a fraction of vesicles is permeabilized, leading to a rapid loss of fluorescence, while a further slow decrease in the fluorescence signal can be observed. We have been able to attribute the latter observation to alpha-synuclein oligomer enhanced lipid flip-flop. Additional experiments showed that the fraction of negatively charged lipids is decisive for membrane impairment. Taken together these results suggest that alpha-synuclein oligomers disturb the lipid packing, therefore impairing membrane integrity. The loss of lipid asymmetry is a novel mechanism that may contribute to or trigger neuronal death in PD.
Finally, using atomic force microscopy in combination with suspended asymmetric phospholipid bilayers, which closely mimic the plasma membrane, we characterize the membrane properties at binding sites of alpha-synuclein oligomers (e.g. penetration depths, membrane thinning or creation of membrane defects) to get further insights about the impact of the oligomers on lipid bilayer integrity.

**1299-Pos Board B69**
**The Physical Motive of Early Tau Oligomer Formation and Aggregation into Fibrils is Seen through Site-Specific Tracking of Hydration Dynamics**
**Anna Pavlova**, Songi Han.
UCSB, Santa Barbara, CA, USA.
The early onset and increase in severity of Alzheimer disease symptoms are often associated with accumulation and toxic effects of the early tau oligomers, rather than highly structured fibrils. These oligomers recently became an important therapeutic target for Alzheimer treatment, yet their structural and physical characteristics are purely understood due to challenges associated with capturing and studying these transient and dynamic species that form under physiological conditions. While the essential role of biological water in mediating the protein aggregation and toxicity of oligomeric intermediates is undeniable, experimental demonstration has been lagging behind. We have recently developed and utilized a novel ultra-sensitive technique, termed Overhauser Enhanced Dynamic Nuclear Polarization (OE-DNP), for temporal tracking of the biological water dynamics at specific protein sites. This methodology has enabled us to determine the role of solvation water on the surface of tau protein as it undergoes transformations into early oligomers and further formation of amyloid fibrils. We find tau oligomers accumulate and dominate the early stages of in-vitro aggregation before appearance of elongated fibrils. Our data shows that these early intermediates are partially structured, but mostly disordered, where the core of the oligomers is filled with slow water. We find that further aggregation proceeds through structural rearrangements of tau as the early oligomers mature into the structured oligomeric blocks, that anneal in a linear manner to form elongated amyloid fibrils. We also observe that the slow water, homogeneously spanning the core of the early oligomers, shifts its signature toward the more polar and charged amino-acid regions of tau as mature fibrils are formed. Thus the loosely bound water plays an immediate role in hydrating the early oligomeric globule of tau and further self-assembly.

**1300-Pos Board B70**
**A Mobile Precursor Determines Amyloid-β Peptide Fibril Formation at the Liquid-Solid Interface**
**Xiaoyang Zhu**, Lei Shen.
University of Texas, Austin, TX, USA.
The aggregation of peptides into amyloid fibrils plays a crucial role in various neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. While extensive work has been devoted to the study of solution fibril formation in vitro, the situation differs fundamentally from that in a living cell where the effect of macromolecular crowding provides a very large surface area surrounding peptide and protein molecules. Here we study the dynamics of fibril formation of Alzheimer's amyloid-β peptide, Aβ42, on nanoscopic patterned block copolymer films and self-assembly monolayer modified surfaces with different hydrophobicities. We report the discovery of that weakly adsorbed peptides with two-dimensional diffusivity is a critifcal precursor to fibril growth. A balance between mobility and transient concentration of the peptide precursor state determines the kinetics of fibril formation.

