STRUCTURE AND DYNAMICS OF MEMBRANOUS INTERFACES

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INTRODUCTION

The initial idea for this book occurred over two decades ago, when this editor was a graduate student in an obscure university located in an obscure city in eastern Canada. While attending various lectures on membrane-related topics and Biophysical Society of America meetings, and having a mentor who was an expert in some fields of membranous system, I was fascinated by membranous system research in the late 1980s. This was mainly due to a graduate project on the development of an instrument to observe domains in monolayer films undergoing phase transitions. Over the last century, membrane research had developed classically from the seminal findings of Langmuir, of Gorter and Grendall, and of Singer and Nicholson's fluid mosaic model; then in the early 1990s, proteins and genes had taken center stage in biomolecular research. Lipids had taken a back seat despite the fact that the chemical diversity of lipids in membranes was unknown. Even with the development of enormously sophisticated biophysical technology, only a few membrane protein structures are known to date, and we know very little about membrane structures. I was also quite intrigued with the use of protein analysis and molecular biology techniques in researching the "elusive" proteins such as flippases (which are supposed to maintain lipid asymmetry) when we knew so little about the structure-dynamics aspect of one (cholesterol) or more membrane components or details of their physicochemical behavior. Later, I came to understand that a major hurdle in studying membranes is their physical property of being liquid crystalline material, and thus general biochemical approaches to study such systems were limited. The available tools for studying the physicochemical behavior of membranes, their dynamics, and atomic structures were only accessible to elite "atom smashing scientists" and others doing research in diverse nonbiological fields such as chemical physics or condensed matter. Observing the behavior of various amphipathic molecules using physicochemical methods also required an integrated theoretical framework, which could suggest the molecular structure and dynamic properties of membranous systems such as surfactants, colloids, thin films, liquid crystals, and soft condensed matter. Some of these properties of amphipathic molecules, although complex, had some similarity to the physical properties of "soap." This integrated theoretical framework of membranes, surfactants, liquid crystals, and self-assembling systems being "soft materials" was described by Professor P. DeGennes in the early 1990s. To quote this master of analogies (and a 1992 Nobel laureate): "it is perhaps amusing to note there is similarity in thought between those who study string theories and those of description of soaps." This framework brought about

a revolution in our theoretical understanding of soft-material and liquid crystal membranous systems, as well as in defining the polymorphic behavior of such materials.

Although there are a number of excellent review books in the field of cell membrane research as well as specialized books and monographs on surfactants and liquid crystalline materials, the idea that some of these diverse systems can be observed from the viewpoint of "membranous systems" had not yet been developed to date. There are a number of "membranous systems" that are diverse in composition and function compared to cell bilayers. Some are extracellular secretions such as lung surfactant; others, such as lipoproteins, skin barrier and prokaryotic double membranes, and colloidal surfactants, have similarities to the self-assembly and polymorphic behavior of membrane components. For example, lung surfactant, a highly evolutionary conserved lipid–protein secretion from type II lung cells, helps in normal respiration for all air-breathing vertebrates. The internal structures found in the postsecretory lung surfactant suggest a fascinating array of lipid polymorphic structures such as bilayer vesicles, planar multilayers, multilamellate vesicles, and tubular hexagonal type I phases. Others, such as lipoprotein particles, which carry cholesterol and triglycerides in plasma, are membranous in the sense that lipid–protein spherical monolayers hold these structures intact. Other systems, such as the neural axons, have peculiar coiled membranous sheaths and conduct electrical impulses via ion exchange. Bacterial membranes consist of some ubiquitous glycolipids that form double bilayers, remnants of structures of mitochondrial membranes. It has been proposed that mitochondria developed from bacterial insertion in primordial cells. Some of the lipid-associated proteins in these systems, although diverse in function, have similar types of lipid–protein organizations, membrane orientations, structure–function domain organization, physical properties, and lipid dynamics as those observed in studies of membrane models. I believe that research on such diverse membranous systems has greatly enhanced our understanding of not only cellular membranes, but also surfactant, colloids, and other soft materials. Most research on membranes conducted using state-of-the-art physicochemical techniques is also regularly utilized in studies of the physicochemical behavior of soft and hard materials. This book was originally planned to incorporate as many diverse membranous systems as possible, but focusing on recent developments in the field of cellular membrane research. Certain fields such as lipoproteins have not been studied in detail as general membranous systems; also, some authors balked at contributing to a book based heavily on biophysics and structure–function studies of membranous interfaces. The word "interface" as used in the title of this book means mainly the lipid–protein bilayer and film interface with a polar medium, as well as the "interface" between various membranous systems.

