Laboratory 3
What Makes Enzymes Work?

One hallmark of effective scientific inquiry is focus on solving a problem, rather than on using a particular method to study a problem (1). You should already have read Strong Inference by John R. Platt. But if not, please be sure to read this important paper before you come to the laboratory this week. You can download it at www.bio.miami.edu/dana/151/gofigure/Strong_Inference.pdf.

In most biological disciplines, hypotheses are constructed to provide alternative possible explanations for an observed phenomenon. This helps to prevent bias on the part of the investigators, and helps to keep things objective.

Today you will consider biological catalysts, known to drive specific chemical reactions. You will try to re-imagine a time in our history when little was known about how enzymes work. With your understanding of protein structure and enzyme function from lectures, text readings and primary literature you have already found and studied, you should be ready to embark on this endeavor and use strong inference to pose competing hypotheses about why enzymes do what they do, and then design experiments to test those hypotheses.

I. Introduction to Biological Catalysts: Enzymes

A catalyst is a substance that affects the rate of a chemical reaction without being consumed or permanently changed in the reaction. Catalysts in biological systems belong to a special class of proteins called enzymes. The substance upon which an enzyme operates is known as its substrate.

A. Protein Structure

Like other proteins, enzymes have a

- primary structure - the order of amino acids in their polypeptide chains)
- secondary structure - coiling or pleating of the polypeptide chain)
- tertiary structure – three-dimensional shape formed by spatial relationships of the secondary components
- quaternary structure – shape formed by the combination of multiple protein subunits joined to form a single, functional enzyme.

Secondary protein structures (helices, sheets, ribbons, etc.) often comprise distinct domains of a protein, each of which has a specific function. The combination of and interactions between its domains determine how a particular enzyme functions.

B. Forces Determining Protein Structure

In addition to the covalent peptide bonds that bind individual amino acids together in a protein’s primary structure, several non-covalent forces may contribute to the form (and hence, function), of an enzyme. Consider these when constructing your hypotheses.

1. Hydrogen bonds

The functional groups of amino acids may be either proton donors or acceptors, and their attraction to one another can facilitate protein coiling, folding, and pleating. In addition, the medium in which the protein exists can contain proton donors and acceptors, and can affect the shape of the active enzyme, as well as maintain its affinity for it’s the matrix in which it is embedded.
2. Hydrophobic forces
Protein functional groups may be polar (hydrophilic) or non-polar (hydrophobic). Since proteins are most often found in an aqueous matrix, non-polar regions of the molecule are repelled by the environment, and may fold inwards, leaving polar regions on the surface of the molecule.

3. Electrostatic forces
Protein functional groups may form a dipole (i.e., having equal and opposite charges at each end), or ionic (having either an overall negative or positive charge). Attractions between opposite charges of dipoles and charged regions of functional groups can have a strong effect on protein configuration. Charged regions of amino acid functional groups interacting with charged regions of the protein’s environment can also affect protein form and function.

4. van der Waals forces
These weak repulsive or attractive forces between the opposite ends of dipoles contribute to protein folding not because of their strength, but because of their numbers. Protein dipole interactions are the main source of van der Waals forces in these large molecules.

Because of their structure and the forces governing their shape, enzyme conformation can readily change. This malleability is a critical functional property of an enzyme, of course. But this also means that changes in an enzyme’s environment can alter its efficacy and efficiency in driving its particular reaction.

C. Enzyme Behavior: The Michaelis-Menten Hypothesis
In 1913, Leonor Michaelis and Maud Menten published their work on what was to become one of the most important breakthroughs in biochemistry: a mechanism for the catalysis of chemical reactions in biological systems. Their publication provided a general explanation of the general mechanism of enzyme-catalyzed reactions, as well as the relationship between enzyme/substrate concentrations and speed of reaction.

An enzyme is a complex protein with tertiary and/or quaternary structure forming one or more three-dimensional active sites. The enzyme works at its maximum speed when all active sites are occupied by substrate molecules, meaning when substrate concentration is very high. An enzyme working at top speed in a high-enzyme solution is said to be saturated.

Enzyme reaction rate can be expressed with the Michaelis-Menten equation:

$$v_0 = \frac{v_{\text{max}}[S]}{K_M + [S]}$$

In which:
- $v_0$ = rate of substrate conversion at a given substrate concentration
- $v_{\text{max}}$ = maximum rate of substrate conversion (at saturation)
- $[S]$ = substrate concentration
- $K_M$ = Michaelis constant

This can be represented graphically as shown in Figure 1.
The Michaelis constant is equal to the substrate concentration at which the reaction rate is half of $V_{\text{max}}$. Thus, the $K_M$ constant is an indirect measure of enzyme/substrate affinity (i.e., just how attractive substrate and enzyme are to each other). The lower the value of $K_M$ for a particular enzyme/substrate reaction, the higher the affinity between that particular enzyme and substrate. (The substrate concentration doesn’t have to be very high to saturate the enzyme if the two molecules are very friendly with each other). The higher the $K_M$ value, the lower the affinity of enzyme and substrate.

