

2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

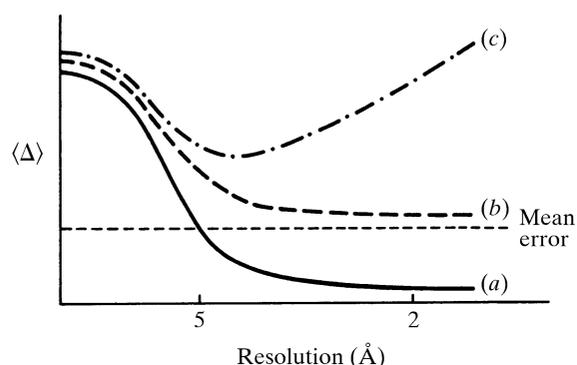


Fig. 2.3.3.6. A plot of mean isomorphous differences as a function of resolution. (a) The theoretical size of mean differences following roughly a Gaussian distribution. (b) The observed size of differences for a good isomorphous derivative where the smaller higher-order differences have been largely masked by the error of measurement. (c) Observed differences where 'lack of isomorphism' dominates beyond approximately 5 Å resolution.

Crick & Magdoff (1956) also derived the approximate expression

$$\sqrt{\frac{2N_H}{N_P}} \cdot \frac{f_H}{f_P}$$

to estimate the r.m.s. fractional change in intensity as a function of heavy-atom substitution. Here, N_H represents the number of heavy atoms attached to a protein (or other large molecule) which contains N_P light atoms. f_H and f_P are the scattering powers of the average heavy and protein atom, respectively. This function was tabulated by Eisenberg (1970) as a function of molecular weight (proportional to N_P). For instance, for a single, fully substituted, Hg atom the formula predicts an r.m.s. intensity change of around 25% in a molecule of 100 000 Da. However, the error of measurement of a reflection intensity is likely to be around 10% of I , implying perhaps an error of around 14% of I on a difference measurement. Thus, the isomorphous replacement difference measurement for almost half the reflections will be buried in error for this case.

Scaling of the different heavy-atom-derivative data sets onto a common relative scale is clearly important if error is to be reduced. Blundell & Johnson (1976, pp. 333–336) give a careful discussion of this subject. Suffice it to say here only that a linear scale factor is seldom acceptable as the heavy-atom-derivative crystals frequently suffer from greater disorder than the native crystals. The heavy-atom derivative should, in general, have a slightly larger mean value for the structure factors on account of the additional heavy atoms (Green *et al.*, 1954). The usual effect is to make $\sum |\mathbf{F}_{NH}|^2 / \sum |\mathbf{F}_N|^2 \simeq 1.05$ (Phillips, 1966).

As the amount of heavy atom is usually unknown in a yet unsolved heavy-atom derivative, it is usual practice either to apply a scale factor of the form $k \exp[-B(\sin \theta / \lambda)^2]$ or, more generally, to use local scaling (Matthews & Czerwinski, 1975). The latter has the advantage of not making any assumption about the physical nature of the relative intensity decay with resolution.

2.3.4. Anomalous dispersion

2.3.4.1. Introduction

The physical basis for anomalous dispersion has been well reviewed by Ramaseshan & Abrahams (1975), James (1965), Cromer (1974) and Bijvoet (1954). As the wavelength of radiation approaches the absorption edge of a particular element, then an atom will disperse X-rays in a manner that can be defined by the

complex scattering factor

$$f_0 + \Delta f' + i\Delta f''$$

where f_0 is the scattering factor of the atom without the anomalous absorption and re-scattering effect, $\Delta f'$ is the real correction term (usually negative), and $\Delta f''$ is the imaginary component. The real term $f_0 + \Delta f'$ is often written as f' , so that the total scattering factor will be $f' + if''$. Values of $\Delta f'$ and $\Delta f''$ are tabulated in *IT IV* (Cromer, 1974), although their precise values are dependent on the environment of the anomalous scatterer. Unlike f_0 , $\Delta f'$ and $\Delta f''$ are almost independent of scattering angle as they are caused by absorption of energy in the innermost electron shells. Thus, the anomalous effect resembles scattering from a point atom.

The structure factor of index \mathbf{h} can now be written as

$$\mathbf{F}_{\mathbf{h}} = \sum_{j=1}^N f'_j \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j) + i \sum_{j=1}^N f''_j \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j). \quad (2.3.4.1)$$

(Note that the only significant contributions to the second term are from those atoms that have a measurable anomalous effect at the chosen wavelength.)

