7 Lipid Diversity and Its Implications for Membrane Organization

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7.1 INTRODUCTION

Biological membranes are complex, mesoscopic assemblies that possess functions, which are far more elaborate than a simple permeability barrier (or a passive matrix) in which proteins reside and carry out their associated functions. Instead, biomembranes are complex, highly functional dynamic machines that are central to a host of biological processes, including the transport of materials, cell defense, recognition, adhesion, and signaling. Although the exact composition of biomembranes varies among different types of organisms—and even within individual organisms—all cell membranes share a generic lipid bilayer architecture.

Membrane lipid molecules contain a polar (hydrophilic) headgroup and typically, two long nonpolar (hydrophobic) hydrocarbon fatty acid chains (Figure 7.1). In a lipid bilayer, two sheets (leaflets) of lipids align to form a pseudoplanar structure with their polar headgroups exposed to water, and their hydrocarbon chains packed in an oil-like phase. For membrane models, bilayers can be prepared as vesicles (Iqbal et al. 2011), which are closed, spherical systems—ranging from nanometers to micrometers in diameter—or as supported bilayers (Katsaras 1997, 1998), which are planar and therefore, highly suitable for experimental techniques such as x-ray and neutron diffraction (both in-plane and out-of-plane), and different types of microscopies. Monolayers prepared at the air–water interface using a Langmuir–Blodgett trough are simplified models of bilayers, and are useful experimental platforms all by themselves (Abraham et al. 2008).

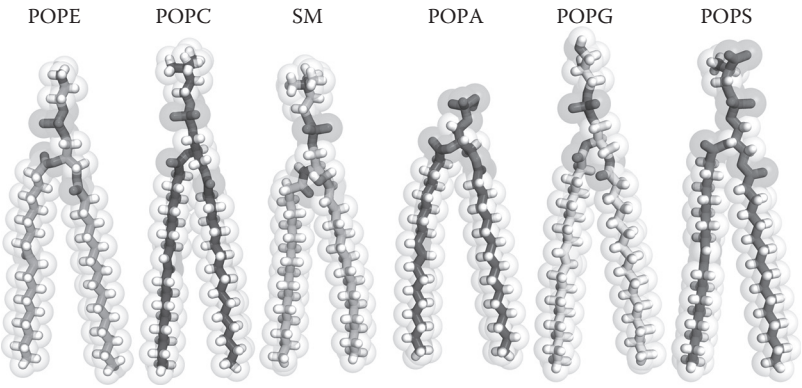

FIGURE 7.1 Molecular structures of common lipid species with different headgroup and backbone moieties.

7.2 MODELS OF BIOLOGICAL MEMBRANES

Danielli and Davson developed what is arguably the first widely accepted model of a biological membrane (Danielli and Davson 1935). In that model of the membrane, the lipid bilayer proposed by Gorter and Grendel (1925) was coated with a layer of protein, which was used to address Danielli's observations regarding the surface tension of lipid bilayers. However, the Danielli–Davson model proved to be inadequate (Hendler 1971), and was later modified by Danielli to address some of its obvious shortcomings (Danielli 1975). Later, using electron microscopy data, J. D. Robertson proposed a variant of the Danielli–Davson model of the membrane (Robertson 1957). More importantly, however, was his proposal that all biological membranes share a common three-layered structure (two protein layers adsorbed to the lipid bilayer), and are about 7.5-nm thick (Robertson 1959). This notion of universality was, and is a significant contribution to membrane biology.

On the basis of three-dimensional (3D) x-ray crystallographic structures of water-soluble proteins, electron micrographs of membranes, the role of hydrophobic amino acids, and their own studies of proteins with a high α-helical content, Lenard and Singer proposed the possibility that some proteins may be capable of spanning the lipid bilayer (Lenard and Singer 1966). Eventually, this notion was refined into the now well-known Singer–Nicolson or "fluid mosaic" model of the membrane (Singer and Nicolson 1972). Basically, this model retained the core lipid bilayer structure, but instead of proteins coating it, they were now permitted to reside within the two-dimensional (2D) fluid matrix, with lipids and proteins diffusing freely. Of course, no model is perfect and neither is the fluid mosaic model of the membrane. Since 1972, there have been a number of new ideas that have been added to it. For example, free diffusion of lipids and proteins within the membrane is often limited to a few tens of nanometers because of interactions with the cytoskeleton (Marguet et al. 2006). Moreover, functional domains (the so-called "rafts") may also exist within membranes. These domains may limit the diffusion of biomolecules and impart functionality. Although this topic of functional domains has been well scrutinized for more than three decades (Lingwood and Simons 2010), many questions still remain.

7.3 LIPID BILAYER STRUCTURE AND SDP MODEL

Currently, many individual lipid species have been identified along with their synthetic pathways and physiological relevance (Shevchenko and Simons 2010)—a few of the commonly studied lipids are shown in Figure 7.1. Lipids display a great range of chemical diversity, and the human plasma membrane, alone, is made up of thousands of lipid species (Quehenberger et al. 2010). As a result, fundamental questions regarding the origins of such lipid diversity have been the focus of much research (van Meer et al. 2008).

Lipid bilayers are supramolecular assemblies that can affect the functionalities of various membrane-associated proteins, either through specific chemical recognition or regulation of their bulk physical properties (e.g., hydrophobic thickness, curvature, and lateral pressure) (Phillips et al. 2009). Thus, the need to accurately quantify the structural properties of lipid bilayers is clear. In this regard, the scattering density profile (SDP) model that was developed by Kučerka et al. (2008a, 2012) using multiple contrast scattering data sets (i.e., different contrast small-angle neutron (SANS) and x-ray (SAXS) scattering data), in conjunction with molecular dynamics (MD) simulations, has proven especially useful in achieving this goal. Compared to traditional Fourier reconstruction of quasi-Bragg peaks, or model analysis using a single data set (i.e., neutron or x-ray), the data obtained from SDP model analysis have resulted in robust structural parameters for a number of different lipid bilayers (Heberle et al. 2012).

