BIBC 103: Biochemical Techniques

Fall Quarter, 2020

Instructor: Aaron Coleman, Ph.D.
abcoleman@ucsd.edu

Lecture: Mon/Weds/Fri 1 – 1:50 PM on Zoom. Log into Canvas and join each lecture through the link on the Zoom LTI page or in the Calendar. The link contains an embedded password, so you must join the lecture through this link.

You are strongly encouraged to attend lecture. While it is not mandatory, in lecture we will discuss the background to the labs and strategies for approaching the lab work and assignments. The lectures will be recorded and will be available on Canvas, but attending live gives you the opportunity to ask questions.

Labs: Mon/Weds 2:30 – 5:00 PM on Zoom. Log into Canvas and join each lecture through the link on the Zoom LTI page or in the Calendar. You will be directed to a waiting room when you enter, and your IA will admit you to the lab. You are required to use your full, real name on Zoom.

You are required to attend lab on Zoom with your video on (please contact me if you are not set up for this). You will work in groups of four to complete the lab work, and during the lab sessions you will go back and forth between your lab class of 24, and breakout rooms where you will work in your lab groups. The lab sessions will not be recorded. Some of the lab work is for credit for what is completed during the lab sessions.

Office Hours: Fridays 3:00 – 4:00 PM. Enter through Zoom link on Canvas Zoom LTI page.

BIBC 103 Remote Course Learning Objectives:
This course will introduce some of the experimental methods used in biochemistry and molecular biology, with an emphasis on those techniques used to study proteins. You will gain conceptual understanding of various protein purification techniques and methods for analyzing the different properties of proteins. The laboratory work will consist of two big multi-week projects and some shorter side projects. The lab work will emphasize the analytical and quantitative reasoning skills that are essential to work independently in a biochemistry lab.

More importantly, this course is designed to give an appreciation of what science is and how it works. Science is not just a bunch of random facts…it is a process! It is easier to understand biology, or any field, when you understand how we know what we know about it. Understanding how information in biology is brought to light is just as important as the information itself. Through the laboratory projects we will develop the skills necessary to interpret data from experiments in order to answer questions about biological systems, and to design experiments to ask new questions.

Required Textbook: BIBC 103 Biochemical Techniques Laboratory Manual, 2020-2021 edition. An eBook version of the lab manual is available for fall quarter.

Remote Course Structure **(Point values for in-lab work indicated in blue.)**

Module 1 – Quantifying Concentration of Solutions and Spectrophotometry

Lab manual pp. 5 – 11; Lab 1 part E

Lecture
- review molar concentration
- mass per volume concentration
- percent concentration
- dilutions
- components of spectrophotometer
- derive Beer's Law

Lab Calculations for Lab 1 part E **10 pts.**

Assessment Quiz on calculations for making solutions and spectrophotometry **50 pts.**

Module 2 – Electrophoresis and SDS-PAGE; Identify fluorescent proteins

Lab manual Lab 2 parts A, B, and part C through step 2; Lab 13 part A

Videos
- Running an SDS-PAGE gel
- Fluorescent protein expression and purification

Lecture
- Introduction to electrophoresis
- Introduction to fluorescent proteins

Lab
- Calculations for preparing SDS-PAGE samples **10 pts.**
- You will be assigned two unknown fluorescent proteins to identify, and will be provided absorption spectra and pictures of SDS-PAGE gels to do this. **40 pts. (20 pts. each)**

Assessment Quiz on electrophoresis, SDS-PAGE, and lab work **50 pts.**

Module 3 – Enzyme purification: You will choose an enzyme for which you will design a purification strategy. You will compare and contrast the purification strategy for your enzyme to the purification strategy for lactate dehydrogenase (LDH), the enzyme purified in the hands-on BIBC 103 labs.

