Maggie L. Walker
GOVERNOR'S SCHOOL
for Government and International Studies

# Summer Assignment 2020-21

## Course: AP Biology

| | |
|---|---|
| **Assignment title** | Video Notes & Biochemistry Worksheets |
| **Date due** | Part 1 – Video Notes – due 1st day/block of class<br>Part 2 – Biochemistry Worksheets – due 2nd day/block of class |
| **Estimated time for completion** | 6 – 7 hours |
| **Resources needed to complete assignment** | ☒ Textbook - *OpenStax Biology for AP Courses*<br>☒ Notes in packet<br>☒ Other supplies: device(s) with internet capabilities. |
| **How the assignment will be assessed** | The **Video Notes** and the **Biochemistry Worksheets** will be scored using the accompanying rubric and guidelines. Both assignments will be averaged together and will be counted as a **project grade** for the 1st quarter. |
| **Purpose of assignment** | ☒ Review of foundational material/concepts/skills.<br>☒ Expose students to required material/concepts/skills/texts that will not be covered during the academic year.<br>☒ Have students read material that will be discussed or used in class at the beginning of the year. |

# AP Biology Summer Assignment

Welcome to AP Biology! This course is designed to be the equivalent of a two-semester introductory biology course usually taken in the first year of college. In other words, it is a little like drinking from a fire hose. It will be a rewarding experience, but as with most things that are, it will also be challenging. Throughout the course, you will become familiar with major recurring ideas that persist throughout all topics and material.

## The 4 Big Ideas of AP Biology

**Big Idea 1:** The process of evolution drives the diversity and unity of life.
**Big Idea 2:** Biological systems utilize free energy and molecular building blocks to grow, to reproduce and to maintain dynamic homeostasis.
**Big Idea 3:** Living systems store, retrieve, transmit and respond to information essential to life processes.
**Big Idea 4:** Biological systems interact, and these systems and their interactions possess complex properties.

On the pages that follow, you will find instructions for the two assignments that comprise your summer work for AP Biology. Both assignments will review biological chemistry concepts that you learned in freshman biology as well as foundational chemistry concepts you learned your sophomore year in chemistry. The first part of the assignment involves watching several assigned videos and taking video notes. The second part of your summer assignment consists of completing several sets of questions focusing on biological chemistry.

**Your video notes are due on the 1st day of AP Biology and your biochemistry worksheets will be due on the 2nd day. Both will be averaged together and counted as a project grade for 1st quarter. No late summer assignments will be accepted!**

Included in this packet are the following documents:

# Assignment #1 – Video Notes – due 1st day of AP Biology

Watch the videos listed below and take ***hand-written*** notes on each of them. The notes should be your ***original work.*** EACH note sheet will be scored 0 to 5 based on completeness and thoroughness as shown in the rubric below. Note pages **will not** be accepted late nor will they be accepted typed.

| # | Video Content | Links |
|---|---|---|
| 005 | Essential Characteristics of Life | https://bit.ly/2HUpsES |
| 010 | Abiogenesis | https://bit.ly/2U6a7Yg |
| | Molecules of Life | https://bit.ly/2IwqLXK |
| | Carbohydrates | https://bit.ly/2L7RADv |
| | Lipids | https://bit.ly/2IqVDJh |
| | Proteins | https://bit.ly/2IJHWIS |
| | Water – A Polar Molecule | https://bit.ly/2TUsfnQ |

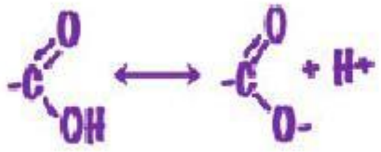
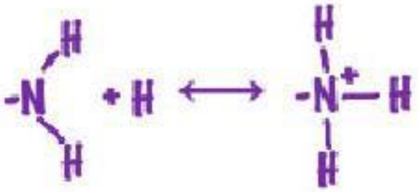
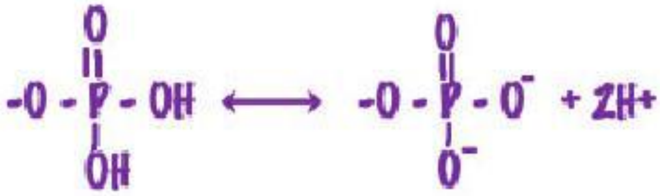
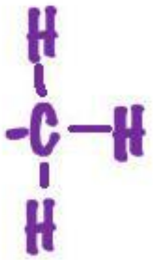
| 0<br>No Credit | 2<br>Below Expectations | 3 – 4<br>Complete | 5<br>Meets Expectations |
|---|---|---|---|
| No notes ***OR*** copied from a peer | Several criteria are missing from entry | All criteria are met, but there is room for improvement within criteria ***OR*** one criterion is missing from entry | All criteria listed below are met ***OR*** have been exceeded for each entry. |

**What does work that "meets expectations" have?**

- ✓ Each video's notes are on a different page.
- ✓ The video's title is written as it appears in the video on the top line of the paper.
- ✓ The notes are legibly written.
- ✓ Highlighting or colors are used to emphasize key points, new vocabulary, and/or important concepts.
- ✓ Examples are documented in some way when given in the video.
- ✓ Pictures, charts, or graphs are used to display details provided in the video.
- ✓ A summary of the video content is provided at the end of the notes. Please emphasize the summary in some way (title it, star it, highlight it, etc.)
- ✓ Each entry is pledged.

Notes are to be ***original work*** and are not to be copied from a peer – these serve as a log of what you have learned from the video. Copying them from a peer and not watching the video does you no good. You will receive zero credit if you are found submitting work that is too closely aligned with a classmate's work.

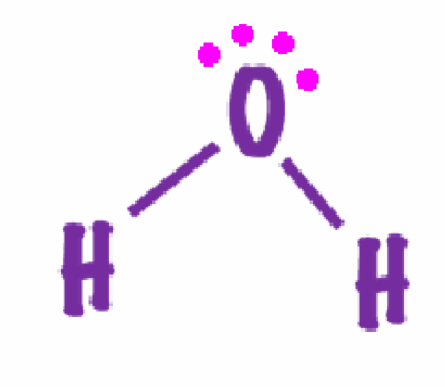
# ORGANIC CHEMISTRY BASICS NOTES

| **FUNCTIONAL GROUP** | **DRAWING/ FORMULA** | **PROPERTIES** |
|---|---|---|
| **Carboxyl** | $-COOH$<br> | • Polar<br>• Water soluble<br>• Acid |
| **Amino** | $-NH_2$<br> | • Polar<br>• Water soluble<br>• Weak base |
| **Sulfhydral** | $-SH$ | • Form disulfide bridges<br>• Stabilize protein shape |
| **Phosphate** |  | • Polar<br>• Water soluble<br>• Acid<br>• Important in energy transfer |
| **Methyl** | $-CH_3$<br> | • Nonpolar<br>• Not water soluble |

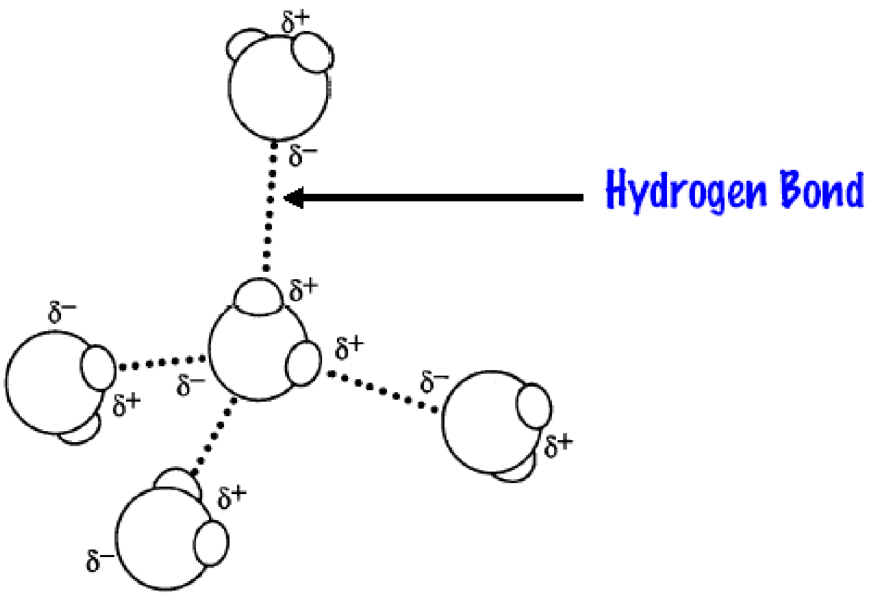
# WATER, ACIDS, BASES, BUFFERS

## STRUCTURE & GEOMETER OF WATER:

Water is polar



Maximum number of H bonds = 4
Each water molecule can form a max. of 4 hydrogen bonds with 4 other water molecules



