Solution Key - 2010 7.012 Problem Set 1

Question 1

The major macromolecules present in the cells of all living organisms are proteins, nucleic acids, carbohydrates and lipids. These macromolecules (with the exception of most lipids) are formed by the joining of specific monomers.

a) Name the four elements commonly found in the major types of biological macromolecules.
Carbon, hydrogen, Oxygen and Nitrogen

b) From the following, **circle** the type of bond or interaction that joins the monomers to form the polymers.

(Covalent bonds) ionic bonds hydrophobic interactions van der Waals forces

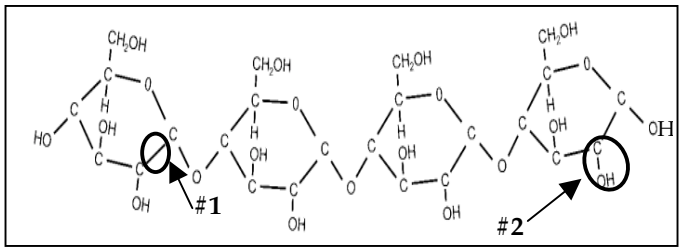
c) What is produced as a **byproduct** of a condensation reaction?
Water

d) Proteins are among the major classes of biological macromolecules. They show organelle –specific location and have defined functions. List the **subcellular location** of each of the following **eukaryotic** proteins in their **active (functional) state.** Choose from:
Lysosomes, Extracellular matrix (ECM), cytoplasm, nucleus, mitochondria, endoplasmic reticulum, golgi body, cell wall.

Protein	**Location in active (functional) state**
A protein that binds to deoxyribonucleic acid (DNA)	*Nucleus*
A protein that glues the cells together into tissues	*ECM*
A protein involved in the synthesis of ATP via oxidative phosphorylation	*Mitochondria*
A protein involved in the synthesis of ATP via Fermentation	*Cytoplasm*

Question 2

a) Consider the following carbohydrate that is a part of a **protein**.



i. Name each bond or interaction that is circled in the schematic above. **Explain** why you selected these options. Choose from *polar covalent, non-polar covalent, ionic, hydrophobic, van der Waals*.

- Bond #1 (C-C): *This is a non-polar covalent bond formed by equal sharing of electrons between the two carbon atoms.*
- Bond #2 (C-OH): *This is a polar covalent bond formed by unequal sharing of electrons between the carbon and oxygen atoms. Oxygen being more electronegative has a greater affinity for the shared pair of electrons compared to carbon.*

Question 2 continued

ii. Would you expect this polymer shown in 2 (a) on the previous page to be soluble in the aqueous environment? **Explain** your answer.

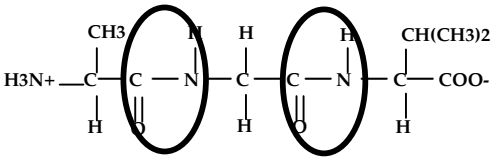
Yes, the schematic on page 1 represents a polar molecule that can form hydrogen bonds with the surrounding water molecules, which allow it to dissolve in the aqueous environment.

iii. What type of bonds or interactions occur between two such long polymers? Choose from: *covalent, hydrogen, hydrophobic, ionic, van der Waals*. **Explain** your answer.

The multiple hydroxyl groups (-OH groups) that are a part of this polymer allow it to hydrogen bond or covalently bond (through an enzyme catalyzed condensation reaction) with an identical polymer.

b) Proteins are composed of amino acids.

i. Draw a peptide **N-ala-gly-val-C** in the form it would be at pH=7.



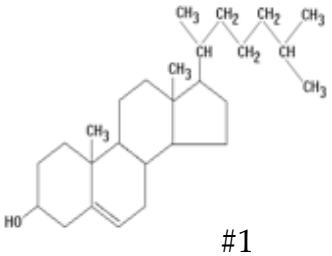
ii. Circle each peptide bond in your drawing above.

iii. The side-chains of the amino acids that make this peptide would be classified as… *Polar, non-polar, charged, uncharged, hydrophilic, hydrophobic.* Circle **all** the correct options.

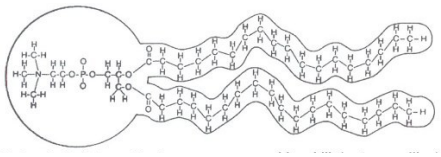
iv. How does the amino acid glycine (gly) differ from **all** the other amino acids?

