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The Molecular Replacement Method. I. The Rotation Function Problem, Application to Bovine Liver Catalase and STNV

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A method to predict all peaks of the rotation function corresponding to a given molecular crystal is formulated in a rigorous mathematical manner. The applicability of this method is shown both in the analysis of rotation function data and in testing the validity of model molecular crystals proposed on the basis of limited rotation function data. Possible models of crystalline bovine liver catalase are determined assuming that all peaks of the rotation function are contained in the set of peaks determined by Eventoff and Gurskaya. All peaks of the rotation function corresponding to a model of crystalline satellite tobacco necrosis virus (STNV) proposed by Klug and Akervall *et al.* are determined and tabulated. Experimental determination of all these peaks would be a final test of the validity of this generally accepted model of crystalline STNV.

1. Introduction

In this paper the word *molecule* will refer to any biological macromolecule which is made up of identical subunits. In particular, we will be considering protein molecules with the property of being made up of identically folded polypeptide chains, and such aggregates of protein molecules as constitute the polyhedral shell of small viruses. The crystalline form of such molecules will be called a *molecular crystal*.

To determine the structure of such molecules from X-ray diffraction data of a molecular crystal, the 'molecular replacement method' has been developed (Rossmann, 1972). This method consists of three parts:

(1) The rotation problem: determining the orientation of the molecules in a molecular crystal and the relative orientation of the subunits of each molecule.

(2) The translation problem: determining the translation vectors between molecules in a molecular crystal.

(3) The phase problem: Using the results of the first two steps in determining the phases of the structure factors of the X-ray diffraction data, and subsequently in determining the structure of the molecules of a molecular crystal.

We will limit our discussion in this paper to the first of these three problems, *i.e.* to the rotation problem, to determining the orientation of the molecules in a molecular crystal, and of more importance to determining the relative orientation of a molecule's constituent subunits. The method which is used in solving the rotation problem is based on the use of the rotation function defined by Rossmann & Blow (1962). Peaks of this function have been shown to correspond to rotations which leave a molecule invariant or rotate a molecule into the orientation of some other molecule of the molecular crystal. A collection of reprints on the molecular replacement method and in particular on the rotation problem, including the paper by Rossmann & Blow (1962), can be found in the book titled *The Molecular Replacement Method* (Rossmann, 1972).

The computational time of the rotation function is quite considerable, and information on all peaks of the rotation function is not always available for the analysis of the structure of a molecular crystal. Because no method to predict all peaks of the rotation function corresponding to a given molecular crystal has been formulated (Rossmann, 1973), no method has been available for use in a systematic analysis of rotation function data, nor to verify the validity of molecular crystal structures proposed on the basis of limited rotation function data. Consequently, rotation function data has been misinterpreted (Akervall *et al.*, 1971*a*).

It is the purpose of this paper to formulate in a rigorous mathematical manner a method to predict all peaks of the rotation function corresponding to a given molecular crystal. The applicability of this

method will be shown both in the analysis of rotation function data and in testing the validity of molecular crystal structures proposed on the basis of limited rotation function data.

In § 2 we first review some fundamental concepts of crystallography, pointing out that a molecular crystal of space-group symmetry F can be partitioned into a set of *simple molecular crystals*. We then show that all rotations which leave a molecule of a simple molecular crystal invariant or rotate it into the orientation of some other molecule of the simple molecular crystal can be determined from the rotational part of elements of the space group F and the *symmetry point group*, the set of all rotations which leaves a molecule invariant, of any one molecule of the simple molecular crystal.

We determine in § 3 all peaks of the rotation function corresponding to a given molecular crystal, and a method to determine, assuming that all peaks of the rotation function are known, the orientation and symmetry point group of the molecules of a molecular crystal. This method is then applied to the case of bovine liver catalase. In addition we discuss the information on the molecular crystal which may be obtained from an analysis of the height of the rotation function peaks.

The analysis of the rotation function data of satellite tobacco necrosis virus (STNV) is discussed in § 4. It is shown that the limited available rotation function data indicate a molecular crystal with molecules of icosahedral point-group symmetry. All peaks of the rotation function corresponding to this generally accepted model of STNV proposed by Klug (1971) and Akerval *et al.* (1971*b*) are determined and tabulated.

2. Molecular crystals

Consider a molecular crystal whose symmetry group is the space group F. The elements of a space group F are denoted by $F = [R \mid \tau(R) + t]$ where R is a proper or improper rotation, $\tau(R)$ the non-primitive translation associated with R, and t a primitive translation. Let r_i, $i = 1, 2, \ldots$ denote the *molecular position vectors*, the position vectors of the centre of mass of the molecules. The molecules of the molecular crystal can be partitioned into *simple molecular crystals* consisting of identical molecules whose molecular position vectors can all be obtained by applying all elements of the space group F to any one molecular position vector r_1. We will say that the molecular position vectors of a simple molecular crystal are generated by F from r_1. If no two molecular position vectors of the molecular position vectors generated by F from r_1 are equal, the molecular position vectors are called *general position vectors*; otherwise they are called *special position vectors*.

A molecular position vector r_1 can be characterized by specifying its *site space group* $F(r_1)$. The site space group $F(r_1)$ is defined as the subgroup of all elements of F which generate the set of molecular position vectors

$r_1 + t$, for all primitive translations t of the subgroup of primitive translations T of F. The point group $R(r_1)$ of $F(r_1)$ is called the *site point group* of r_1 (Opechowski & Guccione, 1962).

We decompose F into left cosets relative to $F(r_1)$:

$$F = F(r_1) + [R_2 \mid \tau(R_2)]F(r_1) + \ldots + [R_n \mid \tau(R_n)]F(r_1) . \quad (1)$$

The coordinates of the set of n molecular position vectors

$$r_1; \ [R_2 \mid \tau(R_2)]r_1 = R_2 r_1 + \tau(R_2); \ \ldots;$$
$$[R_n \mid \tau(R_n)]r_1 = R_n r_1 + \tau(R_n) \quad (2)$$

are the coordinates of the molecular position vectors of the molecules in the primitive unit cell of a simple molecular crystal whose molecular position vectors are generated by F from r_1. This set of coordinates is given for each F and r_1 in *International Tables for X-ray Crystallography* (1952) and these coordinates are called there the *coordinates of equivalent positions*, while the site point group $R(r_1)$ is called the *point-group symmetry* of each of the equivalent positions. If r_1 is a general position vector, $F(r_1) = T$ and $R(r_1)$ consists of the identity element only.

