Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 7848–7849, July 1998

Commentary

A new twist on protein crystallization

*Seth A. Darst**

The Rockefeller University, 1230 York Avenue, New York, NY 10021

Electron microscopy (EM), combined with image processing, has become an increasingly powerful tool for structural biology. Advances in cryopreservation and imaging, along with increasingly sophisticated computational tools for image processing, allow three-dimensional (3D) structure determination of macromolecules or macromolecular assemblies trapped in native states (1, 2). Structures from noncrystalline specimens of large macromolecular assemblies that are inaccessible to NMR and extremely challenging for x-ray crystallography can be determined to resolutions approaching 7 Å for highly symmetric structures such as icosahedral viruses (3, 4) or around 15 Å for asymmetric structures (5). Currently, for higher resolution information, ordered specimens are required to facilitate orientation and averaging of views. Crystalline specimens have generally come in two flavors, either very thin, usually single-layer thick or two-dimensional (2D), crystals, or helical assemblies. The largest impact of EM and image processing to date has come from the analysis of 2D crystals, which has sometimes yielded near atomic resolution structures of membrane proteins and tubulin, each presenting formidable problems for preparing suitable 3D crystals for x-ray analysis (6–10). A method specifically designed to generate 2D crystals of soluble macromolecules has been introduced, facilitating the application of EM and image processing to a wider range of systems (11–13). While helical specimens can sometimes provide advantages over 2D crystals, studies have been limited to naturally occurring helical assemblies or to a few instances where helical crystals were obtained (probably accidentally) when 2D crystals were being sought. The method presented by Wilson-Kubalek *et al*. (14) in the current issue of the *Proceedings* promises to have a significant impact on structural biology by making the relative ease and rapidity of structure determination from helical crystals accessible to a wider range of systems.

To place the helical crystallization method of Wilson-Kubalek *et al*. (14) in context, one needs to understand the method for generating 2D crystals introduced by Uzgiris and Kornberg (11). This method, which has been called lipid layer crystallization, depends on constraining proteins in two dimensions without loss of mobility. For this purpose, proteins are adsorbed from aqueous solution onto the surface of a lipid monolayer generated at the air/water interface. Binding and crystallization of the protein at the lipid surface can be achieved by the use of specific ligands derivatized to the head groups of the lipids (11) or by nonspecific, electrostatic interactions with charged lipids (15, 16). The method has been further generalized through the introduction of Ni^{2+}-chelating moieties to the head groups of lipids, expanding the application of the lipid layer crystallization method to highly popular and well characterized hexahistidine-tag affinity labels (17). The method has allowed the application of EM and image processing to a wide range of new systems, and low- to medium-resolution (30–10 Å) structures of a number of macromolecules and macromolecular assemblies have been determined from 2D crystals prepared in this way (18). Moreover, the potential for structural studies at high resolution (<3 Å) has been demonstrated for at least one system (19, 20).

In the lipid layer crystallization method, display of ligands on a flat, sheet-like surface gives rise to the formation of 2D crystals with sheet-like morphology. In at least two interesting cases, however, incubation of a protein/lipid-ligand system under conditions designed to induce the formation of 2D crystals at the air/water interface resulted instead in the formation of cylindrical, helical crystals in solution or associated with the interface (21, 22). In these helical crystals, a lipid bilayer formed into a cylindrical tube, and the protein crystallized with helical symmetry around the outside of the tube. The lipids used in these studies do not form tubule structures on their own, indicating that tube formation was induced by forces provided by the crystallization of the proteins themselves. This observation indicates that at least in some cases, the formation of a curved lattice can be powerfully favored over a flat lattice demanded by 2D crystals.

The clever trick introduced by Wilson-Kubalek *et al.* (14) was to display protein ligands not on a flat, sheet-like surface but on a cylindrical or tubular template, with the idea that this might coax the formation of helical crystals. For tubular templates on the size scale useful for EM and image processing (typically on the order of 10 to 100 nm diameter), one needs only to draw from the extensive literature on "nanotubes" made from amphiphiles that spontaneously form tubular structures (23, 24). While several lipid systems give rise to tubular structures only under conditions where the acyl chains are in a solid, crystallized phase, these are conditions known to be disfavorable for the crystallization of proteins on the surfaces of the lipids because of restricted mobility of the bound protein. Wilson-Kubalek *et al.* (14) used galactosylceramides with either an unsaturated acyl chain (25) or, later, mixtures of galactocerebrosides, either of which would be expected to inhibit lipid crystallization, even at low temperatures.

As with lipid layer crystallization, a range of methods can be used for attracting and concentrating the protein to the surface of the lipid tubule. Doped into the tube-forming lipids can be specific lipid-ligands, charged lipids, or the generalized lipid-ligand with the Ni^{2+}-chelating moiety attached to the head group. What is particularly striking in the Wilson-Kubalek *et al*. (14) report is the apparent robustness of the technique; helical crystals were formed from an impressively wide range of protein/lipid-ligand systems. In other words, the formation of helical crystals was not a lucky fluke of a few specific cases.

