NAME:_____ **EXAM III**

1.) Previously, we utilized ethidium bromide (EtBr) to visualize DNA on an agarose gel. This question concerns the binding of EtBr to an oligonucleotide of double stranded DNA that is 12 base pairs in length. In 1 M NaCl, this oligonucleotide exists exclusively in the B-form conformation where binding of EtBr was found to be noncooperative.

$$region_{between\ adjacent\ \text{bases}} + EtBr \overset{K_A}{\Leftrightarrow} region_{between\ adjacent\ \text{bases}} : EtBr \qquad \text{(eq. \#1)}$$

We want to determine the total number of EtBr molecules that can bind one DNA oligomer since each region between adjacent bases may be able to bind an EtBr.
SHOW EVERY STEP OF YOUR WORK!

A. Write an equation for the equilibrium association constant K_A in terms of concentrations of products and reactants from eq.#1

B. Write an equation for the total number of DNA regions ($[region_{DNA}]_{tot}$) and rearrange this equation to isolate $[region_{DNA}]_{free}$ on one side.

C. Plug your equation from B into your equation from A.

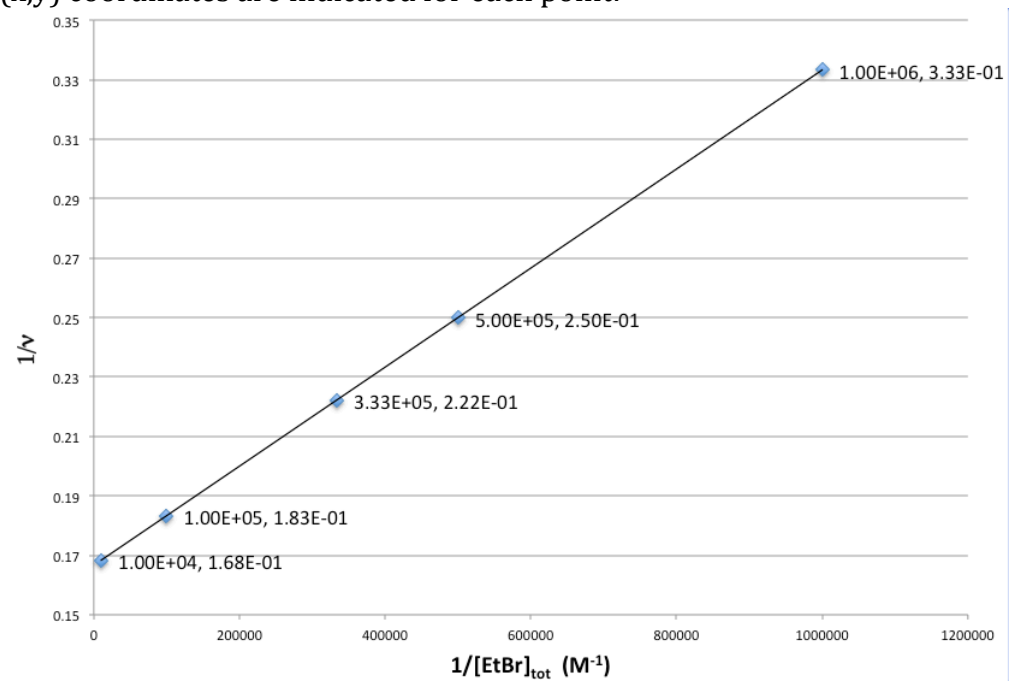
D. Solve for θ, where $\theta = \dfrac{[region_{DNA} : EtBr]}{[region_{DNA}]_{tot}}$

E. The average number of EtBr molecules bound to an individual DNA oligomer is represented by ν, where $\nu = N\theta$. N is the total number of EtBr molecules that can bind one DNA oligomer (what we set out to determine!). Write an equation for ν using your equation from D (leave in the term "N", do not simplify).