The underlying principle of the SDP model is schematically shown in Figure 7.2. The lipid bilayer is parsed into several components (Figure 7.2a), whose volume distributions are represented by analytical functions, such as Gaussians and an error function (Figure 7.2b). In addition, the volume distribution of the aqueous medium is determined through complementarity with the total distribution of the lipid bilayer. The one-dimensional (1D) electron density (ED) and neutron scattering length density (NSLD) profiles are then readily obtained by summing the products of the different volume distributions with the corresponding x-ray or neutron scattering amplitudes (i.e., the number of electrons or the neutron scattering length, respectively). By Fourier's transform, the 1D ED and NSLD profiles can then be directly compared to experimentally obtained form factors. The best estimated structural parameters are obtained when the difference between the model and the experimental data is minimized (Kučerka et al. 2008a). (For a detailed description of the SDP and other models used to describe scattering data, the reader is referred to the review by Heberle et al. (2012).)

It is known that the electrostatic potential at or near the membrane surface is instrumental to a number of biological processes (Mclaughlin 1989, Cevc 1990). For example, the enzymatic activity

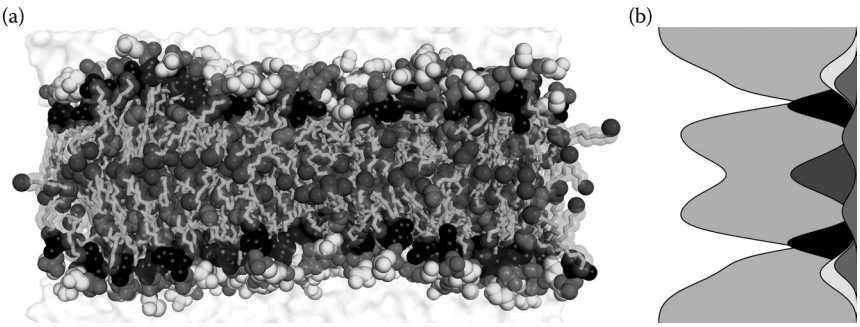

FIGURE 7.2 (**See color insert.**) Schematic description of the palmitoyl oleoyl phosphatidylglycerol (POPG) bilayer SDP model derived from MD simulations. (a) A snapshot of an equilibrated POPG bilayer obtained from MD simulations. In the hydrocarbon chain region, the methyl (CH_3), methylene (CH_2), and methine (CH) groups are depicted by the blue, cyan, and red spheres, respectively. The PG lipid polar headgroup is decomposed into three components, namely, the carbonyl–glycerol backbone (black spheres), the phosphate (magenta spheres), and the terminal glycerol (yellow spheres). The two layers of water flanking the lipid bilayer are depicted by the semitransparent surface representation. (b) Component volume distributions along the bilayer normal. Three Gaussians are used to describe the lipid headgroup, one each for the carbonyl–glycerol (black), phosphate (magenta), and terminal glycerol (yellow) moieties. The total hydrocarbon chain region (i.e., the sum of the CH_2, CH, and CH_3 groups) is represented by an error function. The CH (red) and CH_3 (blue) groups are each described by single Gaussians, which are then subtracted from the error function to obtain the CH_2 distribution (cyan). The distribution of water (gray) is obtained through the complementarity with the total distribution of the lipid bilayer, by requiring that all volume probabilities add up to unity at each point along the bilayer normal.

of Ca^{2+} lipid-dependent protein kinase C is strictly associated with the lipid's headgroup moiety (Newton 1993). Moreover, the orientation and topology of membrane proteins are found to depend on the total amount of anionic lipids present in a membrane (van Klompenburg et al. 1997). Other examples include the role of anionic lipids in the activation of the adenosine triphosphate (ATP)-sensitive potassium channel (Fan and Makielski 1997), in providing the most energetically favorable environment for voltage-sensing proteins (Schmidt et al. 2006), and in promoting peptide fibril formation (Olofsson et al. 2007). Suffice it to say, the charge state of the lipid headgroup is crucial in maintaining proper membrane function.

To systematically compare the specialized structural and functional properties of neutral and charged lipids, Kučerka et al. used the SDP model to look at the commonly studied phosphatidylcholine (PC, neutral) and phosphatidylglycerol (PG, monoanionic) lipids in their biologically relevant fluid phase (Kučerka et al. 2008a, Pan et al. 2012). They found that the area per lipid—a key structural parameter obtained from SDP model analysis, and which is used extensively in the validation of MD simulations—decreased with increasing hydrocarbon chain length, primarily the result of enhanced attractive van-der-Waals interactions between neighboring hydrocarbon chains. However, the introduction of an unsaturated bond in PC bilayers disrupted the packing of the hydrocarbon chains, resulting in increased lipid areas. An unexpected result was that despite their smaller headgroup volume, monoanionic PG lipids possessed larger areas per lipid than their zwitterionic PC counterparts, implying that the long-range electrostatic interactions between charged headgroups play a prominent role in governing lipid lateral packing. By extrapolating to infinite chain length, Pan et al. discovered that areas per lipid of equivalent PC and PG lipids differed very little between the two different headgroup lipids, suggesting that the same steric interactions are experienced by both (Pan et al. 2012). In fact, the data indicated that the glycerol–carbonyl backbone is primarily responsible for the lateral packing of lipids at infinite chain length. This notion is particularly informative when one considers the broad spectrum of membrane properties that are most likely affected by the glycerol backbone, a moiety that effectively delineates the membrane–water interface.

