Computational Modeling of PROTAC-Induced Targeted Degradation of Tau Protein in Alzheimer's Disease

Akhil Chamarti

ABSTRACT

Alzheimer's disease (AD) is a progressive disease that destroys memory and other mental functions. It is a type of dementia primarily affecting older adults, causing a decline in cognitive abilities, behavior, and social skills severe enough to interfere with daily life. Tau is a protein that plays a key role in AD by forming abnormal clumps called neurofibrillary tangles inside brain cells when it becomes misfolded and accumulates, which disrupts the normal function of neurons and contributes significantly to the cognitive decline associated with the disease. PROTAC is a small molecule that degrades harmful proteins by binding to a target protein, recruiting E3 ubiquitin ligase, labeling the protein with a ubiquitin tag, and then degrading the protein. We hypothesize that a chemical alteration in the PROTAC 3D structure could make a stronger binding PROTAC. PROTAC binds to the tau protein and the E3 ubiquitin ligase, disintegrating the fibrils. The HDOCK web server allowed protein-protein and protein-DNA/RNA docking based on a hybrid strategy. Two molecular docking simulations were performed to understand the interactions between the fibril-PROTAC and E3 Ligase-PROTAC. Finally, the complete Fibril-PROTAC-E3 Ligase complex was formed. A PLIP interaction analysis was performed to understand interactions between the PROTAC and protein (tau and E3 Ligase). Finally, based on the docking analysis, the PROTAC was chemically modified to enhance its binding affinity. The application of PROTAC research for AD lies in developing targeted therapies to degrade disease-related proteins, potentially improving treatment outcomes.

Keywords: PROTAC, Alzheimer's Disease, Tau Protein Degradation, Molecular Docking, Targeted Protein Degradation.

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioral changes (Lanctôt et al., 2024). Symptoms include difficulty recalling recent events, disorientation, confusion, and impaired reasoning (Lanctôt et al., 2024). Fibrils in Alzheimer's disease (AD) are formed when beta-amyloid peptides aggregate, creating plaques, and tau proteins form tangles, thereby disrupting neuronal function. Current treatments like cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists only manage symptoms and do not stop disease progression. New methods are crucial because treatments fail to target the underlying causes, such as fibril accumulation. PROTAC (Proteolysis Targeting Chimeras) offers promise by degrading these fibrils, potentially addressing disease progression (Pujari, Bhatt, Soni, Sharma, & Patil, 2024). The tau protein is a microtubule-associated protein that forms the neurofibrillary tangles associated with AD and related tauopathies. However, the tau proteins are not always harmful; they maintain neuronal health by stabilizing microtubules and facilitating intracellular transport. AD triggers abnormal phosphorylation, which alters tau's shape, causing it to lose its ability to stabilize. This causes the detached protein to clump together and form neurofibrillary tangles, which disrupt cellular transport, trigger inflammation, and ultimately lead to the death of neurons.

PROTAC (Proteolysis Targeting Chimera) is a protein degradation tool that can remove specific unwanted proteins (Pujari et al., 2024). PROTAC utilizes the cell's ubiquitin-proteasome system to selectively degrade target proteins (Xu et al., 2023). PROTAC binds a target protein and an E3 ubiquitin ligase, which helps tag the target protein for destruction by the proteasome. PROTAC consists of three essential parts: a ligand for the target protein, a ligand for an E3 ubiquitin ligase, and a chemical linker that connects the two (Gao, Sun, & Rao, 2020). PROTACs are being explored in various diseases, particularly oncology, targeting proteins such as androgen receptors and BRD4. They are also being developed for the treatment of neurodegenerative diseases and immune-related conditions. Because PROTAC uses degradation rather than inhibition, it can target a broader range of proteins. It can also be used at a smaller dosage and is less susceptible to resistance mechanisms.

