Enzyme Substrate Reaction

Teacher Guide

BIOBRIDGE

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Table of Contents

1 Program and Lab Overview

BioBridge Program

About BioBridge

BioBridge is a Science Outreach Initiative based at UCSD (University of California, San Diego) that serves secondary school teachers and students by connecting students to current and relevant scientific research through classroom activities, university experiences and community events. The foundation of the BioBridge program is our Professional Development and Curriculum program, from which the following activity was developed at UCSD in collaboration with local science teachers and is now offered as a training and implementation package for the high school classroom.

One primary goal of BioBridge is to create very affordable and accessible labs that engage students with authentic science experiences. We work to optimize each activity to minimize the dependency on expensive equipment and other resources sometimes lacking at a school site. In doing so, we have created activities that can be implemented in virtually ANY classroom, but are also able to be "ramped up" or have added complexity to challenge more advanced students or to utilize available classroom resources. BioBridge also supports and is helping to optimize student-run biotechnology sites within specific school districts that will allow materials to available and sustainable over time, eliminating dependency on external resources.

Professional development & curricula

BioBridge's unique phase training model strives to create connections between teachers and scientists, increase teachers' and students' access to current scientific information and resources, as well as encourages the engagement of students as leaders in the classroom. Each BioBridge teacher is trained to use the materials and lab protocol created at UCSD and also brings with them a handful of students from their science classrooms that will also learn to use the resources and will serve as teaching assistants and resident "experts" in the classroom during activity implementation. All student and teacher input is encouraged and considered at all times, such that our training sessions, curriculum, and resources are the most effective and useful to the audience.

We are very pleased to offer these resources to you and hope you have a great experience with this lab activity!

For more information and program updates, visit: www.biobridge.us

Lab Description

Enzymatic reactions

Enzymes are critical to biochemical reactions inside every living thing on Earth, from the tiniest viruses and simplest bacteria to the most complex of mammals. Enzymes are proteins with particular structures that provide specific functions within cells. For instance, our food is broken down by enzymes (e.g. milk protein by enzyme *lactase*, starch by *amylase*), our DNA is "unzipped" during transcription by the enzyme *helicase*, and our body defends itself from bacterial infection by destroying the bacterial cell membrane with *lysozyme*. Like all biological proteins, enzymes are difficult to see--their interactions with substrates and the products they help create are not easily observed. This BioBridge lab allows students to visualize enzymatic reactions using a common enzyme that is easily obtained, manipulated and observed in the classroom.

The enzyme *tyrosinase* is widely distributed throughout the living world—it is present in bacteria, plants, animals, and fungus—and is responsible for the browning of fruit, the fur color of certain animals, and skin pigmentation in human beings. It is easily extracted from mushrooms and, when reacting with the substrate L-DOPA, will create a yellow/brownish product that can be seen within a test tube and measured against a colorimetric chart. From this reaction, students can measure reaction rate and compare enzymatic rates under different conditions such as change in pH level, temperature, and in the presence of an inhibitor.

After completing the lab, students will have a better understanding of enzyme structure and function and will have gained experience with both quantitative and qualitative methods of data collection and analysis, including graphing. Students will also be able to describe enzyme-substrate reactions as it relates to their everyday lives, including how understanding enzyme function is critical to drug development and the effectiveness of these drugs within the body.

The BioBridge Enzymatic Reactions lab is useful to both Biology and Chemistry teachers, as it covers the biological function of enzymes, protein structure and function, and reaction rates. It is an affordable and highly visual lab that will engage any level of student and can be adapted to allow for extended investigation and experimentation.

2 Biology Curriculum

Lab Goals and Objectives

In this lab, students will extract the enzyme tyrosinase from portabella mushrooms. They will learn about it's role in melanin production, and it's potential social and economic applications. When tyrosinase reacts with the substrate L-DOPA it forms the pigment Dopachrome. By measuring the amount of Dopachrome (reddish color) students can measure the rate of the reaction. After adding three different variables, student will see how pH, inhibitors and temperature affect enzyme function through the graphs that are produced from the data collected. Students will explain and provide examples of how pH, temperature and inhibitors relate to enzyme function in organisms, especially humans and explore how understanding enzymatic activity contributes to the discovery, development, and dissemination of drugs and pharmaceuticals.

Baseline reaction

Tyrosinase + L-DOPA (substrate)

Test the effect of pH

Tyrosinase + HCL + L-DOPA

Test the effect of an inhibitor

Tyrosinase + Sodium Benzoate (Inhibitor) + L-DOPA

Test the effect of temperature

Tyrosinase + Boiling water + L-DOPA

Lab Objectives

After completing this activity students will be able to:

1. Define, identify and explain the function and activity of biological enzymes.
2. Explain how enzymes catalyze reactions.
3. Draw graphs of an enzyme catalyzed reaction, under suitable and non-suitable conditions.
4. Draw a graph of the rate of reaction of an enzyme.
5. Diagram/Demonstrate how an inhibitor functions in an enzyme-substrate reaction.

CA State Standards Addressed

The following CA state Biology standards are addressed in the Enzyme/Substrate Reaction lab.

Cell Biology

The fundamental life processes of plants and animals depend on a variety of chemical reactions that occur in specialized areas of the organism's cells. As a basis for understanding this concept:

- Students know cells are enclosed within semi permeable membranes that regulate their interaction with their surroundings.
- Students know enzymes are proteins that catalyze biochemical reactions without altering the reaction equilibrium and the activities of enzymes depend on the temperature, ionic conditions, and the pH of the surroundings.

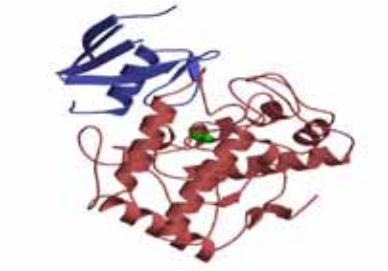
- Students know most macromolecules (polysaccharides, nucleic acids, proteins, lipids) in cells and organisms are synthesized from a small collection of simple precursors.

Content Information

Background information

Enzymatic Reactions using Mushroom Tyrosinase

Section 1: Lab Synopsis/Overview

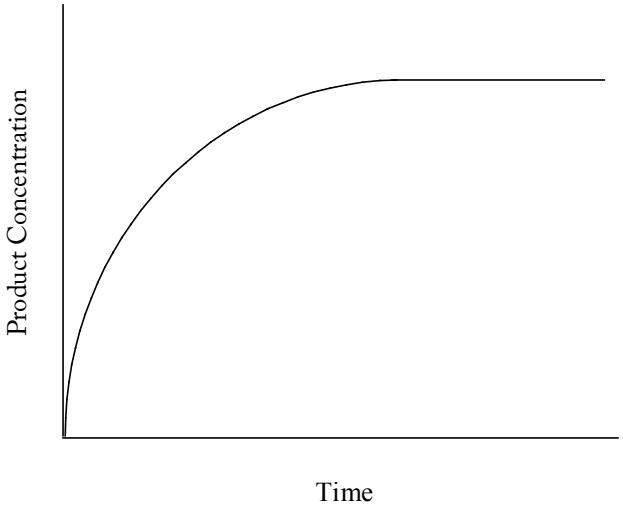


In this lab activity, the enzyme Tyrosinase will be extracted from mushrooms and observed as it catalyzes a substrate and creates a colored product.

Enzyme + Substrate —> Enzyme-Substrate —> Enzyme + Product

The rate of reaction will be determined under normal conditions (room temperature, neutral pH). This will be expressed as amount of product over time.

Rate of Enzyme-Substrate Reaction



This is a typical enzyme-substrate reaction curve. In the beginning, the rate of reaction is very fast since there is a great deal of substrate available. The reaction rate decreases substantially as the enzyme converts all the substrate to product.

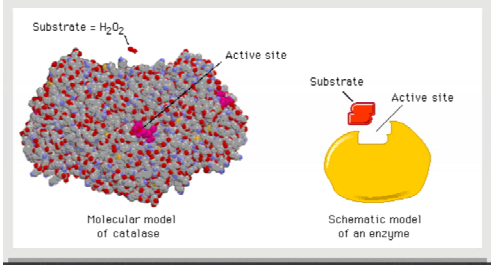
The reaction will be done again under differing pH (acidic) and temperature (very hot) conditions to observe if the enzyme activity is affected and how.

The enzyme Tyrosinase is widely distributed throughout the living world—it is present in bacteria, plants, animals, and fungus—and is responsible for the browning of fruit, the fur color of certain animals, and skin pigmentation in human beings.

Tyrosinase + L-Dopa —> Intermediate —> Tyrosinase + Dopachrome

Because people desire to find ways of slowing down or stopping the production of pigment, such as the browning fruit and vegetables or the darkening of skin, much research has been done and continues to be done to identify substances that will inhibit the function of the tyrosinase enzyme. In this lab, you will be provided a known tyrosinase inhibitor to test the effects on rate of reaction.

Section 2: Enzyme Characteristics



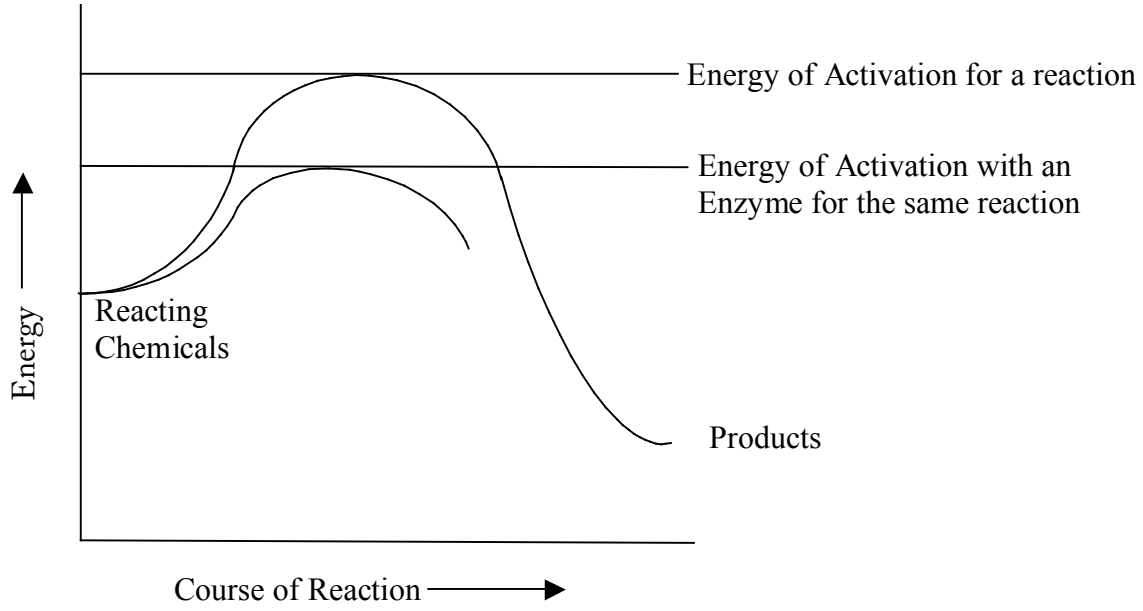
A. Enzymes are special Proteins

Proteins are macromolecules created from a chain of amino acids that is folded upon itself into a unique 3-dimensional shape. Your body is made up of proteins (examples: Keratin in hair and nails, Hemoglobin in blood). Enzymes are proteins too, but are different from other proteins because the have an "active site" where that give enzymes the unique ability to do its job of as a catalyst. **See appendix #1**

B. Enzymes act as Biological Catalysts

A catalyst is something that speeds up a chemical reaction without being consumed. Enzymes speed up chemical reactions within living cells. Without enzymes, it would take a very long time for products to be broken down or built up within the cell--we would not be able to grow, think, or move without enzymes. Enzymes lower the activation energy required to complete biochemical reactions in a timely manner. **See appendix #2**

Energy of Activation



C. Enzymes are Reaction and Substrate Specific

Enzymes catalyze particular reactions and only work with specific molecules called substrates. For example, lactase is an enzyme that breaks down milk sugar, lactose. Lactase does not interact with any other protein or substance. (and, vice versa--lactose, the milk protein, is unable to be broken down/digested without the enzyme lactase being present). **See appendix #3**

D. Enzymes are sensitive to changes in pH, temperature

Most enzymes are only functional within a specific, narrow range of pH and temperature. Enzymes in humans and other mammals will not function if the body temperature rises or drops too much. Digestive enzymes in our stomachs function in the highly acidic environment, and will not work outside of the stomach where the pH higher and less acidic. **See appendix #4**

E. Enzymes can be slowed or ceased by the presence of Inhibitors

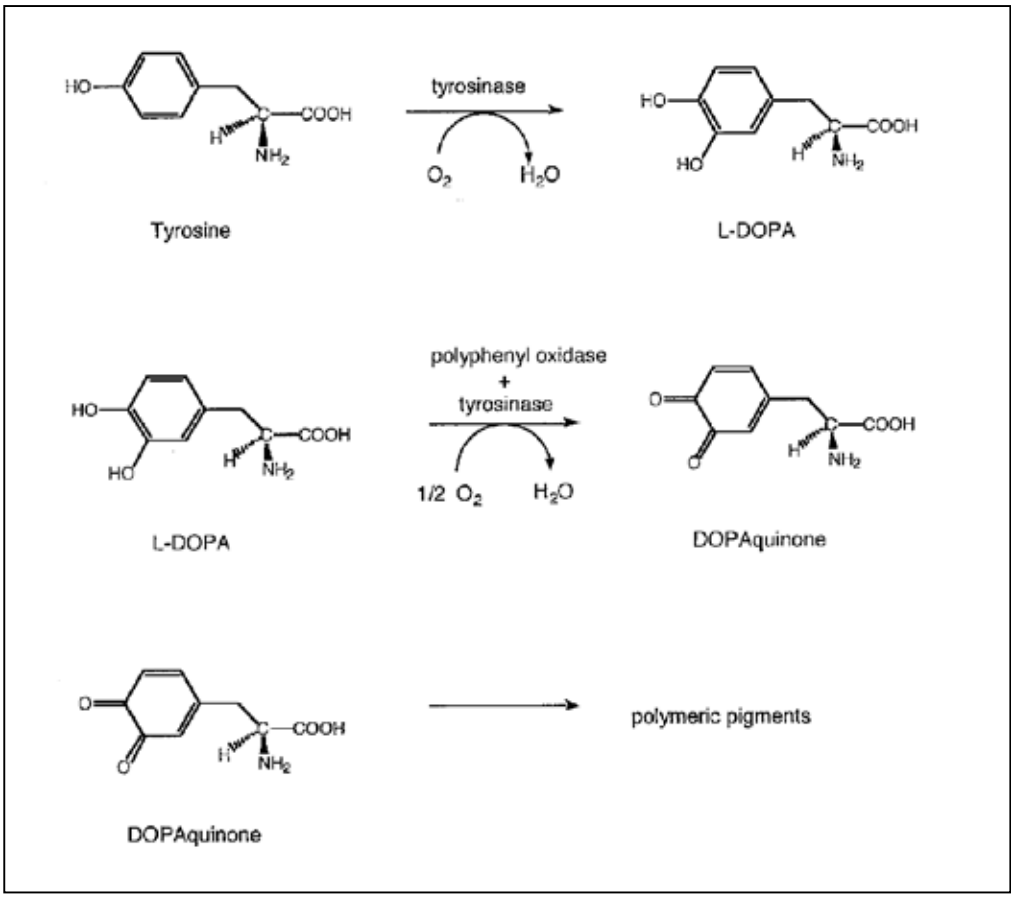
Inhibitors are natural or synthetic molecules that block the active site of the enzyme or remove the substrate before the enzyme can react with it. Inhibitors may slow down or prevent enzyme function. Many drugs are created to interact with or act as inhibitors to alter the level of activity of certain enzymes. **See appendix #5**

Tyrosinase the enzyme

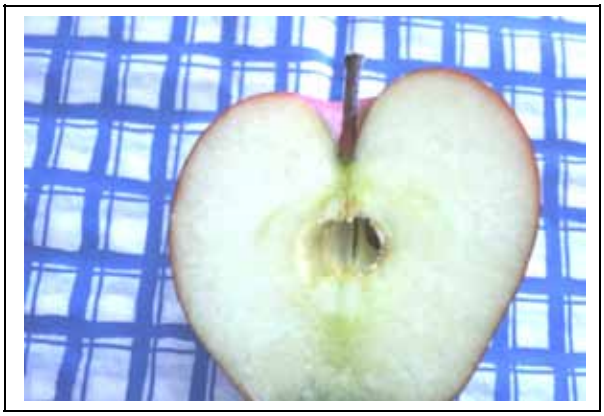
Tyrosinase is an enzyme found in a variety of organisms and plays a significant role in the production of pigment within cells and tissues. It acts as a catalyst in the creation of pre-cursors to melanin and is responsible for the browning of apples and other fruits and vegetables as well as the color of eyes, skin, and hair/fur of mammals.

