Mechanical manipulation at the single molecule level of proteins exhibiting mechanical stability poses a technical challenge that has been almost exclusively approached by atomic force microscopy (AFM) techniques. However, due to mechanical drift limitations, AFM techniques are restricted to experimental recordings that last less than a minute in the high-force regime. Here we demonstrate a novel combination of electromagnetic tweezers and evanescent nanometry that readily captures the forced unfolding trajectories of protein L at pulling forces as low as 10~15 pN. Using this approach, we monitor unfolding and refolding cycles of the same polyprotein for a period of time longer than 30 minutes. From such long lasting recordings, we obtain ensemble averages of unfolding step sizes and rates that are consistent with single molecule AFM data obtained at higher stretching forces. The unfolding kinetics of protein L at low stretching forces confirms and extends the observations that the mechanical unfolding rate is exponentially dependent on the pulling force within a wide range of stretching forces spanning from 13 pN up to 120 pN, thereby excluding the presence of curvature in the rate-versus-force plot. Our experiments demonstrate a novel approach for the mechanical manipulation of single proteins for extended periods of time in the low-force regime, providing an ideal complement to force-clamp AFM, expanding the accessible regions of the unfolding energy landscape of a mechanically stable protein.

2860-Plat
Magnetic Tweezers Measurement of Single Molecule Torque
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Helical duplex DNA continually experiences torque from translocating macromolecular complexes and helix unwinding proteins. We have developed a magnetic tweezers methodology using a cylindrical magnet and magnetic nanorods to directly measure torsional stress, or resistive torque, as twists are introduced at low pulling forces. We demonstrate the utility of this method by measuring the resistive torque of single DNA molecules and, for the first time, single chromatin fibers.
Figure: New and conventional magnetic tweezers configurations. (a) In conventional magnetic tweezers, the field orients the induced dipole of the superparamagnetic bead horizontally, producing a strong horizontal angular trap that prevents angular fluctuations of the probe. (b) In the new configuration consisting of a vertical magnetic field and nanorod-bead construct, the magnetic field and the probe dipole align vertically, thus horizontal angular movements are not constrained. A weak horizontal force generates a weak horizontal angular trap allowing us to measure the torque applied to the molecule. (c) Scanning electron micrograph of magnetic Ni-Pt nanorods (bar = 1μm). (d) Bright-field image of nanorod-bead probe. Nanorod and bead self-assemble by magnetic attraction.

2861-Plat
A Novel Way To Combine Magnetic Tweezers and Fluorescence Microscopy For Single Molecule Studies
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Single-molecule experiments make it possible to look at and manipulate individual molecular systems in real time and do not suffer from averaging over a large number of unsynchronised events. In magnetic tweezers, one traps a single DNA molecule between a glass surface and a magnetic bead, and exerts controlled force and topological constraint on the double helix. However, visualization of events occurring along the molecule is made difficult by the geometry of the device, in which the trapped molecule is pulled perpendicular to the observation plane. We developed a new generation of magnetic tweezers that addresses this problem, while keeping the mechanical control of the trapped molecule. The DNA-bead system is injected into a microfluidic channel placed on a home-made epifluorescence microscope. The channel's top surface is coated with a reflective metallic layer and is tilted so that the whole length of the stretched tether's reflection will be located at the focus of the objective. We applied this instrument to the study of homologous recombination, a DNA double-strand break repair pathway playing an essential role in vivo in genome maintenance and replication. The functional form of the central protein in this process, RecA in bacteria and Rad51 in eukaryotes, is a nucleoprotein complex in which protein monomers assemble into a helical filament on single-stranded DNA. This filament promotes homologous pairing and strand exchange with duplex DNA. Despite the extensive literature on homologous recombination, several key aspects of the detailed molecular mechanism remain quite controversial, especially concerning how the nucleoprotein filament interacts with homologous DNA and exchanges strands. For the first time, our instrument allowed us to observe individual nucleoprotein filaments, labeled with quantum dots, interacting with a double-stranded DNA molecule held by the tweezers.

2862-Plat
First-principles Calculation Of DNA Looping In Tethered Particle Experiments
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We show how to calculate the probability of DNA loop formation mediated by regulatory proteins such as Lac repressor, using a mathematical model of DNA elasticity. Our approach has new features enabling us to compute quantities directly observable in Tethered Particle Motion (TPM) experiments; e.g. it accounts for all the entropic forces present in such experiments. Our model has no free parameters; it characterizes DNA elasticity using information obtained in other kinds of experiments. It can compute both the ''looping J factor'' (or equivalently, looping free energy) for various DNA construct geometries and repressor concentrations, as well as the detailed probability density function of bead excursions. We also show how to extract the same quantities from recent experimental data on tethered particle motion, and compare to our model's predictions. In particular, we present a new method to correct observed data for finite camera shutter time.
The model successfully reproduces the detailed distributions of bead excursion, including their surprising three-peak structure, without any fit parameters and without invoking any alternative conformation of the repressor tetramer. However, for short DNA loops (around 95 bp) the experiments show more looping than is predicted by the linear-elasticity model, echoing other recent experimental results. Because the experiments we study are done in vitro, this anomalously high looping cannot be rationalized as resulting from the presence of DNA-bending proteins or other cellular machinery. We also show that it is unlikely to be the result of a hypothetical ''open'' conformation of the repressor.
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2863-Plat
Single Molecule Investigations of HSP70 Proteins
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Heat Shock Proteins 70 (HSP70) are a class of proteins involved in protein folding and are highly upregulated when a cell is under stress. The protein consists of three functional domains: the N-terminal ATP binding domain where ATP and ADP can bind, the substrate binding domain which can bind small residues and the C-terminal domains which acts as a lid for the substrate binding domain. It is thought that the binding of ATP/ADP drives conformational changes in this protein.
Using burst analysis with pulsed interleaved excitation, we have performed single-pair Förster resonance energy transfer (FRET) experiments to investigated the distribution of conformations in HSP70 molecules under different conditions. The FRET efficiency is very sensitive to the distance between donor and acceptor on the scale of 2-10 nm and thus provides information over the conformation of the different HSP70 domains as they diffusion through the focus of our confocal microscope. As experiments are performed on single molecules, subpopulations can be directly observed. We have investigated the conformation of HSP70 by cloning a number of mutants that allowed specific labeling of the different domains. Experiments were performed with or without ATP, ADP, and nonhydrolysable ATP analogs as well as in the absence or presence of peptide substrates or cochaperons. We will present how the conformation and flexibility of HSP70 is influenced by these various interactions.

2864-Plat
Single-molecule Observations of Replisome Structure and Function
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DNA replication requires the coordinated activity of a large number of enzymes at the replication fork. Understanding the mechanisms controlling this organization requires a direct probing of the dynamics of fully functional replisomes during replication. Observations at the single-molecule level provide the most direct way to visualize the complex biochemistry of the replisome and to quantify the many transient intermediates essential to replication. We present a novel assay that combines the observation of individual fluorescently labeled proteins with the mechanical manipulation of DNA. Surface-tethered DNAs labeled