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Thermal Biophysics of Membranes

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Cover

The cover picture shows confocal microscopy

images of giant lipid vesicles containing domains

of ordered and disordered lipid (in red and green).

The bottom shows the snapshot of a Monte-Carlo

simulation describing the same lipid system. The

large objects represent proteins.

Contents

Preface

Biological membranes display a wealth of physical phenomena including phase transitions, propagating voltage pulses, variable permeability, structural transitions (as seen in endo- and exocytosis), and domain formation that is thought to have an important influence on signal cascades. The title of this book "Thermal Physics of Membranes" indicates that it deals in particular with the thermodynamics of such systems. Thermodynamics is always true because it is based on only two basic and intuitive laws: the conservation of energy and the maximum entropy principle. Beyond that it is free of any approximations and assumptions. One therefore finds thermodynamics as a basis for physics on all length scales from atomic dimensions up to cosmological scales. Naturally, thermodynamics is also true on the level of biological membranes. We wish to introduce the reader to some of these principles and their consequences concerning the behavior of membranes. Important topics in this book are "phase diagrams" including domain formation and rafts, elasticity and the related changes in vesicular shape, pulse propagation, permeability as well as protein binding and electrostatics.

Biology deals with complex ensembles of organic molecules including proteins, nucleic acids, and lipids, but also salts and water. Proteins often display unique molecular surfaces that give rise to specific interactions. Much of biophysical research therefore has been dedicated to the study of structures and interactions between individual molecules. Cells and their compartments are defined by a large variety of membranes that not only surround the cell as a whole but also each organelle as the nucleus, mitochondria, or the endoplasmic reticulum. On average 50% of the biomembrane mass stems from proteins. The human genome contains about 30,000 genes encoding at least as many proteins, many or most of those being membrane proteins.

The major building blocks of membranes, however, are hundreds or thousands of different lipid species. The human body contains several kg of membrane lipids with a total surface on the order of 0.4 km^2 per kg. The plasma membranes of one eucariot cell contains about 10^{10} lipid molecules. Although

the diversity of lipids is seemingly smaller than that of proteins, lipid membranes contain many molecules and are thus large ensembles.

Biological molecules usually do not only interact with one specific binding partner but also with the abundant lipid surfaces, with protons (because macromolecules contain protonable groups), ions and, very importantly, with water. Therefore one typically deals not with one interaction but rather with many. Even if only a few of these interactions have a strength that is of interest and even if one takes into account that one cell usually does not express all the proteins that are encoded in the genome, it is immediately obvious that it is a impossible to investigate all possible interactions. One further has to take into account that the molecules may have different orientations and different conformations further increasing the complexity. We leave it to the reader to figure out how many different arrangements of, say, 200 lipid species in variable concentrations and conformations in an ensemble of 10^{10} molecules are possible—but the number is beyond any range that can ever be accessed by computers. One must come to the conclusion that life will never be understood on the basis of binary molecular interactions alone. In particular, many cooperative phenomena such as the melting of lipid membranes are beyond the scope of single molecule physics.

Thermodynamics is a fundamental discipline of physics that describes the behavior of assemblies of molecules. It solely relies on two basic principles: the law of the conservation of energy (first law) and the seemingly tautological principle that a most likely state exists that is assumed with the highest probability (second law). The latter principle is also known as the principle of maximum entropy. These two principles are so general and universal that the thermodynamic relations that are derived from them are also fundamentally true. In the case of biological systems, the variety of proteins, lipids, and ions is taken into account by their chemical potentials that are a function of the concentrations of other molecules as well as of temperature, pressure, voltage, or other intensive variables. In thermal equilibrium a multimolecular ensemble like a membrane fluctuates around the state of maximum entropy. If the system is not in equilibrium, the first derivative of the entropy constitutes the thermodynamic forces, which are the forces that drive a system back to equilibrium. The second derivatives of the entropy are related to susceptibilities, for example, to the heat capacity or the elastic constants of membranes. These properties of membranes are often easier to measure, for example with calorimeters (heat capacity), ultrasonic velocity measurements (volume compressibility) or by vesicular shape fluctuations (bending elasticity). Even though in thermal equilibrium the thermodynamics forces are zero, the susceptibilities generally assume nonzero values. Since the different susceptibilities are all second derivatives of the same thermodynamic function (the entropy), they are not independent of each other, but one can find surprising

