CONTENTS

Contributors xi
Preface xv
Volumes in Series xvii

1. Analyses of Subnanometer Resolution Cryo-EM Density Maps 1
   Matthew L. Baker, Mariah R. Baker, Corey F. Hryc, and Frank DiMaio
   1. Introduction 2
   2. Features in a Subnanometer Resolution Density Map 3
   3. Tools: Analyzing a Subnanometer Resolution Density Map 4
   4. Protocol: From Density Map to Atomic Model 9
   5. Case Study: Mm-cpn 21
   6. Discussion 24
   Acknowledgments 25
   References 25

2. Methods for Segmentation and Interpretation of Electron Tomographic Reconstructions 31
   Niels Volkmann
   1. Introduction 32
   2. Noise Reduction 35
   3. Segmentation 36
   4. Detection and Mapping of Macromolecular Assemblies 38
   5. Classification and Averaging 39
   6. Validation 40
   Acknowledgments 41
   References 42

3. Integration of Cryo-EM with Atomic and Protein–Protein Interaction Data 47
   Friedrich Förster and Elizabeth Villa
   1. Introduction 48
   2. The Problem of Placing Assembly Subunits into Cryo-EM Maps 49
   3. Structure Prediction of Subunits 50
4. **Protein–Protein Interaction Data**

5. **Model Building of a Complex Using Cryo-EM and Additional Data**

6. **Refinement of Atomic Models Using High-Resolution Maps**

7. **Conclusion and Outlook**

References

<table>
<thead>
<tr>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Unified Data Resource for Cryo-EM</td>
</tr>
<tr>
<td>Catherine L. Lawson</td>
</tr>
<tr>
<td>1. Introduction</td>
</tr>
<tr>
<td>2. EM Structural Data Archives</td>
</tr>
<tr>
<td>3. Deposition and Content</td>
</tr>
<tr>
<td>4. Access</td>
</tr>
<tr>
<td>5. Examples of Use</td>
</tr>
<tr>
<td>6. Future Prospects</td>
</tr>
<tr>
<td>7. Guide to Deposition of EM Structural Data</td>
</tr>
<tr>
<td>Acknowledgments</td>
</tr>
<tr>
<td>References</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Electron Crystallography and Aquaporins</td>
</tr>
<tr>
<td>Andreas D. Schenk, Richard K. Hite, Andreas Engel, Yoshinori Fujiyoshi, and Thomas Walz</td>
</tr>
<tr>
<td>1. Electron Crystallography</td>
</tr>
<tr>
<td>2. Contributions of Electron Crystallography to the Structural Biology of Aquaporins</td>
</tr>
<tr>
<td>3. Contributions of Structural Studies on Aquaporins to Advances in Electron Crystallography</td>
</tr>
<tr>
<td>Acknowledgments</td>
</tr>
<tr>
<td>References</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Cryoelectron Microscopy Applications in the Study of Tubulin Structure, Microtubule Architecture, Dynamics and Assemblies, and Interaction of Microtubules with Motors</td>
</tr>
<tr>
<td>Kenneth H. Downing and Eva Nogales</td>
</tr>
<tr>
<td>1. Introduction: The Role of Electron Microscopy in Tubulin Studies</td>
</tr>
<tr>
<td>2. Tubulin Studies by Electron Crystallography</td>
</tr>
<tr>
<td>3. Microtubule Structure</td>
</tr>
<tr>
<td>4. Structure of Microtubule Assembly/Disassembly Intermediates</td>
</tr>
<tr>
<td>5. Mechanism of Kinesin Movement Along Microtubule</td>
</tr>
<tr>
<td>6. Drug Binding Studied by Diffraction and Modeling</td>
</tr>
<tr>
<td>7. Tomography for Larger Structures</td>
</tr>
<tr>
<td>8. Conclusion</td>
</tr>
</tbody>
</table>
Acknowledgments
References

7. Helical Crystallization of Two Example Membrane Proteins: MsbA and the Ca^{2+}-ATPase
John Paul Glaves, Lauren Fisher, Andrew Ward, and Howard S. Young
1. Helical Crystallization of the Bacterial Integral Membrane Protein, MsbA
2. Helical Crystallization of the Sarcoplasmic Reticulum Ca^{2+}-ATPase
3. Discussion
4. Conclusions
Acknowledgments
References

8. Multiparticle Cryo-EM of Ribosomes
Justus Loerke, Jan Giesebrecht, and Christian M. T. Spahn
1. Introduction
2. Dealing with Heterogeneity: 3D Sorting
3. Subnanometer Multiparticle Cryo-EM of Ribosomes in Practice
4. Interpretation
5. Conclusions
Acknowledgments
References

9. Single-Particle Electron Microscopy of Animal Fatty Acid Synthase: Describing Macromolecular Rearrangements that Enable Catalysis
Edward J. Brignole and Francisco Asturias
1. Electron Microscopy and the Next Frontier in Structural Biology
2. The Catalytic Cycle of FAS Requires Conformational Changes
3. Methods and Rationale Employed in the Conformational Analysis of FAS
4. EM and FAS: A Versatile Tool for a Flexible Macromolecule
Acknowledgments
References

10. Tomography of Actin Cytoskeletal Networks
Dorit Hanein
1. Introduction
2. Testing the Lamella Hypothesis
3. Challenges  
Acknowledgments  
References  

206  
212  
212  

11. Visual Proteomics  
Friedrich Förster, Bong-Gyoon Han, and Martin Beck  

1. Introduction  
2. Data Acquisition  
3. Templates  
4. Template Matching  
5. Assessment of Performance  
6. The Spatial Proteome of \textit{L. interrogans}  
7. Outlook  
Acknowledgments  
References  

215  
216  
217  
221  
227  
232  
237  
241  
241  
242  

12. Cryoelectron Tomography of Eukaryotic Cells  
Asaf Mader, Nadav Elad, and Ohad Medalia  

1. Introduction  
2. Specimen Preparation  
3. Relying on Correlative Light and Electron Microscope for Cellular Structural Study of Eukaryotic Cells  
4. Cryoelectron Tomography of Cytoskeleton-Driven Processes  
5. Cryotomography of Midbodies  
6. Structural Analysis of the Nuclear Pore Complex by Cryo-ET  
7. Concluding Remarks  
Acknowledgments  
References  

245  
246  
248  
252  
254  
256  
257  
260  
260  
260  

13. 3D Visualization of HIV Virions by Cryoelectron Tomography  
Jun Liu, Elizabeth R. Wright, and Hanspeter Winkler  

1. Introduction  
2. Cryoelectron Tomography  
3. 3D Visualization of Intact HIV Virion  
4. Conclusions and Perspective  
Acknowledgments  
References  

267  
268  
270  
278  
286  
286  
286  
286  
286  
286


1. Introduction 292
2. Specimen Preparation 295
3. Autoloading and Robotic Screening 300
4. Microscopy 304
5. Image Processing 311
6. Assessment and Integration 322
7. The Future of Automation 327
Acknowledgments 330
References 330

Author Index 339
Subject Index 355