BIBC 103: Biochemical Techniques

Spring Quarter, 2023

Instructor: Aaron Coleman, Ph.D.

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Office Hour: Mondays 2 – 3 pm in York 3080D (my office) and York 2300 (conference room

on ground floor). We will start in my office and move to the conference room if

we need more room.

Lecture: Sequoyah Hall 147 Tu/Th 12:30 – 1:50 pm.

Labs: York Hall 3306 and 3406; Tu/Th 2 – 5:50 pm

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 two 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, 2022 2023 Edition (available from the Bookstore)
- 2) Safety glasses
- 3) Lab coat

Course Structure

| Activity | Point Value |
|--|-------------|
| LDH Purification: Lab notebook | 35 |
| LDH Purification: Purification table analysis | 100 |
| Drosophila Alcohol Metabolism: Lab notebook and statistical analysis | 55 |
| Sea Urchin Fertilization Signaling: Lab notebook | 30 |
| Sea Urchin Fertilization Signaling: Lab report | 220 |
| Bioinformatics: Lab manual questions | 50 |
| Bioinformatics: PyMOL protein structure | 50 |
| Lysozyme crystallization: Lab notebook | 20 |
| Lysozyme crystallization: Improve crystals | 20 |
| Quizzes: Four at 30 points each | 120 |
| Exam 1 | 100 |
| Exam 2 | 200 |
| Total | 1000 |

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

| 910-1000 | Α | 790-799 | C+ |
|----------|----|---------|----|
| 900-909 | A- | 705-789 | С |
| 890-899 | B+ | 695-704 | C- |
| 810-889 | В | 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 <u>Canvas</u>. 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 Notebooks:

Careful documentation of your experiments is critical to working effectively in science. You will keep an electronic lab notebook that is a Google doc. It should be a record of everything you are doing in lab. It will contain all of your raw data as well as any analysis you do. All of your data and analysis needs to go into your notebook, even if you are also presenting it in a separate written assignment. Read the lab notebook instructions on Canvas carefully. You will create your lab notebook and submit the URL to Canvas after the first lab session. You will be provided with an MS Word document template that you can copy and paste into your Google doc for the first project. After that, you should follow the same format for the other projects.

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 <u>based on what you have actually done in lab</u>. 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 6 questions. <u>The topics that will be covered on each quiz will be posted in an announcement on Canvas on the Monday prior to the quiz.</u>

The two exams are cumulative and are focused on having you use what you are learning in the class at a level that requires application and analysis. They may include some basic

questions on the concepts we have covered, but will emphasize using the knowledge and skills you have developed for more in-depth problem solving. 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 caught up 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 reports 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 reports 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

| | Dates | Experiment/Activity | Lab Manual Chapter |
|-------|--|--|------------------------------------|
| Wk 1 | Apr. 4 | Enrollment and safety orientation; Lab skills and equipment exercises | Safety information and Lab 1 |
| | Apr. 6 | Introduction to SDS-PAGE | pp. 1 - 11 Lab 2 |
| Wk 2 | Apr. 11 | LDH 1: Initial purification of LDH from crude homogenate: centrifugation, ammonium sulfate precipitations; prepare size exclusion column | Lab 3 |
| VVK Z | Apr. 13 | LDH 2: Affinity chromatography Quiz 1 | Lab 4 |
| Wk 3 | Apr. 18 | LDH 3: Size exclusion chromatography | Lab 5 |
| VVK 3 | Apr. 20 | LDH 4: Activity assays; Bradford protein assays | Lab 6 |
| | Apr. 25 | LDH 5: SDS-PAGE of LDH purification fractions | Lab 7 |
| Wk 4 | Apr. 27 | LDH 6: Native gel electrophoresis of LDH with activity stain; Set up lysozyme crystallization 1 Quiz 2 | Lab 8 Lab 18 |
| | May 1 | LDH Purification Table Analysis Due Monday at 11:59 PM | |
| Wk 5 | May 2 | Sea urchin fertilization, prepare cell lysates | Lab 9A |
| | May 4 | Exam 1 in Lab | |
| Wk 6 | May 9 | MAPK Western blot—SDS PAGE and electroblotting | Lab 10 |
| VVK | May 11 MAPK Western blot—Immunodetection | | Lab 11 |
| Wk 7 | May 16 | ELISA for phospholipase C activity; Examine lysozyme crystals 1; set up lysozyme crystallization 2 | Lab 12 Lab 18 |
| | May 18 | Work up ELISA data; make figures for lab report Quiz 3 | Lab 12 |
| | May 22 | Fertilization Signaling Lab Report Due Monday at 11:59 PM | |
| Wk 8 | May 23 | Fly Lab 1: Sort flies and prepare assays | Canvas pdf |
| | May 25 | Fly Lab 2: Ethanol Mobility Behavior Assay; alcohol dehydrogenase activity assays; Bradford assays | Canvas pdf |
| Wk 9 | May 30 | Fly Lab 3: Statistical analysis of data; determine substrate specificities for fly and yeast ADH; Examine lysozyme crystals 2 | Canvas pdf Lab 18 |
| | June 1 | Bioinformatics 1 Quiz 4 | Lab 19 part A |
| Wk | Jun. 6 | Bioinformatics 2; Begin PyMOL protein structure assignment | Lab 19 pts B-D |
| 10 | Jun. 8 | Exam 2 in Lab; finish PyMOL protein structure | |