How Science Works

Student Notebook

High School Biology

Module 1

Class Question:

Scientist (Your Name): ______________________________

Teacher's Name: ______________________________

SciTrek Volunteer's Name: ______________________________
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OVERVIEW & BACKGROUND INFORMATION

Complex Chemical Reagent Info for the Curious:

**Benedict's test for Simple Sugars** - Benedict's is a hydrated solution of copper (II) sulfate (CuSO₄). In the presence of reducing sugar, copper sulfate reacts to become copper (I) oxide (Cu₂O).

\[
+ 2\text{Cu}^{2+}(aq) + 5\text{OH}^-(aq) \rightarrow \text{Cu}_2\text{O(s)} + 3\text{H}_2\text{O(l)}
\]

This interaction changes the Cu²⁺ ions which appear blue into Cu⁺ ions which appear red. The transition of color creates a gradient from clear light blue to green to orange to red precipitate. Benedict’s test is used to quantify glucose levels in blood and urine and test for diabetes.

**Biuret test for Proteins** - Biuret is a mixture of sodium hydroxide (NaOH), hydrated copper (II) sulfate (CuSO₄ x H₂O), and potassium sodium tartrate to stabilizes the copper ions. Cu²⁺ ions from the copper (II) sulfate gives off a blue color. In the presence of protein, the Cu²⁺ ions interact with peptide bonds, bonds that link amino acid monomers together to make a protein polymer.

This interaction changes the Cu²⁺ ions which appear blue into a copper complex with a purple color. The deeper the purple color, the more copper-peptide complexes have been formed.
Welcome to SciTrek!

We’re glad to have you onboard! Before we start, check out this video on how BP (British Petroleum, one of the biggest energy companies) is using bananas and converting it to jet fuel. How do you think BP makes jet fuel out of bananas? HINT: it converts molecules in the banana into other molecules (metabolism!!).

Link to video: [https://twitter.com/bp_america/status/1087520154815250433?lang=en](https://twitter.com/bp_america/status/1087520154815250433?lang=en)
[https://tinyurl.com/yxhned9d](https://tinyurl.com/yxhned9d)

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 examples of some of the topics you have studied or think you will study in biology.

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

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

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

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.

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
Demonstration: Water & Polarity

1. 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?

Set-up:

________________________________________________________________________________________________________
________________________________________________________________________________________________________
________________________________________________________________________________________________________
________________________________________________________________________________________________________

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: http://lab.concord.org/embeddable.html#interactives/sam/intermolecular-attractions/3-1-oil-and-water.json (https://tinyurl.com/yyjdxz24)

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

Compounds can be organic or 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: ___________________  Polymer: ___________________

**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): ______________________
Not really a polymer.. repeating unit: ___________________

**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, and other various tasks.

Monomer: _______________  Polymer: _______________

**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. A really important nucleic acids called DNA stores genetic information. Another important nucleic acid called RNA makes proteins.

Monomer: _______________  Polymer: _______________
Organism: The Trading Card Game

**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:**

<table>
<thead>
<tr>
<th></th>
<th>To make</th>
<th>You need</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sugar (Stage 2)</strong></td>
<td></td>
<td>2 hydrogens + 1 carbon + 1 oxygen</td>
</tr>
<tr>
<td><strong>Lipid (Stage 3)</strong></td>
<td></td>
<td>2 sugars</td>
</tr>
<tr>
<td><strong>Carbohydrate (Stage 3)</strong></td>
<td></td>
<td>3 sugars</td>
</tr>
<tr>
<td><strong>Protein (Stage 3)</strong></td>
<td></td>
<td>2 sugars + 2 nitrogens</td>
</tr>
<tr>
<td><strong>DNA (Stage 3)</strong></td>
<td></td>
<td>2 sugars + 2 nitrogens</td>
</tr>
<tr>
<td><strong>Organism (Stage 4)</strong></td>
<td></td>
<td>1 lipid + 1 carbohydrate + 1 protein + 1 DNA</td>
</tr>
</tbody>
</table>

**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.

Questions after the Game

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?

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

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

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

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

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

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 on the following page, box and redraw the repeating monomer unit.

![Carbohydrate and Protein Chains](image)

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?

____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
DAY 2

Testing for Biomolecules

(Caution: Concentrated acids should only be used by the volunteers 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?

Pay attention to your lead and answer the following questions below.

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?

<table>
<thead>
<tr>
<th>Untouched Milk</th>
<th>Acidified Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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:

\[
\text{Mass of Protein (undiluted sample)} = \text{Mass of Protein (diluted sample)} \times (\text{dilution factor})
\]
Record all of your data in the tables on the next page.

