

Molecules of Life

1. Pre-Lab Reading

Be sure to complete the following reading assignment before lab:

Chapter 3 Sections 2-4 “Carbohydrates,” “Lipids,” “Proteins” Biology2e, OpenStax College

Watch this video from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank:

<http://pdb101.rcsb.org/learn/videos/what-is-a-protein-video>

2. Purpose and Objectives

The purpose of this lab is to:

1. Learn about polar, nonpolar, hydrophilic and hydrophobic molecules.
2. Become familiar with the general structural features and chemical properties of carbohydrates, proteins, and lipids.
3. Explore a simple method for separating molecules based on their polarity.

Upon completion of this investigation, you should be able to:

- use appropriate terms for the components of a solution
- recognize common polar and nonpolar bonds in biological molecules
- predict whether or not a molecule will interact with water
- describe the general composition and properties of lipids, proteins and carbohydrates.

3. Laboratory Safety Guidelines for Molecules Lab

- **NO FOOD OR DRINK IN LAB!!**
- Only wear shoes that cover your **entire** foot (**NO SANDALS OR SLIDES**).
- Wear long pants and/or a safety apron.

- Wear goggles and use care when handling glassware and chemicals. Report all accidents, including minor ones, to your instructor immediately.
- **WASH YOUR HANDS BEFORE YOU LEAVE CLASS!**

4. Background Information for solutions and polarity

A **solution** is defined as a mixture of substances having a uniform composition. Solutions can be found in nature and are also common in the Biology laboratory. A solution consists of at least two components: the **solvent**, which is the dissolving medium of a solution and usually present in the greatest amount, and the **solute** or substance dissolved in the solvent. There are many types of solutions. Some are mixtures made up only of gasses. Some are mixtures of liquids. A common type of solution in the laboratory is one made up of a liquid acting as a solvent to dissolve a solid. An example is a salt solution, which is made by mixing sodium chloride and water.

Water is an important solvent in biological systems. In fact, it is fair to say that life could not exist (or at least would be radically different) without water. Water can be found both inside and outside organisms' cells and dissolved in this water are many solutes including ions, sugars, and enzymes. The chemical properties of a solvent determine what types of molecules it can dissolve. Water, which is composed of one oxygen atom and two hydrogen atoms, is described as a **polar** molecule. Bonds between the oxygen atom and the hydrogen atoms are **polar covalent bonds**. This means that the atoms in water do not share electrons equally and this results in partial positive and negative charges on the molecule. Due to these partial charges, water molecules associate very closely with each other by means of chemical bonds called **hydrogen bonds**. This "cohesion" of water molecules results in some unusual properties including high surface tension, capillary action, ability to absorb large amounts of heat energy with minimal temperature change, and the fact that water's frozen form (ice) is less dense than its liquid form.

Molecules that can associate with water and/or be dissolved by water are described as being **hydrophilic**, which means water loving. Molecules that do not interact with water are described as being **hydrophobic**, or water fearing. Whether or not molecules dissolve in water depends on a number of properties including molecular size, charge, and polarity. In general, charged

molecules and molecules with polar covalent bonds dissolve well in water because they interact successfully with the partial charges on the water molecules. Molecules with primarily nonpolar bonds are hydrophobic and will not dissolve in water.

In biological systems, some molecules have a mixture of polar and nonpolar covalent bonds and the relative number of these determines whether they are water soluble, although predicting this is not always straightforward. Some of these molecules are **amphipathic**, having hydrophilic and hydrophobic regions. These molecules are sometimes called **emulsifiers**, meaning that they allow hydrophilic and hydrophobic materials to form a stable mixture because they can interact with both.

While water is a polar solvent, some solvents used in laboratories are best described as **nonpolar solvents**. These liquids may not mix with water because of their nonpolar nature. They can, however, form solutions with other nonpolar, uncharged, molecules. The nonpolar solvent that we will use in lab today is known as **mineral oil**. It is derived from petroleum and is a mixture of **hydrocarbon** molecules. These are molecules composed of only carbon and hydrogen. Mineral oil contains primarily long, unbranched hydrocarbon molecules called alkanes with chain lengths ranging from about 15 to as many as 40 carbons. Mineral oil is hydrophobic and will form a separate layer or “phase” that floats on top of water when mixed with it.

