Chapter 1

Introduction

During evolution, nature has developed an intriguing variety of complex organisms containing a broad variety of molecular structures. One major class of components allowing the existence of life are lipids. Several interesting molecular entities in lipids play thereby a crucial role allowing the survival of organisms under harsh conditions like extreme temperatures and pressure. Also, the viability in toxic environment is enabled through increased density and decreased permeability of the membrane provided by the constituting lipids. Nowadays, synthetic lipids are applied for the understanding and mimicking of the naturally developed systems allowing the creation of artificial membranes with impressive properties. Moreover, those amphiphilic structures are applied for medical purposes as well.
1.1 Composition and Physiological Properties of Bio-Membranes

1.1.1 Lipid: Properties and Classification

The formation of compartments, namely cells and organelles, through bio-membranes is required to enable the existence of living organisms. This compartmentalization allows the formation of concentration gradients, protection of the interior and can result in enrichment and active transport.\(^1\) As shown in Figure 1.1.1a, the major components forming the membrane are proteins and lipids. Thereby, the latter compounds enable the stability and high flexibility at the same time. To fulfill these requirements, noncovalent interactions between membrane constituents are present. The chemical architecture of lipids comprises a hydrophilic head group and a hydrophobic tail resulting in the amphiphilic character (Figure 1.1.1b).\(^2\) Whereas the head group is pointing towards the aqueous medium, the hydrophobic tail undergoes clustering by hydrophobic interactions.\(^2\) As a result of those interactions lipid bilayers can be formed (Figure 1.1.1a).

Lipids can be divided in three major classes.\(^3,4\) The first kind of lipids is represented by phospholipids which contain a phosphate ester unit. As shown in Figure 1.1.2, this lipid class has two members exhibiting different lipid backbones: glycerophospholipids carrying glycerol and sphingophospholipids carrying a sphingosine moiety.\(^3,5\) These molecular entities serve as connection between the aliphatic tail and the polar phosphate ester group. Furthermore, additional polar residues at the phosphate unit are increasing the hydrophilic property of the head group. Common substituents are serine, ethanolamine and choline which are charged

![Figure 1.1.1: (a) Schematic representation of the bio-membrane. (b) Schematic representation of a lipid.](image-url)
under physiological pH (pH = 7.4) (Figure 1.1.2). The hydrophobic tail consists mainly of long hydrocarbon chains which can carry a variety of highly interesting moieties (see Chapter 1.1.2). Furthermore, dependent on the organism two different chemical connections have been observed tying the aliphatic chain on the lipid backbone, namely ester or ether groups. The more common esters are mainly found in eukaryotes and bacteria. In contrast, ether bonds are typically present in archaeal membranes.

Figure 1.1.2: Chemical structure of phospholipids.

The second class of lipids is represented by glycolipids. These sugar-containing lipids possess mono- or oligo-saccharides as hydrophilic head group which are directly connected to the lipid backbone. As shown in Figure 1.1.3, one distinguishes between glyceroglycolipids and sphingoglycolipids. These amphiphiles are generally located at the outer surface of the cell membrane reaching into the extracellular fluid with the carbohydrate unit (Figure 1.1.1a).

Figure 1.1.3: Chemical structure of glycolipids carrying glucose as representative head group moiety.

The third lipid class is represented by sterol derivatives which incorporate several cycloalkane moieties. These lipids are present to a large extent in eukaryotic membranes (20 to 40%), whereas they are absent in prokaryotes. The structure of the most common amphiphilic sterols are shown in Figure 1.1.4. It should be noted that cholesterol 1 is present in animals, ergosterol 2 is found in fungi and β-sitosterol 3 in plants.

Figure 1.1.4: Chemical structure of cholesterol 1, ergosterol 2 and β-sitosterol 3.
1.1.2 Intriguing Aliphatic Acids and Alcohols in Nature

In nature the hydrophobic moiety of lipids is mostly represented by aliphatic alcohols or acids. The most common fatty acids contain an aliphatic chain consisting of an even number of 14 to 24 carbon atoms (Figure 1.1.5). Thereby, unsaturation occurs resulting in up to four double bonds per chain. These mostly cis-configured bonds result in a bended tail as shown for stearic acid in Figure 1.1.5.