relationships between various thermodynamic susceptibilities that can provide insights into the behavior of membranes that one would never be able to predict on the basis of single molecule interactions. Many such relations stem from the so-called Maxwell relation. We show two examples:

$$\left(\frac{dS}{dp}\right)_{T,n_i} = -\left(\frac{dV}{dT}\right)_{p,n_i} \tag{0.1}$$

where S and V are the entropy and the volume of an ensemble, respectively, including all their proteins and lipids—and all their conformations. This equation implies that the term on the left-hand side that is experimentally difficult to access is identical to the volume expansion coefficient that is very easy to measure. A second example is

$$\left(\frac{d\mu_i}{dn_j}\right)_{S,V,n_{i\neq j}} = \left(\frac{d\mu_j}{dn_i}\right)_{S,V,n_{j\neq i}} \tag{0.2}$$

This relation couples the chemical potential of one component to the variation of another and demonstrates the symmetry of the coupling. In biochemical textbooks such couplings usually do not play a role. This implies that the findings shown in such books are not necessarily incorrect but definitely incomplete. However, there are also examples where the molecular textbook models are clearly in conflict with the laws of thermodynamics. The application of thermodynamics therefore should not be considered as a method averaging out the molecular details (and thereby losing information) but rather as a means to gain considerable insight into all the couplings between seemingly different processes.

In this textbook we will introduce the reader to the thermodynamic concepts. Overall, our intention is to show the beautiful manner by which thermodynamics can link seemingly unrelated membrane processes resulting in a unified picture of the behavior of membranes as a whole. Our aim therefore is to present a coherent concept rather than achieving a complete presentation of the field. This approach takes the risk that important results of respected colleagues are not presented to the extent that they deserve.

Copenhagen, April 2007 *Thomas Heimburg*

1
Membranes—An Introduction

In the second half of the 19th century it became evident that an osmotic barrier separates the inside and the outside of cells (Nägeli and Cramer, 1855; de Vries, 1871, 1884; Pfeffer, 1877). Plant cell protoplasts were permeable to water but not to larger macromolecules like sucrose (de Vries, 1871). Pfeffer was the first to study the osmotic pressure within cells and formulated the idea that the protoplasm of cells is surrounded by a thin layer, which he called the plasma membrane. In fact, Pfeffer proposed that this membrane does not only cover the outer surface of cells but also separates all aqueous environments of different composition from each other. One may therefore consider Pfeffer as the father of membrane theory. The developments in biology and botany coincided with a rapid development in the theory of thermodynamics of solutions. In particular, based on Pfeffer's work van't Hoff found the formal analogy of concentrations of solutes in water and the partial pressures of ideal gases (van't Hoff, 1887). Ostwald formulated descriptions for the osmotic pressure across semipermeable walls and the related electrical properties (Ostwald, 1887, 1890).[1]

1.1
Overton (1895)

Charles Ernest Overton is a very important figure in the development of a picture of cell membranes. He investigated the osmotic properties of cells and noticed in the late 19th century that the permeation of molecules through membranes is related to their partition coefficient between water and oil (Overton, 1895). Overton's findings led to the hypothesis that the thin membranes surrounding cells have the properties of oil. In his book on anesthesia (Overton, 1901. Jena, Germany. English translation: Studies of Narcosis, Chapman and Hall, 1991, R. Lipnick, Ed., 1991) he called the layers surrounding cells "lipoids" made from lipids and cholesterol. The properties of lipids are described in detail in Chapter 3 and theory of anesthesia is treated in Chapter 19.

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1) The history of biomembrane research is nicely reviewed in Ling (2001).