It is the only amino acid that lacks an asymmetric central carbon atom i.e. two of the four covalent bonds of the central carbon atom is with hydrogen atoms. The central carbon atom of all the other amino acids forms four covalent bonds with four different groups, which makes them asymmetric.

c) Consider the structures below to answer the following questions.



#1



#2

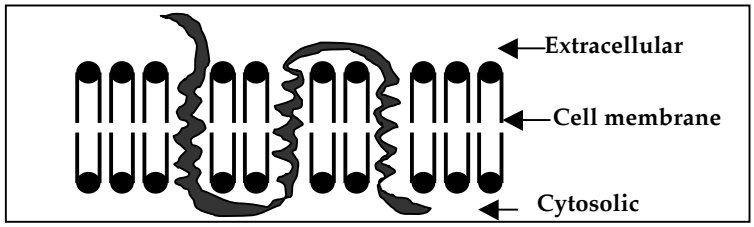
i. Which structure (#1 or #2) can assemble to form a lipid bilayer? What property of this molecule allows it to form a lipid bilayer?

Structure #2, unlike structure #1 is an amphipathic molecule i.e. it has a hydrophilic head that can be exposed to the aqueous environment and a hydrophobic tail that remains hidden within the non-aqueous / hydrophobic environment. Such molecules can assemble in an aqueous environment to form a lipid bilayer.

ii. Which bond or interaction is **most likely** to stabilize the lipid bilayer? Choose from: *covalent, hydrogen, hydrophobic, ionic, van der Waals*.

Question 2 continued

iii. Drawn below is a schematic of a transmembrane protein.



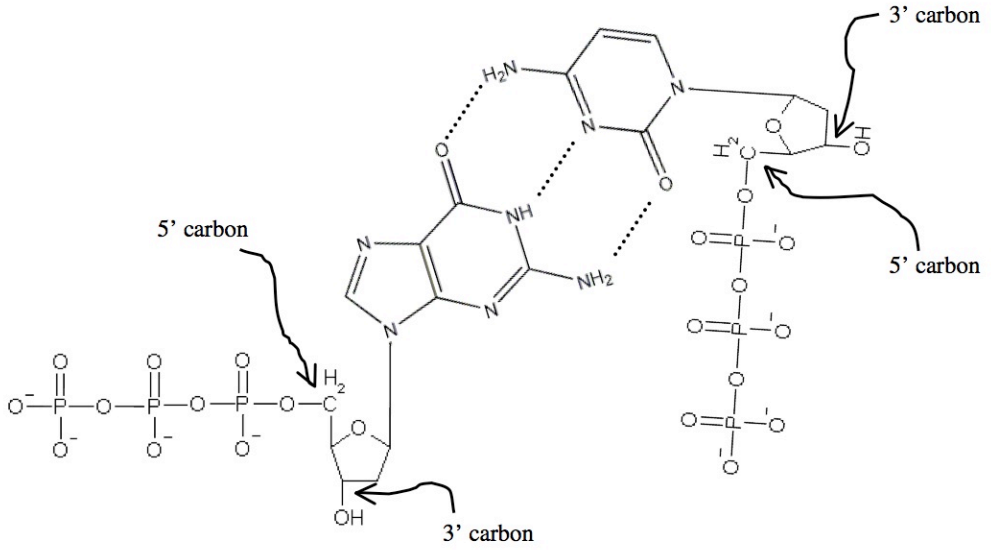
Of the following amino acids, **circle** those that may be located in a linear stretch that is **embedded** in the lipid bilayer? **Explain** why you selected these amino acids.

leucine *serine* *glutamine* *lysine* *threonine* *alanine*

The selected amino acids have non-polar/hydrophobic side-chains that may be embedded in the non-polar, hydrophobic environment at the interior of the lipid bilayer.

d) Nucleotides are the monomers of nucleic acids.

i. Draw a G nucleotide triphosphate (dGTP) **base-pairing** with a C nucleotide triphosphate (dCTP).



ii. Label all the 5′ and 3′ carbons in your drawing above.