7.4 NONUNIVERSAL BEHAVIOR OF CHOLESTEROL'S EFFECT ON BILAYER BENDING MODULUS

A mechanical property that is essential for proper membrane function (e.g., cell endocytosis and exocytosis) is the membrane's bending modulus, which is defined as the energy required to deform a membrane from its intrinsic curvature. Spontaneous, highly curved membranes are often formed during viral infection (e.g., stalk formation) and during the initial steps of cell division. To facilitate and maintain these highly curved structures, modifications to the membrane's bending modulus are made.

Membrane softening accompanied by enhanced fluctuations has been experimentally observed in a number of model membranes upon the introduction of small peptides (Tristram-Nagle and Nagle 2007, Pabst et al. 2007, Pan et al. 2009a). In contrast, cholesterol, a rigid molecule with a flexible hydrocarbon tail (Figure 7.3), is thought to order neighboring, fluid hydrocarbon chains, resulting in thicker and more rigid bilayers. Examining palmitoyl oleyol phosphatidylcholine (POPC) (16:0–18:1 PC) bilayers with different sterols, Henriksen et al. suggested a simple relationship between a bilayer's hydrocarbon thickness and its bending modulus (Henriksen et al. 2006). Studies carried out by Pan et al., however, found that the Henriksen et al. relationship broke down when lipids with a different number of unsaturated chains were considered (Figure 7.3) (Pan et al. 2008, 2009c). Specifically, they found that although cholesterol increases the bilayer thickness of all fluid bilayers, its effect on bilayer bending modulus was intimately associated with the degree of fatty acid chain unsaturation. For example, when both hydrocarbon chains were saturated (e.g., dimyristoyl phosphatidylcholine (DMPC), di-14:0 PC), the addition of cholesterol dramatically increased the bilayer's bending modulus. This increase was, however, less pronounced in the case of lipids with only one monounsaturated chain (e.g., POPC), and was nonexistent in bilayers made up of lipids with two monounsaturated hydrocarbon chains (e.g., dioleoyl phosphatidylcholine (DOPC), di-18:1

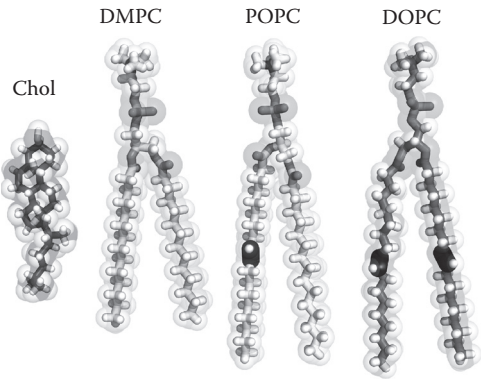

FIGURE 7.3 Molecular structures of cholesterol (Chol), DMPC (di-14:0 PC), POPC (16:0–18:1 PC), and DOPC (di-18:1 PC). Unsaturated bonds are indicated by dark sticks.

PC)—a result that was later reproduced by others (Sorre et al. 2009, Gracia et al. 2010). In contrast to conventional wisdom (i.e., that cholesterol always increases a bilayer's bending modulus), the Pan et al. results made the point that cholesterol's effect depends on the type of bilayer in which it resides in (Pan et al. 2009c).

7.5 LIPID COMPOSITION DETERMINES CHOLESTEROL'S ORIENTATION IN MODEL MEMBRANES

Unsaturated fatty acids contain one or more carbon–carbon double bonds. The presence of double bonds affects the membrane in two ways: first, in the typical *cis* configuration, double bonds introduce a rigid bend in the acyl chain, making it difficult for hydrocarbon chains to tightly pack, with the end result being a more fluid (disordered) membrane—it should be emphasized that membrane fluidity is thought to be critical for proper cell function. In everyday experience this effect is reflected in the difference between butter (a solid) and vegetable oil (a liquid), for example. In microbes, altering lipid composition is an important stress–response mechanism, for example, when organisms adapt to changes in temperature and/or their surrounding environment (e.g., biofuels) (Heipieper et al. 2007, Torres et al. 2011). In plants, changes in membrane fluidity are associated with temperature sensing (Saidi et al. 2011, McClung and Davis 2010, Ruelland and Zachowski 2010) and acclimation (Upchurch 2008, Zhou et al. 2010), while in humans they have been associated with cancer (Baritaki et al. 2007), neurodegenerative (Maccarrone et al. 2011), and amyloid diseases (Eckert et al. 2010); second, double bonds introduce chemically reactive sites in membranes, which affect not only the double bond but also its neighboring (allylic) carbon–hydrogen bonds. These bonds are the source of controlled reactions involving cellular enzymes, and uncontrolled reactions involving reactive oxygen species (ROS). The cellular pathways are often associated with signaling, while the ROS pathways are associated with cytotoxic defense mechanisms, as well as aging and disease (Cheng and Li 2007).

The importance of polyunsaturated fatty acids (PUFAs)—hydrocarbon chains with more than one double bond—is becoming increasingly evident in biology (Soni et al. 2008, Wassall and Stillwell 2008). For example, PUFA-containing lipids are found in neural and retinal membranes, where they are essential for proper function (Aveldano 1989, Stillwell and Wassall 2003). In addition, many chronic conditions and disease states can be alleviated through the consumption of foods rich in PUFAs, resulting in increased levels of PUFA-containing lipids in the plasma membrane.

