International Tables for Crystallography (2012). Vol. F, Section 14.1.8, pp. 370–371.

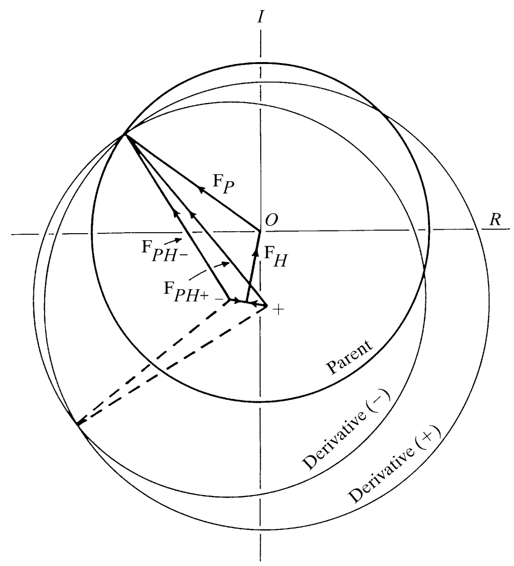

Figure 14.1.7.2
Harker construction for a single isomorphous replacement with anomalous scattering, in the absence of errors.

$$F_H(\mathbf{h}) + iF''_H(\mathbf{h}) = \sum_{n=1}^{N} f'_n(\mathbf{h}) \exp(2\pi i\mathbf{h} \cdot \mathbf{r}_n) + i\sum_{n=1}^{N} f''_n(\mathbf{h}) \exp(2\pi i\mathbf{h} \cdot \mathbf{r}_n). \quad (14.1.7.1)$$

If the heavy atoms are all of the same type, *i.e.* they all have the same ratio of f'_n/f''_n $(= k)$, then F_H and F''_H are orthogonal, and $F''_H = F_H/k$.

The relation between the structure factors of the reflection *hkl* and its Friedel mate $\bar{h}\bar{k}\bar{l}$ is illustrated in Fig. 14.1.7.1(*a*). The situation can be conveniently represented (Fig. 14.1.7.1*b*) by reflecting the $\bar{h}\bar{k}\bar{l}$ diagram through the real axis onto the *hkl* diagram. In cases such as this, where Friedel's law breaks down, we shall refer to the difference $\Delta_{PH} = (F_{PH+} - F_{PH-})$ as the Bijvoet difference, or simply the anomalous-scattering difference. The Harker phase circles corresponding to Fig. 14.1.7.1(*b*) are shown in Fig. 14.1.7.2. It will be seen that, as in the case of single isomorphous replacement, and similarly with the anomalous-scattering data alone, there is an ambiguous phase determination.

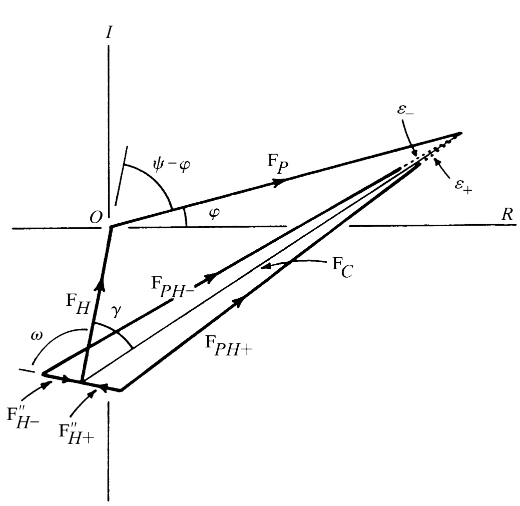

Figure 14.1.8.1
Vector diagrams illustrating lack of closure in the anomalous-scattering method.

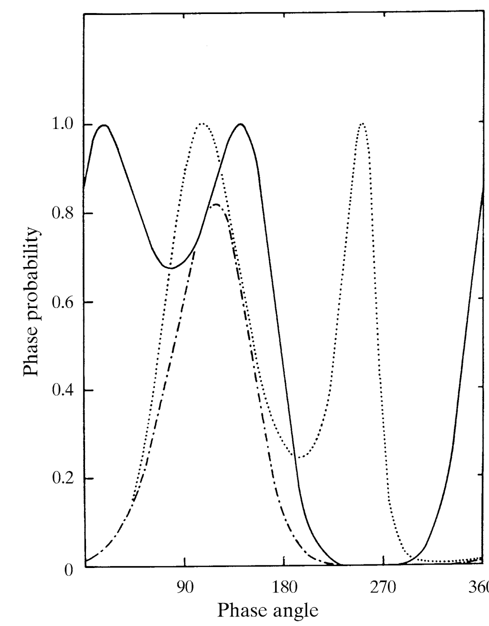

Figure 14.1.8.2
Combination of isomorphous replacement and anomalous-scattering phase probabilities for a single isomorphous replacement. $P_{\rm iso}(\varphi)$ is drawn as a solid line, $P_{\rm ano}(\varphi)$ as a dotted line, and the combined probability distribution is drawn as a dotted-and-dashed line.

