

BOOK REVIEW

Reducing the energy barrier to crystallization

Crystallization of nucleic acids and proteins, edited by A. Ducruix and R. Giegé. 1992.
Oxford, UK: IRL Press. 331 pp. \$50.00 (paper).

Macromolecular X-ray crystallography has benefited from an explosion in the sensitivity and availability of area detectors and in the power and affordability of computers for number crunching and graphical modeling. Meanwhile, the exponential solution and deposition of atomic coordinates have made the likelihood of structural similarity between a newly crystallized molecule and one in the Protein Data Bank ever greater. Such similarity is prerequisite for molecular replacement to assign initial phases to observed X-ray diffraction intensities, a process that can eliminate the need for heavy-atom methods. More than ever, macromolecular crystallography laboratories are limited by the availability of suitable crystals, i.e., crystals that diffract X-rays to a resolution sufficient to address the biological or biochemical problem at hand.

It is important to know that one does not need to be a crystallographer to crystallize macromolecules or even to begin assessing their suitability. The knowledge accrued by the person who has purified and characterized a protein provides advantages that are often unavailable to those in a crystallography lab to whom a protein is shipped. Working knowledge of a protein's solubility and stability and intellectual devotion to the project are key ingredients for growth of crystals.

Crystallographic expertise is needed once crystals are obtained. Although experts may disagree on whether it is worth mounting 100- μ m crystals or collecting data on crystals that diffract poorly, essentially all crystallographers will be far more available to the biochemist who has grown a protein crystal of any quality than to the biochemist who has not. Historically, biochemists have worked to enlist crystallographers by convincing them of the biological significance of their proteins. But I can cite 4 examples of biochemists who grew crystals and were shown directly to the area detectors while the crystallographers eagerly asked about the biology. Difficult as solving a crystal structure can be, it is true that once palpable crystals are grown, most of the remaining problems are real physics or, occasionally, real chemistry, and can be assayed in straightforward ways.

There are immediate and lasting rewards of growing a new protein or nucleic acid crystal. The best way to obtain training in structural biology is to appear in an X-ray laboratory with such a crystal or with an unpublished technique for growing one. Students and fellows working around the X-ray equipment detect new crystals with the acuity of salmon swimming to the place of their birth. A motivated novice should be able to sit in the driver's seat and choose collaborators to teach data collection, solution of Patterson or molecular replacement problems, refinement, chain tracing, and rebuilding. This is how to make friends and be influenced by people.

The activation energy barriers are high. First, one must prepare tens of milligrams of purified protein, nucleic acid, or com-

plex and concentrate it into a 100 μ M to mM solution. Second, one must test hundreds of conditions under which the protein may crystallize and examine them over time in the light microscope. Third, one must evaluate crystals by crushing them, taking still or precession X-ray photographs of them, and recording a few frames of data on an area detector. Largely to help the newcomer get to this point in a crystallography project, the point at which one needs and easily obtains the help of professional crystallographers, A. Ducruix and R. Giegé have edited a new volume of The Practical Approach series entitled *Crystallization of Nucleic Acids and Proteins*. This series, published in trade paperback format by IRL Press, has already published a number of volumes on cloning, expression, and purification, some of which bear renewed examination in order to produce the necessary quantity and quality of starting material.

Crystallization is an activity that still feels alchemical, standing at the crossroads of chemistry and mineralogy and at the gateway to diffraction methods. Crystallization of proteins and biologically significant nucleic acids—by their nature, large, irregular, and highly hydrated compounds—is less well understood than crystallization of small molecules, a process that can be as simple as evaporation of solvent. Nonetheless, *Crystallization of Nucleic Acids and Proteins* provides a framework for understanding the few salient concepts that exist, such as salting-out, salting-in, supersaturation, nucleation, growth, and mosaicism. Appreciating that most readers are driven more to determine and understand structures than to grow macromolecular crystals per se, the editors devoted most of the text to the practice rather than the theory of growing crystals.

Ducruix, Giegé, and co-authors B. Lorber, M. Reis-Kautt, and V. Mikol are responsible for chapters that introduce crystallization and discuss preparation and handling of macromolecules, phase diagrams, physical chemistry of protein crystallization, and methods of crystallization. The latter chapter is useful to the professional crystallographer who may be confined to a single method such as hanging drop vapor diffusion by force of habit. There are numerous examples of crystal forms that have only been obtained by other methods, such as batch evaporation or dialysis. For the novice, however, the chapter may not have emphasized enough the versatility and the economies of time and material afforded by hanging drops.

