CLASSROOM COPY

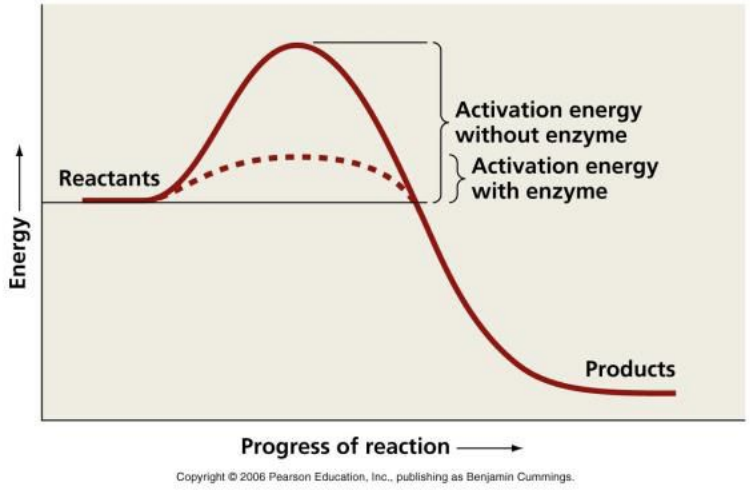
"Toothpickase" Activity

INTRODUCTION

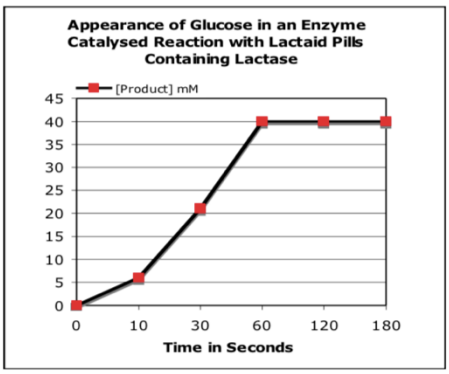
This is a "hands-on" lesson in enzyme action, demonstrating the natural increase in reaction rate, the leveling off of the reaction and the subsequent drop in products produced as the substrate is used up. You are to pretend that toothpicks are the substrate to be broken down and your hands are an enzyme, complete with an "active site" (between your fingers and thumb.) Notice that the enzyme (your hand) is much larger than the substrate (toothpicks.) As you will be performing the activity with your eyes closed, this simulates the random contact made between substrate and enzyme. The object of the activity is to break as many toothpicks in half as possible in two minutes to test the "enzyme".

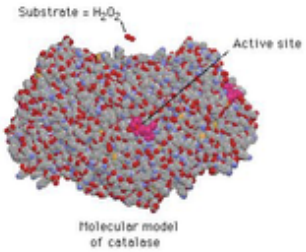
During the activity, you will also notice that the substrate will not break unless you find just the right spot (the bonding site) and that you will naturally find a maximum rate of reaction, the top speed at which your hands can find and break an enzyme. This speed may lower during the activity as your hands become tired, the pieces are all too small to break and the substrates get more and more scattered in the "solution" (your playing field.) Throughout the activity, notice that the enzyme (your hands) remains unchanged throughout the reaction.

Recall that **enzymes** are globular proteins which act as organic catalysts for biochemical reactions. Remember that a catalyst is a substance that speeds up a chemical reaction without being used up during the reaction. Catalysts work to speed up or slow down a chemical reaction by increasing or decreasing the activation energy of a chemical reaction. Activation energy is the energy needed for a chemical reaction to start. Enzymes can lower the amount of **activation energy** needed for a



biochemical reaction to start by placing physical stress on the bonds holding the substrate together. Enzymes are specific as to the substrate they break up. The structure of an enzyme includes an **active site**, which is where the substrate binds (usually through weak hydrogen bonding) when a substrate-enzyme complex is formed. The amino acids that make up the active site are configured in such a way that only certain substrates can fit into it. This is commonly referred to as the "lock and key" theory of enzyme binding. However, because some enzymes are quite flexible and can bind around their substrates, an alternative theory about enzyme binding called **induced-fit theory** states that the enzyme can wrap itself around the substrate for a more snug fit.





