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LIFE – AS A MATTER OF FAT

The Emerging Science of Lipidomics

Springer
To Myer Bloom, mentor in science and life
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The research work underlying the picture of fats, lipids, and membranes advocated in the present book derives from many different scientists and students in several laboratories across the world. The specific examples and data presented are however biased toward the work carried out by the author and his collaborators over the last almost twenty years. Therefore, the book should not be considered an authoritative monograph but more a personal perspective on a diverse and rapidly expanding field of science. The work has been supported by a number of public and private funding agencies, notably the Danish Natural Science Research Council, the Danish Technical Research Council, the Danish Medical Research Council via the Center for Drug Design and Transport, the Villum Kann Rasmussen Foundation, the Carlsberg Foundation, the Velux Foundation, the Hasselblad Foundation, and the Danish National Research Foundation. The author has over the years benefited from stimulating interaction and fruitful collaboration with a large number of colleagues and students, in particular from the Center for Biomembrane Physics (MEMPHYS) and from the Canadian Institute for Advanced Research’s program on the Science of Soft Surfaces and Interfaces under the directorship of Professor Myer Bloom. The author is greatly indebted to the following: Thomas Andresen, Luis Bagatolli, Rogert Bauer, Thomas Baekmark, Gerhard Besold, Rodney Biltonen, Thomas Bjørnholm, Myer Bloom, David Boal, Thomas Hønger Callisen, Robert Cantor, Bernd Dammann, Jesper Davidsen, Lars Duelund, Evan Evans, Sven Frøkjær, Tamir Gil, Henriette Gilhøj, Per Lyngs Hansen, Jonas Henriksen, Pernille Høyrup, John Hjort Ipsen, Ask Jacobsen, Morten Ø. Jensen, Claus Jeppesen, Kent Jørgensen, Thomas Kaasgaard, Daniellle Keller, Lars Kildemark, Dennis Kim, Paavo Kinnunen, Beate Klösgen, Per Knudsen, Mohamed Laradji, Chad Leidy, Jesper Lemmich, Ling Miao, Kell Mortensen, Mohan Narla, David Needham, Morten Nielsen, Jaan Noolandi, Adrian Parsegian, Tina Pedersen, Günther Peters, Amy Rowat, Jens Risbo, Mads C. Sabra, Erich Sackmann, Adam C. Simonsen, Maria M. Sperotto, Jennifer Thewalt, Christa Trandum, Ilpo Vattulainen, Peter Westh, Matthias Weiss, Michael Wortis, and Martin Zuckermann. Olaf Sparre Andersen is thanked for collaboration concerning a conference book on which part of Chap. 7 is based. Michael Crawford shared with me his insight into the relationships between lipids, nutrition, brain
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# Abbreviations

## Fatty acids
- **AA**: arachidonic acid
- **DHA**: docosahexaenoic acid
- **DPA**: docosapentaenoic acid
- **EPA**: eicosapentaenoic acid

## Lipid polar head groups
- **DGDG**: digalactosyl diglyceride
- **PC**: phosphatidylcholine
- **PE**: phosphatidylethanolamine
- **PG**: phosphatidylglycerol
- **PI**: phosphatidylinositol
- **PS**: phosphatidylserine

## Lipids
- **DAG**: di-acylglycerol
- **DAPC**: di-arachioyl PC
- **DCPC**: di-decanoyl PC
- **DLPC**: di-laureoyl PC
- **DMPC**: di-myristoyl PC
- **DMP**: di-myristoyl PE
- **DMPG**: di-myristoyl PG
- **DOPC**: di-oleoyl PC
- **DP**: di-palmitoyl PC
- **DPPE**: di-palmitoyl PE
- **DSPC**: di-stearoyl PC
- **DSP**: di-stearoyl PE
- **POPC**: palmitoyl-oleoyl PC
- **POPE**: palmitoyl-oleoyl PE
- **SM**: sphingomyelin
- **SOPC**: stearoyl-oleoyl PC

## Others
- **ATP**: adenosine triphosphate
- **DNA**: deoxyribonucleic acid
- **ER**: endoplasmic reticulum
- **HDL**: high-density lipoprotein
- **LDL**: low-density lipoprotein
- **RNA**: ribonucleic acid
- **ROS**: reactive oxygen species
Prologue:
Lipidomics – A Science
Beyond Stamp Collection

To most people, fats are something vicious that are dangerous to our health and well-being and therefore should be avoided. Some people know that fats are essential ingredients of the diet and, furthermore, provide for tasty meals. Others acknowledge that certain fats like cholesterol are required for the body’s production of important hormones, such as sex hormones, as well as vitamin D and the bile you need in your stomach to break down the food. Many of us appreciate that the unsaturated fats present in seafood provide for good health and longevity. However, few realize that fats are as important for life as proteins and genes. And probably very few people know that in terms of mass, fat is the most important part of our brain and the second most important of all other soft tissue.

In the sciences, fats are called lipids. Lipids are studied by nutritionists who investigate how the intake of fats in our diet affects the composition of various parts of the body, e.g., the heart, the liver, and the brain. You are what you eat! Lipids are also studied by biochemists who investigate the synthesis and breakdown of lipids as a source of energy and building material for cells. Among molecular biologists and physical scientists, lipids are less appreciated, although lipids are listed along with proteins, genes (DNA and RNA), and carbohydrates (sugars) as the fundamental building blocks of all living matter. Proteins and genes are known to be very specific to the functions they perform. The same is true for the carbohydrates that are used by the living cell to recognize foreign substances and to identify other cells.

Lipids as structure builders and fat depots are, however, most often characterized by terms like variability, diversity, plasticity, and adaptability. Hence, lipids appear to play a fairly nonspecific role, being rather dull and anonymous compared to fashionable stuff like the proteins that catalyze all biochemical reactions and the genes that contain the information needed to produce the proteins. There are no genes coding for lipids as such, only for the enzymes that build and modify the lipids which, for example, we obtain from our food.

There is a particular reason why lipids are considered dull and less interesting. This reason has to do with the powerful concept of molecular structure that has permeated life scientists’ way of thinking throughout the twentieth century. The central dogma is that molecular structure controls function.
Lipids apparently do not have the intriguing molecular structures which important molecules like proteins and DNA have. Moreover, lipids do not as easily as proteins and DNA lend themselves to revealing the secrets of how molecular structure leads to biological function. In fact, lipids are often tacitly assumed to constitute a structureless fatty material which at best is organized in a membrane structure that plays the role of a passive container of the cell and an appropriate solvent or template for the important molecules of life: Lipids grease the functional machinery of the cells controlled and run by proteins and DNA.

The traditional view of lipids as dull molecules has changed considerably over the last decade, and their importance for cell function and health is becoming more recognized. This is what the present book is about. Lipids are proposed to be as important for life as proteins and genes. The book will point out that lipids lead to interesting and intriguing structures with very unusual and subtle materials properties that have been optimized by evolutionary principles over billions of years. These properties are consequences of the fundamental physical principles of self-organization that rule when many molecules act in concert. A key player in this concert is water, which functions as the unique biological solvent. Water is omnipresent in all functioning biological systems. The peculiar properties of water force lipid molecules to self-assemble and organize into subtle structures. In particular, lipids in water can form lipid bilayers and membranes. Lipid membranes are extended thin layers that are only two molecules thick. These layers constitute the backbone of all biological membranes. Membranes are an ubiquitous structural element in all living cells.

And cells are literally packed with membranes. In a human being, which is composed of about $10^{14}$ cells, the total surface of the membranes has been estimated to cover an area of about 100 km$^2$. An important membrane function is purely topological: Membranes compartmentalize living matter into cells and subcellular structures. Furthermore, membranes present themselves to macromolecules as highly structured interfaces at which important biochemical processes are carried out and catalyzed. Obviously, the structure and molecular organization of the lipid-bilayer component of membranes hold the key to understanding the functioning of membranes.

Due to the fact that lipids form membranes by self-assembly processes that do not involve strong chemical forces, membranes are pieces of soft matter. Softness is a materials feature that lipid membranes share with other forms of condensed matter like polymers and liquid crystals. During evolution, nature has evolved biological membrane structures as an optimal form of micro-encapsulation technology that, on the one hand, imparts the necessary durability to the particular soft condensed matter that membranes are made of and, on the other hand, sustains the lively dynamics that are needed to support and control the mechanisms of the many essential cellular functions associated with membranes.
Lipids are now known not only to be structural builders of the cell and an energy source for the cell functioning. In the form of membranes, lipids are crucial for controlling indirectly a great variety of biological functions that take place at or are mediated by membranes. Evidence emerging from recent research has shown that the functional role of membrane lipids may be as important as that of proteins. The lipids not only act as a passive solvent for the proteins and as a means of compartmentalization, but are also an integral part of cellular function. Many lipid species are now known to play a very active role, serving as so-called second messengers that pass on signals and information in the cell. Lipids also play the roles of enzymes, receptors, drugs, as well as regulators of, e.g., neurotransmitter activity. Lipids are known to modulate the expression of genes. Disorder in the lipid spectrum of cells has recently been related not only to atherosclerosis but also to major psychiatric illnesses. Finally, poly-unsaturated lipids via the diet have been proposed to provide an evolutionary driving force supplementing Darwinian natural selection. All these factors emphasize a necessary shift from a one-eyed genocentric approach to membrane science toward a more balanced organocentric view. This puts the study of the physical properties of membranes at a very central position.

We have just witnessed a scientific revolution that at the end of the twentieth century took us through the genomics era with focus on sequencing genes and mapping gene products. We are now in the middle of the proteomics era in which the multitude of proteins that are coded for by the genes are being identified and their functions unveiled. In front of us we have a new era of recognizing the role of lipids. A new science termed lipidomics is emerging. In a not-too-distant future, we can imagine all the “omics” to converge into the grand metabolomics era in which the interplay between all the constituents of living matter is being studied. This development mirrors a gradual anticipation of the actual complexity of the problem.

Some have claimed that life sciences, due to the strong focus on sequencing and the mapping of genes and proteins, have been turned into a data-driven information science. It is certainly true that modern biology has for ever been changed by the tremendous wealth of information that has been derived from the genomics and proteomics programs. However, a deep insight into the workings of living systems requires more than collection of data and information processing. Science is by nature driven by hypotheses, and it goes beyond stamp collection. This is certainly also true of lipidomics. A mapping of all lipid species in all cell types is undoubtedly useful. However, a new lipidomics science has to go beyond that and approach cellular functioning from a more holistic perspective. And it will have to be hypothesis-driven. Lipidomics must involve a quantitative experimental and theoretical study of, e.g., lipid and membrane self-assembly, lipid-protein interactions, lipid-gene interactions, and the biophysical properties of lipid structure and dynamics.
The term lipidomics has only appeared in the scientific literature within the last few years. The name of this most recent member of the ‘omics’ family seems to have been brought forward and discussed at a number of international conferences during 2001 and was first suggested in the scientific literature by Leif Rilfors and Goran Lindblom in 2002 in the context of functional lipidomics. A debate at the 2002 congress of the International Society for the Study of Fatty Acids and Lipids decided to use the term lipidomics in much the same manner as it is being used in the present book, that is in a way that goes considerably beyond mapping out all lipids and their functions in a traditional systems-biology fashion.

A deeper knowledge of the physical properties of lipids is essential for understanding the living world and its modes of functioning. It may also be useful in revealing the causes of malfunctioning and diseased conditions and how such malfunctioning can be restored by medical treatment or by altered living conditions. The insights into the physics and the inner workings of the cellular machinery, including the role of lipids, can furthermore be lessons for technology. Examples include the development of futuristic soft and biocompatible materials, functionalized surfaces and sensors, the design of new and effective drugs and drug-delivery systems for, e.g., cancer therapy, as well as the design of new enzymes for the cheap and clean production of drugs and chemicals.

This book presents a personal and multidisciplinary perspective on the physics of life and the particular role played by lipids and the lipid-bilayer component of cell membranes. The emphasis is on the physical properties of the lipid membrane seen as a soft and molecularly structured interface. By combining and synthesizing insights obtained from a variety of recent studies, an attempt is made to clarify what membrane structure is and how it can be quantitatively described. Furthermore, it will be shown how lipid membrane structure and organization can control functional properties of membranes. The strategy of the book is to provide a bridge between, on the one side, the microscopic world of membranes, i.e., the world of the molecules, and, on the other side, the macroscopic world, i.e., the world as we observe and sense it. This involves unravelling the organizational principles that govern the many types of structure that arise on length scales from the size of the individual molecule, across molecular assemblies of proteins and lipid domains in the range of nanometers, to the meso- and macroscopics of whole cells.

The book provides a Bibliography containing a selected list of references to other books, review papers, and research articles accounting for most of the factual statements made in the book. These references to the literature have been selected according to a minimal principle. The rule has been adopted that references are made to recent publications and not necessarily to the original work. From the references given, the interested reader should be able to track down the original literature. The reader is furthermore referred to the list of specialized books and review papers for a more comprehensive list
of references. I apologize in advance to those authors and colleagues who may feel that their original work should have been referenced and discussed in more detail.

A Note on Length Scales, Forces, Energy, and Temperature

The picture of lipids described in this book takes its starting point in the molecular world where the molecules move around due to influence of temperature. It is hence useful to describe the various entities on scales described in units of nanometers. One nanometer (nm) is $10^{-9}$ m, i.e., one millionth of a millimeter. Cell sizes are conveniently given in units of micrometers. One micrometer ($\mu$m) is 1,000 nm. The appropriate unit of force for biological molecules on the nm scale is pico-Newton (pN). One pN is $10^{-12}$ N. These units sound terribly small. It is helpful to combine them in the form of the energy that corresponds to the thermal energy at room temperature ($T_{\text{room}} = 293K = 20^\circ C$)

$$\text{Thermal energy} = k_B T_{\text{room}} = 4.1 \cdot \text{nm} \cdot \text{pN},$$  

(1)

where $k_B$ is Boltzmann’s constant, which is a universal number. Most of the phenomena characteristic of biomolecular structure and dynamics are strongly influenced by temperature, and it is therefore of importance whether the involved energies are smaller or larger than the thermal energy in (1). As a rule of thumb one would say that if a characteristic energy of some association, e.g., a binding between two molecular-scale objects, is of the order of a few $k_B T_{\text{room}}$ or less, thermal agitation should be significant, and the lifetime of the association can be short. A couple of examples can serve as an illustration. A covalent chemical bond C–C between two carbon atoms represents an energy of the order of $100 k_B T_{\text{room}}$ (with a force equivalent of around 5,000 pN) and is therefore very stable at room temperature. A typical hydrogen bond amounts to about $10 k_B T_{\text{room}}$ (with a force equivalent of around 200 pN) and is therefore often influenced by thermal agitation. Turning then to weak physical interactions, the van der Waals interaction between two methane molecules, or other hydrocarbon moieties, represents about $1 k_B T_{\text{room}}$ (with a force equivalent of around 40 pN). Even lower energies and correspondingly lower forces govern the weak molecular associations in biological systems, e.g., the binding of a small enzyme to a membrane surface amounts to about 20 pN, the force required to pull out a single lipid molecule of a membrane is only about 2 pN, and finally the motor proteins that function, e.g., in muscle contraction, exert forces as small as 1 pN. In all these cases, thermal agitation is of major importance.
Part I

The Overlooked Molecules
1 Life from Molecules

1.1 The Three Kingdoms of Life

Living organisms are divided into three kingdoms: the eukaryotes, the eubacteria, and the archaeabacteria. The eubacteria and the archaeabacteria, which among themselves differ as much as they do from eukaryotes, are conventionally grouped together as prokaryotes. The bacteria common to most people, e.g., coli bacteria or the bacteria in sour milk, are eubacteria. Archaeabacteria are typically found in rather hostile environments, such as in hot springs, at the bottom of deep sea, or in the very acidic milieu of the cow’s stomach. These bacteria do not tolerate oxygen and they often present a health hazard to humans. Eukaryotes are animals, plants, and fungi and also include single-cell organisms like yeast. Figure 1.1 gives examples of single cells from the three kingdoms.

![Fig. 1.1a–c. Examples of cells from the three kingdoms of life. (a) An archaeabacterium: Methanococcus jannischiiwas. Diameter about 2µm. (b) A eubacterium: Escherichia coli. Size about 2–3µm. (c) Eukaryotes: human red and white blood cells shown together with a platelet. The diameter of the red blood cell is about 6µm. The cells from the three kingdoms are not drawn on the same scale.]

Despite their difference in appearance and functioning, archaeabacteria, eubacteria, and eukaryotes are all made from the same basic molecular building blocks, and they are all based on the same chemistry. Although it is generally believed that all cells have a common ancestor, the ancient evolutionary history of the different cell types is subject to considerable dispute. The three
Fig. 1.2. Phylogenetic tree with the three kingdoms of life: eubacteria, archaebacteria, and eukaryotes. The relative distance between the organisms is proportional to the evolutionary distance as determined by ribosomal-RNA nucleotide sequencing.

Although the origin of life on Earth is a controversial and unresolved problem, it is a reasonable assumption that the first cellular living systems on Earth were assembled from four types of molecular building blocks: (i) information-storing molecules capable of reproduction, (ii) enzyme-like catalysts encoded by that information and able to enhance reproduction rates, (iii) molecules capable of storing energy and using this energy to convert molecules into organized assemblies of biologically active molecules, and (iv) special boundary-forming molecules capable of encapsulating and protecting the former three types of molecules. The last category of molecules is the focus of the present book.

1.2 The Molecules of Life

All cells are built from small organic molecules that are based on the chemistry of carbon. These small molecules belong to essentially four classes: the
1.2 The Molecules of Life

Fig. 1.3a–d. Examples representing the four classes of small organic molecules that are the building blocks of all living matter. (a) Sugar: glucose. (b) Amino acid: alanine. (c) Nucleotide: adenosine. (d) Fatty acid: oleic acid

sugars, the amino acids, the nucleotides, and the fatty acids. Examples of these small elementary building blocks of living matter are given in Fig. 1.3.

The small organic molecules are combined with other molecules from the same class or with molecules from the other classes to make larger entities, so-called macromolecules or macromolecular assemblies. There are basically four classes of these larger entities, the polysaccharides, the proteins, the nucleic acids, and the fats (lipids and membranes), as illustrated in Fig. 1.4.

Proteins are also called poly-amino acids (or polypeptides), and nucleic acids are called polynucleotides, reflecting the fact that proteins and nucleic acids, just like polysaccharides, are biopolymers, i.e., long-chain molecules composed of many monomers that are bound together by strong chemical bonds.

Since there are about twenty different types of amino acids in nature and since a protein can consist of up to several hundred amino acids, a very large number of different proteins can be perceived. Similarly, the five different nucleotides used by nature allow for an immense richness in different nucleic acids that make up DNA (deoxyribonucleic acids) and RNA (ribonucleic acids). Like proteins, the nucleic acids are linear molecules, and it is the particular sequence of the monomers that determines the properties of both proteins and nucleic acids. DNA and RNA contain the genetic information that is organized in genes. The entire DNA string of an organism is termed the genome, which can contain millions of nucleotides. For example,
the human genome includes about 20,000–25,000 genes composed of a little less than three billion nucleotides. In addition to encoding genetic information, nucleotides also perform functions as energy carriers (ATP, adenosine triphosphate), catalysts, and messengers.

When it comes to the sugars, living organisms exploit a large number of different monosaccharides. Hence, it is not uncommon to find hundreds of different polysaccharides in a cell. Sugars allow for additional complexity in the type of materials that can be built because they can combine into branched macromolecular networks. Such networks are responsible for forming biological fibers and scaffolding and are also an important part of the cell’s recognition system.

We are then left with the fatty acids and the lipids. In contrast to the sugars, the amino acids, and the nucleotides, lipids do not link chemically to form “poly-lipids.” No such thing exists under natural conditions. Instead, they form “loose” macromolecular (or supramolecular) assemblies, of which
the lipid bilayer membrane, shown in Fig. 1.4d, is the most prominent example. In some cases, lipids combine chemically with proteins and sugars. However, when forming living matter, lipids usually maintain their molecular integrity. The lipid bilayer is the core of all biological membranes. A lipid bilayer membrane contains billions of lipid molecules and a cell membrane often contains hundreds of different kinds of lipids.

