Name:_____ Period_____

Student Background Information

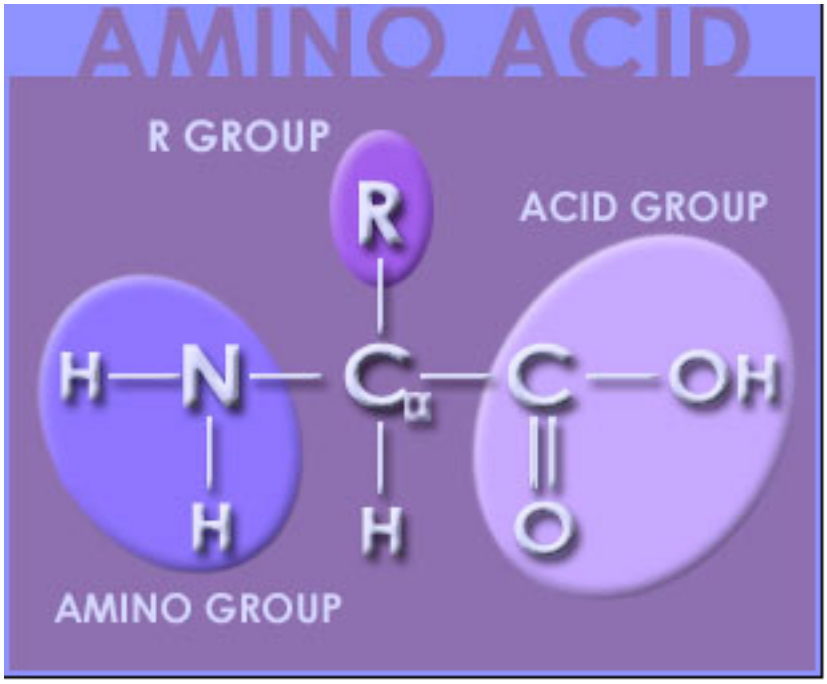
DNA ⇒ RNA ⇒ PROTEIN is the central **dogma** of molecular biology. The **DNA** stores the information; following the **DNA** instructions three different types of **RNAs** (messenger, transfer and ribosomal) assemble the proteins, which do much of the actual work. **Proteins** play a key role in almost everything that organisms do, and carry out most of the work in the cell.

Amino acids are the building blocks of **proteins**. There are 20 types of **amino acids** coded for in the **Universal Genetic Code**. The **Universal Genetic Code** shows the sequence of nucleotides, coded in triplets (**codons**), along the **mRNA**, that determines the sequence of **amino acids** during **protein synthesis**. The **DNA** sequence of a **gene** can be used to predict the **mRNA** sequence, and the **Universal Genetic Code** can in turn be used to predict the corresponding **amino acid** sequence. Your Biology Textbook should have a diagram of the Universal Genetic Code.

All **amino acids** share a basic structure: a central carbon atom (α)with a carboxyl (acid) group, a hydrogen atom, an amino group and a variable side chain (R). The nature of the 'R' chain determines the amino acid. Your biology textbook should provide a reference for the structure of all the amino acids. See Figure 1.

Amino acids are held together by **peptide bonds**. **Peptide bonds** form when the amino group of one **amino acid** chemically binds to the carboxyl group of an adjacent **amino acid**. During this process a molecule of water is lost. This type of chemical bonding is also referred to as '**dehydration synthesis'**.

Figure 1. General structure of amino acids



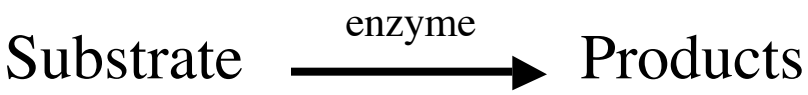
http://www.stanford.edu/group/hopes/basics/proteins/p3.html

Long chains of **amino acids** are called **polypeptides**. A **protein** is one or more **polypeptides** folded into a particular 3-D shape, or conformation. For most **proteins** there is a single 3-D shape that is most stable and at which the protein works best.

There are four different levels of **protein** structure. Each level plays a crucial role in the final 3-D configuration of the **protein**. The first, or primary structure is determined by the sequence of **amino acids**.

