Supporting Information

Partitioning of Fatty Acids into the Lipid Bilayer. Partitioning of oleic acid into a bilayer can be described by an effective dissociation constant of 17 μM at pH 7.2 and 100 mM KCl.[1] The estimated proportion of oleic acid partitioned into a bilayer of DOPC is then 64 % at 30 μM DOPC, 95 % at 300 μM DOPC and 97 % at 600 μM DOPC.

Figure S1 shows quenching of the Trp fluorescence of wild type KcsA by BrSA at DOPC concentrations of 30, 300 and 600 μM; the lower levels of fluorescence quenching observed at 30 μM DOPC and the equal levels of fluorescence quenching observed at 300 and 600 μM DOPC are consistent with partial partitioning of BrSA into the bilayer at low DOPC concentrations but almost total partitioning at DOPC concentrations of 300 μM and above. When the quenching data for 30 μM DOPC are plotted as a function of the bound concentration of BrSA, with correction for partitioning as above, the agreement with the quenching data obtained at high concentrations of DOPC is good (Figure S1). This also suggests that the partitioning of BrSA and oleic acid is similar. Fluorescence quenching experiments to determine binding constants to KcsA were performed at lipid concentrations of 300 μM or above to ensure that all the added fatty acid had partitioned into the membrane.

The partition coefficient K_p for 14-SASL can be estimated from EPR measurements of the partitioning of 14-SASL as a function of pH.[2] As a function of pH, the experimentally observed partition function, K_p, is given by:[2]

$$K_p = K_{LH}\frac{10^{-pH} + 10^{-pK_a^i}}{10^{-pH} + 10^{-pK_a^o}} \qquad (1)$$

where K_{LH} is the partition coefficient of the protonated fatty acid, and $pK_a^i = 7.0$ and $pK_a^o = 4.85$ are the pK_as of the fatty acid in the membrane and in bulk water, respectively.

For spin-labelled myristic acid $K_{LH}\bar{v}_L = 2570$ ml/g^2 which extrapolated to stearic acid with four additional CH_2 groups becomes ~ $2.6.10^5$ ml/g (see ref. 3). Hence from eq 1, $K_p\bar{v}_L \approx$ 3000 ml/g for partitioning of 14-SASL at pH 7.2.

REFERENCES

(1) Froud, R. J., East, J. M., Rooney, E. K., and Lee, A. G. (1986) Binding of long-chain alkyl derivatives to lipid bilayers and to (Ca^{2+}-Mg^{2+})-ATPase. *Biochemistry 25*, 7535-7544.

(2) Miyazaki, J., Hideg, K., and Marsh, D. (1992) Interfacial ionization and partitioning of membrane-bound local anaesthetics. *Biochim. Biophys. Acta 1103*, 62-68.

(3) Cevc, G. and Marsh, D. (1987) *Phospholipid bilayers. Physical principles and models* Wiley-Interscience, New York.

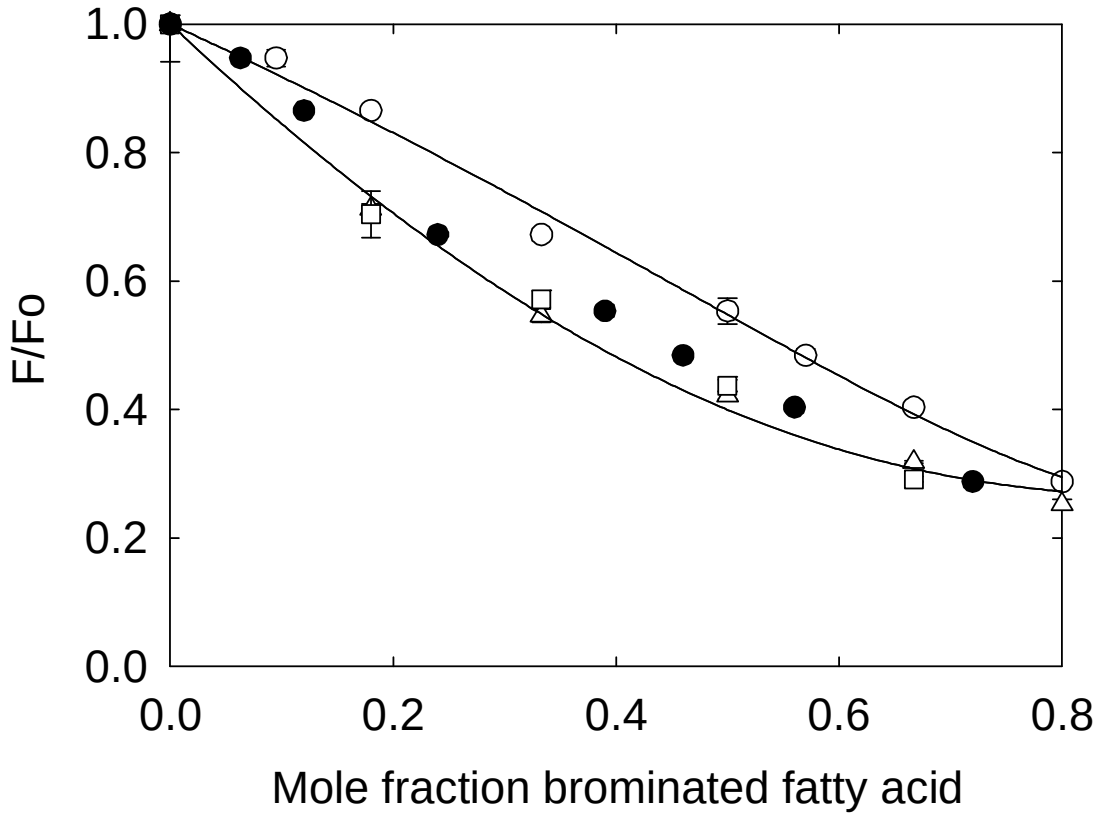

Figure S1. Effect of lipid concentration on fluorescence quenching of wild type KcsA by BrSA. KcsA was reconstituted into mixtures of DOPC and BrSA, and fluorescence intensities were expressed as F/F_o where F_o is the fluorescence intensity in DOPC and F is the fluorescence intensity at the given mole fraction of BrSA. Experiments were performed at DOPC concentrations of 30 (○), 300 (Δ) and 600 (□) μM, at molar ratios of DOPC:KcsA monomer of 100:1 (○) and 1000:1 (Δ, □). The buffer was 20 mM Hepes, 100 mM KCl, pH 7.2. Also shown are the data recorded at 30 μM DOPC corrected for partitioning of BrSA into the membrane, as described in the text (●).