5. D. L. Galson *et al.*, *Mol. Cell. Biol.* **13**, 2929 (1993).
6. R. Hromas *et al.*, *Blood* **82**, 2998 (1993).
7. B. Nelsen *et al.*, *Science* **261**, 82 (1993).
8. J. M. Pongubala *et al.*, *Mol. Cell. Biol.* **12**, 368 (1992); C. F. Eisenbeis, H. Singh, U. Storb, *ibid.* **13**, 6452 (1993).
9. J. Hagman and R. Grosschedl, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8889 (1992); A. L. Feldhaus *et al.*, *Mol. Cell. Biol.* **12**, 1126 (1992).
10. M. K. Shin and M. E. Koshland, *Genes Dev.* **7**, 2006 (1993).
11. D. E. Zhang, C. J. Hetherington, H. M. Chen, D. G. Tenen, *Mol. Cell. Biol.* **14**, 373 (1994); H. L. Pahl *et al.*, *J. Biol. Chem.* **268**, 5014 (1993).
12. S. Schuetze, P. E. Stenberg, D. Kabat, *Mol. Cell. Biol.* **13**, 5670 (1993).
13. S. L. Mansour, K. R. Thomas, M. R. Capecchi, *Nature* **336**, 348 (1988).
14. H. te Riele, E. R. Maandag, A. Berns, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5128 (1992).
15. CCE.1 ES cells were cultured under standard conditions [E. J. Robertson, *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach* (IRL Press, Oxford, 1987)]. We electroporated ES cells (1×10^7) with Not I–linearized pES-PUT (30 μg) using a Bio-Rad Gene Pulsar apparatus (250 V, 500 μF) and placed them under selection [G418 (0.4 mg/ml; BRL) and gancyclovir (1 μM; Ross)] 24 hours after electroporation. Resistant colonies were picked and expanded in 24-well tissue culture plates. Genomic DNA was prepared and subjected to Southern blot analysis [F. M. Ausubel *et al.*, *Current Protocols in Molecular Biology* (Greene and Wiley-Interscience, New York, 1989)]. Hybridization and wash conditions were as described [E. W. Scott and H. V. Baker, *Mol. Cell. Biol.* **13**, 543 (1993)]. The *PU.1* probe used for hybridization corresponds to an 800-bp Kpn I fragment adjacent to the Kpn I fragment used in the targeting construct (Fig. 1A). Targeted clones representing a single integration event were injected into C57BL/6J blastocysts that were then implanted into CD-1 pseudopregnant female mice. Germline transmission of the ES cell genome was achieved with male chimeras derived from two independent clones. Analysis of tail DNA isolated from agouti progeny showed that 50% were heterozygous for the *PU.1* mutation. No apparent defects were manifest in the heterozygous animals when they were compared to wild-type littermates.
16. E. W. Scott and H. Singh, unpublished material.
17. RNA was isolated from fetal livers with RNAzol (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. Ribonuclease protection assays were performed as described [C. L. Miller *et al.*, *Mol. Cell. Biol.* **11**, 4885 (1991)]. The β^M-globin riboprobe was as described [M. H. Baron and T. Maniatis, *Cell* **46**, 591 (1986)]. RT-PCR was performed as follows. First, we performed strand complementary DNA (cDNA) synthesis using 5 μg of RNA and random primers with a Pharmacia kit as per the manufacturer's instructions. PCR reactions for Ig gene transcripts were performed with either D_μ-J_μ primers (*28*) or V_κ-J_κ primers (*29*). PCR conditions were 30 cycles at 94°C for 30 s, 62°C for 30 s, and 72°C for 50 s. PCR products were resolved in 2% agarose gels and subjected to Southern blot analysis as described (*28*, *29*).
18. R. R. Hardy *et al.*, *J. Exp. Med.* **173**, 1213 (1991).
19. G. G. Lennon and R. P. Perry, *J. Immunol.* **144**, 1983 (1990); R. Palacios and J. Samaridis, *Mol. Cell. Biol.* **12**, 518 (1992).
