

# **Chapter 2:**

# **Biochemistry Problems**

# Biochemistry Problems

*If you were a biochemist, you would study chemical substances and vital processes that occur in living organisms.* You might study macromolecules such as lipids and phospholipids, carbohydrates, proteins, or nucleic acids. You might study pathways such as glycolysis or photosynthesis, or any other metabolic pathway. In this chapter, we begin with problems that review the bonds and forces that hold these macromolecules together. We briefly touch on macromolecules that are not proteins, but the majority of this chapter asks you to explore the structure and function of proteins.

## (1) BONDS AND FORCES

### (1.1) Covalent bonds

For the purposes of this book, we have simplified the covalent bonding properties of the atoms most commonly found in living organisms. For this book, we will use the bonding properties given in the following chart:

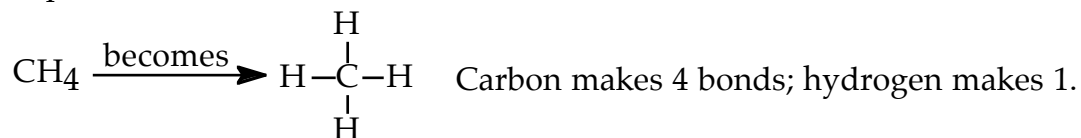
Number of covalent bonds						
Element	0	1	2	3	4	5
H	+	neutral				
O		–	neutral			
N				neutral	+	
C					neutral	
S			neutral			
P						neutral

The shaded boxes indicate configurations that do not appear in this book (for example, a sulfur atom making three covalent bonds). These approximations are sufficient for the problems in this book and most introductory biology courses. As you take further courses in biology and chemistry, you will learn about additional possibilities.

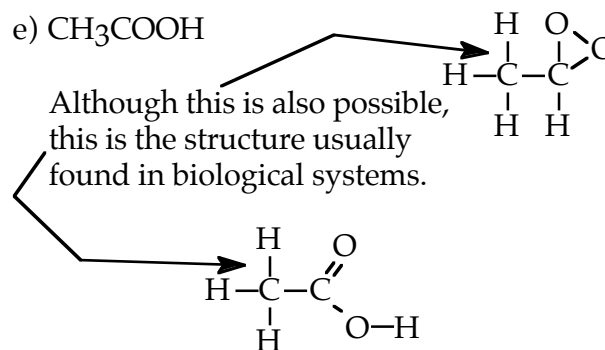
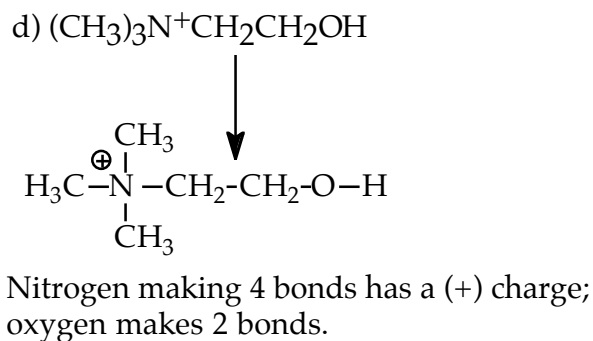
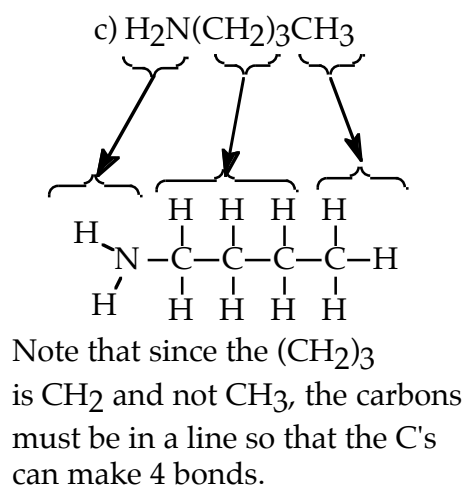
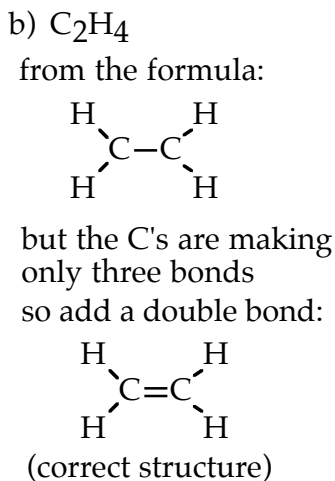
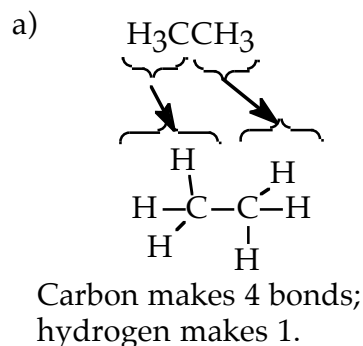
### Diagnostic Question:

Convert the following shorthand formulas to correct structural formulas.

For example:

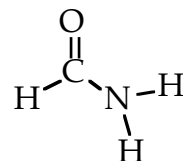
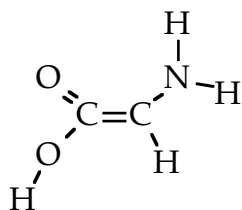
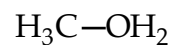
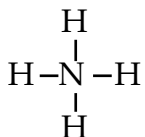
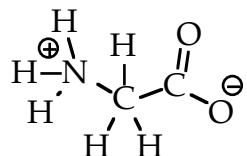


## Answer to Diagnostic Question:



## Problems:

(1.1.1) Check the following structures and correct any mistakes you find. There may be more than one way to correct the structure.



Covalent Bonds

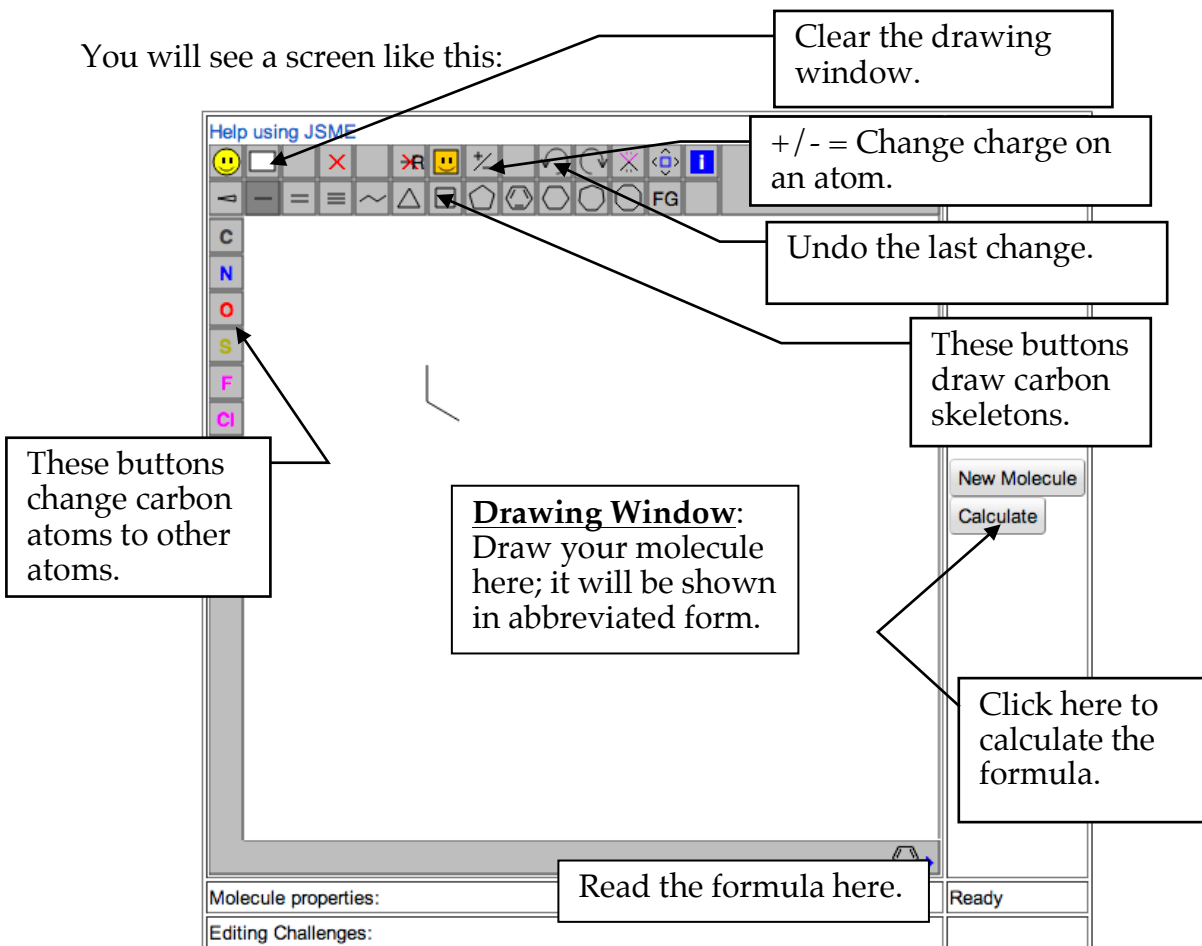
(1.1.2) For each of the functional groups given, draw a structural formula.

- Amino
- Hydroxyl
- Carboxyl
- Methyl
- Phosphoryl
- Aldehyde

### (C1) Computer-Aided Problems 1

These problems use the Molecular Calculator that allows you to practice drawing structural formulas and working with simplified structures. The program, "Molecular Calculator," can be accessed from this link:

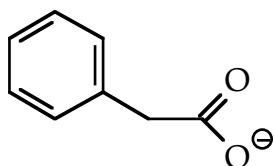
<http://intro.bio.umb.edu/MOOC/jsMolCalc/JsMolCalc.html>




This program will let you draw molecules that follow the basic rules for covalent bonding shown in the table below.

Element	Number of covalent bonds					
	0	1	2	3	4	5
H	+	neutral				
O		-	neutral			
N				neutral	+	
C					neutral	
S			neutral			
P						neutral

This first practice exercise is designed to familiarize you with the Molecular Calculator. You will build a molecule and calculate its formula. The molecule is shown below:



The following steps show you how to draw this molecule and give you practice with the software.

1) Draw propane ( $\text{H}_3\text{CCH}_2\text{CH}_3$  or ). To do this, you:

a) Click on the “hydrocarbon chain” button as shown below:



Hydrocarbon chain button.

b) Put the cursor in the middle of the screen and drag quickly to the right. You will see a zigzag line forming near the cursor, and a number will appear in the lower left part of the Drawing Window. The line is the chain of carbons, and the number tells you how many carbons long it is. Stop when you get to 2. When you release the mouse, you should see something like this:

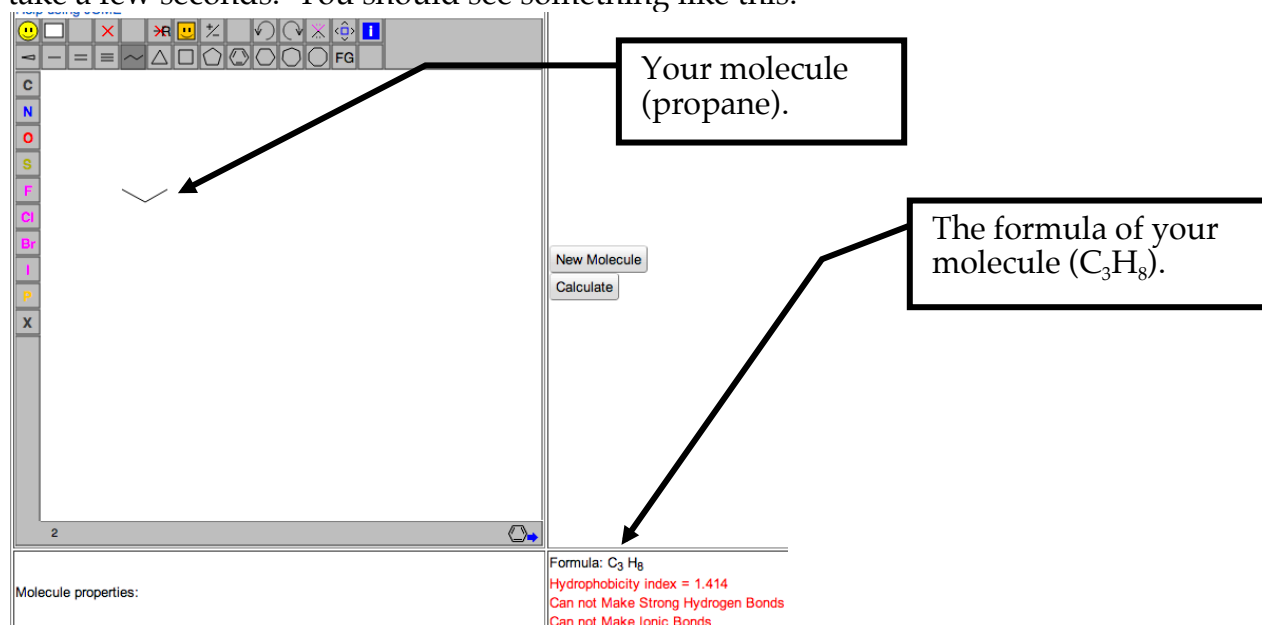


If you make a mistake, you can either:

- Clear it all by clicking the Undo button at the top of the Drawing Window.
- Click the red “X” (delete) button at the top of the Drawing Window. This will delete whatever you click on.

If you want to move your molecule, click near the molecule but not on an atom, and drag the molecule to a more convenient place.

2) Calculate the formula of propane. Click the “Calculate” button. The calculation may take a few seconds. You should see something like this:



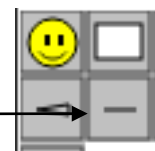
Propane's formula should be C<sub>3</sub>H<sub>8</sub>.

3) Change propane to phenyl propane by adding a benzene ring.

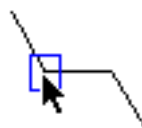
a) First, add a single bond from the left-most carbon using the “bond” tool.

b) Click on the “bond” tool until it turns dark gray.

c) Move the cursor over the left-most carbon until you see a blue square appear.



d) Click once to add a carbon and you should see:

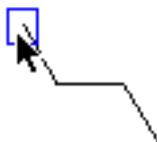


e) Now add a benzene ring with the benzene ring tool. First, click on the benzene ring tool:

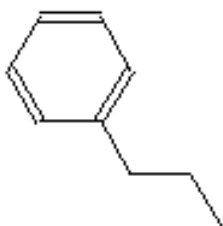


Covalent Bonds

f) Move the cursor until a blue square appears at the left-most carbon in the chain you made.



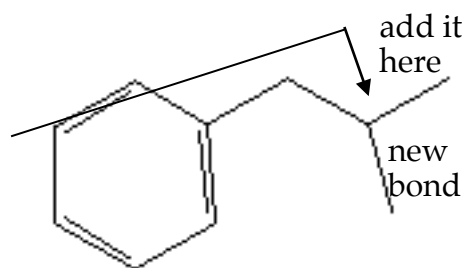
g) Click the mouse and you should see:



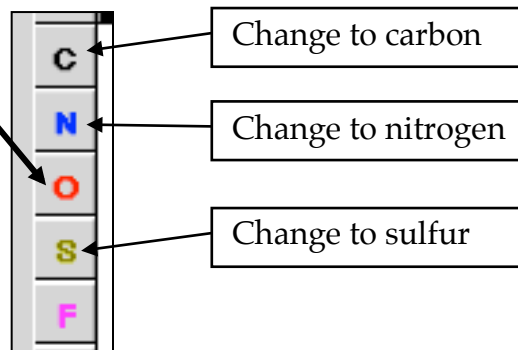
4) Calculate the formula of phenyl propane as you did for propane (step 2). The formula should be  $C_9H_{12}$ .

5) Change the molecule one last time.

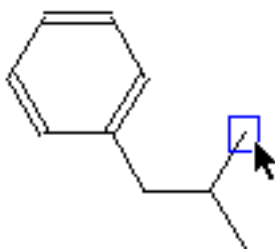
a) Use the “bond” tool as you did in step 3 (a) through (d) to add a carbon to the second carbon from the right-hand end of the chain. Your molecule should look like this:



b) Select the “Change to Oxygen atom” tool.

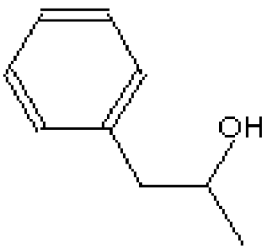


c) Move the cursor to the end of one of the branches at the end of the chain until you see a light-blue square appear.

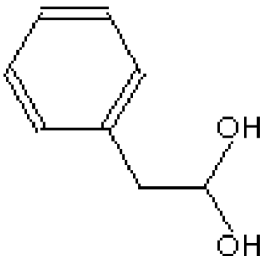




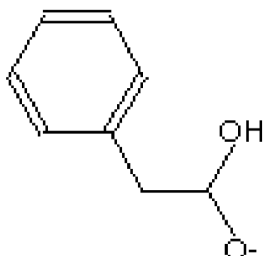
d) Click on the atom to change the carbon to oxygen. You should see:



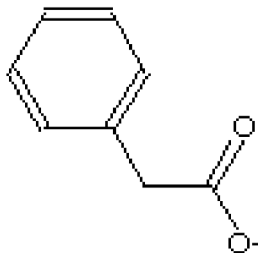
e) Do the same at the other branch end and you should see:



f) Change one of the OH's to O<sup>-</sup>. Click on the "+/-" tool and click on one of the OH's. You should see:



g) To make the structure complete, you must make a double bond between the O (not the O<sup>-</sup>) and the carbon. Do this by selecting the "bond" tool and moving it over the bond between the O and the carbon until you see a blue rectangle appear. Click once to make it a double bond. You should see:



6) Calculate the formula of your new molecule as you did for propane (step 2). The formula should be C<sub>8</sub>H<sub>7</sub>O<sub>2</sub> (-).

7) Draw several molecules on the screen and calculate their formulas by hand. Check your work by clicking the "Calculate" button.

Note that it is possible to draw molecules that the software cannot process properly. Some of these molecules are chemically possible, but their chemistry is beyond the scope of this book. If you attempt to calculate the logP and formula of a molecule containing any of the following atoms, the program will tell you that "It is not possible to calculate logp...". These "illegal" atoms are:

- A carbon atom with any charge.
- A (+)-charged S or O atom.
- An N-atom with a (-) charge or with a charge greater than (+1).
- An S-atom making 3 or 5 bonds.
- A charged P-atom or a P-atom making more or less than 5 bonds.
- A charged F, Cl, Br, or I atom.
- An "X" atom.

**(1.1.3)** For each of the following formulas, draw a molecule with the same formula.

a)  $\text{C}_3\text{H}_8\text{O}$

b)  $\text{C}_3\text{H}_6$

c)  $\text{C}_3\text{H}_5\text{NO}$

d)  $\text{C}_2\text{H}_4\text{NOS}(-)$  (This molecule has a single negative charge on one atom.)

e)  $\text{C}_5\text{H}_8\text{N}(+)$  (This molecule has a single positive charge on one atom.)

## (C2) Computer-Aided Problems 2

For the next problems, you will use computer software that allows you to manipulate a two-dimensional (2-d) representation of a three-dimensional (3-d) molecule. This software is called molecular visualization (MolVis) software. The MolVis software you will use is called “**Molecules in 3-d**” and can be found at this site <http://intro.bio.umb.edu/MOOC/jsMol/>.

### Objectives:

To familiarize you with:

- The structures of some important biomolecules that you will see again and again.
- Translations between the 2-d representations you see in this and other books and the 3-d reality of biomolecules.
- The kind of representation used by the MolVis software that you will use in this book.
- The user interface of the MolVis software that you will use in this book.

