BIOMOLECULES LAB

Objectives

1. Properly identify and distinguish between the molecular structure, types of atoms, and functions of carbohydrates, lipids, proteins, and nucleic acids.

2. Properly identify the monomers and polymers of carbohydrates, proteins, and nucleic acids and the two main components of triglycerides.

3. Relate the four different biomolecules to different foods. In what foods would you find carbohydrates? Lipids? Proteins?

4. Understand the meaning, importance, and proper use of a positive control and a negative control.

5. Execute simple experiments using both a positive and negative control and an unknown to properly identify carbohydrates, lipids, proteins.

6. Interpret results of tests for carbohydrates, lipids, proteins.

What is in my food?

You are learning about biomolecules in your biology class and think about the ingredients found on food labels and what group of biomolecules they could belong to. You find a few household ingredients and decide to test your newfound knowledge.

Part 1: Review of the four biomolecules

What are the four main types of biomolecules and how can you differentiate between them? In this activity, you will explore the properties and features of the four different types of biomolecules: carbohydrates, lipids, proteins, and nucleic acids. You will first examine the similarities and differences in their structures so that you can recognize and identify them by their structural and molecular formulas.

Most biomolecules are composed of **monomers**, repeating units (like bricks of a building) that join chemically to form larger molecules called **polymers** (poly = many, mer = parts). To allow new chemical bonds to form between two monomers, an OH- is removed from one monomer and an H+ from the other. This is called a **dehydration reaction** (removal of water) and it allows electrons between the two monomers to form covalent bonds (bonds that share electrons.) See **Figure 1**.

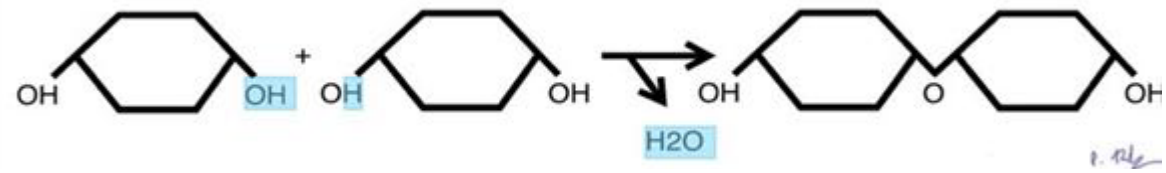


Figure 1: Dehydration reaction of two monomers joining together to form a polymer.

Just as monomers can be joined together, they can also break a large polymer down into its monomers. In this case, water is added to the molecule to break the covalent bond. This type of reaction is called **hydrolysis**. See **Figure 2**.

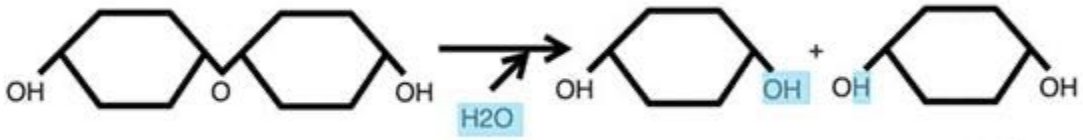


Figure 2: Hydrolysis reaction showing a monomer being released from a polymer with the addition of water.

Activity 1: Exploring the Structures and Functions of Biomolecules

In this activity, you will learn to recognize carbohydrates, lipids, proteins, and nucleic acids based on their structure, arrangement of atoms, function, and monomer and polymer forms. You will discover which monomers are paired with which polymer for the carbohydrates, proteins, and nucleic acids, and study the component parts of a lipid. You will also learn the names of several examples of specific members of each class.

Exploring Carbohydrates

The building block (or monomer) of carbohydrates is a **monosaccharide**. Examples of monosaccharides include glucose, fructose, and galactose. Monosaccharides are sometimes known as simple sugars. Monosaccharides are made of rings of five or six carbons with an abundance of oxygen and hydrogen present. There are approximately two hydrogens for every one oxygen atom in the molecule. This leads to the general formula for carbohydrates: CH_2O.