Part I of this book is focused on direct experimental studies on the polymorphic structures of models and some natural membranous systems. This area is quite extensive since there has been an explosion of recent research based on the discovery of real structure–function domains or lipid rafts in cell membranes. Over

the last three decades, there has been speculation on membrane domains; however, they were finally demonstrated to exist and were imaged in the plasma membranes of cells (see details in Chapter 15). These structure–function rafts were formed by phase-segregated association of sphingomyelin–lipid–cholesterol into nanoscale domains on which membrane proteins reside during function. The chapters in Part I discuss the supramolecular arrangement of lipids and protein in membranes. Although structural domains of specific lipids were first observed in monolayer films using fluorescence techniques, their demonstration and imaging in native and model bilayer membranes was of critical importance to understanding lipid–protein interactions in membranous systems. Thus Chapter 1 discusses the structured domains in model membranes, as well as some functional implications of such structures toward enzyme function. One of the coauthors of this chapter (Dr. Bagatolli) was the first to apply a two-photon bilayer imaging technique for structural observation of domains in freestanding bilayers using a unique giant unilamellar vesicle system. Since I was involved in observing such structures in monolayer films over a decade earlier I was relieved to find such structures were actually found in bilayers as well. The domains (rafts) in freestanding bilayers can be imaged using the fluorescence methods employed for monolayer films; however, due to the extremely complex composition of most natural membranous systems, these "domain-raft" structures are difficult to analyze compositionally. In Chapter 2, a relatively new technique of time-of-flight secondary ion mass spectrometry (ToF-SIMS), which is used to chemically map the domains, is discussed in detail. The method uses sputtering of the surface or interface of a membranous system with an inert ion beam, and thus generating secondary ion fragments or sometimes the complete molecule. Each single secondary ion from a lipid or protein or their fragments is then processed through a mass spectral analyzer and mapped. This allows for compositional analysis of membrane domains and recently has been utilized to understand nanoscale organization of lipids and proteins in rafts. These structures, formed in membranes of different compositions, may allow for specific functional flexibility and are discussed in Chapter 3, where approaches from microscale supramolecular organization to those at the molecular scale are covered. Chapter 4 gives a description of the molecular and atomic scale approach in studying noncellular lung surfactant (LS) membranous structures using X-ray diffraction. The LS system also shows some specific lipid–protein polymorphism and unique phase segregation of lipids, such as hexagonal type I phase structures called tubular myelin as imaged directly at the lung air–water interface and studied using computer simulations. Using cryotransmission electron microscopy and X-ray scattering, the molecular arrangement of lipids of tubular nonlamellar structures can be deciphered and modeled; the role of cholesterol in such systems is discussed in Chapter 4. Although X-ray diffraction has been applied over the last four decades in membrane structural studies, the use of neutron diffraction in such studies in relationship to the atomic scale models developed by X-ray techniques is discussed in detail in Chapter 5. I have purposely allowed the authors of Chapter 5 to include some images of their neutron diffraction (Chalk River, Ontario, Canada)

grand scale facility and meta-complex instrumental details ("atom smashing") involved in studying such mundane materials as membrane lipid organization, to suggest at what level researchers are using subatomic particles to comprehend membrane structures.