Very high concentrations of substrate are usually needed to reach $V_{\text{max}}$. But one need not reach $V_{\text{max}}$ in order to study differences in reaction rate when environmental conditions are varied. Sometimes it’s better to use a lower concentration of substrate so that the reaction proceeds at a comfortably measurable rate, and the substrate is not all explosively consumed in a few seconds! Consider this when you decide what standardized substrate and enzyme concentrations you will use for your experimental trials. (We have conveniently provided a usable standard concentration for you. But if your team feels it is important to change this, we want you to at least know what the consequences might be!)

D. Catalase and Hydrogen Peroxide

Peroxidases are a class of enzymes that catalyze the breakdown of peroxide compounds. One of the most ubiquitous and important peroxidases is catalase. In living plants and animals, its function is to catalyze the breakdown of hydrogen peroxide (a toxic byproduct of many metabolic reactions) into harmless water and oxygen via the following reaction.

\[
\text{catalase} \quad 2\text{H}_2\text{O}_2 \quad \xrightarrow{\text{catalase}} \quad 2\text{H}_2\text{O} + \text{O}_2
\]
The physical and chemical properties of enzymatic proteins are affected by the physical conditions under which they must operate. Temperature, pH, relative concentrations of enzyme and substrate and other factors can affect the physical structure of the protein, and hence, the rate and/or efficacy of enzymatic reaction. Therefore, measuring the change in the rate of a catalytic reaction when environmental variables are changed in a controlled fashion can be used to determine whether a particular aspect of enzyme structure is crucial to its function.

II. Experimental Protocol: Reagents and Equipment

One simple way to determine how well an enzyme is working is to measure the rate of its reaction. Note the reactants and products of the hydrolysis of hydrogen peroxide by catalase. To determine reaction rate, one could measure the amount of H$_2$O$_2$ decomposed or, equally well, the amount of H$_2$O or O$_2$ produced, per unit time (i.e., the rate of this decomposition). Since O$_2$ is a gas, we can conveniently calculate the rate of its production with an oxygen gas sensor. And we just happen to have some for you to use. The following section is a brief overview of the chemical reagents and equipment you will have available.

A. Chemical Reagents

You will be provided with reactant (hydrogen peroxide in aqueous buffer) and enzyme (catalase in living yeast cells).

1. Yeast catalase

Catalase is an antioxidant enzyme made up of four interlocked polypeptide subunits linked to an iron-containing heme complex. Yeast catalase has a total molecular weight of 248,000 g/mole (Seah and Kaplan, 1972). Under optimum conditions, this highly efficient enzyme catalyzes the breakdown of millions of hydrogen peroxide molecules per second (Goodsell 2004, UniProt 2008). Yeast are living, metabolically active fungi. Even dry and dormant, yeast cells are protected by antioxidants, including catalase.

The stock yeast suspension we have provided for you contains 70g of yeast per 1L of stock pH 7.0 sodium phosphate buffer solution.

2. Hydrogen peroxide

Hydrogen peroxide (H$_2$O$_2$) is a powerful oxidizing agent produced as a toxic byproduct of aerobic metabolism. Without rapid enzymatic catalysis, H$_2$O$_2$ would quickly destroy essential biomolecules in a living cell, resulting in cell damage and death.

You will have two different solutions of H$_2$O$_2$ available for your experiments. If you are planning to manipulate any factor other than the pH of your system, then you will use the stock solution of H$_2$O$_2$ we have prepared for you. It consists of 33ml of 30-volume (9.1%) H$_2$O$_2$ added to 1L of pH 7.0 sodium phosphate buffer. You will need to calculate the percentage H$_2$O$_2$ of this solution to present in your presentation.

If you plan to manipulate the pH of your system, then you will use commercial 30-volume H$_2$O$_2$ to mix your own peroxide solutions in buffers of varying pH. Recipes and instructions for different types of buffer and peroxide solutions can be found in the appendix at the end of this lab chapter.

3. Phosphate buffer

Buffers maintain constant pH in solution. Buffer systems are widespread in living cells and organisms, where they help maintain homeostasis. To mimic the conditions of enzymes.
a living cell as closely as possible, buffers are often used in experiments examining biological functions.

