Chapter 18

Differential Scanning Calorimetry Studies of Phospholipid Membranes: The Interdigitated Gel Phase

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Additional information is available at the end of the chapter

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1. Introduction

DSC is a versatile technique and has been used for decades to study hydrated phospholipid membranes [1-4]. It can even be used to analyze whole cell samples [5]. For pure lipids, DSC can accurately determine the phase transition temperatures and the associated enthalpies. As a consequence, how the chemical structure of lipids translates into thermodynamic properties can be systematically studied. In addition to determining the physical properties of pure lipids, the miscibility and phase behavior of lipid mixtures can be determined.

The detailed review of the interdigitated phase written by Slater and Huang in 1988 provides an excellent outline of the properties of the interdigitated phase and the relevant analytical techniques [6]. Furthermore, the meticulous studies of Koynova and Caffrey describe how systematic changes in lipid chemistry can affect their phase behavior [7-9]. Lipids with asymmetrical acyl chains that form either mixed- or partially-interdigitated phases have also been thoroughly investigated [7,10-12]. This review focuses on the interdigitated phase of fully hydrated phospholipids with hydrocarbon chain lengths of equal size. We pay special attention to recently discovered interdigitated systems and the chemicals that can induce or inhibit lipid interdigitation.

For simplicity, we have centered our review around the extensively studied lipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). DPPC is naturally occurring and has thermodynamic phase behavior that is typical for saturated phosphatidylcholines (PCs) [7]. Although DPPC does not spontaneously interdigitate when hydrated, it can be reliably transformed into the fully interdigitated gel phase (Tables 1 and 2). Alterations in the lipid hydrocarbon chains (Figure 1) and the lipid head group (Figure 2) substantially affect spontaneous interdigitation (Figure 3). The predisposition for interdigitation is a finely

tuned balance of interactions among hydrocarbon chains, between polar head groups, and at the interfacial area with the aqueous phase.

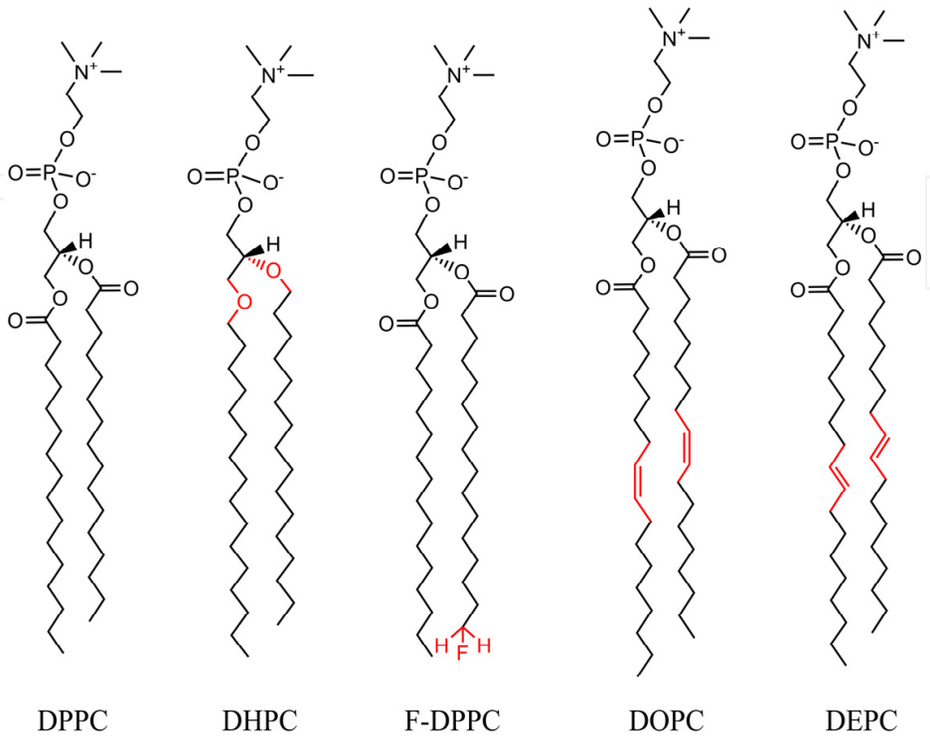

Figure 1. Representative examples of fatty acid modifications of DPPC. The segments in red identify modifications to the structure of DPPC.

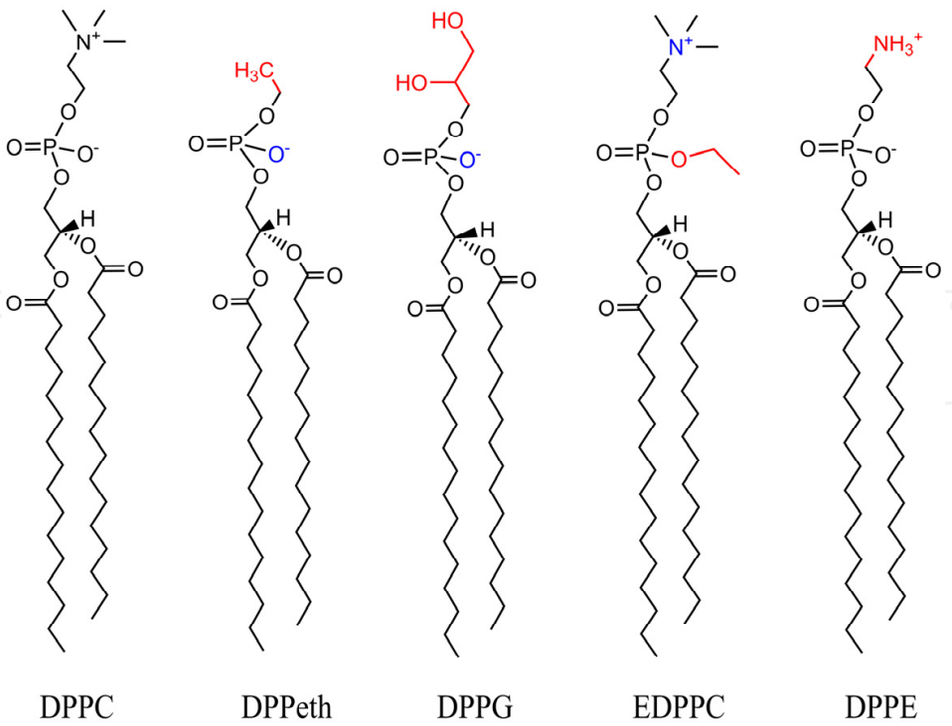

Figure 2. Representative examples of head group modifications of DPPC. The segments in red identify modifications to the structure of DPPC. The segments in blue demonstrate the resulting change in charge.

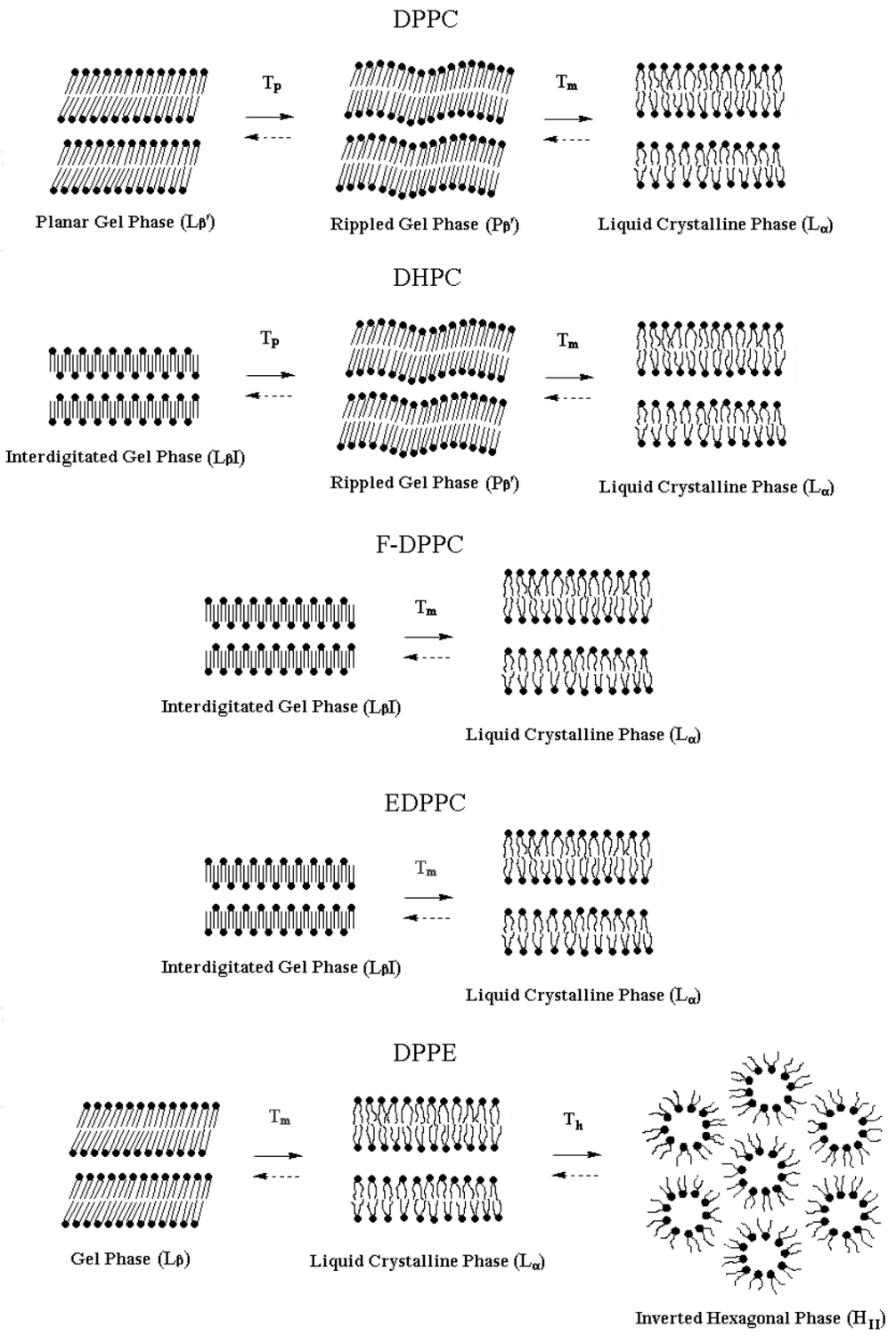

Figure 3. Phase transitions of representative lipids. Solid arrows indicate heating transitions and dotted arrows indicate cooling transitions. Note: subgel phase transitions are not shown.

DPPC/alcohol	Threshold concentration (M)	References
Methanol	2.75 ± 0.35	[13]
Ethanol	1.10 ± 0.10	[13]
1-propanol	0.39 ± 0.03	[14]
2-propanol	0.52 ± 0.03	[14]
1-butanol	0.16 ± 0.02	[15]
Isobutanol	0.17 ± 0.02	[15]
sec-butanol	0.22 ± 0.02	[15]
tert-butanol	0.27 ± 0.02	[15]
1-pentanol	0.07 ± 0.01	[16]
2-pentanol	0.10 ± 0.01	[16]
3-pentanol	0.11 ± 0.01	[16]
3-methyl-2-butanol	0.10 ± 0.01	[16]
2-methyl-1-butanol	0.08 ± 0.01	[16]
3-methyl-1-butanol	0.08 ± 0.01	[16]
2-methyl-2-butanol	0.13 ± 0.01	[16]
neopentanol	0.08 ± 0.01	[16]

Table 1. Threshold concentrations for some alcohol-induced interdigitation.