About 2500 years ago an Eastern ascetic on his enlightened view of the world stated that all "forms are emptiness, and emptiness is nothing other than form." The statement can be justified in the sense that all structures (form) observed in any system are dynamic and eventually dissolve into other forms and thereby are only transitory. I feel this gives a view of the dynamics with which all membrane structures are "formed," evolving into other superstructures as well as finally dissolving into some others or into a general membrane environment. As shown by the "snapshots" of membrane domain structures in Part I, these domains are created by dynamic conditions that exist in the bilayer due to the fast and slow motions of the membrane constituents. The question has always arisen as to what the "equilibrium structures" of domains actually are. At what time frames do the membranous structures such as domains exist for them to function, and how do we study these dynamic events? Recent studies have utilized quantum dot imprinting and single molecule techniques to understand events at different time scales. In Part II, from understanding how soluble plasma proteins may interact with the membranous interface in models of lung disease, as well as how cytoplasmic proteins may interact with the lipid interface (Chapter 6), or how specific enzyme activity leads to single molecular events (Chapter 7), we can get some idea about how membranous structures are related to dynamics at least at the supramolecular level. However, since infrared spectroscopy has allowed us to measure hydrocarbon and amide vibrations that occur at very short bond vibrational time scales, the dynamics of other average motions in lipid and protein molecules in model membranes can also be measured (Chapter 7). In some cases, the chirality of the lipids and amino acids in proteins may be an important factor in membrane organization; domain formation and their dipolar orientation at an interface helps in further understanding how such structurally organized interfaces can be modeled (Chapter 8). One of the most important developments in membrane function has been a model of how various ions (and specifically water) cross the membrane hydrophobic barrier. As the recent discovery of aquaporins (water pores) has demonstrated, a basic understanding of membranes as a "semipermeable" barrier cannot be complete without understanding how such water and ion (pH) balance is maintained across membranes (Chapter 9). All biological energetics or the final formation of the high energy phosphate compounds (adenosine triphosphate) occur at an inner mitochondrial membrane interface via the rotation and dynamics of simple proton pumps, which makes understanding the revised ion barrier dynamics of membranes important even after four decades. Finally, dynamic events and molecular motions at the membranous interfaces are probably best studied using computer simulations, since this method allows for a more atomistic model organization of lipids and proteins, while incorporating the various force fields and degrees of freedom of the molecules at very small time scales, which cannot be deciphered by using a single experimental method

(Chapter 10). Although molecular dynamics simulations are models created by specialized programs, because of the speed with which molecular motions occur at a membranous interface— from single vibrations of bonds in a lipid to lateral and spinning motions of specific or groups of molecules— these highly dynamic events can only be understood via computation (Chapter 10). The snapshots of such dynamic events are thus imaged and modeled using high performance computers and slowed down to our optical ten frames per second understanding of membrane dynamics. With parallel processors, supercomputers, fuzzy logic, and quantum mechanics in MD simulations field calculations, eventually a relatively dynamic view of the membranous system may soon be emerging, which may allow for future evaluation of the specific functional domains.

Part III of this book deals mainly with many complex membranous systems, such as bacterial and neural membranes as well as lung surfactant and other colloidal systems. There are several lipid–protein systems of diverse functionality, which are similar in structure and dynamic behavior to those observed in the cellular bilayers or their models. Some of the earlier studies of plasma membrane models have contributed immensely to our understanding of diverse noncellular membranous systems. Perhaps one of the first models of lung surfactant function was developed from using Langmuir films of extracted (lavage or washed) materials from mammalian lungs. Surfactant is packaged and secreted by type II pneumocyte cells and supposedly forms a highly surface active "mono-molecular layer" or film at the air–water interface. These films reduce the surface tension of the lung interface during respiration and prevent lung collapse. During the late 1950s, when the material was first extracted, Langmuir films were the only tool available to study thin films of LS and the films' low surface tension reducing ability (see details in Chapter 15 for discovery).

