The Determination of the First-Order Phase in Membrane Diffraction Using Electron Density Strip Models

BY C. R. WORTHINGTON
Departments of Biological Sciences and Physics, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213, USA

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Abstract

The problem of assigning the phase to the first-order reflection in membrane diffraction is treated. Biological membrane assemblies with two membranes per unit cell and the special case of lipid bilayers are treated. Phase assignment is dependent on the origin value of the minus fluid or the minus lipid model and on the actual asymmetry of the membranes. The conditions for phase assignment are derived from a study of single- and three-strip electron density models.

Introduction

The study of cell membranes by X-ray diffraction has provided information on the lamellar structure of certain membranes at moderate resolution. The case of multilayered membrane-type systems which contain fluid layers is first treated but the method (to be described) can also be used with lipid bilayers when it can be assumed that lipid chains in the central part of the bilayer have uniform electron density. If the multilayered specimen contains a number of unit cells with a well defined repeating unit, the diffraction pattern will show a series of discrete reflections. The integrated intensities are experimentally measured for each order of diffraction. In order to interpret the low-angle X-ray diffraction intensities in terms of lamellar structure, knowledge of the Fourier transform of the unit cell is required. The assignment of the phase of the first-order reflection can often be made on considerations of density alone. The validity of this assignment is critically examined and a method of procedure is described.

The lamellar structure of certain membrane systems can be determined with deconvolution methods (Worthington, King & McIntosh, 1973; McIntosh & Worthington, 1974; Worthington, 1976). Deconvolution of the autocorrelation function provides two solutions, \( \pm s(x) \). The \( \pm \) ambiguity can sometimes be removed using density considerations (Worthington et al., 1973). The choice between the two solutions follows once the phase of the first-order reflection has been found.

The correctness of an electron density membrane profile is dependent on the choice of phases as well as on the data processing of the X-ray intensities. An incorrect first-order phase leads to a Fourier profile with the wrong asymmetry. It is important for the continuing progress of membrane research that no such errors occur. Unfortunately, incorrect first-order phases of membrane systems have been used in the synthesis of Fourier profiles (Worthington, 1976). Such errors can be eliminated or, at least, minimized by the use of the present methods (to be described).

Electron density models

We treat the case when the membrane assemblies contain fluid layers. The simplest case is when there is...
one membrane per unit cell. The unit cell has width \( d \) while the membrane has width \( m \). The electron density of the membrane is \( m(x) \) and the fluid has electron density \( F \). A drawing of this electron density model is shown in Fig. 1. However, in membrane diffraction, it is convenient to study the minus fluid model (Worthington, 1969; Worthington et al., 1973) which is shown in Fig. 2. The membrane density in the minus fluid model is denoted \( \Delta m(x) \) and \( \Delta m(x) = m(x) - F \). The symbol \( \Delta \) is used to denote the minus fluid model. The Fourier transform of \( \Delta m(x) \) is \( \Delta M(X) \) where \( x, X \) are real- and reciprocal-space coordinates. \( \Delta M(X) \) is also the Fourier transform of the unit cell of the minus fluid model (with one membrane per unit cell).

An important case is when there are two membranes per unit cell and when the unit cell is centrosymmetrical. The electron density of this unit cell is denoted \( t(x) \) and \( T(X) \) is its Fourier transform. The Fourier transform \( T(X) \) can be expressed in terms of \( M(X) \), but the form of \( T(X) \) depends on the asymmetry of the membrane. If \( m(x) \) is asymmetric about its center then \( M(X) \) is complex and
\[
M(X) = A(X) + iB(X). \tag{1}
\]
On the other hand, if \( m(x) \) is symmetric (as in lipid bilayers) then \( M(X) \) is real and \( M(X) = A(X) \). The Fourier transform of \( t(x) \), the electron density of the centrosymmetrical unit cell with two membranes of electron density \( M(X) \) is \( T(X) \) and
\[
T(X) = 2A(X) \cos 2\pi \eta x - 2B(X) \sin 2\pi \eta x, \tag{2}
\]
where \( \eta \) is the distance from the origin of the unit cell (\( x = 0 \)) to the center of the membrane.