	Chemicals	References
Anesthetics	bupivacaine	[17]
	dibucaine	[17]
	lidocaine	[17]
	procaine	[17]
	tetracaine	[17-19]
Drugs	labdanes	[20]
	chlorpromazine	[19]
	valsartan	[21]
Organic solvents	glycerol	[22-24]
	ethylene glycol	[25]
	acetone	[26,27]
	acetonitrile	[27,28]
	propionaldehyde	[27]
	petrahydrofuran	[27]
Salts	KSCN	[29,30]
Pressure	N/A	[31-34]

Table 2. Induced interdigitation of PC membranes.

There are multiple types of interdigitation (Figure 4). The type of interdigitation that forms is heavily dependent on the structure and symmetry of the hydrocarbon chains [6]. Interdigitated lipid systems can further be separated into two classes: spontaneous and induced. We use "spontaneous" to describe lipids that self-assemble into the interdigitated

gel phase when fully hydrated under typical preparation procedures and at ambient pressure. Some notable recent examples are highlighted, such as cationic lipids and lipids with monofluorinated acyl chains. Whether or not a particular lipid spontaneously interdigitates is determined by the balance of properties that favor and disfavor interdigitation. Lipids often have conflicting characteristics regarding the ability to form the interdigitated phase. Consequently, there is no simple formula for determining which lipids will spontaneously interdigitate without relying on experimental data.

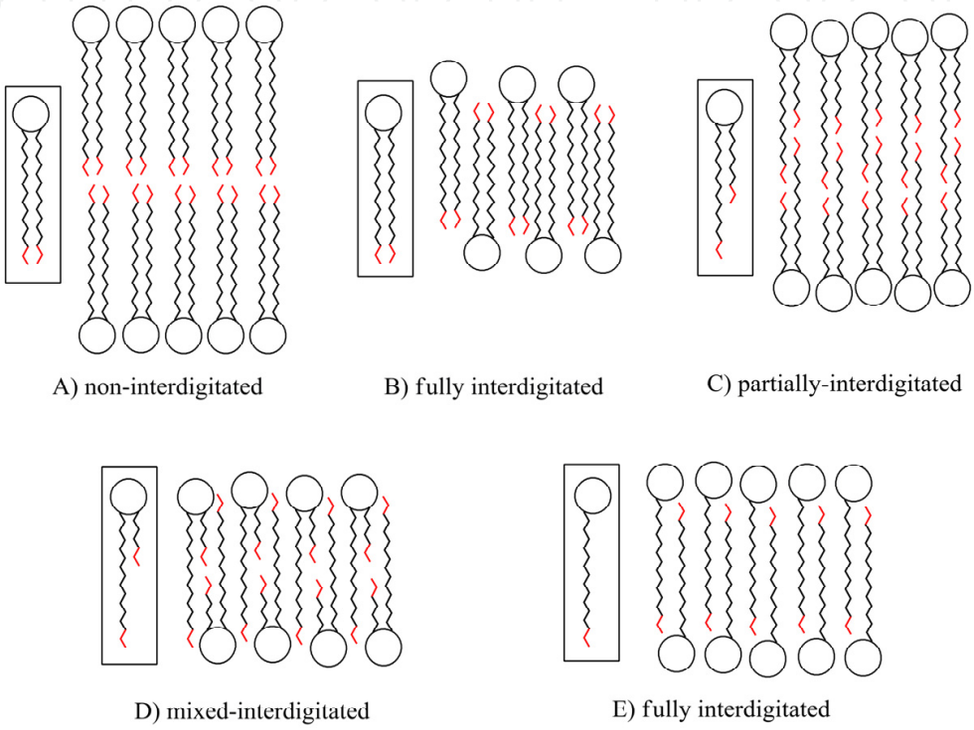

Figure 4. Schematic representation of the different types of membrane interdigitation: A) symmetrical lipid, non-interdigitated; B) symmetrical lipid, fully interdigitated; C) asymmetrical lipid, partially interdigitated; D) highly asymmetrical lipid, mixed-interdigitated; E) lysolipid, fully interdigitated. For clarity, the terminal ends of the hydrocarbon chains are labeled in red.

As can be seen in Figure 4, the structural difference between the interdigitated and non-interdigitated phases can be substantial. In the non-interdigitated membrane, both ends of the hydrocarbon chains meet in the membrane midplane (Figure 4A). Two well-defined leaflets are formed and there is a thick hydrophobic core. In the fully-interdigitated membrane, the thickness of the interdigitated phase is greatly reduced and there is the loss of the membrane midplane. There is an increase in the spacing between the polar lipid head groups and the ends of the lipid hydrocarbon chains become more exposed to the aqueous interface [6]. The difference is most dramatic in the fully interdigitated phase compared to the non-interdigitated membrane (Figure 4A and 4B). In the partially-interdigitated system, the longer chain extends to the other side of the membrane and aligns with the apposing shorter chain (Figure 4C). In the mixed-interdigitated membrane, the short hydrocarbon chains line up with each other and the full-length chain extends to the other side of the membrane (Figure 4D). Lyso lipids also form a fully interdigitated structure (Figure 4E) [35].

2.

2.1. Thermodynamics of phosphatidylcholine membranes

PCs are common in mammalian membranes and have well known phase transitions [7]. The most ubiquitous of these is the gel-to-liquid crystalline transition, often referred to as the melting or main transition. This transition is relatively rapid and is highly reversible [36]. It is characterized by the co-operative melting of the hydrocarbon chains and a high enthalpy DSC peak [2]. The liquid crystalline (L_α) phase has an increased number of gauche conformers and a large increase in membrane fluidity and disorder [2,37]. The pre-transition from the planar gel (L_β') to the rippled gel phase (P_β') has a low enthalpy and is sensitive to sample preparation and the presence of impurities [7,36]. It is also more sensitive to the scan rate, with lower scan rates resulting in lower T_p temperatures [3]. Some PCs also have subgel phases. The subgel transition is slow and is dependent on sample preparation, especially incubation temperature and time [36]. All of the above phases are strongly affected by changes in lipid structure. This review will show that such alterations also have a profound effect on the interdigitated phase.

2.2. Chemically-induced interdigitation of phosphatidylcholines

The most widely studied chemical inducer of interdigitation is ethanol. In non-interdigitated phospholipid membranes, ethanol tends to adsorb to the head groups, especially the region near the hydrocarbon chains [38,39]. In particular, the carbonyl groups of the glycerol backbone of phospholipids are thought to be the favored hydrogen bonding sites for ethanol [40]. Ethanol displaces water when it adsorbs to the head group, which increases the head group volume and decreases the order of the hydrocarbon chains [13,41,42]. The increase in head group volume leads to increased chain tilting and creates energetically unfavorable voids in the hydrocarbon region of non-interdigitated membranes, encouraging the creation of the L_βI phase at high concentrations [13,39,42-44]. Once the L_βI phase is formed, ethanol can bind to the exposed hydrocarbon chains, replacing the unfavorable interaction of the acyl chains with water [45]. Also, it is typical for alcohols to increase the main transition enthalpy above the threshold concentration for interdigitation [15,16].

There are three main characteristics of the chemically-induced interdigitated phase in the DSC thermograms of saturated PCs: the presence of biphasic phase behavior, an increase in T_m hysteresis, and the suppression of the pre-transition. The combination of these can be used to determine the threshold concentration for interdigitation.

The "biphasic effect" indicates two independent interactions within different concentration ranges [46,47]. The biphasic effect is most strongly characterized by an initial decrease in the T_m, but an increase in or stabilization of the T_m once the L_βI phase is formed. The first interaction lies below the threshold concentration. Here, ethanol preferentially partitions into the liquid crystalline phase, lowering the phase transition temperature [47]. The secondary interaction above the threshold concentration stabilizes the interdigitated gel phase. The main transition co-operativity (sharpness of transition peak) can also be enhanced above the threshold concentration [47].

The shape and magnitude of the T_m biphasic effect is also dependent on the alcohol isomer used as shown in Figure 5 [15,16]. For example, *tert*-butanol is an effective inducer of interdigitation and has a pronounced biphasic effect [15]. Other alcohols, such as the pentanol isomers have more "stunted" biphasic behavior [16]. While a difference in trend can still be observed above and below the threshold concentration, the distinction is less pronounced.

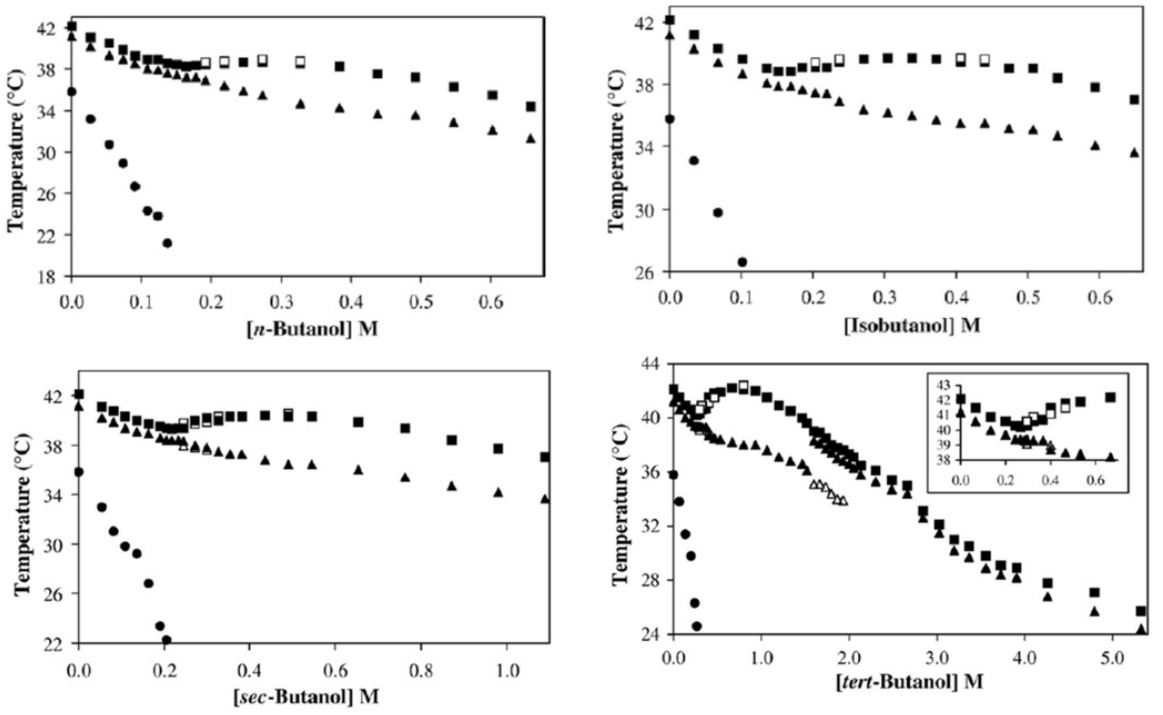

Figure 5. Effects of *n*-butanol, isobutanol, *sec*-butanol, and *tert*-butanol on DPPC phase transition temperatures. (■, heating scan main peak; □, heating scan shoulder peak; ▲, cooling scan main peak; Δ, cooling scan shoulder peak; ●, pre-transition peak). Reprinted from Biophys. Chem., 128, Reeves MD, Schawel AK, Wang W, Dea PK, Effects of butanol isomers on dipalmitoylphosphatidylcholine bilayer membranes, Pages No. 13-18, Copyright (2007), with permission from Elsevier [15].

