Edited by
Gerhard Gompper, Michael Schick

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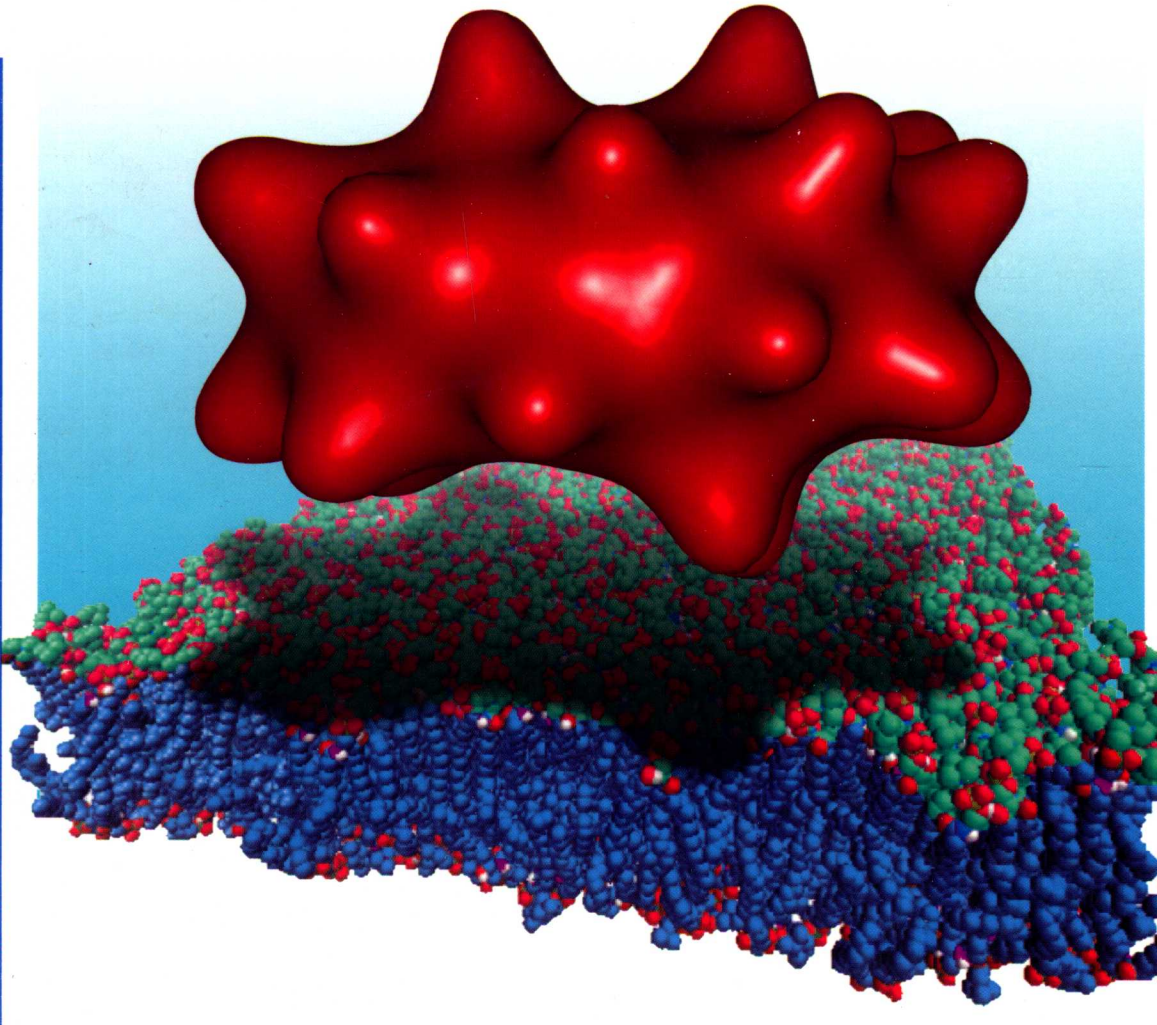
Volume 4
Lipid Bilayers and Red Blood Cells



Soft Matter

Volume 4: Lipid Bilayers and Red Blood Cells

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Cover illustration:

