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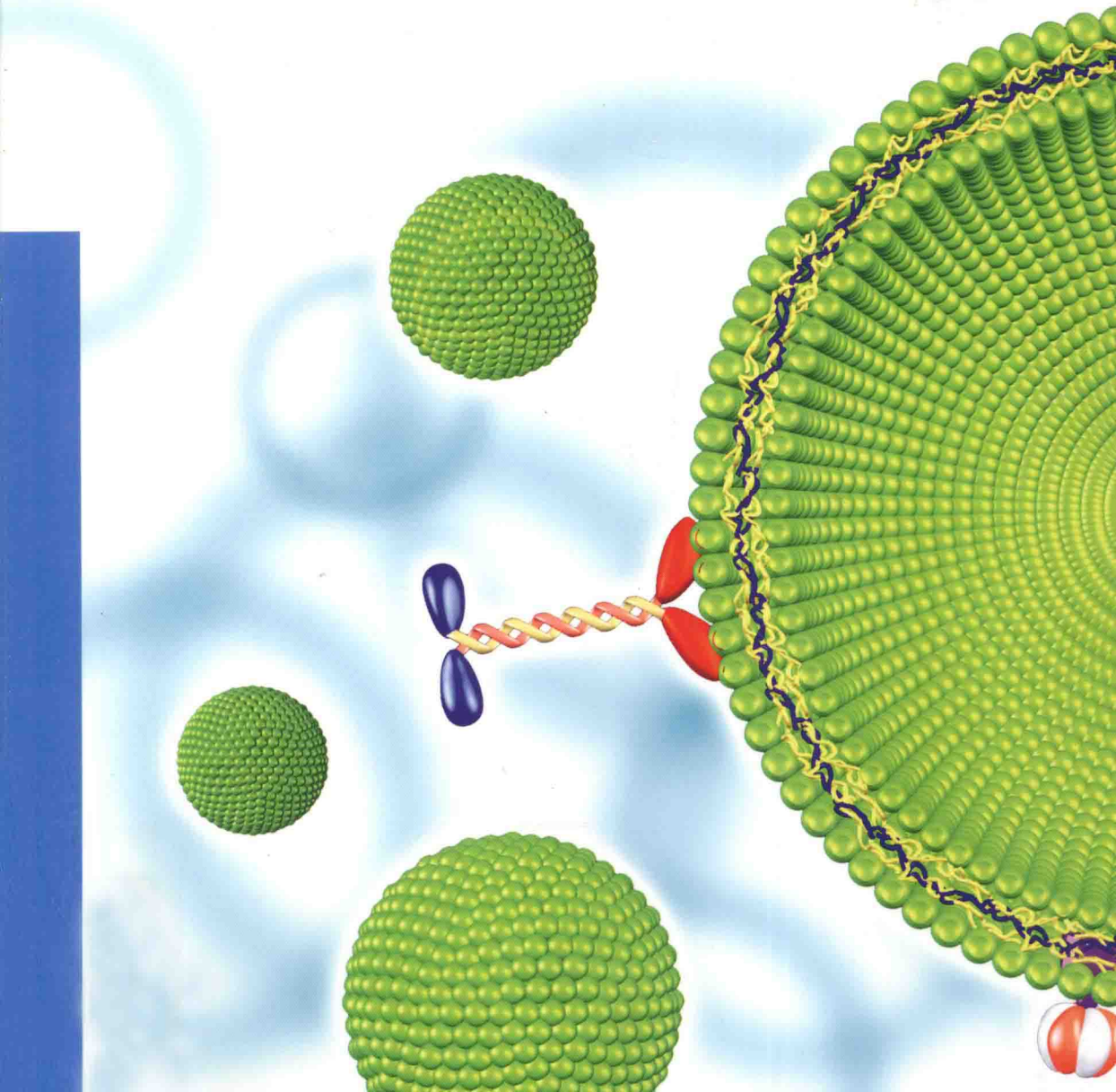

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Preface

We are frequently asked how much we can learn from nature. In most case, we can get the answers by biomimetics. With the development of nanosciences, biomimetics is staring at the molecular level. This is based on the fact that many bioactive molecules like DNA, lipid, peptide, proteins can self-assemble into well-defined structures and further to a supramolecular architecture while combining with other organic, inorganic or metal oxide compounds. It is therefore considered the promising method to fabricate novel materials. The obvious feature of such biomimetic systems are their artificial structures which can be inspired by biology. A major advantage of these assembled systems is that they keep their biochemical and physical parameters and properties in a controlled manner. Thus the intense interest in this field is clearly evident.

