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