How Science Works

Teacher Notebook

High School Biology

Module 1

Class Question:

How do living organisms change the physical properties of compounds?

Scientist (Your Name): _____

Teacher's Name: _____

SciTrek Volunteer's Name: _____

Key:
Answers
Information for Lead/Teachers
The student packet

TABLE OF CONTENTS

OVERVIEW & BACKGROUND INFORMATION

The following is from the Next Generation Science Standards document for High School Biology. These represent topics addressed in the "Ripening" module being developed for Mr. Sifontes, San Marcos High School.

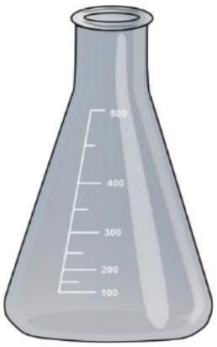
LS1-6 Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules. [Clarification Statement: Emphasis is on using evidence from models and simulations to support explanations.] [*Assessment Boundary: Assessment does not include the details of the specific chemical reactions or identification of macromolecules.*]

LS1-7 Use a model to illustrate that cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and the bonds in new compounds are formed, resulting in a net transfer of energy. [Clarification Statement: Emphasis is on the conceptual understanding of the inputs and outputs of the process of cellular respiration.] [*Assessment Boundary: Assessment should not include identification of the steps or specific processes involved in cellular respiration.*]

HS-LS2 [Cross cutting concepts] Cause and effect: empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects (HS-LS-8)

EXPERIMENTAL MATERIALS

Weighing Balance



Erlenmeyer Flask

Beaker

Graduated Cylinder¶

Timer

Heat Plate

Test Tubes

Plastic Pipettes

REAGENTS

Benedict's - an indicator used to detect simple sugars in a sample

Biuret - an indicator used to detect protein in a sample

Iodine (Lugol's Test) - an indicator used to detect starch in a sample

DAY 1 The Building Blocks of Life

Introduction of SciTrek - 5 minutes

The SciTrek team will give a short introduction about what SciTrek is and what we do. Starting with the lead, each member of the team will introduce themselves, their year and what they are studying. This should take no more than 5 minutes. The students are going to watch a video on how British Petroleum (BP, one of the biggest energy companies) is using bananas to make jet fuel. Don't directly answer questions posed by students -- this video is meant to simply have an idea rolling in their minds that there is some process to convert one substance to another.

Link to video: https://twitter.com/bp_america/status/1087520154815250433?lang=en

Let's see what you've learned so far! Take a few minutes to discuss the following questions with your classmates.

1. What is biology? Give some examples of some of the topics you have studied or think you will study in biology.

Biology is the study of life and living organisms Some of the topics the students cover in biology are biochemistry, cell biology, genetics, anatomy and physiology, ecology, evolution, and plant, animal & microbial diversity. Some students have personal experience with the subject; use this as an opportunity for the students to share.

2. Biologists sometimes call their field, "the study of life and living organisms". What are some examples of the "living organisms" that biologists study?

Bacteria, Fungi, Plants, Animals, and even just "cells" are all examples of living organisms that biologists study. The students will have just had a debate on whether viruses count as living organisms or not; ask them what they think.

3. What makes living organisms different from non-living things? What are some of the characteristics that make something living? HINT: they can change the composition of the molecules within their cells.

Living organisms are often characterized by their complexity in structure and function, by having ways to take, transform, and use energy from the environment, by being able to sense and respond to changes in their surroundings, by having mechanisms to either self-replicate or have offspring, and by the ability to change over time by gradual evolution. You typically don't see this in non-living organisms.

Demonstration: Water & Polarity - 10 minutes

This is a DEMO, which means that the lead will be at the front of the class. Volunteers will have minimal participation in this activity. Students should learn from this activity that the miscibility (ability of one liquid to mix with another) can be used to test for polarity.

Materials: *4 x 100 mL Beakers, DI Water, 1 gram each of the following:*
Vegetable Oil, Whey Protein, Sugar, Salt

Instructions:

1. *Fill up all four beakers with 50-60 mL of DI water.*
2. *While lecturing to the class, individually add all of the four 'solutes' to the water. All should dissolve except for the oil.*
3. *Show the chemical structure and polarity diagrams of all molecules and H_2O.*
4. *Ask the class what patterns they see and how they can use the patterns to determine the polarity of a molecule.*
5. *Check out this simulation from the Concord Consortium:* *http://lab.concord.org/embeddable.html#interactives/sam/intermolecular-attractions/3-1-oil-and-water.json*

Questions for the students to answer:

Draw the setup of the tank in front of the classroom. Did the water and vegetable oil mix or did they form layers? What happened to the sugar, protein, and salt as they were added to the mixture. Did they spread evenly throughout the mixture, or did they go completely into either the water layer or the oil layer?

(insert drawing of the tank setup here)

The water and vegetable oil form two distinct layers, proving that water and oil have different polarities. When the salt, protein, and sugar are added, they should not dissolve in the oil layer, but in the water layer.

Water is polar because it has an unequal sharing of electrons between atoms. That means one side of a water molecule is more positively charged, and the other side is more negatively charged. Have a look at this simulation from the Concord Consortium.

In the simulation, what happens to the mixture of polar molecules with nonpolar molecules over time?

The polar molecules and nonpolar molecules rearrange to form two separate groups of polar and nonpolar molecules. They don't mix together or bond together.

Introduction to Macromolecules - 25 minutes

After introductions and vocabulary activity, the lead will explain to the students what macromolecules are using slides 4-6 on the powerpoint. They will then help the students answer the following questions with the help of the PowerPoint presentation. These questions are found in the student notebook, so remind students to write down the answers. The following strategies are recommended for leading the discussion: 1) ask for volunteers, 2) ask students to discuss in pairs or groups of 4-5 before answering, 3) if no one has an answer, use the answers below to guide your explanations to the class.

Compounds can be organic, inorganic, or both

Organic - compounds that contain both carbon and hydrogen atoms

Inorganic - compounds that DO NOT contain both carbon and hydrogen

There are four classes of organic compounds that are central to life on earth.

1. Carbohydrates 2. Lipids 3. Proteins 4. Nucleic Acids

Carbohydrates (Sugars and Starches) - compounds made of C, H, and O which give us energy. Carbohydrates can be **simple sugars** that give us quick energy, like fruit and white bread, or they can be **complex carbohydrates** that give us long-term energy, like starch found in potatoes, rice, corn. Carbohydrates can also be used for structural support in some organisms: cellulose is a carbohydrate that makes up the cell wall of plants (wood), and glycogen is a food storage compound in animals.

monomer: _____*monosaccharide*_____ polymer: _____*polysaccharide*_____

Lipids (Fats, Oils, Waxes) - compounds made of C, H, and O which store energy. Some lipids have structural functions: Plant wax is a lipid that keeps plants from dehydrating, and cholesterol is a lipid found in membranes of cells and organelles (your cells have this). Lipids are mostly **nonpolar**, meaning they do not mix well with polar molecules. Lipids are not polymers; A common lipids called a triglyceride consists of 3 fatty acids and one molecule of glycerol.

