



THE REVOLUTION WILL NOT BE CRYSTALLIZED

**MOVE OVER X-RAY CRYSTALLOGRAPHY.
CRYO-ELECTRON MICROSCOPY IS
KICKING UP A STORM IN STRUCTURAL
BIOLOGY BY REVEALING THE HIDDEN
MACHINERY OF THE CELL.**

BY EWEN CALLAWAY

In a basement room, deep in the bowels of a steel-clad building in Cambridge, a major insurgency is under way.

A hulking metal box, some three metres tall, is quietly beaming terabytes' worth of data through thick orange cables that disappear off through the ceiling. It is one of the world's most advanced cryo-electron microscopes: a device that uses electron beams to photograph frozen biological molecules and lay bare their molecular shapes. The microscope is so sensitive that a shout can ruin an experiment, says Sjors Scheres, a structural biologist at the UK Medical Research Council Laboratory of Molecular Biology (LMB), as he stands dwarfed beside the £5-million (US\$7.7-million) piece of equipment. "The UK needs many more of these, because there's going to be a boom," he predicts.

In labs around the world, cryo-electron microscopes such as this one are sending tremors through the field of structural biology. In the past three years, they have revealed exquisite details of protein-making ribosomes, quivering membrane proteins and other key cell molecules,

ILLUSTRATION BY VIKTOR KOEN

discoveries that leading journals are publishing at a rapid clip. Structural biologists say — without hyperbole — that their field is in the midst of a revolution: cryo-electron microscopy (cryo-EM) can quickly create high-resolution models of molecules that have resisted X-ray crystallography and other approaches, and labs that won Nobel prizes on the back of earlier techniques are racing to learn this upstart method. The new models reveal precisely how the essential machinery of the cell operates and how molecules involved in disease might be targeted with drugs.

“There’s a huge range of very important biological problems that are now open to being tackled in a way that they could never before,” says David Agard, a structural cell biologist at the University of California, San Francisco.

Scheres was recruited to the LMB several years ago to help push cryo-EM technology to its limits — and he and his colleagues have done just that. Last month, they reported one of the burgeoning field’s most impressive feats: a startlingly clear picture of an enzyme implicated in Alzheimer’s disease, showing the position of its 1,200 or so amino acids down to a resolution of a few tenths of a nanometre¹.

Biologists are now pushing the technique further to deduce ever more detailed structures of small and shape-shifting molecules — a challenge even for cryo-EM. “Whether you call it revolution or a quantum leap, the fact is that the gates have opened,” says Eva Nogales, a structural biologist at the University of California, Berkeley.

CRYSTAL COAXING

Spend a bit of time with a structural biologist and they will probably mention their field’s unofficial motto: ‘structure is function’. Only by knowing the atom-by-atom arrangement of a biomolecule can researchers grasp how it works — how, for instance, the ribosome reads strands of messenger RNA to manufacture proteins, or how molecular pores flip open and shut. For decades, one technique enjoyed a near monopoly in elucidating protein structures to this level of detail: X-ray crystallography, in which scientists persuade proteins to form into crystals, then blast X-rays at them and decipher the protein’s structure from patterns that the X-rays make when they bounce off (see ‘Structure solvers’). Of the more than 100,000 entries in the Protein Data Bank, a popular repository of protein structures, about 90% were solved by this technique. It has contributed to more than a dozen Nobel prizes, including the one awarded in 1962 for revealing DNA’s double helix.

But although X-ray crystallography has been structural biologists’ best tool, it also has major limitations. It can take researchers years to find ways of forming some recalcitrant proteins into large crystals that are suitable for analysis, and many fundamentally important molecules — such as proteins that are embedded in cell membranes or that make up complex molecular machines — have defied crystallization.

X-ray crystallography was certainly king when biologist Richard Henderson arrived at the LMB in 1973 to study a protein called bacteriorhodopsin, which uses light energy to pump protons across a membrane. Henderson and his colleague Nigel Unwin had managed to make two-dimensional crystals from the protein, but they were unsuitable for X-ray diffraction. So the pair decided to try electron microscopy instead.

