7.02/10.702
Protein Biochemistry Study Questions
Spring 2005

The problems in this packet are taken from exams from the past few semesters. As always, these questions are not meant to be exhaustive, but to give you an idea of what topics to study. We strongly urge you to work through the questions **BEFORE** looking at the answer key, and to bring any questions to your TAs or Instructors.

Question 1

You have a crude lysate sample (CL) containing a mixture of six proteins (1, 2, 3, 4, 5, and β-galactosidase), and your goal is to obtain purified β-galactosidase. Some characteristics of these proteins are shown in the table below:

Protein	Concentration of ammonium sulfate (AS) required for precipitation	Molecular Weight (kDa)	Isoelectric point (pI)
1	45%	38	3.7
2	80%	22	4.8
3	65%	4	5.3
4	20%	75	6.8
5	30%	55	9.50
β-galactosidase	45%	115	5.3

You begin your purification by performing an ammonium sulfate (AS) precipitation. You add the appropriate concentration of AS to your CL sample, incubate overnight at 4˚C, then centrifuge to generate a supernatant (AS-S) and pellet (AS-P).

a) What concentration of AS will you use to precipitate β-galactosidase? _____

b) After addition of that concentration of AS and centrifugation, which protein(s) will be in the supernatant (AS-S)? _____

c) Which protein(s) will be in the pellet (AS-P)? _____

d) After resuspending the AS-P in column buffer, you should use a PD-10 column to _____ your sample.

One way to purify β-galactosidase away from any contaminating proteins in the AS-P sample would be to separate the proteins based on their molecular weight.

e) What type of column separates on this basis? _____

f) Which protein (from your AS-P) would elute <u>first</u> from this type of column? _____

Instead, you decide to use ion exchange chromatography to further purify β-galactosidase away from other proteins in your **<u>AS-P</u>** sample. You first run an **<u>anion</u>** exchange column equilibrated using column buffer with a **<u>pH of 5.0</u>.**

g) What charge does the matrix of an anion exchange column have? _____

h) At pH 5.0, which protein(s) from the AS-P will stick to the column? _____

i) Explain your answer to part i) **<u>in one or two sentences.</u>**

Question 1 (continued)

j) State how you would elute a protein bound to an anion exchange column, and explain how this elution method works **in one or two sentences**.

You identify the fraction containing β-gal from your anion exchange column, and decide to run it over a **cation** exchange column to complete your purification.

k) Describe how you would use a **cation** exchange column to purify β-galactosidase away from any remaining contaminating protein(s). Be specific about: 1) the pH at which you'd equilibrate the column; 2) why you chose this pH; and 3) which protein(s) would bind and which protein(s) would flow through the column under these conditions, and why.

Finally, you decide to run an SDS-PAGE gel to analyze your purification. You prepare your samples for loading as in lab, run your crude lysate (CL) sample (containing proteins 1-5 and β-galactosidase) on the gel, stain with Coomassie, and observe 6 bands.

l) Which protein will migrate closest to the dye front of the SDS gel? _____
Explain your answer in one or two sentences.

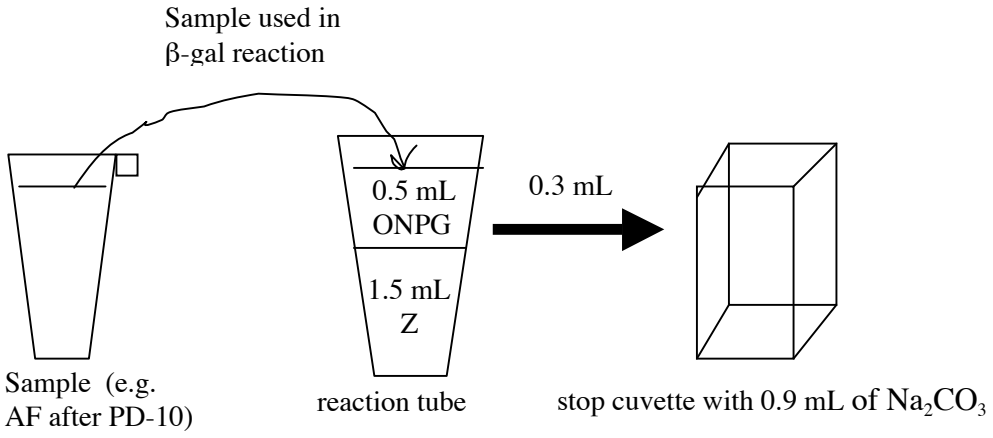
You analyze a second sample of CL by SDS-PAGE, but this time, you do not add β-mercaptoethanol (β-ME) to your sample buffer. You find that protein 2 has the **same** relative mobility in both the presence and absence of β-ME.

