suggest that native disulfide bonds after allosterically the transition state and modulate the internal flexibility of the stressed protein prior the breakage of its mechanical clamp motif.

2273-Plat
Quantifying the Resolution of Single-Molecule Torque Measurements by Allan Variance
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Combined single-molecule manipulation techniques have provided unprecedented insights into the structure, function, interactions, and mechanical properties of biological macromolecules. While many single-molecule manipulation techniques naturally operate in the space of (linear) extension and force, recently a number of techniques have been developed that enable measurements of rotation angle and torque. Examples include the rotor bead tracking assay, the optical torque wrench (OTW), and magnetic torque tweezers (MTT). While systematic analyses of the position and force resolution of single-molecule techniques have attracted considerable attention (see e.g. [1,2]), detailed analysis of the angle and torque resolution is currently lacking. Here, we propose Allan variance as a criterion to systematically quantitate the angle and torque resolution in single-molecule measurements. We apply the Allan variance method to experimental data from our implementations of MTT [3,4,5] and an OTW [6]. Both magnetic and optical torque tweezers can achieve a torque resolution of better or equal to 1 pN nm. We find that our state-of-the-art OTW outperforms MTT for short measurement times. However, for measurement times >10 s, drift becomes a limiting factor in the OTW and the superior stability of MTT accomplishes higher ultimate torque resolution for long measurement times.

In summary, our Allan variance criterion enables to critically assess the torque resolution as a function of measurement time and across different measurement modalities that rely on distinct physical principles. In addition, the Allan variance method provides a tool to optimize the measurement protocol for a given instrument and system.


2274-Plat
Combined Single Molecule Force and Fluorescence Spectroscopy of the Unfolding and Refolding of Green Fluorescent Protein
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We have used optical tweezers to study the free energy surface for unfolding-refolding of the green fluorescent protein (EGFP) and simultaneously monitored the loss and recovery of its fluorescence. This conformational landscape shows unfolding intermediates, molten glob refolding intermediates, as well as misfolded states. As force is used to drive transitions between conformational substates, single molecule fluorescence is probed. In its native state, the emission of EGFP is punctuated by transient dark states ("blinking"); this emission is lost and regained as the protein is unfolded and subsequently refolded. These results provide a full understanding of the unfolding-refolding energy landscape of EGFP and how the conformational state affects the environment of the fluorophore, which reconciles three classes of previous experiments: (1) bulk unfolding/refolding with fluorescence (2) single molecule force-unfolding and (3) single molecule fluorescence intensity fluctuations. This investigation is relevant to efforts at developing EGFP as a genetically encoded force sensor, as well as its use in single molecule imaging and fluorescence recovery after photobleaching experiments.

2275-Plat
VWF - Collagen Interactions Studied with Single Molecule Force Spectroscopy
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Von Willebrand factor (VWF) is a huge multimeric protein that plays a key role in hemostasis. Sites for collagen binding, an initial event of hemostasis, are located in domains A1 and A3 of VWF. Collagen III is believed to interact with the A3-domain, and collagen VI with the A1-domain. The forces and the dynamics of these interactions were investigated with molecular recognition force spectroscopy (MRFS), using substrates with a dense layer of poly (ethylene glycol) chains and terminal benzaldehyde functions for covalent immobilization of collagen. The bond between collagen III and the A3-domain of VWF domain construct A1-A2-A3 was more stable than the bond between collagen VI and the A1-domain of A1-A2-A3, suggesting that A3 is the main binding domain for collagen. We also investigated a mutation in the A3-domain of A1-A2-A3 (S1731T) that shows a slight decrease of collagen III binding determined by ELISA. In MRFS interactions between collagen III and the S1731T mutant showed no significant difference in stability compared to the wild type construct. These data are consistent with our observation that persons with mutation S1731T exhibit only a mild or no significant bleeding tendency. We further compared the collagen VI binding capability of A1-A2-A3 and A1-A2. The bond between collagen VI and A1 was stronger when the A3-domain was missing. In addition, the injection of free A2-domains disturbed the collagen VI - A1 interaction, but had no effect on interactions between collagen III and the A3-domain, indicating that domain A1 might also interact with A2. Our data allow deriving a detailed molecular picture on the interplay of collagen-VWF-domain interactions. This work was supported by the German Research Foundation (DFG Research Unit FOR 1543 - SHEN) and the Austrian Science Fund (Project I 767-B11).

2276-Plat
Resolving the Molecular Determinants of Cadherin Catch Bond Formation
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Classical cadherin Ca2+-dependent cell-cell adhesion proteins play key roles in embryogenesis and in maintaining tissue integrity. Cadherins mediate robust adhesion by binding in multiple conformations. We recently showed that one of these conformations, called an x-dimer, forms catch bonds that strengthen and become longer lived in the presence of mechanical stress. Here we use single molecule force clamp spectroscopy with an Atomic Force Microscope along with Molecular Dynamics and Steered Molecular Dynamics simulations to identify key interactions that mediate x-dimer catch bond formation and to resolve the role of Ca2+ ions in this process. We show that tensile force bends the cadherin extracellular region such that they form force-induced hydrogen bonds that lock x-dimers into tighter contact. When Ca2+ concentration is decreased, these hydrogen bonds are eliminated and catch bond formation is abolished. Based on these results, we formulate a simple ‘flex and lock’ kinetic scheme that quantitatively describes x-dimer catch bonds.

2277-Plat
Catch Bond Interaction Between Glycosaminoglycans and Cell Surface Sulfatase Sulf1
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In biological adhesion, the biophysical mechanism of specific, non-covalent, biomolecular interaction can be divided in slip- and catch-bonds, respectively. Conceptually, slip bonds exhibit reduced bond lifetime under increased external loads whereas catch-bonds, in contrast, increased lifetime for a certain force interval. Since 2003, a handful of biological systems such as the adhesive proteins P-Selectin and FimH have been identified to display catch-bond properties. Upon investigating the specific interaction between the unique hydrophilic domain (HD) of human cell-surface sulfatase Sulf1 against the native glycosaminoglycan (GAG) target heparan sulfate (HS) by single-molecule force spectroscopy (SMFS), we found clear evidence of catch-bond behavior in this system. The HD, about 320 amino acids long and strongly positive charged, and the GAG-polymers, composed of up to 200 disaccharide units, were quantitatively investigated with atomic force microscopy (AFM) based dynamic force spectroscopy (DFS) as well as force-clamp spectroscopy (FCS). The observed catch-bond character of HD against HS was found to be specifically related to the GAG 6-O-sulfation site. Therefore, this behavior can also be found in HS-related GAGs like heparin and (to a lesser extent) dermatan sulfate, whereas in contrast, only slip-bond binding can be observed in a GAG system where these sites are explicitly lacking. Our observed catch-bond binding data can be interpreted within the theoretical framework of a force mediated transition between two slip-bond regimes. Interestingly, the transition between the two states occurs in a force interval of only 5 Piconewtons while the lifetime of the catch-bond interaction increases approximately 5-fold for heparan sulfate and dermatan sulfate.