13.4. Noncrystallographic symmetry averaging of electron density for molecular-replacement phase refinement and extension

BY M. G. ROSSMANN AND E. ARNOLD

13.4.1. Introduction

Electron-density averaging for phasing crystal structures has become a widespread and nearly routine technique. High noncrystallographic symmetry (NCS) permits the solution of structures using relatively poor and even *ab initio* phasing starts, while lower NCS electron-density averaging can significantly improve initial phases obtained by techniques such as isomorphous replacement, anomalous scattering, or molecular replacement. Implicit in any averaging is solvent flattening in the regions not available for NCS averaging. Indeed, if all parts of the unit cell were consistent with the NCS, the symmetry would be crystallographic and valid throughout the crystal lattice.

A number of generalized averaging programs and software packages have been developed for macromolecular crystal structure analyses. Ease of use, coupled with relatively convenient definition of molecular envelopes, as well as enormous advances in computer technology, have facilitated the application of symmetry averaging to a diverse set of crystallographic problems. Averaging of separate domains in multidomain protein structures that can be divided into segments and averaging among multiple crystal forms is becoming increasingly common.

Extension of phases to higher resolution by symmetry averaging of electron density, coupled with solvent flattening, has been applied to numerous problems. The power of phase extension has been especially impressive in many cases involving high NCS, such as icosahedral virus structures. The overall power for phase improvement of averaging, combined with other density-modification techniques, such as solvent levelling, has been found to depend upon the degree of NCS, the solvent content of the crystals, and the quality and completeness of experimental data. Similar averaging methodology can be used for structure analysis by other imaging techniques, such as electron microscopy.

This chapter discusses the underlying principles of electron-density averaging for macromolecular crystallographic phase improvement and describes procedures for computer implementation of these techniques.

13.4.2. Noncrystallographic symmetry (NCS)

Crystallographic symmetry is valid for the infinite crystal lattice. Any crystallographic symmetry element relates all points within the crystal to equivalent points elsewhere. In contrast, an NCS operator is valid only locally within a finite volume (Fig. 13.4.2.1); if a periodic structure is superimposed on itself after operation with an NCS operator, it will superimpose only within the envelope* defining the limits of the local symmetry.

A product of superimposed periodic structures will be non-periodic, containing only the point symmetry of the noncrystallographic operators (Fig. 13.4.2.2). This fact can frequently be used to select a molecular envelope that was not obvious prior to noncrystallographic averaging [see *e.g.* Buehner *et al.* (1974) or Lin *et al.* (1986)]. Although no knowledge of the crystallographic envelope is needed for this first averaging, it is necessary to determine it for the averaged molecular structure within the crystallographic cell to permit Fourier back-transformation.

There must be space between the confining envelopes governed by the local symmetry. Only the crystallographic symmetry is valid within this space. In the limit, when this space has diminished to zero, the local symmetry will have become a true crystallographic operator.

The definition of NCS can be extended to symmetry that relates similar objects in different crystal lattices. An operation that relates an object in one lattice to an equivalent object in another lattice will apply only to the chosen objects in each lattice. Beyond the confines of the chosen objects, there will be no coincidence of pattern.

Two kinds of NCS elements may be defined: proper and improper. The former satisfies a closed point group [*e.g.* a 17-fold rotation as occurs in tobacco mosaic virus disk protein (Champness *et al.*, 1976)]. Here, it does not matter whether a rotation axis is applied right- or left-handedly; the result is indistinguishable. On the other hand, the relationship between different molecules in a crystallographic asymmetric unit is unlikely to be a closed point group. Thus, a rotation in one direction (followed by a translation) might achieve superposition of the two molecules, while a rotation in the opposite direction would not. This is called an improper NCS operator. An operation which takes a molecule in one unit cell to that in another unit cell (initially, the cells are lined up with, say,
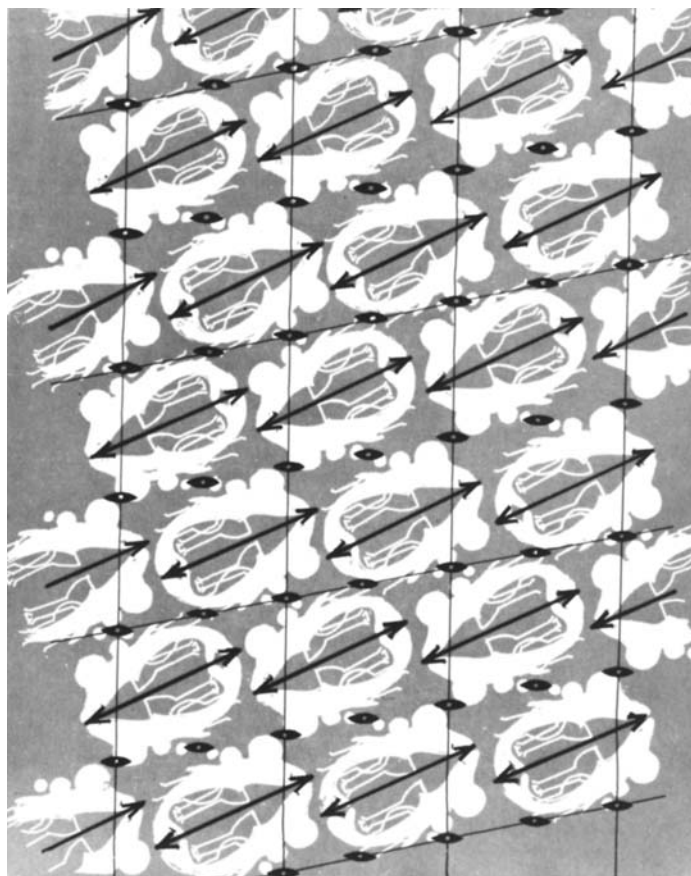

