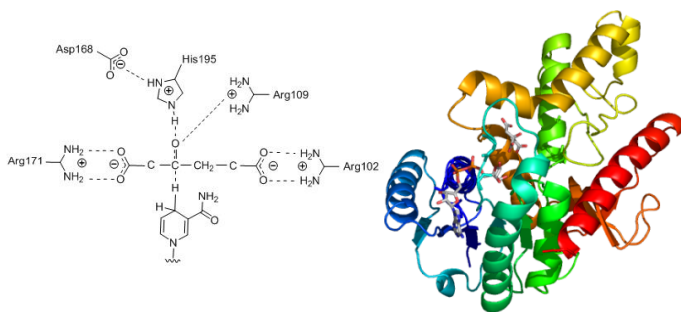




CHEM 435 Biochemistry Laboratory



Instructor: Dr. Joseph Provost
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Lab Hours: Tues/Thurs 2:30 – 6:30 pm SCST 427
Office Hours: Mon 10-11, Wed 3-4, Thurs 11-12, Fri 11-12 (in office) Tues 8-9 Zoom by appt
Contact me by text 701-306-1586 to be escorted into office. Email to make Tues appointments

Required Text: None – Assigned materials on Blackboard and on the class website

Required Materials: Lab coat, safety glasses, laptop or tablet for access to electronic lab notebook.

Course Description: The course is an advanced laboratory course that focuses 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. You will learn DNA purification and analysis, protein expression and quantification, enzyme purification, enzymatic characterization, chromatography, electrophoresis, and spectroscopic analysis. While you become skilled in all of these techniques, you will be designing your own experimental procedures and continually analyzing and evaluating your results. You will do all of this while demonstrating safe laboratory practices and keeping a complete and organized lab notebook.

Expected Learning Outcomes (Revised Summer 2020):

1. Identify and effectively use primary scientific literature.
2. Develop a testable and falsifiable hypothesis, and design appropriate experiments and controls to test hypothesis.
3. Explain and demonstrate bioinformatic and computational approaches to visualize and analyze macromolecular primary sequence and structure.
4. Explain, demonstrate, and practice various standard techniques in biochemistry.
5. Explain, calculate, and evaluate kinetic parameters of an enzyme.
6. Explain the importance of and keep an accurate laboratory notebook.
7. Use visual and verbal tools to explain concepts and data.
8. Communicate scientific results in formal written format following the guidelines of a primary literature article.
9. Work with peers to evaluate data, apply knowledge to data, and interpret data. Give and take directions to be an effective team member.

Advanced Writing Student Learning Outcomes:

Students will:

1. Write with the mastery of a student advanced in an area of study by distinguishing and responding to audiences, occasions, and discursive contexts. (*Contexts and purposes*)
2. Apply relevant and compelling content, based on mastery of assigned subjects, in order to write effectively within the area of study. (*Content*)
3. Use credible sources to develop ideas and arguments that are effective within the area of study. (*Sources and evidence*)
4. Cite sources accurately according to the conventions of the area of study. (*Sources and evidence*)
5. Write clearly and fluently in formats relevant to the area of study, with few errors in syntax and grammar. (*Mechanics*)

Course Research Project: In this course we will be conducting a research project to study the interactions between two proteins well known in carbohydrate metabolism: malate dehydrogenase (MDH) and citrate

synthase (CS). You will research, create a hypothesis and design your own experiments to test the interactions of these two interesting proteins.

Resources:

- (a) 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. This where you will find the laboratory manuals associated with each block. All assignments are posted to Bb under their respective tabs (Pre-lab, Homework, Project, Writing Portfolio). All assignments will be turned in electronically through the Bb site or the electronic lab 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.
- (b) LabArchives electronic notebook. Lab preparations, 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 are also posted. In order to access the ELN website during lab, you are required to bring a laptop or tablet with you each day.

Group work: You will be assigned to a team for the semester. In this team, you will develop a hypothesis, then 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, and debate data/ethical issues pertaining to the course materials.

