Name:_____ Period_____

Student Background Information

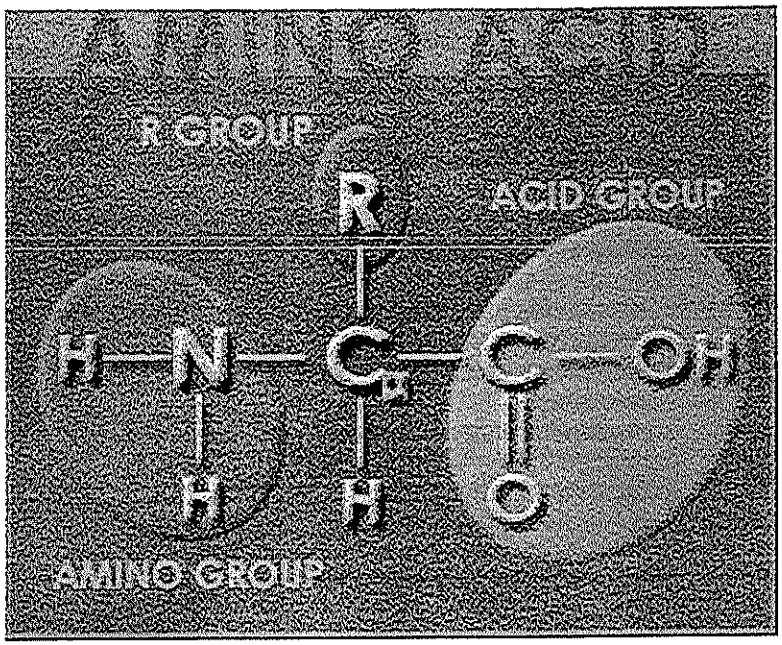
DNA ⇒ RNA ⇒ PROTEIN is the central **dogma** of molecular biology. The **DNA** stores the information; following the **DNA** instructions three different types of **RNAs** (messenger, transfer and ribosomal) assemble the proteins, which do much of the actual work. **Proteins** play a key role in almost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

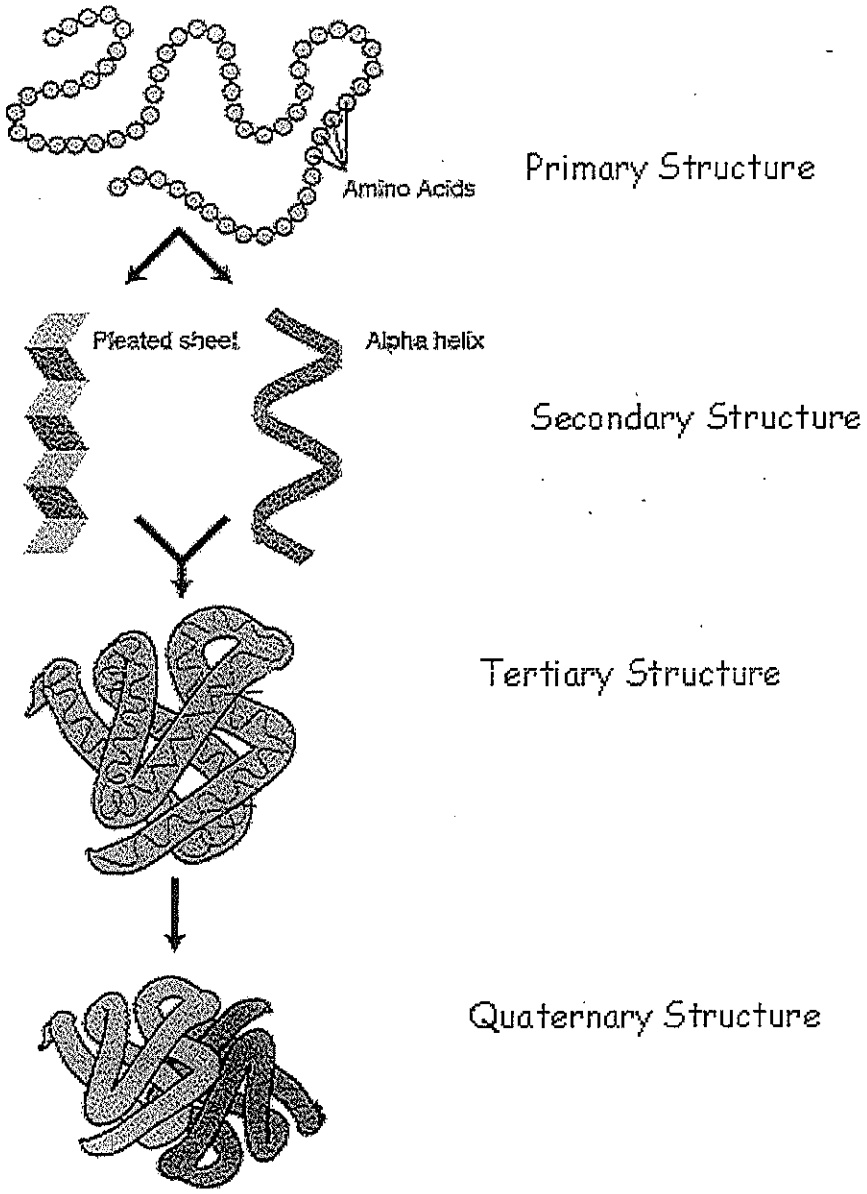
The **amino acids** in the chain interact with each other: there are intramolecular and intermolecular hydrogen bonds formed among the amino groups; these give the chain a very specific geometric shape called the secondary structure.

