SAMPLE PROBLEMS

1. The properties of the three proteins are listed below.

	Molecular Weight	Solubility in Ammonium Sulfate[1]	# of Asp and Glu residues. ($pK_a = 4.0$)	# Lys and Arg Residues ($pK_a = 9.0$)
A	12,000 Da	2.0M	5	10
B	12,000 Da	2.0M	5	8
C	34,000 Da	2.0M	0	10

[1]This is the concentration of ammonium sulfate that will precipitate 50% of the protein, 75% will precipitate when the concentration is 0.5M higher than this value.

Which one of the following three purification schemes will provide pure protein A? Briefly explain which proteins are separated at *each* step in the purification scheme.
Scheme A: Separation by gel filtration, followed by the addition of 1.5 M ammonium sulfate.
Scheme B: Separation by gel filtration, followed by anion exchange chromatography at pH 7.0.
Scheme C: Separation by gel filtration, followed by cation exchange chromatography at pH 7.0

2. The data below describe the binding of ligand A to a macromolecule P. Select an appropriate graphical method, calculate both *n* (the number of ligands bound) and the equilibrium dissociation constant, K_d. What can you conclude about the ligand binding sites?

[A] (μM)	0.5	1.0	2.0	5.0	10.0	20.0
Fraction P bound (FrP)	1.6	2.5	3.2	4.0	4.1	4.8

3. A solution of protein at a concentration of 1.00×10^{-3} M is placed inside a semi-permeable membrane. The membrane bag is then placed in a large volume of a 1.00×10^{-4} M solution of a small molecule, X. The concentration of X outside the membrane is measured at various times and is 1.00×10^{-4}, 9.2×10^{-5}, 8×10^{-5}, 6.25×10^{-5}, 6.12×10^{-5} and 6.12×10^{-5} M at times 0, 1, 3, 7, 15, and 24 hours respectively. After 24 hours the concentration of X inside the membrane is 6.54×10^{-5} M.
(a) Does X bind to the protein and, if so, what is the dissociation constant?
(b) A second molecule, Y is added to the solution outside of the membrane at an initial concentration of 1.00×10^{-3} M. At equilibrium, the concentration of Y is the same on both sides of the membrane but the concentrations of X are 9.72×10^{-5} M (outside) and 9.78×10^{-5} M inside. What can you say about the interaction of Y and X?

4. The data given below describe the variation of sedimentation coefficient, s, and diffusion coefficient, D for a protein as a function of pH. Explain what happens to the protein at low and high pH, assuming that the protein adopts its native structural state (i.e. structural conformation) between pH 5 and pH 6.

pH	2	3	4	5	6	7	8	9	10
s (S)	2.93	3.02	3.89	4.41	4.40	4.15	3.60	2.25	2.20
D ($\times 10^7$) cm^2s^{-1}	7.91	8.00	8.00	5.90	5.92	5.61	4.86	3.08	2.97

5. Plot a Scatchard Curve (Fluor/Free vs. Fluor) and comment on the nature of the curve. Determine the Hill coefficient and association constant. What does the value of the Hill coefficient indicate?

Free ligand	Fluor
9.00E-07	1.62E+00
2.70E-06	5.84E+00
8.10E-06	1.74E+01
2.43E-05	3.94E+01
7.29E-05	4.51E+01
2.19E-04	4.97E+01
6.56E-04	5.29E+01

6. Given the following information, fill in the missing information. What can you conclude about the properties of myosin from your results? Assume T = 20^{o}C, **η = 0.01 g/(cm-sec)**.

Substance	Mol. Wt.	S ($x10^{13}$) sec	v (cm3/g)	D(x106) cm2/s	F (g/s)	fmin	f/fo Ratio
Myosin	440K	5.4	0.73	0.11			

7. In a **sedimentation equilibrium** experiment, a protein is centrifuged at 8000 rpm. The temperature of the system is maintained constant at 20 ^{o}C and the density of the colloid is 1.25 kg/L. Knowing that 1.00 g of sediment displaces 0.752 mL of water. Using absorption optics we determine that the concentration at r_1 (7.08 cm) to be 0.123 mg/mL and the concentration at r_2 = 7.54 cm to be 0.532 mg/mL. Calculate the molecular weight of this protein.

8. The two proteins **A** and **B** were also investigated by dynamic light scattering at a temperature of *T* = 25 °C. These experiments resulted in diffusion coefficients D_A = 13.2 × 10-11 m^2 s^{-1} and D_B = 1.2 × 10-11 m^2 s^{-1} for protein **A** and protein **B**, respectively. Based on the relationships for the diffusion coefficient D and sedimentation coefficient ***s***, calculate the molecular weights M_A and M_B for protein **A** and protein **B**, respectively. Consider the partial specific volume to be 0.73 x 10^3 m^3 kg^{-1}
(ii) Assuming that hydration of the two proteins is negligible, what can you deduce from these experiments concerning the shapes of the two proteins?