Chapter 3
Water and Lipid Bilayers

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Abstract *Water is crucial to the structure* and function of biological membranes. In fact, the membrane's basic structural unit, i.e. the lipid bilayer, is self-assembled and stabilized by the so-called hydrophobic effect, whereby lipid molecules unable to hydrogen bond with water aggregate in order to prevent their hydrophobic portions from being exposed to water. However, this is just the beginning of the lipid-bilayer-water relationship. This mutual interaction defines vesicle stability in solution, controls small molecule permeation, and defines the spacing between lamella in multi-lamellar systems, to name a few examples. This chapter will describe the structural and dynamical properties central to these, and other water- lipid bilayer interactions.

Keywords Permeation • Water distribution • Dynamics

3.1 Water at the Interface of Model Membranes

Lipid-water interactions are ubiquitous in biological systems; our goal is to discuss lipid bilayer-water interactions described in the literature, and more precisely, the ways in which water and lipid bilayers mutually define the structure and dynamics of the lipid-water interface. In fact, the importance of water is such, that bilayer-bilayer interactions have been modeled as the interaction of their associated water shells (Leikin et al. 1993). Keeping this in mind, we begin our discussion with the properties of water at the interface of model membranes.

The structure of interfacial water can be described in a number of ways – depending on how one chooses to approach the system. The classical double layer (Debye and Hückel 1923) description of a lipid bilayer in an aqueous solution, the

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solvent is treated as a continuum and the ions in solution as point charges. Some of these ions are attracted to the bilayer surface, where they act as immobile point charges, screening the membrane. The free ions and solvent molecules near the membrane's surface make up the so-called diffuse layer, and are influenced by the bilayer's net electric field. Within this diffuse layer is the slip plane, a boundary separating the region dynamically associated with the lipid bilayer surface from the mobile region which intermingles with the bulk solvent. The electrical potential at the slip plane – the bulk boundary interface – is known as the zeta potential (ζ). Although ζ is not a true measure of surface charge, it is useful in estimating particle stability. In the case of lipid vesicles, ζ is usually in the range of +/−100 meV, with highly charged particles (−25 mV > ζ or ζ > 25 mV) being more stable in solution.

The spatial extent to which a lipid bilayer electrostatically influences an aqueous solution is defined as the Debye length, κ^{-1}, and is expressed as:

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_B T}{2 N_A E^2 I}}, \tag{3.1}$$

where ε_r is the dielectric constant, ε_0 is the permittivity constant, I is the ionic strength, N_A is Avogadro's number, $k_B T$ is the temperature and Boltzman constant, and E is the elementary unit of charge. The Debye length is typically on the order of one to several nanometers for vesicle and bilayer systems. This is an important consideration when it comes to understanding the effects of ions in screening the surface charge of lipid bilayers. For example, large amounts of salt increase the ionic strength, thus decreasing the Debye length. This decrease in κ^{-1} allows adjacent bilayers to approach closer to each other prior to experiencing electrostatic repulsion. This notion has been borne-out by numerous experiments studying multiple lamellae and vesicle suspensions.

The resulting forces (e.g., van der Waals attractive) between charged surfaces interacting through a liquid medium are quantitatively described by the DLVO theory (Verwey et al. 1948). In the case of two vesicles of radius, r, and separated by a distance, D, one can calculate the interaction energy between the two vesicles through the approximation of the attractive van der Waals force as follows:

$$\Phi_{\mathrm{vdW}}(\mathrm{D}) = \frac{-\mathrm{Ar}}{12\mathrm{D}}, \tag{3.2}$$

where A is the Hamaker constant. Similarly, one can define the repulsive electrostatic force as:

$$\Phi_{\mathrm{R}}(\mathrm{D}) = 2\pi\varepsilon_{\mathrm{r}}\varepsilon_0 \mathrm{r}\mathrm{E}^2 \mathrm{e}^{-\kappa \mathrm{D}}. \tag{3.3}$$

By combining these two equations, we get the interaction energy between the two spheres:

$$\Phi(\mathrm{D}) = \Phi_{\mathrm{vdW}}(\mathrm{D}) + \Phi_{\mathrm{R}}(\mathrm{D}). \tag{3.4}$$

This treatment is useful in providing an intuitive feel for the role of ions and water, and how they interact at the interface of a lipid bilayer, influencing its stability and enabling the formation of other aggregate morphologies (e.g., stalks, vesicles, etc.).

Beyond this, ions screening the bilayer surface are also known to bind to the head group carbonyl oxygen, as was shown in POPC (16:0–18:1 phosphatidylcholine) bilayers, creating larger interacting lipid complexes (Böckmann et al. 2003). This aggregation of lipid molecules increases the ordering of the acyl chains, and results in thicker bilayers with reduced lipid in-plane mobility. Ions in solution have also been proposed to affect water structure and diffusional rates within the bilayer (Kausik and Han 2011), consistent with what has been observed in the bulk (Ishai et al. 2013). Perhaps the most notable example of this is the role of the water-ion complex in the selectivity of ion channels (Doyle et al. 1998).

It is clear, however, that descriptions treating the interface in a manner similar to the bulk fail to describe many of the molecular details regarding ions, lipids, and more importantly, the solvent. Alternative perspectives of understanding the interface, such as the introduction of water sub-phases to which one can ascribe anomalous properties (Disalvo et al. 2008), can be useful. In doing so, one can develop models which better predict the structural changes of water and the resultant modified hydrogen bond network (Lelkes and Miller 1980; Gawrisch et al. 1992; König et al. 1994; Zhou and Schulten 1995; Swenson et al. 2008).