Whether you will choose to run your experiments in a neutral (pH = 7.0), acidic (pH < 7.0) or basic (pH > 7.0) solution, you should mix your reagents in a buffer solution so that the products of your reaction will not change the pH of the reaction’s environment (which could certainly affect your results!).

There are many different kinds of buffers, each with its own components. In this lab, you’ll learn how to make and use your own buffer solutions, which are useful for many procedures in cellular and molecular biology. Recipes and instructions for mixing buffers can be found at the end of this lab chapter.

B. Experimental Workstation and Equipment

Before you begin, check your laboratory workstation for cleanliness, and to be sure all the materials listed below are present and in good working order. If something is not right, check with your TA for replacements.

1. Your Lab Workstation

Before you begin your data collection, check your lab workstation to be sure all the following equipment is present. Check it for cleanliness. Consider: Do you trust the students who used this workstation before you to have left it perfect for the next team? Neither do I. So before using any of the equipment, wash every piece well, and rinse with distilled water. You can then use with confidence that traces of reagents left by others will not affect your results. This is a good rule to use for every lab.

   1. Check to be sure that you have on the tray at your workstation:
      a. one 250mL beaker labeled “H₂O₂”
      b. one 60cc syringe labeled “H₂O₂” (NOTE: 1cc = 1mL)
      c. one 150ml beaker labeled “Yeast”
      d. one 10mL syringe labeled “Yeast”
      e. one 250mL beaker (DRY) labeled “Probe”
      d. one 25mL or 50mL graduated cylinder
      e. one spring-clamp test tube holder
      f. one fingerbowl labeled “water bath”
      f. one thermometer

   2. Beside the tray, you should have (1) one Vernier O₂ sensor probe in its box (stored UPRIGHT) and (2) one plastic Vernier respiration chamber (Figure 3-1) closed with a plastic cap. To connect the probe to your laptop computer, you will need the Vernier adaptor cable. This can be found either in the box with your probe, or on the TA desk at the front of the lab.

   3. Before you leave lab today after finishing your experiments, be sure all the items listed above are neatly replaced on your tray and that all spills are wiped up. Teams who leave lab workstations dirty will be docked points.

2. Your Oxygen Sensor Equipment

You will use an electrochemical O₂ sensor (Vernier Software, www.vernier.com) to detect one of the products of the H₂O₂ hydrolysis reaction, O₂ gas. Before designing experiments, you must first become familiar with using the O₂ sensor and data logger, and then with the basic procedure for measuring the rate of decomposition of hydrogen.
peroxide by yeast catalase. **You will work in teams of four.** Each team member should participate in all aspects of all activities, and should be able to explain the rationale for each step of the methods.

You may wish to assign other specific duties to each team member (such as measuring substrate or enzyme, rinsing glassware, ordering pizza, etc.), but you are wise to have the same team member perform the same duties each time. Why do you think this is important?

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### 3. Setting up the O\textsubscript{2} sensor and data logger software

One team member with a laptop computer will kindly volunteer it for data collection.

1. Holding it only by the edges, place the Logger Lite CD in the CD drive and follow the installation directions that appear.
2. *Only after you have installed the software*, connect the O\textsubscript{2} gas sensor and interface to the USB port on your computer. *Keep the O\textsubscript{2} sensor upright at all times! Treat it with extreme care or you may damage it and will be penalized!*
3. Start the Logger Lite 1.4 software by clicking on the icon on the desktop.
4. The software will detect the sensor and load a data table and graph. You are now ready to collect data!

### 4. Collecting data with the O\textsubscript{2} sensor

As an exercise to practice using the O\textsubscript{2} sensor, use the following instructions to compare the O\textsubscript{2} concentration of your exhaled breath with that of the atmosphere.

1. **Carefully and gently**, place the O\textsubscript{2} gas sensor into the plastic reaction chamber as shown in Figure 3-1. Gently push the sensor down until it stops. The sensor is designed to seal the chamber without unnecessary force.
2. Click “**Collect**” on the toolbar at the top of the Logger Lite window. The sensor will start measuring, 1x per second, the O\textsubscript{2} concentration (as %O\textsubscript{2}) of the air in the chamber. Note that the current %O\textsubscript{2} is displayed in the lower left corner of the window, while the readings over time are displayed on the data table and graph.
3. When the %O\textsubscript{2} value has stabilized, click “**Stop**” on the toolbar.
4. Record the %O\textsubscript{2} value.
5. Click “**Store**” on the toolbar to store this data run, and ready the software for the next.
6. Gently remove the O\textsubscript{2} sensor and place it upright in its dry 250mL holding beaker.
7. Breathe several times into the reaction chamber. Try to replace the air in the chamber with your exhaled breath.
8. Quickly, but still carefully and gently, place the O\textsubscript{2} sensor into the chamber as in step #1 above.
9. Collect data as in steps 2-6 above.
10. Click **“Save”** to save the results of this exercise.

enzymes-6
11. Gently remove the O$_2$ sensor from the chamber and place it upright in its beaker.