## PROPERTIES OF WATER:

Liquid water is cohesive

Cohesion = H bonds between water molecules; $H_2O$ molecules tend to stick tog.
Importance = Transport $H_2O$ against gravity in plants
Higher surface tension

Water has a high specific heat

Takes a lot of energy to raise 1 gram of $H_2O$ 1 °C
Why? Must break H bonds
Liquid $H_2O$ can absorb large amounts of heat with small changes in temperature

Water has a high heat of vaporization

Takes a lot of energy to convert liquid $H_2O$ into vapor
Why? Must break H bonds
Keeps water in liquid state

Water expands with it freezes

Solid $H_2O$ is less dense than liquid $H_2O$
Why? In solid state $H_2O$ locked into max. number of H bonds; takes up more space

Water is a versatile solvent

Will dissolve polar covalent and ionic compounds

## DISSOCIATION OF WATER:

$$H_2O + H_2O \leftrightarrow H_3O+ + OH-$$

$$H_2O \leftrightarrow H+ + OH-$$

Hydronium ion Hydroxide ion

1 out of 554,000,000 water molecules dissociates
At equilibrium in pure water at 25°C
[H+] = [OH-] = $1.0 \times 10^{-7}$ M

If add [H+] to pure water
- Removes OH-
- Equilibrium shifts left
- [H+] > [OH-]

If add [OH-] to pure water
- Removes H+
- Equilibrium shifts left
- [OH-]>[H+]
- reduces H+ indirectly

If add $NH_3$
- $NH_3 + H+ \rightarrow NH_4+$
- Reduces H+ directly

## PH SCALE:

pH = $-\log_{10}$[H+]
if [H+] = $10^{-7}$
then pH = 7

[H+] x [OH-] = $10^{-14}$
If [H+] = $10^{-9}$
Then [OH-] = $10^{-5}$
pOH = 5
pH = 9

**BUFFERS:**

| Description | Function | Importance |
|---|---|---|
| Weak acids or bases | Minimize changes in pH | Controls chemical reactions<br>Maintains homeostasis |

**BICARBONATE BUFFER SYSTEM:**

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

$HCO_3$- = Bicarbonate (weak base)
$H_2CO_3$ = Carbonic acid (weak acid)

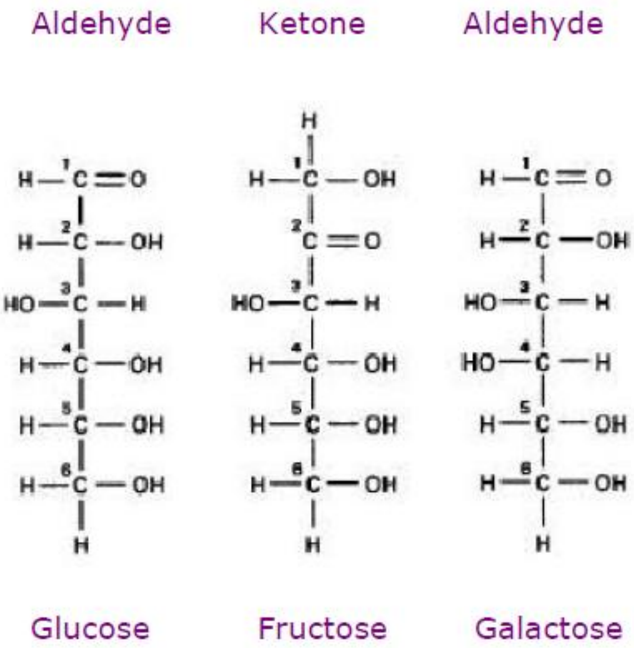
Major buffer system in blood
Maintains blood pH between 7.38 and 7.42

| Action: | Effect: |
|---|---|
| Increase $[H^+]$<br>How?<br>Fat metabolism<br>OD on acidic drug | Increase [H+]<br>Equilibrium shifts left<br>H+ + $HCO_3$- → $H_2CO_3$ → $CO_2$ + $H_2O$<br>Increase $[CO_2]$<br>Increase rate and depth of respiration |
| Increase Rate & Depth of Respiration<br>Hyperventilate | Decrease $[CO_2]$<br>Equilibrium shifts left<br>H+ + $HCO_3$- → $H_2CO_3$ → $CO_2$ + $H_2O$<br>Blood pH increases |

# CARBOHYDRATES NOTES

## GENERAL CHARACTERISTICS:

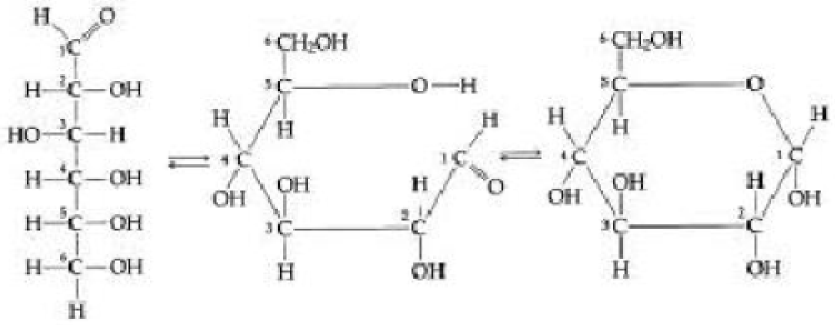
- Polymers of simple sugars
- Classified according to number of simple sugars
- Sugars
  - 3 to 7 carbons
  - -OH attached to each carbon except one
- Aldehydes or ketones



$C_6H_{12}O_6$

## MONOSACCHARIDES:

- Simple sugars
- Monomers of di- and polysaccharides
- Store energy in chemical bonds



Glucose
Linear form (dry)

Glucose
ring form (in sol.)

Trioses
- 3 carbon sugar
- glyceraldehyde

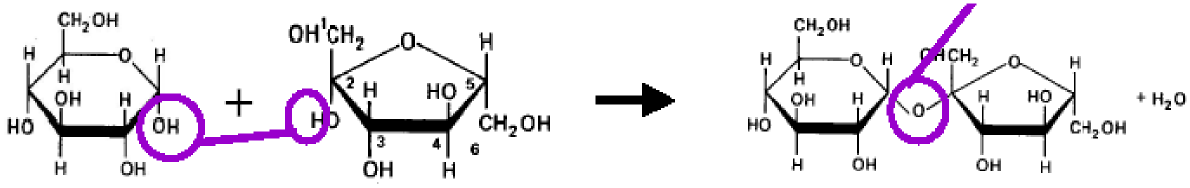
Pentose
- 5 carbon sugar
- Ribose
- Deoxyribose

Hexose
- 6 carbon sugar
- Glucose
- Galactose
- Fructose

## DISACCHARIDES:

Double sugars

Condensation Synthesis: removal of water molecule to form bond between monomers


Glycosidic linkage

Glucose + Fructose → Sucrose + water

Glucose + Glucose → Maltose + water

Glucose + Galactose → Lactose + water

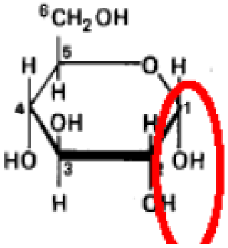
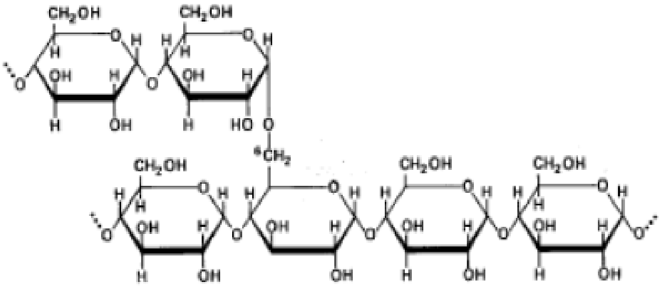
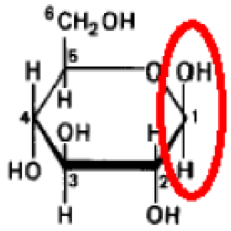
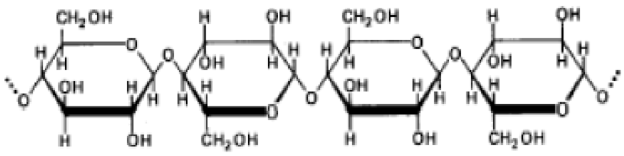
## POLYSACCHARIDES:

