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 $T_C = 45°C$, 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.

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**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.

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**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