m) What do these results allow you to conclude about protein 2? Justify your answer **in one or two sentences**.

Question 2

You are interested in purifying β-galactosidase from the bacterium *S. typhimurium,* and decide to use a purification scheme similar to the one you used in 7.02 laboratory.

At each step of the purification, you perform a quantitative β-galactosidase by assaying a sample of your protein in a "reaction tube" containing 1.5 mL of Z buffer and 0.5 mL of 10 mM ONPG. When the color has developed sufficiently, you transfer 0.3 mL of your reaction to a cuvette containing 0.9 mL of Na_2CO_3. This assay setup is diagrammed below:



You obtain the following data for your AF after PD-10 sample:

sample name	total sample volume (mL)	[protein] (mg/mL)	volume of sample used in reaction tube (μL)	stop time (min.)	A_{420} at stop
AF after PD-10	1.5	0.133	30	6	0.333

a) Calculate the <u>total activity</u> of the "AF after PD-10" sample. **SHOW YOUR CALCULATIONS, and be sure to include UNITS <u>at each step</u>!**

Question 2 (continued)

sample name	total sample volume (mL)	[protein] (mg/mL)	volume of sample used in reaction tube (μL)	stop time (min.)	A_{420} at stop
AF after PD-10	1.5	0.133	30	6	0.333

b) **Define** and **determine** the specific activity (SA) of the "AF after PD-10" sample. **SHOW YOUR CALCULATIONS, and don't forget UNITS!**

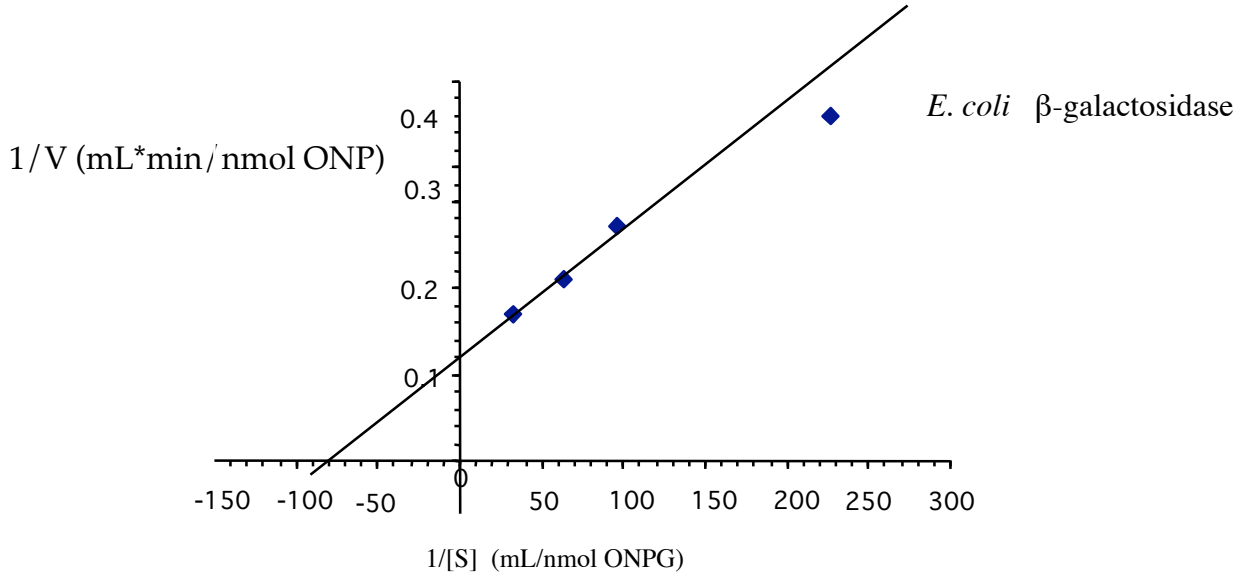
c) You have obtained a 12.5X fold purification and a yield of 2% at the "AF after PD-10" step. What was the protein concentration of your crude lysate (CL) sample, if its volume was 20 mL? **SHOW YOUR CALCULATIONS, and don't forget UNITS!**

