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,

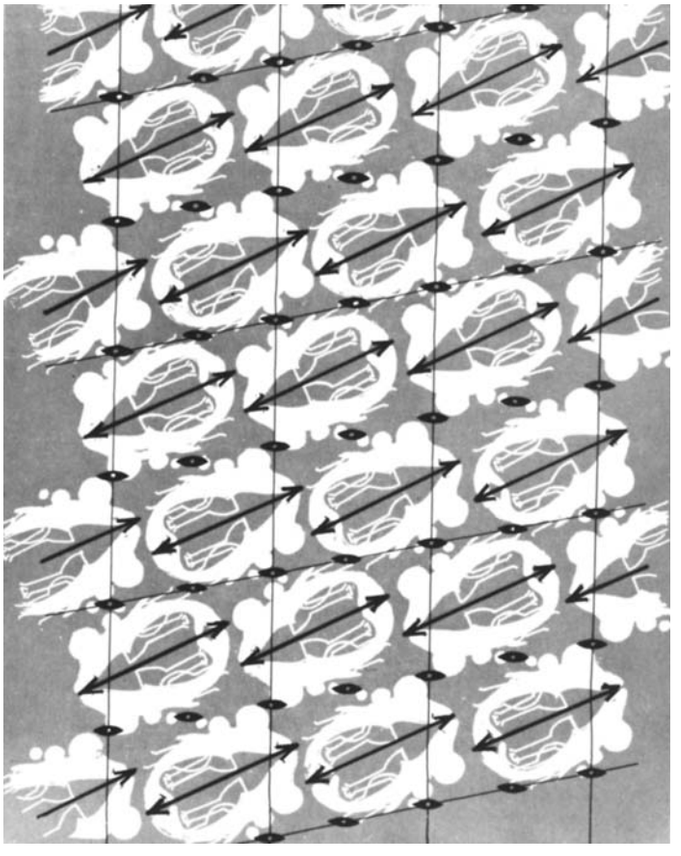

Fig. 13.4.2.1. The two-dimensional periodic design shows crystallographic twofold axes perpendicular to the page and local noncrystallographic rotation axes in the plane of the paper (design by Audrey Rossmann). [Reprinted with permission from Rossmann (1972). Copyright (1972) Gordon & Breach.]

* In this chapter, 'envelope' will be used to describe the external surface or boundary of a molecule, while 'mask' will be used to denote the three-dimensional distribution of grid points that have been assigned within the molecular surface.

references