F. Write an equation for the reciprocal of ν (i.e. $1/\nu$). This is called the Klotz equation.

G. What does the y_{int} of a Klotz plot (see below) indicate? What does the slope (m) of a Klotz plot indicate?

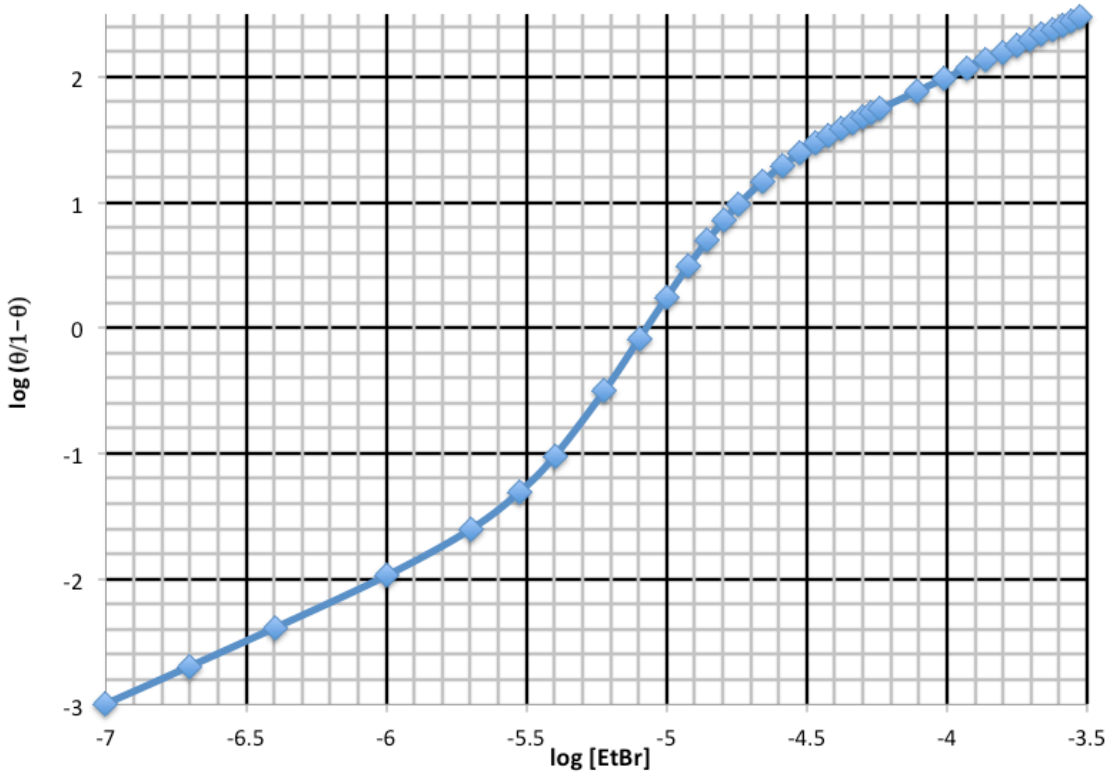
Below is a Klotz plot for EtBr binding to the DNA oligomer in 1.0 M NaCl. The (x,y) coordinates are indicated for each point:



H. Determine the value of N from the Klotz plot. This value should be very close to an integer.

I. Determine the value of K_A from the Klotz plot for EtBr binding to B-form DNA.

In 4.4 M NaCl in the absence of EtBr, this DNA oligomer exists exclusively in the Z-form conformation. Data for the binding of EtBr to the DNA oligomer in this conformation did not produce a line on the Klotz plot. Below is a Hill plot for EtBr binding to the DNA oligomer in 4.4 M NaCl.



Analyze the Hill plot using the MWC model.

J. Determine the value of the weak binding <u>association</u> constant (K_{site}^{T}).

K. Determine the value of the strong binding <u>association</u> constant (K_{site}^{R}).

L. Compare your values from I and K. Describe in words the nature of the conformational change as the T state converts to the R state.

