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 enzymes, biological catalysts that drive specific chemical reactions in living organisms. Review protein structure and enzyme function from your BIL 150 lecture material, textbook readings, and primary literature. This will prepare you to 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.

(c) 2013 Dana Krempels, PhD
**Figure 1.** As substrate concentration increases (on the abscissa (x axis)), reaction rate increases until the enzyme is completely saturated and working at its maximum possible rate (1.0 on the ordinate (y) axis).

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.

\[
\text{catalase} \\
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]
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. Assignment: Literature Search and Review

An essential part of undertaking a research project is to find out what is already known about your area of interest, why it is important and relevant. Previous research might give you ideas about what questions still need to be addressed in this area.

For example, you might be interested in finding out if a particular environmental factor (e.g., pH) affects enzyme activity, but it’s quite possible that research in this area has already been done. In order to conduct a study that is not simply a repeat of someone else’s work, it is important to conduct a literature search and review (http://www.slideshare.net/featherr/how-to-conduct-a-literature-search) before embarking on your experimental project.

A literature search is an organized search for published material on a selected topic. For our purpose, you will be required to use databases that retrieve academic sources of high quality and reliability. Literature databases allow you to search a wide array of journals and other sources, and enable you to collect scholarly references. Some of these may be in the form of an abstract, whereas others might be the full text of a journal article. You can usually save these to your own computer for future reference.

An effective literature search takes an organized approach:

1. Decide on a search topic
With your team, formulate a question to narrow and define the topic. For example, if you wish to determine the effect of pH on enzyme activity, you might ask “Does lower pH change the rate of reaction of an enzyme?”

2. Use appropriate keywords to use in your search
Identify important keywords. In the example above, you might include “enzyme, pH, catalase, acid, alkaline,” or any number of related terms. Also consider:

   • When to use broad terms, and when to use narrower terms to refine your search
   • Use synonyms for your keywords to find every possible variant of the vocabulary used in the research on this topic.
   • Use dictionaries to check spelling and find keyword synonyms
   • Using online encyclopedias (e.g., Wikipedia) to find initial background information that might help you refine your search or choose an area for your research topic.

3. Choose a Database
GoogleScholar (http://www.goglescholar.com) is an excellent place to start, but other databases are available through the UM library system, as well.

(c) 2013 Dana Krempels, PhD -4
4. Perform your Search

- Use **Boolean operators** (AND, OR, NOT) (always enter them in upper case) to combine search keywords.
- **Truncate** (shorten) your keywords to make your search broader.
- If you are not sure how to spell a keyword, use **wildcards**.
  
  A wildcard is a character (either a ? or a *) used to stand for unknown letters.
  
  - A question mark (?) can be used to represent any one single character.
  - An asterisk (*) can be used to represent any number of characters or no character.
  - For example, c?t will find **cat, cot, cut** whereas c*t finds **cat, caught, commencement, conflict, consent, cot, cut**, etc.

- To narrow your search, use phrases enclosed on quotation marks. For example, “pH effect on species richness”.
- Use the database to search for keywords in different places, such as “title” or “abstract”.
- If you find a useful article by a particular author, search that author’s name to find papers on the same topic.
- If you find a useful article, search its Literature Cited section to find additional, related sources.
- Make sure the literature you are citing is recent and current.
- Make sure the literature you use is from a peer-reviewed, scientific journal.
- Make sure to identify whether your source is a journal article, a book, a thesis, etc.

5. Determine the availability of the material you wish to reference.

If the paper you wish to read is not available online, you may be able to get a copy by contacting the people at the Richter Library Help Desk. If our library does not have the paper you need, they may be able to get it via interlibrary loan. Since this requires turnaround time, this is one very good reason to start this assignment immediately, and not find yourself hamstrung by time constraints.

Each student will be assigned the task of finding **at least three relevant papers** from a refereed scientific journal on experiments with catalase. See Section III below for important information about narrowing your search to articles that will help you design an experiment with available reagents.

Once you find a paper of interest, read it completely and analytically. Your assignment is to turn in (1) the journal paper and (2) complete the Literature Search Template linked to the syllabus (right below this chapter). All materials should be submitted electronically to your Laboratory Instructor, who will give you details on his/her preferred venue (email, Blackboard, etc.).

**III. Narrowing Your Literature Search**

If you do a broad literature search on chemicals that affect the activity of catalase, you will no doubt notice that many catalase-inhibiting (or enhancing) compounds seem to have the capability to strike everyone within 15 feet dead. This means that these compounds are not particularly nice to have in a laboratory full of frisky first year students. To prevent death and mayhem, we have narrowed the list of reagents you will be allowed to use in your experiments to those shown below.

(c) 2013 Dana Krempels, PhD -5
Have a look at the list, and do a literature search on any of these compounds with the appropriate keywords. Confer with your teammates when you design your project, and know you will be limited to using the following reagents.

- Ascorbic acid
- Acetyl salicylic acid (in stock aqueous solution)
- Copper sulfate (in stock aqueous solution of less than 0.5M for safety)
- Ethanol
- Isopropanol
- Succinic acid (in stock aqueous solution)
- Salts to make buffers of various pH

We may have provided a list of available chemicals, but that does not mean that we’re going to tell you how they interact with catalase (or hydrogen peroxide) or why it would be interesting to test their effects. That is the job your team will now undertake with literature search and experimental design.

**Literature Cited**

Goodsell, D. S. 2004. Catalase. RCSB Protein Data Bank,  


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)