Our preliminary results find growth rates greater than previously observed by DIC microscopy. We can also measure the growth rate of bundles by observing a mass of fibers that subsequently grow along the channel, and these grow at rates comparable to those determined by DIC microscopy. Implications of the measurements and the method will be discussed.

457-Pos Board B336

A Unified Theory of Liquid-Liquid Demixing and Polymer Formation Kinetics

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Sickle hemoglobin is a natural hemoglobin mutation with a hydrophobic replacement of a charged aminoacid on the molecular surface. This leads to aggregation into rigid helical structures (‘‘polymerization’’), the underlying cause of sickle cell disease. It has also been shown that polymerization occurs in close correspondence with the phase transition of liquid-liquid demixing , or with the critically diverging fluctuations of local concentration occurring in its proximity. Due to this correspondence, polymerization kinetics remarkably appear to exhibit, with respect to demixing temperature, the same universal scaling features shown by amplitudes and lifetimes of fluctuations occurring in proximity of phase transitions. Thus, it is important to understand the relation between polymer formation and liquid-liquid demuxing (LLD). Nucleation kinetics have been described by a relatively complete theory, that until now does not include LLD. We present here a way to incorporate LLD seamlessly into such theory, so as to have a description in terms of the concurrence and interaction of the two processes. In addition, we present new light scattering data supporting the theory. The theory provides a more in-depth understanding of aggregation and crystallization.

458-Pos Board B337

Amyloid-like Aggregation Of A Human Apolipoprotein A-I Variant

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Amyloidosis are characterized by extra cellular deposits of anomalous fibrilar proteins. Human apolipoprotein A-I (apoA-I) is not normally involved within these pathologies. However, one case of severe amyloidosis associated with atherosclerosis was observed when apoA-I shows a deletion of a lysine residue in a central region of the protein (apoA-I Lys107-0). In order to get insight on the local cellular environment that promotes this anomalous aggregation, we studied the folding of the deletion mutant, as compared with wild type apoA-I (Wt). Analysis of chemical denaturation and by using hydrostatic pressure show that apoA-I Lys107-0 is more unstable and has a stronger tendency to form β sheet structure as incubation time increases, specially at acidic pH. Under these conditions, mutant denaturation is less cooperative, suggesting intermediate states folding. In order to confirm that these states prone protein aggregation, we followed protein folding by two-Photon Fluorescence Correlation Spectroscopy. Our results clearly show that, even at very low concentrations, protein aggregation is detected, under acidic conditions, after incubation for a few hours at 37°C. Interestingly, also Wt suffers conformational chances that favor some insoluble states. These results suggest that the anomalous aggregation of apoA-I Lys 107-0, is mediated by intermediate folded states and β sheet conformation, induced by an acidic pH. Protein misfolding is concentration-dependent, but can occur under diluted solutions. We discuss our results in terms of the pathological landscape of atherosclerosis.

459-Pos Board B338

Endogenous Formaldehyde Is Related To Sporadic Alzheimer’s Disease

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Formaldehyde is produced in human body at every moment. Our previous work has showed that formaldehyde at a low concentration induces tau protein to misfolding and aggregation, resulting in cytotoxicity to SH-SY5Y cells and rat hippocampus cells[1,2]. Therefore, concentrations of endogenous formaldehyde of human have been investigated to suggest that endogenous formaldehyde plays an important role in the pathology of sporadic Alzheimer’s disease. Investigation of 420 Chinese people of different ages shows that blood formaldehyde is increased with aging (over 60 years old). The human blood formaldehyde is dynamically kept approximately around 0.087 ± 0.014 mM under physiological conditions. The endogenous formaldehyde is gradually increased and accumulated in human body especially in the central nerve system as aging, resulting in chronic aldehyde damage to human brain including white matter and grey matter. The chronic aldehyde damage is thought as one of the important factors related to sporadic neurodegeneration. According to this viewpoint, clinical investigation has been carried out that the concentration of morning uric formaldehyde is found to be positively related to the degree of dementia: the more severe the dementia, the higher the concentration of uric formaldehyde of the patients. Furthermore, to regulate endogenous formaldehyde as clinical treatment (methods or drugs) for Alzheimer’s patients is suggested in the light of this work.

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460-Pos Board B339

Intramolecular Diffusion of the Amyloidgenic Protein HypF

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The mechanism of amyloid fibril aggregation is as fundamental a process in protein dynamics as folding. Our goal of studying the N terminal domain of E.coli HypF (HypF-N) is to seek the intrinsic intramolecular properties of amyloidgenic proteins. We measured two mutants (W81F and W27F) using Trp-Cys contact quenching to explore the microsecond loop formation of two chain segments of HypF (27-65 and 65-81). Using Szabo, Schulten and Schulten theory and a worm-like chain model to determine an effective intramolecular diffusion coefficient, *D,* we observed different behaviors for these two segments. The short loop (C65-W81) collapsed into a very vicious and compact state under low denaturant conditions while the long loop (W27-C65) is relatively more diffusive. Finally chronological measurements in 3%-6% (v/v) TFE (trifluoroethanol) show that the long loop appears to become unstructured and highly diffusive before aggregating.

