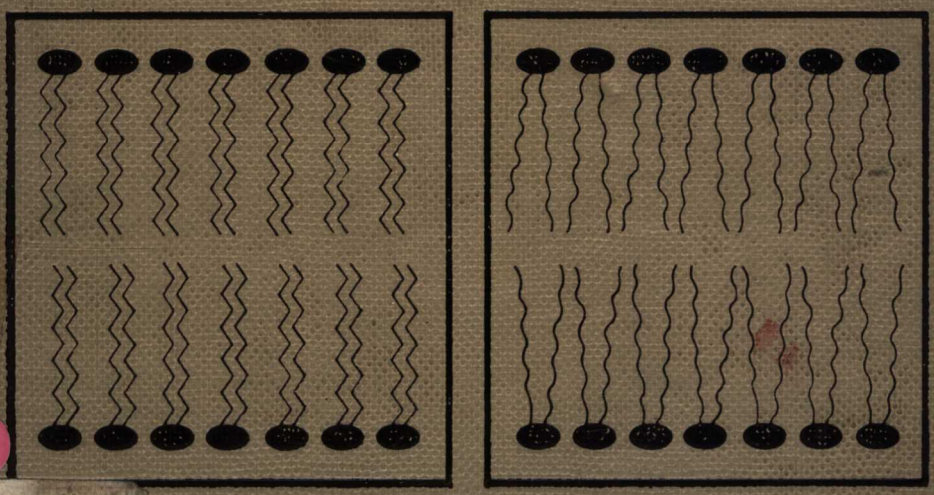
Membrane Fluidity in Biology

VOLUME 1

Concepts of Membrane Structure

Edited by

Roland C. Aloia



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Concepts of Membrane Structure

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Preface

Over the past several years, hundreds of books have been published on many facets of cell and membrane function. However, only a few of these have contained discussions of the various aspects of membrane fluidity and membrane structure–function relationships. Throughout the past decade it has become increasingly clear that alterations in membrane lipid composition and membrane fluidity can be influenced by diet, environmental factors, exogenous agents to which the cell membranes are exposed (e.g., ions, pH, drugs, hormones), and various pathological states. Furthermore, such alterations in membrane composition and fluidity have been shown to influence important cellular functions such as the transport of substances across the cell membrane, immunological recognition, protein–membrane binding, the activity of key membrane-bound enzymes, and the number and affinity of cell receptors. For example, the modulations of integral membrane enzymes such as Na^+/K^+-ATPases, adenylate cyclase, and Ca^{2+}/Mg^{2+}-ATPase, and peripheral enzymes such as acetylcholinesterase have been correlated with alterations in membrane lipid composition and fluidity. Alterations in membrane fluidity have been shown to influence a myriad of cell surface-related phenomena and, hence, to modify cellular and organ function. Conversely, alterations of metabolic state in various physiological processes such as cellular development are reflected in alterations in membrane fluidity.

Although the extensive relationship of membrane fluidity to membrane and cellular function has been documented in numerous journal publications, there has been no published treatise to review the tenets of membrane fluidity and to analyze and evaluate critically the relationship of fluidity to cellular activity. This set of volumes entitled *Membrane Fluidity in Biology* is intended to provide that function. The contributors to these volumes will examine the many membrane properties influenced by alterations in membrane lipid compositions and/or other organizational parameters that are encompassed by the term fluidity. This treatise will serve as a comprehensive source within which the precepts of membrane fluidity are elaborated and the significance of fluidity changes in both normal and pathological cellular functions is discussed. Each volume will represent a state-of-the-art

review and should be a valuable reference source and a springboard for future research.

The present volume, entitled *Membrane Fluidity in Biology: Concepts of Membrane Structure,* is the first volume in this series. I am beginning the series with a work on membrane structure because our perception and understanding of membrane fluidity are ultimately based on our understanding of membrane architecture and organization. I have selected authors who present many new ideas about membrane structural organization and who provide unique and challenging ways to think about the composition and arrangement of the molecular components of cell membranes. Although sometimes controversial, the concepts presented are elaborated with detail and clarity. The authors provide a lucid account of recent evidence, a re-evaluation of older evidence, and a discussion of new perspectives in our understanding of the diversity and complexity of membrane lipids and structure–function relationships. They elaborate structural principles that should provide a sound conceptual framework for evaluating the facets of membrane fluidity discussed briefly in this volume and more extensively in subsequent volumes. Perhaps more importantly, the authors provide new insight into membrane packing principles and constraints that may herald a new era of questioning the architectural basis governing current, popular membrane models. This volume should be valuable and essential reading for all scientists and researchers concerned with an understanding of the molecular principles of cell function.