Let $M(r_i)$, $i = 1, 2, \ldots$ denote the molecule of a molecular crystal whose molecular position vector is r_i. The invariance of the molecular crystal under elements $[R \mid \tau(R) + t]$ of its symmetry space group F means that for every molecule at molecular position r_i there is an identical molecule at molecular position $[R \mid \tau(R) + t]r_i = R r_i + \tau(R) + t$. The orientations of the molecules at r_i and $[R \mid r(R) + t]r_i$ relative to the crystallographic axes are not necessarily the same. The mutual orientation of these two molecules is determined by the rotation R of the space-group element $[R \mid \tau(R) + t]$ and we shall write

$$M([R \mid \tau(R) + t]r_i) = RM(r_i) , \quad (3)$$

that is, the orientation relative to the crystallographic axes of the molecule $M([R \mid \tau(R) + t]r_i)$ is identical to the orientation of the molecule $M(r_i)$ after the latter has been rotated about r_i, its centre of mass, by the rotation R.

It follows from equation (3) that no two simple molecular crystals have molecules in common, and the elements of F permute the molecules of each simple molecular crystal among themselves. It also follows that the position and orientation of all molecules of a simple molecular crystal whose molecular position vectors are generated by F from r_i can be determined from the position and orientation of the molecule $M(r_i)$. We will therefore denote by $[F; M(r_1)]$ a simple molecular crystal whose molecular position vectors are generated by F from r_1, and will say that the simple molecular crystal $[F; M(r_1)]$ is generated by F from the molecule $M(r_1)$. r_1 will always be taken as a position vector of a molecule in the primitive unit cell of the molecular crystal. A molecular crystal of space-group symmetry F and consisting of q simple molecular crystal $[F; M(r_1)]$, $[F; M(r_2)]$, $\ldots$, $[F; M(r_q)]$ will be

denoted by [F; $M(\mathbf{r}_1)$, $M(\mathbf{r}_2)$, ..., $M(\mathbf{r}_q)$] and said to be generated by F from the molecules $M(\mathbf{r}_1)$, $M(\mathbf{r}_2)$, ..., $M(\mathbf{r}_q)$. This notation for a molecular crystal can be used as a classification label of all molecular crystals and is analogous to the classification label of spin arrangements in crystals (Opechowski & Dreyfus, 1971).

We shall see in the following section that the peaks of the rotation function are related to rotations which leave a molecule of a molecular crystal invariant or rotate it into the orientation of some other molecule of the molecular crystal. We therefore now determine what are the distinct molecular orientations of molecules in a molecular crystal and then the rotations which leave a molecule of a molecular crystal invariant or rotate it into the orientation of some other molecule of the molecular crystal. We will first consider the case of a molecular crystal consisting of a single simple molecular crystal.

The orientation of the n molecules in the primitive unit cell of a simple molecular crystal [F; $M(\mathbf{r}_1)$] relative to the molecule $M(\mathbf{r}_1)$ is determined using equation (3) with $[R|\tau(R)+\mathbf{t}]=[R_i|\tau(R_i)]$, $i=1, 2, \ldots, n$, the coset representatives of the decomposition of F relative to $F(\mathbf{r}_1)$ given in equation (1) where $[R_1|\tau(R_1)]=(E|0)$. Since $M(\mathbf{r}+\mathbf{t})=M(\mathbf{r})$, the number of distinct molecular orientations is at most equal to n, the number of molecules in the primitive unit cell. We will now determine the number m of distinct molecular orientations.

Let R denote the point group of the space group F, and P the symmetry point group of the molecule $M(\mathbf{r}_1)$, i.e. the group of all rotations such that $PM(\mathbf{r}_1)=M(\mathbf{r}_1)$, rotations about $\mathbf{r}_1$ which leave the molecule $M(\mathbf{r}_1)$ invariant. We define $X=R \cap P$, the intersection of the point groups R and P, and decompose R into left cosets relative to X:

$$R = X + R_2 X + \ldots + R_m X . \qquad (4)$$

Since elements of X are symmetry elements of the molecule $M(\mathbf{r}_1)$, elements of each coset when applied to $M(\mathbf{r}_1)$ rotate this molecule into the same orientation, and elements of different cosets rotate the molecule into different molecular orientations. Consequently, there are exactly m distinct molecular orientations of molecules in [F; $M(\mathbf{r}_1)$]. We shall denote the m distinct orientations as M_k, $k=1, 2, \ldots, m$ where $M_1 = M(\mathbf{r}_1)$ and $M_k = R_k M_1$ where R_k is the kth coset representative in the coset decomposition of R relative to X given in equation (4).

Setting $|G|$ to denote the order of the group G, from equations (1) and (4) we have that $n=|F|/|F(\mathbf{r}_1)|=|R|/|R(\mathbf{r}_1)|$, $m=|R|/|X|$, and consequently that $n=(|X|/|R(\mathbf{r}_1)|)m$. Since all rotations of the site point group $R(\mathbf{r}_1)$ are necessarily contained in X, n is equal to or an integral multiple of m. The number of molecules in the primitive unit cell in a specific orientation is n/m which is an integer and is independent of the orientation. There are then $n|T|/m$ molecules in [F; $M(\mathbf{r}_1)$] in each of the m distinct orientations. Only if $X=R(\mathbf{r}_1)$ is

$n=m$ and the number of distinct molecular orientations equal to the number of molecules in the primitive unit cell.

The distribution of the n molecules in the primitive unit cell among the m distinct orientations is determined as follows: The position vector of the jth molecule $M(\mathbf{r}_j)$ in the primitive unit cell is, see equation (2), $\mathbf{r}_j = [R_j | \tau(R_j)]\mathbf{r}_1$. R_j is an element of R, and if this rotation R_j is contained in the kth coset of equation (4), then $M(\mathbf{r}_j)=M_k$, i.e. the jth molecule in the primitive unit cell is in the kth distinct molecular orientation.

As an example consider the simple molecular crystal [F; $M(\mathbf{r}_1)$] of space-group symmetry $F=D_4^1(P422)$ where $\mathbf{r}_1$ is a general position vector. The site space group $F(\mathbf{r}_1)=C_1^1(P1)$ and the coset decomposition of F relative to $F(\mathbf{r}_1)$ is:

$$F = F(\mathbf{r}_1) + (2_x|0)F(\mathbf{r}_1) + (2_y|0)F(\mathbf{r}_1) + (2_z|0)F(\mathbf{r}_1)$$
$$+ (2_{xy}|0)F(\mathbf{r}_1) + (2_{\bar{x}y}|0)F(\mathbf{r}_1) + (4_z|0)F(\mathbf{r}_1)$$
$$+ (4_z^3|0)F(\mathbf{r}_1) .$$

The $n=8$ molecules in the primitive unit cell have molecular position vectors which we denote as follows:

$$
\begin{array}{ll}
\mathbf{r}_1 & \mathbf{r}_5 = 2_{xy}\mathbf{r}_1 \\
\mathbf{r}_2 = 2_x\mathbf{r}_1 & \mathbf{r}_6 = 4_z\mathbf{r}_1 \\
\mathbf{r}_3 = 2_y\mathbf{r}_1 & \mathbf{r}_7 = 4_z^3\mathbf{r}_1 \qquad (5) \\
\mathbf{r}_4 = 2_z\mathbf{r}_1 & \mathbf{r}_8 = 2_{\bar{x}y}\mathbf{r}_1 .
\end{array}
$$

The site point group $R(\mathbf{r}_1)=E$.

