The film (Ilford Industrial G) was assumed to contain 1.45 mg cm^{-2} of silver bromide and 25 mg cm^{-2} of a base (40% C, 10% N, 40% O and 10% H) (Morimoto & Uyeda, 1963).

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Best Density Maps in Low-Resolution Crystallography with Contrast Variation

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Abstract

A method is presented of merging experimental diffraction data measured at different contrasts and structure factors calculated with models defined (and refined) at some of these contrasts (or at any other) in order to calculate an optimal density map at any contrast. It is based on a probabilistic approach which uses a joint probability distribution, for each $[h, k, l]$, of the measured intensities and the calculated structure factors.

1. Introduction

The technique of low-resolution single-crystal diffraction with contrast variation has been applied in several protein-structure determinations. It is especially useful in cases where, for instance, a part of the molecule is disordered in the crystal, like the

RNA in viruses (Bentley, Lewit-Bentley, Liljas, Skoglund, Roth & Unge, 1987, or the detergent in membrane-protein crystals (Zulauf, Timmins & Garavito, 1986). It has also been used to initiate the structural study of systems where no isomorphous derivative is yet available (Bentley, Lewit-Bentley, Finch, Podjarny & Roth, 1984; Zulauf, Timmins & Garavito, 1986). The technique consists of the collection of several diffraction data sets to a given resolution with crystals of the same molecule soaked in solvents with different scattering-length density. For the case of neutrons, the scattering-length density of the solvent is usually modified by changing its concentration of D_2O/H_2O. For X-rays, the scattering-length density can be modified by changing some salt concentration, for instance (Bragg & Perutz, 1952).

Often, in using this technique, one has to solve the problem of interpolating or extrapolating structure factors from one or a few contrasts for which model densities have been determined to another contrast for which the density has to be determined, using the

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structure-factor relationship between contrasts characteristic of linear contrast variation. This is one application of the calculations presented here. From another point of view, these calculations allow the incorporation of the contrast-variation phase relationships as a constraint in density-map refinements starting from density or model refinements made with intensities alone. Finally, the method also allows one to make use of partial knowledge of a structure to determine the whole structure, a situation which is rather common in the contrast-variation technique, where a model for part of the molecule, valid at the contrast where the rest of the molecule is matched out, represents a known part of the structure at any other contrast if one takes into account the change in contrast conditions.

The originality of the method compared with similar calculations is that it includes in a unique probability distribution function (a) the joint probability distribution function of the measured data, as it results from the statistics of the background corrected intensities, the application of the constraint of linear dependence of the structure factors with respect to contrast and the relative scaling of the intensities measured in different contrasts, and (b) the probability distribution functions of the F_{calc}'s of the models.

2. Best density map

Following Blow & Crick (1959) and Sage & Melsa (1971), we consider as best density map the map which minimizes the errors in the calculated density map with respect to the true map for any contrast c at any point r of the lattice cell. This error can be defined as

$$R(\mathbf{r}, c) = \int C[\hat{\rho}(\mathbf{r}, c) - \rho(\mathbf{r}, c)] P(\{F\}/\{I\}, \{F_c\}) \, d\{F\}. \tag{1}$$

Here $C[\hat{\rho}(\mathbf{r}, c) - \rho(\mathbf{r}, c)]$ is an error cost function of the difference between the true density $\rho(\mathbf{r}, c)$ and the density $\hat{\rho}(\mathbf{r}, c)$ calculated from the measured data and the models, these densities being given respectively by

$$\rho(\mathbf{r}, c) = (1/V) \sum_{h,k,l} F(h, k, l, c) \exp(-i2\pi\mathbf{H} \cdot \mathbf{r}), \tag{2}$$

where $\mathbf{H}$ is the reciprocal-lattice vector with components $[h, k, l]$ and V is the volume of the unit cell, and

$$\hat{\rho}(\mathbf{r}, c) = (1/V) \sum_{h,k,l} \hat{F}(h, k, l, c) \exp(-i2\pi\mathbf{H} \cdot \mathbf{r}), \tag{3}$$

where $\hat{F}(h, k, l, c)$ is the best estimate of $F(h, k, l, c)$ (i.e. the estimate obtained by minimization of R with respect to $\hat{\rho}$).

The function $P(\{F\}/\{I\}, \{F_c\})$ is the a posteriori probability density function of the structure factors $F(h, k, l, c)$ at contrast c. It is the conditional joint probability density function of these F's (ensemble $\{F\}$) with the following factors taken into account: (1) the result of the measurement of the intensities $I(h, k, l, c_i)$ (ensemble $\{I\}$) at different reciprocal-lattice points h, k, l and contrasts c_i, and (2) the values of the calculated structure factors $F_{cj}(h, k, l)$ at different contrasts c_j (ensemble $\{F_c\}$) obtained from model fittings and map refinements at these contrasts.

