# On a Physical Interpretation of Direct-Methods Relationships

By A. F. Mishnev and S. V. Belyakov

*Institute of Organic Synthesis, Latvian Academy of Sciences, 226006 Riga, Latvia*

## Abstract

On the basis of Laue's dynamical theory of X-ray diffraction the non-negativity condition for the Karle-Hauptman determinant is obtained. For the three-beam case the validity of the triplet phase relation of direct methods is displayed. The unitary relation and dynamical X-ray scattering amplitude from crystals are used to derive a Sayre-type equation, which for identical atoms reduces to Hughes's equation. These results allow the interpretation of relations between structure amplitudes in direct methods on the basis of dynamical theory.

## Introduction

The theory of direct methods (DM) has proved very successful in crystal-structure determination since its development in the early 1950s. There are now a great number of papers on DM in the literature. The most fundamental contributions to the theory of DM are discussed in the review by Woolfson (1987), in which the evolution of DM is examined 'from birth to maturity'. The theory of DM is purely mathematical. Therefore the physical meaning of DM relationships is an important problem.

Let us consider the basic assumptions forming the foundations of DM. Firstly, it is a hypothesis of the atomicity of matter. The real crystal, with continuous electron density $\rho(\mathbf{r})$, is replaced by an ideal one, the unit cell of which consists of $N$ discrete non-vibrating point atoms located at the maxima of $\rho(\mathbf{r})$ (Hauptman, 1986). This allows, instead of the structure factor $F(\mathbf{H})$, the use of the normalized structure factors $E(\mathbf{H})$ and $U(\mathbf{H})$. The second principle, the condition of non-negativity of the electron density function, is exploited in determinantal inequalities (Karle & Hauptman, 1950; Goedkoop, 1950) and also in the derivation of Sayre's equation (Sayre, 1952). Thirdly, the main assumption of DM is that the values of $xh + yk + zl$ are uniformly distributed on a trigonometric circle, where $h$, $k$ and $l$ may take any integer values for fixed $x$, $y$ and $z$ or $x$, $y$ and $z$ may run over all values in the unit cell for given integers $h, k, l$ (Kitaigorodskii, 1961). The presence of a crystal as a scattering object is taken into account in DM through integer values of Miller indices.

Since DM use a geometrical structure factor and its modifications it is of no importance how intensities are measured and how the $|F(\mathbf{H})|$ are calculated from them. Additionally, if DM do not use any assumptions about the size and perfection of a crystal then they are also valid for a large ideal crystal. Therefore we can use dynamical theory more generally even though DM are used mainly for the structure determination of crystals under conditions of kinematical X-ray diffraction. In this paper an attempt is made to derive some fundamental relationships of DM and to explain them on the basis of the dynamical theory of X-ray diffraction.

## Derivation of DM relations on the basis of dynamical theory

Up to now the interpretation of phase relations has been limited to a graphical explanation by the consideration of electron density waves in a crystal (Golovastikov & Belov, 1955; Stothard, 1978; Schenk, 1979, 1981). Such a geometrical approach gives a visible result but in no way represents the real process of X-ray diffraction in a crystal.

It is known that the most important relation used in DM is the triplet or $\Sigma_2$ relation

$$\varphi(\mathbf{H}_1) + \varphi(\mathbf{H}_2) + \varphi(\mathbf{H}_3) \simeq 0 \qquad (1)$$

for large values of $E_3 = N^{-1/2}|E(\mathbf{H}_1)E(\mathbf{H}_2)E(\mathbf{H}_3)|$ with the condition

$$\mathbf{H}_1 + \mathbf{H}_2 + \mathbf{H}_3 = 0. \qquad (2)$$

Relation (1) specifies a three-phase structure invariant as it is independent of the choice of origin. At the same time, (2) is a condition for double diffraction or the three-beam case in dynamical X-ray diffraction theory (Pinsker, 1978; Chang, 1984). The analogy between triplet relations of DM and three-beam dynamical diffraction was noticed by Schagen, Schenk & Post (1981) and recently was used to calculate the number of triplet relations (Mishnev & Belyakov, 1992).

