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. 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 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. Express proteins in bacteria and purify recombinant proteins by ion exchange chromatography methods
3. Characterize proteins using methods that determine molecular weight, quaternary structure, cofactor content, and protein identification by mass spectrometry
4. Characterize enzymes using equilibrium ligand binding, steady-state kinetics, spectroscopy, and redox titration
5. Apply chemical principles to developing assays for small molecules
6. Maintain an accurate laboratory notebook appropriate for biochemistry research
7. Read the biochemical research literature critically
8. Develop the written communication skills necessary to be a successful scientist.

Professor:	Joseph Jarrett Bilger 245 jtj@hawaii.edu 956-6721	Lab Schedule Mon Wed or Tues Thurs 1:30 – 5:30 pm	Teaching Assistants M W: Giacomo (Jack) Mazzotti & Don Tran T Th: Julie Cramer

TEXTBOOKS

Handouts will be given out prior to each lab. You are expected to read them thoroughly before lab.

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. 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; up to 2 weeks late, 1/4 of the points).

LAB NOTEBOOKS

The TAs will sign your notebooks before and after the experiment. The student will NOT be allowed to perform the laboratory exercises if pre-lab calculations and step-by-step protocols are not included in the lab notebook prior to the experiment. 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 (10 – 15 pages)	40% (20% each) (a grading rubric will be provided)
Final Exam	20% (no make-up exams; will cover both skills and theory)
Lab Notebook	20% (1% for each experiment; both pre-lab and post-lab are required)
TA Evaluations	10% (skills, theory, care of instruments, safety, attendance, general attitude, etc.)

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

Plagiarism will not be tolerated. This includes plagiarism of journal articles and reviews, and also plagiarism of another student's work. If two lab reports contain essentially the same wording, we will assume the students worked together and both students will be penalized. Note that it is expected that lab partners will work together to collect data, analyze data, and discuss the results. However, each student must write their own separate report. Penalty: First offense, student 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 refered 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 Flavodoxin and Flavodoxin Reductase

1	Bacterial expression of flavodoxin and flavodoxin reductase
2	Lysis, centrifugation, and ion exchange chromatography of FLD
3	Lysis and Ni-NTA chromatography of FNR
4	Concentration of the protein and dialysis
5	Bradford protein assay; polyacrylamide gel electrophoresis
6	Determination of the identity of the cofactors in FLD and FNR
7	FNR enzyme assay with NADPH and O_2; Determination of K_M
8	FNR enzyme assay with NADPH and FLD; Determination of k_{cat}
9	Chemical reduction of FLD; estimation of electrochem. potential
10	Electron paramagnetic resonance of FLD, ferredoxin, and cytochrome C (these additional proteins will be provided)

Project 2 – Purification and Characterization of Chicken Lactate Dehydrogenase

1	Purification of LDH; tissue homogenization and $(NH_4)_2SO_4$ 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 $K_M(NAD^+)$ and k_{cat}
6	Development of a coupled assay; Determination of K_M(lactate)
7	Enzyme inhibition: Determination of K_I for an Inhibitor
8	Tryptic digest of a protein; HPLC analysis of peptides
9	LCMS analysis of tryptic peptides; use of proteomics to identify a protein