You repeat the quantitative assay shown above, but use 60 μL of protein in your reaction tube instead of 30μL.

d) Assuming that ONPG is in excess in these reactions, how long will it take (in minutes) to obtain an A420 of 0.500 **in the cuvette**? **Justify your answer.**

Question 2 (continued)

After purifying the *S. typhimurium* β-galactosidase, you decide to compare its kinetic parameters to those of *E. coli* β-galactosidase. You perform enzyme kinetics experiments with both *E. coli* and *S. typhimurium* β-galactosidase using varying concentrations of ONPG as in 7.02 lab. You obtain the following plot for *E. coli* β-galactosidase:



You compare *S. typhimurium* β-galactosidase to *E. coli* β-galactosidase , and find that *S. typhimurium* β-galactosidase can bind to ONPG **exactly as efficiently** as *E. coli* β-galactosidase.

e) What must be the Km of *S. typhimurium* β-galactosidase? **Show your calculations, and include units!**

You performed both the *S. typhimurium* and *E. coli* β-galactosidase enzyme kinetics experiments using the **same** quantity of β-galactosidase: 2.5×10^{-11} moles/mL.

f) Determine Kcat for *E. coli* β-galactosidase. **Show your calculations, and include units!**

g) On the plot above, draw the results you'd expect if *S. typhimurium* β-galactosidase is **more efficient** at catalyzing the conversion of ONPG to ONP + galactose than *E. coli* β-galactosidase.

Question 3

In the Protein Biochemistry module, you analyzed the results of your β-galactosidase purification using SDS-PAGE and Western blotting.

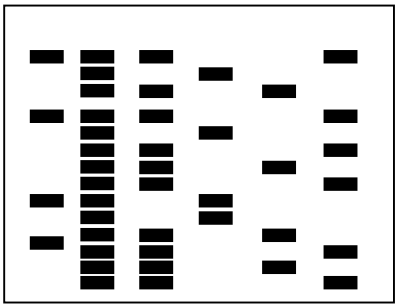
The diagrams on the next few pages indicate "expected results" for two different steps in the SDS-PAGE and Western blotting process, along with examples of "bad" results obtained by different student groups at each step. For simplicity, only the following lanes of the gel or Western are shown: MW standards, CL, CL-S, CL-P, AS-S, AS-P.

For each "bad" result:

1. **Identify the reagent or experimental step that was forgotten/performed incorrectly ("Problem")**
2. **State the specific observations that led you to this conclusion ("Evidence")**
3. **State how this reagent/step—when used/performed correctly—allows you to obtain the "expected results" ("Explanation")**

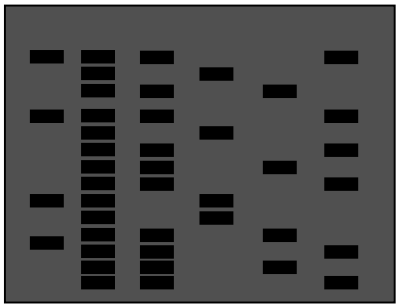
SDS-PAGE gel (Day 4)
(you may assume that all samples were prepared and loaded correctly on the gel)

