Making Lipid Membranes Rough, Tough, and Ready to Hit the Road

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Abstract

Solid-supported lipid bilayers hold strong promise as bioanalytical sensor platforms because they readily mimic the same multivalent ligand–receptor interactions that occur in real cells. Such devices might be used to monitor air and water quality under real-world conditions. At present, however, supported membranes are considered too fragile to survive the harsh environments typically required for non-laboratory use. Specifically, they lack the resiliency to withstand air exposure and the thermal and mechanical stresses associated with device transport, storage, and continuous use over long periods of time. Several successful strategies are now emerging to make supported membranes tougher. These strategies incorporate mimics of the cytoskeleton and glycocalyx of real cell membranes. The promise of these more robust lipid bilayer architectures indicates that future materials should be designed to more fully resemble the actual structure of cell membranes.

Keywords: *biological, biomimetic, cellular, sensor.*

Introduction

Even with all the tireless efforts of modern medical researchers to thwart deadly viral infections, society is still plagued by that indefatigable viral strain, influenza. Viral mutants such as the one responsible for the pandemic of 1918 have a vast history of killing populations, yet to date we have minimal, if any, protection against new and ever-increasing variations of pathogenic mutants. These mutants, whether developed naturally through evolution or synthesized for the purpose of terrorism, present a very real threat to society. Imagine a terrorist, toting a backpack with a landscaping atomizer filled with a lethal strain of influenza, spraying the corridors of a large city subway at rush hour and subsequently infecting a significant portion of the population before it can be detected.[1] An even more likely scenario may be a deadly mutant of the avian influenza virus developing in Southeast Asian chickens that is easily transported to other parts of the world via human vectors.[2] Probably the biggest threat is to those in the armed forces, who could be exposed to biological agents through poisonous aerosols and sprays.

One could imagine many different scenarios for how a fatal flu virus or other malignant pathogen might contact the human population. Without the option of a vaccine for all possible variants, our only defense might be the development of a biomimetic early-warning detection device that is robust enough to function in harsh environments for extended lengths of time (see Reference 3). Such a device is essentially a "canary-on-a-chip"[4] that will continuously monitor the conditions and alert us to contaminants in our environs before toxic amounts find their way to our skin, lungs, and eyes. "Canary" here is a reference to the practice of coal miners having a caged canary alongside them as they worked. A canary is particularly sensitive to toxic gases and thus served as an early warning to miners of a health threat. Like a canary, the device should be easily transportable to the location of detection, robust enough to monitor the area reliably and continuously for months or even years, require little maintenance, and give few false positives. These new platforms will be tailored to mimic human responses and monitor pollutants that are specifically lethal to us.

One promising detection platform uses solid-supported lipid bilayers[5–8] as its central sensing component. Artificial membranes are unique materials that are especially suited for biomimetic devices. For example, they are composed of the same lipid and protein molecules that can be found in the plasma membranes of living cells. These synthetic bilayers preserve the lateral fluidity of their lipid constituents, just like natural cells.[9–14] This is critical for the ability to carry out multivalent ligand–receptor interactions, whereby an incoming protein binds to multiple membrane-associated ligands via lateral rearrangement of the surface moieties (Figure 1). Multivalent binding can be especially critical for viruses like influenza for which each individual interaction is quite weak.[15] Indeed, quite a few interactions are probably required in order to trigger infection.

Supported membranes within lab-on-a-chip–type devices could be used for monitoring multiple toxins in parallel by

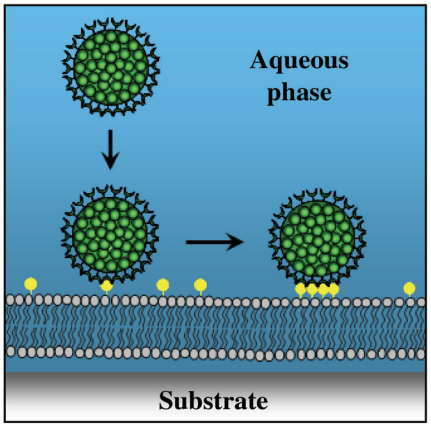

Figure 1. Schematic illustration of a generic virus (in green) binding initially to one membrane-associated ligand (in yellow) on a solid-supported lipid bilayer, followed by the lateral rearrangement of other ligands and their subsequent binding to receptors on the particle surface.

