**1119-Plat**
**Single-Molecule Constant-Force (Force-Clamp) AFM Measurements Confirm Catch-Bonds and Multiple Binding States in Bacterial Adhesin FimH**
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Bacteria can adhere to mannose present on the surface of epithelial cells via the FimH-mannose bond. Surprisingly, they have been shown to enhance their adhesion to the cells when fluid flow is increased. These conditions would normally weaken traditional 'slip' bonds. Catch bonds have been defined as non-covalent bonds whose lifetime increases with tensile force on the bond, instead of decreasing as expected for slip bonds.
Here we present results confirming catch bonds in FimH-mannose complexes at the single molecule level using the Atomic Force Microscope (AFM). This is the first AFM measurement of catch bond lifetimes using a constant force mode. In constant force mode (or force clamp), a feedback loop maintains the force at a predefined level, correcting for changes in conformation of the molecule. We observe multiple lifetimes and that force enhances the proportion of bonds with a long lifetime. This is the first measurement that resolves multiple lifetimes in catch bonds while verifying that only a single molecule is tethered. We discuss the implications of these findings in the context of the mechanism giving rise to bacterial catch bonds.

**1120-Plat**
**Characterizing Fibrin 'A-a' Interactions By Single Molecule Force Spectroscopy**
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Blood vessel injury triggers the conversion of soluble fibrinogen to insoluble fibrin polymer that serves as the structural scaffold of a blood clot. Understanding the biophysical forces involved in maintaining fibrin structure is of great interest to the biomedical community. Previous reports have identified the 'A-a' interaction as a main contributor to the structural integrity of fibrin. Herein, we present the use of single molecule force spectroscopy to study the forced dissociation of 'A-a' interactions between fibrin molecules. The rupture of the 'A-a' interaction is accompanied by a characteristic force pattern previously unreported in fibrin force spectroscopy, reminiscent of the forced unfolding of other proteins in the literature. We propose that the characteristic pattern represents structural deformation of fibrinogen prior to the rupture of the 'A-a' interaction. Several analysis techniques are employed to characterize each unfolding event of the pattern. First, the polymeric nature of each event is examined using the worm-like chain model. We find that the first three events may be fit with one persistence length, but the significantly larger persistence length of the final event suggests a fundamentally different type of molecular extension than the previous three. Next, the energy landscape of each event is investigated by varying the loading rate. The first event is characterized by an ill-defined force probability distribution, indicating that it might correspond to initial reorientation of the substrate-bound molecule. In contrast, the other events have profiles characteristic of well-defined single-bond ruptures. While high-force events two and three have strikingly similar kinetic parameters implying similar molecular nature, the fourth, low-force event likely represents the final dissociation of the weakened 'A-a' interaction. Characterization of the forced dissociation of 'A-a' interactions may provide insight into the biomechanical properties of fibrin fibers held together via these interactions.

**1121-Plat**
**Mechanisms of ATP-dependent Chromatin Remodeling Revealed by Single-molecule Manipulation Studies**
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Genome-wide chromatin structures, such as nucleosome positioning and various histone modifications, have recently been mapped relative to the underlying DNA sequences, revealing an exquisite and dynamic organization of chromatin. The chromatin structures are established and remodeled mainly by a large family of highly conserved and specialized ATP-dependent chromatin remodeling complexes (remodelers) in cooperation with histone-modifying enzymes. The core of these remodelers is a DNA translocase, a molecular motor capable of actively moving along DNA. It remains unclear how the energy of ATP hydrolysis is converted to the mechanical work for DNA translocation and in turn to nucleosome remodeling by remodelers, and how the remodeling process is regulated by different protein subunits of remodelers, nucleosome substrates, and histone modifications. Using high-resolution optical tweezers, we studied the nucleosome remodeling process by SWI/SNF and RSC, two prototypes of remodelers containing 11 and 15 subunits, respectively. We found that both remodelers can translocate along DNA at rates of ~13 bp/s and generate forces up to ~12 pN, producing DNA loops of a broad range of sizes (5-1200 bp) in a nucleosome-dependent manner. Interestingly, when attached by a strong DNA binding domain and anchored to a bare DNA molecule, the isolated ATPase subunit of RSC alone can efficiently translocate along DNA to produce DNA loops in a nucleosome-independent manner. Surprisingly, the tethered translocase can now move against forces as high as 26 pN, making the remodeler translocase one of the strongest molecular motors. Our single-molecule experiments revealed a powerful and versatile DNA translocase engine for remodelers, which may be crucial for their role of disrupting DNA-histone interactions in a regulatory fashion.

**1122-Plat**
**Revisiting Protein Folding at the Single Molecule Level**
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Determining the mechanism by which a protein folds remains a primary goal in biology. Statistical theories of protein folding have long predicted plausible mechanisms for reducing the vast conformational space through distinct ensembles of structures. However, these predictions have remained untested experimentally, since the multiplicity of trajectories and folding structures is averaged out using bulk techniques. Moreover, most intermediate conformations are only transiently present, rendering their isolation and characterization difficult by commonly used spectroscopic methods. Owing to recent advances in single molecule force-clamp spectroscopy, we are now able to probe the structure and dynamics of the small protein ubiquitin by measuring its length, mechanical stability and effect of solvent environment during each stage of folding. Here we discover that upon hydrophobic collapse, the protein rapidly selects a subset of non-native like, minimum energy structures that are mechanically weak and insensitive to the solvent environment. From this much reduced ensemble, the native state is acquired through a barrier-limited transition. The existence of such heterogeneous ensemble of minimum energy collapsed states was theoretically proposed by lattice simulations to be a milestone in the process of narrowing the available conformational space of a protein during its journey to the native fold, and a general feature of proteins that are naturally designed through evolution to fold on biological timescales. Here we demonstrate that such ensemble of collapsed states is also apparent in our experiments in the well-characterized I27 and Protein L proteins, albeit on different timescales, thus suggesting that their presence is ubiquitous to other mechanically stable proteins with a well-defined fold. Our results present the first experimental evidence for the validity of statistical mechanics models in describing the folding of small proteins on biological timescales.

**1123-Plat**
**The Anisotropic Response of Ubiquitin Unfolded by Periodic Forces**
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Biological forces govern essential molecular processes in all living organisms. Many cellular forces, e.g. those generated in cyclic conformational changes of biological machines, have repetitive components. In apparent contrast, little is known about how dynamic protein structures respond to repetitive mechanical information. The small protein ubiquitin is found in all eukaryotes and serves as cellular signaling tag e.g. in the forceful degradation of misfolded proteins. Here, we probed the nano-mechanical stability of single and multimeric ubiquitins unfolded by periodic forces. Using coarse-grained molecular dynamics simulations, we were able to model repetitive forces with periods about two orders of magnitude longer than the relaxation time of the protein. We found that even a moderate periodic force weakened ubiquitin and shifted its unfolding pathways in a frequency- and amplitude-dependent manner. Our results also showed that the dynamic response of a small protein can be complex with transient refolding of secondary structures and an increasing importance of local interactions in asymmetric protein stability. We tested the geometry dependence of ubiquitin's mechanical stability and found that the ubiquitin linkage involved in protein degradation is remarkably insensitive to periodic forces. These observations are qualitatively and quantitatively explained using kinetic energy landscape models which can be in turn used to predict the dynamic response of proteins. Our results are also discussed in light of dynamic

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.
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**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**
**Matthias Meier**, Julia Kennedy-Darling, See Hoon Choi,
Sangram S. Sisodia, Rustem F. Ismagilov.
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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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