51-Plat
Assembly and Regulation of the Human RNA Polymerase II Preinitiation Complex: A Single-Molecule Approach to a Complex Biological System
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Transcription of all protein-coding genes in human cells begins with the assembly of the RNA polymerase II preinitiation complex (PIC). The PIC consists of Pol II itself and at least six general transcription factors (more than forty polypeptides, total molecular weight of ~ 3MDa). Due to the high complexity and the dynamic nature of the PIC, the mechanism of its assembly and regulation remains elusive after decades of biochemical studies. We have developed a surface-based, promoter-specific Pol II transcription system suitable for single-molecule analysis. The system consists of (a) an imaging surface and a fluidics system supporting Pol II transcription from immobilized DNA templates; (b) fluorescently labeled transcription factors (recombinant and highly purified from nuclear extracts); (c) novel probes capable of rapid real-time detection of mRNA synthesis at the single-molecule level. We have begun to use this system to dissect the PIC assembly process coupled to a fully reconstituted single molecule transcription reaction. We use two separate multi-color fluorescence imaging instruments capable of simultaneous detection of thousands of individual PIC assembly and transcription events at sub-second time resolution for hour-long time periods: (a) actively stabilized (drift of <1 nm over hours) temperature-controlled total internal reflection (TIR) microscope; (b) zero mode waveguide multiplex confocal imaging system developed by Pacific Biosciences. Using these first generation reconstituted single molecule RNA Pol II transcription systems, we have gained new insights into the mechanisms of human transcriptional regulation not attainable by the conventional in vitro biochemical assays that have been the gold standard for over 30 years.

52-Plat
Scanning Evanescent Fields in TIRF Microscopy Using a Single Point-Like Light Source and a DNA Worm Drive
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Total internal reflection fluorescence (TIRF) microscopy is an elegant optical technique that limits the dimension of the volume, in which fluorophores are excited, along the z-direction to the hundred nanometer-scale. The method makes use of the evanescent field, which arises when light is totally internally reflected at the boundary to a medium of lower refractive index. Often the penetration depth of this exponentially decaying field is left undetermined, which sets limits to the reproducibility in different experiments. Here, we present a novel method to measure this quantity: We use a quantum dot as a point-like light source and a Holliday junction as a drive to move the fluorescent probe with nanometer precision perpendicular to the substrate surface. The junction serves as a worm drive, which couples rotation into translational movement, while the DNA pitch serves as a precise intrinsic ruler. The Holliday junction is forced to migrate by adding negative turns to the DNA stretched perpendicular to the surface using magnetic tweezers. This causes the quantum dot, which is attached upstream of the junction, to decrease its height above the surface by 3.4 nm per turn. Thus it can be moved continuously through the excitation field while monitoring its height-dependent fluorescent signal. Since the quantum dot is a point-like light source, the intensity decay of the evanescent field can be obtained by dividing the signal recorded in TIRF illumination by the one recorded in conventional epi-illumination without further corrections. Our assay should allow for probing the axial intensity profile of any electromagnetic field distribution with a point-like light source, without the necessity of attaching it to an interfering surface.

53-Plat
Catch, Hold, and Release: Manipulating Single DNA Molecules in Solution
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The ability to manipulate matter on submicron length scales has revolutionized biophysical research and fueled important scientific and technological advances in past decades. For example, larger dielectric particles can be trapped free in solution by steep electromagnetic field gradients produced by a strongly focused laser beam (optical tweezers).
Pushing the limits to the nanometer level, however, has proven challenging. The only known method for trapping fluorescent nanoparticles such as single molecules uses a time-varying DC field in a feedback loop to counteract Brownian motion. This trap, however, can only operate when the particle can be located through fluorescence detection, which is problematic in the case of single molecules where intermittent fluorescence emission is often observed, and requires a complex hardware and software setup.
In this contribution, we will discuss a novel and elegant approach for the trapping and manipulation of single molecules and other particles over extended periods of time: the electrostatic corral. The proposed trapping scheme has distinct characteristics which set it apart from other trapping techniques, such as a trapping efficiency that scales favorably with particle size (down to the single molecule level), a stable potential well that does not require any imaging for particle trapping, and multi-particle trapping capabilities. The feasibility of the corral trap approach will be demonstrated with experiments on micro- and nanoscale particles, with particular emphasis on the trapping of single-stranded DNA molecules.

54-Plat
Dual Molecule Assay to Investigate the Mechanics of Homology Search and Recognition in Recombination
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Central to homologous recombination in E. Coli is the interaction of a RecA nucleoprotein filament formed on DNA with a second DNA molecule. The filament-DNA interaction governs the search for sequence homology, sequence recognition, and eventually the exchange of homologous strands. Here, we employ a dual molecule assay to study the mechanics of this two-molecule interaction.
In our experiments, a RecA filament is manipulated in 3D by dual-bead optical tweezers, and brought into contact with a second dsDNA molecule tethered in a magnetic-tweezers configuration. The strength of the interaction is studied using a force-spectroscopy technique with the magnetic bead as the sensitive force probe (resolution 10 fN/sqrt(Hz)). We present results on the interaction between supercoiled dsDNA and RecA filaments formed on dsDNA. We find that the strength of interaction depends sensitively on the supercoiling state of the dsDNA with stronger interactions recorded for negatively supercoiled DNA. This observation is interpreted as enhanced binding of DNA in a denatured state to the secondary binding site of the RecA filament. The strong affinity of the RecA filament for denatured dsDNA could lead to a favoring of the search process to regions of denatured dsDNA where base sampling is possible, a feature that is expected to be important in governing a fast and efficient homology search process.

55-Plat
Measuring the Folding Landscape of a Harmonically Constrained Biopolymer
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Pioneering studies have shown that the probability distribution of opening length for a DNA hairpin, recorded under constant force using an optical trap, can be used to reconstruct the energy landscape of the transition. However, measurements made under constant force are subject to some limitations. Under constant force a system with a formidable energy barrier, such as a long hairpin, spends most of its time in the closed or open state, with relatively few statistics collected in the transition state region. We describe a measurement scheme in which a harmonic constraint is imposed on the opening length of the hairpin whose origin can be chosen to enhance the collection of statistics in a given region. An algorithm has been developed which extracts the native energy landscape of the hairpin from a series of data runs in which the system is constrained at various regions of the energy landscape. We illustrate this technique in simulations and demonstrate its effectiveness in experiments on a DNA hairpin. We find that the combination of harmonic constraint with the use of extremely short DNA handles facilitates a high resolution measurement of the folding landscape of the hairpin.

56-Plat
Excitable Particles in an Optical Torque Wrench
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The optical torque wrench is a laser trapping technique capable of applying and directly measuring torque on microscopic birefringent particles via spin momentum transfer, and has found application in the measurement of static torsional properties of biological molecules such as single DNAs. Motivated by the potential of the optical torque wrench to access the fast rotational dynamics of biological systems, a result of its all-optical manipulation and detection, we focus on the angular dynamics of the trapped birefringent particle, demonstrating its excitability in the vicinity of a critical point. This links the optical torque