having each species bind to its own surface-associated ligand or combination of ligands. Since it is easy to create spatially addressed arrays of lipid bilayers on a single chip,[7,8,16–18] one could even create platforms for screening applications in a clinical setting. There are, however, several requirements for early-warning detection devices to function in real-world environments. First, the device must be robust enough to survive rough transport and storage conditions—that is, the lipid bilayer film must resist mechanical and thermal stresses associated with transit to point of use, as well as possible prolonged storage before implementation. Second, for protection during shipping and storage, the devices should be able to withstand current preservation techniques[19,20] such as dehydration or freezing. Once at the point of use, the lipid bilayer film must recover full function upon rehydration or, ideally, be permanently air-stable. Third, the devices should be exceedingly resistant to contamination outside the laboratory setting to avert environmental fouling or bacterial growth during long-term monitoring. Finally, the devices must be highly sensitive and specific to the intended analytes.

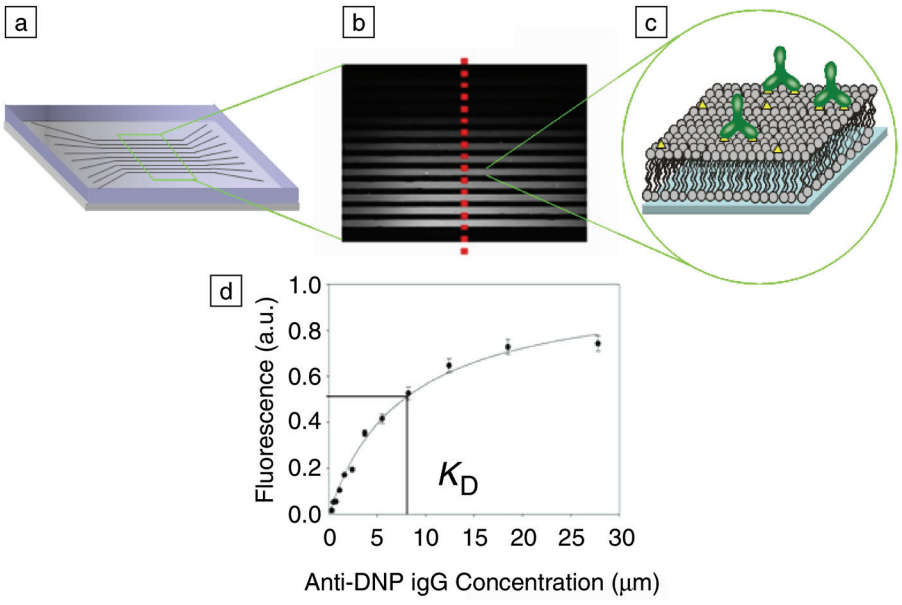

Figure 2. (a) Schematic illustration of a microfluidic device to perform one-shot binding assays. (b) Total internal reflection microscopy image of the channels in a working microfluidic device. Each channel has a different concentration of fluorescently tagged protein flowing through it. (c) Schematic representation of a bilayer coated on the surface of the microchannel and the binding of a protein to a ligand presented on it. (d) Binding curve obtained from the data in (b). K_D is the dissociation constant of the ligand–receptor binding event for anti-dinitrophenyl (DNP) immunoglobulin G (IgG) to DNP-conjugated lipids in the bilayer.

Making Highly Sensitive Biodetection Devices by Exploiting Multivalency

As with influenza, nature's general solution for generating both high sensitivity and specificity at the membrane surface has been to employ multivalency. Lateral diffusion of the recognition components allows more sensitive detection. Indeed, the organism's immune system can detect the presence of foreign elements at much lower concentrations than possible with monovalent binding and therefore induce a faster immunoresponse.