Molecular interactions based on polarity are very important in biological systems. Polar molecules, for example, do not enter cells easily because cell membranes have a nonpolar lipid layer in which polar molecules are not soluble. Though polar molecules are often transported across membranes and into cells by membrane proteins, they also tend to be found in extracellular body fluids (which contain water) in animals and are eliminated relatively quickly via urine. Thus, for humans, the water-soluble vitamins such as vitamin C (a polar molecule) need to be consumed daily. The fat-soluble vitamins, such as vitamin A (nonpolar) can be stored in fat cells and are not eliminated quickly. Because of this, the fat-soluble vitamins can build up to toxic levels in the body when they are consumed in excess, usually as supplements.

Movement and persistence of molecules in the environment is also influenced by whether they are polar or nonpolar. For example, toxins that bio accumulate (become concentrated in organisms) tend to be nonpolar.

5. Background Information for Carbohydrates and Proteins

Carbon is a very versatile element that forms the backbone of four important groups of organic molecules found in the cells and tissues of living things: **carbohydrates, lipids, proteins and nucleic acids**. Having four valence electrons allows carbon to form a vast array of molecules exhibiting a variety of shapes and sizes.

As described above, carbon molecules that consist of carbon and hydrogen are called **hydrocarbons**. Many organic molecules also contain other elements, such as oxygen, nitrogen, phosphorus and sulfur. How these elements bind to carbon and to each other determines the properties of the molecules that they form.

Some biological molecules are described as **polymers**. These **macromolecules** are constructed from smaller building blocks or structural units called **monomers**. Monomers are joined together in a type of condensation reaction called a **dehydration synthesis**. In this reaction, one monomer covalently bonds to another. In the process, a hydrogen from one monomer and a hydroxyl (-OH) group from another are removed. In effect, the equivalent of a molecule of water is removed when two monomers are bonded together.

Macromolecules can be broken down into smaller molecules in a process called **hydrolysis**. When a specific bond between two adjacent monomers is broken, a hydrogen atom attaches to one monomer and a hydroxyl group to another, again, the equivalent of a water molecule.

Carbohydrates are molecules made up primarily of carbon, hydrogen, and oxygen (C:H:O) in a ratio of approximately 1:2:1 (e.g., chemical formula for glucose is $C_6H_{12}O_6$ and for sucrose is $C_{12}H_{22}O_{11}$). **Monosaccharides**, the simplest of sugars, are the monomers used to build more complex carbohydrates. Examples of monosaccharides include glucose, fructose, galactose and

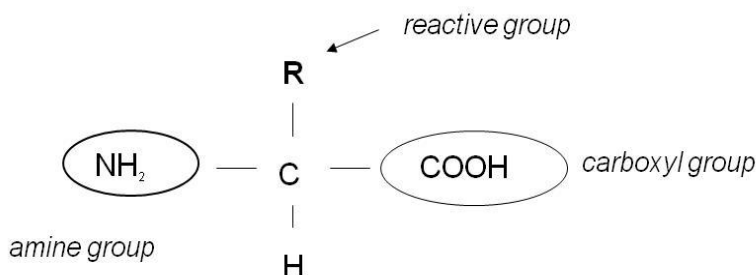
ribose. Maltose, sucrose and lactose are examples of **disaccharides** and are composed of two monosaccharides bonded together. Sugars often function as a quickly available source of energy for cells.

Polysaccharides are macromolecule polymers formed by the linkage of three or more monosaccharide monomer subunits. Examples of polysaccharides include glycogen, cellulose and amylopectin, a form of **starch**. These three polysaccharides are all polymers composed of the monosaccharide glucose. Glycogen functions as an energy storage molecule in animal cells, and is found in liver and muscle tissue in humans. Starch also functions as an energy storage molecule and is found in plants. Structurally, starch and glycogen differ in the amount of branching in the polysaccharide chains, with glycogen being more highly branched. Cellulose differs from glycogen and starch both in function and structure. Cellulose is a rigid polymer that is an important component of plant cell walls. Its function is primarily structural support.

Proteins

Proteins are the most versatile molecules in nature. They are made up primarily of carbon, hydrogen, oxygen and nitrogen (C:H:O:N) Proteins are polymers of **amino acids**. All amino acids have the generalized structure shown in Figure. 2.

