

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/228418913>

# Active Quality Factor Control in Liquids for Force Spectroscopy

ARTICLE *in* LANGMUIR · OCTOBER 2000

Impact Factor: 4.46 · DOI: 10.1021/la000766c

---

CITATIONS

101

---

READS

9

3 AUTHORS, INCLUDING:



Andrew D. L. Humphris

Infinitesima Ltd

32 PUBLICATIONS 1,476 CITATIONS

SEE PROFILE



Mervyn J Miles

University of Bristol

141 PUBLICATIONS 4,537 CITATIONS

SEE PROFILE

# Active Quality Factor Control in Liquids for Force Spectroscopy

A. D. L. Humphris,<sup>\*,†</sup> J. Tamayo,<sup>†,‡</sup> and M. J. Miles<sup>†</sup>

*H. H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol, BS8 1TL, United Kingdom, and Centro Nacional de Microelectrónica (IMM-CNM-CSIC) Isaac Newton, 8 28760 Tres Cantos (PTM), Madrid, Spain*

*Received May 31, 2000. In Final Form: August 14, 2000*

Molecular processes are both dynamic and energetic in nature and thus exhibit both conservative (elastic) and dissipative (viscous) forces. We present a method suitable for application to a conventional atomic force microscope (AFM) that is capable of measuring this complex quantity and use it to study the force-extension response of a single molecule. The dynamic force sensitivity of the AFM cantilever was increased with a positive feedback system producing a stable effective quality factor of over 300 in a liquid environment compared to the nominal value of  $\sim 1$ . This facilitated tracking of the resonant frequency and the separation of conservative (elastic) and dissipative (viscous) forces. With access to this extra information, not available by conventional static force spectroscopy, it is possible to estimate the effective viscosity of a single dextran molecule to be 6000 Pa s.

## Introduction

Atomic force microscopy (AFM) was first developed for imaging purposes.<sup>1</sup> A very sharp tip attached to a cantilever is brought into contact with the surface of interest. By use of a raster scan, the topography is mapped by detecting the repulsive force between the atoms of the tip and the surface due to hard core repulsion. This contact mode has provided topographic information about organic and inorganic materials at the nanometer scale in air, ultrahigh vacuum (UHV), and liquid environments. The invention of a dynamic mode of operation produced a significant improvement in the AFM capabilities. Here, the cantilever is set into oscillation by an externally driven force and the tip-sample interaction measured through changes in the amplitude, phase, and/or resonant frequency of the oscillation. This dynamic technique is most frequently used in "tapping mode"<sup>2</sup> in which the tip intermittently makes contact with the specimen surface minimizing lateral forces that can disrupt and displace molecules weakly adsorbed on a substrate or damage soft samples when using traditional contact mode. This has extended the use of AFM to a wider range of biological samples, polymers, and other organic materials.

When a dynamic mode of operation is used, noncontact forces can be measured with an increased sensitivity of up to 4 orders of magnitude compared to the static mode.<sup>3</sup> Dynamic mode operation in noncontact has been mainly applied to magnetic and electrostatic imaging and, recently, to obtain true atomic resolution in ultrahigh vacuum. However, it has not been applied to single molecule force spectroscopy. In this rapidly expanding field, the mechanical properties of a biomolecule chemically anchored between the tip and the surface or the interaction between a ligand and a receptor are studied by measuring the deflection of the cantilever as a function

of the separation. This has provided new information not available before. The implementation of a dynamic mode of operation in single molecule force spectroscopy can have two advantages. First, the higher sensitivity would enable greater force resolution of the interaction. Second, the interaction can be decoupled to its conservative (elastic) and dissipative (viscous) components, allowing a true understanding of the nature of the interaction.

An elastic interaction acts as a spring, changing the effective resonant frequency of the cantilever by a factor  $(1 - f'/k)^{1/2}$ , where  $f'$  is the force gradient of the interaction and  $k$  the spring constant of the cantilever. On the other hand, a dissipative force changes the damping constant of the cantilever,  $\gamma$ , and therefore the effective quality factor of the cantilever, defined by  $Q = m\omega_0/\gamma$ , where  $\omega_0$  is the free resonant frequency of the cantilever. The change of  $Q$  is reflected in the change of the amplitude at resonance, defined by  $A = QF/k$ , where  $F$  is the excitation force.

