Chapter

The Role of Water in the Responsive Properties in Lipid Interphase of Biomimetic Systems

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Abstract

The lack of details in the hydration properties of lipid bilayers hinders the design of biomimetic systems that, as liposomes and vesicles, may be used for biotechnological and medical purposes. In this chapter, studies indicate water as a membrane dynamic component determining the affinity and response of lipid membranes to amino acids, peptides and others stimuli. Based on thermodynamic analysis in lipid monolayers and its comparison with swelling shrinkage processes in liposomes and vesicles, it is concluded that: (1) the interphase of a lipid bilayer in a bidimensional solution of hydrated polar groups imbibed in labile water can be exchanged with the media by osmosis and or expansion-compression. (2) Excess water beyond the hydration shell (confined water) has solvent properties for additives in the bulk water phase and confers free energy that is in excess for binding of amino acids and peptides. (3) Dissolution in the water membrane phase changes the water activity (a_w) and affects the surface pressure. (4) Defects may be formed by the compression of bilayers in which carbonyl groups organized water differently. These studies indicate that a deeper understanding of the role of lipid bilayers in cellular biology and support the development of future lipid-based biotechnology that should necessarily include the role of water as a membrane dynamic component.

Keywords: lipid bilayers, hydration, osmotic stress, responsive membranes, curvature, defects

1. Introduction: cell membranes and lipid membranes

Cell membranes are complex systems composed by lipids and proteins [1, 2]. They have structural and functional properties that are essential for life. The lipid bilayer is the backbone of cell membranes and is mostly composed by amphiphilic molecules such as phospholipids, cholesterol and glycolipids among others [3–7] (**Figure 1**).

A qualitative step in describing the membrane properties in terms of the lipid composition was the isolation and purification of lipids and its stabilization in water (**Figure 1**-Part B). After Bangham's discovery in the 1960s [8, 9], lipids were found to form closed particles (liposomes) that are able to trap in controlled conditions such as ions, macromolecules and polar molecules of different nature in the inner aqueous space (**Figure 2**). With this information, it was thought reliable the possibility to modulate such trapping properties by changing membrane composition and to orient filled liposomes to specific organ targets. The changes were in the

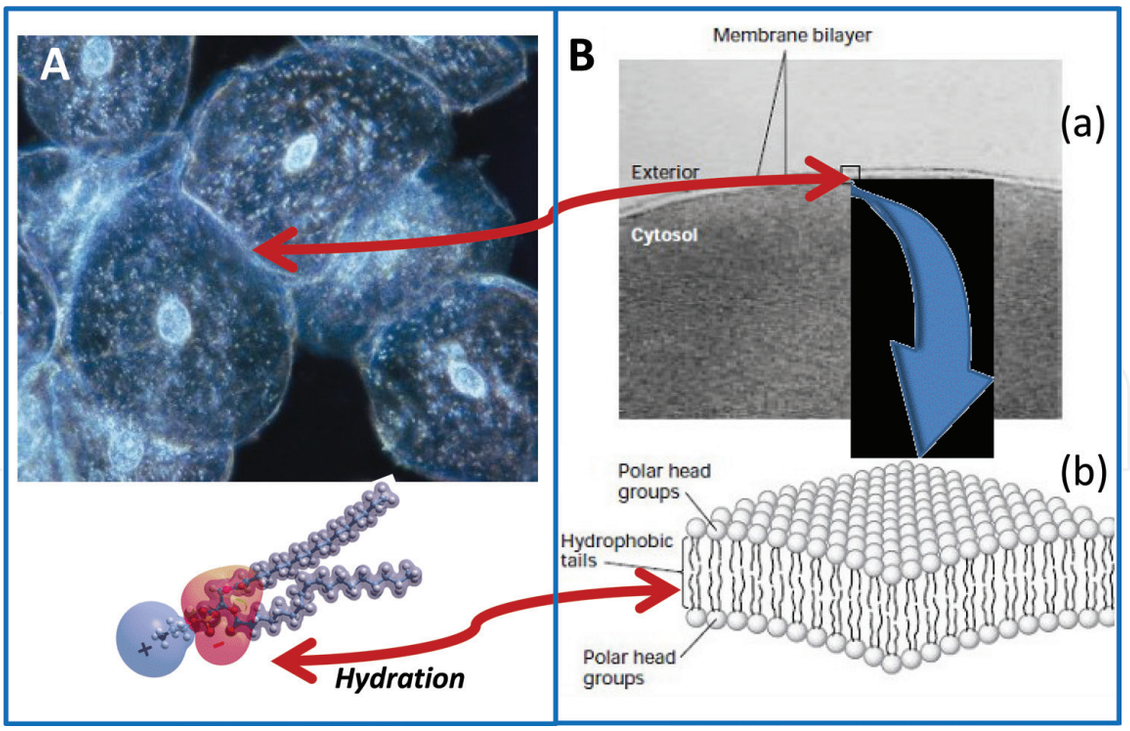

Figure 1.
(A) Electronic microscopic of cells; (B) cell membrane backbone is the lipid bilayer (a) which is formed by amphiphilic compounds such as lipids (single phospholipid) dispersed in water (b) facing the polar head group to water and segregating the acyl chains.

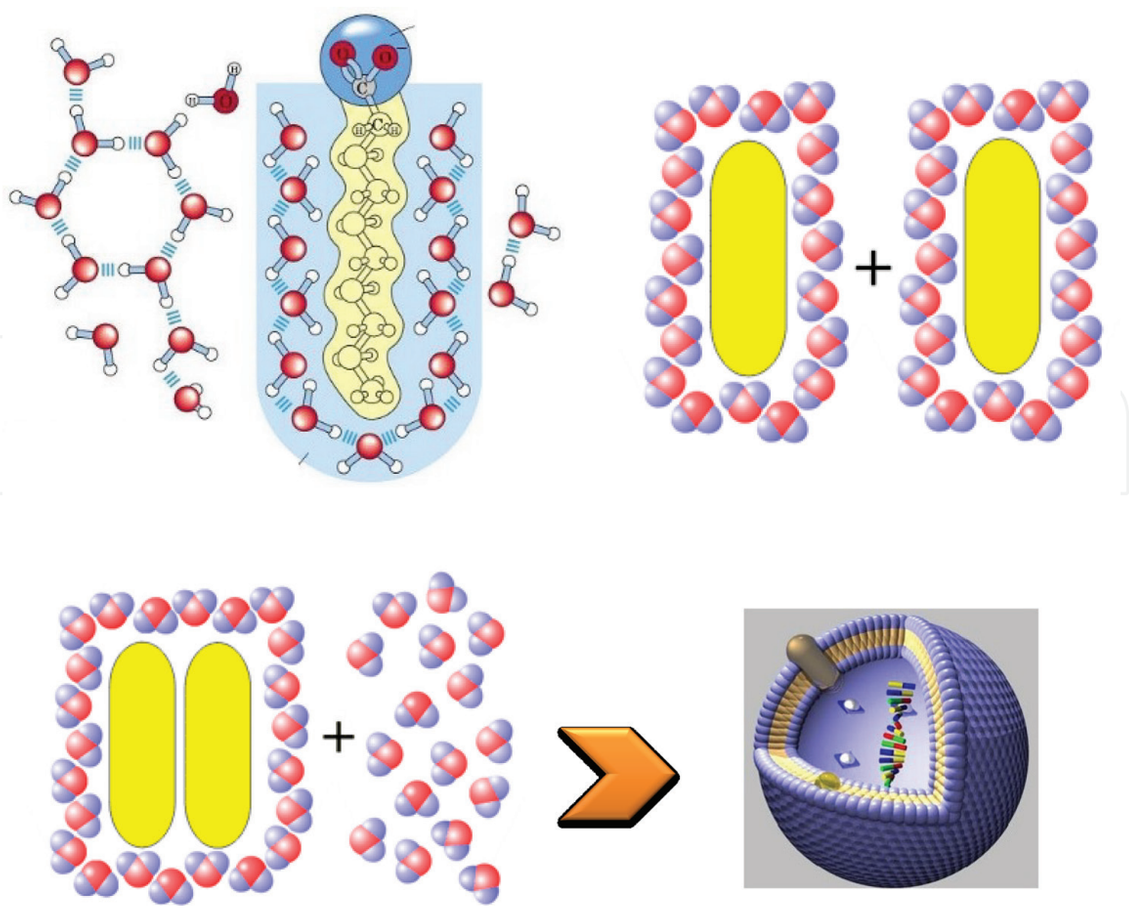

Figure 2.
Organized water around the acyl chains is displaced promoting aggregation by an increase in entropy of the system (upper part). Lipids self-aggregate in water due to the hydrophobic interaction of the nonpolar chains forming closed particles such as liposomes (lower part).

