

Cornell Institute for Biology Teachers

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Lab review date: January, 2009

Title:

**The Building Blocks of Life:
Examining the Importance of Enzyme Shape**

Authors:

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Revised by Nancy Harrison, Vestal High School and Florianna Blanton, Cornell University

**Appropriate
Level:**

Living Environment or Advanced Placement Biology

Abstract:

The shape of a protein determines its function. In this lab, students will be given a hypothetical DNA sequence for part of an enzyme. Using the Universal Genetic Code, they will then determine the amino acid sequence coded for by the DNA. Students will examine a “substrate” and predict the shape of an enzyme that could interact with that substrate. Differently shaped Lego® blocks will represent different amino acids. Next they will construct the “enzyme” using Legos® as specified in the code and see if it a) matches their prediction and b) fits the substrate. Effects of mutant DNA sequences on the enzyme structure will be examined to see how the interaction between the enzyme and substrate are affected.

**Time
Required:**

A double period or two to three 40 minute class periods. The ‘supplemental background reading’ can be completed in one period including a full class discussion, or can be assigned as homework depending on the students’ level.

**NYS Learning
Standards:**

Living Environment: 2.1g-i, 2.2e, 5.1f, 5.1g

Additional Teacher Information

Objectives

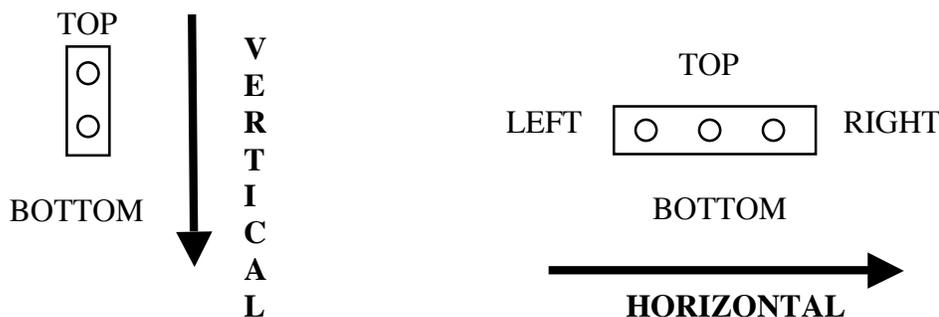
Students will be able to understand:

- the lock and key model of enzyme-substrate interactions
- the order of amino acids in a polypeptide is determined by the DNA sequence
- the shape of a protein depends upon the chemical properties of the amino acids
- changes in DNA affect the primary structure of a polypeptide, and how this can affect the shape (and thus the function) of the enzyme

Prerequisite Information

- DNA \Rightarrow RNA \Rightarrow Protein
- Triplet code
- The basic structure of amino acids as the building blocks of proteins. The levels of protein structure.
- Hydrophobic and hydrophilic interactions
- The role of enzymes in biological systems
- The role of enzymes in metabolic diseases

You will need to demonstrate to your students what you mean by “vertical” and “horizontal” when building with Legos® before turning them loose on the lab.



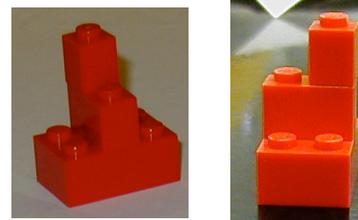
Recommendations:

- Copy the *Lego® Shapes* page on a different colored paper for easy reference.
- Direct students to separate the pieces beforehand and to return to the bag the ones they are not immediately using. Each bag should contain the same amount and types of blocks.
- Use the ‘Packing List’ (the last page in this document) and paste it in the bags that contain the Lego® pieces for easy clean up.

Materials

Each working group of four students should have **two** plastic bags of assorted Lego® pieces. Each bag should contain:

- A bag of Legos® containing at least the makings for the “substrate” and the “enzyme”: two 1x1, five 2x1, one 3x1, one 3x2, one L (corner piece), one 2x2, one 4x1, one 6x1, one 4x2 sheet, and one 2 wide roofing piece. See *Lego® Shape* handout in the Student Section.
- Assemble the “substrate”: using these pieces from the bag of Legos® described above: a 3x2 (rectangle) with a 2x1 over of the middle two circles and a 1x1 over the top circle of the 2x. To save class time, we suggest to assemble this before hand and permanently gluing it in place with Duco® cement or a similar glue. Make sure the substrate pieces fit properly. See picture.
- Handouts as follows:
 1. Chart listing DNA codons, with corresponding RNA codons, amino acids, and Lego® piece (included –see *Table 1, The Universal Genetic Code and Lego Code for Select Amino Acids*)
 2. *Table 2: ‘The Blue Print’* for constructing the “enzymes”
 3. *Lego® Shape* handout

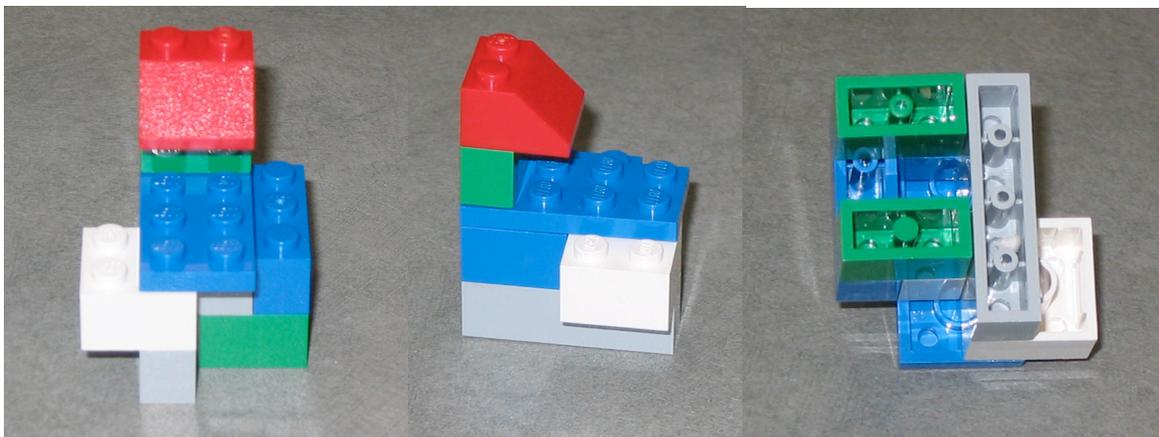


We suggest having a class set of copies plus one additional on the lab benches for each working group.

