Amyloidogenic Proteins: Identifying Early-stage Aggregation Mechanisms

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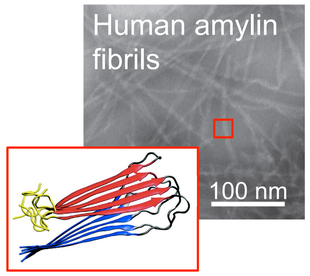
Introduction and Motivation

Millions of people worldwide are afflicted by diseases which share a common feature: certain proteins misfold and aggregate into a fibrillar structure, accumulating in a specific part of the body. These diseases include type II diabetes and numerous neurodegenerative illnesses (ex. Alzheimer's, Parkinson's, and Huntington's diseases)

Although aggregates consist of disease-specific proteins, they all share this fibrillar architecture known as amyloid. Amyloid is toxic to cells, and early-stage aggregates are most responsible for this cytotoxic effect.



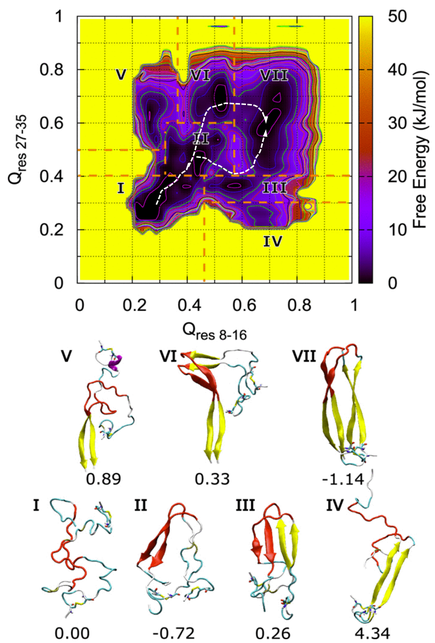
In order to improve our fundamental understanding of these diseases and design successful methods for diagnosis and treatments, it is crucial to understand the mechanism of early-stage fibril formation and to detect early species.

Atomistic molecular simulations have the potential to identify early aggregation mechanisms and facilitate the study of relevant intermediate species.

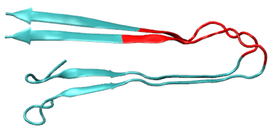
Key questions include:
- What are the mechanisms behind the first steps of amyloid formation?
- Can we identify structures, pathways, or transition states to potentially target therapeutically?

System: Human Amylin (hIAPP)

We aim to understand the early stages of amyloid formation, which are most relevant to disease pathology. We use a variety of molecular simulation techniques to study the mechanisms through which early aggregates form and their thermodynamic properties. Here we focus on human amylin (hIAPP), implicated in type II diabetes. This 37 residue polypeptide forms fibrils where individual hIAPP molecules stack in a parallel U-shape; an example of this U-shape aggregate is shown in the proposed hIAPP dimer structure below.

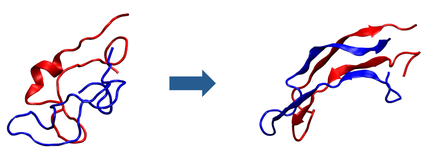


Right: U-shaped hIAPP dimer exhibiting two parallel β-sheets (proposed from ssNMR experiments). Residues highlighted in red (20-29) are proposed to form a β-sheet intermediate during fibril formation.



Prior work applied metadynamics to the hIAPP dimer to map out a free energy landscape (**Left**); the proposed β-sheet intermediate (structure II) has been supported by 2D IR experiments. However, metadynamics has disadvantages: much simulation time is spent in regions irrelevant to the transition mechanism. Additionally, metadynamics would require an unreasonable amount of resources to study higher order aggregates.