Not really a monomer.. repeating unit(s): _____*3 fatty acids and glycerol*_____

Not really a polymer.. repeating unit: _____*triglyceride*_____

Proteins (long chains of amino acids) - compounds made of C, H, O, and N which have many functions and are an energy source. The monomers of proteins are called **amino acids**, and the bonds that hold amino acids together are called **peptide bonds**. Proteins are used to build and repair hair, nails, and muscle tissues. A special class of proteins, called **enzymes,** are used to speed up the rate of chemical reactions. Enzymes are used for digestion, respiration, reproduction, vision, movement, thought, and other various tasks.

monomer: _____*amino acids*_____ polymer: _____*proteins*_____

Nucleic Acids (made up of nucleotides) - compounds made of C, H, O, and N which include DNA and RNA. The monomers of nucleic acids are called **nucleotides** which are composed of a nitrogenous base, a 5-carbon sugar, and a phosphate group. Really important nucleic acids called DNA stores genetic information. Another important nucleic acid called RNA makes proteins.

monomer: _____*nucleotides*_____ polymer: _____*nucleic acids*_____

Trading Card Activity - 15 minutes

The next activity the class will do is a trading card game in which they trade with their classmates to produce organisms. The objectives and rules are described below:

Objective: You and your group are working together as a plant cell. You have limited resources (element cards) that you need to build another organism (reproduce). Trade with the other cells in your class so that you have enough biomolecules to survive and reproduce.

Types of cards:

Stage 1 – elements - hydrogen, carbon, oxygen, nitrogen
Stage 2 – sugar
Stage 3 – lipids, carbohydrates, protein, DNA
Stage 4 – organism

Recipes:

To make:	You need:
Sugar	2 hydrogens + 1 carbon + 1 oxygen
Lipid	2 sugars
Carbohydrate	3 sugars
Protein	2 sugars + 2 nitrogens
DNA	2 sugars + 2 nitrogens
Organism	1 lipid + 1 carbohydrate + 1 protein + 1 DNA

Rules:

1. Shuffle the element cards.
2. Each group is provided 25 random element cards and an instruction card to begin (take out one nitrogen card to make 125 element cards total). During this time, the groups can sort out their cards.
3. With the cards given to each group, consult the formula on the instruction card to use your elements to construct your sugar molecules first.
4. After constructing sugar molecules, the groups can trade in their sugar molecules for larger molecules from a SciTrek volunteer (the volunteer will have the sugar and macromolecule cards)
5. Groups are allowed 45 seconds to discuss trading plans to trade any cards for other cards with another group (ex. Give up nitrogen for 2 hydrogens). Start a timer for 45 seconds.
6. Once the 45 seconds are up, have each group send one representative to the front of the room. The representatives will trade with each other for 1 minute, then return to their groups.
7. Repeat steps 5 and 6 until a group forms an organism. The first group to form an organism wins.
8. To push the game along if the trading dies down and no organisms are formed:
    a. At 7 minutes, photosynthesis occurs! Give each group 2 sugar cards.
    b. At 11 minutes, fertilization occurs! Give each group 2 nitrogen cards.

*(*A volunteer/lead should prepare a 70^0C water bath at the front of the class during the game*)*

Questions after the Game - 15 minutes

1. Circle the following macromolecules that are considered "building blocks" of organisms.

Carbohydrates ***Proteins*** ***Lipids*** **LEGO** ***Nucleic Acids***

2. Why does your body need carbohydrates and where can you find them?

Carbohydrates are an immediate source of fuel. The body uses carbs (through processes like glycolysis and respiration) to make a molecule called adenosine triphosphate [ATP], a major energy source. We get carbs by eating things like grains, rice, and bread. Without carbs, our bodies will have to break down fats and proteins for energy, which occurs when you are starving. .

3. Why does your body need proteins and where can you find them?

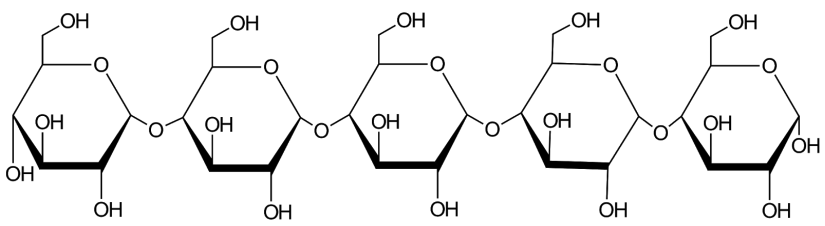
Proteins do a lot for the body. We need protein to build and repair the body's structures like our nails, hair, cartilage, and muscles. We also have a special case of proteins called enzymes, that help the body do chemical reactions like digesting food. Proteins are made from

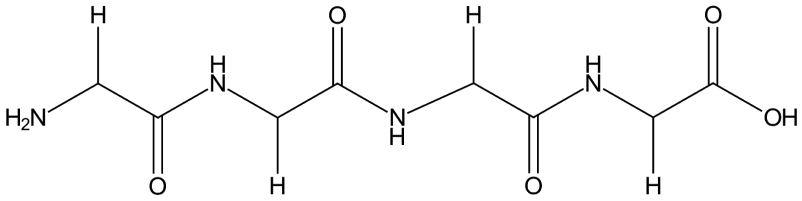
monomers called amino acids, and since the body call make all the amino acids, we need to get them from the food we eat. We get protein from milk, eggs, meat, some plant-sources, rice, corn, and beans.

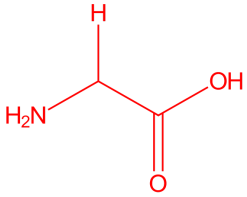
4. Why does your body need fats and where can you find them?

Fats are essential for giving the body energy and supporting growth. They also insulate the body. We can get fats from avocados, cheese, dark chocolate, oily fish, nuts, and chia seeds.

5. It takes a lot more than one molecule each of carbohydrates, proteins, fats, and DNA to make an organism. What actually happens is that carbs and proteins will form long chains called **polymers** made of individual repeating units called **monomers.** Given the pictures of a carbohydrate chain and a protein chain respectively, box and redraw the repeating monomer unit.







If the students ask, not all lipids are polymers, and DNA they will discuss sometime later.

6. A SciTrek volunteer is having a hard time trying to get lipids to dissolve in water. Why would lipids and water typically not want to mix together: What might you add to help the lipids dissolve in water? Hint: why do you use shampoo to wash your hair? What are you trying to get rid of?

   Lipids are nonpolar but water is polar, so the two will not mix without the help of additional molecules. You can add soap or detergents to help dissolve lipids in water.

DAY 2 Testing for Biomolecules

Caution: Concentrated acids should only be used by the lead and placed in a safe area when not in use. Affected areas should be washed with soap and water for 10 minutes.

It's going to be Legend- wait for it- Dairy

Demonstration: What's in my Milk? - 10 minutes

The students should now be familiar with the concepts of carbohydrates, proteins, and fats, and recognize that these macromolecules are the building blocks of living organisms. The next question to ask the students is how we are able to know that. The lead will show a jug of perfectly homogenized milk and ask the students how we can tell that this completely white liquid is actually made of different macromolecules. The students will need a way to separate out the protein from the milk. In other words, the students need some "analytical tests" that are used to detect the presence of these molecules in a sample.

The lead will perform a demonstration in front of the class. The lead will pour a small amount of milk into a 100 mL beaker, making observations about the physical properties of the milk. (It's homogenous, white, and free flowing). Then the lead will add about 1.5 mL of concentrated acid (6 M HCl) into the milk and mix the contents of the beaker. The beaker will then be passed around the class for the students to make notes and observations.

