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Separation of Membrane-Bound Compounds by Solid-Supported Bilayer Electrophoresis

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Separation, purification, and detection of biomembrane species such as lipids and transmembrane proteins are difficult tasks. The processing conditions are often harsh, which can result in alteration of native structures or complete loss of material.[1,2] Furthermore, it is difficult to detect subtle post-translational changes in these molecules that occur on the cell surface.[3−5] Typical purification procedures often require one to dissolve the membrane in detergent, sonicate, filter through chromatographic columns, and separate into bands using gel electrophoresis. Procedures that circumvent such drawbacks would represent an attractive alternative and could significantly impact transmembrane proteomics.

Herein, we describe a new method to rapidly separate membrane components without exposing the molecules to harsh environments. We employ a solid-supported lipid bilayer (SLB) made of POPC[6] and cholesterol as the separation medium to laterally separate membrane-bound species. This procedure is somewhat analogous to gel electrophoresis, except that the SLB replaces the gel. It is well-documented that membrane components can be manipulated in SLBs using electrophoresis, including lipids,[7−11] vesicles tethered to the bilayer using DNA hybridization,[12,13] and GPI-linked proteins.[14] To conduct separations, however, it is necessary to tune the bilayer chemistry to attenuate the diffusion coefficient of the lipids and, therefore, reduce the diffusive mixing. Cholesterol significantly decreases the lipid diffusion coefficient[15,16] and increases the band resolution one can obtain. As will be shown, this analytical-scale separation technique is powerful enough to separate isomers of fluorescently labeled lipids.

In a first set of experiments, we demonstrate the ability of cholesterol to decrease band broadening during the separation process. We compared the behavior of a band of 1 mol % of Texas Red DHPE[17] lipids migrating in a plain POPC bilayer and in a POPC bilayer with 25 mol % of cholesterol. To begin an experiment, supported bilayers were formed on a planar glass substrate by the vesicle fusion method.[18] Next, a thin line of bilayer (∼80 μm width) was removed using a sharp piece of glass coated with Teflon (see Supporting Information). Vesicles containing POPC and 1 mol % of Texas Red DHPE were then introduced into the aqueous phase above the surface. The vesicles fused to the bare portion of the substrate, creating a thin bilayer strip with Texas Red DHPE in it. A 100 V potential (DC) was applied parallel to the plane of the bilayer. Because the fluorophores were negatively charged, they migrated toward the positive electrode. We monitored the lateral movement and band broadening of the Texas Red bands as a function of time using an inverted epifluorescence microscope (Figure 1).

The bilayer without cholesterol showed substantial band broadening after 30 min of applied voltage (left images). By contrast, the band in the bilayer containing 25 mol % of cholesterol (right images) remained much more compact. Moreover, this band resolved into two distinct chromatographic features with an area ratio of ∼70:30.

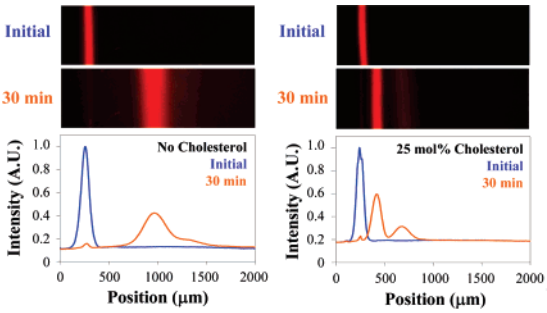

Figure 1. Comparison of the band broadening of Texas Red labeled lipids migrating in either pure POPC (left) or POPC doped with 25 mol % of cholesterol (right). The upper two images are epifluorescence micrographs of the systems immediately after formation of the Texas Red containing strips. The lower two images show band migration after applying 100 V across the sample for 30 min. The positive and negative electrodes were located on the right and left sides of the frame, respectively. The faint line to the left in the bottom panels represents a 2% immobile fraction of lipids. The plots below the images show the corresponding linescans initially and after 30 min. The linescans have been corrected for vignetting and normalized to the fluorescence level of the initial image to account for photobleaching. The length of the images matches the scale of the *x*-axis in the plots.

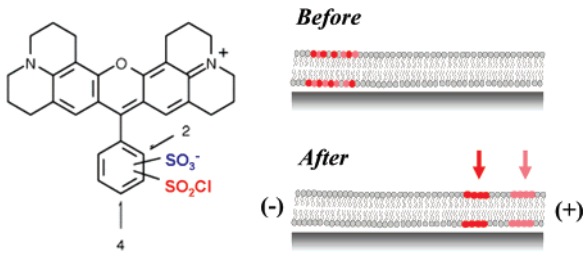

Figure 2. Left: Chemical structure of Texas Red sulfonyl chloride.[22] The lower ring contains the reactive sulfonyl chloride group (red), which can reside at either the 2 (ortho) or 4 (para) position. Right: Illustration of the separation of a mixture of Texas Red DHPE lipid isomers into distinct bands in a supported bilayer.

