Cryo-EM Revolutionizes the Structure Determination of Biomolecules
Stefan Raunser*

cryo-EM · drug discovery · electron microscopy · protein structures · structural biology

The Royal Swedish Academy of Sciences has awarded the Nobel Prize in Chemistry 2017 to Jacques Dubochet, Joachim Frank, and Richard Henderson for developing cryo-electron microscopy (cryo-EM) for the high-resolution structure determination of biomolecules in solution. This is the third Nobel Prize to be given for electron microscopy in 35 years, which highlights the general importance of the method.

In 1931, Ernst Ruska (Nobel Prize winner in Physics, 1986) and Max Knoll built the first transmission electron microscope, initially called the “Übermikroskop”.[1] The subsequent serial models produced ever-increasing magnification and higher resolution. Their use in material and life sciences opened up completely new perspectives. And in 1938, Helmut Ruska, Ernst’s brother, visualized viruses by electron microscopy for the first time.[2]

Until the 1970s, transmission electron microscopy was mainly used to examine tissue sections embedded in plastic in the life sciences. Aaron Klug and his team at the Laboratory of Molecular Biology in Cambridge developed the method of electron crystallography,[3] for which he was awarded the Nobel Prize in Chemistry in 1982. The method is used to determine the three-dimensional structures of biomolecules that are arranged in the form of two-dimensional crystals, filaments, or viral capsids by electron microscopy.

Despite these advances, the commonly used technique of negative staining of biological samples with heavy metals for electron crystallography limited the resolution of the structures obtained. Nigel Unwin and Richard Henderson therefore developed a method to study two-dimensional crystals of bacteriorhodopsin, the retinal protein of halobacteria, without resolution constraints. They embedded the crystals in sugar and recorded not only electron micrographs but also electron diffraction patterns at different tilt angles, and were thereby able to determine the three-dimensional structure of a membrane protein at 7 Å for the first time.[4] Richard Henderson continued the work on bacteriorhodopsin, optimized the data quality, and recorded datasets at cryogenic temperatures. In 1990, Henderson and his co-workers determined in their pioneering work the first high-resolution three-dimensional structure of a protein by means of cryo-EM.[5]

Since it is difficult to obtain well-diffracting two-dimensional crystals, electron crystallography is rarely used nowadays. Instead, single-particle cryo-EM has become a major technique for determining the structures of proteins. For this purpose, the samples are plunge-frozen in liquid ethane or propane, thereby ensuring that the hydration of complex biological structures is maintained.[6,7] The result is a collection of macromolecules, or single particles, arranged in random orientations and embedded into a layer of amorphous ice under near-native conditions. This technique was pioneered, developed, and refined by Jacques Dubochet.[7]

Many two-dimensional images of the individual proteins are recorded in the electron microscope under low electron dose and at liquid-nitrogen temperature. The randomly oriented particles are then digitally aligned, classified, superposed, and back-projected to obtain a three-dimensional structure of the protein. Joachim Frank provided the decisive impetus and has been the driving force behind the development of such computer-based image processing for many years.[8]

Physical limits in classical image recording resulted, however, in suboptimal signal-to-noise ratios and long recording times. Near-atomic resolution structures could only be obtained for highly symmetrical specimens, such as virus capsids. The recent development of a new generation of detectors capable of directly measuring incident electrons has finally revolutionized the field over the past four years and has enabled the determination of highly resolved structures of non-symmetric proteins. The read-out speed of the detectors has been tremendously improved, allowing the recording of movies instead of single images. Since the signal-to-noise ratio of the single movie frames is high, they can be aligned to correct for the movements of the particles in the vitrified ice layer, which is induced by the interaction of the electron beam with the specimen. The result is a tremendous increase in resolution to even better than 2 Å in some cases (Figure 1).

Insight into biological processes at the atomic level is essential to understand how organisms are built and work. Single mutations can lead to serious disruption of the complex interactions in a cell and often result in severe diseases. It is therefore important to determine the three-dimensional structures of biological...
and to perform local refinements in the case of protein complexes with flexible regions.

Cryo-EM has now become a major technique in structural biology. In the last four years, high-resolution structures of many protein complexes have been determined that could have not been solved by X-ray crystallography, amongst them, spliceosome complexes, ribosomes, cation channels, respiratory supercomplexes, actomyosin complexes, and many others. The resolutions generally obtained by single-particle cryo-EM allow not only exact fitting of side chains, but in many cases also exact positioning of small molecules (Figure 2). The technique therefore has the potential to become a key tool for drug discovery research.\textsuperscript{9}

However, cryo-EM is not yet ready for high-throughput approaches. Many steps in sample preparation, image acquisition, and image processing still require the input of an experienced user. Conditions have to be adjusted individually for every specimen. It will take the joint efforts of academia and industry to optimize all of the steps in cryo-EM in order to fully automate the workflow. In addition, there is still room for improvement of technology. The recent breakthrough development of the Volta phase plate\textsuperscript{10} is a good example. This device tremendously reduces the problems of limited signal-to-noise ratios and low contrast by enhancing the phase contrast of the object. The improved contrast in turn improves the alignment and classification of particles. It also makes it possible to solve the structures of relatively small proteins (\textless 100 kDa) by cryo-EM. The continuous upgrading of the detectors to give even higher signal-to-noise ratios, faster read-out speeds, and larger fields of view will improve the data quality and reduce the number of particles needed to obtain high-resolution structures. Time-resolved electron microscopy that allows mixing of samples prior to plunge freezing will allow trapping of intermediate states of biochemical reactions and solution of the corresponding structures.

The future of single-particle cryo-EM is bright, and many new exciting discoveries using this technique are expected in the near future. For biological applications at a larger scale and in the physiological environment of the cell, cryo-electron tomography will be the method of choice. This technique, pioneered by Wolfgang Baumeister and colleagues, uses the same equipment as single-particle cryo-EM, but instead of recording single particles in random orientations, images of the same specimen at different tilt angles are taken and later back-projected to obtain a three-dimensional reconstruction. Cryo-electron tomography profits as much from the recent development of the direct electron detectors and the Volta phase plate as single-particle cryo-EM. Especially in combination with subtomogram averaging, resolutions previously thought unreachable are obtained. The recently introduced technique of cryo-focused ion-beam (FIB) micromachining paves the way for deep insight into cells at unprecedented resolution, opening up completely new perspectives. Taken together, cryo-EM has become a versatile key technique in structural biology and, in my opinion, the future of structural biology belongs to cryo-electron microscopy.
Conflict of interest

The authors declare no conflict of interest.

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