
Aligned Lipid-Water Systems

John Katsaras and V. A. Raghunathan

1 Introduction

Under physiologically relevant conditions, membrane lipids normally form self-assembled bilayers where the lipids are usually in the disordered liquid crystalline or L_α phase. In this phase the lipid molecules are undergoing rapid translational diffusion and their fatty acid chains experience *trans-gauche* isomerizations [1–3]. Furthermore, ‘physiological relevance’ also implies that the lipid bilayers are ‘fully hydrated’ i.e. in excess water conditions. Although the condition of full hydration is easily met in liposomal preparations, by immersing the lipids in bulk water, this has not been the case for lipid bilayers aligned on solid supports and hydrated from water vapour [4,5]. The result of this was aligned bilayers exhibiting repeat spacings (d -spacings) which were less than their liposomal counterparts.

Despite this discrepancy in hydration, aligned lamellar arrays of lipids and biological membranes, have proven to be of great use especially when the elucidation of structure has been the primary objective [6–31]. Compared to isotropic or commonly referred-to liposomal preparations, aligned membrane systems give access to a variety of powerful physical techniques for extracting unambiguous structural information. For example, techniques such as, x-ray diffraction [6–19], neutron diffraction [13,20,21], optical [10,21,22], spectroscopic [23–29] and other methods [6,30,31] have been effectively used to study both natural and model aligned membrane systems. More importantly, in certain cases structural solutions have only been made possible when an aligned sample became available [15,16,18,19].

Over the years a variety of techniques have been employed in aligning lipid bilayers and biological membranes e.g. [16,18,4,32–34]. However as mentioned previously, whenever L_α aligned multibilayers were hydrated from water vapour at equilibrium with liquid water, they consistently and without exception achieved d -spacings much less than those observed in liposomal preparations [35,36]. With the exception of two studies [37,38], this has also been the case for rigid, $L_{\beta'}$ bilayers [18,37,39,40]. Only in a couple of cases, when in contact with liquid water, have aligned multibilayer systems in other phases exhibited repeat spacings comparable to their liposomal counterparts [4,34].

Why then is water (liquid and vapour) at equilibrium conditions and having the same chemical potential behaving so differently when it comes to

hydrating the same material? This commonly observed and widely accepted discrepancy has over the years come to be known as the vapour pressure paradox (VPP) [41]. Only most recently has it been shown not to exist [5].

In this review we will describe contributions that aligned systems have made to the structure of biomimetic membranes, especially to lipid-water phases such as the subgel ($L_{c'}$) and gel ($L_{\beta'}$) phases. Also, we will deal with the recent developments in the fabrication of physiologically relevant aligned systems where the lipids are in the L_{α} phase and which can be fully hydrated. From our view, as a result of these recent developments aligned systems will prove to be an indispensable ‘tool’ with regard to the study of biological relevant model membrane systems.

2 The Subgel Phase of DPPC Multibilayers

Dipalmitoyl phosphatidylcholine (DPPC) is the most studied and possibly best understood lipid-water system [4,9,15,16,20,37–40,42–48]. Prior to 1980, it was known that with increasing temperature fully hydrated DPPC multilamellar suspensions exhibited two thermotropic phase transitions; the ‘main transition’ ($P_{\beta'}$ - L_{α}) at ≈ 41 °C corresponding primarily to a melt of the hydrocarbon chains and at a lower temperature ($T_p = 35$ °C), the commonly referred-to ‘pretransition’ ($L_{\beta'}$ - $P_{\beta'}$) where the stiff planar bilayers become corrugated [46]. However in 1980, scanning calorimetric evidence for a third phase transition centered at ≈ 18 °C and dubbed the subtransition was obtained [45]. The subgel phase went undetected because unlike the main transition and pretransition, the subgel phase is sensitive to sample history and was only observed after a DPPC multilamellar suspension was incubated at 0 °C for 3.5 days [45].

Once this low temperature subgel or $L_{c'}$ phase was discovered, its structure became an open issue and was the subject of numerous x-ray diffraction studies [47–50]. It was found that compared to the higher temperature $L_{\beta'}$ phase, $L_{c'}$ bilayers exhibit a smaller lamellar periodicity (59 Å *vs* 63 Å) and the diffraction pattern from $L_{c'}$ bilayers contained reflections, namely those at $1/10$ and $1/6.8$ Å⁻¹, that were not as a result of the lamellar structure [47–50]. However, despite the various experimental efforts the structure of the subgel phase remained unresolved. Why then this difficulty in elucidating the structure of the $L_{c'}$ phase from powder diffraction patterns when the $P_{\beta'}$ phase, which gives rise to more a complicated diffraction pattern (e.g., more diffraction maxima), was solved for both DPPC and dimyristoyl phosphatidylcholine (DMPC) bilayers using similar experimental strategies [46]? Fig. 1 shows the diffraction pattern from aligned DPPC multibilayers in the subgel phase [15,16]. Unlike diffraction patterns from powder samples, visual examination of a diffraction pattern from aligned samples easily reveals reflections arising from structure both parallel and perpendicular to the plane of the bilayer. For example, with the exception of the lamellar reflections

