Bio 5312 Spring 2016

Problem Set 5 **Due February 29**

1. A protein (M) is an enzyme that binds substrate (S). M can dimerize to form M_2 and each monomer can bind one substrate, hence the dimer can bind two substrate molecules.

a.) Write the thermodynamic cycles describing the equilibrium binding of M, M_2 and S.

b.) Derive an expression describing the average moles of substrate bound per mole of total protein concentration, where the protein concentration is in total monomer units.

c.) Derive an expression for the observed dimerization constant, L_{obs}, as a function of [S].

d.) The results of equilibrium titrations of protein with substrate are shown in Table 1 below for titrations performed at two protein concentrations (71 and 401 nM). Given that the equilibrium constant for protein dimerization of protein with no substrate bound is $L_0 = 4 \times 10^4\ M^{-1}$, (see below), determine the remaining equilibrium constants by performing a non-linear least squares analysis of these data and report the uncertainties for the equilibrium constants at the 68% confidence level.

$$L_0 = [M_2]/[M]^2$$

Table 1

Total Substrate Concentration (M)	S_{bound}/M_{total} (71 nM)	S_{bound}/M_{total} (401 nM)
1.0×10^{-10}	0.0036	0.0094
1.8×10^{-10}	0.0047	0.0043
3.2×10^{-10}	0.0039	-0.0036
5.7×10^{-10}	0.0071	0.0001
1.0×10^{-9}	0.0211	-0.0003
1.8×10^{-9}	0.0378	0.0078
3.3×10^{-9}	0.0401	0.0043
5.8×10^{-9}	0.0813	0.0166
10.4×10^{-9}	0.1424	0.0254
18.6×10^{-9}	0.2403	0.0404

33.1×10^{-9}	0.3887	0.0816
59.2×10^{-9}	0.4692	0.1437
105.8×10^{-9}	0.5270	0.2589
189.0×10^{-9}	0.5854	0.4635
337.7×10^{-9}	0.6487	0.5655
603.4×10^{-9}	0.7265	0.6693
1.1×10^{-6}	0.8078	0.7707
1.9×10^{-6}	0.8717	0.8509
3.4×10^{-6}	0.9258	0.9158
6.1×10^{-6}	0.9555	0.9541
1.10×10^{-5}	0.9738	0.9757
1.96×10^{-5}	0.9874	0.9816
3.51×10^{-5}	0.9851	0.9878
6.27×10^{-5}	0.9924	0.9905
1.1×10^{-4}	0.9941	0.9982
2.0×10^{-4}	0.9993	0.9930

e.) The following enzyme activity data were obtained as a function of substrate concentration at a total protein concentration of 400 nM (total monomer concentration). Use these data to determine which form(s) of the enzyme have activity. Present quantitative evidence supporting your conclusion.

Total Substrate Concentration (M)	Percent Activity
1.00×10^{-8}	2.46
1.22×10^{-8}	3.43
1.49×10^{-8}	4.63
1.82×10^{-8}	3.65
2.23×10^{-8}	5.70
2.72×10^{-8}	6.62
3.33×10^{-8}	7.78
4.07×10^{-8}	10.56
4.97×10^{-8}	12.70
6.07×10^{-8}	15.53
7.42×10^{-8}	17.99
9.07×10^{-8}	23.10
1.11×10^{-7}	28.17
1.35×10^{-7}	32.86
1.65×10^{-7}	40.73
2.02×10^{-7}	45.07
2.47×10^{-7}	45.60
3.02×10^{-7}	42.96
3.69×10^{-7}	40.48
4.51×10^{-7}	37.96
5.51×10^{-7}	34.05
6.73×10^{-7}	31.17
8.22×10^{-7}	25.90
1.0×10^{-6}	23.03
1.2×10^{-6}	19.67

2. A report is published that describes a study of the equilibrium binding of a protein to an oligodeoxynucleotide. The researcher has evidence that the protein is dimeric and assumes that the protein is dimeric under all conditions and when bound to the DNA. Equilibrium titrations are performed by titrating protein (at a total concentration of 10^{-9} M (monomers)), from which an apparent equilibrium association constant, K_{app}, is obtained by assuming that the only protein species present in solution are unligated dimers and DNA-bound dimers. Experiments performed at two temperatures (4˚C and 25˚C) are used to calculate ΔH°_{app}= -25 kcal/mol. However, you have independent evidence that the protein also undergoes dissociation to monomers in the absence of DNA, with a temperature-dependent dimerization constant, $L_0=[P_2]/[P]^2$, and that protein monomers do not bind DNA.

a.) Obtain an expression for the average extent of DNA binding per "protein dimer" in terms of K_1, the true equilibrium association constant for DNA binding to the protein dimer, and the monomer dimer equilibrium constant, L_0.

b.) Obtain an expression for K_{app} in terms of K_1 and L_0.

c.) Obtain a more accurate estimate of the ΔH° for binding of protein dimer to the DNA if $L_0=10^9$ M^{-1} at 4˚C and 10^8 M^{-1} at 25˚C.

d.) Suggest another set of experiments that can be used to test your hypothesis that DNA binding is linked to protein dimerization.