Solid supported lipid bilayers\(^1\) possess a unique combination of physical properties, which make them well suited to serve as cell membrane mimics.\(^2\) Chief among these is the two-dimensional fluidity of the individual lipid molecules.\(^6\) Such mobility is crucial for studies of cell signaling, pathogen attack, trafficking of lymphocytes, as well as the inflammatory response.\(^9\) Mobility is required because all these processes involve multivalent ligand–receptor attachment, which relies on the reorganization of cell surface constituents.\(^12\) When fully hydrated, supported lipid bilayers can be employed as sensor platforms; however, these systems are quickly destroyed upon exposure to the air/water interface\(^17\) and therefore must remain underworld at all times. Several attempts have been made to overcome this limitation. Hybrid bilayers,\(^18\) with a bottom leaflet consisting of a self-assembled thiol monolayer on gold and a top leaflet of lipids, can be formed in air and then hydrated. However, the lipids probably reorient during air exposure.\(^19\) Bolaamphiphile monolayers and hybrid bilayers prepared by the Langmuir–Schaefer method were stabilized with a crystalline sheet of S-layer proteins,\(^20\) which strongly chemisorbs to lipids. Such films could be pulled through the air/water interface without disruption, although the lipid mobility was greatly reduced. Cross-linked bilayers prepared by polymerization of synthetic lipids produced air-stable membranes, but also with very low lateral mobility.\(^21\) Furthermore, glass modified with \(\gamma\)-aminopropylsilane has been used as a substrate for bilayers that can be dried and rehydrated.\(^24\) These bilayers possessed some long-range lateral mobility as observed by fluorescence recovery after photobleaching (FRAP). However, the degree of recovery was only 50% even before drying, whereas glass supported bilayers typically recover more than 90% of their original fluorescence intensity.\(^25,26\) It would be highly desirable to create solid supported bilayers which could be insensitive to the air/water interface, yet still maintain complete fluidity. In addition, if these bilayers could be dried and stored, it would substantially increase their utility as sensing platforms. Here, we introduce a step toward the goal of rugged bilayer formation and provide some mechanistic insights into the process.

Solid supported bilayers were prepared by fusing vesicles containing 5 mol % 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (biotin-cap-PE), 0.05 mol % Texas-Red dihexadecanoylphosphatidylethanolamine (TR-DHPE) and ~95 mol % egg phosphatidylcholine (egg-PC) to a four-channel polydimethylsiloxane (PDMS)/glass microfluidic device. Every other supported bilayer was incubated with a solution of 0.25 mg/mL streptavidin in pH 7.2 phosphate buffered saline (PBS) and then rinsed with PBS. While under water, the PDMS channels were peeled off the glass and the bilayers imaged (Figure 1a). The sample was then drawn through the air/water interface five times and re-imaged under water. Figure 1b clearly shows that the bilayers bound with streptavidin were still present, while the unprotected bilayers were completely removed. FRAP measurements revealed that the sample through the air/water interface did not reduce the lateral lipid mobility. When drawn through the interface, a thin layer of water was clearly visible over the lanes that were protected with streptavidin. This water layer clung tenaciously; therefore, during the withdrawal and re-immersion cycles, some bulk water was constantly present. In a separate experiment, bilayers containing the same constituents as above were prepared on glass, bound with streptavidin, and rinsed with PBS. At this point, the sample was dried under a stream of \(\text{N}_2\). Remarkably, the entire bilayer was still present and in excellent condition as seen by fluorescence microscopy. A 13-\(\mu\)m diameter spot was bleached on this bilayer while in air but did not recover. However, when the sample was placed in a high-humidity environment (see Supporting Information (SI)) the bleached spot recovered quite well. Figure 2 shows fluorescence micrographs of a bleached and recovered spot, as well as the associated FRAP curve. The diffusion coefficient of the bilayer in humid air was \(2.9 \times 10^{-9}\) cm\(^2\)/s. When fully rehydrated in bulk aqueous solution (see SI) the diffusion coefficient returned to the more typical value of \(1.9 \times 10^{-8}\) cm\(^2\)/s.

While the exact mechanism by which the protein protects bilayers from destruction is not completely understood, two factors are considered here. First, the close packing of the specifically bound...
streptavidin monolayer may stiffen the bending elastic modulus of the bilayer (Figure 3).27 A separate experiment was performed to examine the effect of lipid density vs streptavidin protection. At low lipid densities significant damage was done to the bilayer upon drawing through the air/water interface (see SI), and the effect of the protein layer is probably maximized at full coverage. We have repeated this experiment with other bound proteins such as IgGs, and it seems that the protein’s identity is not the crucial factor. A mechanism involving a change in the bending modulus would be reminiscent of the forces which lead to the formation of inside-out vesicles when Escherichia coli bacteria are forced through a French press.28–30 In that case, proteins normally on the inside of the bacteria relocate to the outside of the smaller vesicle membranes, presumably due to the mechanical and electrostatic forces between the surface membrane proteins. Similar forces31 may serve to stabilize these supported bilayers upon drying.

Second, it should be noted that in the absence of a protein layer one might expect the lipid molecules in dry bilayers to reorganize to have their alkyl chains point toward the air. This destructive effect is probably avoided by the presence of the protein layer. Indeed, a thin layer of water is likely bound to the protein-coated bilayers, thereby keeping the system partially hydrated even when blown dry with a stream of nitrogen. Therefore, although the bulk water is removed very quickly, surface-bound water, including water which may have penetrated slightly into the membrane, remains behind. This water may later evaporate under sufficiently low-humidity conditions, leaving the bilayer mostly dehydrated but still intact because of the difficulty of reorganizing the alkyl chains in the presence of the protein film. Also, it is interesting to note that the bilayer could not recover in ambient (dry) air but could recover in humid air. This supports the idea that the presence of some interfacial water molecules imparts fluidity.

This method of bilayer preservation requires no substrate modification and can be performed using commercially available reagents. The bilayer retains its property of lateral fluidity, even when removed from bulk water. This has potentially important implications in the field of biosensing. If rugged supported bilayers could be preserved and stored for later rehydration, complex sensor arrays could be manufactured at dedicated facilities and then later employed in the field. Of course, the protective protein layer would need to be removed first. This might be accomplished by the use of a photo or chemically cleavable linker. Another tantalizing possibility is the use of this method to preserve black lipid membranes. Traditionally, these are very delicate systems, which in combination with single ion channels extracted from cell membranes can serve as specific single-molecule sensors.32 Adding a monolayer of specifically bound protein could potentially strengthen BLMs for creating rugged single-molecule sensor platforms.33–36

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Supporting Information Available: Procedures for bilayer preparation, humid FRAP experiments, and rehydration. This material is available free of charge via the Internet at http://pubs.acs.org.

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