In light of these developments, others have sought to understand the repulsive force between bilayers microscopically, i.e., based on the local restructuring of water by the bilayer. Experimental evidence of structured water was first observed using NMR in the studies of Finer and Darke (1974), and was later theoretically interpreted by the order parameter model of Marčelja and Radić (1976). This model uses a free energy approach, relating the imposed structural order of water at the interface to disordered bulk water to the repulsive hydration pressure, P_h, between two surfaces. This is written as;

$$P_h = -\partial G\left(d_f\right)/\partial\left(d_f\right) \tag{3.5}$$

where d_f is the fluid space between adjacent bilayers and $G(d_f)$ is the excess Gibbs free energy per unit volume. The minimum of this expression for repulsive pressure can be used to define the equilibrium spacing of adjacent bilayers when evaluated fully (Rand and Parsegian 1989).

The order parameter is defined by the geometrical and dynamical restrictions imposed by the bilayer, thereby bringing in to play the presence of hydrogen bond partners, hydrogen bond lifetimes, and dynamical retardation factors. Interestingly, this result is equivalent to the Debye model when evaluated at distances much greater than the order parameter, λ, which is expressed below using the nomenclature of McIntosh and Simon:

$$P_h \approx 4P_o \mathrm{e}^{-d_f/\lambda} \tag{3.6}$$

Here λ is the length scale of the order parameter (McIntosh and Simon 1994) and P_0 is a function of the solvent and surface properties. P_0 can be expanded as follows:

$$P_o = 2\chi \frac{\Psi_h}{\lambda}^2 \tag{3.7}$$

where Ψ_h is the hydration potential and χ is the orientational susceptibility of the solvent. Hydrated lipid bilayers exhibit a variety of interactions (e.g., water-water, lipid-water, and lipid-lipid), and this model can be extended to include repulsive pressures such as those arising from water-water interactions, as well as steric lipid-lipid interactions, including thermally induced undulations (Helfrich and Servuss 1984; Kornyshev and Leikin 1989; Cevc 1991; Parsegian and Rand 1991; Israelachvili 2011).

Early investigators lacked information about the actual energies and geometries of hydration water, and initially the structure of ice was seen as a system from which one was able to glean information regarding the orientational biases of water (Bernal and Fowler 1933; Jorgensen et al. 1983). However, ice is far from being an accurate description of hydration water, failing to include more complicated collective features that had been reported in bulk water (Frank and Wen 1957; Fecko et al. 2003; Tielrooij et al. 2010) (*Note of the Editor: see Chap. 7 by Alarcon et al.).

New models are still being proposed to explain the reorientation of water (Torre et al. 2004; Laage and Hynes 2006; Taschin et al. 2013), which will undoubtedly provide some new insights about the nature of hydration water at the bilayer surface.

We must also recognize that in biological membranes, the system is not uniformly distributed. Complex lipid and sterol compositions, the inclusions of proteins, glycosylation, as well as the formation of lipid domains all result in inhomogeneous local properties of the bilayer and the solvent. This complexity is critical for the function of biological membranes, but it also makes the analysis of real systems non-trivial.

3.2 Water Distribution Within the Bilayer

Water not only associates with lipid head groups (Griffith et al. 1974), but also with many of the membrane's other functional groups – in addition to occupying free volume. Early measurements of lecithin bilayers by Zaccai et al., mapped out the distribution of water across the bilayer (Zaccai et al. 1975). They were able to quantify water molecules in equilibrium near the glycerol and phosphorylcholine (PC) head group regions, even at low hydration and below the gelation temperature.

Up to this point we have focused on water outside the formal bilayer volume. However, there is a finite amount of water that can reside deep within the lipid-water interface. In this region, water can directly affect the membrane's structural and dynamical ordering. Ultimately, we will consider the role that the hydrocarbon region plays in defining the amount of water associated with the head group, and consider the limited, but necessary presence of water inside the bilayer's hydrocarbon core.

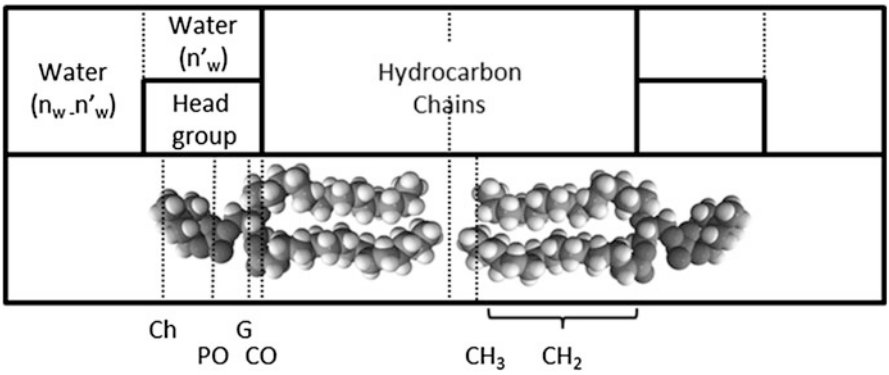

Fig. 3.1 The *upper panel* shows the parsing scheme of a DPPC bilayer in the L_α fluid phase and its waters of hydration, as depicted by Nagle and Wiener (1988). In the *lower panel*, the relative positions of the different head group moieties are illustrated, with the abbreviations Ch, PO, G, CO, CH_2, and CH_3 representing the choline, phosphate, glycerol, carbonyl, methylene, and methyl groups, respectively

The upper panel of Fig. 3.1 shows an idealized lipid bilayer (Nagle and Wiener 1988), emphasizing the substantial presence of water in the region of the polar head group, as well as recognizing that approximately 40 % of the total bilayer thickness is due to this hydrated region (*see Chap. 2 by Tristram-Nagle). (For illustrative purposes we will use the structure of a DPPC bilayer, which has been extensively studied over a number of decades, using both neutron and x-ray scattering techniques; and more recently, molecular dynamics simulations). For all intents and purposes, the DPPC bilayer is considered the prototypical model membrane system (Büldt et al. 1978; Katsaras 1995; Nagle et al. 1996; Petrache et al. 1997; Nagle and Tristram-Nagle 2000; Kučerka et al. 2008a).

