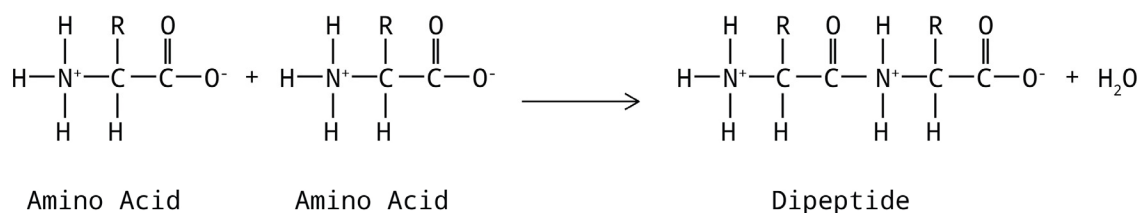


## PRELIMINARY ACTIVITY FOR Investigating Protein: The Bradford Assay

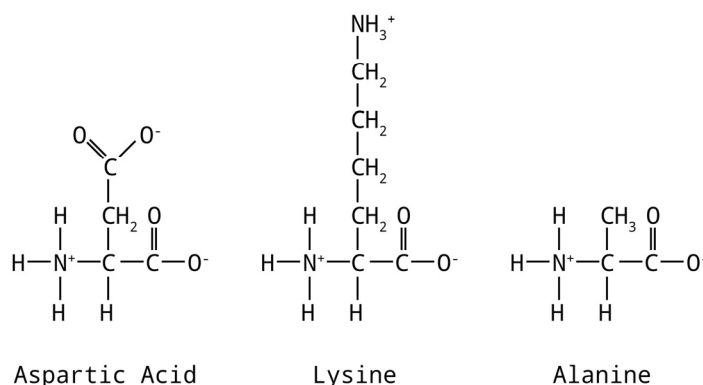
### Open Inquiry Version

This investigation is designed to introduce you to the study of macromolecules. Proteins, DNA, RNA, and polysaccharides such as starch, glycogen, and cellulose are all macromolecules. Macromolecules are formed by connecting many smaller molecules together. The individual components of a macromolecule are referred to as monomers. Proteins are composed of monomers called amino acids. All amino acids have a carboxyl group, an amino group, and a central or alpha carbon. A side chain, often referred to as an R group, is bonded to the central carbon of each amino acid. Amino acids form polymers when the carboxyl group and amino group of two amino acids form a peptide bond, as shown in Figure 1. Water and a dipeptide are formed in the reaction. More amino acids can be added to the carboxyl group of this dipeptide until a polypeptide is formed.



**Figure 1**

There are 20 different amino acids that are found in proteins, and each one has a different R-group. These side chains are very important because they impart each amino acid with different characteristics. Amino acids can be characterized as polar, nonpolar, or charged. Charged amino acids are further characterized as acid or basic. Uncharged amino acids can be considered neutral. Three different amino acids are shown in Figure 2. Aspartic acid is acidic, lysine is basic, and alanine is neutral. The sequence of amino acids that makes up a polypeptide is referred to as the primary structure of the protein. The primary structure determines how the protein will fold, which will determine its function. The shapes within a polypeptide are referred to as the secondary structure. The three dimensional structure of an entire polypeptide is referred to as its tertiary structure.

