also related to the intensity-based domain refinement (Yeates & Rini, 1990).

In the presence of noncrystallographic symmetry, the locked self rotation function can be used to define the orientation of the noncrystallographic symmetry point group in the crystal. If an atomic model is available for the monomer but not for the entire oligomer, the locked cross rotation function can be used to determine the orientation of this monomer in the oligomer. The locked translation function can then be used to determine the position of this monomer relative to the centre of the noncrystallographic symmetry point group (Tong, 1996*b*, 2001*a*), which will produce a model for the entire oligomer. The centre of this oligomer in the crystal can be defined by a simple translation search.

With the knowledge of the orientation of one monomer of the oligomer, the first term of (2.3.7.5) is dependent on the position of this monomer relative to the centre of the noncrystallographic symmetry oligomer (Tong, 1996*b*). The atomic positions of the entire noncrystallographic symmetry oligomer in the standard orientation are given by

$$\mathbf{X}_{n,j} = [\mathbf{I}_n]\big([\mathbf{F}]\mathbf{X}_j^0 + \mathbf{V}_0\big),$$

where $\mathbf{X}_j^0$ are the atomic positions of the monomer model, centred at (0, 0, 0); [**F**] is the orientation of this model in the oligomer in the standard orientation; $\mathbf{V}_0$ is the position of this monomer relative to the centre of the oligomer; and $[\mathbf{I}_n]$ is the nth noncrystallographic symmetry rotation matrix in the standard orientation. The atomic positions of the noncrystallographic symmetry oligomer in the crystal unit cell, centred at the origin, are given by

$$\mathbf{x}_{n,j} = [\boldsymbol{\alpha}][\mathbf{E}]\mathbf{X}_{n,j} = [\boldsymbol{\alpha}][\mathbf{E}][\mathbf{I}_n]\big([\mathbf{F}]\mathbf{X}_j^0 + \mathbf{V}_0\big),$$

where [**E**] is the orientation of the noncrystallographic symmetry in the crystal unit cell and [$\boldsymbol{\alpha}$] is the deorthogonalization matrix.

By incorporating the calculated structure factors based on this noncrystallographic symmetry oligomer into the first term of (2.3.7.5), the locked translation function is given by

$$\begin{aligned} T_{\rm L}(\mathbf{V}_0) &= \sum_{\mathbf{h}} |\mathbf{F}_{\mathbf{h}}^{\rm o}|^2 |\mathbf{F}_{\mathbf{h}}|^2 \\ &= \sum_{\mathbf{h}} \sum_{n} \sum_{m \neq n} |\mathbf{F}_{\mathbf{h}}^{\rm o}|^2 \mathbf{F}_{\mathbf{h},n} \mathbf{F}_{\mathbf{h},m}^{*} \exp\{-2\pi i \mathbf{h}([\boldsymbol{\theta}_m] - [\boldsymbol{\theta}_n])\mathbf{V}_0\}, \end{aligned} \tag{2.3.7.6}$$

where $[\boldsymbol{\theta}_n] = [\boldsymbol{\alpha}][\mathbf{E}][\mathbf{I}_n]$ and $\mathbf{F}_{\mathbf{h},n_o} = \sum_j f_j \exp(2\pi i \mathbf{h}[\boldsymbol{\theta}_n][\mathbf{F}]\mathbf{X}_j^0)$. A constant term $\sum_{\mathbf{h}} \sum_n |\mathbf{F}_{\mathbf{h}}^{\rm o}|^2 |\mathbf{F}_{\mathbf{h},n}|^2$ has been omitted from this equation.

Conceptually, the locked translation function is based on the overlap of intermolecular vectors within the noncrystallographic symmetry oligomer and the observed Patterson map (Tong, 1996*b*). The equation for the locked translation function, (2.3.7.6), bears remarkable resemblance to that for the ordinary Patterson-correlation translation function, (2.3.7.5), with the interchange of the crystallographic ([$\mathbf{T}_n$]) and noncrystallographic symmetry ([$\boldsymbol{\theta}_n$]) parameters.

