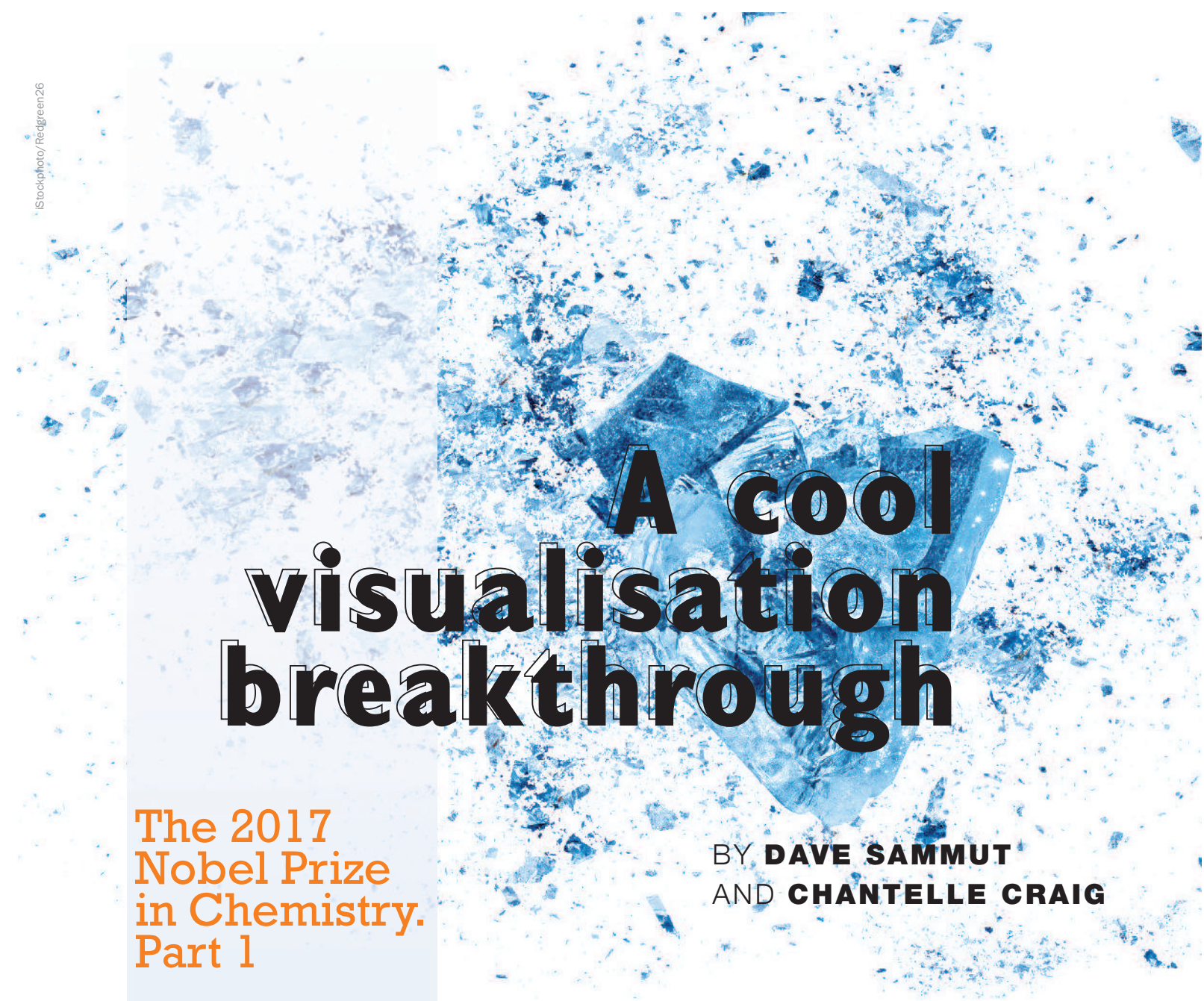

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A cool visualisation breakthrough

The 2017 Nobel Prize in Chemistry. Part 1

BY **DAVE SAMMUT**
AND **CHANTELLE CRAIG**

The limits of microscopy resolution have been smashed as part of work recognised by the 2017 Nobel Prize in Chemistry.

