holo, PLP-bound form. We also determined the crystal structures of the three apo mutants at 1.9Å resolution and compared them to the known structure of holo, wt *P. vulgaris* Trpase in order to unravel the mechanism of reversible cold dissociation of Trpase. Crystal structure analysis revealed that the mutants in their apo form are found in an "open" conformation compared to the "closed" conformation of *P. vulgaris* in its holo form. We show here for the first time that the cold inactivation and dissociation of *E. coli* Trpases is primarily affected by PLP release. The main cause for the enhanced loss of activity of the three mutants is due to the change in the size of the amino acid side chain. This prevents the tight assembly of the active tetramer, making it more susceptible to the cold driven changes in hydrophobic interaction which facilitates the PLP release.

2794-Pos Abnormal Formation And Remodeling Of Fibers Containing Type I Collagen Homotrimers

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Board B97

The normal form of type I collagen is an $\alpha1(I)_2\alpha2(I)$ heterotrimer. In fetal tissues, tumors, and several heritable disorders, $\alpha1(I)_3$ homotrimers have been found as well, but their role remains unclear. Here we report abnormal formation and remodeling of fibers containing the homotrimers. Turbidity measurements show five fibrillogenesis steps in hetero- and homotrimer mixtures: (i) early growth, (ii) low plateau, (iii) lag phase, (iv) delayed growth, and (v) high plateau. Only (i) and (ii) occur in homotrimers and only (iii)–(v) in heterotrimers. Confocal microscopy reveals that the homotrimers form spikes emanating from a common center while heterotrimers form an entangled, thread-like fibril network. In mixtures, initial homotrimer spike formation appears to be followed by heterotrimer fibrillogenesis at spike surfaces, resulting in intermediate fibril morphology. Hetero- and homotrimers copolymerize into fibrils, but they segregate at a sub-fibril level, e.g., into separate microfibrils. With increasing homotrimer fraction in the mixture, the solubility of the heterotrimers in equilibrium with fibrils remains constant whereas the solubility of the homotrimers increases, consistent with the segregation and presence of pure heterotrimer microfibrils. The segregation may affect tissue remodeling by matrix-metalloproteinases (MMP-1 and MMP-13). Indeed, we find that the homotrimers are cleaved ~10 times slower than the heterotrimers. The corresponding Km are similar. The lower cleavage rate of homotrimers is associated with a reduced Vmax, indicating a preferential MMP interaction with the α2(I) chain at a rate-limiting step. The uneven cleavage rates may cause accumulation of aberrant homotrimer microfibrils upon remodeling of segregated fibrils by MMP. This may explain, e.g., how a small fraction of the homotrimers can cause a predisposition to osteoporosis, which was recently linked to the α1(I) chain overproduction in individuals with an SP1 polymorphism in the α1(I) promoter region.

Protein Aggregates

2795-Pos Structure of α-synuclein Oligomers by Site-directed Spin Labeling

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Board B98

The misfolding and aggregation of α-synuclein is central to the pathology of Parkinson's disease (PD) and other neurodegenerative disorders. Growing evidence indicates that the primary pathogenic species are the nonfibrillar oligomer rather than mature fibrils. Numerous studies on the aggregation pathway of α-synuclein have described different oligomeric structures, which include spheres, rings, chains and amorphous structures in addition to the fibril. Although various biophysical and biochemical techniques have been used to examine the structure of these oligomeric states of α-synuclein, a direct comparison of the structural conformation between these oligomer types and the fibril has not been shown. In this study, we used electron paramagnetic resonance (EPR) spectroscopy combined with site-directed spin labeling to obtain residue-specific structural information for oligomeric α-synuclein. Using EPR spectroscopy, two different types of oligomeric structures were identified. In addition, we observed that the structures of both types of oligomers do not match the fibril structure, indicating that these oligomers are conformationally unrelated to the fibril. Based on these results, we are testing the hypothesis that these oligomers are not structural precursors to the fibril, but are instead "off-pathway" to fibril formation, providing further support for the suggestion that fibrils may be protective and that the oligomers are the toxic species. This has important implications for the understanding of PD pathology and for the development of disease modifying therapeutics for the treatment of PD and other α-synuclein related diseases.

2796-Pos Elucidating the Mechanism of Amyloid Formation by Horse Apomyoglobin

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Board B99

Myoglobin is a 153 residue long globular protein responsible for transporting oxygen in muscle tissue. In its native form, myoglobin is composed of eight well characterized α-helices, which collapse into a spherical tertiary structure stabilized by hydrophobic interactions. However, in spite of the high helical content of native

myoglobin, skeletal horse apomyoglobin can be induced to form amyloid-like aggregates at high temperature and pH. In this work, a combination of FTIR spectroscopy, thioflavin T fluorescence assays and AFM were used to elucidate the mechanism by which horse apomyoglobin forms amyloid-like aggregates. We found this mechanism to be a complex, multi-step process. In a first step, native apomyoglobin can be induced to form a partially unfolded intermediate by increasing the pH. If the concentration of the sample is higher than ~40 μ M, this unfolded intermediate polymerizes and precipitates out of solution and amyloid-like aggregates are never formed. However, if the concentration of the apomyoglobin sample remains below ~40 μ M, the majority of the unfolded intermediate remains in solution (likely as a mono- or oligomer) and can be induced to form amyloid-like aggregates when the temperature of the sample is raised to 55° C or above. Once the temperature is raised, the partially unfolded intermediate rapidly undergoes a conformational change in a second step forming amyloid like-aggregates within 30 minutes. These aggregates continue to grow slowly over time in a third and final step. The fact that the temperature has to be raised above 55° C for the formation of amyloid-like aggregates to proceed seems to point to the existence of an energy barrier between the aggregated intermediate and the amyloid-like aggregates. These results show that the formation of amyloid-like aggregates by apomyoglobin is a complex process finely regulated by sample concentration, temperature and pH.

2797-Pos Folding of Polyglutamine Chains

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Board B100

Long polyglutamine chains have been associated with a number of neurodegenerative diseases. These include Huntington's disease, where expanded polyglutamine (PolyQ) sequences longer than 36 residues are correlated with the onset of symptoms. In this talk we compare the folding pathways of long PolyQ chains into several meta-stable folded structures, including a beta-helical structure and a beta-sheet structure. Transition path sampling Monte Carlo simulations are used to generate unbiased reactive pathways between the random coil and the folded structures of the polyglutamine chain. Our simulations for folding to the beta-helical structure reveal that the formation of a few critical contacts is necessary and sufficient for the molecule to fold. Once the primary contacts are formed, the fate of the protein is sealed and it is largely committed to fold. We find that, consistent with emerging hypotheses about PolyQ aggregation, a stable "rich" structure could serve as the nucleus for subsequent polymerization of amyloid fibrils. Our results for beta-helical structure indicate that PolyQ sequences shorter than 36 residues cannot form that nucleus, and it is also shown that specific mutations dictated by our folding pathway analysis exacerbate its stability.

2798-Pos Thermodynamic Stability Of Amyloid Cross-β Spine Patterns As A Guide For Structural Selection And Molecular Polymorphism

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Board B101

Crystal structures of the amyloid cross-β spine have been found recently, which possess the characteristic dehydrated bilayer interface, referred to as the steric zipper. Subsequent computational studies probed their intrinsic properties such as the helical pitch and the size-dependent stability of oligomers. However, the selection mechanism of the native bilayer structure over other possible patterns has not been addressed yet. We developed a systematic procedure for generating potential steric zipper patterns and calculating the binding free energy of inserting a monomer into them. This method requires minimal experimental input in constructing candidate bilayer patterns, namely the β-sheet type and the inter-peptide distance in a β-sheet. The patterns differ in the relative β-sheet orientation and the registry of side chains in the interface. The binding free energy was calculated using a thermodynamic cycling method that combines molecular dynamics simulations in explicit and implicit solvents. In addition, normal mode analysis was used to account for the vibrational free energy. We examined possible filaments formed by the peptide GNNQQNY whose x-ray structure is available (PDB ID: 1YJP). The pattern with the lowest binding free energy was compatible with the x-ray structure; the RMSD deviation was less than the crystal resolution. Analysis of the non-bonded contribution to the free energy by individual residues revealed that Q4 has the largest stabilizing role while G1 played a minimal role, in agreement with previous experimental and computational results. When we tested another peptide NNQQ, there were multiple minima with similar stability that corresponded to two known crystal structures of the peptide. The present approach thus can be used to predict the cross-β structure and to identify key stabilizing residues, as well as suggest the possibility of molecular polymorphism.