The present book attempts to introduce the aspects and practical techniques of molecular assembly of biomimetic systems, especially, the layer-by-layer assembly, self-assembly, microcontact printing, electron beam lithography and chemical lithography.

We have benefited from many efforts of our co-workers in making this book reality. We sincerely acknowledged them, notably Weixing Song, Zhihua An, Liqin Ge, Cheng Tao. We have to say that we have learned a lots about molecular assembly from Profs. H. Möhwald, H. Rinsdorf, T. Kunitake and Jiacong Shen who have done much of the pioneering work and we are grateful to all of them for their motivated and skillful helps and contributions.

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Introduction

In nature, biological systems and physiological processes have evolved over millions of years to improve their properties and functions. Biomimetics, simply, is the attempt to mimic these properties and functions. It involves studying structures and mechanisms of tissue formation in the organisms. Using biology as a guide, we can now understand, engineer, and control bioactive molecular interactions, and assemble them into novel systems or materials. The molecular biomimetic approach opens up new avenues for the design and utilization of multifunctional molecular systems with a wide range of applications in nanotechnology and biotechnology. Molecular assembly of biomimetic systems is now regarded as one of the promising methods to fabricate well-defined nanostructures and materials, and its importance is now generally recognized.

Biomimetic systems are artificial structures that are inspired by biology. A major advantage of these systems is that both biochemical and physical parameters can be controlled precisely. Therefore, it is feasible to utilize biomimetic systems as experimental models for guiding research on biological mutation and evolution in organisms. Some bioactive molecules such as peptides, proteins, nucleic acids, and lipids can undergo self-assembly into well-defined structures similar to the assembly in living organs. Biomimetics is not limited to just copying nature because, with the development of modern biology, scientists can directly utilize biological units themselves to construct new types of systems sometimes as hybrid nanostructured materials. In this way, some of the manufacturing difficulties of biomimetics can be avoided. As will be illustrated in this book, natural molecular machines such as motor proteins are integrated into the engineering of active biomimetic systems so that new functionalized systems can be constructed.

This book covers fundamental aspects and practical techniques of the molecular assembly of biomimetic systems; in particular, layer-by-layer (LbL) assembly, self-assembly, microcontact printing, electron beam lithography (EBL), and chemical nanolithography. It also presents an overview of the molecular assembly of biomimetic systems that consists of the following six topics covered in individual chapters.

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Biomimetic Membranes

Biological membranes are key components in biological systems, forming the natural boundary of cells to separate inner components from outer environment. A number of cell actions and functions relevant to the environment are fulfilled via membrane processes, in most cases depending on the interactions of membrane proteins and carbohydrates. Owing to the complexity of biological membranes, it is very important to design and engineer artificial model membranes to overcome the difficulty of investigating membrane function directly in living cells. In this regard, biomimetic membranes as either a lipid monolayer or lipid bilayers are created by simple artificial strategies, such as spread at air/water or oil/water interfaces, or vesicle fusion. A lipid monolayer fixed at the air/water interface is a popular model membrane for investigation of the hydrolysis process catalyzed by enzymes at the interface. More attention is being paid to lipid bilayers supported on a planar surface or a curved surface because supported membranes, especially cushioned by polymers, are an ideal model to unravel the physical and chemical properties of biomembranes and their contribution to membrane functions.

In Chapter 1, we primarily focus on the fabrication of a lipid monolayer as a simplified model for studying the dynamic adsorption and interfacial behavior as well as membrane hydrolysis process catalyzed by enzymes at the interface. In addition, we briefly summarize the most recent developments and applications on supported lipid bilayers at polyelectrolyte multilayers, including at planar and curved surfaces. The introduction of such a biomimetic membrane will enhance greatly our understanding of the function and property of biological membranes, and will also significantly help to develop advanced characterization tools or techniques for a better understanding of the biological membrane system.

Layer-by-Layer Assembly of Biomimetic Microcapsules

Biomimetic microcapsules are a class of artificial hollow sacs with controllable size and versatile function such as tunable physicochemical properties and permeability. They can be regarded as a promising cell mimic to simulate some functions of cell membranes. Quite different from conventional liposomes, such a biomimetic hollow sac contains a large enough compartment so that the natural environment of membrane-bound proteins can be recreated. The materials making up microcapsules are a variety of polymers that are beneficial for the affinity and embedment of membrane proteins on supported biological membranes. The LbL assembly technique, which was first developed by Decher for the fabrication of ultrathin multilayers, is effective in the preparation of a hollow shell upon colloidal templates. Biogenic microcapsules prepared by the LbL technique are of great interest due to their potential application in medicine, catalysis, cosmetics, and biotechnology. By the conversion of liposomes into lipid bilayers, the coating of active lipid bilayers on polymer microcapsules can readily be achieved. Such lipid-decorated microcapsules can serve as an ideal supported biomimetic membrane

system to mimic functions of the cell membrane. This new hybrid system also enables the design and application of new biomimetic structured materials.

