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Preface

Approximately 15 years ago, a volume of similar title, Protein and Nucleic Acid Crystallization Methods, appeared in this series, Methods: A Companion to Methods in Enzymology, edited by my colleague, Charles Carter. Its contents covered in a comprehensive and lucid fashion the state of the science up until that time. That volume also addressed many of the problems which then appeared most pressing and some of the opportunities and new ideas that were emerging. This volume attempts to take off where that one ended, though a quick perusal of Volume 1 of this series and the Table of Contents here would show that though many of the same challenges still remain, some of the nascent ideas of that earlier time have now reached fruition. It would also show that many novel and intriguing ideas, strategies, and approaches have developed in the meantime.

The scientific rationale for this volume remains essentially the same as that of the one 15 years ago, though accentuated by the structural genomics initiative that is currently gathering force. X-ray crystallography has virtually transformed our understanding and application of molecular biology, including its influence on medicine, industry, and agriculture. Structural studies now dominate enzymology, pharmacology, the genetic engineering of proteins, and many of the other crucial disciplines in biochemistry and biophysics. Application of X-ray crystallography, however, continues to be absolutely dependent on our ability to grow crystals of target proteins, nucleic acids, viruses, macromolecular assemblies, and complexes. Thus, crystallization remains the linchpin in the process of structure determination and analysis, and the major sticking point in an even greater exploitation of the enormously powerful X-ray diffraction technique. Recent evidence from the structural genomics program has clearly indicated that once proteins are crystallized in forms suitable for diffraction analysis, their structures follow, often quickly, but inevitably. Only protein expression and crystallization remain as substantive obstacles, and the latter certainly dominates.

The success of X-ray crystallography is largely a product of technological advances that have converged in a timely manner to provide us with a methodology of unparalleled power and precision. The formerly tedious and meticulous processes of data collection have been superceded by the rapid acquisition of structure amplitudes using synchrotron radiation, CCD detectors, variable wavelengths, and cryogenically frozen crystals. The phase problem that once dominated crystal structure solution has been significantly reduced by a host of new phasing techniques such as multiple or single anomalous dispersion (MAD and SAD), or improved applications of old approaches, multiple isomorphous replacement (MIR), and molecular replacement (MR). While these advances have greatly reduced the size of crystals needed for analysis (to 0.1 mm, and sometimes less), and the number of crystals required (frequently only one), they can never entirely eliminate the fundamental problem of obtaining crystals, nor the need for the degree of perfection required to obtain high-resolution data. Furthermore, implementation of all of the new methods generally requires that we be able to efficiently freeze the crystals, form complexes with ligands, and grow isomorphous samples of modified molecules. Demanding tasks certainly remain.

The contributions of the authors included in this volume are directed toward alleviating the problems remaining in macromolecular crystallization, and equally important, bringing to the fore novel ideas and innovative approaches that have emerged since the previous volume. The first chapter attempts a succinct summary of some of the fundamental principles and methods in protein crystallization, and is largely remedial. There are many good books now available that deal with this material in far greater detail and with greater elegance. The editor recommends, for example, books by Bergfors [1], Ducruix and Giége [3], and that by McPherson [4]. There is also a wealth of useful information for beginning students and practitioners from the purveyors of commercial supplies and apparatus, and in the set of volumes constituting the proceedings of the now 10 symposia of the International Conferences on the Crystallization of Biological Macromolecules (ICCBM).

Chapters by Asherie, and by Malkin and Thorne describe the progress that has been made in understanding the physical aspects of protein crystallization. This new knowledge was acquired by application of novel analytical techniques, often borrowed from other disciplines or fields of endeavor. These include principally elastic and inelastic light scattering, in Chapter 2, and atomic force microscopy along with X-ray topography and other physical methods in

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Chapter 3. If anything can be said with certainty, it is that the last 15 years have seen a dramatic and profound increase in our knowledge of the physics of protein crystallization. This point is amply illustrated in these two chapters.

In spite of our progress in understanding the physical aspects of protein crystallization, the behavior of macromolecules in solution and their interaction with the great variety of molecules in their environment, and with one another, remains ambiguous. This is particularly true in concentrated protein solutions and those containing high concentrations of the precipitating agents necessary to effect nucleation and growth. Collins and Bolen, the authors of Chapters 4 and 5, respectively, are among the foremost authorities in this area and both offer original insights into the effects of ions, solvents, and osmolytes on protein stability and solubility. This is an area of protein crystallization that has previously received but scant attention, and these chapters will likely prove seminal works.

