Dynamical Properties of Supported Membrane Junctions

Yoshihisa Kaizuka and Jay T. Groves

Department of Chemistry, University of California, Berkeley
103 Lewis Hall, University of California, Berkeley CA, 94720
JTGroves@lbl.gov

ABSTRACT

We have recently introduced a supported membrane junction system : supported membrane-based platform for studying membrane-membrane interactions [1]. The junction consists of a conventional supported bilayer membrane, on top of which a second bilayer membrane is deposited by rupture of a giant vesicle. This second (upper) membrane is stably associated with the lower supported membrane and the solid support. By incorporating °uorescently labeled lipid molecules in the membranes, two °uorescence imaging methods with nanometer resolution are made possible : intermembrane °uorescence resonance energy transfer (FRET) [2] and optical standing wave interferometry [3]. Both techniques reveal the topography of the membrane junction - FRET for separation less than 10nm, and interferometry for separations up to hundreds of nanometers.

In addition to providing a means of studying intermembrane interactions, this supported membrane junction system allows examination of lipid bilayers in a more natural three-dimensional setting than provided by conventional supported bilayer membrane. We examine various thermal phenomena of two apposing membranes, such as topographic °uctuations, unbinding transition and demixing of lipids, and discuss their meanings.

Keywords: lipid bilayer membrane, FRET, interferometry, °uctuation, phase transition

1 Introduction

Supported membranes, consisting of a lipid bilayer assembled on a solid substrate, have proven useful in a broad collection of physical and biological investigations, ranging from thermodynamic studies of membrane structure [4] to the creation of phantom cell surfaces used to resolve mechanisms of immune cell recognition [5]. An important characteristic of the supported membrane con¯guration is that a thin (» 1 nm) layer of water separates the lipid bilayer from the underlying substrate. The natural bilayer structure and lateral mobility of individual molecule is well preserved in this con¯guration. However, due to strong adhesion between the membrane and substrate, collective motions in both lateral and perpendicular dimensions are signi¯cantly damped. Furthermore, singular supported membranes don't readily allow the study of membrane-membrane interactions. Many important biological events happen at the intermembrane zone, including immunological and neural synapses, therefore physical study and development of new experimental tools for examining two apposing membranes are important.

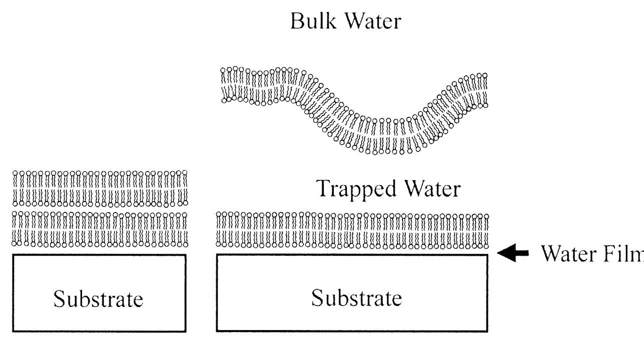

Figure 1: Schematic of the Supported Membrane Junction System. The lower membrane is a conventional supported membrane. The upper membrane is deposited by rupture of a giant vesicle and shows various topographical features. Two membranes are bound in the left-hand side image and are loosely associated in the right-hand side image.

One such tool we have developed is a membrane junction system consisting of a conventional supported bilayer membrane, on top of which a second bilayer membrane is deposited by rupture of a giant vesicle (Figure.1). The two planar membranes are stably associated with each other in the membrane junction. Since the scale of interaction and °uctuation phenomena between membranes is as small as nanometers, imaging methods which have nanometer resolution are necessary.

Intermembrane FRET provides sub-nanometer resolution when the spacing between two membranes is within 10nm [1,2]. Two di®erent °uorescent dyes conjugated to lipid molecules of the two apposing membranes work as donor and acceptor molecules. Resonance energy transfer between the two molecules quenches one of the °uorophores and creates a footprint in the supported membrane, mapping the contact zone between two membranes (Figure 2a,b). We have shown that the e±ciency of resonance energy transfer yields quantitative measurements of intermembrane separation distances that agree precisely with structural data of molecules in the intermembrane region [2]. Besides a simple uniform interaction between two membranes, intermembrane FRET can reveal topographical patterns of the intermembrane region in the lateral direction. Patterns formed by polyethylene glycol(PEG) conjugated lipids which are not visible in conventional °uorescence images are clearly imaged [2].

