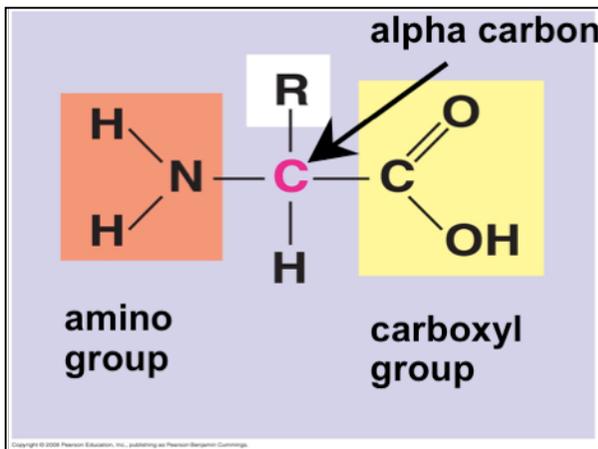


Activity 1 – Build Your Own Protein Adapted from 3-D Molecular Designs Student Handout 1

Introduction:

Amino Acids – the Building Blocks of Proteins

Amino acids are small molecules that link together in long chains to form proteins. They are often referred to as the "building blocks" of proteins. The sequence of amino acids in a protein, and hence the protein function, are determined by the **genetic code**, defined by the sequence of nucleotides in DNA or RNA determines the amino acid sequence of a protein (*definition adapted from National Human Genome Research Institute.*).



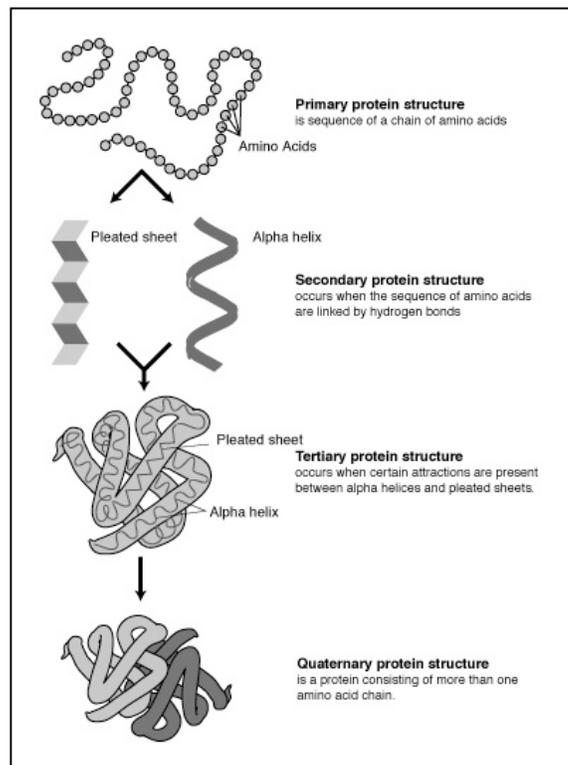
There are 20 amino acids and each one consists of two parts – a **backbone** and a **sidechain**. The backbone, shown in the figure to the left, is the same in all 20 amino acids, and the sidechain is different in each one.

Each sidechain consists of a unique combination of **atoms**, which determine the side chain's 3-D shape and its chemical properties.

Figure (above) from Campbell and Reece, 8th ed, 2008

When different amino acids join together to make a protein, the unique properties of each amino acid determine how the protein folds into its final 3-D shape. The shape of the protein allows it to perform a specific function in our cells. There are four levels of structure that describe a protein as it folds, as shown in the diagram below: primary, secondary, tertiary, and quaternary.

Figure (right) from www.accessexcellence.org.



Learning Objectives:

You will explore how the unique chemical properties of the 20 amino acids determine the final shape of a protein. Specifically,

- You will be able to identify amino acids according to their chemical properties and create a short polypeptide of 15 amino acids (the primary structure).
- You will be able to fold your polypeptide into a 3-D shape (the tertiary structure) while satisfying the chemical properties of all of the amino acid sidechains.

****Note:** A protein generally folds into its secondary structure (of alpha helices and beta-sheets) prior to assuming its tertiary structure. For the purpose of this activity, you will skip the secondary structure folding.

Activity Instructions:

1. Check the kit to make sure that you have each of the following:
 - a. Chemical properties circle
 - b. Amino acid chart
 - c. Mini-toober (foam covered wire) with a blue capped end and red capped end and 15 U-shaped metal clips spaced 3 in apart
 - d. 21 foam amino acid sidechain models with magnets and blue, red, yellow and green dots
 - e. Hydrogen bond connectors
2. Place each foam amino acid sidechain on the magnetic Chemical Properties Circle according to its chemical properties. You will need to consult the Amino Acid Sidechain List. Be sure to look at the colored dots (which represent different atoms) to help you distinguish between similar sidechains) – see step #3 below
 - a. **Hydrophobic** sidechains are yellow
 - b. **Hydrophilic** sidechains are white
 - c. **Acidic** sidechains are red
 - d. **Basic** sidechains are blue
 - e. **Cysteine** sidechains are green
3. Note the colored dots on the grey side of the amino acid sidechains. These represent different atoms.
 - a. **Carbon** is gray
 - b. **Oxygen** is red
 - c. **Nitrogen** is blue
 - d. **Hydrogen** is white
 - e. **Sulfur** is yellow

Observations:

- A. Hydrophobic sidechains are composed primarily of which atoms?
_____ What does the term “hydrophobic” tell you about the chemical properties of these side chains and their respective amino acids?”