Cholesterol is found in all mammalian cells and is obtained either through diet (e.g., animal fats), or manufactured by various cells, predominantly those in the liver. It is required for building and maintaining cell membranes, regulates their fluidity, and may even act as an antioxidant (Smith 1991). A role often associated with cholesterol in membranes is the organization of their molecular

structure. It is well known that the introduction of cholesterol to gel phase disaturated PC bilayers (e.g., di-16:0 PC, DPPC) disrupts the regular packing of their fatty acid chains, but restricts their reorientation in liquid crystalline bilayers (Vist and Davis 1990). Nominally, in membranes, cholesterol is oriented in its understood upright orientation, with its 3β-hydroxyl group locating just below the lipid–water interface (Leonard et al. 2001). In biological membranes, dynamic, functional domains (i.e., rafts) are thought to exist, and cholesterol has been identified as a key component of lipid rafts in mammalian cell membranes (Silvius 2003).

Although it has been shown that cholesterol interacts differentially with a number of membrane lipids—for example, interacting vigorously with high-transition temperature sphingolipids and phospholipids, and less so with lipids containing unsaturated fatty acid chains (McMullen and McElhaney 1996, Brown and London 2000)—cholesterol's interaction with PUFA-containing phospholipids is less well understood. Over the past few years, Harroun et al. (2006b, 2008) and Kučerka et al. (2009, 2010) have studied the orientation of cholesterol in different PC bilayers, including those composed of PUFAs. Using neutron scattering in conjunction with "headgroup" and "tail" deuterated cholesterol, the studies by Harroun et al. demonstrated that although cholesterol assumes its nominal upright orientation (Leonard et al. 2001) in POPC (16:0–18:1 PC), DOPC (di-18:1 PC), and 18:0–20:4 PC bilayers (Figure 7.4a), it sequesters itself into the middle of PUFA (di-20:4 PC) bilayers (Figure 7.4b) (Harroun et al. 2006b, 2008). This differential behavior by cholesterol in the different PC bilayers was rationalized in terms of the high degree of disorder commonly associated with PUFAs, whereby they created an environment that was not amenable to cholesterol's rigid steroid moiety to remain near the lipid–water interface. Subsequent coarse-grained MD simulations confirmed the Harroun et al. result, albeit with a slight twist (Marrink et al. 2008). Namely, while the neutron scattering studies located cholesterol exclusively in its nominal upright orientation in all bilayers—the exception being the PUFA bilayer, where cholesterol sequesters itself into the bilayer's center (i.e., flat orientation)—the MD simulations noted that cholesterol experienced flip-flop rates in all bilayers, ranging from nanoseconds to microseconds in PUFA and POPC bilayers, respectively (Marrink et al. 2008).

Kučerka et al. (2009, 2010) followed up on the Harroun et al. (2006b, 2008) studies by doping cholesterol-containing PUFA bilayers with increasing amounts of either POPC or DMPC (di-14:0 PC). They reported that it took the addition of almost 50 mol% of POPC into cholesterol-containing PUFA bilayers to induce cholesterol to flip from its location in the bilayer center to its upright orientation. Conversely, only 5 mol% of DMPC achieved the same result (Kučerka et al. 2009). Moreover, MD simulations performed on similar systems predicted the formation of DMPC-rich domains in which cholesterol preferentially locates in its upright orientation, while in DMPC-depleted domains, cholesterol was found predominantly in its flat orientation (Kučerka et al. 2010). These experimental and simulation studies (Harroun et al. 2006b, 2008, Kučerka et al. 2009, 2010)

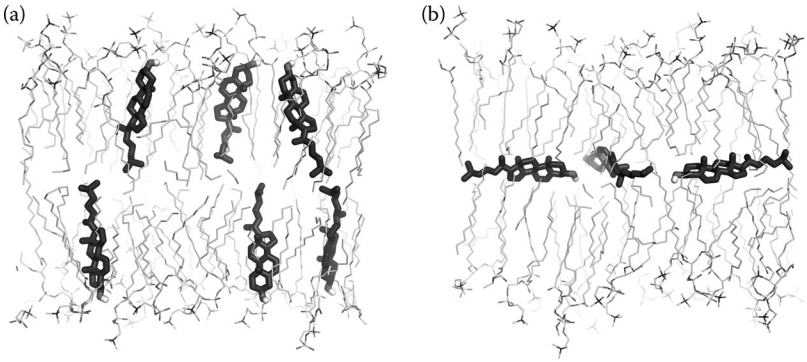

FIGURE 7.4 Two orientations of cholesterol in a lipid bilayer. (a) The nominal upright orientation. (b) The flat orientation where cholesterol sequesters itself into the middle of the bilayer.

are a clear evidence that a biological membrane's lipid composition not only affects the membrane's in-plane organization (i.e., domains), but that a molecule's orientation (i.e., function) in a membrane may depend on the domain in which it resides, highlighting the importance of lipid diversity as it pertains to the proper functioning of biological membranes.

