

13.1. NONCRYSTALLOGRAPHIC SYMMETRY

accurately in certain cases, and the results are difficult to interpret and are imprecise (Blow *et al.*, 1964; Rossmann *et al.*, 1964). In practice, when dealing with a totally unknown structure, translational relationships are more frequently discovered by using specific markers on the subunit (intense scattering centres or 'heavy atoms', or anomalous scattering centres).

13.1.4. Interpretation of generalized noncrystallographic symmetry where the molecular structure is partially known

13.1.4.1. The cross-rotation function

The rotation function can be used in the generalized case to compare the Patterson functions of different crystals. When used in this way, it is called the cross-rotation function. In the most usual case, information providing some kind of structural model is available for one of the crystals.

The power of the cross-rotation function may be greatly improved by removing all intermolecular vectors from the 'model' Patterson function. This may be done by constructing an imaginary crystal structure in which a single copy of the structural model is placed in a unit cell that is large enough for all intermolecular vectors to be longer than the longest intramolecular vector of the model. In this cell, the self-Patterson vectors may be completely isolated and used for comparison with the Patterson function of a crystal containing a molecule of unknown orientation.

13.1.4.2. The cross-translation function

In searching for the position of a molecule in the generalized case of noncrystallographic symmetry, a molecular model defines an origin of coordinates in the model structure, and the corresponding position can be sought in an unknown structure (Nordman & Nakatsu, 1963; Tollin & Cochran, 1964; Huber, 1965; Crowther & Blow, 1967). The procedure is to calculate a three-dimensional function whose peaks should lie at the inter-subunit vectors. In

some procedures (Tollin & Cochran, 1964; Crowther & Blow, 1967), this function may be calculated as a Fourier series.

The translation functions will fail if the corresponding rotation is incorrect, or even if it is insufficiently accurate to give a good overlap between the structures. To avoid this danger, Brünger (1997) recommends computing translation functions using rotations corresponding to many (*e.g.* 200) high values of the rotation function. Though this is a huge increase in computing load, it still compares favourably with a full six-dimensional search.

These methods are considered further in Chapter 13.3.

13.1.4.3. Structure determination

Table 13.1.4.1 distinguishes a number of different situations in which noncrystallographic symmetry can be used to aid structure determination. The most frequent application of molecular-replacement methods is to cases where a structure is partially known, but is not yet susceptible to refinement by standard techniques.

Two types of situation arise in the standard case, where noncrystallographically related subunits exist in the same crystal. Most frequently [type (2)], the noncrystallographic symmetry allows the electron density to be improved at the given resolution. Occasionally, high-order noncrystallographic symmetry may be used to extend the resolution to the point where conventional structural refinement becomes possible (Schevitz *et al.*, 1981; McKenna *et al.*, 1992). In the most favourable case, high-order noncrystallographic symmetry constraints may allow direct structure determination [type (1)], starting from the position of a symmetric particle in the asymmetric unit (Jack, 1973).

In the generalized case, most often, similarities with a known molecular structure can be employed to improve an unknown structure [types (5) and (6)]. Such techniques were first used by Tollin (1969) (before structural refinement was possible) and by Fehlhammer & Bode (1975).

It is also possible that a refinable structure could be generated from intensity data observed from several different crystal forms,

Table 13.1.4.1. Structure determination using noncrystallographic symmetry

Starting structural information	Relationships within the same crystal (standard case)	Relationships between different crystals (generalized case)
None	(1) Subunit arrangement defined by relation between noncrystallographic and crystallographic symmetry. Resolution extended by noncrystallographic symmetry constraints	(3)* Subunit arrangement defined by relation between noncrystallographic and crystallographic symmetry in at least one crystal. Cross-rotation and translation functions applied to other crystals. Resolution extended by noncrystallographic symmetry constraints
Poorly resolved structure, unsuitable for refinement	(2) Electron density improved or resolution extended by noncrystallographic symmetry constraints	(4)* Resolution extended by noncrystallographic symmetry constraints
Similar structure known		(5) Subunit orientation found by cross-rotation and translation functions. Phases derived from structural model and may be improved by noncrystallographic symmetry constraints
Part of unknown structure resembles a known structure		(6) Subunit orientation found by cross-rotation and translation functions. Phases derived from structural model and may be improved by noncrystallographic symmetry constraints

* Structure determinations of this kind have not been reported.

using noncrystallographic symmetry constraints, but this is not known to have been done in practice [types (3) and (4)].

13.1.5. The power of noncrystallographic symmetry in structure analysis

13.1.5.1. Relevant parameters: standard case

A poorly determined structure, if known at sufficient resolution and accuracy, can be improved by structural refinement of an atomic model to fit the observations. These methods often use existing structural knowledge (of bond lengths and angles, for example) to improve the convergence of the refinement process.

The most important contributions of noncrystallographic symmetry arise before this point of structure determination is reached. In this stage of structural analysis, the distribution of scattering density may be constrained by the requirements of noncrystallographic symmetry. The density may be improved by imposing noncrystallographic symmetry on poorly defined scattering density, and, in favourable cases, cyclical improvement leads to a unique corrected structure. To avoid any confusion with refinement of the atomic structure, this process will be referred to as ‘symmetry correction’ or ‘correction’ (Hoppe & Gassmann, 1968).

