1. Refer to figures 11-1 in the textbook.
   a. Show the conversion of the Fisher projection of D-idose to the Haworth projection of β-D-idose. (4 points)

   ![Fisher to Haworth projection](image)

   b. Raffinose, also called melitose, is a trisaccharide that is found in beans, cabbage, brussels sprouts, and broccoli. Humans cannot digest this saccharide and it is fermented in the large intestine by gas-producing bacteria. Give the systematic name for this sugar. (5 points)

   ![Structure of Raffinose](image)

   \( \alpha\text{-D-galactopyranosyl-(1→6)}\alpha\text{-D-glucopyranosyl-(1→2)}\beta\text{-D-fructofuranoside} \)

   c. Proteins called lectins reversibly bind to specific carbohydrate residues in glycoproteins. Peanut lectin binds specifically to the disaccharide composed of galactose and N-acetylgalactosamine. The two monosaccharides are linked by a \(\beta(1\rightarrow3)\) glycosidic bond. Draw the structure of this disaccharide. Hint: the non-reducing end is galactose. (4 points)

   ![Structure of Peanut Lectin](image)
2. a. Given the following changes to a lipid membrane, would you expect the melting temperature of the membrane to increase or decrease? Explain each answer. (3 points each)
   i. The lengths of the acyl tails are increased.
      Increase. More free energy (and a higher temperature) is required to disrupt the more extensive van der Waals interactions in longer acyl chains.
   ii. Further sites of unsaturation in the hydrocarbon tails are introduced.
      Decrease. Cis double bond produces a kink in the acyl chain and so it is less able to pack efficiently against it neighbors and disrupts van der Waals interactions.

b. In a membrane, would you expect a cis double bond or a trans double bond to cause a greater change in the membrane transition temperature? Explain your answer. (4 points)

A cis double bond would cause a greater change in membrane transition temperature. A trans double bond does not produce a kink in the molecule as cis double bonds do, therefore, its geometry more closely resembles that of a single bond.

c. The distance between the Cα atoms in a β sheet is approximately 3.5 angstroms. Can a single 9-residue protein segment with a β-conformation reasonably serve as the transmembrane portion of an integral membrane protein? Explain your answer. (4 points)

No. Although the 9-residue β-strand could theoretically span the membrane, a single strand would be unstable because its backbone could not form the hydrogen bonds it would form with water in an aqueous environment.

d. Does the phosphatidyl glycerol “head group” of cardiolipin (see Table 12-2) project out of a lipid bilayer like other glycerophospholipid head groups? Explain your reasoning. (3 points)

No. The two acyl chains of the head group, being hydrophobic, would bury themselves in the lipid bilayer interior, leaving the head group of diphosphoglycerol.

e. What properties of triacylglycerols make them unsuitable to be major components of lipid bilayers, such as those found in membranes? (3 points)

Triacylglycerols lack polar head groups so they do not orient themselves in a bilayer with their acyl chains inward and their glycerol moiety toward the surface.
3. a. A kinetics experiment was performed with a particular enzyme and substrate A or substrate B. A Lineweaver-Burk plot was constructed from the data collected to give a graph similar to the following:

![](image)

i. Which substrate has a higher $V_{\text{max}}$ value? Explain. (3 points)

B
The value of the y-intercept is equal to $1/V_{\text{max}}$ for each substrate. Due to this inverse relationship, the substrate corresponding to the line with the lower $1/v_0$ value, has the higher $V_{\text{max}}$. In this case, substrate B has the higher $V_{\text{max}}$ value.

ii. Which substrate has a higher $K_{\text{m}}$ value? Explain. (3 points)

A
The value of the x-intercept is equal to $1/K_{\text{M}}$ for each substrate. Due to this inverse relationship, the substrate corresponding to the line with the lower $1/[S]$ value, has the higher $K_{\text{M}}$. In this case, substrate A has the higher $K_{\text{M}}$ value.

iii. Assuming the same amount of enzyme was used in both experiments, with which substrate does the enzyme attain the highest catalytic efficiency? Explain your answer. (5 points)

B
catalytic efficiency $= k_{\text{cat}}/K_{\text{M}} = V_{\text{max}}/(K_{\text{M}}[E]_T)$, so the substrate with the higher $V_{\text{max}}$ value and the lower $K_{\text{M}}$ value represents the substrate with which the enzyme has the highest catalytic efficiency.

b. For an enzyme that follows simple Michaelis-Menten kinetics, what is the value of $V_{\text{max}}$ if $v_0$ is equal to 1 µmole/min at 1/10 $K_{\text{m}}$? (4 points)

11 µmoles/min
3. c. Protein phosphatase 1 (PP1) catalyzes a reaction which yields products that are important in regulating cell division. Consequently, PP1 is a possible drug target to treat certain types of cancers. The PP1 enzyme acts to hydrolyze a phosphate group from a specific substrate. One of PP1’s substrates is myelin basic protein (MBP). The reaction is shown below:

\[
\text{PP1} \quad \text{MBP-phosphate} \quad \rightarrow \quad \text{MBP} + P_i
\]

The activity of PP1 was measured in the presence and absence of the inhibitor phosphatidic acid (PA). The concentration of PA was 300 nM.