We consider the case where the molecule $M(\mathbf{r}_1)$ has the symmetry point group P:

$$P = \{E, 2_y, 2_{xy}, 2_{\bar{x}z}\} . \qquad (6)$$

Therefore

$$X = P \cap R = \{E, 2_y\}$$

and:

$$R = X + 2_x X + 2_{xy} X + 2_{\bar{x}y} X$$
$$= \{E, 2_y\} + \{2_x, 2_z\} + \{2_{xy}, 4_z^3\} + \{2_{\bar{x}y}, 4_z\} . \qquad (7)$$

There are then $m=4$ distinct molecular orientations among the $n=8$ molecules in the primitive unit cell, two molecules in each of the four orientations. The four orientations are, see equation (7), $M_1 = M(\mathbf{r}_1)$, $M_2 = 2_x M_1$, $M_3 = 2_{xy}M_1$, and $M_4 = 2_{\bar{x}y}M_1$. With M_1 represented by a rectangular block, these four orientations are shown in Fig. 1. Since E and 2_y, 2_x and 2_z, 2_{xy} and 4_z^3, and $2_{\bar{x}y}$ and 4_z, are pairs of rotations belonging to the same cosets in equation (7), we have from equations (5) and (7) that the distribution of the eight molecules in the primitive unit cell among the four distinct molecular orientations is:

$$
\begin{aligned}
M(\mathbf{r}_1) &= M(\mathbf{r}_3) = M_1 \\
M(\mathbf{r}_2) &= M(\mathbf{r}_4) = M_2 = 2_x M_1 \\
M(\mathbf{r}_5) &= M(\mathbf{r}_7) = M_3 = 2_{xy}M_1 \\
M(\mathbf{r}_6) &= M(\mathbf{r}_8) = M_4 = 2_{\bar{x}y}M_1 .
\end{aligned}
$$

We will now determine the rotations which leave a molecule of a simple molecular crystal invariant or rotate it into the orientation of some other molecule of the simple molecular crystal. Since there are exactly m distinct molecular orientations M_k, $k = 1, 2, \ldots, m$, we determine all rotations $R(jk)$ which satisfy

$$R(jk)M_j = M_k \qquad (8)$$

for $j, k = 1, 2, \ldots, m$. The set of rotations $\{R(jk)\}$ for specific j and k is the set of all rotations which rotate molecules of the jth orientation into molecules of the kth orientation. We shall determine these rotations in terms of the rotations R_k, $k = 1, 2, \ldots, m$ the coset representatives of equation (4), and the rotations P of the symmetry point group P of the molecule $M(\mathbf{r}_1)$ which has been taken as the molecule of molecular orientation M_1.

The set of $|P|$ rotations $R_k P R_j^{-1}$ for every element P of P belongs to the set $\{R(jk)\}$, since:

$$
\begin{aligned}
R_k P R_j^{-1} M_j &= R_k P M_1 \\
&= R_k M_1 \\
&= M_k \ .
\end{aligned}
$$

There are no additional rotations in $\{R(jk)\}$ as one can show that every rotation $R(jk)$ which satisfies equation (8) must be of the form $R_k P R_j^{-1}$ for some element P of P: from equation (8)

$$R(jk)R_j M_1 = R_k M_1$$
$$R_k^{-1} R(jk) R_j M_1 = M_1$$

and $R_k^{-1} R(jk) R_j$ is thus a rotation which leaves molecules of the orientation M_1 invariant. Consequently this rotation belongs to the group P, and for some element P of P

$$R_k^{-1} R(jk) R_j = P$$

and therefore:

$$R(jk) = R_k P R_j^{-1}.$$

Consequently:

$$\{R(jk)\} = \{R_k P R_j^{-1}\} \qquad (9)$$

is the set of all rotations which rotates a molecule of the jth orientation into the orientation of a molecule of the kth orientation.

The set of rotations $\{R(jj)\}$ constitutes a group, the symmetry point group of molecules of the jth orientation. The set of rotations $\{R(jk)\}$ $j \neq k$ does not in general constitute a group of rotations. We note that $\{R(kj)\} = \{R(jk)^{-1}\}$.

The $|P|m^2$ rotations $\{R(jk)\}$ $j, k = 1, 2, \ldots, m$ contain all rotations which leave a molecule invariant or rotate it into the orientation of some other molecule of a simple molecular crystal; and conversely, every one of the $|P|m^2$ rotations corresponds to such a rotation. The number of times a distinct rotation appears in this set of $|P|m^2$ rotations is proportional to the number of molecules of the simple molecular crystal which when rotated by the rotation are left invariant or rotated into the orientation of some other molecule. If a specific

rotation appears d times among the $|P|m^2$ rotations, d taking the possible integral values of between 0 and m, then d/m of the molecules of the simple molecular crystal are left invariant under this rotation or rotated into the orientation of some other molecule. All rotations of the point group R of the space group F of a simple molecular crystal are each contained $d = m$ times in this set of $|P|m^2$ rotations.

In the example considered above, there are four distinct molecular orientations. The symmetry point group P of M_1 is given in equation (6), and from equation (7) we have:

$$R_1 = E; \quad R_2 = 2_x; \quad R_3 = 2_{xy}; \quad R_4 = 2_{\bar{x}y} \ .$$

The 64 rotations $\{R(jk)\}$ $j, k = 1, 2, 3, 4$ are found using equation (9) and are given in Table 1. The 24 distinct rotations constitute the point group O (432). The crystallographic rotations, which constitute the point group $R = D_4^{(x, y, z)}$, each appear four times since $m = 4$, and each of the remaining rotations of O (432) appear twice. For example, see Table 2, the rotation 4_x interchanges the orientation of molecules of orientation M_3 and M_4, i.e. $4_x M_3 = M_4$ and $4_x M_4 = M_3$, but neither leaves invariant nor rotates into the orientation of some other molecule, molecules of orientation M_1 and M_2.

In the case of a molecular crystal [F; $M(\mathbf{r}_1)$, $M(\mathbf{r}_2)$, $\ldots$, $M(\mathbf{r}_q)$], consisting of identical molecules, the rotations which leave molecules of the molecular crystal invariant or rotate them into the orientation of other molecules are determined as follows. Let M_1^α be the orientation of the molecule $M(\mathbf{r}_\alpha)$ from which the αth simple crystal is generated by F. The symmetry point group of $M(\mathbf{r}_\alpha)$ will be denoted by P^α, and R_j^α,
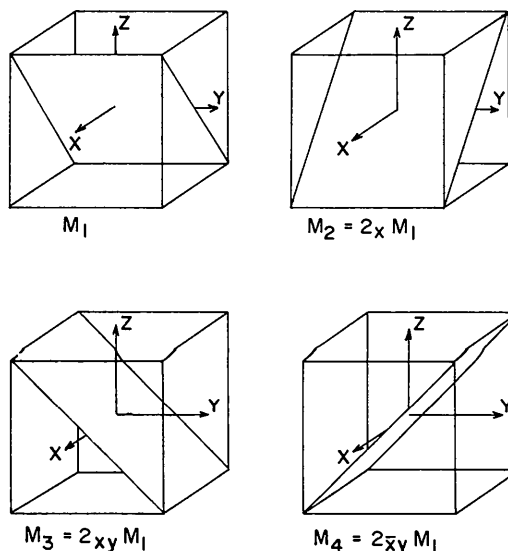

Fig. 1. The four distinct molecular orientations of the simple molecular crystal [F; $M(\mathbf{r}_1)$] where $F = D_4^1$ ($P422$), $\mathbf{r}_1$ is a general position vector, and the symmetry point group of $M(\mathbf{r}_1)$ is $D_2^{(y, xz, \bar{x}z)}$. The molecules are represented schematically by flat rectangular plates.

