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Assembling, Visualizing and Improving DNA Motors using Single Molecule Fluorescence

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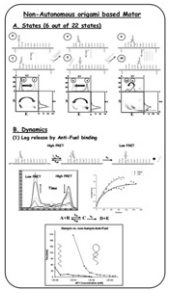
A major obstacle in developing and improving complex DNA-machines, such as motors and robots, is the difficulty of characterizing the integrity, structural dynamics and function of the assembly intermediates and of the final product. Typically, non in-situ Gel and AFM and in-situ bulk fluorescence methods are used, but these methods often failed to provide detailed structural dynamic information sufficient for device rational improvements.

We will present two different DNA-motors studied using single-molecule fluorescence techniques. One motor is made of DNA-track embedded in a DNA-origami on which bipedal DNA-walker is striding upon interacting with fuel/anti-fuel, while the second motor is design to adapt autonomous behavior.



The single-molecule FRET method provides detailed information about the motors structure, and the ALEX, provides information about the motor integrity and helps cleaning the data, monitors the motor assembly process and demonstrates activity in real time. The binding rate of different fuel/anti-fuel type and sequence and the efficiency of their individual steps are compared to achieve maximum operation efficiency, speed, and reliability. The detailed structural dynamic information extracted allows deeper theoretical understanding of the system behavior, and as a consequence, leading to motor improvement.

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Insights on the Nature of the DNA Overstretching Transition from Experiments and Simulations

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We study the kinetics of the overstretching transition in lambda-phage double-stranded (ds) DNA from the basic conformation (B state) to the 1.7-times longer and partially unwound conformation (S state). Using a dual-laser optical tweezers with unprecedented time resolution we have recently demonstrated that millisecond force steps of 0.5-2 pN applied to the dsDNA at 25 °C in the range 62-72 pN (the overstretching transition region) induce elongation responses with exponential time course, suggesting a two-state nature of the B-S transition (Bianco et al., Biophys. J. 101, 866-874, 2011). The load-dependence of the rate constant of the elongation allowed to define the elementary elongation step (~5.8 nm) and thus the degree of cooperativity (~25 base pairs). Here we expand the investigation determining the effect of temperature (range 10-25 °C) on the kinetics of the overstretching transition. The U-shaped relation between the rate constant of elongation and the force is progressively shifted to higher forces by the reduction in temperature, so that at 10 °C the force for the minimum rate is 70 pN, 4 pN higher than at 25 °C. Instead, both the minimum value of the rate constant and the degree of cooperativity are temperature independent, suggesting that the transition barrier between the compact and the extended state is basically entropic. All-atoms molecular dynamics simulations support these conclusions and quantitatively reproduce the enthalpic profile determined by fitting the experimental data. According to simulations, overstretched DNA is characterized by a limited amount of residual base pairing and a rather efficient hydrophobic screening of apolar regions.

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Single-Molecule Elasticity Measurements Reveal Swelling Transition in PEG and Certain ssDNAs

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Although long, flexible polymers form self-avoiding random walks when free in solution, most single-molecule stretching experiments elongate the polymer into a highly-aligned geometry, preventing the long-range interactions that lead to polymer swelling. Here, we report single-molecule stretching data at low force and quantify the effects of swelling in synthetic PEG molecules and various sequences of single-stranded DNA (including heterogeneous ssDNA and poly(dA)). These data will be discussed in light of classic polymer scaling theory and electrostatic effects. Synthetic PEG molecules show multiple elastic transitions as force is increased: 1. at low force the polymer forms swollen coils, 2. at intermediate force it forms smaller, ideal coils, and 3. at high force it forms an elongated chain. In contrast, charged, denatured single-stranded DNA, in mono- and divalent salt solutions, shows an immediate transition from a swollen chain at low forces to an extended chain at high forces, lacking the intermediate ideal coils regime. Single-stranded DNA composed entirely of adenine bases (poly(dA)) cooperatively base stacks. Thus, at low forces, poly(dA) has stiff base-stacked domains interspersed with domains of swollen coils, indicated by an elastic response that is intermediate between ideal and self-avoiding. These data permit estimation of microscopic, intensive properties such as the Kuhn length and excluded volume, which have value in understanding the polymer's low (to zero) force structure and how it varies with polymer size and solvent conditions (e.g., salt concentration).

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Dynamics and Multiple Binding Modes of DNA Intercalators Revealed by Single-Molecule Force Spectroscopy

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By combining single molecule force spectroscopy with simple buffer exchange, we isolated the effects of binding and intercalation of YOYO and YOPRO. We showed that force-enhanced intercalation can occur from a reservoir of bound dye that was not fully intercalated, yet remained out of equilibrium with free dye for periods long compared to many single molecule experiments (>5 min for YOPRO and >2 hr for YOYO). Moreover, for YOYO, this reservoir of polycyclic moieties that is not bis-intercalated accounts for the most of the decay due to the force-enhanced intercalation. Our work highlights that binding/unbinding and intercalation/de-intercalation are distinct processes that can occur on very different time scales.

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Bubbles, Free Ends and the Kinetics of Force-Induced DNA Melting

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Application of a stretching force to the opposite ends of torsionally unconstrained double stranded (ds) DNA leads to its abrupt 1.7-fold elongation at a force of roughly 65 pN. While the disruption of base stacking is understood to drive the length change, the extent to which base pairing remains intact continues to be controversial. Here we present kinetic data on the overstretching transition including new results on the pulling rate dependence of the DNA overstretching transition at different solution ionic strengths. It is now clear that the observed DNA overstretching transition indeed occurs in two very different modes, though bases are unpaired in both. In the first mode, DNA peels sequentially from any free end. In the second mode, bubbles of several hundred cooperatively melted base pairs appear along heterogeneous DNA. Kinetic data further reveals that the transition state is much closer to dsDNA than to the melted form. Our results also show that only the rate of base pair closing is strongly affected by force, which destabilizes each base pair not by facilitating melting but by inhibiting closing. Finally, we discuss how the switch between the peeling and internal FIM modes is affected by solution ionic strength, temperature, DNA sequence heterogeneity and the DNA pulling rate.

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Using a Light-Activated Culture Matrix to Determine the Microenvironmental Cues that Initiate Breast-Cancer Tumor Metastasis

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Many types of gels (e.g. collagen, alginate, matrigel, hyaluronate, and polyacrylamide) are currently being employed to study how cells interact with their environment. The mechanical and chemical properties of these gels are established by the degree of polymer crosslinking and the ECM proteins covalently attached to the matrix using bifunctional crosslinkers, respectively. Once synthesized, these gels have static mechanical and chemical properties that cannot be changed. Thus, they cannot be used to study how evolving microenvironmental conditions affect cell behavior and signalling. Furthermore, because of how the gels are synthesized, removal of cells for analysis is usually not feasible. Here, we describe a light-activated culture matrix for studying how cells adapt to dynamic microenvironmental conditions. Using a reversible, light-mediated interaction to crosslink biocompatible polymers (e.g. PEG or PVA), we can synthesize, reversibly and controllably, a 3D-culture environment whose mechanical and chemical properties can be modulated with near-IR light. By employing light that is not phototoxic to the cells, we can quickly decompose the gel, thereby releasing the cells for immediate analysis. We are employing this dynamically controllable culture matrix to investigate how microenvironmental cues facilitate tumor progression in breast cancer. In particular we are investigating the role of the microenvironment in controlling the behavior (e.g. quiescence vs tumor initiation and proliferation) of CD24-/CD44+ tumor-initiating cells.