

Biochemistry Lab II

Biochem Lab Project Ideas.



INSTRUCTIONS: This is a short list of possible starting points for you to consider for your semester projects. 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. **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 each clone may do.

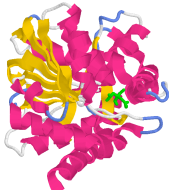
See the web page for a full list of clones. A few relevant MDH papers are also found on the web page. **DO NOT LIMIT YOURSELF TO THESE PAPERS.** Doing so will ensure an average effort and result in an average grade.

Projects must also compare results to the amino acids in the active site and use modeling to predict and explain the results.

TASK: Each group will meet with your instructor provide your hypothesis, the observations (from papers and other sources) for 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.

IDEAS: Each group can use one or a number of wild-type or mutant MDH clones to study.

- **Kinetics** – Several of these mutants and wild types will have different K_m and V_{max} values. Some of the mutants are specifically designed to alter the affinity of the substrate from the wild-type.
 - Compare V_{max} and K_m between different mutants and wild type
 - Compare kinetic values between wild types
 - Examine the kinetics of MGH. Nothing has been done with this protein!
- **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 K_m for each.
- **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.
 - This project might look at wild-type vs mutant or mixtures binding to each other.
 - 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.
- **Stability** - Several of the wild types and mutants are suggested to have structures which keep the protein native at extremes of temp and pressure.



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- **Crystal Structures** – A quick study of most of these wild types and almost all of the mutants will show that there are no crystal structures on 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. There is also a biochemist that is willing to assist and shoot the crystals we make.
- **MGH Characterization** – Nothing has been done to characterize the protein you worked on last semester, 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
- **Other** – use your imagination and the papers published on MDH to come up with an interesting and novel experiment to work on.