One of the major developments in analyzing the complexity and diversity of lipids in membranous systems was the study of lipidomics using mass spectrometry. The method has been refined to a point where whole membranes can be literally "extracted" using organic solvents and the lipidome can be analyzed, where each single lipid species is analyzed to a mass resolution of less than one unit. The bacterial membranes are unusual in the sense that certain species contain a double bilayer as well as a large number of glycolipids and some unusual structured lipids with four to six fatty acyl chains (Chapter 11). Previous analyses of these complex systems were possible by complicated acylation–deacylation reactions, esterification, and fragmentation using gas chromatography. With the development of tandem and "soft" ionization mass spectrometers, the complete lipidomes are mapped; in addition, single species are detected at mass resolutions where the isotropic distribution of carbon-13 in a single species can be discriminated (Chapter 11). The mass spectral method has also been modified to actually map or image the localization of specific lipids or proteins in domains in model and natural membranes as discussed in Chapter 2, thus helping in chemically mapping membrane structures.

The other complex membranous systems dealt with in Part III are on nerve signal conducting pathways (Chapter 12), lung surfactant (Chapters 13 and 15),

and general inorganic and organic detergents, which form some of the most peculiar nonbilayer phase structures, due to their general "surfactant-like" nature (Chapter 14). The nerve ("ous"!) systems are dealt with from the viewpoint of thermodynamics, since the phase states of lipids as well as some of the structures involved with these phases are critical for the functioning of the myelin-covered cellular membranous extensions of axons and dendrons.

As editor, I must profess a heavy handedness in dealing with a number of chapters on lung surfactant, since this complex system has recently been rediscovered by membrane physical chemists, physicists, engineers, and nanotechnologists. In Chapter 15, we have tried to speculate on how normal cell membrane research as well as its discoveries regarding phase states, lipid-raft structures, and lipid–protein interactions can easily be compared to a completely functionally different system present in all air-breathing species lungs. After all, fascination with lung surfactant research was planted in my mind by cell membrane studies performed over a century ago. I have also been more fascinated by recent developments in the fields of soft materials, nanotechnology, biophysics, surface and interface science, and many other still emerging fields over the last decade, as pertaining to membranous systems.

My humble hope is that this book will contribute to the reader's imagination in diverse fields and in the broader overview of dynamic yet structured soft materials in nature. If that happens, my work on this volume will not have been in vain. If not, then I humbly apologize for the many mistakes, lack of knowledge, and naivety with which I may have pursued this project from a simple fascination with a system that perhaps played a critical role in the evolution of life on our planet.

I would like to thank a number of people with whom I have had the opportunity to meet and discuss membranes over the last two decades. My initial interest in the area developed about three decades ago, when I was an undergraduate student at Presidency College, in Kolkata, India. Dr. Haripada Chattopadhyay, in a dark, mosquito-infested chronobiological physiology laboratory, described to his students the methods to analyze bilateral respiratory rhythms of humans, and ignited my initial curiosity of things wonderful but yet unknown and their role in the evolution of life. Incidentally, this room was one floor above the laboratory where the Nobel Laureate C. V. Raman had worked on the development of the first Raman spectroscope, as well close to an office space used by Dr. Satyen Bose, who had developed the ideas on Higgs–Boson condensates. I was also fascinated by a lecture on cosmology by Dr. A. K. Roychowdhury of the same institution, which led to my pursuing degrees in physics and human physiology at this institution. Later, in Canada, working in a biochemistry laboratory under Dr. Kevin Keough for a second master's degree and later a doctorate, my scientific imagination was allowed to run wild in trying to develop an instrument to visualize Langmuir films via fluorescence. In fact, this laboratory was the first in the world to synthesize "real" cell membrane phospholipids that had chain melting transitions well below 0 °C and develop theories on interdigitated phases in membranes as well as study them using calorimetry. I spent about seven years

