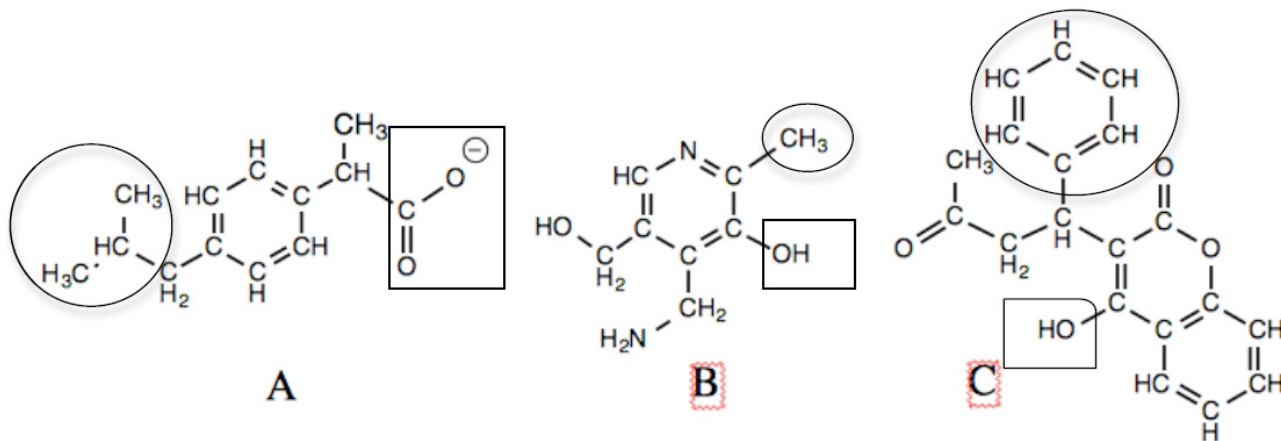


# Solution key-7.016 Problem Set 1

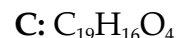
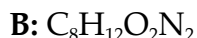
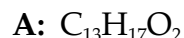
## Question 1

The following "line-angle" drawings represent three chemical structures. On each drawing, the hydrogen atoms that should be bonded to the NON-carbon atoms are missing.



a) For each structure, show the position of **All** carbon (C) and all hydrogen (H) atoms. Note: If there is a charge present make sure you take it into account.

b) Give the **chemical formula** of each of the structures shown by the line angle drawings.



c) Circle a **nonpolar** functional group for each of the above structures.

Please note that there are many correct answers but only one has been circled for each of the structures above.

d) Box a **polar functional group** for each of the above structures.

Please note that there are many correct answers but only one has been circled for each of the structures above.

e) Which of the above structures is most soluble in water? **Explain** why you selected this option.

Structure B is most soluble in water. It has several polar functional groups that can hydrogen bond with the surrounding water molecules.

f) Which of the above "line-angle" drawings represent a chemical structure that is amphiphilic? **Explain** why you selected this option(s).

Amphiphilic by definition refers to a molecule that has a polar group attached to a nonpolar hydrocarbon. Since structure A has a polar carboxyl group (-COO<sup>-</sup>), structure B has polar amino (-NH<sub>2</sub>) and hydroxyl groups (-OH) and structure C has multiple oxygens that can acquire charge, all three structures can be regarded as amphiphilic.

## Question 2

a) The biological macromolecules present in the cells of all living organisms are proteins, nucleic acids, carbohydrates and lipids.

i. Which **three** elements (choose from C, H, O, N, S, P) are found in **all** biological macromolecules? They are Carbon, Hydrogen and Oxygen.

ii. Which radioactive element (choose from C, H, O, N, S, P) is the **best option** to detect **the nucleic acids** in a cell? **Explain** why you selected this element.

You would use radioactive phosphorous ( $P^{32}$ ), which is a part of all nucleotides that are the monomers of nucleic acids. It also participates in forming the sugar phosphate backbone of nucleic acids.

iii. Which radioactive element (choose from C, H, O, N, S, P) is the **best option** to detect **the proteins** in a cell? **Explain** why you selected this element.

You would use radioactive sulphur ( $S^{35}$ ), which is in the side-chain of methionine. This essential amino acid is usually the first amino acid in most newly synthesized proteins. Sulphur is also present in the side-chains of cysteine, which is one of the 20 essential amino acids and is likely present in most proteins. Other macromolecules are highly unlikely to contain sulphur.

b) Which of the following represent a condensation reaction? Place an **X** next to all that apply.

