

are performed at symmetry points in the Brillouin zone, in electron diffraction theory this is not the case. For example, in conventional 100 keV electron microscopy the crystal orientation normally used, because this provides the best electron transmission in bright field (Hashimoto, Howie & Whelan, 1962) is with the crystal set slightly positive of a Bragg reflecting position; the corresponding Bloch waves usually have no particular symmetry at such a setting. With 1 MeV incident electrons the situation is different and although the crystal orientation which maximizes the electron transmission is often a point of symmetry (Humphreys & Lally, 1970), in many cases it is a point of no symmetry (Humphreys, Lally, Thomas & Fisher, 1970).

It is possible that in electron diffraction calculations for points of very high symmetry a group representation system may be desirable. However these points have in the past been of little interest to experimental electron microscopists and only a few theoretical calculations have been made. The authors feel that in general the labelling scheme for electron diffraction theory which is the most logical, simple and consistent with schemes in related fields is the system which labels the Bloch waves in terms of the dispersion surface in order of the magnitude of their  $k_{\perp}$  component wave vectors, as shown in Fig. 1. Following Phillips (1956) it is suggested that this system be called the ordered labelling scheme. The use of such a scheme is entirely straightforward, and the authors suggest that this scheme be generally adopted in electron diffraction theory.

#### Summary and conclusions

(1) The essential mathematical unity of all wave propagation in periodic structures is illustrated and emphasized.

(2) A brief discussion of the critical voltage effect

is given and equivalent effects in other forms of wave propagation are stated.

(3) It is suggested that the Bloch wave labelling scheme for electron diffraction which is the simplest and most logical, and which is consistent with accepted notations in other forms of Bloch wave propagation, is an ordered labelling scheme in which the top branch of the dispersion surface corresponds to wave 1, the second branch to wave 2, the third branch to wave 3, and so on.

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## A Note on the Correlation of the Heavy-Atom Positions in Different Isomorphous Protein Crystals Using Both Isomorphous-Replacement and Anomalous-Dispersion Data

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In this note more accurate expressions are obtained for the Fourier coefficients of the iso-ano correlation function and the origin correlation function of Kartha & Parthasarathy.

#### Introduction

After obtaining the intensity data from the native protein and its isomorphous heavy atom derivatives

the first stage involved in the determination of the protein structure is the location of the heavy atoms in the derivative crystals. This is usually carried out in two steps, namely, (i) to obtain the coordinates of the heavy atoms in each derivative crystal and (ii) to correlate the positions of the heavy atoms in the different derivatives to a common origin. Kartha & Par-

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thasarathy (1965a) and Matthews (1966) have obtained methods for combining isomorphous-replacement and anomalous-dispersion data to tackle the first step and they have further shown that such a combination of the two sets of data leads to better results than could be obtained by the use of either individually. Matthews (1966) has further made a comparative study of his method and that of Kartha & Parthasarathy (1965a) in the case of  $\alpha$ -chymotrypsin and has shown that his method obtains better results. The second step could be tackled by making use of the iso-ano or the origin correlation function of Kartha & Parthasarathy (1965b) (hereafter referred to as KP, 1965b) and here again it has been shown that a combined use of the isomorphous-replacement and anomalous-dispersion data leads to better results than could be obtained by using either of the sets of data alone. However, they have derived these correlation functions by making use of the simplifying assumption that the phases of the reflexion ( $hkl$ ) from the native protein and the derivatives are the same. They have remarked that this assumption is less justifiable in their anomalous dispersion correlation function than in the isomorphous case (see KP, 1965b). In this note we shall show that their final results for the iso-ano and the origin correlation functions can be arrived at, without using this assumption, provided we redefine the Fourier coefficients for these correlation functions in a different way.

It is relevant to point out here that the second step can be carried out only after the first step has been carried out successfully (see the *Discussion* section later). We shall therefore assume in this note that the positions of the heavy atoms in each derivative are known and the problem is one of correlating the heavy atom configurations in the different derivatives with respect to a common origin.

