with this model, we found previously that isolated htt^{NT} peptide added in trans inhibits the aggregation of htt NTF peptides, presumably by co-assembling with htt NTFs into mixed oligomers with decreased local polyQ concentrations. In a further test of this model, we found that the aggregation inhibitory activities of a set of ten scrambled htt^{NT} sequences correlate with their α-helical potential. We have now selected three of these scrambled sequences - (a) one that inhibits htt NTF aggregation ($_{sc}htt^{NT}{}_{ASSQ}$), (b) one that does not inhibit ($_{sc}htt^{NT}{}_{FAKF}$), and (c) one that readily forms amyloid fibrils itself (unlike htt^{NT} or the other sequences) but does not inhibit ($_{sc}htt^{NT}{}_{SAFM}$) - and used them in place of htt^{NT} to make synthetic analogs of htt NTF by adding a $Q_{37}P_{10}K_2$ sequence. Consistent with expectations, we found that the peptide with the inhibitory, high α-helical potential, leader sequence ($_{sc}htt^{NT}{}_{ASSQ}$) exhibits an aggregation profile similar to that of the htt^{NT}-containing control peptide. In contrast, despite their identical amino acid compositions, htt NTF analogs containing the other two scrambled sequences (low aggregation inhibition, low α-helix) exhibit aggregation behavior more typical of simple polyQ sequences, hence deriving no kinetic benefit from their N-terminal sequences. Our results support a strong role for α-helix formation within htt^{NT} in greatly enhancing the kinetics of formation of the polyQ core of htt NTF amyloid.

1289-Pos Board B59

Water Penetration into a Dry Amyloid Fibril

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Amyloid formation is a key feature of Alzheimer's disease. Amyloid is composed of 39-42 residue proteins that have aggregated into fibrils. Experimental and theoretical studies of amyloid fibril structure have suggested a model in which two different parallel in-register beta sheets pack against each other with a dry interface. However, recent 2D-IR results have detected water molecules within a mature folded fibril. To explore this discrepancy, we created a molecular dynamics simulation of a dry amyloid fibril in water. We observed that water enters the space between beta sheets through hydrogen bonding interactions with the side chain or main chain of Asp23, and the main chain carbonyl group of Gly25. The involvement of main chain carbonyl groups in water transport down the interior of an amyloid fibril suggests that individual hydrogen bonds comprising the beta sheet may form and transiently unform, facilitating events like water transport while maintaining overall fibril structure.

1290-Pos Board B60

Observation of Secondary Nucleation on Specific Aβ 42 Fibril Type

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Amyloid-β (Aβ) fibril formation is the pivotal phenomenon in Alzheimer's disease (AD). However, the understanding of amyloid fibril mechanism has been hindered by its heterogeneous morphology. Further, recent studies point out the existence of new growth sites on pre-existing fibrils, called secondary nucleation, but there is no report of secondary nucleation for Aβ 42. Here we present a detailed inter-conversion scheme of Aβ42 from the study of Aβ fibrillogenesis by means of electron microscopy and atomic force microscopy. We indentified that the secondary nucleation events occur more favorably on specific fibril type during monomer fibrillization. Further, we proved the stability of the most homogeneous and stable Aβ42 fibril type and analyzed the structural elastic properties by using the statistical theory of semi-flexible polymer. The result suggests that lateral association mechanism gives the most structural stability to certain fibril type with a twisted ribbon structure. The extracted elastic modulus of fibrils is ~ 1.4 GPa on mica and highly ordered pyrolytic graphite. We suggest that elucidation of secondary nucleation events and elastic modulus value will help progress in our understanding of the aggregation mechanism of Aβ 42 fibrillogenesis in AD.

