inhibitors are sought to depopulate these transient intermediates and thus diminish their toxicity.
We have identified ezrin, a microtubule-associated protein that belongs to the ezrin radixin moesin family, as a potent inhibitor of aS aggregation at sub-stoichiometric concentrations. Evidence showed that binding of ezrin to aS causes conformational rearrangements and sequesters the protein in amorphous aggregates thus preventing fibril formation and rescuing toxicity in primary neurons and yeast cells, thereby acting as a non-conventional chaperone.
We have produced and characterized alternate aS oligomers by size exclusion chromatography, circular dichroism, TEM, dot blotting and ThT fluorescence. One group of oligomers is associated with a higher β-sheet content and can be internalized in human neuronal model cells. Following treatment with these oligomers prepared with incorporation of fluorescently labeled aS, we observed their uptake into human SH-EP cells. The other group of oligomers is A11 antibody reactive, mainly unstructured and result in significant cell death.
We hypothesize that binding of ezrin may alter the biophysical, biological characteristics and aggregation properties of aS oligomers. Current efforts are concentrating on investigating the interaction of ezrin with these alternate oligomeric species.

2645-Pos Board B75
Electrostatics Promotes Molecular Crowding and Selects the Fibrillation Pathway in Fibril-Forming Protein Solutions
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The role of intermolecular interaction in fibril-forming protein solutions and its relation with molecular conformation is a crucial aspect for the control and inhibition of amyloid structures. Here, we use optical spectroscopies, x-ray and light scattering to study the fibril formation and the protein-protein interactions of different model proteins. In the case of lysozyme, the monomeric solution is kept in a thermodynamically metastable state by strong electrostatic repulsion, even in denaturing conditions. At high temperature proteins are driven out of metastability through conformational sub-states, which are kinetically populated and experience lower activation energy for fibril formation. This explains how electrostatic repulsion may act as a gatekeeper in selecting the appropriate pathway to fibrillation. The protein density fluctuations are measured by light scattering and exhibit a regime dominated by protein concentration. Highly charged colloidal solutions are characterized by damped density fluctuations and mimics locally a crowded environment. Further, by photon correlation spectroscopy (PCS) and fluorescence correlation spectroscopy (FCS) we measure protein collective and self-diffusion. Both PCS and FCS evidence how an increase of concentration hinders protein self-diffusion, likely due to both hydrodynamics and crowding effect.

2646-Pos Board B76
Role of Side-Chains in Forming Peptide Aggregates and Fibrils. IR and VCD Spectroscopic Studies. Theory and Experiment
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Formation of peptide aggregates and fibrils must ultimately be driven by interactions of side chains. Typically the lower solubility of hydrophobic sequences favors desolvation by formation of interchain contacts which are then stabilized further by various means. One way is development of ordered interdigitization of the side chains, which is particularly evident in poly-glutamic acid at low pH. The Glu side chain -COOH groups form bifurcated H-bonds with the amide C=O groups that are cross-strand H-bonded in what is termed a β2 sheet. This extra H-bond interaction leads to large shifts of the amide I vibrational frequencies and formation of stacked arrays of β-sheets. In other systems, hydrophobic interactions can results in similar stacking interactions. Vibrational spectroscopic methods have been used to establish β-sheet structures underlying many of these and isotope labeling has been used to differentiate parallel vs. antiparallel forms. Our DFT-based calculational model of β-sheet vibrational spectra was originally based on Ala-oligopeptides, forming normal β1 sheets, and focused on IR, VCD, and Raman spectra for isolated and stacked sheet structures, with variations in structure, alignment of strands, isotope labeling, twisting of single sheets and relative rotation of multiple sheets in the stack. In particular, our analyses of the spectra of Glu_{10} based peptides that form β2 sheets, are shown to be antiparallel but out of register by one residue, most likely in a step-wise pattern. Alternate $(Glu\text{-}Aaa)_n$ variants, where Aaa is a hydrophobic residue, can also show this pattern of intersheet side-chain intercalation. We extended these calculations by computing IR and VCD for Glu_n structures along MD trajectories that show evidence of sidechain ordering in qualitative agreement with experiment.

