CICLO DE SEMINARIOS 2012

SMAT-C Soft Matter Research and Technology Center

"A hybrid TIRF-magnetic tweezers instrument for studying sub-nanometer effects of force on proteins and DNA"

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Single-molecule manipulation has increasingly become a useful method for studying macromolecular dynamics. We have built a novel instrument for force spectroscopy that combines the capabilities of magnetic tweezers and single molecule Förster resonance energy transfer (smFRET). A magnet exerts force on 2.1 μm antidigoxigenin-coated paramagnetic beads tethered to specific proteins under study. These fluorescently labeled proteins are functionalized with DNA handles containing biotin and immobilized on the surface of a flow chamber in a total internal reflection fluorescent (TIRF) microscope via streptavidin interactions. Because the FRET from each molecule in the microscope's field of view can be measured simultaneously, the extension between dyes of individual molecules as a function of force can be monitored. The model system that we are currently studying is adenylate kinase (AK). This enzyme was successfully labeled with fluorescent dyes and DNA using click chemistry and cysteine chemistry. AK was first characterized in optical tweezers and was found to unfold around 25 pN during force-extension experiments with a fast 4 nm intermediate at 15 pN. This intermediate could correspond to the ATP binding domain unfolding independently of the rest of the protein. Preliminary fluorescence data from our instrument confirms the existence of this intermediate under force. HHMI-CB; NIH-SM

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