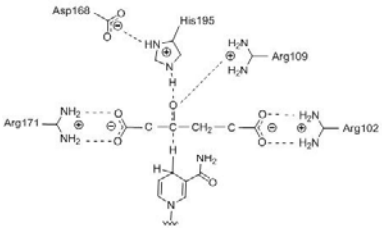
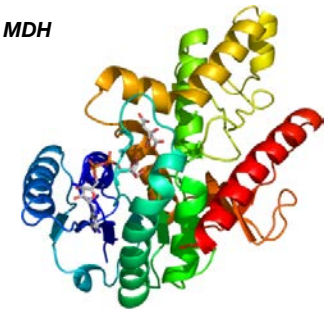
CHEM 335W Fall 2018
Section 02
Biochemistry



Active site and structure of MDH



Instructors: Dr. Ellis Bell
Office: SCST 491
Email: jbell@sandiego.edu
Phone: 260-7527 (office)/ 260-8972 (lab)
Lab Hours: T/Th 2:30-6:30 pm SCST 427
Office Hours: TR: 11.30am-12.30pm; F: 3-5 pm or by appointment

Required Text: *Assigned Pre-Lab Readings on Blackboard*

Course Description: The course is an advanced laboratory course that is built around a full semester research project. The project will require a focus on techniques for the preparation and quantitative analysis of proteins and other macromolecules, presenting students with a broad spectrum of techniques, approaches and concepts of contemporary biochemistry in the context of their application to research. You will learn aspects of DNA purification and analysis, protein expression and quantification, enzyme purification, enzymatic characterization, chromatography, electrophoresis, immunological techniques, and spectroscopic analysis. While you become skilled in all of these techniques, **you will be designing your own experimental procedures to address a research question that you will develop, continually analyzing and evaluating your results**. **You will do all of this while demonstrating safe laboratory practices and keeping a complete and organized notebook.**

This course has a series of specific learning goals: i) Find, use and present relevant primary literature, protein sequences, structures and bioinformatics tools ii) Understand the various roles that non-covalent interactions may play in the structure and function of an enzyme, and. iii) Create/develop and present a testable and falsifiable hypothesis and appropriate experiments to interrogate the hypothesis. iv) Work Safely & Keep an accurate lab notebook. v) Successfully follow a protocol, explain the basis for the steps involved and understand the variables that must be controlled for. & vi) Be able to obtain and use quantitative data and appreciate the need for reproducibility and appropriate statistical analysis.

Expected Learning Outcomes:

1. Identify, locate and use the primary literature.
2. Develop a testable and falsifiable hypothesis based upon review of related primary literature and bioinformatics approaches, and design appropriate experiments and controls to test hypothesis.
3. Design, construct and validate one or more mutants to interrogate your hypothesis
4. Use various biochemical and biophysical approaches to characterize, compare and contrast, mutant and wild type proteins.
5. Calculate kinetic parameters of an enzyme from experimental data and use kinetic parameters to compare wild type and mutant enzymes.
6. Explain the importance of and keep an accurate laboratory notebook.
7. Communicate scientific results in formal written format following the guidelines of a primary literature article. Use visual and verbal tools to explain concepts and data
8. Work with peers to evaluate data, apply knowledge to data & interpret data. Give and take directions to be an effective team member.

Resources:

(a) *Assigned Readings:* Although the theme of this course is original research, and you will find and explore the primary literature as a critical part of your project, the overall project is organized around four modules, i) Hypothesis Development and Proposal, ii) Construction & Expression, iii) Kinetics and Ligand Binding, and iv) Conformation & Stability. These modules are found on Blackboard and will be the subject of the in class quizzes that occur

(b) *Blackboard course site.* Class materials will be available on the course Blackboard (Bb) site. The laboratory is broken down into lab blocks, as listed in the lab block section of the Bb site. In addition to the modules listed above you will find a variety of protocols that you may or may not need depending upon the direction your project takes. You will also find the rubrics for the various graded assignments- these will give you a guide to the level of knowledge you need to be successful in the course. All assignments are posted to Bb under their respective tabs (Pre-lab, Homework, Research Project, Writing Portfolio). All assignments will be turned in electronically through the Bb site or the electronic notebook (see below). Several video tutorials and websites are embedded in the Bb site. These are resources that provide information that are REQUIRED to be prepared for class and to complete your assignments. Ignore them at your own academic peril.

