmitter and the protein. The glide score is the sum of LipophilicEvdW rewards, hydrophobic enclosure rewards, HB interaction rewards, Electrostatic rewards and Low MW rewards, this glide score includes rotational penalty for freezing of rotatable bonds. Dopamine can exist in the anionic, neutral, cationic, or zwitterionic form, depending on the ambient pH. Here in physiological pH dopamine exist in cationic form as C_6H_3 (OH) $_2$-CH_2-CH_2-NH_3^+. From our molecular docking studies it is observed that, the protonated NH_3^+ group of Dopamine interact with Asp76,Phe 320, Ala 77, Ser 321 residue of DAT via hydrogen bonding interaction which is weaker than covalent bond, but is stronger than vdw's forces. The cationic dopamine bind to the π faces of phe 320 of the dopamine transporter, producing non covalent cation - π interactions. The π electron cloud in the phenyl ring of dopamine recognizes the phenyl-ring side chains of the residues phe326 and phe 76 through π-π interaction. The Carbon atoms in the dopamine are hydrophobic in nature and made hydrophobic interaction with the residues of Ser 422, Val 38, Phe 326, Phe 76, Tyr 156.Because of such interactions, a large number of aromatic residues of the DAT, including most of the phenylalanine and tryptophan residues in Trans membrane regions, have been mutated which in turn makes physiological response. From the above observation it is inferred that phenethylamine appears to be the most important structural element accommodated by the DAT protein. Almost all dopamine carrier substrates are phenethylamine derivatives, and are positively charged at physiological pH. These features have been used as a guide to find residues of the substrate-

binding site at the carrier. Thus, charged, aromatic, and polar residues becomes the cause of physiological response and phenyl ethylamine moiety is a functional part of the dopamine in effective docking.

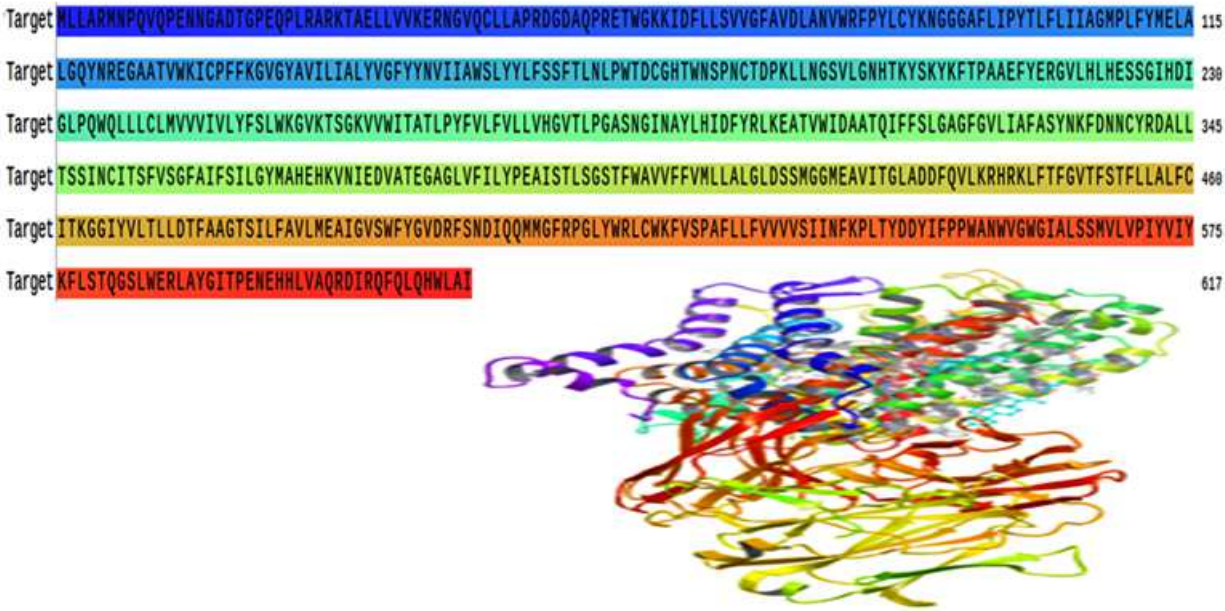


Fig. 6. Fasta sequence and Modeled norepinephrine transporter Protein.

