Scientific Method
Part 1: Experimental Design

Learning Objectives
1. Be able to explain the process of the scientific method.
2. Use the scientific method to formulate a hypothesis and design an experiment investigating aspects of enzyme function
3. Be able to describe enzyme function and factors that affect enzyme activity
4. Understand how to collect and present data
5. Be able to describe the parts of a scientific report and write a scientific report

This lab will continue over several weeks. Ensure you have read the appropriate material and completed any pre-lab requirements before each part.

Part 1: Scientific Method and Experimental Design
You will learn about the scientific method and the principles of good experimental design. You will design your own experiment to test aspects of enzyme function.

Part 2: Data Collection
You will complete your experiment and collect all the data you will need for analysis and to write your report.

Part 3: Writing a Scientific Report
You will learn about the components of a scientific report and complete a written report in class.

Part 1: Experimental Design
Introduction
Scientific Method

Science is both a body of knowledge and a process. Scientists use the scientific method to answer questions and provide explanations about natural phenomena. The scientific method is a logical process based on careful observation and experimentation. In following the scientific method scientists practice evidence based decision making. The information collected contributes to a comprehensive understanding of how the natural world works.

The scientific method is not always a strict step-by-step process, however there are certain components that are always found (see Figure 1). The scientific method begins with an observation that leads to a question about the observed phenomena. Biologists ask questions about the way the natural world works. Based on observations and evidence scientists generate a hypothesis, or a tentative explanation for the observed phenomena. Hypotheses are written as statements and they should always be testable. A good hypothesis offers only one explanation and leads to predictions (often written as an “if-then” statement). The predictions are tested using carefully designed experiments in which measurements are made and data is collected. Data collected from the experiments are analyzed and evaluated in respect to the original hypothesis statement, and conclusions are drawn. To contribute to the body of knowledge scientists report and share their findings. The scientific method does not end here. If the experimental analysis leads to a rejection of the hypothesis, then a new or revised hypothesis statement is constructed and the process starts over. If the hypothesis is supported, further experimentation is still warranted. Repetition strengthens the evidence for hypotheses. It is important to remember that scientific inquiry is an ongoing process and
hypotheses are never considered to be proven. Data can support or reject the hypothesis and often new data may alter or even reject previously supported scientific explanations.

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**Figure 1: Overview of the scientific method**

Look at the following example of an observation and hypothesis/prediction:
Observation: The leaves on trees change color when the weather is cold.
Hypothesis: Leaf color change is related to temperature and low temperatures result in changes in leaf color.
Prediction: If leaf color change is related to temperature, then exposing plants to low temperatures will result in changes in leaf color.

Now write your own hypothesis and prediction for the following observation:
Observation: Eating breakfast increases a student’s performance in school.

Hypothesis: ____________________________________________________________________________________

Prediction: ____________________________________________________________________________________

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**Experimental Design**

An observational or experimental study needs to be designed so that it is capable of testing the predictions made from a hypothesis. If possible, experiments should only test one factor (variable) at a time, with all other conditions being kept constant. It should be fair, and without bias, and outside factors should not affect the outcome of the experiment. If the experiment has been designed properly, the data should either support or reject the hypothesis. A hypothesis is *never proven*. If you cannot support or reject the hypothesis statement with the data collected, then the experiment has been poorly designed.
In designing an experiment there are many factors to consider. To address the hypothesis you need to make the right comparisons and this requires appropriate controls. For example, to know whether your treatments (experimental groups) have an effect you need to compare to an untreated group (control group). An experiment is always set up knowing which comparisons will be made and how the data will be collected. There are many variables that can influence the outcome of an experiment. In an experiment the independent variable is the factor that you are manipulating (what did you change) and the dependent variable is the factor that you are measuring (what you are effecting). There are always many other factors that could influence the outcome of an experiment and it is important to keep these constant throughout the experiment. Note that these controlled variables (or constant variables) are not the same as a control group in the experiment, controlled variables are kept the same in both the experimental and control groups.

