RETROSPECTIVE: STRUCTURAL BIOLOGY AND BIOCHEMISTRY

Max Perutz (1914–2002)

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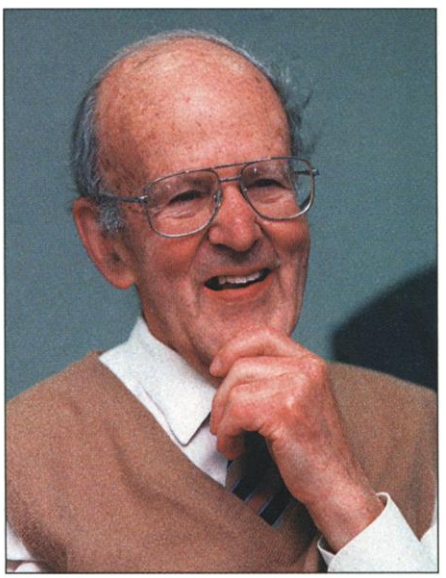
Max Perutz died on 6 February in Cambridge, UK, where he had worked for 65 years, with only a single interruption during World War II. He was a scientist of great distinction, internationally recognized as the founding father of modern x-ray protein crystallography, through his demonstration that the introduction of a heavy atom into a protein molecule allowed the solution of the x-ray "phase problem" for proteins. This led to the first determinations of the three-dimensional (3D) structure of a protein: of hemoglobin by Perutz, and of myoglobin by John Kendrew, for which they shared the Nobel Prize in Chemistry in 1962. Now, protein structures are solved at the rate of two or three a day, and the power of the method extends to cellular organelles and megadalton molecular structures. Perutz is commonly thought of as a crystallographer, but to him solving the molecular structure was the way to understand the mechanism behind the function. He was, above all, a chemist.

Perutz was born in Vienna, and studied chemistry at the University. In 1936, he moved to Cambridge to undertake doctoral work with J. D. Bernal, who had shown 2 years earlier that good x-ray diffraction photographs could be obtained from protein crystals provided they were kept wet. Perutz set out "to solve a great problem in biochemistry" and chose the structure of hemoglobin because, despite its large size, it was abundant, easy to crystallize, and was the key protein in the respiratory transport of oxygen. He obtained excellent diffraction patterns of hemoglobin, showing thousands of x-ray reflections to 3 Å resolution. On the strength of these, the head of the Cavendish Laboratory, Sir Lawrence Bragg, the founder of x-ray crystallography, obtained a grant for Perutz from the Rockefeller Foundation.

By the standard of the time, when structures had been determined only for molecules of 100 or so atoms, the prospect of solving the structure of hemoglobin with its four subunits—two α's and two β's, 17,000 daltons each—seemed vanishingly small. Yet Bragg knew that if Perutz were to succeed, the reward would be enormous, an entry into the "secret of life," as Bernal had called it. It took Perutz 22 years of hard work, frustration, and inspiration to do so.

The work was interrupted by the war, during which Perutz was interned as an "enemy alien." He was later released to work on a project to create large floating ice fields to serve as staging posts for aircraft crossing the Atlantic or hunting U-boats. Perutz's task was to make strengthened ice by growing it with wood pulp, his qualification being that he was an experienced Alpinist who had spent several months in 1938 on the Jungfrau studying ice-crystal texture. Perutz succeeded, but the project



was abandoned when the range of aircraft was increased, and he returned to civilian life at the Cavendish, and to hemoglobin.

Next came lean years, with little to show to the outside world, but much was learned about handling and understanding protein crystals. In a classic paper in 1946, Perutz demonstrated, by swelling experiments, the existence of a layer of "bound water" surrounding a protein molecule, an important concept that is now taken for granted. In 1951, angered by having missed the α helix when enumerating (with Kendrew and Bragg) helical configurations into which the polypeptide chain of a protein might fold, he took appropriate x-ray diffraction photographs to confirm the existence of Pauling's α helix in both proteins and polypeptides. He took the first photograph the very day that Pauling's paper appeared.

