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can mature into fibrillar bundles due to hydrophobic interactions between the fibrils. Besides genetic modifications, our study stands for the first example of formation of protein fibrils under native conditions through posttranslational modification. Our finding suggests that long time exposure of organisms to UV radiation may cause damage of protein functions.

2930-Pos Board B35
A 2DCOS Infrared Study of Fibril Formation
Igor de la Arada, Nerea Andraka, **Jose Luis**, R. Arrondo.
Amyloid fibrils are proteinaceous aggregates that can be formed in the process of degenerative diseases. Insulin is a model of fibril formation that has produced a wealth of biochemical and structural data. The time-course of fibril formation can be followed by infrared spectroscopy looking at the appearance of a characteristic band in the lower region of extended structure. The kinetics is triggered by temperature at 70 °C and pH 2.3.The infrared spectrum shows, that after a lag time (concentration-dependent), the α-helical band decreases and the random coil component increases subsequently. Random coil increases up to a percentage and later a band at 1626 cm-1, associated with extended chains, replaces the random coil component. Infrared 2D-COS has been applied to different stages of the process. Maps have been formed at different incubation times: before random coil formation and at different stages in the random coil-fibril change. Synchronous two-dimensional IR map shows that the process occurs in a two step mode. At pH 7.0, heating of insulin for long periods does not produce the random coil structure and subsequently no fibrils are formed. Human insulin, with a different aminoacid in the N-terminal segment, forms a fibril formation in a lower time than bovine or porcine. The bandshape corresponding to the fibril is different if bovine insulin is compared with human and porcine; what can be associated with a difference in aminoacids 8 and 10 that are located in the intrachain disulfide bond loop. The results show that small changes in protein sequence makes the kinetics different. Lipids have been proposed as one factor influencing fibril formation. The effect of different lipid composition, including anionic lipids, sphingomyelin and cholesterol has also been studied to see changes in kinetics looking at the lipid charge.

2931-Pos Board B36
General *In-Vitro* Catalysis of Amyloid Formation by the Bacterial Curli Protein
Kevin Hartman, Jeffery Brender, Nataliya Popovych, Matthew R. Chapman, Ayyalusamy Ramamoorthy.
Proteins misfolded into insoluble, fibrillar aggregates known as amyloid are a pathological feature of many common and devastating diseases. Amyloid formation is typically a slow process that can be strongly affected by extrinsic factors, among the most critical being the presence of a small amount of preformed seeds that serves to nucleate aggregation. Amyloid nucleation is often considered a highly specific process dependent on a high degree of similarity in both peptide sequence and fiber morphology. However, we show here that amyloid fibers known as curli that are produced in E. coli and related bacteria catalyze amyloid formation of a variety of dissimilar amyloidogenic peptides and proteins, including PAP248-286 (SEVI), insulin, and calcitonin. The preformed curli fibers appear to act as a nucleation site for amyloidogenic proteins and as such, can decrease the induction time, sometimes drastically, and induce the formation of fibers. In particular, cross-seeding of SEVI amyloid formation by curli was more effective than seeding the reaction with SEVI amyloid fibers obtained under a different reaction condition. The elongation rate of fiber formation is also increased for some (but not all) of the proteins tested, indicating curli can also increase in some circumstances the rate of addition of proteins to the ends of amyloid fibers. Curli and curli-like amyloid fibers are ubiquitous in mammalian hosts, in fact, the innate immune response common to almost all amyloids has been proposed to have evolved as a response to curli amyloid formation by E. Coli. Given that certain bacteria that express a curli-like protein colocalize with amyloid deposits in Alzheimer?s patients, the induction of amyloid formation by curli may be a factor of high clinical importance.

