EXPLORING THE STRUCTURAL CONFORMATIONS OF AMYLOID-BETA OLIGOMERS IN WATER UNDER CONFINEMENT, A MOLECULAR DYNAMICS STUDY

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Abstract

Alzheimer's disease (AD) is a devastating neurological disorder, affecting millions of people worldwide. The aggregation of amyloid-beta (Aβ) peptides and the intermediate protein tau into soluble oligomers and amyloid fibrils is believed to be a critical event in the pathogenesis of AD. Amyloid fibrils represent the endpoints of aggregation, and it is widely believed that their precursors, the soluble oligomers, are the more important neurotoxins. However, there is limited structural information available on Aβ oligomers. Here, we apply the enhanced sampling algorithm REST2 (replica-exchange with solute tempering), implemented in the molecular dynamics NAMD package, to efficiently sample the conformations of five Aβ peptides using the TIP4P explicit water model. To increase the likelihood of oligomerization, we additionally utilize a harmonic wall to confine the peptides. Our simulations have resulted in cross β-sheet formation between Aβ monomers, similar to that observed experimentally. In particular, we find that residues 17-21 and 35-40 are involved in β-sheet formation, the former of which have been observed in the Aβ fibril structure. We also observe the prominence of the intramolecular Asp23–Lys28 salt bridge, which has been found to play a significant role in the formation of Aβ fibrils. Confinement has been found to increase the conformational stability of proteins, suggesting an increased probability of Aβ formation in the crowded environment of the cell.

Overview of Amyloid-Beta Oligomers

The aggregation of amyloid-beta oligomers is widely believed to be the precursor to Alzheimer's disease. However, there is limited structural information about the oligomers and how they interact to aggregate to form amyloid fibrils.

Our primary goals are to:

- Investigate the dynamic interactions between amyloid-beta oligomers and their mechanisms for aggregation.
- Explore the use of harmonic wall confinement to induce faster secondary structure formation in molecular dynamic simulations.

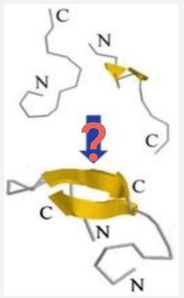


Image Source: Itoh, S.G., Okumura, H. (2022). All-Atom Molecular Dynamics Simulation Methods for the Aggregation of Protein and Peptides. In: Methods in Molecular Biology, vol 2340. https://doi.org/10.1007/978-1-0716-1546-1_10

Self-Assembled Monolayers

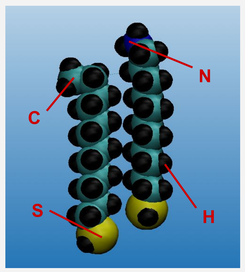


Figure 1. *Two SAM molecules.*

We construct self-assembled monolayers (SAMs) to mimic the surface properties of mica, a substance used in Atomic Force Microscopy to study conformations of amyloid-beta oligomers.

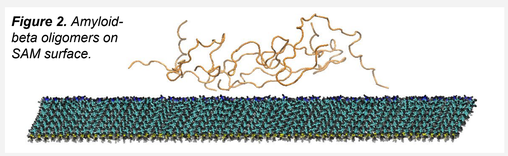


Figure 2. *Amyloid-beta oligomers on SAM surface.*

Structural Metrics of Ubiquitin Under Confinement

The function of a protein is largely influenced by its structure. The RMSD and Rg values enable a quantification of the structure. We can observe that expanding the wall too much is less favorable to expanding the wall by a modest amount and reducing it to the same final relative distance.