All cells of living beings are confined and compartmentalized by a number of membranes, as illustrated in Fig. 1.5 in the case of eukaryotes. Common for all cells is a cell-surface membrane called the plasma membrane. The plasma membrane is a very stratified and composite structure whose central element is the lipid bilayer, as illustrated in Fig. 1.6 in the case of a very simple unicellular microorganism, *Escherichia coli*. The lipid bilayer is extremely thin in comparison to the size of the cell it encapsulates. A schematic cartoon of the plasma membrane of a eukaryotic cell with all the other components

![Schematic illustration of a generic eukaryotic cell that is drawn artificially to compare an animal cell (left) and a plant cell (right). Plant cells, as well as bacterial cells, have an additional outer cell wall](image)
it contains in addition to the lipid bilayer is shown in Fig. 1.7. The plasma membrane is a unique composite of all the types of macromolecules described above except nucleic acids. Its molecular composition depends on the type of cell. Carbohydrates are a minor component with less than 10% of the dry mass. The weight ratio of proteins and lipids can vary from 1:5 to 5:1.

Whereas prokaryotic cells only have a plasma membrane and some less structured internal membrane systems, the eukaryotic cells have in addition a number of well-defined internal membranes associated with the cell nucleus and the organelles (cf. Fig. 1.5). The cell nucleus, which contains the cell’s genetic material, is wrapped in a porous double membrane (the nuclear envelope). This membrane is topologically connected to the membranes of the endoplasmic reticulum (ER), which is the major site of synthesis of lipids and proteins. The Golgi apparatus contains a very convoluted agglomerate of membranes. This is where the newly synthesized molecules are modified, sorted, and packaged for transport to other organelles or for export out of the cell. The membranes of both Golgi and the ER are morphologically very complex and exhibit substantial curvature. The mitochondria, which contain their own DNA and RNA and produce their own proteins, contain two intertwined membranes, an inner and an outer membrane. The outer membrane acts as a sieve, retaining the larger proteins within its compartment. Lysosomes are rather small organelles bound by a membrane. The lysosomes operate as the
Fig. 1.7. Schematic model of the plasma membrane of a eukaryotic cell that highlights the membrane as a composite of a central lipid bilayer sandwiched between the carbohydrate glyocalyx (which consist of polysaccharides) on the outside and the rubber-like cytoskeleton (which is a polymeric protein network) on the inside. Intercalated in the lipid bilayer are shown various integral proteins and polypeptides. The membrane is subject to undulations, and the lipid bilayer displays lateral heterogeneity, lipid domain formation, and thickness variations close to the integral proteins. Whereas the lipid molecules in this representation are given with some structural details, the membrane-associated proteins remain fairly featureless. In order to capture many different features in the same illustration, the different membrane components are not drawn to scale.

cell’s garbage and recycling system, performing digestion, degradation, and export of unwanted molecules. Finally, there is a bunch of vesicles that support the extended trafficking needed by the cell to transport material within the cell and across the plasma membrane.

Membranes thereby become the most abundant cellular structure in all living matter. They can be considered nature’s preferred mode of micro-encapsulation technology, developed as a means of compartmentalizing living matter and protecting the genetic material. The biological membrane is the essential capsule of life. Many important biological processes in the cell either take place at membranes or are mediated by membranes, such as transport, growth, neural function, immunological response, signalling, and enzymatic activity. An important function of the lipid bilayer is to act as a passive permeability barrier to ions and other molecular substances and leave the transmembrane transport to active carriers and channels.

Judging naively from Fig. 1.7, the role of lipids is much less glorious than that played by proteins and nucleic acids. Whereas proteins possess a specific molecular structure and order supporting function, and DNA has a
very distinct molecular structure and order encoding the genetic information, lipids appear to be characterized by disorder and a lack of any obvious structural elements. The lipid bilayer is a molecular mess and it is hard to imagine any structural order in and among the lipids that is specific enough to control a delicate biological function.

This is the reason why lipids were not among the favorite molecules of the twentieth century’s molecular and structural biologists. The lipids became the most overlooked molecules in biology.

In many ways, this is a paradox. Without comparison, lipids are the most diverse class of molecules in cells. Prokaryotic cells typically contain a hundred different types of lipids, and the larger eukaryotes cells many hundred different kinds. Moreover, the results of genomic research have revealed that more than 30% of the genome codes for proteins embedded in membranes. A possibly even larger percentage codes for proteins that are peripherally attached to membranes. If one adds to this the observation that the membrane-spanning parts of the proteins contain some of the most evolutionarily conserved amino-acid sequences, it becomes clear that lipids are indeed very important molecules for life. This insight calls for a deeper understanding of how membrane proteins and their function are related to the properties of the lipid bilayer membrane.

In order to understand the implications of disregarding lipids in the study of the physics of life and which challenges it has left us with at the beginning of the new millennium, we have to make a status on life sciences upon the entry to what has been called the post-genomic era. This era is characterized by an almost complete knowledge of genomes of an increasing number of different species ranging from bacteria, fungi, insects, worms, and mammals, including mouse and man.

1.3 The Post-Genomic Era

Molecular and structural biology have been some of the most successful sciences of the twentieth century. By focusing on the concept of structure – from the genes to the workhorses of living beings, the proteins – first revealing the genetic code, then the structure of many proteins, and very recently the whole genome of several species, including that of man, these sciences have had an enormous impact on life sciences and society. Structure, in particular, well-defined atomistic-level molecular structure, has been the lodestar in the quest for unravelling the genetic code and the properties of DNA, for understanding transcription of the code into protein synthesis, and for determining the properties of the proteins themselves. The relationship between macromolecular structure and function is simply the key issue in modern biology. The human genome project is probably the most monumental manifestation of the conviction among life scientists that gene structure is the Holy Grail of life.
Knowing the genome of an organism implies information about which proteins the cells of this organism can produce. The genome is so to speak the blueprint of the proteins. Since we know how information is passed on from the genome to the proteins, we can also unravel the relationships between possible defects in a protein on the one side and errors and modifications in the genome on the other side. Such errors can lead to genetically determined diseases, such as cystic fibrosis. This insight can be of use in gene-therapeutic treatment of serious diseases, as well as in the production of plants and animals with desirable properties, e.g., plants that can better cope with poor weather conditions or are resistant to the attack of insects and microorganisms. Results of the genome research can also be used technologically to alter the genes of microorganisms like bacteria. Gene-modified bacteria can be exploited to produce useful chemicals and drugs.

Knowing the genome of an organism does not imply that one necessarily knows which function a given protein can carry out. It is not to be read in the genome why and how proteins carry out their various tasks, not even in the case where their function is known. Furthermore, it is not written in the genome how a cell and its various parts are assembled from the molecular building blocks. Neither can one read in the genome how biological activity is regulated or how cells are organized to become multi-cellular organisms of specific form and function. As a striking example, one cannot read in the genome why our fingers are almost equally long, how the leopard gets its spots, or what determines the width of the zebra’s stripes.

The information contained in the genome is in this sense not complete, and additional principles have to be invoked in order to describe and understand the complex organization of the molecules of life. Complexity in living organisms does not come from the genome alone. One way of expressing this fact is to say that the genome provides the limitations and the space within which the biology can unfold itself. Biological function and pattern formation are emergent phenomena that arise in this space. This is the point where physics and the physics of complex systems come in. Physics is the generic discipline that in principle has the tools to predict and describe the emergent properties that are the consequences of the fact that many molecules are interacting with each other. The grand challenge in the post-genomic era consists of formulating and completing a program that combines results of genomic research with basic physics in a sort of biophysical genomics combined with proteomics and lipidomics.

The tremendous complexity of the problem we are facing perhaps becomes obvious when it is considered that the 30,000 or more genes in humans code for millions of different proteins. Each protein in turn can be in several different molecular conformations each of which may have its specific function. Moreover, many of the proteins often become post-translationally modified, e.g., installed with hydrocarbon chains. In addition to this, the functioning
of a protein is modulated by its environment and how this environment is structured in space and time.

These challenges imply the provision of hypothesis-driven paradigms for understanding how cellular and subcellular structures of enormous complexity are formed out of their molecular building blocks, and how living systems are organized, regulated, and ultimately function. The old problem of bridging the gap between the genotype and phenotype still remains: Complete knowledge of a genome does not alone permit predictions about the supramolecular organization and functioning of a complex biological system.

Solving this problem is intellectually far more difficult than determining the genome of a species. For this purpose, principles from fundamental physics and chemistry are needed. Moreover, these principles will have to be developed and explored in a truly multidisciplinary setting. However, if this can be achieved, the post-genomic era will not only furnish the greatest challenges but also comprise some of the largest opportunities.

**Biological membranes** are outstanding examples of molecular assemblies of extreme complexity whose structure and function cannot be determined from the genome alone and which present some grand challenges to science. Due to the immense importance for life processes and not least the well-being of human beings, the study of biological membranes has for a long time been a central and very active field of research within medicine and biochemistry. Scientific disciplines like physiology, pharmacology, molecular biology, and nutritional science have all contributed to our current knowledge about biological membranes and their functions. However, the progress in the fundamental understanding of membranes has not been impressive compared to that related to proteins and DNA.

It is somewhat paradoxical that the preoccupation with well-defined molecular structure, which has led to so many successes in structural biology, may be the reason why an advance in the understanding of lipid membrane structure, and structure-function relationships for membranes, has been rather slow. The problem is that if one searches for well-defined structure in membranes in the same way as investigations are made of the structure of genes and proteins, one is going to utterly fail. The reason for this is that membranes are self-assembled molecular aggregates in which subtle elements of structure arise out of a state of substantial disorder, and where entropy consequently plays a major role. Disordered and partly ordered systems are notoriously difficult to characterize quantitatively. The challenge is to ask the right questions and to identify the hidden elements of order.

Although membranes consist of molecules (lipids, proteins, carbohydrates) of a well-defined chemical structure that are coded for in the genes, these molecules organize among themselves by physical principles that are nowhere to be found in the structure of the genetic material. The big question is then what these principles are and how they can operate to produce the robustness and specificity necessary for biological function. It is
thought-provoking that the lipids in the biological membrane are not linked by strong and specific chemical forces in contrast to the amino acids in proteins and the nucleotides in DNA. Instead, they are kept together by weak and nonspecific physical forces, which we shall return to in Chap. 3. It is striking that nature has used a technology based on self-assembly processes in the construction of the essential capsule of all known life forms. Related to this question is the big mystery of lipid diversity. Why is it that membranes are composed of such large numbers of different lipid species?

As we shall see in Chap. 5, membranes seen as physical states of matter are fluid and soft interfaces, and they possess all the subtle structures of liquids and liquid crystals. Elucidation of the structure of membranes therefore requires concepts from the physics and physical chemistry of disordered materials and soft condensed matter. An increased understanding of the subtle physical properties of membranes viewed as soft biological materials is likely to lead to new insights as well as surprises. This insight is a prerequisite in the post-genomic era for effectively exploiting the wealth of structural information that becomes available. The goal is to understand the regulation of entire systems of cell organelles and whole cells, which involve complex, dynamic, and self-organized structures of membranes, biological fibers, and macromolecules that are constantly being transported, translated, and inserted into various parts of the cell.

1.4 A Call for Physics

The study of the physics of membranes is not an easy one and requires challenging experimental and theoretical approaches. Several circumstances have in recent years stimulated an interest in the physics of biological membranes.

Firstly, modern experimental techniques have provided quantitative information about the physical properties of well-defined model-membrane systems, seen as large self-organized assemblies of interacting molecules. This information has shown that the properties of membranes and aspects of their biological function are controlled by basic physical principles. Revealing and understanding these principles, along with a clarification of the nature of the feedback mechanism between physical properties and function, open up for rational ways of manipulating membrane function and malfunction.

Secondly, physicists and physical chemists have realized that biological systems, in particular membranes and proteins, are interesting objects of study in their own right: Membranes are structured and functional materials (soft interfaces) with unique material properties that are designed by nature during evolutionary times over billions of years. These natural materials are therefore in most respects functionally superior to man-made materials. In particular, natural materials are designed to be mechanically stable and to function on small scales (from nanometers to micrometers) and are therefore
promising candidates for a whole new generation of micro- and nanotechnol-
ogy. One example of an important biomedical application of membrane sys-
tems is the use of liposomes as biocompatible microcapsules in targeted drug
delivery and gene therapy. Another example includes biosensors and medical
micro-devices composed of immobilized enzymes or proteins attached to sup-
ported lipid membrane interfaces. We shall return to the various technological
applications of lipids in Chap. 20.

In an attempt to understand, in molecular detail, how the functioning of
biological membranes is related to their physical properties on different time
and length scales, and how this relationship may be influenced by pharma-
ceutical drugs and environmental conditions, it is essential to characterize
different membrane systems by means of a variety of powerful experimental
and theoretical physical techniques.

In particular, it is necessary to achieve knowledge about the lateral struc-
ture and molecular organization of lipid membranes on length scales that are
relevant to the particular membrane phenomenon in question. This puts focus
on the nanometer scale and makes membrane science a truly nano-science. In
fact, biological membranes as a micro-encapsulation technology can be seen
as nature’s preferred nanotechnology. Membrane science is concerned with
an object that is 5 nm thick and has delicate structural features over scales
from 1–1,000 nm. Indeed, biological membranes are optimized by evolutional
processes to function on the nanometer scale.

This perspective should be kept in mind when research strategies are cho-
sen to investigate membranes and membrane models. Whereas many biologi-
cal systems are accurately characterized on the molecular and atomic scale, as
well on the large, cellular, and super-cellular level, there is a gap of knowledge
at the intermediate subcellular scales that constitutes precisely the nanome-
ter regime. It is in this regime where the workings of the complex cellular
machinery is manifested. The advent in recent years of powerful theoretical
methods and novel experimental techniques based on physical principles has
opened a window to the nanometer world that calls for a renewed extensive
study of membranes.

The nanoscopic organization of membranes is a key factor in various
biological events governing the binding of molecules to the membrane,
penetration and permeation of peptides and drugs, as well as insertion of
membrane proteins. Moreover, the lateral structure controls the mechanical
properties of the membrane and thereby its interaction with other mem-
branes. The mechanical properties in turn are of crucial importance for the
shape of cells, for cell cytosis and fusion, as well as for cell motility. In order
to understand how proteins and enzymes function in membranes, e.g., in rela-
tion to transport, biochemical signaling, energy transduction, receptor-ligand
interactions, and nerve activity, it is necessary to determine the ways in which
proteins interact with the lipid bilayer, specifically how the proteins influence
the local structure and composition of the bilayer, on the one hand, and how
changes in the lipid-bilayer physical properties modulate the functional state of the proteins on the other hand.

Answering these questions is the challenge to the emerging science of lipidomics, and the answers hold the key to understanding fundamental aspects of the nanoscopic “machinery” of the cell.
2 Head and Tail

2.1 Fat Family: Fats and Fatty Acids

Oils and fats refer to a large and diverse group of chemical compounds that do not easily dissolve in water. There is no strict distinction between oils and fats; fats usually refer to materials like wax, lard, and butter that are solid at room temperature, whereas oils like olive oil and fish oil are liquid. As is well known, butter can melt upon heating and olive oil solidify by freezing. Fats are just frozen oils.

The main part of a fat or an oil is a hydrocarbon moiety, typically a long-chain hydrocarbon, as shown in Fig. 2.1. Hydrocarbon chains can contain different numbers of carbon atoms, and the bonds between the carbon atoms can be single bonds (saturated) or double bonds (unsaturated). Hydrocarbons are said to be hydrophobic since they do not easily dissolve in water.

![Hydrocarbon chains](image)

**Fig. 2.1.** Hydrocarbon chains shown in three different representations. *Top:* all atoms and all bonds. *Middle:* bonds between invisible carbon atoms placed at the vertices. *Bottom:* space-filling models. (a) Saturated hydrocarbon chain with fourteen carbon atoms. (b) Mono-unsaturated hydrocarbon chain with eighteen carbon atoms. The double bond is here positioned in the middle of the chain.
A hydrocarbon chain can be turned into a fatty acid by attaching a \(-\text{COOH}\) (carboxyl) group at the end, as shown in Fig. 2.2a. The carboxyl group is said to be hydrophilic since it can be dissolved in water. Fatty acids, therefore, more easily dissolve in water than pure hydrocarbons. The fatty acids are the fundamental building blocks of all lipids in living matter. Plants and animals use a variety of fatty acids with chain lengths ranging from two to thirty-six. The most common chain lengths fall between fourteen and twenty-two. As we shall see in Sect. 15.1, this is likely to be controlled by the need for cells to have membranes with a certain thickness in order to function properly. Some bacteria have been found to have fatty-acid chains as long as eighty. The simplest way of classifying fatty acids is to write the number of carbon atoms followed by the number of double bonds. For example, myristic acid is denoted 14:0, oleic acid 18:1, and docosahexaenoic acid 22:6. Obviously, a more elaborate notation is needed to specify where double bonds are located along the chain. We shall return to this in Sect. 16.1.

It is most common to find chains with an even number of carbon atoms, although odd ones are found in rare cases. In animals and plants, most of the fatty-acid chains are unsaturated, most frequently with a single double bond (e.g., oleic acid shown in Fig. 2.2b) and in some cases with as many as six double bonds (docosahexaenoic acid, DHA), as shown in Fig. 2.2c. Unsaturated fatty acids with more than one double bond are called polyunsaturated. Those with as many as five and six are called super-unsaturated. The occurrence of poly- and super-unsaturated fatty acids and how they are synthesized are described in Sect. 16.1.

Short-chain fatty acids can be produced by electrical discharges, e.g., lightening, out of inorganic compounds like carbon dioxide and methane. Intermediate- and long-chain fatty acids are believed only to be produced by biochemical synthesis in living organisms. Therefore, these fatty acids, along with amino acids, are taken as signs of life and are hence looked for in the exploration of extraterrestrial life, e.g., in comets and on Mars.

Fatty acids are rarely found free in the cell, except when they transiently appear in the course of chemical reactions or are transported from cell to cell while attached to certain transporter proteins, so-called lipoproteins. Instead, they are chemically linked to a hydrophobic group, e.g., glycerol, which is an alcohol that can be esterified in up to three positions, as illustrated in Fig. 2.2d and e in the case of a di-acylglycerol derived from myristic acid and a tri-acylglycerol derived from stearic acid. This process leads to the formation of a lipid molecule, in this case a non-polar lipid. The fatty-acid chains at the different positions can be different and most often they are, typically with a middle one that differs from the other two. Glycerol acts as the backbone of the lipid molecule. For comparison, a polar lipid molecule, DMPC, is shown in Fig. 2.2f (cf. Sect. 2.2).

When an ester bond is formed, a water molecule is released. The reverse process, where an ester bond is broken, is referred to as hydrolysis.
Fig. 2.2a–h. Fats. The polar and aqueous region is shown to the left and the hydrophobic region to the right. The interfacial region is highlighted in grey. (a) Fatty acid (myristic acid, 14:0) corresponding to the hydrocarbon chain in Fig. 2.1a. (b) Oleic acid (18:1, with one double bond) corresponding to the hydrocarbon chain in Fig. 2.1b. (c) Docosahexaenoic acid (DHA) with six double bonds (22:6). (d) Diacylglycerol (DAG) of myristic acid in (a). (e) Triacylglycerol (triglyceride) of stearic acid. (f) Lipid (di-myristoyl phosphatidylcholine, DMPC) made of the diacylglycerol in (d) and phosphatidylcholine. (g) Lysolipid. (h) Phosphatidic acid
(i.e., breaking water) or lipolysis (i.e., breaking lipids). Certain enzymes can perform lipolysis and we shall return to this in Chap. 18. The result of the lipolysis can be the formation of a so-called lysolipid which is a lipid missing one of the fatty-acid chains as shown in Fig. 2.2g. The effect of other enzymes to be described in Sect. 18.1 is to produce di-acylglycerol as shown in Fig. 2.2d or phosphatidic acid as shown Fig. 2.2h.