2.) Imagine that you are purifying the following proteins from one another:

Glutamate dehydrogenase
447 amino acids (48,580 Da)
pI 6.0
54 basic residues
50 acidic residues

Glucokinase
321 amino acids (34,700 Da)
pI 6.6
43 basic residues
37 acidic residues

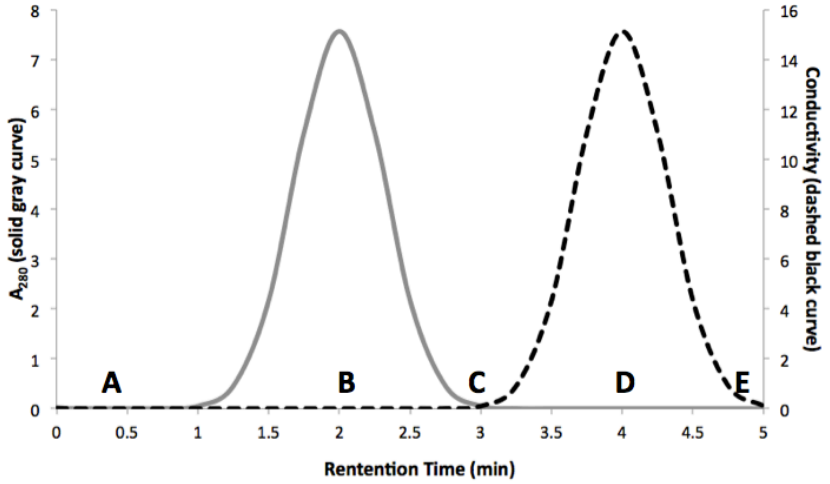
Lac repressor
363 amino acids (39,000 Da)
pI 7.5
37 basic residues
32 acidic residues

Beta-galactosidase
1024 amino acids (116,000 Da)
pI 5.5
120 basic residues
126 acidic residues

RNA polymerase omega subunit
91 amino acids (10,000 Da)
pI 4.5
13 basic residues
17 acid residues

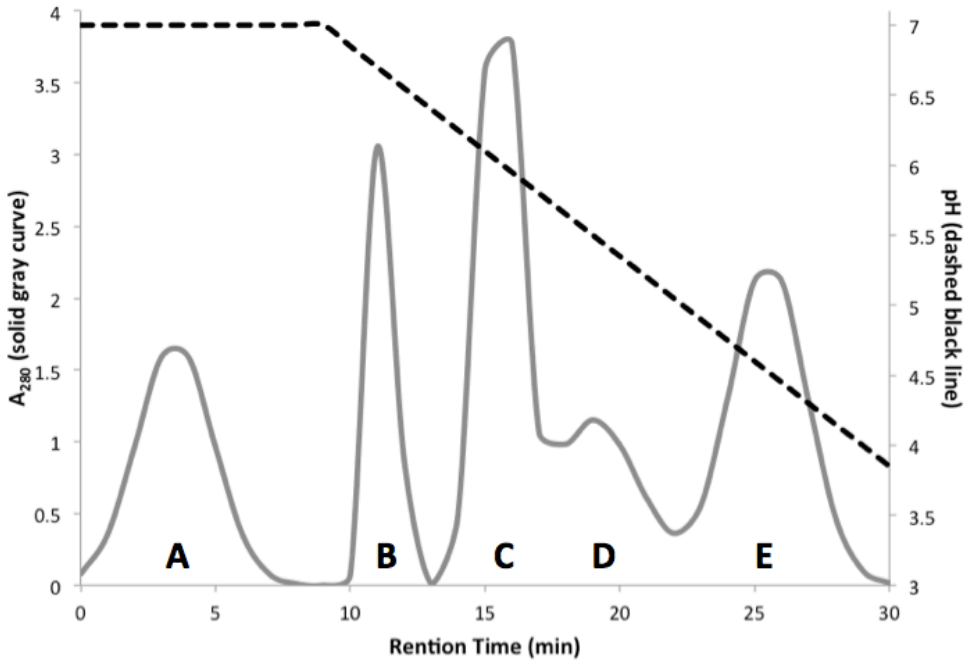
A. After several initial purification steps, you have 50 mL of a solution containing the above proteins. You would like to separate the proteins using an FPLC with an anion exchange column; however, 3 mL is the most that you can load on the column at one time. Describe in words how you could efficiently reduce the total volume of your sample to 3 mL without loosing most of the proteins.