The membranes of almost all cells include a plasma membrane consisting of a lipid bilayer with protein inclusions. The background shows a snapshot of a typical lipid bilayer generated by a Molecular Dynamics simulation. The outer regions (green, red) typically charged or dipolar, while the interior region (blue) consists of non-polar hydrocarbon chains. The highly flexible and disordered nature of the lipid bilayer is essential for proper biological functioning of membrane protein.
The membrane of red blood cells is more complex, since it is composed of a plasma membrane plus a closely-associated elastic protein net – called the membrane skeleton – attached on the inside. Normal human red blood cells are soft bi-concave disks. Chemical and physical stresses can induce modification of this shape. Shown in the foreground is a so-called echinocytic shape. Echinocytes occur as a minority population in normal blood. (Original pictures courtesy of Sagar Pandit, See-Wing Chiu, and Gerald Lim H. W.)

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Preface

This is the fourth volume in the series "Soft Matter" and the first to be devoted to biological systems, the study of which has become one of the most intense activities in soft condensed matter in recent years.

Both chapters of this volume address the properties of lipid bilayers, a system which forms the basis of all biological membranes. At first glance, however, the two contributions are very different.

The first chapter, authored by Sagar Pandit and H. Larry Scott, is concerned with the behavior of the bilayer on the scale of the individual lipid molecules. They review, therefore, the numerous microscopic models which are used to describe bilayers, and the methods by which they are simulated, in particular molecular dynamics, Monte Carlo, and Langevin dynamics. *Thermal fluctuations are important* because the hydrocarbon chains are very flexible, and accordingly their conformations are dominated by entropy. The focus is on the dynamics of the several different components which make up the bilayer. Here, the three major players are cholesterol, lipids whose chains are fully saturated, and other lipids whose chains often contain one double bond, but occasionally as many as six. *Chemical details matter.* For example, cholesterol contains a rigid multi-ring structure of which one face is "smooth" the other "rough". These interact differently with the chains. Some lipids, particularly those readily synthesized and available commercially, have two identical tails. In contrast, biological lipids often have one tail which is saturated and the other mono-unsaturated. What are the differences expected between laboratory and biological systems? How do the differences in the structures of the membrane components account, if at all, for "rafts", the putative agglomeration of cholesterol and saturated lipids which float, like rafts, in a sea of unsaturated lipids?

In contrast to the above, the chapter by Gerald Lim H. W., Michael Wortis, and Ranjan Mukhopadhyay, treats the membrane, consisting of the lipid bilayer and its associated skeleton, as a continuum surface described by various elastic modulii. *Details of the bilayer components do not matter.* They are relevant only in determining the actual values of the elastic modulii. Attention here is on the *equilibrium* shape of the red blood cell, whose characteris-

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tic size is on the order of several microns, a thousand times larger than the characteristic size of the lipid components. For the most part, *thermal fluctuations are not important* as the characteristic energy of the system is that of the bending modulus, about 50 kT. The focus of this chapter is the fascinating behavior of the shape of red blood cells, which is normally that of a flattened, biconcave disc, a "discocyte", under varying conditions. By the application of suitable chemical agents, this shape can be made to undergo several transformations: to become either more concave and invaginated (shapes denoted stomatocytes, from the Greek for "mouth"), or to exhibit external perturbations and protrusions (denoted echinocytes, from the Greek for "hedgehog"). What the authors show conclusively is that an energy functional, which accounts for curvature and stretching elasticity as well as the effect of a difference in area between the bilayer leaves, leads to the sequence of shapes normally observed, and makes many predictions about others.

There are also several striking similarities between the chapters however. First one notes the central role of Newton's laws. Of course molecular dynamics is the sequential application of Newton's laws to the components of the system. Similarly elasticity theory is the application of Newton's laws to a continuous body. What makes the application to membranes so fascinating is that, because the membrane can change shape, one must implement elasticity theory in a manner applicable to arbitrarily curved surfaces. This immediately brings us to the applications of differential geometry, which are often relegated to courses on general relativity and astrophysics. It is a pleasure to see them applied here to more terrestial problems. The necessary material is clearly presented in a masterful series of appendices. Both chapters also show that, to extract useful results, one has to rely on numerical solutions. Indeed the explication of the means to undertake this forms a large part of the chapter by Pandit and Scott.

