Analyzing Amyloid-β Pathogenesis with AlphaFold

Gideon Mitchell
gideonm@uchicago.edu
Professor Esmael Haddadian
Quad Undergraduate Research Scholars Program

Abstract

In this project, I am working directly under Professor Esmael Haddadian, whose research involves computational modeling of protein systems. Our aim is to construct a clearer picture of how Alzheimer's disease affects the brain by focusing on a specific pathogenic agent called amyloid-β (Aβ) peptides. These small proteins aggregate to form complexes known as *oligomers*, that finally merge into large insoluble fibers called *fibrils*. Historically, Aβ fibrils (AβFs) were believed to be the principal pathogenic agent in Alzheimer's disease, but recent research has identified the intermediate Aβ oligomers (AβOs) as the more important neurotoxins. Despite their clinical significance, relatively little is known about AβOs as their innate instability makes them resistant to traditional imaging techniques. The purpose of our research is to elucidate the structure and the cell-membrane interaction of these AβOs. To accomplish this, we employed the machine-learning model AlphaFold to generate potential AβOs and then further refined them using molecular dynamics (MD) simulations.

Methodology

Over the summer, I worked with Dr. Haddadian to elucidate the potential structure of AβOs using Alphafold2's complex prediction algorithm for structure determination. I built models of 2- through 15-peptide AβOs using the most common pathogenic amyloid-β peptides *(Aβ 40- & 42-residues, see Fig. A for a typical example)*. To determine the viability of these generated structures we relied on a combination of dynamic and static analysis. Broadly, the static analysis consisted of comparing the structural features and motifs of AlphaFold-generated complexes with existing experimental research, while the dynamic analysis consisted of simulating the complexes using molecular dynamics (MD) programs and assessing their stability.

We began our static analysis by using AlphaFold's built-in confidence metric, the predicted local distance difference test (pLDDT), to rank the structures. AlphaFold's pLDDT america has been shown to accurately predict the instability of a generated structure, so we applied it in order to rank the generated structures. We next assessed their similarity to the experimentally available Aβ fibril structures using a novel metric to compare the secondary structure of the AlphaFold-generated AβO against the aggregate of Aβ secondary structures. We theorized that correspondence between generated secondary structures and experimental secondary structures suggests viability *(see Fig. B)*.

In our dynamic analysis, the highest-ranked structures were then run through MD simulations to assess stability. MDs are Newtonian physics simulations that can illustrate the