Volume 2 will cover such topics as phase transitions, hydrophobic and electrostatic effects of proteins, lateral phase separations, phospholipid transfer proteins, calcium and magnesium ion effects, sphingolipids, and cell-associated water. Volume 3 will examine the relationship of membrane fluidity to disease processes. Thus, the entire treatise should serve as a primary source for research scientists and teachers interested in cellular membrane fluidity phenomena.

I wish to express my sincerest thanks to the Department of Anesthesiology at Loma Linda University and the Anesthesiology Service at the Pettis Memorial Veterans Hospital for allowing me the time to devote to this effort. I am also indebted to Drs. George Rouser, Gene Kritchevsky, and William Thomson for kindling my interest in membranes and for their continued support, and to Darla Leeper, Gizete Babcock, Hilda McClure, Julie Porter, and Helen Mayfield for their dedicated and patient secretarial assistance.

Contents

Chapter 1

Nonrandom Lateral Organization in Bilayers and Biomembranes

Mahendra Kumar Jain

"The question is" said Alice, "whether you can make words mean so many different things."

"The question is which is to be master, that's all." Humpty Dumpty continued in a scornful tone, "when I use a word, it means just what I choose it to mean . . . neither more nor less."

"Contrariwise," said Tweedledee, "if it was so, it might be; and if it were so, it would be; but as it isn't, it ain't. That's logic."

"Tut, tut, child," said the Duchess. "Everything has got a moral if only you can find it."

Taken somewhat out of context from
Through the Looking Glass

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Introduction

Considerable progress in our understanding of biomembrane structure and function has resulted from structural generalizations invoked and articulated in the various models proposed over the last 50 years (Jain and White, 1977). Admittedly, models are meant to articulate consensus, to consolidate and formalize collective thinking, and to polarize the dialectics. Thus the generalizations implicit in various membrane models do provide a framework within which we can generate structural correlates of membrane function. The following structural and organizational generalizations are inherent in the various models in the literature and incorporated into the *plate-tectonics* model of membrane organization presented elsewhere (Jain and White, 1977).

Organization of phospholipids and proteins is in a two-dimensional matrix that arises from a bilayer arrangement of phospholipids. In this matrix the various components are held together by noncovalent forces; formation and stability of the matrix is due to hydrophobic effects arising from the amphipathic nature of the membrane components whereby the free energy gain is 750–800 cal/mole for the transfer of a methylene residue from the aqueous phase to bilayers; energetic contribution from polar groups is minimum; however, their shape and size determines the polymorphic behavior of the aggregate. It follows that the concentration of the membrane components in the surrounding aqueous medium is negligibly low (less than 10^{-10} M for phospholipids), and therefore the uncatalyzed or nonmediated exchange and transfer of components between different membranes is rather slow for phospholipids (half-time more than several hours). Proteins in membranes interrupt bilayer organization; however, factors governing the state of proteins in bilayers are not understood. It is generally agreed that membrane proteins contain segments of hydrophobic residues that dip into or protrude across the bilayer; the aqueous phase concentration and the intermembrane exchange rate of these proteins is immeasurably low; and the phase properties of bilayers influence properties of membrane proteins.

Molecular motions (rotational, segmental) of methylene chains of phospholipids give rise to a gradient of motional freedom ("fluidity") across the thickness of a phospholipid bilayer. Flexing of the acyl chains (segmental motion) increases the "disorder" from the aqueous phase to the center of the bilayer. Similarly, the dielectric constant (a bulk property) is expected to be 2 in the center of the bilayer and 80 in the aqueous phase. Thus both the disorder and the polarity do not necessarily change abruptly at the interface. One of the consequences of such gradients is existence of activation energy

barriers for partitioning and diffusion of solutes in and across the bilayer.

