Problem sets (Section 1): Problems such as these may be on the first midterm. Problems marked “GG” are from Garrett and Grisham.

Lecture 1 (Introduction)

GG1-1. The nutritional requirements of *E. coli* cells are far simpler than those of humans, yet the macromolecules in bacteria are about as complex as those of animals. Do bacteria have more biosynthetic capacity and hence more metabolic complexity than animals?
What does it mean to have simpler nutritional requirements? How do you measure biosynthetic capacity and metabolic complexity?

GG1-3. *E. coli* cells are about 2 µm long and 0.8 µm in diameter. Glucose, a major energy-yielding nutrient, is present in bacterial cells at a concentration of about 1 mM. What is the concentration of glucose expressed as mg/mL? How many glucose molecules are contained in a typical *E. coli* cell? (Recall Avogadro’s number = 6.023 x 10^23.)
e. A number of regulatory proteins are present in *E. coli* at only one or two molecules per cell. Assume one molecule per cell: what is the molar concentration of this protein in the cell? If the molecular weight of this protein is 40 kD, what is its concentration, expressed as mg/mL?

GG1-10. Why does the central role of weak forces in biomolecular interactions restrict living systems to a narrow range of environmental conditions?

Lecture 2 (Thermodynamics)

GG3-1. An enzymatic hydrolysis of fructose-1-P,

\[ \text{fructose-1-P} + \text{H}_2\text{O} \rightleftharpoons \text{fructose} + \text{Pi}, \]

was allowed to proceed to equilibrium at 25°C. The original concentration of fructose-1-P was 0.2 M, but when the system had reached equilibrium the concentration of fructose-1-P was only 6.52 x 10^-5 M. Calculate the equilibrium constant for this reaction and the standard free energy of hydrolysis of fructose-1-P.

GG3-3. The standard state free energy of hydrolysis for acetyl phosphate,\n
\[ \text{acetyl-P} + \text{H}_2\text{O} \rightleftharpoons \text{acetate} + \text{Pi}, \]

is \(\Delta G^\circ = -42.3\ \text{kJ/mol}\). Calculate the free energy change for acetyl phosphate hydrolysis in a solution of 2 mM acetate, 2 mM phosphate, and 3 nM acetyl phosphate.

GG3-6. For the process \(A \rightleftharpoons B\), \(K_{eq} (AB)\) is 0.02 at 37°C. For the process \(B \rightleftharpoons C\), \(K_{eq} (BC) = 1000\) at 37°C.
a. Determine \(K_{eq} (AC)\), the equilibrium constant for the overall process \(A \rightleftharpoons C\), from \(K_{eq} (AB)\) and \(K_{eq} (BC)\).
b. Determine standard-state free energy changes for all three processes, and use \(\Delta G^\circ (AC)\) to determine \(K_{eq} (AC)\). Make sure that this value agrees with that determined in part a of this problem.
GG3-8. Write the equilibrium constant, $K_{eq}$, for the hydrolysis of creatine phosphate and calculate a value for $K_{eq}$ at 25°C from the value of $\Delta G^0 = -43.3 \text{ kJ/mol}$.

GG3-10. Calculate the free energy of hydrolysis of ATP in a rat liver cell in which the ATP, ADP, and Pi concentrations are 3.4, 1.3, and 4.8 mM, respectively.

**Lectures 3, 4 (pH)**

1. The weak acid imidazole, $\text{C}_3\text{H}_2\text{N}_5^+$, has a pK$_a$ of 6.99. At pH 6.99, what is the ratio of the concentrations of the acid form and the conjugate base? Use the Henderson-Hasselbalch equation to show a general solution.

2. For an acid HA, the concentrations of HA and $\text{A}^-$ are 0.075 M and 0.025 M, respectively, at pH 6.0. What is the pK$_a$ value for HA?

GG2-1. Calculate the pH of the following:
- a. $5 \times 10^{-4}$ M HCl
- b. $7 \times 10^{-5}$ M NaOH
- c. 2 µM HCl
- d. $3 \times 10^{-2}$ M KOH
- e. 0.04 mM HCl
- f. $6 \times 10^{-9}$ M HCl

GG2-3. The pH of a 0.02 M solution of an acid was measured to be 4.6.
- a. What is the [$\text{H}^+$] of the solution?
- b. Calculate the acid dissociation constant $K_a$ and pK$_a$ for this acid.

GG2-4. The $K_a$ for formic acid is $1.78 \times 10^{-4}$ M.
- a. What is the pH of a 0.1 M solution of formic acid?
- b. 150 mL of 0.1 M NaOH is added to 200 mL of 0.1 M formic acid, and water is added to give a final volume of 1L. What is the pH of the final solution?