<table>
<thead>
<tr>
<th>Nonfat Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of filter paper</td>
</tr>
<tr>
<td>Mass of the filter paper and protein (after 5 min of drying)</td>
</tr>
<tr>
<td>Mass of the filter paper and protein (after 7.5 min of drying)</td>
</tr>
<tr>
<td>Mass of the filter paper and protein (after 10 min of drying)</td>
</tr>
<tr>
<td>Mass of protein (diluted sample)</td>
</tr>
<tr>
<td>Mass of Protein (undiluted sample)</td>
</tr>
</tbody>
</table>

1. Create a graph that compares the grams of protein of milk and yogurt in the grid below: (Hint: What type of graph do you use to compare things between different groups?)
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.

Average Value = \( \frac{\text{Sum of all your values}}{\text{How many values you have}} \) =

Range of Values = \( \text{Maximum Value} - \text{Minimum Value} \) =

Standard Deviation = \( \sqrt{\frac{(\text{your protein mass} - \text{mean average protein mass})^2}{n-1}} \) =

The actual expected values 0.6 grams for milk and 2.2 grams for yogurt.

a. Calculate the percent error between your experimental values and the expected values.

\[
\text{Percent Error} = \frac{|\text{your value} - \text{expected value}|}{\text{expected value}} =
\]

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

____________________________________________________________________________________________________

____________________________________________________________________________________________________

Testing for the Other Macromolecules

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 spectrophotometry, where the concentration can be determined using chemical indicators.

When light is passed through a sample, some of the light is absorbed by the sample, and the intensity of light that passes through will change. With higher concentrations of a substance
that absorbs light of a particular wavelength, the absorbance of the sample increases. This phenomena can be used to quantify how much of a substance is present in the sample.

**Demonstration: Bradford Reagent for Testing Protein Amount**

Which has more protein, chicken or butter?

A few drops of Bradford reagent were added on top of the chicken (left) and butter (right). What do you notice about the color of the Bradford drops on the apple when compared to the iodine on the potato?

1. The test tube with 0% protein is called a **control**. What color is the control? Why do we need a control in the test tube rack?

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

```
0.01%  0.001%  0.0001%  0.00001%  0%
protein  protein  protein  protein  protein
```
Percents are given in mass per volume, meaning that 0.1% protein solution was made by adding 0.1g (one tenth of one gram) to 100mL water.

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

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

4. What are some limitations to this test (ex. what if the sample is already colored)? What are some problems in determining the quantity of each solution?

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

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 the one below. Copy the values into your notebooks.

The solution concentrations are given in percent by volume, meaning that 1% protein = 1 gram of protein/100 mL of water.

<table>
<thead>
<tr>
<th>Concentration of Protein</th>
<th>Control (0%)</th>
<th>0.0001</th>
<th>0.00001</th>
<th>0.000001</th>
<th>0.0000001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of Sample @ 610 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What is the shape of the graph formed when the absorbance is plotted against concentration? Paste or redraw the graph and write down the equation corresponding to it. What is the R-squared value for this line? What does that tell you about the tightness of your data points.
The Separation Problem
The SciTrek lead will add Bradford reagent directly to the milk to see if we can use it to make a calibration curve for protein concentration. There’s however a little problem that might come up.

<table>
<thead>
<tr>
<th>Milk without Bradford reagent</th>
<th>Milk with Bradford reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Day 2 Waiting Activities

Vocabulary Activity
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.

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Macromolecules</th>
<th>Monomers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleic Acids</th>
<th>Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Nutrition Facts Activity
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: ___ Protein: ___ Fat: ___
Brown Rice: Carbohydrate: ___ Protein: ___ Fat: ___
Butter: Carbohydrate: ___ Protein: ___ Fat: ___
Tofu: Carbohydrate: ___ Protein: ___ Fat: ___
DAY 3 Calibration Curves

Colorimetric Calibration Curve Activity

How do we know that living things (i.e. food and ourselves) are made of varying amounts macromolecules? Is it possible for us to quantify the macromolecule content in a sample?

Test #1: Benedict’s Reagent for Carbohydrates

(Caution: Hot objects ~ test tubes and hot plate ~ can lead to painful burns. Be careful!)

The first reagent that we will be using is the Benedict’s test, which tests for reducing sugars.