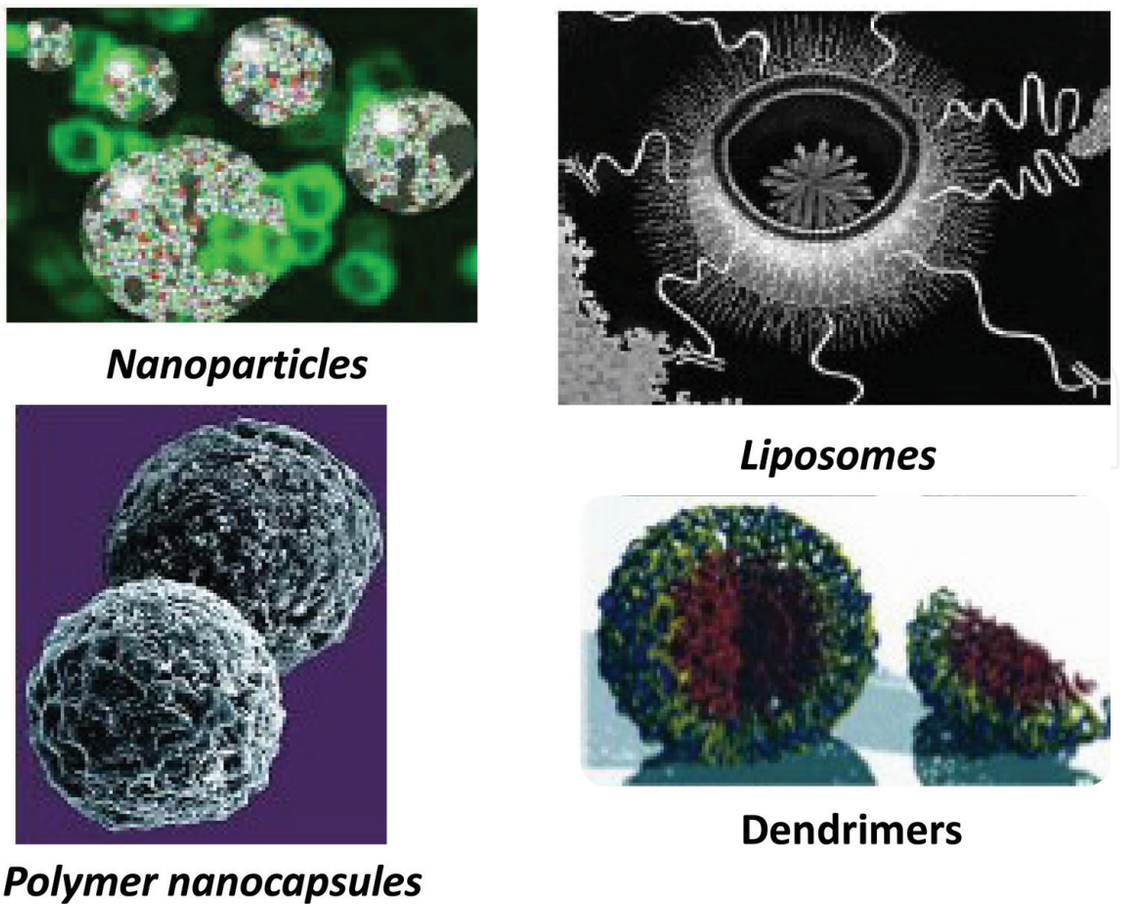

Figure 3.
Liposomes are one of the most attractive biomimetic systems because its preparation is done with lipids extracted from cells. In addition, other biomimetic nanoparticles can include lipids in its matrix.

direction to modulate the surface properties, the excluded aqueous volume, the water permeability and the mechano-elastic properties of the particles.

In addition, different methodologies have been designed, afterwards, in order to obtain suspension of homogeneous size distribution of different magnitudes [10–12]. With this wide range of possibilities, it was immediate to infer that liposomes and its different versions of covered or uncovered unilamellar vesicles would be the ideal tools to trap, vehiculate specific compounds to drive them to specific targets and deliver drugs to organs and tissues, specifically for human beings pathologies (**Figure 3**).

However, there are a number of difficulties for the direct use of these preparations that are mostly derived by limited knowledge of the physicochemical properties of the lipid bilayers. These include the presence of water as a major component in the membrane matrix; the thermodynamic properties derived from it in relation to the response from physicochemical stimuli and the interphase properties.

The purpose of this chapter is to analyze these points in order to propose new strategies for designing biomimetic lipid particles more efficiently.

2. Lipid hydration and bilayer stabilization

When dry phosphatidylcholines (PCs) of chain length above 12 hydrocarbon atoms are dispersed in water above their transition temperature as described in **Figure 2**, they form lamellar onion-like structures in which bilayers are separated by aqueous spaces that are available to trap the compounds of interest to vehiculize and deliver (**Figure 4**).

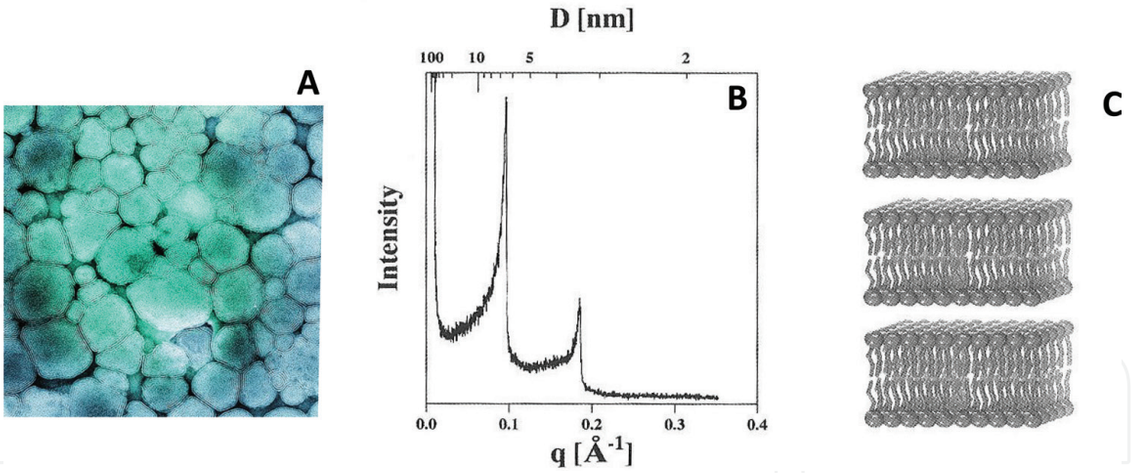

Figure 4.
(A) Electronic microscopic traditional image of multilamellar liposomes; (B) the diffraction pattern illustrates the separation between bilayers; (C) water solution trapped in between bilayers is schematically represented.

After Bangham, Luzzati and others [13–15] determined the thickness of lipid bilayers by SAXS. Different thermal profiles were obtained according to the lipid features and head group structure was found [16–18]. Lipids stabilize differently according to its geometry. Phosphatidylcholines, that have similar areas in the head group region and the acyl chain, form bilayers by stacking molecules in cylindrical shape. In contrast, phosphatidylethanolamine (PE) forms hexagonal phases due to the conical shape of molecules [19, 20].

The process of lipid hydration that derives the formation of liposomes consists of different stages as described in **Figure 5**.

The hydration step consists of the increase in area and decrease in thickness up to around 20% c.a. 22–24 water molecules per lipid. After this stage, area and thickness remain constant and the swelling of liposomes starts by the increase of water in the interlamellar space. This description of lipid swelling illustrates about several structural and physicochemical properties of the bilayers. The first observation is that there is a defined number of water molecules per lipid that determines the area per lipid and the bilayer thickness. This number is around 7–8 below the phase transition temperature and 22–24 above as derived by DSC [17, 18, 21, 22]. Thus, water is a component of the structure of the lipid bilayer, determining its thermodynamic stability.

