Ultrafast Force-Clamp Spectroscopy of Single Molecular Motors and DNA Binding Proteins

by
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Abstract:

Force plays a fundamental role in a wide array of biological processes, regulating, for example, enzymatic activity, kinetics of molecular bonds, and molecular motors mechanics. Single molecule force spectroscopy techniques have enabled the investigation of such processes, but they are inadequate to probe short-lived (millisecond and sub-millisecond) molecular complexes. Here, we present a novel force-clamp laser trap that is capable of applying constant loads to molecular complexes with lifetimes above ~10 μs [Capitanio et al., Nature Methods 9, 1013-1019 (2012)]. Such capability is enhanced by a detection strategy allowing the investigation of very short interactions (~100μs). Moreover, the high temporal and spatial resolution of the method enables us to probe sub-nanometer conformational changes with a time resolution of few tens of microseconds. We tested our method on molecular motors and DNA-binding proteins. We could apply constant loads to a single motor domain of myosin before its working stroke was initiated (0.2–1 ms), thus directly measuring its load dependence. We found that, depending on the applied load, myosin weakly interacted (<1 ms) with actin without production of movement, fully developed its working stroke or prematurely detached (<5 ms), thus reducing the working stroke size with load. Our technique can be applied to a wide variety of non-processive molecular motors, single domains of processive motors, protein-DNA and protein-RNA interactions, and conceivably to any short-lived protein-protein interaction.

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