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### **Single Molecule Force Spectroscopy using Optical Traps and AFMs**

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Force spectroscopy is an important single-molecule technique to study the energetics and dynamics of biological systems. Both optical traps and atomic force microscopes (AFMs) can measure the dynamics of individual molecules. My talk will focus on two intellectually distinct ways to improve these experiments: passive force clamps and an optically stabilized AFM. To increase measurement precision, feedback is used to maintain a constant force on a molecule – often called a force clamp. Precise yet rapid active feedback is limited by Brownian motion. This limited bandwidth leads to significant fluctuations in force that are particularly pronounced for the rapid, large changes in extension seen in nucleic acid structures (e.g. DNA hairpins, ribozymes, riboswitches). Here, we show that the dynamics determined in active force clamps are five-to-seven fold different than in a passive force clamp, which has a  $\sim 30$ -fold faster control of force. Thus, the dynamics of biological molecules can be significantly altered by the mechanism of force feedback. In AFM-based force spectroscopy experiments, force versus extension curves are generated by retracting the tip using a PZT stage while measuring force via cantilever deflection. Extension is inferred, not measured, and therefore convolved with drift in the AFM assembly ( $\sim 10$  nm/min). We developed an ultrastable AFM by scattering a laser off the apex of a commercial AFM tip to measure and thereby stabilize the tip in 3D. A second laser stabilized the sample, leading to a 100-fold improvement in tip-sample stability compared to the previous state-of-the-art at ambient conditions (in air at room temperature). We next demonstrated simultaneous and independent measurement of extension and force in liquid. Preliminary studies of bacteriorhodopsin, a model membrane protein, highlight this instrument's unique force- and position-clamp modes.