Computational Modeling of PROTAC-Induced Targeted Degradation of Alpha-Synuclein in Parkinson's Disease

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Parkinson's disease is a neurodegenerative disease that takes a heavy toll on the patient's motor skills as the protein alpha-synuclein aggregates the susbstania niagra in the brain, causing symptoms like tremors, speech and writing difficulties, and muscle stiffness. The alpha-synuclein protein attacks brain cells and obstructs the brain's ability to perform everyday functions effectively. Despite the magnitude of this neurological disorder, PROTACs (Proteolysis Targeting Chimera), designed to degrade targeted proteins, can act as a potential solution for controlling the abundance of alpha-synuclein. We hypothesize that the alpha-synuclein protein binds to the E3 Ligase protein with the help of PROTAC. Our second hypothesis is that chemical alteration in the PROTAC structure could potentially develop more potent PROTACs. We have used the HADDOCK software to dock the α-Synuclein and the E3 Ligase to form an α-Synuclein_E3 Ligase complex. In the next step, we chemically alter the PROTAC to obtain 10 PROTACs. Combine the α-Synuclein-E3 Ligase complex with the 10 PROTACS to create a final complex—α-Synuclein-E3 Ligase-PROTAC complex analysis using PLIP software. We successfully modeled α-Synuclein and E3 Ligase binding and got α-Synuclein-E3 Ligase complex. We found that PROTAC P1 and P2 were the most appropriate PROTAC for binding to both proteins by interacting with them. The work helps in understanding the α-Synuclein and E3 Ligase interaction mechanism. Found appropriate PROTAC binding to α-Synuclein-E3 Ligase complex.

1. INTRODUCTION

Parkinson's disease (PD) is a degenerative brain disorder that is centered around the loss of dopamine-producing neurons in the substantia nigra.(1) The substantia nigra, although a minuscule structure in comparison to the rest of the brain's anatomy, is responsible for the body's control over movement and cognitive processes.(2) Concerning damage to the neurons of this essential aspect of the brain, symptoms such as tremors, rigidity, bradykinesia (slowness of movement), and postural instability arise due to the substantia nigra's impairment of control.(2) The body's needs are not adequately met without the neuron's consistent acceptance of neurotransmitters and messages that carry information.(1) The havoc embedded in the substantia nigra's neurons is believed to involve a combination of genetic and environmental factors, yet scientists cannot accurately justify this claim.(1) Affecting millions worldwide, Parkison's not only imposes a profound burden on patients' quality of life but to their caregivers and devoted yet unsuccessful healthcare systems looking for an antidote.(3) The prevalence and impact of PD will only continue to affect the elderly population if intervention is not established soon. Explain the steps of Lewy body formation is 2-3 lines, as shown in Figure 2. The formation Lewy bodies begins with monomors which misfold into oligomers due to changes in their structure. From there, the oligomers continue to aggregate into fibrils. The fibrils clump together to form the Lewy body which is prone to cause the cellular dysfunction that defines the symptoms of Parkinson's Disease in the neurodegenerative process.(1)

PROTAC (PROteolysis TArgeting Chimeras) is a unique drug that destroys specific cell proteins (4). Unlike traditional small-molecule medicines that block a protein's function, PROTACs harness the cell's natural degradation machinery (4). This domination of power manipulates the cell to destroy the protein. (4). Each PROTAC has three main parts: a targeting ligand that binds to the bad protein, an E3 ligase ligand that binds to an E3 ubiquitin ligase (which tags proteins for destruction), and a linker that connects these two components. (5). When the PROTAC binds to its target protein and thus expels the E3 ligase, the ligase tags the protein with ubiquitin molecules, signaling the cell to degrade the protein (4). Removing the fatal protein comes with sustained therapeutic effects, highlighting PROTAC's significant impact in the pharmaceutical field for proteins traditionally considered "undruggable." Its promising approach to incurable diseases, like cancer and neurodegenerative disorders, highlights its potential as a new frontier in drug discovery regardless of its experimental stage (4).