**Results:**
What is the % concentration of O$_2$ in the atmosphere? _________________
What is the % concentration of O$_2$ in your exhaled breath? _________________
How long did it take the O$_2$ sensor to detect fully the %O$_2$? _________________

**OPTIONAL: Customizing data collection and graphical display**
To make your subsequent work easier, familiarize yourself with the Logger Lite software.

1. Click “Open” on the toolbar at the top of the window.
2. Click “Tutorials” in the “Experiments” folder that opens.
3. Click on “Tutorial 3 – Customizing.” The first page will tell you that a Go Temp probe should be attached. Ignore this; you will still be able to use the information in the tutorial. Click on “Pages” on the toolbar to go to other pages.
4. Using the information in this tutorial, be able to do the following on your saved graph (your instructor may check to be sure that you know how to do this):
   · Change the rate and duration of data collection
   · Give the graph a title
   · Un-connect the points of the graph
5. Now open “Tutorial 4 – Working with graphs.” Be able to:
   · Change the scale of the x and y axes
   · Stretch the x and y axes
   · Autoscale the axes

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**Figure 2. Vernier O$_2$ gas sensor probe and reaction chamber ready to collect data.**

**5. Variability in Enzyme Reaction Rate**
You are now ready to study the rate of reaction of catalase in living yeast cells. First, you will do three preliminary trials under the same conditions, both to gain experience with the method and to study experimental variability.

1. Click “New” on the top toolbar to open a new data table and graph.
2. Take the small beaker labeled “yeast” (from your workstation) to the metal tray in the middle of your lab table where the stock solutions are located. Mix the yeast suspension well with a glass stirring rod, then decant about 40ml into the beaker. Bring it back to your workstation.
3. Take the small beaker labeled “H₂O₂” (from your workstation) to the stock solution tray. Decant about 80ml of stock H₂O₂ in pH 7 buffer (9.1% solution) into the beaker and bring it to your workstation.

4. Using the 10cc syringe labeled “Yeast” on your tray, draw up 10cc of yeast suspension. Carefully squirt the yeast suspension into the plastic Vernier chamber.

5. Draw up 20cc of H₂O₂ solution into the labeled H₂O₂ syringe, but DO NOT ADD IT TO THE REACTION CHAMBER UNTIL YOUR TEAM IS READY TO BEGIN TAKING MEASUREMENTS.

   **NOTE:** H₂O₂ is somewhat caustic, so handle with care. Avoid contact with skin and eyes. Be careful to avoid spills and splashing.

6. DO NOT hold the chamber by the body. Use the spring-handle test tube holder clamped around the neck of the chamber as a handle. (Why?)

7. When your team is ready to begin collecting data,
   a. Gently squirt your 20cc of H₂O₂ into the reaction chamber
   b. Quickly and gently stopper the chamber with the probe assembly
   c. Hold the neck of the chamber and **slowly** swirl the contents.
   d. As quickly as possible after you have started swirling, click “**Collect**” to begin collecting data.
   e. Continue swirling the chamber as you record the data.
   f. **DO NOT SPLASH THE PROBE.** *If you start to get erratic readings, it is very likely that the probe has become humid, and needs time to dry out.* There is a small fan available in the lab (ask your TA) to help speed this process. But always handle the probe gently, keep it dry, and keep it upright.

8. When %O₂ readings start to plateau, click “**Stop.**” Otherwise, data collection will continue for a total of 300 seconds, then stop automatically.

9. Click “**Store**” to store the data from this run.

10. Gently remove the O₂ sensor and place it upright in its DRY beaker.

11. Discard the contents of the chamber in the front sink, and rinse down the drain with plenty of tapwater until the sink is clear.

12. Rinse the chamber well with tap water, then with distilled water. Shake out excess water and dry the neck of the chamber.

13. Repeat steps 6-12 above four more times.

14. Find the rate of reaction for each trial using the Logger Lite software as explained below. Express the rate of reaction as %O₂/g/s, and volume of the reaction chamber as mL/O₂/g/s.

15. Find the mean rate of reaction and the standard deviation for the 5 trials.
6. Calculating rate of reaction

Use the Vernier software to calculate and compare the rates of reaction for the five trials you just ran. Examine the curves on your graph. Each curve shows the %O₂ as it changes over time (the time course of the reaction). At first, there may be a lag period, as substrate molecules enter the cell and enzyme molecules in the cells gradually come into contact with the substrate. Then there is an initial, steadily rising linear portion of the curve that represents the maximum rate of reaction. As substrate is used up, the increase in %O₂ will slow over time, and the curve will gradually flatten, forming an S-shaped curve. This means that all the H₂O₂ has been broken down, and the reaction has stopped. No more oxygen is being generated.