The main limitation for the development of single molecule dynamic force spectroscopy is the low quality factor of a cantilever in a liquid environment, of the order of 1. This is between 3 and 5 orders of magnitude lower than the respective values in gas and UHV, preventing the detection of the change in resonant frequency and quality factor with high sensitivity. The slope of the resonant peak of the cantilever is proportional to the quality factor and, therefore, the sensitivity to track a resonant frequency change. A low quality factor also implies that the damping factor is dominated by the hydrodynamic interaction of the cantilever. Thus, dissipative interactions hardly affect the observed quality factor. The potential of the dynamic force spectroscopy in fluids has been previously demonstrated<sup>4</sup> with a transverse probe such as a STM tip oscillating parallel to the sample. This study determined the complex stiffness of a single molecule with true tip-surface distance control.

In this Letter we present a method that increases the effective quality factor of a cantilever in a liquid environ-

\* To whom correspondence may be addressed. Electronic mail: andy.humphris@bristol.ac.uk.

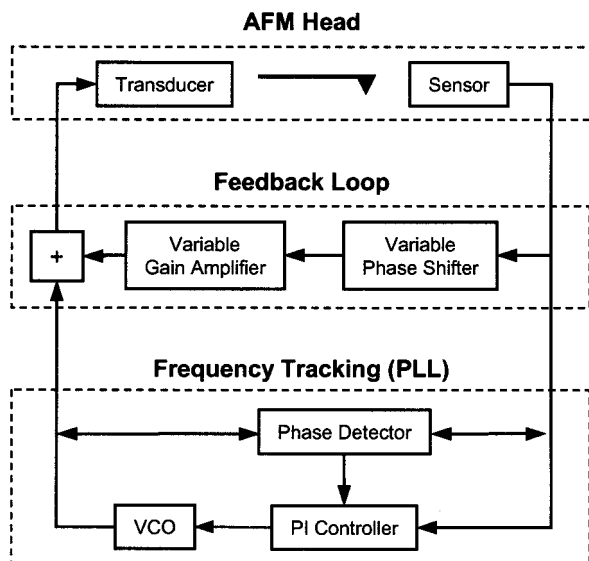
<sup>†</sup> University of Bristol.

<sup>‡</sup> Centro Nacional de Microelectrónica Isaac Newton.

(1) Binnig, G.; Quate, C. F.; Gerber, C. *Phys. Rev. Lett.* **1986**, *56*, 930.  
(2) Zhong, Q.; Innis, D.; Kjoller, K.; Elings, V. B. *Surf. Sci.* **1993**, *290*, L688.

(3) Martin, Y.; Williams, C. C.; Wickramasinghe, H. K. *J. Appl. Phys.* **1987**, *61*, 4723.

(4) Humphris, A. D. L.; Antognozzi, M.; McMaster, T.; Miles, M. J. Submitted.



**Figure 1.** Schematic of control electronics including AFM head.

ment via positive feedback.<sup>5,6</sup> A quality factor of several hundreds can be reached, accessing the piconewton force regime in tapping mode in a liquid environment.<sup>7</sup> Here, this method is applied to dynamic force spectroscopy of the polysaccharide dextran, a polymer formed by  $\alpha(1-6)$  linked glucose residues. In addition to the active quality factor control, the resonant frequency of the cantilever is tracked with a phase-locked loop (PLL) arrangement. Thus, the elastic component of the interaction is measured by the resonant frequency shift and the dissipative interaction is measured by the amplitude at resonance that it is proportional to the quality factor. A comparison between this new information and the total force produces a better understanding of the mechanical properties of the dextran molecule.

A schematic of the experimental setup is shown in Figure 1. It consists of two main parts: (i) a positive feedback loop to increase the effective quality factor and (ii) a phase-locked loop to track the resonant frequency of the system. Using a PLL not only provides knowledge of the resonant frequency and thus the elastic component of the interaction but is critical for the measurement of the tip-sample interaction with the high  $Q$  generated here by the positive feedback loop. Thus, for the slope detection method, in which amplitude and phase changes are measured at a fixed frequency, the maximum detectable interaction corresponds to a resonant frequency change smaller than the line width  $\Delta\omega \approx \omega_0/Q$ .