461-Pos Board B340

Amyloid Formation By Peptides From Yeast Adhesins

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Candida albicans adhesins bind to peptides and lead to cell aggregation with amyloid properties. We therefore searched in other fungal adhesins for β-aggregation forming sequences with the TANGO algorithm and synthesized three peptides. The *C. albicans* Als5 peptide, SNG (SNGIVIVATTRTV), has a 90% β-aggregation potential and forms amyloids (Otoo, et al, Euk. Cell, 7(5): 776-782, 2008). The two additional peptides, from *C. albicans* Eap1 adhesin (HTA VTTGVTIITVTTND) and *Saccharomyces cerevisiae* Flo1 adhesin (TDETVIV IRTP), have aggregation potentials of 90% and 42% respectively, and were studied for amyloid formation.

Peptide interactions were analyzed by circular dichroism (CD), absorbance and fluorescence spectroscopy to monitor secondary structure and amyloid formation. CD spectra showed unstructured random coil for both Eap1 and Flo1 peptides in buffer, changing to β-aggregate with an ellipticity minimum at 230-235nm after stirring to induce amyloid formation. The stirred solutions of both Flo1 and Eap1 peptides showed an increase in Congo Red absorbance with a shoulder near 550nm and also had enhanced thioflavin-T fluorescence. Thioflavin-T emission intensity is much greater when it is bound to amyloids and the emission spectra of both amyloid peptides show a significant fluorescence intensity increase. These results with the Flo1 and Eap1 peptides suggest a change from non-amyloid characteristics when initially dissolved in buffer to amyloid formation with stirring. Therefore, sequences from two additional adhesins show conformational changes leading to amyloid formation.

462-Pos Board B341

Conformational Instability, Aggregation, and Hydrogel formation of a 16-Residue Alanine-Based Peptide in Aqueous Media

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Table of Contents

Polyalanine peptides doped with a small number of charged residues typically adopt alpha-helical conformations in aqueous solution if the number of residues exceeds a certain threshold value. Helical wheel projections of peptides based on the repeating unit $(AAKA)_n$ clearly illustrate the amphipathic nature of the peptides, with all the lysine residues residing on the same side of the helix. The amphipathic nature of these peptides distinguishes both them and their behavior in solution from alanine peptides of similar length and composition. At sub-millimolar concentrations, Ac-$(AAKA)_4$-NH_2 shows conformational instability over time upon dissolution in aqueous media. UV-Circular Dichroism (UV-CD) spectra indicate the presence of some α-helical structure at concentrations below ~ 100 μM, with increasing β-structural content as the concentration enters the millimolar regime. Above a certain threshold concentration (single digit millimolar), the peptide adopts a mostly β-like structure, which, upon salt addition, undergoes hydrogelation to form a network of fibers, as evidenced by Atomic Force Microscopy (AFM) images. Electronic and vibrational studies will be presented of both the gelled and un-gelled state, as will rheological and imaging studies of the hydrogel.

463-Pos Board B342

Study of Misfolding and Aggregation for Short Peptide from the Yeast Prion Sup35 Using AFM Imaging and Force Spectroscopy

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Protein misfolding is a fundamental pathway of protein self-assembly into nanoaggregates of various morphologies. However, our knowledge of this phenomenon is very limited. Protein aggregation is the cause of many conformational diseases justifying further study of protein misfolding phenomenon. We hypothesized earlier that the protein misfolded conformation is characterized by elevated intermolecular forces ultimately leading to aggregation. In this work we studied a short fragment (-GNNQQNY) of the yeast prion Sup35 which is critical for aggregation of the entire protein and presumably in the protein misfolding. By using force spectroscopy we measured forces for the interactions between a single pair of peptides. We established a correlation between aggregate morphology and strength of inter-peptide interactions as well as their pH dependence. The results of this study provide additional support for the importance of single molecule force spectroscopy for elucidating mechanisms of protein misfolding and aggregation. Using AFM imaging we also show that aggregates formed at different conditions (pH) for this short peptide exhibit distinctly different morphologies that cannot be predicted from the kinetics of aggregation study with ThT fluorescence. We found a dramatic difference of fibrils properties and their structure depending on the aggregation conditions. The difference in the aggregate properties was reflected in their adsorption to the surfaces having different properties: negatively or positively charged surface, PEG modified and hydrophobic surface. Salt concentration has also strong influence on kinetics and aggregate morphology with faster kinetics at higher salt concentration. We also show that the replacement of one amino acid residue in the sequence of this short peptide (Q4P) completely abolishes aggregation. Thus, the primary structure of the peptide is a critical determinant of aggregation propensity.