Note that Molecules in 3-d can sometimes take a little while to load. Click the link marked “Biochemistry C2” to see the page for this problem.

You will see something like the following:

The screenshot shows the JSmol web interface. On the left, a sidebar titled "Choose A Problem Here" contains a list of links: Biochemistry C2, Biochemistry C4, Biochemistry C5, Biochemistry C6, Biochemistry C7, Molecular Bio C1, and Integration 2. On the right, a sidebar titled "Biochemistry Problem C2" contains five buttons: "Load the linear form of Glucose", "Load the linear form of Fructose", "Load the circular form of Glucose", "Load the first amino acid", "Load the second amino acid", and "Load the third amino acid". The central area is a large black rectangle representing the molecular structure. Three white callout boxes with black text provide instructions: the first points to the problem links, the second points to the loading buttons, and the third points to a color key at the bottom. The color key shows colored squares for Carbon (grey), Hydrogen (white), Oxygen (red), Nitrogen (blue), and Sulfur (yellow). The JSmol logo is in the bottom right corner of the central area.

Choose A Problem Here

Biochemistry C2  
Biochemistry C4  
Biochemistry C5  
Biochemistry C6  
Biochemistry C7  
Molecular Bio C1  
Integration 2

Biochemistry Problem C2

Load the linear form of Glucose  
Load the linear form of Fructose  
Load the circular form of Glucose  
Load the first amino acid  
Load the second amino acid  
Load the third amino acid

These links allow you to select the card for a particular problem.

Once you have selected a particular problem, these buttons change the molecules or view as appropriate.

This color key indicates how the atoms are color coded.

Carbon Hydrogen Oxygen Nitrogen Sulfur JSmol

We will use this software throughout this part of the book, so we will take some time now to describe its use in detail. This software allows you to get information from the image in several ways:

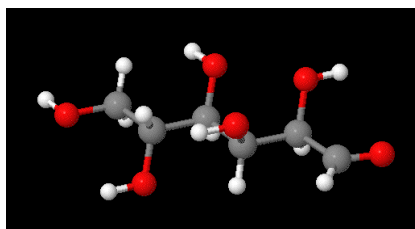
- Rotating the molecule: This is the best way to get an idea of the molecule's 3-d structure. You can click and drag on any part of the molecule and it will rotate as though you had grabbed it.
- Zooming in or out: This helps to get close-up or "big-picture" views of the molecule. Hold the shift key down while dragging the cursor up (to zoom out) or down (to zoom in) the image.
- Identifying the atom you are looking at: You can find information on the atoms in the molecule in one of two ways:
  - By putting the cursor over the atom you are interested in and waiting a few seconds for the information to pop up. The program will then display information on the atom in a little pop-up window. The information in the pop-up is more detailed than the first one above but rather cryptic. If you put the cursor over the left-most carbon atom (gray), the pop-up reads "1.C. #7." The most important part of this is the "C"; this says that you clicked on a carbon atom. Try putting the cursor over some other atoms to see what you get. Note that this does not always work, especially on Macintosh computers.

In addition to the above, atoms are also identified by their color. The color scheme is shown to the right of the molecule images.

Atoms are indicated by spheres; covalent bonds are shown by rods; noncovalent bonds are not shown at all.

**Important note:** This software does not distinguish between single, double, and triple covalent bonds. All covalent bonds are shown as single rods. You have to decide whether a bond is single, double, or triple based on your knowledge of covalent bonding and the structures of known biological molecules.

Click the tab for this problem “Biochemistry C2.” Click the button marked “Load the linear form of glucose” and you should see this in the large black window (the molecule window):



This is a 2-d representation of glucose. Since glucose is really 3-dimensional, you can't see all the details of its structure from a single 2-d image.

Each of the following questions applies to the structures shown by the program.

a) Click the button marked “Load the linear form of glucose.” Note that the top line of text below the structure now shows “Load the linear form of glucose”; this is to remind you which structure you are looking at. The structure shown is the sugar glucose in its linear form. Based on the image shown, draw the structure of the linear form of glucose. You should use letters to represent atoms and lines to represent covalent bonds. Be sure to include all hydrogens. Compare this structure with the structure of glucose given in your textbook.

b) Click the button marked “Load the linear form of fructose.” This shows the sugar fructose in its linear form. Based on the image shown, draw the structure of the linear form of fructose. Compare this structure with the structure of fructose given in your textbook. How does fructose differ from glucose?

c) Click the button marked “Load the circular form of glucose.” This shows the structure of glucose in its circular form. Based on the image shown, draw the structure of the circular form of glucose. Which parts of the linear glucose molecule were connected to give the circular form? Hint: it involves attaching one atom to another and moving one hydrogen atom; no carbon-carbon bonds are made or broken.

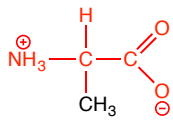
A chart of the amino acid structures can be found on the next page.

d) Click the button marked "Load the first amino acid." This shows an amino acid. Draw its structure and determine which amino acid it is.

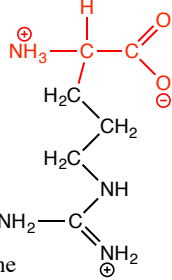
e) Click the button marked "Load the second amino acid." This shows an amino acid. Draw its structure and determine which amino acid it is.

f) Click the button marked "Load the third amino acid." This shows an amino acid. Draw its structure and determine which amino acid it is.

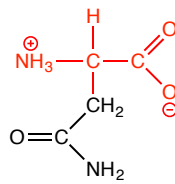
## Structures of Amino Acids



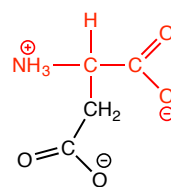
Alanine  
(Ala A)



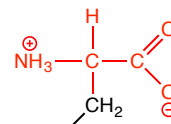
Arginine  
(Arg R)



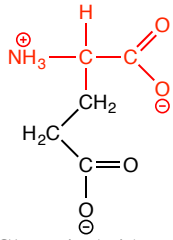
Asparagine  
(Asn N)



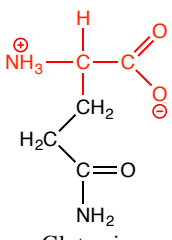
Aspartic Acid  
(Asp D)



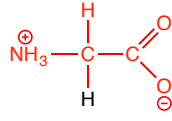
Cysteine  
(Cys C)



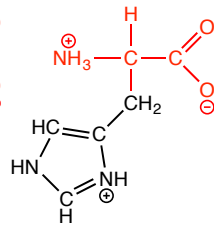
Glutamic Acid  
(Glu E)



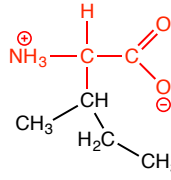
Glutamine  
(Gln Q)



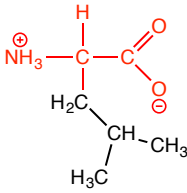
Glycine (Gly)



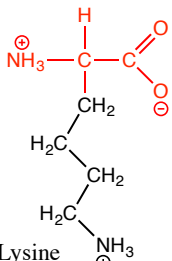
Histidine  
(His H)



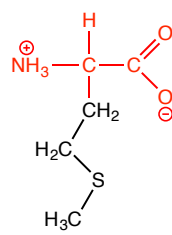
Isoleucine  
(Ile I)



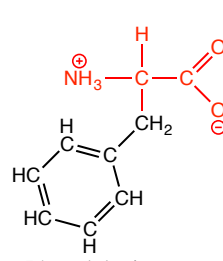
Leucine  
(Leu L)



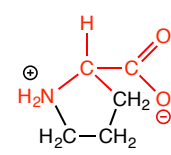
Lysine  
(Lys K)



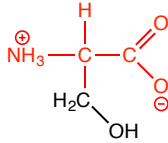
Methionine  
(Met M)



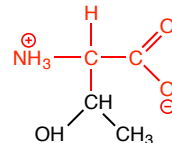
Phenylalanine  
(Phe F)



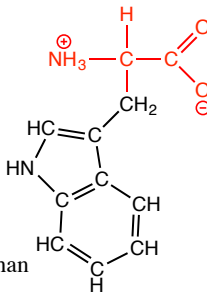
Proline  
(Pro P)



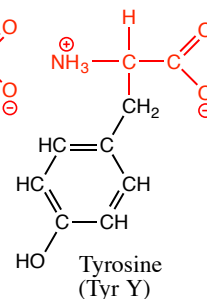
Serine  
(Ser S)



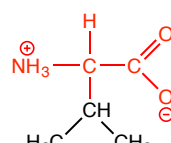
Threonine  
(Thr T)



Tryptophan  
(Trp W)



Tyrosine  
(Tyr Y)



Valine  
(Val V)

## Covalent Bonds

## (1.2) Noncovalent bonds and forces

In these problems, you will be given the covalent bonds (these are shown as solid lines) and must *infer* their noncovalent bonding properties. Noncovalent bonds/interactions are shown by dotted lines (etc.). These two types of “bonds” are entirely separate; for example, an oxygen (which can make only two covalent bonds) can make several hydrogen bonds *in addition to* the covalent bonds. That is, noncovalent “bonds” do not count toward an atom’s covalent bond total.

As a reminder, here are the types of noncovalent interactions we will use in this book. They are listed from strongest to weakest:

- Ionic/electrostatic bonds (also known as “salt bridges”): These are the strongest noncovalent bonds. They occur between *fully charged* (that is, + or –; not partially charged) atoms or groups.
- Hydrogen bonds: These require a “hydrogen donor”: a hydrogen atom covalently bonded to an oxygen or nitrogen (–OH or –NH) and a “hydrogen acceptor”: a lone pair of electrons on an oxygen or nitrogen atom (O: or N:).
- Hydrophobic interactions: These occur when several or many hydrophobic atoms or groups clump together to avoid contact with water. Hydrophilic groups cannot form hydrophobic interactions. Unlike an ionic or a hydrogen bond that occurs between two molecules, hydrophobic interactions are not true bonds, but involve nonpolar molecules that cluster together to avoid the water that surrounds them. The effect of clustering nonpolar molecules or chemical groups to shield them from water is a significant force.
- van der Waals bonds: These occur between any two nonbonded atoms and are the weakest interactions possible. Although they are always present, they are not significant unless large surface areas are positioned very closely together. In this case, the combined van der Waals forces can play a significant role.

There are several ways you will be asked to apply this information depending on the nature of the question.

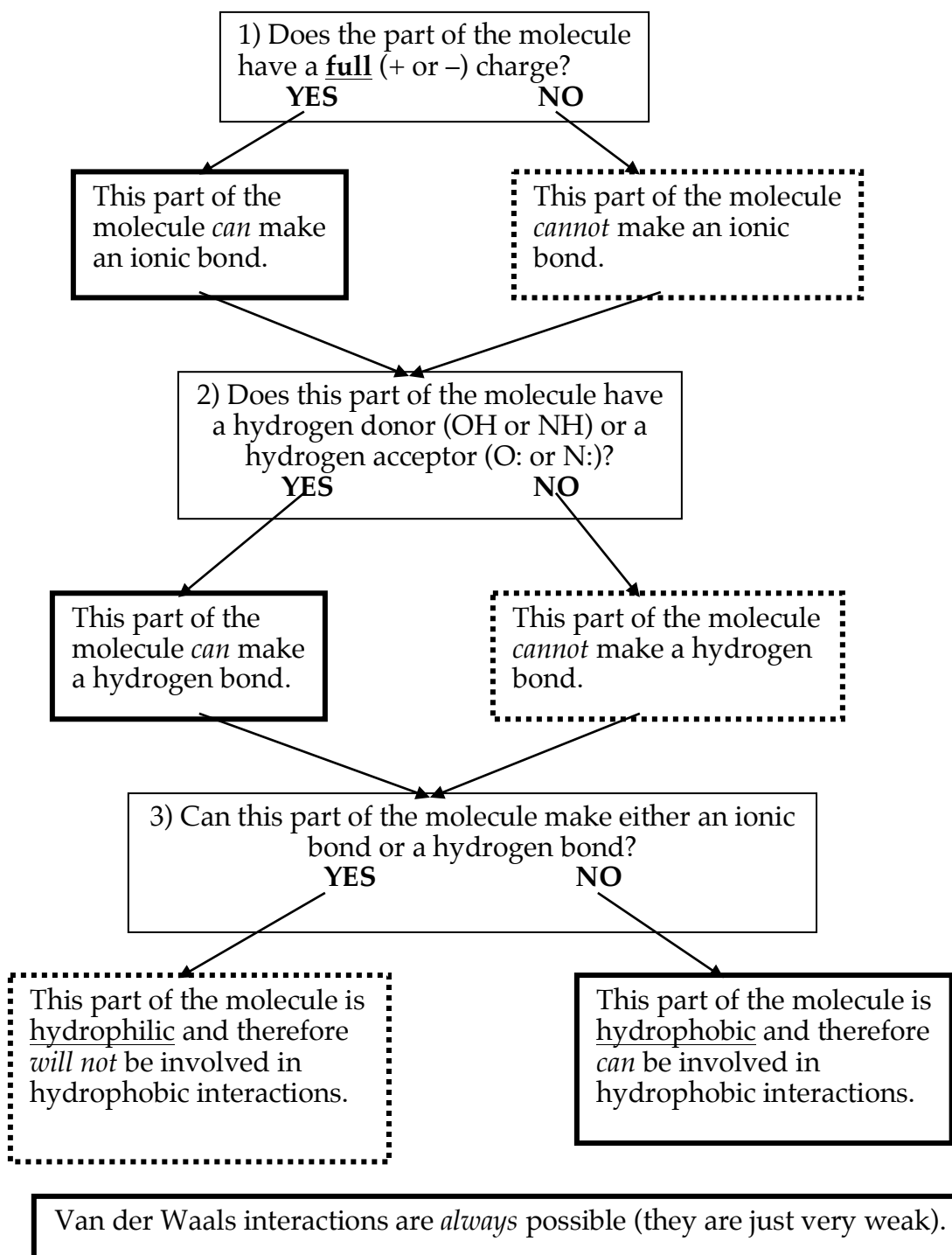
First, the question can be asked in one of two ways:

- 1) What types of interactions are possible? In this case, there can be more than one answer. You should specify all the types that could occur.
- 2) What is the *strongest* interaction between two molecules? In this case, we assume that the strongest noncovalent interaction is an ionic bond, followed by a hydrogen bond, and finally a van der Waals interaction. If there are several nonpolar interacting species, then hydrophobic interactions should be considered.

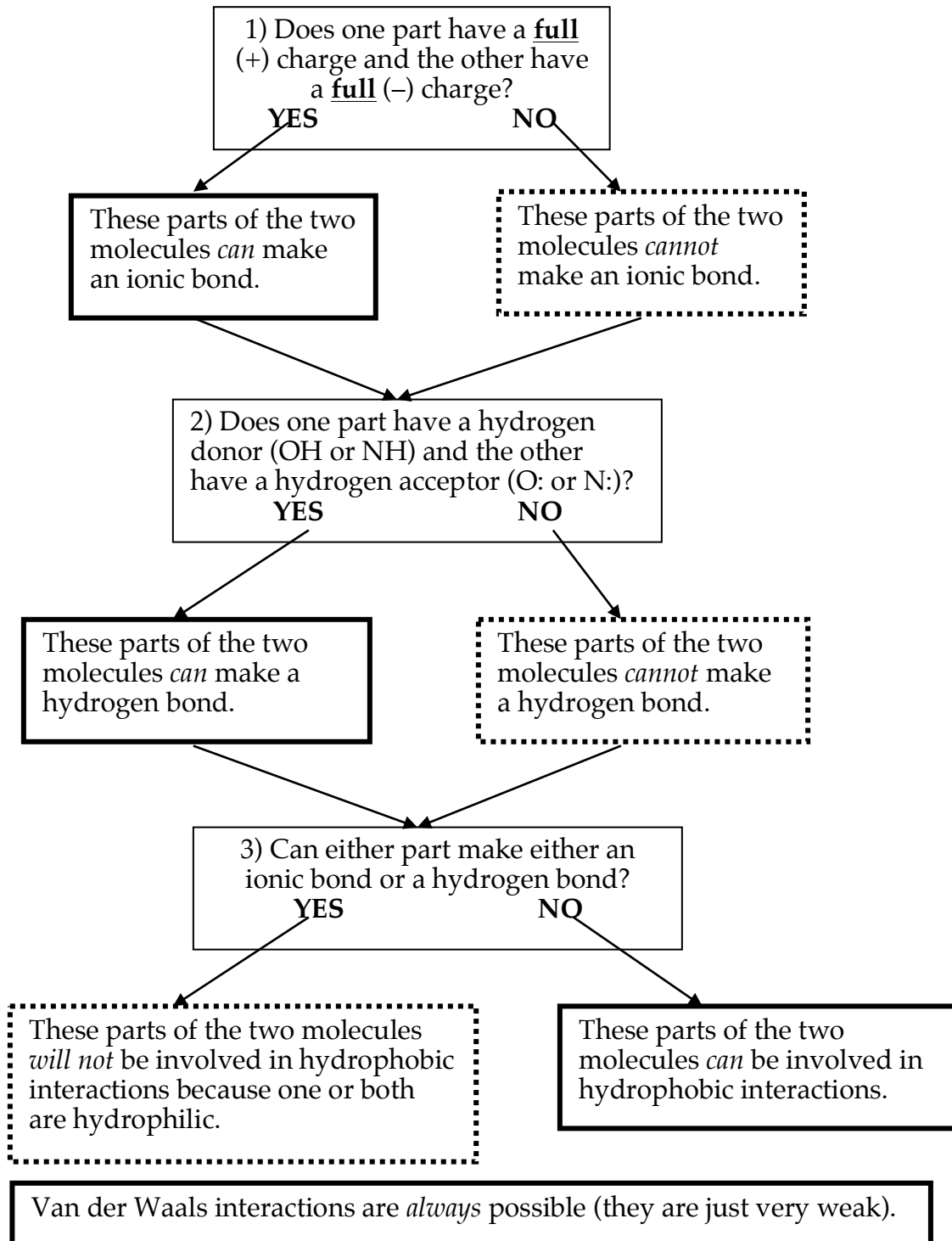


Second, the question can be asked in one of two contexts:

1) What kind(s) of interaction(s) can this part of a molecule make? Since it takes two items to make a bond, the bond couldn't form without a "suitable partner." Either explicitly or implicitly, this question assumes the existence of a suitable partner. For these, the following flowchart applies:



2) What kind(s) of interactions are possible between these two (parts of) molecules? In this case, you have to determine whether the other molecule is a suitable partner. This is a slightly more restrictive question than (1). The flowchart below applies in this case. Note that the questions now ask about the other molecule(s).



You will also be asked to compare the relative hydrophobicity/hydrophilicity of different molecules. For these problems, the following rules are useful:

1. The more hydrophilic atoms or groups of atoms that a molecule has, the more hydrophilic the molecule is. Hydrophilic groups are:
  - Charged (+) or (-)
  - Hydrogen bond donors (NH or OH)
  - Hydrogen bond acceptors (N: or O:)
2. Charged groups are more hydrophilic than hydrogen bond donors or acceptors.
3. The more hydrophobic atoms or groups that a molecule has, the more hydrophobic the molecule is. Hydrophobic groups are any not listed above (for example, C-H, S-H, C-C, C-S, C=C, etc.).
4. Per atom or group of atoms, hydrophilic groups contribute more than hydrophobic groups to the overall hydrophobicity of a molecule. That is, one hydrophilic group will make a molecule more hydrophilic than one hydrophobic group will make it hydrophobic. Put another way, imagine putting parts of a molecule on a scale with hydrophobic parts on one side and hydrophilic parts on the other. Each of the hydrophilic groups will “weigh” more than each of the hydrophobic groups. Thus, it takes more hydrophobic atoms to “balance out” a single hydrophilic atom.

