

452-Pos Board B331**Amyloid Peptide Conformations In Soluble Oligomers**

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Fluorescence Resonance Energy Transfer (FRET) was used to study protein structures within soluble oligomer intermediates of amyloid and non-amyloid aggregates. Three protein aggregation systems were studied using fluorescence donor and acceptor near the N and C terminus:

- (1) Alzheimer's $A\beta_{1-40}$ peptide.
- (2) 20-residue polyglutamine $K_2Q_{16}K_2$.
- (3) 20-residue polyglutamic acid E_{20} . (non-amyloid)

The aggregation of each peptide showed different conformational changes: $A\beta_{1-40}$ partially compacted into a structure consistent with solid-state NMR structures, $K_2Q_{16}K_2$ extended to form β -sheets, and E_{20} compacted heavily into β -hairpins. However, based on donor-acceptor distances, soluble oligomers conformations of all three peptides show a remarkable degree of similarity to their monomeric precursors, albeit with a slightly expanded conformation for $A\beta_{1-40}$ and E_{20} . These findings support assembly models of small soluble oligomers which largely consist of monomer-like structures, with some increase in α -helical content for $A\beta_{1-40}$. These donor-acceptor distances are used to directly assess the accuracy of different molecular dynamics force fields in the study of these soluble oligomers.

453-Pos Board B332**Real-Time Monitoring of Heat Induced Unfolding and Aggregation of β -lactoglobulin in the Presence of Chaperones Using High Resolution Ultrasonic Spectroscopy**

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Industrial processing of proteins often exposes them to different stress conditions (elevated temperature, pH, salts, presence of hydrophobic interfaces, etc.) where protein molecules undergo conformational changes, aggregate and lose their functionality. The aggregation can be suppressed partially or completely by blocking the atomic groups of proteins involved in intermolecular 'bridging'. This blocking can be achieved with a help of host molecules, chaperones. Selection of optimal chaperones and optimisation of protein processing requires effective real-time monitoring of structural protein rearrangements and formation of protein aggregates, which is a difficult task for most analytical techniques. In our talk we present the results of an application of high-resolution ultrasonic spectroscopy for real-time monitoring of conformational changes and evolution of particle sizes during thermal denaturation, aggregation and subsequent gelation of β -lactoglobulin in the absence and presence of chaperones suppressing the aggregation process. Continuous measurements of ultrasonic velocity and attenuation in the frequency range 2 to 20 MHz performed with the HR-US 102 spectrometer in a temperature ramp mode (up and down temperature ramps between 35-120°C, various ramp speed) allowed us monitoring of thermal transitions and formation of protein particles in aqueous β -lactoglobulin solutions. The results provided information on protein partial unfolding and transition to molten globule state (50-75), formation of protein nano-particles (75-80°C) and formation of particle gel network above 80°C. The data was used to evaluate reversibility of β -lactoglobulin aggregation, the effects of heating rate, pH, ionic strength and action of chaperones on the denaturation and aggregation profiles. Kinetics of unfolding and evolution of size of protein aggregates under a broad range of conditions were analyzed.

454-Pos Board B333**Detecting Protein Aggregation on Cells Surface: Concanavalin A Oligomers Formation**

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A number of neurodegenerative diseases involve protein aggregation and amyloid formation. Recently evidence has emerged indicating small-transient prefibrillar oligomers as the primary pathogenic agents. Noteworthy, strict analogies exist between the behaviour of cells in culture treated with misfolded non-pathogenic proteins and in pathologic conditions, this instance together with the observation that the oligomers and fibrils are characterised by common structural features suggest that common mechanisms for cytotoxicity could exist and have to be perused in common interactions involved in aggregation.

We here report an experimental study on ConcanavalinA (ConA) aggregation and its effects on cells. *In vitro*, close to physiological temperature, this protein readily forms fibrils involving secondary structure changes leading to β -aggregate structures.