1.2
Langmuir (1917) and Gorter and Grendel (1925)

Langmuir (1917) developed an apparatus in which molecular layers of lipids were spread at the air–water interface. With this monolayer trough (see Section 6.7 and Fig. 6.14) the lateral pressure of the monolayer films could be measured. Langmuir proposed that in the molecular film the polar head groups were directed toward the water whereas the hydrophobic hydrocarbons are pointed toward the air phase.

Gorter and Grendel (1925) experimentally investigated the surface area of lipids. For this purpose they extracted the lipids from red blood cells of man, dog, rabbit, sheep, guinea pig, and goat in acetone. The lipids were spread on a water surface and the area was measured using a Langmuir film balance. From the same blood preparations they measured the surface area of the red blood cells from the microscopic images. They found that the surface area of the monofilms was within error exactly two times that of the cells. They concluded that cell membranes are made of two opposing thin molecular layers, and they proposed that this double layer is constructed such that two lipid layers form a bilayer with the polar head groups pointing toward the aqueous environment (Fig. 1.1). This is the picture of the lipid membrane we know today. As Robertson (1959) noted later, the attractive simplicity of Gorter's and Grendel's pictures is also its greatest weakness since it fails to account for the manifold of functions attributed to cell membranes.

H₂O

lipid double layer

H₂O
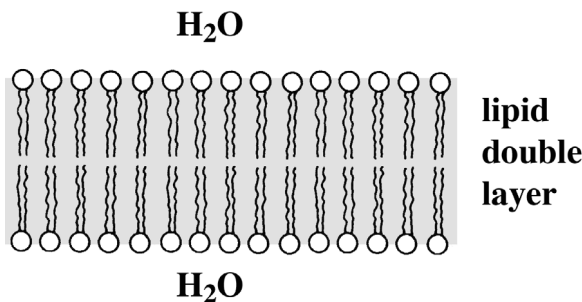
Fig. 1.1 The cell membrane according to Gorter and Grendel (1925). They proposed the lipid bilayer structure.

1.3
Danielli and Davson (1935)

The earliest molecular model for the biomembrane structure including proteins was the model from Danielli and Davson (1935). They took into account that the layers surrounding cells had a significant content of proteins adsorbed
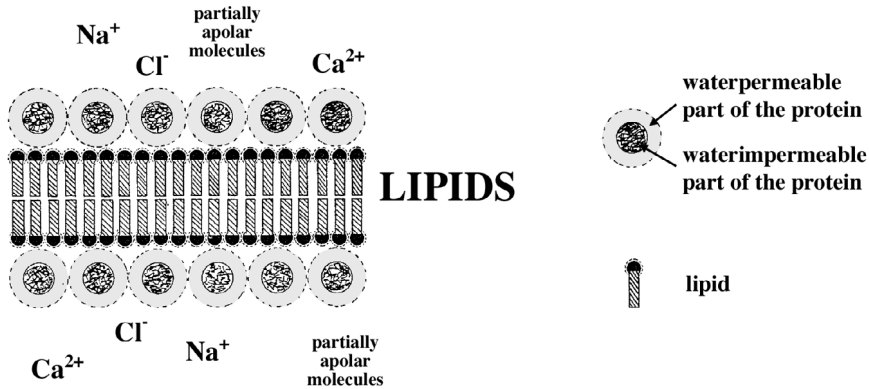
Fig. 1.2 Danielli and Davson (1935) proposed a membrane model including proteins. They used their model to interpret the observation of different membrane permeabilities of ions and hydrocarbons. In particular, they assumed that the membrane has both a lipophilic and a hydrophilic character. Water-containing regions in the membrane give rise to ion transport depending on water content of the membrane and its charge; lipophilic parts are responsible for the transport of water-insoluble molecules. Figure adapted from Danielli and Davson (1935) with permission.

to the layers. It was known that phospholipids have an amphiphilic nature. Furthermore, proteins investigated were mostly water soluble but nevertheless often adsorbed to membranes. Jim Danielli and Hugh Davson thus proposed a model of the cell membrane consisting of a lipid bilayer, with which a protein layer is tightly associated (Fig. 1.2, left). As in earlier membrane studies (e.g., by Overton) they were in particular interested in the permeation properties of membranes. In a theoretical paper they made the following consideration.