2932-Pos Board B37
Structure, Dynamics and Surface Hydrophobicity of the Cataract-Associated Mutant, Pro23Thr of Human Gamma D-crystallin: Molecular Basis of Cataract Formation
Priya R. Banerjee, Swamy Puttamadappa, Ajay Pande,
Alexander Shekhtman, Jayanti Pande.
The cataract-associated Pro23Thr (P23T) mutation in human gammaD-crystallin (HGD) is geographically widespread - thus there is considerable interest in determining the molecular basis of opacity. In an earlier study (1), we found that the mutant showed markedly lowered, retrograde solubility compared to wild-type HGD, leading to the conclusion that the aggregation of P23T was mediated by hydrophobic protein-protein interactions. Subsequently, using NMR (2) and a binding assay with the fluorescent dye Bis-ANS (3), we showed that, in fact, hydrophobic patches were generated on the surface of the mutant protein. Those studies (3) also suggested that the binding site for Bis-ANS on P23T may coincide with the self-association site of the mutant and result in its lowered, retrograde solubility. Here we present new NMR-evidence to identify the Bis-ANS binding sites, and using independent NMR dynamics studies, also show that there is a measurable reduction in the flexibility of the peptide backbone near the hydrophobic patches. These two factors, namely the creation of hydrophobic patches and the lowered peptide backbone flexibility, taken together make a compelling argument that the hydrophobic patches may indeed facilitate the nucleation of aggregates of P23T which are held together by net hydrophobic interactions. Such aggregation would explain the retrograde solubility as well as the lens opacity *in vivo*.
1. Pande et al (2005) *Biochemistry*, **44**, 2491-2500.
2. Pande et al (2009) *Biochem. Biophys. Res. Commun.,* **382**, 196-199.
3. Pande et al (2010) *Biochemistry,***49,** 6122-6129.
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2933-Pos Board B38
Polymorphism of Amyloid Fibrils Formed by a Short Peptide from Yeast Prion Protein Sup35: AFM and Tip Enhanced Raman Scattering Study
Alexey V. Krasnoslobodtsev, Alexander M. Portillo, Tanja Deckert-Gaudig, Volker Deckert, Yuri L. Lyubchenko.
Misfolding of prion protein and its subsequent aggregation is the cause of various prion related diseases. Prions are composed of aggregates of a misfolded prion protein. Although primary structure of prion protein is the same, its infectious form, amyloid fibrils, exist as multiple strains. The ability of prions to generate multiple strains poses an immediate health threat, which renders studies of this phenomenon very important. The strains are thought to represent structurally different prion protein molecules packed into amyloid fibrils. The biophysical properties of the fibrils, such as fragility, represent a major mechanism of prion amplification. Here, we demonstrate that variations in environmental conditions such as pH, salt concentration, temperature and mechanical stress (stirring) produces a variety of fibrillar polymorphs for a short peptide CGNNQQNY from yeast prion protein Sup35. The fibrils differ by their length and diameter as well as their ability to bundle together. We have used Tip-Enhanced Raman Scattering (TERS) in combination with AFM to study underlying conformational peculiarities of peptides within individual aggregates. Two types of fibrils were investigated, one formed in water and another one at pH 5.6. These conditions produce morphologically distinct fibrils in terms of their length and diameter. They also exhibited different kinetics of aggregation. The Raman spectra obtained with TERS also revealed the peptide conformational differences between these two types of fibrils. The observed differences are mostly manifested in the positions of the characteristic amide bands (I and III), suggesting that peptides in these two types of fibrils have different conformational states. This study demonstrates potentials of such a combined method as TERS/AFM for structural analysis of individual protein aggregates.

2934-Pos Board B39
Amyloid Aggregates Alter the Membrane Mobility of GM1 Gangliosides
Martino Calamai, Francesco S. Pavone.
Neuronal dysfunction in neurodegenerative pathologies such as Alzheimer's disease is currently attributed to the interaction of amyloid aggregates with the plasmamembrane. Amongst the variety of toxic mechanisms proposed, one involves the binding of amyloid species to GM1 gangliosides. GM1 takes part into the formation of membrane rafts, specialized microdomains responsible for the compartmentalization of cellular processes such as signalling and protein trafficking. GM1 has antineurotoxic, neuroprotective, and neurorestorative effects on various central neurotransmitter systems. In this study, we investigated the effect of amyloid-like aggregates formed by the yeast prion Sup35 on the membrane mobility of GM1. Although Sup35 is not associated to any disease, it contains a highly amyloidogenic structural motif (Sup35NM) and has been used extensively as a model peptide for studying amyloid aggregation. Preformed Sup35 and Sup35NM aggregates were incubated with neuroblastoma cells and GM1 molecules were subsequently labeled with biotinylated CTX-B and streptavidin quantum dots (QDs). Single QDs bound to GM1 were then tracked. The trajectories of QDs labeled GM1 molecules were used to calculate their mean square displacement (MSD) and extrapolate their diffusion coefficients (D). The diffusion behavior of GM1 in the absence or in the presence of full length Sup35 aggregates was found to be substantially identical. By contrast, the mobility of GM1 decreased dramatically in the presence of Sup35NM aggregates. In this case, the median D of GM1 was found to be approximately one order of magnitude lower. Furthermore, the motion of GM1 appeared to change from mostly Brownian in the absence of the Sup35NM aggregates to mainly confined in their presence. The considerable interference of amyloid-like aggregates with the lateral diffusion of GM1 might imply a consequent loss of function of GM1, thus contributing to explain the toxic mechanism ascribed to this particular interaction.