B. Before loading the sample on an anion exchange column, you decide to make sure the salt concentration of your protein sample is very low by running a gel filtration desalting column. Indicate at which retention time (A, B, C, D, or E) each protein eluted.



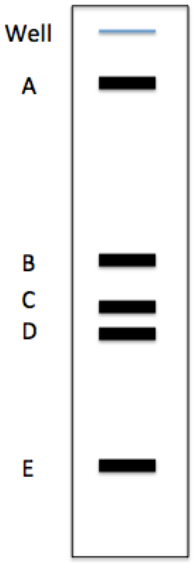
Glutamate dehydrogenase:
Glucokinase:
Lac repressor:
Beta-galactosidase:
RNA polymerase-omega:

C. Indicate at which retention time (A, B, C, D, or E) each protein eluted from the anion exchange column as the pH was varied.



Glutamate dehydrogenase:
Glucokinase:
Lac repressor:
Beta-galactosidase:
RNA polymerase-omega:

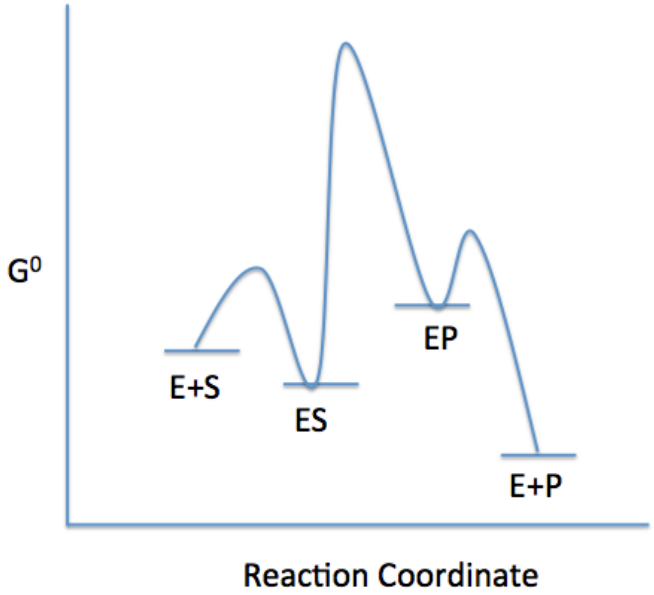
D. You decide to run an SDS-PAGE analysis of the original solution containing five proteins.



a. Indicate the charge and position of the electrodes utilized to separate the proteins in this gel.
b. Identify (A, B, C, D, or E) which protein composes each band

Glutamate dehydrogenase:
Lac repressor:
RNA polymerase-omega:
Glucokinase:
Beta-galactosidase:

3.) Below is the reaction coordinate diagram for an enzyme catalyzed reaction:



$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \underset{k_{-2}}{\overset{k_2}{\Leftrightarrow}} EP \underset{k_{-3}}{\overset{k_3}{\Leftrightarrow}} E + P$$

A.) Fill in the blanks:

k_1_____k_{-1}

k_2_____k_{-2}

k_3_____k_{-3}

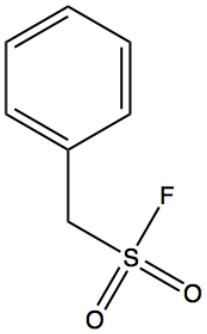
$\Delta G^{\circ}_{ES \rightarrow (ES-EP)^{\mp}}$ _____ $\Delta G^{\circ}_{EP \rightarrow (ES-EP)^{\mp}}$

$\Delta G^{\circ}_{E+S \rightarrow E+P}$ _____ $\Delta G^{\circ}_{E+P \rightarrow E+S}$

B.) What is the rate limiting step for this reaction?

