

## 2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

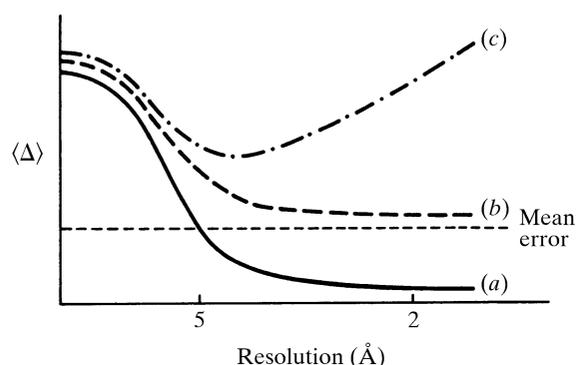


Fig. 2.3.3.6. A plot of mean isomorphous differences as a function of resolution. (a) The theoretical size of mean differences following roughly a Gaussian distribution. (b) The observed size of differences for a good isomorphous derivative where the smaller higher-order differences have been largely 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,