Mass spectral analysis showed that Texas Red DHPE had a molecular weight of 1381.85 Da and was relatively pure (Supporting Information). It is well-known, however, that the sample should consist of an isomeric mixture[19] (Figure 2, left side). Indeed, phosphatidylethanolamine lipids can conjugate to Texas Red sulfonyl chloride at either the ortho or para positions of the lower aromatic ring. This accounts for the presence of two bands in Figure 1 and is illustrated schematically in Figure 2 (right side).

Two other possible origins for the band splitting need to be considered. First, a difference in the mobilities of the lipids in the upper and lower leaflets might lead to band separation. Also, it is

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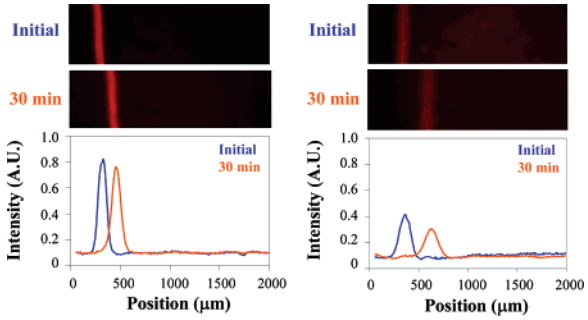

Figure 3. Images of Texas Red DHPE migrating through a 75 mol % of POPC/25 mol % of cholesterol bilayer after TLC purification. Left: Texas Red from the large mol fraction isomer after TLC purification, initially (top) and after 30 min of 100 V applied potential (middle). Right: Corresponding images for the other isomer. The plots below the images show the associated linescans, initially and after 30 min, corrected for vignetting.

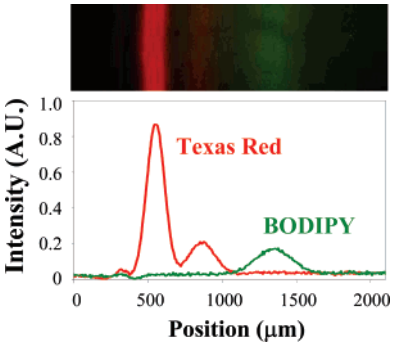

Figure 4. Top: Composite image of the separation of Texas Red DHPE and BODIPY DHPE in a POPC bilayer containing 25 mol % of cholesterol after 35 min of applying a 100 V potential. Bottom: Corresponding linescan of the image, corrected for vignetting. The small peak at ~300 μm represents an approximately 2% immobile fraction of total dye-labeled molecules. The areas of the large and small peaks of Texas Red are ~75 to 25%, which is slightly different than the areas in Figure 1 and probably represents a small amount of batch-to-batch variability in the dye formulation of the manufacturer.

conceivable that the separated bands represent species in different microdomains or lipid rafts.[20,21] We ruled out both of these possibilities by purifying the Texas Red DHPE using thin layer chromatography (see Supporting Information). Each pure lipid isomer was separately mixed into POPC vesicles and fused into supported bilayer strips adjacent to a separation matrix consisting of a bilayer made from 75 mol % of POPC and 25 mol % of cholesterol. As expected, 30 min of electrophoresis gave rise to only one band in each case (Figure 3). As a control, UV−vis spectroscopy was performed on the isolated compounds, as well. These experiments confirmed that the isomers existed in an approximately 70:30 mole ratio (Supporting Information).

In a final set of experiments, we demonstrated that a more complex mixture can be separated. For this purpose, we fused POPC vesicles containing 1 mol % of Texas Red DHPE and 1 mol % of BODIPY DHPE[23] into a bilayer strip as performed above. Figure 4 (top) shows the three bands that were resolved after 30 min of electrophoresis at 100 V. As can be seen, the Texas Red lipid bands move more slowly than the single green BODIPY band. The linescan (bottom) shows that the bands are resolved.

The methods described herein could be extended to the purification and separation of membrane proteins by incorporating an appropriate polymer cushion[24] into our supported bilayer system to ensure two-dimensional mobility of the transmembrane species. Protein bands in the bilayers could be detected by Western blotting with appropriate protein-specific antibodies, or the bilayers could be freeze-dried and imaged via mass spectrometry for label-free detection.[25] Such an advance might even have impact in the analysis of whole cell membranes. In fact, cell membranes could be analyzed to follow protein expression or post-translational modifications.

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Supporting Information Available: Experimental procedures and analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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