

**The
Physical Chemistry of
MEMBRANES**

An Introduction to the
Structure and Dynamics
of Biological Membranes

Brian L. Silver

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TECHNION—ISRAEL INSTITUTE OF TECHNOLOGY
HAIFA, ISRAEL

ALLEN & UNWIN
Boston · London · Sydney

The SOLOMON PRESS
New York

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Permission requests should be addressed to: Publishers Creative Services Inc.,
89-31 161 Street, Suite 611, Jamaica, New York 11432, USA

First published in 1985

This book is a joint publication of Allen & Unwin and The Solomon Press (a division of Publishers Creative Services Inc.)

It is distributed by Allen & Unwin:

Allen & Unwin Inc., Fifty Cross Street, Winchester, Mass. 01890, USA

George Allen & Unwin (Publishers) Ltd,
40 Museum Street, London WC1A 1LU, UK

George Allen & Unwin (Publishers) Ltd,
PO Box 18, Park Lane, Hemel Hempstead, Herts HP2 4TE, UK

George Allen & Unwin Australia Pty Ltd, 8 Napier Street, North Sydney, NSW 2060, Australia

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British Library Cataloguing in Publication Data

Silver, Brian

The physical chemistry of membranes : an introduction to the structure and dynamics of biological membranes.

1. Membranes (Biology) 2. Biological chemistry

I. Title

574.8'75 QH601

ISBN 0-04-574028-3

Library of Congress Cataloguing in Publication Data

Silver, Brian L.

The physical chemistry of membranes.

Bibliography: p

Includes index

1. Cell Membranes. 2. Bilayer lipid membranes.

3. Cytochemistry. 4. Chemistry, Physical organic.

I. Title. [DNLM: 1. Cell Membrane. 2. Chemistry, Physical. QH 601 S587p]

QH601.S48 1985 547.87'5 84-24407

ISBN 0-04-574028-3 (alk. paper)

Book design by Raymond Solomon

Set by Mathematical Composition Setters Ltd, Salisbury, UK

Printed and bound in Great Britain by

Anchor Brendon Ltd, Tiptree, Essex

Preface

This book is an account of what physical chemistry has to say about the structural, electrical and transport properties of biological membranes and their simplest model—the lipid bilayer. The accent throughout is on basic ideas. In contrast to the essentially descriptive approach characteristic of texts on membrane biochemistry, our underlying themes are the role of force and entropy in maintaining membrane organization, in determining the electric fields and ionic environment of membranes, and in regulating the passage of molecules and ions across membranes. Although experimental findings will always be the touchstone against which theory will be tried, no attempt is made to present an exhaustive survey of experimental data. On the other hand, there is discussion of the nature and limitations of the results obtainable by the major laboratory techniques. The treatment is at the level of an advanced undergraduate course or an introductory survey suitable for post-graduate students carrying out research in biochemistry, biophysics, or physiology. The mathematical demands on the reader are trivial. The few forbidding equations appearing in Chapter 7 are soon whittled away to simple practical expressions. Although the current–voltage characteristics of nerves are traditionally the province of biophysics rather than physical chemistry, certain aspects relevant to the electrical activity of nerves are nevertheless included in this text, namely, membrane and diffusion potentials and conductivity fluctuations.

Where rival theories exist, conflicting convictions have been presented, but not necessarily accorded equal approbation. The author has a viewpoint.

The overwhelming majority of original research work referred to in the book was published after 1970. In general older papers are evoked

when they fall within the categories of unavoidable classics or seminal studies. In the early papers, the student will meet angstroms, kilocalories, and other relics of the past. I have retained these units in quoting experimental results rather than destroy their antique charm by conversion to SI units.

Part of this book was written while I enjoyed the hospitality of the Department of Chemistry of the Imperial College of Science and Technology, London. In particular I wish to thank Dr. John Gibson. Professor J. Barber of the Department of Pure and Applied Biology contributed greatly to my knowledge of matters photosynthetical.

The manuscript was typed by an international bevy of impressively accurate typists, the bulk of the work being shared by Norma Jacobs, Charlotte Diamant, and Doreen Walsh in Haifa and Nadine Green in London.

Raymond Solomon of Publishers Creative Services was patient and helpful far beyond the call of duty as deadline after deadline bit the dust.

