

Link to Order: http://fivephoton.com/index.php?route=product/product&path=37&product_id=67

Price: \$119.95

Website: www.fivephoton.com. **Tel:** 800-462-4507

METHODS AND RESULTS IN CRYSTALLIZATION OF MEMBRANE PROTEINS

edited by
SO IWATA

Table of Contents:

Preface **xiii**
List of Contributors **xv**

Part I. INTRODUCTION 1

1. How to Use This Book 3

So Iwata and Bernadette Byrne

1.1. Solubilization and Purification of Membrane Proteins	5
1.2. Crystallization of Membrane Proteins	7
1.3. Detergent Selection	8
1.4. Antibody Approach	9
1.5. Where Do We Start?	9
1.6. Conclusions	10

Part II. PRINCIPLES AND TECHNIQUES IN MEMBRANE PROTEIN CRYSTALLIZATION 13

2. Solubilizing Detergents for Membrane Proteins 15

Melvin H. Keyes, Don N. Gray, Ken E. Kreh, and Charles R. Sanders

2.1. Introduction/Background	17
2.2. Need for Detergents	18
2.3. Requirement for Many and Varied Detergents	19
2.3.1. Different for Each Membrane Protein	19
2.3.2. Sugar-Based Detergents	19
2.3.3. Lipid-Like Detergents	24
2.3.4. Cyclohexyl Alkyl Detergents	26
2.3.5. Polyoxyethylene Detergents	27
2.4. Biochemical Testing of CYFOS TM , FOS-MEA TM , and FOS-CHOLINE [®] Detergents	27
2.4.1. Lipid Solubilization	27
2.4.2. Reconstitutive Refolding of a Misfolded Membrane Protein Facilitated by FOS-CHOLINE [®] , CYFOS TM , and FOS-MEA TM Detergents	28
2.5. Strategies and Criteria for Detergent Selection	30
2.5.1. Extraction	30
2.5.2. Crystallization and Molecular Studies	31
2.5.3. Practical Observation Regarding Detergent Solubility and Purity	31

2.5.4. Price versus Risk 33

3. Crystallization of Membrane Proteins in Lipidic Cubic Phases 39

Ehud M. Landau

3.1. Introduction 41

3.2. Crystallization in Lipidic Cubic Phases 42

 3.2.1. Preparing Cubic Phases 42

 3.2.2. Preparing Cubic Phases for Crystallization of Membrane Proteins 45

 3.2.3. Crystal Handling 50

3.3. Summary 50

4. Practical Aspects of Membrane Protein Crystallization in Lipidic Cubic Phases 57

Peter Nollert

4.1. Operation Principle and General Considerations 59

4.2. Preparation of LCP Crystallization Setups 60

 4.2.1. Glass Tubes 60

 4.2.2. Preparing the LCP in a Glass Tube 61

 4.2.3. Mixing and Dispensing Using Syringes 61

 4.2.4. Variable Parameters in LCP Crystallization Screens 63

4.3. Inspection of Crystals Grown in LCP 65

 4.3.1. General Remarks 65

 4.3.2. Instrumentation for Inspection 66

 4.3.3. Colored Protein Crystals 67

 4.3.4. Non-Colored Protein Crystals 68

 4.3.5. Potential Problem Areas, False Positives 69

 4.3.6. Guidelines for Observing LCP Crystallization Setups 70

5. Antibody Fragment-Mediated Crystallization of Integral Membrane Proteins: A Review 73

Christian Ostermeier

5.1. Introduction 75

5.2. Membrane Proteins 76

5.3. How can Membrane Proteins Form Well-Ordered Type II Crystals? 80

5.4. The Critical Role of the Polar Surface 81

5.5. Enlargement of the Polar Surface Area 81

5.6. Antibody Fragments 82

5.7. Production of FV Fragments 83

 5.7.1. Cloning 83

 5.7.2. Expression 83

5.8. FV Fragments as a Tool for Isolation and Crystallization 84

 5.8.1. FV Fragments as a Tool for Purification 84

 5.8.2. Conformational Epitopes 84

 5.8.3. FV Fragments as a Tool for Crystallization: They Really Work! 85

5.9. Alternatives to FV Fragments 86

6. Generating Antibody Fragments for Structural Studies: A Guide 89*Bernadette Byrne and So Iwata*

6.1. Introduction	91
6.2. Hybridoma Cell Line Production	92
6.3. Procedure	93
6.3.1. Immunization	93
6.3.2. Fusion	93
6.3.3. Screening of Hybridoma Cell Lines	94
6.4. Fab Fragments	95
6.5. Fv Fragments	97
6.6. Summary	98

**7. Crystallization of Bacterial Outer Membrane Proteins from Detergent Solutions:
Porin as a Model 101***Gabriele Rummel and Jurg P. Rosenbusch*

