Preface

This volume is aimed at biological scientists intending to gain structural information on membrane proteins or soluble proteins by electron crystallography. Membrane proteins may be reconstituted and induced to form two-dimensional (2D) crystals in a near-native lipid environment. Soluble proteins may also be induced to form ordered 2D arrays. From such 2D crystals, both images and electron diffraction patterns can be acquired with an electron microscope. The basic principle of electron crystallography is to calculate a 3D density map by combining the amplitudes obtained from electron diffraction patterns with the experimental phases calculated from images. The technology for studying membrane proteins is very well developed and has produced a number of atomic models of membrane proteins in a lipid environment. The book is focused on comprehensive experimental protocols, and its chapters cover the entire range of techniques used in electron crystallography, from protein sample preparation, 2D crystallization, and screening in negative stain over electron cryo-cryomicroscopy (cryo-EM) and data processing to modeling of conformational changes. The reader will be guided through each step, including sample preparation and initial low-resolution studies, which require only a minimum of equipment and in-house expertise, to an overview of planning and using a state-of-the-art cryo-EM facility for high-resolution data collection and processing. Additional chapters provide perspective on past, present, and future challenges as well as complementary methods.

It is our hope that this book will guide both laboratories new to the methods as well as state-of-the-art facilities in the nontrivial techniques used in electron crystallography. Protocols will be applicable to laboratories wishing to gain initial structural information on, for example, the number of subunits on a protein, over secondary structural features to atomic resolution information with the aim of structure–function studies.

In addition to biological scientists, a considerable number of physicists have moved to apply their skills to biological problems. This book will provide valuable guidelines for understanding the differences encountered when working with beam-sensitive organic samples from specimen preparation, handling, and analysis to low-dose techniques.

The introduction (Chapter 1) covers the importance of the method to structural biology and provides a historical overview of the development of cryo-EM and electron crystallography in terms of both advances in methods development as well as biological applications and findings. At the foundation of all structural studies lies a sample that is properly purified in large-enough quantities. Furthermore, the structural and functional integrity needs to be maintained, which still poses a challenge for membrane proteins. Chapter 2 describes protocols for membrane protein overexpression and purification based on three challenging examples. Following the biochemical sample preparation, the next step is to induce 2D crystallization, which is most commonly achieved through dialysis and described in Chapter 3. Here, considerations important to strategy as well as basic protocols are discussed. Chapter 4 describes the method of 2D crystallization by the monolayer technique, which provides a valuable extension of electron crystallography to soluble proteins. Chapter 5

then focuses on the screening by transmission electron microscopy of negatively stained samples, essential to identifying and optimizing 2D crystallization conditions. Once large and well-ordered 2D crystals are produced, data collection by Cryo-EM is the next step (Chapter 6). For this, the specimen needs to be frozen in a thin layer of vitreous buffer under the most favorable circumstances (Chapter 7) to collect the best quality image and electron diffraction data. Careful image data collection (Chapter 8) is absolutely critical to obtaining the highest resolution information possible and plays an equal role in the number of useful images collected, controlling the timeframe of the project significantly. Chapter 9 outlines detailed protocols for collecting electron diffraction patterns. Next is the extraction of the structural information from the cryo-EM data by processing. Image processing is described in Chapters 10 and 11, providing many critical details to the understanding of the individual steps. As for any method in structural biology, data obtained needs to be comprehensively and meticulously assessed, showing the validity of the model of the structure (Chapter 12). Once a model is available, structures may be modeled in terms of higher resolution and conformational changes (Chapter 13). Comparison of structures of related proteins and docking, allow for further analysis and interpretation on the structural and functional level. One of the newest methodological advances in terms of phasing electron diffraction data by molecular replacement is described in Chapter 14.

In addition to protocols for these essential steps of electron crystallography, the book also covers a number of recent technological development efforts, particularly the technology of automation. Such technological advances are critical for the future of electron crystallography. Chapter 15 describes applying automated methods to 2D crystallization and a promising cyclodextrin protocol as well as several additional, detailed procedures that give insights adding to and complementing Chapter 3. Chapters 16 and 17 outline automation of grid handling and imaging at the electron microscope, and Chapter 18 provides guidelines on the automation of image processing.

The strength of electron crystallography is always related to the instrumentation. A variety of set-ups of minimal to state-of-the-art cryo-EM facilities are available and can be arranged, depending on needs, funding, and space. Chapter 19 gives guidance on selection and maintenance of all critical instrumentation. Chapter 20 provides a detailed overview of past and possible future developments of instrumentation benefiting electron crystallography.

Further chapters describe methods that may be used on their own or in conjunction with electron crystallography. Tubular crystals and helical arrays may be obtained by methods similar to 2D crystallization and provide the advantage of not having a missing cone as well as that images of tilted samples are not necessary. An overview of the handling of such samples is given in Chapter 21. Single particle electron microscopy (Chapter 22) is a sister cryo-EM technique to electron crystallography and has contributed a wealth of structural information on both soluble and membrane proteins. Electron tomography can reveal essential details on ordered structures that might not be accessible by electron crystallography, due to, e.g., paracrystalline samples such as described in Chapter 23. Atomic force microscopy provides structural information on membrane proteins within 2D crystals that is highly complementary to electron crystallography and can reveal a wealth of additional information (Chapter 24). X-ray crystallography and nuclear magnetic resonance spectroscopy (NMR) are traditional structural methods that are continuing to break new ground and often go hand in hand with the cryo-EM methods. X-ray crystallography and NMR are covered in Chapters 25 and 26, respectively. Chapter 27 outlines just a few examples of the wealth of structural and functional information revealed by electron crystallography of

soluble and membrane proteins. Chapter 28 provides a comprehensive description of protocols to induce 2D crystallization of the two soluble protein complexes, carboxysome shell proteins, and HIV CA. Chapter 29 describes the processing of electron diffraction data.

While an impressive amount of methods development has been accomplished and automation is in the process of breaking further ground for electron crystallography, Chapter 30 introduces and highlights the great potential of electron crystallography in terms of biological information that cannot be revealed by other methods.

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