- Proteins are adsorbed to the lipophilic layers surrounding cells. The proteins possess hydrophobic interiors and a water-containing outer layer.

- The lipid layer possesses amphiphilic or charged head groups. This implies that the lipid membrane also contains some water.

- The water-containing regions of protein layers adsorped on lipid layers are permeable for charged solutes, e.g., ions.

- Divalent cations as calcium form complexes with lipids or proteins that reduce their interaction with water. Therefore membranes containing calcium are less permeable for ions.

- Hydrophobic molecules such as ether penetrate the membranes through their lipophilic lipid part.

They included some theoretical considerations about the different dependencies of the permeabilities of membranes to ions and hydrophobic molecules as a function of temperature.

> Danielli and Davson concluded that the permeabilities of membranes for solutes are explainable within the concepts of the physical chemistry of the hydrophilic and lipophilic regions of the cell membranes and that no particular chemical reactions including the solutes are needed to explain the transport properties.

Unfortunately, this very sober view is nowadays not in the focus of much of the biochemical membrane research due to the emphasis of the localized function of ion- and solute-specific transport channel proteins. In the chapter on permeability (Chapter 17) we will return to the quite realistic physical view of Danielli and Davson.

Danielli and Davson did not exclude the possibility that the proteins may span the membrane such that a "mosaic" of protein-rich and lipid-rich regions is formed. However, they did restrain themselves from speculating about such a structure due to the lack of experimental evidence. The term "mosaic membrane" was later used again by Singer and Nicolson (1972).

1.4
Robertson (1958)

So far most evidence about the structure of cell membranes was indirect. The resolution of light microscopy is restricted to the regime above 200 nm, which is not sufficient for revealing the bimolecular structure of the biological membrane that is between 5 and 10 nm thick. This changed with the progresses in electron microscopy. In 1959, J. David Robertson wrote a review in which

Fig. 1.3 Two opposing plasma membranes showing the double-layer character of the membranes. Picture taken from Bloom and Fawcett (1994) © Springer. Such images support the view of Gorter and Grendel (1925) and of Danielli and Davson (1935).
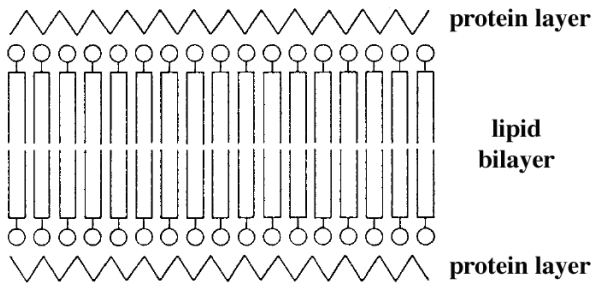
Fig. 1.4 Robertson (1959) collected electron microscopy images of many cells and organelles. His picture of a biological membrane resembles that of Danielli and Davson (1935). However, it is now based on microscopic evidence. Reproduced with permission from Robertson (1959) © The Biochemical Society.

he collected his evidence for a unique membrane structure obtained from the then advanced electron microscopy (Robertson, 1959). He basically confirmed the models of Gorter and Grendel (1925) and Danielli and Davson (1935). In his review he carefully described the membrane structures of the different organelles including the double membrane layers of mitochondria and the cell nucleus (Fig. 1.3). He also described the membranes of nerve cells and recognized that the Schwann cells form membrane layers surrounding the nerve membranes. Robertson's conclusion was that all evidence points at a common construction principle for all membranes of biological cells. They form a three-layered structure and are about 7.5 nm thick. In Robertson's view two protein layers are adsorbed to the lipid bilayer (see Fig. 1.4). As he noted himself this picture is in agreement with that of Danielli and Davson (1935). Remember, however, that the aim of Danielli was rather to explain selective transport of ions and apolar molecules. Robertson's model was sometimes incorrectly interpreted as that all membranes have the same composition. However, Robertson's statement was merely meant to describe a common structure.