Table 1. *The sets of rotations* $\{R(jk)\}=\{R_k PR_j^{-1}\}$, *j,k* $=1,2,3,4$ *are tabulated for* $P=\{E,2_y,2_{xz},2_{\bar{x}z}\}$ *and* $R_1=E$, $R_2=2_x$, $R_3=2_{xy}$, *and* $R_4=2_{\bar{x}y}$

jk		{R(jk)}		
11	E	2_y	2_{xy}	$2_{\bar{x}z}$
21	2_x	2_z	4_y^3	4_y
31	2_{xy}	4_z	$3^2_{\bar{x}yz}$	$3^2_{\bar{x}\bar{y}z}$
41	$2_{\bar{x}y}$	4_z^3	$3^3_{xy\bar{z}}$	3_{xyz}
12	2_x	2_z	4_y	4_y^3
22	E	2_y	$2_{\bar{x}z}$	2_{xz}
32	4_z^3	$2_{\bar{x}y}$	$3^3_{xy\bar{z}}$	3_{xyz}
42	4_z	2_{xy}	$3^2_{\bar{x}yz}$	$3^2_{\bar{x}\bar{y}z}$
13	2_{xy}	4_z^3	$3_{\bar{x}yz}$	$3_{x\bar{y}z}$
23	4_z	$2_{\bar{x}y}$	$3_{xy\bar{z}}$	3^2_{xyz}
33	E	2_x	$2_{\bar{y}z}$	2_{yz}
43	2_z	2_y	4_x	4_x^3
14	$2_{\bar{x}y}$	4_z	$3_{xy\bar{z}}$	$3^2_{x\bar{y}z}$
24	4_z^3	2_{xy}	$3_{\bar{x}yz}$	$3_{x\bar{y}z}$
34	2_z	2_y	4_x^3	4_x
44	E	2_x	2_{yz}	$2_{\bar{y}z}$

Table 2. *In the simple molecular crystal* [F; $M(\mathbf{r}_1)$] *where* $F=\underline{D}_4^1(P422)$, $\mathbf{r}_1$ *is a general position vector, and* $P=D_2^{(y,xz,\bar{x}z)}$, *the intersection of the ith row and the jth column of this table gives the distinct molecular orientation into which molecules of distinct orientation* M_i *are rotated by rotations* R_j *given in Table* 1

If the (*ij*)th entry is blank, then the rotation R_j neither leaves invariant molecules of the *i*th distinct orientation, nor rotates them into the orientation of any other molecule of the simple molecular crystal.

	E	2_x	2_y	2_z	3^2_{xyz}	$3_{xy\bar{z}}$	$3_{\bar{x}yz}$	$3_{x\bar{y}z}$
M_1	M_1	M_2	M_1	M_2	M_4	M_4	M_3	M_3
M_2	M_2	M_1	M_2	M_1	M_3	M_3	M_4	M_4
M_3	M_3	M_3	M_4	M_4				
M_4	M_4	M_4	M_3	M_3				

	3_{xyz}	$3_{x\bar{y}z}$	$3^2_{xy\bar{z}}$	$3^2_{\bar{x}yz}$	$2_{\bar{x}y}$	4_z	4_z^3	2_{xy}
M_1					M_4	M_4	M_3	M_3
M_2					M_3	M_3	M_4	M_4
M_3	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1
M_4	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2

	$2_{\bar{y}z}$	2_{yz}	4_x	4_x^3	$2_{\bar{x}z}$	4_y^3	2_{xz}	4_y
M_1					M_1	M_2	M_1	M_2
M_2					M_2	M_1	M_2	M_1
M_3	M_3	M_3	M_4	M_4				
M_4	M_4	M_4	M_3	M_3				

$j=1, 2, \ldots, m^\alpha$ will denote the coset representatives of equation (4) when $X=R \cap P^\alpha$. We then determine for each α a rotation R^α such that $R^\alpha M_1^\alpha = M_1^\alpha$. The set $\{R(\alpha j, \beta k)\}$ of all rotations which rotate a molecule of the orientation M_j^α into the orientation of a molecule of the orientation M_k^β is then given, in terms of P^1, R^α, and R_j^α as follows:

$$\{R(\alpha j, \beta k)\}=\{R_k^\beta R^\beta P^1 (R_j^\alpha R^\alpha)^{-1}\}. \qquad (10)$$

3. The rotation function

The Patterson function of a molecular crystal consists of two parts: sets of *self-vectors* representing the inter-

atomic distances of atoms of each of the molecules, and *cross-vectors* representing interatomic distances of atoms on different molecules. The symmetry and orientation of each set of self-vectors is related to the symmetry and orientation of the corresponding molecule: The number of distinct sets of self-vectors is equal to the number of distinct molecular orientations of molecules in the molecular crystal. If the symmetry point group of a molecule is P, a group of proper rotations, then the symmetry point group of the corresponding set of self-vectors is P × I, since the inversion I is always a symmetry element of the Patterson function. (As our interest is in the structure of proteins and protein aggregates, and as proteins consists only of levo amino acids, rotations of P, of the point group of F, and of rotations R corresponding to peaks of the rotation function defined below will be proper rotations.) In addition, if the orientations of two molecules are related by a rotation R then the orientations of the corresponding sets of self-vectors are also related by the same rotation R.

Because of these relationships between the symmetry of the molecules and the symmetry of the corresponding sets of self-vectors, and between the relative orientation of two molecules and the relative orientation of the corresponding sets of self-vectors, the rotation function $\mathscr{R}(R)$ has been defined (Rossmann & Blow, 1962) to determine from the Patterson function of molecular crystals the symmetry point group and relative orientation of molecules in molecular crystals. In the case of a molecular crystal consisting of identical molecules, the rotation function is defined as

$$\mathscr{R}(R)=\int_V P(\mathbf{x})P(R\mathbf{x})d\mathbf{x}, \qquad (11)$$

the integral of the product of a Patterson function $P(\mathbf{x})$ with a rotated image of itself. The integration is over a volume V about the origin, chosen as to minimize contributions from cross-vectors. The rotation function $\mathscr{R}(R)$ will have peaks for those values of R corresponding to rotations which leave a set of self-vectors invariant or rotate a set of self-vectors into the orientation of some other set of self-vectors.

