MAR11-2011-020246

Abstract for an Invited Paper
for the MAR11 Meeting of
the American Physical Society

Overview of single-molecule methods including high-force, force-fluorescence, and dual-trap studies for probing molecular and cellular machinery

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High force optical trapping, including double trap geometry and simultaneous visualization with single molecule fluorescence imaging enables a wide range of measurement capabilities applicable for probing molecular and cellular machinery. A series of single molecule measurement methods will be presented. Force-fluorescence microscopy enables visualizing amyloid fibers while physically probing their structures including direct unfolding and rupture of fibers with a high force optical trap. Force spectroscopy is employed to probe the strength of single peptide aptamer bonds. A dual-trap geometry allows for direct tracking of unfolding and translocation machinery of the biological motor ClpXP. Force fluorescence microscopy directly visualizes T-cell activation. Automation and flexibility in our instruments coupled with advances in physical assay design strategies are leveraged to access a broad set of molecular and cellular measurement targets.