Nobel Prize for Chemistry 2017 (Edited)[1]

The Royal Swedish Academy of Sciences award Jacques Dubochet, Joachim Frank and Richard Henderson the Nobel Prize for Chemistry 2017 for "Developing cryoelectron microscopy for the high-resolution structure determination of biomolecules in solution"

Introduction

Just a few years ago scientists could still only dream of being able to use the electron microscope to zoom in further into cells and organelles, in order to uncover the atomic details of the biomolecules that underpin their architecture and function.

This dream became reality recently when a series of critical developments made it possible to take full advantage of the pioneering discoveries and improvements made by Jacques Dubochet, Joachim Frank and Richard Henderson. These advances now allow structural determination of non-crystalline biomolecules in solution at high resolution (see Fig.1), using single-particle cryo-electron microscopy (EM).

Challenges in structural studies of biological material

Short after the experimental demonstration of an electron microscope by Ernst Ruska, for which he was honoured with the Nobel Prize for Physics in 1986, Ladislaus Marton published a paper that commented on Ruska's discovery. Marton noted that the new instrument unfortunately could not be used to study biological material without the "*destruction of the organic cells by the intense electronic bombardment*". Preventing such destruction would require a new sample-preparation technique. Thus Marton proposed the use of an approach similar to negative staining or cooling the biological material. Another major problem was how to preserve water in the biological sample in the vacuum maintained inside the electron microscope chamber.

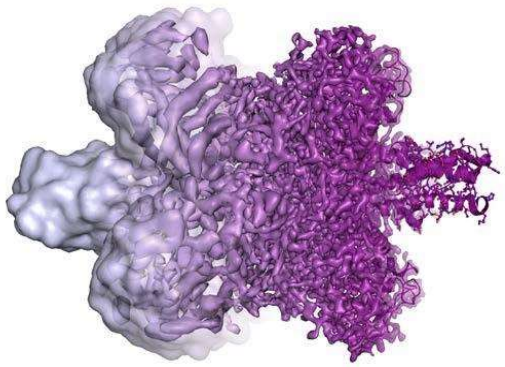

Figure 1 *The resolution progression of cryo-EM, illustrated by a representation of glutamate dehydrogenase with an increasing level of detail from left (approx. 2nm resolution) to right. For a protein of this size, 334 kDa, the 1.8 Å resolution to the right could only be achieved after 2012/13.*

Many additional problems prevailed; intact biological material has very low image contrast as most high-energy electrons pass straight through the specimen. At the same time, the electron dose must be kept low enough to prevent damage. The probability for multiple electron scattering events must be negligible at the electron energy used; i.e. samples must be thin, ideally comprising a single layer of the particles of interest. Furthermore, the studied objects often move both upon interacting with electrons and due to drifts in temperature; the movement reduces information content, especially when using film or slow detectors to record images. As a result, until recently the resolution was typically limited to a few nanometres for biological molecules.

Negatively stained biological material

The necessity to use the lowest possible electron intensities to study low-contrast samples stimulated the development of new sample-preparation methods when recording images of biological material. The first

[1] Full article is available from https://6702d.https.cdn.softlayer.net/2017/10/sciback_ke_en_17.pdf accessed December 2017. Full and edited article available on cooperativelearning,works.

commonly and successfully employed method was negative staining, established in the 1940s and refined during the following 20 years.

When using this approach, the biological material is embedded in a thin amorphous film of a heavy-metal salt, which generates a cast around the object. The cast scatters electrons more strongly than the encapsulated material, is more resistant to electron damage, and prevents collapse of the biological material during drying in the vacuum within the electron microscope.

The approach offered detailed information about the morphology of bacteria, viruses and organelles. However, for studies of single molecules or molecular complexes, in the best case the pictures could reveal only the envelope of the covered particles with a resolution that is limited by the granularity of the stain. Nevertheless, the use of this sample-preparation technique offered important low-resolution structural information.

Native protein crystals at cryogenic temperatures

Techniques were also needed to preserve intact biomolecules in the hydrated state in the electron microscope and to determine conditions for non-destructive irradiation. Cooling the specimen was expected to reduce water evaporation and to protect the biological material from radiation-induced damage. Starting in the 1950s, Humberto Fernández-Morán explored the possibilities for freezing samples and preparing thin cryo-sections for studies using cryo-EM. However, upon freezing, water typically nucleates to form crystalline ice, which strongly diffracts electrons, thereby effectively obliterating signals originating from the sample. Furthermore, formation of ice crystals may change the specimen structure.

Basile Luyet had already noted the problems associated with the formation of crystalline ice when cooling cells in the 1940s. Luyet realized that the solution to the problem could be to cool the biological material sufficiently rapidly to preserve water in a liquid amorphous state, referred to as vitrified water. Cooling techniques were also introduced early for studies of protein crystals in the field of X-ray crystallography, where the formation of crystalline ice was prevented by using sucrose or glycerol as cryo-protectants.

