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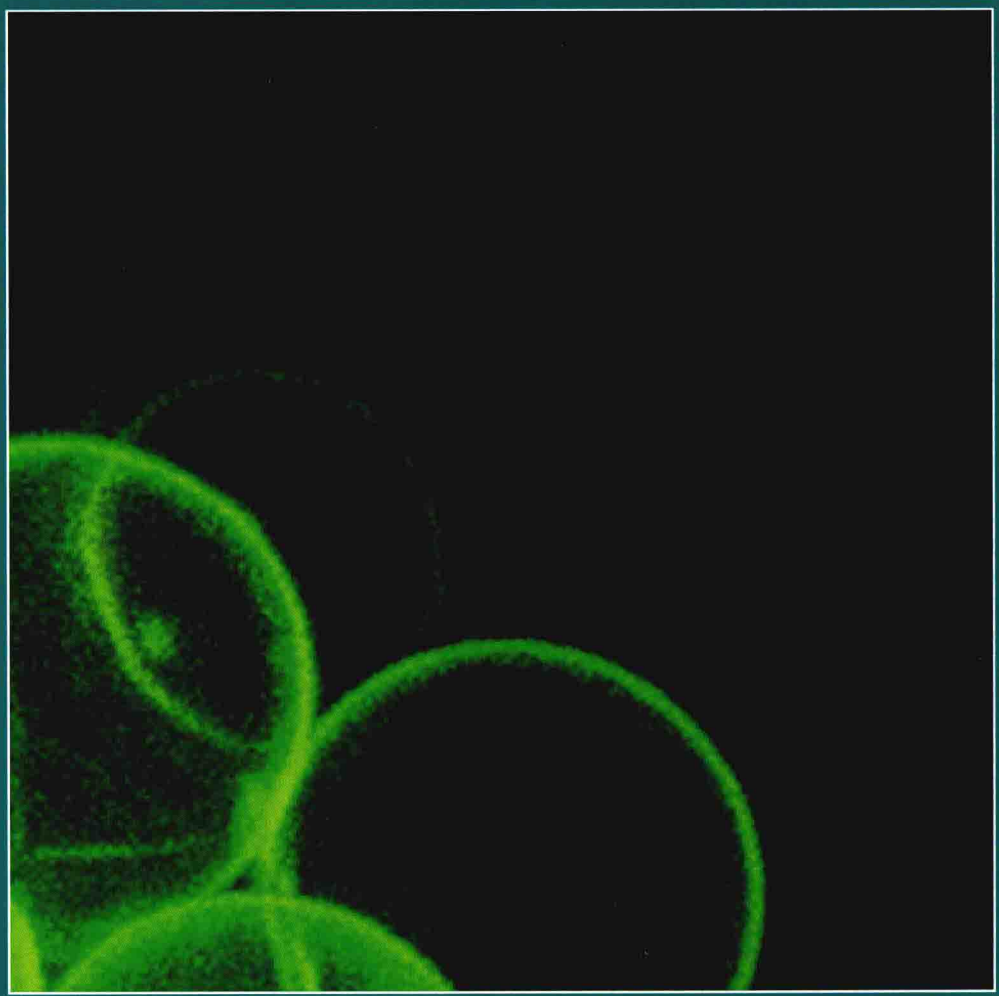

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PREFACE

"Model Membrane Systems" is a central theme of Volume 20 of *Advances in Planar Lipid Bilayers and Liposomes* (APLBL) which includes eight chapters. An assortment of subjects is covered under this theme such as chemical and electrostatic interactions of biomolecules (DNA, antimicrobial peptides) with model biomembranes, structural evolution and phase transitions in self-assembling systems, and biological significance of self-assembling systems. Model systems comprise lipid monolayers, Langmuir–Blodgett films, supported lipid bilayers, vesicles, as well as nonlamellar nanostructures. Recent advances in these fields are nicely presented by an amalgamation of theoretical and experimental approaches. The overall content of this volume is thus potentially useful for wide scientific community working on model lipid systems and their biotechnological implications.