Starting from the bilayer center, the terminal methyl groups are followed by 28 methylene groups – 14 in each of DPPC's hydrocarbon chains. Taken together, the methyl and methylene groups represent the bilayer's hydrophobic core. Interactions between hydrocarbon chains are predominantly van der Waals, and there is no possibility for hydrogen bonding with neighboring chains.

The two acyl chains and the PC head group are attached, via ester bonds, to the glycerol backbone's sn-1 and sn-2, and sn-3 positions, respectively (Fig. 3.1). Variations to this structure involve substitutions to the choline head group or the acyl chains. Typical head groups include serine, ethanolamine, glycerol, and inositol, to name a few. Acyl chains can also vary in length and degree of unsaturation (number of double bonds). In fact, biologically relevant lipids contain a saturated hydrocarbon chain at the sn-1 position and a longer unsaturated chain at the sn-2 position (e.g., POPC). These variations in acyl chain length and unsaturation have a major impact on the thickness and packing density of the bilayer's hydrophobic core, with longer saturated chains trending to thicker bilayers, and increased unsaturation leading to more disordered hydrophobic cores.

The lipid head group region intimately associates water, as we have discussed in the previous section. The carbonyl oxygen atoms participate, on average, one hydrogen bond with water. Non-ether phosphate oxygen atoms average about 4 hydrogen bonds per lipid, of which 1.7 are involved in bridging two lipid molecules together (Pasenkiewicz-Gierula et al. 1997). These water molecules are considered to be "tightly" bound, and are structurally and dynamically distinct from bulk water (Ghosh et al. 2007; Nagata and Mukamel 2010).

Simulations have studied the role that head group chemistry plays in determining the structure of water in the vicinity of the bilayer (Murzyn et al. 2006). For example, phosphatidylethanolamine (PE) and phosphotidylglycerol (PG) head groups hydrogen bond with only 1 or 2 water molecules each, while PC head groups affect ∼11 water molecules by inducing a "clathrate" structure to accommodate the three methyl groups of choline. This water structuring scenario is consistent with experimental data from amphiphilic molecules in solution (Perticaroli et al. 2011).

In DPPC bilayers, up to 16 water molecules per lipid have been found to be affected by the presence of the bilayer (i.e., $\sim 5H_2O$ molecules tightly bound to CO and PO, $\sim 11H_2O$ molecules associated with the choline methyls). Åman and Lindahl suggest that a second shell of ∼6 additional water molecules exhibit some lesser ordering imposed from contact with the more ordered inner shell of water (Åman et al. 2003). This is consistent with the picture of a double layer. In the context of the order parameter model, it is the work required to orient these additional water molecules that gives rise to the so-called 'hydration force' which manifests itself when two, apposing, hydrated bilayers approach each other (Rand and Parsegian 1989; Leikin et al. 1993).

We will now go on to consider how the bilayer's hydrophobic core contributes to the water structure at the bilayer surface. Primarily this affects water in the choline region and the 'second shell', which we referred to in the previous paragraph. The latter population of waters appears to be highly sensitive to the area per lipid (Table 3.1), a parameter known to vary, for a given phase (e.g., gel or liquid crystalline) and as a function of acyl chain length. Thickening of the bilayer with increasing hydration or a thermodynamic phase change (e.g., gel to liquid crystalline) are two ways that this effect can be observed.

In purple membranes for example, upon full hydration the bilayers thicken by about 0.5 nm, even below the gel transition (Fitter et al. 1999). In this rigid structure, the water molecules associate with the lipid's glycerol backbone, and each water molecule is able to hydrogen bond with multiple neighboring phospholipids. Upon heating through the gel transition, bilayer thickness expands from 5.3 to 6.8 nm. This change is associated with incoming hydration water in to the newly available volume around the head group. This picture of bilayer thickening with increasing levels of hydration has been found to be consistent with results from NMR (Ulrich and Watts 1994) and infrared spectroscopy (Binder 2007). Changes in hydration have been shown to play a role in determining the phase of gel DMPC (Sirota et al. 1988) and DPPC (Raghunathan and Katsaras 1995) bilayers, where increasing humidity was shown to lower the transition temperature for the L_α phase as well as the transition temperatures of the other sub phases (Katsaras et al. 2000).

Table 3.1 Number of water molecules associated with the lipid head group region, n_w' and the area per lipid molecule

	n_w'	Area [Å^2]	Reference
DOPC	10.7	72.4	Kučerka et al. (2006)
DOPC	11.0	72.2	Tristram-Nagle et al. (1998)
DOPC	11.1	72.5	Nagle and Tristram-Nagle (2000)
POPC	9.4	68.3	Kučerka et al. (2006)
di22:1PC	9.7	69.3	Kučerka et al. (2006)
DMPC	7.2	60.3	Kučerka et al. (2006)
DMPC	7.2	59.6	Nagle and Tristram-Nagle (2000)
EPC	10.2	69.4	Nagle and Tristram-Nagle (2000)
DPPC (Gel)	3.7	47.9	Nagle and Tristram-Nagle (2000)
DPPC	8.6	64.0	Nagle and Tristram-Nagle (2000)
DPPC	11.9	62.9	Kučerka et al. (2004)
DLPE (Gel)	2.0	41.0	Nagle and Tristram-Nagle (2000)
DLPE	4.7	51.2	Nagle and Tristram-Nagle (2000)
DLPE (Gel)	7	41.0	McIntosh and Simon (1986)
DLPE	9	49.1	McIntosh and Simon (1986)