Read the scenario described below and answer the following questions.
A group of students decide to test their hypothesis that leaf color change is related to temperature and that low temperatures result in changes in leaf color. They buy plants of the same species, which are all approximately the same age and height. The students divide the plants into two groups, one group of plants is kept at 10°C and the other at 30°C for 2 weeks. All of the plants are planted in Miracle-Grow and given 20 mL of water once a day for 2 weeks. After the two weeks the students record their observations describing the leaf colors of the plants.

What is the independent variable? ____________________________________________________________
What is the dependent variable? _____________________________________________________________
What factors have been controlled for? _______________________________________________________________________________________
Are there other factors that should be controlled for? ____________________________________________________________________________

What is the control group? ________________________________________________________________________________
What is the experimental group? ____________________________________________________________________________
Would you design this experiment differently? How? ____________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________

Activity: Investigation of enzyme (sucrase) activity

Background to problem
Today you will design your own experiment to investigate aspects of enzyme function. You should have reviewed enzymes in detail prior to lab. 
Enzymes are usually protein molecules that act as biological catalysts. A catalyst greatly increases the speed of a chemical reaction by lowering the activation energy necessary to get the reaction started. Most biological reactions would not occur fast enough in a cell without enzymes, thus enzymes are critically important to cellular function.

The function of enzymes is tightly linked to their structure. Enzymes are typically protein molecules, and the molecular shape of the enzyme is determined by primary, secondary, tertiary and quaternary protein structure. On the surface of the enzyme is an active site that temporarily binds the reactants or substrates forming an enzyme-substrate complex.
The catalytic action of the enzyme then converts the substrate to a product or products. This conversion can take the form of a synthesis (building more complex molecules), a decomposition (splitting of the substrate), an oxidation/reduction (addition or removal of electrons), or an isomerization (rearrangement of atoms within a molecule).

When the product or products are released, the enzyme emerges unchanged and available to convert more substrate into more products. Since enzymes can be used again and again, they are effective even at low concentrations.

Each enzyme is highly specific; that is, it catalyzes only a single chemical reaction or small group of related reactions. An enzyme can distinguish its substrate from even closely related isomers. For example, the enzyme maltase will catalyze the breakdown of the disaccharide maltose into its two glucose subunits. However, the disaccharides sucrose and lactose are unaffected by maltase.

The activity of an enzyme is affected by many factors including temperature, pH and substrate concentration. Today you will design an experiment to assess the effect of these factors on enzyme activity. The enzyme you will investigate is sucrase. Sucrase is an enzyme that breaks down sucrose into glucose and fructose as described in the following reaction:

\[
\text{Sucrose (C}_{12}\text{H}_{22}\text{O}_{11}) + \text{water (H}_2\text{O}) \xrightarrow{\text{sucrase}} \text{glucose (C}_6\text{H}_{12}\text{O}_6) + \text{fructose (C}_6\text{H}_{12}\text{O}_6)
\]

In this exercise you will design your own experiment to examine the effects of temperature, pH or substrate concentration on sucrase activity.

**Materials:**
The following materials will be available to you to design your experiment:

- Sucrase (enzyme) stock solution (3ml per tube)
- Benedicts reagent (2ml per tube)
- Sucrose (substrate) stock 10% solution (2ml per tube)
- Acid – Concentrated (12N HCL) and weak (lemon juice)
- Base - Concentrated (14N NH₄OH)
- Ice
- Water
- Hot plates
- Room temperature water bath
- Thermometer
- pH strips
- Test tubes
- Test tube racks
- Test tube holders
- Test tube markers
- Pipettes/pipetman
- Glassware (beakers/measuring cylinders etc)
Sucrase is present in a wide range of organisms including humans and even yeast. The sucrase stock solution provided was prepared by placing dry yeast cells in warm water for 10 minutes which allowed them to become metabolically active. The yeast suspension was then homogenized in a blender for three minutes which ruptured the cells and released sucrase into solution. The homogenate was filtered through cheesecloth and the resulting filtrate became the sucrase stock solution.

**Benedict’s reagent** is a blue solution of sodium bicarbonate, sodium citrate, and copper sulfate. When this solution is mixed and heated with a monosaccharide like glucose or fructose, a precipitate forms. The color of the precipitate will vary from green to red depending upon the quantity of monosaccharide present.