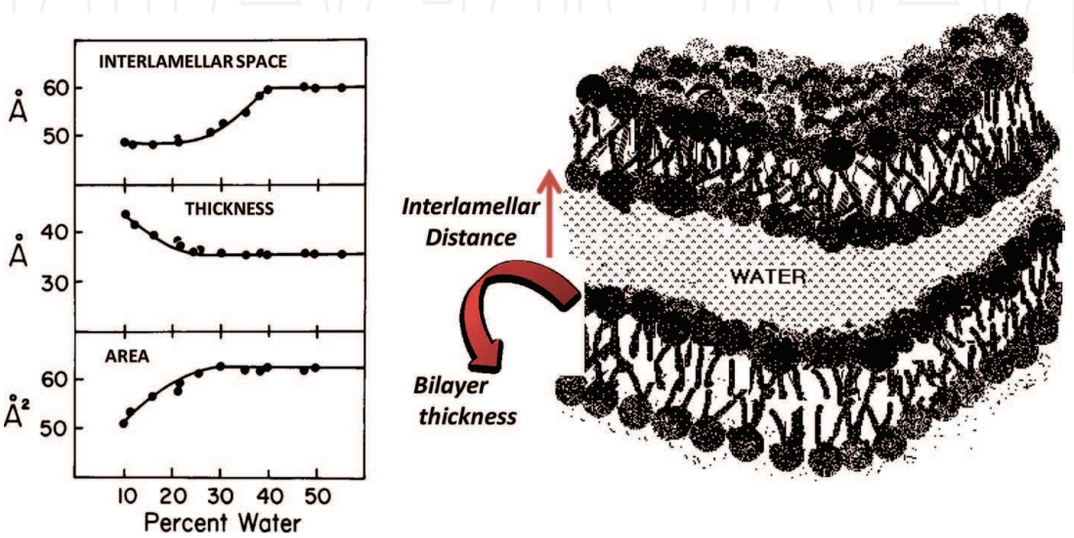

Figure 5.
Hydration of lipids and swelling of liposomes.

At least four features deserve discussion. At equilibrium in fully hydrated state, the membrane thickness is composed by the excluded volume of the lipid molecules and the excluded volume of the water organized by them (the hydration number denoted above). Thus, the barrier properties do not only merge with the head group and the acyl chain region per se but also of the packing and arrangement of water molecules in the hydration shell of the phospholipids. This means that for any solute, to overcome the bilayers, that is, releasing the trapped solute or incorporating some of them must permeate or alter the hydration shell. This can, in principle, be done by some of these mechanisms: insertion in the water network removing or replacing water molecules in the hydration shell or changing the area per lipid by expansion or compression.

The second feature of lipid bilayers is derived from the first one. The interbilayer space consists of water not bound to the membrane, that is, it can be displaced by changing the osmotic gradient between the inner spaces and the outer media of the liposome. Water can permeate the lipid bilayer with certain facility depending on the phase state of the lipids, the presence of double bonds or ramifications in the acyl chains [23, 24]. In contrast, membrane is completely or partially impermeable to some solutes, such as sugars, ions, depending on its size and molecular structure. The differences in permeation rates between water and any of these solutes means that at least in the beginning of the process, a gradient of water chemical potential can be built with a difference in solute concentrations between the two sides of the bilayer. Let us consider the interbilayer space described in **Figure 5**. If solute is more concentrated between the bilayers, water will be driven to enter due to a difference in osmotic pressure and them the spacing (and hence the trapped volume) will be larger. An opposite effect can be caused, if the solute is concentrated in the outer media of the liposomes. In this case, liposomes shrink and the interbilayer space decreases.

The third feature is represented by an area per lipid for a membrane thickness of 60 Å, when lipids hydrate with 22–24 water molecules per lipid. Thus, any change in the number of these water molecules will affect thickness and area with concomitant effect on permeability.

Finally, the fourth feature is defined by the limit of the volume decrease. This is given by the steric repulsion of the groups in the surface of the bilayer, in which water plays a significant role. Water associated with the lipids is oriented at the bilayer surface constituting an electrical potential that hinder bilayers approach. This repulsive force is named as dipole potential or hydration forces [24–27] (**Figure 6**).

It is immediate to derive that the presence of these forces hinders the adhesion or fusion of membranes with different kinds of surfaces (inorganic and organic materials, other membranes, proteins, tissues, etc.). On the other way round, those processes will be feasible if water of hydration is totally or partially removed. This point is essential to understand the role of water in terms of membrane response to biologically relevant effectors.

Entering details of the dynamic properties of membranes in relation to water, we may again inspect **Figure 5**. *The equilibrium point* corresponding to an area per molecule of 75 Å and 20 water molecules per lipid could be modified by the inclusion or exclusion of water molecules. So, the question is which perturbations can trigger *changes in hydration* that can be dominated in order to promote controlled changes in permeation. In this direction, let us focus on the mechanical and chemical forces at constant temperature. By mechanical forces, we refer to processes that led the membrane to expand or contract and by chemical forces, the competition with water by membrane sites of compounds may form hydrogen bonds. To analyze these points, previous considerations about water as a component of membranes must be done.

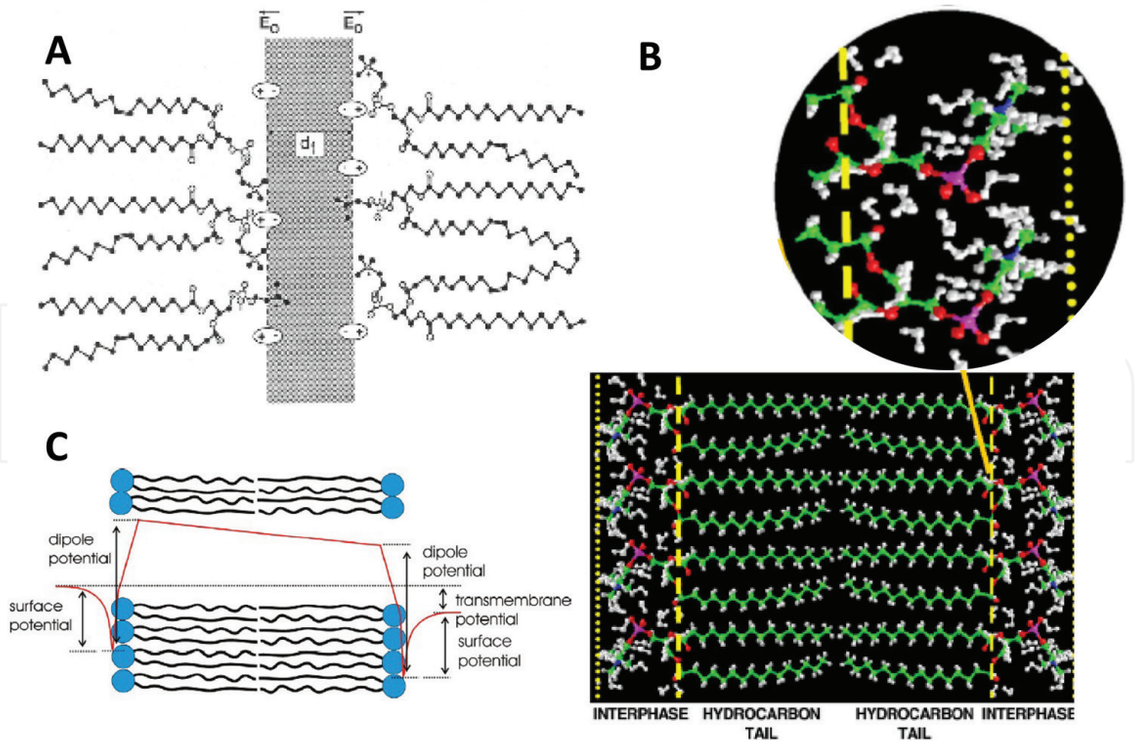

Figure 6.
(A) The limit of approach of lipid bilayers, (B) water organized at the interphase determining the repulsion forces, (C) dipole potential at the lipid interphase. This potential makes the bilayer interior positive and has important consequences in the binding and penetration of charged peptides and proteins.

3. The bilayer structure and the water ratio

The understanding of the structural role of water in lipid membranes received considerable attention after Luzzatti et al. and efforts were addressed to give a defined location to water and to determine the appropriated values for area per lipid molecule [22, 27, 28]. Today, it is generally accepted that PCs admit up to a limit of around 22–24 water molecules per lipid above the phase transition temperature to stabilize in a bilayer with an area per lipid of 64 $Å^2$ and a thickness of around 40 A [29, 30]. In the first stages of the hydration process, the water molecules interact with the phosphate group and, at a second step, with the carbonyl groups [31–33]. The formation of hydrogen bonds between these groups and water molecules can be monitored by observing the frequency shift of their stretching frequencies [34, 35]. It is expected that upon hydrogen-bonding, these frequencies decrease as a consequence of the elongation of the chemical bonds in the relative functional groups. Furthermore, the conformation of the acyl chains changes with the hydration [36].