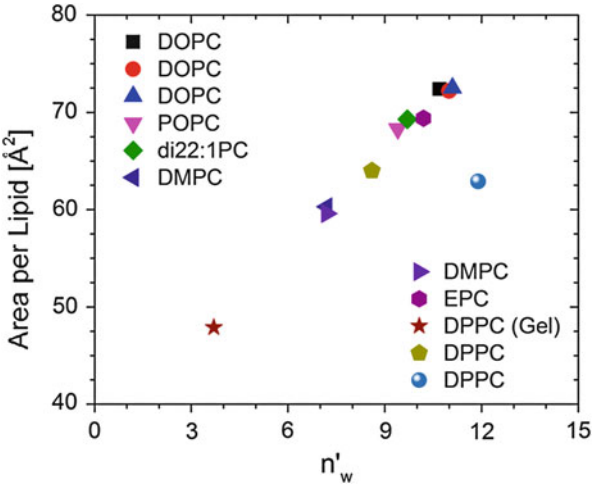

Fig. 3.2 Here we illustrate the number of head group associated water molecules, n_w', versus area per lipid for the different PC lipids in Table 3.1. We see that the available volume between head groups, as implied from area per lipid, is directly correlated to the water content in the head group region

A basic parameter that is used to describe interfacial water is n_w', the number of water molecules within the volume defined by the head group – i.e., the head group thickness, D_{HH}. This parameter is obtained through X-ray and neutron scattering studies of bilayers (Nagle and Tristram-Nagle 2000). It should be noted that new joint refinement models based on molecular volumes do not explicitly compute n_w' (Kučerka et al. 2008a). Also, as summarized in Table 3.1, a strong correlation can be implied between the number of water molecules and the area per lipid (see Fig. 3.2).

It is interesting that the number of water molecules associated with each PC head group is correlated to the packing of acyl chains in the hydrophobic core. This means that the free volume in the head group region is a determining factor in the number of hydration waters. It follows then that this relationship would also be observed when pressure is used to modulate area per lipid. In the study by Tristram-Nagle et al., the number of water molecules per DOPC lipid indeed decreased as a function of increasing hydrostatic (or osmotic stress) pressure – unsurprisingly, the area per lipid molecule also decreased with increasing pressure (Tristram-Nagle et al. 1998). This has implications in how one considers permeation of water through the bilayer (Mathai et al. 2008), as will be discussed in a later section of this chapter.

It should also be pointed out that the experimentally reported head group associated water molecules are fewer than those reported from simulations. At this point we should mention a few caveats regarding the water estimates from liquid crystallographic studies. Firstly, the number of head group associated water molecules, $n_w^{'}$, are determined from the excess of the fit functions used for lipid volumes, i.e. there is no explicit term in these models for water. This brings us to the second caveat, namely that the volumes of all water molecules (associated and bulk) are the same, thus enabling one to determine $n_w^{'}$. However, it is clear that the order parameters (and hence the volume) of hydration water is different from those in the bulk. These obvious deficiencies clearly show the need to further refine the structural models used to interpret scattering data.

These deficiencies are important to keep in mind when one considers water within the hydrophobic region. So, while NMR (Griffith et al. 1974) and a few scattering studies (Kučerka et al. 2008b) have shown water penetration into the bilayer's hydrophobic core, the absence of a measureable probability for water in most scattering models is simply a reflection of how the structural model deals with the exceedingly low statistical probability of finding water in this region.

Generically, trace water content decreases from the acyl chain carbonyls to the bilayer center, with a slight increase at the center of the bilayer, where there is an increase in free volume. This is illustrated by the free energy calculations of Marrink and Berendsen for a number of small molecules in DPPC (Marrink and Berendsen 1994). The penetration of water into the bilayer is also dependent on acyl chain length, degree of unsaturation, and the presence of cholesterol (Subczynski et al. 1994). For example, Subczynski et al. demonstrated increased hydrophobicity with increasing fatty acid chain length and the introduction of a double bond at C9–C10 position. The introduction of cholesterol, however, increased water penetration to a depth of C7–C9, the approximate depth of the cholesterol steroid ring.

3.3 Permeation

Water and other small neutrally charged solute molecules are known to passively cross lipid bilayers. As discussed in Sect. 3.2, water is present, to some degree, at all depths within the bilayer. It is therefore unsurprising that water is transiting the

bilayer. In fact, this is a vital feature of biological membranes, allowing for osmotic equilibrium, while at the same time, maintaining the separation of ions, electrolytes, and large biomolecules.

Classically, Overton's rule informs us that the permeability of a small molecule, such as water, through a hydrocarbon layer is defined by the partition coefficient (Overton 1899). However, Overton's rule is known to overestimate the thickness of a bilayer by nearly a factor of four (Finkelstein 1987; Nagle and Tristram-Nagle 2000), inviting new assumptions (Mathai et al. 2008) and models (Deamer and Bramhall 1986) in order to ameliorate this overestimate.

Kedem and Katchalsky (1958) quantitatively describe the passive transport of water, and any uncharged permeable solute, in terms of classical diffusion plus a convection model. (We must recognize that the presence of a convective term highlights the importance of water permeation in defining all types of transport across the bilayer.) The three parameters that describe the transport of water across a bilayer are: (i) hydraulic conductivity, L_P; (ii) solute permeability, ω; and (iii) the reflection coefficient, σ. The hydraulic conductivity, L_P, is defined as the volume flux of water, J_v, per pressure differential across the membrane, ΔP, and is evaluated for an infinitely thin membrane per unit area. This is expressed as follows:

$$L_P \equiv \left(\frac{J_V}{\Delta P} \right)_{\Delta x=0} \tag{3.8}$$

The bilayer solute permeability, ω, which is defined in terms of the solvent flux, J_s, and the osmotic pressure, $\Delta\pi$, at zero flux of water (per unit area) can be written as:

$$\omega \equiv \left(\frac{J_s}{\Delta \pi} \right)_{J_v=0} \tag{3.9}$$

where the osmotic pressure is,

$$\Delta \pi \equiv RT \Delta c_s \tag{3.10}$$

where R is the gas constant, T the temperature, and Δc_s is the concentration difference of the solute across the membrane. Finally, the reflection coefficient, σ, is used to characterize interactions of the solute with the bilayer, or solvent, that reduce transport. It is confined to values $0 \leq \sigma \leq 1$.

