Advanced Biological Chemistry

The University of Toledo
Department of Chemistry and Biochemistry
CHEM 4500/6500/8500 and MBC 6550/8550
CRN - 44955, 46401, 44970, 44975, 41568, 41648

Instructor:	Matthew Wohlever	**Offered**:	Fall 2021
Email:	matthew.wohlever@utoledo.edu	**Course Website**:	Blackboard Learn
Office Hours:	MW 2:00 – 3:50 P.M. (remote)	**Class Location**:	Bowman-Oddy 2059
Office Location:	Wolfe Hall 3210B	**Class Day/Time**:	MW 10:00 – 11:50 A.M.
Instructor Phone:	419-530-8401	**Credit Hours**:	4

CATALOG/COURSE DESCRIPTION

The chemistry of cellular and molecular transformations in biochemical systems. Molecular structure of proteins, nucleic acids, and membranes.

COURSE OVERVIEW/ TEACHING METHODOLOGY

This course will teach you how to use chemistry and biochemistry to address problems of biological significance. I will challenge you in this course to move beyond the memorization and simple application of facts to the point where you can use your knowledge to evaluate arguments in the primary literature and design experiments to advance or disprove this model.

There are things you need to memorize (such as amino acids, basics of protein structure, key experimental techniques etc.) as they are fundamental to the analysis we will be doing, but that is not the focus of the course. A good analogy is that you need to have a decent vocabulary in order to write a novel. The focus of this course is on "writing a novel", not on learning the "vocabulary". If you are struggling with "vocabulary", you will need to bring yourself up to speed with this outside of class. Use your resources (office hours, text books, the library, Wikipedia, review articles, primary literature). Learning to teach yourself this "vocabulary" is an incredibly important skill that you will use throughout your life and research career. The course will be difficult not because I want to be mean, lazy, or unfair (I will provide the resources necessary for you to succeed in this course) but because this is the best way to prepare you for the tribulations of scientific research and life in general. I am training you to be my peer, and you will be treated as such. Therefore, we will be on a first name basis in the classroom.

This is an advanced course that is designed to prepare you for academic research in a biochemical lab. To receive a top grade in this course, you must move beyond learning facts and instead creatively combine what you have learned to solve research problems (i.e. design and/or trouble shoot experiments). At the end of this course, you should know how to use biochemical tools to address questions of biological significance. You should also be comfortable reading and critically evaluating the primary literature. This will require you to know the underlying assumptions with experiments and determine what conclusions can properly be made from experimental results. This course will be divided into five sections. Section 1: Molecular structure of proteins. Section 2: Kinetics and thermodynamics of proteins. Section 3: Enzymes. Section 4: Lipids and membrane proteins. Section 5. Nucleic acids

STUDENT LEARNING OUTCOMES

A. Summarize key points in a biochemistry research article

*Required

B. Describe assumptions underlying experimental approach and evaluate if conclusions are adequately supported by data
C. Determine if experimental logic justifies the conclusions of a biochemistry research article
D. Use macromolecular structure, kinetics, thermodynamics, and molecular biology techniques to design logical experiments to test a hypothesis
E. Develop a hypothesis/model for how a biological system functions based on data from macromolecular structure, kinetics, thermodynamics, and molecular biology experiments
F. Use molecular viewing software (PyMOL) to examine a protein structure and generate a hypothesis for molecular function
H. Develop appropriate controls to show that the experiment is working as intended
I. Apply "rules of thumb" for quickly determining experimental feasibility
J. Describe expected results for experiments conducted in bulk solution compared to single molecule experiments.

PREREQUISITES AND COREQUISITES

None. However, this course will be challenging for anyone who had not passed an undergraduate biochemistry course.

TEXTS AND ANCILLARY MATERIALS

There is not a required text for this course. Most of the reading will be available as handouts that will be posted on Blackboard. Any general biochemistry text can be purchased if you have not had a biochemistry course previously. The syllabus will occasionally reference the Lehninger biochemistry text book. You can get this either from the University of Toledo bookstore or buy it online from a variety of retailers. The library also has many versions of biochemistry texts available.

TECHNOLOGY REQUIREMENTS

Due to the Covid-19 pandemic, we may need to do small group activities remotely using blackboard collaborate ultra. Office hours will also take place on Blackboard collaborate ultra. There will be some graded quizzes on blackboard to gauge comprehension of the journal club articles.