![Figure 1.1.5: Molecular structures and space filling molecular models of three C₁₈-fatty acids, namely stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). (Number of carbon atoms: number of double bonds).](image)

The most common aliphatic alcohols present in the archaeal lipids contain 20, 25 or 40 carbon atoms. In contrast to fatty acids, no double bonds are present in those aliphatic compounds. Instead methyl branched chains in repetitive units are common (Figure 1.1.6).

![Figure 1.1.6: Molecular structure and space filling molecular model of common archaeal aliphatic alcohols.](image)

It was suggested that the molecular structure of the hydrophobic tail can influence significantly the stability and permeability of bio-membrane. Archaea which incorporate a high degree of methyl branched lipids and the more stable ether linkage can survive at extreme temperatures without showing significant increase in membrane permeability. In contrast, the membrane permeability of eukaryotes and bacteria is often strongly influenced by temperature changes. However, to enable bacteria to survive harsh conditions, e.g. high temperatures, low pH and high salt concentration, different modifications of the fatty acids occurred during evolution. In this regard, in 1929 Anderson et al. isolated the methyl branched fatty acids tuberculostearic acid 4 (10-methylstearic acid, Figure 1.1.7a) from *Mycobacterium tuberculosis*. The structure of this acid was established in 1934 by Spielman followed by determination of the absolute configuration in 1948 by Prout et al. First approaches studying the biosynthetic pathway revealed that the methylation of the double bond of oleic...
Introduction

Acid resulted in fatty acid 4. Further studies showed that an enzymatic reaction mechanism which is involved in a variety of double bond modifications catalyzes methylation, cyclopropanation and double bond migration. During the following decades a large variety of fatty acids carrying not only non-polar methyl branched moieties but also polar methoxy- and ketone units were isolated from Mycobacteria. Fatty acids with those polar functionalities are known as mycolic acids and some representative compounds are shown in Figure 1.1.7b and c.

Figure 1.1.7: (a) Molecular structure of tuberculostearic acid 4. (b) General molecular structure of mycolic acids 5. (c) Molecular structure of an α-mycolic acid 6.

After the discovery of cyclopropane moieties in mycolic acids 5 more naturally occurring cis- and trans-cyclopropane-containing fatty acids (CFAs) have been found. One representative of the more common cis-isomer is lactobacillic acid 7, which was discovered in 1950 by Hofmann et al. (Figure 1.1.8). Also the trans-cyclopropane ring was found in several fatty acids like lyngbyoic acid 8, grenadadiene 9 and majusculoic acid 10, which were isolated from cyanobacteria. The latter one was isolated by MacMillan et al. revealing the presence of a cyclopropane moiety and a brominated olefinic moiety (Figure 1.1.8).

Figure 1.1.8: Molecular structures of lactobacillic acid 7, lyngbyoic acid 8, grenadadiene 9 and majusculoic acid 10.

Other molecular architectures like modifications carrying cycloalkane moieties have been found in several membrane components. Unique examples are the ladderane lipids.
In 2002 Sinninghe Damsté et al. discovered these lipids which are carrying three to five linear fused cyclobutane rings at the hydrophobic tail of the fatty acid. As shown in Figure 1.1.9, ladderane lipids incorporating a cyclohexane ring were described as well. At this point it is noticeable that not only the for bacteria common ester bonds are present in ladderane lipids. Instead many aliphatic alcohols are incorporated resulting into ether linkages.

![Molecular structures of fatty acids and alcohols containing ladderane units.](image)

Furthermore, it needs to be mentioned that cycloalkane moieties are also found in aliphatic alcohols that are present in archael membranes. As shown in Figure 1.1.10, several cyclopentane and occasionally also cyclohexane moieties can be present.

![Molecular structures of aliphatic alcohols occurring in archael membranes.](image)

1.1.3 Lipids: The Key for Survival

The above discussed broad range of lipids has been found in several organisms like bacteria, eukaryotes and archaia. These organisms have in common that they are able to survive under extreme conditions. Thereby, even direct adaptation to environmental changes has been observed.

The multiplicity of modifications has a direct influence on the physiological properties of the membrane and the phase transition states, permeability, molecular packing, fluidity and density of the membrane can be varied. This response to the environment can result in higher chemical tolerance and stability of the organism.