20. Flow cytometry analysis was performed on single-cell suspensions of the fetal liver or thymus prepared by standard methodology [J. E. Coligan *et al.*, *Current Protocols in Immunology* (Greene and Wiley-Interscience, New York, 1993)]. Cells were stained with fluorescein isothiocyanate (FITC)– or phycoerythrin (PE)-conjugated antibodies and scanned on a FACScan (Becton Dickinson). Propidium iodide uptake and scatter-gating for size were used to exclude dead cells from analysis. Monoclonal antibodies [RA 3-6B2 (B220), S7 (CD43), 53-2.1 (Thy-1.2), RM2-5 (CD2), RM4-5 (CD4), 53-5.8 (CD8), RB6-8C5 (Gr-1), C71/16 (CD18), and M1/70 (CD11b)] were used according to the supplier's (PharMingen) instructions. Adult mouse spleen, bone marrow, and thymus suspensions were used as positive controls in setting staining gates.
21. E. V. Rothenberg, *Adv. Immunol.* **51**, 85 (1992).
22. M. L. Mucenski *et al.*, *Cell* **65**, 677 (1991).
23. Embryos were fixed in 4% paraformaldehyde and embedded in paraffin for sectioning. Hematoxylin and eosin staining was performed on embryo sections by standard methodology [W. J. Williams *et al.*, *Hematology* (McGraw-Hill, New York, 1990)]. Immunostaining for lysozyme was performed on fetal liver sections with a commercially available antibody as per the manufacturer's instructions (Dayco). Fetal liver touch preparations were stained for myeloperoxidase-positive cells by standard methodology.
24. Clonogenic assays were performed on single-cell suspensions of the fetal liver prepared as described for flow cytometry. Cells (2×10^5) were plated in 1.25 ml of complete MethoCult medium (Stemcell Tech., Vancouver, B.C., Canada) and incubated at 37°C in 5% CO_2. Colonies were counted and scored by morphology beginning on day 5 of culture. Colony identification was confirmed by histological staining of cytospin preparations.
25. P. Mombaerts *et al.*, *Cell* **68**, 869 (1992); Y. Shinkai *et al.*, *ibid.*, p. 855.
26. R. Palacios and J. Samaridis, *Blood* **81**, 1222 (1993).
27. A. Cumano, C. J. Paige, N. N. Iscove, G. Brady, *Nature* **356**, 612 (1992); F. Hirayama *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5907 (1992).
28. M. S. Schlissel *et al.*, *J. Exp. Med.* **173**, 711 (1991).
29. M. S. Schlissel and D. Baltimore, *Cell* **58**, 1001 (1988).
30. A targeting vector cassette was created by cloning the *PGK::neo* gene from plasmid KJ-1 [M. W. McBurney *et al.*, *Nucleic Acids Res.* **20**, 5755 (1991)] into the Eco RI and Hind III sites of pTK-AB to generate pTK-neo. A *PU.1* cDNA (*2*) was used to isolate a 40-kb *PU.1* genomic DNA clone from a murine 129-SV cosmid library (Stratagene). A 1.7-kb Kpn I fragment containing exons 3 and 4 and a portion of exon 5 was inserted into pUC19. This *PU.1*–Kpn I fragment deletes 73 amino acids from the COOH-terminus of PU.1 (amino acids 200 to 272), which are required for DNA binding. The fragment was then inserted as an Xba I–Eco RI segment upstream of the *PGK::neo* gene of pTK-neo to generate pTK-5′-neo. A 14-kb Eco RI fragment, containing sequences immediately 3′ to exon 5 of *PU.1*, was subcloned into pBluescript SK (Stratagene). This *PU.1*–Eco RI fragment was then inserted as a Xho I–Not I segment into pTK-5′-neo to generate pES-PUT (Fig. 1A).
31. Peripheral blood was obtained from aortic bleeds. Hematocrit assays were performed essentially as described [M. D. Collins *et al.*, *Am. J. Physiol.* **257**, 542 (1989)].
32. We thank R. Maki for a *PU.1* cDNA clone; J. Bermingham, University of California, San Diego, for pTK-AB; F. Alt for low-passage CCE.1 cells; E. B. Crenshaw for experimental advice; P. Shah, J. Leiden, and C. Thompson for their critical review; and M. Min for histological preparations. Supported by the Howard Hughes Medical Institute (E.W.S. and H.S.). E.W.S. also acknowledges support from NIH grant F32 AI08933. M.C.S. was supported by an institutional American Cancer Society grant and the Cancer Foundation of Chicago. J.A. is a special fellow of the Leukemia Society of America.

