This test is take-home and open book, and it is intended that all members of the group contribute to completing it. Only one copy is to be submitted by the group, and all members who participated should sign their names below. **Test is due by noon on Monday, July 17.**

Please use dark pencil or ink and write legibly.

________________________________  ________________________________
________________________________  ________________________________
________________________________  ________________________________

Page  Points
1    ______
2    ______
3    ______
4    ______
5    ______

Total   ______

Points

(8)  1. To illustrate the importance of tautomeric structure in the Watson-Crick base pairing, draw base pair structures showing how cytosine in the less stable tautomer can base pair with adenine, and how guanine in a less stable tautomer can base pair with thymine.

(4)  2. Explain why RNA is sensitive to hydrolysis in base, but DNA is not.

(8)  3. You have prepared DNA from two organisms isolated from the swamps of south Georgia, designated culture A and culture B. DNA from culture A contains 24% G, while DNA from culture B contains 30% G. Complete the following table for the expected composition of the other purine and pyrimidine bases.

<table>
<thead>
<tr>
<th></th>
<th>%G</th>
<th>%A</th>
<th>%T</th>
<th>%C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture A</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Culture B</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

DNA from which organism will have the higher melting temperature?
4. Denaturing DNA by increasing the temperature is sometimes called "melting". What changes occur upon melting in:

(a) the secondary structure of DNA?

(b) absorbance at 260 nm?

(c) viscosity of the DNA solution?

5. Subtilisin (MW = 27,600) is a bacterial protease that can catalyze hydrolysis of certain amino acid esters and amides. For the synthetic substrate N-acetyl-L-tyrosine ethyl ester (Ac-Tyr-OEt), subtilisin exhibits Km and \( k_2 \) values of 0.15 M and 550 s\(^{-1}\), respectively.

(a) What is the V\(_{\text{max}}\) for the hydrolysis of Ac-Tyr-OEt when the subtilisin concentration is 0.4 mg ml\(^{-1}\)?

(b) Indole is a competitive inhibitor of subtilisin with a Ki of 0.05 M. What is the V\(_{\text{max}}\) for Ac-Tyr-OEt hydrolysis by 0.40 mg ml\(^{-1}\) subtilisin in the presence of 6.25 mM indole?

(c) What is the velocity (v) when 0.40 mg ml\(^{-1}\) subtilisin is incubated with 0.25 M Ac-Tyr-OEt?
6. From the following DNA sequences, write the complementary sequence under it (in the 3’ to 5’ direction), and circle the bases of the resulting double stranded DNA which are palindromic sequences at least four base pairs in length.

(a) \(5’-\text{GCTTCGAAC}-3’\)  \(3’-\text{CTACTACTA}-5’\)

7. A biochemist studying the properties of a newly isolated metabolic enzyme obtains the following rate data during kinetic experiments in the absence and presence of two different inhibitors, A and B, one a substrate analogue.

<table>
<thead>
<tr>
<th>[S] (M (\times 10^4))</th>
<th>(v) (\mu\text{mol/min})</th>
<th>(v) (\mu\text{mol/min with A})</th>
<th>(v) (\mu\text{mol/min with B})</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1.25</td>
<td>0.74</td>
<td>0.48</td>
</tr>
<tr>
<td>2.5</td>
<td>0.87</td>
<td>0.45</td>
<td>0.33</td>
</tr>
<tr>
<td>1.7</td>
<td>0.67</td>
<td>0.32</td>
<td>0.25</td>
</tr>
<tr>
<td>1.2</td>
<td>0.54</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>1.0</td>
<td>0.45</td>
<td>0.21</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Inhibitor A concentration is 5 \(\times 10^{-4}\) M; that of B is 3.2 \(\times 10^{-6}\) M.

a. Determine \(V_{\text{max}}\) and \(K_M\) for this enzyme using the Lineweaver-Burk reciprocal plot. Plot the inhibitor data on the same graph. (Note: Use graph paper. Pick your axes and scales carefully so that the lines may be extrapolated to the negative x intercept. It would be a good idea to draw the graph on scratch graph paper first, then do a clean finished copy.)

b. Classify the inhibitors as competitive, not-competitive, non-competitive, or uncompetitive. Which one is likely to be the substrate analogue?

c. Plot the data for the uninhibited reaction on a separate graph using the Eadie-Hofstee plot (\(v\) plotted versus \(v/S\)). Determine \(V_{\text{max}}\) and \(K_m\) for the uninhibited reaction from this graph, and compare with part a. (Note: This is a different plot than the Haynes-Woolf plot described in your text.)

(Show your calculations on a blank sheet of paper, and plot your graphs on the graph paper, and attach them to the exam. Put your group name on the calculation sheet and the graph paper.)
8. Following are three models for reversible inhibition of a simple one-substrate enzyme reaction, three rate laws which are derived from the models, expressed in reciprocal form, and four terms describing types of reversible inhibition. For the graphs which follow, indicate in the blank below the graph the model (a, b, or c), the rate law (d, e, or f), and the term(s) (g, h, i, or j) which apply to that graph.

Model:

(a) \( E + S \rightleftharpoons ES \rightarrow E + P \)  
(b) \( E + S \rightleftharpoons ES \rightarrow E + P \)  
(c) \( E + S \rightleftharpoons ES \rightarrow E + P \)  
\( E + I \rightleftharpoons EI \)  
\( ES + I \rightleftharpoons ESI \)  
\( E + I \rightleftharpoons EI \)  
\( ES + I \rightleftharpoons ESI \)

Rate Law:

(d) \( \frac{1}{v} = \frac{1}{V_m}(1 + I/K'_1) + \frac{K_m}{V_m}(1/S) \)  
(e) \( \frac{1}{v} = \frac{1}{V_m}(1 + I/K'_1) + \frac{K_m}{V_m}(1/S)(1 + I/K_1) \)  
(f) \( \frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}(1/S)(1 + I/K_1) \)  

Term:

(g) competitive  
(h) not-competitive (mixed noncompetitive)  
(i) "pure" noncompetitive  
(j) uncompetitive

9. The fraction of ligand binding sites \( Y \) on myoglobin and hemoglobin can be expressed by the relationship

\[
Y = \frac{(pO_2)^n}{(pO_2)^n + (p_{50})^n}
\]

where \( pO_2 \) is the pressure of oxygen, \( p_{50} \) is the oxygen pressure required to occupy 50% of the sites, and \( n \) is the Hill coefficient. For myoglobin, \( p_{50} = 1 \) torr and \( n = 1 \). For hemoglobin, \( p_{50} = 26 \) torr and \( n = 2.8 \).

Calculate \( Y \) for the two proteins in tissues where \( pO_2 = 30 \) torr.
The mechanism of chymotrypsin illustrates several of the factors that are believed to contribute to the rate acceleration obtained by enzymes. Describe each of the following aspects of the chymotrypsin mechanism.

(a) A reaction model that shows ping-pong kinetics. (i.e., specify the identity of A, B, P, Q, E, and E', the components of the ping pong mechanism as given on page 452).

(b) Transition state stabilization by bonds formed between the enzyme and the transition state that are not found in the binding of substrate or product.

(c) Acid-base catalysis mediated through a "catalytic triad". Describe how the triad assists in the formation of the covalently bound intermediate.

(d) Substrate specificity provided by the nature of the substrate binding site. (Explain how chymotrypsin differs from trypsin in the binding site.)