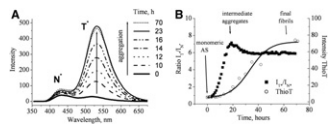
such amyloid fibrils *in vitro*. However, it exhibits poor sensitivity and reproducibility, requires sampling, and is insensitive to the early stages of aggregation. We introduced a new sensor molecule for the *continuous* monitoring of AS aggregation, denoted AS-140HC, consisting of the AS mutant (A140C,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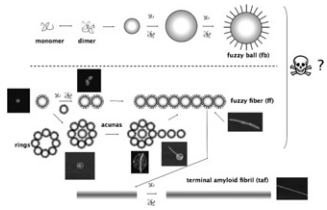
αlpha-synuclein (AS) is a key player in the development of Parkinson's disease. Neither the mechanism of its aggregation nor its role in neurotoxicity have been established as yet. However, it has been proposed that early oligomeric species may be the most cytotoxic [1].

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 $82 \pm 2$ /mM/Sec. The monomer off-rate is $751 \pm 79$ molecules/sec in agreement with earlier DIC observations of $850 \pm 170$ molecules/sec. The method predicts a solubility of $16.0 \pm 1.1$ 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

Hb concentration. The same cell is oscillated back and forth along the channel by changing pressure, and tracking of the cell determines its frictional coefficient. Light from an Argon ion laser is imaged on the cell, causing it to lose CO and subsequently rigidify. The functional effect of the rigidity is seen as the cell's ability to oscillate becomes impaired. This can be compared with the mass of polymer that forms within the cell. Such information is critical for understanding the details of how polymer formation results in vaso-occlusion.

**1322-Pos**

**Miccrorheology of Sickle Hemoglobin Gels**

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Sickle cell disease is a rheological disease, yet no quantitative rheological data exists on microscopic samples. We have developed a novel method for probing the microrheology of sickle hemoglobin gels, based on magnetically driven compression of 5-8 μm thick emulsions containing hemoglobin droplets of ~100 μm diameter. By observing the expansion of the droplet area as the emulsion is compressed, our method can resolve changes in thickness of a few nm with temporal resolution of ms. Carbon monoxide bound to sickle hemoglobin was dissociated by laser illumination allowing the resulting deoxyhemoglobin to form gels in target droplets. The amount of polymer formed was determined by observing, in the target droplet, the residual concentration in a small region that was unilluminated by the laser. Thickness was monitored by observing a non-photolyzed reporter droplet adjacent to the target droplet.

Gels were formed at different initial concentrations, temperatures and fractional saturation with CO. In addition, some gels were formed in small spatial regions which then were allowed to grow to the full extent of the target droplet, to contrast with the same sample gelled completely in the target droplet ab initio, thereby creating a different domain structure in the gel. We find that all the gels behave as Hookean springs with linear and repeatable dependence of thickness on force. This allowed us to determine Young's modulus, which ranged from 300 to 1500 kPa for the gels which varied in polymerized hemoglobin concentration from 6 g/dl to 12 g/dl. A highly simplified model for the gel, treating it as a simple lattice with fixed junctions, describes the observed quadratic concentration dependence of Young's modulus data. These measurements provide a quantitative rationale for pathophysiology in the disease.

**1323-Pos**

**Unraveling the Pressure Effect on Nucleation Processes of Amyloidogenic Proteins**

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Fully or partially unfolded proteins may undergo non-native self-assembly as a competing pathway to native functional folding, and are the first steps in the nucleation and fibrillation process of proteins, which can lead to a series of diseases including Alzheimer's and type II Diabetes Mellitus. Up to date, still little is known about the nucleation event initiating fibril formation of proteins and how it is influenced by thermodynamic variables, such as temperature, pressure and the activity of cosolutes, although such factors are often responsible for the polymorphic nature of the fibrils formed. Pressure tuning in combination with calorimetric, spectroscopic and structural techniques revealed new insights into the pre-aggregated regime as well as mechanistic details about concurrent aggregation pathways and the differential stability of insulin aggregates [1-4]. Here we focus now on a simple model within the framework of classical nucleation theory that is able to shed light on the effect of pressure on the nucleation process of amyloidogenic proteins. With the input parameters determined and the pV-corrected free energy term of the classical nucleation theory, the experimental data follow the theoretical predictions remarkably close. The negative activation volume observed suggests that the transition state for nucleation and subsequent growth is less hydrated and more densely packed than the partially unfolded insulin monomers entering the nucleation pathway. The insights provided by the model presented will be very helpful to quantify the influence of pressure on protein aggregation/fibrillation reactions in general.