In Chapter 2, we describe how LbL-assembled polyelectrolyte microcapsules can be interfaced with biological components such as phospholipid membranes and proteins. LbL assembly has attracted extensive attention for the fabrication of biomimetic microcapsules because it provides engineered features including size, shape, thickness, composition and permeation, and the capability of incorporating different types of biomolecules. The applications of these biomimetic microcapsules in drug delivery, biosensors, and hybrid nanodevices are also addressed.

F_oF_1-ATP Synthase-Based Active Biomimetic Systems

ATP synthase (ATPase) is one of the most popular molecular machines and has been extensively studied. It can act as a rotary motor in the design of novel nanodevices, continuously synthesizing ATP in the artificial environment. Production of ATP is one of the most important chemical reactions in living biology. With regard to the production of ATP, ATPase is the primary enzyme to catalyze the reaction where the generation of ATP from ADP and inorganic phosphate is performed upon the induction of proton gradients. The functionality of ATPase has attracted great interest over the last decade. Many potential applications have been suggested, from the generation of bioenergy to the fabrication of nanodevices. Lipid membranes have been widely used as models for biological membranes and ATPase is particularly selected as a model membrane protein, since it is a major ATP supplier in the cell. As a membrane-bound protein, ATPase can be reconstituted *in vitro* into liposomes via detergent mediation. Nevertheless, the limitations of the size and instability of the assembled liposome complexes result in difficulties in understanding and analyzing the system. Instead, lipid-coated polymer microcapsules exhibit extensive advantages as a biomimetic vehicle having a similar function to liposomes, but controllable in size and robust in structure.

In Chapter 3, we explore how biomimetics can be applied to engineering functional nanomaterials, particularly to assembling ATPase in artificial containers and mimetic cellular systems with cellular processes. Much effort has been focused on assembling ATPase in biomimetic systems so that a complex cellular process can be constructed in a controllable manner. Recently, LbL-assembled microcapsules have proven to be a suitable cellular mimetic structure and have been applied to engineering active biomimetic systems with cellular processes. An added benefit is that these assembled microcapsules can be used as bioenergy containers and thus supply ATP on demand.

Kinesin–Microtubule-Driven Active Biomimetic Systems

Linear motor proteins such as kinesin and myosin can transport cargoes inside cells with both spatial and temporal precision. These linear motor proteins provide

the inspiration of the design and build-up of novel biomimetic functional nanomaterials. Kinesins are a family of proteins that can be divided into 14 classes based on sequence similarity and functional properties. Over the past decades, efforts to use linear motor proteins as nanoactuators have made rapid progress. In general, these motor proteins consume chemical energy to power the movement of targeted components into devices engineered at the micro- and nanoscale. The design of such hybrid nanodevices requires suitable synthetic environments and the identification of unique applications. Linear cytoskeletal kinesin motors have dominated the emerging field of protein-powered devices because they are relatively robust and readily available. Tubulin can be commercially purchased, while the motor proteins can be purified from cells or expressed in recombinant bacterial systems and harvested in large quantities.

In Chapter 4, the recent progress of assembling kinesin–microtubule–cargo systems in a synthetic environment is presented. In particular, we discuss the selection, loading, and unloading of cargoes, and also highlight our ongoing work–LbL-assembled microcapsules serving as cargoes driven by kinesin motors.

Biomimetic Interface

Biomimetic interface engineering modifies the interfaces between biological and nonbiological systems to gain valuable insight into the biological interactions at these interfaces. The main advantage of biomimetic interface strategies is the ability to influence biological interactions by modifying the interfaces, while still retaining the vital physical properties and to some extent improving the biocompatibility of the materials. A number of methods or techniques, including optical lithography, nanoimprint lithography, dip-pen nanolithography, and microcontact printing, are available for the engineering or patterning of interfaces. These biologically functionalized interfaces, generally as biomimetic interfaces, have a wide range of applications in biology and nanotechnology (e.g., for drug delivery, biosensors, biochips and medical implants, etc.).

In Chapter 5, we provide a brief overview of the advances in the application of microcontact printing for lipid micropatterning, and EBL for lipid nanopatterning and polymer gradient structures. In particular, a relatively new technique, chemical nanolithography, which is based on radiation-induced changes in organic self-assembled monolayers, is addressed.

