

BIBC 103: Biochemical Techniques

Spring Quarter, 2021

Instructor: Goran Bozinovic, Ph.D.

gbozinovic@ucsd.eduOffice Hours: by appointment *via* Zoom

Lecture: Mon*, Wed, Fri 9-9:50 AM

Labs: CO1,02: Wed, Fri 10AM – 1:50 PMCO3,04: Wed, Fri 2:30 - 6:20 PMLog in to “live” lectures and labs *via* Zoom:<https://ucsd.zoom.us/j/96388829260?pwd=ZkxOT2I5eTVmYUF2WkE2S0RENlhtQT09>

Meeting ID: 963 8882 9260

Password: sp103c

Lab Section	Instructional Assistant	Email
C01	Hoda Ahmed	hahmed@ucsd.edu
C02	Enlin Wei	enwei@ucsd.edu
C03	Runtian Jiang	ruj016@ucsd.edu
C04	Armando Vazquez	arv012@ucsd.edu

Lab Sections Virtual Lab Participation: Regardless of your Section enrollment (C01/2 or C03/4), please use the respective Zoom link above to join the virtual Labs. IAs and the instructor will be available to answer questions during virtual lab sessions.

Course Objectives:

This course will introduce various laboratory techniques used in biochemistry and molecular biology. These will include methods for purifying proteins, expressing recombinant proteins in bacterial cells, and analyzing biological molecules by electrophoresis, Western blotting, and enzyme activity assays. Laboratory work will consist of three multi-day projects, as well as some smaller, single-day experiments. The importance of good experimental design, including the use of appropriate controls, will be highlighted in all experiments. Also, as this is an introductory lab course, all lab work will emphasize the learning of basic lab skills (including dilutions, good pipetting technique, and basic statistical analysis) and good lab practices (such as good notebook keeping).

Lectures will be held live *via* Zoom beginning Monday, March 29th @ 9AM. Note that after the 1st lecture on Monday, Jan 4th, the lectures will be held on Wednesdays and Fridays, since we have enough time to cover conceptual topics and continue discussions during lab sessions. Hence, the only Monday we will meet is the March 29th. Recordings of each Zoom lecture and .pdf lecture slides will be available throughout the quarter on Canvas in weekly modules. You are encouraged to attend “live” lectures as they will be interactive, you will be able to ask to questions / participate in discussion and the material covered often goes beyond the laboratory manual information.

Virtual Laboratory Sections will begin on Wednesday, March 31st @ either 10AM or 2:30PM. *via* Zoom. During the virtual labs you will work on experimental designs and protocols outlined in the laboratory manual, perform calculations, data analysis and bioinformatics modules that will help you master the course material. Use the same zoom link for lectures to access the virtual lab sections.

Online Classroom and Virtual Lab Zoom Etiquette: Keep your line muted during classes unless the instructor / IA calls on you to ask a question. If you have a question, please use the “raise hand”

function under the “participants” tab. Enabling your video is optional during lectures. Zoom etiquette for section discussions/labs are up to the discretion of your IA.

- Make sure your line is muted unless you are prompted to ask a question.
- Asking questions: Please ask questions! Student discussion during lectures is vital to course effectiveness. Use the “Raise Hand” option on zoom to notify me that you have a question. Lectures will be “paused” periodically to allow for your questions and/or clarification
- We may not have an opportunity for traditional class discussions on an online platform. If you have a comment or question, please be considerate of class time. To make sure all the questions are addressed, the last 10-15’ of each lecture will be reserved for review and discussion.