2799-Pos Time-Resolved Infrared Spectroscopy of pH-Induced Aggregation of the Alzheimer Aβ 1–28 Peptide

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Board B102

Aggregation of the Alzheimer's disease related Aβ 1–28 peptide has been induced by a rapid, sub-millisecond pH jump and monitored by time-resolved infrared spectroscopy. The release of a proton induced by the photolysis of a caged compound (NPE-sulfate) and the

pH-dependent structural transition of the peptide have been monitored on the millisecond to second time-scale. The aggregation of the Aβ 1–28 peptide induced by the pH jump from 8.5 to below 6 yields an antiparallel β-sheet structure. The kinetics of the structural transition is biphasic, showing an initial rapid phase with a transition from random coil to an oligomeric β-sheet form with approximately 5–10 strands during 3–4 s. This phase is followed by a second slower transition, which yields larger aggregates during 30–50 s. The pH jump generated in our experimental setup is used to model the Aβ peptide structural conversions that may occur in the acidic endosomal/lysosomal cell compartment system.

2800-Pos Aβ 40 Protects Nontoxic Aβ 42 Monomer from Aggregation

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Board B103

Aβ 40 and Aβ 42 are the predominant Aβ species in the human body. Toxic Aβ 42 oligomers and fibrils are believed to play a key role in causing Alzheimer's disease (AD). However, the role of Aβ 40 in AD pathogenesis is not well established. Emerging evidence indicates a protective role for Aβ 40 in AD pathogenesis. Although Aβ 40 is known to inhibit Aβ 42 fibril formation, it is not clear whether the inhibition acts on the nontoxic monomer or acts on the toxic Aβ 42 oligomers. In contrast to conventional methods that detect the appearance of fibrils, in our study Aβ 42 aggregation was monitored by the decreasing NMR signals from Aβ 42 monomers. In addition, differential NMR isotope labelling enabled the selective observation of Aβ 42 aggregation in a mixture of Aβ 42 and Aβ 40. We found Aβ 40 monomers inhibit the aggregation of nontoxic Aβ 42 monomers, in an Aβ 42/Aβ 40 ratio dependent manner. NMR titration revealed that Aβ 40 monomers bind to Aβ 42 aggregates with higher affinity than Aβ 42 monomers. Aβ 40 can also release Aβ 42 monomers from Aβ 42 aggregates. Thus, Aβ 40 likely protects Aβ 42 monomers by competing for the binding sites on pre-existing Aβ 42 aggregates. Combining our data with growing evidence from transgenic mice and human genetics, we propose Aβ 40 plays a critical, protective role in Alzheimer's by inhibiting the aggregation of Aβ 42 monomer. Aβ 40 itself, a peptide already present in the human body, may therefore be useful for AD prevention and therapy.

2801-Pos Anti-prion Properties of Prion-protein Derived Cell Penetrating Peptides

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Board B104

In prion diseases, the cellular prion protein (PrP^{C}) becomes misfolded into the pathogenic isoform of the prion protein called scrapie (PrP^{Sc}) via a conversion mechanism triggered by PrP^{Sc}. Cell-penetrating peptides (CPPs) are short peptides, less than 30 residues and usually with a high positive charge, which are capable of penetrating cellular membranes and translocate attached macromolecules into cells. We show that peptides derived from the prion protein N-terminus which are CPPs, have potent anti-prion effects. Healthy and scrapie-infected mouse neuronal hypothalamic cells were treated with various CPPs. Lysates were analyzed by Western blot for levels of PrP^{C} or PrP^{Sc} compared to control cultures. We show that treatment with the prion protein-derived CPPs $mPrP_{1–28}$ from mouse and $bPrP_{1–30}$ from cow, reduce PrP^{Sc} levels in prion-infected cells, but have no effect on PrP^{C} protein levels in non-infected cells. The peptides are composed of a hydrophobic signal sequence (residues 1–22 in mouse PrP) followed by a basic segment (residues 23–28 in mouse PrP). Further, when non-infected cells were infected with scrapie, the prion protein-derived CPPs dramatically prolonged the prion course of infection. Treatment with other CPPs (penetratin, transportan-10 and poly-L-arginine) or other prion protein-derived peptides lacking a CPP function ($mPrP_{23–28}$ and $mPrP_{23–50}$) has no effect on PrP^{Sc} levels. The results suggest a mechanism by which the observed effects are accomplished: the signal sequence guides the prion-derived peptide into a cellular compartment where the scrapie conversion takes place, whereupon the basic sequence binds to the aggregated scrapie material and hinders further accumulation of PrP^{Sc}.

2802-Pos Amyloid Formation and Membrane Disruption by Islet Amyloid Polypeptide are Separate Processes Localized in Different Regions of the Peptide

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Board B105

One of the hallmarks of Type II diabetes is the presence of highly aggregated amyloid deposits of human Islet Amyloid Polypeptide Protein (hIAPP). Because the ability of the IAPP peptide to form amyloid fibers has been shown to strongly correlate with the peptide sequence within the 20–29 region of the peptide, variances of the sequence within this region have been used to explain the relative toxicity of different versions of the peptide. However, despite the strong tendency of the $IAPP_{20–29}$ fragment to form amyloid fibers, the toxicity of this fragment is significantly less than the full length

peptide. Our results indicate that the N-terminal region of the peptide (residues 1–19), rather than the 20–29 region, is primarily responsible for the membrane disrupting effect of the hIAPP peptide. Our liposome leakage experiments confirm that the $hIAPP_{1-19}$ fragment at low peptide concentration induces membrane disruption to an identical extent as the full length peptide. At higher peptide concentrations, the $hIAPP_{1-19}$ fragment induces a greater extent of membrane disruption than the full-length peptide due to the full-length peptide's tendency to form inert aggregates. Similar to the full length peptide, $hIAPP_{1-19}$ exhibits a random coil conformation in solution and adopts an α-helical conformation upon binding to lipid membranes. However, the formation of amyloid fibers by the $hIAPP_{1-19}$ fragment is inhibited by at least two orders of magnitude compared to the full-length peptide. These results indicate that membrane disruption can occur independently from amyloid formation in IAPP, and the sequences responsible for amyloid formation and membrane disruption are located in different regions of the peptide. The stability of the $hIAPP_{1-19}$ fragment enabled the study of the membrane-peptide system at atomistic resolution using two-dimensional solid-state NMR spectroscopy.

2803-Pos Microfluidic Optimization of Protein Crystallization

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Board B106

X-ray crystallography depends on the ability to grow protein crystals with a high degree of order, and understanding the phase behavior of aggregating protein solutions can lead to new strategies for growing protein crystals. The ability to control a thermodynamic trajectory through these phases would be invaluable. We used dynamic light scattering to monitor microfluidic protein crystallization reactions. Implementing dynamic light scattering in a microfluidic device combines the ability to retrieve real time aggregate size data with fast and precise reagent control, thus allowing for active alteration of phase transition trajectories in nucleating protein solutions. The low Grashof number in nanoliter volumes allows phase transition kinetics to be explored in free interface diffusion conditions. Growth kinetics were measured while varying both protein and precipitant concentration.

