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Cryo-EM

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What is cryo-EM?

If you have been paying any attention, you should have noticed an explosive growth in the number of macromolecular structures visualized using 'cryo-EM' (cryo-electron microscopy). For cryo-EM, biological macromolecules are prepared in a frozen-hydrated state by very fast freezing (Figure 1) and kept at liquid nitrogen temperature during storage, transfer to the microscope and data collection. Cryo-EM has been around for several decades, but it has only recently gained widespread attention. Low-dose images (to minimize radiation damage) are now obtained using 'direct detectors', resulting in a dramatic improvement in contrast and resolution and leading to a 'quantum leap' in cryo-EM studies. Cryo-EM overcomes the need for crystallization, it requires very little amount of sample — and I mean very little — and recent developments in image processing software can both deal with and characterize heterogeneous samples. It has thus dramatically expanded the range of molecules that can be studied and the functional insight gained from the resulting structures. Thanks to recent technical developments in detector technology and software tools, cryo-EM is now a high-resolution (atomic or near-atomic) and high-throughput technique within reach of many structural biologists.

What are the sample requirements?

As for any other structural biology technique, the better your starting material, the more likely it is that you will succeed. Thankfully, even minute amounts are sufficient for a resourceful cryo-EM practitioner to pursue structural studies. While a biochemically pure and well-behaved sample is ideal when aiming for atomic resolution, cryo-EM is more forgiving than X-ray crystallography when it comes to sample requirements. The reason is that it is possible to computationally remove the images of individual objects that are incompatible with the rest because they correspond to either impurities or damaged molecules (post-imaging purification *in silico*). This is particularly important because the process of preparing the sample for cryo-EM can be damaging in and of itself for a certain population of molecules, especially if the sample is physically fragile. The process relies on objective, statistical measures of self-consistency, both to eliminate 'bad particles' from the analysis and to characterize functionally relevant conformational and compositional mixtures.

Which equipment is required?

Electron microscopes have been constantly improved and adjusted to the needs of the growing cryo-EM community. Improvements include the quality of the optical system, the stability of the stage holding the frozen samples or the vacuum that allows for long hours, even days of operation without contamination. Most scopes used for cryo-EM analysis operate at a voltage of either 200 or 300 keV. Some would say that each energy has its perks, and while 300 keV has the potential for high resolution, higher energy does come with significantly higher costs. A highly coveted feature is the ability to insert up to 12 samples into the scope, so that EM grids with different sample preparations can be screened faster, without disturbing the vacuum in the scope with multiple insertions. By far the most important feature of competitive cryo-EM work today is to use a direct detector. Scopes and detectors can now be operated using automated data collection packages and your data will come out fast and furious, so be prepared for large-scale data storage and some serious computation.

Can you guarantee I will get atomic resolution?

You got your purified sample, the right scope–detector combo, and are setup for some serious computational work. Shouldn't you have an atomic model and start writing your paper within weeks? One of the major bottlenecks is the cryo-EM sample preparation. All newcomers are baffled by how often apparently biochemically sound samples break apart, aggregate or simply disappear when they are placed on an EM grid, blotted to a thin layer, and quickly frozen; or when all your molecules end up in the same orientation, making it impossible to produce a 3D structure. There are a number of strategies that you can try to overcome these problems. The solution, unfortunately, may be different for different samples, and a lot more effort is needed to address the physical origin of these problems — likely due to the way biomolecules interact with an air–water interface (Figure 1). In addition, while available software packages can detect and characterize mixtures in your sample, conformational changes of a continuous nature still pose a challenge. Today's solutions require some compromises, some ingenuity and very large data sets; yet another area where innovation and funding are badly needed.

What if I want the structure of my favorite macromolecular complex inside the cell?

