Biochemistry Lab
MDH Project & Lit Search Guide

Introduction: *In this lab activity, you will be introduced to the possible MDH projects and to hypothesis development, the scientific method, and oriented to literature search using a number of sources.*

Project Ideas: As introduced at the beginning of the semester, your group will generate a hypothesis, design experiments and test your hypothesis. Your choice of project must meet three criteria; 1) the project must be hypothesis driven and tested by your experimental design, 2) your project must be based on an observation(s) from the literature (not a random idea), and 3) must be research. The Council on Undergraduate Research (CUR) defines research as, "*an inquiry or investigation conducted by an undergraduate student that makes an original intellectual or creative contribution to the discipline*". For your project, CUR's statement is reflected in that you will work to contribute new knowledge to biochemistry. In specific and simplified language, you will create a hypothesis-driven project that is not a repeat of existing experiments but will instead answer a new unknown question. That question can be a twist on something published using a new and different isozyme of MDH or another dehydrogenase. It should be something non-incremental and potentially (with a few additional experiments) publishable.

The general flow to your project design will be to learn about the specific characteristics of activity, regulation, structure and other elements of MDH. You will read the literature, look at the clones of MDH and LDH available to you and propose a hypothesis and experimental design that will include designing and making amino acid mutations of MDH.

To help you define projects that might be interesting to you, a list of possible experiments / projects and a list of MDH/LDH clones available for use have been posted to the class website. This list is a general description of ideas to consider. None of these projects are hypothesis driven, turn-key ideas. The projects are there to inspire and help you think of something as you learn more about MDH.

Generating a hypothesis-driven project: Most students are not used to defining a hypothesis. Doing an experiment is not a hypothesis. It is how one tests a hypothesis. A few things a hypothesis is not:

- Let's see if… doing an experiment to see if a change occurs is not a hypothesis.
- Changing an amino acid without support or observations from your own experiments or published experiments is a fishing expedition
- Testing to see if one "thing" is (better, faster, larger… fill in the blank) than another "thing" is not a hypothesis driven project. An example is to see if soap A kills more bacteria than soap B. That is consumer testing but is not hypothesis-based science.
- A hypothesis is not a "hunch", an unsupported idea, educated guess or a random concept.
- Questions – a proper hypothesis is not formed as a question, it should be a statement.

What is a hypothesis? A hypothesis is part of the scientific method you've learned about many times since middle school/junior high. There are three steps to creating a good hypothesis.

1. <u>*Observations*</u> - A hypothesis is a testable statement based on a previous observation from a published experiment or a personal observation from an experiment.

2. <u>*Scientific Questions*</u> - After researching the background of a scientific problem you will have a question that you wish to answer. And the hypothesis will be the testable prediction of your question.

3. <u>*Generation of a testable and falsifiable hypothesis*</u> - A good hypothesis is an ***IF/THEN*** statement. If x (I do this/this happens), then Y will result. A good hypothesis is testable and falsifiable. Science doesn't prove anything. It tests and supports a hypothesis, science can falsify the hypothesis with testing, but science doesn't prove a hypothesis.

The rationale supporting the hypothesis will follow a good hypothesis. Publications supporting your ideas are critical. Next determine the variables needed to test the hypothesis. THEN decide on the correct approach (experimental design) to test and possibly falsify the hypothesis.

Example: Reading the literature and looking at the structure of an enzyme you find three amino acids are critical to reduce the activation energy of the enzyme-catalyzed reaction. From these observations, you generate the question: if you change one of the amino acids will the reaction have less specificity or have a higher rate? A hypothesis is then created; If the Asp involved in stabilizing the reactive intermediate of oxalate ion is neutralized, the reaction will take place at a significantly higher rate. Determine the variables needed for the experiment. Mutational analysis of MDH from Asp to small-uncharged amino acid or a neutral charged amino acid (based on literature and inspection of the structure of the active site) define the variables. Finally design the experimental approach by creating specific experiments (with controls) needed to test and falsify the experiment.

By searching the literature about MDH and learning the structure of the enzyme, you can create a good scientific question, design a strong hypothesis, determine variables involved to test the hypothesis and then design the experiments needed to test and falsify your hypothesis.