During the first phases of melanin production, tyrosinase converts a protein building block (amino acid) called tyrosine to

another compound called dopaquinone. A series of subsequent chemical reactions converts dopaquinone to melanin. In humans (and other mammals), the enzyme tyrosinase is located in the melanocytes, specialized cells responsible for producing the pigment melanin which gives our skin, hair, and eyes their color. Melanin also plays a role in vision, as it is found in the light-sensitive tissue in the retina of the eye. Without tyronsinase, the products needed to form melanin cannot be produced causing albinism. **For more information on albinism see appendix #8**



The browning of mushrooms, fruits and vegetables is the result of the oxidation of phenolic compounds by tyrosinase when cells are broken or damaged 2. In fungi such as mushrooms, the role of tyrosinase and subsequent melanin production is related to reproductive organ differentiation and spore formation as well as protection of tissues after injury 3.

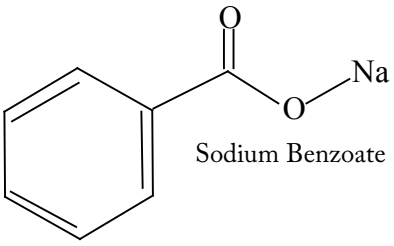


Fresh apple 10 seconds after cutting.

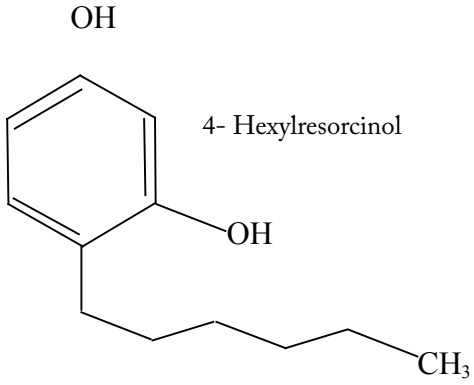
The same apple 4 hours later.

Tyrosinase is a copper-containing enzyme whose activity may be inhibited by a variety of natural and synthetic compounds. Natural inhibitors (substances that block the enzyme active site or out-compete the enzyme for substrate) of mushroom tyrosinase include ascorbic acid, Green Tea, Aloe vera, and some sulfur compounds, to name a few 2,3. Other synthetic sources have been identified as well 3. There is much interest in tyrosinase inhibitors within the food and restaurant industries—they are used widely to prevent unwanted discoloration of fruits and vegetables. The cosmetic industry has recently invested in research and applied as creams and other substances containing a tyrosinase inhibitor, marketing to consumers within cultures that desire lighter skin 3.

Examples of tyrosinase inhibitors are sodium benzoate and 4-hexylresorcinol. They are pictured below. Some inhibitors of tyrosinase are marketed as skin whitening agents because they prevent the formation of melanin, the pigment which causes color in human beings.



Sodium Benzoate



4- Hexylresorcinol

While tyrosinase can easily be obtained from several types of fruits and vegetables, it has been found to be quite concentrated in and easily extracted from mushrooms. Mushroom tyrosinase has been widely studied by researchers as it is inexpensive, readily available, and requires simple tools and protocols to study 3.

References

1. TYR-tyrosinase (oculocutaneous albinism IA) – Genetics Home Reference, http://ghr.nlm.nih.gov, September 15, 2008
2. Zhang, Xiaodong, et.al, "Characterization of Tyrosinase from the Cap Flesh of Portabella Mushrooms", Journal of Agricultural Food Chemistry, 1999, vol. 47, pp. 374-378.
3. Seo, Sung-Yum, Vinay Sharma and Niti Sharma, "Mushroom Tyrosinase: Recent Proposals", Journal of Agricultural Food Chemistry, 2003, vol. 51, pp. 2837-2853.

PowerPoint notes

The PowerPoint presentations that were given at both the teacher and student BioBridge training is available on our website for your use in the classroom. Embedded in the notes section of the phase II PowerPoint presentation are talking points for the presentation that can printed out to use for your classroom lecture.

Download the files at www.biobridge.us. Log on and then select Programs=>BioBridge Curricula=>Content Areas=>ENZYMES=> Resources=>Teaching resources for your classroom. *You must be logged on to download the files!*

Classroom Implementation

Teacher preparation - **Prior to implementation**

Buy mushroom

Buy portabella mushroom - one mushroom should be enough for at least two classes - use fresh (within 4 days - do not freeze).

Protocols

Print out protocols for each student/station - the protocol is a part of this teacher guide and there is also a Word version for you to modify to fit your classroom needs.

Defrost materials

Transfer 15ml tubes of L-Dopa from -20˚C (freezer) into 4˚C (refrigerator)

Label assembly

To save time - label the materials (using included stickers) before the lab day. If you have the time - it is highly beneficial to have students label and assemble their group's materials for the entire activity into a plastic bag. This acquaints them with the materials and helps expedite set up.

Distribute station materials

pH group

(1) pipette "GB"
(1) pipette "ACID"
(1) pipette "ENZ"
(1) pipette "SUB"
(1) test tube "ENZ"
(1) test tube "CTRL"
(1) test tube "EXP"
(1) Weigh boat
(1) cut filter paper
(1) 5mL tube of grinding buffer
(1) 5mL tube of grinding buffer
(1) 15mL tube of L-Dopa
(1) tube of HCl
(4) pieces of pH paper
(1) colormetric chart
(1) pH chart

Stickers
(1) "GB"
(2) "ENZ"
(1) "SUB"
(1) "CTRL"
(1) "EXP
(1) "ACID"

Inhibitor group

(1) pipette "GB"
(1) pipette "INH"
(1) pipette "ENZ"
(1) pipette "SUB"
(1) test tube "ENZ"
(1) test tube "CTRL"
(1)test tube "EXP"
(1) Weigh boat
(1) cut filter paper
(1) 5mL tube of grinding buffer
(1) 15mL tube of L-Dopa
(1) tube of Inhibitor (INH)
(1) colormetric chart

Stickers
(1) "GB"
(2) "ENZ"
(1) "SUB"
(1) "CTRL"
(1) "EXP
(1) "INH"

Temperature group

(1) pipette "GB"

(1) pipette "ENZ"

(1) pipette "SUB"

(1) test tube "ENZ"

(1) test tube "CTRL"

(1) cut filter paper

(1)test tube "EXP"

(1) Weigh boat

(1) colormetric chart

(1) 15mL tube of L-Dopa

Stickers

(1) "GB"

(2) "ENZ"

(1) "SUB"

(1) "CTRL"

(1) "EXP

Day of Activity Preparation

Add 5% SDS to grinding buffer

1. Add 1 ml of 5% SDS to each 15 ml tube containing 5ml of Grinding Buffer.
2. Invert the tube multiple times to mix, then place tube on ice or in the refrigerator for later use.

***NOTE:** Do this the day of the activity - if mixed too early it will precipitate out of solution.

Prepare mushroom samples

1. Using a razor blade or small kitchen knife, dissect out a 1 cm3 Mushroom cube for each station. The mushroom cubes should be taken from the cap area as it contains the highest levels of the tyrosinase enzyme. Refrain from using pieces containing the gills of the mushroom as they contain very little tyrosinase enzyme.
2. Hand out mushroom pieces to each station.

***NOTE:** We recommend dissecting the mushroom at the beginning of class to allow the students to make a connection between the whole mushroom and the small piece they receive.

Set-up hot water bath

Place a large beaker full of water on a hot plate. Heat the water until it reaches a rolling boil at 90C or above. Only one hot water bath is required per class. Groups assigned to the variable temperature experiments can share the bath.

Glossary

Baseline experiment

An assessment designed to measure the normal function of an enzyme under normal conditions. In the Enzyme Kinetics activity, the baseline experiment measures tyrosinase activity at room temperature and pH 6.

Grinding Buffer

Contains phosphates, ascorbate (vitamin C), and sodium dodecyl sulfate (SDS). Used to lyse and break open the cell wall and plasma membrane of the mushroom cells to help release the tyrosinase enzyme into the grinding buffer solution.

Hydrocholric Acid (HCl)

A strong mineral acid used in many industrial and research settings. It's also a natural component of gastric acid in the human stomach. In the tyrosinase kinetics activity it's used to lower the pH of the solution to approximately 3.

L-dopa

A naturally occurring amino acid found in food and made from L-Tyrosine in the human body. In the enzyme kinetics activity, L-dopa is converted to the redish-brown dopachrome molecule in a reaction catalyzed by the enzyme tyrosinase.

Sodium Benzoate

A competitive inhibitor of the tyrosinase enzyme. It binds to the active site on the enzyme, preventing the catalysis of L-dopa —> Dopachrome. It's also a commonly used preservative for acidic foods.

Sodium dodecyl sulfate (SDS)

Acommon detergent used to lyse open cells. It acts to break apart the cell wall and plasma membrane of the mushroom cells to help release the tyrosinase enzyme into the grinding buffer solution.

Variable experiment

An assessment designed to measure enzyme activity under irregular conditions. In the Enzyme Kinetics activity, the variable experiments included effect of pH, effect of sodium benzoate (inhibitor), effect of heat on substrate, and effect of heat on enzyme.

Enzyme Kit Checklist

Store at room temperature

___ (10) Weight Boats

___ (10) 15mL tubes of GB (5mL each)

___ (10) Colormetric Charts * (Note: one set/teacher — not 1 set/kit – keep to reuse each year)

___ (3) pH charts * (Note: one set/teacher — not 1 set/kit – keep to reuse each year)

___ (6) tubes of HCl (extra for expanding lab)

___ (6) tubes of INH (inhibitor) -(extra for expanding lab)

___ (10) pieces filter paper

___ (12) pieces of pH paper

___ (1) 10mL tube 5% SDS

___ (40) Transfer Pipets

___ (50) Test Tubes

Bag of Sticker Labels

___ (10) GB
___ (20) ENZ
___ (10) SUB
___ (20) CTRL
___ (20) EXP
___ (3) ACID
___ (3) INH

Store at 4°C (refrigerated)

___ (10) 15mL tubes of L-Dopa (***LIGHT SENSITIVE)

Strategies

We suggest that you organize students in groups of 4 and have no more than 8 lab groups. We give you enough materials for 10 groups, though to have extra materials using only 8 groups of 3 is best.

Before implementation

Take time to walk students through the experimental steps

- Have them practice timing and understand how quickly the reactions will start.
- Practice pipeting and collecting small amounts of liquid.
- Assign roles to the lab group members.

The curriculum is designed to be completed in three, 50 minute periods.

- Day 1 – Prepare the students with the PowerPoint, pre-lab material, organize lab groups and prepare station materials
- Day 2 – Lab protocol
- Day 3 – Results, analysis and conclusion

Student leader preparation

- Meet with your student leaders before the implementation to answer any questions and review the lab procedure with them.
- Also discuss how to guide their fellow classmates rather than just taking over the step or telling them the answer.
- If you are not having your classes prepare their station materials, this a good task for your student leaders to assist you with.
- If you have student leaders assisting you in the classroom for implementation, we suggest assigning them to certain lab stations in order to guide those students through the protocol.

Protocols

There are three versions of the enzyme protocol.

- **P1:** This protocol has pre and post lab questions that are directly aligned with the biology standards.
- **P2:** This protocol is adapted to have the questions embedded in the protocol to keep the students engaged in the material and thinking about the processes behind each step.
- A third protocol is available on our website in an MS Word version including pictures for you to modify into a lab format that works for your classroom.

Assessment Strategies

Constructive feedback is essential for learning. It is also essential for you to best understand what your students know and do not know in regard to science content and processes. We have provided you a pre-assessment sheet that displays a modified Frayer model so that students can explain (within their group) what they know about bacteria. If you have not used a Frayer model before, then we recommend that you use one with a familiar concept and work with your students together or provide it as homework. Then, once the students have done a Frayer model, then they will be primed to use the one concerning our topic – enzymes.

The second sheet contains specific questions related to scientific content that is important for demonstrating understanding of the enzymatic reaction activity.

A third assessment tool designed to be similar to the Frayer model, focuses on the process that students are learning about – enzymatic reactions. It is suggested that this tool be used after implementation to check for understanding.

A final assessment tool is designed to have students reflect on their learning through this activity and can be given as a homework assignment individually.

Group Members _____

Enzyme

Definition	Characteristics
Examples	Examples of non-bacteria

Name _____ Date _____

1. What does an enzyme do?

2. How does altering the pH affect an enzyme's activity?

3. How does altering the temperature affect an enzyme's activity?

4. What does an inhibitor do to affect an enzyme's activity?

Name _____

Enzyme

Definition	Necessary steps of protocol
How does the (pH, temperature, or inhibitor) affect the enzyme?	Provide an everyday example of enzymes.

Name _____

About enzymes...

I used to think…	But now I know...

Name _____

About enzymes...

I used to think…	But now I know...

3 Chemistry Curriculum

Lab Goals and Objectives

In this lab, students will be able to explain the rate of chemical reactions as the decrease in the amount of reactant/ increase in the amount of product over time. They will understand this through analyzing the oxidation reaction of the substrate Levodihydroxyphenlyanine (L-DOPA) to form the product Dopachrome by measuring the amount of Dopachrome (reddish color) produced over time. Furthermore, they will understand how enzymes work to catalyze reactions through the use of the enzyme tyrosinase. Furthermore, they will see how enzyme function is affected by pH, inhibitors and temperature. Tyrosinase will be presented through its extraction from portabella mushrooms, it's role in melanin production, and it's potential social and economic applications. The overall importance of enzymes relating to the discovery, development, and dissemination of drugs and pharmaceuticals will be discussed.

Baseline reaction

Tyrosinase + L-DOPA (substrate)

Test the effect of pH

Tyrosinase + HCL + L-DOPA

Test the effect of an inhibitor

Tyrosinase + Sodium Benzoate (Inhibitor) + L-DOPA

Test the effect of temperature

Tyrosinase + Boiling water + L-DOPA

Lab Objectives

After completing this activity students will be able to:

1. Explain how reaction rates are found by calculating the change in the concentration of reactant or product over time.
2. Define, identify and explain the function and activity of biological enzymes.
3. Explain how enzymes catalyze reactions.
4. Draw a graph of the rate of reaction with an enzyme and calculate the reaction rate
5. Draw a graph of the rate of reaction with an enzyme when it has been put under various conditions (inhibitor, temperature change, and pH change) and calculate the rate.
6. Explain how enzyme function will change through changes in temperature, pH, and inhibitors.

CA State Standards Addressed

The following CA state science standards are addressed in the Enzyme/Substrate Reaction lab.

Conservation of Matter and Stoichiometry

The conservation of atoms in chemical reactions leads to the principle of conservation of matter and the ability to calculate the mass of products and reactants. As a basis for

understanding this concept...

