solvent flattening only operates on the solvent region of the map. The same envelope that was used for isolating the solvent region can be used to determine the protein region of the cell. An alternative approach is to define separate solvent and protein masks, with uncertain regions excluded from either mask and allowed to keep their unmodified values.

15.1.2.2.4. *Scaling the observed structure-factor amplitudes according to the ideal density histogram*

In the process of density modification, electron density or structure factors from different sources are compared and combined. It is, therefore, crucial to ensure that all the structure factors and maps are on the same scale. The observed structure factors can be put on the absolute scale by Wilson statistics (Wilson, 1949) using a scale and an overall temperature factor. This is accurate when atomic or near atomic resolution data are available. The scale and overall temperature factor obtained from Wilson statistics are less accurate when only medium- to low-resolution data are available. A more robust method of scaling non-atomic resolution data is through the density histogram (Cowtan & Main, 1993; Zhang, 1993).

The ideal density histogram defines the mean and variance of an electron density, as shown in equations (15.1.2.15) and (15.1.2.16). We can scale the observed structure-factor amplitudes to be consistent with the target histogram using the following formula, obtained from the structure-factor equation and Parseval's theorem. The mean density and the density variance of the observed map can be calculated as

$$\overline{\rho}' = (1/V)F(000), \tag{15.1.2.19}$$

$$\sigma'(\rho) = (1/V)\left[\sum_{\mathbf{h}}|F(\mathbf{h})|^2\right]^{1/2}. \tag{15.1.2.20}$$

The mean and variance of the electron-density map at the desired resolution are calculated using the target histogram, the mean value of the solvent density, $\overline{\rho}_{\rm solv}$, and the solvent volume of the cell, $V_{\rm solv}$. The $F(000)$ term can then be evaluated from equations (15.1.2.15) and (15.1.2.19):

$$F(000) = (V - V_{\rm solv})\overline{\rho} + V_{\rm solv}\overline{\rho}_{\rm solv}. \tag{15.1.2.21}$$

The scale of the observed amplitudes can be obtained from equations (15.1.2.16) and (15.1.2.20),

$$F'(\mathbf{h}) = KF(\mathbf{h}), \tag{15.1.2.22}$$

where

$$K = \left[(\overline{\rho^2} - \overline{\rho}^2)\right]^{1/2} \Big/ \left\{(1/V)\left[\sum_{\mathbf{h}}|F(\mathbf{h})|^2\right]^{1/2}\right\}. \tag{15.1.2.23}$$

This method is adequate for scaling observed structure factors at any resolution.

15.1.2.3. *Averaging*

The averaging method enforces the equivalence of electron-density values between grid points in the map related by noncrystallographic symmetry. The averaging procedure can filter noise, correct systematic error and even determine the phases *ab initio* in favourable cases (Chapman *et al.*, 1992; Tsao *et al.*, 1992).

15.1.2.3.1. *Introduction*

Noncrystallographic symmetry (NCS) arises in crystals when there are two or more of the same molecules in one asymmetric unit. Such symmetries are local, since they only apply within a subregion of a single unit cell. A fivefold axis, for example, must be noncrystallographic, since it is not possible to tessellate objects with fivefold symmetry. Since the symmetry does not map the crystal lattice back onto itself, the individual molecules that are related by the noncrystallographic symmetry will be in different environments; therefore, the symmetry relationships are only approximate.

Noncrystallographic symmetries provide phase information by the following means. Firstly, the related regions of the map may be averaged together, increasing the ratio of signal to noise in the map. Secondly, since the asymmetric unit must be proportionally larger to hold multiple copies of the molecule, the number of independent diffraction amplitudes available at any resolution is also proportionally larger. This redundancy in sampling the molecular transform leads to additional phase information which can be used for phase improvement.

15.1.2.3.2. *The determination of noncrystallographic symmetry*

The self-rotation symmetry is now routinely solved by the use of a Patterson rotation function (Rossmann & Blow, 1962). The translation symmetry can be determined by a translation function (Crowther & Blow, 1967) when a search model, either an approximate structure of the protein to be determined or the structure of a homologous protein, is available. The searches of the Patterson rotation and translation functions are achieved typically using fast automatic methods, such as *X-PLOR* (Brünger *et al.*, 1987) or *AMoRe* (Navaza, 1994). In cases where no search model is available or the Patterson translation function is unsolvable, either the whole electron-density map, or a region which is expected to contain a molecule, may be rotated using the rotation solution and used as a search model in a phased translation function (Read & Schierbeek, 1988).

Once the averaging operators are determined, the mask can be determined using the local density correlation function as developed by Vellieux *et al.* (1995). This is achieved by a systematic search for extended peaks in the local density correlation, which must be carried out over a volume of several unit cells in order to guarantee finding the whole molecule. The local correlation function distinguishes those volumes of crystal space which map onto similar density under transformation by the averaging operator. Thus, in the case of improper NCS, a local correlation mask will cover only one monomer. In the case of a proper symmetry, a local correlation mask will cover the whole complex (Fig. 15.1.2.4*a*,*b*).

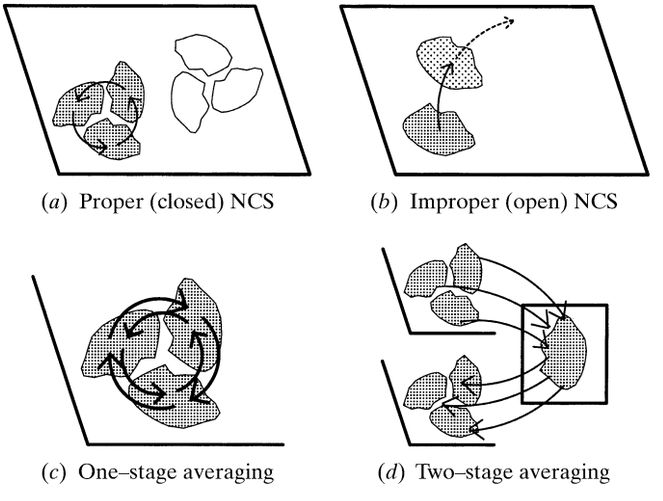

Fig. 15.1.2.4. Types of noncrystallographic symmetry and averaging calculation.

references