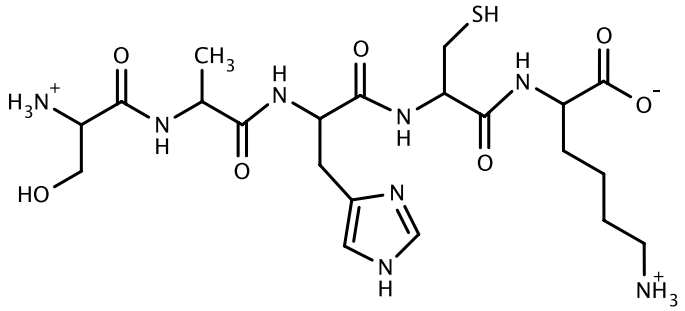
Name:_____

Biology 3515/Chemistry 3515
Biological Chemistry Laboratory
Spring Semester 2017
Quiz 3 - 25 April 2017

Please write your name on each page.
Be sure to show your work and include correct units in all of your answers!
You do **not** need to fill all of the space provided for all of the questions! Short clear answers are better than long fuzzy ones.

50 points total.

1. The peptide with the structure drawn below happens to contain several of the amino acid residue types that we have discussed at various times through the semester.



The structure is drawn to represent the ionization state that would predominate at pH 7. For the following, assume that the terminal amino group of the peptide has a pK_a of 8, and the pK_a of the terminal carboxyl groups is 4. You should know the approximate pK_as of the side chains.

(a) (3 pts.) Write the sequence of this peptide, using the full amino acid names or 3-letter abbreviations. Follow the usual convention of writing sequences from N- to C-terminus.

(b) (2 pts.) In the drawing above, circle the atoms that will change ionization state if the pH is decreased from 7 to 2.

(c) (2 pts.) What will the net charge of the peptide be at pH 2?

(d) (5 pts.) In the table below, write the expected net charge of the peptide at the indicated pH values from 3 to 9. Indicate the charge to the closest half-integer value. Also identify the functional group(s), if any, that undergo a change in ionization when the pH is increased from that indicated in the row above (*e.g.,* from 3 to 5).

pH	Charge	Functional group with changed ionization
3		_____
5		
7		
9		

(e) (4 pts.) At pH 8.3, in what fraction of the molecules would you expect the terminal amino group to be charged?

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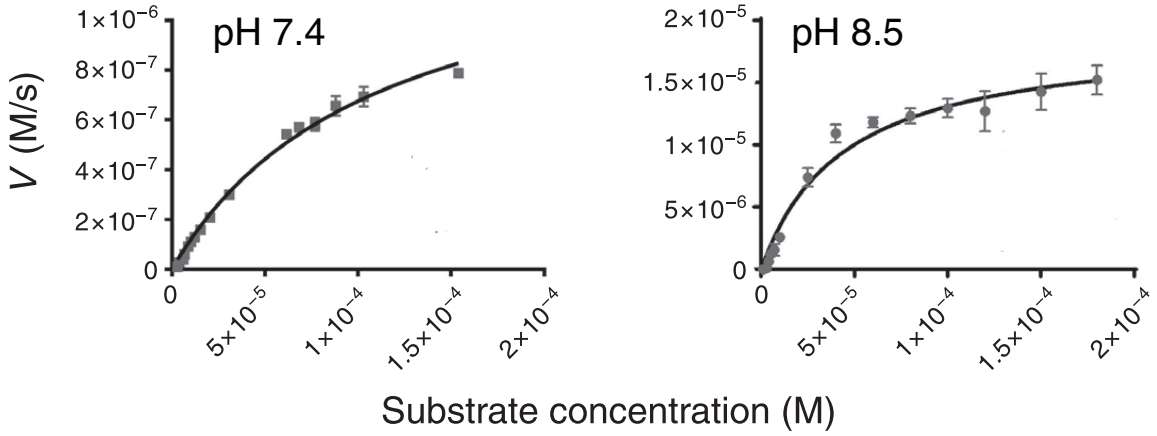
2. In class, we discussed the development of inhibitors of the Zika virus protease, as a possible first step in the development of pharmaceuticals to treat infections by this virus. Before, inhibitors could be studied, it was necessary to develop a suitable substrate for in vitro assays. A paper published in 2016[1] Describes the screening of different peptide substrates to identify sequences recognized by the Zika virus protease. One of substrates, based on the sequence Val-Lys-Lys-Arg, was chosen for more detailed study. The plots below show the results of velocity versus substrate concentration experiments carried out using two buffer solutions:



In both experiments, the enzyme concentration was 5.5×10^{-8} M.

(a) (2 pts.) From the graph on the left, estimate the values of $V_{\max}$ and K_{m}, with units of μM/s and μM, respectively, in the pH 7.4 buffer.

(b) (2 pts.) From the graph on the right, estimate the values of $V_{\max}$ and K_{m}, with units of μM/s and μM, respectively, in the pH 8.5 buffer.

[1]Gruba, N., Martinez, J. I. R., Grzywa, R., Wysocka, M., Skorenxi, M., Burmistrz, M., Lecka, M., Lesner, A., Sienczyk, M. & Pyrc, K. (2016). Substrate profiling of Zika virus NS2B-NS3 protease. *FEBS Lett.*, 590, 3459–3468. `http:\\dx.doi.org/10.1002/1873-3468.12443`

(c) (4 pts.) The enzyme appears to be substantially more active in the pH 8.5 buffer than in the pH 7.4 buffer. From the data, do you think that this difference is due primarily to a difference in the binding of the substrate in the two buffers or to a difference in the rate of catalysis? Briefly explain your answer and any assumptions you have made.

(d) (3 pts.) At pH 8.5, what value of $V_{\max}$ would you expect when the enzyme concentration is $1\,\mu$M?

(e) (3 pts.) Calculate the expected reaction rate at pH 8.5 if the enzyme concentration is $1\,\mu$M and the substrate concentration is $50\,\mu$M.

(f) (3 pts.) Under the conditions described in part (e) above, what fraction of the enzyme will have substrate bound to it?

3. (5 pts.) The irreversible protease inhibitor we studied in class, 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), is commonly used in biochemical research to inhibit the activities of proteases in biological samples, thereby protecting other proteins of interest. Typically, AEBSF is added to a sample before or shortly after the cells are disrupted to release the proteins. From our experiments, we know that the second-order rate constant for the inactivation of trypsin with AEBSF is approximately $100\,\mathrm{min^{-1}M^{-1}}$ at 4°C and pH 8.

   Suppose that you are preparing a cell extract and you want to be sure to inactivate 99% of the protease in the sample. The concentration of proteases in the extract is estimated to be no more than $10\,\mu$M. If you use 1 mM AEBSF, how long will you need to incubate the reaction?

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4. In the laboratory, we used gel electrophoresis to compare three forms of ribonuclease A (RNAse A):

   - The native protein
   - The protein with its disulfides reduced and the thiols modified with iodoacetic acid (RCM)
   - The protein with its disulfides reduced and the thiols modified with iodoacetamide (RCAM)

   Suppose that you were to subject these three samples to gel-permeation chromatography. Assume that you use a chromatography matrix for which K_{ave} lies between 0 and 1 for all three forms. Be sure to explain your reasoning in answering each of the following questions:

   (a) (3 pts.) Which form (or forms) of RNAse A would you expect to elute from the column first?

   (b) (3 pts.) For which form (or forms) would you expect K_{ave} to be largest?

(c) (3 pts.) Suppose that you wanted to separate the native and RCAM forms of RNAse A by affinity chromatography. Suggest a type of molecule that you might attach to a chromatography matrix that would enable you to specifically bind one of the two forms to the matrix. Which form would be bound?

(d) (3 pts.) Suggest a chromatographic method that could be used to separate the RCAM and RCM forms of RNAse A. Briefly explain how this method would separate the two forms.