Phase transitions of lipid bilayers
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Phase behavior of monotail lipids

Abstract

In this chapter we study the phase behavior of the simplest mesoscopic model of a phospholipid, that consists of a hydrophilic head group and one hydrophobic tail. With this mesoscopic lipid-water model we observe the formation of the liquid crystalline phase ($L_\alpha$) and gel phases in which the tails are interdigitated ($L_{\beta_1}$) or non-interdigitated ($L_\beta$). For double-tail lipids experiments show all three phases, while for single-tail lipids only $L_\beta$ and $L_\alpha$ are observed. We show that at sufficiently high head-head repulsion the $L_{\beta_1}$ phase is stable for single-tail lipids. This suggests that it might be possible to induce an $L_\beta \rightarrow L_{\beta_1}$ transition by adding chaotrope salts.
3.1 Introduction

In the previous chapter, we discussed the technique to study the self-assembly and the phase behavior of lipid bilayers. An important question in the development of a mesoscopic model is how much chemical detail should be included in the model. Often, a model consisting of a hydrophilic head group and a single hydrophobic tail is used as a model of a phospholipid. In this chapter, we investigate if such a model correctly describes the (phase) behavior of double tail phospholipids. A second goal of this chapter is to compare the results of our simulations with experiments that are performed on monotail lipids.

The phase behavior of different PC's has been determined experimentally (see [9] for a review). All PC's have a low temperature $L_\beta'$ phase (see figure 3.1). In this phase the bilayer is a gel: the chains of the phospholipids are ordered and show a tilt relative to the bilayer normal. At higher temperature the $L_\alpha$ phase is the stable phase. This phase is the liquid crystalline state of the bilayer in which the chains are disordered and tail overlap due to this thermal disorder is possible. This phase is physiologically the most relevant [63].

Figure 3.1: Schematic drawings of the various bilayer phases. The characteristics of these phases are explained in the text. The filled circles represent the hydrophilic head group of a phospholipid and the lines represent the hydrophobic tails.

Under normal conditions the two monolayers of a bilayer contact each other at the terminal methyl group of their hydrophobic chains, while their hydrophilic head groups are in contact with water. However, it is known experimentally that at low temperatures an interdigitated state, in which the terminal methyl groups of one monolayer interpenetrate the opposing layer, is also possible. This $L_{\beta1}$ phase does not spontaneously form in bilayers of symmetrical chain phospholipids, but has to be induced by changes in the environment or in the molecular structure (see chapter 6 for more details). Interdigitation reduces the bilayer thickness, and this can, for example, affect the diffusion of ions across the bilayer or influence the activity of membrane proteins. It has been proposed [65, 66] that specific interactions are not important in the formation of an interdigitated phase, and that the main driving force that induces interdigitation is an increase in the head group surface area, which results in the creation of voids between the molecules. Since voids in the bilayer core are energetically unfavorable, they are filled up by molecules of the opposite mono-
layer.

This mechanism suggests that the formation of an interdigitated phase should be a general phenomenon. This would imply that an interdigitated phase could also be induced in bilayers of, for example, single-tail lipids. The fact that for single-tail lipids the interdigitated phase has not been observed experimentally, is one of the motivations to investigate the molecular aspects underlying the formation of an interdigitated phase in more detail.

Our simulations correctly describe the hydrophobic tail length dependence of this transition and the effect of adding salt. In addition, the simulations predict that both the interdigitated and non-interdigitated phases can be formed in systems with single-tail lipids.

3.2 Computational details

In this investigation we consider lipids with one head segment connected to a single tail with variable length (see figure 3.2). Two consecutive beads are connected by harmonic springs with spring constant \( k_r = 100.0 \) and \( r_0 = 0.7 \). A harmonic bond bending potential between three consecutive beads is added with a bending constant \( k_\phi = 10 \) and an equilibrium angle \( \phi_0 = 180^\circ \).

![Figure 3.2: Models used in this study with their nomenclature. The black particles represent the head beads and the white particles the tail beads.](image)

The repulsion parameters used are \( a_{ww} = a_{tt} = 25 \), \( a_{wh} = 15 \), and \( a_{wt} = 80 \) (see equation 2.6, chapter 2). In addition, we vary the head-head interaction parameter \((a_{hh})\) to study the effect of changing the interactions between the hydrophilic segments of a lipid. In a real system the head-head interactions can be changed by, for example, adding salt to the system.

