2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

Table 2.3.5.1. Possible types of vector searches

<table>
<thead>
<tr>
<th>Self-vectors</th>
<th>Cross-vectors</th>
<th>Dimension of search, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Locate single site relative to particle centre</td>
<td></td>
<td>n = 3</td>
</tr>
<tr>
<td>(2) Use information from (1) to locate particle centre</td>
<td></td>
<td>n = 3</td>
</tr>
<tr>
<td>(3) Simultaneous search for both (1) and (2). In general this is a six-dimensional search but may be simplified when particle is on a crystallographic symmetry axis</td>
<td>3 ≤ n ≤ 6</td>
<td></td>
</tr>
<tr>
<td>(4) Given (1) for more than one site, find all vectors within particle</td>
<td></td>
<td>n = 3</td>
</tr>
<tr>
<td>(5) Given information from (3), locate additional site using complete vector set</td>
<td></td>
<td>n = 3</td>
</tr>
</tbody>
</table>

heavy-atom position was assigned (atom A₂ at x, y, z). At this juncture, the known noncrystallographic symmetry was used to obtain a full interpretation. From Table 2.3.5.2 we see that molecular axis 2 will generate a second heavy atom with coordinates roughly \( \frac{1}{2} + y, -\frac{1}{2} + x, 2Z - z \) (if the molecular centre was assumed to be at \( \frac{1}{2}, \frac{1}{2}, Z \)). Starting from the tentative coordinates of site A₂, the site A₁ related by molecular axis 1 was detected at about the predicted position and the second site A₁ generated accessible cross-vectors with the earlier determined site A₂. Further examination enabled the completion of the set of four noncrystallographically related heavy-atom sites, such that all predicted Patterson vectors were acceptable and all four sites placed the molecular centre in the same position. Following refinement of these four sites, the corresponding SIR phases were used to find an additional set of four sites in this compound as well as in a number of other derivatives. The multiple isomorphous replacement phases, in conjunction with real-space electron-density averaging of the noncrystallographically related units, were then sufficient to solve the GDPAH structure.

When investigators studied larger macromolecular aggregates such as the icosahedral viruses, which have 532 point symmetry, systematic methods were developed for utilizing the noncrystallographic symmetry to aid in locating heavy-atom sites in isomorphous heavy-atom derivatives. Argos & Rossman (1974, 1976) introduced an exhaustive Patterson search procedure for a single heavy-atom site within the noncrystallographic asymmetric unit which has been successfully applied to the interpretation of both virus [satellite tobacco necrosis virus (STNV) (Lentz et al., 1976), southern bean mosaic virus (Rayment et al., 1978), alfalfa mosaic virus (Fukuyama et al., 1983), cowpea mosaic virus (Stauffacher et al., 1987)] and enzyme [catalase (Murthy et al., 1981)] heavy-atom difference Pattersons. A heavy atom is placed in turn at all plausible positions within the volume of the noncrystallographic asymmetric unit and the corresponding vector set is constructed from the resulting constellation of heavy atoms. Argos & Rossman (1976) found a spherical polar coordinate search grid to be convenient for spherical viruses. After all vectors for the current search position are predicted, the vectors are allocated to the nearest grid point and the list is sorted to eliminate recurring ones. The criterion used by Argos & Rossman for selecting a solution is that the sum

\[
S = \sum_{i=1}^{N} P_i - NP_{av}
\]

of the lookup Patterson density values \( P_i \) achieves a high value for a correct heavy-atom position. The sum is corrected for the carpet of cross-vectors by the second term in the sum.

An additional criterion, which has been found useful for discriminating correct solutions, is a unit vector density criterion

\[
U = \frac{N}{\sum_{i=1}^{N}} \left( \frac{P_i}{n_i} \right)
\]

where \( n_i \) is the number of vectors expected to contribute to the Patterson density value \( P_i \) (Arnold et al., 1987). This criterion can be especially valuable for detecting correct solutions at special search positions, such as an icosahedral fivefold axis, where the number of vector lookup positions may be drastically reduced owing to the higher symmetry. An alternative, but equivalent, method for locating heavy-atom positions from isomorphous difference data is discussed in Section 2.3.3.5.

Even for a single heavy-atom site at a general position in the simplest icosahedral or \( T = 1 \) virus, there are 60 equivalent heavy atoms in one virus particle. The number of unique vectors corresponding to this self-particle vector set will depend on the symmetry but may be as many as \( (60)(59)/2 = 1770 \) for a virus particle at a general crystallographic position. Such was the case for the STNV crystals which were in space group C2 containing four virus particles at general positions. The method of Argos & Rossman was applied successfully to a solution of the \( K_2HgI_4 \) derivative of STNV using a 10 Å resolution difference Patterson. Application of the noncrystallographic symmetry vector search procedure to a \( K_2Au(CN)_2 \) derivative of human rhinovirus 14 (HRV14) crystals (space group \( P2_13, Z = 4 \)) has succeeded in establishing both the relative positions of heavy atoms within one particle and the positions of the virus particles relative to the crystal symmetry elements (Arnold et al., 1987). The particle position was established by incorporating interparticle vectors in the search and varying the particle position along the crystallographic threelfold axis until the best fit for the predicted vector set was achieved.

2.3.6. Rotation functions

2.3.6.1. Introduction

The rotation function is designed to detect noncrystallographic rotational symmetry (see Table 2.3.6.1). The normal rotation function definition is given as (Rossman & Blow, 1962)

\[
R = \int \frac{P_1(u) \cdot P_2(u)}{U} \, du, \tag{2.3.6.1}
\]

where \( P_1 \) and \( P_2 \) are two Pattersons and \( U \) is an envelope centred at the superimposed origins. This convolution therefore measures the degree of similarity, or ‘overlap’, between the two Pattersons when \( P_2 \) has been rotated relative to \( P_1 \) by an amount defined by