14.1. HEAVY-ATOM LOCATION AND PHASE DETERMINATION

where N is a normalizing factor such that the sum of all probabilities is unity. The un-normalized probability distribution corresponding to Fig. 14.1.4.1 (and Fig. 14.1.2.1a) is shown in Fig. 14.1.2.1(b). The two most probable phase angles ($\varphi = \varphi_1$ and $\varphi = \varphi_2$) are the alternative phases of F_P for which the phase triangle is closed.

Individual probability distributions for the additional heavy-atom derivatives are derived in an analogous manner and may be multiplied together to give an overall probability distribution. The joint probability distribution corresponding to Fig. 14.1.3.1(a) is shown in Fig. 14.1.3.1(b), and in this case the most probable phase is that which simultaneously fits best the observed data for the two isomorphous derivatives.

The main objection which may be made to the Blow & Crick treatment is that it assumes that there is no error in F_P. In practice, however, this is not a serious limitation.

14.1.5. The best Fourier

A protein crystallographer desires to obtain a Fourier synthesis that can most readily be interpreted in terms of an atomic model of the structure. One synthesis which could be calculated is the 'most probable Fourier', obtained by choosing the value of $F_P(\mathbf{h})$ for each reflection which corresponds to the highest value of $P(\varphi)$. Blow & Crick pointed out that although this Fourier is the most likely to be correct, it has certain disadvantages. In the first place, it might tend to give too much weight to uncertain or unreliable phases, and, in the second place, for cases where $P(\varphi)$ is bimodal, there is a strong chance of making a large error in the phase angle. Blow & Crick suggested that in cases such as this, a compromise is needed, and that the centroid of the phase probability distribution provides just the required compromise. They showed that the corresponding synthesis is the 'best Fourier', which is defined to be *that Fourier transform which is expected to have the minimum mean-square difference from the Fourier transform of the true F's when averaged over the whole unit cell.*

The centroid of the phase probability distribution may be defined as a point on the phase diagram with polar coordinates (mF_P, φ_B), where φ_B is the 'best' phase angle. The quantity m, which acts as a weighting factor for F_P, is called the 'figure of merit' of the phase determination. Its magnitude, between 0 and 1, is a measure of the reliability of the phase determination.

14.1.6. Anomalous scattering

All atoms, particularly those used in preparing heavy-atom isomorphs, give rise to anomalous scattering, especially if the energy of the scattered X-rays is close to an absorption edge. The atomic scattering factor of the atom in question can be expressed as

$$\mathbf{f} = f_0 + \Delta f' + if'' = f' + if'', \qquad (14.1.6.1)$$

where f_0 is the normal scattering factor far from an absorption edge, and $\Delta f'$ and f'' are the correction terms which arise due to dispersion effects. The quantity $\Delta f'$, in phase with f_0, is usually negative, and f'', the imaginary part, is always $\pi/2$ ahead of the phase of the real part ($f_0 + \Delta f'$). It may be noted that by using different wavelengths, the term $\Delta f'$ is equivalent to a change in scattering power of the heavy atom and produces intensity differences similar to a normal isomorphous replacement, except that in this case the isomorphism is *exact* (Ramaseshan, 1964).
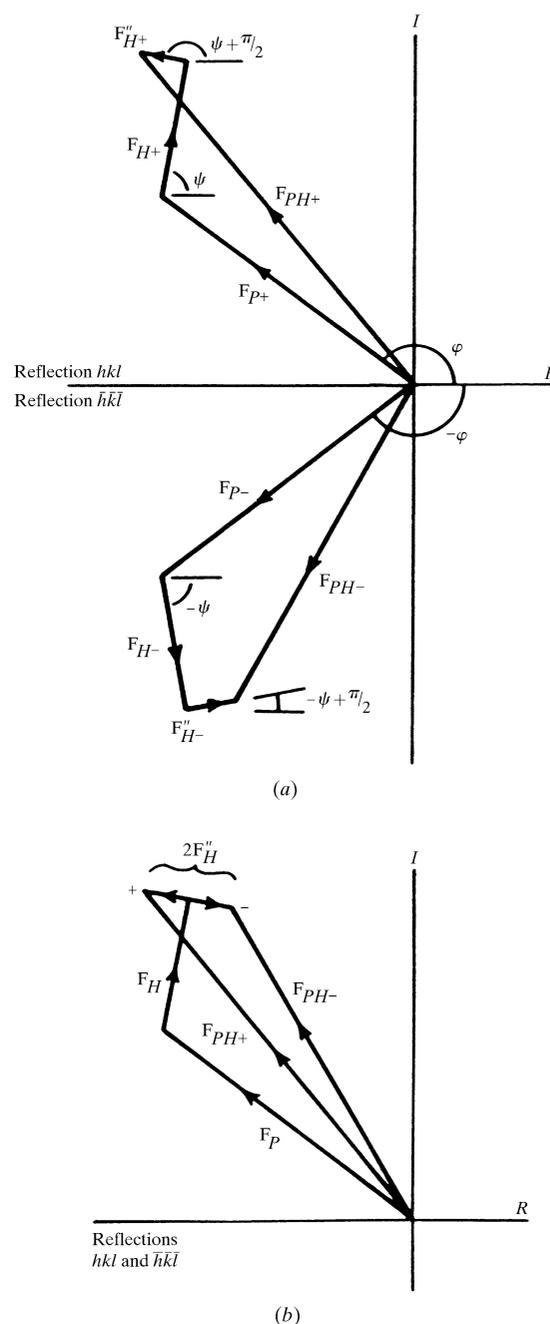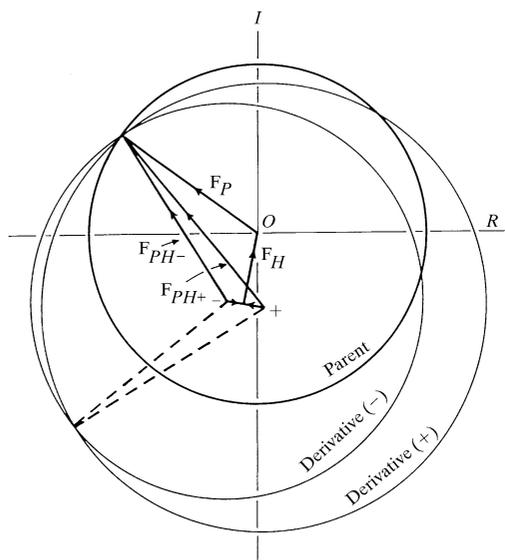