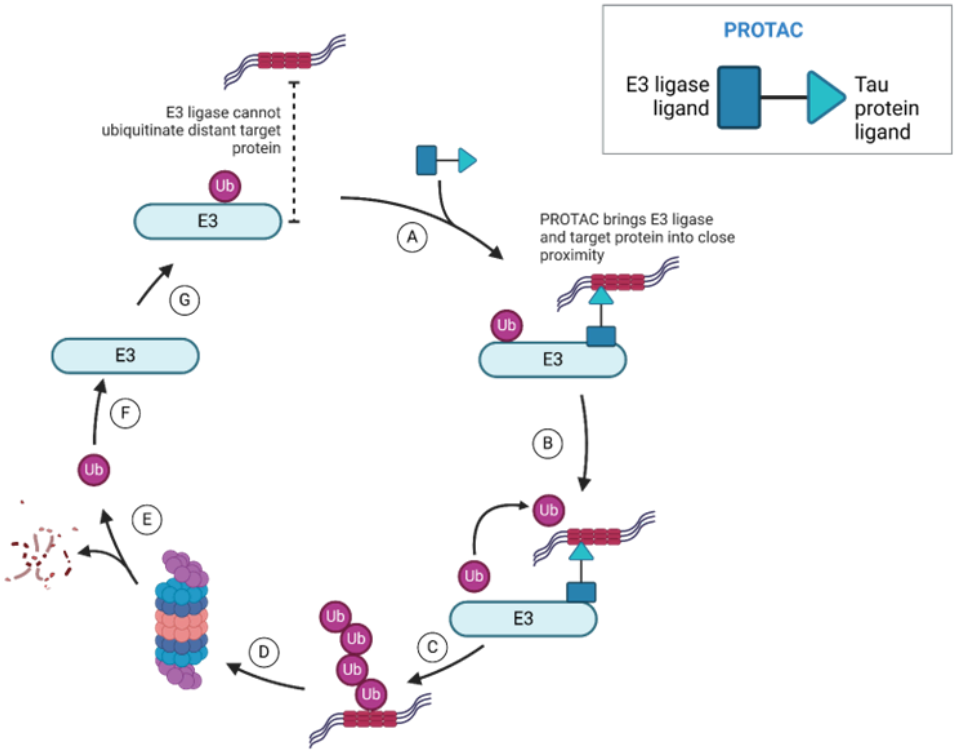

Figure 1. *Schematic of PROTAC-Mediated Tau Protein Degradation. (A) The PROTAC molecule binds to the E3 ligase and the target protein (e.g., tau protein), bringing them into close proximity. (B) The E3 ligase transfers ubiquitin molecules (Ub) to the target protein. (C) Multiple ubiquitin molecules are attached to the target protein, forming a polyubiquitin chain. (D) The polyubiquitinated target protein is recognized and taken to the proteasome (a protein degradation complex). (E) The proteasome breaks down the target protein into small fragments, effectively removing it. (F) The E3 ligase detaches from the PROTAC and can be reused. (G) The cycle restarts, and E3 ligase remains available to ubiquitinate other target proteins with the help of PROTAC.*

Molecular docking is the study by which two or more molecular structures fit together (Fan, Fu, & Zhang, 2019). Docking is a molecular modeling technique to predict how a protein interacts with small molecules. Molecular docking is essential because it is a crucial tool in drug

discovery, as it allows researchers to predict how small molecules can interact with a target protein. Molecular docking helps identify potential drug candidates by analyzing their binding affinity and orientation within the active site of a protein. This technique can save time and resources by narrowing down viable compounds for experimental testing. Additionally, it provides valuable insights into the molecular mechanisms of diseases, aiding in the design of more effective and specific therapeutic agents (Fan et al., 2019). Our study demonstrates the potential of PROTACs in degrading tau fibrils, offering a novel therapeutic approach for AD, with future applications focusing on optimizing PROTACs for clinical efficacy and overcoming challenges like blood-brain barrier permeability. PROTAC also has the potential for similar uses in other diseases such as Cancer, Parkinson's, and Huntington's.