7.6 ALIGNABLE MODEL MEMBRANES BY EXTERNAL FIELDS AND SOME OF THEIR APPLICATIONS

Lipid heterogeneity affects not only the conformation of biomolecules in membranes, but also the aggregate morphology in which the lipids find themselves in. An example of this is the long- and short-chain lipid mixture, known as "bicelles" (bilayered micelles), and first referred to as such by Sanders and Landis (1995). For the most part, bicellar mixtures have been commonly used for the structural determination of membrane-associated proteins (Ujwal and Bowie 2011, Faham and Bowie 2002, Andersson and Mäler 2002, Zandomeneghi et al. 2003, Seddon et al. 2004, Marcotte and Auger 2005, Prosser et al. 2006, Diller et al. 2009, Halskau et al. 2009, Matsumori and Murata 2010, Warschawski et al. 2011), and there are several unique properties which make bicelles attractive for studying such proteins. For example, they can be uniformly dispersed in water at a much higher total lipid concentration, C_{lp} (>30 wt.%) than pure long-chain lipid solutions (Ram and Prestegard 1988, Sanders and Prestegard 1990), thus greatly enhancing the concentration of membrane proteins. Moreover, since a biological membrane's underlying structure is a lipid bilayer, bicellar mixtures lend themselves as a better membrane mimic for membrane-associated proteins than commonly used detergent-based substrates.

One of the most attractive features of bicellar mixtures, especially for nuclear magnetic resonance (NMR) studies, is their ability to align in the presence of a strong magnetic field. In fact, magnetically aligned lipid mixtures have been reported as early as the late 1970s (Sanders and Schwonek 1992, Forrest and Reeves 1979, 1981). It is well known that the alignment of bicellar mixtures is strongly dependent on temperature (T). If T is lower than the melting transition temperature, T_M, of the long-chain lipid, the mixture is in a morphology that is not capable of being aligned in the presence of an external magnetic field. However, as T approaches, or is even slightly greater than T_M, the system's viscosity increases and it aligns in a manner that its bilayer normal ($\mathbf{N_B}$) is perpendicular to the external magnetic field ($\mathbf{M}$) (i.e., $\mathbf{N_B} \perp \mathbf{M}$) (Forrest and Reeves 1979, 1981, Sanders and Prestegard 1990, Ram and Prestegard 1988, Vold and Prosser 1996)—lipid molecules are diamagnetic. While such an alignment restricts $\mathbf{N_B}$ in one plane, the membranes form a powder with respect to the magnetic field, as shown in Figure 7.5a. However, Prosser et al. found that by doping small amounts of lanthanide (paramagnetic) ions, such as Tm^{3+}, Er^{3+}, Yb^{3+}, or Eu^{3+} into bicellar mixtures, they were able to alter the orientation of $\mathbf{N_B}$ from $\perp\mathbf{M}$ to $\parallel \mathbf{M}$, as shown in Figure 7.5b (Prosser et al. 1998, 1996). As a result, the freedom of $\mathbf{N_B}$ is reduced to one orientation, making the system a more utile "goniometer" for

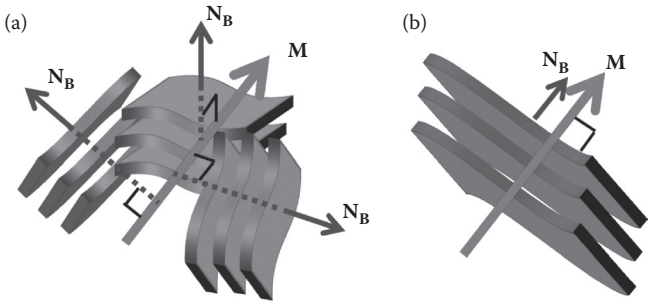

FIGURE 7.5 Bilayers align their normals, $\mathbf{N_B}$ either (a) perpendicular ($\mathbf{N_B} \perp \mathbf{M}$) to or (b) parallel ($\mathbf{N_B} \parallel \mathbf{M}$) with the external magnetic field, $\mathbf{M}$ depending on whether or not they are doped with paramagnetic lanthanide ions—lanthanide ions result in the $\mathbf{N_B} \parallel \mathbf{M}$ scenario.

structural studies of membrane-associated proteins. It has been reported that the temperature range in which these lipid mixtures are magnetically alignable depends on the charge density of the membrane, that is, mixtures with a higher molar ratio of charged lipids or paramagnetic ions (Nieh et al. 2002).

Compared with solid substrate aligned bilayers, magnetically aligned membranes provide a more biomimetic membrane environment for integral proteins. However, the requisite strong magnetic fields can only be realistically implemented using only a few characterization techniques (e.g., NMR and SANS). (It should be mentioned that a 0.9 T fixed field device was developed by Harroun et al. for use with standard optical microscopes (Harroun et al. 2006a).) Nieh et al. also examined a lanthanide-free charged bicellar mixture and found that the presence of an oscillating shear flow induced good alignment in membranes, with their $\mathbf{N_B}$ aligning parallel to the direction of the shear (Nieh et al. 2003). Importantly, alignment persisted for a period of hours after shear flow ceased, thus greatly simplifying the apparatus needed for inducing alignment. In doing so, this method of alignment may enable a number of other physical techniques to interrogate these interesting and versatile systems.

In bicellar mixtures, membrane alignability is closely associated with aggregate morphology. Over the years, the so-called alignable morphology has evolved from disk-like micelles (Vold and Prosser 1996, Sanders and Schwonek 1992, Forrest and Reeves 1979, 1981, Sanders and Prestegard 1990, Ram and Prestegard 1988), to bilayered ribbons (Nieh et al. 2004, Soong et al. 2010, Harroun et al. 2005, van Dam et al. 2004), to perforated lamellae in the case of charged systems (Katsaras et al. 2005, Nieh et al. 2001)—these results were derived from a combination of cryogenic transmission electron microscopy (van Dam et al. 2004), NMR (Gaemers and Bax 2001), and SANS studies (Harroun et al. 2005, van Dam et al. 2004, Katsaras et al. 2005). The currently accepted structural diagrams for both zwitterionic and charged bicellar mixtures, derived from SANS data, are shown in Figure 7.6 (Nieh et al. 2005). Generally speaking, and with the exception of zwitterionic systems at low C_{lp} where multilamellar vesicles (MLVs) are observed (Figure 7.6a), bilayered micelles are found at low temperatures. In a zwitterionic system (Figure 7.6a), MLVs and ribbons, or nonswellable lamellae composed of ribbons (Nieh et al. 2001, 2005), are found at low and high C_{lp} samples, respectively (Gaemers and Bax 2001), while further increases in temperature result in the presence of MLVs at all C_{lp}. In charged bicellar mixtures (Figure 7.6b), as T increases, unilamellar vesicles (ULVs) and perforated lamellae that are capable of taking up water are found in low and high C_{lp} samples, respectively (Gaemers and Bax 2001). These morphological transitions are closely related to the location of the short-chain lipid in the aggregate morphology. Presumably, segregation of the short-chain lipid from the long-chain lipid (as a result of the immiscibility between liquid

