exclusively in brain and testis. CaMKIIN proteins are highly specific inhibitors of CaMKII activity, but the physiological role and oligomeric state of these proteins are not known. It is also not known if interactions with the holoenzyme are stable or transient, or if these interactions are stoichiometric with kinase subunits. To address these questions we used Fluorescence Polarization and Fluctuation Analysis (FPFA), a hybrid method that simultaneously measures Homo-FRET and fluorescence correlation spectroscopy (FCS). FPFA studies reveal that C-terminal Venus-tagged CaMKIIN is a monomeric protein that can bind stably to Ca/CaM-activated CaMKII. Up to six CaMKIIN monomers can bind to the holoenzyme, suggesting that one CaMKIIN inhibitory protein interacts with two CaMKII subunits in the holoenzyme. Furthermore, Homo-FRET analysis of Venus-tagged CaMKII holoenzyme co-incubated with untagged CaMKIIN revealed the separation of catalytic domain pairs, but only after activation with Ca/CaM. This is consistent with our previous observation of catalytic domain unpairing in hippocampal neurons upon calcium influx through NMDA receptors. The C-terminus of CaMKIIN is thought to play a key role in inhibition of CaMKII because a reduction on inhibitory potency was observed when its C-terminal was tagged with Venus. Our results suggest that in addition to inhibiting the kinase, CaMKIIN regulates catalytic domain pairing.

Assemblies and Aggregates II

3444-Pos Board B172

Two-Dimensional Infrared Spectroscopy and Electron Microscopy of Seeded and Non-Seeded Amyloid β Peptide Fibrils

Jianqiang Ma[1], **Hiroaki Komatsu**[2], Paul H. Axelsen[2].
[1]Chemistry, University of Pennsylvania, Philadelphia, PA, USA,
[2]Pharmacology, University of Pennsylvania, Philadelphia, PA, USA.

Seeded and non-seeded fibrils from amyloid β 40-residue (Aβ40) peptides have been studied with two-dimensional infrared (2D-IR) spectroscopy and electron microscopy (EM). For the former, $^{13}C{=}^{18}O$ isotope labels were placed in various residues to probe residue-specific amide-I' vibrations of Aβ40 peptides. 2D-IR spectroscopy (2D-IR photon echo) of both kinds of fibrils shows distinct linear chain excitons of amide units due to the interamide vibrational coupling. Structural differences between the seeded and the non-seeded fibrils vary across the sequence. However, seeded fibrils tend to have a better aligned tertiary structure than non-seeded fibrils. Negatively stained EM images indicate that non-seeded fibrils had no discernible narrowing or nodes. In comparison, seeded fibrils tend to appear twisted like a ribbon, with periodic narrowing or nodes. The distances between nodes (the ''internodal'' distances) were relatively homogeneous distributions (~75 nm) and the same in all isotopically labeled peptides. The apparent width of the fibrils in these images indicate that seeded fibrils are two kinds and that the width of the narrower fibrils is the same as the width of non-seeded fibrils. The mass-per-length evaluated from dark-field EM images indicates that the most prevalent number of filaments in seeded fibrils is 6, which is twice as many as the number in non-seeded fibrils (3 filaments per fibril). Overall, these results demonstrate that seeded and non-seeded fibrils have distinctly different tertiary structures.

3445-Pos Board B173

A New Quantitative Bead Aggregation Assay for Determining the Association Rates of Protein-Protein Interactions

Nagamani Vunnam, Natalie René Phongam, Ashton Walters, Nathan Hammer, Susan Pedigo.
Chemistry and Biochemistry, University of Mississippi, University, MS, USA.

Developing new quantitative methods for studying the interactions of cell surface proteins is imperative to advance understanding of the molecular determinants of cell adhesion. Our interest in the dimerization kinetics of cadherin, a calcium-dependent cell-cell adhesion molecule, has motivated us to develop a quantitative bead aggregation assay to study the effects of calcium concentration on the kinetics of association. Although bead aggregation has been used for some time to study protein-protein interactions, the data are usually only interpreted qualitatively. Here we establish a quantitative method for studying the aggregation of beads that are coated with neural-cadherin. Data indicated exponential growth in aggregate size as a function of time. Time constants of association ranged from 18 to 3 minutes over a 100 fold increase in calcium concentration. We used this method to study the slow association kinetics of a mutant cadherin (NCAD1/R14E). These studies yielded a time constant of 28 minutes, which indicates that the association rate of mutant is slower than wild type (9 minutes). Experimental factors were controlled to ensure precision in the data including protein binding to beads, incubation time in the presence of calcium, mixing of the beads prior to placing on the slide and the residence time on the slide before data were acquired. This method is the first report of experimental determination of the kinetics of the association of proteins tethered to a surface, and is broadly applicable to study the kinetics of protein-protein interactions.

