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Mechanism Of DNA Translocation By SpoIIIE/Ftsk
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During cell division, chromosomal DNA is segregated both rapidly and specifically. In bacteria, chromosomal segregation is conducted by the DNA translocases FtsK (*Escherichia coli*) and SpoIIIE (*Bacillus subtilis*).These enzymes assemble at the division septum and translocate the chromosome (~400 kb and ~3 Mbp, respectively) unidirectionally by using the energy of ATP hydrolysis by means of their AAA+ motor domains. Despite functional and sequence similarities, SpoIIIE and FtsK were proposed to use drastically different mechanisms: SpoIIIE was suggested to be a unidirectional DNA transporter that exports DNA from the compartment in which it assembles, whereas FtsK was shown to establish translocation directionality by interacting with highly skewed chromosomal sequences. Here, we use a combination of single-molecule, bioinformatics and *in vivo* fluorescence methodologies to unveil the mechanism of DNA translocation by SpoIIIE, and to show that its wirestripping activity is largely responsible for compartment-specific gene expression during sporulation. We propose a sequence-directed DNA exporter model that reconciles previously proposed models for SpoIIIE and FtsK, and constitutes the first unified model for directional DNA transport by the SpoIIIE/FtsK/Tra family of AAA+ ring ATPases.

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VirE2: A Unique ssDNA-Compacting Molecular Machine
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The translocation of single-stranded DNA (ssDNA) across membranes of two cells is a fundamental biological process occurring in both bacterial conjugation and *Agrobacterium* pathogenesis. Whereas bacterial conjugation spreads antibiotic resistance, *Agrobacterium* facilitates efficient interkingdom transfer of ssDNA from its cytoplasm to the host plant cell nucleus. These processes rely on the Type IV secretion system (T4SS), an active multiprotein channel spanning the bacterial inner and outer membranes. T4SSs export specific proteins, among them relaxases, which covalently bind to the 5′ end of the translocated ssDNA and mediate ssDNA export. In *Agrobacterium tumefaciens*, another exported protein_VirE2_enhances ssDNA transfer efficiency 2000-fold. VirE2 binds cooperatively to the transferred ssDNA (T-DNA) and forms a compact helical structure, mediating T-DNA import into the host cell nucleus. We demonstrated_using single-molecule techniques_that by cooperatively binding to ssDNA, VirE2 proteins act as a powerful molecular machine. VirE2 actively pulls ssDNA and is capable of working against 50-pN loads without the need for external energy sources. Combining biochemical and cell biology data, we suggest that, in vivo, VirE2 binding to ssDNA allows an efficient import and pulling of ssDNA into the host. These findings provide a new insight into the ssDNA translocation mechanism from the recipient cell perspective. Efficient translocation only relies on the presence of ssDNA binding proteins in the recipient cell that compacts ssDNA upon binding. This facilitated transfer could hence be a more general ssDNA import mechanism also occurring in bacterial conjugation and DNA uptake processes.

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Experimental Evidence Of Polymer Hydrophobic Collapse Due To Water Density Fluctuation
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Hydrophobic collapse of protein chain is a major driving force for its folding in water. However, the detailed mechanism of hydrophobic collapse is not clear. One compelling theory explains the hydrophobic collapse due to the density fluctuation of water creating local voids in the vicinity of a hydrophobic polymer chain that drives its collapse. This density fluctuation happens rapidly in the time scale of picoseconds and is therefore difficult to detect directly. However, a result of the fluctuation is the much longer lasting collapsed structure of hydrophobic polymer chains, which can be detected by single molecule force spectroscopy (SMFS). We performed experiments to investigate the effect of water density fluctuation in the collapse of single polystyrene (PS) chains using the atomic force microscope (AFM). In particular, we applied a constant tension to single PS chain and detected end-to-end distance fluctuation, which directly probes the collapsed state of the PS chain and provides us insight to the hydrophobic collapse of the molecule.

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Non-ideal Elasticity Of Single Stranded DNA At Low Forces
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Single molecule force-extension experiments on dsDNA and ssDNA are usually compared to the worm-like chain (WLC) or freely-jointed chain (FJC) models. The WLC and FJC are ideal polymer models: they account for local stiffness through the persistence or Kuhn length, but ignore long-range interactions between monomers, accounted for by, e.g., the excluded-volume parameter in the Flory scaling theory, that determine the self-avoiding random-walk structure of polymers not under tension. To attempt to bridge the gap between ideal force/extension models and the classic scaling picture of real polymers, we explore the low-force elasticity of chemically-denatured single-stranded DNA (ssDNA) using magnetic tweezers. We find a low-force regime where extension grows as a non-linear power-law with force, in contradiction with ideal predictions, but in agreement with scaling predictions made by P. Pincus (Macromolecules 9, 386 (1976)). By analyzing this power-law regime, we extract the dependence of the Kuhn length of ssDNA on monovalent salt concentration, and find that it is linearly proportional to the Debye screening length. This result is in contrast to the quadratic dependence predicted in the well-known OSF theory, but agrees with other theories and simulations. Finally, we identify a high-salt point ([NaCl] ~ 3 M) where ideal polymer behavior returns, and the data is well-fit by the WLC model at all forces. We identify this as a theta point of the polymer, and show that, beyond 3M salt, the ssDNA aggregates in a manner consistent with a polymer in poor solution.

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Single Molecule Force Spectroscopy of Guanine Quadruplex DNA
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A single molecule force spectroscopy study was performed to examine the formation and stability of guanine quadruplexes using various G-rich oligomers. The study examined the differences in interactions of four-stranded and two-stranded G-quadruplexes. The two-stranded G-quadruplex system was used for dynamic force spectroscopy measurements. Rupture force dependency on the log loading rate was found to be non-linear. Using recently developed microscopic models, the apparent kinetic and thermodynamic parameters for the G-quadruplex system were estimated. The microscopic models predict barrier widths that are consistent with the bond length of the quadruplex strand.

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Kalman Filter Estimates of the Contour Length of an Unfolding Protein in Single-Molecule Force Spectroscopy Experiments
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Force spectroscopy measurements of single molecules using AFM have enabled the study of a range of molecular properties not accessible with bulk methods. These properties of interest must typically be inferred by manually fitting models to selected portions of measured data. As manual intervention in the fitting process easily introduces a bias in the analysis, there is a need for more sophisticated analysis methods capable of interpreting data in an unbiased and repeatable ''hands-off'' manner. Here we apply an extended Kalman filter to the estimation of protein contour length (L_c) during mechanical unfolding, based on force and extension data from an AFM experiment. This filter provides an online and fully automated estimate of L_c based on a system model, the experimental measurements, and noise statistics. The system model comprises a physical model of the cantilever and a nonlinear WLC approximation of the extended protein. When manually fitting the WLC model to force-extension data from ubiquitin proteins, the estimate of the change in contour length during unfolding is distributed normally as N (22.7 nm, 6.59 nm^2). Testing the Kalman filter on the same protein yields ΔL_c ~ N (24.54 nm, 0.24 nm^2). As the variance limits resolution in estimating the number of amino acids released by unfolding, it is clear that the Kalman filter presents a substantial improvement over the conventional method. We thereby demonstrate that the Kalman filter provides a powerful unbiased approach to interpreting force spectroscopy data, capable of increasing resolution beyond the traditional experimental limit. Due to the flexibility of this approach, it can be extended to monitoring other state variables of molecular systems observed by various forms of force spectroscopy, including optical and magnetic tweezers.