In the electron microscopy field, Kenneth Taylor and Glaeser demonstrated electron diffraction patterns from unprotected frozen catalase crystals to higher than 3-Å resolution. They showed that hydration was maintained in the electron microscope at cryogenic temperatures and contrast was improved compared to when using glucose. The available technology at that time only allowed studies above approximately -120°C, which is above the transition temperature from amorphous to crystalline ice. However, the authors did not observe crystalline ice in the protein crystals, which they attributed to interactions between water molecules and the protein surface.

Taylor and Glaeser also developed technical solutions for specimen handling at cryogenic temperatures. They showed that cooling results in improved resistance to radiation damage, such that longer exposure times or larger electron intensities are possible. In other words, they concluded that cooling the specimen would increase the information content.

A sample-preparation method for cryo-EM

As discussed above, cooling was expected to solve many of the complications that limited the use of electron microscopy for structural studies of biomolecules. Problems associated with formation of crystalline ice could, in principle, be overcome by cooling liquid water into a vitrified state.

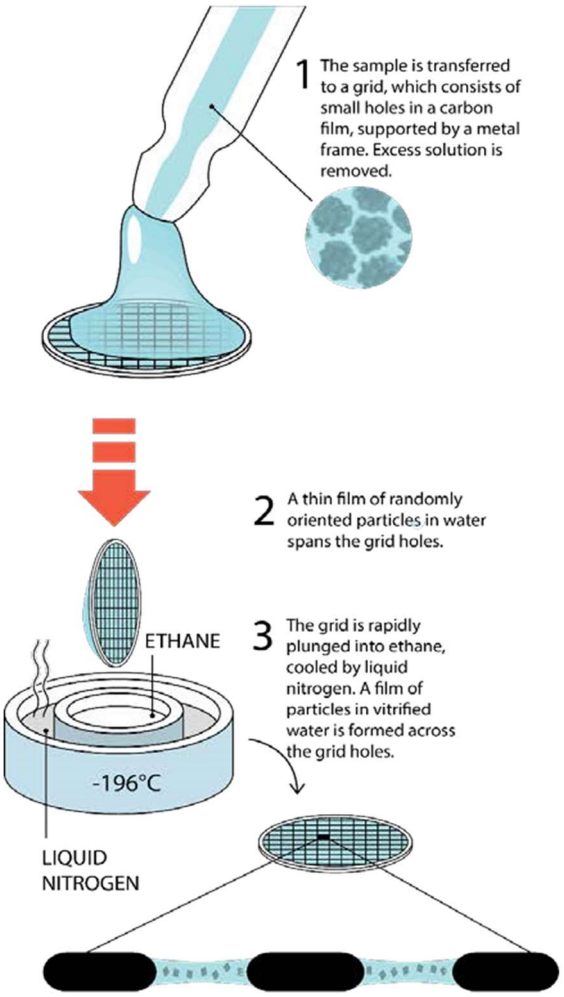

Figure 2 *Sample-preparation procedure for cryo-EM. © Johan Jarnestad/The Royal Swedish Academy of Sciences.*

However, before 1980 whether bulk water could be transformed into a vitrified solid state was still controversial because theory predicted that the required cooling rate would be practically unattainable. The phenomenon had been demonstrated, but only for condensation of water vapour at cold metal surfaces.

In 1980 the discussions were brought to an end with the demonstration that vitrified water could be formed by rapid cooling of micrometre-sized droplets of bulk water. It is also interesting to note that vitrified water may be the most common form of water in the universe. In 1981 Dubochet and Alasdair McDowall finally presented a method that allowed formation of a film of non-crystalline solid water on a specimen grid for observation in the electron microscope.

The full potential of Dubochet's sample preparation method was realized in 1984, when the group presented electron micrographs of virus suspensions, cooled using an improved method that allowed preparation of thin, unsupported water layers in the vitrified state. The new technique made it possible to prepare unsupported water layers that could be made sufficiently thin to allow rapid vitrification, but thick enough to accommodate a single layer of randomly oriented molecules or molecular complexes in their native state.

Dubochet and colleagues showed that the sample-preparation method is generally applicable in cryo-EM studies of other biological particles as well. The resulting images showed an impressive contrast. Finally, in the beginning the 1980s, Dubochet had solved the problem that had cast a shadow over the first 5o years since the invention of the electron microscope: “the most abundant constituent of living things, water, has invariably been excluded”. The new preparation method was immediately adopted by Dubochet’s colleagues and it is now used universally within the cryo-EM field, both in studies of assemblies of single particles and in cryo-tomographic studies of single objects.

The year 1990 marked another critical milestone when Henderson and colleagues showed for the first time that it is possible to obtain high-resolution structures of biomolecules using cryo-EM through averaging over many copies of the same object. Frank developed many of the important mathematical tools used for image analysis, which form the basis for single particle cryo-EM. He gathered them together in a suite of computer programs called SPIDER, making them readily available and useable for the scientific community.

This progress would not have been achieved without the contributions of the three Laureates.