- *Students know how to identify reactions that involve oxidation and reduction and how to balance oxidation-reduction reactions.

Reaction Rates

Chemical reaction rates depend on factors that influence the frequency of collision of reactant molecules. As a basis for understanding this concept...

- Students know the rate of reaction is the decrease in concentration of reactants or the increase in concentration of products with time.
- Students know how reaction rates depend on such factors as concentration, temperature, and pressure.
- Students know the role a catalyst plays in increasing the reaction rate.
- *Students know the definition and role of activation energy in a chemical reaction.

Acids and Bases

Acids, bases, and salts are three classes of compounds that form ions in water solutions. As a basis for understanding this concept...

- Students know how to use the pH scale to characterize acid and base solutions.
- *Students know buffers stabilize pH in acid-base reactions.

Solutions

Solutions are homogeneous mixtures of two or more substances. As a basis for understanding this concept

- *Students know how molecules in a solution are separated or purified by the methods of chromatography and distillation.

Content Information

Background information

Reaction rate

A reaction rate is the speed at which reactants are converted into products in a chemical reaction. Over time the concentration of reactants will decrease and the concentration of products will increase. If you find the derivative of the line, then it would give you the instantaneous rate of change for any reactant and product (e. g. d[A]/dt). You can also find the rate over a period of time. This is calculated by finding the change in the amount of product formed over the change in time. This will differ based on whether it is at the beginning of end of the reaction- as the reactants are used up, the reaction will happen slower.

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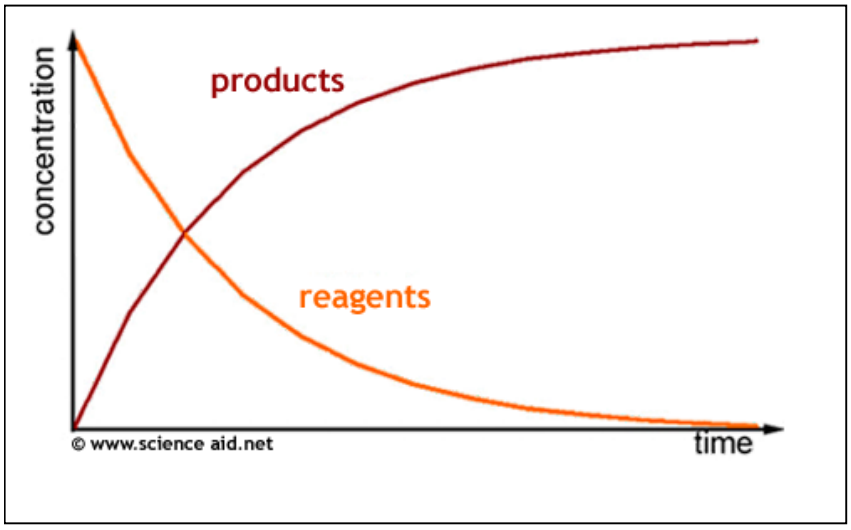


$$\text{rate} = \frac{\Delta[\text{product}]}{\Delta t}$$

Factors that affect reaction rates

Rates of reaction depend on many factors:

- **temperature of the reaction**- usually by increasing the temperature of a reaction you increase the reaction rate. This is because an increase in temperature means an increase in the kinetic energy/movement of the molecules so there are more collisions (about double for each 100C increase in temperature)
- c**oncentration of reactants**- higher concentration of reactants means more collisions which will increase the reaction rate. Likewise a higher concentration of products will decrease the reaction rate. (for a gas can use partial pressure of gas as concentration)
- **presence of a catalyst**- catalysts lower the activation energy of a chemical reaction and increase the reaction rate of the reaction without being consumed.

Enzymes as catalysts

Enzymes are critical to biochemical reactions inside every living thing on Earth, from the tiniest viruses and simplest bacteria to the most complex of mammals. Enzymes are proteins with particular structures that provide specific functions within cells. For instance, our food is broken down by enzymes (e.g. milk protein by enzyme *lactase*, starch by *amylase*), our DNA is "unzipped" during transcription by the enzyme *helicase*, and our body defends itself from bacterial infection by destroying the bacterial cell membrane with *lysozyme*. Like all biological proteins, enzymes are difficult to see--their interactions with substrates and the products they help create are not easily observed. This BioBridge lab allows students to visualize enzymatic reactions using a common enzyme that is easily obtained, manipulated and observed in the classroom.

The enzyme *tyrosinase* is widely distributed throughout the living world—it is present in bacteria, plants, animals, and fungus—and is responsible for the browning of fruit, the fur color of certain animals, and skin pigmentation in human beings. It is easily extracted from mushrooms and, when reacting with the substrate L-DOPA, will create a yellow/brownish product that can be seen within a test tube and measured against a colorimetric chart. From this reaction, students can measure reaction rate and compare enzymatic rates under different conditions such as change in pH level, temperature, and in the presence of an inhibitor.

Sodium benzoate C6H5COONa

A sodium salt of benzoic acid. It is used as a food preservative because it acts as an inhibitor for enzymes that cause food spoilage.

PowerPoint notes

The powerpoint presentations that were given at both the teacher and student BioBridge training is available on our website for your use in the classroom. Embedded in the notes section of the phase II PowerPoint presentation are talking points for the presentation that can printed out to use for your classroom lecture.

Download the files at www.biobridge.us. Log on and then select Programs=>BioBridge Curricula=>Content Areas=>ENZYMES=> Resources=>Teaching resources for your classroom. *You must be logged on to download the files!*

Classroom Implementation

Teacher preparation - **Prior to implementation**

Buy mushroom

Buy portabella mushroom - one mushroom should be enough for at least two classes - use fresh (within 4 days - do not freeze).

Protocols

Print out protocols for each student/station - the protocol is a part of this teacher guide and there is also a Word version for you to modify to fit your classroom needs.

Defrost materials

Transfer 15ml tubes of L-Dopa from -20˚C (freezer) into 4˚C (refrigerator)

Label assembly

To save time - label the materials (using included stickers) before the lab day. If you have the time - it is highly beneficial to have students label and assemble their group's materials for the entire activity into a plastic bag. This acquaints them with the materials and helps expedite set up.

Distribute station materials

pH group

(1) pipette “GB”
(1) pipette “ACID”
(1) pipette “ENZ”
(1) pipette “SUB”
(1) test tube “ENZ”
(1) test tube “CTRL”
(1) test tube “EXP”
(1) Weigh boat

(1) cut filter paper
(1) 5mL tube of grinding buffer
(1) 5mL tube of grinding buffer
(1) 15mL tube of L-Dopa
(1) tube of HCl
(4) pieces of pH paper
(1) colormetric chart
(1) pH chart

Stickers
(1) “GB”
(2) “ENZ”
(1) “SUB”
(1) “CTRL”
(1) “EXP
(1) “ACID”

Inhibitor group

(1) pipette “GB”
(1) pipette “INH”
(1) pipette “ENZ”
(1) pipette “SUB”
(1) test tube “ENZ”
(1) test tube “CTRL”
(1)test tube “EXP”

(1) Weigh boat
(1) cut filter paper
(1) 5mL tube of grinding buffer
(1) 15mL tube of L-Dopa
(1) tube of Inhibitor (INH)
(1) colormetric chart

Stickers
(1) “GB”
(2) “ENZ”
(1) “SUB”
(1) “CTRL”
(1) “EXP
(1) “INH”

Temperature group

(1) pipette “GB”
(1) pipette “ENZ”
(1) pipette “SUB”
(1) test tube “ENZ”
(1) test tube “CTRL”

(1) cut filter paper
(1)test tube “EXP”
(1) Weigh boat
(1) colormetric chart
(1) 15mL tube of L-Dopa

Stickers
(1) “GB”
(2) “ENZ”
(1) “SUB”
(1) “CTRL”
(1) “EXP

Day of Activity Preparation

Add 5% SDS to grinding buffer

1. Add 1 ml of 5% SDS to each 15 ml tube containing 5ml of Grinding Buffer.
2. Invert the tube multiple times to mix, then place tube on ice or in the refrigerator for later use.

***NOTE:** Do this the day of the activity - if mixed too early it will precipitate out of solution.

Prepare mushroom samples

1. Using a razor blade or small kitchen knife, dissect out a 1 cm3 Mushroom cube for each station. The mushroom

cubes should be taken from the cap area as it contains the highest levels of the tyrosinase enzyme. Refrain from using pieces containing the gills of the mushroom as they contain very little tyrosinase enzyme.

2. Hand out mushroom pieces to each station.

***NOTE:** We recommend dissecting the mushroom at the beginning of class to allow the students to make a connection between the whole mushroom and the small piece they receive.

Set-up hot water bath

Place a large beaker full of water on a hot plate. Heat the water until it reaches a rolling boil at 90C or above. Only one hot water bath is required per class. Groups assigned to the variable temperature experiments can share the bath.

Glossary

Baseline experiment

An assessment designed to measure the normal function of an enzyme under normal conditions. In the Enzyme Kinetics activity, the baseline experiment measures tyrosinase activity at room temperature and pH 6.

Grinding Buffer

Contains phosphates, ascorbate (vitamin C), and sodium dodecyl sulfate (SDS). Used to lyse and break open the cell wall and plasma membrane of the mushroom cells to help release the tyrosinase enzyme into the grinding buffer solution.

Hydrocholric Acid (HCl)

A strong mineral acid used in many industrial and research settings. It's also a natural component of gastric acid in the human stomach. In the tyrosinase kinetics activity it's used to lower the pH of the solution to approximately 3.

L-dopa

A naturally occurring amino acid found in food and made from L-Tyrosine in the human body. In the enzyme kinetics activity, L-dopa is converted to the redish-brown dopachrome molecule in a reaction catalyzed by the enzyme tyrosinase.

Sodium Benzoate

A competitive inhibitor of the tyrosinase enzyme. It binds to the active site on the enzyme, preventing the catalysis of L-dopa —> Dopachrome. It's also a commonly used preservative for acidic foods.

Sodium dodecyl sulfate (SDS)

Acommon detergent used to lyse open cells. It acts to break apart the cell wall and plasma membrane of the mushroom cells to help release the tyrosinase enzyme into the grinding buffer solution.

Variable experiment

An assessment designed to measure enzyme activity under irregular conditions. In the Enzyme Kinetics activity, the variable experiments included effect of pH, effect of sodium benzoate (inhibitor), effect of heat on substrate, and effect of heat on enzyme.

Enzyme Kit Checklist

Store at room temperature

___ (10) Weight Boats

___ (10) 15mL tubes of GB (5mL each)

___ (10) Colormetric Charts * (Note: one set/teacher — not 1 set/kit – keep to reuse each year)

___ (3) pH charts * (Note: one set/teacher — not 1 set/kit – keep to reuse each year)

___ (6) tubes of HCl (extra for expanding lab)

___ (6) tubes of INH (inhibitor) -(extra for expanding lab)

___ (10) pieces filter paper

___ (12) pieces of pH paper

___ (1) 10mL tube 5% SDS

___ (40) Transfer Pipets

___ (50) Test Tubes

Bag of Sticker Labels

___ (10) GB

___ (20) ENZ

___ (10) SUB

___ (20) CTRL

___ (20) EXP

___ (3) ACID

___ (3) INH

Store at 4°C (refrigerated)

___ (10) 15mL tubes of L-Dopa (***LIGHT SENSITIVE)

Strategies

We suggest that you organize students in groups of 4 and have no more than 8 lab groups. We give you enough materials for 10 groups, though to have extra materials using only 8 groups of 3 is best.

Before implementation

Take time to walk students through the experimental steps

- Have them practice timing and understand how quickly the reactions will start.
- Practice pipeting and collecting small amounts of liquid.
- Assign roles to the lab group members.

The curriculum is designed to be completed in three, 50 minute periods.

- Day 1 – Prepare the students with the PowerPoint, pre-lab material, organize lab groups and prepare station materials
- Day 2 – Lab protocol
- Day 3 – Results, analysis and conclusion

Student leader preparation

- Meet with your student leaders before the implementation to answer any questions and review the lab procedure with them.
- Also discuss how to guide their fellow classmates rather than just taking over the step or telling them the answer.
- If you are not having your classes prepare their station materials, this a good task for your student leaders to assist you with.
- If you have student leaders assisting you in the classroom for implementation, we suggest assigning them to certain lab stations in order to guide those students through the protocol.

Protocols

There are three versions of the enzyme protocol.

- **P1:** This protocol has pre and post lab questions that are directly aligned with the chemistry standards.
- **P2:** This protocol is adapted to have the questions embedded in the protocol to keep the students engaged in the material and thinking about the processes behind each step.
- A third protocol is available on our website in an MS Word version including pictures for you to modify into a lab format that works for your classroom.

Assessment Strategies

Constructive feedback is essential for learning. It is also essential for you to best understand what your students know and do not know in regard to science content and processes. We have provided you a pre-assessment sheet that displays a modified Frayer model so that students can explain (within their group) what they know about bacteria. If you have not used a Frayer model before, then we recommend that you use one with a familiar concept and work with your students together or provide it as homework. Then, once the students have done a Frayer model, then they will be primed to use the one concerning our topic – enzymes.

The second sheet contains specific questions related to scientific content that is important for demonstrating understanding of the enzymatic reaction activity.

A third assessment tool designed to be similar to the Frayer model, focuses on the process that students are learning about – enzymatic reactions. It is suggested that this tool be used after implementation to check for understanding.

A final assessment tool is designed to have students reflect on their learning through this activity and can be given as a homework assignment individually.

Group Members _____

Enzyme

Definition	Characteristics
Examples	Non-examples

Name _____ Date _____

1. What does an enzyme do?

2. How does altering the pH affect an enzyme's activity?

3. How does altering the temperature affect an enzyme's activity?

4. What does an inhibitor do to affect an enzyme's activity?

Name _____

Enzyme

Definition	Necessary steps of protocol
How does the (pH, temperature, or inhibitor) affect the enzyme?	Provide an everyday example of enzymes.

Name _____

About enzymes...

I used to think…	But now I know...

Name _____

About enzymes...

I used to think…	But now I know...

Biology

Protocol 1

Experimental Group (circle one)	pH Inhibitor Temperature
Group # _____	

Each group will run 4 trials. Each student should do each job at least once.

Write the name of the mixer, reader, timer and recorder for each trial in a table in your notebook.

	Mixer	Timer	Reader	Recorder
Trial 1 (control)				
Trial 2 (experimental)				

Central question

What are enzymes and how can we cause changes in enzyme function?

Overview of experiment

In this lab you will observe changes in enzyme activity when you change the environment of the reaction. You will either introduce an inhibitor, alter the temperature or alter pH of the surroundings. In this experiment, the enzyme tyrosinase catalyzes the conversion of a non-colored molecule into a reddish-brown molecule. You will determine the activity of the enzyme by recording the color change of the reaction solution and graphing your data using a standard curve.

