BIOLOGICAL MEMBRANES

Biological Membranes

A Molecular Perspective from Computation and Experiment

Kenneth M. Merz, Jr. and Benoît Roux
Editors

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Kenneth M. Merz, Jr.
Department of Chemistry
Pennsylvania State University
152 Davey Laboratory
University Park, PA 16802
USA

Benoît Roux
Groupe de Recherche en Transport
Membranaire (GRTM)
Départements de physique et de chimie
Université de Montréal
C.P. 6128, succ. Centre-Ville
Montréal, Québec
Canada H3C 3J7

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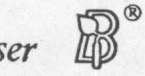

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Preface

The interface between a living cell and the surrounding world plays a critical role in numerous complex biological processes. Sperm/egg fusion, virus/cell fusion, exocytosis, endocytosis, and ion permeation are a few examples of processes involving membranes. In recent years, powerful tools such as X-ray crystallography, electron microscopy, nuclear magnetic resonance, and infra-red and Raman spectroscopy have been developed to characterize the structure and dynamics of biomembranes. Despite this progress, many of the factors responsible for the function of biomembranes are still not well understood. The membrane is a very complicated supramolecular liquid-crystalline structure that is largely composed of lipids, forming a bilayer, to which proteins and other biomolecules are anchored. Often, the lipid bilayer environment is pictured as a hydrophobic structureless slab providing a thermodynamic driving force to partition the amino acids of a membrane protein according to their solubility. However, much of the molecular complexity of the phospholipid bilayer environment is ignored in such a simplified view. It is likely that the atomic details of the polar headgroup region and the transition from the bulk water to the hydrophobic core of the membrane are important. An understanding of the factors responsible for the function of biomembranes thus requires a better characterization at the molecular level of how proteins interact with lipid molecules, of how lipids affect protein structure and of how lipid molecules might regulate protein function. Computer simulations of detailed atomic models based on realistic microscopic interactions represent a powerful approach to gain insight into the structure and dynamics of complex macromolecular systems such as a biomembrane. At the present time, even qualitative information gained from such computer simulations is valuable. Nevertheless, extension of current computational methodologies to simulate biomembrane systems still represents a major challenge. However, this field is just in its infancy, and it is likely that both experimental and theoretical tools will be needed to solve these problems. It is the goal of the present volume to provide a concise overview of computational and experimental advances in the understanding of lipid bilayers and protein/lipid interactions at the molecular level.

It can be reasonably expected that molecular simulations will play an increasingly important role in the future. While most trajectories to date are confined to the 1 ns time regime, this clearly will not be the case in the coming years. Just what kinds of time scales will we be able to simulate in the next five years? As an illustration, let us consider the following analysis. A typical simulation for a biological system now consists of ~15,000 atoms, which on modern parallel and vector supercomputers requires approximately 1 hour to generate 1 ps of a trajectory. Thus, one individual running MD simulations continuously can generate at most ~9 ns of trajectories in one year utilizing modern vector and parallel hardware resources available at many universities and supercomputer centers. Furthermore, it can be expected that algorithmic capabilities will be enhanced by a factor of 2 to 10-fold in the next year. This suggests that in one year we will go from a 9 ns capability to a 18 ns–90 ns capability just by improving our computational algorithms. In addition, it is generally agreed that there is a twofold speed increase every 18 months in computer technology. Thus, by the end of five years we can estimate a tenfold increase in computer power alone. Hence, the 9 ns/year we estimate now will increase to 90 ns based only on an increase in computer performance. Including an algorithmic improvement of 2 to 10-fold on top of this leads to an estimate of a 180 ns to 0.9 micros/year capability for one individual only. Obviously, this analysis is just an estimate and neglects many factors (e.g., increased system sizes, increased potential function complexity, etc.), but we think it is clear that molecular simulations on phospholipid bilayers will reach near microsecond capabilities in the next five years.

It is clear that theoretical methods will evolve to the point where we can address very long time scale issues. How about experimental techniques? Clearly, new approaches to solve experimental problems involving biomembranes will be developed in the coming years. Furthermore, we can also expect significant improvements in the techniques used to study biomembranes. For example, NMR has enjoyed tremendous growth over the years and this will continue in the coming years. The field strength of magnets have continued to grow, which provides higher resolution information that can be used to analyze biomembrane structure and dynamics. Moreover, new Solid State NMR techniques for oriented samples continue to be developed that will improve the ability to analyze biological membrane systems. Similarly, other experimental techniques like neutron scattering, X-ray, IR, CD, etc., will also continue to be improved upon in the coming years. Hence, the combination of improved computational and experimental techniques indicate that there is a bright future for the continued investigation of the structure, function, and dynamics of biological membranes at the molecular level.