Noncrystallographic symmetry can also be used to improve the accuracy and convergence of atomic structural refinement by increasing the number of observations to a given resolution (Section 13.1.5.5).

The power of correction methods in improving an unknown structure in the standard case (Section 13.1.2.1) depends on:

- (1) the resolution of the analysis, d ;
- (2) the number, N , of subunits per asymmetric unit;
- (3) the volume fraction, NU/V_a , of the asymmetric unit over which the noncrystallographic symmetry operation applies;
- (4) whether the density between subunit volumes is constant;
- (5) the degree of similarity of the subunits being matched; and
- (6) the extent to which the noncrystallographic symmetry operations differ from the crystal symmetry operations.

The first three parameters are expressed in quantitative terms; parameter (4) might be true or false, but more often lies between these; and parameters (5) and (6) are not easily expressed in measurable form.

The resolution d should ideally be matched to the level of similarity of the subunits. The root-mean-square displacement between an atom in one subunit and the rotated and translated position of the corresponding atom from another subunit (or model subunit) provides an order of magnitude for the resolution d which can be used effectively. In many cases, the resolution is worse than this for practical reasons of crystal disorder and data collection.

This limit was encountered by Huber *et al.* (1974) working at 1.9 Å resolution. They found that a model structure with a mean coordinate difference of 1.9 Å was not usable for molecular replacement, while another model agreeing to 0.75 Å gave results which allowed the structure to be refined. This suggests that agreement significantly better than the resolution is required.

13.1.5.2. Information gain from ideal noncrystallographic symmetry

Rossmann & Blow (1962) wrote, “The effect of noncrystallographic symmetry . . . results in decreasing the size of the structure to be determined, while the number of observable intensities remains the same. This ‘redundancy’ in information might be used to help solve a structure.” This idea is developed below.

First, it will be shown that (in the absence of noncrystallographic symmetry) there is a constant ratio between the number of

independent measurements required to specify the scattering density at a chosen resolution and the volume of the asymmetric unit. Then the effect of noncrystallographic symmetry on this ratio is discussed. The importance of the ratio (volume of symmetry-constrained unit/volume of asymmetric unit) ($= U/V_a$) is stressed. Another ratio is developed – available no. of measurements/ideally required no. of measurements – and this is referred to as the overdetermination ratio.

Consider a noncentrosymmetric crystal whose asymmetric unit volume is V_a and whose diffraction data have been measured to a resolution d . If the multiplicity of the space group (number of asymmetric units in the primitive unit cell) is Z , the volume of reciprocal space, V^* , per point of the primitive reciprocal lattice is given by

$$V^* = 1/V = 1/ZV_a,$$

where V is the volume of the primitive unit cell.

The number of independent orders of diffraction (the number of independent intensities) within the resolution sphere of radius $1/d$ is given by

$$N_{\text{ref}} = \left(\frac{4\pi}{3d^3}\right) \left(\frac{1}{V^*}\right) \left(\frac{1}{2Z}\right) = \frac{2\pi V_a}{3d^3}. \quad (13.1.5.1)$$

In this formula, Friedel’s law is supposed to apply. A set of $2Z$ reflections have identical intensity due to the combined effects of Friedel’s law and crystal symmetry. When N_{ref} is expressed in terms of V_a , the multiplicity factor disappears.

To calculate the scattering density over the volume V_a at resolution d , $2N_{\text{ref}}$ independent quantities need to be specified (say, the real and imaginary parts of each of the N_{ref} independent structure factors). The required number of measurements is

$$R = 2N_{\text{ref}} = (4\pi/3d^3)V_a. \quad (13.1.5.2)$$

If only the diffracted intensities can be measured, they provide exactly half the $2N_{\text{ref}}$ measurements required to calculate the density at resolution d .

In what follows, it is assumed that the required number of measurements, R , to specify the scattering density at the chosen resolution is proportional to the volume over which the density must be specified. This is true when the volume is a crystallographic asymmetric unit [equation (13.1.5.2)], and it agrees with another analysis discussed below. Following this argument, the overdetermination ratio

$$\frac{\text{available no. of measurements}}{\text{ideally required no. of measurements}} = \frac{N_{\text{ref}}}{R} = \frac{V_a}{2X}, \quad (13.1.5.3)$$

where X is the volume whose density is unknown.

Next, consider that ideal noncrystallographic symmetry applies. The crystal asymmetric unit contains N identical subunits and no other scattering matter. Since the symmetry is noncrystallographic, it is never possible to fit the subunit volumes together so as to fill the unit cell exactly. The volume assigned to each subunit, U , has to be less than V_a/N , leaving some parts of the unit cell not assigned to any subunit. In the case of ideal noncrystallographic symmetry, these regions are necessarily empty. In this case $X = U$, which is less than V_a/N , so from equation (13.1.5.3)

$$\text{overdetermination ratio} = (V_a/2U) > N/2.$$

Even where N is only 2, more intensity data are available than the number of measurements ideally required to specify the electron density at resolution d .

A more sophisticated analysis of the number of variables required to define a structure with noncrystallographic symmetry has been made in terms of sets of orthogonal ‘eigendensity