forces, confirming specificity. SPR yielded k_{on} ~ 7000 L/mol-sec and k_{off} ~ 0.01 sec^{-1} at 25 °C. SPR equilibrium and transition state thermodynamic data, obtained at 15 - 37 °C, show that αIIbβ3:cHArGD interactions must overcome an entropy-unfavorable activation energy barrier ($\Delta G_a^{o\ddagger}$ 12 kcal/mol) before gaining a favorable ΔH and ΔS for binding (ΔG° - 8 kcal/mol).
Conclusions: SPR and DFS gave comparable dissociation rates for αIIbβ3:-cHArGD interactions and a critical rupture distance that agrees with the dimensions of the complementary electrostatic contacts shared by all integrin:RGD complexes. Our energy landscape adds a nanoscale to the mechanisms that regulate αIIbβ3's interactions with pharmacological and physiological ligands.

3082-Pos
Stressed and Compressed Molecular Bonds Revealed in Footprints of Rolling Neutrophils using Total Internal Reflection Fluorescence Microscopy
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Neutrophil recruitment to the sites of inflammation involves selectin-mediated rolling followed by chemokine-induced activation and beta2 integrin-mediated arrest. PSGL-1, a ligand for endothelial P-selectin is presented on the tips of neutrophil microvilli. It has been predicted that P-selectin-PSGL-1 bonds form when the microvillus tip approaches the P-selectin expressing substrate to within 70 nm, but this prediction has not been tested experimentally. A PDMS based microfluidic device with a glass substrate coated with P-selectin/ICAM-1 was perfused with blood from an anesthetized mouse expressing green fluorescent protein (GFP) in neutrophils. Rolling interactions were studied at wall shear stress of 6-8 dynes/cm2 using TIRF microscopy which provides high resolution in z-direction. The contact zones of rolling neutrophils were revealed as footprints which were 3-6 μm in diameter, about twice as large as what would be expected for spherical cells. Following bond formation, microvilli in the footprint undergo compression, approaching the substrate to within 25 nm near the center of the cell. At the trailing edge, the P-selectin-PSGL-1 bonds stretch to a length of 125-150 nm before they dissociate. Adding the chemokine CXCL1 to the substrate induced neutrophil arrest and formation of single, long, branched tethers that stretch for up to 10 μm behind the arrested cells. The closest contact between the arrested neutrophil and the substrate is always found in front of the cell center and covers 1-3 μm2. Its distance from the substrate (44 nm) corresponds to the length of the ICAM-1-LFA-1 bond. These results identify the molecular and cellular dimensions of rolling neutrophils and provide a framework for the biomechanical analysis of this fundamental process.
This work was supported by a postdoctoral fellowship 09POST2230093 from American Heart Association (P.S) and NIH EB 02185 (K.L).

3083-Pos
Kinetics of DNA Force-Induced Melting
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Once stretched beyond its B-form contour length, double-stranded DNA reveals a sudden increase in length over approximately constant force at about 65 pN. During this conformational transition, DNA base pairing and base stacking are disrupted, converting double stranded DNA (dsDNA) into single stranded DNA (ssDNA). While thermodynamic and recent chemical labeling and fluorescence imaging experiments indicate that this transition is analogous to thermal melting, the kinetics of DNA force-induced melting have not been characterized. We present a predictive model of force-induced melting in which thermal fluctuations induce local melting and re-annealing of DNA. These fluctuations are stabilized by the application of tension during the overstretching transition, favoring the conversion to ssDNA as the applied force is increased. This model quantitatively predicts small changes in the melting force as the pulling rate is varied. We verify that the DNA melting force varies with pulling rate, consistent with this model, and that DNA force-induced melting depends only weakly on pulling rate at slow pulling rates, as melting occurs cooperatively with a domain size of 100-200 base pairs. As the pulling rate is increased beyond the natural duplex opening rate, the melting force depends strongly on pulling rate and the melted domain size decreases to 5-10 base pairs, as the DNA is ripped sequentially from the free ends (or any boundary). The final strand separation occurs at much higher forces, representing the nonequilibrium ripping of the most stable regions that remain at the end of the low force transition. The results indicate that force only weakly enhances base pair opening, while strongly inhibiting base pair closing.

