

## 14. ANOMALOUS DISPERSION

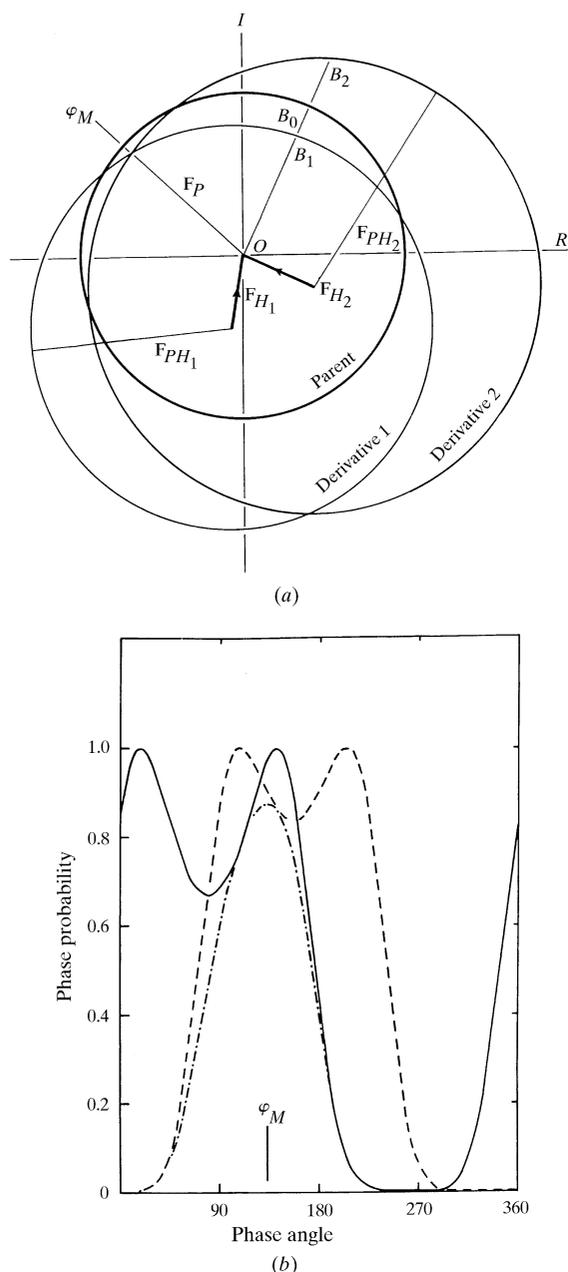


Fig. 14.1.3.1. (a) Harker construction for a double isomorphous replacement.  $\varphi_M$  is the ‘most probable’ phase for  $\mathbf{F}_P$ . (b) Phase probability distribution corresponding to the double isomorphous replacement shown in part (a). The curve for derivative 1 is solid, that for derivative 2 is dashed, and that for the combined distribution is drawn as a dotted-and-dashed line.

symmetrical about  $\mathbf{F}_H$ . This ambiguity may in principle be resolved in two ways: (a) by using a second heavy-atom isomorphous derivative or (b) by utilizing the anomalous-scattering effects for the first isomorph.

### 14.1.3. The method of multiple isomorphous replacement

The phase information provided by a second isomorph is illustrated in Fig. 14.1.3.1(a). In theory, the three phase circles will intersect at a point and the phase ambiguity will be resolved. In practice, there will be errors in the observed amplitudes  $F_P$  and  $F_{PH}$  and in the heavy-atom parameters (and thus in  $\mathbf{F}_H$ ). Also, the isomorphism may be imperfect. As a result, the intersections of the three phase

circles may not coincide. Another complication arises from the fact that for reflections where  $\mathbf{F}_H$  is small, the circles will be essentially concentric and will not have well defined points of intersection. In other words, the phase determination will become indeterminate. The method of Blow & Crick (1959) was introduced as a way to take all these factors into account. It has had an extraordinary impact, not only as a practical method for protein phase determination, but also in influencing all subsequent thinking in this area.

#### 14.1.4. The method of Blow & Crick

Blow & Crick pointed out that in practice the phase angle  $\varphi$  can never be determined with complete certainty. Rather, there is a finite probability that any arbitrary phase angle may be the correct one. Consider the vector diagram shown in Fig. 14.1.4.1, in which  $\mathbf{F}_H$  is known and we wish to determine the probability  $P(\varphi)$  that the arbitrary phase angle  $\varphi$  is the correct phase of  $\mathbf{F}_P$ . Strictly, one should allow for the possibility of errors in  $\mathbf{F}_H$ ,  $F_P$  and  $F_{PH}$ , and should consider the probability that the vector  $\mathbf{F}_P$  occupies all possible positions in the Argand diagram. However, Blow & Crick suggested that the analysis might be considerably simplified by assuming that  $F_P$  and  $\mathbf{F}_H$  are known accurately and that all the error lies in the observation of  $F_{PH}$ . In other words, it was assumed that the vector  $\mathbf{F}_P$  must lie on the circle of radius  $F_P$ , and the probability distribution of  $F_P$  could be evaluated as a function of  $\varphi$  only.

