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Platform: Force Spectroscopy and Scanning Probe Microscopy

1780-Plat
Imaging and Three-Dimensional Reconstruction of Chemical Groups in a Protein Complex using DNA Labels
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Atomic force microscopy (AFM) is a powerful tool for imaging and chemical characterization of bio-samples at molecular resolution in physiologically-relevant environments. However, the localized tip-sample interactions limit high-resolution images to the topmost layer of surfaces. Consequently, characterizing the three-dimensional (3-D) inner structures of molecules has been a challenge. Here, we demonstrate three-dimensional (3-D) localization of chemical groups within a single protein complex using AFM. We employ short DNA sequences to label specific chemical groups inside a protein complex. T-shaped cantilevers functionalized with complementary probe DNAs allow locating each label with sequence specificity and sub-nanometer resolution. We also measure pairwise distances between labels and reconstruct the 3-D loci of the target groups using simple geometric calculations. Experiments with the biotin-streptavidin complex showed that the 3-D loci of carboxylic acids of biotins are within 2-Angstroms of their respective 3-D loci in the corresponding crystal structure, suggesting AFM may complement existing structural biological techniques in solving structures that are difficult to study due to their size and complexity. This technique maybe finds applications in studying structure of DNA or RNA binding proteins.

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Acoustic Force Spectroscopy
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Single-molecule force spectroscopy has become an indispensable tool to unravel the structural and mechano-chemical properties of biomolecules. In most force-spectroscopy instruments only a limited number of biomolecules can be studied simultaneously, which reduces experimental throughput and limits statistics. At the same time, many independent measurements are required to distinguish the intrinsic stochasticity of the process of interest from heterogeneity.
With Acoustic Force Spectroscopy (AFS) we extend the force-spectroscopy toolbox with an acoustic manipulation device that allows exerting acoustic forces on tethered molecules. AFS is a Lab-on-a-chip device consisting of a flow cell of two glass plates with a fluid chamber in between and a piezo element glued on top. While applying an alternating voltage to the piezo element, forces from sub-pN to hundreds of pNs are exerted to thousands of biomolecules in parallel, with sub-millisecond response time and inherent stability.
As a proof of concept we performed force-extension measurements on DNA and RecA-coated DNA. These experiments demonstrate that AFS can be used to apply highly controlled forces up to at least 120 pN, with a force ramp speed between 10^{-4} - 10^2 pN/s and showing inherent stability over tens of hours over an observation area of at least 1 mm^2. AFS experiments are highly parallel, allowing the simultaneous measurement of thousands of biomolecules simultaneously, in a single field of view. We demonstrate the use of this by mapping the energy landscape of the DIG/anti-DIG antibody-antigen bond over 6 orders of magnitude of force loading rates within 2 days of experimentation.
AFS distinguishes itself by its relative simplicity, low cost and compactness, which allow straightforward implementation in lab-on-a-chip devices. These aspects will help to spread single-molecule methods from the realm of fundamental research in specialized laboratories towards more wide-spread applications in for example molecular biology and medical diagnostics.

1782-Plat
Revisiting the Free Energy of Modular Proteins under Force
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The elasticity of muscle tissue relies on tandem modular proteins such as titin, which gives muscles passive elasticity. A free-energy model is the only way to understand and predict the behavior under force of such complex proteins, composed of hundreds of individual domains. Here we use magnetic tweezers to measure the dynamics of single tandem modular proteins under constant force conditions during hour-long recordings. At forces between 4 to 17 pN we measure unfolding/refolding of individual domains as upward/downward steps in the end-to-end protein length, while higher forces yield unfolding steps exclusively. We find a strong force dependency of the step size of proteins undergoing folding/unfolding reactions with the applied force. This finding contradicts current free energy models of proteins that typically do not consider the polymeric nature of a denatured polypeptide chain under force and simply scale the free energy of a protein with the mechanical work. To explain the measured step size dependency with force we propose a new free energy model that also considers the entropic work needed to extend the molecule. Brownian dynamics simulations over the proposed free energy landscape accurately reproduce our experimental benchmarks. The experimental and theoretical advances demonstrated in this work provide a novel view on the free energy of proteins under force, now permitting a more realistic modeling of tissue elasticity.

1783-Plat
Directly Observing the Reversible Unfolding and Refolding of an Alpha/Beta Protein by Single-Molecule Atomic Force Microscopy
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Single-molecule force spectroscopy based on atomic force microscopy (AFM) has evolved into a powerful tool to study protein folding-unfolding dynamics and mechanism. Direct observation of protein refolding in real time using AFM has been challenging. Proteins that have been observed to refold using AFM are mainly limited to all-alpha proteins, such as ankyrin and calmodulin. Here we report the use of AFM to directly monitor the folding of an alpha/beta protein, NuG2. Our results indicate that at slow pulling speeds (<50 nm/s), the refolding of NuG2 can be clearly observed. Lowering the pulling speed reduces the difference between the unfolding forces and refolding forces, bringing the non-equilibrium unfolding-refolding towards equilibrium. At very slow pulling speeds (~2 nm/s), reversible unfolding and refolding were observed. Based on Crooks Theorem, we measured the equilibrium free energy change between the folded and unfolded states of NuG2, which is in good agreement with values reported using bulk chemical denaturation method. Our results demonstrate the utility of AFM in elucidating the unfolding-refolding dynamics of proteins close to equilibrium.

1784-Plat
Electromagnetic Tweezers with Independent Force and Torque Control
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Mechanical forces and torques play an important role in many biological processes. A range of instruments have been developed for direct and precise measurements of force and torque applied by biomolecules ranging from proteins to nucleic acids. However, simultaneous force and torque applications in which the two components are decoupled and controlled independently remain challenging and have only been implemented by recently-developed hybrid magnetic tweezers combining electro- and permanent magnets. Our goal was to develop bona fide electromagnetic tweezers (eMT) that can apply force and torque on single biomolecules or polymer molecules conjugated via superparamagnetic microspheres and can control the two components independently simply by changing the currents applied to different coils of the eMT. We implemented our eMT by combining a monopole that generates a force and a set of quadrupoles that generate a torque. To demonstrate the capability of our tweezers we attached Janus beads to single DNA molecules. We show that tension in the piconewton force range can be applied to single DNA molecules and simultaneously the molecule can be twisted with torques in the piconewton-nanometer regime. Our results also demonstrate that the two components are independently controlled. At various force levels applied to the Janus bead, the trap torsional stiffness can be changed simply by varying the current magnitude applied to the quadrupole torque tweezers. Our eMT voids the need of complex positioners to translate and rotate permanent magnets or frequency modulation of current to decouple the force and torque used in previous works. We believe that the flexible control of the mechanics of biomolecules with our eMT will enable novel studies of DNA-protein interactions and DNA conformation dynamics.