Di-acylglycerol (DAG) with two fatty acids is a key lipid molecule in certain signaling pathways which we shall describe in Sect. 19.3. Tri-acylglycerols are the typical storage lipid or fat, used for energy production, and saved in certain fat cells (adipocytes) and specialized fat (adipose) tissues.

2.2 The Polar Lipids – Both Head and Tail

Tri-acylglycerols are strongly hydrophobic which means that they cannot be dissolved in water. The affinity for water can be improved by replacing one of the fatty acids with a polar group. The resulting polar lipid molecule, called a glycerophospholipid, then appears as a molecule with a hydrophobic tail and a hydrophilic head (see also Sect. 3.2). An example is given in Fig. 2.3a. In this case the polar head group is phosphatidylcholine (PC). Other head groups are phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidyglycerol (PG), and phosphatidylinositol (PI) as shown in Fig. 2.3. While PC and PE lipids are neutral (zwitter-ionic), PS, PG, and PI lipids can be electrically charged. This difference has an important consequence for the capacity of the lipids, when incorporated into a lipid membrane, to bind proteins and drugs. The examples of phospholipids shown in Fig. 2.3a–f have two fatty-acid chains that are the same. The lipids in natural membranes usually contain two different chains and most often one of them is unsaturated. In the following, we shall for convenience represent polar lipids by the simple schematic illustration in Fig. 2.3g.

Nature also uses another strategy to construct lipids with head and tails. Instead of using glycerol to bind the fatty acids, sphingosine, which is a long-chain amine, is bound to the fatty acid. The simplest version of the resulting sphingolipid is ceramide, shown in Fig. 2.4a. Ceramide is an important component of the skin, as we shall discuss in Sect. 19.1. It is also involved in programmed cell death, which is the topic dealt with in Sect. 19.4. Ceramide is a rather hydrophobic molecule. A head group can be attached to ceramide, e.g., PC, leading to sphingomyelin, shown in Fig. 2.4b.

The phospholipids, both those based on glycerol and sphingosine, can be installed in the head group with sugar groups of varying degrees of complexity. Examples are shown in Fig. 2.3f and Fig. 2.4c and d, respectively. Such lipids are called glycolipids.

Phospholipids can be broken down into their different parts by specific enzymes just like tri-acylglycerols can be hydrolyzed. These enzymes are
2.2 The Polar Lipids – Both Head and Tail

Fig. 2.3a–g. Different polar head groups of glycerophospholipids. The polar and aqueous region is shown to the left and the hydrophobic region to the right. The interfacial region is highlighted in grey. (a) Di-myristoyl phosphatidylcholine (PC). (b) Di-myristoyl phosphatidylserine (PS). (c) Di-myristoyl phosphatidylethanolamine (PE). (d) Di-myristoyl phosphatidylinositol (PI). (e) Di-myristoyl phosphatidylglycerol (PG). (f) A glycolipid. (g) Schematic representation of a polar lipid with a hydrophilic head group and a hydrophobic tail consisting of two hydrocarbon chains

called phospholipases, and their modes of action will be described in Chap. 18. Another type of enzyme, sphingomyelinase, can hydrolyze sphingomyelin.

Whereas the tri-acylglycerols are storage and fuel lipids, phospholipids and sphingolipids are structural and functional lipids. An enormous range of possible lipids can be perceived by varying, e.g., fatty-acid chain length, degree of saturation, polar head group, and type of glycosylation. It is hence not surprising that lipids are the most chemically diverse group of molecules
in cells. The question naturally arises as to what is the reason and need for this richness and diversity?

### 2.3 Cholesterol – A Lipid of Its Own

*Cholesterol* is a lipid that is quite different from the phospholipids and sphingolipids we discussed above. Rather than having a fatty-acid chain as its hydrophobic part, cholesterol has a steroid ring structure and a simple hydroxyl group (–OH) as its polar head. The steroid skeleton has a small hydrocarbon chain at the end. Hence, cholesterol can be characterized as a lipid molecule with a bulky and stiff tail and a small head, as shown in Fig. 2.5a.
2.4 Strange Lipids

The molecular structure of cholesterol is very similar to that of bile salt, vitamin D, and sex hormones. Cholesterol is one of several members of the sterol family that play similar roles in different types of organisms, e.g., ergosterol in fungi and sitosterol in plants, cf. Fig. 2.5.

Fig. 2.5a–e. Sterols related to cholesterol. (a) Cholesterol. (b) Ergosterol. (c) Sitosterol. (d) Testosterone (male sex hormone). (e) Vitamin D

2.4 Strange Lipids

Some lipids appear to have rather strange structures that may suggest that they are useful for optimizing the physical properties of membranes that have to work under unusual conditions, for example, at deep sea or in hot springs, as described in Sect. 19.2. These lipids are either very bulky, very long, or based on ether chemistry rather than ester chemistry. The fact that we consider these lipids as strange is likely to reflect that the current fashion of research is biased toward eukaryotic, in particular, mammalian membranes, and that the world of, e.g., the eubacteria and the archaeabacteria is much less explored.
In Fig. 2.6 are listed several lipids with unusual structures. Cardiolipin in Fig. 2.6a is basically a dimer lipid that has four fatty-acid chains and is found in the inner mitochondrial membrane, in plant chloroplast membranes, as well as in some bacterial membranes. Lipids based on ether bonding of fatty acids rather than ester bonding are frequently found in archaebacterial membranes. As an example, a di-ether lipid with branched fatty-acid chains is shown in Fig. 2.6b. *Bolalipids* refer to a class of bipolar lipids, i.e., lipids with a polar head in both ends, that can span across a bacterial membrane. Figure 2.6c shows an example of a bolalipid being a tetra-ether lipid, which is a basic component of the membranes of halophilic archaebacteria.

Finally, poly-isoprenoid lipids, as illustrated in Fig. 2.6d, are commonly associated with both prokaryotic and eukaryotic membranes and can act as lipid and sugar carriers.

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Fig. 2.6a–d. A selection of strange lipids. (a) Cardiolipin. (b) Di-ether lipid. (c) Tetra-ether lipid (bolalipid, or di-biphytanyl-diglycerol-tetraether). (d) Poly-isoprenoid lipid
2.5 Lipid Composition of Membranes

As suggested by the description above, an enormous range of different lipids can be constructed. Obviously, nature only exploits some of the possibilities, although the number of different lipid species found in a given kingdom of life, and even within a single cell type, is surprisingly large. Furthermore, a given type of cell or organism can only synthesize a limited range of lipids. For example, human beings only produce a few types of fats and lipids themselves. Most of the fats and lipids in our bodies come from the diet. We shall discuss in Chap. 16 this issue in the context of the fats of the brain and in the visual system.

Without making an attempt to give an overview of the lipid contents in different organisms and cell types, we quote some striking observations for mammalian plasma membranes that shall turn out to be relevant when discussing the physics of lipid membranes. It should be noted that there are characteristic differences between the lipid composition of plasma membranes and that of the various organelles.

Cholesterol is universally present in the plasma membranes of all animals (sitosterol in plants) in amounts ranging between 20–50% of total lipids. In contrast, the organelle membranes contain very little: mitochondrial membranes, less than 5%; Golgi membranes, about 8%; and ER membranes around 10%. In contrast, sterols are universally absent in the membranes of all prokaryotes. These striking numbers can be related to the role played by cholesterol in the evolution of higher organisms, as described in Sect. 14.2.

The amount of charged lipids is about 10% of total lipids in plasma membranes, but there is a substantial variation in the ratio between PS and PI lipids. It is a remarkable observation that nature only uses negatively charged and not positively charged lipids in membranes. It is generally found that the longer the fatty-acid chain, the more double bonds are present. For example, lipids with eighteen carbon atoms have typically one double bond, those with twenty have four, and those with twenty-two have six. PC lipids have typically short chains, whereas SM often have very long chains. PE, PS, and PI lipids typically carry a high degree of unsaturation, whereas PC carry less.

There is a remarkable lipid asymmetry in the lipid composition of the two monolayers of the bilayer of the plasma membrane. Whereas SM, PC, cholesterol, and glycolipids are enriched in the outer monolayer, PS, PI, and PE are enriched in the inner layer.
3 Oil and Water

3.1 Water – The Biological Solvent

Water is necessary for life of the form we know. In fact, it is so essential that when NASA goes into space and looks for signs of extraterrestrial life, the search is concentrated on water and features of planetary surfaces that may reflect that water is present or has been present. Moreover, it is mandatory for the evolution of life and maintenance of life processes that water be present in its liquid state. This is why water is called the biological solvent.

No other solvent can substitute for water in supporting life. The reason is found in the peculiar properties of the water molecule and how it interacts with other water molecules in condensed phases like liquid water and ice. Water molecules have a unique capacity for forming hydrogen bonds in addition to ordinary dipole-dipole interactions. A hydrogen bond is formed when a hydrogen atom in one water molecule is attracted by a nonbonding pair of electrons from the oxygen atom of a neighboring water molecule, as illustrated in Fig. 3.1. Since each oxygen atom can contribute to two hydrogen bonds, every water molecule can participate in up to four hydrogen bonds with its neighbors. By this mechanism, liquid water is said to form a loose network of hydrogen bonds. It is the peculiar properties of this network that provide the driving force for forming the organized structures and self-assembled materials that are the basis of all life, whether it be the specific molecular structure of a protein or the self-assembly of lipids into a bilayer membrane.

In liquid water, the hydrogen bonds are constantly formed and broken, leading to a very dynamic situation. Due to the many different ways the hydrogen network can be dynamically arranged, the network is strongly stabilized by entropy. This is the reason why water is such a coherent liquid with both a high surface tension and a high boiling point compared with other hydrogen compounds with a similar molar mass, e.g., methane or ammonia.

The hydrogen bonding network is a property that emerges because many water molecules act in concert. The entropy that stabilizes the structure is a nonlocal quantity, i.e., it is not a property of the individual water molecule but emerges from the cooperative nature of a large collection of water molecules.
3.2 The Hydrophobic Effect

The stability of the hydrogen bonding network in water makes it difficult to dissolve oil and oil-like compounds in liquid water. Materials that are difficult to dissolve in water are called hydrophobic compounds, i.e., compounds that are “water haters.” It is well known that oil and water do not mix. This is referred to as the hydrophobic effect. Typical oil molecules are simple hydrocarbon chains, as shown in Fig. 3.2. Oil molecules cannot form hydrogen bonds, and liquid oil is therefore only held together by dipole-dipole interactions.

The reason why oil does not mix with water is not so much that the individual parts of the hydrocarbon molecules do not interact favorably with the water molecules via dipole-dipole interactions but that oil is not capable of forming hydrogen bonds. Hence, when an oil molecule is put into water, the hydrogen bonding network in water suffers. As a consequence, the entropy is lowered and the stability of the whole system is decreased. The hydrophobic effect, which will act to drive the oil molecules together in order to diminish the contact with water, is therefore to a large extent of entropic origin.

Compounds that can be dissolved in water are called hydrophilic, i.e., “water lovers.” Examples are polar or ionic compounds, which due to their charges can form hydrogen bonds.
3.3 Mediating Oil and Water

It is well known that water and oil can be made to mix if appropriate additives are used. For example, olive oil and vinegar can be mixed to mayonnaise if a stitch of egg yolk or egg white is applied. Similarly, greasy fat in textiles or on our skin can be removed by water if soaps or detergents are brought into use. The ability of these compounds to mediate oil and water can be appreciated if one considers the energetics of the interface that are formed between oil and water as a result of the hydrophobic effect. The interface is characterized by an interfacial tension (or surface tension), \( \gamma \), which is a measure of the free energy that is required to increase the interface between oil and water (cf. Sect. 5.2). Obviously, the larger \( \gamma \) is, the more unfavorable it is to form an interface.

The interface between oil and water can be mediated, and the interfacial tension lowered, if one introduces compounds that are water-like in one end and oil-like in the other end. Molecules with these combined properties are called amphiphilic or amphiphatic, i.e., they love both oil and water and therefore have mixed feelings about water. The stuff in egg yolk and egg white has such qualities; in fact, egg yolk consists of amphiphilic proteins and lecithin, which is a mixture of different lipid molecules. Similarly, soaps and detergents are also amphiphilic, typically salts of fatty acids.
Due to their mixed feelings about water, amphiphilic molecules tend to accumulate in the oil-water interface, as shown in Fig. 3.3. This leads to a lowering of the interfacial tension, which, in turn, facilitates the mixing of oil and water. The resulting mixture is called an emulsion or dispersion. Amphiphilic molecules are also called interfacially active compounds, emulgators, or surfactants. They are of extreme technological importance not only in detergency, but also for processing foods and for making cosmetics, paints, and surface modifiers. Examples of interfaces in oil/water emulsions are shown in Fig. 3.3c–g. The organization of amphiphilic molecules at the interfaces of the emulsion is a consequence of many molecules acting in unison.

Nature has long ago discovered that amphiphilic molecules in the form of lipids are indispensable for “emulgating” living matter. Lipids are nature’s own surfactants. It is the amphiphilic character of lipids that give them a unique position among the molecules of life.
3.4 Self-Assembly and the Lipid Aggregate Family

When mixing lipids with water, the hydrophobic effect acts to make sure that the oily chains of the lipid molecules are screened as much as possible from water. This leads to a whole family of supramolecular aggregates that are formed spontaneously by self-assembly. The self-assembly process is a many-molecule effect, and it requires that many lipid molecules act together. The family of lipid aggregates is illustrated in Fig. 3.4. In all these lipid aggregates, the polar head of the lipids is hydrated by water and the fatty-acid chains are tugged away from the water.

The simplest and most ideal lipid aggregate form is the lipid monolayer in Fig. 3.4a. The lipid monolayer is a monomolecular film of lipids formed on the interface between water and air (or another hydrophobic substance, such as oil). We shall describe lipid monolayers in more detail in Chap. 10.
The lipid bilayer in Fig. 3.4b can be considered as two monolayers back to back like in a skinny oil-in-water mixture (cf. Fig. 3.3d). Several lipid bilayers often organize among themselves to form multi-lamellar structures, as shown in Fig. 3.4c. The forces that stabilize a stack of bilayers are of a subtle origin and will be dealt with in Sect. 5.3. Obviously, open ends cannot be tolerated, and the lipid bilayers have to close onto themselves and form closed objects, as shown in Fig. 3.4e and f. Such structures are called respectively uni-lamellar and multi-lamellar lipid vesicles or liposomes.

Images of vesicles and liposomes obtained by microscopy techniques are shown in Fig. 3.5. A uni-lamellar liposome constitutes the simplest possible model of a cell membrane. It should be noted that lipids extracted from biological membranes when mixed with water will self-assemble and can form lamellar lipid aggregates, as in Fig. 3.5, although they quite often form the non-lamellar structures described in Sect. 4.3.

The lipid aggregates in Fig. 3.5 are all characterized by being of planar or lamellar symmetry. This requires that the lipid molecules have a shape that is approximately cylindrical in order to fit in. If the shape is more conical, other aggregate symmetries may arise of curved form as described in Chap. 4. Nonpolar lipids like triglyceride oil do not form aggregates in water, whereas all polar lipids, except cholesterol, form aggregates in water.

The most important lesson from the observation of lipid self-assembly is that lipid aggregates, e.g., lipid bilayer membranes and hence biological membranes, owe their existence to water as the biological solvent. The aggregate and the solvent are inextricably connected. Lipid bilayers do not exist on their own in the absence of water. Moreover, the fact that lipid aggregates are formed and stabilized by self-assembly processes implies that they possess self-healing properties. If they are subject to damage, e.g., hole- or pore-formation in lipid bilayers, the damage is often repaired automatically by filling in holes and by annealing various defects.

It is instructive at this point to compare the self-organization and formation of lipid structures in water with another important self-assembly process of immense importance in biology, the process of protein folding, as illustrated in Fig. 3.6a–b. Most proteins are composed of both hydrophobic and hydrophilic amino acids. When exposed to water, proteins will undergo a folding process, which leads to a molecular structure that is a compromise between minimizing the exposure of the hydrophobic amino acid residues to water on the side and maximizing the interactions between the various amino acids in the sequence. These interactions involve electrostatic forces, hydrogen bonds, as well as sulphur bridges. The resulting structure is a delicate balance between these forces. Hence, the structure of the protein, and therefore also its function, is very sensitive to shifting this balance by changes in external conditions, e.g., temperature or pH. Such changes can induce a complete or partial unfolding of the structure, also termed denaturation. Many proteins are water soluble and exert their function in water. The folding process of
Fig. 3.5a–d. Uni-lamellar and multi-lamellar liposomes as obtained by microscopy techniques. (a) Small uni-lamellar liposomes of approximately 100 nm diameter. The picture is obtained by cryo-electron microscopy. Smaller vesicles are seen to be trapped inside some of the liposomes. (b) A large uni-lamellar liposome with a diameter around 70 µm. Smaller uni-lamellar liposomes are trapped inside. (c) A large multi-lamellar liposome with an outer diameter of 40 µm. (d) Cross section through an agglomerate of multi-lamellar vesicles shown from different angles.
trans-membrane proteins is subject to the extra complication that the folded protein has to come to terms with an environment that is both hydrophilic and hydrophobic. We shall return to this in Sect. 13.3.

### 3.5 Plucking Lipids

The stability of liquid water provided by the dynamic hydrogen bonding network has as a consequence that liquid water is more dense than solid water where the hydrogen bonding network is forced to be more fixed. This explains the well-known observation that ice flows on the top of liquid water. In this respect, water is very different from most other substances where the solid state usually is more dense than the liquid state. This highlights the special role of water as the only possible biological solvent. If ice would sink
to the bottom of lakes and oceans, life as we know it could not have evolved and survived under the climate conditions of planet Earth.

When dissolving amphiphilic and hydrophobic material like lipids and proteins in water, the water molecules closest to the hydrophobic material will be unable to engage in all four possible hydrogen bonds and will suffer from a change in the hydrogen bonding dynamics. This implies that the part of the water that can “feel” the hydrophobic material will be more structured and less dense than bulk liquid water. In other words, the water is depleted from the hydrophobic surface, which becomes effectively de-wetted. The density of this structured water is similar to that of amorphous ice, which is about 90% of bulk liquid water. This reduction in water density has, in fact, been observed in an experiment that can measure the density of water close to a layer of hydrocarbons. The results have been confirmed by computer-simulation calculations, as shown in Fig. 3.7. Figure 3.7 also leads to an estimate of the range over which water is perturbed by a hydrophobic surface. This range is about 1–1.5 nm.

The hydrophobic effect is accompanied by subtle changes in the ordering of the water dipole moments. In contrast to common belief, recent work

**Fig. 3.7.** A slab of water between two solid surfaces made of (a) a long-chain alcohol and (b) a long-chain hydrocarbon. The density of water near the hydrophobic surface in (b) is seen to be smaller. To the left are shown enlarged versions of the interfacial region illustrating how the hydrophilic alcohol surface is wetted by water and the hydrophobic hydrocarbon surface is de-wetted. The de-wetting is a direct manifestation of the hydrophobic effect
has shown that the water dipoles are more ordered near hydrophilic surfaces than hydrophobic surfaces. The higher degree of water ordering at hydrophilic surfaces implies a slowing down of the water diffusion along the interface.