For an arbitrary phase angle  $\varphi$ , the phase triangle (Fig. 14.1.4.1) will not close exactly. If we define  $F_C$  to be the vector sum of  $\mathbf{F}_H$  and  $F_P \exp(i\varphi)$ , then the lack of closure of the phase triangle is given by

$$\varepsilon = F_C - F_{PH}. \quad (14.1.4.1)$$

Following Blow & Crick, if  $E$  is the r.m.s. error associated with the measurements, and the distribution of error is assumed to be Gaussian, then the probability  $P(\varphi)$  of the phase  $\varphi$  being the true phase is

$$P(\varphi) = N \exp(-\varepsilon^2/2E^2), \quad (14.1.4.2)$$

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.

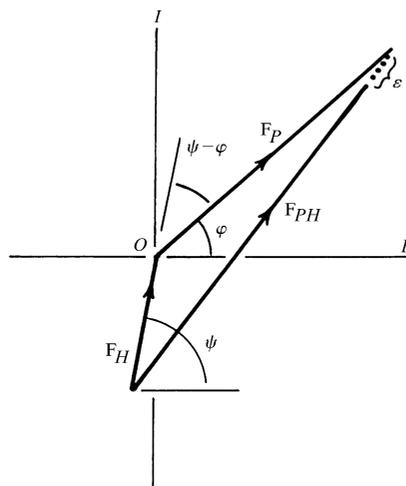


Fig. 14.1.4.1. Vector diagram illustrating the lack of closure,  $\varepsilon$ , of an isomorphous-replacement phase triangle.

## 14.1. HEAVY-ATOM LOCATION AND PHASE DETERMINATION

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  $(mF_P, \varphi_B)$ , where  $\varphi_B$  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

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

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). 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).

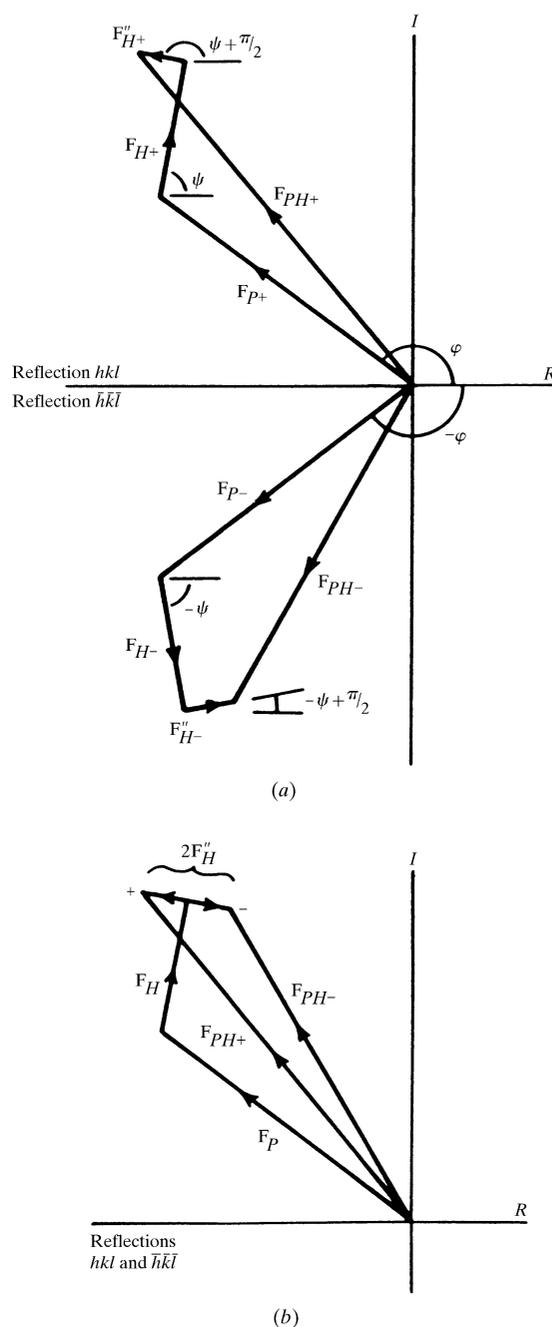


Fig. 14.1.7.1. (a) Vector diagrams illustrating anomalous scattering for the reflections  $hkl$  and  $\bar{h}\bar{k}\bar{l}$ . (b) Combined vector diagram for reflections  $hkl$  and  $\bar{h}\bar{k}\bar{l}$ .

### 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

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

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  $F_H'' = F_H/k$ .