At least three regions of differential hydration can be identified according to the rate of exchange between membrane phase and the aqueous environment: the phosphate group, the carbonyl groups and the hydrocarbon chains. Each of them has different level of water organization in regard to coordination water by H bonding [30]. From them, CO and acyl chains seem to be important to evaluate the role of water in regulatory and functional response [37, 38].

Figure 7 shows the lipid bilayer denoting the water region (upper part figure) and the profile of water distribution along the lipid bilayer (lower part).

It is observed that water covers all the phosphate and choline regions and partially the carbonyl groups. Moreover, it is also observed that water may penetrate beyond the carbonyl region, that is, the first methylene groups of the hydrocarbon chains. This denotes that different arrangements of water can be found along the membrane thickness each of one with a different energetic profile, that is, the ability

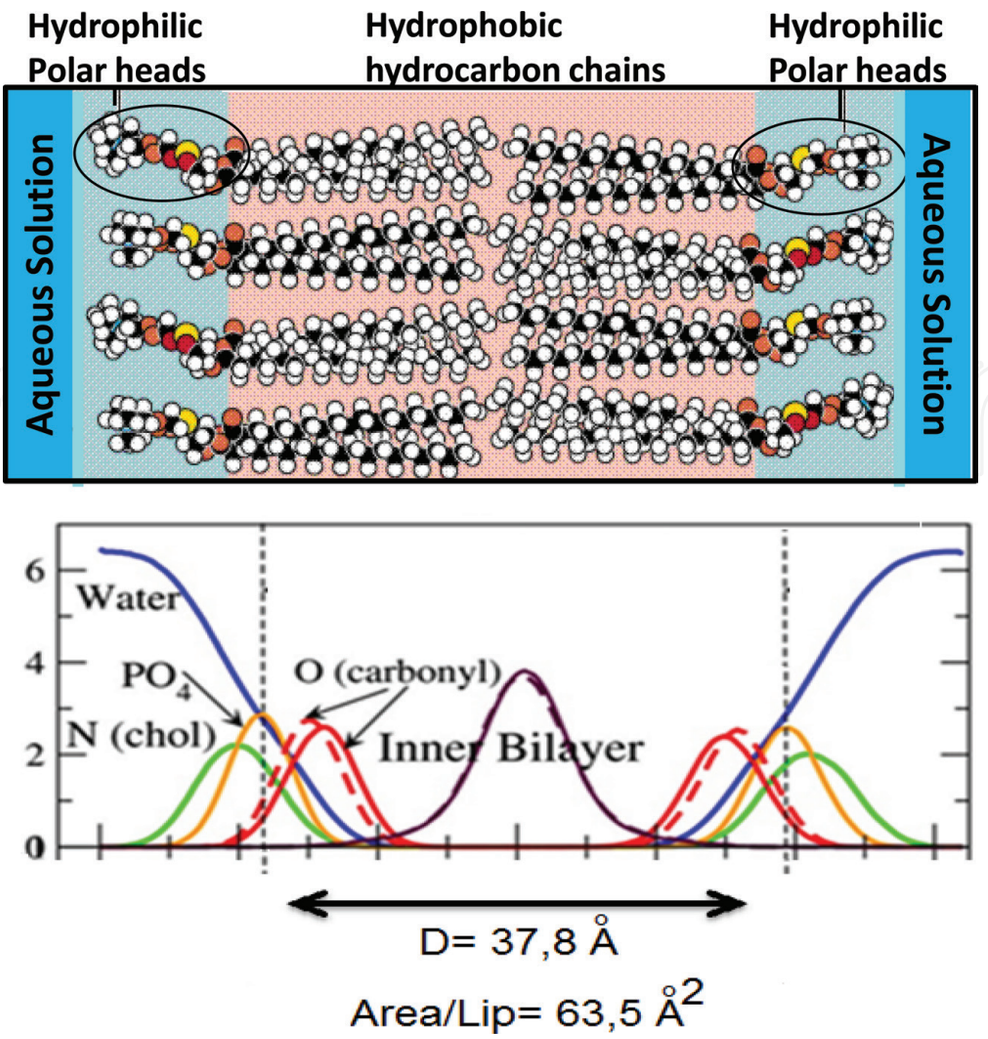

Figure 7.
The lipid bilayer considering the water interphases. In the lower diagram, it is observed that the water profile (blue line) penetrates up to the region of the carbonyl groups (red solid and dashed lines), being phosphates (orange line) and cholines (green line) groups completely covered by water.

to react with polar or ionic solutes. This reactivity can be ascribed to the residual capacity the water molecules have to form H bonds depending on the surface groups with which its interacts.

In **Figure 8**, water populations are visualized using infrared spectroscopy. Water bands at different frequencies denote the presence of non bonded to tetracoordinated waters [35, 39].

A closer analysis by molecular simulation allows to explain that the broad bands can be ascribed to subpopulations of water molecule in four of the five groups described (**Figure 9**) [30].

Hence, in a population of molecules with three hydrogen bonds, the following combinations can be possible: www, ppp, ccc, wwp, wwc, wpp, wcc, wcp, ppw, ppc, etc. This means that the water distribution and the energy of the surface are extremely heterogeneous and hence a great versatility in reaction can be expected, even more if we consider that these combinations have a mean life time. Hence, the sites can be modified by fluctuations and these can in turn affect the presence of solute from the media and the lateral interaction at different lateral pressure of the bilayer. In this regard, the presence of proteins can alter this picture.

For a better understanding of the influence of the lateral pressure on the membrane properties, we first deal with thermodynamic aspects of lipid monolayer. We will connect these properties with bilayer in the last part of the next section.

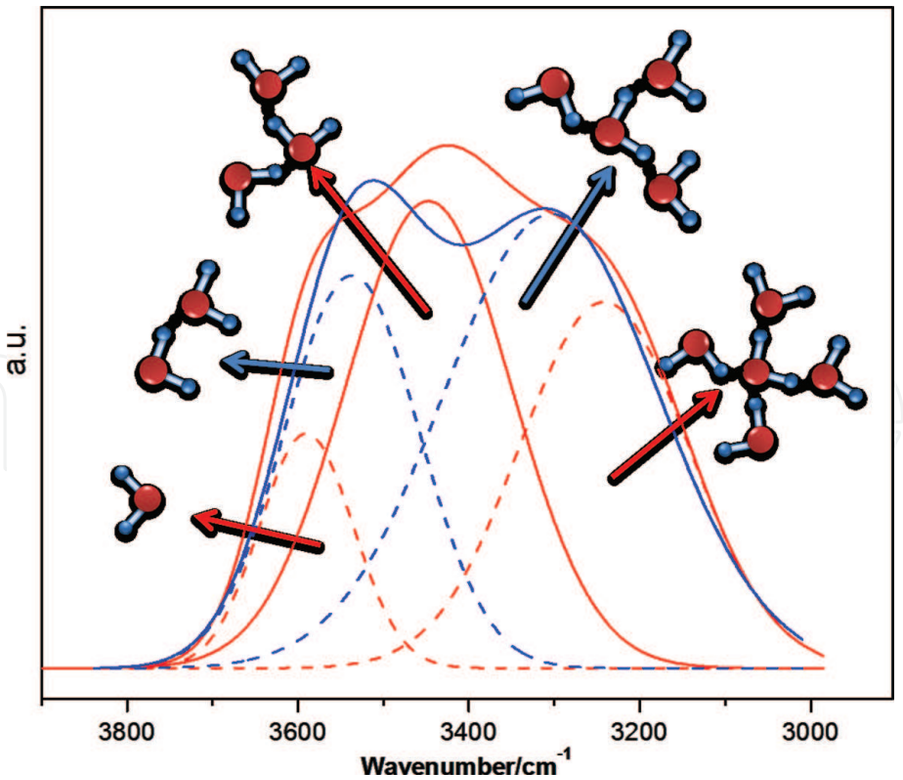

Figure 8.
Water bands in the adjacencies of lipid membranes in the gel (red line) and in the liquid crystalline state (blue line). Dotted lines denote the populations of water with none, one, two, three and four H-bonds. Central band at approximately 3500 cm^{-1} (red solid line) corresponds to bulk water without lipids.

