

Posters

Protein Assemblies & Aggregates I**232-Pos Board B1****Molecular Dynamics Simulations of Gamma-Crystallin**

J. Alfredo Freitas, Eric Wong, William D. Brubaker, Carolyn N. Kingsley, Amanda J. Brindley, Rachel W. Martin, Douglas J. Tobias.

University of California, Irvine, Irvine, CA, USA.

Cataract, a leading cause of blindness worldwide, is caused by a loss of solubility of the eye lens structural proteins, called crystallins, that leads to spatial fluctuations in density and the formation of aggregates that scatter light. gammaS-crystallin is the most abundant structural protein in the human eye lens cortex and has been linked to an autosomal dominant early-onset cataract. It consists of two double Greek key domains which, although not identical in sequence, are highly symmetric, making this protein essentially a structural homodimer. Previously (Brubaker et al. *Biophys. J.* (2011) 100:498), we have shown that although the cataract-related G18V variant aggregates more readily than either wild-type or the symmetry-related G106V variant, it is not the most strongly destabilized. NMR structural studies of human gammaS wild-type and G18V revealed that the cataract-related variant remains fully folded and monomeric under experimental conditions with local structural changes around the point mutation (Brubaker and Martin, *Biomol. NMR Assign.* (2012) 6:63). These results suggest that aggregation propensity in gammaS-crystallin is not mediated purely by thermodynamic stability, but by a more complex interplay of protein-protein and solvent-protein interactions. Here, we report on atomistic MD simulations using recently determined human gammaS NMR structures of the wild-type and G18V variant as initial configurations to gain detailed insight into the changes in structural and hydration dynamics that result from cataract-related modifications in protein sequence.

233-Pos Board B2**Exploring the Interactions between a Coiled-Coil Peptide Model System and Metallated Porphyrins towards the Design of Photoelectronically Active Biomaterials**

James C. Taggart, Elizabeth Z. Taggart, Bashkim Kokona, Robert Fairman.

Haverford College, Haverford, PA, USA.

Porphyrin-peptide structures hold great potential for design of environmentally responsive nanowires, and porphyrin metallation may modulate these structures' electronic properties. To better understand these effects, the binding of metallated *meso*-tetrakis(4-sulfonatophenyl)porphine (TPPS₄) to the peptide Cp3K-N was investigated. Cp3K-N is a non-polymerizing model for the polymerizing peptide Cp3K, allowing for quantitative experiments that would be impossible in an aggregating system. Cu(II)TPPS₄, Ni(II)TPPS₄, Pd(II)TPPS₄, and Zn(II)TPPS₄ were studied using circular dichroism (CD) spectroscopy, UV-Visible spectroscopy, and sedimentation velocity analytical ultracentrifugation (SV-AU). CD and UV-Vis titrations of the ratio of Cp3K-N to porphyrin were performed, and binding constants for the complexes were determined. A blue-shifted Soret Band absorbance in the porphyrin, concomitant with induction of Cp3K-N alpha-helicity, was observed for all metals but zinc, which demonstrated a red-shifting of its Soret Band. Such spectroscopic characteristics suggest specific porphyrin-peptide binding. Titration experiments for the copper, nickel, and palladium porphyrins indicated equal peptide:porphyrin stoichiometry, while SV-AU experiments suggest higher order complex formation. These results confirm that metallated TPPS₄ can bind Cp3K-N with the specificity and tightness of TPPS₄, suggesting that such porphyrins have potential for use in polymerizing systems, towards the goal of designing novel photoelectronically active biomaterials.

234-Pos Board B3**A Kinetic Study of Amyloid Formation**

John Schreck, Jian-Min Yuan.

Drexel University, Philadelphia, PA, USA.

We propose a kinetic model for amyloid protein aggregation. The problem of protein aggregation is important for the understanding and treatment of neurodegenerative diseases and also for the development of bio-macromolecules as new materials. By extending several well-known models for protein aggregation, we write the time evolution of aggregate concentrations containing r proteins, denoted $c(r,t)$, in terms of Smoluchowski kinetics. With this approach we take into account all possible aggregation and fragmentation reactions involving clusters of any size. For example, two aggregates of sizes x and y could merge to form a larger aggregate of size $x+y$. Correspondingly, an aggregate of size $x+y$ could break-up into two smaller constituent aggregates of sizes x and y , respectively. The rates of each aggregation or fragmentation reaction,

called kernels, are specified in terms of the aggregate size, and we solve $c(r,t)$ for large cluster sizes using advanced numerical techniques. We show that by using Smoluchowski kinetics many pathways to fibrillation are possible and quantities, such as the aggregate length distribution at a given time, can be calculated. More importantly, the model shows good agreement with experimental results.