Hypothesis

Student pre-lab questions

1. What does the enzyme tyrosinase do?

2. What is the purpose of grinding the mushroom?

3. What is the function of L-DOPA in the reaction?

4. Describe the difference between the control reaction and the experimental reaction.

Material Checklist

pH groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) 1.5mL tube containing acid (HCl)
___ (2) pieces of pH paper
___ (1) pH color chart
___ (3) glass test tubes
___ (1) weighing boat
___ (4) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Temperature groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) weighing boat
___ (3) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (3) glass test tubes
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Inhibitor groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) 1.5 ml tube of sodium benzoate/inhibitor (INH)
___ (1) weighing boat
___ (4) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (3) glass test tubes
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Lab Procedure

Label your test tubes and pipettes as described

___ (1) Test Tube: "CTRL" for control trial

___ (1) Test Tube: "EXP" or for experimental trial

___ (1) Test Tube "ENZ" for enzyme extract

___ (1) Pipette: "GB" for use with grinding buffer

___ (1) Pipette: "SUB" for use with L-DOPA substrate

___ (1) Pipette: "ENZ" for use with enzyme

pH groups only

___ (1) Pipette: "ACID" for the HCl

Inhibitor groups only

___ (1) Pipette: "INH" for the Sodium Benzoate inhibitor

Preparing enzyme extract

1. Obtain a mushroom piece and place it in the weighing boat.
2. Using the "GB" pipette, add 4mL of cold grinding buffer on top of the mushroom.
3. Grind up the mushroom using the bottom of a test tube for 3 minutes or until it is mixed well.
4. Make a filtering cone from the coffee filter by folding it three times, creating a cone shape. Place the cone into the test tube labeled "ENZ" for enzyme.
5. Pour the liquid from the weighing boat through the filter and into the test tube. This is your tyrosinase enzyme extract! Place it in your test-tube holder at room temperature.

Each team member should be prepared to do his or her job immediately. The reaction moves very quickly!

Perform control trial (Trial 1)

In this portion of the experiment, you are determining the activity of your enzyme under standard conditions.

1. Using the pipette labeled "SUB", Pipette 2mL of the L-DOPA substrate solution into the test tube labeled "CTRL".
   - Immediately begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the enzyme.
2. Using the transfer pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "CTRL" tube and swirl the test tube to mix.
3. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.
4. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

EXPERIMENTAL TRIALS

Determine the effect of pH (HCl) on tyrosinase activity

1. Using the pipette labeled "SUB", Pipette 2mL of the L-DOPA substrate solution into the test tube labeled "EXP".
2. From the sample, use the "SUB" pipette to draw up one small drop of substrate and place it on pH paper. Using the pH color chart, determine pH of substrate and record pH on the data sheet.
3. Using the "ACID" pipette, transfer the entire contents of the HCl (acid) tube into the substrate solution and mix. DO NOT DISCARD PIPETTE.
4. With the "ACID" pipette , add 0.1 ml (~one drop) of substrate and HCl mix onto a NEW piece of pH paper. Using the pH color chart, determine pH of substrate and record on the data sheet.
   - Immediately begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds. as soon as you add the enzyme.
5. Using the transfer pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube and swirl the test tube to mix.
6. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.
7. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

Determine the effect of an inhibitor on tyrosinase activity

1. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into your first test tube labeled "EXP" .
2. With the "INH" pipette, place the entire contents of the tube of inhibitor (sodium benzoate "INH") into the substrate and swirl the test tube to mix.
    - **Immediately** begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the enzyme.
3. Using the transfer labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube and mix. Continue mixing during the entire trial.
4. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.
5. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

Determine the effect of temperature on tyrosinase activity

1. Using the pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube.
2. Place the "EXP" tube containing the enzyme in hot (90°C) water for 1 minute.
    - **Immediately** begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the substrate.
3. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into your test tube labeled "EXP " and swirl the test tube to mix.
4. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.
5. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container

After completing all trials, convert colorimetric letters to mM concentration of substrate and graph your data.

Experimental Trial

Substrate pH:_____

Substrate + acid pH:_____

Graphing data

Use a different color pencil for each condition.

Analysis questions

1. What does color change tell you about enzyme activity?
2. What differences did you observe between the control and the experimental trials?
3. For your experimental condition (temperature, pH or inhibitor), explain how that condition affected enzyme activity.
4. How does the control reaction help you interpret your experimental results? How would you know if enzyme activity was changed in the experimental reaction if you had no control reaction data?

Conclusion / summary (revisit hypothesis)

		Trial # 1 Control		Trial # 2 Experimental:_____	
Time (Minutes: Seconds)	Convert time to seconds	Color Chart Letter	DOPA-chrome (mM)	Color Chart Letter	DOPA-chrome (mM)
10 second intervals					
6:50 0:10					
6:40 0:20					
6:30 0:30					
6:20 0:40					
6:10 0:50					
6:00 1:00					
5:50 1:10					
5:40 1:20					
5:30 1:30					
5:20 1:40					
5:10 1:50					
5:00 2:00					
20 second intervals					
4:40 2:20					
4:20 2:40					
4:00 3:00					
3:40 3:20					
3:20 3:40					
3:00 4:00					
1 minute intervals					
2:00 5:00					
1:00 6:00					
0 7:00					

pH Group

Substrate pH:_____

Substrate + acid pH:_____

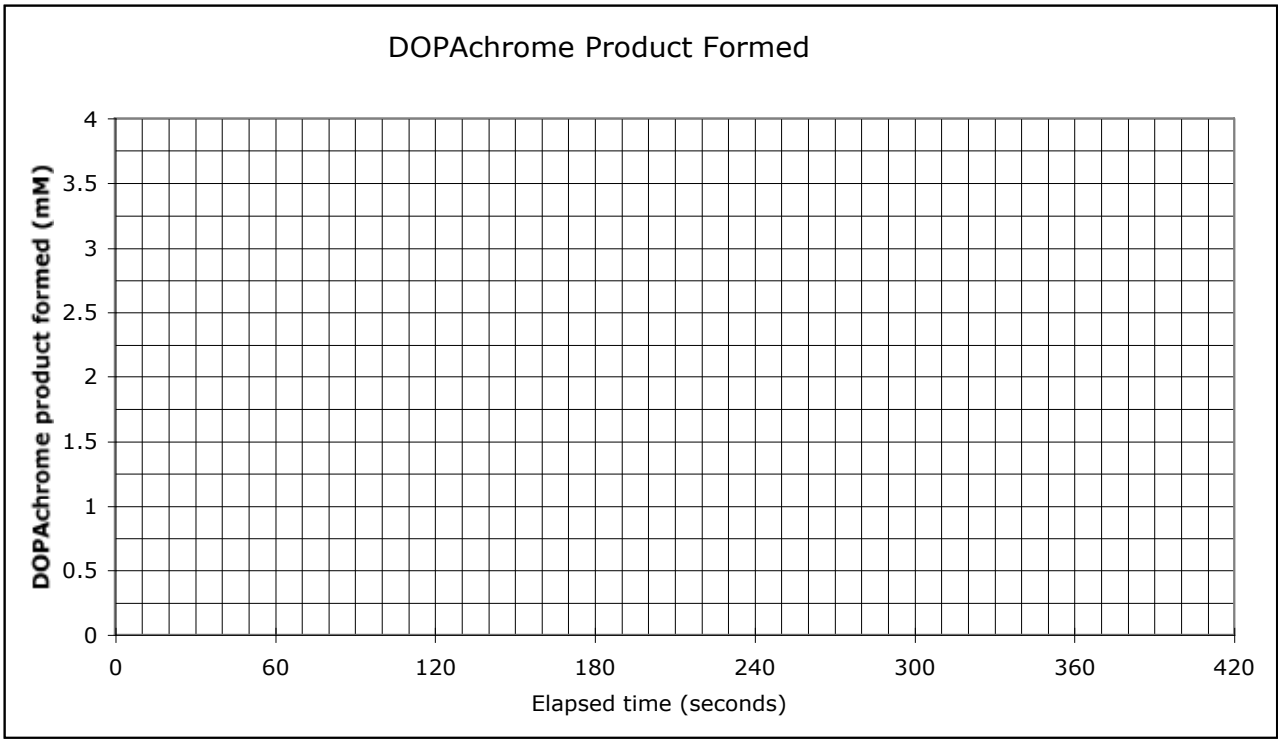
Graphing data

Use a different color to graph each condition and list the color below.

Color key:

Control:_____

Circle your experimental group: pH, Temperature, Inhibitor: _____



Biology

Protocol 2

Experimental Group (circle one)	pH Inhibitor Temperature
Group # _____	

Each group will run 2 trials.

Write the name of the mixer, reader, timer and recorder for each trial in a table in your notebook.

	Mixer	Timer	Reader	Recorder
Trial 1 (control)				
Trial 2 (experimental)				

Central question

What are enzymes and how can we cause changes in enzyme function?

Overview of experiment

In this lab you will observe changes in enzyme activity when you change the environment of the reaction. You will either introduce an inhibitor, alter the temperature or alter pH of the surroundings. In this experiment, the enzyme tyrosinase catalyzes the conversion of a non-colored molecule into a reddish-brown molecule. You will determine the activity of the enzyme by recording the color change of the reaction solution and graphing your data using a standard curve.

Hypothesis

Student pre-lab questions

1. What does the enzyme tyrosinase do?

2. What is the purpose of grinding the mushroom?

3. What is the function of L-DOPA in the reaction?

4. Describe the difference between the control reaction and the experimental reaction.

Material Checklist

pH groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) 1.5mL tube containing acid (HCl)
___ (2) pieces of pH paper
___ (1) pH color chart
___ (3) glass test tubes
___ (1) weighing boat
___ (4) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Temperature groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) weighing boat
___ (3) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (3) glass test tubes
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Inhibitor groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) 1.5 ml tube of sodium benzoate/inhibitor (INH)
___ (1) weighing boat
___ (4) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (3) glass test tubes
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Lab Procedure

Label your test tubes and pipettes as described

___ (1) Test Tube: "CTRL" for control trial

___ (1) Test Tube: "EXP" or for experimental trial

___ (1) Test Tube "ENZ" for enzyme extract

___ (1) Pipette: "GB" for use with grinding buffer

___ (1) Pipette: "SUB" for use with L-DOPA substrate

___ (1) Pipette: "ENZ" for use with enzyme

pH groups only

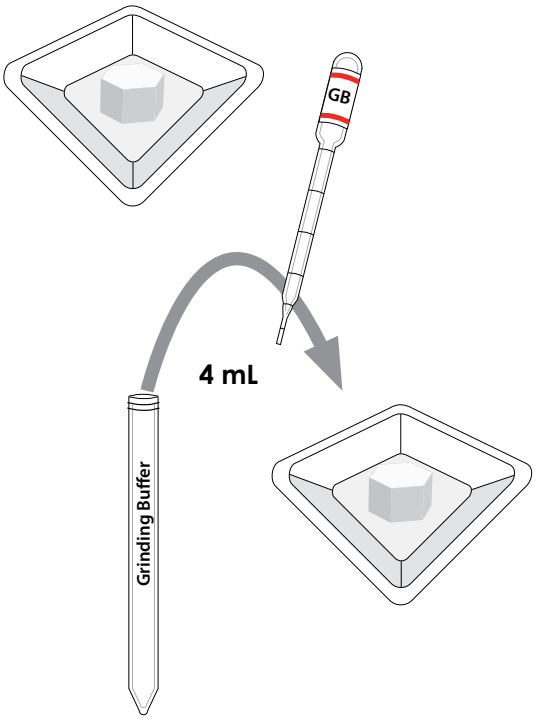
___ (1) Pipette: "ACID" for the HCl

Inhibitor groups only

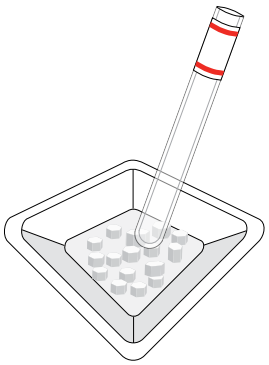
___ (1) Pipette: "INH" for the Sodium Benzoate inhibitor

Preparing enzyme extract

1. Obtain a mushroom piece and place it in the weighing boat.

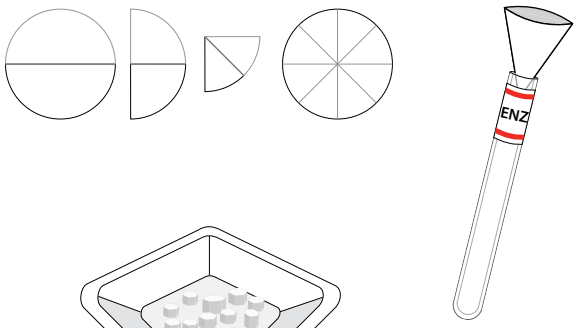


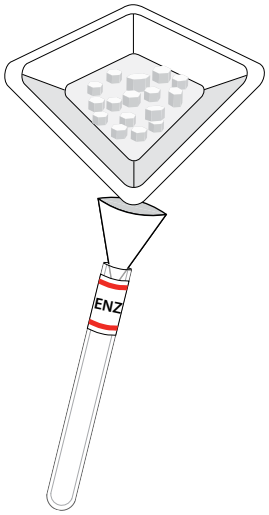
2. Using the "GB" pipette, add 4mL of cold grinding buffer on top of the mushroom.



3. Grind up the mushroom using the bottom of a test tube for 3 minutes or until it is mixed well.

4. Make a filtering cone from the coffee filter by folding it three times, creating a cone shape. Place the cone into the test tube labeled "ENZ" for enzyme.





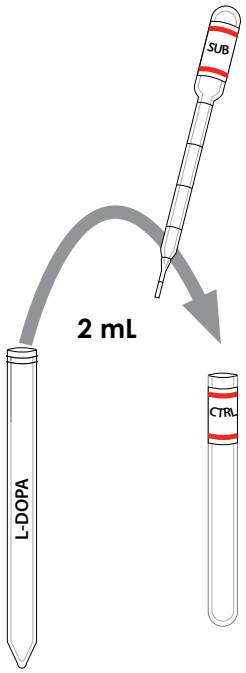
5. Pour the liquid from the weighing boat through the filter and into the "ENZ" test tube. This is your tyrosinase enzyme extract! Place it in your test-tube holder at room temperature.

Each team member should be prepared to do his or her job immediately. The reaction moves very quickly!

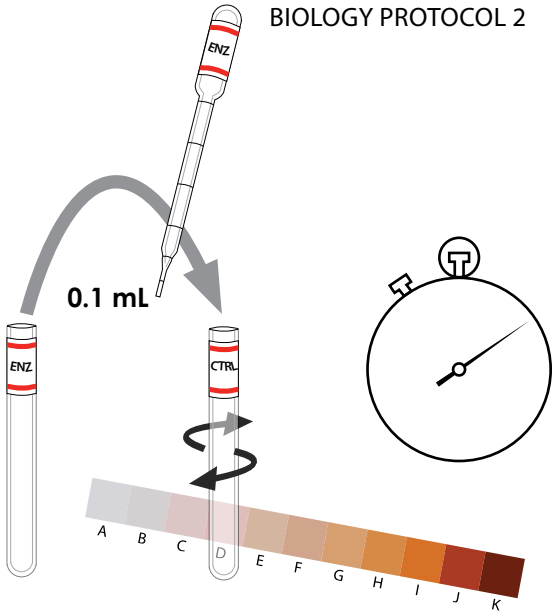
As a team, you will determine and record the color change of your solution for a TOTAL of SEVEN MINUTES for each trial.

Perform control trial (Trial 1)

1. Using the pipette labeled "SUB", Pipette 2mL of the L-DOPA substrate solution into the test tube labeled "CTRL".
    - **Important!** Get your data chart, timer and color metric chart ready before you add the enzyme in the next step.



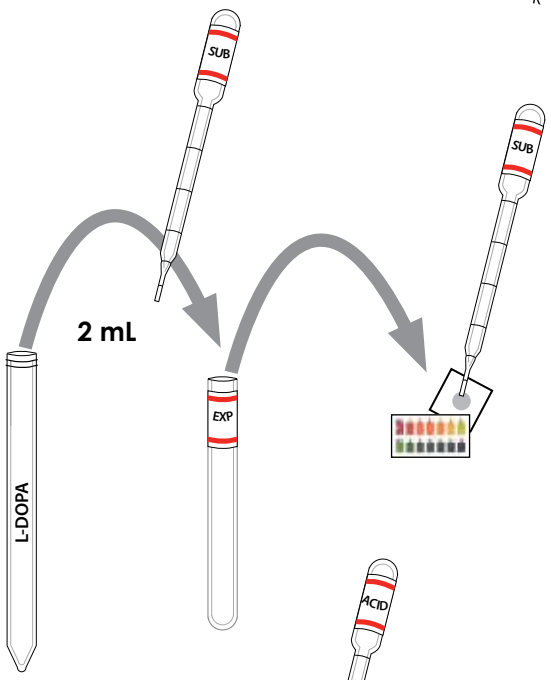
2. Using the transfer pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "CTRL" tube and swirl the test tube to mix.
    - **Immediately** start your interval recordings. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.