Many monosaccharides covalently bonded together

### FUNCTIONS:

| | |
|---|---|
| Storage<br>Starch: storage carbohydrate in plants<br>Glycogen: storage carbohydrate in animals | Structural<br>Cellulose: plant cell wall component<br>Chitin: polymer of amino sugar building block of exoskeletons |

### STARCH VS CELLULOSE

| | |
|---|---|
| Starch<br>Polymer of α - glucose<br>Branched α 1-4 linkages<br><br> | Cellulose<br>Polymer of β - glucose<br>Linear<br>Unbranched β 1-4 linkages<br>Most animals lack enzyme to break β 1-4 linkages<br><br> |

# LIPIDS NOTES

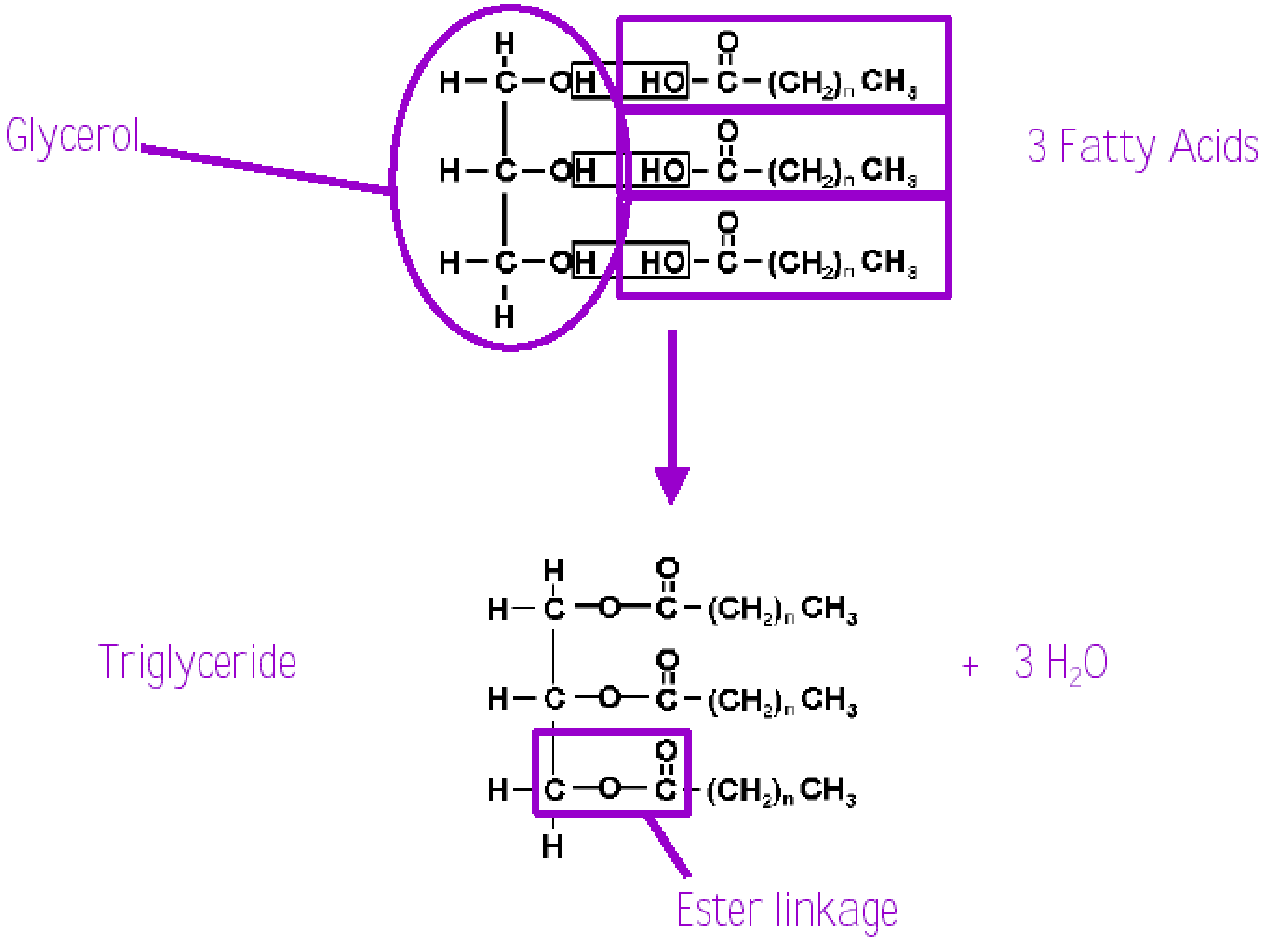
**General Characteristics:**

Not soluble in water

Mostly hydrocarbon chains

Fats, steroids, phospholipids

**Building Blocks:**



**Fats:**

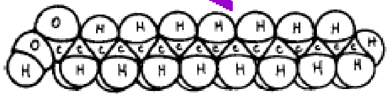
Glycerol + fatty acids
Triglycerides have 3 fatty acids
Fatty acids present may vary

Compact energy source
Cushions vital organs
Provides insulation

**Saturated:**

No double bonds between carbons
Straight chain

Fatty acid

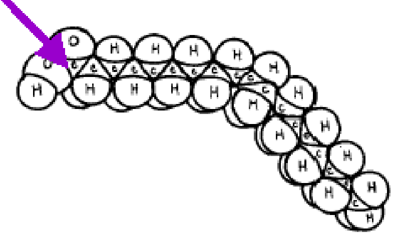


Usually solid at room temperature
Straight chains allow for tight packing
Most animal fats

**Unsaturated:**

At least 1 double bond between carbons
Hydrocarbon chain is bent

Fatty acid



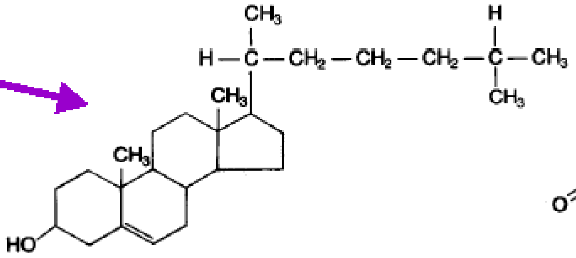
Usually liquid at room temperature
Bent chain prevents tight packing
Most plant fats

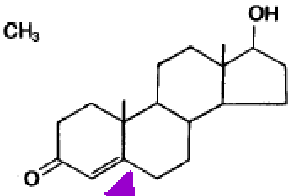
**STEROIDS:**

Consist of 4 fused carbon rings
Three are 6-sided
One is 5-sided
Attached functional groups vary

