1951-Plat

Angstrom-Scale Chemical Microscopy: Multicolor Single-Molecule Imaging with Energy Landscape Engineering

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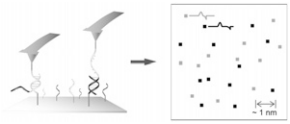
We explored physical mechanisms that might allow transducing Angstrom scale chemical information about biomolecules in a solution environment. Here we present multicolor imaging of chemical identities of biomolecules with Angstrom-scale resolution using single-molecule force spectroscopy (SMFS). We first demonstrate that SMFS can probe intrinsically non-equilibrium interactions among short complementary DNA strands whose melting points are below the room temperature. We observed a significant enhancement in the chemical selectivity of these far from equilibrium DNA interactions. The enhanced selectivity allowed us to discriminate molecular identities by designing probes that can hybridize to multiple short DNA targets, with each target creating a unique energy landscape. Due to the small dimensions of these molecules, images based on their interactions exhibited Angstrom scale resolution. Intrinsically non- equilibrium biomolecular interactions are difficult to characterize with methods relying on ensemble averages of equilibrated systems. The presented single-molecule approach harnesses non-equilibrium interactions to discriminate and map chemical identities on the Angstrom scale. The resulting imaging platform holds promise for both sub-molecular chemical characterization like sequencing and protein structure determination, and for studying biomolecular complexes and subcellular structures by using short conjugated DNA strands as Angstrom scale labels.



1952-Plat

Antibody Movement on Regular Antigen Clusters: Fab Arms are made for Walking

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Antibodies are key molecules for the immune system of vertebrates. The Y-shaped IgGs exhibit C2-symmetry; their Fc stem is connected to two identical Fab arms binding antigens. The Fc part is recognized by the complement system and by phagocytic cells. Antibodies can be considered molecular calipers; bivalent binding of the two Fab arms to adjacent antigens can only occur within a distance of roughly 6 to 12 nm. This leads to much higher avidity and slower dissociation rates as compared to monovalent binding. Here we show that antibodies exhibit ''bipedal'' walking on antigenic surfaces and static binding of both Fab arms of an antibody may hold true only for a time scale of ~ 0.04 s. The walking speed depends on the lateral spacing and symmetry of the antigens. On 2D-crystalline surfaces, such as found on bacteria and viruses, steric strain thus appears to be the main reason for short-lived bivalent binding. Importantly, the collision between randomly walking antibodies was seen to reduce their motional freedom. It leads to formation of transient antibody clusters even at low antibody density. Interestingly, such assemblies are known nucleation sites for docking of the complement system and/or phagocytes.

1953-Plat

High-Speed AFM Force Spectroscopy Unfolds Titin at the Speed of Molecular Dynamics Simulations

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The mechanical unfolding of muscle protein titin by atomic force microscopy (AFM) was a landmark experiment in single molecule biophysics (1). Computational molecular dynamics simulations aimed at deciphering the measured phenomena at the atomic level. The experimental mechanical unfolding by conventional AFM approaches was typically performed at velocities ranging from 10 nm to 10 μm per second. In contrast, full-atomic simulations can only be done over nano- to microseconds, resulting in typical pulling velocities ranging from 1 mm to 10 m per second (2). To bridge experiment to simulation, we have developed high-speed AFM single molecule force spectroscopy and carried out measurements at velocities reaching the mm/s range. With small cantilevers of ~1 MHz resonance frequency we unfold titin I91 domains (formerly I27) across the three orders of magnitude of velocity that separated so far experiment from simulation. This first example of a biophysical experiment that meets time scales of simulation allows us to evaluate the mechanical behavior of molecules at unprecedented loading rates and directly compare experimental data to atomistic predictions.

1. Rief, M., M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub. 1997. Reversible unfolding of individual titin immunoglobulin domains by AFM. Science 276:1109-1112.
2. Lee, E. H., J. Hsin, M. Sotomayor, G. Comellas, and K. Schulten. 2009. Discovery Through the Computational Microscope. Structure 17:1295-1306.

1954-Plat

Mechanically Untying a Protein Slipknot by Single Molecule Force Spectroscopy

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Protein structure is highly diverse when considering a wide range of protein types, helping to give rise to the multitude of functions that proteins perform. In particular, certain proteins are known to adopt a knotted or slipknotted fold. How such proteins undergo mechanical unfolding was investigated utilizing a combination of single molecule atomic force microscopy (AFM), protein engineering, and steered molecular dynamics (SMD) simulations to show the mechanical unfolding mechanism of the slipknotted protein AFV3-109. Our results reveal that the mechanical unfolding of AFV3-109 can proceed via multiple parallel unfolding pathways that all cause the protein slipknot to untie and the polypeptide chain to completely extend. These distinct unfolding pathways proceed via either a two- or three-state unfolding process involving the formation of a well-defined, stable intermediate state. SMD simulations predict the same contour length increments for different unfolding pathways as single molecule AFM results, thus providing a plausible molecular mechanism for the mechanical unfolding of AFV3-109. These SMD simulations also reveal that two-state unfolding is initiated from both the N- and C-termini, while three-state unfolding is initiated only from the C-terminus. In both pathways, the protein slipknot was untied during unfolding, and no tightened slipknot conformation was observed. Detailed analysis revealed that interactions between key structural elements lock the knotting loop in place, preventing it from shrinking and the formation of a tightened slipknot conformation. Our results demonstrate the bifurcation of the mechanical unfolding pathway of AFV3-109 and point to the generality of a kinetic partitioning mechanism for protein folding/unfolding.

1955-Plat

Dependence of the Most Probable and Average Bond Rupture Forces on the Force Loading Rate in Single Molecule Dynamic Force Spectroscopy Experiment: First Order Correction to the Bell - Evans Model

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The Bell-Evans model which predicts the linear dependence of the most probable bond rupture force on the logarithm of the force loading rate is usually used to discuss the dynamic force spectroscopy experiment data. This model is consistent with the Kramers' theory of the bond dissociation rate only if one presupposes an independence of the pre-exponential factors in the Kramers relation on the acting force F and a linear decrease of the dissociation barrier height on this same force, and for this to be true a rather special shape of the interaction landscape is required. By this reason, different attempts to generalize the model have been undertaken. They are usually based on certain specific dissociation rate first used in 1995 by Garg for quite different purposes in superconductivity researches. These models, however, are *not* a first order Taylor expansion correction to the Bell-Evans model and their applicability range is limiting.

Here we present a first order correction to Bell-Evans model where the first terms of corresponding Taylor expansions for Kramers' dissociation rate are taken into account. Both the most probable and average bond rupture forces are considered. All approximations made and the range of applicability of the obtained results are carefully described and compared with those for some other models in the field as well as with the experimental data. It is shown that in many cases the use of both Bell-Evans and ''Garg-like'' models can lead to misinterpretation of experimental data. In particular, the use of these models may sometimes lead to appearance of an ''artificial'' two-barrier picture.