MCB102 Midterm 1 Fall 2007

NAME with LAST NAME FIRST [redacted]

STUDENT ID [redacted]

Section Day Tues Time 10:00Am TA [redacted]

	Maximum Points	Your Points
Question 1	15	8
Question 2	25	24
Question 3	15	13
Question 4	25	+22
Question 5	10	+10
Question 6	10	10

87/100

regrade

88/100

REFERENCE INFORMATION:

Henderson-Hasselbalch $pH = pK_a + \log([\text{proton acceptor}]/[\text{protein donor}])$

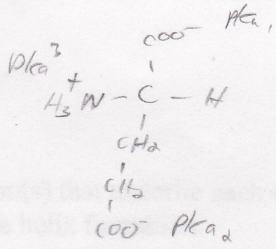
Glutamate: $pK_1 = 2.2$; $pK_2 = 4.3$; $pK_3 = 9.7$

$A = \varepsilon cl$

Name: [redacted]

8

9.7
6.3
3.4



Question 1

A. What percentage of glutamate molecules have no net charge at pH 6.3? **NOTE: reference information is provided on the cover page!**

$6.3 = 9.7 + \log\left(\frac{A^-}{HA}\right)$

$-3.4 = \log\left(\frac{A^-}{HA}\right)$

$10^{-3.4} = \frac{A^-}{HA}$

$A^- : HA$

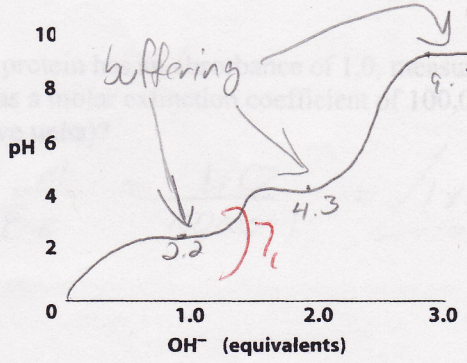
$10^{-3.4} : 1$

about 0% have no net charge

B. What net charge do the charged glutamate molecules carry at pH 6.3?

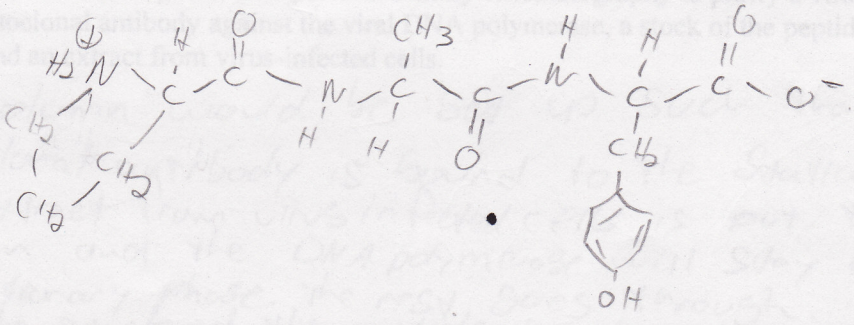
−1

C. Sketch the general shape of the titration curve of glutamate and indicate the region(s) in which buffering occurs. **NOTE: reference information on the cover page!**