-----The formation of covalent disulfide bonds between two cysteines.

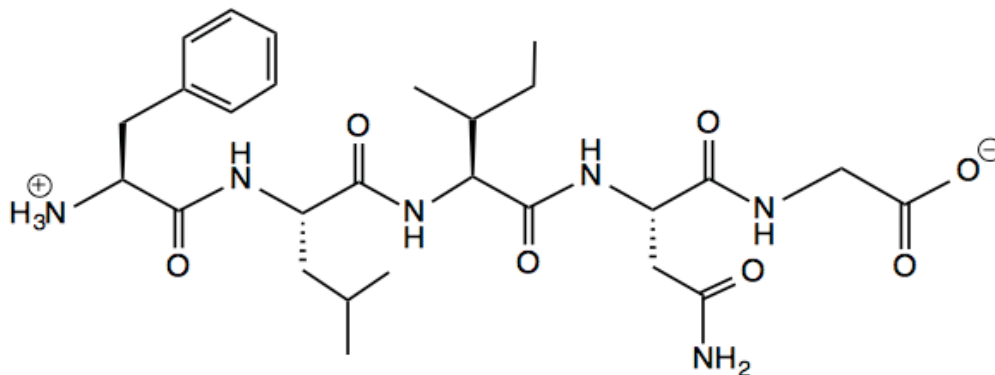
-----X-----The formation of a peptide bond.

-----X-----The formation of a glycosidic linkage to form a disaccharide.

-----The formation of glucose from lactose.

-----The cleavage of double-stranded DNA by an enzyme into small DNA fragments.

c) The following is the amino acid sequence of a growing peptide in a cell.



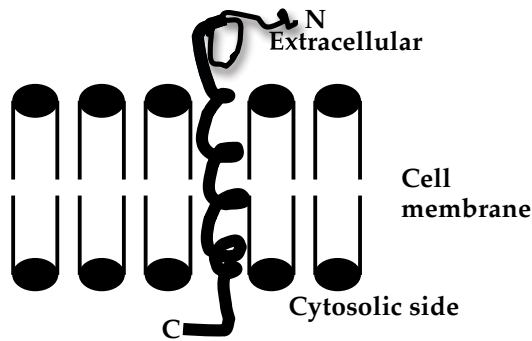
i. Add the amino acid **glycine** to the growing end of the peptide drawn above.

ii. What level of protein structure does the amino acid sequence in the schematic above represent (choose from primary, secondary, tertiary and quaternary)?

It represents the primary level of protein structure.

### Question 2 continued

d) Drawn below is a schematic of a transmembrane protein.



- i. When this transmembrane protein is embedded in the lipid bilayer, what is the highest order of structure (choose from *primary, secondary, tertiary and quaternary*) of this protein? **Explain** why you selected this option.

*This protein is comprised of a single polypeptide chain. Therefore it should have tertiary as the highest order of protein structure.*

- ii. From the list below, circle the amino acid(s) that might be more common in the **extracellular domain** of this membrane protein and whose side-chain can form hydrogen bonds with the surrounding water molecules. **Explain** why you selected this option(s).

Lysine

Serine

Phenylalanine

Methionine

*Both Lysine and serine have polar, hydrophilic side-chains that makes them compatible to the aqueous environment.*

- iii. From the list below, circle the amino acid(s) that would likely be found in the **transmembrane/membrane-spanning domain** of this protein and whose side-chain interacts with the lipid bilayer.

Lysine

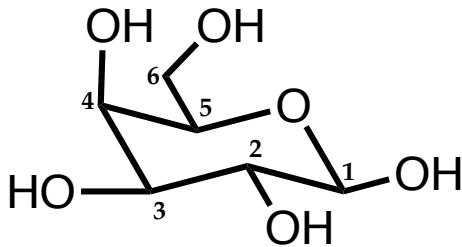
Serine

Leucine

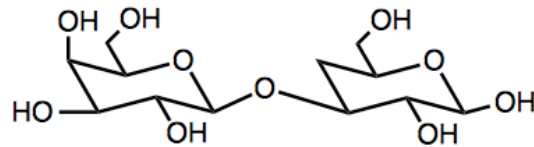
Methionine

### Question 3

a) Consider the following structure that represents a galactose molecule ( $C_6H_{12}O_6$ ).



