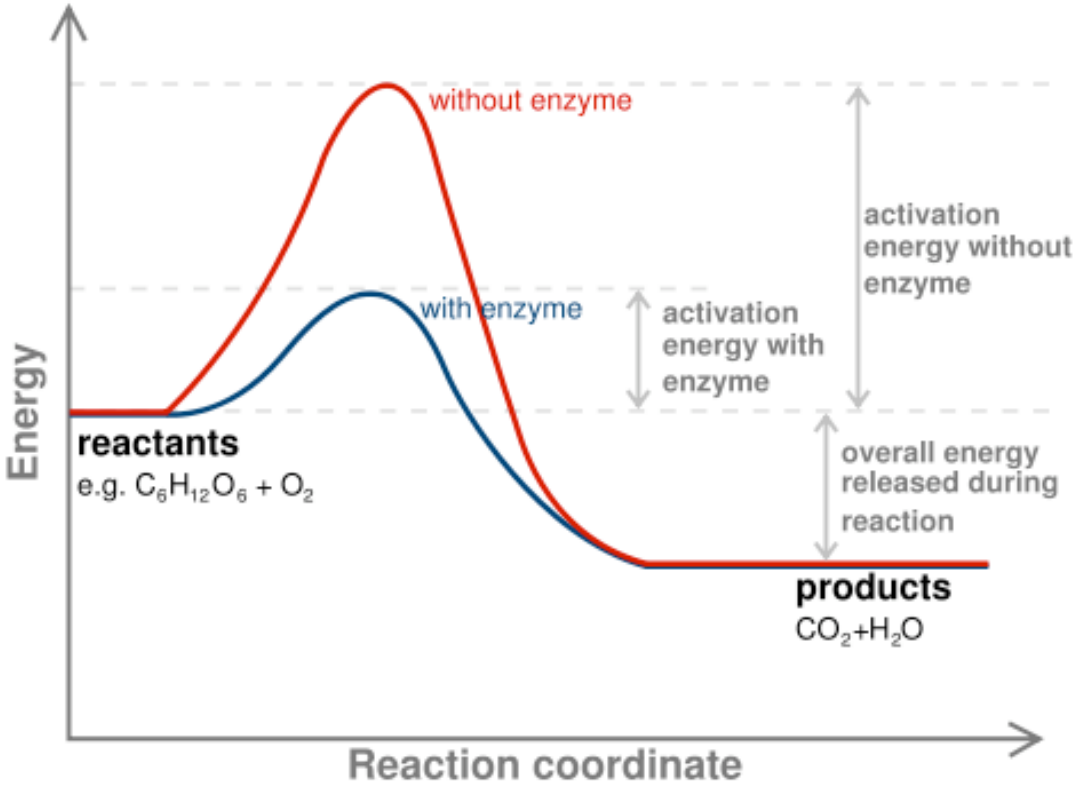
Enzyme Activity

Introduction

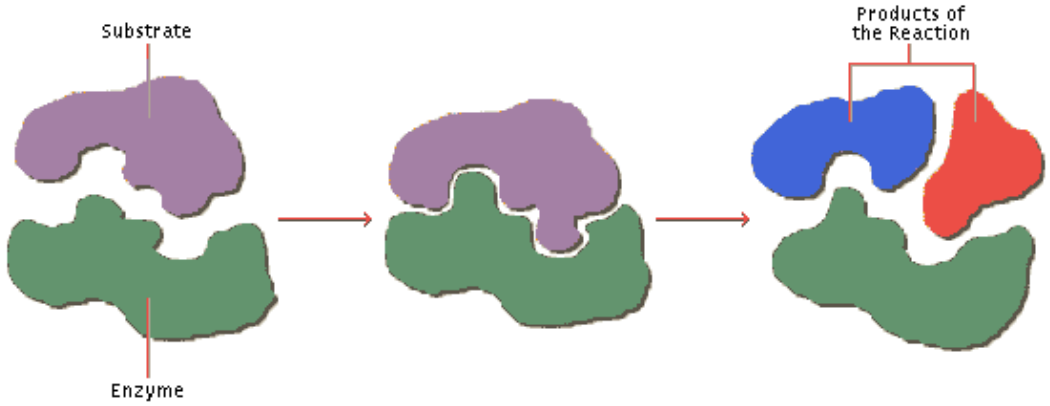
Enzymes allow many chemical reactions to occur within the constraints of a living system. Without enzymes, reactions would still occur, but they would be too slow to benefit living systems. Enzymes function as organic **catalysts**. A catalyst is a chemical involved in, but not changed by, a chemical reaction. Many enzymes function by lowering the **activation energy** of reactions. By bringing the **reactants** closer together, chemical bonds may be weakened and reactions will proceed faster than without the catalyst.



