single-molecule measurements and physiological forces. We believe that our work provides first steps towards a theoretical framework to better understand dynamic and cellular protein biomechanics and biological force generation.
We have been supported by a grant N202 0852 33 of the Ministry of Science and Higher Education in Poland (to P.S.) and a fellowship of the European Molecular Biology Organization (to H.J.).

1124-Plat
Probing Protein Folding Kinetics with High-resolution, Stabilized Optical Tweezers
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Single-molecule techniques provide a powerful means of exploring molecular transitions such as the unfolding and refolding of a protein. However, the quantification of bi-directional transitions and near-equilibrium phenomena poses unique challenges, and is often limited by the detection resolution and long-term stability of the instrument. We have developed unique optical tweezers methods that address these problems, including an interference-based method for high-resolution 3D bead tracking (~1 nm laterally, ~0.3 nm vertically, at $>$ 100 Hz), and a continuous autofocus system that stabilizes the trap height to within 1-2 nm longterm [1-3]. We have used our instruments to quantify the force-dependent unfolding and refolding kinetics of single protein domains (e.g. spectrin [3,4]). These single-molecule studies are presented, together with the accompanying probabilistic analysis that we have developed.
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Platform W: Anyloids from Multiple Perspectives

1125-Plat
Possible Mechanism Of Amyloid Formation By Apomyoglobin Mutants
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It is known that even proteins, not involved into diseases, are able to form amyloid-like structures similar in final architecture of their fibrils. This fact suggests that formation of aggregated cross-beta structure is a common property of a polypeptide chain under appropriate conditions. Sperm whale apomyoglobin was used to investigate amyloid formation because the properties and folding process of this protein are well known. Process of the apomyoglobin mutant aggregation was monitored under conditions close to physiological ones (40°C, pH 5.5) by ThT binding, turbidity, FTIR spectroscopy and electron microscopy. Mutated proteins contained a single point substitution at positions Val10 and Met131 by Ala, Phe and Trp. It was shown that the WT apomyoglobin formed aggregates not containing beta-structure, while variants of apomyoglobin have shown significant increase of ThT fluorescence intensity and changes in a form of FTIR spectra. These changes evidenced appearance of beta-structured aggregates, and EM images showed fibril-like aggregates. Kinetics of amyloid formation monitored by turbidity and ThT binding allowed to calculate three rate constants of amyloid formation and to distinguish three stages of this process. Obtained results suggest that the rate of the first stage is affected by a position of substitution, and is not influenced by its type. In contrast, the rate of the second stage depends on a type of substitution: it is slower for mutants with aromatic amino acid substitutions. This work was supported by INTAS grant 05-1000004-7747, partly by the Howard Hughes Medical Institute Award 55005607 to A.V. Finkelstein and by the RAS Program on ''Molecular and Cellular Biology''.

1126-Plat
Investigations of Amyloid Fiber Formation of Alpha-Synuclein and Amyloid-beta Using Newly Synthesized Small Molecules
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Alpha-Synuclein and Amyloid-beta are amyloid forming proteins which aggregate in Parkinson's and Alzheimer's disease brain, respectively. We designed and synthesized a novel boronic acid- and chromene-based small molecule library, and tested the molecules' *in vitro* activity against alpha-Synuclein and Amyloid-beta by examining the effect on the aggregation process. The aggregation was monitored using the amyloid-specific Thioflavin T fluorescence, as well as by native gel electrophoresis, and transmission electron microscopy. We observed that some compounds were effective at stabilizing the initial species, while others appear to stabilize a ring-like oligomeric intermediate as observed by electron microscopy combined with single particle analysis. Furthermore, some compounds were able to promote the formation of amyloid fibers. Together, these results serve as a foundation for the future design of small molecule inhibitors and diagnostic agents (PET-agents) for amyloid fibers. In addition, they provide insights into the mechanism of aggregation in many neurodegenerative diseases.

1127-Plat
Using Pressure Perturbation for Studying the Free Energy and Conformational Landscape of Proteins Upon Aggregation and Amyloid Formation
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Pressure tuning in combination with calorimetric, spectroscopic and structural techniques (DSC, PPC, FTIR, SAXS, AFM) revealed new insights into the pre-aggregated regime as well as mechanistic details about concurrent aggregation pathways and the differential stability of insulin aggregates. A thorough thermodynamic approach has provided a coherent and precise description of changes of the partial specific volume, heat capacity, the coefficient of thermal expansion, as well as the adiabatic and isothermal compressibility of the protein upon unfolding and aggregation. This was only possible due to a novel application of ultrasound velocimetry and pressure perturbation calorimetry. Besides pressure, also solvational perturbations, accomplished by the addition of various salts and cosolvents such as glycerol, ethanol and TFE, have been explored. They exert pronounced and diversified effects on the unfolding, non-native assembly and fibril formation, which ultimately manifest in morphological variations of mature aggregates and fibrils (strains). The phenomenon of strains easily fits to a generalized protein energy landscape picture involving an alternative comb-shaped aggregation funnel. The pressure variable has also been explored to study more disease related amyloidogenic proteins, such as PrP and IAPP. Several examples will be given.