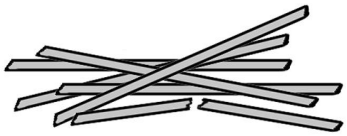
MATERIALS

Toothpicks (approx. 50 per group), Stopwatch, Calculator, Other supplies as needed

PRE-LAB QUESTIONS (answer before doing the lab)

1. How can the rate of the reaction be measured?
2. What factors can affect the reaction rate?
3. Would the rate be faster or slower if...
    a) the room was filled with toothpicks (saturated)?
    b) there were only a few toothpicks located on the other side of the room? (low substrate concentration)
    c) the toothpicks were mixed in with look-alikes (competitive inhibitors) d) two enzymes were working at the same time (enzyme concentration)

PROCEDURE



The Rules:

1. You must break each toothpick one at a time
2. You must break each toothpick with one hand ONLY. -Sit on your other hand
3. You must break each toothpick completely in half.
4. You cannot begin before your partner says "Go!"
5. You must stop precisely when your partner says "STOP!"
6. You must keep your eyes closed throughout the entire activity. Enzymes don't have eyes!☺

The Activity Part 1:

1. Place 50 toothpicks in a tray. Blindfold the toothpickase (if you don't trust them to keep their eyes closed).
2. The toothpickase will pick up one toothpick at a time, break it in half and place the halves back in the bowl. They will do this as fast as they can continuously for 180 seconds. Breaking toothpicks that have already been broken is prohibited.
3. As you break, one partner will count the number of toothpicks broken aloud.
4. Your other partner(s) will be the timer, the listener and the recorder. After 10 seconds has elasped on the stopwatch, record the number of toothpicks broken, keep timing and listening and record after 30 seconds, 60 seconds, 120 seconds, and 180 seconds.
5. Determine the rate of reaction (number of toothpicks broken down per second) for each time interval.
6. Plot the rate of reaction vs. time interval.
7. Compile a class average on Google Sheets.
8. For Part 2, each group should perform a variable as assigned by Mrs. Hale and share the data afterwords. See table below for how to perform variable.

Part 1: How fast toothpickase works

Time (seconds)	Number of toothpicks broken	Initial/Reaction Rate
0	0	----------
10		Initial Rate:
30		
60		
120		
180		

ANALYSIS

1. Calculate the initial rate of enzyme activity by dividing the number of toothpicks broken by the change in time (10 seconds). Record the initial rate in the chart above. Remember units!!

   Formula: $\frac{\Delta M}{\Delta t} = \frac{\Delta \text{ in amount}}{\Delta \text{ in time}} = \frac{\text{Change in amount}}{\text{Change in time}}$

2. Now, calculate the rate of enzyme activity for the other times (30, 60, 120, and 180). Remember, the rate is the # broken/change in time in seconds. Record this rate in the chart above. Remember units!!
3. What happens to the reaction rate as the supply of unbroken toothpicks runs out?
4. How does the "active site" of toothpickase fit the concept of induced fit? The induced fit concept says that there is a change in the shape of the active site of an enzyme so that it binds more snugly to the substrate, induced by the entry of the substrate.

Part 2:

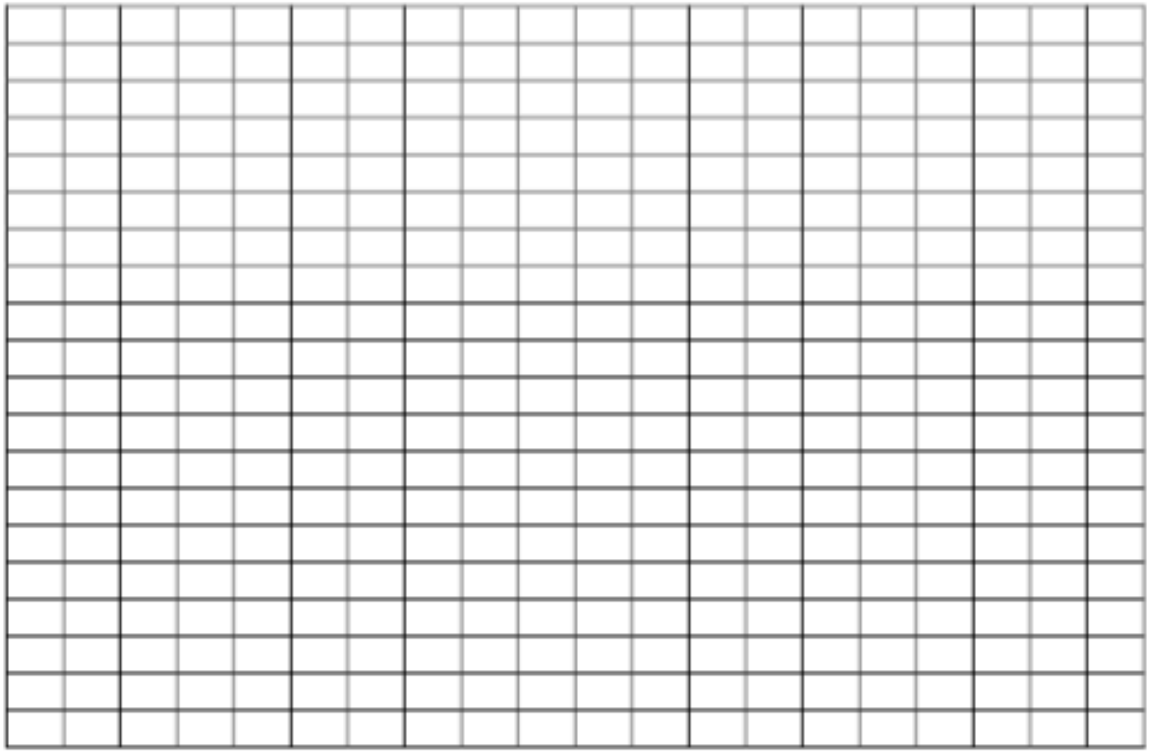
Time (sec)	Your own Data	[Substrate] Rate	[Enzyme] Rate	Decr T Rate	pH/salt Rate	Competitive Inhibitor RAte	Non competitive inhibitor Rate	Allosteric Regulator Rate
0								
10								
30								
60								
120								
180								

Part 2: Class rate and your assigned variable

Lab Simulation	Factors affecting enzyme function
Amount of Substrate	If more toothpicks are added, the enzyme's rate does not change however it takes longer for the entire reaction to come to completion. The rate of the enzyme unaffected but the rate of reaction is slower with more substrate.
Amount of enzyme	If two student enzymes are breaking 1000 toothpicks they should finish twice as fast as one student enzyme breaking 1000 toothpicks. The reaction will come to completion faster with more enzyme, however the enzyme's rate is not affected
pH	Changes in pH from optimal levels slow enzyme reaction. Student enzyme wears gloves or crosses fingers to simulate denatured protein
Temperature	Changes in temperature from optimal levels slow enzyme reaction. Student enzyme puts hands in cold water for 1 minute
Cofactors/coenzymes	These increase enzyme's ability to function. Student enzyme is handed 2 or 3 toothpicks at a time to break (otherwise without a coenzyme or cofactor the toothpickase enzyme can only break one at a time)
Competitive inhibitors	Slows reaction rate. Twisty ties are the same size and shape as the toothpicks, so therefore can fit into the active site of the enzyme and thus slow the rate of reaction by occupying the active sites of the enzymes.
Non-competitive inhibitors	These also slow the rate of reaction by binding to the enzyme, but NOT in the active site. The noncompetitive inhibitors cause the shape of the protein to change and thus the active site to be less functional or possibly not at all functional. Student enzymes are given two balls to put under their arms or between their ears and shoulders thus slightly changing the protein's shape (Allosteric!) and hindering its function.
Allosteric regulation (activators or inhibitors)	Another student will tap the enzyme on the shoulder, when he or she does that, the enzyme will cross his or her arms and thus be inactivated by a shape change. When tapped by another student on the head, the enzyme will resume functioning by changing its shape into the active form.

Line Graph (proper title, labels, and numbering):

Graph your data from Part 1 and connect the dots to make a line. Then, graph your data from Part 2 and connect the dots to make a line. Use different colors for each line and make a key to the graph.



Key:

Conclusion Questions (answer in complete sentences):

1. What is an enzyme? What is the enzyme in this activity?
2. What is a substrate? What is the substrate in this activity?
3. Predict what would happen if we used double the amount of toothpicks per person.
4. Predict what would happen if the toothpicks were more spread out over a big table. Scattered randomly across the room.
5. Predict what would happen if two pairs of hands acted as enzymes (double the amount).
6. Was this reaction anabolic or catabolic? Was it exergonic or endergonic? positive or – ΔG?