In the process of double scattering by a crystal, two different crystallographic planes $\mathbf{H}_1$ and $\mathbf{H}_2$ are simultaneously in a reflecting position. At the same time, a beam, reflected from plane $\mathbf{H}_2$ hits another plane where the Bragg condition is again fulfilled and a double-reflected beam arises (from plane $\mathbf{H}_3$), which coincides in direction with the beam from plane $\mathbf{H}_1$. Moreover, the indices of the reflection planes are

related by condition (2). Fig. 1, taken from the paper of Post (1979), illustrates the double-reflection geometry in direct and reciprocal space. The three-beam case can always be realized by rotating a crystal around the reciprocal vector $H_1$.

The fundamental equations of the dynamical theory of X-ray diffraction on crystals were given by von Laue (1960):

$$[(k_H^2 - k^2)/k_H^2]D_H = \sum_L \chi_{H-L} D_L, \qquad (3)$$

where $k$ and $k_H$ are the wave numbers of the incident and reflected beams, $\chi_{H-L} = -e^2 F(H-L)/(\pi m \nu^2 V_0)$ are coefficients in the expansion of the dielectric susceptibility in a Fourier series, $\nu$ is the frequency of the X-rays in vacuum, $V_0$ is the unit-cell volume, $D_H$ is the electric displacement and $F(H-L)$ is a structure amplitude. If polarization is not considered the condition for nontrivial solution of the fundamental equations (3) is

$$\begin{vmatrix} F(0)-\lambda & F(-H_1) & F(-H_2) & \cdots & F(-H_n) \\ F(H_1) & F(0)-\lambda & F(H_1-H_2) & \cdots & F(H_1-H_n) \\ F(H_2) & F(H_2-H_1) & F(0)-\lambda & \cdots & F(H_2-H_n) \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ F(H_n) & F(H_n-H_1) & F(H_n-H_2) & \cdots & F(0)-\lambda \end{vmatrix} = 0$$

(4)

where

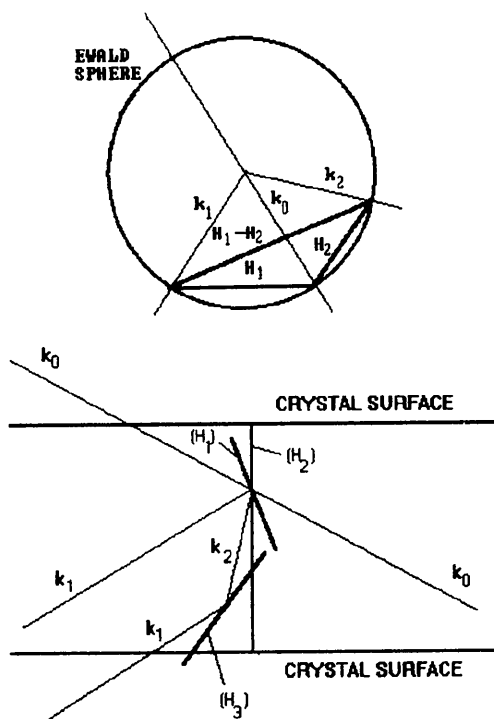$$\lambda = (\pi m \nu^2 V_0/e^2)[(k^2/k_H^2) - 1].$$



Fig. 1. Simultaneous three-beam diffraction in direct and reciprocal space.