Perutz was joined in 1945 by Kendrew, who chose myoglobin, a monomolecular cousin of hemoglobin, for his research. In 1947, the Medical Research Council set up the MRC Unit for the Study of Molecular Structure of Biological Systems, with Perutz as its head and Kendrew as a staff member. Francis Crick soon joined the unit, followed by Hugh Huxley in 1948, Jim Watson in 1951, and Sydney Brenner in 1957. As the unit's successes mounted, it was relocated and renamed the Laboratory of Molecular Biology, then being joined by Fred Sanger and myself. Perutz was styled as Chairman, rather than Director, of the Laboratory, with Crick, Kendrew, and Sanger as respective heads of the three Divisions of Cell Biology, Structural Studies, and Protein (later to include Nucleic Acid) Chemistry.

In 1953, Perutz made the great breakthrough in protein crystallography. Bernal had pointed out in his 1939 Tilden Lecture to the (British) Chemical Society that, in principle, a protein structure might be solved by the method of isomorphous replacement, which had been used to solve small organic structures. In this method, a light atom is replaced by a heavier atom, preferably a metal; by measuring the changes in the x-ray intensities between the native and derivatized crystals, the position of the heavy atom, and the phases of the diffracted x-ray waves, could be determined. But this method did not seem to be applicable to large molecules, because the x-ray scattering contribution by a metal atom was thought to be too weak to observe, and moreover, inserting sufficient heavy atoms would distort the protein structure. Perutz did some preliminary work in which he measured the absolute x-ray intensities of hemoglobin, and this gave him confidence that the method would work in a protein. When he attached a mercury atom to a cysteine group in hemoglobin, he found measurable changes in the intensities of the diffraction spots, but no changes in their positions, indicating no change in crystal lattice structure, just as he had hoped. The phase problem could be solved! The first application was to solve a 2D projection of the protein, but it took another 5 years before the full 3D structure was obtained at 5.5 Å resolution. Meanwhile, Kendrew had made swift progress with the smaller and simpler myoglobin, and for the first time the world knew what the outline of a protein looked like.

For Perutz, however, it was only the beginning, because the hemoglobin model did not reveal its inner workings. Indeed, it provided a paradox. Hemoglobin (unlike myoglobin) has distinctive binding properties for oxygen, which biochemists and physiologists interpreted in terms of interactions between the four heme groups and so expected them to be in contact. Yet, in Perutz's model, the heme groups lay in deep pockets, distantly separated from one another. It took Perutz another decade to solve the molecular basis of hemoglobin activity, his original goal.

Hemoglobin is no simple oxygen carri-

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er because such a carrier would not allow enough of the oxygen carried in the red blood cells to be unloaded in the tissues. The binding of oxygen to the simpler myoglobin shows a hyperbolic binding curve of uptake versus concentration, typical of a single-site Langmuir isotherm. In contrast, the binding curve of hemoglobin is sigmoid shaped. This allows the maximum amount of oxygen to be loaded in the lungs, and at the same time enough oxygen to be unloaded in the tissues, where the oxygen concentration is low. The more pronounced the sigmoid shape, the greater the fraction of oxygen that can be released. Increased acidity and carbon dioxide produced in metabolically active tissues make the curve more sigmoid, promoting the release of still more oxygen (the Bohr effect). The sigmoid shape signifies that oxygen-free molecules of hemoglobin (deoxyhemoglobin) bind the first of the four oxygen molecules weakly, but the affinity increases as more oxygen molecules bind, rising steeply for the addition of the last oxygen. In other words, the binding of oxygen is highly cooperative. But, how could one heme sense that any of the others had bound oxygen, when they were isolated from each other in Perutz's structure?

Perutz realized that he must solve the structure of both oxyhemoglobin and deoxyhemoglobin at a resolution high enough to reveal atomic detail. In 1970, the first step toward this goal was realized with the acquisition of good electron density maps that showed the differences between the two structures. It took several more years, including increasing the resolution to 2 Å, to interpret fully the changes in structure upon oxygenation, and to fight the many controversies that followed.

The changes in quaternary structure on oxygenation were large, leading Perutz to call hemoglobin the "molecular lung." The distance between the hemes of the β subunits decreases by 7 Å, and one pair of α and β subunits rotates by 15° relative to the other pair in the $\alpha_2\beta_2$ tetramer. The interface between α and the other β switches from one dovetailed position to another, reducing the area of contact. The deoxyhemoglobin molecule is stabilized by four pairs of salt bridges (electrostatic interactions) that are broken in the oxy structure. These features explain why deoxyhemoglobin has a lower affinity for oxygen compared with myoglobin or an isolated hemoglobin subunit. The binding of oxygen requires the expenditure of energy to disrupt the stabilizing interactions present in the deoxy state. Perutz also showed that the making of the salt bridges in the deoxy state accounts for most of the Bohr effect, because there is a concomitant uptake of the protons in them.

