Macromolecular Crystallography Protocols

Volume 1: Preparation and Crystallization of Macromolecules

Edited by

Sylvie Doublié

Department of Microbiology and Molecular Genetics, University of Vermont, Burlingon, VT
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 shear 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.
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 Doublié*
Contents

Preface .............................................................................................................. v
Contributors ..................................................................................................... ix
Contents of Volume 2 ................................................................................... xiii

1. A Generic Method for the Production of Recombinant Proteins in Escherichia coli Using a Dual Hexahistidine-Maltose-Binding Protein Affinity Tag
   Joseph E. Tropea, Scott Cherry, Sreedevi Nallamsetty, Christophe Bignon, and David S. Waugh ......................................... 1

2. Cloning, Production, and Purification of Proteins for a Medium-Scale Structural Genomics Project
   Sophie Quevillon-Cheruel, Bruno Collinet, Lionel Trésaugues, Philippe Minard, Gilles Henckes, Robert Aufrère, Karine Blondeau, Cong-Zhao Zhou, Dominique Liger, Nabila Bettache, Anne Poupon, Ilham Aboulfath, Nicolas Leulliot, Joël Janin, and Herman van Tilbeurgh ................. 21

3. Baculoviral Expression of an Integral Membrane Protein for Structural Studies
   Dean R. Madden and Markus Safferling ..................................................... 39

4. Protein Engineering
   Sonia Longhi, François Ferron, and Marie-Pierre Egloff ......................... 59

5. Production of Selenomethionyl Proteins in Prokaryotic and Eukaryotic Expression Systems
   Sylvie Doublié ............................................................................................ 91

6. How to Use Dynamic Light Scattering to Improve the Likelihood of Growing Macromolecular Crystals
   Gloria E. O. Borgstahl ............................................................................... 109

7. Screening and Optimization Methods for Nonautomated Crystallization Laboratories
   Terese Bergfors ......................................................................................... 131

8. Improving Marginal Crystals
   Charles W. Carter, Jr. and Madeleine Riès-Kautt .................................. 153

9. Optimization Techniques for Automation and High Throughput
   Naomi E. Chayen ...................................................................................... 175
10. Three-Dimensional Crystallization of Membrane Proteins
   James Féthière .......................................................................................... 191

11. Crystallization of Protein–DNA Complexes
   Thomas Hollis .......................................................................................... 225

12. Preparation and Crystallization of RNA
   Barbara L. Golden .................................................................................. 239

13. Crystallization of RNA–Protein Complexes
   Eiji Obayashi, Chris Oubridge, Daniel Pomeranz Krummel,
   and Kiyoshi Nagai ............................................................................... 259

Index ............................................................................................................. 277
Contributors

ILHAM ABOULFATH • Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France
ROBERT AUFRÈRE • Institut de Génétique et Microbiologie, Université de Paris-Sud, Orsay, France
TERESÉ BERGEFORS • Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden
NABILA BETTACHE • Institut de Génétique et Microbiologie, Université de Paris-Sud, Orsay, France
CHRISTOPHE BIGNON • Glycogenomics and Biomedical Structural Biology, Architecture et Fonction des Macromolécules Biologiques, CNRS/Université de Provence/Université de la Méditerrané, Marseille, France
KARINE BLONDEAU • Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France
GLORIA E. O. BORGSTAHL • Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE
CHARLES W. CARTER, JR. • Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC
NAOMI E. CHAYEN • Biological Structure and Function Section, Division of Biomedical Sciences, Imperial College, London, United Kingdom
SCOTT CHERRY • Protein Engineering Section, Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute–Frederick, Frederick, MD
BRUNO COLLINET • Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France
SYLVIE DOUBLIÉ • Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT
MARIE-PIERRE EGLOFF • Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique and Universités Aix–Marseille I et II, Marseille, France
FRANÇOIS FERRON • Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique and Universités Aix–Marseille I et II, Marseille, France
JAMES FÉTHIÈRE • European Molecular Biology Laboratory, Heidelberg, Germany and Department of Pharmacology, University of Montreal, Montreal, Canada
BARBARA L. GOLDEN • Department of Biochemistry, Purdue University, West Lafayette, IN