Fig. 13.4.2.1. The two-dimensional periodic design shows crystallographic twofold axes perpendicular to the page and local noncrystallographic rotation axes in the plane of the paper (design by Audrey Rossmann). [Reprinted with permission from Rossmann (1972). Copyright (1972) Gordon & Breach.]

279

(*a*)   (*b*)   (*c*)
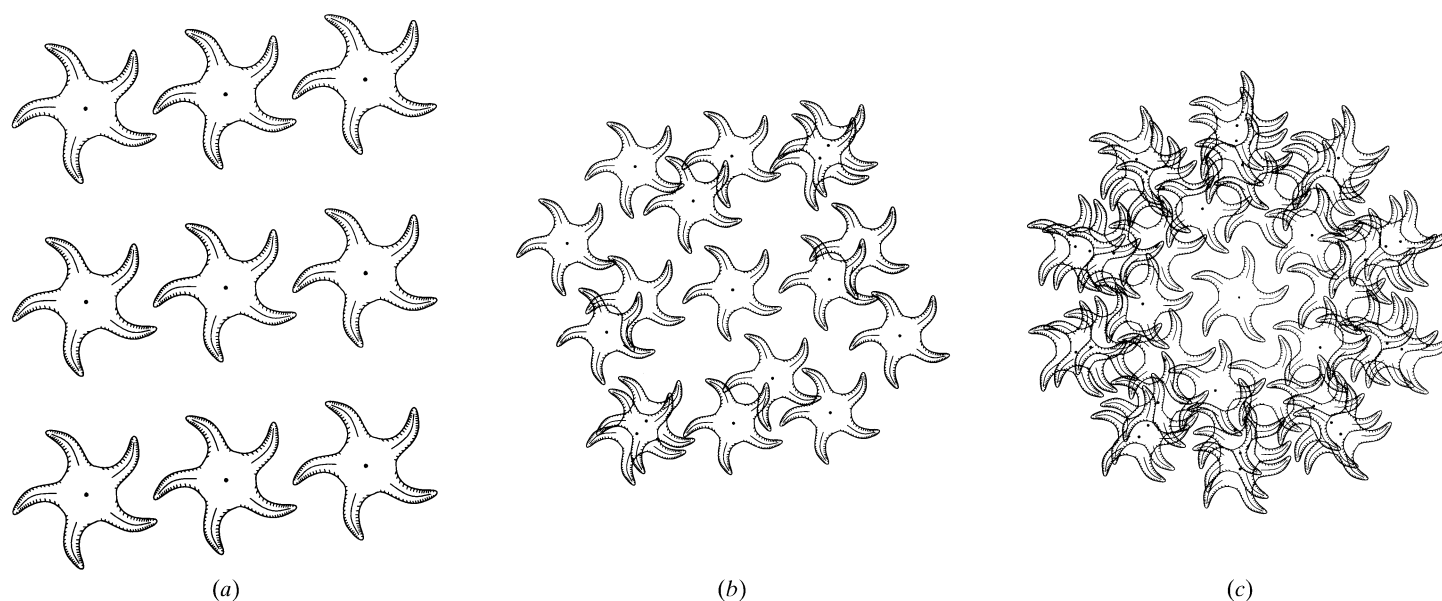
Fig. 13.4.2.2. (*a*) NCS in a triclinic cell. (*b*) Superposition of the pattern in (*a*) on itself after operation with the noncrystallographic fivefold axis. (*c*) Superposition of the pattern in (*a*) on itself after a rotation of one-fifth, two-fifths, three-fifths and four-fifths. Note that the sum or product of periodic patterns is aperiodic and in (*c*) has the point symmetry of the noncrystallographic operation. [Reprinted with permission from Rossmann (1990). Copyright (1990) International Union of Crystallography.]

their orthogonalized *a*, *b* and *c* axes parallel) must equally be an improper rotation.