Methods

We downloaded a tau protein and an E3 ubiquitin ligase from the Protein Data Bank (Abramson et al., 2024; Consortium, 2014). The Protein Data Bank is an open-access, global database for the 3D structural data of biological macromolecules. From this database, we downloaded a tau protein (PDB ID: 8fug) and an E3 ubiquitin ligase (PDB ID: 5t35). We used the HDOCK Software to visualize a hybrid molecular docking pattern (Yan, Zhang, Zhou, Li, & Huang, 2017). Then, we put that into ScanNet to identify possible binding sites (Tubiana, Schneidman-Duhovny, & Wolfson, 2022). We utilized HDOCK software to perform hybrid molecular docking, which combines template-based and free docking methods to predict and visualize molecular interactions. This helped identify the most probable docking patterns between the target molecules. Once the docking process is complete, we input the resulting complex into ScanNet, a tool specifically designed to detect potential binding sites and key interaction regions. This combined approach provided a more detailed and accurate understanding of molecular binding and interaction mechanisms. The tool used to create the figures in this research paper is UCSF ChimeraX, a sophisticated molecular visualization tool designed for rendering high-resolution 3D images of biomolecular structures (Meng et al., 2023). Specifically, we used ChimeraX to generate detailed visualizations of the PROTAC molecule, including surface representations, electrostatic potential maps, and ribbon models, to depict the structural features and interactions of PROTACs in a visually comprehensive manner.

Results

We performed molecular docking simulations to model the interactions between PROTAC, tau protein, and E3 ubiquitin ligase. We identified binding sites and chemically modified the PROTAC structure to improve its binding affinity for targeted degradation of tau fibrils linked to AD. We performed binding site prediction to identify optimal areas on the tau protein and E3 ubiquitin ligase where PROTAC can effectively bind, ensuring targeted degradation of tau fibrils in AD.

A binding site is a region on a molecule, such as a protein, where another molecule, known as a ligand, can bind. It is typically lined with amino acids, and drugs are usually designed to bind to a specific site. To identify the binding site, we employed ScanNet, a software that utilizes a deep learning model to learn patterns in protein structures associated with binding sites. Figures 2a and 2d show binding sites predicted by ScanNet. Figure 2a shows an E3 Ligase, while Figure 2d shows a tau protein. The colors signify the location of a binding site. The

red regions indicate a high predicted binding probability, the blue areas denote a low binding probability, and the white or pale regions represent a neutral or intermediate binding likelihood. In Figures 2b and 2e, we used ESP (electrostatic surface potential). ESP measures the charge distribution on the protein's surface. As we can see in the figures, some parts are shaded with red, some are shaded with blue, and some are shaded with white. The red parts signify a negative charge, the blue parts are positive, and the white parts are neutral. Finally, Figures 2c and 2f show the molecules as ribbons. This highlights the overall structure of the protein and makes it easier to compare the protein to other proteins.

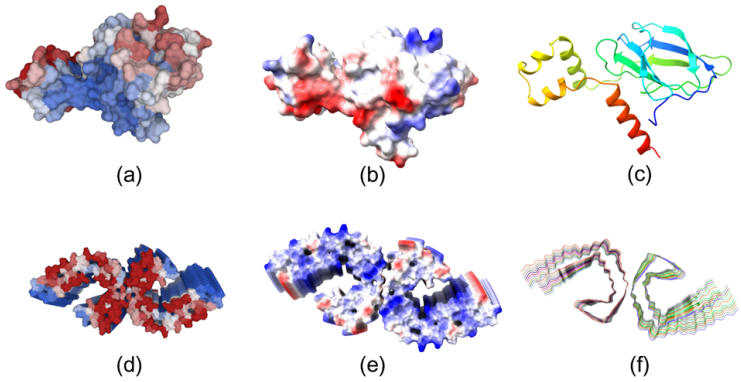

Figure 2. *Protein structures visualized using different representation techniques. (a) and (d) display binding sites predicted by ScanNet for an E3 ligase and a tau protein, respectively. (b) and (e) show the electrostatic surface potential (ESP), where red regions indicate a negative charge, blue regions indicate a positive charge, and white regions indicate a neutral charge. (c) and (f) present the proteins as ribbon diagrams, illustrating the overall fold of the molecules for easier comparison to other proteins.*

