The Liquid-Crystallographic Structure of Fluid Lipid Bilayer Membranes

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Introduction

Knowledge of the structure of lipid bilayers in the fluid liquid-crystalline state is important for understanding the permeability and stability of membranes and the insertion and folding of membrane proteins. Quantitative structural models are especially important at the present time for validation of Monte Carlo and molecular dynamics simulations of lipid bilayers (Pastor, 1994). Diffraction studies of phospholipid crystals at low hydrations can provide atomic-resolution images of the phospholipid molecules of membranes (Pascher et al., 1992) but such images are of marginal value for understanding membrane bilayers for the obvious reason that the phospholipids are in a noncrystalline state. Fluid bilayers present special problems to the structural biologist because their inherent thermal motion and disorder exclude entirely the possibility of obtaining three-dimensional structural information. The only kind of structural image that can be obtained by diffraction methods is a one-dimensional one consisting of the time-averaged transbilayer distributions of the multilamellar submolecular groups comprising the lipids such as the phosphate, carbonyl groups, double-bonds, etc. (Figure 1). Such projections have become a standard method for describing the results of bilayer simulations (Damodaran and Merz, 1994; Egberts et al., 1994; Fattal and Ben-Shaul, 1994; Heller et al., 1993). The changes in mean intergroup distances obtainable from the images (called bilayer profiles) are invaluable for understanding how lipid composition and proteins affect membrane organization (White and Wimley, 1994). However, because the profiles represent long-time averages projected onto a line normal to the membrane plane, important information such as instantaneous three-dimensional atomic positions and trajectories are lost from view.
Figure 1. Concept of quasimolecular structure. The inherent thermal motion of lipids in fluid bilayers precludes the possibility of three-dimensional atomic-level crystallographic structures like that of dimyristoylphosphatidylcholine (DMPC) (Pearson and Pascher, 1979) illustrated in the molecular graphics image. However, one can obtain by combined X-ray and neutron diffraction measurements useful structures that consist of time-averaged transbilayer probability distributions for the principal structural groups such as the phosphate group shown here (Wiener and White, 1991b,c).

Monte Carlo and molecular dynamics simulations of membranes can, in principle, provide this information and are thus important endeavors. The evolution of computer hardware and software technologies in the coming years will make simulations increasingly useful and practical tools. Direct bilayer structural information obtained by diffraction methods combined with simulations thus have the potential for providing comprehensive descriptions of complex membrane systems.

We consider in this chapter the structure of a fluid bilayer membrane obtained by what we call liquid-crystallography in which X-ray and neutron diffraction measurements are combined through joint-refinement procedures (Wiener and White, 1992a,b; 1991a,b,c). We have recently summarized the liquid-crystallographic method in a comprehensive review (White and Wiener, 1995). Here we provide an overview of the nature of the fluid bilayer structure determination problem, our composition-space structural refinement method for combining the X-ray and neutron diffraction data, and the fully resolved structural image of fluid bilayers formed from dioleoylphosphatidylcholine (DOPC).
Nature of the Fluid Bilayer Structure Problem

Diffraction studies of fluid bilayers are generally accomplished using multilamellar bilayer arrays (multilayers) formed from phospholipids by either dispersal in water or deposition on glass substrates. The resulting one-dimensional lattice of thermally disordered bilayer unit cells typically yields five to ten orders of lamellar diffracted intensities from which the bilayer profile can be constructed (Blaurock, 1982; Franks and Levine, 1981; Levine and Wilkins, 1971). The profiles can be expressed as transbilayer electron density, scattering-length density, or probability density depending upon the scale factors used in the Fourier transformation of the phased structure factors obtained from the diffracted intensities. Examples of X-ray and neutron scattering-length density profiles for bilayers formed from dioleoylphosphocholine (DOPC) bilayers are shown in Figure 2. These profiles leave the impression that they contain only modest amounts of information. In fact, they contain a great deal of information because all of the atoms in the unit cell contribute to the profile. The object of liquid-crystallography is to decompose these rather smooth profiles into subprofiles that represent the transbilayer distributions of submolecular groupings such as the phosphates, cholines, and carbonyl groups.

