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Biology 3515/Chemistry 3515
Biological Chemistry Laboratory
Spring Semester 2016
Quiz 3 - 21 March 2016

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. Two students have been given a solution of ATP and asked to determine its concentration by measuring the absorbance at 260 nm. The extinction coefficient of ATP at this wavelength is 15,400 $cm^{-1}M^{-1}$. When they attempt to measure the absorbance of the solution, they discover that it exceeds the maximum value that can be measured by their spectrophotometer. So, they dilute the sample by 10-fold. Using a cuvette with a path length of 1 cm, they determine that the diluted sample has an absorbance of 0.673,

   (a) (4 pts) What fraction of the incident light is absorbed by the ATP in the solution?

Name:_____

(b) (4 pts) What is the concentration of the undiluted solution of ATP (in mM units)?

2. Angiotensin converting enzyme (ACE) plays a major role in regulating blood pressure, and inhibitors of this enzyme are widely used as pharmaceuticals in the treatment of hypertension and other conditions. ACE is a protease, and one of the reactions it catalyzes is the cleavage of angiotensin I, which is a 10-residue peptide, to produce an 8-residue peptide, angiotensin II. Angiotensin II is a potent hormone with several activities, including the stimulation of vasoconstriction.

   Using a synthetic substrate, the kinetic parameters of ACE have been determined to be $K_{\mathrm{m}} = 300\,\mu\mathrm{M}$ and $k_{\mathrm{cat}} = 19,000\,\mathrm{min}^{-1}$.

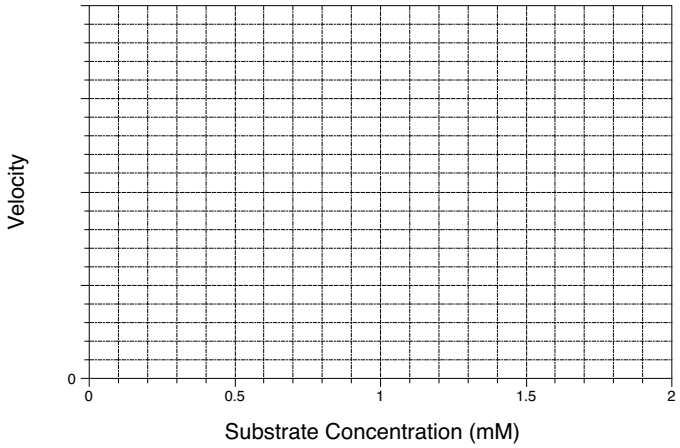
   (a) (4 pts) Suppose that you want to carry out a series of kinetic experiments with ACE, under conditions where V_{max} will be $15\,\mu\mathrm{M/min}$. Calculate the concentration of enzyme that you should use for your experiments.

Name:_____

(b) (4 pts) What reaction velocity would you expect to observe using the enzyme concentration you calculated above and a substrate concentration of 1.6 mM?

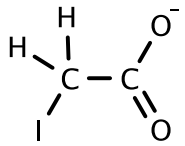
(c) (3 pts) Under the conditions specified for part *b*, what fraction of the enzyme molecules would have substrate bound to them?

(d) (3 pts) On the coordinate axes below, sketch out the graph that you would expect to generate if you were to measure the reaction velocity as a function of substrate concentration, using the enzyme concentration you calculated in part *a* and the range of substrate concentrations indicated on the horizontal axis. Add your own labels to the vertical axis, with the units identified, and indicate the point on the curve where $[S] = K_m$. You should not need to do any additional calculations for this part.



Name:_____

3. In the lab we used iodoacetate, with the structure shown below, to irreversibly block the thiols of ribonuclease A after reducing the disulfides of the native protein.



When this reagent reacts with a thiol, it is actually the thiolate ion that is the reactive species. As a consequence, the rate of reaction depends on the fraction of the thiols that are in the ionized form. For the following, assume that the pK_a of a cysteine thiol is 8.3 and that the second-order rate constant for the reaction of the **ionized form** of a thiol is $10\,s^{-1}M^{-1}$.

(a) (5 pts) Suppose that you want to carry out a cysteine-modification reaction with a protein known to contain a single Cys residue. The reaction will be carried out at pH 8. At this pH, what fraction of the Cys thiols will be in the reactive form?

(b) (4 pts) If you were to determine the second-order rate constant for this reaction at pH 8, based on the total concentration of iodoacetamide in the reaction, what value would you expect to find?

(c) (4 pts) In your reaction, the protein concentration will be 50 μM. Suggest a reasonable concentration for the iodoacetate that will ensure that the reaction kinetics will be well approximated as pseudo first-order. Briefly explain how you decided on this concentration.

(d) (3 pts) What will the value of the pseudo first-order rate constant be under these conditions.

(e) (5 pts) Using the iodoacetate concentration you chose in part c, calculate the minimum time for the reaction that will ensure that 99% of the protein molecules will have reacted with iodoacetamide.

Name:_____

4. (7 pts) After carrying out the reaction described in the previous problem, you want to determine whether or not it proceeded as expected. Suggest an experiment, based on a technique that we used in class, that you could carry out to determine the extent to which the reaction has gone to completion. Be sure to describe the possible results of the experiment and how they would be interpreted. Also describe any important conditions of the experiment that must be specified and any important controls.