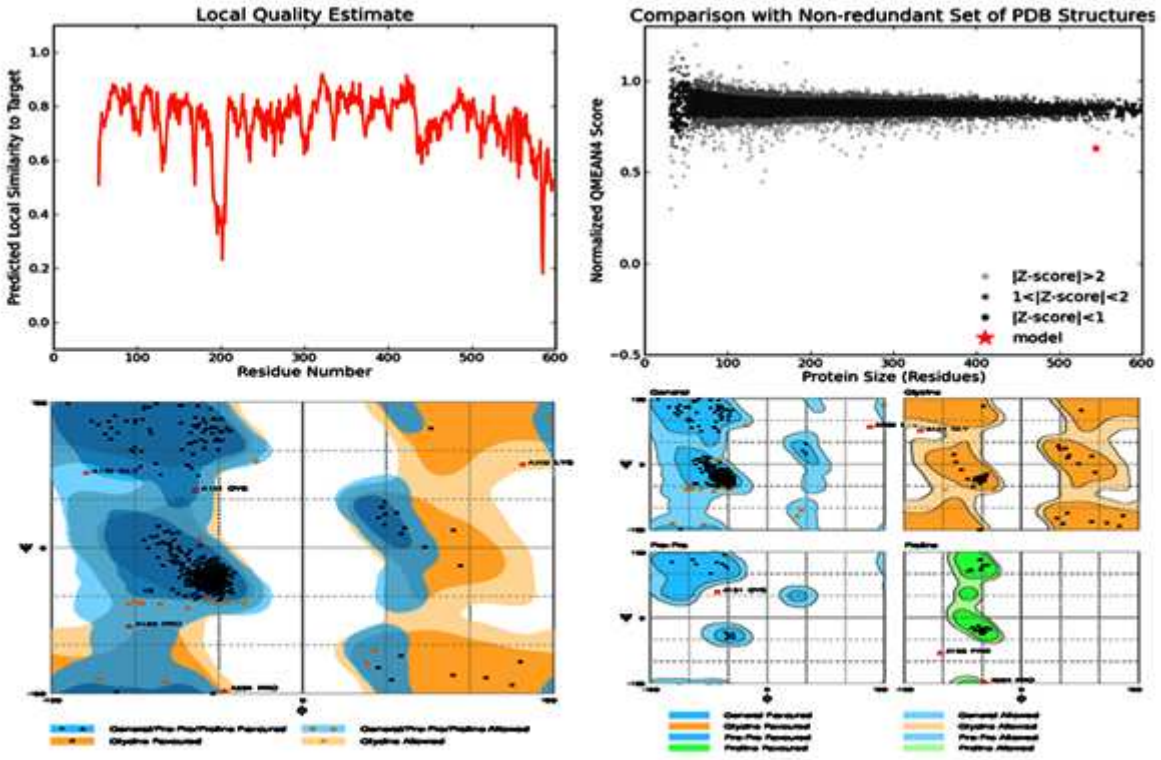


Fig. 7 Z-Score and Rampage Validation of Modeled NET Protein

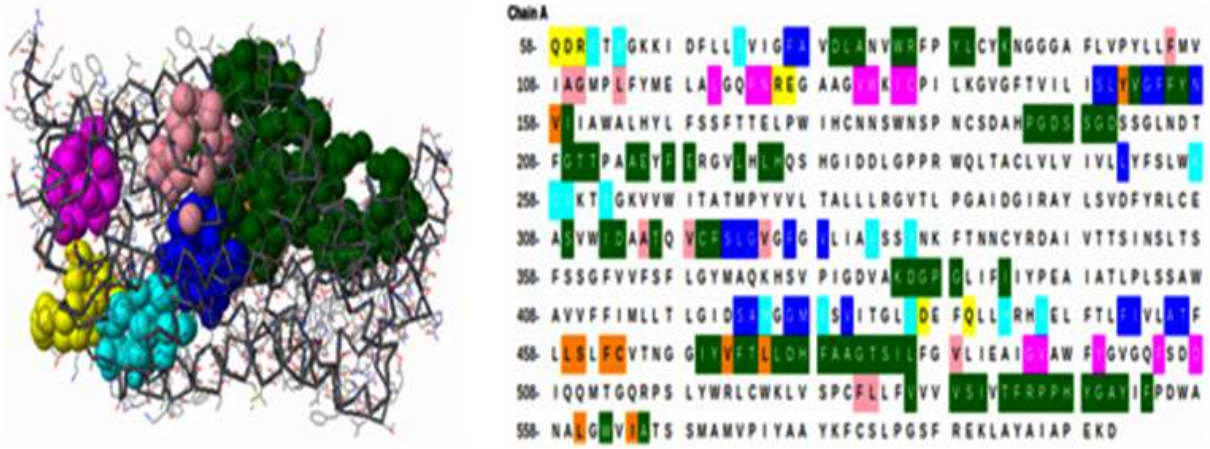