To be useful, distributions of the submolecular groups must represent fully resolved images. These can be obtained only when: (1) the one-dimensional lattice is perfect; and (2) all \( h_{\text{max}} \) of the observable diffracted intensities are recorded. The canonical resolution of the experiment is \( d/h_{\text{max}} \) where \( d \) is the one-dimensional Bragg spacing. The Bragg spacing for bilayer systems is typically 50 Å with \( h_{\text{max}} = 5 \) to 10 so that the canonical resolution is 5–10 Å. This resolution is frequently assumed to be the limit on the accuracy with which the separation of structural features can be determined. That assumption is incorrect; the positions of resolvable features can be determined with a precision that greatly exceeds the canonical resolution (Wiener and White, 1991b). For example, the high electron density peaks in the bilayer profiles obtained from X-ray diffraction (Figure 2B) are assigned to the phosphate moieties, and the distance between them (\( d_{p-p} \)) is frequently cited to a precision of 1 Å or better (Inoko and Mitsui, 1978; Janiak et al, 1979; McIntosh and Simon, 1986; Ranck et al, 1977). We call this aspect of resolution, resolution precision. The apparent conflict between the canonical resolution and the resolution precision can be resolved by a careful consideration of the nature of the disorder found in multilamellar fluid bilayer systems and its effects on the images obtained by Fourier transformation of the phased structure factors.

Resolution and resolution precision are controlled by three types of disorder (Blaurock, 1982; Hosemann and Bagchi, 1962; Schwartz et al, 1975) in diffraction experiments. Disorder of the first kind is thermal disorder in which the atoms or molecular fragments oscillate about well-defined positions within the unit cell. A sample with only this type of disorder will have a unit cell of well-defined composition and a lattice with a high degree of long-range order. All of the diffraction peaks will be perfect images of the incident beam so that
Figure 2. Observed eight-order absolute scattering-length density profiles of DOPC bilayers at 23°C and 66% RH (Wiener and White, 1992a). A. Profile determined by neutron diffraction. Hydrogen atoms have negative values of scattering length so that the average scattering length density is close to zero. B. Profile determined by X-ray diffraction. Notice the difference in the locations of the dominant headgroup peaks in two profiles. This is because the dominant structural feature for neutron scattering is the carbonyl group whereas for X-ray scattering the phosphate group dominates.
the widths of diffraction peaks will be independent of diffraction order \( h \). The intensities of the peaks, however, will decrease more rapidly with increasing values of \( h \) compared to unit cells with less thermal disorder. Disorder of the second kind, lattice disorder, results when long-range order and/or uniform unit cell composition are lacking. This is typically the case for compacted stacks of biological membranes. The membranes have constant composition but the water spacing between membranes varies and thus causes a loss of spatial coherence. When lattice disorder is present, the decreased long-range order causes the diffracted peaks to increase in width as \( h \) increases. A third type of disorder is orientational disorder which is related solely to the macroscopic features of a particular sample. For example, a single crystal of salt will produce discrete diffraction spots at well-defined angular positions relative to the X-ray beam. If the crystal is broken up to form a powder, the numerous small crystallites will be oriented at various angles with respect to one another and the X-ray beam so that ring-like diffraction spots are produced. The diffracting lattices can be nearly perfect in both cases.

The nature and quality of images obtained in diffraction experiments is governed by: (1) the thermal disorder of the unit cell, which determines the maximum number of diffraction orders \( h_{\text{max}} \) that can be obtained under ideal conditions; (2) the number \( h_{\text{obs}} \) of diffraction orders which are observable as result of the disorder of the lattice or other experimental conditions; and (3) the number of diffraction orders \( h_{\text{for}} \) actually used in the Fourier reconstruction of the image (Wiener and White, 1991b). Thermal disorder sets the ultimate upper limit on the image obtainable. If thermal disorder is very low, atomic-level structures, referred to as high-resolution structures, can be obtained. If thermal disorder is high, then only polyatomic-level structures can be obtained. These are called low-resolution structures. In either case, with an excellent lattice and good experimental technique, \( h_{\text{obs}} = h_{\text{max}} \), and Fourier transformation with \( h_{\text{for}} = h_{\text{max}} \) yields a fully resolved image of the unit cell. If \( h_{\text{for}} < h_{\text{max}} \), the resulting image will be a partially resolved image of the high- or low-resolution structure. A fluid bilayer structure is inherently a low-resolution structure because thermal motion causes the atoms of the molecules to be broadly distributed over distances of 5–10 Å so that \( h_{\text{max}} \) is limited to 5 to 10 diffraction orders regardless of the care with which samples are prepared, the sensitivity of the detector, or the intensity of the source. In such a case, Fourier transformation using \( h_{\text{for}} = h_{\text{max}} \) yields a fully resolved image of the low-resolution structure.

