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BICH 440, EXAM 3, Wednesday, November 19, 2008, 100 points total

You MUST sign the following academic integrity statement:

On my honor, I have neither given nor received unauthorized aid on this academic work.

Signed: _____

1. Write your name and UID# on the cover page ONLY.
2. Write your student UID# on page 2 (at least). If you do not remember your UID#, make up a random 8 digit number. DO NOT use your social security number.
3. Write concise answers to demonstrate effectively your mastery of the subject. In order to obtain maximum credit, you need to show your work.

gas constant R 8.315 J/mol-K
Faraday constant F 96.5 kJ/mole-volt

Exam 3 Score _____

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1. (12 pts) Draw the mechanism of Ribonuclease A. Include the entire sidechains (R-groups) of important amino acid residues in the active site, the phosphodiester backbone of RNA at the site cleaved by the enzyme, and the movement of electrons and protons in the reaction.

2. (10 pts) 2 micrograms of an enzyme of molecular weight 60,000 are used for kinetic assays. It is determined that the turnover number of this enzyme is 60 min^{-1} and the catalytic efficiency (k_{cat}/K_m) is $10^6 \text{ M}^{-1}\text{sec}^{-1}$. Sketch the double reciprocal plot that describes the Michaelis-Menten kinetics of this enzyme on the graph paper below. The units on the x-axis should be $1/\text{micromolar}$ and the units on the y-axis min/nmole . **IMPORTANT:** To receive full credit, and partial credit if necessary, you **MUST** show your work in the white space below.

3. (15 pts) Draw the mechanism presented in your book (and lecture) for aspartic proteases. Include the important functional groups for important amino acid residues used in this mechanism, an abbreviated version of the peptide bond to be cleaved, important hydrogen bonds, and the movement of electrons and protons. (I do not care whether you depict hydrogen bonds as “low barrier” or “normal”.)

4. (5 pts) What is the substrate concentration for an enzyme-catalyzed reaction (that follows Michaelis-Menten kinetics) when the initial velocity is 10% of the maximal velocity? The K_m for this enzyme is 50 micromolar.

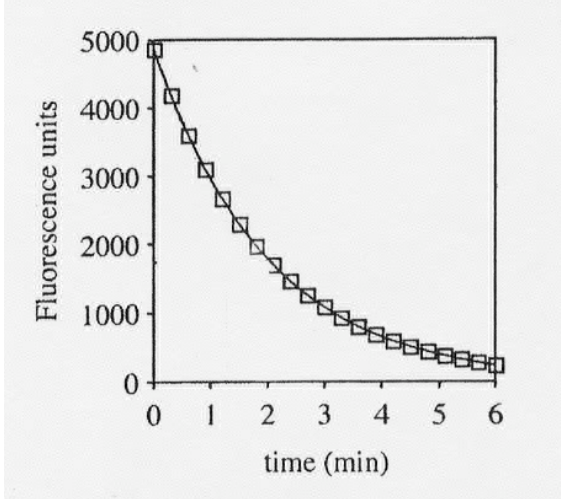
5. (15 pts) Given the following double-stranded fragment of DNA:

5'-GGCCTCAGATTACGCGAGTCCTTAGCCGGTACGCCTGCGAATCGC-3'
3'-CCGGAGTCTAATGCGCTCAGGAATCGGCCATGCGGACGCTTAGCG-5'

Using a primer with the sequence 5'-GGCGTACCGGCTAAGGACT-3', DNA polymerase, and the nucleotide combinations given below, write the DNA fragment(s) produced with each reaction mixture. Do not write out the sequence of the primer each time; simply write 5'-primer- followed by the correct sequence(s).

- A) dGTP, dTTP, dATP, ddATP
- B) dGTP, dTTP, dATP, dCTP, ddATP (at much lower concentration than dATP)
- C) dGTP, dATP, dCTP, ddTTP
- D) dCTP, dGTP, dTTP, ddCTP (at much lower concentration than dCTP)
- E) dGTP, dCTP, dTTP, dATP, ddTTP (at much lower concentration than dTTP)

6. (6 pts) The graph below shows the dissociation of a bimolecular complex, AB. The amount of AB complex was measured by a fluorescence assay such that when the fluorescence is zero, there is no complex. What is the dissociation rate constant (k_{-1}) for this system? Is this a first-order or second-order rate constant? Make sure to show your reasoning.



7. (8 pts) In lecture I discussed the QuickChange protocol for site-directed mutagenesis of the DNA sequence within a plasmid DNA. Briefly, describe this technique.

8. (29 pts) Shorter answer questions.

A)(4 pts) Name the four general classes of proteases

B)(2 pts) What are two different proteins produced by HIV retrovirus that are effectively targets for control of this infection by drugs?

C)(8 pts) What four distinct regions of the active site of chymotrypsin did I discuss in lecture and BRIEFLY describe the function of each region.

D)(4 pts) Name four different amino acid residues that can be part of covalent intermediates in enzymatic mechanisms.

E)(3 pts) What is a transition state analog of an enzyme? What type of inhibition would be caused by addition of such a compound?

F)(2 pts) Name two examples of ribozymes.

G)(2 pts) What anti-inflammatory medication is an irreversible inhibitor of cyclooxygenase?

H)(1 pt) Name an example of a coenzyme (not a metal ion).

I)(3 pts) If a competitive inhibitor is added to an enzyme-catalyzed reaction, is the V_{max} changed? The effective K_m changed? The k_{cat} changed? (No explanation necessary; just yes or no for each question.)