Molecular docking is a computational technique to predict how a potential drug binds to a target protein (6). Simulating

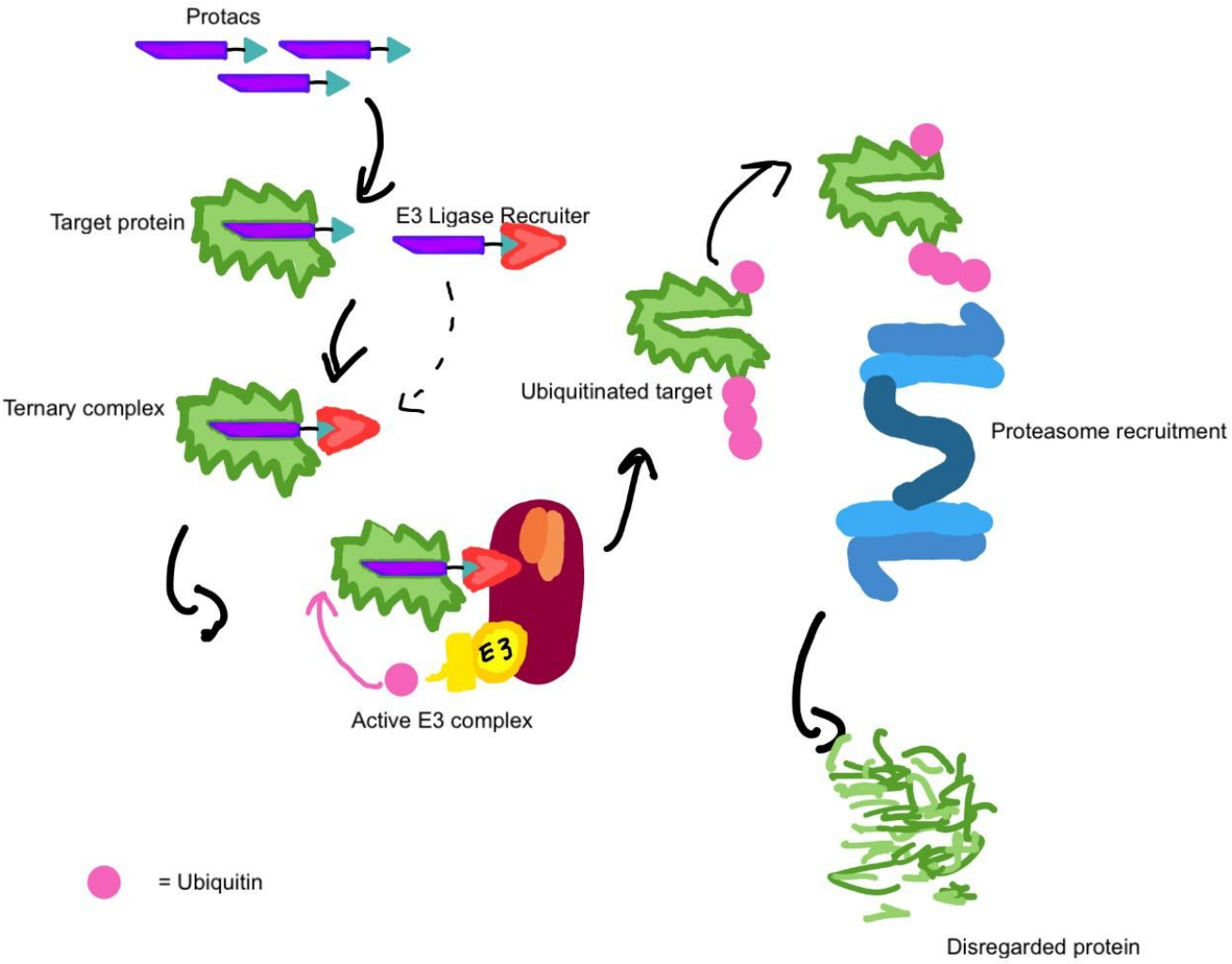

Fig. 1. Schematic of PROTACs: The mechanism of targeted protein degradation using PROTACs (Proteolysis Targeting Chimeras) is shown in the illustration. PROTACs are bifunctional molecules with two binding domains that bind to a target protein and attract an E3 ubiquitin ligase. A ternary complex is created upon interaction, which makes it easier for the E3 ligase to transfer ubiquitin molecules—pink circles—to the target protein. The target protein is then successfully eliminated from the cell by the proteasome's recognition and degradation of the ubiquitinated protein. This method offers a precise way to break down proteins that cause sickness.

the binding process finds potential bonding sites, to which deducting the perfect location depends on the bond's strength (6). Thousands of algorithms that create different shapes of ligands that align with several possible sites ultimately lead to the ideal combination of ligand structure and targeted site alignment (6). Docking is critical in efficiently creating a functioning solution to destroying the fatal protein (6).

Recently, Zhu et al. have designed PROTAC, which can degrade alpha-synuclein fibril formed in Parkinson's disease.