For example, some bacteria developed the ability of double bond isomerization. Studies demonstrated that the amount of trans-unsaturated lipids increases under influence of organic...
solvents or higher temperatures resulting in lower fluidity of the membrane. Other modifications are important to influence the packing behavior resulting in an increase of lipid density and the dependent permeability. Also, it was shown that modifications can lead to an increase of the chemical stability of the membrane without influencing the physiological properties considerably.

A representative example are mycolic acids which are able to influence the membrane properties. For instance, the presence of fatty acids in the cell wall of mycobacteria reduces the permeability for small hydrophilic compounds up to 10.000-fold in comparison to Escherichia coli. Hence, cell walls containing mycolic acids represent a great barrier against antibiotics like β-lactams and aminoglycosides. Furthermore, it reduces the vulnerability by chemicals.

Additionally, it has to be emphasized that the composition of the cell wall, in particular the degree of cis/trans isomerization, is directly influenced by the increasing temperature resulting in a direct adjustment of the affiliated fluidity. The same correlation was shown for other bacteria like Pseudomonas. The thermal adaptation is required to maintain the viscosity at low temperature and simultaneously increasing the stability at higher temperatures by controlling the cis/trans ratio of double bonds and cyclopropyl moieties.

Furthermore, it has been shown that not only the temperature can influence the membrane composition. In 1965, Knivett et al. demonstrated already that changes in pH, temperature, carbon source, salt concentration and air composition can influence the amount of cyclopropane fatty acids (CFA) in the membrane. Also, it was reported that cell cultures growing at low pH, high temperatures and in presence of an oxygen rich environment incorporate a larger amount of CFAs. Several studies trying to understand the role of cyclopropane units in lipids report contradictory results. However, it was clearly shown that the cyclopropyl unit exhibits lower reactivity compared to olefinic moieties resulting in higher stability of the membrane.

In this regard, it was shown that cyclopropanes are chemically inert against oxidation reagents like hydrogen peroxide and ozonolysis. Hence, through the presence of cyclopropyl moieties an increased chemical tolerance is observed without changing the physiological properties of the membrane. Furthermore, it was reported that the presence of organic solvents like toluene results in a change of the degree of cycloproponation.

A further unique modification of membranes is the incorporation of ladderane fatty acids exhibiting several linear fused cycloalkane moieties. It is suggested that the presence of these molecular entities results in an increase of the density of the bio-membrane. The increased density prevents diffusion of small molecules through the membrane and therefore, enables the surviving of the organism in a toxic environment.

Despite that the reason for the presence of the large variety of molecular moieties is not fully understood, studies clearly reveal a relation between the degree of the change of membrane composition and changes of the environment.
1.2 Lipid Aggregation in Aqueous Media

The ability of amphiphilic compounds, such as lipids, to aggregate in aqueous media driven by non-covalent interactions has been studied intensively during the last forty years. First attempts to understand the parameters that effect aggregation and to find general rules predicting the resulting aggregate have been published by Israelachvili et al. and Tanford. Tanford considered mainly hydrophobic effects, repulsive interactions and thermodynamic factors which all together result in micelle formation and a lower chemical potential for the amphiphile incorporated in the assembly, in comparison to the free amphiphile in solution. In this regard, the formation of spherical micelles is energetically preferred in comparison to aggregates like vesicles and sheets. At around the same time Israelachvili et al. demonstrated the relationship between the molecular structure, size and shape of the amphiphile as well as non-covalent interactions. To explain the existence of thermodynamic disfavored aggregates he introduced a simple concept defining a packing parameter based on geometric and mathematic relations. As shown in Figure 1.2.1, the packing parameter \( P \) is dependent on the length \( l_c \), volume \( v \) of the hydrophobic tail and the surface area \( a_0 \). Thereby, \( P \) is defined as \( v/a_0 l_c \). It has to be emphasized that the surface area \( a_0 \) is strongly depended on charge repulsion effects, resulting in an effective area larger than the physical size of the head group.

\[
\text{equation 1: } \frac{V}{v} = \frac{A}{a_0} = N
\]
\[
\text{equation 2: } r \leq l_c
\]
\[
\text{equation 3: } P = \frac{v}{a_0 l_c} \leq \frac{V}{A r}
\]

Figure 1.2.1: Schematic representation of an amphiphile and its parameters: length \( l_c \), surface area \( a_0 \) and volume \( v \).