<table>
<thead>
<tr>
<th>[MBP] (mg/mL)</th>
<th>(v_0) without PA (nmol P(_i) released·mL(^{-1})·min(^{-1}))</th>
<th>(v_0) with PA (nmol P(_i) released·mL(^{-1})·min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.010</td>
<td>0.0209</td>
<td>0.00381</td>
</tr>
<tr>
<td>0.015</td>
<td>0.0335</td>
<td>0.00620</td>
</tr>
<tr>
<td>0.025</td>
<td>0.0419</td>
<td>0.00931</td>
</tr>
<tr>
<td>0.050</td>
<td>0.0838</td>
<td>0.0140</td>
</tr>
</tbody>
</table>

i. Use the data provided to construct a Lineweaver-Burk plot on the attached sheet of graph paper. (5 points) What kind of inhibitor is PA? Explain your answer. (3 points)

Graphs should resemble the graph above.

PA is a mixed inhibitor – it has a different x- and y-intercept, corresponding to a change in \(V_{\text{max}}\) and \(K_M\).

ii. What are the \(K_M\) and \(V_{\text{max}}\) values for PP1 in the presence and the apparent \(K_M\) and apparent \(V_{\text{max}}\) values in the absence of the inhibitor? (8 points)

Without inhibitor: \(K_M = 0.102\ \text{mg/mL};\ \ V_{\text{max}} = 0.239\ \text{nmol·mL}^{-1}·\text{min}^{-1}\)

With inhibitor: \(K_M = 0.152\ \text{mg/mL};\ \ V_{\text{max}} = 0.064\ \text{nmol·mL}^{-1}·\text{min}^{-1}\)
d. For a one-substrate enzyme catalyzed reaction, double-reciprocal plots were determined for 3 different enzyme concentrations. Which of the following three families of curve would you expect result? Explain. (5 points)

If $E_T$ is increased, $V_{\text{max}}$ will also increase since $V_{\text{max}} = K_2[E_T]$. But $K_M = (k_{-1} + k_2/k_1)$; that is, it is independent of substrate concentration.
4. a. What specific aspect of a protein structure can be derived from a quantitative measurement of the nuclear Overhauser effect (NOE) involving a pair of $^1$H nuclei? (4 points)

Identify interacting nuclei that are separated by distances less than 6 Å

b. In x-ray crystallography the “unit cell” is defined as ______________________

the minimal entity, which by translation alone, can produce the entire crystal. (2 points)

c. What is the “phase problem” as applied to the x-ray crystallography of proteins? (2 points)

The problem that arises in determining the electron density function of a crystal from x-ray diffraction data, namely that a complete determination requires knowledge of both the magnitudes and phases of the structure factors, but experimental measurements yield only the magnitudes.

Using a good estimate of the phase with each measured amplitude allows for the direct visualization of the protein via its electron density map.

Give the name of one method to resolve the phase problem. (2 points)

Possible answers:
1. MIR (multiple isomorphous replacement)
2. MR (molecular replacement)
3. MAD (multiple anomalous dispersion)
4. d. (4 points) The diagram below is typically associated with the derivation of the Bragg equation in which the crystal planes \( P \) are considered to be reflectors. Reinforcement in the reflected beam will occur only when the path difference is some multiple of the wavelength, \( \lambda \)

so that \( \theta \) must satisfy the Bragg equation \( n\lambda = 2d \sin \theta \).

![Diagram showing the Bragg equation derivation](image)

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e. As a protein structure determination method, what are two significant advantages that **NMR spectroscopy** has relative to **x-ray crystallography**? Please try to be neat and concise in your answer. (6 points)

1. You don't need crystals for NMR.
2. NMR can be used to obtain information about internal motions in a protein or nucleic acid.
3. NMR determines the structure of the molecule in solution, rather than the solid state.

f. As a protein structure determination method, what are two significant advantages that **x-ray crystallography** has relative to NMR spectroscopy? Please try to be neat and concise in your answer. (6 points)

1. X-ray methods often provide a more precise view of the structure.
2. X-ray methods can be used to study very high MW molecules and complexes.
5. A high-affinity receptor for interleukin-13 (IL-13) is over-expressed in disease-related fibroblasts and neoplastic cells and is involved in cancer, allergic, and inflammatory diseases. When the extracellular domain of the receptor is cleaved, it acts as a decoy receptor. Researchers at the FDA expressed and purified the extracellular domain of the IL-13 receptor in both E. coli and mammalian systems as a soluble fragment and studied its biological activities. Although both products showed IL-13 inhibitory activities, mammalian cell-derived protein appeared to be superior compared with purified protein from E. coli. (Kioi M, Seetharam S, Puri RK., FASEB J., Oct 2006) Suggest a simple explanation for the discrepancy in biological activity. (5 points)

Eukaryotic proteins, but not prokaryotic proteins, are subject to glycosylation. Glycosylation can affect protein properties in many ways, including protein folding, oligomerization, physical stability, specific bioactivity, rate of clearance from the bloodstream, and protease resistance.

Bonus Question: (4 points)
A “starch blocker” protein extracted from beans was once marketed as a diet pill for oral administration. This material inhibited α-amylase in vitro but did not do so in the human alimentary tract. Explain why this might be so. If the pills had worked as advertised, what would have been the likely unwanted side effects?

The ingestion of an effective intestinal α-amylase inhibitor with a starch-containing meal would result in the starch being transported, in undigested form, to the colon. There, the bacterial flora, with are equipped with a large variety of carbohydrate-hydrolyzing enzymes, would readily digest it, with a resulting digestive upset similar to but probably more severe than that suffered by individuals with lactose intolerance who drink milk. However, since the purported α-amylase inhibitor is a protein, it would probably be denatured and partially degraded by the high acidity and (acid-resistant) proteolytic enzymes in the stomach before it enters the intestines.