Fig.8. Active sites on DAT protein predicted using CASTp server.

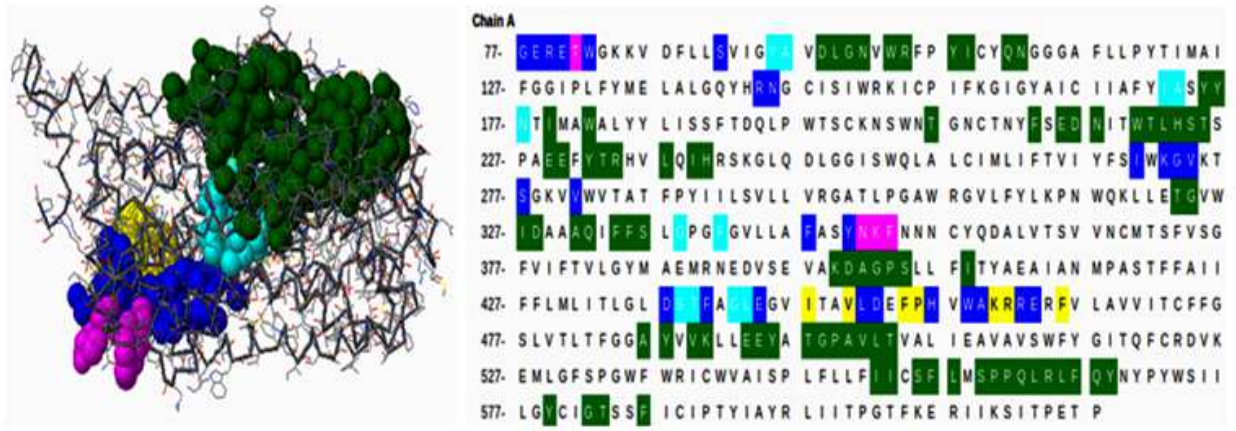


Fig.9. Active sites on SERT protein predicted using CASTp server.