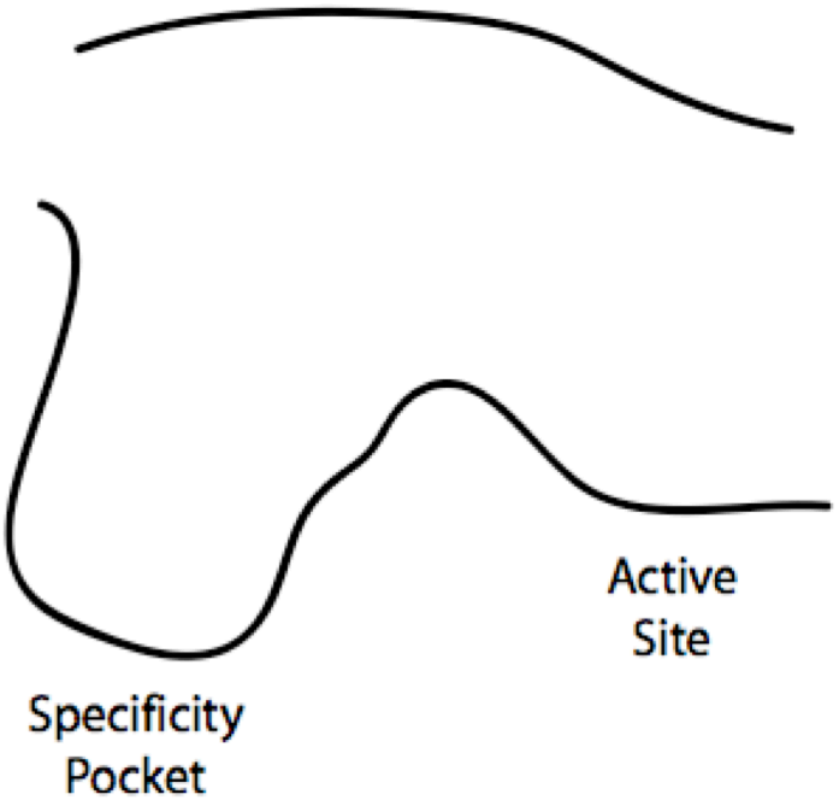
C.) Add a curve or curves to the graph to describe the reaction coordinate diagram for the uncatalyzed reaction: $E + S \Leftrightarrow E + P$.

4.) To inhibit serine proteases during protein purification, many protocols include the use of phenylmethylsulfony fluoride (PMSF). PMSF acts as a suicide inhibitor of serine proteases by forming an irreversible covalent linkage to an active site residue. <u>The initial mechanism is the same as the peptide cleavage reaction.</u>



PMSF

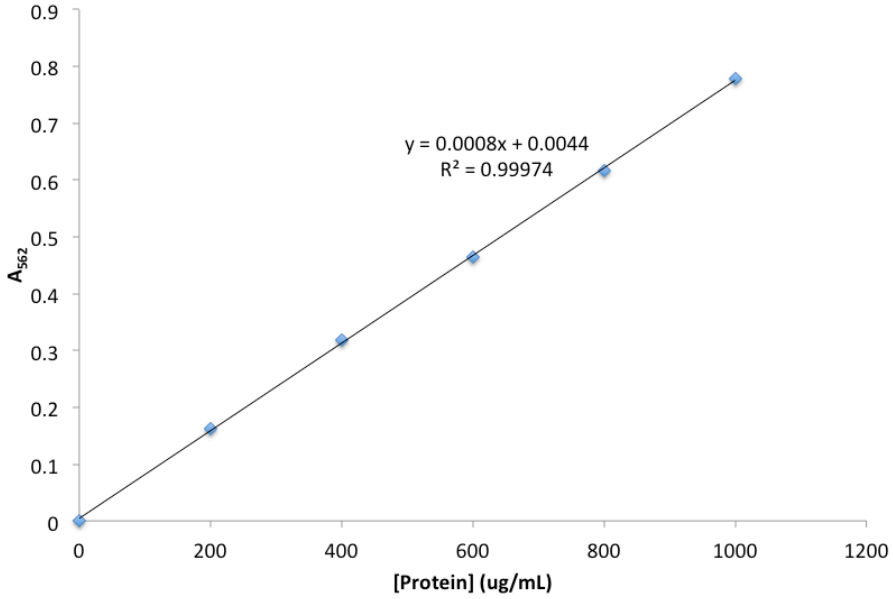
A. Draw PMSF in the cartoon of a protease below as you might expect it orientate.
B. Draw the chemical structure and identify at least three unique amino acid side chains that you might find projected into the specificity pocket of a protease with high affinity for PMSF.



