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
Prithu Sundd[1], Edgar Gutierrez[2], Maria Pospieszalska[1], Alexander Groisman[2], Klaus Ley[1].
[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA.
[2]UCSD, La Jolla, CA, USA.
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
Micah J. McCauley[1], Leila Shokri[1], Ioulia Rouzina[2], Mark C. Williams[1].
[1]Northeastern University, Boston, MA, USA, [2]University of Minnesota, Minneapolis, MN, USA.
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
Shulin Zhuang, Qing Peng, Yi Cao, Hongbin Li.
University of British Columbia, Vancouver, BC, Canada.
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
Laurel E. Averett, Oleg V. Gorkun, Mark H. Schoenfisch.
UNC - Chapel Hill, Chapel Hill, NC, USA.
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
Mónica Fernández-Sierra, Violeta Delgado-Martí, Edwin Quiñones.
University of Puerto Rico, San Juan, PR, USA.
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