Method of the Year 2015

The end of 'blob-ology': single-particle cryo-electron microscopy (cryo-EM) is now being used to solve macromolecular structures at high resolution.

The three-dimensional structure of a protein or protein complex provides crucial insights into its biological function. As a structure-determination technique, cryo-EM has played second fiddle to the higher-resolution approaches of X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. This is rapidly changing, however, thanks to recent technical advances that now allow near-atomic-resolution structures to be solved using cryo-EM. The time is right to celebrate single-particle cryo-EM as our Method of the Year.

For decades, X-ray crystallography has been the go-to approach for solving protein structures. However, many proteins—especially membrane proteins and protein complexes—are stubbornly difficult, if not impossible, to crystallize. Alternatives to traditional crystallography have had different limitations. For example, the technique of serial femtosecond crystallography, carried out using an X-ray free-electron laser (XFEL), requires a slew of easier-to-produce microcrystals rather than single large protein crystals, but the competition for beamtime at a highly specialized XFEL is fierce. NMR spectroscopy is useful for solving the structures of small proteins, but it remains quite difficult to apply it to larger ones.

In contrast to crystallography, cryo-EM is particularly well suited for obtaining structural information for large protein complexes and for systems that exhibit multiple conformational or compositional states. Researchers in this initially small field have made steady advances to improve the resolution and, by extension, the biological applicability of cryo-EM over the past few decades. A brief history of the key milestones in cryo-EM is given in a Historical Commentary by Eva Nogales on page 24.

This once small field is now exploding. A new type of highly sensitive direct-detection camera that captures electrons directly is making leaps in achievable resolution possible. The first papers to exploit these new detectors were published in 2013, and 2014 saw the publication of several important, high-resolution structures solved by cryo-EM. In 2015, the 3-Å resolution barrier was breached in multiple studies—an unprecedented feat that has surprised even long-time cryo-EM practitioners.

A good detector is not everything, however. A successful cryo-EM study depends heavily on good sample preparation as well as sophisticated image-processing software tools, as discussed in a Primer on page 23.

The cryo-EM resolution revolution is really just beginning, as discussed in a Commentary by Robert Glaeser on page 28. The sensitivity enhancements in detector technology have spurred opportunities for the development of new and improved methods that will push resolution, applicability and usability even further. Though cryo-EM is especially suited for large protein complexes, those that have been studied so far represent mainly the low-hanging fruit. Practical, reproducible and general methods for sample preparation are strongly needed to extend cryo-EM's applicability to structures that have so far resisted determination by any structural technique. Data analysis is also ripe for improvement: researchers want simple, reliable computational methods to go from raw two-dimensional images to three-dimensional protein structures, especially for examining structurally heterogeneous systems.

Like any scientific field undergoing a period of rapid growth, cryo-EM has not been without growing pains, as discussed in a News Feature on page 19. Encouragingly, many countries are setting up national user facilities with high-end instrumentation, but the demand for the top instruments is currently outstripping their availability. Many researchers are eager to take advantage of the technology, but cryo-EM is not a push-button technique (at least, not yet). There are many complex steps in sample preparation and in data analysis, which researchers must take care to properly apply, document and validate in order to avoid making mistakes. Proper training of new cryo-EM practitioners is crucial. The field must also come to a consensus about what raw data should be made available to reviewers and upon publication of a cryo-EM structure, as well as devise practical solutions for storing such large data sets.

Do these developments in cryo-EM signify the end of crystallography? X-ray crystallography will remain a robust technique for solving structures of proteins that can be readily crystallized. But the recent advances in cryo-EM open up exciting opportunities to access previously unexplored biological space. This is where future development efforts are likely to be concentrated.

To our readers, a very happy new year!