- Colored pencils

Note: You can purchase individual Lego® pieces at www.shop.lego.com.

The completed normal enzyme:



Answers to Post-Lab Questions

Worksheet Page #1

Amino Acid Sequence of Normal Enzyme

Alanine-Isoleucine-Serine-Isoleucine-Valine-Leucine-Tryptophan-Histidine-Isoleucine-Aspartate

Lego® Sequence of Normal Enzyme

1x1- 2x1 - 3x1- 2x1 - L - 4x1 - 2x2 - 4x2 sheet -2x1 – roof piece

Amino Acid Sequence of Mutant Enzyme #1 (does not fit substrate)

Alanine-Isoleucine-Isoleucine-Isoleucine-Valine-Leucine-Tryptophan-Histidine- Isoleucine -Aspartate

Lego • Sequence of Mutant Enzyme #1

1x1- 2x1 – 2x1 - 2x1 - L - 4x1 - 2x2 - 4x 2 sheet -2x1 – roof piece

Amino Acid Sequence of Mutant Enzyme #2 (fits substrate)

Alanine-Isoleucine-Cystine-Isoleucine-Valine-Leucine-Tryptophan-Histidine- Isoleucine -Aspartate

Lego • Sequence of Mutant Enzyme #2

1x1- 2x1 - 6x1 - 2x 1- L - 4x1 - 2x2 (square) - 4x2 sheet -2x1 – roof piece

Amino Acid Sequence of Mutant Enzyme #3 (fits substrate)

Alanine-Isoleucine-Serine-Isoleucine-Valine-Leucine-Tryptophan-Histidine- Serine -Aspartate

Lego® Sequence of Mutant Enzyme #3

1x1- 2x1 - 3x1- 2x1 – L - 4x1- 2x2 - sheet -3x1 – roof piece

Amino Acid Sequence of Mutant Enzyme #4 (does not fit substrate)

Alanine-Isoleucine-Serine-Isoleucine-Aspartate-Leucine-Tryptophan-Histidine- Isoleucine -Valine

Lego® Sequence of Mutant Enzyme #4

1x1- 2x1 - 3x1- 2x1 –roof piece - 4x1 - 2x2 -4x2 sheet -2x1 - L

Post-Lab Questions

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



Write a short paragraph that explains what this diagram represents.

Protein synthesis is the process in which the DNA directs the production of amino acids and proteins. The information stored in the molecule of DNA is transcribed into mRNA. A ribosome binds to the mRNA and tRNA translates the message into an amino acid sequence.

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

The physical and chemical properties of the amino acid, which are coded for by the DNA sequence.

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

A change in DNA sequence can change the primary amino acid sequence, and as a result change the shape of the enzyme.

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

No. If the DNA change doesn't cause a change in the ability of the enzyme to interact with its substrate, then it won't affect the enzyme activity. Some DNA changes do NOT change the sequence of amino acids, due to redundancy in the Genetic Code. Some DNA changes will not alter the active site. Keep in mind though that mutations may change activity without directly affecting the conformation of the active site, e.g. Affect the stability of the mRNA or of the enzyme.

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

- A. changing a hydrophobic amino acid to a hydrophilic amino acid *or*
B. changing a hydrophobic amino acid to a hydrophobic amino acid

A. is likely to have a greater effect. One of the key causes of folding is hydrophobic interaction, changing a hydrophobic amino acid to a hydrophilic amino acid will disrupt this interaction.

6. Of the 4 mutants you modeled, which do you think are most likely to result in an abnormal phenotype? Why?

Mutants # 1 and #4 are most likely to result in a disease phenotype. In both cases, the active site of the enzyme was altered so it was no longer a complementary shape to its substrate. Since it can't bind to its substrate it won't catalyze the reaction and may result in a disease phenotype. (Note: though that even with a change in active site, may not have abnormal phenotype if recessive mutation, and only one copy is altered or if genetic redundancy (more than one protein carries out the function).

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

Changing the pH or raising the temperature may disrupt the chemical bonds that stabilize the 3-D structure of the protein. If the bonds are disrupted, the protein shape will fall apart (denature). This will cause the rate at which the enzyme works to decrease.

8. Read the case study “The Fish-Odor Syndrome” from Mange and Mange “Basic Human Genetics” 1999, Sinauer Associates, Inc. pg. 361.

A mis-sense mutation is a mutation that alters 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 cause someone to smell like rotting fish?

The mis-sense mutation may alter the 3-D shape of the FMO3 enzyme such that it will no longer be able to interact with trimethylamine (its substrate) causing a build up of this smelly substrate.

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

If you know the shape of the enzyme, you can design a drug to interact with the active site of the enzyme and block it from interacting with its substrate.

References

BBC:

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.contexto.info/DNA_Basics/images/proteinstructuresweb.gif

Elmhurst College

<http://www.elmhurst.edu/~chem/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>

Teacher Background Information

DNA ⇒ RNA ⇒ PROTEIN is the central **dogma** of molecular biology. The **DNA** stores the information; following the **DNA** instructions the **RNA** (messenger, transfer and ribosomal) assembles 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.

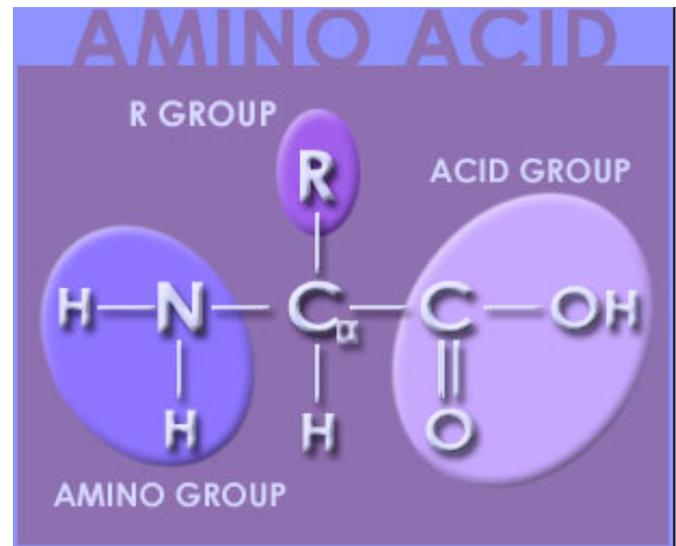
Amino acids are the building blocks of **proteins**. There are 20 types of **amino acids** coded for in the **Universal Genetic Code**. The **Universal Genetic Code** shows the sequence of nucleotides, coded in triplets (**codons**) along the **mRNA**, that determines the sequence of **amino acids** 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. See Figure 1 (from the Utah Genetics webpage, <http://learn.genetics.utah.edu/>).

