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# This Week's Citation Classic®

**Blow D M & Crick F H C.** The treatment of errors in the isomorphous replacement method. *Acta Crystallogr.* 12:794-802, 1959.
[Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, Cambridge, England]

A method is described for assessing the errors that arise in the isomorphous replacement method, both due to observational error and nonisomorphism. The relative probabilities of different phases may be used to calculate a "best" Fourier in which the errors of electron density are minimized. [The *SCI*® indicates that this paper has been cited in over 435 publications.]

## Phasing X-ray Diffraction Data for Proteins

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When I became Max Perutz's graduate student in the Cavendish Laboratory, Cambridge, in October 1954, I was assigned a desk near the door in a large, bare room, whose walls were formed of uncovered brickwork. This room was shared by the junior members of the group, often as many as eight of us.

Perutz was tackling the "impossible" problem of finding the molecular structure of the haemoglobin molecule, using crystallographic methods. Within this problem was the simpler puzzle of assigning signs to a few hundred observed X-ray intensities to make a projection down the twofold symmetry axis of the molecule, at low resolution. Perutz[1] had already shown how, by introducing mercury into the crystals, most of the required signs could be found. I was convinced that this correct solution showed how to tackle the bigger problem.

When a structure is viewed down a twofold axis, it looks symmetrical, and this symmetry simplifies the puzzle. In this case each X-ray intensity only needs to have its *sign* determined (+ or −). Any other view of the molecule is asymmetrical, and for the vast majority of X-ray intensities the *phase angles* can have any value between 0 and $2\pi$.

My PhD problem, to find a general method for determining these phase angles, was a simple geometrical problem.[2] But there were two snags. One was that no isomorphous replacement could solve the problem on its own, because it would always give two alternative answers: the results from several different derivatives had to be combined somehow.[3] The other was that Perutz's results had shown that the effect of errors was very serious: the errors were almost as large as the differences caused by the heavy atom.

One day an extrovert new arrival appeared in our room, returned from a postdoctoral year at Brooklyn Polytechnic. At first sight he was just going to be a nuisance, as his method of working was to talk loudly all the time. To me, a diffident physics student who knew next to nothing about biology, most of his conversation was gibberish. Although Francis Crick's aim was to solve the genetic code (a concept beyond me at the time), Perutz had taken him back into his laboratory to advise on theoretical aspects of X-ray diffraction.

In due course I had to overcome my diffidence and explain to Francis what I was doing. With amazing rapidity he grasped the essence of the problem. In an hour he had shown me a new geometrical picture of the errors in phase angles and their relation to errors in an electron density map. He had suggested a criterion for minimizing these errors and invented the name "best" for a map using this criterion.

Following this talk, I spent months trying to generate explicit probability functions for the sine or cosine of a phase angle that could be integrated using this criterion to generate a "best" phase and weight. I failed, and instead built an analogue computer, a "triangular slide rule," from which I could laboriously derive an overall phase probability curve, which could finally be used to calculate a "best" phase and weight. Computer programming was then in its infancy, and it was some years before I was able to make a robust computer algorithm for the method.

For my PhD in 1957, I applied this method to make one view of the haemoglobin molecule; the method has been used widely since its publication in 1959. Greater generality and rigour are quite possible,[4] but even with today's abundant computing power, the improved accuracy is small and the added complication can be confusing. Surprisingly, the original method is still most popular.[5] Francis won a few prizes for his other work, but he got none for this. It helped me to share the Charles Leopold Meyer Prize of the French Academy of Sciences (1979) and the Wolf Prize for Chemistry (1987).

1. **Green D W, Ingram V M & Perutz M F.** The structure of haemoglobin. IV. Sign determination by the isomorphous replacement method. *Proc. Roy. Soc. London Ser. A* 225:287-307, 1954. (Cited 160 times.)
2. **Bokhoven C, Schoone J C & Bijvoet J M.** The Fourier synthesis of the crystal structure of strychnine sulphate pentahydrate. *Acta Crystallogr.* 4:275-80, 1951. (Cited 60 times.)
3. **Harker D.** The determination of the phases of the structure factors of non-centrosymmetric crystals by the method of double isomorphous replacement. *Acta Crystallogr.* 9:1-9, 1956. (Cited 55 times.)
4. **Sygusch J.** Minimum-variance Fourier coefficients from the isomorphous replacement method by least-squares analysis. *Acta Crystallogr. A—Found. Crys.* 33:512-8, 1977. (Cited 10 times.)
5. **Watenpaugh K D.** Overview of phasing by isomorphous replacement. *Meth. Enzymology* 115:3-15, 1985.




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