time, as well as a model with which to fit these data to determine the kinetic rate constants for pausing and release during termination under different conditions. The rates for two different terminators have been determined in the presence or absence of transcriptional co-factors, NusA and N, and further on-going experiments will be described.

Platform M: Protein Aggregates

132-Plat AFM Imaging Of Recombinant Human Prion Aggregation Reveals Multi-step Formation Process Involving Stable Oligomeric Intermediates

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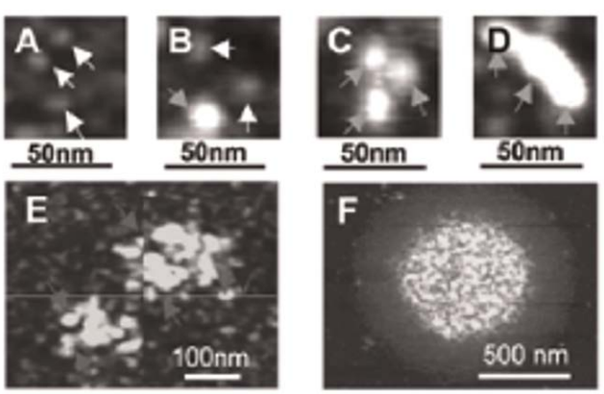
The key event in the prion disease is oligomerization of cellular prion protein (PrPc) into scrapie prion protein (PrPsc) with high beta sheet content and subsequent aggregation of PrPsc. To explore physical controls on this process, we used AFM to study aggregation pathways of recombinant human prion proteins, specifically comparing differences between normal (PrPcwt) and a mutant (PrPc10or) protein. Experiments were carried out in partly denaturing acid solutions that trigger oligomerization and aggregation. The results showed that both PrPcwt and PrPc10or followed the same pathway to formation of nonfibrillar aggregates, but did not follow a conventional growth process (Fig. 1): First, trimeric-to-tetrameric units formed from the monomers. These then combined to form larger oligomeric units of 8 and 10 monomers. These further aggregated to form micron-scale globular structures, which were clearly still comprised of the oligomers. The difference between PrPcwt and PrPc10or was in the rates of oligomerization and oligomers aggregation. PrPc10or exhibited higher oligomerization and aggregation rates and larger aggregates. The results support a hypothesis of multi-step aggregation in which mutant proteins are unstable and spontaneously form PrPsc oligomers, which slowly accumulates over time.

Fig. 1 AFM images showing prion aggregatation. A–E show evolution from monmers to 8–10mer units. The large aggregate in F is comprised of the same oligomeric units as in E. White, yellow and red arrows point to monomers, trimers and 8–10mers respectively.



133-Plat Sequential Conformational Changes Occurring During Aggregation of Amyloidogenic Proteins

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Many proteins or protein fragments can aggregate into cross ß-amyloid fibrils. These fibrils accumulate into insoluble plaques in vivo and are associated with the progression of neurodegeneration in Alzheimer's, Parkinson's and Creutzfeldt-Jakob's diseases. Transmissible spongiform encephalopathies in cows (mad cow), deer and elk (chronic wasting syndrome), sheep and hamsters (scrapie) also show accumulation of ß-amyloid during their progression.

Many mechanistic intermediates have been proposed for the assembly of amyloid fibrils from soluble precursor proteins and peptides. However few of these proposed intermediates have been observed directly. We will discuss the aggregation of ß-lactoglobulin and alpha-synuclein into ß-amyloid fibrils and our efforts to characterize the structural and size distributions through all stages of the assembly using a combination of bulk and single molecule methods.

We have observed that upon aggregation into a non-fibrillar aggregate, a conformational change occurs in the amyloidogenic proteins we have studied. Once in the colloidal aggregate, another conformational change occurs that is nearly irreversible and leads to the growth phase of amyloid formation.

134-Plat Mechanism of Lipid Bilayer Disruption by Oligomeric Alpha-Synuclein

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Parkinson's disease is characterized by the deposition of aggregated fibrillar α-synuclein in Lewy bodies within the brain. The nature of the pathogenic species in Parkinson's disease is not unequivocally established, but current thinking points towards early oligomeric species rather than the mature fibrillar forms of the protein. A possible mechanism by which cytotoxicity occurs is through permeabilization and disruption of lipid membranes by these early intermediates. A pore-like mode of action has been suggested for the increase in membrane permeability upon interaction with oligomeric α-synuclein. However such a mechanism has never been unequivocally proven. We have characterized lipid bilayer disruption by monomeric, oligomeric and fibrillar α-synuclein using a dequenching assay on liposomes. We found that vesicle leakage was

concentration dependent and independent of vesicle size. Monomeric, oligomeric and fibrillar α-synuclein all disrupted the vesicles, however the oligomeric species induced leakage at a much lower concentration. We have also found that this process shows a remarkable specificity for vesicles composed of POPG and POPA. The disease related mutants A30P, E46K and A53T all showed similar properties. Furthermore using single channel recordings across planar lipid bilayers we have tried to established the mechanism of α-synuclein membrane permeabilization. No conclusive evidence of the elusive pore-like mechanism has been found. Our findings contribute to the understanding of α-synuclein lipid interaction in both its monomeric and aggregated forms and its potential role in Parkinson's disease.