### Diagnostic Question:

Complete the table below. When evaluating the bond or interaction, assume that a suitable partner is nearby.

Part of molecule	Is the bond polar or nonpolar?	Hydrophobic or hydrophilic?	Ionic bond?	Hydrogen bond?	Hydrophobic interactions?
C-C					
C-H					
C-N					
C-O					
S-H					
O-H					
N-H					

# Answer to Diagnostic Question:


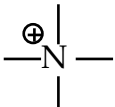
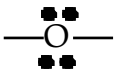
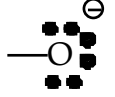
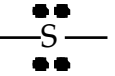
Part of molecule	Is the bond polar or nonpolar?	Hydrophobic or hydrophilic?	Ionic bond?	Hydrogen bond?	Hydrophobic interactions?
C–C	<i>nonpolar</i>	<i>hydrophobic</i>	<i>no</i>	<i>no</i>	<i>yes</i>
C–H	<i>nonpolar</i>	<i>hydrophobic</i>	<i>no</i>	<i>no</i>	<i>yes</i>
C–N	<i>polar</i>	<i>hydrophilic</i>	<i>a</i>	<i>≠</i>	<i>no</i>
C–O	<i>polar</i>	<i>hydrophilic</i>	<i>a</i>	<i>≠</i>	<i>no</i>
S–H	<i>nonpolar</i>	<i>hydrophobic</i>	<i>no</i>	<i>no</i>	<i>yes</i>
O–H	<i>polar</i>	<i>hydrophilic</i>	<i>a</i>	<i>yes</i>	<i>no</i>
N–H	<i>polar</i>	<i>hydrophilic</i>	<i>a</i>	<i>yes</i>	<i>no</i>

<sup>a</sup> If the O or N is charged, “yes”; if not, “no.”

≠ Yes, if the N or O has a lone pair available.

## Problems:

(1.2.1) Complete the table below. When evaluating the bond or interaction, assume that a suitable partner is nearby.

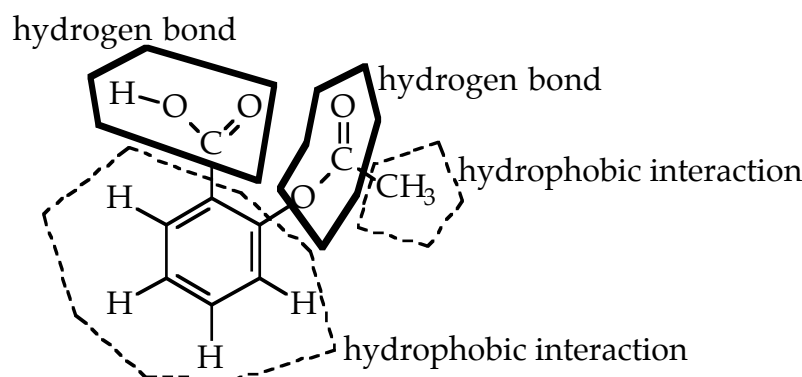
Part of molecule	Is the bond polar or nonpolar?	Hydrophobic or hydrophilic?	Ionic bond?	Hydrogen bond?	Hydrophobic interactions?
C–S					
P–O					
S–O					
					
					
					
					
					

**(1.2.2)** A gecko can stick to just about any surface and walk with its feet over its head. The sole of a gecko's foot is covered with perhaps a billion tiny hairs that put the gecko in direct physical contact with its environment. In experiments, the toes of geckos adhered equally well to neutral, strongly hydrophobic, and strongly hydrophilic surfaces. As the number of tiny hairs decreases, the adhesive properties decrease. What noncovalent force or bond might explain the gecko's acrobatics?

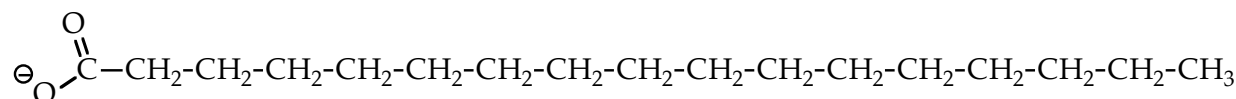
**(1.2.3)** For each molecule, draw a solid line around each hydrophilic group of atoms; draw a dotted line around each hydrophobic group of atoms. For each group you circle, give the type(s) of bonds that this group could make (ionic bond, hydrogen bond, hydrophobic interaction).

For example:

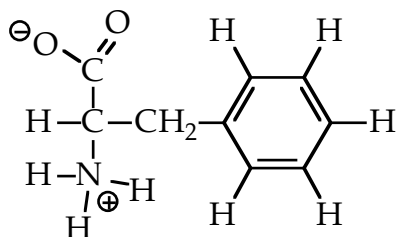
aspirin



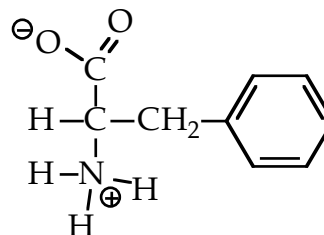
a) Soap



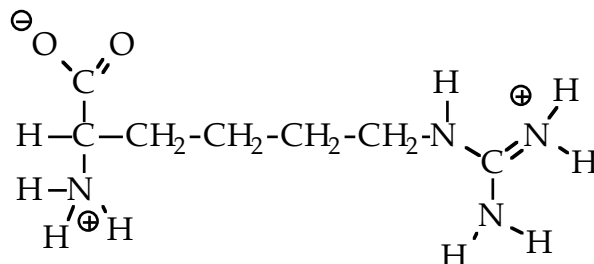
b) Phenylalanine (an amino acid)



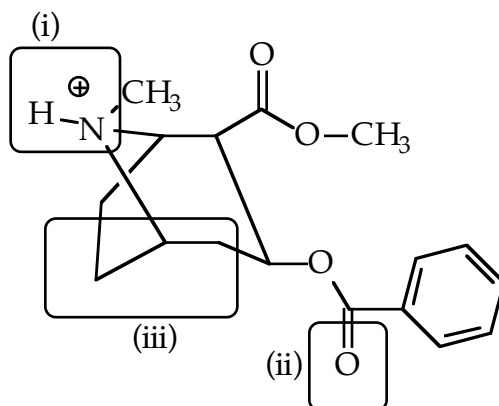
The hydrogens are often off of the ring for simplicity.



(1.2.4) Draw the hydrogen bonds that could form between water molecules and the appropriate regions of arginine. Indicate the hydrogen bonds with dashed lines.



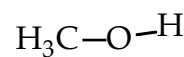
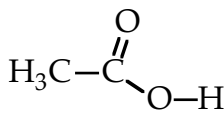
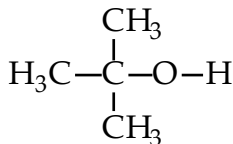
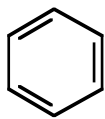
(1.2.5) Shown below is the structure of cocaine. For each of the circled regions, indicate which bonds that part of cocaine could form with another molecule, given a suitable partner. Assume that the circled parts remain attached to the rest of the molecule. Fill in the table with “yes” if that type of bond is possible, “no” if it is not.



Part	Could this part form <u>ionic bonds</u> with another molecule?	Could this part form <u>hydrogen bonds</u> with another molecule?	Could this part form a <u>hydrophobic interaction</u> with another molecule?
(i)			
(ii)			
(iii)			

(1.2.6)

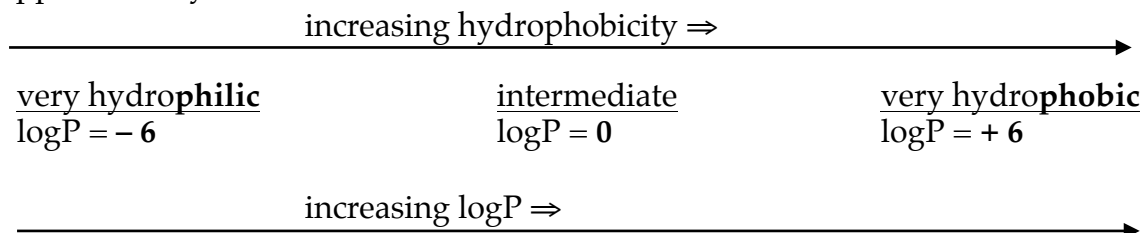
Rank these in order from most hydrophobic to most hydrophilic and explain.



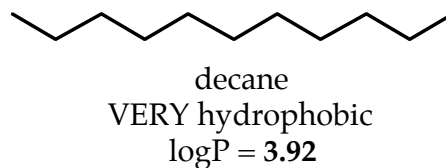
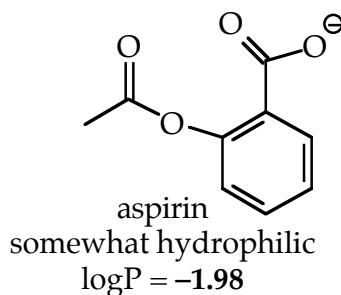
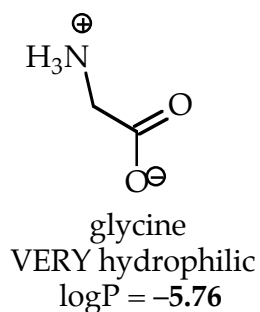
(C3) Computer-Aided Problems 3

The **Molecular Calculator** is a computer program that calculates the relative hydrophobicity of a molecule. The program calculates the hydrophobicity of a molecule in terms of its logP (short for “log  $P_{O/W}$ ”). You will draw molecules and the program will calculate the approximate hydrophobicity of the molecule.

The value of logP tells you how hydrophobic a molecule is. For more detail, see the end of this problem. The higher the logP value, the more hydrophobic the molecule is. And, approximately:



For example:

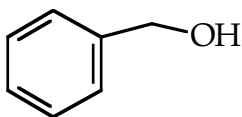


You will use the Molecular Calculator to check your own estimations of relative hydrophobicity as a way to practice with this material.

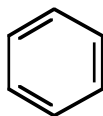
You will use the Molecular Calculator as you did in problem (C1) to work through the following problems. To calculate the logP value of a molecule, click “Calculate Formula and logP.” Look at the “logP” value shown at the bottom of the window.

1) Consider the following three molecules:

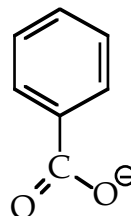
Molecule #1



Molecule #2



Molecule #3



a) Rank them in order from most hydrophobic to least hydrophobic using what you know about chemical properties. Explain your choices.

Most hydrophobic

Intermediate

Most hydrophilic

b) Use the Molecular Calculator to check your answer.

Molecule	logP
1	
2	
3	

c) Make a molecule more hydrophobic than the most hydrophobic molecule from part (1a). Check your work with the Molecular Calculator.

logP: \_\_\_\_\_

d) Make a molecule more hydrophilic than the most hydrophilic molecule from part (1a). Check your work with the Molecular Calculator.

logP: \_\_\_\_\_

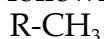
e) Make a molecule that is in between two of the molecules from part (1a) in terms of hydrophobicity. Check your work with the Molecular Calculator.

logP: \_\_\_\_\_



2) Different groups of atoms contribute differently to the logP of a molecule. This question compares the contributions of four different groups of atoms. In organic chemistry "R" is shorthand used to represent "the rest of the molecule." To answer this question, you can use the "R group" of your choice; just be sure that you use the same "R group" for all four molecules.

Consider the following four molecules:



For any given R group, two have high logP values and two have low logP values.

a) Choose an R group of your own design, draw the four variations of this molecule ( $\text{R-CH}_3$ ,  $\text{R-OH}$ ,  $\text{R-SH}$ , and  $\text{R-NH}_2$ ), and give their logP values. Note that you can calculate the formula to be sure that you have done this correctly. Suppose that you started with a particular R group. If you add a  $-\text{CH}_3$ , one of the H's will be replaced by a  $\text{CH}_3$ ; so the new formula should be "R" minus one H (for the one that was replaced) plus one C and three H's. Overall, this would be " $\text{R} + \text{C} + \text{H}_2$ ." Likewise for  $\text{R-OH}$ , the new formula should be " $\text{R} + \text{O}$ "; for  $\text{R-SH}$ , " $\text{R} + \text{S}$ "; and for  $\text{R-NH}_2$ , " $\text{R} + \text{N} + \text{H}$ ."

b) In terms of the polarity of the bonds involved, explain why the two molecules with high logP are more hydrophobic and why the two with low logP are more hydrophilic.

3) Ethanol ( $\text{H}_3\text{CCH}_2\text{OH}$ ) and di-methyl-ether ( $\text{H}_3\text{COCH}_3$ ) have the same number of carbons, hydrogens, and oxygens ( $\text{C}_2\text{H}_6\text{O}$ ) but differ in the following important way. In ethanol, the O is bonded to a carbon and a hydrogen, but in di-methyl-ether, the O is bonded to two carbons.

Create a similar pair of molecules; you can check these features by having the program calculate the formula for you.

- Both members of this pair should have the same number of carbons, hydrogens, and oxygens.
- Both members should have only one oxygen.
- One member should have the oxygen bonded to a carbon and a hydrogen; the other should have the oxygen bonded to two different carbon atoms.

a) Draw the two molecules.

b) In terms of their capability of forming bonds with water, predict which will be more hydrophobic and explain your reasoning.

c) Give the logP values for your two molecules. Do they agree with your prediction?

4) Adding an -OH (hydroxyl) group makes a molecule more hydrophilic; adding a -CH<sub>3</sub> (methyl) makes a molecule more hydrophobic. Approximately how many -CH<sub>3</sub>'s are required to counterbalance the effect of an -OH? Note that this will depend on many factors and will not be the same for all molecules.

a) Start with a molecule of your choosing. Draw it below and calculate its logP:\_\_\_\_\_

b) Add an -OH to the molecule from part (4a). Draw it below and calculate its logP:\_\_\_\_\_

c) Keep adding -CH<sub>3</sub>'s to the molecule from part (4b) until it has approximately the same logP as the original molecule (4a). Draw the molecule below, fill in the number of -CH<sub>3</sub>'s you had to add, and give the logP.

# of -CH<sub>3</sub>'s required:\_\_\_\_\_

logP:\_\_\_\_\_

5) Adding a charged group  $\text{-O}^-$  or  $\text{-NH}_3^+$  group makes a molecule much more hydrophilic; adding a  $\text{-CH}_3$  (methyl) makes a molecule more hydrophobic. Approximately how many  $\text{-CH}_3$ 's are required to counterbalance the effect of a charged group? Note that this will depend on many factors and will not be the same for all molecules.

a) Start with a molecule of your choosing. Draw it below and calculate its logP:\_\_\_\_\_

b) Add a charged group to the molecule from part (5a). Draw it below and calculate its logP:\_\_\_\_\_

c) Keep adding  $\text{-CH}_3$ 's to the molecule from part (5b) until it has approximately the same logP as the original molecule (5a). Draw the molecule below, fill in the number of  $\text{-CH}_3$ 's you had to add, and give the logP.

# of  $\text{-CH}_3$ 's required:\_\_\_\_\_

logP:\_\_\_\_\_

## **Appendix:** What does logP mean?

Many researchers, especially drug designers, need to be able to estimate how hydrophobic a drug is. If it is too hydrophobic, it will not dissolve well enough in blood (which is mostly water) to get to the target. If it is too hydrophilic, it may have trouble passing through the hydrophobic core of the cell membranes. They could just make the drug and see, but synthesis is very expensive and they'd like to be able to at least estimate its hydrophobicity beforehand.

If they were able to make the drug, they would measure its hydrophobicity by adding it to a flask containing water and octanol ( $\text{H}_3\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$  – a very hydrophobic molecule). Since water and octanol don't mix appreciably, you get two layers. If the drug is very hydrophilic, you will find all of it in the water layer and none in the octanol. If the drug is very hydrophobic, you will find all of it in the octanol layer and none in the water layer. If the drug is in between, you will find some in the water and some in the octanol. The ratio of the amount found in the octanol divided by the amount found in the water is called the octanol-water partition coefficient; this is abbreviated  $P_{\text{OW}}$  and is higher the more hydrophobic a molecule is. Since  $P_{\text{OW}}$  varies over a large range, it is convenient to take the base-10 logarithm of  $P_{\text{OW}}$  or  $\log(P_{\text{OW}})$ .

For example, consider a drug that is moderately hydrophobic. Suppose that if you put 10 grams of the drug into the octanol/water flask, shake it up, and let it come to equilibrium, you find 9.09 grams of the drug in the octanol and 0.909 gram of the drug in the water. The  $P_{\text{OW}} = 9.09/0.909$  or 10 (10 times more of the drug goes into the octanol than the water). The  $\log(P_{\text{OW}})$  would be  $\log(10)$  or 1. So, the logP would be 1, what you'd expect for a moderately hydrophobic molecule. The table below shows some other values.

<b>logP</b>	<b>-2</b>	<b>-1</b>	<b>0</b>	<b>1</b>	<b>2</b>
$P_{\text{OW}}$	0.01	0.1	1	10	100
% of molecule in octanol	0.99	9.09	50	90.9	99
% of molecule in water	99	90.9	50	9.09	0.99

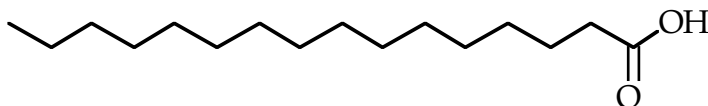
The Molecular Calculator examines the structure you submit to it and estimates the  $\log(P_{\text{OW}})$  using a variety of measured and calculated factors.

## (2) MACROMOLECULES

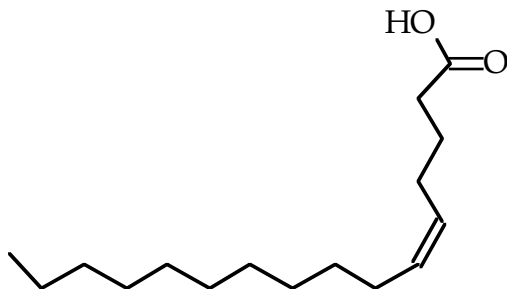
### (2.1) Lipids and phospholipids

(2.1.1) Organisms use fats and lipids as an energy reserve. Fats are important in transporting other nutrients such as the vitamins A, D, E, and K, which are not water soluble. Fats also form an essential part of the cell membrane. Some fatty acids, like those in Crisco or butter, form a solid at room temperature, whereas others, like those in corn oil, are liquid at room temperature. A saturated fatty acid contains no C=C bonds, as shown below.

$\text{CH}_3-(\text{CH}_2)_{14}-\text{COOH}$ :



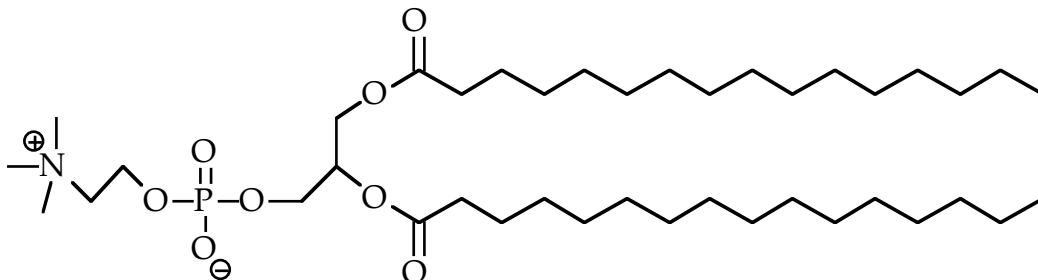
An unsaturated fatty acid has one or more C=C bonds.