If one considers as cost function the squared difference $[\hat{\rho}(\mathbf{r}, c) - \rho(\mathbf{r}, c)]^2$, one obtains the following best estimate of $F(h, k, l, c)$:

$$\hat{F}(h, k, l, c) = \int F(h, k, l, c) P(\{F\}/\{I\}, \{F_c\}) \, d\{F\}, \tag{4}$$

that is,

$$\hat{F}(h, k, l, c) = \int F(h, k, l, c)$$
$$\times p[F(h, k, l, c)/\{I\}, \{F_c\}] \, dF(h, k, l, c), \tag{5}$$

where $p[F(h, k, l, c)/\{I\}, \{F_c\}]$ is the marginal probability density of $P(\{F\}/\{I\}, \{F_c\})$ with respect to the random variable $F(h, k, l, c)$. This type of best density map (least-squares type) was first proposed by Blow & Crick (1959). Another definition of the cost function C leads to a value of $\hat{\rho}$ given by a Fourier series identical to (3), using for each $\hat{F}$ the value which maximizes the function $p(F/\{I\}, \{F_c\})$; this defines the maximum a posteriori probability best map (see Sage & Melsa, 1971). As already discussed by Blow & Crick (1959), this approach may raise some difficulties if the probability distribution has more than one maximum; in particular, if it has two maxima of the same height.

3. Marginal a posteriori probability density functions

The main approximation we make in deriving these functions is to neglect the correlations existing between structure factors from different h, k, l's. That is to say we consider the function $p[F(h, k, l, c)/\{I\}, \{F_c\}]$ as a function $pF(h, k, l, c)/[I], [F_c])$, where $[I]$ and $[F_c]$ are the subsets of $\{I\}$ and $\{F_c\}$ corresponding to the single reciprocal-lattice point h, k, l under consideration (for instance, $[I]$ is the ensemble of intensities measured at that h, k, l in the different contrasts). We thus neglect the probabilities of multiplet phase relationships or other causes of correlation.

The structure factor $F(h, k, l, c)$ is actually a function of the two partial structure factors F_0 and F_d according to the relation (Worcester & Franks, 1976)

$$F(h, k, l, c) = F_0(h, k, l) + cF_d(h, k, l). \tag{6}$$

F_0 and F_d are the relevant random complex variables

of the problem. Then, from Bayes' theorem,

$$p(\mathbf{F}_0, \mathbf{F}_d/[I], [\mathbf{F}_c]) = p'([I], [\mathbf{F}_c]/\mathbf{F}_0, \mathbf{F}_d)p_a(\mathbf{F}_0, \mathbf{F}_d),$$
(7)

where p' is the joint conditional probability density function of the measured intensities $[I]$ and the calculated structure factors $[\mathbf{F}_c]$, if one knows the values of $\mathbf{F}_0$ and $\mathbf{F}_d$ for the given h, k, l; $p_a(\mathbf{F}_0, \mathbf{F}_d)$ is the a priori probability density of these two structure factors $\mathbf{F}_0$ and $\mathbf{F}_d$. The conditional probability density function of the calculated structure factors $[\mathbf{F}_c]$ which has to be introduced here is the probability of having 'found' $[\mathbf{F}_c]$ if one knows $\mathbf{F}_0$ and $\mathbf{F}_d$. This function can be given some reality by looking for a function which measures the degree of accuracy of a given $\mathbf{F}_c$ with respect to its true value $\mathbf{F}$, given by (6), that is based on a statistical model for the errors.

We are going to make the following additional assumptions:

(1) the probability density functions of the $\mathbf{F}_{cj}$ for the different models are independent;

(2) for a given model (i.e. a given contrast) the distribution of $\mathbf{F}_{cj}(h, k, l)$ is independent of the probability distribution of the intensities $[I]$ measured at this h, k, l in all contrasts.

The first assumption is justified because the different models are supposed to be fitted and refined independently on independently measured data sets. The second may be justified by the fact that although each $\mathbf{F}_{cj}$ is a function of the measured intensities in the given contrast via the model refinement, this relation is very complex, so that the errors in the measured intensity and the errors in the $\mathbf{F}_{cj}$ for the same given h, k, l are random and uncorrelated.

With these assumptions one finally obtains

$$p(\mathbf{F}_0, \mathbf{F}_d/[I], [\mathbf{F}_c])$$

$$= p_I([I]/\mathbf{F}_0, \mathbf{F}_d)p_a(\mathbf{F}_0, \mathbf{F}_d) \prod_j p_F(\mathbf{F}_{cj}/\mathbf{F}_0, \mathbf{F}_d).$$
(8)

From here on, we shall consider in the equations only intensities and structure factors corresponding to the same reciprocal-lattice point; therefore, the reference to h, k, l will be omitted in the notation.