Figure 2: The Basic Structure of Amino Acids



The 20+ different amino acids found in proteins differ only in what chemical group occupies the reactive group (R-group) position. Amino acids can be grouped into three broad categories based on R groups: **nonpolar**, **polar**, and **charged**. Proteins are synthesized by the formation of **peptide bonds** (C – N linkages) between adjacent amino acids to form linear chains called polypeptides. Polypeptides vary in length but typically contain at least 200-300 amino acids. Some proteins consist of more than one polypeptide.

Protein function depends on shape and each protein folds in a unique three-dimensional pattern. Folding patterns are difficult to predict from amino acid sequence, but are known to depend on a number of factors including polarity of the amino acids in the polypeptide chain. Hydrophobic amino acids tend to be found in the interior of a folded protein, away from the surrounding fluids, which are often mostly water.

Proteins have a variety of functions in cells. Some provide structural support; others are involved in functions such as movement, transport or even protection from infectious disease. An important group of proteins is the enzymes. They function as catalysts, speeding up metabolic reactions in cells.

6. Experiment 1: Polar and Nonpolar Bonds in Plant Pigments

Background

Plants produce a number of types of pigments, molecules that absorb some wavelengths of visible light and therefore appear to have particular colors. Functions of these vary. Some examples are absorption of light energy for photosynthesis, protection of plant tissues from excess light, and attraction of pollinators such as bees.

We will examine solubility of two plant pigments. **Betanin** is a red pigment found in beet plants. **Beta-carotene** is an orange pigment found in many plants but particularly abundant in carrots. The structures of these pigment molecules are shown below.

Safety

- Wear goggles when working with any chemicals.
- Follow instructions for disposal of materials at the end of the lab period.

Procedure

Record all results and observations in your lab book.

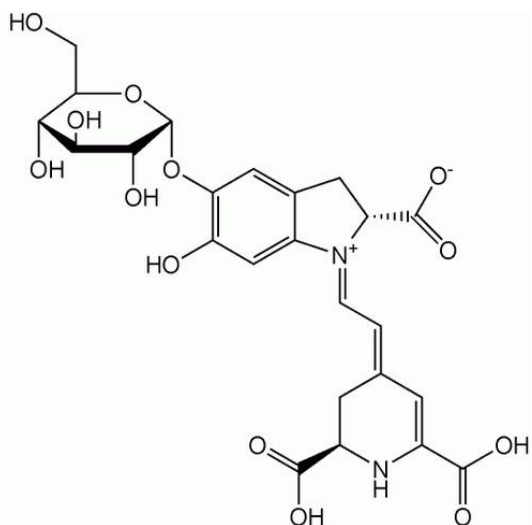
1. Prepare your lab notebook by recording the **lab title**, **date**, **lab section**, and your **lab partner's name**. Write a **purpose** and a **procedure** statement. Remember that the procedure statement refers to the appropriate pages in the lab manual. See the "Lab Assignments and Expectations" pages for more information.
2. Read the instructions for constructing and labeling graphs and tables in "Lab Assignments and Expectations". Construct a table in your lab book for recording your results. The table should have 4 rows (one column label, one control and 2 samples) and should include the following columns: description, soluble in water, soluble in oil, hydrophilic or hydrophobic. **Don't forget to include a table number and title.**
3. Obtain 3 1-2 mL test tubes and place them in a test tube rack. Label the tubes 1-3.
4. Fill each tube about $\frac{1}{4}$ full with baby (mineral) oil. Add $\frac{3}{4}$ mL water using a transfer pipette ($\frac{3}{4}$ ml is the third mark from the tip of the pipette).
5. Add the following to each tube:
Tube 1: 5 drops of water (this is your control tube)
Tube 2: 5 drops of beet juice
Tube 3: 5 drops of beta-carotene
6. Close the lids on the tubes tightly and shake for 10 seconds to mix.
7. Place the tubes back in the rack and let them sit for one minute or until the control tube separates. Since oil is less dense than water, the oil phase (layer) will float on top of the water.

8. Record in your table what happened in each tube. Did the water and oil separate? Did the components added (tubes 2-3) dissolve in the water phase or oil phase of your tubes? Record whether each component added (tubes 2-3) is hydrophilic, hydrophobic, or amphipathic.

Remember that hydrophilic molecules should dissolve in the water phase in the bottom of your tubes, hydrophobic molecules should mix with the oil phase in the top of your tubes and amphipathic molecules may act as emulsifiers, allowing oil and water to form a stable mixture.

9. Look at the molecular structures below and make a conclusion about which bonds are polar covalent bonds (which tend to make molecules hydrophilic) and which bonds are nonpolar. Include C-C, C-H, C-O and O-H bonds in your statement.