1291-Pos Board B61

Carboxymethylation of Cysteines Impedes Aggregation of Hen Lysozyme in Alkaline pH

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In our previous work, we have shown that hen lysozyme spontaneously forms globular and fibrillar aggregates which are stabilized later through formation of intermolecular disulphide bond in pH 12.2. Therefore, focus of the present work was on suppression of HEWL aggregation by carboxymethylation of free -SH groups with iodoacetamide that inhibits further formation of disulphide bonds. Changes in structure and dynamics of aggregates were monitored using various biophysical techniques like fluorescence spectroscopy and atomic force microscopy (AFM). Thioflavin T (ThT) fluorescence showed that in comparison to free thiol containing HEWL control, carboxymethylated HEWL was unable to form fibrils. However presence of moderate ThT intensity in modified HEWL indicated presence of oligomers. Increased fluorescence intensity and marginal red shift of ANS spectra in modified HEWL compared to unblocked control revealed that modified HEWL possessed exposed hydrophobic residues compared to free -SH containing HEWL. Fluorescence anisotropy of carboxymethylated dansyl-labeled HEWL were significantly lower compared to control. The fluorescence anisotropy decay kinetics revealed that carboxymethylated HEWL-dansyl conjugates possessed shorter global rotational correlation time (~11.7 ns) compare to control (~22 ns) after 30 hours of incubation in pH 12.2 at room temperature. Taken together, these investigations suggest that blocking thiol groups in HEWL suppresses formation of amyloid fibrils and bigger aggregates but not oligomers, in pH 12.2 at room temperature. This work can be helpful for food industries in making aggregate-free lysozyme preservatives in future.

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1292-Pos Board B62

Investigating the Factors Affecting the Aggregation of Alpha-Synuclein using Single Molecule Fluorescence and Fast Flow Microfluidics

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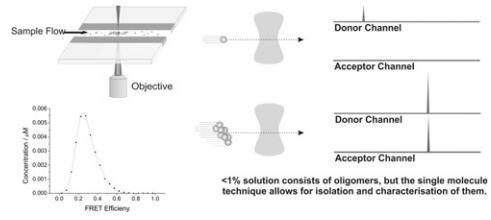
The conversion of α-synuclein (αs) into oligomeric and fibrillar species and its deposition into Lewy bodies is the pathological hallmark of Parkinson's disease (PD). It is therefore of great importance to understand the mechanism of αs aggregation and its relationship to PD pathogenesis. We use single molecule fluorescence techniques with fast flow microfluidics to follow the early stages of this process *in vitro*. The methodology is based on the detection of fluorescent bursts from red or blue fluorophore-tagged αs species as they flow through a blue confocal laser volume. Förster Resonance Energy Transfer (FRET) occurs between blue and red fluorescently-tagged αs within the oligomers, giving rise to a signal in both the acceptor and donor channels. As only oligomers generate a coincident signal, they can be isolated from a solution which is >99% monomer, allowing their size and structure to be determined. By taking regular time-points, the kinetics of the aggregation can be deduced.

This technique has allowed us to gain a unique insight into the effects of nanobodies and molecular chaperones on the aggregation of both the wild-type and pathological mutants of αs.



1293-Pos Board B63

Inhibition of Lysozyme Amyloidogenesis by Osmolytes

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Report of systemic amyloidosis by point mutants of human lysozyme paved way towards more systematic studies into general principles of amyloidosis and subsequently development of broad spectrum drugs rather than individual searches for various amyloidogenic diseases. Osmolytes are well-known protein stabilizers and known to be found in incredibly large quantities in animals living under extreme conditions. The fact that they occur in huge quantities in deep-sea animals gives hope that they alone or as cocktails can be administered to terminally ill patients (regardless of the type of amyloid disease) after preliminary safety trials. However there are not many studies in this direction and they are on different proteins thus making comparison difficult. Hen lysozyme forms amyloid under various conditions. So far we have employed alkaline and acidic conditions. We have utilized residual enzymatic activity (REA) to quantify extent of folded protein, fluorescence steady state anisotropy (r_{ss}) to quantify mean oligomer size and fluorescence based Thioflavin-T (Th-T) assay kinetics to quantify amyloid content. We have studied many osmolytes in parallel,viz, Arginine, Betaine, Trehalose, TMAO, Taurine, Ectoine, Putrescine, Spermidine & Spermine. Since majority of data is kinetic in nature, an attempt is being made to tabulate all this data in the form of a mathematical matrix so as to facilitate concise presentation and facile comparison. RESULTS (with alkaline condition): (a) Different osmolytes affect different steps with their own concentration dependence profile. (b) Ectoine and Polyamines are effective