1128-Plat
Amyloid Peptide Aggregation In Plugs Formed By Microfluidics
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We present a novel microfluidic device for amyloid peptide aggregation research. The device relies on the control of interfacial chemistry, which allows miniaturizing of aggregation measurements to nanoliter volumes. In traditional in vitro aggregation experiments, adsorption of amyloid peptides to various interfaces has been shown to nucleate and to enhance peptide aggregation. The problem of adsorption is even more pronounced upon miniaturization of aggregation experiments. Miniaturization leads to an increase of the surface-to-volume ratio, and concomitantly to an increase of amyloid peptide aggregation if the surface is not controlled. Nevertheless, miniaturization of aggregation experiments is desirable for samples available only in small volumes, as for example cerebrospinal fluid (CSF) from mice. CSF has recently gained interest in Alzheimer research, however CSF analytics has been hampered to due the small available volume.
In order to miniaturize and control the interfacial chemistry of aggregation experiments we used a plug based microfluidic approach. Plugs are nanoliter sized aqueous droplets formed in the flow of immiscible fluids inside microfluidic channels. Upon peptide encapsulation into plugs, the unfavorable interfaces are exchanged for an adjustable liquid/liquid interface. We show for one prominent amyloid peptide, the Alzheimer's peptide Aβ(1-40), that aggregation in plugs has kinetics of orders of magnitude slower than under standard conditions. Further we show the applicability of this miniaturized system to aggregation experiments by testing the inhibitory potency of CSF from wild type and ceAPPswePS1ΔE9/TTR-/- mice on Aβ aggregation. Using the plug-based approach, we were able to perform over 750 experiments with a single mouse CSF sample of 5 μl in volume. The plug system offers many new opportunities to investigate in vitro aggregation studies, as for example time controlled aging of amyloid peptides, nucleation in a confined environment, and screening of drug components.

1129-Plat
Unraveling the Polyglutamine Aggregation Pathway in Huntington's Disease by Small-Angle Neutron Scattering
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The presence of an abnormally expanded polyglutamine (polyGln) sequence in huntingtin protein ultimately results in beta-sheet-rich fibrillar aggregates, a hallmark of Huntington's disease. Current challenges are to map out the polyGln aggregation pathway by identifying the various precursor structures and establish their pathological roles. We are using time-resolved small-angle neutron scattering (SANS) to probe the aggregates formed by peptides having the protein context of huntingtin exon 1 (HD protein) and with varying polyGln lengths. SANS is a particularly useful technique for following structural changes on the nanometer length-scale in solution. From the time-resolved scattering data, we obtain snapshots of the polyGln structures as the kinetics reaction ensues, which yields quantitative information on the size and shape of precursors and the internal structure of the resulting fibrils. Measured changes in the radius of gyration and mass per length illustrate multiple growth regimes with a transition from early aggregates to fibril elongation and association. Our SANS results on mature polyGln fibrils are consistent with the Perutz beta-helix structural model. This research is providing new insights into the pathway of polyGln aggregation and should later assist in determining the role that precursors play in neuronal toxicity.

1130-Plat
Solid State NMR Studies Of Structural And Motional Complexity In Amyloid-Like Fibrils Of The Peptide GNNQQNY
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Solid state NMR allows the site-specific characterization of structure and dynamics in a variety of immobilized biomolecules, and thus allows a unique structural view on amyloid fibrils. These fibrils are common to various human disorders and appear to share a number of characteristic features, both in terms of their structure and formation. In the hope of delineating the biophysical details of the fibrillization process and the fibrils themselves, various groups have focused on the experimental and theoretical study of small peptide fragments of amyloid-forming proteins. One prominent system is the $GNNQQNY_{7\text{-}13}$ fragment of the yeast prion protein Sup35p, since it was found to form not just amyloid-like fibrils, but also seemingly amyloid-like microcrystals. X-ray diffraction based structures from the latter have inspired numerous theoretical analyses and generalizations regarding the biophysics and structures of amyloid fibrils.
We have instead applied biological solid state NMR methods to characterize the GNNQQNY fibrillar aggregates. Magic angle spinning (MAS) solid state NMR was used for various structural measurements, aimed at both the intramolecular as well as intermolecular structural motifs of the fibrils (as well as the crystalline aggregates). Our studies have revealed a remarkable complexity in these fibrils, despite the relatively small size of the peptide building blocks. This is in marked contrast with the rigid and homogeneous nature of the crystalline structures, as revealed by X-ray crystallography and solid state NMR. These observations provide further insights into the structure of the fibrils of this peptide model system and should also be of importance as input to numerous theoretical studies that rely on the crystal structure data.