The position in space of a noncrystallographic rotation symmetry operator can be arbitrarily assigned. The rotation operation will orient the two molecules similarly. A subsequent translation, whose magnitude depends upon the location of the NCS operator, will always be able to superimpose the molecules (Fig. 13.4.2.3). Nevertheless, it is possible to select the position of the NCS axis such that the translation is a minimum, and that will occur when the translation is entirely parallel to the noncrystallographic rotation axis.
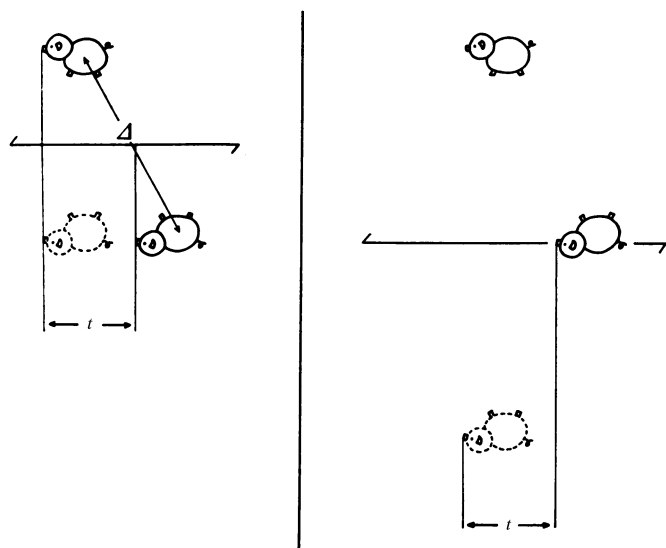

Fig. 13.4.2.3. The position of the twofold rotation axis which relates the two piglets is completely arbitrary. The diagram on the left shows the situation when the translation is parallel to the rotation axis. The diagram on the right has an additional component of translation perpendicular to the rotation axis, but the component parallel to the axis remains unchanged. [Reprinted with permission from Rossmann *et al.* (1964). Copyright (1964) International Union of Crystallography.]

The position of an NCS axis, like everything else in the unit cell, must be defined with respect to a selected origin. Consider the noncrystallographic rotation defined by the 3×3 matrix [C]. Then, if the point $\mathbf{x}$ is rotated to $\mathbf{x}'$ (both defined with respect to the selected origin and axial system),

$$\mathbf{x}' = [C]\mathbf{x} + \mathbf{d},$$

where $\mathbf{d}$ is a three-dimensional vector which expresses the translational component of the NCS operation. The magnitude of the components of $\mathbf{d}$ is quite arbitrary unless the position of the rotation axis is defined. If the rotation axis represents a proper NCS element, there will exist a point $\mathbf{x}$ on the rotation axis, when positioned to eliminate translation, such that it is rotated onto $\mathbf{x}'$. It follows that for such a point

$$\mathbf{x} = [C]\mathbf{x} + \mathbf{d},$$

from which $\mathbf{d}$ can be determined if the position of the molecular centre is known. Note that $\mathbf{d} = 0$ if, and only if, the noncrystallographic rotation axis passes through the crystallographic origin.

The presence of proper NCS in a crystal can help phase determination considerably. Consider, for example, a tetramer with 222 symmetry. It is not necessary to define the chemical limits of any one polypeptide chain as the NCS is true everywhere within the molecular envelope and the boundaries of the polypeptide chain are irrelevant to the geometrical considerations. The electron density at every point within the molecular envelope (which itself must have 222 symmetry) can be averaged among all four 222-related points without any chemical knowledge of the configuration of the monomer polypeptide. On the other hand, if there is only improper NCS, then the envelope must define the limits of one noncrystallographic asymmetric unit, although the crystallographic asymmetric unit contains two or more such units.

13.4.3. Phase determination using NCS

The molecular replacement method [*cf.* Rossmann & Blow (1962); Rossmann (1972, 1990); Argos & Rossmann (1980); Rossmann & Arnold (2001)] is dependent upon the presence of NCS, whether it

280