Both of these chapters represent contemporary studies in biological systems, but they also represent complementary qualities that we hope to showcase in this series. Simulations of large biological systems are changing rapidly as the capabilities of computers increase. The chapter by Pandit and Scott presents a snapshot of the state of such simulations at this moment in time. In several years, the applications illustrated in such a chapter, and some of the underlying methodology, will probably be significantly different. The chapter by Lim, Wortis, and Mukhopadhyay on the other hand is a definitive monograph. There may be some adjustment of parameters in the future, and further comparison with experiment, but it is likely that this work will remain the definitive text. We are both pleased, and proud, to present these two outstanding contributions to the community.

March 2008 Gerhard Gompper and Michael Schick

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Simulations and Models of Lipid Bilayers

Sagar A. Pandit and H. Larry Scott

Abstract

Atomistic level molecular dynamics can provide insights into the structure, dynamics and thermodynamic stability of lipid membranes and of localized raft-like regions in membranes. However the challenges in the construction and simulation of accurate models of heterogeneous membranes are great. In this chapter we outline the steps needed to carry out and analyze atomistic simulations of hydrated lipid bilayers. While molecular dynamics is a method that is simple in its conceptual content, there are many subtle challenges that must be addressed in the construction of a simulation of a lipid bilayer in water. These include simulation algorithms, forcefields, boundary conditions, equilibration and others. We will discuss all of the basic requirements for the construction and running of a molecular dynamics simulation of a lipid bilayer. We then discuss how one analyzes the data presented by a simulation, in terms of experimental results and detailed structural and dynamical predictions of the simulation. In the final part of the chapter we show how the data from a molecular dynamics simulation can be used to construct a coarse grained model for the heterogeneous bilayer that can predict the lateral organization and stability of rafts at up to millisecond timescales.

1.1
Introduction

There are many well documented fields of research in structural biology to which physicists and physical chemists regularly contribute; equally interesting are fields of biological research for which the reverse is true. The emergence of order in complex systems, from basic bio-molecular building

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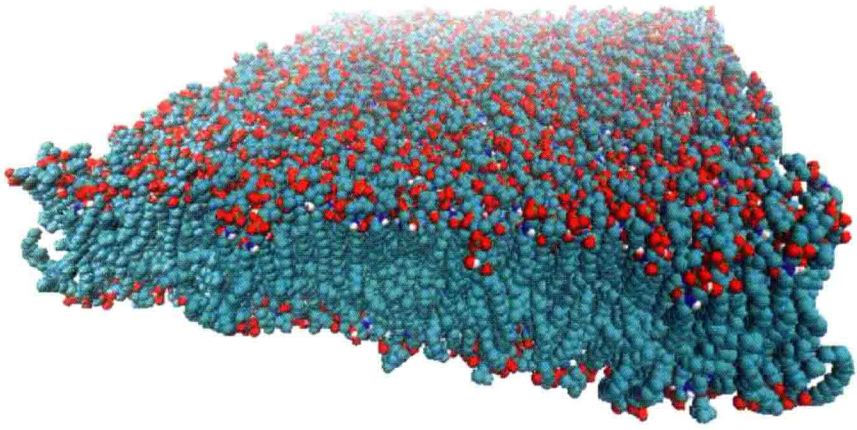

Fig. 1.1 Snapshot of a sphingomyelin lipid bilayer produced in a simulation. The lipid is 18:0 sphingomyelin, a common brain lipid. Color coding is: gray: hydrocarbon chains; red: oxygen atoms, orange: phosphorous atoms: white: hydrogen atoms and blue: nitrogen atoms. Water molecules and hydrogen atoms on hyrdocarbon chains and outermost choline groups have been excluded for clarity.

blocks (proteins, lipids, carbohydrates, sterols and others), suggests new levels of emergent material properties that hold fundamental insights in basic soft matter physics and chemistry. One such biologically inspired field in soft matter science is the study of the structure, thermodynamics, dynamics and functional behavior of biological membranes. The physics of biological membranes is uniquely interesting for multiple reasons including, but not restricted to, the following: they self-assemble spontaneously in solution, they are quasi-two-dimensional, they are composed of relatively large but flexible molecules with many intramolecular degrees of freedom, they exhibit complex phase behavior and they are capable of incorporating larger biomolecules, like proteins, without compromising their basic structural integrity. This level of structural diversity presents many challenges to those desiring to use modeling to dissect the underlying physical and chemical properties of biomembranes, and it also presents the possibility that new physics may emerge from biomembrane modeling.

