Interpreting atomic force microscopy measurements of hydrodynamic and surface forces with nonlinear parametric estimation

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A nonlinear parameter estimation method has been developed to extract the separation-dependent surface force and cantilever spring constant from atomic force microscope data taken at different speeds for the interaction between a silica colloidal probe and plate in aqueous solution. The distinguishing feature of this approach is that it exploits information from the velocity dependence of the force-displacement data due to hydrodynamic interaction to provide an unbiased estimate of the functional form of the separation-dependent surface force. An assumed function for the surface force with unknown parameters is not required. In addition, the analysis also yields a consistent estimate of the in situ cantilever spring constant. In combination with data from static force measurements, this approach can further be used to quantify the extent of hydrodynamic slip.

Single-molecule imaging reveals target-search mechanisms during DNA mismatch repair

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The ability of proteins to locate specific targets among a vast excess of nonspecific DNA is a fundamental theme in biology. Basic principles governing these search mechanisms remain poorly understood, and no study has provided direct visualization of single proteins searching for and engaging target sites. Here we use the postreplicative mismatch repair proteins MutS\textsubscript{α} and MutL\textsubscript{α} as model systems for understanding
Regulation of actin-myosin interaction by conserved periodic sites of tropomyosin

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Cooperative activation of actin–myosin interaction by tropomyosin (Tm) is central to regulation of contraction in muscle cells and cellular and intracellular movements in nonmuscle cells. The steric blocking model of muscle regulation proposed 40 y ago has been substantiated at both the kinetic and structural levels. Even with atomic resolution structures of the major players, how Tm binds and is designed for regulatory function has remained a mystery. Here we show that a set of periodically distributed evolutionarily conserved surface residues of Tm is required for cooperative regulation of actomyosin. Based on our results, we propose a model of Tm on a structure of actin–Tm–myosin in the “open” (on) state showing potential electrostatic interactions of the residues with both actin and myosin. The sites alternate with a second set of conserved surface residues that are important for actin binding in the inhibitory state in the absence of myosin. The transition from the closed to open states requires the sites identified here, even when troponin + Ca\textsuperscript{2+} is present. The evolutionarily conserved residues are important for actomyosin regulation, a universal function of Tm that has a common structural basis and mechanism.

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Single-Particle Tracking Demonstrates that Actin Coordinates the Movement of the Ebola Virus Matrix Protein

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The Ebola virus causes severe hemorrhagic fever and has a mortality rate that can be as high as 90%, yet no vaccines or approved therapeutics, to our knowledge, are available. To replicate and egress the infected host cell the Ebola virus uses VP40, its major matrix protein to assemble at the inner leaflet of the plasma membrane. The assembly and budding of VP40 from the plasma membrane of host cells seem still poorly understood. We investigated the assembly and egress of VP40 at the plasma membrane of human cells using single-particle tracking. Our results demonstrate that actin coordinates the movement and assembly of VP40, a critical step in viral egress. These findings underscore the ability of single-molecule techniques to investigate the interplay of VP40 and host proteins in viral replication.

**In Vivo Biochemistry in Bacterial Cells Using FRAP: Insight into the Translation Cycle**

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In vivo measurements of the mobility and binding kinetics of cellular components are essential to fully understand the biochemical processes occurring inside cells. Here, we describe a fluorescence recovery after photobleaching-based method that can be easily implemented to the study of reaction-diffusion processes in live bacteria despite their small size. We apply this method to provide new, to our knowledge, quantitative insight into multiple aspects of the bacterial translation cycle by measuring the binding kinetics and the micrometer-scale diffusive properties of the 50S ribosomal subunit in live Caulobacter cells. From our measurements, we infer that 70% of 50S subunits are engaged in translation and display, on average, limited motion on the micrometer scale, consistent with little mixing of transcripts undergoing translation. We also extract the average rate constants for the binding of 50S subunits to 30S initiation complexes during initiation and for their release from mRNAs when translation is completed. From this, we estimate the average time of protein synthesis and the average search time of 50S subunits before they engage in the next initiation event. Additionally, our experiments suggest that so-called free 50S subunits do not diffuse freely; instead their mobility is significantly slowed down, possibly through transient associations with mRNA.

**Fibers with Integrated Mechanochemical Switches: Minimalistic Design Principles Derived from Fibronectin**

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Inspired by molecular mechanisms that cells exploit to sense mechanical forces and convert them into biochemical signals, chemists dream of designing mechanochemical switches integrated into materials. Using the adhesion protein fibronectin, whose multiple repeats essentially display distinct molecular recognition motifs, we derived a computational model to explain how minimalistic designs of repeats translate into the mechanical characteristics of their fibrillar assemblies. The hierarchy of repeat-unfolding within fibrils is controlled not only by their relative mechanical stabilities, as found for single molecules, but also by the strength of cryptic interactions between adjacent molecules that become activated by stretching. The force-induced exposure of cryptic sites furthermore regulates the nonlinearity of stress-strain curves, the strain at which such fibers break, and the refolding kinetics and fraction of misfolded repeats. Gaining such
computational insights at the mesoscale is important because translating protein-based concepts into novel polymer designs has proven difficult.

The Conformational Ensembles of α-Synuclein and Tau: Combining Single-Molecule FRET and Simulations

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Intrinsically disordered proteins (IDPs) are increasingly recognized for their important roles in a range of biological contexts, both in normal physiological function and in a variety of devastating human diseases. However, their structural characterization by traditional biophysical methods, for the purposes of understanding their function and dysfunction, has proved challenging. Here, we investigate the model IDPs α-Synuclein (αS) and tau, that are involved in major neurodegenerative conditions including Parkinson’s and Alzheimer’s diseases, using excluded volume Monte Carlo simulations constrained by pairwise distance distributions from single-molecule fluorescence measurements. Using this, to our knowledge, novel approach we find that a relatively small number of intermolecular distance constraints are sufficient to accurately determine the dimensions and polymer conformational statistics of αS and tau in solution. Moreover, this method can detect local changes in αS and tau conformations that correlate with enhanced aggregation. Constrained Monte Carlo simulations produce ensembles that are in excellent agreement both with experimental measurements on αS and tau and with all-atom, explicit solvent molecular dynamics simulations of αS, with much lower configurational sampling requirements and computational expense.

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Abstract Top

Single molecule studies have expanded rapidly over the past decade and have the ability to provide an unprecedented level of understanding of biological systems. A common challenge upon introduction of novel, data-rich approaches is the management, processing, and analysis of the complex data sets that are generated. We provide a standardized approach for analyzing these data in the freely available software package SMART: Single Molecule Analysis
Research Tool. SMART provides a format for organizing and easily accessing single molecule data, a general hidden Markov modeling algorithm for fitting an array of possible models specified by the user, a standardized data structure and graphical user interfaces to streamline the analysis and visualization of data. This approach guides experimental design, facilitating acquisition of the maximal information from single molecule experiments. SMART also provides a standardized format to allow dissemination of single molecule data and transparency in the analysis of reported data.