Figure 14.1.7.1
(*a*) Vector diagrams illustrating anomalous scattering for the reflections hkl and $\overline{hkl}$. (*b*) Combined vector diagram for reflections hkl and $\overline{hkl}$.

This is the basis of the multiwavelength-anomalous-dispersion (MAD) method (Hendrickson, 1991) discussed in Chapter 14.2. Here we focus on measurements based on a single wavelength, traditionally referred to as the 'anomalous-scattering method'.

The anomalous scattering of a heavy atom is always considerably less than the normal scattering (for Cu $K\alpha$ radiation, $2f''/f'$ ranges from about 0.24 to 0.36), but there are several factors which tend to offset this reduction in magnitude (*e.g.* see Blow, 1958; North, 1965).

14.1.7. Theory of anomalous scattering

Suppose that two isomorphous crystals are differentiated by N heavy atoms of position $\mathbf{r}_n$ and scattering factor ($\mathbf{f}'_n + i\mathbf{f}''_n$). Then, for the reflection hkl, the calculated structure factor of the N atoms is
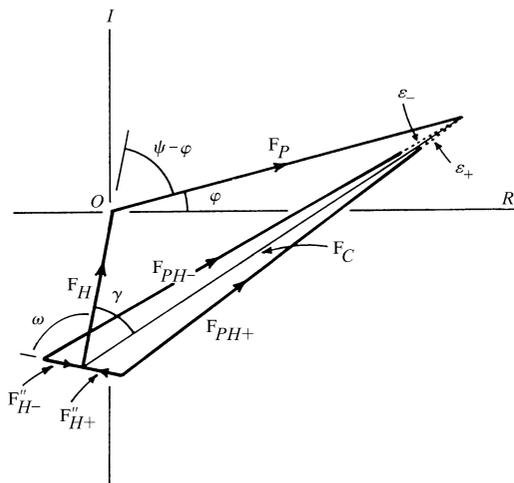
Figure 14.1.7.2
Harker construction for a single isomorphous replacement with anomalous scattering, in the absence of errors.

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

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

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 $\overline{hkl}$ is illustrated in Fig. 14.1.7.1(*a*). The situation can be conveniently represented (Fig. 14.1.7.1*b*) by reflecting the $\overline{hkl}$ 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.
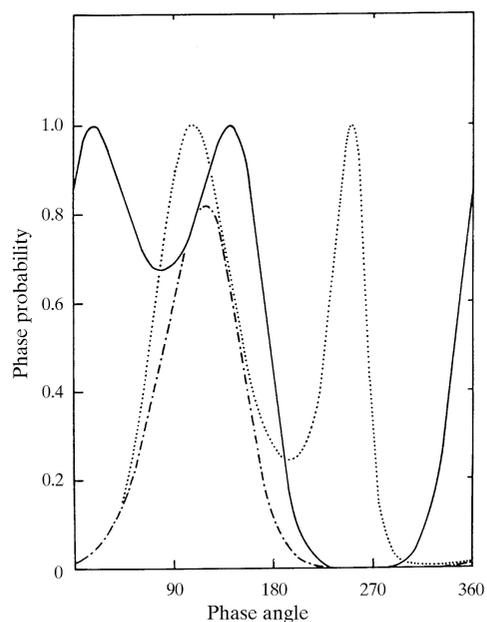
Figure 14.1.8.2
Combination of isomorphous replacement and anomalous-scattering phase probabilities for a single isomorphous replacement. $P_{\text{iso}}(\varphi)$ is drawn as a solid line, $P_{\text{ano}}(\varphi)$ as a dotted line, and the combined probability distribution is drawn as a dotted-and-dashed 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 $P_{\text{ano}}(\varphi)$ 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

references