2804-Pos How Does SDS, CTAB and DTT Affect the Size, Rotational Dynamics and Growth Kinetics of Soluble Lysozyme Aggregates?

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Board B107

Protein aggregation plays an important role in neurodegenerative disease. The biophysical features of 'soluble aggregates', identified as crucial early intermediates in the aggregation pathway, are however poorly understood. Halting the growth of soluble aggregates is a promising strategy to arrest the progress of aggregation. We have previously monitored the size/dynamics of soluble oligomers in hen eggwhite lysozyme (HEWL) using the fluorescence anisotropy of covalently tagged dansyl probe (*FEBS Lett.*, *580*:2097–2101, 2006). Here, we investigate the size, rotational motion and aggregation kinetics of soluble oligomers of HEWL over a time span of several weeks, in presence and absence of additives like, SDS, CTAB and DTT, using steady-state & nanosecond time-resolved fluorescence anisotropy of dansyl probe, size-exclusion chromatography, thioflavin T fluorescence and free -SH group estimation. We show that in contrast to hindered and slow segmental motion (5 ns) in the absence of additives, free and fast dansyl segmental motion (1–2 ns) is a key indicator of reduced aggregation propensity amongst soluble oligomers in presence of additives. A ten-fold reduction in thioflavin T fluorescence and higher gel-filtration elution volumes were observed in presence of CTAB or DTT compared to control. The gel-filtration data clearly revealed a heterogeneous aggregate population with appearance of a prominent peak at 120h that coincided with increased thioflavin T fluorescence at same period. Estimation of free sulfhydryl groups revealed an increase in free -SH group population over the first three days followed by saturation and decrease later, suggesting that intermolecular disulphide bonds may be stabilizing the aggregates beyond 120 h. Our findings highlight the role of disulphide bonds in promoting aggregation, apart from offering novel insights on the size and dynamics of soluble aggregates during inhibition of aggregation by additives.

2805-Pos Novel Coarse-Grained Model for Protein Aggregation

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Board B108

We introduce a novel mid-resolution off-lattice coarse-grained model to investigate the self-assembly of beta-sheet forming peptides. The model retains most of the peptide backbone degrees of freedom as well as one interaction center describing the side chains. The peptide consists of a core of alternating hydrophobic and hydrophilic residues, capped by two oppositely charged residues. Non-bonded interactions are described by Lennard-Jones and Coulombic terms. The model is simple enough to allow the simulation of systems consisting of hundreds of peptides, while remaining realistic enough to successfully lead to the formation of chiral, ordered beta-tapes, ribbons as well as higher order fibrillar aggregates.

Both the thermodynamics and the kinetics of aggregation of a seven residue peptide were investigated using Langevin Dynamics simulations considering (a) different levels of *hydrophobic* and *steric* forces between the side chains of the peptides and (b) different degrees of beta-sheet *propensity*. The coarse-grained model was also applied to the study of fibrillogenesis inhibition by N-methylated peptides (D.J. Gordon *et al.*, *Biochemistry 40*, 8237–8245, 2001). A simple representation of the N-methylated peptide, based solely on the *steric effect*, was successful in reproducing the basic

mechanisms of both fibril growth inhibition and disassembly of pre-existing protofilaments.

2806-Pos Direct Numerical Fitting of Protein Protein Interaction Potentials From SAXS Experiments

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Board B109

We present a method for computing interaction potentials of solvated proteins directly from SAXS data, by computing the Guinier plot from Monte Carlo or Molecular Dynamics simulation of model proteins in implicit solvent. This obviates the need for certain approximations, including the low concentration approximation, made in simplified analytical models. We apply the method to lambda repressor fragment 6-85 and fyn-SH3. Several potential forms not easily treatable analytically are tested. With the increased availability of fast computer clusters, molecular dynamics analysis using residue-residue potentials may soon become feasible.

2807-Pos Adsorption of α-Synulcein on Lipid Bilayers containing Phosphatidic Acid: Effects of Lipid Demixing and Protein-protein Interactions

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Board B110

α-Synuclein is a small natively unfolded pre-synaptic protein. It is the major component of intracellular inclusions that are the pathological hallmarks of Parkinson's disease (PD) and mutations of α-synuclein (A53T, A30P, E46K) are associated with familial PD. The interaction of α-synuclein with lipid bilayers was examined using supported lipid bilayers, epi-fluorescence microscopy and fluorescence recovery after photobleaching. The membranes contained phosphatidylcholine (PC) and phosphatidic acid (PA), which in the absence of protein mix uniformly. Upon protein adsorption, the lipids de-mix and PC-rich and PA-rich regions form. The protein was observed to bind avidly to the PA-rich regions, suggesting that the protein is in a conformation that promotes protein-protein contacts, while on the PC-rich regions, such contacts are inhibited. Results will be presented highlighting the effect of lipid organization on the aggregation of wild-type and mutant (A53T, A30P, and E46K) α-synuclein at the membrane surface.

2808-Pos Binding of Wild Type, A53T, A30P, and E46K α-Synuclein to Supported Lipid Bilayers

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Board B111

α-Synuclein is the main component of Lewy body inclusions in the brains of Parkinson's Disease (PD) patients. Three α-synuclein mutants, A53T, A30P, and E46K are associated with familial PD, a rare, early-onset form of the disease inherited in an autosomal dominant manner. Several studies indicate that α-synuclein aggregation is increased in the presence of vesicles having a net negative charge. A53T and A30P both display greater tendency to self-associate than wild-type α-synuclein, while not much is known about E46K. We have examined the binding of wild type and three mutants to bilayers containing different amounts of negatively charged lipids using epi-fluroscence microscopy and bulk fluorescence techniques. Results will be presented showing a change in protein morphology and lipid rearrangement as a function of protein concentration and lipid composition with variable amounts of anionic lipid. Results will also highlight and compare membrane binding between wild type and the mutants.

2809-Pos Membrane Binding of α-Synuclein Depends on its Aggregation State as Probed by Plasmon Waveguide Resonance Spectroscopy

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Board B112

α-Synuclein is a presynaptic protein whose fibrillar and β-sheet rich aggregates are implicated in several neurodegenerative diseases such as Parkinson's disease. Different lines of evidence suggest that oligomer intermediates rather than mature fibrillar deposits constitute the toxic species, probably by membrane incorporation and pore formation [1]. We used Plasmon Waveguide Resonance (PWR) spectroscopy to characterize the binding of various α-synuclein oligomers to planar lipid membranes [2]. The binding isotherms yielded affinity constants for the membrane-active aggregation species of α-synuclein. The binding persisted even after washing the membrane for 45 min. with buffer, indicating the complete insertion of the aggregated protein into the bilayer. In addition, using different lipid mixtures, we studied the role of the lipid composition for membrane insertion of the α-synuclein oligomers. The lipid bilayers, consisting of glycerophospholipids, sphingolipids, and cholesterol, were designed so as to mimic the compositional and structural heterogeneity of neuronal membranes. Using PWR, we studied the effect of neurotoxic factors such as Zn^{2+} on the interaction of monomeric or oligomeric α-synuclein with lipid membranes.

The results show that binding and membrane insertion of α-synuclein is highly dependent on the aggregation state of the protein. Our data suggest that the lateral segregation into lipid domains strongly promotes the insertion of the toxic aggregation species. We therefore propose that the pathogenicity of α-synuclein is highly dependent on the lipid composition of intracellular membranes, most notably the membranes of synaptic vesicles.