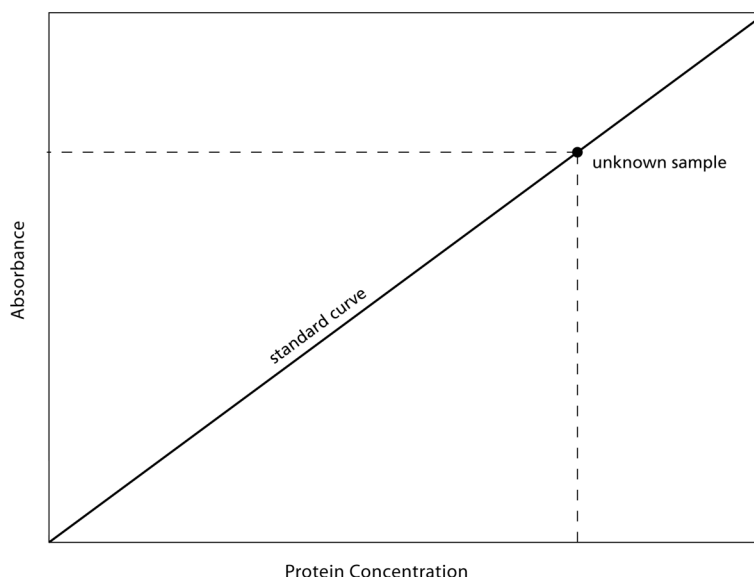


**Figure 2**

In the Preliminary Activity, you will use a spectrophotometer and the Bradford protein assay to determine the protein content of milk. The Bradford protein assay is an extremely sensitive assay for protein. The Bradford

## Investigation 5

reagent contains a dye called Coomassie G-250 that can interact with the R-group of specific amino acids. The dominant protein in milk is called casein, which is composed of 224 amino acids. Thirteen of these amino acids react with the dye in the Bradford reagent. These amino acids include one tryptophan, four arginines, four tyrosines, and four histidines.



**Figure 3**

When the dye in the Bradford reagent interacts with these specific amino acids it turns the solution blue. The greater the concentration of protein in solution the deeper the color will be. If a set of known protein concentrations are allowed to react with a known concentration of Bradford reagent, we can measure the absorbance of the resulting solutions to create a standard curve. When a graph of absorbance vs. concentration is plotted for the standard solutions, a direct relationship should result, as shown in Figure 3. The direct relationship between absorbance and concentration for a solution is known as Beer's law. To determine the protein concentration of an unknown solution, we can measure its absorbance and see where it falls on the standard curve. Because the relationship is linear, we could also calculate the protein concentration using the formula for the standard curve.

After completing the Preliminary Activity, you will first use reference sources to find out more about protein before you choose and investigate a researchable question. Some topics to consider in your reference search are:

- macromolecule
- protein
- monomer
- polymer
- amino acid
- alpha carbon
- carboxyl group
- primary structure
- amino group
- R group
- peptide bond
- polypeptide
- Bradford protein assay
- Beer's law

## PROCEDURE

1. Obtain and wear goggles and gloves.
2. Obtain two 15 mL centrifuge tubes.
  - Label one tube **PB** and add 3 mL of PBS (phosphate buffered saline).
  - Label the other tube **BR** and add 12 mL of Quick Start™ Bradford Dye Reagent.
3. Obtain a 1.5 mL microtube. Label it **M**, and add 980 µL of PBS. Then add 20 µL of nonfat milk. **Note:** You are thus diluting the nonfat milk sample by a factor of 50.
4. Obtain seven empty cuvettes with lids and a set of Bovine γ-globulin standards to create a set of protein standards.
  - a. Fill each cuvette with 1 mL of Bradford reagent.
  - b. Label one cuvette **2.0** and add 20 µL of solution from the 2.0 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
  - c. Label the next cuvette **1.5** and add 20 µL of solution from the 1.5 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
  - d. Label the next cuvette **1.0** and add 20 µL of solution from the 1.0 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
  - e. Label the next cuvette **0.75** and add 20 µL of solution from the 0.75 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
  - f. Label the next cuvette **0.5** and add 20 µL of solution from the 0.5 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
  - g. Label the next cuvette **0.25** and add 20 µL of solution from the 0.25 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
  - h. Label the next cuvette **0.125** and add 20 µL of solution from the 0.125 mg/mL standard. Cap the cuvette and gently invert the cuvette three times.
5. Obtain an empty cuvette with a cap.
  - a. Fill the cuvette with 1 mL of Bradford reagent.
  - b. Label the cuvette cap with an **M** and add 20 µL from microtube M. Cap the cuvette and gently invert the cuvette three times.
6. Use a USB cable to connect the SpectroVis Plus spectrophotometer to your computer or LabQuest. Start the data-collection program, and then choose New from the File menu.
7. Prepare a blank by filling an empty cuvette with 1 mL of Bradford reagent and 20 µL of PBS. Label it **B**. Place the blank in the cuvette slot. To correctly use cuvettes, remember:
  - Wipe the outside of each cuvette with a lint-free tissue.
  - Handle cuvettes only by the top edge of the ribbed sides.
  - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
  - Always position the cuvette so the light passes through the clear sides.
8. Calibrate the spectrophotometer.
  - a. Choose Calibrate from the Sensors menu of LabQuest or the Experiment menu of Logger Pro.
  - b. When the warmup period is done, select Finish Calibration. When the calibration procedure is complete, select OK.