All our simulations are performed on a tensionless bilayer of 200 lipids. The total number of particles was 3500. The overall density of the system is \( \rho = 3 \). We initialize our system by distributing lipids randomly in water and we observe the self-assembly of a bilayer using DPD simulation only. After the bilayer is formed, we perform, in ad-
dition to the DPD moves, Monte Carlo moves in which we change the area as well. A typical simulation required 100,000 cycles of which 20,000 cycles were needed for equilibration. All the results are expressed in the usual reduced units, *i.e.* using $r_c$ as the unit of length and repulsion parameter $\alpha = 1$ as unit of the energy.

### 3.3 Results and Discussion

In this section we first describe in detail the different phases of a bilayer formed by single tail lipids consisting of one head bead and nine tail beads ($ht_9$), which we study at different reduced temperatures, from $T^* = 0.8$ to $T^* = 1.5$. At a fixed head-head repulsion of $\alpha_{hh} = 35$ an interdigitated gel phase is formed at low temperatures, while at $\alpha_{hh} = 15$ the non-interdigitated $L_\beta$ phase is formed. We then investigate the influence of changing the interactions between the head groups and we show that we obtain the non-interdigitated $L_\beta$ phase or the interdigitated $L_{\beta1}$ phase dependent on the head-head repulsion parameter (see figure 3.3). Finally, we investigate the influence of tail length and we compare our results with experimental data on single-tail lipids.

![Figure 3.3](image-url) **Figure 3.3:** Computed phase diagram of the lipid $ht_9$ as function of head-head repulsion parameter $\alpha_{hh}$ and reduced temperature $T^*$. At high values of the head-head repulsion parameters the interdigitated phase $L_{\beta1}$ phase is formed, while at low values the non-interdigitated $L_\beta$ phase is formed. Increasing temperature causes the melting of the bilayer to the $L_\alpha$ phase.
3.3 Results and Discussion

3.3.1 The lipid h₉₉

**Head-head repulsion** $a_{hh} = 35$

In figure 3.4 the average area per lipid $A_t$ and the bilayer thickness, $D_{bb}$, are plotted as function of temperature. The error bars have been calculated with the block averages method [32,67]. In all the other plots of the area per lipid or bilayer thickness, we will not include error bars, which, however, have been estimated as $\leq 5\%$.

![Figure 3.4: Area per lipid $A_t$ and (b) bilayer thickness $D_{bb}$ as function of reduced temperature $T^*$ for lipid type h₉₉.](image)

In both figures 3.4(a) and 3.4(b) we can distinguish two regions: at low temperatures, the area per lipid decreases with increasing temperature and the thickness is increasing, while at high temperatures the area is increasing with increasing temperature, and the thickness is decreasing. At the lowest temperature studied ($T^* = 0.8$) the area is larger than at the highest temperature studied ($T^* = 1.5$) while the thickness at $T^* = 0.8$ is smaller than the thickness at $T^* = 1.5$. This different temperature dependence of $A_t$ and $D_{bb}$ suggests that the bilayer undergoes a phase transition. Before discussing this transition in detail we will first characterize the low and the high temperature phases.

To characterize the ordering of the lipids in the bilayer we use the order parameters $S_{tail}$ and $S_n$. In figure 3.5 the values of both $S_{tail}$ and $S_n$ are plotted as a function of temperature. The high values of $S_n$ at temperatures below $T^* = 0.95$ indicate that the bonds are ordered along the bilayer normal. This order persists even for bonds far from the head-group region, decreasing slightly with increasing temperature. Above $T^* = 0.95$ the values of $S_n$ further decrease with increasing temperature, and the order along the chain is lost.

The overall order of the tails ($S_{tail}$) shows a similar behavior (figure 3.5(b)). Also here we can distinguish two regions: below $T^* = 0.95$ where $S_{tail}$ has values higher than 0.5 indicating that the chains are ordered along the bilayer normal, and above
Figure 3.5: (a) Local order parameter $S_n$ and (b) tail order parameter $S_{tail}$, as function of reduced temperature $T^*$. 