3084-Pos
Modulating the Mechanical Stability of Extracellular Matrix Protein Tenascin-C in a Controlled and Reversible Fashion
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Tenascin-C is a large extracellular matrix protein and is subject to stretching force under its physiological condition. Regulating the mechanical properties of the fibronectin type III domains of tenascin-C will alter its response to mechanical stretching force and thus may provide the possibility of regulating the biological activities of tenascin-C in living cells. However, tuning the mechanical stability of proteins in a rational and systematic fashion remains challenging. Combining steered molecular dynamics simulations, protein engineering and single-molecule atomic force microscopy, we have rationally engineered a bihistidine-based metal chelation site into the third fibronectin type III domain (TNfn3) of tenascin-C. We used its metal chelation capability to selectively increase the unfolding energy barrier for the rate-limiting step during the mechanical unfolding of TNfn3. The resultant TNfn3 mutant exhibits enhanced mechanical stability. Using a stronger metal chelator, one can convert TNfn3 back to a state of lower mechanical stability. This is the first step toward engineering extracellular matrix proteins with defined mechanical properties, which can be modulated reversibly by external stimuli, and will provide the possibility of using external stimuli to regulate the biological functions of extracellular matrix proteins.

3085-Pos
Effects of Solution Chemistry on Fibrin Nanomechanics
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Fibrin, the polymerized protein that provides the structural scaffold of blood clots, is critical to hemostasis and wound healing. The complexity of the fibrin network has left many questions regarding its formation unanswered, including how temperature, metal concentration, and pH, factors known to mediate of fibrin polymerization, influence the mechanics of the interactions between fibrin monomers. Previously, we used the atomic force microscope to examine the mechanical properties of the 'A-a' knob-hole interaction, the most significant bond between fibrin monomers. Force applied to this bond was shown to cause stepwise unfolding of the hole-bearing region of fibrinogen as evidenced by force curves exhibiting a characteristic pattern of events. The dependence of environmental effects such as pH, temperature, ionic strength, and divalent ion (i.e., Ca2+ and Mg2+) concentration on this characteristic pattern remains unknown. Herein, we examine the force, spacing, and probabilities of each force event in the characteristic pattern as a function of solution chemistry. Calcium concentration significantly influenced incidence of the last event in the characteristic pattern, but otherwise had no effect on the knob-hole interaction. Such behavior, attributed to the high-affinity γ1 calcium-binding site, was found to be reversible and specific. The force data indicate that the γ1 site has no effect on the strength of the knob-hole bond prior to unfolding but makes the hole more resilient to unfolding. Our results may explain previous paradoxical findings that calcium had no affect on the affinity of knob 'A' for hole 'a', but was critical to 'A-a' driven fibrin polymerization. Likewise, understanding the dependence of fibrin mechanics on solution conditions may help resolve other questions surrounding the relationship between the fibrin polymerization and the local solution environment.

3086-Pos
A Rotor Driven by the Torque Stored in Braided DNA Molecules
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We present a direct measurement of the torque exerted by braided DNA molecules undergoing spontaneous unwinding while attached to a paramagnetic dumbbell. A magnetic tweezers setup was employed to stretch and braid immobilized lambda DNA molecules. The free end of each immobilized DNA molecule was covalently attached to a paramagnetic microsphere through a single bond. When single DNA molecules were bound to a sphere, they followed a constant circular trajectory. Conversely, when multiple DNA molecules were bound to the same microsphere, they underwent braiding and the trajectory of the sphere spiraled down to the point of attachment on the surface. The braiding process displayed two reversible regions of DNA compaction. We hypothesized that the energy stored in the braids due to the exerted torque could be used to unwind the DNA molecules. In order to observe if unwinding occurred, we used pairs of spheres bound together forming a paramagnetic dumbbell. Upon removal of the magnetic field, the braided DNA molecules underwent spontaneous unwinding, converting the stored torque into enough mechanical energy to rotate the tethered dumbbells for periods as long as 30 minutes. In most cases observed, the number of spontaneous rotations