(7) However, the mechanism of alpha-synuclein-PROTAC-E3 Ligase has yet to be discovered. We hypothesize that the PROTAC should bind to alpha-synuclein and E3 ligase at a specific site, resulting in alpha-synuclein-E3 Ligase interaction. In the current investigation, we have used computer tools to understand the structural mechanisms involved in complex formation. The research will give us a deeper insight into developing novel PROTAC that can degrade the alpha-synuclein and prevent the disease.

2. METHOD

We downloaded the alpha-synuclein fibrils 3D structure from a protein data bank (PDB ID 7YNM) to model the alpha-synuclein fibrils. The PROTAC mutations were created through the following steps: the PROTAC 3D structure, in .mol2 format and sourced from the PROTAC-DB database, is uploaded on the Crem webserver to create the PROTAC library, and we converted SMILES to 3D PDB structures using the NovoPro web server.(8) Through this process, 10 PROTACs were formed and ready for molecular docking under the HADDOCK web server.(9) In contrast, the protein-ligand interactions were identified under the Protein-Ligand Interaction Profiler (PLIP) web server.(10) The ChimeraX software was used to comprehend the complexes, visualize, and look beyond the model.(11) The research process

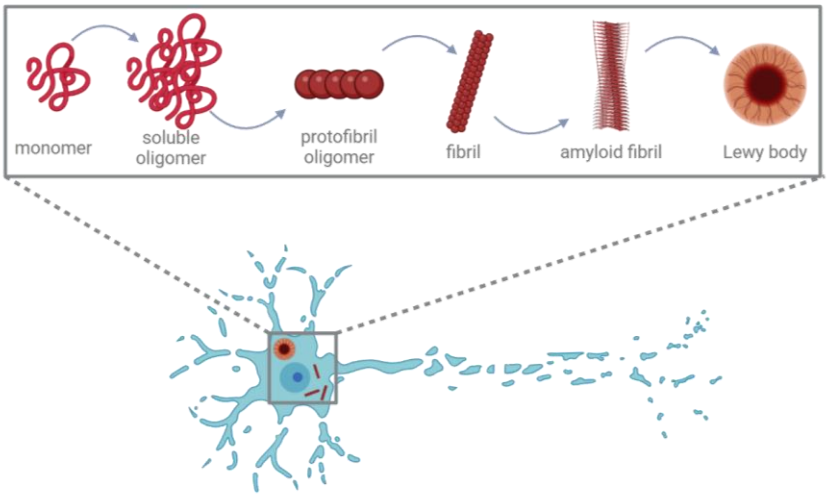

Fig. 2. Formation of Lewy Body in Parkinson's disease. In Parkinson's disease, sticky monomers (alpha-synuclein) are formed, which stick together to form soluble oligomers. These oligomers aggregate to form amyloid fibrils, producing toxic Lewy body formation.