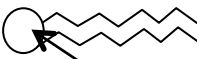


Which fatty acid do you predict will be solid at room temperature? Explain your answer.

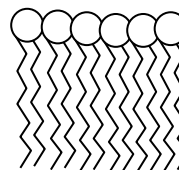
(2.1.2) An example of a phospholipid is shown below. Phospholipids are a major component of \_\_\_\_\_.

A phospholipid contains both polar and nonpolar domains. Circle the polar domain. Box the nonpolar domain.



A schematic of a phospholipid can be drawn like this:  Polar head

Explain why you would not find phospholipids arranged like this →

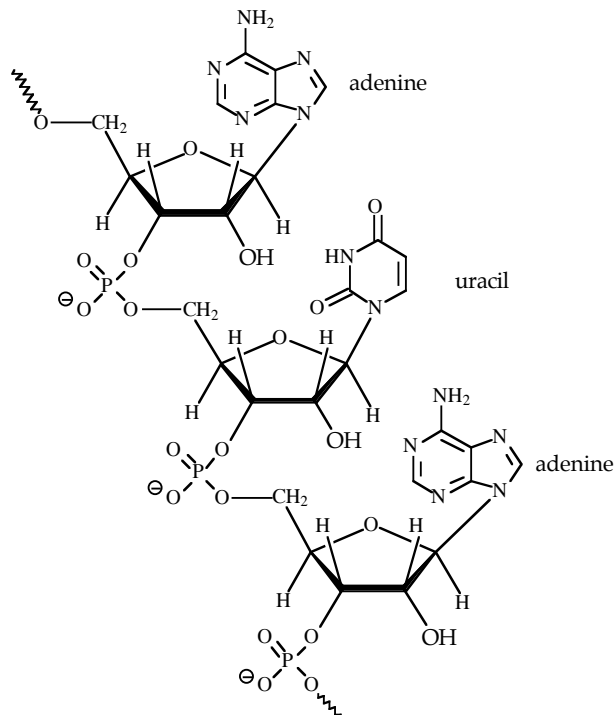
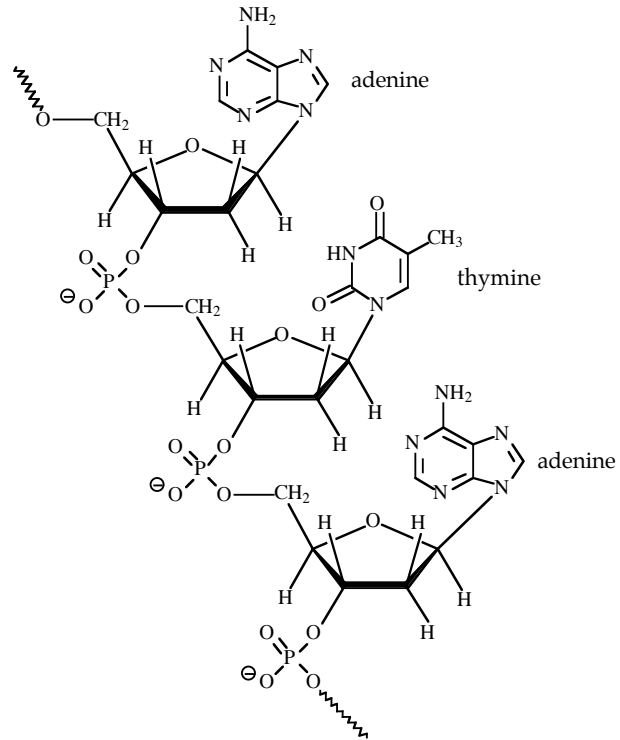


**(2.1.3)** Phospholipids can spontaneously form three different structures in aqueous environments. Draw the three possible structures that can be formed by phospholipids. Explain why the phospholipid molecules form these structures.

## (2.2) Nucleic acids

A more in-depth treatment of nucleic acids can be found in Chapter 3.

(2.2.1) Consider the two molecules shown below. Which is DNA and which is RNA? Describe the purpose(s) each serves in the cell.



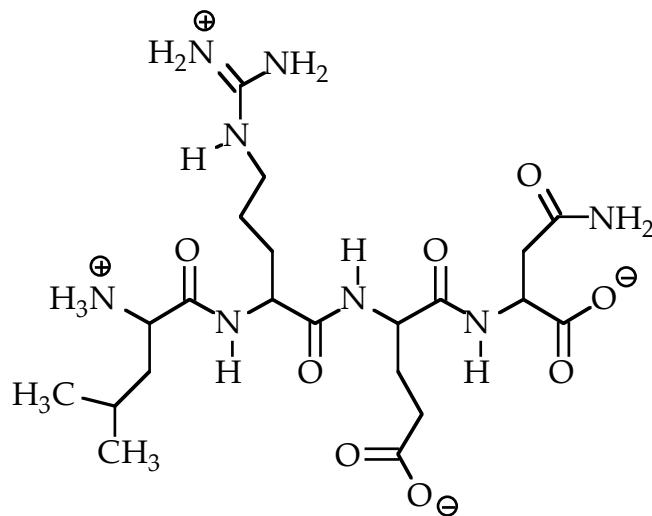
Nucleic Acids

## (2.3) Polypeptides and proteins, background

Diagnostic Problem:

Below is a small polypeptide.

- It is composed of \_\_\_\_\_ amino acids.
- Give the sequence of the amino acids in this polypeptide (the primary structure) and label the N and C termini.
- Circle the peptide bonds. Are these bonds covalent or noncovalent?





d) For the pairs of amino acids given below, **circle** each side chain. Give the strongest type of interaction that occurs between the side-chain groups of each pair.

Amino Acids	Interaction
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <math display="block">\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{O}^- \\   \\ \text{NH}_3^+ \end{array}</math> <p>Glycine</p> </div> <div style="text-align: center;"> <math display="block">\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{CH}_2-\text{CH}_2-\text{C} \\   \qquad \qquad \qquad \parallel \\ \text{NH}_3^+ \qquad \qquad \text{NH}_2 \end{array}</math> <p>Glutamine</p> </div> </div>	
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <math display="block">\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{CH}_2-\text{C}_6\text{H}_4-\text{OH} \\   \\ \text{NH}_3^+ \end{array}</math> <p>Tyrosine</p> </div> <div style="text-align: center;"> <math display="block">\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{CH}_2-\text{C} \\   \qquad \qquad \qquad \parallel \\ \text{NH}_3^+ \qquad \qquad \text{NH}_2 \end{array}</math> <p>Asparagine</p> </div> </div>	
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <math display="block">\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{CH}_2-\text{CH}_2-\text{C} \\   \qquad \qquad \qquad \parallel \\ \text{NH}_3^+ \qquad \qquad \text{O}^- \end{array}</math> <p>Glutamic Acid</p> </div> <div style="text-align: center;"> <math display="block">\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_3^+ \\   \\ \text{NH}_3^+ \end{array}</math> <p>Lysine</p> </div> </div>	

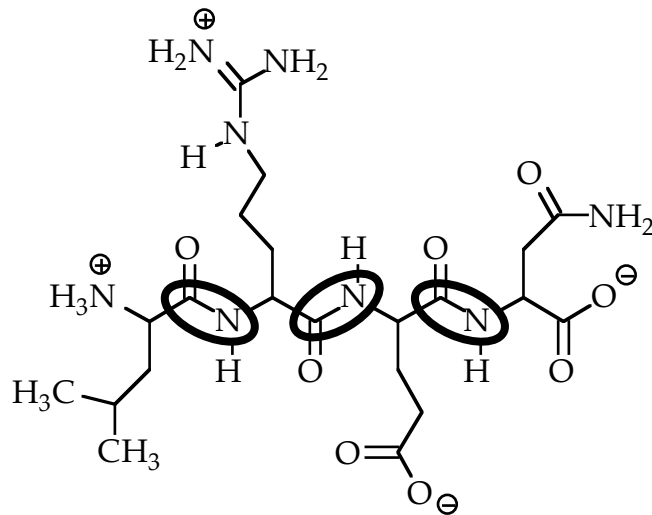
e) What are the types of structural organization in a polypeptide?

## Answer to Diagnostic Problem:

- a) It is composed of *four* amino acids.  
 b) Give the sequence of the amino acids in this polypeptide and label the N and C termini.

*N-leucine-arginine-glutamic acid-asparagine-C*

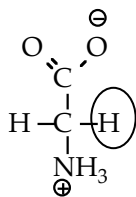
- c) Circle the peptide bonds. Are these bonds covalent or noncovalent?



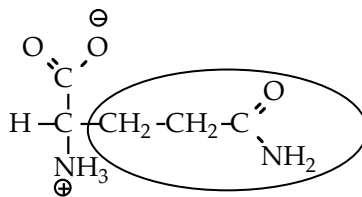
- d) The side chains or R groups of the amino acids are circled, and the interactions described refer to interactions between the side-chain groups.

### Amino Acids

### Interaction



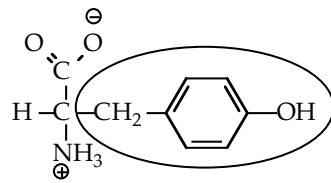
Glycine



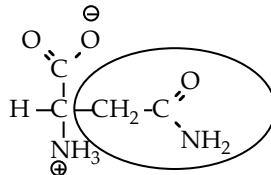
Glutamine

Van der Waals

*Glycine is nonpolar, glutamine is polar, and the strongest interaction is van der Waals forces.*



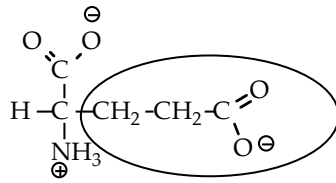
Tyrosine



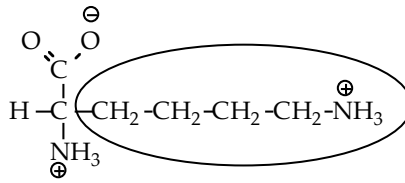
Asparagine

Hydrogen bond

*Tyrosine has a polar O–H group, asparagine is polar, and there is both a hydrogen donor and a lone pair of electrons, so a hydrogen bond could form.*



Glutamic Acid



Lysine

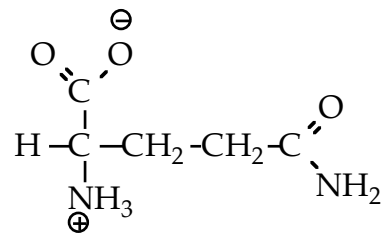
*Both side chains are polar and fully charged. One is positively charged, the other negatively charged, so an ionic bond could form.*

e) What are the types of structural organization in a polypeptide?

- **Primary:** The linear order of the amino acids.
- **Secondary:** Regions of local structure ( $\alpha$ -helix or  $\beta$ -sheet) mostly due to hydrogen bonding of one portion of the polypeptide backbone to another portion of the polypeptide backbone.
- **Tertiary:** The three-dimensional shape of a polypeptide.
- **Quaternary:** The interactions between subunits.

(2.3.1)

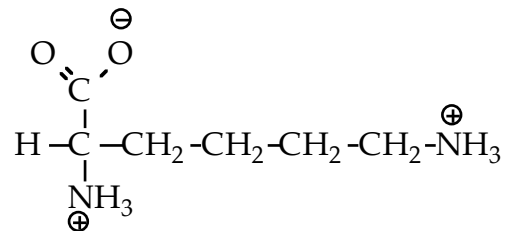
a) The structure of the amino acid glutamine is shown below.



i) Give an amino acid whose side chain can form a hydrogen bond with the side chain of glutamine. There may be more than one correct answer here; give only one.

ii) Next to the structure of glutamine shown above, draw the side chain of the amino acid you selected in part (i) making a hydrogen bond with glutamine. Indicate the hydrogen bond with a dashed line. There may be more than one correct answer here; give only one.

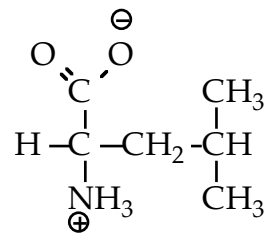
b) The structure of the amino acid lysine is shown below.



i) Give an amino acid whose side chain can form an ionic bond with the side chain of lysine. There may be more than one correct answer here; give only one.

ii) Next to the structure of lysine shown above, draw the side chain of the amino acid you selected in part (i) making an ionic bond with lysine; indicate the ionic bond with a dashed line. There may be more than one correct answer here; give only one.

c) The structure of the amino acid leucine is shown below.

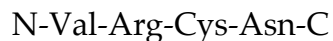


i) Give an amino acid whose side chain can form a hydrophobic interaction with the side chain of leucine. There may be more than one correct answer here; give only one.

ii) Next to the structure of leucine shown above, draw the amino acid you selected in part (i) making a hydrophobic interaction with the side chain of leucine. Indicate the hydrophobic interaction by circling the interacting parts of the two side chains. There may be more than one correct answer here; give only one.

**(2.3.2)** Researchers have found that some bacteria communicate with one another by releasing small peptides into their growth media.

Consider the sequence of the peptide shown below:



Draw the structure of the peptide (including the side chains of each amino acid) as it would be found at pH 7.0.

#### (C4) Computer-Aided Problems 4

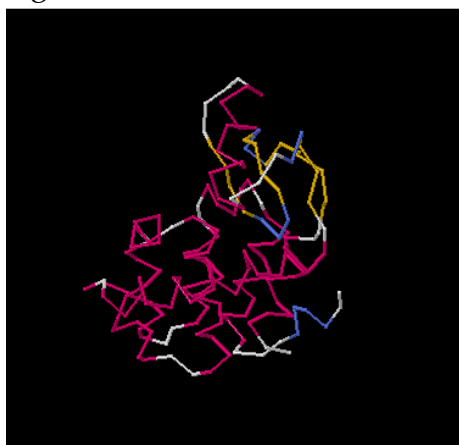
Because secondary structure is a 3-dimensional concept, there will be no problems on paper in this section.

##### Objectives:

- To observe the three major types of protein secondary structure.
- To see how they can be fitted together to form a protein.
- To introduce you to the complex 3-d structures of proteins.

##### Procedure:

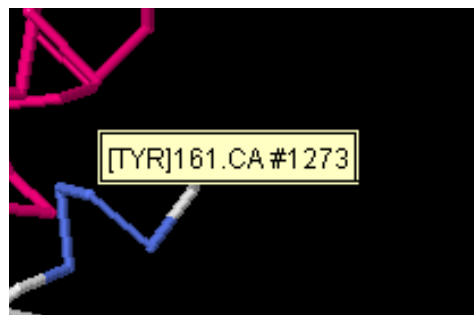
1) Access “**Molecules in 3-d**” at this site <http://intro.bio.umb.edu/MOOC/jsMol/> and click on the tab for this problem “Biochemistry C4.” Then, click the “Load lysozyme and show backbone” button (note that it may take a few seconds to load the structure). You will see something like this:



The image on the black screen shows the backbone of the lysozyme molecule. You can rotate or zoom in on this just as you did with the small molecules. Different amino acids have been colored based on their secondary structure.

- alpha helix = red or purple
- beta sheet = yellow
- turn = blue (this is a specifically shaped turn of the backbone)
- random coil = white (none of the above)

In addition to being able to rotate and zoom in on a molecule, this program also allows you to identify the amino acid over which you have placed the cursor. This works much the same as it did for the earlier molecular visualization exercises. The program will then display information on the atom in a pop-up window (this does not always work on Macintoshes). The information in the pop-up window is rather cryptic.



This image shows “[TYR]161.CA #1273.” This can be broken down into:

- “[TYR]” means that you clicked on a tyrosine.
- “161” means that the tyrosine you clicked on was amino acid number 161. Amino acids are numbered starting with #1, the amino terminus.
- “CA” means that you clicked on the alpha carbon of the lysine in the polypeptide chain.
- “#1273” means that the alpha carbon of amino acid #161 is atom number 1273, the overall protein molecule. This information is not particularly useful; do not confuse this number with the amino acid number.

Note that, when using either of these methods, it can be tricky to be sure what you have clicked on. Often, you can get a clearer “click” by rotating the molecule until the desired amino acid is clearly separated from the others.

a) Using this, describe the secondary structure of all the amino acids in the enzyme lysozyme.

- Start by finding one of the ends of the backbone chain. Interestingly, both ends are quite close together.
- Put the cursor over it or click on it. If it is number 1, you have found the amino terminus. Start here.
- Trace the backbone as it coils and twists. It may be difficult to be sure what you are clicking on; try rotating the molecule as you work. Determine the secondary structure of each amino acid.

Here is how it should look for the first 20:

#1 to #2: random coil

#12 to #13: random coil

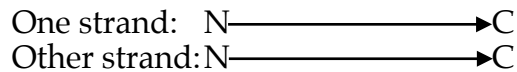
#3 to #11: alpha helix

#14 to #20: beta sheet

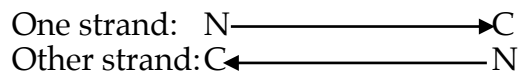
You should complete this for the rest of the protein.

b) Look closely at a short segment of alpha helix. You may need to zoom in to see it in detail (shift-drag up or down on the molecule). Each sharp bend in the backbone corresponds to one amino acid. Roughly how many amino acids are there per turn of the alpha helix? Hint: you may find it easier to count the number of amino acids in two or more turns.

c) Beta sheets are composed of two or more parallel backbone segments. In some cases, the backbone segments run from amino to carboxyl terminus in the same direction ("parallel beta sheet"):



In other cases, the backbone segments run amino to carboxyl terminus in opposite directions ("antiparallel beta sheet"):

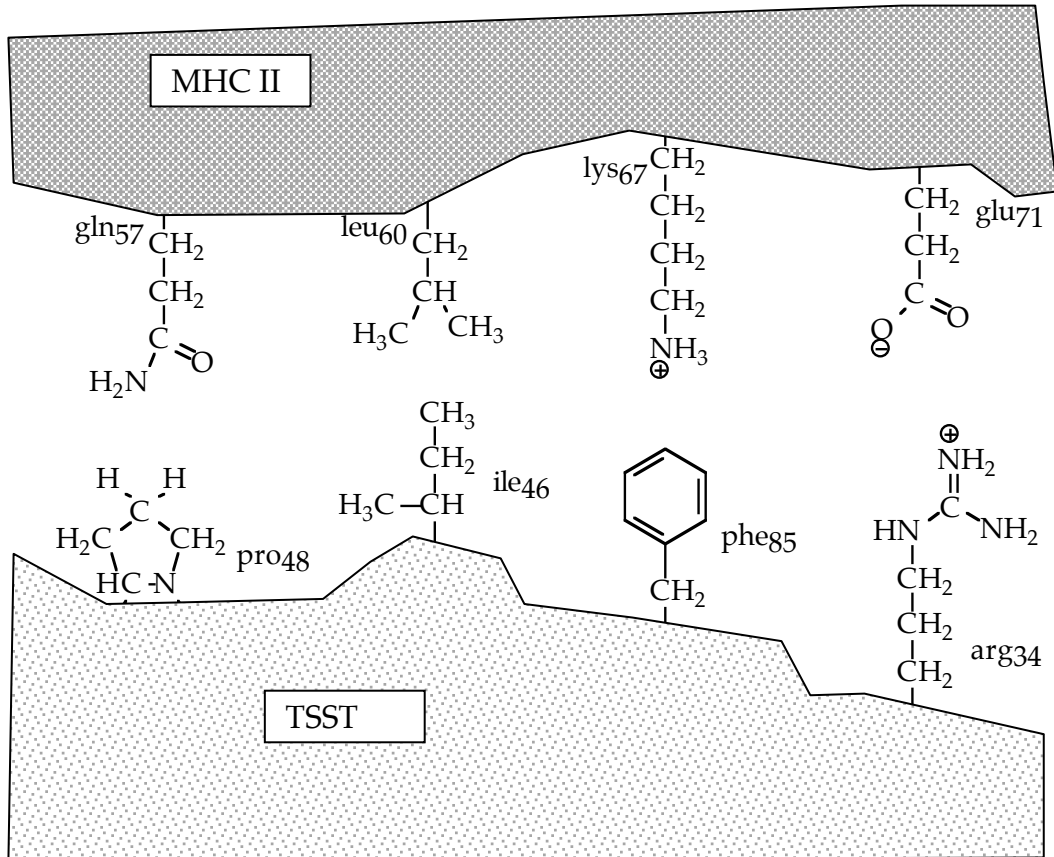


There are four regions where the backbone of lysozyme is in the beta sheet form where you can clearly see the interacting strands: 15 to 17, 24 to 27, 31 to 34, and 56 to 58. For each of the sections of beta sheet, determine which sections are interacting and whether they are parallel or antiparallel. You can find the direction of a given part of the protein by clicking on the amino acids; if the numbers increase, it means that you are moving toward the carboxyl terminus.