4. Joint probability density function of the measured intensities

Correlations in the statistical distributions of the intensities measured at the same h, k, l in different contrasts result from the relative scaling of these different data sets based on the parabolic variation of the intensities, and from the least-squares fit of the measured intensities to (6) for each h, k, l, made in order to determine the values of the parameters $\mathbf{F}_0, \mathbf{F}_d$ and the cosine of their phase difference $\Delta\varphi$ (Roth, Lewit-Bentley & Bentley, 1984).

In terms of intensities, (6) can be rewritten as

$$I = I_0 + cI_1 + c^2 I_2$$
(9)

with

$$I_0 = F_0^2; \quad I_1 = 2F_0 F_d \cos(\Delta\varphi); \quad I_2 = F_d^2.$$
(10)

The variance-covariance matrix **B** of these three parameters after least-squares determination is obtained from the variance-covariance matrix of the measured reflections $[I]$ after relative scaling and from the variance-covariance matrix of the scaling factors, as shown by Roth, Lewit-Bentley & Bentley (1984). The scaling can be performed either by use of the parabolic variation with respect to c of the intensity of each reflection (Roth, Lewit-Bentley & Bentley, 1984) or by absolute scaling of the sums of the intensities for each contrast (Roth, 1986). If one assumes that the scaled intensities are Gaussian variables, the same is then true for I_0, I_1 and I_2 obtained by least squares, and the unnormalized conditional probability density is

$$p_I([I]/\mathbf{F}_0, \mathbf{F}_d) = \exp(-\Delta\mathbf{I}'\mathbf{B}^{-1}\Delta\mathbf{I}/2),$$
(11)

where $\Delta\mathbf{I}'$ is the three-dimensional vector

$$\Delta\mathbf{I}' = [\hat{I}_0 - F_0^2, \hat{I}_1 - 2F_0 F_d \cos(\Delta\varphi), \hat{I}_2 - F_d^2].$$
(12)

Here $\hat{I}_0$, $\hat{I}_1$ and $\hat{I}_2$ are the least-squares estimates of I_0, I_1 and I_2, and $\Delta\mathbf{I}'$ is the transpose of $\Delta\mathbf{I}$. This joint probability density function of the measured intensities replaces in the present approach the probability density functions based on lack of closure used in the case of isomorphous replacement (Blow & Crick, 1959; Hendrickson & Lattman, 1970).

5. A priori probability density function of F_0 and F_d

In our problem nothing is known a priori concerning the moduli of $\mathbf{F}_0$ and $\mathbf{F}_d$. For a non-centrosymmetric reflection the phase φ_0 of $\mathbf{F}_0$ is also undetermined a priori. The phase of $\mathbf{F}_d$ is related to φ_0 by

$$\varphi(\mathbf{F}_d) = \varphi_0 - \varepsilon\Delta\varphi$$
(13)

where $\Delta\varphi$ is the absolute phase difference between $\mathbf{F}_0$ and $\mathbf{F}_d$ restricted to the range $[0, \pi]$ whose value is also undetermined a priori, and ε is a variable equal to $+1$ or -1 with probability q and $1 - q$ respectively (a priori $q = \frac{1}{2}$). The a priori probability density $p_a(\mathbf{F}_0, \mathbf{F}_d)$ is thus just the probability distribution of this Bernoulli random variable ε.

For a centrosymmetric reflection, the phase of a given h, k, l can have only two values differing by π (i.e. $\Delta\varphi$ is equal to 0 or π). The a priori probability density $p_a(\mathbf{F}_0, \mathbf{F}_d)$ is therefore given by

$$p_a(\varphi_0, \Delta\varphi) = [\delta(\varphi_0 - \beta) + \delta(\varphi_0 - \beta + \pi)]$$

$$\times [\delta(\Delta\varphi) + \delta(\Delta\varphi - \pi)]/4,$$
(14)

where β depends on h, k, l and is fixed by the space group.

6. Conditional probability density function of the F_{calc}'s

The conditional probability density function of a given F_c with respect to its true value F given by (6) is obtained, as already mentioned, on the basis of a stochastic model for the errors of the F_c density map with respect to the true density map. Rather than going through the process of model fitting to define that error distribution, we use a method formally very similar to that used in predicting structure factors for partially known structures [see, for instance, Sim (1959) and Jauch (1983)]. The model is based on a partition of the lattice cell into small identical contiguous parallelepipeds (called cubes in the rest of the text for the sake of brevity) with edges parallel to the crystal axes and with an edge length equal to a fraction of the resolution at which the density map is calculated. The difference between the average F_c density and the average true density in each of these cubes is treated as a random variable with different values from place to place. It is assumed that it has a zero mean and a variance v_j for the contrast j. In this model the value of F_c is deduced from the value of F by adding to F the amplitudes scattered by all these elementary error densities. If one assumes that these elementary errors are distributed at random through the lattice, the distribution of F_c in the complex plane is given as a function of F by application of the central-limit theorem as

$$p_F(\mathbf{F}_{cj}/\mathbf{F}_0, \mathbf{F}_d) = \exp\left[-|\mathbf{F}_{cj} - \mathbf{F}_0 - c_j\mathbf{F}_d|^2/(pNv_j')\right],$$

