lipid bilayer to protect and exchange their contents with the cell. We have developed methods primarily based on atomic force microscopy that allows precise investigation of the mechanical properties of liposomes and that could be applied to study other related organelles/viruses. The mechanical properties of small, spherical vesicles were probed by applying very low forces (~ 0.1 nN), which led to a maximum 10 % deformation. The effects of lipid composition, temperature, osmotic pressure and the radius of curvature were studied for liposomes with diameters between 30 and 150 nm. The liposome deformation was modeled using finite element methods in order to extract the lipid bilayer elastic properties. For the larger liposomes we find a very good agreement with previously reported experiments on micrometer sized giant vesicles.

3071-Pos

Probing the Mechanical Properties of Single Scleroproteins with Optical Tweezers

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Stretching and relaxing single proteins provides quantitative information on their elasticity and other mechanical properties. This can be done with optical tweezers, a technique in which the ends of the protein are chemically attached to micron-scale spheres, used to manipulate the protein and measure its response. We are working on the application of this technique to scleroproteins, nonglobular proteins whose mechanical properties are of direct relevance to their physiological roles. These proteins self-assemble into hierarchically organized load-bearing structures, often found in the extracellular matrix. The ability of optical tweezers to manipulate single molecules and higher-order structures suggests their application to probing the mechanical response at different hierarchies of assembly. Applying this technique to stretch these single proteins presents many challenges, including the production of constructs with appropriate labels for attachment to microspheres, relatively short contour lengths which can introduce experimental artifacts, and self-aggregation and binding interactions of these predominantly insoluble proteins, which make it difficult to isolate and manipulate single molecules. We discuss our work to overcome these challenges, with a specific focus on elastin.

3072-Pos

Elastic Behavior of ssDNA in Salty Solutions

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The interaction between highly charged poly-ions, such as DNA, and the smaller ions in salty solutions is of fundamental importance to the basic processes of molecular biology (e.g., ion-mediated nucleic acid folding, collapse and stabilization of proteins). Despite its importance, this phenomenon is poorly understood, particularly for multivalent ions where mean-field theories (e.g. Debye-Huckel) break down. By stretching single denatured ssDNAs in monovalent salt solutions, we have established that force-extension measurements directly and quantitatively probe electrostatic effects on charged polymers in solution (O.A. Saleh *et al.*, PRL **102**, 068301 (2009)). We exploited access to the 'tensile blob' regime to show that, for a broad range of NaCl concentrations, ssDNA behaves as a real polymer in good solvent with a Kuhn length linearly proportional to the Debye length. Here, we present data on the effects of cations with different valences and chemistries on ssDNA structure. We find that the effects of divalent ions greatly exceed those predicted by simple Debye-Huckel calculations and discuss our data in the context of more realistic theories.

3073-Pos

Mechanical Unfolding of Cardiac Myosin Binding Protein-C by Atomic Force Microscopy

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Cardiac myosin binding protein-C (cMyBP-C) is a member of the immunoglobulin (Ig) superfamily of proteins and consists of 8 Ig- and 3 fibronectin (Fn)-like domains along with a unique regulatory sequence referred to as the M-domain. Domains near the C-terminus bind tightly to myosin and mediate the association of MyBP-C with thick (myosin-containing) filaments, whereas N-terminal domains of MyBP-C, including the M-domain, bind reversibly to myosin S2 and/or actin. The ability of MyBP-C to bind to both myosin and actin raises the possibility that MyBP-C cross links thick and thin (actin-containing) filaments and thereby imposes a drag that regulates shortening velocity during contraction. To investigate the mechanical properties of the proposed thick-thin filament linkage, we used atomic force microscopy (AFM) and electron microscopy (EM) to assess the single molecule elasticity and mechanical stability of full-length mouse cardiac (c) MyBP-C expressed in sF9 cells. Force-extension curves showed that cMyBP-C is extensible via unfolding of individual domains evident as "saw tooth" peaks in force spectra. Spectra with up to 12 peaks were obtained. The force required to unfold the domains varied, with the least and most stable domains unfolding at forces <50 pN and >100 pN, respectively, suggesting that a mechanical hierarchy exists along cMyBP-C. EM images of purified, rotary shadowed cMyBP-C showed that molecules were frequently V- or U-shaped with lengths ~44 nm. These data indicate that cMyBP-C is extensible and contains regions with variable resistances that could slow sarcomere shortening or limit lattice expansion. Supported by NIH HL080367.

3074-Pos

Active Force Clamp Control of Optical Tweezers

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In a typical high-resolution optical tweezers (OT) experiment a molecular motor changes the contour length of a trapped dumbbell-construct. Unless the inter-trap distance is actively controlled the OT increases the load on the molecular motor as it steps along the template. To counter this phenomenon we implement a real-time controller for the OT to be used in constant force measurements.

We trap a dumbbell construct (bead-DNA-bead) in an inverted microscope by dividing a CW laser beam into a stationary trap and a steerable trap. Separate low power detection lasers and position sensitive detectors in the back-focal plane measure the position of both beads. The position of the bead in the stationary trap is used for constant-force feedback control. The feedback algorithm runs a Proportional-Integral-Derivative-controller on a field programmable gate array, and acousto-optical deflectors update the steerable trap position at a rate of 200 kHz.

We test the force clamp control with a 10kb dsDNA molecule and present a theory explaining the power spectrum of the force clamped bead's position. We study the effect of controller bandwidth by digitally filtering the signal used for feedback control, and test the response time of our real-time controlled optical tweezers with a RNA hairpin opening/closing reaction.

3075-Pos

Protein-DNA Interactions Studies using Magnetic Tweezers

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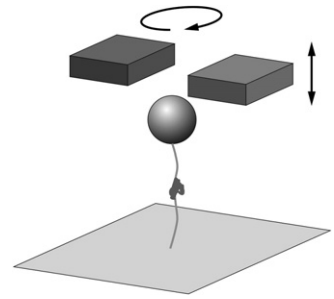
Single molecule force spectroscopy techniques allow the forces, energy barriers, and mechanisms of biologically important structural transitions to be manipulated and observed at the single molecule scale. A major advantage of magnetic tweezers is the ability to manipulate not only the force in such systems, but also the torque, which is extremely important in processes involving the DNA double helix, since such processes frequently involve rotational motion. We will present recent results studying protein-DNA interactions using a newly constructed magnetic tweezer.



3076-Pos

Single-Molecule Atomic-Force Spectroscopy Captures a Novel Class of Molecular Nanosprings with Robust Stepwise Refolding Properties

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Biological systems are constantly under mechanical stress either during movement or when acted upon by external forces. The identification of proteins motifs that behave as biological springs will be important for understanding how cells respond to mechanical stimuli and can also propel the design of non-biological nanomaterials. We report here identification of a large class of alpha-helical spiral or solenoid-shaped proteins comprised of ANK-R, ARM, or HEAT repeats that rapidly and forcefully refold following stretching. Each of these repeats unfolds and refolds in equilibrium through discrete events involving