Protein Assemblies II

2647-Pos Board B77
Assembly of the RIG-I 2CARD Signaling Complex
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The innate immune system of mammals utilizes pattern-recognition receptors to differentiate between pathogen associated molecular patterns and host molecules. The retinoic acid inducible-gene-I (RIG-I) like receptors (RLRs) bind viral dsRNAs, triggering an innate immune response. RLRs contain two N-terminal caspase activation and recruitment domains (2CARD), a DExD/H-box helicase-like domain, and a C-terminal regulatory domain. In the absence of RNA, the 2CARD region is sequestered by the helicase domains in an autoinhibited state. Upon binding to the 5′-ends of activating viral dsRNAs a conformational change exposes 2CARD. The CARD domains then interact with MAVS, a mitochondrial outer membrane protein, leading to downstream signaling and interferon expression. Signaling is modulated by K63-linked polyubiquitin chains (polyUb). It has been reported that polyUb binding to 2CARD induces formation of 2CARD tetramer. However, the nature of the assembly process is not well understood and we are investigating the thermodynamic linkage between polyUb binding and 2CARD oligomerization using sedimentation velocity analytical ultracentrifugation. 2CARD alone exists as a monomer; however, in the presence of polyUb several high molecular weight complexes are formed. The assembly process is dependent on salt, polyUb concentration, and polyUb chain length.

2648-Pos Board B78
Characterization of the MDA5 2CARD Signaling Complex
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The mammalian innate immune system is the first line of defense against invading pathogens. The RIG-I like receptors (RLRs), RIG-I and MDA5 are critical for host recognition of viral RNAs. These receptors contain a helicase-like core domain, two caspase activation and recruitment domains at the N-terminus (2CARD) and a C-terminal regulatory domain. In RIG-I, RNA binding leads to exposure of the 2CARD domain and 2CARD oligomerization. The 2CARD signaling domain associates with the CARD domain of MAVS, a mitochondrial outer membrane protein, leading to oligomerization of MAVS, downstream signaling and interferon induction. K63-linked polyubiquitin chains (polyUb) play a role in RLR signaling by interacting with the 2CARD domain. PolyUb binding to RIG-I 2CARD induces formation of 2CARD tetramer. However, the nature of the signaling complex of MDA5 and the role of polyUb binding in MDA5 activation are not well understood. We have used sedimentation velocity analytical ultracentrifugation to characterize MDA5 2CARD oligomerization in absence and presence of polyUb. MDA5 2CARD forms a broad distribution of oligomers. The size distribution is concentration dependent, indicating reversibility. MDA5 2CARD forms complexes with polyUb, and complex formation is highly dependent on NaCl concentration. Multisignal sedimentation velocity experiments will probe the nature of the polyUb-2CARD complexes.

2649-Pos Board B79
Detailed Mechanism of Rapid Amyloid Fibril Self-Assembly Due to Surface Diffusion
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Surface-mediated fibrillization has been considered as an alternative pathway to form amyloid fibrils under physiological conditions. In order to develop new probes and therapies for neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, it's important to explore the detailed mechanism of the surface effects. However, the surface morphologies change with time and are hard to image without staining or labeling, which may alter the process of self-assembly. In recent work, we demonstrated a label-free procedure via controlling incubation time and the drying process to monitor the kinetics of fibril growth on surfaces across many length scales. We propose an anisotropic growth model to fit the time-evolution of morphologies of amyloid fibrils forming near a surface as measured using Atomic force microscopy (AFM). Two important variables, the deposition rate and the two-dimensional diffusion coefficient, can be used as fitting variables and can be characterized by the combination of experimental data and the theoretical approach presented here.