Supplementary Information

Figures:

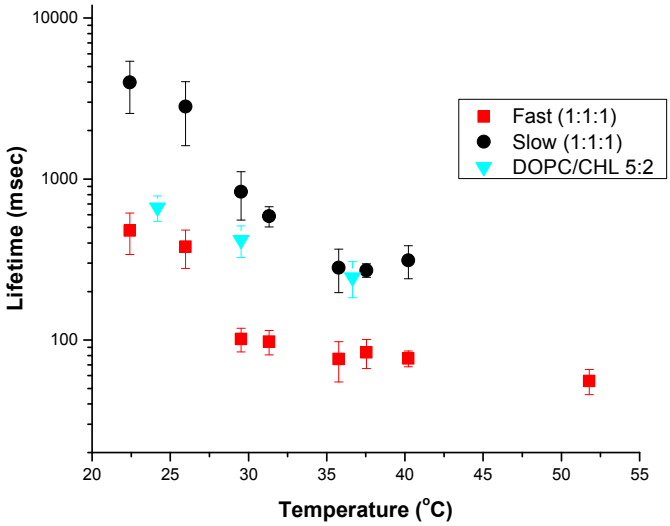

Figure S1: Lifetimes for fast (red squares) and slow (black circles) gramicidin channels in DOPC/SPM/CHL 1/1/1 plotted against temperature. Lifetimes for channels in DOPC/CHL5/2 (blue triangles) are plotted for comparison. Error bars represent standard error. Miscibility transition is approximately 27-28° C.

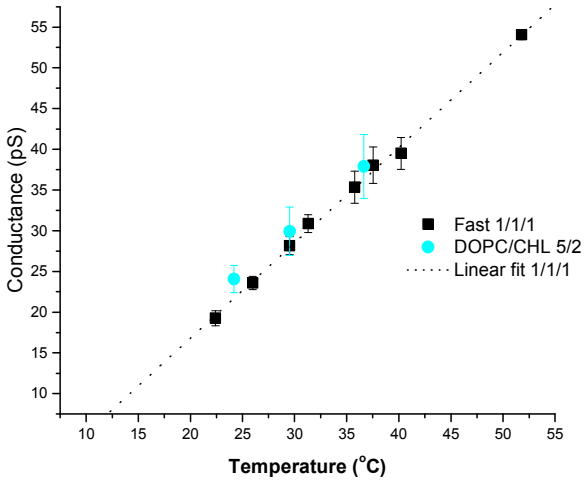

Figure S2: Conductance for fast channels in the 1/1/1 mixture (black squares) and channels in the DOPC/CHL 5/2 mixture (blue circles) plotted against temperature. Dotted line is a linear fit to the fast channels. Error bars represent standard error.

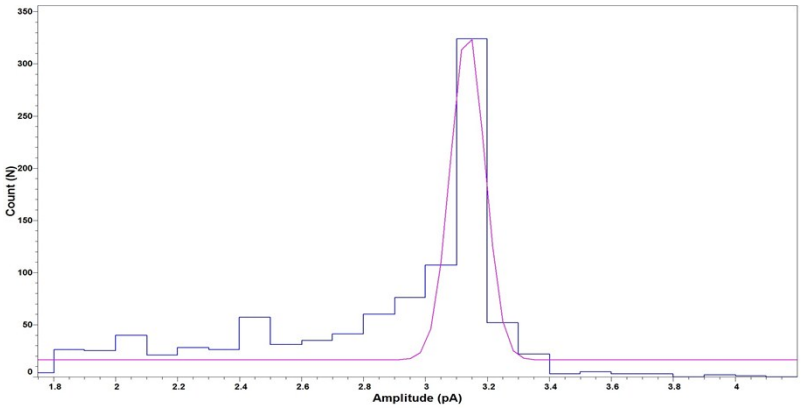

Figure S3: Amplitude histogram for all channels in the 1/1/1 mixture in an experiment at 32° C, 100 mV. Red trace indicates a fit to a single Gaussian. Two Gaussians did not improve the fit even with a more relaxed criterion ($p < .05$). Mean conductance 31 pS.