Don't we all? This dream is now coming within reach. *In situ* structural studies, or 'cellular structural biology', requires unique sample preparation procedures, which are far from being mainstream yet, as well as an alternative way of data collection and 3D reconstruction: cryo-electron tomography or cryo-ET. Cryo-ET studies objects that cannot be initially averaged, such as organelles, and a reconstruction is obtained from a tomographic series of the unique object, where each image receives only a fraction of the total electron dose allowed. As a result, the signal is low and so is the resolution. However, when the reconstruction contains many identical objects (say, ribosomes), sub-tomogram averaging can provide a much-improved reconstruction of that object. While cryo-ET followed by sub-tomogram averaging applied to cell-free systems that can be blotted to a thin layer has been demonstrated to

achieve near-atomic resolution (better than 4 Å), it is the problem of having a thin ‘section’ of a cell that stands as the major technical hurdle today. Cryo-sectioning of frozen-hydrated cells remains the realm of a handful of labs and is burdened by dramatic cutting artifacts. A promising alternative, especially when coupled to fluorescence imaging for localization of the features of interest, is cryo-FIB (focused ion-beam) milling. While, again, this is today still an elitist practice in a few labs, and the yield is still low, the ‘lamella’ of the cell is free from the artefacts in cryo-sections. The latest results applying sub-tomogram averages to cryo-ET reconstructions of cryo-FIB samples warrant further investment in making this methodology more robust and generally applicable. Maybe atomic resolution of macromolecules inside their cellular context will cease being sci-fi before I retire!

Where can I find out more?

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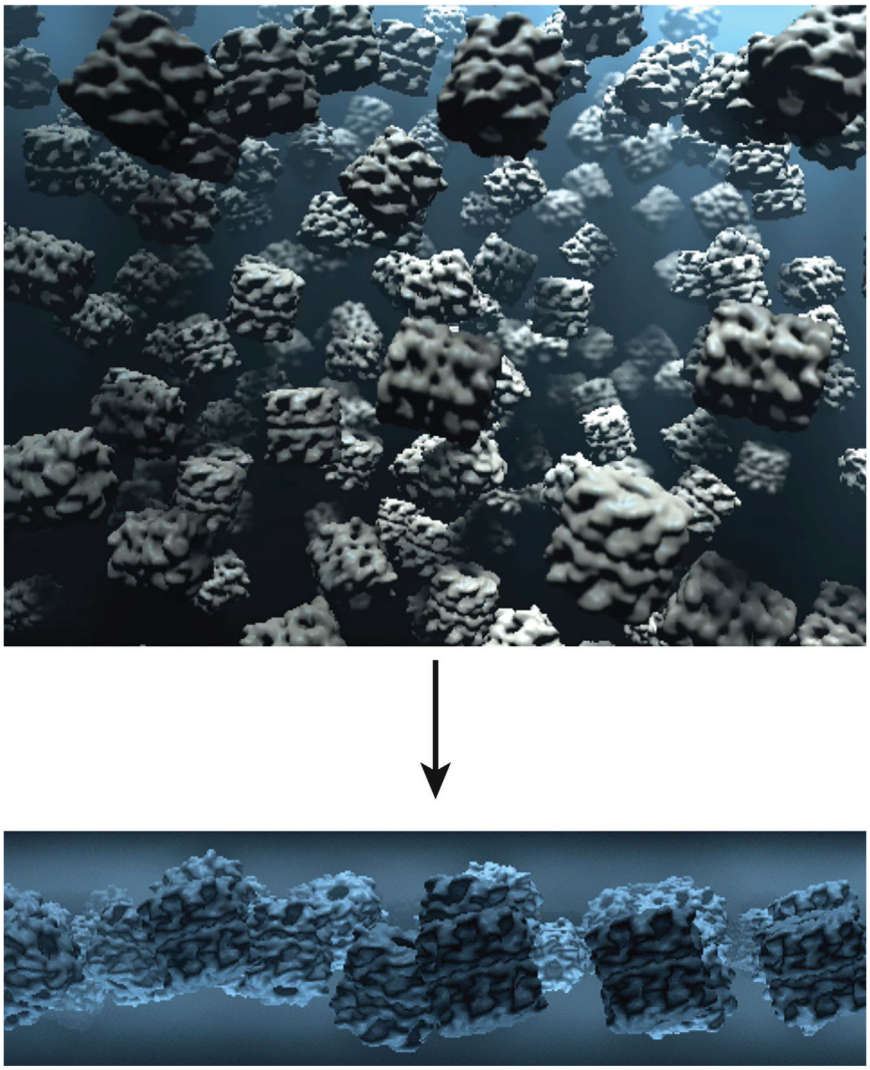

Figure 1. Cryo-EM visualization.

In cryo-EM, biological samples that were in solution (top) are restrained to a small layer that is quickly frozen (bottom), and then visualized in a transmission electron microscope using low electron doses (image: Gabriel Lander, The Scripps Research Institute).