corresponded to the number of induced rotations. We observed initial unwinding frequencies ranging from 0.02 Hz to 0.1 Hz, which decreased exponentially as the unbraiding process progressed. Using hydrodynamic equations, we estimated torques of up to 140 pN nm.

3087-Pos
Investigating the Interaction Between Folic Acid and Folate Binding Protein at the Single Molecule Level
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The folic acid-folate receptor recognition interaction has been exploited for targeted drug delivery, yet the detailed mechanism of this binding is unknown. Here, atomic force microscopy was employed to measure the folic acid-folate binding protein interaction. After conjugating a flexible poly(ethylene glycol) linker to folic acid and attaching this to an AFM tip, a controlled mechanical force was applied to disrupt the binding interaction. The amount of force required to cause rupture at various force loading rates were measured. The rupture force dependency on the loading rate characterizes the energy landscape of the single molecule interaction between folic acid and folate binding protein. Control experiments were performed to ascertain the specificity of the FA-FBP single molecule interaction. The use of dynamic force spectroscopy to investigate the details of this binding interaction provides new insight to guide the design of folate receptor-targeting molecules.

3088-Pos
Isopeptide Bonds Block the Mechanical Extension of Pilins in Gram-Positive Bacteria
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Pathogenic bacteria use long, fibrous structures known as pili to attach to host cells. In Gram-positive bacteria, the pili shaft is formed by the covalent polymerization of the so-called major pilin protein. Accessory pilins, usually with adhesive properties, are located along the pilus shaft interspersed with the major pilin, and/or at the tip of the pilus. In this work, the mechanical behavior of Spy0128, the major pilin from *Streptococcus pyogenes* (M1 serotype), has been studied by single-molecule force spectroscopy. Spy0128, which is composed of two domains, was sandwiched between I27 protein modules, and the resulting heteropolyprotein was pulled using an atomic force microscope. In this kind of experiments, the mechanical response of the I27 modules produces a characteristic fingerprint that identifies the traces where the pilin must have been subject to force. In those successful traces, no unfolding events corresponding to the pilin modules were detected, even when the protein was pulled at forces close to 1 nN. The possibility of the pilin modules extending at very low forces was ruled out by estimating the initial extension before the first I27 unfolding event. Therefore, Spy0128 is the most mechanically stable protein identified so far. In order to test whether the mechanical resilience of Spy0128 is caused by two intramolecular isopeptide bonds (one per domain) described by X-ray crystallography and mass spectroscopy, mutant variants of Spy0128 that block the formation of the isopeptide bonds were also studied. Different to wild-type, the mutant domains unfolded at around 200 pN, implying that the isopeptide bonds further stabilize protein domains that are mechanically stable *per se*. The findings presented here provide new insights into the mechanical architecture of pili from pathogenic Gram-positive bacteria.

3089-Pos
Multi-Scale Modeling of Force Propagation in Proteins Under Mechanical Stress
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Single-molecule measurements of how proteins respond to applied force can provide valuable clues to their structure-function relationship, particularly for proteins whose role *in vivo* relies on an ability to resist or sense force. Interpretation of such measurements relies heavily on theoretical and computational modeling; however, the brute-force approach, molecular dynamics simulation at atomic resolution, is only feasible for timescales orders of magnitude shorter than those appropriate to experiments. Thus coarse-graining is essential for accessing experimentally relevant timescales. However, almost all coarse-grained protein models to date have been designed for the explicit purpose of studying protein folding or normal mode flexibility, and are not capable of supplying quantitative predictions about response to large applied forces. We develop a new procedure for using force measurements from all-atom molecular dynamics simulations to parameterize a coarse-grained model specifically designed for studying force response. This model has the novel feature of using the flexible Morse potential as a basis function for describing non-bonded interactions. We test the model by using it to study the kinetics of ubiquitin rupture under quasi-equilibrium forcing, and compare with experimental results.