MW CL CLS CLP ASS ASP



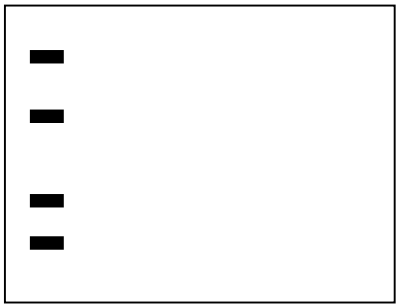
"expected" results

MW CL CLS CLP ASS ASP



Group 1

MW CL CLS CLP ASS ASP



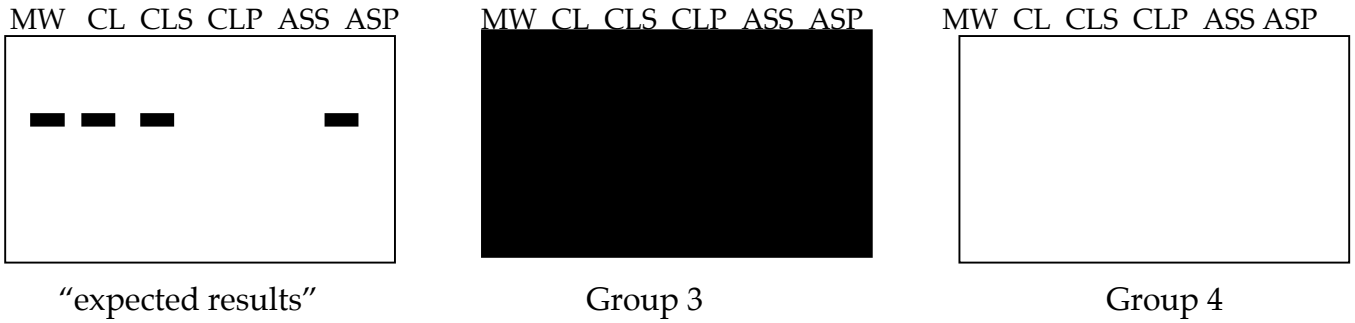
Group 2

Group	"Problem"	"Evidence"	"Explanation"
1			
2			

Question 3 (continued)

Western blotting (Day 6): Nitrocellulose membrane after addition of NBT/BCIP

(you may assume that all three groups loaded the same amount of protein in each lane, and that transfer to the membrane was performed successfully)



"expected results" Group 3 Group 4

Group	"Problem"	"Evidence"	"Explanation"
3			
4			

Question 4

Below are seven amino acids. Indicate all characteristics that apply to each amino acid by writing letter(s) in the blanks provided.

Amino acid	
lysine	_____
arginine	_____
proline	_____
aspartic acid	_____
tryptophan	_____
cysteine	_____
glutamic acid	_____

Characteristics
a) forms disulfide bonds
b) is charged at pH=7
c) non-polar/hydrophobic
d) ring of side chain covalently linked to peptide backbone
e) has a ring
f) absorbs light at wavelength 280 nm
g) contains sulfur
h) polar/hydrophilic

Question 5

In 7.02 this term, you purified and characterized β-galactosidase from *E. coli* cells. Some groups purified the wild type enzyme (CSH36), while others purified the mutant enzyme (HIS461). Next semester, we would like to have students purify a mutant *E. coli* β-galactosidase (ARG388) that is known to be severely deficient in substrate binding.

Which two experiments that we did this year in class will reveal to the students the nature of the ARG388 defect? Explain your reasoning.

(HINT: A complete answer to this question will name the 2 experiments and identify the observation(s) you'd make/data you'd collect in each that would help you identify the ARG388 defect as "substrate binding.")

Experiment 1: _____

Experiment 2: _____

Question 6

After 7.02 lab, you decide to do a UROP in a protein biochemistry lab. Your lab is interested in the AB complex, which is formed by two proteins ("A"+"B"). Your project is to see how mutations in A or B affect complex formation.

You first decide to purify the wild type complex. After a number of purification steps, you have two bands left in your most pure sample (as judged by Coomassie staining of an SDS-PAGE gel). To identify the bands, you decide to perform a western blot.

You probe your western blot with primary antibody (rabbit anti-"A"), then a secondary antibody (goat anti-rabbit IgG) conjugated to alkaline phosphatase. Your NBT-BCIP reaction worked great—and you see that one of the bands in your most pure sample is definitely protein A.

Next, you want to check if the other band is protein B.

a) Your postdoc had protein B injected into chickens to obtain protein B-- specific antibodies (IgG). Antibodies made in chickens are concentrated in the egg yolk. What is a simple way to purify protein B—specific IgG molecules away from other proteins in the egg yolk? (Hint: this can be accomplished in ONE column).