The relatively low-resolution diffraction obtained from most of the helical crystals prepared by Wilson-Kubalek *et al*. (14) remains promising in light of the powerful computational tools being developed for image processing of helical specimens. Helical processing methods are developing rapidly and have resulted in published structures in the 10-to 8-Å resolution range of acetylcholine receptor (26, 27), the Ca^{2+}-ATPase of sarcoplasmic reticulum (28), and bacterial flagella (29, 30). Higher-resolution structures are anticipated in the near future.

The companion to this commentary is published on pages 8040–8045.

*To whom reprint requests should be addressed. e-mail: darst@rockvax.rockefeller.edu.

We should expect this list to grow as a result of the helical crystallization on nanotubes introduced by Wilson-Kubalek *et al*. (14).

1. Taylor, K. A. & Glaeser, R. M. (1974) *Science* **186,** 1036–1037.
2. Dubochet, J., Adrian, M., Chang, J.-J., Homo, J.-C., Lepault, J., McDowall, A. W. & Schultz, P. (1988) *Q. Rev. Biophys.* **21,** 129–228.
3. Bottcher, B., Wynne, S. A. & Crowther, R. A. (1997) *Nature (London)* **385,** 88–91.
4. Conway, J. F., Cheng, N., Zlotnick, A., Wingfield, P. T., Stahl, S. J. & Steven, A. C. (1997) *Nature (London)* **385,** 91–94.
5. Malhotra, A., Penczek, P., Agrawal, R. K., Gabashvili, I. S., Grassucci, R. A., Jünemann, R., Burkhardt, N., Nierhaus, K. H. & Frank, J. (1998) *J. Mol. Biol.,* in press.
6. Henderson, R., Baldwin, J. M., Ceska, T. A., Zemlin, F., Beckmann, E. & Downing, K. H. (1990) *J. Mol. Biol.* **213,** 899–929.
7. Jap, B. K., Walian, P. J. & Gehring, K. (1991) *Nature (London)* **350,** 167–170.
8. Kühlbrandt, W., Wang, D. N. & Fujiyoshi, Y. (1994) *Nature (London)* **367,** 614–621.
9. Kimura, Y., Vassylyev, D. G., Miyazawa, A., Kidera, A., Matsusha, M., Mitsuoka, K., Murata, K., Hirai, T. & Fujiyoshi, Y. (1997) *Nature (London)* **389,** 206–211.
10. Nogales, E., Wolf, S. G. & Downing, K. H. (1998) *Nature (London)* **391,** 199–203.
11. Uzgiris, E. E. & Kornberg, R. D. (1983) *Nature (London)* **301,** 125–129.
12. Kornberg, R. D. & Ribi, H. O. (1987) in *Protein Structure, Folding, and Design 2*, ed. Oxender, D. L. (Liss, New York), pp. 175–186.
13. Kornberg, R. D. & Darst, S. A. (1991) *Curr. Opin. Struct. Biol.* **1,** 642–646.
14. Wilson-Kubalek, E. M., Brown, R. E., Celia, H. & Milligan, R. A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 8040–8045.
15. Darst, S. A., Ribi, H. O., Pierce, D. W. & Kornberg, R. D. (1988) *J. Mol. Biol.* **203,** 269–273.
16. Darst, S. A., Kubalek, E. W., Edwards, A. M. & Kornberg, R. D. (1991) *J. Mol. Biol.* **221,** 347–357.
17. Kubalek, E. W., Le Grice, S. F. J. & Brown, P. O. (1994) *J. Struct. Biol.* **113,** 117–123.
18. Brisson, A., Olofsson, A., Ringler, P., Schmutz, M. & Stoylova, S. (1994) *Biol. Cell* **80,** 221–228.
19. Kubalek, E. W., Kornberg, R. D. & Darst, S. A. (1991) *Ultramicroscopy* **35,** 295–304.
20. Avila-Sakar, A. J. & Chiu, W. (1996) *Biophys. J.* **70,** 57–68.
21. Schmid, M. F., Robinson, J. P. & DasGupta, B. R. (1993) *Nature (London)* **364,** 827–830.
22. Polyakov, A., Severinova, E. & Darst, S. A. (1995) *Cell* **83,** 365–373.
23. Schnur, J. M., Price, R., Schoen, P., Yager, P., Calbert, J. M., Georger, J. & Singh, A. (1987) *Thin Solid Films* **152,** 181–206.
24. Schnur, J. M. (1993) *Science* **262,** 1669–1676.
25. Kulkarni, V. S., Anderson, W. H. & Brown, R. E. (1995) *Biophys. J.* **69,** 1976–1986.
26. Unwin, N. (1993) *J. Mol. Biol.* **229,** 1101–1124.
27. Unwin, N. (1995) *Nature (London)* **373,** 37–43.
28. Zhang, P., Toyoshima, C., Yonekura, K., Green, N. M. & Stokes, D. L. (1998) *Nature (London)* **392,** 835–840.
29. Mimori, Y., Yamashita, I., Murata, K., Fujiyoshi, Y., Yonekura, K., Toyoshima, C. & Namba, K. (1995) *J. Mol. Biol.* **249,** 69–87.
30. Morgan, D. G., Owen, C., Melanson, L. A. & DeRosier, D. J. (1995) *J. Mol. Biol.* **249,** 88–110.