Increasing the alcohol content well above the threshold concentration lowers the T_m [15,16]. At these concentrations, additional alcohol destabilizes the $L_\beta I$ phase relative to the L_α phase. The membrane bilayer structure can also break down for alcohols that are highly soluble in water [15]. For example, above 2.00 M *tert*-butanol in DPPC, the main transition hysteresis is absent (Figure 5). Additionally, the heating main transition DSC peaks above 2.00 M *tert*-butanol become increasingly broad. Changes in the ^{31}P-NMR spectra at high concentrations confirm the loss of lamellar structure [15].

The biphasic behavior is also reflected in the increase in the main transition enthalpy as the alcohol concentration increases [15,16]. Often, the rate of change in the transition enthalpy above and below the threshold concentration is different (Figure 6). This effect also depends on the alcohol chain length and isomer used. For instance, this difference is clear with *n*-butanol but not *tert*-butanol [15].

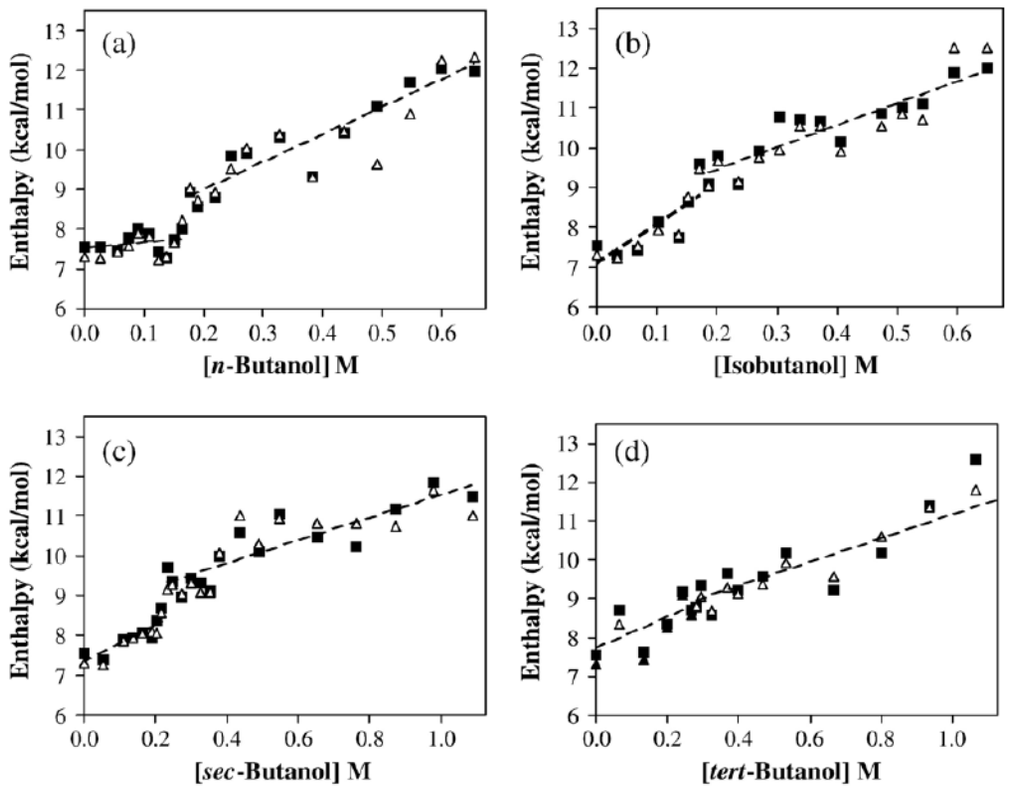

Figure 6. Effects of (a) *n*-butanol, (b) isobutanol, (c) *sec*-butanol and (d) *tert*-butanol on DPPC main transition enthalpies (■, heating scan main transition enthalpy; Δ, cooling scan main transition enthalpy). Reprinted from Biophys. Chem., 128, Reeves MD, Schawel AK, Wang W, Dea PK, Effects of butanol isomers on dipalmitoylphosphatidylcholine bilayer membranes, Pages No. 13-18, Copyright (2007), with permission from Elsevier [15].

A property that accompanies the biphasic effect is the emergence of hysteresis in the main transition [15,16,48,49]. The hysteresis as it relates to DSC is defined as the difference in the transition temperature between heating and cooling scans. This corresponds to the reversibility and kinetics of the transition. The return to the interdigitated phase with a decrease in temperature is a slow process and is therefore less reversible [48,51]. For systems that do not interdigitate, such as phosphatidylethanolamine (PE) lipids, the addition of alcohol does not affect the transition hysteresis [48].

The disappearance of the pre-transition is another consistent property of alcohol-induced interdigitation of saturated PCs. The decrease in the T_p follows a well defined trend below the threshold concentration until it is finally abolished (Figure 5). The rate at which the T_p is depressed depends on the efficacy of the chemical inducer.

By comparing the threshold concentrations of different chemicals, they can be ranked on their effectiveness at inducing the interdigitated phase. For instance, the threshold concentrations for alcohol-induced interdigitation systematically decreases as the lipid hydrocarbon chain length increases (Table 1). The isomers with the most solubility in water are the least effective at inducing interdigitation, as shown by the increase in threshold concentrations [15]. Additionally, the more soluble an isomer is in water, the less effectively

it depresses both the temperature of the pre-transition and the main transition prior to the threshold concentration [15].

The ether-linked analogue of DPPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (DHPC), is also a useful model membrane for studying interdigitation. DHPC goes through a low temperature pre-transition from the L_βI phase to the non-interdigitated rippled gel phase P_β' (Figure 3) [52,53]. Therefore, chemicals that stabilize the L_βI phase increase the T_p until it merges with the main transition into the L_α phase [49]. This process occurs at lower concentrations for more effective inducers of interdigitation.

2.3. Pressure-induced interdigitation

It is well established that the application of hydrostatic pressure favors interdigitation in a multitude of lipid systems (Tables 2 and 3). As hydrostatic pressure is applied, the intermolecular distance between adjacent lipids is reduced and molecular packing becomes denser [34]. By changing the packing structure of the membrane, interdigitation can relieve the stress caused by the increased steric hindrance.

Pressure-induced interdigitation is dependent on lipid hydrocarbon chain length and the chemical structure, much like chemically-induced interdigitation. Ether- and ester-linked lipids with longer chains require less pressure to interdigitate [51,54]. Under high temperature and pressure conditions, ester-linked lipids behave similarly to the equivalent ether-linked lipids at normal pressure [51]. Pressure-induced interdigitation is not universal, however. As with chemically-induced interdigitation, certain lipids do not interdigitate even under high pressure [34,55,56].

2.4. Spontaneous interdigitation in ether-linked lipids and 1,3-DPPC

The type of bond that connects the hydrocarbon chain to the lipid head group also affects the thermodynamic properties. Switching either or both of the ester bonds of DPPC with ether linkages results in a small increase in the T_m (<4 °C) and enthalpy (<1 kcal/mol) [57]. A single ether linkage can be sufficient to allow the formation of the interdigitated gel phase [57,58]. Furthermore, in ether lipids that spontaneously interdigitate, the interdigitated phase is stable up to higher temperatures as the chain length increases (Figure 7) [51]. There is an increased amount of head group repulsion in DHPC, which favors the interdigitated phase [51,59,60]. Conversely, the stronger interactions in the head groups of ester lipids hinder interdigitation [34,51]. DHPC is an especially useful lipid for studying the interdigitated phase because its transition from the interdigitated gel to non-interdigitated ripple gel phase is highly sensitive to its environment.

The similarity of DHPC to DPPC also allows for the comparison between ether- and ester-linked lipids. It is consistently easier to interdigitate ether-linked lipids whether through chemical means [17,48,49] or by the application of pressure [51,61]. Furthermore, the ether-linked 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE) demonstrates the result of competing influences on the interdigitated gel phase. In DHPE, the ether linkages

favor interdigitation, but the PE head group is more strongly opposed to interdigitation [62]. Therefore, while DHPC spontaneously interdigitates, the PE head group of DHPE prevents interdigitation.

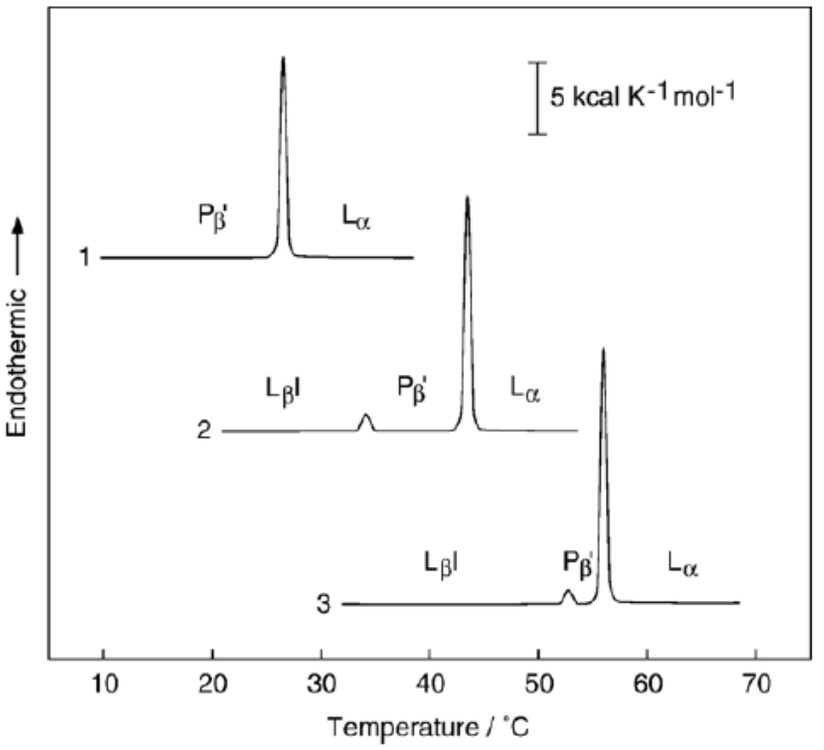

Figure 7. DSC heating thermograms of ether-linked PC bilayer membranes: (1) *O*-14:0-PC, (2) *O*-16:0-PC, (3) *O*-18:0-PC Reprinted from Biochim. Biophys. Acta, 1768, Matsuki H, Miyazaki E, Sakano F, Tamai N, Kaneshina S, Thermotropic and barotropic phase transitions in bilayer membranes of ether-linked phospholipids with varying alkyl chain lengths, Pages No. 479-489, Copyright (2007), with permission from Elsevier [51].