The number of diffraction orders observed from crystalline and liquid-crystalline phases is a direct consequence of the spatial distribution of matter resolvable over the time course of a diffraction experiment. Individual atoms or small groups of atoms are discernible in the high-resolution structure of a crystal while the thermal disorder of the liquid crystal causes these distributions to overlap, producing a low-resolution structure. As discussed below, the physically appropriate structural subunits of the liquid crystal are these overlapping multiatomic quasimolecular pieces which have Gaussian shapes (King and White, 1986; Wiener and White, 1991b,c; 1992a). For both crystalline and
liquid-crystalline materials, the intensities of the diffracted X-rays can be accurately measured, and in both cases models of appropriate structural resolution can be constructed that allow one to refine the structural image with a resolution precision which is considerably better than the canonical resolution. Thus, it is not correct to assume that the low canonical resolution of the bilayer diffraction experiment makes it impossible to determine distances and distributions to better than \( d/h_{\text{max}} \). On the contrary, if thermal motion is the only cause of disorder, very accurate fully resolved images of the low-resolution structure can be obtained. This frequently is the case for oriented multilamellar bilayer arrays (Franks and Lieb, 1979; Smith et al, 1987; Wiener and White, 1991b).

**Determination of Fully-Resolved Images of Fluid Bilayers**

*Joint-Refinement of X-ray and Neutron Data*

The structural resolution of bilayer diffraction experiments can be increased using neutron diffraction and specific labeling with deuterium at various positions within a lipid molecule (Blasie et al, 1975; Schoenborn, 1975; Worcester, 1975). The transbilayer position and distribution of labels can be determined with a precision of better than 1 Å (Büldt et al, 1979; 1978; Worcester and Franks, 1976; Zaccari et al, 1979). The general difficulty with such experiments is the heroic amount of chemical and diffraction work that must be done (Büldt et al, 1979; 1978; Zaccari et al, 1979). To avoid heroism, we developed the so-called composition-space joint refinement method that combines X-ray and neutron data in a way that minimizes the amount of neutron data required to obtain a detailed image of a fluid bilayer.

The refinement method is possible because of the significant differences in the neutron and X-ray scattering density profiles (Figure 2) observed for phospholipid bilayers (Franks and Lieb, 1979). The neutron scattering-length density profile is different from the X-ray scattering-length density profile because neutrons interact with nuclei whereas X-rays interact with electrons. Because there is no specific relation between X-ray scattering length (determined by atomic number) and neutron scattering length (determined by nuclear scattering), X-ray and neutron diffraction data sets are independent of each other. Thus, the use of both kinds of diffraction doubles the amount of data available for structure refinement. Each experimental method sees a different representation of the molecule in its own scattering space, and each method has different sensitivities to various regions of the molecule. Neutrons scatter most strongly from the carbonyl groups of phospholipids because this part of the molecule lacks hydrogens whereas X-rays scatter most strongly from the electron-dense phosphate moiety.
Quasimolecular Models and the Multi-Gaussian Representation of Bilayers

