Question 1
(a) Below is a list of biological functions. For each macromolecule listed, choose two functions that the macromolecule is involved in.

<table>
<thead>
<tr>
<th>membrane component</th>
<th>catalyst</th>
<th>structural support</th>
<th>hormone</th>
</tr>
</thead>
</table>

(ai) Lipid
- membrane, hormone, energy store

(aii) Protein
- catalyst, membrane, structural, hormone, signaling, pump

(aiii) Carbohydrate
- membrane, structural, energy store, signaling

(aiv) Nucleic Acid
- catalyst, genetic material, structural, signaling molecule

(b) Choose two of the following chemical forces and describe how they contribute to the structure of a double-stranded DNA molecule (note: some of these forces might not be involved in DNA structure): hydrogen bonds, van der Waals, ionic bonds and covalent bonds

Force #1: hydrogen – between base pairs; keeps strands together
Force #2: hydrogen – between sugar-phosphate backbone and aqueous environ.; keeps bases “inside” the helix
Force #3: covalent – between nucleotides; creates the strands
Force #4: van der Waals – between stack of bases; helps keep bases stacked correctly; lowers the energy level.

(c) We know that in DNA adenine bases always pair with thymine bases and guanine bases always pair with cytosine bases. Sometimes, the DNA double helix is represented using the following schematic:

(ci) Using a similar schematic, draw what you expect the double helix to look like if adenine paired with guanine and cytosine paired with thymine (label 5’ and 3’ ends and hydrogen bonds):

Adenine and guanine are both 2-ringed bases while cytosine and thymine each have only 1 ring. Pairing adenine and guanine would put four rings across one stack and pairing cytosine with thymine would put only 2 rings across the stack. Therefore, A:G pairs would be wider than C:T pairs, causing the helix to bulge in and out.

(cii) Using a similar schematic, draw what you expect the double helix to look like if adenine paired with cytosine and guanine paired with thymine (label 5’ and 3’ ends and hydrogen bonds):

Pairing A with C and G with T would result in three rings per stack, so the distance between the backbones of the two strands should remain fairly constant. This alternate pairing, however, would probably also result in alternate bond angles which would affect the shape of the helix.
(d) While the rule of base pairing is generally followed, in rare cases alternative base pairing occurs. Below are two sets of nucleotides that are involved in alternate base pairing (dR represents the deoxyribose and phosphate groups). For each of the alternate pairs, draw a line between the appropriate atoms to indicate where hydrogen bonds would form.

(e) Erwin Chargaff isolated double-stranded DNA from a variety of organisms and discovered that the amount of adenine (A) equals the amount of thymine (T) and the amount of cytosine (C) equals the amount of guanine (G). Would you expect the amount of G to equal the amount of C for single-stranded RNA? Explain. The reason why G is equal to C in double-stranded DNA is because a G on one strand pairs with a C on the other strand. RNA is single-stranded so there is no reason why the amount of G should equal the amount of C. There are times that RNA can be double-stranded, but enough of the RNA remains single-stranded such that G and C do not equal each other.

(f) Consider a situation where a DNA molecule has A paired with C.

After two rounds of semi-conservative replication starting with this one cell, what is the base pair at this position in each of the four DNA molecules (note: assume that there is no “repair” of the mutation)? Drawing a diagram might help you think about this problem!

\[
\begin{array}{cccc}
\text{#1: } & \text{AT} & \text{#2: } & \text{AT} \\
\text{#3: } & \text{GC} & \text{#4: } & \text{GC}
\end{array}
\]
**Question 2**
You are an undergraduate researcher working in a lab that studies transport proteins. You have just identified a novel protein from human cells, Protein X, which you believe is involved in intracellular transport. After careful investigation, you determine the amino acid sequence of Protein X. Below is a portion of the sequence that contains the part of the site where Protein X binds the molecule that it transports:

Val-Ala-Phe-Gln-Gly-Leu-**Asp**-**Asp**-Gly-Glu-Ala-Trp-Tyr-Met-Leu-Lys-Leu

*Bold* represents amino acids in the binding site.