The hydrophobic effect is usually quantified by assigning a so-called transfer free energy to the process of transferring hydrophobic molecules from a hydrophobic, oily phase into water. The Canadian biophysicist Evan Evans has succeeded in monitoring this transfer process on the level of a single molecule by “plucking” a single lipid molecule from a lipid bilayer membrane. This unique type of experiment is illustrated in Fig. 3.8. A single lipid molecule is targeted by binding of a receptor molecule (avidin) to a ligand (biotin) that is chemically bound to the head group of the lipid molecule. The receptor molecule, in turn, is linked to a micrometer-sized glass bead. The glass bead, in turn, is attached to the surface of a soft body, such as a swollen red blood cell or a liposome. This soft body acts like a spring whose spring constant can be varied by changing the tension of the body. This is done by pressurization, using a micro-pipette as shown in the figure. The resulting mechanic transducer, which is called a bioprobe force spectrometer, can be used to measure the force exerted on the soft body, e.g., when it becomes distorted during extraction of the lipid molecule from the target membrane, as illustrated in Fig. 3.8b. The force it takes to extract the lipid molecule depends on the rate by which the pulling is performed. Except when extracted extremely rapidly, the anchoring strength of a lipid molecule is very weak, typically 2–4 pN, thus providing a quantitative measure of the hydrophobic effect.

![Fig. 3.8. Extracting a single lipid molecule from a lipid bilayer using a bioprobe single-molecule force spectrometer developed by Evan Evans. (a) The spectrometer involves a micro-pipette, a soft body, e.g., a red blood cell or a liposome, and a glass bead (here shown with a cross). (b) The target lipid molecules in the membrane are associated with biotin moieties that can be chemically bound to the avidin receptor molecules linked to the glass bead](image-url)
4 Lipids Speak the Language of Curvature

4.1 How Large Is a Lipid Molecule?

The dimensions of a lipid molecule is determined by several factors. Firstly, there are obvious geometric factors like the size of the polar head, the length of the fatty acid tail, and the degree of unsaturation of the fatty-acid chains. Figure 4.1 shows examples where the molecules are inscribed by cylinders. Obviously, the longer the fatty acid tail is, the longer is the hydrophobic part of the molecule. The chains in this figure are stretched out as much as they can be. In the case of one or more double bonds, the end-to-end length of a chain will be shorter than for chains with fewer double bonds and the same number of carbon atoms. Double bonds will make the chain depart from the linear arrangement, as illustrated in Fig. 4.1c, and the approximation by a cylinder will be less good. For a given number of double bonds, the length of the hydrophobic part of a lipid molecule (and consequently the thickness of the lipid bilayer it may form, cf. Sect. 8.3) is linearly proportional to the number of carbon atoms in the chains. To illustrate this fact and for later reference, Fig. 4.2 shows a homologous family of di-acyl PC with two identical saturated chains.

The actual conformation of the molecule will influence its effective size. A conformation refers to the actual spatial arrangements of the atoms of the molecule. In Figs. 4.1 and 4.2 are drawn the conformations that have the lowest conformational energy, i.e., very ordered conformations in which all the C–C–C bonds occur in a zigzag arrangement (all-trans). However, temperature effect will lead to rotations, so-called excitations, around the C–C bonds and consequently to more disordered conformations. In this sense, lipids are qualitatively different from the other energy-producing molecules of the cell, the carbohydrates, which frequently are composed of stiff ring structures that allow for limited flexibility.

An example of a series of excited conformations of lipid molecules in a bilayer is shown in Fig. 4.3. It is clear from this figure that the long flexible chains of the lipids imply that the effective size and shape of the lipid molecules are very dependent on temperature. This property is of immense importance for the use of lipids in biological membranes. It is the source of the softness of lipid membranes, which is needed for their function.
Fig. 4.1a–c. Schematic illustration of the dimensions of lipid molecules. (a) Di-stearoyl phosphatidylethanolamine (DSPE). (b) Di-stearoyl phosphatidylcholine (DSPC). (c) Stearoyl-oleoyl phosphatidylcholine (SOPC)

Fig. 4.2. The homologous family of di-acyl PC lipids with two identical saturated chains. The figure also serves to define the acronyms traditionally used for these lipids. The numbers at the bottom denote the number of carbon atoms in the fatty-acid chains.
Only lipids with a limited degree of disorder will fit into a bilayer structure. In general, the average molecular shape has to be close to that of a cylindrical rod, as we shall see in Sect. 4.2 below. In a lipid-bilayer membrane in the physiological state, the typical cross-sectional area of this cylinder is about 0.63 nm$^2$ and its average length from 1.0–1.5 nm, depending on the chemical nature of the fatty-acid chains, in particular, the number of carbon atoms and the degree of saturation. The average length of the chains determines the hydrophobic thickness of the bilayer membranes it can form, as described in Sect. 8.3.

In this context, it should be remarked that there are two ways of forming double C–C bonds: cis-double bonds and trans-double bond. Nature usually makes cis double bonds in fatty acids. The trans-double bond leads to a less jagged chain, which has a significant ordering effect on the membrane lipids. This difference is part of the reason why trans-fatty acids in foods are less healthy than cis-fatty acids.
4.2 Lipid Molecules Have Shape

It may already have been noticed from Fig. 4.3 that temperature has an effect not only on the size of a lipid molecule but possibly also on its shape. The effective molecular shape is important for the ability of a lipid to form and participate in a bilayer structure. It is a matter of fitting. A word of caution is in order at this point. The use of the term shape can be misleading if taken too literally. A lipid molecule, when incorporated into a lipid aggregate like a bilayer, does not occupy a well-defined volume of a well-defined shape. At best the effective shape of a lipid molecule describes how its average cross-sectional area depends on how deeply it is buried in the lipid aggregate. Therefore, the effective shape is a property that is influenced by the geometrical constraints imposed by the aggregate. This will become more clear when in Sect. 8.2 we describe the various forces that act in a lipid bilayer. With this caveat we shall take the liberty to assign an effective shape to lipid molecules.

It has in recent years become increasingly clear that lipid shape is important for functioning. We shall demonstrate this by a specific example in Sect. 4.4 below and return to the mechanism of coupling curvature to protein function in Sect. 15.2.

The effective shape of a lipid molecule is determined by the compatibility between the size of the head group and the size of the hydrophobic tail. Full compatibility implies an effective cylindrical form. The effective shape of a lipid molecule, as a measure of its ability to fit into a particular lipid aggregate, is conveniently described by a packing parameter

$$P = \frac{v}{a \cdot l},$$

(4.1)

where $v$, $a$, and $l$ are defined in Fig. 4.4. Since the volume of a cylinder-shaped molecule is $a \cdot l$, a deviation of $P$ from unity suggests that non-lamellar aggregates can be expected. $P > 1$ corresponds to a shift from a cylindrical shape toward an inverted cone, whereas $P < 1$ corresponds to a shift toward a normal cone.

There are various ways of changing the effective shape of a lipid molecule by varying the relative sizes of the head and tail. A small head and a bulky tail and a large head and a skinny tail will produce conical shapes of different sense, as illustrated in Fig. 4.5. As we shall see later, this variability, which is peculiar for lipids, is used in a wide range of membrane processes.

4.3 Lipid Structures with Curvature

The effective shape of lipid molecules determines their ability to form a stable bilayer. The more non-cylindrical their shapes are, the less stable a bilayer they will form. This is illustrated in Fig. 4.6, where the two monolayers possess separately an intrinsic tendency to elastically relax toward a state of
finite curvature. The monolayers display a so-called spontaneous curvature. We shall return with a fuller description of spontaneous curvature in Sect. 6.2 in which we consider membranes as mathematical surfaces. When a bilayer is made of monolayers with nonzero spontaneous curvature, it becomes subject to a built-in frustration termed a curvature stress field. If the spontaneous curvatures of the two monolayers are different, the bilayer becomes asymmetric and itself assumes a nonzero spontaneous curvature. This is similar to a normal plastic tube, which, when cut open, will maintain its curved shape, whereas a piece of paper wrapped unto itself will not.
Fig. 4.5a–g. Effective shapes of lipid molecules. (a) Cylindrical: similar sizes of head and tail. (b) Cone: big head and skinny tail. (c) Inverted cone: small head and bulky tail (e.g., with unsaturated fatty-acid chains). (d) Going conical by increasing temperature. (e) Going conical by changing the effective size of the head group, e.g., by changing the degree of hydration or by changing the effective charge of an ionic head group. (f) Going conical by chopping off one fatty-acid chain, e.g., by the action of phospholipase A<sub>2</sub>, which forms a lysolipid molecule and a free fatty acid. (g) Going conical by chopping off the polar head group, e.g., by the action of phospholipase C.

Fig. 4.6. Illustration of the destabilization of a lipid bilayer composed of lipids with conical shapes that promote a tendency for the two monolayers to curve. Bilayers made of monolayers with a nonzero curvature have a built-in curvature stress.
If, however, the cohesion of the bilayer cannot sustain the curvature stress, the stress will force non-lamellar structures to form, as shown in Fig. 4.4. Less regular structures known as emulsions and sponge structures formed by lipids at curved interfaces in water will be described in Sect. 5.2. In all these curved structures, the lipids speak the language of curvature. The variety of lipid structures of different morphology is referred to as lipid polymorphism.

A particularly interesting structure is the inverted hexagonal structure in Fig. 4.4, which in the following will be called the $\text{H}_{\text{II}}$ structure. This structure is characterized by long cylindrical rods of lipids arranged as water-filled tubes. The diameter of the tubes can be varied by changing the type of lipid and by varying environmental conditions such as temperature, degree of hydration, as well pH. Despite their exotic appearance, both the inverted hexagonal structure, as well as the cubic structure described below, turn out to be of significance for the functioning of biological membranes, as described specifically in Sects. 4.3 and 4.4 and in several other places throughout the book. The other hexagonal structure, $\text{H}_{\text{I}}$, in Fig. 4.4 is not very common, except for very polar lipids and for lipids with very big head groups like lysolipids.

Cubic structures are much more complex than lamellar and hexagonal structures, and there are several types of them. They are bicontinuous in the sense that the water is divided into disconnected regions, one on each side of the lipid bilayer, which is curved everywhere. They are in a mathematical sense so-called minimal surfaces, i.e., surfaces that everywhere are in the form of a saddle with zero mean curvature (cf. Sect. 6.1). Even if cubic structures are subtle and appear exotic, they are related to biological function. It is an interesting observation that cubic phases can dissolve amphiphilic proteins, which can adapt locally to the curvature and, in certain cases, form micro-crystallites. This can be exploited for elucidation of membrane protein structure, as described in Sect. 15.2.

The propensity of the lipids for forming and stabilizing the $\text{H}_{\text{II}}$ structure is increased by shifting the balance between $l$ and $a$ in 4.1 (cf. Fig. 4.5) – for example, by decreasing the effective size of the head group (by dehydrating the system or screening the charge of the polar head by adding ions), increasing the temperature, increasing the degree of unsaturation of the fatty-acid chains, increasing the length of the fatty-acid chains, or decreasing the hydrostatic pressure. The reason for the last effect, which is important for deep-sea bacteria, is that the volume of lipids in water decreases slightly upon the application of pressure, corresponding to a stretching-out of the fatty-acid chains (cf. Sect. 19.2).

Since the self-assembly process of lipid molecules into aggregates of different morphology implies a subtle competition between forces of different origin and since many of the forces are of a colloidal and entropic nature, the relative stability of the resulting structures is intimately dependent on temperature, composition, and environmental conditions. In particular, increasing
temperature can drive a lamellar structure into an inverted hexagonal or cubic structure. Furthermore, incorporation of various hydrophobic and amphiphilic solutes such as hydrocarbons, alcohols, detergents, as well as a variety of drugs, can shift the equilibrium from one structure to another in the series shown in Fig. 4.4. For example, mono-acylglycerols, di-acylglycerols, tri-acylglycerols, alkanes, and fatty acids promote the \( \text{H}_2 \) structure, whereas detergents, lyso-phosphatidylcholine, digalactosyl diglyceride, certain antiviral peptides, as well as detergents inhibit the formation of the \( \text{H}_2 \) structure.

The shape of the cholesterol molecule in relation to membrane curvature deserves a special remark. Compared to the small head group, which is just a –OH group, the steroid ring structure shown in Fig. 2.5a, although hydrophobically rather smooth, is bulky, thus providing cholesterol with an inverted conical shape. Cholesterol, therefore, displays a propensity for promoting \( \text{H}_2 \) structures.

It should be remarked that the curvature of large lipid bilayer objects like the vesicles and liposomes shown in Fig. 3.5, is not caused by intrinsic, curvature stress, as described in the present section. The curvature in these cases is simply caused by the boundary conditions. Due to the hydrophobic effect, the bilayer would have to close onto itself, just like biological membranes have. Apart from very small vesicles, which have a large built-in tension, the curvature of liposomes is typically in the range of micrometers, which is much larger than the radii of curvature involved in hexagonal and cubic structures. For these structures, the radii of curvature are of the same order as the size of the lipid molecules.

It is a remarkable observation that lipid structures formed in water by the lipid extract from real biological membranes that are lamellar, often turn out to form non-lamellar phases despite the fact that the composition of these lipid extracts varies tremendously from organism to organism and among the different cell types for the same organism. In addition, more than half of the lipids naturally present in biological membranes, when studied as individual pure lipids, do not form bilayer phases, but rather cubic or inverted hexagonal structures. This may partly be related to the fact that non-lamellar-forming PE-lipids are abundant in both prokaryotic and eukaryotic cells. For example, 70% of the phospholipids in \textit{Escherichia coli} are PE-lipids. Another striking observation is that the lipid bilayer of natural membranes in many cases is found to be close to a transition from a lamellar structure to a non-lamellar structure. This transition, which is a phase transition, as described in Sect. 9.2, can be triggered globally in bilayers made of lipid extracts from the real membranes by using the principles of shape changes illustrated in Fig. 4.5.

It is not desirable for functional biological membranes to deviate globally from a lamellar symmetry, possibly with the exception of the very curved and convoluted membranes of Golgi and the ER, cf. Fig. 1.5. However, the presence of non-lamellar structures as virtual states leads to a curvature stress
field in the membrane. The stress field can in fact be changed enzymatically by specific enzymes that change the H$_{11}$ propensity of the lipids while they reside in the bilayer, e.g., by enzymatically cleaving off the polar head or removing one of the fatty-acid chains. The resulting stress may be released locally, e.g., by changes in the local molecular composition, by binding a protein or hormone, or by budding of the membrane as an initiation of a fusion process. We shall in Sect. 15.2 return to these phenomena in connection with the way lipids can control membrane function.

4.4 Microorganisms’ Sense of Curvature

An interesting series of studies have been performed on a simple unicellular organism, *Acholeplasma laidlawii*, which show that the lipid composition of this organism is regulated to preserve spontaneous curvature under diverse living conditions. The results suggest that the propensity for forming an H$_{11}$ structure may be a signal for cell growth.

*Acholeplasma laidlawii* is a so-called mycoplasma that is even simpler than bacteria. It is deficient in synthesizing fatty acids and therefore has to do with the fatty acids it feeds on. The fatty acid composition of its membrane will therefore reflect which fatty acids the mycoplasma selects from its food. However, *Acholeplasma laidlawii* contains specific enzymes that are able to change the polar head group of the lipids. In particular, it can choose to vary the size of the head group by using different sugar groups. It turns out that the organism regulates, given a specific diet of fatty acids, the ratio between glycolipids with different head group sizes in order to compensate for fatty acids that do not pack well into the membrane due to their shape.

In a series of experiments performed by the Swedish chemists Åke Wieslander, Göran Lindblom and their collaborators, *Acholeplasma laidlawii* was fed mixtures of saturated (palmitic) and mono-unsaturated (oleic) acids in different compositions. Being the tail of a phospholipid, oleic acid is expected to have a larger propensity for forming an H$_{11}$ structure as compared to palmitic acid. These fatty acids were found to be effectively incorporated into the lipids produced by the organism and constituted more than 90% of the fatty acid content of the plasma membrane after adaptation. The total lipid content was then extracted, and when mixed with water it was found to form an H$_{11}$ structure. The spontaneous curvature, $C_0$, of this structure was measured by X-ray analysis. The result showed that $C_0$ was nearly constant for a wide range of different compositions of the food. In contrast, the corresponding $C_0$-values of the pure lipids, which are believed to dominate the spontaneous curvature, were found capable of varying within a much larger region. Moreover, it was found that the ratio of large head groups to small head groups adopted by *Acholeplasma laidlawii* varied almost proportionally to the ratio of oleic acid to palmitic acid. Hence, large contents of bulky oleic chains were compensated by similarly large amounts of large head groups,
and vice versa. Consequently, the particular value of the spontaneous curvature must in some way be optional for the functioning and growth conditions of the cell.

Hence, it appears that *Acholeplasma laidlawii* is an organism that is able to homeostatically regulate the lipid composition of its membrane in order to maintain a constant spontaneous curvature and hence a constant propensity for forming non-lamellar structures. This is achieved simply by playing around with the compatibility between the head group size and tail size of the lipids in order to obtain the right molecular shape for packing effectively into the lipid bilayer membrane.

Other microorganisms, including *Escherichia coli*, have been proposed to regulate their membrane properties by a similar principle based on lipid molecular shape and optimal packing. It is at present unknown by which mechanism the lipid synthesis is regulated by the curvature stress field of the membrane and which membrane-bound proteins are involved. We shall in Sect. 15.2 discuss possible physical mechanisms of coupling membrane curvature to the functioning of proteins.
5 A Matter of Softness

5.1 Soft Matter

Most biological matter, like membranes, are soft materials. Soft materials have a number of unusual properties that are very different from those of traditional hard materials such as metals, ceramics, semi-conductors, and composites. Lipid membranes are soft because they are basically structured liquids made of molecules with substantial conformational complexity. At the same time, they have tremendous durability and toughness over ordinary liquids due to the fact that they owe their existence to the self-assembly principles described in Sect. 3.4. As we shall see throughout the remainder of this book, the softness is a requirement of the various modes of function that membranes engage in. Technological applications of soft-matter systems made of lipids will be described in Chap. 20.

Soft materials refer to a vast and ubiquitous class of structured and complex systems that include polymers, supramolecular aggregates, emulsions, colloids, liquid crystals, as well as membranes. Examples from daily life are syrup, ketchup, glue, paint, toothpaste, egg white, and silly putty. All these systems exist in a condensed phase, but none of them can be described unambiguously as a liquid or a solid. As opposed to conventional solid materials, the physical properties of soft materials are largely determined by soft and fluctuating interfaces, the physics of which is dominated by entropy. Softness implies high deformability, but not necessarily high bulk compressibility. Furthermore, soft matter is usually anisotropic and constructed in a hierarchical manner with structure occurring on several different length scales that are connected in subtle ways.

5.2 Soft Interfaces

Figure 5.1 shows a gallery of examples of systems with soft interfaces of increasing complexity. All of these systems are structured liquids, and they are basically a collection of soft fluid interfaces. The interfaces are fluid in the sense that there is no fixed relationship between nearest-neighbor molecules within the interface. They exert no resistance to shear forces. A particularly peculiar structure is the sponge phase, which can be considered a disordered
Fig. 5.1a–e. Examples of systems with fluid soft interfaces. (a) Liquid-liquid interface enriched in interface-active molecules (e.g., amphiphiles like soaps, detergents, or lipids). (b) Di-block co-polymers in a lamellar phase. A di-block co-polymer consists of two incompatible polymers that are chemically linked together. (c) Micro-emulsion, which is a complex collection of convoluted and fluctuating interfaces covered by interface-active molecules like lipids. (d) Sponge phase, which is a disordered variant of the bicontinuous cubic lipid phase in Fig. 4.4. (e) A collection of lipid vesicles.

variant of the cubic phase. The sponge phase is also bicontinuous and can be compared with a complex arrangement of tubes. This has led to its nickname, “the plumber’s nightmare.” It consists of curved lipid bilayers, but it is not a liquid crystal. We shall have a closer look at this phase in Sect. 6.1.

For comparison, Fig. 5.2 shows a related set of soft matter systems. These systems are also characterized by interfaces. However, in contrast to the interfaces in Fig. 5.1, the interfaces in Fig. 5.2 share some of their internal
5.2 Soft Interfaces

**Fig. 5.2a–c.** Examples of soft matter systems with tethered interfaces. (a) A polymer chain characterized by a tethered string of beads with fixed connections. (b) A tethered two-dimensional membrane resembling a cytoskeleton with fixed connectivity attached to a fluid lipid bilayer with dynamically changing connectivity. (c) Electron microscopy image of the spectrin network, which is part of the cytoskeleton of the red blood cell. The size of the image is about 500 nm × 500 nm.