We use the convention that \( n \)-strip electron density models (Worthington, 1969) refer to the membrane density. Thus, the electron density model for the unit cell contains a total of \( n + 2 \) strips when there is one membrane per unit cell and a total of \( 2n + 3 \) strips when there are two membranes per unit cell. An \( n \)-strip electron density model for \( n = 3 \) in the case of two membranes per unit cell is shown in Fig. 3. The membranes in Fig. 3 have width \( m \) and are symmetrical. The membrane pair has width \( c \) such that \( c = 2m + c \), where \( c \) is the width of the (cytoplasmic) fluid layer between the two membranes. The origin of the unit cell is at the center of the cytoplasmic fluid layer. The distance \( \eta \) in (2) is given by \( \eta = (c + c)/4 \). Although the Fourier transforms \( T(X) \) of \( n \)-strip electron density models with one and two membranes per unit cell have been previously given (Worthington, 1969), in this paper it is necessary to present the appropriate formulas for the single- and three-strip models.

**Single-strip models**

The membrane has electron density \( m(x) \) and average electron density \( M \). In the single-strip model \((n = 1)\), the membrane of width \( m \) has uniform electron density \( M \). The Fourier transform of the single-strip minus fluid model with one membrane per unit cell is \( \Delta M(X) = \Delta A(X) \) and
\[
\Delta A(X) = (M - F)m \sin \pi n X, \tag{3}
\]
where \( \sin \theta = \sin \theta /\theta \) and where the origin value \( X = 0 \) of the Fourier transform is \( (M - F)m \). The Fourier transform of the single-strip minus fluid model with two membranes per unit cell is \( \Delta T(X) \) and
\[
\Delta T(X) = 2\Delta A(X) \cos 2\pi \eta X, \tag{4}
\]
where \( \Delta A(X) \) is defined by (3) and where the origin value of the Fourier transform is \( 2(M - F)m \).

In general, membranes have a non-uniform structure as demonstrated by X-ray diffraction and electron microscopy. The electron density models which have been used in X-ray studies on membranes to describe the diffraction at very small angles of diffraction (Worthington, 1969) are nevertheless exact for \( X = 0 \) and remain valid for small values of \( X \). The models however become invalid at moderate values of \( X \). It is, therefore, appropriate to consider membrane models with more than one strip of electron density. The next simplest model consistent with the known bilayer structure contains three strips per membrane.

**Three-strip models**

A symmetric bilayer model \((n = 3)\) with electron densities \( P, L, P \) and widths \( p, l, p \) respectively is shown in Fig. 3. The Fourier transform of the three-strip minus fluid model with one symmetric bilayer per unit cell is \( \Delta M(X) = \Delta A(X) \) and
\[
\Delta A(X) = (P - F)m \sin \pi MX - (P - L)l \sin \pi lX. \tag{5}
\]
The origin value of the Fourier transform \( \Delta A(X) \) is \( (M - F)m \), the same as in the single-strip case. The Fourier transform of the three-strip minus fluid model with two symmetric bilayers per unit cell is \( \Delta T(X) \) and \( \Delta T(X) \) is given by (4) but where \( \Delta A(X) \) is defined by (5). The origin value of the Fourier transform \( \Delta A(X) \) is \( 2(M - F)m \), the same as in the single-strip case.

Biological membranes are, in general, asymmetrical about their centers. In the special case of two
membranes per unit cell there is often a plane of symmetry at the origin of the unit cell owing to the mode of formation. This situation frequently occurs in biology; for example, the unit cells of nerve myelin and retinal rod photoreceptors each contain two asymmetric membranes but the unit cells are centrosymmetrical. The asymmetry \( \sigma \) is defined arbitrarily as the outer electron density (O) minus the inner electron density (I) and thus \( \sigma = O - I \). It is convenient to add or subtract \( \sigma \) on the outer strip centered at \( \pm (v - p)/2 \) and to define a symmetric membrane \( Q, L, Q \) with widths \( p, l, p \), as before, but where \( Q = P - \sigma/2 \). The Fourier transform of the three-strip minus fluid model with one symmetric bilayer \( (Q, L, Q) \) per unit cell is denoted \( \Delta M(X) = \Delta A(X) \) and

\[
\Delta A(X) = (Q - F) m \, \text{sinc} \, \pi MX - (Q - L) l \, \text{sinc} \, \pi LX.
\]  

(6)

The Fourier transform of the three-strip minus fluid model with two asymmetric membranes (with asymmetry \( \sigma \)) per centrosymmetrical unit cell is denoted \( \Delta T(X) \) and

\[
\Delta T(X) = 2 \Delta A(X) \cos 2\pi \eta X + 2\sigma p \, \text{sinc} \, \pi px \cos \pi(v - p)X,
\]