It is clear that the values $\lambda$ are the eigenvalues of the Karle-Hauptman matrix $\|F(H_i - H_j)\|$. The ratio $k_H/k$ is a refractive index, which for X-rays is less than unity. Hence the eigenvalues $\lambda$ are positive. Since the determinant of a matrix is equal to the product of its eigenvalues,

$$\det \|F(H_i - H_j)\| = \prod_{k=1}^{n} \lambda_k, \qquad (5)$$

the Karle-Hauptman determinant cannot be negative. This confirms the result of Karle & Hauptman (1950). Thus one can interpret the non-negativity of the determinant (5) as a consequence of the X-ray refractive index being less than unity.

For the case of three strong waves, expanding the determinant (4) and denoting $F(0) - \lambda$ by $\tau$, we get a dispersion equation

$$\tau^3 - \tau[|F(H_1)|^2 + |F(-H_2)|^2 + |F(H_2 - H_1)|^2]$$
$$+ 2|F(H_1)F(-H_2)F(H_2-H_1)|$$
$$\times \cos[\varphi(H_1) + \varphi(-H_2) + \varphi(H_2-H_1)] = 0. \qquad (6)$$

Equation (6) represents a condition for a combination of three phases, the sum of indices of which is zero, i.e. it determines the value of the three-phase structure invariant. Unknown magnitudes of $\tau$ do not permit the use of (6) immediately for phase determination. Nevertheless, (6) can be used for qualitative confirmation of the triplet formula (1). To do this, let us rewrite (6) in the form

$$\cos \Phi_3 = \{\tau[|F(H_1)|^2 + |F(-H_2)|^2$$
$$+ |F(H_2-H_1)|] - \tau^3\}$$
$$\times [2|F(H_1)F(-H_2)F(H_2-H_1)|]^{-1} \qquad (7)$$

where $\Phi_3 = \varphi(H_1) + \varphi(-H_2) + \varphi(H_2-H_1)$. We shall fix the values of $|F(H_1)|$ and $\tau$ and map the function $\cos \Phi_3$ relative to $|F(-H_2)|/F(0)$ and $|F(H_2 - H_1)|/F(0)$. Since $|\cos \Phi_3| \leq 1$ one obtains a limited region of allowed values of variables. Fig. 2 shows the $\cos \Phi_3$ map for $\tau/F(0) = 0.4$ and $|F(H_1)|/F(0) = 0.3$. The symbols $-, K, H, \ldots, A, 0, \ldots, 9, +$ represent the values of $\cos \Phi_3$ from $-1$ to $+1$ in increments of 0.1. The boundary separating positive values (region $A$) from negative (region $B$) is an arc of the circle of radius $[\tau^2 - |F(H_1)|^2]^{-1/2}$ with center at the origin. Fig. 2 shows that for large magnitudes of structure factors $\cos \Phi_3 \cong 1$. If the sign of $\tau$ is reversed, the sign of $\cos \Phi_3$ in regions $A$ and $B$ will be changed too. However, it is easy to show from the non-negativity condition (5) that the case $\cos \Phi_3 \cong -1$ for large values of $|F(H)|$ cannot be realized. Thus the dynamical theory equations for strong reflections give a similar result to that of triplet relation (1) of DM.

Further evidence in favor of the suggested physical interpretation of DM is the direct derivation of a Sayre-type equation from the dynamical effects of

diffraction. It is well known that the unitary relation of classical electromagnetic theory (Saxon, 1955; Gerber & Karplus, 1972)

$$\tilde{A}(\mathbf{k}', \mathbf{k}) - \tilde{A}^{\dagger}(\mathbf{k}, \mathbf{k}')$$

$$= (ik/2\pi) \int \tilde{A}^{\dagger}(\mathbf{k}'', \mathbf{k}') A(\mathbf{k}'', \mathbf{k}) \, d\Omega \qquad (8)$$

is valid for the dynamical scattering amplitude but not for the kinematical one. In (8), $\tilde{A}(\mathbf{k}', \mathbf{k})$ is a scattering-amplitude tensor and the integration on the right-hand side is over all the directions of $\mathbf{k}''$, the length of this vector being kept fixed at the value $|\mathbf{k}''| = |\mathbf{k}'| = |\mathbf{k}|$. Gerber & Karplus (1973) suggested that the X-ray scattering amplitude of a crystal be written so that the radiation reaction of free electrons was accounted for. In this case the scattering amplitude for a crystal can be written in the following approximation, which satisfies the unitary relation.