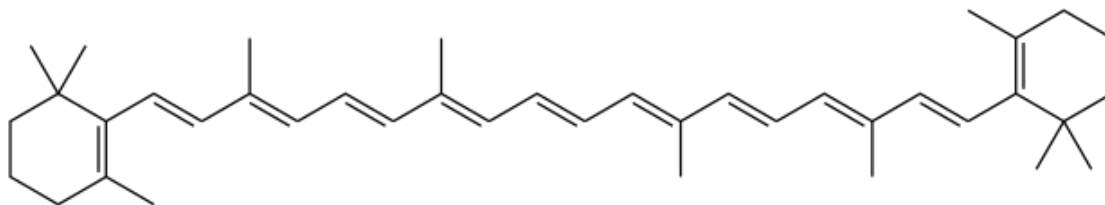
Figure 1: Betanin Structure



Betanin, $C_{24}H_{26}N_2O_{13}$, pigment in beet juice

Public Domain, <https://commons.wikimedia.org/w/index.php?curid=442301>

Figure 2: Beta-carotene Structure



Beta-carotene, $C_{40}H_{56}$, pigment in carrot juice

ByNEUROtiker-Ownwork,PublicDomain,

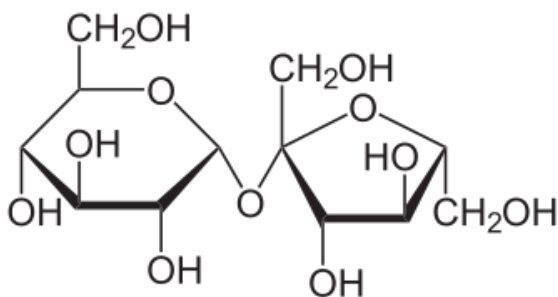
<https://commons.wikimedia.org/w/index.php?curid=2330317>

7. Experiment 2: Carbohydrates and Proteins

Introduction

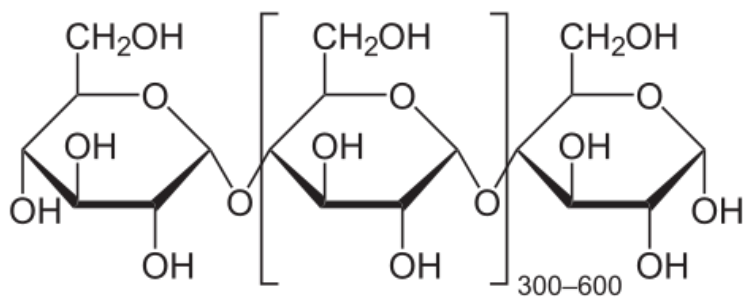
It is evident from your results in the first experiment that plant pigments differ in structure and water solubility. Other molecules in cells also vary in water solubility with some being hydrophilic and found in the cytoplasm and others hydrophobic and primarily associated with membranes. Today we will test some carbohydrates and a protein for water solubility. Remember from above that both contain carbon, hydrogen and oxygen and that proteins also contain nitrogen. Sucrose is a **disaccharide** composed of one glucose and one fructose bonded together. Starch is a **polysaccharide** composed of many glucose molecules. The structures are shown below:

Figure 3: Sucrose Structure



The disaccharide sucrose $C_{12}H_{22}O_{11}$, Public domain

Figure 4: Starch Structure



Starch, public domain

Phycocyanin is a protein made by some photosynthetic bacteria from the Cyanobacteria group including *Arthrospira*, from which the nutritional supplement spirulina is made. The protein consists of two polypeptide chains and, when bound to a small “chromophore” molecule, has a blue color. In cells, the protein assembles into “hexamers”, ring-like structures composed of six proteins. Phycocyanin is one of several pigments that these organisms use to absorb light for photosynthesis.

Procedure

1. Set up a table to record your results. It should be the same as the table for experiment one, except it should have enough rows for the samples listed (see below).
2. Write a hypothesis that answers the following question: Are sucrose, starch, and phycocyanin hydrophilic or hydrophobic? Predict whether these molecules will dissolve in water or oil.
3. Because sucrose and starch are colorless, we will test their solubility in water and oil in **separate tubes**. Obtain two tubes and label one H₂O and the other oil. Add about ½ mL of water to one tube and ½ mL of oil to the other.
4. Add ½ of a small spatula of sucrose from the sample provided to each tube and mix. A clear solution means that the sucrose dissolved, a cloudy solution means that it did not. Record your results in a table.

