Serotonin Transport Protein and SSRI Docking: A Computational Exploration of SERT Wild-type and Neurodevelopmental Disorder Variants

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Introduction: In our computational project, we will be exploring whether or not people suffering from neurodevelopmental disorders, such as autism and OCD, experience similar effectiveness of various serotonin reuptake inhibitors (SSRIs) in treating depressive symptoms. To do this, we simulated the docking interactions between the human serotonin reuptake transporter (SERT) and different common SSRI drugs. Specifically, we tested the effects of SERT mutations common in subjects with certain disorders on SSRI drug/ligand binding. Hopefully this can help us create more effective therapeutics and drug regimens for patients.

Background: SSRIs, or selective serotonin reuptake inhibitors, is a class of antidepressant drugs that prevent the reabsorption of serotonin into the presynaptic neuron. This allows serotonin levels to remain high in the synaptic cleft, and higher overall throughout the brain [1]. Serotonin, or 5HT (5 HydroxyTryptamine), is an integral neurotransmitter that is researched to influence a variety of physiological functions including mood, appetite, digestion, sleep, memory, and even sexual desire. Low 5HT levels have been linked to depression; however, it is still unclear whether low levels of the neurotransmitter contribute to depression or result from it.

The key part of the pathway which SSRIs target are presynaptic membrane proteins labeled SLC6A4 below, which transport 5HT present in the synaptic cleft back into the presynaptic terminal. This class of proteins, serotonin reuptake transporters (SERTs), are an integral transport protein that rely on a Na+/Cl- ion gradient [1]. Thus, as shown in the figure, SLC6A4 is the molecular target for an SSRI drug, which results in an inhibition of 5HT reuptake, leading to more serotonin in the synapse cleft.

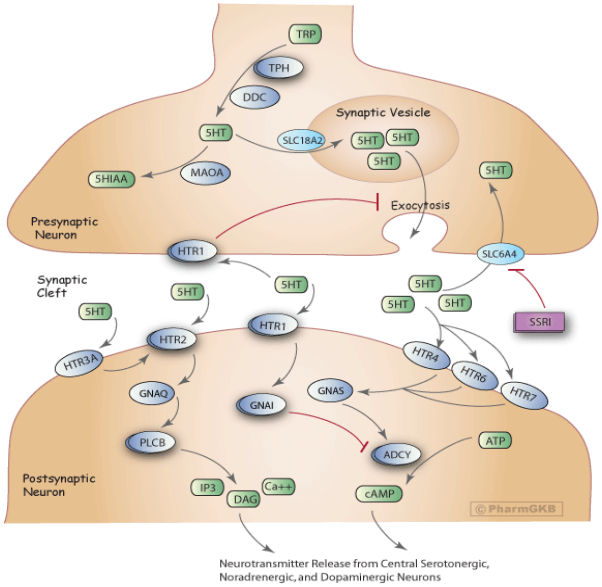

Figure 1: Serotonin synapse and reuptake pathway [1]

Although there has not been a comprehensive conclusion about the best situational treatment action for depression, SSRIs are one of the first choice therapies because of their relatively safe side-effect profile. Furthermore, there are many different FDA approved SSRIs which inhibit serotonin reuptake in slightly different ways. The five typical SSRIs that we will be exploring are: Citalopram (Celexa), Escitalopram (Lexapro), Fluoxetine (Prozac), Paroxetine (Paxil, Pexeva), and Sertraline (Zoloft) [2].

