Make MicroED an efficient tool for ultrahigh-resolution structural determination

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Microcrystal electron diffraction (MicroED) is becoming a powerful tool in determining the crystal structures of biological macromolecules and small organic compounds. However, wide applications of this technique are still limited by the special requirement for radiation-tolerated movie-mode camera, and preparing high-quality sample is still a challenge. Herein, we developed a stage-camera synchronization scheme to minimize the hardware requirements and enable the use of the conventional electron cryo-microscope with single-frame CCD camera, which ensures not only the acquisition of ultrahigh-resolution diffraction data but also low cost in practice. This method was demonstrated by the structure determination of both peptide and small organic compounds at ultrahigh resolution up to ~0.60 angstrom with unambiguous assignment of nearly all hydrogen atoms. Importantly, this work also demonstrates the capability of the low-end 120kV microscope with a CCD camera in solving ultra-high resolution structures of both organic compound and biological macromolecules.

To develop a routinely-available method for high-quality MicroED sample, we used the focused ion beam (FIB) equipped on the scanning electron microscope (SEM) to mill a large crystal to thin lamella. The influences of the milling on the crystal lamella were observed and investigated, including radiation damage on the crystal surface during the FIB imaging, deformation of the thin crystal lamella, and variation in the diffraction intensities under electron radiation. These observations provide important information to optimize the FIB milling, and hence is important to obtain high-quality crystal samples for routine structure determination of protein crystals using the electron cryo-microscope.