

Imagine a crystal's inner life

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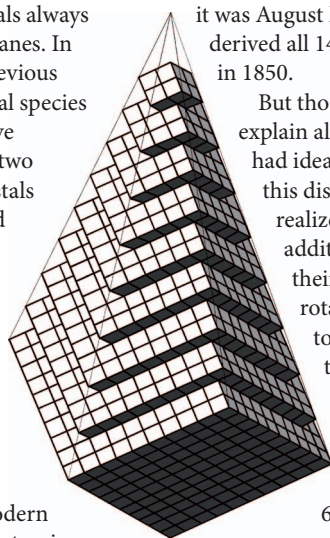
As the story goes, one of the most defining events for crystallography was a mishap. Rene-Just Haüy, a Parisian priest, had been invited to look at a friend's latest acquisition, a beautiful prismatic calcite crystal. In a careless moment, the crystal slipped out of Haüy's hands and shattered on the floor. At this time, in 1781, characterizations of crystals were solely based on their outer morphology. But Haüy's mishap led to a deeper understanding of the essential inner characteristics of the crystalline state of matter: periodicity.

On examination of the crystal's fragments, Haüy noticed that it "had a single fracture along one of the edges of the base... I tried to divide it in other directions and I succeeded, after several attempts, in extracting its rhomboid nucleus." In other words, Haüy realized that crystals always cleave along crystallographic planes. In addition, it was known from previous discoveries that in a given crystal species the interfacial angles always have the same value. Based on these two clues, Haüy concluded that crystals must be periodic and composed of stacks of little polyhedra, which he called *molécules intégrantes*. This theory could conveniently explain why all crystal planes are related by small rational numbers, a principle we nowadays refer to as the law of rational indices.

Considering how closely Haüy's theory resembles the modern concept of periodicity, it is a masterpiece of imagination. But it posed two major questions. The first one again relates to outer morphology: What is the complete

list of symmetries that a crystal can in principle possess? It was clear that only 2, 3, 4 and 6-fold rotational axes were consistent with Haüy's laws, and eventually Moritz Frankenheim (in 1826) and Johann Hessel (in 1830) concluded that this restriction results in 32 possible crystal classes.

The second question concerns the exact nature of the *molécules intégrantes*, which in Haüy's drawings look like little bricks. But this proved to be incompatible with the observation that crystals are elastic. What was missing was the concept of a space lattice. That a crystal is best described by an array of discrete points generated by defined translational operations was independently devised by Ludwig Seeber in 1824 and Gabriel Delafosse in 1840. And it was August Bravais who then famously derived all 14 possible lattice symmetries in 1850.



Haüy's concept of periodicity. Construction of a scalenohedron by stacking *molécules intégrantes*. Figure reprinted with permission from A. Authier *Early Days of X-ray Crystallography* p12 (Oxford Univ. Press, 2013).

But those 14 lattices could not explain all 32 crystal classes. Bravais had ideas about how to reconcile this discrepancy but did not realize his crucial oversight: In addition to pure translations, their combination with rotations and reflections had to be considered. It then took geometrical group theory to elaborate all possible combinations. Leonhard Sohncke took on this task, presenting 65 space groups in 1879,

but left out certain symmetry operations. The two scientists who independently sought to extend Sohncke's result were Arthur Schönflies and Evgraf Fedorov. After learning about each other's work, they started a lively correspondence, eliminating mistakes and finally, in 1891, agreeing on a catalogue of 230 space groups.

Compared with the 32 crystal classes, these concepts seemed like an unnecessary complication. And there was no means of testing the notion of a space lattice or space groups. Consequently, neither of their inventors got due credit at first. "Somehow I did not think that I would live to see the day when the distribution of atoms as I predicted it in my papers would actually be determined," Fedorov commented on the first X-ray diffraction experiments. But thanks to Max von Laue (Milestone 2) and the Braggs (Milestones 3 and 4), the concepts of the space lattice and space groups were verified earlier than Fedorov had ever hoped for.

Leonie Mueck, Associate Editor, Nature

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MILESTONE 2

A photograph of crystal order

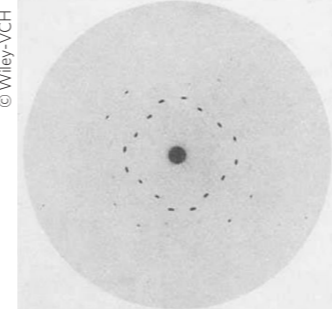
“What would happen if you assumed very much shorter waves to travel in the crystal?” This was the question posed by Max von Laue, then an associate professor at the Institute of Theoretical Physics in Munich, to Paul Ewald who was a student of Arnold Sommerfeld, director of that institute, during a discussion on the propagation of light in crystals. As it turns out, that conversation laid the basis for modern X-ray crystallography. A few months later, in April 1912, the first demonstration of X-ray diffraction from a crystal lattice was achieved.

The ‘shorter waves’ mentioned by von Laue were the X-rays discovered by Röntgen 17 years before. At the time, the nature of these rays was the subject of intense debate. The photoelectric effect showing that gas molecules are ionized by an X-ray beam indicated a corpuscular nature, whereas the observations that X-rays are polarized and can be diffracted by fine slits supported a wave-like interpretation. Several researchers

also estimated the wavelength of these rays to be around 0.5 Å, orders of magnitude smaller than light. When Ewald, who was developing a theoretical model to explain the double refraction of light passing through a crystal, described crystalline structures as a regular arrangement of resonators having a distance comparable to this short wavelength, von Laue resolved that the characteristic X-ray fluorescence emitted from these particles had to produce diffraction patterns.

At the beginning, this idea received some opposition; indeed, both Sommerfeld and Wilhelm Wien doubted that the emission coming from these atoms would be coherent and thought that the interference would be destroyed by thermal motion of the crystal. Nevertheless, in April 1912 von Laue was able to secure the help of two brilliant experimentalists, Walter Friedrich and Paul Knipping, to test his hypothesis. The two physicists used a powerful X-ray bulb and collimated a narrow primary beam on several

X-ray diffraction pattern from a zinc-blende (ZnS) crystal. Figure reprinted with permission from W. Friedrich et al. *Annalen der Physik* **346**, 971–988 (1913).



crystals (copper sulphate pentahydrate and zinc sulphide, in particular) that, according to previous studies, contained metallic species showing strong X-ray fluorescence. Looking for interference from an isotropic radiation, they first positioned a collecting photographic plate parallel to the primary X-ray beam, but detected no signal. When they added a photographic plate behind the crystal, Friedrich and Knipping finally recorded traces of the diffracted beam, proving that the intuition of von Laue was true, though only in part (Milestone 3).

The results of the experiment and their theoretical interpretation were published in August 1912. Yet even before the papers were out, the success of the experiment spread around Europe: Max Planck recalled that scientists in Berlin “felt that a remarkable feat had been achieved” and Albert Einstein defined the experiment as “among the most glorious that physics has seen so far”. The interference patterns supported the

Society. During the previous summer, W. H. Bragg discussed at great length with his son William Lawrence — who was just a 22-year-old student at Cambridge University at the time — possible explanations for von Laue’s experiment. That the son eventually succeeded in dissuading the father from his particle-based theory is evident in a later correspondence with Alfred Tutton.

“Dr. Tutton suggests that the new experiment may possibly distinguish between the wave and corpuscular theories of the X-rays,” writes W. H. Bragg in 1912. “...the properties of X-rays point clearly to a quasi-corpuscular theory, and certain properties of light can be similarly interpreted. The problem then becomes, it seems to me, not to decide between two theories of X-rays, but to find, as I have said elsewhere, one theory which possesses the capacities of both.” This, of course, is another story.

W. L. Bragg rejected von Laue’s assumption that the atomic structure of zinc-blende was a simple cubic structure with atoms sited at each corner. Instead, he considered a face-centred cubic lattice. And whereas von Laue believed the X-ray radiation in his experiments comprised five distinct wavelengths, W. L. Bragg assumed that the incident beam was a continuous spectrum that reflected from successive planes in the atomic structure.

“A minute fraction of the energy of a pulse traversing the crystal will be reflected from each

interpretation of X-rays as electromagnetic waves. Remarkably, these findings also had an exceptional resonance among crystallographers: those well-defined spots were seen as conclusive evidence that atoms arrange in a space-lattice configuration in crystals. As Alfred Tutton — an English crystallographer — stated in November 1912 “the space-lattice structure of crystals . . . is now rendered visible to our eyes” (Milestone 1).

Luigi Martiradonna, Associate Editor, Nature Materials

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plane in succession, and the corresponding interference maximum will be produced by a train of reflected pulses. The pulses in the train follow each other at intervals of $2d \cos \theta$, where θ is the angle of incidence of the primary rays on the plane, d is the shortest distance between successive identical planes in the crystal. Considered thus, the crystal actually ‘manufactures’ light of definite wavelengths...” And so, Bragg’s equation was born, albeit in a slightly different form to the expression used today, and a bridge established between the atomic and the macroscopic.

David Gevaux, Senior Editor, Nature Communications

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MILESTONE 3

The equation to bridge worlds

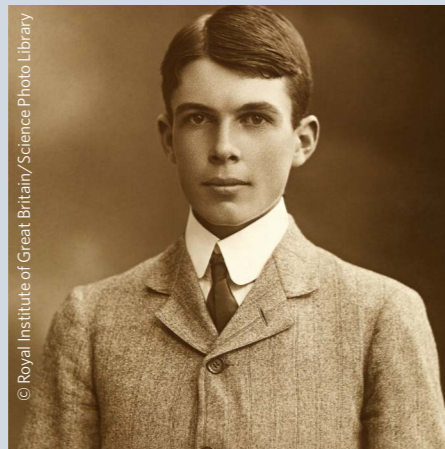
It was immediately clear from the photographic plates of Max von Laue, Walter Friedrich and Paul Knipping that X-rays opened a link between atomic structure and the macroscopic world (Milestone 2). What was missing was a way to quantify this link. But in 1912, the nascent field of X-ray crystallography had two main handicaps: little knowledge of the atomic structure of crystals and even less about the nature of X-rays.

Von Laue himself made the connection between his results and optical diffraction: if X-rays were a form of electromagnetic radiation with a wavelength similar to the space between atoms, then such a pattern could be formed just like visible light reflecting from a ruled grating. Where he went wrong, however, was to overstate the role of the atoms themselves. He believed deflected X-rays were emitted from the atoms after they had been excited by the primary incoming beam.

Whereas von Laue’s “optical feeling” led him, and others, to look to diffraction for answers, William Henry Bragg and Johannes Stark separately subscribed to the idea that X-rays were particles. They believed that X-rays incident

on a crystal were siphoned off into “avenues” or channels created by the atomic lattice.

But whether corpuscular or wave-based, none of these theories could quantifiably predict the positions of the spots as seen by von Laue in his experiment. On 11 November 1912, a solution that accounted for them all was presented to the Cambridge Philosophical



W. L. Bragg as an undergraduate student at Cambridge University.

MILESTONE 4

A new crystallography is born

The adrenaline rush accompanying the thrill of a scientific discovery was palpable in the summer of 1913 in the laboratory of William Henry Bragg in Leeds, UK. “It was a glorious time, when we worked far into every night with new worlds unfolding before us in the silent laboratory,” recalls his son William Lawrence Bragg. In rapid succession, father and son solved the structures of several inorganic crystals and of diamond. Underpinning this flurry of activity was the intuition that X-rays were reflected from planes of atoms in crystals and the discovery of Bragg’s law (Milestone 3). Also crucial was the development of an X-ray spectrometer.