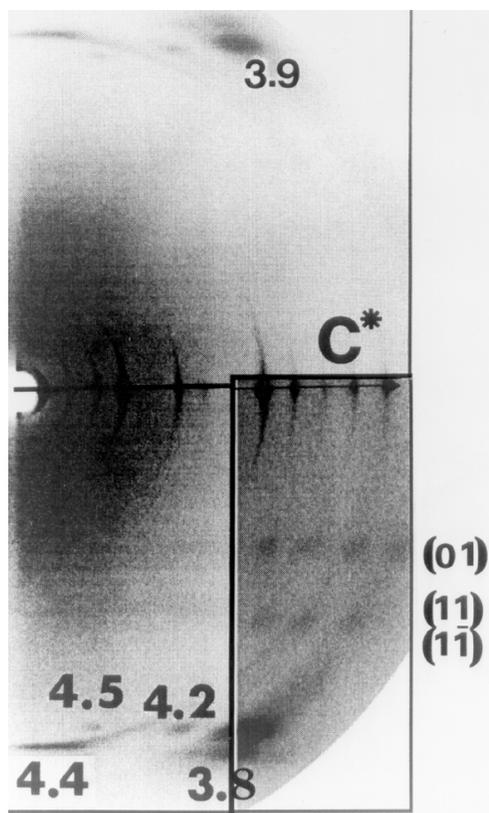


Fig. 1. Diffraction pattern from an aligned DPPC stack of subgel or L_c' bilayers. The reflections along the c^* axis correspond to a lamellar periodicity of 56.4 Å, while all the other reflections are due to in-plane structure. The reflections in the 4 Å range correspond to the various spacings between the hydrocarbon chains. The weak (0 1), (1 1) and (1 $\bar{1}$) reflections, parallel to c^* , are due to the molecular lattice and are clearly seen in the enhanced portion of the pattern. It can also be seen that the (0 1), (1 1) and (1 $\bar{1}$) reflections have their intensities modulated. This modulation in the intensity corresponds to a length scale of approximately 40 Å, the separation between the headgroups across the thickness of the bilayer.

lying along the c^* axis all others are the result of structure within the plane of the bilayer. Most importantly, and the primary reason that the structure of the subgel phase could not be determined from powder samples, are the reflections corresponding to d -spacings of 4.2 and 4.5 Å. They happen to be collinear with the 3.9 Å reflection and are the result of secondary maxima in the form factor of the hydrocarbon chains [18]. Thus the 4.2 and 4.5 Å reflections are not the result of any lattice constant found in the structure of the subgel phase! This ‘detail’ could not be taken into account by previous

studies using powder samples. As a result, proposed models formulated to account for all of the diffraction maxima were unsuccessful in determining the structure of the $L_{c'}$ phase [49,50]. It should be mentioned that for off-equatorial reflections, in other words, maxima lying along the c^* axis and those reflections which do not lie or cut across the axis perpendicular to c^* , their lattice parameters are obtained by projecting them onto the equatorial axis, the axis perpendicular to c^* . As such, the lattice constant of the $1/3.9 \text{ \AA}^{-1}$ reflection is not 3.9 \AA but rather 4.65 \AA . On the other hand, the lattice constant of the $1/4.4 \text{ \AA}^{-1}$ reflection corresponds to that reflection's repeat spacing, namely 4.4 \AA (Tab. 1).

Table 1. Experimentally observed and calculated d -spacings^a of DPPC bilayers in the $L_{c'}$ phase

$(h k)$	Calculated d -spacings (\AA)	Experimental d -spacing ^c (\AA)
0 1	10.0^b	9.8-10.14
1 0	9.4^b	9.3-9.4
1 1	6.8^b	6.75-6.84
$1 \bar{1}$	6.8^b	6.75-6.84
0 2	5.0^b	4.9-5.0
$\bar{1} 2$	4.4	4.4-4.46
2 0	3.9	3.8-3.9
1 2	3.8	

^aThese d -spacings are in addition to the lamellar spacings.

^b d -spacings as a result of DPPC molecules 'ordering'.

^cThe range of d -spacings in the experimental data is the result of a compilation of data from various experiments on the subgel phase of DPPC over the years [16,47,48,50–52].