Introduction

Our image of the cell membrane has progressed, in this century, from a structureless, almost functionless packaging for the cytoplasm to a complex organ responsible for many of the most fundamental processes characterizing the living cell. Up to the late 1950s, the spectacular advances were made on the biochemical front. Membrane-bound proteins were found to be capable, among other things, of effecting the active transport of ions and molecules, to be implicated in immunological reactions, to be the site of the initial steps in photosynthesis, and to be involved in oxidative phosphorylation. Although our understanding of protein function has deepened immensely over the past decade, the molecular structure of membrane proteins remains on the whole obscure. By contrast it was early suspected that the proteins swim in a ubiquitous sea—a bilayer of lipid molecules.

The building blocks of the bilayer were long ago identified as amphiphilic molecules; that is, molecules containing both hydrophobic and hydrophilic regions. Standard chemical structure determination has established their primary structure, showing them to be, in the main, phospholipids with long hydrocarbon chains attached to polar head groups. By the mid-1930s it was already fashionable to depict membranes as flexible bilayers impregnated with lumps of protein. This book takes up the story from that point but not from that date. It was not until the 1950s that real progress was made in understanding the behavior of membranes in terms of their structure. The upsurge in membrane research and the torrent of papers now devoted to membranes are very largely a result of technical innovations in the laboratory. The separation and characterization of complex mixtures has undergone a revolution, and molecular structure and dynamics are under attack from a range of

powerful techniques, such as nuclear magnetic resonance, electron spin resonance, fluorescence spectroscopy using lasers, and high-sensitivity calorimetry. Physical chemistry has driven deep into membrane science and has enabled us to discuss the stability of the lipid bilayer in terms of intermolecular forces, to analyze its electric field in terms of classic electrochemistry, to explain intermembrane forces with the aid of the modern theory of dispersion forces, to account for the transport of ions in terms of diffusion equations or activated rate theory, and to understand the time-dependent electrical conductivity of nerve membranes in terms of the theory of stochastic processes. The following chapters contain a general view of the physical chemistry of membranes. We keep well away from biochemical aspects since these have been the subject of several monographs. Our concern will be with structure, forces, thermodynamics, and kinetics. We will see how the traditional concepts of physical chemistry are rapidly laying a rational foundation for the observed physical properties and biological functions of membranes.

Our starting point is the crystal structure of pure phospholipids, which will be seen to have strong similarities to the arrangement and molecular conformation of the bilayer portion of natural membranes. Anhydrous lipids undergo conformational changes on heating that are understandable mainly in terms of the disordering or "melting" of all-trans hydrocarbon chains.

Since natural membranes exist in an aqueous milieu, it is necessary to examine some of the many phases adopted by hydrated phospholipids. Hydrated phospholipids can be induced to adopt a rich variety of phases by a change in temperature and variation in water content. Hydrated phospholipids at physiological temperatures exist in a number of forms, including bilayers, micelles, and the closed spherical structures known as vesicles or liposomes. It will be shown that by using simple considerations of molecular shape, it is possible to predict the particular phase formed by a given phospholipid as a function of temperature, pH, and the nature of the cations in the ambient solution.

In biological membranes the phospholipids have melted chains and the chain conformations are continually changing. Diffraction and magnetic resonance methods have provided a means of quantitatively specifying the average conformational state of the individual chain segments in melted chains. The state of the chains in bilayers is strongly affected by the addition of proteins and the interactions between lipids and other membrane components are a major area of experimental and theoretical investigation. There is rapidly mounting evidence that the properties of lipids are very relevant to the functioning of membrane proteins and to the interactions between cells. The complex phase diagrams of hydrated lipids and the vital biological importance of protein-lipid interactions

have prompted theoretical attacks on the structure and dynamic properties of bilayers and membranes. A great range of techniques has been employed, from the use of cardboard disks to represent molecules to quantum mechanical calculations. Any realistic model of a molecular ensemble needs to take account of geometry and forces. In Chapter 7 we consider the forces that maintain the integrity of bilayers, control their ionic environment, and determine the forces between membranes. We will find it necessary to enter the modern theory of van der Waals' forces and to accept the existence of huge repulsive forces between adjacent hydrated bilayers. In selecting for perusal theoretical studies on bilayers or membranes, I have concentrated on classical statistical thermodynamics, on calculations based on the topology of simple geometrical models, and on the recent use of molecular dynamics. Inevitably quantum mechanics has been mobilized by some investigators; however, I barely mention these efforts as they are so few and as yet are based on oversimplified models. Every theory outlined in the text suffers from its own approximations and enjoys its own success. Among the areas that theory has illuminated to date are the nature of the lipid chain-melting transition, the average conformational state of the melted chains, and the effect of proteins on these chains.