By combining these relations with the appropriate driving forces, i.e., ΔP, $\Delta\pi$, and Δc_s, we can formulate the Kedem-Katchalsky equations which describe the net solvent volume flux, J_v, and the net solute flux, J_s across a lipid bilayer;

$$J_v = L_P \Delta P - \sigma L_P \Delta \pi \tag{3.11}$$

$$J_s = \overline{c}_s \left(J_v - \sigma L_P \Delta P \right) + \omega \Delta \pi \tag{3.12}$$

Expressed in this way, it becomes clear that water traversing a bilayer plays a critical role in defining the net flux of most molecules going through a membrane.

Essentially there are two perspectives from which to consider the passive transport of water across a bilayer. The solubility diffusion model considers water to be homogenously solubilized in the lipid bilayer, and implies that solute partitioning and diffusivity are responsible for the rates of permeation of solute molecules across the bilayer (Hanai and Haydon 1966; Träuble 1971; Finkelstein 1976; Nagle et al. 2008). Alternatively, water can cross a membrane through nanometer scale pores spanning the thickness of the membrane (Finkelstein 1987; Tepper and Voth 2005), or transient pore diffusion, where clusters of bulk-like water within the bilayer diffuse across (Deamer and Bramhall 1986; Jansen and Blume 1995; Leontiadou et al. 2004; Kausik and Han 2011). These pore-based models essentially connect permeability to bilayer stability. In biological systems, protein structures such as aquaporin, also allow for the passive transport of water (Murata et al. 2000; de Groot and Grubmüller 2001; Sui et al. 2001).

The solubility-diffusion model is based on a few key assumptions. For example, it considers the membrane as a homogenous slab, or series of homogeneous slabs in which water is partitioned and diffuses through. Additionally, it assumes that the slab is homogeneous in the lateral dimension, a condition that is not true for lipid compositions which spontaneously form raft domains. In such systems, not only does lipid composition change laterally, and hence the diffusivity and partition coefficients, but bilayer thickness is also known to vary as well (Heberle et al. 2013).

In the context of the Kedem-Katchalsky definitions, the permeability can be equated to hydraulic conductivity. The permittivity of each slab can then be expressed as:

$$L_P = P = \frac{KD_C}{d_c} \tag{3.13}$$

where K is the partition coefficient, D_C is the diffusion coefficient in the membrane, and d_C is the bilayer thickness. From this, it is clear to see how bilayer thickness is overestimated when using Overton's rule.

As one might suspect, real systems cannot be fully described by the single slab model, as it fails to capture the membrane's structural and chemical heterogeneity. The head group region and hydrocarbon core, for example, have vastly different properties as they relate to water. Both simulation (Marrink and Berendsen 1994; Bemporad et al. 2004) and experimental results (Kausik and Han 2011) illustrate this point by showing that D_C varies along the bilayer; as does the amount of water (Xiang and Anderson 1998; Bemporad et al. 2004).

Using simulations, it is possible to evaluate the integral definition of the permeability, which is given by:

$$P = \int_0^d \frac{K(Z)\, D_c(Z)}{dz} \tag{3.14}$$

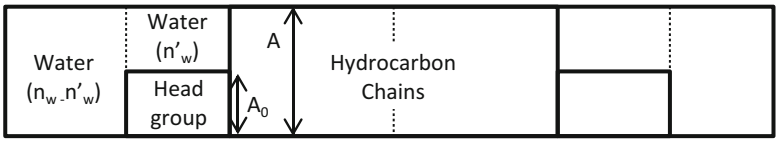

Fig. 3.3 A representation of the reduction in lipid area as a result of the head group encountering a diffusing water molecule as it permeates the bilayer (Nagle and Wiener 1988)

where the partition and diffusion coefficients can be evaluated as functions of the their position along a bilayer of thickness d (Fig. 3.3).

It is convenient to parse the bilayer into multiple slabs with distinct properties. The rational for this approach is laid out in detail by Nagle and coworkers (Nagle et al. 2008), where they advocate that the bilayer be divided into three slabs, namely: (1) two slabs describing the head group regions (one on each side); and (2) a slab representing the acyl chain region. In this model, the permeability of the two head group regions, P_H, and hydrocarbon region, P_C, are related as follows:

$$\frac{1}{P_f} = \frac{2}{P_H} + \frac{1}{P_C} \tag{3.15}$$

From this, one can account for water partitioning, diffusion constants, and relative bilayer thickness which result from inhomogeneities of the membrane. Nagle et al. (2008) also advocate for a fractional area term, $(A - A_0)/A$, when calculating the permeability of the head group region, P_H, where A_0 is then the head group barrier area (Mathai et al. 2008). As a result, permeability tends to zero as A_0 tends to A, as is evident from:

$$P_H = \left(\frac{K_H D_H}{d_H}\right)\left(\frac{A - A_0}{A}\right) \tag{3.16}$$

where D_H and K_H are, respectively, the diffusion and partition coefficients in the head group region, and d_H is the thickness of the head group region. This is somewhat reminiscent of the reflection coefficient used by Kedem and Katchalsky, except that in the Nagle et al. case the correction accounts for water/head group interactions, rather than water/solute interactions. In the hydrocarbon chain region, area is not thought to be important. As such, P_C is defined as:

$$P_C = \frac{K_C D_C}{2\, d_C} \tag{3.17}$$

where D_C and K_C are, respectively, the diffusion and partition coefficients in the bilayer's hydrophobic core, and d_C is the hydrophobic core thickness. These two relations make it clear that the rate controlling step in this model is the effective partition coefficient in the head group region, which is being modulated by the area of the lipid head group relative to the area per lipid (Mathai et al. 2008). This

is an important insight which arises from a deep structural understanding of lipid hydration mentioned in a previous section. From those studies, it was clear that the area per lipid scales with the number of water molecules associated with the head group region. It is only natural then, to apply this knowledge to modeling water permittivity.