Figure 1.

Universal Genetic Code (mRNA format)					
	U	C	A	G	
U	UUU--Phe	UCU--Ser	UAU--Tyr	UGU--Cys	U
	UUC--Phe	UCC--Ser	UAC--Tyr	UGC--Cys	C
	UUA--Leu	UCA--Ser	UAA--stop	UGA--stop	A
	UUG--Leu	UCG--Ser	UAG--stop	UGG--Trp	G
C	CUU--Leu	CCU--Pro	CAU--His	CGU--Arg	U
	CUC--Leu	CCC--Pro	CAC--His	CGC--Arg	C
	CUA--Leu	CCA--Pro	CAA--Gln	CGA--Arg	A
	CUG--Leu	CCG--Pro	CAG--Gln	CGG--Arg	G
A	AUU--Ile	ACU--Thr	AAU--Asn	AGU--Ser	U
	AUC--Ile	ACC--Thr	AAC--Asn	AGC--Ser	C
	AUA--Ile	ACA--Thr	AAA--Lys	AGA--Arg	A
	AUG--Met	ACG--Thr	AAG--Lys	AGG--Arg	G
G	GUU--Val	GCU--Ala	GAU--Asp	GGU--Gly	U
	GUC--Val	GCC--Ala	GAC--Asp	GGC--Gly	C
	GUA--Val	GCA--Ala	GAA--Glu	GGA--Gly	A
	GUG--Val	GCG--Ala	GAG--Glu	GGG--Gly	G

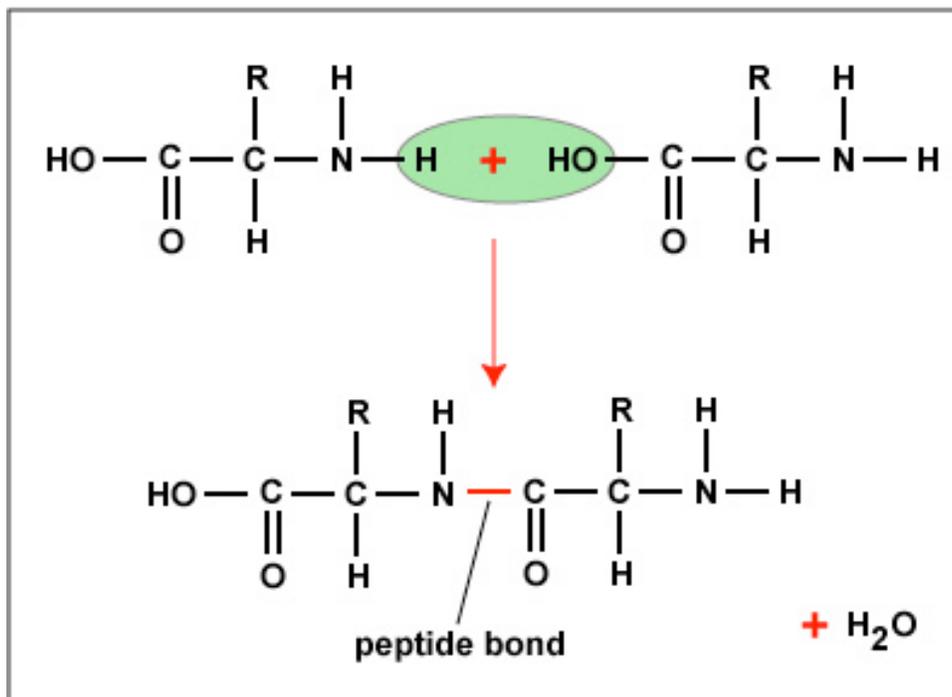
All **amino acids** share a basic structure: a central carbon atom (α) with a carboxyl (acid) group, a hydrogen atom, an amino group and a variable side chain (R). The nature of the 'R' chain determines the amino acid. Your biology textbook should provide a reference for the structure of all the amino acids, and of the **Universal Genetic Code**. See Figure 2 (from <http://www.stanford.edu>).

Figure 2. General structure of amino acids



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**'. See Figure 3.

Figure 3. Peptide bond formation by dehydration synthesis



<http://student.ccbcmd.edu/~gkaiser/biotutorials/proteins/images/peptidebond.jpg>

For animations of peptide bond formation and dehydration synthesis please visit:

- <http://science.nhmccd.edu/biol/dehydrat/dehydrat.html> (scroll down to Protein Metabolism)
- http://www.bbc.co.uk/education/asguru/biology/02biologicalmolecules/01proteins/12polymers/06polymers_b/index.shtml (interactive tutorial)

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**.

