



**Chemistry 3560 – Introduction to Biochemistry Laboratory**  
**The University of Toledo**  
**College of Natural Sciences and Mathematics**

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**Instructor:** Timothy C. Mueser  
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**Office Hours:** M, W, F 11:00 am - 12:00 p.m. or  
by appointment  
**Office Location:** WO4211  
**Office Phone:** 419-530-1510  
**Term:** Spring 2022  
**Lab Location:** BOL 2089  
**Credit Hours:** 2

**CHEM 3560-001 CRN:14351**  
8:30 a.m. - 11:20 a.m., Tues.-Thur.  
Teaching Assistant: Veronica Martin  
**CHEM 3560-002 CRN:11172**  
1:00 p.m. - 3:50 p.m., Tues.-Thur.  
Teaching Assistant: Tomi Idowu  
**CHEM 3560-004 CRN:11175**  
1:00 p.m. - 3:50 p.m., Wed.-Fri  
Teaching Assistant: Matt Lohman

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#### **COURSE/CATALOG DESCRIPTION**

Practice of biochemistry laboratory techniques.

#### **STUDENT LEARNING OUTCOMES**

The laboratory is designed to provide the necessary skills for independent research. Students completing Chemistry 3560 will gain proficiency in techniques used in modern biochemistry research laboratories and the theory behind them. Skills include preparation of solutions, use of UV/visible spectrophotometry in assays of protein concentration and enzyme activity, various types of gel electrophoresis, analysis of ligand binding, collection and analysis of enzyme kinetic data, and protein purification techniques (centrifugation, fractional precipitation, ion exchange chromatography, affinity chromatography, size exclusion chromatography).

New this year is the use of LabArchives Electronic Lab Notebook (ELN). The course will be migrating to the new format during the semester and changes may occur to the scheduling of quizzes, homework, etc. The ENL is cloud based and can be accessed outside the lab.

#### **PREREQUISITES AND COREQUISITES**

C grade or better in CHEM 3510

#### **REQUIRED TEXTS AND ANCILLARY MATERIALS**

Lehninger Principles of Biochemistry 8<sup>th</sup> Ed. David L. Nelson; Michael M. Cox. ISBN-13: 978-1319228002, ISBN-10: 1319228003

LabArchives Electronic Lab Notebook

Approved safety glasses.

#### **ACADEMIC ACCOMMODATIONS**

The University of Toledo is committed to providing equal access to education for all students. If you have a documented disability or you believe you have a disability and would like information regarding academic accommodations/adjustments in this course, please contact the [Student Disability Services Office](#). The



University is an equal opportunity educational institution. University Policy # 3364-50-03 Nondiscrimination on the Basis of Disability Americans with Disability Act Compliance. [https://www.utoledo.edu/policies/administration/diversity/pdfs/3364\\_50\\_03\\_nondiscrimination\\_o.pdf](https://www.utoledo.edu/policies/administration/diversity/pdfs/3364_50_03_nondiscrimination_o.pdf)

## LABORATORY REQUIREMENTS AND POLICIES

**1. Attendance and absences** - As this is a laboratory course, attendance, preparation and participation are required. Students must attend all laboratory sessions, arrive on time, and be ready to begin by the start time. Some sessions will require the entire three hours, while others may be shorter. Students arriving late may not have time to complete the day's procedures and may not be permitted to work in the lab that day at the instructor's discretion. To accommodate unavoidable excusable absences (as well as inclement weather), some lab periods may be designated as make-up sessions (as indicated on the schedule). If you know in advance of a schedule conflict that requires you to miss all or part of a lab, inform Dr. Mueser and your TA as early as possible. In the case of an emergency or other unanticipated absence, contact Dr. Mueser and your TA by phone or email as soon as you can. The availability of makeup for labs missed for reasons other than excusable absences will be decided on a case-by-case basis. University Missed Class policy # 3364-71-14. <https://www.utoledo.edu/policies/academic/undergraduate/pdfs/3364-71-14%20Missed%20class%20policy.pdf> )

**2. Preparation** - Students must be prepared prior to the lab. You must read the experimental handouts and assigned papers before the lab session. As indicated on the schedule, there will be brief quizzes based on this material at the beginning of the session.

**3. Resources** - Handouts for the occasional lectures, assigned reading, experimental handouts and other background material on the experimental techniques will be available in LabArchives ELN. Students are expected to use other resources (including looking up research articles from the literature and obtaining sequence and structural data from on line databases) to prepare for experiments and in the writing of lab projects and reports. The first week of class will include a brief introduction to the on-line biochemical literature and databases, and some useful links will be posted.