To describe the operation of the experiment, it is necessary to consider the external signal processing and control electronics shown in Figure 1, namely, the feedback loop and PLL. The PLL produces a signal of the form  $F_1(t) = F_0 \exp(i\omega t)$  and monitors the system response adjusting the drive frequency,  $\omega = 2\pi f$  to maintain a phase difference of  $90^\circ$  compared to the drive signal, i.e., resonance. The feedback loop monitors the response of the cantilever,  $x(t) = A \exp(i(\omega t - \varphi))$ , shifts its phase by  $90^\circ$ , amplifies it with a gain  $G$ , and adds this processed signal,  $F_2 = Gx(t)$

$\exp(i\pi/2) = GA \exp(i(\omega t - \varphi + \pi/2))$ , to the drive signal of the cantilever. Thus the motion of the cantilever can be described by the differential equation

$$m\ddot{x} + \gamma\dot{x} + kx = F_1 + F_2$$

expanding  $F_1$  and  $F_2$

$$m\ddot{x}(t) + \gamma\dot{x}(t) + kx(t) = F_0 e^{i\omega t} + G e^{i\pi/2} x(t)$$

The velocity of the cantilever can be expressed as

$$\dot{x} = \omega A e^{i(\omega t - \varphi + \pi/2)} = \omega e^{i\pi/2} x$$

Thus

$$m\ddot{x}(t) + \gamma\dot{x}(t) + kx(t) = F_0 e^{i\omega t} + \frac{G}{\omega} \dot{x}(t)$$

which produces

$$m\ddot{x}(t) + \gamma_{\text{eff}}\dot{x}(t) + kx(t) = F_0 e^{i\omega t} \quad \text{and} \quad \gamma_{\text{eff}} = \gamma - \frac{G}{\omega}$$

where  $\gamma$  is the damping constant of the cantilever as a result of its internal friction and the viscous interaction with the environment and  $\gamma_{\text{eff}}$  is the effective damping resulting from the active feedback control. The effective damping of the system may be increased or reduced, which changes the quality factor according to the relation  $Q_{\text{eff}} = m\omega/\gamma_{\text{eff}}$ .

## Experimental Methods

A commercial atomic force microscope (Nanoscope IIIa with Multi Mode head, Veeco Digital Instruments) with liquid cell equipped for both using acoustic<sup>8,9</sup> and magnetic<sup>10</sup> cantilever excitation methods was used for all experiments. To enable magnetic excitation a coil was wound into a recess in the fluid cell normally used to retain a rubber O-ring. Both methods used silicon nitride cantilevers with a nominal spring constant of 0.37 N/m. Cantilevers to be driven via a magnetic field were produced by coating with chromium and then cobalt using thermal evaporation. A magnetic moment was induced in the thin film with a strongly oriented magnetic field. Typical oscillation amplitude used was 10 nm with resonant frequencies of 12.5–17.0 kHz.

The polysaccharide sample was prepared by drying down onto a glass cover slip from a 10% (weight/volume) pure water (18 M $\Omega$ ) solution. Washing with excess quantities of water left a monolayer of the molecules on the glass surface. Attachments between the tip of the atomic force microscope and the surface layer were made by pressing them together with a force of several nanonewtons. All experiments were carried out in a controlled fluid environment of phosphate-buffered saline with a pH 7.1.

## Results and Discussion

The effect of the quality factor control on the resonant peak is shown in Figure 2 using acoustic and magnetic excitation. Without  $Q$  control, the acoustic excitation displays many resonant peaks as the cantilever response is convoluted with the fluid cell response (Figure 2b). By use of magnetic excitation (Figure 2a), the cantilever is excited directly and the spectrum shows the response which has a quality factor of approximately 3. With

(5) Anczykowski, B.; Cleveland, J. P.; Krüger, D.; Elings, V.; Fuchs, H. *Appl. Phys. A* **1998**, *66*, S885.