Science is not the lonely profession that it used to be. Gone are the days of the misanthrope academics locked away in their ivory towers, in single-minded pursuit of arcane science. In the modern world, whole groups of academics cram into their ivory towers, collaborating to create the next leaps forward in scientific understanding. In 1675, Sir Isaac Newton wrote to Robert Hooke: 'If I have seen further than others, it is by standing upon ye sholders of giants'. Even then, he was adapting words that were already centuries old.

How, then, do we recognise achievement in the new era of science? Over the last 50 years, there has been a steady growth of the Nobel Prize in Chemistry being awarded jointly, and most particularly in the laurels being shared by the mandated maximum of three researchers. From 1968 to 1977, three researchers shared the prize just once. By contrast, the 2017 winners collected the eighth instance of a triple-shared prize in the last decade.

In 2017, the world's most famous prize for scientific merit was awarded to three researchers – Jacques Dubochet (University of Lausanne, Switzerland), Joachim Frank (Columbia University, USA) and Richard Henderson (MRC Laboratory of

Molecular Biology, UK) – 'for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution'.

This relatively new science for the visualisation of active molecules is creating significant excitement at the biochemical frontier. As just one example, the technique has already been used to create high-resolution, 3D images of the Zika virus in its natural state, identifying targets for a vaccine.

As is becoming common in modern science, the story of this breakthrough is one of separate, complementary developments coming together to create something outstanding.

The science

Let's start with the basics. For the purposes of this article, I'm going to follow the Royal Swedish Academy of Sciences in describing a fairly broad range of molecules, biological macromolecules and complexes under the generic term 'particle'.

The structure of particles influences their activity. This is particularly the case with biologically active particles, for which the structures can get *really* complex. Much more than just the issue of potential chirality of simple organic molecules, the complexity of particles only grows when you start to consider issues such as the folding of proteins (see May 2017, p. 26), or the physical interactions between particles and their environment.

An early breakthrough in microscopy came when it was found possible to produce an image of an object by using an electron beam.

Learning about the structure of these particles tells us a great deal about their behaviours. Perhaps most famously, the 1962 Nobel Prize for Physiology or Medicine was awarded to Watson and Crick 'for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material'. Their discovery crucially relied on X-ray crystallography results generated by Rosalind Franklin, who was only posthumously recognised for her contribution to this seminal work (see August 2014, p. 19).

Conventional microscopes fail at resolutions less than 400 nanometres, because molecules such as proteins are smaller than the wavelength in the visible spectrum. As demonstrated by Watson, Crick and Franklin, X-ray crystallography can assist with the visualisation of particles. X-ray crystallography was also key to the 1962 Nobel Prize in Chemistry, for Max Perutz and John Kendrew's studies of the structures of globular proteins.

However, X-ray crystallography suffers two substantial drawbacks. First, it only works for materials that will form crystals, which eliminates a significant portion of biologically active samples. And second, even when a crystal can be formed, this is often only possible by changing the matrix and/or state of the particle, so that the biological function or activity can't be assessed in context.

Nuclear magnetic resonance spectroscopy can assist in the characterisation of samples that are non-crystalline, but it is limited to very small particle sizes. Anything so large as a ribosome or an ion channel is prohibitive, let alone full cells.

Over the last few years, researchers have published atomic structures of numerous complicated protein complexes. From top to bottom: a protein complex that governs the circadian rhythm; a sensor of the type that reads pressure changes in the ear and allows us to hear; the Zika virus.

So an alternative technique was still needed. An early breakthrough in microscopy came when it was found possible to produce an image of an object by using an electron beam. With a significantly shorter wavelength, electrons offer a theoretical resolution as fine as about 0.1 nanometres, subject to quantum effect limitations.

Ernst Ruska constructed the first electron microscope in 1933, for which he (jointly) won the Nobel Prize in Physics in 1986. He went on to take part in the development of the first commercial mass-produced electron microscopes, which many consider to be the major breakthrough for this analytical technique.

However, electron microscopy (EM) also has a number of significant drawbacks. It has suffered long-term problems with sample preparation and stability, signal quality, and data analysis, and many of these problems were widely considered to be potentially insoluble.

Samples must be very thin to limit multiple electron scattering events, preferably only a single layer of the particles of interest. As well as this, even in crystalline samples the particles themselves can move due to temperature changes or interaction with the electron beam, and the movement further limits the resolution of the images. This effect is then influenced or limited by the speed of the detector.

And the problem is exacerbated for non-crystalline, asymmetric, randomly oriented particles.