Typical biomembranes are highly non-homogeneous in composition but the basic underlying structural matrix, the lipid bilayer, is the same in almost all prokaryotic and eukayrotic cells. Figure 1.1 shows a snapshot of a lipid bilayer from a simulation. The commonly accepted conceptual model for a biological membrane is such a double layer of lipid molecules, within which are embedded sterols and a bewildering variety of membrane proteins. Lipid bilayers are back-to-back monomolecular layers of phospholipid molecules. A typical phospholipid molecule, generally of a molecular weight

around 750, consists of two distinct parts: a water-soluble, or hydrophilic part, and a water-insoluble, or hydrophobic part. Figure 1.2 shows a diagram of a commonly studied phospholipid, dipalmitoylphosphatidylcholine (DPPC). Figure 1.2 shows that a typical biological lipid consists of three distinct chains linked through a "backbone". In phospholipids the backbone is a three-carbon glycerol link while for sphingolipids the link is a sphingosine group. In all cases of biological interest two of the chains are made of CH_2 groups connected by single or, in some cases, double bonds and terminated by a methyl CH_3. These two chains are highly hydrophobic, with very low solubility in water. The third chain contains phosphate (PO_4) and choline ($N(CH_3)_3$) fragments connected by a methylene (CH_2). At neutral pH the phosphate carries a net negative charge that is balanced by a net positive charge on the choline. The dipolar nature of the polar chain (referred to as the "head group") renders the head group highly soluble in water. As a consequence of the amphiphilic (half water-hating and half water-loving) nature of the molecule, when dispersed in excess water lipids like DPPC self assemble into structures that shield the hydrophobic regions from water and maximize contact between the polar regions and water. One class of structures are lipid bilayers. In a lipid bilayer in an aqueous solution, the hydrophobic parts make up the interior while the hydrophilic parts make up the interface with the water. Lipid bilayers can spontaneously form closed spherical structures, or vesicles, when mixed in excess water. Vesicles are the natural "compart-

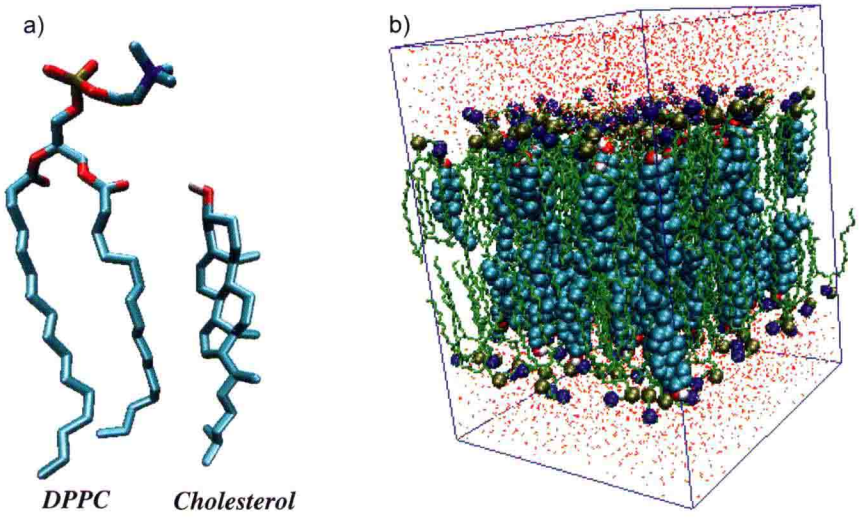

Fig. 1.2 (a) Stick models of DPPC and cholesterol molecules. In DPPC, the upper chain is the hydrophilic polar head group, and the two lower chains are the hydrophobic part of the molecule. (b) A snapshot of a simulated bilayer made up of DPPC and cholesterol. This snapshot also shows water molecules above and below the bilayer.

ments" that separate the interior from the exterior of a cell. Cholesterol, also shown in Fig. 1.2, is another biologically important lipid that contains hydrophobic and hydrophilic parts. In the case of cholesterol the hydrophobic part has four fused rings and a short tail, while the hydrophilic part consists of a single hydroxyl attached to the first ring. Of particular interest, as we will discuss later in this chapter, are the three methyls that protrude from one side of the ring portion of cholesterol. They are represented by three horizontal sticks in Fig. 1.2.

