Determining Fatty Acid – Membrane Partition Coefficients

Synopsis

ADIFAB2 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 ADIFAB2 and a membrane and measure the fluorescence ratio (550/457 upon excitation at 375 nm).

Procedure

For details on measuring the ADIFAB2 ratio and calculating [FFA] see Determining the ADIFAB2 Ratio. To determine R_0, add ADIFAB2 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 (550/457 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 ADIFAB2 or bound to the walls.

Example

For an example experiment using *ADIFAB* see Determining Fatty Acid – Membrane Partition Coefficients:ADIFAB.