Bio 5312 January 29, 2016
Problem Set-3 **due Feb.5**
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1. The following equilibrium binding data (<X> = moles of ligand bound per moles of total protein) were obtained for the binding of a ligand, X, to a protein. The titration data were obtained at a total protein concentration of 5 μM.

X_T [total ligand] (μM)	<X>
2.0 μM	0.40
2.4	0.48
2.9	0.56
3.5	0.71
4.2	0.84
5.1	1.05
6.2	1.20
7.4	1.46
8.9	1.77
10.8	2.10
13.0	2.58
15.7	3.07
18.9	3.55
22.8	3.92
27.5	3.95
33.2	4.0
40.0	3.98

a.) Plot these data in the following forms: <X> vs. X_T and <X> vs. log X_T.

b.) Use an "n independent and identical binding sites" model to analyze these data. Estimate the number of binding sites, n, and the equilibrium association constant, k, by simulating a series of theoretical binding isotherms (varying n and k) and comparing these to the experimental isotherm. **DO NOT use non-linear least squares to analyze the data in this exercise.**

c.) The following set of data were obtained for the same system, under the same solution conditions, but at a total protein concentration of 10 nM. Using these data and the same "n independent and identical sites model", estimate the number of binding sites, n, and the equilibrium association constant, k, by simulating a series of theoretical binding isotherms (varying n and k) and comparing these to the experimental isotherm.

X_T [total ligand] (nM)	<X>
10	0.27
13.3	0.36
17.8	0.44
23.7	0.63
31.6	0.76
42.1	0.98
56.2	1.2
75	1.5
100	1.75
133	2.1
178	2.35
237	2.75
316	2.95
421	3.14
562	3.3
750	3.5
1000	3.64

d.) Explain why one set of data has advantages for determining the equilibrium binding constants for this system and why one set of data is more useful for determining the stoichiometry (n)?

2. The intrinsic tryptophan fluorescence of a protein, P, decreases upon binding a ligand, X. The following two sets of titration data (percent fluorescence quenching *vs*. total ligand concentration) were collected for the binding of X to P at two different total protein concentrations.

[total protein]=0.274 μM	
total ligand concentration (M)	Quenching
6.30×10^{-8}	0.05
8.51×10^{-8}	0.09
1.12×10^{-7}	0.15
1.90×10^{-7}	0.20
2.95×10^{-7}	0.30
3.71×10^{-7}	0.35
4.46×10^{-7}	0.42
5.24×10^{-7}	0.46
5.89×10^{-7}	0.49
6.76×10^{-7}	0.52
8.31×10^{-7}	0.57
1.04×10^{-6}	0.615
1.25×10^{-6}	0.65
1.44×10^{-6}	0.67
1.99×10^{-6}	0.70
2.51×10^{-6}	0.73
4.36×10^{-6}	0.795
6.30×10^{-6}	0.82
8.31×10^{-6}	0.835
1.17×10^{-5}	0.85
1.51×10^{-5}	0.87
1.86×10^{-5}	0.88

[total protein] =0.945 μM	
total ligand concentration (M)	Quenching
7.24×10^{-8}	0.025
1.41×10^{-7}	0.05
2.08×10^{-7}	0.070
2.81×10^{-7}	0.100
3.54×10^{-7}	0.125
4.16×10^{-7}	0.145
4.89×10^{-7}	0.16
6.60×10^{-7}	0.21
8.31×10^{-7}	0.27
9.55×10^{-7}	0.30
1.12×10^{-6}	0.35
1.32×10^{-6}	0.40
1.54×10^{-6}	0.45
1.78×10^{-6}	0.50
1.99×10^{-6}	0.52
2.18×10^{-6}	0.57
2.69×10^{-6}	0.63
3.16×10^{-6}	0.68
4.27×10^{-6}	0.73
5.01×10^{-6}	0.75
7.08×10^{-6}	0.795
8.91×10^{-6}	0.82
1.26×10^{-5}	0.85
1.58×10^{-5}	0.86
1.99×10^{-5}	0.87

Analyze these data to determine the correlation between protein fluorescence quenching and <X> and estimate the total number of sites for binding of X to P.