2.3.7.6. Computer programs for rotation and translation function calculations

Several programs are currently in popular use for the calculation of rotation and translation functions. These include *AMoRe* (Navaza, 1994, 2001*a*), *BEAST* (Read, 2001*b*), *CCP4* (Collaborative Computational Project, Number 4, 1994), *CNS* (Brünger *et al.*, 1998), *COMO* (Jogl *et al.*, 2001), *EPMR* (Kissinger *et al.*, 1999), *GLRF* (part of the *Replace* package) (Tong, 1993, 2001*a*; Tong & Rossmann, 1990, 1997), *Molrep* (Vagin & Teplyakov, 2000) and *Phaser* (Storoni *et al.*, 2004).

The correct placement of an atomic model in a crystal unit cell is generally a six-dimensional problem, with three degrees of rotational freedom and three degrees of translational freedom. Systematic examination of all six degrees of freedom at the same time is computationally expensive and cannot be used routinely (Fujinaga & Read, 1987; Rabinovich & Shakked, 1984; Sheriff *et al.*, 1999). On the other hand, directed sampling of the six degrees of freedom, driven by a stochastic or genetic algorithm (Chang & Lewis, 1997; Glykos & Kokkinidis, 2000; Kissinger *et al.*, 1999), has been successful in solving structures.

Traditionally, the calculations are divided into a rotational component (the rotation function) and a translational component (the translation function). Only a few rotation angles (for example the top few peaks of the rotation function) are manually passed to the translation function for examination (Fitzgerald, 1988). With the power of modern computers, it is now possible to perform limited six-dimensional searches, with the sampling of the rotational degrees of freedom guided by the rotation function. For example, the top peaks of the rotation function (Navaza, 1994) and their neighbours (Urzhumtsev & Podjarny, 1995) can be automatically examined by the translation function. A more general approach is to examine all rotation-function grid points with values greater than a certain threshold (Tong, 1996*a*). Such combined molecular replacement protocols have been found to be very powerful in solving new structures.

2.3.8. Molecular replacement

2.3.8.1. Using a known molecular fragment

The most straightforward application of the molecular replacement method occurs when the orientation and position of a known molecular fragment in an unknown cell have been previously determined. The simple procedure is to apply the rotation and translation operations to the known fragment. This will place it into one 'standard' asymmetric unit of the unknown cell. Then the crystal operators (assuming no further noncrystallographic operators are present in the unknown cell) are applied to generate the complete unit cell of the unknown structure. Structure factors can then be calculated from the rotated and translated known molecule into the unknown cell. The resultant model can be refined in numerous ways.

More generally, consider a molecule placed in any crystal cell (**h**), within which coordinate positions shall be designated by **x**. Let the corresponding structure factors be $\mathbf{F}_{\mathbf{h}}$. It is then possible to compute the structure factors $\mathbf{F}_{\mathbf{p}}$ for another cell (**p**) into which the same molecule has been placed N times related by the crystallographic symmetry operators $[\boldsymbol{C}_1], \mathbf{d}_1; [\boldsymbol{C}_2], \mathbf{d}_2; \ldots; [\boldsymbol{C}_N], \mathbf{d}_N$. Let the electron density at a point $\mathbf{y}_1$ in the first crystallographic asymmetric unit be spatially related to the point $\mathbf{y}_n$ in the nth asymmetric unit of the **p** crystal such that

$$\rho(\mathbf{y}_n) = \rho(\mathbf{y}_1), \tag{2.3.8.1}$$

where

$$\mathbf{y}_n = [\boldsymbol{C}_n]\mathbf{y}_1 + \mathbf{d}_n. \tag{2.3.8.2}$$

From the definition of a structure factor,

$$\mathbf{F}_{\mathbf{p}} = \sum_{n=1}^{N} \int_U \rho(\mathbf{y}_n) \exp(2\pi i \mathbf{p} \cdot \mathbf{y}_n)\, \mathrm{d}\mathbf{y}_n, \tag{2.3.8.3}$$

where the integral is taken over the volume U of one molecule. But since each molecule is identical as expressed in equation (2.3.8.1) and since (2.3.8.2) can be substituted in equation (2.3.8.3), we have

$$\mathbf{F}_\mathbf{p} = \sum_{n=1}^{N} \int_U \rho(\mathbf{y}_1) \exp[2\pi i \mathbf{p} \cdot ([\boldsymbol{C}_n]\mathbf{y}_1 + \mathbf{d}_n)] \, \mathrm{d}\mathbf{y}_1. \quad (2.3.8.4)$$