Enzymes can act rapidly, as in the case of **carbonic anhydrase** (enzymes typically end in the -ase suffix), which causes the chemicals to react 107 times faster than without the enzyme present. Carbonic anhydrase speeds up the transfer of carbon dioxide from cells to the blood. There are over 2000 known enzymes, each of which is involved with one specific chemical reaction. Enzymes are **substrate** specific. The enzyme peptidase (which breaks peptide bonds in proteins) will not work on starch (which is broken down by amylase found in the human mouth).

Enzymes are proteins. The shape of the protein determines the function of the enzyme. The arrangement of molecules on the enzyme produces an area known as the **active site**

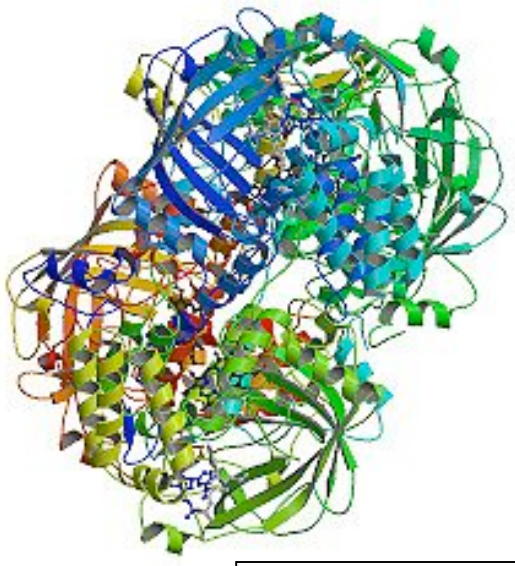
within which the specific substrate(s) will "fit". It recognizes, confines and orients the substrate in a particular direction. Where is the active site in the following diagram? (label it)



In today's experiment, you will be working with the enzyme **catalase**.

Catalase is a common enzyme found in nearly all living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second. Looking at the chemical equation of the reaction that catalase catalyzes, what could you observe to determine the rate of the reaction? _____

$$2H_2O_2 + \text{catalase} \text{ ----------> } 2H_2O + O_2 + \text{catalase}$$



Structure of an enzyme

Procedure

Prepare Enyme

1. Cut a cube of liver ~5 cm^2
2. Add 1 medium-sized scoop of ice
3. Add 100 ml pH 7 phosphate buffer
4. Grind until smooth in blender (~30-60 seconds)
5. Pour mixture through a double layer of cheesecloth into a beaker
6. This stock solution should be diluted for class use and stored 4°C
7. Dilute 10 ml of stock solution in 450-500 ml distilled water (each class)
8. Keep enzyme suspension on ice

Prepare Computers for Data Collection – Instructor will give instructions on how to do this.