As each DPPC molecule consists of two aliphatic chains connected to a glycerol backbone carrying the hydrophilic phosphorylcholine headgroup, it is possible to construct various molecular lattices from a given hydrocarbon chain sublattice, each leading to a distinct set of reflections. Only the superlattice shown in Fig. 2 was found to be consistent with the reflections observed from the diffraction pattern obtained using aligned DPPC multibilayers (Fig. 1). However, even for this unique molecular lattice there exist a variety of packing arrangements shown in Fig. 2. Presently the molecular arrangement within the plane of the bilayer is an unresolved issue. The pa-

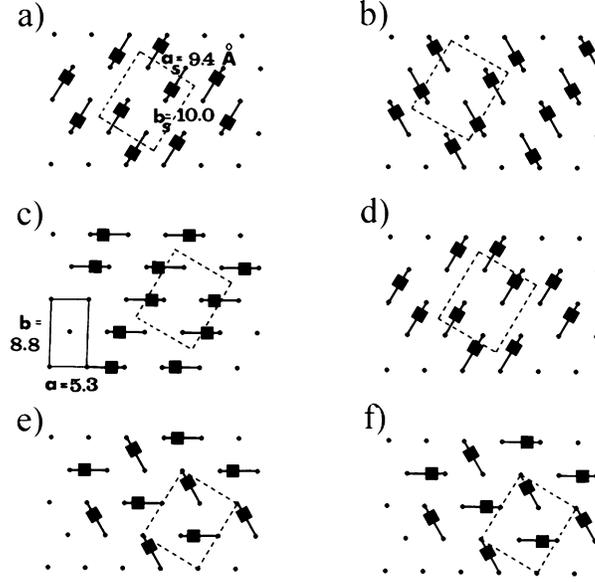


Fig. 2. The six possible two dimensional molecular arrangements of DPPC molecules in the subgel phase. The phosphorylcholine headgroups are denoted by ■'s, and the hydrocarbon chains by ●'s. One lipid molecule is made up of one headgroup and two hydrocarbon chains. The molecular lattice contains two lipid molecules and has dimensions $a_s = 9.4 \text{ \AA}$, $b_s = 10.0 \text{ \AA}$ and $\gamma = 90^\circ$ while the hydrocarbon chain sub lattice has dimensions of $a = 5.3 \text{ \AA}$, $b = 8.8 \text{ \AA}$ and $\gamma = 94^\circ$. The molecular and hydrocarbon chain lattices are denoted by broken and solid lines, respectively.

parameters for the molecular lattice or superlattice can be expressed in terms of the hydrocarbon chains sublattice by the following relations:

$$a_s^2 = \frac{1}{4}(9a^2 + b^2) - \frac{3}{2}ab \cos \gamma \quad (1)$$

$$b_s^2 = a^2 + b^2 + 2ab \cos \gamma \quad (2)$$

$$\cos \gamma_s = \frac{3a^2 - b^2 + 2ab \cos \gamma}{2a_s b_s} \quad (3)$$

$$(4)$$

Using the hydrocarbon sublattice values ($a = 5.3 \text{ \AA}$, $b = 8.8 \text{ \AA}$ and $\gamma = 94^\circ$) the superlattice parameters were found to be $a_s = 9.4 \text{ \AA}$, $b_s = 10.0 \text{ \AA}$ and $\gamma_s = 90^\circ$ with the unit cell of the superlattice containing two DPPC molecules compared to only one lipid molecule for the chain sublattice.

The use of an aligned sample made it possible to unambiguously understand the ‘origin’ of the various diffraction maxima thus enabling the extraction of the structural features which make the subgel phase unique and can be summarized as the following:

- the in-plane arrangement of lipid molecules can be described by a molecular lattice or superlattice, containing two lipid molecules and a hydrocarbon chain sublattice having one DPPC molecule.
- besides being ordered in the plane of the bilayer, DPPC molecules are positionally correlated across a single bilayer but not with those in adjacent bilayers making the subgel phase a highly ordered two dimensional phase. From the diffraction pattern the thickness of the bilayer, $\approx 40 \text{ \AA}$, is given by the periodicity of modulation of the $(0\ 1)$, $(1\ 1)$ and $(1\ \bar{1})$ reflections.

3 In-Plane Structure of the Gel Phase

In a landmark paper, Tardieu et al. [43] described the structure and polymorphism of a variety of lipid-water systems. In it, the gel phase is shown to be comprised of lipids which have, with respect to the bilayer normal, untilted hydrocarbon chains (L_{β} phase $\theta = 0^\circ$) and others whose hydrocarbon chains are tilted ($\theta \neq 0^\circ$). This latter gel phase is commonly denoted as the $L_{\beta'}$ phase and is usually observed in synthetic lecithins with identical saturated fatty acid chains. Furthermore, the magnitude of θ was described as being dependent on the water content of the lipid [43].