$$\tilde{A}(\mathbf{k}', \mathbf{k}) = \begin{pmatrix} \dfrac{-e^2 \cos 2\Theta}{(1+iq)mc^2} & 0 \\ 0 & \dfrac{-e^2}{(1+iq)mc^2} \end{pmatrix} U(\mathbf{k}', \mathbf{k}), \qquad (9)$$

where $\Theta$ is the Bragg angle, $q = 4\pi\nu e^2/3mc^3$, $U(\mathbf{k}', \mathbf{k})$ is a unitary structure amplitude. The Bragg condition $\mathbf{k}' - \mathbf{k} = 2\pi\mathbf{H}$ leads to the dependence of amplitude only on the difference of wave vectors and we have $U(\mathbf{k}', \mathbf{k}) = U(\mathbf{H})$. Substituting the expression for $A_{22}(\mathbf{k}', \mathbf{k})$ into (8) after renormalization we obtain the Sayre-type equation

$$U(\mathbf{H}) = \left[ U(0) \bigg/ \sum_{\mathbf{K}} |U(\mathbf{K})|^2 \right] \sum_{\mathbf{K}} U(\mathbf{K}) U(\mathbf{H} - \mathbf{K}), \qquad (10)$$
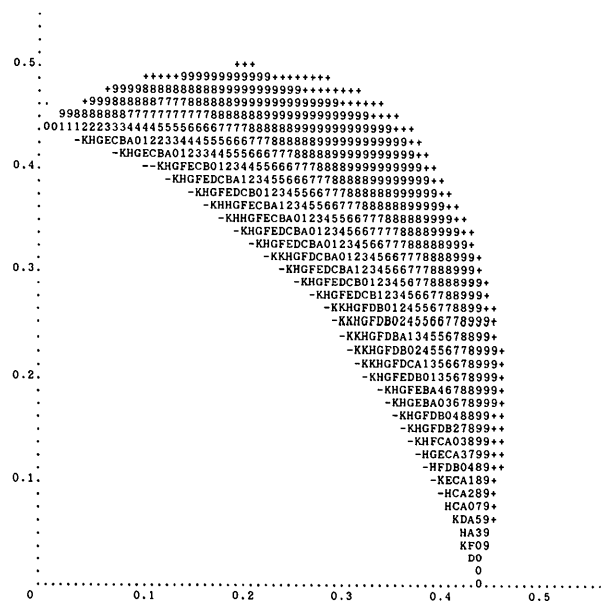
where $U(\mathbf{H})$ corresponds to the primary, $U(\mathbf{K})$ the secondary and $U(\mathbf{H} - \mathbf{K})$ the cooperative reflection. The summation in (10) is performed over all possible simultaneous reflections for a given primary reflection $U(\mathbf{H})$. For crystal structures with identical atoms $E(\mathbf{H}) = N^{1/2} U(\mathbf{H})$, then

$$\sum_{\mathbf{K}} |U(\mathbf{K})|^2 = n\langle E^2 \rangle / N,$$

where $N$ is the number of atoms in the unit cell and $n$ is the number of terms in the summation. For $\langle E^2 \rangle = 1$, (10) reduces to

$$E(\mathbf{H}) = N^{1/2} \langle E(\mathbf{K}) E(\mathbf{H} - \mathbf{K}) \rangle_{\mathbf{K}} \qquad (11a)$$

or

$$U(\mathbf{H}) = N \langle U(\mathbf{K}) U(\mathbf{H} - \mathbf{K}) \rangle_{\mathbf{K}}. \qquad (11b)$$

Equations (11a,b) correspond to Hughes's (1953) equation.