1.5
The Fluid Mosaic Model of Singer and Nicolson (1972)

In the 1960s, the structures of a number of soluble proteins were solved by X-ray crystallography. Lenard and Singer (1966) found that many membrane proteins have a high α-helical content. Also, electron micrographs revealed that labeled proteins form isolated spots in some membranes. Furthermore, they considered the role of hydrophobic amino acids in α-helices. From this Singer and Nicolson concluded that proteins may also span through mem-

branes. This led to the famous Singer–Nicolson model (Singer and Nicolson, 1972) also known as the "fluid mosaic model." This model can be summarized as follows: Membranes are constructed from lipids and proteins. The proteins form mainly two classes. Peripheral proteins are those proteins that are only loosely attached to the membrane surface and can easily be separated from the membrane by mild treatment (e.g., cytochrome c in mitochondria or spectrin in erythrocytes). Integral proteins, in contrast, cannot easily be separated from the lipids. They form the major fraction of membrane proteins. The structure forming unit (matrix) is the lipid double layer (bilayer). Proteins may be either adsorbed to the membrane surface or span through the membrane (Fig. 1.5). The term "fluid mosaic model" used by Singer and Nicolson probably originates from Danielli and Davson (1935) although their paper was not cited.
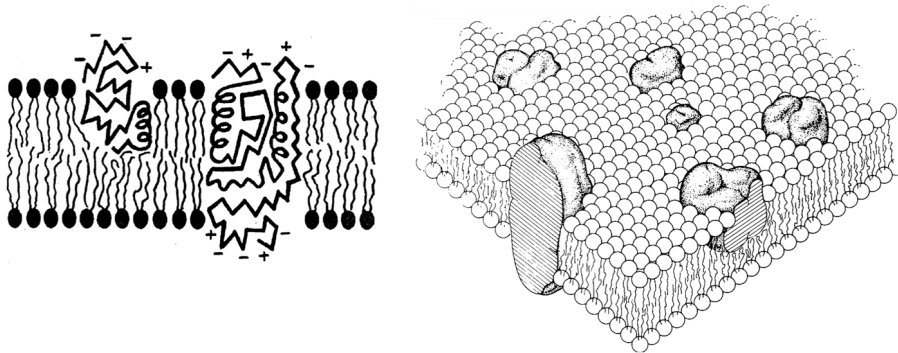
Fig. 1.5 The "fluid mosaic model" of Singer and Nicolson (1972). The left side shows the lipid bilayer including globular proteins intercalated with the membrane, and transmembrane proteins. The membrane proteins are not all distributed homogeneously. Reprinted with permission from AAAS.

Singer and Nicolson (1972) underlined that some proteins seem to interact with the surrounding lipids and that protein function may depend on the presence of specific lipids (see Fig. 1.6). They proposed that the proteins are surrounded by a layer of strongly interacting lipids while most of the remaining lipids are hardly influenced by the presence of proteins. This implies that the lipids form a matrix and no long-range order of proteins exists within the matrix. Short range order due to protein–protein interactions (possibly mediated by specific lipids) was considered as a possibility (see Fig. 1.6). Such interactions are discussed in Chapter 9. It was postulated that the lipid membranes of biological cells are in the fluid lipid state (with exceptions, e.g., the myelin) in which proteins can freely diffuse. In this respect an interesting paper by Frye and Edidin (1970) showed that when two different cells with different proteins are forced to fuse, the proteins redistribute over the whole surface within 40 min. This finding supports the view of freely diffusing pro-
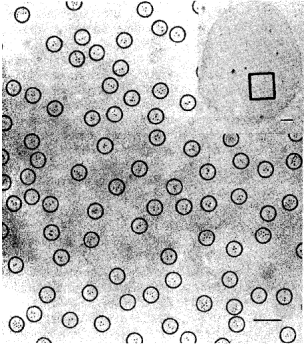
Fig. 1.6 Protein distribution in erythrocyte membranes from Singer and Nicolson (1972). Specific proteins were labeled with antibodies. The circles indicate protein clusters with a diameter of about 30 nm. Reprinted with permission from AAAS.

teins in cell membranes. Singer and Nicolson also noted that the fluid mosaic membrane is most likely asymmetric and displays distinctly different features on the inside and the outside of cells. Asymmetry of membrane lipids has in fact been found in experiments (Rothman and Lenard, 1977; Rothman and Kennedy, 1977). Also, proteins are now known to display preferential orientations in membranes.