10 May 1994; accepted 19 July 1994

Direct Observation of Enzyme Activity with the Atomic Force Microscope

Manfred Radmacher,* Monika Fritz,* Helen G. Hansma, Paul K. Hansma

The height fluctuations on top of the protein lysozyme adsorbed on mica were measured locally with an atomic force microscope operated in tapping mode in liquid. Height fluctuations of an apparent size of 1 nanometer that lasted for about 50 milliseconds were observed over lysozyme molecules when a substrate (oligoglycoside) was present. In the presence of the inhibitor chitobiose, these height fluctuations decreased to the level without the oligoglycoside. The most straightforward interpretation of these results is that the height fluctuations correspond to the conformational changes of lysozyme during hydrolysis. It is also possible, however, that the height fluctuations are, at least in part, the result of a different height or elasticity of the transient complex of lysozyme plus the substrate.

Since its invention, the atomic force microscope (AFM) (*1*) has been a promising tool for biological applications. In the last few years, proteins under physiological conditions (*2*) and live cells (*3*) have been imaged. In some instances, the AFM demonstrates true atomic resolution (*4*) and can detect the small forces of specific binding between individual molecules (*5*, *6*).

Recent progress in measuring and imaging the forces between proteins has been achieved with several techniques and systems (*6*, *7*). These measurements suggested that the state of the art of detecting small movements at very low forces would be sufficient for observing the motion of proteins at the molecular level. The advantage of the AFM is its combination of sensing small forces and its ability to position with nanometer accuracy and to detect objects at a molecular resolution. Enzymes are a very promising system for watching protein mo-

M. Radmacher, H. G. Hansma, P. K. Hansma, Department of Physics, University of California, Santa Barbara, CA 93106, USA.
M. Fritz, Department of Physics and Marine Science Institute, University of California, Santa Barbara, CA 93106, USA.
*To whom correspondence should be addressed.

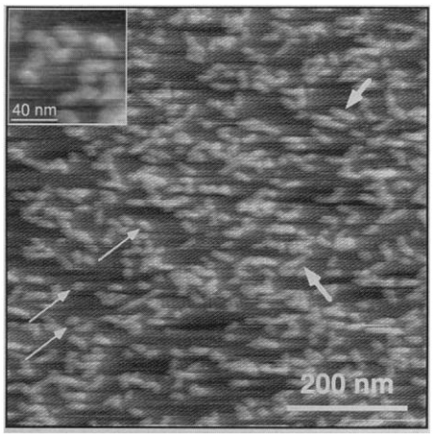

Fig. 1. AFM image of lysozyme molecules adsorbed on mica (*24*). There are single molecules visible (thin arrows) as well as small, chainlike clusters of two or three molecules (thick arrows). The inset shows an enlargement of the area near the topmost thin arrow. (The modulation of the tapping results in a moiré background that has been removed by Fourier filtering in the inset.) Because of tip broadening, the molecules appear about 15 to 20 nm in diameter (*25*).