135-Plat Computational Approaches To Understand Mechanisms Of Fibrillation

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Proteins can misfold and aggregate, which is believed to be the cause of a variety of diseases, affecting very diverse organs in the body. Many questions about the nature of aggregation and the proteins that are involved in these events are still left unanswered. We have investigated two different proteins which both form fibrils, namely Transthyretin and Glucagon. Both proteins have been studied in experimental labs for decades, which has provided us with a vast amount of knowledge, however, structural insights of the amyloidogenic process are still lacking. Through our all-atom molecular dynamics studies we have proposed a mechanism for fibrillation of Transthyretin and investigations into the fibrillation pathway of Glucagon are well underway. We are using free energy calculations, as well as principal component analysis to investigate and understand the processes involved this aberrant behavior. Knowledge of these pathways is a necessary first step in designing drugs to prevent fibrillation.

136-Plat Quantitative Atomic Force Microscopy Establishes Free Energy Parameters of Amyloid Fibril Formation

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The nanoscale properties and behavior of intrinsically disordered proteins in human brain cells are key to understanding the molecular biophysics of neurodegenerative disorders such as Parkinson's disease (PD). Intracellular inclusions that consist mainly of fibrillar aggregates of the protein α-synuclein are the pathological hallmark of PD.

We use quantitative atomic force microscopy[1] (AFM) to measure the length distribution of *in vitro* fibrillar α-synuclein aggregates as a function of initial monomeric protein concentration. A recent statistical-mechanical model of amyloid formation[2] predicts this fibril length dependence, in addition to other properties of amyloid fibril formation such as the weight fractions of the various aggregate species, based on assumptions of the free energies of protein association.

Our quantitative AFM data confirms the model predictions both qualitatively and quantitatively, and allows us to estimate the magnitude of the free energies associated with the intermolecular interactions that govern fibril formation[3]. These fundamental insights into the energetics of amyloid formation will be valuable in establishing quantitative knowledge of the biophysics of fibril formation both in α-synuclein and in other amyloid-forming proteins.

References

1. Van Raaij, M. E., Segers-Nolten, I. M. J. & Subramaniam, V. (2006). Quantitative morphological analysis reveals ultrastructural diversity of amyloid fibrils from alpha-synuclein mutants. *Biophys. J.* ***91***, L96–98.
2. Van Gestel, J. & de Leeuw, S. W. (2006). A statistical-mechanical theory of fibril formation in dilute protein solutions. *Biophys. J.* ***90***, 3134–3145.
3. Van Raaij, M. E., Van Gestel, J., Segers Nolten, I. M. J., De Leeuw, S. & Subramaniam, V. (2007). Concentration dependence of a-synuclein fibril length assessed by qualitative atomic force microscopy and statistical-mechanical modeling. *Submitted.*

137-Plat Amyloid Fibrils Of α-Synuclein Mutants Exhibit Distinctive Dual-Emission Fluorescence Signatures Upon Binding Of 3-Hydroxyflavones

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Parkinson's disease is a movement disorder characterized by the presence in the mid-brain of amyloid deposits of the 140-aa protein α-synuclein (AS). Three genetic mutants, A53T, A30P and E46K, have been linked with early onset Parkinson's. The amyloid fibrils consist of interwound protofilaments with a cross β-sheet secondary structure. Although highly conserved among different aggregopathies, mature fibrils exhibit a certain degree of polymorphism, which is poorly characterized and understood.

In the present work we used 3-hydroxyflavone derivatives as novel fluorescent probes to distinguish structural features of amyloid fibrils formed by AS and its familial mutants. These dyes exhibit two intensive well-separated emission bands reflecting excited state redistributions. Their intensity ratio constitutes an ultrasensitive and tunable reporter of microenvironment properties such as polarity, hydrogen bonding, and polarizability. The dyes are nonfluorescent in the presence of the wt and mutant monomeric AS proteins but exhibit distinctive fluorescence spectra upon binding to the respective fibrils formed in vitro. The dyes do not simply absorb at the surface but are integrated into the internal fibrillar structures, as indicated by spectral changes corresponding to decreased polarity and partial disruption of hydrogen bonds to water. It is remarkable that each point mutant is clearly distinguishable by the spectral signatures of these external dyes. We anticipate that the 3-hydroxyflavone derivatives may serve as useful tools, including diagnostic, for detecting polymorphism in other amyloid systems as well as for uncovering oligomeric intermediates currently identified as the neurotoxic species.

138-Plat Real-time Monitoring of Hb S Fiber Formation by UV Resonance Raman

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Sickle cell disease is caused by a single point mutation (Glu β6 Val), creating a hydrophobic patch on the surface of the Hb S tetramer. This hydrophobic region interacts with the hydrophobic EF corner pocket, resulting in the formation of long polymers of Hb S in the red blood cell. In this study, we used time-resolved UV resonance Raman (UVRR) spectroscopy to observe both the homogeneous nucleation step and the heterogeneous nucleation step of Hb S polymerization.