Now let the molecule in the **h** crystal be related to the molecule in the first asymmetric unit of the **p** crystal by the noncrystallographic symmetry operation

$$\mathbf{x} = [\boldsymbol{C}]\mathbf{y} + \mathbf{d}, \quad (2.3.8.5)$$

which implies

$$\rho(\mathbf{x}) = \rho(\mathbf{y}_1) = \rho(\mathbf{y}_2) = \ldots. \quad (2.3.8.6)$$

Furthermore, in the **h** cell

$$\rho(\mathbf{x}) = \frac{1}{V_\mathbf{h}} \sum_\mathbf{h} \mathbf{F}_\mathbf{h} \exp(-2\pi i \mathbf{h} \cdot \mathbf{x}), \quad (2.3.8.7)$$

and thus, by combining with (2.3.8.5), (2.3.8.6) and (2.3.8.7),

$$\rho(\mathbf{y}_1) = \frac{1}{V_\mathbf{h}} \sum_\mathbf{h} \mathbf{F}_\mathbf{h} \exp[-2\pi i(\mathbf{h}[\boldsymbol{C}] \cdot \mathbf{y}_1 + \mathbf{h} \cdot \mathbf{d})]. \quad (2.3.8.8)$$

Now using (2.3.8.4) and (2.3.8.8) it can be shown that

$$\mathbf{F}_\mathbf{p} = \frac{U}{V_\mathbf{h}} \sum_\mathbf{h} \mathbf{F}_\mathbf{h} \sum_{n=1}^{N} G_{\mathbf{hp}_n} \exp[2\pi i(\mathbf{p} \cdot \mathbf{S}_n - \mathbf{h} \cdot \mathbf{S})], \quad (2.3.8.9)$$

where

$$UG_{\mathbf{hp}_n} = \int_U \exp[2\pi i(p[\boldsymbol{C}_n] - \mathbf{h}[\boldsymbol{C}]) \cdot \mathbf{u}] \, \mathrm{d}\mathbf{u}. \quad (2.3.8.10)$$

S is a chosen molecular origin in the **h** crystal and $\mathbf{S}_n$ is the corresponding molecular position in the nth asymmetric unit of the **p** crystal.

2.3.8.2. Using noncrystallographic symmetry for phase improvement

The use of noncrystallographic symmetry for phase determination was proposed by Rossmann & Blow (1962, 1963) and subsequently explored by Crowther (1967, 1969) and Main & Rossmann (1966). These methods were developed in reciprocal space and were primarily concerned with *ab initio* phase determination. Real-space averaging of electron density between noncrystallographically related molecules was used in the structure determination of deoxyhaemoglobin (Muirhead *et al*., 1967) and of α-chymotrypsin (Matthews *et al*., 1967). The improvement derived from the averaging between the two noncrystallographic units was, however, not clear in either case. The first obviously successful application was in the structure determination of lobster glyceraldehyde-3-phosphate dehydrogenase (Buehner *et al*., 1974; Argos *et al*., 1975), where the tetrameric molecule of symmetry 222 occupied one crystallographic asymmetric unit. The improvement in the essentially SIR electron-density map was considerable and the results changed from uninterpretable to interpretable. The uniqueness and validity of the solution lay in the obvious chemical correctness of the polypeptide fold and its agreement with known amino-acid-sequence data. In contrast to the earlier reciprocal-space methods, noncrystallographic symmetry was used as a method to improve poor phases rather than to determine phases *ab initio*.