We would like to thank all authors who contributed their chapters to the Volume 20—Jacek Lipkowski, Natalia Wilke, Sarah Rachel Dennison, Daniela Uhríková, Vernita D. Gordon, Tanja Pott, Theyencheri Narayanan, Marko Marhl, and their coauthors. We would like to thank all members of the Editorial Board. We also thank our Technical and Publishing Team of *APLBL* Volume 20, especially Shellie Bryant, Kate Newell, and Preeta Kumaraguruparan.

Aleš Iglič and Chandrashekhar V. Kulkarni

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CHAPTER ONE

Biomimetic Membrane Supported at a Metal Electrode Surface: A Molecular View

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Abstract

This chapter reports on recent advances in the application of spectroscopic and surface imaging techniques to provide molecular level information about the structure of gold-supported phospholipid bilayers. It describes methods used to deposit biomimetic membrane at the gold electrode surface. It provides information about the structure of the membrane deposited at the gold electrode surface and its changes as a function of the applied potential obtained with the help of techniques such as scanning electron microscopy or atomic force microscopy, neutron reflectivity, and infrared reflection absorption spectroscopy. These experimental approaches provided unique molecular level information about the interactions of the membrane components with the metal, orientation, and conformation of molecules within the membrane, water content in the supported bilayer, and the structure of water molecules within the supported bilayer. The interactions of the bilayer with the metal restrict mobility of the membrane. From biomimetic point of view, this is an unwelcomed effect. However, the ability to

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immobilize phospholipid matrix on a conductive support provides unique opportunity to employ scanning tunneling microscopy to acquire molecular resolution images of channels formed by antibiotic peptides and in this way to provide direct evidence and molecular information of their action and their biocidal activity. The metal-supported model membranes find applications as biosensors. Proteins incorporated into such membranes constitute the sensing element and act as transducers of chemical to electrical information. This chapter includes a review of IRRAS studies of the potential induced changes in the orientation and conformation of membrane and peripheral proteins incorporated into the gold-supported bilayers.

1. INTRODUCTION

Model lipid membranes supported at a metal electrode surface allow transduction of chemical changes taking place in the membrane to electrical signal such as current or changes of the membrane capacitance and resistance. The transduction of chemical to electrical information allows development of biosensors that find applications for fast drug screening and selective detection of ions and molecules in general [1,2]. These systems constitute also ideal platforms for a broad range of biomedical research such as studies of implant biocompatibility, cell adhesion and fusion, drug screening, and amyloid plaque formation [3,4].

The supported bilayer lipid membrane (sBLM) is a planar bilayer with one leaflet physically adsorbed to a solid surface and the other leaflet freely exposed to solution. The planar geometry and long-term mechanical stability of this design allow one to investigate the relationship between the structure and properties of the bilayer using a wide range of surface sensitive techniques, such as IR spectroscopy [5–20], scanning tunneling microscopy (STM) [20–22], atomic force microscopy (AFM) [23,24], Raman spectroscopy [25] and neutron reflectivity (NR) [26–28].

There are several recent reviews that report on properties of sBLM at metal surfaces [29–34]. Therefore, the scope of this chapter is to describe recent advances in the application of spectroscopic and surface imaging techniques to provide molecular level information about the structure of gold-supported bilayers and on how the structure depends on the potential applied to the electrode surface. We also discuss how the structure and properties of sBLM depend on the interaction between the lipid molecules and the substrate and describe distribution of water molecules within the supported bilayer. The interactions of the bilayer with the metal restrict

mobility of the membrane. From biomimetics point of view, this is an unwelcomed effect. However, the ability to immobilize phospholipid matrix on a conductive support provides unique opportunity to employ STM to acquire molecular resolution images of channels formed by antibiotic peptides and in this way to provide direct explanation of their biocidal activity. At metal surface linearly polarized IR photons are interacting with metal surfaces, the incident and reflected beams enter into destructive interference when the electric field of the photon is oriented parallel to the surface and into constructive interference when the electric field is located in the plane of incidence that is normal to the surface. Therefore, by taking a difference between the two signals one is able to determine the absorption spectrum of molecules in the supported bilayer. We discuss how to use such polarization modulation to determine orientation and conformation of molecules in the supported membrane and how these properties are affected by the potential drop across the membrane.