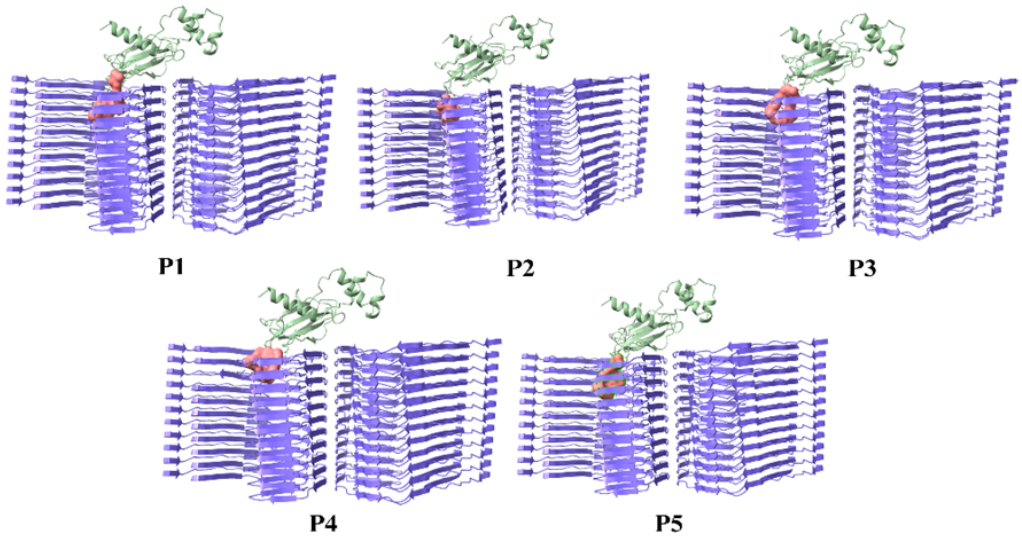

Figure 3. *Docking structures of the fibril and the PROTAC obtained from HDOCK.*

Molecular Docking Simulation:

In the next step, we performed molecular docking simulations to model the interactions between PROTAC, tau protein, and E3 ubiquitin ligase, identified binding sites, and chemically modified the PROTAC structure to improve its binding affinity for targeted degradation of tau fibrils linked to AD. A molecular docking simulation is a computational technique for predicting how two molecules, typically a small molecule, like a ligand, and a large molecule, like a protein, interact at the atomic level (Ferreira, Santos, Oliva, & Andricopulo, 2015). According to our results from ScanNet (Tubiana et al., 2022), the PROTAC binds firmly with both the E3 Ligase and the tau protein. We used the HDOCK server for docking, and we received successful results (Yan et al., 2017). The 2D Image shows PROTAC, as shown by the purple shape, bonding with the surrounding amino acids. The 3D Image shows the completed bond of PROTAC, E3 Ligase, and the tau protein, as displayed in Figure 3.