The monosaccharides you will see most often are six-sided hexagon rings with the molecular formula of $C_6H_{12}O_6$. Examples of monosaccharides include glucose, fructose, and galactose. These monosaccharides are used as energy sources in cells.

A Note on Interpreting Molecular Structures

All Biomolecules contain carbon, but sometimes they are not drawn in images, as a sort of molecular shorthand. In the image below, carbon is indicated at all of the junctions between two lines. Compare the left image with the right image. Can you spot 6 carbons in both structures?

When two monosaccharides combine (by a dehydration reaction), they form a **disaccharide**. Examples of disaccharides are sucrose (table sugar) and lactose (milk sugar). Like the monosaccharides, disaccharides serve as energy sources for cells. Not surprisingly, a disaccharide like sucrose has twice the number of carbon atoms as a monosaccharide. The typical molecular formula for a disaccharide is $C_{12}H_{22}O_{11}$.

Three or more monosaccharides linked together form a polymer called a **polysaccharide**, also known as a complex carbohydrate. Examples of polysaccharides are starch, cellulose, and glycogen. These are long and sometimes branching chains of repeating monosaccharide units. Functionally, some polysaccharides serve as different ways to store energy. For example, starch is the sugar storing molecule found in plants and glycogen is the sugar-storing molecule found in animals. Cellulose, another polysaccharide, is a critical structural component of plant cell walls, making them rigid. Cellulose fibers make up cotton fabric and paper, both products deriving from plants.

Procedure: (Work together as a table)

1. In the bag of molecules, find three monomers with 6 carbons arranged in a ring. These are a monosaccharide.

2. Observe the laminated image of these monosaccharides, and use the reading above, to fill in the chart below.

Biomolecule	Atoms Present (C, H, O, N, S, P?)	Monomer name	Function of Monomer
Carbohydrate			

3. You should have found three different examples of this monomer. What are the names of these three molecules?
    -
    -
    -

Exploring Lipids

Lipids are made of carbon, hydrogen, and oxygen and are usually large molecules with many more carbon and hydrogen atoms than oxygen atoms. All lipids are non-polar and do not dissolve in water.

Here we will focus on the **triglyceride** group. This group is formed by combining two different components. One, called glycerol, has a short 3-carbon backbone; each of the three carbons is attached to an "–OH" (alcohol) group. The second component is a **fatty acid**. Fatty acids are long chains consisting almost entirely of carbon and hydrogen. They are therefore called hydrocarbon chains. Three fatty acids combine with one glycerol molecule at the "-OH" end of each of glycerol's three carbons to form a large, non-polar molecule called a triglyceride. (**Figure 3**)

Triglycerides can come in two forms: saturated fats or unsaturated fats, depending on the structure of the fatty acid chains. Triglycerides serve as a major energy source in cells and are generally stored in fat cells for later use as energy. Fat is also an excellent insulation and serves as a cushion to protect internal organs.

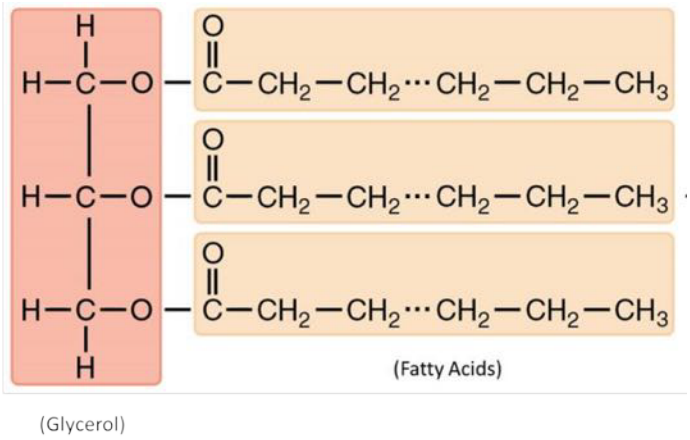


Figure 3: The two components of a triglyceride are glycerol and 3 fatty acids.

There are two other types of lipids: **phospholipids** and **steroids**. Like all lipids, these groups are non-polar and do not dissolve in water. Phospholipids are a major component of cell membranes and serve a structural purpose. Steroids have an

interlocking four ring structure with very little oxygen. These large molecules often serve as hormones or to suppress the immune response.