Cholesterol

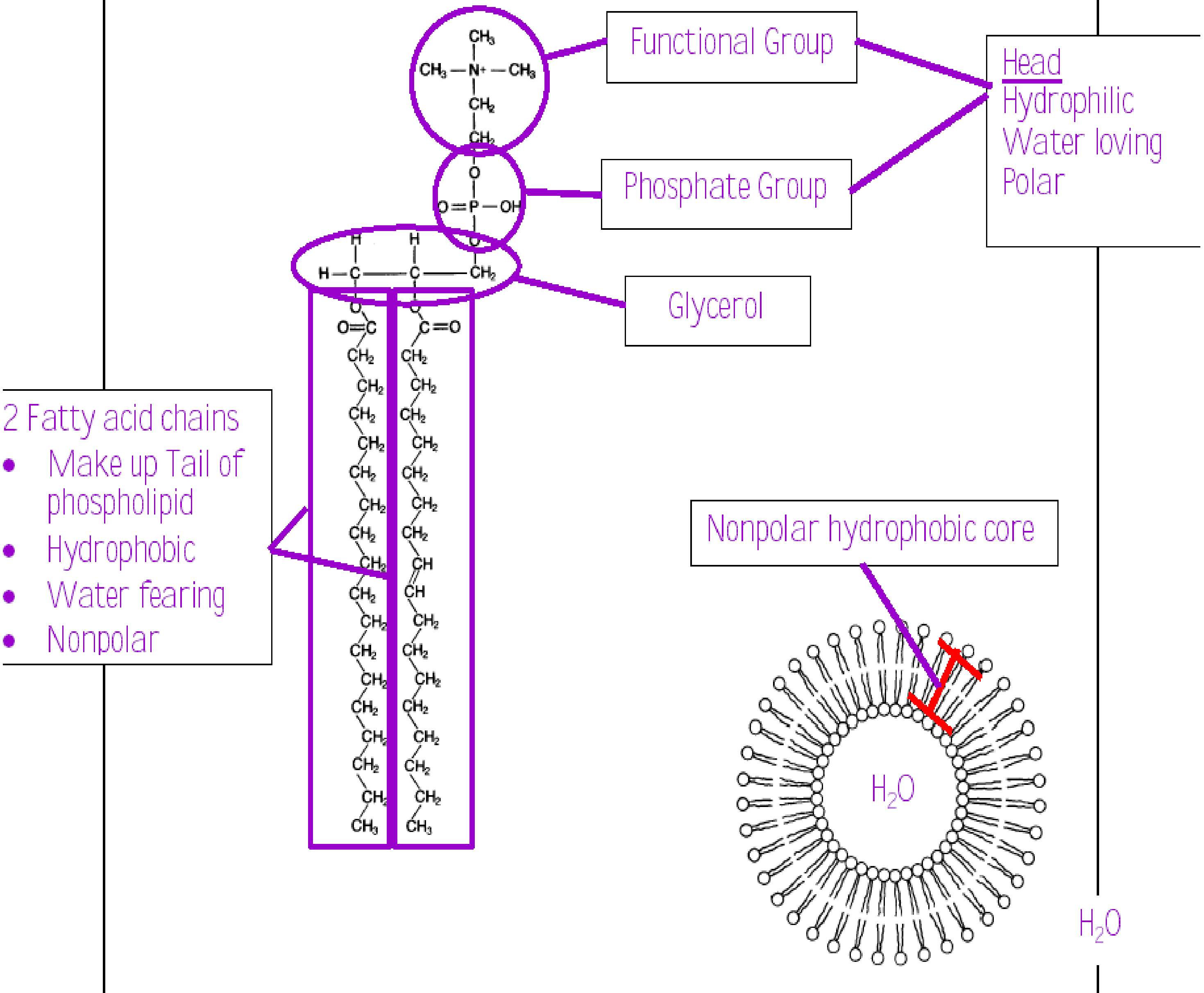
- Precursor of other steroids
- Component of animal cell membranes
- Contributes to artereosclerosis





Testosterone

**PHOSPHOLIPIDS:**



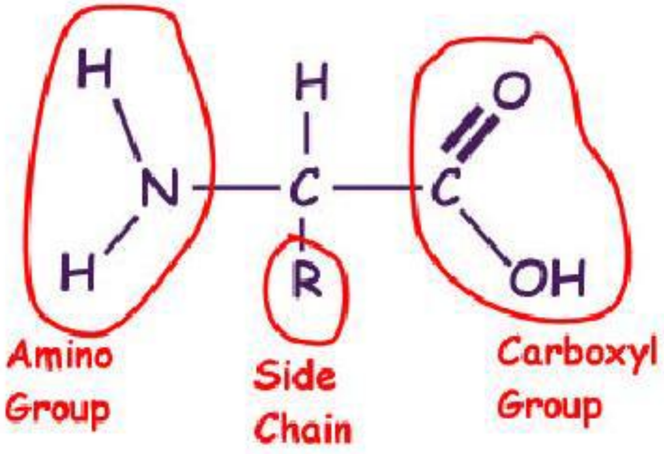
# PROTEINS NOTES

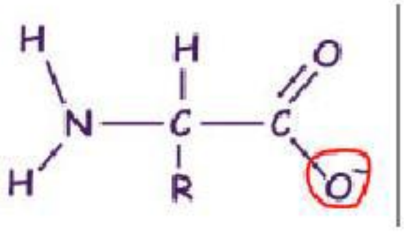
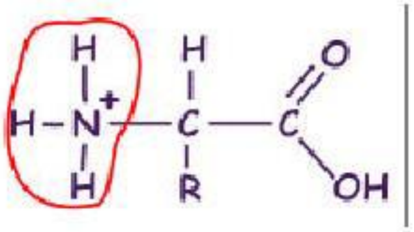
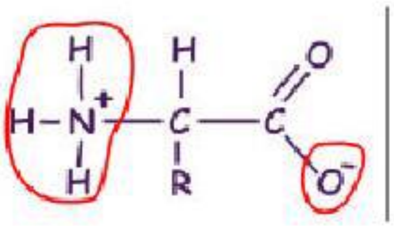
**GENERAL CHARACTERISTICS AND IMPORTANCES:**

- Polymers of amino acids
- Each has unique 3-D shape
- Vary in sequence of amino acids
- Major component of cell parts
- Provide support
- Storage of amino acids
- Receptor proteins; contractile proteins; antibodies; enzymes

**BUILDING BLOCKS:**

Amino acids
20 different
amino acids



| ANION | CATION | DIPOLAR ION |
|---|---|---|
|  |  |  |

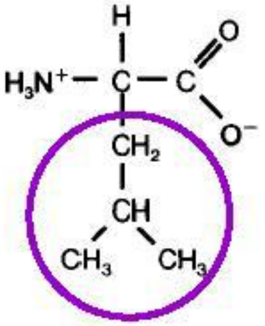
**CLASSIFICATION:**

Based on properties of side chain

**NONPOLAR:**

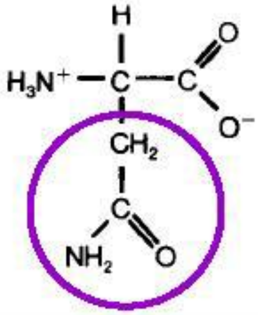
Hydrocarbon
Chains

No oxygen



**POLAR:**

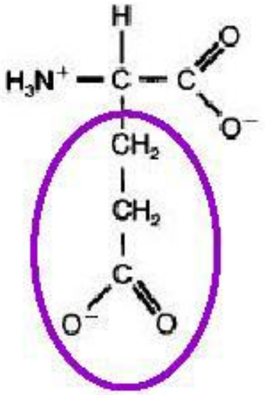
Oxygen present
Sometimes sulfur
No charge