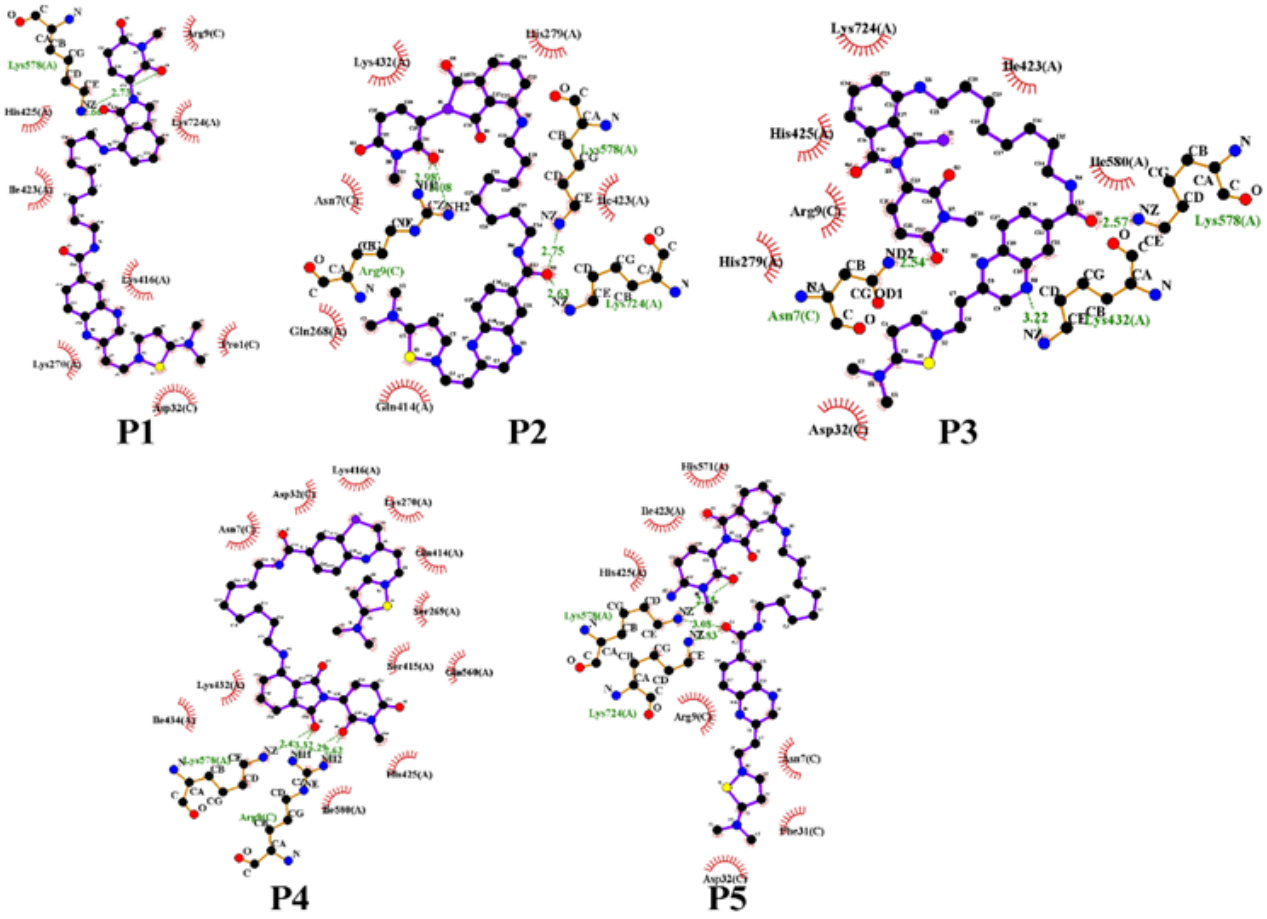

Figure 4. *Deeper atomic-level interactions between fibril and PROTAC. The green lines are the hydrogen bonds, and the red eyelashes are the hydrophobic interactions.*

Molecular Docking Analysis:

The appropriate PROTAC binding to the proteins was selected based on the following criteria. Using ChimeraX gives us a visual view of the structural and molecular interactions within the PROTAC and protein docking. It allows us to ensure proper binding between the PROTAC, tau protein, and E3 Ligase. By proper binding, we mean the PROTAC binds to the predicted binding site of both E3 Ligase and tau protein. In the next step, we used the PRODIGY web server to compute the binding energy between the protein-PROTAC complex to find the strongest PROTAC binder. The mutated PROTAC (P2) is stronger than the control (P) because of its higher number of stabilizing interactions. This makes it a better candidate for targeted protein degradation in AD.