during this phase looking at structures, domains, and black "strings and galactic spirals" (Chapter 15) formed in Langmuir films for every possible compositional mixture of lung surfactant. However, to my utter dismay, we discovered that the natural porcine lung surfactant does not behave like any of its single- or multi-component models; instead, it acts as an alloy. My biochemist's interest in the physics of soft materials, of membranous systems, of fractal dimension, of quantum jitters, of chaos, and of superstrings was highly encouraged by Dr. David Pink (St. Xavier University, Antigonish, Nova Scotia), who unfortunately could not contribute a chapter due to physical reasons. I feel immense gratitude toward him and basically this book is indirectly a product of his initial encouragement. (Thank you, David; live long and prosper!). Recently, I developed doubts (more questions?) regarding the real functional role (and not the "laboratory assigned" one) of LS in the lungs as only a "surface-active" agent. Perhaps this material is a unique extracellular membranous system with some yet unknown functions, some components and structures having similarities to the cell membranes. I am not sure anymore after two decades of research.

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St. John's, Newfoundland and Labrador, Canada
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CONTENTS

PART I

MEMBRANE STRUCTURE

CHAPTER 1

The Membrane Interface as a Structured Compartment and a Substrate for Enzyme Action

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CONTENTS

1.1 INTRODUCTION

Our picture of the biological membrane has changed considerably over the last few decades mainly due to the advancements in instrumentation that allow us to image membranes with an increasing resolution in space and time. Whereas the picture of the membrane since the introduction of the celebrated Singer–Nicolson fluid-mosaic model [1] has always been one imparting the membrane assembly with considerable dynamics and disorder, the current picture is more refined, describing the membrane as a structured bimolecular and fluid flexible sheet with a certain degree of local lateral organization [2–4] in terms of differentiated lipid domains, in some cases called rafts [5]. Although the lipid-bilayer component

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of the membrane is only about 5 nm thick, it is furthermore associated with a distinct transmembrane structure that is described by the so-called lateral pressure profile [6], which displays variations of local stresses corresponding to hundreds of atmospheres across the 5-nm thick bilayer. It is this highly dynamic and still structured and stressful environment that the proteins and enzymes associated with the membranes have to come to terms with in order to carry out their function.

This insight has led to an increasing understanding of the importance of lipids and lipid structure for cell function in general and for protein and enzyme function in particular. By adding to this picture that certain lipids are now also known to act as signaling molecules for a large range of biochemical processes, it becomes clear why lipids and the emerging fields of lipidology and lipidomics have now moved center stage, and the importance of lipids for life sciences is considered to be similar to that of genes and proteins.

This has led to a revival of the study of lipid–protein interactions and of the mutual influence of lipids and proteins on each other. Questions have arised not only as to how proteins influence the lipid matrix but also as to how the lipids influence protein structure and function. Specific questions cover the insertion and folding of integral membrane proteins, the oligomerization of the protein segments in the plane of the membrane, the structural stability of membrane proteins, the anchoring of proteins in membranes and at membrane surfaces, and the requirement of specific lipids and a certain lipid structure for optimal protein functionality [4, 7].

However, the study of lipids and lipid membranes is complex because of the subtle elements of order that characterize lipid assemblies. Whereas the properties of genes and proteins are described in terms of well-defined molecular structure, the properties of lipids are characterized by terms like variability, diversity, plasticity, adaptability, fluidity, and complexity. The particular role played by lipids is most often determined by their collective properties—that is, properties that cannot be associated with the individual lipid molecule but are consequences of their interactions and cooperative behavior. Examples of such properties are membrane curvature and curvature stress, transbilayer pressure profile, acyl-chain order parameter, packing density, diffusional motion, phase state, and small-scale lateral organization in space and time characterized by a coherence length or equivalently by an average lipid-domain size. Properties like these require quantitative characterization by use of powerful biophysical techniques.

In this present chapter we review some of the results that have been obtained in our laboratories with regard to characterization of lateral order in model bilayer membranes as well as biological membranes, with particular focus on membrane domains in the submicron regime. The results are based mainly on fluorescence microscopy and two-photon laser scanning microscopy as well as atomic force microscopy (AFM). We then show by a specific example how a small enzyme, secretory phospholipase A_2 (s-PLA_2) becomes activated at membrane interfaces in a way that is controlled by the lateral structure of the lipid-bilayer substrate.