- i. Draw a disaccharide composed of two galactose monomers that are linked between C1 of one monomer and C3 of the other monomer.



- ii. The molecular formula for galactose is  $C_6H_{12}O_6$ . Which of the following options represents an oligosaccharide composed of five galactose monomers?

$C_{30}H_{60}O_{30}$

$C_{48}H_{50}O_{22}$

$C_{30}H_{52}O_{26}$

$C_{36}H_{56}O_{30}$

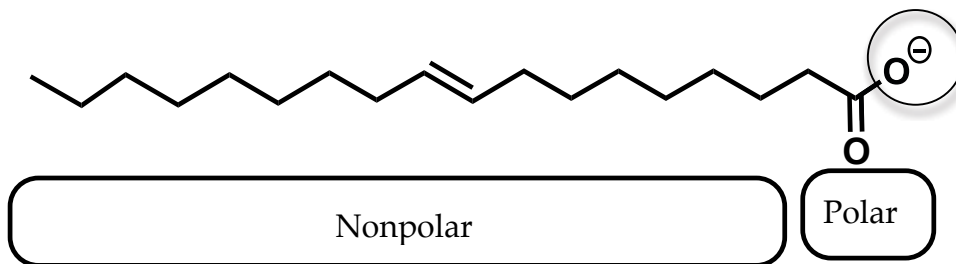
*One water molecule is produced as a byproduct for every glycosidic bond formed. In this case, four glycosidic bonds are formed between five galactose monomers producing four water molecules as byproduct.*

- iii. What types of **non-covalent bonds** or forces (choose from *hydrogen bonds, ionic bonds, hydrophobic interactions, vanDer Waals (VDW) forces*) **most likely** occur between the two oligomers of galactose? **Explain** your answer.

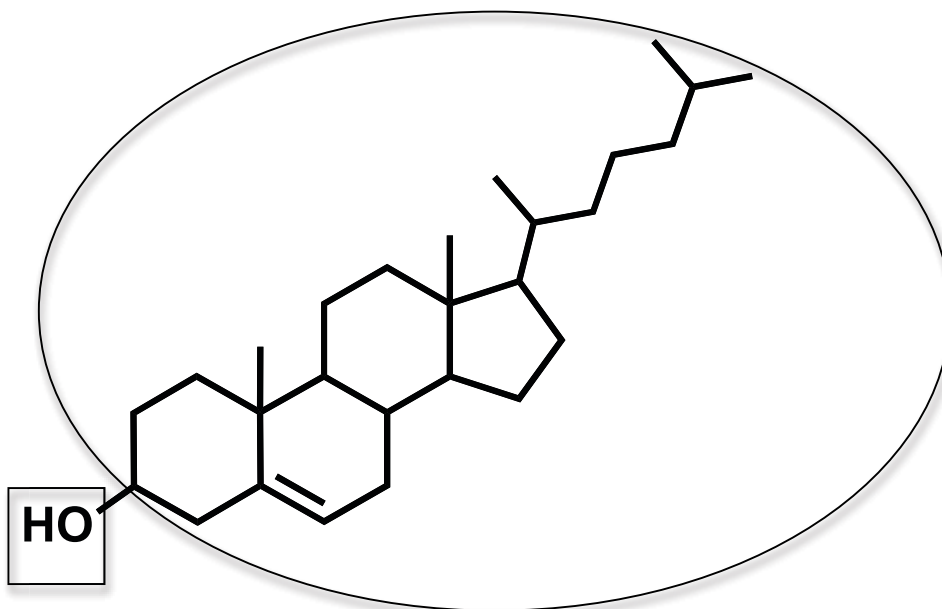
*The many hydroxyl groups (-OH groups) of the galactose monomers that make the polymer may form hydrogen bonds with the -OH groups of other galactose monomers of the same polymer.*

### Question 3 continued

b) Consider the structure of the fatty acid below to answer the following questions.



