

CHEM 463L

ADVANCED BIOCHEMISTRY LABORATORY

COURSE OVERVIEW

Biochemistry is a field that is primarily developed through experiment and observation. Conducting successful experiments requires specialized skills that can often only be acquired through practice. In this course, we will develop many of the hands-on skills that will be essential for you to be successful in a research career. Even if you don't pursue a research career, you will find that a knowledge of how experiments are conducted will help you appreciate how medicines are developed, or how experiments can help define the way organisms live.

In this course students will work together in small groups (2-3 students per group) to purify and characterize proteins from two different sources: animal muscle tissue and recombinant bacteria. Based upon these proteins, a variety of experiments will be conducted to learn more about the behavior of each protein and how it might function inside the respective organism in addition to understanding the thermodynamics of protein stability. The experiments will be conducted in a manner similar to how a graduate student might approach their thesis research, with protocol development, data collection and analysis, and extensive use of notebooks, reports, and papers.

Overall the course will be separated into two groups of experiments (projects) that last about two months each. Towards the end of each project, the students will write a draft and final lab reports. The final lab reports are expected to be 12-15 pages, including data tables, figures, and extensive referencing of journal articles and reviews. Reports must include the student's own data, and where appropriate, might include experiments that did not work correctly with a discussion of possible reasons and solutions. Grades will be heavily based upon the quality of these reports.

STUDENT LEARNING OBJECTIVES

1. Isolate proteins from animal tissues and purify proteins by affinity chromatography methods.
2. Characterize proteins using methods that determine molecular weight, quaternary structure, cofactor content, and protein identification by mass spectrometry
3. Characterize enzymes using equilibrium ligand binding, steady-state kinetics, and spectroscopy
4. Apply chemical principles to developing assays for small molecules

5. Understand how recombinant expression is used to make large quantities of protein.
6. Use circular dichroism and fluorescence to understand protein stability.
7. Use bioinformatic tools to analyze DNA sequences and Protein characteristics.
8. Maintain an accurate laboratory notebook appropriate for biochemistry research
9. Read the biochemical research literature critically
10. Develop written communication skills necessary to be a successful scientist.

Professor

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Lab Schedule

Tues Thurs
 1:30 – 5:30 pm

Teaching Assistants

Jennifer Simien
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 Bilger 208

Matt Lam
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 BilAd 302

Paulina Panek
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TEXTBOOKS

Chem 463 Lab Manual

The following books are not required, but helpful for reference. Copies will be available in Bilger 202.

1. Modern Experimental Biochemistry, R. Boyer. Benjamin Cummings 2000.
2. Biochemistry Laboratory, R. Boyer. Prentice Hall 2012.
3. Fundamental Laboratory Approaches for Biochemistry and Biotechnology, A.J. Ninfa et al. Wiley 2010.

LECTURES

There will be short lectures on important experimental topics at the beginning of each week. Attendance is required. There will also be 3-4 longer lectures throughout the course that are intended to give background on important concepts that will be applied in the lab. These lectures will be held in Bilger 242 Conference Room.

ATTENDANCE AND PARTICIPATION

Attendance at all class sessions is expected. Each unexcused absence will affect the lab notebook portion of the grade and may also affect the TA evaluation. TAs will also be asked to note when a student arrives late or leaves early, and to consider these work habits in their evaluations.

Collaboration with your lab partners is not only expected, but absolutely required. However, all students must keep their own lab notebook which will be checked and signed by the TAs each week. All students must write their own separate lab report. Extensive plagiarism from another student's lab report will result in a rejection and rewrite.

WRITING INTENSIVE COURSE

There will be two major lab reports of 12-15 pages due after each project is complete. However, the writing of these reports will take place throughout the semester.

There will be two lectures on scientific writing included in the schedule. These will discuss the major sections of a report, and will offer suggestions for style and for inclusion of published research.

Each student will write a draft lab report (at least 7 pages) due approximately two weeks before the end of each project. TAs will read these reports and offer both written and oral suggestions for improvement. Students will then revise and add additional references, data or figures. Final lab reports (12-15 pages) will have a strict deadline. Reports turned in late will receive reduced credit (1 day late, 3/4 credit; up to 1 week late, 1/2 credit; NO DRAFTS WILL BE ACCEPTED AFTER 1 WEEK).

LAB NOTEBOOKS

The TAs will sign your notebooks before and after the experiment. You should take detailed notes on experimental procedures, and record data in your notebook at every class. At the end of the semester, TAs will collect the notebooks for grading, and any experiments that are not signed will be an automatic deduction from the notebook portion of the grade.

