BIBC 103: Biochemical Techniques

Winter Quarter, 2021

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Lecture: Tues/Thurs 12:30 – 1:50 PM Lectures will be synchronous 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: Tues/Thurs 2:00 – 4:30 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: Mondays 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 three multi-week projects and some shorter activities and exercises. 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. The print edition of the lab manual is available at the UCSD Bookstore.

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

Module 1 – Quantifying Concentration of Solutions and Spectrophotometry

Lab manual pp. 5 – 11; Lab 1 part E

Relevant Concepts

- review molar concentration
- mass per volume concentration
- percent concentration
- dilutions
- spectrophotometry
- Beer's Law

Lab

- **Pipetting video**
- Calculations for Lab 1 part E; this involves converting absorbance values to molar concentration using Beer's Law, and then converting these to percent concentration. **10 pts.**

Assessment

- Quiz 1: Calculations for making solutions and spectrophotometry **50 pts.**

Module 2 – Electrophoresis and SDS-PAGE

Lab manual Lab 2 parts A, B, and part C through step 2; Lab 8 parts A and B

Video (watch before lab)

- SDS-PAGE; YouTube BioRad Mini-Protean video

Relevant Concepts

- General properties of electrophoresis
- SDS-PAGE
- Isoelectric point and protein net charge

Lab

- **Live demo sample prep for SDS-PAGE with calculations**
- Do calculations to complete table for Lab 2 part C, step 2 **10 pts.**
- Analysis of native, agarose gel electrophoresis to determine LDH isozymes **20 pts.**

Assessment

- Quiz 2: Electrophoresis, SDS-PAGE, and lab work **50 pts.**

Module 3 – Lactate dehydrogenase (LDH) purification

Lab 3

Lab manual Lab 3 parts A and B

Relevant Concepts

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

Lab

- **Live demo of homogenization, initial centrifugation, and ammonium sulfate precipitation**

Lab 5 first and then Lab 4

Lab manual Lab 4 parts A and C; Lab 5 part A

Relevant Concepts

- Size exclusion chromatography
- Affinity chromatography

Lab

- **Live demo size exclusion chromatography**

Lab 6

Lab manual Lab 6 all (skip part G)

Video (watch before lab)

- LDH activity assay

Relevant Concepts

- 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

Assessment

- Written assignment—LDH purification table analysis **150 pts.**
- Quiz 3: Protein purification techniques **50 pts.**

Module 4 – Analysis of FGF signaling in NIH 3T3 cells

Lab manual Lab 9B all; ; Lab 10 parts A and B; Lab 11 parts A, C, and D; Lab 12 parts A and B

Video (watch before lab)

- YouTube BioRad Trans-turbo electroblotting video

Relevant Concepts

- What are the unanswered questions about FGF signal transduction?
- 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 Lab 9B data **10 pts.**
- Interpret Lab 9B part B data, come up with questions about the data **10 pts.**
- 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.**
- Western blot sample calculations
- Receive Western blot and ELISA data; interpret, work on lab report
- Work on proposal presentation

Assessment

- Written assignment—Research article-style lab report on FGF signal transduction in NIH 3T3 cells **225 pts.**
- Group presentation of research proposal **150 pts.**
- Quiz 4: Antibodies, Western blotting, ELISA and lab work **50 pts.**

Module 5 – Bioinformatics, analysis of protein structures and protein crystallization

Lab manual Lab 18 and Lab 19

Relevant Concepts

- X-ray crystallography
- B-Raf structure and functional domains
- Kinase domains

Lab

- Lab 19 part A
- **Live demo of hanging drop preparation**
- Lab 18 part C; prepare crystals round 1 (staff will prepare the drops)
- Lab 18 part D; examine crystals round 1
- Lab 18 part C; prepare crystals round 2 (staff will prepare the drops)
- Lab 18 part D; examine crystals round 2 **Improve crystals 20 pts.**
- Lab 19 parts C and D

Assessment

- Quiz 5: Bioinformatics, protein crystallization, and kinase domains **50 pts.**
- Bioinformatics Lab 19 questions and PyMOL images **125 pts.**

Point values for grade determination

Activity	Point Value
Quizzes (5 x 50 points each)	250
Lab activities	100
Written work: LDH purification table analysis	150
Written work: FGF signaling lab report	225
Research project: Group presentation of FGF research proposal	150
Bioinformatics module	125
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 five 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 taken 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:
The two written assignments for the class (LDH Purification Table Analysis and the FGF Signaling Lab Report) will be submitted through Canvas, and are 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 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	Tu 1/5	Organize groups; Module 1: Pipetting video, Lab 1 part E calculations
	Th 1/7	Module 2: **Live demo SDS-PAGE sample prep.;** Calculations for preparing electrophoresis samples (Lab 2, part C, table for step 2);
2	Tu 1/12	Module 2: Determine LDH isozymes from agarose gel electrophoresis
	Quiz 1 window Weds 9 am – Fri 9 am	
	Th 1/14	Module 5: Bioinformatics Lab 19 part A
3	Tu 1/19	Module 5: **Live demo hanging drop preparation**; Prepare crystals round 1 (Lab 18 part C)
	Th 1/21	Module 3: **LDH Lab 3 live demo**
4	**Quiz 2 window Mon 9 am – Weds 9 am**	
	Tu 1/26	Module 3 **Live demo size exclusion chromatography**
	Th 1/28	Module 3: Analyze activity assay and Bradford assay data; prepare purification tables
5	Tu 2/2	Module 3: Work on LDH purification table analysis
	Th 2/4	Module 5: Examine crystals round 1; prepare crystal round 2
	Fri 2/5	**LDH Purification Table Analysis due**
6	**Quiz 3 window Mon 9 am – Weds 9 am**	
	Tu 2/9	Module 4: Quiz on Lab 9B data; examine lab manual data, come up with questions
	Th 2/11	Module 4: Form hypothesis and design Western blot and ELISA experiments
7	Tu 2/16	Module 4: Calculations for Western blot samples; receive and interpret data
	Th 2/18	Module 4: Receive and interpret ELISA data
8	Tu 2/23	Module 5: Bioinformatics Lab 19 part C
	Th 2/25	Module 5: Examine crystals round 2
	Quiz 4 window Fri 9 am – Sun 9 am	
9	Tu 3/2	Module 5: Bioinformatics Lab 19 part D
	Wed 3/3	**FGF Signaling Lab Report due**
	Th 3/4	Module 4: Work on research proposal presentations
10	**Quiz 5 window Mon 9 am – Weds 9 am**	
	Tu 3/9	Module 4: Group presentations on research proposals
	Th 3/11	Module 4: Group presentations on research proposals