Bio 5312 Exam 2 -Spring 2014
Due Friday, March 21 before class
Start the answer for each question on a new page.

1. (25 points) A DNA binding protein, L, can bind to a specific DNA sequence that is 35 base pairs long, with equilibrium site binding constant K_S. However, it can also bind non-specifically to the DNA with equilibrium intrinsic (site) binding constant K_N. In the non-specific binding mode the protein occludes 20 base pairs and can bind with cooperativity, described by an equilibrium cooperativity parameter, ω. Experiments are performed to measure the binding isotherm for this protein to an oligodeoxynucleotide duplex DNA that is 40 bp long. The 35 bp site for specific binding is contained within the 40 bp DNA.

A.) Write an expression for the Binding Polynomial for protein binding to the oligodeoxynucleotides, explicitly including all statistical factors.

B.) Obtain expressions for the average number of proteins bound per DNA molecule in both the specific binding mode, $\langle L \rangle_S$, and the non-specific binding mode, $\langle L \rangle_N$.

C.) Obtain an expression for the fraction of protein that is bound, L_{bound}/L_{total}.

D.) What conditions (relative concentrations of DNA and protein), do you expect would favor specific binding? Why?

E.) Given: $K_S = 1.6 \times 10^8\ M^{-1}$, $K_N = 1.6 \times 10^5\ M^{-1}$, and $\omega = 1 \times 10^5$, at 0.1 M NaCl, simulate the expected binding isotherm and plot this in Scatchard form, $\langle L \rangle / L_f$ vs. $\langle L \rangle$. Discuss the shape of the plot and explain why it has this shape even though the protein binds with high cooperativity.

F.) How might you modify the DNA used in the above experiments to increase the population of protein bound to its specific site relative to non-specific binding? Will this modification likely affect the values of K_S and K_N?

2. (15 points) Duplex DNA can undergo a transition from a right-handed B-form (10.5 base pairs per 36 Å) to a left-handed Z form (12 base pairs per 45 Å). The two forms are in equilibrium, described by equilibrium constant $K_{obs}=[Z]/[B]$, and high (molar) monovalent salt concentrations facilitates the transition from B DNA to Z DNA. Other experiments show that increasing concentrations of sucrose (a neutral solute) also facilitates the transition from B form to Z form.

A.) Based solely on considerations of the axial charge spacing of B-form and Z-form DNA, and using counterion condensation theory, what would you predict to be the effect of increasing [NaCl] on the stability of Z-form, relative to B-form DNA? Explain this and make quantitative predictions based on counterion condensation theory of polyelectrolytes.

B.) Under one set of solution conditions, the midpoint of the B to Z transition occurs at 2.5 M NaCl and $(d\log K_{obs}/d\log a_{\pm}) = 4.3$. Provide a qualitative explanation of these observations including why increasing sucrose also facilitates the transition to the Z form.

C.) Provide a quantitative analysis of the value of $d\log K_{obs}/d\log a_{\pm} = 4.3$.

3. (40 points) The equilibrium binding of a ligand (L) to a protein (P) was examined by labeling the protein with a fluorophore and monitoring the enhancement of the fluorescence intensity of the labeled protein upon binding L. Two titrations were performed at protein concentrations of 2 nM and 20 nM and the resulting data are given in the Table below. The fractional change in fluorescence intensity, $\Delta F_{obs} = (Fi - Fo)/Fo)$, where Fo is the fluorescence intensity in the absence of ligand and Fi is the fluorescence intensity measured at each total ligand concentration (L_{tot}).