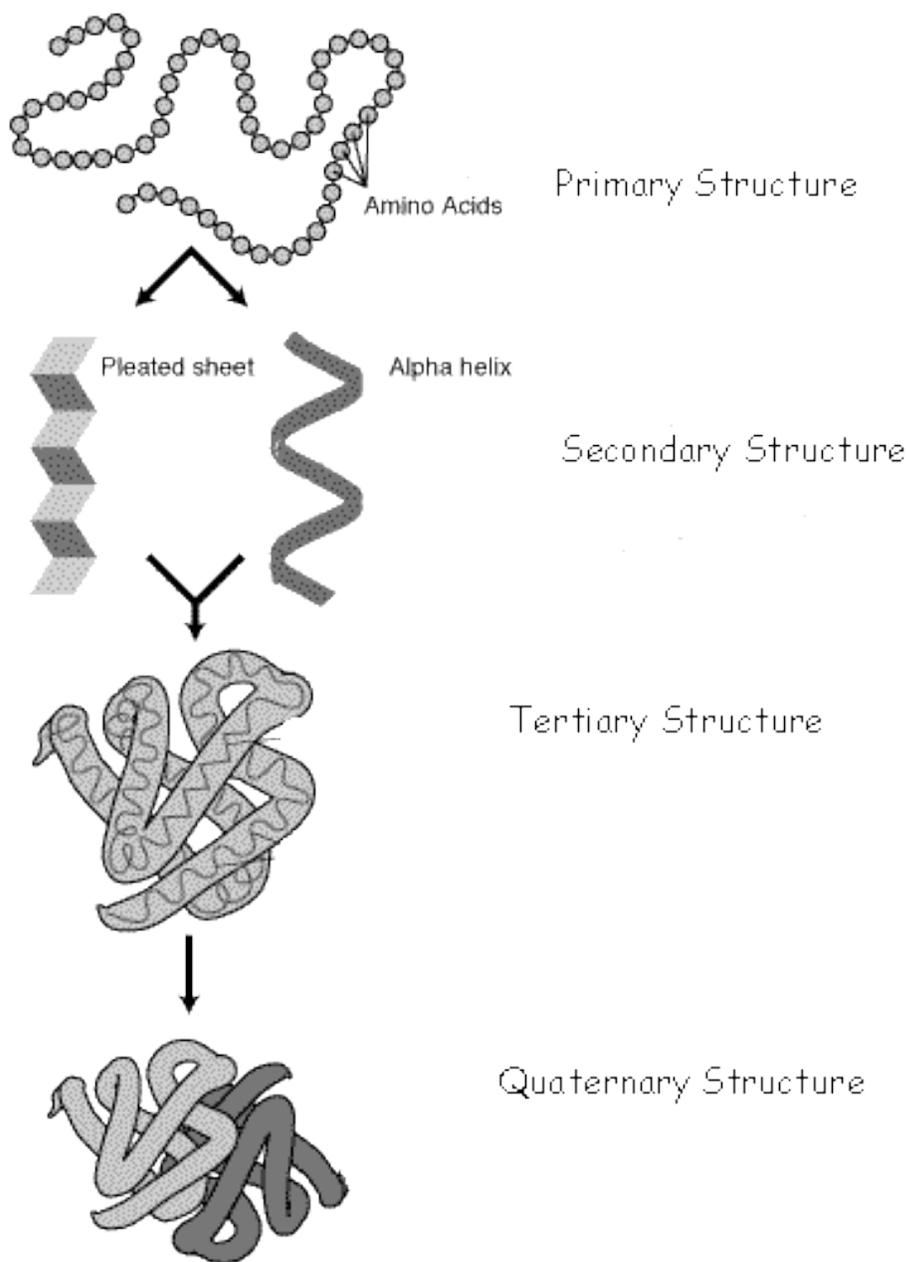
The first, or primary structure is determined by the sequence of **amino acids**.

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.

The tertiary structure is determined by 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 4).

Figure 4 . Different levels of protein structure



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

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.

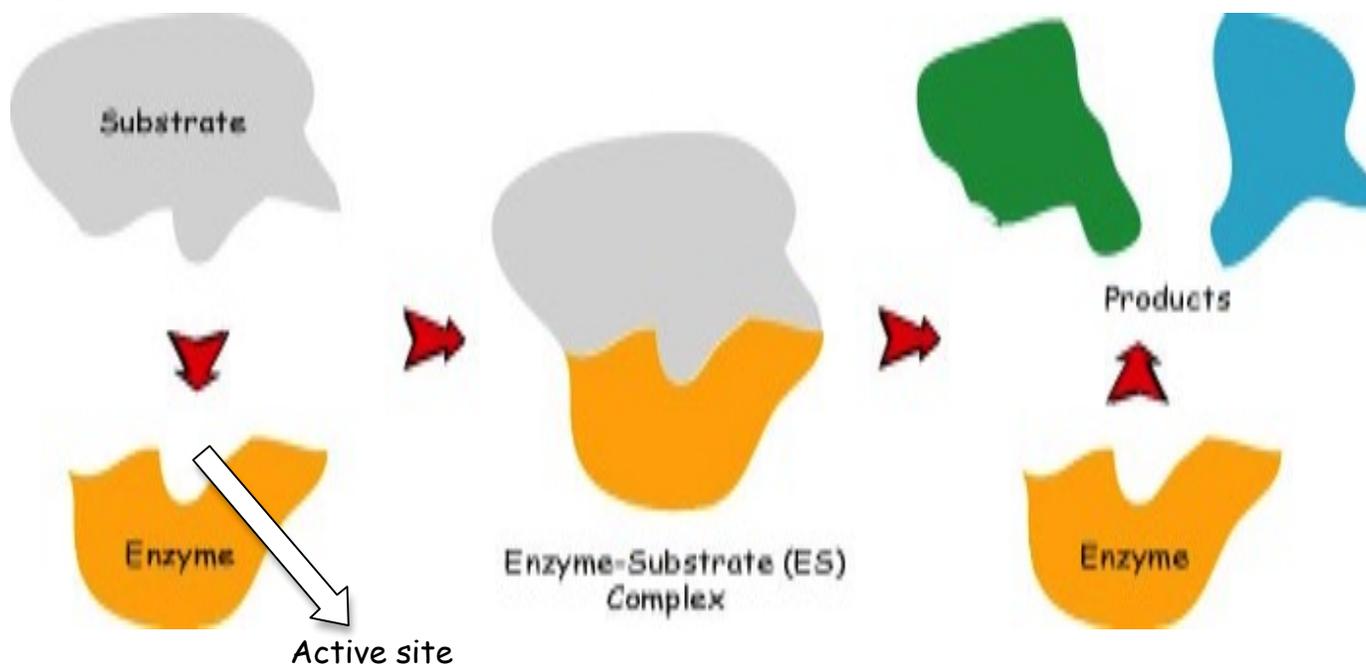
One of the most important properties of the side chain is whether it is polar (hydrophilic) or non-polar (hydrophobic). One of the key determinants of **protein** shape is the hydrophobic interaction. **Proteins** fold in a way that maximizes having polar amino acids on the outside and non-polar on the inside. The shape of the **protein** has 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 its function.