Table 1: Protein-PROTAC binding energy computed using the PRODIGY software.

PROTAC ID	Binding Energy (Kcal/mol)
P1	-12.26
P2	-11.37
P3	-10.71
P4	-10.98
P5	-9.94
P6	-10.00
P7	-9.72
P8	-9.53
P9	-10.00
P10	-9.10

also required using GrASP, a graph neural network (GNN).(12) GNN can predict the most druggable site of the receptor for the aptamers using a pre-trained graph neural network model, which aids in protein bonding. This confirms GNN's credibility and reaffirms the sites' vulnerability.

3. RESULTS

In the current work, we have designed and analyzed PROTACs that target the alpha-synuclein formed in Parkinson's disease. Researchers had to guess possible binding spots on the alpha-synuclein and E3 ligase to find an ideal binding spot. A binding site is a specific region on the protein that the PROTAC can attach to and prove effective, which is why locating potential sites is critical in determining the efficiency of the PROTACs.

This estimate explains the PROTAC's role in developing the alpha-synuclein and E3 ligase connection. See Figures 3a, b, c, and d. A binding site is a specific region on the protein that the PROTAC can attach to and prove effective, which is why locating potential sites is critical in determining the efficiency of the PROTACs. As seen in Figures 3e and f, the proteins' electrostatic potential (ESP) was found. The results show that the binding site of E3 ligase was mainly positive, while the site on the alpha-synuclein was neutral primarily and partially positive in some nature. In the next step, we have performed molecular docking simulations. Docking is a technique that predicts how well a ligand might interact with the hypothesized bonding sights, which assesses a drug's efficiency.

Through thousands of simulations, docking allows the researcher to find the ideal shape and bonding site that coordinates with it.4).performed the docking analysisanalysis tool visualizes the binding between the E3 Ligase and alpha-synuclein with the assistance of the PROTAC. 2,3,4,6 are the strongest because their hydrogen bonding is the strongest, which makes them suitable PROTAC.

Figure 5: Alpha-Synuclein-PROTAC-CRBN interaction graph.

4. DISCUSSION

The experiment faced limitations since computational research requires experimental validations. Therefore, experiments confirming the PROTAC's success rate must be conducted and further studied. Also, we have just screened only 10 PROTACs, and in the future, we will be increasing the number of PROTACs to increase the potential range of PROTACs that could be used in this work. Since molecular docking simulation only involves protein and PROTAC, there is some limitations of molecular

