THE PROTEIN SOCIETY

EDITORIAL

A snapshot of cryo-EM

The recent explosion of electron cryo-microscopy (cryo-EM) has dramatically reshaped the landscape of structural biology by providing stunningly detailed snapshots of biological assemblies at multiple complexities and scales. Using cryo-EM we can now calculate near-atomic resolution structures from relatively few thousand images of macromolecular complexes with sizes ranging from ~150 kDa to several MDa. Crucially, powerful image classification algorithms can facilitate data sorting to not only enable the determination of 3D maps with higher resolution, but also to explore the multiple conformational and compositional states that are often present in a single sample. This simultaneous characterization of both structure and dynamics creates unprecedented opportunities to illuminate the inner workings of macromolecular complexes with a level of detail we have never seen before. In conjunction with electron cryo-tomography (cryo-ET), which employs tomographic imaging to obtain the overall architecture of large cellular organizations (and requires its own dedicated Special Issue), cryo-EM can provide a continuous structural framework for understanding cell biology all the way to the atomic level. This aspect will be key in our efforts to visualize more complex organizations and interactions in native-like environments with multiple components present.

The spectacular results obtained by cryo-EM have spurred additional efforts related to the application, including improvements in cryo-sample preparation, data collection and processing workflows, and the modeling and refinement of structures in cryo-EM 3D maps. The extensive crosstalk between biophysicists, computational biologists, biochemists, and cell biologists accelerates method development and brings the experience and tools developed in other disciplines to cryo-EM. Further fine-tuning and standardization of various components in the workflow will enable cryo-EM to increase both the quality and throughput of data it can provide, and also become even more accessible to the non-expert user.

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This Special Issue aims to provide a glimpse into the current state of cryo-EM as a standalone field and in relation to other methodologies, exploring current challenges as well as new directions. The issue includes both reviews and original research papers, giving an overview of distinct aspects of the methodology and its application in addressing biological questions. Besides describing cryo-EM studies on biologically important systems, this collection of papers aims to illustrate the breadth of ways that very useful information can be extracted at various resolutions, the current challenges in the field, and some of the new directions opening up in an effort to fully explore cryo-EM capabilities. In addition, this issue serves as a snapshot of the developing social network around cryo-EM, reflecting the interest from a diverse scientific community and a range of perspectives on the field as it moves forward.

Joachim Frank and co-workers contribute a review of the considerations and parameters that allowed them to obtain a 2.5-Å cryo-EM structure of ribosomes from *Trypanosoma cruzi*, a eukaryotic pathogen affecting many thousands of people worldwide [DOI: 10.1002/pro.3068]. The ribosomes of these parasites are promising antibiotic targets and thus high-resolution information is invaluable for the design of selective agents. The paper provides a methodological overview that can serve as a general guide to the application of single-particle cryo-EM on any macromolecular complex. Furthermore, the review highlights multiple aspects of instrumentation, data collection, and image processing that are crucial for near-atomic resolution structure determination.

The high resolution maps now attainable by cryo-EM pose new challenges, such as the need of solid procedures for the refinement of structural models according to the Coulomb potentials represented by the 3D density map. Currently, this is primarily accomplished with the application of tools developed for X-ray crystallography. Jimin Wang and Peter Moore caution on the differences between cryo-EM electric potential (EP) maps and electron density (ED) maps produced by X-ray crystallography [DOI: 10.1002/pro.3060]. EP maps include

negative features due to the presence of negative charges in the structure, such as carboxyl groups in amino acids or the phosphate backbone of nucleic acids. The authors examine such regions in published atomic resolution cryo-EM maps and compare them with simulated ED and EP maps. The results clearly show the effect of charge in the appearance of cryo-EM maps at high resolution, necessitating the development of the theoretical and experimental framework to fully interpret these data and facilitate appropriate structure modeling.

Cryo-EM is an ideal platform for the integration and modeling of data from other biophysical and biochemical methods. Hong-Wei Wang and co-workers discuss the complementarity of cryo-EM with X-ray crystallography [DOI: 10.1002/pro.3022]. Reliable docking of X-ray structures, even in low resolution cryo-EM maps, can provide invaluable information for the architecture and interaction surfaces within macromolecular complexes. The authors further describe how cryo-EM may be employed to provide intermediate resolution maps that can direct the design of constructs for atomic resolution structure determination of parts of the complex by X-ray crystallography. In addition, intermediate resolution cryo-EM maps can be successfully employed to provide the phases for diffraction-based techniques when the lack of homologous structures precludes the application of molecular replacement.

Even in the case of cryo-EM structures with overall high resolution, the identification of individual components in a large and complicated macromolecular assembly can be challenging in the absence of any prior high resolution structures. This is particularly the case for peripheral protein components that usually involve flexible parts or disordered regions, rendering density connectivity and backbone tracing a challenging and often unattainable task. Ning Gao and colleagues report on the modeling of five non-ribosomal proteins in complex with a pre-60S ribosome biogenesis intermediate from yeast [DOI: 10.1002/pro.3045]. The authors used a hybrid approach involving the combination of structure prediction, proximity mapping based on chemical crosslinking followed by mass spectrometry (CX-MS), and identification and modeling of the corresponding segment in the high-resolution cryo-EM map.