The conceptual view of biomembranes based on the lipid bilayer concept, called the fluid mosaic model, was first proposed in 1972 (Singer and Nicholson 1972). In the fluid mosaic picture, membrane proteins, sterols (such as cholesterol) and other biologically essential molecules reside in, on or penetrate the lipid bilayer, performing essential biochemical functions required by the cell. Since 1972 much progress has been made in understanding the properties of a fluid mosaic membrane at a molecular level. It has become clear that the fluid mosaic picture describes a highly dynamic structure of extremely hetreogeneous composition that can fluctuate in its lateral organization and in the ordering of the lipid chains in response to stimuli from the interior and the exterior of the cell. Within the plane of the lipid bilayer there is rapid lateral diffusion and dynamical fluctuations in structure on a sub-nanometer scale. Since a typical membrane is made up of perhaps a dozen different lipids, and contains sterol and proteins, to dissect the underlying physical interactions is a formidable problem. To better understand the many complex physical and chemical reactions and interactions which drive biological functions, a tractable approach is to first gain insight into the molecular interactions within simple lipid bilayers, such as shown in Fig. 1.1. In model membranes the composition is greatly simplified compared to that of biological membranes. Typically model membranes contain only one or two different lipids, a controlled amount of cholesterol and/or one or no membrane proteins. Figure 1.2 shows the structure of two commonly studied lipid molecules. Figure 1.2 also shows a snapshot that illustrates a typical distribution of lipids and water in a small part of a model bilayer which was generated by a simulation. The highly disordered, fluid nature of the bilayer can be seen in this figure.

Model membranes have been quantitatively studied experimentally by a wide variety of methods (Merz and Roux 1996; Nagle and Tristram-Nagle 2000; Tristram-Nagle and Nagle 2004). Over the years an interplay has developed between experiment and simulation, wherein experimental data revise and improve the quality of simulations and in turn simulations are used to interpret experimental data. As a consequence, there is now a sizable and growing data base of structural and dynamical data from which it is possible to construct theoretical models for lipid bilayers. The goal of theoretical

models is to understand how the microscopic intermolecular interactions in lipid bilayers lead to the experimentally observed structures. As an insight is gained into simple lipid bilayers through this process, the goal of future modeling work is to apply the new information to the expanded study of lipid bilayers of a more complex and biological composition. Unfortunately the complex structure of even the simplest lipid molecules (see Figs. 1.1 and 1.2) makes modeling especially difficult. Each of the three chains (two in the hydrophobic region and one hydrophilic chain) can change shape by rotations about atomic bonds (dihedral rotations), so that the conformation space of a single molecule is huge. A lipid bilayer is not just a simple two-dimensional fluid but a two-dimensional fluid of molecules, each of which has a large number of internal degrees of freedom. Hence, the interaction between pairs of lipid molecules depends not only on their separation, but also in some complex fashion on the conformational shapes of the molecules.

Theoretical models for lipid bilayers that concentrated on the main chain melting phase transition have been proposed (Nagle 1973; Scott 1975; Nagle and Scott 1978). However, it is generally very difficult to realistically use the analytical tools of statistical mechanics to model a complex system such as a lipid bilayer without major approximations. The best approximation schemes are those that are guided by experimental data. This difficulty was a severe limitation in the early development of this field due to a lack of detailed understanding of atomic level interactions between lipid molecules in bilayers, an essential requirement for the construction of realistic models. However over the past 10–15 years this situation has begun to change as atomistic simulations of lipid bilayers have progressed. A major motivation for doing simulations for lipid bilayers is that by doing reliable simulations (where reliability means the simulations are of sufficient scale in size and simulation time, and that they agree with all available experimental data) one gains atomic level structural and dynamical coordinates of all atoms in the system. The basic prediction of any simulation is a trajectory or a set of system configurations consisting of atomic coordinates, velocities (for molecular dynamics simulations) and interaction potentials which can be directly linked to the macroscopic behavior observed in experiments. This wealth of atomic resolution, structural and dynamical data is then available to test hypotheses used by experimentalists in interpreting measurements and for the design of new experiments. It can also be used by theorists to formulate better coarse grained statistical mechanical models for membrane phase behavior.

In this chapter we discuss the current state of atomistic simulations of model membranes. We will also describe some ways by which information from atomistic simulations can be directly employed for improved statistical mechanics modeling of model membranes at scales that greatly exceed