Ice-cold realities

Cryo-electron microscopy is currently one of the most productive structural techniques, especially for large protein complexes such as photosystems. This success is built on a very long history of technological advances.

Without the use of tools to objectively observe the world influenced as little as possible by the observer, science could never have evolved beyond the study of subjective reality, the proper domain of philosophy. The most iconic of scientific technologies, which allow us to observe what we cannot see unaided, are the telescope, the first patent for which was filed in 1608, and its conceptual relative, the compound microscope. Without Galileo's telescopic observations of moons orbiting Jupiter, Nicolaus Copernicus's theories from the previous century would have had no power to place the sun at the centre of the solar system. Equally, without Robert Hooke's microscopic work later in the seventeenth century on the structure of cork, the idea that living creatures were made up from individual cells would not have taken hold.

It may be over 500 years since their invention, but the development of telescopes and microscopes continues to provide discoveries at the very edges of our understanding. Every increase in their sophistication and power promises a step change in the knowledge they can provide. By using a network of radio telescopes across the globe, astronomers have been able to image a black hole at the centre of galaxy M87, 55 million light years away and 6.5 billion times the mass of the Sun[1]. Meanwhile, advances in cryo-electron microscopy (cryo-EM) in the last few years have enabled unprecedented views of the most complicated machinery within cells, including the photosynthetic apparatus — some of which have appeared in *Nature Plants* in recent months[2,3].

Creating a microscope using a beam of electrons rather than a beam of light, with magnets rather than glass as its lenses, is surprisingly old technology. Ernst Ruska designed the first electron microscope in the 1930s, although he had to wait over 50 years before receiving the Nobel Prize in Physics for this achievement in 1986. Equally, by the 1950s the flash freezing of biological samples as a way to prepare them for observation was being experimented with. However, it wasn't until the 1990s that high-resolution structures began to be obtained on the back of technological improvements in instrumentation, sample preparation and image processing for which Jacques Dubochet, Joachim Frank and Richard Henderson received the 2017 Nobel Prize in Chemistry.

Even at these early stages, photosynthesis was a focus for cryo-EM research as the protein complexes involved had proved difficult to image by other means. In 1990, Richard Henderson and colleagues published the structure of bacteriorhodopsin at 3.5 Å resolution[4]. This was followed in 1994 by a structure of light-harvesting complex II (LHC-II) in pea plants at 3.4 Å from Werner Kühlbrandt and colleagues[5]. In fact, neither of these two studies are cryo-EM as we understand it today. In both studies, it was necessary for the proteins under investigation to be arranged as two-dimensional (2D) crystals of many hundreds of individual proteins. Electron diffraction patterns could be recorded from these, from which 3D structures were constructed using the same techniques as employed in x-ray crystallography.

The current surge of cryo-EM studies is based on collecting hundreds of thousands of images of individual protein particles from thousands of individual electron micrographs. These are combined computationally to create atomic-level resolution structures. The ability to achieve this level of detail from pictures, that to the untrained eye look like amorphous blobs, is the result of three separate technological developments.

About five years ago, several direct detector cameras came on the market, the most commonly used being the K2 from Gatan and the Falcon from FEI, both of which companies are now part of Thermo Fisher Scientific. These cameras capture every electron reaching the complementary metal oxide semiconductor chip that forms the detector. This has resulted in an order of magnitude increase in data quality from the charge-coupled device cameras previously used for this application.

Such cameras are now routinely coupled to the Titan Krios transmission electron microscope (also from FEI), which became available a few years earlier. This microscope is very stable and generally regarded to be user-friendly. This coupling of microscope and detector can collect enough data for a 3 Å structure in under a week. The set-up has proved extremely popular, initially in European and American laboratories, and more recently in China and Japan, to the point that Titan Krios microscopes can now be found in well over 100 research institutes worldwide.

But getting high quality raw images of individual complexes is not particularly useful without the means of processing them. Luckily, there has also been a similarly rapid improvement in data analysis software. Using packages such as RELION and cryoSPARC, naïve users can, with little training, use deep-learning algorithms to pick all the useable particles out of their micrographs and take them through all the steps of model building, alignment, classification and 'polishing' in under a month. Always assuming that the initial samples are good enough (with biochemistry remaining the limiting factor), experienced researchers can go from raw data to finished structure in less than a week.

Advances in cryo-EM have also had knock-on effects for related techniques, such as cryo-electron tomography. Here, 3D images of cellular structures are obtained by tilting samples during data collection. An example of this approach is a study in which the thylakoids of the cyanobacterium *Synechocystis* were imaged; published in *Nature Plants* last month[6]. "With this new technology", confesses Benjamin Engel of the Max Planck Institute of Biochemistry and lead author on the paper, "we can routinely get structures of complexes within the cell at 10–30 Å resolution".

These are exciting times, with new structures of photosystem components appearing almost monthly. They also serve to demonstrate that what was true half a millennium ago is still true today: technology drives discovery. ❐

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