319-Pos Board B89
Studying α-Synuclein Misfolding through Förster Resonance Energy Transfer
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One of the goals of the Peterson laboratory is to develop new methods to study the misfolding of amyloidogenic proteins. Due to their inherent structural heterogeneity, these proteins can be difficult to characterize by traditional structural methods (e.g. crystallography). Fluorescence spectroscopy provides a powerful tool to study protein folding and misfolding in real-time, but its employment requires labeling the protein of interest with two or more fluorophores. We have developed methods to combine multiple orthogonal labeling techniques to produce homogenous double-labeled α-synuclein for Förster resonance energy transfer (FRET) studies. Ultimately, these studies will aid in understanding protein misfolding implicated in neurodegenerative disease.

320-Pos Board B100
Investigating the Trimethylamine N-Oxide (TMAO) Induced Structure of α-Synuclein
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Trimethylamine N-oxide (TMAO) is a naturally occurring osmolyte that is known to stabilize protein structure. Previous studies have shown that the addition of TMAO can induce folding of thermodynamically unstable proteins, causing them to regain high functional activity. In solution, monomeric α-synuclein (α5S) is intrinsically disordered. Our laboratory and others have shown that α5S undergoes significant compaction in the presence of TMAO. Previously, we have demonstrated that p-cyanophenylalanine and a thioamide can serve as a minimally perturbing probe pair for Förster Resonance Energy Transfer (FRET) experiments. Despite the utility of this pair in measuring short intramolecular distances, inclusion of the thioamide is synthetically intensive, rendering it difficult to generate a large library of double-labeled mutants for FRET studies. As an alternative, we have expressed a library of double-labeled α5S mutants containing the genetically encodable FRET pair, Cys and tryptophan (Trp). This set of double-labeled proteins will allow us to obtain a more comprehensive description of the TMAO-induced morphology of αS.

321-Pos Board B101
From Monomers, Dimers to Oligomers: How Metal Ions Regulate Amyloid Beta Porteins in Amyloid Formation?
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Amyloid β protein is associated with the pathology of Alzheimer’s disease. Metal ions can regulate the self-assembly pathway of amyloid peptides, leading to polymeric non-fibrillar oligomers that are more neurotoxic than mature fibrils. It is still challenging to investigate the interactions between them at a molecular level by various experimental approaches. Computational simulations, notably molecular dynamics simulations and free energy calculations, enable us to examine the binding affinity of metal ions to amyloid peptides, and to delineate the metal ions’ effects on conformational populations of monomers, dimers, and oligomers of Aβ peptides. We have conducted systematic studies of Zinc-βA peptides interactions. One of important observations from our large-scale simulations reveals that metal ions like Zn can selectively stabilize the classic β-hairpin motif of Aβ peptides, rather than Aβ peptide fibril-like motif. Zinc-bound β-hairpins are only transiently sampled by monomers, however, zinc-bound β-hairpin dimers are relatively stable in aqueous solution, and such stabilization effects may increase as the size of amyloid oligomers increases, leading to off-pathway amyloid aggregates.

322-Pos Board B102
Transient Binding of Zn(II) Redirects Amyloid Beta Peptide from Fibril Formation
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Metal ions have emerged to play a key role in the aggregation process of amyloid β (Aβ) peptide which is closely related to the pathogenesis of Alzheimer’s disease. A detailed understanding of the underlying mechanistic process of peptide-metal interactions has however been difficult to obtain. Applying a combination of NMR relaxation dispersion and fluorescence based aggregation kinetics methods enabled us to investigate quantitatively thermodynamic Aβ-Zn(II) binding features as well as the nucleation mechanism of the aggregation process. Our results reveal that Zn(II) binds to the N-terminus of the peptide which transiently forms a marginally stable fold that encloses the metal ion. Aggregation kinetics studies show that Zn(II) inhibits generation of amyloid fibrils probed by the Thioflavin T fluorescence assay. Kinetic analyses of aggregation half times in the absence and presence of Zn(II) reveal mechanistic details of the Aβ(1-40) aggregation processes. The inhibition impact by Zn(II) on the aggregation process shows an exponential dependence on the Zn(II) ion concentration, and exerts its main effect on the secondary nucleation. Taken together, these findings suggest that transient binding of Zn(II) to the N-terminus of the peptide prevents Aβ(1-40) monomers to participate in secondary nucleation reactions and thereby decelerates Aβ(1-40) self-assembly.