We shall now determine the set $\{R\}$ of rotations R corresponding to all peaks of the rotation function $\mathscr{R}(R)$ of a molecular crystal consisting of a single simple molecular crystal [F; $M(\mathbf{r}_1)$], where the symmetry point group of the molecule $M(\mathbf{r}_1)$ is P. The sets of rotations $\{R(jk)\}$, $j,k=1, 2, \ldots, m$, see equation (9), consist of all rotations which leave a molecule of a simple molecular crystal invariant or rotate the molecule into the orientation of some other molecule of the simple molecular crystal. Because of the correspondence between the symmetry and orientation of molecules of a molecular crystal and the symmetry and orientation of the corresponding sets of self-vectors of the molecular crystal's Patterson function, the set $\{R\}$ of rotations corresponding to all peaks of

the rotation function of a simple molecular crystal is

$$\{R\} = [\{R(jk)\}|, j,k = 1, 2, \ldots, m], \qquad (12)$$

the set of distinct rotations contained in the $|P|m^2$ rotations $\{R(jk)\}, j,k = 1, 2, \ldots, m$.

In the example of the previous section, the set of 64 rotations $\{R(jk)\}, j,k = 1, 2, 3, 4$ contained 24 distinct rotations, rotations of the point group O (432). In general, the set of rotations $\{R\}$ of equation (12) does not constitute a group, as will be seen in the example of § 4. In the case of a molecular crystal consisting of identical molecules and more than a single simple crystal, the sets of rotations given in equation (10) are used in place of those of equation (9), and the rotations corresponding to all peaks of the rotation function are found in the same manner as above.

A second problem which we shall investigate is how to determine from rotation function data information on the point-group symmetry and orientation of molecules in a molecular crystal. Consider a molecular crystal consisting of a single simple molecular crystal [F; $M(\mathbf{r}_1)$], and let $\{R\}$ denote the set of rotations corresponding to all peaks of the rotation function $\mathscr{R}(R)$ of this simple molecular crystal. Let P be a subset of $\{R\}$ which constitutes a group and contains as a subgroup the site point group $R(\mathbf{r}_1)$. P will be called a *possible symmetry point group* of $M(\mathbf{r}_1)$ if equation (12) is satisfied, *i.e.* if the set of rotations $[\{R(jk)\}j,k = 1, 2, \ldots, m]$, where $\{R(jk)\}$ is defined by equation (9), is identical with the set of rotations $\{R\}$. With the knowledge of the symmetry point group P of $M(\mathbf{r}_1)$ and the space group F one can then determine the orientation of all molecules of the simple molecular crystal [F; $M(\mathbf{r}_1)$]. From each possible symmetry point group P one derives then a *possible model* of the simple molecular crystal. A possible model of a molecular crystal is to be understood only as a model of the molecular crystal which specifies the symmetry point group and orientation of the molecules and which gives rise to a rotation function with peaks corresponding to a given set of rotations. Additional information on, *e.g.*, the structure of the molecule or on the relative peak heights, can as will be seen below, place additional conditions on the admissibility of such possible models as actually representing the real molecular crystal.

A simple molecular crystal [F; $M(\mathbf{r}_1)$] can be generated by F from any molecule $M(\mathbf{r}_i)$ of the simple molecular crystal. The position vector $\mathbf{r}_1$ in [F; $M(\mathbf{r}_1)$] has been taken as an arbitrary position vector of a molecule in the primitive unit cell of the simple molecular crystal. Because of this arbitrariness in the choice of the position vector $\mathbf{r}_1$ two possible models of a simple molecular crystal corresponding to two possible symmetry point groups P_1 and P_2 will be identical if P_1 and P_2 belong to the same equivalence class of point groups and are such that $RP_1R^{-1} = P_2$ where R is a rotation of the point group of the space group F. That is, a simple molecular crystal [F; $M(\mathbf{r}_1)$]

where P_1 is the symmetry point group of $M(\mathbf{r}_1)$ contains in its primitive unit cell a molecule $M(\mathbf{r}_j)$ whose symmetry point group is P_2; and the simple molecular crystal [F; $M(\mathbf{r}_1)$] can be alternatively denoted by [F; $M(\mathbf{r}_j)$]. Consequently, the number of distinct possible models of a simple molecular crystal will in general be less than the number of possible symmetry point groups.

In the case of a molecular crystal consisting of more than a single simple molecular crystal, the same method is used. However, the sets of rotations $\{R(jk)\}$ of equation (9) are replaced by the sets of rotations $\{R(\alpha j, \beta k)\}$ of equation (10).

As an example of this we consider the rotation function data of bovine liver catalase. X-ray diffraction studies have shown that the catalase molecule has at least one twofold axis of symmetry (Glauser & Rossmann, 1966; Gurskaya, Labanova & Vainshtein, 1971). Electron microscopy studies (Barynin & Vainshtein, 1971) indicate that the catalase molecule consists of four subunits and has the symmetry point group D_2 (222). A rotation function study of the trigonal crystalline form of catalase was then performed to determine the symmetry point group of the catalase molecule (Eventoff & Gurskaya, 1975).

Trigonal crystalline catalase is a single simple molecular crystal [F; $M(\mathbf{r}_1)$] whose space-group symmetry is $F = D_3^4$ ($P3_121$) and where $\mathbf{r}_1$ is a general position vector. The rotation function data shows that a set of rotations $\{R\}$ corresponding to peaks of the rotation function constitute the point group O (432) (Eventoff & Gurskaya, 1975). In Fig. 2 we give the cubic coordinate system in which the rotations of $\{R\}$ and of the space group F will be taken, and the relation of the trigonal coordinate system with respect to this cubic coordinate system. The molecular position vectors of the six molecules in the primitive unit cell will be denoted by

$$\mathbf{r}_1$$
$$\mathbf{r}_2 = (3^2_{xyz} | 2\tau)\mathbf{r}_1$$
$$\mathbf{r}_3 = (3_{xyz} | \tau)\mathbf{r}_1$$
$$\mathbf{r}_4 = (2_{\bar{x}y} | 0)\mathbf{r}_1$$
$$\mathbf{r}_5 = (2_{\bar{y}z} | 2\tau)\mathbf{r}_1$$
$$\mathbf{r}_6 = (2_{\bar{x}z} | \tau)\mathbf{r}_1$$

where τ is the non-primitive translation associated with the threefold rotation of the space group $F = D_3^4$ ($P3_121$). We assume that the set of rotations $\{R\} = O$ (432) corresponds to all peaks of the rotation function, and now determine all possible models of trigonal crystalline catalase. There are 30 groups P contained in the set of rotations $\{R\} = O$ (432). Of these, six groups $D_3^{(xyz)}$, $C_3^{(xyz)}$, $C_2^{(xy)}$, $C_2^{(xz)}$, $C_2^{(yz)}$ and C_1 give rise to a set of rotations $[\{R(jk)\}|j,k = 1, 2, \ldots, 6] = D_3^{(xyz)}$ and can be eliminated as possible symmetry point groups of the molecule $M(\mathbf{r}_1)$. One finds that not all of the 24 possible models of the simple molecular crystal determined from the remaining possible symmetry point groups P are distinct. One finds that there are