2. sBLM PREPARATION METHODS

The most common procedures used to form sBLMs at solid surfaces are the vesicle fusion (VF) and Langmuir–Blodgett (LB) and Langmuir–Schaefer (LS) transfer methods, which are discussed below.

2.1. Vesicle fusion

Vesicles are closed lipid bilayers that encapsulate an aqueous solution. The procedure for VF consists of the adhesion and fusion of small unilamellar vesicles (~50 nm in diameter) at a solid substrate from aqueous vesicle dispersion. At hydrophilic surfaces such as glass, quartz, or mica, VF involves adsorption, deformation, and rupture followed by sliding of a single bilayer or rolling of two juxtaposed bilayers in a tank tread-type motion on a thin lubricating film of the solvent. A theory depicting the adhesion, fusion, and rupture of vesicles at solid surfaces was developed by Lipowsky and Seifert [35]. The validity of this theory was confirmed by Reviakine and Brisson [36] who with the help of AFM showed images of adsorbed and ruptured vesicles at a solid surface. Unilamellar vesicles also fuse at an atomically smooth surface of gold to form a bilayer [20,21]. STM studies of pure 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) and mixed DMPC–cholesterol vesicles fusion at a Au(111) surface [20] demonstrated that the mechanism of the bilayer formation at the gold surface is distinctly different from that on hydrophilic surfaces of glass or quartz. The molecules

released by rupture of a vesicle initially self-assemble at the metal surface into a well-ordered monolayer. The self-assembly is controlled by the interaction between the acyl chain and the metal surface. When more molecules accumulate at the surface, the monolayer is transformed into a hemimicellar state. In solutions with high vesicle concentrations, the hemimicellar state is transformed further into a bilayer. This point is illustrated in Fig. 1.1 which shows STM images of the Au(111) surface; image (A) 3 min and image (B) 30 min after addition of vesicles into the solution of an electrochemical STM (EC-STM) cell. Figure 1.1A shows rows formed by acyl chains of DMPC molecules lying flat on the surface. However, the images of flat-lying molecules could be observed only in dilute vesicle solutions and during a short period of time after the injection of vesicles to the cell. Figure 1.1B shows that after about 30 min, the film transforms into totally different structure. The nature of this structure was identified with the help of complementary AFM experiments. Figure 1.2A is an AFM image of the gold surface acquired after about 70 min of incubation in a solution of DMPC vesicles. The contrast in this image shows film with a corrugated surface with the periodicity of the corrugation similar to that in the film imaged in Fig. 1.1B by STM. In the case of