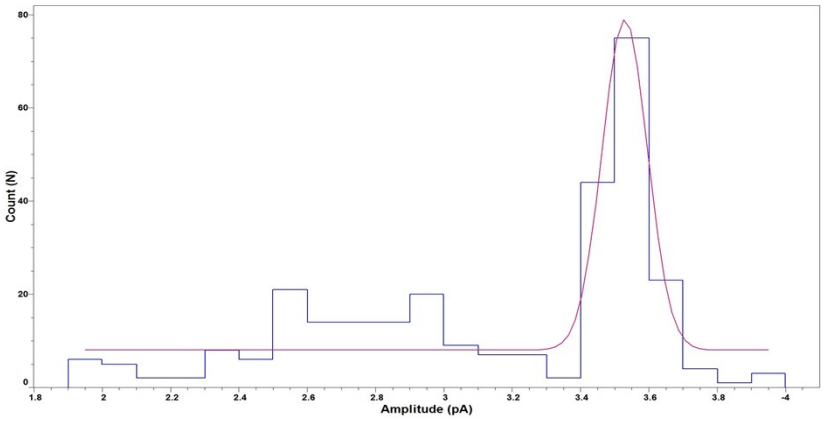

Figure S4: Amplitude histogram for channels in DOPC/CHL 5/2 at 30° C, 100 mV. Red trace is a Gaussian. Mean conductance of 35 pS is significantly higher than that of the 1/1/1 mixture.