Optical standing wave interferometry has proven to be very useful to observe larger topographical features (Figure 2c). When the membrane system is supported on an oxidized silicon wafer, optical standing waves created by the silicon surface provide contour mapping of the membrane topography with nanometer resolution and a range of a few hundred nm.

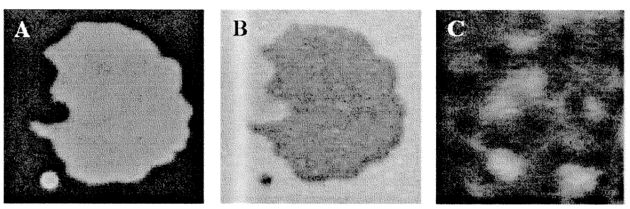

Figure 2: Images of the upper membrane(a) and the lower membrane(b) in the membrane junction system. Intermembrane FRET footprint is observed (b). (c) The image of upper membrane on an oxidized silicon wafer. The intensity variance caused by optical standing wave interferometry re°ects the topographical features of the membrane. The length scale of membrane is about 10^{1}m in (a) and (b), and 5^{1}m in (c).

Since the upper membrane is not directly supported by the substrate, we can expect that it shows di®erent physical properties from a conventional supported membrane. Physical characterization of upper membrane and interaction between the upper membrane and the supported membrane will be discussed.

2 Problems

Two classes of biophysical problems can be studied with membrane junction systems. One is the interaction between two apposing planar membranes. The other is behavior of upper membranes loosely associated to supported membrane and substrate.

As for the interaction of two membranes, we can examine how two membranes start or stop associating , and how intermembrane molecules work once two membranes are associated. The ¯rst problem relates to existing theoretical study of unbinding transitions of two vesicles [6]. Experimental and theoretical studies of immunological synapses are examples of the latter problem [5,7].

An upper membrane in the membrane junction is not supported but rather is freely associated with a bottom membrane and a substrate. The upper membrane, therefore, is more laterally °uid than a supported membrane. Because of this °uidity, the molecules in the membrane are in more native state - more similar to that of a cell membrane than those in a supported membrane.

In its rupture, a giant vesicle doesn't make uniformly °at membrane patches but rather creates blisters [1] and other structures that don't appear in supported membranes. Intermembrane FRET and optical standing wave interferometry are two methods to study di®erent aspects of the structure. The following biophysical problems are studied.

2.1 Thermal Fluctuation of Membrane

We examine thermal °uctuations of membranes. Thermal °uctuation of vesicles or erythrocytes have been observed and analyzed in the past [8]. As for planar membrane, some theoretical work has been done [9], but experiment has been impossible. Since planar membranes are easier to handle with in experiments and to be used for applications with biological molecules, the possibility of observing °uctuations in a planar membrane with this membrane junction system, and possible applications of °uctuation imaging are studied.

2.2 Unbinding Transition

Transition from the bound state to the unbound state of two membranes can be studied in membrane junctions, especially in the context of °uctuation-induced repulsion (Helfrich repulsion).

2.3 Lateral Demixing Phase Separation

Structures of coexisting phases in lipid membranes are of great biological interest [10]. When phospholipid is mixed with cholesterol, lipids are separated into a phospholipid rich liquid-disordered phase and a cholesterol rich liquid-ordered phase [11]. The experimental study of phase separation for planar lipid bilayers, however, has been limited, as the interaction between a membrane and a substrate doesn't allow phase separated domains to grow to mesoscopic sizes which are optically visible. We explore the use of membrane junction systems to study the demixing phase separation.

3 Summary

Supported membrane junction system provides the platform to study intermembrane interactions and behaviors of relatively free planar membrane. Two imaging methods, intermembrane FRET and optical standing wave interferometry, resolves nanometer scale topographical features of membranes. Combining of the system and imaging methods are useful for many biophysical investigations which cannot studied by conventional systems.

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