**4. Laboratory Safety** - Students must comply with all departmental, University, State and Federal laws and policies regarding safety. All individuals in the lab must wear appropriate safety equipment. No food or drink is allowed in the laboratory. Tobacco and related products are banned from anywhere on campus. A closed bottle of water or another beverage may be kept in the cubbies by the door where coats and bags are stored, but you must go out into the hall to drink from it. Ensure that you wear clothing appropriate for a laboratory. You will not be allowed into the lab if you are wearing clothing that creates a safety concern. Shorts and open-toed shoes are not permitted. Failure to adhere to these policies or follow the directions of your instructors will lead to you being dismissed from the laboratory for the day without the opportunity to make up the work; repeated violations may lead to an F in the course.

<https://www.utoledo.edu/depts/safety/>

**Covid19 Precautions** - To prevent the transmission of Covid19, you must wear a face mask that covers the mouth and nose at all times; details of the requirements for mask and social distancing can be found at:

([https://www.utoledo.edu/depts/hr/pdfs/Masks\\_and\\_Face\\_Coverings\\_Requirement.pdf](https://www.utoledo.edu/depts/hr/pdfs/Masks_and_Face_Coverings_Requirement.pdf)).

This is a requirement for all areas of the campus, but is particularly important in this course since maintaining distance in the lab will be difficult. To the extent possible, stay at least 6 feet from your coworkers in the laboratory. If you have symptom such as a dry cough, fever, loss of taste or smell, or feel unwell, you should report this to me immediately and get tested; there is additional information on p. 13 of this handout regarding testing; you will not be allowed in the laboratory until your test comes back negative. If you think you have symptoms or if you think you have been

exposed, you should get tested. To minimize the chance of exposure you will work in pairs for most assignments with two groups per bench.

**5. Laboratory records.** Each student will be provided with a laptop for access to the electronic lab notebook. The laptops can be carried to the workstations taking extreme care and returning them to the cabinet for recharging after each session. The ELN entries should be a record of procedures, data and observations in the laboratory. Data and calculations should be written directly in your ELN which will provide a timestamp (do not write elsewhere and copied into your notebook later). Photos and screen captures (of chromatograms or photographs of gels, for example) should also be uploaded into your notebook. The notebook should include calculations associated with the experiments. For example, volumes of stock solutions used to prepare working solutions at various concentrations. Preparations of buffers, reaction mixtures and other solutions with multiple components can be given in a table with each component and the volume/amount required. Include enough narrative so that you or someone else can repeat what you did several weeks after doing the experiment. Write the description of experiments such that a different person could repeat what you did based on what is recorded in the notebook.

Useful links on lab notebooks are listed below:

<http://colinpurrington.com/tips/academic/labnotebooks>

<http://www.ruf.rice.edu/~bioslabs/tools/notebook/notebook.html>

**6. Lab Partners** All experiments will be carried out in groups of two as assigned by the teaching assistant. You will be responsible for equitable division of labor for the laboratory procedures. Each group will be assigned bench space, storage, and lab equipment including pipettes, a pH meter, chromatography columns with tubing and a peristaltic pump, test tube racks, etc. Collaboration between lab partners should be limited to completing procedures and collect data in class, and discussion of the data outside of class. You must do your own processing and analysis of data, and complete homework assignments and lab reports independently. The same text in two reports will be treated as plagiarism.

**7. Academic honesty** – As is customary, cheating and plagiarism that violate principles of academic integrity will not be tolerated. Copying or adaptation of the text, data, and figures of another person without proper attribution is plagiarism and is unacceptable. You will work with partners to carry out experiments and will collect your data as a team. However, your ELN will require separate entries for data collected and analyzed. Data tables and graphs that required some form of analysis must be completed in each separate ELN. Students who turn in the work of another student as their own or plagiarize published sources may receive a grade of 0 for the assignment; a repeat offense may lead to a grade of F for the course. Anyone who cheats on a quiz will get an F for the quiz; a second offence may result in an F in the course. Plagiarism on one of the two major projects or cheating on the final exam will result in a grade of F for the course. See the university Academic dishonesty policy 3364-71-04.

<https://www.utoledo.edu/dl/students/dishonesty.html>

**8. General Laboratory Tidiness** – Each group will be assigned a drawer for storage of materials. The workstation should be left in proper order. Make sure that you put away all materials and supplies when you finish working in an area and that you **clean up any spilled liquid or chemicals**. Do not leave cuvettes in spectrophotometers or tubes and racks next to instruments. Be sure to **turn off the lamps** on spectrophotometers when you finish; the lamps have a limited life and are expensive to replace. You may have points deducted for slovenly technique or behavior.