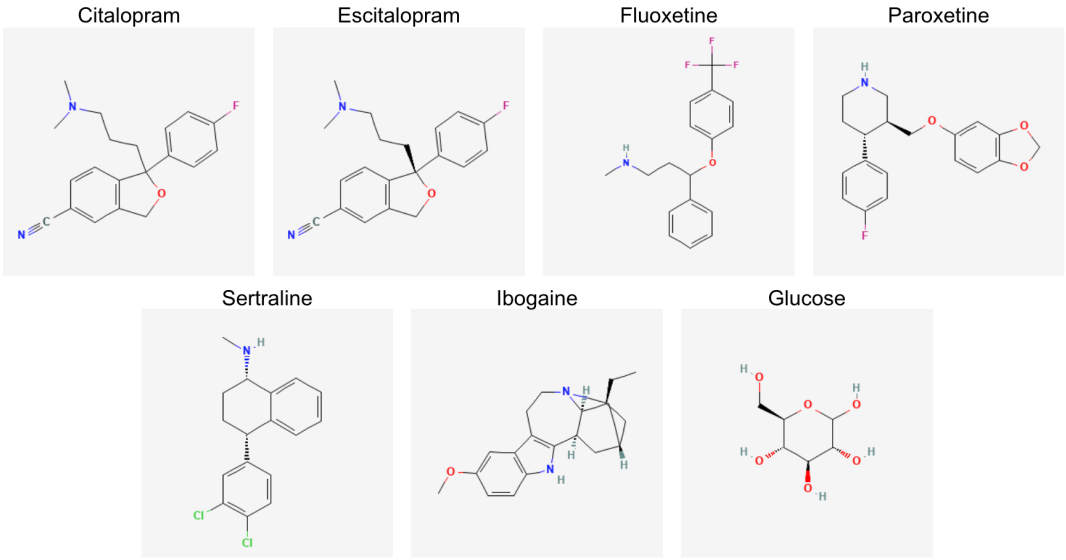

Figure 2: Chemical structure of common SSRIs, ibogaine and glucose (images from [3])

Additionally, we included two chemicals that are not considered SSRIs in our computational project: ibogaine and glucose. Ibogaine is a psychoactive drug found in West Africa with claims of attenuating dependence and withdrawal symptoms of opiates, alcohol, nicotine, and other stimulants [4]. We wanted to explore ibogaine to confirm if it acted in the addiction reward pathway as well as observe if it would do so as other well-documented SSRIs. We included glucose as a form of negative control, as we didn't expect for glucose to have tight binding with the active site on SERTs.

Link between autism, OCD and SERT mutations: When researching the link between autism and depression we found that "rare, functional, non-synonymous variants in the human serotonin (5-hydroxytryptamine, 5-HT) transporter (hSERT) gene (*SLC6A4*) have been identified in both autism and obsessive–compulsive disorder (OCD)." And within subjects with autism, these SERT variants are associated with obsessive-compulsive disorder (OCD) traits "suggesting both phenotypic overlap with OCD and a shared relationship with disrupted 5-HT signalling" (Prasad et al. 2009) [5]. This paper analyzed 24 unrelated subjects with autism and found five SERT mutations: Ile425Val, Ile425Leu, Phe465Leu, Leu550Val, Lys605Asn. Ile425Val, specifically, is a distinct variant that has already conclusively been associated with OCD and Asperger's syndrome (Ozaki et al. 2003) [6]. In our GLIDE simulations we performed these mutations on the SERT protein to see the effects of the mutations on SSRI binding.

Methodology: For our docking simulations we ended up using Shrödinger's receptor-ligand software GLIDE: Maestro seen below. We started trying to use Autodock Vina, however its newest update is no longer compatible with the latest MacOS. We got Autodock Vina downloaded with a Windows VM, but ran into other bugs successfully converting our modified

PDBs to PDBQT format and so we switched to GLIDE. We also tried SwissDock, but quickly realized it does not allow us to complete the necessary modifications to proteins for our project.