**POLAR CHARGED ACIDIC:**

Negative
charge

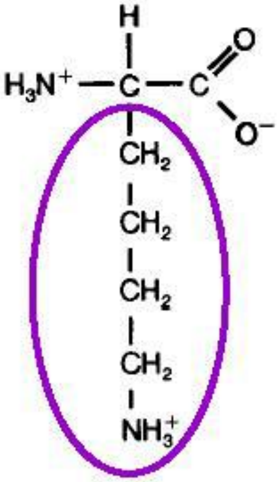
Donate H+
to solution



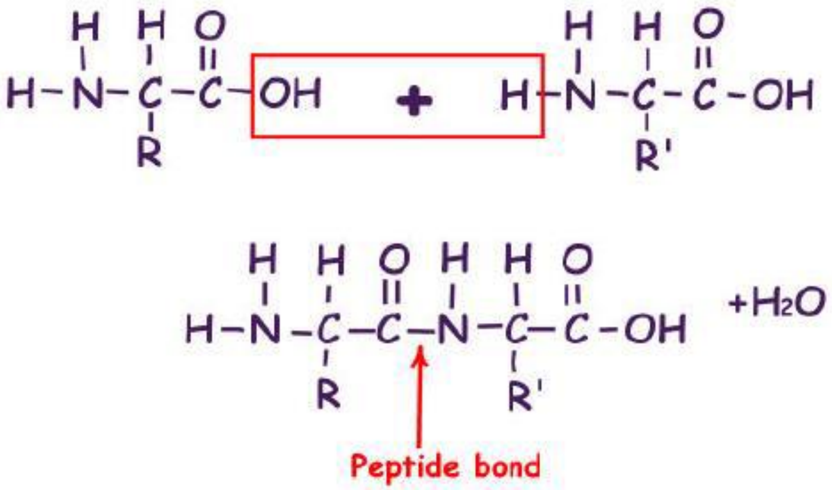
**POLAR CHARGED BASIC:**

Positive
charge

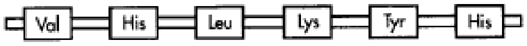
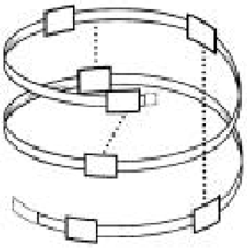
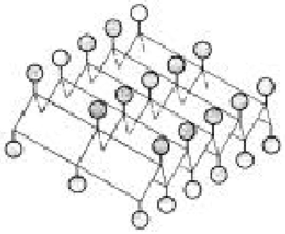
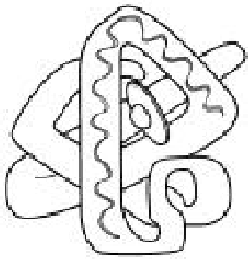
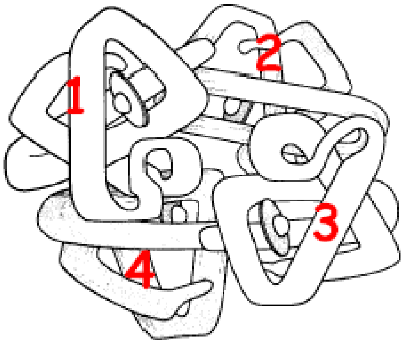
Gain H+ from
solution



**PEPTIDE BONDS:**



**PROTEIN CONFORMATION:**
Unique 3-D shape

| | |
|---|---|
| **PRIMARY:**<br> | - Sequence of amino acids<br>- Determined by genes (sequence of bases in DNA) |
| **SECONDARY:**<br>α helix<br><br>β pleated sheet<br> | - Regular repeated folding of peptide chain<br>- Folding stabilized by hydrogen bonds |
| **TERTIARY:**<br> | - Globular proteins<br>- Irregular contortion<br>- Shape stabilized by H bonds, ionic bonds, hydrophobic interactions, disulfide bridges<br>- Enzymes |
| **QUATERNARY:**<br> | - Interaction of several polypeptides<br>- Hemoglobin<br>- Collagen<br>Hemoglobin<br>4 polypeptide chains |

**DENATURATION:**

Changing protein's native conformation

Change shape = change in activity

How?

1. High temperature
2. Chemical agent (acid or base) change in pH
3. Organic solvent

AP BIOLOGY
P. Edgerton

NAME ______________________________

BLOCK ________ DATE ______________

# Summer Assignment Part #2

## Biochemistry Worksheets

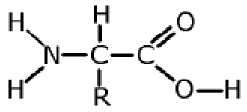
*I have neither given nor received, nor will I give or receive, unauthorized aid on this assignment.*

______________________________________________
*Student Signature*

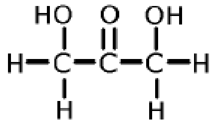
## I. ORGANIC CHEMISTRY QUESTIONS

***Circle and identify*** the functional group(s) found in each of the following illustrated molecules.

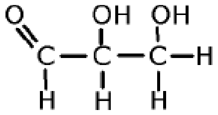
**Molecule #1**



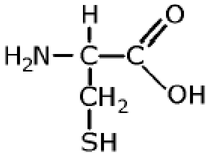
**Molecule #2**



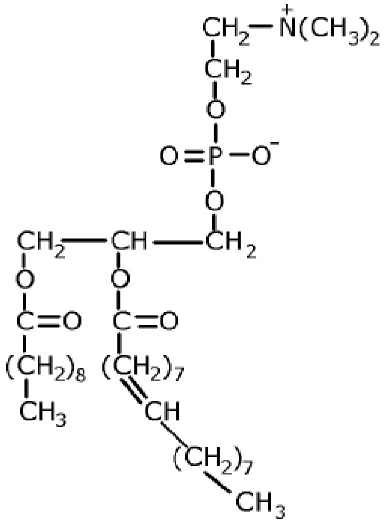
**Molecule #3**



**Molecule #4**



**Molecule #5**



## II. WATER, ACIDS, BASES, & BUFFERS QUESTIONS:

1. ***Fill out*** the following table. ***Name and explain*** five of water's unique properties, and ***discuss*** the biological importance/significance of each of these properties.

| Property of Water | Explanation of Property | Biological Importance/Significance |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |

2. ***Match*** the description/definition with the correct term.

______ Homogenous mixture of 2 or more substances

______ Dissolving agent

______ Material being dissolved

______ Solution where water is solvent

______ Water loving; molecules with an affinity for water

______ Water fearing; molecules that do not have an affinity for water

A. Aqueous solution

B. Hydrophilic

C. Hydrophobic

D. Solute

E. Solution

F. Solvent

3. ***Explain WHY*** water is a versatile solvent. Include water's structure in your explanation.

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

4. In general, what kinds of materials will not dissolve in water? ______________________________

5. At equilibrium in pure water at 25$^{O}$C:

a. How does the [$H^+$] compare to the [$OH^-$]? ______________________________________

b. What is the [$H^+$]? ______________________________________________________

6. Each of the following will affect the equilibrium established in pure water during the dissociation of water. ***Describe*** what effect each will have on the equilibrium by completing the following chart.

| **Addition of:** | **Effects on [$H^+$]** | **Effect on [$OH^-$]** | **Direction Equilibrium Shifts** |
|---|---|---|---|
| $H_2SO_4$ | | | |
| KOH | | | |
| $NH_3$ | | | |

7. How does the [H+] compare to the [OH-] in each of the following:

a. A neutral solution: ______________________________________________________

b. An acidic solution: ______________________________________________________

c. A basic solution: ________________________________________________________

8. What is the pH range for most biological fluids? ____________________________________

What biological fluid is the exception to this range? ____________________________________

9. ***Complete*** the following chart.

| **[$H^+$]** | **pH** | **[$OH^-$]** | **pOH** |
|---|---|---|---|
| $10^{-2}$ | | | |
| | 4 | | |
| | | $10^{-4}$ | |
| | | | 2 |

10. A patient has been vomiting for a prolonged period of time.

a. What effect would this have on the [H+] in the blood? ____________________________________________

b. How will the bicarbonate buffer system respond to this change?

________________________________________________________________________________

________________________________________________________________________________

________________________________________________________________________________

c. What effect will the buffer system response have on the rate of respiration?

________________________________________________________________________________

________________________________________________________________________________

________________________________________________________________________________

d. If the buffer system does not return the blood pH to within the normal range or if the vomiting continues, how will the kidneys respond?

Will the kidneys excrete or reabsorb $H^+$? ____________________________________________________

Will the kidneys excrete or reabsorb $HCO^{3-}$? _________________________________________________

## III. CARBOHYDRATES QUESTIONS:

1. ***Match*** the definition with the correct term.

A. Condensation Synthesis B. Hydrolysis C. Monomer D. Polymer E. Polymerization

______ Large molecule that consists of many subunits called monomers

______ Identical or similar subunits of a polymer

______ Process of linking monomers to form a polymer

______ Loss of a water molecule between two monomers to form a covalent bond between the monomers

______ Breaking the covalent bond between monomers by adding a water molecule

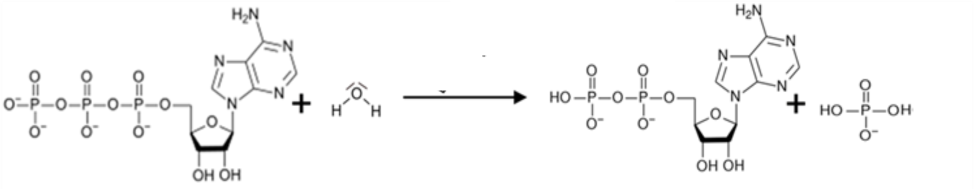
______ A.K.A. dehydration synthesis

2. ***Indicate*** if each of the following is an example of condensation synthesis or hydrolysis.

