Comments on direct visualization of protein complexes by scanning tunneling microscopy

Edstrom et al. (1) have observed the formation of phosphorylase–phosphorylase kinase complexes by scanning tunneling microscopy (STM). Concerning strictly biological information, the main point of the paper is to show that both size and shape of phosphorylase kinase are modified when it is bound to phosphorylase b. Though this actually may happen, the authors' results do not support that claim.

The authors report that the height of phosphorylase kinase is increased by a factor of three when bound to phosphorylase b (from 0.7 to 2.3 nm). This factor represents a considerable change if only noncovalent interactions are involved. But either value seems to be smaller than the one that it should be expected for a protein of this molecular weight (~ 5 nm).

A reanalysis of the authors' data, together with previous STM results suggests another explanation for the observed changes in phosphorylase kinase. At this stage in the development of the STM field on biology, two factors make it hard to draw definite conclusions about the dimensions of biomolecules with the STM. (*a*) The influence of the geometry of the tip, and (*b*) the still unknown tip-biomolecule interaction that allows the formation of the image.

The relationship between the STM image and the real object is an open question. For instance, for metallic samples with features of several tens of Angstroms or higher, it is known that the geometry of the imaging tip affects both the resolution and the image (2). In fact, the image should always be considered as a convolution between tip and object geometries (3–5). These distortions are particularly important when imaging biologicals, where the difficulty of achieving the desired tunneling current usually do not allow testing for the influence of the tip's geometry on the images. This effect seems to be present in the authors' images (Figs. 5 and 6), where all the reported phosphorylase kinase molecules present slightly different geometries.

But there is one more fundamental reason why the dimensions of biologicals from STM images should be taken with caution. It is known (and the authors acknowledge this) that the heights measured from STM images of biomolecules are systematically smaller than they should be. This could be partially due to the tendency of the STM tip to squeeze the biomolecule to achieve the fixed current. It is not unreasonable to suppose then, that the lateral dimensions of the image should be at least different than those of the molecule. Because the details of the interaction between tip and biomolecule remain unknown, conclusions about changes in dimensions and geometries should be made tentatively, at least until more images with a statistical relevance are provided.

A close view to the authors' results provide data that reflects the inaccuracy in the dimensions taken from STM images (Table 1). Also, the cover figure illustrates some artifacts that can appear when measuring heights. The height of phosphorylase b is ~3 Å if measured from the left, whereas from the right could amount to 20 Å. Another illustration of those problems is given in Fig. 5, where the heights of three phosphorylase b chains seem to be different, even though they are imaged by the same tip.

Finally, Fig. 4 poses a subtle question: how to discriminate between long rods that are biomolecules on the substrate from long rods that are defects in the substrate. In fact, it has been reported that features can appear in a graphite surface with a DNA-like appearance, even though no DNA was deposited on the substrate (6–8).

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REFERENCES

1. Edstrom, R. D., M. H. Meinke, X. Yang, R. Yang, V. Elings, and D. F. Evans. 1990. Direct visualization of phosphorylase–phosphorylase kinase complexes by scanning tunneling and atomic force micrscopy. *Biophys. J.* 58:1437–1448.
2. Bartolomé, A., R. Garcia, L. Vazquez, and A. M. Baro. 1988. Imaging an optical disc by the combined use of scanning tunneling microscopy and scanning electron microscopy. *J. Microscopy.* 152:205–211.
3. Chicon, R., M. Ortuno, and J. Abellan, 1987. An algorithm for surface reconstruction in scanning tunneling microscopy. *Surface Sci.* 181:107–111.
4. Niederman, P., and O. Fisher. 1988. Imaging of granular high-T_c thin films using a scanning tunneling microscope with large scan range. *J. Microscopy.* 152:93–101.
5. Keller, D. 1991. Reconstruction of scanning tunneling microscope and atomic force microscope images distorted by finite size tips. *Surface Sci.* In press.
6. Salmeron, M., T. Beebe, J. Odriozola, T. Wilson, D. F. Ogletree, and W. Sickhaus. 1990. Imaging of bimolecules with the scanning tunneling microscope: problems and prospects. *J. Vac. Sci. Technol. A* 8:637–641.
7. DeRose, J. A., S. M. Lindsay, L. A. Nagahara, P. I. Oden, T. Thundat, and R. L. Rill. 1991. Electrochemical deposition of nucleic acid polymers for scanning probe microscopy. *J. Vac. Sci. Technol.* B9:1166–1170.
8. Garcia, R. 1991. Scanning tunneling microscopy in biology: changing the pace. *Microscopy and Analysis.* In press.

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