- B. Acidic sidechains contain two _____ atoms. This is called a carboxylic acid functional group. Draw a carboxylic acid functional group in the space below.
- C. Basic sidechains contain _____ atoms. This is called an amino functional group. Draw an amino functional group in the space below.
- D. Hydrophilic sidechains have various combinations of which atoms?

- What does the term “hydrophilic” tell you about the chemical properties of these side chains and their respective amino acids?”

Predictions: What causes proteins to fold into their 3-D shapes?

- A. Which sidechains (e.g., hydrophobic, hydrophilic, acidic, basic) might position themselves on the interior of a protein, where they are shielded from water?

- B. Which sidechains (e.g., hydrophobic, hydrophilic, acidic, basic) might be attracted to one another? Give an example of two amino acids that might form an ionic bond.

- C. Would the final shape of a protein be a high energy or low energy state for the atoms in the structure? _____ Why?

Activity Instructions, Continued:

4. Select the following sidechains from your chemical properties circle (15 total):
 - a. 1 Methionine
 - b. 6 hydrophobic (non-polar) sidechains
 - c. 2 acidic sidechains
 - d. 2 basic sidechains
 - e. 2 Cysteine sidechains
 - f. 2 additional hydrophilic (polar) sidechains

5. Place the Methionine on the metal clip of your mini-toober closest to the Blue End Cap (called the **N-terminus**). Mix the other sidechains together and place them in any order (randomly) on the 14 remaining metal clips of your mini-toober. The sequence of your amino acid sidechains on the mini-toober is called the **Primary Structure** of your protein. As a general rule, the final shape of a protein is determined by its primary structure.
 - a. What amino acids define the primary structure of your protein (give the single letter abbreviation of your protein from the N-terminal to the C-terminal)?

 - b. Why do we call the beginning of the protein the “N-terminus” and the end of the protein the “C-terminus” (hint: look at the functional groups on the backbone of each amino acid)?

6. Now you will fold your 15-amino acid protein according to the chemical properties of the sidechains. Remember that all of the chemical properties affect the protein at the same time.
 - a. Start by folding your protein so that all of the hydrophobic, nonpolar sidechains are buried on the inside of your protein, where they will be hidden from polar water molecules.
 - b. Next, fold your proteins so the acidic and basic (charged) sidechains are on the outside surface of the protein and pair one negative sidechain with one positive sidechain so that they come within one inch, thereby neutralizing each other.
 - c. Continue to fold your protein making sure that your hydrophilic, polar sidechains are also on the outside surface of your protein where they can hydrogen bond with water.
 - d. Last, fold your protein so that the two Cysteine sidechains are positioned opposite each other on the inside of the protein where they can form a

disulfide bond that helps stabilize your protein. Disulfide bonds are covalent bonds that form between the sulfur groups of cysteines, thus stabilizing the tertiary shape.

Note: As you continue to fold your protein, applying each new property on the list, you will probably find that some of the sidechains you previously positioned are no longer in place. Keep folding until you have a shape in which all properties above apply. The final shape of your protein is called the **Tertiary Structure**.

Post Activity Questions:

- A. Why should Methionine be next to the Blue End Cap, which represents the N-terminus of the protein?

- B. Were you able to fold your protein so that all of the chemical properties were in effect at the same time? If not, why do you think this was the case?

- C. Did your protein look like the proteins that other students folded? Should it have? Why or why not?

- D. How many different proteins, 15 amino acids long, could you make given an unlimited number of each of the 20 amino acids?

- E. How many nucleotides would be required to generate a polypeptide that is 15 amino acids long? This requires knowing how many nucleotides of DNA code for one amino acid.

- F. Assuming that there are between 20,000-25,000 genes in the human genome, do you think there are 1) fewer, 2) approximately the same number, or 3) more proteins in the human genome? Explain your answer.

Homework: What causes Sickle-Cell Anemia?

Objective: At the end of this activity you will be able to describe the effect that a missense mutation has on a protein's function.

Part I: Introduction to Sickle-Cell Anemia

Description:

Sickle cell anemia is a disease in which red blood cells that carry oxygen throughout the body assume a rigid, abnormal ("sickle") shape that restricts their movement through blood vessels. This, in turn, reduces the oxygen that is delivered to the tissues of the body. Under normal conditions, the hemoglobin molecule is composed of 2 alpha-globin and 2-beta globin polypeptides (an example of a quaternary structure). In individuals with sickle-cell anemia, the beta-globin gene has a mutation at the 17th nucleotide of DNA, which leads to a substitution in the 6th amino acid (not counting the initial Methionine, which is removed during processing). A **missense mutation** is one in which a change in the DNA sequence results in a change in the amino acid sequence. Sometimes, this can result in a change in protein folding and/or activity.

Sequence Details:

- Nucleotide #17 - Adenine (A) is replaced by Thymine (T)
- The codon, GAG, which codes for Glutamic Acid (Glu or E) is changed to GTG, which codes for Valine (Val or V) in the 6th position of the beta-hemoglobin protein.

Questions - To be answered before proceeding to Part II.

1. Draw the side chains of glutamic acid and valine below.

2. Describe the difference in the chemical properties of the side chains of glutamic acid and valine (look at their structures, think to which group of amino acids they belong, and discuss how they would behave as part of a protein).

3. How do you think this mutation (from a glutamic acid to a valine) affects the structure and function of the hemoglobin protein?