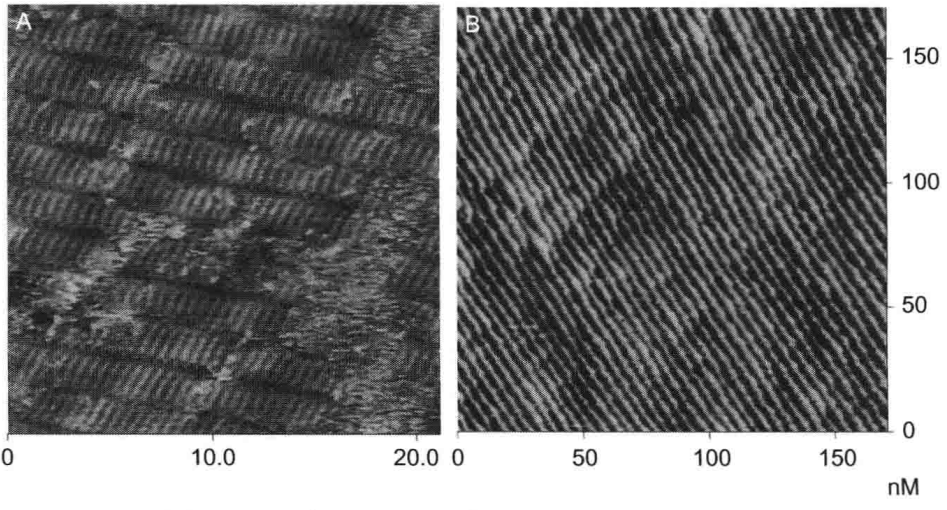

Figure 1.1 (A) STM image of a Au(111) surface acquired 3 min after injection of a solution of DMPC vesicles showing individual DMPC molecules flat lying on the surface. (B) The corrugated structure of a film of DMPC molecules at a Au(111) electrode acquired 30 min after injection of vesicles (electrode potential +200 mV vs. Ag/AgCl electrode saturated with KCl; supporting electrolyte in 50 mM $KClO_4$ with in 0.04 M total DMPC concentration). Imaging conditions: $I_t = 1.00$ nA, $E_{tip} = -150$ mV. *Adapted from Ref. [20].*

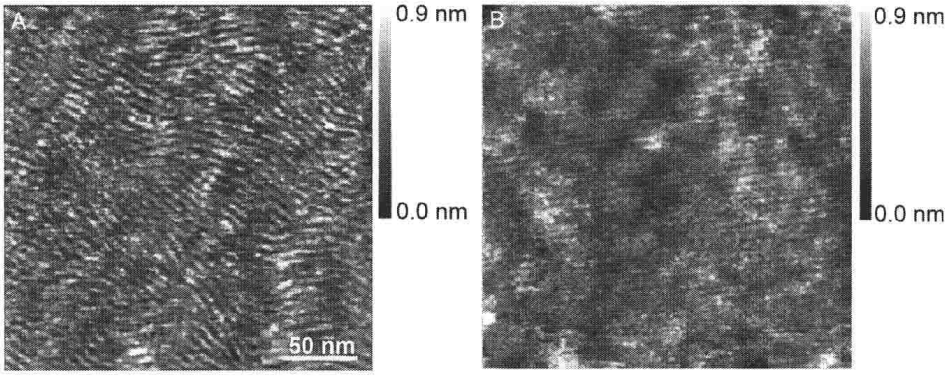

Figure 1.2 Comparison of AFM images of a Au(111) electrode surface covered by bilayer formed by vesicle fusion: (A) pure DMPC bilayer in 50 mM NaF solution containing 0.1 mg/mL DMPC vesicles and (B) DMPC–Chol (7:3) DMPC–Chol bilayer in 1 mM NaF solution containing 0.07 mg/mL of DMPC. Images acquired ~70 min after injection of vesicles to the AFM cell at a temperature of 20 ± 1 °C. *Adapted from Ref. [24].*

AFM, the film can also be characterized by the force–distance curves recorded in approach of the AFM tip to the film covered surface.

Representative force–distance curves are shown in Fig. 1.3A. Curve 1 displays a characteristic discontinuity when DMPC molecules are present in the solution. This discontinuity is absent in curve 2 when it is recorded in the pure supporting electrolyte. The discontinuity corresponds to the penetration of the tip across the film of DMPC molecules and could be used as a measure of the film thickness. The measured values of the film thickness are plotted in Fig. 1.3B as a function of temperature. The thickness of the film formed by fusion of unilamellar vesicles is equal to about 4.5 nm at 20 °C. This corresponds to the expected thickness of DMPC bilayer in the gel state [37–39]. At temperatures above 24 °C, the thickness decreases to a value ~3.8 nm which is expected for the liquid-crystalline state [37]. These data indicate that at sufficiently long incubation times, the bilayer of DMPC is formed at the gold electrode surface by fusion of unilamellar vesicles.

The temperature dependence of the bilayer thickness shown in Fig. 1.3B indicates that transition between gel and liquid-crystalline states is observed between 20 and 22 °C. In DMPC vesicles the phase transition is observed at 24 °C [37]. IR experiments on hybrid bilayers with one leaflet composed of hydrogen and the second with deuterium-substituted acyl chains indicated that in the bilayer supported at gold the two leaflets are poorly coupled [10]. This poor coupling explains the observed shift of the phase transition to lower temperatures.