Measure Enzyme Activity

1. Place five test tubes in a rack and label them 1, 2, 3, 4 and 5
    a. *Each group member should prepare one of the test tubes below:*
    b. Fill test tube #1 with 4 mL of water
    c. Fill test tube #2 with 1 mL of 3.0% H_2O_2 and 3 mL of water
    d. Fill test tube #3 with 2 mL of 3.0% H_2O_2 and 2 mL of water
    e. Fill test tube #4 with 3 mL of 3.0% H_2O_2 and 1 mL of water
    f. Fill test tube #5 with 4 ml of 3.0% H_2O_2
2. Using a clean pipette, add 10 ml of enzyme suspension to your 250 ml Nalgene bottle
3. Pour H_2O_2/ water solution from the test tube into your 250 ml Nalgene bottle and immediately connect the sensor and begin data collection
    a. *To connect the sensor*: place the O_2 Gas Sensor into the bottle. Gently push the sensor down into the bottle until it stops. The sensor is designed to seal the bottle without the need for unnecessary force.
4. As soon as the sensor is connected to the bottle, click Collect to begin data collection
    a. Do this as quickly as possible to collect as much data as you can
    b. Gently swirl the bottle during data collection
5. Allow data collection to continue for 2 minutes. To finish data collection, click the Stop button, which is the same as the Collect button.
6. When data collection has finished, remove the O_2 gas sensor from the 250 ml bottle.
7. Rinse the bottle with water and dry with a paper towel.
8. Using the mouse, select the initial linear region of your data on the graph. Click on the Linear Fit button, click "ok" and a best-fit linear regression line will be shown for each run selected.
9. In your data table, record the value of the slope, m, for each of the three solutions. (The linear regression statistics are displayed in a floating box for each of the data sets.) The slope is equal to the rate of the reaction.
10. *Do this before moving on to the next experimental test tube – when you move on to the next experiment, your data will be lost!*
11. Collect data for test tubes #2, #3, #4 and #5: Repeat steps 2-11.
12. *Graph reaction rate vs. substrate concentration*

Which variable was measured in this first experiment? _____

Measure Effect of Temperature on Enzyme Activity

1. Place four test tubes in a rack and label them 1, 2, 3 and 4.
2. Using a clean pipette, add 10 ml of enzyme suspension to each test tube.
    a. Place test tube #1 in a 5°C ice bath
    b. Leave test tube #2 in the test tube rack at room temperature (~25°C)
    c. Place test tube #3 in a 34°C water bath

   d. Place test tube #4 in a 44°C water bath
3. Allow test tubes to incubate at each temperature for 5 minutes before proceeding with the next step of the experiment.
4. Add 2 ml of 3.0% H_2O_2 and 2 ml of water to your 250 ml Nalgene bottle.
5. Pour enzyme suspension from the test tube into your 250 ml Nalgene bottle and immediately connect the sensor and begin data collection
   a. *To connect the sensor*: place the O_2 Gas Sensor into the bottle as shown by your instructor, or in Figure 1. Gently push the sensor down into the bottle until it stops. The sensor is designed to seal the bottle without the need for unnecessary force.
6. As soon as the sensor is connected to the bottle, click Collect to begin data collection
   a. Do this as quickly as possible to collect as much data as you can
   b. Gently swirl the bottle during data collection
7. Allow data collection to continue for 2 minutes. To finish data collection, click the Stop button, which is the same as the Collect button.
8. When data collection has finished, remove the O_2 gas sensor from the 250 ml bottle.
9. Rinse the bottle with water and dry with a paper towel.
10. Using the mouse, select the initial linear region of your data on the graph. Click on the Linear Fit button, click "ok" and a best-fit linear regression line will be shown for each run selected.
11. In your data table, record the value of the slope, m, for each of the four trials. (The linear regression statistics are displayed in a floating box for each of the data sets.)
12. *Do this before moving on to the next experimental test tube – when you move on to the next experiment, your data will be lost!*
13. Collect data for test tubes 2-4: Repeat steps 3-12.
14. *Graph reaction rate vs. temperature*.

Measure Effect of pH on Enzyme Activity

1. Spend a few minutes designing an experiment to measure the effect of pH on enzyme activity.
2. Check with your experimental design with your instructor before beginning.
3. Carry out your experiment and record all your data
4. *Graph reaction rate vs. pH*.