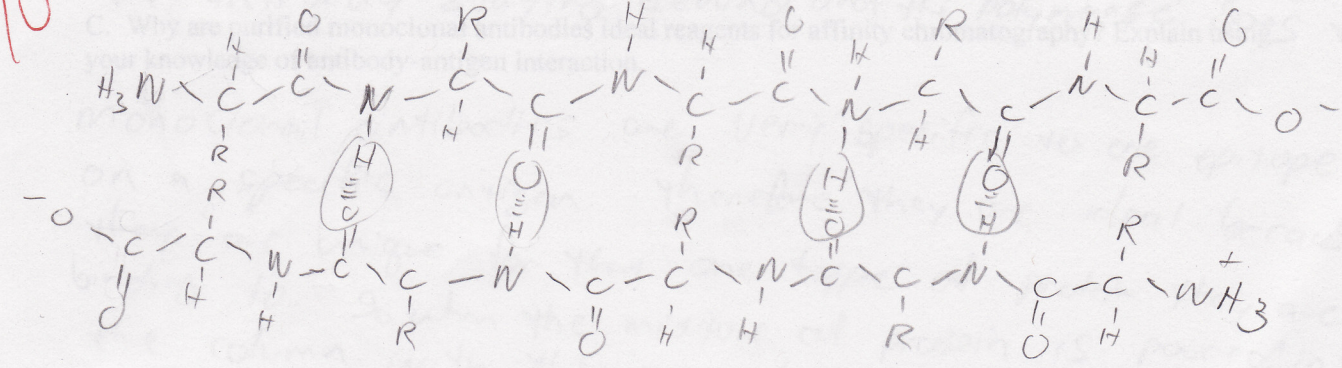


Question 2

A. Draw the structure of the peptide PAY at pH = 7.0. Make sure to indicate all atoms, bonds and charges.



B. Draw the polypeptide backbone (label side groups = R) and hydrogen bonding of two 5 amino acid beta strands paired together in an **anti-parallel** beta sheet (omit the connector between them).



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C. Indicate the energetic motivation(s) that underlie each of the following protein folding steps: (1) molten globule formation, (2) alpha helix formation.

1.) hydrophic collapse to remove H_2O from non-polar regions of the protein reduce the penalty of constricting the free H-bonds of the H_2O. Entropy + energy decrease ✓ 2

* 2) Increased stability from the added H-bonds and reduced steric interference by putting all the R groups to the outside of the helix VDW

Question 3

A. A sample of your purified protein has an absorbance of 1.0, measured in a cuvette with 1 cm path length and 280 nm light. It has a molar extinction coefficient of 100,000. What is the concentration of your protein (make sure to give units)?

$A = \varepsilon c l \quad c = \frac{A}{\varepsilon e} = \frac{1.0}{100{,}000 \cdot 1} = 1 \times 10^{-5} M$ 4

B. Describe in brief how you would perform affinity chromatography to purify a viral DNA polymerase using a monoclonal antibody against the viral DNA polymerase, a stock of the peptide recognized by the antibody, and an extract from virus-infected cells.

The column would be set up such that the monoclonal antibody is bound to the stationary phase. The extract from virus infected cells is put through the column and the DNA polymerase will stay behind bound to the stationary phase. the rest goes through. next you would put the stock of the peptide in the column to displace the DNA polymerase from the stationary phase while the peptides bind to the antibody staying behind and the polymerase goes through 7

C. Why are purified monoclonal antibodies ideal reagents for affinity chromatography? Explain using your knowledge of antibody-antigen interaction.

monoclonal antibodies are very specific to one epitope on a specific antigen. therefore they are ideal because they are unique for that one type of protein they are binding to. So when the mixture of protein is poured into the column with the monoclonal antibody bond to the beads, the only protein that will be left behind is the one of interest 2

Name: [redacted]

Question 4

+3 A. How can you discriminate cooperative ligand binding from a plot of ligand concentration [L] versus concentration of protein-ligand complex [PL]?

when [L] and [PL] are plotted, a sigmoidal curve will indicate cooperative ligand binding.

+5 * B. Define the specificity constant, including units, and how the two terms in the specificity constant combine to provide a ranking of enzyme perfection.

specificity constant = $\frac{k_{cat}}{K_m}$ $\frac{+2 \text{ units/s}}{M^{-1}}$ $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} EP$

$K_m = \frac{(k_2 + k_{-1})}{k_1}$

the larger k_{cat} is, the better the enzyme is at displacing the product. So large k_{cat} = fast enzyme. The smaller K_m is, the better the enzyme binds to the substrate. So when these numbers are combined k_{cat}/K_m → the larger this ratio = Enzyme perfection

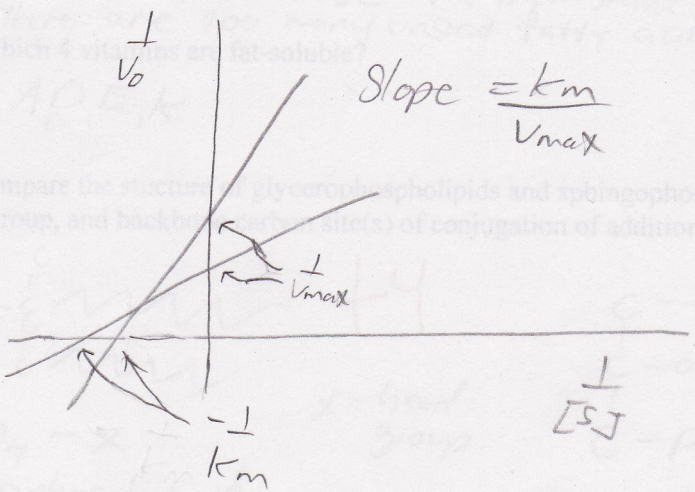
explain!