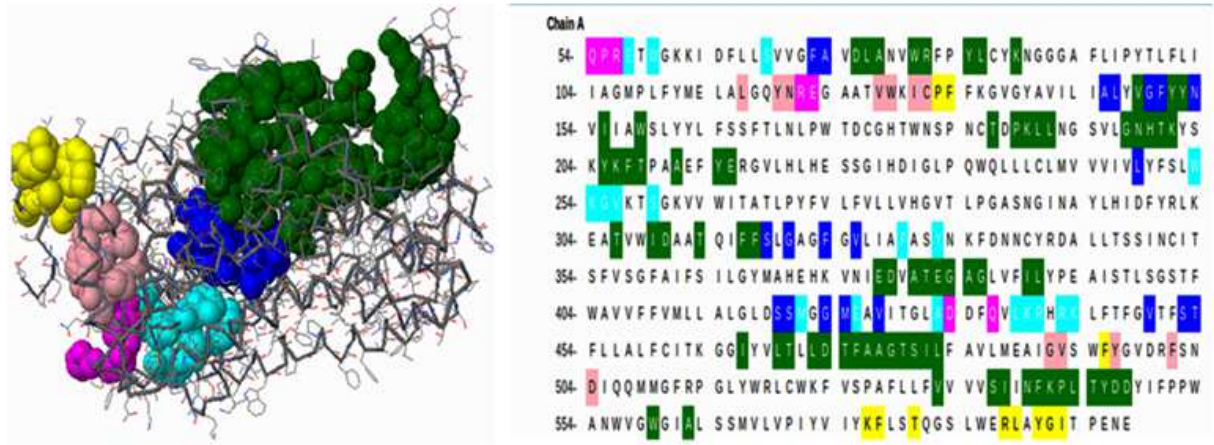


Fig.10. Active sites on NET protein predicted using CASTp server.

2.2 Docking of serotonin into serotonin transporter (SERT) protein.

The Molecular dynamics study performed using the Glide Module of Schrodinger Suite displays the interaction of the serotonin with its transporter. The numerical values, which describe the magnitude of interaction is given in Table 1.The mode of interaction and the species responsible for are presented in the Table.2 and illustrated in Fig.5. The glide score or docking score is an empirical scoring function is the estimate of the binding affinity. The glide score for serotonin transporter-serotonin docking is -4.1 kcal mol^{-1}, illustrate the moderate binding between the neurotransmitter and the protein. The glide score is the sum of LipophilicEvdW rewards, hydrophobic enclosure rewards, HB interaction rewards, Electrostatic rewards and Low MW rewards, this glide score includes rotational

penalty for freezing of rotatable bonds. At ambient pH, Serotonin can exist in the anionic, neutral, cationic, or zwitterionic form. Here in physiological pH, serotonin exist in its cationic form as C_8H_4 NH (OH) -CH_2-CH_2-NH_3^+. From our molecular docking studies it is observed that, the protonated NH_3^+ group of serotonin interact with Glu493, Gln332 residue of SERT via hydrogen bonding interaction which is weaker than covalent bond, but is stronger than vdw's forces. Because of such interactions residues of the SERT, including Glu493, Gln332 in transmembrane regions, has been altered, which in turn produces physiological response. Generally glutamate induces an excitatory function in the brain. May because of the availability of high serotonin level, the electronic structure of Glu493 gets perturbed and it's the excitatory action perhaps suppressed. From the above docking results, it is inferred that, indol-3-ethylamine, the core structural element accommodated by the SERT protein and the perturbation of electronic cloud of Glu493 is recognized as a cause for the physiological response. Glutamic acid is a polar negatively charged aliphatic amino acid that contains a α-amino group and a α-carboxylic acid and a chide chain carboxylic acid. Thus, it has arrived that, negatively charged and polar residues become the most attractive targets for effective docking and indol-3-ethylamine moiety is the indispensable functional part involved in effective docking.

