Name _____

I.D. _____

Score _____

Exam 3, BICH 440, Section 500, Monday, November 19, 2001

Write your name on each page. 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. (10 pts) An enzyme exhibits V-system allosteric regulation. On the same set of velocity vs. [S] axes, draw plots illustrating the kinetics of this enzyme: a) without the addition of a regulatory molecule, b) with the addition of a positive regulator molecule, and c) with the addition of a negative regulator molecule. Also, demonstrate on the graph how you could estimate the $K_{0.5}$ for one of these plots.

2. (6 pts) Circle the double-stranded DNA in each pair with the lowest melting point:

a) 200 bp, 45% G+C, in 0.1M NaCl OR 200 bp, 45% G+C, in 0.01M NaCl

b) 200 bp, 45% G+C, in 0.01M NaCl OR 20 bp, 45% G+C, in 0.01M NaCl

c) 200 bp, 45% G+C, in 0.01M NaCl OR 200 bp, 70% G+C, in 0.01M NaCl

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3. (6 pts) You are studying an important protein kinase enzyme. Your goal is to determine the phosphorylation site on a protein substrate of this enzyme. The phosphorylation site has been narrowed down to the following tryptic peptide:

glu-asn-pro-ser-gly-ala-tyr-ala-met-ser-gln-leu-ile-gly-lys

a) What possibilities remain for the precise site of phosphorylation?

b) Given that you have a cloned version of the cDNA encoding this protein substrate and can express recombinant protein, suggest an experimental strategy to determine the exact site of phosphorylation.

4. (10 pts) Draw the Hill plot describing the binding of oxygen to hemoglobin. Label the axes. Demonstrate how the index of cooperativity is measured from this plot. Demonstrate how the plot shows the difference in affinity for the binding to hemoglobin of the first oxygen molecule and the last oxygen molecule.

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5. (10 pts) Describe, using structures and words, why RNA is subject to hydrolysis under basic conditions, whereas the DNA phosphodiester backbone is stable to this condition.

6. (6 pts) Write the pathway of equilibria for the bisubstrate reaction ($A + B \leftrightarrow P + Q$) in which the enzyme catalyzing this reaction employs a ping-pong (double displacement) mechanism.

7. (7 pts) Diagram what features are necessary for a plasmid expression vector (i.e., the signals on the plasmid that are required for plasmid maintenance and selection, and for protein expression from the inserted DNA).

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8. (15 pts) Michaelis-Menten enzyme kinetics

a) Write out the equilibria for the enzymatic conversion of a single substrate, S, to a product, P. Label the arrows with the appropriate rate constants. DO NOT make ANY assumptions yet.

b) Write the rate equation for the initial velocity of the reaction. What assumption is made here?

c) To derive the equation, what assumption is made about the rate of change of the concentration of the enzyme-substrate complex, [ES]? Write the rate equation for the overall rate of change of [ES].

d) What is the ratio of rate constants combined to form the constant K_m?

9. (9 pts) (a) Calculate the superhelical density of a 4000 bp plasmid DNA containing 20 positive supercoils (normal B-form DNA).

(b) If this plasmid is restricted at a single location by *Eco*RI, would the linking number change? Explain.

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10. (12 pts) Write the equation that describes the Lineweaver-Burk double-reciprocal plot. Draw an example of such a plot, demonstrating how K_m and V_{max} can be determined. On the same axes of this plot, draw another plot where the same enzyme-catalyzed reaction is subjected to inhibition by a mixed, noncompetitive inhibitor in which the K_m is increased.

11. (9 pts) Short-answer

a) Write an equation to describe the fractional saturation, Y, of a protein, P, bound by a ligand, L.

b) What is the difference between a genomic library and a cDNA library?

c) What are possible units for a second-order rate constant?

d) (3 pts) Draw the structure of cyclic AMP (cAMP). You do not need to draw out the structure of the adenine base.