323-Pos Board B103
Site-Specific Dynamics of Aβ1-23 Amyloid Formation and Fibrillar Configuration using an Unnatural Amino Acid
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Amyloid diseases, such as Alzheimer’s and Parkinson’s, are linked to a poorly understood progression of protein misfolding and aggregation that form tissue-selective fibrillar deposits. Elucidation of site-specific dynamics of protein aggregation is crucial for understanding the mechanistic details of protein amyloidogenesis. Hence, using Aβ1-23 as a model molecule, we identified distinct site-specific dynamics over the course of the aggregation and amyloid formation, and defined the structural characteristics of amyloid fibrils by using an unnatural amino acid, p-cyanophenylalanine, as a sensitive fluorescent and Raman probe. Our results reveal distinct local environmental changes of specific side chains during the aggregation of Aβ1-23. In addition, our results suggest that an edge-to-face aromatic interaction between the Phe4 and Phe19 residues from the adjacent in-register β-strands plays a key role in the conformational conversion to form and stabilize β-sheet structure. Moreover, the alignment of the flip-over antiparallel pattern of β-sheet in the amyloid fibrils at the molecular level is proposed on the basis of the PheCN probing results.

324-Pos Board B104
Surface Interactions Restricts Amyloid-β Peptides Movements Resulting in their Rapid Self-Assembly into β Sheets; a Molecular Dynamics Study
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Alzheimer’s Disease (AD) is an important and increasingly prevalent neurodegenerative disease in the United States. According to the amyloid hypothesis, formation of soluble monomers and eventually fibrils composed of neurodegenerative amyloid beta (Aβ) peptides in the brain is the etiological agent of AD. While the macro level causes of AD are somewhat understood, the molecular interactions that lead to the formation of fibrils from individual Aβ peptides are not well understood. It has been shown experimentally that Aβ peptides self assemble to form fibrils on 2D surfaces at much faster rates than they form fibrils in the 3D environment of solutions. However, the molecular mechanism of monomer self-assembly and the effect of amyloid chemistry on the speed and extent of fibril formation remain unknown. We have run long molecular dynamics simulations of Aβ (1-40) monomers on Alkanethiol self-assembled monolayers (SAM) with different functional head groups (-CH3, -OH) and also in bulk water solution. Our simulations showed that SAM-CH3 adsorbs Aβ monomers quickly, restricting their motion and resulting in inter-monomer β-sheet formation in orders of 10s of nanoseconds. The same effect was not observed in bulk solution, even though those simulations ran for a time order of magnitude longer than SAM-monomer simulations. Unlike the SAM-CH3 surface, Aβ-monomer adsorption on SAM-OH surface was weak, leading the monomers to leave the surface and move into the solution. These results indicate the importance of hydrophobic interactions for mediating Aβ self-assembly on the SAM. These simulations help us to better understand the aggregation and toxicity mechanism of Aβ peptides in more physiologically relevant surfaces such as lipid bilayers, which have been shown to interact with amyloids to modulate fibrillation.

325-Pos Board B105
Binding of Aβ Monomer to DMPC Bilayer using Isotopic-Isotropical Replica Exchange Molecular Dynamics
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Interactions between Aβ peptides and cellular membranes have been implicated as a possible cause of the cytotoxicity associated with Alzheimer’s disease; however, little is known about the molecular mechanisms predominating Aβ and