Current microfluidic devices already provide us with information about multivalent binding, for example, in investigations of the effect of ligand density on bivalent antibody binding in fluid supported bilayers.[21–23] Such microfluidic devices employ solid-supported lipid membranes intermingled with antigens as coatings on the channel walls. The channels are made from polydimethylsiloxane and glass and are fully enclosed to maintain an aqueous environment above the bilayers. The advantage of these devices is that it is possible to obtain simultaneously all the data points for a single binding curve as a function of ligand density, as shown in Figure 2. Here, each channel is coated with a bilayer containing the appropriate ligands, and the level of binding within the channel is correlated to the level of fluorescence. From a plot of fluorescence intensity versus bulk protein concentration, we can obtain the dissociation constant, K_D, of the ligand–receptor binding event. While these kinds of devices easily yield thermodynamic and even kinetic information, they are primarily limited to laboratory use because of the fragility of the underlying lipid film.

Current Membrane Pitfalls

Simple lipid membrane architectures must be in an aqueous environment at all times. In fact, the lipid bilayer will delaminate from the support if the thin film is exposed to the air/water interface.[24–31] This detachment occurs because it is energetically unfavorable to remove the hydrophilic lipid head groups from solvation waters. Therefore, when an air bubble arrives at the surface, the membrane must reorganize to expose some of its lipid chains to the nascent air/water interface, while the rest of the lipid material becomes part of newly formed vesicles in the aqueous solution, as depicted in Figures 3a and 3b.

A number of attempts to modify supported bilayers in order to protect them upon exposure to air are present in the literature. One strategy involves the use of tethered or hybrid bilayer systems.[32] These systems are generally prepared via the Langmuir–Blodgett deposition of the upper lipid leaflet,[27] and the bottom monolayer is covalently attached to the underlying support. The type of modification depends on the substrate. Thiol or silane monolayers are employed for gold and SiO_2 substrates, respectively. These chemical modifications help anchor the thin lipid film to the support.[33,34] Other methods to preserve membrane stability employ bolaamphiphile monolayers. Bolaamphiphiles consist of two hydrophilic moieties attached by a hydrophobic functionality, resulting in a bilayer that is resistant to reorganization upon air exposure.[35]

Polymerizable synthetic lipids have also been used to create a new class of stable lipid bilayers.[36] These lipids usually contain two triple bonds within their hydrophobic tail region and can be either chemically polymerized or photopolymerized and have been found to be resistant to air and chemical solvent exposure.[28,29] Photopolymerization has also been used to spatially address lipid membranes for sensing applications. Finally, other attempts to stabilize lipid membranes have been achieved by employing charged lipids, thus relying on the electrostatic interactions between the bilayer and substrate. Although these