235-Pos Board B4**Amyloid Formation in Heterogeneous Environments: Islet Amyloid Polypeptide Glycosaminoglycan Interactions**

Hui Wang, Ping Cao, Daniel Raleigh.

SUNY-Stony Brook, Stony Brook, NY, USA.

Amyloid formation plays an important role in a broad range of diseases and the search for amyloid inhibitors is an active area of research. Amyloid formation takes place in a heterogeneous environment *in vivo* with the potential for interactions with membranes and with components of the extracellular matrix. Naturally occurring amyloid deposits are associated with sulfated proteoglycans and other factors. However, the vast majority of *in vitro* assays of amyloid formation and amyloid inhibition are conducted in homogeneous solution where the potential for interactions with membranes or sulfated proteoglycans is lacking and it is possible that different results may be obtained in heterogeneous environments. We show that variants of islet amyloid polypeptide, which are non-amyloidogenic in homogeneous solution can be readily induced to form amyloid in the presence of glycosaminoglycans and glycosaminoglycans are found to be more effective than anionic lipid vesicles at inducing amyloid formation. Several known inhibitors of IAPP amyloid formation are found to be less effective in the presence of glycosaminoglycans. The results indicate that peptide-GAG interactions may play important roles in amyloid formation *in vivo* and highlight the importance of considering these effects in the design and screening of inhibitors.

236-Pos Board B5**The Role of SDH Assembly Factor 2 and YgfY in Flavinylation of Succinate Dehydrogenase Flavoprotein**

Lala Zafreen, Li-Shar Huang, Nancy Walker-Kopp, Edward A. Berry.

SUNY Upstate Medical University, Syracuse, NY, USA.

A mutation of the mitochondrial protein SDH assembly factor 2 (AF2) has been linked to hereditary paraganglioma in humans (Hao et al.(2009), *Science* 325,1139-42). These authors showed that AF2 is required for flavinylation of the succinate dehydrogenase flavoprotein (SDHA) in human cells or yeast, and that yeast SDHA heterogeneously expressed in *E. coli* is flavinated only if yeast AF2 is coexpressed. We have extended these observations to the human protein: hSDHA is flavinated in *E. coli* only if coexpressed with human AF2. Flavinylation does not occur when purified recombinant hSDHA and AF2 are mixed with FAD in the presence or absence of succinate, fumarate, and MgATP, so some other factor provided by *E. coli* is required. We will report results fractionating *E. coli* cell lysate to determine the factors required and elucidate the role of AF2 in flavinylation. The *E. coli* protein YgfY is a distant homolog of mitochondrial AF2, and *E. coli* has SDH, the flavoprotein of which is very similar to that of mitochondrial SDH. We show here that a strain of *E. coli* with YgfY deleted is unable to grow with succinate as carbon source or to accumulate flavinylated SDH flavoprotein in its membranes. These observations are consistent with but do not prove a role for YgfY in maturation of SDH flavoprotein.

237-Pos Board B6**The Conformation of Monomeric Aggregation Precursor States Control the Aggregation of Lysozyme**

Mohanish Borana, Basir Ahmad.

UM-DAE- Centre for Excellence in Basic Sciences, Mumbai, India.

Protein aggregation, the process by which native proteins convert into insoluble fibrillar structures, has implication in human health, biotechnology and material science. Most studies on aggregation kinetics of proteins associated with diseases indicated accumulation of critical nucleus during the process. However, little is known about the conformation of aggregation nucleus and its precursor monomer. In spite of their importance these states are difficult to characterize mainly because they form transiently in the process. In this study, we have designed the conditions and method to stabilize the monomeric aggregation precursor state of lysozyme. We report that conformation of monomeric aggregation precursor state of lysozyme regulate the accumulation and conformation of critical nucleus of the aggregation. We would also show that the polymorphism in fibrils/protofibrils thus formed is ultimately controlled by monomeric aggregation precursor state. Small molecule inhibitor and accelerator of aggregation act by binding to native state of the protein and modifying the unfolding pathway of native state and conformation of aggregation precursor state. Our study offers opportunities to investigate the small differences existing in the mechanism of formation of different kind of aggregates.