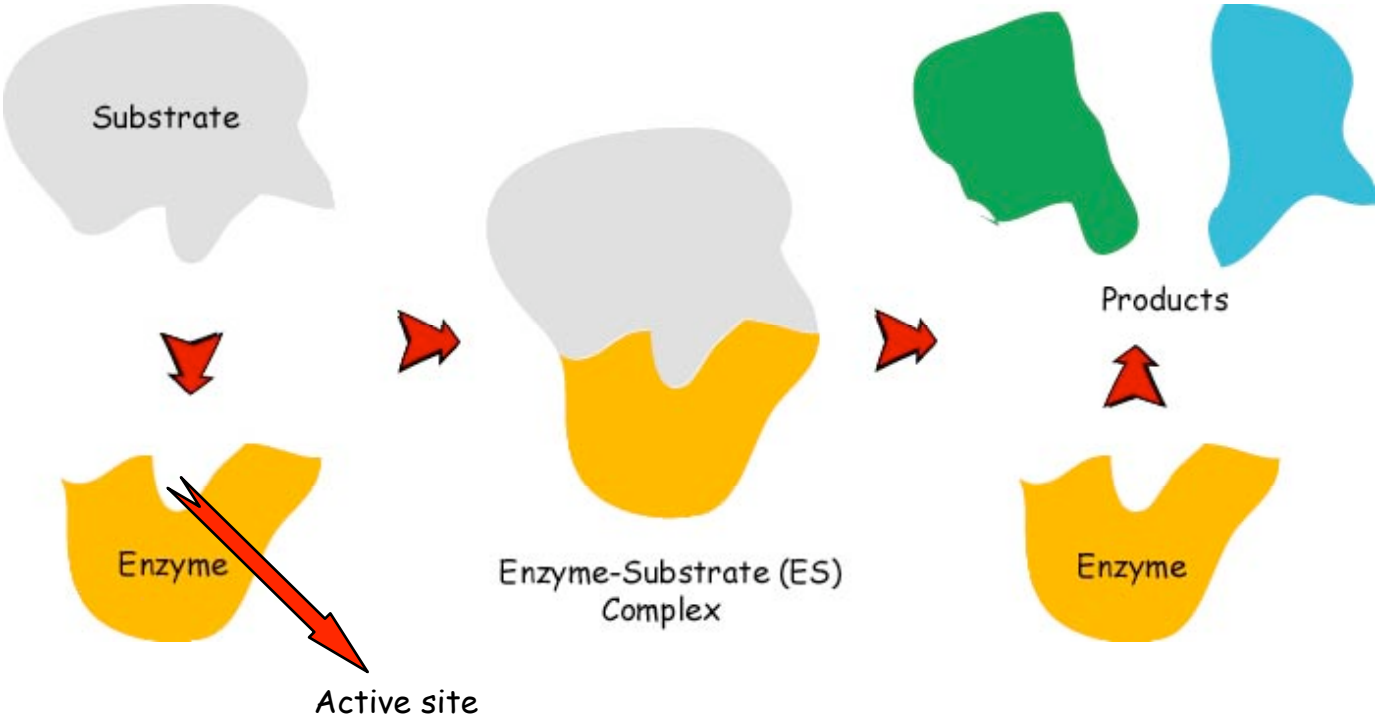
The **amino acids** in the chain interact with each other: there are intramolecular and intermolecular hydrogen bonds formed among the amino groups; these give the chain a very specific geometric shape called the secondary structure.

In this lab we will focus on the relationship between a **protein enzyme** and its **substrate**.



Enzymes are active proteins that catalyze chemical reactions. **Catalysts** are molecules or substances that make chemical reactions go faster. Many of the chemical reactions in your body wouldn't happen at all, or would occur too slowly, without the presence of a **catalyst**. In the course of the chemical reaction the **catalyst** is not changed –thus **enzymes** can be used by your body over and over and over. **Substrates** are what the **enzymes** work on, and are chemically changed into a product by the reaction. The specific point in the **enzyme** where the **substrate** binds is called the **active site**. See Figure 3 below. Notice that the enzyme is not changed in the course of the reaction.

