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Review Article

Toward High-Resolution Cryo-Electron Microscopy: Technical Review on Microcrystal-Electron Diffraction

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Cryo-electron microscopy (cryo-EM) is arguably the most powerful tool used in structural biology. It is an important analytical technique that is used for gaining insight into the functional and molecular mechanisms of biomolecules involved in several physiological processes. Cryo-EM can be separated into the following three groups according to the analytical purposes and the features of the biological samples: cryo-electron tomography (cryo-ET), cryo-single-particle reconstruction, and cryo-electron crystallography. Cryo-tomography is a unique EM technique that is used to study intact biomolecular complexes within their original environments; it can provide mechanistic insights that are challenging for other EM-methods. However, the resolution of reconstructed three-dimensional (3D) models generated by cryo-ET is relatively low, while single-particle reconstruction can reproduce biomolecular structures having near-atomic resolution without the need for crystallization unless the samples are large (>200 kDa) and highly symmetrical. Cryo-electron crystallography is subdivided into the following two categories according to the types of samples: one category that deals with two-dimensional (2D) crystalline arrays and the other category that uses 3D crystals. These two categories of electron-crystallographic techniques use different diffraction data obtained from still diffraction and continuous-rotation diffraction. In this paper, we review crystal-based cryo-EM techniques and focus on the recently developed 3D electron-crystallographic technique called microcrystal-electron diffraction.

Key Words: Cryo-electron microscopy, Cryo-electron tomography, Single-particle analysis, Microcrystal-electron diffraction, High-resolution protein structure

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INTRODUCTION

Cryo-electron microscopy (cryo-EM) is arguably the most powerful and promising tool used in structural biology. It is an important analytical technique that is used for gaining insight into the functional and molecular mechanisms of biomolecules involved in several physiological processes (Dubochet, 2012; Frank, 2016; Henderson, 2004). Cryo-EM can be subdivided into the following three groups according to the analytic purpose and the features of the biological samples: cryo-electron tomography (cryo-ET), cryo-single-particle reconstruction, and cryo-electron crystallography (Asano et al., 2016; Dubochet, 2012; Frank, 2009; Henderson, 2004; Nannenga & Gonen, 2014). Cryo-ET is a unique EM technique that is used to study intact biomolecular complexes within their native environments; it can provide mechanistic insights that are challenging for other EM-methods (Asano et al., 2016). However, the resolution of reconstructed three-dimensional (3D) models generated by cryo-ET is low, while single-particle reconstruction can reproduce biomolecular structures having near-atomic resolution without the need for crystallization unless the samples are large (>200 kDa) and highly symmetrical (Frank, 2016). Cryo-electron crystallography is subdivided into the following two categories according to the types of samples: one category that deals with two-dimensional (2D) crystalline arrays (2D

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electron crystallography) and the other category that uses 3D crystals (3D electron crystallography). These two types of electron-crystallographic techniques use different diffraction data obtained from still diffraction and continuous-rotation diffraction (Nannenga & Gonen, 2016). In this paper, we review crystal-based cryo-EM techniques and focus on the recently developed 3D electron-crystallographic technique called microcrystal-electron diffraction (micro-ED).