Procedure: (Work together as a table)

1. In the bag of molecules, find a triglyceride. This molecule has three long chains made only of carbon and hydrogen (a hydrocarbon chain), called fatty acids. These three fatty acid chains are attached to a short 3-carbon backbone called glycerol.

2. Use the laminated image of a triglyceride and the reading and figure above, to fill in the chart below.

Biomolecule	Atoms Present (C, H, O, N, S, P?)	Three Types of Lipids	Functions of Lipids
Lipid (triglyceride)		1. 2. 3.	1. 2. 3.

3. Triglycerides are not made of monomers but are made of two components. Name them.
    -
    -

Exploring Proteins

The monomer of proteins is called an **amino acid**. Amino acids are made of carbon, hydrogen, oxygen, and nitrogen. One specific amino acid (cysteine) contains sulfur as well. All amino acids are composed of a carboxylic acid (COOH group), an amine group (NH_2), and a variable portion called the “R” group. Our DNA only codes for 20 “R” groups. Examples of amino acids include alanine, serine, lysine, cysteine, tyrosine, and proline. When they are linked together by repeated dehydration reactions, they form long chain polymers called **polypeptides**. Much like balling up a wad of yard, polypeptides then fold into unique three-dimensional blobs or ribbons called **proteins**. Proteins have many different functions. (**Table 1**)

Table 1: Proteins have a wide array of functions!

Cell membrane transport: carrier proteins, sodium-potassium pump of nerve cells
Communication: cellular receptors like the spike protein in COVID, an insulin receptor, an opioidreceptor, or a receptor for a neurotransmitter
Immunity: antibodies against Measles, Smallpox, and Chickenpox
Structural proteins: keratin, which makes up hair and nails, and collagen that strengthens skin and bones
Homeostatic proteins: hormones like insulin, glucagon, and adrenaline, that regulate sugar levels, metabolism and flight or fight reactions.
Catalytic proteins: enzymes like amylase, pepsin, and dehydrogenases that speed up chemical reactions within cells, and organs within the digestive system that contain enzymes to help rapidly digest food

One important protein, hemoglobin, carries oxygen. Insulin and glucagon are hormones responsible for regulating blood sugar levels. Many proteins are enzymes that speed up the rate of metabolic reactions. Other proteins, like actin, keratin and collagen, are structural proteins that make up muscles, tendons, and hair. Proteins can be found in all cells from bacteria to fungi, to plants, and to animals.

Procedure: (Work together as a table)

1. In the bag of molecules, find the five monomers called an amino acid. These molecules have a carbon in the middle, and different groups sticking off the carbon in four directions. One group will be an amine group (NH_2), one group will be a carboxyl group (-COOH), another is bound to hydrogen (H). The fourth bond of the central carbon will be the "R" (or variable) group. This changes, depending on what amino acid it is.

2. Observe a laminated image of an amino acid (pick any amino acid) along with the reading above to complete the table.

Biomolecule	Atoms Present (C, H, O, N, S, P?)	Monomer name
Proteins		

3. Identify the “R” group for the five amino acids you find in your collection of images and fill in the chart below.

Amino Acid Name	Draw the structure of the “R” group

4. Look at the structure of the “R” groups you drew above.
   - Which amino acid(s) has/have a charge?

   - Lysine is an amino acid with a positive charge. Which of the amino acids above would be attracted to lysine?

Exploring Nucleic Acids

The last group, the **nucleic acids**, include **DNA** and **RNA**. The building blocks (monomers) of nucleic acids are **nucleotides**. All nucleotides are composed of three elements: a phosphate group (PO_4^{3-}), a sugar (usually a 5-carbon sugar called either ribose or deoxyribose), and one of 5 nitrogen containing (nitrogenous) bases.

DNA and RNA are slightly different in their structure. DNA is composed of unique combinations of four distinct nucleotides: adenine, cytosine, guanine, and thymine. RNA is composed of adenine, cytosine, and guanine, but thymine is replaced by uracil. DNA is the instruction manual for making proteins in living cells. While different types of RNA play different roles in cells, RNA is almost always an intermediary for DNA, assisting DNA by acting as a messenger molecule between the nucleus and the ribosomes. Other RNA molecules are structural in nature.

