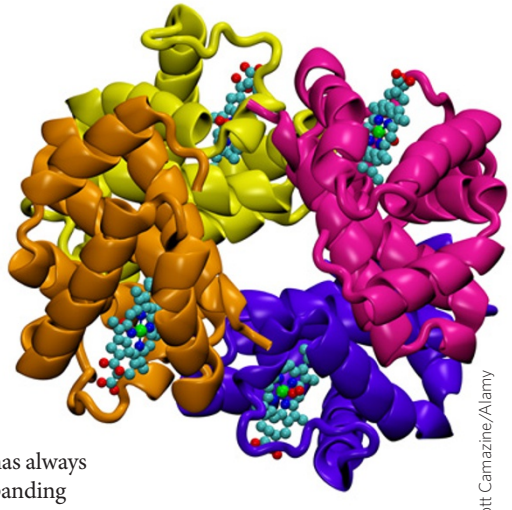
MILESTONE 12

# The first of its kind



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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 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 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.

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**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)