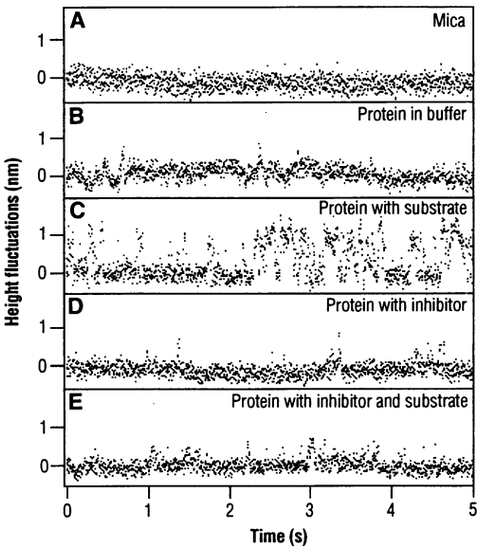

Fig. 2. Height fluctuations measured with the AFM tip on top of a monolayer of lysozyme molecules adsorbed on mica (*26*). The data were recorded on mica (**A**) or on lysozyme in buffer (**B**), on lysozyme in buffer containing the substrate 4-methylumbelliferyl-*N*,*N*′,*N*″-triacetyl-chitotriose (~10 μM) (Calbiochem, La Jolla, California) (**C**), in buffer containing the inhibiting substance *N*,*N*′-chitobiose (~20 μM) (Sigma) (**D**), and in buffer containing both substances (**E**). In (C), spikelike jumps appear in the height signal. The apparent height of these jumps is on the order of 1 nm.

tion (*8*) because most of them undergo a conformational change during their biological function at a time scale accessible to the AFM (*9*).

Lysozyme is found in a variety of vertebrate cells and secretions, such as placenta, spleen, milk, tears, and egg white. Its natural function is to hydrolyze β(1→4) glucosidic bonds in the proteoglycan layer of bacteria at a maximum turnover rate of about two per second (*10*). It is a compact, single chain molecule with a molecular mass of about 17 kD, with four disulfide bonds imposing rigidity to the molecule. The slightly egg-shaped protein has dimensions of about 4.5 nm by 3.0 nm by 3.0 nm. Although the interior of the molecule is hydrophobic, the active cleft is negatively charged and the outside carries extensive positive charges, resulting in an isoelectric point at pH 11.1. The active cleft binds and hydrolyzes not only the polysaccharide component of bacterial cell walls but also a variety of short oligomers of chitin (*11*). A conformational change due to hydrophobic interactions during hydrolysis has been proposed (*12*). In recent studies, the activity of lysozyme adsorbed on glass has been verified (*13*). We checked the activity of the enzyme here by light adsorption measurements (*14*).

Single lysozyme molecules adsorbed to mica were investigated with the AFM in tapping mode (*15*) under buffer (Fig. 1). In tapping mode, the sample can be vibrated at a high frequency (typically, 17 to 20 kHz). The response of the cantilever of the AFM to this oscillation depends on the distance between tip and sample and can therefore be used to control the separation of tip and sample. In the case of small, adsorbed single molecules, tapping mode seems to be much less destructive than normal imaging modes (*16*). Height fluctuations under various conditions were recorded while the AFM tip sat on top of a monolayer of lysozyme without scanning (Fig. 2) (*17*). We found spikelike height fluctuations of an apparent height of 1 nm while the substrate was present in the buffer. In all other cases, these spikes were missing. In particular, they were missing when the inhibitor and the substrate were present in the buffer. We recorded and analyzed a total of 271 data sets taken on several spots of a total of six different samples (*18*). Each data set shows the height fluctuations while the tip was sitting at one spot of the sample for a period of 32 s. Not all data sets in all categories showed the pronounced differences of the data sets shown in Fig. 2.

To quantify the noisiness or the number of spikes in each data set and to compare these quantities for all data sets of all categories, we used several methods that all yielded essentially the same results. The most straightforward standard measure is the root mean square value of the data, but we also created a custom measure: the counts of values that are beyond some certain threshold (0.5 nm) compared to the local mean of the data (Fig. 3). There were essentially no spikelike fluctuations while the tip was sitting on bare mica; the enzyme without any substrate in the buffer or in the presence of the inhibitor showed slightly more movement as can be seen by the increased number of spikes. The data sets with the highest frequency of spikes were recorded while the substrate was present in the buffer and the enzyme was in its active state (no inhibitor present). But there is a large spread in the data, so that even in the presence of substrate we could find data sets that were essentially identical to the data sets in the absence of substrate. However, even the differences among the mean numbers of spikes in each category are statistically highly significant even if all data sets are included in the analysis. Therefore, we conclude that these spikelike height fluctuations are due to enzymatic activity. It is not surprising that not all data sets (corresponding to all areas on the sample) showed these signs of activity. The activity of lysozyme is reduced when the molecule is adsorbed on a surface (*11*, *19*), probably because of steric hindrance: because of their orientations, not every enzyme molecule on a surface is accessible to the substrate.