Figure 2. Different levels of protein structure

Tertiary structure is determined by the interactions between the "side chains" of the **amino acids**. These interactions are caused by a variety of bonds that cause a number of folds, bends, and loops in the **protein** chain.

The quaternary **protein** structure occurs when different chains of **polypeptides** in the **protein** interact with one another and fold the already folded structure into an specific shape (see Figure 2).

Scientists have not yet learned how to accurately predict the 3-D structure of a particular sequence of **amino acids**. However, we do know that the different **amino acids** have distinct chemical properties determined by their variable side chains. It is important to remember that the **amino acids** are 3-D structures themselves. Although the structural formulas for **amino acids** are 2-D on paper, all molecules have a 3-D shape that is determined by chemical bonds. One of the most important properties of the side chain is whether it is polar (hydrophilic) or non-polar (hydrophobic).



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

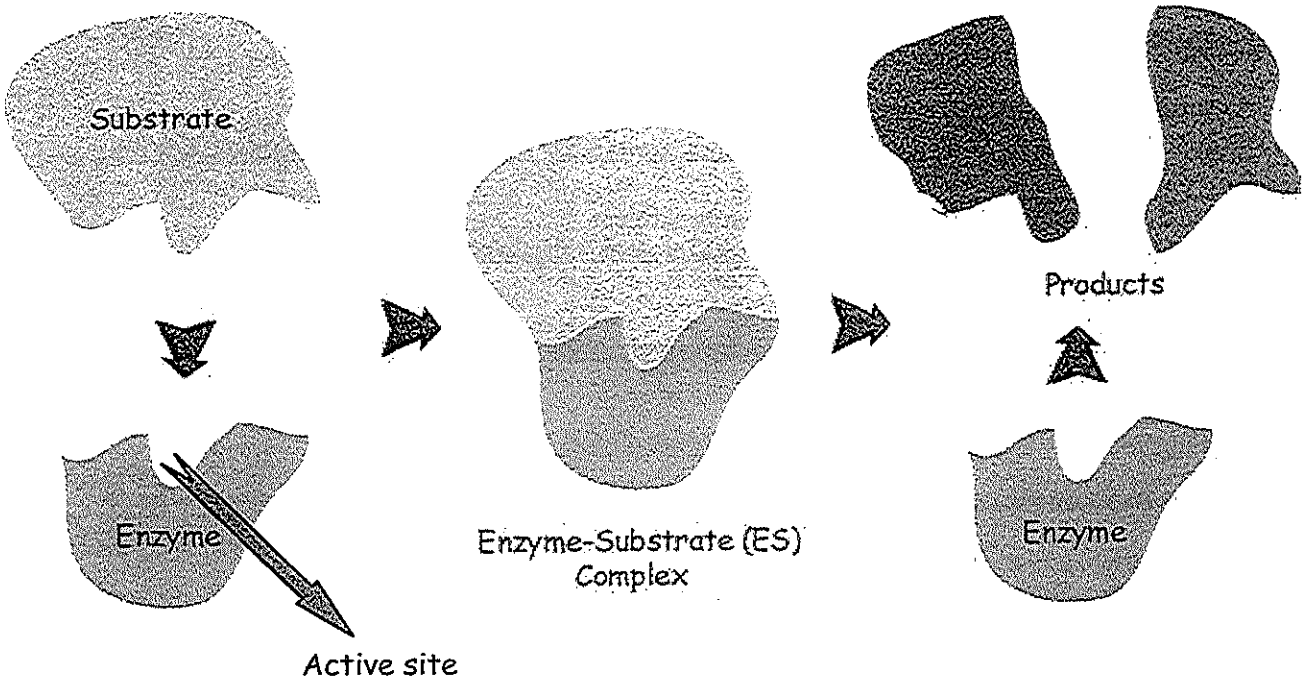
One of the key determinants of **protein** shape is the hydrophobic interaction. **Proteins** fold in a way that maximizes having polar amino acids on the outside and non-polar on the inside. The shape of the **protein** gives it chemical properties that allow the **protein** to perform specific functions in the cell. **Mutating** the sequence (changing even one **amino acid**) may disrupt this 3-D structure and may, therefore, affect the function.