However, due to the quality of the powder patterns the in-plane interactions of the hydrocarbon chains could not be properly evaluated even though in the case of smectic liquid crystals it was known that molecules tilted in the plane of the hexagonal net could exhibit both nearest neighbour ($\phi = 30^\circ$) and next nearest neighbour ($\phi = 0^\circ$) interactions [53].

Using well aligned (mosaic $\simeq 0.1^\circ$) freely suspended samples, Smith et al. [18] demonstrated that the $L_{\beta'}$ phase is in fact, three distinct 2D phases (Fig. 3), one of which, namely the $L_{\beta L}$ phase, is unique to the lipid-water system (Fig. 3b). Constant-intensity contours in the (q_r, q_z) plane are shown in Figs. 3a-c and their real-space structures of hydrocarbon chains are shown in Figs. 3d-f, respectively. The data clearly demonstrates that at a constant temperature and as a function of increasing hydration the tilt direction changes continuously from next nearest neighbour ($\phi = 0^\circ$) to nearest neighbour ($\phi = 30^\circ$). It should also be noticed that the principal maxima are accompanied by weaker, subsidiary peaks which are understood as resulting from the molecular form factor of a pair of fatty acid chains sitting end-to-end. These maxima are similar to those observed in the subgel phase of DPPC. The widths along q_r and q_z of the main reflections, which are wider than the instrumental resolution, correspond to an in-plane correlation length of $\simeq 200 \text{ \AA}$. There is also an absence of any out-of-plane correlations. The absence

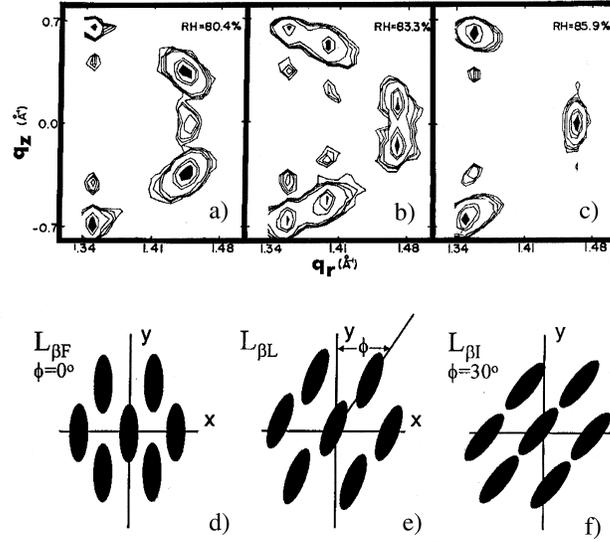


Fig. 3. Contour plots in the (q_r, q_z) plane at a temperature of 23.5 °C of DMPC multibilayer stacks in the (a) $L_{\beta F}$, RH = 80.4%; (b) $L_{\beta L}$, RH = 83.3%; and (c) $L_{\beta I}$, RH = 85.9% phases and their corresponding (d)-(f) real space structures. The azimuthal angle is denoted by ϕ , q_r is the cylindrical in-plane coordinate and q_z the reciprocal-space coordinate normal to the bilayers. For the $L_{\beta L}$ case ϕ was found to be 17.2°.

of an out-of-plane correlation length clearly shows that the $L_{\beta'}$ phases are not three dimensional phases, but rather two dimensional structures similar to the subgel phase. A distinct difference between the two phases is that in the gel phase the lipid molecules are not positionally correlated across the bilayer.

The $L_{\beta'}$ phase can thus be characterized by the following features:

- the $L_{\beta'}$ phase is not, as previously believed, one but three distinct phases differentiated by the direction in which the hydrocarbon chains are tilted with respect to the in-plane lattice.
- each distinct phase has been shown to be a 2D phase with no out-of-plane correlations.
- the tilt angle θ , as a function of hydration, increases up to a limiting maximum. The increase in θ can also be correlated to the transformation of the in-plane structure, e.g., $L_{\beta F} \rightarrow L_{\beta L} \rightarrow L_{\beta I}$.

4 The Ripple Phase

The ripple or $P_{\beta'}$ phase characterized by a one-dimensional height modulation of the bilayers is seen in some phospholipids under high hydration [43,46]. In the phase diagrams of these systems, it is sandwiched between the high temperature L_{α} phase and the low-temperature $L_{\beta'}$ phase. Both these transitions are first order, with the enthalpy change at the $P_{\beta'} \rightarrow L_{\alpha}$ transition (often called the main transition) being almost an order of magnitude larger than that of the $L_{\beta'} \rightarrow P_{\beta'}$ transition [54]. Most of the studies on the ripple phase have been in homoacyl disaturated phosphatidylcholines, in particular, in DMPC and DPPC [17,34,46,55–59]. This phase has also been observed in saturated mixed-chain PC derivatives [60,61], some PC derivatives with unsaturated chains [62–64] and in various other systems [65–69].

