1311-Pos
Molecular Basis for the Catalysis of B-Sheet Formation by Water-Nonpolar Interfaces
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Recent studies suggest that the toxicity of several neurodegenerative pathologies such as Alzheimer's and Parkinson's diseases involves the interaction of oligomeric aggregates of amyloidogenic proteins with the neuronal membrane. We examine the physical and structural basis of peptide adsorption and aggregation in a model membrane using molecular dynamics simulations. Blocked amphipathic octapeptides with simple, repetitive sequences, $(Gly\text{-}Ala)_4$ and $(Gly\text{-}Val)_4$, are used as models of β-sheet-forming polypeptide chains found in the core of amyloid fibrils. Placed in aqueous solution in the presence of an n-octane phase mimicking the nonpolar core of lipid membranes, the peptides spontaneously partition at the octane-water interface. The adsorption of nonpolar sidechains displaces the conformational equilibrium of the peptides from a heterogeneous ensemble characterized by a high degree of structural disorder towards a more ordered ensemble favoring β-hairpins and elongated β-strands. Once adsorbed at the interface, peptides spontaneously aggregate and rapidly evolve β-sheet structure on a 10-to100-ns time scale, while aggregates of the same peptides in water remain amorphous. The catalysis of β-sheet formation at the water-membrane interface results from the combination of the hydrophobic effect and of reduced conformational entropy of the polypeptide chain. While the former drives interfacial partition and displaces the conformational equilibrium of monomeric peptides, the planar interface further facilitates β-sheet organization by increasing peptide concentration and reducing the dimensionality of self-assembly from three to two dimensions. These findings have general implications to the formation of β-sheets on the surface of globular proteins and to amyloid self-organization on the surface of biological membranes.

1312-Pos
Oligomerisation, Fibrillation and Activity of Hen Lysozyme in Alkaline Medium: A Concentration Dependent Investigation
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Oligomerisation and fibrillation are hallmarks of protein misfolding diseases like Alzheimer's, Prion, Parkinson's, systemic amyloidosis and so on. Hen egg-white lysozyme (HEWL) which spontaneously aggregates at pH 12.2 under room temperature as shown by us previously is an excellent model protein for aggregation studies. The present work was focussed on investigating the role of protein monomer concentration on the size and morphology of aggregates. For this purpose we employed HEWL concentrations ranging from 120 μM to 300 nM. Our findings reveal that A) FRET efficiency between dansyl labelled HEWL and dabcyl labelled HEWL in the aggregate, monitored over 12 hours was inversely proportional to HEWL monomer concentration B) Size exclusion chromatography after 12 hours showed that while all aggregates of 50 & 120 uM HEWL eluted much later, a small population of aggregates from 10 uM HEWL eluted early through the void volume C) Binding of ANS revealed fluorescence spectra that were gradually blue shifted and more intense, over 12h, in the following order with aggregates of 120 > 50 > 3 uM, while for 300 nM, these spectra were fairly invariant in emission wavelength and intensity over 12 hours. D) HEWL enzymatic activity decreased almost uniformly with time in alkaline pH for all concentrations suggesting a concentration independent early unfolding step E) AFM images showed extensive fibrils in 3 & 0.3 μM HEWL within 12 hours but predominantly large globular aggregates with 120 μM and 50 μM HEWL samples in the same time period. The above results clearly suggest that size and morphology of HEWL aggregates at alkaline pH are critically dependent on the initial monomer concentration.

1313-Pos
The Addition of an Osmolyte, Trimethylamine N-Oxide Promotes Secondary Structure Heterogeneity Among Amyloid Fibrils
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We demonstrated that secondary structure heterogeneity was observed among mature amyloid fibrils synthesized by the addition of an osmolyte, called trimethylamine N-oxide (TMAO) by promoting the formation of anti-parallel β-sheet structure along with parallel β-sheet structure. In regards to amyloid fibrils formed in the absence of TMAO, only parallel β-sheet secondary structure was observed. This structural effect was observed regardless of pH. The amyloid fibrils were investigated via Fourier-transform infrared spectroscopy and near-field microscopy, and the characterized by circular dichroism and UV-Vis spectroscopy. Using FTIR spectroscopy, the peak observed at 1630 cm^{-1} indicates the presence of parallel and/or anti-parallel β-sheet structure and a peak at 1692 cm^{-1} denotes the presence of anti-parallel β-sheet structure. This latter peak, which appears only in the TMAO-containing fibril solutions, indicates that TMAO promotes anti-parallel β-sheet fibrils in solution.