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2810-Pos Amyloid Fibers in Parkinson's Disease: Alpha-Synuclein's Journey

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Board B113

Intracellular aggregates of alpha-Synuclein (αSyn) are present in Lewy bodies in the brains of individuals with Parkinson's disease (PD) and other neurodegenerative diseases. αSyn is a 140 amino acid protein with unknown function. In aqueous solution, it is unstructured, but in association with lipids it adopts an α-helical conformation. In addition, in Lewy bodies found *in vivo*, it can display a crossed β-sheet structure, typical of all amyloid fibrils. It remains a fundamental question as to which state of the protein causes neuronal damage. Several lines of recent evidence suggest that off-pathway oligomeric intermediates of αSyn populated during the fibrillation process are the toxic species responsible for neurodegeneration in PD. In this work, we use various analytical methods, including fluorescence spectroscopy, transmission electron microscopy and non-denaturing gel electrophoresis, to investigate the molecular basis for αSyn fibril formation and its possible prevention by small molecules. Our data show that αSyn fibrillation follows a nucleation-dependent mechanism. We found that certain small molecules can prevent αSyn amyloid fiber formation and stabilize αSyn intermediate species.

2811-Pos Tryptophan Environmental Diversity in Human Gamma Crystallins

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Board B114

Crystallins are a family of structural proteins that comprise the lens of the human eye. Aggregation of these proteins is thought to be the cause of cataracts; however, the mechanism by which these proteins aggregate is unclear. There are four highly conserved Trp residues in Hγ D-Crys, and it has been suggested that fluorescence quenching of one or more Trp residues in Hγ D-Crys may protect the Trp residues from UV damage. In this study, UV resonance Raman spectroscopy was used to investigate Trp residue environment, using single Trp mutants to examine the contribution of each residue to the UVRR spectrum. The frequency of the W3 vibrational mode was compared to the predicted W3 frequency obtained from $\xi^{2,1}$ torsion angle analysis. The discrepancy between the predicted and observed W3 frequencies suggests that all four Trp residues are in distinct solvent environments. The frequencies of the single Trp mutants of the homologous pair W42-W130 are significantly upshifted from the predicted frequencies, and these two residues appear to dominate the W3 signal in the wild-type UVRR spectrum. UVRR spectra of thermal and chemical aggregate forms of Hγ D show a further upshift of the W3 frequency, suggesting an increase in the hydrophobicity of the environment around the W42 and W130 residues upon aggregate formation. These results have implications for elucidating the mechanism of Hγ D-Crys aggregation.

2812-Pos Computational Modeling of the Toxic β-Amyloid (Aβ) Ion Channels in the Membrane

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Board B115

Alzheimer β-sheet ion channels embedded in a fully solvated DOPC lipid bilayer were modeled and simulated by using U-shaped Aβ peptides with the β-*strand*-*turn*-β-*strand* motif. Two Aβ monomer conformations, the NMR-based $A\beta_{17–42}$ (2BEG) and $A\beta_{9–40}$ (R. Tycko, Pers. Comm.), were used to construct channels of different sizes (12, 24 and 36 monomers/channel) in an annular shape. The explicit MD simulations provided that in particular the 24-mer simulations in the DOPC bilayer obtain channels with similar subunit organization and dimensions as imaged by AFM. The 24-mer channels spontaneously break into three to five subunits in the bilayer. The outer diameters (~8 *nm*, $A\beta_{17–42}$; ~7.8 *nm*, $A\beta_{9–42}$) are equal or slightly smaller than AFM channels (8–12 *nm*); the pore sizes (~2.5 *nm*, $A\beta_{17–42}$; ~2.2 *nm*, $A\beta_{9–42}$) are slightly larger than AFM channels (~2 *nm*). In addition, the 24-mer channels conduct solvent through the pore and sign the ion selectivity. On the other hand, smaller (12-mer) channels collapse and larger (36-mer) channels are not supported by the bilayer. Examination of the shapes and dimensions of our channels and comparison with AFM images presents a clear picture that the model 24-mer channels can suggest a candidate structure of the toxic Aβ channels in the membrane.

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2813-Pos Probing The Origins Of Misfolding And Toxicity Of The Amyloid Beta Peptide With Amyloid Beta Fragments And Other Small Molecules

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Board B116

Spontaneous protein misfolding and aggregation are responsible for many debilitating degenerative diseases such as Alzheimer's and Parkinson's. Here we study the stability and toxicity of the soluble aggregates of Amyloid beta 1–40 (Aβ , which is believed to be a causative agent of Alzheimer's disease) in presence of small molecules such as Aβ fragments and Curcumin. We also investigate the possible nucleation sites of the misfolding transition. We use fluorescence correlation spectroscopy, electron microscopy, and fluorescence microscopy to assay the effect of the smaller molecules on the population and toxicity of soluble Aβ aggregates. We find that Curcumin, which has been reported to inhibit amyloid aggregation, destabilizes the soluble aggregates and decreases the toxicity of Aβ to cultured neurons. We also find that none of the fragments investigated (Aβ 12–28, and Aβ 22–35) form the soluble aggregates characteristic of $A\beta_{1-40}$ (size >20 nm) but they do form small soluble aggregates (size <10 nm). We have constructed a series of peptides which constitute various parts of the suggested beta hairpin structure, and are characterizing the basic unit responsible for the stabilization of the misfolded structure. Their solubility and their propensity to form soluble aggregates are also being investigated as a function of concentration and as a function of the solution conditions.

2814-Pos Amyloid oligomers exhibit memory

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Board B117

Although many intermediate agents have been attributed to amyloid diseases such as Huntingtons, Parkinsons, prion, type 2 diabetes, a molecular basis for their onset is still unknown. Attention is increasingly being focused on the fibrillar precursors, also called oligomers and protofibrils, and on the reaction kinetics of the formation of the amyloid fibrils. Insulin is a favored model for amyloid formation, not only because amyloidosis can be a major problem in diabetes, but also because aggregation and fibrillation causes problems during production, storage and delivery. The typical insulin fibril formation process at 10 mg/ml, pH 1.6 and T=65Ã'Â°C is characterized by a lag phase in which soluble oligomers are formed (0–1 h), followed by a fibril growth phase characterized by a time period often shorter than the lag phase (1–2 h). Eventually, the process reaches equilibrium when most soluble proteins are converted into fibrils. The main question we address here is as follows: do the oligomers, formed during the lag-phase, display memory and under what conditions is memory retained. By memory, we mean do the oligomers remember their original state, after a temperature perturbation. Hence, a series of experiments were run by removing samples after 1.5 h during the oligomer formation period (lag-phase), cooling them from 65 to 20Ã'Â°C and then storing them for a series of cooling times, tau varying from 1 to 28 days. Then, the temperature of these samples was elevated to 65Ã'Â°C and the reaction allowed to proceed to fibril growth. The results showed a time-dependent memory of the aggregates: they were very stable after 24 hours and showed no evidence that they had been cooled. However, as tau increased, they appeared to lose memory of their previous state, resulting in longer and longer lag-times, eventually reverting back to the reference curve.

2815-Pos All-atom Monte Carlo Simulations Of Abeta(16–22) Oligomerization: Thermodynamics And Beta-barrel Formation

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Board B118

The formation of small oligomers in association with amyloid diseases has recieved much interest in recent years. Many studies have pointed out oligomers as a possible pathogenic agent in several diseases. Here we study oligomerization of an amyloid-forming 7-residue fragment of Alzheimer's Abeta peptide, Abeta(16–22). For a system of six chains, we investigate the thermodynamics of cluster formation by all-atom Monte Carlo simulations. We find that at high temperature small clusters dominate and as temperature is lowered the cluster-sizes increase. At the lowest temperatures studied we also see ordering of the clusters into beta-sheet rich oligomers. In agreement with experiments, we see a preference for antiparallel beta-sheets. A beta-barrel with six antiparallel strands is a stable structure that occurs spontaneously in our simulations.