(15)

where N is the number of these cubes per unit cell in the lattice, v_j' is the variance of the amplitude scattered by these cubes, the scattering length of each cube being equal to the product of the density difference (density error) in the cube and the volume of the cube, and p is equal to 1 or 2 for non-centrosymmetric or centrosymmetric reflections respectively (de Rango, Tsoucaris & Zelwer, 1974). Because of the small size of these cubes with respect to the resolution, this variance v_j' is proportional to v_j:

$$v_j' = \langle A^2 + B^2 \rangle v_j/N'$$

(16)

where A and B are the trigonometric functions of h, k, l, x, y, z, given for every space group by *International Tables for X-ray Crystallography* (1952) for the calculation of $F(h, k, l)$. The average $\langle \ldots \rangle$ is taken over x, y, z in the unit cell and N' is the number of asymmetric units per unit cell.

To determine the value of v_j for a given F_{calc} map we shall equate its value to the average of the square of the difference between this calculated map and the true map. In a first approximation one can use the average of the square of the difference map,

$F_{calc} - (F_{obs}, \varphi_{calc})$:

$$v_j = (v/V)^2 \sum_{H(\neq 0)} [F_{cj} - F_{obs}(c_j)]^2$$

(17)

with

$$F_{obs}(c) = (I_0 + cI_1 + c^2 I_2)^{1/2},$$

(18)

v being the volume of one of the cubes. Then, by iteration, the square of the difference map $\hat{\mathbf{F}}_{cj} - \hat{\mathbf{F}}_0 - c_j\hat{\mathbf{F}}_d$ can be used instead, where $\hat{\mathbf{F}}_0$ and $\hat{\mathbf{F}}_d$ are the best estimates of $\mathbf{F}_0$ and $\mathbf{F}_d$ and are taken from the preceding iteration. As can be seen in applications, however, this iteration is not very useful.

The value of N is given by

$$N = V/v \quad \text{with } v = (d/m)^3,$$

(19)

where d is the minimum d spacing considered and m is an integer larger than 1.

The variance of F_c resulting from this model of density error is independent of h, k, l. It is proportional to the average of the squared difference $|F_{calc} - F_{obs}|^2$ over all h, k, l's for the contrast under consideration. Our method is analogous to some extent to that proposed for a similar case by Bricogne (1976), which consists of the use of the probability distribution of Sim (1959) based on the identification of the imperfectly known F_{calc}'s with the structure factors of a partially known structure and proposes to use as an empirical variance the average of the absolute intensity differences $|I_{calc} - I_{obs}|$, an average calculated as a function of the resolution in different resolution shells. With neutrons the atomic form factor is independent of the resolution and therefore there is no need to consider a resolution-dependent average. In the present method the variance depends on the overall resolution, d.

Stochastic models

In some cases, a model can by itself allow for an F_c distribution because of the stochastic nature of some of the parameters, like the position of the totality or of a part of the molecule, distributed around a mean position or distributed at random through the lattice [see, respectively, Luzzati (1955), Jauch (1983), Giacovazzo (1983)]. Relation (1) then reads

$$R(\mathbf{r}, c) = \int C[\hat{\rho}(\mathbf{r}, c) - \rho(\mathbf{r}, c)]$$
$$\times P(\{F\}/\{I\}, \{F_c\})p_c(\{F_c\})\, d\{F\}\, d\{F_c\}, \quad (20)$$

where $p_c(\{F_c\})$ is the joint probability density function of the F_c's of the model. By repeating the derivation and assuming independent Gaussian distributions for the F_c's of these statistical models, one finds finally an expression like (15) where F_{cj} is now the expectation value corresponding to the Gaussian distribution of F_c for the model at contrast c_j. The new variance

w_j of the distribution of F_{cj}, (15), is now

$$w_j = Nv_j' + m_j, \qquad (21)$$

where m_j is the variance of the distribution of F_c.

An example of such a stochastic model is given in §8.

7. Integration

By combining everything, one obtains the joint probability density functions of F_0 and F_d which follow.