(7)

where \( \Delta A(X) \) is given by (6) and where \( \sigma \) can be positive, negative or zero. The asymmetric membrane has average electron density \( M \), as before, irrespective of the asymmetry:

\[
M = (2pP + lL)/m.
\]

(8)

Conditions for phase assignment

A. Biological membranes

The special case of two asymmetric membranes per unit cell with a plane of symmetry at the origin is treated. The conditions for the validity of the three-strip minus fluid model are examined. The Fourier transform for this model is given by (7) and we note that the extra term in (7) vanishes at \( X = \left[2(v - p)\right]^{-1} \). Although the repeat periods in the range \( d \geq (v + c) \) are of direct interest, it is convenient first to examine the repeat periods in the reduced range \( d \geq 2(v - p) \). We write \( M - F = \varepsilon \) and let \( \varepsilon > 0 \) so that \( \Delta T(0) \) is positive. In particular, we require to find the smallest value of \( \varepsilon \) so that \( \Delta T(Z) \) is positive, where \( Z = \left[2(v - p)\right]^{-1} \). The condition that \( \Delta T(Z) \) is positive reduces to the condition that \( \Delta A(Z) \), as defined by (6), is positive. This latter condition is

\[
Q - (Q - L)(g/m) - F > 0,
\]

(9)

where \( Q = P - \sigma/2 \) and where \( g \) is defined by

\[
g = \text{sinc} \, \pi LZ/\text{sinc} \, \pi mZ,
\]

(10)

and \( g > 1 \). The average electron density of the membrane \( M \) can be expressed in the form

\[
M = P - (P - L)(l/m).
\]

(11)

By combining (9) and (11) the condition that \( \Delta T(Z) \) is positive in the three-strip minus fluid model is when

\[
\varepsilon \geq (P - L)(l/m)(g - 1) + \sigma/2(1 - \log m).
\]

(12)

Similarly, the condition that \( \Delta T(Z) \) is negative in the same model is when

\[
\varepsilon < (P - L)(l/m)(g - 1) + \sigma/2(1 - \log m).
\]

(13)

These conditions, expressed by (12) and (13), are valid for the case when repeat periods \( d \) are in the restricted range \( d \geq 2(v - p) \).

Typical values of model parameters are listed in Table 1. From Table 1, it follows that \( v = 165, v + c = 180 \) and \( 2(v - p) = 280 \AA \). The value of \( g \) for \( Z = 1/280 \AA^{-1} \) is 1.114. Note that \( \sigma = 0.03 \) \( \text{e} \AA^{-3} \) represents a comparatively large asymmetry. The numerical values of \( \varepsilon \) which are listed in Table 2 were computed from the parameters of Table 1. The values of \( M \) follow from the particular case when the electron density of the fluid \( F = 0.34 \text{ e} \AA^{-3} \). When an upper limit is used for the asymmetry, the Fourier transform \( \Delta T(Z) \) is positive for \( M \geq 0.353 \text{ e} \AA^{-3} \) and negative for \( M < 0.353 \text{ e} \AA^{-3} \). This result is of interest. For example, the Fourier transform \( \Delta T(Z) \) for sarcoplasmic reticulum membranes in a fluid medium of \( F = 0.34 \text{ e} \AA^{-3} \) is certainly positive as \( M > 0.37 \text{ e} \AA^{-3} \) (Worthington & Liu, 1973). We note, however, with some surprise that certain workers (Dupont, Harrison & Hasselbach, 1973) have assigned a negative phase to the first-order reflection of sarcoplasmic reticulum membranes and so obtained an incorrect electron density profile. On the other hand, from Table 2, when \( F = 0.34 \text{ e} \AA^{-3} \) and when \( M \) is intermediate between 0.334 and 0.353 \text{ e} \AA^{-3}, \) the phase of \( \Delta T(Z) \) is uncertain. For example, the phase of the first-order reflection for nerve myelin with \( M = 0.343 \text{ e} \AA^{-3} \) (McIntosh & Worthington, 1974) cannot be directly assigned.