2816-Pos Effects of Ca^{+2} Ions and Lipids on Aggregation and Conformational Changes of Synthetic Amyloid β-peptides Associated with Alzheimer's Disease

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Board B119

Amyloid-β peptide (Aβ) has been directly linked to Alzheimer's disease. Starting with a soluble monomeric form, Aβ undergoes conformational changes under a variety of *in vitro* conditions to produce a range of oligomeric species collectively named protofibrils. In recent years it has been demonstrated that oligomeric and protofibrillar forms of Aβ are the most neurotoxic and inhibit the electrophysiological activity of neurons.

It has been noted recently that high extracellular concentrations of calcium in the brain, together with disruption in the regulation of Ca^{+2} homeostasis in the aging brain and in neurodegenerative disorders, may play an important role in the onset of protein aggregation. In this work we showed formation of protofibrils and fibrils of Aβ 40 and its arctic variant Aβ 40 (E22G), enhanced by the presence of physiological concentration of Ca^{+2}. Although it has

been shown by several researchers that protofibrils exhibit higher toxicity than fibrils, it is not yet clear which species causes the disruption of neuronal function. It was proposed that structural features rather than protein sequence are responsible for the toxicity. Using FTIR spectroscopy, we assessed secondary structure elements and small conformational changes in the different Aβ conformations.

There is an increasing amount of evidence suggesting that the interaction of Aβ peptide with the neuronal membrane is a necessary step in the development of neurotoxicity. A variety of lipids was reported to have contradictory effects on the aggregation of Aβ peptide, depending on the experimental conditions used. Here, we attempt to show *in vitro* the effects of physiologically relevant constituents of the neuronal lipid membranes on aggregation patterns of Aβ 40, as well as of Aβ peptide carrying the arctic mutation (E22G).

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2817-Pos New Insights into the Mechanism of Alzheimer Amyloid-beta Fibrillogenesis Inhibition by N-methylated peptides

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Alzheimer's disease is a debilitating neurodegenerative disorder associated with the abnormal self-assembly of amyloid-beta(Ab) peptides into fibrillar species. N-methylated peptides homologous to the central hydrophobic core of the Ab peptide are potent inhibitors of this aggregation process. In this work, we use fully atomistic molecular dynamics simulations to study the interactions of the N-methylated peptide inhibitor Ab16–20m (Ac-Lys16-(Me) Leu17-Val18-(Me)Phe19-Phe20-NH2) with a model protofilament consisting of Alzheimer Ab16-22 peptides. Our simulations indicate that the inhibitor peptide can bind to the protofilament at four different sites:

1. at the edge of the protofilament,
2. on the exposed face of a protofilament layer,
3. between the protofilament layers and
4. between the protofilament strands.

The different binding scenarios suggest several mechanisms of fibrillogenesis inhibition:

1. fibril inhibition of longitudinal growth (in the direction of monomer deposition),
2. fibril inhibition of lateral growth (in the direction of protofilament assembly),
3. fibril disassembly by strand removal and perturbation of the periodicity of the protofilament (disruption of fibril morphology).

Our simulations suggest that the Ab16–20m inhibitor can act on both prefibrillar species and mature fibers and that the specific mechanism of inhibition may depend on the structural nature of the Ab aggregate. Disassembly of the fibril can be explained by a mechanism through which the inhibitor peptides bind to disaggregated or otherwise free Ab16–22 peptides in solution, leading to a shift in the equilibrium from a fibrillar state to one dominated by inhibitor-bound Ab16-22 peptides.

2818-Pos MD Simulations of Force-Induced Unbinding of Aβ Peptides from Amyloid Fibrils

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Using All-atom Steered Molecular Dynamics, we study the force-induced unbinding of Aβ peptides from the experimentally determined Aβ amyloid fibril structure. We show that the mechanical dissociation of Aβ peptides is highly anisotropic and proceeds via different pathways when force is applied in parallel or perpendicular direction with respect to the fibril axis. The threshold forces associated with lateral unbinding of Aβ peptides exceed those observed during the mechanical dissociation along the fibril axis. In addition, Aβ fibrils are found to be brittle in the lateral direction of unbinding and soft along the fibril axis. We rationalize these findings by identifying the different types of fibril interactions that are loaded in each of these pathways. Lateral unbinding is primarily determined by the cooperative rupture of the fibril backbone hydrogen bonds. The unbinding along the fibril axis largely depends on the interpeptide Lys-Asp electrostatic contacts and the hydrophobic interactions formed by the Aβ C terminal. Due to universality of the amyloid β structure, the anisotropic mechanical dissociation observed for Aβ fibrils is likely to be applicable to other amyloid assemblies. The estimates of equilibrium forces required to dissociate Aβ peptide from the amyloid fibril suggest that these supramolecular structures are mechanically stronger than most protein domains.

2819-Pos Decomposition of Time-resolved IR Spectra of Amyloid Aggregates

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Board B122

The aggregation of Aβ , a 40 – 42 residue polypeptide produced by cleavage of the amyloid precrusor protein, into amyloid fibers is associated with the onset of Alzheimer's disease. The mechanism of this process, especially the role of early intermediates in aggregation, is the topic of intense study and speculation. Small peptides derived from the Aβ sequence have been studied as models of the aggregation process. In this work, we use infrared spectroscopy (IR), particulary of the amide I band, in order to characterize the

conformational changes and dynamics associated with aggregation of fragments of Aβ . Analysis of the kinetic data is performed using singular value decomposition (SVD). SVD can be very useful when analyzing large sets of spectroscopic data collected as a function of time. A time series of FTIR spectra is regarded an m x n matrix (where $m \geq n$) in which each row corresponds to a frequency at a fixed time. The matrix is then decomposed into a sum of terms by SVD. This allows a purely mathematical operation to isolate spectral changes. Other contributions to the spectra such as buffer and water vapor that are typically subtracted during data processing are also isolated into individual terms. Overall, the SVD analysis is interpreted in terms of two main components of the aggregate dynamics, perhaps corresponding to inter- and intra-aggregate processes.

2820-Pos Kinetics Of Misfolding And Aggregation Of Amyloid Proteins

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Board B123

Understanding amyloid misfolding and aggregation is an important biophysical problem as many amyloidogenic proteins like amyloid-beta, and alpha-synuclein are responsible for several neurodegenerative diseases. Resolving the early steps in the aggregation of amyloid proteins (e.g. amyloid-beta, and alpha-synuclein) is important for determining the concentration-dependent free energy landscape governing the aggregation process. Misfolding of proteins lead to aggregate formation which are believed to grow through a nucleation mediated pathway. However important aggregation parameters, such as the nucleation radius, the surface tension of the aggregate, and the free energy barrier towards aggregation have remained difficult to measure. Homogeneous nucleation theory, if applicable, can directly relate these parameters to measurable quantities, but accurate measurement of homogeneous nucleation rates have remained a challenge. There are two major hurdles:

1. the misfolding kinetics are fast.
2. various surface effects drive the system to inhomogeneous aggregation.

We adopt the strategy of creating a spatial pH jump by multiphoton excitation of a photo-acid in an optically limited volume. This not only allows us to create an aggregating micro-environment for the protein, keeping the rest of the sample volume in physiological condition, but also allows accurate tuning of the pH and the dwell time of the protein. Characterization of this optical system with fluorescein as a reporter molecule proves the feasibility of this approach. Preliminary results on the protein barstar shows that pH induced conformational changes can be investigated. Experiments with other amyloid forming proteins such as amyloid-beta, and alpha-synuclein are underway.