This volume is separated into four sections. In section I, the basic theoretical and computational issues regarding biomembrane structure and dynamics are addressed. These issues range from basic statistical mechanics to force field development and evaluation. Thus, this section contains the necessary information required for anyone interested in attempting to model biomembranes using

molecular dynamics or Monte Carlo methods. Section II then moves onto a series of chapters describing experimental probes that can be used to assess biomembrane structure. These include X-ray, IR and NMR techniques, and all are capable of providing microscopic or macroscopic insights that can be used to enhance our understanding of biomembrane structure and dynamics. Moreover, these experimental techniques generate information that can be used to assess and verify theoretical studies. Section III gives both a theoretical and experimental perspective on the interaction of peptides with biomembranes. Many peptides are membrane active and deserve study in their own right, but these systems can also serve as powerful models of protein/lipid interactions. Hence, by understanding these smaller (and hopefully less complicated) systems we will increase our understanding of the larger integral membrane class of proteins. Finally, Section IV gives a broad theoretical and experimental perspective of protein/lipid interactions. In this section the chapters give insights into the thermodynamics of protein lipid interactions as well as provide structural details of these systems.

The editors would like to thank the staff at Birkhäuser and the contributors for helping us produce an outstanding volume on recent advances towards understanding the structure, function and dynamics of biomembranes and lipid/protein interactions.

List of Contributors

Donna Bassolino, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, NJ 08543-4000, USA

Jürgen Brickmann, Institut für Physikalische Chemie, Technische Hochschule Darmstadt, Petersenstraße 20, D-6100 Darmstadt, Federal Republic of Germany

Michael F. Brown, Department of Chemistry, University of Arizona, Tucson, AZ 85721, USA

Timothy A. Cross, Center for Interdisciplinary Magnetic Resonance at the National High Magnetic Field Laboratory, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, Fl 32306-3016, USA

K.V. Damodaran, Department of Chemistry, Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802, USA

Scott E. Feller, Biophysics Laboratory, Center for Biologics Evaluation and Research, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD, 20852-1448, USA

Kenneth Hamer, Theoretical Biophysics, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Thomas Heimburg, Abteilung Spektroskopie, Max-Planck-Institute für Biophysikalische Chemie, Postfach 2841, D-37018 Göttingen, Germany

Xiche Hu, Theoretical Biophysics, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Huey W. Huang, Department of Physics, Rice University, 6100 Main Street, Houston, TX 77005-1892, USA

Eric Jakobsson, National Center for Supercomputing Applications, 4039 Beckman Institute, University of Illinois, 405 North Mathews Ave, Urbana, IL 61801, USA

Martin Karplus, Department of Chemistry, Harvard University, 12 Oxford St, Cambridge, MA 02138, USA; and Laboratoire de Chimie Biophysique, Institut le Bel, Université Louis Pasteur, 67000 Strasbourg, France

Randal R. Ketchem, Center for Interdisciplinary Magnetic Resonance at the National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, Fl 32306-3016, USA

Paavo K.J. Kinnunen, Department of Medical Chemistry, Institute of Biomedicine, POB 8, FIN-00014, University of Helsinki, Finland

Juergen Koepke, Max-Plank-Institut für Biochemie, Abteilung Molekulare Membranbiologie, 6000 Frankfurt, Germany

Alexander D. MacKerell, Jr., Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland at Baltimore, 20 North Pine Street, Baltimore, MD 21201-1180, USA; and Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA

Derek Marsh, Abteilung Spektroskopie, Max-Planck-Institute für Biophysikalische Chemie, Postfach 2841, D-37018 Göttingen, Germany

Richard Mendelsohn, Department of Chemistry, Newark College, Rutgers University, 73 Warren Street, Newark, NJ 07102, USA

Kenneth M. Merz, Jr., Department of Chemistry, Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802, USA

Mariusz Milik, Department of Molecular Biology, Scripps Research Institute, 10666 N. Torrey Pines Road, MB1, La Jolla, CA 92037, USA

Hartmut Michel, Max-Plank-Institut für Biochemie, Abteilung Molekulare Membranbiologie, 6000 Frankfurt, Germany

Ole G. Mouritsen, Department of Physical Chemistry, Technical University of Denmark, Bldg 206, DK-2800 Lyngby, Denmark

