ANSWERS to Sample Problems

1. The properties of 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

ANSWER:
Scheme A cannot work since the solubility is the same in Ammonium sulfate
Schemes B and C will separate proteins A and B from C based on the molecular weight difference.

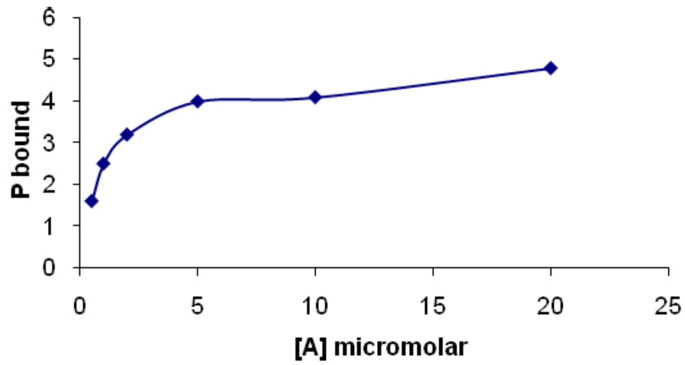
At pH 7.0, acidic residues are ionized completely and basic residues are protonated. Charge on protein A = -5+10=+5
Charge on protein B=-5+8=+3

Cation exchange resins will bind both proteins that can then be eluted from the column.

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?

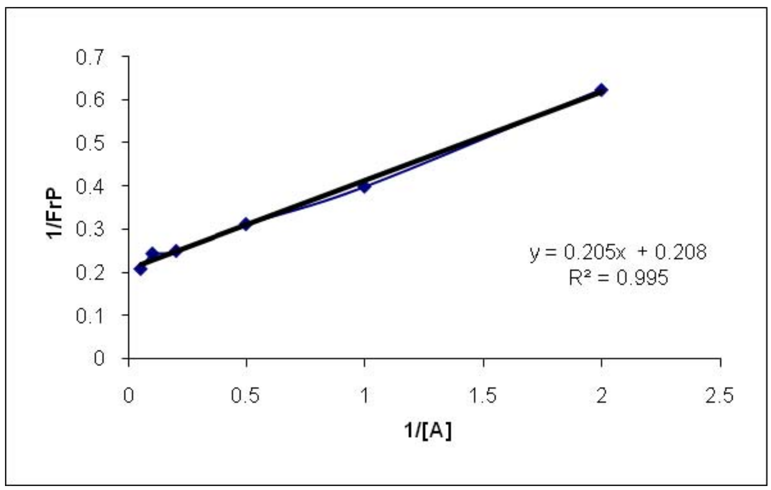
[A] (μM)	0.5	1.0	2.0	5.0	10.0	20.0
Fraction P bound (FrP)	1.6	2.5	3.2	4.0	4.1	4.8

[A] (μM)	Fraction P bound (FrP)
0.5	1.6
1	2.5
2	3.2
5	4
10	4.1
20	4.8



1/A	1/FrP
2	0.625
1	0.4
0.5	0.3125
0.2	0.25
0.1	0.243902
0.05	0.208333

Intercept 1/n
Slope 1/nK



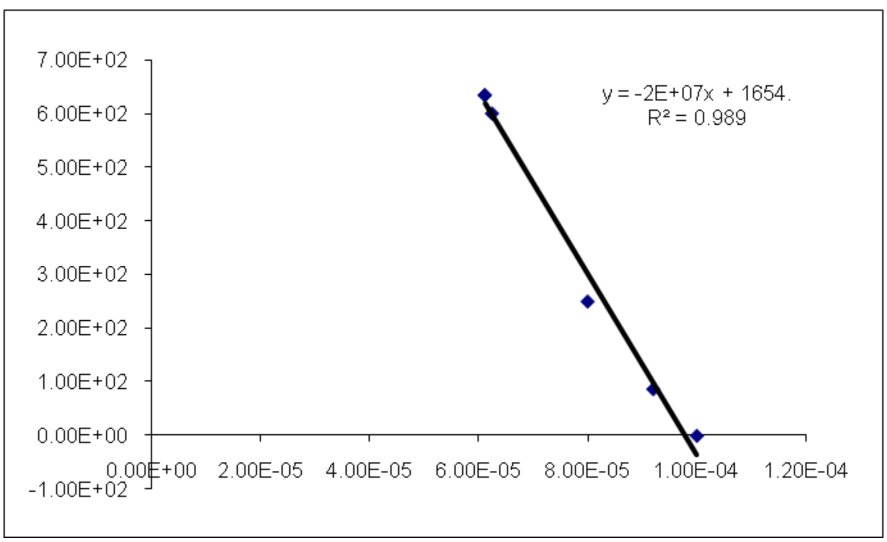
This indicates that the sites are identical and independent.

