
Preface

In the decade since the publication of the first edition of *Crystallographic Methods and Protocols*, the field has seen several major developments that have both accelerated the pace of structure determination and made crystallography accessible to a broader range of investigators. As evidence of this growth, this new work, *Macromolecular Crystallography Protocols*, encompasses two volumes: volume 1, *Preparation and Crystallization of Macromolecules*, and volume 2, *Structure Determination*.

Fanning the fire are the large number of synchrotron beamlines dedicated to macromolecular crystallography and the availability of inexpensive desktop supercomputers. Expression systems for proteins and nucleic acids have greatly improved as well. Several improvements come to mind: ligation-independent cloning, the development of N-terminally fused expression tags that help protein solubility, and the use of eukaryotic expression systems. In addition, structural genomics has increasingly changed the way we go about solving crystal structures, not only because of the sheer increase in the number of deposited structures, but more importantly because of the new tools the structural genomics centers have developed and are making available to the community at large.

The first five chapters of *Macromolecular Crystallography Protocols: Preparation and Crystallization of Macromolecules* are concerned with the production of proteins for structural biology. In the first chapter, Tropea and collaborators describe the use of a dual affinity tag for the production of recombinant proteins. The chapter by Quevillon-Cheruel and colleagues describes the cloning, expression, and purification of yeast proteins in a medium-sized structural genomics group. When eukaryotic proteins do not express in bacterial cells, one usually turns to a eukaryotic system. Madden and Safferling describe in Chapter 3 the baculovirus expression of integral membrane proteins. Very often, one has to tinker with a protein in order to crystallize it. Longhi and collaborators share the tricks they have employed to cajole proteins into adopting a crystalline form. One of the ways to solve a crystal structure *de novo* is to incorporate selenomethionine into proteins. In Chapter 5, Doublé reviews the different methods to achieve selenomethionine substitution in bacteria and eukaryotic cells.

No fewer than eight chapters are dedicated to crystallization and the ways to increase the odds of obtaining crystals. In Chapter 6, Borgstahl describes how to use dynamic light scattering to increase the probability of obtaining crystals. Screening and optimization methods are described in Chapter 7 by Bergfors. In

Chapter 8, Carter and Riès-Kautt share their expertise in transforming marginal crystals into diffraction-worthy crystals. Optimization techniques are also presented in Chapter 9 by Chayen, this time in a high-throughput format. The last four crystallization chapters are more specialized. In Chapter 10, Féthière focuses on the crystallization of membrane proteins. Hollis presents methods to grow protein–DNA crystals in Chapter 11. The RNA world is represented in the last two chapters. Golden describes methods to prepare and crystallize RNA in Chapter 12, whereas Obayashi and collaborators tackle the task of explaining how to obtain crystals of protein–RNA complexes in Chapter 13.

The second volume of *Macromolecular Crystallography Protocols: Structure Determination* complements this first volume by addressing laboratory techniques for crystal handling and structural characterization, as well as computational techniques for data collection, phasing, and refinement. Readers will also benefit from a survey of the available crystallographic software.

It is my sincere hope that students will find the two *Macromolecular Crystallography Protocols* volumes useful, as it was they that I had in mind when I put this book together. I essentially designed a book I wished I could have had available when I was a student. May these two volumes help all crystallographic apprentices obtain crystals and guide their steps along the all too often rugged path of structure determination.

I would like to express my sincere thanks to Anne MacLeod for her help during the final phase of manuscript handling. Finally, I am grateful to all the authors for carving out time to write their manuscripts, for being so cooperative, and for their patience throughout the different stages of the book production.

Sylvie Doublé

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