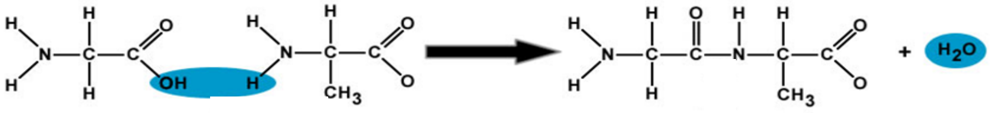
Reaction #1: ________________________________________

Reactions that digest proteins, carbohydrate, or lipids

Reaction #2:________________________________________



Reaction #3:________________________________________



Reaction #4: ________________________________________

Reactions that create proteins, carbohydrates, or lipids

3. How can you tell if a chemical equation represents:

a. condensation synthesis? ________________________________________________________________________

b. hydrolysis? ___________________________________________________________________________________

4. What characteristic is used to classify/group carbohydrates? ***Give an example.***

___________________________________________________________________________________________

___________________________________________________________________________________________

___________________________________________________________________________________________

5. ***Match*** the description with the correct term.

A. Disaccharides
B. Lactose
C. Maltose
D. Monosaccharides
E. Polysaccharides
F. Sucrose

______ Simple sugar

______ General term used to describe a molecule that consists of 2 simple sugars covalently bonded

______ General term used to describe a molecule that consists of 100s or 1000s of simple sugars covalently bonded

______ Molecule that consists of 2 glucose molecules covalently bonded

______ Molecule that consists of a glucose and a galactose covalently bonded

______ Molecule that consists of a glucose and a fructose covalently bonded

6. ***Identify*** each of the following as either a **M**onosaccharide, a **D**isaccharide, or a **P**olysaccharide.

______ Sucrose

______ Galactose

______ Chitin

______ Glyceraldehyde

______ Cellulose

______ Maltose

______ Ribose

______ Deoxyribose

______ Glycogen

______ Amylopectin

______ Glucose

______ Lactose

______ Starch

______ Amylose

______ Fructose

7. ***Draw*** a glycosidic linkage between two glucose molecules.

8. Listed below are characteristics of four biologically important polysaccharides. Use the key below to indicate the polysaccharide described in each characteristic.

A. Cellulose B. Chitin C. Glycogen D. Starch

______ Polymer of α-glucose

______ Polymer of β-glucose

______ Polymer of an amino sugar

______ α 1-4 glycosidic linkages

______ β 1-4 glycosidic linkages

______ Linear and unbranched

______ Branched chain

______ Storage polysaccharide in animals

______ Storage polysaccharide in plants

______ Component of plant cell walls

______ Forms the exoskeleton in arthropods; building material of cell walls in some fungi

9. ***Describe*** how α-glucose is different from β-glucose (structurally & functionally).

________________________________________________________________________________

________________________________________________________________________________

________________________________________________________________________________

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10. ***Discuss*** how two polysaccharides, starch and cellulose, each having the same subunit (glucose), have completely different properties. Why can we digest starch but not cellulose?

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## IV. LIPID QUESTIONS:

1. ***Explain*** why lipids are insoluble in water. In your explanation relate their chemical structure to their insolubility.

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2. ***Discuss*** three ways cholesterol is important for biological structure/function.

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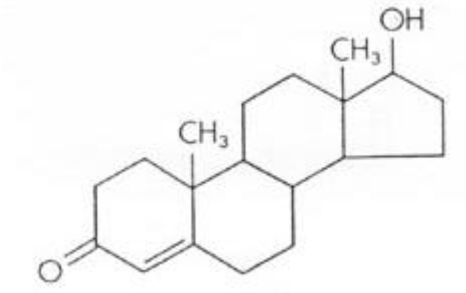
3. ***Indicate*** if each of the following is true of a **F**at, a **P**hospholipid, or a **S**teroid.

______ Consists of glycerol and three fatty acids

______ Energy source

______



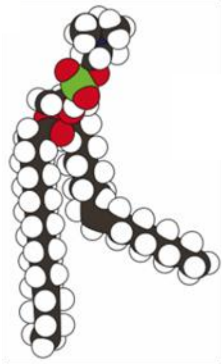
______ Cushions and insulates

______ Consists of glycerol, 2 fatty acids, and a phosphate group

______ Triglycerides

______ Part of the molecule is hydrophilic and the other part is hydrophobic

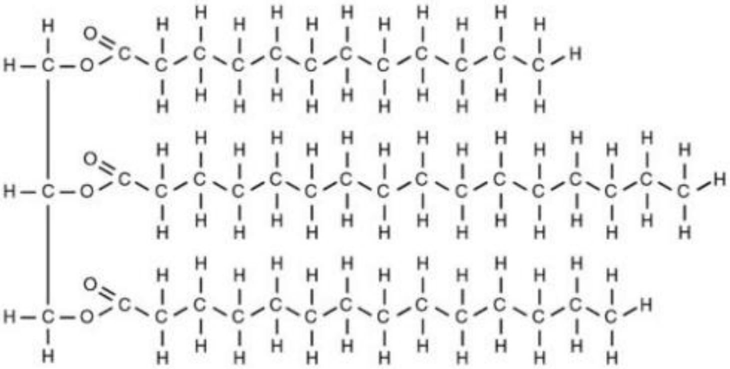
______



______ Major component of cell membranes

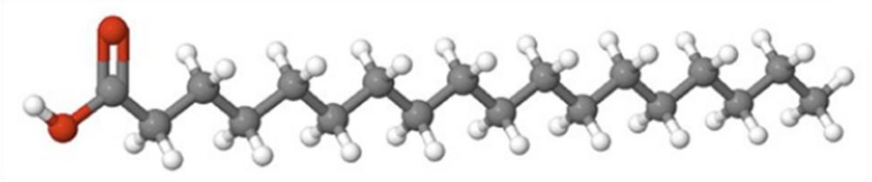
______ Consists of four fused carbon rings – three 6-sided rings and one 5-sided

______



4. ***Indicate*** if each of the following is true of **S**aturated or **U**nsaturated fats.

_______



______ 1 or more double bonds within the carbon chain

______ Usually solid at room temperature

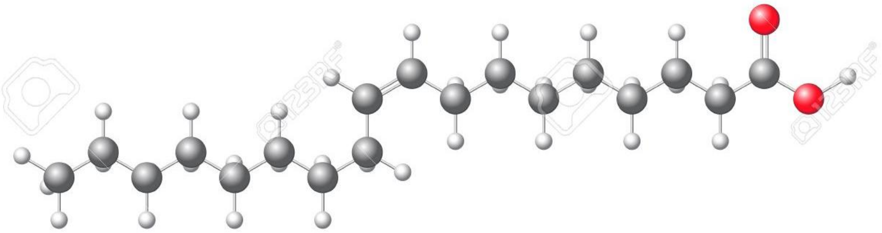
______ Molecules are tightly packed together

______ Usually liquid at room temperature

______ Most plant fats

______ Most animal fats

______



5. ***Discuss*** why unsaturated fats are not solid at room temperature. ***Include their structure in your discussion.***

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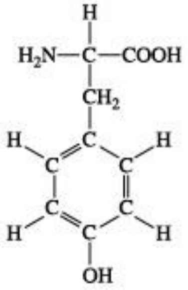
## V. PROTEIN QUESTIONS:

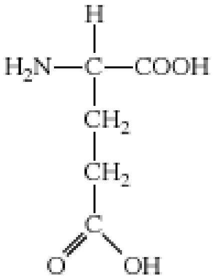
1. ***Classify*** each of the following amino acids as **nonpolar**, **polar uncharged**, **polar charged acidic** or **polar charged basic**. ***Justify your classification.***

a._________________________



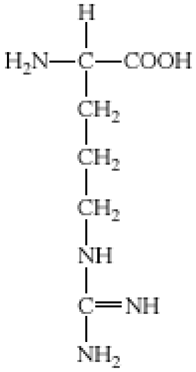
Justification:

b.___________________________



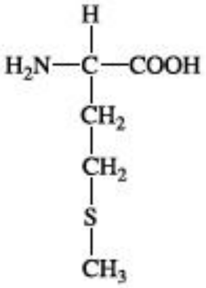
Justification:

c._________________________



Justification:

