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

possible beginning of nucleation. The study has provided a base for continuation of the study of oligomerization and subsequent fibrillation of BLG, which may provide a fundamental mechanism of formation transferable to other proteins in vivo.

1327-Pos

Amyloid Gels: Formation of an Insulin Fibrillar Network

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The formation of insulin amyloid fibrils is important not only for the development of reliable drugs and drug delivery systems, but also for modeling the basic properties of protein self-assembly. Fibrillation kinetics are typically characterized by an initial apparent lag-phase, related to the formation of oligomer, protofibrils and aggregation nuclei. Afterwards, aggregation proceeds over a wide range of length scales via fibril elongation, thickening and/or flocculation, and eventual gelation. Here, we focus on the formation of such a gel, made of insulin amyloid fibrils, upon incubation at high temperature and low pH. By light scattering and rheological techniques, we monitor the development of the structural, dynamical and mechanical properties of fibrillar aggregates, up to the dynamic arrest of the sample and the appearance of a non-ergodic behavior, which marks the occurrence of gelation. Atomic force microscopy imaging on incubated samples highlight the existence of a fibrillar network , as well as a complex hierarchy of different morphologies. Also, small and large angle dynamic light scattering experiments clearly show a non-diffusional dynamic behavior. Our experiments were able to reveal the structural details hidden in the apparent lag-phase, displaying the slow fibril nucleation and elongation. We confirm that this initial stage is followed by an exponential growth of structures of different sizes. These two kinetic stages of structural growth are mirrored by the kinetics of the viscoelastic properties and, in particular, by the growth of the elastic modulus. Our results show that the appearance of a noteworthy elastic network is associated with the initial fibril nucleation and elongation rather than with the formation of larger structures which cause the eventual gelation.

1328-Pos

Hybrid Amyloid Fibril Genesis with Components of Sporadic and Familial Alzheimer's Disease

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Generally, Alzheimer's Disease (AD) develops spontaneously. Nevertheless, many families with an inheritable occurrence were identified. Of these, a considerable number shows mutations in the Alzheimer Precursor Protein (APP) gene concerning regions of the Aß1-40 peptide, as e. g. the ''Flemish'' A21G [APP A692G] or the ''Iowa'' mutant D23N[APP D694N]. All except two of today's known Aß1-40 mutations in familial AD were reported heterozygously dominant. Therefore, an interaction of wild type (WT) and mutated (MUT) Aß peptide is likely to occur in the brain of affected patients.

Here we report on the extended co-fibrillation analysis of WT and MUT Aß1-40 peptide. Thioflavin-T fluorescence data of cofibrillation kinetics indicated a collateral aggregation process, correlated with the MUT:WT ratio. Coherently, the fibril morphologies of cofibrillates as observed in negative contrast transmission electron micrographs also appear to correlate with the MUT:WT peptide ratio.

We interpret these results that MUT peptide with a preferred fibril conformation can template the fibril formation of the morphologically pluripotent WT dose-dependently. This may explain the strain-like symptomatic of different familial AD cases.

1329-Pos

Iapp Preamyloid Oligomers Accumulate in the Heart and Contribute to Cardiac Dysfunction in Type-2 Diabetes

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Islet amyloid polypeptide (IAPP), a hormone co-secreted with insulin by pancreatic β-cells, forms amyloids when overexpressed. IAPP amyloids accumulate in pancreatic islets and are a hallmark of type-2 diabetes mellitus (T2DM). Recently, IAPP amyloids were found in kidneys of T2DM patients, suggesting that IAPP can deposit in organs other than pancreas. Apparently, the toxic effects to cells are mediated by the preamyloid oligomers. Here, we investigated whether IAPP preamyloid oligomers are present in the heart in T2DM. Using immunochemistry, we found significantly increased levels of IAPP oligomers (by $18 \pm 3\%$) in failing hearts from T2DM humans vs. non-diabetics and in hearts from T2DM rats transgenic for human IAPP (by $88 \pm 15\%$) vs. T2DM rats expressing only rat non-amyloidogenic IAPP. Most likely, IAPP accumulation in the heart occurs in the pre-diabetic state, **when** IAPP is oversecreted in the blood. To investigate the acute effect of IAPP oligomers on cardiac function, we incubated isolated rat cardiac myocytes with exogenous IAPP (5 and 50 μM) for 1-2 h. 50 μM IAPP significantly increased Ca transient amplitude (by $73 \pm 19\%$) in myocytes contracting at 2 Hz. For rapid screening and analysis of sites susceptible for IAPP accumulation we designed a noninvasive water proton NMR protocol. We deciphered the magnetic signal of water surrounding preamyloid oligomers. The variation of this signal was correlated with population distributions of oligomers and fibrils by using immunochemistry and electron microscopy. We found that embryonic amyloids generate hyper-intense magnetic signals, which are distinct from hypo-intense magnetic signals induced by amyloid plaques. These results suggest that IAPP preamyloid oligomers contribute to cardiac dysfunction in T2DM. Water proton NMR may prove a useful non-invasive method for detecting these molecular entities in the heart.