Let us now write the first term as $A + iB$ and the second as $a + ib$. Then, from (2.3.4.1),

$$\mathbf{F} = (A + iB) + i(a + ib) = (A - b) + i(B + a). \quad (2.3.4.2)$$

Therefore,

$$|\mathbf{F}_{\mathbf{h}}|^2 = (A - b)^2 + (B + a)^2$$

and similarly

$$|\mathbf{F}_{\bar{\mathbf{h}}}|^2 = (A + b)^2 + (-B + a)^2,$$

demonstrating that Friedel's law breaks down in the presence of anomalous dispersion. However, it is only for noncentrosymmetric reflections that $|\mathbf{F}_{\mathbf{h}}| \neq |\mathbf{F}_{\bar{\mathbf{h}}}|$.

Now,

$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}}^{\text{sphere}} \mathbf{F}_{\mathbf{h}} \exp(2\pi i \mathbf{h} \cdot \mathbf{x}).$$

Hence, by using (2.3.4.2) and simplifying,

$$\rho(\mathbf{x}) = \frac{2}{V} \sum_{\mathbf{h}}^{\text{hemisphere}} [(A \cos 2\pi \mathbf{h} \cdot \mathbf{x} - B \sin 2\pi \mathbf{h} \cdot \mathbf{x}) + i(a \cos 2\pi \mathbf{h} \cdot \mathbf{x} - b \sin 2\pi \mathbf{h} \cdot \mathbf{x})]. \quad (2.3.4.3)$$

The first term in (2.3.4.3) is the usual real Fourier expression for electron density, while the second term is an imaginary component due to the anomalous scattering of a few atoms in the cell.

2.3.4.2. The $P_s(\mathbf{u})$ function

Expression (2.3.4.3) gives the complex electron density expression in the presence of anomalous scatterers. A variety of Patterson-type functions can be derived from (2.3.4.3) for the determination of a structure. For instance, the $P_s(\mathbf{u})$ function gives vectors between the anomalous atoms and the 'normal' atoms.

From (2.3.4.1) it is easy to show that

$$\begin{aligned} \mathbf{F}_{\mathbf{h}} \mathbf{F}_{\mathbf{h}}^* &= |\mathbf{F}_{\mathbf{h}}|^2 \\ &= \sum_{i,j} (f'_i f'_j + f''_i f''_j) \cos 2\pi \mathbf{h} \cdot (\mathbf{x}_i - \mathbf{x}_j) \\ &\quad + \sum_{i,j} (f'_i f''_j - f''_i f'_j) \sin 2\pi \mathbf{h} \cdot (\mathbf{x}_i - \mathbf{x}_j). \end{aligned}$$

Therefore,

2.3. PATTERSON AND MOLECULAR-REPLACEMENT TECHNIQUES

$$|\mathbf{F}_h|^2 + |\mathbf{F}_{\bar{h}}|^2 = 2 \sum_{i,j} (f'_i f'_j + f''_i f''_j) \cos 2\pi \mathbf{h} \cdot (\mathbf{x}_i - \mathbf{x}_j)$$

and

$$|\mathbf{F}_h|^2 - |\mathbf{F}_{\bar{h}}|^2 = 2 \sum_{i,j} (f'_i f'_j - f''_i f''_j) \sin 2\pi \mathbf{h} \cdot (\mathbf{x}_i - \mathbf{x}_j).$$

Let us now consider the significance of a Patterson in the presence of anomalous dispersion. The normal Patterson definition is given by

$$\begin{aligned} P(\mathbf{u}) &= \int_V \rho^*(\mathbf{x}) \rho(\mathbf{x} + \mathbf{u}) \, d\mathbf{x} \\ &= \frac{1}{V^2} \sum_{\mathbf{h}}^{\text{sphere}} |\mathbf{F}_h|^2 \exp(-2\pi i \mathbf{h} \cdot \mathbf{u}) \\ &\equiv P_c(\mathbf{u}) - iP_s(\mathbf{u}), \end{aligned}$$

where

$$P_c(\mathbf{u}) = \frac{2}{V} \sum_{\mathbf{h}}^{\text{hemisphere}} (|\mathbf{F}_h|^2 + |\mathbf{F}_{\bar{h}}|^2) \cos 2\pi \mathbf{h} \cdot \mathbf{u}$$

and

$$P_s(\mathbf{u}) = \frac{2}{V} \sum_{\mathbf{h}}^{\text{hemisphere}} (|\mathbf{F}_h|^2 - |\mathbf{F}_{\bar{h}}|^2) \sin 2\pi \mathbf{h} \cdot \mathbf{u}.$$

The $P_c(\mathbf{u})$ component is essentially the normal Patterson, in which the peak heights have been very slightly modified by the anomalous-scattering effect. That is, the peaks of $P_c(\mathbf{u})$ are proportional in height to $(f'_i f'_j + f''_i f''_j)$.