Graph all of your data. Be sure to give each graph a **title** and label each **axis.**

In each case, which variable is the **Independent** (X-axis) variable? _____

In each case, which variable is the **Dependent** (Y-axis) variable?_____

Name _____

Enzyme Activities

Results and Data Analysis (Be sure to attach *all 3 graphs*)

Substrate (H_2O_2) Volume	**0 mL**	**1 mL**	**2 mL**	**3 mL**	**4 mL**
Slope (m)					

Temperature	**5°C**	**22°C**	**34°C**	**44°C**
Slope (m)				

pH	**pH _____**	**pH _____**	**pH _____**	**pH _____**
Slope (m)				

Questions

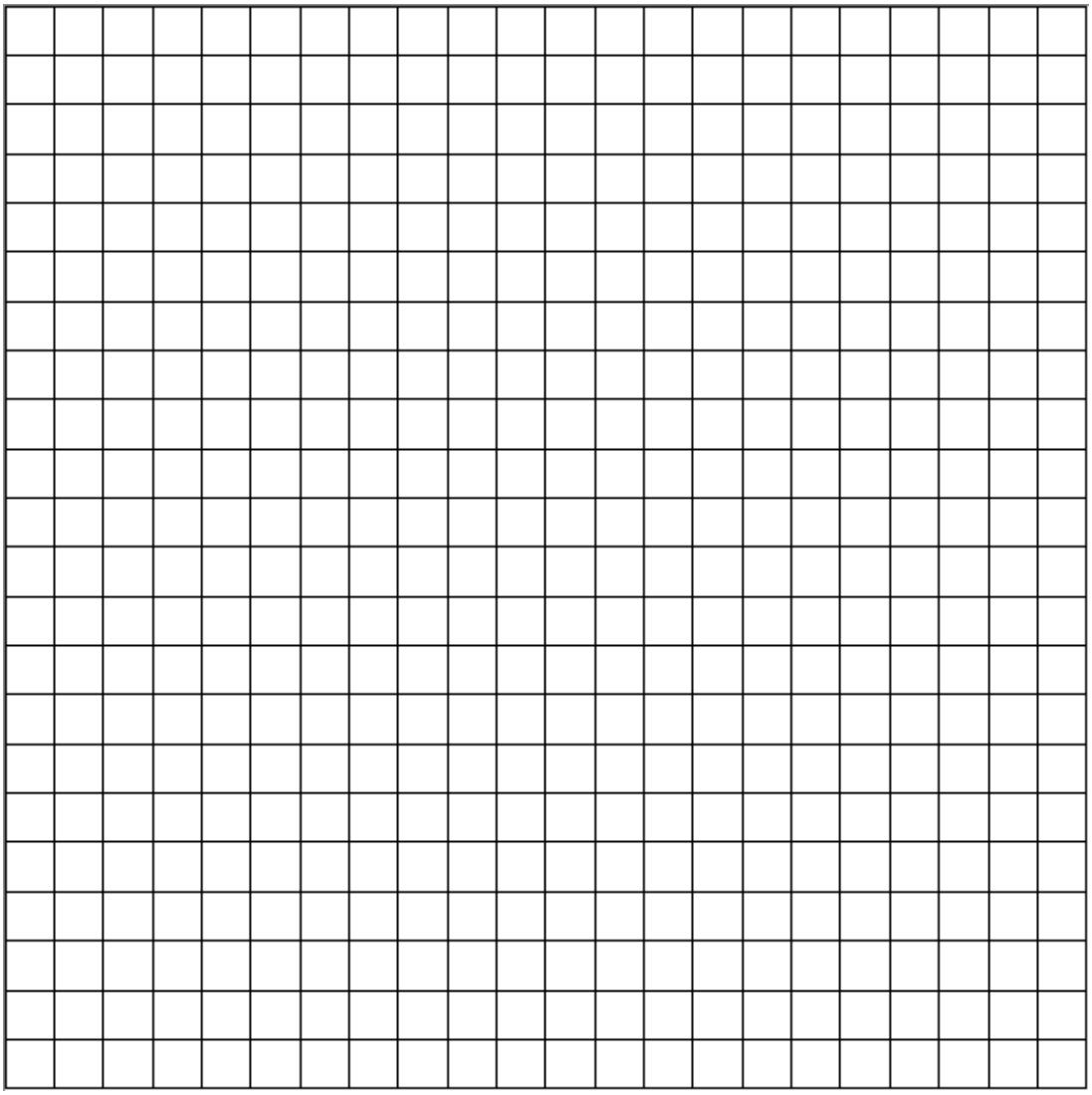
1. According to your results, what is the relationship between substrate concentration and enzyme activity? How might you explain this result?