Materials:

- Hot plate + beaker of hot water (~250mL H₂O @ boiling) + stir bar (prepared beforehand)
- Thermometer
- Benedict’s solution
- 0.05% dextrose solution prepared beforehand
- DI water
- 10mL graduated cylinder + 50mL beaker
- Plastic pipette
- Labeled test tube rack + 5 labeled test tubes (A, B, C, D, and E)
- Spectrophotometer + cord
- Laptop
- Cuvettes

Prepare a test tube rack labeled like the diagram on the previous page.
1. To prepare the calibration curve, start by adding 0 mL of H₂O to test tube A, 5 mL of H₂O into tube B, 8 mL of H₂O into tube C, and 9 mL of H₂O 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 newly made 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 who will help you measure the absorbance of your sample (at 750 nm) and will record your data in a table on the same page. The concentrations corresponding to the dilutions you’ve made can be found in the table at the end of this section. 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, squeezing and releasing the pipette bulb 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.

<table>
<thead>
<tr>
<th>Sugar Solution</th>
<th>Test Tube A</th>
<th>Test Tube B</th>
<th>Test Tube C</th>
<th>Test Tube D</th>
<th>Test Tube E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A =</td>
<td>Solution B =</td>
<td>Solution C =</td>
<td>Solution D =</td>
<td>Solution E =</td>
<td></td>
</tr>
<tr>
<td>10 ml stock</td>
<td>5 ml stock</td>
<td>2 ml stock</td>
<td>1 ml stock</td>
<td>0 ml stock</td>
<td></td>
</tr>
<tr>
<td>0 ml H₂O</td>
<td>5 ml H₂O</td>
<td>8 ml H₂O</td>
<td>9 ml H₂O</td>
<td>10 ml H₂O</td>
<td></td>
</tr>
<tr>
<td>(most sugar)</td>
<td></td>
<td></td>
<td></td>
<td>(least sugar)</td>
<td></td>
</tr>
</tbody>
</table>

Benedict's Amount

- Test Tube A: 1 mL
- Test Tube B: 1 mL
- Test Tube C: 1 mL
- Test Tube D: 1 mL
- Test Tube E: 1 mL

Color Display

- Test Tube A: 0.050%
- Test Tube B: 0.025%
- Test Tube C: 0.010%
- Test Tube D: 0.005%
- Test Tube E: Control

Sample Absorbance

Enter your data on Excel and make a scatterplot of the data points with concentration of sugar on the abscissa (x-axis) and absorbance of the sample on the ordinate (y-axis). Paste or redraw a copy of the graph below.

**Test #2: Biuret Reagent for Proteins**

The second test that we will be doing is testing for proteins using Biuret solution.

**Materials:**

- Labeled test tube rack, Biuret solution, Plastic pipettes, DI water
- Albumin Solution (200 mg/ml)
- 10mL graduated cylinder
- Plastic pipette
- Labeled test tube rack + 6 labeled test tubes (A, B, C, D, and E)
- Spectrophotometer + cord
- Laptop
- Cuvettes
Procedure for Biuret Assay:
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. 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.
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.

<table>
<thead>
<tr>
<th></th>
<th>Test Tube A</th>
<th>Test Tube B</th>
<th>Test Tube C</th>
<th>Test Tube D</th>
<th>Test Tube E</th>
<th>Test Tube F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20 mg/mL</td>
<td>10 mg/mL</td>
<td>4 mg/mL</td>
<td>0.8 mg/mL</td>
<td>0.16 mg/mL</td>
<td>0 mg/mL</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Control)</td>
</tr>
<tr>
<td>Biuret Amount</td>
<td>8 drops</td>
<td>8 drops</td>
<td>8 drops</td>
<td>8 drops</td>
<td>8 drops</td>
<td>8 drops</td>
</tr>
<tr>
<td>Color Display</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorbance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enter your data on Excel and make a scatterplot of the data points with concentration of protein on the x-axis and absorbance of the sample on the y-axis. Paste or redraw an image of the graph below.
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
- Plastic pipettes
- Labeled test tube rack + 6 labeled test tubes (A, B, C, D, E, and F)
- Spectrophotometer + cord
- Laptop
- Cuvette

Procedure:

1. To prepare the calibration curve, start by adding 9 mL of H₂O to test tube A, 9 mL of H₂O into tube B, 9 mL of H₂O into tube C, 9 mL of H₂O into tube D, and 9 mL of H₂O to test tube E. Add 10 mL of H₂O.
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. Repeat steps 1-3 for test tubes C, D, and E. Mix using the pipette.
5. Add only 10 mL of water to test tube F.
6. 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.
7. Carefully remove the tubes (only grasp the top of each tube since the bottom may be hot) and replace them on their rack.
8. 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.
9. Rename the table that is generated by pressing the three dots in the upper right hand corner next to the table’s title.
10. 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.
11. 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.

