Counting and filtering macromolecular electron diffraction data

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Cryogenic electron microscopy (cryo-EM) has transformed structural biology, enabling high-resolution structure determination of macromolecules through both imaging and diffraction. Among these methods, electron diffraction (MicroED) provides accurate structural models from nanocrystalline samples, typically of smaller biomolecules, that are difficult to solve by more conventional methods [1, 2]. A major challenge with any structural technique is sample preparation. Recent advances, particularly the use of focused ion beam (FIB) milling, now make a much broader range of target systems accessible for structure determination [3]. In parallel, data collection has improved significantly. Electron counting using hybrid pixel detectors [4] and direct electron detectors [5] enables fast and accurate recording of the diffracted intensities, while energy filtering eliminates noise from inelastic scattering, boosting the signal-to-noise ratio and enabling recovery of higher-resolution information [2, 6]. Together, these advances have increased the accuracy and reliability of electron diffraction experiments. Meanwhile, automation of both data acquisition and processing pipelines, including the use of high-throughput serial [7] and 4D-STEM approaches, is making the technique increasingly accessible and scalable, enabling time-resolved experiments, investigation of protein dynamics, and efficient ligand screening in drug discovery efforts. Together, these developments are transforming electron crystallography into a practical and versatile tool for routine structural analysis of nanocrystalline samples, extending its impact even beyond structural biology to address fundamental questions in materials science and chemistry.

1. B. L. Nannenga, D. Shi, A. G. W. Leslie, T. Gonen, High-resolution structure determination by continuous-rotation data collection in MicroED. *Nat Methods* **11**, 927–930 (2014).
2. K. Yonekura, K. Kato, M. Ogasawara, M. Tomita, C. Toyoshima, Electron crystallography of ultrathin 3D protein crystals: Atomic model with charges. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 3368–3373 (2015).
3. M. W. Martynowycz, A. Shiriaeva, M. T. B. Clabbers, W. J. Nicolas, S. J. Weaver, J. Hattne, T. Gonen, A robust approach for MicroED sample preparation of lipidic cubic phase embedded membrane protein crystals. *Nat Commun* **14**, 1–15 (2023).
4. M. T. B. Clabbers, E. Van Genderen, W. Wan, E. L. Wiegers, T. Gruene, J. P. Abrahams, Protein structure determination by electron diffraction using a single three-dimensional nanocrystal. *Acta Crystallogr D Struct Biol* **73**, 738–748 (2017).
5. M. W. Martynowycz, M. T. B. Clabbers, J. Hattne, T. Gonen, Ab initio phasing macromolecular structures using electron-counted MicroED data. *Nat Methods* **19**, 724–729 (2022).
6. M. T. B. Clabbers, J. Hattne, M. W. Martynowycz, T. Gonen, Energy filtering enables macromolecular MicroED data at sub-atomic resolution. *Nat Commun* **16**, 2247 (2025).
7. R. Bücker, P. Hogan-Lamarre, P. Mehrabi, E. C. Schulz, L. A. Bultema, Y. Gevorkov, W. Brehm, O. Yefanov, D. Oberthür, G. H. Kassier, R. J. Dwayne Miller, Serial protein crystallography in an electron microscope. *Nat Commun* **11**, 996 (2020).