188-Pos Board B67
Unfolding, refolding and proteolysis of the von Willebrand Factor A2 domain under tensile force
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von Willebrand Factor (vWF) is a plasma protein essential to the early stages of blood coagulation. Shear induced proteolysis at the A2 domain of vWF is an important mechanism to convert the highly thrombogenic, ultra large vWF multimers to smaller multimeric forms and, consequently, to prevent overgrown thrombus. It has been hypothesized that the A2 domain undergoes conformational changes in response to tensile force and exposes its Tyr842-Met843 scissile bond for cleavage by ADAMTS13, a metalloprotease found in the circulating blood. In this work, the unfolding and folding kinetics of the A2 domain is studied using optical tweezers under pulling forces that mimicked the tensile forces exposed to vWF multimers in the vasculature. We demonstrate that A2 domain is unstable upon pulling and unfolds at between 7 to 14 pN at loading rates ranging from 0.3 to 300 pN/s. Once unfolded, stress-free refolding of A2 domain takes 1.9 seconds, increasing dramatically with tensile force. Unfolded A2 domain was cleaved by physiological concentration of ADAMTS13 with a catalytic rate constant of 0.14/s. The results suggest that the A2 domain is unraveled at physiological tensile forces in vivo and its slow refolding process ensures the enzymatic reaction of ADAMTS13. Hence, the A2 domain acts as a force sensor that triggers ADAMTS13 cleavage at the pico-newton force range.

189-Pos Board B68
The Temperature Dependency of Disulfide Bond Reduction Events Measured by Single-molecule Force Clamp Spectroscopy
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An emerging application of single-molecule force clamp spectroscopy is the ability to explore the chemical kinetics of disulfide bond cleavage under different stretching forces at the single-bond level. Our previous work demonstrated that the rate of thiol/disulfide exchange reaction in proteins is force-dependent, and well described by an Arrhenius term of the form: $r = A(exp((F\Delta x_r\text{-}E_a)/k_BT)[nucleophile])$. From the force dependency of the reduction rate we could measure the bond elongation, Δx_r (0.2-0.6 Å), that occurs at the transition state of the SN2 reaction cleaved by different chemical reagents and enzymes, never before observed by any other technique. However, our estimates of E_a (53-60 kJ/mol) were uncertain and dependent on our choice of value for A (10^{12} $M^{-1}s^{-1}$). Here, we show that by carrying out the force-dependent disulfide bond reduction experiments at a series of well-controlled temperatures, A can be measured independently. We demonstrate that the reaction rate of the disulfide bond cleavage by nucleophilic attack of tris(2-carboxyethyl)phosphine (*TCEP*) increases monotonically with temperature and, A is measured to be at the order of 10^7 $M^{-1}s^{-1}$, which is far lower than that predicted by the transition-state theory, in which A is given by k_BT/h and around 10^{12} $M^{-1}s^{-1}$ at room temperature. Factoring in the much lower value of A, E_a is calculated to be 35 kJ/mol, which is much lower than 58 kJ/mol that we had reported previously. For thioredoxin (*Trx*)-catalyzed disulfide bond cleavage, obtaining the A, E_a and Δx_r values can help elucidating the reaction mechanisms and the role of temperature in the regulation of *Trx* activity, which is of special interest when comparing enzymes from different species. These measurements demonstrate the power of single-molecule force spectroscopy approach in providing unprecedented access to protein based chemical reactions.

