THE FRONTIERS COLLECTION

Series editors

Avshalom C. Elitzur

Unit of Interdisciplinary Studies, Bar-Ilan University, 52900 Ramat-Gan, Israel

e-mail: avshalom.elitzur@weizmann.ac.il

Laura Mersini-Houghton

Department of Physics, University of North Carolina, Chapel Hill,

NC 27599-3255, USA

e-mail: mersini@physics.unc.edu

T. Padmanabhan

Inter University Centre for Astronomy and Astrophysics (IUCAA), Pune, India

Maximilian Schlosshauer

Department of Physics, University of Portland, Portland, OR 97203, USA

e-mail: schlossh@up.edu

Mark P. Silverman

Department of Physics, Trinity College, Hartford, CT 06106, USA

e-mail: mark.silverman@trincoll.edu

Jack A. Tuszynski

Department of Physics, University of Alberta, Edmonton, AB T6G 1Z2, Canada

e-mail: jtus@phys.ualberta.ca

Rüdiger Vaas

Center for Philosophy and Foundations of Science, University of Giessen,

35394 Giessen, Germany

e-mail: ruediger.vaas@t-online.de

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A.C. Elitzur L. Mersini-Houghton T. Padmanabhan M. Schlosshauer
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Ole G. Mouritsen · Luis A. Bagatolli

LIFE – AS A MATTER OF FAT

Lipids in a Membrane Biophysics Perspective

Second Edition

Ole G. Mouritsen
Department of Physics, Chemistry
and Pharmacy
MEMPHYS-Center for Biomembrane
Physics
Odense
Denmark

Luis A. Bagatolli
Department of Biochemistry and Molecular
Biology
MEMPHYS-Center for Biomembrane
Physics
Odense
Denmark

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Preface

The first English edition of the present book was published in 2005 and although still in use around the world the text could be much improved by a timely update on several counts. Since 2005 the field of membrane biophysics has expanded tremendously and moved center stage in the study of biological membranes, not least due to a tremendous development in the experimental techniques that can be applied to membranes. It was deemed useful to prepare a revised edition of the book by incorporating some of the advances in the field over the last decade. In addition, the book now includes more tutorial material on the biochemistry of lipids and the principles of lipid self-assembly.

The second edition greatly owes its materialization to the efforts of one of the authors (LAB), who undertook a revision of the first edition (single-authored by OGM) for preparation of a Spanish edition that was published in 2014. The present second English edition is to a great extent a revised version of the Spanish edition.

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 authors and their collaborators. 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 book is provided with a Bibliography containing a selected list of references to 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 mostly 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. We apologize in advance to those authors and colleagues who may feel that their original work should have been referenced and discussed in more detail.

The authors have 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) at the University of Southern Denmark.

The authors have drawn on the help from a number of colleagues and the permission to use some of their graphic material for preparing many of the figures of this book. A list of the sources for the figures is provided at the end.

Odense
May 2015

Ole G. Mouritsen
Luis A. Bagatolli

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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
PA	Phosphatidic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PS	Phosphatidylserine

Lipids

DAG	Di-acylglycerol
DAPC	Di-arachidoyl PC
DCPC	Di-capryl PC
DLPC	Di-laureoyl PC
DMPC	Di-myristoyl PC
DMPE	Di-myristoyl PE
DMPG	Di-myristoyl PG
DOPC	Di-oleoyl PC
DPPC	Di-palmitoyl PC
DPPE	Di-palmitoyl PE
DSPC	Di-stearoyl PC

DSPE	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
GUV	Giant unilamellar vesicle
HDL	High-density lipoprotein
LAURDAN	6-Dodecanoyl-2-Dimethylaminonaphthalene
LDL	Low-density lipoprotein
RNA	Ribonucleic acid
ROS	Reactive oxygen species

Prologue: Lipidology—The Science of Lipids

To many 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 the 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, nucleic acids (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 we, for example, obtain from our food.

There is a particular reason why lipids are considered as being 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 viewpoint on lipids as dull molecules has changed considerably over the last couple of decades and their importance in 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* that 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 which are only two molecules thick. These layers constitute the backbone of all biological membranes. Membranes are a ubiquitous structural element in all living cells.

And cells are literally packed with membranes. In a human being which is composed of about 4×10^{13} cells, the total surface of all plasma cell membranes can be estimated to cover an area of about 12,000 m², corresponding to almost two football fields. Counting also internal membranes, the total area is probably a couple of orders of magnitude larger. 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 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; they are 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 been related not only to atherosclerosis but also to major psychiatric illnesses. Finally, polyunsaturated 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, which at the end of the twentieth century took us through the *genomics* era with focus on sequencing genes and mapping gene products. We are passing through the *proteomics* era where the multitude of proteins that are coded for by the genes are identified and their functions being unveiled. We are in the middle of the *lipidomics* era. In a not too distant future we can imagine all the ‘omics’ to converge into the grand *metabolomics* era where 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 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 and scientific thinking 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, the real fundamental science of lipids, i.e., *lipidology*, has to go beyond that and approach cellular functioning from a more holistic perspective. And it will have to be hypothesis-driven. Lipidology 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.

A deeper knowledge of the physical properties of lipids is obviously useful for understanding the living world and its modes of functioning. It may also be useful for 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 development of futuristic soft and biocompatible materials, functionalized surfaces and sensors, design of new and effective drugs and drug-delivery systems, for e.g., cancer therapy, as well as design of new enzymes for cheap and clean production of drugs and chemicals.

The present 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 which 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.

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 (μm) is 1000 nm. The appropriate unit of force for biological molecules on the nanometer 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 ($T_{\text{room}} = 293 \text{ K} = 20^\circ\text{C}$)

$$\text{Thermal energy} = k_{\text{B}}T_{\text{room}} = 4.1 \cdot \text{nm} \cdot \text{pN}, \quad (1)$$

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 Eq. (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 $k_{\text{B}}T_{\text{room}}$ or less, thermal agitation should be significant, and the lifetime of the association can be short. A couple of examples can serve as illustration. A covalent chemical bond C–C between two carbon atoms represents an energy of the order of $100 k_{\text{B}}T_{\text{room}}$ (with a force equivalent around 5000 pN) and is therefore very stable at room temperature. A typical hydrogen bond amounts to about $10 k_{\text{B}}T_{\text{room}}$ (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, represent 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.