At the time, electron microscopy was used to study viruses or slices of tissue that had been treated with heavy-metal stains. A beam of electrons is fired at a sample, and the emerging electrons are detected and used to map out the structure of the materials they smashed into. This approach produced the first detailed image of a virus — a tobacco pathogen — but the stain made it difficult to see individual proteins, let alone the atomic details that the X-rays were revealing. “It was blobby stuff or negative-stained, and you would see outlines of molecules,” says Agard.

In a pivotal step, Henderson and Unwin omitted the stain when they used electron microscopy to image crystal sheets of bacteriorhodopsin — instead, they placed the crystals on metallic grids to make the protein stand out. “You were looking at the atoms in the protein,” says Henderson, who, with Unwin, published² the structure of bacteriorhodopsin in 1975. “That was

➔ **NATURE.COM**
See a selection of
stunning cryo-EM
structures at:
go.nature.com/cehow8

such a huge step forward,” Agard says. “That said, ‘OK, it will be possible to solve protein structures by EM.’”

The cryo-EM field developed through the 1980s and 1990s; a key advance was the use of liquid ethane to flash-freeze proteins in solution and hold them still³, which is how the ‘cryo’ came to cryo-EM. But still the technique could generally resolve structures only to more than 10 Ångströms (1 Å is one-tenth of a nanometre) — nothing to rival the

“WE SHOULD GO FOR GLOBAL DOMINATION OF CRYO-EM OVER ALL THE STRUCTURAL METHODS.”

better than 4-Å models of X-ray crystallography, and nowhere near what was needed to use the structures for drug design. While funders such as the US National Institutes of Health were ploughing hundreds of millions of dollars into ambitious crystallography initiatives, support for cryo-EM lagged far behind.

In 1997, when Henderson attended the annual Gordon Research Conference on 3D electron microscopy, a colleague opened the meeting with a provocative statement: cryo-EM was a “niche” method, he said, unlikely to ever supplant X-ray crystallography. But Henderson could see a different future, and he fired back a salvo in the next talk. “I said we should go for global domination of cryo-EM over all the structural methods,” he recalls.

THE REVOLUTION STARTS HERE

In the years that followed, Henderson, Agard and other cryo-EM evangelists worked methodically on technical improvements to electron microscopes — in particular, on better ways to sense electrons. Long after digital cameras had taken the world by storm, many electron microscopists still preferred old-fashioned film because it recorded electrons more efficiently than did digital sensors. But, working with microscope manufacturers, the researchers developed a new generation of ‘direct electron detectors’ that vastly outperforms both film and digital-camera detectors.

Available since about 2012, the detectors can capture quick-fire images of an individual molecule at dozens of frames per second. Researchers such as Scheres, meanwhile, have written sophisticated software programs to morph thousands of 2D images into sharp 3D models that, in many cases, match the quality of those deciphered with crystallography.

Cryo-EM is suited to large, stable molecules that can withstand electron bombardment without jiggling around — so molecular machines, often built from dozens of proteins, are good targets. None has proved more suitable than ribosomes, which are braced by rigid twists of RNA. The solution of ribosome structures by X-ray crystallography won three chemists the 2009 Nobel Prize in Chemistry — but those efforts took decades. In the past couple of years, ‘ribosomania’ has gripped cryo-EM researchers, and various teams have quickly determined and published dozens of cryo-EM structures of ribosomes from a multitude of organisms, including the first high-resolution models of human ribosomes^{4,5}. X-ray crystallography has largely fallen by the way-side in the LMB laboratory of Venki Ramakrishnan, who shared the 2009 Nobel. For large molecules, “it’s safe to predict that cryo-EM will largely supersede crystallography”, he says.

The rocketing number of cryo-EM publications suggests this to be true: in 2015 alone, the technique has so far been used to map the structures of more than 100 molecules. And, unlike X-ray crystallography, in which crystals lock proteins in a single, static pose, researchers can use cryo-EM to calculate the structure of a protein that has been flash-frozen in several conformations and so deduce the mechanisms by which it works.