Table 2.3.8.1. *Molecular replacement: phase refinement as an iterative process*

(*A*)	$\mathbf{F}_\mathrm{obs}, \alpha'_n, m'_n \rightarrow \rho_n$
(*B*)	$\rho_n \rightarrow \rho_n$(modified) (i) Use of noncrystallographic symmetry operators (ii) Definition of envelope limiting volume within which noncrystallographic symmetry is valid (iii) Adjustment of solvent density† (iv) Use of crystallographic operators to reconstruct modified density into a complete cell
(*C*)	ρ_n(modified) $\rightarrow \mathbf{F}_{\mathrm{calc},\,n+1}; \alpha_{\mathrm{calc},\,n+1}$
(*D*)	$(\mathbf{F}_{\mathrm{calc},\,n+1}, \alpha_{\mathrm{calc},\,n+1}) + (\mathbf{F}_\mathrm{obs}, \alpha_0) \rightarrow \mathbf{F}_\mathrm{obs}, \alpha'_{n+1}, m'_{n+1}$ (i) Assessment of reliability of new phasing set α_{n+1} in relation to original phasing set $\alpha_0(w)$ (ii) Use of figures of merit m_0, m_{n+1} and reliability w to determine modified phasing set α'_{n+1}, m'_{n+1}‡ (iii) Consideration of α_{n+1} and m_{n+1} where there was no prior knowledge of (*a*) $\mathbf{F}_\mathrm{obs}$ (*e.g.* very low order reflections or uncollected data) (*b*) α_0 (*e.g.* no isomorphous information or phase extension)
(*E*)	Return to step (*A*) with α'_{n+1}, m'_{n+1} and a possibly augmented set of $\mathbf{F}_\mathrm{obs}$.

† Wang (1985); Bhat & Blow (1982); Collins (1975); Schevitz *et al*. (1981); Hoppe & Gassmann (1968). ‡ Rossmann & Blow (1961); Hendrickson & Lattman (1970).

Many other applications followed rapidly, aided greatly by the versatile techniques developed by Bricogne (1976). Of particular interest is the application to the structure determination of hexokinase (Fletterick & Steitz, 1976), where the averaging occurred both between different crystal forms and within the same crystal.

The most widely used procedure for real-space averaging is the 'double sorting' technique developed by Bricogne (1976) and also by Johnson (1978). An alternative method is to maintain the complete map stored in the computer (Nordman, 1980*b*). This avoids the sorting operation, but is only possible given a very large computer or a low-resolution map containing relatively few grid points.

Bricogne's double sorting technique involves generating real-space non-integral points (D_i) which are related to integral grid points (I_i) in the cell asymmetric unit by the noncrystallographic symmetry operators. The elements of the set D_i are then brought back to their equivalent points in the cell asymmetric unit (D'_i) and sorted by their proximity to two adjacent real-space sections. The set I'_i, calculated on a finer grid than I_i and stored in the computer memory two sections at a time, is then used for linear interpolation to determine the density values at D'_i which are successively stored and summed in the related array I_i. A count is kept of the number of densities received at each I_i, resulting in a final averaged aggregate, when all real-space sections have been utilized. The density to be assigned outside the molecular envelope (defined with respect to the set I_i) is determined by averaging the density of all unused points in I_i. The grid interval for the set I'_i should be about one-sixth of the resolution to avoid serious errors from interpolation (Bricogne, 1976). The grid point separation in the set I_i need only be sufficient for representation of electron density, or about one-third of the resolution.

Molecular replacement in real space consists of the following steps (Table 2.3.8.1): (*a*) calculation of electron density based on a starting phase set and observed amplitudes; (*b*) averaging of this density among the noncrystallographic asymmetric units or molecular copies in several crystal forms, a process which defines

a molecular envelope as the averaging is only valid within the range of the noncrystallographic symmetry; (*c*) reconstructing the unit cell based on averaged density in every noncrystallographic asymmetric unit; (*d*) calculating structure factors from the reconstructed cell; (*e*) combining the new phases with others to obtain a weighted best-phase set; and (*f*) returning to step (*a*) at the previous or an extended resolution. Decisions made in steps (*b*) and (*e*) determine the rate of convergence (see Table 2.3.8.1) to a solution (Arnold *et al*., 1987).

