Values of some useful constants:
the Boltzman constant, $k_B = 1.38 \times 10^{-16}$ erg/degree•molecule
the gas law constant, $R = 1.98$ cal/mole•degree
Planck's constant, $h = 6.627 \times 10^{-27}$ erg•s

1. Give a brief description of the principles behind the following techniques or methods: (16 points)
   a. size exclusion chromatographic separation (gel filtration) of proteins
   
   b. precipitation of proteins by addition of a miscible organic solvent
   
   c. measurement of protein concentration using the method of Bradford.
   
   d. a photoaffinity label

2. List five ways in which protein can differ from one another that can be used as means to separate and/or purify them and give one technique that makes use of each of the properties you list. (20 points)
3. Classify the enzymes that catalyze the following reactions according to the EC nomenclature numbering system by assigning the first digit (18 points).

a. ________________

\[
\begin{align*}
\text{Glucose-6-Phosphate} & \quad \text{Fructose-6-Phosphate} \\
\end{align*}
\]

b. ________________

\[
\begin{align*}
\text{Fructose-6-Phosphate} & \quad \text{Phosphoenolpyruvate} \\
\end{align*}
\]

c. ________________

\[
\begin{align*}
\text{Phosphoenolpyruvate} & \quad \text{Pyruvate} \\
\end{align*}
\]

d. ________________

\[
\begin{align*}
\text{ADP} & \quad \text{ATP} \\
\end{align*}
\]
4. Sugar (i.e., sucrose) is hydrolyzed by the enzyme invertase:

\[
\text{Sucrose} \xrightarrow{\text{Invertase}} \text{Glucose} + \text{Fructose}
\]

It can be assumed that the hydrolysis of simple disaccharides proceeds by attack of water on the anomeric carbon atom of the monosaccharide on the non-reducing end of the disaccharide, to cleave the acetal bond. But, sucrose is a non-reducing sugar - both monosaccharides employ their anomeric carbon atoms in the glycosidic bond, so that both sides of the glycosidic linkage are actually acetal bonds. In principle, either the fructose or the glucose moieties could be attacked by the incoming water molecule. Suggest an experiment to determine which monosaccharide unit is actually attacked by water when the reaction is catalyzed by a particular enzyme (8 pts.).

5. The plot below shows results obtained from kinetic inhibition experiments in which \([S]_0\) was varied in the absence of inhibitor and at two levels of inhibitor. Initial velocities were measured (velocity units were \(\mu\)moles/mL•min and substrate concentrations were in mM).

![Kinetic inhibition plot](image)

a. What kind of reversible inhibition is observed? (5 points)
b. The data in the table were obtained from the double-reciprocal plot.
What are $K_M$ for the substrate, $V_{\text{max}}$ for the reaction and $K_I$ for this inhibitor? (13 pts.)

c. Draw a diagram (i.e., of the reactions) that shows what form(s) of the enzyme bind substrate and/or inhibitor and which forms proceed to product. (5 points)

6. The temperature dependence of an enzyme-catalyzed reaction is important for a number of reasons, both mechanistic and practical. The following questions concern the temperature dependence of an enzyme-catalyzed reaction.

a. If the reaction velocity at 37$\degree$C is twice that observed at 20$\degree$, what is the Arrhenius activation energy for the reaction? (9 points)

b. What is the enthalpy of activation ($\Delta H^\dagger$) for this reaction? (9 points)
c. If the velocity of the reaction at 20°C is approximately equal to that observed at 45°C, what would you conclude? (7 points)

7. The following classes of reaction are catalyzed by enzymes that require certain coenzymes or prosthetic group. For each class of reaction, indicate the coenzyme or prosthetic group most commonly associated with the class. (21 points)

a. acyl transfer reactions

b. oxidative decarboxylations with NADH as electron acceptor

c. one-electron transfer reactions

d. transamination

e. hydride transfer reactions (2-electron reductions)

f. one-carbon transfer reactions (methylene, formyl or methyl)

g. methylation reactions
8. The following diagram shows the ionizations relevant to the kinetics of a particular enzyme-catalyzed reaction:

\[
\begin{align*}
E_{H2} + S & \quad \rightarrow \quad EH_2S \\
\text{pKa} = 4.4 \\
EH^{-} + S & \quad \rightleftharpoons \quad EHS^{-} \\
\text{pKa} = 9.5 \\
E^{2-} + S & \quad \rightarrow \quad ES^{2-} \\
& \quad \rightarrow \quad E^{2-} + P
\end{align*}
\]

a) Sketch the initial velocity versus pH profile for this reaction under conditions where \([S]_o \ll K_m\) and \([S]_o \gg K_m\). (Be sure to indicate which line is which). (8 points.)