The sheer numbers of different salts, buffers, polymers, alcohols, and detergents that are used for macromolecular crystallization are daunting. One compound, or more frighteningly, a combination of compounds at specific concentrations might be crucial to crystallizing a new protein. To screen a protein with various concentrations of 1 salt against a concentration gradient of 1 polyethylene glycol is to be exhaustive in just 1 region of the multidimensional reagent space in which protein crystals

have been grown. C.W. Carter, Jr.'s chapter considers the problem of crystallization to be similar in form to problems of industrial quality control in which a wide variety of factors might contribute to successful manufacture. Given a limited number of crystallization set-ups to be tried, he argues that it is better to sample more variables in combination with each other even if many of the possible combinations are randomly skipped. By scoring the results of such "incomplete factorial analyses," reagents that contribute to success may be identified early in the search for crystallization conditions. Carter's arguments have been so influential in macromolecular crystallization that his chapter has become somewhat dated by publication and dissemination by fax and e-mail of specific incomplete factorial screening regimens for soluble proteins, nucleic acids, and membrane proteins.

After Carter called for design of crystallization trials by random recombination of a series of reagents that have proven to be useful in previous crystallizations, J. Jancarik and S.-H. Kim found that even greater historical bias was warranted. They observed that some combinations of reagents that work for one protein work for many others. For example, 0.2 M Na citrate with 0.1 M Tris Cl, pH 8.5, and 30% polyethylene glycol 400 was found to be an extremely effective cocktail for protein crystallization, producing crystals of 4 out of 15 previously crystallized proteins and 4 of 31 proteins that had not been crystallized previously. As published, the screen sampled conditions from pH 4.6 to 8.5 by combining 6 buffers, 16 salts, 2 alcohols, and 4 polyethylene glycols in a total of 50 solutions (Jancarik & Kim, 1991). Factorial screens are empirically derived and subject to improvement. After screening hundreds of proteins, the few original solutions that never produced crystals have been eliminated and 8 additional reagents are sampled in a total of 58 solutions (J. Jancarik, pers. comm.).

From the biased nature of these formulations, it may follow that macromolecules that are very distinct from those that have been crystallized are less likely to crystallize under these conditions. To address this limitation, a screen for crystallization of ribonucleic acids based on conditions from the RNA crystallization literature (Doudna et al., 1993), a screen utilizing a number of promising reagents that are new to crystallographers (Cudney et al., 1994), and a screening regimen for membrane proteins (M.H.B. Stowell & D.C. Rees, in prep.) have been developed. These screens have become the first things to try in crystallization and form an essential supplement to the Ducruix and Giegé text. To reduce further the energy barriers to novices, factorial screening solutions can now be ordered from kits (Hamp-ton Research, Riverside, California).

Success in initial screening is declared when crystals of any size or quality are identified that are not salt. It is relatively straightforward to improve the size, habits, and regularity of crystals by modification of one's conditions. Oftentimes, however, no condition produces obvious crystals and a variety of

conditions produce precipitates that appear light and granular, whereas others look like scrambled eggs. A great deal of time and material can be spent modifying conditions that produce precipitates in the hope of producing single crystals. Time and material are more prudently spent after reading E.A. Stura and I.A. Wilson's chapter on crystal seeding. Because dull precipitates can hide diamonds in the rough, streaking can assay whether similar-looking precipitates are actually microcrystalline. Seeding can also assay whether different solutions support the growth of established crystalline nuclei. This chapter reinforces the fact that nucleation and crystal growth may occur under disparate conditions and that careful manipulation of crystals can be highly beneficial. In photographs, the authors chronicle obtaining large hexagonal crystals by seeding from a shower of microcrystals that were obtained, in turn, by streaking from a very unimpressive-looking precipitate. In learning to seed, one learns that good crystal growers have good hands.

Chapters on crystallization in gels, crystallization of nucleic acids and nucleoprotein complexes, crystallization of membrane proteins, automating crystallization, and preparation of selenomethionyl protein crystals are useful guides for these applications. Additionally, 2 chapters are provided for those that have hurdled the step of obtaining crystals. E.A. Stura and P. Chen discuss soaking crystals for incorporation of ligands and heavy atoms or for transfer to low temperatures. L. Sawyer and M.A. Turner's chapter on X-ray analysis refers one to the important texts of diffraction methods, introduces key concepts of symmetry and reciprocal space, and provides detailed protocols for mounting crystals and performing precession photography.

Ducruix and Giegé's book has already been discovered and used extensively by crystallographers. It is time for it to be discovered by the larger audience of all those engaged in the study of particular macromolecules. This book may help bring about an era in which biologists attract crystallographers with crystalline bait and crystallographers respond with symmetry.

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References

- Cudney B, Patel S, Weisgraber K, Newhouse Y, McPherson A. 1994. Screening and optimization strategies for macromolecular crystal growth. *Acta Crystallogr* 50:414-423.
- Doudna JA, Grosshans C, Gooding A, Kundrot CE. 1993. Crystallization of ribozymes and small RNA motifs by a sparse matrix approach. *Proc Natl Acad Sci USA* 90:7829-7833.
- Jancarik J, Kim SH. 1991. Sparse matrix sampling: A screening method for crystallization of proteins. *J Appl Crystallogr* 24:409-411.

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