Searching scientific literature: There are several ways to search the literature to find information about MDH. The success of any search is to first understand the basics and key terms of the scientific project. If little is known about an enzyme or system, a simple search of a textbook will get you warmed up. There are a couple of good, simple sources to start out with if you have very little background on a topic, concept or theory. Google-ing malate dehydrogenase will bring up the Wiki page for MDH. A read through such a page will help you begin to understand key words or phrases needed to refine a more comprehensive search of the literature. Another page to look at is Wikigenes. Wikigenes is a collaborative approach to gather information for genes and proteins. Searching this webpage will give you some ideas of papers and information about the enzyme. A quick search and read through this page will provide lots of good information to help refine and deepen your literature search.

Once you've gotten a handle on terms, concepts and important points about an enzyme or topic, you should search for publications using PubMed or Google scholar. PubMed is a government run website which searches the Medline database. Another search engine to consider when looking for scientific journal articles is ScienceDirect. Any of these search engines will allow the user to inquire for a journal/publication from a number of databases. A less efficient way to search is to use individual journal websites. However more and more of the journals, such as the Journal of Biological Chemistry, and the American Chemical Society publications will allow you to search for publications and link your search to similar searches in PubMed or other databases. Many journal articles will be available as both a PDF and web (html) versions. Be certain to print out and save only the PDF versions. Save the html option for reading online or on your iPad/tablet device. Access to articles will depend on the publisher. Some are fully open access. These journals allow all publications to be freely accessible to the public. Others have limited access. These will often have the most recent issue open, then the newest year or two issues will only be accessible to those with a paid subscription. Many of these journals have made older journals freely available. While it is tempting to expect all journals to provide free and full access to their publications, it is important to recognize that there are significant costs associated with providing a stable historical record of publications as well as costs associated with running and maintaining various journals. Some societies use funds to further science in a variety of ways. Free access journals often charge a significant fee to the scientist when publishing. This cost can range from a few hundred to several thousand dollars! Subscription based access reduces the financial burden on the individual research and spreads it out amongst individuals and institutions utilizing the journals.

Note: The databases mentioned (and more!) can be accessed from the Copley Library webpage Databases tab. From here you can see whether the database provides citation-only information, or full-text articles. The library does have subscriptions to a number of scientific journals, meaning you will be able to access the full text of the subscribed journal articles.

When starting a comprehensive literature search it is helpful to first search for review articles about the topic. Including the term "review" will bring journal articles that will provide a larger picture about the topic. Most will not include primary data, but a good review will point the reader to trends and ideas about the future of the scientific problem and even be critical of current work. This kind of analysis helps to get a scientist up to speed on what is cutting edge and historically relevant for a gene, protein, cell, or system of interest. With this kind of information in hand, it becomes easier to search the primary literature (journal articles with results of experiments) to help you understand the topic and define your hypothesis. The class webpage includes links for a few important review and primary journal articles to help get you started.

Getting Started: Here are a few questions and points to consider as you begin to understand MDH, its functions and developing your hypothesis. The answers to these questions are found in the scientific literature.

- Know your enzyme. What is the reaction catalyzed by MDH? What is its molecular weight? Where is the protein expressed? Is the protein active as a monomer, dimer or some other oligomer? Is there a difference in different species?
- Learn the key amino acids in binding and involved in the reaction. What is the reaction mechanism for the enzyme?
- What are the key kinetic values for MDH? If there are isozymes within a species or cell are there differences in the kinetic constants? If there are multiple isozymes between cell types or species (i.e. plant vs yeast vs mammalian) what are the important features of the protein?
- Are there interesting small molecule regulators of the enzyme? Activators, inhibitors…
- Are there allosteric regulators of the enzyme? Do other proteins bind or interact with the protein?

Once you have a handle on the questions above, refine your literature and begin to create your question to build the hypothesis. Use the list of potential MDH / LDH clones to assist your search. Don't limit yourself to this list of clones in your background search. There is a lot of interesting information about MDH from other sources that you can use to create your project. Finally, look at the list of potential project for MDH. See what sparks your interest and let that inform your process as you develop the hypothesis and experimental approach/design.