Reply to "comments on direct visualization of protein complexes by scanning tunnelling microscopy"

To the Editor:

We, too, are concerned about the appropriate use of these new techniques. It was from our concern that we have chosen to compare our results by scanning tunneling microscopy (STM) and atomic force microscopy (AFM) with those obtained by others using scanning transmission microscopy, x-ray crystallography and x-ray scattering. In general, the measurements we made by STM and AFM are in agreement with those obtained by the other techniques. Where they are not, we have taken care to point out the discrepancy and discuss possible causes. This is an exciting new area of research. It is not surprising that one obtains results that cannot be explained easily based on the limited understanding we all have of the image generating mechanisms and probe sample interactions in these techniques. Questions concerning these points are thoroughly discussed in the *Biophysical Journal* paper (1) and in our other publications (2–7).

Two points mentioned by Garcia are of concern to all experimentalists in this field. The first of them is the well known discrepancy between height measured by STM and the equivalent dimension obtained by other techniques. Until the mechanism for contrast development is elucidated (8, 9), it will not be possible to understand the basis for the discrepancy. As we point out in our paper, the STM images of biomolecules are generally only 20–40% as thick as expected, a result routinely observed by others in the field.

The second question concerns the ability to distinguish between images of the molecules being studied and background noise including dirt and graphite mimics (10). As with all scientific investigation, one must use good judgment when claiming to have observed something. There is nothing unusual about our criteria for observational validity. All competent experimenters use such criteria. Does the observation of the structure depend on application of a sample to the substrate? Is the observation reproducible? Is the observed structure comparable, if not identical, to what might be expected based on independent information about the molecule? Because these customary criteria were followed in the case of our paper in the *Biophysical Journal,* we are fully confident of the work as presented.

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