W. H. Bragg, in his 50s at the time of von Laue’s experiment (Milestone 2), had already been working on X-rays for several years and had become a master in handling X-ray tubes and ionization chambers. Although these pieces of equipment were extremely delicate to work with, he managed to assemble an instrument that became an essential tool for the nascent technique of X-ray crystallography. It was developed from an optical spectrometer, in which the diffraction element was replaced by the crystal under study. X-rays emanating from a tube were collimated to the sample and the reflected radiation was collected in an ionization chamber with a gold-leaf electroscope. The main difference of this design with respect to von Laue’s photographic plate set-up was that the X-rays were measured in reflection rather than in transmission — a key advance that followed the discovery of specular reflection of X-rays from mica in 1912. This geometry gave the Braggs the flexibility to detect the reflected X-rays for different angles of incidence one at the time, and single out individual angles of reflection from the layers of atoms in the crystal. Using Bragg’s law, the crystal structure of the sample could then be derived.

In the beginning, assigning a structure was largely a matter of imagining atoms in space; those that could be solved were the simplest ones, mostly limited to face-centred cubic crystals. Nevertheless, at a time in which even the notion of an ionic crystal was not well established, the structural characterization of a salt as simple as NaCl was of major significance. Perhaps the most striking demonstration of the power of the new analysis was the determination of the structure of diamond, which conclusively

confirmed the tetravalency of carbon, as postulated for many organic compounds.

Bragg’s X-ray spectrometer required fairly large crystals, often only accessible through the goodwill of mineralogist friends. In 1916–1917, however, Paul Debye, Paul Sherrer and, independently, Alfred Hull showed that powder (polycrystalline) samples also diffracted X-rays, opening up X-ray analysis to many more types of crystal. Experiments with powders were conceived from the idea that the diffraction of X-rays was due to their interaction with the electrons of the atoms in the lattice. Because, according to the Bohr model, electrons are in a defined spatial location relative to the nucleus, diffraction should also occur in randomly oriented crystals (a coherent theory behind this phenomenon would be due to Arthur Compton). An important upshot of this insight was that powder diffraction spectra provided a means to directly compare scattered intensities coming from all crystal planes, something that was not possible using the Bragg spectrometer.

As more and more crystal types could be measured, the complexity of the data analysis increased. Already in 1915, W. H. Bragg had proposed to use Fourier transform to convert the two-dimensional crystallographic patterns obtained at different angles into a three-dimensional map of electron density, an idea that greatly assisted the blossoming of powder diffraction experiments (Milestone 15).

Alberto Moscatelli, Senior Editor, Nature Nanotechnology

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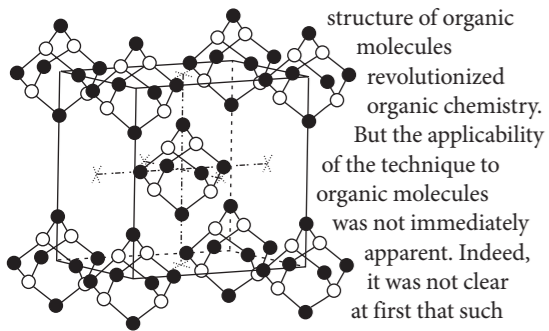
The X-ray spectrometer built by W. H. Bragg.

MILESTONE 5

Molecular visions

For today's organic chemists, analytical techniques have reached a level of sophistication that enables the structures of even the most complicated molecules to be determined quickly. By comparison, chemists in the 1920s were working in the dark: structural analysis required painstaking experiments and logical deductions; and the validation of proposed structures usually involved several supporting chemical syntheses.

The discovery that X-ray crystallography enabled the direct determination of the structure of organic molecules revolutionized organic chemistry. But the applicability of the technique to organic molecules was not immediately apparent. Indeed, it was not clear at first that such



Crystal lattice of hexamethylenetetramine (Carbon atoms, black; nitrogen atoms, white). Figure reprinted with permission from R. Dickinson and A. L. Raymond *J. Am. Chem. Soc.* **45**, 22–29 (1923).

molecules would retain their own identity within crystals.

The first direct evidence came in 1923, when two groups — one at Caltech and the other in Berlin — independently reported the first complete and accurate crystal structures of hexamethylenetetramine ($C_6H_{12}N_4$). They opted to study this compound because it was one of the few without salt character to form crystals of cubic symmetry, greatly simplifying the structural determination. What's more, there are only two molecules of hexamethylenetetramine per unit cell, which meant that the positions of the carbon and nitrogen atoms could be determined from just two parameters. The work confirmed the chemical formula of the compound, proved its structure and demonstrated that molecules could indeed pack to form crystals.

The next real breakthrough was the remarkable work of Kathleen Lonsdale, who used X-ray crystallography to solve one of the biggest mysteries of chemistry: the structure of the benzene ring. Chemists had long depicted the benzene ring as a flat hexagon, but always entertained the possibility that it wasn't planar. Several scientists proposed that

benzene's carbon atoms were arranged in a puckered hexagon, in two superposed planes. X-ray crystallography spurred attempts to study benzene, but although the size of the unit cell, the space group and the number and approximate positions of molecules in the cell had been determined, the actual arrangement of atoms remained elusive.

These problems stimulated others to study benzene derivatives, perhaps most notably William Henry Bragg, who worked on naphthalene and anthracene. Although his findings allowed the width of a benzene ring to be calculated, they did not settle the issue of whether benzene rings are flat or puckered. The structure of graphite, fully determined in 1924, conclusively proved that the atoms of this carbon allotrope are arranged in flat sheets of hexagons. The findings put a new complexion on the benzene problem, but did not solve it.

Lonsdale chose to study hexamethylbenzene — not an easy subject, because the crystals are triclinic, and therefore not of high symmetry. But hexamethylbenzene did offer several advantages compared with benzene and other benzene derivatives: it is solid at room temperature, and has only one molecule per unit cell, which avoided the problem of accounting for relative orientations.

Lonsdale found that the (001) plane of the crystal gave exceptionally strong reflections, the higher orders of which fell off uniformly

in intensity, in the same way as those from the basal plane of graphite. This observation led unavoidably to the conclusion that hexamethylbenzene is flat. Lonsdale went on to perform a detailed analysis comparing her data with those of several models, but found that only a planar hexagonal model provided a satisfactory fit. Her accomplishment underpinned the analysis of all aromatic molecules to this day.

These early findings eventually led to a reversal of the roles of structural determination and organic synthesis: crystal structures could be used to validate chemical syntheses, rather than syntheses being used to validate proposed structures.

Andrew Mitchinson, Chief Editor, News & Views, Nature

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MILESTONE 6

Fingerprinting minerals

Before the discovery of X-ray diffraction, the most powerful tool for analysing minerals was the polarized light microscope, which, despite providing valuable morphological data, was unable to deliver accurate information about the structural arrangement of atoms within crystals. The very first crystal structures to be determined by X-ray crystallography were those of minerals, and with the invention of X-ray powder diffraction in 1916–1917 (Milestone 4), structural mineralogy had its boom in just a couple of years.

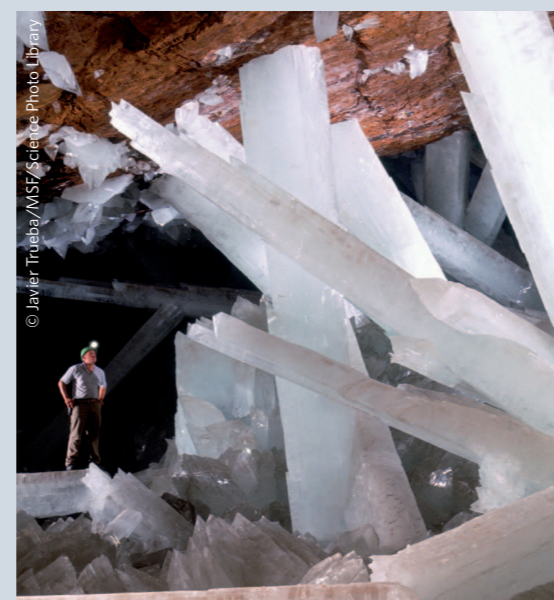
When William Henry Bragg and R. E. Gibbs started to study quartz, many other simpler structures had already been disentangled, but quartz kept baffling scientists because of its complexity. It was only in 1925 that the structures of α - and β -quartz became known. This marked the beginning of extensive work on silicates by many researchers, with the main input coming from the Braggs' school.

As the number of crystal structures being determined kept growing, the need emerged

to rationalize some theoretical principles to interpret the data. In 1926, Victor Goldschmidt distinguished between atomic and ionic radii, and postulated some rules for atom substitution in crystal structures. Inspired by his work, Linus Pauling realized that those principles were not always sufficient to describe the structure of complex ionic crystals and formulated a new set of rules of his own. These rules accounted for the importance of coordination polyhedra and were first put in practice in the study of zeolites.

This was a very fruitful period for structural mineralogy. In 1928, Felix Machatschki, who was working with Goldschmidt, showed that silicon could be replaced by aluminium in feldspar structures, an observation reinforced by the work of William Taylor some years later.

Finally, in 1930, with all the information gathered thus far, William Lawrence Bragg put together the first comprehensive classification of silicates, describing their structure in terms of grouping of SiO_4 tetrahedra, isolated as in olivine;



Gypsum crystals inside the Cave of Crystals in Naica, Mexico.

or in chains, rings or sheets as in diopside, beryl or mica, respectively; or in frameworks as in zeolites and feldspars.

Meanwhile, mineralogists had turned their attention to the study of crystal defects and

imperfections. As these microstructures can be related to the natural processes involved in their formation, these studies provide useful hints for understanding the growth environment in which many natural minerals are found.

Nowadays, X-ray crystallography remains a valuable tool in Earth and planetary science. The structure and behaviour of minerals under extreme conditions, such as those found in the deep Earth, are routinely investigated using high-pressure crystallography. And with the X-ray spectrometer installed in NASA's rover Curiosity, the composition and past environmental conditions of the surface of Mars is being uncovered.

Mara Silva, Associate Editor, Nature

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MILESTONE 7

Getting directly to the structure

During diffraction experiments, X-ray radiation is scattered by the atoms present in the crystal and the intensity of the scattered X-rays are measured as reflections. However, the phase differences for the scattered X-rays are not known. Recovering this phasing information has been the focus of many efforts by many researchers.

In the early structure determinations, where there were only a few atoms in the unit cell, the atoms usually sat on symmetry elements and it was possible, with some guesswork, to deduce the positions of the atoms, given the space group was known. Indeed, for many years, researchers put pencil to paper, using trial and error as they tested their assumptions about the reflections' phase to determine the structure of their molecule of interest.

In 1934, Lindo Patterson published his keen insight on the use of Fourier theory to narrow the phasing search. His equation, called the Patterson function, used diffraction intensities to determine the interatomic distances within a crystal, setting limits to the possible phase values. Shortly thereafter, David Harker found that symmetry-related atoms produced peaks in the Patterson function at certain crystal planes. These findings cut down on manual computation time and allowed researchers to examine structures of even greater complexity, creating a boon for organometallic crystallography, which had heavy atoms that provided stronger diffraction intensities as guideposts. Organic compounds as well as molecules with more than -50 atoms remained a challenge.

In the early 1950s, David Sayre suggested that the phase problem could be more easily solved if you had at least one more intensity measurement beyond those of the Bragg peaks (Milestone 3) in each dimension. This idea was inspired by Claude Shannon's work on communication theory and is a concept understood today as oversampling. William Lawrence Bragg and Max Perutz, building on earlier work examining the dehydration of haemoglobin crystals, came to similar, but perhaps less precise, conclusions using Fourier analysis.

Meanwhile, Jerome Karle and Herbert Hauptman inferred that relationships must exist between the diffracted waves, as there were usually more measured reflections than atoms in the crystallized molecule. In

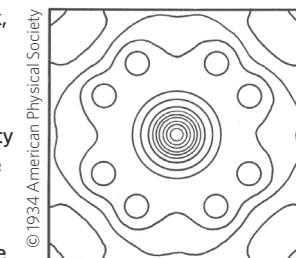
Nobel prize-winning work, they were able to deduce such relationships based on the idea that a molecule's electron density can never have a negative value. Using probability theory, they developed extremely useful formulae for phase determination, known as direct methods. Sayre later developed an equation that gave rise to dominant relationships in triplets of strong reflections that produced similar results.