The power of the molecular replacement procedure for either phase improvement or phase extension depends on the number of noncrystallographic asymmetric units, the size of the excluded volume expressed in terms of the ratio $(V - UN)/V$ and the magnitude of the measurement error on the structure amplitudes. Crowther (1967, 1969) and Bricogne (1974) have investigated the dependence on the number of noncrystallographic asymmetric units and conclude that three or more copies are sufficient to ensure convergence of an iterative phase improvement procedure in the absence of errors on the structure amplitudes. As with the analogous case of isomorphous replacement in which three data sets ensure reasonable phase determination, additional copies will enhance the power of the method, although their usefulness is subject to the law of diminishing returns. Another example of this principle is the sign determination of the *h*0*l* reflections of horse haemoglobin (Perutz, 1954) in which seven shrinkage stages constituted the sampling of the transform of a single copy.

In an analysis of how phasing errors propagate into errors in calculations of electron density, Arnold & Rossmann (1986) concluded that the 'power' of phase determination could be related to the noncrystallographic redundancy, *N*, the ratio of the molecular envelope volume, *U*, to the unit cell volume, *V*, the fractional error of the structure-factor amplitudes, *R* and the fractional completeness of the data, *f*, by (Arnold & Rossmann, 1986)

$$P = \frac{(Nf)^{1/2}}{RU/V}. \qquad (2.3.8.11)$$

This semiquantitative result makes intuitive sense in that the noncrystallographic redundancy and solvent content terms can be directly related to over-sampling of the molecular transform in reciprocal space, and, thus, are analogous in providing phasing information. The phasing power of solvent flattening/density modification was further analysed and shown to lead to Sayre's equations (Sayre, 1952) at a limit where the molecular envelope is sufficiently detailed and shrunken to cover sharpened and separated atoms (Arnold & Rossmann, 1986). This result suggests that more detailed definitions of molecular envelopes than are traditionally used could be advantageous for phase improvement and extension procedures.

Procedures for real-space averaging have been used extensively with great success. The interesting work of Wilson *et al*. (1981) is noteworthy for the continuous adjustment of molecular envelope with increased map definition. Furthermore, the analysis of complete virus structures has only been possible as a consequence of this technique (Bloomer *et al*., 1978; Harrison *et al*., 1978; Abad-Zapatero *et al*., 1980; Liljas *et al*., 1982). Although the procedure has been used primarily for phase improvement, apparently successful attempts have been made at phase extension (Nordman, 1980*b*; Gaykema *et al*., 1984; Rossmann *et al*., 1985). *Ab initio* phasing of glyceraldehyde-3-phosphate dehydrogenase (Argos *et al*., 1975) was successfully attempted by initially filling the known envelope with uniform density to determine the phases of the innermost reflections and then gradually extending phases to 6.3 Å resolution. Johnson *et al*. (1976) used the same procedure to determine the structure of southern bean mosaic virus to 22.5 Å resolution. Particularly impressive was the work on polyoma virus (Rayment *et al*., 1982; Rayment, 1983; Rayment *et al*., 1983) where crude initial models led to an entirely unexpected breakdown of the Caspar & Klug (1962) concept of quasi-symmetry. *Ab initio* phasing has also been used by combining the electron-diffraction projection data of two different crystal forms of bacterial rhodopsin (Rossmann & Henderson, 1982).

2.3.8.3. Update on noncrystallographic averaging and density-modification methods

Since this article was originally written, molecular replacement has been subject of a number of reviews (Rossmann, 1990), including a historical background of the subject (Rossmann, 2001). A series of chapters pertaining to molecular replacement have been published in *IT* Volume F (Rossmann & Arnold, 2001*a*), reviewing noncrystallographic symmetry (Chapter 13.1; Blow, 2001), rotation (Chapter 13.2; Navaza, 2001*b*) and translation (Chapter 13.3; Tong, 2001*b*) functions, and noncrystallographic symmetry averaging for phase improvement and extension (Chapter 13.4; Rossmann & Arnold, 2001*b*). Chapters on phase improvement by density modification (Chapter 15.1; Zhang *et al.*, 2001), optimal weighting of Fourier terms in map calculations (Chapter 15.2; Read, 2001*a*) and refinement calculations incorporating bulk solvent correction (Chapter 18.4; Dauter *et al.*, 2001) are also recommended reading.