In order to assign values for the packing parameter Israelachvili et al. treated different aggregates as simple geometrical structures like sphere, cylinder and cuboid for micelles, wormlike micelles and bilayer, respectively. However, further assumptions were necessary to apply basic mathematic relations. Therefore, the total volume \( V \) of an aggregate was introduced and was defined by the volume \( v \) of one amphiphile multiplied with the number of amphiphilic molecules \( N \) present in an assembly (equation 1). The same relation was assumed for the total surface area \( A \) (equation 1). Furthermore, the radius \( r \) of an aggregate has to be smaller or equal to the total length of the unfolded hydrocarbon chain \( l_c \) of one amphiphile (equation 2). Combining equation 1 and 2 with the above mentioned definition of the packing parameter \( P \) result in equation 3 allowing the determination of \( P \) (Figure 1.2.3).
Additionally, as shown in Figure 1.2.2 the total surface area $A$ can be further simplified, since it is highly dominated by the larger surfaces of the assembly. As examples the simplified surface $A$ of sheets and wormlike micelles are shown in Figure 1.2.2.

\[
A = 2ab + 2bc + 2ac \\
2ac \approx 2bc \ll 2ab \\
\Rightarrow A \approx 2ab
\]

\[
A = 2\pi rh + 2\pi r^2 \\
2\pi r^2 \ll 2\pi rh \\
\Rightarrow A \approx 2\pi rh
\]

**Figure 1.2.2:** Simplified equation to determine the surface area $A$ of a thin cuboid and a long cylinder.

Having in mind equation 1, the packing parameter of aggregates can be calculated for each individual amphiphilic molecule. Hence, now a relation between amphiphile shape, packing parameter and assembly can be drawn. As shown in Figure 1.2.3, different aggregates can be expected dependent of the shape of the molecule.

<table>
<thead>
<tr>
<th>$V = Nv =$</th>
<th>$V = Nv =$</th>
<th>$V = Nv =$</th>
<th>$V = Nv =$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$= (4/3)\pi r^3$</td>
<td>$= \pi r^2 h$</td>
<td>$= ab2r$</td>
<td>$= \pi r h$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$A = Na_0 \cong$</th>
<th>$A = Na_0 \cong$</th>
<th>$A = Na_0 \cong$</th>
<th>$A = Na_0 \cong$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$= 4\pi r^2$</td>
<td>$= 2\pi rh$</td>
<td>$= 2ab$</td>
<td>$= 2\pi rh$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$P = v/a_0 c$</th>
<th>$P = v/a_0 c$</th>
<th>$P = v/a_0 c$</th>
<th>$P = v/a_0 c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq 1/3$</td>
<td>$\leq 1/2$</td>
<td>$\leq 1$</td>
<td>$\leq 1$</td>
</tr>
</tbody>
</table>

**Figure 1.2.3:** Illustration of the relationship between geometrical structures, calculated packing parameter, amphiphile shape and aggregates. Figure adapted from literature.37,39

Micellar phases are dominantly formed by single tailed amphiphiles and lamellar phases are preferably consisting of double tailed compounds. However, parameters like temperature, salt concentration and chain composition have a large influence on the observed aggregates. For instance, with increasing temperature more flexibility is expected for the hydrophobic tail
which would result in an increased packing parameter. In contrast, an extension of the chain would result in higher transition temperatures reducing $P$. Also, the addition of counter ions could lead to a variety of results. An example is the presence of positively charged ions like Ca$^{2+}$ which can have a reducing effect on the charge repulsion of negatively charged head groups.

It can be concluded that the above described packing parameters have to be used with caution. Certainly, they can give an estimation which aggregate is to be expected, but packing parameters do not include surrounding influences.