$T^* = 0.95$ where the values of $S_{tail}$ decrease below 0.5, showing an increase in the disorder of the chains.

To further characterize the structure of the bilayer in the low and high temperature regions, we compare in figure 3.6 the in-plane radial distribution function $g(r)$ of the head beads of the lipids at one interface, for two different temperatures: $T^* = 0.8$ and $T^* = 1.5$. At $T^* = 0.8$, the radial distribution function shows more pronounced peaks compared to the $g(r)$ at $T^* = 1.5$, which corresponds to a more structured organization of the lipids head groups in the bilayer plane. The structure in the radial distribution function and the high values of the order parameters for low temperatures, suggest that the low temperature phase is the ordered gel phase, while at high temperatures the bilayer is in the disordered liquid crystalline phase.

Figure 3.6: Two dimensional radial distribution function $g(r)$ in the bilayer plane for the head groups at $T^* = 0.8$ and $T^* = 1.5$. 
3.3 Results and Discussion

Figure 3.7: Density profiles $\rho(z)$ along the bilayer normal $z$ for different reduced temperatures $T^\ast$. Each line is the density profile for a different bead: full lines are the densities of the tail beads, dashed lines are the densities of the head beads, and the thin solid line is the density of water. The black lines correspond to the lipids in one monolayer, while the grey lines correspond to the lipids in the opposite monolayer. The big dots correspond to the maxima in the bead density distributions and illustrate the position of the beads in the bilayer. The full circles correspond to tail beads and the open circles to head beads.

In figure 3.7 we show the density profiles in the direction normal to the bilayer for the system components at different reduced temperatures. Figures 3.7(a) and 3.7(b) correspond to a bilayer in the gel phase, while 3.7(c) and 3.7(d) correspond to a bilayer in the liquid crystalline phase. It is clearly visible that, in the low temperature region, the two monolayers are interdigitated. At $T^\ast = 0.8$ the overlap extends up to the 8th bead in the tail and the peaks of the density profiles for the lipids tail beads in one monolayer (black full lines) are exactly alternating with the peaks of the opposite monolayer (red full lines), showing an optimal packing of the tails. This structure resembles the experimentally observed interdigitated phase $L_{\beta_1}$.

We can now explain the temperature dependence of the area per lipid (figure 3.4). The low temperature phase is the interdigitated gel $L_{\beta_1}$. In this phase the ordering
of the chains is the dominating effect. The lipids stretch out in the direction normal to the bilayer, inducing interdigitation. This packing results in a larger average distance between the lipids head groups in each monolayer and in a larger area. In this region an increase of temperature reduces the values of the order parameter (figure 3.5(b)), but along the chain the order persists (figure 3.5(a)). Thus interdigitation is still present, but is decreasing in depth, resulting in an increase of the bilayer thickness and a decrease of the area per lipid. Above the transition temperature, the chains loose the persisting order and are not interdigitated. Only the terminal tail beads overlap, due to thermal disorder. In this temperature region an increase in temperature increases the effective volume occupied by the molecules, but the extent of tail overlap does not depend significantly of temperature. As a result the area per molecule increases while the bilayer thickness decreases.

*Head-head repulsion* $a_{hh} = 15$

In the previous section we have seen that single tail lipids spontaneously form an interdigitated phase at low temperatures, while the most common organization of (symmetric) phospholipids in membranes is a bilayer formed by two separate monolayers [68]. It is therefore interesting to investigate whether we can adapt the single tail model to reproduce the phase behavior of real membranes, and in particular if we are able to obtain a non-interdigitated gel phase. If the main cause of interdigitation is an increase in the head groups surface area [65,66], we can test this mechanism by changing the value of the head-group repulsion parameter, $a_{hh}$, in our model. Taking as initial condition the interdigitated bilayer at $T^* = 0.85$, we decrease the head-group repulsion parameter from $a_{hh} = 35$ to $a_{hh} = 15$, the latter being the same repulsion parameter as between an hydrophilic bead and a water-bead. Experimentally, changing the head-head interactions corresponds to, for example, adding salt to the system. It is important to recall that, with the zero surface tension scheme, the system can evolve to the optimum area per lipid even if the bilayer undergoes structural rearrangements.