It is known that lysozyme undergoes a conformational change during binding of the substrate (*20*). This conformational change might, depending on the orientation, lead to a fluctuation in the height of the molecule. This motion can be detected in principle with a soft enough cantilever sitting on top of the enzyme. A simple estimation shows that the cantilevers used could detect the small proposed conformational changes. The force needed to bend the cantilever by 1 nm is 50 pN, and the energy related with this bending is 2×10^{-20} J, corresponding to about 0.1 eV. Because the enthalpy of the hydrolysis is about 0.5 eV per oligoglycoside molecule (*21*), this hydrolysis can supply enough energy for moving the cantilever. The inertial forces for pushing

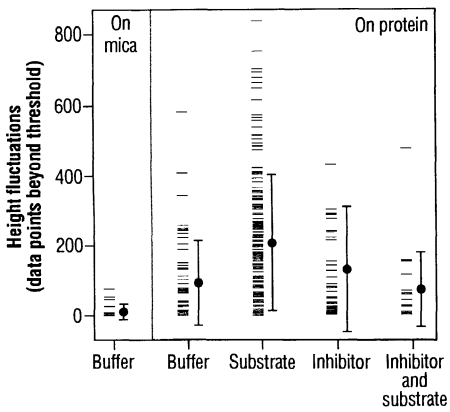

Fig. 3. Comparison of all 271 data sets recorded in six different preparations. To quantify the number of spikes in the data sets shown in Fig. 2, we counted the number of data points that were larger than 0.5 nm above the mean value of the data. Accounting for some slight drift in the height, which is probably thermal in nature, the mean was calculated in a local window of 0.5-s length. The mean value and standard deviation are indicated by the filled circle and vertical line. The significance of the differences between the categories was tested by the measure of Kolmogorov-Smirnoff (*27*). This test showed that the data with the substrate are highly significantly different from all others ($P < 0.01$).

a cantilever up by 1 nm in less than 1 ms are several orders of magnitude smaller than the bending forces. One may also neglect the viscous forces of the water.

Our measurements show that the observed spikes in the measured height with the substrate present are related to the enzymatic activity of the lysozyme. They disappear if the enzyme is inhibited. However, care has to be taken in the interpretation of these data, because even in the established-contact-mode AFM, elasticity, surface charges, and lateral forces can have an influence on the apparent height. The most straightforward explanation of our data is a height change that is a result of a conformational change of the molecule or a movement of the entire molecule during hydrolysis (that is, a rotation of the egg-shaped molecule). It is also possible that the energy release of the hydrolysis created an acoustic shock wave at the molecular level, which could be detected by the sensing tip. Because single molecules of lysozyme appear about twice as high in tapping mode as they really are (*22*), some properties of the sample other than topography may influence their appearance.