While the majority of PC lipid studies use lipids with the hydrocarbon chains on the *sn*-1 and *sn*-2 positions, there are some examples of experiments using synthetic lipids with the chains located at *sn*-1 and *sn*-3. One intriguing example is the positional isomer of DPPC, 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (1,3-DPPC or *β*-DPPC), which has unique properties including the ability to spontaneously interdigitate [63-65]. However, the phase diagram is different from the ether-linked lipids that spontaneously interdigitate [51,66]. At lower temperatures, 1,3-DPPC exists in a non-interdigitated "crystalline" bilayer phase termed (L_c). At higher temperatures, but below the T_m, 1,3-DPPC can form a fully interdigitated structure [63]. The ability to interdigitate may be due to greater head group repulsion resulting from a different phosphocholine tilt or conformation relative to the glycerol backbone [63-65]. As with most interdigitated systems, 1,3-DPPC converts into a non-interdigitated structure during the heating transition into the L_α phase. The cooling transition from the L_α phase into the interdigitated phase has considerable hysteresis [63].

2.5. The monofluorinated analogue of DPPC: F-DPPC

The monofluorinated analogue of DPPC, 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine (F-DPPC), spontaneously forms the L_{β}I phase below the main transition temperature (T_m) [67-69]. The main transition temperature of F-DPPC also occurs at a higher temperature (~50 °C) and with a higher transition enthalpy (9.8 kcal/mol) compared to DPPC [67]. The endothermic peak of F-DPPC is also broader than DPPC. The transition can be split into two overlapping peaks, with the peak centered at 50.6 °C accounting for 36% of the area and the peak at 52.0 °C accounting for 64% of the area [67]. The lower transition peak component does not correspond to a change in the hydrocarbon chains as detected by

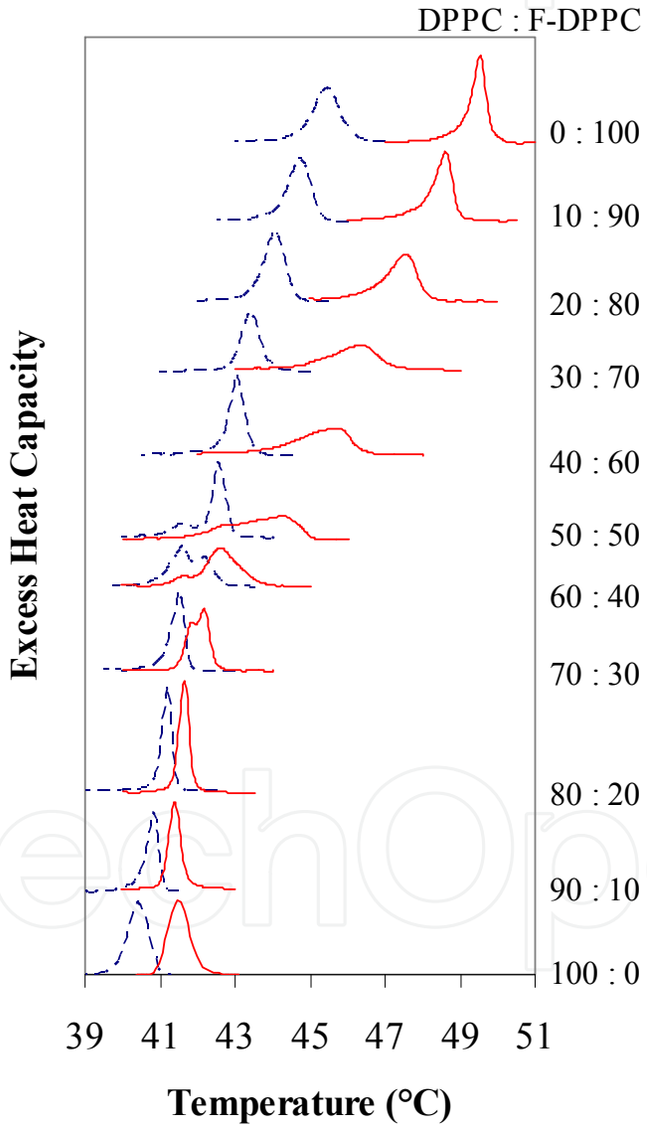

Figure 8. Heating (red lines) and cooling (dashed blue lines) DSC thermograms of the T_m of the DPPC/F-DPPC system are shown. The cooling scans have been inverted to allow comparison with the heating thermograms. For clarity, the thermograms are also offset vertically. Reprinted from Biophys. Chem., 147, Smith E.A., van Gorkum C.M., Dea P.K., Properties of phosphatidylcholine in the presence of its monofluorinated analogue, Pages No. 20-27, Copyright (2010), with permission from Elsevier [69].

FTIR spectroscopy. It is possible that this relates to a conversion from interdigitated to non-interdigitated gel right before the transition into the liquid crystalline phase [67]. The main transition is also characterized by a large main transition hysteresis (Figures 8 and 9) [67,69]. Additionally, the L_{β}I phase has high conformational order and tight lipid packing [68].

It appears that the fluorine must be located on the terminal hydrocarbon chain to have a dramatic effect on interdigitation. When the fluorine substitution is not located on the terminal carbon, DSC data reveal that the physical properties are only modestly changed and they are largely miscible with the non-fluorinated parent lipid [70]. Lipids with more fluorine, such as when the 13-16 carbons are perfluorinated, do not spontaneously interdigitate either [71,72]. Therefore, it is the interaction of the polar terminal C-F bond with the aqueous interface that encourages interdigitation [67]. The large dipole moment is the most likely culprit for stabilizing the interdigitated phase by reducing the unfavorable exposure of the hydrophobic acyl chains to water. However, the slightly larger van der Waals radius and the possibility of weak hydrogen bonding may also play a role [73-77].

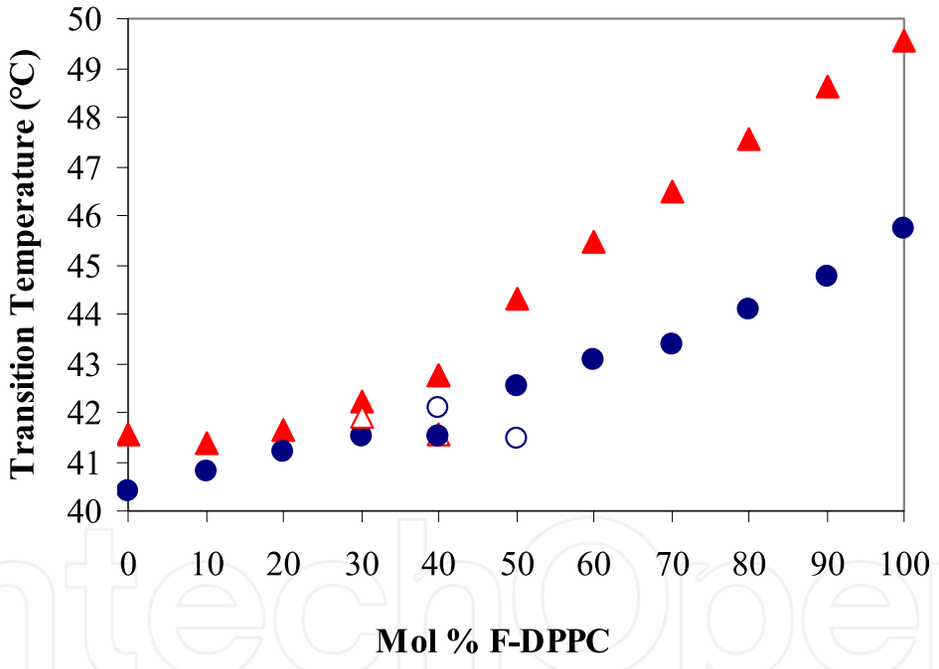

Figure 9. The main transition temperature (T_m) of DPPC/F-DPPC mixtures determined by DSC. Heating scans shown by filled red triangles (▲). Cooling scans shown by filled blue circles (●). Shoulder peaks indicated by unfilled triangles for heating scans (Δ) and unfilled circles for cooling scans (○). Reprinted from Biophys. Chem., 147, Smith EA, van Gorkum CM, Dea PK, Properties of phosphatidylcholine in the presence of its monofluorinated analogue, Pages No. 20-27, Copyright (2010), with permission from Elsevier [69].

2.6. The interdigitated gel phase in anionic lipids

As with PCs, di-saturated long chain phosphatidylglycerols (PGs) have a strong propensity towards interdigitation (Table 3) [78]. The negatively charged PGs are commonly found in

microbial membranes [90]. The interaction of some peptides with lipids is heavily dependent on the composition of the membrane [82]. This contributes to the ability of antimicrobial peptides to selectively target microbial membranes [91]. Recently, it was found that DPPG has the ability to form a quasi-interdigitated gel phase with the addition of the human multifunctional peptide LL-37 [81,82]. The antimicrobial peptide peptidyl-glycylleucine-carboxyamide (PGLa) has a similar effect below the main transition temperature of saturated PGs [83]. In these instances, the peptide shields the acyl chains of the interdigitated lipid from the aqueous layer by orienting in the interfacial region below the T_m (Figure 10).

Furthermore, other chemicals such as Tris-HCl induce interdigitation in DPPG by binding between lipids, resulting in the increased area per head group that favors interdigitation [85]. As in zwitterionic lipids, interdigitation relieves head group repulsion in charged lipids by allowing for a larger area per head group [84]. Charge repulsion in DPPG leads to tilted acyl chains in the non-interdigitated bilayer [85]. This is similar to the ethanol-induced interdigitation of DPPC, where the increased head group size increases the tilt in the gel phase and which ultimately results in the interdigitated gel phase [43]. Ethanol further enhances interdigitation in DPPG, most likely by partitioning into the interfacial region and reducing the exposure of the terminal methyl groups to water [84].