Richard W. Pastor, Biophysics Laboratory, Center for Biologics Evaluation and Research, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD, 20852-1448, USA

Mary F. Roberts, Merkert Chemistry Center, Boston College, Chestnut Hill, MA 02167, USA

Benoît Roux, Groupe de Recherche en Transport Membranaire (GRTM), Départements de physique et de chimie, Université de Montréal, C.P. 6128, succ. Centre-Ville, Montréal, Québec, Canada H3C 3J7

Michael Schlenkrich, Institut für Physikalische Chemie, Technische Hochschule Darmstadt, Petersenstraße 20, D-6100 Darmstadt, Federal Republic of Germany; and Silicon Graphics, Erlenstraesschen 65, CH-4125 Riehen, Switzerland; and Department of Chemistry, Harvard University, 12 Oxford St, Cambridge, MA 02138, USA

Klaus Schulten, Theoretical Biophysics, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

H. Larry Scott, Department of Physics, Oklahoma State University, Stillwater, OK 74078-3072, USA

Barbara A. Seaton, Structural Biology Group, Department of Physiology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118, USA

Jeffrey Skolnick, Department of Molecular Biology, Scripps Research Institute, 10666 N. Torrey Pines Road, MB1, La Jolla, CA 92037, USA

Robert G. Snyder, Department of Chemistry, University of California, Berkeley, CA 94720-1460, USA

Terry R. Stouch, Bristol-Myers Squibb Pharmaceutical Research Institute, Room H3812, P.O. Box 4000, Princeton, NJ 08543-4000, USA

Shankar Subramaniam, National Center for Supercomputing Applications, 4041 Beckman Institute, University of Illinois, 405 North Mathews Ave, Urbana, IL 61801, USA

Stephen H. White, Department of Physiology and Biophysics, School of Medicine, University of California at Irvine, Irvine, CA 92717-4560, USA

Michael C. Wiener, Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, CA 94143-0448, USA

Thomas B. Woolf, Department of Physiology, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA

Dong Xu, Theoretical Biophysics, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Contents

Part I

Computational Issues Regarding Biomembrane Simulation

This section describes the basic background material for molecular simulations as they relate to biomembrane containing systems. Biomembrane modeling, while in many respects very similar to modeling other biomolecules (e.g., proteins, DNA, etc.), has its own set of technical vagaries that must be considered prior to beginning a simulation. MD trajectories based on atomic models are typically limited to a few ns, while many membrane phenomena take place over much longer time scales. In Chapter 1, Rich Pastor and Scott Feller describe the time scales of lipid bilayer motions and how they affect the outcome of molecular dynamics (MD) simulations. This first chapter provides a critical discussion of the limitations of current computational models. In Chapter 2, Michael Schlenkrich, Jurgen Brickmann, Alex Mackerell, and Martin Karplus describe the parametrization of the all-atom CHARMM PARAM 22 molecular mechanical force field for phospholipid molecules. They stress the factors that need to be considered and indicate the limitations of this type of approach. Larry Scott, in Chapter 3, gives an overview of the basic statistical mechanics of lipid bilayers and also presents details regarding how Monte Carlo (MC) methods can be used to advantage when studying the configurations of lipid assemblies. MD and MC methods have strengths and weaknesses that can be used to advantage when trying to understand the dynamics of lipid containing phases. In the present volume, calculations based on MD are described in Chapters 1, 2, 3, 8, 11, 15, and 17; calculations based on MC are described in Chapters 2, 3, 10, 13, 14, and 16. Further methodological issues, such as application of current simulation methods to model a lipid bilayer under constant pressure are also addressed by Eric Jakobsson, Shankar Subramanian, and Larry Scott in Chapter 4. Their chapter also illustrates how a molecular simulation provides insight into the origin of the membrane surface potential.

1

Time Scales of Lipid Dynamics and Molecular Dynamics

RICHARD W. PASTOR AND SCOTT E. FELLER

Introduction

It is finally possible to carry out a molecular dynamics (MD) computer simulation of a protein or peptide in a lipid bilayer. Simulation programs with reasonable potential energy parameters are readily available, computer workstations are affordable, and plausible initial conditions can be constructed by combining the polypeptide with lipid configurations taken from simulations of pure lipid bilayers. Clearly, there are many questions to ask. Does the protein somehow order the nearby lipids or perturb the water structure at the headgroup/solution interface? If the membrane contains a mixture of lipids, do some selectively condense around the protein? What are the lateral diffusion constants and isomerization rates for the lipids and protein, and are they perturbed from the pure state? These sorts of effects might be important to the protein's function, or they might modulate the rate that substrates pass through the bilayer. They could change the interfacial tension, making it easier for the membrane to bend or even fuse with another. A peptide with potential drug applications might disrupt the bilayer, aggregate to form channels, or bind to a membrane protein.