REFERENCES AND NOTES

1. G. Binnig, C. F. Quate, C. Gerber, *Phys. Rev. Lett.* **56**, 930 (1986); D. Rugar and P. K. Hansma, *Phys. Today* **43**, 23 (1990).
2. B. Drake *et al.*, *Science* **243**, 1586 (1989); H. G. Hansma and J. H. Hoh, *Annu. Rev. Biophys. Biophys. Chem.* **23**, 115 (1994); J. Yang, L. K. Tamm, T. W. Tillack, Z. Shao, *J. Mol. Biol.* **229**, 286 (1993); S. Karrasch, R. Hegerl, J. H. Hoh, W. Baumeister, A. Engel, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 836 (1994).
3. E. Henderson, *Nucleic Acids Res.* **20**, 445 (1992); M. Radmacher, R. W. Tillmann, M. Fritz, H. E. Gaub, *Science* **257**, 1900 (1992); M. Fritz, M. Radmacher, H. E. Gaub, *Biophys. J.* **66**, 1328 (1994); D. Keller, L. Chang, K. Luo, S. Singh, M. Yorgancioglu, *Proc. Soc. Photo. Opt. Instrum. Eng.* **1639**, 91 (1992); E. Henderson, *Prog. Surf. Sci.* **46**, 39 (1994).
4. F. Ohnesorge and G. Binnig, *Science* **260**, 1451 (1993).
5. G. U. Lee, D. A. Kidwell, R. J. Colton, *Langmuir* **10**, 354 (1994).
6. E.-L. Florin, V. T. Moy, H. E. Gaub, *Science* **264**, 415 (1994).
7. A. Ishijima, T. Doi, K. Sakurada, T. Yanagida, *Nature* **352**, 301 (1991); K. Svoboda, C. F. Schmidt, B. J. Schnapp, S. M. Block, *Ibid.* **365**, 721 (1993); J. T. Finer, R. M. Simmons, J. A. Spudich, *ibid.* **368**, 113 (1994).
8. A. Keaton, J. F. Holzrichter, W. J. Siekhaus, Proceedings of the NATO Advanced Study Institute (ASI), Schluchsee, Black Forest, Germany, 7 to 18 March 1994.
9. M. B. Jackson, *J. Chem. Phys.* **99**, 7253 (1993).
10. T. Imoto, L. N. Johnson, A. C. T. North, D. C. Phillips, J. A. Rupley, in *The Enzymes*, P. D. Boyer, Eds. (Academic Press, New York, 1972), vol. VII, pp. 665–868.
11. J. A. Rupley, *Proc. R. Soc. London Ser. B* **167**, 416 (1967).
12. I. Amdur and G. G. Hammes, *Chemical Kinetics: Principles and Selected Topics* (McGraw-Hill, New York, 1966).
13. C. G. Golander, V. Hlady, K. Caldwell, J. D. Andrade, *Colloids Surf.* **50**, 113 (1990).
14. We verified the enzymatic activity of the lysozyme adsorbed on mica by light adsorption measurements with a natural substrate (*Micrococcus lysodeikticus*; Sigma). In these tests, we checked also the efficiency of the inhibiting substance *N,N'*-chitobiose (*19*) (the enzymatic degradation of the bacteria was stopped irreversibly) and in a competitive study the activity against the substrate 4-methyl-umbelliferyl-*N,N',N''*-triacetyl-chitotriose (*11*, *21*, *23*) used in this study (the degradation of the bacteria was slowed down in the presence of the competing substrate).
15. P. K. Hansma *et al.*, *Appl. Phys. Lett.* **64**, 1738 (1994).
16. The reasons for this are the lack of lateral forces that push aside weakly adsorbed molecules and the possibility of applying low loading forces. Frequently in the AFM, a mechanical instability occurs and the tip jumps to the sample. This instability sets a lower limit for the imaging force that can be used. This instability is not present in tapping mode, however, and therefore the only limit of the loading force is the accuracy of the electronics and of the detection system itself. Thus, soft samples can be imaged at very low forces. In tapping mode, there is no direct way of calibrating the loading forces, which can easily be done in contact mode. From the elastic indentation of soft molecules, we could estimate that the loading forces are in our case less than 200 pN.
17. To ensure that the tip was always sitting on top of a molecule, even when the tip moved sideways because of thermal drift, the surface density of the samples used for recording the data of Fig. 2 was higher than the density of the sample presented in Fig. 1.
18. The five data sets presented here were taken from the same sample but from different areas. Because of lateral drift, which is at best on the order of 0.1 nm s^{-1} in the AFM, it is not possible to take the data for all categories while the tip sits on the same molecule.
19. C. C. F. Blake *et al.*, *Proc. Roy. Soc. London Ser. B* **167**, 378 (1967).