**1301-Pos Board B71**
**Can We Prevent the Beta Amyloid Peptide, the Characteristic Peptide Found in Alzheimer's Disease, from Becoming Neurotoxic?**
**Sandra Chimon Peszek**.
DePaul University, Chicago, IL, USA.
At the early-stages of amyloid research, amyloid fibrils were believed to formed from direct conversions of unstructured monomeric amyloid beta peptide, Aβ, the amyloid protein which characterizes Alzheimer's disease (AD). However, recent structural and kinetic results describe a model for the molecular kinetic level. First, monomeric Aβ molecules in random coil conformations self- assemble into a non-beta sheet intermediate, $I_{N\beta}$, which is an aggregate with no amyloid-like β-sheet structures. Then, $I_{N\beta}$ assembles into a β-sheet intermediate, $I_{\beta}$, a spherical intermediate that is surrounded by well ordered β-sheets.
Great interest is focused on the ''hair-pin'' region, between residues 16 and 21 also known as the ''KLVFFA'' region. This region has been shown to contribute to both the secondary and tertiary structure of the peptide; containing the transition portion of the peptide which moves from extracellular to transmembrane regions; and it contains the salt bridge from residue D23 to K28. In spite of the growing importance of these intermediates as a therapeutic target for AD, there is little structural information about the intermediates of the shorter region between resides 22 and 35, Aβ(22-35), because of their non-crystalline and unstable nature. The primary objective of this study is to determine the structure of the hair-pin region of these interval sites without the presence of the ''KLVFFA'' residues.
The examination of the intermediate species for various single point mutations will be explained because not only do they have higher neurotoxicity than the wild type, but also promote the formation of subfibrilliar intermediates. The obtained structures will be compared to those of the WT Aβ intermediates and fibrils, based on a hypothesis that the molecular level structural change caused by the mutation also change the kinetics of misfolding.

## Protein Assemblies

**1302-Pos Board B72**
**Application of Split-GFP System in Biophysical Research and in Cell Biology**
**Keunbong Do**, Steven G. Boxer.
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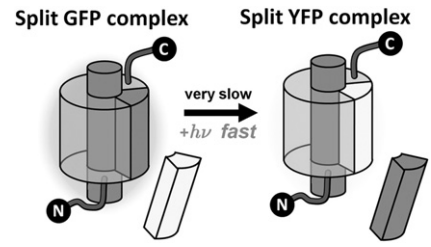
Truncated green fluorescent protein (GFP) that is refolded after removing the 10th beta strand can readily bind to a synthetic strand to fully recover the absorbance and fluorescence of the whole protein. 1) The spontaneous reassembly allows rigorous experimental determination of thermodynamic and kinetic parameters of the split system including the equilibrium constant and the association/dissociation rates, which enables residue-specific analysis of peptide-protein interactions. 2) Furthermore, the split-GFP scheme, with its complete synthetic control over the split peptide, is ideal to study the biophysics of color tuning in fluorescent proteins by gradually changing the local solvation environment of the chromophore to selectively stabilize or destabilize a certain resonance contributor that leads to different degree of color shift. 3) The dissociation rate of the noncovalently-bound strand is observed through the strand exchange process that is accompanied by a color change, and surprisingly, the rate is greatly enhanced by light irradiation. This peptide-protein photodissociation is a very unusual phenomenon and can potentially be useful for introducing spatially and temporally well-defined perturbations to biological systems as a genetically encoded caged protein.




**1303-Pos Board B73**
**Mechanisms of Molecular Assembly for the PDZ and LIM Domains in the Adaptor Protein LMO7**
Justin C. Baker, Jun Li, Bryce C. Hilburn, Cassandra N. Meadows, Pradeep R. Rajasekaran, Janelle M. Owens, Yuanxiufu Cao, Fernando F. Cuadrado, Shannon C. Banning, **Gabriela C. Perez-Alvarado**.
Southern Illinois University, Carbondale, IL, USA.
The cadherins and nectin transmembrane proteins are connected indirectly to each other and to the cytoskeleton through their interactions with cytoplasmic proteins. The adaptor protein LIM domain only-7 (LMO7) has a role in stabilizing the communication between the cadherin and nectin associated complexes in the adherens junctions through its association with alpha-actinin and afadin.[1] LMO7 is also associated with the regulation of transcriptional activity of myocardin-related transcription factors.[2] In the nucleus, LMO7 interacts at the nuclear envelope with emerin, and regulates the transcription of genes important for heart, muscle and retina formation.[3] LMO7 interacts with afadin through a C-terminal LIM domain and interacts with alpha-actinin through a region N-terminal to a PDZ domain. We have characterized these domains by SDS-PAGE electrophoresis, analytical reverse-phase HPLC, mass spectrometry and NMR spectroscopy. The oligomerization states of these