Grades:

Homework, Prelabs, Quizzes 200 pts (20% of total grade)

Homework and pre-lab assignments will be turned in throughout the first half of 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. Notice of quizzes is not provided in advance.

Laboratory Notebook 200 pts (20% 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 you're 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.)

NOT MAINTAINING A LAB NOTEBOOK WILL RESULT IN AN AUTOMATIC "F."

Research Project 300 pts (30% of total grade)

For the project, you will work in groups of 2-4 to learn the structure, function protein-protein interactions and important characteristics of human mitochondrial malate dehydrogenase (hMDH2). You will research MDH, create a hypothesis based on the known structure/function of MDH, understand amino acid mutations, express and purify your proteins, and test your hypothesis. (See research project guidelines for detailed information.)

Writing Assignments 300 pts (30% of total grade)

This is a writing course and fulfills the advanced writing requirement in the major. In this course, you will develop a formal research paper based upon your research project. The components of the paper: Introduction and Hypothesis, Results, and Discussion, will be written over the course of the semester. The Materials and Methods component will 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. If revisions are offered: Must be completed within one week of graded work being returned to student.

Safety: Safety regulations require that all students working in a 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.

Course Schedule:

<u>Block I:</u>	Sept 2, 7 & 9	Basic Lab Skills (lab math, buffers, pipetting, dilutions, lab notebook)
<u>Block II:</u>	Sept 14 Sept 16	Introduction to MDH, CS, protein-protein interactions/metabolons Advanced Protein Recombinant Protein Expression, Affinity Tags, Purification and Characterization workshop
<u>Block III:</u>	Sept 21, 23, Oct 5	Bioinformatics and Protein Modeling MDH-CS Interaction Hypothesis and Mini Presentation
<u>Block IV:</u>	Oct 7, 12 & 14	Recombinant CS and MDH protein purification and characterization
<u>Block V:</u>	Oct 19 & 21	Experimental Design and Assay Development – mini presentation
<u>Block VI:</u>	Oct 26, 28 Nov 2 & 4	Determination of MDH specific activity (1 ml continuous assay) Km and Vmax determination of wild-type and mutant MDH using plate assay
<u>Block VII:</u>	Nov 9 - Dec 9	Project – Assay development of MDH-CS interactions and investigation of MDH-CS regulation.

Assignments Schedule:

	Due Date	Points
Pre-lab Assignments		
Pre-lab 1 (PL1) – Purification	Oct 7	12.5
Pre-lab 2 (PL2) – MDH Assay	Oct 26	12.5
	<i>Subtotal</i>	25
Quizzes		
Pop quizzes	N/A	50
	<i>Subtotal</i>	50
Homework		
HW1 – Solutions + dilutions	Sept 7	20
HW2 – Protein assay expt.	Sept 14	20
HW3 – Protein Expression and Purification	Sept 21	20
HW4 – Bioinformatics	Oct 14	40
HW5 – MDH kinetic assays	Nov 9	25
	<i>Sub-total</i>	125
Laboratory Notebook		
Daily Notebook Checks (5 pts ea)	N/A	100
Notebook Check I (NB1)	Oct 12	20
Notebook Check II (NB2)	Nov 16	30
Notebook Check III (NB3)	Dec 14	50
	<i>Sub-total</i>	200
Project-related Assignments		
P1 – Basic Hypothesis – presentation, group	Oct 5	20
P2 – Experimental design – written and presentation Group	Oct 21	40
P3 – Short presentation – In class, group	Nov 9	40
P4 – Progress report – Group (+ instr/peer evals)	Nov 30	100
P5 – Final presentation	Dec 14	100
	<i>Sub-total</i>	300
Writing Assignments		
W1 – Introduction & hypothesis	Oct 7	40
W2 – Introduction for Peer review	Oct 21	5
W3 – Figure Preparation	Nov 2	40
Peer Review	Nov 11	25
W4 – Results	Nov 18	40
W5 – Results/Discussion	Dec 7	50
W6 – Final paper	Dec 14	100
	<i>Sub-total</i>	300
Total Points		1000