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

$$\text{Substrate} \xrightarrow{\text{enzyme}} \text{Products}$$

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.

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The Building Blocks of Life Lab:

Examining the Importance of Enzyme Shape

CIBT
Student Laboratory Exercise

Name:_____ **Period:**_____ **Date:**_____

Introduction:

Proteins do much of the work in the cell. The shapes of proteins are critical in determining their function. **Proteins** consist of a linear chain of **amino acids** and fold into a specific 3-D shape, or conformation. The pattern of folding is largely determined by whether the amino acids are hydrophobic (water hating) or hydrophilic (water loving). In this lab we will focus on the interaction between a protein enzyme (molecules that **catalyze** chemical reactions) and its **substrate** (the molecules that the enzymes act upon). You will often hear of the "**lock and key**" model to describe the way in which enzymes and substrates interact. The **active site** of an enzyme often has a shape that is complementary to the **substrate**.

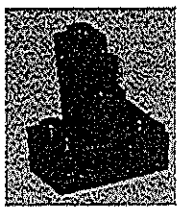
DNA is the genetic material. The sequence of **DNA** will ultimately determine the sequence of **amino acids** in a **protein**. First the information in the **DNA** must be copied into a **messenger RNA** molecule. The **RNA** is complementary to the **DNA** molecule such that G always pairs with C and T with A. However, RNA contains U instead of T, so where there is an A(adenine) in the DNA, the RNA will have a U (uracil). The **Universal Genetic Code** is the key used to decode the relationship between the sequence of bases in the **messenger RNA** and the sequence of **amino acids**.

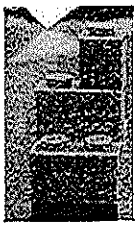
In this lab you will build a model of an **enzyme** using Lego® pieces and you will then examine how a **mutation** (a change in the **amino acid** sequence) can lead to a change in the shape, and thereby the function, of the **enzyme**.

PART I: THE NORMAL ENZYME

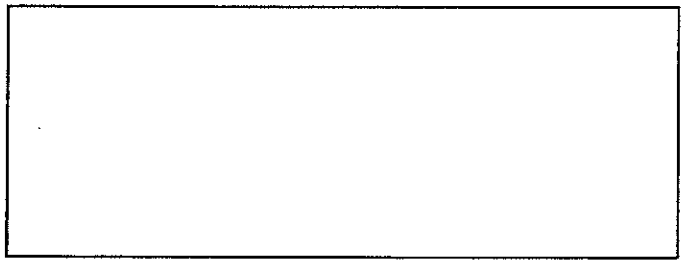
Procedure:

1. Obtain a Lego® kit from your teacher. This contains an assembled structure (the substrate) and Lego® building blocks which represent amino acids that will be used to assemble the enzyme.





2. Observe the substrate and **predict** the shape of an enzyme that could interact (fit) with the substrate. Then use all, or at least most of the Legos ® to create an enzyme that would interact with your substrate. Fit the enzyme and the substrate together to create the **enzyme-substrate complex**. Use the box below to sketch the enzyme as it interacts with the substrate. Color the substrate only, and label both substrate and enzyme. **Keep this structure. Do not take it apart until you are directed to do so.**

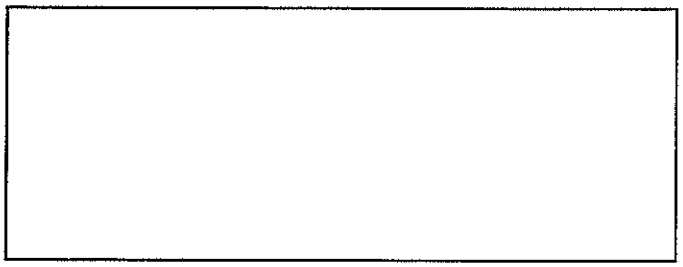


3. Using the DNA sequence of the **normal enzyme** given below and the information on **TABLE 1**, determine the primary structure (amino acid sequence) of the enzyme. Transcribe the sequence and record the amino acid and Lego® sequence on your **Worksheet Page** for future reference.