5. Set up an additional two tubes, one with oil, and one with water. Pipette several drops of starch solution into each and mix. Record your results.
6. Label and set up **one tube** (it should contain both oil and water) as described for experiment one.
7. Add two drops of phycocyanin solution to this tube and mix. Let settle and record your result.

8. Experiment 3: Using Polarity to Separate a Mixture

Separation of a mixture of molecules is a common task in the Biology laboratory and in industry. Examples include purification of blood proteins from plasma (the liquid portion of blood), separation of petroleum components to produce gasoline and motor oil, or purification of various natural compounds with medicinal value from plant tissues.

Methods for separating molecules from each other exploit differences in molecules' chemical properties. A number of methods separate molecules based on differences in polarity. One approach is to place material in a column-shaped tube and pour the mixture through the column, allowing some components to bind. Components of the mixture can then be eluted (washed off the column) using various solvents.

The mixture that you will separate today is grape Kool-Aid, which contains two dyes, one red in color and the other blue. The column used is called a Sep-Pak[®] C18 cartridge. It contains nonpolar beads. The relatively nonpolar dyes in the Kool-Aid should stick to the column. You will then elute the dyes from the column using a series of increasingly nonpolar solvent mixtures made by adding isopropyl alcohol to water.

Procedure

1. Do this experiment as a table (usually 4 people). **Wear goggles.**

2. Obtain a 6 well microplate, 3 10 mL syringes, and 50 mL tubes of grape soda, 5% isopropyl alcohol, and 25% isopropyl alcohol. Label 4 wells of the plate 1-4.
3. Place a few mL of the grape soda in the top left well of the plate (well 1).
4. Label a syringe “grape” and fill it with 5 mL of the Kool-Aid. Attach the syringe firmly to the long end of the column using a twisting motion. Push the soda through the column, collecting the liquid in well 2 of the plate. Note the color of the eluent and of the column.
5. Label a syringe ‘5%’. Fill with 10 mL of 5% isopropyl alcohol. Attach carefully to the column (make sure you are wearing goggles) and push through the column, collecting the eluent in well 3 of the plate. Note the color of the eluent and the column.
6. Repeat the above procedure except use 25% isopropyl alcohol and collect the eluent in well 4 of the plate. Note the color of the eluent and the column.
7. Describe the color of the liquid in each of your wells 1-4 if you have not already done so and list what liquid was placed into each.
8. Wash the column. Working in the fume hood, first push 10 mL of 70% isopropyl alcohol through the column. Repeat with 10 mL deionized water. Collect these eluents in the beaker provided.

9. Conclusion and Summary

Write a conclusion and summary for your investigation that addresses the following questions. Please number your answers.

1. Write a conclusion regarding your hypothesis for experiment 2 (sucrose, starch and phycocyanin).

2. If a molecule is nonpolar, is it most likely to be hydrophilic or hydrophobic?
3. Organic molecules in biological systems typically contain C-C, C-H, C-O, and O-H covalent bonds. Based on your results, which of these are nonpolar?
4. Describe two ways in which polar/nonpolar interactions are important in biological systems.
5. Including last week's experiment, you have worked with lipids, carbohydrates and proteins. Which of these is typically hydrophobic? Which is typically hydrophilic?
6. Because amino acids vary in polarity, proteins can be hydrophilic, hydrophobic or a mix of both. If a protein is found in an environment that is mostly water (cytoplasm, blood etc), would you expect hydrophilic amino acids to be on the inside or outside of the folded protein?
7. Describe two ways in which these experiments could be extended, i.e. what other experiments could you do to gain more information about biological molecules?
8. **Extra Credit:** Look up the molecular structures of FD&C Blue 1 and FD&C Red 40 dyes. Explain why Red 40 is eluted from the C18 column in 5% isopropyl alcohol but Blue 1 requires 25% isopropyl alcohol for elution.

References

Separation of Food Dyes Via Column Chromatography, University of Minnesota,
http://www1.chem.umn.edu/services/lecturedemo/info/Column_chromatography.html
Biology, Answering the Big Questions of Life/Organic Chemistry,
https://en.wikibooks.org/wiki/Biology,_Answering_the_Big_Questions_of_Life/Organic_Chemistry