The principal objective of molecular modeling in bilayer diffraction studies is to construct a real-space model for the distribution of matter across the bilayer. The one-dimensional projection of a perfect crystalline lipid structure along the bilayer normal will result in a series of sharp (approximately δ-function) peaks representing the individual atoms. Thermal disorder, described by the Debye-Waller factors of the atoms in crystallographic refinements, broadens these peaks to produce a disordered crystalline model. The Gaussian quasimolecular model is a logical extension of the disordered crystal model in that Debye-Waller factors for small crystals are rigorously derived by considering the Gaussian-distributed deviations of atoms from their equilibrium positions (Warren, 1969). If the thermal disorder is very high, the broadened adjacent atomic peaks will overlap and thus make it impossible to resolve them individually. These overlapping atomic distributions merge into a single Gaussian function representing a multiatomic grouping. The quasimolecular model thus consists appropriately of a family of Gaussians that accounts for all of the atomic mass of the unit cell. It should be emphasized that the use of Gaussians is more than a mathematical convenience because direct structural determinations demonstrate that the transbilayer profiles of specific multiatomic groupings are in fact Gaussian (Wiener and White, 1991a; Wiener et al., 1991). The positions of the Gaussians represent the time-averaged positions of the submolecular pieces while their widths describe the range of thermal motion of the pieces (Willis and Pryor, 1975). Because the quasimolecular model accounts for thermal motion from the start, Debye-Waller terms are not included in the transform. The use of Gaussian distributions implies that the motions of these multiatomic distributions are primarily harmonic. In crystal structures, some atoms probably undergo anharmonic motion, but molecular dynamics calculations suggest that these regions are best described by a series of Gaussians rather than a single non-Gaussian distribution (Kuriyan et al., 1986).

The number of Gaussians necessary for modeling the bilayer is related to the number of observable diffraction orders (Wiener and White, 1991b). The canonical resolution, \( d/h_{\text{max}} \), is the most appropriate length scale for describing the bilayer because it represents the characteristic sizes of molecular subunits that are discernible in the diffraction experiment. If ten diffraction orders are observable from a bilayer with a \( d \)-spacing of 50 Å, the principal scattering centers will be about 5 Å wide. Therefore, appropriate quasimolecular models will have Gaussian distributions with 1/\( e \)-halfwidths of about 2.5 Å. Because of the importance of the canonical resolution in the determination of the appropriate length scale, all of the observable diffraction orders must be recorded, as noted earlier. Furthermore, the experimental errors of the structure factors must be carefully estimated so that the limits on spatial resolution can be determined (Wiener and White, 1991b). A model based upon an imperfect data set, i.e.,
one that excludes significant higher order structure factors, will result in an incorrect model of the bilayer.

Composition Space

Our joint refinement procedure is based upon the obvious fact that, for thermally disordered liquid-crystalline bilayers, there is a single time- and space-averaged bilayer structure that is invariant with respect to the type of beam used in the diffraction experiment. We therefore used a composition-space representation in which the quasimolecular Gaussian distributions describe the number or probability of occupancy per unit length across the width of the bilayer of each component (Wiener and White, 1991c). This representation permits the joint refinement of neutron and X-ray lamellar diffraction data by means of a single quasimolecular structure that is fit simultaneously to both diffraction data sets (Figure 3). Scaling of each component by the appropriate neutron or X-ray scattering length maps the composition space profile to the appropriate scattering-length space for comparison to experimental data. Other extensive properties, such as mass, can also be obtained by an appropriate scaling of the refined composition-space structure. Based upon simple bilayer models involving crystal and liquid crystal structural information (Wiener and White, 1991c), we estimate that a fluid bilayer with \( h_{\text{max}} \) observed diffraction orders will be accurately represented by a structure with approximately \( h_{\text{max}} \) quasimolecular components.

The time-averaged Gaussian probability distribution of each quasimolecular piece projected onto the bilayer normal can be described by:

\[
n_i(z) = \left( \frac{N_i}{A_i \sqrt{\pi}} \right) \exp \left[ - \left( \frac{z - Z_i}{A_i} \right)^2 \right] \tag{1}
\]

where \( n_i(z) \) is the fraction of the piece located at position \( Z_i \) with 1/e-halfwidth \( A_i \) (Figure 3). The distribution can be viewed as the convolution of the hard-sphere or steric distribution of the quasimolecular fragment with an envelope of thermal motion (Wiener and White, 1991a; Wiener et al, 1991). In general, each piece \( i \) consists of \( N_i \geq 1 \) identical subpieces. The \( n_i(z) \) include the water molecules associated with the lipid and any other molecules contained within the unit cell. The distribution of matter across the bilayer can be represented in terms of neutron scattering length or X-ray scattering length by multiplying Equation (1) by, respectively, the neutron scattering length \( b_n \) or X-ray scattering length \( b_x \) of piece \( i \) so that the scattering length per unit length is:

\[
\rho_j^*(z) = b_j \cdot n_i(z) \tag{2}
\]

where \( j = n \) or \( x \). Thus, the neutron or X-ray scattering length per unit length at any point in the bilayer is given by:

\[
\rho_j^*(z) = \sum_{i=1}^{P} \rho_j^i(z) \tag{3}
\]
where \( p \) is the number of quasimolecular pieces per lipid. The neutron and X-ray structure factors \( F_j(h) \) of the model consisting of the set of \( p \) quasimolecular pieces are then given analytically by the Fourier transform of Equation (2) summed over all of the pieces:

\[
F_j(h) = 2 \sum_{i=1}^{p} b_{ji} N_i \cdot \exp \left[ - \left( \frac{\pi A_i h}{d} \right)^2 \right] \cos \left( \frac{2\pi Z_i h}{d} \right) \tag{4}
\]
The rule-of-thumb for the number of quasimolecular components required is that the number of Gaussians \( p \) is approximately equal to the number \( h_{\text{max}} \) of observed lamellar diffraction orders (Wiener and White, 1991c). There are many ways to divide a lipid molecule into \( p \) fragments, but two important guidelines simplify the process. The first is to parse the hydrated molecule into \( p \approx h_{\text{max}} \) pieces that have widths \( 2A_i \approx d/h_{\text{max}} \). The second guideline is inherent in the composition-space refinement method. Namely, the positions \( Z_{ji} \) of the pieces must be the same in both X-ray and neutron scattering-length spaces. This entails parsing the atoms among the pieces so that the weighting by the scattering lengths, Equation (2), leads to model scattering-length profiles consistent with the observed ones. The appropriate parsing is ultimately determined by experimental sensitivity and the relative widths and scattering lengths of the distributions (Wiener and White, 1991b). The parsing must be done largely by trial and error in specific cases.

**Model Refinement**

The structural model is obtained by finding the set of composition-space models that yield the best agreement to both the neutron and X-ray data (Wiener and White, 1991c; 1992a). Nonlinear minimization with the standard Levenberg-Marquardt algorithm (Bevington, 1969; Press et al, 1989) is carried out to determine the parameters \( Z_i \) and \( A_i \) of Equation (4) which minimizes the joint crystallographic R-factor defined here as:

\[
R = \sum_{j=m,n} R_j
\]  

where:

\[
R_j = \frac{\sum_h |F_j(h)| - |F_j^*(h)|}{\sum_h |F_j^*(h)|}
\]  

\( F_j^*(h) \) are the experimental structure factors scaled to the appropriate relative absolute scale (Jacobs and White, 1989; Wiener and White, 1991c; Wiener et al, 1991). A composition-space structure is judged to be satisfactory if it provides fits to both the neutron and X-ray data sets that were below the experimental noise or “self-R” (Wiener and White, 1991b). The robustness of the structure determination and the uncertainties in the parameters are examined by introducing Gaussian-distributed noise into the data sets. Each of the absolute neutron and X-ray structure factors has an associated uncertainty which is used to define the width of a normal distribution centered at the best value of the structure factor. Monte Carlo methods (Press et al, 1989) are used to select data sets from these distributions which are in turn used as the input for the structural calculations (Wiener and White, 1991a; Wiener et al, 1991). Generally, about fifty different data sets are selected in this way, which result in an equal number of suitable
models. The mean values of the positions and widths of the Gaussians provide the best estimates of the actual positions and widths. The standard deviations give the estimated experimental uncertainties of the final model.