Chapters 6, 7, and 8 deal with more practical aspects of protein crystallization, both the screening for initial crystallization conditions and their optimization. D'Arcy et al. describe an old technique recast for the modern world, microbatch methods, while Bard et al. extend conventional approaches to very large problems, and very large screening matrices using robotic systems and computer automation. Nollert shows how a novel technique for growing crystals of membrane proteins, the cubic lipidic phase, can be used in a manner similar to those we normally apply to conventional proteins.

One of the most promising new approaches to growing crystals of otherwise refractile proteins is the application of genetic engineering, or as Dale et al. [2] suggested, making the protein a major variable. The problem, of course, is knowing how best to modify the amino acids of the target protein to achieve a crystalline result. Derwenda, in Chapter 9, combines our accumulated wisdom and experience with novel ideas based on the physical properties of amino acid residues to gain insight into this problem. Use of recombinant techniques is a rich and promising area that is only now being investigated in a rational and systematic manner. It is the area that seems most likely to provide important advances in both fundamental knowledge, and ultimately in practical applications.

Perhaps the most pressing, yet seemingly intractable, domain of macromolecular crystallization has been the growth of suitable crystals of membrane proteins and other lipophilic macromolecules. The problems here are not simply in devising appropriate crystallization conditions, but in producing, purifying, and solubilizing membrane proteins even before crystallization can be attempted. The conditions too are more complex because of the detergents and amphiphiles that must be incorporated to achieve success. Nonetheless, progress has been and continues to be made. The more recent developments in this regard are presented in Chapter 10 by Weiner, an experienced practitioner of the techniques and a knowledgeable user and developer of the reagents involved. This is also an area where we may expect significant progress to be made in coming years as the necessity for membrane protein structural information increases from both the structural genomics and drug design perspectives.

The structural genomics initiative, which was initiated about three years ago, has already begun to produce results in plenty. In addition to new protein structures, it has also provided an initial sampling of the expected massive flow of new data regarding crystallization conditions, both successful and otherwise. These data, if systematically accumulated, cataloged, and managed, have the potential of transforming our approaches to crystal growth. Only time will tell. Chapter 11 by Page and Stevens shows what can be done with only a segment of those data, and it is impressive. Rupp, taking an analytical view in Chapter 12, considers how we had best address the use of those data, and what reasonable expectations we might have.

Among the most attractive objectives for X-ray diffraction analysis in coming years will be not proteins, but nucleic acids and protein–nucleic acid complexes. Because our library of structural information in the area of nucleic acids is so small, and because its richness is only now becoming evident from new structures like ribozymes and ribosomes, nucleic acids will undoubtedly occupy a central place in future crystallographic research. Chapter 13 by Doudna and Ke addresses this area of discovery and describes the current state of the art.

Finally, it must be recognized that the best and largest crystal is of no real value unless it can be exposed to X-rays and have its diffraction pattern recorded. In current times, this generally means that the crystal must be frozen and maintained under cryogenic conditions for the duration of data collection. This is neither easy nor assured in a great many cases, as the methodology of cryocrystallography is still work in progress. Chapter 14 by Pflugrath addresses this issue from a practical standpoint and illustrates many of the important considerations for carrying out the process successfully.

An examination of the contents of this volume suggests that macromolecular crystallization still remains largely empirical, and still problematic. It is also evident, however, that much progress has been made in the past 15 years, and that the field remains alive with innovation and novel ideas. Perhaps in another 15 years there may remain little need for a further update of the field. That should be our aspiration.

References

[1] T.M. Bergfors, Protein Crystallization: Techniques, Strategies and Tips, In International University Line, La Jolla, CA, 1999.
[2] G.E. Dale, C. Oefner, A. D'Arcy, J. Struct. Biol. 142 (2003) 88–97.
[3] A. Ducruix, R. Giége, Crystallization of Nucleic Acids and Proteins, A Practical Approach, IRL Press, Oxford, 1992.
[4] A. McPherson, Crystallization of Biological Macromolecules, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999.

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