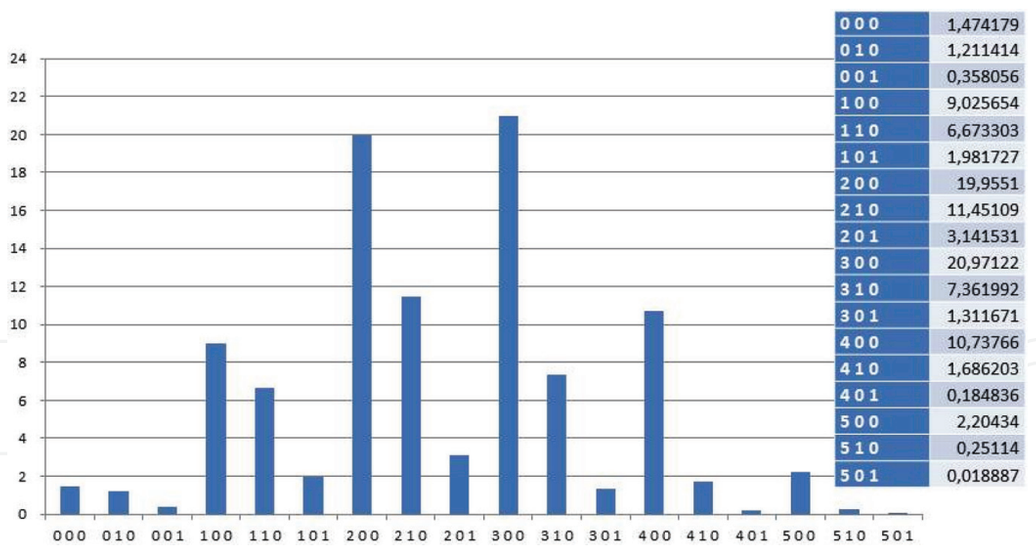

Figure 9.
Distributions of subpopulations of water forming none, one, two, three and four H bonds. Numbers correspond to water (w), carbonyl (C) and phosphate (P). Thus, 2 1 0 means two H-bonds with water, one with carbonyl, none with phosphates.

4. Stability and membrane response: a dynamic picture

The thermodynamic stabilization of the lipids in water to form bilayers as a consequence of the hydrophobic interaction between acyl chains has been discussed in several previous paper and will not be analyzed here [40–42].

Instead, the thermodynamic aspects of the bilayer as a reactive surface considering its *mechanical properties* will specifically be discussed. This approach is based

on the proposals presented earlier by Damoradan et al., Cevc, Disalvo et al. [37, 38, 43–45]. In terms of interfacial properties, it is important to consider lipid interphases results that may give complementary information to present a more rigorous picture in terms of thermodynamic response. This implies to focus on the interfacial properties of the lipid arrangements both in bilayers and monolayers in which water is a component of the structure. For this reason, some general properties of lipid monolayers will be discussed to relate them in response with lipid bilayers of liposomes and vesicles to have a general formalism that can explain the behavior of both systems.

The main property inherent to membrane stabilization as a bilayer or as a monolayer is the *surface tension of water*. In the formation of bilayers, the surface tension between the acyl chain and water as described in **Figure 2** is the main force driven the *hydrophobic interaction*. The increase in entropy is expressed by the decrease in the surface tension, that is, of the surface free energy. Hence the process is spontaneous.

When lipids spread on the air-water surface (**Figure 10**), monolayers are formed spontaneously by the same reason: decrease of the surface tension with respect to that of pure water (γ^0) [46]. This is generally expressed as the *surface pressure* (Π) which is given by:

$$\Pi = \gamma^0 - \gamma \tag{1}$$

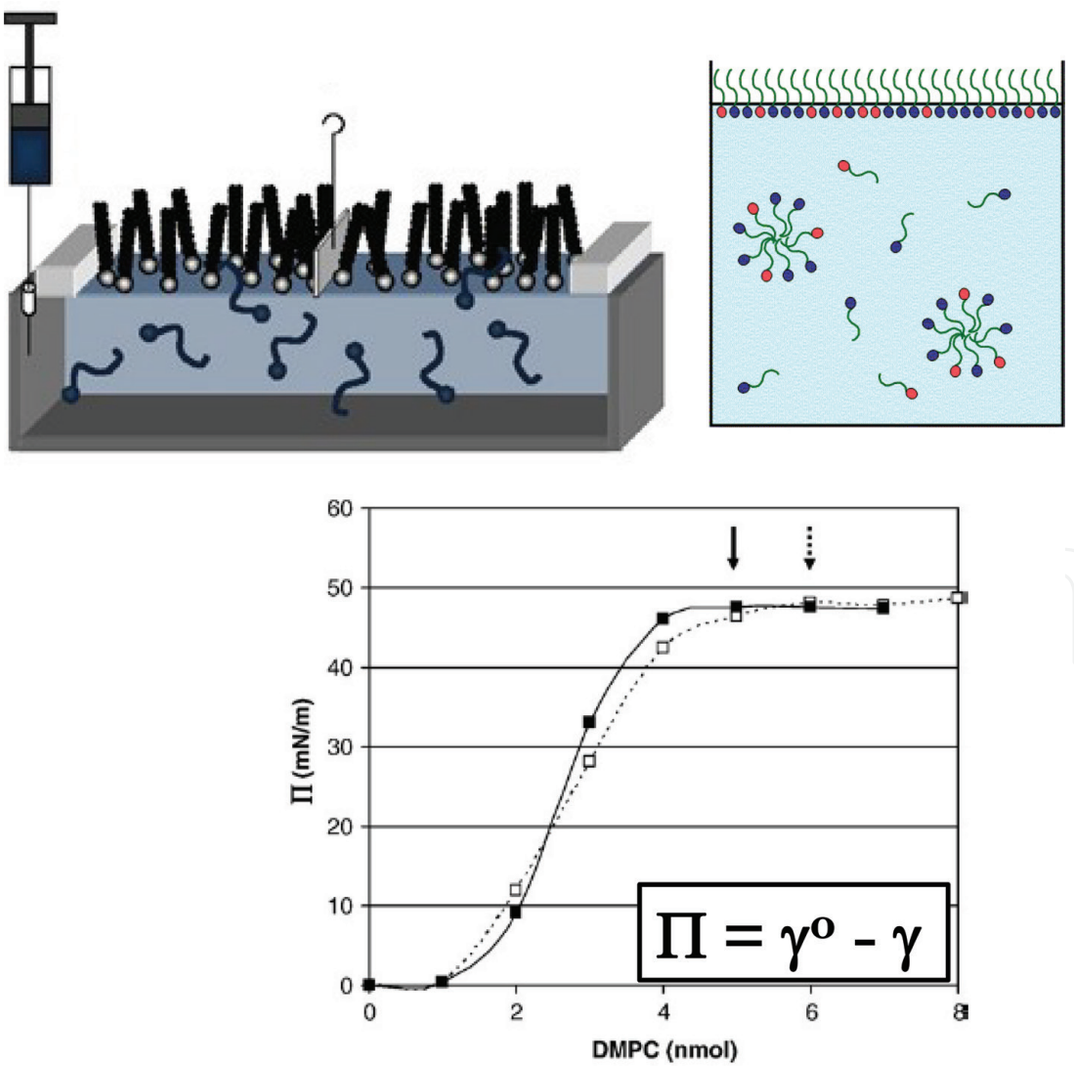

Figure 10.
The addition of lipids to the air water surface in a Langmuir though at constant area increases the surface pressure (Π) by decreasing the water surface tension (γ^o) until a limit value is reached. From this point, the area per lipid molecule can be calculated.