For a non-centrosymmetric reflection there is a double determination. For $\varepsilon > 0$,

$$p(F_0, F_d/[I], [F_c])$$
$$= q \exp\left[-\{\Delta I' B^{-1} \Delta I + \sum_j |F_{cj} - F(c_j)|^2/w_j\}/2\right], \qquad (22)$$

and for $\varepsilon < 0$, the same expression holds with q replaced by $(1-q)$. The summation over j concerns all contrasts c_j for which a model exists. The value of $F(c)$ is given in both cases by

$$F(c) = F_0 \cos(\varphi_0) + cF_d \cos(\varphi_0 - \varepsilon \Delta \varphi)$$
$$+ i[F_0 \sin(\varphi_0) + cF_d \sin(\varphi_0 - \varepsilon \Delta \varphi)]. \qquad (23)$$

The calculation of the least-squares best estimate $\hat{F}$ [(5)] requires the calculation of the first moments of the probability density function (22). One has to sum the contributions of the two determinations. The integration of the function (22) itself (*i.e.* of the sum of the two determinations) must also be performed because the expressions for the probability densities given in this paper have not been normalized to 1.

The integration variables for a non-centrosymmetric reflection are F_0, φ_0, F_d and $\Delta \varphi$. The integration with respect to φ_0 can be performed analytically. The result involves the zero- and first-order modified Bessel functions of the first kind, $I_0(s)$ and $I_1(s)$, of the variable s defined by

$$s = (S_c^2 + S_s^2)^{1/2} \qquad (24)$$

[see relations 3.937.1 and 3.937.2 in Gradshteyn & Ryzhik (1965)], where

$$S_c = \sum_i [F_0 F_{cj} \cos(\varphi_{cj}) + c_j F_d F_{cj} \cos(\varphi_{cj} + \varepsilon \Delta \varphi)]/w_j \qquad (25)$$

$$S_s = \sum_j [F_0 F_{cj} \sin(\varphi_{cj}) + c_j F_d F_{cj} \sin(\varphi_{cj} + \varepsilon \Delta \varphi)]/w_j, \qquad (26)$$

with φ_{cj} equal to the phase of F_{cj}. With the further definitions

$$S_0 = \sum_j [F_{cj}^2 + F_0^2 + 2c_j F_0 F_d \cos(\Delta \varphi) + c_j^2 F_d^2]/w_j \qquad (27)$$

and

$$1/K = \int I_0(s) \exp[-(S_0 + \Delta I' B^{-1} \Delta I)/2] dF_0 dF_d d\Delta \varphi, \qquad (28)$$

one obtains

$$\hat{F}_0 = K \int F_0 \{q[S_c(\varepsilon = +1) + iS_s(\varepsilon = +1)] + (1-q)[S_c(\varepsilon = -1) + iS_s(\varepsilon = -1)]\}$$
$$\times [I_1(s)/s] \exp[-(S_0 + \Delta I' B^{-1} \Delta I)/2]$$
$$\times dF_0 dF_d d\Delta \varphi \qquad (29)$$

$$\hat{F}_d = K \int F_d \{q[S_c(\varepsilon = +1) \cos(\Delta \varphi)$$
$$+ S_s(\varepsilon = +1) \sin(\Delta \varphi)$$
$$+ iS_s(\varepsilon = +1) \cos(\Delta \varphi)$$
$$- iS_c(\varepsilon = +1) \sin(\Delta \varphi)]$$
$$+ (1-q)[S_c(\varepsilon = -1) \cos(\Delta \varphi)$$
$$- S_s(\varepsilon = -1) \sin(\Delta \varphi)$$
$$+ iS_s(\varepsilon = -1) \cos(\Delta \varphi)$$
$$+ iS_c(\varepsilon = -1) \sin(\Delta \varphi)]\}$$
$$\times [I_1(s)/s] \exp[-(S_0 + \Delta I' B^{-1} \Delta I)/2]$$
$$\times dF_0 dF_d d\Delta \varphi. \qquad (30)$$

The other three integrations have to be carried out numerically. In our application program (*PROPHA*), this numerical integration is not performed on F_0, F_d and $\Delta \varphi$ but actually on the three components of the vector ΔI [defined by (12)], calculated in the eigenvector system of axes of B and integrated between $-3b$ and $+3b$ along each of these axes, b being the eigenvalue of B for this axis, with the restriction $I_1^2 < I_0 I_2$. The same method of integration is used to calculate the variance of $\hat{F}$ (*i.e.* the variance–covariance matrix of its real and imaginary parts). The calculation involves the second moments of the density function with respect to F_0 and F_d and therefore the second-order modified Bessel function of the first kind, $I_2(s)$.

In the case of centrosymmetric reflections, there are also two determinations, corresponding to $\varphi_0 = \beta$ and $\varphi_0 = \beta + \pi$ [see (14)]. The numerical integration is made directly on I_0 and I_2 [(12)], and the integration over I_1 degenerates into the sum of only two terms corresponding to $I_1 = +(I_0 I_2)^{1/2}$ and $I_1 = -(I_0 I_2)^{1/2}$ [*i.e.* $\Delta \varphi = 0$ and $\Delta \varphi = \pi$ in (14)].