+6 C. Why are proteases like chymotrypsin classified as serine proteases? What residues other than serine participate in the catalytic mechanism and what are their roles in general terms (do NOT list specific reaction steps; this is supposed to have only few-word answers)?

They are serine proteases because serine acts as a nucleophile to attack the carbonyl carbon in the process of cleaving the peptide. Aspartate increases the ~~pKa~~ of histadine so it takes the proton from serine making it a good nucleophile. Glycine helps stabilize the acyl intermediate with H-bonds

+2

+8 D. Draw an example of data that would indicate mixed inhibition using a double-reciprocal (Lineweaver-Burk) plot. You can draw only two lines. Make sure to label both axes and also label both intercepts and the line slope in V_{max} and K_m. Units on the axes are not required.



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Question 5

A. Proteins can be modified to carry covalently attached oligosaccharide or lipid.
(1) Which protein side chain can be the site of attachment of a sugar or a lipid?

Serine +2

A few other reactive sites can be coupled to sugar or to lipid but not to both. Give one example of a sugar-specific and one example of a lipid-specific site of covalent protein modification.
(2) sugar-only?

asparagine asn +2

* (3) lipid-only?

cystein +2

B. (a) Explain in molecular terms why cattle but not humans can use the plant polysaccharide cellulose as a nutrient, while humans can use the plant polysaccharide starch. (b) What polysaccharide do humans produce for sugar storage?

a) a cow has an enzyme that can cleave a $\beta(1-4)$ linkage in cellulose that humans don't have. humans have an enzyme that can cleave the $\alpha(1-4)$ linkage in starch to produce glucose units

+4

b) glycogen

Question 6

A. The bacterium *E. coli* can grow at 20 °C or at 40 °C. At which growth temperature would you expect the membrane phospholipids to have a higher ratio of saturated to unsaturated fatty acids, and why?

At 40°C, unsaturated fatty acids have a cis bond allowing them to be more fluidic when packed against eachother in the membrane. At a high temp, there is no need for a lot of unsaturated fatty acids because the membrane will be fluid enough. If there are too many unsat fatty acids at high temp, the membrane may break.

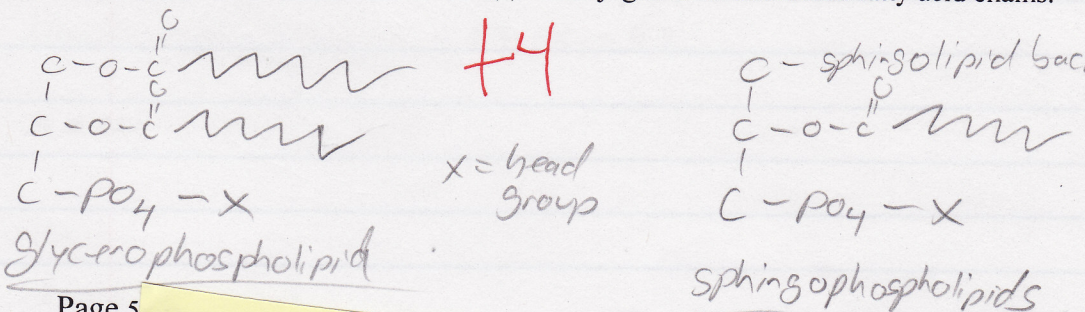
+2

B. Which 4 vitamins are fat-soluble?

A, D, E, K

+4

C. Compare the stucture of glycerophospholipids and sphingophospholipids including the backbone, headgroup, and backbone carbon site(s) of conjugation of additional fatty acid chains.



+4

glycerophospholipid

sphingophospholipids

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