It took some time for the statistical methods proposed by Karle and Hauptman to gain traction, but the rapid increase in the strength of computing power helped the methods and their derivatives obtain widespread acceptance. Computationally intensive iterative techniques use a very simple mathematical framework developed from the above-mentioned oversampling methods.

Direct methods and the Patterson function are most effectively applied to the determination of small-molecule structures. Macromolecular crystallographers had to await the development of isomorphous replacement (Milestone 12), molecular replacement (Milestone 13) and anomalous diffraction (Milestone 19) techniques to address their phasing problems. However, direct methods and the Patterson function are routinely used as part of these structure solution efforts.

Michelle Montoya, Senior Editor, Nature Structural & Molecular Biology

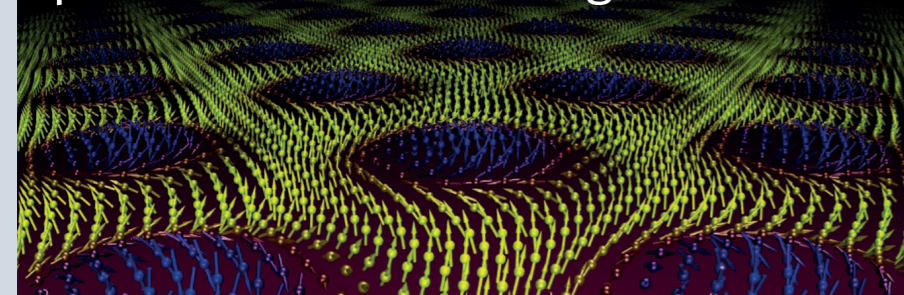
ORIGINAL RESEARCH PAPERS Patterson, A. L. A Fourier series method for the determination of the components of interatomic distances in crystals. *Phys. Rev.* **46**, 372–376 (1934) | Karle, J. & Hauptman, H. The phases and magnitudes of the structure factors. *Acta Crystallogr.* **3**, 181–187 (1950) | Sayre, D. Some implications of a theorem due to Shannon. *Acta Crystallogr.* **5**, 843 (1952) | Bragg, L. & Perutz, M. F. The structure of haemoglobin. *Proc. R. Soc. Lond.* **213**, 425–435 (1952) | Hauptman, H. & Karle, J. *Solution of The Phase Problem. I. The Centrosymmetric Crystal* (ACA Monograph No. 3, Polycrystal Book Service, 1953) **FURTHER READING** Harker, D. The application of the three-dimensional Patterson method and the crystal structure of proustite, Ag_3AsS_3 , and pyrrargyrite, $Ag_2Sb_2S_5$. *J. Chem. Phys.* **4**, 381–390 (1936) | Bernal, J. D., Fankuchen, I. & Perutz, M. F. An X-ray study of chymotrypsin and hemoglobin. *Nature* **141**, 523–524 (1938) | Shannon, C. E. Communication in the presence of noise. *Proc. Inst. Radio Eng. NY* **37**, 10 (1949) | Sayre, D. The squaring method: a new method for phase determination. *Acta Crystallogr.* **5**, 60–65 (1952)



A map section presented in Patterson's 1934 publication. Figure reprinted with permission from A. L. Patterson *Phys. Rev.* **46**, 372–376 (1934).

MILESTONE 8

Spins and arrows of outrageous fortune



By the time neutron radiation was discovered in the 1930s, X-ray diffraction was already widely used and highly successful in revealing the inner structure of crystals (Milestone 6). It was soon suggested that neutron rays could be put to similar use. After all, particles can be treated like waves, as the previous decade had taught, and free neutrons would have a wavelength comparable to the spacing of atoms in a crystal. However, it was thought at the time that neutron sources were too weak for neutron diffraction to be a practical tool.

This radically changed during the grim and purposeful years of the Second World War, which saw the completion of the first nuclear reactors. When the war came to an end, scientists such as Ernst Wollan rallied to make the best possible

A lattice of skyrmions — whirlpools of magnetic moments that can be revealed with neutron diffraction.

use of the newly available facilities. In 1946, he started his first neutron scattering experiments at the Oak Ridge nuclear reactor in Tennessee, a large graphite block measuring 7 m on each side and pierced with uranium rods. Clifford Shull joined him and they set to work developing the principles of neutron diffraction.

In 1949, a modest one-page report by Shull and James Samuel Smart appeared in *Physical Review*, reporting just a few neutron scattering peaks for manganese oxide, a material with a simple cubic crystal. The paper did no less than confirm an outstanding prediction in fundamental magnetism. In 1932, Louis Néel suggested that

some materials can be magnetically ordered, yet are not overall magnetic. In these so-called antiferromagnets, the elementary magnetic moments, or spins, would have alternating orientations and cancel each other out. Neutrons have spin themselves so they are tiny magnets. Smart therefore suggested that neutron diffraction might be able to directly detect antiferromagnetism. He was right. The paper simply shows that an additional peak appears in the neutron scattering pattern for manganese oxide at low temperature (80 K) that is absent at room temperature. This is because at the lower temperature, antiferromagnetic order sets in and the magnetic unit cell, which has to contain an 'up' and a 'down' spin, is twice the size of the chemical cell.

This opened up a new field of magnetic crystallography, with a unique role for neutron scattering. But early on, Shull and Wollan identified another advantage of neutrons. Whereas X-rays scatter against electrons and barely notice a light element such as oxygen, neutrons interact equally well with light and heavy elements. This proved very useful in unravelling the structure of complex oxides, such as the high-temperature superconductor yttrium barium copper oxide in the late 1980s.

When the field of neutron scattering began in the 1940s, the availability of sources, which was limited to nuclear reactors, hindered progress. However, this was overturned with

the introduction of new collaboration schemes (Milestone 16), where a large community of scientists have access to a national facility. Nowadays, many neutron facilities exist over the world and neutron scattering is an essential tool in materials research with wide impact, such as in optimizing energy-storage materials and unravelling the structure of viruses and proteins.

In fundamental science, neutron scattering remains invaluable as a probe of unusual forms of magnetism. A unique capability of the technique is that it can also measure fundamental excitations to structural and magnetic ground states, and therefore how the magnetic moments are correlated to each other. In 2009 for example, a neutron diffraction experiment revealed a striking type of magnetic order — that of stable whirlpools of spins called skyrmions. These particle-like entities are examples of topological order in materials, an emerging research theme where neutron scattering will no doubt play an important role in the decades to come.

Liesbeth Venema, Senior Editor, Nature

ORIGINAL RESEARCH PAPERS Shull, C. G. & Smart, J. S. Detection of antiferromagnetism by neutron diffraction. *Phys. Rev.* **76**, 1256 (1949) | Muhlbauer, S. *et al.* Skyrmion lattice in a chiral magnet. *Science* **323**, 915–919 (2009) **FURTHER READING** Mason, T. E., Gawne, T. J., Nagler, S. E., Nestor, M. B. & Carpenter, J. M. The early development of neutron diffraction: science in the wings of the Manhattan Project. *Acta Crystallogr. A* **69**, 37–44 (2013)

MILESTONE 9

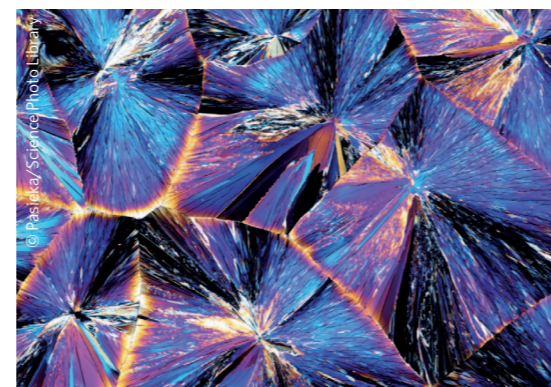
Spatial awareness

In the late 1800s, chemists started to develop ways to depict the spatial arrangements of atoms and groups in molecules possessing one or more stereogenic centres in two dimensions, and sought a means to distinguish between stereoisomers. At this time, however, only relative stereochemistry could be determined. More specifically, it was possible to recognize that two molecules with the same atom connectivity, but which rotate the plane of polarized light in opposite directions, are enantiomers (that is, mirror images of each other). The absolute configuration of the atoms and groups around a stereogenic centre, however, remained uncertain. In 1951, Bijvoet *et al.* removed this uncertainty using X-ray crystallographic techniques.

About 50 years before this revelation, Emil Fischer suggested a way of representing stereoisomers by drawing a projection in two dimensions. These drawings, named Fischer projections, were used originally to depict

carbohydrate stereochemistry but became steadily more widely used with amino acids and other organic molecules. In a completely arbitrary assignment, Fischer classified the configuration of glucose to be the + isomer (more specifically, the isomer that rotates the plane of polarized light in a clockwise direction) and the L configuration to be the - isomer. This connection was further developed by Martin Rosanoff in the classification of other chiral molecules including the assignment of D-(+)-glyceraldehyde and L-(-)-glyceraldehyde.

Bijvoet and his colleagues confirmed that this arbitrary assignment of absolute configuration, as depicted in Fischer projections, in relation to the direction of optical rotation of polarized light, was correct after all. They arrived at this conclusion by X-ray diffraction analysis of the sodium-rubidium salt of the natural (+)-form of tartaric acid using a phenomenon called anomalous scattering, which relies on the



A light micrograph of tartaric acid crystals.

ability of certain atoms to absorb X-rays strongly. The development of tunable X-ray sources would make anomalous scattering a useful technique for protein crystallography (see Milestone 19), but at the time of Bijvoet's experiment, a heavy atom (the rubidium atom of the sodium-rubidium double salt) similar in atomic weight to the element used to generate the X-rays (here, zirconium) was needed. The ability to excite solely the rubidium ion, and not the rest of the atoms in the crystal, made it possible to determine the absolute configuration of all the atoms in the crystal rather than just the

relative stereochemistry, which is typically obtained using X-ray crystallographic methods. Bijvoet *et al.* revealed that the natural (+)-form of tartaric acid indeed has the L-configuration as was assumed by Fischer.

The confirmation of the absolute configuration of atoms or groups around stereogenic centres using anomalous X-ray diffraction gave chemists the possibility to establish, with certainty, the absolute stereochemistry of a molecule they had isolated or synthesized. In addition, the chemical community could breathe a sigh of relief — the configurations of the many chiral molecules depicted in textbooks and in the chemical literature during more than half a century leading up to 1951 were correct, and there was no need to go back to the drawing board.

Alison Stoddart, Senior Editor, Nature Materials

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MILESTONE 10

An iron-clad structure

In 1951, Peter Pauson and his student Tom Kealy set out to make an unusual hydrocarbon called pentafulvalene, in which two cyclopentadiene rings are joined together through a carbon-carbon double bond. Although this particular target eluded them, their experiments resulted in the formation of a remarkably stable compound made of carbon, hydrogen and iron — a compound that arguably started a revolution in organometallic chemistry.

Analytical data revealed that the elemental composition of this unexpected product was consistent with the formula $C_{10}H_{10}Fe$, but what was the structure? Because the reaction used a starting material with a five-membered ring, it was reasonable to conclude that the final product simply contained two of these rings — the harder part was figuring out how they were bonded to the iron atom.

Pauson and Kealy suggested a linear structure in which the two rings were bonded to opposite sides of the metal, each through a single carbon atom. The same structure was proposed by Miller, Tebboth and Tremaine in a paper published just a couple of months later (although it had been submitted for publication almost a month earlier) describing a different synthesis of the same compound.