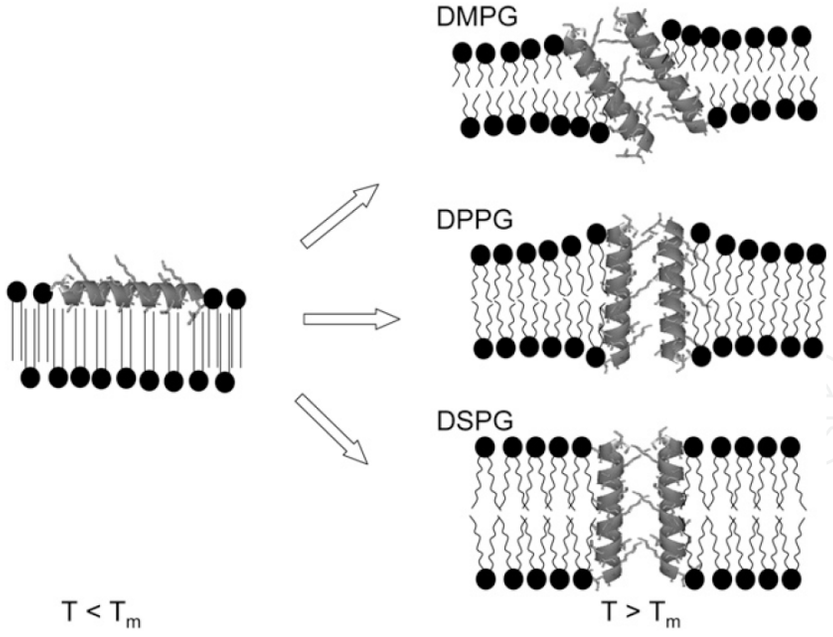

Figure 10. Schematic representation of the peptide PGLa-associated structural changes in PG bilayers. Below the main phase transition ($T < T_m$), the lipids of different hydrocarbon chain lengths exhibit a quasi-interdigitated phase in the presence of PGLa. Reprinted from Biophys. J. 95, Pabst G, Grage SL, Danner-Pongratz S, Jing W, Ulrich AS, Watts A, Lohner K, Hickel A, Membrane thickening by the antimicrobial peptide PGLa, Pages No. 5779-5788, Copyright (2008), with permission from Elsevier [83].

Inducer	References
polymyxin B	[24,79,80]
peptide LL-37	[81,82]
peptide PGLa	[83]
myelin basic protein	[80]
Tris HCl	[79,84,85]
choline and acetylcholine	[86]
atropine	[87,88]
anisodamine	[89]
Pressure	[50]

Table 3. Induced interdigitation of PG membranes

When ethanol substitutes for water in the transphosphatidylation reaction catalyzed by phospholipase D, phosphatidylethanols (Peth) are formed [84,92]. Peth lipids are unique because they have a small anionic lipid headgroup (Figure 2). These lipids are biologically relevant since Peths accumulate in membranes of animal models of alcoholism [93]. Like DPPG, DPPeth can be chemically induced to interdigitate with Tris-HCl and the interdigitated phase is stabilized with the addition of ethanol [84].

2.7. The interdigitated gel phase in cationic lipids

Cationic lipids with modified head groups can spontaneously form interdigitated gel phases below the main transition. One recent example is the positively charged lysyl-DPPG, which is DPPG with a lysine moiety attached. Lysyl-DPPC forms an interdigitated phase primarily due to the large repulsion between head groups [94].

Another modification is the esterification of the phosphate head group, which increases the steric bulk and changes the molecule from zwitterionic to positively charged, allowing interdigitation [95,96]. For example, the P-O-ethyl ester analogue of DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (EDPPC or Et-DPPC), is fully interdigitated in the gel phase and has a main transition at 42.5 °C with an enthalpy of 9.6 kcal/mol [95-97]. These values are slightly higher than those for DPPC, which has a T_m around 41.3 °C and a corresponding enthalpy of 8.2 kcal/mol ([7] and references therein). The thermodynamic behavior of these cationic triesters of phosphatidylcholine can be attributed to the net positive charge and the absence of intermolecular hydrogen bonding [98]. Furthermore, the overall polarity of the lipid is decreased, which may decrease the interfacial polarity. This would reduce the energetic cost when the ends of the hydrocarbon chains are exposed to the polar head group and the aqueous phase in the interdigitated phase [96]. The additional ethyl group in the head group may also mitigate the unfavorable exposure of the acyl chains [96]. Infrared spectroscopic

data lend some support to this conclusion, since the polar/apolar interfaces of cationic PCs are less polar than the parent PC lipids [96]. These lipids have complex phase diagrams that are dependent on temperature, mechanical agitation, and kinetics (Figure 11) [97].

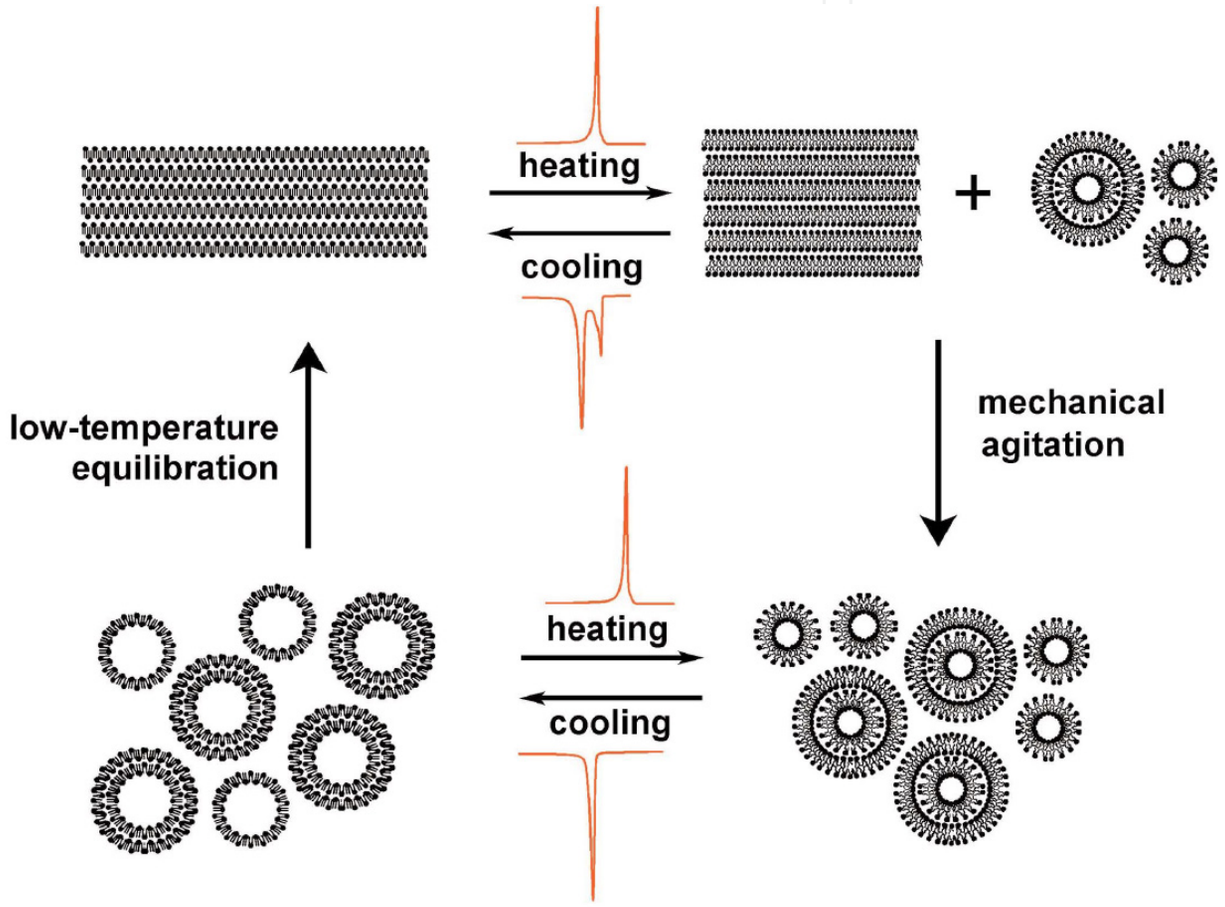

Figure 11. Diagram of the morphological changes in EDPPC dispersions. The equilibrium low temperature arrangement appears to be lamellar sheets, with chain interdigitation. Upon heating, liposomes and lamellar sheets (both non-interdigitated) coexist, whose mixture fully converts into liposomes (apparently the equilibrium liquid crystalline phase arrangement) only after mechanical treatment. Cooling back to the gel phase produces gel-phase liposomes which convert back into lamellar sheets only after prolonged low-temperature exposure. Reprinted from Biochim. Biophys. Acta., 1613, Koynova R, MacDonald RC, Cationic *O*-ethylphosphatidylcholines and their lipoplexes: phase behavior aspects, structural organization and morphology, Pages No. 39-48, Copyright (2003), with permission from Elsevier [97].

Vesicles formed from cationic triester lipids readily fuse with anionic lipids [98]. This may help explain why lipoplexes made from cationic *o*-ethylphosphatidylcholines with di-saturated hydrocarbon chains are effective transfection agents [98,99]. The structure and transfection capability of cationic phospholipid-DNA complexes are dependent on preparation conditions and ionic strength [98,100]. These lipids confer multiple advantages: they are non-viral, metabolized by cells, have low toxicity, and closely resemble naturally occurring phospholipids [101].

In some instances, DNA can be sandwiched between interdigitated gel phase lipid sheets into a rectangular columnar two-dimensional superlattice [97,99]. The gel-to-liquid crystalline phase transition results in the contraction of the DNA strand arrays so that the mean charge density is balanced with the increased positive charge of the non-interdigitated lipid. Additionally, in the non-interdigitated liquid crystalline phase, the interlamellar correlation in DNA ordering is no longer observed [99].

2.8. Phosphatidylethanolamines

Phosphatidylethanolamines (PEs) are distinct due to their strong reluctance to interdigitate. PEs are not susceptible to alcohol-induced interdigitation [48] or pressure-induced interdigitation [55]. Even the ether-linked DHPE does not interdigitate with pressure [34,56]. A major reason for this is that the PE headgroup can form hydrogen bonds [34]. PC headgroups interact through a weaker electrostatic attraction between the positively charged quaternary nitrogen and the negatively charged oxygen of a neighboring lipid headgroup [34]. Additionally, the smaller size of the PE headgroup also allows for closer interaction (less repulsion) [2,8,102].

2.9. Unsaturated phospholipids

Unsaturated lipids with common head groups and acyl chains of nearly equal length are strongly disfavored to interdigitate spontaneously. In general, unsaturated lipids are also resistant to both pressure- and chemically-induced interdigitation [44,103,104]. Even under high pressure, unsaturated lipids typically retain the transition from the non-interdigitated lamellar gel phase (L_β) into the liquid crystalline phase (L_α) [104,105]. Pressure does stabilize the L_β phase to the detriment of the L_α phase, but it is not sufficient to induce interdigitation [105].

Furthermore, unsaturated lipids have substantially lower main transition temperatures [7,10]. As interdigitation is highly unfavorable in the liquid crystalline phase, the relevant temperature range of the gel phase where interdigitation is likely to occur is much smaller. The main transition tends to be lowered the most when the double bond is located near the middle of the fatty acid chain [2,10,36]. Although double bonds that are *trans* usually have less influence than *cis* bonds [7,36], no ethanol-induced interdigitation was found in the *trans* lipid 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC) [106]. It was postulated that the increased cross-sectional area due to the double bond and the restriction in sliding motions contributes to the lack of interdigitation [106].

The inhibition of interdigitation also applies to lipid mixtures involving unsaturated lipids. In a model membrane of DPPC/DOPC/ergosterol, increasing the unsaturated lipid or sterol component co-operatively hinders the formation of the interdigitated phase [107]. DOPC is known to result in a more disordered and less tilted gel phase and can lead to phase separation at higher concentrations [108]. As a consequence, it was hypothesized that changes in the plasma membrane composition may play a role in the ethanol tolerance of yeast cells during fermentation ([107] and references therein).