only ten distinct possible models corresponding to the possible symmetry point groups:

$$O; \; T; \; D_3^{(\overline{xyz})}; \; C_3^{(\overline{xyz})}; \; D_4^{(x)}; \; C_4^{(x)};$$

$$D_2^{(x,y,z)}; \; D_2^{(x,yz,\overline{yz})}; \; C_2^{(x)}; \; C_2^{(xy)}.$$

The possible point group $P = C_2^{(y)}$ does not give rise to an additional distinct possible model, since taking $P = C_2^{(y)}$ as the symmetry point group of $M(\mathbf{r}_3)$, $\mathbf{r}_3 = (3_{xyz} | \tau) \mathbf{r}_1$, gives rise to a possible model identical with the possible model found by taking $P = C_2^{(x)}$ as the symmetry point group of $M(\mathbf{r}_1)$.

The catalase molecule consists of four subunits (Barynin & Vainshtein, 1971), and, following Eventoff & Gurskaya (1975), we assume that the subunits have no intrinsic rotational symmetry other than the trivial identity rotation. Consequently, we eliminate as possible models of the molecular crystal those models corresponding to all possible symmetry point groups not belonging to the classes of point groups $C_2(2)$, $D_2(222)$, and $C_4(4)$. The remaining possible models are those with the possible symmetry point groups:

$$C_4^{(x)}; \; D_2^{(x,y,z)}; \; D_2^{(x,yz,\overline{yz})}; \; C_2^{(x)}; \; C_2^{(xy)}. \qquad (13)$$

Three of these possible models, those corresponding to the possible symmetry point groups $C_4^{(x)}$, $D_2^{(x,y,z)}$, and $C_2^{(x)}$ have been considered by Eventoff & Gurskaya (1975).

In the case of $P = D_2^{(x,yz,\overline{yz})}$ the molecules of the simple molecular crystal are of three distinct orientations; these orientations and the orientations of the molecules in the primitive unit cell are:

$$M(\mathbf{r}_1) = M(\mathbf{r}_5) = M_1$$
$$M(\mathbf{r}_2) = M(\mathbf{r}_6) = M_2 = 3_{xyz}^3 M_1$$
$$M(\mathbf{r}_3) = M(\mathbf{r}_4) = M_3 = 3_{xyz} M_1 .$$

In the remainder of the cases there are six distinct orientations:

$$M(\mathbf{r}_1) = M_1$$
$$M(\mathbf{r}_2) = M_2 = 3_{xyz}^2 M_1$$
$$M(\mathbf{r}_3) = M_3 = 3_{xyz} M_1$$
$$M(\mathbf{r}_4) = M_4 = 2_{\overline{xy}} M_1$$
$$M(\mathbf{r}_5) = M_5 = 2_{\overline{yz}} M_1$$
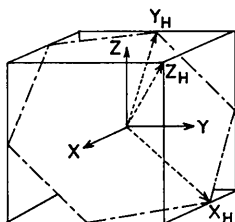$$M(\mathbf{r}_6) = M_6 = 2_{\overline{xz}} M_1 .$$

Fig. 2. The hexagonal coordinate system (X_H, Y_H, Z_H) of the bovine liver catalase molecular crystal is shown relative to the cubic coordinate system (X, Y, Z) in which are defined the rotations corresponding to the peaks of the associated rotation function.

A method to distinguish between the five possible models corresponding to the five possible symmetry point groups P given in equation (13) is as follows: There are two contributions to a peak of the rotation function $\mathscr{R}(R)$ and we shall write:

$$\mathscr{R}(R) = \mathscr{R}^s(R) + \mathscr{R}^k(R) .$$

$\mathscr{R}^s(R)$ is the contribution due to overlapping of sets of self-vectors, and $\mathscr{R}^k(R)$ is the contribution due to overlapping of cross-vectors of the Patterson function of the molecular crystal. In general, the volume of integration V in equation (11) cannot be chosen as to eliminate completely all contributions $\mathscr{R}^k(R)$. For a crystallographic rotation R_c, a rotation of an element of the space group F of the molecular crystal, since for every cross-vector $\mathbf{k}$ there exists a cross-vector $R_c\mathbf{k}$, one anticipates a non-negligible contribution $\mathscr{R}^k(R_c)$ to $\mathscr{R}(R_c)$. However, for non-crystallographic rotations R_{nc}, rotations corresponding to peaks of the rotation function but not a rotation of an element of the space group F of the molecular crystal, and a cross-vector $\mathbf{k}$, there is in general no cross-vector $R_{nc}\mathbf{k}$. Consequently we shall assume that $\mathscr{R}^k(R_{nc})$ can be neglected with respect to $\mathscr{R}^s(R_{nc})$. That is, we assume

$$\mathscr{R}(R_c) = \mathscr{R}^s(R_c) + \mathscr{R}^k(R_c)$$

and:

$$\mathscr{R}(R_{nc}) = \mathscr{R}^s(R_{nc}) .$$

The ratio $\mathscr{R}(R_c)/\mathscr{R}(R_{nc})$ can be experimentally determined as this represents the ratio of peak heights. Since $\mathscr{R}^s(R)$ is proportional to the number of molecules of the molecular crystal invariant under R or rotated into the orientation of some other molecule by the rotation R, the ratio $\mathscr{R}^s(R_c)/\mathscr{R}(R_{nc})$ can be calculated from a possible model of the structure of the molecular crystal. Therefore one can determine the ratio of the two contributions to $\mathscr{R}(R_c)$:

$$\frac{\mathscr{R}^k(R_c)}{\mathscr{R}^s(R_c)} = \frac{\mathscr{R}(R_c)/\mathscr{R}(R_{nc}) - \mathscr{R}^s(R_c)/\mathscr{R}(R_{nc})}{\mathscr{R}^s(R_c)/\mathscr{R}(R_{nc})} . \qquad (14)$$

In the case of catalase, the crystallographic rotations R_c are the rotations of the group $D_3^{(xyz)}$, and R_{nc} are rotations $O - D_3^{(xyz)}$. From Table 2 of Eventoff & Gurskaya (1975) one finds that $\mathscr{R}(R_c)/\mathscr{R}(R_{nc}) \simeq 3$. One has for the possible models corresponding to $P = C_4^{(x)}$ and $D_2^{(x,y,z)}$ that $\mathscr{R}^s(R_c)/\mathscr{R}(R_{nc}) = 1$, and therefore, from equation (14) that $\mathscr{R}^k(R_c)/\mathscr{R}^s(R_c) \simeq 2$. For the possible models corresponding to $P = D_2^{(x,yz,\overline{yz})}$, $C_2^{(x)}$, and $C_2^{(xy)}$, one finds that $\mathscr{R}^s(R_c)/\mathscr{R}(R_{nc}) = 3$, and from equation (14) that $\mathscr{R}^k(R_c)/\mathscr{R}^s(R_c) \simeq 0$. Since we have assumed that $\mathscr{R}^k(R_c)$ is not negligible relative to $\mathscr{R}^s(R_c)$, these calculations indicate that the only possible models which are admissible are the two corresponding to the possible symmetry point groups $C_4^{(x)}$ and $D_2^{(x,y,z)}$, in agreement with the conclusions of Eventoff & Gurskaya (1975).

