LETTER TO THE EDITOR

Structure Determination of Membranes in Swollen Lamellar Systems

Dear Sir:

Since its original application to nerve myelin (Finean and Burge, 1963; Moody, 1963), considerable use has been made of the technique of phasing the X-ray diffraction patterns of lamellar membrane systems by the method of swelling stages (Boyes-Watson and Perutz, 1943). The resulting data were first analyzed (Finean and Burge, 1963) by the principle of "minimum wavelength" (Bragg and Perutz, 1952), or by a more quantitative version of this principle (Moody, 1963) which uses the Whittaker-Shannon sampling theorem (whose crystallographic implications were first pointed out by Sayre, 1952). Besides this principle, later workers have used the deconvolution methods of Hosemann and Bagchi (1962) (see, for example, Lesslauer and Blasie, 1972). Worthington et al. (1973) have recently surveyed the applicability of all these different techniques. The purpose of this letter is to point out two additional methods, and to discuss their usefulness in relation to the deconvolution and sampling theorem approaches.

Method 1. Deconvolution procedures operate on the autocorrelation function $A(x)$ (also known as the Q_0 function) of the density $\rho(x)$ of an isolated membrane. (Densities are taken relative to the density of the uniform fluid layer which separates the membranes in swollen lamellar systems.) However, the experimental data give, after cosine transformation, a Patterson function consisting of $A(x)$ convoluted with the autocorrelation function of the lattice function of the lamellar system. When the periodicity d of the lattice function is smaller than the width w of $A(x)$ (i.e. less than twice the width of $\rho(x)$), then different copies of $A(x)$ are at least partially superposed by the convolution. The outer parts of $A(x)$ are thereby obscured, which interferes with the application of deconvolution procedures, the recursion method (Hosemann and Bagchi, 1962) being the most sensitive in this respect. Worthington et al. (1973) have drawn attention to this problem and have concluded that deconvolution methods are inapplicable when $w > d$ (or, in an extreme limit, when $w > 4d/3$).

It is nevertheless possible, in principle, to extract $A(x)$ by adaptation of a procedure devised for use in the image analysis of electron micrographs (Crowther et al., 1970). The experiment yields $A(x)$ convoluted with a lattice of equidistant points of spacing $d < w$; consequently we know its transform

$$F^2(X_j) = \int_{-w/2}^{w/2} A(x) \exp(2\pi i X_j x)\, dx, \tag{1}$$

only at the points $X_j = j/d$, where j is an integer. In order to isolate the function $A(x)$ we need to have it convoluted instead with a lattice of spacing $D \geqslant w$. This convoluted $A(x)$ can be expanded to give

$$\sum_{n=-\infty}^{\infty} A(x + nD) = (1/D) \sum_{k} F^2(k/D) \exp(-2\pi i x k/D), \tag{2}$$

where k is an integer. Eq. 1 now gives (since $w \ll D$)

$$F^2(j/d) = \int_{-w/2}^{w/2} \left\{ \sum_{n=-\infty}^{\infty} A(x + nD) \right\} \exp(2\pi i x j/d) dx$$

$$= (1/D) \sum_k F^2(k/D) \int_{-w/2}^{w/2} \exp\{2\pi i x(j/d - k/D)\} dx$$

$$\therefore F^2(j/d) = (w/D) \sum_k F^2(k/D) \left[\frac{\sin\{\pi w(j/d - k/D)\}}{\pi w(j/d - k/D)} \right]. \quad (3)$$

This is a form of the Whittaker-Shannon sampling theorem. It constitutes a set of simultaneous equations which can in principle be solved, provided we restrict the number[1] of unknown $F^2(k/D)$ to no more than the number of $F^2(j/d)$ we have measured. If we have only one set of $F^2(j/d)$, this entails a restriction in resolution of the calculated $F^2(k/D)$ by a factor of d/D or less. However, if we have several sets of experimental $F^2(j/d)$ with different values of d (as we would, if the data derived from a series of lamellae with different degrees of swelling), then we may be able to calculate $F^2(k/D)$ up to essentially the same resolution as the experimental data. Eq. 3 is then more appropriately written

$$F^2_{obs}(X_j) = (w/D) \sum_k F^2_{calc}(k/D) \left[\frac{\sin \pi w(X_j - k/D)}{\pi w(X_j - k/D)} \right], \quad (3A)$$

since the reciprocal coordinates X_j are no longer multiples of $1/d$, and since F^2_{calc} will usually differ slightly from F^2_{obs} at the same reciprocal spacing (indeed, there may be several different F^2_{obs} at the same spacing, deriving from different swelling experiments). Under these circumstances, where the number of knowns considerably exceeds the number of unknowns, the equations would be solved by the method of least squares, as on pp. 323–324 of Crowther et al. (1970). In either case, the critical stage of the solution is the inversion of a matrix. This is the matrix of coefficients if only one data set is available; when the least squares method is used, it is the normal matrix. In both cases the order of the matrix equals the number of $F^2_{calc}(k/D)$ we are calculating, and this is so small as to present no computational problems. But the stability of the solution is extremely important, and an estimate of the magnitude of the errors introduced by the deconvolution procedure can be obtained by studying the eigenvalue spectrum of the matrix (see pp. 330–331 of Crowther et al., 1970). If the matrix should be nearly singular, it would be necessary to obtain more data or to restrict further the number of $F^2_{calc}(k/D)$ to be calculated; for it would rarely be possible, in subsequent calculations, to confine attention to only those linear combinations of $F^2_{calc}(k/D)$ which happen to be accurately determined by a nearly singular normal matrix. Once a well-conditioned set of equations has been obtained, the least squares method gives not only the required $F^2_{calc}(k/D)$, but also the error matrix for these quantities.