GILLES HENCKES • Institut de Génétique et Microbiologie, Université de Paris-Sud, Orsay, France

THOMAS HOLLIS • Department of Biochemistry, Center for Structural Biology, Wake Forest University Health Science Center, Winston Salem, NC

JOEL JANIN • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Équipe Génomique Structurale, Université de Paris-Sud, Orsay, France

NICOLAS LEULLIOT • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France

DOMINIQUE LIGER • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France

SONIA LONGHI • Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique and Universités Aix–Marseille I et II, Marseille, France

DEAN R. MADDEN • Department of Biochemistry, Dartmouth Medical School, Hanover, NH and Max Planck Institute for Medical Research, Heidelberg, Germany

PHILIPPE MINARD • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France

KIYOSHI NAGAI • Structural Studies Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom

SREEDEVI NALLAMSETTY • Protein Engineering Section, Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute–Frederick, Frederick, MD

EIJI Oabayashi • Structural Studies Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom

CHRIS OUBRIDGE • Structural Studies Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom

DANIEL POMERANZ KRUMMEL • Structural Studies Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom

ANNE Poupon • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Équipe Génomique Structurale, Université de Paris-Sud, Orsay, France

SOPHIE QUEVILLON-CHERUEL • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France

MADELEINE RIES-KAUTT • Laboratoire de Cristallographie et RMN Biologiques, Université René Descartes, Paris, France

MARKUS SAFFERLING • Ion Channel Structure Group, Max Planck Institute for Medical Research, Heidelberg, Germany and Bayer CropScience AG, Monheim, Germany
Lionel Trésaugues • Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, Université de Paris-Sud, Orsay, France
Joseph E. Tropea • Protein Engineering Section, Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute–Frederick, Frederick, MD
Herman van Tilbeurgh • Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Équipe Génomique Structurale, Université de Paris-Sud, Orsay, France
David S. Waugh • Protein Engineering Section, Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute–Frederick, Frederick, MD
Cong-Zhao Zhou • National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, China
Contents of Volume 2

1. Cryocrystallography of Macromolecules: Practice and Optimization
   Elspeth Garman and Robin L. Owen
2. Determination of Reaction Intermediate Structures in Heme Proteins
   Kelvin Chu
3. Annealing Macromolecular Crystals
   B. Leif Hanson and Gerard J. Bunick
4. First Analysis of Macromolecular Crystals: Biochemistry and X-Ray Diffraction
   David Jeruzalmi
5. X-Ray Data Collection From Macromolecular Crystals
   Elspeth Garman and Robert M. Sweet
6. Characterizing a Crystal From an Initial Native Dataset
   Michael R. Sawaya
7. Molecular Replacement
   Eric A. Toth
8. Phase Determination Using Halide Ions
   Miroslawa Dauter and Zbigniew Dauter
9. The Same But Different: Isomorphous Methods for Phasing and High-Throughput Ligand Screening
   Mark A. Rould
    G. David Smith, Christopher T. Lemke, and P. Lynne Howell
11. Substructure Determination in Isomorphous Replacement and Anomalous Diffraction Experiments
    Ralf W. Grosse-Kunstleve and Thomas R. Schneider
12. Automated Structure Solution With autoSHARP
    Clemens Vonrhein, Eric Blanc, Pietro Roversi, and Gérard Bricogne
13. Introduction to Macromolecular Refinement
    Dale E. Tronrud
14. Quality Control and Validation
    Gerard J. Kleywegt
15. Crystallographic Software: A Sustainable Resource for the Community
    Stephen J. Everse and Sylvie Doublié