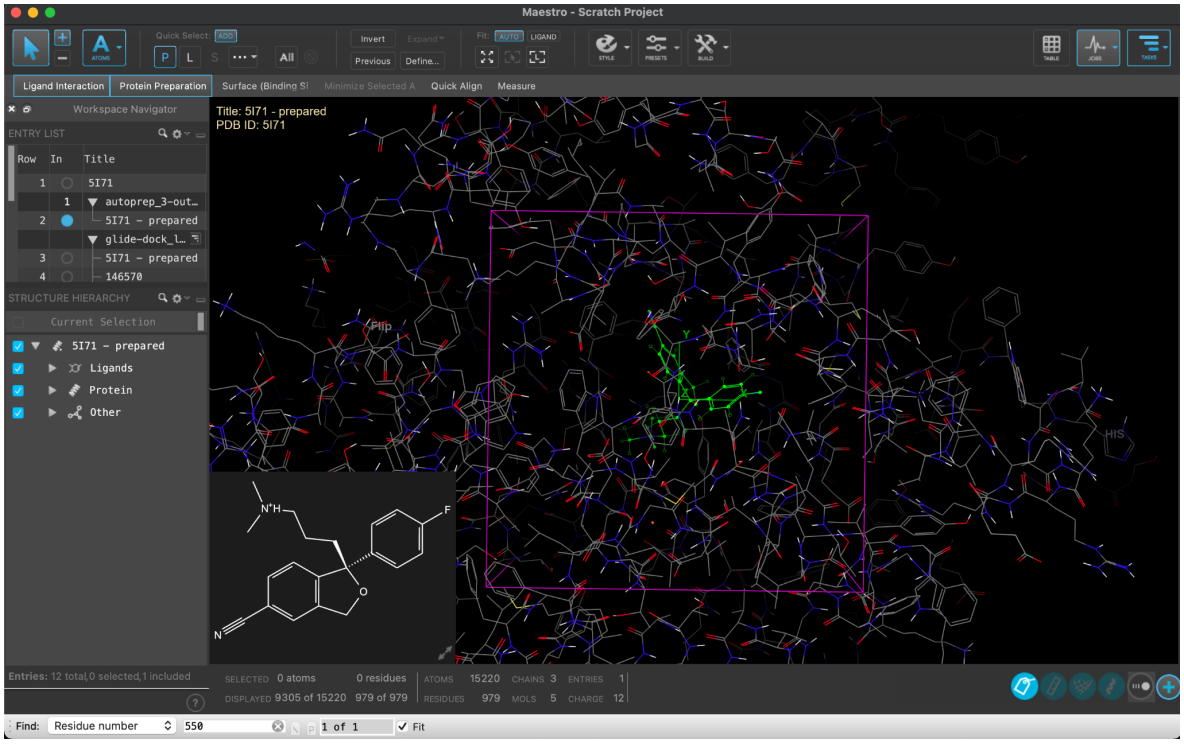

Figure 3: S-Citalopram ligand in SERT protein on Maestro Interface

GLIDE Procedure (modified from [7]):

We repeated these steps for the wild type 5I71 as well as the five other mutations mentioned above: Ile425Val, Ile425Leu, Phe465Leu, Leu550Val, Lys605Asn.

Step 1: Open PDB in GLIDE

- For our simulations we used 5I71: X-ray structure of the ts3 human serotonin transporter complexed with s-citalopram at the central site
- Once the PDB is opened, we removed s-citalopram to be left with the protein only
- **Mutation**: we performed the amino acid mutations in the Mastro application by simply searching for the specific residue then clicking mutate residue and selecting the new amino acid.

Step 2: Preparing Protein Structure for Docking

- GLIDE luckily automates many of the typical operations. In the Protein Preparation Preprocess settings we made sure to select: Fill in missing side chains, Assign bond order using CCD database, Replace hydrogens, Create: Zero-order bonds to metals, Create: Disulfide bonds, Generate het states (using Epik): pH: 7.4 +/- 2.0.

- We also selected Optimize H-bond Assignments which performs automatic optimization to address overlapping hydrogens and finally Clean Up which optionally removes water molecules that do not impact ligand binding.

Step 3: Prepare Ligands to be Docked

- The LigPrep must be done for each .sdt ligand file individually. The ligands we ran were escitalopram, citalopram, fluoxetine, paroxetine, sertraline, ibogaine, and glucose.
- In LigPrep the settings we used were Generate possible states at target pH: 7.0 +/- 2.0 (using Epik), Desalt, Generate tautomers, Retain specified chiralities, Output format: Maestro.
- The prepared ligand is saved at a .maegz file and will be used later during the docking step.