The $P_s(\mathbf{u})$ component is more interesting. It represents vectors between the normal atoms in the unit cell and the anomalous scatterers, proportional in height to $(f'_i f'_j - f''_i f''_j)$ (Okaya *et al.*, 1955). This function is antisymmetric with respect to the change of the direction of the diffraction vector. An illustration of the function is given in Fig. 2.3.4.1. In a unit cell containing N atoms, n of which are anomalous scatterers, the $P_s(\mathbf{u})$ function contains only $n(N - n)$ positive peaks and an equal number of negative peaks related to the former by anticommetry. The analysis of a $P_s(\mathbf{u})$ synthesis presents problems somewhat similar to those posed by a normal

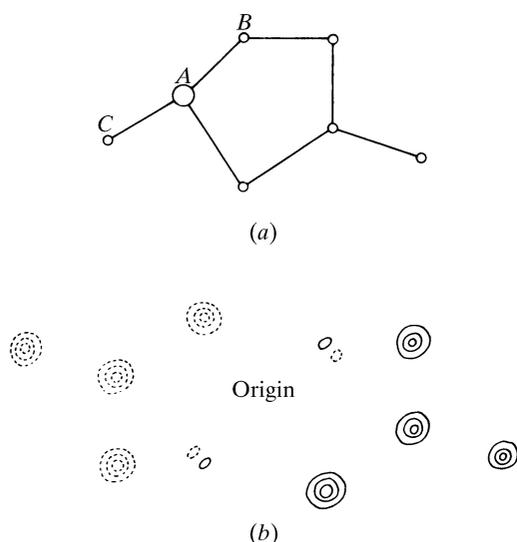


Fig. 2.3.4.1. (a) A model structure with an anomalous scatterer at A. (b) The corresponding $P_s(\mathbf{u})$ function showing positive peaks (full lines) and negative peaks (dashed lines). [Reprinted with permission from Woolfson (1970, p. 293).]

Patterson. The procedure has been used only rarely [*cf.* Moncrief & Lipscomb (1966) and Pepinsky *et al.* (1957)], probably because alternative procedures are available for small compounds and the solution of $P_s(\mathbf{u})$ is too complex for large biological molecules.

2.3.4.3. The position of anomalous scatterers

Anomalous scatterers can be used as an aid to phasing, when their positions are known. But the anomalous-dispersion differences (Bijvoet differences) can also be used to determine or confirm the heavy atoms which scatter anomalously (Rossmann, 1961a). Furthermore, the use of anomalous-dispersion information obviates the lack of isomorphism but, on the other hand, the differences are normally far smaller than those produced by a heavy-atom isomorphous replacement.

Consider a structure of many light atoms giving rise to the structure factor $\mathbf{F}_h(N)$. In addition, it contains a few heavy atoms which have a significant anomalous-scattering effect. The non-anomalous component will be $\mathbf{F}_h(H)$ and the anomalous component is $\mathbf{F}_h''(H) = i(\Delta f''/f')\mathbf{F}_h(H)$ (Fig. 2.3.4.2a). The total structure factor will be \mathbf{F}_h . The Friedel opposite is constructed appropriately (Fig. 2.3.4.2a). Now reflect the Friedel opposite construction across the real axis of the Argand diagram (Fig. 2.3.4.2b). Let the difference in phase between \mathbf{F}_h and $\mathbf{F}_{\bar{h}}$ be φ . Thus

$$4|\mathbf{F}_h''(H)|^2 = |\mathbf{F}_h|^2 + |\mathbf{F}_{\bar{h}}|^2 - 2|\mathbf{F}_h||\mathbf{F}_{\bar{h}}| \cos \varphi,$$

but since φ is very small

$$|\mathbf{F}_h''(H)|^2 \simeq \frac{1}{4}(|\mathbf{F}_h| - |\mathbf{F}_{\bar{h}}|)^2.$$

Hence, a Patterson with coefficients $(|\mathbf{F}_h| - |\mathbf{F}_{\bar{h}}|)^2$ will be