Biomembranes exhibit functional and compositional asymmetry (Op den Kamp, 1979; Van Deenen, 1981). The phospholipid composition of the two monolayer halves are generally quite different; some proteins are exposed only on one side, or different parts of the same protein are exposed on the two sides, thus giving rise to a characteristic orientation to membrane proteins; asymmetric distribution of proteins is absolute and that of the phospholipids is relative, that is only a quantitative difference between the phospholipid composition of the two monolayer halves is noted; glycolipids and the carbohydrate conjugates of glycoproteins are always exposed to the outside surface of the plasma membrane. Existence of asymmetry implies that the exchange of the components between the two halves of a bilayer is very slow (half-time of several hours) or nonexistent.

The ability of phospholipids in aqueous media to adopt a variety of organized forms (bilayer, micelle, hexagonal) has been used to explain membrane functions such as endocytosis, secretion, fusion, and transbilayer exchange ("flip-flop"). Although such polymorphs are not widely recognized to be present in biomembranes, it is becoming increasingly obvious that the nonbilayer polymorphs such as hexagonal and micellar phases can be generated under physiological conditions (Cullis and DeKruijff, 1979; Verkleij and DeGier, 1981). The role of nonbilayer polymorphs in the lateral organization of biomembranes is not considered in this chapter, but their potential importance in certain membrane processes deserves serious consideration. Constraints of molecular geometry that may lead to the generation of one polymorph over the others have been discussed (Israelachvilli *et al.*, 1977). Generally speaking relative cross-sectional areas of the polar group and the acyl chain are important. A cylindrical molecule would favor a planar bilayer type of organization, whereas a wedge or cone shaped molecule would give rise to polymorphs with a highly curved lipid–water interface as seen in hexagonal and micellar phases. Obviously effective sizes of polar and nonpolar groups are determined not only by their structure, but also by temperature, the state of ionization, ion binding, and association with other membrane components.

Selective ordering and segregation of components in the plane of a membrane can lead to a nonrandom organization (Jain and White, 1977). Such ordering arises from favorable constraints of packing and specific interactions between membrane conponents. Nonrandom lateral organization gives rise to phase separation, patching, phase boundaries, multiple relaxations, and anomalous temperature dependence of membrane processes. Such aspects of lateral organization in bilayers and biomembranes are considered in this chapter.

Domains of One Phase in Single Component Bilayers

Intuitively, all the apparent and emergent properties of materials are governed by the spatiotemporal patterns generated by the components. The role of spatial heterogeneity and coexistence of discrete iso- and polymorphic clusters and domains in apparently homogeneous systems is crucial to our understanding of a wide variety of aggregate systems. According to the third law of thermodynamics a perfect crystal cannot exist at temperatures above absolute zero. Thus, in analogy with any organized structure, the bilayer lattice of single phospholipid species would have its organization interrupted with defects and imperfections. Such imperfections locally disturb the regular arrangement of the atoms and molecules, and they accommodate trace impurities and other constraints of organization and thermal motion. The phenomenon of lattice defects and imperfections is exhibited by all types of crystals, metals, magnetic materials, and even liquid crystals and amorphous glassy solids (Adams, 1974; Hull, 1975). Segregation into domains is facilitated not only by specific interactions within domains, but also by the fact that the strain (thermal and geometrical) can be relieved by "plating-out" of the impurities and by creation of defect regions (dislocations, defects, voids, cluster–cluster interfaces).[1] A visual demonstration of such a phenomenon is presented in Fig. 1, where spontaneous close packing of identical steel ball bearings under the force of gravity into a two-dimensional lattice always leads to the formation of domains of close-packed hexagonal lattice interrupted by point, line, and nodal defects, as well as the grain boundaries. *Point defects* arise from a vacancy in the lattice, and at all temperatures above absolute zero there is a thermodynamically stable number of vacancies. *Line defects* or dislocations or subgrain boundaries arise primarily due to plastic deformation, and they describe the registry of atoms or molecules across a line. Dislocation lines can end in closed loops, or branch into other dislocations, or terminate in grain boundaries. Two or more line dislocations can meet at a point or a node. Crystalline solids consist of ordered

[1]Several types of imperfections in organization are described in the literature (see Hull, 1975; Lee, 1975). One of the interesting analogies may be found in the "polymorphic" structure and consequent imperfections in the organization of human society, where segregation into nations, communities and clans results from economic and social factors (interests, ties, affiliations, inequalities and webs of countless other trivia of everyday life that separates or brings us together!). The "biological advantage" of such patches and their imperfections are that the individuals exploit the local environment and resources, facilitate competition and predation, and relieve internal strains and external pressures often at the cost of some patches (see Levin and Paine, 1974).