## (2.4) Polypeptides and proteins, interactions

(2.4.1) Toxic Shock Syndrome Toxin (TSST) is a protein produced by the bacterium *Staphylococcus aureus*. During an *S. aureus* infection, the TSST protein binds to MHC Class II proteins (MHC II) found on the surface of antigen-presenting cells of the patient's immune system. Binding of TSST to MHC II results in hyperactivation of the immune cells, which leads to the symptoms of toxic shock syndrome. A simplified version of the structure of both proteins has been determined; part of the binding interface of TSST as it binds to MHC II is shown below. (Note that "gln<sub>47</sub>" is shorthand for "the 47th amino acid, starting from the amino terminus, is a glutamine.")



a) Classify each of the eight side chains shown above as "hydrophobic," "hydrophilic and charged," or "hydrophilic and polar."

b) Each side chain of MHC II interacts with an opposite side chain in TSST (for example, Gln<sub>57</sub> of MHC II interacts with Pro<sub>48</sub> of TSST). What type(s) of interactions (covalent, hydrogen, ionic, or van der Waals) are possible between side chains of the MHC II protein and the opposite TSST side chain?

MHC II side chains

Gln<sub>57</sub>  
Leu<sub>60</sub>  
Lys<sub>67</sub>  
Glu<sub>71</sub>

Interaction with opposite side chain of TSST

c) Suppose you wanted to design an altered version of either MHC II or TSST that would make the interaction between TSST and MHC II stronger than in the normal situation. What amino acid would you change and what would you change it to? There are many possibilities; give one and explain how your change would strengthen the binding.

The remainder of this question deals with some hypothetical (possible but not yet studied) altered versions of the TSST protein and how they would interact with the MHC II protein.

d) Version 1 of TSST (TSST<sub>1</sub>; normal TSST is called TSST<sub>Norm</sub>) has a glutamine at position 34 instead of an arginine. Under conditions where TSST<sub>Norm</sub> would bind to MHC II, TSST<sub>1</sub> **does not bind**. Provide a reasonable explanation for why TSST<sub>1</sub> does not bind.

e) Version 2 of TSST (TSST<sub>2</sub>) has a glutamic acid at position 34 instead of an arginine. Under conditions where TSST<sub>Norm</sub> would bind to MHC II, TSST<sub>2</sub> **does not bind**. Provide a reasonable explanation for why TSST<sub>2</sub> does not bind.

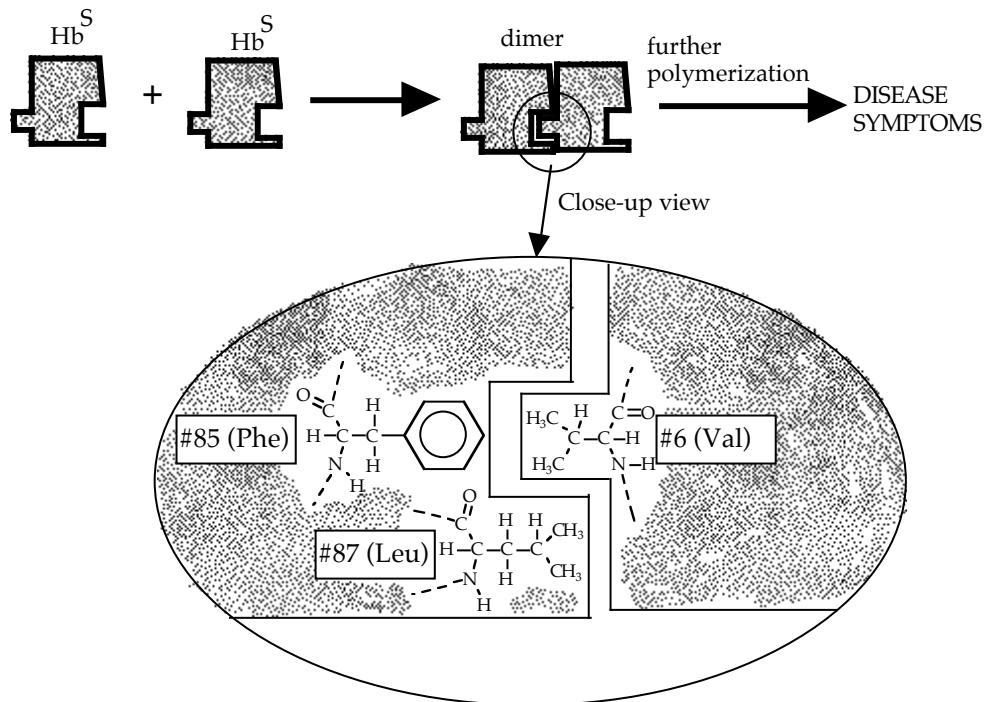
f) Version 3 of TSST (TSST<sub>3</sub>) has a leucine at position 46 instead of an isoleucine. Under conditions where TSST<sub>Norm</sub> would bind to MHC II, TSST<sub>3</sub> **does bind**. Provide a reasonable explanation for why TSST<sub>3</sub> does bind.

g) Version 4 of TSST (TSST<sub>4</sub>) has a glutamine at position 34 instead of an arginine **and** a serine at position 48 instead of proline. Under conditions where TSST<sub>Norm</sub> would bind to MHC II, TSST<sub>4</sub> **does bind**. Provide a reasonable explanation for why TSST<sub>4</sub> does bind.

h) Version 2 of TSST (TSST<sub>2</sub>) does not bind to normal MHC II. What amino acid substitution could you make in MHC II that would allow it to bind to TSST<sub>2</sub>? There are several possibilities; describe one and explain your reasoning briefly.

**(2.4.2)** Sickle-cell anemia is a genetic disease involving hemoglobin, the protein which carries  $O_2$  in the red blood cells. The disease symptoms are caused by the presence of abnormal hemoglobin molecules ( $Hb^S$ , S for sickle-cell; normal hemoglobin molecules are designated  $Hb^+$ ) which aggregate under certain conditions, preventing proper red blood cell function.

Aggregation of  $Hb^S$  begins with an interaction between two molecules of  $Hb^S$ ; the resulting dimers then aggregate to form the disease-causing long polymers. The aggregation is driven by an interaction between the side chain of amino acid #6 (valine) of one hemoglobin molecule with a pocket formed by the side chains of amino acids #85 (phenylalanine) and #87 (leucine) of another hemoglobin molecule. This is shown below.



Only the three relevant amino acids are shown; the peptide backbone is indicated with a dashed bond.

a) What type of bond/interaction exists between the side chain of valine #6 and the side chains of phenylalanine #85 and leucine #87 (ionic bond, hydrogen bond, hydrophobic interaction)?

- b) Wild-type hemoglobin does not form dimers or polymers of any kind. The only difference between wild-type ( $\text{Hb}^+$ ) and sickle-cell ( $\text{Hb}^S$ ) hemoglobins is:
- Amino acid #6 in  $\text{Hb}^S$  (sickle-cell) is valine (shown on the preceding page).
  - Amino acid #6 in  $\text{Hb}^+$  (wild-type) is glutamic acid.

Based on these data, provide a plausible explanation for why  $\text{Hb}^+$  does not form polymers.

- c) Consider the completely hypothetical case of a mutant form of hemoglobin that is identical to wild-type hemoglobin ( $\text{Hb}^+$ ), except that amino acid #6 in the mutant hemoglobin ( $\text{Hb}^{\text{Phe}}$ ) is phenylalanine instead of glutamic acid. There are two possibilities:

i) Suppose that  $\text{Hb}^{\text{Phe}}$  **does not** form polymers under any circumstances. Provide a plausible explanation for this observation, based on the structures of the molecules involved.

ii) Suppose that, under circumstances where  $\text{Hb}^S$  forms polymers,  $\text{Hb}^{\text{Phe}}$  **does** form polymers with the same general structure as polymers of  $\text{Hb}^S$ . Provide a plausible explanation for this observation, based on the structures of the molecules involved.

**(2.4.3)** The structure of the enzyme tryptophan synthetase has been studied extensively by a variety of methods. In a series of studies, Yanofsky and coworkers examined the effect on enzyme activity of various amino acid changes in the protein sequence (*Federation Proceedings*, **22**:75 [1963] and *Science* **146**:1593 [1964]). Altered amino acids are shown in **bold**. “Wild-type” is the normal strain isolated from the wild.

<u>Strain</u>	<u>Amino Acid at Position A</u>	<u>Enzymatic Activity</u>
wild-type	Gly	full
mutant 1	<b>Glu</b>	none
mutant 2	<b>Arg</b>	none

Here are two possible explanations for these results:

- The Gly  $\Rightarrow$  Glu and Gly  $\Rightarrow$  Arg changes introduce a **charge** (+) or (–) into a region of the protein that requires an uncharged amino acid like glycine.
- The Gly  $\Rightarrow$  Glu and Gly  $\Rightarrow$  Arg changes introduce much **larger** amino acid side chains into a space in the protein that requires a small amino acid like glycine.

Yanofsky and coworkers collected more mutants and examined their proteins to determine which of the above explanations was more likely to be correct:

<u>Strain</u>	<u>Amino Acid at Position A</u>	<u>Enzymatic Activity</u>
wild-type	Gly	full
mutant 3	<b>Ser</b>	full
mutant 4	<b>Ala</b>	full
mutant 5	<b>Val</b>	partial

a) Which of their models is supported by these data? Why?

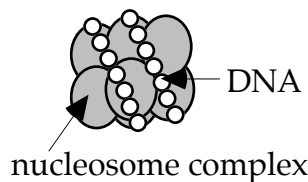
Alterations of amino acids at another location in the protein were found to interact with alterations at position A.

<u>Strain</u>	<u>Amino Acid at Position A</u>	<u>Amino Acid at Position B</u>	<u>Enzymatic Activity</u>
wild-type	Gly	Tyr	full
mutant 1	<b>Glu</b>	Tyr	none
mutant 6	<b>Glu</b>	<b>Cys</b>	partial
mutant 7	Gly	<b>Cys</b>	none

b) Explain the behavior of mutant 6 in terms of your model of part (a).

c) Given your model above, explain the lack of activity found in mutant 7.

**(2.4.4)** Nucleosomes are protein complexes formed by eight interacting subunits. These complexes aid in the orderly packing of DNA by acting as a spool around which the DNA double helix is wound.



a) How many polypeptides compose the nucleosome complex?

b) What is quaternary structure? Does the nucleosome complex have quaternary structure?

c) The following sequence of amino acids is found as part of the primary structure of the nucleosome complex:

Val-Leu-Ile-Phe-Val-Val-Ile-Ile

i) In what general region of the nucleosome complex would you expect to find this stretch of amino acids?

ii) Why did you choose this region?

d) Some regions of the nucleosome complex have high percentages of lysine and arginine. Given the function of the nucleosome:

i) Where might these regions be found?

ii) What might be the role of these regions?

Your friend wants to examine the interactions between nucleosome complexes and DNA double helices. He prepares three identical samples of nucleosome complexes associated with DNA and treats each sample with an agent that disrupts a different type of molecular force. He disrupts hydrogen bonds in sample 1, ionic bonds in sample 2, and peptide bonds in sample 3.

You know that all of the treatments eliminate the binding between nucleosome complexes and DNA double helices and also disrupt other interactions.

e) Indicate how each treatment affects the nucleosome complexes by listing the appropriate number(s) in the table below. Choose any or all that apply.

- 1) No change, complex intact.
- 2) Disrupt tertiary structure.
- 3) Disrupt disulfide bonds.
- 4) Disrupt secondary structure.
- 5) Disrupt primary structure.

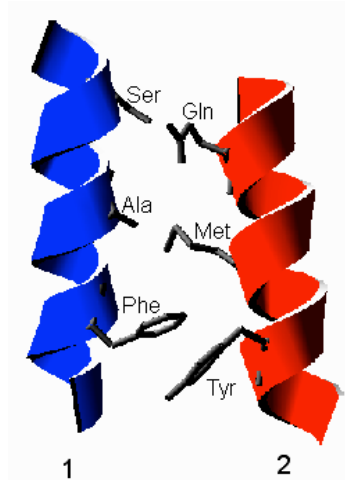
Treatment	Effect on nucleosome complexes (list appropriate number(s) from above)
Disrupt hydrogen bonds	
Disrupt ionic bonds	
Disrupt peptide bonds	

f) Indicate how each treatment affects the structure of DNA double helices by listing the appropriate number(s) in the table below. Choose any or all that apply.

- 1) No change, double helices intact.
- 2) Disrupt base pairing.
- 3) Disrupt hydrophobic interactions.
- 4) Disrupt sugar-phosphate backbone.

Treatment	Effect on structure of DNA double helices (list appropriate number(s) from above)
Disrupt hydrogen bonds	
Disrupt ionic bonds	
Disrupt peptide bonds	

(2.4.5) Suppose you have two different protein  $\alpha$ -helices that bind to one another. A variety of amino acids are seen at the binding interface between these helices. At the binding surface of helix 1 is a serine, an alanine, and a phenylalanine. On the binding surface of helix 2 is a glutamine, a methionine, and a tyrosine. (Note the binding interface in the figure below.)



a) Interactions between these amino acids hold the helices together. What is the strongest possible interaction between each of the following pairs of amino acids? Choose from covalent bonds, van der Waals forces, ionic bonds, and hydrogen bonds.

	Amino acids	Strongest interaction
i)	Ser and Gln	
ii)	Ala and Met	
iii)	Phe and Tyr	

If a little heat is applied to these helical proteins, you observe that the helices no longer bind one another and instead are free helices in solution. If even more heat is applied, you no longer even see helices. Only elongated peptides with no defined structure are observed.

b) Explain why at low heat the proteins maintain a helical structure but fail to interact, while higher heat produces elongated peptides.

You replace both the phenylalanine of helix 1 and the tyrosine of helix 2 with cysteine.

c) How does changing both these residues to cysteine affect the stability of the interaction? Why?



### (C5) Computer-Aided Problems 5

The problems in this section deal with the enzyme lysozyme. Lysozyme catalyzes the breakdown of bacterial cell walls. Lysozyme is used by the bacterial virus called bacteriophage T4 to break out of the host cell.

#### 1) Hydrophobic/Hydrophilic

In general, you would expect to find amino acids with hydrophobic side chains in the interior of a protein and amino acids with hydrophilic side chains on the outside of the protein. In this problem, you will explore a simple real-world protein to see how these principles are applied in nature.

Access “**Molecules in 3-d**” at this site <http://intro.bio.umb.edu/MOOC/jsMol/> and click on the tab for this problem “Biochemistry C5.” Click the “Load lysozyme and show exterior; red = phobic” button; it may take a little while to load the structure. You should see a black window with a collection of red and white spheres displayed. The red and white spheres are individual atoms of the protein lysozyme. Atoms in amino acids with hydrophobic side chains are red; hydrophilics are white.

a) Look at the view you just loaded. You should see the red and white protein. Use the mouse to rotate it to see all sides. How would you characterize the amino acid side chains on the surface (all hydrophobic, mostly hydrophobic, equally hydrophobic and hydrophilic, mostly hydrophilic, all hydrophilic)? How well does this fit with your expectations? Provide a plausible explanation for why this might be so.

b) Click the button marked “Show interior; red = phobic” to show a brief animation. The display will rotate lysozyme to a specific position, pause briefly, and then show the interior of the enzyme. The view shows what you would see if you sliced the protein in a vertical plane parallel to the screen and removed the front section – like slicing an orange down the middle and looking inside. How would you characterize the amino acid side chains in the interior (all hydrophobic, mostly hydrophobic, equally hydrophobic and hydrophilic, mostly hydrophilic, all hydrophilic)? How well does this fit with your expectations? Provide a plausible explanation for why this might be so.

c) Click the button marked “Show valines.” The display will show most of the atoms in the protein as balls made of tiny yellow dots; this allows you to see through them into the interior of the protein. Several other atoms are shown as solid spheres; these are the atoms in the nine valines in the protein. The atoms in the valines are colored according to what element they are (see the color scheme on the web page).

Valine has one of the most hydrophobic side chains of any amino acid. Valine's side chain is composed entirely of carbon (gray) and hydrogen (not shown).

For each valine, determine (to the best of your ability) whether the side chain is inside the protein or exposed to the water at the protein's surface. The best way to do this is to pick an individual valine (you can identify which one it is by putting the cursor over it or by clicking on it as in previous problems), then rotate the protein carefully while trying to see if any of the side chain is not covered by yellow dots. If you can see parts of the side chain that are not covered by yellow dots, then that side chain is exposed to the water surrounding the protein. If there is no way to see the side chain without looking through yellow dots, then the side chain is buried.

How many of the nine valines are completely buried? How does this match with your expectations? Why might this be so?

d) Click the button marked "Show lysines." The display will show the bulk of the atoms in the protein as balls made of tiny yellow dots; this allows you to see through them into the interior of the protein. Several other atoms are shown as solid spheres; these are the atoms in the 13 lysines in the protein. The atoms in the lysines are colored according to what element they are (see the color scheme on the web page).

Lysine has one of the most hydrophilic side chains of any amino acid. Lysine's side chain ends with a single positively charged nitrogen atom (blue).

For each lysine, determine (to the best of your ability) whether the side chain is inside the protein or exposed to the water at the protein's surface. You should focus on the most hydrophilic part – the blue nitrogen atom at the tip of the side chain. You can use the same method you used for part (c).

How many of the 13 lysines are completely buried? How does this match with your expectations? Why might this be so?

## 2) Side-chain interactions

We will next consider interactions between side chains of different amino acids in the protein lysozyme. These interactions contribute to the tertiary structure of the protein.

Click the “Load Lysozyme” button. You must click this button first to load the structure for the other parts of this problem.

You will see a black window with the protein lysozyme shown in “ball and stick” mode – atoms are shown as balls and the covalent bonds connecting them are shown as rods. You can click on the “Show atoms as spacefill” button to change the representation “Spacefill” where atoms are shown as solid spheres at their actual sizes. You will find it useful to switch back and forth between the two views. Note that you may sometimes need to click this button three times to get the view to change.

- The ball and stick view shows covalent bonds as rods and is most useful for determining which atoms are *covalently bonded* to each other. The small size of the atoms can sometimes make it hard to tell which atoms are close together for noncovalent interactions.
- The spacefill view shows atoms as joined spheres of their approximate actual size in the molecule. It is most useful for determining which atoms are *closest together*. Because it does not show covalent bonds, it can sometimes be hard to figure out what atoms are covalently or noncovalently bonded.