Only the early, linear portion of the curve reflects the maximum reaction rate; this is the region of the curve you should use to compare your different reaction rates. Follow the steps below for each curve.

1. Highlight the maximum rate of reaction portion of the curve using the mouse.

2. Click on “Analyze” on the top menu, then “Linear Fit.” In the dialog box that appears, be sure to check the curve you've just highlighted. A best fit linear regression line will now appear for your highlighted points, along with a floating box containing the equation of the line. The correlation statistic (r) shows how well your actual data points fit the line, with a correlation of 1.0 showing a perfect fit. Using the mouse, you can grab and move the brackets to change the highlighted points, to see if there is a better fit—the line, correlation and equation will automatically update.

3. Examine the equation of the best fit line you chose. The slope (m) shows the change in %O₂ over the change in time, which is the rate of reaction. Record this value as the rate (%O₂/sec) in the appropriate cell of Table 1.

4. The volume of the reaction chamber, with the O₂ gas sensor inserted, is approximately 280 mL. Your solutions take up about 30mL of this space. Use this information to calculate the mL O₂/sec produced as a percentage of the total working volume.

5. Now recall that your stock yeast suspension contains 70g/L of yeast. From this information, you should be able to calculate the mL O₂/g/sec generated by the yeast. Record this adjusted rate in Table 1.

<table>
<thead>
<tr>
<th>tube #</th>
<th>Volume of yeast suspension</th>
<th>mL 6% H₂O₂</th>
<th>rate (%O₂/sec)</th>
<th>adjusted rate (mL O₂/g/sec)</th>
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</table>

mean
standard deviation
6. Cut and paste your data table and graph into a Word, Excel or similar document of your choice. **Be sure each team member gets a copy.**

7. **Experimental Error vs. Human Error**
   Your team used the same quantities of reagents in each trial, and (presumably) used the same temperature and pH—but did you get exactly the same reaction rate each time? What might account for slightly different results among trials?
   Slight variation in results in carefully run trials is known as **experimental variability** or **experimental error**. Note that this “natural” variability is NOT the same as variability caused by actual mistakes (**human error**) made during the experiment. If you made accidental mistakes that could affect your results, you should re-do the experiment.
   DO NOT include human error in this or any future discussions of experimental variability. **Experimental error ≠ mistakes!** When contemplating your results, your fellow scientists will assume you have done your experiments as carefully as possible, and have minimized inaccuracies due to human error.

8. **Translating These Techniques to Your Own Experiment**
   In the next section, your team will design an experiment to exclude one of your hypotheses about enzyme function. To collect your data, use the procedures outlined above for all of your experimental runs, manipulating only one variable.
   Until you understand how to perform and use the equipment and software, DO NOT CONTINUE TO THE REST OF THE LAB EXERCISE. You will be able to analyze the data from your own original experiment only if you understand (1) how to calculate the rate of any given trial, (2) how to calculate the mean rate of multiple trial runs under the same environmental conditions, and (3) how to meaningfully compare two or more means calculated for reaction rate under different environmental conditions.

III. **Experimental Design: The Fun Part!**
   Now that you are intimately familiar with the use of the oxygen sensors and software, you can use the available chemical reagents as models to try to discover what it is about enzymes that make them able to drive their particular reactions.

A. **Identifying the Problem and Formulating Hypotheses**
   What is the physical structure of a typical enzyme? Consider primary, secondary, tertiary, and quaternary structure.
   Next consider: Why do enzymes work? What is it about their physical structure that allows them to drive the chemical reaction of their substrate(s)? Is it the sequence of amino acids alone? Or is it something to do with the three-dimensional structure? How could you find out “from scratch”, knowing what you know about proteins and how they are constructed?
   Reaction rate depends on many different factors, including concentration of enzyme, concentration of substrate, and the pH, osmolarity, and temperature of the reaction environment. The catalase you used for your preliminary trials was inside yeast cells. Consider the natural environment of yeast, which is ubiquitous. Cultivated, “domestic” strains of yeast have been artificially selected by humans to function well under certain conditions (e.g., those suitable for making beer, wine, or other products). Think about what those conditions might be. Also think about how—at the molecular level—different enzyme or substrate concentrations, enzyme inhibitors, pH, or temperature might affect the function of catalase. This will help you interpret your results with more precision.
In the space below, give a brief introduction to the problem you are addressing. Give as much background as possible, considering what you know about enzymes and why they work.