- Classify the above fatty acid as saturated or unsaturated? **Explain** your choice.  
*This is an unsaturated fatty acid as shown by double bond between the carbon atoms of the fatty acid chain.*
  - Identify whether the boxed regions of the molecule are polar and non-polar (**fill in the boxes**).
  - This fatty acid can undergo a condensation reaction with glycerol to form mono-, di- or triglycerides. In the schematic above, **circle the group** that participates in the condensation reaction.
  - The fatty acid chains are an integral part of a lipid bilayer. Which bonds or interactions of these chains (*choose from ionic, hydrogen, covalent, hydrophobic, VDW*) stabilize the lipid bilayer?  
*The hydrophobic interactions between the nonpolar fatty acid chains stabilize the lipid bilayer.*
- c) Below is the structure of an important component of the plasma membrane, cholesterol, which is also a precursor for the steroid hormones and vitamin D.

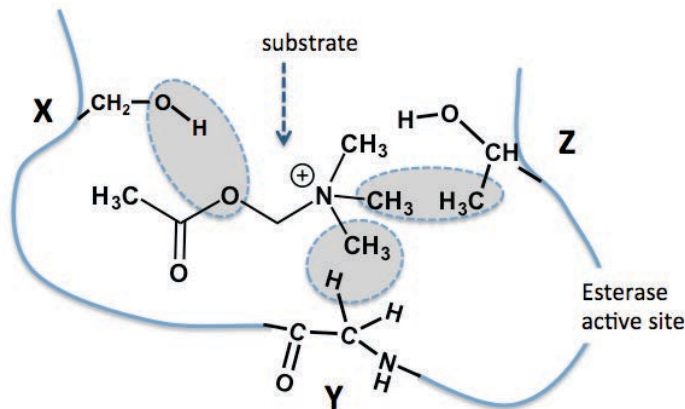


On the diagram above...

- Box the entire hydrophilic region** of this molecule.
- Circle the entire hydrophobic region** of this molecule.

### Question 4

The following schematic represents a substrate that is bound to the active site of an esterase enzyme. Esterases hydrolyze ester bonds. For simplicity, only the amino acids whose side-chains interact with the substrate are shown. Each circled interaction is important for the binding of the substrate to the enzyme.



a) In the table below, give the **strongest interaction** that can occur between the substrate and the side-chains of amino acids X, Y and Z at the active site of the enzyme.

Amino acid	Hydrogen-bond / Ionic bond / Hydrophobic bond?
X	Hydrogen bond
Y	Hydrophobic interaction
Z	Hydrophobic interaction

b) Which of the above amino acids, (X, Y or Z) is **closest to the N terminus** of the primary sequence of the enzyme?

*Amino acid Z is closest to the N terminus of the primary amino acid sequence of this enzyme.*

c) You create two mutants (1 & 2) of the enzyme, each of which has two amino acid substitutions at the active (substrate-binding) site. You observe that one mutant can bind to the substrate similarly to the wild-type version of the enzyme, whereas the other cannot. Which of the two mutant versions (1 or 2) will probably **not** bind to the substrate? **Explain, in terms of the characteristics of amino acids**, why you selected this version. Note: A table showing the structures of amino acids is given on the last page of this problem set.

Enzyme	Amino acids at the active site		
<b>Wild-type</b>	X	Y	Z
<b>Mutant 1</b>	Asp	Y	Tyr
<b>Mutant 2</b>	X	Ala	Z

*Mutant 1 will not bind to the substrate unlike mutant 2 and normal esterase. In mutant 1, although the side-chain of Asp (that replaces amino acid X) will not form a hydrogen bond with the substrate and the bulky benzene ring in the side-chain of tyrosine (that replaces amino acid Z) will generate steric hindrance thereby disrupting the 3D- conformation and the substrate- binding site of esterase. In comparison, in Mutant 2, the small non-polar hydrophobic side-chain of alanine (that replaces amino acid Y) can undergo VDW interaction similar to the original esterase. Therefore mutant 2 binds to the substrate unlike mutant 1.*