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?

time hrs	X outside	X bound	v=X bound/Protein
0	1.00E-04	0.00E+00	0.00E+00
1	9.20E-05	8.00E-06	8.00E-03
3	8.00E-05	2.00E-05	2.00E-02
7	6.25E-05	3.75E-05	3.75E-02
15	6.12E-05	3.88E-05	3.88E-02
24	6.12E-05	3.88E-05	3.88E-02

	6.54E-05
Protein conc	1.00E-03

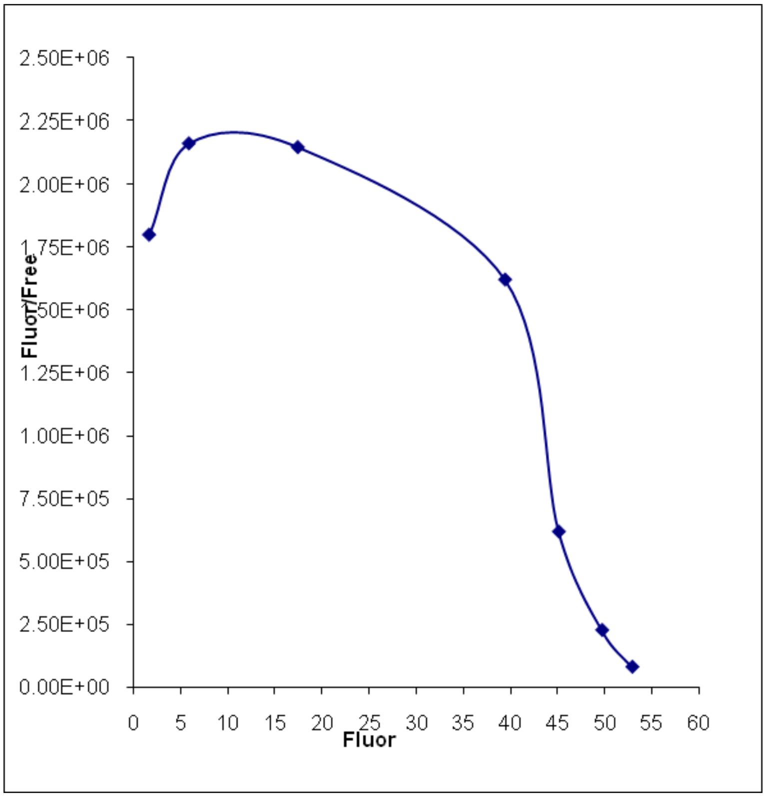
X	v/X outside
1.00E-04	0.00E+00
9.20E-05	8.70E+01
8.00E-05	2.50E+02
6.25E-05	6.00E+02
6.12E-05	6.34E+02
6.12E-05	6.34E+02



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?

x	y
1.62E+00	1.80E+06
5.84E+00	2.16E+06
1.74E+01	2.15E+06
3.94E+01	1.62E+06
4.51E+01	6.19E+05
4.97E+01	2.27E+05
5.29E+01	8.06E+04

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



The upward curvature indicates positive cooperativity.

In a Y/[A] vs. Y Scatchard plot like the one above – the Hill coefficient is obtained by taking the x value corresponding to the maximum and minimum points – say x1 and x2. The Hill coefficient equals 1/[1-(x1/x2)]

Thus, the Hill coefficient equals 1/[1-(7/54)] = 1.15

The other problems are formula based.

For density values of diluted macromolecular solutions take the density of water.