3446-Pos Board B174

Structural and Hydration Properties of Huntingtin Aggregates Determined by Small-Angle Neutron Scattering

Christopher B. Stanley[1], Tatiana Perevozchikova[2], Helen P. McWilliams-Koeppen[2], Valerie Berthelier[2].
[1]Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [2]Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN, USA.

Huntington's disease involves an abnormally expanded polyglutamine sequence in huntingtin protein (Htt-exon1) making it highly susceptible to aggregate formation. A current challenge is to map out the aggregation pathway by identifying the various precursor structures and establishing their roles in the disease. Here, we are using time-resolved small-angle neutron scattering (SANS) to follow the aggregation kinetics of both wild type and pathological Htt-exon1, where we obtain snapshots of the structures formed as the kinetic reaction ensues. An advantage of neutrons for these long time-scale measurements is that they are non-damaging to the biological sample. The scattering data can be fit using a multiple Guinier-Porod empirical model to account for the presence of multiple species, and this provides quantitative information on the size and shape of the precursors and internal structure of the resulting fibrils. Importantly, both the early-formed oligomers and mature fibrils of pathological Htt-exon1 exhibit distinct structural differences compared to wild type. Neutron contrast variation affords us with the ability to also probe corresponding hydration differences. This research is providing new insights into the pathway of Htt-exon1 aggregation and should later assist in determining the role that precursors play in neuronal toxicity.

3447-Pos Board B175

Enhancement, Equal Fluorescence Efficiency, and Quenching in the Interpretation of Fluorescence Anisotropy Data

Zahra Zolmajd-Haghighi, **Quentin Hanley**.
School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom.

Decreases in fluorescence anisotropy from homo-FRET processes can report on the number of dyes on a protein or the size of a cluster. Fluorescent dyes exhibit a range of behaviours from fluorescence enhancement to quenching when assembled into clusters. The degree of enhancement and/or quenching depends on the number of dyes in close proximity. When interpreting anisotropy, an assumption of equal fluorescence intensity is widely applied. This assumption predicts, for example, that 3 fluorophores in a cluster have the same fluorescence intensity as the same three fluorophores outside of a cluster. This assumption will give incorrect predictions in cases where either quenching or enhancement of fluorescence occurs. Additionally, existing theory typically assumes that all positions within a cluster are equivalent.

Application of these assumptions affects interpretation in two ways. It will tend to under-predict the anisotropy of a stochastic mix of individual species and, depending on whether the dye system is quenched or enhanced, will either under-predict or over-predict cluster sizes.

Based on computations exploring the impact of enhancement, quenching, and inhomogeneous clustering, a number of conclusions may be reached. As fractional labeling approaches one all models converge to the same value. Inhomogeneous labeling tends to increase anisotropy at low fractional labeling. Applying equal fluorescence intensity assumptions to a fluorescence enhanced system of dyes will over-predict cluster size. Applying it to a quenched system will under-predict cluster size. These cases will be illustrated with simulations and experimental data.

3448-Pos Board B176

Charge Crowding Promotes Self-Assembly of Collagen Hetrotrimers

Nida F. Hasan, Avanish S. Parmar, Mihir Joshi, Patrick Nosker, Vikas Nanda.
Department of Biochemistry and Molecular Biology, Center of Advanced Biotechnology and Medicine, Robert Wood Johnson Medical School, Rutgers Univeristy, Piscataway, NJ, USA.

