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Spotlight

Shrinking a bottleneck in cryo-EM sample preparation

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In a recent issue of *Nature Methods*, Eluru et al.[1] describe a microfluidic approach that integrates protein purification with cryogenic electron microscopy (cryo-EM) grid preparation to enable high-resolution single-particle cryo-EM structure determination from minute amounts of starting material.

Cryogenic electron microscopy (cryo-EM) is, at its core, a remarkably sensitive technique. High-resolution structures can be obtained from as few as 10^3–10^5 particles, corresponding to femtogram quantities for many macromolecular complexes.[2] This sensitivity sets cryo-EM apart from macromolecular X-ray crystallography and nuclear magnetic resonance spectroscopy, where a single experiment typically requires on the order of 10^{13}–10^{17} molecules and where the preparative scale of biological starting material is dictated by these requirements. Yet, despite this fundamental difference, cryo-EM has largely inherited the preparative conventions of these techniques. Proteins are typically expressed and purified at scales many orders of magnitude larger than ultimately required for imaging, with only a vanishingly small fraction of the material ever contributing to a final three-dimensional reconstruction. While recent progress in sample dispensing and blot-free vitrification has reduced the volume required for grid preparation to the nanoliter regime,[3–5] the upstream requirement for large-scale purification has remained largely unchanged.

In a recent issue of *Nature Methods*, Eluru et al. present a microfluidic workflow that directly addresses this mismatch between analytical sensitivity and preparative scale. Their method, termed MISO (microisolation method), integrates affinity purification and size separation with cryo-EM grid preparation on a single microfluidic platform.[1] The core idea behind MISO is to treat protein purification as a process that should be tailored to the true material requirements of cryo-EM. This realization is not entirely new. Earlier pioneering work demonstrated that coupling of nanoliter dispensing onto cryo-EM grids with inline affinity capture enables direct cryo-EM analysis from sub-microliter cell lysates.[6] MISO advances this line of work by increasing the biochemical sophistication of on-chip purification while maintaining the drastically reduced scale. Rather than relying on a single affinity capture step, the platform integrates affinity purification with on-chip size-exclusion chromatography. This two-step workflow enables buffer exchange and removal of aggregates or low-molecular-weight contaminants prior to grid preparation, more closely mirroring conventional preparative protocols used for challenging cryo-EM targets. In this sense, MISO closes an important gap between earlier demonstrations of microfluidic purification and the multistep workflows commonly required in practice. Although the microfluidic columns necessarily operate at lower resolving power than analytical liquid chromatography, their performance is sufficient for the requirements of single-particle cryo-EM. High-resolution reconstructions were obtained from minute protein amounts isolated from a single bacterial colony or from small fractions of mammalian cell extracts, including integral membrane proteins that typically require multistep purification and reconstitution. The resulting density maps are comparable in quality to those derived from standard large-scale preparations of the same protein targets, underscoring that the integrated workflow is sufficiently robust for routine high-resolution cryo-EM analysis. At the same time, even with MISO, the amounts of material required remain several orders of magnitude above the theoretical minimum implied by single-particle cryo-EM. This naturally raises the question of whether further reductions in preparative scale are possible and what new constraints emerge when approaching that limit. Moreover, while the proof-of-principle experiments described by Eluru et al. rely on overexpressed proteins, the exciting prospect of extending this approach to native source purification will further tighten abundance of starting material. Addressing both challenges requires considering the physical consequences of operating at ever smaller dimensions.

The shrinking of workflows to increasingly smaller dimensions exposes physical effects that are largely negligible at the macroscale. In microfluidic systems such as MISO, the increased surface-to-volume ratio exacerbates non-specific protein adsorption, and maintaining a concentrated elution becomes increasingly sensitive to dispersion effects. As cryo-EM sample preparation is further reduced in scale, it approaches dimensions at which interfacial forces play an increasingly important role. In nanofluidic systems, defined here as having at least one dimension below a few hundred nanometers, the surface-to-volume ratio becomes sufficiently large such that electrostatic and confinement effects strongly influence solute behavior at the solid-liquid interfaces near device walls. In this regime, surface charges become dominant and biomolecule transport becomes strongly dependent on molecular charge, size, and conformation. Proteins and nucleic acids themselves carry heterogeneous surface charge, such that confinement can bias molecular mobility and partitioning near interfaces even in the absence of irreversible adsorption. These effects introduce new constraints, as uncontrolled surface interactions can compromise yield or structural integrity.

Nonetheless, recent advances alongside the development of MISO indicate that specific steps in the cryo-EM sample

preparation pipeline are beginning to approach the theoretical limit for single-particle analysis. Nanofluidic sample supports demonstrate that high-resolution structure determination can be performed from picoliter volumes of enclosed material.[7] Alternatively, femtoliter-scale dispensing approaches suggest that sample deposition onto conventional cryo-EM grids can, in principle, approach the material limit required for single-particle structure determination.[8] Together, these developments point toward a promising convergence of microfluidic purification workflows such as MISO with ultrasmall volume sample carriers or dispensing techniques.

Viewed in this context, the proof of principle demonstrated by MISO represents an important stepping stone toward cryo-EM workflows that are scaled to the actual analytical requirements of the method. Achieving this will require careful consideration of surface and confinement effects that become influential at small scales, but doing so holds the potential to extend cryo-EM analysis to increasingly scarce or difficult-to-access biological assemblies.[9] By shrinking the preparative bottleneck, approaches such as MISO move cryo-EM closer to a future in which limited sample availability is no longer a barrier to structural insight.

DECLARATION OF INTERESTS

The author declares no competing interests.

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