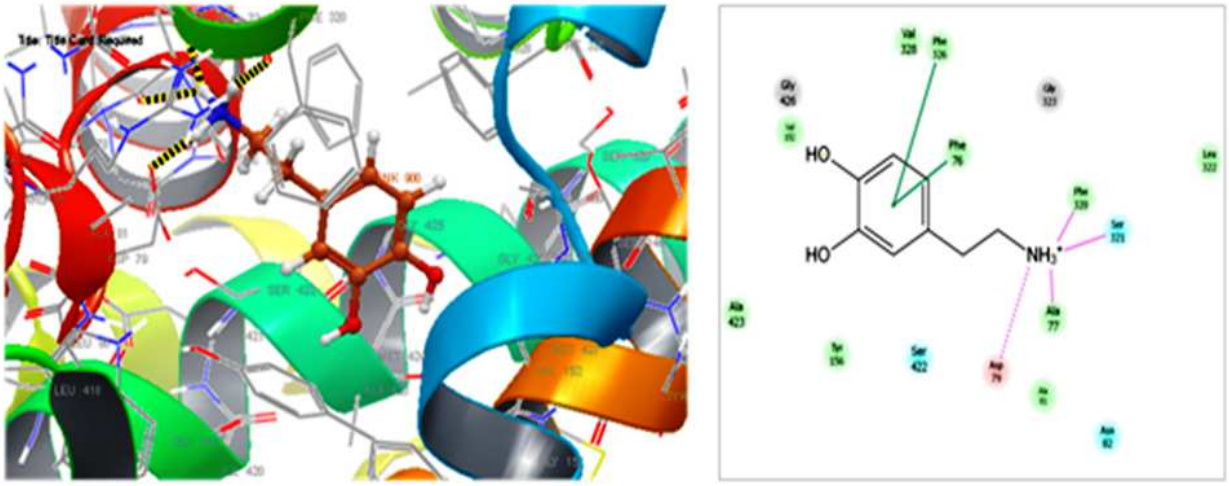


Fig .11. Docking of Dopamine with Dopamine Transporter Protein.

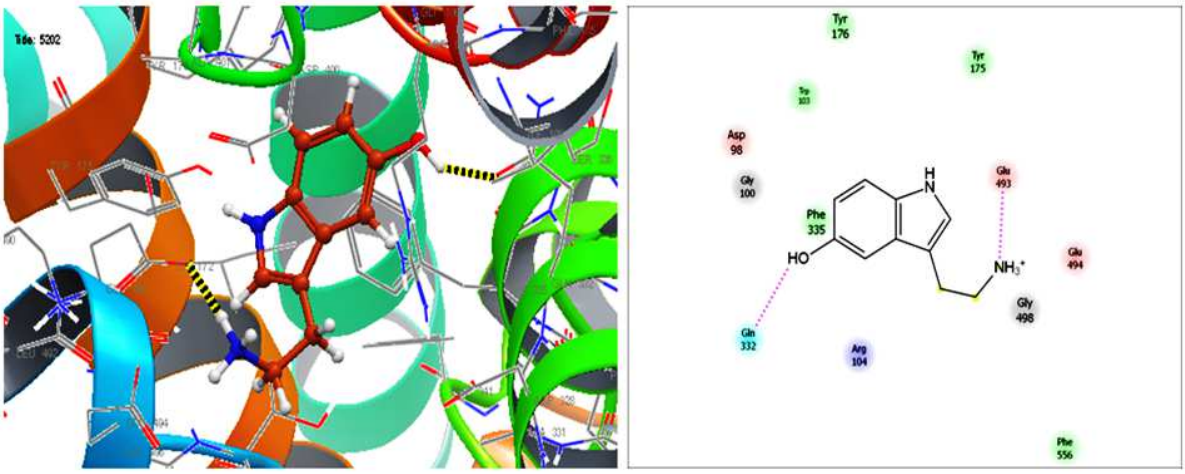


Fig .12 Docking of serotonin with serotonin transporter Protein.

