1. Molecular design of 3D-structures of amyloids:

1.1. Design and prediction of 3D-structure of amyloids

Protein aggregation of amyloids is associated with numerous incurable diseases, including amyloid β (Aβ) and Tau protein in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD), and human islet amyloid polypeptide (hIAPP) in type 2 diabetes (T2D). Previously, I investigated the self-assembly of Aβ oligomers at the molecular level, using computational molecular modeling tools.

One of the research goals in our lab is to investigate the self-assembly of other amyloids, such as Tau protein, hIAPP and α-synuclein, that similarly to Aβ, also display a general cross-β structure with β-strands perpendicular to the fibril axis and hydrogen bonds parallel to the fibril axis: **1)** AFM, FT-IR, CD and X-ray fiber diffraction experiments have shown that fragments of the β-structure in Tau protein repeats can stably interact with each other and with themselves and can aggregate into fibrils, however, how the Tau protein repeats self-assembled remain elusive. Based on these experimental data our lab will predict the self-assembly of the Tau protein repeats. **2)** Two ssNMR models available for hIAPP oligomer, yet it is unknown which one of these two models is the preferred model. Using MD simulations for these models and comparing their energies our lab envisions determining the preferred model. **3)** The recent ssNMR of α-synuclein only proposed a three-dimensional folding structure of five β-strands, but did not provide a set of PDB coordinates. Based on this ssNMR data, our lab will predict the self-assembly of a segment in α-synuclein that is responsible on the self-assembly process.

1.2. Design and predicting the molecular structure of cross-amyloid interactions

Experimental evidence led to the hypothesis that cross-amyloid interactions (e.g., interactions between Aβ and hIAPP) also play a critical role in protein aggregation. Structure-based characterization of the interactions between two types of amyloids is fundamental to understanding the self-assembly mechanism that exists between them and may pave the way to elucidate the link between two diseases (e.g. T2D and AD). Experimentally solved structures of the interactions between two types of amyloids at the molecular level have remained elusive. The lab's overall goal of this project is to investigate cross-amyloid interactions in incurable diseases through structure-based computational characterization using two model systems: Aβ–hIAPP oligomers and Aβ–α-synuclein oligomers. Preliminary results for the cross-amyloid interactions between oligomers of Aβ and of hIAPP indicate that Aβ–hIAPP interactions are indeed stable. Some of the structural models are illustrated in Fig. 1.

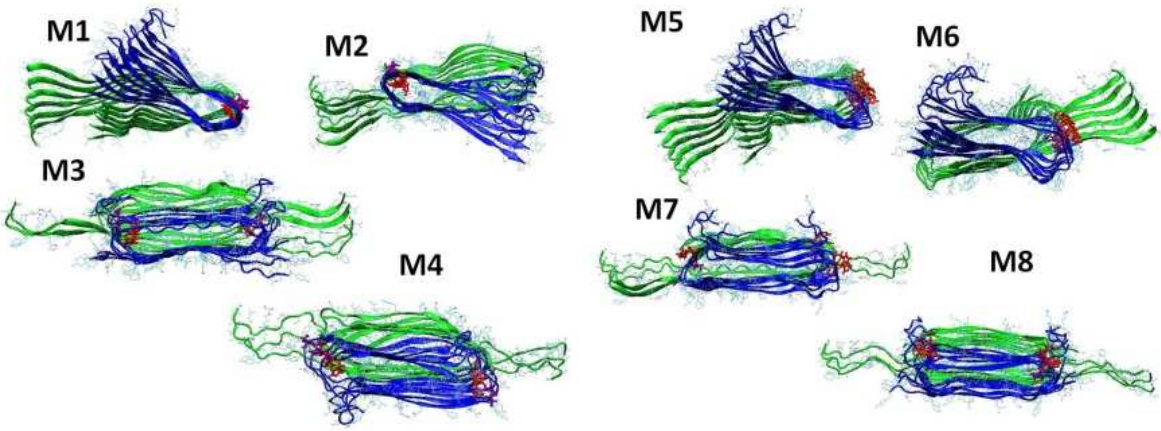

Fig. 1: Some of the Aβ-hIAPP predicted in our lab, illustrate various organizations between Aβ peptides (green) and hIAPP peptides (blue) and between themselves. One ssNMR for hIAPP illustrates His18 (red) oriented inside the core domain and Ser19 (purple) oriented outside the core domain (as illustrated in M1-M4). Second ssNMR illustrates His18 (red) oriented outside the core domain and Ser19 (purple) oriented inside the core domain (as illustrated in M5-M8).

To further test our predicted models, we are collaborating with Prof. Aphrodite Kapurniotu, Technische Universität München (TUM), Germany. Prof. Kapurniotu is an expert on amyloid–amyloid interactions.

