Determining ADIFAB Fatty Acid Dissociation Constants: K_d

Synopsis
This protocol outlines how to calibrate ADIFAB for a particular fatty acid in order to determine the binding constant K_d. ADIFAB K_d values for some common fatty acids are listed in Determining the ADIFAB Ratio.

Procedure
For details on measuring the ADIFAB ratio and calculating [FFA] see Determining the ADIFAB Ratio. To determine R_0, add 0.2 μM ADIFAB to a cuvette containing buffer, and measure the fluorescence ratio (505/432 upon excitation at 386 nm). Titrate the cuvette with known concentrations of FA (to measure the concentration of the FA stock see Determining the Concentration of Fatty Acid in an Aqueous Solution) and measure R after each addition—be sure to allow 5 – 10 minutes for equilibrium before measuring R. Continue the titration until R decreases or no longer significantly changes with additional fatty acid aliquots. Plot R vs. [FA] and fit this titration curve with Eq. (1) by the method of least squares:

$$R = R_0 + \frac{(R_{max} - R_0) \bullet (Q \bullet \sqrt{FA^2 + 2 \bullet FA \bullet (K_d - AD) + K_d^2 + AD \bullet (2 \bullet K_d + AD)} + FA \bullet (Q - 2) - Q \bullet (K_d + AD))}{2 \bullet (FA \bullet (Q - 1) + K_d \bullet Q^2 - Q \bullet (K_d + AD))} \quad (1)$$

or, written linearly for ease of plugging into a fitting program:

R = Ro-((Ro-Rm)*(Q*(FA^2+2*FA*(Kd-AD)+Kd^2+AD*(2*Kd+AD))^0.5+FA*(Q-2)-Q*(Kd+AD))/(2*(FA*(Q-1)+Kd*Q^2-Q*(Kd+AD)))) (1)

where:
R = measured ADIFAB ratio (505/432 upon excitation at 386 nm)—from titration data
R_0 = ADIFAB ratio in the completely unbound state (with no FA present)—from titration data
R_{max} = ADIFAB ratio in the completely bound state (saturated with FA)—hold R_{max} constant at 11.5
Q = intensity of ADIFAB at 432 nm in the *unbound* state (no fatty acid present) divided by the intensity at 432 nm in the *bound* state (completely saturated with fatty acid0—hold Q constant at 19.5
FA = total fatty acid concentration—from titration data, correct for wall binding (see Determining Wall Binding)
K_d = ADIFAB dissociation constant—allow K_d to vary
AD = ADIFAB concentration—hold constant at 0.2 μM

Notes

- K_d is dependent on buffer conditions—changes in pH, temperature and ionic strength will alter K_d.
- R_{max} and Q cannot be determined from this experiment because fatty acid aggregates at concentrations lower than ADIFAB saturation. When the fatty acid begins to aggregate, R will no longer increase with FA, even though ADIFAB is not fully saturated, because ADIFAB binds only monomeric fatty acids. Values of R_{max} = 11.5 and Q = 19.5 have been calculated numerically from titration data, and we recommend that these values be used in all ADIFAB data analysis.

Example

Example experiment coming soon.