   DNA Sequence of Normal Enzyme:

   3'CGATAATCATAACAAGATACCGTGTAACTA5'

4. Get a second set of Lego® pieces. Using **TABLE 2: *"The Blueprint"***, assemble the 3D structure of the normal enzyme. Draw it here; colors are not necessary.



   How does it compare to the enzyme you had created in step 2? List two similarities and two differences in the structure (**not** the colors).

   _____

   _____

   _____

5. How does the normal enzyme bind to the substrate? Try to fit the substrate into the enzyme **but do not snap** together (the enzyme might become undone easily when trying to pull the substrate away and can be quite frustrating). Set the predicted enzyme, the normal enzyme and the substrate aside. To help you keep track of these three structures , take a blank piece of paper and write, at three different points on the paper: 'Predicted Enzyme', 'Normal Enzyme' and 'Substrate'. Place the corresponding structures on the paper accordingly.

PART II: MUTANT ENZYMES

Procedure:

1. Observe the DNA sequence for the 4 mutant DNA sequences on the *Worksheet Page.*
2. Using the DNA sequences and TABLE 1, determine the primary structure (amino acid sequence) of each of the mutant enzymes. Transcribe these sequences and record the amino acid and Lego® sequence on your Worksheet Page. Circle or highlight the location of the amino acid substitutions in each mutant enzyme.
3. In genetics, a normal sequence (or individual) is called a '**wild-type**' and any sequences (or individuals) exhibiting changes are called **mutants**. Compare the primary structure of each mutant to the normal "**wild-type**" amino acid sequence. *Predict* which mutants will *still* be able to bind to the substrate and which mutants will *not* be able to bind to the substrate. Record your predictions on the *Prediction Chart* below.
4. Using TABLE 2 (the *Blueprint),* and the amino acid sequence on the Worksheet Page, assemble the 3-D structure of mutant enzyme #1. Determine whether or not the enzyme can bind to the substrate, as the normal enzyme does. Use the building blocks that you used to build the predicted enzyme (the first enzyme that you built). Don't forget to substitute the amino acid according to the mutation. Record whether it binds or not on the *Prediction Chart* below.
5. Repeat step 4 for mutants #2, 3 and 4.

> *****To construct your mutant enzymes, follow the directions in the blueprint and insert or substitute alternative pieces when necessary- use the same orientation as directed for the normal enzyme.**** A useful idea is to line up the Lego® pieces in the corresponding order according to the Building Blocks sequence on the Worksheet Page.*

PREDICTION CHART

	PREDICTION	**ACTUAL RESULT**
Mutant Enzyme	*Will* bind to substrate (Y or N)	*Did* bind to substrate (Y or N)
#1		
#2		
#3		
#4		

Worksheet Page

The Normal Enzyme

3'CGATAATCATAACAAGATACCGTGTAACTA5'

Messenger **RNA** ____-____-____-____-____-____-____-____-____-____

Amino Acid Sequence of Normal Enzyme ____-____-____-____-____-____-____-____-____-____

Building Blocks Sequence ____-____-____-____-____-____-____-____-____-____

The Mutant Enzymes

DNA Sequence of Mutant #1 3'CGATAATAATAACAAGATACCGTGTAACTA5'

Messenger **RNA** ____-____-____-____-____-____-____-____-____-____

Amino Acid Sequence of Mutant Enzyme #1 ____-____-____-____-____-____-____-____-____-____

Building Blocks Sequence ____-____-____-____-____-____-____-____-____-____

DNA Sequence of Mutant #2 3'CGATAAACATAACAAGATACCGTGTAACTA5'

Messenger **RNA** ____-____-____-____-____-____-____-____-____-____

Amino Acid Sequence of Mutant Enzyme #2 ____-____-____-____-____-____-____-____-____-____

Building Blocks Sequence ____-____-____-____-____-____-____-____-____-____

DNA Sequence of Mutant #3 3'CGATAATCATAACAAGATACCGTGTCACTA5'