Step 4: Generate the Receptor Grid

- In this step we specify the receptor and ligand structures and generate a grid centered around a selected X, Y, Z point to represent the active site of the receptor
- We used a Scaling factor of 1.0 and a Partial charge cutoff of 0.25.
- We run this job which creates grid files we will use for the next docking step

Step 5: Rigid Docking

- We open the Ligand Docking task. For the grid we select the receptor grid .zip file we just generated in step 4, and for ligands we input the seven .maegz files for our seven prepped ligands. We used the Dock rigidly, Standard precision docking, and other base settings.
- We ran the docking and after about a minute had a csv with the GLIDE scores.
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Glide and GlideScore: Glide is an approach that allows us to do a search of the conformational, orientation, and positional space of a docked ligand, as represented by the figure below [8].

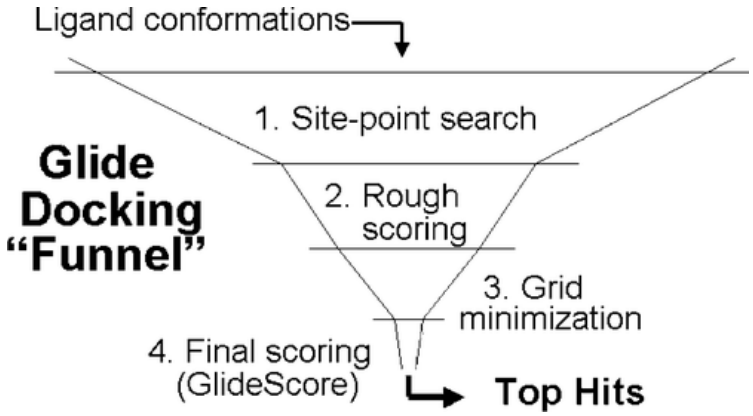

Figure 4: Glide Ligand Docking Overview

The GlideScore is a measure that attempts to provide an assessment of the quality of the fit of the ligand to the protein. The GlideScore takes into account various aspects of ligand-protein interactions during the docking process and is represented by the equation below:

$$\begin{aligned}\Delta G_{\text{bind}} = {} & C_{\text{lipo-lipo}} \sum f(r_{\text{lr}}) + \\ & C_{\text{hbond-neut-neut}} \sum g(\Delta r)\ h(\Delta\alpha) + \\ & C_{\text{hbond-neut-charged}} \sum g(\Delta r)\ h(\Delta\alpha) + \\ & C_{\text{hbond-charged-charged}} \sum g(\Delta r)\ h(\Delta\alpha) + \\ & C_{\text{max-metal-ion}} \sum f(r_{\text{lm}}) + C_{\text{rotb}} H_{\text{rotb}} + \\ & C_{\text{polar-phob}}\, V_{\text{polar-phob}} + C_{\text{coul}} E_{\text{coul}} + \\ & C_{\text{vdW}} E_{\text{vdW}} + \text{solvation terms}\end{aligned}$$

Figure 5: GlideScore Equation

The first term is a function of distance between the hydrophobic atoms of the ligand and the protein; the second term characterizes ligand-atom/receptor-atom pairs that are lipophilic; the third term characterizes hydrogen-bonding interactions; the remaining terms also take into account the other aspects of ligand-receptor interactions [8]. As for Glide scores, the actual value and cutoff for a good Glide score vary system to system. For Glide SP (standard precision), scores of -10 represent great binding, and for some specific proteins a score of -9 to -8 will be good [9]. In the next section, we will discuss the results from the docking simulations completed and their resulting Glide Scores.