There are two important things to note about these views:

- They show only the covalent bonds; you must *infer* the noncovalent bonds based on your knowledge of amino acids and their properties.
- These views show only the amino acids listed on the button; the remaining amino acids are shown as dark lines.

Because these problems deal with amino acids in an actual protein, it is important to consider the relative positions and conformations of the side chains. Put another way, “Even if a particular interaction is possible based on the structures on paper alone, the side chains must be arranged properly in order for the interaction to actually occur in the protein.”

Each part of this problem involves looking at the interaction between the side chains of two amino acids.

For each problem, click the appropriate button and answer the following questions. You will find it useful to rotate, zoom in, and/or change from ball and stick to spacefill views. The questions are:

i) Look up the structures of each amino acid in your textbook. Based on these structures only, what interaction(s) are possible between their side chains?

- Ionic bond
- Hydrogen bond
- Hydrophobic interaction
- van der Waals interaction

ii) Which of the interactions you selected above is the strongest?

iii) Look at how these side chains are arranged in lysozyme and sketch their relative arrangement on paper. Be sure to add in the hydrogen atoms.

iv) Based on the structure you drew in (iii), what is the strongest interaction between the side chains in the actual protein?

a) Glu<sub>11</sub> and Arg<sub>145</sub> – click the button labeled “Show Glu 11 and Arg 145” and answer the four questions above.

b) Asp<sub>10</sub> and Tyr<sub>161</sub> – click the button labeled “Show Asp10 and Tyr 161” and answer the four questions above.

c) Gln<sub>105</sub> and Trp<sub>138</sub> – click the button labeled “Show Gln 105 and Trp 138” and answer the four questions above.

d) Met<sub>102</sub> and Phe<sub>114</sub> – click the button labeled “Show Met 102 and Phe 114” and answer the four questions on page 134.

e) Tyr<sub>24</sub> and Lys<sub>35</sub> – click the button labeled “Show Tyr 24 and Lys 35” and answer the four questions on page 134. This is a challenging one.

### 3) Effects of mutations on protein structure

In a truly heroic series of experiments, Rennell, Bouvier, Hardy, and Poteete (*Journal of Molecular Biology* **222**:67-87 [1991]) generated a huge set of mutant versions of lysozyme. In each individual mutant, only one amino acid was changed; all the others were the same. Each individual mutant was checked to determine whether it had full activity. In their studies, each of the 164 amino acids in lysozyme was individually changed to 13 alternatives.

We have chosen mutants that affect the amino acids you explored in problem (C2). In addition, the “Molecules in 3-d” program in the “Biochemistry” folder on the CD-ROM also contains a set of views of lysozyme specifically arranged for this problem “Lysozyme III.”

Provide a plausible explanation for each of the following results in terms of your findings from problem (C2), keeping in mind the properties of different amino acid side chains. This first is given as an example:

Question: “If Glu<sub>11</sub> is changed to Ser, the resulting protein is not fully active.”

Complete answer: “Based on problem (C2), Glu<sub>11</sub> normally makes an ionic bond with Arg<sub>145</sub>. If the Glu at position 11 were replaced with Ser, an ionic bond would no longer be possible. Although an H-bond is possible, this would be weaker than an ionic bond. This weaker bond must not be strong enough to hold the protein in the correct shape; thus it is nonfunctional.”

Your answers should be structured similarly.

- a) If Glu<sub>11</sub> is replaced with Arg, the resulting protein is not fully active.
- b) If Glu<sub>11</sub> is replaced with Phe, the resulting protein is not fully active.
- c) If Glu<sub>11</sub> is replaced with Asp, the resulting protein is not fully active.
- d) If Arg<sub>145</sub> is replaced with Ser, the resulting protein is not fully active.
- e) If Arg<sub>145</sub> is replaced by His, the resulting protein is fully active; if it is replaced by Lys, the resulting protein is not fully active.
- f) If Tyr<sub>161</sub> is replaced with Ser, the resulting protein is not fully active.
- g) If Asp<sub>10</sub> is replaced with Glu, the resulting protein is fully active.
- h) If Gln<sub>105</sub> is replaced with Glu, the resulting protein is fully active.

i) If Gln<sub>105</sub> is replaced with Leu, the resulting protein is fully active. Why is this surprising?

j) If Met<sub>102</sub> is replaced with Glu, Arg, or Lys, the resulting protein is not fully active.

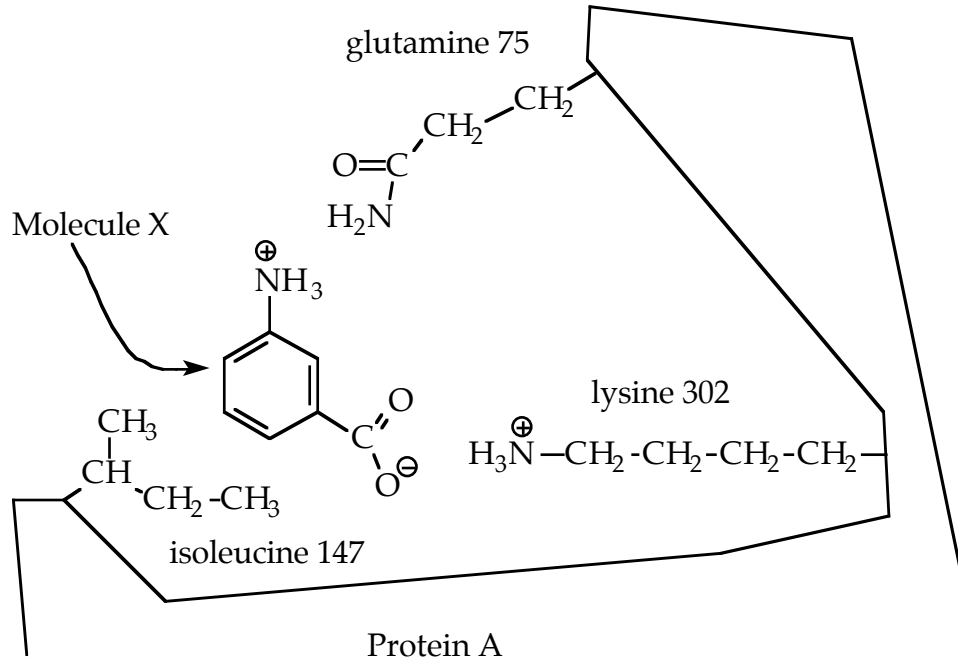
k) Lys<sub>35</sub> can be replaced with any amino acid and all the resulting proteins are fully active.

l) If Phe<sub>67</sub> is replaced with Pro, the resulting protein is not fully active. You should go back to “Molecules in 3-d” problem “Lysozyme III” and look at the view of Phe<sub>67</sub> and the secondary structure of the protein (click the button “Show Phe 67 and secondary struct.”). In this view, Phe<sub>67</sub> is shown as spheres; the rest of the protein is shown as backbone only. The backbone of Pro is slightly but significantly different from the backbone of all the other amino acids; you should check your textbook for details.

## (2.5) Polypeptides and proteins, binding sites

One of the most important functions of proteins is to act on smaller molecules. Proteins do this by binding these smaller molecules via the noncovalent interactions we have already discussed.

(2.5.1) You are studying a protein, Protein A, which binds a small molecule, Molecule X. The binding site is shown below with Molecule X bound.



Molecule X binds to Protein A via the side chains of three amino acids in Protein A: glutamine 75, isoleucine 147, and lysine 302.

- What is the strongest possible interaction between the side chain of **glutamine 75** and the nearest part of Molecule X?
- What is the strongest possible interaction between the side chain of **isoleucine 147** and the nearest part of Molecule X?
- What is the strongest possible interaction between the side chain of **lysine 302** and the nearest part of Molecule X?

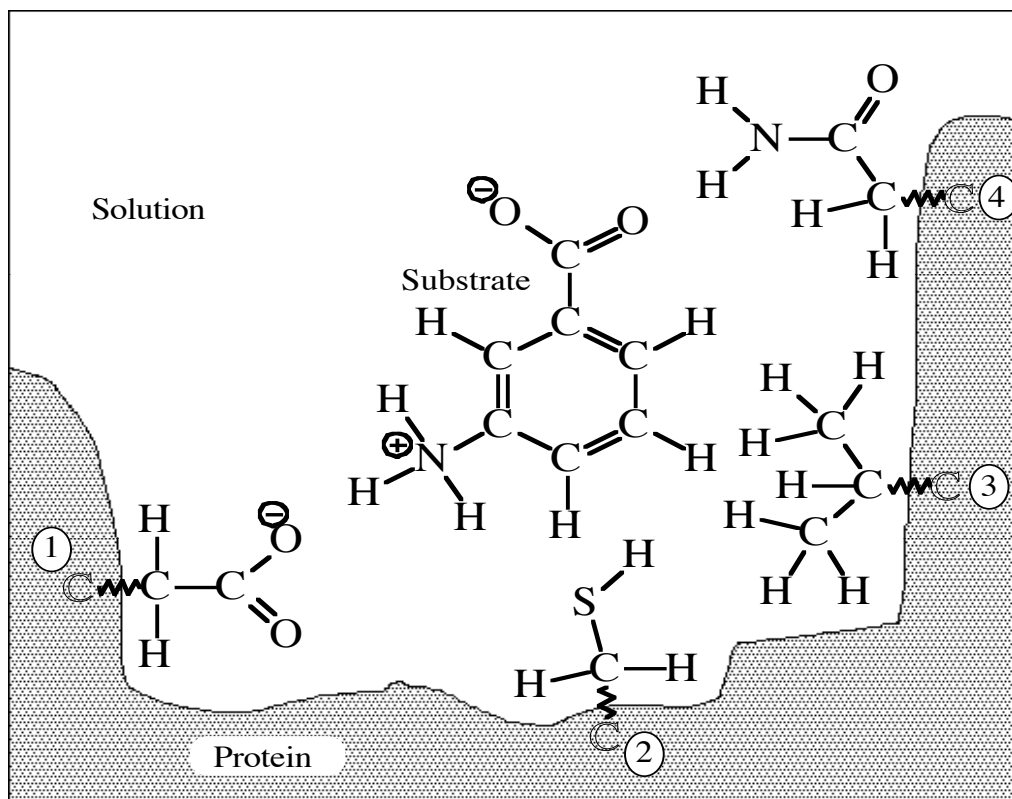


Consider each of the following changes to the protein separately.

d) A mutant version of Protein A differs from the normal Protein A in only one amino acid: glutamine 75 is replaced by asparagine. This mutant protein no longer binds Molecule X. Explain why this change has this effect.

e) A different mutant version of Protein A differs from the normal Protein A in only one amino acid: lysine 302 is replaced by glutamic acid. This mutant protein no longer binds Molecule X. Explain why this change has this effect.

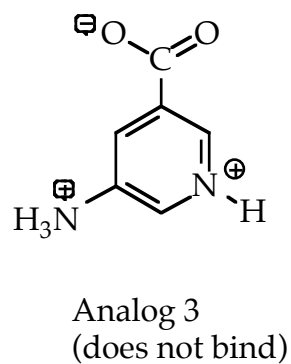
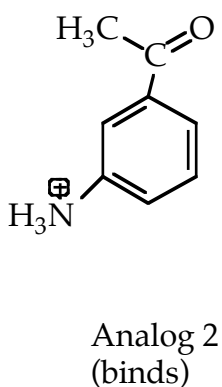
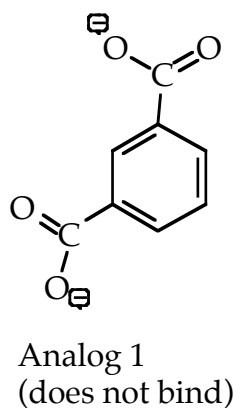
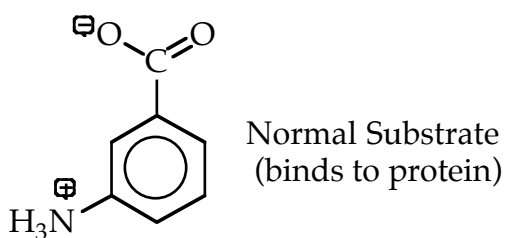
(2.5.2) Shown on the right is a hypothetical substrate molecule binding to a hypothetical protein. The substrate binds to the enzyme via noncovalent (hydrogen, ionic, hydrophobic) interactions. Below is a close-up of the substrate and substrate-binding region of the protein.



a) Each of the numbered groups is a side chain of a particular amino acid. For each side chain, give the amino acid.

b) Each of the four side chains of the protein that interact with the substrate are numbered on the figure above. For each side chain, state which type(s) of interactions it could have with the substrate in the configuration shown above. Also classify each side chain as hydrophobic, hydrophilic-polar, or hydrophilic-charged.

c) One way to study the noncovalent interactions between substrate and protein is to synthesize molecules similar to the substrate and see if they bind to the protein. Shown below are three "substrate analogs" along with the normal substrate. Explain, in terms of the interactions you described in part (a), why each of the analogs binds or fails to bind to the protein.



d) Suppose you wanted to strengthen the binding of the substrate to the original enzyme by altering one of the four amino acids that you labeled in part (a) above. Which amino acid would you change, what would you change it to, and why?

### **(C6) Computer-Aided Problems 6**

It is very important for cells to be able to repair DNA once it gets damaged. There are many mechanisms that cause DNA damage; one of these is “alkylation,” where a foreign molecule becomes covalently attached to the DNA. Cells have specific enzymes that recognize this damaged DNA and begin the process of repair; one of these is alkyl adenine glycosylase (AAG). AAG recognizes adenine (A) bases in DNA that have inappropriate molecules covalently bonded to them. This enzyme and its interaction with DNA will be used as an example of enzyme-substrate interactions in 3-d.

a) Access “Molecules in 3-d” at this site <http://intro.bio.umb.edu/MOOC/jsMol/> and click on the tab for this problem “Biochemistry C6.” Click the “Load AAG and DNA” button; it may take a little while to load the structure. You will see a view of AAG binding to some damaged DNA. You should rotate it to see all the parts of the molecule:

- The two DNA strands (the intertwined purple and yellow strands)
- The protein (light blue or gray)
- The damaged DNA base (red – buried at the interface between the DNA and protein)

Notice how the protein grabs onto the DNA and how the damaged DNA base is recognized by a pocket on the surface of the protein. The remainder of the problem will focus on the interactions between the DNA and the protein.

The next four parts involve interactions between particular side chains of the protein and particular parts of the DNA. The amino acids in the protein are identified as they have been throughout this book. The DNA bases are designated similarly: “T8” means “the 8<sup>th</sup> base in a particular chain, which is a Thymine (T).” Consult your textbook for structures of DNA and related molecules. When you click a particular button, the selected parts of the protein and DNA are shown as spheres connected by lines; the rest of the protein and DNA are shown as dark gray lines.

The program includes one important feature to make it easier for you to solve these problems: “Show atoms as spacefill” and “Show atoms as ball and stick” buttons – these switch back and forth between “spacefill” and “ball and stick” representations. For descriptions of “spacefill” and “ball-and-stick,” see problem (C5) part (2).

For each of the interacting parts below, you should do the following:

i) Click the corresponding button (for example, for part (a), click “Show Arg 182 in the protein and T 8 in the DNA”). Draw the interacting parts of the amino acid and the DNA. You do not have to draw the complete amino acids or nucleotides; draw only the few atoms that are interacting and their immediate neighbors.

ii) What are the possible interactions between these parts of the molecule? Which is the strongest?

b) Arg<sub>182</sub> and T<sub>8</sub>

c) Thr<sub>143</sub> and G<sub>23</sub>

d) Met<sub>164</sub> and T<sub>19</sub>

e) Tyr<sub>162</sub> and T<sub>8</sub>

The next parts involve particular mutations in AAG. These were explored in another large study by Lau, Wyatt, Glassner, Samson, and Ellenberger (*Proceedings of the National Academy of Sciences of the United States* 97(25):13573-13578 [2000]). In these experiments, they determined which amino acid substitutions are “tolerated.” That is, the set of mutations that result in AAG proteins that are still fully active.

For each mutation, use your findings from previous parts (b) through (e) and your understanding of amino acids and protein structure to provide a plausible explanation for these observations. Here is an example:

Statement: “No substitutions of Arg<sub>182</sub> are tolerated.”

Complete explanation: “Arg has a medium-length side chain with a (+) charge as well as H-bond donors and acceptors; it cannot make a hydrophobic interaction. It makes an ionic bond with the (–)-charged oxygen on the phosphate group of T<sub>8</sub> in the DNA. Both Lys and His should be capable of making an ionic bond with a negative oxygen atom. Since neither His nor Lys is tolerated at this position, the amino acid at this position must be a specific size as well as (+)-charged.”

f) Thr<sub>143</sub> can be replaced by Gln to produce a fully functional protein.

g) Met<sub>164</sub> can be replaced by Ile or Phe to produce a fully functional protein.

h) No substitutions of Tyr<sub>162</sub> are tolerated.

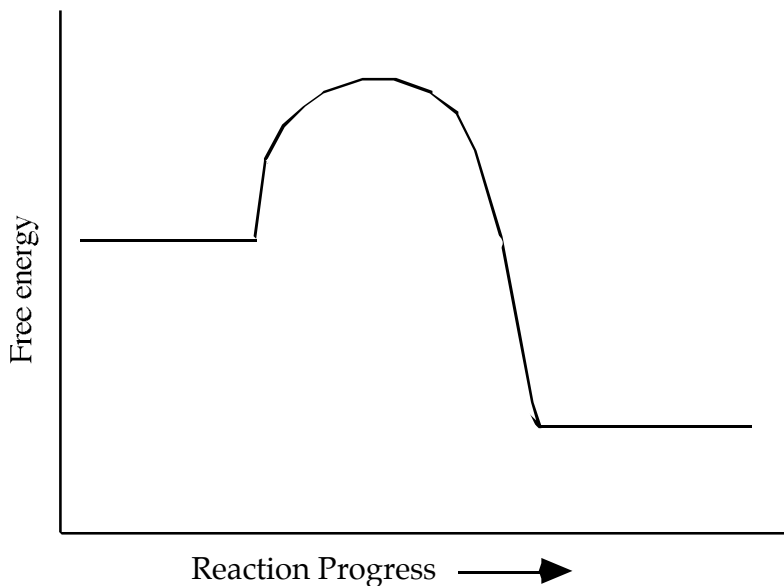
### (3) ENERGY, ENZYMES, AND PATHWAYS

#### (3.1) Energy and enzymes

Diagnostic Problem:

Graphs like that below are called “reaction coordinate diagrams”; they are one way of following the energetics of a reaction from start to finish. Energy of a compound at any given point is “ $G$ ,” and the free energy “ $\Delta G$ ” of a reaction or portion thereof is the difference between two points ( $\Delta G = G_{\text{product}} - G_{\text{reactant}}$ ). For more on this, see your textbook.