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



Enzymes are biological **catalysts**. **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 5 below. Notice that the enzyme is not changed in the course of the reaction.

Figure 5. Lock and key model of enzyme action



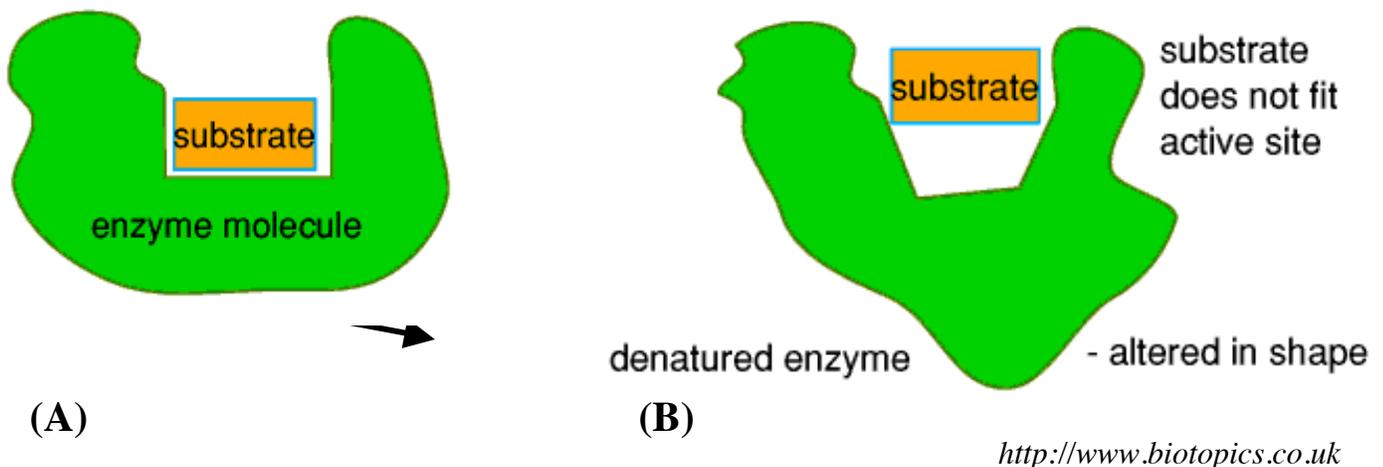
Adapted from: http://stzlab1.unl.edu/reu1999/dputn226/ChemHelp/RET_Web_Pages/Enzymes/lock_key1.gif

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 work together. A slight change in the grooves 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 stronger the chemical bonding or level of interaction between the two. Chemical reactions occur because of the chemical interactions taking place in the **active site** between the **substrate** and the **enzyme**. The bonds in the **substrate** are rearranged and the **substrate** is changed into the **product**s of the reaction. The **products** are then released from the **active site** and the **enzyme** can be used to **catalyze** the same chemical reaction by acting upon left over **substrates**. 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 rate. 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**'. Look at Figure 6, for a graphic representation.

Figure 6. Representation of an enzyme before (A) and after being denatured (B)



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.

Extended topics:

- **Metabolic Disorders:** missing or defective enzymes cause over 200 human diseases including PKU and Tay-Sachs. Collectively they are termed metabolic diseases. Individuals with the disease either don't produce any enzyme at all or they have a mutated enzyme with an altered shape such that it does not interact well with its substrate. Either way there is a build up of precursors and/or a lack of end products. Understanding the cause of the disease may lead to a treatment. For example people with PKU have a mutation in the enzymes needed to convert phenylalanine to tyrosine. A low phenylalanine diet bypasses this problem and is a treatment for the disease.

- **Proteomics:** The proteome is the protein equivalent of the genome: it consists of all of the proteins expressed in a cell. A key component of this new and growing field is determining the 3D structure of proteins as well as determining what proteins interact with one another. Proteomics will play an important role linking genomics with pharmacogenetics and other medical applications.
- **Drug Design:** Knowing the shape of enzymes and substrates can lead to rational drug design. In the case of the HIV virus, drugs can be designed to block the active site of viral proteins, slowing down replication of the virus.

Student Background Information

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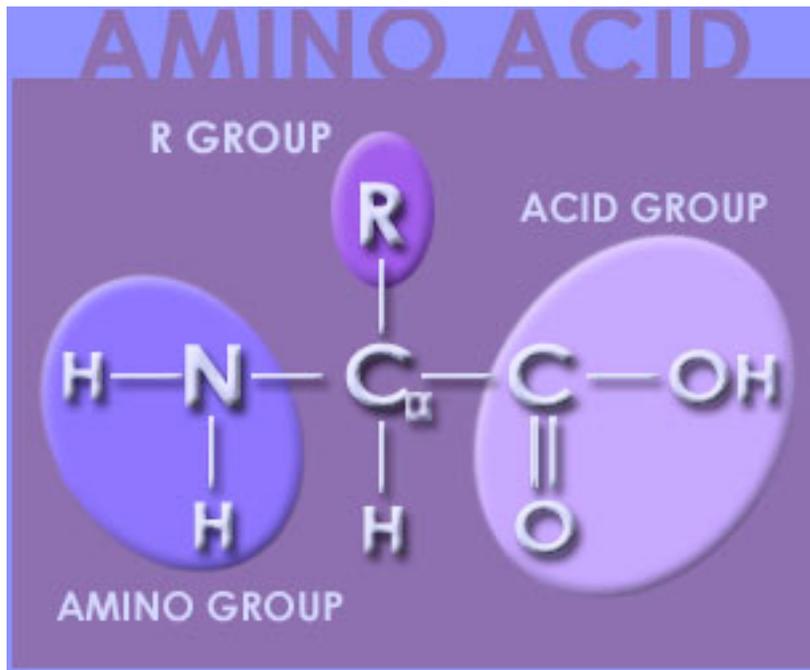
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Figure 1 . General structure of amino acids