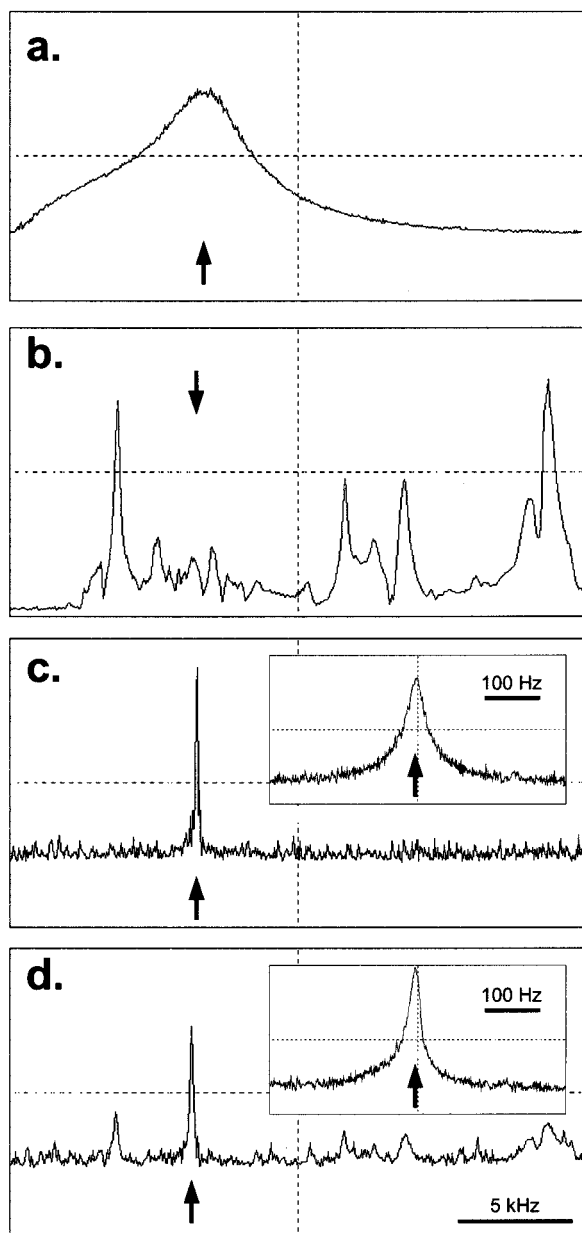
(6) Sulchek, T.; Hsieh, R.; Adams, J. D.; Yaralioglu, G. G.; Minne, S. C.; Cleveland, J. P.; Atalar, A.; Adderton, D. M. *Appl. Phys. Lett.* **2000**, *76*, 1473.

(7) Tamayo, J.; Humphris, A. D. L.; Miles, M. J. *Appl. Phys. Lett.* **2000**, *77*, 582.

(8) Hansma, P. K.; Cleveland, J. P.; Radmacher, M.; Walters, D. A.; Hillner, P. E.; Benzanilla, M.; Fritz, M.; Vie, D.; Hansma, H. G.; Prater, C. B.; Massie, J.; Fukunaga, L.; Gurley, J.; Elings, V. *Appl. Phys. Lett.* **1994**, *64*, 1738.

(9) Putman, C. A. J.; van der Werf, K. O.; de Grooth, B. G.; Van Hulst, N. F. *Appl. Phys. Lett.* **1994**, *64*, 2454.

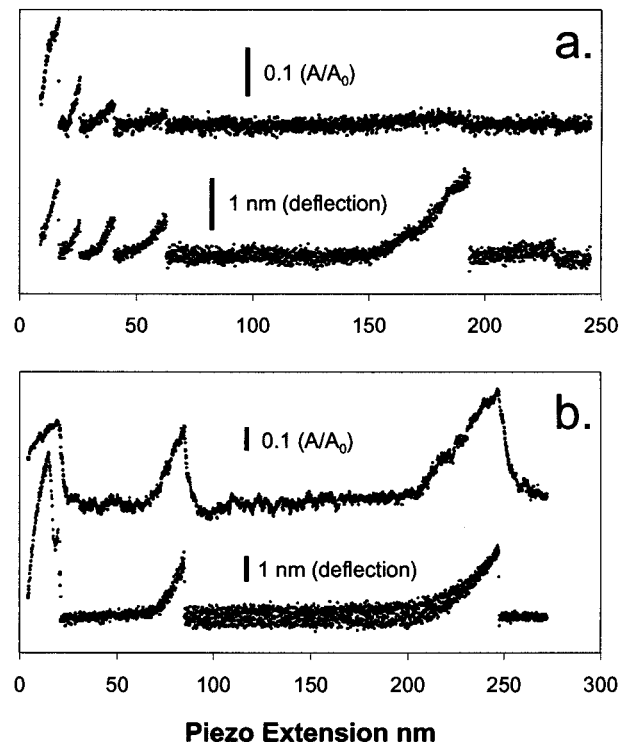
(10) Han, W.; Lindsay, S. M.; Jing, T. *Appl. Phys. Lett.* **1996**, *69*, 4111.