(c) LabArchives electronic notebook. Lab prep, data, and analyses will be recorded in an electronic notebook. You will be enrolled in the electronic laboratory notebook (ELN) associated with the class. You will receive an email notification of this enrollment. An access fee will be assessed by LabArchives. A video tutorial on ELN usage is posted on the Bb site under the Notebook tab. Guidelines on maintaining your laboratory notebook also posted.

Group work: You will be assigned to a team for the semester. As part of this team, you will develop a hypothesis, design and complete experiments to test the group's hypothesis. As part of your team work, you will evaluate your team and your team will evaluate you. Your group work reflects the real-world experience of scientists – that is - team-based studies and interdisciplinary cohorts. From your group work, you will gain experience working with peers to evaluate, interpret & debate data/ethical issues pertaining to the course materials.

Grades:

Homework, Prelab Quizzes 250 pts (25% of total grade)

Homework assignments, Pre-Lab reading and associated quizzes will be turned in throughout the semester and must be completed on time to be adequately prepared for the next lab period. See the detailed grade breakdown below and refer to the schedule of due dates provided. Late homework and assignments will not be accepted for a grade.

Laboratory Notebook 50 pts (5% of total grade)

Your laboratory notebook should be an accurate record of what you do in the lab, and should contain notes and calculations as well as appropriate comments to the lab your working on. You should enter the lab with your notebook prepared for the day's experiments. A major function of a lab notebook is to allow another competent scientist to reproduce exactly your experiment. (See lab notebook format document for detailed information.)

Research Project Related Topics 250 pts (25% of total grade)

For the project, you will work in groups of two or three to perform original research related to the structure, function and important characteristics of malate dehydrogenase (MDH). You will research MDH, create a hypothesis on the structure or function of MDH, design an amino acid mutation, write a proposal, critique proposals, express and purify your protein and conduct experiments to test your hypothesis. Depending on the project and progression of the project, you could even publish your work! This may take a few semesters for that to happen. (See research project guidelines for detailed information.)

Writing Assignments 450 pts (45% of total grade)

To report research results and secure research funding, scientists must be able to clearly and succinctly communicate their science to peers and lay people. In this course, you will develop a formal research proposal for your research project. Over the course of the semester you will refine the proposal to incorporate further components of the final paper, Introduction, Hypothesis, Materials and Methods, Results, and Discussion, that will be written by the end of the semester. The Materials and Methods component will also be evaluated in the context of your laboratory notebook. Through instructor feedback, revision and peer review, you will hone your scientific writing skills. You will also learn how to prepare publication quality figures and figure legends, prepare a literature review of relevant primary literature, and master proper usage of references.

The grade cut offs are A-90%, B-80%, C-70%, D-60% F-<60%. The +/- grades will be awarded within each range, typically top 2% of each range corresponds to "+," bottom 2% of each range corresponds to "-." If revisions are offered: Must be completed within one week of graded work being returned to student. **NOT MAINTAINING A LAB NOTEBOOK WILL RESULT IN AN AUTOMATIC "F."**

Safety: Safety regulations require that all students working in laboratory receive training in the safe handling of any potentially dangerous chemicals or biohazards. The first day in lab will cover refresher training in the safe handling of these materials. For the most part this laboratory poses very little risk, however we will be using several chemicals that are potentially dangerous. A safety training sheet will be signed at the beginning of the semester.

Attendance Policy: Attendance is mandatory. Excused absences can only include: 1) Official University or academically-related event approved by your instructor, or 2) Illness, if you provide a doctor's note. For non-illness related absences you must inform the instructors at least 48 hours in advance, and provide supporting documentation. In the event of an unexcused absence from the laboratory, you will not be allowed to make up the session and you will not receive credit for that part of the lab. Continued, unexcused absence from class may result in a failing grade or you will be asked to withdraw from the class.