Adenosine triphosphate (ATP) is actually a modified nucleotide because it contains three phosphate groups, in addition to its nitrogenous base (adenine) and sugar (ribose). ATP is an immediate, usable energy source for cells.

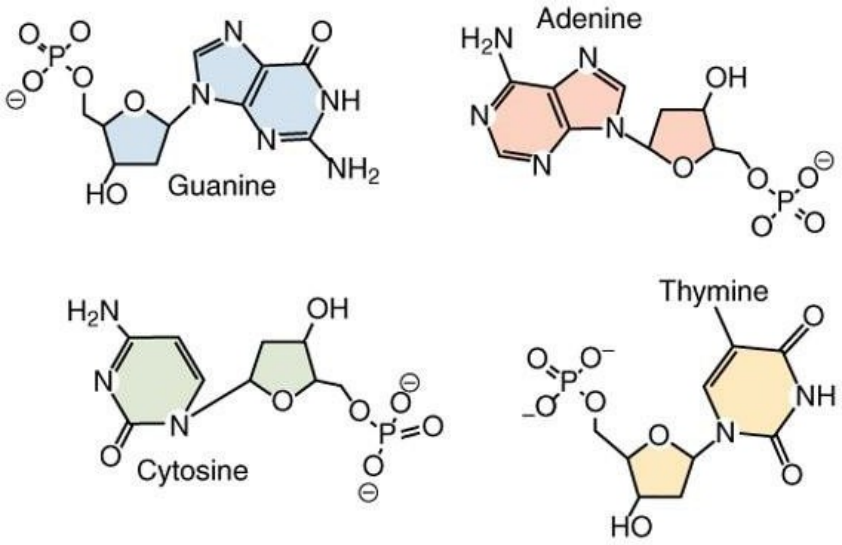
Procedure: (Work together as a table)

1. In the bag of molecules, find a nucleotide monomer. Look for a molecule that has a five-carbon sugar ring, a phosphate group that contains phosphorus surrounded by oxygen, and a single or double ring that contains a lot of nitrogen (nitrogenous base).

2. Observe the image and use the reading above to fill in the chart below.

Biomolecules	Atoms Present (C, H, O, N, S, P?)	Monomer name	Name five nitrogenous bases found in nature
Nucleic Acid			1. 2. 3. 4. 5.

3. From the reading above, review the structure of the nucleotides. Look at the image below and circle and label the phosphate group, the nitrogenous base, and the sugar components of each nucleotide.



4. Use the dry erase markers to write on the laminated part of the poster board as you review the atoms and functions of the biomolecules on the upper half of the poster board.

5. In the bag of laminated images of monomers and polymers, find the appropriate monomer and polymer pair, along with a lipid. Place the pairs on the lower half of the poster board, in the correct categories.

6. Look at the labels for the 5 samples that you will be testing. Based on what you learned in this activity, make a prediction for which samples will be positive for the different biomolecules. Put a + in the boxes that you hypothesize will be positive for the biomolecule.

Sample #	Mono or Disaccharides	Polysaccharides	Lipids	Protein
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				

Instructor Initials for completed poster board and hypothesis: _____

Part 2: Test for the different biomolecules

NOTE: Positive and negative controls:
When doing tests, we need to ensure that the test itself is not faulty and might give us false results. To confirm that the test is working, we often use something we KNOW will work. This is called the "positive control." We expect that the test will show us a positive result. We also set up a negative control, usually water, to give us an indication what a negative test should look like. If either the positive or negative control does not behave as we expect it should, we call the test into question. If the positive or negative controls are unreliable, then the experiment must be scrapped and redone, perhaps with new reagents, new test tubes, or other sources of possible contamination.

