Chem 135: First Midterm

September 21st, 2012

Please provide all answers in the spaces provided. You are allowed to use a calculator for this exam, and you may use (previously disassembled) molecular model kits. Including the title page, there should be **4** total questions spread over **5** pages. There is also a sixth page that should be blank. You can use this last page for scratch paper if you need it, but please remember to copy your answers into the appropriate exam question space.

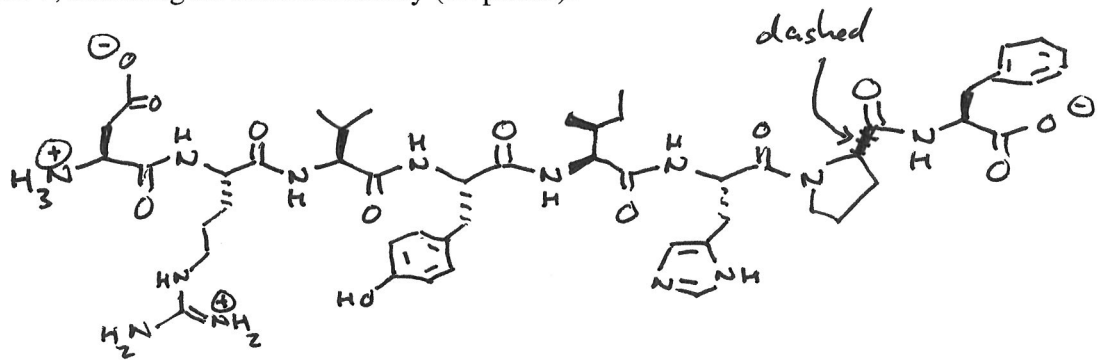
Name: Key

(1)	_____	(45 points)
(2)	_____	(15 points)
(3)	_____	(25 points)
(4)	_____	(15 points)
TOTAL	_____	(100 points)

1. Angiotensin II is a short peptide hormone that is involved in the regulation of blood pressure. It is an octapeptide of the following composition, with free N- and C-termini:

DRVYIHPF

a. Provide a full chemical structure of the angiotensin II peptide in its most abundant protonation state at pH 7, including all stereochemistry (12 points).



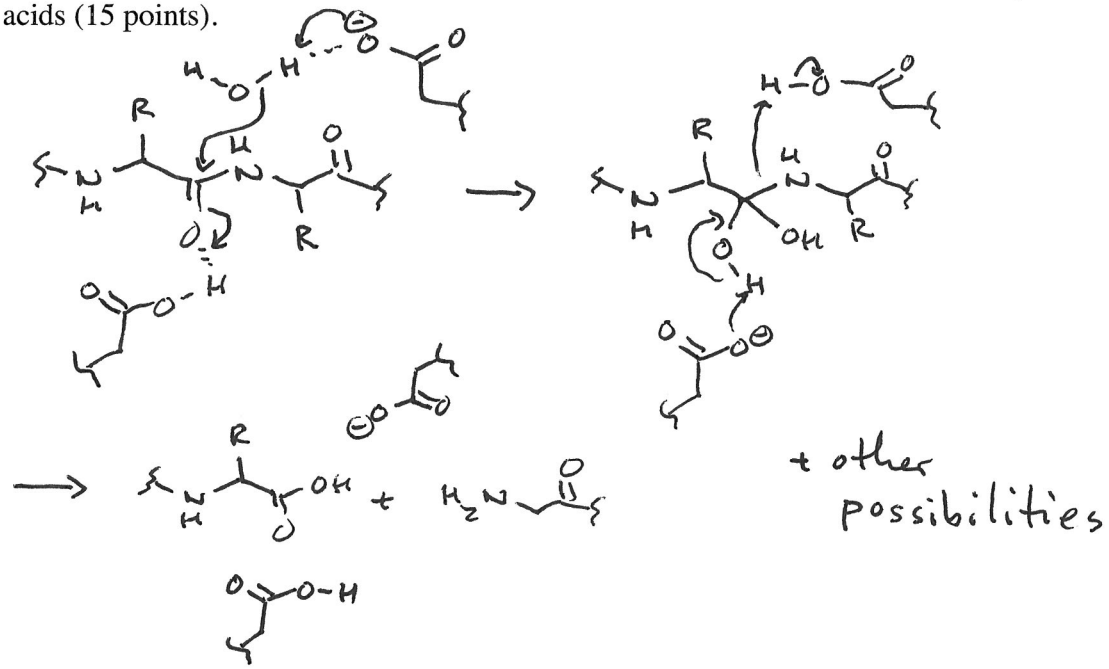
b. Give an example of a pH value at which the net charge of the peptide will be +1 (4 points).