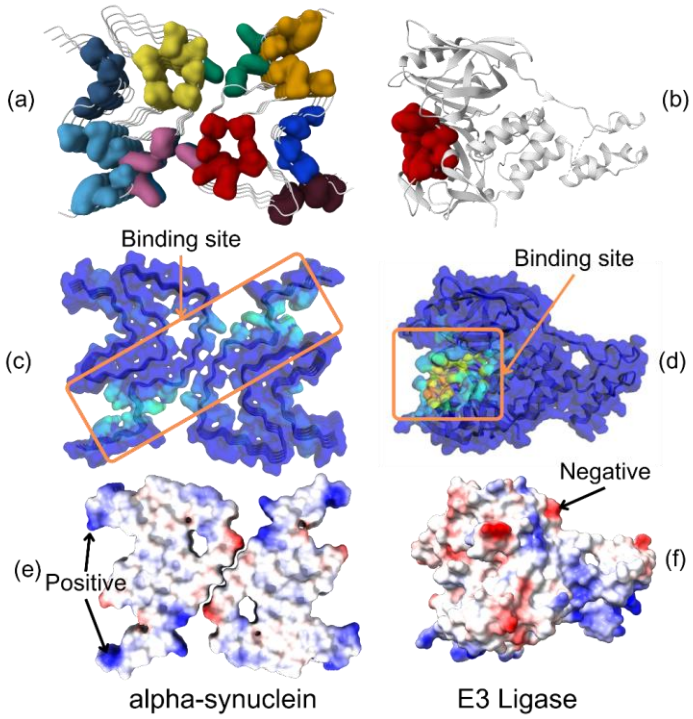

Fig. 3. Structure of alpha-synuclein and E3 ligase.(a) Binding site on the surface of alpha-synuclein protein predicted by P2Rank; (b) Binding site on the surface of E3 ligase predicted by P2Rank; (c) Binding site on the surface of alpha-synuclein protein predicted by graph neural network; (d) Binding site on the surface of E3 ligase protein predicted by graph neural network; (e) electrostatic surface potential (ESP) of alpha-synuclein; and (f) ESP of E3 ligase.

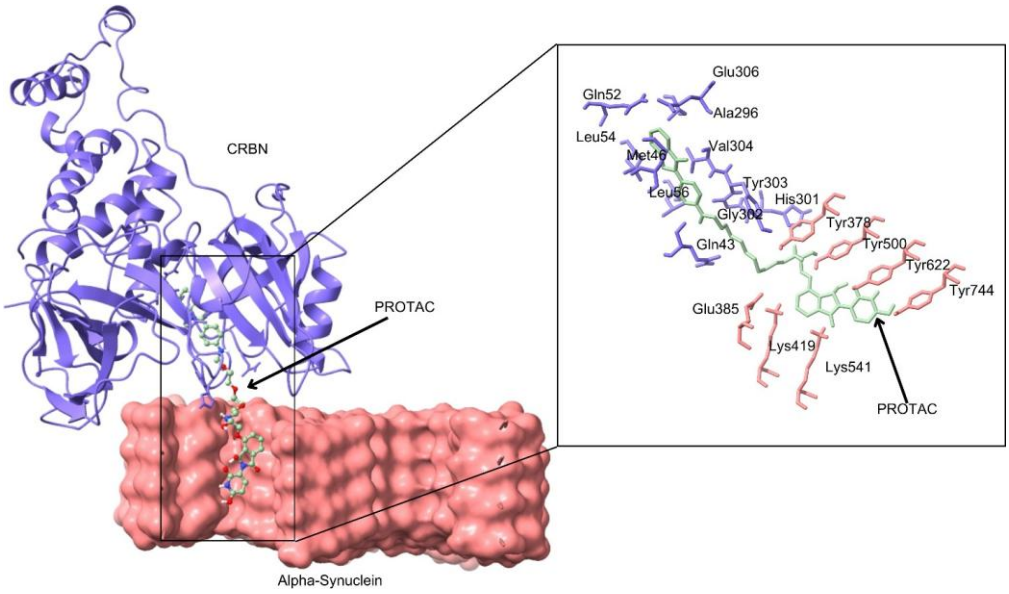

Fig. 4. Alpha-Synuclein-PROTAC-CRBN bound structure.