The design of heterospecific collagen like peptides presents a large challenge in the field of protein design. Many rational and computational approaches have been used to achieve this goal. Our model suggests that crowding of charged

amino acids could help in driving the desired heterospecificity. Stability and specificity of the desired state was targeted using positive and negative design. With this approach, we have targeted the folding of the heterotrimer state by destabilizing homotrimer states via electrostatic repulsion. In this study, we sought to investigate the specific impact of repulsive interactions at the molecular level. Three peptides have been designed by introducing charge crowded acidic amino acids either at N-terminal, central, or at C-terminal end of the Pro-Hyp-Gly sequence repeats. Our CD and NMR results demonstrate that charge crowding may destabilize the homotrimer states in our triple helical peptide system and can be utilized to promote the formation of the heterotrimer. Increase in salt concentration or decrease in pH result in an increase of homotrimer stability, which confirms the role of charge crowding on the destabilization of homotrimers via electrostatic repulsion. Further investigation is required to understand the molecular role of charge crowding and can be used in conjunction with other approaches to create specific collagen heterotrimers. We further extended our study to create higher order structure by designing basic amino acids sequences in place of acidic amino acids. Mixing of basic and acidic amino sequences resulted in higher order structures.

3449-Pos Board B177

Urea, Guanidine Hydrocloride and 2,2,2-Trifluoroethanol Can Change the Amyloid Fibril Formation of Model Proteins: A Spectroscopic Study

Leandro R.S. Barbosa.
General Physics - DFGE, Institute of Physics of University of Sao Paulo, Sao Paulo, Brazil.

The influence of external agents on proteins function and structure is essential to elucidate the unfolding pathways and the protein self-assemble properties. The knowledge of the protein amyloid fibril formation process is important due to the fields that this subjected is related, in particular for the neurodegenerative disorders as Parkinson's and Alzheimer's diseases. In the present study we evidenced the influence of different external agents on the Bovine Serum Albumin (BSA) and Lysozyme amyloid fibril formation by means of UV-Vis and Fluorescence spectroscopy. Concerning BSA, the presence of urea (< 3M) was able to induce the formation of amyloid fibrils at 328 K and increasing urea concentration the amount of protein in the amyloid form also increases. Moreover, a different effect was evidenced by the presence of TFE, where BSA underwent an amorphous aggregation process, leading even to the flocculation of BSA. Interestingly the presence of both urea and TFE up to 5% induces the appearance of amyloid fibrils, instead of amorphous aggregation. Regarding GndHCl, it was not able to induce the formation of amyloid fibrils in neither BSA nor Lysozyme. It is interesting that GndHCl and Urea are well-known as protein denaturant agents, however, their interaction in the protein surface is quite different, such a difference could lead the protein to different final conformations, including the amyloid fibril one. This study indicates that the hydration shell can play an important role in the amyloid aggregation process.

Acknowledgements: FAPESP (grant # 2012/01953-9) CNPq (grant # 479229/2011-2, # 303048/2012-3).

3450-Pos Board B178

The Oligomeric State of Human Alpha-Defensin 1 in Solution

Grzegorz Piszczek.
Biophysics and Biochemistry Center, NHLBI/NIH, Bethesda, MD, USA.

Defensins are small (18-45 amino acids) proteins present in organisms from plants to humans. They function as host defense peptides, assisting the immune system cells in killing phagocytosed bacteria, and are also active against fungi and viruses. The defensins mode of action is membrane permeabilization - forming fatal, pore-like membrane defects in microbial cells. Since membrane permeabilization models require formation of protein dimers or higher oligomers to open pores in the membrane, the defensins propensity to oligomerize is important to their function and is recently a subject of many research projects. Studying oligomerization of small proteins and peptides presents many challenges. Several methods are suitable to detect oligomeric species, especially after chemical cross-linking, but only the light scattering and analytical ultracentrifugation based measurements allow estimating the equilibrium oligomerization constants without chemical modification of proteins. Still, low scattering light intensity, high diffusion and slow sedimentation rates of peptides make obtaining accurate results difficult. We will present a detailed study of the human alpha-defensin 1 (HNP1) oligomerization. HNP1, prevalent in human neutrophil granulocytes, is not only a potent antimicrobial agent, but was also shown to have cytotoxic activity against many mammalian tumor cells in vitro.

3451-Pos Board B179

Aggregation of Transforming Growth Factor β Induced Protein Studied by Protein-Protein Docking

Ole J. Andersen, Heidi Koldsø, Birgit Schiøtt.
inSPIN, iNANO and Department of Chemistry, Aarhus University, Aarhus, Denmark.