Under standard conditions, the reaction  $A + B \Rightarrow C + D$  has a positive  $\Delta G_0$ .  
The reaction  $C + D \Rightarrow A + B$  has a negative  $\Delta G_0$ .



a) On the energy diagram above, label the following:

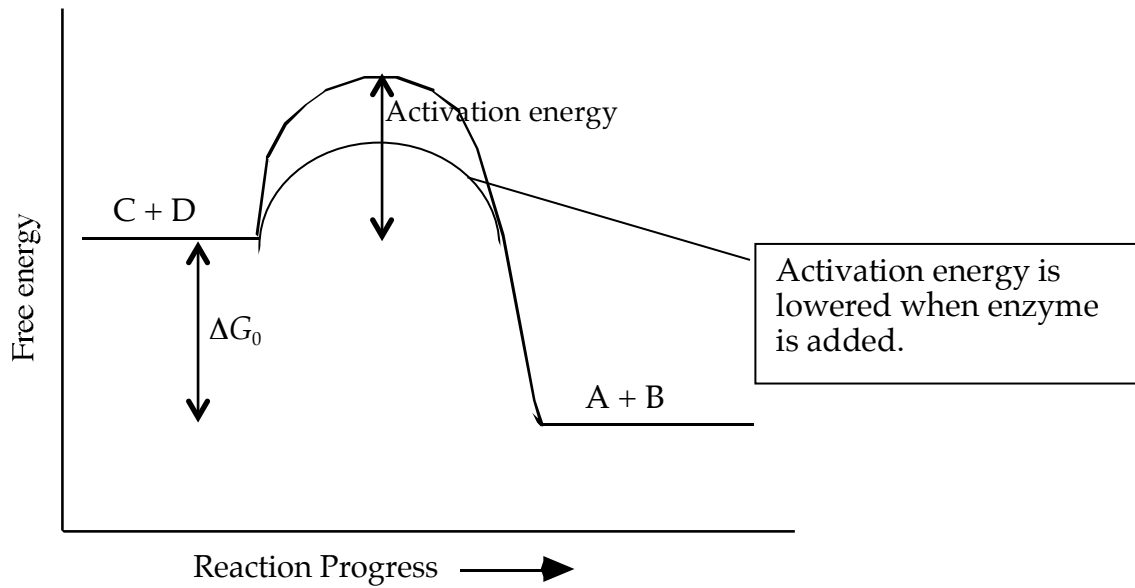
- Activation energy
- $\Delta G_0$
- C + D
- A + B

b) Modify the diagram above to show how it would change if an enzyme that catalyzed the reaction is added.

c) What effect, if any, would adding an enzyme that catalyzed this reaction have?

## Solutions to Diagnostic Problem:

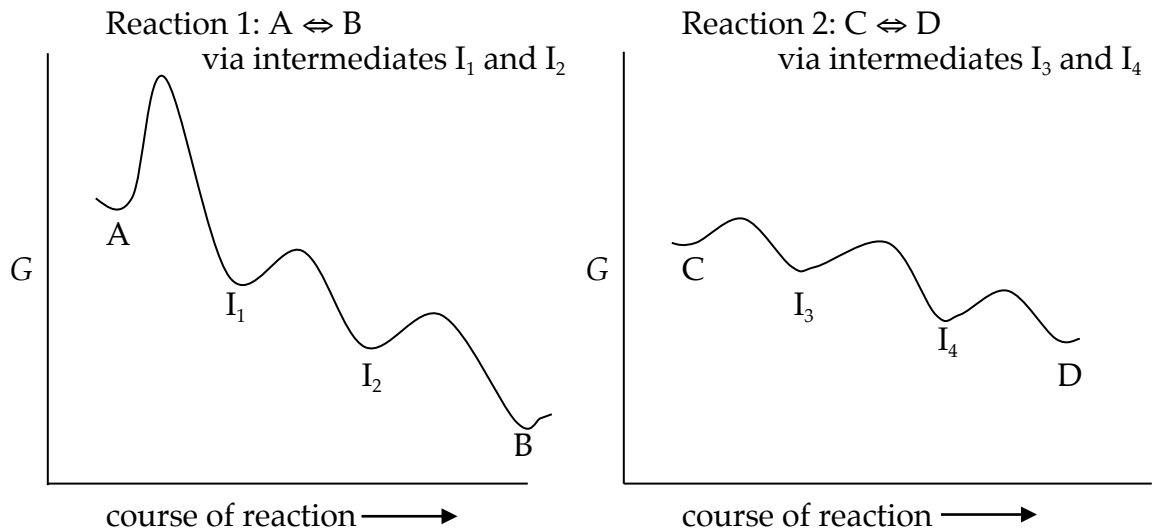
a) and b)



c) What effect, if any, would this have on the reaction?

*An enzyme does not change the  $\Delta G_0$  of the reaction, but by lowering the activation energy it speeds up the reaction  $C + D \Rightarrow A + B$ .*

**(3.1.1)** Virtually all of the reactions we will consider in this book will have multiple steps and several intermediates.



a) Which of the above reactions ( $A \Rightarrow B$  or  $C \Rightarrow D$ ) yields the most free energy? Which reaction is the most thermodynamically spontaneous? Explain your reasoning.

b) We have described free energy as a way of predicting the likelihood of a reaction. However, even if thermodynamics says that a reaction is spontaneous ( $\Delta G$  is negative), it is not necessarily true that the reaction will be fast (low activation energy). Given the diagram above, which reaction ( $A \Rightarrow B$  or  $C \Rightarrow D$ ) will proceed at the greater rate? Explain your reasoning.

c) Would adding an enzyme that catalyzes the first step of reaction 1 ( $A \Rightarrow B$ ) change the spontaneity ( $\Delta G$ ) and rate (activation energy) of the reaction  $A \Rightarrow B$ ? Explain your reasoning.



**(3.1.2)** The first step in the pathway of glycolysis is the transfer of a phosphate group from ATP to glucose, giving glucose 6-phosphate (reactions of this type are phosphorylations). This step is shown in Reaction 1.

Reaction 1:  $\text{Glucose} + \text{P}_i \Rightarrow \text{Glucose 6-phosphate}$        $\Delta G_0 = + 3.3 \text{ kcal/mol}$

a) Under standard conditions, which is more favorable energetically, breakdown of glucose 6-phosphate (net reaction to the left) or formation of glucose 6-phosphate (net reaction to the right)? Why?

Reaction 2 is the hydrolysis of ATP to ADP:

Reaction 2:  $\text{ATP} \Rightarrow \text{ADP} + \text{P}_i$        $\Delta G_0 = -7.3 \text{ kcal/mol}$

b) Under standard conditions, which is more favorable energetically, formation of ATP (net reaction to the left) or breakdown of ATP (net reaction to the right)? Why?

c) Using the two reactions given, draw the overall reaction that makes the formation of glucose 6-phosphate a favorable, spontaneous reaction. In other words, what reaction do you add to (1) to make a reaction where glucose 6-phosphate is one of the products and the  $\Delta G$  is (-)? What is the  $\Delta G_0$  for the overall reaction? In which direction does the reaction proceed?

(3.1.3) Given that the reaction  $\text{H}_2 + \text{O}_2 \Rightarrow \text{H}_2\text{O}$  is spontaneous, answer the following questions.

a) Rank the following states of hydrogen and oxygen in order from the highest free energy to the lowest free energy.

<u>State</u>	<u>Description</u>
1	$\text{H}_2\text{O}$ molecules
2	Free H and O atoms not bonded to anything
3	$\text{H}_2$ and $\text{O}_2$ molecules

Highest free energy \_\_\_\_\_

Middle free energy \_\_\_\_\_

Lowest free energy \_\_\_\_\_

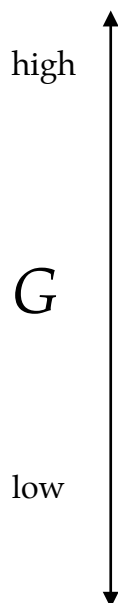
b) What type(s) of bonds are being broken when you go from state (3) to state (2)?

covalent bonds      ionic bonds      hydrogen bonds      hydrophobic interactions

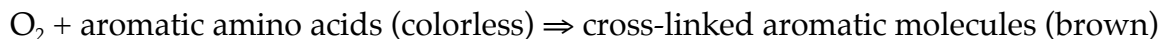
c) The reaction  $\text{H}_2 + \text{O}_2 \Rightarrow \text{H}_2\text{O}$  proceeds only very slowly at room temperature. Draw a reaction coordinate diagram for this reaction on the graph below. Be sure to include:

- $\text{H}_2 + \text{O}_2$
- $\text{H}_2\text{O}$
- transition state
- appropriate line(s) connecting the three states

Note that only the relative levels are important here; you do not need to worry about the spacing between the levels.



**(3.1.4)** Fresh raw potatoes contain an enzyme that rapidly produces an unwanted discoloration when the potato is peeled. This reaction can be thought of as:



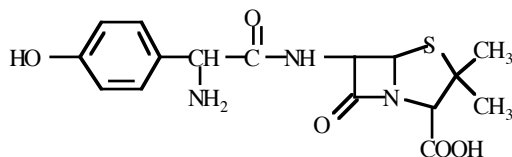
Provide a plausible explanation for each of the following phenomena.

a) Putting cut potatoes under water slows the rate of browning. Previously submerged potatoes brown at the usual rate once they have been removed from the water.

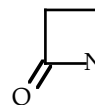
b) Cooked (heated above 100°C for several minutes) potatoes do not brown at all even when they cool down (like in potato salad).

c) Sprinkling lemon juice over potatoes prevents browning, but sprinkling plain water does not. Hint: lemon juice has a very low pH.

**(3.1.5)** Transpeptidation is an essential step in the synthesis of the cell walls of certain bacteria. It is catalyzed by the enzyme transpeptidase. The active site of transpeptidase can also bind the  $\beta$ -lactam ring of penicillin (shown below). When this happens, the  $\beta$ -lactam ring opens and covalently binds to transpeptidase, permanently inactivating the enzyme.



Penicillin G



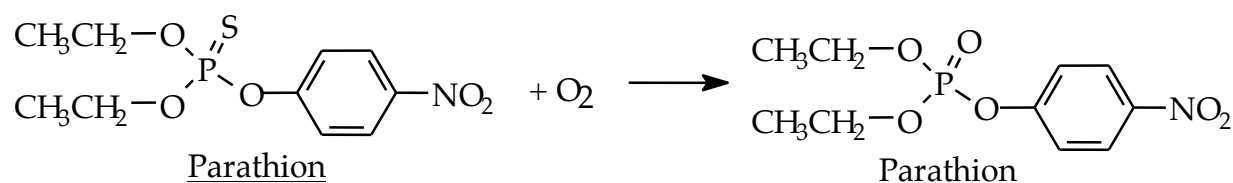
$\beta$ -lactam ring

a) Based on this information and the structure of bacteria (consult your textbook), (i) explain the role of transpeptidase in bacterial cells, and (ii) how penicillin results in bacterial killing.

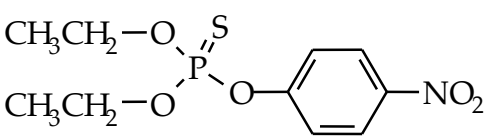
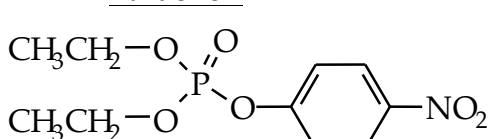
b) Based on this information and the structure of human cells (consult your textbook), why doesn't penicillin kill human cells?

c) Certain strains of bacteria contain an enzyme,  $\beta$ -lactamase, that catalytically opens the  $\beta$ -lactam ring of penicillin, rendering the penicillin nontoxic to bacteria.  $\beta$ -Lactamase therefore renders these cells resistant to penicillin. Transpeptidase is located outside the cell membrane, in the cell wall. Where do you expect  $\beta$ -lactamase to be located? Explain briefly, based on the function of  $\beta$ -lactamase in the cell.

(3.1.6) Insects have an enzyme that catalyzes the following reaction:



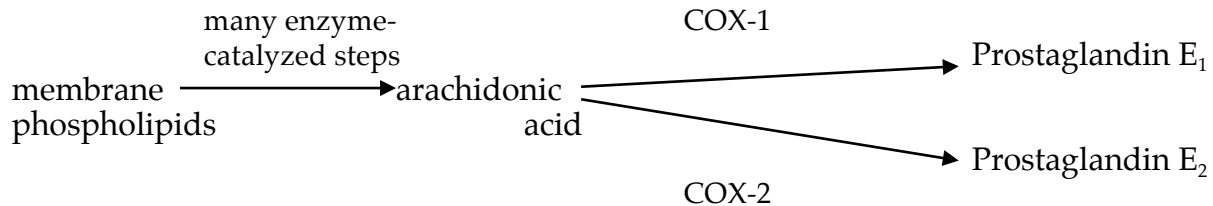
Humans lack this enzyme. The toxicity of parathion in insects and humans is given below.

<b>Compound:</b>	<u>Parathion</u>	<u>Paraoxon</u>
<b>Structure:</b>		
<b>Human Toxicity:</b>	low	high
<b>Insect Toxicity:</b>	high	high

Explain how exposure to parathion can be more toxic to insects than humans.

**(3.1.7)** A class of drugs called nonsteroidal anti-inflammatory drugs (NSAIDs for short) are used to reduce pain and inflammation. Aspirin was the first drug of this group; it was developed more than 100 years ago. Aspirin is a very effective pain killer, but long-term use can lead to stomach ulcers. A stomach ulcer is where the lining of cells in the stomach is breached.

Aspirin acts by inhibiting two enzymes, cyclo-oxygenase 1 and cyclo-oxygenase 2 (COX-1 and COX-2). COX-1 and COX-2 have very similar structures. These enzymes are involved in the following pathway (you may want to look up prostaglandins in your textbook):



Membrane phospholipids are converted to arachidonic acid by a series of enzyme-catalyzed reactions. Arachidonic acid is converted to Prostaglandin E<sub>1</sub> by COX-1; it is converted to Prostaglandin E<sub>2</sub> by COX-2. In the absence of any drugs, arachidonic acid is converted to both prostaglandins. The prostaglandins are hormones that circulate in the blood. They have very different effects in the body:

Prostaglandin E<sub>1</sub> is required to maintain the layer of cells that line the stomach. Without Prostaglandin E<sub>1</sub>, these cells are not replaced as they die.

Prostaglandin E<sub>2</sub> binds to receptors on the surface of pain-sensitive cells and increases their sensitivity to pain. That is, in the presence of Prostaglandin E<sub>2</sub>, pain-sensitive cells send stronger and more frequent pain messages to the brain.

a) Aspirin inhibits both COX-1 and COX-2 by covalently binding to the -OH of a serine at the active site of both enzymes.

i) Based on this, why does aspirin act as an analgesic (pain reliever)?

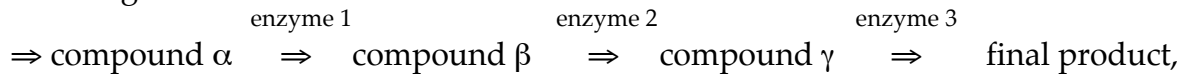
ii) Based on this, why does extended use of aspirin lead to stomach ulcers?

b) Newer NSAIDs, like Celebrex and its relatives, are designed to inhibit COX-2 only. Why would Celebrex and other COX-2 inhibitors be better pain relievers than aspirin?

### (3.2) Biochemical pathways, general

#### Diagnostic Problem:

In an organism where:



you could assume that an organism lacking only enzyme 1 would not form  $\beta$  and could not therefore make the final product. However, if this organism had an exogenous supply of  $\beta$ , enzyme 2 could convert  $\beta$  into  $\gamma$  and enzyme 3 could convert  $\gamma$  into the final product.

- a) What compound or compounds could be made if an organism lacked both enzyme 1 and enzyme 2?
- b) If an organism that lacked both enzyme 1 and enzyme 2 had an exogenous supply of  $\beta$ , would it be able to make the final product?
- c) Given the pathway above, an organism lacking enzyme 2 might accumulate which compound?
- d) Given the pathway above, an organism lacking both enzyme 1 and enzyme 2 might accumulate which compound?

#### Solutions to Diagnostic Problem:

- a) What compound or compounds could be made if an organism lacked both enzyme 1 and enzyme 2?

*Only compound  $\alpha$  could be made.*

- b) If an organism that lacked both enzyme 1 and enzyme 2 had an exogenous supply of  $\beta$ , would it be able to make the final product?

*Even if this organism had an exogenous supply of  $\beta$  it could not make  $\gamma$ , so it could not make the final product. Indeed, the organism lacking both enzyme 1 and enzyme 2 would need an exogenous supply of  $\gamma$  to complete the pathway and make the final product.*

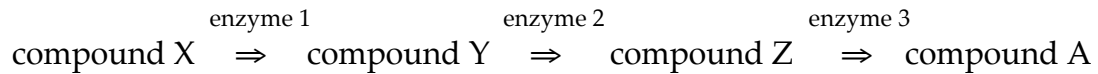
- c) Given the pathway above, an organism lacking enzyme 2 might accumulate which compound?

*An organism lacking enzyme 2 would accumulate  $\beta$ .*

- d) Given the pathway above, an organism lacking both enzyme 1 and enzyme 2 might accumulate which compound?

*An organism lacking both enzyme 1 and enzyme 2 would accumulate  $\alpha$ .*

(3.2.1) The following represents a pathway for the synthesis of the essential compound A in a bacterial cell.



Bacterial cells with defective enzyme 1, 2, or 3 will grow only if compound A is available (added to the growth media).

a) What compound will build up in the cells defective in the following enzymes?

enzyme 1:

enzyme 2:

enzyme 3:

b) What compound(s) must be available to cells defective in the following enzymes?

enzyme 1:

enzyme 2:

enzyme 3:

c) What compound will build up in the cells defective in the following enzymes?

enzyme 1 and enzyme 2:    enzyme 2 and enzyme 3:    enzyme 1 and enzyme 3:

d) What compound(s) must be available to cells defective in the following enzymes?

enzyme 1 and enzyme 2:    enzyme 2 and enzyme 3:    enzyme 1 and enzyme 3:

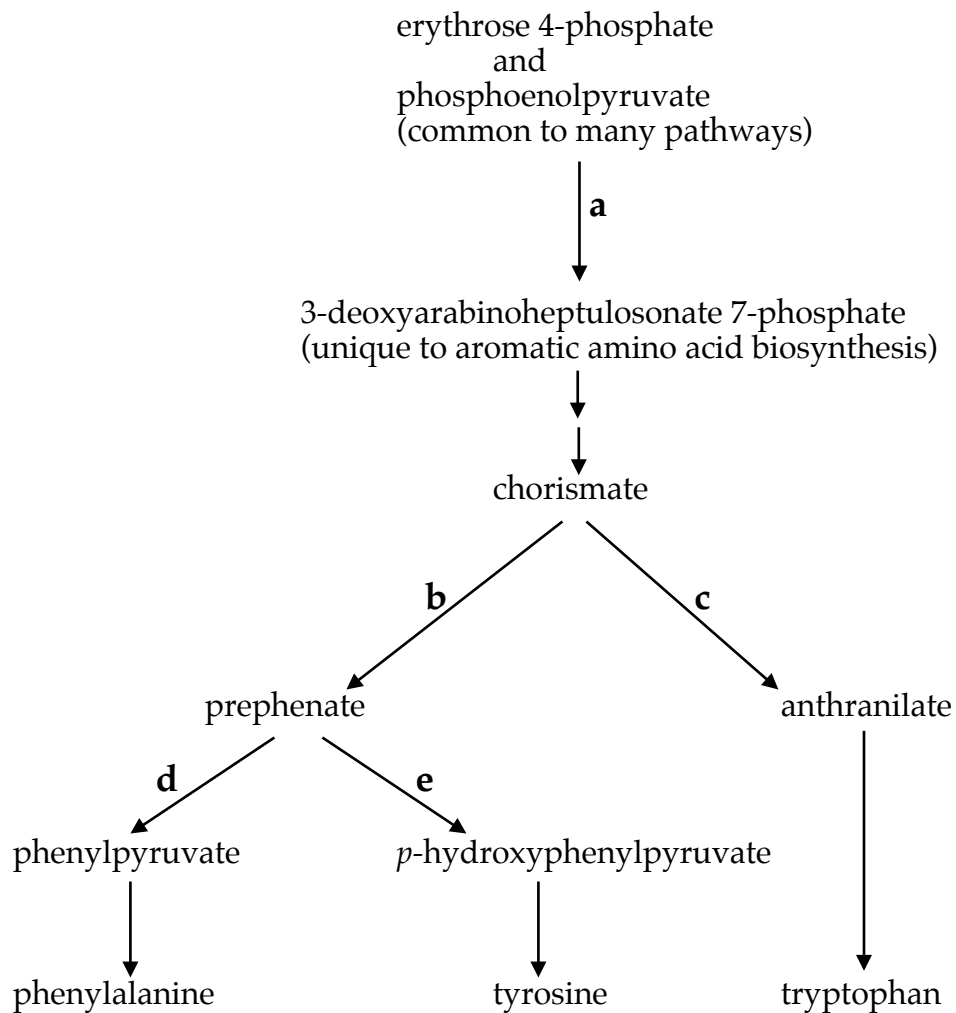
**(3.2.2)** You are studying the biosynthesis of the amino acid arginine. You have cells that are missing different enzymes needed in this pathway. You can add three potential compounds (A, B, C) as a supplement to the medium. You test your cells to determine whether they grow (+) or not (-).