You probe your Western blot with your purified chicken anti—"B" primary antibody. You then use the same secondary antibody as before (goat anti-rabbit IgG, alkaline phosphatase conjugate) and develop your blot. You see no bands on your blot.

b) Explain what went wrong, and how you would change your protocol to fix the problem. (Assume that the second protein in your most pure sample is indeed protein B, and that both antibodies are "functional"—i.e. not denatured.)

Question 7

After 7.02 ends, you decide to do a UROP in the lab of Dr. Jean Tics. Dr. Tics wants to see how much you learned about protein purification in 7.02, so she asks you to purify a well characterized protein (PHD Isomerase) using the lab's standard protocol.

Starting from crude lysate (CL), you performed the following steps (and created these samples to be assayed):

1. Precipitation by ammonium sulfate (yielding AS-P)
2. Desalting on PD-10 column (yielding DEAE-Load)
3. DEAE column (eluted fractions pooled as DEAE total)
4. Affinity column followed by PD-10 desalting (yielding AF after PD10)

You then assayed each sample for PHD Isomerase activity, and obtained the following results:

sample	Volume (ml)	Protein conc. (mg/ml)	Total protein (mg)	Total Activity (U)	Specific Activity	Fold Purification
CL	25	4	100	200000		
AS-P	4	10	40	120000		
DEAE-Load	2	17.5	35	105000		
DEAE-Total	2	11	22	68200		
AF after PD-10	1	3	3	60000		

a) Fill in the specific activity and fold purification for each step of the purification on the chart above (include units where appropriate).

b) Which step of the protocol gave the best purification? How do you know?

c) If Dr. Tics asked you to improve this protocol, which step might you choose to eliminate? Why?

Question 8

As part of your UROP you decide to try to purify human β-galactosidase (β-gal) from gut epithelial cells. You first need to develop an assay to track your human β-gal through the purification process. Prior experiments by a postdoc in your lab have indicated that human β-gal is present at ~0.5 mM in crude lysate from these cells.

a) You plan to set up your human β-gal assay by placing a sample of protein in Z buffer, then adding an appropriate substrate. Which of the following would be the most useful substrate for <u>following the purification</u> of human β-gal? Circle one and explain your reasoning.

i. lactose at 10mM ii. ONPG at 10 mM

iii. Xgal at 0.5 mM iv. ONPG at 0.5 mM

Armed with your assay, you are ready to begin your purification. You detect enzyme activity in your CL-S sample, and decide to next perform an Ammonium Sulfate precipitation. You don't know the concentration of AS at which human β-gal precipitates, so you decide to try three different concentrations: 10%, 35%, and 55%.

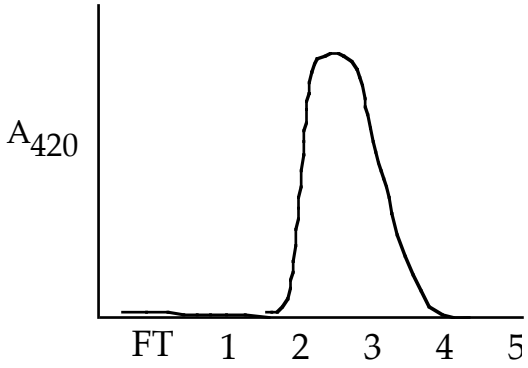
b) You start with 15 ml of CL-S. How much 100% ammonium sulfate would you need to add to bring the concentration of the CL-S to 55% AS? **Show your calculation**.

c) Another UROP in your lab (who didn't take 7.02!) thinks you are wasting your time trying to determine how much AS to use. He asks: "Why don't you just use 90% AS?" What would you tell him?

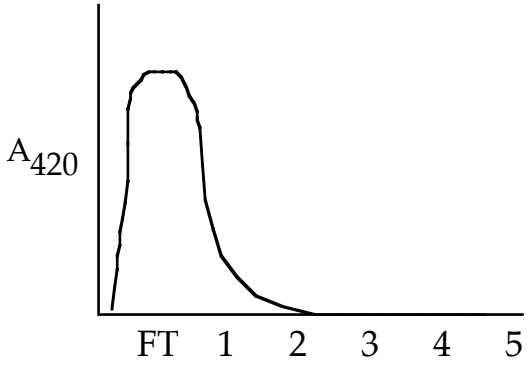
Question 8 (continued)

You decide to try anion exchange chromatography (DEAE column) next. To do this, you first equilibrate your column with column buffer (0.1M NaCl, pH 7.5). You then apply your desalted AS-P sample to the column, and collect the flowthrough (FT). Next, you elute the column with increasing concentrations of NaCl, and collect fractions (#1-5). Finally, you perform a quantitative assay of each fraction for activity.