It should be noted that $F^2(0)$ is not directly measurable, and must therefore be included among the unknowns on the right-hand side of Eq. 3 or 3 A. The present method thus provides a least squares approach for calculating this important quantity.

[1]This number is strictly infinite for a function $A(x)$ which vanishes outside $-w/2 < x < w/2$. When the number is curtailed, $A(x)$ will not quite vanish outside these limits, but the resulting error can be made small if D is chosen carefully in relation to the available resolution.

After they have been calculated, the $F^2_{calc}(k/D)$ are substituted into Eq. 2, giving a series of copies of $A(x)$ separated by an adequate distance $(D - w)$.

Method 2. When the autocorrelation function $A(x)$ of an even function $\rho(x)$ is known, it may be deconvoluted to give $\rho(x)$ by the methods in Hosemann and Bagchi (1962) or by the following procedure which does not appear to have been described before. The moments

$$p_k = \int_{-w/2}^{w/2} x^k A(x) dx \tag{4}$$

of $A(x)$ are first calculated, and its transform can be expressed in terms of these:

$$F^2(X) = \int_{-w/2}^{w/2} A(x) \exp(2\pi i X x)\, dx = \sum_{k=0}^{\infty} \frac{(2\pi i X)^k}{k!} p_k, \tag{5}$$

where k must be even since $A(x) = A(-x)$. Moreover, $F^2(X)$ can also be expressed as a power series in terms of the moments m_k of $\rho(x)$:

$$\begin{aligned} F^2(X) &= \left\{ \int_{-w/4}^{w/4} \rho(x) \exp(2\pi i X x) dx \right\}^2 \\ &= \left\{ \sum_{k=0}^{\infty} \frac{(2\pi i X)^k}{k!} m_k \right\}^2, \end{aligned} \tag{6}$$

where k must again be even since the even function $\rho(x) = \rho(-x)$, and where the moments m_k are defined by

$$m_k = \int_{-w/4}^{w/4} x^k \rho(x) dx. \tag{7}$$

Expanding the square in Eq. 6, and equating the resulting series with the series in Eq. 5,

$$\begin{aligned} p_0 - 2\pi^2 p_2 X^2 + (2\pi)^4 p_4 X^4/4! - (2\pi)^6 p_6 X^2/6! + \ldots\ldots \\ = m_0^2 - 4\pi^2 m_0 m_2 X^2 + 4\pi^4 (m_0 m_4/3 + m_2^2) X^4 \\ - (8\pi^6/3)(m_0 m_6/15 + m_2 m_4) X^6 + \ldots\ldots \end{aligned}$$

Equating the constant terms gives $m_0 = \pm\sqrt{p_0}$, with the well-known sign ambiguity in deconvoluting an autocorrelation function. Equating the coefficients of successive equal powers of X allows each successive m_k to be calculated without further ambiguity. (This procedure, like all others, fails when $\rho(x)$ has no symmetry since there are then two new unknowns to determine at each step, once the coefficient of X^4 is passed.)

The calculated higher moments m_k should grow progressively more inaccurate, for two reasons. First, there is the accumulation of errors in the sequential solution of the successive equations. Second, there is the fact that, by Eq. 4, the higher the order k of p_k, the more it depends on the outermost parts of $A(x)$. The higher moments are thus particularly sensitive to errors in these small outer parts, and such errors would be especially large if deconvolution

were required to isolate $A(x)$. Successful use of the method must take account of the increasing inaccuracy of the higher moments. The calculated m_k must be tested to determine the point where the errors become unacceptably large. This point could be identified, for example, by making use of the fact that, by Eq. 7,

$$m_k < (w/4)^k m_0. \tag{8}$$

The first moment m_K which significantly exceeds this limiting value should be rejected, and likewise all the subsequent moments, whose values depend on m_K through the sequential method of calculation. The power series is thereby truncated:

$$F(X) \doteq \sum_{k=0}^{K-2} \frac{(2\pi iX)^k}{k!} m_k \quad (k \text{ even}). \tag{9}$$

The effect of this truncation is to limit the resolution of the calculated $\rho(x)$. For, if the (unknown) correct value of the Kth moment is denoted by m'_K, the Kth term (which is the first to be ignored) is small when

$$X < X_K = (1/2\pi)(m_0 K!/m'_K)^{1/K}.$$

Combining this with Eq. 8,

$$X_K \geqslant (2/\pi w)(K!)^{1/K}.$$

Using Stirling's formula,

$$X_K \geqslant (2K/\pi w)(2\pi K)^{1/2K} \exp\{-1 + (1/12K^2) - (1/360K^4) + \ldots\},$$

so that the higher is the order $(K - 2)$ of the accurate moments, the greater is the resolution $X_K(A^{-1})$ to which $F(X)$ is known.