Next, you find mutant versions of Protein X that have single amino acid substitutions.

(a) A mutant in which one of the Asp residues is mutated to a Glu residue. Even with this mutation, Protein X is still functional. Explain why.

*The two Asp amino acids are both in the binding site, so they are important for binding the molecule to be transported. Asp and Glu are both negatively charged amino acids. Asp and Glu are also fairly similar in size. Assuming that the negative charge is important for binding, Glu can substitute for Asp because the charge is conserved.*

(b) A mutant in which the Glu residue is mutated to a Lys residue. This mutation causes Protein X to be non-functional. Explain why.

*The Glu amino acid is in the binding site, so it is important for binding the molecule to be transported. Glu and Lys are amino acids with opposite charge. If the negative charge on Glu is important for Protein X to bind its transport molecule (i.e. – be functional), the change to a positively charged amino acid would inhibit the function of Protein X. Additionally, Lys is longer than Glu, which might also affect structure.*

(c) A mutant in which the Val residue is mutated to a Phe residue. This mutation causes Protein X to be non-functional. Explain why.

*Unlike the Asp amino acids and the Glu amino acid, Val is not in the binding site, so it is not directly involved in binding the transport molecule. Val is changed to Phe. Both amino acids are hydrophobic or non-polar. Phe, however, is a very large bulky amino acid compared to Val. Thus, it is possible that the change to Phe causes some disruption in the tertiary structure that prevents Protein X from being functional.*

(d) What level of protein structure do you know is changed in all of these mutant proteins?

*primary*
Question 2 continued

After further analysis of your protein, you find that α-helices and β-sheets are extremely important for maintaining the structure of the binding site.

(e) What level of structure do α-helices and β-sheets represent?
    secondary

(f) What region of the polypeptide (backbone or side-chains) is involved in forming α-helices and β-sheets?
    the backbone

(g) What type of bond holds together the shape of α-helices and β-sheets?
    hydrogen

(h) You boil Protein X at 100 °C for 15 minutes. Would you expect Protein X to still be functional and, if not, what level(s) of protein structure would be affected: primary, secondary or tertiary?
    Functional? – no  Levels of structure? – secondary and tertiary
    Boiling Protein X adds a lot of energy to the system that could disrupt hydrogen and possibly ionic bonds of Protein X. You would not expect Protein X to be functional if these bonds were disrupted because the structure of Protein X would no longer exist. The levels of structure that are disrupted are secondary and tertiary.

(i) You put Protein X in methane, a hydrophobic solution. Would you expect Protein X to still be functional and, if not, what level(s) of protein structure would be affected: primary, secondary or tertiary?
    Functional? – no  Levels of structure? – secondary and tertiary
    You would not expect that Protein X would be functional in methane. Part of the tertiary structure is a result of hydrophobic interactions between nonpolar amino acids. In an aqueous environment (the cell is an aqueous environment), the majority of the hydrophobic residues fold into the interior of the protein. In a hydrophobic solution such as methane, it would be more energetically stable to have the hydrophobic amino acids on the outside of the structure and the polar amino acids on the inside of the structure. Thus, the protein would be turned “inside out” and no longer functional. Because some of these hydrophobic residues might also be involved in secondary structure, this level of protein structure would also be disrupted.

(j) Based up your knowledge of Protein X, would you predict that it transports positively charged molecules, negatively charged molecules or uncharged molecules?
    positive
Question 2 continued

Doing some more analysis of the function of Protein X reveals that in addition to binding the molecule it transports, it also binds ATP (at a different site). You have a transport assay that measures the activity of Protein X. You perform the transport assay under different conditions:

<table>
<thead>
<tr>
<th>Transport Molecule Added?</th>
<th>Nucleotide Added</th>
<th>Nucleotide Binding?</th>
<th>Transport Activity Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>ATP</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Yes</td>
<td>ADP</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>GTP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>ATP-γS**</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

** - ATP-γS is an analog (or version) of ATP that has an S atom in place of one of the O atoms attached to the third, or γ, phosphate atom. ATP-γS can not be cleaved between the second and third phosphates.