Table 1. Typical membrane model parameters

<table>
<thead>
<tr>
<th>Electron densities (e \AA^{-3})</th>
<th>Widths (\AA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P - L = 0.09 )</td>
<td>( m = 75 )</td>
</tr>
<tr>
<td>( F = 0.34 )</td>
<td>( p = l = 25 )</td>
</tr>
<tr>
<td>( \sigma = 0.03 )</td>
<td>( c = 15 )</td>
</tr>
</tbody>
</table>

Table 2. Conditions for the assignment of the first-order phase when \( d \geq 2(v - p) \) \AA; \( M \) is the average electron density of the membrane when \( F = 0.34 \text{ e} \AA^{-3} \)

<table>
<thead>
<tr>
<th>Asymmetry</th>
<th>( \varepsilon ) and ( M ) (e \AA^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Positive phase</td>
<td>a. Positive: ( \varepsilon \geq 0.013, M \geq 0.353 )</td>
</tr>
<tr>
<td>b. Zero: ( \varepsilon = 0.003, M \geq 0.343 )</td>
<td></td>
</tr>
<tr>
<td>c. Negative: ( \varepsilon \geq 0.006, M \geq 0.334 )</td>
<td></td>
</tr>
<tr>
<td>B. Negative phase</td>
<td>a. Positive: ( \varepsilon &lt; 0.013, M &lt; 0.353 )</td>
</tr>
<tr>
<td>b. Zero: ( \varepsilon &lt; 0.003, M &lt; 0.343 )</td>
<td></td>
</tr>
<tr>
<td>c. Negative: ( \varepsilon &lt; -0.006, M &lt; 0.334 )</td>
<td></td>
</tr>
</tbody>
</table>

B. Lipid bilayers

The case of one symmetric bilayer per unit cell and the case when there is a fluid layer between adjacent head groups is considered. In the three-strip model the
lipid bilayer has the same symmetric structure as the bilayer shown in Fig. 3. The Fourier transform \( \Delta A(X) \) of the three-strip minus fluid model is given by (5). We write \( M - F = e \) and let \( e > 0 \) so that \( \Delta A(0) \) is positive. In particular, we require to find the smallest value of \( e \) so that \( \Delta A(X) \) is positive for \( X = 1/d \) where \( d > m \). The analysis follows that given in part A. Thus, \( \Delta A(X) \) is positive in the three-strip minus fluid model when

\[
e \geq (P-L)/(m/g - 1),
\]

where \( g \) as defined by (10) is a function of \( X \), the reciprocal spacing. The conditions for the particular case of \( d = 2(v-p) \) Å are the same as for the biological membranes when there is no asymmetry. From Table 1, \( \Delta A(X) \) is positive for \( X = 1/280 \) Å\(^{-1} \) when \( e \geq 0.003 \) e Å\(^{-3} \). The condition expressed by (14) is valid for repeat periods \( d > m \) but \( e \) increases for the small \( d \) values as the value of \( g \) increases as a function of \( X \). For example, when \( d = 82.5 \) Å and using the parameters of Table 1, \( g = 5 \) so that \( \Delta A(X) \) is positive for \( X = 1/82.5 \) Å\(^{-1} \) when \( e \geq 0.12 e \) Å\(^{-3} \).

The case when the lipid bilayers contain no appreciable water layers between head groups such that repeat period \( d \) becomes equal to \( m \) is treated differently. It is convenient to consider the minus \( L \) model (Worthington & Khare, 1978) where the electron density is denoted \( \Delta l(x) \) and is defined by

\[
\Delta l(x) = m(x) - L.
\]

The origin of the minus \( L \) model \( (x = 0) \) is moved to the interface of the head groups so that the minus \( L \) model resembles the membrane model shown in Fig. 3, except that \( P - L \) replaces \( M - F \) and \( 2p \) replaces \( m \). The Fourier transform of the minus \( L \) model is denoted \( \Delta L(X) \) and, from (3), \( \Delta L(X) \) is given by

\[
\Delta L(X) = 2(P - L)p \text{sinc} 2\pi p X.
\]

If the model was strictly valid over a range of \( X \) where \( 0 \leq X \leq (2p)^{-1} \) then diffraction orders \( h \) have positive phases provided that \( h \leq d(2p)^{-1} \). Thus, if \( d = 60 \) Å and \( p = 12 \) Å then \( h \leq 5/2 \) so that the first two diffraction orders, \( h = 1 \) and \( h = 2 \), have a positive phase. The phase assignment for the first-order reflection is straightforward and, moreover, the assignment of phases may also be extended to the higher orders. An example of an oriented lipid bilayer assembly with no appreciable water layers is sphingomyelin with \( d = 68.5 \) Å and \( p = 12 \) Å (Khare & Worthington, 1978). From the above theory based on the three-strip minus \( L \) model the \( h = 1 \) and \( h = 2 \) diffraction orders have positive phases. This phase assignment is in agreement with a previous X-ray analysis using a different method (Khare & Worthington, 1978).