Pore models describe water transport by very different physical phenomena. To understand pore mediated water transport, one is not concerned with how much water exists in the bilayer at equilibrium, and how it diffuses, but rather in the following: (1) the number of water containing pores; (2) how long do they last; and (3) how does water diffuse within the pores during their lifetime. These notions can be expressed as a partition coefficient for the bilayer as follows:

$$P = \frac{n}{A} P_p \tag{3.18}$$

where P_p is the permeability per pore, and *n/A* is the number of pores per unit area of bilayer. We can further define P_p as:

$$P = \frac{v_w N D_w}{L^2} \tag{3.19}$$

where v_w is the molar volume of water, D_w and N are the diffusion constant of water and the average number of water molecules in a pore, respectively, and L is the pore length.

Thermodynamically, pore formation and stability can be thought of in terms of free energy. To form a pore of radius, r, in a bilayer, one considers the line tension, γ, which stabilizes the bilayer, and the surface tension, Γ, which stabilizes a pore. These quantities can be expressed as:

$$E(r) = 2\pi r\gamma - \pi r^2 \Gamma \tag{3.20}$$

The differences between these competing forces give us some understanding of pose size and the frequency of pore formation. Line tension and surface tension are parameters relating to bilayer stability, implying that pores appear more frequently in unstable bilayers. Indeed, large hydrophilic pores require a significant structural rearrangement of the bilayer, and can approach a nanometer in diameter, frequently spanning the entire bilayer (Leontiadou et al. 2004).

Hydrophobic pores, on the other hand, are thought to be smaller and more transient, with sizes smaller than a nanometer in diameter and lifetimes on the order of tens of picoseconds (Kausik and Han 2011). Some experimental evidence for this notion has recently been put forth, which was based on the mismatch in the temperature dependence of the activation energy of water diffusion and the observed internal water diffusion rate. One would expect these two quantities to scale with each other above and below the phase transition. However, the local diffusion constant of internal water was found to vary only slightly in the different phases,

while the activation energy experienced a discrete change at the phase transition (Kausik and Han 2011). The reasoning for this was that water diffuses freely within a pore, but that the pore diffuses much slower in the plane of the bilayer below the gel temperature. This is good evidence for the lack of dynamical coupling between the water and the bilayer, as implied by the solubility-diffusion model. However, the use of a spin probe in these studies may have also perturbed the system, as has been previously observed for fluorescent probes (Ackerman et al. 2013). Such a perturbation can affect bilayer properties, allowing for local, dynamically decoupled water regions near the spin probe.

Most physical evidence points to solubility-diffusion as the predominant mechanism for water permeation (Al-Awqati 1999). This was demonstrated in the dependence of the water permeation rate as a function of bilayer thickness. One would expect a dependence of d_C^{-1} in the case of the solubility diffusion model, but a much stronger, exponential dependence as a function of bilayer thickness in a pore model. Indeed this observation is borne-out by experiments from Paula et al. (1996), where they showed that water permeation does not follow the predicted exponential dependence. Instead, their data were consistent with transport through pores of charged and ionic solutes. This seems reasonable as such molecules must traverse the bilayer as a water cluster, and clearly indicates that pores are not the predominant mode of action for water permeation. Another possibility is that both mechanisms are taking place, but that the majority of water permeation takes place through a solubility-diffusion mechanism.

3.4 Dynamics

As has been discussed in previous sections of this chapter, water molecule dynamics are highly sensitive to the relative location of the molecule to the lipid bilayer. For example NMR studies using a spin labeled PC system at 300 K demonstrated a fourfold reduction in the diffusion constant of choline associated water (0.69×10^{-9} m^2 s^{-1}), compared to the bulk (2.3×10^{-9} m^2 s^{-1}) (Kausik and Han 2011). This retardation of the water's diffusion constant was increased by an additional 20 % in the vicinity of the glycerol/carbonyl, and water motions slowed yet further in the acyl chain region. These results are in agreement with earlier experimental (Griffith et al. 1974; Marsh 2002) and simulation studies (Marrink and Berendsen 1994; Pasenkiewicz-Gierula et al. 1997; Åman et al. 2003). Diffusion, however, is not the only relevant dynamical feature of water. Many of its vibrational, librational, stretching, and bending modes are also modified when in the presence of a lipid bilayer. These reorientational motions of water can be highly informative when it comes to order parameters, as retardation of this process directly relates to the change in free energy of the bound molecule versus the bulk (Åman et al. 2003).