Students will also need to download PyMOL for class. You can download the software at the links below.

https://pymol.org/2/#download

License File is available on Blackboard. This is an education only license and should only be used during class. Please do not share the license or pymol builds with anyone else.

The website for this course is available on blackboard, so you can access it with your UTAD credentials. The main page can be found at: https://blackboard.utdl.edu
You can download copies of the syllabus (if you happen to lose this one).

ACADEMIC POLICIES

Academic Misconduct

I take academic misconduct very seriously. Graded problem sets are, by their very nature, open note/book. However, **students must work individually on graded problem sets**. **You are NOT allowed to consult with others for graded problem sets FOR ANY REASON**. If you need clarification on a question, please contact me. Do not put yourself or your fellow students in a compromising position by asking questions about the problem sets. All students will be required to sign an honor code for each graded assignment stating that the work is his/her own and that s/he will report any cases of academic dishonesty.

One of the most rewarding parts of college, especially graduate school, is the connections you form with your classmates. Your classmates are your future colleagues and having a broad network is vital to your success in any career, especially academic research. Therefore, you are strongly encouraged to work together on the UNGRADED problem sets. Students may also meet to discuss the papers prior to our in class journal clubs. **However, students may NOT discuss the research article or the written peer review of the article that will serve as the final exam**. Assignments will be checked for plagiarism using Safe Assign.

Undergraduate Policies: http://www.utoledo.edu/policies/academic/undergraduate/
Graduate Policies: http://www.utoledo.edu/policies/academic/graduate/

COURSE EXPECTATIONS

Attendance, participation, and make-ups

Students are expected to attend every lecture and pay attention. Students are expected to carefully read any reading material administered during the semester. Attendance will not be formally taken, but pop quizzes and classroom participation are part of your grade.

There will be **NO** make-up examinations. An excused absence will be allowed only for justified and ***thoroughly documented purposes***. Lack of preparation, vacation, personal problems, transportation difficulties, job conflicts, alien abduction, etc. do not constitute acceptable reasons for missing an examination.

Discussion boards

If you have a question about the course material, it's likely that your classmates do too. Rather than e-mailing me with a question about material, please post your questions in the group discussion section on blackboard. If you e-mail me a question about the course material, I will answer it by posting in the discussion section of blackboard. For more personal matters, such as grades, students are of course welcome to contact me by e-mail. The purpose of this policy is to allow me to be a better teacher by reducing my e-mail burden. Furthermore, one of the best ways to master material is to teach it to others. You are strongly encouraged to monitor class discussions on blackboard and respond when you know the answer.

OVERVIEW OF COURSE GRADE ASSIGNMENT

Your final grade will be determined by the total number of points earned on graded problem sets, quizzes, literature discussions, exams, and peer review of an article in the primary literature. I will also provide ungraded problem sets for students. While these do not need to be turned in, it is *strongly recommended* that the students complete *all* of the problem sets. Below is a tentative breakdown of points for the semester, but this is subject to change.

For your final exam you will be assigned a paper from the current literature to evaluate as if you are a peer reviewer for a journal that is considering a paper for publication. You will critically evaluate the quality of the experiments and determine if the author's claims are fully supported by the data. This will be turned in as a short (~ 2 page) written assignment.

Late Assignments

Assignments are due at the beginning of class. Assignments turned in after the beginning of class are accessed a 10% penalty for being late. Assignments are then accessed an additional 10% penalty for each day that it is late. For example, if a 100 point assignment is due in class on Monday and is submitted on Tuesday, a 20 point penalty is accessed. If you need to miss class, you may submit your assignment electronically or in person BEFORE the start of class.

Points

Graded problem set 1 (Sections 1 & 2)	100 points
Graded problem set 2 (Sections 2 & 3)	100 points
Flash Talks	100 points

*Required

Quizzes and participation in literature discussion	100 points
Literature Review (Comprehensive)	100 points

*Additional graded assignments may be assigned as needed. For example, pop quizzes or participation in classroom discussion may become a larger portion of the final grade if there is insufficient participation in literature days or the class shows a lack of preparation.