To address this, we use <u>string method</u>, a technique built for identifying and studying transition pathways, which has not been applied to amyloid formation or protein aggregation in general. Through this iterative method, a transition pathway is approximated for the dimerization process between the disordered state and the highly β-sheet dimer state:



Disordered → High β-sheet dimer

Applying String Method to Amylin Aggregation

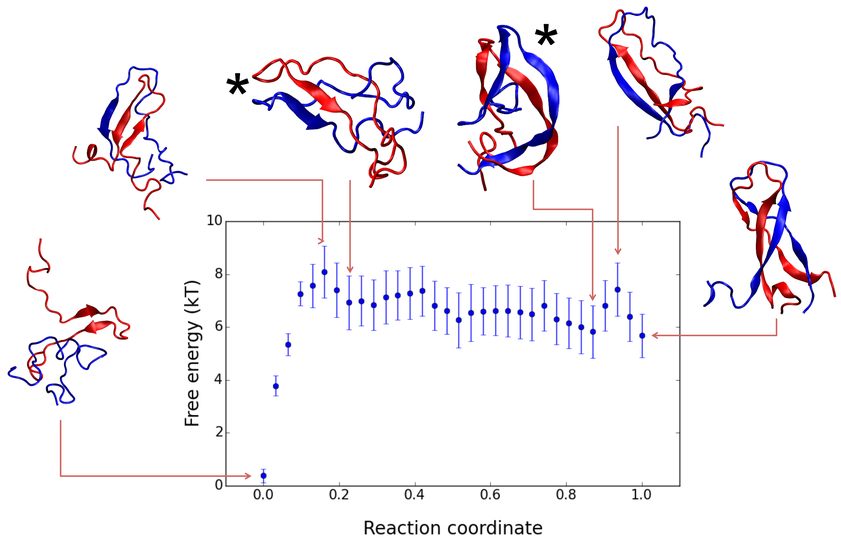
Finite Temperature String Method was performed on the human amylin dimer; as string methods have not been available in previously available simulation packages, the string method calculations were carried out using a combination of GROMACS 4.6.7, the PLUMED 2.1 plugin, and custom codes written in Python. **This work has been used as a basis for developing string methods in SSAGES (Software Suite for Advanced Generalized Ensemble Simulations, MICCoM)**, the first instance of string methods made available in a molecular simulation package.

Amylin Dimerization:

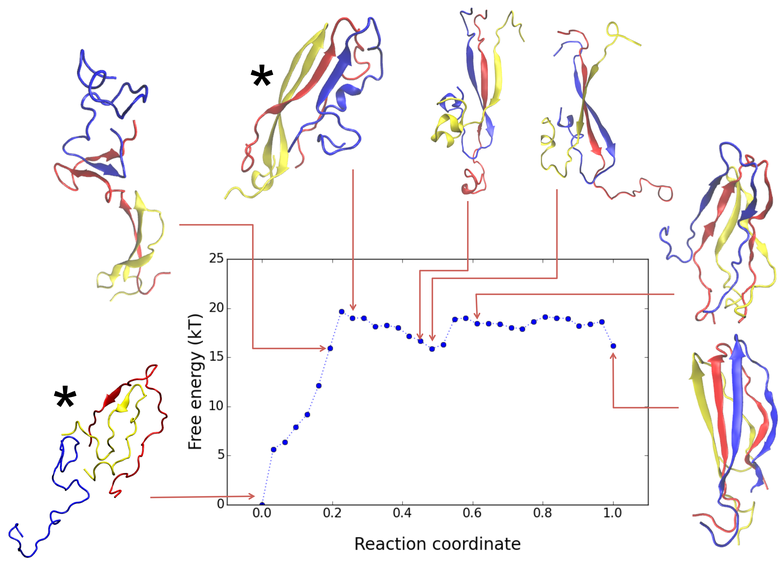
* Configurations obtained from string method match with two previously predicted hallmarks of human amylin aggregation:

1. β-sheets are first formed in the intermediate turn region

2. β-sheets are then formed in the C-terminus before the N terminus



Amylin Trimerization:



* Configurations obtained from string method calculations for the trimer show:

1. Disordered end of the string find a configuration containing the dimer intermediate – suggests stepwise aggregation mechanism.

2. Again, β-sheets are formed in the intermediate turn region.

Next steps:
- Will the aggregation process be different in more physiologically relevant environments?

References: 1. Buchanan et al., *Proc. Natl. Acad. Sci.* (2014). 2. Middleton et al., *Nature Chem.* (2012). 3. Luca et al. Biochemistry (2007). 4. Chiu, C-c., de Pablo, J.J. AIP Advances (2015).