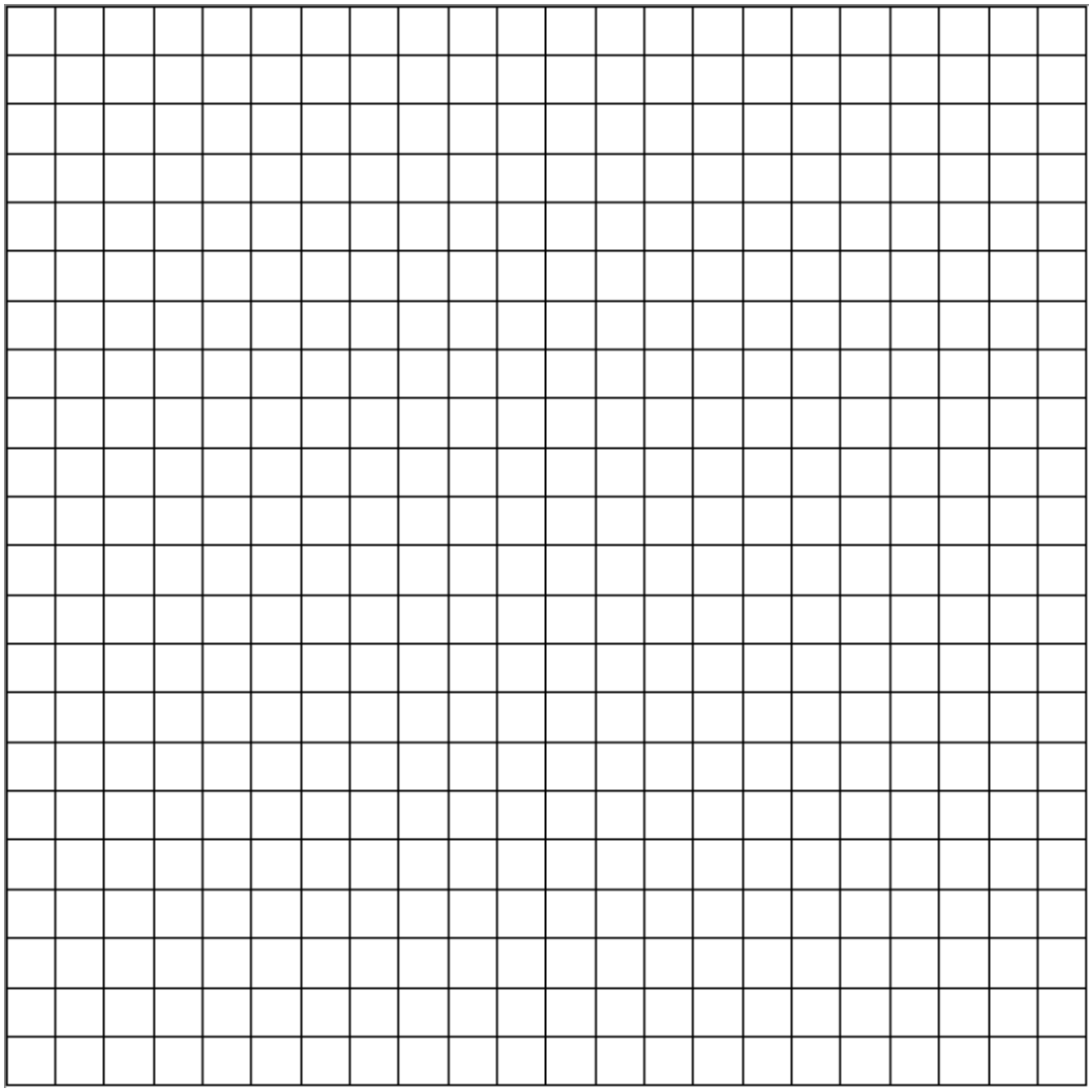
2. According to your results, what is the relationship between temperature and catalase enzyme activity? How might you explain this result?