It wasn't long before other chemists started to question the linear structure suggested in these first two reports, most notably Robert Woodward and Geoffrey Wilkinson at Harvard University and Ernst Fischer at the Technische Hochschule in Munich. Woodward and Wilkinson were the first to suggest a sandwich-like structure (although they did not use that term), whereby the iron atom sits nestled between the faces of two five-membered rings stacked on top of one another. The supporting experimental evidence was, however, still indirect, with the strongest hint being that the infrared absorption spectrum revealed that all of the C-H bonds were equivalent — which would not be the case in the linear structure.

The first use of X-ray crystallography to help decipher the structure of what had now been christened 'ferrocene' by Woodward and co-workers was described by Fischer and Wolfgang Pfab later in 1952. Preliminary data about the symmetry of the molecule and the size of the unit cell were much more consistent with the sandwich structure than the linear one. Two further X-ray crystallographic

An early drawing of the chemical structure of ferrocene. Figure reprinted with permission from E. O. Fischer and R. Jira. *J. Organomet. Chem.* **637-639**, 7–12 (2001)

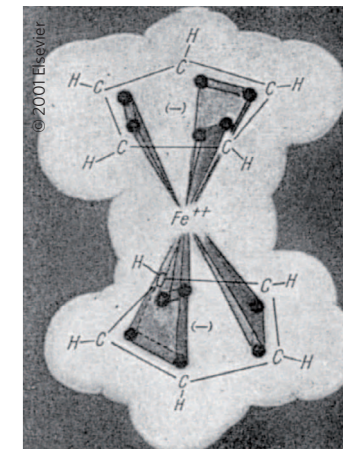
studies finally confirmed the sandwich structure of ferrocene, with the iron atom, indeed providing the metallic filling in a pentagonal antiprism defined by two parallel cyclopentadienyl rings.

These studies finally laid to rest any scepticism surrounding the true three-dimensional arrangement of the 21 atoms in ferrocene, and three years later more precise structural details — including more accurate C-C and C-Fe bond distances — were reported.

It soon became apparent that ferrocene was just the tip of the iceberg and many other metals were found to be suitable fillings for molecular sandwiches. And that wasn't the only part of the sandwich that could be varied — other aromatic systems could be used in the place of the five-membered cyclopentadienyl rings. The importance of the discovery of ferrocene and other sandwich compounds for the field of organometallic chemistry was underlined by the award of the Nobel Prize in Chemistry in 1973 to Fischer and Wilkinson.

Stuart Cantrill, Chief Editor, Nature Chemistry

ORIGINAL RESEARCH PAPERS Kealy, T. J. & Pauson, P. L. A new type of organo-iron compound. *Nature* **168**, 1039–1040 (1952) | Miller, S. A., Tebboth, J. A. & Tremaine, J. F. Dicyclopentadienyliron. *J. Chem. Soc.* 632–635 (1952) | Wilkinson, G., Rosenblum, M., Whiting, M. C. & Woodward, R. B. The structure of iron bis-cyclopentadienyl. *J. Am. Chem. Soc.* **74**, 2125–2126 (1952) | Woodward, R. B., Rosenblum, M. & Whiting, M. C. A new aromatic system. *J. Am. Chem. Soc.* **74**, 3458–3459 (1952) | Fischer, E. O. & Pfab, W. Cyclopentadien-metallkomplexe, ein Neuer Typ Metallorganischer Verbindungen. *Z. Naturforsch. B* **7**, 377–379 (1952) | Eiland, P. F. & Pepinsky, R. X-ray examination of iron bis-cyclopentadienyl. *J. Am. Chem. Soc.* **74**, 4971 (1952) | Dunitz, J. D. & Orgel, L. E. Bis-cyclopentadienyl iron: a molecular sandwich. *Nature* **171**, 121–122 (1953) | Dunitz, J. D., Orgel, L. E. & Rich, A. The crystal structure of ferrocene. *Acta Crystallogr.* **9**, 373–373 (1956) **FURTHER READING** Wilkinson, G. The iron sandwich. A recollection of the first four months. *J. Organomet. Chem.* **100**, 273–278 (1975) | Pauson, P. L. Ferrocene — how it all began. *J. Organomet. Chem.* **637-639**, 3–6 (2001) | Fischer, E. O. & Jira, R. How metallocene chemistry and research began in Munich. *J. Organomet. Chem.* **637-639**, 7–12 (2001) | Werner, H. At least 60 years of ferrocene: the discovery and rediscovery of the sandwich complexes. *Angew. Chem. Int. Ed.* **51**, 6052–6058 (2012)



MILESTONE 11

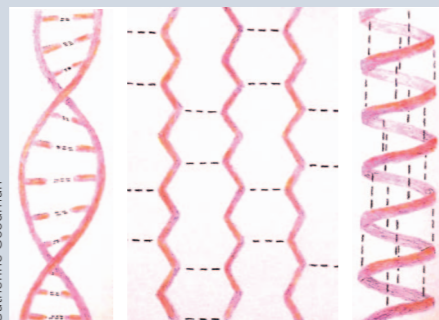
A tale of two twists

In the middle of the twentieth century, there was general consensus that a protein consisted of a single polypeptide chain that formed a unique structure that could be reversibly denatured. In 1936, Alfred Mirsky and Linus Pauling had also put forward their concept that “the importance of the hydrogen bond in [this] protein structure can hardly be overemphasized,” yet the structures themselves remained unknown.

Over the next 15 years, Pauling set himself and his colleagues to the careful task of identifying “all hydrogen bonded structures for a single polypeptide chain,” aided by crystal structures of amino acids and short peptides to define bond lengths and angles. They were inspired by the work of others from the field, including previously abandoned efforts by William Astbury that included a description of a helical ‘ α -form’ and a stretched ‘ β -form’. Pauling and Corey, in 1950, announced the conclusions of their efforts: the existence of a ‘plane’ and two ‘spiral’ structures as the primary elements of protein configurations. A series of publications in 1951 provided extensive details of these structures,

defining the β -sheet and α -helix, along with their atomic coordinates; this body of work contributed to Pauling’s receipt of the Nobel Prize in Chemistry in 1954.

The structure of DNA and its biological importance were also unresolved in the first half of the 1900s. Although DNA had been identified as the basis for bacterial transformation, leading some to argue that it was the hereditary material, others were convinced other molecules were responsible. At one point, DNA’s structure was believed to be a ‘tetranucleotide’ (in which each ‘molecule’ of DNA contained one of each of the four nucleotide bases), and only in the 1930s was it established that DNA was a macromolecule. Scientists also had different predictions of its structure, including Pauling’s suggestion of a triple helix.



MILESTONE 12

The first of its kind

In 1937, an ambitious project was initiated with the aim of determining the molecular structure of haemoglobin, the protein in red blood cells that transports oxygen. Haemoglobin forms crystals that diffract X-rays, but even a beautiful diffraction pattern still requires phases to solve the three-dimensional structure (see Milestone 7) and it was this problem that Max Perutz and his colleagues were confronted with.

In 1954, David Green, Vernon Ingram and Perutz published the seminal paper describing how, in principle, X-ray diffraction could be used for the direct determination of a protein structure.

To obtain the crucial phases, Perutz and his colleagues used the isomorphous replacement method, whereby they introduced

‘heavy’ atoms (in this case mercury) into the haemoglobin crystal, taking advantage of the complexes formed between mercury-containing compounds and the free sulphur groups present in haemoglobin. Comparing the differences in intensities between the diffraction spots from a heavy-atom-containing crystal and the normal crystal allowed them to determine the location of the mercury atoms and from that information the phases of the X-rays — solving the so-called ‘phase problem’. As William Lawrence Bragg pointed out, this heavy-atom technique works because “the molecule takes no more notice of such an insignificant attachment than a maharaja’s elephant would of the gold star painted on its forehead”.

It would have seemed from this breakthrough that the structure determination of haemoglobin was just around the corner. In fact, it took six years of hard work before Perutz was able to publish the structure of haemoglobin, at a resolution of 5.5 Å. To obtain the structure, it was necessary to analyse thousands of reflections and, as

In 1950, the pieces started to fall together when Erwin Chargaff reported that adenine and thymine, and separately guanosine and cytosine, were present in approximate 1:1 ratios. Jerry Donohue, a crystallographer who had worked with Pauling, helped James Watson and Francis Crick understand the significance of Chargaff’s result by correcting the chemical structures of the nucleotide bases they were using, leading to the correct A–U and G–C pairings. The crystallographic work on DNA fibres was initiated by Maurice Wilkins and perfected by Rosalind Franklin. It was Franklin’s unpublished diffraction patterns, seen without her knowledge, that provided key features to Watson and Crick, who built a model that was consistent with the existing data. The three groups then published back-to-back papers describing DNA as a double helix with the phosphates on the outside of the helix, containing strands running in reverse directions and pairing purine and pyrimidine bases across the helix. Wilkins’s and Franklin’s manuscripts primarily focused on the basic crystallographic details, whereas Watson and Crick laid out the full model and hinted at the functional implications of their proposal. Once they were able to study the crystallographic data in full, Watson and Crick then published a more daring paper just a month later, describing in detail how “the precise sequence of the bases is the code

Perutz pointed out, “we have in fact been very fortunate, because the development of computers has always just kept in step with the expanding needs of our X-ray analyses.” After completing this complex raw-data analysis, the four polypeptide chains of haemoglobin could be traced into the calculated electron density, which were described as resembling the vapour trails of an airplane. Intriguingly, each of these four chains resembled the structure of the much smaller iron- and oxygen-binding muscle protein myoglobin, for which a preliminary 6-Å resolution structure had been reported in 1958 by John Kendrew.

Myoglobin had presented the researchers with different challenges from haemoglobin. Importantly, myoglobin did not have the sulphur atoms required to bind to mercury atoms in the same way as haemoglobin. Several hundred possible heavy-atom-containing ligands were empirically tested, and finally mercury- and gold-containing ligands were found to bind isomorphously to myoglobin, allowing its structure to be determined. In 1960, John Kendrew and his colleagues reported the structure of sperm whale myoglobin to a resolution of 2 Å, which required the assessment of 10,000 reflections. Crucially, in this high-resolution structure the details of the atomic interactions could

which carries the genetic information.” The 1962 Nobel Prize in Physiology or Medicine was awarded to Crick, Watson and Wilkins (Franklin’s untimely death made her ineligible for the award) for this discovery, which both galvanized the emerging field of molecular biology at that time and has led to extensive knowledge of the genetic basis of heredity and disease since then.

Catherine Goodman, Senior Editor, Nature Chemical Biology

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FURTHER READING Astbury, W. T. & Woods, H. J. X-ray studies of the structure of hair, wool, and related fibres. II. The molecular structure and elastic properties of hair keratin. *Phil. Trans. R. Soc. Lond. A* **232**, 333–394 (1934) | Pauling, L. & Corey, R. B. Two hydrogen-bonded spiral configurations of the polypeptide chain. *J. Am. Chem. Soc.* **72**, 5349 (1950) | Chargaff, E. Chemical specificity of nucleic acids and mechanism of their enzymatic degradation. *Experientia* **6**, 201–209 (1950) | Watson, J. D. & Crick, F. H. C. Genetical implications of the structure of deoxyribonucleic acid. *Nature* **171**, 964–967 (1953)

be seen, including the right-handed α -helices and the position of the haem group within the structure.

The advances represented by this structural work were rapidly recognized within the scientific community, and resulted in the award of the Nobel Prize in Chemistry in 1962 to Perutz and Kendrew “for being the first to successfully identify the structures of complex proteins.” In his acceptance speech, Kendrew looked forward to a day in the future when structural predictions would allow X-ray crystallographers to “go out of business, perhaps with a certain sense of relief”; this day is yet to come.

