Single-particle cryo-electron microscopy

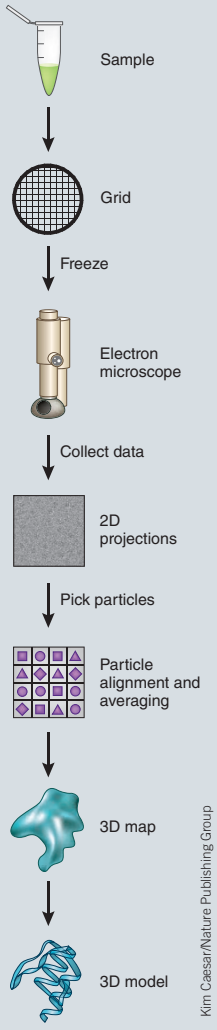
A brief overview of how to solve a macromolecular structure using single-particle cryo-electron microscopy (cryo-EM).

For decades X-ray crystallography reigned as the dominant technique for obtaining high-resolution information about macromolecular structure. Single-particle cryo-EM was traditionally used to provide insights into the morphology of large protein complexes that resisted crystallization, albeit at substantially lower resolutions than crystallography. Though the overall strategy has not changed appreciably over the years, very recent technological advances in sample preparation, computation and especially instrumentation are now allowing researchers to use cryo-EM to solve near-atomic-resolution macromolecular structures.

The first step: sample preparation

A cryo-EM experiment begins with a purified protein sample. The protein solution is applied to a special sample grid consisting of tiny holes in a film (conventionally made of amorphous carbon) supported by a metal frame. Ideally the protein particles distribute evenly within the grid holes in a variety of orientations. The grid is then plunged into a cryogen such as liquid ethane, flash-freezing it and trapping the particles in a thin film of vitreous ice. In addition to capturing the protein structure at the moment of freezing, this process protects the sample to some degree from radiation damage and prevents evaporation of buffer in the high-vacuum conditions of a transmission electron microscope.

Researchers have explored various ways to improve sample preparation, including optimizing protocols for purifying fragile protein complexes, automating the preparation of sample grids and improving the grids themselves. Such seemingly incremental optimizations can together have a large impact on the success of a cryo-EM experiment.



The overall single-particle cryo-EM workflow, from protein sample to 3D model.

From 2D images to 3D model

The moniker 'single-particle' cryo-EM comes from the fact that 2D electron micrographs are snapped of individual protein particles on the sample grid. Because very low electron doses must be used in order to avoid damaging radiation-sensitive samples, such 2D projections are too noisy to allow structures to be resolved in atomic detail. The signals can be improved, however, by averaging of a large number of individual particles.

Particles are often frozen in random orientations on the sample grid, so averaging is not a straightforward process. This is beneficial, however, because many different 2D views of a protein are needed to reconstruct its 3D structure. Sophisticated image-processing methods are used to align the images and merge the data. Next, an initial 3D map is constructed. This map is iteratively refined and validated using dedicated software tools. Finally, the protein sequence is fitted into the 3D map to build a 3D model of the protein.

In the past, millions of individual particle images were required to solve a high-resolution structure. Now, however, the development of highly sensitive, direct-detection cameras is making it possible for structures to be solved from far fewer particle images, helping to save both time and precious samples while also providing higher resolution.

The dawn of direct detectors

In the early days of cryo-EM, the 2D particle images were recorded on photographic film. Film provided relatively high resolution but in practice was tedious to use. Many researchers in the field thus switched to charge-coupled device (CCD) cameras for the convenience of a digital readout, but the resolution achievable with such cameras was relatively poor.

The recent development and commercialization of direct-detection cameras have been major advances for cryo-EM. Whereas CCD cameras convert electrons into photons in order to record images, direct detectors do just what their name suggests: they detect the electrons directly. This allows particle images to be collected with much greater sensitivity than with a CCD camera.

Direct detectors are also fast, which allows images to be recorded in 'movie' mode. Exploiting this ability, researchers have devised methods to correct for the image blurring that occurs as a result of tiny electron beam–induced movements of samples during imaging. This new mode of data collection has been key for obtaining near-atomic-resolution information.

Heterogeneity: a blessing and a curse

Whereas crystallization typically locks a protein into its most stable orientation, proteins in cryo-EM samples are free to move around until the moment of flash-freezing. Because cryo-EM is a single-particle technique, such conformational transitions can be captured and studied, and ultimately lead to deeper biological insights about protein function and mechanism.

However, such conformational heterogeneity can also make high-resolution 3D reconstruction a major challenge. Computational algorithms have come a long way in enabling heterogeneous data sets to be classified into structurally homogeneous subsets, but there is still much room for improvement.

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