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Direct Identification of Two Distinct Transition State Structures in Reduction of a Disulfide Bond Revealed by Single Bond Force-clamp Spectroscopy
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Disulfide bonds are common to many extracellular proteins, where they serve to stabilize the native conformation. Indeed, the thiol/disulfide exchange mechanism is involved in important and complex biological processes. Much experimental and theoretical work seems to support the idea that the reaction proceeds through an uncomplicated S_N2. While in gas phase a double minimum potential model describes the potential energy surface governing the chemical reaction, in solution phase only unimodal, concerted profiles without intermediates have been identified. However, the detailed shapes of the energy surfaces of these reactions are largely unknown, because the collisions with solvent control the trajectories of the molecules. The combination of molecular engineering techniques with single molecule force-clamp spectroscopy has made it possible to monitor the reduction of single disulfide bonds, allowing us to experimentally measure the bond elongation at the reaction transition state with sub-Ångström resolution. Such an experimental approach provides an unprecedented experimental platform to directly probe the energy landscape of a simple chemical reaction in solution at the single bond level. By greatly expanding the range of pulling forces up to 1.5 nN, where covalent bonds are not yet broken, here we demonstrate that the disulfide bond cleavage by hydroxyl occurs through a double-barrier energy landscape. Whereas at low pulling forces (100-500pN) the reaction rate is limited by a first energy barrier exhibiting a distance to the transition state Δx ~0.5 Å, at higher forces (500-1500 pN) a second energy barrier exhibiting a shorter transition state of Δx ~0.1 Å becomes dominant. Our experimental approach allows us to probe regions of the energy landscape that were previously experimentally inaccessible, revealing signatures of unanticipated complexity.

191-Pos Board B70
Nebulin Elasticity Pre-loads Thin Filaments of Skeletal Muscle: Unfolding of Transient a-helices
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Nebulin, a giant modular protein (600 - 900 kDa), acts as a thin filament ruler and regulator of contraction. The bulk of the protein consists of ~200 tandem repeats of ~35 residue actin-binding modules arranged as two single-repeat regions and 22 sets of seven-module super-repeats. The nanomechanics of nebulin were investigated with atomic force microscopy by tethering and stretching full length molecules between a pair of site-specific antibodies to either N or C terminus of nebulin, with one attached covalently to a functionalized self-assembled monolayer and the other to a functionalized cantilever. Upon stretch, single nebulin molecules extend to well over 1 μm and yield force curves with variable numbers and heights of peaks. An underlying periodicity at ~22 and ~15 nm was observed by periodogram analysis. Major force peaks appear to result from the re-orientation and unfolding of short α-helical bundles of nebulin modules, as suggested by circular dichroism, steered molecular dynamics simulations of computed structures as well as experimental force-extension curves of nebulin single and super repeats.
The nebulin molecule exerts a compressive force at the ends and alongside the 1 μm long thin filaments. In the sarcomere, nebulin's compressive stress may stiffen actin filaments and assist the re-annealing of severed actin filaments under undue stress. In the skeletal muscle sarcomere, the elastic nebulin associates helically around actin filaments, thus behaving as a length regulating ruler only under stress and upon binding to the actin filaments. It may also act as a reversible and elastic tether between the myosin heads and actin during contraction. We propose the elasticity of nebulin as an integral component of its ruler function and acto-myosin regulation in preloaded thin filaments.

192-Pos Board B71
Effects of Cantilever Stiffness on Unfolding Force in AFM Protein Unfolding
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Direct mechanical unbinding of ligand-receptor pairs and the similar process of mechanically unfolding single protein molecules are common methods for exploring the energy landscape of unbinding/unfolding interactions. In these experiments, mechanical force applied through a cantilever and linking molecule causes the test system to transition between a bound/folded state and unbound/unfolded state. The effective linker stiffness k depends on both the chemical linker and the cantilever and is of central importance in calculating the loading rate $df/dt = kv$. This effect is well appreciated in standard analysis of unbinding/unfolding results. However, the effect of effective linker stiffness on the underlying energy landscape itself is often overlooked. Recent experiments on biotin-streptavidin by Walton et al. have shown surprisingly strong effects on average unbinding force under standard conditions, suggesting that future work should take the stiffness-modified landscape into account. We carry out force spectroscopy measurements on I27 octomers using cantilevers with a range of spring constants and discuss our findings and their effect on the analysis of mechanical protein unfolding experiments.

193-Pos Board B72
E-selectin/sLea Form Catch-Slip Bonds Without Force-History Dependence
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It has been established that P- and L-selectins form catch-slip bonds with their ligands, with initially increasing and subsequent decreasing bond lifetimes with