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.

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**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