Students will be introduced to this process as curdling. Curdling occurs naturally in cows' milk if left open to air for a few days in a warm environment. (We are accelerating the process of milk rotting in order to finish this module in the span of two days). The protein in milk is normally suspended in a colloidal solution, which means that the protein molecules float around freely and independently. Normally these protein molecules will repel each other, allowing them to float about without clumping, but as the pH drops and becomes more acidic, the protein (carding and others) molecules attract one another and become curdles, clumps floating in a solution of translucent whey.

The solid mass of protein will be filtered and dried with a heat gun to quantitatively determine the concentration of protein in the sample. This is a nice exercise for the students to recognize that biological samples can be separated into different macromolecules. Once the students make observations of the sample in their notebooks, they will have the chance to test this process on their own with both milk and yogurt sample. Yogurt is a fermented dairy product made from by certain acid-producing bacteria like Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. The bacteria produce lactic acid in milk, causing

the milk protein to curdle. The yogurt samples are made from the same milk samples that are provided by the SciTrek volunteers.

Questions for students:

In the boxes below, draw a picture and write a short description of the milk before and after acid is added to it. What does the milk look like in each case? Is the milk transparent or does it have a solid color? Is the milk a perfectly uniform liquid, or can you see chunks floating in the milk?

Untouched Milk	**Acidified Milk**
The milk looks homogenous. It does not seem to have any solids floating within it. It is impossible to see anything while looking through this liquid.	*After 1.5 mL of 6.0 M HCl is added to the milk, there is some clumps of white stuff floating within the Erlenmeyer flask. The milk no longer seems to be a homogenous liquid as it is possible to see through some of the liquid.*

How does the milk change after acid is added to it? Why do you think the milk changes this way in the presence of acid? Talk with your classmates and SciTrek volunteer and see if you can come up with an explanation for what is happening. Hint: what do you think happens when you hard boil an egg? It was liquid and then…?

_____

_____

_____

_____

Experiment: Getting the protein out of milk

1. Pour 15 milliliters (mL) of diluted milk into a 100 mL beaker; the milk is diluted five fold (one part milk, four parts water)
2. Ask the SciTrek volunteer to add acid (2.5 mL HCl) to your beaker
3. Swirl the mixture and observe what happens to the protein. Record your observation in the margins of this page. Let solution sit for 5 minutes.
4. Fold 2 pieces of your filter paper together as directed by the lead/volunteers.
5. **-Important-** Weigh both of your filter papers before proceeding with step 6 and record the weight in the chart below.
6. Place the folded filter paper into a plastic funnel and place the funnel into a vacuum flask.
7. Filter the samples by pumping air out with a hand pump until all liquid has passed the filter or after 20 minutes have passed. Switch off with another student to keep pumping if student gets tired.
8. Place the wet filter paper + protein on a paper towel.
9. Begin drying the filter paper with a heat gun on high. For best results, have the heat gun 5 cm (~2 inches) away from the filter paper. *(While you wait go to page 20 for the wait activities!)*
10. Weigh the wet filter paper after drying 5 minutes, then continue drying and record the mass in intervals of 2.5 minutes (150 seconds). If a greater than 0.05 gram change was observed, continue to dry for 2.5 minutes and weigh again until a change no larger than 0.05 grams is reported.
11. Weigh the final dry filter paper + protein and then calculate the mass of the protein for each dry sample.
12. Use this information to calculate the percent mass of protein in the original **undiluted** solution. Ask the lead or volunteers for help if needed. The equation to find this is:

*Mass of Protein (undiluted sample) = Mass of Protein (diluted sample) * (**dilution factor**)*

Record all of your data in the tables on the next page.

Dairy Type	**Nonfat Milk x20**
Mass of filter paper	
Mass of the filter paper and protein (dry: 5 min)	
Mass of the filter paper and protein (dry: 7.5 min)	
Mass of the filter paper and protein (dry: 10 min)	
Mass of protein (diluted sample)	0.00265g
Mass of protein (undiluted sample)	0.053g

The students should be collecting data in their notebooks while they follow the procedure. The students can then compare the mass of protein in the milk and yogurt and discuss observations and conclusions that they make. The lead should encourage the sharing of data between the groups to see if everyone got similar results. The most consuming parts of this procedure are the filtration and drying. During these steps, the students can help themselves to the "While you wait..." activities on the following pages.

Once the milk is done filtering and drying, the students should be able to analyze their data using the following questions.

1. The expected values for the mass of protein are *0.6 grams for milk* and *2.2 grams for yogurt*.
    a. Calculate the percent error between your experimental values and the expected values.

$$Percent\ Error = \frac{|your\ value - expected\ value|}{expected\ value}$$

    b. Were your values above or below the expected values? What could explain your variation?

- *For data above expected values:*
    - *Filter paper not completely dried.*
    - *Impurities in the protein sample (could still contain some carbs)*

    - *Inconsistent measuring of reagent volumes.*
- *For data below expected values:*
    - *Lost protein material during the drying process.*
    - *Impurities in the protein sample (could still contain some carbs)*
    - *Inconsistent measuring of reagent volumes.*

2. With the help of the lead in the front of the classroom, compare your data to other groups. Calculate the average value for protein mass, range of values, and standard deviation.

Mean Average = Sum of all of the numbers / How many numbers there are
Range of Values = Maximum Value - Minimum Value
Standard Deviation = $\sqrt{\frac{(your\ protein\ mass - mean\ average\ protein\ mass)^2}{n-1}}$

Introduction to Analytical Spectroscopy - 30 minutes

Congratulations! What you just did was used for a long time to determine protein amounts in dairy and other foods. It works with large samples but takes a bit of time. Modern methods used for example in the food industry make use of spectroscopy, where the concentration can be determined using chemical indicators.

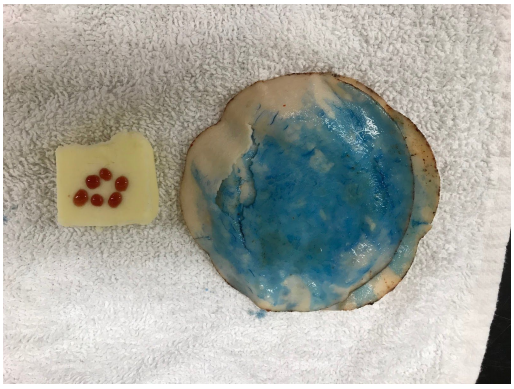
Gravimetric analysis is great and effective when you have a relatively large amount of sample, but if you look at the weigh scales we have, they only go up to two decimal points. What if I want to measure out something in milligrams or track a small change in carbohydrates, protein, or fat content? Let me introduce you to my little friend…..

Lead plunks ThermoScientific Spectronic 15 in front of the class and everyone says ooo
SciTrek Lead: *slaps roof of spec* this bad boy can fit so many mgs of sugar over it.

An Introduction to Calibration Curves
The students will have a link to Colby's slide deck. The link can be found here:
https://docs.google.com/presentation/d/14wuZGweie8ZcU_SIgUMmfT_Usb4VuXGv_gK2KrwC1z8/edit#slide=id.p

Demonstration: Bradford Reagent Test for Detection of Proteins

The SciTrek lead will call attention to the chicken and butter that they have on the front desk. As the name of the test suggest, Bradford tests for proteins. This can be easily visualized by adding Bradford's reagent to protein heavy food (chicken and non-protein food (butter).



Note the change of the Bradford to blue on the chicken (right) but not the butter (left). Chicken contains proteins, but butter does not.

A SciTrek volunteer will show the class 5 test tubes that contain varying concentrations of starch (%Starch: 0.01%, 0.001%, 0.0001%, 0.00001%, 0 (control)). Percents are given in mass per volume, meaning that 0.1% starch solution was made by adding .1g to 100mL water.