Melanie Ohi and co-workers provide an overview of key steps in single-particle cryo-EM analysis of macromolecular complexes with an emphasis on aspects of specimen preparation [DOI: 10.1002/pro.3054]. The authors discuss the extensive fine-tuning that may often be needed in the biochemical purification and specimen preparation for cryo-EM, especially for challenging macromolecular complexes characterized by inherent compositional and conformational heterogeneity. The paper provides many practical tips for visualizing unstable and dynamic complexes, including extracting information from low-resolution maps. The authors also illustrate the merits of a relatively simple and quick screening of the specimen by negative stain EM followed by 2D classification, a solid tool for the assessment of sample quality and overall characteristics.

Montserrat Samso provides a guide to the structure of the Ryanodine receptor, an important calcium release channel and membrane protein that has been studied by cryo-EM over many years [DOI: 10.1002/pro.3052]. RyR has favorable characteristics for single-particle cryo-EM, such as large size and symmetry, and has been pursued as a showcase specimen from early years of the technique. The review constitutes a valuable roadmap into the complex structure of this receptor, which includes several distinct binding sites for biological ligands affecting its function. The manuscript also provides an account for the gradual improvements in understanding receptor structure using a combination of cryo-EM and X-ray crystallography.

The value of intermediate resolution cryo-EM maps, especially when homologous structures of components are available, is illustrated in the research paper of Justin Kolmann and colleagues [DOI: 10.1002/pro.2979]. The group examined actin-like filaments composed of the protein MamK, an actin homologue that is necessary to the function of magnetotactic bacteria. Using iterative helical real space reconstruction routines the group obtained a 3D cryo-EM map of the MamK filament at 6.5-Å resolution, in which they refined a homology model for the MamK protomer. The final model reveals an unusual non-staggered double stranded arrangement of MamK protomers with large gaps between adjacent strands, providing valuable insights to the determinants of filament assembly and helical organization.

Along the same lines, David Stokes and co-workers prepared and imaged by cryo-EM helical tubular arrangements of Bor1p, a yeast boron transporter, within lipid bilayers. Bor1p, belongs to a family of proteins that plays an important role in pH regulation in animals [DOI: 10.1002/pro.3061]. After determining helical parameters, the authors applied a combination of single-particle analysis and helical reconstructions to obtain a ~6-Å cryo-EM structure of the transporter revealing the arrangement of transmembrane α-helices. The authors further applied molecular dynamics (MD) simulations to flexibly fit a homology model of Bor1p in the cryo-EM map. The results, besides providing insights into the transport mechanism, also reveal the interplay of cryo-EM with homology modeling and MD simulations to obtain structural information.

David Veesler and colleagues describe the various challenges they had to address in order to obtain the cryo-EM structure of a coronavirus transmembrane

glycoprotein S, a trimeric protein spike responsible for cell binding and membrane fusion in deadly viruses such as SARS and MERS [DOI: 10.1002/pro.3048]. The structure of these spikes has great biomedical interest given the potential of various strategies for intercepting viral fusion with host cells. The group's breakthrough in structure determination involved fine-tuning a number of factors, including successfully engineering stable ectodomain constructs, applying extensive 2D and 3D classification procedures to sort out particle heterogeneity, and employing advances in automated computational procedures to build and refine atomic models.

Huilin Li and co-workers present their efforts in extracting information on dynamic and unstable complexes involved in DNA replication [DOI: 10.1002/pro.3033]. The authors employed several approaches to capture EM images of such assemblies, including direct mixing of the associated components, which allowed them to identify complexes after 2D classification and averaging. The researchers also used various protein labeling approaches that are of great value for the relative localization of individual subunits in low resolution EM reconstructions. This paper illustrates how single-particle EM, including both cryo-EM and negative stain EM, provides a comprehensive visualization arsenal that can be integrated after tailored specimen preparation protocols to image challenging complexes.

But cryo-EM includes additional, currently more exotic tools for structure determination. Tamir Gonen and co-workers provide a review of key steps in the application of micro electron diffraction (MicroED), a method they recently developed and which relies on extremely small but well ordered 3D crystals of the macromolecular target [DOI: 10.1002/pro.2989]. Electron diffraction data from these nanocrystals can be recorded in the electron microscope while the specimen is rotated, resulting in series of diffraction patterns that can be processed with available X-ray crystallography software. MicroED has recently produced impressive results, especially in the case of α-synuclein and prion peptides, while suitable nano-crystals may be readily available in the process of crystallogenesis for X-ray diffraction experiments. As the technique is further tested and established we will obtain a more thorough understanding of its advantages and limitations as they apply to individual biological problems.

Finally, Hong-Wei Wang, Jianlin Lei, and Yigong Shi provide an account on the exciting biological cryo-EM landscape in China [DOI: 10.1002/pro.3018]. This contribution includes a historical perspective on the development of biological EM in the country and the scientific connections amongst Chinese researchers, many of whom are leaders in their field. China is investing unparalleled resources in upgrading academic facilities with state-of-the-art cryo-TEMs and recruiting well-trained scientists in various aspects of the application. The country has become a powerhouse for biological cryo-EM and will continue to have major contributions in structural biology.

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