Part 1

Lab manual Lab 3 parts A and B

Lecture

- Introduction to protein purification strategies
- Isolation of cellular compartments and organelles by centrifugation
- ammonium sulfate precipitation

Lab

- Choose enzyme to purify; why is this enzyme interesting to you? What is the starting material? **5 pts.**
- Decide on initial purification and centrifugation steps for your enzyme. How and why is this different from the LDH purification (or why the same)? **10 pts.**

Part 2

Lab manual Lab 4 parts A and C, Lab 16 part B

Video Running a size exclusion chromatography column

Lecture

- introduction to column chromatography
- brief introduction to affinity chromatography
- quantifying binding affinity by dissociation constant
- Cibacron blue affinity column purification of LDH; column loading, elution, and collection of fractions
- Epitope tag (His-tag) purification of recombinant proteins

Lab Come up with affinity purification for your enzyme; compare to LDH affinity purification; try to find affinity resin **10 pts.**

Part 3

Lab manual Lab 8 parts A and B

Lecture

- What determines net charge on proteins? Isoelectric point
- LDH isozymes

Lab

- Look up pI values for LDH isozymes
- Identify LDH isozymes from pictures of native gel electrophoresis **20 pts.**
- Find amino acid sequence for your enzyme
- Look up pI value for your enzyme **10 pts.**

Part 4

Lab manual Lab 5 part A; Lab 8 part D

Lecture

- Ion exchange chromatography

Lab

- Design size exclusion chromatography purification for your enzyme; which resin/why? **10 pts.**
- Design ion exchange chromatography to separate LDH isozymes **10 pts.**
- Design ion exchange chromatography to purify your enzyme **10 pts.**

Part 5

Lab manual Lab 6 all (skip part G)

Video LDH activity assay

Lecture

- Enzyme activity assays
- Bradford protein assay
- Calculations for LDH purification table

Lab

- Analyze activity assay and Bradford protein assay data from LDH purification; do calculations for purification table
- Design an activity assay for your enzyme (does not have to be spectrophotometric) **20 pts.**

Part 6

Lab Come up with overall purification strategy for your enzyme

Assessment

- Write up purification strategy for your enzyme with rationale for each step, including starting material and details for each step **100 pts.**

- Purification table from LDH purification data; critique how successful it was and suggest changes to improve the strategy **150 pts.**
- Quiz on protein purification techniques **50 pts.**

Module 4 – Analysis of fibroblast growth factor (FGF) signaling in NIH 3T3 cells

Lab manual Lab 9B parts A – D; Lab 11 parts A – E; Lab 12 parts A – D

Lecture

- Project introduction; unanswered questions about FGF signaling
- Ras-Erk signaling from FGFR; activation of Erk by phosphorylation
- Introduction to antibodies; polyclonal vs. monoclonal; primary vs. secondary; signal-producing conjugates
- Overview of Western blotting
- Phospholipase C signaling from FGFR
- Competition ELISA detection of IP1

Lab

- Synchronous quiz on interpreting Lab 9B data **10 pts.**
- Interpret the data presented in Lab 9B part B, come up with questions
- Form hypothesis to explain signal transduction leading to effects of FGF-2 in NIH 3T3 cells
- Come up with predictions and design Western blot (p-Erk) and ELISA (PLC) to test **20 pts.**
- Receive Western blot and ELISA data; interpret, work on lab report
- Work on proposal presentation

Assessment

- Lab report; testing hypothesis and interpretation of data **250 pts.**
- Group presentation of research proposal **105 pts.**
- Quiz on antibodies, ELISA, and Western blotting **50 pts.**

Point values for grade determination

Activity	Point Value
Quizzes (4 x 50 points each)	200
Lab activities	195
Written work: LDH purification table analysis	150
Written work: Write-up of purification strategy for your enzyme	100
Written work: FGF signaling lab report	250
Research project: Group presentation of FGF research proposal	105
Total	**1000**

Point Cutoffs for Grade Assignments: (Cutoffs may be lowered at the instructor's discretion.)

910-1000	A	790-799	C+
900-909	A-	705-789	C
890-899	B+	695-704	C-
810-889	B	600-694	D
800-809	B-	0-599	F

Course Web Site:
All course materials will be accessed through the course webpage on Canvas. Be sure to check Canvas frequently for announcements and updates on assignments.