**9. Resources** – There are a number of programs and online resources that you will need to use.

**National Center for Biotechnology Information, NCBI**

This site has links to a variety of sites with reference information, data bases and various tools for structural and sequence analysis. <https://www.ncbi.nlm.nih.gov/>



**Protein Data Base** - This site is a source of proteins sequences with links to other sites with additional structural information. <https://www.ncbi.nlm.nih.gov/protein>

**RCSB PDB Protein Data Bank** -This site is a source of protein structures determined primarily by X-ray diffraction. <https://www.rcsb.org/>

**Expasy Bioinformatics Resource Portal and Protein Chemistry** These sites has links to a number of different sites for a wide range of analyses.

<https://www.expasy.org/>  
[https://molbiol-tools.ca/Protein\\_Chemistry.htm](https://molbiol-tools.ca/Protein_Chemistry.htm)

**Peptide Property Calculator and Protein Calculator v3.4** - These are sites that have applications that can be used to calculate molecular weight, isoelectric point, charge, extinction coefficients and a number of other molecular properties of proteins.

<http://biotools.nubic.northwestern.edu/proteincalc.html>  
<http://protcalc.sourceforge.net/>

**Prism/Graph Pad** This a program for plotting fitting data; you will have access to a copy that you may load on one computer. You will receive an email GraphPad-Prism inviting you to access to the program; it will only be active for this semester for students enrolled in the course; it will cease to work on May 14, 2022. You can make simple plots, fit data, perform simple statistical analysis and considerably more complicated analyses. During the course your TAs will show you how to use the program for your assignments; you will need it to plot and analyze data you collect in your experiments.

**Pymol** - This program allows one to use PDB coordinate files to make representations of macromolecules. It is available on computers on room 2051.

**Rasmol** - This is another program for viewing structures using PDB coordinate files. It has fewer features compared to Pymol, but is quite simple to use. It can be downloaded from:

<http://www.openrasmol.org/>

## LAB EXERCISES

There will be several experiments focusing on mastering a specific technique or set of techniques. Each exercise will have a short quiz and a lab reports to be turned in (5 points for the quiz, 5 points for the lab report).

**1. Buffers and Solutions** - Students will prepare a variety of solutions from stock solutions prepared by your teaching assistant. To gain access to the solutions, you will need to have approved calculations for the preparation of both stock and diluted solutions. The pH meters and pipettes will be verified for accuracy. Students will train in the use of micropipettes to practice handling of small volumes of liquid and do basic data analysis.

**2. UV/Visible Spectrophotometry** - Students will review principles of spectrophotometry and operation of the available spectrophotometers. Students will generate and plots relating for relating

absorbance to concentration, record absorbance spectra of various proteins and determine extinction coefficients.

**3. The Protein Determination** - Students will learn assays for the quantitation of total protein in a sample of material of unknown concentration. You will also prepare standards for SDS PAGE electrophoresis to estimate the size and purity of the proteins using SDS PAGE.

**4. Ligand binding** - Students will learn how to perform ligand binding experiments and analyze the data.

## Projects

There will be three major projects that will take several weeks to complete.

**1. Enzyme assays and Enzyme kinetics – (alcohol dehydrogenase)** – Students will learn how to perform various types of enzyme assays and perform a detailed analysis of the kinetics of commercially available. Data will be collected using spectrophotometers and plate readers.

**2. Protein Purification (lactate dehydrogenase and aspartate amino transferase)** - Students will learn various chromatographic and batch methods for purification of proteins from a.) a natural source (lactate dehydrogenase from beef heart) using fractionations and gel chromatography and b.) a recombinant source (aspartate amino transferase expressed in *E. coli*) using affinity chromatography.

**3. Protein Analysis** – Students will analyze their purified proteins for purity using SDS PAGE, activity using enzyme kinetics, identity using MALDI-MS peptide fingerprinting, molecular weight using size exclusion chromatography on the FPLC, and isoelectric focusing if time permits.

## Course Expectations

**Quizzes** - At the beginning of each lab, students will verify their preparedness for that day including calculations, videos, handouts, etc. Quizzes based on the content of assigned reading, handouts and protocols for the exercise. are indicated on the schedule; there will no make-up quizzes. The lowest score will be replaced by the average of the highest and lowest score; legitimate excused absences will not be included in the grade calculation. However, the missed work must be completed to continue.

**Lab Reports** - There will be lab reports (due as indicated on the schedule) that summarize what was done during that session including the results. A specific template will be provided. Laboratory reports will be graded on content and clarity as well as the precision and accuracy of your data. The reports on lab projects must be typed using a text editor and the final version submitted into your ENL. The content of the lab report should be based on the information contained in your laboratory notebook.

**Project Reports** – There will be projects that will be more extensive, requiring up to three weeks to complete. A much longer, detailed report will be required for these projects. There will be detailed instructions for writing these reports.

**Grading:** Total points for the semester will be based on scores on lab reports, project reports, quizzes, technique as perceived by your TA, and the final exam. Scores for individual components of the grade will



be weighted as indicated in what follows. There will be quizzes (5 points), lab reports (15 points), and project reports (45 points). The score for assignments (except with an appropriate excuse) will be decreased 20% for each lab day they are late; they will not be accepted beyond two lab days from the due date.