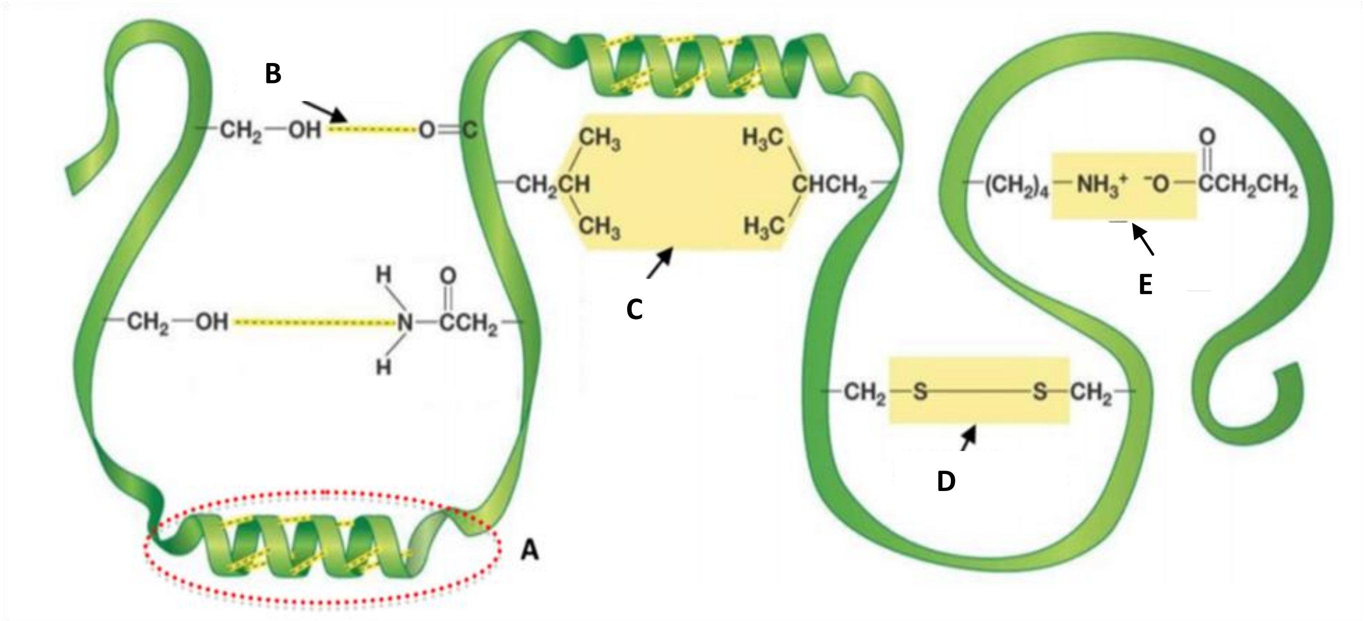
d.___________________________



Justification:

2. ***Draw*** two amino acids - cysteine and aspartic acid - with a peptide bond between them.

3. Use the diagram below to answer the questions that follow.



a. What level of protein structure/organization is shown in the diagram? _____________________________________

b. ***Match*** the following with the correct letter from the diagram.

______ Hydrogen bonding

______ Disulfide bridge

______ α helix

______ Hydrophobic interaction

______ Salt bridge

4. ***Indicate*** the level of protein structure (1, 2, 3, or 4) described in each of the following.

______ α helix

______ β pleated sheets

______ Collagen and hemoglobin

______ Form stabilized by hydrogen bonds

______ Interaction among several polypeptide chains

______ Regular, repeated folding of the peptide chain

______ Form stabilized by hydrogen bonds, ionic bonds, hydrophobic interactions, and disulfide bridges

______ Sequence of amino acids in a protein

______ Most enzymes

______ Determined by the sequence of DNA bases

______ Globular proteins

5. ***Describe*** what happens to a protein (i.e. its structure) when it is ***denatured***.

________________________________________________________________________________

________________________________________________________________________________

________________________________________________________________________________

6. ***Explain*** how denaturation affects the ***function*** of a protein.

________________________________________________________________________________

________________________________________________________________________________

________________________________________________________________________________

________________________________________________________________________________

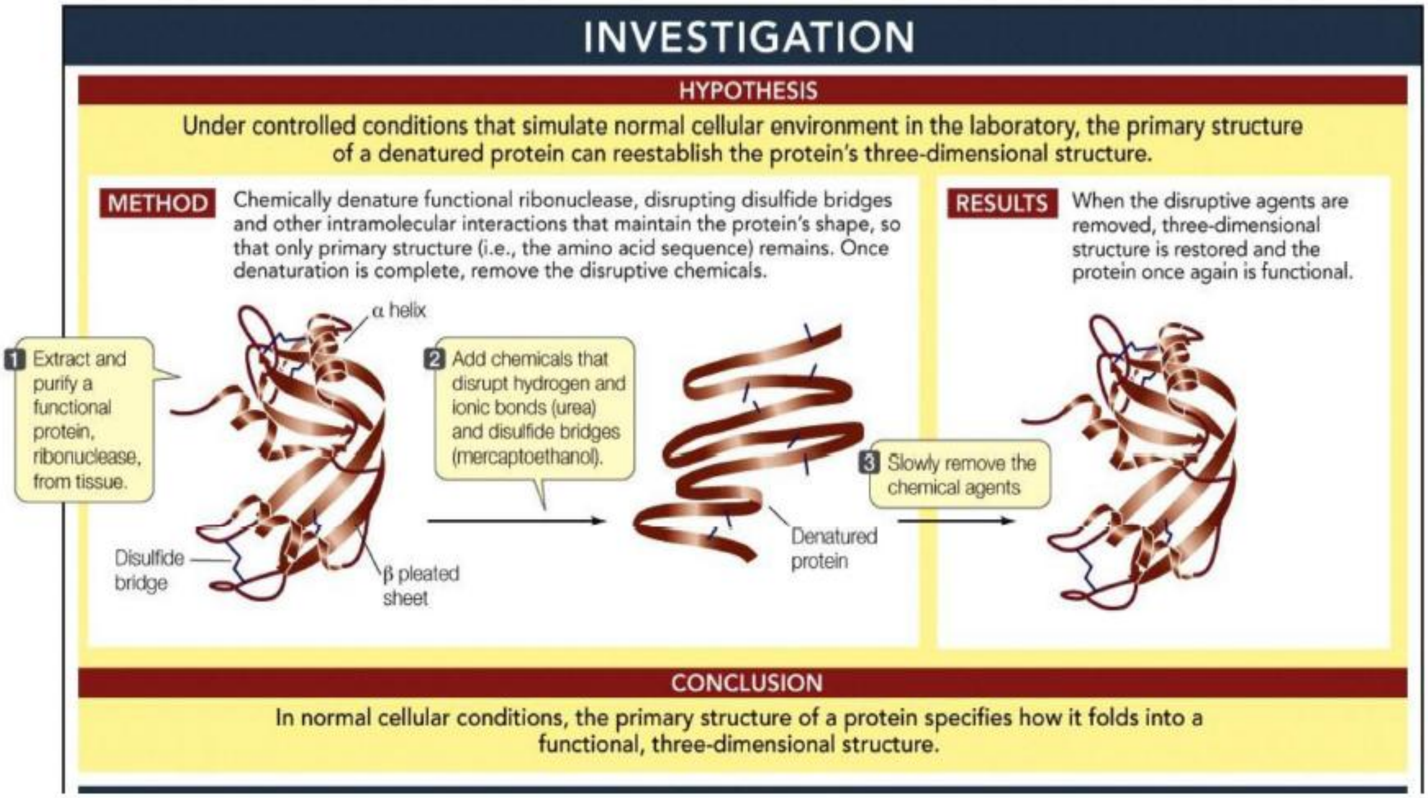
7. ***Explain how/why*** each of the following conditions causes a protein to denature.

| | |
|---|---|
| **Subjecting the protein to high temperatures** | |
| **Placing the protein in a strong acid** | |
| **Placing the protein in an organic solvent** | |

## VI. EXPERIMENTAL RESEARCH QUESTIONS

### Introduction

After the tertiary structures of proteins were first shown to be highly specific, the question arose as to how the order of amino acids determined the three-dimensional structure. The second protein whose structure was determined was ribonuclease A, an enzyme from cows that was readily available from pancreases at slaughterhouses. Because it works in the highly acidic environment of the cow stomach, RNase A was stable compared to most proteins and easy to purify. RNase A has 124 amino acids, among which are eight cysteine residues which form four disulfide bridges. Were these covalent links between amino acids essential for the three dimensional structure? Christian Anfinsen and his colleagues set out to answer this question by first destroying these links by reducing the S-S bonds to SH and SH. With the links destroyed, they measured the three-dimensional structure of the protein (was it denatured?) as well as the effect of denaturation, the loss of enzyme activity. They then removed the mercaptoethanol and allowed the S-S bonds to reform by bubbling in oxygen gas and looked again at the structure and function of the enzyme. They found that indeed, the disulfide bonds between amino acids (primary structure) were essential for protein structure and function. Anfinsen was awarded the Nobel Prize in chemistry in 1973.