**Figure 2.** Amplitude response of the system as a function of frequency in normal operation (a and b) and with  $Q$  enhancement (c and d), acoustic (b and d), and magnetic (a and c) excitation methods used to drive the cantilever. Insets show magnified plot of the resonant peak. Arrow marks 16.05 kHz.

active- $Q$  control, quality factors in excess of 1000 were reached in a fluid environment. However, for stability reasons the effective quality factor was reduced to approximately 300 (Figure 2c, magnetic, and Figure 2d, acoustic excitation), 2 orders of magnitude higher than standard operation in fluid environments. The system starts to become unstable at high quality factors as the cantilever approaches self-sustained oscillation, which is caused by a negative effective damping factor. When indirect, acoustic excitation is used, the "true" resonance of the cantilever can be found using quality factor enhancement in a liquid environment.

Single molecules of dextran were extended while tracking the resonant frequency and recording amplitude, deflection, and phase of the probe as a function of piezo movement. Phase is the error signal and should be constant if the PLL is functioning correctly. It displays



**Figure 3.** Single molecule extension curves in normal operation (a) and with  $Q$  enhancement (b): top trace, normalized amplitude; lower trace, dc deflection of cantilever.

information occurring on a shorter time scale than the time constant of the PLL.

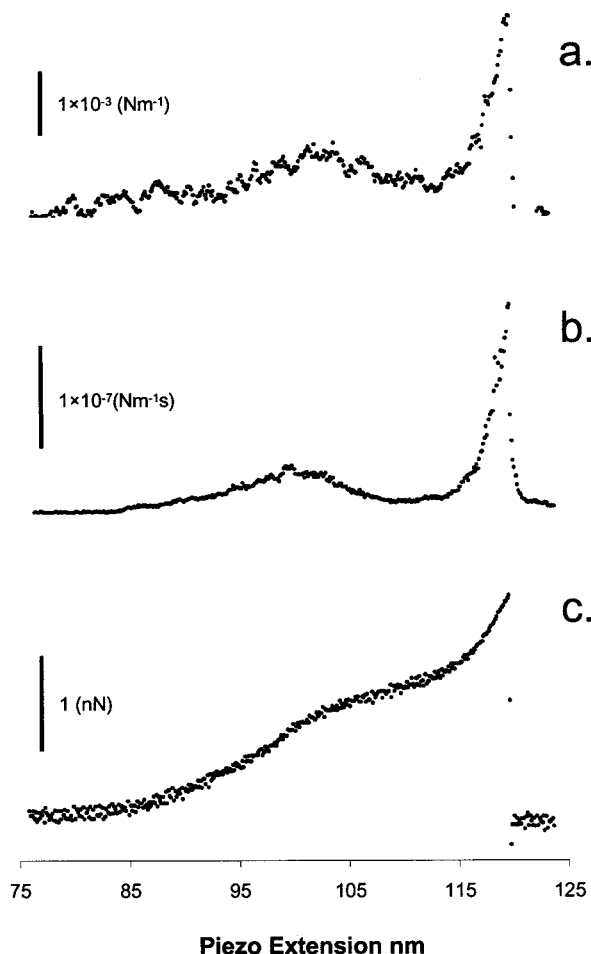
Figure 3 shows a comparison between the total force measured, which is measured by the average deflection of the cantilever, and the amplitude at resonance with and without quality factor control enabled. The amplitude sign has been changed to ease the comparison with the stretching force, thereby an increase of the relative amplitude means an increase of the dissipative force. Without quality factor enhancement (Figure 3a) the amplitude sensitivity of stretching events decreases with distance from the surface, the longest shown stretching of a dextran molecule, about 200 nm, does not produce a detectable change in the oscillation amplitude. However, the amplitude sensitivity is increased by  $\sim 20$  times when the quality factor is tuned to a value of  $\sim 160$ , this is  $\sim 50$  times the quality factor of the cantilever without enhancement and was observed at all distances from the surface (Figure 3b). The significant nonharmonic response of a cantilever near a surface<sup>11</sup> causes difficulties for the PLL to track the resonant frequency. So for small extensions near the surface the elastic and dissipative components will be coupled increasing the observed change in amplitude. At larger distances the movement of the tip is small compared to the length of the molecule and does not present any problem.