The Singer–Nicolson model still is the widely accepted model. In particular, due to progresses in the crystallization of membrane proteins it is nowadays known that membrane proteins display α-helical or β-barrel-like membrane spanning segments of predominantly apolar amino acids.

1.6
The Mattress Model by Mouritsen and Bloom (1984)

The fluid mosaic model of Singer–Nicolson has nowadays experienced some refinement, which takes into account that lipids and proteins may distribute inhomogeneously and that domains and clusters may form within the membrane. Without explicitly saying so, the Singer–Nicolson model considered the lipid membrane as a homogeneous fluid in which the proteins diffuse in two dimensions. In 1984, Mouritsen and Bloom (1984) proposed the mattress model (Fig. 1.7) that suggests that proteins and lipids display interactions with a positive free energy content due to variations in the hydrophobic length of the molecules (see Section 9.1). The typical thickness of a lipid bilayer is about 5 nm. If the hydrophobic core of a membrane protein is longer or shorter than this length, either some hydrophobic protein or lipid segments are exposed to water, or the lipid membrane has to be deformed to compensate for the

a   **b**
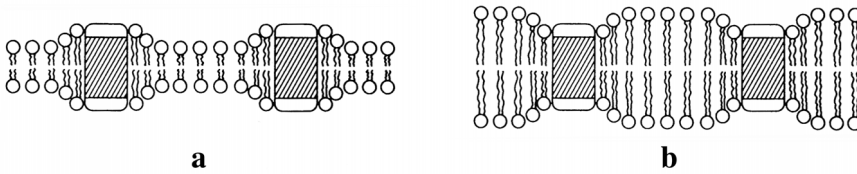
Fig. 1.7 The mattress model from Mouritsen and Bloom (1984). This model takes into account that the hydrophobic core of proteins may not match the bilayer thickness. This leads to interfacial tensions and capillary forces between protein and lipids. With permission from Biophys. J.

unfavorable hydrophobic interactions. This effect is called the "hydrophobic matching." The hydrophobic matching gives rise to interfacial tensions between lipids and proteins. These tensions may result in the accumulation of certain lipid species around the proteins (see Fig. 1.8), and in the mutual attraction of proteins due to capillary forces, leading to aggregation and clustering of proteins.
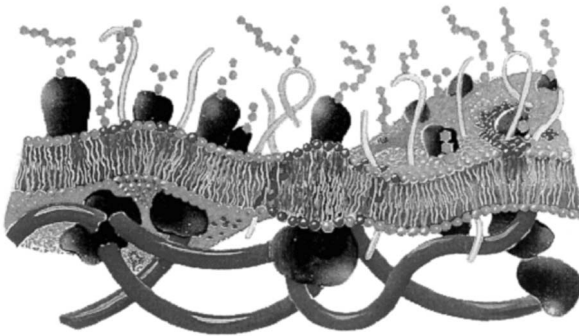
Fig. 1.8 In the mattress model the proteins may influence the lipids in their vicinity. Picture courtesy to O. G. Mouritsen.

1.7
Domain Formation and Protein Clusters

Similar arguments as for the matching of lipids and proteins lead to the assumption that also different lipid species may not match perfectly. Biological membranes contain hundreds of different lipid species with variable head group and chain composition (Chapter 3). Most lipids possess two apolar hydrocarbon chains with variable length. Furthermore, lipid membranes undergo melting transitions, which are accompanied by changes in the effective

lipid chain length (Chapter 6). Thus, also the lipids within the membrane plain can form various clusters, domains, and aggregates. A modern view of biological membranes has to include the phase behavior of the lipid and protein components (Chapters 7–9).