The standard college grading scale will be used and adjustments, if necessary, will be based on the performance of the three lab sections:

	A $\geq$ 93%	92% > A- $\geq$ 90%	
89% > B+ $\geq$ 87%	86% > B $\geq$ 82%	81% > B- $\geq$ 79%	
78% > C+ $\geq$ 76%	75% > C $\geq$ 71%	70% > C- $\geq$ 68%	
67% > D+ $\geq$ 65%	64% > D $\geq$ 60%	60% > D- $\geq$ 58%	57% > F

<b>Scoring for Quizzes, Lab Reports and Project Reports</b>	
<b>Lab Reports and Quizzes</b>	
Problems, lab report Week 1	20 points
UV/Visible spectrophotometry, Quiz and lab report Week 2	20 points
Protein concentration determination, Quiz and lab report Week 3	20 points
Ligand binding, Quiz and lab report Week 4	20 points
Enzyme assays, Quiz and lab report Week 5	20 points
<b>Project Reports</b>	
Enzyme Kinetics, Quiz Week 6	50 points
Protein Purification, Quiz Week 10	50 points
Protein Characterizations and Analysis, Quiz Week 12	50 points
<b>Final Exam</b>	30 points
<b>Attendance, Preparation, Lab Technique</b>	20 points
<b>Total</b>	300 points

<b>Chemistry 3560 Schedule, Spring, 2020</b>		
	<b>Experiment</b>	<b>Assignments Due</b>
<b>Week 1, January 18 – 22</b>		
Day 1	Introduction to Biochemistry Lab Safety Training The ELN laboratory note book Solution preparation	
Day 2	Buffers and solutions Pipetting, balances, pH measurement	<b>Problem Set #1</b>
<b>Week 2, January 25 – 28</b>		
Day 1	<b>Quiz 1</b> , Introduction to GraphPad/Prism UV/Vis Spectrophotometry: review Beer-Lambert law	
Day 2	UV/Vis Spectrophotometry: taking spectra Plotting and analyzing data	<b>Problems set #2</b> <b>Lab report Week 1</b>
<b>Week 3, February 01 – 04</b>		
Day 1	<b>Quiz 2</b> , Protein concentration determination	
Day 2	Protein concentration determination	<b>Lab report Week 2</b>
<b>Week 4, February 08 – 11</b>		
Day 1	<b>Quiz 3</b> , Dye binding to BSA	
Day 2	Dye binding to BSA	<b>Lab report Week 3</b>
<b>Week 5, February 15 – 18</b>		
Day 1	Enzyme assays	
Day 2	<b>Quiz 4</b> , Enzyme Assays	<b>Lab report Week 4</b>
<b>Week 6, February 22 – 25</b>		
Day 1	<b>Quiz 5</b> , Enzyme Kinetics	
Day 2	Enzyme kinetics	<b>Lab report Week 5</b>
<b>Week 7, February 28 – March 04</b>		
Day 1	Enzyme kinetics	
Day 2	Enzyme kinetics and Data analysis	
<b>Week 8, Spring Break, March 07 – 11</b>		
<b>Week 9, March 15 – 18</b>		
Day 1	Purification of MW markers and SDS PAGE electrophoresis	
Day 2	<b>Quiz 6</b> Purification of lactate dehydrogenase	<b>Enzyme Kinetics Report</b>
<b>Week 10, March 22 – 25</b>		
Day 1	Purification of lactate dehydrogenase	
Day 2	Purification of lactate dehydrogenase	
<b>Week 11, March 29 – April 01</b>		
Day 1	Purification of lactate dehydrogenase	
Day 2	Purification of lactate dehydrogenase	
<b>Week 12, April 05 – 08</b>		
Day 1	Purification of lactate dehydrogenase	
Day 2	Purification of aspartate amino transferase	

<b>Week 13, April 12 – 15</b>		
Day 1	<b>Quiz 7</b> , Purification of aspartate amino transferase MALDI protein finger printing	
Day 2	FPLC Size exclusion chromatography and Isoelectric focusing (IEF)	
<b>Week 14, April 19 – 26</b>		
Day 1	FPLC Size exclusion chromatography and Isoelectric focusing (IEF)	<b>Purification of Protein Report</b>
Day 2	FPLC Size exclusion chromatography and Isoelectric focusing (IEF)	
<b>Week 15, April 26 – 29</b>		
Day 1	FPLC Size exclusion chromatography and Isoelectric focusing (IEF)	
Day 2	<b>Lab Closeout, Final exam</b>	
<b>Week 16, May 03 – 04, Final exam week</b>		
Day 1		<b>Characterization of Protein Report</b>