1.3 Application of Amphiphilic Compounds during Everyday Life

The knowledge obtained studying the phenomena observed in nature can be applied for a variety of different materials. Thereby, fatty acids can be found during daily life for example in cleaning reagents, cosmetic products, food supplements and drugs. The amphiphilic character of fatty acids results after mixing with aqueous media to a decrease of the surface tension of water and an increase of the solubility of hydrophobic compounds. These advantages were already recognized and used by ancient cultures like Babylonians and Egyptians. Simple recipes mixing animal fat or vegetable oil with alkaline salt in water resulted in soap-like mixtures which were used for cleaning, medical and cosmetic purposes. The first known scientific literature mentioning acid-soaps was published in 1823 by Chevreul. During the following decades Ekwall et al. and McBain et al. studied the acid-soaps described by Chevreul proving their existence and analyzing the stoichiometric ion composition.

Furthermore, it was observed that solutions incorporating surfactants are able to form foams in the presence of gas. Thereby, gas bubbles are surrounded by surfactant aggregates which are stabilized through noncovalent interactions. As shown in Figure 1.3.1, an orientation of the amphiphilic compound takes place wherein the hydrophilic head group is pointing to the polar aqueous phase and the hydrophobic tail is facing towards the apolar gas phase. This behavior is widely observed for different amphiphiles. Some well known examples observed in everyday life are soap foam, beer foam and fire extinguisher foam.
Another industrial application is the use of surfactant in emulsions. Here, amphiphiles are employed to increase the miscibility of hydrophobic material with water and hydrophilic compounds in oil. As shown in Figure 1.3.2, the minor compound is encapsulated by the surfactants resulting in an emulsion. This principle is widely applied in paint-, cosmetic-, food- and pharmaceutical-industry.

For most applications simple saturated and unsaturated fatty acids are sufficient whereas fatty acids, carrying a variety of modifications, are often applied during drug development. Studies using artificial membranes which incorporate molecules based on natural occurring fatty acids can lead to drug systems that overcome chemical resistance, increase stability and decrease permeability. Also they might results in controlled release mechanisms, allow influencing the circulation life times and give access to higher target selectivity. Thereby, the amphiphilic character of fatty acids enables their application as transport systems in aqueous media. An example are DNA carrier systems in which an aggregate complex (lipoplex, Figure 1.3.3a) between the negatively charged DNA phosphate backbone and a positively charged surfactant is allowing efficient transportation of DNA molecules into a broad range of eukaryotic cells (Figure 1.3.3b). Thereby, the lipoplex is preventing the DNA from degradation through nucleases and increases the half-time of DNA in vivo.
Figure 1.3.3: (a) Schematic representation of the lipoplex formation stabilized by electrostatic interactions. (b) Schematic representation of DNA transfer through endocytosis using an lipoplex as DNA-carrier. 1) Interaction of liposome with bio-membrane 2) Invagination of membrane 3) Endosome formation 4) Encapsulation of endosome carrying a liposome 5) Degradation of endosome. Figure adapted from literature.\textsuperscript{52}
1.4 Outline of this Thesis

The above mentioned unique features of amphiphiles allow the survival of different organisms under extreme conditions and in the presence of toxic chemicals. However, the low availability of most of these amphiphilic compounds isolated from natural sources and the often complicated cultivation process makes organic synthesis to an irreplaceable tool giving not only access to the desired molecule but also to a variety of derivatives.

Therefore, the first part of my thesis is focusing on the synthesis of natural fatty acids and their derivatives. In Chapter II and III, the synthesis of fatty acids carrying a hydrophobic tail containing linear fused cyclobutane rings is described. The in Chapter II via [2+2] photocycloaddition obtained building blocks are used in Chapter III giving access to ladderane fatty acids. Furthermore, a brominated, unsaturated fatty acid carrying a cyclopropane moiety, namely majusculoic acid, is synthesized in Chapter IV. The synthesis described in this thesis is the first successful total synthesis of majusculoic acid. Key steps applied in this synthesis are selective reduction and isomerization of double bonds, ozonolysis and asymmetric cyclopropanation.

In the second part of this thesis a simplified lipid system containing only naturally occurring building blocks is reported. First, the optimized synthesis of N-phosphoamino acids composed of just three synthetic steps is reported in Chapter V. This efficient three-step synthesis is applied to obtain a collection of more than 15 amphiphilic acids. Subsequently, these amphiphilic compounds are used in Chapter VI for aggregation and biocompatibility studies. Finally, further modifications at the hydrophilic head group of N-phosphoamino acids is performed and their influence on the aggregation and biocompatibility is demonstrated in Chapter VII.
1.5 References