The phase behavior of membranes becomes especially interesting if one considers cooperative transitions in the biological membrane. Native membranes show several cooperative events in direct proximity of growth or body temperature. As an example a calorimetric experiment on native *E. coli* membranes grown at 37° is shown in Fig. 1.9. Slightly below growth temperature a cooperative lipid melting peak is found. Above growth temperature several protein unfolding peaks can be seen.
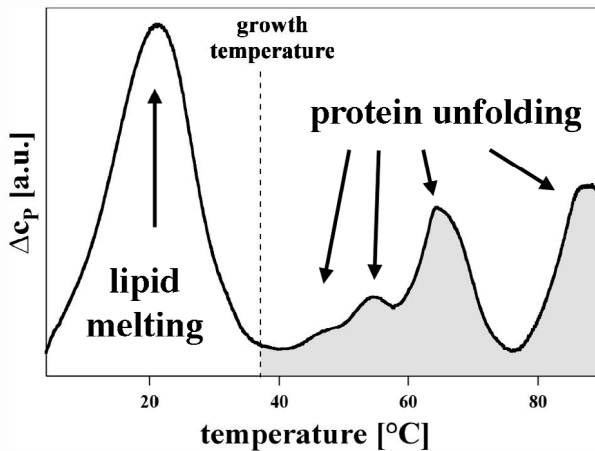
Fig. 1.9 A calorimetric experiment on a native *E. coli* membrane shows that lipid melting events take place slightly below growth temperature. Above growth temperature a number of protein unfolding events take place. Adapted from Heimburg and Jackson (2007a).

During lipid melting transitions the thickness of lipid membranes and the lateral lipid distribution changes. This is of extreme interest for regulation and signal transduction purposes in such membranes (Section 9.5 and Chapter 10). The mattress model implied that the matching of the dimensions of lipids and proteins influences the lipid recruiting around proteins and the lateral arrangement of proteins due to attractive forces from capillary effects. Thus, such transitions are linked to the formation of domains and clusters. Some examples are shown in Fig. 1.10. The left-hand panel shows a fluorescence microscopy image of a monolayer consisting of one single lipid. The dark regions represent ordered lipid domains while the bright regions represent disordered chains. The center panel shows domain formation phenomena in the fluorescence microscopy image of a giant lipid vesicle made from a lipid mixture. Giant vesicles are lipid bilayer vesicles that have similar dimensions to
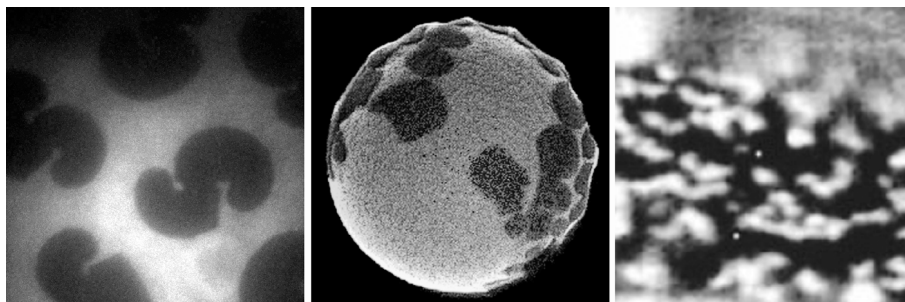
Fig. 1.10 Domain formation in lipid mono-layers, bilayers, and in biological cells. Left: Domain formation in the phase coexistence regime of DPPC monolayers. The dimension of the panel is about 100 μm. From Gud-mand/Heimburg, NBI Copenhagen. Center: Confocal fluorescence microscopy image of domain formation in a giant lipid vesicle (DLPC:DPPC = 30:70 at room tempera-ture). The size of the vesicle is about 30 μm in diameter. From Fidorra/Heimburg, NBI Copenhagen. Right: Placental alkaline phos-phatase distribution in fibroblast. The size of the segment is about 4 μm. From Harder et al. (1998).

biological cells. The right-hand panel shows the formation of protein clusters (placental alkaline phosphatase=PLAP) in a fibroblast cell form (Harder et al., 1998). In this paper it was shown that different proteins species tend to colo-calize in different regions of the cell membrane. In biomembranes a special kind of domain called "raft" is presently highly discussed. Rafts are thought to be microdomains consisting predominantly of sphingolipids, cholesterol and certain GPI-anchored proteins. These phenomena are discussed in much more detail in Chapters 8 and 9.