Current models of water dynamics seek to bring together the translational and reorientational motions of water through collective or concerted mechanisms

related to hydrogen bond cleavage and large amplitude angular jumps (Torre et al. 2004; Laage and Hynes 2006; Taschin et al. 2013). While these models have been evaluated primarily for bulk water, or in the presence of some small solute molecules, they may be informative regarding water interactions with a lipid bilayer. So what insights do these models provide? The rotational and translational diffusion coefficients may be governed by the rate of hydrogen bond cleavage and reformation. In support of this, is the example of protein hydration water, where the rotational retardation factors ($D_{R,bulk}/D_{R,hyd}$) are comparable to translational retardation ($D_{T,bulk}/D_{T,hyd}$), but with the added advantage that rotational relaxations can be probed locally using NMR techniques (Marchi et al. 2002). This is quite elegantly described for protein hydration dynamics (Halle 2004), and is applicable to lipids. This similarity in retardation data between rotational and translational motions means that both processes use the lifetime of the hydrogen bond as a rate limiting step. When compared to the bulk, the hydrogen bond lifetime of hydration water is more than 5 times longer (Balasubramanian et al. 2002), and even structural perturbations imposed by non-hydrogen bonding head groups, such as choline, reduce reorientation times of local water by a factor of 4 (Murzyn et al. 2006). Having said this, an extended residence time for water near the bilayer is not indicative of strong bonding interactions, but could also be the result of a local topography/geometry which prevents water participation in the cooperative rearrangement of the bulk (Laage et al. 2009). The importance of a topographical/geometric restriction interfering with the cooperative mechanism becomes clearer when one considers the restricted geometry near the carboxyl region of the bilayer.

Before moving on, we would like to briefly return to the local translational diffusion rate within the hydrophobic core of the bilayer, where water motions are thought to be distinctly different. This is a limiting case where there is a lack of hydrogen bonding and a very low barrier to reorientation, resulting in rapid rotational diffusion, but a restricted translational diffusion – based on the available free space (Kausik and Han 2011). A diffusion mechanism in this environment requires free volume (Almeida et al. 1992), a volume that happens to be occupied by the acyl chains. As a result, water motions in the bilayer become connected to the motions of the acyl chains despite the lack of a shared hydrogen bond network. This part of the lipid bilayer undergoes a range of processes from structural relaxations, to vibrations, rotations and lateral translations (Rheinstädter 2012). Collective dispersions also appear to play a prominent role in the mechanisms governing the dynamical processes of the bilayer (Chen et al. 2001; Rheinstädter et al. 2004).

Experimentally, a wide range of techniques have been applied to the water-interface problem. It must be emphasized that different techniques probe different physical properties; i.e. spin, polarization, dipole, neutron scattering cross section, etc. As such, the identity of the perturbation, the influence of length scale, and the

sensitivity to the bilayer will be highly technique dependent. For a more detailed discussion regarding this, the reader is referred to the excellent review by B. Halle (2004).

NMR has long been used to study the structure of water at the interface (Seelig 1977), and a number of NMR dynamical techniques have been applied to this problem, such as pulsed field gradient (Devaux and McConnell 1972; Volke et al. 1994; Wassall 1996), Overhauser dynamic nuclear polarization (Armstrong and Han 2009; Kausik and Han 2011), and residual magnetic relaxation profile (Victor et al. 2013). This powerful tool has yielded important information on highly localized dynamical processes on the picosecond to nanosecond timescale. Depolarized light scattering techniques, Raman, Kerr-Effect, IR and 2D-IR are all also suited to studying the dynamics of water (Auer et al. 2007; Binder 2007; Mazur et al. 2010). However, separating the water signal at the interface from that of the bulk is often difficult and requires careful fitting of the data in order to properly assign spectral features to corresponding hydration numbers and retardation factors (Perticaroli et al. 2013). New techniques based on two dimensional infrared methods (Kolano et al. 2006) provide detailed information about the vibrational modes of water and how they are affected in the presence of a lipid bilayer (Nagata and Mukamel 2010). Dielectric spectroscopy has also been used to investigate lipid bilayers, with time-domain terahertz techniques showing promise in the study of reorientational dynamics in aligned bilayers (Tielrooij et al. 2009).

Quasielastic neutron scattering (QENS) is a probe-free, ensemble technique (Bee 1988) that has been used to directly study the dynamics of bilayer associated water. (* N of E: for details see Chap. 4 by Pfeiffer) The advantages of neutron scattering are twofold: (1) the incoherent scattering cross-section of hydrogen and deuterium differ by a factor of ~40 (Sears 1992), making isotopic substitution of the bilayer an attractive strategy for separating the dynamics of water from those of the bilayer. (2) Neutron scattering offers the possibility to directly observe dynamics as a function of scattering wave vector, Q. This important feature allows one to assign the dynamic process being observed, i.e., a true diffusive process scales as the length scale squared,

$$D_w = \frac{\Gamma\,(\mathrm{Q})}{Q^2} \tag{3.21}$$

where D_w is the diffusion coefficient of water and $\Gamma(Q)$ is the decay constant in units of s^{-1}, which can be determined from a fit of the observed dynamic structure factor, S(Q,E). This fitting of neutron data is typically performed by convoluting Lorentzians, representing the contribution of particles in motion, with delta functions, representing stationary particles on the timescale of the instrumental resolution (Fitter et al. 1999). It is also possible, and arguably more appropriate, to utilize the dynamic susceptibility formalism of the neutron scattering spectrum, and then fit the data with a Cole-Cole distribution to account for the stretching of water relaxation spectra at the interface of a weakly hydrated biological sample

(Settles and Doster 1996; Nickels et al. 2012). The conversion to dynamic susceptibility is accomplished by removing the Bose occupation number, $n_B(E)$, from the observed dynamic structure factor,

$$\chi''(Q,E) \propto \frac{S(Q,E)}{n_B(E)} = \frac{S(Q,E)}{\left(e^{E/k_BT}-1\right)^{-1}} \tag{3.22}$$

The Cole-Cole distribution is typically fit in the frequency domain, which is accessible through Plank's constant, $\hbar$;

$$\frac{\chi''(Q,E)}{\hbar} = \chi''(Q,\omega) \tag{3.23}$$

and can then be used to fit at all measured values of Q according to:

$$\chi''(Q,\omega) = \frac{\chi_0(\omega\tau)^{1-\alpha}\sin\left(\frac{\alpha\pi}{2}\right)}{1+2(\omega\tau)^{1-\alpha}\cos\left(\frac{\alpha\pi}{2}\right)+(\omega\tau)^{2-2\alpha}} \tag{3.24}$$

where α is the stretching parameter, χ_0 is the scaling factor, and τ is the central relaxation time.