Properties with solids. They are all tethered, implying that they have a fixed relationship between neighboring molecules and therefore display shear resistance. The polymer chain in Fig. 5.2a is a one-dimensional tethered string in which neighboring monomers are bound by chemical bonds. Although flexible on long length scales, the polymer displays some rigidity at a shorter scale, referred to as the persistence length. The tethered network in Fig. 5.2b is a two-dimensional generalization of a polymer. Each node in this network is tethered to a certain number of its neighbors. Also, this sheet is flexible but provides a certain shear resistance. A tethered interface is like a sheet of vulcanized rubber. The mechanical, conformational, and statistical properties of tethered interfaces are very different from those of fluid interfaces. In Fig. 5.2c is shown a biological realization of such a tethered network, the spectrin skeleton of a red blood cell. Spectrin is a protein that can cross-link with other spectrin molecules into a two-dimensional network. It serves here to provide the blood cell with its characteristic shape. We shall come back to this in Sect. 6.4.

The properties of fluid interfaces and surfaces are most often controlled by the interfacial tension

\[ \gamma = \left( \frac{\partial G^s}{\partial A} \right)_V, \tag{5.1} \]

where \( G^s \) is the Gibbs excess free energy, \( V \) is the volume, and \( A \) is the area of the interface. The interfacial tension, which acts so as to make the interface as small as possible, imparts a certain stiffness to the interface. The interface can be softened by introducing interfacially active molecules, e.g., amphiphiles like lipids, which accumulate in the interface and lower the interfacial tension. If there is a sufficient amount of amphiphiles, the interface can be fully covered, as shown in Fig. 5.1a and c. This implies that the area is essentially fixed and the interfacial tension tends toward zero. In that case,
which is true for many fluid membranes, the stability and conformation of the interface is controlled by conformational entropy and the elasto-mechanical properties of the interface.

An interface can be considered soft in several ways. It can be easy to bend, as illustrated in Fig. 5.3a; it can be easy to compress or expand, as illustrated in Fig. 5.3b; or it can be easy to shear, as illustrated in Fig. 5.3c. In the case of a fluid interface, which is the case pertaining to the lipid bilayer of a biological membrane, the resistance to shearing is nil, and we can neglect that mode. The two other ways of deforming the interface are associated with two parameters, two elasto-mechanical modules, termed the bending modulus, $\kappa$, and the area compressibility modulus, $K$, respectively. The area compressibility modulus is defined via the energy per unit area, $E_K$, one has to spend in order to uniformly stretch an interface of area $A_o$, to produce an area change of $\Delta A$ according to the well-known Hooke’s law for an elastic spring:

$$E_K = \frac{1}{2} K \left( \frac{\Delta A}{A_o} \right)^2.$$  
(5.2)

The bending modulus for a flat interface (we shall in Sect. 6.1 describe the case of curved interfaces) is defined via the energy per unit area, $E_\kappa$, that is required to produce a mean curvature, $H$, of the interface according to

![Fig. 5.3. Bending (a) and stretching (or compressing) (b), or shearing (c) a soft interface like a membrane. The curvature of an interface is characterized geometrically by the two radii of curvature, $R_1$ and $R_2$, indicated in (d)](image-url)
where the mean curvature is given by the two principal radii of curvature, \( R_1 \) and \( R_2 \), defined in Fig. 5.3d, as

\[
H = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right). \tag{5.4}
\]

In the definition of the bending energy, \( E_\kappa \), we have assumed that there is no internal structure of the interface and that there are no constraints imposed by boundaries, i.e., that the interface has to close onto itself. We shall come back to this constraint in Chap. 6. Obviously, the two modules \( \kappa \) and \( K \) must be related. It can be shown that this relation in the simplest case can be written as \( \kappa = d_L^2 K \), where \( d_L \) is the thickness of the interface.

In Fig. 5.4 are shown the contours of two closed soft interfaces, a giant liposome and a red blood cell membrane. Both of these bodies are soft, but their ability to bend is different. The length scale over which they appear flat and smooth, i.e., the persistence length, \( \xi \), is different. The persistence length is indeed related to the bending modulus via the relation

\[
\xi \sim \exp \left( \frac{cK}{k_B T} \right), \tag{5.5}
\]

where \( c \) is a constant. According to this equation, it is the ratio between the bending modulus and the thermal energy, \( k_B T \), that determines the persistence length. Hence, \( \xi \) depends exponentially on the bending modulus.

**Fig. 5.4a–b.** Examples of two closed soft interfaces. (a) The contour of a giant liposome of 60 \( \mu \)m diameter imaged by fluorescence microscopy. (b) A red blood cell of 5 \( \mu \)m diameter
Liposomal membranes can easily be prepared to be very soft and exhibit low values of $\kappa$, as described in Sect. 5.4 below. This allows for very substantial thermally driven fluctuations and surface undulations. Plasma membranes of cells usually have bending modules that are considerably larger than the thermal energy, $\kappa \gg k_B T$, so the persistence length for the membrane is larger than the size of the object. Hence, the plasma membrane in Fig. 5.4b appears smooth. It is interesting to note that some internal membranes in eukaryotic cells, e.g., Golgi and endoplasmic reticulum, as shown in Fig. 1.5, appear to be very soft and strongly convoluted, sometimes exhibiting nonspherical topologies. As we shall discuss in Sect. 9.4, the absence of cholesterol in these membranes may partly explain their apparent softness. Cholesterol tends to increase the value of the bending rigidity $\kappa$.

### 5.3 Forces Between Soft Interfaces

The softness of interfaces in general and membranes in particular has some striking consequences for the colloidal forces that act between them. A colloidal force is a thermodynamic force. In contrast to a mechanical force, which is determined by the gradient of a mechanical energy (or enthalpy, $H$) the colloidal force, $F$, is a spatial derivative of a free energy, $G = H - TS$, given by

$$F = -\left(\frac{\partial G}{\partial r}\right) = -\left(\frac{\partial H}{\partial r}\right) + T\left(\frac{\partial S}{\partial r}\right),$$

(5.6)

where $r$ is the distance between the objects. Hence, the colloidal force involves the entropy, $S$. This implies that there is always an entropic repulsion between soft interfaces, even in the extreme case (like an ideal gas) where there are no direct mechanical forces in effect and the first term of the right-hand side of (5.6) tends to zero. It is the reduction in configurational entropy due to the confinement that produces the repulsive force.

Several examples of this scenario are illustrated in Fig. 5.5. The extreme case is that of a micro-emulsion, which is basically a dense gas of very soft, strongly repelling and fluctuating interfaces, as shown in Fig. 5.5f. A special version of a colloidal particle covered by polymers is a liposome incorporated with special lipids, so-called lipopolymers, to whose head groups are attached polymer chains. Such liposomes are called *stealth liposomes* for reasons that will be revealed in Sect. 20.3. These liposomes can be used as drug carriers to circumvent the immune system, partly because of the entropic repulsion that results from the softness of the polymer cushion on their surface.

An explicit expression for the entropic *undulation force* (sometimes referred to as an osmotic pressure) acting between a stack of soft interfaces like in Fig. 5.5e with spacing $d$ was derived by the German physicist Wolfgang Helfrich to be

$$F \sim \frac{(k_B T)^2}{\kappa d^3}.$$  

(5.7)
5.4 Lipid Membranes are Really Soft

A visual impression of the softness of a lipid bilayer in the form of a unilamellar liposome can be obtained from Fig. 5.6a, which shows a series of contours of a giant liposome observed in a microscope at different times. A substantial variation in the contour is seen over time. This is a manifestation of thermally induced surface fluctuations or undulations. The intensity of the fluctuations, considering the size of the liposome in relation to the thickness of the bilayer, shows that lipid membranes are really soft. The softness in terms of the bending modulus, $\kappa$, can be extracted from an analysis of the spectrum of fluctuations.

The softness of a lipid bilayer in terms of its area compressibility modulus, $K$, can be studied using micro-mechanical techniques. Using a glass pipette with a very small diameter, of about 1 to 10 µm, it is possible by aspiration to apply a stress, $\tau$, to the membrane and subsequently measure the resulting area strain, $\Delta A/A_0$, as illustrated in Fig. 5.6b, simply by measuring the expansion of the membrane into the pipette. Area strains of up to a couple of percent can typically be obtained before the membrane is broken. $K$ can subsequently be determined from (5.2), since $\tau = K(\Delta A/A_0)$.

According to this equation, the repulsive force increases as the bending rigidity, $\kappa$, is diminished. This has important consequences for the interaction between lipid membranes, as shown in Sect. 5.4 below.
Fig. 5.6. (a) Fluctuating liposomes of diameters around 50 µm. (b) Aspiration of a giant vesicle of diameter 32 µm into a micro-pipette. Suction pressure is increased from top to bottom, leading to a change in vesicle area.

To get a feeling of the softness of natural materials in the form of membranes and lipid bilayers it is instructive to compare it to the softness of a man-made material, e.g., a simple soft plastic like polyethylene. When it comes to bending, a DMPC lipid bilayer is about five times softer than a red blood cell membrane, which, in turn, is 50,000 times softer than a film of polyethylene of the same thickness. No wonder a closed bag of polyethylene cannot do what a red blood cell must do in its life span of 120 days in circulation: it travels 400 km, and during its excursions into the fine and narrow blood capillaries it has to stretch and bend to change its shape by a very large amount, more than 100,000 times without falling apart. In terms of the area compressibility, a DMPC bilayer is about ten times softer than a red blood cell membrane, which, in turn, is about five times softer than a film of polyethylene of the same thickness.
The reason why the membrane of the red blood cell is less soft than a DMPC bilayer is that the red cell has a cytoskeleton. If the lipids are extracted from the red cell membrane and re-formed as a lipid bilayer, this bilayer is considerably softer but is still less soft than DMPC bilayers. The reason for this is that the red blood cell, being an eukaryote (although without a cell nucleus), has a plasma membrane that contains large amounts of cholesterol, typically 30%. Cholesterol tends to make membranes less soft, both in terms of bending stiffness and area compressibility. Other factors that influence the softness are fatty-acid chain length and degree of saturation of the chains.

The general trend is that shorter and more unsaturated chains provide for greater softness. Furthermore, various solutes can influence the softness quite dramatically. The typical values of the elasto-mechanical modules for lipid bilayers correspond to energies that are in the range of the thermal energy, $k_B T$, e.g., $\kappa$ for DMPC is around $10 k_B T$. Hence, the elastic membrane fluctuations are expected to be very sensitive to temperature. This will have some dramatic consequences at membrane phase transitions, as discussed in Chap. 12.

A conspicuous consequence of undulation forces experienced by bilayers that become soft is that a lipid bilayer or a membrane that is adsorbed to a solid surface should be repelled from the surface if the bilayer is softened. This effect, which may be of importance for cell-cell adhesion and possibly for the motility of unicellular organisms, has indeed been observed. Two examples are illustrated schematically in Fig. 5.7. Figure 5.7a shows soft lipid bilayers that are being repelled from a hard surface and from each other by undulation forces, leading to unbinding. Figure 5.7b shows a vesicle or cell-like object that, due to renormalization of its bending modulus, is made to hop off the surface to which it adheres.

The strong effects on the softening of lipid bilayers discussed above is due to fluctuations in density. Bilayers can also be made softer by compositional fluctuations. In this case, the softening is due to local variations in the

![Fig. 5.7.](image)

(a) Lifting off bilayers from a stack by undulation forces. (b) Lifting off a vesicle or cell from a surface by undulation forces
composition. Close to so-called critical mixing points, the membrane composition can fluctuate strongly, as described in Sect. 9.3. Hence, at any given time, the local composition at a given place in the bilayer can be very different from the average global composition of the bilayer. We shall have a closer look at this phenomenon in Sect. 11.2. Both fluctuations in density and composition tend to lower the bending modulus, $\kappa$.

One of the major questions we shall address in the following chapters is the microscopic and molecular origin of membrane softness and how it is manifested in membrane structure on the nanometer scale. This may provide some clues as to how the softness eventually can be controlled. It is the hypothesis that the lipid-bilayer softness, the dynamic structure of the membrane, and the corresponding lipid organization are important regulators of membrane function and the ability of the membrane to support biological activity. A consequence of this hypothesis is that the generic effects of peptides, proteins, and drugs on membrane structure and function, on the one side, and the influence of bilayer structure on these compounds, on the other side, may be understood in part by the ability of these compounds to alter lipid-bilayer softness and molecular organization.
6 Soft Shells Shape Up

6.1 Bending Interfaces

It is instructive to consider the spatial dimensions of a membrane system like a uni-lamellar vesicle, as shown in Fig. 3.5. Whereas the lipid bilayer itself is only about 5 nm thick, the diameters of vesicles and liposomes are orders of magnitude larger, typically in the range of 50 nm to 50,000 nm. Hence, lipid bilayers are extremely thin films of tremendous anisotropy. It is therefore to be expected that some of the generic properties of vesicles and possibly cells can be understood and described by considering the membranes as infinitely thin shells associated with unique material characteristics. Many theoretical approaches to determine membrane conformations, topology, and shapes therefore assume the membrane to be a two-dimensional liquid interface imbedded into a three-dimensional space.

A liquid interface exhibits no resistance to shearing. Therefore, when it is mechanically deformed, there are only the two possible modes of deformation illustrated in Fig. 5.3a and b: bending and stretching/compressing. If we for a moment assume that the interface is infinitely thin with no internal structure and its area furthermore is fixed, we can neglect area compressibility and are then left with the sole possibility of bending. Bending leads to curvature, and the curvature of the interface at any given point is described by two principal radii of curvature, $R_1$ and $R_2$, as illustrated in Fig. 6.1.

In order to determine the most likely shape of an interface that is left alone, we need an expression for the energy that is involved in the bending. The German physicist Wolfgang Helfrich formulated in 1973 the most general expression for the elastic bending energy, $dE_{\text{surface}}$, that is required for deforming an element of area $dA$ to a shape described by $R_1$ and $R_2$ (cf. (5.3) and (5.4))

$$dE_{\text{surface}} = \left[ \frac{\kappa}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right)^2 + \frac{\kappa_G}{R_1 R_2} \right] dA. \quad (6.1)$$

The total bending energy for deforming the entire interface called $S$ is then the sum (i.e., the integral) over all the elements of area, i.e.,

$$E_{\text{surface}} = \int_S dE_{\text{surface}} = E_{\kappa} + E_{\kappa_G}. \quad (6.2)$$
Fig. 6.1. (a) The two principal radii of curvature describing the local curvature of a mathematical interface. (b) From left to right: “Sphere”, where $R_1$ and $R_2$ have the same sign, in this case positive. The mean curvature, $H = 1/2 (1/R_1 + 1/R_2)$, is nonzero. “Cylinder”, where $R_1 = \infty$ and $R_2 > 0$. The mean curvature is nonzero. “Saddle”, where $R_1$ and $R_2$ have opposite sign. For the special case of a minimal surface, $R_1 = -R_2$, and the mean curvature is zero. (c) Closed interfaces of different topology characterized by different values of the genus number $g$.

From these expressions we see that, in addition to the normal bending modulus $\kappa$ (also called the mean curvature bending modulus), which we considered in Sect. 5.2 and in (5.3), there is an additional property of the interface, $\kappa_G$, that will determine the bending energy and hence the shape of the interface. $\kappa_G$ is the so-called Gaussian curvature modulus (or the saddle-splay modulus).

The Gaussian curvature modulus, $\kappa_G$, controls the topological complexity of the interface. This is most easily seen by noting that for a closed interface, the contribution from the Gaussian curvature to the energy of the interface is proportional to a topological invariant, $4\pi(1 - g)$, according to
\[ E_{\kappa_G} = \kappa_G \oint_S \frac{1}{R_1 R_2} \, dA = \kappa_G 4\pi (1 - g). \] (6.3)

This relationship is purely mathematical and is known as the Gauss-Bonnet theorem. \( g \) is the so-called genus number, which describes the topology of the closed interface, as illustrated in Fig. 6.1c. For a sphere, \( g = 0 \). More complex surfaces with holes have higher values of \( g \). The value of the Gaussian curvature modulus is difficult to determine experimentally. \( \kappa_G \) is generally believed to be of the same order of magnitude as the mean curvature modulus, \( \kappa \).

In order to get an intuitive feeling about the contribution of these two conceptually very different terms to the bending energy of an interface, let us consider a special class of interfaces or surfaces that are called minimal surfaces. Minimal surfaces have zero mean curvature everywhere, i.e., \( H = 0 \).

A flat lamellar interface and the interface defined by the cubic structure in Fig. 6.2 are examples of minimal surfaces. For lamellar and cubic structures, which are perfectly ordered at very low temperatures, as shown to the left in Fig. 6.2, the mean curvature is identically zero, \( H = 1/2 (1/R_1 + 1/R_2) = 0 \), and \( E_{\kappa} = 0 \). For the lamellar structure, \( E_{\kappa_G} = 0 \), whereas it is different from zero for the cubic structure. Obviously, the cubic structure, which is a bunch

![Diagram of minimal surfaces](image)

**Fig. 6.2.** Minimal surfaces with zero mean curvature: the lamellar (a) and the cubic structure (b). The surface structures have perfect order corresponding to low temperatures. Fluctuations at elevated temperatures lead to the fluctuating lamellar structure in (c) and the sponge structure in (d), respectively. The associated elastic bending energy is related to the bending modulus, \( \kappa \). For negative values of the Gaussian curvature modulus, \( \kappa_G \), the lamellar structure becomes unstable toward formation of the ordered cubic structure and the sponge structure, which have a more complex topology than the lamellar structure.
of saddles, will be stabilized for large negative values of $\kappa_G$, whereas the lamellar structure will be stabilized for large positive values of $\kappa$.

When fluctuations are introduced, e.g., by increasing the temperature, both the lamellar and the cubic structure will be able to assume some local mean curvature. As shown to the right in Fig. 6.2, this leads to undulations on the lamellar interface and possibly to a disordering of the cubic structure into a sponge structure. The mean curvature modulus, $\kappa$, serves to control the amplitude of the thermal fluctuations by assuring that the deviation from the zero mean curvature is as small as possible.

6.2 Spontaneous Curvature

In the description of the bending of fluid interfaces in Sect. 6.1 above, we assumed that the interfaces had no internal structure and were infinitely thin. For real interfaces like lipid monolayers and bilayers, the internal structure of the interface and its thickness have to be taken into account. As described in Sects. 4.2 and 4.3 and illustrated in Fig. 4.6, lipid monolayers can exhibit spontaneous curvature due to the fact that lipid molecules have shape.

When two symmetric lipid monolayers have to live together in a bilayer, the bilayer itself has no intrinsic tendency to curve and its spontaneous curvature, $C_0$, is zero. However, if the two monolayers are chemically different, the bilayer prefers to curve and has a finite spontaneous curvature $C_0 = R_0^{-1}$. As we shall see shortly, there are several other possibilities of inducing an asymmetric condition for the bilayer to induce a spontaneous curvature. The description of the bending energy of a membrane has to take into account that the bilayer can have an intrinsic desire to bend when left alone. This is readily done by rewriting (6.1) as

$$dE_{\text{surface}} = \left[\frac{\kappa}{2} \left(\frac{1}{R_1} + \frac{1}{R_2} - \frac{2}{R_0}\right)^2 + \frac{\kappa_G}{R_1 R_2}\right] dA.$$  \hspace{1cm} (6.4)

For fixed topology, the bending energy will then be minimal when the bilayer assumes a curvature corresponding to its spontaneous curvature. We shall in the following restrict ourselves to considering only membranes of spherical topology.

