

5.2: Supported and Tethered Membranes

Model lipid bilayers or synthetic lipid bilayers are membranes that are created *in vitro*. *In vivo* studies of lipid bilayers and the proteins embedded within them can be difficult due to the complexity of the cellular dynamics of membranes. Model bilayers use biological membranes, along with artificial constraints, to investigate the structure and function of lipid bilayers. Supported and tethered membranes are two types of model bilayers frequently used in model studies. Importantly, these hybrid systems are not employed as vesicles, but, instead, maintain a non-curved structure placed against or physically attached to a non-fluid surface. This method of studying membrane biology is valuable in that it allows for the utilization of many techniques are not as applicable to “freestanding” membranes, since supported and tethered membranes are more stable [1]. Furthermore, the complexity of natural membranes makes them difficult to study, especially *in vivo*. Model bilayers provide an excellent model for studying the natural functions of lipids and membrane proteins in a simple manner. Moreover, many techniques require a physical interaction with the membrane, which would be difficult to perform on freestanding membranes that are capable of floating through fluid.

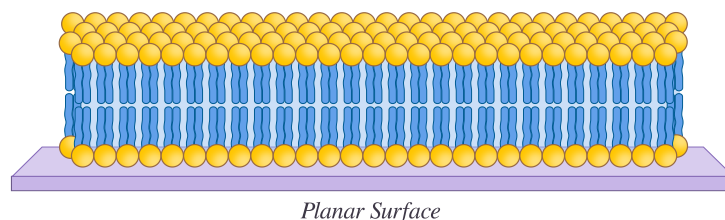


Figure 5.2.1: Model lipid bilayers consist of biological or artificial membranes and potentially an interacting solid substrate. (CC BY-NC; Ümit Kaya)

Supported Membranes

Supported lipid bilayers (SLBs) are biological or synthetic membranes with one side of the membrane exposed to fluid environment, while the other side is lined against a flat solid surface. There is typically a very thin fluid layer separating the membrane from the surface (Figure 5.2.1). This type of configuration allows SLBs to maintain their fluid integrity and to diffuse laterally in 2D space. However, this small distance between the membrane and its surface (1-2nm) could potentially lead to interactions/friction between the lipids and the surface [2]. Another type of model bilayer has been designed to relieve this issue.

Regardless, SLBs usually remain stable for long periods of time as well [3]. In addition to the new techniques developed from using SLBs (see **Scientific Applications**), established methods have been used with SLBs to further understand lipid bilayer dynamics and function [4]. In one of the first examples, researchers employed fluorescence microscopy to examine the diffusion of two different fluorescent labeled lipids (Figure 5.2.2).

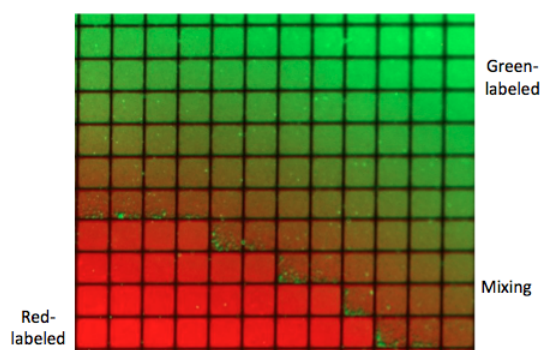


Figure 2. Fluorescence microscopy studies using supported membranes show the mixing of two different fluorescent labeled lipids.

Preparation of Supported Membranes

SLBs can be built in a number of different ways. One of the first methods for synthesizing SLBs used alkylated glass slides and applied only a monolayer of lipids to an air-water interface (Figure 5.2.3) [5]. This first type of configuration was mostly used to study the lateral movement of monolayers via fluorescence microscopy. Since this approach was employed, many different surface types have been used, including glass, mercury, and silicon-oxide [5]. Silicon oxide is the most popular surface type [6]. Self-

assembly of lipid bilayers is central for maintaining biological integrity. Self-assembly is also important for maintaining the biology of any embedded proteins. A more recent technique for forming supported membranes uses a previously constructed lipid monolayer that is supported on a surface as a template to fuse an additional monolayer from a vesicle [7]. In this technique, the monolayer from the vesicle fuses only with the already supported monolayer to form a new lipid bilayer. This prevents any preservation issues that might arise from the monolayer and proteins being exposed directly to a planar surface. For instance, exposing a membrane protein directly to the support surface can prevent any lateral movement of the protein. This issue would prevent characterizing the native movements and functions of the membrane protein. An additional technique for preparing supported membranes is called vesicle deposition [8]. In this method, either unilamellar vesicles (SUVs) or giant unilamellar vesicles (GUVs) are fused with the solid support surface. This is achieved by increasing the temperature at which the reaction is taking place to above the melting temperature of lipids. At the temperature, the vesicles will naturally fuse with the surface.

The number of surface-constricted membrane systems has greatly expanded. Solid-supported lipid bilayers, polymer-cushioned lipid bilayers, hybrid bilayers, tethered lipid bilayers, suspended lipid bilayers, and supported vesicular layers have all become well-established models [9].