The structure of this phase has been extensively studied using a variety of techniques [17,34,43,46,55–60,70–73] and have established the existence of three types of ripple phases with the vast majority of scattering experiments showing a phase with an oblique unit cell (Type I). The wavelength of the ripples in this phase usually lies somewhere in the range of 120 to 160 Å while the peak-to-peak amplitudes of the ripples varying between 10 to 20 Å [70,71,74,75]. Freeze fracture electron microscopy studies have shown that the shape of these ripples is more saw-tooth like than sinusoidal. The electron density map of the ripple phase of DMPC, shown in Fig. 4, confirm such a shape [70,71]. However, the details of the packing of the hydrocarbon chains in the bilayer are presently under debate. The ordering in the plane of the bilayer seems to be at least hexatic if not crystalline. Furthermore, spectroscopic [76] and diffusion studies [77] have shown the existence of a significant fraction of disordered chains (i.e., chains that are not in the fully stretched all-trans conformation) in this phase. Recent analysis of the electron density maps indicates that the chains are tilted in the direction of the ripple [78]. This seems to offer a more consistent interpretation of the various features in the electron density map, than an earlier interpretation in terms of coexisting L_{α} and $L_{\beta'}$ regions in the bilayer [70]. These Type I ripples are usually referred to as asymmetric.

The second type of structure corresponds to a rectangular unit cell (Type II). The shape of these ripples has not been unambiguously determined, though freeze fracture electron microscopic studies indicate that the shape is not a simple sinusoidal [79]. This structure often occurs as a metastable phase on cooling some lipids like DPPC across the main transition [56–58]. The wavelength of these ripples is slightly less than twice that of the ripples of Type I. The larger lamellar spacing found in the case of ripples of Type II, compared to those of Type I, indicates that the chain packing in these two systems are probably very different. Similar ripples have also been seen in some lipids in the presence of cations which are adsorbed on the bilayers. This structure is usually referred to as symmetric ripples. The third structure, as far as we know, has been seen only in one x-ray scattering study of

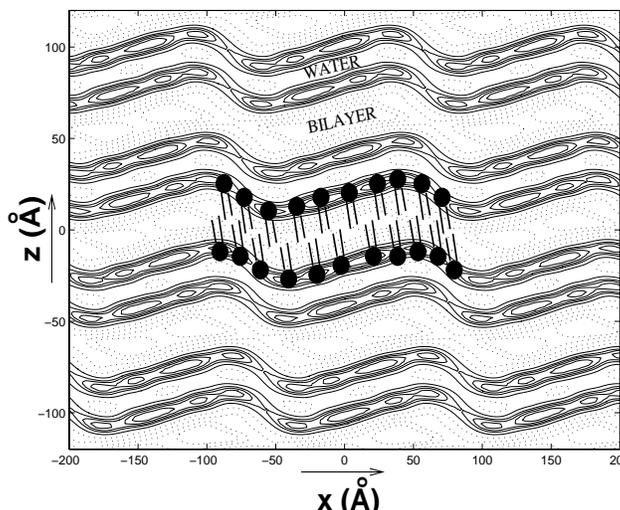


Fig. 4. Electron density map showing the asymmetric ripples produced by a stack of DMPC multibilayers in the $P_{\beta'}$ phase.

aligned DPPC multibilayers [34]. The wavelength and lamellar periodicity of these ripples is comparable to those of Type I. However, the unit cell in this case is rectangular. From this and from the absence of any $(0,k)$ reflections (which correspond to modulations in the electron density of the rippled bilayer projected on to its mean plane) the authors of this study concluded that these ripples are also symmetric, with the chains tilted in a direction normal to the rippling direction.

A prerequisite for a given lipid to form the ripple phase seems to be the tendency for its hydrocarbon chains to tilt from the layer normal below the chain melting transition. Thus in almost all the systems that show the ripple phase, an $L_{\beta'}$ phase occurs at lower temperatures. In some systems, like DHPC an L_{β} phase is present [80], but is a result of hydrocarbon chain interdigitation. Further, it is possible to induce a ripple phase in a system by increasing the interaction between the head groups, for example, by changing the pH [66,80], which also induces a chain tilt in the gel phase [65]. It is also possible to suppress the ripple phase by tuning the headgroup interactions using salt solutions [67]. Another aspect of this phase that is now well established is the fact that it occurs even in unilamellar vesicles [72], which indicates that intra-bilayer interactions are responsible for the rippling.