Activity 1: Testing for Carbohydrates

In this activity you will test for monosaccharides and disaccharides (simple sugars) using **Benedict's test**, and you will test for starch using iodine. The Benedict's test uses Benedict's solution, a bright blue solution which changes color in the presence of monosaccharides and disaccharides (simple sugars). (See the laminated image of this test in your lab tray.) A low concentration of a mono- or disaccharide in a sample will result in a green color, and higher concentrations of sugar in a sample will turn yellow, orange, or even red!

The **Iodine test** uses a reddish-brown iodine solution to identify polysaccharides like starch and cellulose. In the presence of either of these polysaccharides, iodine turns from a reddish color to a black/dark blue color.

Procedure: (Work together as a table)
Goggles, gloves, and lab coat are required for this experiment. Hot water will be used.

Note: Both Benedict's solution and Biuret's reagent are blue. Make sure you use the correct solution!

1. Label seven clean test tubes '+ Control,' '- Control,' 'Sample 1,' 'Sample 2,' 'Sample 3,' 'Sample 4,' and 'Sample 5.'

2. Use a ruler to mark where the 2 cm mark is on each of the seven test tubes with the wax pencil. Then, add a second line indicating the 3 cm mark.

3. In the test tube labeled '+ Control,' add the sugar solution to the 2 cm mark to the. (This is the positive control.)

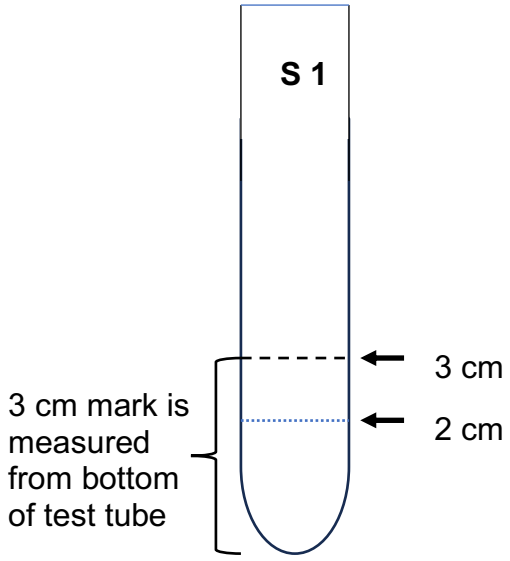


Figure 4: How to measure the test tube.

4. In the test tube labeled '- Control,' add deionized (DI) water to the 2 cm mark. (This is the negative control.)

5. Continue to fill the other test tubes with 'Sample 1,' 'Sample 2,' 'Sample 3,' 'Sample 4,' and 'Sample 5 to the 2 cm mark.

6. Then, use a clean pipet to add Benedict's solution to each of the seven test tubes to the 3 cm mark. You should have the same volume in all three test tubes.

7. Put on your goggles and use test tube holders to carefully place each of the three tubes into boiling water for 2 minutes. A positive result is a color change from blue to green, yellow, orange, or red, depending on how much monosaccharide or disaccharide is in the sample.

8. Looking at your controls:
    a. What was the starting color of the '- Control' tube? _____
    b. What was the ending color of the '- Control' tube? _____
    c. What was the starting color of the '+ Control' tube? _____
    d. What was the ending color of the '+ Control' tube? _____

9. Fill in the data table on the last page of the lab with your results for the samples.

10. Label seven clean test tubes '+ Control,' '- Control,' 'Sample 1,' 'Sample 2,' 'Sample 3,' 'Sample 4,' and 'Sample 5.'

11. Use a ruler to mark where the 2 cm mark is on each of the three test tubes with the wax pencil.

12. Use seven clean pipets to place starch ('+ Control), DI water ('- Control), and samples 1-5 in the appropriately labelled test tubes to the 2 cm mark.

13. Add 2-3 drops of iodine solution to each of the test tubes. A positive result is a color change from brown to blue-black that occurs immediately.

14. Looking at your controls:
    a. What was the starting color of the '- Control' tube? _____
    b. What was the ending color of the '- Control' tube? _____
    c. What was the starting color of the '+ Control' tube? _____
    d. What was the ending color of the '+ Control' tube? _____

15. Fill in the data table on the last page of the lab with your results for the samples.

Activity 2: Testing for Lipids

In this experiment you will test your unknown food sample for the presence of lipids using an emulsion test. This test positively identifies lipids.