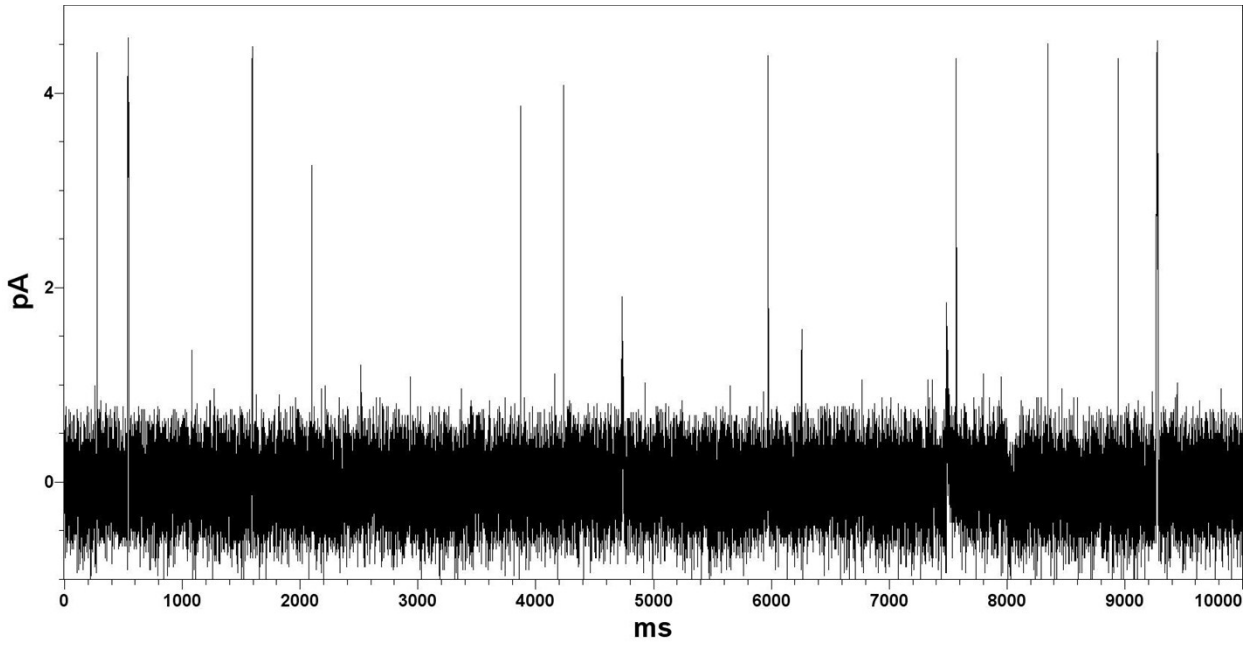

Figure S5: Trace of gramicidin channels inserted into SPM/CHL 5/2 at 42.3 ^{o}C. Gramicidin channels did not appear until the membrane was heated to 42 ^{o}C.

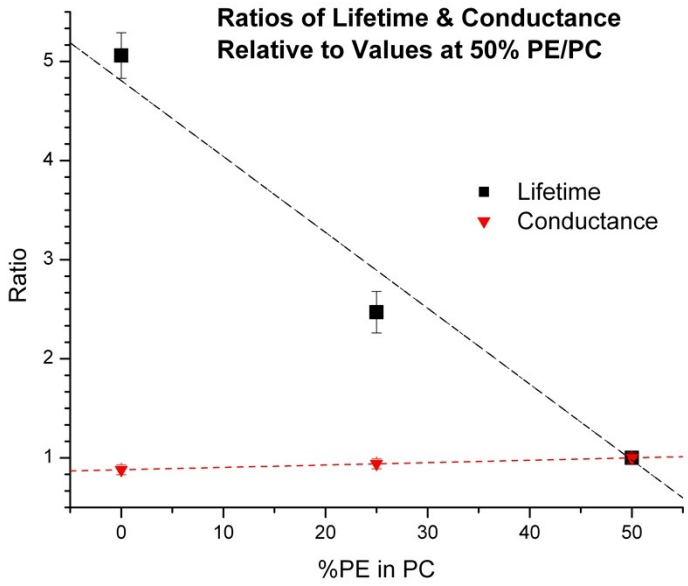

Figure S6: Ratios expressed relative to the values at 50% PE/PC for lifetime and conductance of gramicidin as a function of phosphatidylethanolamine concentration. Dashed lines are linear fits. Experiments at 1 M KCl, 23 ± 1 °C.

Notes:

The proportion of fast conducting channels increases with increasing temperature (r^2 = .55, data not shown); however, there is considerable variation between experiments, and we were not able to maintain the stability of single membranes over a wide enough temperature range to demonstrate significant change in the distribution of channel lifetimes while controlling for membrane variability.

We estimated the maximum number of gramicidin channels in the lipid bilayer as follows: Assuming a 60 μm hole and an average area per lipid of 60 Å^2, there are 6 x 10^9 lipid molecules in each leaflet of the bilayer. Adding 1 μl of a 10^{-9} M solution of gramicidin to each side of the chamber is equivalent to 10^{-15} mol of gramicidin to each side. The ratio of the area of the hole to the area of the lipid air interface is 3.6 x 10^{-5}. Assuming that all of the gramicidin partitions into the monolayers and bilayer, we have 3.6 x 10^{-20} mol of gramicidin in the hole leaflet, or 2 x 10^4 gramicidin molecules. The ratio of gramicidin to lipid is thus 3 x 10^{-6}. Note that the gramicidin in these experiments is not at true equilibrium between the solvent and the membrane, so that it is not possible to use the rate constants derived by Bamberg and Lauger.[1] Moreover, the amount of lipid in the chamber actually exceeds the amount in the monolayers considerably, and we do not know what proportion of gramicidin partitions into the nonlamellar portion of the lipid. Thus, the above estimate constitutes an upper bound for the actual gramicidin concentration in the membrane bilayer.

1. E. Bamberg and P. Lauger, *J Membr Biol*, 1973, **11**, 177-194.