Messenger **RNA** ____-____-____-____-____-____-____-____-____-____

Amino Acid Sequence of Mutant Enzyme #3 ____-____-____-____-____-____-____-____-____-____

Building Blocks Sequence ____-____-____-____-____-____-____-____-____-____

DNA Sequence of Mutant #4 3'CGATAATCATAACTAGATACCGTGTAACAA5'

Messenger **RNA** ____-____-____-____-____-____-____-____-____-____

Amino Acid Sequence of Mutant Enzyme #4 ____-____-____-____-____-____-____-____-____-____

Building Blocks Sequence ____-____-____-____-____-____-____-____-____-____

TABLE 1: The Genetic Code and "Lego® Code" for Select Amino Acids

DNA	RNA	Amino Acid	Hydrophilic or Hydrophobic?	Lego® code
$_3$,TCA$_5$,	$_5$,AGU$_3$,	Serine (Ser)	Hydrophilic	3x1
$_3$,TAA$_5$,	$_5$,AUU$_3$,	Isoleucine (Iso)	Hydrophobic	2x1
$_3$,CGA$_5$,	$_5$,GCU$_3$,	Alanine (Ala)	Hydrophobic	1x1
$_3$,CAA$_5$,	$_5$,GUU$_3$,	Valine (Val)	Hydrophobic	L
$_3$,GAT$_5$,	$_5$,CUA$_3$,	Leucine (Leu)	Hydrophobic	4x1
$_3$,ACC$_5$,	$_5$,UGG$_3$,	Tryptophan (Try)	Hydrophobic	2x2 square
$_3$,GTG$_5$,	$_5$,CAC$_3$,	Histidine (His)	Hydrophilic	4x2 sheet
$_3$,CTA$_5$,	$_5$,GAU$_3$,	Aspartate (Asp)	Hydrophilic	Roof piece
$_3$,ACA$_5$,	$_5$,UGU$_3$,	Cysteine (Cys)	Hydrophilic	6x1
$_3$,TGC$_5$,	$_5$,ACG$_3$,	Threonine (Thr)	Hydrophilic	6x2 block

BOX 16B *The Fish-Odor Syndrome*

"What have we here? a man or a fish? dead or alive? A fish: he smells like a fish; a very ancient and fish-like smell; a kind of, not of the newest, Poor-John.* A strange fish!" (William Shakespeare, 1611)

So cried the jester Trinculo when he discovered the savage and deformed slave Caliban, in Shakespeare's *The Tempest* (II.ii.24–27). This quote could also apply to some unfortunate individuals who actually smell like rotting fish or, to some people, like stale urine (Mitchell 1996). They suffer from an unusual condition called *trimethylaminuria*, more simply known as the *fish-odor syndrome*. Although they show no other clinical symptoms, their extremely offensive body odor usually makes them social outcasts from an early age, experiencing ridicule, rejection, isolation, and employment difficulties: "Affected persons may be deeply disturbed, depressed, and even suicidal, with psychosocial problems in school" (McKusick et al. 1997).

The syndrome results from a defect in the liver enzyme FMO3 (flavin-containing *mono-oxygenase*), which normally converts the smelly protein trimethylamine to nonsmelly trimethylamine *N*-oxide. The trimethylamine is produced by bacteria in the gut, acting on breakdown products from the digestion of certain substances, including fish and other seafoods, eggs, liver, soybeans, milk, and choline- or lecithin-containing health foods or drugs. In affected individuals, the unprocessed trimethylamine is excreted in their breath, sweat, and urine. Although the resulting stench cannot be completely eliminated, it may be reduced by avoidance of the trimethylamine-producing substances, by using acidic soaps and lotions, and perhaps by intermittent suppression of gut bacteria through limited antibiotic treatment. In addition, psychiatric counseling may help affected people to deal with their serious social and psychological problems.

The trait is inherited as an autosomal recessive, with the *FMO3* locus residing on chromosome 1q. Homozygotes for the mutant allele are affected, and heterozygotes exhibit reduced levels of the FMO3 enzyme activity. In 1997, Dolphin et al. sequenced the coding regions of the *FMO3* DNA from an affected individual, identifying a single missense mutation possessed by all affected members of that pedigree.

*A Poor-John is a salted, dried hake (a fish related to Atlantic cod).

"The Fish-Odor Syndrome" from Mange and Mange, Basic Human Genetics 1999, pg. 361.

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?