There has been remarkable progress in the general area of density modification, involving improvement of real-space methods for averaging and reconstruction, and treatment of solvent for iterative phase improvement and refinement calculations. The use of real-space averaging between noncrystallographically related electron density within the crystallographic asymmetric unit has become an accepted mode of extending phase information to higher resolution, particularly for complex structures such as viruses [Acharya *et al.*, 1989; Arnold & Rossmann, 1988; Gaykema *et al.*, 1986; Hogle *et al.*, 1985; Luo *et al.*, 1989; Rossmann & Arnold, 2001*b* (*IT* F Chapter 13.4); Rossmann *et al.*, 1985, 1992]. *Ab initio* phase determination based on noncrystallographic redundancy has become fairly common (Chapman *et al.*, 1992; Lunin *et al.*, 2000; Miller *et al.*, 2001; Rossmann, 1990; Tsao *et al.*, 1992). General programs in common use for noncrystallographic symmetry averaging include *BUSTER-TNT* [Blanc *et al.*, 2004; Roversi *et al.*, 2000; Tronrud & Ten Eyck, 2001 (*IT* F Section 25.2.4)], *CNS* [Brünger *et al.*, 1998; Brunger, Adams, DeLano *et al.*, 2001 (*IT* F Section 25.2.3)], *DM*/*DMMULTI* [Cowtan & Main, 1993; Cowtan *et al.*, 2001 (*IT* F Section 25.2.2); Schuller, 1996; Zhang, 1993], *PHASES* [Furey, 2001 (*IT* F Section 25.2.1); Furey & Swaminathan, 1997], *RAVE*/*MAVE* (Jones, 1992; Kleywegt, 1996) and *SOLVE*/*RESOLVE* [Terwilliger, 2002*b*, 2003*c*; Terwilliger & Berendzen, 2001 (*IT* F Section 14.2.2)].

Solvent flattening has been formulated in reciprocal space for greater computational efficiency (Leslie, 1987; Terwilliger, 1999) and solvent 'flipping' is a powerful extension of solvent density modification (Abrahams, 1997; Abrahams & Leslie, 1996). Bulk-solvent corrections are now commonly used in crystallographic refinement, allowing for better modelling and phase determination of low-resolution data [Brünger *et al.*, 1998; Dauter *et al.*, 2001 (*IT* F Chapter 18.4)]. The problem of phase error estimation and analysis and bias removal has been treated extensively (Cowtan, 1999; Cowtan & Main, 1996), including extension of methods to include maximum-likelihood functions and iterative bias removal procedures [Brunger, Adams & Rice, 2001 (*IT* F Chapter 18.2); Hunt & Deisenhofer, 2003; Lamzin *et al.*, 2001 (*IT* F Section 25.2.5); Perrakis *et al.*, 1997; Terwilliger, 2004]. Histogram matching [Cowtan & Main, 1993; Lunin, 1993; Nieh & Zhang, 1999; Refaat *et al.*, 1996; Zhang, 1993; Zhang *et al.*, 2001 (*IT* F Chapter 15.1)] and skeletonization [Baker *et al.*, 1993;

Zhang *et al.*, 2001 (*IT* F Chapter 15.1)], and structural fragment matching procedures (Terwilliger, 2003*a*) have been added to the arsenal of density-modification methods. Automated mask and molecular-envelope definition has helped to remove the tedium and increase the efficiency and quality of density-modification and symmetry-averaging procedures. Noncrystallographic symmetry averaging among different crystal forms (Perutz, 1954) has become increasingly common, and exploitation of the unit-cell variation among flash-cooled and noncooled forms of the same crystal is a broadly applicable method for phase determination (Das *et al.*, 1996; Ding *et al.*, 1995); soaking crystals in a series of different solvents and buffers can produce an analogous effect (Ren *et al.*, 1995; Tong *et al.*, 1997). Phases from noncrystallographic symmetry averaging and other 'experimental' sources have been incorporated into crystallographic refinement procedures using a number of formalisms (Arnold & Rossmann, 1988; Rees & Lewis, 1983) including maximum likelihood (Pannu *et al.*, 1998).

