individual repeats or their alpha-helical subunits. We also present evidence for the capture of unfolding/refolding transient events while stretching or relaxing by AFM and analyze differences in refolding lengths and forces for each repeat. This class of stacked helical-repeats behave as molecular nanosprings, are likely important for cellular mechanosensation, and can be used as platforms for structural elements of nanomechanical systems based on proteins. Supported by the NIH (PEM) and HHMI (VB).

3077-Pos

High Force Elastic Profiles of Single and Double Stranded Polynucleotides Probed with AFM Force Spectroscopy

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Elasticity is an important property of nucleic acids. During cellular processes, DNA and RNA are subjected to various mechanical forces which greatly deform their original structure. Also, in the field of DNA nanotechnology, an understanding of how DNA will react to mechanical loading will allow for the design of novel nanostructures with different forms and functions. The elastic response of nucleic acids subjected to very high loadings on the order of 1 nN has not been previously studied. We use AFM-based single-molecule force spectroscopy to, for the first time, compare and contrast the elasticity of different sequences of double and single stranded polynucleotides, including single stranded poly(A), poly(dA), poly(dT), poly(C), and poly(dC); and double stranded poly(dA)poly(dT), poly(dA-dT), poly(dG)poly(dC), and poly(dG-dC). We found that even up to forces as high as 800 pN poly(dA) is stiffer than the other single stranded structures. We have also observed marked differences in the behavior of double stranded poly(dA)poly(dT) and poly(dG)poly(dC) with poly(dA-dT) and poly(dG-dC), respectively. Despite their different elasticities, these double stranded polynucleotides exhibit striking features similar to those exhibited by poly(dA) when stretched. We investigate the origin of these differences and similarities in terms of base-base and base-backbone interactions.

3078-Pos

Adhesion Mechanisms of the Mussel Foot Proteins mfp-1 and mfp-3

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Mussels adhere to a variety of surfaces by depositing a highly specific ensemble of 3,4-dihydroxyphenyl-L-alanine (DOPA) containing proteins. The adhesive properties of Mytilus edulis foot proteins mfp-1 and mfp-3 on mica (a common alumino-silicate clay mineral) and TiO2 surfaces were directly measured at the nano-scale by using a surface forces apparatus (SFA). The adhesion energy between mfp-3 and mica was on the order of W=3×10-4 J/m2 which corresponds to an approximate force per plaque of ~100 gm - more than enough to hold a mussel in place if no peeling occurs. In contrast, no adhesion was detected between mica surfaces bridged by mfp-1. AFM imaging and SFA experiments showed that mfp-1 can adhere well to a single mica surface, but in order for bridging to occur between two mica surfaces the protein must be sheared or allowed extended contact time with the opposing surface. On TiO2 surfaces the mfp-1 interaction is 10-fold stronger than with mica, presumably due to capability of DOPA to form coordination bonds with the TiO2 surface. The results are consistent with the apparent function of the proteins, i.e., mfp-1 is disposed as a "protective" coating and mfp-3 as the adhesive or ''glue'' that binds mussels to surfaces. While mussel foot protein is capable of making strong adhesive bonds with TiO2, the adhesion to mica is actually weak and likely due to weak physical interactions rather than chemical bonding. However, strong adhesion forces of mussel plaques can arise as a consequence of plaque geometry (i.e., their inability to be peeled off) even on surfaces such as mica that do not have a high intrinsic surface or adhesion energy, W.

3079-Pos

Motor-Substrate Interactions in a Ring ATPase

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Many processes in biology, including DNA recombination, prokaryotic cell-segregation, gene transposition, and viral DNA packaging, involve the translocation of DNA or RNA by ATP-driven ring motors belonging to the ASCE/AAA superfamily. While the mechanism by which these motors convert the chemical energy from ATP hydrolysis to mechanical work is beginning to be understood, little is known about how these motors engage their nucleic acid substrates. Do motors contact a single DNA element, such as a phosphate or a base, or are contacts distributed over multiple parts of the DNA? In addition, what role do these contacts play in the mechanochemical cycle? Here we use a single-molecule assay for the genome packaging motor of the *Bacillus subtilis* bacteriophage phi-29 to address these questions. The full mechanochemical cycle of the motor involves two phases–an ATP loading dwell followed by a translocation burst of four 2.5-bp steps. By challenging the motor with a variety of modified DNA substrates, we show that during the dwell phase important contacts are made with adjacent phosphates every 10-bp on the 5'-3' strand in the direction of packaging. In addition to providing stable, long-lived contacts, these phosphate interactions also regulate the chemical cycle. In contrast, during the burst phase, we find that DNA translocation is driven against large forces by extensive contacts, some of which are not specific to the chemical moieties of DNA. Such promiscuous, non-specific contacts may reflect common translocase-substrate interactions for both the nucleic acid and protein translocases of the ASCE superfamily.