4. Satellite tobacco necrosis virus

In practice, due to the quite considerable computation time, not all peaks of the rotation function of a molecular crystal are calculated from the experimental data. Consequently not all peaks of the rotation function are known and the procedure of § 3 to determine the symmetry point group and orientation of molecules in a molecular crystal cannot be applied. Instead, using the incomplete available data one can use the following procedure: Based on the available data and possibly additional criteria, one chooses a symmetry point group P and with the knowledge of the symmetry space group F and molecular position vectors, one constructs a model of the molecular crystal. From this model one then calculates, using the method of § 3, the set of all peaks of the corresponding rotation function. These peaks are then compared with the incomplete available data. For this model to be a possible model of the molecular crystal, the set of calculated peaks must include all known peaks, and in general will include additional peaks. It must then be determined if these additional peaks are in fact contained in the rotation function of the actual molecular crystal. If so, one concludes that the model is a possible model of the molecular crystal. In this section we will consider as an example of such a procedure the analysis of the rotation function data of crystalline satellite tobacco necrosis virus (STNV), a small 'spherical' virus consisting of a core of nucleic acid and a polyhedral protein shell. The symmetry point group of the virus is assumed to be the symmetry point group of the protein shell.

Crick & Watson (1956) proposed that the protein shell of small 'spherical' viruses consist of a number of identical subunits in a structure of cubic point-group symmetry. Casper & Klug (1966) concluded that icosahedral point-group symmetry was preferred, and this conclusion has been confirmed by electron microscopic and X-ray diffraction studies on small viruses (Klug, Longley & Leberman, 1966).

A rotation function analysis of 15 Å X-ray diffraction data of STNV crystals was performed by Akervall et al. (1971a) and interpreted to imply a cubic point-group symmetry of the STNV molecule. These results were reinterpreted (Klug, 1971; Akervall et al., 1971b) as being consistent with icosahedral point group symmetry of the STNV molecule. In these rotation function studies of crystalline STNV the concluded models were based on peaks of the rotation function associated with the anticipated icosahedral symmetry of the STNV molecule and additional peaks corresponding to rotations not among the rotations of the icosahedral point group. However, this analysis was based on only limited rotation function data, as all peaks of the rotation function were not experimentally determined. While the concluded model indicating icosahedral point-group symmetry of the STNV molecules does give rise to all of the known peaks of the

rotation function, no attempt was made to calculate all peaks of the rotation function corresponding to this model and then to determine the existence of these peaks. We will now calculate all such peaks.

The space group of monoclinic crystalline STNV is $F = C2$ (C_2^3) a base-centred monoclinic space group with the twofold rotation axis along the b direction. The generators of this space group are $\left(E \mid \frac{a}{2} \frac{b}{2} 0\right)$, $\left(E \mid \frac{a}{2} \frac{-b}{2} 0\right)$, $(E \mid 0 0 c)$, and $(2_b \mid 0 0 0)$ where (Rossmann, Akervall, Lentz & Strandberg, 1973): $a = 319$, $b = 304$, $c = 185$ Å, and $\beta = 94°22'$, and where the primitive unit cell contains two molecules whose molecular position vectors are $+(0.23a, 0, 0.26c)$. The crystalline STNV then consists of a single simple molecular crystal $[F; M(\mathbf{r}_1)]$ whose symmetry space group is $F = C2$ and where $\mathbf{r}_1$ is a general position vector.

The rotation function analysis of Akervall et al. (1971a,b) determined a set of peaks which contained two subsets of peaks corresponding to rotations of icosahedral point groups J (532), and a single subset of peaks corresponding to rotations of the cubic point group O (432). These subsets of peaks are not mutually exclusive, and in what follows we will refer to these subsets of peaks as, respectively, the icosahedral peaks and the cubic peaks. These results point to

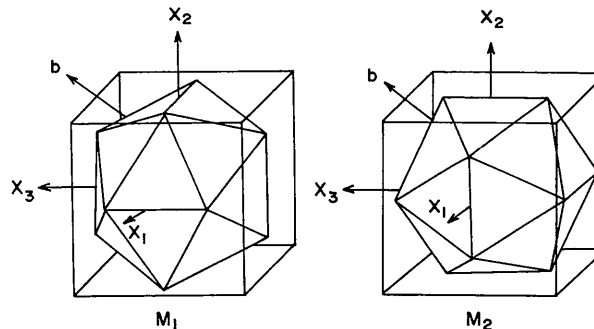(1) An icosahedral symmetry point group of the STNV molecules.

Fig. 3. The two distinct molecular orientations of icosahedral molecules in a model of crystalline STNV relative to the cubic coordinate system (X_1, X_2, X_3). The peaks of the rotation function corresponding to fourfold rotations are along the X_1, X_2, and X_3 axes. The crystallographic b axis is also shown.
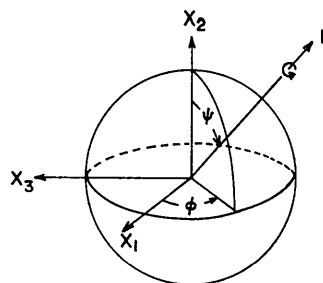

Fig. 4. The polar coordinates ψ and φ which specify the direction of the rotation axis of a rotation through an angle κ.

(2) Two distinct molecular orientations in the primitive unit cell related by the crystallographic twofold rotation about the b axis.

Since there is only one subset of peaks corresponding to rotations of the point group T (32) and this subset of peaks is common to both subsets of icosahedral peaks and the subset of cubic peaks, the orientation of the two distinct molecular orientations relative to the cubic peaks is determined. These two orientations of the icosahedral molecules are denoted by M_1 and $M_2 = 2_b M_1$ and are shown in Fig. 3 relative to a cube whose symmetry axes are the directions of the cubic peaks. The b axis, about which a twofold rotation interchanges the two distinct molecular orientations, is also shown. The orientation of these molecules with respect to the crystallographic a and c axes is not shown, but may be determined from the orientation of the cubic peaks relative to these axes (Akervall *et al.*, 1971*a,b*).