iii. How many hydrogen bonds (1/2/3/none) are present in your drawing above? *3*

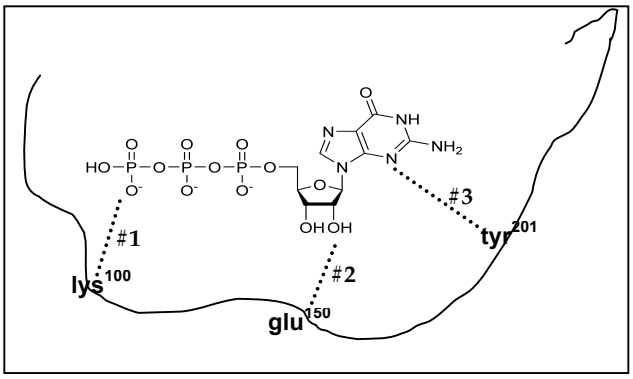
iv. How many phosphodiester bonds (1/2/3/none) are present in your drawing above? *None*

v. Is the overall charge on a DNA double helix at pH7 neutral, negative, positive, or inconclusive? **Explain** your choice.

It has an overall negative charge due to the negatively charged phosphate groups that are a part of each deoxy-ribonucleotide base.

Question 3

The following is a schematic of the GTP bound to the active site of an enzyme E1. For simplicity only the amino acids that interact with the GTP are shown.



a) In the table below, write the **strongest interaction** at positions #1, #2 and #3 **(shown by the dotted lines)** between the GTP and the side-chains of the amino acids that are shown in the schematic.

Position	Strongest interaction
#1	*Ionic*
#2	*Hydrogen*
#3	*Hydrogen*

b) You create two mutant versions (mutant 1 and mutant 2) of this enzyme each of which has mutations in its active site. These mutations prevent the binding of the enzyme to GTP. For each mutant version of the enzyme, **explain** how the mutation(s) alter the bonding seen with the normal version and why this prevents the enzyme from binding to GTP.

Enzyme	Amino acids at the active site		
Normal	**lys**^{100}	**glu**^{150}	**tyr**^{201}
Mutant 1	**lys**^{100}	**gly**^{150}	**thr**^{201}
Mutant 2	**leu**^{100}	**val**^{150}	**trp**^{201}

- **Mutant 1:** *This mutant will not bind to GTP since gly^{150}, which has a non-polar, hydrophobic side-chain, can't form a hydrogen bond with GTP at position #2 unlike the normal functional version of the enzyme.*

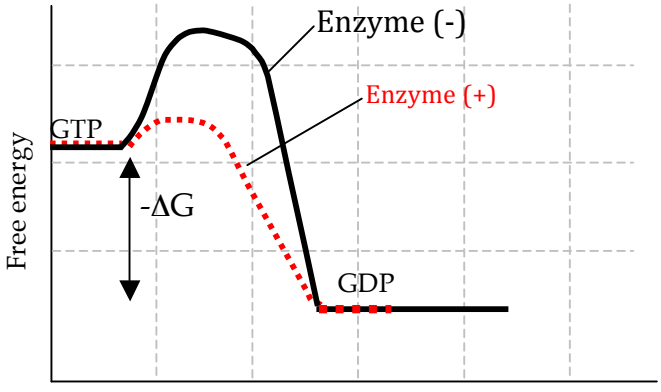
- **Mutant 2:** *Mutant 2 will not bind to GTP since val^{150}, which has a non-polar, hydrophobic side-chain, cannot form a hydrogen bond with GTP at position #2 unlike the normal functional version of the enzyme. Also, trp^{201} being a non-polar, hydrophobic amino acid with an aromatic ring in its side-chain cannot form a hydrogen bond with GTP at position #3 unlike the normal functional version of the enzyme. The aromatic ring of trp^{201} may also create steric hindrance and may disrupt the active three-dimensional conformation of the enzyme.*

c) The enzyme E1 catalyzes the hydrolysis of GTP to GDP as shown below.

GTP $\xleftrightarrow{\textbf{E1}}$ **GDP**

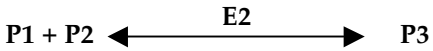
i. Draw the energy profile of the forward **E1 catalyzed reaction** on the axes below. Label the reactants and the products and indicate the overall free energy change.

ii. Draw the energy profile of the same reaction **in the absence of E1** on the axes below. Label the reactants and the products and indicate the overall free energy change.



Question 3 continued

d) The following is a reaction catalyzed by a different enzyme E2.



In a living cell, the coupling of GTP hydrolysis with the reaction above increases the rate of E2 catalyzed reaction. **Explain** why this is so.