Figure 3.8(a) shows the temperature dependence of the area per lipid for the repulsion parameters $a_{hh} = 35$ and $a_{hh} = 15$. We observe that the behavior in temperature of the area per lipid for the two values of $a_{hh}$ is very different. At low temperatures the area at $a_{hh} = 35$ is almost twice the value of the area at $a_{hh} = 15$. The decrease of the head-group surface area is also shown in figure 3.8(b), where we compare the radial distribution functions of the head groups in the bilayer plane at $T^* = 0.85$ (see also figure 3.9 for snapshots of the two systems). The peaks in the radial distribution function for the system with $a_{hh} = 15$ (solid line) are shifted to the left compared to the system with $a_{hh} = 35$ (dashed line), showing a decrease of the distance between the head groups. This is a strong indication that at low temperature, with the lower repulsion parameter, the bilayer is in the $L_\beta$ phase.
3.3 Results and Discussion

Figure 3.8: Comparison of (a) the area per lipid \( A \) as function of reduced temperature \( T^* \), and (b) two dimension radial distribution function \( g(r) \) in the plane of the bilayer at \( T^* = 0.85 \), for two different repulsion parameters between the lipid head groups: \( a_{hh} = 15 \) (circles, solid line) and \( a_{hh} = 35 \) (squares, dashed line).

Figure 3.9: Snapshots of the simulations of a bilayer consisting of the lipid \( h_{19} \) at \( T^* = 0.85 \). (a) The non-interdigitated gel phase \( L_\beta \) at \( a_{hh} = 15 \) and (b) the interdigitated gel phase \( L_\beta \) at \( a_{hh} = 15 \). Black represents the hydrophilic head group and grey represents the hydrophobic tails.

To further characterize the bilayer structure for \( a_{hh} = 15 \), we study the order parameters \( S_n \) and \( S_{\text{tail}} \), which are plotted in figure 3.10. At temperatures \( T^* \leq 0.95 \) the chains are locally ordered (values of \( S_n \) above 0.5), and the order does not decrease significantly going through the hydrophobic core. Also the overall order of the chains \( S_{\text{tail}} \) is high in this temperature region. Above \( T^* = 0.95 \) we observe a decrease in both the order parameters. The chains become disordered and the persistence of order along the chain is lost. This trend is analogous to the one observed for \( a_{hh} = 35 \). In both cases the low temperature region is characterized by the ordering of the chains, while at high temperatures the chains are disordered. However, while for \( a_{hh} = 35 \) the two monolayers are interdigitated in the ordered phase, for \( a_{hh} = 15 \) the ordered phase is a bilayer formed by two separated leaflets. This can clearly be seen from the density profiles, which we plot as function of reduced temperature in figure 3.11. This figure shows that the melting of the bilayer results in a broader shape of the den-
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**Figure 3.10:** (a) Local order parameter $S_n$ and (b) tail order parameter $S_{\text{tail}}$, as function of reduced temperature $T^*$ for a bilayer formed by lipids with $a_{hh} = 15$.

sity profiles. The increase of disorder in the chains (see figure 3.10) results in a partial overlap of the two monolayers. This transition upon heating is also reflected in the trend of the area per lipid with temperature (figure 3.8(a)), which shows a sharp increase between $T^* = 0.95$ and $T^* = 1.0$. We can then conclude that a transition from an ordered to a disordered phase takes place at a temperature $0.95 < T_m < 1.0$.

We have shown that the bilayer structure in the low temperature region depends on the repulsion between the lipid head groups. By tuning this parameter, we can obtain both the gel phase $L_\beta$ and the interdigitated gel phase $L_{\beta 1}$. Experimentally, both in the liquid crystalline phase [69] and in the gel phase [70], a monotonic increase of the area per lipid is observed when the temperature is increased. This is caused by an increase in the disorder of the tails [69]. For the low repulsion parameter of $a_{hh} = 15$ we reproduce the experimental observed trends. It is worth mentioning that, in most cases, in the gel phase the phospholipid chains are tilted with respect to the bilayer normal [9]. While for single tail lipids we do not observe any tilt, we will see in the next chapter that the double tail lipids are tilted in the gel phase ($L_\beta$ phase).