1314-Pos
Structural and Functional Analysis of Amyloid Fibril Formation by Two Closely Related Light Chains
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Light chain amyloidosis (AL) is a hematological disorder in which a clonal population of B cells expands and secretes enormous amounts of immunoglobulin light chain protein. These light chains misfold and aggregate into amyloid fibrils, leading to organ dysfunction and death. In AL the sequence of the light chain is unique for each individual, giving rise to a highly variable course of disease. We are studying two proteins, designated AL09 and AL103, derived from the κI O18:O8 germline that are highly similar in sequence, yet had significant differences in the disease phenotype. We have studied the *in vitro* kinetics of fibril formation and the structure of the resulting fibrils in order to explain this phenomenon.
We have begun by undertaking a systematic study of different solution properties and co-solutes that may affect fibril formation in these two proteins. We find that even though the proteins have similar thermodynamic properties, their fibril formation behavior is very different. AL09 readily forms fibrils under virtually every condition studied, while AL103 forms fibrils both more slowly and under fewer conditions. We have also explored the potential role of different glycosaminoglycans (GAGs) in the fibril formation process, specifically looking at the role of the size and charge of the GAG molecules. Furthermore, we have analyzed fibrils formed by these two disease proteins using limited proteolysis and mass spectrometry and have determined that in spite of their different phenotype the fibrils share a significant portion of their amyloid-forming core residues. Further structural studies are ongoing to determine how proteins with such different fibril formation kinetics can share a common amyloid structure.
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1315-Pos
Instantaneous Fibril Formation of α-Synuclein by Lateral Association of the Preformed Oligomeric Granules
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Elucidation of underlying mechanism of amyloidogenesis is a central issue not only for developing prophylactic and therapeutic means against amyloid-related diseases including Parkinson's and Alzheimer's diseases, but also for utilizing protein fibrils as nanomaterials for future nanobiotechnology. While the nucleation-dependent fibrillation is the prevailing notion to illustrate the amyloid fibril formation, we demonstrate an alternative mechanism of amyloidogenesis with α-synuclein, a pathological component of Parkinson's disease, in which the amyloid fibrils are formed via lateral association of the preformed oligomeric species of α-synuclein by acting as an assembling unit. Homogeneous oligomeric granules of α-synuclein were isolated in the middle of the lag period during the fibrillation kinetics. In the presence of an organic solvent of hexane, the granules instantaneously turned into the fibrillar structures which are indistinguishable from the amyloid fibrils obtained without the hexane treatment. Moreover, accelerated fibril formation of α-synuclein was also observed by imposing shear force on the granular structures by either centrifugal filtration or rheometer. Both organic solvent and physical influence might cause granules to experience structural rearrangement, resulting in the granular assembly into amyloid fibrils. As consequence, we propose a double-concerted fibrillation model to explain the *in vitro* fibrillation of α-synuclein, in which two consecutive associations of monomers and subsequent oligomeric granules are responsible for the eventual amyloid fibril formation.