Based on this introduction, create at least two, mutually exclusive hypotheses stating what it is about the enzyme's structure that drives the reaction. Focus on the problem ("What is it about the enzyme that allows it to act upon its substrate?"), rather than the experimental procedures ("What will happen if we subject the enzyme to condition X?").

Hypothesis 1: 

Hypothesis 2: 

What experiment(s) could you perform to determine, by process of elimination (falsification), which of these hypotheses is correct? Consider all possibilities, and, as a team, decide what you will manipulate in your experimental set up to test your hypotheses. List all possible outcomes of your experiments. Make predictions about what you expect to observe if Hypothesis 1 is correct. Do the same for Hypothesis 2.

If Hypothesis 1 is true, we predict ____________________________________________

(HINT: Create an if/then statement such as “If Hypothesis 1 is true, then if we do XXX, then we should observe XXX. Do the same for Hypothesis 2, and so on.)

If Hypothesis 2 is true, we predict ____________________________________________

Before you are able to actually devise the methods to use to test these hypotheses, you will need to familiarize yourself with the experimental apparatus and reagents we have provided. Read the next section before you proceed to design your experiment.

**B. Methods**

Once you have constructed your hypotheses, you must determine what you will measure in order to test them. For example, if you have predicted that a particular enzymes-11
property of an enzyme’s structure is responsible for its activity, you may wish to try and chemically disrupt that particular property to see if this affects its rate of reaction. In this case, we already have determined that your parameter will be rate of reaction. Remember that you must hold all experimental conditions as constant as possible except for the one thing designed to test your hypotheses. In the spaces provided below, describe the experimental protocols you will use to test your hypotheses. How many different manipulations will you perform, and what will they be? How many replications of each?

Also remember that you are using living organisms, not a stock solution (of catalase) of known molarity. How might this introduce variability in your trials?

The materials available to your team include:
• Catalase in living yeast
• 9.1% hydrogen peroxide in aqueous solution
• Phosphate buffers of varying pH (You will mix these yourself, if you need them.)
• Thermometers, ice and hotplates.
• One or more potential inhibitors of catalase, such as salicylic acid, acetylsalicylic acid (aspirin), succinic acid, or ascorbic acid (vitamin C)

NOTE: If your team uses any of these inhibitors, you must know the details of HOW and WHY they inhibit catalase on a molecular level, or they will not help you choose between your competing hypotheses. Be sure to do your background research thoroughly!

1. List the materials your team will use

2. Briefly describe your experimental set-up and procedure. (Remember: you need not give a detailed explanation of equipment here, unless it is unique to your experiment and really requires an explanation for the educated reader to understand what you will be doing. Simply refer to the lab manual as a literature cited for equipment specifics.)

3. If you are testing several different values of a particular environmental variable (for example, several different temperatures or pH values) in your experimental vessel, do you think it is sufficient to run only one experiment at each value of that variable? Or should you run multiple trials at each value? Explain your answer: ___
3. How many times will you replicate your experiment at each environmental value?

If you plan to run multiple trials at each value of your variable—a wise choice—then you will need to calculate the mean rate of reaction of the runs. (For example, if you ran your experiment five times at five different pH levels, then you must calculate the mean reaction rate for each pH from your five runs at that pH. If you did five runs at pH 4.5, then average the rate of those five runs and report that mean as your rate of reaction at pH 4.5.). An explanation of how to do this can be found below.

C. Parameters and Data Analysis
What type of data are you collecting? Is it attribute, discrete numerical, or continuous numerical data, as described in Appendix 2.
1. What parameter will you measure?

2. How will you analyze your data? What statistical test/analysis will you perform?

D. Predictions and Interpretations
In the spaces below, list all possible outcomes of your experimental trials. (As before, we have given you more spaces than you might need for this.) Each outcome should be accompanied by an explanation of how it supports or refutes each hypothesis and its companion predictions. Remember that the result is not the same as the interpretation. The outcome is the summary of the results themselves, whereas the interpretation is the logical, reasoned explanation for the observed results. The explanation of your outcome can serve as the basis for the next level of posing hypotheses, so be prepared to go to that next level.

Outcome 1:

Explanation:

Outcome 2:

Explanation:
Outcome 3:

Explanation:

Outcome 4:

Explanation:

IV. Conducting Your Experiment

Once you have designed your experiment and are confident that it has a good chance of yielding meaningful results, collect the materials necessary from the materials supplied in lab and set up your trials.