Test the class by asking what the purpose of the control is. What is in the color? What color should it turn when we add iodine indicator? Why do we need a control?

100% water, the control solution turns clear with bluish tint with the addition of the Bradford Reagent.

The chemical the students will be using in this test is Bradford reagent. It forms a complex with protein which gives off a blue color. The greater the concentration of protein, the darker blue the solution will be. ***Students will observe the volunteer add 14 drops of Bradford to the samples****.*

Is there a pattern of how the solution color changes with increasing starch concentration? Draw and color the series of solutions below.

The other test tubes with protein turn blue.

Control = clear , 1/1000 protein = blue, 1/10000 protein = light turquoise, 1/100000 = turquoise, 1/1000000 = dark turquoise

Ask the students how they could use this series of solutions to figure out how much protein is in an unknown solution? We typically call a series of solutions a **gradient**. There's a different name that the students have learned before, a **calibration curve.**

You need to know the concentrations that were used in the gradient, then perform the Bradford's test on a sample and match the resulting color to the colors in your gradient.

Ask the students, what are some limitations to this test? What are some problems in determining the quantity of each solution?

Color of the sample will interfere with the color produced from the reagent. Small variations in concentration are difficult to observe. Hard to quantify the exact concentration, that is if you are eyeballing it.

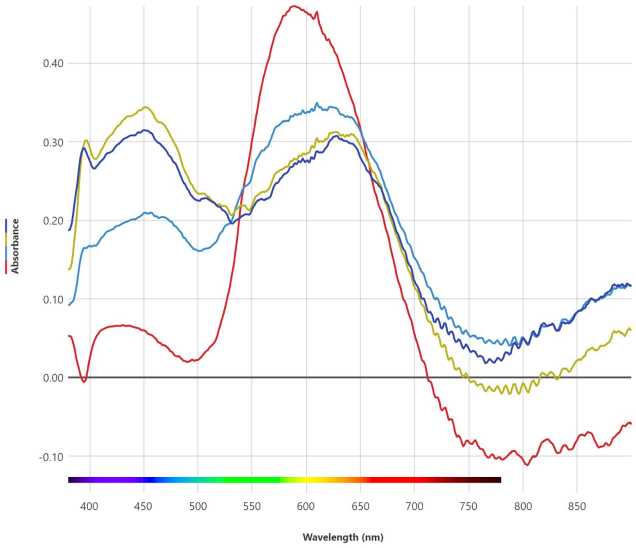
Ah, but what if we had a way to do better than simply eyeballing the color? This one is for all you color-blind people out there. The lead will take samples of each solution into a cuvette and measure their absorbance at 610 nm. After getting a series of absorbances, the lead will make a table on the board that looks like this:

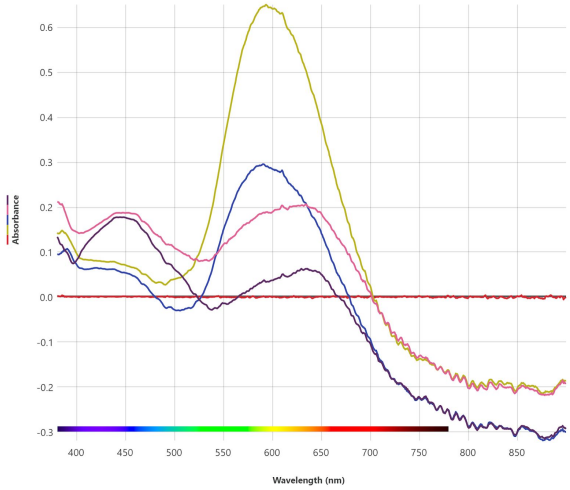
Concentration of Protein	**Absorbance of Sample @ 610 nm**
Control	*0*
0.01%	*~0.465-0.633*
0.001%	*~0.282-0.349*
0.0001%	*~0.204-0.305*

0.00001%	~0.050-0.288

The students will see that there is a linear relationship between the concentration of the starch and the absorbance on the spectrophotometer. This is called a ***standard curve*** *or calibration curve. The lead will start a presentation on how to use calibration curves to help determine the concentrations of unknown samples.*

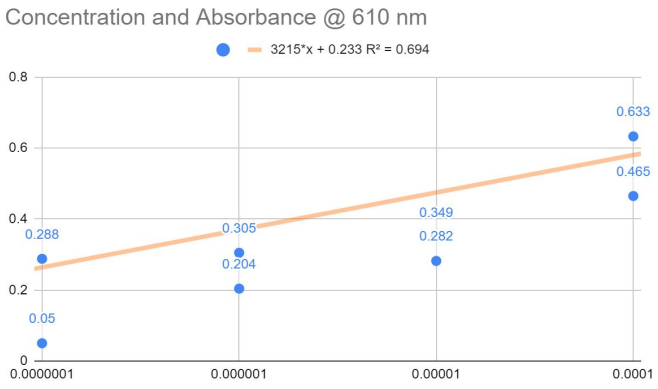
While this presentation is happening, Bradford is going to be added to the milk sample to determine its protein concentration. However, a problem that the students will run into is that when the Bradford reagent is added to the milk, it will immediately react with the milk, but it will be too opaque to get a good reading on the spec. And it will not mix well do due this reaction.





Here is what the spec graphs should look like:

Here is what the best line of fit should be around:



Day 2 Waiting Activities

Vocabulary Activity

Have students review concepts and vocabulary terms. Maybe have a quiz for the next day for review before Day 3. Explain how organic molecules and atoms (C, H, N, and O) are conserved to make different organic molecules and are reused in biological processes.

It is important that you (the lead) have drawn this box on Mr. Sifontes's whiteboard as the kids are working on extracting protein from milk.

First, the class will be divided into 8 groups (One for each definition). Make a table with eight boxes on the whiteboard; each box will contain one of the terms found below. One volunteer should be assigned to each group. Each group will then be given a card with multiple ways to identify which term they are looking for and will try to match the definitions to the corresponding term. Cards will include definitions, pictures of the molecule, and real-life examples. After each group has decided which definition, picture, and an example for a term, they will compare their answers with other groups and ask each other why they believe their answers are correct, explain their reasoning, and have time to change their answers. After each group has finalized their answers, the lead and volunteers will go over the right definitions for each of the vocab terms, and students will write down the answers in their notebooks.

Together with your group, come up with a thorough but concise definition of the following vocabulary terms on the table. Use no more than ten words per definition.

Carbohydrates	*Compounds with the molecular formula $(CH_2O)_n$ composed of small subunits called monosaccharides or sugars*
Lipids	*Can be a fat or oil depending on whether they are solid or liquid at room temperature, respectively*
Macromolecules	*A macromolecule is a very large molecule made from smaller molecules*
Monomers	*Single subunits that can be strung together to make large molecules*
Nucleic Acids	*Biomolecules with subunits that are composed of a phosphate group, a sugar, and an identifying molecule.*
Polymers	*A chain made of monomers. The common polymers of life (biopolymers) are nucleic acids, proteins, and carbohydrates*
Proteins	*Polymers made of amino acids.*
Starch	*A large and complex type of carbohydrate. polymer of many sugars bonded together.*

Nutrition Facts Activity

While the class continues their work on extracting proteins from milk, the lead will utilize the PowerPoint to assess each student's knowledge of everyday food and their compositions (acc. Nutrition Facts). On the PowerPoint, there will be slide introducing the activity, followed by multiple slides with images of food. There are two slides for each type of food. The first slide of a food is an image alone, while the second slide contains the image of the food with a nutrition label for the food. The task for the students is to identify which macromolecule is most abundant in these foods prior to looking at the nutrition facts.