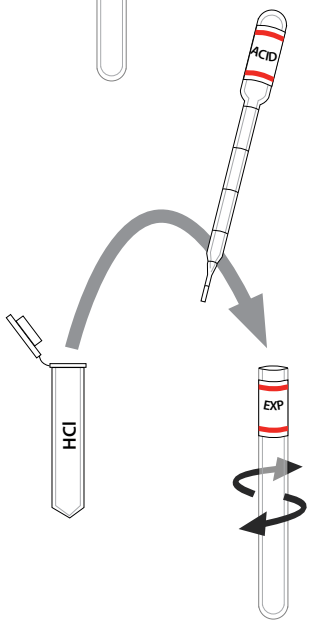
3. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

Determine the effect of pH (HCl) on tyrosinase activity

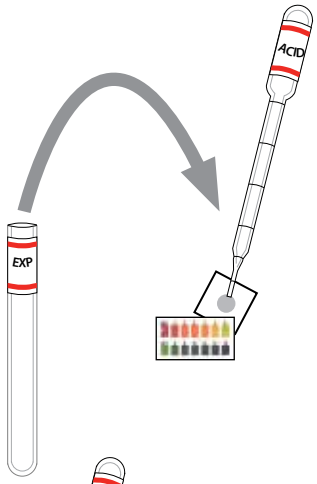
1. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into the test tube labeled "EXP".
2. From the same test tube labeled "EXP", use the "SUB" pipette to draw up one small drop of substrate and place it on pH paper. Using the pH color chart, determine pH of substrate and record pH on the data sheet.



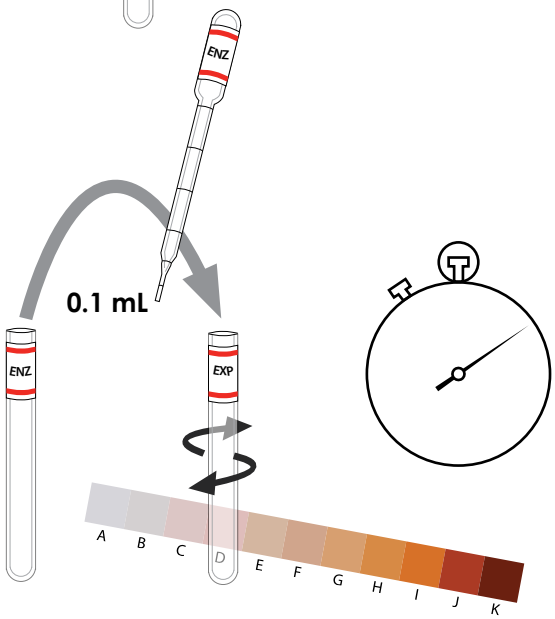
3. Using the "ACID" pipette, transfer the entire contents of the HCl (acid) tube into the same "EXP" test tube and mix. DO NOT DISCARD PIPETTE.



4. With the "ACID" pipette, add one drop from the "EXP" test tube onto a NEW piece of pH paper. Using the pH color chart, determine pH of substrate and record on the data sheet.
    - **Important!** Get your data chart, timer and color metric chart ready before you add the enzyme in the next step.

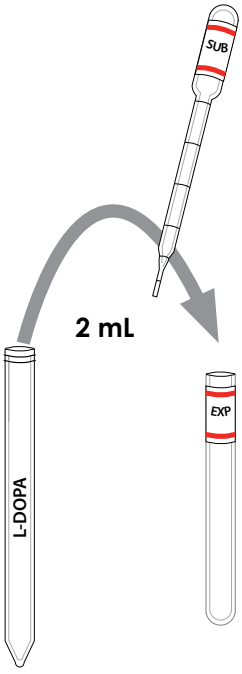


5. Using the transfer pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube and swirl the test tube to mix.
    - **Immediately** start your interval recordings. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.
6. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

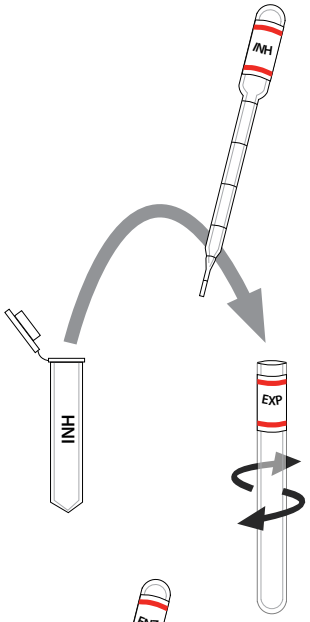


Determine the effect of an inhibitor on tyrosinase activity

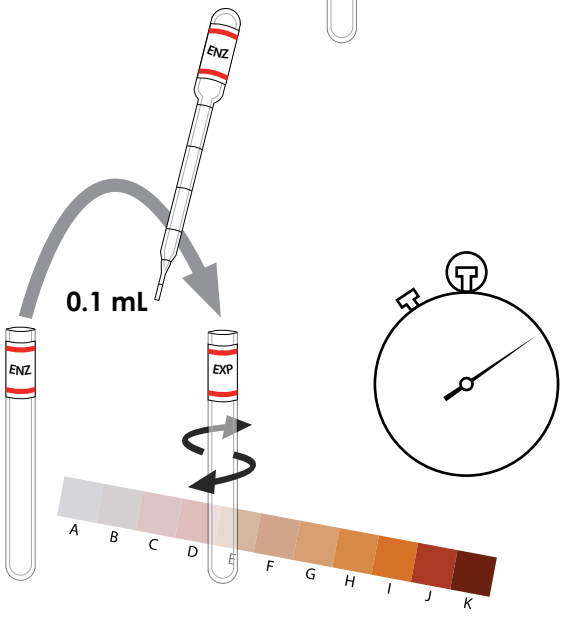
1. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into your first test tube labeled "EXP".



2. With the "INH" pipette, place the entire contents of the tube of inhibitor (sodium benzoate "INH") into the "EXP" test tube and swirl the test tube to mix.
    - **Important!** Get your data chart, timer and color metric chart ready before you add the enzyme in the next step.



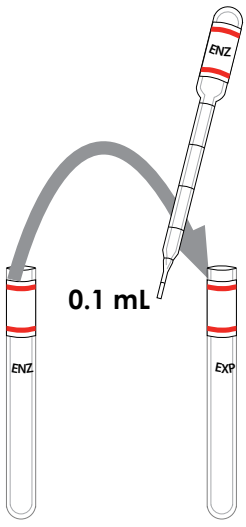
3. Using the pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube and mix.
    - **Immediately** begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the enzyme. Make sure you are recording data for each time point in your data table.



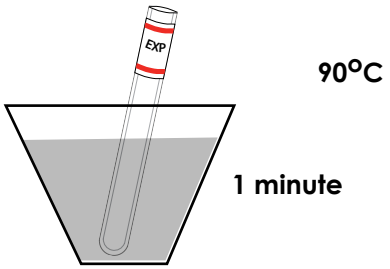
4. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

Determine the effect of temperature on tyrosinase activity

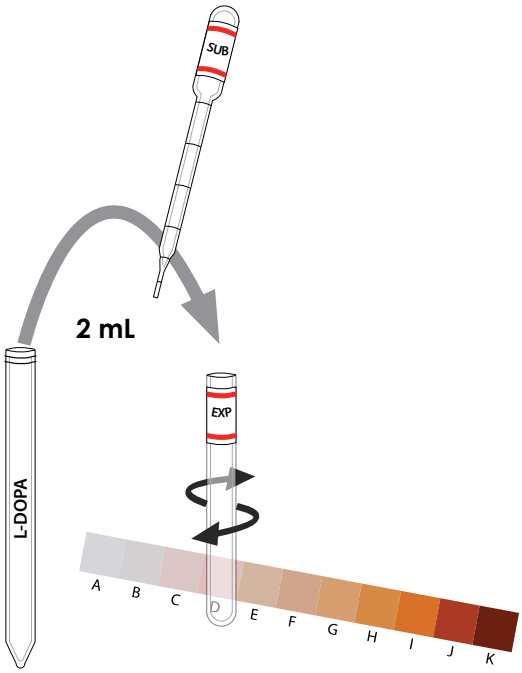
1. Using the pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube.



2. Place the "EXP" tube containing the enzyme in hot (90°C) water for 1 minute.
    - **Important!** Get your data chart, timer and color metric chart ready before you add the substrate in the next step.



3. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into your test tube labeled "EXP" and swirl the test tube to mix.
    - **Immediately** begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the substrate. Make sure you are recording data for each time point in your data table.



4. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

After completing all trials, convert colorimetric letters to mM concentration of substrate and graph your data.

		Trial # 1 Control		Trial # 2 Experimental:_____	
Time (Minutes: Seconds)	Convert time to seconds	Color Chart Letter	DOPA-chrome (mM)	Color Chart Letter	DOPA-chrome (mM)
10 second intervals					
6:50 0:10					
6:40 0:20					
6:30 0:30					
6:20 0:40					
6:10 0:50					
6:00 1:00					
5:50 1:10					
5:40 1:20					
5:30 1:30					
5:20 1:40					
5:10 1:50					
5:00 2:00					
20 second intervals					
4:40 2:20					
4:20 2:40					
4:00 3:00					
3:40 3:20					
3:20 3:40					
3:00 4:00					
1 minute intervals					
2:00 5:00					
1:00 6:00					
0 7:00					

pH Group

Substrate pH:_____

Substrate + acid pH:_____

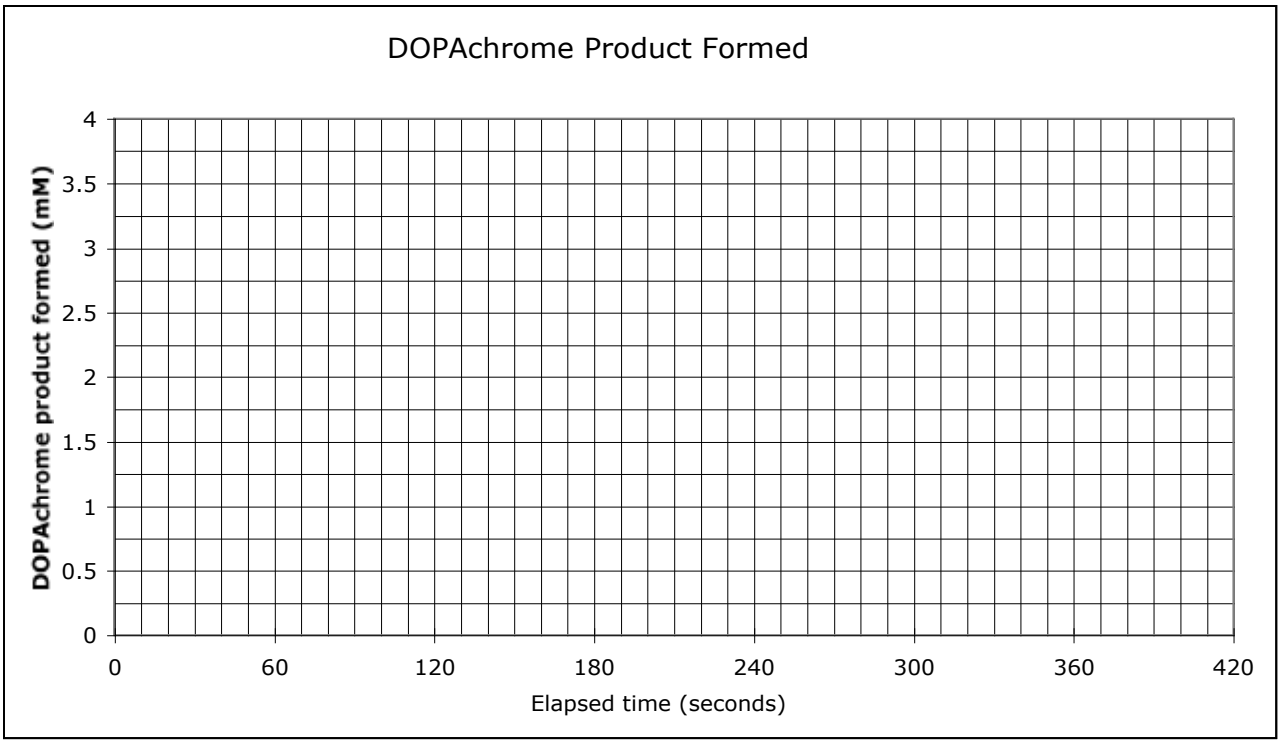
Graphing data

Use a different color to graph each condition and list the color below.

Color key:

Control:_____

Circle your experimental group: pH, Temperature, Inhibitor: _____



Analysis questions

1. What does color change tell you about enzyme activity?

2. What differences did you observe between the control and the experimental trials?

3. For your experimental condition (temperature, pH or inhibitor), explain how that condition affected enzyme activity.

4. How does the control reaction help you interpret your experimental results? How would you know if enzyme activity was changed in the experimental reaction if you had no control reaction data?

Conclusion / summary (revisit hypothesis)

Chemistry

Protocol 1

Experimental Group (circle one)	pH Inhibitor Temperature
Group # _____	

Each group will run 2 trials.

Write the name of the mixer, reader, timer and recorder for each trial in a table in your notebook.

	Mixer	Timer	Reader	Recorder
Trial 1 (control)				
Trial 2 (experimental)				

Central question

How do enzymes effect reaction rates? What factors would influence the effect of enzymes?

Overview of experiment

In this experiment you will observe and calculate the effects that an enzyme (tryrosinase) has on a chemical reaction (L-DOPA —> Dopachrome). The enzyme tyrosinase catalyzes the conversion of a non-colored molecule (L-DOPA) into a reddish-brown molecule (Dopachrome). You will determine the activity of the enzyme by recording the color change of the reaction solution, graphing your data using a standard curve, and performing a reaction rate calculation. Furthermore, you will observe how the function of the enzyme and thus the reaction rate will change when you alter the environment of the reaction by either introducing an inhibitor, altering the temperature or altering the pH of the surroundings.