Any pH below 6.5 and above 4.0.

c. Give an example of a pH value at which the net charge of the peptide will be –1 (4 points).

Any pH above 8 and below 10.

d. The biosynthesis of angiotensin II begins with the cleavage of the ten N-terminal amino acids of a protein called "antiotensinogen". The remaining large protein fragment is then discarded, and the 10-residue sequence becomes "angiotensin I". The enzyme responsible for this transformation is called "renin", and is produced by the kidneys. The active site of renin possesses two aspartic acid residues that are required for catalytic activity. *Provide a detailed, arrow pushing mechanism* that shows how this pair of aspartate residues could lead to the hydrolysis of a peptide bond. You may abbreviate your peptide substrate using 'R' groups, so long as your structures are clear. For the purpose of this question, you do not have to indicate the stereochemistry of the amino acids (15 points).



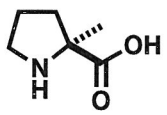
e. Suppose you generate a mutant of renin in which one of the aspartate resides has been switched to an asparagine residue. Would you expect this change to have a larger effect on the value of K_M or k_{cat}? Briefly explain your answer (5 points).

Since this residue is directly involved in catalysis, k_{cat} should be lowered substantially.

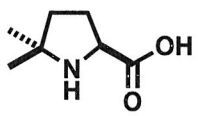
f. Once angiotensin I has been produced by renin, it is converted to angiotensin II by the "angiotensin converting enzyme" (ACE). Suppose you are studying a sample of the ACE protein that originally came from a fruit fly (*Drosophila melanogaster*). The protein you obtain from this organism is catalytically active and consists of 602 amino acids. Using mass spectrometry, you determine that the molecular weight of the protein is 69665 Daltons. In an attempt to make things simpler, you decide to express the protein in *E. coli* bacteria, and find that you can do so successfully. However, the protein you obtain from *E. coli* has no catalytic activity. Upon analysis, you find that the protein still has 602 amino acids, but now the mass is 69671 Daltons. Propose a hypothesis that could explain why the two samples have different masses and reactivity. Also provide a brief description of an experiment that you could perform to test your hypothesis (5 points).

The mass change is +6 Da, which could correspond to the loss of 3 disulfide bonds that were structurally important. One could compare the proteins using SDS-PAGE, or treat the active one w/ DTT.

2. As a way to probe the role of proline conformation in biology, the two proline analogs shown below (2-Me-Pro and Dmp) have been incorporated into synthetic peptides to bias the conformations. Draw each of these compounds as a dipeptide with a valine residue at the N-terminus (i.e. val-2-Me-Pro and val-Dmp), including all stereochemistry. Provide the *trans* and *cis* peptide conformations for each, and circle the conformation that would be preferred in each case (15 points).



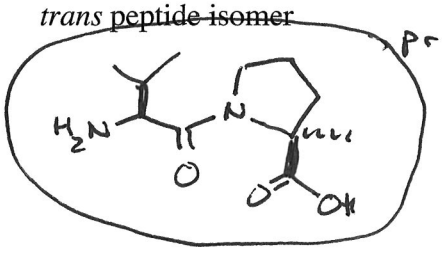
2-Me-Pro



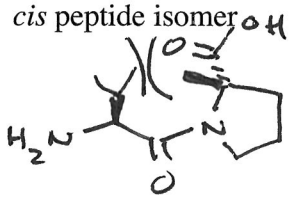
Dmp

val-2-Me-Pro dipeptide:

trans peptide isomer

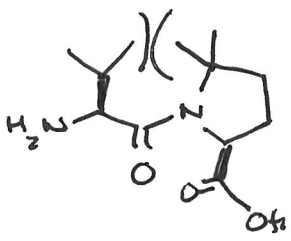


cis peptide isomer

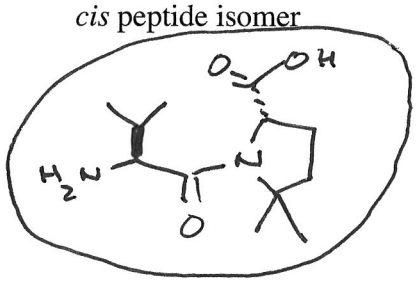


val-Dmp dipeptide:

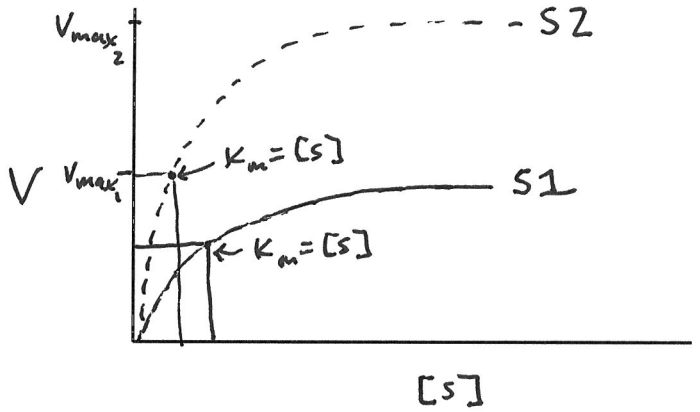
trans peptide isomer



cis peptide isomer



3. The following question refers to a hypothetical enzyme that obeys Michaelis-Menten kinetics.
   a. Using the axes below, plot the rate (V) of an enzymatic reaction as a function of substrate concentration ([S1]). Show how your plot can be used to determine the values of k_{cat} and K_M. Next, suppose an equal concentration of the enzyme is exposed to a different substrate (S2), which corresponds to a 2-fold lower (improved) value of K_M and a 2-fold higher value of k_{cat}. Plot the V vs. [S] curve for this second enzyme using the same axes. Clearly label the two different curves (10 points).



for both,

$V_{max} = [E]_T k_{cat}$

   b. Now consider the situation in which the enzyme will encounter an equimolar mixture of the two substrates. Which substrate will be processed more quickly by the enzyme and by how much when S1 and S2 are present in very high concentrations? Briefly show how you arrived at your answer (5 points).

$[S2]_p$ denotes "Product of S2"

At high conc, S2 will be processed to $[S2]_p$ more quickly

$$\frac{[S2]_p}{[S1]_p} = \frac{k_{cat(S2)}}{k_{cat(S1)}} = 2$$

   c. For an equimolar mixture of the two substrates, which substrate will be processed more quickly by the enzyme and by how much when S1 and S2 are present in very low concentrations? Briefly show how you arrived at your answer (5 points).

S2 is still processed more quickly

$$\frac{[S2]_p}{[S1]_p} = \frac{(k_{cat}/K_m)_{S2}}{(k_{cat}/K_m)_{S1}} = 4$$

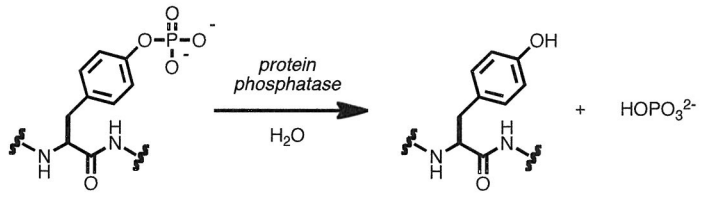
   d. Considering different possible values of k_{cat} and K_M, can you devise a scenario in which one substrate will be processed with 10-fold selectivity at high concentrations, but also in which both will be processed with equal rates at very low concentrations? Provide specific values (including units) for k_{cat} and K_M for the two substrates (5 points).