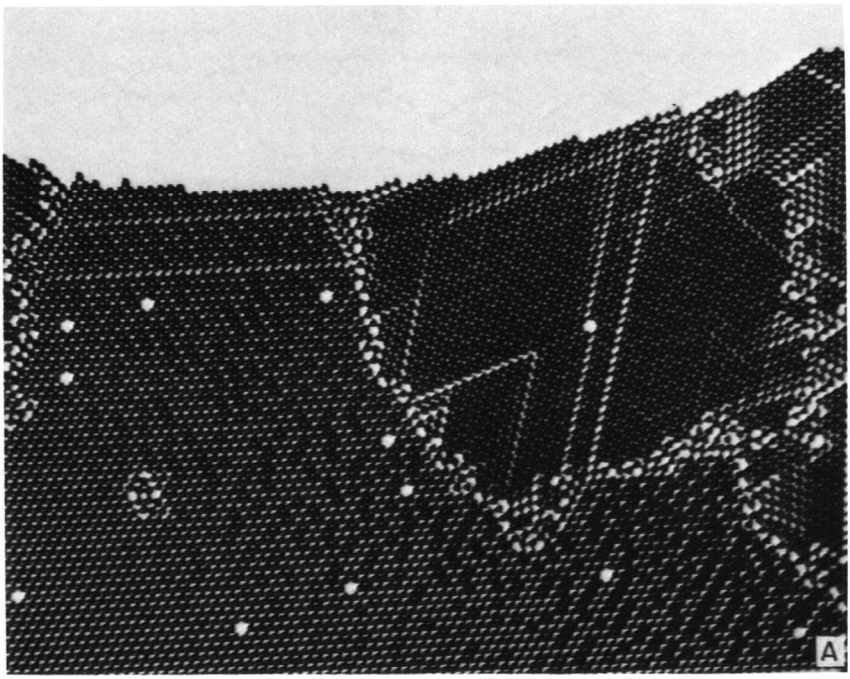

Fig. 1. Photograph of back illuminated panel of a randomly organized "monolayer" of identical ball bearings (between two lucite sheets) close packed under the force of gravity. Although the same arrangement cannot be reproduced by shaking the ball bearings, one always observes several types of defects that interrupt the hexagonally close-packed lattice. The most common types of defects are: *point defects* that appear as white dots; *line defects* which appear as sharp straight lines; *grain boundaries* that appear as diffuse regions between hexagonally close-packed domains. These terms for the various types of defects are used in the same general sense as they are used to describe defects in three-dimensional solid crystals.

domains (grains) separated by grain boundaries where such order does not exist. Each domain is a single close-packed lattice that is separated from other domains by defects. Random orientation of molecules at *grain boundaries* gives rise to disordered regions that are several molecules wide.

The existence of imperfections in bilayers of a single phospholipid species is indicated by a variety of techniques. The organization of bilayers is largely a consequence of acyl chain interactions, and the conformation of acyl chains is determined by temperature. Therefore, the temperature-dependent changes in the organization of bilayers are of considerable interest. Probably the most important thermotropic change in acyl chain conformation is due to rotation around C—C bonds which gives rise to two energetically favorable

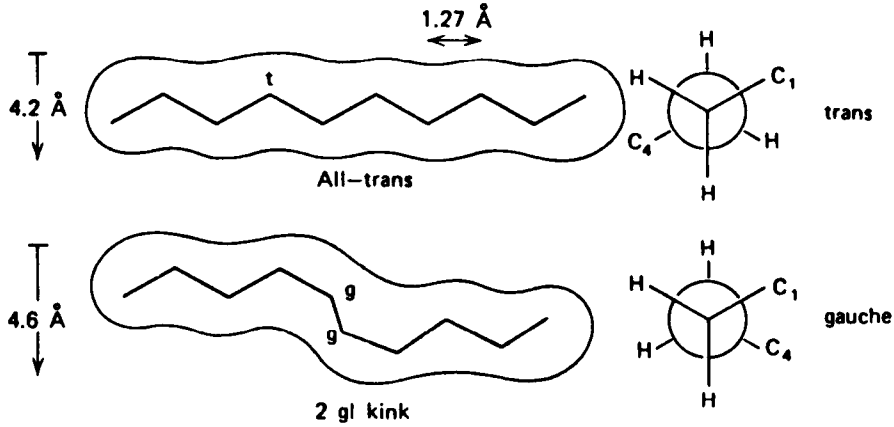

Fig. 2. Alkyl chains in an all-trans(t) and mixed trans–gauche(g) conformation. The Newman projections for the trans and gauche conformations are also shown. The dihedral angle (C_1—C_4) is 180° for the trans conformation and 120° or 240° for the gauche conformation. A kink is formed by rotating one C—C bond by an angle of 120° and then rotating either of the two next-nearest neighboring bonds by −120°.

