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Commentary
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Challenges and exultation in cryo-electron tomography

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Introduction

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 often 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 field 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 . The 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 field of biology by storm, and there is not a clear end in sight [1].

The fight 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 different angles. Using this tilt series of projections, a 3D image can then be computationally reconstructed into a volume known as a tomogram. The power of tomography is that it can achieve a relatively high resolution (~3 nm), with no averaging, and is especially good for studying unique biological specimens such as whole cells or organelles. Cryo-ET allows for views of cellular structures at unprecedented resolution and clarity. The lack of chemical fixatives and stains allow for direct imaging of the biological molecules in their hydrated state and often provides fresh new details about well-studied cellular ultrastructure . High-resolution molecular structures can also be elucidated, particularly with the use of subtomogram averaging. Here subvolumes within a tomogram containing an object of interest are aligned and averaged in an iterative fashion to produce density maps, often with resolutions below 1 nm. Further information on subtomogram averaging can be found in recent reviews written on the topic [2].

Identifying structures and targeted milling

As mentioned earlier, one of the great challenges of cryo-ET is identifying specific structures within the greyscale tomogram. Without prior knowledge of what specific molecular components look like or how they are arranged, you may not be able to say with confidence that you know what you are looking at, or which subtomogram densities should be averaged together to get to higher resolution. This is the exact opposite challenge associated with fluorescence microscopy, which produces spatial information about where specific molecules are, but often without the context of the surrounding molecular environment. Initially, there was much enthusiasm for the development of a "GFP-like" molecule for use in EM. There were serious attempts to use metal-chelating proteins as genetically encoded electron-dense tags, but success has been limited to those particular proofs of principle. This is likely because the addition of large densities to the object of interest often interferes with the assembly or visualization of the structure and begins to negate the reason for using cryo-ET in the first place [3].

So far, the most broadly successful approach to identifying molecular components within ET data has been correlated fluorescence . It combines the best of both techniques to overcome the challenges in each, while creating some of its own. This approach is reviewed elsewhere so we will only describe its development briefly. In this approach, the protein of interest is tagged with a fluorescent probe and the cells expressing the fluorescent protein are plunge-frozen on the surface of an EM grid. The vitrified sample is loaded onto a specialized fluorescent microscope outfitted with a cryo-stage capable of maintaining liquid nitrogen temperatures. Once the fluorescence signal is located and imaged, the sample is withdrawn and transferred under liquid nitrogen to the cryo-TEM, where the fluorescence data guide target selection for tomographic data collection. If high-precision correlation is needed, fiducial markers that are visible across both imaging modalities and multiple magnifications must be added to the sample prior to plunge-freezing and postprocessing must be done to provide high precision correlation [4].

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Conflict of Interest

None

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