Below are the structures for each of the nucleotides that you added to the reaction:

![ATP Structure](image1)

ATP

![ADP Structure](image2)

ADP

![ATP-γS Structure](image3)

ATP-γS

![GTP Structure](image4)

GTP

(k) ADP can not substitute for ATP to transport the molecule. Is this because of a problem with the nucleotide binding Protein X or a problem with deriving energy to transport the molecule? Provide a reason for your choice based on the structure or energy capacity of the nucleotide (i.e. – do not simply refer to the data above).

The data show that ADP binds to Protein X, but transport does not happen. Therefore, ADP must not be able to substitute for ATP because it lacks the energy capacity. Indeed, ADP is missing the third phosphate group. It is the bond between the second and third phosphate on ATP that is cleaved to generate energy. Because ADP does not have the third phosphate group, there is no bond to be cleaved and thus no energy can be derived for the reaction.
(l) ATP-γS can not substitute for ATP to transport the molecule. Is this because of a problem with the nucleotide binding Protein X or a problem with deriving energy to transport the molecule? Provide a reason for your choice based on the structure or energy capacity of the nucleotide (i.e. – do not simply refer to the data above).

The data show that ATP-γS binds to Protein X, but transport still does not happen. Therefore, ATP-γS must not be able to substitute for ATP because it lacks the energy capacity. As indicated below the table, ATP-γS can not be cleaved because one of the oxygen atoms on the third phosphate groups has been replaced by a sulfur atom. Because it can not be cleaved, ATP-γS cannot provide energy for the transport reaction.

(m) GTP can not substitute for ATP to transport the molecule. Is this because of a problem with the nucleotide binding Protein X or a problem with deriving energy to transport the molecule? Provide a reason for your choice based on the structure or energy capacity of the nucleotide (i.e. – do not simply refer to the data above).

The data show that GTP does not bind Protein X. This is most likely because ATP and GTP have different nitrogenous bases. Enzymes (and other proteins as well) have binding sites that are very specific. Due to the different structures of the nitrogenous bases, the ATP-binding site on Protein X can not bind GTP. Additionally, even if Protein X could bind GTP, it probably still could not substitute for ATP because GTP is not used as an energy source by the cell.

You are interested in determining the thermodynamic role of ATP in your assay, so you measure the concentration of ATP, ADP and Pᵢ before and after your experiment.

(n) Your bench-mate, who also took 7.014 with you, mentions that there is no need to measure the concentrations. He says to you “Professor Walker already told us that in the cell the ΔG’ of ATP hydrolysis is -12 kcal/mol.” Explain why ΔG’ of ATP hydrolysis for your specific reaction would be different.

The ΔG’ of a reaction is dependent on the concentration of the products and the reactants. The concentration of ATP, ADP and Pᵢ in the cell is likely to be different than the concentration of these molecules in your reaction. Other reaction conditions also are likely to be different,

(o) You measure the concentration of ATP, ADP and Pᵢ in your reaction and find that ΔG’ of ATP hydrolysis is -1.4 kcal/mol. Do you think that in the absence of Protein X this value would (circle the appropriate answer):

- decrease
- increase
- stay the same

(p) What cellular process(es) provides the ATP for this reaction?

glycolysis, citric acid cycle (Krebs Cycle) and oxidative phosphorylation. (photosynthesis)
**Question 3**

(a) If a cell is under anaerobic conditions (no $O_2$) and has a mutant form of the enzyme responsible for converting pyruvate to lactic acid such that the enzyme could not perform the conversion, the cell will die because it can not make ATP. **Explain** why the cell must convert pyruvate to lactic acid under anaerobic conditions to live.