GG2-5. The pK$_a$ for acetic acid is 4.76. Given 0.1 M solutions of acetic acid and sodium acetate, describe the preparation of 1 L of 0.1 M acetate buffer at pH 5.4.

GG2-6. The pK$_a$ for the dissociation of $\text{H}_3\text{PO}_4^-$ is 7.2. If the internal pH of a muscle cell is 6.8, what is the [$\text{HPO}_4^{2-}$]/[$\text{H}_2\text{PO}_4^-$] of the cell?

GG2-7. The pK$_a$s for the dissociation of $\text{H}_3\text{PO}_4$, $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ are 2.15, 7.2, and 12.40, respectively. Given 0.1 M solutions of Na$_3$PO$_4$ and $\text{H}_3\text{PO}_4$, describe the preparation of 1 L of a phosphate buffer at a pH of 7.5. What are the molar concentrations of the ions in the final buffer solution, including Na$^+$ and H$^+$?

GG2-10. Citric acid, a tricarboxylic acid important in intermediary metabolism, can be symbolized as $\text{H}_3\text{A}$. Its dissociation reactions are:
- $\text{H}_3\text{A} \rightleftharpoons \text{H}_2\text{A}^- + \text{H}^+$  \hspace{1cm} pK$_1$ = 3.13
- $\text{H}_2\text{A}^- \rightleftharpoons \text{HA}^{2-} + \text{H}^+$  \hspace{1cm} pK$_2$ = 4.76
- $\text{HA}^{2-} \rightleftharpoons \text{A}^{3-} + \text{H}^+$  \hspace{1cm} pK$_3$ = 6.40
If the total concentration of the acid and its anion forms is 0.02 M, what are the individual concentrations of $H_3A$, $H_2A^-$, $HA^{2-}$, and $A^{3-}$ at pH 5.2?

**Lecture 5 (Amino acids)**


2. What are the names, three letter abbreviation and one-letter symbols of the four amino acids below? (This is an example. You need to know this for all 20.)

3. Which of the amino acids shown in problem 2 are associated with the following characteristics:
   a. hydrophobic side chain   b. basic side chain   c. three ionizable groups
d. $pK\alpha$ of approximately 10 in proteins   e. modified form of phenylalanine

4. Match each amino acid in the left-hand column with the appropriate side-chain type in the right-hand column.
   a. leu  1. hydroxyl-containing
   b. glu  2. acidic
c. lys  3. basic
d. ser  4. sulfur-containing
e. cys  5. non-polar aromatic
f. trp  6. non-polar aliphatic

5. For each of the following pairs of amino acids, identify which would be most soluble in water:
   a. ala, leu   b. tyr, phe   c. ser, ala   d. trp, his
6. Which of the following amino acids have R groups that have hydrogen-bonding potential? ala, gly, ser, phe, glu, tyr, ile, and thr.

7. For an amino acid such as alanine, the major species in solution at pH 7 is the zwitterionic form. Assume a pKₐ value of 8 for the amino group and a pKₐ value of 3 for the carboxylic acid. Estimate the ratio of the concentration of the neutral amino acid species (with the carboxylic acid protonated and the amino group neutral) to that of the zwitterionic species at pH 7.

GG4-5. Draw an appropriate titration curve for aspartic acid, labeling the axes and indicating the equivalence points and the pKₐ values.

**Lecture 6 (Protein structure)**

1. Proteins are quite stable. The lifetime of a peptide bond in aqueous solution is nearly 1000 years. However, the free energy of hydrolysis of protein is negative and quite large. How can you account for the stability of the peptide bond in light of the fact that hydrolysis releases much energy?

2. Draw the structure of the dipeptide gly-his. What is the charge on the peptide at pH 5.5? pH 7.5?

3. How many different polypeptides of 50 amino acids in length can be made from the 20 common amino acids?

4. An enzyme called protein disulfide isomerase (PDI) catalyzes disulfide-sulfhydryl exchange reactions. PDI rapidly converts inactive scrambled ribonuclease into enzymatically active ribonuclease. In contrast, insulin is rapidly inactivated by PDI. What does this observation imply about the relation between the amino acid sequence of insulin and its three-dimensional structure?

GG6-7. A new protein of unknown structure has a molecular weight (Mr) of 240,000. In the presence of 6M guanidine hydrochloride (denaturing agent), chromatography yields a single peak at Mr 60,000. In the presence of 6M guanidine hydrochloride plus 10 mM β-mercaptoethanol, there are two peaks, at Mr 34,000 and 26,000. What can be determined about the structure of the protein from these data?

