Determining the Fatty Acid Binding Affinity of a Protein

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

Procedure
For details on measuring the ADIFAB ratio and calculating [FFA] and [$ADIFAB_{bound}$] see Determining the ADIFAB Ratio. To determine R_0, measure the fluorescence ratio (505/432) of a cuvette containing ADIFAB, 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):

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

where $[FA]_{total}$ is the *total* fatty acid concentration in the cuvette after each addition, [FFA] is the *free* fatty acid concentration and [$ADIFAB_{bound}$] is the concentration of fatty acid *bound to ADIFAB*. (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):

$$\frac{\frac{[FA]_{bound}}{[Protein]_{total}}}{[FFA]} = \frac{1}{K_d'} \bullet \frac{[FA]_{bound}}{[Protein]_{total}} + \frac{n}{K_d'} \quad (2)$$

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.

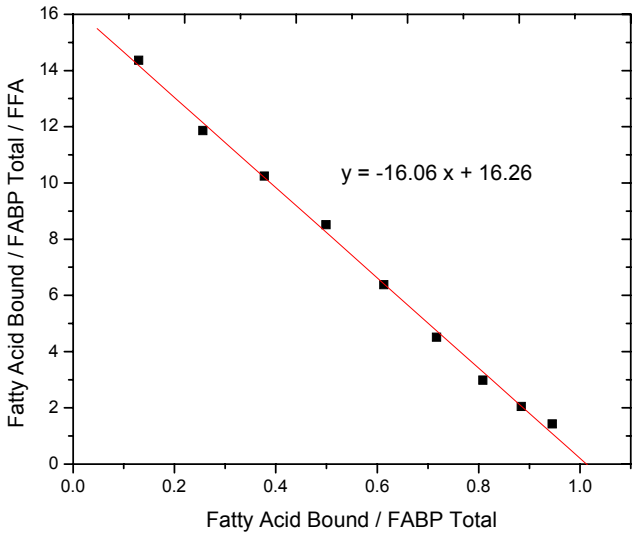
Example
In order to determine the affinity of oleic acid to murine adipocyte fatty acid binding protein (mAFABP), 4.0 μM of AFABP and 0.2 μM ADIFAB were added to a cuvette containing 1.5 ml buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, at pH 7.4 and 37°C). The R_0 value was measured and found to be 0.2671. Sodium oleate

was added so that the OA concentration in the cuvette was 0.53 μM, and after waiting 10 minutes for equilibrium, the R value was measured and found to be 0.2855. Additional aliquots of sodium oleate were added and after each addition R values were measured. The results and analysis of these measurements are listed in Table 1. A Scatchard plot (Fig.1) of this data (column G vs. column F) resulted in a straight line with a slope of –16.06 ($-1/K_d'$) and an x-intercept of 1.01 (*n*). Therefore the oleic acid binding affinity of mAFABP is 62 nM at a single binding site.

Table 1. Analysis of ADIFAB measurements to determine the affinity of oleic acid and murine adipocyte FABP. All concentrations in μM.

Column A	*Column B*	*Column C*	*Column D*	*Column E*	*Column F*	*Column G*
[FA] Added	Measured R Value	[FFA]	$[ADIFAB]_{bound}$	$[FA]_{bound}$	$\frac{[FA]_{bound}}{[mAFABP]_{total}}$	$\frac{\frac{[FA]_{bound}}{[mAFABP]_{total}}}{[FFA]}$
0	0.2671	0				
0.053	0.2855	8.96E-03	6.20E-03	0.515	0.129	14.368
1.06	0.3113	2.16E-02	1.43E-02	1.024	0.256	11.870
1.57	0.3424	3.68E-02	2.33E-02	1.510	0.377	10.244
2.09	0.3865	5.87E-02	3.46E-02	1.997	0.499	8.510
2.6	0.4612	9.60E-02	5.11E-02	2.453	0.613	6.387
3.1	0.5846	1.59E-01	7.24E-02	2.869	0.717	4.516
3.6	0.7977	2.71E-01	9.83E-02	3.231	0.808	2.984
4.09	1.09	4.32E-01	1.21E-01	3.537	0.884	2.049
4.58	1.477	6.59E-01	1.40E-01	3.781	0.945	1.434

Figure 1: Scatchard plot of OA binding to mAFABP.


y = -16.06 x + 16.26
Fatty Acid Bound / FABP Total / FFA
Fatty Acid Bound / FABP Total
0
2
4
6
8
10
12
14
16
0.0
0.2
0.4
0.6
0.8
1.0