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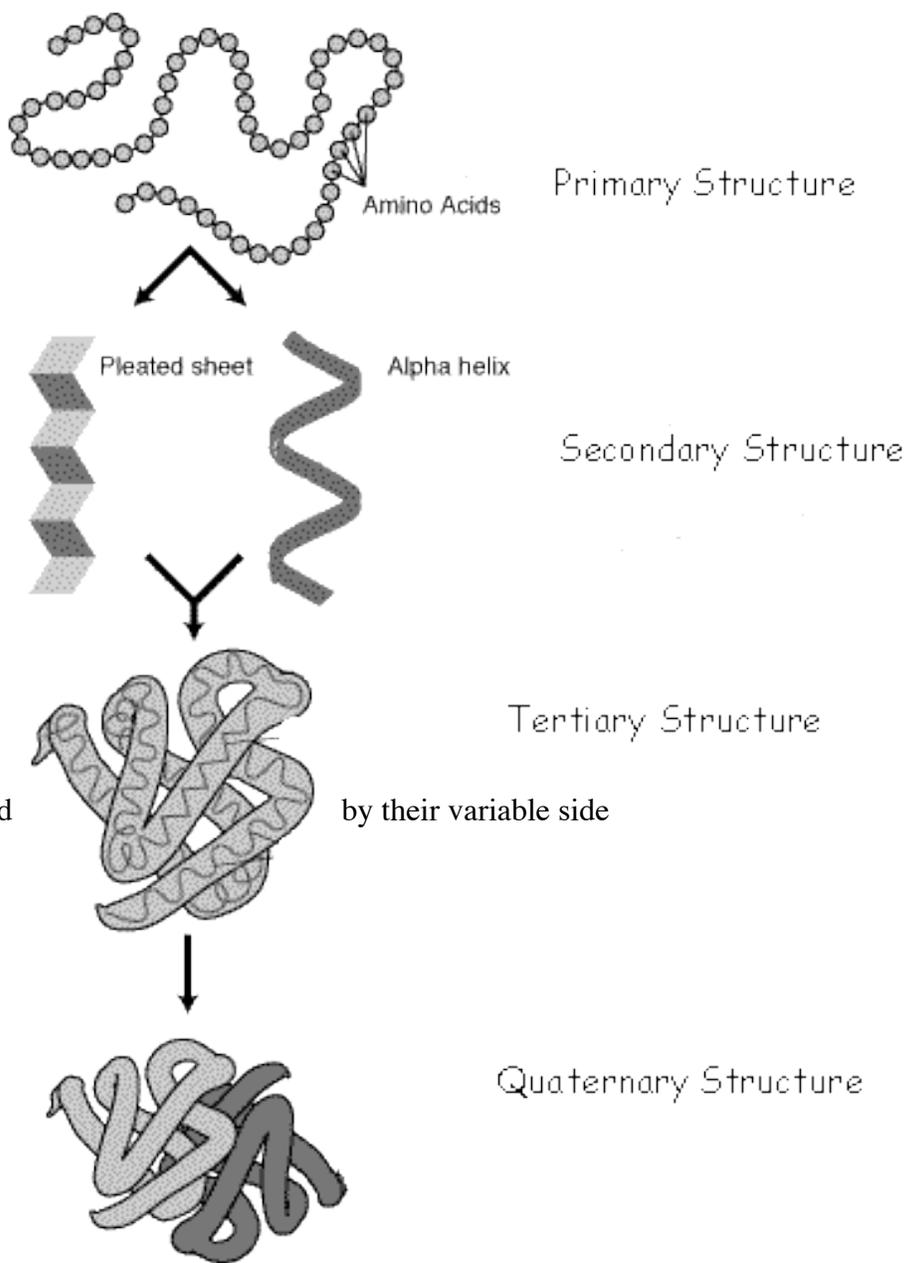
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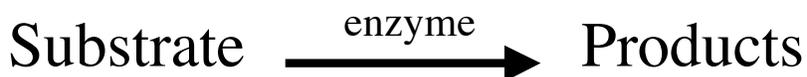
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Figure 2. Different levels of protein structure



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

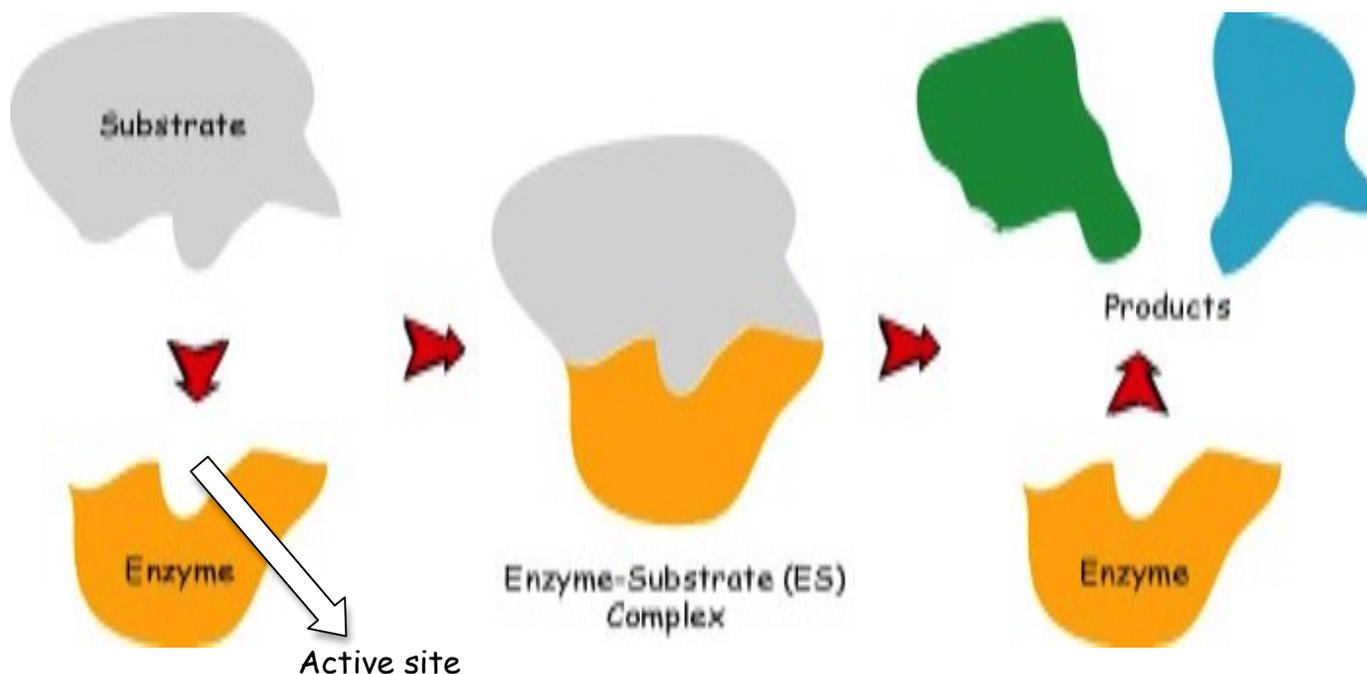
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Figure 3. Lock and key model of enzyme action