GG6-9. The hemagglutinin protein in influenza virus contains a remarkably long α-helix, with 53 residues.
   a. How long is this α-helix (in nm)?
   b. How many turns does this helix have?
   c. Each residue in an α-helix is involved in two H bonds (except the ones at the ends). How many H bonds are present in this helix?
GG6-11. Which amino acids would be capable of forming H bonds with a lysine residue in a protein?

GG6-12. Poly-L-glutamate adopts an α-helical structure at low pH but becomes a random coil above pH 5. Explain this behavior.

**Lecture 7 (Protein purification)**

1. A. In each of the three tables below, calculate the specific activity, fold-purification, and % yield for each step, compared to the initial starting material.
   - Specific activity: divide total enzyme activity by total protein.
   - Fold purification: divide sp. act. by initial sp. act. (8.2).
   - % yield: divide total enzyme activity by starting total activity x 100.

<table>
<thead>
<tr>
<th>Your Steps</th>
<th>Protein (mg)</th>
<th>Enzyme (units)</th>
<th>Specific Activity</th>
<th>Fold purification</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Starting Material</td>
<td>511</td>
<td>4200</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40% Ammonium Sulfate Precipitation</td>
<td>181</td>
<td>3991</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE Flow Through, pH 7</td>
<td>67.6</td>
<td>3787</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Isoelectric Focusing (pH 9.7)</td>
<td>3.8</td>
<td>2949</td>
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<table>
<thead>
<tr>
<th>Jim’s Steps</th>
<th>Protein (mg)</th>
<th>Enzyme (units)</th>
<th>Specific Activity</th>
<th>Fold purification</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Starting Material</td>
<td>714</td>
<td>5850</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40% Ammonium Sulfate Precipitation</td>
<td>253</td>
<td>5584</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE Flow Through, pH 9</td>
<td>5.1</td>
<td>5174</td>
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</table>

<table>
<thead>
<tr>
<th>Sue’s Steps</th>
<th>Protein (mg)</th>
<th>Enzyme (units)</th>
<th>Specific Activity</th>
<th>Fold purification</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Starting Material</td>
<td>410</td>
<td>3360</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40% Ammonium Sulfate Precipitation</td>
<td>146</td>
<td>3192</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM, pH 7.0, NaCl elution</td>
<td>1.9</td>
<td>2516</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

B. After swallowing your pride you realize your friends have improved upon your method. What factors would you consider in choosing between Sue or Jim’s protocol?

C. What information in your purification scheme prompted Sue to switch to cation exchange chromatography?
D. Why did switching the pH from 7.0 to 9.0 result in a better fold-purification at the DEAE chromatography step?

E. If your protein is approximately 0.1% of the initial starting material, what percentage of the total protein in your last purification step is probably your protein? In other words, how pure is your protein?

2. The picture below shows the results of a 2-D gel of a protein extract.

[Image of 2-D gel]

a. How might the information in this gel help design a method for purifying the protein marked by the arrow?
b. The line of spots that are circled may represent related proteins (or related forms of one type of protein). How might these be related; put another way, what structural features might distinguish them?

**Lecture 8 (Enzyme mechanisms)**

1. Match the $K_{eq}$ values with the appropriate $\Delta G^\circ$ values.

<table>
<thead>
<tr>
<th>$K_{eq}$</th>
<th>$\Delta G^\circ$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. $1$</td>
<td>$28.53$</td>
</tr>
<tr>
<td>b. $10^{-5}$</td>
<td>$-11.42$</td>
</tr>
<tr>
<td>c. $10^4$</td>
<td>$5.69$</td>
</tr>
<tr>
<td>d. $10^2$</td>
<td>$0$</td>
</tr>
<tr>
<td>e. $10^{-1}$</td>
<td>$-22.84$</td>
</tr>
</tbody>
</table>

2. Assume that you have a solution of 0.1 M glucose-6-phosphate. To this solution, you add the enzyme phosphoglucomutase, which catalyzes the reaction:

$$\text{glucose-6-phosphate} \rightleftharpoons \text{glucose-1-phosphate}$$

The $\Delta G^\circ$ for the reaction is $+7.5$ kJ/mol.

a. Does the reaction proceed as written, and if so, what are the final concentrations of glucose-6-phosphate and glucose-1-phosphate?

b. Under what cellular conditions could you produce glucose-1-phosphate at a high rate?

GG14-1. Tosyl-1-phenylalanine chloromethyl ketone (TPCK) specifically inhibits chymotrypsin by covalently labeling his_{57}.