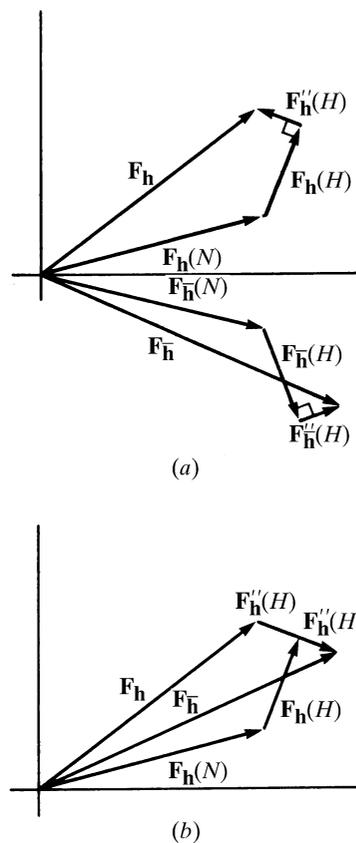


Fig. 2.3.4.2. Anomalous-dispersion effect for a molecule whose light atoms contribute $\mathbf{F}_h(N)$ and heavy atom $\mathbf{F}_h(H)$ with a small anomalous component of $\mathbf{F}_h''(H)$, 90° ahead of the non-anomalous $\mathbf{F}_h(H)$ component. In (a) is seen the construction for \mathbf{F}_h and $\mathbf{F}_{\bar{h}}$. In (b) $\mathbf{F}_{\bar{h}}$ has been reflected across the real axis.

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equivalent to a Patterson with coefficients $|\mathbf{F}_h''(H)|^2$ which is proportional to $|\mathbf{F}_h(H)|^2$. Such a Patterson (Rossmann, 1961a) will have vectors between all anomalous scatterers with heights proportional to the number of anomalous electrons $\Delta f''$. This 'anomalous dispersion' Patterson function has been used to find anomalous scatterers such as iron (Smith *et al.*, 1983; Strahs & Kraut, 1968) and sulfur atoms (Hendrickson & Teeter, 1981).

It is then apparent that a Patterson with coefficients

$$\Delta F_{\text{ANO}}^2 = (|\mathbf{F}_h| - |\mathbf{F}_h|)^2$$

(Rossmann, 1961a), as well as a Patterson with coefficients

$$\Delta F_{\text{ISO}}^2 = (|\mathbf{F}_{NH}| - |\mathbf{F}_H|)^2$$

(Rossmann, 1960; Blow, 1958), represent Pattersons of the heavy atoms. The ΔF_{ANO}^2 Patterson suffers from errors which may be larger than the size of the Bijvoet differences, while the ΔF_{ISO}^2 Patterson may suffer from partial lack of isomorphism. Hence, Kartha & Parthasarathy (1965) have suggested the use of the sum of these two Pattersons, which would then have coefficients $(\Delta F_{\text{ANO}}^2 + \Delta F_{\text{ISO}}^2)$.

However, given both SIR and anomalous-dispersion data, it is possible to make an accurate estimate of the $|\mathbf{F}_H|^2$ magnitudes for use in a Patterson calculation [Blundell & Johnson (1976, p. 340), Matthews (1966), Singh & Ramaseshan (1966)]. In essence, the Harker phase diagram can be constructed out of three circles: the native amplitude and each of the two isomorphous Bijvoet differences, giving three circles in all (Blow & Rossmann, 1961) which should intersect at a single point thus resolving the ambiguity in the SIR data and the anomalous-dispersion data. Furthermore, the phase ambiguities are orthogonal; thus the two data sets are cooperative. It can be shown (Matthews, 1966; North, 1965) that

$$F_N^2 = F_{NH}^2 + F_H^2 \mp \frac{2}{k}(16k^2 F_P^2 F_H^2 - \Delta I^2)^{1/2},$$

where $\Delta I = F_{NH}^{+2} - F_{NH}^{-2}$ and $k = \Delta f''/f'$. The sign in the third-term expression is $-$ when $|(\alpha_{NH} - \alpha_H)| < \pi/2$ or $+$ otherwise. Since, in general, $|\mathbf{F}_H|$ is small compared to $|\mathbf{F}_N|$, it is reasonable to assume that the sign above is usually negative. Hence, the heavy-atom lower estimate (HLE) is usually written as

$$F_{\text{HLE}}^2 = F_{NH}^2 + F_H^2 - \frac{2}{k}(16k^2 F_P^2 F_H^2 - \Delta I^2)^{1/2},$$

which is an expression frequently used to derive Patterson coefficients useful in the determination of heavy-atom positions when both SIR and anomalous-dispersion data are available.