<table>
<thead>
<tr>
<th>Starch Amount</th>
<th>1.0%</th>
<th>0.1%</th>
<th>0.01%</th>
<th>0.001%</th>
<th>0.0001%</th>
<th>0% (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of Potassium Iodide</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Color Displayed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enter your data on Excel and make a scatterplot of the data points with concentration of fat on the abscissa (x-axis) and absorbance of the sample on the ordinate (y-axis). Paste or redraw an image of the graph below.
Questions:

1. What happens to the color of the solution as the protein, sugar, complex carbohydrate concentration increases? (*Three different answers*)

__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________

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

__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________

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

__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________

4. What is the purpose of a spectrophotometer?

__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________

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 behind the Biuret reagent)

__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________

6. What is the purpose of making calibration curves?

__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
__________________________________________________________________________________________________________________
Linear Regression: Making the Best of Everyone’s Data

Take the data you and your group have collected and add it to the Google Sheets for the class found here:

https://docs.google.com/spreadsheets/d/1ns-Atrs80IqBD1PSMGio8S5lj5yEfvgDlrD7gxAcuA/edit#gid=1158093814
https://tinyurl.com/y2wav293

Paste or redraw images of the combined class data graphs below and use it to answer the questions on the next page.

Compare the R-squared value of your group’s data to the R-squared value of the overall class data. What happened to the tightness of the linear fit as more groups added data to the scatterplot? Which line do you think is more accurate to use, your original line or the overall class line?

On the graph, notice the vertical lines passing through each data point. These are called error bars, and they represent the uncertainty or deviation from a given data point. Check out this article for some of the uses of error bars in biology:

https://www.biologyforlife.com/interpreting-error-bars.html
From the overlap error bars, is the mean value a representative number for the data set? How spread out is the data around the mean value for each point? Are these differences statistically significant?

_________________________________________________________________________________________________________

_________________________________________________________________________________________________________

_________________________________________________________________________________________________________

_________________________________________________________________________________________________________

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!

<table>
<thead>
<tr>
<th>Class Average Equations</th>
<th>Simple Sugars</th>
<th>Complex Carbohydrates</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Squared value</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 your 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

<table>
<thead>
<tr>
<th></th>
<th>Color</th>
<th>Absorbance Value</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex Carb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Identity of Unknown Solution:

### Synthetic Sample #2

<table>
<thead>
<tr>
<th></th>
<th>Color</th>
<th>Absorbance Value</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex Carb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Identity of Unknown Synthetic:
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**

<table>
<thead>
<tr>
<th></th>
<th>Color</th>
<th>Absorbance Value</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex Carb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Yogurt**

<table>
<thead>
<tr>
<th></th>
<th>Color</th>
<th>Absorbance Value</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex Carb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.
Feedback for SciTrek!

Please tear this page out and turn it into the lead at the end of the day.

1. What was your favorite part about this week’s module?

2. Have you done a SciTrek module before in elementary or middle school? If so how does this experience compare to other modules you have done with us?

3. After participating in this module, I feel that I can 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.

   Strong Disagree    Disagree    Somewhat Neutral    Agree    Strongly Agree

   Please explain your response to the prompt above.

4. What was something you did in this module that you think scientists do regularly?

5. Do you have any suggestions on how we can improve this module?

Thank you for inviting us to your classroom! We hope you enjoyed this experience and wish you the best throughout the school year.    ~ The SciTrek Team
GLOSSARY

● **Macromolecules** - A macromolecule is a very large molecule made from smaller molecules. Some common macromolecules in biochemistry are nucleic acids, proteins, and carbohydrates.

● **Monomers** - Single units that can be strung together to make large molecules (polymers). Monomers do not need to be identical, but they must have a similar structure. (Figure 1)

● **Polymers**: A chain made of monomers. The common polymers of life (biopolymers) are nucleic acids, proteins, and carbohydrates.

● **Carbohydrates**: Organic compounds with the molecular formula \( (CH_2O)_n \) composed of small subunits called monosaccharides (ex. glucose). These combine in different ways to form chains of polysaccharides with different properties and functions. (Carbs give us energy to do science!) (Figure 2)

● **Starch**: A large and complex type of carbohydrate. Starch is a polymer of many sugars bonded together. (As an aside, we give a carbohydrate a different name based on the sugars and types of bond linkages that it has. Starch is only one of thousands of carbohydrates!). (Figure 2)

● **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 with to form cell membranes

● **Nucleic Acids**: Biomolecules with subunits that are composed of a phosphate group, a sugar, and an identifying molecule. DNA is responsible for genetic inheritance and RNA helps to translate DNA into protein, which is responsible for genetic expression.