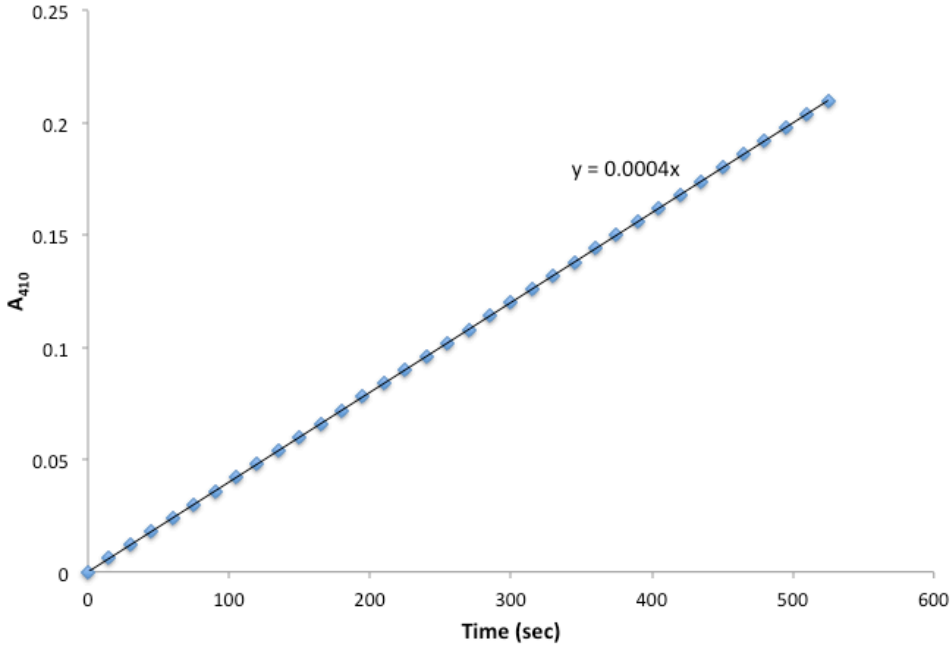
C. Propose an electron pushing mechanism for the inhibition of a serine protease with PMSF. [Make sure to include the residues of the catalytic triad in your figures.]

5.) In an effort to determine the specific activity of an alkaline phosphatase preparation, you collect the following pieces of data:

BCA standard curve:



Activity assay:



All assays contained 3 mL total volume.

A BCA assay of a 1:3 dilution of the original protein prep yielded an A_{562} of 0.4125.

250 uL of the original protein prep was added to the activity assay.

The molar absorptivity of the PNP product is 18,000 M^{-1} cm^{-1}.

A. Determine the concentration of PNP produced per minute (rate) in units of nM min^{-1}.

B. Determine the total number of nanomoles of PNP produced per minute in the assay (units: nmole min^{-1} which is equal to mU).

C. Determine the activity of the protein prep per mL (units: mU mL^{-1}).

D. Determine the mass of protein per mL of purified protein solution (units: mg mL^{-1}).

E. Use the values from *D* and *E* to determine the specific activity of the purified protein solution (units: mU mg^{-1}).

1.)	36 pts	(3 pts/question)
2.)	20 pts	(5 pts/question)
3.)	12 pts	(4 pts/question)
4.)	18 pts	(6 pts/question)
5.)	20 pts	(4 pts/question)

106 total points