An understanding of the static properties of membranes is an essential prelude to the study of the movement of molecules within the membrane. The lateral and rotational diffusion of proteins and lipids in the plane of the membrane has been observed via a number of techniques, and although the quantitative prediction of diffusion rates is still beyond us, satisfying qualitative explanations are beginning to emerge for the effects of protein and cholesterol concentration on lipid diffusion. The lateral diffusion of proteins and other membrane components is implicated in a number of important phenomena, such as the intramembranal transfer of electrons in photosynthesis and the redistribution of proteins on the cell surface in some immunological reactions. The diffusion of one or more membrane components may be a rate-determining step in these processes. In Chapter 10 we present a survey of the principle observations and theories concerning lateral and rotational diffusion in membranes.

The movement of molecules through membranes has not surprisingly attracted perhaps more experimental and theoretical attention than any other aspect of membrane science. Nineteenth century theories of the transport of matter through liquids were based on phenomenological diffusion equations, such as Fick's first law, which assumed the medium to be a continuum and defined a diffusion constant the magnitude of which was taken to be an indication of the frictional resistance to molecular motion through the medium. It was natural to use a similar

formalism when the problem of ion transport through membranes was first attacked. The Nernst-Planck equation, the central result of the approach, takes into account both simple diffusion and the effect on ion transport of electric potential gradients across the membrane. The first major application of the theory to real systems was the famous Goldman-Hodgkin-Katz (GHK) expression, which accounted successfully for the relationship between the extra-membranal concentrations of ions and the potential difference across the membrane. In Chapter 12 the GHK equation is derived and its failures are also hinted at. The apparent inapplicability of diffusional equations to a growing number of experimental systems prompted an increased appeal to activated rate theory in which ions passing through membranes are presumed to move over a hilly potential energy surface. In Chapter 13 the basic formalism of the rate theory is described and developed. One need not know how to swim to cross a river; one alternative is to row across. Molecular boats, capable of ferrying ions and molecules across membranes, have been isolated and characterized. In Chapter 14 there is a discussion of the kinetics of transport by carriers in which it is shown that a number of at first-sight puzzling phenomena are explicable in terms of simple equations and ideas.

Thermodynamics appears throughout our story, often implicitly, but explicitly in Chapters 8 and 15. Natural membranes are nonideal mixtures but contain too many components to be suitable subjects for theory, and thermodynamic analysis of membrane models and bilayers has concentrated on binary mixtures. The treatment for mixtures of lipids is followed through using standard equilibrium thermodynamics. The effect of proteins on the free energy of lipid bilayers has been analyzed in terms of the effect of rigid impurities on the average conformational state of the lipid chains. The results have been used to account for the nature of the thermal transition in bilayers and also to suggest the presence of lipid-mediated forces between protein molecules. In the 1950s irreversible thermodynamics, which deals with systems not at equilibrium, was directed at biophysics. The two main targets were the "passive" transport of water and the "active" transport of ions and molecules through membranes. The theory promised somewhat more than it delivered. In Chapters 15 and 16 we take a look at the methodology of irreversible thermodynamics, some of its successes, and one or two misapplications. The reader will again be conscious of the gap between our primitive knowledge of membrane protein structure and our ability to predict or account for the general characteristics of the dynamic membrane processes presided over by protein molecules.

Macroscopic equilibrium does not preclude microscopic fluctuation. In membranes one of the clearest symptoms of fluctuations is the time-

varying electrical conductivity of membranes, which is often a consequence of the random swinging to and fro of the molecular "gates" at the entrances to the channels through which ions carry charge. In Chapter 17 enough of the elementary theory of random processes is presented to allow the reader to appreciate the way in which kinetic information can be extracted from experimental data. Basically the same theory is shown to be relevant to the interpretation of certain spectroscopic observations on lipid chains.

The theories and laboratory methods of physical chemistry have reached beyond the problems presented by model systems intended to replicate some of the properties of real membranes. The membranes of cells are increasingly the subject of experimental probing and the structural and dynamic features of simple models have been demonstrated to be replicated in living systems. The physical chemistry of membranes is coming of age.

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Introduction

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