2.3.5. Noncrystallographic symmetry

2.3.5.1. Definitions

The interpretation of Pattersons can be helped by using various types of chemical or physical information. An obvious example is the knowledge that one or two heavy atoms per crystallographic asymmetric unit are present. Another example is the exploitation of a rigid chemical framework in a portion of a molecule (Nordman & Nakatsu, 1963; Burnett & Rossmann, 1971). One extremely useful constraint on the interpretation of Pattersons is noncrystallographic symmetry. Indeed, the structural solution of large biological assemblies such as viruses is only possible because of the natural occurrence of this phenomenon. The term 'molecular replacement' is used for methods that utilize noncrystallographic symmetry in the solution of structures [for earlier reviews see Rossmann (1972) and Argos & Rossmann (1980)]. These methods, which are only partially dependent on Patterson concepts, are discussed in Sections 2.3.6–2.3.8.

Crystallographic symmetry applies to the whole of the three-dimensional crystal lattice. Hence, the symmetry must be expressed both in the lattice and in the repeating pattern within the lattice. In contrast, noncrystallographic symmetry is valid only within a limited volume about the noncrystallographic symmetry element. For instance, the noncrystallographic twofold axes in the plane of the paper of Fig. 2.3.5.1 are true only in the immediate vicinity of each local dyad. In contrast, the crystallographic twofold axes perpendicular to the plane of the paper (Fig. 2.3.5.1) apply to every point within the lattice. Two types of noncrystallographic symmetry can be recognized: proper and improper rotations. A proper symmetry element is independent of the sense of rotation, as, for example, a fivefold axis in an icosahedral virus; a rotation either left or right by one-fifth of a revolution will leave all parts of a given icosahedral shell (but not the whole crystal) in equivalent positions. Proper noncrystallographic symmetry can also be recognized by the existence of a closed point group within a defined volume of the lattice. Improper rotation axes are found when two molecules are arbitrarily oriented relative to each other in the same asymmetric unit or when they occur in two entirely different crystal lattices. For instance, in Fig. 2.3.5.2, the object A_1B_1 can be rotated by $+\theta$ about the axis at P to orient it identically with A_2B_2 . However, the two objects will not be coincident after a rotation of A_1B_1 by $-\theta$ or of A_2B_2 by $+\theta$. The envelope around each noncrystallographic object must be known in order to define an improper rotation. In contrast, only the volume about the closed point group need be defined for proper noncrystallographic operations. Hence, the boundaries of the repeating unit need not correspond to chemically covalently linked units in the presence of proper rotations.

Translational components of noncrystallographic rotation elements are said to be 'precise' in a direction parallel to the axis and

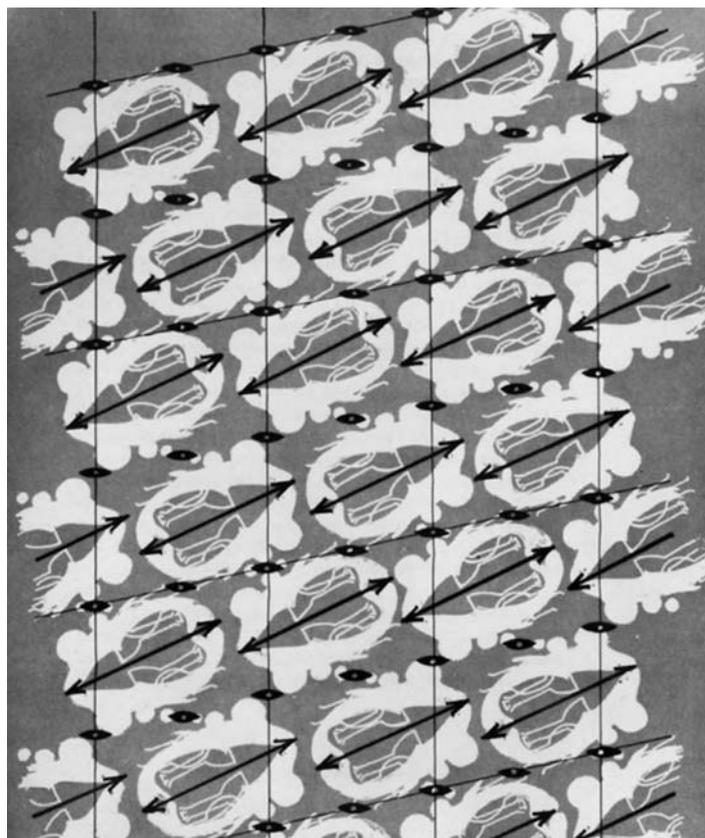


Fig. 2.3.5.1. The two-dimensional periodic design shows crystallographic twofold axes perpendicular to the page and local noncrystallographic rotation axes in the plane of the paper (design by Audrey Rossmann). [Reprinted with permission from Rossmann (1972, p. 8).]