1330-Pos

Small Molecule Inhibitors of Islet Amyloid Polypeptide Fibrillogenesis and Cytotoxicity

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Protein fiber formation is associated with diseases ranging from Alzheimer's to type II diabetes. The fiber formation is a complex reaction, which includes a number of conformational and oligomeric intermediate states. In recent years, it has become clear that it is these states, and not the end product (i.e. fibers) of amyloid formation that are the toxic agents associated with disease. These insights indicate that small molecule screens must to be directed at assembly mechanism in order to maximize the prospects for success. Islet amyloid polypeptide (IAPP) is a 37 residue peptide hormone co-secreted with insulin by the ß-cells of the pancreas. In patients with type II diabetes, this protein aggregates as amyloid in a process that is correlated with ß-cell dysfunction and the loss of ß-cell mass. In in vitro studies, the addition of soluble IAPP has been shown to be toxic to many ß- and non-ß-cell lines. IAPP fibrillogenesis, as is the case for many other amylodiogenic proteins, can be catalyzed by lipid bilayers. Paradoxically, while amyloid fibers are ß-sheet rich, membrane-stabilized states are α-helical. We have identified a small molecule alpha helix mimetic, IS5, which inhibits bilayer catalysis of fibrillogenesis, and rescues IAPP-induced toxicity in cell culture. Importantly, IAPP:IS5 interactions localize to the putative α-helical region of IAPP, revealing that α-helical states are on pathway to fiber formation. Normally, IAPP is not amyloidogenic as its cosecreted partner, insulin, prevents self-assembly. Here, we show that IS5 inhibition is synergistic with insulin. IS5 therefore represents a new approach to amyloid inhibition as the target is an assembly intermediate that may additionally restore functional IAPP expression.

1331-Pos

Zinc Inhibits Human Islet Amyloid Polypeptide (IAPP) Amyloidogenesis

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Human Islet Amyloid Polypeptide (hIAPP) is a highly amyloidogenic protein found in islet cells of patients with type II diabetes. Because hIAPP is highly toxic to beta-cells under certain conditions, it has been proposed that hIAPP is linked to the loss of beta-cells and insulin secretion in type II diabetics. One of the interesting questions surrounding this peptide is how the toxic and aggregation prone hIAPP peptide can be maintained in a safe state at the high concentrations found in the secretory granule where is stored. We show here through a combination of NMR, ITC, CD, and Thioflavin T fluorescence that zinc, which is found at millimolar concentrations in the secretory granule, binds to hIAPP with a Kd of approximately 100 nM and inhibits hIAPP amyloid fibrillogenesis in the micromolar range. NMR spectroscopy shows that zinc interacts with hIAPP through coordination to His18 and a probable cation - pi stacking interaction with Phe15. ITC binding experiments with the rat variant of IAPP, which lacks His18, indicated an additional binding site with approximately 1 mM affinity. The lower affinity binding site was localized to Arg11 by NMR. The binding of zinc also alters the structure of hIAPP, rigidifying the N-terminal region of the protein in a near-helical conformation stabilizing the non-amyloid form of the peptide. The inhibition of the aggregated and toxic forms of hIAPP by zinc provides a possible mechanism between the recent discovery of linkage between deleterious mutations in the SLC30A8 zinc transporter, which transports zinc into the secretory granule, and type II diabetes.