In the absence of error, the three phase circles (Fig. 14.1.7.2) will meet at a point, resolving the phase ambiguity and giving a unique solution for the phase of $\mathbf{F}_P$. The isomorphous-replacement method gives phase information symmetrical about the vector $\mathbf{F}_H$, whereas the anomalous-scattering phase information for $\mathbf{F}_{PH}$ is symmetrical about $\mathbf{F}''_H$, which, for heavy atoms of the same type, is at right angles to $\mathbf{F}_H$. In other words, the two methods complement each other, one method providing exactly that information which is not given by the other.

On average, the experimentally measured isomorphous-replacement difference, $(F_{PH} - F_P)$, will be larger than the anomalous-scattering difference, $(F_{PH+} - F_{PH-})$. The former, however, relies on measurements from different crystals and is also susceptible to errors due to non-isomorphism between the parent and derivative crystals. The latter can be obtained from measurements on the same crystal, under closely similar experimental conditions, and is not affected by non-isomorphism. Therefore, it is desirable to use methods that take into account the different sources of error in the respective measurements (Blow & Rossmann, 1961; North, 1965; Matthews, 1966*b*). One method is as follows.

14.1.8. The phase probability distribution for anomalous scattering

From Fig. 14.1.8.1, it can be seen that the most probable phase angle will be the one for which $\varepsilon_+ = \varepsilon_-$. At any other phase angle, there will be an 'anomalous-scattering lack of closure' which we define to be $(\varepsilon_+ - \varepsilon_-)$. The value of $(\varepsilon_+ - \varepsilon_-)$ can readily be calculated as a function of φ (Matthews, 1966*b*; Hendrickson, 1979). Thus, if the r.m.s. error in $(\varepsilon_+ - \varepsilon_-)$ is E', and the distribution of error is assumed to be Gaussian, then from measurements of anomalous scattering, the probability $P_{\rm ano}(\varphi)$ of phase φ being the true phase of $\mathbf{F}_P$ can be estimated using an equation exactly analogous to equation (14.1.4.2).

An example of an anomalous-scattering phase probability distribution is shown by the dotted curve in Fig. 14.1.8.2. The asymmetry of the distribution arises from the fact that $P_{\rm ano}(\varphi)$ is the phase probability distribution for F_P rather than that of F_{PH}, which would be symmetrical about the phase of F''_H. The overall probability distribution obtained by combining the anomalous-scattering data with the previous isomorphous-replacement data (Fig. 14.1.2.1*b*) is given by

$$P(\varphi) = NP_{\rm iso}(\varphi)P_{\rm ano}(\varphi) \qquad (14.1.8.1)$$

and is illustrated in Fig. 14.1.8.2.

14.1.9. Anomalous scattering without isomorphous replacement

The treatment outlined above of phase determination by anomalous scattering assumed that data were available for a parent crystal devoid of anomalous scatters and an anomalously scattering isomorphous heavy-atom derivative. It is not uncommon that the native protein itself contains atoms which scatter anomalously or has been engineered to contain such scatterers. In such cases, measurements will usually be made at multiple wavelengths in order to exploit MAD phasing (Hendrickson, 1991). If, however, measurements are available only at a single wavelength, they can be utilized to obtain some phase information (*e.g.* Matthews, 1970).

14.1.10. Location of heavy-atom sites

During the development of protein crystallography, it was understood that heavy-atom sites might be located from difference Patterson functions, but there was substantial debate as to the type of function that was preferable (Perutz, 1956).

Blow (1958), and also Rossmann (1960), advocated a Patterson function with amplitudes $(F_{PH} - F_P)^2$. It relies on the admittedly crude assumption that the desired scattering amplitude of the heavy atoms, $|F_H|$, can be approximated by

$$|F_H| \simeq |F_{PH} - F_P|. \qquad (14.1.10.1)$$

The approximation does have one very helpful characteristic, namely, that it tends to be most accurate when $|F_{PH} - F_P|$ is large, *i.e.* when F_H is parallel or antiparallel to F_P (*cf.* Fig. 14.1.4.1). Thus, the numerically largest coefficients in the Patterson function tend to represent $|F_H|^2$ correctly. Given a well behaved isomorphous heavy-atom derivative, and accurately measured data, experience has shown that a map with coefficients $(F_{PH} - F_P)^2$ can give an excellent representation of the desired heavy-atom–heavy-atom vector peaks.