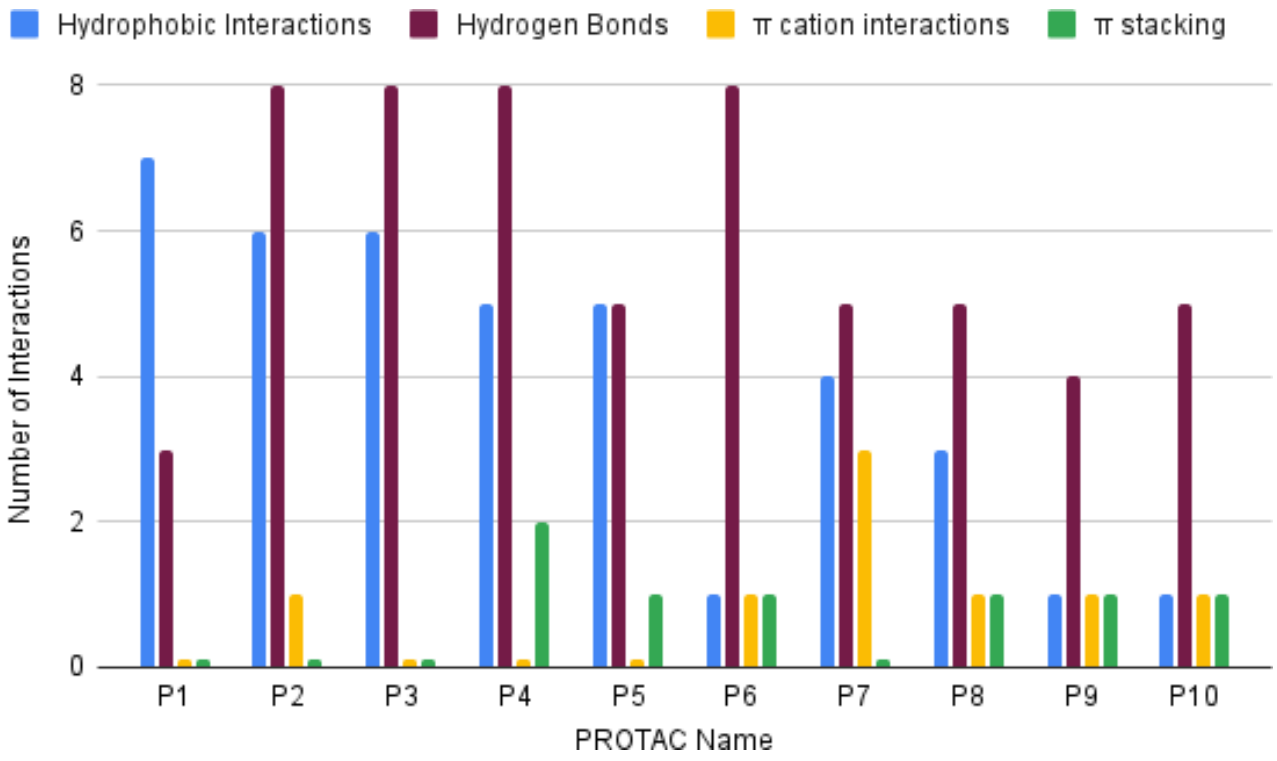

Fig. 5. Protein-PROTAC binding energy computed using the PRODIGY software.

dynamics.

In docking simulation, for instance, the water is not considered in molecular dynamic simulation. However, all the proteins and proteins are inside our body, surrounded by water molecules. Therefore, in future studies, we will perform molecular dynamics simulation, which includes protecting proteins surrounded by water molecules and creating the actual water environment. However, this research involves complex computations, simulations, and resources. Therefore, it was not considered in this research paper; in future studies, we will also be interested in performing experiments to validate our results by using experimental techniques like surface plasmon resonance (SPR) and isothermal calorimetry (ITC). These experimental techniques will further validate the PROTAC interaction and can lead to optimized, most appropriate protective candidates.

5. CONCLUSION

In this work, we have performed a computational simulation to design a PROTAC that can help in alpha-synuclein degradation through an E3 Ligase, thus hindering Parkinson's disease development. This research involved molecular docking between alpha-synuclein and E3 Ligase to understand their binding interactions. Then, another molecular docking simulation was run through the ideal complex and the PROTAC. Variations of PROTACs ran under the docking process on the alpha-synuclein and the E3 Ligase protein to get the complex structure. The binding energy and the number of interactions calculated were performed to find the ideal PROTAC used for the complex. P1 and P2 had the strongest binding energy and thus were selected for the research process. This current research will, therefore, pave the way for a current study on the degradation of alpha-synuclein proteins associated with Parkinson's Disease.

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