20. Crystallographic data are available only for the enzyme and for the enzyme-inhibitor complex. Because the inhibitor is chemically very similar to (part of) the substrate, it is reasonable to assume that the conformation of the enzyme-substrate complex will be similar to the known conformation of the enzyme-inhibitor complex. The difference in diameter between the two structures is up to 0.5 nm (*12*).
21. Y. Yang and K. Hamaguchi, *J. Biochem.* **87**, 1003 (1980).
22. We calibrated the height sensitivity of the piezo in contact mode and in tapping mode by imaging steps on mica. The step size corresponds to the distance between two cleavage planes and is about 1 nm. The calibration was [within our accuracy (20%)] the same in both operating modes. However, in tapping mode the lysozyme appeared to be 6 to 8 nm in height, which is twice its expected height.
23. M. Wenzel, H. P. Lenk, E. Schütte, *Z. Physiol. Chem.* **327**, 13 (1961).
24. Hen egg white lysozyme (Sigma) was adsorbed on freshly cleaved mica. A solution of lysozyme (1 μg/ml or ~0.1 μM) in buffer containing 5 mM KH_2PO_4 at pH 6 was incubated for 30 min. The amount of solved protein was chosen to create a monolayer coverage of protein after all molecules had adsorbed on the mica. Then the fluid was exchanged against the same buffer without lysozyme. Atomic force microscopy was performed with a modified version of a commercial microscope (Nanoscope III, Digital Instruments, Santa Barbara, CA), which could be operated in a novel tapping mode under liquids (*17*). In this mode, less damage is done to soft samples. The sample height was modulated sinusoidally at high frequencies (20 kHz; amplitude about 5 nm). The cantilever response depends on the distance between tip and sample. A custom-made electronic device measures the cantilever response and uses it as the input to a feedback loop, adjusting the piezo height such that this amplitude stays constant. This mode is analogous to the constant deflection mode in normal atomic force microscopy. Before our experiments, the tips were checked in terms of adhesion forces; only tips that showed very low adhesion (<0.5 nN) were used. Low adhesion correlated with good atomic resolution on mica and with good resolution on the adsorbed proteins, and vice versa. Therefore, we believe that tips with a high adhesion are either tips with a large radius of curvature at the end or, more likely, contaminated tips.
25. In the AFM, structures like proteins appear broadened because of the finite size of the tips. With a typical radius of curvature of 10 to 20 nm, the apparent size of a structure 3 to 4 nm high will be 15 to 25 nm. This is in good agreement with the apparent size measured here. Frequently molecules appear to be much broader than this because of mechanical deformation or lateral displacement by the tip during scanning. There is no sign of these artifacts in our case.
26. Data were recorded at a sampling rate of 1 kHz while the AFM was operated in tapping mode, with no lateral motion (scan size was 0). The data were transferred to another computer (Macintosh Quadra AV, Apple, Cupertino, CA), read in a data analysis program (IGOR, Wavemetrics, Lake Oswego, OR), rearranged so that a continuous time sequence of data was achieved, and analyzed. Four adjacent data points were averaged before analysis.
27. W. H. Press, B. P. Flannery, S. A. Teukolsky, W. T. Vetterling, *Numerical Recipes* (Cambridge Univ. Press, Cambridge, 1981).

28. Supported by the Office of Naval Research (M.R. and M.F.), the Deutsche Forschungsgemeinschaft (M.F.), and the NSF under grants DMR 9221781 (P.K.H.) and DIR-9018846 (H.G.H.). Equipment was supplied by Digital Instruments. We thank H. E. Gaub, H. Ringsdorf, D. Keller, E. Evans, K. Foltz, J. Cleveland, P. E. Hillner, D. A. Walters, and J. Hoh for helpful discussions. We are especially grateful to H. J. Butt for his collaboration on looking for protein motion several years ago and to G. Binnig, J. Holzrichter, and W. Siekhaus for inspiring us to try again with improved instrumentation.

21 April 1994; accepted 26 July 1994