The relative amount of glucose and fructose present can be estimated using the following scale:

<table>
<thead>
<tr>
<th>Color</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>0</td>
</tr>
<tr>
<td>Green</td>
<td>+</td>
</tr>
<tr>
<td>Yellow</td>
<td>++</td>
</tr>
<tr>
<td>Orange</td>
<td>+++</td>
</tr>
<tr>
<td>Red</td>
<td>++++</td>
</tr>
</tbody>
</table>

**HINT:** In a typical control experiment 3 ml enzyme stock solution + 2 ml of a 5% sucrose solution would be added to a test tube and kept at room temperature for 10 minutes. 5 ml of Benedict’s reagent would then be added to the test tube and the solutions mixed. The test tube would be heated in a boiling water bath for two minutes. After 2 minutes the solution in the test tube would be orange/red indicating a high amount of monosaccharides (glucose and fructose). This indicates that the sucrase (enzyme) was highly active since the sucrose (substrate) was converted to glucose and fructose (products).

**Experimental Design**

Determine which factor(s) your group will investigate. Before designing your experiment answer these important questions:

What factors will you be testing?

What is your hypothesis and prediction?

____________________________________________________________________________________

____________________________________________________________________________________

____________________________________________________________________________________

____________________________________________________________________________________
A well designed experiment should only test one factor (variable) at a time, with all other conditions being kept constant and the data collected should either support or reject the hypothesis.

As a group you should consider:
- What is your independent variable?
- What is your dependent variable?
- What variables need to be constant?
- Do you have a control group? Positive control/negative control?
- How many treatments do you have (replicates?) and what comparisons will you make? (How many test tubes will you need?)
- What volumes of solutions will you use?
- How long will you incubate solutions for?
- What will you measure?

After taking into account all the factors and materials available you will work with your group and design an experiment. It is important to include all necessary details such that you will be ready to conduct the experiment in part 2 of this lab. You will need to submit a materials list and detailed experimental protocol to your instructor by the end of lab today. Use your osmosis and diffusion lab handout as a guide for writing an experimental protocol.
Pre-Lab Activity

Prior to this lab, you should:

1. Read Section 1.6 Doing Biology, and review The Big Picture Doing Biology in your Biological Science textbook (Freeman 6th edition pages 9-13 and pages 16-17)
2. Review the UC Berkeley website [http://undsci.berkeley.edu/article/intro_01](http://undsci.berkeley.edu/article/intro_01) which has a good overview of the scientific method.
3. Read Chapter 3 Protein Structure and Function, and Section 8.3 How Enzymes Work of your Biological Science textbook (Freeman 6th edition pages 78-91 and pages 179-184)
4. Review the pre-lab PowerPoint presentation.
5. Answer the following questions BEFORE lab:

**What are the monomers that make up proteins?** _______________________________________________________________________

**Describe the 4 levels of protein structure** _______________________________________________________________________

**What is a catalyst?** _______________________________________________________________________

**What is an enzyme?** _______________________________________________________________________

**What is the substrate of an enzymatic reaction?** _______________________________________________________________________

**Is an enzyme consumed during an enzymatic reaction?** _______________________________________________________________________

**Where does a substrate bind to an enzyme?** _______________________________________________________________________

**Why are enzymes highly specific?** _______________________________________________________________________

**Why are enzymes important for cells?** _______________________________________________________________________

**What factors can change the speed of enzymatic reactions?** _______________________________________________________________________


**Post Lab Activity**

1. After lab you should be able to answer the following questions:

   What color precipitate would you expect in a test tube in which the enzyme was not functional? ________
   ___________________________________________________________

   What color precipitate would you expect in a test tube in which the enzyme was at optimal activity? ________
   ___________________________________________________________

   What color precipitate would you expect in a negative control? ___________________________________________

   If your treatment alters enzyme activity so it is no longer optimal what color precipitate would you expect?
   __________________________________________________________________________________________

2. During the next lab you will conduct your experiment and collect your data. Create a data entry table for
   the experiment you have designed. Look at the tables provided in the osmosis and diffusion lab handout as a
   guide.