b) Suggest which amino acid sidechain could be responsible for each of these ionizations. (4 points)

9. \(V_o\) for an enzyme-catalyzed reaction was found to be 85 \(\mu\)moles/mL•min at \([S]_o = 1\) mM. If \(K_M\) for the substrate is 5 mM, what is \(V_{max}\)? (8 points)
10. Several kinds of reactions are catalyzed by oxidoreductases. Some examples are given below.

<table>
<thead>
<tr>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. ( \text{AH}_2 + B \rightarrow \text{A} + \text{BH}_2 )</td>
</tr>
<tr>
<td>b. ( \text{AH}_2 + 0_2 \rightarrow \text{A} + \text{H}_20_2 )</td>
</tr>
<tr>
<td>c. ( 2\text{AH}_2 + O_2 \rightarrow 2\text{A} + 2\text{H}_20 )</td>
</tr>
<tr>
<td>d. ( \text{A} + \text{B} + \text{H}_20 \rightarrow \text{A}0 + \text{BH}_2 )</td>
</tr>
<tr>
<td>e. ( \text{A} + \text{BH}_2 + 0_2 \rightarrow \text{A}0 + \text{B} + \text{H}_20 )</td>
</tr>
</tbody>
</table>

Assume that you have evidence for the presence of oxidoreductase activity in a particular sample and you want to determine exactly which type of oxidoreductase reaction it catalyzes.

a) How could you distinguish experimentally among reactions a, b and c? (9 points)

b) How could you distinguish experimentally between reactions d and e? (6 points)

11. Complete the following purification balance table: (12 points)

<table>
<thead>
<tr>
<th>step</th>
<th>Volume (mL)</th>
<th>Protein (mg/mL)</th>
<th>Activity (units/mL)</th>
<th>Specific Activity (units/mg)</th>
<th>Recovery (%)</th>
<th>Fold Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>crude extract</td>
<td>1000</td>
<td>10.0</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium Sulfate ppt'n (20-35%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE Cellulose</td>
<td>30.0</td>
<td>0.2</td>
<td>4.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12. Match the chemical modification reagent in the column on the left with the number (from the column on the right) of the amino acid side chain or group it commonly modifies or reacts with. (12 points)

<table>
<thead>
<tr>
<th>Chemical Modification Reagent</th>
<th>Amino Acid Side Chain or Group Commonly Modifies or Reacts With</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. $p$-hydroxymercuribenzoate</td>
<td>1. heme iron</td>
</tr>
<tr>
<td>b. diethylpyrocarbonate</td>
<td>2. bound metal ions</td>
</tr>
<tr>
<td>c. EDTA</td>
<td>3. His imidazole</td>
</tr>
<tr>
<td>d. DTNB (Ellman's reagent)</td>
<td>4. Cys-SH</td>
</tr>
<tr>
<td>e. N-ethyl maleimide</td>
<td>5. Leu-CH$_3$</td>
</tr>
<tr>
<td>f. cyanide ion</td>
<td>6. Lysine -NH$_3^+$</td>
</tr>
</tbody>
</table>

13. Biological systems normally employ L-amino acids for precursors and to obtain energy. Organisms occasionally encounter D-amino acids and must convert them to the L isomer before they can be metabolized or incorporated into proteins:

\[
\text{CH}_3\text{C}^{\text{NH}_2}\text{H} \rightleftharpoons \text{CH}_3\text{C}^{\text{NH}_2}\text{H}
\]

Many of these enzymes (D-amino acid racemases) utilize pyridoxal phosphate as cofactor. Write a reaction scheme that shows how this transformation might take place. The structure of pyridoxal phosphate is given below. (10 points)