## Investigation 5

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9. Determine the optimum wavelength for examining the absorbance of the Bradford reagent when it is bound to protein.
  - a. Remove the blank cuvette. Gently invert cuvette M twice and then place it into the spectrophotometer.
  - b. Start data collection. A full spectrum graph of the solution will be displayed. Note that one area of the graph contains a peak absorbance. Stop data collection.

10. Set up the data-collection mode.

### Logger Pro

- a. Click Configure Spectrometer Data Collection, . Select Abs vs. Concentration as the Collection Mode. The wavelength of maximum absorbance ( $\lambda$  max) will be selected. Verify that the maximum absorbance is close to 595 nm.
- b. Enter **Concentration** as the Column Name, **Conc.** as the Short Name, and **mg/mL** as the Units.
- c. Select OK. Remove the cuvette from the spectrophotometer and proceed to Step 11.

### LabQuest App

- a. The peak absorbance is automatically selected. On the Meter screen, tap Mode and change the data-collection mode to Events with Entry.
  - b. Enter **Concentration** as the Column Name and **mg/mL** as the Units.
  - c. Select OK. Remove the cuvette from the spectrophotometer and proceed to Step 11.
11. You are now ready to collect absorbance data for the seven protein standards. Start data collection. Obtain the cuvette labeled 2.0. Wipe the outside with a tissue and place it in the spectrophotometer. Wait for the absorbance value displayed on the screen to stabilize, and then Keep the data point. Enter **2.00** as the concentration. The data pair you just collected will now be plotted on the graph. Remove the cuvette from the spectrophotometer.
  12. Obtain the cuvette labeled 1.5. Wipe the outside and place it in the spectrophotometer. When the absorbance value stabilizes, keep the data point, enter **1.50** as the concentration.
  13. Repeat the Step 12 procedure for the remaining protein standards. When you have finished with the 0.125 mg/mL standard solution, stop data collection.
  14. Record the absorbance values in your data table.
  15. Examine the graph of absorbance vs. concentration. To see if the curve represents a direct relationship between these two variables, apply a Linear Fit to the data. The best-fit linear regression line should pass near or through the data points.
  16. Record the equation of this line in your data table.
  17. You are now ready to collect absorbance data for your unknown. Obtain cuvette M. Wipe the outside of the cuvette and place it in the spectrophotometer. When the displayed absorbance value stabilizes, record the value in your data table. **Important:** The reading on the screen is live, so it is **not** necessary to start data collection to read the absorbance value.

18. To determine the concentration of the unknown, interpolate along the regression line to convert the absorbance value of the unknown to the concentration value.

*Logger Pro*

- a. Choose Interpolation Calculator from the Analyze menu.
- b. Check that the absorbance value that you recorded is displayed. If it is not, enter the absorbance value in the correct space. The protein concentration in mg/mL will be displayed. Record the concentration value in your data table.
- c. Select OK. A point on the graph will be displayed. Proceed to Step 19.

*LabQuest App*

- a. Tap the Graph tab and choose Interpolate from the Analyze menu.
- b. Find the absorbance value that is closest to the absorbance reading you obtained for cuvette M. The protein concentration in mg/mL will be displayed to the right of the graph.
- c. Record the concentration value in your data table and proceed to Step 19.

19. Print the graph as directed by your instructor.

## **QUESTIONS**

1. Determine the actual protein concentration of your milk sample. Remember that you diluted your original milk by a factor of 50 before conducting the Bradford protein assay.
2. Obtain the published protein value for the milk sample from your instructor. This value is typically found on the nutrition label of the milk container. Convert the published value to mg/mL of protein.
3. List three protein-containing liquids whose protein concentration might be determined using this protocol.
4. List at least one researchable question concerning protein concentration.