Grading

Your final grade will be based on your percentage of the total points possible. A breakdown of the grade cut-off percentages are as follows:

A - Total score is ≥ 85%
B - Total score is between 75 and 84%
C - Total score is between 65 and 74%
D - Total score is between 55 and 64%
F - Total score is < 55

The cut-off percentage for grades at the end of the semester may decrease, but will not increase.

Midterm Grading will reflect your performance grades when the midterm grades are due. This usually includes the first problem set and a couple of journal clubs.

UNIVERSITY POLICIES

Institutional Classroom Attendance Policy

Please be aware that the university has implemented an attendance policy, which requires faculty to verify student participation in every class a student is registered at the start of each new semester/course. For this course, if you have not attended/participated in class (completed any course activities or assignments) within the first 14 days, I am required by federal law to report you as not attended. Unfortunately, not attending/participating in class impacts your eligibility to receive financial aid, so it is VERY important that you attend class and complete course work in these first two weeks. Please contact me as soon as possible to discuss options and/or possible accommodations if you have any difficulty completing assignments within the first two weeks.

Policy Statement on Non-Discrimination on the Basis of Disability (ADA): The University is an equal opportunity educational institution. Please read The University's Policy Statement on Nondiscrimination on the Basis of Disability Americans with Disability Act Compliance. Students can find this policy along with other university policies listed by audience on the University Policy webpage (http://www.utoledo.edu/policies/audience.html/#students).

Academic Accommodations: The University of Toledo embraces the inclusion of students with disabilities. We are committed to ensuring equal opportunity and seamless access for full participation in all courses. For students who have an Accommodations Memo from the Office of Accessibility and Disability Resources, I invite you to correspond with me as soon as possible so that we can communicate confidentially about implementing accommodations in this course.

For students who have not established accommodations with the Office of Accessibility and Disability Resources and are experiencing disability access barriers or are interested in a referral to health care resources for a potential disability, please connect with the office by calling 419.530.4981 or sending an email to StudentDisability@utoledo.edu.

ACADEMIC AND SUPPORT SERVICES

Please follow this link to view a comprehensive list of Student Academic and Support Services (http://www.utoledo.edu/studentaffairs/departments.html) available to you as a student

SAFETY AND HEALTH SERVICES FOR UT STUDENTS

Please use the following link to view a comprehensive list Campus Health and Safety Services available to you as a student.

INCLUSIVE CLASSROOM STATEMENT

In this class, we will work together to develop a learning community that is inclusive and respectful. Our diversity may be reflected by differences in race, culture, age, religion, sexual orientation, gender identity/expression, socioeconomic background, and a myriad of other social identities and life experiences. We will encourage and appreciate expressions of different ideas, opinions, and beliefs so that conversations and interactions that could potentially be divisive turn, instead, into opportunities for intellectual and personal development.

COURSE SCHEDULE

#	Date	Topic	Examples & Case study	Experimental Techniques & Rules of Thumb	Activities Graded & Ungraded	References:
Section 1 – Molecular Structure of Proteins						
1	8/30	Introduction/review of basic biochemistry • Syllabus, clicker • Instructions for downloading PyMOL • Introduction to the primary literature • Basics of protein structure • Amino acids • Basics of protein folding				Key #s in Biology Slides Lehninger 1-142 (focus on 115-142)
2	9/1	In vitro study of proteins • Cloning • Protein expression • Protein purification PyMOL tutorial		Protein Cloning Protein Expn Protein Purify	Clicker - syllabus Pymol tutorial	Pymol tutorial handout
	9/6	No Class, Labor Day				
3	9/8	Protein structure techniques • NMR • X-ray crystallography • Cryo-EM • Co-variation analysis • XL-MS • Integrative Structure Determination	YidC	NMR X-tal XL-MS Cryo-EM Co-variation m, u, n, p prefixes		Lehninger 313-331 Rout & Sali Cell 2019
Section 2 – Kinetics & Thermodynamics						

4	9/13	1st Order Reactions, Energy & Equilibrium
		• Protein folding
		• Rate laws & kinetic constants
		  ○ Rate constant graph from data
		  ○ Derive integrated sltn
		• Boltzman distribution
		• Reaction coordinate diagrams
		• Kinetics (k) vs. Equilibrium (K)
		Experimental techniques for measuring 1st order reactions
			Protein G, B1 domain	How to measure kf/ku
				Limited proteolysis
				Diffusion limit=10^9
				W fluorescence
				Extrapolate for $K_{u/f}$
				If K=1
				Then $\Delta G = 1.4$ kcal/mol
						Simple kinetics & equilibrium Handout #1
						Lehninger 143-151