**Phase behavior as a function of head-head repulsion**

It is now interesting to do a more systematic study of these phase transitions for a range of repulsion parameters. The phase transitions we consider are:

1. transition from interdigitated gel to gel ($L_{\beta 1} \rightarrow L_\beta$)
2. transition from interdigitated gel to liquid crystalline ($L_{\beta 1} \rightarrow L_\alpha$)
3. transition from gel to liquid crystalline ($L_\beta \rightarrow L_\alpha$).

As we have shown, the first transition is induced by a decrease in the repulsion parameter $a_{hh}$, while the latter ones are temperature dependent.
We use three quantities to distinguish among the different phases: the area per lipid $A_l$, the extent of tail overlap $D_{\text{overlap}}$, and the ordering of the tails $S_{\text{tail}}$. By studying the behavior of these quantities as function of temperature and head-head repulsion parameter we can determine the phase diagram of $ht_c$ as shown in figure 3.3.

In figure 3.12 we plot the area per lipid $A_l$, the extent of tail overlap $D_{\text{overlap}}$, and the chain order parameter $S_{\text{tail}}$ as function of temperature and head-head repulsion parameter. For repulsion parameters $a_{hh} \leq 18$, the low temperature phase is the bilayer gel $L_\beta$ phase, while for repulsion parameters $a_{hh} > 18$, the low temperature phase is the interdigitated gel $L_{\beta 1}$.

By increasing temperature all bilayers melt from an ordered into a disordered phase. For bilayers in the $L_\beta$ phase, the area per molecule and chain overlap increase upon melting, while for bilayers in the $L_{\beta 1}$ phase the area per molecule and chain overlap decrease.

The curves in figure 3.12(c) show that the transition from an ordered phase to a disordered one is very gradual. Much larger systems might be required to observe
3.3.2 Phase behavior as a function of tail length

Besides investigating the effect of changing the head-head repulsion parameter, it is also interesting to vary the tail length of the lipid. A similar analysis, as was presented for the lipid h_{t9}, has been carried out for lipid types h_{t6}, h_{t7}, and h_{t8} (see figure 3.13).
3.3 Results and Discussion

Depending on the repulsion parameter we obtain two gel phases $L_{\beta 1}$ and $L_{\beta}$ for all tail lengths. For high head-head repulsion the system can gain energy by adding water particles in between the heads. As a result the distance between the head groups increases and the interdigitated phase is stabilized. For low values of $a_{hh}$ the head groups expel water and the stable phase is the non-interdigitated phase. In between we find $a_{hh}^*$ for which the transition from $L_{\beta 1}$ to $L_{\beta}$ occurs. The difference between the two phases is that in the $L_{\beta 1}$ phase the tail ends are in direct contact with water, whereas in the $L_{\beta}$ phase the tail ends face each other. Therefore, the critical value $a_{hh}^*$ to induce interdigation is higher than the value of $a_{hw}$.

We observe hysteresis if we change $a_{hh}$ at a constant temperature: the bilayer can be both in the $L_{\beta}$ or in the $L_{\beta 1}$ phase, depending on the initial dimension of the area. The range of $a_{hh}$, in which hysteresis occurs, increases with decreasing temperature (see figure 3.14). This suggests that the transition $L_{\beta}$ to $L_{\beta 1}$ is a first order transition. In the phase diagrams of figure 3.13 we define the phase found during decreasing temperature at a constant head-head repulsion parameter as the stable phase.

As we increase the tail length the gel phases are stabilized and the transition shifts to higher temperatures. The effect of increasing the head-head repulsion on the gel to liquid crystalline transition temperature is much more pronounced for the $L_{\beta} \rightarrow L_{\alpha}$ compared to $L_{\beta 1} \rightarrow L_{\alpha}$. This can be understood from the fact that in the interdigitated phase the average distance between the heads is already much larger compared to the non-interdigitated phase, and a further increase in this distance does not have a dramatic effect on the stability of the gel phase.

For lipids $ht_8$ and $ht_9$ the $L_{\beta 1}$ phase occurs at slightly lower repulsion parameters than for lipids $ht_6$ and $ht_7$. This is consistent with experimental results [66]. Since the interdigitated phase is more closely packed than the non-interdigitated phase, the vanderWaals energy is greater. This energy gain is proportional to the number of carbon atoms in the phospholipid chain and thus interdigation becomes energetically more favorable for longer chains. Also in our simulations we observe that the interdigitated phase is more compact and hence $a_{hh}^*$ decreases slightly with increasing tail length.