As the cantilever movement is known to be of a harmonic nature, it is straightforward to calculate the elastic force gradient,  $F'$ , from the observed frequency shift,  $\Delta\omega$ , using the nominal spring constant of the cantilever,  $k$

$$F' = k \left( 1 - \left( \frac{\omega_0 + \Delta\omega}{\omega_0} \right)^2 \right)$$

Energy dissipated per cycle can be expressed by the damping constant and is related to the observed change

(11) Tamayo, J. *Appl. Phys. Lett.* **1999**, *75*, 3569.



**Figure 4.** Forces acting on a probe during a single molecule extension curve with quality factor enhancement, elastic force gradient (top trace), dissipative force represented as effective damping constant (middle trace), and total force (lower trace).

in the oscillation amplitude by ( $\Delta\omega \ll \omega_0$ )

$$\gamma \approx \frac{k}{\omega_0 Q_{\text{eff}}} \frac{A_0}{A}$$

By subtracting the damping constant of the free system  $\gamma_{\text{sys}}$ , it is possible to evaluate the intrinsic damping of the molecule,  $\gamma_{\text{m}}$

$$\gamma_{\text{m}} = \gamma_{\text{eff}} - \gamma_{\text{sys}} = \frac{k}{Q_{\text{eff}}} \left( \frac{A_0}{\omega A} - \frac{1}{\omega_0} \right)$$

The forces acting on the probe, which had an effective quality factor of 160, are shown in Figure 4. Trace (a) is the conservative (elastic) component, (b) dissipative component described by the effective damping constant of the molecule and (c) total force derived from the dc deflection multiplied by the spring constant of the cantilever. These extension curves show a conformation

transition that has been previously observed for the dextran molecule using conventional dc force spectroscopy. This transition can be explained by a chair-to-boat transition of the glucose ring<sup>12,13</sup> that increases the contour length of the molecule when the transition force is reached. Dynamic differential force spectroscopy enables the separation of elastic and dissipative components of force during the transition. The elastic component is actually due to the local change in force with tip oscillation and so results in the elastic force gradient. It shows a broad maximum in the differential elastic force response (Figure 4a) corresponding to an inflection in the total elastic force as seen by dc AFM force spectroscopy. The damping constant of the molecule also increases showing that there is a large dissipative force, energy supplied to the molecule, which would be expected during a conformational change.

It is possible to calculate the viscosity of a single dextran molecule by including the geometry of the system. The viscosity of an isotropic system is defined as  $\gamma/lS$ , where  $S$  is the area and  $l$  is the length of the system under strain. Radius of the molecule is difficult to measure, but a reasonable estimate is of the order of 1 nm. From Figure 4, the damping constant and length are  $2 \times 10^{-7} \text{ N m}^{-1} \text{ s}$  and 100 nm, respectively, at full extension of the molecule. With these values, the effective viscosity of the molecule is estimated to be 6000 Pa s. AFM has previously been used to estimate the effective viscosity of the lysozyme molecule with a value of 800 Pa s.<sup>14</sup>

### Conclusion

We have shown that the use of a positive feedback system can increase the effective quality factor by 2 orders of magnitude in a liquid environment. This improves the force sensitivity of the AFM and can be used in conjunction with a PLL to track the resonant frequency of the probe thus separating the conservative (elastic) and dissipative forces.

It has been demonstrated that this technique can be applied to single molecule force spectroscopy with significant improvement over conventional dynamic and dc methods. The elastic and dissipative force required to extend a single dextran molecule has been evaluated and a phase transition observed; this information is not accessible with conventional dc force spectroscopy. From this information, the effective viscosity of a single dextran molecule has been estimated to be 6000 Pa s at high extension.

**Acknowledgment.** A.D.L.H. thanks the EPSRC and IACR, Long Ashton for jointly funding this work. This work benefited from valuable discussions and support concerning electronic considerations from R. Murphy and R. Harris.

LA000766C

(12) Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E. *Science* **1997**, *275*, 1295.

(13) Marszalek, P. E.; Oberhauser, A. F.; Pang, Y.; Fernandez, J. M. *Nature* **1998**, *396*, 661.

(14) Radmacher, M.; Fritz, M.; Cleveland, J. P.; Walters, D. A.; Hansma, P. K. *Langmuir* **1994**, *10*, 3809.