The Structure of a Fluid Bilayer

Quasimolecular Model

We obtained the complete structure of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in the liquid-crystalline phase (66% RH, 23°C) by the joint refinement of neutron and X-ray lamellar diffraction data (Wiener and White, 1992a). The requirement that a successful quasimolecular model fit two independent sets of data strongly constrains the ways in which the molecule can be divided. Figure 4 depicts the quasimolecular model of DOPC and its associated water molecules that was used in the structural determination. This one was chosen initially because it logically identified the obvious molecular fragments. Subsequent examination of more than 30 other parsing schemes did not lead to successful refinements, suggesting that the chosen model may be unique. The methylene region (part 2) in Figure 4 is represented by three Gaussians so that ten quasimolecular fragments are required to obtain the complete structure of the DOPC bilayer. Each piece requires three parameters: position $Z_i$, 1/e-halfwidth $A_i$, and area $N_i$. The water and double-bond distributions were determined independently from neutron diffraction experiments (Wiener et al, 1991) which reduced the number of parameters from thirty to twenty-four. The terminal methyl distribution was determined from a direct combination of neutron and X-ray data prior to the full joint refinement so the parameter set was further reduced to twenty-one (Wiener and White, 1992b). The contents of each of the remaining pieces of the model, except for the methylene envelope, were fixed by the parsing so that only the positions and 1/e-halfwidths were determined during the nonlinear minimization. Specifically, the contents of the carbonyl, glycerol, phosphate, and choline fragments were fixed so that the number of parameters was reduced to seventeen. We estimated previously that a fluid bilayer yielding $h_{max}$ diffraction orders would require $p \approx h_{max}$ quasimolecular Gaussian distributions to describe it adequately (Wiener and White, 1991c). We had $h_{max} = 8$ orders for both X-rays and neutrons and $p = 10$ Gaussian fragments consistent with the approximation.

The Complete Structure of DOPC Bilayers at 66% RH

The complete and fully-resolved image of a DOPC bilayer is shown in Figure 5. The positions and widths of the Gaussians and their experimental uncertainties may be found in the original publication (Wiener and White, 1992a). The positions were determined with precisions ranging from 0.02 Å for the carbonyl group (the most strongly scattering feature in neutron experiments) to 0.77 Å.
Figure 4. The parsing of DOPC into the quasimolecular parts used in the structure determination by the joint refinement of X-ray and neutron data. Redrawn from Wiener and White (1992a). Used with permission.

for the water. Because this real-space image is physically meaningful, there is useful and interesting information in the widths of the Gaussian distributions that characterize each quasimolecular fragment. The positions of the distributions denote the most likely place to locate the center of scattering of each fragment whereas the widths describe the range of thermal motions projected onto the bilayer normal assuming undulatory motions are insignificant in our system. The 1/e-halfwidth $A_i$ of a quasimolecular Gaussian fragment can be viewed as the convolution of a hard-sphere of van der Waals radius $D_H$ located at $Z_i$ with a Gaussian envelope of thermal motion describing the range over which that piece moves within the bilayer. The observed 1/e-halfwidth is
Figure 5. The structure of a fluid dioleoylphosphocholine (DOPC) bilayer determined by the joint refinement of X-ray and neutron diffraction data (Wiener and White, 1992a). The structure consists of the time-averaged distributions of the principal structural groups of the lipid projected onto an axis normal to the bilayer plane. The distributions are Gaussians whose areas equal the number of structural groups represented by them; the distributions therefore represent the probability of finding a structural group at a particular location. A Gaussian distribution is appropriate because that distribution is invariably observed in direct structural determinations of the individual fragments (Wiener and White, 1991a; Wiener et al, 1991). The positions and widths of the distributions can generally be determined with a precision of better than 0.5 Å. (a) A representation of the length of a 27 amino acid transbilayer helix. (b) The distributions of the methyl (CH₃), methylene (CH₂), double-bonds (C=C), carbonyls (COO), glycerol (GLYC), and water. The interfaces of the bilayer are defined as the regions occupied by water. Notice that an α-helix that is parallel to the bilayer can be comfortably accommodated in the interfaces. (c) The distributions of the glycerol, choline (CHOL), and phosphate (PO₄) groups. Note that the interfaces of the bilayer, each about 15 Å thick, account for 50% of the bilayer thickness. These regions are highly heterogeneous chemically and can therefore host a wide variety of non-covalent interactions with peptides and proteins. The figure is slightly modified from White (1994) and used with permission.
given approximately by $\sqrt{D_{H}^2 + D_{T}^2}$ where $D_T$ describes the envelope of thermal motion (Wiener and White, 1991a). Because of the approximate nature of this crude expression and the ambiguity in estimating hard-sphere widths of each of the quasimolecular fragments, we did not explicitly repeat the calculation for all of the fragments. The narrowest thermal distribution is that of the glycerol region ($A_{GLYC} = 2.46 \pm 0.38\text{Å}$). The 1/e-halfwidths of the quasimolecular pieces on either side of the glycerol backbone increase as shown graphically in Figure 5c. The general image is a gradient of thermal motion within the interface zone in which the regions bounding the relatively rigid glycerol backbone undergo increasing ranges of motion that are roughly proportional to the distance from the glycerol fulcrum. This is consistent with NMR results (Braach-Maksvytis and Cornell, 1988; Strenk et al, 1985) and crystallographic measurements (Elder et al, 1977; Hitchcock et al, 1975) which indicate that the glycerol backbone is the most rigid portion of the liquid-crystalline phospholipid bilayer on DMPC.