1. N. Javid, R. Winter, et al., Phys. Rev. Lett. 99 (2007) 028101
2. S. Grudzielanek, V. Smirnovas, R. Winter, J. Mol. Biol. 356 (2006)
3. V. Smirnovas, R. Winter, Biophys. J. 94 (2008) 3241-32462
4. R. Mishra, R. Winter, Angew. Chem. Int. Ed. 47 (2008) 6518-6521

**1324-Pos**

**Point Substitution in Albebetin Sequence Accelerates the Amyloid Structure Formation**

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It is suggested that partially folded states play a key role in amyloid formation. So, it was of special interest to investigate a protein the ''wild'' type of which is initially in this state. Albebetin, a de novo protein, is an example of such proteins and can form amyloid structures during long incubation at high temperature. On the other hand, it was predicted theoretically that single point substitution His65 by Phe may strengthen amyloid formation by this protein. Properties of the obtained mutant protein were investigated by far UV CD. The amyloid formation was monitored by ThT fluorescence and electronic microscopy under various conditions. Interaction with phospholipids vesicles was also studied. It was shown that the His65Phe mutant was able to form amyloid structures even at more moderate conditions than the ''wild'' type did. Additionally, the amyloid growth rate for the mutant protein was substantially higher of that for the ''wild'' type. Temperature decrease led to reduced rate of amyloid structure growth, while enhancing of ionic strength accelerated amyloid formation and increased its yield. EM images showed fibrillar morphology of formed aggregates. Investigation on amyloid formation by the de novo protein may shed light on common features of amyloid structures. This work was supported by RFBR 09-04-01348, partly by the Howard Hughes Medical Institute Award 55005607 to A.V. Finkelstein, by the RAS Program on ''Molecular and Cellular Biology'', by Federal Agency for Science and Innovations 02.740.11.0295, and Program of Scientific Schools 2791.2008.4.

**1325-Pos**

**Amino Acid Modifications in the N terminal Sequence of htt Exon-1 Modulate In Vitro Aggregation**

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Huntington's disease (HD) is one of ten neurodegenerative diseases caused by expanded CAG repeats. A characteristic feature of postmortem HD brains is the presence of intra-nuclear inclusions comprising N terminal mutant Huntingtin (htt) fragments. Based on these and other results, it was posited that protein aggregation might play a crucial role in mediating disease pathologies. Using exon-1 peptide models, we have been able to delineate a clear link between polyglutamine expansion and aggregation propensities as modulated by the first 17 residues adjacent to polyglutamine in the N terminus (httNT). Here we investigate the effect of httNT amino acid modifications - in particular mutations designed to block or mimic putative post-translational modification (PMTs) - on the aggregation of these exon-1 peptides. A particularly striking result was that exon-1 peptides in which both httNT serine residues are mutated to the phospho-Ser mimic aspartate aggregate more slowly and form irregular/immature aggregates, compared to peptides with WT httNT sequence. These results nicely correlate with results in a tg mouse model of HD, in which the Ser->Asp double mutant produces no aggregates and does not develop HD symptoms (X. W. Yang, personal communication). Analysis of single Ser to Asp mutants suggests that these mutations act in concert to produce these effects. Over the PMT mutations studied, we observed a correlation between net hydrophobicity and aggregation propensity. This observation was further corroborated in two multiple mutants containing mutations not associated with PMTs that are designed to either increase or suppress net hydrophobicity. We believe our data to date support our hypothesis that one to a few mutations or PMTs in the N terminal segment can have significant effects on the development of HD pathology, possibly mediated largely by biophysical effects.

**1326-Pos**

**Prefibrillar Formation Conditions of *f*À-Lactoglobulin by Titration and Chaotropes Urea and KSCN Under Thermal Load**

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The harmful growth of toxic oligomers in the formation of protein amyloid fibrils have been connected to degenerative diseases like Alzheimer's and Huntington's diseases. Understanding the fundamental mechanisms behind protein unfolding and subsequent fibrillogenesis may provide a way to stop the process from occurring. The purpose of this study was to identify favorable fibril growth conditions for a globular model protein β-lactoglobulin using the chaotropes urea and KSCN, along with titration of a pH 7.04 phosphate buffer solution at 40°C over five days. Time-resolved and steady-state fluorescence was used to examine the shift in emission of the tryptophan amino acids over the applied denaturation ranges. BLG, a dimer in native form, monomerized and partially unfolded at 5 M Urea, 2 M KSCN and at pH 2 in phosphate buffer in vitro. Exposure of the solutions to continuous heat over time caused a increase in the lifetimes and red shift in the emission spectra , indicating the