K2 Direct Detection Camera

2017年 Nobel Prize in Chemistry 2017
GatanはクライオEMの研究を長年に渡って推し進めてきた全ての研究者と共に、ジャック・デュボシェ、ヨアヒム・フランク、リチャード・ヘンダーソンの3氏に対しお祝いを申し上げます。
この偉業に貢献した世界初、そして唯一のリアルタイム電子カウンティングカメラ、Gatan社製K2 Summit直接検出型カメラをお求め易い価格にてご提供いたします。

今回ご提供するカメラは、使用暦の浅い良好な状態のシステムを工場にて完全メンテナンスの上出荷いたします。

安心してお使い頂くために
工場にてメンテナンスを行い、さらに厳重な動作チェックを行った上で出荷いたします。
センサー部は新しいものに全て交換を行います。
カメラに付属するサーバーOSは最新の物に更新されます。
本システムは1年間の無償保証が受けられます。

仕様、装着の可否、お値段の詳細に関しては、下記にお問い合わせください。

株式会社クレイラ
〒350-1177
埼玉県川越市かわつる三芳野1番地47-105
TEL：049-231-6966, Fax：049-298-8771
E-mail：sakaki@cleyera.co.jp

GATAN

株式会社日本ローパー　ガタン事業本部
〒135-0033
東京都江東区深川2-8-19　サクライビル3F
www.gatan.com
Tel：03-5639-2772　　Fax：03-5639-2763

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

4 OCTOBER 2017

Scientific Background on the Nobel Prize in Chemistry 2017

THE DEVELOPMENT OF CRYO-ELECTRON MICROSCOPY

BOX 50005 (LILLA FRESCATIVÄGEN 4 A), SE-104 05 STOCKHOLM, SWEDEN
TEL +46 8 673 95 00, KVA@KVA.SE ⋆ WWW.KVA.SE

The Royal Swedish Academy of Sciences has decided to award **Jacques Dubochet**, **Joachim Frank** and **Richard Henderson** the Nobel Prize for Chemistry 2017 for "developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution".

Introduction

In 1968, George Gamow, a physicist, and Martynas Yčas, a microbiologist, published a popular-science book, *Mr. Tompkins Inside Himself: Adventures in the New Biology*, which tells a story about Mr. Tompkins as he explores the cellular architecture of his body on a dream journey through his bloodstream, guided by his doctor. While inspecting the structural details of single cells and organelles, Mr. Tompkins' guide enthusiastically informs him that this knowledge is based on studies using the electron microscope.

Mr. Tompkins' tour makes it obvious that at that time the instrument had already brought studies of biological material to a previously unimaginable level of detail. However, until 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, using single-particle[1] 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 (1), Ladislaus Marton published a paper (2) that commented on Ruska's discovery. In this short report, 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. Marton proposed visionary solutions to the problem: cooling the biological material or the use of an approach similar to negative staining. Another major problem was how to preserve water in the biological sample in the vacuum maintained inside the electron microscope chamber.

And there were even more challenges to face. To mention only the most basic ones, 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

[1] The term "particle" is used for biological macromolecules or complexes. The term "single particle" is used to indicate that non-crystalline specimens are analyzed. When using this approach, an ensemble of a large number of individual particles in solution is analyzed.

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 (**Fig. 1**).

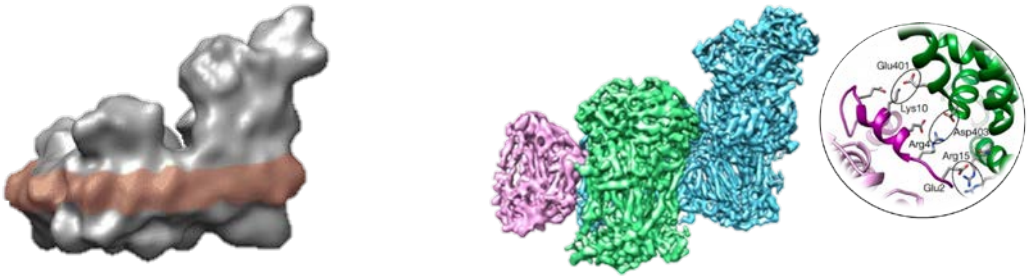

Fig. 1. Models of the electron-transport chain components in a mitochondrial supercomplex $I_1III_2IV_1$, determined in 2011 (left, from (3), resolution ~2 nm) and 2016 (right, from (4), resolution ~6 Å), respectively. On the left, the coloured shape indicates the position of amphipols used for solubilization. On the right, complexes I, III and IV are shown in blue, green and pink, respectively. The encircled inset shows a model of a putative CIII-CIV interface.

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 commonly and successfully employed method was negative staining, established in the 1940s and refined during the following 20 years (5-7).

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. The experimental and theoretical tools used for calculation of three-dimensional (3D) structures from two-dimensional (2D) projections in the electron microscope established the basis for today's advancements.

The first high-resolution model of bacteriorhodopsin (31) was based on analysis of millions of protein molecules in a 2D crystal, which allowed the spread of the total electron dose over a large number of particles.