Using QENS, and other physical characterization techniques, a picture of dynamical decoupling for the most tightly associated water molecules emerges that is consistent with the removal of the cooperative relaxation mechanism, in low hydration samples, and the carboxyl associated water molecules. Swenson and coworkers (Swenson et al. 2008) have built on the works by König and Pfeiffer (Pfeiffer et al. 1989; König et al. 1994) to experimentally determine the slowdown in translational diffusion of hydration water. They looked at the dynamics of interfacial water in low-hydration, aligned DMPC and DPPC bilayers, and noted that the onset of water translation begins at ~295 K, which coincides with the gel-to-liquid transition of the DMPC lipid bilayer – 20 K higher than bulk water (Swenson et al. 2008). It must be pointed out, however, that these QENS observations of water dynamics were made at hydration levels far below what is considered "full" hydration – i.e., the amount of water where the lamellar repeat spacing ceases to change with the further addition of water (Katsaras 1997; Fitter et al. 1999). This implies a predominant role of acyl chain dynamics in facilitating water translation in the low hydration condition, i.e. in the tightly associated water region.

The dramatic slowdown of water in the hydration layer is not an isolated result. A study focusing on the hydration water of purple membranes at higher concentration of water also illustrated the dynamical decoupling of hydration water from the membrane (Wood et al. 2007). Wood et al. demonstrated that transitions in hydration water had little or no influence on the dynamics of the membrane itself. Separate transitions, assigned to the onset of collective dynamics of the acyl region and the freezing of bulk-like heavy water, respectively,

were also observed in hydrated, aligned DMPC-d54 bilayers in D_2O at 295 K and 271 K (using an instrumental resolution of ~1 ns), indicating a lack of dynamical coupling between water and the collective dynamics of the membrane (Rheinstädter et al. 2005). Other studies demonstrated that the lateral diffusion constants of water and those of individual lipid molecules were separated by two orders of magnitude at full hydration condition, with water diffusing at a rate of 4.7×10^{-10} m^2s^{-1} and lipids diffusing at a rate of 8.6×10^{-12} m^2s^{-1} (Gaede and Gawrisch 2003).

Experiments using NMR have shown the presence of a small, dynamically distinct, water population at the surface in a number of different PC bilayers (Volke et al. 1994). More recently, 2D-FTIR on weakly hydrated DMPC membrane fragments demonstrated a wide distribution of stretching and vibration relaxation times for water (Volkov et al. 2007). While the latter is perhaps an artifact of isolated water molecules at these weakly hydrated surfaces, it may also be typical of how geometrically restricted some of the carbonyl associated waters are, i.e., isolated from the collective bilayer relaxational mechanism. Independent simulations on fully hydrated systems have clearly illustrated how decoupled the small water population bound to the glycerol region of the head group is from the population of water molecules associated with the choline region, or the bulk (Nagata and Mukamel 2010).

A slowdown in local water dynamics near the bilayer, equivalent to a threefold increase in local viscosity, has recently been invoked to explain anomalously rigid measurements of bending moduli from neutron spin echo experiments (Yi et al. 2009). However, alternative explanations of this anomaly have cited internal bilayer friction as a possible explanation (Watson and Brown 2010; Woodka et al. 2012).

The dynamics of water in the hydration shell of lipid bilayers are an interesting counter point to what has been observed in proteins. In proteins, the solvent fluctuations drive the dynamics of the macromolecule – a phenomenon termed as ‘slaving’ (Fenimore et al. 2002). Lipid bilayers, despite their close contact with water, do not appear to behave in this manner. This stems from the lack of an extensive hydrogen bond network and a large solvent excluded volume in the acyl chain region of lipid bilayers. This is not meant to suggest that there is no influence of water in the protruding region of the head group (the choline region), as evidence clearly shows a role for water in this region (Ulrich and Watts 1994), and a precipitous reduction in lateral lipid diffusion rates occurs at hydration levels below 30 % (Filippov et al. 2003). (* N of E: This point is discussed by Arsov in Chap. 6) Nonetheless, further modeling of the diffusive properties of interfacial water in DPPC illustrates how the diffusivity in this population of interfacial water follows the phase behavior of the lipids (Debnath et al. 2013). Indeed, membrane proteins also seem to follow the phase behavior of the bilayer, as studies on purple membrane have shown that protein dynamics are substantially different when incorporated into the lipid bilayer (Fitter et al. 1999).

3.5 Concluding Remarks

In this chapter we have described some of the basic concepts defining the interaction of lipid bilayers and water. Fundamentally, it is the energetic cost of perturbing the equilibrium disorder of bulk water that dictates many lipid bilayer properties. Repulsive interactions between bilayers define solution stability of vesicles and multilamellar spacing. These repulsive forces are sensitive to properties such as ionic strength, and lipid chemistry. Water is also important within the bilayer, and water permeation is critical to osmotic equilibrium. Yet, the distribution of water within a bilayer is still a subject of current study, with new nanopore models being put forth, aided by liquid crystallography and NMR techniques. These methods also give important estimates of the amounts of water associated at the interface of the bilayer. Finally, we described the dynamics of water in and near a lipid bilayer, along with the primary techniques used to study this system. Taken together, structural and dynamical studies have advanced our understanding of this important biological interface, which we hope will lead us to a deeper understanding as to how the membrane/water interface defines and modulates biological functions.

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