1131-Plat
Computational Study of Assembly and Toxicity Inhibition of Amyloid Beta-Protein and Its Arctic Mutant
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Amyloid b-protein (Ab) exists in two main alloforms, Ab40 and Ab42, of which Ab42 is linked particularly strongly to Alzheimer's disease (AD). Prior computational work demonstrated that the ab initio discrete molecular dynamics approach with an intermediate-resolution protein model captures biologically relevant differences between Ab40 and Ab42 folding and oligomerization. In the present work we apply the same approach to explore the relationship between the structure and toxicity. Assuming that Ab42 oligomers are more toxic than oligomers formed by Ab40, our structural analysis indicates that the solvent accessible surface area (SASA) in the N-terminal region of Ab42 oligomers is significantly higher than that of Ab40 oligomers. We then investigate effects of the C-terminal fragment (CTF), which was shown to attenuate Ab42 oligomer toxicity in a cell culture, on Ab42 oligomerization. Our results indicate that CTFs associate with Ab42 to form heterooligomers, consistent with quasielastic light scattering data. We show that the presence of CTFs significantly reduces SASA in the N- terminal region of Ab42 compared to the same region in Ab42 oligomers formed in the absence of CTFs.
We further explore folding and oligomer formation of the Arctic mutants, [E22G]Ab40 and [E22G]Ab42, associated with a familial form of AD. Our results demonstrate that the substitution E22G disrupts the folding structure and oligomerization pathways of both Arctic mutants and results in increased SASA at the N-terminus of the Arctic Ab40 mutant. These findings suggest that Ab oligomer neurotoxicity might be directly or indirectly associated with the degree of solvent exposure of the N-terminal region of Ab.

1132-Plat
Discrete Molecular Dynamics simulations on hexameric amyloid-β (1-40) and (1-42) models
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Aggregation of amyloid-β peptides (Aβ) may play a pivotal role in neurotoxicity of Alzheimer's disease. Two major alloforms of Aβ are the 40-residue long Aβ40 and the 42-residue long Aβ42. Though Aβ42 has only two more residues at the end of C-terminus, Aβ40 and Aβ42 show different characteristics in early aggregation: Aβ40 aggregates to exist from monomers up to tetramers while Aβ42 exists from monomers to hexamers, dodecamers, or even octadecamers. However, the molecular mechanism of the different aggregation between Aβ40 and Aβ42 is not clearly understood since their oligomeric structures are not available from experiments due to their meta-stable characteristics. Here, we simulated nine hexameric Aβ40 and nine hexameric Aβ42 models with Discrete Molecular Dynamics (Discrete MD). The hydrophobic core of these models is a six-stranded β-barrel formed by residues 30-40 that has three-fold symmetry about its axis. This core is shielded from water by residues 1-28. The models differ by the relative positions of the core β strands, and whether the other segments surrounding the core contain α helices or β-strands. The potential energy of Aβ40 measured by Miyazawa-Jernigan interaction matrix were considerably lower than the potential energy of Aβ42 in all of 18 models tested, probably because more hydrophobic residues are exposed to water in theAβ42 models. In two of nine models of Aβ42, dangling hydrophobic β-strands emerged on the surface. This implies that the association of these hexamers may be possible, which could lead to the formation of larger assemblies.

Platform X: Exploring the Unfolded State of Peptide & Proteins

1133-Plat
Concentration Dependent Instability of ß-sheet aggregates of Ac-(AAKA) 4NH2 in solution
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The amphiphatic polyalanine peptide, Ac-(AAKA)4-NH2, has recently been shown to aggregate into a hydrogel at high chloride concentration or alkaline pH. It forms soluble ß-sheet type aggregates at neutral pH and centimolar concentrations. In order to further characterize the transition from the monomeric to the aggregated state, we measured the far UV-ECD spectrum at different concentrations between 35 - 700 μM. At very low concentration (i.e. 0.05 mg/mL or 35 μM) the observed spectrum is indicative of a stable mixture of right handed α-helical and ß-strand (sheet) conformations. At higher concentrations (> 1 mM) we observed a spectrum reflecting a very stable ß-sheet aggregate. However, at concentrations between 70 - 700 μM the peptide shows a very strange, and totally unexpected, behavior. Upon dissolving it in solution, a statistical coil-like mixture comprising polyproline II (PPII), α-helical and ß-sheet-like conformations is formed. Subsequently, most of the ß-sheet fraction decays into a conformation which exhibits a PPII-type ECD spectrum on a time scale of 104-105 s. The kinetics of the process follows a power law at low concentrations and becomes mono-exponential at higher concentration. Generally, the relaxation slows down with increasing peptide concentration until the ß-sheet becomes stable on the time scale of our experiments (<105 s). We hypothesize that aggregation involves multiple steps with the formation of a rather unstable ß-sheet as the first step. The second step involves the formation of stable fibers. This step competes with the formation of collagen like coil-coil state, rather, which is known to exhibit a PPII-like ECD spectrum.

1134-Plat
Characterization of the Disordered Regulatory Domain from Calcineurin
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Calcineurin (CaN) is a highly-conserved, ubiquitous Ser/Thr phosphotase that plays vital roles in memory development and retention, cardiac growth, and