1316-Pos
New Fluorescent Probe for Continuous Monitoring of Alpha-Synuclein Aggregation
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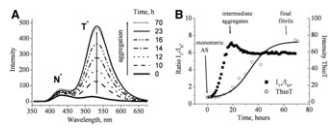
The aggregation of the presynaptic protein alpha-synuclein (AS), associated with Parkinson's disease, results in fibrils with a cross-beta-amyloid structure. Thioflavin T (ThT) is widely used for the identification and quantification of

such amyloid fibrils *in vitro*. However, it exhibits poor sensitivity and reproducibility, requires sampling, and is insensitive to the early stages of aggregation. We introduced a new sensor molecule for the *continuous* monitoring of AS aggregation, denoted AS-140HC, consisting of the AS mutant (A140C, C-terminus) labeled with the 3-hydroxychromone dye MFC[1]. MFC exhibits two fluorescence bands (N* and T*) arising from Excited State Intramolecular Proton Transfer (ESIPT)[2]. The intensity ratio of (I_{T*}/I_{N*}) reflects the microenvironment of the probe. Addition of AS-140HC in the range of 0.5-5% to wild type AS allows the monitoring of aggregation via the strong increase of I_{T*}/I_{N*} (panel A), which occurs at a much earlier stage of aggregation than the ThT response (panel B). See also refs [3-6].



[1] manuscript in preparation; [2] Demchenko et al., Biophys J., 2009, 3461; [3] poster Fauerbach et al.; [4] poster Shvadchak et al.; [5] Caarls et al., J. Fluor, 2009, DOI 10.1007/s10895-009-0536-1; [6] Celej et al., Biochemistry 2009, 7465.

1317-Pos

Characterization of Alpha-Synuclein Early Aggregates by Atomic Force Microscopy

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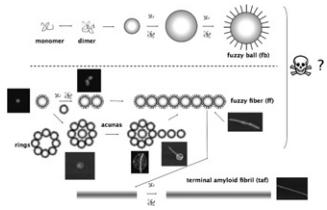
αlpha-synuclein (AS) is a key player in the development of Parkinson's disease. Neither the mechanism of its aggregation nor its role in neurotoxicity have been established as yet. However, it has been proposed that early oligomeric species may be the most cytotoxic [1].

Through the use of a covalently attached dual fluorescent emission ESIPT dye [2], we are able to monitor continuously the entire aggregation process *in vitro* [3]. Examination of samples by AFM has revealed a new pantheon of supramolecular species varying greatly in size and form. We identify a progression of structures starting from the unstructured monomer and proceeding through (i) spherical microaggregates (''fuzzy balls''); (ii) concatenated linear beaded fibrils (''fuzzy fibrils''); (iii) ring-like assemblies; (iv) circular ''platforms'' supporting nascent fibers [''acunas'' = amyloid cunas (Spanish for cradle)]; and (v) terminal amyloid fibers.



[1] A.L.Fink, **Acc. Chem. Res.**, 2006, **39**, 628-634; V.N.Uversky, **Curr. Prot. Peptide Sci.**, 2008, **9**, 507-540.
[2] V.V.Shynkar et al., **J. Phys. Chem. A.,** 2004, **108**, 8151-8159.
[3] Manuscripts in preparation; poster Yushchenko et al.

1318-Pos

Sickle Hemoglobin Fiber Kinetics Revealed by Optical Pattern Generation

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Sickle hemoglobin (HbS), a mutant of normal adult hemoglobin (HbA), will polymerize at concentrations above a well-defined solubility. HbS polymerization occurs by a double nucleation mechanism. A fundamental element of the mechanism is the growth of individual fibers, whose diameter (20 nm) precludes direct optical visualization. We have developed a photolytic method to measure the HbS fiber growth speed in HbS carbon monoxide derivative (COHbS) solutions. The idea of this method is that a single fiber entering a region of concentrated deoxyHbS will generate large numbers of additional fibers by heterogeneous nucleation, allowing the presence of the first fiber to be inferred even if it is not directly observed optically. We implement this method by projecting an optical pattern consisting of three parts: a large incubation circle, a small detection area, and a thin channel connecting the two. The connecting channel is turned on for just a short time; only if fiber growth is fast enough will the detection circle polymerize. Our fiber growth rates obtained from pure HbS, HbS/HbA mixtures, and partial photolysis of HbS validate a simple growth rate equation including any non-polymerizing species in the activity coefficient calculation. The monomer on-rate is determined to be 82 ± 2 /mM/Sec. The monomer off-rate is 751 ± 79 molecules/sec in agreement with earlier DIC observations of 850 ± 170 molecules/sec. The method predicts a solubility of 16.0 ± 1.1 g/dl in good agreement with 17.2 g/dl from sedimentation methods. The preceding values are for 25°C. Our measurements also rationalize the observed growth rate of the dense mass of fibers that grows more slowly along the channel and which can be visualized directly. Future uses of this method include HbS fiber bending and HbS solution fluctuations.