Rebecca Kirk, Senior Editor, Nature Communications

ORIGINAL RESEARCH PAPERS Green, D. W., Ingram, V. M. & Perutz, M. F. The structure of haemoglobin. IV. Sign determination by the isomorphous replacement method. *Proc. Royal Soc. Lond. A* **225**, 287–307 (1954) | Perutz, M. F. et al. Structure of haemoglobin: a three-dimensional Fourier synthesis at 5.5-Å resolution, obtained by X-ray analysis. *Nature* **185**, 416–422 (1960) | Kendrew, J. C. et al. Structure of myoglobin: a three-dimensional Fourier synthesis at 2 Å resolution. *Nature* **185**, 422–427 (1960)

FURTHER READING Kendrew, J. C. et al. A three-dimensional model of the myoglobin molecule obtained by X-ray analysis. *Nature* **181**, 662–666 (1958) | Bragg, W. L. in *Fifty Years of X-ray Diffraction* Ch. 8 (ed. Ewald, P. P.) 120–136 (IUCR, Oosthoek, 1962) | Perutz, M. F. Nobel Lecture: X-ray Analysis of Haemoglobin (Nobel Foundation, 1962) | Kendrew, J. C. Nobel Lecture: Myoglobin and the Structure of Proteins (Nobel Foundation, 1962)

MILESTONE 13

A method ahead of its time

In the 1950s and early 1960s, interest in using X-ray diffraction to solve protein crystal structures was growing. But determining the missing phase information for protein crystals proved to be much more of a challenge than for simple small molecules, for which direct methods (Milestone 7) could be applied. At the time, the sole method available for recovering phases for protein crystals was multiple isomorphous replacement using heavy-atom doping (Milestone 12), a challenging and cumbersome approach. A paper published by Michael Rossmann and David Blow in 1962 laid the foundation for the molecular replacement approach, which would grow to provide crystallographers with a powerful option for solving the phase problem without requiring any additional experimental effort. (A third class of phasing methods, including single-wavelength and multiwavelength anomalous dispersion, would later follow; Milestone 19.)

Rossmann and Blow’s fundamental insight was that the phenomenon of non-crystallographic symmetry — structural similarity found in different parts of the asymmetric unit of a crystal lattice’s unit cell — could be exploited to recover the phases required for structure determination. They derived a rotation function that could be applied to orient molecules relative to one another. Even in this early report, Rossmann and Blow astutely recognized that the concept could also be applied to find relationships between similar molecules in different crystal lattices. By applying additional translation procedures — reported a few years later — to superimpose the molecules, the missing phase information could be obtained.

The use of non-crystallographic symmetry to recover phases would not be called ‘molecular replacement’ for another ten years, when Rossmann published a book, collecting and reviewing the early papers, entitled *The Molecular Replacement Method*. The definition of molecular replacement grew to cover all methods exploiting non-crystallographic symmetry within or between crystals to obtain phase information.

The true power of the concept, however, did not really ‘crystallize’ until decades after Rossmann and Blow’s seminal report. Today, the term molecular replacement is most often used to refer to the particular, though most common, practice of using a known



A two-dimensional illustration of non-crystallographic symmetry. Figure reprinted with permission from M. G. Rossmann *The Molecular Replacement Method* (Gordon & Breach, 1972).

homologous atomic-resolution structure as a search model to interpret the phases of an X-ray diffraction pattern of an unknown protein structure. Two crucial developments that helped solidify the central importance of the approach were (1) advances in computation, including both hardware improvements and software tools to automate molecular replacement calculations, and (2) the growing availability of high-quality, atomic-resolution protein structures, helped along by structural genomics efforts, to serve as search models. If a search model with more than 30% sequence homology can be identified, then there is a good chance that the phase information can be recovered for the unknown structure.

Most protein crystal structures today are solved using modern molecular replacement methods. The continual development and improvement of software tools that identify structural homology and automate molecular replacement calculations and model refinement have made it a key method in the crystallographer’s toolbox.

Allison Doerr, Senior Editor, Nature Methods

ORIGINAL RESEARCH PAPERS Rossmann, M. G. & Blow, D. M. The detection of sub-units within the crystallographic asymmetric unit. *Acta Cryst.* **15**, 24–31 (1962) **FURTHER READING** Crowther, R. A. & Blow, D. M. A method of positioning a known molecule in an unknown crystal structure. *Acta Cryst.* **23**, 544–548 (1967) | Rossmann, M. G. *The Molecular Replacement Method* (Gordon & Breach, 1972) | Bricogne, G. Geometric sources of redundancy in intensity data and their use for phase determination. *Acta Cryst.* **A30**, 395–405 (1974) | Rossmann, M. G. The molecular replacement method. *Acta Cryst.* **A46**, 73–82 (1990) | Scapin, G. Molecular replacement then and now. *Acta Cryst.* **D69**, 2266–2275 (2013)

MILESTONE 14

Electrons for crystallography

The early realization that electrons diffract as waves provided a clear basis for their use in diffraction experiments. It was by exploiting their charge, however, that electrons eventually came into their own with the invention of the electron microscope.

In 1927, Hans Busch calculated that the magnetic field of a short coil acts on an electron beam just as a convex glass lens does on light, and that the focal length of such a magnetic lens can be tuned by the current coil. Ernst Ruska and Max Knoll pursued this notion further, and reasoned that it should be possible to obtain enlarged images of objects by focusing an electron beam. In 1931, they succeeded in making an apparatus based on this principle — the first electron microscope. The magnification of their instrument was extremely modest, but Ruska and Knoll estimated a resolution limit of 2.2 Å. In 1937, they teamed up with industrial partner Siemens, who in 1939 delivered the first serially manufactured instrument capable of magnifications of over 20,000 times.

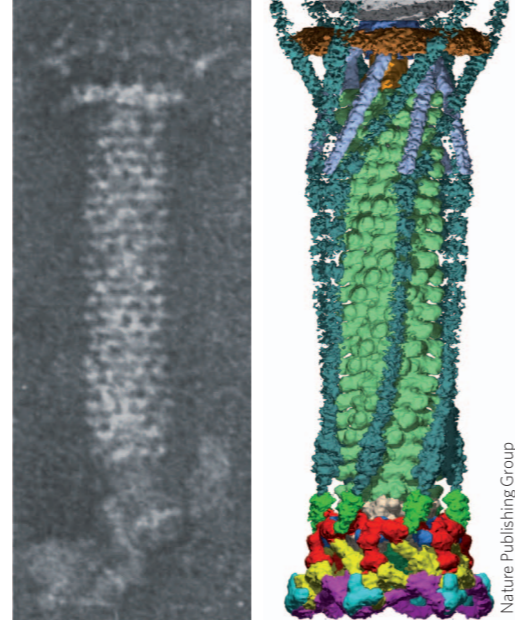
The electron crystallography pioneers used only diffraction patterns to solve crystal structures. Compared with X-ray diffraction, the method required samples to be extremely

thin to avoid multiple scattering artefacts. This made the technique perfect for the study of surfaces and nanoscale structures — a classic example being the discovery of quasicrystals (Milestone 20).

From the intensities in diffraction patterns, only scattered amplitudes can be directly deduced; phases are generally lost. Various techniques to overcome the notorious 'phase problem' known from X-ray crystallography (Milestone 7) were also adapted to electron diffraction.

One aspect, however, was waiting to be exploited. The phase information present in diffracted electron beams can be recovered by focusing them back into a two-dimensional, real-space projection by means of the magnetic lenses of an electron microscope. In 1968, David DeRosier and Aaron Klug demonstrated that starting from a limited set of electron microscopy images, they could reconstruct the original structure in three dimensions. They successfully applied this procedure to the tail of bacteriophage T4, a common virus, thus signalling a major breakthrough in macromolecular structure determination.

Klug would eventually be awarded the Nobel Prize in Chemistry for his role in the



The tail of bacteriophage T4 as recorded in an electron micrograph in 1968 (left) and in a reconstruction from cryo-electron microscopy in 2005 (right). Figures reproduced from: Left, D. J. DeRosier and A. Klug *Nature* **217**, 130–134 (1968); right, V. A. Kostyuchenko *et al.* *Nature Struct. Mol. Biol.* **12**, 810–813 (2005).

development of crystallographic electron microscopy. The great power of the technique lies in its ability to form images of atoms and molecules directly. Subsequent work on two-dimensional protein crystals and electron tomography led to the modern field of cryo-electron microscopy, a crucial tool for determining the structure of biomolecules that cannot be crystallized.

Other ways for going from sample to structure with electrons have been explored over the years — for example, by making comparisons of high-resolution electron micrographs and simulated images, an

information to be obtained and analysed, well beyond the simple structure of a material. The method is widely used in metallurgy, mineralogy, forensic science, archaeology, condensed-matter physics, and the biological and pharmaceutical sciences, and it can provide information on quantitative phase analysis, strain and defect distribution. The technique also played a central role in the recent geochemical analyses carried out by the Curiosity rover on the surface of Mars.

As Rietveld himself put it in 2002, "I am totally amazed looking at the ever increasing use that is being made of the method. [...] What began as a solution for a particular problem, turned out to be a tool of much broader value."

Fabio Pulizzi, Chief Editor, Nature Nanotechnology

ORIGINAL RESEARCH PAPERS Knoll, M. & Ruska, E. Das Elektronenmikroskop. *Z. Phys.* **78**, 318–319 (1932) | DeRosier, D. J. & Klug, A. Reconstruction of three dimensional structures from electron micrographs. *Nature* **217**, 130–134 (1968) | O'Keefe, M. A., Buseck, P. R. & Iijima, S. Computed crystal structure images for high resolution electron microscopy. *Nature* **274**, 322–324 (1978) | Hövmöller, S., Sjögren, A., Farrants, G., Sundberg, M. & Marinder, B.-O. Accurate atomic positions from electron microscopy. *Nature* **311**, 238–241 (1984)

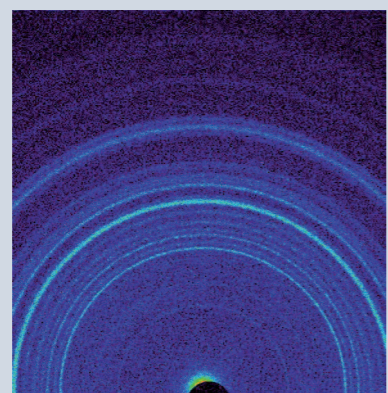
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MILESTONE 15

Powder struggle

"The powder method has gained a new importance in neutron diffraction owing to the general lack of large specimens for single-crystal methods". With this brief opening sentence of his landmark paper in 1969, Hugo Rietveld had explained very clearly the scenario of solid-state crystallography at the time.

In a powder diffraction experiment, the sample is composed of a large number of randomly oriented crystalline grains, so that the peaks from scattering by diffraction of all lattice planes can in principle be detected at the same time. The technique originated several decades before Rietveld's work, when Peter Debye and Paul Scherrer performed X-ray diffraction experiments that led them to discover the structure of graphite in 1916 (Milestone 4). Around 30 years later, the first neutron powder diffraction experiments appeared. Although it had proved useful to solve relatively simple structures, by the 1960s it was realized that powder diffraction was unwieldy to



Powder diffraction pattern of a sample of the soil of Mars, collected by the Curiosity rover. Figure reprinted with permission from D. L. Bish *et al.* *Science* **341**, 6153 (2013).

use with structures of increasing complexity. The presence of multiple phases, different grain sizes, and reflections due to the experimental conditions, for example the sample or detector geometry, were generating a large number of overlapping and intermixing peaks that were difficult to separate.

Hugo Rietveld was a crystallographer at the Netherlands Energy Research Foundation, and one of his tasks was to unveil the structures of possible fuels for nuclear reactors, for which only

powder samples existed. He had experience in using computer software to model diffraction patterns from single-crystalline samples, and he set off to extend his previous work to powder diffraction patterns. The software he generated used some of the parameters that could be extracted from the actual data, like the peaks' positions, intensities and widths, to calculate a theoretical diffraction profile. This was used as a starting point for an iterative least-squares fit to the measured spectrum. In Rietveld's method, a curve-fitting procedure for the whole diffraction pattern replaces a comparison of individual peak intensities.