Transforming growth factor β induced protein (TGFBIp) is an extracellular matrix protein that has been linked to several types of corneal dystrophies. Nearly fifty percent of the mutations leading to corneal dystrophies affect two major single residue hotspots, namely R124 and R555, located in the first and the fourth FAS1 like domain of TGFBIp, respectively (1). The remaining mutations are primarily located in the fourth FAS1 like domain (FAS1-4). Three mutations in the FAS1-4 domain of TGFBIp are investigated in the present study: R555W, leading to granular corneal dystrophy; R555Q, leading to Thiel-Behnke corneal dystrophy; and A546T, leading to lattice corneal dystrophy. Of the three mutations, A546T is the only one resulting in deposits of amyloid fibrils *in vivo* and *in vitro.* The aim of this study is to investigate whether or not the three pathogenic mutations give rise to distinct association poses discerning between amorphous and amyloid phenotypes. Protein-Protein docking calculations have been performed using the docking algorithm DOCK/PIERR (2). Two highly abundant poses involving direct contact between the suspected fibrillating core regions of the FAS1-4 domain have been identified. Runager et al. has shown the A546T mutant to be the least stable of the three mutants (3). Combining the decreased stability with the docking poses could give an explanation to the amyloidogenic nature of the A546T mutant.

1. Munier et al. *Invest. Ophthalmol. Vis. Sci.* **2002***, 4,* 949-954.
2. Ravikant et al. *Proteins* **2010***, 2,* 400-419.
3. Runager et al. *J. Biol. Chem* **2011***, 7,* 4951-4958.

3452-Pos Board B180

Stable Amyloid Oligomers can Seed Fibril Growth Near Physiological Conditions

Mentor Mulaj, Tatiana Miti, Joseph Foley, Martin Muschol.
University of South Florida, Tampa, FL, USA.

Spontaneous formation and deposition of protein fibrils with cross beta-sheet structure are associated with an increasing number of human disorders including Alzheimer's disease, senile systemic or dialysis-related amyloidosis. The morphological and structural similarities of amyloid deposits and their various intermediates suggest that amyloid diseases share basic aspects of their etiology. Elucidating the molecular mechanisms underlying these similarities has been challenging since biophysical studies of fibril formation *in vitro* require partial denaturation of native proteins. These conditions are far from the physiological environment relevant to fibril growth *in vitro*. Here we show that metastable amyloid oligomers and protofibrils of lysozyme, transiently formed under denaturing conditions, can become stabilized at physiological conditions. In addition, they are capable of inducing the conversion of natively folded, monomeric lysozyme into aggregated β-sheet rich fibrils *in vitro*. There was no discernible threshold concentration of lysozyme monomers below which seeding didn't induce growth.

Our data suggest that only the nucleation of amyloid oligomers is a low-probability event requiring partially denaturing conditions. Once formed, these seeds can become stabilized under near physiological conditions and induce seed elongation and fragmentation. The stabilization of amyloidogenic intermediates away from nucleation conditions, their ability to remain soluble or to seed subsequent fibril growth of native monomers appear to be protein-specific. The oligomer/protofibril-induced growth of native lysozyme observed in our experiments bears intriguing similarities with the ability of prion proteins to drive the autocatalytic conversion of their native counterpart into amyloid fibrils.

3453-Pos Board B181

Phase Boundaries for Fibril and Metastable Oligomer Formation of Lysozyme

Tatiana Miti, Mentor Mulaj, Joseph Foley, Martin Muschol.
University of South Florida, Tampa, FL, USA.

Deposition of protein fibers with a characteristic cross-beta sheet structure is the molecular marker for many human disorders including Alzheimer's disease, type II diabetes and rheumatoid arthritis. Given the large number of proteins and peptides beyond those associated with diseases that have been shown to form amyloid fibrils in vitro, it has been suggested that amyloid fibril formation represents a generic protein phase transition. Mapping out the corresponding phase boundaries is complicated by the presence of least two distinct fibril assembly pathways. One pathway is characterized by the nucleation of long, rigid fibrils common to the late stages of amyloid diseases. A second pathway involves the formation of globular oligomeric species and curvilinear protofibril.