Hypothesis

Student pre-lab questions

1. What happens in a chemical reaction?

2. How can you calculate the rate of a chemical reaction?

3. What are the various factors that can increase the rate of a chemical reaction?

4. In the reaction of L-DOPA —> Dopachrome what is the reactant (substrate)? What is the product?

5. What does the enzyme tyrosinase do?

6. What is the purpose of grinding the mushroom?

7. Describe the difference between the control (baseline) reaction and the experimental reaction.

8. How do inhibitors, low pH and temperature affect enzyme activity?

9. How is sodium benzoate? How does this relate to the activity of tyrosinase and the rate of the chemical reaction?

Material Checklist

pH groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) 1.5mL tube containing acid (HCl)
___ (2) pieces of pH paper
___ (1) pH color chart
___ (3) glass test tubes
___ (1) weighing boat
___ (4) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Temperature groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) weighing boat
___ (3) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (3) glass test tubes
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Inhibitor groups

___ (1) "CTRL" label
___ (1) "EXP" label
___ (2) "ENZ" labels
___ (1) "INH" label
___ (1) "GB" label
___ (1) "SUB" label
___ (1) 1.5 ml tube of sodium benzoate/inhibitor (INH)
___ (1) weighing boat
___ (4) disposable transfer pipettes
___ (1) filter paper (cut coffee filter)
___ (3) glass test tubes
___ (1) test tube rack
___ (1) $1cm^3$ mushroom cube
___ (1) colorimetric chart
___ (1) liquid waste container
___ (1) stopwatch/ timer
___ Safety Goggles for each person
___ (1) Styrofoam cup with ice

on ice

___ (1) 5mL tube of Grinding Buffer
___ (1) 15mL tube of L-DOPA

Lab Procedure

Label your test tubes and pipettes as described

___ (1) Test Tube: "CTRL" for control trial

___ (1) Test Tube: "EXP" or for experimental trial

___ (1) Test Tube "ENZ" for enzyme extract

___ (1) Pipette: "GB" for use with grinding buffer

___ (1) Pipette: "SUB" for use with L-DOPA substrate

___ (1) Pipette: "ENZ" for use with enzyme

pH groups only

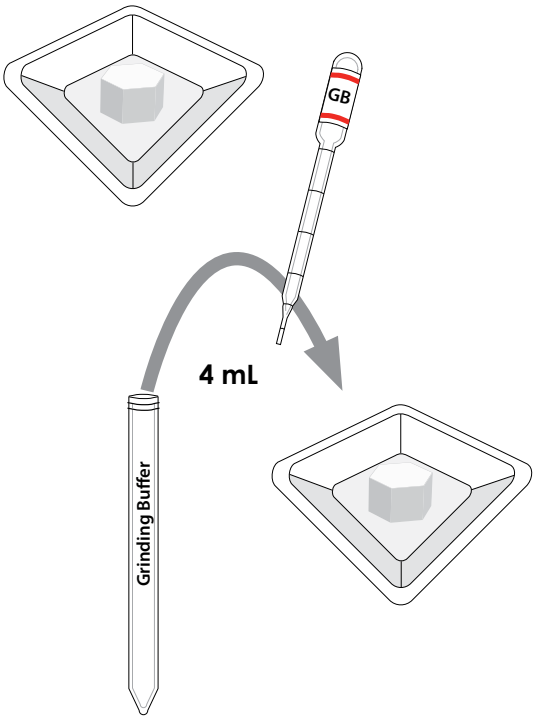
___ (1) Pipette: "ACID" for the HCl

Inhibitor groups only

___ (1) Pipette: "INH" for the Sodium Benzoate inhibitor

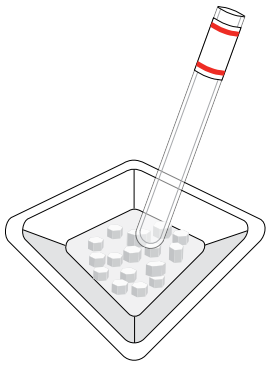
Preparing enzyme extract

1. Obtain a mushroom piece and place it in the weighing boat.

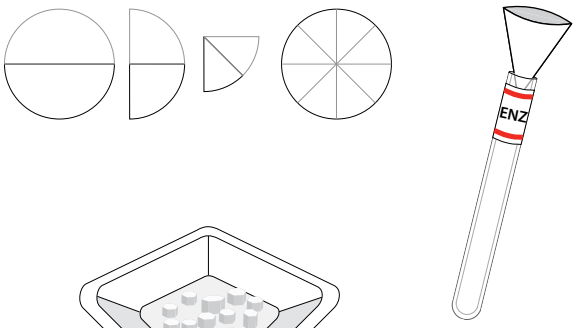


2. Using the "GB" pipette, add 4mL of cold grinding buffer on top of the mushroom.

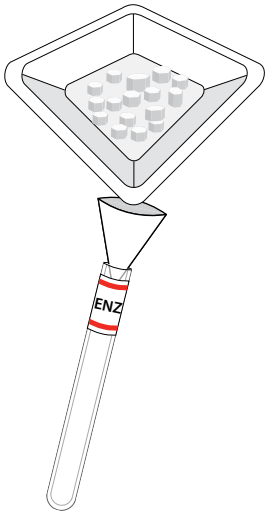
3. Grind up the mushroom using the bottom of a test tube for 3 minutes or until it is mixed well.



4. Make a filtering cone from the coffee filter by folding it three times, creating a cone shape. Place the cone into the test tube labeled "ENZ" for enzyme.



5. Pour the liquid from the weighing boat through the filter and into the "ENZ" test tube. This is your tyrosinase enzyme extract! Place it in your test-tube holder at room temperature.

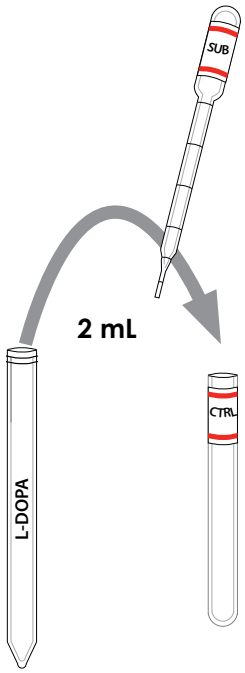


Each team member should be prepared to do his or her job immediately. The reaction moves very quickly!

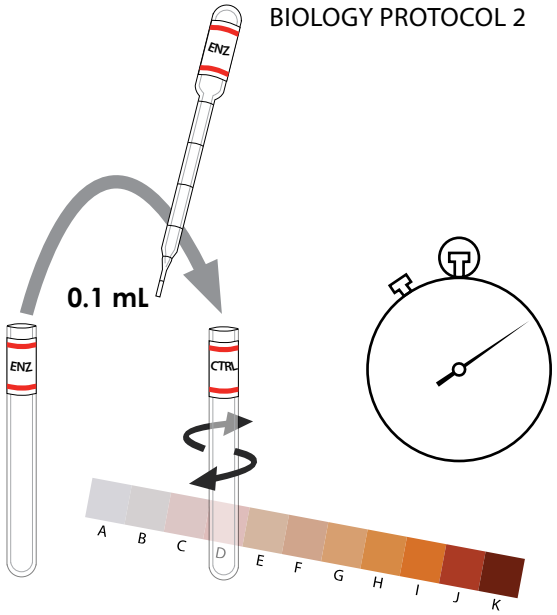
As a team, you will determine and record the color change of your solution for a TOTAL of SEVEN MINUTES for each trial.

Perform control trial (Trial 1)

1. Using the pipette labeled "SUB", Pipette 2mL of the L-DOPA substrate solution into the test tube labeled "CTRL".
    - **Important!** Get your data chart, timer and color metric chart ready before you add the enzyme in the next step.



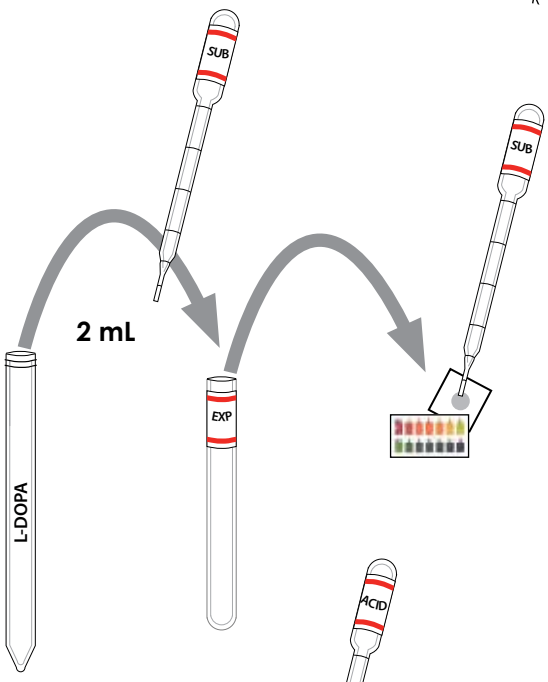
2. Using the transfer pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "CTRL" tube and swirl the test tube to mix.
    - **Immediately** start your interval recordings. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.



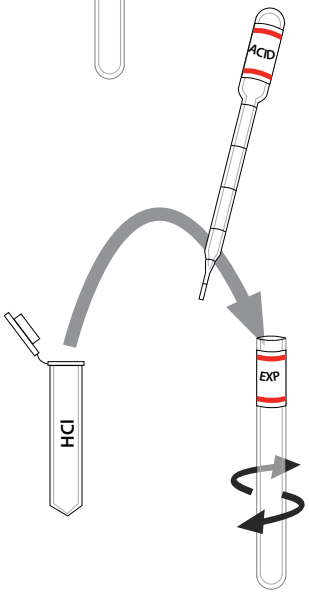
3. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

Determine the effect of pH (HCl) on tyrosinase activity

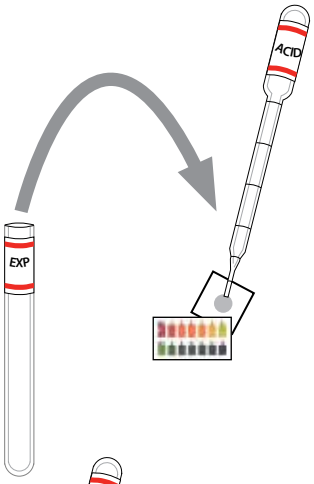
1. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into the test tube labeled "EXP".
2. From the same test tube labeled "EXP", use the "SUB" pipette to draw up one small drop of substrate and place it on pH paper. Using the pH color chart, determine pH of substrate and record pH on the data sheet.



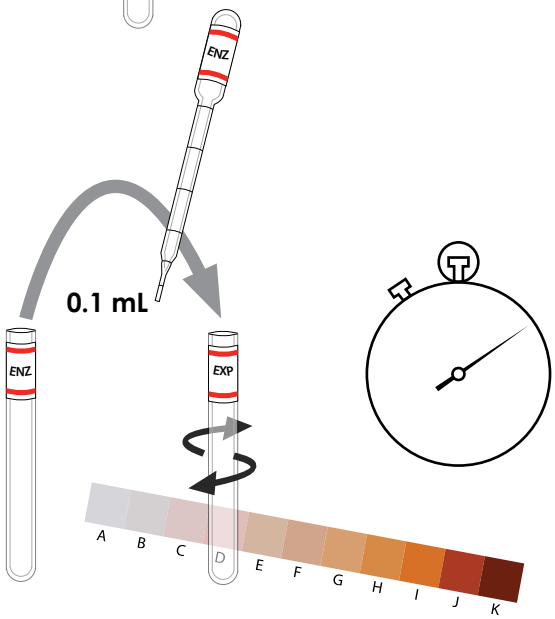
3. Using the "ACID" pipette, transfer the entire contents of the HCl (acid) tube into the same "EXP" test tube and mix. DO NOT DISCARD PIPETTE.



4. With the "ACID" pipette, add one drop from the "EXP" test tube onto a NEW piece of pH paper. Using the pH color chart, determine pH of substrate and record on the data sheet.
    - **Important!** Get your data chart, timer and color metric chart ready before you add the enzyme in the next step.

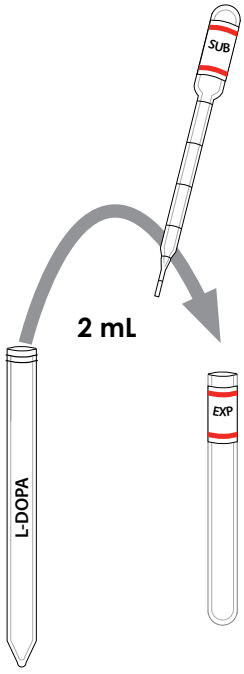


5. Using the transfer pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube and swirl the test tube to mix.
    - **Immediately** start your interval recordings. At each time interval, write the letter that is reported. Make sure you are recording data for each time point in your data table.
6. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

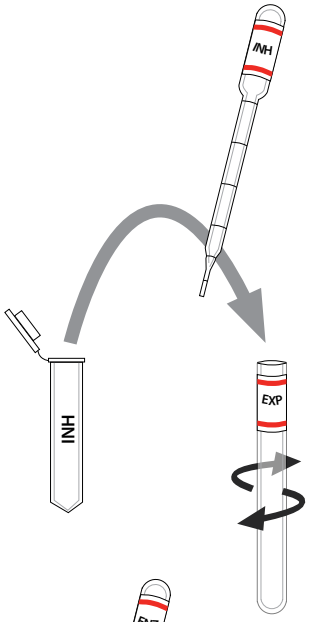


Determine the effect of an inhibitor on tyrosinase activity

1. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into your first test tube labeled "EXP".

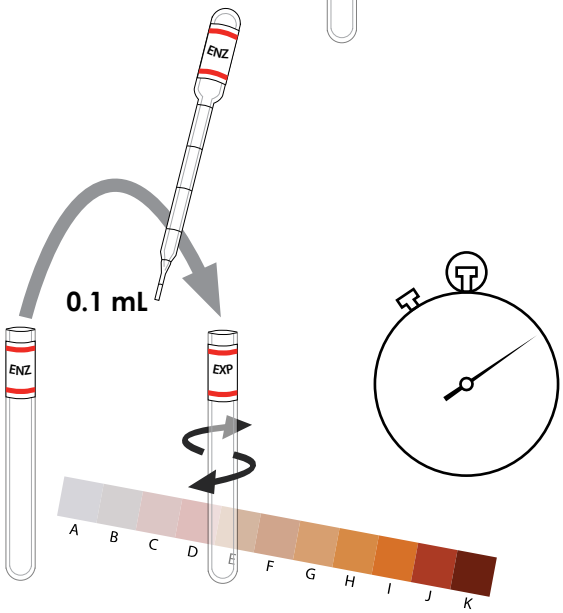


2. With the "INH" pipette, place the entire contents of the tube of inhibitor (sodium benzoate "INH") into the "EXP" test tube and swirl the test tube to mix.
    - **Important!** Get your data chart, timer and color metric chart ready before you add the enzyme in the next step.



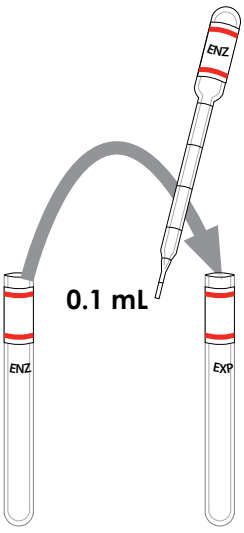
3. Using the pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube and mix.
    - **Immediately** begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the enzyme. Make sure you are recording data for each time point in your data table.

4. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

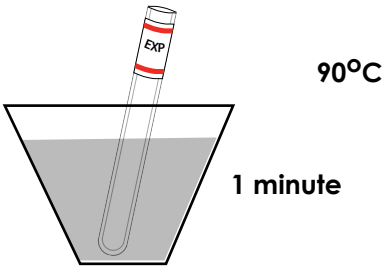


Determine the effect of temperature on tyrosinase activity

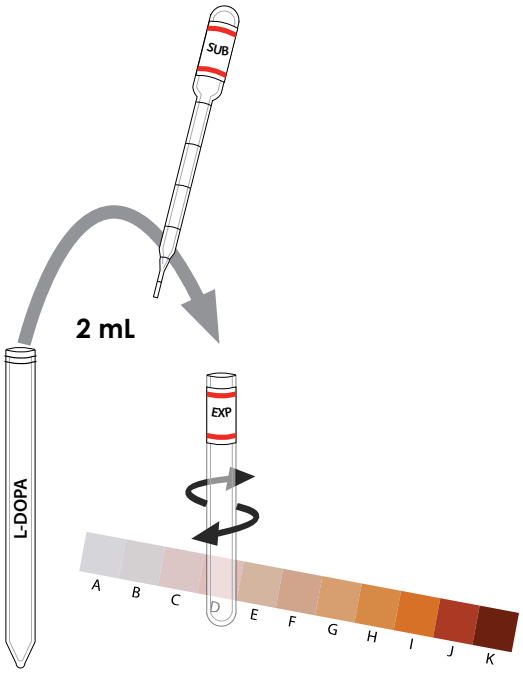
1. Using the pipette labeled "ENZ", add 0.1 mL of the tyrosinase enzyme extract to the "EXP" tube.



