Phase transitions of lipid bilayers
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Introduction
1.1 The cell membrane

The cell is the most important unit in life. In 1665 Hooke observed that the tissue of a cork plant was divided into tiny compartments, which he called cells (*cellulae* means rooms). In 1840 improved observations on many tissues led to the hypothesis that all organisms exist either as single cells or aggregates of cells. More than a century of study later this hypothesis was confirmed. Moreover, there is no relation between the size of the cells and the size of an organism. All cells are of about the same size; a larger organism has just more cells than a small one.

An (eukaryotic) cell can be regarded as a factory, in which different processes take place. The cell is surrounded by the so-called cell membrane, in which the cytosol, a semi fluid, is contained (see figure 1.1) \[1,2\]. In this cytosol the organelles are located: membrane-surrounded structures in which specialized functions are carried out. The major organelles are the nucleus, the mitochondria, the endoplasmic reticulum and the Golgi complex. Most cells of higher organisms are specialized in function, for example in the production and the export of one or a few molecular products. However, despite their different function, they are all composed of the same kinds of lipids, proteins, nucleic acids, and polysaccharides.

![Figure 1.1: Schematic overview of an eukaryotic cell with the most common organelles of both plants and animals](image)

The membranes play an important role in the protection of the cell or organell from its surroundings. The membrane, however, is more than just a protective wall. It contains highly selective gates (proteins), that regulate the transport of nutrients in certain directions. In this way, a cell or organell can create its own environment and thus it can perform its specific function.

The common representation of a membrane of an eukaryotic cell is known as the Fluid Mosaic Model, that was proposed by Singer and Nicolson (see figure 1.2) \[3\]. The essence of the model is that membranes are two-dimensional solutions of oriented (phospho)lipids and globular proteins. The basis of the membrane are the lipids that are arranged in a bilayer. This bilayer has a dual role: it is a solvent for the integral membrane proteins and it is a permeability barrier. Both the lipids and the
proteins can undergo lateral movement (in the plane of the bilayer) and rotational movement (around its molecular axis perpendicular to the membrane). A small proportion of the lipids interact specifically with some membrane proteins and may thus be essential for the function of these proteins.

![Figure 1.2: The Fluid Mosaic model of a cell membrane: a mosaic of numerous protein molecules dissolved in a fluid bilayer of lipids](image)

## 1.2 Lipids and lipid bilayers

From the previous section it is clear that the lipid bilayer plays an important role in the cell membrane. Before discussing the properties of a bilayer, we will first look at a lipid, the building block of a bilayer, in more detail.

Lipids are so-called amphipathic molecules, meaning that one molecule contains both a polar, hydrophilic ("water-loving") head group, which tends to associate with water, and one or more hydrophobic ("water-fearing"), water repelling, tails. Due to this amphipathic character, lipids associate together in water, a process called self-assembly. The hydrophobic parts stick together, while the hydrophilic head groups are in contact with water. Dependent on the shape of the lipid and the concentration of lipids in water, different structures can form (see figure 1.3) [4–7], of which the lipid bilayer is a particular one. If the head group is large with respect to the hydrophobic part, the lipids will form a micelle: a globular structure in which the head groups are surrounded by water and the hydrophobic tails are sequestered inside. The opposite is the formation of the inverse micelle, formed if the tails are bulky and the head group is relatively small. The third and most important structure in biology is the lipid bilayer. The lipids are comprised of a large head group and mostly two hydrophobic tails. This yields a roughly cylindrical molecule, which can easily pack in parallel to form extended sheets. The various structures can transform from one to another by changing the solution conditions such as the electrolyte concentration, the pH, or temperature.

The formation of lipid bilayers is a rapid and spontaneous process, with the hydrophobic interactions as the main driving force (i.e. the hydrophobic effect). Water
molecules are released from the hydrophobic tails as these tails become sequestered in the interior of the bilayer. Additionally, the vanderWaals attractive forces between the tails favor a close packing. And finally, the lipid bilayers are stabilized by the electrostatic interactions and the formation of hydrogen bonds in the head group region [2, 8].