2.3.8.4. Equivalence of real- and reciprocal-space molecular replacement

Let us proceed in reciprocal space doing exactly the same as is done in real-space averaging. Thus

$$\rho_{\rm AV}(\mathbf{x}) = \frac{1}{N}\sum_{n=1}^{N}\rho(\mathbf{x}_n),$$

where

$$\mathbf{x}_n = [\boldsymbol{C}_n]\mathbf{x} + \mathbf{d}_n.$$

Therefore,

$$\rho_{\rm AV}(\mathbf{x}) = \frac{1}{N}\sum_{N}\frac{1}{V}\left[\sum_{\mathbf{h}}\mathbf{F}_{\mathbf{h}}\exp(2\pi i\mathbf{h}\cdot\mathbf{x}_n)\right].$$

The next step is to perform the back-transform of the averaged electron density. Hence,

$$\mathbf{F}_{\mathbf{p}} = \int_U \rho_{\rm AV}(\mathbf{x})\exp(-2\pi i\mathbf{p}\cdot\mathbf{x})\,{\rm d}\mathbf{x},$$

where U is the volume within the averaged part of the cell. Hence, substituting for $\rho_{\rm AV}$,

$$\mathbf{F}_{\mathbf{p}} = \int_U\left[\frac{1}{NV}\sum_{N}\sum_{\mathbf{h}}\mathbf{F}_{\mathbf{h}}\exp(2\pi i\mathbf{h}\cdot\mathbf{x}_n)\right]\exp(-2\pi i\mathbf{p}\cdot\mathbf{x})\,{\rm d}\mathbf{x},$$

which is readily simplified to

$$\mathbf{F}_{\mathbf{p}} = \frac{U}{NV}\sum_{\mathbf{h}}\mathbf{F}_{\mathbf{h}}\sum_{N}G_{\mathbf{hp}n}\exp(2\pi i\mathbf{h}\cdot\mathbf{d}_n).$$

Setting

$$\mathbf{B}_{\mathbf{hp}} = \frac{U}{NV}\sum_{N}G_{\mathbf{hp}n}\exp(2\pi i\mathbf{h}\cdot\mathbf{d}_n),$$

the molecular replacement equations can be written as

$$\mathbf{F}_{\mathbf{p}} = \sum_{\mathbf{h}}\mathbf{B}_{\mathbf{hp}}\mathbf{F}_{\mathbf{h}} \qquad (2.3.8.12)$$

(Main & Rossmann, 1966), or in matrix form

$$\mathbf{F} = [\boldsymbol{B}]\mathbf{F},$$

which is the form of the equations used by Main (1967) and by Crowther (1967). Colman (1974) arrived at the same conclusions by an application of Shannon's sampling theorem. It should be noted that the elements of $[\boldsymbol{B}]$ are dependent only on knowledge of the noncrystallographic symmetry and the volume within which it is valid. Substitution of approximate phases into the right-hand side of (2.3.8.12) produces a set of calculated structure factors exactly analogous to those produced by back-transforming the averaged electron density in real space. The new phases can then be used in a renewed cycle of molecular replacement. The reciprocal-space molecular replacement procedure has been implemented and tested in a computer program (Tong & Rossmann, 1995).

Computationally, it has been found more convenient and faster to work in real space. This may, however, change with the advent of vector processing in 'supercomputers'. Obtaining improved phases by substitution of current phases on the right-hand side of the molecular replacement equations (2.3.8.1) seems less cumbersome than the repeated forward and backward Fourier transformation, intermediate sorting, and averaging required in the real-space procedure.

2.3.9. Conclusions

Complete interpretation of Patterson maps is no longer used frequently in structure analysis, although most determinations of heavy-atom positions of isomorphous pairs are based on Patterson analyses. Incorporation of the Patterson concept is crucial in many sophisticated techniques essential for the solution of complex problems, particularly in the application to biological macromolecular structures. Patterson techniques provide important physical insights in a link between real- and reciprocal-space formulation of crystal structures and diffraction data.

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