The 2017 Nobel Prize in Chemistry

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The 2017 Nobel Prize in Chemistry was awarded to Dr. Joachim Frank of Columbia University, Dr. Jacques Dubochet of Lausanne University and Dr. Richard Henderson of MRC Molecular Biology Institute for "developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"[(1)].

High-resolution atomic level structural analysis of proteins is essential for understanding life and for identifying and treating the causes of associated diseases. Particularly in the development of pharmaceutical drugs, the high-resolution structure is the most powerful information at the stage of optimization of a candidate compound. This is because it provides guidelines for improvement of the compound once interactions between it and the target protein have been identified, and the environment and space around the compound can be closely examined.

Conventional structural analysis of protein molecules at the atomic level has been mainly performed by single crystal X-ray structure analysis and NMR. Structural analysis methods using electron beams, such as electron diffraction and computed tomography (CT), have been conducted; however, they have never been major analytical methods. Both methods have decisive drawbacks because they require that protein molecules be kept hydrated in a vacuum and they must withstand damage from an electron beam. There are some additional problems: low contrast between solvent regions mainly composed of water and a protein molecule consisting only of light elements, movement of protein molecules due to interaction with electrons, and increasing sample temperature caused by electron beam irradiation. The latter problem becomes severe with relatively small proteins.

However, structural analysis using electron microscopes made a major turnaround in 2013. Now this has become a star technique in the field of protein structure analysis. This is because the development of numerous software, hardware and measurement methods has made it possible to perform so-called single-particle cryo-electron microscopy (Cryo-EM), a third electron microscopy method in addition to electron diffraction and CT. In single-particle analysis, electron beams are simultaneously irradiated onto multiple molecules frozen in a solvent thin layer, and the obtained 2D transmission images are grouped and used for reconstruction to derive a 3D structure. It has the advantage that a protein having multiple conformations can be directly analyzed. Another reason for the current popularity of this technique is that single crystal analysis by electron microscopy is good at large protein complexes whereas single crystal X-ray structure analysis is rather weak. This is because large protein complexes are often difficult to crystallize, which is a requirement for single crystal X-ray structural analysis. Even when a single crystal can be obtained, difficulties such as spatial resolution of diffraction spots and optical resolution due to a long crystal lattice and crystallinity, phase determination and ambiguities in model building intrinsically exist. Single-particle analysis by cryo-EM does not have these problems, in principle.

The three recipients of the 2017 Nobel Prize in Chemistry received the award for pioneering research on single-particle structure analysis by electron microscopy and developing methods essential for single-particle structural analysis.

The fundamental problem in determining the structure of asymmetric protein molecules randomly dispersed in water is in capturing the features of the shape from images with poor signal-to-noise (S/N) and classifying those images. Dr. Joachim Frank and colleagues showed that by using a cross-correlation function, 2D images of molecules having different structures can be classified and, by averaging numerous classified 2D images, one can obtain a high-resolution 3D structure. Additionally, Dr. Frank made a significant contribution to protein structure analysis by cryo-EM by developing a series of basic software tools commonly used in single-particle cryo-EM analysis, which were released in the form of a program package called SPIDER.

As mentioned above, the main problem with single-particle cryo-EM analysis of protein molecules is that the protein must be kept hydrated in a vacuum and must withstand damage caused by electron beam irradiation. Dr. Jacques Dubochet made a great contribution to solving these problems. When a protein solution forming a thin film is frozen rapidly, it becomes vitrified. This not only improves contrast, but also keeps the protein hydrated even in vacuum and suppresses damage from the electron beam. The method of freezing a protein solution developed by Dr. Dubochet has been accepted rapidly and adopted not only for single-particle structure analysis but also for CT.

Dr. Henderson's accomplishment is to prove that when averaging a large number of transmission electron microscopic images of the same molecule, a high-resolution structure is obtained. The method established by Dr. Henderson provides the basis of single-particle analysis. Additionally, Henderson et al. identified problems with electron microscopes around the world and demonstrated that when these problems are addressed,

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cryo-EM will be capable of determining high-resolution structures of biological macromolecules.