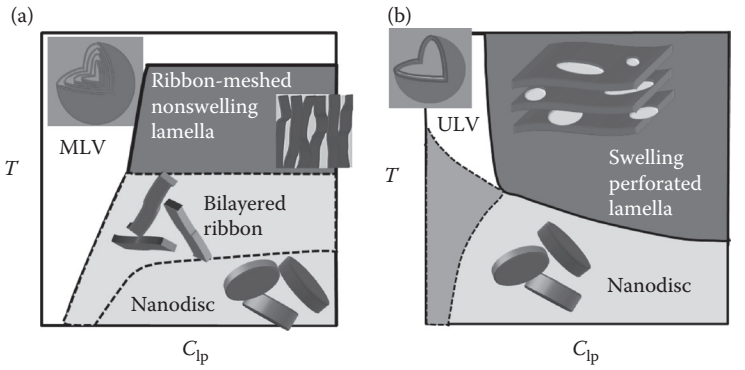

FIGURE 7.6 (**See color insert.**) Structural diagrams of (a) zwitterionic and (b) charged bicellar mixtures. The range of magnetically alignable structures is indicated by the red regions. The dashed lines represent not well defined boundaries, and the gray region in (b) consists of either bilayered micelles or ULVs, depending on the system's charge density.

disordered and gel phases) favors structures with a larger total circumference, as the short-chain lipid, which has a larger spontaneous curvature, is able to stabilize the high curvature edge.

Besides aligning membrane proteins, bicellar mixtures have also been used as substrates to crystallize membrane-associated proteins. For example, Fahama and Bowie successfully used a bicellar mixture to crystallize bacteriorhodopsin extracted from *Halobacterium salinarum*. They took advantage of the inherent low-viscosity solution formed by bilayered micelles at low *T*, which allowed the application of general screening methods (Faham and Bowie 2002). For further details regarding this method of crystallizing proteins, the reader is referred to the review by Ujwal and Bowie (2011), while other applications of bicellar mixtures, as studied by NMR and other spectroscopic techniques, are summarized in Table 7.1.

TABLE 7.1
Composition of Bicellar Mixtures and Their Applications

Long Chain/Short Chain	Applications	References
DMPC/DHPC	Structural determination of membrane-associated molecules by NMR	Andersson and Mäler (2002), Zandomeneghi et al. (2003), Marcotte and Auger (2005), Prosser et al. (2006)
	Aligning water-soluble proteins	Ottiger and Bax (1998a, b, 1999), Martin-Pastor and Bush (2000)
	Protein crystallization	Faham and Bowie (2002), Ujwal and Bowie (2011)
	Separation and sensor devices	Mills and Holland (2004), Pappas and Holland (2008), Luo et al. (2010)
	Application to skin	Barbosa-Barros et al. (2008a), Rodríguez et al. (2010, 2011)
DMPC/CHAPS	Structural determination of membrane-associated molecules	Booth et al. (1997), Sugiyama et al. (1999), Kim et al. (2001), Renthal and Velasquez (2002), Andersson et al. (2007), McKibbin et al. (2007), Gayen and Mukhopadhyay (2008), Krishnamani et al. (2012)
DMPC/CHAPSO	Structural determination of membrane-associated molecules	Sanders and Prestegard (1990, 1991), Aubin et al. (1993), Salvatore et al. (1996), Chen and Gouaux (1999), Kawaguchi et al. (2003), Wang et al. (2012)
	Protein crystallization	Faham et al. (2005)
DPPC/DHPC	Structural determination of membrane-associated molecules	Lind et al. (2008)
	Protein/drug carrier	Nieh et al. (2006), Rubio et al. (2011)
	Application to skin	Barbosa-Barros et al. (2008b, 2009), Rodríguez et al. (2010, 2011)
	Carbon nanotube assembly	Wallace and Sansom (2009)
DLPC/DHPC	Structural determination of membrane-associated molecules	Lind et al. (2008)
DLPC/CHAPSO		Wang et al. (1998)
POPC/DHPC		Chou et al. (2004), Wang et al. (2004)
DPC/SDS		Baek et al. (2011)
DMLPC/DHPC	Structural characterization of bicellar mixtures	Aussenac et al. (2005)
DIOMPC/DIOHPC		Evanics and Prosser (2005)
SM/DHPC		Yamaguchi et al. (2012)
TBBPC/DHPC		Loudet et al. (2010)

7.7 LIPID BILAYER DETERMINES ANTIMICROBIAL PEPTIDE ORGANIZATION

Antimicrobial peptides (AMPs) are a class of small molecules which are capable of disrupting biological membranes through a number of different mechanisms. However, their ability to differentiate between eukaryotic and prokaryotic membranes makes them promising therapeutic agents against certain pathogens importantly, without inducing drug resistance—an often occurring problem with drugs targeting a specific protein or gene.