The students will vote for a specific macromolecule (protein, lipid, carbohydrate, or nucleic acid) by raising their hands for the macromolecule of their choice. Have the students predict the main component prior to showing the answer. Their options are:

- *Proteins, Lipids, Carbohydrates, nucleic acids (This answer is always wrong)*

After students have cast their votes, the lead should ask for volunteers to justify their answers. (i.e. "Could someone that voted for protein explain their reasoning please?") The lead can ask for explanations from people with conflicting votes to hear multiple perspectives. Once discussions come to a close, the lead can change the slide to show the nutrition label and explain the major macromolecule in each food.

- *For the first nutrition label slide, point out that the correct answer is the one with the highest number of grams, which is why it is circled in green*

On the last food slide (tofu + nutrition label), there is a note that points out the fact that even though nucleic acids are not shown on the nutrition facts, they are still present in our food. Here is an interesting calculation that can help us think about this topic.

Roughly 1% of the mass in the human body is DNA and RNA. These numbers are likely lower in refined food, which leads to the assumption that the quantities of nucleic acids are too small to make it onto the nutrition facts label.

Together with your group, rank the following foods based on their macromolecule composition. Rank the macromolecule that is highest abundance as 1, and the lowest abundance as 3. If you think the food does not contain any of a particular macromolecule, write a 0 on the line.

Banana:	Carbohydrate: __1__	Protein: __2__	Fat: __3__
Brown Rice:	Carbohydrate: __1__	Protein: __2__	Fat: __3__
Butter:	Carbohydrate: __2__	Protein: __2__	Fat: __1__
Tofu:	Carbohydrate: __3__	Protein: __1__	Fat: __2__

DAY 3 Calibration Curves

Colorimetric Calibration Curve Activity

Today we are going to bring the unknown food samples to the students. The students will go around station-by-station to get hands-on practice doing the Benedict's (sugar), Biuret (protein), and Lugol's (starch) tests. They will take their solutions to the front and measure the absorbances on the spectrophotometer.

How to use the SpectroVis Plus Spectrophotometer

1. *Download the SpectroVis Plus Spec software onto a device (computer, laptop, iphone even, requirement: must have a USB port)*
2. *Plug the spectrophotometer into your device and open the downloaded software*
3. *Once the program window appears, an option list should pop up. Under the "Absorbance" drop-down select "vs. Concentration (Beer's Law)". This will allow you to measure the absorbance of a sample at the wavelength of your choice.*
4. *Wait for the spectrophotometer to warm up. This should take less than a minute. In the meantime, prepare a blank cuvette with H2O, which the program will prompt you to insert for calibration when it is finished warming up. When the spectrophotometer is ready for the blank, put in the cuvette and press "finish calibration."*
5. *A window called "Choose a Wavelength" will pop up. Type your desired wavelength to the nearest whole number and press enter. You are now ready to start measuring samples!*
6. *Load your sample into the cuvette (must fill to at least above the V shape) and insert the cuvette into the spectrophotometer. Press "collect", wait a few seconds, then press "keep" to collect a data point. Type in the corresponding concentration and press "keep point" to save it. Press "stop" to collect.*
7. *Repeat until you have created a graph.*

Test #1 – Benedict's Reagent for Carbohydrates

(Caution: Hot objects ~ test tubes and hot plate ~ can lead to painful burns. Be careful!)
The first station that the students will have set-up is the Benedict's test, which tests for reducing sugars. This station will require a water bath on a hot plate (~100^0C) and materials necessary for the experiment.

Materials

- Hot plate + beaker of hot water (~250mL H_2O @ boiling) + stir bar (prepared beforehand)
- Thermometer
- Benedict's solution
- 0.05% dextrose solution prepared beforehand (100-fold dilution of 5% dextrose solution)
- DI water
- 10mL graduated cylinder + 50mL beaker
- Plastic pipettes
- Labeled test tube rack + 5 labeled test tubes (A, B, C, D, and E)
- Spectrophotometer + cord
- Laptop
- Cuvettes

Calibration Curve Procedure:
Prepare the hot water bath beforehand (takes ~30 minutes to heat). Plug the spectrophotometer into your device and open the Vernier Spectrovis Plus software. Choose the "vs. Concentration" option of data collection. The spectrophotometer will take a few seconds to calibrate so in the meantime prepare a blank by filling a cuvette with pure water. Insert the blank when prompted and press "finish calibration" to blank the spectrophotometer. When prompted, input the desired wavelength (750 nm) for the Benedict's assay.

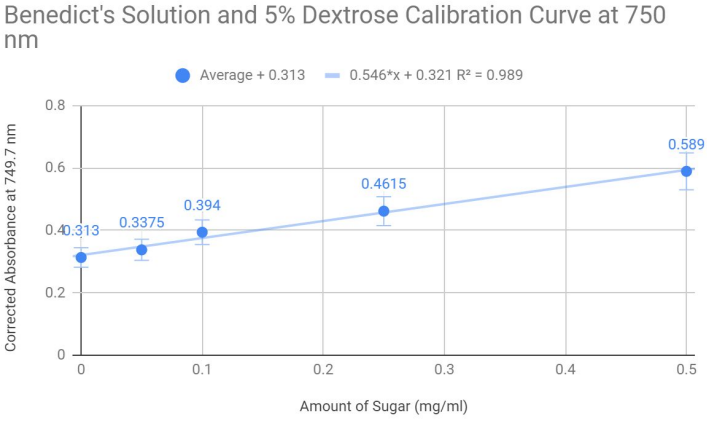
1. To prepare the calibration curve, start by adding 0 mL of H_2O to test tube A, 5 mL of H_2O into tube B, 8 mL of H_2O into tube C, and 9 mL of H_2O into tube D. Add 10mL of H2O to test tube E.
2. Obtain 18 mL of 0.05% dextrose stock solution from your Scitrek volunteer.
3. Take 10mL of the dextrose stock and add this to tube A.
4. Take 5 mL stock and add it to test tube B. Mix by swirling.
5. Add 2 mL of stock to tube C. Swirl to mix.
6. Add 1 mL of stock to tube D and mix.
7. Tube E will just have water.
8. Add 1 mL of Benedict's solution to each of the test tubes, then carefully lower them into the pre-prepared water bath. For ~ 3 minutes.

9. Carefully remove the tubes (only grasp the top of each tube since the bottom may be hot) and replace them on their rack.
10. To measure the absorbance of each sample, use a plastic pipette to fill a clean cuvette with sample until the sample volume reaches at least ⅔ of the cuvette height. Give the cuvette to your table's designated lead. *Remove the blank and insert the sample. Press "collect" on your device screen and wait for a few seconds before pressing "keep." Type in the corresponding concentration of your sample in the table that pops up (the concentrations are the same as in the example table at the bottom of this section). Press "keep point" to save your data.* Repeat this for each of your samples.
11. Rename "Data Set __" with "Benedict's Calibration Curve" and your table number. Press the three dots in the upper right hand corner next to the table's title to change the table name. If you've labeled your table well, you'll be able to determine the corresponding absorbance values for each sample.
12. Clean out your cuvette by emptying it into a waste beaker. Add water to it, pipetting up and down to flush out any remaining sample, and empty out the cuvette into the waste beaker once more. Do not leave sample in your cuvette since precipitate will get stuck inside it.
13. Report your absorbances to the lead at the front of the classroom to plot your data.

