# Biochemistry Lab
# MDH Project Ideas

**INSTRUCTIONS**: *This is a short list of possible starting points for you to consider for your semester project. The only limits to the project is 1) the project must be hypothesis driven – meaning you must have found some reason to support your hypothesis and 2) the project must have something to do with the MDH (protein or DNA) clones that we have. LDH is there as an interesting possibility. 3) Your project MUST be research. Meaning you will create new knowledge, answering new questions. No direct repeat from published work will be allowed. NOTE – the ideas given below are just ideas and not given as a hypothesis. You must use the skills from the first weeks of your work to determine what you can do.*

*See the web page for a full list of clones. A few relevant MDH papers are also found on the web page. DO NOT LIMIT YOURSELF TO THESE PAPERS. Doing so will ensure an average effort and result in an average grade.*

**TASK**: *Each group will meet with your instructor provide your hypothesis, the observations (from papers and other sources) used to develop your hypothesis and a fairly well thought out experimental plan. Regardless of the project, each member of the group will be responsible for purifying one or more of the proteins and ensuring that they are fully engaged throughout the semester.*

**NOTE:** The ideas below are just ideas and not presented as a hypothesis based on observations. You must still use published literature to make your hypothesis from. These are just guidelines/suggestions/ideas.

- **Kinetics** – Several of these mutants and wild types will have different Km and Vmax values. Some of the mutants are specifically designed to alter the affinity of the substrate from the wild-type.
    - Compare Vmax and Km between different mutants and wild type
    - Compare kinetic values between wild types
    - Examine the kinetics of MGH. Nothing has been done with this protein!
    - TM LDH shows a very high Km for its substrate unless fructose 1-phosphate is present. Why? Does MDH or other LDH isoforms show similar properties? Can an LDH or MDH be made to bind and interact with F1P in the same way? Are there other compounds which drive MDH kinetics?
    - There are two regions on watermelon MDH that significantly alter the kinetics in two ways…
    - Allosteric interactions – MDH and LDH will oligimerize to dimers and larger changing kinetics. Can you make MDH active as a monomer? What forces the oligmerization? Is there a difference between the dehydrogenases that are dimers or tetramers or other? Will mutations help define the interactions?

- **Substrate Specificities** – Some of the mutants have been created to change the specificity of the enzyme from Malate to another carboxylic acid.
    - Find the mutants that might change MDH to LDH or an NADPH enzyme and test for substrate specificity between several selected substrates. Consider how one examines for substrate specificity.
    - Compare and contrast the binding pocket of the enzyme for the substrate. Are there key differences between some of the wild type enzymes? Test between several potential substrates and their Km for each.
    - NADPH vs NADH binding?
    - Inhibitors – what inhibition takes place with MDH vs LDH? Can you alter the impact of an inhibitor without changing the kinetics of MDH substrate?
    - On of our clones is an LDH with MDH activity. Lots to think about with that clone. What about making a different LDH do the same? Or the reverse?

- **Informatics** – If a group or individual, liked the first part of the project, they could work to complete the sequence (DNA and aa), create alignment, search for protein structures and even add DNA sequences that haven't been published to the database. Only one group can do this project.

- **Protein interactions** – There are several published papers which indicate that some MDH proteins must dimerize to be active. There are several ways to look for protein-protein interactions that include chromatography, molecular crowding agents, native gels and other techniques.

University of San Diego

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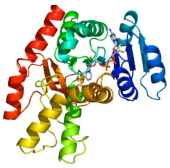

- This project might look at wild-type vs mutant or mixtures binding to each other. Use size exclusion chromatography or immunoprecipitation (IP) to analyze interactions.
- Another set of publications point out that MDH interacts with citrate synthase (CS) in the mitochondria. We have purified enzyme to test for this CS vs various MDH interactions.
- Is the "substrate channel" or "substrate shuttling" seen with MDH and CS limited to mitochonrdria MDH? Is the interaction just change kinetics? Lots of possibilities here!
- Crosslinking experiments with or without substrates in wild-type or mutants might be interesting.

- **Stability** - Several of the wild types and mutants are suggested to have structures which keep the protein native at extremes of temp and pressure. Some are cold tolerant (have activity and remain stable at reduced temperatures) while two are heat stable. Can you reduce the tolerance/stability? Can you re-create that in another enzyme?

- **Impact of important mutations on protein structure and stability** – Can examine stability of protein conformations using various spectroscopic methods, which may include fluorescence, circular dichroism, and absorbance spectroscopy.

- **Crystal Structures** – A quick study of most of these wild types and or mutants will show that there are few crystal structures in the database. This project would require a larger scale purification resulting in a pure protein that can be made into crystals. There are several papers describing the process for watermelon MDH structures and other MDH isoforms to help.

- **MGH Characterization** – Nothing has been done to characterize a novel clone using a His tagged, GFP – MDH protein called (MGH). All of these projects could be done comparing MGH to other wild type or commercially obtained and purified protein.

- **In-Gel Assays** – There is a way to run the protein on a gel and then soak the protein in a solution that turns color where NADH utilizing protein is located in the gel. This is not a western blot. This project would start to define how to study the enzyme activity of pure protein after developing this technique.

- **pH studies** – Use different buffers to study the protein's activity and compare two or more wild type and or mutants. This project will need to focus on the amino acids in the active site and make predictions on the pH max based on the mechanism of the enzyme. There are several papers and reviews that discuss this.

- **Protease Studies** - A group what is interested in this may purify several proteins and based on on the amino acid sequence predict how and where two or more proteases cleave the various purified MDH proteins. Then measure the resulting protease products using reverse phase chromatography or two dimensional gel electrophoresis.

- **Rapid Protein Folding Assay using MGH** –(GFP folding paper and GFP as a control).

- **Metabolite Inhibition** -Pyruvate, lactate or other carboxylic acid as an inhibitor

- **Heat shock protein interactions** - look for inhibitors of MDH and see if mono/di-carboxylic acids act as an inhibitor

- **Tryptophan fluorescence** - open possibilities to look at protein conformation using tryptophan fluorescence.

- **Impact of important mutations on protein structure stability** – CD spectral analysis to measure folding energies of various wild-type and mutant enzymes. With or without substrate.

- **Other –** use your imagination and the papers published on MDH to come up with an interesting and novel experiment to work on.