**Conditions for phase assignment using additional phase information**

**A. Biological membranes**

So far, conditions for the assignment of the first-order phases were found for a restricted range of repeat periods \( d \geq 2(v-p) \). The range of repeat periods \( v+c < d < 2(v-p) \) remain and, from Table 1, this range is \( 180 \leq d \leq 280 \) Å and it refers to fluid layers of width \( \leq 100 \) Å which frequently occur in membrane assemblies. It is possible (but somewhat awkward) to extend the previous treatment so as to derive conditions that the Fourier transform is positive when \( d \) falls in this restricted range. A special case is treated when additional phase information is available, namely, the phases for diffraction orders \( h = 2 \) to 7 or 8. It is convenient to write the Fourier transform \( \Delta T(X) \) at sampling intervals of \( X = h/d \), an integer in the form

\[
\Delta T(h) = S(h)\langle \Delta T(h) \rangle,
\]

where \( S(h) \) is the phase (or sign) of the reflection and, as the unit cell is centrosymmetrical, \( S(h) = +1 \) or \( -1 \). The repeat period \( d \) is in the range \( (v+c) < d < (2v-p) \). Let \( e > 0 \) so that the Fourier transform \( \Delta T(Z) \) for \( Z = [2(v-p)]^{-1} \) Å\(^{-1} \) is positive. It is assumed that all phases \( S(h) \) from \( h = 0 \) to 7 or 8 are known except \( S(1) \). The Fourier transform \( \Delta T(Z) \) can be expressed in terms of the observed X-ray data \( |\Delta T(h)| \) sampled at intervals of \( 1/d \) using the sampling theorem of communication theory:

\[
\Delta T(Z) = \sum_{h} S(h)\langle \Delta T(h) \rangle \text{sinc}(\pi dZ - \pi h).
\]

A factor \( \alpha(h) \) is defined according to

\[
\alpha(h) = [1 - (h/dZ)^2]^{-1},
\]

so that (18) becomes

\[
\Delta T(Z) \text{sinc}(\pi dZ)^{-1} = S(0)\langle \Delta T(0) \rangle
\]

\[
-2 \sum_{h} (-1)^{h}S(h)\langle \Delta T(h) \rangle \alpha(h).
\]

As the Fourier transform \( \Delta T(Z) \) is positive by definition an inequality is obtained:

\[
S(0)\langle \Delta T(0) \rangle - 2 \sum_{h} (-1)^{h}S(h)\langle \Delta T(h) \rangle \alpha(h) > 0.
\]

The only unknown in this inequality is \( S(1) \). Now (21) can be expressed as

\[
S(1) + c > 0,
\]

where \( c \) is the numerical value given by

\[
c = [2\langle \Delta T(1) \rangle \alpha(1)]^{-1}
\]

\[
\times [S(0)\langle \Delta T(0) \rangle - 2 \sum_{h} (-1)^{h}S(h)\langle \Delta T(h) \rangle \alpha(h)].
\]

Note that \( c \) can be readily evaluated for \( |\alpha(h)| \) becomes very small for \( h \geq 7 \) or 8. It is evident that if \( c < 1 \) the \( S(1) = +1 \). The application of the inequality to membrane diffraction requires knowledge of X-ray data on the same relative scale. The particular case of sarcoplasmic reticulum membranes where the Fourier transform has
been traced out (Worthington & Liu, 1973) is considered. The experimental X-ray data with a repeat period \( d = 220 \, \text{Å} \) is used. The origin value \( \Delta T(0) \) on a relative scale was obtained by extrapolation and the value of the numerical constant was found to be \( c = 0.4 \) using the published phases for order \( h = 2 \) to 7. Thus, \( S(1) = +1 \) for an assembly of sarcoplasmic reticulum membranes with repeat period \( d = 220 \, \text{Å} \).

**Discussion**

It has been demonstrated that the phase assignment for the first-order reflection is dependent on the origin value of the minus fluid model and also on the actual asymmetry of the membranes. Conditions for phase assignment have been derived as a function of the average density of the membrane relative to the density of the fluid and as a function of the asymmetry. It is evident from this study that phases can be rigorously assigned in the case of biological membranes which have an average electron density considerably larger than the fluid density. The special case of lipid bilayers has been treated. The assignment of the first-order phase is straightforward for lipid bilayers which do not contain water layers and, in this case, it has been suggested that the assignment of phases can often be extended to the second and third order of diffraction.

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**References**