The effect of the truncation in Eq. 9 can be smoothed out by multiplying this polynomial approximation to $F(X)$ by a temperature factor $\exp(-B^2X^2)$, where B is adjusted to make $F(X \geqslant X_K)$ suitably small. The resulting $F(X)$ would finally be cosine transformed to give the calculated $\rho(x)$.

Errors in the peripheral parts of $A(x)$ have the effect of restricting the resolution of $\rho(x)$ with this method, rather than of making the entire deconvolution uncertain as with the recursion method (Hosemann and Bagchi, 1962). Furthermore, the method should be computationally rapid, since calculation of the moments m_k is direct. Calculation of the moments p_k can be made rapid if $A(x)$ is to be calculated from a cosine series (e.g. through application of the first method described in this letter). In that case the evaluation of p_k involves only integrals of the form

$$\int_{-w/2}^{w/2} x^k \cos(2\pi x X_j) dx,$$

and these can be expressed in terms of simple functions by successive partial integrations.

Discussion. When the method of swelling stages is applied to lamellar membrane systems (or

to any[2] structure in which variations in the degree of hydration change only the size of the aqueous spaces between the particles), analysis of the data poses two problems. First, we must calculate the electron density that best accounts for the diffraction data (the "best" estimate for the electron density presumably satisfying the usual criteria of being unbiased and of minimum variance). Second, we need to know the errors involved, to give us a clear idea of how superior is the best solution to its nearest competitor. How well are these two problems solved by the deconvolution and sampling theorem approaches? The problem of calculating the electron density curve is more directly tackled by the deconvolution approach (which thus has an advantage when the number of possible sign combinations is very large), but at present there appears to be no good statistical treatment of stability and error propagation in any deconvolution method. It is, therefore, necessary to test the reliability of the solution by Monte Carlo methods, for which purpose it is important that the deconvolution calculation be as rapid as possible. The method proposed in this paper may have an advantage here, since it involves neither iterative procedures (as does the relaxation method; Worthington et al., 1973), nor matrix inversion (as does the Fourier series technique; Pape, 1974). But the problem of error analysis is perhaps better approached through the sampling theorem, by adapting the first method described in this paper. Each possible sign combination would be tested as follows. The exact positions of the transform nodes would be chosen, and a signed $F_{obs}(X)$ calculated from each observed $F^2_{obs}(X)$. We can then use Eq. 3 *A* in the form

$$F_{obs}(X_j) = \sum_k F_{calc}(2k/w)\left[\frac{\sin \pi(wX_j/2 - k)}{\pi(wX_j/2 - k)}\right], \tag{10}$$

where $F(X)$ is the transform of $\rho(x)$ whose width is $w/2$. The least squares solution gives the values of $F_{calc}(2k/w)$ (including $F_{calc}(0)$), and also the error matrix whose elements are

$$P_{mn} = E\{\Delta F_{calc}(2m/w)\,\Delta F_{calc}(2n/w)\},$$

where ΔF denotes an error in F. Substitution of the least squares $F_{calc}(2k/w)$ back into the right-hand side of Eq. 10 gives at each X_j a new calculated $F'_{calc}(X_j)$ for comparison with $F_{obs}(X_j)$. The variance of each $F'_{calc}(X_j)$ is

$$\sigma^2\{F'_{calc}(X_j)\} = \sum_m \sum_n P_{mn}\left[\frac{\sin \pi(wX_j/2 - m)}{\pi(wX_j/2 - m)}\right]\left[\frac{\sin \pi(wX_j/2 - n)}{\pi(wX_j/2 - n)}\right].$$

A measure of the agreement between the $F'_{calc}(X_j)$ and the $F_{obs}(X_j)$ could be obtained by calculating either

$$U = \sum_j [F'_{calc}(X_j) - F_{obs}(X_j)]^2/\sigma^2\{F'_{calc}(X_j)\}$$

or

$$V = \sum_j [F'_{calc}(X_j) - F_{obs}(X_j)]^2.$$

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[2] The methods discussed here could be used with two- or three-dimensional data by applying them to each central line of the transform.

For each sign combination, we would choose the positions of the nodes so as to minimize U or V, and then the different sign combinations could be evaluated by comparing the corresponding minimum values of U or V. Finally, knowledge of the error matrix P_{mn} allows confidence intervals to be established for the different linear combinations of $F_{calc}(X_j)$ which are formed when calculating the Fourier series to obtain the electron density distribution (see Rust and Burrus [1972] for a discussion of confidence intervals of different linear combinations of least squares solutions).

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M. F. MOODY
Biology Department
Brookhaven National Laboratory
Upton, New York 11973