amyloidogenesis leads to an aggregation free energy landscape. We define the roles of and propose a classification scheme for different oligomeric species based on their location on the aggregation free energy landscape. We relate the different types of oligomers to the amyloid cascade hypothesis and the toxic oligomer hypothesis for amyloid-related diseases. We discuss existing kinetic mechanisms in terms of the different types of oligomers. We provide a possible resolution to the toxic oligomer-amyloid coincidence.

2189-Plat
Amyloid-Like Cross-Beta Structure Polymorphism: An Energetic Point of View
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Amyloid fibrils are highly ordered protein aggregates involved in numerous pathological conditions, including neurodegenerative diseases. A single mature unbranched fibril is formed from at least several interacting protofilaments, which share a common structural feature - a cross-β spine, in which β-sheets are aligned with the fibril's main axis. It has been observed that amyloid fibrils may exist with different morphologies and twists depending on their mode of preparation, even within a single sample. However, the precise etiology and pathological implications of such twist-polymorphism are unclear. We present here the results of a series of molecular dynamics (MD) simulations of a protofilament model formed by 40 copies of the GNNQQNY peptide fragment of the yeast prion protein, Sup 35. The planar protofilament observed in the crystal structure displays no free energy barrier against twisting in the absence of crystal packing interactions. Umbrella sampling simulations, in which the twist between consecutive peptides is used to control the overall protofilament's twist, confirm that the free energy minimum is observed at a 7.5 degree left-handed twist conformation. There is little apparent free energy penalty derived from twisting the cross-β structure in the range of −12 to 0 degrees. Moreover, the twist of the cross-β structure is enthalpy-driven, and while the backbone favors the straight form of the protofilament, side chains favor the twisted form. We propose that the twist of a protofilament might easily adapt to external stresses such as interactions with other protofilaments. This hypothesis is further illustrated by our characterization of different morphologies of protofilament assemblies composed of one to four protofilaments. Taken together the data support an energetic basis for the different twist-morphology states observed in amyloid fibrils.

2190-Plat
Dissecting the Membrane Dynamics of Amyloid Oligomers at a Single Molecule Level
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Fibrillar deposits of proteins are the hallmark of amyloid diseases, amongst which Alzheimer's disease stands out as the most widespread neurodegenerative pathology of the brain. Neuronal dysfunction is currently attributed to the interaction of A-beta oligomers with the plasma membrane. Several scenarios have been proposed, but the mechanisms of binding of the oligomers to the cell membrane and their subsequent toxicity is still unclear. Distinct results indicate that oligomers may insert non-specifically into the lipid bilayer, or bind to specific targets, such as post-synaptic structures or gangliosides characteristic of lipid rafts. In general, these studies have investigated the averaged features of an ensemble of molecules.
Here, we have been able to successfully monitor the mobility of single A-beta oligomers on the plasmamembrane of living neuroblastoma cells. Preformed oligomers were incubated with cells and subsequently labelled with monoclonal primary antibodies and secondary Fab fragments coupled to quantum dots (QDs). Single QDs bound to the oligomers were then tracked.
The analysis of the trajectories reveals that most of the oligomers show a highly confined membrane mobility, suggesting a potential involvement of the cytoskeleton, while some diffuse laterally following a free Brownian motion. Strikingly, we found that other amyloid aggregates sharing a similar conformational structure but composed of different proteins (amylin and prion Sup35) display comparable dynamics. Moreover, we discovered that the presence of amyloid aggregates decreases dramatically the membrane diffusion of GM1 gangliosides labelled with biotinilated cholera toxin coupled to streptavidin-QDs.
Overall, these results enable a better understanding of the basic mechanisms underlying Alzheimer's diseases and other amyloid pathologies.

2191-Plat
Physical Properties of Yeast Prion Proteins Studied with Optical Tweezers
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Formation of amyloid fibers plays a vital role in both natural biological processes and neurodegenerative disease. Recently, amyloid formation has been shown to be a general property of proteins and peptides. Their impressive mechanical properties, which are comparable to spider silk, combined with their ease of assembly in synthetic preparations make amyloid fibers particularly suited for nanomaterials applications, including as templates for conducting nanowire formation, as scaffolds for cell growth, and as functionalized biosensors. Prion proteins are a special class of amyloid fiber forming proteins which are self-templating and thereby transmissible as disease vectors. This work combines optical tweezers force spectroscopy with fluorescence imaging to study the physical properties of amyloid fibers formed from polymorphic variants of a 253 amino acid N-terminal fragment (NM) of the yeast prion protein Sup35. Experiments revealed that fibers associated with a ''weak'' NM prion strain have an approximately 2-fold larger bending stiffness than those associated with a ''strong'' NM prion strain. We further subjected NM fibers to multiple cycles of forces up to 250 pN resulting in unfolding of individual prion subdomains and rupture of intermolecular interactions. Our results have implications for the physical basis of prion strain diversity and give important insights into the underlying structure of Sup35 prions.

2192-Plat
Probing the Conformational Ensemble of Polyglutamine During the Initial Stages of Aggregation
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Nine different neurodegenerative diseases, including Huntington's disease, are associated with the aggregation of proteins whose only commonality is a repeating stretch of glutamine. Experiments and computer simulations have demonstrated that monomeric forms of polyglutamine molecules sample heterogeneous sets of collapsed structures in water. Molecular simulations have predicted that these molecules spontaneously associate at conditions approaching those of typical in vitro experiments for chains of length N>15. Moreover, the spontaneity of these homotypic associations increases with increasing chain length. These results suggest that polyglutamine aggregation is unlikely to follow a homogeneous nucleation mechanism, which is currently the most widely accepted mechanism by which polyglutamine aggregation is thought to occur. In this work, we test these predictions using both steady state and time resolved Förster Resonance Energy Transfer (FRET). Hopefully this work, along with the simulation results, will allow a better understanding of how monomeric polyglutamine assembles into soluble oligomers and, eventually, insoluble aggregates.

2193-Plat
Changing the Kinetics of Amyloid Beta Plaques Formation: Implications for Alzheimer's Disease Immunotherapy
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Clear evidence exists linking the presence of neuritic amyloid beta (Aß) peptides plaques with the brain's tissue deterioration and cognitive impairment in patients with Alzheimer's disease (AD). Removing these plaques results a logic approach to treat patients with AD. Understanding the plaque formation mechanisms is key to developing strategies to remove them. In previous studies our group has investigated the plaque formation process using attenuated total reflection Fourier infrared (ATR-FTIR) spectroscopy and atomic force microscopy (AFM), observing how different Aß peptide aggregates formed and under what kinetic conditions they assembling into mature fibrils. We have been able to determine the changes in the secondary structure of the peptide molecules during this process. In this work, we combined the same analytical techniques to investigate the use of different anti-Aß monoclonal antibodies to analyze the process of destabilization and prevention of AD plaque formation. We compared the changes of kinetic rates of fibrillization when the peptides were incubated with different antibodies from the early stage of aggregation, at pH 7.4 and 37°C. The molar ratio of antibodies to peptide used was 1:1000. We found that some antibodies considerable decrease the formation of parallel beta sheets structures increasing the formation of alpha helix structures or unordered,