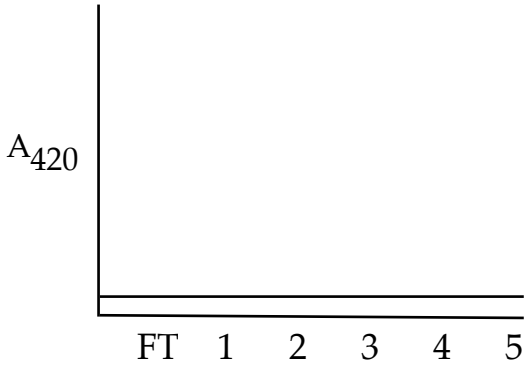
d) Below are three possible (independent) outcomes of this experiment. Explain what each outcome tells you about the properties of human β-gal under the conditions you ran the column. (You may assume that the AS-P fraction had activity before you loaded it.)



Properties of human β–gal indicated:



Properties of human β-gal indicated:

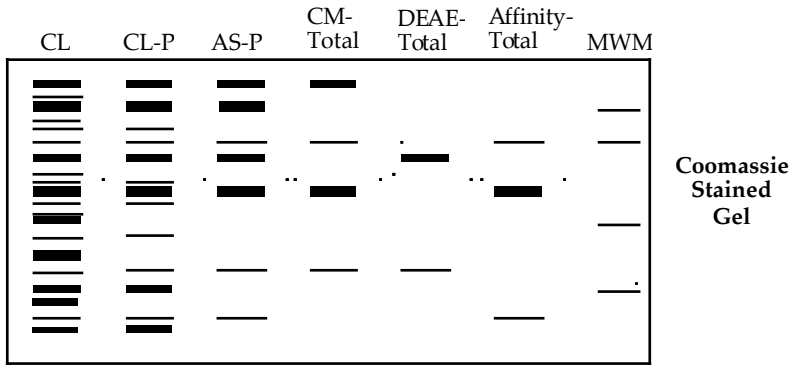


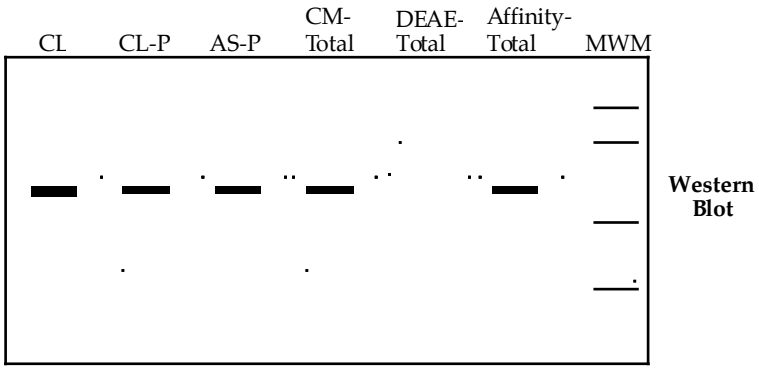
Properties of human β-gal indicated:

Question 8 (continued)

e) After finishing your purification, you pour an SDS polyacrylamide gel containing both stacking and separating portions, prepare your samples as in 7.02 lab, and load the gel. While the gel is running, you notice that the sample band is very **diffuse**. What might have gone wrong in the preparation of your gel, leading to the observation of a diffuse band?