The first concept and results were published in 1967, and a second publication two years later provided a more comprehensive description of the procedure now known as the Rietveld refinement method. The 1969 paper is specifically about neutron diffraction but it speculates about extending the method to X-ray powder diffraction, which would eventually be achieved almost a decade later, and is still widely used today.

The secret of the method's longevity and widespread use is its strong computational nature. With time, ever-growing computational power has allowed increasing amounts of

approach initiated by Sumio Iijima and colleagues in the 1970s — and electron crystallography has now become a vast field. Modern inventions include electron precession diffraction and aberration-corrected electron microscopy; the latter is now extensively used in materials science.

It is fair to say that with DeRosier and Klug's triumph in 1968, electrons secured their place in the history of crystallography. The countless developments in electron microscopy since only make Ruska's words in 1986, when accepting his Nobel Prize in Physics, more relevant than ever: "It is a miracle that by now the difficulties have been solved to an extent that so many scientific disciplines today can reap its benefits."

Bart Verberck, Associate Editor, Nature Physics

ORIGINAL RESEARCH PAPERS Knoll, M. & Ruska, E. Das Elektronenmikroskop. *Z. Phys.* **78**, 318–319 (1932) | DeRosier, D. J. & Klug, A. Reconstruction of three dimensional structures from electron micrographs. *Nature* **217**, 130–134 (1968) | O'Keefe, M. A., Buseck, P. R. & Iijima, S. Computed crystal structure images for high resolution electron microscopy. *Nature* **274**, 322–324 (1978) | Hövmöller, S., Sjögren, A., Farrants, G., Sundberg, M. & Marinder, B.-O. Accurate atomic positions from electron microscopy. *Nature* **311**, 238–241 (1984)

FURTHER READING Klug, A. *Nobel Lecture: From Macromolecules to Biological Assemblies* (Nobel Foundation, 1982) | Ruska, E. *Nobel Lecture: The Development of the Electron Microscope and of Electron Microscopy* (Nobel Foundation, 1986) | Glaeser, R., Downing, K., DeRosier, D., Chiu, W. & Frank, J. *Electron Crystallography of Biological Macromolecules* (Oxford Univ. Press, 2007) | Zou, X., Hövmöller, S. & Oleynikov, P. *Electron Crystallography: Electron Microscopy and Electron Diffraction* (Oxford Univ. Press, 2011) | Spence, J. C. H. *High-Resolution Electron Microscopy* 4th edn (Oxford Univ. Press, 2013)

As Rietveld himself put it in 2002, "I am totally amazed looking at the ever increasing use that is being made of the method. [...] What began as a solution for a particular problem, turned out to be a tool of much broader value."

Fabio Pulizzi, Chief Editor, Nature Nanotechnology

ORIGINAL RESEARCH PAPERS Rietveld, H. M. Line profiles of neutron powder-diffraction peaks for structure refinement. *Acta Crystallogr.* **22**, 151–152 (1967) | Rietveld, H. M. A profile refinement method for nuclear and magnetic structures. *J. Appl. Crystallogr.* **2**, 65–71 (1969)

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MILESTONE 16

More than the sum of the parts

When France and Germany agreed to build a joint research centre for neutron science at the 1964 Geneva Conference on the Peaceful uses of Atomic Energy, it was celebrated as a historic symbol of post-war collaboration between the two countries. By the time the Institut Laue-Langevin (ILL) eventually opened in 1972 in Grenoble, however, few could have anticipated that it would also go on to redefine the very meaning of international scientific collaboration.

The pioneering neutron scattering experiments carried out in the late 1940s (Milestone 8) made clear the immense potential of the technique for addressing fundamental physics problems. However, it was not immediately obvious how this potential could be unlocked: in contrast to X-ray science, there was very little familiarity with neutrons in the wider research community outside the national laboratories that housed the nuclear reactors built in North America, Europe and the Soviet Union from the 1950s onwards.

Collaborations with university scientists were initially set up informally, but as these started to grow in number there was an inevitable need for more formal agreements. In the mid-1950s, a seemingly sensible, but ultimately far-reaching development took place in the United Kingdom. The national body responsible for funding university research and graduate training agreed to pay the Atomic Energy Research Establishment, which operated the neutron facilities, to allocate a part of its neutron scattering facilities and some 'beamtime' — literally the time required to use the beam of neutrons to perform the scattering experiments — to be used by university scientists. Other countries quickly followed suit, and by the early 1960s, a community of neutron scatterers had established itself internationally.

The ILL opened in 1972 as the world's first independent user facility, funded and dedicated entirely for fundamental research. Although initially focused on achieving technical excellence in neutron instrumentation and technology, under the visionary leadership of Rudolf Mössbauer, the ILL successfully brought the user system to the international arena. Mössbauer understood that it was not enough for the ILL to be a world leader in science and technology; it also needed to provide an important service to the wider scientific community.



G. Stoltenberg, the German Minister for Research & Technology (left) and A. Peyrefitte, the French Minister for Research (right) agree to build the Institut Laue-Langevin.

In parallel to the developments in neutron science, large-scale X-ray facilities were also set up using the synchrotron technology spawned by high-energy physics research programmes such as CERN. As for neutrons, atomic and solid-state physicists had understood the enormous potential intense and tunable beams of X-rays had for their research. Significantly, however, the power of synchrotron radiation also got noticed by the community of life scientists, especially for resolving the structure of proteins. By the 1980s, the demand for access to large-scale facilities, be they neutron or X-ray sources, came from scientists across all the disciplines.

Formal user programmes are now the template on which almost all national and international central facilities around the world are based on, and they have massively increased the collaborative nature of research. To perform their experiments, researchers can tap into the expertise of dedicated instrument scientists based locally at the facility, so they do not need to be specialists themselves and can focus on the science at hand.

Without the proper organizational structure, the scientific potential of modern-day large-scale facilities would go unrealized. Their spectacular success across the scientific disciplines is a testament to the efficiency of the modern user programme.

Andrea Taroni, Senior Editor, Nature Materials

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MILESTONE 17

Sharing the structures

In 1971, protein crystallographers attending the Cold Spring Harbor Symposium on 'Structure and Function of Proteins at the Three Dimensional Level' began to discuss the idea of a central, open repository for protein structural data. Later that year, a short statement in *Nature New Biology* officially announced the establishment of the Protein Data Bank (PDB), a repository for protein crystallographic data initially run as a collaboration between the Brookhaven National Laboratory and the Cambridge Crystallographic Data Centre.

At the time, sharing crystallographic data was a fundamentally challenging endeavour. Receiving and distributing structural data required shipping paper punch cards or magnetic tape through the mail, and computer hardware and software needed to visualize or analyse these data were

still rare. In 1974, three years after launch, the PDB had less than twenty structures available for distribution in its repository.

Working at Brookhaven, with the collection of protein structures that would grow into the PDB, Edgar Meyer developed the first general software tools for handling and visualizing protein structural data. In 1971, he published a description of the first software for interactive three-dimensional visualization of protein structures, and then, in 1974, a software for storing and searching protein structures in the PDB. The latter included a brief description of a system that permitted remote computers to connect and search data stored at Brookhaven — an early forerunner to the web-based systems we now take for granted.

These early tools, and the PDB itself, were part of a long-standing tradition within the crystallography community of openly sharing code and software. Building on this tradition in 1979, scientists in the UK, including David Blow, Tom Blundell and Eleanor Dobson, founded the Collaborative Computational Project Number 4 (CCP4) to provide protein crystallographers with software tools for processing and analysing crystallographic diffraction data. CCP4 evolved into a suite of programs that are still used to this day.

In the 1980s, as techniques for structure determination improved and supporting computer technologies became more widely available, the number of structures deposited at the PDB began to grow dramatically. By the end of the decade, the value of the PDB had become sufficiently evident that structural biologists, led by Fred Richards, began to argue that deposition of structural data to the PDB should be required of all scientists in the field.

As a testament to the success of these early efforts, the PDB now hosts more than 100,000 structures, of which more than 87,000 are derived from X-ray crystallography. Today, the PDB is mirrored and distributed from centres on three continents: the Research

Laboratory of Structural Bioinformatics (RCSB PDB) in the US, EMBL-EBI's Protein Data Bank in Europe (PDBe) and PDB Japan (PDBj). All structures are provided freely and without restriction, and many journals routinely require deposition of protein structures and the associated experimental data to the PDB as a prerequisite for manuscript publication.

Andrew L. Hufton, Managing Editor, Scientific Data

ORIGINAL RESEARCH PAPERS Protein Data Bank *Nature New Biol.* **233**, 223 (1971) | Meyer, E. F. Jr Interactive computer display for the three dimensional study of macromolecular structures. *Nature* **232**, 255-257 (1971) | Meyer, E. F. Jr Storage and retrieval of macromolecular structural data. *Biopolymers* **13**, 419-422 (1974) | Meyer, E. F. Jr et al. CRYNET, a crystallographic computing network with interactive graphics display. *Fed. Proc.* **33**, 2402-2405 (1974) | Collaborative Computational Project. The CCP4 suite: programs for protein crystallography. *Acta crystallogr. D* **50**, 760-763 (1994)

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MILESTONE 18

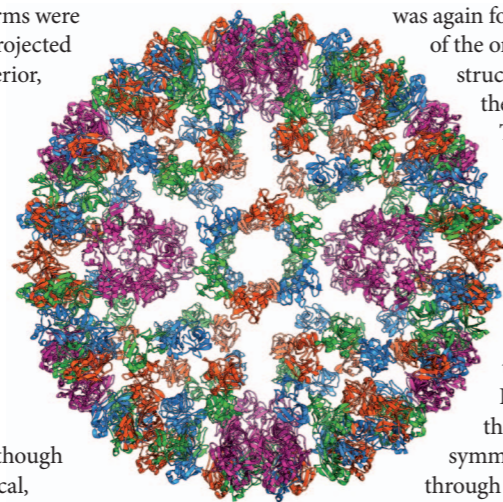
From chemistry and physics to biology

The early 1980s marked the expansion of crystallographic analysis from organic materials and isolated proteins to more complex biological samples. The first crystal structures of plant RNA viruses revealed the inherent symmetry of viral capsid assemblies, and the subsequent elucidation of a surface antigen of influenza virus provided the first structure of pathological relevance.

Tomato bushy stunt virus (TBSV) consists of a single RNA packaged in an icosahedral capsid of 180 identical coat protein subunits. The virus self-assembles *in vitro* from its RNA and protein components, and electron microscopy images had revealed the spherical capsid shape. In 1978, Harrison and colleagues provided the first high-resolution structural view of how the coat protein subunits are arranged to produce a spherical virus. The TBSV coat protein was found to be composed of two linked domains and a flexible N-terminal arm. Surprisingly, the N-terminal regions of only 60 of the coat subunits were ordered, and these interacted with each other in groups of three to form a β -annulus structure with a three-fold axis of symmetry. In the remaining 120 subunits,

the N-terminal arms were disordered and projected into the virus interior, presumably making contacts with the RNA. These subunits made distinct interprotein contacts with their neighbours to form a combination of pentamers and hexamers on the capsid surface; although chemically identical, they formed different three-dimensional structures.

Two years later, Abad-Zapatero and colleagues solved the crystal structure of the southern bean mosaic virus (SBMV) capsid. The capsid was found to share the same quasi-equivalent arrangement of protein subunits in the viral shell as TBSV, despite the absence of one of the two domains of the TBSV coat protein, and a β -annulus structure



was again formed by interactions of the ordered N-terminal structures of one third of the constituent subunits. Together, the symmetric arrangement of hexamers and pentamers within these two capsid structures exemplified the "quasi-equivalence" theory of Caspar and Klug, which predicted that icosahedral symmetry could be achieved through identical protein subunits whose specific interactions are determined by their position within the viral capsid.