The calculation of the maximum *a posteriori* probability estimate of F for non-centrosymmetric reflections requires the solution of the system of four equations

$$d\left[\Delta I' B^{-1} \Delta I + \sum_j (|F_{cj} - F(c_j)|^2/w_j)\right]\Big/ du = 0, \qquad (31)$$

where $u = F_0$, φ_0, F_d or $\Delta \varphi$, for both determinations of $F(c_j)$ (*i.e.* for $\varepsilon = +1$ and $\varepsilon = -1$); the 'best' solution is the one which corresponds to the highest value of $p(F_0, F_d/[I], [F_c])$.

For centrosymmetric reflections one has only to consider two variables, F_0 and F_d, and thus a system

of only two equations. It has to be solved in the four possible cases:

(1) $\varphi_0 = \beta$, $\quad \Delta\varphi = 0$; $\quad$ (2) $\varphi_0 = \beta$, $\quad \Delta\varphi = \pi$;

(3) $\varphi_0 = \beta + \pi$, $\Delta\varphi = 0$; $\quad$ (4) $\varphi_0 = \beta + \pi$, $\Delta\varphi = \pi$.

The probability of each case has to be evaluated and the case with highest probability selected.

8. Centroid maps

When only one model is known, corresponding to a certain contrast c_1, the only way to derive a map in another contrast c is to calculate the centroid map. In the usual method, one uses as structure factor for the contrast c the projection of $F(c)$ on F_{c1}, i.e.

$$\tilde{F}(c) = F(c) \cos(\Delta\varphi_c) F_{c1}/F_{c1} \qquad (32)$$

with

$$\cos(\Delta\varphi_c) = [F(c_1)^2 + F(c)^2 - (c - c_1)^2 F_d^2]$$
$$\times [2F(c_1)F(c)]^{-1} \qquad (33)$$

and $F(c)$ given by (18).

It can be shown that by applying the relations (29) and (30) to that case where the sum over j is restricted to just one term, $j = 1$, one obtains a kind of weighted average of (32),

$$F(c) = K \int \tilde{F}(c) I_1(s) \exp\left[-(S_0 + \Delta I' \boldsymbol{B}^{-1} \Delta I)/2\right]$$
$$\times \, dF_0 \, dF_d \, d\Delta\varphi. \qquad (34)$$

Because the Bessel functions I_1 and I_0 satisfy $I_1(s) < I_0(s)$ for any $s > 0$, the modulus of $F(c)$ is smaller than the modulus of $\tilde{F}(c)$; this is especially true if the reflection is weak, the overall agreement for the F_{c1} map is bad, or the measured value $F_{obs}(c)$ [(18)] is not very accurate.

This situation may be improved somewhat by making use of the *orthogonality of some contrasts*. By orthogonality we mean the following: let us consider a macromolecule made of two moieties A and B, like the core protein and nucleic acid in a simple virus. If $\rho_A(\mathbf{r})$ and $\rho_B(\mathbf{r})$ are the densities of each component in the lattice, then the relation

$$\rho_A(\mathbf{r}) \rho_B(\mathbf{r}) = 0 \qquad (35)$$

holds everywhere in the lattice and the corresponding structure factors are related by

$$\sum_{\mathbf{H}'} \mathbf{F}_A(\mathbf{H}') \mathbf{F}_B(\mathbf{H} - \mathbf{H}') = 0 \qquad (36)$$

for any $\mathbf{H}$. This relation is also valid if one or both densities are model densities, provided that the orthogonality is preserved. The use of this relation can be applied only to the case where the contrast c_1 for which one has defined a model is the contrast c_B where the density A is matched out by the solvent, and for the evaluation of the density A corresponding to the contrast c_A where B is matched out. But this

could be generalized to other cases (see Bentley, Finch, Lewit-Bentley & Roth, 1984).

Then one can write

$$\mathbf{F}(\mathbf{H}, c) = -[1/\mathbf{F}(0, c_1)] \sum_{\mathbf{H}'(\neq \mathbf{H})} \mathbf{F}(\mathbf{H}', c) \mathbf{F}(\mathbf{H} - \mathbf{H}', c_1),$$
$$(37)$$

because $\mathbf{F}(\mathbf{H}, c)$ and $\mathbf{F}(\mathbf{H}, c_1)$ are proportional respectively to $\mathbf{F}_A(\mathbf{H})$ and $\mathbf{F}_B(\mathbf{H})$. The values of $\mathbf{F}(0, c)$ and $\mathbf{F}(0, c_1)$ which have to be considered here are those corresponding to the scattering-density difference with respect to the solvent.

