Determining Fatty Acid – Membrane Partition Coefficients

Synopsis
ADIFAB can be used to determine the partition coefficient of a fatty acid between a membrane phase and aqueous solution. Simply add fatty acid to a cuvette containing ADIFAB and a membrane and measure the fluorescence ratio (505/432 upon excitation at 386 nm).

Procedure
For details on measuring the ADIFAB ratio and calculating [FFA] see Determining the ADIFAB Ratio. To determine R_0, add ADIFAB and a membrane of known lipid concentration to a cuvette containing measuring buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, at pH 7.4), and measure the fluorescence ratio (505/432 nm). Titrate the solution with fatty acid aliquots of known concentration and measure the R value after each addition—be sure to wait at least 5-10 minutes for equilibrium before measuring R. For each R measured, calculate the partition coefficient, K_p, using Eq. (1):

$$K_p = \frac{\frac{V_a}{V_m} \bullet ([FA]_{total} - [FFA])}{[FFA]} \quad (1)$$

where $[FA]_{total}$ is the *total* fatty acid concentration in the cuvette after each addition, [FFA] is the *free* fatty acid concentration, and V_a and V_m are the volumes of the aqueous and membrane phases, respectively. (Note: to accurately report $[FA]_{total}$, measure the concentration of the fatty acid stock according to Determining the Concentration of Fatty Acid in an Aqueous Solution.) With sufficient membrane present, no correction for wall binding to the cuvette walls is necessary because for typical conditions >95% of the fatty acid will be bound to the membrane; very little will be free, bound to ADIFAB or bound to the walls.

Example
In a cuvette containing 2 ml buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, at pH 7.4 and 37°C), 100 μM egg phosphatidylcholine vesicles (EPC) and 0.2 μM ADIFAB, the R_0 was measured and found to be 0.279. 3 μM of sodium palmitate was added, and after waiting 10 minutes for equilibrium, the R value was measured and found to be 0.335. Using the R value to determine that [FFA] = 88.6 nM and substituting V_a/V_m = 10000 and $[FA]_{total}$ = 3 μM into Eq. (1), K_p was calculated to be 3.29×10^5. Additional aliquots of sodium palmitate were added and after each addition R values were measured and K_p values were calculated. The average value of K_p for the titration was 3.47×10^5. The complete set of R, [FFA] and K_p values are listed in Table 1.

Table 1. Titration data from palmitate and EPC vesicles K_p determination.

$[FA]_{total}$ (uM)	Measured R Value	[FFA] (nM)	$K_p \times 10^{-5}$
0	0.279	0	
3	0.427	89	3.29
5	0.516	143	3.40
7	0.606	199	3.42
10	0.718	270	3.60
15	0.920	402	3.63
20	1.150	558	3.48
25	1.345	696	3.49
		average =	3.47