3080-Pos

Mapping Micro-Mechanical and Micro-Structural Changes in the Ageing Aorta

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In healthy individuals, arterial function is critically dependent on the biomechanical properties of stiff fibrillar collagens, resilient elastic fibre proteins and contractile smooth muscle cells. Although age-related reductions in arterial compliance (arteriosclerosis) are associated with chronic hypertension and hence with the development of aortic aneurysms, heart failure and stroke, the differential role played by each of these vascular components in the progression of disease remains unclear.

The scanning acoustic microscope (SAM), when operated at frequencies close to 1GHz, is capable of measuring acoustic wave speeds (which are related to tissue stiffness) with a spatial resolution of ~1 micrometer. Using unfixed tissue cryo-sections, we mapped variations in wavespeed from the intimal surface of young (less than 1.75years) and old (more than 8.00years) sheep aortas. Whilst there was a significant age-related increase in mean wavespeed, across the tissue (young: 1.847km/s, SEM 0.004km/s; old: 1.882km/s, SEM 0.003km/s; Mann Whitney U test, $p<0.001$) the increase was most pronounced in the inter-lamellar (IL) regions located between large elastic lamellae (EL) (wavespeed increase; IL: 0.047km/s, EL: 0.021km/s). Atomic force microscopy of ovine aorta cryo-sections identified both fine elastic fibres and collagen fibril bundles within this IL space. Collagen and elastin contents of young and old aortas were determined (as a percentage of tissue section area) using light microscopy of picrosirious red and Miller's stained sections respectively. Although collagen content increased significantly in old compared with young sheep (young: 30.97%, SD 2.63%; old: 44.86%, SEM 5.00%; Student's t-test $p<0.05$) there was no significant change in elastin content (young: 49.75%, SD 4.86%; old: 49.98%, SEM 4.27%; Student's t-test $p=0.97$).

These observations suggest, therefore, that gross mechanical stiffening of the ageing aorta, may occur primarily as a result of localised collagen remodelling in the space between elastic lamellae.

3081-Pos

Integrating Dynamic Force Spectroscopy and Surface Plasmon Resonance to Define the Energy Landscape for Integrin:Ligand Binding

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Background: Blood clots, aggregates of platelets trapped in a mesh of fibrin fibers, can impede normal blood flow, causing heart attacks and strokes. Therapeutic interventions use drugs with Arg-Gly-Asp (RGD) sequences to disrupt interactions between platelet αIIbβ3 integrins and the fibrin network's subunits. Determination of the αIIbβ3-ligand energy landscape will elucidate the successes and limitations of integrin antagonists.

Objectives: Integrating surface plasmon resonance (SPR) and dynamic force spectroscopy (DFS), we studied the energetics of αIIbβ3: ligand interactions. We focused on cHArGD, a cyclic peptide structurally similar to eptifibatide, a cardiovascular disease drug, as well as to fibrinogen's KQAGDV integrin-recognition sequence.

Methods: DFS determined single bond rupture forces, the dissociation constant k_{off}, and the rupture distance x_{-1} for αIIbβ3: cHArGD interactions. SPR determined the kinetic and thermodynamic parameters for αIIbβ3: cHArGD binding.

Results: DFS performed at three different pulling rates (14000, 42000, and 70000) pN/s) yielded rupture forces of 77, 86 and 88 pN; Bell model analysis yielded a dissociation constant, k_{off} ~ 0.03 sec^{-1} and rupture distance x_{-1}~ 0.6 nm. Excess cHArGD in solution dramatically reduced the rupture

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.
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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