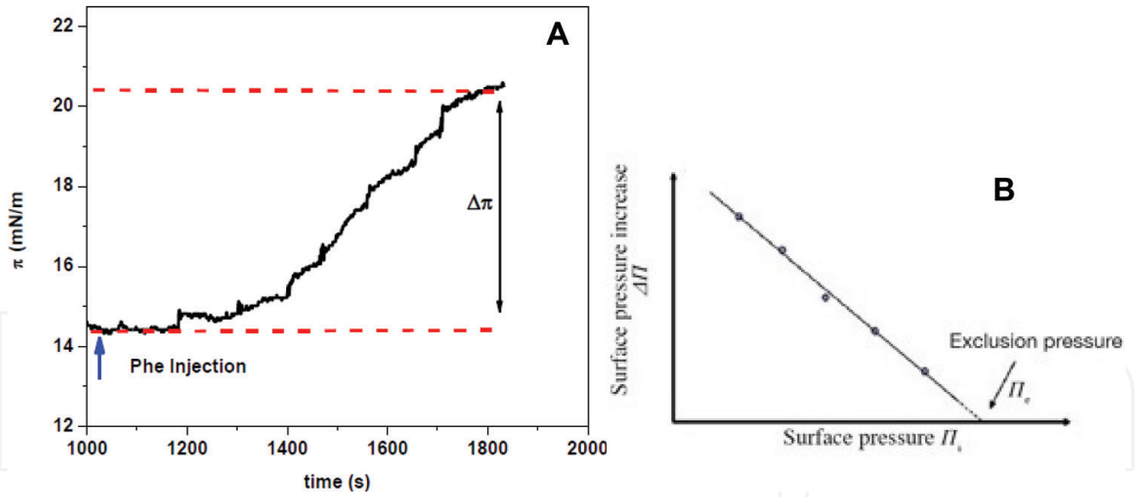

Figure 11.
(A) Kinetic of surface pressure changes after the injection of the effector to the subphase. The extent of the change reflects the affinity of the effector by the monolayer, (B) surface pressure increase versus initial pressure plot used for determining the exclusion pressure of the lipid monolayer.

Once the monolayer is stabilized at different lateral pressures (i. e. different surface excess of lipids), the response to perturbations promoted by solutes in the aqueous subphase can be tested (**Figure 11**).

As observed in **Figure 12**, *the exclusion pressure or cut off*, that is, the surface pressure at which no further effect on the monolayer is observed depends on the head group region for a given acyl chain length. DMPE (mean hydration ratio, 7 water molecules per lipid) shows a lower exclusion pressure (cut off), than DMPC (water ratio 22–24 water per lipid), although the slope of the curves remains constant. Thus, in the whole range of pressure, PE monolayers are less reactive than those of PC in the same conditions [47].

In addition, the magnitude of the surface pressure increase is also dependent on the hydrocarbon composition for similar head group regions. In **Figure 13**, it is

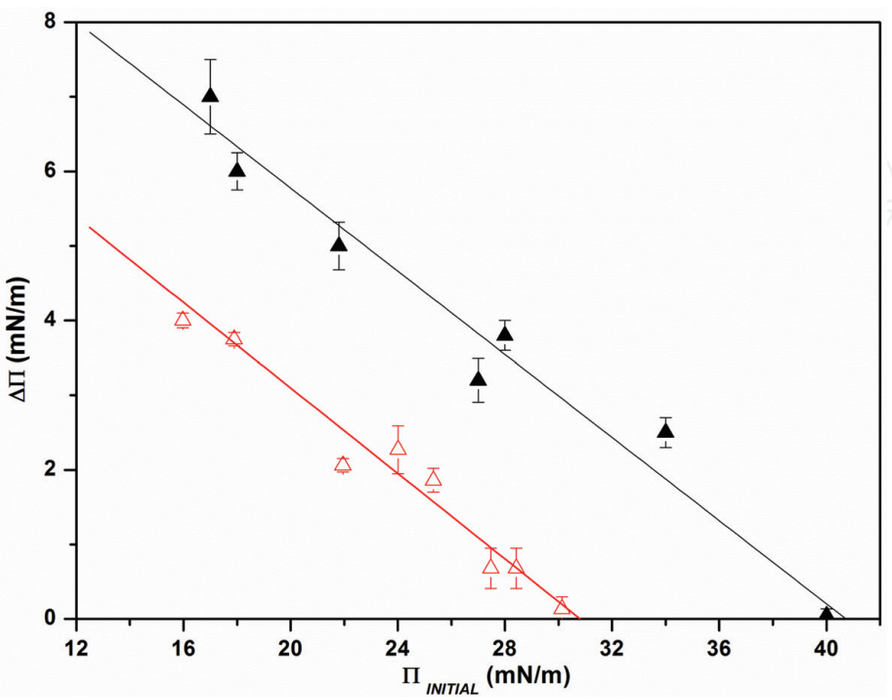

Figure 12.
The effect of a soluble protease on the surface pressure of pure DMPC (black full triangles) and DMPE (red empty triangles).

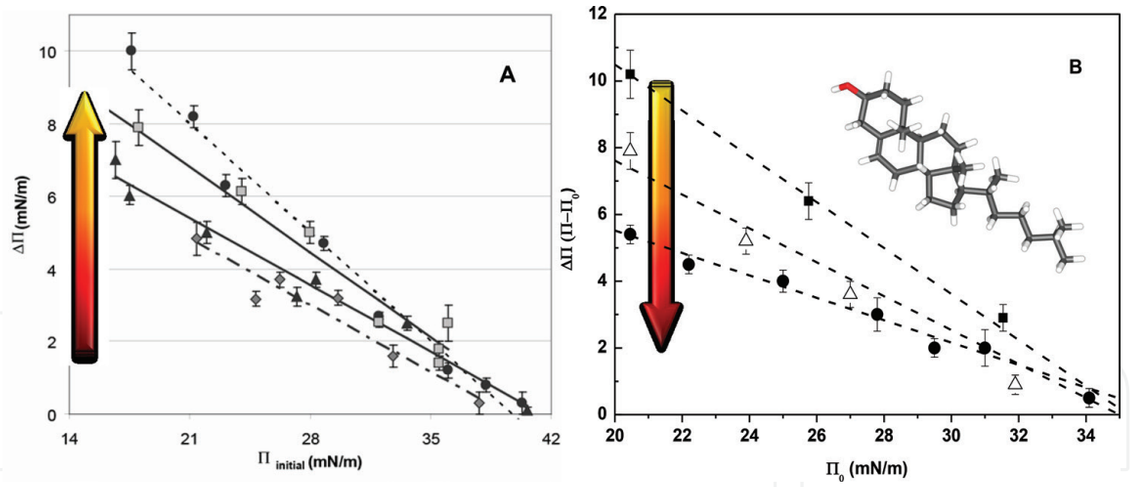

Figure 13.
Effect on the magnitude of the surface pressure response to a soluble protease of (A) unsaturation of the acyl chains in phosphatidylcholines, (B) inclusion of cholesterol in phosphatidylcholine monolayers.

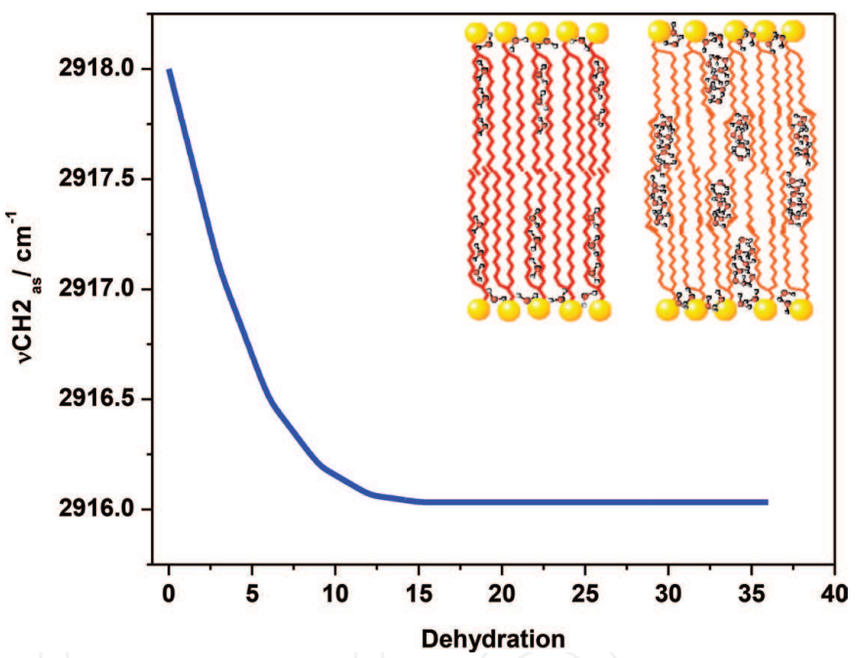

Figure 14.
Dehydration produces a decrease in confined water between acyl chains.

observed that the response at a given pressure increases with the unsaturation or ramification of the acyl chains and decreases with the presence of cholesterol [47, 48].