Result/Analysis

Data (units: kcal/mol):

Drug/Mutation	Wild type	Ile425Val (OCD associated)	Ile425Leu	Phe465Leu	Leu550Val	Lys605Asn	Average of the mutation condition
Escitalopram	-9.68504	-9.88556	-10.1977	-9.4727	-10.0012	-9.9694	-9.90532
R-Citalopram	-9.35531	-9.36605	-10.0765	-9.4315	-9.4397	-9.4531	-9.5537
Fluoxetine	-8.02344	-8.28	-8.1955	-8.9042	-8.5251	-8.5082	-8.4826
Paroxetine	-8.63761	-8.54487	-8.98409	-8.4972	-8.3291	-8.7623	-8.623512
Sertraline	-8.0588	-8.0537	-8.87402	-8.0440	-7.9686	-8.0649	-8.201044
Ibogaine	-9.10224	-9.25612	-9.29215	-9.2558	-9.1349	-9.2817	-9.244134
Glucose	-5.96186	-5.13386	-4.76808	-5.6948	-4.9836	-5.9606	-5.308188

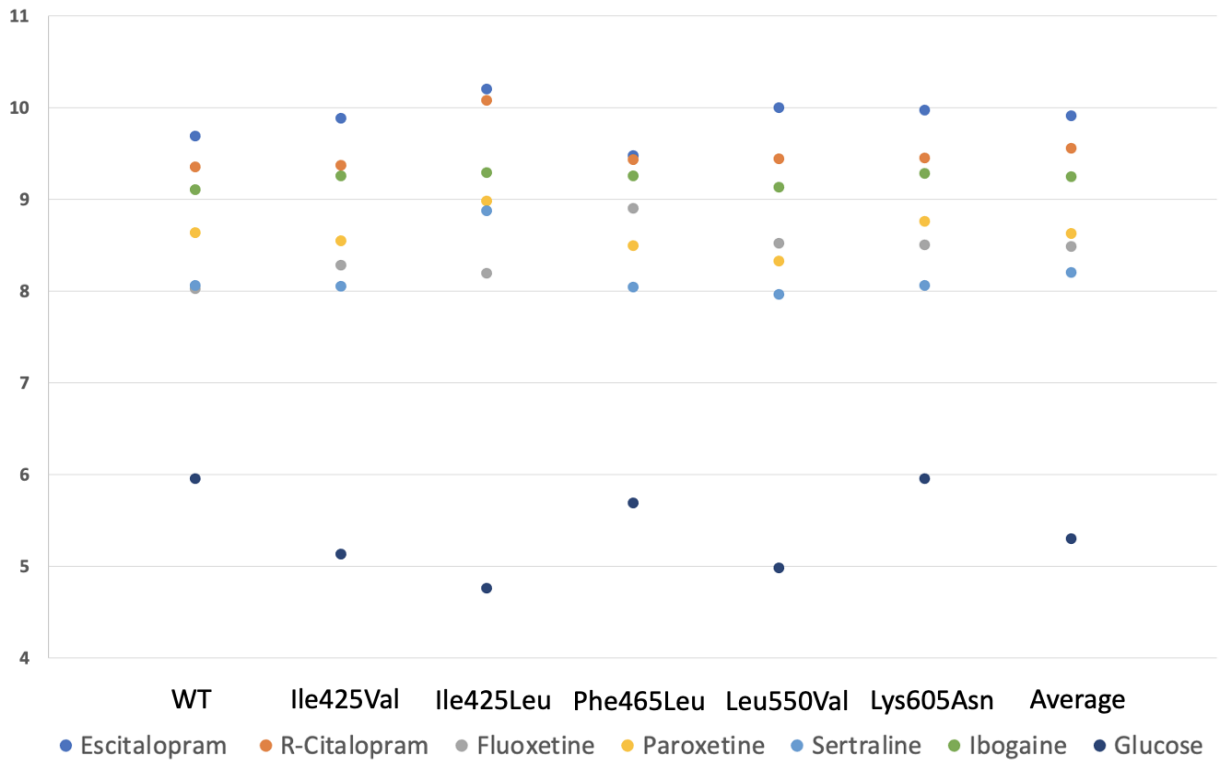

Figure 6: Table and Graph of Docking Data

Upon the completion of the WT simulation, we observed that S-Citalopram had the highest GlideScore of -9.68504, indicating strong binding, with R-Citlopram following close behind with a GlideScore of -9.35531 (3.46% increase), indicating slightly weaker binding affinity. The lowest binding that we observed was Fluoxetine and Sertraline with a GlideScore of -8.02344 and -8.0588, respectively. However, the binding was not as low as the negative control of glucose which was expected (GlideScore -5.96186). Additionally, we were surprised to see such a high score for ibogaine of -9.10224 which means it significantly inhibits the SERT protein as the literature suggests.

