Attaching molecules to surfaces for scanning probe microscopy

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Since their invention in the early and mid 1980's, Scanning Probe Microscopes, chiefly the Scanning Tunneling Microscope (STM) and the Scanning Force Microscope (SFM), have excited biochemists and biophysicists with the possibility that, at long last, a quick, reliable, widely applicable method can be found to obtain high resolution structural information on biomolecules in their native environments. Unlike conventional light or electron microscopes, Scanning Probe Microscopes form images without either lenses or radiation. Instead, images are formed by scanning a very sharp probe tip over the sample surface to "read" the sample topography, much as a phonograph needle "reads" the topography of a record. The STM and SFM differ only in the nature of the tip-sample interaction (a small tunnel current for the STM, and a small force of contact for the SFM) that makes it possible to detect the sample surface. Both STM and SFM are capable of atomic resolution on samples in a wide variety of environments, including aqueous, physiological solutions. Initial efforts to apply STM to biomolecular structure began almost immediately after it was invented in 1981 (1), and have continued with the SFM (or Atomic Force Microscope (AFM), as it is sometimes called) since its invention in 1986 (2).

As is well known, the main existing high resolution structural techniques, successful as they have been, suffer from a variety of limitations: x-ray diffraction requires good crystals, often difficult to obtain, and is very labor intensive even when crystals are available. Transmission Electron Microscopy (TEM) is usually quick and straightforward, but has limited resolution. It also often involves drying and staining, and can damage the sample. This means, among other things, that it is impossible to view the same field of molecules both before and after a change has been made. Hence, the enthusiasm for probe microscopy, which could in principle provide straightforward, high resolution images of biomolecules under physiological conditions.

At the present state of the art, all major types of biomolecules (proteins, nucleic acids, membranes) can be routinely and reliably imaged by probe microscopy, but resolution is still somewhat below that of TEM. Two technical problems have so far stopped the full promise of probe microscopy from being realized. First, the resolution of probe microscopes depends crucially on the geometry and physical properties of the probe tips used to form the images. Up to now these probes have generally not been well characterized and are often very non-uniform from tip to tip. For example, a common procedure for making STM probe tips is to simply cut a thin wire at an angle with a pair of scissors. By chance, atomically sharp tips can result, but the actual shape of the tip is completely unknown. Tips for the SFM are made commercially by semiconductor microfabrication techniques, and are usually more controlled, but are still not characterized on the atomic scale. Work on this "tip problem" is intense in both academic labs and on the part of commercial manufacturers of probe microscope equipment, and satisfactory solutions appear to be near (3, 4).

The second problem is that in most probe microscope experiments there is a tendency for the probe tip to physically move biomolecules that are not firmly attached to the substrate surface. Molecules can be damaged or even swept entirely out of the field of view. This is especially true in aqueous environments, where molecules are often "dissolved off" the substrate as soon as they are touched by the probe. Unlike the problem of probe tip geometry, this is not a problem that can be solved once and for all: for each sample a method must be found which attaches the molecules to the substrate strongly enough to prevent movement but not so strongly that molecules are denatured (5–7). Therefore, at this stage in the development of probe microscopy, each new successful deposition technique widens the range of samples that can be treated and deepens our general understanding of what sorts of approaches are likely to be useful for untried systems.

Two papers in this issue of *Biophysical Journal* address this "deposition problem." Ill et al. have engineered a nickel-binding peptide sequence into the constant region of an antibody. They then show that the antibody binds strongly (via the peptide, not its intrinsic binding sites) to a nickel-treated mica surface. It can then be imaged by SFM with good stability under buffer solutions. It is particularly noteworthy that the antibodies are not moved even at very high tip-sample forces (100 nN). Presumably, this approach or variants of it could be used for a wide range of proteins. The fact that the adsorbed antibodies are bound by their constant region tails, leaving their intrinsic binding sites free to react with molecules in solution, is also important for technological applications of the technique.

In the paper by L. Haggerty and A. M. Lehnhoff the deposition problem almost solves itself. The authors make use of the fact that concentrated solutions of cer-

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tain proteins, hen egg-white lysozyme in the present case, spontaneously form ordered surface layers on hydrophobic substrates. In these ordered layers the protein molecules are restricted in their motion by the packing of the crystalline lattice, and need not be as strongly bound to the surface. In fact, Haggerty and Lehnhoff find that at low surface coverage only large, ordered patches of protein molecules can be visualized: single molecules and small patches are apparently swept away. This is similar in spirit (though very different in scale) to the earlier imaging of ordered surface layers of liquid crystal molecules by STM, in which crystal packing was also found to be necessary for reliable imaging. Using the STM, the authors are then able to follow the process of surface adsorption directly from solution, and monitor changes in the ordering and lattice parameters of the protein layer as functions of protein concentration and ionic strength.

Both of these results illustrate the potential range of applications of probe microscopy, and the rapid progress presently taking place in applications to biomolecules. Indeed, it appears that the major conceptual and technical hurdles can be overcome and probe microscopy will soon be making routine important contributions to structural problems in molecular biophysics.

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REFERENCES

1. Binnig, G., H. Rohrer, Ch. Gerber, and E. Weibel. 1982. Surface studies by scanning tunneling microscopy. *Phys. Rev. Lett.* 49:57–61.
2. G. Binnig, G., Quate, C. F., and Ch. Gerber. 1986. Atomic force microscopy. *Phys. Rev. Lett.* 56:930–933.
3. Wolter, O., Th. Bayer, and J. Greschner. 1991. Micromachined silicon sensors for scanning force microscopy. *J. Vac. Sci. Technol.* B9:1353–1357.
4. Keller, D. J., and C. C. Chou. 1992. Sharp, vertical-walled tips for SFM imaging of steep or soft samples. *Ultramicroscopy.* 42:1481–1489.
5. Hansma, H. G., A. L. Weisenhorn, S. A. C. Gould, R. L. Sinsheimer, H. E. Gaub, G. D. Stucky, C. M. Zaremba, and P. K. Hansma. 1991. Progress in sequencing deoxyribonucleic acid with an atomic force microscope. *J. Vac. Sci. Technol.* B9:1282–1284.
6. Lindsay, S. M., N. J. Tau, J. A. De Rose, P. I. Oden, Y. L. Lyubchenko, R. E. Harrington, and L. Shlyakhtenko 1992. Potentiostatic deposition of DNA for scanning probe microscopy. *Biophys. J.* 61:1570–1584.
7. J. Vasenka, M. Guthold, C. L. Tang, D. Keller, E. Delaine, and C. Bustamante. 1992. Substrate preparation for reliable imaging of DNA molecules with the scanning force microscope. *Ultramicroscopy.* 42–44:1243–1249.