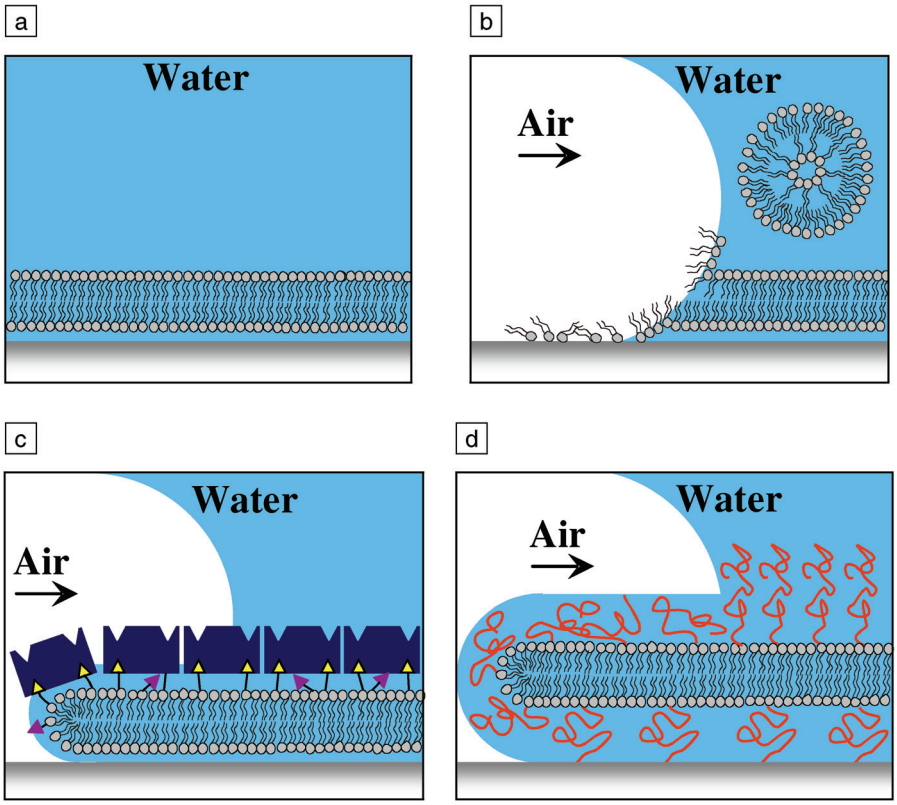

Figure 3. (a) A bilayer formed on a solid substrate submerged in an aqueous environment. A thin water layer beneath the bottom leaflet and the substrate preserves the lipid mobility. (b) The introduction of an air interface destroys the solid-supported bilayer by peeling it away from the surface in vesicle sections. Some lipids may also form patches of monolayers at the air surface. (c) Protein coatings, such as streptavidin (dark blue rectangles) bound to biotinylated lipids (yellow triangles), help to reduce delamination and also cover other ligands (magenta triangles) so that they are unavailable for binding with the target antigens. (d) PEGylated lipids protect the bilayer from delamination and provide more space between the bottom leaflet and the support.

methods yield air-stable supported bilayers, the systems generally lack or experience a loss in the lateral mobility of their lipid components.[26,27,37] Consequently, any multivalent interactions dependent on two-dimensional ligand rearrangement are hindered, and thus their performance as biomimetic sensors is impaired.

Making Fluid Membranes Tough

Our laboratory has developed several unique strategies to prevent delamination of fluid bilayers during air exposure.[38,39] The first approach involves specifically binding a protein monolayer of streptavidin to a biotin-presenting phospholipid surface.[38] Coating the bilayer with streptavidin has two effects on membrane stability. First, it increases the bending elastic modulus (stiffness) of the membrane and thus increases the energy barrier for lipid patches to roll up into sheets and peel away as vesicles,[40,41] as depicted in Figure 3c. Second, the presence of the protein coat makes it difficult for the upper leaflet of the bilayer to rearrange to form a monolayer film at the nascent air/water interface. This strategy works well for preventing delamination from occurring. It was also shown that the lipid molecules remained mobile when the system was placed in air. The diffusion constant D of the lipids in the bilayer before removal from water was 1.9×10^{-8} cm^2/s, while in air near 100% relative humidity, it was 2.9×10^{-9} cm^2/s. The diffusion constant returned to its original value upon subsequent rehydration in bulk water. Of equal significance was the finding that the mobile fraction of labeled lipids in the membrane was greater than 90% after rehydration. Despite the success of this approach, it is impractical for use in biosensors, because it is necessary to blanket the entire bilayer with a relatively close-packed streptavidin film to afford air stability. Therefore, any additional ligands incorporated into the film for sensing would be unavailable for binding with target analytes, because of the protective streptavidin layer covering them.

A second approach to protecting the bilayers from delamination allowed modest-sized proteins to penetrate through the coating. This method involved the use of lipopolymer lipids.[39] Lipopolymers were chosen as an alternative membrane coating because in a primitive way they resemble the elaborate chemistries found on bacterial and eukaryotic cell surfaces. Cell surfaces are often terminated with a variety of glycosylated proteins, glycolipids, and polymeric structures, collectively called the glycocalyx, that can extend tens of nanometers above the plasma membrane.[42] The glycocalyx affords cell stability and plays a role in cell signaling and cell–cell interactions. To mimic such a protective architecture on solid-supported lipid bilayers, the coating must be porous enough to permit access of proteins, toxins, and other analytes of interest while still affording the required air stability.[43] Poly(ethylene glycol)-conjugated lipids were found to be excellent materials for this purpose.[44]

The PEG conformation in a phospholipid bilayer depends on the concentration of PEG-conjugated lipids within the membrane.[45–48] The appropriate polymer conformation was found to be key to affording stability upon air exposure. At low surface densities, the polymer assumes a mushroom-like configuration[46] (Figure 4a) that does not fully carpet the surface, thus leaving some areas exposed to the surrounding medium. Consequently, bilayers sustain damage when bulk water is removed. However, at higher surface densities, the polymers are more crowded on the surface, and therefore they assume brush-like configurations (Figure 4b).[49] In this configuration, the polymer extends out farther into the surrounding medium, creating a thicker, more hydrated protective layer, as shown in Figure 3d.[50]