There are some exceptions, however. While lipids with double bonds on both chains are particularly unlikely to interdigitate, there are a few examples of interdigitation where only one chain has a double bond. For example, McIntosh et al. tested the ethanol-induced interdigitation of five positional isomers of 1-eicosanoyl-2-eicosenoyl-*sn*-glycero-3-phosphocholine (C(20):C(20:1Δ^n)PC) with a single *cis* bond on the *sn*-2 chain at position n = 5, 8, 11, 13 and 17 [109]. Ethanol-induced interdigitation can be induced when the position of the *cis* bond is at n= 5 or 8, but not at n= 11, 13, or 17 [109]. In contrast, the fully saturated lipid with the same chain length can easily be interdigitated with a small amount of ethanol [47].

Additionally the *cis* mono-unsaturated 1-stearoyl,2-oleoyl-phosphatidylcholine (SOPC) can be interdigitated with glycerol [24]. The PG lipid, 1-palmitoyl,2-oleoyl-phosphatidylglycerol (POPG), can also be interdigitated with the addition of polymyxin B [24]. Certain mixtures of unsaturated zwitterionic and charged lipids, such as 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and POPG, can form the interdigitated phase at high concentrations of ethanol and at low hydration [110].

Therefore, it can be concluded that while it is possible to induce the interdigitated gel phase in unsaturated lipids, they are more resistant to interdigitation compared to the equivalent saturated lipid. Additionally, when the interdigitated phase does occur in unsaturated lipids, the phase appears to be less stable and less ordered than in saturated lipids [24].

2.10. Membrane curvature and interdigitation

The curvature of the membrane due to the macromolecular size and shape affects the thermodynamic properties [36]. For instance, on DSC scans, small unilamellar vesicles (SUVs) have lower enthalpic peaks and greater widths compared to multilamellar vesicles (MLVs) [2,3]. SUVs also have more mobility and less order in the hydrocarbon chains [2].

The degree of membrane curvature also affects the ability to interdigitate. Bending in the membrane causes increased steric interference in opposing lipid monolayers [44]. As a consequence, ethanol-induced interdigitation is dependent on curvature, with the more highly curved vesicles requiring more ethanol to interdigitate [111,112]. Sonicated DPPC SUVs are not stable in the presence of ethanol above the threshold concentration for interdigitation [112]. Furthermore, SUVs have a tendency to fuse into large unilamellar vesicles (LUVs), which have properties more similar to MLVs [36,113]. The more planar MLVs allow interdigitated lipids to slide by each other with low steric interference and therefore have the lowest threshold concentrations [44,112].

2.11. Inhibition of the interdigitated gel phase by cholesterol

Just as there are chemicals that induce interdigitation, there are chemicals that inhibit the formation of the L_{β}I phase. Cholesterol is a chemical inhibitor of interdigitation in a wide variety of lipid systems (Table 4).

Interdigitated Lipid System	References
DPPC/EtOH	[114]
DPPC/Pressure	[31]
DPPeth/Tris-HCl	[115]
DPPG/Tris-HCl	[115]
DHPC	[66,116]
EDPPC	[95]
F-DPPC	[117]
3:7 ratio of 16:0 LPC:DPPC	[118]

Table 4. The inhibition of interdigitation by cholesterol

In non-interdigitated membranes, cholesterol increases the fluidity of the gel phase, broadens the main transition, and decreases the main transition enthalpy [119]. Figure 12 demonstrates that these effects are also seen in membranes where cholesterol eliminates the interdigitated phase [115-117]. The amount of cholesterol required to prevent interdigitation is related to the stability of the L_{β}I phase. For DHPC, only ~5 mol% cholesterol is required to eliminate interdigitation [66,116]. However, the amount of cholesterol required to prevent interdigitation is approximately quadrupled for F-DPPC, which exists in the L_{β}I phase around 15 °C higher than DHPC [117]. At high cholesterol concentrations the L_{β}I phase of F-DPPC is replaced by a non-interdigitated liquid-ordered (l_o) phase with properties similar to DPPC/cholesterol [117]. On DSC scans, this effect can be observed by the broadening of the main transition peak and a reduction in the T_m hysteresis (Figure 12). The interdigitated phase of cationic EDPPC is especially resilient in the presence of cholesterol, with interdigitated domains still present at 30 mol% cholesterol [95].

There are multiple reasons why cholesterol-rich membranes disfavor interdigitation. For example, lipid head group crowding is mitigated by cholesterol serving as a spacer between lipids [115]. If cholesterol is placed within an interdigitated membrane, the increased spacing also increases the likelihood that the terminal lipid methyl groups will be exposed at the aqueous interface. Since the interdigitated phase lacks the thick membrane midplane region of non-interdigitated membranes, hydrophobic cholesterol located within the interdigitated phase is more likely to come in contact with water [115]. Furthermore, cholesterol significantly disrupts the lattice structure of gel phase lipids [108,117,120]. Lastly, in the interdigitated phase of highly asymmetrical lyso-lipids, cholesterol can take the place of the missing acyl chain thereby compensating for the size mismatch between the head group and the hydrocarbon chains [118,121].

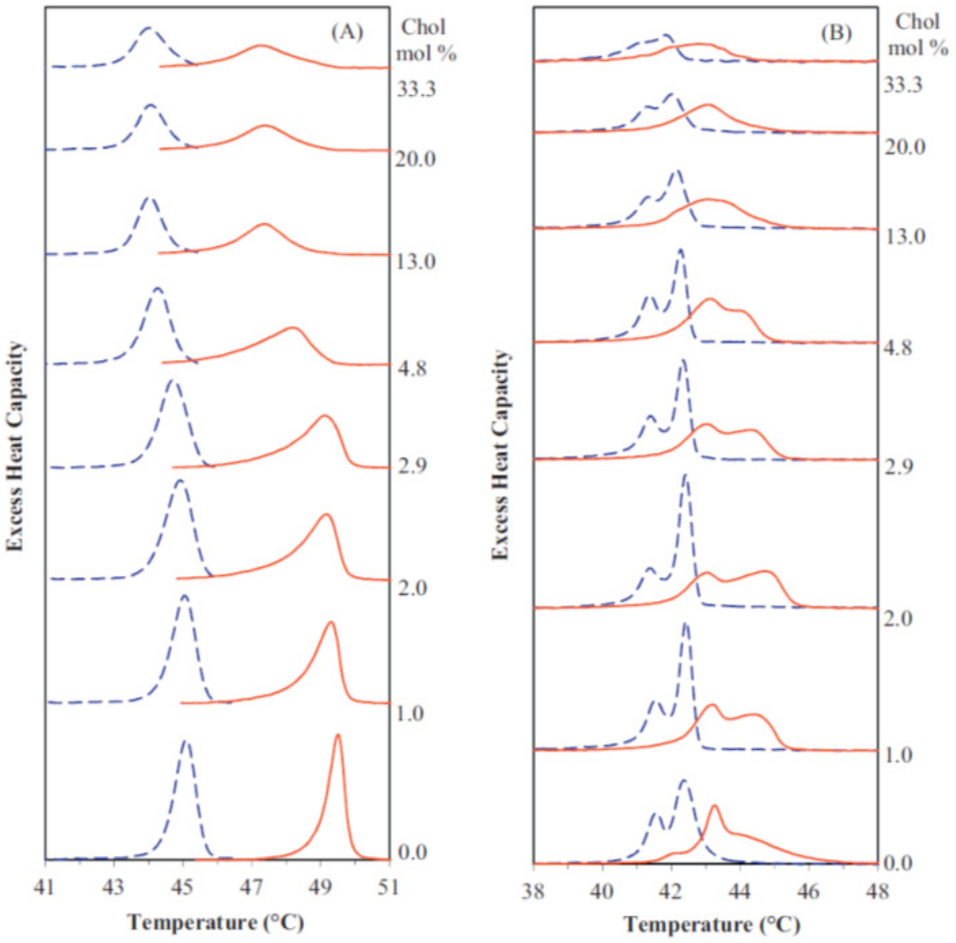

Figure 12. Heating (solid red lines) and cooling (dashed blue lines) DSC thermograms of: (A) F-DPPC and (B) 1:1 F-DPPC/DPPC with various concentrations of cholesterol. The cooling scans have been inverted to allow comparison with the heating peaks. For clarity, the thermograms are also offset vertically. Reprinted from Chem. Phys. Lipids, 165, Smith EA, Wang W, Dea PK, Effects of cholesterol on phospholipid membranes: Inhibition of the interdigitated gel phase of F-DPPC and F-DPPC/DPPC, Pages No. 151-159, Copyright (2012), with permission from Elsevier [117].

2.12. Chemical inhibition of the interdigitated gel phase

In a general sense, solvent inhibitors of interdigitation work in the opposite fashion as chemical inducers. Some researchers have focused on the difference of how kosmotropic and chaotropic solutes interact with lipid membranes [122-124]. Kosmotropes deplete the solution at the interface and increase the interfacial tension whereas chaotropes accumulate in the interface and decrease surface tension [125]. The changes in the structure of water due to these types of chemicals can be attributed to alterations in the hydrogen bonding network of water [126,127]. Kosmotropic substances are classified as water-structure makers, meaning that they stabilize the structure of bulk water. When kosmotropes interact with hydrated lipids, they tend to reduce the interfacial area and inhibit interdigitation [122]. Chaotropic chemicals are classified as water-structure breakers and increase the surface area per lipid, favoring interdigitation [122,124].

The differences between chemicals that induce or inhibit interdigitation have also been illustrated according to the interaction free energy of the lipid membrane interface with solvents ([128] and references therein). The solvent free energy relationship can be further split into interactions with the polar head groups and interactions with the hydrophobic lipid chains. In this model, when "good" solvents are added, the interfacial area swells to increase the total contract with the solvent. For organic solvents that are water-miscible and have a high solubility for alkanes, such as acetone and ethanol, the interaction increases the interfacial area by reducing the interaction free energy between the solvent and the interfacial alkyl chains [128]. On the other hand, the interaction of "poor" solvents with lipids is unfavorable and has larger free energy penalty. As a result, the interfacial segments shrink in size to prevent contact with the solvent. Consequently, "good" solvents will favor interdigitation while "poor" solvents will destabilize the L_{β}I phase.

The inhibition of interdigitation has also been described in terms of osmotic stress. Chemicals that apply osmotic stress, such as poly(ethylene glycol) tend to inhibit interdigitation [60]. As in the other models described above, this has been proposed to occur because of a decrease in the repulsive interaction between the lipid head groups.

Dimethyl sulfoxide (DMSO) is an example of a solvent inhibitor of interdigitation. The interaction of DMSO with membranes is of great interest because it can be used as a cryoprotectant for biological material, such as stem cells [129]. DMSO can also enhance the permeability of membranes [130]. The mechanism by which DMSO inhibits interdigitation is by decreasing the repulsion between head groups [131]. The ability of DMSO to form unusually strong hydrogen bonds may explain this effect [132]. This phenomenon can be clearly seen in the phase behavior of DHPC. Just as chemicals that favor interdigitation shift the pre-transition of DHPC to a higher temperature; factors that disfavor interdigitation shift the pre-transition to a lower temperature. The suppression of the pre-transition clearly demonstrates that DMSO destabilizes the L_{β}I phase (Figure 13) [131].

