

Name \_\_\_\_\_

Score \_\_\_\_\_

**Exam 3, BICH 440 Honors, Monday, November 22, 2004**

You MUST sign the following academic integrity statement:

On my honor, as an Aggie, I have neither given nor received unauthorized aid on this academic work. Signed: \_\_\_\_\_

Write concise answers to demonstrate effectively your mastery of the subject. Show your work in order to receive maximum credit where applicable.

gas constant  $R$  8.315 J/mol-K

Faraday constant  $F$  96.5 kJ/mol-volt

- 1) (12 pts) Draw the mechanism of Ribonuclease A. You will need to show the phosphodiester backbone of the RNA substrate at the cleavage site, the sidechains of the two important active site residues of the enzyme that are involved in catalysis, and the movement of protons and electrons in the reaction.

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2) (12 pts) Draw the structures of deoxyguanosine and deoxycytidine at pH 7 when oriented in a standard Watson-Crick base-pair. Clearly indicate the hydrogen bonds with dotted lines.

3) (7 pts) What is meant by the steady state assumption in Michaelis-Menten enzyme kinetics? Write out the rate equation used to derive the Michaelis-Menten equation that describes the steady state assumption for a simple single substrate reaction catalyzed by an enzyme. Assume you are dealing with the initial stages of the reaction.

4) (6 pts) Name the six general classes of enzymes according to the international classification scheme.

5) (12 pts) Given the following double-stranded fragment of DNA:

5' -CCAGTTGGCGTAAAGCTGATCCCAGAGTTGATC-3'

3' -GGTCAACCGCATTTTCGACTAGGGTCTCAACTAG-5'

Using the oligonucleotide primer, 5' -GTTGGCGTAAAGCTGATC-3', write the sequence of ALL the DNA fragments that would be generated in each reaction after adding DNA polymerase and the following sets of nucleotides. You do not have to write the sequence of the primer for each DNA fragment – just write “primer.”

- a) dGTP, dATP, dTTP, dCTP (1 mM of each); plus ddGTP (0.03 mM)
- b) dGTP, dTTP, dCTP (1 mM of each); plus ddTTP (0.03 mM)
- c) dGTP, dCTP, dATP, ddTTP (1 mM of each)

6) (4 pts) Name two examples of ribozymes.

7) (9 pts) Fill in the following table to differentiate parameters of A, B and Z forms of DNA:

type of DNA	handedness of helix	approx. # of bp/turn	bp significantly tilted relative to axis? (yes or no)
A form			
B form			
Z form			

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- 8) (15 pts) READ CAREFULLY! (a) Draw the catalytic triad of chymotrypsin at the stage in the mechanism where there is a covalent acyl intermediate. Structures of all three amino acid sidechains ARE necessary. (b) Then, draw the steps in the mechanism for the deacylation phase (hydrolysis of acyl intermediate by water). In the latter steps you need only draw structures of sidechains that are directly involved in proton or electron transfers. Be sure to illustrate movement of electrons in the mechanism. DO NOT draw the entire mechanism, just the deacylation phase. If you draw the entire mechanism, you will LOSE points, as it infers that you do not understand the chemistry.

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- 9) (12 pts) Calculate how much (in grams) of enzyme X was added to generate the following data. X follows Michaelis-Menten kinetics. Use the graph paper to plot the data to determine kinetic constants.

Catalytic efficiency ( $k_{\text{cat}}/K_m$ ) of X =  $5 \times 10^7 \text{ M}^{-1}\text{sec}^{-1}$

Molecular weight of X = 50,000

Reaction volume = 100 microliters

Table of  $v_0$  vs. [S] for X:

[S] (mM)	$v_0$ (nM/sec)
0.1	45.5
0.5	166.7
1.0	250
2.0	333.3
5.0	416.7

10) (9 pts) A laboratory researcher tried to carry out a PCR amplification of a segment of DNA but was unsuccessful. Upon discussing the protocol with you, the following issues became focal points. For each statement below: (i) Tell whether that step in the protocol is wrong (write "correct" or "incorrect"), and (ii) Suggest a solution if you believe the protocol was erroneous.

A) 2', 3'-dideoxynucleoside triphosphates were used in the reaction.

(i)

(ii) solution?

B) The protocol used a two-step cycle, varying the temperature back-and-forth between 50C and 72C.

(i)

(ii) solution?

C) Two oligodeoxynucleotide primers were used in the reaction that annealed to opposite strands of the DNA.

(i)

(ii) solution?

11) (2 pts) Give an example of a nonreversible enzyme inhibitor.