Finally, you are interested in performing enzyme kinetics on the human β-gal protein. After attempting to purify the protein using anion exchange (DEAE), cation exchange (CM) and affinity columns (Affinity), you run your samples on a Coomassie stained gel and perform a Western blot. You observe the following:

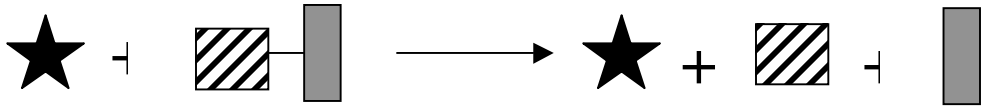




f) For enzyme kinetics, you want to use the sample with the highest β-gal specific activity--but you've run out of BioRad assay reagents! Which sample do you predict will have the highest β-gal specific activity? Explain your reasoning.

Question 9

The fictitious enzyme NMDase carries out the following reaction:



NMDase XY NMDase X Y (BLUE)

Experimentally, the progress of the reaction can be measured by looking at the production of product Y. Product Y is BLUE, and its accumulation can be measured in the spectrophotometer at A_{595}.

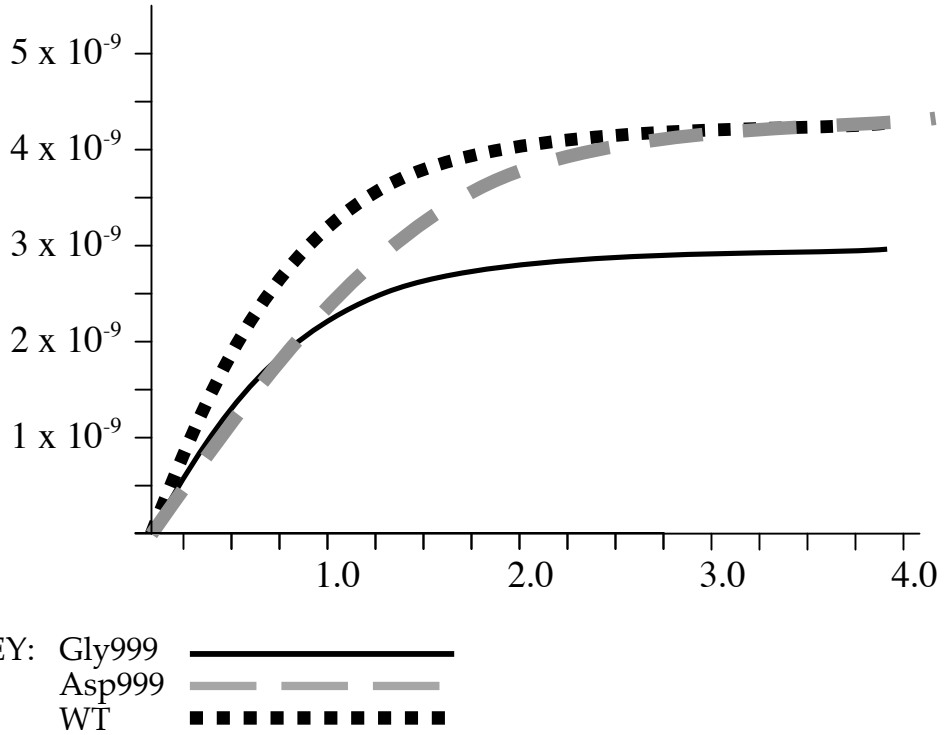
An important first step in the conversion of XY to X + Y by NDMase is the binding of enzyme to substrate. One residue suspected to be important for substrate binding is Trp999. To test this hypothesis, you have created mutant NMDase enzymes with the following amino acid substitutions at position 999: His999 (Trp999◊His999); Asp999 (Trp999◊Asp999); Gly999 (Trp999◊Gly999); and Lys999 (Trp999◊Lys999).

a) Draw the **side chains** of the following amino acids at pH 7.0. Indicate any charges expected in aqueous solution (positive, negative, neutral).

Tryptophan Charge _____	Histidine Charge _____	Aspartic Acid Charge _____
Glycine Charge _____	Lysine Charge _____	Any additional aa Name: _____ Charge _____

Question 9 (continued)

You perform enzyme kinetics on wild type NDMase and two of the mutants (Asp999 and Gly999) that have been purified for you by a colleague in your laboratory. You obtain the following Michaelis-Menton curves for the three enzymes:



b) Compare the **kinetic parameters** of the Asp999 and Gly999 enzymes to those of the wild type NDMase. If a parameter has changed, be sure to say **how** it has changed (increased or decreased). If a parameter is the same as wild type, indicate this as well.

c) Which part(s) of the enzymatic reaction (generalized as E + S $\longrightarrow$ ES$\longrightarrow$ E + P) is affected in the Asp999 mutant? In the Gly999 mutant? Explain your reasoning.