L_{tot} (nM)	ΔF_{obs} (P = 2 nM)	ΔF_{obs} (P = 20 nM)
0	0	0
0.2	0.025454	0.006608
0.37	0.046333	0.012205
0.7	0.084964	0.023018
1.2	0.139023	0.039266
2.3	0.241323	0.074434
3.1	0.303626	0.099496
4.2	0.375962	0.133228
5.7	0.45519	0.177819
7.7	0.53567	0.234653
10.5	0.61652	0.308978
14.2	0.689049	0.397509
19.2	0.752765	0.499624
26.1	0.807448	0.609917
35.4	0.851727	0.7134
47.9	0.886747	0.798729
65	0.914401	0.862684
88.1	0.935628	0.906582
119.5	0.951854	0.935961
162	0.9641	0.955486
219.6	0.973303	0.96865
297.7	0.980188	0.977676
403.7	0.985325	0.983968
547	0.989134	0.988399
742	0.99197	0.991572
1006	0.994066	0.993851
5000	0.998801	0.998793
8000	0.99925	0.999247

I. A.) Plot the relationship between ΔF_{obs} and the average moles of ligand bound per mole of protein.

B.) Determine the binding stoichiometry when the protein is saturated with ligand.

C.) Write an equation relating ΔF_{obs} in terms of the free ligand concentration (L_f), site binding constant(s) (κ_i), and the average fluorescence signal change (ΔF_i) upon binding ligand to each site on the protein.

D.) Use non-linear least square analysis to determine the site binding constant(s) for ligand binding to the fluorescently labeled protein. Show all equations used in the analysis.

II. This same ligand, L, is known to bind to another protein (C). In order to examine the binding of L to the non-fluorescent protein (C), two additional titrations were performed. Ligand was titrated into solutions containing protein (C) at concentrations of 30 nM and 120 nM. However, these samples also contained the fluorescent protein (P) at a total concentration of 20 nM (the same total concentration used in one of the experiments performed in part (A)). The results of these titrations are given in the Table below.

Ltot (nM)	ΔFobs (P = 20 nM, C = 30 nM)	ΔFobs (P = 20 nM, C = 120nM)
0	0	0
0.2	0.000991	0.000279
0.37	0.001835	0.000516
0.7	0.003478	0.000977
1.2	0.005981	0.001676
2.3	0.011539	0.003219
3.1	0.015627	0.004346
4.2	0.021312	0.0059
5.7	0.029182	0.008031
7.7	0.03989	0.010892
10.5	0.055296	0.014934
14.2	0.076401	0.020344
19.2	0.106275	0.027779
26.1	0.150027	0.038279
35.4	0.213394	0.052883
47.9	0.305304	0.07335
65	0.438049	0.102967
88.1	0.610049	0.146075
119.5	0.783258	0.210611
162	0.893859	0.308899
219.6	0.944044	0.459333
297.7	0.967026	0.673655
403.7	0.979032	0.887463
547	0.985994	0.964543
742	0.990364	0.983211
1006	0.99323	0.990303
5000	0.99877	0.998698
8000	0.999239	0.999212

E.) Determine the stoichiometry of ligand bound to the unlabeled protein C at saturation. Discuss the rationale for your answer including any necessary equations.

4. **(20 points)** Two proteins, A and B, can interact to form a complex, AB, with equilibrium constant, K_{AB}, defined as:

$$K_{AB} = \frac{[AB]}{[A][B]}$$

A.) A positive value of the observed heat capacity change, $\Delta C_{p,obs}$, is measured for this reaction. A colleague suggests that this could result if the product, AB, actually exists in two conformations, AB_1 and AB_2, where $[AB_1] + [AB_2] = [AB]$, with $K_0 = [AB_2]/[AB_1]$, where A and B interact to form AB_1 with equilibrium constant

$$K_1 = \frac{[AB_1]}{[A][B]}$$

Determine whether this proposal is correct. Your answer should include a derivation of the expression for the heat capacity change for formation of AB in terms of K_0, K_1, the enthalpy changes, ΔH_1 and ΔH_0, and the intrinsic heat capacity changes, ΔCp_1 and ΔCp_0.

B.) If both $\Delta Cp_1 = 0$ and $\Delta Cp_0 = 0$, how is the sign (+/-) of $\Delta C_{p,obs}$ affected by the signs of ΔH_1 and ΔH_0?

C.) Briefly, describe some other possible explanations for a positive $\Delta C_{p,obs}$.