Determining ADIFAB2 Fluorometric Constants: K_d, Q, and R_{max}

Synopsis
This protocol outlines how to calibrate ADIFAB2 for a particular fatty acid in order to determine the fluorometric constants K_d, Q, and R_{max}. ADIFAB2 constants for some common fatty acids are listed in Determining the ADIFAB2 Ratio.

Procedure
For details on measuring the ADIFAB2 ratio and calculating [FFA] see Determining the ADIFAB2 Ratio. To determine R_0, add 0.5 μM ADIFAB2 to a cuvette containing buffer, and measure the fluorescence ratio (550/457 upon excitation at 375 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 ADIFAB2 ratio (550/457 upon excitation at 375 nm)—from titration data
R_0 = ADIFAB2 ratio in the completely unbound state (with no FA present)—allow R_0 to vary
R_{max} = ADIFAB2 ratio in the completely bound state (saturated with FA)—hold R_{max} constant
Q = intensity of ADIFAB2 at 457 nm in the *unbound* state (no fatty acid present) divided by the intensity at 457 nm in the *bound* state (completely saturated with fatty acid), calculate Q from the titration data by dividing I_{457} of the R_0 by I_{457} of the R_{max}—hold Q constant at calculated value
FA = total fatty acid concentration—from titration data, correct for wall binding (see Determining Wall Binding)
K_d = ADIFAB2 dissociation constant—allow K_d to vary
AD = ADIFAB2 concentration—hold constant at 0.5 μM

Notes

- K_d is dependent on buffer conditions—changes in pH, temperature and ionic strength will alter K_d.