Question 9 (continued)

In order to compare the **Kcats** of the three enzymes (wt, Asp999, Gly999). you need information from the enzyme kinetics experiment described above as well as **two** additional experiments.

d) **Name** these two additional experiments, and describe what piece(s) of information needed for your Kcat calculation each provides.

After all these experiments, you've run out of your enzymes, and need to purify some more protein.

e) Using the Michaelis-Menton graphs on the previous page, identify the best concentration(s) of XY to use to **follow the purification** of wild type NDMase, Asp999, and Gly999. Justify your answer.

Question 10

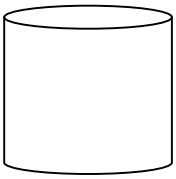
P22 phage adheres to the surface of the bacteria *Salmonella typhimurium* (*S. tymphimurium)* by binding to a membrane bound protein on the cell surface. In order to understand more about this P22/*Salmonella* interaction, you decide to purify this membrane protein using a density gradient centrifugation technique.

a) Name the type of density gradient centrifugation you would use to purify this protein and state the composition of the gradient (material and density range).

b) Draw the relative positions of membrane bound proteins and soluble proteins in the gradient tube below.

top of tube

bottom of tube



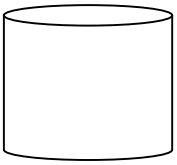
c) Name two characteristics that determine where a protein will migrate in this density gradient.

1._____ 2._____

d) The expression of the *S. tymphimurium* membrane protein that P22 attaches to is regulated by a DNA binding protein. What type of gradient would you use if you wanted to purify this DNA binding protein? Draw the relative positions of DNA and soluble proteins in this gradient.

top of tube

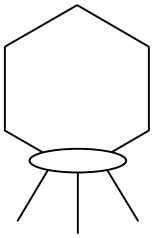
bottom of tube

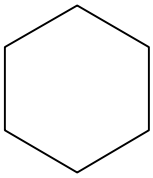


Question 10 (continued)

One of your colleagues has identified a mutant P22 ("tailless") that cannot lyse bacterial cells on its own. When she looks at these mutant P22 under the electron microscope, she sees that they have no tails!

Wild type P22





"Tailless" P22

Your colleague asks you to help her determine what P22 structural protein(s) are missing in the "tailless" P22. She gives you two tubes--one containing "tailless" P22 and one containing wild type P22--to work with, and you may assume that you have any equipment/reagents used in the PBC module at your disposal.

e) Describe an experiment that will allow you to determine how many protein(s) are missing in the "tailless" P22 mutant. **(BE BRIEF—3 sentences MAX!)**

f) In your experiment described above, what observation(s) will you make that let you know that a protein is "missing" in the "tailless" P22.

g) What additional piece of information can you gain about the P22 structural protein(s) from this experiment? _____

h) From your experiment in part e), you find that there are four proteins missing in the P22 tailless mutant. Name **five** properties that will likely differ between these proteins, and which could possibly be used to separate them during purification.

1. _____ 4. _____
2. _____ 5. _____
3. _____

Question 11

a) In the 7.02 PBC module, you used two different colorometric assays: the Bradford (BioRad) assay and the βgal assay. Complete the following chart to compare and contrast these two assays:

	Bradford (BioRad) Assay	βgal Assay
What is this assay used for?		
What are the components of each reaction? (i.e. what goes in the reaction tube?)		
Which of the above components was used to "start" the reaction?		
What did you observe after the reaction had proceeded for a while? What happened to give this result?		
How was the assay quantitated (wavelength)?		
What did you use as a standard, and what did you do with this data?		
What reagent did you use to stop the reaction, and how did this work?		

b) Two other experiments performed in 7.02 lab give you similar information as the Bradford and βgal assays, but do so in a qualitative rather than quantitative way. Name these two experiments, and explain your choices in a sentence or two.