A major caveat with these solvent models is that the interactions with lipids are often concentration-dependent. For instance, in the DPPC/DMSO/water system, three distinct effects are found within different DMSO concentration ranges [133]. Perhaps the most remarkable is at above mol fractions of ~0.9 DMSO, the T_m temperature is greatly elevated and an interdigitated gel phase is formed [133].

The disaccharide trehalose is another example of a chemical inhibitor of interdigitation [124]. Like DMSO, the interactions of trehalose with membranes show promise in the cryopreservation of biological material [129]. In the yeast *Saccharomyces cerevisiae*, trehalose appears to increase viability during ethanol fermentation and provide protection against oxidative stress [134-137]. Similar to DMSO, trehalose disfavors interdigitation by increasing the packing density of the lipid head groups [124,138,139]. However, there is disagreement over the exact molecular interaction with lipids. The main dispute is over whether or not sugars are directly bound to or excluded from the membrane surface [140,141]. Recently, Andersen et al. have tried to explain this discrepancy by proposing that there are two concentration-dependent interactions. In this explanation, trehalose binds strongly to the bilayer at low concentrations, but is gradually expelled above ~0.2 M [141].

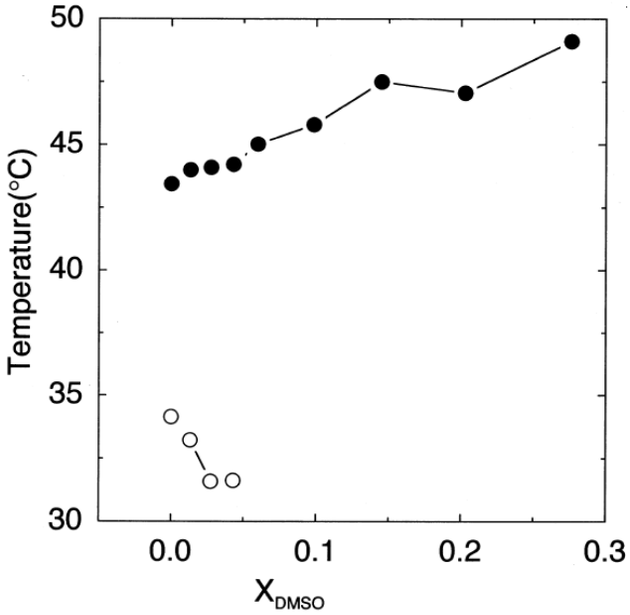

Figure 13. Phase transition temperatures of DHPC-MLV at various concentrations of DMSO (mole fraction) determined by DSC. (●) shows gel to liquid-crystalline phase transition temperatures and (○) shows L_βI to P_β' phase transition temperatures. Reprinted from Biochim. Biophys. Acta., 1467, Yamashita Y, Kinoshita K, Yamazaki M, Low concentration of DMSO stabilizes the bilayer gel phase rather than the interdigitated gel phase in dihexadecylphosphatidylcholine membrane, Pages No. 395-405, Copyright (2000), with permission from Elsevier [131].

2.13. The interdigitated gel phase versus the inverted hexagonal phase

A clear inverse relationship exists between the interdigitated phase gel phase and the inverted hexagonal phase (H_{II}) [56,128]. The major structural factor is the relative size of the lipid headgroup and the attraction/repulsion between headgroups. A lipid that forms the inverted hexagonal phase is unlikely to interdigitate and vice versa. The temperature dependence of these phases is also opposite. For example, with DHPC, the interdigitated phase is present only below the pre-transition. The interdigitated phase requires predominately *trans* confirmations in the hydrocarbon chains, so it is unlikely to form in the liquid crystalline phase where there are abundant *gauche* confirmations and a high degree of disorder [2,37]. In contrast, the inverted hexagonal phase typically forms well above the main transition into the liquid crystalline phase [8].

This relationship also extends to environmental factors that encourage or discourage interdigitation (Table 5). Chemicals that favor the interdigitated phase such as ethanol tend to destabilize the H_{II} phase [128,124 and references therein]. Interdigitation is favored because the surface area per lipid head group in the L_βI phase is substantially larger versus non-interdigitated membranes [124]. The H_{II} is the opposite because it requires a small head group area. Solvents that stabilize the H_{II} phase like dimethyl sulfoxide therefore also inhibit interdigitation [56,122,128]. This relationship appears to apply to hydrostatic pressure as well. While increased pressure favors interdigitation (Tables 2 and 3), pressure destabilizes the inverted hexagonal phase in PE lipids [56].

L_βI	H_{II}
Large head group repulsion	Small head group repulsion
Chaotropic chemicals	Kosmotropic chemicals
High hydrostatic pressure	Low hydrostatic pressure
Low Temperatures	High Temperatures

Table 5. Factors that stabilize the interdigitated gel (L_βI) phase and the inverted hexagonal (H_{II}) phase.

2.14. Influence of hydration and pH on the L_βI phase

While most interdigitated systems are studied in excess water, interdigitation can be affected at less than full hydration. For instance, interdigitation of DHPC is reliant on hydration, as coexisting interdigitated and non-interdigitated phases are found at low hydration [142,143]. However, the cationic EDPPC may be interdigitated in the dry state [97].

Furthermore, substituting deuterium oxide (D_2O) for water slightly disfavors the spontaneous interdigitated phase of DHPC [144]. Using D_2O also increases the threshold concentration for the chemically-induced interdigitation of DPPC [27] and increases threshold pressure for interdigitation [145]. These phenomena are explained by the different hydrophobic interactions and interfacial energies in H_2O versus D_2O [27,144,145].

Changing the pH of the aqueous solution can also affect interdigitation. In DHPC membranes a low pH will inhibit interdigitation [59]. As the pH is lowered the phosphate groups are protonated and ultimately the total repulsive force between head groups is decreased, disfavoring interdigitation [59]. The pH is also highly relevant to the interdigitated phase in charged lipids, such as PGs. At a high pH, the electrostatic repulsion between head groups that encourages interdigitation in PGs is increased [78].

2.15. Lipid mixtures and interdigitated/non-interdigitated gel phase coexistence

Under certain circumstances, interdigitated and non-interdigitated phases can coexist within a membrane even though the boundaries between these domains are considered to be energetically unfavorable [115,116]. The uneven structure between these domains can significantly increase the membrane permeability [146,147]. With the variety of lipids now known to interdigitate, there are many possible lipid systems that will have complex phase diagrams involving the L_βI phase.

Gel phase coexistence can often be found in binary mixtures of a lipid that can spontaneously interdigitate (e.g. F-DPPC or EDPPC) and one that cannot (e.g. DPPC or PE lipids). For example, at equimolar amounts of F-DPPC and DPPC, interdigitated F-DPPC-rich domains create a phase-segregated system [69,117]. On DSC scans this manifests itself as multiple peaks (Figure 8). The peaks with the greatest transition hysteresis likely correspond to interdigitated domains rich in F-DPPC. When the F-DPPC molar fraction is large, the hysteresis is also increased [69]. Additionally, gel phase coexistence occurs in the mixture of 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine (DEPE) and EDPPC [95].

However, this is not true of all such binary mixtures. Outside of the phase transition regions in DPPC/EDPPC, for instance, there is no gel phase segregation [95].

Mixing an interdigitated lipid with cholesterol can also produce gel phase coexistence. Cholesterol-poor interdigitated domains and cholesterol-rich non-interdigitated domains have been found in DHPC/cholesterol [116], F-DPPC/cholesterol [117], and EDPPC/cholesterol [95]. For these mixtures, the lipids with the most stable interdigitated phase tend to have a larger region of phase coexistence within the phase diagram.

Alternatively, a lipid such as DPPC that can be chemically induced to interdigitate can be mixed with lipids that cannot, such as PE lipids [147]. The DPPC-rich domains will interdigitate with ethanol, but domains composed of mostly PE lipid will not. A similar result can be achieved in mixtures of DPPC/cholesterol/ethanol, where the cholesterol-rich domains remain non-interdigitated in the presence of ethanol [39,146].

It is also possible to have coexistence in membranes with only one lipid. For instance, coexisting interdigitated and non-interdigitated phases form in supported F-DPPC membranes where the lateral expansion of the lipid film is restricted [68]. This results in a "frustrated" state, where the energetically favorable interdigitated phase cannot fully form due to constraints in topology and the available surface area [68]. Additionally, while the 16-carbon chain length DPPG does not spontaneously interdigitate, the 18-carbon chain DSPG spontaneously forms an interdigitated gel phase that coexists with a non-interdigitated gel phase [78]. This two-phase coexistence was attributed to a kinetically trapped system that is not at thermal equilibrium [78].

2.16. Applications of the interdigitated gel phase

One of the most promising applications for the interdigitated gel phase is the creation of large unilamellar vesicles termed interdigitation-fusion (IF) vesicles [44,148]. Figure 14 demonstrates the process for the creation of IF liposomes using ethanol [148]. Below the main transition, the ethanol causes the formation rigid and flat interdigitated sheets [149]. These sheets are surprisingly stable under the T_m, even when ethanol is removed [149]. When the temperature is raised above the main transition, the sheets fuse into large vesicles. This fusion encapsulates particles from the surrounding solution [149,150]. These materials include other small vesicles, biological macromolecules, colloids, and nanoparticles [149,150]. The amphiphilic nature of lipids allows for the capture of hydrophobic materials [151]. Transmembrane insertion of protein into IF vesicles has also been achieved using electropulsation [152].

The IF procedure can also be used to create multicompartment vesicle-in-vesicle structures called "vesosomes" [150]. These multicompartment vesicles should be closer replicas of eukaryotic cells than regular vesicles [150,153]. Therefore, vesosomes have the potential to more closely mimic biological conditions and reactions in artificial cells [150,154,155]. Furthermore, the retention of encapsulated material can be substantially increased in vesosomes [151,156,157]. These vesicles are highly customizable because the composition of

the inner and outer components can be varied [149,150,154]. As a result, it is theoretically possible to use vesosomes as controlled nanoreactors [153,155]. For complex and expensive chemistry such as enzyme reactions, vesosomes should be able to optimize reaction conditions and drastically reduce the amount of reagents needed [155].