One can use (37) in several ways. The simplest is probably to consider it as a particular way of defining a model at contrast c with respect to a model at contrast c_1 and to use it as a second model ($j = 2$) in (29) and (30). Following Makowski (1986), one could try then to use (37) in an interative way and apply the central-limit theorem to the sum on the right-hand side of (37) to calculate the variance of $\mathbf{F}(\mathbf{H}, c)$ (left-hand side). This method is, however, not adequate in the present case. The reason is that orthogonality of densities does not apply to approximate density maps (even in ideal matching-out conditions and after subtraction of the solvent) because the scattering-density errors are spread all over the unit cell for both densities, i.e.

$$\hat{\rho}_A(\mathbf{r}) \hat{\rho}_B(\mathbf{r}) \neq 0. \qquad (38)$$

Relation (37) thus does not apply rigorously to approximate structure factors and, in particular, not to the model structure factors $\mathbf{F}_{cj}$ characterized by the statistical distribution of errors defined in this paper. If one nevertheless defines a model at contrast c (with $j = 2$) by

$$\mathbf{F}_{c2}(\mathbf{H}) = -[1/\mathbf{F}_{c1}(0)] \sum_{\mathbf{H}'(\neq \mathbf{H})} \mathbf{F}_{c2}(\mathbf{H}') \mathbf{F}_{c1}(\mathbf{H} - \mathbf{H}'), \quad (39)$$

the statistics of $\mathbf{F}_{c2}$ will depend not only on the statistics of $\mathbf{F}_{c1}$ but also on errors due to the lack of complete orthogonality of the density errors just described, to series-truncation effects or to the use of centroid phases as starting phases, and it will be sound to apply to it the method of random-density error distribution based on mean squares of the difference map [in an iterative manner starting with the centroid phases for the $\mathbf{F}_{c2}$'s on the right-hand side of (39)].

Another possibility for improving centroid maps, when an envelope of the missing part of the molecule is known, is to use Wilson's (1949) *random model restrained to this envelope*. By envelope we mean any part of the lattice cell where the missing part of the molecule is likely to be found. If we divide this missing part into N small identical pieces (with respect to the resolution), each with a scattering length b (defined with respect to the solvent) and distributed at random inside the envelope, the proba-

bility density function $p_c(\mathbf{F}_c)$ can be derived as

$$p_c(\mathbf{F}_c) = \exp\left(-|\mathbf{F}_c(\mathbf{H}) - (Nb/fV)\mathbf{F}_s(\mathbf{H})|^2\right.$$
$$\left.\times \{pNb^2[1 - fi_s(\mathbf{H})]\}^{-1}\right), \qquad (40)$$

where $\mathbf{F}_s(\mathbf{H})$ is the structure factor of the envelope [with $F_s(0) = fV$, f being the ratio of the volume of the envelope to the volume V of the lattice cell] and $i_s(\mathbf{H})$ is equal to $F_s(\mathbf{H})^2/F_s(0)^2$. The scattering-density factor Nb/fV represents the contrast of the missing part of the molecule 'diluted' over the volume of the envelope.

9. Application and discussion

This calculation has been developed as a Fortran program called *PROPHA* available from the author. It has given very satisfactory results up to now. The effectiveness of the use of the orthogonality of densities or the use of an envelope-restrained random model for improving centroid maps has not been tested. Nevertheless, before the present joint probability approach was developed, density orthogonality [(36)] was tested on non-stochastic models as a tool for predicting the sign ε of the phase difference $\Delta\varphi$, and the results appeared to be rather good except for weak reflections. Application programs of relation (36) exist for space groups $C2$, $P2_12_12_1$ and $I432$.

Partially known structures

A very important application is the case where part of the investigated structure is already known. This concerns, for instance, partly disordered structures, like those mentioned in the *Introduction*, where the structure of the ordered part has been determined by X-rays.

The method was thus applied to the refinement of the STNV (satellite tobacco necrosis virus) (Bentley, Lewit-Bentley, Liljas, Skoglund, Roth & Unge, 1987) and the TBSV (tomato bushy stunt virus) structures (Wild, Timmins & Witz, private communication), with neutron low-resolution diffraction data and with X-ray phases as starting point. In these cases, the method was applied only at the end of a refinement procedure which consisted mainly of extensive icosahedral symmetry averaging in different contrasts.

It appeared more recently that the most straightforward and efficient way of using this method is to calculate in two or more different contrasts the partial structure factors corresponding to the known part of the structure, using for each amino-acid residue its known position in the lattice and its proper contrast with respect to the solvent, and to incorporate these partial structure factors as F_{calc}'s at the different contrasts (*i.e.* as F_{cj}'s) in the equations. This method was first applied to the case of the determination of the structure of the detergent in a membrane-protein crystal and proved to be very successful (publication

in preparation with H. Michel, H. Deisenhofer, R. Huber and A. Lewit-Bentley).