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

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References

BBC:

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

Bio Topics

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North Harris College

<http://science.nhmccd.edu/biol/dehydrat/dehydrat.html>

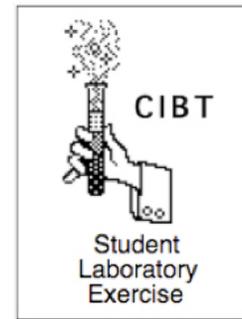
Stanford University *HOPES* – Huntington's Outreach Project for Education at Stanford:

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

Utah Genetics:

<http://learn.genetics.utah.edu/units/disorders/mutations/mutatedna.cfm>

The Building Blocks of Life: Examining the Importance of Enzyme Shape



Name: _____

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**.

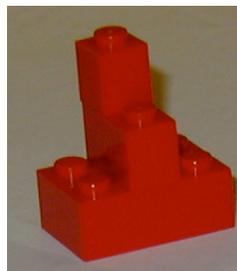
DNA is the genetic material. The sequence of **DNA** will ultimately determine the sequence of **amino acids** in a **protein**. First the information in the **DNA** must be copied into a **messenger RNA** molecule. The **RNA** is complementary to the **DNA** molecule such that G always pairs with C 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**.

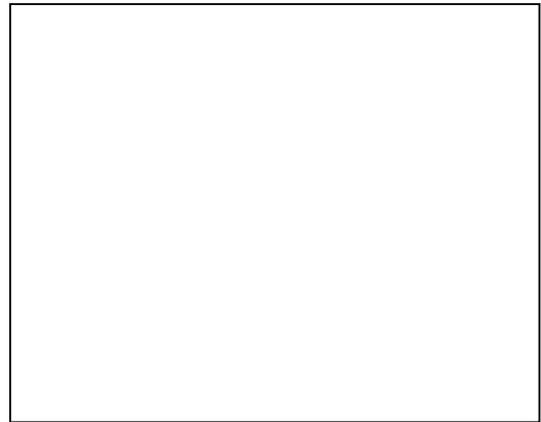
PART I: THE NORMAL ENZYME

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 at right 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.**

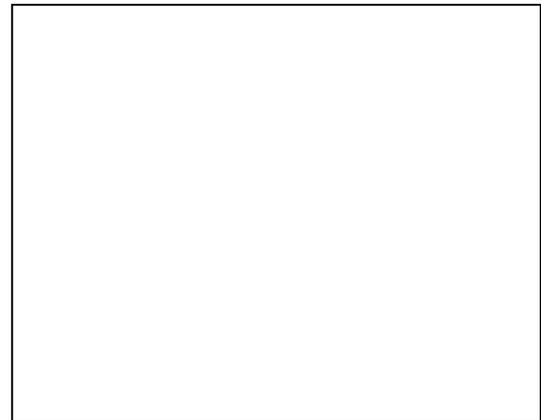


3. Using the DNA sequence of the **normal enzyme** given below and the information on **TABLE 1**, determine the primary structure (amino acid sequence) of the enzyme. Transcribe the sequence and record the amino acid and Lego® sequence on your **Worksheet Page** for future reference.

DNA Sequence of Normal Enzyme:

3'CGATAATCATAACAAGATACCGTGTA ACTA5'

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



5. 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).

6. 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.

****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.*

5. Repeat step 4 for mutants #2, 3 and 4.

PREDICTION CHART

	PREDICTION	ACTUAL RESULT
Mutant Enzyme	<i>Will bind to substrate (Y or N)?</i>	<i>Did bind to substrate (Y or N)?</i>
#1		
#2		
#3		
#4		

Worksheet Page

THE NORMAL ENZYME (WILD TYPE)

Messenger RNA

Amino Acid Sequence of Normal Enzyme

Building Block Sequence

3'CGA - TAA - TCA - TAA - CAA - GAT - ACC - GTG - TAA - CTA5'

____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____

THE MUTANT ENZYMES

DNA Sequence of Mutant #1

Messenger RNA

Amino Acid Sequence of Mutant Enzyme #1

Building Block Sequence

3'CGA - TAA - TAA - TAA - CAA - GAT - ACC - GTG - TAA - CTA5'

____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____

DNA Sequence of Mutant #2

Messenger RNA

Amino Acid Sequence of Mutant Enzyme #2

Building Block Sequence

3'CGA - TAA - ACA - TAA - CAA - GAT - ACC - GTG - TAA - CTA5'

____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____

DNA Sequence of Mutant #3

Messenger RNA

Amino Acid Sequence of Mutant Enzyme #3

Building Block Sequence

3'CGA - TAA - TCA - TAA - CAA - GAT - ACC - GTG - TCA - CTA5'

____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____

DNA Sequence of Mutant #4

Messenger RNA

Amino Acid Sequence of Mutant Enzyme #4

Building Block Sequence

3'CGA - TAA - TCA - TAA - CTA - GAT - ACC - GTG - TAA - CAA5'

____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____
____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____ - ____

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

“**W**hat 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

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

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 elimi-

nated, 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.

“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:



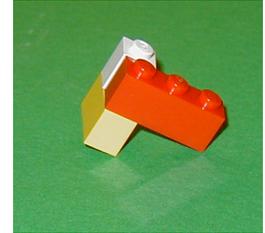
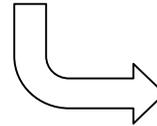
Write a short paragraph that explains what this diagram represents.