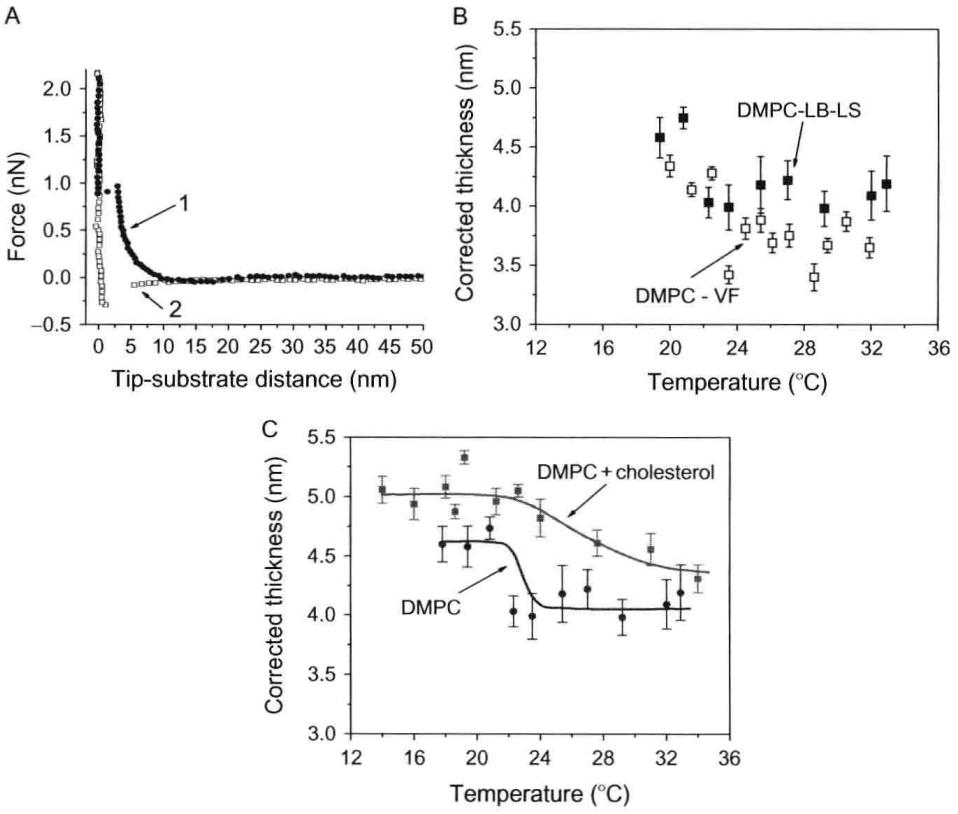

Figure 1.3 (A) Force–distance curves recorded at $E = 0.2$ V, curve 1 and solid circles for 0.1 M NaF + 0.1 mg/mL DMPC vesicle solution; curve 2 and open squares for pure supporting electrolyte; (B) Dependence of the thickness of the film of DMPC at the gold electrode surface as a function of temperature; solid squares: DMPC bilayer formed by a combination of the Langmuir–Blodgett and Langmuir–Schaefer methods; open squares: DMPC bilayer formed by a spontaneous fusion of small unilamellar vesicles from 0.1 M NaF + 0.2 mg/mL DMPC vesicle solution; $E = 0.2$ V versus Ag/AgCl electrode saturated with KCl; (C) Temperature dependence of the bilayer thickness, squares for the mixed 70% DMPC + 30% cholesterol and circles for pure DMPC bilayer formed by the LB-LS method. *Adapted from Ref. [23].*

The differences between mechanisms of spreading unillamelar vesicles at hydrophilic surfaces and at a gold are particularly well illustrated by the example of spreading mixed DMPC–cholesterol vesicles [21]. When vesicles rapture and their material is released onto the surface, strong lipid–metal interactions are causing segregation of the film into pure cholesterol and pure DMPC domains seen in the STM image (Fig. 1.4A) recorded 36 min after injection of vesicles. The zigzag-like features in this contrast correspond to an ordered domain formed by flat-lying cholesterol