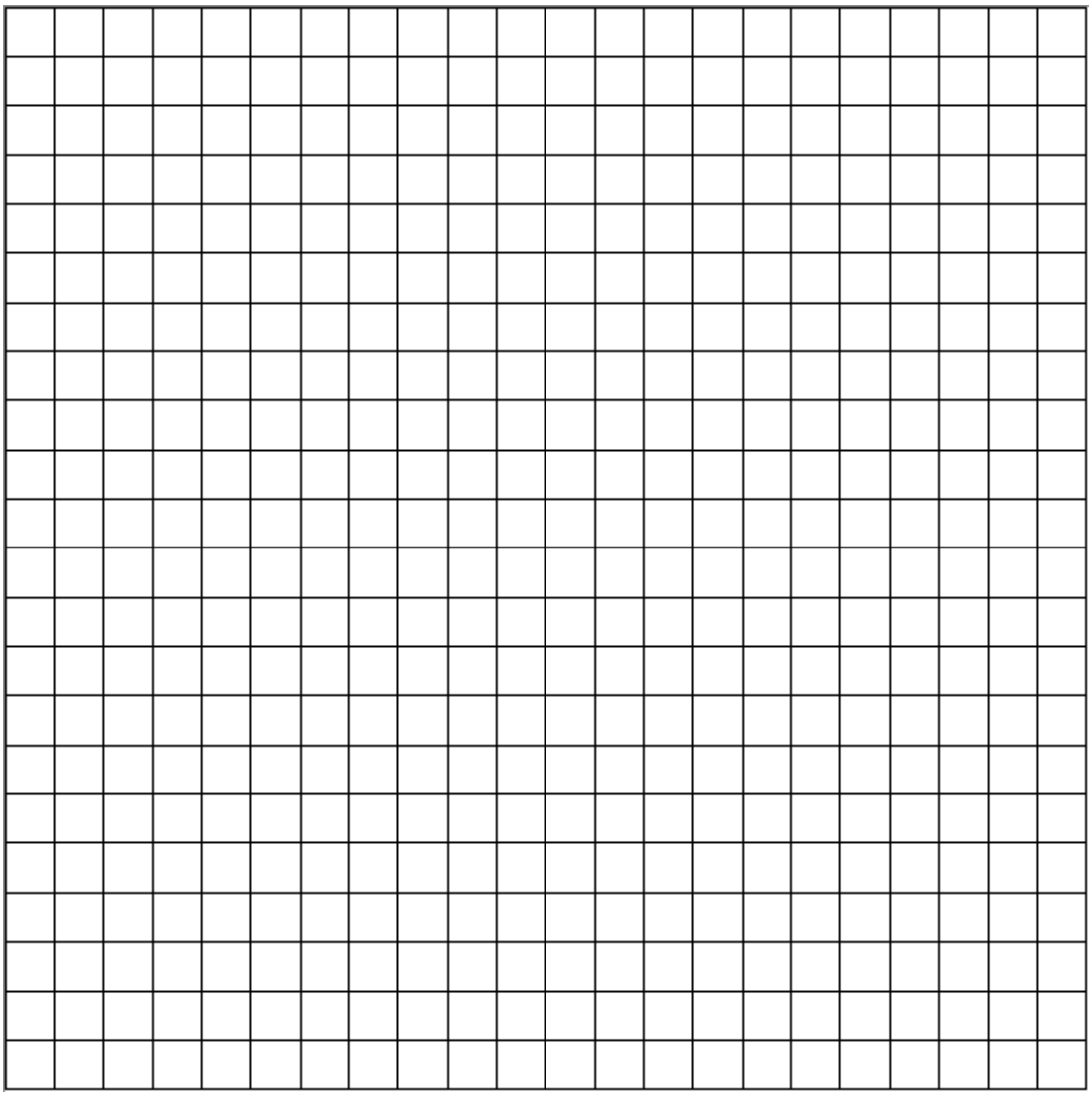
3. Briefly describe the procedure you used to test the effect of pH on enzyme activity. You may include a labeled diagram as part of your explanation.

4. According to your results, what is the relationship between pH and enzyme activity? How might you explain this result?

5. Chorionase is an enzyme that induces hatching of fish eggs. Studies have shown that this enzyme functions best at a pH of 6-7. How would acid rain affect populations of fish? In what part of the country is this problem most likely to occur?







Be sure to check your instructor's website for extra credit questions and announcements.