The most abundant lipids in biological membranes are the phospholipids. In figure 1.4 a schematic drawing of a phospholipid is given, together with an example. The backbone of a phospholipid is glycerol. To this glycerol unit two hydrocarbon tails, derived from the fatty acids, are connected. These fatty acid chains contain an even number of carbon atoms, typically between 12 and 24, of which the 16, and 18 carbon fatty acids are the most common ones. The tails can be both saturated or unsaturated, meaning that one or more double bonds between carbon atoms are present. At the remaining carbon atom of the glycerol the hydrophilic head group is attached. This head group consists of a phosphate group and an alcohol group. Different alcohols lead to a variation in head groups and thus a variation in properties of the lipid bilayer.

Figure 1.3: Self-assembly of lipids in water gives different structures, dependent on the molecular structure of the lipid: (a) micelle, (b) inverse micelle, and (c) bilayer. The filled circles represent the hydrophilic head groups of the molecules and the wavy lines the hydrophobic tails.

Figure 1.4: Schematic drawing of the components of a phospholipid (left) and the phospholipid DiMyristoylPhosphatidylCholine (right). 'Gly' denotes the glycerol group.
Lipids and lipid bilayers

Figure 1.5: Schematic representations of the lipid bilayer phases during a heating process. \( T_{m_1} \) and \( T_{m_2} \) denote the pretransition and the main transition, respectively.

The most common phospholipids are the PhosphatidylCholines (PC's), in which the alcohol group is a choline (see the example in figure 1.4). The bilayers of these lipids undergo three phase transitions within the temperature range of 10°C to 80°C [9]. In figure 1.5 these phases are drawn in order of increasing temperature. The lowest temperature phase is the \( L_c \) phase, also called the subgel phase, which transforms to the gel phase or \( L_{\beta'} \) phase upon heating. In both phases the hydrocarbon tails are tilted with respect to the bilayer normal, but in the \( L_{\beta'} \) phase the head group is more hydrated (i.e. surrounded by water). The transition from the \( L_c \) phase to the \( L_{\beta'} \) phase is called the subtransition. Increasing temperature further leads to the formation of the the rippled (\( P_{\beta'} \)) phase. The transition is called the pretransition and in this \( P_{\beta'} \) phase, the bilayer is not flat, but corrugated. Finally, the bilayer undergoes the transition to the liquid crystalline or fluid \( L_\alpha \) phase, which is called the main transition or the chain order/disorder transition. In this phase, the bilayer is a two-dimensional fluid, meaning that the lipids are free to move in the plane of the bilayer. The hydrocarbon chains become disordered and therefore the transition to the \( L_\alpha \) phase is regarded as the melting of the bilayer.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>( n_C )</th>
<th>( T_{m_1} )</th>
<th>( T_{m_2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>14</td>
<td>15.3</td>
<td>24.0</td>
</tr>
<tr>
<td>DPPC</td>
<td>16</td>
<td>35.5</td>
<td>41.5</td>
</tr>
<tr>
<td>DSPC</td>
<td>18</td>
<td>51.0</td>
<td>54.3</td>
</tr>
<tr>
<td>DAPC</td>
<td>20</td>
<td>62.1</td>
<td>64.1</td>
</tr>
</tbody>
</table>

Table 1.1: Pretransition (\( T_{m_1} \)) and main transition (\( T_{m_2} \)) temperatures (in °C) of various PhosphatidylCholines (PC's) dependent on the number of carbon atoms in the hydrocarbon chains \( n_C \) [10].

The various transition temperatures are characteristic for the lipid of which the bilayer consists. The pre- and main- transition temperatures increase with increasing tail length of the lipid (see table 1.1), but decrease with increasing head group hydration and unsaturation of the alkyl chains. In the latter case the transition temperatures are not only influenced by the number of double bonds in the chains, but also by position. In case of a pure phospholipid bilayer the transition to another phase is very sharp and takes place over a temperature range of 0.8 – 1.5°C. How-
ever, this temperature range varies in the presence of cholesterol, proteins, cations, or small molecules that interact with the bilayer.