Scale factors

Normally the calculation presented here should include, to be complete or fully consistent, the refinement of the scale factors F_{calc} *versus* F_{obs} for the different models. In fact, in the present approach, the F_{obs}'s cannot be considered as fixed constants but are random variables whose values are deduced from the values of the random variables F_0 and F_d by means of (6). Since the variables F_0 and F_d are the integration variables of the joint probability density function in (5), these scale factors should be recalculated at each step of the numerical integration. Scale factors can actually be estimated in different ways. What is required here is a method that provides a scale factor such that the expectation value of each scaled structure factor $s_j\mathbf{F}_{cj}$ is unbiased (s_j = scale factor of the $\mathbf{F}_{cj}$'s with respect to the corresponding F_{obs} values $F_0 + c_j\mathbf{F}_d$). This is necessary because the random-error model which is used here to derive the probability distribution of $\mathbf{F}_{cj}$ is based on the assumption that the expectation value of $\mathbf{F}_{cj}(\mathbf{H})$, *i.e.* in practice $s_j\mathbf{F}_{cj}(\mathbf{H})$, is equal to $F_0(H) + c_j\mathbf{F}_d(H)$. The recalculation of the scale factors s_j as a function of the F_0's and $\mathbf{F}_d$'s during the integration of the joint probability function is not yet incorporated into the application program. In its present state the program treats each reflection separately, one after another. To take into account this recalculation, it would be necessary to perform simultaneously the numerical integration of the joint probability density function of all reflections selected for the calculation of the scale factors. No great improvement of the accuracy of the calculation is expected from the introduction of such a modification.

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A New Description of Pentagonal Frank–Kasper Phases and a Possible Structure Model of the Icosahedral Quasicrystal

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Abstract

The structure of pentagonal Frank-Kasper phases can be recovered from the projection of a common $MgCu_2$ cube described in a six-dimensional space. From the close structural relationship between the newly discovered icosahedral quasicrystal and the Frank-Kasper phases, a structure model has been proposed for the former.

1. Introduction

Penrose (1974) first pointed out that a two-dimensional (2D) plane can be tiled by two rhombi, 72-108 and 36-144° respectively, with a fivefold symmetry. Later, this was developed to the three-dimensional (3D) case by Mackay (1982a, b), who used two rhombohedra, 63·43 and 116·57° respectively, displaying the icosahedral $2/m\bar{3}5$ symmetry. This has attracted much attention lately on account of the discovery of an icosahedral quasicrystal (Shechtman, Blech, Gratias & Cahn, 1984).

An icosahedron has 12 vertices and six fivefold axes passing through its centre and vertices. The 3D Penrose lattice has been described by using a six-axes system and it was therefore called a quasilattice by Mackay (1982a, b). Consequently, this can also be described in terms of the projection of a 6D simple cubic lattice onto a 3D hyperplane [see, for example, Kramer & Neri (1984) and Elser (1986)]. If the projection is carried out onto an incommensurate hyperplane, a 3D quasilattice is recovered.

The structure of pentagonal Frank-Kasper (FK) phases was known to consist mainly of icosahedra (Frank & Kasper, 1958, 1959) and this close relation-

ship between FK phases and the icosahedral phase has guided some investigators to obtain new icosahedral phases in Ti_2Ni (Zhang, Ye & Kuo, 1985), $Mg_{32}(Al, Zn)_{49}$ (Ramachandrarao & Sastry, 1985) and V-Ni-Si (Kuo, Zhou & Li, 1987). Moreover, structure models of 2D and 3D quasicrystals have already been derived from their close relationship to the FK phases (Yang & Kuo, 1986).

According to Anderson (1978), the structure of the pentagonal FK phases can be recovered by applying various symmetry operations, such as twinning, reflection, rotation, inversion *etc.*, to the rhombohedral unit (60°) of the f.c.c. $MgCu_2$ structure and the Zr_4Al_3 unit. Since this 60° rhombohedral unit is quite like the acute rhombohedron (63·43°) of the icosahedral phase, it is natural to inquire into the possibility, by projection of the 6D $MgCu_2$ onto a 3D hyperplane, of obtaining the structure of both the pentagonal FK phases and the icosahedral phase. The present investigation is devoted mainly to a new description of the FK phases, and in the meantime a possible structure model for the quasicrystal is proposed. A preliminary report has already been published (Yang & Kuo, 1986) and a similar study on the structure of the $Mg_{32}(Al, Zn)_{49}$ icosahedral phase has also appeared (Henley & Elser, 1986).

2. Projection method

In the case of the projection of a 2D lattice onto a 1D space (Fig. 1), Elser (1986) pointed out that when $\tan \alpha$ is an irrational number an incommensurate structure will result. This applies only to the case where the direction of projection is normal to the