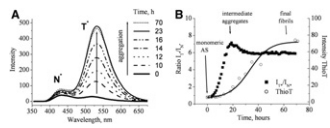
such amyloid fibrils *in vitro*. However, it exhibits poor sensitivity and reproducibility, requires sampling, and is insensitive to the early stages of aggregation. We introduced a new sensor molecule for the *continuous* monitoring of AS aggregation, denoted AS-140HC, consisting of the AS mutant (A140C, 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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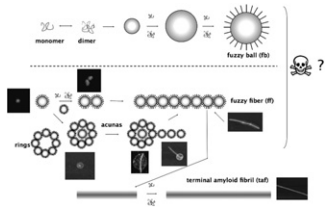
αlpha-synuclein (AS) is a key player in the development of Parkinson's disease. Neither the mechanism of its aggregation nor its role in neurotoxicity have been established as yet. However, it has been proposed that early oligomeric species may be the most cytotoxic [1].

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