Supporting Information

Materials and Methods

Chloroform solvated phospholipids (>99% purity) were purchased from Avanti Polar Lipids, stored at -20°C, and used without further purification. Lipids used in these experiments were 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA), 1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-sn-glycero-3-phosphocholine (NBD-PC), and 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC).

Glass coverslips (22×30 mm) purchased from Fisher Scientific were cleaned in a 7:1 solution of 7X® detergent that had been heated to clarity for 20 minutes. Slides were then rinsed extensively with distilled water, dried with nitrogen, baked for 4 hours at 400 °C and used within 24 hours.

Round-bottom flasks were rinsed consecutively with ethanol, methanol, and then chloroform to remove any water, and appropriate aliquots of each lipid were added to achieve a final concentration of 2.5 mM. The lipid solution was dried to a thin film under nitrogen, and placed under vacuum for 45 minutes. The lipids were rehydrated with buffer containing 50 mM MES, 0.1 mM EDTA, and 250 mM KCl at pH 5.0 to form vesicles. The solution was then extruded through a 50 nm polycarbonate membrane to obtain large unilamellar vesicles (LUVs) of uniform size, and centrifuged at 14,000 rpm for 5 min.

CoverWell perfusion chambers (Invitrogen-Molecular Probes, Inc) were affixed to slides and filled with 60µL of the vesicle solution. After 5 min (to allow for fusion and bilayer formation) the bilayer was rinsed with 3 1-mL aliquots of buffer solution to remove excess lipid material. In all experiments, the perfusion chamber was kept on the slide to allow exchange of the bulk solution above the bilayer.

Bilayers were imaged using a Nikon TE2000-U inverted epi-fluorescence microscope equipped with a Cascade 512B CCD camera. An X-Cite 120 B short arc lamp was used with a FITC HQ filter cube (Chroma) to illuminate the sample. For the fluorescence recovery after photobleaching experiments a silicon avalanche photodiode (APD) Single Photon Counting Module (SPCM-AQR-16-FC,PerkinElmer, Inc., Vaudreuil, Quebec) was used collect and count the emitted fluorescence. A 25 mW Argon ion laser (488 nm Melles Griot), which was attenuated to 250 nW using a 5X (focal transmission of 1x10^5) neutral density filter (NE50B, Thorlabs, Inc., Newton, NJ), was used to illuminate the bilayer. The neutral density filter was removed from the beam path for one second and the bilayer was bleached to background. The neutral density filter was then replaced and the counts monitored.