### Iso-ano correlation function

The structure factor equation for a reflexion ( $hkl$ ) in the  $j$ th heavy atom derivative of the protein can be written as (Fig. 1)

$$F_{PHj} = F_P + F_{Hj} \quad (1)$$

where the contribution to the structure factor for the reflexion ( $hkl$ ) from the native protein is denoted by  $F_P$  while that from the heavy atoms alone in the  $j$ th derivative by  $F_{Hj}$ . From (1) we have

$$|F_{PHj}|^2 = |F_P|^2 + |F_{Hj}|^2 + 2|F_P| |F_{Hj}| \cos(\alpha_P - \alpha_{Hj}). \quad (2)$$

We can rewrite (2) as

$$|F_P| |F_{Hj}| \cos(\alpha_P - \alpha_{Hj}) = [|F_{PHj}|^2 - |F_P|^2 - |F_{Hj}|^2] / 2 = I_j, \text{ say.} \quad (3)$$

In (3), the quantities  $|F_P|^2$  (which denotes the intensity

of the reflexion ( $hkl$ ) from the native protein) and  $|F_{PHj}|^2$  (which denotes the intensity of the reflexion ( $hkl$ ) from the  $j$ th derivative) are known. By making use of the synthesis proposed by Matthews (1966) the coordinates of the heavy atoms in the  $j$ th derivative can be determined and from this the value of  $|F_{Hj}|^2$  (which is independent of the choice of origin in the  $j$ th derivative) for each reflexion can be calculated. Since the values of  $|F_P|^2$ ,  $|F_{PHj}|^2$  and  $|F_{Hj}|^2$  are known for each reflexion, the value of  $I_j$  can be obtained from (3).

The Bijvoet difference in the  $j$ th derivative will be given by [see equation (22) of Kartha & Parthasarathy, (1965a)]

$$(\Delta I)_j = 4|F_{PHj}| |F_{Hj}^*| \sin(\alpha_{PHj} - \alpha_{Hj}) = 4k_j |F_{PHj}| |F_{Hj}| \sin(\alpha_{PHj} - \alpha_{Hj}) \quad (4)$$

where we have assumed, as in the earlier paper, that all the anomalous scatterers are of the same type. It is clear from Fig. 1 that

$$|F_{PHj}| \sin(\alpha_{PHj} - \alpha_{Hj}) = |F_P| \sin(\alpha_P - \alpha_{Hj}) = AB. \quad (5)$$

Making use of (5) in (4) we obtain

$$|F_P| |F_{Hj}| \sin(\alpha_P - \alpha_{Hj}) = \frac{(\Delta I)_j}{4k_j} = A_j, \text{ say.} \quad (6)$$

It is clear from (6) that from the measured value of the Bijvoet difference  $(\Delta I)_j$  in the  $j$ th derivative and the known value of  $k_j$  (see Matthews (1966) for an experimental procedure for obtaining the value of  $k_j$ ) the value of  $A_j$  can be calculated.

From (3) and (6) we obtain

$$I_j + iA_j = |F_P| |F_{Hj}| \exp\{i(\alpha_P - \alpha_{Hj})\} = F_P F_{Hj}^*. \quad (7)$$

It is clear from (7) that

$$I_1 + iA_1 = F_P F_{H1}^*$$

$$I_2 + iA_2 = F_P F_{H2}^*$$

so that

$$\begin{aligned} F_{H1}^*/F_{H2}^* &= (I_1 + iA_1)/(I_2 + iA_2) \\ &= (I_1 + iA_1)(I_2 - iA_2)/(I_2^2 + A_2^2). \end{aligned}$$

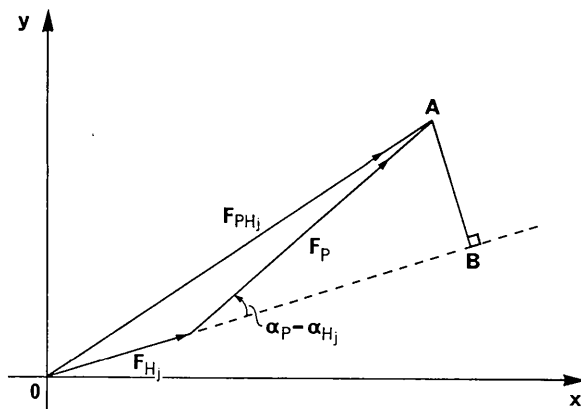


Fig. 1. Argand diagram showing the structure factor relation of a reflexion ( $hkl$ ) in the  $j$ th isomorphous derivative of the native protein.