There have been many theoretical attempts at modeling the ripple phase, some of them being phenomenological in nature and the others more microscopic. Brief reviews of earlier theories are presented in the papers by Carlson and Sethna [82], Chen et al. [83] and Scott and McCullough [84]. Recently there have been some more papers on the subject [85–87]. It is fair to say

that none of the current models are satisfactory since they do not account for all the salient structural features of this phase. There has also been some interest in the influence of molecular chirality on the structure of the ripple phase, following the suggestion by Lubensky and MacKintosh [88,83] that chirality is responsible for the occurrence of asymmetric ripples. However, experiments using aligned samples have ruled out this possibility as identical asymmetric ripples have been observed in both chiral and racemic mixture of DMPC [17]. Thus it is clear that more detailed structural information, most likely obtained using aligned lipid multibilayers, is needed to develop a satisfactory theoretical model of the ripple phase.

5 Hydration, Aligned Lipid Systems and the Vapour Pressure Paradox

Given the evidence in the preceding sections, why choose to study liposomal preparations? The answer can be summarized in one word, hydration! The physiologically meaningful condition is one in which the membrane mesophase has access to an ‘unlimited’ supply of water. For almost three decades it was widely recognized, and accepted, that lipid bilayers hydrated from water vapour at 100% relative humidity (RH) did not take up as much water as those same bilayers hydrated in direct contact with liquid water [13–18,35–37,39–41,89]. In fact on a weight percent basis, water in liposomal phosphatidylcholine multibilayers immersed in the liquid accounts for 45% to 55% of the total and up to 30% or so, when these same systems are hydrated from a saturated water vapour at equilibrium with bulk water [90]. At equilibrium conditions, the chemical activity of liquid water and water vapour at 100% RH should be the same implying that regardless of whether lipid bilayers are hydrated from saturated water vapour or liquid water their level of hydration should be identical. This discrepancy between theory and observation has come to be known as the vapour pressure paradox (VPP). [41]

Over the past three decades, there has been no experimental evidence which compromised the integrity of the VPP. Thus it became necessary to gain some physical understanding into this phenomenon. Recently, a theoretical description was provided which described that bounded surfaces (e.g., vapour/multibilayer or multibilayer/substrate), under tension, can suppress bilayer fluctuations leading to long-range attractive forces [91]. This type of pseudo-Casimir attraction had previously been proposed in smectic liquid crystals [92,93]. Furthermore, this description was consistent with the aligned, fully hydrated, $L_{\beta'}$ multibilayer data [37,38] since it is commonly believed that the intrinsically stiffer $L_{\beta'}$ bilayers do not depend on bilayer fluctuations in order to achieve full hydration.

Using neutron diffraction, and a newly designed sample chamber [4] it was shown that both phosphoryl choline (PC) and phosphoryl ethanolamine (PE) multibilayers (Fig. 5), aligned on rigid silicon substrates, achieved full

hydration in all mesophases including the physiologically relevant L_α phase [4]. The experimental results were seemingly in contradiction to the theoretical explanation given for the VPP. However, it was explained that once immersed in water, the multibilayer stack may not have remained adsorbed to the substrate. At this point the silicone wafers simply acted in confining the bilayers which would be free to undulate. From a practical point of view the question of routinely preparing fully hydrated aligned samples was answered. Nevertheless, the question of the VPP remained.