Furthermore, EM requires vacuum conditions, which tends to vaporise water from samples, and which in turn can completely change their structure and/or chemistry. For EM to have wide applicability, it needed a technique to preserve water in the particles.

In transmission EM (of which cryo-EM is an improved version), only a portion of the high-energy electrons interact with the particle, yielding low contrast and in turn requiring high-intensity electron beams. But such intensive beams commonly destroy biological samples, and so the researcher was faced with the trade-off

Microscopy through the ages

13th–14th centuries Lenses developed for use in spectacles.

1590 First microscope made by Dutch lens grinders Hans and Zacharias Janssen by placing two lenses in a tube.

1609 Compound microscope developed by Galileo Galilei.

1667 Robert Hooke published his microscope studies, including of cork, in *Micrographia*.

Scheme I, *Micrographia*, 1665

1675 Living cells seen by Anton van Leeuwenhoek using a simple, single-lens microscope. He achieved up to 300 times magnification of blood, insects and microorganisms.

18th century Technical advances improved microscopes and they became more common.

1830 Spherical aberration reduced by Joseph Jackson Lister, who combined several weak lenses to give good magnification with no blurring.

1878 Ernst Abbe's mathematical formula correlating resolution to the wavelength of light made it possible to calculate theoretical maximum resolution.

$$d = \frac{\lambda}{2n\sin\theta}$$

1903 Ultramicroscope developed by Richard Zsigmondy, which could study objects below the wavelength of light.

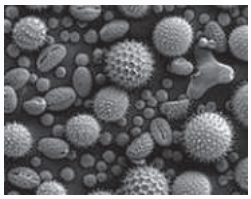
1932 Phase-contrast microscope invented by Frits Zernike, which could study colourless and transparent biological materials.

J. Brew / CC BY-SA 3.0

First transmission electron microscope built by Ernst Ruska. Using electrons, rather than light, greatly improves resolution.

1930s

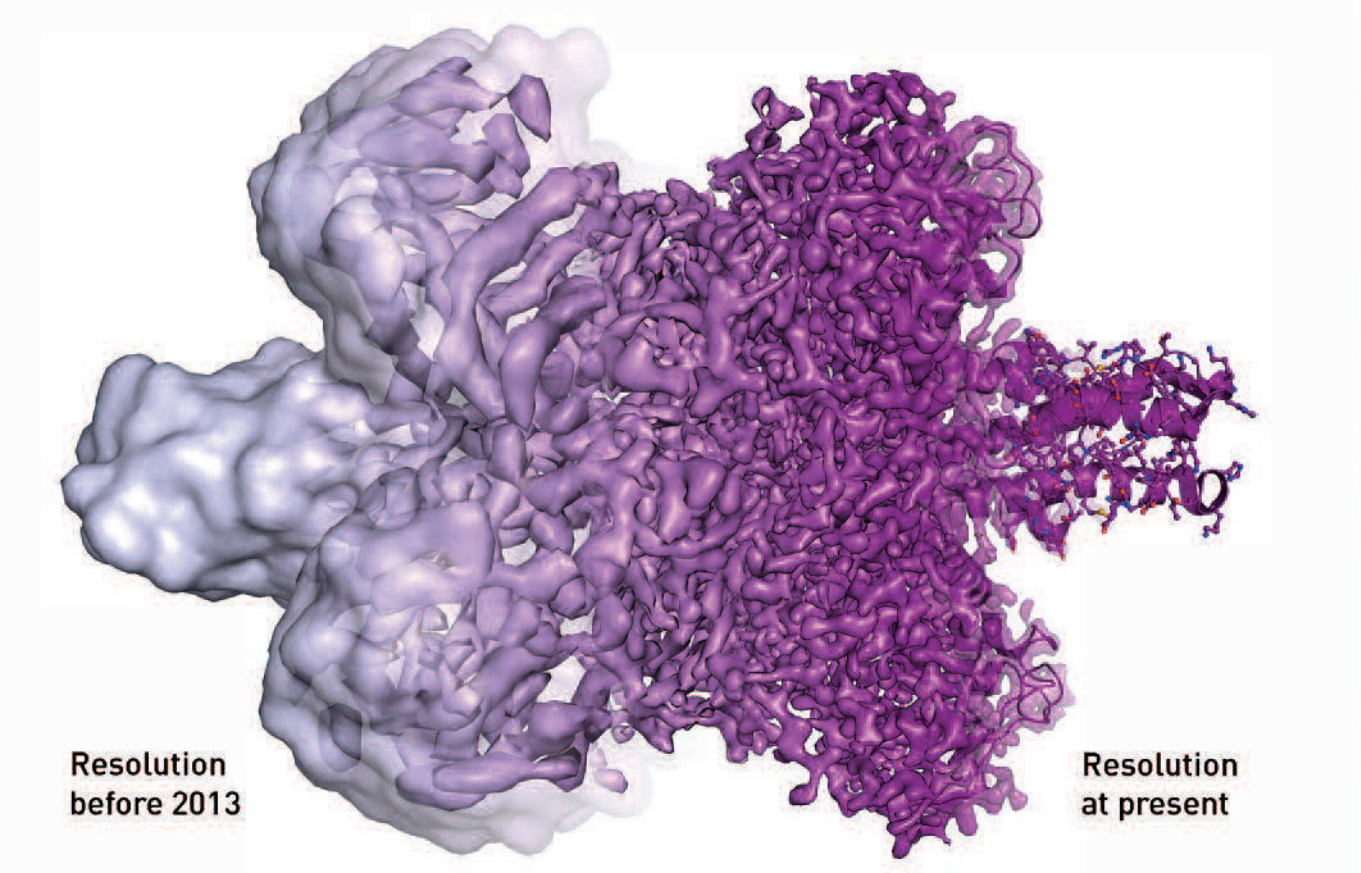
1942 First scanning electron microscope built by Ernst Ruska. It transmits a beam of electrons across specimen surface.