states (Fig. 2): trans (continuous rotation angle $\theta = 0°$), and gauche ($\theta = \pm 120°$). The free energy change associated with such a change is about 0.3 to 0.5 kcal/mole in the favor of trans conformation and the activation energy is about 3 kcal/mole. Therefore at 25°C one gauche conformer is expected to be present for every five trans conformers in the acyl chain. The all-trans acyl chains can pack closely. Introduction of gauche conformers bends acyl chains (Fig. 2) so that they cannot close-pack unless two or more gauche conformers form a kink which mainimizes the bend of the acyl chain. The kinked chains, however, introduce some disorder in an otherwise close-packed bilayer. The acyl chains containing gauche conformers and kinks can plate out and collate into grain boundaries, and the all-trans chains can form close-packed two-dimensional domains (grains). Thus a conformational disorder of acyl chains can be translated into an organizational imperfection or defect. The thermodynamic and geometrical constraints of such an ensemble containing localized disorder would show up in the sizes and life times of the ordered domains. Other types of imperfections (point and line defects) could still exist in such ordered close-packed domains separted by the grain boundaries.

Diacylphospholipid bilayers exhibit thermotropic transitions. With phosphatidylcholines three transitions have been reported: (*a*) the main transition due to increased disorder resulting from increased gauche conformers in acyl chains; (*b*) the pretransition arising probably from a change in the orien-

tation of acyl chains; and (*c*) the subtransition at a still lower temperature whose origin is yet unknown (Chen *et al.*, 1980).

Bilayers of pure phospholipids below their main transition are said to be in "solid," "gel," or "ordered" phase; and in "liquid," "fluid," "disordered," or "liquid-crystalline" phase above their main transition. Although all these terms have some historical or mechanistic significance, in this review I shall use only solid and liquid phase to characterize bilayers below and above their main transition. The terms are not meant to imply any similarity to the bulk solid and liquid phases. The bilayers between the pre- and main transitions are said to be in $P\beta'$ phase. All the three types of transitions exhibit a hysteresis in the heating and cooling cycles. The subtransition has been reported only in the first heating cycle of bilayers stored for several days at 0°C. The pretransition is seen in the heating cycles only when the sample is preequilibrated at low temperature for more than 15 min. The main transition is seen both in the heating and in the cooling cycles, however the exact shape and the transition temperature is not the same for the heating and the cooling cycles. These transitions also exhibit a finite width, that is the phase transition occurs over a finite temperature range. Both the finite width and hysteresis of transitions are most probably due to structural imperfections in bilayers. It should be noted that there is a tendency among the theoreticians to assume that the main transition is first order, and it is argued that the width and hysteresis are probably due to trace impurities that cause imperfections in the solid phase and influence nucleation in the liquid phase. It is also possible that the heating and cooling rates may be too fast to attain an equilibrium during the scan for a transition profile.

The presence of imperfections or organizational disorders in bilayers can be demonstrated by freeze-fracture electron microscopic studies. Bilayers quenched from the solid or the liquid phase exhibit a characteristically smooth fracture face, whereas, the bilayers quenched from the $P\beta'$ phase exhibit a characteristically rippled pattern (Fig. 3). The periodicity of these ripples probably arises from internal strain in the bilayer organization induced by a change in the orientation of phospholipid molecules. The linearity of the ridge of the ripple probably arises from a distortion of the symmetry properties in one dimension (singularly oriented strain in an array). The cumulative strain of domains with rippled patterns could give rise to secondary ripples of larger periodicity of the type seen in mixed lipid systems (see Fig. 4 and the discussion in the next section).

The rippled bands in such fracture faces of bilayers in $P\beta'$ phase are seldom, if ever, uniform over the whole bilayer surface. In fact such disorders in the long range organization of bilayers can be introduced simply by manipulating the history of the sample. The freeze-fracture electron micrographs of ditetradecylphosphatidylcholine (the ether analog of dimyristoyl-