Multiplying both sides of the above equation by  $F_{H_2}F_{H_2}^* = |F_{H_2}|^2$  and making use of the result that  $I_1^2 + A_1^2 = |F_P|^2 |F_{H_1}|^2$  [see equation (7)] we obtain

$$F_{H_1}^* F_{H_2} = [I_1 I_2 + A_1 A_2 + i(A_1 I_2 - A_2 I_1)] / |F_P|^2. \quad (8)$$

It is clear that the left-hand side of (8) is the same as the right-hand side of equation (11) of KP (1965*b*). The Fourier synthesis making use of the known quantities in the right-hand side of (8) therefore gives peaks at the ends of the vectors ( $\mathbf{r}_{H_2} - \mathbf{r}_{H_1}$ ) [for details of this synthesis see KP (1965*b*)]. Thus by defining the Fourier coefficient for the iso-ano correlation synthesis as in the right-hand side of (8) it is possible to avoid the less valid simplifying assumption that  $\alpha_{PH_1} = \alpha_{PH_2} = \alpha_P$ . From (8) we also obtain

$$F_{H_1} F_{H_2}^* = [I_1 I_2 + A_1 A_2 - i(A_1 I_2 - A_2 I_1)] / |F_P|^2 \quad (9)$$

which can be compared with equation (12) of KP (1965*b*). A map with coefficients, as in the right-hand side of (9), gives peaks at the ends of the vectors  $\mathbf{r}_{H_1} - \mathbf{r}_{H_2}$ .

### Origin correlation function

This function gives the vector  $\mathbf{R}_{12}$  relating the origin of derivative 2 with respect to that of 1 and the Fourier coefficients for this synthesis are given by  $\exp(i\mathbf{R}_{12} \cdot \mathbf{S})$  (see KP, 1965*b*). Following Kartha & Parthasarathy (1965*b*), if we refer all the coordinates to the origin of derivative 1, we will have

$$\mathbf{r}_{H_2} = \mathbf{R}_{12} + \mathbf{r}_{H_2}' \quad (10)$$

where the prime over  $H$  denotes that the quantities involved are referred with respect to the origin of derivative 2.

Making use of (10) we obtain

$$F_{H_1}^* F_{H_2} = F_{H_1}^* F_{H_2}' \exp(i\mathbf{R}_{12} \cdot \mathbf{S}). \quad (11)$$

The Fourier coefficient for the origin correlation function can be obtained from (8) and (11) as

$$\exp(i\mathbf{R}_{12} \cdot \mathbf{S}) = \frac{I_1 I_2 + A_1 A_2 + i(A_1 I_2 - A_2 I_1)}{|F_P|^2 F_{H_1}^* F_{H_2}'}. \quad (12)$$

A Fourier synthesis using the right-hand side of (12) as coefficient gives a peak at the end of the vector  $\mathbf{R}_{12}$  [for further details of this synthesis see KP (1965*b*)].

### Discussion of the correlation functions

It is relevant to note the following points regarding the computation of the Fourier coefficients for the cor-

relation functions as obtained here and by KP (1965*b*):

(i) For the interpretation (but not the computation) of the iso-ano correlation function of KP (1965*b*) it is necessary to know the configurations of the heavy atoms in one of the derivatives only. However, for both the computation *and* the interpretation of the iso-ano correlation function from (8), it is necessary to know the configurations of the heavy atoms in each of the derivatives. The greater accuracy in the Fourier coefficient obtained in (8) might possibly be worth the trouble of obtaining the configurations of the heavy atoms in each of the derivatives and this can be easily achieved by making use of the synthesis proposed by Matthews (1966).

(ii) The computation of the origin correlation function obtained here and by KP (1965*b*) also pre-supposes that the configurations of the heavy atoms in both the derivatives are completely established and that the only unknown parameter to be determined is the vector  $\mathbf{R}_{12}$ .

(iii) Whereas the iso-ano synthesis contains a number of peaks ( $H_1 H_2$  in number) corresponding to the correlation vectors, the origin correlation function gives rise to single peak at  $\mathbf{R}_{12}$ . In spite of this apparent advantage of the origin correlation function over the iso-ano function obtained in (8) (both these functions requiring a knowledge of the configurations of the heavy atoms in both the derivations) the iso-ano function might probably be more advantageous since in the origin correlation function there is a greater chance for a number of Fourier coefficients to become unduly large due to a very small heavy contribution in either of the derivatives. KP (1965*b*) suggest the use of some arbitrary weighting function to tackle such a situation. However, the actual superiority of one of the correlation functions over the other is yet to be determined by experience.

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