	Test tube A	**Test tube B**	**Test tube C**	**Test tube D**	**Test tube E**
Sugar Solution Volume	Solution A = $\frac{10\ ml\ stock}{0\ ml\ H_2O}$ (most sugar)	Solution B = $\frac{5\ ml\ stock}{5\ ml\ H_2O}$	Solution C = $\frac{2\ ml\ stock}{8\ ml\ H_2O}$	Solution D = $\frac{1\ ml\ stock}{9\ ml\ H_2O}$	Solution E = $\frac{0\ ml\ stock}{10\ ml\ H_2O}$ (least sugar)
Benedict's Amount	1 mL	1 mL	1 mL	1 mL	1 mL
Expected Observation	*Orange*	*Brownish Orange*	*Brown*	*Brownish Blue*	*Blue*

For those of you who are interested in the specifics behind the reagent, Benedict's reagent is made with copper (II) sulfate. When reacting with a reducing sugar, the copper(II) ions which appear blue are reduced to copper(I) ions which appear red. This is the cause of the color transition you see above.

Concentration of Sugar	Absorbance of Sample @ 749.7 nm
0.050%	*~0.5890*
0.025%	*~0.4615*
0.010%	*~0.3940*
0.005%	*~0.3375*
Control	*~0.3130*
*stock solution is 0.05% dextrose (simple sugar)	



Test #2: Biuret Test for Proteins

The second test that the students will be doing uses Biuret solution, which tests for proteins. Show the class 6 test tubes that contain varying of albumin (Protein: (0 mg/ml (control), 0.16 mg/ml, 0.8 mg/ml, 4mg/ml, 10 mg/ml, 20 mg/ml). Add Biuret reagent to the samples and mix. Aid the class in answering the questions in their notebooks.

Materials:

- Labeled test tube rack, Biuret solution, Plastic pipettes, DI water
- Albumin solution (200 mg/ml)
- 6 labeled test tubes of varying albumin protein concentration (A, B, C, D, E, F)
- 10mL graduated cylinder
- Plastic pipette
- Spectrophotometer + cord
- Laptop
- Cuvettes

Procedure for Biuret Assay and Calibration Curve:

1. To begin the calibration curve, first take 2 ml from the albumin solution using a pipette and add it to test tube A. Then add 18 ml of H2O to test tube A. Mix solution in test tube by swirling.
2. Next, add 8 ml of H2O each to test tubes C, D, and E.
3. Take 2 ml from test tube A and add to test tube C. Swirl solution.

4. After test tube C is mixed, take 2 ml from test tube C and add to test tube D. Swirl solution.
5. Take 2 ml from test tube D and add to test tube E then swirl.
6. For test tube B, take 5 ml from test tube A and add 5 ml of water. Swirl to mix
7. Add 10 ml of H2O for test tube F. This will be your control test tube.
8. After the test tube solutions are prepared, add 8 drops of Biuret solution to each test tube and observe the change in color. Record observations of color in the chart.

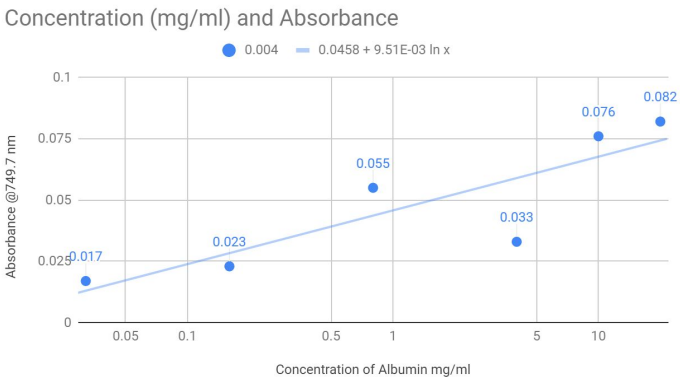
The spectrophotometer should be plugged into your device and the Vernier Spectrovis Plus software should be open. Choose a New Experiment by clicking the File Menu on the upper left corner. Choose the "vs. Concentration" option of data collection. The spectrophotometer will take a few seconds to calibrate so in the meantime prepare a blank by filling a cuvette with pure water. Insert the blank when prompted and press "finish calibration" to blank the spectrophotometer. When prompted, input the desired wavelength (590 nm) for the Biuret assay.

9. Get a clean pipette and pipette out a sample from test tube A into a clean plastic cuvette until the cuvette is filled ⅔ of its total volume. Give cuvette with sample to your lead so the lead can insert your cuvette into the spectrophotometer at the wavelength 590 nm.
10. Click "Collect" to measure the absorbance of the sample. Wait for the Absorbance Value to stop changing after a few seconds and click "Keep." Type in the concentration of the sample you're testing. For example, if you're testing test tube A, name the concentration as "20 mg/ml." Label your tables well so you'll be able to keep track of all the corresponding absorbance values for each sample.
11. Record the absorbance value for test tube A in your notebooks. After you saved the absorbance value and the concentration, click "Stop" to reset for measuring absorbance for another sample.
12. After your lead gives you back your cuvette, dump the sample into a waste beaker. Add water to the cuvette and pipette up and down to flush out any remaining sample. Rinse again with water to clean the cuvette.
13. Repeat steps 9-11 for each remaining test tube to get absorbance values for all your samples until you generated an Absorbance vs. Concentration graph from all the data points you recorded.
14. After getting absorbance values for all your test tube samples, report your data to the lead at the front to be plotted.

	Test tube A	Test tube B	Test tube C	Test tube D	Test tube E	Test tube F
Protein Concentration	20 mg/ml	10 mg/ml	4 mg/ml	0.8 mg/ml	0.16 mg/ml	0 mg/ml (control)
Biuret Amount	8 drops	8 drops	8 drops	8 drops	8 drops	8 drops
Expected Observation	*Light Pink Yellow over time*	*Pink-purple*	*Purple*	*Dark purple*	*Light blue*	*clear*

For those of you who are interested in the specifics of the reagent, proteins have a unique type of bond called a peptide bond. The copper(II) ions in the Biuret solution turns into copper(I) ions when there are peptide bonds in the solution, resulting in the color change.

Concentration of Protein (mg/ml)	**Absorbance of Sample @ 590 nm**
20	*~0.082*
10	*~0.076*
4	*~0.033*
0.8	*~0.055*
0.16	*~0.023*
Control	*0.0000~0.004*



Test #3: Testing for Complex Carbohydrates with Potassium Iodide

Materials:

- Labeled test tube rack,Potassium Iodide solution, Plastic pipettes, DI water
- Starch solution (200 mg/ml)
- 10mL graduated cylinder
- Labeled test tube rack + 6 labeled test tubes (A, B, C, D, E and F)
- Spectrophotometer + cord
- Laptop
- Cuvettes

Procedure:

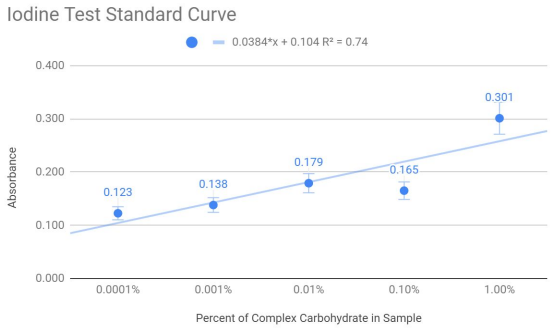
1. To prepare the calibration curve, start by adding 9 mL of H_2O to test tube A, 9 mL of H_2O into tube B, 9 mL of H_2O into tube C, and 9 mL of H_2O into tube D, and 9 mL of H2O to test tube E. Add 10 mL of H_2O to test tube F.
2. Add 1 mL of the starch stock solution to test tube A. Mix the test tube by squirting the pipette a few times until the solution looks evenly distributed.
3. Add 1 mL of the solution in test tube A and add it to test tube B. Mix using the pipette.
4. Do the same to test tube C. Mix using the pipette.
5. Do the same to test tube D. Mix using the pipette.
6. Do the same to test tube E. Mix using the pipette.
7. Test tube F will just have 10 mL of water in it.
8. Add 1 mL of the potassium iodide solution to each of the test tubes, then gently swirl the test tubes without spilling any of the liquid over the edge
9. Carefully remove the tubes (only grasp the top of each tube since the bottom may be hot) and replace them on their rack.
10. To measure the absorbance of each sample, use a plastic pipette to fill a clean cuvette with sample until the sample volume reaches at least ⅔ of the cuvette height. Give the cuvette to your table's designated lead. *Remove the blank and insert the sample. Press "collect" on your device screen and wait for a few seconds before pressing "stop."*
11. Rename the table that is generated by pressing the three dots in the upper right hand corner next to the table's title.
12. Clean out your cuvette by emptying it into a waste beaker. Add water to it, pipetting up and down to flush out any remaining sample, and empty out the cuvette into the

waste beaker once more. Do not leave sample in your cuvette since precipitate will get stuck inside it.

13. Repeat with each sample until each sample has its own absorbance spectrum. Click or press on the graph above the desired wavelength (~503 nm) for the corresponding absorbances to appear for each sample. If you've labeled your graphs well, you'll be able to determine the absorbance values for each sample. Report your absorbances to the lead at the front of the classroom to plot your data.

Starch Amount	1.0%	0.1%	0.01%	0.001%	0.0001%	0% (control)
Potassium Iodide Amount	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Expected Observation	*Purple*	*Dark Blue*	*Blue*	*Light Blue*	*Bluish Yellow*	*Yellow*

Concentration of Fat	**Absorbance of Sample**
Control	*~0*
1.0%	*~0.28-0.322*
0.1%	*~0.146-0.184*
0.01%	*~0.179*
0.001%	*~0.160*
0.0001%	*~0.117-0.128*



Questions:

1. What happens to the color of the solution as the protein, sugar, complex carbohydrate concentration increases? (*Three different answers)*? How does this change in color affect absorbance?

As the concentration of the solution is increased, the color of the solution turns darker and the color intensifies. Because there is an increased concentration as the color darkens, the absorbance value goes higher because more light is absorbed through the solution.

2. Why do we need a control such as a sample of water?

The control can be used as a qualitative comparison to see if a sample contains starch, protein or reduced sugars.

3. Why is it important to only test one variable at a time?

It is important to only test one variable at a time so that if we see a change in our results, we can be absolutely sure of what caused the change. If multiple variables are changed, we cannot be sure whether any one of the alterations caused the differing results or if a combination of those results was what caused the change.

4. What is the purpose of a spectrophotometer?

The spectrophotometer is included in this module to quantitatively measure the amount of change seen in each sample when each reagent is added. This allows for greater accuracy in measurements and more meaningful results. For instance, in the Benedict's test, we know that a green sample has more sugar in it than a blue sample, but if someone were to ask how much more sugar was in the green sample, the only way you would be able to tell them is by quantitatively measuring the sugar content in both samples. A spectrophotometer is one of the ways to do just that.

5. What type of interaction is the Biuret solution causing to the protein in the sample to make a color change? (Refer to the background information page about the Biuret reagent)

The copper ion from the Biuret solution interacts with the peptide bond in the protein to form a complex that gives off the purple color. As the protein concentration increases, the more peptide bonds the copper ion can interact with and the color deepens and has a higher absorbance value.

6. What is the purpose of making calibration curves? What are the steps to make one?

Calibration curves are used to help quantify the concentration or how much stuff is in a sample accurately. For instance, you make different amounts of samples with varying concentrations as your independent variable and measure the absorbance of each sample as your dependent variable. Then, plot the values or variables to get a linear relationship between your variables. The goal is to use this curve to help quantify the concentration of an unknown sample by using the known values for comparison.

Linear Regression: Making the Best of Everyone's Data (20 minutes)

By this point everyone should have some data points for all three tests. The students will share the data up on the board, and the lead will be transcribing the data into Google Sheets as the students are writing. The link to the Google Sheets used for this can be found here: https://docs.google.com/spreadsheets/d/1ns-Atrs8OJqBD1PSMGio8S5Jy5yEfvGbDIrD7gxAcuA/edit#gid=1158093814

Some students might get outlier data which can be discarded. The average of all the student's data points should form a line.

Similar to the previous exercise, copy down the combined class calibration curves to compare the concentration of sugar, protein, and fat to the spectroscopic absorbance on the grid below.

Below, please record all of the linear equations of the class average for each of the different macromolecule concentration curves. These equations will be very helpful for us in the future!

Class Average Equations	Simple Sugars	Complex Carbohydrates	Proteins
Equation			
R^2 value			

DAY 4 The Ripening Finale

In front of you are two synthetically made solutions containing each of the three macromolecules you tested on Day 3. How would you go about testing the concentration of these molecules? Discuss with your group and come up with an accurate way to measure the concentration of you synthetic samples. Record all data below:

Materials:

- Two synthetic samples
- 6 test tubes
- 6 pipettes
- 6 cuvettes
- 1 spectrophotometer
- 1 hot plate
- 1 beaker (400 mL)
- 1 stir bar
- 20 mL of each indicator
- 100 mL of DI water

Synthetic Sample #1

	Color	Absorbance Value	Concentration
Protein			
Sugar			
Complex Carb			

Identity of Unknown Solution: *The answers to these tables will vary between synthetic sample. We will have a cheat sheet that goes along with all the synthetics!*

Synthetic Sample #2

	Color	Absorbance Value	Concentration
Protein			
Sugar			
Complex Carb			

Identity of Unknown Synthetic: *The answers to these tables will vary between synthetic sample. We will have a cheat sheet that goes along with all the synthetics!*

As the lead and or teacher in this situation, bring back up the data tables that had a summary of all of the student's tests from the previous day. In the event that they did not write the equations from the class average, they can use this to help them compare the results they get and figure out the concentration of their substance!

That didn't seem too difficult, right? Now let's take a look at a real biological sample that you have worked with before, and see if you can spot any differences in the macromolecule concentration as milk transitions to yogurt.