7.1. Stating the Problems	103
7.2. Considering Potential Remedies	104
7.3. Porin as a Model	106
7.4. Criteria for Rational Detergent Selection	106
7.5. Polymorphism of Detergents and Membrane Proteins in Solution: The Significance of the Phase Diagram	114
7.6. Crystallization of Porin and Other Bacterial Outer Membrane Proteins: A Brief Survey	118
7.7. Monodispersity of Building Blocks versus Micelle Dynamics: Lessons from Protein-Detergent Contacts in Crystals	118
7.8. Porin as a Paradigm: Is It a Valid Model?	121
7.9. Conclusions and Perspectives	123

8. Crystallization of Membrane Proteins in Oils 131*Naomi E. Chayen*

8.1. Introduction	133
8.2. The Automated Microbatch Technique	134
8.3. Examples of Membrane Proteins Crystallized under Oil	134
8.4. Advantages of Crystallization in Oils	135
8.5. Harvesting the Crystals	136
8.6. Summary and Future Developments	137
8.7. Exercises	137
8.7.1. Materials Required	137
8.7.2. Screening Procedure	138
8.7.3. Optimization	138

**PART III. EXAMPLES OF SUCCESSFUL CRYSTALLIZATION OF
MEMBRANE PROTEINS 141****A. Complexes in Photosynthesis 143****9. Crystallization of Photosystem I 145***Petra Fromme*

9.1. Introduction	147
9.2. Results and Discussion	148
9.3. Biological and Biochemical Parameters	149
9.3.1. The Organism	149
9.3.2. The Physiological Status of the Organism	150
9.3.3. The Quaternary Structure and the Subunit Composition	151
9.3.4. Proteolytic Digestion and Ageing of the Protein	153
9.3.5. Binding of "Ligands"	153
9.4. Physical and Chemical Parameters	155
9.4.1. Ionic Strength	155
9.4.2. Nature of Salts	159
9.4.3. pH	160
9.4.4. Temperature	161
9.4.5. Detergent	162
9.4.6. Crystallization Agents	166
9.4.7. Diffusion and Convection (Gravity/Microgravity)	167
9.5. Nucleation and Seeding Techniques—Micro- and Macroseeding	168

B. Respiratory Complexes **175****10. Crystallization of Wolinella succinogenes Quinol:Fumarate Reductase in Three Crystal Forms** **177***C. Roy D. Lancaster*

10.1. Introduction	179
10.2. Preparatory Steps	181
10.2.1. Growth of Wolinella succinogenes	181
10.2.2. Isolation of Quinol:Fumarate Reductase	182
10.3. Crystallization of Quinol:Fumarate Reductase	183
10.4. Characterization of the Crystals	185
10.5. Structure Determination	187

11. Crystallization of the Respiratory Complex Formate Dehydrogenase-N from *Escherichia coli* **193***Mika Jormakka*

11.1. Introduction	195
11.2. Expression and Purification of Fdn-N	196
11.3. Crystallization and X-ray Data Collection of Fdn-N	196
11.4. Structure Determination of Native Fdh-N	198
11.5. Determination of the Quinone Binding Site	200

12. Crystallization of the Cytochrome bc₁ Complex **203***Li-Shan Huang, David Cobessi, and Edward A. Berry*

12.1. About the Protein	205
12.2. Purification Procedures	206
12.3. Early bc ₁ and b6f Crystallization Studies	207
12.4. Three-Dimensional Crystals of Mitochondrial Cytochrome bc ₁	208
12.5. Large Tetragonal Crystals from the Yu Preparation	209

12.6. Monoclinic, Tetragonal, and Hexagonal Crystals from the Rieske Preparation	210
12.7. Crystallization of the bc1 Complex from Various Vertebrate Organisms in Berkeley	211
12.8. Improved Beef bc1 Crystals from the Jap Group at Berkeley and the Iwata Group in Uppsala	213
12.9. Higher Resolution Crystals of the Fungal bc1 Complex from MPI-Frankfurt	213
12.10. Unpublished Observations from the Berkeley Group	214
12.10.1. Needle Crystals of the Bovine bc1 Complex	214
12.10.2. Hexagonal Bipyramid Crystals ($P6_522$)	215
12.10.3. Conditions for Growth	216
12.10.4. Hexagonal Bipyramid Crystals without Seeding	217
12.10.5. Precrystallization	218
12.10.6. Rabbit Cytochrome bc1 Crystals	218
12.10.7. Orthorhombic Chicken bc1 Crystals	219
12.11. Conclusions	221
13. Crystallization of Cytochrome bo_3 Ubiquinol Oxidase from <i>E.coli</i>	227
<i>Jeff Abramson, Bernadette Byrne, and So Iwata</i>	
13.1. Purification, Crystallization, and X-Ray Data Collection for Cytochrome bo_3	229
13.1.1. Crystal Form 1: Wild-Type Cytochrome bo_3	229
13.1.2. Crystal Form 2: Cytochrome bo_3 -Fusion Complex	233
13.2. Structure Determination of Cytochrome bo_3	235
13.2.1. Structure Determination for Crystal Form 1: Wild-Type Cytochrome bo_3	235
13.2.2. Structure Determination and Interpretation for Crystal Form 2: Cytochrome bo_3 -Protein Z Fusion	236
C. Channel and Receptor	239
14. Crystallization and Structure Determination of MscL, a Gated Prokaryotic Mechanosensitive Channel	241
<i>R. H. Spencer, G. Chang, R. B. Bass, and D. C. Rees</i>	
14.1. Introduction	243
14.2. Target Identification	244
14.3. Protein Expression	245
14.4. Protein Purification	246
14.5. Protein Crystallization	248
14.6. Crystallographic Analysis	248
14.7. Conclusions	250
15. Crystallization of Bovine Rhodopsin, a G Protein-Coupled Receptor	253
<i>Tetsuji Okada</i>	
15.1. Introduction	255
15.2. Purification Procedure	256