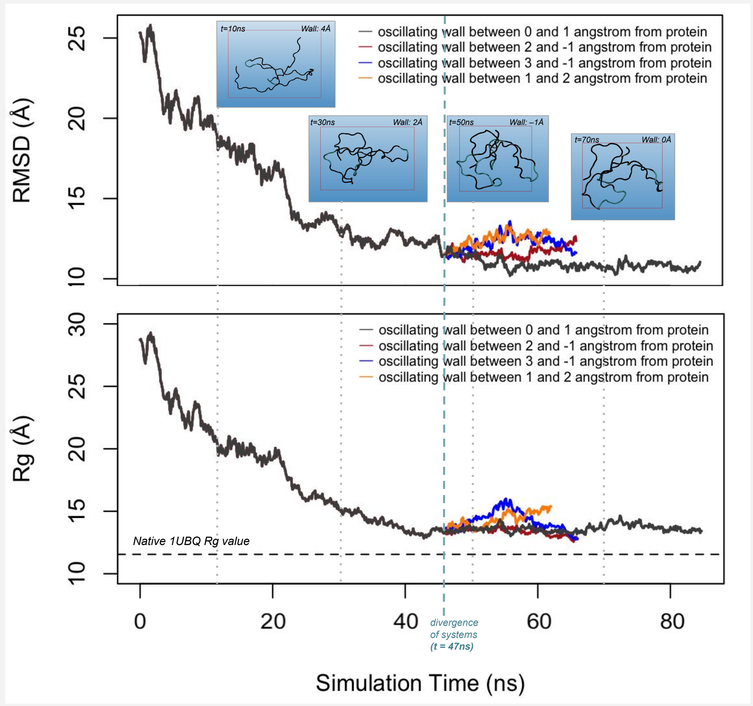


Figure 3. (top) *The RMSD (root mean square deviation) values, aligned to 1UBQ, during simulation.* (bottom) *The Rg (radius of gyration) values throughout the simulation, compared to the Rg of 1UBQ. The snapshots of the protein show its degree of foldedness at a given timestep, as well as the imposed wall (in red) with respect to the protein backbone.*

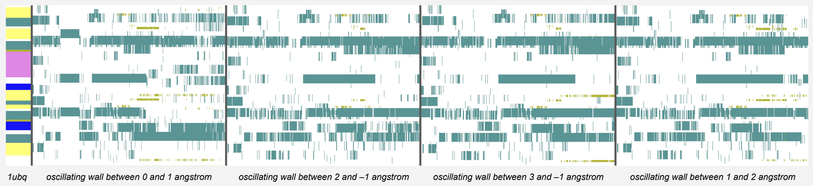


Figure 4. Ubiquitin secondary structure throughout simulation. We observe some transient beta sheet formation in residues expected by 1UBQ's structure. While the oscillating wall between 3 and -1 angstroms varies relatively more in terms of RMSD and Rg, it has the most consistent beta sheet structure, particularly in the latter parts of the simulation.

SAM Charge Distribution and Sec. Structure

We compare Aβ structural formation on a SAM with a more uniform surface charge distribution with formation on a SAM with a nonuniform distribution. Both surfaces have a net surface charge $\sigma = -0.2e^{-}/100A^{2}$. We observe:

- An uneven surface charge distribution tends to be more conducive to beta sheet formation.
- The formation of helices (alpha and 3-10 helices) shows no significant difference between the charge distributions.

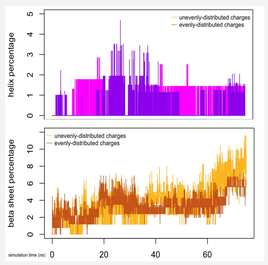
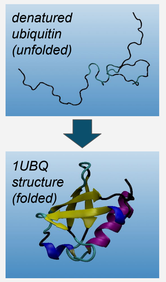


Figure 5. *A comparison of the percentage of percentage of Aβ secondary structure.*

A Preliminary Analysis of Ubiquitin Protein Structure Formation Under Confinement

Experimental data has suggested that confinement can expedite protein folding speeds. Since protein folding occurs on the timescale of milliseconds in molecular dynamic simulations, we are interested in finding the optimal combination of variables to induce secondary structure formation. However, since the aggregation of amyloid-beta oligomers is mostly unknown, we test our variables using the procedures below on ubiquitin (PDB ID: 1UBQ), a protein with extensive structural information available.



1. Denature protein by applying heat to remove structure.
2. Perform NPT production runs on the system, and apply harmonic walls using NAMD's Collective Variables module.
3. Readjust wall positions relative to protein's position every 0.5ns.
4. Allow relative distance of wall from protein to oscillate every 1ns.
5. Analyse the structural formation and compare to 1UBQ structure.

Conclusions

- A small expansion in the harmonic wall, followed by a reversion of this expansion, is the most effective method of oscillating the wall to accelerate the convergence of the RMSD and Rg values.
- Although confinement drastically increases the speed with the RMSD and Rg values of a denatured protein approach their native values, more rapid convergence of values seems to correlate with less permanence in secondary structure formation.
- Asymmetric surface charge distributions on SAM surfaces seem to be more favorable to β-sheet formation in amyloid-beta oligomers.

Further Research: Confinement and REST

- Continue experimenting with expanding and relaxing the wall to find an optimal combination of variables that enable an increased rate of RMSD and Rg convergence, in addition to permanence of structure.
- Apply the conclusions from the preliminary confinement analysis with ubiquitin to the amyloid-beta system to increase the rate of structural formation between the amyloid-beta oligomers, in addition to replica-exchange solute tempering.
- Augment the protein folding time improvements under confinement with the increased conformational efficiency of replica-exchange solute tempering by introducing confinement to the REST algorithm.

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