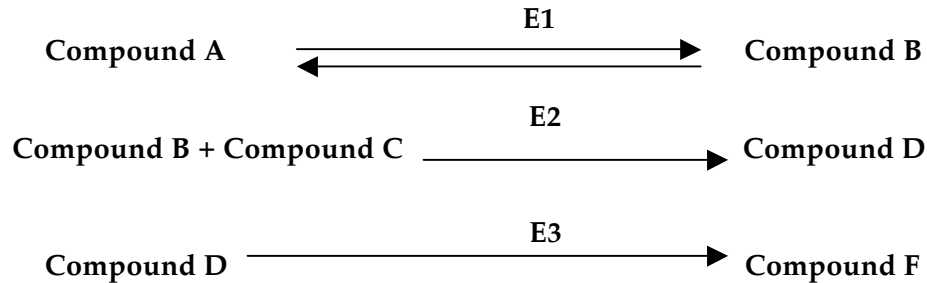
#### Question 4 continued

d) Full activity of esterase is observed at 37°C and a pH of 7.4. You isolate the **active form of esterase**, subject it to a **high salt (high sodium chloride) concentration** and show that it cannot bind the substrate. You then return the sample to the original conditions and measure the substrate binding ability of the esterase again. Would the esterase bind to its substrate when returned to the pre-treatment conditions (Yes/No)? **Explain.**

*Yes the esterase will most likely bind to the substrate when returned to the pre-treatment conditions. The bonds disrupted in response to an increase in the salt concentration will be the non-covalent bonds (Hydrogen, ionic, van der waals) that regulate the higher order of protein structure. But the primary structure of esterase will remain the same even after this treatment. Once the conditions are brought back to normal, the esterase can refold and be functional.*

#### Question 5

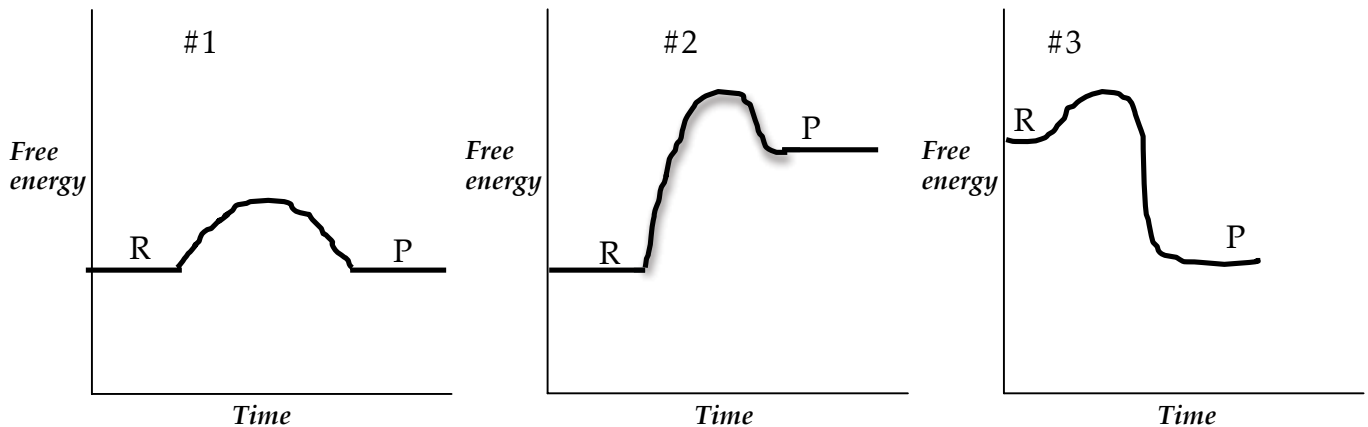
You are looking at the following biological reactions that are catalyzed by specific enzymes (E1, E2, E3).



#### Note:

- *In Reaction#1, Enzyme E1 catalyzes the conversion of Compound A to Compound B. This reaction is **truly reversible** and the reverse reaction is as likely as the forward reaction.*
- *In Reaction#2, Enzyme E2 catalyzes an **energy requiring reaction** that uses Compounds B and C as substrates to produce Compound D.*
- *In Reaction#3, Enzyme E3 catalyzes an **energy producing reaction** that converts Compound D to Compound F.*

a) Draw the energy profiles of reactions #1, #2 and #3 on the axes given below. On the diagram, please label the reactants and the products.



### Question 5 continued

b) Summarize the effect of each of these enzymes on the following reaction parameters. Note: Your choices are increases/decreases/remains unchanged.