Alamethicin (Alm) is a small AMP that spontaneously aggregates to form a membrane-spanning bundle (Figure 7.7). To compensate for the hydrophobic mismatch between the bilayer's hydrophobic core and the protein's embedded hydrophobic domain, the membrane is deformed—this is because the peptide is stiffer than the bilayer. The energy cost associated with such deformation depends on the membrane's thickness, its bending and area stretch moduli, and its intrinsic curvature.

Pan et al. studied how the Alm bundle structure behaves in two lipid bilayers, namely, di-18:1 PC and di-22:1 PC (Pan et al. 2009b). These bilayers have similar physical properties, except that the di-22:1 PC bilayer is about 7 Å thicker than di-18:1 PC. It was found that Alm forms a hexametric bundle in di-18:1 PC, while a nonamer structure was discovered in di-22:1 PC. The smaller Alm bundle in di-18:1 PC was the result of hydrophobic thickness matching between di-18:1 PC bilayers and Alm—as mentioned, the hydrophobic thickness of di-22:1 PC bilayers is 7 Å larger. This notion is consistent with the well-known functional cutoff effect (Balgavý and Devínsky 1996) observed, for example, in Ca^{2+}-transporting ATPase incorporated in lipid bilayers (Karlovská et al. 2006). The proper function of a membrane protein in a biological membrane, thus depends on the structural and dynamical properties of the underlying lipid matrix.

The close interplay between the lipid matrix and associated AMPs has also emerged from other studies. Sani et al. reported that lipid composition is an important regulator in controlling maculatin 1.1's conformation and orientation (Sani et al. 2012). In the case of zwitterionic PC lipid bilayers, the peptide's helical content—a good indicator of the peptide's interaction potential with lipid bilayers—was found to depend on lipid hydrocarbon chain length and degree of unsaturation, while in anionic lipid bilayers, maculatin 1.1 interacted strongly and oriented orthogonal to the bilayer normal. In another study involving a PC/PG mixture and the cationic AMP, aurein, Cheng et al. found that AMP–membrane interactions were affected by the amount of charged PG lipid present and the hydrophobic thickness of the lipid bilayer (Cheng et al. 2009). More recently, MD simulations of gramicidin A in different lipid bilayers have shown a radial dependence of lipid bilayer perturbation, induced by the addition of gramicidin A (Kim et al. 2012).

From these studies, one can surmise that AMP organization and function are to a great extent regulated by the host lipid bilayer through a variety of chemical and physical interactions. In-depth studies of AMP interactions with model membranes are paving the way in deciphering how AMPs

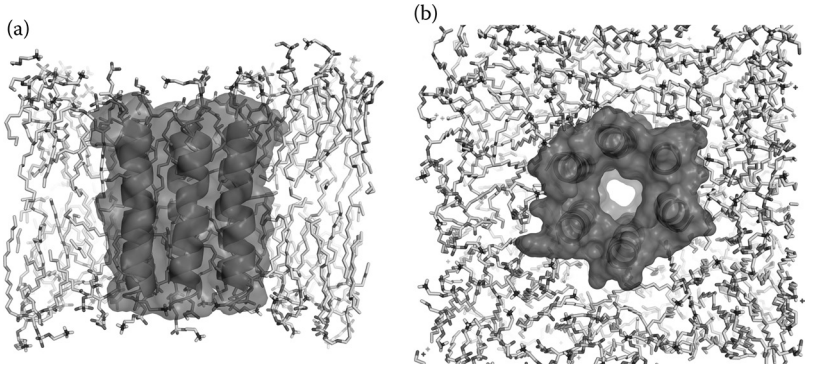

FIGURE 7.7 The Alm hexameric structure in a lipid bilayer. (a) Side view and (b) top view.

interact with the different lipid species that make up biological membranes. We are of the belief that such knowledge will prove to be invaluable when designing and developing more effective peptide-based antibiotics.

7.8 ION-SPECIFIC EFFECTS IN BACTERIAL MEMBRANES

In addition to lipid–peptide (and lipid–protein) interactions, the significance of the aqueous phase for the proper function of biological membranes cannot be overestimated. Biological membranes are surrounded by an electrolytic liquid containing Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- ions. Their interactions with cell membranes are understood to influence, for example, the gating of ion channels, membrane fusion, and membrane fluidity, to name a few. Over the years, there have been copious amounts of biophysical reports demonstrating that ions affect the physical properties of lipid bilayers (see, e.g., Pabst et al. 2010, for a recent review).

The effect of Ca^{2+} cations was recently reported in bacterial mimetic membranes composed of lipopolysaccharides (LPSs) (Kučerka et al. 2008b). LPSs are the major lipid component making up the outermost leaflet of the asymmetric outer membrane (OM) of Gram-negative bacteria (Wilkinson 1996, Nikaido 2003). It contributes to the OM's structural integrity and also protects the bacteria from a variety of toxic molecules, such as certain antibiotics (e.g., penicillin), digestive enzymes (e.g., lysozyme), detergents, heavy metals, bile salts, and some dyes. The passage of nucleotides, disaccharides, amino acids, vitamins, and iron for nutritional growth are usually transported through the OM by porin proteins, but it is LPS that provides the bacteria with its remarkable selectively permeable membrane that is resistant to a variety of deleterious agents. In particular, *Pseudomonas aeruginosa* is well noted for its recalcitrance to conventional antibiotic therapy, partly as a result of its unique surface chemistry (Rocchetta et al. 1999). For this reason, and also due to the ubiquity of *P. aeruginosa* and its impact upon health as both an opportunistic and nosocomial pathogen, this organism represents an attractive candidate for medical and pharmacological studies.