1319-Pos

Do Different Ligands Produce Different Effects in Sickle Hemoglobin Polymer Growth?

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Sickle Hemoglobin (HbS) is a variant of human hemoglobin with a point mutation on two subunits. This mutation causes HbS molecules to grow into polymers when the ligands it transports are released it and changes conformation from an R (relaxed) state to a T (tense) state. The polymer mass that grows inside a red blood cell can cause it to become too rigid to deform to pass through tight capillaries. This causes vaso occlusion and is one of many side effects of sickle cell disease. Polymer growth can be measured by fully photolyzing an HbS sample with a laser, thereby causing the solution molecules to release all their ligands and switch into a T-state. However, in vivo, the partial pressure of oxygen rarely falls below 50% which makes the Hb a combination of fully, partially and un-liganded species. Equilibrium and kinetic measurements were done previously on fractional O2, CO and NO species, although a complete systematic comparison has never been conducted to quantify all of the differing data. A comparison of previous data along with new kinetic results will be presented. Partially ligated crystal protein structures will also be employed to rationalize the results.

1320-Pos

Light Scattering Measurements of Hemoglobin Critical Fluctuation and the Energy Landscape For Polymerization

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We have developed a novel method for measuring light scattering to observe critical fluctuations in hemoglobin (Hb) solutions. A small rectangular cell (0.2 x 4.0 x 30 mm) is filled with 24 μL of Hb solution. An optical fiber with outer diameter of 125 μm (62.5 μm core) is sealed into the cell in contact with the solution, and light scattering is measured at 90°. The flat faces of the cell permit measuring absorbance spectra to ensure sample integrity. The scattering source is a 785 nm laser diode that delivers 1.5 mW to the sample. Scattered light is detected by a Hamamatsu GaAs(Cs) PMT via a LWD microscope objective. Measured scattered light intensity agrees ($\pm 10\%$) with scattered intensity expected from Hb fluctuations. Sample temperature is controlled by a thermoelectric stage and raised in a series of user-controlled steps. Temperature may be conveniently returned to prior values to insure reversibility. Assuming that the divergence in scattering that is observed signifies a spinodal, all our measured experiments agree with published spinodals for deoxyHbS, and deoxyHbA. In addition we have obtained data for COHbA, and cross-linked deoxyHbA. Spinodal lines for COHbA and deoxyHbA are essentially indistinguishable, despite differences in quaternary structure. Spinodal lines for cross-linked deoxyHbA and regular deoxyHbA coincide at high c values, but differ significantly at lower ones. In conclusion, changes of quaternary structure cause alterations to spinodal lines when polymerization is possible. This kind of data can be used to explore free energy landscapes having features inaccessible to techniques based on equilibrium thermodynamic properties.

1321-Pos

Sickle Cell Occlusion in Microchannels

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Sickle Cell disease is the result of a genetic mutation on the surface of the hemoglobin molecule that makes it capable of polymerization upon deoxygenation. Such polymerization leads to impaired circulation and tissue damage due to the rigidity of the polymer mass. A powerful method for studying the reaction is to replace the physiological ligand (oxygen) by CO, which can be readily and reversibly photolyzed by a strong light source such as a laser. This provides a means of initiating and sustaining the reaction for as long as desired, followed by full reversal once the light is turned off. This method has been used with both solutions and cells. Here we have coupled the method to a microfluidic system to observe directly how photolytically sickled cells occlude small channels, of width ranging from 2 to 10 μm. Because the thickness of the channels is 2 to 5 μm, it is completely spanned by the red cell. This fixed path length permits microspectrophotometry of the cell to determine the internal