2821-Pos Probing Amyloid Forming Yeast Prion Structure with Optical Tweezers

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Board B124

The relevance of amyloid fibers to a variety of severe human disorders and in many newly discovered beneficial functions in cell biology demands a better understanding of their molecular structures. It has been demonstrated that a single protein can assemble into amyloid fibers with multiple morphologies with different underlying misfolded structures. In *Saccharomyces cerevisiae,* the structural diversity of the amyloids by Sup35, or its N-terminal fragment (NM), gives rise to a range of yeast prion phenotypes, simply referred to as weak [PSI^+] or strong [PSI^+]. This research focuses on using optical trapping and single molecule techniques to explore the structure and structural diversity of NM amyloid fibers and its relevance to yeast prion biology. Fibers were reconstituted at 4°C and 37°C from purified NM protein. These fibers induce strong [PSI^+] and weak [PSI^+] respectively when transferred in non-prion yeast cells ([psi $^-$]). Tethers were made by attaching one end of these NM fibers to a cover slip and the other end of the fiber to an 800 nm polystyrene bead. Optical tweezers were then used to obtain force versus extension curves. These curves were fit to a modified wormlike chain model in order to characterize contour length, persistence length, and axial extension stiffness of individual fibers. The data have shown differences in the mechanical properties, specifically in the axial extension stiffness, indicating that distinct structures result in different intermolecular and intramolecular interactions of misfolded prion proteins. In order to study these interactions in more detail, a single-molecule assay is being developed to probe a single interaction between two NM proteins with optical tweezers and single molecule flourescence.

2822-Pos The Mechanism of Thermal Dissociation of a Prion Peptide Aggregate

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Board B125

The *in vivo* aggregation of many different polypeptides into β-sheet rich amyloid fibers is associated with a range of diseases. The mechanism of aggregation for many of these proteins is still unknown; small polypeptides are useful models for exploring this process by both physical and computational methods. H1, a peptide derived from residues 109–122 of the Syrian hamster prion peptide (Ac-MKHMAGAAAAGAVV-NH_2) forms antiparallel β-sheet aggregates in solution; these register of the β-sheets, as determined by isotope-edited infrared (IR) spectroscopy, has three overhanging residues at the N-terminus, with residue 117 is aligned in all strands (1). In previous work, we have demonstrated that the mutant of H1

A117L forms fibrous aggregates at low temperatures which fully dissociate upon heating (2). In this work, we have determined the mechanism of this thermal dissociation of the A117A aggregates via application of IR spectroscopy, dynamic light scattering (DLS), and atomic force microscopy (AFM). Upon heating, the first intermediate in the dissociation lacks β-sheet secondary structure (as indicated by changes in the amide I' bands in the IR spectrum); however, the polypeptides remain aggregated in solution (as indicated by hydrodynamic radius). If the sample is recooled from this intermediate point, the fibrous aggregates rapidly reform. Further reheating results in a full dissociation, and recooling from this point results in very slow, incomplete reformation of the fibrous aggregates. The concentration dependence of this energy landscape will also be presented.

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2823-Pos Characterization of Multiple Aggregation Pathways of Human Gamma C Crystallin

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Board B126

Cataract is the opacification of the eye lens and is the leading cause of blindness worldwide. Aggregation of lens proteins has been identified as one of the leading causes for lens opacification. Proteins in the lens may be susceptible to aggregation due to covalent damage from UV radiation and oxidative stress throughout the lifetime. Human Gamma-C Crystallin (Hγ C-Crys) is a member of the γ crystallin family of lens proteins. Aggregation of Hγ C-Crys was studied by characterizing conformational changes in native and partially unfolded states using fluorescence spectroscopy. Partially unfolded intermediates were propagated at pH 3 in 50 mM sodium acetate buffer, while similar species were observed at pH 2 in 50 mM sodium citrate buffer. Fluorescence intensity was monitored as a function of time, and indicated the presence of different partially unfolded states under different buffer conditions. Similar effects were not observed using divalent metal ions, suggesting that the observed effects are the result of differential anion accessibility. UV resonance Raman spectroscopy was used to monitor changes in Trp and Tyr local environment at different pH's, using both sodium citrate and sodium acetate buffers. These results suggest that at low pH, the solvent directly interacts with exposed residues in the partially unfolded proteins. The manner in which these interactions occur may influence the conformation of the partially unfolded intermediate and subsequent aggregate species.

2824-Pos AFM Study of Misfolding and Aggregation for Short Peptide from the Yeast Prion Sup35

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Board B127

Protein misfolding and its subsequent aggregation is the cause of many misfolding diseases. Modulation of adhesive forces in misfolded conformation is an important attribute of protein-protein interactions. The formation of "sticky"-aggregation prone conformation ultimately leads to aggregation. We have shown earlier that the force spectroscopy method is capable of detecting misfolded conformation of a protein.(1, 2) This is the very early stage of entire process leading to the self assembly of the protein into disease-prone nanoaggregates.

We have used a short fragment (-GNNQQNY) of the yeast prion Sup35. This is a core sequence of Sup35 which is critical for aggregation of the entire protein and presumably in the protein moisfolding. We measured forces for the interactions between a single pair of GNNQQNY peptides. We have also established a correlation between aggregate morphology and strength of inter-peptide interactions as well as their pH dependence.(1, 2) Fibrils, large globular and small globular aggregates have been observed at pH values 2, 3.7 and 7 respectively. The strength of interactions has been also observed to increase at low pH suggesting that low pH values favor misfolded - aggregation prone state of peptides. This is consistent with previously observed effect that low pH values facilitate protein aggregation. The obtained data provide additional support for the importance of single molecule dynamic force spectroscopy for elucidating thermodynamic and kinetic parameters of protein misfolding and aggregation.

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2825-Pos Enhanced VCD Signal As A Marker For Tyrosine Ring Coupling In An Aggregated 8-residue Alanine Based Polypeptide

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Board B128

Short alanine based peptides are of growing interest in the development of an understanding of residual structure in the unfolded state of polypeptides and proteins. Among the many techniques currently available which allow for the structural elucidation of these peptides, vibrational spectroscopies, such as Vibrational Circular Dichroism (VCD), FTIR, and Polarized Raman, serve as excellent candidates, especially when used complementarily. Tyrosine residues are commonly inserted into short alanine peptides as an easy and accurate means of concentration determination, due the well-defined extinction coefficient of the tyrosine ring at 275 nm. We applied the above spectroscopies with the intention to explore the conformational manifold of Ac-AAAAKAAY (AY8) in aqueous solution. Surprisingly, at a concentration of 41 mM, we found that its VCD spectrum depicts an unusually strong positive couplet centered at ~1614 cm^{-1}, which is assignable to the A_1 tyrosine C-C(s) ring mode. Its integrated rotational strength exceeds that of typical peptide amide I bands by a ratio of approximately 500:1. This ratio nearly triples at 65°C. We repeated the measurement at a reduced concentration (~10 mM), and found the couplet to be absent. This strongly suggests that it results from ππ stacking of tyrosine groups, most likely with an orthogonal (perpendicular) orientation, which could allow coupling between the electronic and magnetic transition dipole moments associated with the ring mode. It is likely that more than two peptides are involved in the aggregation process. Other peptides with similar compositions but different chain lengths do not display such a VCD couplet in the amide I region. We plan to perform DFT calculations on small Ac-AY-OH dimmers, to check the validity of our interpretation.

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2826-Pos Infrared Studies of Polyglutamine Aggregates

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Board B129

Huntington's disease (HD) is a neurodegenerative disorder that causes uncontrollable movements, emotional disturbance and dementia. HD is caused by a CAG repeat expansion in the Huntingtin gene, which results in an extended stretch of glutamine residues at the N-terminus of the protein. Aggregates of Huntingtin, formed when the glutamine tract exceeds the pathogenic threshold (approximately 35 residues), have been found in neuronal inclusions upon post-mortem examination of the brains of HD patients.

The side chain of glutamine contains amide and carboxyl groups, allowing for hydrogen bonding between side chains which mimics that of the backbone. This additional hydrogen bonding potential is one theory as to the stability of polyglutamine (pQ) aggregates.[i] Using FT-IR spectroscopy, pQ_{32} and pQ_{43} aggregates were analyzed to determine the structural difference between pathogenic and non-pathogenic variants. The amide I band of a protein (primarily due to the C=O stretching vibration and thus affected by strength and alignment of hydrogen bonds) is an excellent marker for secondary structure. Analysis of our data shows notable differences between the secondary structures of pQ_{32} and pQ_{43} although both can form visible aggregates. A band is observed at 1612 cm^{-1} in the spectrum of the pQ_{43} that is absent in that of pQ_{32}; this band is indicative of fibrous β-sheet.