Since you will be recording data on a laptop computer handled by only one team member, remember that it is vital for every team member to get a copy of the data before she or he leaves lab today. You and your teammates should communicate and meet between now and the next lab period to put together a PowerPoint presentation, as described in the “Instructions for Student Presentations” link in the course syllabus at www.bio.miami.edu/dana/151

V. Assistance for the Chemistry Challenged

The following information is provided to teach you how to mix chemical solutions and calculate concentrations and molarities. These should be included in the Methods section of your report in appropriate fashion. Be sure to explain why you are manipulating the particular variable you have chosen, in terms of why the results should be consistent with one or the other of your competing hypotheses.

The acidity or alkalinity of an enzyme’s environment can strongly influence its activity. You should be able to figure out why this is the case (on a molecular level) by studying the factors that interact to help an enzyme maintain its tertiary configuration. Using buffer solutions of known, different pH can help you determine whether your predictions about enzyme structure and activity are correct.

A buffer solution resists changes in pH as a chemical reaction takes place in the solution. Using a buffer as a matrix for your chemical reaction can help prevent the confounding effect that changing pH might have on your reaction. Basic instructions for mixing a buffer appear below, and recipes for specific types of buffer can be found after the instructions.

I. Guidelines for Good Laboratory Technique

RULE #1: ALWAYS LABEL EVERYTHING. You will be using glassware and various types of equipment as you mix buffers. To keep track of what’s what, label everything until you’re done with it, then properly discard leftovers, remove labels, rinse, and replace in the proper location.
RULE #2: DON’T ASSUME IT’S CLEAN. Think about it. Do you really trust that the students who did the lab before you had your experiment in mind when they were leaving the lab? If you’re not sure a piece of glassware is clean, then wash, rinse with distilled water and dry. You can then use it with confidence.

RULE #3. USE THE RIGHT TOOLS. Do not use beakers for measuring reagents. They are not accurate. To measure precise amounts of reagents, always use a graduated cylinder or volumetric flask. For very small liquid volumes, you may use a graduated syringe.

RULE #4. DON’T CONTAMINATE! Never use the same glassware, spatulas, weighing dishes, etc. for more than one type of chemical reagent. Use the same LABELED glassware and other equipment for a particular reagent, and avoid contamination. If you’re not sure, then wash, rinse with distilled water, and then use with confidence.

RULE #5. CLEAN UP AFTER YOURSELF. Wash all glassware and other dirty equipment after use with tapwater, then rinse well with distilled water before setting to dry. NEVER leave dirty glassware or other materials in the sink or on lab benches and then leave the lab, EVER. YOU AND ALL YOUR TEAMMATES WILL BE Docked points for leaving a dirty workstation.

II. Titration: General Instructions.

Titration is defined as the determination of the concentration of an unknown solution with respect to pure water (pH 7). To make a buffer, we slowly add an acid component (analyte) to a known volume of base component (titrant) of known concentration until the desired pH is attained.

1. Mix the acid component and base component in separate containers, as described in the “recipes” section below. The nature of the titrant and analyte will depend on what type of buffer you are making.

2. Use glassware of appropriate/adequate size. We have provided a variety.

Once your acid and base components are ready...

3. Add the base portion of your solution to glassware of appropriate size.
4. Place a magnetic stir bar in the vessel, and turn on the stirrer until a funnel is visible in the solution.
5. Rinse pH meter electrodes with distilled water, and calibrate the pH meter using the instructions posted next to the machine.
6. When the meter is calibrated, place the electrode into your base solution.
7. While monitoring pH, slowly add acid component until the solution reaches the desired pH.
8. Titrate slowly! If you overshoot and have to back titrate, the salt concentration of the buffer could be altered more than necessary.
9. When finished, remove the magnetic stir bar with the magnetic retriever stick. (You’ll have to take the glassware off the stirring plate first.)
10. Wash and rinse all glassware and materials well with tap water, and then with distilled water before setting to dry.
III. Buffer Recipes

There are dozens of different types of buffer recipes, many of which are useful for biological applications. Different buffers can be titrated to a range of pH levels, with some having a narrow range, and others a very broad range. Here, we provide recipes for three different buffers useful in biological systems: sodium phosphate buffer (pH 5.8 – 8.0), citrate buffer (pH 2.5 - 5.6), and glycine-sodium hydroxide buffer (pH 8.6 - 10.6). These should provide a range of pH environments you can use to manipulate your experimental system.

A. Sodium phosphate buffer (0.05 M, pH ~ 5.8 - 8.0)
1. Dissolve 0.69 grams of NaH$_2$PO$_4$.H$_2$O (monobasic sodium phosphate acid) in 100mL of distilled water. This is the ACID component of your buffer.
2. Separately, dissolve 1.06 grams of Na$_2$PO$_4$ (dibasic sodium phosphate base) in 150mL of distilled water. This is the BASE component of your buffer.
3. Titrate to desired pH by slowly adding acid to base. (A pH of 7.0 will require approximately 2 volumes of acid to 3 volumes of base)
4. If you need a greater volume of buffer, adjust the quantities accordingly.