Hydrolysis of GTP is an exergonic reaction (-ΔG) that releases energy. When this reaction is coupled with an endergonic reaction, the energy released following the hydrolysis of GTP may be used to enhance the rate of E2 coupled endergonic reaction.

Question 4

Ras proteins are plasma membrane bound proteins that communicate signals from the outside of the cell to the nucleus. These proteins are essential components of various cell-signaling pathways that regulate growth, cell division and cell death. The Ras proteins function as a molecular switch by cycling between an active GTP-bound state and an inactive GDP-bound state. Conversion from the GTP-bound state to the GDP- bound state is mediated by the intrinsic GTPase activity of Ras, which hydrolyzes GTP to GDP. Mutations that activate the Ras proteins are found in 20-25% of all human cancers.

In this exercise, you will use StarBiochem, a protein 3D- viewer to explore the structure of the Ras protein and understand its active and inactive state.

To begin using StarBiochem, please navigate to: **http://web.mit.edu/star/biochem**. Click on the **Start** to launch the application. In the top menu under Samples, select **"5P21"**. Take a moment to look at the structure from various angles by rotating and zooming on the structure. Instructions for changing the view of structure can be found in the top menu, under **View -> Structure viewing instruction**.

a) How would you classify this protein: monomeric or oligomeric?

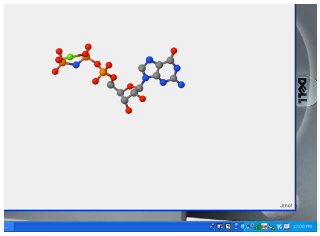
Monomeric since it is comprised of only a single polypeptide chain as evident from the primary and quaternary structures.

b) Based on the primary structure of 5P21, what is the length, in terms of amino acids, of the mature Ras protein?

This protein has 166 amino acid residues.

c) Identify and draw the other structural element(s) (besides the amino acids) shown in 5P21 structure.

I*t is a GTP analog.*



d) What form of Ras (active/inactive) is shown in this structure? **Explain** why you selected this option.

Since the Ras protein is bound to GTP and not GDP it is in its active form.

e) Choose the option from below, which best describes the structural element that you have drawn in part (c) above. **Explain** why you selected this option.

Deoxyribonucleotide *Ribonucleotide* *Lipids* *Carbohydrates*

Question 4 continued

e) We will now analyze the secondary structure of Ras (5P21).

i. From the following, underline the option that best describes the secondary structure of 5P21?

- Beta sheets surrounded by helices and coils.
- Coils surrounded by beta sheets and helices.

ii. Is helix HE located at the N- or the C-terminus of the protein? **Explain.**

It is located at the C- terminus of Ras protein since it is comprised of amino acids #152 - #164 that are close to C-terminus of the protein.

f) The Ras protein has five major motifs that bind to GDP/GTP directly. These motifs are: G1 (amino acids #10-17), G2 (amino acid #35), G3 (amino acids #57-60), G4 (amino acids #116-119) and G5 (amino acids #145-147).

i. Which of these motifs (G1/G4/G5) bind to the phosphate groups of GTP*?*

G1 motif

ii. Which of these motifs (G1/G4/G5) interact with the guanine base of GTP/GDP? What is the most likely mode of interaction?

G4 motif

iii. To which part of GTP (base/sugar/phosphate) does amino acid #30 interact? What is the most likely mode of interaction? *Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces.*

Amino acid #30 is Asp that forms a hydrogen bond with the ribose sugar of GTP.

iv. The interaction of Ras with GTP/GDP also involves Mg^{++} ions. ***Identify the part of GTP/GDP that interacts with Mg^{++} ions.*** Your choices are 'guanine base', 'sugar' or 'phosphates'.

The phosphates of GTP interact with Mg^{2}.

g) Mutations in the genes that encode Ras protein family members are very common in human cancers. The following are two individual amino acid substitutions that result in two mutant forms of the Ras protein.

Substitution 1: amino acid residue #17 substituted by cysteine.
Substitution 2: amino acid residue #119 substituted by glutamine.

Explain which substitution is more likely to have an effect on the activity of Ras protein.

In substitution 1, the side-chain of Ser17 forms a hydrogen bond with the guanine base of GTP unlike cysteine. Therefore this substitution will have an effect on the activity of Ras protein. In contrast, both Asp and glutamine can potentially form hydrogen bond with the GTP. Therefore substitution 2 will have no effect on the interaction of Ras with GTP.