2372-Pos
Modeling Ion-Permeable β-Amyloid (Aβ) Barrels in the Lipid Bilayer
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We modeled Alzheimer's Aβ ion channel in a β-barrel structure embedded in the lipid bilayer using molecular dynamics (MD) simulations. The β-barrel is characterized by the inclination angle of the β-strands with respect to the pore axis. Our Aβ barrels have two β-strand layers, and each layer has 16 or 20 β-strands that are tilted by ~37° relative to the membrane normal. In the Aβ barrel simulations, we employed the U-shaped *β-strand-turn-β-strand* peptides using currently available NMR-based coordinates of non-amyloidogenic p3 ($Aβ_{17-42}$) and $Aβ_{9-42}$ peptides. The non-amyloidogenic Aβs resulting from α-secretase and BACE cleavage are often found in amyloid plaques, but the biophysical properties and functional role of these non-amyloidogenic peptides are not understood. In good agreement with previously modeled Aβ ion channels that aligned the β-strands parallel to the pore axis, all Aβ barrels break into loosely-associated mobile β-sheet subunits, verifying that membranes do not support intact β-sheet conformations. We obtain ion-permeable pore-like Aβ barrels whose subunit morphologies and shapes are consistent with AFM images, suggesting that the Aβ barrels are polymorphic conformations of Aβ ion channels in the membrane. The emerging picture from our large-scale simulations is that toxic ion channels formed by β-sheets spontaneously break into loosely-interacting dynamic units which associate and dissociate leading to toxic ionic flux. Funded by NCI Contract HHSN261200800001E (RN) and NIH (NIA) extramural program (RL).

2373-Pos
The Sevi Precursor Peptide $PAP_{248-286}$, a Dramatic Enhancer of HIV Infectivity, Promotes Lipid Aggregation and Fusion
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Amyloid fibers formed from a peptide ubiquitous in human seminal fluid (SEVI) have been found to dramatically enhance the infectivity of the HIV virus (3-5 orders of magnitude by some measures). To complement these previous *in vivo* studies we have performed *in vitro* assays of $PAP_{248-286}$, the most active precursor to SEVI, and other polycationic polymers to investigate the physical mechanisms by which the $PAP_{248-286}$ promotes the interaction with lipid bilayers. At acidic, but not at neutral, pH freshly dissolved $PAP_{248-286}$ catalyzes the formation of large lipid flocculates in a variety of membrane compositions which may be linked to the promotion of convective transport in the vaginal environment rather transport by a random Brownian motion. Furthermore, $PAP_{248-286}$ is itself fusiogenic and weakens the integrity of the membrane in such a way that may promote fusion by the HIV gp41 protein. A partially α-helical conformation of $PAP_{248-286}$, lying parallel to the membrane surface, is implicated in promoting bridging interactions between membranes by the screening of the electrostatic repulsion that occurs when two membranes are brought into close contact. This suggests non-specific binding of monomeric or small non-fibrillar oligomeric forms of SEVI to lipid membranes may be an additional mechanism by which SEVI enhances the infectivity of the HIV virus. In addition, we show that the curli protein, a strongly amyloidogenic protein used by bacteria to adhere to surfaces, seeds SEVI amyloid formation, indicating a possible role for bacterial infection in HIV transmission.

2374-Pos
Fluorescent Probes of Membrane-Bound α-Synuclein: Insights into the Role of Membranes in Aggregation
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Identifying pathways of α-synuclein misfolding are of great importance because the presence of aggregated α-syn in the brain is a hallmark of Parkinson's disease (PD). Due to the proximity of α-syn to synaptic organelles *in vivo*, the role of phospholipid membranes in modulating α-syn oligomer and aggregate formation is of particular interest. To probe how membranes affect α-syn conformation, we use circular dichroism spectroscopy as a secondary structural probe in conjunction with steady-state and time-resolved fluorescence techniques. Specifically, we employ single Trp-containing and dye labeled (1,5-IAEDANS, IANBD amide) α-syn variants to report on residue-specific environments and intermolecular contacts *via* changes in intrinsic fluorescence and measurements of Förster energy transfer between proteins modified with either donor or acceptor fluorophores. Using these methods, we aim to characterize the protein-protein and protein-membrane interactions that promote protein aggregation.

2375-Pos
Interaction Het-S Prion / Membrane to get a Better Insight of Amyloid Toxicity
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Many neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, spongiform encephalopathies or amyloidopathies, correlate with amyloid formation. Several hypotheses have been proposed to explain the toxicity of amyloids: the existence of "intermediates", from oligomers to protofilaments and/or the ability of amyloids to form pores which would destabilize the membranes but also change the cellular homeostasis.
Our work aims to characterize the interaction amyloid/membrane in relation with the amyloid toxicity. We have chosen the non toxic Prion Forming Domain 218-289 of Het-s from the filamentous fungi *Podospora anserina*, as model. By random PCR mutagenesis we generated a toxic mutant in yeast (M8) that interacts *in vivo* with membrane vesicles. We have demonstrated that *in vitro*, M8 forms very unusual short amyloid fibers contrary to WT, which polymerizes as long fibers. Furthermore, ATR spectra have shown that M8 toxic mutant is essentially assembled into mixed parallel and anti-parallel β-sheets whereas WT displays a predominant parallel organization. Monolayers of phospholipids at the air-water interface were used as membrane model to investigate the interaction with non toxic amyloid (WT) and toxic amyloid (M8). Characterization of the molecular interactions between amyloid (WT and M8) and different lipid monolayers (DOPG, DOPC, DOPS DOPE and DOPI), at the air-water interface were performed by polarization-modulated infrared reflection absorption spectroscopy, Brewster angle microscopy and ellipsometry. BAM images reveal a high selectivity of the M8 toxic mutant for the anionic phospholipid monolayers (DOPG, DOPI and DOPS). In comparison, WT presents less evidences of interaction with the tested phospholipid monolayers. The secondary structure of amyloids (M8 or WT) interacting with a phospholipid monolayer is mainly made of anti-parallel β-sheets (observed by PMIRRAS) but the orientation of the β-sheet differs between toxic and non toxic mutant.

2376-Pos
Effects of Fatty Acids and Phospholipids on Amyloid-β (Aβ) Peptide Aggregation
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The aggregates of Aβ found in brains of Alzheimer's patients are strongly believed to be the cause for neuronal death and cognitive decline. Among the different forms of Aβ aggregates, smaller aggregates called 'soluble oligomers' are increasingly believed to be the primary neurotoxic species responsible for early synaptic dysfunction. Since it is well known that the Aβ aggregation is a nucleation dependant process, it is widely believed that the toxic oligomers are intermediates to fibril formation, or what we call the 'on-pathway' products. Although it is true that there may be toxic oligomers along the fibril formation pathway, it is not obligatory that all toxic oligomers must be 'on-pathway' intermediates. It is important to understand the pathways because if the oligomers are 'off-pathway' products, their half-life can be significantly longer than the 'on-pathway' ones that may result in prolonged toxicity to the neuronal cells. Here, we test this hypothesis in the presence of saturated fatty acids and phosphatidylglycerol lipids by varying their carbon chain lengths. We observed that Aβ aggregation can adopt more than one pathway, and the pathway is dictated by Aβ-fatty acid/lipid ratio. Oligomers generated from lipids and fatty acids were isolated and characterized using thioflavin-T (ThT) fluorescence, immunoblotting and atomic force microscopy (AFM). These data are presented and discussed.