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* affect 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. Think about the effects of these changes on enzymes:
 - a. What effect will changing the pH have on an enzyme?
 - b. What effect will changing the temperature have on an enzyme?
8. Read the case study “The Fish Odor Syndrome,” on page 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)?
9. Research scientists have identified the shape of key proteins coded for by the HIV virus. How could you use this knowledge to treat AIDS?

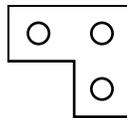
TABLE 2: “The Blueprint”

1. Place 1x1 piece in front of you. Do not rotate 1x1 piece from starting position throughout the building process.
2. Turn 2x1 to vertical on the desk. Place top circle of 2x1 under 1x1.
3. Place 3x1 horizontal. Place 3x1 so that left circle is over bottom circle of 2x1.



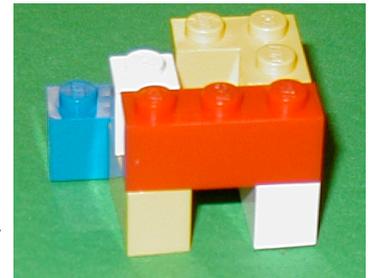
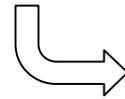
4. Turn a second 2x1 vertical. Attach bottom circle under right circle of 3x1.

5. Turn L piece like this:



Attach bottom circle of L piece over top circle of 2x1.

6. Turn 4x 1 horizontal. Attach right two circles under top two circles of L piece.



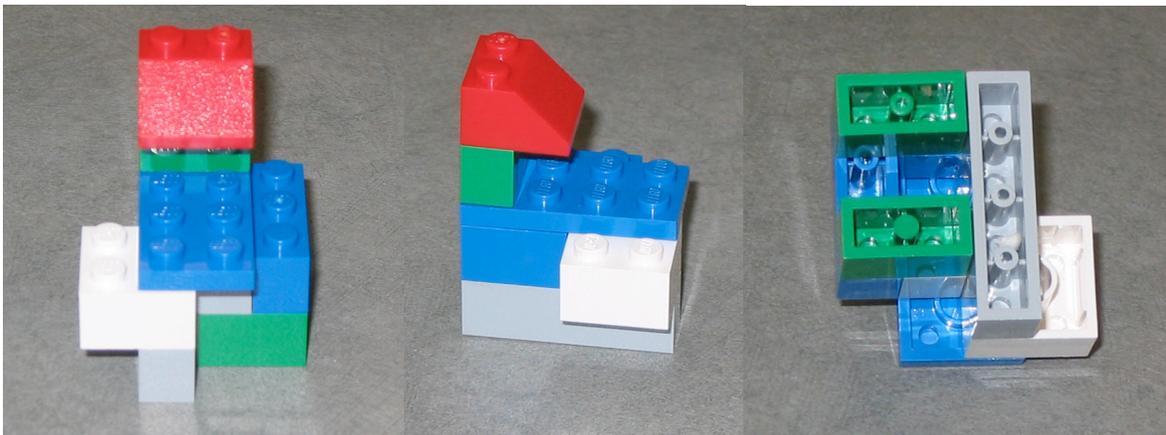
7. Attach 2x2 over left two circles of the 4x1 piece.

8. Place 4x2 sheet horizontally. Place right two circles of 4x2 over the two vertical circles of the L piece, parallel to the 3x1.

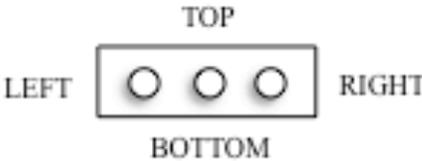
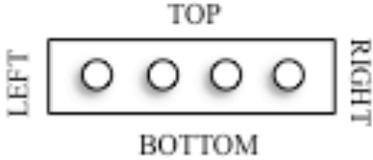
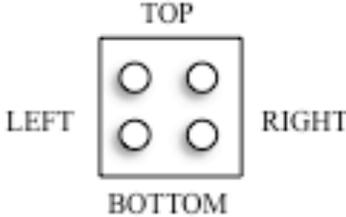
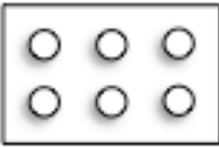
9. Place 2x1 vertically over right two circles of the 4x2 sheet.

10. Place roof piece vertically over top of 2x1, so that the slanted part is towards the center of the molecule (over the third row of the 4x2 sheet).

The completed normal enzyme:

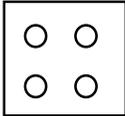


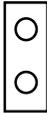
LEGO® SHAPES

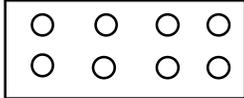
1x	2x1	3x1
		
“L” Piece	4x1	2x2
		
4x2 Sheet	3x2 Sheet	Roof Piece (side view)
		

Packing List – Make sure that you have all these pieces before and after the lab!

One: 1x 

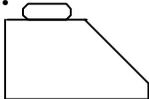
One: 2x2 

Four: 2x1 

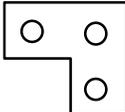
One: 4x2 SHEET: 

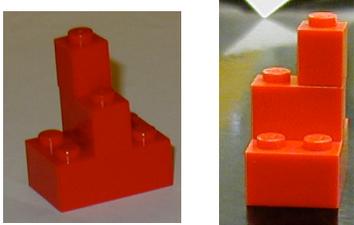
Two: 3x1 

Two: 4x1 

One: Roof piece, side view: 

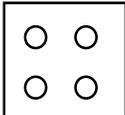
One: 6x1 

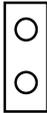
One: “L” PIECE 

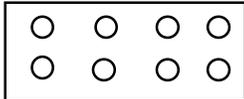
One: assembled substrate: 

Packing List – Make sure that you have all these pieces before and after the lab!

One: 1x 

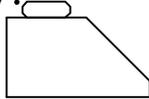
One: 2x2 

Four: 2x1 

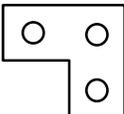
One: 4x2 SHEET: 

Two: 3x1 

Two: 4x1 

One: Roof piece, side view: 

One: 6x1 

One: “L” PIECE 

One: assembled substrate: 