SAFETY

Lab safety is of PARAMOUNT importance! Learning lab safety procedures protects you and your classmates. We will go over lab safety in our first session. Needless to say, but personal protective equipment such as safety

glasses or goggles and disposable gloves are absolutely required when working in the lab.

General rules to follow:

1. Never pipette by mouth or put any lab equipment near or in your mouth, nose, or eyes.
2. Dispose of all waste material properly. Sanitize or autoclave all biological waste.
3. Tell your TA if anything is broken, immediately if this creates a hazard for others.
4. No open-toed shoes in the lab (sandals, slippers, etc.).
5. No eating or drinking in the lab.
6. If you are not sure what to do, ASK!

GRADING

Draft Lab Reports (>7 pages)	10% (5% each)
Final Lab Reports (12 – 15 pages)	40% (20% each)
Final Exam	30% (no make-up exams)
Lab Notebook	10% (prelab and postlab)
TA Evaluations attendance, etc)	10% (skills, care of instruments, safety,

There will be no extra credit assignments to help raise your grade later in the semester. You need to work hard from the beginning and maintain a high standard for your work.

ACADEMIC MISCONDUCT

UH Manoa Student Conduct code Executive Policy 7.208. IV.B.1a

“Acts of dishonesty, including but not limited to the following: Cheating, plagiarism, or other forms of academic dishonesty. Cheating is an act of academic dishonesty and includes, but is not limited to: (1) use of any unauthorized assistance in taking quizzes, tests, or examinations; (2) use of sources beyond those authorized by the instructor in writing papers, preparing reports, solving problems, or carrying out other assignments; (3) the acquisition, without permission, of tests or other academic material belonging to a member of the UH faculty, staff or student body; and (4) engaging in any behavior specifically prohibited by a faculty member in the course syllabus or class discussion.

Plagiarism is also an act of academic dishonesty and includes, but is not limited to, the

use, by paraphrase or direct quotation, of the published or unpublished work of another person without full and clear acknowledgement. It also includes the unacknowledged use of materials prepared by another person or agency engaged in the selling of term papers or other academic materials.”

Plagiarism penalty: First offense, students will be asked to re-write the report without the offending material, and will be given 1/2 credit if they do so. Subsequent offense: student will receive zero credit and the matter will be referred to the Department Chair and Dean for further action.

Likewise, cheating on an exam will be dealt with severely. A student will receive no credit for the entire exam, and the offense will be reported to the Department Chair and Dean for further action.

LIST OF EXPERIMENTS

Introduction and essential skills

- 1 General lab safety, biological safety
- 2 Creation of buffers and stock solutions
- 3 Spectroscopy, use and care of instruments

Project 1 – Purification and Characterization of Chicken Lactate Dehydrogenase

- 1 Purification of LDH; tissue homogenization and $(\text{NH}_4)_2\text{SO}_4$ precipitation
- 2 Purification of LDH; affinity chromatography and dialysis
- 3 Bradford protein assay; polyacrylamide gel electrophoresis
- 4 Determination of quaternary structure by gel filtration chromatography
- 5 Development of a direct enzyme assay; Determ. of $K_M(\text{NADH})$ and k_{cat}
- 6 Determination of $K_M(\text{pyruvate})$
- 7 Enzyme inhibition: Determination of K_i for two inhibitors (oxalate/oxamate)

Project 2 – Analysis of Ribosomal Protein Subunit 6 (S6) by Circular Dichroism and Fluorescence

- 1 Bioinformatics
- 2 Cloning into *E. coli*
- 3 Making an equilibrium titration series.
- 4 Protein stability analysis by CD.
- 5 Protein stability analysis by Fluorescence.
- 6 Protein Thermal melt T_m calculations

Exp#		Topic
	8/27	Introduction to LDH experiment; General lab safety; biological safety Buffer preparation, method development for LDH purification
1-1	8/29	Purification of LDH from chicken breast tissue; ammonium sulfate precipitation
1-2	9/3	Purification of LDH; affinity chromatography
1-3	9/5	Bradford's Assay for determining protein concentration and SDS PAGE analysis of LDH
	9/10	Lecture on enzyme kinetics and inhibition; assay method development First writing workshop and data discussion
1-4	9/12-17	9/17 FIRST DRAFT DUE for LDH report (introduction and experimental sections) Direct spectrophotometric assay to determine K_M (NAD ⁺) and k_{cat}
1-5	9/19-24	9/24 RETURN First Draft of LDH Indirect coupled assay to determine K_M (lactate)
1-6	9/26-10/1	Determination of K_i for oxamate and oxalate 10/1 Second writing workshop and data discussion
	10/3	10/3 SECOND DRAFT DUE for LDH report (All sections) Lecture on recombinant expression of proteins
2-1	10/8	Transform BL21(DE3) cells with S6 DNA Bioinformatics on DNA sequence
	10/10	Lecture on protein folding
	10/15	10/10 Return Second Draft of LDH Lecture on protein stability using CD and fluorescence