Domain formation is also interesting for the electrostatic properties of mem-branes. Many membrane components carry charges. Thus, domain formation leads to inhomogeneities in electrostatic potential and to the preferential bind-ing of proteins.

1.8
Perspectives of this Book

The biological membrane resembles the picture in Fig. 1.11, showing varia-tions in the membrane thickness, the presence of peripheral and transmem-brane proteins, as well as the formation of lipid and protein domains (Chap-ters 8 and 9). The thermodynamics of such phenomena is an essential part of this book. Cooperative transitions also influence the elastic constants (Chap-ter 14). Thus, rearrangement of proteins and lipids is also generally linked to alterations of membrane elasticity and compressibility. Due to the cou-plings in the thermodynamic equations these relations go in both directions meaning that changes in the membrane curvature by necessity have to change
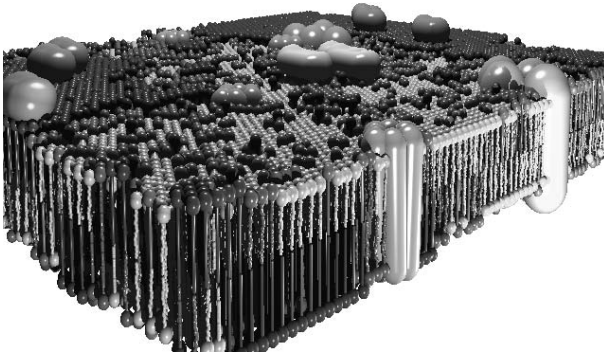
Fig. 1.11 The modern picture of membranes allows for lateral hetero-geneities, cluster and domain formation within the membrane plane. Picture generated by H. Seeger, NBI Copenhagen.

lipid distributions. If the melting of membranes leads to the rearrangement of proteins and a related change of the elastic constants, then conversely a change in protein distribution will lead to a change in the physical state of the membrane, and bending will influence melting and protein distributions. The elastic constants are responsible for many changes in the geometry of membranes (Chapter 15), for the possibility of mechanical excitations propagating in membranes (Chapter 18), and for changes in permeability (Chapter 17). Since some of the membrane components carry charges, generally domain formation also leads to heterogeneities in the electrostatic potential and to a coupling of electrostatic fields to the phase behavior of membranes.

The subjects treated in this book include

- composition and structure of biological membranes (Chapters 2 and 3),

- the role of water and the hydrophobic effect (Chapter 5),

- phase behavior and domain formation (Chapters 6–9),

- lipid–protein interactions (Chapter 9) and protein binding to surfaces (Chapter 12),

- diffusion in membranes containing domains (Chapter 10),

- electrostatics and its influence on protein binding (Chapter 11),

- the elastic constants and how they are influenced by temperature, pressure, protein binding, and other thermodynamic variables (Chapters 13 and 14),

- changes in membrane geometry due to changes in the elastic constants (Chapter 15),

- relaxation phenomena (Chapter 16),

- some considerations on the permeability of membranes for ions and larger molecules and how it is related to the thermodynamics of the membrane (Chapter 17).

- the propagation of density pulses and a related thermodynamic theory for the propagation of nerve pulses (Chapter 18),

- a thermodynamic theory for anesthesia (Chapter 19),

The function of the biological membrane cannot be understood without consideration of its thermodynamics. It is a multicomponent system that sensitively responds to changes in temperature, pressure, and the chemical potentials of its components. Therefore, this book also contains a basic introduction into thermodynamics (Chapter 4). The purpose of this book is to describe the concepts of thermodynamic couplings of seemingly independent properties of membranes. It will be shown that all of the above phenomena are intimately related and fit into a coherent thermodynamic picture.