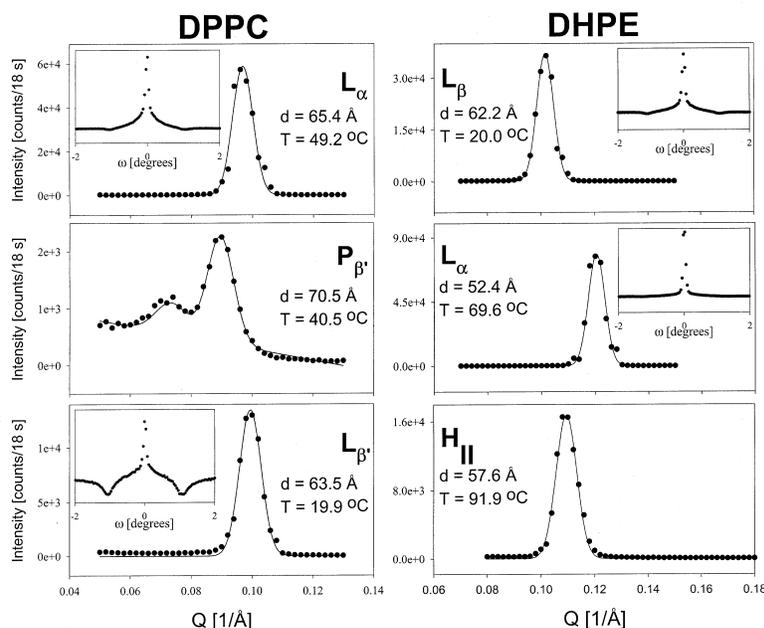


Fig. 5. Resolution-limited (except for H_{II} phase of dihexadecyl phosphatidylethanolamine (DHPE)) Bragg maxima of the various phases of aligned (mosaic $\leq 0.5^\circ$) dipalmitoyl phosphatidylcholine (DPPC) and DHPE. The insets contain the so-called rocking curves or ω scans which are a measure of the angular distribution of the lipid multibilayers with respect to the substrate. All of the d -spacings are in good agreement with those d -spacings exhibited by liposomal preparations.

For egg PC bilayers there is an exponential dependence between percent relative humidity and interbilayer separation [41]. It was therefore not surprising that a mere decrease of 0.1% in humidity resulted in enough osmotic stress to remove between 1/3 to 1/2 the water from the multibilayers. In repeat spacing, this translates to $\approx 6 \text{ \AA}$! Could it then be possible that bilayers

historically were never hydrated in 100% RH environments? How could this be the case even when an aligned lipid multibilayer stack was exposed to a supersaturated water atmosphere [35]?

As mentioned earlier, under equilibrium conditions the chemical activity of water vapour at 100% RH should be the same as that of liquid water. If the substrate is creating these long range attractive forces, as predicted by theory, then adsorbing bilayers to a substrate and immersing them in liquid water should result in bilayers having a reduced d -spacing. Mica was chosen as the substrate as it is known to tenaciously adhere onto the first lipid bilayer preventing the multibilayer stack from detaching from the substrate. From diffraction experiments it was convincingly shown that the highly aligned DMPC bilayers (mosaic $\leq 0.5^\circ$) exhibited d -spacings no different than those of their liposomal counterparts [5]. More importantly, the same result was obtained when L_α DMPC multibilayers were hydrated from water vapour [5]. It was thus shown experimentally that the VPP was the result of inherent deficiencies in the design of previous sample environments (i.e., temperature gradients) where most probably, humidities greater than 99% were never achieved. Was it then possible to reconcile theory and experiment?

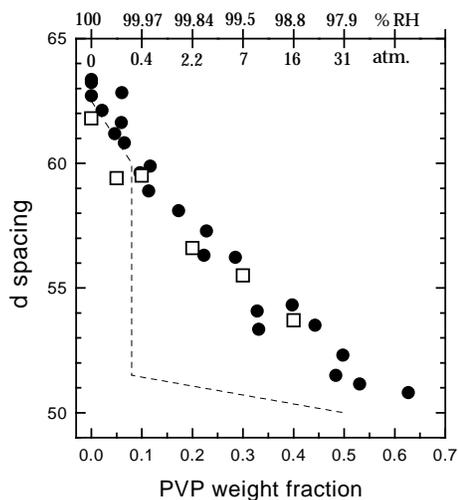


Fig. 6. d -spacing versus concentration (wt.%) of 40,000 MW polyvinylpyrrolidone (PVP) for DMPC multibilayer stacks at 30 °C. At various PVP concentrations, the neutron data from aligned DMPC samples is depicted by \square 's and the x-ray data from DMPC liposomal preparations by \bullet 's. The corresponding osmotic pressures and %RH's are shown in the upper horizontal axis. The dashed curve schematically indicates the behaviour of the repeat spacing for aligned samples if a vestigial vapour pressure paradox existed at 0.3 atm of pressure.

Building on the theory described in Ref. [91], it was shown theoretically that the VPP does not exist [95] and in agreement with the recent neutron diffraction data [5]. This new theory did not contradict the main analysis of the previous one, but instead critically examined the assumption that a multi-bilayer stack will remain adsorbed to the substrate and/or that the bilayer at the bilayer/vapour interface will remain dehydrated. The recent analysis clearly demonstrated that the system will reduce its free energy by hydrating its outermost bilayer thus enabling fluctuations and avoiding the VPP [95]. Interestingly, the recent analysis also suggested the possibility of a ‘vestigial’ VPP which would then explain why the VPP was so persistent. This vestigial VPP would involve a phase transition as a function of applied osmotic pressure. However, as demonstrated in Fig. 6, diffraction experiments using both aligned and liposomal preparations of DMPC bilayers show that although with an increasing concentration of polyvinylpyrrolidone (PVP, MW 40,000) osmotically stressing the bilayers, d -spacing decreases rapidly, it does so in a smooth and continuous manner. There is no indication of a critical pressure. If a vestigial VPP existed the lamellar spacing for aligned samples would resemble that curve schematically drawn in Fig. 6. It was thus concluded that there is no vestigial VPP at least for pressures < 16 atmospheres.