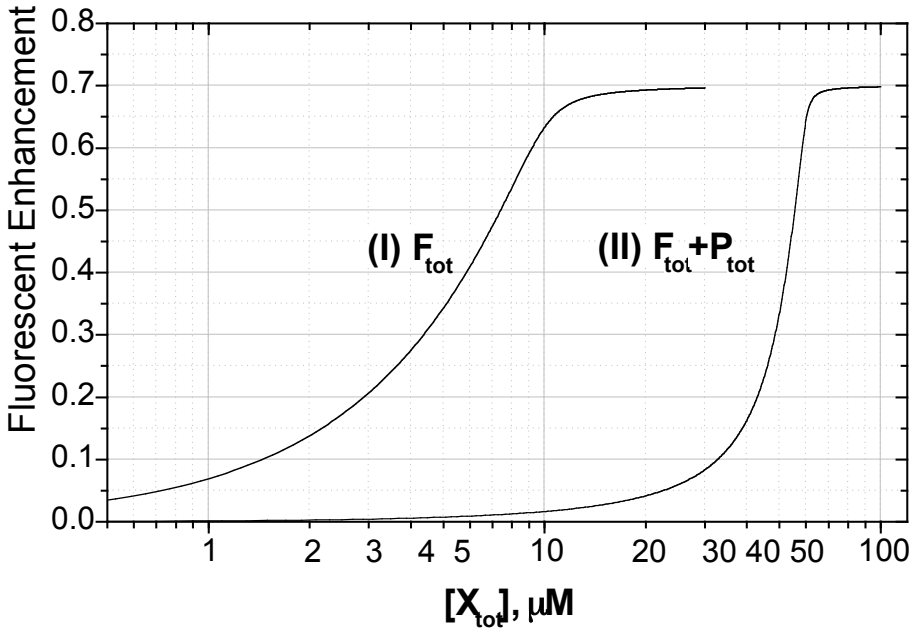
3. The following equilibrium binding data were collected for a ligand, X, binding to a protein, P (at 1 μM total protein concentration). Determine a binding model that provides a good description of this ligand-protein binding system. Choose between an n-independent and identical sites model and an n-independent and non-identical sites model. Use non-linear least squares analysis to estimate the number of sites as well as the equilibrium binding constant(s) for this system along with the 68% confidence limits.

<X>	Xtotal (μM)
0.127	0.10
0.131	0.142
0.163	0.203
0.303	0.289
0.450	0.411
0.648	0.585
0.70	0.833
1.02	1.19
1.10	1.69
1.27	2.40
1.44	3.42
1.62	4.87
1.69	6.93
1.85	9.87
1.82	14.0
1.87	20.0

4. A protein, P, binds multiple ligands, X, without affecting its oligomeric state, although no spectroscopic signal change accompanies binding. However, the protein can be labeled fluorescently to form a modified version, F, which displays a fluorescent enhancement upon binding X. Shown below are plots of the normalized fluorescent enhancement, E, (normalized per F_{tot}) obtained from two titrations:

Curve (**I**) is a titration of the fluorescent protein, F (at total concentration, F_T= 10 μM) as a function of total ligand concentration, X_{tot}.

Curve (**II**) is a titration of a mixture of F (at the same total concentration as in **I**, F_{tot}= 10 μM) and the non-fluorescent wt protein, P (at total concentration, P_{tot} = 50 μM).



a) From these data calculate the value of $\langle X \rangle_P$ = average moles of ligand bound per mole of wt protein for the point at a Fluorescent Enhancement of 0.35 in titration **II**.

b) Briefly describe the basis for your calculation. This should include equations.