In 1981, Wilson *et al.* provided the first glimpse of a surface antigen of a human viral pathogen. The influenza virus adds two glycoproteins, haemagglutinin and neuraminidase, to the lipid membrane envelope that it acquires as it emerges from its cellular host. Haemagglutinin is

a homotrimer that binds host cell surface receptors to initiate viral entry, and the receptor binding site lies within a globular region that is projected from the membrane surface by a triple-stranded bundle of α -helices. The globular domain also contains the variable antigenic determinants that are the targets of neutralizing antibodies. Each protein monomer has both ends anchored in the membrane, forming a distinctive loop that connects a short and a long α -helix. The latter interacts with other subunits of the trimer to form the extended 'stem', and the membrane-proximal region contains the activation peptide whose cleavage triggers membrane fusion.

These studies provided the first correlation of viral structures with the receptor binding and antigenic properties that contribute to their infectivity, and firmly established the relevance of crystallography to biomedical research.

Beth Moorefield, Associate Editor, Nature Structural & Molecular Biology

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MILESTONE 19

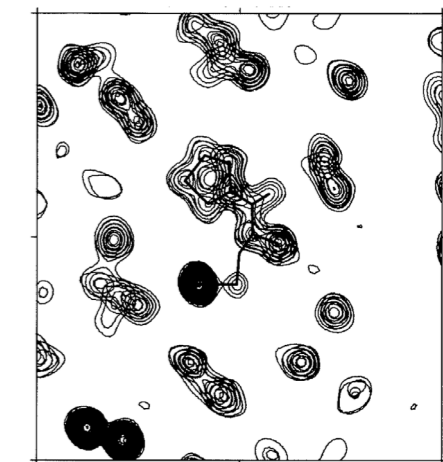
Anomalous diffraction tackles phasing

In 1979, Martha Teeter and Wayne Hendrickson generated crystals of crambin, a small, hydrophobic protein found in the seeds of Abyssinian cabbage. These crystals diffracted to a remarkable 0.88 Å resolution, so Hendrickson and Teeter expected that the structure would reveal a degree of detail on a par with small molecules at the time. However, they had yet to determine the phase of the diffracted beam. Normally, they could make use of differences in the diffraction pattern after isomorphous replacement with heavy metals to help them resolve phases (Milestone 12). Unfortunately, the crambin crystal resisted these attempts. How, then, could its structure be solved?

Hendrickson had previously located the position of the two iron atoms in haemerythrin — and was in the process of determining the structure of trimeric haemerythrin — using the phenomenon of anomalous scattering of native iron using a process that was later called single-wavelength anomalous diffraction, or SAD. As crambin has six sulphur atoms arranged in three disulphide bonds, Hendrickson and Teeter wondered if a similar approach based on the anomalous scattering of sulphur could be used to solve the crambin structure. This was a long shot as anomalous scattering is most useful when the X-ray wavelength is close to the absorption edge of the atom being studied, and their X-ray source had a much shorter wavelength (1.54 Å) than the absorption edge of sulphur (5.02 Å).

Nevertheless, weak anomalous scattering was detected and the positions of disulphide units were determined. With the phase solved, Hendrickson then went to work with pencil and paper, solving the initial structure by hand, working outwards from the sulphur atoms. Further rounds of refinement and revision resulted in the final 1.5-Å structure, published in 1981.

The application of synchrotron radiation to protein crystallography in the 1970s (Milestone 16) offered the possibility of generating X-rays with different wavelengths. As early as 1956 it was predicted that simple protein structures could be solved by collecting data at different wavelengths. The theoretical basis for multiple wavelength anomalous diffraction (MAD) was first laid down by Jerome Karle (for which he was awarded the Nobel Prize in Chemistry in



A portion of the electron density map of crambin. Figure reprinted with permission from W. A. Hendrickson and M. M. Teeter *Nature* **290**, 107-113 (1981).

1985) and further developed by Hendrickson. This new development simplified the collection of data by allowing diffraction data and phase information to be collected from the same crystal. Initial protein structures contained native heavy metals such as iron or copper, but the introduction of methods to replace methionine with selenomethionine allowed MAD (and SAD) to be applied to proteins that do not bind metals. Although the use of selenomethionine is still popular, modern data collection and statistical phasing approaches now make it possible to use MAD and SAD approaches on unlabelled protein, using the anomalous scattering of sulphur just as Hendrickson and Teeter did nearly 35 years ago.

Kyle R. Legate, Assistant Editor, Nature Communications

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MILESTONE 20

What is a crystal?

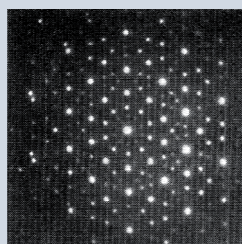
Until the 1980s, scientists felt confident that they knew the answer to this question: a crystal is a regular repeating arrangement of unit cells. Then, in 1984, Daniel Shechtman published work that challenged this definition and sent the crystallography community into turmoil.

Shechtman was investigating an alloy of aluminium that had been rapidly cooled to prevent the material from crystallizing. Electron diffraction allowed him to focus in on small regions of the material. One region of the solid produced a clean diffraction pattern, which suggested 10-fold rotational symmetry and no periodic translational symmetry, that is, the crystal seemed to be aperiodic. This was very odd. In a conventional crystal, only 1-, 2-, 3-, 4- and 6-fold symmetries are possible, with unit cells that completely fill the space available in a periodic manner. In Shechtman's own words "crystals cannot and do not exhibit icosahedral (5-fold) point group symmetry". So these were not crystals and yet they diffracted like a crystal. What on earth was going on?

Towards the end of 1984, a paper from physicists Dov Levine and Paul Steinhardt suggested an answer. The researchers were inspired by the work of mathematician Roger Penrose, who developed two-dimensional aperiodic patterns from pre-designed tiles. In other work, crystallographer Alan Mackay showed that a diffraction pattern from Penrose's aperiodic tiling had 5-fold symmetry. Levine and Steinhardt showed that, at least theoretically, such aperiodicity could also exist in three dimensions. These structures had no unit cell and no periodic translational order but had long-range bond orientational order, which generated a defined diffraction pattern. They called the structures quasicrystals. Levine and Steinhardt acknowledged the compatibility of their findings with Shechtman's experimental data.

Many researchers refused to accept the quasicrystal theory and proposed other explanations for Shechtman's discovery. Two-time Nobel prize winner Linus Pauling

Electron diffraction pattern from a metal alloy displaying a ten-fold rotational symmetry. Reprinted with permission from D. Shechtman *et al.* *Phys. Rev. Lett.* **53**, 1951-1953 (1984).



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was a particularly vocal critic and famously excoriated the researchers: "There are no such things as quasicrystals, only quasi-scientists." In a Letter to *Nature* he challenged Shechtman's analysis of the data, concluding instead that the sample was a multiply twinned cubic crystal. "Crystallographers can now cease to worry that the validity of one of the accepted bases of their science has been questioned," he announced. *Nature's* editor John Maddox suggested that Pauling had put a cat among the pigeons. But Shechtman and his colleagues fought back, citing experimental evidence to undermine Pauling's model. "The pigeons will endure," they retorted.

The quasicrystal theory rapidly gained support through further experimental evidence. In 1992 the International Union of Crystallography changed its definition of a crystal to "any solid having an essentially discrete diffraction pattern" thus formally recognizing quasicrystals.

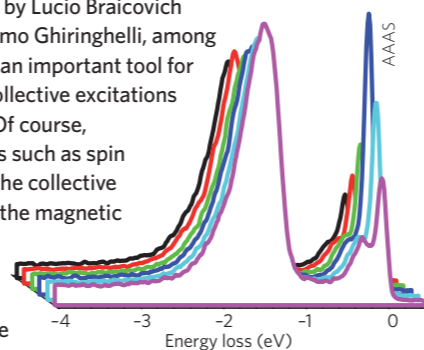
In fact quasicrystals can be considered periodic but in higher dimensions. Analysis of data from normal crystals requires only three integer values (Miller indices) representing the three dimensions of space in which the unit cells are periodic. Quasicrystals require at least five linearly independent vectors. In other words they are periodic in five-dimensional space (or higher). Quasiperiodicity has now been identified in numerous material systems including liquid

absorption edges. This extreme sensitivity also had its drawbacks. For example, for energies at which air and sample absorption is very high, experiments must be done under vacuum and result in rather short penetration depths. But as the interest in nanoscale magnetic structures grew, the availability of a technique that seemed tailor-made for investigating magnetic and electronic surface and interface effects proved invaluable.

Along with the rapid enhancement of instrumental capabilities, resonant scattering techniques have continued to evolve over the past three decades. One especially significant development has been the recent rise of resonant inelastic X-ray scattering, a technique pioneered by Lucio Braicovich and Giacomo Ghiringhelli, among others, as an important tool for probing collective excitations in solids. Of course, excitations such as spin waves — the collective motion of the magnetic moments

in a system — can also be

Resonant inelastic X-ray scattering spectra for thin films of the cuprate superconductor $\text{Nd}_{1-x}\text{Ba}_x\text{Cu}_2\text{O}_7$. Figure reprinted with permission from G. Ghiringhelli *et al.* *Science* **337**, 821-825 (2012).



crystals, nanoparticle superlattices and two-dimensional oxide films, amongst others. A natural quasicrystal has also been identified in mineral samples from the Koryak mountains in Russia.

Shechtman's faith in his science that ultimately undermined one of the presumed basic tenets of crystallography earned him the Nobel Prize in Chemistry in 2011. "Even our greatest scientists are not immune to getting stuck in convention," observed the Nobel Academy. "Keeping an open mind and daring to question established knowledge may in fact be a scientist's most important character trait."

Rosamund Daw, Senior Editor, *Nature*

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measured in neutron scattering experiments (Milestone 8). But the sensitivity of the resonant approach also makes them measurable in very small volume samples and thin films. For example, a series of resonant inelastic X-ray scattering experiments performed on cuprate superconductors have already helped to significantly shape the discourse on their electronic structure and the mechanism for high-temperature superconductivity. Resonant magnetic X-ray diffraction techniques look set to continue to profoundly influence our understanding of correlated states of matter in the future.

Andrea Taroni, Senior Editor, *Nature Materials*

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MILESTONE 22

Porous by design

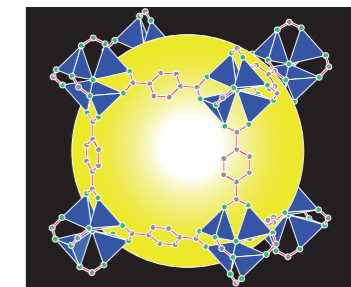
For most of the twentieth century, serendipity was a key ingredient in the synthesis of crystalline solids. The construction of materials with precise architectures was a formidable challenge, but through a subtle combination of chemistry and crystallography, 'designer' crystals — materials with predetermined structures and properties — slowly began to emerge. At the forefront of these endeavours was the field of porous crystals.

Porous crystals have nanometre-sized holes that can selectively trap molecules and ions of different shapes and sizes. The archetypal porous crystals are zeolites: aluminosilicates that form naturally in environments such as volcanic rocks. Reports on creating zeolites in the laboratory appeared as far back as the 1860s, but it was the work of Richard Barrer in the 1940s that kick-started the era of synthetic zeolites.

In 1948, Barrer reported the preparation of a zeolite with no natural counterpart and of a synthetic analogue of the zeolite mordenite. In both cases, X-ray powder diffraction provided the key characterization data. Later, following fundamental work by Robert Milton and Donald Breck, the Union Carbide Corporation commercialized synthetic zeolites. The materials were at first used to dry refrigerant and natural gas, but subsequently found widespread use as hydrocarbon cracking catalysts and as ion exchangers in detergents.