2. Place the "EXP" tube containing the enzyme in hot (90˚C) water for 1 minute.
    - **Important!** Get your data chart, timer and color metric chart ready before you add the substrate in the next step.



3. Using the pipette labeled "SUB", transfer 2mL of the L-DOPA substrate solution into your test tube labeled "EXP " and swirl the test tube to mix.
    - **Immediately** begin the timer and compare color of solution to colorimetric chart and report the letter that corresponds as soon as you add the substrate. Make sure you are recording data for each time point in your data table.

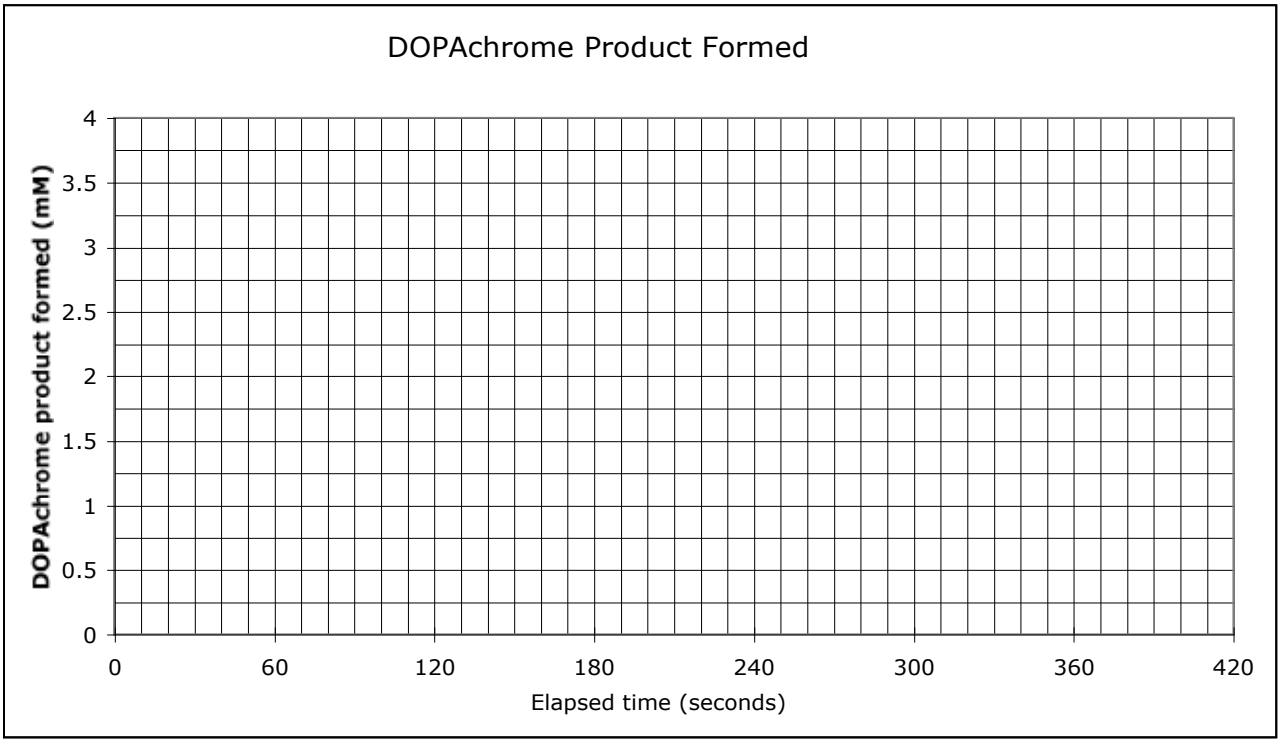


4. Once you have completed the trial, empty the contents of the tube into your liquid waste container and place your test tube in the dry waste container.

		Trial # 1 Control		Trial # 2 Experimental:_____	
Time (Minutes: Seconds)	Convert time to seconds	Color Chart Letter	DOPA-chrome (mM)	Color Chart Letter	DOPA-chrome (mM)
10 second intervals					
6:50 0:10					
6:40 0:20					
6:30 0:30					
6:20 0:40					
6:10 0:50					
6:00 1:00					
5:50 1:10					
5:40 1:20					
5:30 1:30					
5:20 1:40					
5:10 1:50					
5:00 2:00					
20 second intervals					
4:40 2:20					
4:20 2:40					
4:00 3:00					
3:40 3:20					
3:20 3:40					
3:00 4:00					
1 minute intervals					
2:00 5:00					
1:00 6:00					
0 7:00					

Graphing data

Use a different color pencil for each condition.



Analysis

Calculate the following reaction rates- remember, rate of reaction = $\text{rate} = \frac{\Delta[\text{product}]}{\Delta t}$

TRIAL #	Time	Changes in concentration	Change in time	Reaction rate (mM/s)
1	0s - 420s			
2	0s - 420s			

Which of the reactions has the fastest rate? Which has the slowest? What does this mean?

TRIAL #	Time	Changes in concentration	Change in time	Reaction rate (mM/s)
2	0s - 60s			
2	60s - 120s			
2	120s - 240s			
2	240s - 360s			
2	360s - 420s			

Which part of the reaction happens the fastest? The slowest? Why do you think this is?

Analysis questions

1. Why was a buffer used in Part A rather than just water?

2. How did you separate the mushroom extract from the pieces of ground mushroom? What separation technique is this?

3. Is there a difference in reaction rates between the control and experimental? Why do you think this is?

4. Which part of trial 1 or trial 2 had the fastest rate? Why do you think this is?

5. What does color change tell you about enzyme activity?

6. What differences did you observe between the control and the experimental trials?

7. How does the control reaction help you interpret your experimental results? How would you know if enzyme activity was changed in the experimental reaction if you had no control reaction data?

8. ANSWER THESE QUESTIONS FOR EACH EXPERIMENTAL OPTION… IF YOU DIDN'T PERFORM THAT PART, THEN MAKE SURE TO TALK WITH OTHER GROUPS…

a. For the pH experiment did the pH increase or decrease? (if you didn't do this one, then check with the other groups)? Why was this? What happened to the Hydrogen ion concentration? How did this affect the reaction rate?

b. How was the temperature changed? How did this effect reaction rate?

c. What was the inhibitor used? How did it affect the reaction rate? Why would sodium benzoate be used as a food preservative?

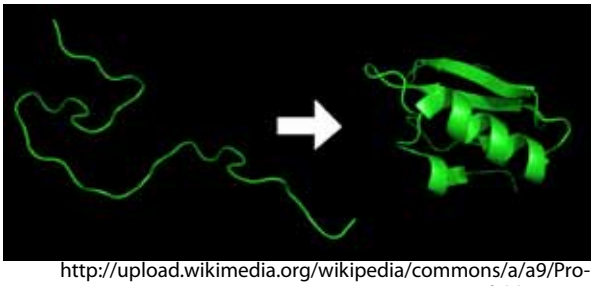
Conclusion

What did you learn about how enzymes affect the rate of reactions? What about how enzymes are affected by the environment? What are two sources of error in this lab? How might they have affected your results? What is one thing you would change if you did this lab again and WHY?

Appendix #1

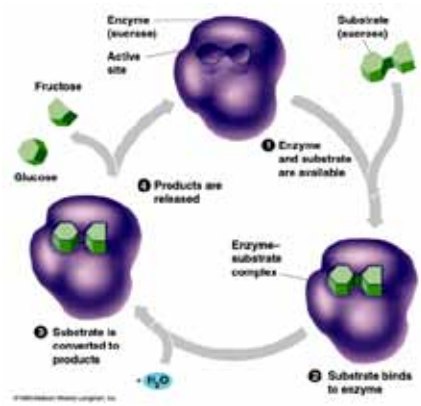
Section 3A – Enzymes are special proteins

All living things are constructed of proteins that are produced by cells using the DNA genetic code. Proteins make up our flesh and blood, carry oxygen to our lungs, and regulate our bodily functions. Enzymes are proteins, too. They are responsible for digesting our food, creating pigment in our skin, and are required for DNA replication, elimination of toxins, and other vital bodily functions. From viruses to mammals, enzymes are required by every living thing to exist and survive.



http://upload.wikimedia.org/wikipedia/commons/a/a9/Protein_folding.png

Enzymes are macromolecules created from a chain of amino acids that is folded upon itself into a unique 3-dimensional shape.



http://www.bio.miami.edu/~cmallery/150/metab/EScomplex.jpg

Enzymes differ from other proteins due to the presence of an "active site"—the location where interactions and reactions between molecules takes place. This unique characteristic allows enzymes to aid in biochemical reactions by speeding up the process of building or breaking down substances. The enzyme acts on molecules by providing the conditions for chemical bond creation or breakage.

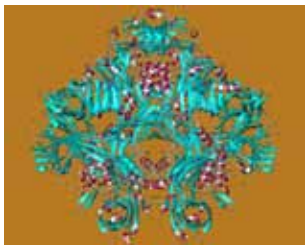
There have been thousands of enzymes identified across the Kingdoms of Life.

A few examples of enzymes are:

Lysozyme

A naturally occurring enzyme, found in our saliva and nasal drippings. Acts like scissors to cut peptide bonds that make up the cell wall of bacteria, thus destroying and killing potentially harmful organisms. Viruses also create and use lysozyme to break into and infect bacterial cells.

http://wiki.symplus.co.jp/lib/exe/fetch.php/rcsb_image/1lyd.jpg



http://160.114.99.91/astrojan/protein/pictures/lactase6.jpg

Lactase

The enzyme found in the digestive system of mammals, including humans that drink milk and/or eat milk products. It helps to break down milk proteins into simple sugars that can be utilized by the body.

Protease

A group of enzymes that breakdown proteins in the body and are utilized in detergents and soaps.

Often, enzymes are named for the substrate they interact with (i.e. protein) and adding the suffix –ase ("prote-ase").

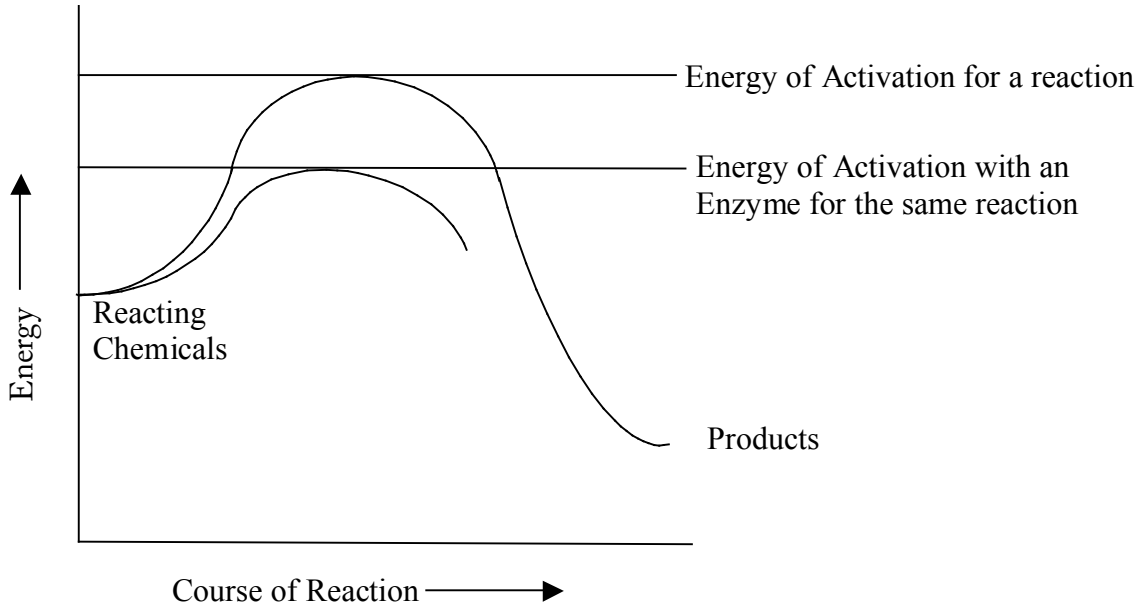
Appendix #2

Section 3B – Enzymes act as a Biological Catalyst to assist and speed up biochemical reactions.

A ***"catalyst"*** is anything that speeds up a chemical reaction without being consumed. Enzymes are biological catalysts—they are required for specific biochemical reactions to occur within living organisms. Enzymes help to create ***"products"*** by either breaking down pre-existing chemical bonds and molecules or building new ones. When the products are created, the substrate has been used up but the enzyme is still present.

Enzyme + Substrate —> Enzyme-Substrate —> Enzyme + Product

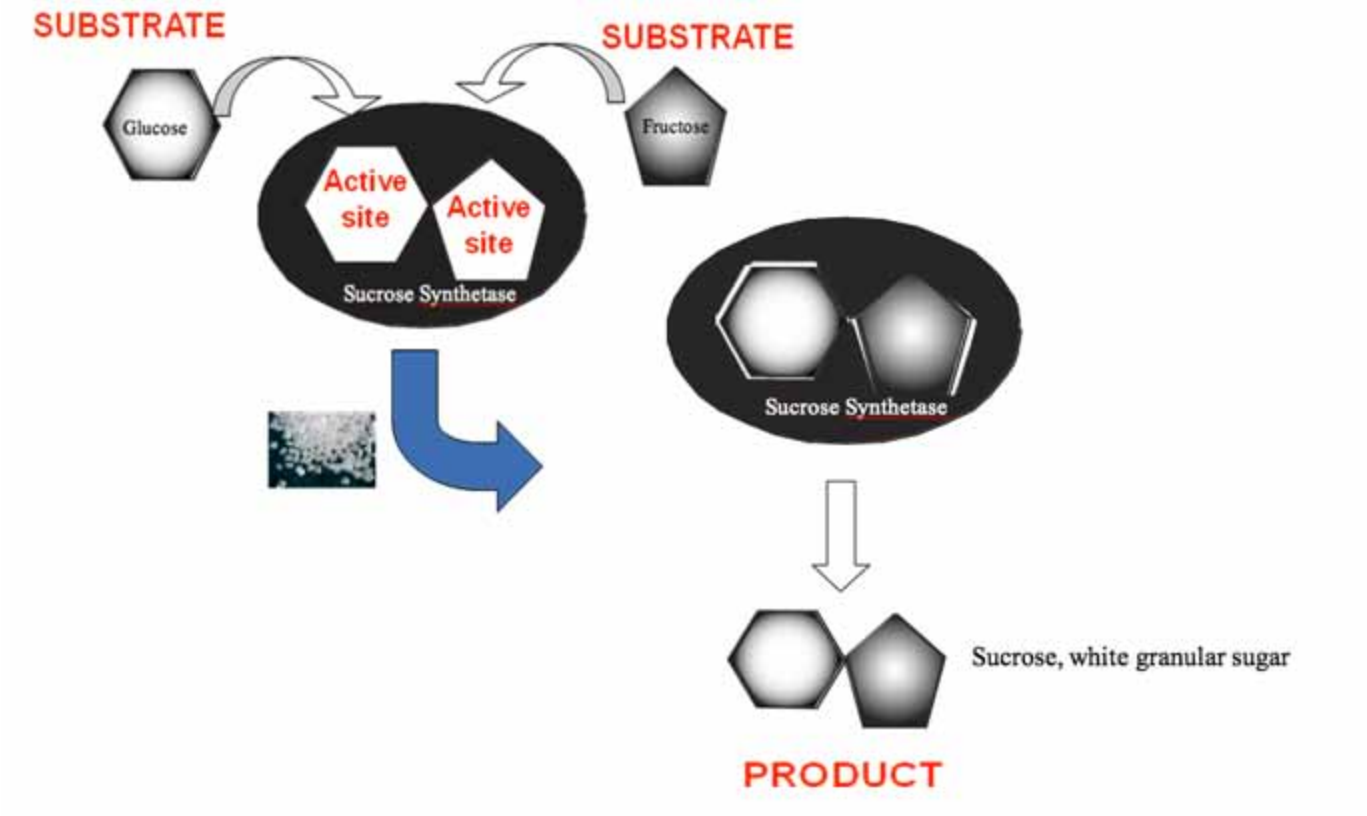
Enzymes lower the "activation energy" of a reaction by creating an environment that allows for reactions to occur faster and with less required energy. They sometimes achieve this state by altering the shape of the substrate to allow for easier breakage or connection of chemical bonds.