Quizzes:
There will be four quizzes, one for each module of the class, and covering the information in that module as described in the course structure section of the syllabus. The quizzes will be delivered through Canvas. They will be asynchronous, and you will have a 48-hour window in which to take the quiz. Once you begin the quiz, you will have 60 minutes to complete it. When taking the quizzes, you may use the BIBC 103 lab manual, and any notes that you have prepared yourself, including your answers to the problem set questions. You may not communicate with other students during the quiz, and you may not utilize the internet (Canvas should be the only site open on your browser). Once you have completed the quiz, you are forbidden from discussing it in any way with other students until the 48-window is over. You are expected to conduct yourself with integrity in completing the quizzes and other assignments for the class.

Lab Attendance Policies:
Attendance on Zoom at each lab session is mandatory. An unexcused absence will result in 10 points being deducted. If you know that you need to miss a lab session, discuss this with the instructor (not the IA, they are not authorized to give you permission) to see if it will be possible to make up the lab session or excuse you from the lab with no consequences. Please bring this to the instructor's attention as soon as you know that it will be an issue. **Only the instructor can excuse an absence. Two unexcused absences will result in the student failing the course.**

Turning in Written Work:
All written work for the class (New Enzyme Purification Strategy, LDH Purification Table Analysis, and the FGF Signaling Lab Report) will be submitted through Canvas, and is due by the end of the day (11:59 PM) on the due date indicated in the lab schedule (see below). Ten points will be deducted for each day that the lab report is late. Students agree that by taking this course all required papers will be subject to review for textual similarity by Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin service is subject to the terms of use agreement posted on the Turnitin site.

Regrade Policy for Written Work: Your work will be graded by your IA, based on specific guidelines. I work closely with all the IAs to ensure that the grading is accurate and equivalent between sections. If you disagree with the grading of your work, discuss this with your IA to get clarification on why points were deducted. If you still disagree with the grading you may submit the report to me for a re-grade. This must be done within one week of receiving the graded report. I will re-grade the entire report and give you a new score, and this is the score that will be recorded.

Lab Schedule

The timing of the scheduled lab exercises is tentative. More time may be given for some exercises if necessary.

Week	Day	Activity
1	Mon 10/5	Organize groups; Module 1: Lab 1 part E calculations
	Weds 10/7	Module 2: Calculations for preparing electrophoresis samples (Lab 2, part C, table for step 2); Determine unknown fluorescent proteins
2	Mon 10/12	Module 2: cont.
	Quiz 1 Window Tues 10/13 9 am to Thurs 10/15 9 am	
	Weds 10/14	Module 3: choose enzyme to purify; determine initial centrifugation steps and compare to LDH
3	Mon 10/19	Module 3: design affinity chromatography purification; compare to LDH affinity purification
	Weds 10/21	Module 3: determine LDH isozymes from native gel electrophoresis; look up new enzyme amino acid sequence and isoelectric point
4	**Quiz 2 Window Mon 10/26 9 am to Weds 10/28 9 am**	
	Mon 10/26	Module 3: design size exclusion chromatography purification, compare to LDH purification; design ion exchange chromatography to separate LDH isozymes; design ion exchange chromatography for new enzyme
	Weds 10/28	Module 3: Get LDH enzyme activity and Bradford assay data, analyze for purification table
5	Mon 11/2	Module 3: Design enzyme activity assay for their enzyme and work on overall purification strategy
	Tues 11/3	**LDH Purification Table Analysis Due**
	Weds 11/4	Module 4: quiz at beginning of lab; interpret Lab 9B part B data; come up with questions
6	Mon 11/9	Module 4: form hypotheses to explain lab manual data, make predictions based on that hypothesis and design Western blot and ELISA experiments to test
	Weds 11/11	Holiday, no labs; **Purification Strategy for Your Enzyme Due**
7	**Quiz 3 Window Mon 11/16 9 am to Weds 11/18 9 am**	
	Mon 11/16	Module 4: receive Western blot data, interpret
	Weds 11/18	Module 4: receive ELISA data, interpret
8	Mon 11/23	Module 4: work on lab reports
	Weds 11/25	Holiday, no labs
9	Mon 11/30	Module 4: work on proposal presentations
	Tues 12/1	**FGF Signaling Lab Report Due**
	Weds 12/2	Module 4: work on proposal presentations
	Quiz 4 Window Weds 12/2 9 am to Fri 12/4 9 am	
10	Mon 12/7	Module 4: group presentations
	Weds 12/9	Module 4: group presentations