14.1.11. Use of anomalous-scattering data in heavy-atom location

A relation exactly analogous to equation (14.1.10.1) can be used to approximate the anomalous heavy-atom scattering amplitude, namely,

$$|F''_H| \simeq \tfrac{1}{2}|F_{PH+} - F_{PH-}| \qquad (14.1.11.1)$$

(see Fig. 14.1.7.1*b*). As noted above, if all the heavy atoms are the same, $F_H = kF''_H$. Thus, a Patterson function with coefficients $(F_{PH+} - F_{PH-})^2$ should also show the desired heavy-atom–heavy-atom vector peaks (Blow, 1957; Rossmann, 1961).

For each individual reflection, however, and as is also the case for phase determination, the information that is provided by the isomorphous-replacement difference $(|F_{PH}| - |F_P|)$ is exactly complementary to that provided by the anomalous-scattering measurement $(|F_{PH+}| - |F_{PH-}|)$. By combining both sets of experimental measurements, it is possible to obtain a much better estimate of the heavy-atom scattering, $|F_H|$, for every reflection (Kartha & Parthasarathy, 1965*a*,*b*; Matthews, 1966*a*; Singh & Ramaseshan, 1966). One formulation (Matthews, 1966*a*) can be written as

$$F_H^2 = F_P^2 + F_{PH}^2 - 2F_PF_{PH}\{1 - [wk(F_{PH+} - F_{PH-})/2F_P^2\}^{1/2}, \qquad (14.1.11.2)$$

where $F_{PH} = (F_{PH+} + F_{PH-})/2$ and w is a weighting factor (from 0 to 1) that is an estimate of the relative reliability of the measurements of $(F_{PH+} - F_{PH-})$ compared with $(F_{PH} - F_P)$.

14.1.12. Use of difference Fourier syntheses

The discussion above has focused on the use of difference Patterson functions to locate heavy-atom sites. Once one or more putative sites have been located, they can be used to calculate approximate protein phases, which, in turn, can be used to calculate difference Fourier series with coefficients in the form

$$m(F_{PH} - F_H)\exp(-i\varphi_B), \qquad (14.1.12.1)$$

where m is the figure of merit and φ_B is the 'best', albeit approximate, phase of the protein structure factor. Putting aside errors due to inaccuracies in φ_B, such maps do not give the true heavy-atom vector, $\mathbf{F}_H$. Rather, they give, essentially, the projection of $\mathbf{F}_H$ along $\mathbf{F}_P$ (*cf.* Fig. 14.1.4.1). Nevertheless, subject to certain limitations, such difference maps are extraordinarily powerful in locating secondary sites in a given heavy-atom derivative, or in using approximate phases from one derivative to search for heavy-atom sites in other putative derivatives. It is in this context, however, that certain limitations of the single-isomorphous-replacement (SIR) method have to be kept in mind. These are noted in the next section.

14.1.13. Single isomorphous replacement

Although phase determination from a single heavy-atom derivative in the absence of anomalous-scattering data is, in principle, ambiguous, it was realized early on that useful phase information can still be obtained (Blow & Rossmann, 1961). As shown in Fig. 14.1.2.1(*a*), the two possible phases for the protein are φ_1 or φ_2. In terms of the analysis of Blow & Crick (1959), the 'best' phase to use for the protein is the average of φ_1 and φ_2. This is also equivalent to using *both* φ_1 *and* φ_2. With this in mind, a situation that is of special concern is one in which the heavy-atom distribution used to determine the phases happens to have a centre of symmetry. One common way in which this can occur is when one has a heavy-atom derivative with a single site in space group $P2_1$. A related situation occurs when there are multiple sites in space group $P2_1$, but all have the same y coordinate. If the origin of coordinates is considered to be at the site of centrosymmetry, then all of the heavy-atom vectors $\mathbf{F}_H$ (Fig. 14.1.2.1*a*) will necessarily have phases of 0 or π. If such phases are used, for example, to try to identify heavy-atom-binding sites in a second derivative, the map will show the correct sites, but will also show spurious peaks of equal height related by the centre of symmetry. Faced with this choice, one must arbitrarily choose one of the alternative peaks which, in turn, will define an overall handedness for the heavy-atom arrangement. In the absence of any anomalous-scattering data, one can proceed with the structure determination in the standard way, but it must be kept in mind that either the correct electron-density map or its mirror image will ultimately be obtained.

references