DNA: bending, stretching or melting. Our goal is to understand the physics behind these processes. To this end, we use optical tweezers in combination with fluorescence microscopy and microfluidics, which allows manipulation of DNA, application and measurement of forces, localization and counting of proteins and ligands binding to it, and rapid switching of buffer conditions. Using this approach, we visualize that DNA melts under high tension. We find that melting occurs from the ends, in bursts of several hundreds of basepairs, which can be readily discerned in force-extension curves. An equilibrium thermodynamic model, with the elastistic energy of single- and double-stranded DNA stretches and the base-pairing energy calculated from the DNA sequence as input parameters, provides an excellent quantitative description of the melting progress. We furthermore demonstrate that for a proper quantitative description of DNA elasticity it is imperative to include tension-induced winding and unwinding of the double helix, resulting in a novel model for DNA elasticity: the ''twistable worm-like chain''.

2137-Plat
Direct Mechanical Measurements Reveal the Material Properties of 3D DNA-Origami
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The recent development of the origami technique has revolutionized DNA-based assembly by allowing the synthesis of arbitrarily shaped and sub-micrometer sized two-dimensional (2D) and three-dimensional (3D) architectures with atomic precision. Such structures offer great potential for the development of nanomechanical elements, mediators and sensors, which however requires detailed understanding of their complex mechanical properties. Using magnetic tweezers, we here present direct mechanical measurements on single DNA origami structures and characterize the bending and torsional rigidity of four- and six-helix bundles assembled by this technique. Compared to duplex DNA, we find the bending rigidities to be greatly increased while the torsional rigidities are only moderately augmented. We present a mechanical model explicitly including the crossovers between the individual helices in the origami structure that can describe the experimentally observed behavior. Our results provide an important basis for future applications of 3D DNA origami structures as rigid scaffolds and force transducers as well as noise suppressors in single-molecule mechanical measurements. Beyond that we show how origami structures can be defined and rigidly interfaced to surfaces, which is an important prerequisite to develop structured and three-dimensional surface modifications with the help of DNA templates.

2138-Plat
Single-Molecule Analysis of the Functional Structure of Telomerase RNP
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Telomerase is a cellular ribonucleoprotein (RNP) complex comprised of telomerase RNA, telomerase reverse transcriptase (TERT) and other protein cofactors. The activity of the telomerase enzyme is essential for the maintenance of genome stability and normal cell development. Despite the biomedical importance of telomerase activity, detailed structural models for the enzyme remain to be established. Here we report the development and application of a single-molecule assay for direct structural analysis of catalytically active telomerase enzymes. In this assay, oligonucleotide hybridization was used to probe the primer-extension activity of individual telomerase enzymes with single nucleotide sensitivity, allowing precise discrimination between inactive, active and processive enzyme binding events. FRET signals from individual enzyme molecules during the active and processive binding events were then used to determine the global organization of telomerase RNA within catalytically active holoenzymes. Using this assay, we have identified an active conformation of telomerase among a heterogeneous population of enzymes with distinct structures. In particular, we have established that the phylogenetically conserved pseudoknot motif within telomerase RNA is properly folded in catalytically active enzymes. Interestingly, the pseudoknot motif is misfolded in the absence of proteins, and the protein subunits of the telomerase holoenzyme counteract RNA misfolding and allow a significant fraction of the RNPs to form the pseudoknot structure. Only those RNP complexes containing a properly folded pseudoknot are catalytically active. These results not only demonstrate the functional importance of various telomerase RNA regions such as the pseudoknot but also establish a direct method for elucidating how the structure of telomerase enables its function.

2139-Plat
Global Structure of a Three-Way Junction in a phi29 Packaging RNA Dimer determined using Site-Directed Spin Labeling
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The condensation of bacteriophage phi29 genomic DNA into its preformed procapsid requires the DNA packaging motor, which is the strongest known biological motor. The packaging motor is an intricate ring-shaped protein/RNA complex, and its function requires an RNA component, called the packaging RNA (pRNA). Current structural information on pRNA is limited, which hinders studies of motor function. Here, we used site-directed spin labeling to map the conformation of a pRNA 3-way junction, which bridges binding sites for the motor ATPase and the procapsid. The studies were carried out in a pRNA dimer, which is the simplest ring-shaped pRNA complex and serves as a functional intermediate during motor assembly. Using a nucleotide-independent labeling scheme, stable nitroxide radicals (R5) were attached to eight specific pRNA sites without perturbing RNA folding and dimer formation, and a total of 17 inter-R5 distances spanning the 3-way junction were measured using Double Electron-Electron Resonance spectroscopy. The measured distances, together with steric chemical constraints, were used to select approximately 3,600 viable 3-way junction models from a pool of 65 billion. The results reveal a similar conformation among all the viable models, with two of the helices (HT and HL) adopting an acute bend. This contrasts to a recently reported pRNA tetramer crystal structure, in which HT and HL stack onto each other linearly. The studies reveal versatility in pRNA conformations that may be beneficial to packaging motor function, and establish a new venue for mapping global structures of complex RNA molecules.

2140-Plat
Visualizing Large RNA Molecules in Solution
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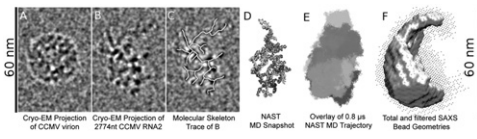
Single-stranded (ss) RNAs longer than a few hundred nucleotides do not have a unique structure in solution. Their equilibrium properties therefore reflect the average of an ensemble of structures. We use cryo-electron microscopy to image projections of individual long ssRNA molecules and characterize the anisotropy of their ensembles in solution. A flattened prolate volume is found to best represent the shapes of these ensembles. The measured sizes and anisotropies are in good agreement with complementary determinations using small-angle X-ray scattering and coarse-grained molecular dynamics simulations. A long viral ssRNA is compared with shorter non-coding transcripts to demonstrate that prolate geometry and flatness are generic properties independent of sequence length and origin. The anisotropy persists under physiological as well as low-ionic-strength conditions, revealing a direct correlation between secondary structure asymmetry and 3D shape and size. We discuss the physical origin of the generic anisotropy and its biological implications.
The attached figure uses experimental and computational visualization methods to demonstrate that a 2774-nucleotide viral RNA has an elongated concave shape under *in vitro* assembly conditions and is physically slightly larger than the virus particle it ultimately packages into.



2141-Plat
The Structure and Function of Highly Bent Toroidal DNA in Bacteriophage φ29
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Tailed bacteriophages are highly efficient machines for infecting a host cell. A single virion must attach to the host surface, penetrate the cell wall, then release its double-stranded DNA genome. In these remarkable DNA delivery machines, controlling how and when the genome is released is a task of paramount importance. Recently, a three-dimensional cryo-electron microscopy (cryo-EM) reconstruction of mature bacteriophage φ29 revealed an intriguing toroidal DNA structure contained in a small cavity below the viral capsid. This highly bent toroidal DNA supercoil is thought to be 30-40 basepairs of dsDNA and its function remains unknown.
In this study, we employ an elastic rod model to simulate highly strained DNA as it is compressed within the protein cavity. The model provides estimates of force and energy required to form the toroid as well as its equilibrium