Recent developments in the hydration of aligned samples can be summarized as follows:

- highly aligned lipid bilayers can be produced under ‘biologically relevant conditions’ (e.g., fully hydrated L_α bilayers and physiologically relevant pH and ionic strength)
- the much accepted vapour pressure paradox was shown to be the result of aligned bilayers being hydrated under humidities that were less than 100% RH
- a newly developed theory has reconciled recent experimental evidence and previous theory

6 Magnetically Alignable Lipid ‘Substrates’

With the exception of the freely suspended samples prepared by Smith et al. [18], the examples given thus far have been of systems aligned using solid substrates. However, it has been shown that biologically relevant molecules possessing sufficient anisotropy in their diamagnetic susceptibility can align in the presence of a magnetic field [21,96–100]. The problem for the most part is, biological systems do not possess sufficient diamagnetic anisotropy to readily align in reasonable magnetic fields. This problem has been circumvented *via* the use of alignable nematic particles and ferrofluids which align biological assemblies regardless of the host molecule’s intrinsic magnetic properties [101,102]. However, it should be pointed-out that these ‘alignable substrates’ are not constituted from biologically relevant materials.

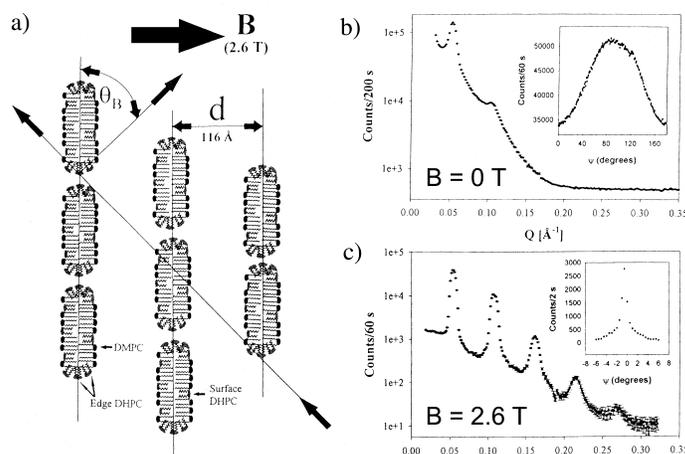


Fig. 7. (a) Schematic and diffraction geometry of the DMPC/DHPC magnetically alignable bilayered micelles, or ‘bicelles’ doped with Tm^{3+} paramagnetic ions, in the presence of an applied magnetic field ($B = 2.6$ T). θ_B is the Bragg angle and d is the repeat spacing. Diffraction patterns in the absence (b) and presence of a magnetic field (c). The insets depict the rocking curves and show that when the magnetic field is applied the system becomes highly oriented (mosaic $\leq 1.0^\circ$ vs 90°) resulting in higher quality data (i.e., more Bragg maxima).

In recent years, biomimetic substrates have been produced using a variety of lipid combinations [103–105]. These particular systems formed nematic phases with the major face of this bilayered disk or so-called ‘bicelle’ being parallel to the magnetic field. However, doping these same bicelles with a paramagnetic ion such as, Tm^{3+} , generated bicelles which were now aligned with their bilayer normals parallel to the applied magnetic field (Fig. 7a) [33]. In addition to ‘flipping’ the bilayered micelles by 90° with respect to the magnetic field, the addition of Tm^{3+} altered the system from a nematic to a poorly aligned smectic in the absence of a magnetic (Fig. 7b) and having a repeat spacing of 120 Å. Application of a magnetic field resulted in the system becoming highly aligned (Fig. 7c).

Besides the interesting physical properties exhibited by these bilayered micelle systems [106], there is presently a great amount of effort to utilise them as biomimetic substrates to align peptides and proteins under physiologically relevant conditions (see contribution by Prosser et al. below).

7 Concluding Remarks

Historically it was thought that the preparation of aligned samples was best left to the ‘experts’. We can unequivocally say that this is not the case. For

the most part, aligned samples are easily prepared and judging from their contributions to model membranes are certainly worth the efforts necessary in procuring them. Many of the traditional drawbacks of aligned samples, especially the attainment of fully hydrated, biologically relevant L_α phase bilayers, have been overcome. Moreover, these days one is not limited to studying aligned systems on solid substrates as the magnetically alignable bicelles are gaining popularity as the 'system of choice' especially with techniques such as nuclear magnetic resonance and electron paramagnetic resonance where an externally applied magnetic field is an intrinsic part of the apparatus.

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