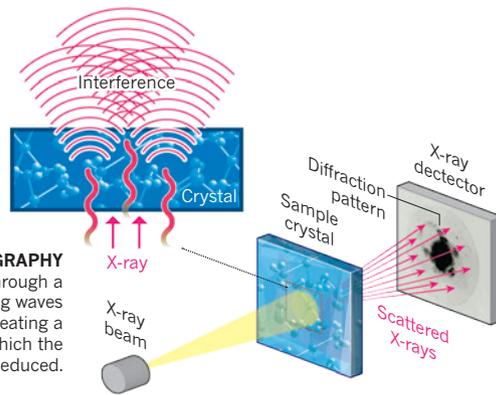
In May, structural biologist John Rubinstein at the University of Toronto, Canada, and his colleagues used around 100,000 cryo-EM images to create a ‘molecular movie’ of a rotor-shaped enzyme called V-ATPase, which pumps protons in and out of cell vacuoles by burning ATP⁶. “What we saw is that everything is flexible,” Rubinstein says. “It’s bending and twisting and deforming.” He thinks that the enzyme’s flexibility helps it to efficiently transmit energy released by ATP to the pump.

STRUCTURE SOLVERS

X-ray crystallography has long been the dominant method for deducing high-resolution protein structures, but cryo-electron microscopy is catching up.

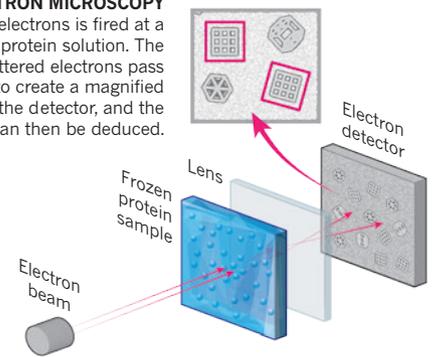
X-RAY CRYSTALLOGRAPHY

X-rays scatter as they pass through a crystallized protein; the resulting waves interfere with each other, creating a diffraction pattern from which the position of atoms is deduced.



CRYO-ELECTRON MICROSCOPY

A beam of electrons is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, and the structure can then be deduced.



X-RAY IMAGE: SPL

And when a team led by Nogales in 2013 pieced together cryo-EM images of a complex that orchestrates the transcription of DNA into RNA, they discovered that an entire arm swings 100 Å around the DNA strand like a crane, potentially influencing whether a gene is transcribed⁷. “I think this is beautiful,” says Nogales. “It’s a true insight into how these biological machines work.”

SMALL AND BEAUTIFUL

Now that cryo-EM has hit its stride, experts are looking for grander challenges. For many, the most coveted targets are smaller proteins sandwiched in cellular membranes. These tend to be linchpins in cellular signalling pathways, as well as popular drug targets. They are also notoriously difficult to crystallize, and imaging individual proteins with cryo-EM is tough because it is harder to extract the signal from the background noise.

These hurdles did not stop Yifan Cheng, a biophysicist at the University of California, San Francisco (UCSF), from attempting cryo-EM on a small membrane protein called TRPV1, which detects the molecule that gives chilli peppers their burn and is closely related to other pain-sensing proteins. A team led by his collaborator David Julius, a UCSF physiologist, had failed to crystallize the protein. The cryo-EM project was slow-going at first, but the same technical advances that drove ribosomania produced a 3.4-Å structure of TRPV1 in late 2013. The report⁸ was a thunderbolt to the field, because it showed that cryo-EM could conquer small, medically important molecules. “I literally lost an entire night’s sleep when I saw that,” says Rubinstein.

More sleepless nights are likely to follow. “There’s going to be a huge explosion in the number of membrane-protein structures that get solved,” says Agard.

One such solution was that published last month¹ by Scheres, structural biologist Yigong Shi of Tsinghua University in Beijing and their team. They produced a model of γ -secretase — a protein that makes the amyloid- β molecule that is linked to Alzheimer’s disease. The 3.4-Å-resolution map reveals that γ -secretase mutations that cause rare inherited forms of Alzheimer’s map to two ‘hotspots’ in the enzyme and seem to influence its ability to form toxic amyloid- β particles. The structure could help researchers to understand why drugs that inhibit the enzyme have failed in past clinical trials, and help them to design new pharmaceuticals. “Stunning” is how Cheng describes the structure.

