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A precision force microscope for biophysics GAVIN KING, University of Missouri, ALLISON CHURNSIDE, JILA: NIST/CU Boulder, THOMAS PERKINS, JILA: NIST/CU Boulder, MCD Biology, CU — In a typical force spectroscopy experiment, an atomic force microscope (AFM) tip is coupled to a surface-adsorbed protein and force-extension curves are generated by retracting the tip using a piezoelectric (PZT) stage. Force is measured by cantilever deflection; extension is deduced from the PZT stage position used to control the vertical tip position. This deduced extension is sensitive to vertical mechanical drift of the AFM assembly. We have previously developed an ultrastable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci in three dimensions. These lasers establish a local reference frame that is insensitive to long-term mechanical drift of the AFM assembly. We have now extended the ultrastable AFM capabilities into liquid and can mechanically unfold proteins very slowly, which allows averaging to increase precision. We can also reduce the pulling velocity to zero and stabilize the tip-sample separation while measuring force. Using these techniques, we are studying the unfolding and re-folding of bacteriorhodopsin, a model transmembrane protein.

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