2-2	10/17	Mixing GdmCl and Eq titration
2-3	10/22-10/29	10/22 FIRST DRAFT of S6 (introduction and experimental sections) 10/29 FINAL DRAFT DUE for LDH report 10/29 Return First Draft of S6 CD/Fluorescence in 2 groups of 2
	10/31	Writing workshop and data discussion
2-3	11/5-12	Thermal melt 2 groups of 2
	11/14	SECOND DRAFT of S6 report due (all sections) Final exam review Writing workshop and data discussion
	11/19	Return Final Draft of LDH
	11/21	Return Second Draft Final Exam Writing workshop and data discussion
	11/26	FINAL DRAFT of S6 report due

*Experiment

Lecture

Due Date

Category	Excellent	Average	Poor	Score
Abstract	10-9 <ul style="list-style-type: none"> • Abstract Explains the importance of the experiments.. • Makes the reader want to read the paper. • Summarizes the data found. 	8-7 <ul style="list-style-type: none"> • Abstract does not grab the attention of the reader. • Has some information on the purpose of the experiments. • Summarizes some of the data found 	6-0 <ul style="list-style-type: none"> • Abstract does not grab the attention of the reader. • Does not have the purpose of experiments. • Does not have data. 	___/10
Introduction	25-23 <ul style="list-style-type: none"> • Gives relevant background on the protein of interest. • Explains the broader impact this research gives. • Explains what experiments were conducted. 	22-20 <ul style="list-style-type: none"> • Has some background on the protein of interest. • Describes little of the broader impacts • Has some information on the experiments conducted. 	19-0 <ul style="list-style-type: none"> • Gives no background on the protein of interest. • Introduces little about experiments conducted. • Has no broader impacts. 	___/25
Methods	40-36 <ul style="list-style-type: none"> • Speaks in third person past tense consistently. • Uses scientific wording to convey how experiments were conducted including reagent concentrations. 	35-32 <ul style="list-style-type: none"> • Speaks in third person past tense some of the time. • Gives some of the important steps in series of experiment. Includes some of the concentrations used. 	31-0 <ul style="list-style-type: none"> • Does not speak in third person past tense. • Gives little methods used in the series of experiment. Does not give any concentrations of reagents used. 	___/40
Results	20-18 <ul style="list-style-type: none"> • Clearly states the data in an organized way. • Has relevant graphs to represent the data with clear 	17-16 <ul style="list-style-type: none"> • Includes some of the data obtained. • Has graphs to represent data. 	15-0 <ul style="list-style-type: none"> • Does not include all of the data obtained. • Has graphs that do not clearly represent the 	___/20

	legends.		data. Or skews the data.	
Discussion	40-36 <ul style="list-style-type: none"> Discusses all of the relevant data that was mentioned in the Results section. Clearly explains what the data means and what implications it may have. 	35-28 <ul style="list-style-type: none"> Discusses some of the data that was mentioned in the Results section. Explains what the data means and what implications it may have. 	27-0 <ul style="list-style-type: none"> Does not discuss all of the relevant data that was mentioned in the Results section. Does not explain what the data means and what implications it may have. 	___/40
Revisions	20-18 <ul style="list-style-type: none"> Critiques have been used in making changes. 	16-15 <ul style="list-style-type: none"> Some critiques have been used in making changes. 	14-10 <ul style="list-style-type: none"> Little to no critiques were used in editing this paper. 	___/20
Reference	5-4 <ul style="list-style-type: none"> Sources were relevant and used well to support the argument of the paper. 	3-2 <ul style="list-style-type: none"> Sources were used for the argument of the paper. 	1-0 <ul style="list-style-type: none"> Sources were not relevant and/or does not support the argument of the paper. 	___/5
Overall	10-9 <ul style="list-style-type: none"> The paper was excellent and could be used as an example. Spelling and grammar were used well. Captured the audience's attention and kept it. Formatting is clean and easy to follow. 	8-7 <ul style="list-style-type: none"> The paper was okay, but needs a little work. Okay spelling and grammar. Formatting is okay. 	6-3 <ul style="list-style-type: none"> The paper needs a lot of work. Needs help with spelling and grammar. Formatting has no system. 	___/10
			Total	___/170

