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BICH 440, EXAM 3, Monday, November 19, 2007, 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 on the cover page ONLY.
2. Write your student UID# on ALL pages. 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/mol-volt

1.(12 pts) Draw the proposed mechanism of catalysis by lysozyme that involves a covalent intermediate. You need to draw the sidechains of the active site amino acids and the two monosaccharide residues of the substrate surrounding the site of hydrolysis.

2.(12 pts) A 200 bp segment of closed circular double-stranded DNA undergoes a transition from the B form to the Z form WITHOUT breakage of the phosphodiester backbone. What is the CHANGE in its linking number (L), twist (T), and writhing number (W)? Your answers need not be integers.

3.(6 pts) Name the six major classes of enzymes according to the systematic classification system by the Enzyme Commission.

UID# _____

4.(12 pts) Briefly describe the steps to follow in order to determine experimentally the forward rate constant (k_1) for the bimolecular binding reaction $A + B \leftrightarrow AB$

5.(15 pts) Draw the mechanism of ribonuclease A. Show the sidechains of the two important active site amino acid residues of the enzyme that are involved in catalysis, the phosphodiester backbone of the RNA substrate at the site of hydrolysis, and the movement of protons and electrons in the reaction. Clearly point out in words every instance of general acid or general base catalysis during the mechanism (write "general acid" or "general base" in each case, while pointing to the exact sites of this type of chemistry).

6.(15 pts) Use the graph paper on the next page for your answer to this problem. Draw all plots using the same set of axes.

The kinetics of an enzyme are measured as a function of substrate concentration in the absence and presence of an inhibitor (inhibitor concentration is 100 μM).

[S] (μM)	velocity ($\mu\text{mol}/\text{min}$), no I	velocity ($\mu\text{mol}/\text{min}$), plus I
3	10.4	2.1
5	14.5	2.9
10	22.5	4.5
30	33.8	6.8
90	40.5	8.1

- A) What are the values for the V_{max} and K_m in the absence and presence of inhibitor? Include units in your answer in order to receive full credit.
- B) What type of inhibitor is exhibited?
- C) A different inhibitor is known to act in a competitive fashion and when added at a concentration of 1mM causes the effective K_m of the enzyme to increase to 25 μM . Sketch the double reciprocal plot showing kinetics upon addition of this competitive inhibitor to the enzyme.

8. Shorter answer (13 pts. total)

A)(3 pts) What are the amino acid residues that comprise the catalytic triad of serine proteases?

B)(2 pts) Name two examples of aspartic proteases

C)(2 pts) What was the first enzyme whose structure was determined by x-ray crystallography?

D)(2 pts) What is the role of the specificity pocket within the active site of a serine protease?

E)(2 pts) Write out the ratio of rate constants that defines the K_m of an enzyme.

F)(2 pts) Define the superhelical density of DNA.