Materials:

- 2 biological samples
- 6 test tubes
- 6 cuvettes
- 6 pipettes
- 1 spectrophotometer
- 1 hot plate
- 1 beaker (400 mL)
- 100 mL of DI water

Milk: *Dilution of 1/625*

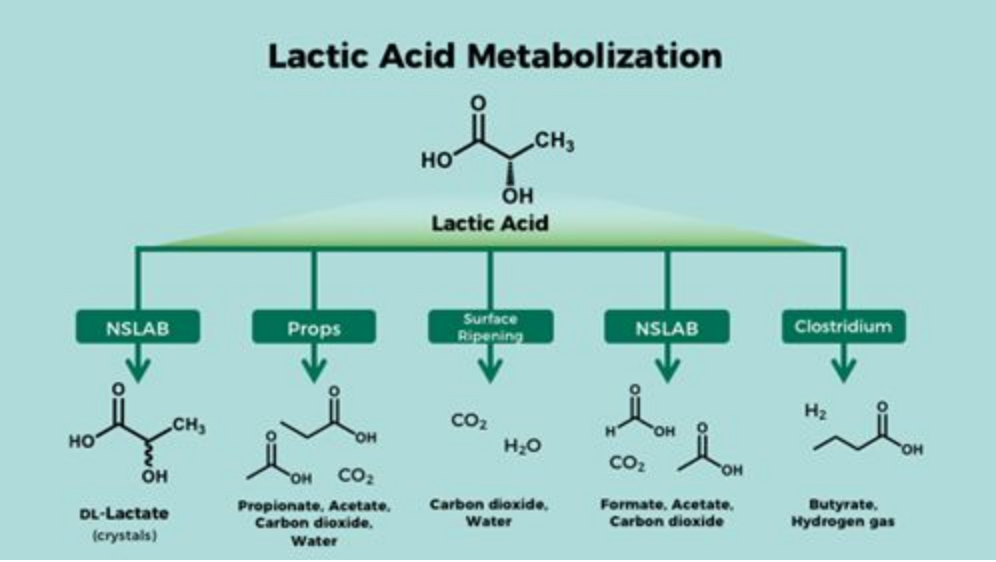
	Color	Absorbance Value	Concentration
Protein	*Purple*		*Same as yogurt*
Sugar	*Brownish*		*Higher than yogurt*
Complex Carb	*Dark Purple*		*Negative response*

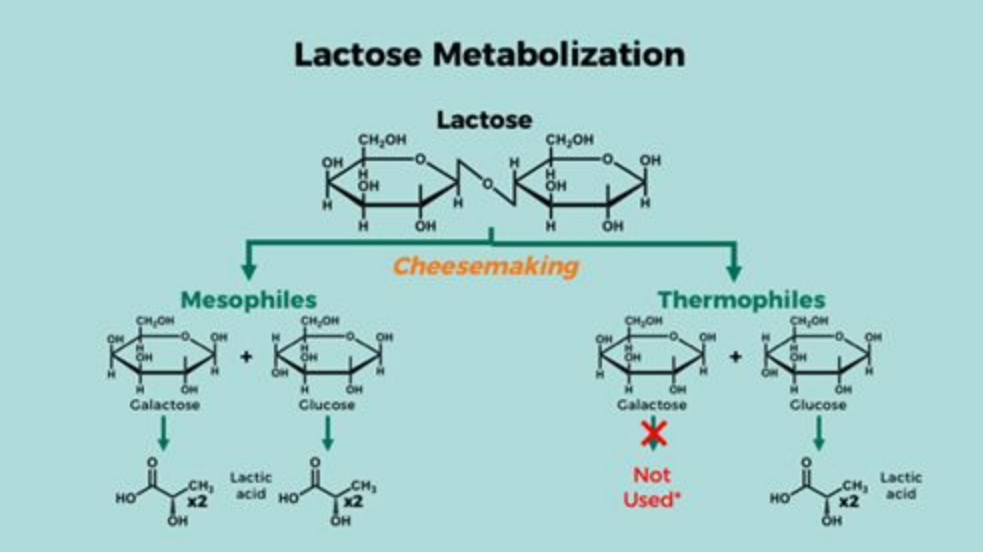
Yogurt: *Dilution of 1/625*

	Color	Absorbance Value	Concentration
Protein	*Purple*		*Same as milk*
Sugar	*Blue*		*Lower than sugar*
Complex Carb	*Dark Purple*		*Negative response*

Did you notice anything different between your two biological samples? If so, why do you think there was a difference in any of the three macromolecules? *Hint: What did we add to the milk on day 2 to force it to coagulate?* Summarize your findings and make a conclusion. Support your conclusion with numerical data from the data above

The protein amount should be the same. The complex carbohydrate test should yield a negative result, indicating that there are no complex carbohydrates within the milk or yogurt. There should be a noticeable difference in absorbance, and therefore, concentration in milk and yogurt. As milk is converted to yogurt, lactose (The main sugar found in milk) *is converted to lactic acid by bacteria! The lactic acid causes a decrease in the pH of the biological sample which causes the proteins to coagulate. This can be seen when eating store bought yogurt. There is a small acid like taste that is covered by the sweet supplements most companies that make yogurt put in to hide it. Pure yogurt without any supplements actually does not taste too sweet.*





Wrapping it Up/Conclusion - 10 minutes

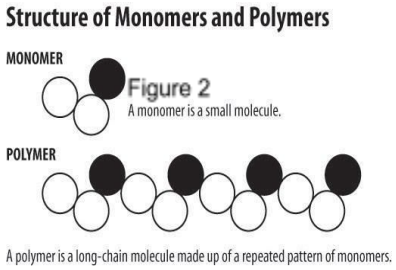
In the final 10 minutes of class, the lead and volunteers will pass out a small 3x5 2 question exit form to the students which will be the exit slip.

1. What was your favorite part about today's module?
2. What are the 4 main elements that make up biological macromolecules?

GLOSSARY

● **Macromolecules -** A macromolecule is a very large molecule made from smaller molecules.

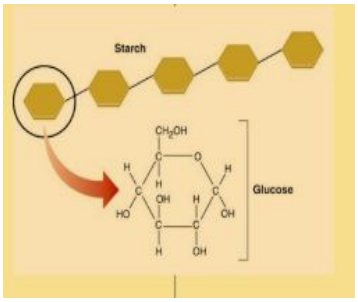
● **Monomers -** Small molecules that can be strung together to make large molecules (polymers) (Figure 1)



● **Polymers:** A chain made of monomers or small molecules

● **Carbohydrates:** Compounds made of small subunits called monosaccharides or sugars (ex. glucose). Carbs give us energy to do science! (Figure 2). Type of polymer**.** Ex. Starch

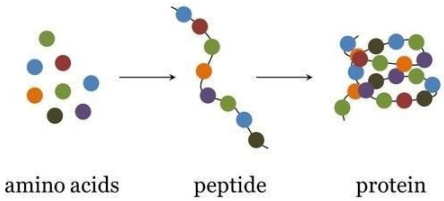
● **Proteins:** Polymers made of amino acids. (Proteins build and repair muscles; they're amazing!)



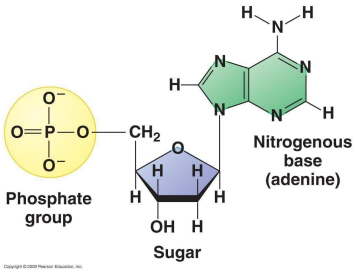
● **Lipids:** Can be a fat or oil depending on whether they are solid or liquid at room temperature, respectively. Lipids aren't polymers, but they can interact together to form cell membranes

● **Nucleic Acids:** Biomolecules with subunits that are composed of a phosphate group, a _____ sugar, and base

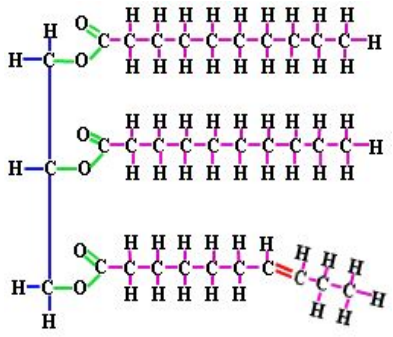
Ex. DNA is responsible for genetic inheritance of traits.



Proteins



Nucleic Acids



Lipids