The nature of the head group and the presence of small molecules interacting with the bilayer do not only influence the transition temperatures, but also the structure of the low temperature phases of the bilayer. If the head group is small, the stable phase is a gel phase ($L_\beta$), in which the tails do not show a tilt with respect to the bilayer normal (see figure 1.6(a)). If small amphiphilic molecules are added to the bilayer, the low temperature phase is the interdigitated $L_{\beta 1}$ phase. In the interdigitated phase, the terminal methyl groups of the lipid chains of two opposing layers do not face each other, but are located near the head group region of the opposing layer (figure 1.6(b)).

![Figure 1.6: Schematical representation of (a) the $L_\beta$ phase and (b) the $L_{\beta 1}$ phase.](image)

Let us now return to the cell membrane. As explained, the basis of the cell membrane is the lipid bilayer, in which the hydrophilic head groups stick into the water and the hydrocarbon tails are sequestered in the middle. Not only the lipids are amphiphilic, but also the proteins in the cell membrane have a hydrophilic and a hydrophobic region. The organization of the lipids around the proteins plays a crucial role in the functioning of the proteins. If this organization changes due to changes in composition, temperatures or additives to water, the lateral pressure on the different parts on the protein will change [11] or the hydrophobic part of the protein will be exposed to water (the so-called "hydrophobic mismatch"), causing the disfunctioning of the protein. Therefore, knowledge of the behavior of lipid bilayers is very important for our understanding of the functioning of the cell.

### 1.3 Computer simulations

In recent years many experimental techniques, such as X-ray crystallography, electron microscopy, infra-red and Raman spectroscopy, have been developed to characterize the structure of a membrane. Despite these developments, the precise functioning of membranes is still not well understood [12]. Therefore, a better characterization of the (phase) behavior of lipid membranes and the interaction between lipids and proteins is needed. This insight can be gained by performing computer simulations on detailed atomistic models based on realistic microscopic interactions.
The most used method to simulate biological systems, like lipid membranes, at an atomistic scale, is Molecular Dynamics (MD). In this method all interactions between the individual atoms are taken into account. Let us take one of the simplest systems, a bilayer of DMPC (see figure 1.4), as an example. To calculate the properties of a fully hydrated bilayer, 64 lipids and 1645 water molecules are needed [13], which gives a total of 12,487 atoms. For all these atoms both the intramolecular interactions (bonds, angles, dihedral angles) and the intermolecular interactions (van-der-Waals forces, hydrogen bonds, electrostatic interactions) must be described and calculated in every time step. This costs a lot of CPU time and thus these MD simulations are restricted to a small part of the bilayer (the length scale) and 1 or 2 nanoseconds (the time scale). Recently the progresses in computational techniques and the increased power of computers have allowed to reach time scales of 100 nanoseconds [14,15], but there are still various phenomena that occurs at longer time and length scales. These time and length scales are still not reachable by all-atom simulations and therefore other methods have been developed.

Although an all-atom model is seen as the realistic description of a biological membrane, such a model assumes that the quantumchemical nature of the interactions is not essential for our understanding of some of the properties of a membrane. Similarly, one can assume that some of the atomic details can be ignored, while preserving the essential aspects of the molecular structure. In such a mesoscopic approach, clusters of atoms are replaced by spheres, which are connected by harmonic springs. A commonly used approach is to apply MD simulations, in which the interactions between the clusters of atoms are described by a Lennard-Jones potential [16–20]. An alternative approach is to use Dissipative Particle Dynamics, which is more efficient compared to MD for these models.