As this chapter shows, only some of these questions can be answered at present with a conventional MD simulation, which, for a bilayer/protein system, can produce a trajectory of about 100 picoseconds (ps) to one nanosecond (ns). The following section provides a brief overview of the molecular dynamics method and some specifics pertaining to simulations of lipid bilayers, including constant pressure algorithms. Lipid motions are then discussed in order of their accessibility on the MD time scale: isomerization (reasonably good); rotational

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relaxation (borderline); and lateral diffusion (just out of reach). We examine these motions using a combination of analytic theory and Brownian dynamics simulations of simple model systems, and molecular dynamics simulations of a dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. The analyses, though restricted to pure lipid systems, should provide a sense of the motions on the ps to ns time scale, and assist readers in assessing simulations and other modeling of more complex membranes.

The Molecular Dynamics Method

Overview

For a system with constant particle number, volume, and energy (*NVE*, or microcanonical ensemble), the molecular dynamics method simply involves numerically solving Newton's equations for each particle (Allen and Tildesley, 1987). First the initial conditions (positions and velocities) and interparticle forces must be specified. The form of the potential function and the type of algorithm then dictate the time step (too large a time step will lead to an unacceptable drift in the total energy). Finally, the nature of the problem and the available computer resources determine the length of the simulation. As such, an MD simulation is similar to a numerical simulation of orbiting planets and moons. Aside from the interparticle forces, there are several important differences:

(1) The initial velocities of the atomic system are typically obtained from a Maxwell-Boltzmann distribution, and are scaled (or rerandomized) until kinetic and potential energies are in equipartition and the target temperature is reached. As a result, the statistical nature of the system is introduced early on.

(2) The assignment of initial positions for simple systems (such as atomic fluids) can proceed from a relatively ordered configuration at the appropriate density, while complex systems like membranes often require artistry. In any case, the system should be well equilibrated before the production phase of the simulation begins.

(3) Periodic boundary conditions are usually imposed in order to eliminate so-called wall effects and thereby better model a bulk fluid. Hence, although the terms *box* or *cell* are commonly used, the walls are completely porous: a particle leaving the cell through one side reenters through the opposite face. This technique can lead to difficulties when the number of particles is small (e.g., the particle interacts strongly with its own image); it also sets an upper limit on the wavelengths of undulations or collective modes that can be studied.

It is not obvious that molecular dynamics simulations should work: one could imagine that it would require a nearly macroscopic number of particles and very accurate potential energy functions to produce anything comparable with experiment. (To verify the assertion that fast computers and complicated

theories do not guarantee good results, check the weather report or business section of today's newspaper). Nevertheless, by the 1960's MD simulations (and a cousin, Monte Carlo) were able to reproduce structural and dynamic properties of fluids using simple potentials and only several hundred particles. These early successes motivated the application to more complex systems, and computer simulation rapidly became an important complement to formal statistical mechanical theory. Simulations of biopolymers (Brooks et al, 1988; van Gunsteren et al, 1993) and lipid bilayers (Brasseur, 1990; Pastor, 1994b) followed, and, despite occasional grumbling by nonpractioners, molecular dynamics has become a central technique in biophysics. The remainder of this section describes some important technical details of bilayer simulations.

Dynamics at Constant Pressure

Unfortunately, it is difficult to simulate membranes using only constant volume algorithms. Both the height normal to the interface and the surface area must be specified correctly because the properties of surfactants are sensitive to both the normal pressure and the surface area per molecule (Cevc and Marsh, 1987; Small, 1986). While quantities such as interlamellar spacing and molecular areas have been determined for some lipids (Nagle, 1993; Rand and Parsegian, 1989), experiments give indirect guidance at best for assigning the appropriate simulation cell dimensions for bilayers made up of mixtures of lipids, or for ones containing peptides and proteins. Consequently, some allowance for volume and/or shape adjustments of the simulation cell is necessary in most cases.