The glycerol region is also interesting because it is at the extreme boundaries of both the methylene and water distributions and thus marks the water-methylene interface (shaded Gaussian in Figure 5b). The net thermal motions within the hydrocarbon region, compared to the interface zone, are qualitatively different in that the 1/e-halfwidth of the terminal methyl groups (2.95 Å) is about the same as the carbonyl (2.77 Å) or phosphate groups (3.09 Å) while the width of the double-bond distribution is significantly larger (4.29 Å). This situation makes it appear as though the flexible acyl chain is tethered at one end to the interface by the carbonyls and at other end to the bilayer center by the terminal methyls. Because the half-thickness of the hydrocarbon is considerably shorter than the length of the fully extended chain, this apparent tethering allows the double bonds to diffuse over a relatively large volume of space relative to the carbonyls and terminal methyls. Tethering also permits some of the methylenes to venture beyond the C(2) carbons into the interfacial zone (Figure 5b). The concept of tethering, used here strictly in a spatial sense, seems to violate the NMR observation that there is a smooth increase in motion along the chain such that the terminal methyl is the most disordered part of the chain (Seelig and Seelig, 1977; 1974). However, deuterium NMR orientational order-parameter measurements are made in the time domain whereas the diffraction measurements are made in the spatial domain. The diffraction results simply indicate that the motion of the terminal methyl groups reported by the NMR experiment occurs in a limited region of the bilayer thickness.

An approximate average tilt-angle of the phosphocholine dipole with respect to the bilayer surface can be estimated from the distance between the centers of the phosphate and choline pieces along the bilayer normal. Assuming that the phosphorus and nitrogen atoms are the centers-of-scattering of each of these roughly spherical fragments and a phosphate-nitrogen distance of 4.5 Å obtained from the crystal structure of DMPC (Hauser et al, 1981), the dipole is calculated to be canted with an angle of $22 \pm 4^\circ$ with respect to the bilayer surface. This compares favorably with the values obtained from crystal structures (Hauser
et al, 1981) and neutron diffraction of oriented multilayers (Büldt et al, 1979) and is in reasonable agreement with the recent value of 18° obtained from 2H-NMR and Raman spectroscopic studies of DPPC (Akutsu and Nagamori, 1991). Similar orientations are observed in several molecular dynamics simulations (Egberts et al, 1994; Heller et al, 1993; Zhou and Schulten, 1995).

Structural Implications for Peptide-Bilayer Interactions

The structure shown in Figure 5 reveals the complexity of the bilayer as the nonpolar phase for the partitioning of peptides and proteins. The structure has been divided into interface and hydrocarbon (HC) core regions based upon the water distribution. Note that the total interfacial thickness is about the same as the HC core thickness. This means that it is not correct to think of the bilayer as a thin slab of hydrocarbon separating two aqueous phases. Furthermore, the interfacial region is a complex mixture of phosphocholine, glycerol, carbonyl, and methyl groups. The interactions of peptides with these region are equally complex. Finally, as shown in Figure 5b, the thermal thickness of the interface is sufficient to accommodate comfortably an α-helix parallel to the bilayer surface. This emphasizes the likely importance of the interface in protein folding and insertion (Jacobs and White, 1989; White and Wimley, 1994).

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REFERENCES


5. LIQUID-CRYSTALLOGRAPHIC STRUCTURE OF FLUID LIPID BILAYER MEMBRANES


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