
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.

Following volume I, which is dedicated to the preparation and crystallization of macromolecules, this second volume, *Structure Determination*, covers both laboratory and computational methods for characterizing crystals and solving structures. The topic of crystal handling, characterization, and data collection is covered in Chapters 1–6. Most crystals are cryocooled in order to increase their lifetime in the X-ray beam. Garman and Owen share their practical experience in optimizing cryocooling techniques in Chapter 1. In Chapter 2, Chu outlines how reaction intermediates can be captured at ultra-low temperatures. Annealing techniques, which can dramatically improve crystal diffraction limits, are reviewed by Bunick and Hanson in Chapter 3. In Chapter 4, Jeruzalmi acquaints us with the first analysis of macromolecular crystals. Garman and Sweet go over the nitty-gritty of macromolecular crystal data collection in Chapter 5. In addition, in Chapter 6 Sawaya tells us everything we need to know about characterizing a crystal from an initial dataset.

Five chapters are dedicated to the subject of phasing. In Chapter 7, Toth presents the different ways to solve a structure by molecular replacement. Isomorphous replacement is covered in Chapter 8 by Dauter and Dauter, who describe the use of halide ions for phasing, and also in Chapter 9 by Rould, who extends the concept of isomorphous replacement to isomorphous difference

Fourier maps and their use. Location of heavy metal positions is the focus of Chapter 10 by Smith and collaborators, who describe the use of *Shake-and-Bake* with anomalous datasets, and Chapter 11 by Grosse-Kunstleve and Schneider, who present anomalous and isomorphous cases. Vonnrhein and co-workers describe automated structure solution by autoSHARP in Chapter 12.

Structure refinement is detailed in Chapter 13 by Tronrud. Kleywegt focuses on quality control and validation in Chapter 14. Finally, Chapter 15 by Everse and Doublé surveys 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.

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