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Observation of DNA Knots Using Solid-State Nanopores

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Long DNA molecules can self-entangle into knots. Experimental techniques to observe such DNA knots (primarily gel electrophoresis) are limited to bulk methods and circular molecules below 10 kbp in length. Here we show that solid-state nanopores can be used to directly observe individual DNA knots in both linear and circular single molecules of arbitrary length. DNA knots are observed as short spikes in the nanopore current traces of traversing DNA molecules. The observation of knots is dependent on sufficiently high measurement resolution, which can be achieved using high-concentration LiCl buffers. We study the percentage of DNA molecules with knots for different DNA molecules, up to 166 kbp in length. We find that the knotting probability rises strongly with length, and compare our experimental data to simulation-based predictions for long polymers. From the translocation time of the knot through the nanopore, we estimate that the majority of the DNA knots are tight, with small sizes below 100 nm. In the case of linear molecules, we observe that knots are able to slide out upon applying high driving forces (voltage). Our results demonstrate that the solid-state nanopore technique can provide a wealth of information about the position and the size of knots, including the number of DNA strands inside DNA knots.

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Enhanced Electrostatic Force Microscopy Imaging Reveals Mechanism of TRF2 Mediated DNA Compaction

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T-loop formation at telomeres is proposed to play an important role in telomere protection through the sequestration of the 3' single-stranded overhang. Previous studies indicated that shelterin protein TRF2 modulates telomere structure by promoting DNA compaction and T-loop formation. To further understand the mechanism underlying TRF2-mediated DNA compaction, we applied Dual-Resonance-frequency-Enhanced Electrostatic force Microscopy (DREEM), a recently developed technique capable of high-resolution imaging of weak electrostatic potentials. DREEM images of nucleosomes clearly reveal DNA strands wrapped around histone proteins in nucleosomal arrays. In contrast, DREEM imaging shows DNA compacted inside TRF2 complexes through a 3-dimensional stacking of TRF2 dimers mediated by collective actions of multiple copies of TRF2 proteins. TRF2-mediated DNA compaction leads to electric potential gradients across the complexes. Surprisingly, while DNA wrapped around histones displays similar electrostatic potential signals compared to bare DNA, TRF2 DNA compaction leads to significant differences in the electrical potential signals inside multi-oligomeric TRF2 complexes compared to protein or DNA alone. These results clearly demonstrate the electrostatic changes in TRF2-DNA complexes upon oligomerizaton of proteins and DNA compaction, and underscore the importance of developing new electrostatic force microscopy imaging techniques for studying biological systems.

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Grab & Watch: Correlative Optical Tweezers-Fluorescence Microscopy (CTFM) as a Versatile Tool for Biological Studies

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The combination of optical tweezers with confocal fluorescence microscopy and microfluidics is a promising approach to investigate biological system because of its unique ability to simultaneously manipulate, sense and visualize individual molecules with exquisite sensitivity and resolution. Here we present our efforts in validating this technology by developing a commercial-grade instrument that allows turn-key operation with ease of operation, without compromising performance. We call this instrument ''Correlative optical Tweezers-Fluorescence Microscope'' (CTFM). Using this instrument, we carry out a series of demonstration experiments, exploring applications of CTFM in the fields of DNA-protein interaction, nano-mechanics of intermediate filaments, synthetic models of signal transduction and molecular motors. These experiments show that the technological advances in hybrid single-molecule methods can be turned into an easy to use and stable instrument that has the ability to open up new venues in many research areas.

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Manipulation with Magnetic Tweezers of Mechanosensitive Ion Channels and Adaptation Motors in Hair Cells of the Inner Ear

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Hair cells, ubiquitously found in the auditory and vestibular systems of vertebrates, transduce mechanical stimuli into electrical responses. The apical surface of each cell sports a hair bundle composed of numerous actin-filled stereocilia. Mechanical stimulation deflects the hair bundle and tenses filamentous cadherin tetramers called tip links that interconnect adjacent stereocilia. Tensing of the tip links is thought to open mechanosensitive ion channels to which the links are connected, leading to depolarization of the hair cell and the subsequent firing of afferent nerve fibers. A motor containing myosin 1c molecules readjusts the tension in the tip links, allowing transduction to adapt during sustained deflections. Because the channels and motors lie deep within the hair bundle, the direct experimental manipulation of the individual components has been challenging: experiments have heretofore been limited to deflections of entire hair bundles. We have devised a scheme for labeling tip links from the sacculus of the bullfrog's inner ear with paramagnetic particles through an epitope-specific anti-cadherin antibody and a long DNA tether. By measuring the potential difference across the tissue, we show that application of a magnetic field elicits inward transduction current_a result that directly confirms the hypothesis that tension in the tip links gates the mechanically sensitive channels. Future experiments that track the position of the magnetic particle may demonstrate the opening and closing of transduction channels and the climbing and slipping of adaptation motors.

Force Spectroscopy and Scanning Probe Microscopy

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Bringing Force Probe Molecular Dynamics Simulations Closer to Experiments

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The interaction between Streptavidin and Biotin is one of the strongest known non covalent bonds in nature. For this reason, many experimental and computational studies have been done on this complex, which revealed many details on binding and unbinding of biotin, which turned out to be an astonishingly complex process.

However, taken all these studies together and despite considerable technical advances over the past years, there is still a time scale gap remaining, i.e., the loading rates possible in force spectroscopy experiments and those achievable in molecular dynamics (MD) simulations hardly overlap.

Recent high speed force spectroscopy (HS-FS) experiments by Scheuring offer the possibility to reach pulling velocities up to 5 mm/s.

Here we present extensive MD simulations covering pulling velocities from 0.5 mm/s to 50 m/s, thus providing an overlap of one order of magnitude with experimental time scales. Further, to resemble the AFM experiment as closely as possible in the simulations, we modeled the PEG-linker between Biotin and the cantilever as a wormlike chain and chose a spring constant of the pulling potential to match the stiffness of the cantilever in HS-FS experiments done by Scheuring.

Over the whole interval, agreement of the probability distributions of unbinding forces is seen. Taken together, these data cover unbinding time scales over 10 orders of magnitude, which offers a unique opportunity to compare against several proposed transition state treatments of this process.

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A Precision Scanning Probe Microscope with Direct Access to the Three Dimensional Tip-Sample Interaction Force Vector

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Single-molecule imaging and force spectroscopy studies have produced rich insights into macromolecular structure and dynamics in biological settings and the atomic force microscope (AFM) has thus emerged as an important complementary apparatus in the structural biologist's toolkit. While conventional AFM has achieved sub-nm resolution imaging of membrane proteins in native membrane, atomic-scale tip-sample stability - which opens promising new