Peptide-Based Biomimetic Materials

Self-assembly of biological building blocks has attracted increasing attention due to their versatility for bottom-up fabrication, biocompatibility, and biodegradability, with a wide range of application in biology and nanotechnology. Many biomolecules including peptides and proteins can interact and self-assemble into highly ordered supramolecular architectures with functionality. In the self-assembly

process the precise control of supramolecular architectures is achieved through synergistic effects of some weak noncovalent interactions such as hydrogen bonds, electrostatic interactions, π–π stacking, hydrophobic forces, nonspecific Van der Waals forces, chiral dipole–dipole interactions, and so on. Although these forces are individually weak, when combined as a whole, they govern self-assembly of molecular building blocks into superior and ordered superstructures. Self-assembly is ubiquitous in nature. By learning from nature one can purposefully devise and synthesize artificial building blocks amenable to self-assembly into superstructures by cooperative interactions of weak noncovalent interactions. Notably, peptides composed of several to dozens of amino acids have been of great interest in the creation of biomimetic or bioinspired nanostructured materials owing to their structural simplicity and tunability, functional versatility, cost-effectiveness, and widespread applications.

In Chapter 6, we first focus on the fabrication of peptide-based nanostructural materials from synthetic building blocks such as lipopeptides, polypeptides, amphiphilic peptides and, particularly, diphenylalanine-based peptides derived from Alzheimer's β-amyloid polypeptide. In addition, we present the experimental results and progress in the integration of peptide biomaterials with functional inorganic components for creating multifunctional materials. We then discuss the potential applications of such assembled peptide-based materials in biological and nonbiological areas, including tissue engineering, gene or drug delivery, bioimaging and biosensors, as well as functional templates for nanofabrication.

1
Biomimetic Membranes

1.1
Introduction

The cell membrane is a selectively permeable lipid bilayer that is a basic structural unit in all cells. It is composed of a variety of biological molecules such as lipids, and proteins and lipopolysaccharides are attached to the membrane surface. A vast array of cellular processes, including cell adhesion, ion channel conductance, and cell signaling, are performed at such biological interfaces [1]. The cell membrane is a natural barrier on a cell that separates the intracellular components from the extracellular environment. The biological functions associated with membranes involve a number of different molecular species. Both the lipid and protein compositions of membranes are primarily responsible for membrane function as well as structure. Membrane proteins embedded in the cell membrane are of particular importance in adjusting and controlling the delivery of substances across the membrane and acting as molecular signals that allow cells to communicate with each other. Additionally, protein function can be influenced by the lipid matrix that surrounds it. Understanding the function and structure of cell membranes remains a critical challenge. Altogether, the biological membrane has proved to be crucial for cell survival. The phospholipid bilayer functions in compartmentalization, protection, and osmoregulation, and the proteins have a wide range of functions including molecular recognition, transport of substances, and metabolic processes [2]. Thus, model membranes have historically been indispensable for the development of our understanding of biological membranes. Such biomimetic systems allow us to study the individual features of these highly complex structures.

Lipid membranes are one of most important self-assembled structures in nature. Lipid monolayers or bilayers prepared artificially provide excellent model systems for studying the surface chemistry of biological membranes. To some extent, such a type of membrane is a biomimetic structure. Their use can help us to understand the structure and function of membranes, and the relationship between them through simplifying experiment processes, reducing complexity in

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data interpretation, and improving greater experimental control [3]. It is well known that biological membranes consist of lipid bilayers where other components such proteins and enzymes are able to penetrate. To understand the biological processes of membranes at a molecular level, a lipid monolayer is often considered as a simplified model of biomembranes (e.g., the self-assembled lipid monolayer at the air/water interface has been used as a model membrane for investigating the hydrolysis process catalyzed by enzymes at the interface) [4, 5]. Supported lipid bilayers [6, 7] have become more and more important biomimetic materials, and are popular models of cell membranes for potential application in biology and nanotechnology. The construction of the model membrane is significantly advantageous to understand the function of biological membranes *in vitro* (e.g., investigating protein ligand–receptor interactions [8–11], cellular signaling events [12–14], biological sensing, and transport roles of biological membranes [15–17]). During the past decade a large number of solid-supported lipid membranes have been developed, including inorganic surfaces, functionalized monolayers, polymers, and others. With the increasing demand for supported membranes, polyelectrolytes are emerging as popular choices for cushioning materials. Usually, polyelectrolytes are self-assembled to a variety of substrates by means of the layer-by-layer (LbL) deposition method, providing potential control over the resulting cushion film thickness, porosity, polarity gradient, and so on. Thus, it is possible to provide a new hydrated environment for the lipid membrane that serves as a versatile biomimetic membrane in which function and property can be varied on demand.

