Methods and areas worth watching

Structures in situ

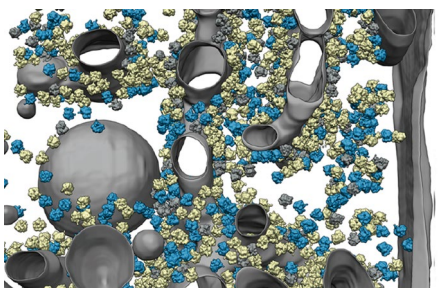
Cryo-electron tomography reveals the structural biology of native macromolecules.

Single-particle cryo-electron microscopy (cryo-EM) has emerged as a transformative approach for determining high-resolution structures of proteins and nucleic acids. Methods for solving structures by cryo-EM have developed and matured, increasing the uptake of this approach by the broader research community.

However, the advantages of electron microscopy for structural biology go well beyond single-particle cryo-EM. For resolving structures as they occur in their native cellular context, cryo-electron tomography (cryo-ET) comes to the fore.

In cryo-ET, a tilt series of 2D images of a sample are taken using a transmission electron microscope; each image is taken at a slightly different angle, after which computational methods are used to create a tomographic, 3D reconstruction of the sample. This 3D reconstruction represents a snapshot of the cell and can capture transient and rare events that can be missed when using purified components. By combining averaging tools from single-particle cryo-EM with images obtained with cryo-ET, structures can currently be obtained with 10–30 Å resolution. These tomograms can also be fitted with high-resolution structures obtained using other approaches to create detailed models of structures and complexes as they occur in cells.

Although cryo-ET has already yielded rich structural information in cellular contexts, better methods are still needed to realize its full potential. Current implementations are often limited to thin samples, such as bacteria; its application has been more limited in mammalian cells. But improved methods for focused ion beam milling of samples for cryo-ET, for example, can give access to thicker cells and tissues (*Nat. Methods* **12**, 634–636, 2015; *Nat. Methods* **16**, 757–762, 2019; *Nat. Methods* https://doi.org/10.1038/s41592-019-0630-5, 2019).



Ribosomes in a cryo-electron tomogram. Adapted with permission from Schaffer et al. *Nat. Methods* **16**, 757–762 (2019), Springer Nature.

There are also challenges of image analysis. The 'missing wedge' plagues many tomographic approaches and reduces resolution; it arises because images cannot be acquired for all tilt angles. Computational approaches are making exciting headway to address this problem (*J. Struct. Biol.* **206**, 183–192, 2019). Another challenge is annotating particles of interest from the crowded intracellular environment, with computational approaches greatly facilitating the process (*Nat. Methods* **14**, 983–985, 2017). Additional processing and computational workflows further improve cryo-ET (*Nat. Methods* **15**, 955–961, 2018; *Nat. Methods* **16**, 1161–1168, 2019).

We anticipate future methods development will improve throughput, sample preparation, analysis, and achievable resolution of cryo-ET. ❐

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Base editors beware

Tools are needed to study off-target mutations introduced by base editors.

Base editors leverage RNA-guided, catalytically inactivated nucleases to target base-modifying enzymes to DNA or RNA. Their great success in correcting point mutations has stimulated the fast-moving field of base editing.

The first DNA base editor was created by fusing the APOBEC1 cytidine deaminase to a dead Cas9, which enables C to U conversion; this class is now known as cytosine base editors (CBEs). Later there emerged adenine base editors (ABEs), which convert A to G, and RNA base editors.

Because they circumvent double strand breaks (DSBs), base editors are considered to be safer editing tools that eliminate undesired indels, translocation or rearrangements resulting from DSBs. Recent studies, however, have revealed that base editors may not be as safe as we had hoped. Sequencing studies performed on genomic DNA have confirmed that off-target mutations are induced by CBEs and ABEs.

Base editors' specificity depends on the targeting nuclease complex and the fused deaminase. In addition to the off-target mutations induced by the nuclease complex, deaminase encounters with transient single-stranded DNA and RNA may also lead to off-target editing outside the editing window, proximally or distally.

The question then becomes how to detect off-targets efficiently and, more importantly, without bias. Whole-genome sequencing of genomic DNA offers a way to detect all types of off-targets in vitro. For example, modified Digenome-seq showed that CBEs, ABEs and unmodified Cas9 produce different off-target profiles, which underline the necessity of assessing genome-wide specificity (*Nat. Biotechnol.* **37**, 430–435, 2019).

Examining base editors' on-target and off-target sites. Credit: Marina Corral Spence/ Springer Nature

The question is further complicated in vivo because of genome background interference. A recent tool uses two-cell mouse embryos, one edited and the other one unedited, to infer the true mutations introduced by base editors (*Science* **364**, 289–292, 2019). There is still a need, however, for tools that enable in vivo off-target detection at large scale. Additionally, computational tools that predict the off-target sites on the basis of sequence similarity or enzyme binding affinity will be helpful to guide sequencing studies. Comprehensive analysis of sequencing data to calculate off-targets would also benefit the community when evaluating the activity and specificity of emerging base editors. ❐

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