# BIBC 103: Biochemical Techniques

**Fall Quarter, 2021**

**Instructor:** Aaron Coleman, Ph.D.
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**Office Hour:** York 2300 conference room, Thursday 2 – 3 pm

**Lecture:** Mon/Weds/Fri 2 – 2:50 pm. Lectures will be held remotely 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:** York Hall 3306 and 3406; Tues/Thur 8 am – 12 noon. Labs are in-person and there is not a remote option for the lab sessions.

**Course 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 and hands-on experience in various protein purification techniques and methods for analyzing the different properties of proteins. The laboratory work will consist of three big multi-week projects and some shorter side projects. All the lab work will emphasize mastery of the skills that are essential to work independently in a biochemistry lab, including hands-on wet-lab and quantitative reasoning skills.

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. In keeping with this, the importance of good experimental design, including the use of appropriate controls, will be highlighted in all experiments.

**Materials Required:**
1) Biochemical Techniques Lab Manual, 2020 – 2021 Edition (available from the Bookstore)
2) Bound laboratory notebook (see Canvas for information on the type of notebook required)
3) Safety glasses
4) Lab coat

**Course Structure**

| Activity | Point Value |
|---|---|
| LDH Purification: Lab notebook | 35 |
| LDH Purification: Purification table analysis | 100 |
| Sea Urchin Fertilization Signaling: Lab notebook | 30 |
| Sea Urchin Fertilization Signaling: Lab report | 240 |
| Bioinformatics: Lab manual questions | 30 |
| Bioinformatics: PyMOL protein image | 40 |
| Fluorescent Proteins: Lab notebook | 30 |
| Fluorescent Proteins: Identify unknowns | 20 |
| Lab Practical: Assemble and load SDS-PAGE gel | 10 |
| Quizzes: Four at 35 points each | 140 |
| Exam 1 | 100 |
| Exam 2 | 225 |
| **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:**
Except for the lab manual, all course materials will be accessed through the course webpage on Canvas. Much of the data you generate in your experiments will be accessed through Canvas, in addition to lab report guidelines and practice problem sets for quizzes and exams. Be sure to check Canvas frequently for announcements and updates on assignments.

**Lab Quizzes and Exams:**
All quizzes and exams will be taken in person during the lab sessions. The purpose of the lab quizzes is to be sure you are mastering the basic concepts behind your experiments as we go through the class. This includes understanding the purpose of the lab projects and how each experiment fits into this, the basic concepts underlying the procedures, and simple mathematical and analytical skills based on what you have actually done in lab. The quiz

dates are given in the lab schedule. Quizzes will be given at the beginning of lab, will take 15 – 30 minutes, and will consist of 5 to 7 questions. The topics that will be covered on each quiz will be posted in an announcement on Canvas on the Monday prior to the quiz.

The two exams are cumulative and will be problem solving-based. They may include some basic questions on the concepts we have covered, but will emphasize taking the information you have learned and extrapolating to solve problems you have not seen before. Practice questions will be provided on Canvas to help you prepare for the exams.

**Lab Attendance Policies:**
In-person attendance at each lab session is mandatory. If you test positive for COVID-19 or feel ill, however, stay home and contact the instructor by email. If you test-positive for COVID-19 and must isolate, we will work with you to keep you in the class. 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. An unexcused absence will result in 10 points being deducted. **Only the instructor can excuse an absence. Two unexcused absences will result in the student failing the course.**

**Turning in the Lab Report:**
Lab report and data write-ups will be submitted electronically on Canvas. Lab reports are due before the end of the day (11:59 pm) on the due date. Ten points will be deducted for each day following the due date 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.

**Lab Report Grading and Regrade Policy:** Your lab report will be graded by your IA, based on the lab report 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 lab report, 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

| Week | Dates | Experiment/Activity | Lab Manual Chapter |
|---|---|---|---|
| 0 | Sep. 23 | Enrollment and safety orientation | |
| 1 | Sep. 28 | Introduction to equipment, pipetting and dilution skills | Lab 1 |
| | Sep. 30 | Introduction to SDS-PAGE<br>**Quiz 1** | Lab 2 |
| 2 | Oct. 5 | Lactate dehydrogenase (LDH) purification 1: Initial purification of LDH from crude homogenate: centrifugation, ammonium sulfate precipitations | Lab 3 |
| | Oct. 7 | LDH 2: Affinity chromatography purification | Lab 4 |
| 3 | Oct. 12 | LDH 3: Size exclusion chromatography purification | Lab 5 |
| | Oct. 14 | LDH 4: Analyze purified LDH: Enzyme activity assays and Bradford protein assays | Lab 6 |
| 4 | Oct. 19 | LDH 5: More analysis: SDS-PAGE of LDH purification fractions<br>**Quiz 2** | Lab 7 |
| | Oct. 21 | Examine sea urchin fertilization, prepare cell lysates for Western blot and ELISA | Lab 9A |
| | Oct 22 | **LDH purification table analysis due on Friday**; submit to Canvas by 11:59 pm | |
| 5 | Oct. 26 | **Exam 1 in lab** | |
| | Oct. 28 | Sea urchins: MAPK Western blot—SDS PAGE and electroblotting | Lab 10 |
| 6 | Nov. 2 | Sea urchins: MAPK Western blot—Immunodetection | Lab 11 |
| | Nov. 4 | Sea urchins: ELISA for phospholipase C activity | Lab 12 |
| 7 | Nov. 9 | Bioinformatics 1: Investigation of an unknown melanoma gene<br>**Quiz 3** | Lab 19, part A |
| | Nov. 11 | Veterans Day Holiday, no lab | |
| 8 | Nov. 16 | Bioinformatics 2: Modeling protein structures using PyMOL;<br>Select protein for PyMOL image project | Lab 19, parts B – D |
| | Nov. 17 | **Sea urchin fertilization signaling lab report due on Weds**; submit to Canvas by 11:59 pm | |
| | Nov. 18 | Purification and analysis of fluorescent proteins (FP 1): Make competent cells and transform with plasmid for recombinant protein expression;<br>Work on PyMOL image project | Lab 13 Intro and parts A and B only; Lab 15 |
| 9 | Nov. 23 | FP 2: His-tag purification of FPs; spectroscopy to determine unknowns<br>**Quiz 4** | Lab 16 |
| | Nov. 25 | Thanksgiving Holiday, no lab | |
| 10 | Nov. 30 | FP 3: SDS-PAGE of purified FPs | Lab 17 |
| | | **PyMOL protein image due on Tues**; submit to Canvas by 11:50 pm | |
| | Dec. 2 | **Exam 2 in lab** | |