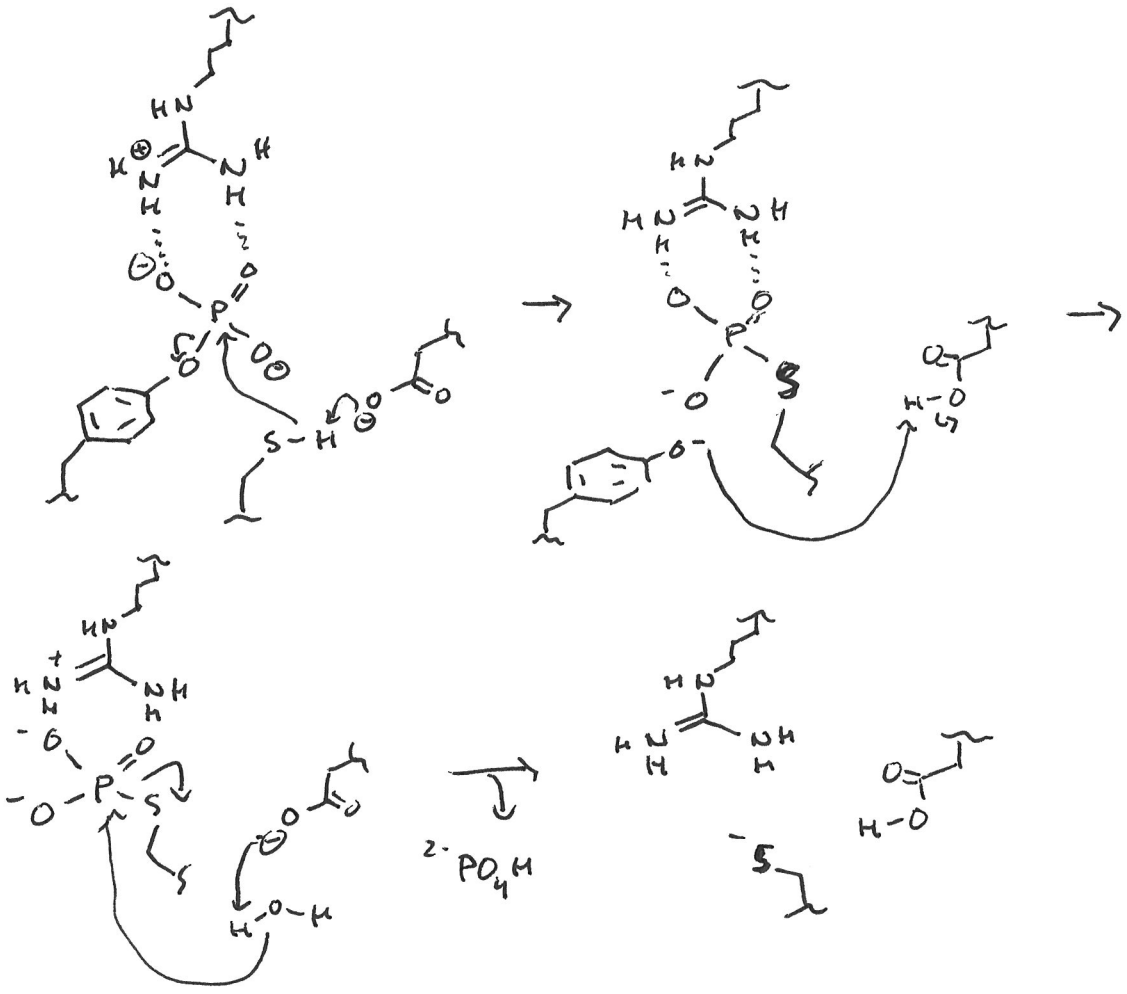
One example:

$(k_{cat})_{S1} = 10\ s^{-1}$ $\quad (K_m)_{S1} = 1 \times 10^{-4}\ M$

$(k_{cat})_{S2} = 100\ s^{-1}$ $\quad (K_m)_{S2} = 1 \times 10^{-3}\ M$

4. One common form of protein regulation is the addition of a phosphate group to serine, threonine, or tyrosine residues. This can change the protein conformation or alter its binding properties—either of which can produce a new signal in a cell. To stop the signal, the phosphate group is removed from the residue by *protein phosphatase* enzymes. Tyrosine phosphatases commonly contain one Arg residue in the active site and no metal ions. They also contain a Cys that is required for catalysis, and an Asp residue that increases activity. By making an analogy to protease enzymes, show how these three residues would work to catalyze the reaction shown below. You do not need to include full amino acid structures or indicate stereochemistry for the purposes of this question (15 points).





This last page should be blank. You may use it as scratch paper, but be sure to recopy your answers into the exam questions so that we can grade them easily.