Homework Set #8

6:27

The dissociation constant can be obtained by plotting $Y/[L]$ vs Y. The slope is $-1/K_d$.

L and Y are obtained using the following relations:

$$[L] = [Ca^{2+}]_{total} - [Ca^{2+}]_{bound}$$

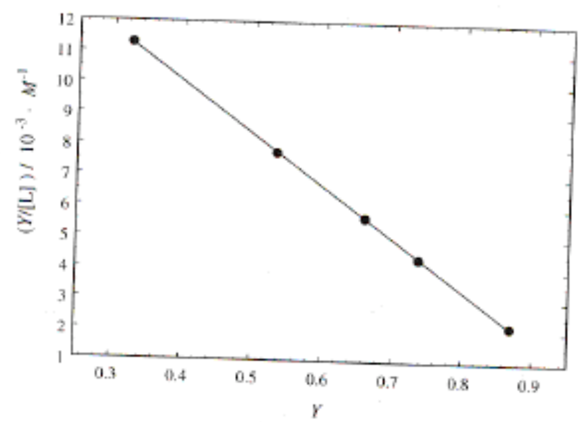
$$Y = \frac{[Ca^{2+}]_{bound}}{96\ \mu M}$$

According to the data given above, the values of [L], Y, and $Y/[L]$ are

$[L]/\mu M$	28.8	68.8	116.6	169.2	396.6
Y	0.3250	0.5333	0.6604	0.7375	0.8688
$(Y/[L])/10^{-3} \cdot M^{-1}$	11.28	7.751	5.664	4.359	2.191

The slope of the plot is -16.72×10^{-3}. Therefore,

$$K_d = \frac{1}{16.72 \times 10^{-3}} = 59.8$$



6.28 An equilibrium dialysis experiment showed that the concentrations of the free ligand, bound ligand, and protein are $1.2 \times 10^{-5}\ M$, $5.4 \times 10^{-6}\ M$, and $4.9 \times 10^{-6}\ M$, respectively. Calculate the dissociation constant for the reaction $PL \rightleftharpoons P + L$. Assume there is one binding site per protein molecule.

Since there is one binding site per protein molecule, $[PL] = [L]_{bound}$.

$$K_d = \frac{[P][L]}{[PL]} = \frac{(4.9 \times 10^{-6})(1.2 \times 10^{-5})}{5.4 \times 10^{-6}} = 1.1 \times 10^{-5}$$

6.46 The following data show the binding of Mg^{2+} ions with a protein containing n equivalent sites:

$[Mg^{2+}]_{total}/\mu M$	108	180	288	501	752
$[Mg^{2+}]_{free}/\mu M$	35	65	115	248	446

Apply the Scatchard plot to determine n and K_d. The protein concentration is 98 μM.

The Scatchard plot follows from the equation

$$\frac{Y}{[L]} = \frac{n}{K_d} - \frac{Y}{K_d}$$

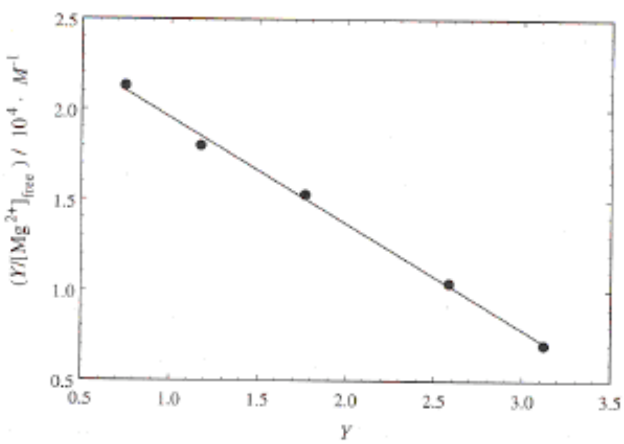
and is a plot of $\frac{Y}{[L]}$ vs Y, where

$$Y = \frac{[Mg^{2+}]_{bound}}{[P]}$$

$$[L] = [Mg^{2+}]_{free} = [Mg^{2+}]_{total} - [Mg^{2+}]_{bound}$$

The slope of the plot is $-\frac{1}{K_d}$ and the intercept is $\frac{n}{K_d}$. The relevant data are given in the table below.

$[Mg^{2+}]_{total}/\mu M$	108	180	288	501	752
$[Mg^{2+}]_{free}/\mu M$	35	65	115	248	446
$[Mg^{2+}]_{bound}/\mu M$	73	115	173	253	306
Y	0.745	1.173	1.765	2.582	3.122
$\frac{Y}{[Mg^{2+}]_{free}}/10^4 \cdot M^{-1}$	2.13	1.80	1.53	1.04	0.700



The equation for the best fit line to the data is $y = -5.86 \times 10^3 x + 2.54 \times 10^4$. Therefore,

$$K_d = -\frac{1}{\text{slope}} = -\frac{1}{-5.86 \times 10^3} = 1.71 \times 10^{-4} = 1.7 \times 10^{-4}$$

$$n = (\text{intercept})\,(K_d) = \left(2.54 \times 10^4\right)\left(1.71 \times 10^{-4}\right) = 4.34 \approx 4$$