There are a number of different sources for spontaneous curvature in lipid bilayers and biological membranes in addition to the simple one due to a possible chemical difference between the two lipid monolayers. Figure 6.3 gives a schematic presentation of a closed membrane vesicle that is subject to various asymmetric conditions leading to a nonzero spontaneous curvature. Some of these conditions can be different for different vesicles in the same preparation, even if they are made of the same lipids. The intrinsic spontaneous curvature can also vary from vesicle to vesicle, and it is usually difficult to measure experimentally.
6.3 Shaping Membranes

Before we are ready to discuss shapes of closed membranes we have to mention another circumstance that can make it necessary to consider different vesicles independently. It has to do with a fairly obvious but often overlooked fact. A lipid membrane in the form of a vesicle has a history in the sense that it is made of a lipid bilayer which, in order to avoid problems with an open boundary, at some stage closes into a closed shape. This implies that the initially unstressed area of the inner monolayer, \( A_{\text{inner}} \), and the initially unstressed area of the outer monolayer, \( A_{\text{outer}} \), have to compress or stretch in order to produce a closed vesicle with a fixed mean area of \( A = \frac{1}{2}(A_{\text{inner}} + A_{\text{outer}}) = \frac{1}{2}(A_{\text{inner}}^{\circ} + A_{\text{outer}}^{\circ}) \). This implies that, although the mean area is fixed, there can be deviations in the differential area, \( \Delta A = A_{\text{inner}} - A_{\text{outer}} \), from its equilibrium value, \( \Delta A_{\circ} = A_{\text{inner}}^{\circ} - A_{\text{outer}}^{\circ} \).

Stressing the two monolayers introduces an extra term in the total energy for the membrane, which includes the area compressibility modulus, \( \kappa \), and the bilayer thickness, \( d_L \). This so-called Area-Difference-Energy (ADE) term, which was first anticipated by the Canadian biophysicist Ling Miao and her collaborators, has the form...
\[ E_{ADE} = \frac{\alpha \kappa \pi}{2 Ad_L^2} (\Delta A - \Delta A_c)^2, \] (6.5)

where \( \alpha \) is a constant that is close to unity for all phospholipids. In contrast to the spontaneous curvature, the differential area is not an intrinsic property of the bilayer but set by the way the closed bilayer membrane happens to be prepared.

The total bending energy, which will determine the equilibrium shape of a closed lipid vesicle, is then given by

\[ E_{\text{total}} = \kappa \frac{1}{2} \int_S \left( \frac{1}{R_1} + \frac{1}{R_2} - \frac{2}{R_0} \right)^2 dA + \frac{\alpha \kappa \pi}{2 Ad_L^2} (\Delta A - \Delta A_c)^2. \] (6.6)

The equilibrium shape of a given fluid vesicle is obtained by minimizing \( E_{\text{total}} \), given the geometrical parameters of the vesicle, i.e., area \( A \) and volume \( V \), and the materials parameters, i.e., spontaneous curvature \( R_0^{-1} \) and preferred differential area \( \Delta A_c \). The effects of temperature are neglected here. This is a reasonable procedure since the bending modulus is typically ten times the thermal energy, \( \kappa \sim 10 k_B T \). The results for the equilibrium shapes are given in the form of a phase diagram as shown in Fig. 6.4.

The phase diagram exhibits an enormous richness of shapes. The theoretically predicted shapes as well as the transitions between different shapes are generally in good agreement with findings in the laboratory. In Fig. 6.5 are shown a series of vesicles with different shapes. For each type of shape, the vesicles will under experimental circumstances exhibit thermally induced fluctuations in shape.

When varying the parameters that determine the shape according to the phase diagram, transitions from one type of shape to another can be observed. This is most easily done by varying the osmotic pressure across the membrane, e.g., by changing the sugar solution on the outside. The transition will take some time, since it takes some time for water to diffuse across the bilayer to establish the new equilibrium. An example of the transition from one shape to another is illustrated in Fig. 6.6. This figure shows a time sequence of the transition across the line from pear-shaped vesicles to a vesicle with a bud, cf. the phase diagram in Fig. 6.4. This transition is called the budding transition.

### 6.4 Red Blood Cells Shape Up

Red blood cells have plasma membranes that are much more complex than the lipid membranes we have considered so far. In addition to the fluid lipid bilayer, the red blood cell membrane has a cytoskeleton that is a cross-linked network of the protein spectrin. Therefore, bending and changing the shape of a red blood cell membrane involve both deformations of the lipid bilayer as well as elastic deformation of the cytoskeleton, which is a tethered membrane.
Fig. 6.4. Phase diagram predicted for closed vesicle membranes. Stable shapes are given as a function of two reduced parameters: the reduced vesicle volume, $v$, where $v = 1$ corresponds to a sphere, and a parameter, $\Delta a$, which is a combination of spontaneous curvature and preferred differential area. $\Delta a$ is a measure of the preferred curvature of the vesicle. The names of several of the shapes refer to the shapes that have been found for red blood cells with a certain resistance to shearing, as illustrated in Fig. 5.2b and c. Our understanding of the relationship between the morphology of lipid bilayer vesicles described above and the shapes of red blood cells is still very premature. However, some of the gross aspects of the red blood cell shapes indicate
that they are governed by a similar physics related to bending elasticity as simple lipid vesicles are.

A healthy blood cell has a biconcave shape, a so-called discocyte, as illustrated in Fig. 6.7. It has been known for many years that this shape can be turned into other shapes by the influence of a number of factors such as osmotic pressure, cholesterol content, pH, level of ATP, as well as various other externally added amphiphatic compounds. Some of these other shapes are characteristic of various diseases. Two shapes are of particular interest, the so-called stomatocyte, shown in the left-hand side of Fig. 6.7, and the echinocyte, shown in right-hand side of Fig. 6.7. The normal discocyte has a shape in between these two extremes. The curious thing is that when the lipids are extracted from the cell membrane, the shape of the spectrin network becomes almost spherical. Hence, the precise red blood cell shape is
a manifestation of a coupling between the lipid bilayer properties and the elastic properties of the cytoskeleton.

The Canadian physicist Michael Wortis and his collaborators have shown that the sequence of human red blood cell shapes, from the stomatocyte, over the normal discocyte, to the spiked echinocyte, can be described theoretically by the ADE-model described in Sect. 6.3 above when including the stretch and shear elasticity properties of the protein-based cytoskeleton. The correlation between the experimental shapes and the theoretical predictions is surprisingly good, as seen by comparing the two panels in Fig. 6.7. Within the model, a single parameter describes the transition between the different shapes. This parameter is related to the preferred differential area $\Delta A_0$ in (6.6), which is a measure of the relaxed area difference between the inner and the outer monolayer leaflets of the lipid bilayer membrane. The beauty of this model lies in the fact that the red blood cell shapes can be described by a general physical model and the detailed biochemistry only enters in the way it determines the actual value of $\Delta A_0$. As an example, it is known that cholesterol is predominantly incorporated in the outer leaflet, leading to an increase in $\Delta A_0$, thereby shifting the shape towards the echinocyte. This is consistent with experimental observations. Similarly, cholesterol depletion of red cells leads to stomatocytes, which correlates to a lowering of the preferred differential area.
7 Biological Membranes – Models and Fashion

7.1 What Is a Model?

A *model* is an abstraction of nature. It can be very concrete and practical, e.g., given by a protocol to construct a particular sample for experimental investigation, or it can be given by a precise mathematical formula that lends itself to a theoretical calculation. A model can also be less well-defined and sometimes even implicit in the mind of the researcher. The concept of a model or *model system* is one of the cornerstones in natural sciences. It is a powerful and necessary tool to facilitate our perception of complex natural phenomena. The model helps us ask some relevant and fruitful questions out of the millions of possible questions that can be asked. It helps us guide experiments and perform theoretical calculations. And it is instrumental in interpreting the results of our endeavors. A good model is a blessing, but it can also be a curse. It may bias our thinking too strongly if we forget that it is just a model and not Nature herself. Models must constantly be scrutinized and questioned, even the most successful ones, not least because models reflect fashion among scientists.

A key element in the formulation of a useful model of such a complex system like a biological membrane is to strike the proper balance between general principles and specific details – or to balance the sometimes conflicting demands for truth and clarity. This can be illustrated by the photo of a ship in Fig. 7.1a. The construction blueprint in (b) seeks to capture as many details as possible of the ship, although it is clearly a model abstraction. Finally, the primitive drawing in (c) of the ship is a very simplified model of a ship. It lacks all sorts of details and is clearly out of proportion. Nevertheless, any kid can tell that this is a ship that can sail on water and is driven by some kind of motor. Depending on what you need, you would choose one of the three representations. They each have their virtues, even the hand-drawn one. You may want to choose that one if you are in the process of investigating a ship that you only know a little about. It contains very little bias and few details. Too many details will render the model applicable only to specific cases, and the details may obscure the generic underlying principles of organization. On the other hand, a too general model may provide little mechanistic insight, which makes the model less useful for the design of further and more penetrating critical investigations.
Fig. 7.1a–c. Ships and models of ships. (a) A real ship – that is, a photograph of a real ship. (b) A construction blueprint of a ship. (c) The author’s drawing of a ship

In the case of biological membranes, the important elements of a model are likely to depend on which length and time scales are relevant for describing the problem of interest. This can imply serious difficulties since many membrane properties are controlled by phenomena that take place over a wide range of scales that are mutually coupled. It is likely that one will be best served by working with a set of membrane models, experimental as well as theoretical ones, chosen according to the particular type of question under consideration – and with due reference to which time and length scales are expected to be relevant.

7.2 Brief History of Membrane Models

In 1972, Singer and Nicolson proposed their celebrated fluid-mosaic model of biological membranes. The Singer-Nicolson model has since been a central paradigm in membrane science. The simple yet powerful conceptual framework it provided continues to have an enormous impact on the field
of membranes. As a key property, the Singer-Nicolson model assigned to the lipid-bilayer component of membranes a certain degree of fluidity. The fluidity concept was meant to characterize the lipid bilayer as a kind of pseudo two-dimensional liquid in which both lipids and membrane-associated proteins display sufficient lateral mobility in order to allow for function. The overall random appearance of this lipid-protein fluid composite made the membrane look like a mosaic. Except in cases where sterols or unsaturated lipid chains might alter the bilayer “fluidity,” the conspicuous diversity in the chemical structures of lipids, which is actively maintained by cells, had little significance in the model. This lipid diversity, together with the varying but characteristic lipid composition of different types of cells and organelles, has become an increasing puzzle, which is exacerbated by the enhanced understanding of the variation in physical properties among different lipids and lipid assemblies.

When Singer and Nicolson proposed the fluid-mosaic model in 1972, membrane modelling already had come a long way, as illustrated in Fig. 7.2. The first important step was taken in 1925 by Gorter and Grendel who showed that the membrane is very thin, being only two molecules thick, as shown in Fig. 7.2a. The experiment behind this remarkable and fundamental insight was surprisingly simple and elegant. Gorter and Grendel made a lipid extract of red blood cells whose surface area was known from microscopy. The lipids were spread on a water surface and compressed to produce a dense lipid monolayer as in Fig. 3.4a. When measuring the area of the resulting monolayer, it was found to be twice that of the surface of the red cells that provided the lipid extract. Hence, it could simply be concluded that the membranes of the cells were only two molecules thick.

The association of membrane proteins with the lipid bilayer was introduced in the Danielli-Davson model. In this model, the proteins appeared as a kind of spread on the lipid polar head groups at the two sides of the lipid bilayer, as illustrated in Fig. 7.2b. A related version of membrane organization appears in Robertson’s unit membrane model in which the proteins are pictured as stratified layers sandwiching the lipid bilayer, as shown in Fig. 7.2c.

In the Singer-Nicolson fluid-mosaic model pictured in Fig. 7.2d, the proteins are grouped into two classes: integral membrane proteins that traverse the bilayer and primarily interact with the bilayer through hydrophobic forces, and peripheral membrane proteins that are peripherally associated with the lipid bilayer and primarily interact with the bilayer through polar (electrostatic and hydrogen bond) interactions. In either case, the proteins “float in a fluid sea.”

Refinements of the fluid-mosaic model have been suggested from time to time, usually inspired by new insights obtained by focusing on some specific, or specialized, membrane feature. One example is the model by Jacob Israelachvili, who refined the Singer-Nicolson model to account for the need of membrane proteins and lipids to adjust to each other. This refined model also
incorporated membrane folding, pore formation, and thickness variations, as well as some degree of heterogeneity, as shown in Fig. 7.2e. Another elaboration of the Singer-Nicolson model, which emphasized the importance of the cytoskeleton and the glycocalyx, was developed by Erich Sackmann and is presented in Fig. 7.2f.

The various refinements of the Singer-Nicolson model represent the fashions in the field of membranes where researchers who investigate certain aspects of membrane complexity are in need of simple and transparent working models that can help them guide their intuition and facilitate the interpretation of experiments.

### 7.3 Do We Need a New Membrane Model?

There are several reasons to expect that we need a new model of biological membranes. Many of these reasons are dealt with in the present book. The notion of membrane fluidity, which was embodied in the Singer-Nicolson fluid-mosaic model in Fig. 7.2d, was important because it served to emphasize
that membranes are dynamic structures. Unfortunately, many subsequent investigators assumed, explicitly or implicitly, that fluidity implies randomness. This assumption neglects that fluids or liquids may be structured on length scales in the nanometer range, which are difficult to access experimentally, as described in Chap. 11. Also, structuring in time, in particular the correlated dynamical phenomena characteristic of liquid crystals, was not appreciated as being important for membrane function in the Singer-Nicolson model.

However, lively dynamics is perhaps the most conspicuous feature of a liquid membrane (Sect. 8.4). The dynamics does not necessarily imply randomness and disorder. In fact, the many-body nature inherent in the molecular assembly of a membrane insures that local order and structure develop naturally from an initially disordered liquid. Finally, the fluid-mosaic model pictured the membrane as a flat, pseudo two-dimensional layer. This may be an artistic simplification. It nevertheless de-emphasizes the transverse dynamical modes of individual lipid molecules, as well as the existence of large-scale excursions into the third dimension with the ensuing curvature-stress fields (Chap. 8), instabilities toward non-lamellar symmetries (Chap. 4), and coupling between internal membrane structure and molecular organization (Chap. 15) on the one hand and membrane shape and shape transformations on the other (Chap. 6). All these phenomena are intimately related to the fact that membranes are pieces of soft condensed matter, as we saw in Chap. 5.

It is now recognized that the randomness implied in the fluid-mosaic membrane model does not exist. This recognition builds on a wealth of experimental results that show that the lateral distribution of molecular components in membranes is heterogeneous, both statically and dynamically—corresponding to an organization into compositionally distinct domains and compartments, as described in Chap. 11. In addition to immobilization and domain formation due to interactions between the cytoskeleton or the extracellular matrix and the membrane, several physical mechanisms generate dynamic lateral heterogeneity of both lipids and proteins in liquid membranes.

This nonrandom organization imposed by the fluid membrane means that membrane functions do not need to depend on random collisions and interactions among reactants, but may be steered in a well-defined manner that allows for a considerable mobility of the individual constituents. This dynamic organization of the membrane makes it sensitive to perturbations by both physical (e.g., temperature and pressure) as well as chemical (e.g., drugs and metabolites) factors (Chap. 17), which thus provides an exceptional vehicle for biological triggering and signalling processes (Chap. 15).

It has been suggested that the Singer-Nicolson model of membranes has been successful because it does not say (too) much. It does not bias the user strongly and hence allows for broad interpretations of new experimental data and novel theoretical concepts. This is the strength of the model. It is also its weakness, as it in many cases is not very helpful when questions are asked about membrane structure and, in particular, about membrane
function. For those purposes, the model is too generic – in part because it provides too little, or no, insight into membrane protein assembly, lipid bilayer heterogeneity, monolayer or bilayer curvature, and bilayer bending and thickness fluctuations. Moreover, the model, by emphasizing stability, tends to de-emphasize dynamics; it does not address the issues relating to conformational transitions in membrane proteins and, just as importantly, the model does not address the conflict between the need for bilayer stability (the membrane must be a permeability barrier and consequently relatively defect-free) and the need for the bilayer to adapt to protein conformational changes. The bilayer must not be too stable because that would tend to limit protein dynamics. A manifestation of this dichotomy may be the widespread occurrence of lipids with the propensity for forming non-bilayer structures, as we discussed in Sects. 4.3 and 4.4.

7.4 Theoretical and Experimental Model Systems

Throughout this book we exploit the concept of models and modelling in our attempt to understand the role of lipids in membrane structure and functioning. In Chap. 6, we started out on the highest level of abstraction, where the membrane was considered a mathematical surface which is associated with mechanical properties and which displays complex conformations, shapes, and topologies. We found that the shapes of red blood cells could be understood and described within this general framework. The simplest experimental model of a lipid membrane will be dealt with in Chap. 10. Here we consider just half a membrane, a lipid monolayer at an air/water interface. Monolayer model membranes as well as the model bilayer membranes dealt with in Chaps. 8, 9, and 11 in the form of vesicles and liposomes are fairly easy to make since they basically self-assemble when dry powders of lipids are exposed to water. Some of the experimental bilayer models described in Chap. 11 are more complicated to manufacture since they require a solid support on which the layer is deposited.

The next level of complication in membrane modelling involves the incorporation of specific molecules in the lipid membranes, such as cholesterol dealt with in Sect. 9.4 and membrane proteins considered in Chap. 13. The experimental preparation of lipid-protein recombinants require special skills, in particular when functioning proteins and enzymes are involved.

We shall take advantage of theoretical models and model concepts throughout the presentation, ranging from very detailed microscopic models with atomistic detail, over various mesoscopic coarse-grained models where atomic and molecular details are hidden, to phenomenological models formulated in terms of macroscopic parameters. A very powerful tool exploited to derive the properties of the theoretical models is computer simulation techniques, using either stochastic principles, as in Monte Carlo simulation, or deterministic principles, as in Molecular Dynamics simulation.
Part II

Lipids Make Sense
8 Lipids in Bilayers – A Stressful and Busy Life

8.1 Trans-bilayer Structure

So far we considered membranes as ultrathin shells in which the internal membrane structure only entered very indirectly through the spontaneous curvature, through the observation that a bilayer consists of two monolayers and by the simple fact that the membrane has a thickness. In order to understand the organizational and functional principles of membranes, it is important to realize that a lipid bilayer is not just a homogeneous thin slap of a dielectric medium immersed in water but that the bilayer is a highly stratified structure with a distinct trans-bilayer molecular profile. This profile determines the membrane both as a barrier, carrier, and target. This is of particular importance for understanding how proteins function in and at membranes and how, for example, drugs interact with membranes.

The trans-bilayer profile is the most well-characterized structural property of bilayers since it most easily lends itself to being monitored by a number of techniques, e.g., X-ray and neutron-scattering techniques, magnetic resonance experiments, molecular-probe measurements, or computer simulation calculations. Magnetic resonance and molecular-probe techniques can give information about the structure and dynamics in various depths of the bilayer by using local reporter molecules or atoms. In addition to determining the thickness of membranes, scattering techniques can determine the probability of finding a specific part of a lipid molecule at a given depth in the bilayer. Computer simulation calculations can, if based on an accurate atomic-scale model with appropriate force fields, provide very detailed information on the structure and dynamics of bilayers.

Figure 8.1 illustrates two representations of the trans-bilayer structure of phospholipid bilayers. One representation (a) is a snapshot in time and the other (b) is an average over time. The fact that two representations are used illustrates the importance of realizing that lipid molecules in lipid bilayers are very lively, as we shall return to below.

