Name:_____

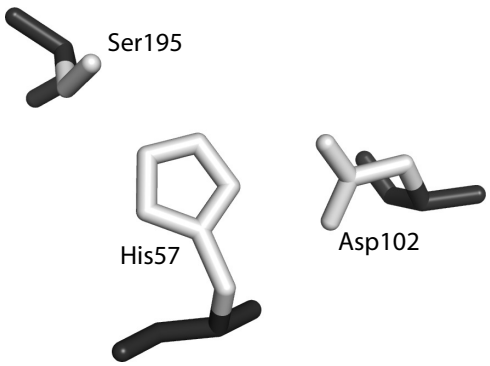
Lab section:_____

Biology 3515/Chemistry 3515
Biological Chemistry Laboratory
Spring Semester 2015
Quiz 2 - 12 March 2015

Please write your name on each page.
Be sure to show your work and include correct units in all of your answers!
25 points total.

Special note: Extra space has been left for some of the answers. Please do not feel compelled to fill all of the space!

1. (6 points) Trypsin, like other members of the serine protease family, contains in its active site three critical residues, referred to as the catalytic triad and shown in the figure at the right. In this drawing, the bonds between backbone atoms are shown in dark gray, and the side-chain bonds are light gray.



Briefly but specifically, describe the role of each of these residues in the catalytic mechanism of a serine protease:

(a) His57

(b) Asp102

(c) Ser195

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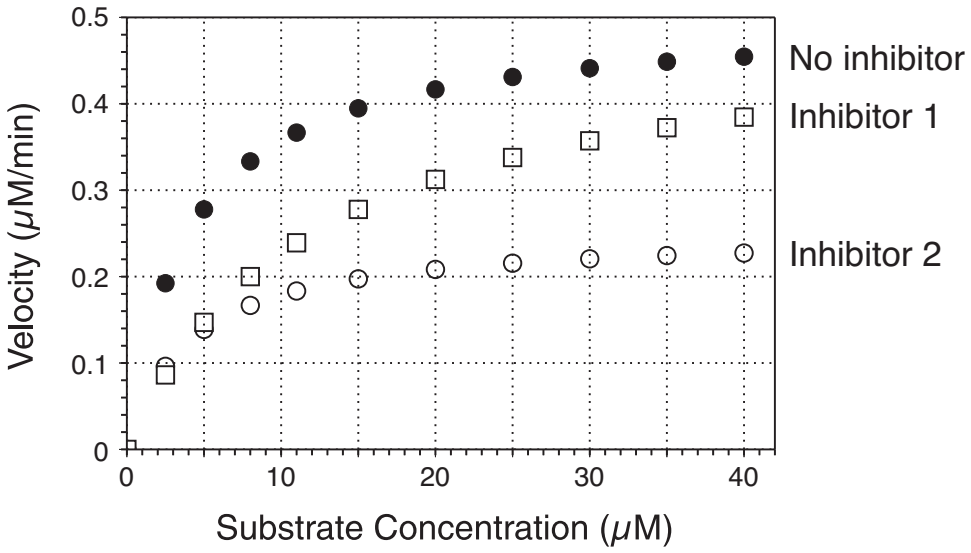
2. (11 pts.) Collagen is a distinctive protein found in the connective tissues of animals. The unusual sequence and structure of collagen make it resistant to many proteases, but there are proteases that effectively degrade it. One of these, found in humans, is called matrix metalloproteinase 13 (MMP-13) and has a molecular weight of 42,300 Da. Elevated levels of MMP-13 are associated with osteoarthritis, and it is believed that the protease contributes to the breakdown of connective tissue in this condition. As a consequence, there is considerable interest in developing inhibitors of MMP-13.

   Scientists at the giant pharmaceutical company, Proteins-R-Us, have purified MMP-13 and developed an assay to measure its activity in vitro. The graph below shows the results of measuring the reaction velocity of the enzyme without any inhibitor present (filled circles) and with two inhibitors that have been discovered (open squares and circles). For each of the experiments, the enzyme concentration was 0.5 μg/mL.



(a) From the graph, estimate K_{m} and V_{max} for the uninhibited enzyme.

(b) Calculate k_{cat} for the enzyme in the absence of inhibitor.

(c) Suppose that, for a specific experiment, you want to use a substrate concentration of $10\,\mu$M and you want the reaction velocity to be $0.1\,\mu$M/min. What enzyme concentration would you use for this experiment? You may express your answer in units of either mass concentration (e.g. μg/mL) or μM concentration.

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3. (8 pts.) Turn now to the data for the two inhibitors.

   (a) One of the inhibitors binds only to the free enzyme, whereas the other binds equally well to the free enzyme and the enzyme-substrate complex. From the kinetic data, which of the inhibitors appears to bind only to the free enzyme?

   (b) Briefly but clearly, justify your answer to the previous question by identifying the mechanisms for the two inhibitors and explaining how these mechanisms give rise to the results shown in the graph.

   - Inhibitor 1

   - Inhibitor 2

Problems 2 and 3 were inspired by a recent paper describing inhibitors of MMP-13: Spicer, T. P., Jiang, J., Taylor, A. B., Choi, J. Y., Hart, P. J., Roush, W. R., Fields, G. B., Hodder, P. S. & Minond, D. (2014). Characterization of selective exosite-binding inhibitors of matrix metalloproteinase 13 that prevent articular cartilage degradation in vitro. *J. Med. Chem.*, 57, 9598–9611. `http://dx.doi.org/10.1021/jm501284e`