Determining the Fatty Acid Binding Affinity of a Protein

Synopsis
ADIFAB2 can be used to determine FA binding constants to any unlabeled protein by monitoring *free* fatty acid levels as a solution of ADIFAB2 and protein is titrated with fatty acid.

Procedure
For details on measuring the ADIFAB2 ratio and calculating [FFA] and [$ADIFAB2_{bound}$] see Determining the ADIFAB2 Ratio. To determine R_0, measure the fluorescence ratio (505/432) of a cuvette containing ADIFAB2, the protein of interest and measuring buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, at pH 7.4). Titrate the solution with small 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 amount of fatty acid bound to the protein using Eq. (1):

$$[\text{Protein}]_{\text{bound}} = [\text{FA}]_{\text{total}} - [\text{FFA}] - [\text{ADIFAB}]_{\text{bound}} \quad (1)$$

where $[FA]_{total}$ is the *total* fatty acid concentration in the cuvette after each addition, [FFA] is the *free* fatty acid concentration and [$ADIFAB2_{bound}$] is the concentration of fatty acid *bound to ADIFAB2*. (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.) In the case of single-affinity binding sites, analyze the data using the Scatchard method, Eq. (2):

$$\frac{\frac{[\text{Protein}]_{\text{bound}}}{[\text{Protein}]_{\text{total}}}}{[\text{FFA}]} = \frac{n}{K_d'} - \frac{1}{K_d'} \bullet \frac{[\text{Protein}]_{\text{bound}}}{[\text{Protein}]_{\text{total}}}$$

where $[Protein]_{total}$ is the concentration of binding protein added, *n* is the number of fatty acid binding sites per protein monomer, and K_d' is the binding affinity of the fatty acid to the protein. Plotting the data as $[FA]_{bound}/[Protein]_{total}/[FFA]$ vs. $[FA]_{bound}/[Protein]_{total}$ yields a straight line with a slope equal to $-1/K_d'$ and a x-axis intercept equal to *n*. For multiple binding sites of different affinities, the Scatchard plot is nonlinear.

Example
For an example experiment using *ADIFAB* see Determining the Fatty Acid Binding Affinity of a Protein:ADIFAB.