PREFACE

In this, the fifty-fourth year of *Methods in Enzymology*, we celebrate the discovery and initial characterization of thousands of individual enzymes, the sequencing of hundreds of whole genomes, and the structure determination of tens of thousands of proteins. In this context, the architectures of multienyzme/multiprotein complexes and their arrangement within cells have now come to the fore. A uniquely powerful method in this field is electron cryo-microscopy (cryo-EM), which in its broadest sense, is all those techniques that image cold samples in the electron microscope. Cryo-EM allows individual enzymes and proteins, macromolecular complexes, assemblies, cells, and even tissues to be observed in a "frozen-hydrated," near-native state free from the artifacts of fixation, dehydration, plastic-embedding, or staining typically used in traditional forms of EM (Chapter 3, Vol. 481). This series of volumes is therefore dedicated to a description of the instruments, samples, protocols, and analyses that belong to the growing field of cryo-EM.

The material could have been organized well by two schemes. The first is by the symmetry of the sample. Because the fundamental limitation in cryo-EM is radiation damage (Chapter 15, Vol. 481), a defining characteristic of each method is whether and how low-dose images of identical copies of the specimen can be averaged. In the most favorable case, large numbers of identical copies of the specimen of interest, like a single protein, can be purified and crystallized within thin "two-dimensional" crystals (Chapter 1, Vol. 481). In this case, truly *atomic* resolution reconstructions have been obtained through averaging very low dose images of millions of copies of the specimen (Chapter 11, Vol. 481; Chapter 4, Vol. 482; Chapters 5 and 6, Vol. 483). The next most favorable case is helical crystals, which present a range of views of the specimen within a single image (Chapter 2, Vol. 481 and Chapter 7, Vol. 483) and can also deliver atomically interpretable reconstructions, although through quite different data collection protocols and reconstruction mathematics (Chapters 5 and 6, Vol. 482). At an intermediate level of (60-fold) symmetry, icosahedral viruses have their own set of optimal imaging and reconstruction protocols, and are just now also reaching atomic interpretability (Chapters 7 and 14, Vol. 482). Less symmetric particles, such as many multienyzme/multiprotein complexes, invite yet another set of challenges and methods (Chapters 3, 5, and 6, Vol. 481; Chapters 8–10, Vol. 482). Many are conformationally heterogeneous, requiring that images of different particles be first classified and then averaged

(Chapters 10 and 12, Vol. 482; Chapters 8 and 9, Vol. 483). Heterogeneity and the precision to which these images can be aligned have limited most such reconstructions to "sub-nanometer" resolution, where the folds of proteins are clear but not much more (Chapter 1, Vol. 483). Finally, the most challenging samples are those which are simply unique (Chapter 8, Vol. 481), eliminating any chance of improving the clarity of reconstructions through averaging. For these, tomographic methods are required (Chapter 12, Vol. 481; Chapter 13, Vol. 482), and only nanometer resolutions can be obtained (Chapters 10–13, Vol. 483).

But instead of organizing topics according to symmetry, following a wonderful historical perspective by David DeRosier (Historical Perspective, Vol. 481), I chose to order the topics in experimental sequence: Sample preparation and data collection/microscopy (Vol. 481); 3-D reconstruction (Vol. 482); and analyses and interpretation, including case studies (Vol. 483). This organization emphasizes how the relatedness of the mathematics (Chapter 1, Vol. 482), instrumentation (Chapters 10 and 14, Vol. 482), and methods (Chapter 15, Vol. 482; Chapter 9, Vol. 481) underlying all cryo-EM approaches allows practitioners to easily move between them. It further highlights how in a growing number of recent cases, the methods are being mixed (Chapter 13, Vol. 481), for instance, through the application of "single particle-like" approaches to "unbend" and average 2-D and helical crystals (Chapter 6, Vol. 482), but also average subvolumes within tomograms. Moreover, different samples are always more-or-less well-behaved, so the actual resolution achieved may be less than theoretically possible for a particular symmetry, or to the opposite effect; extensively known constraints may allow a more specific interpretation than usual for a given resolution (Chapters 2–4 and 6, Vol. 483). Nevertheless, within each section, the articles are ordered as much as possible according to the symmetry of the sample as described above (i.e. methods for preparing samples proceed from 2-D and helical crystals to sectioning of high-pressure-frozen tissues; Chapter 8, Vol. 481). The cryo-EM beginner with a new sample must then first recognize its symmetry and then identify the relevant chapters within each volume.

As a final note, our field has not yet reached a consensus on the placement of the prefix "cryo" and other details of the names of cryo-EM techniques. Thus, "cryo-electron microscopy" (CEM), "electron cryo-microscopy" (ECM), and "cryo-EM" should all be considered synonyms here. Likewise, "single particle reconstruction" (SPR) and "single particle analysis" (SPA) refer to a single technique, as do "cryo-electron tomography" (CET), "electron cryo-tomography" (ECT), and cryo-electron microscope tomography (cEMT).

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