Type of cell	Medium				
	Nothing added	Compound A added	Compound B added	Compound C added	Arginine added
Normal	+	+	+	+	+
Missing enzyme 1	-	-	+	-	+
Missing enzyme 2	-	-	-	-	+
Missing enzyme 3	-	+	+	-	+
Missing enzyme 4	-	+	+	+	+

What is the order of enzymes and compounds in the pathway?



**(3.2.3)** In certain bacteria, the biosynthesis of aromatic amino acids (phenylalanine, Phe; tyrosine, Tyr; and tryptophan, Trp) proceeds by a branched pathway where some intermediates are used to make more than one amino acid. The pathway and some key intermediates are shown below.

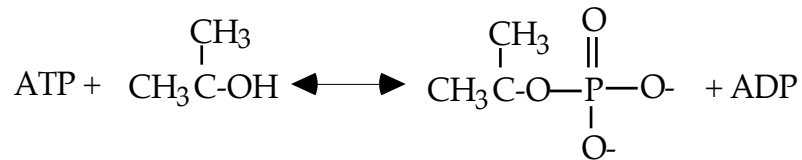


This pathway is regulated by feedback inhibition at enzymes **a** through **e**. For enzymes **a** through **e**, list the intermediate or product shown that you expect to be the most likely inhibitor of each enzyme (for a description of the different types of inhibition, see your textbook). Provide a brief explanation for each answer.

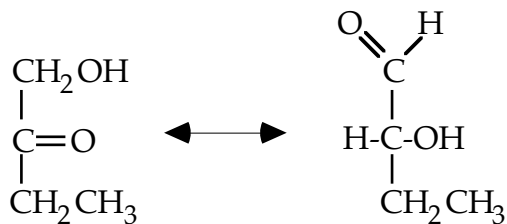
### (3.3) Glycolysis, respiration, and photosynthesis

(3.3.1) There are only a few different classes of enzymes that catalyze the reactions of the glycolytic pathway.

a) Name two enzymatic steps in glycolysis that are similar to the following reaction. Write the names of the reactant, the product, and the enzyme used in each of these two reactions.

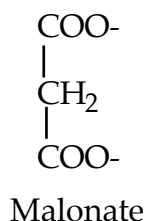


b) Name two enzymatic steps in glycolysis that are similar to the following reaction. Write the names of the reactant, the product, and the enzyme used in each of these two reactions.



c) If you add each of the above substrates (shown above) to the corresponding glycolytic enzymes you indicated above, you will find that these substrates will not necessarily be converted into the given products. Why do you think this is the case?

d) One of the reactions of the citric acid cycle (Krebs cycle) is catalyzed by succinate dehydrogenase. This step is inhibited by adding malonate, shown below, to the solution.



Explain why malonate acts as a competitive inhibitor of the succinate dehydrogenase enzyme.

**(3.3.2)** In the 1860s, Louis Pasteur observed the following phenomenon, which has come to be called “the Pasteur Effect.” If you grow a culture of *Escherichia coli* bacteria (which can grow anaerobically or aerobically) without O<sub>2</sub>, they consume large amounts of glucose as they grow and they produce lactic acid from the glucose. If you now supply this culture with an excess of O<sub>2</sub>, two things happen rapidly.

- (1) Lactic acid is no longer produced.
- (2) The rate of glucose consumption decreases even though the rate of cell growth is constant.

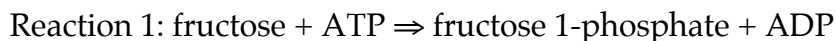
a) Explain (1) in terms of the biochemical fate of glucose and its derivatives; what happens to the glucose if it isn’t converted to lactic acid? (You need not list the entire pathway, just the key intermediates and end products.)

b) How is it that less glucose is required for the same growth rate once O<sub>2</sub> is added?

c) Small amounts of NAD<sup>+</sup> are sufficient to metabolize large amounts of glucose. Explain why this statement is true under both anaerobic and aerobic conditions.

**(3.3.3)** The first step in the pathway of glycolysis (see your textbook) is the transfer of a phosphate group from ATP to glucose, giving glucose 6-phosphate (reactions of this type are phosphorylations). The next step in glycolysis is the isomerization of glucose 6-phosphate to fructose 6-phosphate.

Cells can also use fructose as an energy source. Through a pathway similar to early steps in glycolysis, fructose is converted to an intermediate in the glycolytic pathway (i.e., it enters glycolysis at a middle step). In the first step of fructolysis, fructose is phosphorylated to convert it to fructose 1-phosphate. The phosphorylation of fructose is catalyzed by the enzyme fructokinase. This reaction is shown below:



a) Fructose 1-phosphate is then metabolized to glyceraldehyde and dihydroxyacetone phosphate (DHAP) by the enzyme fructose-1-phosphate aldolase. DHAP enters glycolysis directly. However, the glyceraldehyde must first be phosphorylated to glyceraldehyde 3-phosphate by triose kinase. Which molecule would most likely be used to provide the phosphate group and the energy required to phosphorylate glyceraldehyde? Write the chemical reaction for this phosphorylation. (You need not draw structures, just use chemical names.)

b) Based on the known glycolytic pathway (see your textbook), reaction 1, and the reaction you postulated in part (a), draw the pathway from fructose to two molecules of pyruvate. (You need not draw chemical structures, just use chemical names.)

c) Based on the pathway you drew for part (b), how much ATP is consumed in the conversion of fructose into two pyruvate molecules? How much ATP is generated? How does the net result of converting fructose to two molecules of pyruvate compare with the conversion of glucose to two molecules of pyruvate?

(3.3.4) During fermentation, glucose is broken down into pyruvate via glycolysis (see your textbook); the pyruvate is then converted to CO<sub>2</sub> and ethanol (see your textbook). This is called the Embden-Meyerhoff-Parnas pathway for the researchers who worked it out. The stoichiometry of the overall reaction is:



There is an alternative fermentation pathway for the anaerobic breakdown of glucose: the Entner-Doudoroff pathway. What follows is a list, in random order, of the enzymes of the Entner-Doudoroff pathway and the reactions they catalyze (note that the longer chemical names have abbreviations shown in parentheses):

Notes:

- For the overall reaction: the reactants are glucose, ADP, and P<sub>i</sub>; the products are CO<sub>2</sub>, ethanol, ATP, and H<sub>2</sub>O.
- No NAD<sup>+</sup> or NADH are produced or consumed by the overall reaction. That is, any NAD<sup>+</sup> or NADH produced by one reaction must be recycled by another reaction.

<u>Enzyme</u>	<u>Reaction Catalyzed</u>
A	6-phosphogluconate (6PG) $\longrightarrow$ 2-keto-3-deoxy-6-phosphogluconate (2,3,6PG) + H <sub>2</sub> O
B	glucose 6-phosphate (G6P) + H <sub>2</sub> O + NAD <sup>+</sup> $\longrightarrow$ 6-phosphogluconate (6PG) + NADH + H <sup>+</sup>
C	1,3-bisphosphoglycerate (BPG) + ADP $\longrightarrow$ 3-phosphoglycerate (3PG) + ATP
D	3-phosphoglycerate (3PG) $\longrightarrow$ 2-phosphoglycerate (2PG)
E	glyceraldehyde 3-phosphate (G3P) + P <sub>i</sub> + NAD <sup>+</sup> $\longrightarrow$ 1,3-bisphosphoglycerate (BPG) + NADH + H <sup>+</sup>
F	2-phosphoglycerate (2PG) $\longrightarrow$ phosphoenolpyruvate (PEP) + H <sub>2</sub> O
G	glucose + ATP $\longrightarrow$ glucose 6-phosphate (G6P) + ADP
H	phosphoenolpyruvate (PEP) + ADP $\longrightarrow$ pyruvate + ATP
I	acetaldehyde + NADH + H <sup>+</sup> $\longrightarrow$ ethanol + NAD <sup>+</sup>
J	pyruvate $\longrightarrow$ acetaldehyde + CO <sub>2</sub>
K	2-keto-3-deoxy-6-phosphogluconate (2,3,6PG) $\longrightarrow$ pyruvate + glyceraldehyde 3-phosphate (G3P)

a) Draw the Entner-Doudoroff pathway, indicating the order of reactions, the enzyme that catalyzes each step, and the intermediates. Your diagram should look like the figure in your textbook except that you can use names or abbreviations for the intermediates; be sure to label each reaction with the letter that corresponds to the enzyme that catalyzes it.

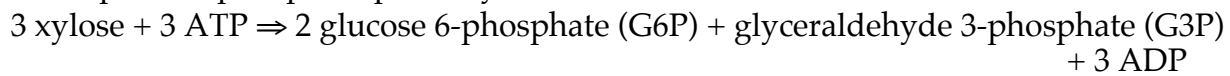
b) What is the stoichiometry of the overall reaction of the Entner-Doudoroff pathway? How many ATPs does a bacterium using this pathway get per glucose consumed; how does this compare with the ATP yield from the Embden-Meyerhoff-Parnas pathway?

c) The two pathways have several identical enzymatic steps. For each step in the Entner-Doudoroff pathway (A through K), either:

(1) Give the identical step in the Embden-Meyerhoff-Parnas pathway; give the figure from your textbook where the corresponding step appears and the step number in that figure.

or: (2) State that there is no equivalent step.

d) The two pathways described above consume hexoses: 6-carbon sugars like glucose. Bacteria can also grow on pentoses (5-carbon sugars) like xylose. First, the xylose must be processed into a usable form by the pentose phosphate pathway. The overall reaction of the pentose phosphate pathway is:



Note that carbon atoms are conserved in these reactions:  $3 \text{ C}_5 \Rightarrow 2 \text{ C}_6 + \text{C}_3$ .

What would be the stoichiometry of the overall reaction if you combined the pentose phosphate pathway with the Entner-Doudoroff pathway to convert xylose, ADP, and  $\text{P}_i$  to ethanol,  $\text{H}_2\text{O}$ , ATP, and  $\text{CO}_2$ ?

e) How many ATPs does a bacterium using these combined pathways get per xylose consumed?

**(3.3.5)** When exposed to light, plant cells show net absorption of  $\text{CO}_2$  and net production of  $\text{O}_2$ . In the dark, they show net production of  $\text{CO}_2$  and net absorption of  $\text{O}_2$ .

a) What biochemical process is responsible for the plant's absorption of  $\text{O}_2$  and production of  $\text{CO}_2$  in the dark?

b) Does this process continue when the plant is exposed to light? If so, why aren't net production of  $\text{CO}_2$  and absorption of  $\text{O}_2$  seen under these conditions? Explain briefly.

**(3.3.6)** Refer to the figure in your textbook that shows photosynthetic electron transport. The Hill reagent is an organic molecule that can bind to NADP-reductase and compete with  $\text{NADP}^+$  as an electron acceptor. However, once it picks up electrons, the Hill reagent cannot transfer them to any biomolecules. When large amounts of the Hill reagent are added to plant cells, all the electrons from noncyclic photophosphorylation are transferred to the Hill reagent instead of to  $\text{NADP}^+$ . Under these conditions and in the presence of  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and light,  $\text{O}_2$  production continues for a short time, but eventually stops.

a) How can  $\text{O}_2$  continue to be produced by these cells for a short time? Explain your answer briefly.

b) Why does  $\text{O}_2$  production stop eventually? Explain your answer briefly.

c) The herbicide DCMU {[3-(3,4-dichlorophenyl)-1,1-dimethylurea]} binds to plastoquinone (also called pQ) and inactivates it so that electrons can no longer be transferred through pQ.

i) Will  $\text{O}_2$  be produced by these cells? Explain your answer.

ii) Will  $\text{ADP} + \text{P}_i$  still be converted to ATP by the chloroplasts of these cells? Explain your answer briefly.

iii) Will  $\text{CO}_2$  still be reduced to glucose? Explain your answer briefly.



**(3.3.7)** It is now possible to determine the DNA sequence of the entire genome of an organism. This allows you to make a list of all the proteins that this organism can produce. From this, it is sometimes possible to deduce an organism's metabolic behavior.

This has been done for a number of bacteria. This problem deals with four:

- *Haemophilus influenzae*, a human pathogen that causes meningitis, among other nasty diseases.
- *Mycoplasma genitalium*, a nonpathogenic bacterium.
- *Mycoplasma pneumoniae*, which causes some cases of pneumonia.
- *Escherichia coli*, a usually harmless bacterium.

This problem also deals with six enzymes that we will use as markers for the pathways they participate in. They are:

<u>Enzyme</u>	<u>Reaction Catalyzed</u>
lactate dehydrogenase (lactate DHase)	pyruvate + NADH $\Rightarrow$ lactate + NAD <sup>+</sup>
alcohol dehydrogenase (Alcohol DHase)	acetaldehyde + NADH $\Rightarrow$ ethanol + NAD <sup>+</sup>
aldolase	fructose 1,6-bisphosphate $\Rightarrow$ dihydroxyacetone phosphate + glyceraldehyde phosphate
proton ATPase	lets H <sup>+</sup> move through membrane to produce ATP from ADP + P <sub>i</sub>
cytochrome <i>c</i> oxidase	transfers electrons from cytochrome <i>c</i> <sub>1</sub> to cytochrome <i>a</i>
citrate synthase	oxaloacetate + acetyl-CoA $\rightarrow$ citrate

a) For each of the enzymes listed above, determine the part of energy metabolism (glycolysis, fermentation, electron transport, etc.) that the enzyme belongs to. You will have to look through the sections of your textbook that deal with glycolysis, fermentation, electron transport, and oxidative phosphorylation. Some of the names may be slightly different in different texts.

In general, if one enzyme in a part of energy metabolism is present, then all the related enzymes are also present. In this way, the presence of each of the enzymes above can be used as a marker for the rest of that part of energy metabolism. Using this, you can figure out the kinds of energy metabolism that an organism can carry out.

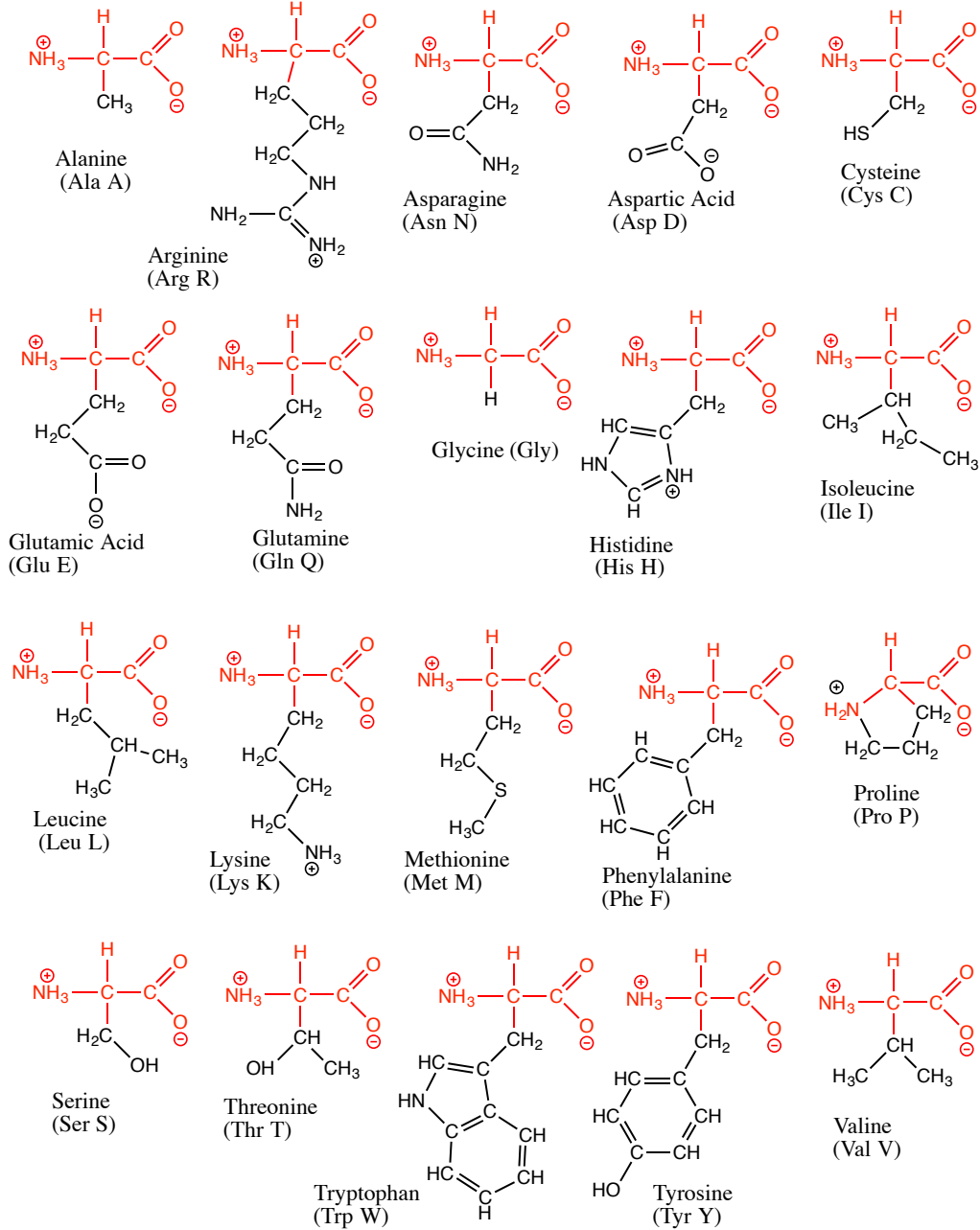
The table below shows the presence (+) or absence (–) of each of the enzymes above in the genomes of the bacteria in this problem:

Organism	Lactate DHase	Alcohol DHase	Aldolase	Proton ATPase	Cytochrome <i>c</i> oxidase	Citrate synthase
<i>Haemophilus influenzae</i>	–	+	+	+	–	–
<i>Mycoplasma genitalium</i>	+	–	+	+	–	–
<i>Mycoplasma pneumoniae</i>	+	–	+	+	+	–
<i>Escherichia coli</i>	–	+	+	+	+	+

b) For each of the four bacteria, answer the following questions based on the data above:

- If this organism is grown on glucose in the **presence** of oxygen, will  $\text{CO}_2 + \text{H}_2\text{O}$ , alcohol +  $\text{CO}_2$ , or lactic acid be produced? Roughly how many ATPs will the cell get from each molecule of glucose under these conditions?
- If this organism is grown on glucose in the **absence** of oxygen, will  $\text{CO}_2$  only, alcohol +  $\text{CO}_2$ , or lactic acid be produced? Roughly how many ATPs will the cell get from each molecule of glucose under these condition

## Structures of Amino Acids



Pathways: General