The formation of the intermolecular $^{1}\beta_1$-$^{2}\beta_2$ contacts is observed by monitoring the change in Phe signal intensity as a function of time, yielding kinetic progress curves similar to those obtained by turbidity. Comparison of individual spectra collected during the homogeneous nucleation step show small Phe intensity changes, which are attributed to the presence of small aggregates. Comparison of the spectra collected during the homogeneous nucleation phase show constant increases in Phe signal intensity, consistent with stabilization of the $^{1}\beta_1$-$^{2}\beta_2$ contacts.

Changes at the $\alpha_1\beta_2$ interface upon fiber formation were observed by monitoring changes in frequency and intensity of the tyrosine and tryptophan vibrational modes. Kinetic progress curves generated by monitoring changes in the frequency of the Tyr modes, and changes in intensity of the Trp modes, exhibit two distinct transitions. The first transition is correlated with the initial stages of fiber formation, while the second transition occurs much later and is associated with the formation of higher order polymer structures. These results suggest that there are multiple steps in the process of Hb S polymerization.

139-Plat Alzheimer Disease: Role of Abeta Peptide for the Cholinergic Deficiency. Molecular Details using NMR Spectroscopy

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A deficiency of the neurotransmitter acetylcholine (ACh) in the brain is associated with Alzheimer's disease (AD). The ACh deficiency significantly contributes to cognitive symptoms and is a target for drug development. ACh is synthesized by choline acetyltransferase (EC 2.3.1.6) and reduced activity of this enzyme has been reported in AD patients. Aβ peptide inhibition of high-affinity choline uptake has been hypothesized. However, the molecular cause of ACh deficiency has not been determined. We now wish to report a possible mechanism for Ab peptide induced ACh deficiency.

We report the hydrolysis of the GPC to PC in aqueous medium by Aβ peptide. This would reduce the generation of choline, which is the major precursor for the synthesis of ACh. Our NMR results indicate that hydrolysis efficiency of Aβ peptide in aqueous environment follow a hierarchal pattern (Aβ42 > Aβ40 > Aβ28). Based on our NMR results, a molecular model will be presented for Aβ induced cholinergic deficiency in AD.

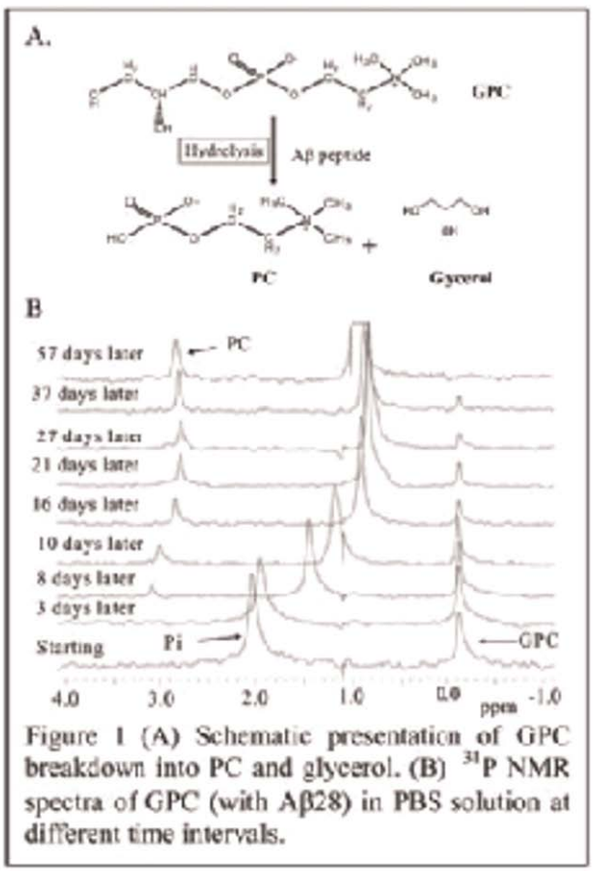

Figure 1 (A) Schematic presentation of GPC breakdown into PC and glycerol. (B) ^{31}P NMR spectra of GPC (with Aβ28) in PBS solution at different time intervals.

Platform N: Actin & Actin-binding Proteins

140-Plat High Resolution Cryo-EM Structure of the F-Actin-Fimbrin/Plastin ABD2 Complex

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Many actin-binding proteins have a modular architecture, and calponin-homology (CH) domains are one such structurally conserved module found in numerous proteins that interact with F-actin. The manner in which CH-domains bind F-actin has been controversial. Using cryo-EM and a single-particle approach to helical reconstruction[1] we have generated 10 Å resolution map of F-actin alone and 12 Å resolution map of F-actin decorated with a fragment of human fimbrin (L-plastin) containing tandem CH-domains. The high resolution allows an unambiguous fit of the crystal structure of