In this chapter, we focus primarily on the fabrication of lipid monolayers as a simplified model for studying their dynamic adsorption and interfacial behavior, as well as membrane hydrolysis processes catalyzed by enzymes at the interface. Additionally, we briefly summarize the most important new developments and applications on the supported lipid bilayers at the polyelectrolyte multilayers, including at planar and curved surfaces. The introduction of such a biomimetic membrane will greatly enhance our understanding to the function and property of biological membranes, and will also help to develop advanced characterization tools or techniques for better investigation of biological membrane systems.

1.2 Lipid Monolayers

1.2.1 Phospholipid Monolayers at the Air/Water Interface

With increasing interest in biological membranes, phospholipid monolayers have been fabricated as a simple model membrane for studying the corresponding interfacial behavior. Lipid monolayers at the air/water interface can be easily formed by spreading an organic solution of phospholipid on the surface of water [18, 19]. The hydrophilic headgroups of the phospholipid face towards the water

while the hydrophobic alkyl chains are exposed to the air. The self-assembly nature of the phospholipid molecule leads to the formation of a two-dimensional lipid phase.

The phase state of this monolayer is pertinent to the phospholipid concentration at interfaces (molecules/area). We can obtain a two-dimensional phase diagram when compressing the phospholipid monolayer at a constant temperature (i.e., decrease the area available to the phospholipid). Surface pressure (π) as a function of the molecular area (A) (i.e., π–A isotherm) may give integral information on the lipid-phase transition [20–22]. For instance, the π–A isotherm of the L-α-dipalmitoylphosphatidylcholine (L-DPPC) monolayer undergoes almost all of the possible phase states of the insoluble monolayer. As shown in Figure 1.1, with the increase of surface pressure, different molecule packing patterns of L-DPPC in a monolayer will appear. At the beginning, the monolayer is in the gas-phase state and the molecular arrangement is completely disordered. When the monolayer is compressed by a lateral surface force, the molecules become closer and subsequently go through a phase change from gas to the liquid-expanded phase, followed by the coexistence region of the liquid-expanded and the liquid-condensed phase. If the given pressure is large enough, the stacking phase state of the monolayer is also likely as the solid state, in which the molecules are closely packed and orderly oriented along a certain direction. However, when the given pressure is over a

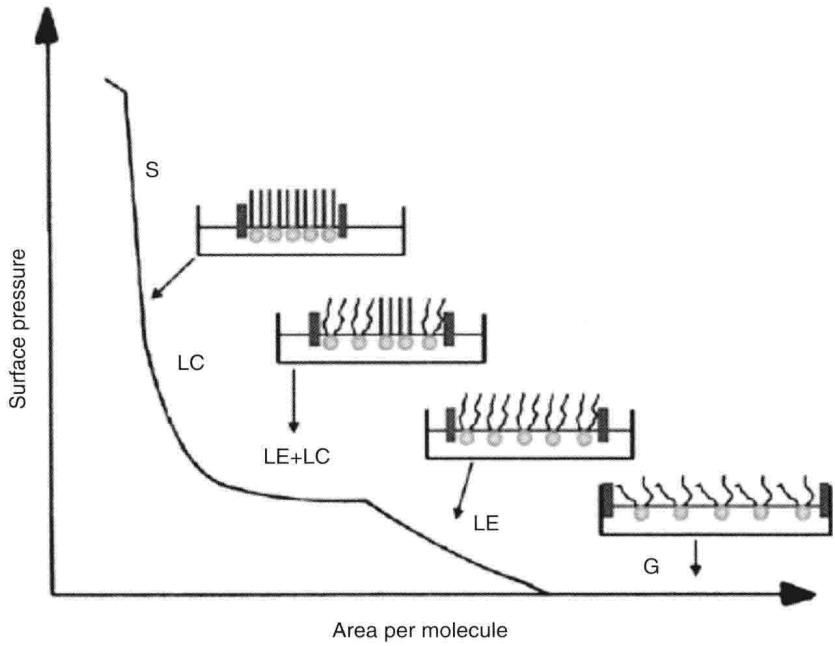

Figure 1.1 Pressure/area diagram of a lipid monolayer: scheme of the compression behavior and packing in the gas (G), liquid-expanded (LE), liquid-compressed (LC), and solid (S) phases. (Reprinted with permission from [5]. © 2007, Elsevier.)