Results such as these are attracting the attention of drug companies hoping to study medically important proteins that have resisted crystallography. Scheres is working with New York-based pharmaceutical giant Pfizer on ion channels, a broad class of membrane protein that includes pain-sensing molecules and neurotransmitter receptors. “I’ve been contacted by almost everybody,” says Nogales of the drug companies lining up at her door.

But despite the advances, many in the field see room for further improvement. They hope to devise better electron detectors and better methods for preparing protein samples. This would allow scientists to image proteins that are even smaller and more dynamic, and at even greater resolution than before. A 2.2-Å structure of a bacterial enzyme

published in May⁹ showed just how sharp cryo-EM structures can get.

Like any burgeoning field, this one has growing pains. Some experts worry that researchers rushing to use the technique could produce problematic results. A 2013 structure of an HIV surface protein¹⁰ was questioned by scientists who said that the images used to build the model were white noise¹¹. Since then, X-ray and cryo-EM models generated by other teams have challenged the original model, but the researchers have stood by their result¹². This June, at the field’s Gordon conference, researchers wanting more quality control passed a resolution urging journals to provide referees with details of how cryo-EM structures were created.

Costs could slow the spread of the technology. Scheres estimates that the LMB spends around £3,000 per day running its cryo-EM facility, plus another £1,000 on electricity, most of it for computers needed to store and process the images. “You’re £4,000 per day lighter if you want to do this. That, for many places, is a very high cost,” he says. To make cryo-EM more accessible, some funders have established shared facilities at which researchers can book time. The Howard Hughes Medical Institute (HHMI) operates a cryo-EM lab on its Janelia Farm Campus in Virginia that is open to HHMI-funded investigators based elsewhere. In the United Kingdom, a national cryo-EM facility funded by the government and the Wellcome Trust opened this year in Didcot, near Oxford. “There is a real tidal wave of people wanting to learn about it,” says Helen Saibil, a structural biologist at Birkbeck, University of London, who helped to establish the UK facility.

Riding the wave is Rod MacKinnon, a biophysicist at Rockefeller University in New York City, who shared the 2003 Nobel Prize in Chemistry for determining the crystal structure of certain ion channels, but who is now deep into cryo-EM. “I’m on a steep slope of a learning curve, which always thrills me,” says MacKinnon, who hopes to use the method to study how ion channels open and close.

Henderson’s tongue may have been firmly in his cheek when he declared back in 1997 that cryo-EM could rule the structural-biology world. But nearly 20 years later, his prediction is looking less like hyperbole than it did then. “If it carries on, and all the technical problems are solved, cryo-EM could indeed become, not just a first choice, but a dominant technology,” he says. “We are probably halfway there.” ■

Ewen Callaway writes for Nature from London.

1. Bai, X.-C. *et al. Nature* <http://dx.doi.org/10.1038/nature14892> (2015).
2. Unwin, P. N. T. & Henderson, R. *J. Mol. Biol.* **94**, 425–440 (1975).
3. Adrian, M., Dubochet, J., Lepault, J. & McDowell, A. W. *Nature* **308**, 32–36 (1984).
4. Amunts, A., Brown, A., Toots, J., Scheres, S. H. W. & Ramakrishnan, V. *Science* **348**, 95–98 (2015).
5. Khatter, H., Myasnikov, A. G., Natchiar, S. K. & Klaholz, B. P. *Nature* **520**, 640–645 (2015).
6. Zhao, J., Benlekhir, S. & Rubinstein, J. L. *Nature* **521**, 241–245 (2015).
7. Cianfrocco, M. A. *et al. Cell* **152**, 120–131 (2013).
8. Liao, M., Cao, E., Julius, D. & Cheng, Y. *Nature* **504**, 107–112 (2013).
9. Bartesaghi, A. *et al. Science* **348**, 1147–1151 (2015).
10. Mao, Y. *et al. Proc. Natl Acad. Sci. USA* **110**, 12438–12443 (2013).
11. Henderson, R. *Proc. Natl Acad. Sci. USA* **110**, 18037–18041 (2013).
12. Mao, Y., Castillo-Menendez, L. R. & Sodroski, J. G. *Proc. Natl Acad. Sci. USA* **110**, E4178–E4182 (2013).