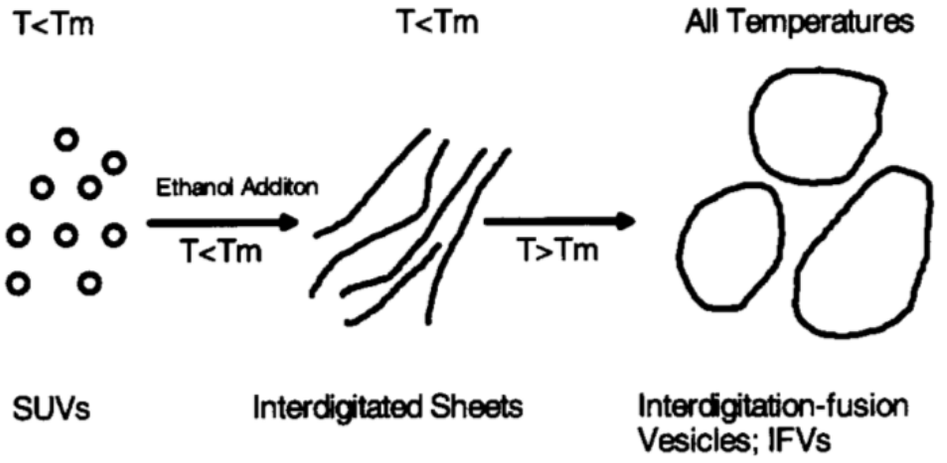

Figure 14. Liposome formation by interdigitation fusion (IF) using ethanol. Reprinted from Biochim. Biophys. Acta., 1195, Ahl PL, Chen L, Perkins WR, Minchey SR, Boni LT, Taraschi TF, Janoff AS, Interdigitation-fusion: a new method for producing lipid vesicles of high internal volume, Pages No. 237-244, Copyright (1994), with permission from Elsevier [148].

As described by Ahl et al. [44], there are four general guidelines for IF liposomes: (1) the lipids must be able to form the interdigitated phase; (2) the precursor liposomes should be small, preferably sonicated SUVs; (3) the temperature of the precursor SUV suspension after the addition of the alcohol must be below the T_m of the phospholipids; and (4) the temperature should be raised above the T_m of the phospholipids after the formation of the interdigitated sheets. Therefore, the creation of these liposomes is dependent on the lipid composition. Adding cholesterol and lipids containing *cis* double bonds can compromise the formation of IF liposomes [103,148]. PE lipids are also unsuitable because of their reluctance to interdigitate [44].

A similar result can be achieved using pressure to create pressure-induced fusion (PIF) liposomes [103]. An advantage of this technique is that no organic solvent is required and it is an effective sterilization method [103]. The captured volume of the IF or PIF vesicles is larger than other techniques for liposome preparation ([44] and references therein).

3. Conclusions

As an analytical instrument, DSC offers many advantages. One advantage is the simplicity of the sample preparation procedure. Samples do not have to be supported or spatially oriented and do not require the insertion of a membrane probe. For sensitive low enthalpy phase transitions, it is a great benefit not to need a probe so that the purity of the sample can

be maintained. The importance of this can be seen in alcohol-induced interdigitation, where the low enthalpy pre-transition is an important aspect of the analysis (Figure 5) [15]. Moreover, the effects of pressure can be measured concomitantly with calorimetry data with the appropriate equipment. This greatly expands the range of the phase diagram that can be experimented with.

We have shown that DSC can accurately measure changes in the thermodynamic properties of phospholipid membranes with the addition of chemicals that either encourage or discourage interdigitation. DSC is particularly well-suited for the study of chemically-induced interdigitation because it is sensitive enough to detect small, incremental changes in phase transition temperatures (Figure 5). With the capability to perform heating and cooling scans at a constant rate, the transition hysteresis can also be easily determined. In addition, the transition enthalpy can highlight the "biphasic" behavior above and below the threshold concentration for interdigitation (Figure 6).

Moreover, DSC can reveal how changes in either the hydrocarbon chains (Figure 1) or in the polar head group (Figure 2) will affect the thermodynamics. Modifications that either encourage or discourage interdigitation are summarized in Figure 15. Understanding the importance of structural differences reveals the importance of lipid diversity in biological membranes. Lipid composition can help explain why, for example, a peptide might interact differently with human versus microbial membranes [81]. With the increasing popularity of liposomes for pharmaceutical applications and research, it also is essential to find suitable lipid candidates. For instance, calorimetry can be applied to screen potential IF vesicles by determining whether interdigitation is present and by determining the T_m temperature.

In addition, more information can be inferred from DSC data than the phase transition temperature. With careful analysis, the nature of the lipid/solvent interaction and the properties of the chemicals themselves can be derived. For example, the characteristics of kosmotropic and chaotropic chemicals are clearly reflected in their effects on lipid membranes (see section 3.12.). This analysis can also increase the understanding of how chemicals interact with biological membranes, such as why chemicals like DMSO and trehalose can protect cells during cryopreservation [129].

However, DSC also has limitations when analyzing phospholipid samples. Perhaps the greatest weakness is the lack of direct structural information. As a consequence, relying solely on DSC data can be misleading. For instance, the pre-transition peaks of DPPC and DHPC look similar on DSC thermograms. However, the actual nature of the transition is substantially different (Figure 3). While the structure can often be reasonably inferred from thermodynamic properties, it is not as robust as other experimental techniques [6]. Additionally, while alterations in the macromolecular structure can be reflected in DSC data (see section 3.10.), the changes are not specific enough to be able to infer the true structure.

Overlapping or multiple transitions can also present a problem. In F-DPPC/DPPC, the multiple peaks reflect the presence of phase segregation (Figure 8), but this is not always the case. Multiple DSC peaks can also indicate separate phase transitions that involve the entire

membrane. In the case of EDPPC, different morphologies result in separate DSC peaks (Figure 11) [97]. Overlapping peaks can also obscure individual transitions, especially when there are multiple components in the membrane and the transition peaks are broad.

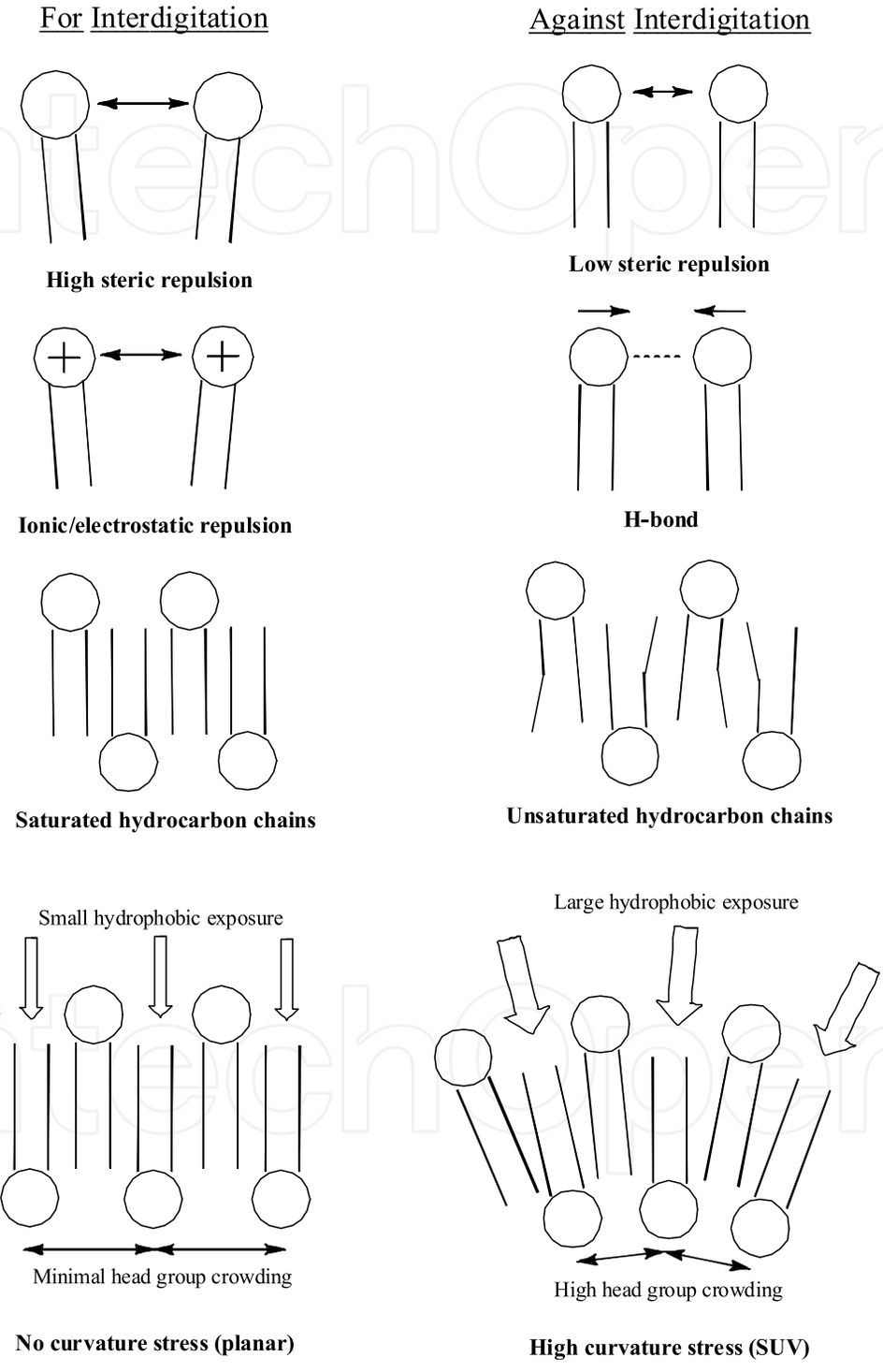

Figure 15. Schematic representation of factors that favor or disfavor interdigitation.

Fortunately, one of the greatest strengths of DSC data is that it is highly compatible with other analytical techniques. In the case of the L_{β}I phase, methods such as x-ray diffraction,

nuclear magnetic resonance, and fluorescence techniques can fill in the gaps ([6] and references therein). Additionally, DSC is highly valuable in determining the relevant temperature range to use for the other experimental techniques.

The stability of the interdigitated phase plainly demonstrates the balance of forces within the membrane. Factors as varied as electrostatic and steric interactions, van der Waals forces, solvent binding at the interface, and the presence of double bonds all contribute to the properties of hydrated phospholipid membranes. DSC provides a way to judge the resulting balance of these forces by measuring the stability of different thermodynamic phases. Consequently, the wealth of information calorimetric analysis provides ensures that DSC will remain an invaluable tool for the study of membrane biophysics.

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Abbreviations

differential scanning calorimetry (DSC)
main transition temperature (T_m)
pre-transition temperature (T_p)
small unilamellar vesicle (SUV)
large unilamellar vesicle (LUV)
multilamellar vesicle (MLV)
interdigitation-fusion vesicle (IFV)
interdigitated gel phase ($L_\beta I$)
planar gel phase ($L_{\beta'}$)
ripple gel phase ($P_{\beta'}$)
liquid crystalline phase (L_α)
inverted hexagonal phase (H_{II})
crystalline bilayer phase (L_c)
liquid-ordered (l_o)
phosphatidylcholine (PC)
phosphatidylglycerol (PG)
phosphatidylethanolamine (PE)
phosphatidylethanol (Peth)
1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)
1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (1,3-DPPC or *β*-DPPC)
1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (DHPC)
1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine (F-DPPC)
1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanol (DPPeth)

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1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DPPG)
1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (16:0 LPC)
1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (EDPPC or Et-DPPC)
1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE)
1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE)
1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE)
1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)
1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC)
1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC)
1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC)
1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG)

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