Static analysis of AlphaFold-generated structures

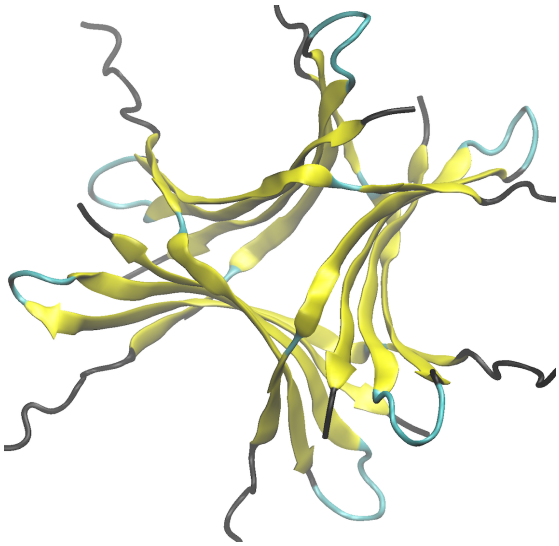

Figure A

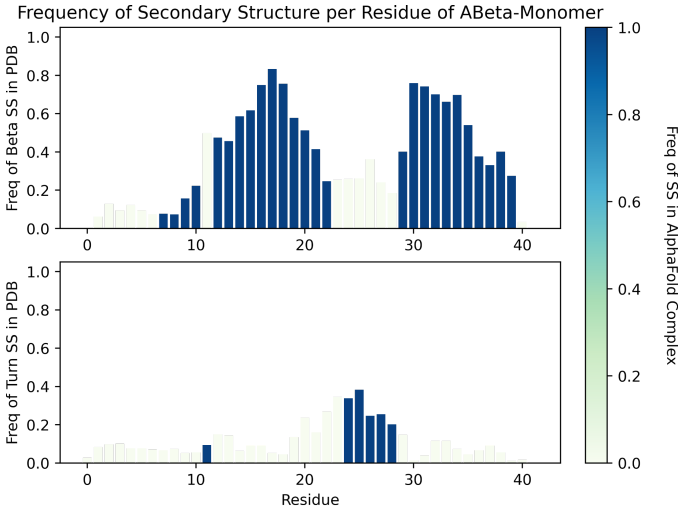

Figure B

Dynamic analysis of AlphaFold-generated structures

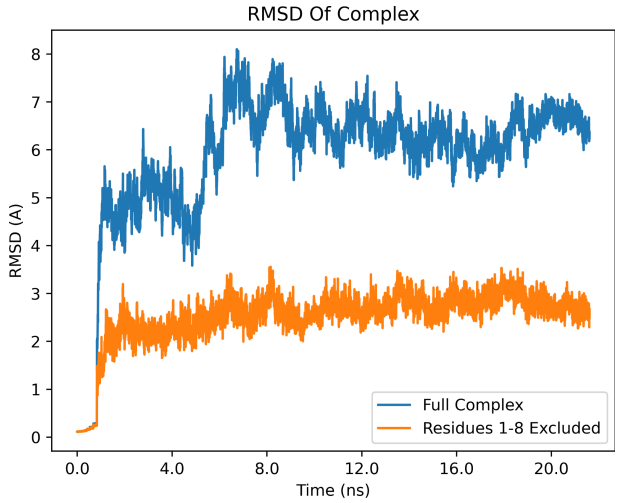

Figure C

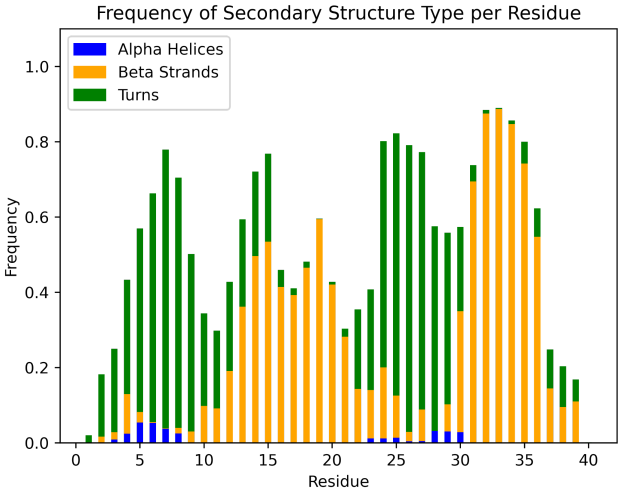

Figure D

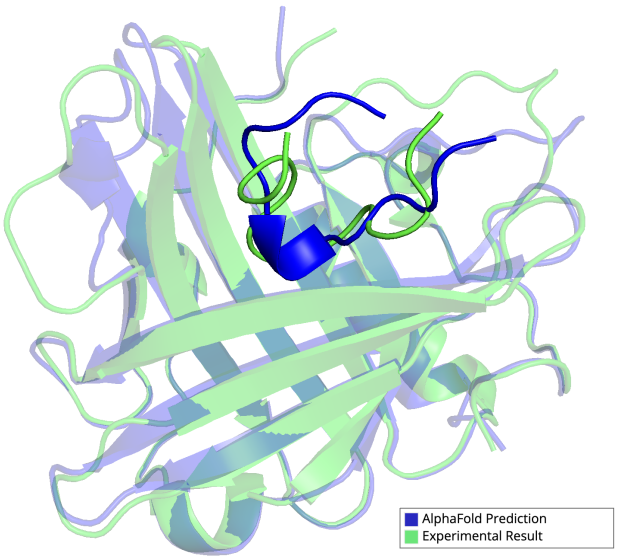

Figure E

dynamic character of a system. In these simulations, we analyzed two metrics: the root mean squared distance (RMSD) of the complex and its aggregate secondary structure. The RMSD measures conformational changes over time, so we applied it to understand the dynamic character of the system — if the RMSD increased or was volatile then the system was unstable *(Fig. C)*. We also measured the secondary structure over the course of the simulation to see how well it agreed with experimental fibril data *(Fig. D)*.

Lastly, to validate AlphaFold's ability to accurately place Aβ monomers, we generated all experimentally-derived Aβ-containing complexes in PDB. We found a high correspondence between the experimental and AlphaFold-generated placement of Aβ monomers, suggesting that AlphaFold was capable of accurately predicting their position *(see Fig. E for a typical example)*.

Using these techniques to determine viable structures we discovered several viable Aβ structures and motifs: among smaller AβOs (3-4-monomers), the most stable structures consisted of 2 antiparallel β-sheets oriented parallel to one another. In AβOs consisting of 5+ monomers, AlphaFold consistently formed a channel composed of either parallel or antiparallel twisted β-hairpins around a hydrophobic center, forming a closed β-barrel. We also observed several structures resembling Aβ-fibrils consisting of stacked, parallel β-strands. The majority of our high-ranking structures contained mostly β-topology and very little (if any) α-topology, which was unexpected.

Work to be Completed During the Fall Quarter

While we have models of viable structures, the pathogenic mechanism of AβOs remains unknown. It has been theorized that AβOs induce neurodegeneration by breaching or impairing the membrane. Hence, our next step involves placing these structures on top of POPC lipid bilayers, with varying the cholesterol content, and studying their interactions through MD simulations. To effectively sample the Aβ oligomer-lipid interactions, we will implement the Gaussian Accelerated Molecular Dynamics (GaMD) enhanced sampling protocol within the NAMD molecular dynamics package.

I intend to present our findings at the upcoming 68th Biophysical Society annual meeting, scheduled for February 2024 in Philadelphia, PA. This would be a good opportunity to share my data with other scientists in the field and receive their critical feedback, as well as learn about the other areas of computational biophysical research.

Background

In order to accomplish these research goals, I will draw on my computer science and physical sciences background. The chemistry, physics, and computer science sequences at the university have given me a solid grounding in molecular chemistry and physics, as well as the practical computer science knowledge required to effectively model the system I plan to research. Additionally, I have taken professor Haddiadan's course sequence *Multiscale Modelling of Biological Systems* (BIOS10602-3), which has given me the specific skills required

in computational biology. I have a strong grasp of the computational techniques used to analyze macromolecules and their properties, behavior, and movement. The sequence has also given me hands-on experience with running programs in the Midway Research Computing Center, which will be beneficial for training the AlphaFold model. I plan to graduate with an undergraduate degree in mathematics and computer science, then attend graduate school for computer science. Contributing to this research project will give me practical hands-on experience in working in an intensive research environment and will greatly enhance my ability to compose academic research.

In all, our research into AβO structure will contribute to the clinical understanding of Alzheimer's pathogenicity and will aid future diagnostic measures and potential drugs designed to inhibit AβOs.