## Concluding remarks

In DM theory reflections are considered separately, which is consistent with the kinematical theory of diffraction. This corresponds to the assumption of the accidental nature of structure factors in DM. The necessity of the existence of relations between structure amplitudes is substantiated in DM theory by (1) the condition of non-negativity of the electron density function which imposes restrictions on the Fourier coefficients in the expansion of $\rho(\mathbf{r})$ and (2) the redundancy of the problem since the number of measured intensities by far exceeds the number of unknown coordinates.

In the present paper, Sayre-type equations and a non-negativity condition for the Karle–Hauptman determinant are derived on the basis of the dynamical theory of X-ray diffraction. Previously, these relationships were obtained by different methods. The approach presented allows a dynamical interpretation of phase relations of DM to be given. In the dynamical theory the existence of relations between structure amplitudes arises naturally by the interference of X-rays in a crystal. This effect is used in experimental methods for direct phase determination from multiple Bragg reflection. However, dynamical theory has not been used until now for the derivation of equations for structure amplitudes. It is possible that further use of dynamical theory will lead to new relations for the phase determination of structure amplitudes.



Fig. 2. The cos $\Phi_3$ map from the dispersion equation.

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# Unusual Diffraction of Type B Influenza Virus Neuraminidase Crystals

BY MING LUO*

*Center for Macromolecular Crystallography and Department of Microbiology,*
*University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294, USA*

W. GRAEME LAVER

*John Curtin School of Medical Research, Australia National University, Canberra, ACT 2601, Australia*

AND GILLIAN AIR

*Department of Microbiology, University of Alabama at Birmingham, University Station,*
*Birmingham, Alabama 35294, USA*

## Abstract

An unusual X-ray diffraction pattern by tetragonal crystals of a type B influenza virus neuraminidase was observed in that the odd-$l$ reflections were missing or diffuse while the even-$l$ reflections were sharp and strong. A statistical analysis showed that an error ($\varepsilon$) in the spacing of successive planes of neuraminidase molecules was randomly distributed along the $c$ direction, which resulted in such an unusual diffraction pattern. The error $\varepsilon$ follows the Bernoullian distribution and may be caused by a flexible loop on the top surface of the neuraminidase.

## Introduction

Neuraminidase (NA) is a glycoprotein found on the lipid envelope of the influenza virus. Upon virus attachment to the host cell receptor, the neuraminidase cleaves off terminal sialic acid residues from the polysaccharides of the cellular receptor *via* hydrolysis of the $\alpha$-ketosidic linkages. This cleavage of sialic acids from its receptor, its virus hemagglutinin and from mucin facilitates transportation of virions from the site of infection and the departure of the mature progeny virions (Burnet & Stone, 1947). The protein has a subunit molecular weight of 50 000 daltons and exists in a tetrameric form both on the viral surface and as free molecules in solution. The enzymatically active heads of NA (missing about 80 amino acids from the N terminus) can be removed from the viral surface by protease cleavage and subsequently purified for crystallization.

## The NA crystals

NA from type B influenza virus was crystallized in different crystalline forms according to Bossart, Babu, Cook, Air & Laver (1988), Lin, Luo, Laver, Air, Smith & Webster (1990) and Air, Laver, Luo, Stray, Legrone & Webster (1990). Among them, crystals from four different virus strains are tetragonal, body centered. Form I was grown with NA from the B/Hong-Kong/73 virus by vapor diffusion of hanging drops against 1.9 $M$ potassium phosphate. Crystals appeared as rectangular prisms. X-ray diffraction photographs were taken using radiation from both a Rigaku K200 rotating anode and the Cornell High-Energy Synchrotron Source (CHESS). By precession and cone-axis photography, the space group of form I was determined as $I422$, with unit-cell dimensions $a = b = 123.0$, $c = 165.0$ Å. Form II was grown with

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* To whom correspondence should be addressed.