The question, however, remained: How does the binding of oxygen to the iron atom of the heme group induce the subunits to change from the deoxy to the oxy structure? As Perutz put it, it is like the flea that makes the elephant jump. Perutz noticed in the high-resolution electron density maps of the deoxy structure that the iron atom was displaced by a small but significant amount from the plane of the heme group, whereas in the oxy structure it lay almost in the plane. Armed with a knowledge of metal-ion field theory, Perutz realized that this conformational change resulted from a change in the electron spin state of the iron atom, from high spin in the deoxy state to low spin in the oxy state, and hence to a reduction in the radius of the iron. Thus, in the oxy state the iron moves closer into the plane of the heme, and drags with it a protein α helix to which it is connected. This is the trigger that sets in motion a set of "molecular levers" that loosen and break the salt bridges, allowing the subunits in the "tense" deoxy structure to rearrange themselves in the new "relaxed" quaternary oxy structure.

It was left to others to confirm this interpretation through the study of additional bound forms of hemoglobin, but the picture Perutz provided is largely accepted. Earlier, Perutz had collected and analyzed abnormal hemoglobins (mostly single mutants), and so opened up the field of "molecular pathology," relating a structural abnormality to a disease. He provided new insights into the evolution and adaptation of hemoglobin in different species, from migrating geese flying long distances to frogs living at different altitudes. With various colleagues he investigated the ability of different compounds to prevent the "sickling" of hemoglobin in red cells from sickle cell patients, and he also studied heme-containing molecules that sense oxygen levels in tissues.

Approaching 80, Perutz changed direction, starting to work on Huntington's disease (HD), a neurodegenerative disorder caused by abnormal expansions of glutamine repeats in the mutant protein (later called huntingtin). Perutz came to this subject through earlier work on a quite different problem in which he had noticed that a run of glutamines would generate alternate positive and negative changes on two sides of a β strand, forming what he called a polar zipper. In 1994, he and his colleagues showed that polyglutamine polymers could form β sheets in which glutamines of neighboring strands are linked by hydrogen bonds between the main-chain and side-chain amides. He suggested that proteins with long runs of glutamines would therefore form aggregates harmful to the cell. Aggregates of huntingtin have been observed in intranuclear inclusions in the brains of HD patients, but it has not yet been established whether they are themselves the cause of neuronal death. Perutz's penultimate paper provides a nucleation model for the formation of these aggregates, in which nucleation is the critical event that leads to neuronal death. His model explains the finding that the age of disease onset is related exponentially to the length of the glutamine repeats in the pathological protein. This is exactly what would be expected, because the free energy required to form the focus of an aggregate would decrease with the length of the repeat. Perutz's last paper (in press), completed the day before he went into hospital in December, presents a model for the structure of polyglutamine fibers and related amyloid filaments.

Head of the MRC Unit and Laboratory for 32 years (1947 to 1979), Perutz's relaxed management style led it to become the most successful laboratory of its kind in the world. His principle was to choose good people, irrespective of the subject, to give them a free hand to pursue an idea, and to facilitate the interchange of ideas in the tea room as well as in seminars. He led by example, and younger people saw him "standing at the laboratory bench or by an x-ray tube, rather than sitting at his desk." He considered this more important than his administrative work, which he did not put first, but to which he dutifully attended. The Laboratory as a community took its tone from its head. In the 1960s and 1970s, it attracted large numbers of postdoctoral visitors, mostly from the USA, and they carried the new science back with great success.

Perutz was the archetypal researcher who preferred the laboratory to committees, but when the occasion demanded, he entered into debates on science policy and wider issues for the greater public good. Indeed, Perutz was a prolific writer about science through critical and scholarly book reviews and through his own books. He brought to his subjects his own varied experience and wisdom, and gift for lucid exposition in lapidary prose. He died full of years and of honors, and a happy man, to the admiration and affection of the many who came to visit him in his last months. He was never a man to rest on his laurels, working to the end, not for further honors, but simply because research is what he liked to do. It could be said of him, as he once wrote of Sir Lawrence Bragg, "Fame never blunted the keen edge of his scientific curiosity, perhaps because he had no vanity or ambition to use it for personal power." Max Perutz will be long remembered as one of the select few who led the revolution in the life sciences we now call molecular biology.