Articles

New Atomic Force Microscopes (AFM) for the Study of Enzymatic Properties and Processes

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New developments in the field of atomic force microscopy (AFM) instrumentation enable novel experiments on proteins and other biological molecules. Smaller cantilevers provide a better ratio of cantilever stiffness and resonance frequency, thereby lowering thermal noise, and improving imaging bandwidth for a given cantilever stiffness. This new capability was used to image time-resolved processes on a soft, fragile Gro-El Gro-Es chaperone sample in vitro, and to perform force spectroscopy measurements to study the mechanical properties of collagen molecules.

1. INTRODUCTION

The AFM is a proven tool for visualizing single biological molecules, such as DNA [1-18] or proteins [19-21], and for studying their mechanical properties and interactions under near-physiological conditions [19, 22].

For studying protein activity at both good time and force resolution, however, it is necessary to optimize the AFM cantilever's ratio between resonance frequency and spring constant. A higher resonance frequency is desirable for two reasons: First, the resonance frequency limits the rate at which information about the sample can be obtained. It therefore puts an upper limit on the measurement bandwidth in general, or the imaging frame rate in particular. Second, a higher resonance frequency decreases the cantilever thermal noise for a given measurement bandwidth [Fig. 1]. The cantilever generates one kT of thermal noise per resonance interval. For a higher resonance frequency, a given measurement bandwidth becomes a smaller fraction of one resonance interval of the cantilever, and thus contains a smaller fraction of one kT of thermal noise.

We micro machined small cantilevers [23-25] which are about a factor of 1000 smaller in mass than commercial cantilevers. Fig. 1 shows a small cantilever with a width of 5 micrometers in front of human red blood cells for scale comparison. The cantilevers are made of silicon nitride, and have spring constants of, for example, 0.064N/m at a resonance frequency of 180kHz in liquid. The factor of 1000 in mass gives a resonant frequency, $\nu \propto (k/m)^{1/2}$ that is larger by a factor of order $\sqrt{1000} \approx 30$ for the same spring constant, k! The result of spreading the kT thermal noise out over a frequency range that is thirty times higher is to decrease the noise per root Hz by $\sqrt{30} \approx 5$. These theoretical estimates are met in real measurements [25].

Fig. 1: SEM image of a small cantilever with an Electron Beam Deposited (EBD) tip, over a field of human red blood cells, for scale.

These new small cantilevers require new AFM hardware which generates a smaller laser spot size comparable to the

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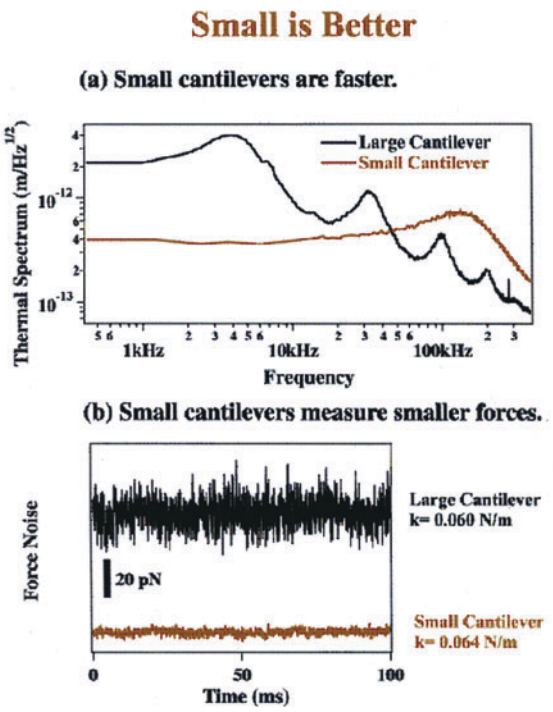

Fig. 2: Small cantilevers are better: the resonance frequency is ~30x higher for a cantilever of comparable spring constant. This leads to smaller thermal noise for a given measurement bandwidth.

size of the cantilever. After several iterations, a small cantilever head was developed that can fit on top of a standard Veeco/ Digital Instruments Multimode [26] AFM.

2. EXPERIMENTS

Imaging biological molecules in their active state requires cantilevers with a stiffness comparable to that of the sample molecules. The increased measurement bandwidth of small cantilevers [25] enabled us to monitor the complex formation of several Gro-El / Gro-Es chaperone molecules simultaneously with a time resolution of ~0.1s [27]. First, a film of Gro-El molecules was imaged with the Gro-Es co-proteins present in solution. Then, the AFMs slow scan axis was disabled, and the height profile of one scan line was plotted over time [Fig. 3]. After adding ATP to the system, sudden changes in height can be seen for individual Gro-El molecules as a function of time. The changes do not occur when either ATP or Gro-Es is not present. The height fluctuations can be attributed to Gro-Es molecules attaching to the Gro-El ring, and their desorption seconds later. The high time resolution of the experiment (~12 line scans/s) allows to histogram the complex lifetime. The result is a complex lifetime function that peaks around 5s.