Procedure: (Work together as a table)

1. Label seven clean test tubes '+ Control,' '- Control,' 'Sample 1,' 'Sample 2,' 'Sample 3,' 'Sample 4,' and 'Sample 5.'

2. Use a ruler to mark where the 2 cm mark is on each of the seven test tubes with the wax pencil. Then, add a second line indicating the 3 cm mark, and a third line indicating the 4 cm mark.

3. Use clean pipets to place oil (lipid, '+ Control), DI water ('- Control), and Samples 1-5 in the appropriately labelled test tubes to the 2 cm mark.

4. To each of the seven tubes, use a pipet to add ethanol to the 3 cm mark.

5. Cover each of the seven tubes with parafilm and shake each tube. Your instructor will show you how to properly use parafilm.

6. Wait for about 3 minutes while the solid settles.

7. Add DI water to the 4 cm mark in each tube and observe if significant layers form. Lipid layers will be cloudy.

8. Fill in the data table on the last page of the lab with your results for the samples.

Activity 3: Testing for Proteins

To test for the presence of proteins or amino acids, we use the Biuret colorimetric test. This test uses a copper-containing reagent that is blue in color. When the reagent binds to the nitrogen of the amine group part of a peptide bond, it turns violet, and thus serves as a positive test for the presence of amino acids or proteins.

Procedure: (Work together as a table)
Gloves and lab coat are required for this experiment.

Note: Both Biuret reagent and Benedict's solution are blue. Make sure you use the correct solution!

1. Label seven clean test tubes '+ Control,' '- Control,' 'Sample 1,' 'Sample 2,' 'Sample 3,' 'Sample 4,' and 'Sample 5.'

2. Use a ruler to mark where the 2 cm mark is on each of the seven test tubes with the wax pencil. Then, add a second line indicating the 3 cm mark.

3. Find albumin protein (+ Control) in the laboratory refrigerator. Use clean pipets to place the albumin ('+ Control), DI water ('- Control), and Samples 1-5 in the appropriately labelled test tubes to the 2 cm mark. Return the protein bottle to the refrigerator.

4. Wear Gloves and lab coat for this step as Biuret reagent is corrosive to skin. Use a clean pipet to transfer Biuret reagent to each of the three test tubes to the 3 cm mark.

5. A positive result is an immediate color change from blue to violet.

6. Looking at your controls:
    a. What was the starting color of the '- Control' tube? _____
    b. What was the ending color of the '- Control' tube? _____
    c. What was the starting color of the '+ Control' tube? _____
    d. What was the ending color of the '+ Control' tube? _____

7. Fill in the data table on the last page of the lab with your results for the samples.

8. Clean all glassware with soap and water. Use a bottle brush to scrub the inside of test tubes. All waste is non-toxic and may be poured down the drain. Please flush with water.

9. Record the results. Indicate the final color of the sample and circle if the result was positive.

	Benedict's	Iodine	Ethanol	Biuret
Sample #	**Mono or Disaccharides**	**Polysaccharides**	**Lipids**	**Protein**
+ Control				
- Control				
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				

10. Was your hypothesis supported or not supported for the following samples:
    a. Sample 1: _____
    b. Sample 2: _____
    c. Sample 3: _____
    d. Sample 4: _____
    e. Sample 5: _____

Name: _____ **Date:** _____

Lab Checkout: When you finish the lab, please clean up your lab space and put away your materials neatly in the tray. Please get your instructor's initials to check-out of lab.

- ❑ Lab bench clean, washed, and dried
- ❑ All test tube solutions are disposed of in the sink with running water
- ❑ Glassware washed and dried
- ❑ Test tubes washed, dried and placed upside down in the test tube rack
- ❑ The mat board has been cleaned off with a dry paper towel
- ❑ Trays neatly put away

Instructor initials:_____ **% Completion of activities:** _____

Figure Citations:
Original artwork by P. Rodgers (NOVA Loudoun faculty): Figures 1, 2, and 3
Original artwork by H. Wangerin (NOVA Loudoun faculty): Figures 4