13.1. NONCRYSTALLOGRAPHIC SYMMETRY

functions', which satisfy the noncrystallographic symmetry (Crowther, 1967). Any structure satisfying the symmetry requirements can be constructed from the appropriate set of eigendensities. Crowther (1969) demonstrated that the number of eigendensities m is approximately $(2N_{ref}U/V_a)$.

The structure is specified by m weights, which are applied to the m allowed eigendensities (which depend only on the symmetry constraints), so the overdetermination ratio

$$\frac{\text{available no. of measurements}}{\text{ideally required no. of measurements}} = \frac{N_{ref}}{m} = \frac{V_a}{2U},$$

the same result as before, showing that the two methods of analysis approximately agree.

13.1.5.3. *Information gain in the non-ideal case*

In the non-ideal case, the definition of volume U assigned to each subunit assumes an important role. It is particularly important that the volumes should not overlap, since this may set up a chain of unrealizable constraints. In imposing noncrystallographic symmetry, the volumes between subunits are often unconstrained, allowing for differences in solvent structure and surface side chains following from their different environments. In addition to the volume U of one subunit, whose structure is to be defined, an additional volume, $V_a - NU$, is left unconstrained. The volume X of unknown electron density is $U + (V_a - NU)$, and, using equation (13.1.5.3), the overdetermination ratio

$$\frac{\text{available no. of measurements}}{\text{ideally required no. of measurements}} = \frac{V_a}{2[V_a - (N - 1)U]}.$$

$$(13.1.5.4)$$

If $N = 2$, U must be less than $V_a/2$, and the overdetermination ratio in equation (13.1.5.4) must be less than 1, so in the non-ideal case there is no chance of convergent correction. This confirms the practical observation that although averaging electron density with $N = 2$ can improve the structure (Matthews *et al.*, 1967), it does not lead to convergent correction (B. W. Matthews, unpublished results). Slowly convergent *ab initio* structure correction was reported at 6.3 Å resolution for $N = 4$ (Argos *et al.*, 1975). In this case, the volume $4U$ of the constrained tetramer was reported to be only about $V_a/2$. Substituting $N = 4$, $U = V_a/8$ in the above expression gives an overdetermination ratio of only 1.6, which was sufficient to allow convergent correction.

An alternative possibility is to constrain the density between subunits to a constant value, even when this may not be precisely correct, in order to improve the convergence of symmetry correction. There is a close analogy to solvent-flattening techniques used in density modification and atomic structural refinement (Schevitz *et al.*, 1981; Wang, 1985). The volume constrained to a constant value is now $V_a - NU$. The volume whose structure is to be determined is only U, and in place of equation (13.1.5.4),

$$\frac{\text{available no. of measurements}}{\text{ideally required no. of measurements}} = \frac{V_a}{2U},$$

as in the ideal case. Such a constraint, while only approximately valid, may allow structure correction to proceed convergently, as found by Rossmann *et al.* (1992). The constraint may be released at a later stage.

This analysis also emphasizes the importance of specifying the size and shape of the subunit volume U as closely as possible (Wilson *et al.*, 1981). Methods of automatic refinement of the chosen volume are available (Rossmann *et al.*, 1992; Abrahams & Leslie, 1996).

13.1.5.4. *Relevant parameters: generalized case*

In the generalized case it is obvious that noncrystallographic symmetry makes more measurements available, since the data from more than one type of crystal are being used. The volume of the subunit U must be less than V_a in each type of crystal. Making a simple assumption of two crystals, each with one subunit in each asymmetric unit, the available number of measurements per volume of the subunit is, in the ideal case,

$$\frac{N_{ref1} + N_{ref2}}{U} = \left(\frac{2\pi}{3d^3}\right)\left(\frac{V_{a1} + V_{a2}}{U}\right) > 2\left(\frac{2\pi}{3d^3}\right).$$

Thus, the overdetermination ratio $= (N_{ref1} + N_{ref2})/(4\pi U/3d^3) > 1$, so even in this case the structure is theoretically overdetermined.

This type of reasoning can be applied to analysis of a crystal which includes a unit of known structure and also another unit whose structure is unknown [type (6), Table 13.1.4.1]. A complex between an enzyme of known structure with an unknown inhibitor provides a familiar example. Note first that the envelope defining the volume U of known structure must be tightly defined, since otherwise unwanted features will be taken over into the unknown structure.

If this known structure of volume U appears once in the asymmetric unit V_a of the partly unknown structure, can noncrystallographic symmetry correction be used to define a unique structure at resolution d?

The unconstrained volume is $V_a - U$. The number of measurements required to define this density at the given resolution is $4\pi(V_a - U)/3d^3$. The partially unknown structure provides $2\pi V_a/3d^3$ measured intensities to this resolution, and specification of the contents of U at resolution d is equivalent to $4\pi U/3d^3$ measurements. Thus the overdetermination ratio is

$$\frac{(2\pi V_a/3d^3) + (4\pi U/3d^3)}{4\pi(V_a - U)/3d^3} = \frac{V_a + 2U}{2(V_a - U)}.$$

The overdetermination ratio is greater than 1 if $U > V_a/4$, that is, if only a quarter of the asymmetric unit represents known structure. Although this relationship applies to an 'ideal' case and is therefore certainly too optimistic, it indicates the remarkable power of the molecular-replacement method. If, for example, half the unit cell is devoted to unknown structure ($U = V_a/2$), the overdetermination ratio is ideally 2.

13.1.5.5. *Noncrystallographic symmetry in atomic coordinate refinement*

In atomic coordinate refinement, noncrystallographic symmetry again provides a useful increase in the ratio of the number of observed quantities to the number of atomic parameters to be refined. As is discussed by Cruickshank in Chapter 18.5, the application of restraints in refinement (on quantities like bond lengths, bond angles and the elimination of short contacts) is formally equivalent to an increase in the number of observational equations. However, if these restraints are tightly applied, they act more like constraints, and their effect is more like a reduction in the number of parameters to be determined. Meaningful refinement is not possible unless the number of observations exceeds the number of parameters, and in practice it usually needs to do so by a factor of 2 or so. If noncrystallographic symmetry is imposed, the number of observations required to define the structure is reduced, because the volume of unknown structure is reduced.

Noncrystallographic symmetry can thus provide a crucial advantage in leading to unambiguous interpretation of structure at relatively poor resolution (say, 3.0 to 3.8 Å), where the ratio of

references