MICROCRYSTAL-ELECTRON DIFFRACTION AND ITS FUTURE DIRECTION

Since the early 1940s, electron diffraction has been used to study the atomic structures of various materials and biological samples (Bendersky & Gayle, 2001). The basic principle used in electron crystallography is similar to that of X-ray crystallography, i.e., the crystalline biological samples scatter the incident beam to generate diffraction patterns that can be eventually used for the reconstruction. This technique has been developed over recent years by many groups by using small-sized and relatively dose-insensitive compounds (Nannenga & Gonen, 2016; Unwin, 2003; Unwin & Henderson, 1975). In electron crystallography, the crystals should be thinner than those used for X-ray crystallography as electrons have much stronger interactions with the atoms and molecules in the sample than the X-ray photons (Chung et al., 2017; Kimura et al., 1997). Owing to this reason, electron diffraction has been restricted to be used with 2D crystals that comprise 1~2 thin layers of a well-ordered 2D crystalline lattice. Over the past decades, 2D electron crystallography has been used successfully to obtain near-atomic-resolution structures of membrane proteins surrounded by lipid layers (Gonen et al., 2005; Hyun et al., 2011). However, most structures analyzed by this technique have only moderate resolution (4~10 A) as electrons have extremely high energies and cause large amounts of radiation damage to the crystals, thus resulting in the loss of structural information (Glaeser, 1971). To overcome this loss, the proposed concept has been developed for using hundreds of individual 3D crystals to generate the electron-diffraction patterns, which are then merged into a single dataset. However, until recently, this technique has been unsuccessful in determining the atomic structure of a sample. A study in 2013 introduced a new 3D electron-crystallography technique called micro-ED that provides a striking ability to determine high-resolution protein structures using 3D crystals (Shi et al., 2013).

Micro-ED is the most recently developed cryo-EM technique that allows the collection of high-quality electron-diffraction patterns from extremely small-sized 3D crystals with thicknesses ranging from 0.1 to 0.4 mm (Shi et al., 2013). Traditional 3D electron crystallography produces a single diffraction pattern for a given 3D protein crystal owing to the radiation-damage issue. Hence, each diffraction pattern has to be merged into a single dataset to reconstruct the 3D model; this causes an indexing problem owing to the lack of sufficient information in a single diffraction pattern (Chung et al., 2017). One previous study solved the indexing and merging problems by using a single nanocrystal under low electron-dose conditions (~10 e^-/A) to collect a complete diffraction dataset (Shi et al., 2013). This initial model of micro-ED used a series of still diffraction patterns (or snapshots) from a single crystal by rotating it through discrete angles among exposures. In this study, a special high-tilt cryo-holder was used to collect the complete tilt-series; it allows the sample to be tilted up to ±70° in 1° increments, thus resulting in the production of a 90° wedge of data from a single crystal. To overcome the "missing wedge" issue, three different crystals were used to produce three sets of diffraction data that were subsequently integrated and merged into one final dataset (Shi et al., 2013). However, the still diffraction method also has the drawback that the rotation must be paused during electron-beam exposure, which leads to a sample-drifting problem. Following this initial proof of the micro-EM principle, an improved data-collection protocol was developed, which is called the continuous-rotation method. This advanced micro-ED data-collection method continuously oscillates the crystal sample during electron-beam exposure, and the diffraction data is recorded using the high frame rate achieved with direct electron detectors (Nannenga et al., 2014b). This improves the data quality by finely sampling reciprocal space (Nannenga et al., 2014b). Because of the many advantages of the continuous-rotation data-collection mode, it has become the standard method for micro-ED, and several high-resolution structures have been generated using this technique (Nannenga et al., 2014a; Rodriguez et al., 2015; Yonekura et al., 2015).

With improvements in the detection hardware and image-processing software, the fundamental bottlenecks of 3D crystallography have largely been solved, and several meaningful studies have been reported using the advanced 3D cryo-EM technique. However, it is still necessary to improve some factors. One of the most interesting current areas of micro-ED is the development of experimental phasing methods because the phasing information that is crucial for crystallography currently cannot be measured from electron-diffraction patterns (Chung et al., 2017). To solve this issue, accurate integrated-diffraction intensities and the development of electron-scattering tables are required (Chung et al., 2017; Nannenga & Gonen, 2016; Scherer et al., 2014). Several strategies have been used to obtain the phase information, such as the use of heavy atoms for isomorphous replacements and changing the electron wavelengths, but unique and interesting approaches are required to solve the phasing problem (Shi et al., 2016). As the technique is

further improved and refined, micro-ED appears to be a highly promising technique in cryo-EM, and it seems likely to become a widely used tool for determining the structures of a handful of important biomolecular samples.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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