Fluctuations in cell dimensions are most naturally accomplished by simulating in ensembles other than the microcononical. Because the appropriate variables are not always obvious, it is useful to start with the thermodynamics. We assume that the bilayer/water interface is planar with surface area A, and normal to the z direction. From the condition of hydrostatic stability, the pressure normal to the interface, P_n, equals the bulk pressure, P. Then, from the First Law of Thermodynamics:

$$dE = TdS - P_n dV + \gamma dA + \sum_{i=1}^{2} \mu_i dN_i, \tag{1}$$

where E is the internal energy, T the temperature, S the entropy, V the volume, γ the interfacial tension, N_i the number of particles of liquid i, and μ_i its chemical potential. We see that there are four pairs of variables (μ, N), (T, S), (P_n, V) and (γ, A); when one is fixed, the other fluctuates. If the system is isolated (i.e., constant particle number and no heat exchange with the surroundings), then $\mu_1 dN_1 = \mu_2 dN_2 = TdS = 0$, and Equation (1) becomes

$$dE = -P_n dV + \gamma dA \tag{2}$$

The consistency of the simulations at NVE is clear from Equation (2): the extensive variables volume and surface area are constant, while their conjugate

intensive variables, normal pressure and surface tension, respectively, can be evaluated by averaging over the trajectory. Surface tensions of liquid/vapor interfaces, including monolayers, are reasonably calculated from simulations in the *NVE* ensemble. Ensembles describing interfaces in which various intensive thermodynamic variables are constant have recently been described (Zhang et al, 1995). Two particulary useful ones are:

(1) *NPAH*, where $H = E + P_n V$. The surface area is fixed, but because the applied normal pressure is constant, the box height (and hence the volume) fluctuates. This ensemble is useful for calculating surface tensions of liquid/liquid systems, including the "microscopic" surface tension of lipid bilayers.

(2) *NPγH*, where $H = E + P_n V - \gamma A$. Both the surface area and volume fluctuate, while the surface tension and normal pressure are constant. This ensemble is useful for expanding or contracting bilayers, or allowing the bilayer to relax under a constant applied surface tension (Feller et al, 1995b).

Developing equations of motions for particles outside the *NVE* ensemble is not particularily straightforward. A method for simulating isotropic systems at constant pressure was introduced by Andersen (1980) and then generalized for solids (where a pressure tensor is required) (Nose and Klein, 1983; Parrinello and Rahman, 1981). The Andersen, or extended system, approach (others are possible, as reviewed in Allen and Tildesley, 1987) is based on incorporating into the Lagrangian an additional degree of freedom, corresponding to the volume but commonly called a piston; the force on the piston is proportional to the difference of the instantaneous and reference pressures. The resulting equations of motion produce trajectories in which the system volume adjusts to and fluctuates about a value consistent with the reference pressure. The extended system equations for the *NPγH* ensemble under an applied normal pressure, P_{n0}, and surface tension, γ_0, are (Zhang et al, 1995):

$$
\begin{aligned}
&\dot{x}_i = \frac{p_{xi}}{m_i} + \frac{\dot{h}_x}{h_x} x_i, \qquad \dot{y}_i = \frac{p_{yi}}{m_i} + \frac{\dot{h}_y}{h_y} y_i, \qquad \dot{z}_i = \frac{p_{zi}}{m_i} + \frac{\dot{h}_z}{h_z} z_i, \\
&\dot{p}_{xi} = f_{xi} - \frac{\dot{h}_x}{h_x} p_{xi}, \qquad \dot{p}_{yi} = f_{yi} - \frac{\dot{h}_y}{h_y} p_{yi}, \qquad \dot{p}_{zi} = f_{zi} - \frac{\dot{h}_x}{h_x} p_{zi}, \\
&M_x \ddot{h}_x = h_y(\gamma_0 - \tilde{\gamma}_{xx}) \\
&M_y \ddot{h}_y = h_x(\gamma_0 - \tilde{\gamma}_{yy}) \\
&M_z \ddot{h}_z = h_x h_y (P_{zz} - P_{n0})
\end{aligned}
\tag{3}
$$

where x_i, p_{xi}, f_{xi} are the position, momentum and force in x for the i^{th} particle, respectively; h_x is the length in x of the simulation cell, and M_x is the mass of this extended degree of freedom. Variables in y and z are defined similarily, and the symbols dot and double dot have their usual meanings as time derivatives. Finally,

$$
\begin{aligned}
\tilde{\gamma}_{xx} &= h_z(P_{n0} - P_{xx}) \\
\tilde{\gamma}_{yy} &= h_z(P_{n0} - P_{yy})
\end{aligned}
\tag{4}
$$