***Comparing Mutation Condition to WT*:** In order to make broader conclusions about the potentially different binding affinities of SSRIs in mutation conditions associated with autism and neurodevelopmental disorders, we first compare the GlideScore averages of the mutant conditions to that of WT. The average GlideScores for all of the ligands show better binding (more negative GlideScores) except for Sertraline and Glucose (our negative control). The better binding could be validated by the observation of "people with autism [being] more likely to have certain side effects from SSRIs, such as impulsive or irritable behavior and trouble sleeping" [10]. These side effects are common and established symptoms of Serotonin Syndrome, a disorder of abnormal accumulation of serotonin in the body. Because SSRIs in mutation conditions representing autism/neurodevelopmental disorders have stronger binding to SERTs, it could lead to better reuptake inhibition, then more serotonin present in the synaptic cleft, and therefore an increased likelihood of experiencing the effects of excess serotonin.

Before analyzing the results, we intuitively expected SSRIs to exhibit stronger binding to mutated SERTs because of the aforementioned clinical symptoms observed in people with autism and other neurodevelopmental disorders.

However, there are some differences that we didn't expect, which are highlighted in orange in the table. A notable unexpected difference exists in the Phe465Leu mutation condition: 3 out of the 6 SSRIs simulated worse binding compared to WT. We predict that replacing the Phenylalanine with Leucine may have led to more drastic conformational changes, from losing the aromatic ring in Phenylalanine, that led to some negative manipulation in the active site of the receptor. Additionally, we observed that both Paroxetine and Sertraline had the most deviance from our expectations; if we were to continue this project, we would like to dive deeper into how the differences in molecular structure and functional group composition influences this deviation, if it's a deviation at all.

Through our results and methodology we learned that people suffering from neurodevelopmental conditions such as OCD or autism could experience stronger or more frequent side effects from taking SSRIs due to their stronger binding to their potentially mutated SERTs. However, we also learned how complex this problem and modeling is, and we can only conclude that SSRI binding strength only generally correlates with mutations associated with neurodevelopmental conditions for certain SSRIs.

***Next Steps*:** A major next step would be to contextualize all of the differences of GlideScore, the strength of binding, through molecular and functional group analysis.

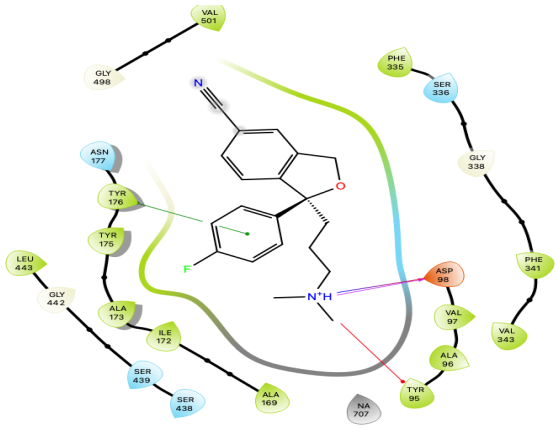

Figure 7: Diagram of Escitalopram residue in SERT active site

Furthermore, the figure above shows the various interactions that residues in, or close to the active site. We would like to conduct further manipulations of these particular residues (if they are observed in human populations) to see how they can impact the binding affinity of the various SSRIs to the active site of SERTs.

Finally, we would like to run the same ligand docking simulations in other software, such as Autodock Vina, SwissDock, and RosettaDock to compare the efficacy of the simulations as well as validate the data that we obtained from Schrodinger Maestro.

Contribution: We both contributed equally to every aspect– ideation, background research, running software, methodology, data analysis, paper writing– of the final project.

Works Cited

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