Dissipative Particle Dynamics (DPD) was originally developed to simulate complex fluids. A fluid particle is to be understood as "being very small compared with the volume of the body under consideration, but large compared with the distances between the molecules" [21]. In many DPD studies, a particle represents three water molecules or three methylene (CH$_2$) groups. Between the particles, there is a soft repulsive potential, in which the characteristics of water and the lipids are described. All particles repel each other, but the repulsive force between, for example, two water particles will be less than the repulsion between a water particle and an oil particle. In this way, a phase separation between oil and water is obtained. In case of a lipid, we end up with a set of repulsion parameters, describing the interactions between the hydrophilic particles, hydrophobic particles and water. The advantage of this technique is that due to the coarse graining much larger length and time scales can be reached. Compared to molecular dynamics simulations on an all-atom system, DPD on such a coarse-grained model can be 4 to 5 orders of magnitude more efficient [16,22]. This gain in CPU time allows us to study longer time and length scales.
than is possible with MD on an all atom model.

1.4 This thesis

Since the structure of the lipid bilayer plays a crucial role in the functioning of the cell, it is interesting to study the properties and the behavior of lipid membranes using computer simulations at longer time and length scales than has been achieved with Molecular Dynamics. Dissipative Particle Dynamics seems to be a very promising method to simulate such bilayers. However, DPD is a relatively new simulation technique and till now, only a few investigations on lipid membranes have been published [22–26].

The aim of this thesis is twofold. First of all, we investigated how much detail should be added to a mesoscopic model of a lipid to reproduce the experimental observations. Questions that arise are, for instance:

- Is it sufficient to have a representation of a lipid, in which the model only contains the basic features of a lipid or should the model be more detailed?
- Related to the previous question: what level of coarse-graining is needed? Should a DPD particle represent one, two, three, or even more water molecules?
- Which set of repulsion parameters should be used and what happens if we change some of the parameters?

Once we have optimized the model, the logical next step is to investigate the properties of a lipid bilayer, which are difficult to answer experimentally. Examples of some experimental questions, that we try to answer in this thesis are:

- The formation of the rippled phase during the heating and cooling process of a lipid bilayer. As can be seen in figure 1.5, the plane of the bilayer in the gel phase and the fluid phase is flat, while in the rippled phase it is corrugated. Since more than 30 years the appearance of this ripple has been puzzling. Why does the ripple appear? What does it look like at a molecular scale? Is the swelling of the bilayer at the main transition coupled to the appearance of the rippled phase?
- It is proposed that the formation of an interdigitated phase is caused by an increased distance between the lipid head groups. This can be achieved in various ways: by changes of the chemical structure of the lipid and by the addition of salts or small amphiphilic molecules, like alcohols or anesthetics, to the bilayer. One may wonder whether the increase in separation of the head groups in itself is sufficient to cause interdigitation. Experimentally this question is difficult to answer, because it is hard to isolate a single cause and effect relation. In silico this is however quite easy as we can turn interactions on and off at will. In case of the addition of small amphiphilic molecules to the bilayer, we
are able to directly count the molecules involved in the interdigitation, which is not possible experimentally.

In chapter 2, we give a description of the simulation technique applied, the model, and the structural quantities of a bilayer, needed to characterize the different phases. With these definitions, we investigate the phase behavior of the most simple model of a lipid, that consists of one hydrophilic head bead connected to a single hydrophobic tail in chapter 3. From the results obtained with this model, we can predict the conditions under which interdigitation in a bilayer consisting of monotail lipids can be induced. In chapter 4 we address the question of the choice of repulsion parameters and of which level of coarse graining is needed to reproduce the experimental characteristics of a bilayer consisting of double tail lipids. We find that a model consisting of a head group of three hydrophilic beads connected to two hydrophobic beads gives the best result. With this model, we study the phase behavior of a lipid bilayer in chapter 5. By changing the interaction between the head groups of the lipid, we observe the formation of various phases and special attention is given to the formation of the rippled phase. In the last chapter we return to the induction of the interdigitated phase, but now in a bilayer containing double tail lipids. Interdigitation is induced in two ways: 1. by changes in the chemical structure of the lipid head group, and 2. by the addition of small amphiphilic molecules to the bilayer.