![TPCK structure](image)

a. Propose a mechanism for the inactivation reaction, indicating the structure of the product(s)

b. State why this inhibitor is specific for chymotrypsin.

c. Propose a reagent based on the structure of TPCK that might be an effective inhibitor of trypsin, which hydrolyzes peptide bonds on the carboxyl side of lysine or arginine.
GG14-2. Replacing asp$^{102}$ with asn in trypsin reduces the proteolytic activity 10,000-fold. Considering the catalytic triad of normal trypsin, suggest a structure for the “uncatalytic triad” of asn-his-ser in the mutant enzyme, and explain how this structure results in reduced activity.

GG14-4. For the reaction $S \rightarrow P$, derive an expression for $k_e/k_u$, the ratio of the rate constants for the catalyzed and uncatalyzed reactions, respectively, in terms of the free energies of activation of the catalyzed ($\Delta G_e^\circ$) and uncatalyzed ($\Delta G_u^\circ$) reactions.

**Lecture 9 (Enzyme kinetics)**

1. Penicillin is hydrolyzed and thereby rendered inactive by penicillinase ($\beta$-lactamase), an enzyme present in some resistant bacteria. The mass of this enzyme in *Staphylococcus aureus* is 29.6 kD. The amount of penicillin hydrolyzed in 1 minute in a 10-ml solution containing $10^{-9}$ g of purified penicillinase was measured as a function of the concentration of penicillin. Assume that the concentration of penicillin does not change appreciably during the assay.

<table>
<thead>
<tr>
<th>Penicillin, $\mu$M</th>
<th>Amount hydrolyzed, nmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.34</td>
</tr>
<tr>
<td>10</td>
<td>0.45</td>
</tr>
<tr>
<td>30</td>
<td>0.58</td>
</tr>
<tr>
<td>50</td>
<td>0.61</td>
</tr>
</tbody>
</table>

a. Plot $V_o$ versus $[S]$ and $1/V_o$ versus $1/[S]$ for these data. Does penicillinase appear to obey M-M kinetics? If so, what is the value of $K_M$?
b. What is the value of $V_{max}$?
c. What is the turnover number of penicillinase under these experimental conditions? Assume one active site per enzyme molecule.
d. Plot $V_o$ versus $V_o/[S]$ (an Eadie-Hofstee plot) and calculate $K_M$ and $V_{max}$. Compare the results with those you found in parts a and b.

2. For an enzyme that follows simple M-M kinetics, what is the value of $V_{max}$ if $V_o$ is equal to 1 $\mu$mol/min when $[S] = 1/10 \ K_M$?

3. For a one-substrate, enzyme-catalyzed reaction, double-reciprocal plots were determined at three different enzyme concentrations. Which of the following three families of curve would you expect to be obtained? Explain.
GG13-1. According to the M-M equation, what is the v/ V_{max} ratio when [S] = 4 K_M?

GG13-2. If V_{max} = 100 \mu\text{mol/mL-s} and K_M = 2 \text{mM}, what is the velocity of the reaction when [S] = 20 \text{mM}?

GG13-10. Triose phosphate isomerase catalyzes the reaction:

\text{glyceraldehyde-3-P} \rightleftharpoons \text{dihydroxyacetone-P}

The K_M of this enzyme for its substrate glyceraldehyde-3-P is 1.8 \times 10^{-5} \text{ M}. when [glyceraldehyde-3-P] = 30 \mu\text{M}, the rate of the reaction, v, was 82.5 \mu\text{mol/mL-s}.

\text{a. What is V}_{\text{max}} for this enzyme?}

\text{b. Assuming 3 nanomoles per mL of enzyme was used in this experiment ([E_{\text{total}}] = 3 \text{ nmol/mL}), what is k_{\text{cat}} for the enzyme?}

\text{c. What is the catalytic efficiency (k_{\text{cat}}/K_M) for triose phosphate isomerase?}

\text{d. Does the value of k_{\text{cat}}/K_M reveal whether triose phosphate isomerase approaches “catalytic perfection” \left(10^8 \text{ mol}^{-1}\text{s}^{-1}\right)?}

\text{e. What determines the ultimate speed limit of an enzyme-catalyzed reaction? That is, what is it that imposes the physical limit on kinetic perfection?}

GG13-11. The citric acid cycle enzyme fumarase catalyzes the conversion of fumarate to form malate,

\text{fumarate + H}_2\text{O} \rightleftharpoons \text{malate}

The turnover number, k_{\text{cat}} for fumarase is 800/sec. The K_M of fumarase for fumarate is 5 \mu\text{M}.

\text{a. In an experiment using 2 nanomole/L of fumarase, what is V}_{\text{max}}?}

\text{b. The cellular concentration of fumarate is 47.5 \mu\text{M}. What is v (reaction velocity) when [fumarate] = 47.5 \mu\text{M}?}

\text{c. What is the catalytic efficiency of fumarase?}

\text{d. Does fumarase approach “catalytic perfection”?}