Title:
Exploring Size and Resolution Limits with Conventional Cryo-EM

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Abstract:
Technical and methodological advances in single-particle cryo-electron microscopy (cryo-EM) have expanded the technique into a resolution regime that was previously only attainable by X-ray crystallography. Although single-particle cryo-EM has proven to be a useful technique for determining the structures of biomedically relevant molecules at near-atomic resolution, nearly 98% of the structures resolved to better than 4 Å resolution have been determined using 300 keV transmission electron microscopes (TEMs). We demonstrate that it is possible to obtain cryo-EM reconstructions of macromolecular complexes to better than 3 Å resolution using a 200 keV TEM. These structures are of sufficient quality to unambiguously assign amino acid rotameric conformations and identify ordered water molecules and bound co-factors, features previously thought only to be resolvable using TEMs operating at 300 keV. Determining the high-resolution structures of sub-100kDa complexes that have been recalcitrant to crystallization has also been a long-term goal of the cryo-EM community. Recently, the Volta Phase Plate has used to solve the structures of numerous small biological specimens, and it is now widely accepted that resolving small-sized samples is only possible with a phase plate. We show that it is possible to solve high-resolution structures of asymmetric, conformationally flexible specimens that are smaller than 100 kDa using conventional cryo-EM methodologies at 200 keV, without the need for a phase plate or energy filter.