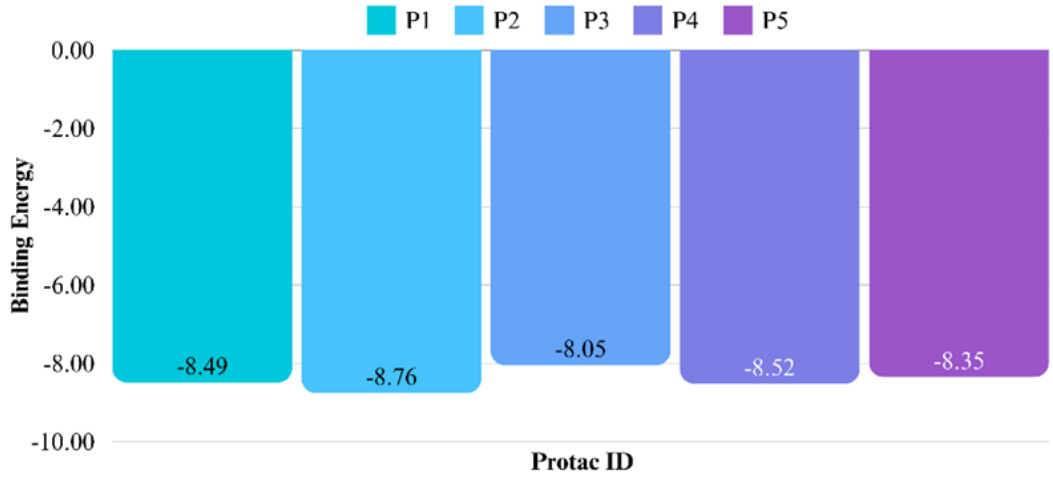

Figure 5. *Binding energies of PROTAC, obtained from PRODIGY Webserver.*

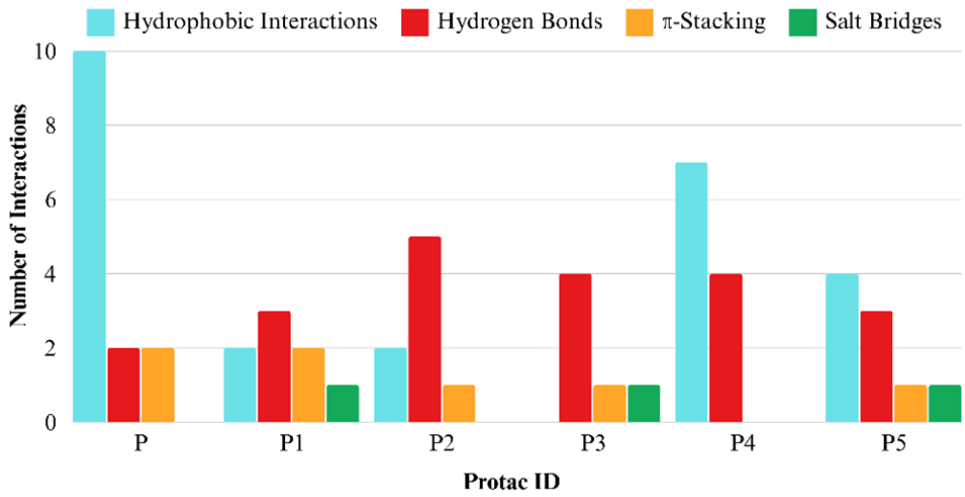

Figure 6. *Number of Protein-PROTAC interactions. Data obtained from Protein-Ligand Interaction Profiler (PLIP)*

Discussion

As of today, there are several AD (Scheltens et al., 2021) treatment strategies, such as Cholinesterase inhibitors, Monoclonal antibodies, and Brexpiprazole (Long & Holtzman, 2019). However, these treatments do not directly degrade the neurofibrillary tangles; they only stop the growth. This is harmful because the existing tangles continue to harm neurons, AD symptoms may continue, and the neurons will not have the potential for regeneration. The benefits of these treatments are only temporary. PROTAC, however, directly targets and degrades the fibrils. This prevents the exacerbation of the disease and provides neurons with the opportunity for functional restoration, offering a chance for improvement rather than stabilization.

Applications:

PROTAC therapy for Alzheimer's disease (AD) targets and removes harmful proteins, such as tau, also known as fibrils. This approach utilizes the body's natural system to break down these proteins, thereby slowing down the disease. In AD, fibrils duplicate rapidly and layer on each other, forming neurofibrillary tangles, leading to mental instability. Precision medicine using PROTAC for AD targets specific proteins like tau that contribute to the disease. By precisely degrading these harmful proteins, it aims to slow progression and reduce damage, offering a more personalized and effective treatment.

Limitations:

Although PROTAC therapy shows promise for AD, it has some challenges. More research is needed to confirm the effectiveness and safety of PROTAC, as it is a relatively new therapy, causing a lack of experimental validation. Administration poses challenges as well. PROTAC is a large molecule, which can lead to difficulty in crossing the blood-brain barrier. Additionally, due to its low lipophilicity, PROTAC struggles to be absorbed into the bloodstream from the stomach. PROTAC also has difficulty dissolving in water because of its high molecular weight.

