A COMBINED ATOMIC FORCE, CONFOCAL, AND TOTAL INTERNAL REFLECTION MICROSCOPE FOR THE STUDY OF SINGLE MOLECULES

William Frederick Heinz, Bertrand Navarro, Kenneth D. Weston, Pernelle Bernardi, Lori S. Goldner: NIST, 100 Bureau Drive, Mail Stop 8442, Gaithersburg, MD 20899-8442

The interactions between biological macromolecules and macromolecular assemblies are central to the proper functioning of biological systems. Historically, these interactions have been characterized by techniques that measure the averaged interactions of large ensembles of particles. Recently, a number of experimental approaches have been developed that enable the study of single molecules, thus the distributions that make up the average behavior and the heterogeneity of a population can be determined as well as the time-dependent behavior of the molecules. Interactions between individual molecules depend on many variables including orientation, conformation, local environment, and separation distance. We propose to monitor molecular configuration or separation as a function of force on the molecule by combining single molecule force measurements with single molecule optical techniques. Here we present an instrument that combines AFM or optical tweezers with confocal and total internal reflection microscopy and report on progress towards the simultaneous measurement of force and orientational and conformational dynamics of single bio-molecules in aqueous conditions.