*Required

		• How to measure K_{eq}				
5	9/15	Experimental techniques for measuring 1st order reactions • How to measure k_f & k_u • Half-life Experimental techniques to study protein folding • Point mutants, transition state, and protein folding • Reaction coordinate diagrams • Chevron plots Kinetics & thermodynamics for protein folding • Populated vs. unpopulated intermediates How to read a paper • You need to think differently • Methods, Supplement • Split into groups, quickly summarize a figure	Protein G, B1 domain Is there an intermediate in protein folding?	Extrapolate for $k_{u/f}$ Chevron plot Single molecule protein folding HD exchange (mass spec & NMR)	Ungraded Pset #1 Post after class	Simple kinetics & equilibrium Handout #1
6	9/20	Journal Club #1 – Cryo-EM of ribosome quality control			Reading Quiz Journal club #1 Post key for Ungraded Pset #1 after class	Shen et al. 2015
7	9/22	Simple binding kinetics & equilibria • How to measure k_{on}/k_{off} ○ Initial rate ○ Pseudo dimerization ○ Pseudo first order ○ How to prevent rebinding • K_D ○ Derive ○ K_D and fraction bound ○ Data from filter binding assay • Stoichiometry binding experiments vs. K_D Effective concentration Review for Pset	Lac Operon Competing models for how repressor works Protein G & Fab	Stoichiometry binding experiments 1 nM = 1 copy per E. coli	Graded Pset #1 given	Multistate kinetics & equilibrium handout #2 Lehninger 158-162
8	9/27	• Experimental techniques for measuring binding ○ Separation ○ Non-separation based techniques		EMSA IP Filter binding FRET Anisotropy SEC ITC	Graded Pset #1 due	Phizicky & Fields Microbio. Rev. 1995
9	9/29	Testing competitive vs. non-competitive inhibition with: • Structure • Kinetics • Equilibrium Double mutant cycles	Lac Operon Competitive vs. non-competit. inhibition			Lehninger 1155-1175

*Required

10	10/4	Journal Club #2 – Single molecule protein folding on the ribosome			Reading Quiz Journal club #2	Nilsson et al. 2015
11	10/6	Complex Binding, Linked Reactions • Law of microscopic reversibility • Linked equilibrium (K1*K2 = K3*K4) • Cooperativity • Hill coefficient	Trp Repressor λ repressor	Cooperativity sigmoidal, curves & semi-log plots Hyperbolic = 90% of binding in 100x of K_D Cooperative = 90% of binding in 10x of K_D		Lehninger 158-174
12	10/11	Allostery • MWC model of Allostery • Allosteric regulation of enzymes • Activation by competitive inhibitors	Hemoglobin pH & BPG Aspartate Transcarbam. & compet. Inhib.			Lehninger 158-174
Section 3 – Enzymes						
13	10/13	Introduction to Enzymes • How enzymes catalyze reactions ○ Stabilize transition state ○ rxn coordinate diagrams ○ Orient substrates ○ Binding energy ○ Acid-base catalysis Michaelis-Menten Kinetics intro assumptions	Abzymes Trypsin Chymotrypsin		Ungraded Pset #2 given	Lehninger 189-199 Lehninger 214-243
14	10/18	Enzyme kinetics • Michaelis-Menten Kinetics ○ Derive • Pre-steady state kinetics ○ Burst & lag phase kinetics ○ Single turnover kinetics • Order of addition experiments • Necessary & sufficient	FOF1 ATPase RNAP	When $K_M = K_D$	Post key for Ungraded Pset #2 after class	Lehninger 200-212
15	10/20	Make up lecture and review			Graded Pset #2 given	
Section 4 – Lipids & Membrane Proteins						
16	10/25	Co-translational membrane protein insertion SEC translocon			Graded Pset #2 due	https://www.ibiology.org/cell-biology/protein-localization-inside-cells/#part-3
17	10/27	Post-translational membrane protein insertion • GET Pathway • EMC • Snd • SecA Twin Arginine			Choose papers for flash talks	Borgese et. al. Protein J. 2019