464-Pos Board B343

On The Mechanisms Regulating Alpha-crystallin Activity

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α-crystallin is a protein that plays several relevant physiological roles (i.e is the major constituent of human lens or help in maintain the correct folding of several protein) all of them affected by the occurrence of aggregation. α-crystallin supramolecular aggregation, induced by generating heat-modified α-crystallin forms, has been investigated over a range of temperature between 30°C and 60°C by means of static and dynamic light scattering and atomic force microscopy. Aggregation, after the formation of first clusters or basic aggregation units, can be described as a cluster-cluster aggregation similar to that of colloidal particles. Below a temperature $T_C = 45^\circ C$, after a large lag time needed to form the first clusters, a fast, diffusion limited, aggregation can be observed. Above T_C we observe a faster lag time followed by a slow aggregation. Correspondingly the temperature dependence of aggregation rates display an abrupt discontinuity at T_C .

This discontinuity and the different kinetics of aggregation shed new light in the pathogenesis of the human eye lens cataract assigning a key role to the heat modified form of α-crystallin that markedly protect from aggregation preserving the transparency of the lens.

Protein Aggregates II

465-Pos Board B344

Effect Of Beta-sheet-breaker Peptides On The Assembly, Morphology And Mechanical Stability Of Oriented aß25-35 Amyloid Fibrils

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Amyloid fibrils are self-associating filamentous structures that play an important role in neurodegenerative and protein misfolding diseases. It has been shown that certain peptides, called beta-sheet-breaker (BSB) peptides, may interfere with amyloid fibril assembly. Although BSB peptides are prospective therapeutic agents in amyloidosis, there is ambiguity about the mechanisms and generality of their action.

In the present work we analyzed the effect of LPFFD, Soto's BSB peptide, on the growth kinetics, morphological and mechanical properties of amyloid ß25-35 (Aß25-35) fibrils assembled in an oriented array on mica surface. Aß25-35 is thought to represent the biologically active, toxic fragment of the full-length beta peptide. Growth kinetics and morphological features were analyzed by using in situ AFM in the presence of various concentrations of LPFFD. The mechanical stability of the fibrils was explored with force spectroscopy methods. We found that the addition of LPFFD did not alter the assembly kinetics of Aß25-35 fibrils. Already formed fibrils did not disassemble in the presence of high concentrations of LPFFD. The nanomechanical behavior of Aß25-35 fibrils is characterized by the appearance of force plateaus which correspond to the force-driven unzipping of protofilaments. We observed that the plateau force did not change in the presence of LPFFD. The lack of significant effects of LPFFD on Aß25-35 fibril assembly and stability may suggest that the beta-sheet-breaking effect of the peptide is not general. Alternatively, the Aß25-35 fibrils formed on mica are in a configuration which is inaccessible to the LPFFD peptide.

466-Pos Board B345

Thermal Stability Of Oriented Aß25-35 Amyloid Fibril Nanoarray

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Amyloid fibrils are filamentous protein deposits in the extracellular space of various tissues in neurodegenerative and protein misfolding diseases. It has been proposed that amyloid fibrils may be used in nanotechnology applications because of their self-assembly properties and stability. Recently we have shown that amyloid beta 25-35 (Aß25-35) forms a highly oriented, potassium-dependent network on mica. The mutant form of the peptide (Aß25-35_N27C), which forms an identically oriented nanoscale network, may be chemically addressed for functionalization in dedicated applications. In order to utilize the amyloid nanoarray in nanotechnology applications, uderstanding its physical and chemical stability is important.

In the present work we investigated thermally induced changes in the morphology of the oriented Aß25-35 fibril network. The fibrils maintained high orientation stability in the temperature range of 30-70 degrees, suggesting that orientational rearrangement of Aß25-35 fibrils on mica is an unfavorable process. Above 45 degree a gradual decrease in fibril length and dissociation from the surface could be observed. In addition, at high temperatures (45-70 degrees) the average fibril thickness increased, indicating changes in the underlying structure or structural dynamics. Possibly, a thermally induced transition in the Aß25-35 peptide around 45 degree leads to structural changes in the fibril as well. The temperature-dependent changes described here need to be considered in the use of amyloid fibrils in nanotechnology applications.

467-Pos Board B346

Fibril formation of Aβ (10-35) studied by UV resonance Raman Spectroscopy

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In Alzheimer's disease the major pathological feature observed is the progressive deposition of insoluble senile amyloid plaques within the cerebral cortex. The major component of these plaques is amyloid beta (Aβ), a 39-43 residue