Parameters	Enzyme E1	Enzyme E2	Enzyme E3
Reaction equilibrium	Unchanged	Unchanged	Unchanged
Reaction rate	Increases	Increases	Increases
Activation energy	Decreases	Decreases	Decreases
Free energy change ( $\Delta G$ )	Unchanged	Unchanged	Unchanged

c) You are told that **Enzyme E1 is the product of a gene** that encodes a protein of molecular weight 50kD. However, this enzyme in its **active form** has a molecular weight of 250kD. Why might the **active form** of Enzyme E1 be **heavier** than the product encoded by its corresponding gene?

*The enzyme, in its active form, is a pentamer (250kD) comprised of five polypeptide chains each of 50kD.*

d) You identify a cell line that **cannot** produce Compound F. You measure the concentration of each metabolite and observe that this cell line shows an excess concentration of Compounds B and C. Based on this observation, which of the three enzymes is **most likely** non-functional in this cell line? **Explain** your choice.

*Enzyme E2 is non-functional. It cannot catalyze the conversion of Compounds B and C to Compound D. This results in the accumulation of Compounds B and C over time.*

You decide to reproduce Reaction #2 in three test tubes. You may assume that this biological reaction occurs at **37°C** and **pH of 7.4** under **normal conditions**.

- **Test tube #1:** You perform the reaction at **70°C** and **pH 7.4** and observe that no Compound D is produced. When the temperature is brought to **37°C**, Compound D is produced at a rate similar to normal condition.
- **Test tube #2:** You perform the reaction at **37°C** and **pH 10.4** and observe that no Compound D is produced. When the pH is brought to **7.4**, Compound D is produced at a rate similar to normal condition.
- **Test tube #3:** You perform the reaction at **37°C** and **pH 7.4** in the **presence of proteases** and observe that no Compound D is produced. The effect of protease treatment persists even after its removal from reaction mixture.

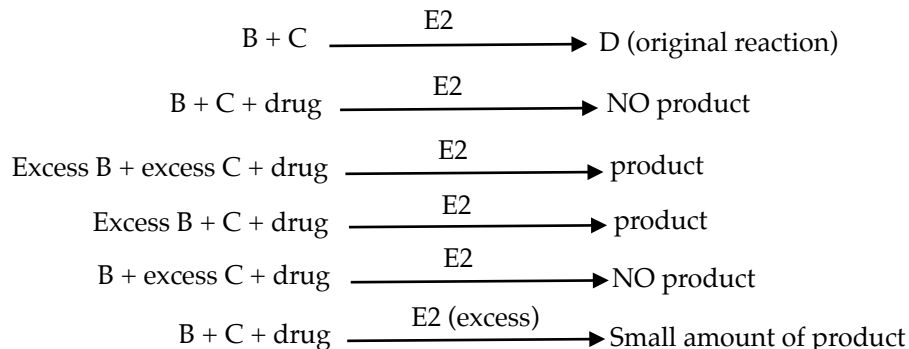
e) Explain the effect of the changed reaction parameters in each of the above test tubes on **structure** and **function** of Enzyme E2.

Reaction parameters	Effect on Enzyme E2	Is the effect reversible/irreversible?
<b>70°C in test tube #1</b>	Non-covalent bonds (hydrogen bonds, ionic bonds, van der Waals forces, hydrophobic interactions) will be disrupted thus disrupting the higher orders of protein structures. Therefore the enzyme will not have an active three dimensional conformation.	A temperature of 70°C denatures and inactivates the enzyme by disrupting the higher order of enzyme structure but has no effect on the primary structure of enzyme. However, if the pretreatment conditions are restored, the enzyme might refold back to its normal form and be active.
<b>pH 10.4 in test tube #2</b>	Same effect as above	Same as above
<b>Protease in test tube #3</b>	This treatment will disrupt the covalent peptide bonds thus disrupting the primary structure of Enzyme E2.	Protease treatment will disrupt the primary structure of Enzyme E2, an effect that cannot be reversed even by restoring the pretreatment conditions.



### Question 5 continued

During an experiment, by mistake, you add a drug to the original Reaction #2 mixture and find that the reaction is completely inhibited. You then try to make the reaction work by increasing the concentration of Compounds B and C. You find that the reaction now works. However as indicated below, if you add an excess of Compound B and a normal amount of Compound C together with drug the reaction works, but if you have a normal amount of Compound B and an excess of Compound C the reaction does not work. The reaction is partially restored if you increase the concentration of Enzyme E2. (These results are summarized schematically below.)



f) Based on this information, briefly **explain** how does the drug most likely inhibit this enzymatic reaction.