It is interesting to compare these results with the experimental data [71]. Misquitta and Caffrey systematically investigate the phase diagrams of monoacylglycerols, a single-tail lipid, and show a similar tail length dependence for the $L_{\beta} \rightarrow L_{\alpha}$ transition. Interestingly, for a similar model of a double-tail lipid we do not observe the formation of an interdigitated phase (see chapter 5). This corresponds to the experimental observation that for the most common double-tail lipids the interdigitated phase does not form spontaneously, but should be induced by the addition of, for example, alcohol [72] (see chapter 6).

The effect of adding salt on the gel to liquid crystalline transition has been studied
Figure 3.13: Phase diagrams as a function of the head-head repulsion parameter $a_{hh}$ and reduced temperature $T^*$ for lipids of different chain lengths: (a) $ht_6$ (b) $ht_7$ (c) $ht_8$, and (d) $ht_9$. 
for double-tail lipids [73] and recently for single-tail lipids [74]. These studies show that adding so-called kosmotropic salts increases the \( L_\beta \rightarrow L_\alpha \) transition temperature, while chaotrophic salts decrease this transition temperature. Similar effects have been observed for nonionic single-tail lipids [75]. Takahashi et al. [74] explain these observations by assuming that kosmotropes tend to be excluded from the interfacial region and hence reduce the amount of interfacial water, while chaotropic salts have the inverse effects, i.e. are adsorbed at the interfacial region and increase the amount of interfacial water. In our model a similar effect can be achieved by changing the head-head interactions; increasing or decreasing \( a_{hh} \) corresponds to adding chaotropes or kosmotropes, respectively. Our simulations show that decreasing the head-head repulsion stabilizes the \( L_\beta \) phase, which corresponds to the case that water is excluded from the interface. Adding chaotropic salts has the reverse effect: it increases the head-head repulsion and stabilizes the \( L_\alpha \) phase. Our simulations show that at sufficiently high head-head repulsion the interdigitated phase \( (L_{\beta 1}) \) is stable. This suggests that it experimentally might be possible to induce the \( L_\beta \rightarrow L_{\beta 1} \) phase transition by adding chaotropic salts to the systems.

3.4 Conclusions

In this chapter, we performed simulations on the most simple representation of a phospholipid. The model consists of a single hydrophilic head bead connected to one tail of hydrophobic beads, which can vary in length. Using the area per lipid, the hydrophobic thickness, the order parameter, and the extent of chain overlap, we are
able to characterize various bilayer phases.

The simulations showed that different stable phases are obtained for a wide range of temperatures. We characterized the low temperature phase as a gel phase, and we reproduced the main order/disorder phase transition from a gel to a liquid crystalline phase. This transition temperature to the $L_\alpha$ phase increases with increasing tail length, as was also found experimentally.

In bilayers consisting of single-tail lipids, only the non-interdigitated $L_\beta$ phase and the fluid $L_\alpha$ phase are observed. However, experiments show that if chaotropic salts are added to the system, the distance between the head groups is increased, stabilizing the $L_\alpha$ phase. We show that at high enough head-head repulsion the $L_\alpha$ phase is indeed stabilized and that the low temperature phase is the interdigitated $L_{\beta,1}$ phase. This suggests that it is possible to induce an interdigitated phase in bilayers of monotail lipids by adding chaotropic salts to the system.

From our results, we can conclude that a model consisting of a head bead connected to a single tail does not describe the phase behavior of a double-tail lipid correctly. The single-tail lipids spontaneously form a low temperature interdigitated phase for high enough values of the repulsion parameter between head groups. Experimentally, this phase is observed in bilayers consisting of double-tail lipids, but should be induced by adding salt to the system. By lowering the value of the head-head repulsion the low-temperature phase is the $L_\beta$ phase. In this phase the tails are ordered parallel to the bilayer normal, while for most common double-tail phospholipids the hydrocarbon tails show a tilt with respect to the bilayer normal (the $L_{\beta,1}$). An option to obtain the correct phase behavior is to make the model more complex by adding the detail of, for instance, two hydrophobic tails to a hydrophilic head group.