INVESTIGATION

HYPOTHESIS

Under controlled conditions that simulate normal cellular environment in the laboratory, the primary structure of a denatured protein can reestablish the protein's three-dimensional structure.

METHOD Chemically denature functional ribonuclease, disrupting disulfide bridges and other intramolecular interactions that maintain the protein's shape, so that only primary structure (i.e., the amino acid sequence) remains. Once denaturation is complete, remove the disruptive chemicals.

RESULTS When the disruptive agents are removed, three-dimensional structure is restored and the protein once again is functional.

1 Extract and purify a functional protein, ribonuclease, from tissue.

2 Add chemicals that disrupt hydrogen and ionic bonds (urea) and disulfide bridges (mercaptoethanol).

3 Slowly remove the chemical agents

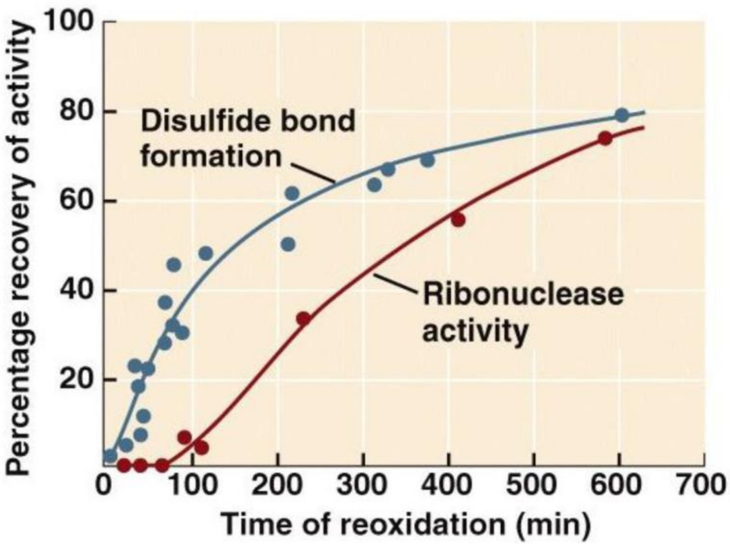
CONCLUSION

In normal cellular conditions, the primary structure of a protein specifies how it folds into a functional, three-dimensional structure.

## Analyze the Data

### Question 1

Initially, disulfide bonds (S—S) in RNase A were eliminated because the sulfur atoms in cysteine were reduced (—SH). At time 0, reoxidation began and at various times, the amount of disulfide bond re-formation (blue circles) and the function of ribonuclease (enzyme activity; red circles) were measured by chemical methods. Here are the data:



A. At what time did disulfide bonds begin to form? ________________________________________________________

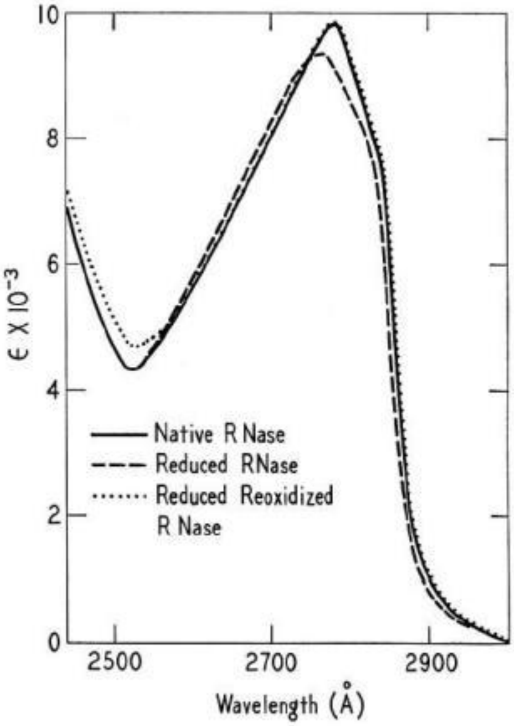
B. At what time did enzyme activity begin to appear? ______________________________________________________

C. ***Explain*** the difference between your answers for the times of (A) and (B).

## Question 2

The three-dimensional structure of RNase A was examined by ultraviolet spectroscopy. In this technique, the protein was exposed to different wavelengths of ultraviolet light (measured in Angstroms [1 A = 10 nm]) and the amount of light absorbed by the protein at each wavelength was measured (E). Here are the results:

A. Look carefully at the graphs. What was the difference between the peak absorbance of native and reduced (denatured) RNase A?



B. When reduced RNase A was reoxidized (renatured), what did its observed spectrum most closely match, that of native RNase A or reduced RNase A?

C. Based on the ***EVIDENCE***, what can you ***conclude*** about the structure of RNase A in these experiments from the data?

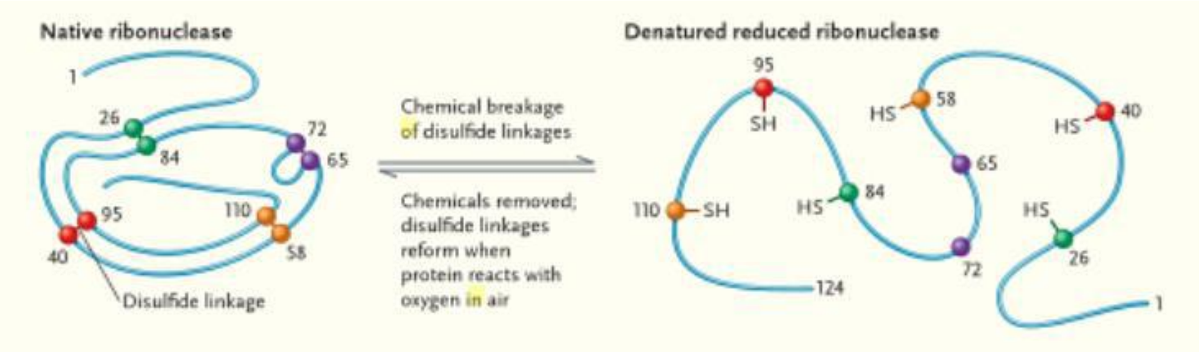
# Question 3

In a second experiment, Anfinsen and Haber investigated the following question.

**Question:** What is the relationship between the amino acid sequence of a protein and its conformation?

**Experiment:** Anfinsen and Haber studied the 124-amino acid enzyme ribonuclease in the test tube. They knew that the native (functional) enzyme has four disulfide linkages between amino acids 26 and 84, 40 and 95, 58 and 110, and 65 and 72 (see figure). They treated the active enzyme with a mixture of urea and β-mercaptoethanol, which breaks disulfide linkages. They then removed the two chemicals and left the enzyme solution in air.

**Results:** The chemical treatment broke the four disulfide linkages, which caused the protein to denature and lose its enzyme activity. After the chemicals had been removed and the enzyme solution exposed to air, Anfinsen made the crucial observation that the protein renatured, slowly regaining enzyme activity. Ultimately the solution showed 90% of the activity of the native enzyme.



He realized that oxygen from the air had reacted with the –SH groups of the denatured enzyme causing disulfide linkages to reform, and that the enzyme had spontaneously refolded into its native, active conformation. All physical and chemical properties of the refolded enzyme the researchers measured were the same as those of the native enzyme, confirming that the same disulfide bridges had formed as in the native enzyme.

**Conclusion:** Anfinsen concluded that the information for determining the three-dimensional shape of ribonuclease is in its amino acid sequence.

If denatured ribonuclease renatures in the presence of a high concentration of urea, the renatured enzyme has physical and chemical properties similar to those of the native enzyme indicating that refolding had occurred, but the enzyme activity is less than 1% of that of the native enzyme. **Interpret this result.**