The effect of a ConA on cell cultures was tested and the formation of protein aggregates in these samples was studied by confocal fluorescence microscopy. We used the N&B analysis method to monitor ConA aggregation in live cells. The N&B analysis shows a rapid and progressive formation of ConA oligomers on cell membrane, even at very low protein concentration; simultaneously, the morphology of the cell changes indicating the progressive cell compaction and death. Cell surface probably provides nucleation sites for aggregation where high local concentration and macromolecular crowding favor aggregation. The formation of small aggregates may stimulate non-specific cellular response as a result of the exposure of reactive regions of protein structure and of the progressive formation of cross- β structures. Moreover, these oligomers could interact with the cell membrane damaging its structural organization and destroying its selective ion permeability. These results show the suitability of using ConA as a model protein and the N&B analysis as a powerful tool to measure aggregation in cells and to give new insights the relation between protein aggregation and disease. P41-RRO3155(EG).

455-Pos Board B334**Protein aggregation in live cells: N&B analysis of Huntingtin**

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The aggregation of the huntingtin (Htt) protein is thought to be responsible for Huntington's disease. One problem is the detection of the different size of aggregates and the stages of aggregation directly in live cells. Recently we develop a method (N&B) to detect the size of aggregates and the number of particles in every pixel of a confocal image. N&B can be performed on the entire cells simultaneously so it is possible to follow the kinetics of aggregation. This method is based on the variance of intensity fluctuations of particles as they diffuse through the excitation volume. We performed experiments in transfected COS7 using different lengths of the polyglutamine sequence (Httex1 97QP-GFP, Httex1 46QP-GFP and Httex1 25QP-GFP). We can determine the presence of units, small aggregates and inclusion bodies in different parts of cell and their time evolution. We observed the presence of Htt throughout the cell and that oligomer formation starts in the cytoplasm. At higher protein concentration aggregation occurs in the nucleus. When an inclusion body forms in the cytoplasm, all Htt including that in the nucleus, is recruited by the inclusion body. We found aggregates, which have a size of 5-10 protein units, diffusing in membrane tubules. We suggest that these aggregates may represent the precursors for protein nucleation events. We estimated that the inclusion body is formed by hundred of protein units. In different cell types the aggregation follows a similar dynamic. Our studies have shown the protein aggregation size distribution in live and how the nucleation events progress in real time. Support by U54 GM064346 CMC (MD, EG), NIH-P41-RRO3155 (EG), P50-GM076516 (EG,GO).

456-Pos Board B335**Sickle Hemoglobin Fiber Growth Rates Deduced Using Optical Channels**

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Sickle hemoglobin (HbS) is a point mutant of normal HbA, and will polymerize at concentrations above a well defined solubility. Polymerization occurs by a double nucleation mechanism in which homogeneous nuclei form in solution following a stochastic delay, and heterogeneous nuclei form on other polymers nucleated by either pathway. A fundamental element is the growth of individual fibers, whose diameter (20 nm) precludes direct optical visualization. Fiber growth and depolymerization have been measured by DIC microscopy, but the heterogeneous pathway makes it possible that bundles rather than single fibers have been observed, in addition to certain technical problems that make interpretation of the results less than simple. We have devised a method in which optical patterns are projected on a COHbS solution by laser photolysis, which creates deoxyHbS that can polymerize only in the illuminated area, easily allowing complex polymer structures to be created optically. In our experiment, polymers first form in an incubation circle. From the circle, a line of deoxyHbS is optically generated along which fibers can grow. Finally, a detection circle is illuminated and the connecting line is extinguished. If a polymer has entered the detection circle, thanks to heterogeneous nucleation it will fill the circle with easily observed polymers, otherwise the detection circle remains monomeric until, at some much later time, homogeneous nucleation occurs. Thus we can measure the elongation of a fiber too small to detect optically.

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.

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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 amino acid 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 demixing (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.

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

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Amyloidosis is characterized by extra cellular deposits of anomalous fibrillar 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 changes 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.

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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 formal-

dehyde 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.

References

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Intramolecular Diffusion of the Amyloidogenic 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 amyloidogenic 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.

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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 VTTGVTTITVTND) and *Saccharomyces cerevisiae* Flo1 adhesin (TDETIV 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.

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

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