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Editorial

AFM Meets Magnetic Tweezers to Probe Protein Dynamics under A Wide Range of Forces

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Magnetic Tweezers

Magnetic Tweezers (MT) is a single-molecule technology that allows researchers to investigate the mechanical characteristics of nucleic acids and protein-nucleic acid interactions in real time. A MT system, contrary to its name, does not manage items in the same way that a pair of macroscopic tweezers does. Because it uses the magnetic field gradient generated by permanent magnets to pull micrometer-size magnetic beads, the position of which can be tracked in three dimensions using pictures given by an inverted optical microscope and CMOS or CCD cameras, an MT is truly a magnetic puller. Fixed beads are used to determine the height of a glass surface as a zero-height reference (Ref-bead).With a DNA molecule with proper functionalization at their ends, DNA beads are anchored to the glass surface. The distance between Reference- and DNA-beads is calculated using the unique diffraction rings that arise from the optical image of the beads. The DNA end-to-end distance as a function of time is calculated by comparing the diffraction rings that arise from the optical image of the beads with a calibration profile. As a result, an MT setup can measure the extension of a DNA molecule in real time at a given force, which is determined by the magnets' distance from the beads.

Protein Dynamics

Proteins involved in processes including mechanotransduction, cell adhesion, and muscle contraction are exposed and forced to perform their functions. Mechanical loads cause conformational changes in these proteins, which lead to downstream actions such as molecular partner recruitment, increased intermolecular bond lifetimes4, and mechanical work delivery. Different nano mechanical properties of these proteins govern the force magnitudes to which they respond, a feature that is inextricably tied to their function and critical for the processes in which they are involved.

Single-molecule force spectroscopy techniques have permitted the mechanical manipulation of proteins for more than 20 years, providing us with unique insights into mechanobiological processes at the nano scale. Atomic Force Microscopy (AFM) is a technique that can apply well-calibrated forces to single proteins over a range of 10 pN to 2000 pN. It was one of the first techniques created and is still one of the most widely used today. The Nano mechanical characteristics of the Ig-like domains of the muscle protein titin, the high mechanical stability of bacterial adhesion proteins, or the extreme mechanostability of specific molecular interactions, to mention a few, have all been determined using AFM. No other single-molecule technique can examine ultra-stable folds and interactions at the force ranges reached by AFM. Unfortunately, despite some good examples, its inadequate resolution at low force ranges and instability has made it impossible to detect protein folding events and slow-kinetic molecular events that occur at forces below 20 pN and in narrow ranges. Magnetic tweezers, on the other hand, have proved that their sub-pN resolution and week-long stability in the 0.1-120 pN range out compete AFM for probing protein dynamics at low forces and for lengthy periods since their first deployment for protein investigations. The inability to produce greater forces, however, limits the scope of its applicability and prohibits the study of highly mechanostable proteins and/or interactions. As a result, there is no single-molecule force spectroscopy approach that can resolve and stabilize the whole protein nanomechanical spectrum.

We describe a magnetic tweezers strategy for achieving AFM-like forces while keeping magnetic tweezers' unique resolution and stability. To achieve higher forces, we employ and calibrate the Dynabeads M-450 super paramagnetic probes in two magnetic tweezers configurations: permanent magnets and magnetic tape head, for the first time. We combine the Halo Tag and split-protein approaches, which allow for end-to-end covalent anchoring of proteins, to achieve long-lasting studies under high strain. We calibrated a force law for our two configurations using force-dependent extension changes and unfolding kinetics of model proteins to identify the force range accessible with the M-450 probes. Our findings show that we can apply forces ranging from 1 to 510 pN in the permanent magnet configuration, whereas the magnetic tape head can apply forces ranging from 0 to 236 pN. We can study the folding and unfolding dynamics of proteins with heterogeneous mechanostability using both tweezers setups while maintaining subpiconewton, sub millisecond, and nanometer resolutions. The capabilities of this improvement are demonstrated by

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mapping the nano mechanics of the Gram-positive pathogen Antinomies oris' mechanostable type 2 pilus proteins FimA. FimA has severe asymmetric dynamics, with unfolding happening at loads more than 300 pN and folding occurring at forces less than 15 pN. The high energy dissipation allowed by the vast force range separation between these two processes, roughly 800 kBT per FimA subunit, shows that A. oris FimA type 2 pili could operate as mega Dalton-scale shock absorbers that protect bacterial adhesion from mechanical difficulties. Despite the substantial increase in force provided by the M-450 super paramagnetic beads to magnetic tweezers, these probes may be adjusted with subpiconewton resolution to address the dynamics of proteins like talin's mechanosensitive R3 domain, which has a folding equilibrium shift of less than 1 pN. Our implementation of the M-450 probes unites the features of AFM and magnetic tweezers without sacrificing their strengths, and establishes an all-in-one force spectrometry technique for the study of most of the proteins and interactions across the force spectrum.