Complexities in the amide I region mean that mathematical deconvolution of all spectra is necessary to make conclusive structural assignments. To further understand the origin of each band, individual amidated glutamine and alanine amino acids were also studied in D_2O and dimethyl sulfoxide (to inhibit hydrogen bonding). Comparison of the peaks allows us to thoroughly analyze the spectra of the aggregates and the effect of hydrogen bonds on the vibrational frequency of the carbonyls.

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2827-Pos High Hydrostatic Pressure Recovery Of Proteins From Formalin-fixed Paraffin-embedded Archival Tissue For Proteomic Studies

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Board B130

If formalin-fixed paraffin-embedded (FFPE) archival tissues could be used for standard proteomic methods, such as 2-D gel electrophoresis and mass spectrometry, these powerful proteomic techniques could be used to both qualitatively and quantitatively analyze large numbers of tissues for which the clinical course of diseases, such as cancer, has been established. However, analysis of archival FFPE tissues by high-throughput proteomic methods has been hampered by the adverse effects of formalin fixation. In this study, we demonstrate the use of high hydrostatic pressure as a method for efficient protein recovery from FFPE tissue surrogates. A tissue surrogates is a gel formed by treating high concentrations of cytoplasmic proteins with formaldehyde, which imparts sufficient physical integrity to the gels that they can be processed by standard histological techniques. Reversal of formaldehyde-induced protein adducts and cross-links at 45,000 psi and 80–100°C was observed when lysozyme tissue surrogates were extracted in Tris buffers containing 2% sodium dodecyl sulfate and 0.2 M glycine at pH 4. These conditions also produced peptides resulting from acid-catalyzed aspartic acid cleavage. Additives such as trimethylamine *N*-oxide or copper (II) chloride decreased the total percentage of these aspartic acid cleavage products, while maintaining efficient extraction of de-modified protein from the FFPE tissue surrogates. Mass spectral analysis of the recovered lysozyme yielded 70% sequence coverage, correctly identified all formaldehyde-reactive amino acids, and demonstrated hydrolysis at all of the expected trypsin

cleavage sites. This study demonstrates that elevated hydrostatic pressure treatment is a promising approach for improving the recovery of proteins from FFPE tissues for proteomic analysis.

2828-Pos A Neutron Reflectivity Study of the Adsorption of Insulin at Model Interfaces

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Board B131

The aggregation and subsequent amyloid formation of proteins is a central phenomenon in a number of diseases, such as Alzheimer's and Parkinson's disease, Type II Diabetes Mellitus, and prion disorders. Upon formation of amyloid fibrils, protein molecules lose their native conformation and generally adopt ordered, stacked cross-β-sheet structures. Despite the recent advances in the theoretical and experimental techniques to understand this protein aggregation process, the underlying mechanisms have proved to be challenging to study. Here, we present neutron reflectivity experiments to understand the relative importance of hydrophobic and electrostatic forces on the interfacial aggregation process of insulin (from bovine pancreas) that cannot easily be separated when using lipid membranes. To achieve this goal we have used model interfaces prepared by modifying silicon-water interfaces with polymers that provide distinct environmental properties for adsorbed insulin molecules. A layer of poly(styrene) (PS) at a silicon-water interface may simulate the hydrophobic nature of lipid chains. On the other hand, the polyelectrolytes poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) can mimic negative and positive charges of lipid headgroups, respectively. The neutron reflectivity data have been analyzed in terms of the density, thickness and roughness of the adsorbed insulin layer, in addition to the penetration depth of insulin molecules into the polymer layers. As a main result it has been found that insulin is strongly adsorbing to all three model interfaces. However, despite its weak negative net charge at pH = 7, there is a thicker adsorbate of insulin at the negatively charged PSS polymer surface. In addition, the effects of pH, temperature and ionic strength on the interfacial structure of insulin adsorbates have been measured and will be presented.

2829-Pos The role of inserted Histidine residues in suppressing Polyglutamine aggregation and toxicity in the SCA 1 Protein

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Board B132

Spinocerebellar Ataxia Type 1 (SCA1) is one of a group of nine expanded polyglutamine (polyQ) neurodegenerative diseases in which expansion of a polyQ tract in a disease protein above a threshold in the 35–45 range is associated with disease. SCA1 is unique in that the disease protein, ataxin1, often contains one or more His residues within the polyQ. There is reason to believe that insertion of the His residues dampens expanded polyQ toxicity, which appears to be associated with the formation of cellular aggregates of the polyQ protein.

Aggregation of simple polyglutamine sequences occurs through nucleated growth polymerization, in which the nucleation phase decreases with increasing glutamine length - consistent with a role of aggregation in disease. Here, we report that His interruptions significantly diminish the tendency of a polyQ sequence to aggregate. First, while the nucleation equilibrium constant (Kn*) for a simple Q_{30} repeat peptide is 3.18×10^{-11}, the Kn* for a similar peptide containing two inserted His residues is a much lower and less favorable value of 8.61×10^{-13}. This effect appears to be associated with the protonation of the His side chain in the monomeric peptide. Second, despite our finding that the His residues are incorporated into turns in the aggregate structure, His residues also appear to destabilize aggregates, shifting the equilibrium toward monomer. Thus, inserted His residues constrain both aggregation kinetics and thermodynamics, and either or both effects may be important in the apparent suppression effect in SCA1.

2830-Pos Stiffness of Sickle Hemoglobin Polymers Alone and in Domains

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Board B133

Sickle hemoglobin (HbS) differs from HbA by a mutation on the surface of the molecule (beta6 glu ->val) which allows concentrated, deoxygenated solutions to form long, 14-stranded polymers. Because of the double nucleation mechanism by which polymers form, they naturally appear in arrays called domains, that become quasi-spherical at long times. The stiffness of the polymers and their domains is fundamental to the pathology of sickle cell disease, which involves occlusion of capillaries by sickled cells. Here we report our efforts to characterize the stiffness of these objects. For individual polymers, we have developed a technique in which a linear region near a polymer domain is photolyzed to allow polymers to grow along the optical track. By creating a track which has an abruptly diverted direction, we can observe the delay as the polymer makes the requisite turn, and thus discover the rigidity of fibers of HbS. For entire domains we have devised a method in which an emulsion of HbS in oil is compressed by electromagnets. The compression enlarges the droplet, and the compression is varied periodically at preset frequencies. Changes on the scale of nm can be tracked by this method. Samples are gelled thermally, and one domain is present on the average. This differs from studies on cells since concentration is accurately known, and from solutions because single domains are being studied, rather than collections of domains. We will present results obtained by both methods and suggest ways in which the information from fibers and domains can be integrated.

2831-Pos Effects Of Dehydration In Ethanol On The Structure Of Formalin-fixed Proteins

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We examined the structural changes associated with increasing ethanol concentration on formaldehyde-treated bovine pancreatic ribonuclease A (RNase A) by circular dichroism (CD) spectroscopy. As we have demonstrated previously (Rait et al. *Laboratory Investigation 84*, 292–299, 2004), incubation of RNase A in 3.7% formaldehyde for one week results in the formation of extensive formaldehyde-induced protein adducts (hydroxymethyl moieties and Schiff's bases) and both intra- and intermolecular protein cross-links. However, these formaldehyde-induced protein modifications do not alter either the secondary structure (as revealed by the far-UV CD spectrum) or the tertiary structure (as revealed by the near-UV CD spectrum) of the protein. In contrast, incubation of formaldehyde-modified RNase A as a precipitate in 100% ethanol for one week produced significant changes in the CD spectrum of the protein. There was a significant decrease in negative and positive band intensities in the far-UV spectrum, which changed to one with a single minimum at ~215 nm, consistent with a conversion from the native $\alpha + \beta$ structure to a mostly β conformation. The near-UV spectrum revealed an almost complete loss of tertiary structure. These results are interpreted to indicate that formaldehyde-treated proteins aggregate and adopt a β-sheet secondary structure, similar to that of amyloid fibrils, at ethanol concentrations above 90%. In this form, the regions of the protein inducing its aggregation are those rich in hydrophobic amino acids, and prone to form β-sheet structures stabilized by intermolecular hydrogen bonds. These findings suggest that a major obstacle to the reversal of protein formaldehyde modifications in formalin-fixed tissues is the inability to fully re-hydrate such protein aggregates.