B. Sodium citrate buffer (0.05M, pH ~ 2.5 - 5.6)
1. Dissolve 0.8 grams of sodium citrate-2 H$_2$O in 100 ml H$_2$O (base)
2. Add 1.25 grams of citric acid (acid)
3. Dissolve 1.1g NaCl into 100mL of distilled water.
4. Add 1ml of this NaCl solution to your buffer
5. Check pH of solution.
6. If pH is greater than 4.5, titrate down with 1M HCl.
7. If pH is lower than 4.5, titrate up with 1M NaOH
8. When solution is the desired pH, add distilled H$_2$O to bring volume to the amount needed.

C. Glycine-sodium hydroxide buffer (0.05 M, pH ~ 8.6 - 10.6)
1. Dissolve 0.38 g of glycine (acid) in 100mL of distilled water
2. Separately, dissolve 0.2 g of NaOH (base) in 100mL of distilled water.
3. Titrate to desired pH.

Hydrogen Peroxide solutions

Hydrogen peroxide (H$_2$O$_2$) solutions can be described in terms of the volume of oxygen gas they generate upon complete breakdown. For example, one milliliter of a 20-volume hydrogen peroxide solution will generate 20 milliliters of oxygen gas when completely decomposed. One milliliter of a 30-volume solution will generate 30 mL of oxygen, and so on.

Our stock solution of hydrogen peroxide is 30-volume strength (which translates to about 9.1% H$_2$O$_2$). You can make a solution that works well in our experimental system by adding 33ml of 30-volume H$_2$O$_2$ to 1 liter of pH buffer. We will let you do the calculations to determine what % solution this is for your presentation.
**VI. Analyzing Your Data**

Once you have determined the mean rate of reaction for each of your experimental variables, you are ready to create a second figure. This time, label the x-axis with the units of your variable (for example, temperature or pH) and the y-axis with units of rate (mLO$_2$/g/sec).

1. On this second graph, plot the rate of each of your experimental trials against the value of your variable at which each experimental trial was performed.

2. Attempt to draw a best-fit curve following your rate vs. variable data points. (HINT: It won't necessarily be a straight line. Examine the data points carefully, and try to understand what is happening to the reaction as you modify the conditions under which you run the experiments.)

3. What you have drawn is a function showing how the rate of H$_2$O$_2$ decomposition by catalase changes when you alter one experimental condition. How did the reaction rate change as you altered your experimental condition?

   How does this relate to your predictions?

   Which hypothesis is supported, and which is not?

Draw conclusions about enzyme function from your results, and be sure to frame your discussion in terms of what you were trying to discover about what features of enzymes allow them to catalyze reactions.

**VII. Your Team’s Presentation**

Before you leave lab today, make sure every team member has a copy of the raw data and any analysis your team has done together, whether on a laptop or flash drive. You should also arrange times and places to meet together during the next week so that you can finish analyzing your data and put together a PowerPoint presentation on your experiment. GET THIS INFORMATION BEFORE YOU LEAVE LAB so there will be no confusion and no lost souls who “can’t get in touch with the team.”

Instructions for creating an effective presentation can be found linked to the syllabus. Be sure to read the instructions carefully, because you and your team will be graded not only on your results and interpretation of data, but also on the effectiveness with which you present your findings to your colleagues.

Rendering your data into clear, analyzable form is only the beginning of a scientific presentation or report. There are no "right" or "wrong" results in an experiment—only valid and invalid explanations for the observations. The most important part of this lab is to explain your results logically, even if they are not what you expected. Think about your results and discuss them intelligently. To improve your presentation, you may use the resources available in the library or online to learn more about catalase and its reaction. Try Google Scholar ([http://scholar.google.com/](http://scholar.google.com/)) for relevant journal articles. Be sure to cite and list all references properly (for example, as we have done here), lest you accidentally become guilty of plagiarism. Read the Appendices [How to Write a Scientific Paper](/) and [Creating Figures and Tables](/) before beginning to make your presentation. In addition, the next lab chapter gives an overview of what is expected of...
your PowerPoint presentation, and how best to create an effective one. Read that chapter completely before you begin your presentation. You are responsible for everything all lab manual readings for this two-part lab exercise, so be prepared.

**Literature Cited**


Sörensen 1986. In Hyatt. In Phosphate Buffer. OpenWetWare, [http://openwetware.org/wiki/Phosphate_buffer](http://openwetware.org/wiki/Phosphate_buffer). (the original reference was cited incompletely by the website)