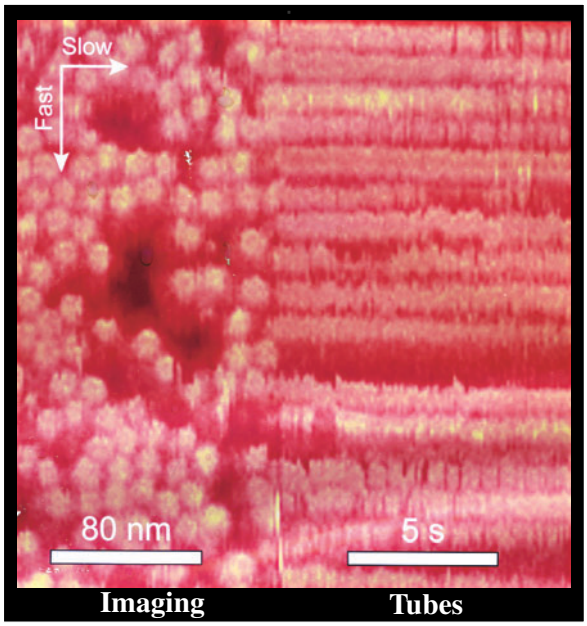

Fig. 3: GroEL chaperones, AFM 2D image (left), and 1D over time (right).

Another practical advantage of the improved stiffness-to-resonance frequency ratio of small cantilevers is their improved imaging speed [28]. Fig. 4 shows an image of DNA on mica in buffer (40 mM HEPES, 10 mM $MgCl_2$, pH 7.4) that was taken in only 20s using small cantilevers. At this point, a further increase of imaging speed depends on the development of new faster scanners and electronics.

The improved noise per measurement bandwidth perfor-

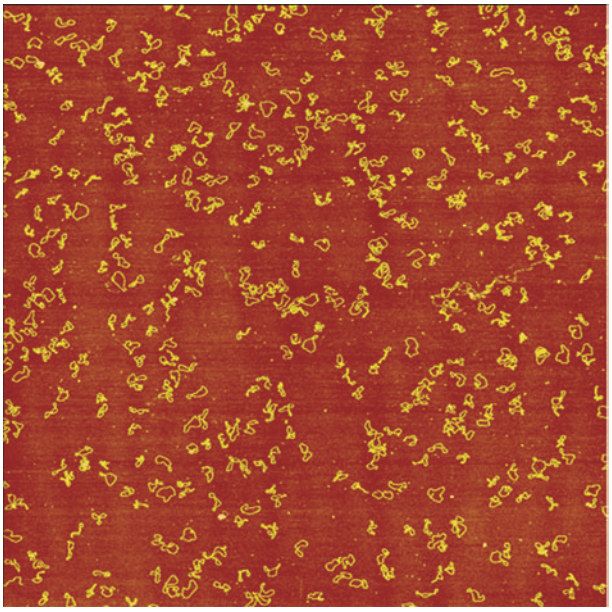

Fig. 4: Image of DNA on mica, scanned with a small cantilever in Buffer in 20s.

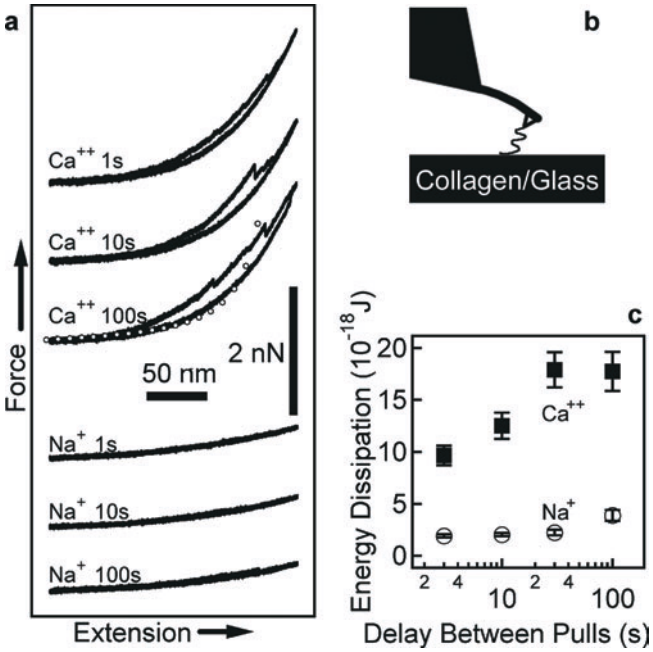

Fig. 5: The recovery of energy dissipation in collagen molecules is modulated by the ionic environment.

mance makes small cantilevers the ideal tool for molecular force spectroscopy (pulling) experiments. We studied the mechanical properties of collagen and rat femur under different ionic conditions [29]. A small cantilever was brought in contact with a collagen [30] coated surface, and force vs. distance curves were obtained with varying time intervals between successive pulls. We observed that the energy dissipated in a pull depends strongly on the time interval since the last pull in a calcium-rich buffer, but not in a buffer only containing sodium ions. [Fig. 5: Fig. 1 Nature paper]. The experiment was then repeated on a polished rat femur, with consistent results. AFM indentation testing on rat femur previously soaked in the two different buffers again showed the same recovery behavior, this time for a larger number of involved molecules.

In summary, small cantilevers provide two major advantages for studying biological samples: fast imaging speed and low noise for force spectroscopy. The future goal is to increase the speed of all components of an AFM setup to perform time dependent biological experiments on time scales a factor of 30 faster than possible with current commercial instruments.

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Acknowledgements

We thank Emin Oroudjev for providing us with the DNA sample. This work was supported by the National Science Foundation under Award No. DMR9988640, the MRL Program of the National Science Foundation under Award No. DMR00-80034, the National Institutes of Health under Award No. GM65354, the NASA/URETI Princeton University under Award No. 00000532, and the Deutsche Forschungsgemeinschaft (project GU 568/2-1).