All peaks of the rotation function corresponding to this model of crystalline STNV correspond to the distinct rotations in the sets of rotations $\{R(jk)\}$ $j,k = 1, 2$ where $R_1 = E$, $R_2 = 2_b$ and where P is the icosahedral point group J (532) of the molecule M_1 of Fig. 3. The rotations of $\{R(11)\}$, rotations through an angles κ about rotation axes defined by the spherical coordinates ψ and φ, see Fig. 4, are listed in Table 3 in terms of (κ, ψ, φ), and are the 60 rotations of the point group P. The set of 60 rotations $\{R(12)\}$ rotations R such that $RM_1 = M_2$ are listed in Table 4. The set of rotations $\{R(21)\}$ are derived from Table 4 by replacing κ by $-\kappa$, and the rotations of $\{R(22)\}$ from Table 3 by replacing φ by $\varphi + 90°$.

Of the 240 rotations, there are 216 distinct rotations, all rotations excepting those belonging to the cubic point group O (432) appear once, while the cubic rotations all appear twice. In Fig. 5 a stereographic projection is given showing the orientation of the axes of rotation corresponding to these 216 rotations. Note that the four axes of the threefold cubic rotations are also the axes of rotation of those rotations where

Table 3. *The set of sixty rotations* $\{R(11)\}$

These rotations leave invariant icosahedral molecules of orientation M_1 shown in Fig. 3.

κ	ψ	φ
0	0	0
72	31·72	0
144	58·28	±90
216	90	±31·76
288	148·28	0
180	0	0
	36	±58·28
	60	±20·91
	72	±58·28
	90	0
	90	90
	108	±58·28
	120	±20·91
	144	±58·28
120	20·91	±90
240	54·74	±45
	69·09	0
	90	±69·09
	110·91	0
	125·26	±45

Table 4. *The set of sixty rotations* $\{R(12)\}$

These rotations rotate icosahedral molecules of orientation M_1, see Fig. 3, into molecules of orientation M_2.

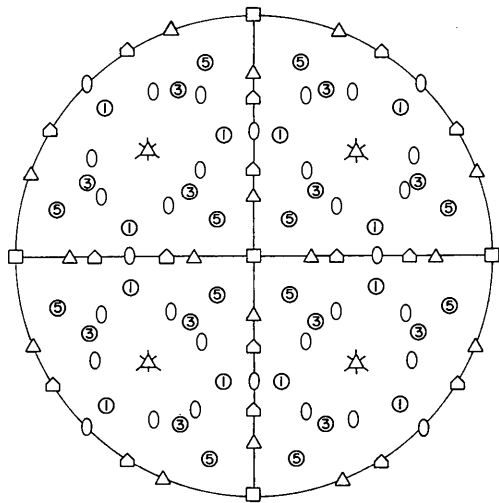| κ	ψ	φ
44·48	54·74	±45
75·52	125·26	±45
164·48		
90	0	0
270	90	0
	90	90
110·21	45·78	±13·28
	45·78	±76·72
	80·52	±45
	99·47	±45
	134·22	±13·28
	134·22	±76·72
138·59	32·31	±45
	67·79	±24·09
	67·79	±65·91
	112·21	±24·09
	112·21	±65·91
	147·69	±45
154·76	18·46	±45
	77·06	±13·28
	77·06	±76·72
	102·94	±13·38
	102·94	±76·72
	161·53	±45
180	45	0
	45	±90
	90	±45
	135	0

Fig. 5. Stereographic projection of the rotation axes of the rotations corresponding to all peaks of the rotation function of a model of crystalline STNV. 0, △, □, and ⬠ denote, respectively, two, three, four, and fivefold rotation axes. The threefold rotation axes denoted by ⚹ are also rotation axes of rotations through $\kappa = \pm 44·48°$, $\pm 75·52°$, $\pm 164·48°$. ①,③,⑤ represent rotation axes of rotations through the angles of, respectively, $\kappa = \pm 110·21°$, $\pm 138·59°$, and $\pm 154·76°$.

$\kappa = \pm 44\cdot48°$, $\pm 75\cdot52°$, and $\pm 164\cdot48°$. Most of the peaks calculated here on the basis of this proposed model of crystalline STNV have been determined from the rotation function data (Akervall *et al.*, 1971*a,b*). However, the 72 peaks corresponding to $\kappa = \pm 110\cdot21°$, $\pm 138\cdot59°$, and $\pm 154\cdot76°$ have as yet to be experimentally verified.

The misinterpretation of the rotation function data of STNV by Akervall *et al.* (1971*a*) was based on the two following erroneous assumptions:* The initial assumption was that if the symmetry point group of the STNV molecule was icosahedral, then only peaks corresponding to two, three, and fivefold rotations would be allowed. Then when a set of strong peaks corresponding to rotations of the cubic point group were found, the assumption that these peaks contradicted the possibility of a icosahedral symmetry point group of the STNV molecule. As we have shown above, in addition to peaks of the rotation function corresponding to rotations of the icosahedral point group, one anticipates additional peaks, and among these additional peaks is a set corresponding to rotations of the cubic point group O (432). Further, using the same considerations concerning relative peak heights as formulated in the previous section, one also anticipates strong peaks corresponding to the cubic rotations. In the set of 240 rotations derived above, each of the cubic rotations appears twice while all other rotations appear only once. Consequently, the peak height of any peak corresponding to a cubic rotation, excluding the crystallographic twofold rotation about the b axis, is twice the height of any peak corresponding to a rotation not in this cubic point group. This fact has been confirmed by Akervall *et al.* (1971*b*). (However, we note that in a recent rotation function study of crystalline STNV, Lentz & Strandberg (1974) found 'no obvious correlation of peak height with the symmetry type of axis'.)

Klug (1971) and Akervall *et al.* (1971*b*) reinterpreted the Akervall *et al.* (1971*a*) data to indicate a icosahedral symmetry point group of the STNV molecules, and explained the strong rotation function peaks corresponding to the point group O (432) as being due to rotations which relate the orientations of the two icosahedral STNV molecules in the primitive unit cell of the molecular crystal. However, this reinterpretation is based on an argument which approximates the STNV molecular crystal as being of cubic space-group symmetry, in particular, as being a crystal of space-group symmetry $F = O^6$ ($P4_332$). Moreover, it was concluded that the analysis could have been carried out in a 'logical' order by starting from the point that the rotation function data indicated the 'approximate' crystal class O (432) of the STNV molecular crystal.

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* It has been pointed out by one of the referees of this paper that with the present knowledge of data collection and computational procedures (data cut-off limits) (see Lentz & Strandberg, 1974) this misinterpretation of the rotation function data of STNV could have been avoided.

While we have shown that the peaks corresponding to the cubic rotations are related to rotations which either leave a molecule invariant or rotate the orientation of one molecule into the orientation of the other molecule in the primitive unit cell of the molecule crystal, at no point is the crystallographic symmetry of the STNV molecular crystal approximated by a cubic space group. Such an approximation procedure is not necessary and in this case is incorrect as it has been shown that the cubic peaks are due to the relative orientation of the two STNV molecules in the primitive unit cell of a molecular crystal of monoclinic space-group symmetry.

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