The analysis of a large number of molecules in the 2D crystal is equivalent to averaging directly in the microscope. For non-periodic assemblies of symmetrical particles, the signal-to-noise ratio can be increased by averaging over the asymmetrical units. However, for the general case of non-periodic asymmetrical particles, the challenge was to determine the position and orientation of each particle in an image from weak signals. Once this could be achieved, averaging would be possible. However, such analyses would require computer power well beyond that available in 1990 (36).

Large virus	300 M	Yes
Small virus	11 M	Yes
Ribosome	3·3 M	Yes
	1·4 M	Yes
Multimeric enzyme	420 K	Possibly
	180 K	Possibly
	52 K	Possibly
Small protein	18 K	No
Very small protein	7 K	No

Fig. 3. Extract from Table 2 in (37), which addresses the question: can single molecule alignment be carried out in practise? The answer is given to the right for a number of example proteins, listed to the left, with molecular weights (Da) in the middle column.

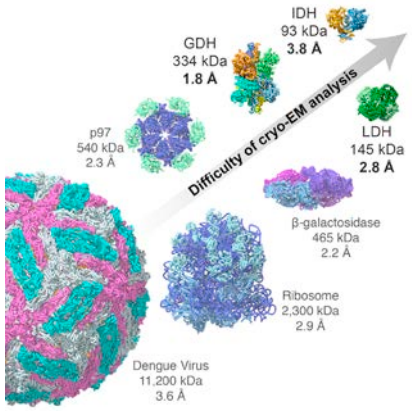

Fig. 4. Examples of structures determined using cryo-EM as of May 2016 [image from (38)]. The figure illustrates the conclusions from (37). Note that the smallest protein (64 kDa) determined to date using cryo-EM is haemoglobin (39).

Five years after the publication of the high-resolution structure of bacteriorhodopsin, Henderson presented a quantitative analysis of the challenges needed to be overcome in order to determine atomic-resolution structures of non-crystalline molecular assemblies (37). He concluded that by using low-intensity, non-destructive electron irradiation in phase-contrast electron microscopy, it would be possible to determine the 2D position and 3D orientation of individual particles, given a sufficiently high molecular weight. The information would allow averaging ensembles of randomly distributed particles, thereby eventually reaching atomic resolution. The conclusion from Henderson's work was that, assuming a molecular weight higher than ~50 kDa (**Fig. 3**), it should be possible to align and average a reasonable number (~10^4) of particles to determine structures at atomic resolution (~3 Å; see **Fig. 4)**. During the coming years the predicted number of particles necessary to reach this resolution was revised to smaller numbers (36,40), and Glaeser presented an analysis that suggested that the size limit could be adjusted to ~20 kDa (36). An analysis along the same lines, but less detailed, was also presented in an earlier paper (41).

Recent and ongoing improvements in image-processing methods and computer programs have also been essential for the current developments. For example, maximum likelihood algorithms (76,77) became particularly important in electron microscopy when better resolution was achieved using the new electron detectors.

Summary

The first high-resolution structure, determined using cryo-EM, was presented in 1990. A decade passed before high-resolution structures of helical and icosahedral particles were imaged, determined based on analysis of data recorded on film. After the introduction of the new Direct Electron Detectors in 2012-13 (see **Fig. 9**) and the first reports of *de novo* atomic structural models of smaller single particles, such as that of the membrane protein TRPV1 ion channel (78), cryo-EM has very rapidly become a major new tool in structural biology.

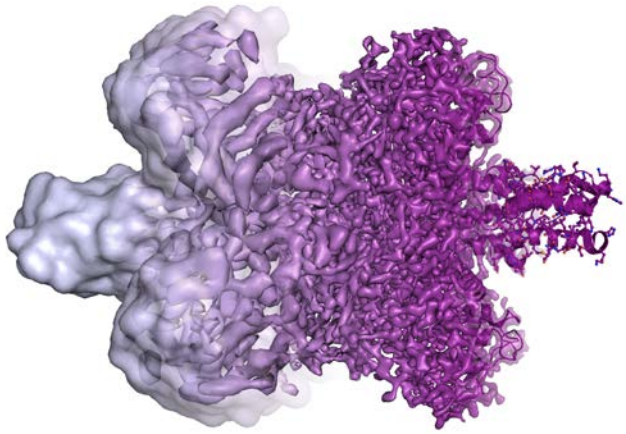

Fig. 9. The resolution progression of cryo-EM, illustrated by a representation of glutamate dehydrogenase with an increasing level of detail from left to right. For a protein of this size, 334 kDa, the 1.8 Å resolution to the right (38) could only be achieved after 2012/13. After an image by V. Falconieri (see ref. 38). Illustration: © Martin Högbom, Stockholm University.

It is captivating to think about the amount of time that has passed before we could get to this point. About six decades after John Kendrew's and Max Perutz's pioneering crystallographic work on myoglobin and haemoglobin (Nobel Prize for Chemistry in 1962 "for their studies of the structures of globular proteins"), and four decades after the first developments that laid the groundwork for single-particle cryo-EM, a high-resolution structure of haemoglobin in solution, determined using cryo-EM, was presented (39).

Single-particle cryo-EM is unique in that it does not require crystallization, uses very small amounts of material, and covers a wide range of sizes, from particles the size of haemoglobin (64 kDa), to very large particles up to several megadaltons. Cryo-electron tomography is used to determine structures of even larger objects, including organelles and cells, with the potential of