*The drug is a competitive inhibitor that competes with Compound B for the active site of Enzyme E2. Since Compound B, in the presence of this drug, cannot bind to the active site of Enzyme E2, the reaction does not proceed and no product formation is observed.*

### Question 6

Hemoglobin (Hb) is a protein present in red blood cells; this protein is responsible for carrying oxygen from the lungs to the tissues and for returning carbon dioxide from the tissues to the lungs. A single amino acid substitution in hemoglobin may distort normal red blood cells into sickle shaped cells. These cells can clog the blood vessels resulting in sickle cell anemia, a genetically inherited disorder.

In this exercise, you will use StarBiochem, a protein 3D viewer, to explore the structure of functional Hb and the abnormal Hb that accounts for sickle cell anemia. To begin using StarBiochem, please navigate to: <http://web.mit.edu/star/biochem>. Click on the Start to launch the application. Click on "Samples-> Select from Samples-> 1A3N". This file represents the PDB structure of normal hemoglobin. Instructions for changing the view of structure can be found in the top menu, under View -> Structure viewing instruction.

a) How would you best classify 1A3N: monomer, dimer, tetramer or two homodimers?

*It is a tetramer of two homodimers i.e. Chains A and C are identical and so are chains B and D.*

b) Based on the primary structure of 1A3N, what is the length, in terms of amino acids, of each polypeptide chain of normal Hb?

*Chains A and C have 141 amino acid residues each. In comparison, chains B and D have 146 amino acid residues each. Thus normal Hb is comprised of  $2(141) + 2(146) = 574$  amino acid residues.*

c) In addition to amino acids, each protein chain in Hb also contains heme groups, which bind to oxygen. How many heme groups do you see in each molecule of Hb?

*There are four heme groups in each molecule of hemoglobin.*

d) What are the different secondary structures you see in 1A3N?

*This structure has helices and coils but it has NO sheets.*



### Question 6 continued

We will now compare the structure of 1A3N with 2HBS, which represents the PDB structure of sickle cell Hb.

Note: In the top menu, click on "Samples-> Select from Samples-> 2HbS". You can now view the PDB structure of sickle cell Hb. A single amino acid substitution at position 6 in two of hemoglobin protein chains to the amino acid valine results in sickle cell anemia.

e) Identify the protein chains (A, B, C, etc) in 2HBS with the above amino acid substitution.

*Val<sup>6</sup> is located in protein chains B, D, F and H in 2HbS.*

f) Name the amino acid present in 1A3N that is substituted to Val<sup>6</sup> in 2HBS. How does the nature of the side-chain of this amino acid differ from Val?

*Normal hemoglobin has glu<sup>6</sup>, which is substituted by val in 2HbS. The amino acid glu has a polar, hydrophilic side-chain in comparison to val<sup>6</sup> which has a non-polar and hydrophobic side-chain.*

g) In the 2HBS, Val<sup>6</sup> in one Hb molecule interacts with Phe<sup>85</sup> and Leu<sup>88</sup> in another molecule of Hb. What is the most likely type of interaction between the side-chain of Val<sup>6</sup> with the side-chains of Phe<sup>85</sup> and Leu<sup>88</sup>? Why is this interaction not observed in 1A3N?

*The val<sup>6</sup> in the H chain of 2HbS interacts with phe<sup>85</sup> and leu<sup>88</sup> of the B chain in 2HbS molecule. The val<sup>6</sup> in the H chain of 2HbS undergoes hydrophobic interaction with phe<sup>85</sup> and leu<sup>88</sup> of the B chain in 2HbS molecule. This is because the side-chains of all the involved amino acids are non-polar and hydrophobic.*

h) Based on what you have learned, briefly explain why the Hb molecules aggregate together in sickle cell anemia patients.

*The substitution of glu<sup>6</sup> by val<sup>6</sup> in 2HbS generates sticky hydrophobic pockets. The val<sup>6</sup> can then undergo hydrophobic interaction with phe<sup>85</sup> and leu<sup>88</sup> of another hemoglobin molecule thus allowing them to cluster together. This clustering makes the RBC more sticky and fragile, allows them to acquire a sickle cell shape and disrupts their ability to transport oxygen in blood.*

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7.016 Introductory Biology  
Fall 2014

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