It is known that PEG increases the bending modulus of the membrane[51–54] and that the modulus varies as a function of density and chain length of PEG. Also, hydration of the bilayer is significantly higher when the PEG is in the brush conformation, compared with the mushroom configuration or naked bilayer. It is this thick hydration layer and higher bending elastic modulus that almost certainly afford air stability. It should also be noted that the PEG moieties themselves do not change the diffusion constant or mobile fraction of lipids present in the bilayer

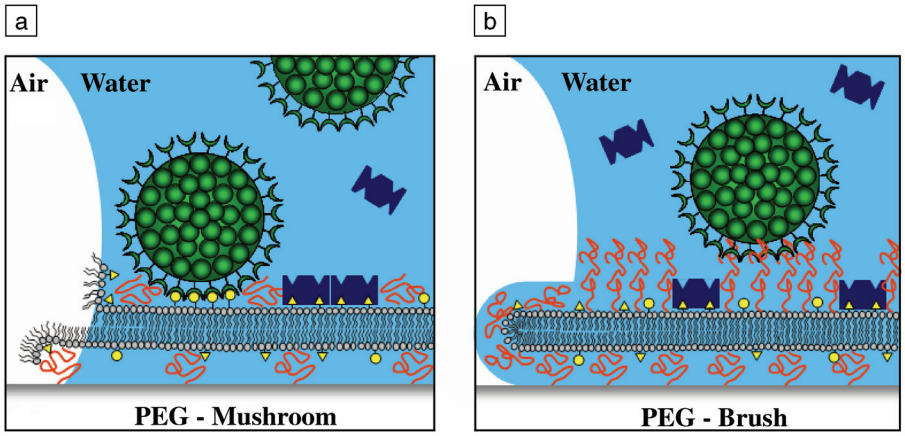

Figure 4. (a) PEG at low surface densities (red) assumes a mushroom configuration that does not protect from delamination upon air exposure, prevent large particles (in green) from fouling the surface, or prohibit aggregation of bound moieties (in dark blue). (b) PEG at higher surface densities assumes a brush configuration that protects well from air exposure and acts as a filter for larger particles (green) so that they do not interrupt the sensing of smaller target analyte (dark blue rectangles), which are still able to pass through the meshwork of the lipopolymers and bind to the membrane surface.

until the PEG concentration is well above that which is necessary to stabilize it against air exposure. Concentrations we employed correspond to the onset of the mushroom-to-brush transition, which is well below this limit. Prior to dehydration, it was found that the mobile fraction of lipids within the bilayer was ~98%, with $D \sim 4 \times 10^{-8}$ cm^2/s for both the mushroom and mushroom-to-brush configurations. Upon rehydration from the dry state, the mobile fraction of lipids was only 40–50% when the bilayer was coated with PEG at densities corresponding to the mushroom configuration. However, at higher PEG coverage (at the mushroom-to-brush transition), there was nearly complete recovery after rehydration. Furthermore, at this surface density, protein binding levels prior to and following rehydration were almost exactly the same, indicating that PEG does indeed afford air stability without adversely hindering ligand–receptor interactions (Figure 4b). Additionally, it is well known that PEG coatings are resistant to nonspecific protein adsorption[55–57] and possibly bacterial growth, both attractive attributes for the design of biosensor devices.

As discussed, the presence of a glycocalyx mimic adds rigidity to the bilayer and the ability to prevent delamination during air exposure. A second reason for inclusion of a model glycocalyx might be to prevent aggregation of receptors and proteins within the 2D membrane plane. A final reason for its inclusion in membrane-based devices might be for protection from larger-sized foulants, such as dust, pollen, and smog particles, that will invariably come into contact with the surface with long-term use. Besides biofouling, solid-supported bilayers can be easily ruined when contaminated with common things like dirt, hydrocarbons, alcohol, or detergents. Bilayers can also be degraded by bacterial growth. The glycocalyx probably also enhances the specificity of the sensor, by allowing only those particles to come to the surface that can infiltrate between the pores of the protective mesh.