3090-Pos
Measuring Adhesion Forces Between Influenza Virus and Living Cells
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Influenza virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cells. This represents the first step of infection and requires multiple simultaneous interactions since the affinity between one single HA-SA pair is estimated to be very low (10-13 M-1). The attachment of influenza virus particles to living host cells was characterised on the level of single molecules using optical tweezers and atomic force spectroscopy. Unbinding events where analysed and revealed a multimodal rupture force distribution. This suggests sequential binding of multiple receptors. Treatment of the cells with neuraminidase (NA) which cleaves terminal sialic acid residues leads to a decrease of the binding probability by >50 %. This indicates a specific interaction between hemagglutinin and sialic acid unravelled by force measurements.

3091-Pos
Fusion of Biomimetic 'Stealth' Probes into Lipid Bilayer Cores
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The ability to specifically and non-destructively incorporate inorganic structures into or through biological membranes is essential to realizing full bio-inorganic integration. However, molecular delivery and interfaces to inorganic objects, such as patch-clamp pipettes, generally rely upon destructive membrane holes and serendipitous adhesion, rather than selective penetration and attachment to the bilayer. In fact, materials greater than a few nanometers have not been shown to penetrate lipid bilayers without disrupting the continuity of the membrane. I will discuss the development of nanofabricated probes that spontaneously insert into the hydrophobic membrane core by mimicking the hydrophobic banding of transmembrane proteins, forming a well-defined bio-inorganic lateral junction. These biomimetic 'stealth' probes consist of hydrophilic posts with 2-10 nm hydrophobic bands formed by molecular self-assembly, and are easily fabricated onto a variety of substrates including silicon wafers, nanoparticles, and AFM tips.
By fabricating this architecture onto AFM probes, we directly measured the penetration behavior and adhesion force of different molecular functionalities within the bilayer. Following insertion, stealth probes remain anchored in the center of the bilayer, while purely hydrophilic probes have no preferred location. The strength of the stealth probe adhesion varies greatly between short and long chain alkane functionalizations, indicating that chain mobility, orientation, and hydrophobicity all contribute to stability within the bilayer. In addition, the consequences of geometric factors such as band thickness and the presence of multiple bands on interface stability have been established. By selectively choosing the desired properties of the hydrophobic band, it is possible to tune the failure tension of the interface from values comparable to that of pristine lipid vesicles to only a fraction of the strength. Finally, the ability to transfer the stealth probe behavior to other platforms (e.g. nanoparticles for drug delivery) will be discussed.

3092-Pos
Effect of Cell Mechanics on the Transient Force Response of a Molecular Bond and the Derived Kinetic Off-Rate
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Molecular force has a fundamental role in cellular motility. The correct knowledge of the transient history of molecular force is essential for accurate estimation of kinetics parameters through force spectroscopy. Currently, it is assumed that the molecular force is instantaneously equal to the externally applied force. In this work we predict via analytical models and simulation of two cells attached by a single bond that cell mechanics and hydrodynamics modulates the externally applied force such that the instantaneous bond force is not equivalent. Various mechanical models (solid, elastic, viscoelastic) of cells and microvilli are considered over relevant ranges of loading rates (10^2-10^5 pN/s) and force magnitudes (0-200 pN). Specifically it is demonstrated that both microvillus extension and tether formation decrease the pulling force imposed on the adhesive bonds leading to a prolonged bond lifetime. It is demonstrated that modulation of molecular force leads to inaccurate estimation of kinetic off-rate. In particular, it is shown that the applied force is not instantaneously