The surface pressures below the critical cut off (Π_c) are those states of the membrane interphase with propensity to react when chased by an effector in the subphase. These states correspond to different water/lipid ratio and hence different surface tension of water in the lipid matrix. The decrease in surface pressure $\Pi_c - \Pi$ is related to the increase of water beyond the hydration water (confined water) which part of it is distributed along the hydrocarbon chains. This can be followed by frequency changes in the methylene region by FTIR spectroscopy (**Figure 14**) [49, 50].

5. Bilayer barrier properties: permeation and controlled release

The studies in lipid monolayers allow to conclude that the response of the membrane to stimuli is due to the excess of surface tension due to the increase

of water beyond the hydration shell, mostly in the acyl chain region. This excess is produced in monolayers by increasing the area per lipid, that is, reducing the surface pressure. In terms of bilayers, the expansion can be achieved by submitting liposomes to a hypotonic swelling. In this condition, membrane expands and liposomes become leaky of solute trapped in the liposome interior in isotonic conditions. Hence, *the modulation of the mechanical properties of the bilayer* by lipid composition, for instance, by including different cholesterol ratios, would allow to have a controlled release. The plots in **Figure 13** help to understand this response. At a given surface pressure, the response decreases with the increase in cholesterol, which can be related to an increase in the membrane rigidity and lower water ratio [16, 51].

Thus, *permeability* (i.e. the ratio between trapped and released solute) depends on the extent of water in the acyl region and in the second shell of phosphate groups. An important derivation of these results is that the properties of permeability can no longer be described by a model that assumes that the membrane is composed of three slabs: *a low dielectric region* (the hydrocarbon chains) and *the two aqueous interphase*s, where the polar groups are located (**Figure 7**). This view predicts that solute permeating the membrane would be those that can partition in a non-polar media [52]. However, *polar amino acids and hydrophilic compounds* such as glycerol, erythritol and urea permeate easily the lipid bilayer. In addition, when swelling is taken place, ions and large molecules such as sucrose trapped in liposomes in isotonic conditions can permeate when they osmotically swell [53, 54].

Therefore, solute permeation involved in the release of trapped solutes is a more complex phenomenon than a simple *partition process*. In terms of water participation at the membrane interphase and in the acyl chain region, the mechanism would involve water reorganization and hence structural changes. To put this complexity into relevance, the interaction of polyarginines (Arg 9) with bilayers of lipids differing in hydration such as phosphatidylcholines and phosphatidylethanolamines will be discussed. In **Figure 15**, the adsorption isotherm of Arg 9 on liposomes composed by PC and PE are shown. Both present typical curves of saturation, denoting a limited number of sites with the affinity to bind Arg 9 are present in each surface [55]. They can be described by Eq. (2):

$$\theta = \frac{\Delta\zeta}{\Delta\zeta\,\max} = \frac{[Arg]^n}{K + [Arg]^n} \tag{2}$$

where θ is the degree of coverage of the liposome by Arg 9, *K* the dissociation constant and *n* is a stoichiometric coefficient of the binding. In the present case, θ has been calculated by the change in the surface potential at each Arg 9 concentration measured by *electrophoretic mobility* from plots as shown in part A of **Figure 15**.

The fitting of the curves of part B gives an affinity constant for DMPC at 18°C, $K = 0.54 \times 10^3 \pm 44\ M^{-1}$ and a value n = 1. For DMPE at 18°C, $K = 2 \times 10^3 \pm 189\ M^{-1}$ and n = 0.74 ± 0.06. According to these results, the affinity of Arg 9 is higher in DMPC than in DMPE. In addition, the interaction of Arg 9 with DMPC is well described by a *Langmuir isotherm*, since n = 1. In contrast, the value of n is different from 1 for Arg 9 in DMPE suggesting different mechanisms of interaction. The strong difference in affinity between PE and PC membrane can be explained recalling the monolayer results. The cut off described in **Figure 12** is much lower in PE than in PC, that is, at pressure above 32 mN/m at which PE is not affected, PC monolayer is still *reactive*. This can be explained by the strong interaction between the polar head groups of the PE molecules that restrict the water excess and hence decreasing responsiveness.

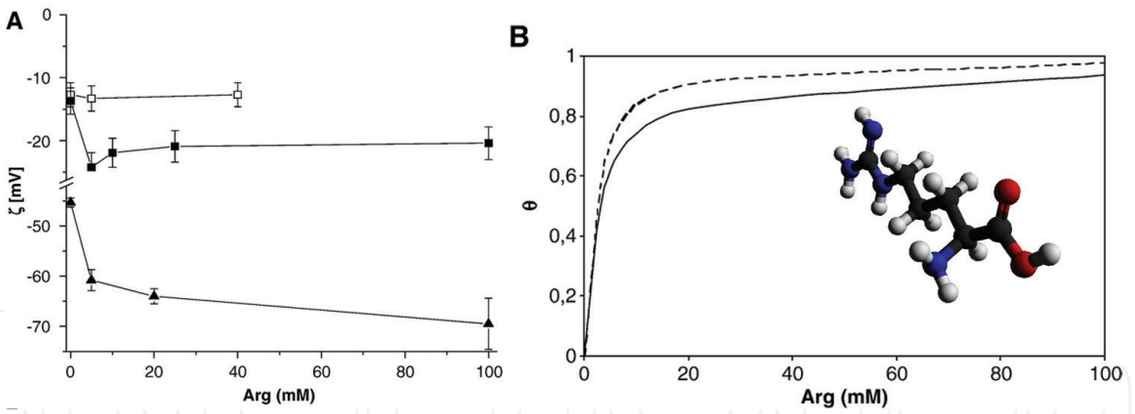

Figure 15.
Adsorption of Arg 9 on DMPC and DMPE liposomes (A) change in zeta potential (ζ) of DMPC (▲) and DMPE (■) liposomes with addition of increasing concentrations of Arg 9. θ is calculated by $\zeta - \zeta^0/\zeta^\infty - \zeta^0$, where ζ is the value at a given Arg 9 concentration, ζ^0 corresponds to liposomes in the absence of Arg 9 and ζ^∞ the value at saturation. (B) Degree of coverage as calculated from part A versus Arg 9 concentration.

6. Topological effects of osmotic shrinkage. Defects in packing

As described above, liposomes and vesicles, as well as cells, respond to *osmometers* [54]. That is, their volumes increase or decrease as a consequence of the entrance or exit of water driven by osmotic gradients. In the previous section, we show that in swelling conditions, bilayer expansion produces changes in its permeability, that is, osmosis does not only affect the liposome volume but also the membrane density. Now we will see that it also affects the surface properties.

In hypertonic media, liposomes shrink, that is, they *expulse the free water in the internal volume*. However, the volume decrease has a limit due to the repulsive forces put in evidence at short distance as described in **Figure 6**. The overcoming of this repulsive barrier derives in adhesion or fusion of the surfaces and implies *water elimination from the interphase*. The compression process due to osmotic shrinkage in bilayers can be compared with the decrease in area in monolayers. In this system, at high pressures (i.e. low areas), monolayer collapses and lipids are lost. In bilayers, the compression has different consequences due to the impossibility to extrude lipids from the bilayer.

Bilayers are soft and dynamic material [56, 57], and as a result, they can bend and deform in response to different stimuli such as dehydration and molecules that may compete with water. So, compression induced by osmotic shrinkage may result in topological changes giving place to regions (domains) of high curvature (invaginations or vesiculations) [58–60]. Thus, bilayer deformation can allow transient defects, when exposed to osmotic gradients (**Figure 16**). These curvature domains have important consequences in the interfacial behavior in the absorption or penetration of peptides composed by different types of amino acids. Many simulation studies showed that defect formation can determine the free energies of many membrane processes [61, 62].

When DPPC LUVs were subjected to hypertonic stress, defects caused by dehydration have more affinity for lytic compounds and amino acids such as phenylalanine [63–66]. The presence of defects in the membrane packing determines the binding and stabilization of peptides containing Arg and Phe motifs [67–69]

The disruption of the water network around the phenyl group and the membrane defect has been invoked to explain the *negative free energy* of the formation a PC-Phe (phosphocholine-phenylalanine) complex in the presence of water. An important observation was that a dipole potential decrease was produced in this interaction which was explained by the orientation of the carboxylate opposing to the CO of the lipids [66, 70].