Popularity of cryo-EM in the field of structural biology since 2013 is mainly due to the advance of single-particle analysis. The theoretical and experimental technologies of single-particle analysis were already established before 2013. Why has the number of structure determinations by cryo-EM increased dramatically since 2013? There are several reasons, but the introduction of a new direct electron detector is definitely one of the final pieces required to complete the technology. Due to the high S/N of direct electron detectors, remarkably high-quality transmission images can be obtained even with a similar dose of electrons to conventional experiments. Conversely, even when the dosage was reduced to its minimum to decrease the damage to protein, images similar to those acquired by conventional experimental conditions can be obtained. Fluctuation of protein molecules upon irradiation with electrons can be overcome by taking a "movie" instead of static photographs. Progress in this component of the instrument played a decisive role.

It seems that when a new but promising analytical method is introduced, it attracts attention at the initial stage and many researchers rush to adopt it. Whether or not the particular analytical method has come to stay depends on whether a critical number of researchers continue to use it after the initial stage. There is no doubt that single-particle analysis by cryo-EM is going to remain as one of the major techniques to determine biological macromolecules. However, as of 2017, it is perhaps attracting more attention than it merits in the initial stage. Prominent crystallographers have adopted cryo-EM already, but the majority view is that it is desirable to use X-ray structural analysis in combination with cryo-EM. However, some researchers are claiming that X-ray crystallography is no longer necessary.

There are some disadvantages to cryo-EM, as with any other analytical method. One is a shortage of experienced researchers and technicians because it is a relatively new but fast-expanding analytical method. Since it requires neither crystallization nor crystallography, people may think anybody can start using cryo-EM without training. Indeed, some articles introducing the 2017 Nobel Prize in chemistry give the impression that anybody can use this technique[2]. However, in reality, at least currently, years of experience and training are necessary to perform structure analysis using cryo-EM appropriately.

There are also indications of potential pitfalls with cryo-EM[3]. It may be hard to imagine, but although cryo-EM actually looks at the structure in real space, there is still a possibility that the images were deformed due to the influence of a skewed template used in extracting 2D images, especially when S/N is low. Analysis of inositol-1,4,5-tris phosphate (IP_3) receptor (IP_3R) can be used as an example in which the reaction mechanism remained elusive using the structure determined by cryo-EM[4] but was determined clearly using X-ray structure analysis[5]. IP_3R is involved in cytosolic Ca^{2+} signaling and is a ubiquitous ion channel essential for a wide range of cellular processes, ranging from muscle contraction and secretion, cell proliferation to cell death. In 2015, a US group posted the structure determined by cryo-EM in *Nature*, but could not clearly identify the allosteric mechanism of ion channeling. Meanwhile, a RIKEN group analyzed the structure of a series of genetically engineered large cytoplasmic domains extending from the IP_3 binding site of IP_3 and IP_3R to the Ca^{2+} channel site, and the structural change upon IP_3 binding was transmitted via three α-helical domains, HD1, HD2 and HD3, and eventually transfers to the Ca^{2+} ion channel through a "leaf type structure" consisting of 21 amino acid residues in HD3 region. The history of the structural analysis of IP_3R warns that the value of single crystal X-ray structural analysis should be reviewed.

The usefulness of the accuracy of high-resolution structures obtained by high-quality single crystal X-ray structural analysis needs no further discussion. Meanwhile, single-particle analysis by cryo-EM is good for large protein complexes and flexible protein molecules that are often difficult to crystallize. Cryo-EM and single crystal X-ray analysis are complementary methods. Both are expected to accelerate future progress in structural biology synergistically.

References

(1) The Royal Swedish Academy of Sciences (2017) "Scientific Background on the Nobel Prize in Chemistry 2017", https://6702d.https.cdn.softlayer.net/2017/10/sciback_ke_en_17.pdf.

(2) For example, https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2017/press.html

(3) Richard Henderson: *Proc Natl Acad Sci USA,* **110** (2013), 18037−18041.

(4) K. Hamada, Hideyuki Miyatake, Akiko Terauchi and Katsuhiko Mikoshiba: *Proc Natl Acad Sci USA*, **114** (2017), E4661−E4666.

(5) G. Fan, M. L. Baker, Z. Wang, M. R. Baker, P. A. Sinyagovskiy, W. Chiu, S. J. Ludtke and I. I. Serysheva: *Nature*, **527** (2015), 336−341.