Supporting Information

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SI Text

The pK_as of DOPG and DOPC SUVs. The pK_as of nonlabeled DOPG and DOPC SUVs were estimated by acid-base titrations. Fig. S1*A* shows a titration curve for DOPG SUVs, indicating a lower pK_a of ∼3 and a higher pK_a of ∼10, in agreement with other reports (1, 2); Fig. S1*B* shows the same curve for DOPC SUVs, with a lower pK_a of ∼3 and a higher pK_a of ∼11. The lower pK_as probably correspond to the protonation of the phosphatic acid groups for the two samples, and the higher is due to the protonation of glycerol and choline, respectively.

Effect of Bulk Ion Concentration on pK_a for Free Oregon Green Fluorophores. The pK_as for Oregon Green fluorophores freely diffusing in an aqueous solution containing 0.90 M NaCl and a pure aqueous solution were determined by a pH titration using a spectrofluorometer (see Fig. S2).

Number of Lipids Per Liposome and Surface Charge Density of DOPG SUVs. The number of fluorescently labeled lipids (DHPE-Fluorescein) per liposome was determined by a series of FCS measurements where the concentration of fluorescently labeled lipids in the membrane was varied between 3.4×10^{-4} mol% and 3.4×10^{-2} mol%. From the linear dependence of the count rate per SUV with increasing fluorophore concentration, the number was determined to be 1/25 fluorescently labeled lipids per SUV for the fluorophore-DHPE to lipid ratio of $1:300,000$ as used in this study. Knowing the concentration of fluorescently labeled SUVs, the number of lipids per SUV is estimated to be $11,800 \pm 500$.

For DOPG SUVs at neutral pH each lipid can be assumed to be negatively charged, due to the low pK_a of the DOPG lipids. Thus, the surface charge density of the membrane equals the surface area of the liposome divided by the number of lipids on each surface. This gives a surface charge density of 1/50 Å^2 for DOPG SUVs.

The average number of protons on the SUV surface for a specific bulk pH can be calculated from the number of lipids per SUV, n_{SUV}, and the pK_a of the lipids as $\frac{n_{\mathrm{SUV}}}{10^{\mathrm{pH}-\mathrm{p}K_a^{\mathrm{lipid}}}}$.

1. Van Dijck PWM, de Kruijff B, Verkleij AJ, van Deenen LLM, and de Gier J (1978) Comparative studies on effects of pH and Ca^{2+} on bilayers of various negatively charged phospholipids and their mixtures with phosphatidylcholine. *Biochimica Et Biophysica Acta* 512(1):84–96.

2. Egorova EM (1998) Dissociation constants of lipid ionizable groups I. Corrected values for two anionic lipids. *Colloid Surface A* 131(1–3):7–18.

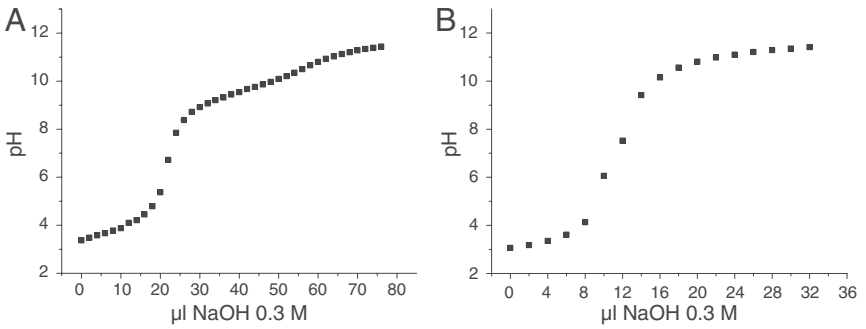

Fig. S1. Titration curves demonstrating the bulk pH response after addition of 0.3 M NaOH in aliquots of 2 µl to a 1.7 ml solution containing DOPG SUVs (*A*) and DOPC SUVs (*B*) in 0.15 M NaCl.

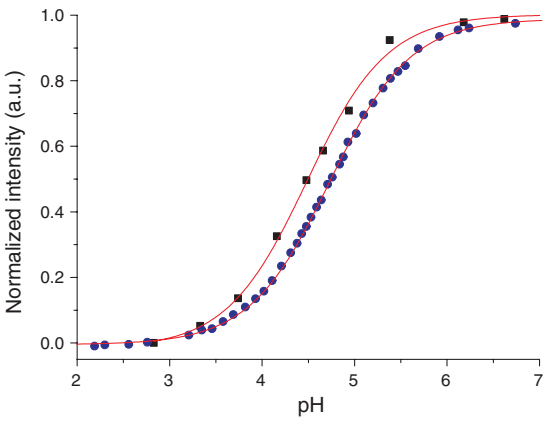

Fig. S2. Normalized fluorescence intensities vs. pH for free Oregon Green in aqueous solution with 0.90 M NaCl (black squares) and a pure aqueous solution (blue circles). Fits to data (red lines) using the Henderson-Hasselbalch equation yields pK_as 4.5 and 4.7 for the 0.90 M NaCl and the pure aqueous solutions, respectively.