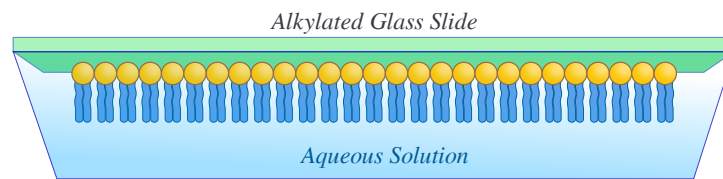


Figure 5.2.3: One of the first formed supported membranes, using a lipid monolayer and alkylated glass slide. (CC BY-NC; Ümit Kaya)

Tethered Membranes

To further increase the stability of hybrid membrane systems, some lipid bilayers are physically attached to a planar surface via a protein or a polymer. Tethered lipid bilayers membranes (tLBMs), also referred to as polymer-supported bilayers, employ a “tether” that is not part of the studied membrane to connect it to a surface (Figure 5.2.4). tLBMs also require the use of membrane proteins for solid-surface support. tLBMs provide some advantages compared to SLBs. Because tLBMs are attached to their surface via a tether, the space between lipids and the surface is much greater than that of SLBs [2]. This feature is more likely to prevent friction between the membrane and the solid. Additionally, SLBs can more easily lose contact with the contact surface or can result in only specific parts of the membrane interacting with the surface, as opposed to the entire membrane [3]. These issues can lead to a system that is no longer biologically relevant. The nature of this type of model lipid bilayer allows the membrane to be exposed to fluids on both sides, more like a naturally occurring membrane.

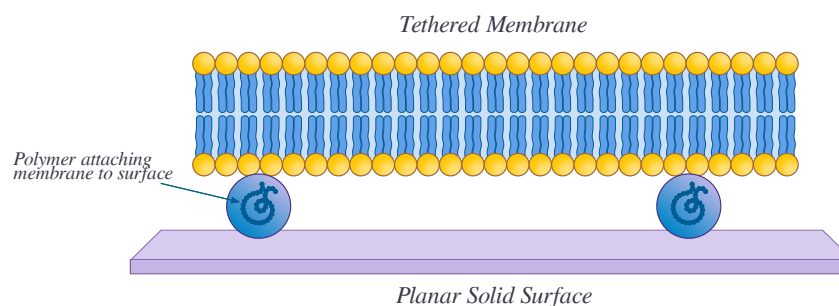


Figure 5.2.4: Tethered lipid bilayer connected to a planar substrate by polymers. (CC BY-NC; Ümit Kaya)

Scientific Applications

One of the most useful applications for tethered and supported membranes is the study of peripheral types membrane proteins. The stable nature of these bilayers causes the proteins embedded within the membrane to remain immobile and well oriented, making it easier to observe protein function. Specifically, studies of the immune and endocrine systems were able to examine lipid embedded T-cell receptors and hormone receptors (Figure 5.2.5). One study used SLBs containing **T-cell receptors** to show that the actual T-cell, when bound to the receptor through the antigen and class II major histocompatibility complex, stabilizes that entire interaction of the complex [10]. Other studies have also employed tLBMs and SLBs in conjunction with antibodies to research cancer. Using a

similar method to the T-cell receptor study, researchers used SLBs chemically linked to antibodies to capture and purify circulating tumor cells (CTCs) [11]. In another application, SLBs and tBLMs are subjected to [atomic force microscopy \(AFM\)](#) and fluorescent imaging as a way to investigate the effects of environmental stressors [12]. In this particular study, the authors used SLBs to show that yeast cells increase the amount of unsaturated lipid content and ergosterol as a means of ethanol tolerance. The authors employed AFM against SLBs to show the change in morphology.

Tethered and supported membranes have been used to investigate many other biological phenomenon including, vesicle/receptor force relationship and effect of undulation forces between a vesicle and a rigid wall [13].

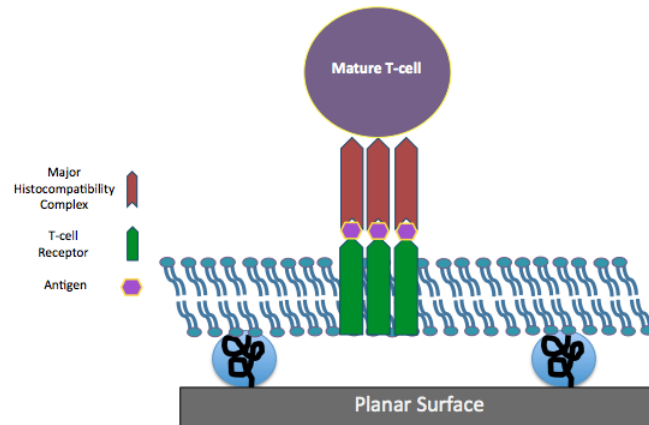


Figure 5.2.1: Supported bilayer containing T-cell receptors bind antigen displayed on a major histocompatibility complex of a mature T-cell.

Figure 5.2.1: Supported bilayer containing T-cell receptors bind antigen displayed on a major histocompatibility complex of a mature T-cell. (Copyright; author via source)

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