Materials Required for lab everyday:

- 1) Biochemical Techniques Lab Manual (available from the Bookstore)
- 2) Calculator

Assignments - Due Dates:

<u>HW 1</u>	Wed, Wk 3 (by midnight – via “Assignments” upload)
<u>HW 2</u>	Fri, Wk 5 (by midnight - via “Assignments” upload)
Bioinformatics	Fri, Wk 9 (by midnight - via “Assignments” upload)
<u>Lab Report</u> – FGF2:	Wed, Wk 10 (by midnight via email and TurnItIn - CANVAS)
<u>Group Presentation</u>	Wed, Fri, Wk 10 via Zoom

EXAMS:

Midterm: Friday, May 7th, 8:30-11AM PST
Final: Friday, June 4th, 8:30—11AM PST

Course Requirements and Grading: Your final grade for the class will be calculated using the following criteria:

Exams – 100, 120	220
Lab Report	120
Bioinformatics – Lab 19	20
Homework (2) – 20, 30	50
Pre-Lab Quizzes (3 at 5 pts. each)	15
Manuscript or method group presentation	40
Total Points	465

% Point Cutoffs for Grade Assignments: (cutoffs may be lowered at the instructor’s discretion)

>92	A	78-79	C+
90-91	A-	72-77	C
88-89	B+	70-71	C-
82-87	B	60-69	D
80-81	B-	<60	F

Course Web Site:

Many of the course materials are available only through the course website CANVAS (<https://canvas.ucsd.edu>). All students will need to access this site. Once you are enrolled in the class you will have access to the site using your ACS username and password. Be sure to check the course website frequently for announcements and updates on assignments. Items such as lab report guidelines and image files of gels and other data will be provided through the website. The

'Additional Materials' folder contains additional background material for some of the experiments. Use the Discussion Board to ask questions on material from lecture or lab. The IAs will check the Discussion Board daily to answer questions, but students are encouraged to answer questions also. This is a handy resource for last minute questions that come up during late night studying for an exam.

Lab Manual and Quizzes: It is important to carefully read the pertinent sections of the lab manual before joining virtual labs via Zoom. The experiments will "proceed" more efficiently, and you will learn more if you have read through the procedure and understand why and what you are doing. To emphasize the importance of reading the lab manual before virtual labs, there will be four scheduled quizzes at the beginning of lab.

Virtual Lab Attendance Policies:

Lab Sections Virtual Lab Participation: Regardless of your Section enrollment (C01,2 or C03,4), please use the same Zoom link to join the virtual Labs. Both section IAs and the instructor will be available to answer questions during virtual lab sessions.

Participating in virtual lab sessions is mandatory. If you are more than 10 minutes late logging in to e-lab, or you leave the lab meeting before your group is finished, you will be counted as absent for the day. An unexcused absence will result in 10 points being deducted from the associated lab report. 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 arrange possible "make up" lab session or be excused 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.**

Participation: everyone is expected to be an active participant in every experimental procedure. Failure to make a meaningful contribution towards "completing" the laboratory experiment/activity will result in points being deducted from the laboratory report score.

Turning in Lab Reports:

We will be using the "Turnitin" *via* CANVAS for lab reports. Lab reports submitted to the TritonEd / Turnitin site do not need to have graphs, tables, or attachments, but you may include them if it is easier. Lab reports must be submitted before midnight of the due date. Lab reports and not submitted via CANVAS by the end of the day will be considered one-day late. Ten points will be deducted for each working day that the lab reports are late (hard copy and Turnitin.com). Students agree that by taking this course all required papers will be subject to review for textual similarity by Turnitin for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com 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.com site.

*Detailed lab report guidelines will be posted on CANVAS two weeks after the course starts.

Lecture Exams: There will be TWO lecture exams this quarter: midterm (week 6) and final (week 10). To minimize the time constraint, you will be allowed 2.5 hrs for the exams. Each exam will be available on CANVAS at 8:30 AM on the scheduled date; your work must be submitted via Gradescope by the stated deadline. Late submissions will not be considered for grading.

Make-up Exams:

Please note that it is extremely burdensome for the instructor and IAs to prepare make-up exams. Missing a scheduled exam will only be excused for medical reasons if documentation is provided. At the instructor's discretion, a missed exam that is excused will either be dropped from the

student's point total for the class or made up by an oral exam scheduled within one week of the original exam.