2832-Pos Amyloid Versus Amorphous Aggregate Structures At The Atomic Level

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Board B135

The atomic-level conformation of polyglutamine peptides and poly-L-glutamic acid peptides within soluble oligomers and insoluble aggregated states were measured using time-resolved fluorescence resonance energy transfer experiments. Molecular dynamics simulations were then used to determine structural ensembles consistent with FRET-measured end-to-end distances. Interestingly, the molecular conformation of polyglutamine peptides within amyloid fibers was more extended than the initial monomer conformations. However, the polyglutamic acid peptides in amorphous aggregates become much more compact than as alpha-helical monomers. Interestingly, soluble oligomers of both peptides are more extended than monomers. These results suggest a similar assembly mechanism early in the aggregation process of both peptides but which later deviates into dissimilar structural routes as further aggregation occurs.

2833-Pos Illuminating the Pathway to Prion Amyloid

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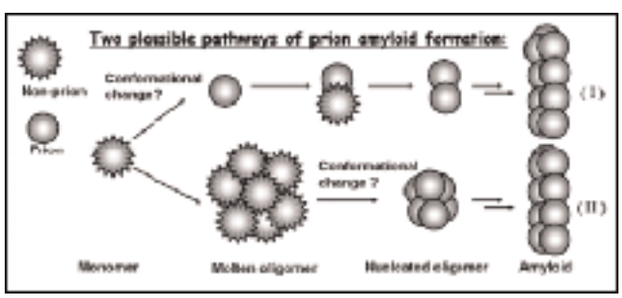
The conformational switch of self-replicating prion proteins is the key step in a range of transmissible neurodegenerative diseases. It is being realized that prions play a much broader, often beneficial, role in biology than was initially anticipated. The yeast prion-state [*PSI*$^+$] is caused by a self-perpetuating conformational change leading to amyloids from a natively unfolded prion-domain of Sup35 protein. We are applying advanced ensemble and single-molecule fluorescence methodologies to understand the pathway to the prion-state. Using single-molecule FRET and FCS, we have recently shown that the monomeric prion-domain exists as a rapidly fluctuating collapsed state. We have now embarked on unraveling the mechanism of prion propagation using fluorescence quenching, anisotropy and correlation measurements. Our findings reveal that monomers rapidly transform into (obligatory) molten oligomers and that the conformational changes within the oligomers convert them into smaller (nucleated) oligomeric species that readily assemble into amyloid fibrils. Together, these intriguing observations strongly support the '*nucleated conformational conversion*' model in which sequestration of amyloid conformers occurs within the context of oligomers (Pathway II) rather than in monomers (Pathway I). This mechanism confronts the traditional views, and is consistent with an emerging polymer physics perspectiveof protein aggregation.



2834-Pos Nanoimaging for Protein Misfolding Diseases

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Board B137

Misfolding and aggregation of proteins is a common theme linking a number of neorodegenerative diseases such as Alzheimer's,

Huntington's and Parkinson's. Protein misfolding is a complex phenomenon that can be facilitated, impeded, or prevented by interactions of the protein with various intracellular metabolites and intracellular nanomachines controlling protein folding. A fundamental understanding of molecular processes leading to misfolding and self-aggregation of proteins will provide critical information to help identify appropriate therapeutic routes to control these processes. Protein misfolding is the very fist link in this long chain of events eventually leading to neurodegeneration. Therefore, availability of methods capable of detecting the disease prone protein conformations facilitates the development of novel tools for diagnostic and treating the diseases at very early stages of development. This presentation summarizes our results on the development and use of the nanoimaging based approaches for detection and analysis of protein misfolding states (e.g., a review article (1)). Our approach for detecting and analyzing transient states based on the fact that misfolded conformations of a protein differ from folded and other protein conformations by their increased propensity to interact with each. We used AFM operating in the single molecule force spectroscopy mode to measure the strength of the interprotein interactions prior the aggregation. We also developed the AFM nanotweezers approach for the single molecule selection of antibodies bound to a particular type of the protein aggregates (2).

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Apoptosis

2835-Pos Mitochondrial Fission During Ceramide-induced Cardiac Myocyte Apoptosis Early Steps

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Mitochondria are organized as a network of interconnected organelles that fluctuate between fission and fusion events (mitochondrial dynamics). The outer mitochondrial membrane protein Fis1 and the GTPase dynamin related protein-1 (Drp-1) are the main elements of the mitochondrial fission machinery. Given that 1) mitochondria fission is associated to apoptotic cell death and 2) ceramides change mitochondrial homeostasis and trigger apoptosis; we investigated here, whether activation of apoptosis with ceramides affects mitochondrial dynamics and promotes mitochondrial fission in neonatal rat cardiac myocytes primary cultures.

Cardiac myocytes mitochondrial network integrity was evaluated by 3D reconstitution of confocal microscopy images of cells loaded with mitotracker green. C2-ceramide, but not dihydro-C2-ceramide, promoted rapid fragmentation of the mitochondria network in a concentration- and time-dependent manner. C2-ceramide also increased mitochondrial Drp-1 and Fis1 content, as well as Drp-1 colocalization with Fis1, studied both, by immunofluorescence and Western blot. C2-ceramide caused a decrease in membrane potential and loss of cytochrome c mitochondria distribution pattern. To decrease the levels of the mitochondrial fusion protein mitofusin 2, we used an antisense adenovirus (AsMfn2). AsMfn2 accentuated the decrease in mitochondria membrane potential and cytochrome c redistribution induced by C2-ceramide.

We conclude that ceramides stimulate mitochondrial fission and this event is associated with early activation of cardiac myocyte apoptosis.

2836-Pos Full Length Bid, A BH3 Only Bcl-2 Family Protein, Forms A Large Pore And Transports Cytochrome C

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The BH3 domain only proapoptotic protein Bid plays a major role in death receptor induced mitochondria dependent apoptosis. Death receptors such as Fas/CD95 activate apical caspases that cleave full-length Bid. The C-terminal part of cleaved Bid, tBid, is believed to subsequently migrate to the mitochondria and promote the release of cytochrome c by regulating the interactions of anti-apoptotic and pro-apoptotic proteins.

In planar lipid bilayers, tBid has been shown to form a pore (1). However, the physiological importance of this pore-forming ability of tBid in cyt c release has not been examined. For that matter, the functional role of uncleaved full length Bid (flBid) in cyt c release has been even more overlooked.

Here we report that the flBid protein forms a long-lasting large conductance pore (~nano Siemens) in planar lipid bilayers and induces cyt c release from liposomes. Since flBid has been shown to bind (or transport) phospholipid (2,3), it is possible that a large conductance flBid pore is a lipid containing pore that shares its native lipidic pore properties where flBid may function to reduce the large energy requirements of lipidic pore formation.

These cyt c conducting and large pore forming abilities previously have been thought to be limited to pro-apoptotic multi-domain Bcl-2 family proteins such as Bax and Bak. This is the first evidence to show that a BH3 domain only protein, Bid, can assemble to form a large pore in lipid membranes and directly contribute to transporting cyt c.

References

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