Instrumentation of the High-speed AFM and Observation of Protein Dynamics

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X-ray crystallography and NMR have been successful in determining the protein 3D structure at atomic level. However, the obtainable structure is a static one averaged over many molecules and hence cannot reveal how protein molecules behave dynamically when they are functioning in solution. Currently-prospering single molecule analysis by fluorescence microscopy can detect dynamic behavior of protein at work but the spatial resolution is not high enough to visualize protein structure. Atomic force microscopy (AFM) does not possess spatial resolution as high as x-ray crystallography and NMR but very unique in its ability to visualize individual protein molecules in solution at (sub) nanometer resolution. However, its imaging rate is too low to capture dynamically moving molecules because of the slow scan speed due to the slow mechanical response of the cantilever and scanner. In addition, the tip-sample interaction force is large, which disturbs weak protein-protein interaction and sometimes leads to destruction of protein. In order to afford AFM an ability to trace moving protein molecules without disturbing their physiological function, we have been developing various devices over the past decade; for example, small cantilevers[1] with a high resonance frequency (0.6-1.2 MHz in water) and a small spring constant (~0.2 N/m), an optical deflection detection system[1,2] compatible with small cantilevers, a high-speed scanner[3] that does not vibrate when operated in z-direction at 150 kHz, a dynamic PID controller[4] that does not lower the feedback bandwidth even when the amplitude set point is adjusted very close to the cantilever's free oscillation amplitude, a high-speed phase detector[5] that allows simultaneous capturing of topography and phase-contrast images. By these devises, it has recently become possible to image at (near) video rate, without disturbing protein's physiological functions[6,7]. For example, hand-over-hand movement of myosin V along actin filaments is clearly imaged. The negatively cooperative binding events between Gro-ES and the two rings of GroEL are successfully captured. These demonstrate that high-speed AFM is truly useful for studying protein's dynamic action and will surely open a new way of elucidating the mechanisms of protein functions.

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