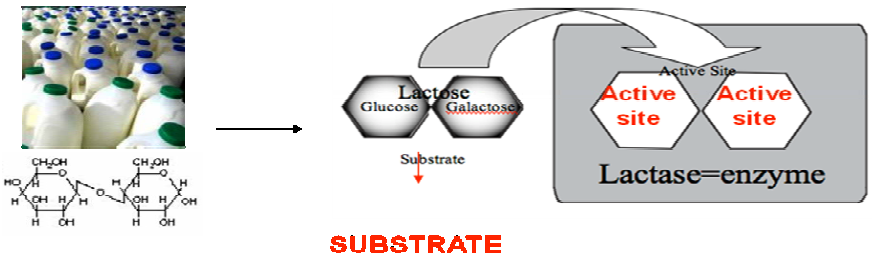
Some chemical reactions will occur without enzymes, but benefit from the catalytic nature of the molecule. An analogy would be a human being attempting to go up and over a mountain pass. Walking may take days, but the journey could be made. By using a bicycle, the time it takes to complete the journey is reduced significantly. A stronger catalyst, such as a car, can speed the process even further. Some enzymes are weak catalysts (like the bicycle) and some are strong (such as the car).

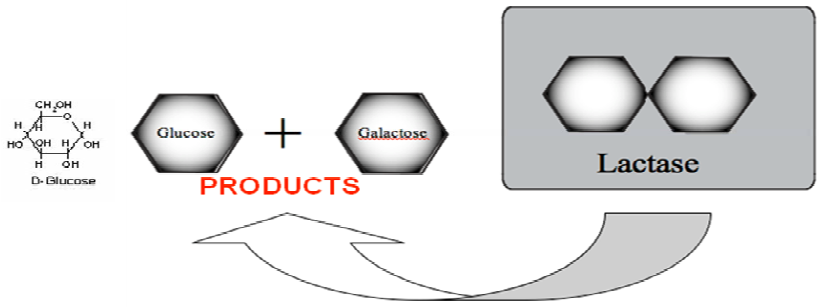
Another analogy could be made regarding the building of a product from two or more existing molecules (***"anabolic"*** reaction) and the breaking down of a molecule into two or more sub-units (***"catabolic"*** reaction). If there are two people who would be a great couple, but they do not know each other, the chances of them meeting are low and the time it may take for that meeting could be long. A matchmaker acts as a catalyst to bring two people together faster and more efficiently than if the match was made on its own. Along the same line, a relationship could be broken up by a rumor or event that was presented to the couple by an outside entity. This person or entity is a catalyst for a breakup, as it weakens the bond between the two people.

An example of an **anabolic** biochemical reaction is the creation of granulated sugar from fruit sugar through bond formation. The enzyme "Sucrose Synthetase" helps to form sucrose sugar (white granular sugar) from glucose and fructose (fruit sugars).



A common catabolic reaction is the digestion or breakdown of milk protein (lactose) by the enzyme "lactase" into simple sugars (glucose and galactose).





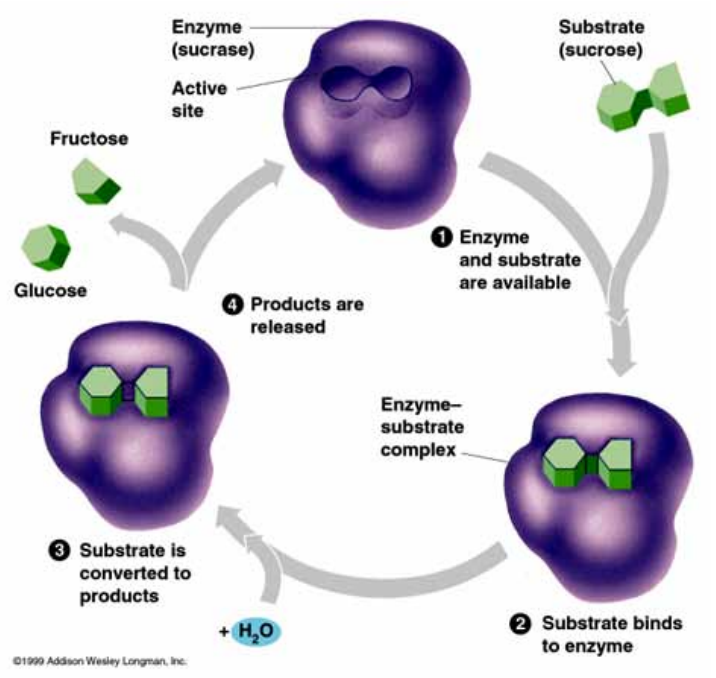
Appendix #3

Section 3B – Enzymes act as a Biological Catalyst to assist and speed up biochemical reactions.

Enzymes catalyze particular reactions and only work with specific substrates. For example, lactase is an enzyme that breaks down milk protein. Lactase does not react with any other protein or substance other than milk protein. (and, vice versa--lactose, the milk protein, is unable to be broken down/digested without the enzyme lactase being present).

The specificity of enzymes is due to different characteristics of the enzymes and substrates including shape, charge, and hydro-phobic/phyllic properties.

The shape of the active site and the fit of the substrate has been referred to as a "lock and key" model, where, like a jigsaw puzzle, the substrate and enzyme connect. However, many scientists believe that that the enzyme will change shape to fit the substrate into the active site. This is known as the "induced fit" model.



http://www.bio.miami.edu/~cmallery/150/metab/EScomplex.jpg

Appendix #4

Section 3D – Enzymes are sensitive to changes in pH and temperature.

Most enzymes are only functional within a specific, narrow range of pH and temperature. Enzymes in humans and other mammals will not function if the body temperature rises or drops too much. Digestive enzymes in our stomachs function in the highly acidic environment, and will not work outside of the stomach where the pH higher and less acidic.

Enzymes can be denatured (rendered useless) by heating or other factors that change the shape, thus the specificity and function, of the enzyme. They can be "unfolded" and can sometimes re-fold to function properly under the right conditions.

An example of an enzyme that is temperature sensitive is Tyrosinase, the enzyme used in the lab activity. It is optimally functional within a specific temperature range in most organisms (e.g. humans at body temperature), but is uniquely dependant on temperature in Siamese Cats. For example, the color pattern observed most frequently is black face, black tail, black paws, with a brown or blonde mid-section

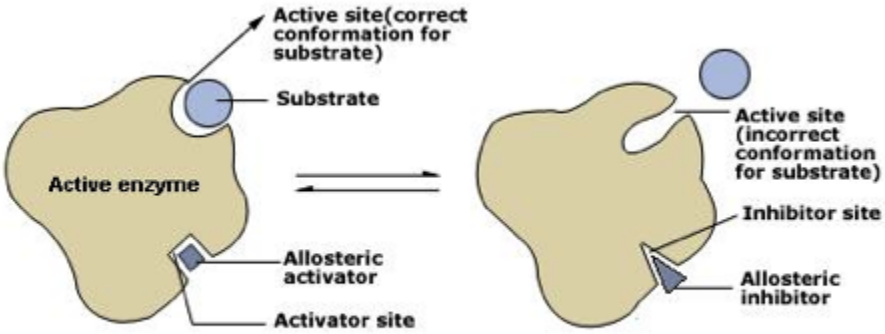
The black pigment is a result of Tyrosinase activity at the extremities, where temperatures are significantly cooler than the rest of the body. At these locations, tyrosinase is actively working to produce melanin, resulting in dark fur.

Appendix #5

Section 3E – Enzymes activity can be slowed or prevented by the presence of inhibitors.

Inhibitors are natural or synthetic molecules that do not allow the enzyme to react with the substrate. In ***"competitive"*** inhibition, the inhibitor and the substrate compete for the same enzyme. If the inhibitor binds to the active site, the enzyme cannot function properly and cannot react with the substrate.

In ***"non-competitive"*** or allosteric inhibition is when the inhibitor binds to the enzymes at another location other than the active site, thus changing the shape of the protein structure and altering the active site enough that the substrate cannot bind. An allosteric activator creates the correct shape for the substrate to bind to the enzyme.



Schematic representation of allosteric enzyme activity

Inhibitors may slow down or completely prevent enzyme function. By understanding inhibitors, scientists can apply what they know to creating and utilizing inhibitors for the benefit of health and medicine, economic growth, and other human concerns. Both naturally occurring and synthetically produced inhibitors are utilized in our everyday lives.

Examples of inhibitors

Common preservatives used in food products are actually enzyme inhibitors. **Sodium Benzoate** is a common preservative used to preserve flavor, prevent browning, and to give a longer shelf life to the product.

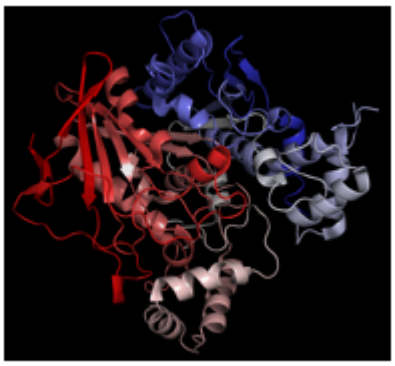
Enzyme Inhibitors are commonly used as insecticides and chemical warfare agents—regular enzyme function within the nervous system can be blocked or slowed, resulting in death. Acetylcholinesterase (AChE) is the enzyme targeted by inhibitors found in insecticides and chemicals such as serum (nerve) gas. AChE is found in many animals and it functions to break down the neurostransmitter acetylcholine in the synaptic cleft. (for more info on Cholinesterases, see Appendix 6). Reversible competitive inhibitors have been identified and manufactured into antidotes used to treat people exposed to nerve gas or other similar agents.

Some common drugs that act as enzyme inhibitors are Viagra, HIV Protease Inhibitors, and some cancer drug treatments (chemotherapy).

Tyrosinase, the enzyme used in this lab, has many inhibitors, both natural and synthetic.

Appendix #6

Cholinesterases



Cholinesterase is an enzyme found in the circulatory and nervous systems of many animals, including humans. This enzyme acts to metabolize a neurotransmitter, acetylcholine, that functions in both the peripheral nervous system (PNS) and central nervous system (CNS). Acetylcholine activates muscles, stimulates the body, when excited, to release substances such as sweat and epinephrine (adrenaline), and plays a significant role in addiction.

Two similar yet distinct cholinesterases exist: "Acetylcholinesterase" and "Butyrylcholinesterase". Both are equally effective, but work on different sizes of choline substrates. (1) [The enzyme acetylcholinesterase converts acetylcholine into the inactive metabolites choline and acetate. This enzyme is abundant in the synaptic cleft, and its role in rapidly clearing free acetylcholine from the synapse is essential for proper muscle function. Certain neurotoxins work by inhibiting acetylcholinesterase, thus leading to excess acetylcholine at the neuromuscular junction, thus causing paralysis of the muscles needed for breathing and stopping the beating of the heart.] wikipedia. The buildup of acetylcholine allows for an increase in the level and duration of activity in the junction.

The cholinesterase active site is buried deep in the globular protein, and the molecular construct of the enzyme creates a streamlined gorge straight to the active site, allowing for very quick in-and-out action on the substrate.

Inhibitors of acetylcholinesterase occur naturally as venom and poisons and are used as nerve agents in terrorism attacks and warfare (e.g. nerve gas or serum gas).

Read more about cholinesterases and the research being conducted at UCSD's School of Pharmacy http://pharmacy.ucsd.edu/faculty/TaylorLab/

Humans have also taken advantage of the work of cholinesterase inhibitors by creating pesticides and insecticides that act on the nervous system of ants, ticks, aphids, and other invertebrates.

Fun fact

- Acetylcholine was the first neurotransmitter to be identified.

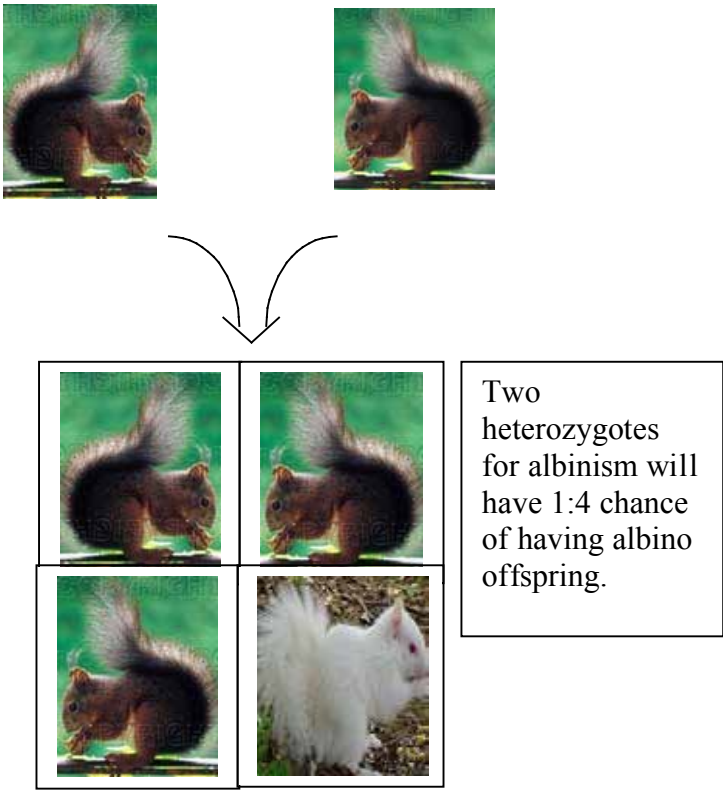
Timeline

1906?	Discovery of cholinesterases
1960's-1970's	Inhibitors and substrates found that bind and interfere with catalytic reaction of cholinesterase
1980's	Protein Purification techniques allowed for identification of cholinesterase active site and gene sequence to allow for cloning of gene that encode for cholinesterase
1990's	Three-dimensional structure of a cholines terase was solved
2000's	Determination of >125 primary structures and >60 three-dimensional structures of cholinesterase

Appendix #7

Albinism and Tyrosinase

Albinism is an autosomal, recessive genetic condition which is characterized by a lack of pigmentation (melanin) in fish, amphibians, reptiles, birds, amphibians, and mammals--including human beings. The lack of pigmentation is due to a mutation in the gene that codes for Tyrosinase. Without tyrosinase, the products needed to form melanin cannot be produced.



From: Fotosearch.com

Because melanin creates color in skin, eyes, and hair, any albinistic organism will appear white or very pale with light blue eyes (or reddish appearing eyes due to the retinal blood vessels shining through).

Approximately 1 in 70 people is a carrier of the albinism gene, leading to an incidence of 1 in 17,000 among human beings. While there are different forms of albinism with various causes, one type with a frequency of 1: 40,000 persons is oculocutaneous Type 1 (OCA1) which is caused by a mutation in the tyrosinase gene, TYR. Albinistic persons with OCA1 produce little or no melanin leading to white, almost translucent hair and skin. They tend to have poor eyesight.

Since albinistic persons produce no melanin as a result of the tyrosinase mutation, they cannot tan and must be particularly careful about long exposure to sunlight. They are particularly susceptible to melanoma--a very deadly form of skin cancer.