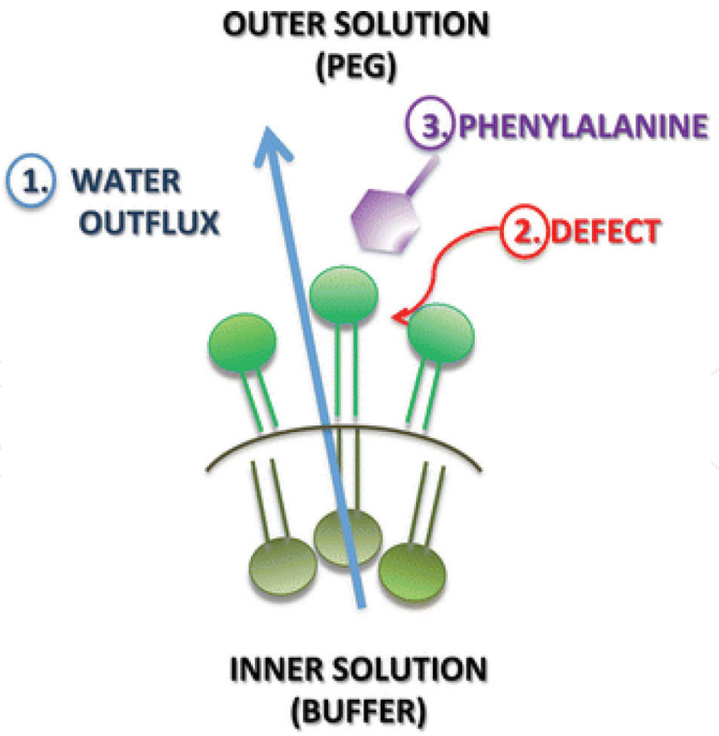

Figure 16.
Defects induced by osmotic shrinkage enhance Phe insertion into lipid bilayers.

As described in **Figure 9**, carbonyl groups are one of the hydration centers in which interfacial water is distributed. The formation of high curvature surfaces is related to changes in the *CO arrangements* [39] (**Figure 17**).

The curvature domains, in fact, increases the bilayer free energy surface by exposing hydrophobic region and carbonyl configuration as observed in part B of **Figure 17**. The stabilization of peptides or amino acid is the result of the decrease of free energy at expense of the bending modulus, that is, the energy cost of topological changes [67, 71, 72]. This last quantity is an important parameter that governs a membrane's tendency for defect formation. Many kinds of defects may be formed, including vesicle budding and fusion, depending on the presence of non-bilayer lipid phases, cholesterol and interactions between lipid bilayers and other biomolecules [19, 73].

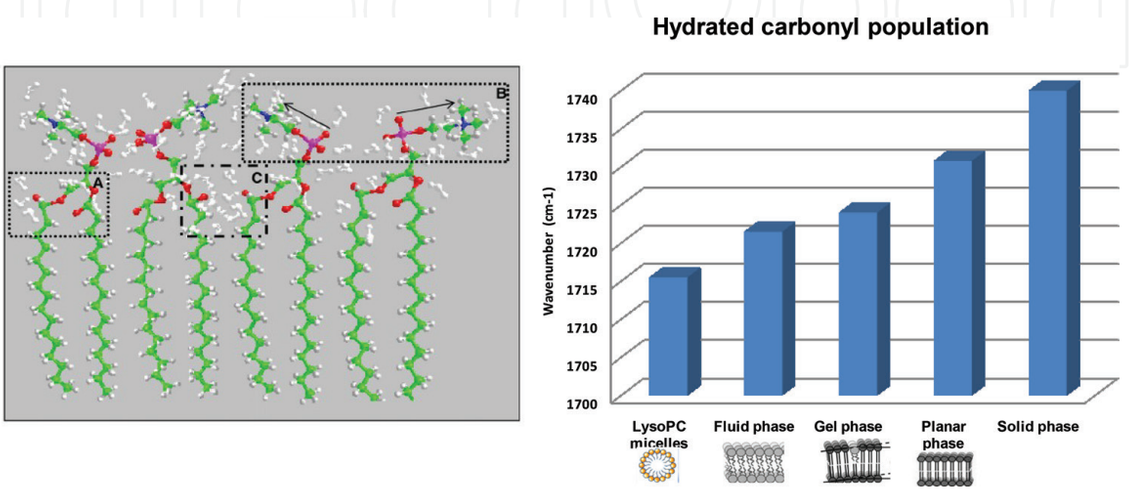

Figure 17.
Orientation of CO groups at the bilayer interphase in relation to curvature.

7. Concluding remarks: design and modulation

The lipid matrix in cell membranes presents a variety of lipids. As shown in **Figure 18**, all of them present a diacyl glycerol structure with acyl chains differing in saturation and length, a phosphate group in position 3 linked to a proton, choline, ethanolamine, glycerol, serine or inositol residues. It is difficult to accept that within the economicity principle of biology, this wide variation does not play a role in *functional properties of the membrane.*

It must be noticed that all lipids may be aligned along a plane containing the glycerol backbone and the carbonyl groups. On one side of this plane, the hydrocarbon region can vary in terms of chain length, saturation and ramification. Each of these variations may give a complex matrix in which water cannot be excluded and constitute a media of a wide range of dielectric properties. On the other, polar groups protrude into the aqueous phase at different magnitudes. Importantly, also these groups are able in different extents to form hydrogen bonds between its lipid neighbors and with water. *In conclusion, it is an oversimplification to reduce the bilayer structure in which lipids organize facing the polar groups to water and segregating the nonpolar chains*.

The lack of details in these regions in relation to their emergent properties is a major limitation in the design of biomimetic systems that, as liposomes and vesicles, may be used for biotechnological and medical purposes. As a consequence, literature is saturated with works in which only "try and error" strategies are employed.

In this chapter, we have briefly discussed that:

- the interface is a bidimensional solution of hydrated polar groups.
- excess water beyond the hydration shell has solvent properties for additives in the bulk water phase and confers free energy that is excess for binding of amino acids and peptides.
- Dissolution in the water membrane phase changes the water activity (a_w) and affects the surface pressure.

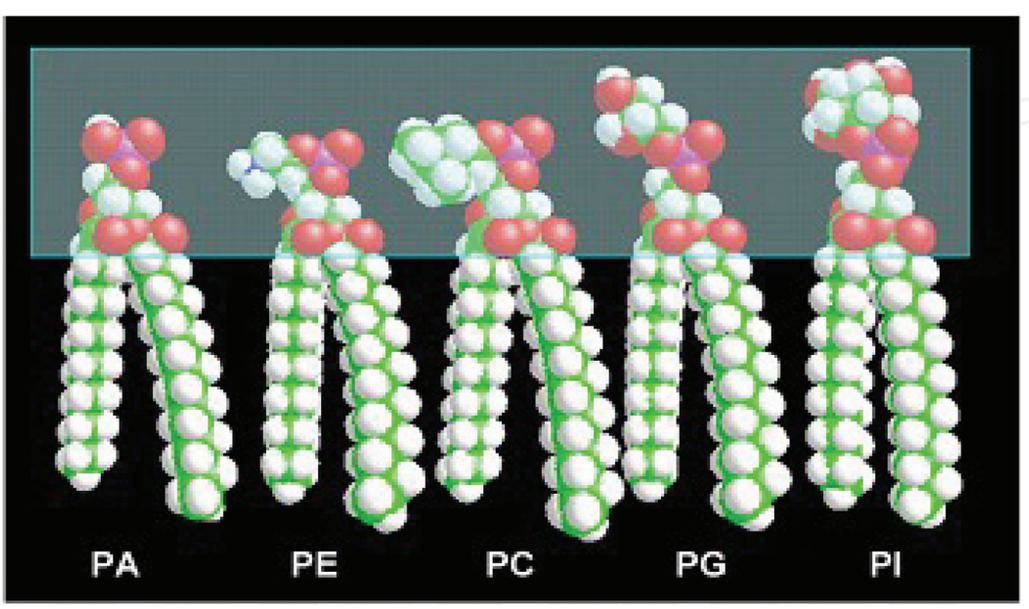

Figure 18.
Protrusion of the groups esterified to the phosphate in the surface of the membrane promotes different water organization.

These studies indicate a deeper understanding of the role of lipid bilayers in cellular biology and support the development of future lipid-based biotechnology that should necessarily include the role of water as a membrane dynamic component.

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