1.3. Molecular design of metal binding sites of amyloids and cross-amyloid interactions

The metal ions Zn^{2+}, Cu^{2+}, and Fe^{2+}, among the most prevalent in biological systems, are known to be essential for normal brain function and development. Disrupted cellular homeostasis of these ions is thought to play a central role in the protein aggregation and neurotoxicity of Aβ. The involvement of heavy metal ions in PD was suggested on the basis of the considerable increase in total Fe^{2+}, Zn^{2+}, and Al^{3+} content observed in the parkinsonian *substantia nigra* and indeed, elevated concentrations of Cu^{2+} were reported in the cerebrospinal fluids of patients with PD. Metal ions are also involved in T2D: the Zn^{2+} content of pancreatic β-cells is among the highest in the body and play a protective role against beta cell destruction. Clinical and epidemiological studies suggest that zinc deficiency is a common symptom of T2D. It has recently illustrated that Zn^{2+} ions inhibit hIAPP aggregation. Finally, a recent study demonstrated that Cu^{2+} inhibits the formation of hIAPP fibrils but accelerates the formation of oligomers.
Based on recent experimental data, our lab constructs Zn^{2+}–hIAPP oligomers. In addition, we construct Cu^{2+}–α-synuclein oligomers, also based on recent experimental data. The Zn^{2+}–hIAPP oligomers will then be combined with my previously constructed Zn^{2+}–Aβ oligomers, and the Cu^{2+}–α-synuclein oligomers will be combined with my earlier constructed Cu^{2+}–Aβ oligomers. Amyloid–amyloid interactions to produce Zn^{2+}–Aβ–hIAPP oligomers and Cu^{2+}–Aβ–α-synuclein oligomers will be set up similar to that between the amyloids without metal ions

2. *In silico* design of novel self-assembling peptides for nanobiotechnology:

2.1 Design of novel self-assembling, metal ion-biding β-hairpin peptides for hydrogel formation

Hydrogels are proving to be an excellent class of materials for biomedical applications, e.g., tissue engineering, drug delivery, and wound healing. In this project, a combination of computational modeling and experimental materials characterization will be used to generate a novel class of hydrogels formed from metal ion binding, self-assembling peptides. The Schneider group, National institute of Health (NIH), US, has developed a class of β-hairpin peptides that self-assemble into fibrillar networks that define a mechanically rigid hydrogel material. Our lab and Schneider's lab test the hypothesis that specific metal ion binding sites can be engineered into the primary sequence of self-assembling peptides and that metal ion binding can be used to govern the assembly process and to influence the resultant hydrogel mechanical properties. Our

group, through computation, has recently generated a detailed molecular-level model of the self-assembled network formed by the peptides (Fig. 2), originally designed by Schneider. Using this model, structure-based design can now be used to engineer metal ion binding sites into the sequence of these self-assembling peptides.

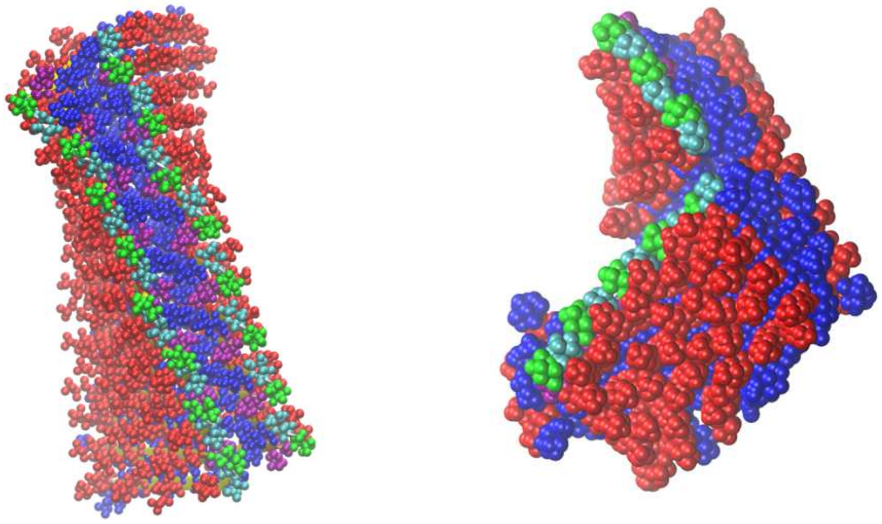

Figure 2: Constructed variants of the sixteen self-assembled β-hairpin peptides (eight peptides in each layer) after molecular dynamics simulations of 60 ns illustrate stable structures.

2.2. Design of self-replicating amphiphilic β-sheet peptides: insight into the mechanisms of the self-assembly process

The research of replicating β-sheet peptides has been studied by Prof. Gonen Ashkenasy, BGU, Israel. Ashkenasy group synthesized a series of amphiphilic peptides that can form soluble, one dimensional β-sheet aggregates in water, which serve to significantly accelerate peptide ligation and self-assembly. Although the experimental studies provided an understanding of the formation of β-sheet structure, a detailed molecular level model of how the peptides arrange themselves with the fibril remained elusive. Moreover, the mechanism of the autocatalytic process of ligation of two fragments of the peptides in reactions that were initially seeded is unknown.

Our lab constructs several variant models of the peptides that have been synthesized by Ashkenasy group and investigating their stabilities. The aim of our lab in this project:

(I) To employ the structure-based self-assembling peptides at the molecular level.

(II) To investigate the mechanism of the autocatalytic process of ligation of two fragments of the peptides in seeded self-assembling peptides.