Figure 8.1 shows the bilayer as a highly disordered liquid system with a distinct stratification. It can grossly be described in terms of four layers: (1) a layer of perturbed water, i.e., water that is structured and deprived of some of its hydrogen bonds, as described in Sect. 3.5, (2) a hydrophilic-hydrophobic region, including the lipid polar head groups as well
Fig. 8.1. (a) Trans-bilayer structure of a fluid DPPC lipid bilayer, as obtained from a Molecular Dynamics simulation. The four structurally different regions are labelled (1) perturbed water, (2) a hydrophilic-hydrophobic interfacial region involving the lipid polar-head groups, (3) a soft-polymer-like region of ordered fatty-acid chain segments, and (4) a hydrophobic core with disordered fatty-acid chain segments. A possible path for permeation of a single water molecule across the bilayer is indicated. (b) Trans-bilayer density profile of a fluid DOPC bilayer obtained from X-ray and neutron-scattering techniques. The curves give the relative probabilities of finding the different molecular segments of the phospholipid molecules. The highlighted region is the extended hydrophilic-hydrophobic interface.
as both water and part of the upper segments of the fatty-acid chains, (3) a soft polymer-like region of ordered fatty-acid chain segments, and (4) a hydrophobic core with disordered fatty-acid chain segments of a structure similar to that of a liquid oil like decane. Although the detailed nature of such profiles depends on the actual lipid species in question, the overall structural stratification is generic for aqueous lipid bilayers.

The most striking and important observation to be made from Fig. 8.1 is that the region of space that makes up the hydrophobic-hydrophilic interface of the membrane, i.e., regions (1) and (2), occupies about half of the entire lipid-bilayer thickness. This is the extended hydrophilic-hydrophobic interface region highlighted in Fig. 8.1. The presence of this layer, its chemical heterogeneity as well as its dynamic nature, is probably the single most important quantitative piece of information on membrane structure and organization that has to be taken into account in the attempt to make a useful membrane model, as discussed in Chap. 7. It has been pointed out by the American biophysicist Steven White that the chemically heterogeneous nature of this extended interface region makes it prone to all sorts of noncovalent interactions with molecules, e.g., peptides and drugs, that bind, penetrate, and permeate membranes. This interface is thick enough to accommodate an α-helical peptide that lies parallel to the bilayer surface. This issue is further discussed in Chap. 17.

8.2 The Lateral Pressure Profile

Lipids in bilayers are kept in place because of the hydrophobic effect discussed in Sect. 3.4. This is a way to keep the oily fatty-acid chains away from the water. It is not an entirely happy situation for the lipid molecules, however. They are subject to large stresses by being confined in a bilayer structure along with their neighbors. In order to appreciate how stressful this can be, we have to examine the various forces that act inside the lipid bilayer. This will lead us to one of the most fundamental physical properties of lipid bilayers, the lateral pressure profile.

Figure 8.2a gives a schematic illustration of a cross section through a lipid bilayer indicating the forces that act to stabilize the layer. When the bilayer is in equilibrium, these forces have to sum up to zero. Since the forces, due to the finite thickness of the bilayer, operate in different planes, the pressures are distributed nonevenly across the bilayer, as shown schematically by the profile in Fig. 8.2b. This profile is called the lateral pressure or lateral stress profile of the bilayer.

The lateral pressure profile is built up from three contributions. A positive pressure resulting from the repulsive forces that act between the head groups, a negative pressure (the interfacial tension) that acts in the hydrophobic-hydrophilic interface as a result of the hydrophobic effect, and a positive pressure arising from the entropic repulsion between the flexible fatty-acid
chains (chain pressure). The detailed form of the pressure profile depends on the type of lipids under consideration. Due to the small thickness of the lipid bilayer, the rather large interfacial tension from the two interfaces of the bilayer has to be distributed over a very short range. This implies that the counteracting pressure from the fatty-acid chains has to have an enormous density, typically around several hundreds of atmospheres. This is easily seen by noting that the interfacial tension at each of the two hydrophobic-hydrophilic interfaces of a lipid bilayer is around $\gamma = 50 \text{ mN/m}$. The lateral pressure of the interior of the lipid bilayer has to counterbalance this tension over a distance corresponding to the bilayer thickness, $d_L$, which is only about 2.5–3 nm. The lateral pressure density (force per unit area) of the bilayer then becomes $2\gamma/d_L$, which amounts on average to about 350 atm. Pressure densities of this magnitude are capable of influencing the molecular conformation of proteins imbedded in the membrane and hence provide a possible nonspecific coupling between the lipid membrane and the function of proteins, as we shall see in Sect. 15.2.

With reference to the discussion in Chap. 4 about the effective shape of lipid molecules, it is now clear from the description of the lateral pressure profile and Fig. 8.2 that it is not possible to assign a well-defined shape to a lipid molecule imbedded in a bilayer. The stressed and frustrated situation that a lipid molecule experiences in a bilayer is better described by the pressure profile, although there is no simple relation between the molecular structure and the actual distribution of stresses in the bilayer. Therefore, it is the lateral pressure profile that is the more fundamental physical property and that underlies the curvature stress field introduced in Sect. 4.3. It is therefore also the lateral pressure profile that determines bilayer spontaneous curvature, as well as the mean curvature and the Gaussian curvature modules described in Sects. 6.1 and 6.2.
8.3 How Thick Are Membranes?

Due to the fact that lipid bilayers are very stratified, the question of how thick a membrane is requires some qualification. Obviously, there are several different average thicknesses one can inquire about. Moreover, the various dynamical modes make it questionable what a thickness measure can be used for. In Sect. 13.3, we shall describe a fundamental principle for the interaction of trans-membrane proteins with lipid bilayers. This principle is based on matching the average hydrophobic thickness of the lipid bilayer and the hydrophobic length of the part of the protein that traverses the lipid bilayer. For this purpose we need an estimate of the hydrophobic thickness.

Obviously, the thickness of a lipid bilayer membrane depends on the length and degree of saturation of the fatty-acid chains of which its lipids are made. The longer the chains and the more saturated they are, the thicker the bilayer will be. The thickness also depends on the degree of hydration. The less hydrated the thicker the bilayer will be, because dehydration causes the head groups and therefore the fatty-acid chains to get closer together and hence stretch out. A very important determinant of lipid bilayer thickness is cholesterol. The reason for this, which is discussed in Chap. 9.4, is related to the fact that cholesterol has a strong tendency to stretch out and order the fatty-acid chains of the phospholipids. Hence, liquid lipid bilayers are usually thickened by cholesterol. Finally, temperature has a dramatic effect on lipid bilayer thickness: The higher the temperature, the thinner the bilayer. Under certain circumstances, to be discussed in Sect. 9.2, the lipid bilayer undergoes a phase transition, the so-called main phase transition. At this transition, the bilayer thickness can vary very abruptly.

The thickness of a lipid bilayer can be measured by X-ray or neutron scattering techniques applied to single bilayers on a solid support (cf. Fig. 11.4a) or to lamellar stacks of bilayers, as schematically represented in Fig. 8.3a. Results for the hydrophobic thickness as a function of temperature for DMPC and DPPC bilayers are shown in Fig. 8.3b. A dramatic reduction of thickness is observed as the bilayers are taken through their respective main phase transition. The thickness is seen to be larger for the lipid species with the longer chains. Moreover, the jump in thickness is more abrupt the longer the chains are. This systematics holds also for other chain lengths.

The hydrophobic membrane thickness of lipid bilayers in the liquid phase is strongly dependent on the amount of cholesterol incorporated into the bilayer. As an example, the thickness of a mono-unsaturated POPC lipid bilayer in its liquid phase can increase as much as 15–20% upon varying the cholesterol concentration from 0 to 30%, which is the level in most eukaryotic plasma membranes.
Fig. 8.3. (a) Schematic illustration of a multi-lamellar stack of lipid bilayers. The lamellar repeat distance is $d$, the hydrophobic lipid bilayer thickness is $d_L$, the thickness of a head group layer is $d_H$, and the water layer thickness is $d_W$. (b) Hydrophobic thickness, $d_L$, as a function of temperature for DMPC and DPPC lipid bilayers in the neighborhood of their respective main phase transition temperatures, $T_m$.

8.4 Lively Lipids on the Move

Lipid molecules in liquid bilayers are extremely lively and undergo a range of different dynamical processes. They are constantly changing intra-molecular conformations, they are wobbling, they are protruding out of the layer, and they are moving around. Figure 8.4 illustrates schematically some of the motions that lively individual lipids perform. These motions range over an enormous time span, from picoseconds to hours. Conformational changes can be fast, since they involve rotations around C–C bonds, which typically take a few picoseconds. The rotation of the lipid molecules are also fast and occur on a time scale of nanoseconds, whereas lateral diffusion is in the range of tens of nanoseconds. A typical lipid will on average rotate once around its axis while it travels a distance corresponding to its own size. The wobbling of the fatty-acid chain, which leads to changes in its direction within the bilayer, is much slower, typically of the order of tens of milliseconds.

The fast lateral mobility of lipids in the plane of the membrane is a typical liquid property. Over time, lipids will be able to explore the entire lipid bilayer or membrane. For a typical cell size, a lipid molecule can travel across the cell membrane within less than half a minute. Lipid molecules furthermore undergo substantial excursions perpendicular to the membrane plane in the form of single-molecule protrusions that take place over time scales of tens of picoseconds. The motion of lipid molecules from one monolayer leaflet
The many kinds of motions that lipid molecules in a lipid bilayer can perform. (a) Conformational change (see also Fig. 4.3). (b) Rotation around the molecular axis. (c) Lateral diffusion. (d) Protrusion out of bilayer plane. (e) Flip-flop between lipid monolayers to the other, the so-called flip-flop process, is, on the other hand, extremely slow, being of the order of hours, possibly days. In real biological membranes, special membrane proteins, so-called flippases, facilitate the redistribution of lipid molecules between the two monolayer leaflets.

The actual values of the rates of the different dynamical processes depend on the type of lipid molecule in question. Furthermore, there is some temperature dependence as well as a significant dependence on the state of matter of the lipid bilayer. If the lipid membrane is taken into a solid phase, all dynamical processes slow down significantly. For example, lateral diffusion is slowed down at least a hundred times. This is probably the single most important reason why membranes stop functioning when taken into solid phases.

The diffusion of lipid and protein molecules in membranes can be monitored by a number of experimental techniques. The motion of single molecules can be detected by either single-particle tracking or by ultra-sensitive single-molecule fluorescence microscopy or fluorescence correlation spectroscopy. In single-particle tracking, a colloidal particle of a typical diameter of 40 nm is linked to the lipid or protein molecule and the particle’s motion is then followed by computer-enhanced video microscopy. Figure 8.5 shows the trace of a fluorescently labeled lipid molecule that diffuses in a lipid bilayer. The spatial resolution of this kind of experiment is about 50 nm, and the time resolution is in the range of about 5 ms.

In addition to the dynamical modes of the individual lipid molecules, collective motion of different kinds involving many lipid molecules take place over a wide range of time scales. These motions include bilayer undulations,
Fig. 8.5. (a) Fluorescence image of a single fluorescence-labelled lipid molecule in a POPE-POPC phospholipid bilayer. The peak in intensity signals a single molecule in the plane of the membrane. (b) Recording of a part of a diffusion trace of a single lipid molecule bilayer thickness fluctuations, as well as collective diffusion of clusters of molecules within the plane of the membrane.

For comparison, integral membrane proteins are less lively. Proteins undergo more slow and restricted internal conformational transitions. Unless they are attached and anchored to the cytoskeleton, the proteins also diffuse laterally in the lipid bilayer. Their diffusion rate is typically a hundred times slower than lipid diffusion. If unrestricted, they would typically need about half an hour to travel over the range of a cell surface. Similarly, because of their larger circumference, they typically rotate around their axis about one full rotation during the time it takes a protein to travel a length corresponding to ten times its size.

The diffusion of lipids and proteins in biological membranes is often hindered because the motion takes place under certain restrictions. As mentioned, proteins can be attached to the cytoskeleton. Moreover, the membrane can be compartmentalized into various domains, which implies that proteins and lipids diffuse in an environment with obstacles, like a ship that navigates in an archipelago. In Sect. 11.3, we shall see how one can learn about lateral membrane structure by tracking the diffusional motion of individual molecules.

The many different dynamical processes that occur in lipid membranes are the reason why a lipid membrane in its liquid state is a very lively place indeed. The presence of lively dynamics in lipid membranes underscores the difficulty in working with a single unified model of biological membranes. Depending on the length and time scales one is interested in, a useful cartoon of a membrane will have different appearances. Since Fig. 8.1a is a snapshot in time and Fig. 8.1b is an average over time, these pictures do not provide the full information about possible dynamical aspects of trans-bilayer structure.
Fig. 8.6. Model of the lipid bilayer component of a cell membrane incorporated with integral membrane proteins (bacteriorhodopsin). The picture is drawn to scale, and it reflects averaging over fast dynamical modes. A 20 nm × 20 nm slab of a 5 nm thick lipid bilayer is shown. The time scale of view is in the range of $10^{-3}$ to $10^{-6}$ s. On this scale most molecular processes will appear blurred but not totally indiscernible. The transmembrane proteins are modelled by use of the X-ray coordinates for bacteriorhodopsin. Consistent with the slow time scale characterizing this picture, the protein surfaces have been slightly blurred that may be relevant, for example, to how proteins and drugs are transported across membranes.

A different membrane model that appreciates the difficulty in presenting a liquid object with all this lively dynamics is presented in Fig. 8.6. This figure highlights the lipid bilayer component and details of the molecular structure of integral membrane proteins (bacteriorhodopsin). The picture is drawn to scale, and it reflects averaging over fast dynamical modes. The time scale of view is in the range of $10^{-3}$ to $10^{-6}$ s. On this scale most molecular processes will appear blurred but not totally indiscernible. For example, the very rapidly moving chains seen on the edges of the lipid bilayer are indicated by subtle texturing parallel to the chain axis. The texture reflects the order of the lipid chains, but the fatty-acid chains themselves are not seen. The membrane edge shading is based on information obtained from X-ray and neutron scattering. The shading used on the head group surfaces suggests the presence of small lipid domains. The picture shows clearly that the lipid bilayer displays large-scale bending fluctuations.
9 The More We Are Together

9.1 Phase Transitions Between Order and Disorder

Some of the most fascinating and spectacular events in nature arise when the matter changes state. Soft matter like lipid bilayers and membranes have their share of these phenomena. Let us, however, start with a simple and well-known example: water. Water in the form of ice melts upon heating into liquid water, which upon further heating turns into vapor. In this case, the matter water has three states, or so-called phases: a solid (ice), a liquid (water), and a gas (water vapor). Although all states are made of the same type of simple H$_2$O molecules, the three phases appear to the naked eye very different. They also turn out to have very different materials properties upon closer investigation.

The two transitions connecting the phases, i.e., melting and boiling, are called phase transitions. The phase transitions are in this case induced by temperature, they are so-called thermotropic phase transitions, and they occur at well-defined temperatures, the melting temperature (0°C) and the boiling temperature (100°C). Obviously, it is possible to go through the transitions in the reverse direction by cooling from the vapor phase. It is well-known that the boiling point of water depends on pressure. Boiling water for a cup of tea in the Himalayas only requires heating up to around 80°C. Similarly, it is well-known that adding something to the water will change its melting point. Adding salt to ice will reduce the melting point.

The description of the phase transitions for water above is rather general and applies basically to any kind of matter. Butter and fat are known to melt upon heating, alcohol evaporates when heated, and olive oil goes solid when frozen. A number of phase transition phenomena are less well-known, e.g., a magnet can lose its magnetization when heated, an insulator can become a conductor upon cooling (possibly even a super-conductor), a liquid crystal display can change color when heated, or a biological membrane can become solid and stop functioning when cooled.

Phase transitions are also called cooperative or collective phenomena and they are highly nontrivial consequences of the fact that many molecules interact with each other. The molecules act in a sort of social manner, and the more they are together the more dramatic are the transitions between the different states of matter. By being many together, the assembly of interacting
molecules assumes properties that no single molecule possesses itself. For example, many water molecules together can form liquid water, although the particular properties associated with a liquid, such as fluidity and density, are not properties of a single water molecule.

The different phases of a material reflect different degrees of order. A solid is very ordered, typical of a crystal, where the molecules are arranged in a regular fashion, as illustrated in Fig. 9.1a. A liquid is more disordered, as shown in Fig. 9.1c, and the molecules in the liquid, although sticking together, diffuse around among themselves. Finally, the molecules in the gas in Fig. 9.1d hardly feel each other and the phase is very disordered. The three phases are distinguished by different densities. A special possibility exists of a solid, a so-called amorphous solid, as shown in Fig. 9.1b. The amorphous solid, which under some conditions is called a glass, has almost the same density as the crystalline solid but the molecules are positioned irregularly and often display very low mobility. This phase is similar to a very viscous liquid of the same density. Hence, this phase is in some sense both a liquid and a solid. As we shall in see in Sect. 9.4, the task carried out by cholesterol in lipid membranes is to produce a special phase of the type in Fig. 9.1b, which is in between order and disorder and where the molecules furthermore have the freedom to move around. In order to see this, we have to introduce an extra, necessary complication concerning the properties of the molecules.

![Fig. 9.1a–d. Two-dimensional representation of solid and liquid phases of matter composed of molecules that are spherically symmetric and have no shape. (a) Crystalline solid, (b) amorphous solid or very viscous liquid, (c) ordinary liquid, and (d) gas](image)

It was tacitly assumed in the description of the phases in Fig. 9.1 that the molecules are isotropic and spherical, i.e., they have no internal degrees of freedom. We now proceed to the next level of complication where the molecules have a nonspherical shape, e.g., prolate and shaped like a cigar. Whereas ordering of the spherical molecules can only involve their positions in space, i.e., the positional (translational) degrees of freedom, prolate molecules have an extra possibility of displaying order and disorder via the direction of their long axis. Order would here imply that the molecules tend to orient their long axes in the same direction. We refer to the direction of the molecules as
an internal degree of freedom, e.g., an orientational or configurational degree of freedom.

A number of new phases, so-called *meso-phases*, in between those of solids and liquids, can now be imagined. Some examples are given in Fig. 9.2. In the liquid phase in Fig. 9.2d, the molecules are disordered with respect to both the translational degrees of freedom and the orientational degree of freedom. In contrast, in the solid (crystalline) phase, order prevails in both sets of degrees of freedom. New phases can arise in between. The meso-phases have elements of order as well as disorder. In Fig. 9.2c, the positions of the molecules are disordered as in a liquid, but their long axes have a preferred direction, i.e., the collection of molecules are ordered orientationally. In Fig. 9.2b, the positions of the molecules have some element of order by being localized in a set of parallel planes that have a fixed distance as in a crystal. Within each of these planes, the positions are however disordered as in a two-dimensional liquid. The molecules are additionally ordered orientationally. Finally, in Fig. 9.2a the positions of the molecules within each plane are also ordered as in a crystal. The two meso-phases are called *liquid crystals*, respectively, a smectic (b) liquid crystal and a nematic (c) liquid crystal. A lipid bilayer is an example of a liquid crystal of the smectic type.

![Fig. 9.2. Prolate molecules exhibiting solid crystalline (a) and liquid (d) phases and two intermediate liquid-crystalline meso-phases: smectic (b) and nematic (c)](image)

Just as for the simpler systems illustrated in Fig. 9.1, the different phases in Fig. 9.2 are connected by phase transitions. The phase transitions can be triggered by changing temperature, i.e., they are thermotropic phase transitions. Increasing temperature will lead to transitions going from left to right in Fig. 9.2.

There is a large number of different liquid crystalline phases possible. More than 10% of all organic chemical compounds known today display one or another type of liquid crystalline phase. Liquid crystals are widespread throughout nature. Living matter is no exception. Being smectic liquid crystals, lipid membranes can therefore be considered nature’s preferred liquid crystals. Hence, living matter is based on liquid-crystal technology.

Phase transitions are conventionally divided into two types: discontinuous transitions (first-order transitions) and continuous transitions (second-order
transitions or critical-point phenomena). At a discontinuous transition, the degree of order in the system changes discontinuously at the transition temperature, whereas it changes in a continuous manner at a continuous transition. Furthermore, at a continuous transition, strong fluctuations prevail. A discontinuous transition can often be driven into a continuous transition by varying a suitable parameter, e.g., pressure. As an example, the boiling of water at 100°C and 1 atm is a first-order transition, and there is a large discontinuous jump in the density (degree of order) going from liquid water to vapor. By increasing the pressure, this jump can be gradually diminished and brought to vanish at 218 atm. At this so-called critical pressure, water evaporates according to a continuous transition at a corresponding critical temperature of 374°C, where there is no density difference between liquid water and vapor. The phase transitions of most thermotropic liquid crystals are of first order, whereas the magnetization of an iron magnet displays a critical-point phenomenon and vanishes continuously at the so-called Curie temperature.

