Thomas Heimburg

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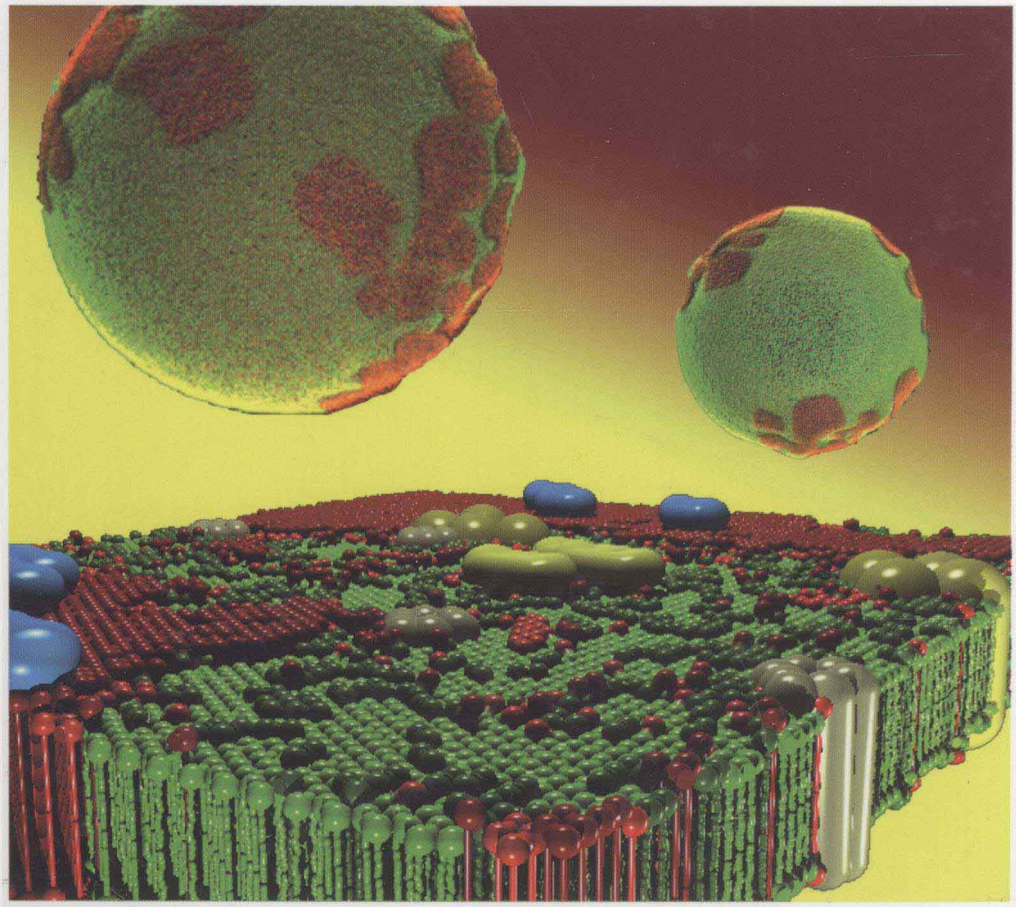
Thermal Biophysics of Membranes



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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.

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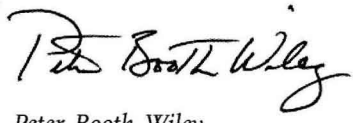

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Preface

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

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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*

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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.

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

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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.

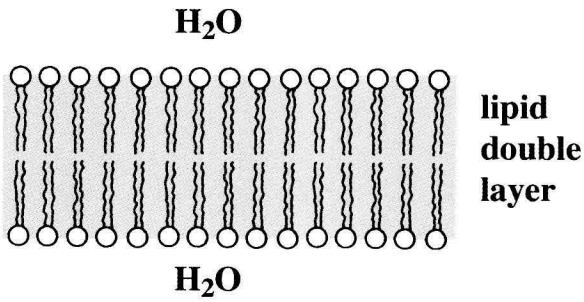

Fig. 1.1 The cell membrane according to Gorter and Grendel (1925). They proposed the lipid bilayer structure.

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