Figure 3. Lock and key model of enzyme action



Adapted from:
http://stezlab1.unl.edu/reu1999/dputn226/ChemHelp/RET_Web_Pages/Enzymes/lock_key1.gif

One model used to explain enzyme action and activity is the "**lock and key**" model. Locks and keys have complementary shapes that allow them to fit and to work together. A slight change in the groves of the key and it won't fit in the lock, or it will fit but it still won't be able to open the door. Similarly **enzymes** and their **substrates** have complementary shapes. According to this model, the **substrate** fits in the **active site** of the **enzyme** and for a brief moment together they form the '**enzyme-substrate complex'**. The better the fit between the **substrate** and the **active site** of the **enzyme**, the faster the reaction will happen. When the reaction is completed the **products** are released from the **active site** and the **enzyme** can be used to **catalyze** the same chemical reaction if there is more **substrate**. This model also illustrates **enzyme specificity**: **enzymes** are specific to a particular reaction and can only **catalyze** one or very few chemical reactions.

Many different factors affect the work of **enzymes**. Temperature and pH are two such factors. All **enzymes** work best at a narrow temperature and pH range. Although a small increase in temperature can serve as a **catalyst** to some chemical reactions, a sharp increase in temperature will affect the chemical bonds within the **enzyme** and can irreversibly distort the **active site**. A malformed **active site** will prevent the **substrate** from binding to the **enzyme** and preclude the reaction from taking place. When **enzymes** are rendered useless they are said to have been '**denatured'**.

Likewise, all **enzymes** will work best at a particular pH. A drastic increase or decrease in the pH surrounding the **enzyme** and **denaturing** can occur.

References:

bbc.co.uk:
http://www.bbc.co.uk/education/asguru/biology/02biologicalmolecules/01proteins/12polymers/06polymers_b/index.shtml

Bio Topics
http://www.biotopics.co.uk

Chemistry of Life's Toolbox
http://stezlab1.unl.edu/reu1999/dputn226/ChemHelp/RET_Web_Pages/Enzymes/lock_key1.gif

The Community College of Baltimore County Student
http://student.ccbcmd.edu/~gkaiser/biotutorials/proteins/images/peptidebond.jpg

Context.info
http://www.contexo.info/DNA_Basics/images/proteinstructuresweb.gif

Elmhurst College
http://www.elmhurst.edu/~c hm/vchembook/566secprotein.html

Mange and Mange. 1999. *Basic Human Genetics*". Sinauer Associates, Inc. Pg. 361.

North Harris College
http://science.nhmccd.edu/biol/dehydrat/dehydrat.html

Stanford University *HOPES* – Huntington's Outreach Project for Education at Stanford:
http://www.stanford.edu/group/hopes/basics/proteins/p3.html

Utah Genetics:
http://learn.genetics.utah.edu/units/disorders/mutations/mutatedna.cfm

Post-Lab Questions: answer in **complete sentences on a separate piece of paper**.

1. Protein synthesis is usually represented by a very simple diagram:

   DNA → RNA → PROTEIN

   Write a short paragraph that explains what does this diagram represent.

2. What determines the 3-D shape of an enzyme?

3. What can cause a change in the 3-D shape of an enzyme?

4. Will a change in the DNA sequence *always* affects enzyme activity?

5. Which is likely to have a greater effect on enzyme activity? *Explain your answer*.

   a. changing a hydrophobic amino acid to a hydrophilic amino acid *or*
   b. changing a hydrophobic amino acid to another hydrophobic amino acid

6. Of the 4 mutants you modeled, which do you think is (are) the most likely to result in an abnormal phenotype? *Explain your answer*.

7. a. What effect will changing pH have on an enzyme?

   b. What effect will changing the temperature have on the enzyme?

8. Read the case study "The Fish Odor Syndrome," on pg. 4 of the lab. ("The Fish-Odor Syndrome" from Mange and Mange, Basic Human Genetics 1999, pg. 361.) Then, answer the following question:

   A mis-sense mutation is a mutation that leads to an alteration of a single amino acid in a protein. Based on what you have learned in this lab, how could changing one amino acid in one enzyme result in such a dramatic phenotypic change (in this example, making someone smell like rotting fish)?

8. Research scientists have identified the shape of key proteins coded for by the HIV virus. How could you use this knowledge to treat AIDS?