9.2 Lipids Have Phase Transitions

As described in Chaps. 3 and 4 and as illustrated in Figs. 3.4 and 4.4, lipids in water form a number of different supramolecular aggregates. Lipid aggregates in water can be considered phases and states of matter. The phases are induced by varying the water concentration, and phase transitions between the different aggregate forms can in many cases be triggered by changing the water concentration under isothermal conditions. Such transitions are called lyotropic transitions.

For example, some lipid lamellar phases can undergo a transition to an H₁ phase by increasing the water content and to an H₁I phase by decreasing the water content. Alternatively, such lyotropic transitions can be triggered by changes in composition, by varying physico-chemical conditions, or by biochemical input, exploiting the fact that lipids speak the language of shape described in Chap. 4. The example described in Sect. 4.4 involving the microorganism Acholeplasma laidlawii, which maintains homeostatic control by changing the lipid composition of its membrane, is an excellent illustration of biological regulation by moving around with a lyotropic phase transition from a lamellar phase to an inverted hexagonal phase. Acholeplasma laidlawii arranges for its membrane to have a composition that positions the lamellar-hexagonal phase transition temperature about ten degrees above the ambient temperature.

In addition to phase transitions between phases of different morphology, lipid aggregates undergo a number of internal phase transitions without changing morphology. We shall here concentrate on lipid phase transitions within lamellar symmetries, specifically, in lipid monolayers as considered in Chap. 10 and in lipid bilayers as dealt with throughout the rest of this
9.2 Lipids Have Phase Transitions

book, and, in particular, in Chaps. 11 and 12. Certain large-scale morphological transitions involving shape changes of large liposomes and whole cells, exemplified by red blood cells, were discussed in Chap. 6.

Aqueous dispersions of phospholipid bilayers in the form of uni-lamellar or multi-lamellar vesicles, as illustrated in Fig. 3.4, display a series of thermotropic phase transitions. This is exemplified in Fig. 9.3, which shows the specific heat as a function of temperature for a dispersion of multi-lamellar bilayers of DPPC. The specific heat is a measure of the heat capacity of the system, i.e., how much heat has to be supplied to raise the temperature one degree. The specific heat has in this case two peaks. A peak in the specific heat is an indication of a phase transition. The heat contained in the peak is a measure of the heat of transition, that is the amount of heat that has to be supplied in order to facilitate the transition. The two peaks separate three phases with trans-bilayer structures, which are illustrated schematically in Fig. 9.3. The presence of these transitions is general for all PC lipids. Other lipids with different fatty-acid chains and different head groups need not have all these transitions. However, the one appearing at the highest temperature in Fig. 9.3 is generally found for all phospholipids. This transition is called the main phase transition. The main phase transition is the intra-bilayer phase

![Fig. 9.3. Phase transitions and phases in DPPC lipid bilayers. Specific heat, C, as a function of temperature. The inserts show schematic illustrations of the trans-bilayer structure in the different phases separated by the phase transitions that are signalled by peaks in the specific heat. The small peak separating the solid (solid-ordered) and the ripple phase corresponds to the pre-transition, and the large peak separating the ripple phase and the fluid (liquid-disordered) phase corresponds to the main transition.](image)
transition that is believed to be of most importance for membrane biology. We shall use PC lipids to illustrate the nature of the main transition and how its properties are reflected in membrane function. The main transition is a first-order transition, although it is often found to be associated with rather strong fluctuations.

The main transition is characterized by a transition temperature, $T_m$, where the specific heat attains its maximum, and a heat (enthalpy) of transition, $\Delta H$, which is a measure of the amount of heat that has to be supplied to the system for the transition to take place. $T_m$ and $\Delta H$ are larger the longer the fatty-acid chains are. For increasing degree of unsaturation, the transition occurs at progressively lower temperatures.

The heat of transition corresponds to a transition entropy

$$ \Delta S = \Delta H / T_m. \quad (9.1) $$

It turns out that this transition entropy is very large, around $15k_B$ per molecule for DPPC, where $k_B$ is Boltzmann’s constant. It is instructive to insert this number into Boltzmann’s formula,

$$ \Delta S = k_B \ln \Omega, \quad (9.2) $$

which gives a rough estimate of how many micro-states, $\Omega$, of the system (per molecule) are involved in the transition. The resulting number is $\Omega \sim 10^5$–$10^6$. This is a very big number. The only available source for all these states is the conformations of the two long, flexible fatty-acid chains of the lipid molecule. An illustration of the richness of these states was given in Fig. 4.3.

We therefore conclude that the main transition is associated with a melting of the lipid molecules in the sense that the molecules below the transition have fairly ordered chains, whereas above the transition the chains are more disordered. This picture of the transition has been confirmed by a variety of spectroscopic measurements. This kind of structural change was indicated in Fig. 9.3 and is illustrated in greater detail in Fig. 9.4. Further structural and rheological studies have shown that the phase below the transition not only has ordered fatty-acid chains; the lipid molecules are at the same time arranged in a regular structure as in a crystalline solid. In contrast, the lipid molecules in the phase above the transition are positionally disordered as in a liquid and subject to rapid lateral diffusion.

Therefore, a complete description of the two phases calls for two labels in the same way as we saw in relation to liquid crystals in Sect. 9.1: one label (ordered, disordered) that refers to the conformational (internal) degree of freedom of the fatty-acid chains and another label (solid, liquid) that refers to the positional degree of freedom. We shall consequently call the phase below the transition the solid-ordered phase and the phase above the transition the liquid-disordered phase. This labelling shall turn out to be very helpful for describing the effect of cholesterol on the lipid bilayer phase transition, as
9.2 Lipids Have Phase Transitions

Fig. 9.4. A slab of a lipid bilayer illustrating the structural changes during the main phase transition. The picture is obtained from a Molecular Dynamics simulation on a DPPC bilayer in water using an atomistic model. To the left is shown the solid-ordered phase and to the right the liquid-disordered phase described in Sect. 9.4. As we shall see, cholesterol is capable of inducing a new membrane phase which is in between the two: the liquid-ordered phase.

In order to illustrate the degree of fluidity and disorder in the liquid-disordered phase, Fig. 9.5 shows a snapshot from a computer simulation calculation on a model using a full-scale atomistic description.

Fig. 9.5. A slab of a lipid bilayer illustrating the liquid-disordered phase. The picture is obtained from a Molecular Dynamics simulation on a DPPC bilayer in water using an atomistic model.

The illustration of the two bilayer phases in Fig. 9.4 suggests that the transition in the bilayer is accompanied by a considerable decrease in bilayer thickness, $\Delta d_L$, and at the same time a substantial area expansion, $\Delta A$, which is typically as large as 10–15%. It turns out that the volume per lipid is only changing a few percent during the transition. Hence, the thickness change and the area change are reciprocally related, i.e., $\Delta A \Delta d_L = \text{a constant}$. In
Sect. 8.3, we discussed how the different hydrophobic and hydrophilic parts of the bilayer contribute to its total thickness.

Figure 9.3 suggests that the solid-ordered phase displays an additional modulated feature over an intermediate range of temperatures. This is the so-called ripple phase, which is characteristic for PC lipids. The ripple structure is pictured in more detail in Fig. 9.6, which shows the surface of a DPPC lipid bilayer in water imaged by atomic force microscopy. The transition between the solid phase and the ripple phase is termed the *pre-transition*.

Fig. 9.6. Atomic force microscopy picture of the surface of a DPPC lipid bilayer in water that forms a ripple phase below the main transition. There are two types of ripples with periodicities of 13 nm and 26 nm, respectively. The picture is 600 nm × 600 nm

### 9.3 Mixing Different Lipids

When more than one type of lipid molecule is present in a bilayer, a more complex behavior results. There is no longer a single transition described by a single transition temperature. The transition now takes place over a range of temperatures where the system separates in more than one phase. One speaks about phase equilibria and phase separation. The social and cooperative behavior of the lipids has developed into a sort of separatism, where lipid molecules over a certain range of temperatures and compositions have a preference for separating out together with other molecules of their own kind.

The underlying physical mechanism for phase separation is simple: Lipid molecules of the same kind often have a stronger attractive interaction than
lipids of different kinds. The resulting phase behavior is conveniently described in a so-called phase diagram, as illustrated for three binary lipid mixtures in Fig. 9.7. A phase diagram of the type shown indicates the actual phase of the mixed system for varying composition and temperature. The three mixtures in Fig. 9.7 were chosen in order to show how an increasing difference between the lipids that are mixed leads to an increasingly complex phase behavior. In the present case, the difference between a pair of lipids is simply the difference in the lengths of their fatty-acid chains. Lipid molecules that are more closely matched in chain length have a larger preference for being together. We saw in Fig. 9.3 that a peak in the specific heat arises at the main transition. In Fig. 9.8, the specific heat is shown for mixtures of DMPC and DSPC. For each composition, the specific heat is seen to have two peaks that occur at the boundaries in the phase diagram in Fig. 9.7.

![Phase diagrams](image)

**Fig. 9.7.** Phase diagrams of lipid bilayers for three binary mixtures of PC lipids with different fatty-acid chain lengths. $f$ denotes the liquid-disordered phase and $g$ denotes solid-ordered phases.

The DMPC-DPPC mixture has the weakest tendency for phase separation of the three mixtures considered in Fig. 9.7. In the phase separation region, the bilayer splits into two phases with different composition. The lipid species with the lowest value of the phase transition temperature, in this case DMPC, is enriched in the liquid-disordered phase, whereas the lipid with the highest
transition temperature, i.e., DPPC, is enriched in the solid-ordered phase. The phase-separation behavior is getting more pronounced for the DMPC-DSPC mixture that has a larger incompatibility in chain lengths. Finally, the DLPC-DSPC mixture develops an added complication in that the solid-ordered phases of the two lipids do not mix at low temperatures. Hence, a region of solid-solid phase separation arises.

Phase separation is in principle a macroscopic phenomenon, i.e., the separating phases are large. We shall in Chap. 11 remark that the phase separation is not necessarily fully developed in these lipid mixtures. Instead, the system is found to be organized in a large number of small lipid domains. As we shall discuss in Sect. 11.4, these domains are a mode of organizing membranes on a small scale, which may be important for many aspects of membrane function, e.g., binding of proteins, fusion, permeability, and enzyme activity.

Lipid mixtures can under appropriate conditions of temperature and composition develop critical-point phenomena, as discussed in Sect. 9.1 above. These conditions are referred to as critical mixing. Below in Sect. 9.4 we shall show that cholesterol when mixed into a phospholipid bilayer can lead to such a situation. At critical mixing, the mixture is subject to strong fluctuations in local composition, and large domains enriched in one of the mixed lipid species are transiently formed, as also discussed in Sect. 11.2.

9.4 Cholesterol Brings Lipids to Order

Being an amphiphilic molecule (cf. Fig. 2.5a), cholesterol easily incorporates into lipid bilayers with its hydrophilic –OH head group at the bilayer-water interface and the steroid skeleton inside the hydrophobic core. The cholesterol molecule barely spans one monolayer leaflet of a typical bilayer, as illustrated in Fig. 9.9. Considering the different types of ordering that pertain to the lipid
molecules in a bilayer, cf. Fig. 9.4, it appears that cholesterol has a problem when coming to terms with the life in a dense lipid bilayer.

On the one side, cholesterol, due to its hydrophobically smooth and stiff steroid ring structure, has a preference for having conformationally ordered lipid chains next to it since they provide for the tightest interactions. From this perspective, cholesterol prefers the solid-ordered lipid phase. On the other side, the solid-ordered phase is a crystalline phase with dense packing order among the lipid molecules. The cholesterol molecule, with its own peculiar size and shape, does not fit well into this packing order, whereas there is plenty of free space to squeeze into in the liquid-disordered phase. From this perspective, cholesterol prefers the liquid-disordered phase. Hence, the cholesterol molecule becomes frustrated when presented with the two different lipid phases. This frustration and the way cholesterol finds a way of releasing the frustration hold the key to understanding not only the effect of cholesterol on the physical properties of lipid membranes, but also the role of sterols in the evolution of higher organisms and their membranes. We shall return to sterols in the context of evolution in Chap. 14.

Cholesterol releases the frustration by introducing a new phase, the liquid-ordered phase, first proposed in 1987 by the Danish biophysicist John Hjort Ipsen. The liquid-ordered phase is in between the two normal lipid bilayer phases. The resulting phase diagram is shown in Fig. 9.10. This diagram
shows that cholesterol stabilizes the liquid-ordered phase over a wide range of compositions and temperatures. A particular feature of the diagram is that it appears to close on the top in a critical mixing point beyond which the liquid-ordered and the liquid-disordered phases become indistinguishable. Around this critical point it is expected that dramatic density and compositional fluctuations will occur. These fluctuations may be a source for the small-scale structures found in membranes containing large amounts of cholesterol, cf. Sect. 11.3.

This liquid-ordered phase is a genuine liquid with positional disorder and high lateral mobility of the membrane molecules. Furthermore, the lipid chains have a substantial degree of conformational order. When introduced into the liquid membrane phase, cholesterol leads to a large increase in membrane thickness. The thickening provides for larger mechanical coherence and less flexible bilayers. It also makes the bilayer tighter, as described in Sect. 12.1.

Consequently, cholesterol has a remarkable dual effect on membranes. It makes the membranes stiffer, but retains the fluidity required for membrane function. In a way, cholesterol acts as an anti-freeze agent. No other molecule is known to have a similar dramatic effect on lipid membrane behavior except the other higher sterols like ergosterol. Based on this insight we can qualify our discussion of what fluidity of membranes actually means.

The liquid membrane phases, whether it be liquid-ordered or liquid-disordered, are the membrane states that should be associated with membrane
fluidity that is so central to the celebrated Nicolson-Singer model described in Sect. 7.2. However, fluidity is not a well-defined physical property. At best it is a loose term that covers the lively dynamics of the liquid phases of membranes. The trouble is that a lipid bilayer can be “fluid” in more than one sense of the word. As we have seen, the description of lipids requires at least two fundamental sets of degrees of freedom, the positional (translational) degrees of freedom and the internal (conformational) degrees of freedom. A lipid bilayer can exhibit fluidity in both sets of degrees of freedom. Hence, if fluidity is meant to imply fast diffusion, it refers to dynamic disorder in the translational variables. However, if fluidity is meant to reflect the fact that the fatty-acid chains can be conformationally disordered or melted, it refers to disorder in the internal degrees of freedom of the chains. A lipid bilayer can exhibit fluidity in both sets of degrees of freedom, as in the liquid-disordered phase, or in one of them, as in the liquid-ordered phase that can be induced by the presence of cholesterol.
Epilogue: Fat for Future

In her beautiful 1998 book *The Fats of Life*, Caroline Pond writes, “Genes contain the information to build the cell, proteins catalyze the necessary chemical reactions, but phospholipids act as the marshals, holding the biochemical machinery together and helping to maintain the right chemical environment.” To this we could add that the marshalling task of the lipids also involves intricate signalling and an amazing ability to support and carry out function. Although the blueprint for the production of all the essential molecules of the cell is provided by the genes, the actual building of the cell and the assembly and functioning of all its molecular machinery are not written in the genes. These phenomena are based on self-organization processes controlled by the laws of physics. In these processes, the lipids play a key role that often has been overlooked or forgotten.

The main aim of this book has been to demonstrate how nature chose a wonderful and versatile class of molecules, the lipids, to structure and organize living matter in a way that provides for unique functions. Lipids and fats are not only foodstuff. Life as we know it could not have evolved or been sustained without lipids. Obviously, revealing the design principles underlying the functioning of lipids in living systems not only provides fundamental insights into biology and the evolution of life, it also holds a strong promise for translating the obtained insights into technologies useful for improving our life conditions.

Lipid-based technologies are inspired by nature’s own nanotechnologies that have been developed over evolutionary time scales to optimize biological function on the cellular and subcellular levels, i.e., precisely on the nanometer scale. The design and function of nature’s materials are a full-blown nano-science using “bottom-up” design principles leading to soft materials of unique function and durability. The study of natural materials and their function is a truly multidisciplinary endeavor. The nano-science based on lipids and other biological molecules operates in a domain where the boundaries between traditional disciplines of science – physics, chemistry, and biology – no longer makes sense. It is in this domain where we may in the future expect real innovative developments within drug discovery and design.

Technology based on lipids and self-assembly processes can also possibly help meet the increasing needs for future sustainability of industrial processes.
As natural materials, lipids are biodegradable and can be reused and recycled. Use of nature’s own bottom-up principles for tomorrow’s nanotechnology and nano-electronics basically puts the factory in a beaker. It does not necessarily presuppose a billion-dollar factory with expensive clean-room facilities that is required for conventional micro- and nano-electronics based on semiconductor chip technology.

There are some issues that make studies of fat and lipids an urgent matter for mankind. We are currently witnessing a rapid increase in non-communicable diseases such as obesity, type II diabetes, hypertension, cardiovascular diseases and stroke, colon and breast cancers, mental ill-health, as well as perinatal conditions. Obesity is increasing globally almost like an epidemic. The burden of ill-health and the number of deaths due to these diseases are now greater than for all infectious diseases combined. The rise in mental ill-health follows the rise in cardio-vascular diseases. It is particularly troublesome that this rise in mental ill-health and behavioral problems is largest in young people. It indicates that a well-protected system like the brain is being affected.

In a paradoxical way, the human genome project actually indirectly showed that the rise in these non-communicable diseases is not genetically determined. Our genes have not changed over the few decades we are talking about. The major cause has to be changes in the diet and environmental conditions. So the question is then how the diet and, in particular, the dietary fats, are involved in the regulation and expression of genetic information. This is again where lipids and biological membranes get involved.

It has been known for some time that the physical non-communicable diseases belong to the so-called metabolic syndrome and are therefore linked to our diet. Special diets like the traditional fish-rich Icelandic and Japanese diets and the special olive-oil rich Mediterranean diet are well-known for promoting longevity and low incidence of heart attacks. It is now gradually becoming clear that a number of mental diseases such as schizophrenia, manic-depression, Alzheimer’s, Parkinson’s, and autism may also be related to changes in the diet. The polyunsaturated fats and their derivatives such as the cell-regulating eicosanoids become key issues here. The general failure of governmental programs aimed at fighting diseases related to the metabolic syndrome, such as coronary heart diseases and obesity, by focusing on fats and basically neglecting carbohydrates in the diet, has highlighted the need for a balanced view of which roles fats and lipids actually play for life.

A large number of mysteries concerning the role of fats and lipids for life are still unresolved. Some of the more obvious mysteries involve the role of lipids in evolution, the need for lipid diversity in membrane function, the physical principles that control cell signalling by lipids, and the relationship between nutrition and health.

Many more mysteries are likely to turn up as we realize how life is a matter of fat.
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GENERAL BOOKS AND REVIEWS ON MEMBRANES AND LIPIDS


**PROLOGUE:**

**LIPIDOMICS – A SCIENCE BEYOND STAMP COLLECTION**


1 LIFE FROM MOLECULES

2 HEAD AND TAIL


3 OIL AND WATER


4 LIPIDS SPEAK THE LANGUAGE OF CURVATURE


5 A MATTER OF SOFTNESS


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7 BIOLOGICAL MEMBRANES – MODELS AND FASHION


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10 LIPIDS IN FLATLAND


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### 13 PROTEINS AT LIPID MATTRESSES


14 CHOLESTEROL ON THE SCENE


15 LIPIDS IN CHARGE


16 BEING SMART – A FISHY MATTER OF FACT


17 LIQUOR AND DRUGS – AS A MATTER OF FAT


18 LIPID EATERS


19 POWERFUL AND STRANGE LIPIDS AT WORK


20 SURVIVAL BY LIPIDS


**EPILOGUE: FAT FOR FUTURE**


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