Under anaerobic conditions, the only source for ATP is from glycolysis. In order for glycolysis to occur, an electron acceptor must be available and the cell uses $NAD^+$. In the absence of $O_2$, the conversion of pyruvate to lactic acid regenerates $NAD^+$ from the NADH that was created during glycolysis. If a cell could not convert pyruvate to lactic acid as it can not in this cell, $NAD^+$ is not regenerated, glycolysis shuts down and ATP is not produced.

(b) Give one reason why respiration is sometimes referred to as the opposite of photosynthesis.

1. photosynthesis = $CO_2 \rightarrow$ glucose  
   respiration = glucose $\rightarrow CO_2$

2. photosynthesis produces $O_2$ and respiration uses it.

(d) In the past few years there have been many reports of athletes (especially cyclists) who “dope” using a drug known as EPO. EPO stands for Erythropoietin, which is a hormone that induces the body to produce more red blood cells. An increase in red blood cells results in an increase in the oxygen level in the blood. In terms of respiration, how is EPO useful for an athlete?

$O_2$ is the final electron acceptor in oxidative phosphorylation. Without $O_2$, oxidative phosphorylation does not occur, so ATP is only generated from glycolysis via fermentation. Glycolysis only produces 2 ATP for every glucose molecule whereas oxidative phosphorylation produces 36 ATP for every glucose molecule. If an athlete uses EPO, more $O_2$ is available for oxidative phosphorylation. This allows the athlete to keep producing maximum amounts of ATP, or energy, despite physical exertion which would normally limit the availability of $O_2$.

(e) For each of the transformations listed, put an “R” if the compound on the left is being reduced, and an “O” if it is being oxidized and a physiological process that mediates this transformation.

<table>
<thead>
<tr>
<th>Transformation</th>
<th>O or R</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_6H_{12}O_6 \rightarrow CO_2$</td>
<td>O</td>
<td>glycolysis, citric acid cycle, respiration</td>
</tr>
<tr>
<td>$NAD^+ \rightarrow$ NADH</td>
<td>R</td>
<td>glycolysis, citric acid cycle</td>
</tr>
<tr>
<td>$NO_3^- \rightarrow N_2O$</td>
<td>R</td>
<td>anaerobic respiration</td>
</tr>
<tr>
<td>$NO_2^- \rightarrow NO_3^-$</td>
<td>O</td>
<td>chemosynthesis</td>
</tr>
<tr>
<td>$O_2 \rightarrow H_2O$</td>
<td>R</td>
<td>oxidative phosphorylation, respiration</td>
</tr>
<tr>
<td>$H_2S \rightarrow S$</td>
<td>O</td>
<td>chemosynthesis, anoxygenic photosynthesis, cyclic photophosphorylation</td>
</tr>
<tr>
<td>$CO_2 \rightarrow C_6H_{12}O_6$</td>
<td>R</td>
<td>photosynthesis, Calvin Cycle</td>
</tr>
</tbody>
</table>
Individual amino acids are linked through these groups to form the backbone of the protein.

Protein Synthesis

Side chain, unique to each different amino acid

Peptide bonds

 STRUCTURES OF AMINO ACIDS

ALANINE (ala)

ARGININE (arg)

ASPARAGINE (asN)

ASPARTIC ACID (asp)

CYSTEINE (cys)

GLUTAMIC ACID (glu)

GLUTAMINE (glN)

GLYCINE (gly)

HISTIDINE (his)

ISOLEUCINE (ile)

LEUCINE (leu)

LYSINE (lys)

METHIONINE (met)

PHENYLALANINE (phe)

PROLINE (pro)

SERINE (ser)

THREONINE (thr)

TRYPTOPHAN (trp)

TYROSINE (tyr)

VALINE (val)