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In the past several decades, cryogenic electron microscopy (cryo-EM) has developed from a niche practice to a fully matured approach for studying biological machinery. In recent memory, it was o en referred to as “blobology,” owing to the low-resolution, blob-like density maps that made their way into the literature. As cryo-microscopists, we were excited by these blobs and the things we were learning, as well as the potential the method held to reveal more biological complexity. Over time, cryo-EM density maps took on more features as both microscopic technique and data processing algorithms developed, bringing more and more to bear on the eld of structural biology. In more recent years, hardware developments have pushed data collection squarely into a regime capable of reaching near-atomic resolution on a routine basis , and just recently to atomic resolution . e battle to have the highest resolution structure by cryo-EM has slowed from making progress on the nanometer scale to fractions of an angstrom, and the number of laboratories with access to high-end cryo-EM facilities has grown exponentially. It is no wonder why cryo-EM has taken the eld of biology by storm, and there is not a clear end in sight [1].

e ght for resolution has moved largely from the test tube into the cell and is slowly making its way into tissue via the use of cryo-electron tomography (cryo-ET). Tomography is a three-dimensional (3D) imaging method that functions by collecting a series of projection images through an object from di erent angles. Using this tilt series of projections, a 3D image can then be computationally r h.tructed

challenges in each, while creating some of its own. is approach is reviewed elsewhere, so we will only describe its development brie y. In this approach, the protein of interest is tagged with a uorescent probe and the cells expressing the uorescent protein are plunge-frozen on the surface of an EM grid. e vitri ed sample is loaded onto a specialized uorescent microscope out tted with a cryo-stage capable of maintaining liquid nitrogen temperatures. Once the uorescence signal is located and imaged, the sample is withdrawn and transferred under liquid nitrogen to the cryo-TEM, where the uorescence data guide target selection for tomographic data collection. If high-precision correlation is needed, ducial markers that are visible across both imaging modalities and multiple magni cations must be added to the sample prior to plunge-freezing and postprocessing must be done to provide high precision correlation.

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None

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None

1. Arnoldetal JArnold, J Mahamid, V Lucić, (2016) Site-specifc cryo-focused