Zeolites are entirely inorganic materials, and despite their industrial significance, their chemistry and composition affords only limited control over the final product. To obtain a greater degree of flexibility, chemists have turned to the use of both organic and inorganic components in the preparation of porous crystals. In particular, since the 1990s, there has been an explosion of research interest in crystals known as metal-organic frameworks (MOFs). These materials form ordered networks by connecting metal units through organic linkers; by adjusting these two building blocks, the size and chemical environment of the crystal pores can, in principle, be tailored for a given application.

A number of MOFs were reported throughout the early 1990s and the term 'metal-organic framework' itself was introduced by Omar Yaghi's group in 1995. It was, however, in 1999 that two key papers appeared. In the February,



Yue-Biao Zhang

The unit cell of MOF-5 forms a cavity represented here by a yellow sphere.

Ian Williams and colleagues reported a MOF known as HKUST-1, a structure made from copper-based clusters and benzene tricarboxylate linkers; in November, Yaghi and colleagues reported MOF-5, a structure made from zinc-based clusters and benzene dicarboxylate linkers. Notably, these robust materials were found to have high surface areas and pore volumes, and, in the case of MOF-5, the values were considerably higher than most zeolites.

Today, tens of thousands of different MOF structures have been synthesized, which have a range of intriguing properties. For example, the porous structure and chemical diversity of MOFs make them attractive for catalysis, an application first explored by Makoto Fujita and colleagues in 1994. Alternatively, MOFs are of potential use in drug delivery, can be used to store hydrogen, and can reversibly adsorb carbon dioxide.

Despite the flurry of research activity and range of potential applications, MOFs are yet to have a commercial impact, and concerns regarding their cost and stability remain. But given their versatility and potential to be built by design, it seems likely that MOFs will, at some point, follow zeolites into the world of practical applications.

Owain Vaughan, Senior Editor, *Nature Nanotechnology*

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MILESTONE 23

Probing the molecular heart of life

Although the structure of DNA had been described by James Watson and Francis Crick in 1953 (Milestone 11), there actually were no crystallographic data within that paper. The first DNA crystal structure — reported 26 years later — was Alex Rich's left-handed Z-form; the first B-form DNA structure was reported in 1982. Instead, the first report of a polynucleotide crystal structure was that of the yeast transfer RNA (tRNA) for phenylalanine, also by Rich's group, published in 1973. The tRNA was found to adopt an L shape, with two right-handed, double-stranded stems. One end of the L harboured the amino acid that was to be added to a growing peptide chain, while the anticodon loop that base-pairs with the messenger RNA (mRNA) in the ribosome was at the other end.

The next major development in nucleic acid structural biology lay in determining protein–nucleic acid complexes. Aaron Klug and colleagues provided a considerable

advance when they solved the structure of the 206-kDa nucleosome core particle in 1984. This 7-Å structure, representative of the basic packing unit of the eukaryotic genome, revealed several novel features, including a periodic kinking of the DNA as it contacted the octamer core and the degree of compression of the inward-facing major and minor grooves of the DNA superhelix.

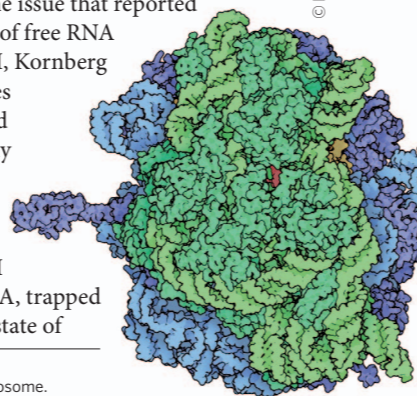
By 2001, technologies had advanced to the degree that the structures of two megadalton-sized complexes were published. The first, by Harry Noller and colleagues, was the 5.5-Å structure of the complete *Thermus thermophilus* 70S ribosome, including tRNAs in the A, P and E sites and a synthetic mRNA. The ability to trace all of the components of the ribosome — the three large ribosomal RNAs, the 50+ proteins — was truly revolutionary. Most importantly, this structure revealed that the major functional regions — the decoding site, the catalytic centre and the interface between

subunits — were based on RNA, establishing the primordial role of RNA in translation.

With the structure of the 70S ribosome having established a wealth of insights for the translation field, the publication of the 2.8-Å structure of *Saccharomyces cerevisiae* RNA polymerase II by Roger Kornberg and colleagues did the same for the transcription field. The structure revealed that the ten-subunit complex was composed of four mobile units, and the high resolution allowed both transcription initiation and elongation mechanisms to be deduced (even though an RNA template was not present).

In the same issue that reported the structure of free RNA polymerase II, Kornberg and colleagues also presented the technically challenging structure of RNA polymerase II bound to DNA, trapped in the active state of

Structure of the *T. thermophilus* ribosome.



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protein that uses light energy to export protons from the cell — by Henderson and Unwin provided the first structural identification of α -helices in membrane proteins. The X-ray crystal structure of this protein, reported in 1997, was the first time the lipidic cubic phase (LCP), a crystalline phase with a toothpaste-like consistency, was used to facilitate the crystallization of a membrane protein; LCP has since been used to obtain the structures of more than 45 unique membrane proteins, including G protein-coupled receptors (GPCRs).

The first crystal structure of an ion channel, determined in 1998, illustrated how the four identical subunits of the KcsA potassium channel assembled into a symmetric, inverted-cone-like architecture. The 12-Å-long selectivity filter comprises oxygen atoms from main-chain carbonyls at discrete locations; the distance between the oxygen atoms leads to the preferential coordination of de-solvated K^+ .

In 2007, two X-ray crystal structures of a GPCR, the human β_2 adrenergic receptor, were published. The authors used an antigen-binding fragment that bound tightly to the GPCR or a T4 lysozyme insert to stabilize the protein for crystallization. Because many drugs elicit their biological effect(s) by binding to a GPCR, the structures of these and other GPCRs may be used to develop highly efficacious drugs with few side effects.

Technological advances (Milestone 25) and an infusion of funding have led to a surge of

elongation. Within this structure, it was possible to see DNA enter the polymerase complex, and to localize nine base pairs of the RNA–DNA hybrid. Later work, also by Kornberg's group, visualized RNA separating from the DNA template.

With these structures, the awe-inspiring details of the essential molecular components of life that store information and convert it into functional readouts were revealed.

Angela K. Eggleston, Senior Editor, Nature

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interesting membrane protein structures in the past few years. In the near future, crystallographers will probably obtain structures of more complex species — for example, heteromeric complexes of multiple membrane proteins or membrane proteins bound to cytosolic regulatory proteins and signalling partners. Although progress has been slow, it has been worth the wait: some of the most interesting structures from the past decade were of integral membrane proteins and even more exciting discoveries are right around the corner.

Joshua M. Finkelstein, Senior Editor, Nature

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MILESTONE 24

Structures of membrane proteins

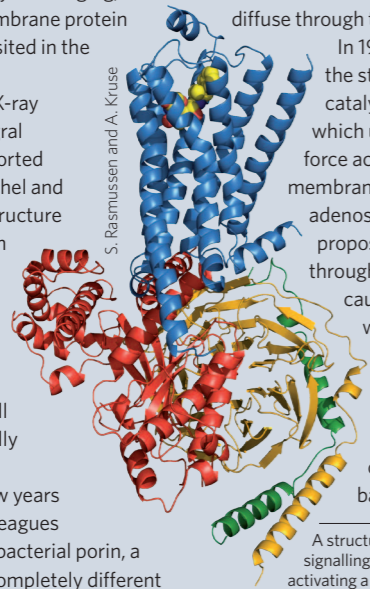
An estimated 20–30% of proteins in the human genome are membrane proteins, which mediate a broad range of biological processes. Obtaining X-ray crystal structures of membrane proteins has been extremely challenging; fewer than 500 unique membrane protein structures have been deposited in the Protein Data Bank.

The first high-resolution X-ray crystal structure of an integral membrane protein was reported in 1985, when Hartmut Michel and colleagues published the structure of a photosynthetic reaction centre. They observed a large hydrophobic surface that could interact with membrane lipids and 11 long α -helices that could span the lipid bilayer, as well as the location of functionally important cofactors of this multiprotein complex. A few years later, Georg Schulz and colleagues reported the structure of a bacterial porin, a membrane protein with a completely different

fold: 16 tilted β -strands formed a cylindrical shape (a 'β-barrel'), with the side-chains of polar amino acids projecting into the centre of the pore. This architecture enables the side-chains to interact with water and solute molecules as they passively diffuse through the protein.

In 1994, Abrahams *et al.* reported the structure of an F_1F_0 -ATPase, the catalytic centre of the F_0F_1 -ATPase, which uses the proton-motive force across the inner mitochondrial membrane to facilitate the synthesis of adenosine triphosphate. The authors proposed that the flow of protons through the F_0 domain somehow causes the F_1 domain to rotate, which advances each of the three nucleotide-binding sites of the F_1 domain to the next step in the catalytic cycle.

The 1975 electron crystallography analysis of bacteriorhodopsin — an archaeal



A structural model of a GPCR (blue) with signalling molecule bound (yellow spheres), activating a G protein (red, gold, and green).

MILESTONE 25

Seeing in a flash

Single-shot diffraction pattern from an individual mimivirus particle taken at the Linac Coherent Light Source in Stanford, California. Image reprinted with permission from M. M. Seibert *Nature* **470**, 78–81 (2011).

at just the energies needed to image individual molecules. A proof-of-principle demonstration on a non-biological sample was carried out at the FLASH soft-X-ray FEL in Hamburg in 2006.

Imaging biological specimens, however, meant new sample delivery technologies, including aerosol sample injection methods developed for single particles and biomolecules, and liquid jets to produce continuous streams of nanocrystals. It also demanded new processing algorithms to piece together the diffraction patterns from all the randomly oriented molecules and produce a complete image. In 2011, these elements coalesced to give a three-dimensional image of photosystem I derived from snapshots of fully hydrated nanocrystals of the complex, and to produce projection images for the giant mimivirus particle captured in the gas phase. Other demonstrations have followed, and other XFEL programmes are underway in Europe and Japan.

In principle, many different structures could be imaged with XFELs, including cells and viruses, while their pulsed nature also offers the promise of creating movies of molecular processes. The short pulses and high energies also make them very appealing for atomic and condensed-matter physics researchers. With more of these light sources turning on across the globe, the future for XFEL science is looking very bright indeed.

Nicky Dean, Senior Editor, Nature Communications

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As its name suggests, crystallography generally requires crystals. Their periodic structure produces Bragg peaks in the X-ray diffraction pattern and these peaks encode and amplify the information about the underlying molecular structure. Unfortunately, many biochemical samples simply can't be crystallized sufficiently to give usable diffraction patterns. In the 1950s, drawing on earlier concepts, David Sayre noticed that a diffraction pattern contained more information than just these peaks, which could be used to reconstruct an image from only a small number of samples, provided enough signal could be captured (Milestone 7). Applying this principle to objects as small as proteins demanded a whole new approach and a whole new light source: the X-ray free-electron laser (XFEL).

In FELs, a highly compressed electron bunch travelling through a periodic magnet array generates photons, which interact back with the electrons, causing microstructuring of the bunch. These microbunches then radiate coherently, creating X-ray pulses of unprecedented brilliance. Infrared FELs have been around for a few decades, but the higher energies of X-ray photons mean that XFELs are much more technically demanding. Yet their production of extremely bright and short pulses of radiation with ångström-scale wavelengths means their inherent advantages are enormous. Their major drawback is that they are so bright that they obliterate most samples placed in their path.

In 2000, Janos Hajdu and colleagues showed how this seemingly intractable problem could be overcome. They calculated that a molecule exposed to an ultrashort X-ray pulse begins to explode on a timescale of around 10 femtoseconds. Light pulses shorter than this can thus pass through the molecule, capturing information about a practically unperturbed structure. And bright enough pulses will give rise to continuous diffraction patterns that are strong enough to be measured.

In principle, the proposed XFELs could provide enough photons to exploit Sayre's ideas, and using this 'diffraction before destruction' concept, they could be designed to have the right pulse duration