Academic Integrity: Review the Student Code of Rights and Responsibilities and Rule of Conduct (http://www.sandiego.edu/conduct/the_code). In particular, familiarize yourself with the Academic Integrity Policy, which is found under "University Policies." You will need your MySanDiego username and password to view the policy.

Assignment Schedule and Associated Points

Pre-Lab Assignment Quizzes and Homework: Total 250 points: Individual

Quizzes*	Date	Points Available
PL1: Enzymes, an Overview	9/11	40
PL2: Hypothesis & Proposal Module	9/25	40
PL3: Construction & Expression Module	10/23	20
PL4: Kinetics & Binding Module	11/15	30
PL5: Conformation & Stability Module	12/04	20
Homework*		
HW1: Specific Activity Calculations, Purity, Yields	9/13	25*
HW2: Characterization & Molecular Weight Calculations etc	10/11	25*
HW3: Kinetics and Inhibitors, Binding Calculations	11/1	25*
HW4: Folding and Stability	11/20	25*

*Upto 50% missed points can be claimed upon correction after return

Laboratory NoteBook: Total 50 Points: Individual

NB1	9/20	12.5
NB2	10/18	12.5
NB3	11/8	12.5
NB4	12/6	12.5

Research Project Related Assignments: Total 250 Points Individual

RP1: Proposal Presentation	10/9	100
RP2: Proposal Critiques	10/18	50
RP3: Project Presentation	12/13	100

*Upto 50% missed points can be claimed upon correction after return

Writing Assignments#: Total 450 Points: Individual

W1:Literature Background	9/15 & 9/25	50
W2: Proposal	10/6 & 10/23	100
W3: Methods & Data Presentation	11/10 & 11/16	50
W4: Discussion & Data Analysis	11/27 & 12/6	100
W5: Final Paper	Finals Week	150

Upto 75% missed points on the draft can be claimed upon Revision

CHEM335W Section 2- Tuesday/Thursday
The schedule is tentative and may change based on the pace of the project and class- be prepared to adjust as appropriate

Day	Block	Activity Description	Date	P-Lab	HWK	NB		Writing
1		Assessments/MDH and Course/Project Overview:	Th:9/6					
2*		What is Known: Finding and Reading Literature Measuring the Activity of the Enzyme: Specific Activity Determination	9/11	PL1				
3*		Characterization of the Wild type etc I	9/13		HW1			
			9/15					W1 Draft
4*		What can we learn from the amino acid sequence: Bioinformatics, clustal analysis etc	9/18					
5*		Exploring the 3D Structure: Developing a Hypothesis I	9/20			NB1		
6*		Primer Design- order Primers- Plasmid DNA preps & Characterization	9/25	PL2				W1Revised
7*		DNA Isolation/Quantitation	9/27					
8		QuikChange	10/2					
9		Transformation etc	10/4					
			10/6					W2Draft
10*		Proposal Presentations	10/9					
11*		DNA Preps for Sequencing	10/11		HW2			
12		Proposal Critiques	10/16					
13*		Expression/Purification	10/18			NB2		
14*		SDS PAGE and Mutant Specific Activity	10/23	PL3				W2Revised
15*		Project Experiments	10/25					
16*		Project Continued	10/30					
17*		Project Continued	11/1		HW3			
18*		Project Continued	11/6					
19		Project Continued	11/8			NB3		
			11/10					W3 Draft
20*		Project Continued	11/13					
21*		Project Continued	11/15	PL4				
			11/16					W3Revised
22*		Project Continued	11/20		HW4			
23*		THANKSGIVING	11/22					
24*		Project Continued	11/27					W4Draft
25		Data Analysis and Presentations II (with collaborators as appropriate)	11/29					
26*		Project Continued	12/4	PL5				
27		Project Continued	12/6			NB4		W4Revised
28		Lab Clean Up etc.	12/11					
		Project Presentations,	12/13					
		Final Paper Due in Finals Week						W5

*Indicates eLN entry required