Conclusion

This study demonstrates the potential of PROTAC-induced targeted degradation of tau protein as a promising therapeutic approach for AD. We identified and optimized PROTAC structures with enhanced binding affinity for both tau fibrils and E3 ubiquitin ligase through computational modeling and molecular docking simulations. The results highlight the feasibility of using PROTACs to facilitate the degradation of neurofibrillary tangles, a key pathological feature of AD. While PROTACs present a novel method for targeting and eliminating disease-causing proteins, challenges such as blood-brain barrier permeability and pharmacokinetic limitations remain to be addressed. Future research should focus on refining PROTAC design to enhance bioavailability, improve central nervous system penetration, and validate findings through experimental studies. Beyond AD, PROTAC technology holds potential for applications in other neurodegenerative diseases like Parkinson's and Huntington's, as well as in oncology and immune-related disorders. As this field advances, PROTAC-based therapies may revolutionize precision medicine, offering more effective and targeted treatment strategies for various protein-related diseases.

References

1. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., . . . Jumper, J. M. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature, 630(8016), 493-500. https://doi.org/10.1038/s41586-024-07487-w

2. Consortium, T. U. (2014). UniProt: a hub for protein information. Nucleic acids research, 43(D1), D204-D212. https://doi.org/10.1093/nar/gku989

3. Fan, J., Fu, A., & Zhang, L. (2019). Progress in molecular docking. Quantitative Biology, 7(2), 83-89. https://doi.org/10.1007/s40484-019-0172-y

4. Ferreira, L., Santos, D. R., Oliva, G., & Andricopulo, A. (2015). Molecular Docking and Structure-Based Drug Design Strategies. Molecules, 20(7), 13384-13421. https://doi.org/10.3390/molecules200713384

5. Gao, H., Sun, X., & Rao, Y. (2020). PROTAC Technology: Opportunities and Challenges. ACS Medicinal Chemistry Letters, 11(3), 237-240. https://doi.org/10.1021/acsmedchemlett.9b00597

6. Lanctôt, K. L., Hahn-Pedersen, J. H., Eichinger, C. S., Freeman, C., Clark, A., Tarazona, L. R. S., & Cummings, J. (2024). Burden of Illness in People with Alzheimer's Disease: A Systematic Review of Epidemiology, Comorbidities and Mortality. The Journal of Prevention of Alzheimer's Disease, 11(1), 97-107. https://doi.org/10.14283/jpad.2023.61

7. Long, J. M., & Holtzman, D. M. (2019). Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. Cell, 179(2), 312-339. https://doi.org/10.1016/j.cell.2019.09.001

8. Meng, C. E., Goddard, D. T., Pettersen, F. E., Couch, S. G., Pearson, J. Z., Morris, H. J., & Ferrin, E. T. (2023). UCSF ChimeraX: Tools for structure building and analysis. Protein science, 32(11). https://doi.org/10.1002/pro.4792

9. Pujari, R., Bhatt, S., Soni, U., Sharma, S., & Patil, S. (2024). Advantages and Disadvantages of PROTACs. In M. Nandave & P. Jain (Eds.), PROTAC-Mediated Protein Degradation: A Paradigm Shift in Cancer Therapeutics (pp. 67-88). Singapore: Springer Nature Singapore.

10. Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., . . . van der Flier, W. M. (2021). Alzheimer's disease. The Lancet, 397(10284), 1577-1590. https://doi.org/10.1016/S0140-6736(20)32205-4

11. Tubiana, J., Schneidman-Duhovny, D., & Wolfson, H. J. (2022). ScanNet: an interpretable geometric deep learning model for structure-based protein binding site prediction. Nature Methods, 19(6), 730-739. https://doi.org/10.1038/s41592-022-01490-7

12. Xu, Y., Yuan, Y., Fu, D.-Q., Fu, Y., Zhou, S., Yang, W.-T., . . . Tang, Z. (2023). The aptamer-based RNA-PROTAC. Bioorganic & Medicinal Chemistry, 86, 117299. https://doi.org/10.1016/j.bmc.2023.117299

13. Yan, Y., Zhang, D., Zhou, P., Li, B., & Huang, S. Y. (2017). HDOCK: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy. Nucleic Acids Res, 45(W1), W365-w373. https://doi.org/10.1093/nar/gkx407