Atomic Force Microscope: The Crystallographer's Best Friend?

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The pioneering days of biological scanning probe microscopy were filled with high hopes that this new tool would revolutionize structural biology. I doubt that many protein crystallographers feared unemployment in the face of the new tools. It soon became clear that scanning probe microscopes suffer from limited resolution, sample damage, and an inability to probe internal structure. The strength of the new methods lies in monitoring processes at the nm scale. This is demonstrated beautifully in the work of Yip and Ward (1996; published in this issue).

These investigators describe a careful atomic force microscopy (AFM) investigation of the growth of insulin crystals. By exploiting evaporation to bring about supersaturation in situ in the microscope liquid cell, the layer-by-layer growth of the crystal was studied in real time. In addition to monitoring the quality of the crystal surface (out to the micrometer scale), these investigators studied the role of defects, measured the growth rate, and used the measured-step radius to estimate the step-free energy. Molecular resolution images permitted confirmation of the space group, direct measurement of the lattice parameters, and identification of polymorphs. This paper constitutes a striking proof-of-principle for the use of in situ AFM in the study of protein crystal growth. Because the growth of large, high quality crystals remains the main impediment to x-ray crystal structure determination, it appears that the AFM will play a role in high-resolution structure determination after all.

The in situ study of dissolution and growth was initiated some years ago in the Hansma laboratory (Gratz et al., 1991; Giles et al., 1995) and carried on by others cited in Yip and Ward's paper. However, this pioneering work used inorganic crystals (or samples with a high mineral content), which are much more robust and easier to handle than most protein crystals.

It is worth recalling that some protein structures have been imaged at rather high resolution by AFM, notably by the groups of Shao (Shao and Yang, 1995) and Engel (Schabert and Engel, 1994). To date, much of this work has been carried out using contact-mode AFM in solution, working on self-assembled two-dimensional films of the protein. In the case of the insulin crystal, Yip and Ward found that the normal contact mode, even operated in solution, was too destructive to permit imaging of the crystal surface. They used the so-called tapping mode, in which the cantilever is oscillated at with an amplitude of some nm and the approach to the surface is detected as a fall in this amplitude as the cantilever interacts transiently with the surface. This has the advantages that the average contact force is smaller, and that the tip delivers an impulse predominately normal to the surface, a direction in which these samples are generally stronger.

Despite the quality of the present work, it is clear that there is much room for improvement. To begin with, the work is tricky, as anyone who has tried AFM in a fluid cell will testify. More importantly, the probe obstructs the free-diffusion of molecules onto the surface and this must play some role in modifying growth as studied in situ. Yip and Ward have been careful to test for this possibility, and it does not appear to be a problem in the present work. However, our (unpublished) in situ studies of electrochemical corrosion and deposition show that mass-transport is often modified by the presence of the probe. Finally, tapping mode AFM, as presently practiced in solution, leaves a lot to be desired. When the probe is acoustically driven, coupling through the fluid medium to resonances of the sample cell leads to spurious responses that make the microscope hard to operate and characterize (Florin et al., 1993). And whereas tapping offers a great improvement on contact mode, it still involves rather violent encounters between the probe and sample. If, for example, a 10% change in amplitude is detected using a free-oscillation amplitude of 10 nm and a cantilever of 0.1 N/m spring constant, elementary mechanics leads to the result that $\sim 10^{-18}$ J is deposited into the sample at each strike of the probe. This is several hundred times thermal energy. Yip and Ward have done beautiful work with relatively primitive tools. It gives a hint of what may come in the future as better methods and microscopes are developed.

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Shuffling Protons in Bacteriorhodopsin: Long-Distance Coupling Between the pK_as of Two Carboxylic Groups

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How are protons transported across membranes? This question can be more specifically asked of the light-driven proton pump, bacteriorhodop-

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