15.2.1. Membrane Preparation	256
15.2.2. Selective Solubilization	257
15.3. Detail of the Crystallization Experiments	258
15.3.1. Protocol	258
15.3.2. The Case History	259
15.4. Characterization of the Crystals	260
15.5. Summary of the Structural Determination	261
15.6. Remarks	261

D. Outer Membrane Proteins **263****16. Crystallization of Phopholipase A in Two Biological Oligomerization States**
265*Arjan Snijder, Thomas Barends, and Bauke W. Dijkstra*

16.1. Introduction	267
16.2. Purification	268
16.3. Crystallization	269
16.3.1. Monomeric OMPLA	269
16.3.2. Crystal Packing of Monomeric OMPLA	271
16.3.3. Dimeric OMPLA	273
16.3.4. Crystallization under Oil	274
16.3.5. Crystal Packing of Dimeric OMPLA	279
16.4. Conclusion and General Message	276

Part IV. CRYSTALLIZATION INFORMATICS OF MEMBRANE PROTEINS
279**17. Crystallization Informatics of Membrane Proteins** **281***So Iwata*

17.1. Introduction	283
17.2. Design of a Kit for Membrane Protein Crystallization	284
17.2.1. Selection of Precipitants	286
17.2.2. Selection of Buffers	288
17.2.3. Selection of Salts	288
17.2.4. Design of the Screening Kit	291
17.3. User Instruction of the Kit	291
17.3.1. Protein Concentration	292
17.3.2. Selection of Detergent	292
17.3.3. Sample Buffer	293
17.3.4. Temperature	294
17.3.5. Additives	294
17.3.6. Observation	294
17.4. Summary	294

APPENDICES **299****Questionnaire** **302**

A1. Porin (OmpF) from <i>Escherichia coli</i>	304
A2. Porin from <i>Rhodobacter capsulatus</i>	305

A3. Porin from <i>Rhodopseudomonas blastica</i>	306
A4. Porin from <i>Paracoccus denitrificans</i>	307
A5. Porin Omp32 from <i>Comamonas acidovorans</i>	308
A6. Phosphoporin from <i>Escherichia coli</i>	309
A7. Osmoporin from <i>Escherichia coli</i>	310
A8. Osmoporin from <i>Klebsiella pneumoniae</i>	311
A9. Maltoporin from <i>Escherichia coli</i>	312
A10. Maltoporin from <i>Salmonella typhimurium</i>	313
A11. Sucroseporin from <i>Salmonella typhimurium</i>	314
A12. Exoporin from <i>Escherichia coli</i>	315
A13. Siderophore translocator (FhuA) from <i>Escherichia coli</i>	316
A14. Siderophore translocator (FhuA) from <i>Escherichia coli</i>	317
A15. Siderophore translocator (FepA) from <i>Escherichia coli</i>	318
A16. Truncated transmembrane domain of OmpA-protein from <i>Escherichia coli</i>	319
A17. OmpX-protein from <i>Escherichia coli</i>	320
A18. Phospholipase A from <i>Escherichia coli</i> outer membrane, monomer	321
A19. Phospholipase A from <i>Escherichia coli</i> outer membrane, dimer	322
A20. MscL from <i>Escherichia coli</i>	323
A21. Photosystem I	324
A22. Cytochrome c oxidase from <i>Paracoccus denitrificans</i>	325
A23. Cytochrome bc ₁ complex, P6 ₅ form	326
A24. Cytochrome bc ₁ complex, P6 ₅ 22 form	327
A25. Ubiquinol oxidase from <i>Escherichia coli</i>	328
A26. Formate dehydrogenase from <i>Escherichia coli</i>	329
A27. Succinate dehydrogenase from <i>Escherichia coli</i>	330
A28. Cytochrome c oxidase from <i>Rhodobacter sphaeroides</i>	331
A29. Halorhodopsin	332
A30. Bacteriorhodopsin	333
Index	339