Dartmouth College Electron Microscope Facility

1981 Scanning tunneling microscope invented by Gerd Binnig and Heinrich Rohrer. It can give 3D images down to the atomic level.

2017 Cryo-electron microscopy allows high-resolution structure determination of biomolecules. Nobel Prize in Chemistry awarded to Jacques Dubochet, Joachim Frank and Richard Henderson.



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 (Merk A. et al. *Cell* 2016, vol. 165, pp. 1698–1707). Illustration: © Martin Högbom, Stockholm University.

between potential sample damage and limitation of resolution. EM has until now been generally only considered to be applicable to 'dead' samples.

Ultimately, this is a problem of signal-to-noise ratio. These effects typically limited EM to resolutions of a few nanometres, whereas cryo-EM potentially offers an extra order of magnitude of resolution.

Lastly, there are all the limitations of the data capture and analysis. The structure of the particles being analysed is three dimensional, and so the techniques that have been developed have had to be able to capture and then transform the electron scattering data, particularly given the complexities of low signal-to-noise ratio, randomly oriented particles, and the transformation of 2D sections into 3D visualisations.

The instrument

Collectively, the 2017 Nobel Prize represents the pinnacle of accumulated developments over decades, with individual inspiration and dedication that has combined to create a new analytical capability with revolutionary implications.

Cryo-EM uses the previously-unknown 'vitrified' state of water below around –160°C to effectively encapsulate and protect biological particles, both preserving them during analysis, and allowing virtually 'in situ' states to be analysed. It then applies some very sophisticated data capture and modelling to create very high-resolution visualisations.

One of the most important aspects of cryo-EM is its ability to analyse very small sample quantities, effectively taking it closer to single particle analysis. But the technique is also versatile across a wide particle size distribution, from nano-scale to macromolecule complexes well over 500 000 daltons. In 1995, Henderson anticipated that enzymes might be analysed to as little as 52 000 daltons, and this has improved with ongoing developments in the technology.

Having come far from its early description as 'blobology', cryo-EM offers an unprecedented degree of resolution. In 2015, Dr Sriram Subramaniam, of the National Cancer Institute's Center for Cancer Research, imaged the enzyme beta galactosidase at a resolution of just 0.22 nanometres (bit.ly/2jPTway).

The technique is already proving to be a valuable tool across a range of biological fields: botany, biotechnology, zoology, pharmaceuticals, health care and cosmetics. Understanding how biomolecules function and interact is fundamental to the development of new innovations in any of these sciences. At this level of detail and resolution, it offers a powerful tool for understanding biochemistry in its dynamic states.

In the next article, we'll look at the individual contributions from Dubochet, Frank and Henderson.

Dave Sammut FRACI CChem and **Chantelle Craig** are the principals of DCS Technical, a boutique scientific consultancy, providing services to the Australian and international minerals, waste recycling and general scientific industries. Part 2 of this article will be published in the March issue.