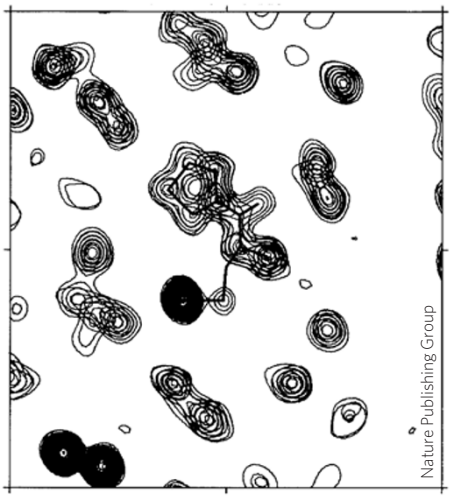
MILESTONE 19

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).



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).

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 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.

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Nature Communications

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