We have tested the ability of PEGylated bilayers[39] to resist bacterial and mold growth. Resistance to such contamination is especially important for bilayer devices that will be used continuously in water or humid environments where the possibility of biofouling by mold, algae, and bacteria is quite high. We observed that bilayers without lipopolymer constituents did not resist the growth of mold very well after two weeks, while bilayers that contained a dense coating of lipopolymers held up quite well (Figure 5).

In the preceding paragraphs, we discussed the possibility of using PEG to deter bilayer delamination and prevent biological fouling; however, in order to approach a real cell's crowded environment, and thus better mimic its function, we need to not only include a glycocalyx, but also overcome the challenge of incorporating functional transmembrane proteins within the system. Because there are often strong interactions between membrane proteins and the substrate, proteins often denature on the support and lose function and mobility.[58] Surmounting this problem is especially important for devices that will use membrane-spanning proteins as detection elements. Polymer cushion

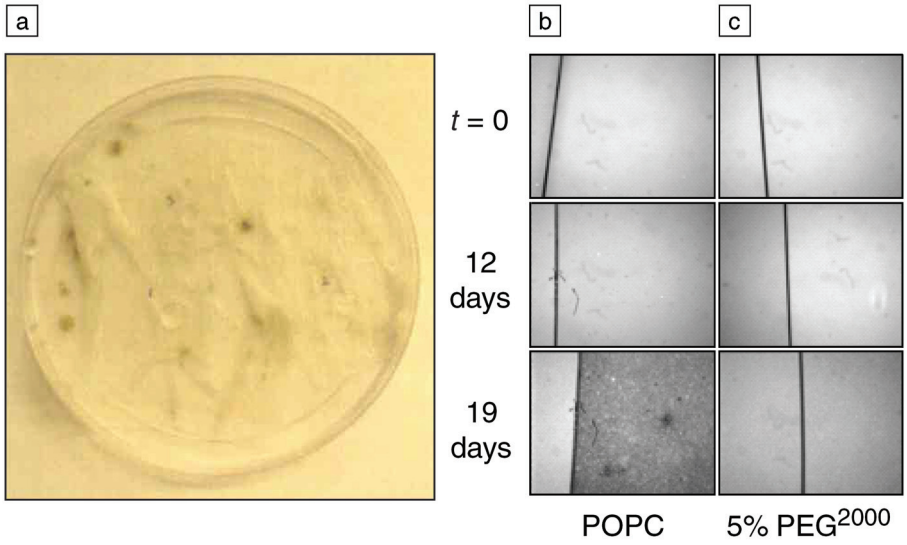

*Figure 5. (a) Unprotected POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) supported bilayer was exposed to mold spores in a petri dish. (b) Initially, the bilayer appeared normal, but after 12 days, mold and/or bacteria seemed to infiltrate the bilayer. After 19 days, the growth was quite substantial and whole quadrants were destroyed. (c) POPC bilayer protected by 5 mol% (molecular weight, 2000) PEG-conjugated lipids. This bilayer showed complete growth inhibition, even after 19 days. Note: the dark lines were scratches purposely made in the bilayer to determine the background fluorescence level and identify different chip regions.*

technologies aimed at solving this problem by trying to create a thicker space between the bottom leaflet and the support have met with limited success, as discussed in a recent review by Tanaka and Sackmann.[59] However, with no universal solution for incorporating transmembrane proteins into solid-supported bilayers, this area is still wide open in terms of new materials development. Recent progress with one class of membrane proteins (G protein-coupled receptors) is further discussed by Fang et al. in this issue of *MRS Bulletin*.

Conclusions

The inclusion of materials such as mimetic cytoskeletons and glycocalyces in supported membranes should make superior devices that will not only better mimic cell membranes, but also substantially improve performance characteristics needed to survive harsh sensing environments. Such requirements could include transport to a remote farm in Southeast Asia, the ability to remain functional upon rehydration after months of dry storage in a desert battlefield, or the ability to resist fouling while strapped to a buoy in the Hudson River for continuous sampling of water quality. Materials that closely resemble what nature has already fabricated are likely to provide an excellent pathway to platform design. It is now the current task of scientists and engineers to create such bioinspired materials.

Acknowledgments

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