Although LPS molecules are structurally diverse, they share a common architecture composed of three basic units. The first is a lipid A moiety that anchors the LPS molecule into the hydrophobic domain of the OM. It consists of two phosphorylated glucosamine units that are typically acylated with four to six fatty acids and is considered to be responsible for most of the toxicity associated with LPS. Second, the LPS' core oligosaccharide is made up of 8–12 monosaccharide units, and is connected to lipid A by a 2-keto-3-deoxyoctonoic acid (Kdo). Finally, the third part is formed by repetitive monosaccharide subunits (i.e., O-side chain), which are responsible for much of the bacterium's immunospecificity (Caroff and Karibian 2003). However, recent experiments revealed a determining effect of counterions involved in the system.

Small-angle neutron diffraction (SAND) data showed that water penetrates Ca^{2+}–LPS bilayers to a lesser extent than Na^+- and Mg^{2+}–LPS bilayers (Kučerka et al. 2008b). While Ca^{2+} cations make LPS bilayers more compact and less permeable to water, a significant amount of water penetrates deep into Mg^{2+}–LPS and Na^+–LPS bilayers, including the bilayer's hydrophobic core (Figure 7.8). It is believed that such increased levels of hydration could be associated with enhanced biological activity in these bacterial membranes. As such, a more accurate determination of the membrane's structure may allow for a better understanding of membrane function. For example, differences in a bilayer's permeability to water could have implications with regard to how small molecules permeate through the OM of Gram-negative bacteria, aiding in the development of more effective antibiotics.

7.9 CONCLUSIONS

Cell membranes possess a ubiquitous bilayer architecture that is vital for proper biological function. Lipids make up the underlying membrane scaffold enabling proteins to carry out their various

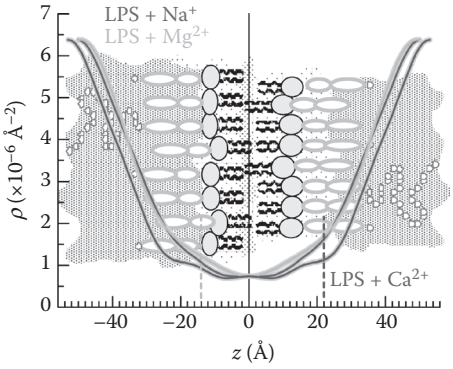

FIGURE 7.8 (**See color insert.**) 1D NSLD profiles obtained from the Fourier reconstruction of diffraction data from oriented LPS bilayers hydrated in 100% D_2O. The solid red and green lines correspond to Na^+- and Mg^{2+}–LPS bilayers, respectively, whereas the solid blue line corresponds to Ca^{2+}–LPS bilayers. The schematic shows the structural model of Na^+- and Mg^{2+}–LPS bilayers on the left, and Ca^{2+}–LPS on the right. The dashed lines demarcate the limits of water penetration.

functions. Membrane heterogeneity (e.g., lateral compartmentalization and leaflet asymmetry) that is essential for cell signaling and trafficking, can be achieved by dynamically recruiting/expelling specific lipids into/from functional compartments. Thus, to better understand the underpinning driving force of lipid homeostasis and to enhance our predictability of more complex biomembrane events, we presented a few examples illustrating how lipid diversity affects membrane organization. These ranged from pure lipid bilayer systems to ones containing other biomolecules.

Specifically, we presented a transverse lipid bilayer model that deconstructs a disordered fluid lipid bilayer into a mathematically manageable number of moieties. The resultant SDP model enabled us to precisely determine the structural properties of bilayers, including the much sought-after area per lipid. From such physical studies, biologically relevant insights can be obtained. For example, net-charged bilayers highlighted the importance of electrostatic interactions in governing lipid lateral packing. Importantly, as naturally occurring lipids have different headgroup moieties with a different net charge (e.g., neutral PE and PC, monoanionic PG and PS, and dianionic cardiolipin), distinct localized structures, and therefore functions, can be elicited by compartmentalizing compositional diverse lipids with differing headgroups. We then went on to describe the close interplay between lipid matrix and a well-known membrane modulator, namely, cholesterol. The prevailing concept of cholesterol's rigidifying effect on membrane flexibility (i.e., bending modulus) was challenged when different degrees of hydrocarbon chain unsaturation were considered. For example, lipid bilayers with dimonounsaturated chains were found to be equally stiff in the presence or absence of 30 mol% cholesterol. In an extreme case where lipid hydrocarbon chains possess multiple unsaturated bonds (i.e., PUFA), cholesterol was found to segregate in the middle of the lipid bilayer. However, the nominal upright orientation of cholesterol was retrieved by doping with less unsaturated lipids. This clearly implies that the composition of a lipid's hydrocarbon chains is important in modulating the orientation of biomolecules. A unique lipid mixture (i.e., micelle) was included in this chapter to illustrate how simple lipid mixtures can assume a number of different morphologies, simply altering temperature or lipid concentration. The micellar system has served as an important platform for a broad spectrum of biochemical and biophysical studies by providing a biomimetic membrane environment for membrane peptides and proteins. The active role played by the lipid matrix in regulating peptide/protein organizations was illustrated by reporting on the Alm bundle size in two lipid bilayers. It was found that by altering lipid bilayer thickness, Alm assumes a different membrane-spanning bundle structure. As each lipid has its own unique structure (e.g., thickness), different membrane thicknesses required for proper

membrane function can be achieved by varying lipid composition. Finally, we showed that not only the membrane, but also ions in the aqueous medium surrounding the biomembrane play an important role in modulating membrane structure and function.

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