Students' Group Manuscript Presentation via Zoom: Your lab partner and you will present a 10-12 min seminar (+ 5 min for questions) during week 10. The topic will be an original science manuscript or a molecular biology method(s) of your choice, relevant to a course content and pre-approved by the course instructor. The detailed information about the format of your seminar will be posted on CANVAS after the midterm exam.

Academic Integrity

Cheating will not be tolerated. The administrative policy on Academic Dishonesty outlined by UCSD will be followed. Students caught cheating during an exam or quiz will be given a "zero" for that assignment. A report will also be filed with the Academic Integrity Coordinator. Cheating includes (but is not limited to) plagiarism and making use of forbidden materials during the test. Tampering with graded exams will result in a failing grade for that exam.

During laboratory sessions, student cooperation and collaboration is highly encouraged. This includes discussion of experimental data with fellow students during lab hours. After the virtual laboratory session is over, however, you are required to work on your own. ***Each student must submit an independently written and independently thought-out data analysis for each lab report / homework assignment.***

It is NOT acceptable to use any old lab reports to assist you in any way. If you happen to be in possession of old copies of lab reports for this class, it is best that you do not even look at them, since they could unintentionally influence the way that you write your own report. If we discover that you have used an old lab report in any way, you will automatically receive a "zero" for that lab report, and you might be reported to the Academic Integrity Coordinator.

While your lab reports will be returned to you, you are NOT permitted to share them with anyone for any reason. If we find that you have shared your lab report with anyone, you will be reported to the Academic Integrity Coordinator, even if you have already completed the class. You are required to read, understand, and sign the "BIBC103 Integrity Policy" which governs the way the academic work in this class is completed.

See the Virtual Lab Schedule on Next Page.

Virtual Lab Schedule:

Week	Day	Activity
1	W	Organize groups; Module 1: Pipetting video, Lab 1 part E calculations
	F	Module 2: demo SDS-PAGE sample prep. ; Calculations for preparing electrophoresis samples (Lab 2, part C, table for step 2);
2	W	Module 2: Determine LDH isozymes from agarose gel electrophoresis
	F	Module 5: Bioinformatics Lab 19 part A
3	W	Module 5: demo hanging drop preparation ; Prepare crystals round 1 (Lab 18 part C) Homework 1 Due
	F	Module 3: LDH Lab 3 demo
4	W	Module 3 demo size exclusion chromatography
	F	Module 3: Analyze activity assay and Bradford assay data; prepare purification tables
5	W	Module 3: Work on LDH purification table analysis
	F	Module 5: Examine crystals round 1; prepare crystal round 2 Homework 2 Due
6	W	Module 4: Quiz on Lab 9B data; examine lab manual data, come up with questions
	F	Module 4: Form hypothesis and design Western blot and ELISA experiments Midterm Exam
7	W	Module 4: Calculations for Western blot samples; receive and interpret data
	F	Module 4: Receive and interpret ELISA data
8	W	Module 5: Bioinformatics Lab 19 part C
	F	Module 5: Examine crystals round 2
9	W	Module 5: Bioinformatics Lab 19 part D
	F	Module 4: Work on research proposal presentations Bioinformatics Answers Due
10	W	Module 4: Group presentations FGF2 Lab Report Due by Midnight
	F	Module 4: Group presentations Final Exam – Friday June 4th

BIBC103_Spring Quarter 2021 Curriculum

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.

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

Videos

- [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
- Analysis of native, agarose gel electrophoresis to determine LDH isozymes

Module 3 – Lactate dehydrogenase 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

- [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

- [demo size exclusion chromatography \(combine with lecture\)](#)

Lab 6

Lab manual Lab 6 all (skip part G)

Video [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 (sequential); do calculations for purification table

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

- [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

- Interpret Lab 9B part B data, come up with questions about the data
- 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
- Western blot sample calculations
- Receive Western blot and ELISA data; interpret, work on lab report
- Work on proposal presentation

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
- **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 (step 1 only; pictures sent to students)
- Lab 18 part C; prepare crystals round 2 (staff will prepare the drops)
- Lab 18 part D; examine crystals round 2 (step 1 only; pictures sent to students)
- Lab 19 parts C and D