2.3 Docking of norepinephrine into norepinephrine transporter (NET) protein.

The Molecular Dynamics study done using the Glide Module of Schrodinger Suite reveals the interaction of the norepinephrine with its transporter. The numerical values, which describe the magnitude of interaction is given in Table 1.The mode of interaction is presented in the Table.2 and illustrated in Fig.5. The glide score or docking score are the estimate of the binding affinity that approximates the ligand binding free energy. The glide score for norepinephrine transporter-norepinephrine docking is -9.9 kcal mol^{-1}, shows the effective binding between the neurotransmitter and the protein. The glide score is the sum of LipophilicEvdW rewards, hydrophobic enclosure rewards, HB interaction rewards, Electrostatic rewards,Low MW rewards and includes rotational penalty for freezing of rotatable bonds. Norepinephrine can exist in the anionic, neutral, cationic, or zwitterionic form, depending on the ambient pH. Here in physiological pH norepinephrine exist in cationic form as C_6H_3 $(OH)_2$-

$CH(OH)\text{-}CH_2\text{-}NH_3^+$. From our molecular docking studies it is observed that, the protonated NH_3^+ group of norepinephrine interact with Ala73, Asp75, phe317 residues of NET via hydrogen bonding interaction which is weaker than covalent bond, but is stronger than vdw's forces. A Cation –π πinteraction of $NH3^+$... Phe317 and π-π Interactions between aromatic ring of norepinephrine and Tyr152, Phe72, Phe323 of NET also observed. Because of such interactions, the residues Ala73, Asp75, phe317 in trans membrane regions, has been perturbed which in turn makes physiological response. From the above observation it is inferred that phenyl(2-hydroxy)ethylamine is the most essential functional part accommodated by the NET protein and perturbation of Ala73,Asp75,phe317 is essential for the physiological response. These observations have been used as a guide for drug designing process.

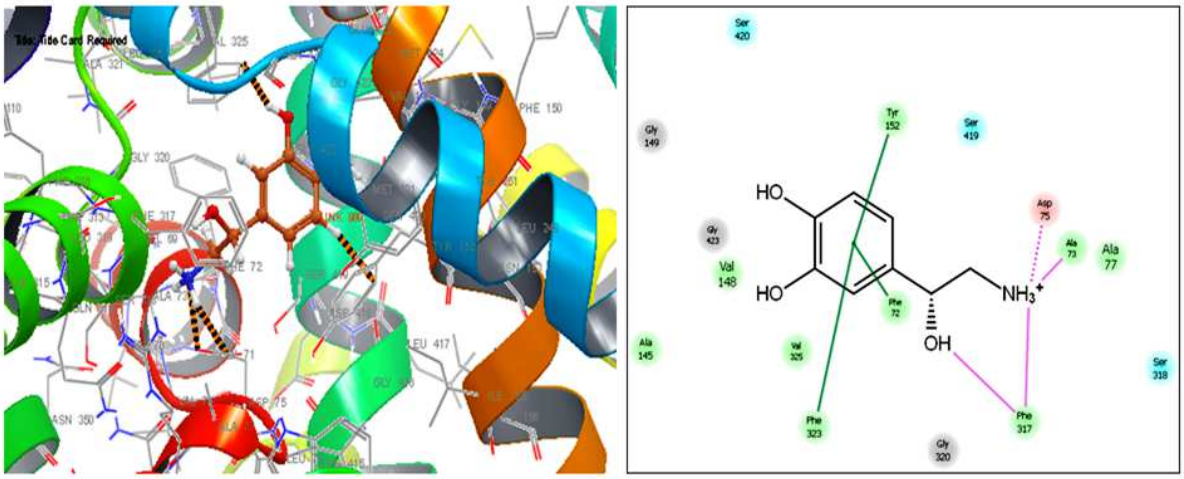


Fig .13 Docking of norepinephrine with norepinephrine transporter Protein

CONCLUSION

The neurotransmitters dopamine, serotonin and norepinephrine were docked with modelled DAT,SERT and NET proteins respectively. Their reported glide scores show signs of the effective binding between them. They all have core ethylamine moiety in their structures. At physiological ph all neurotransmitters becomes cationic by means of protonation occurs at the amine group.In all three nts the cationic amine group makes cation –pi interactions and hydrogen bonding interactions with the residues of transporter proteins. Along with some pi-pi interactions also observed.These interactions are responsible for the reuptake of the neurotransmitters in the synaptic cleft.

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