*Required

18	11/1	Journal Club #3 – Membrane Protein Insertion			Reading Quiz Journal Club #3	O'Donnell et al. 2020
19	11/3	Lipids and membrane protein reconstitution • Lipids • Detergents Reconstitution techniques				Lehninger 362-369 Lehninger 377-402
20	11/8	Membrane protein quality control • ERAD • Ubiquitin proteasome system • Bag6				Wu & Rapoport Curr. Opin. Cell Bio 2018 Juszkiewicz & Hegde, Mol. Cell 2018
21	11/10	Journal Club #4 – Reconstitution of ERAD			Reading Quiz Journal Club #4	Baldridge & Rapoport, Cell 2016
22	11/15	Flash Talks			Flash Talks	
23	11/17	Flash Talks			Flash Talks	
Section 5 – Nucleic acids						
24	11/22	• DNA/RNA structure • Ribosozymes • Sanger sequencing • Chemistry of Next Generation Sequencing				Lehninger 281-306
	11/24	No Class, Thanksgiving Break				
25	11/29	Journal Club #5 – NGS identifies non-canonical proteins			Reading Quiz Journal Club #5	Chen et al. 2020
26	12/1	Next generation sequencing – Guest Lecture				Lehninger 339-351
27	12/6	Liquid-Liquid Phase Separation				
28	12/8	Journal Club #6 – RNA and stress granules			Reading Quiz Journal Club #6	Guillen-Boixet et al 2020
Final	11/30	10:15 AM - 12:15 PM			Peer Review of research article	

*Required

SPECIAL COURSE EXPECTATIONS DURING COVID-19

Maintaining a safe campus during the ongoing COVID-19 pandemic remains a top priority. UToledo continues to follow the guidance of the U.S. Centers for Disease Control and Prevention and Ohio Department of Health to keep our campus safe.

ATTENDANCE

The University of Toledo has a missed class policy. It is important that students and instructors discuss attendance requirements for the course. Before coming to campus each day, students should take their temperature and complete a self-assessment for symptoms of COVID-19, such as cough, chills, fatigue or shortness of breath. Anyone with a temperature at or above 100.0 degrees Fahrenheit or who is experiencing symptoms consistent with COVID-19 should not come to campus and contact their primary care physician or the University Health Center at 419.530.5549. For more information on the symptoms of COVID-19, please go to https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

COVID-19 testing for sick students is available on both Main Campus and Health Science Campus. Call 419.383.4545 for an appointment. Absences due to COVID-19 quarantine or isolation requirements are considered excused absences. Students should notify their instructors and follow the protocols summarized in this document on Navigating COVID-Related Course Concerns.

In the event that you have tested positive for COVID-19 or have been diagnosed as a probable case, please review the CDC guidance on self-isolation and symptom monitoring, and report the disclosure to the Division of Student Affairs by emailing StudentAffairs@utoledo.edu or by connecting with their on-call representative at 419.343.9946. Disclosure is voluntary and will only be shared on a need to know basis with staff such as in the Office of Student Advocacy and Support, The Office of Residence Life, and/or the Office of Accessibility and Disability Resources to coordinate supportive measures and meet contact tracing requirements.

FACE COVERINGS

Face coverings are required while on campus, except while eating, alone in an enclosed space, or outdoors practicing social distancing. Students will not be permitted in class without a face covering. If you have a medical reason preventing you from wearing a face covering due to a health condition deemed high-risk by the CDC, submit an online application to request an accommodation through the Office of Accessibility and Disability Resources. Students will need to provide documentation that verifies their health condition or disability and supports the need for accommodations. Students already affiliated with the Office of Accessibility and Disability Resources who would like to request additional accommodations due to the impact of COVID-19, should contact their accessibility specialist to discuss their specific needs. You may connect with the office by calling 419.530.4981 or sending an email to StudentDisability@utoledo.edu.

VACCINATION

Doctors and other health care professionals agree that the best way to protect ourselves and each other is to get vaccinated. Case data clearly show that vaccines remain highly effective at preventing serious illness from COVID, including the highly contagious delta variant. If you have not yet received your COVID vaccine, the University encourages you do so as soon as possible. No appointment is needed to get the shot at the UTMC Outpatient Pharmacy, University Health Clinic or Main Campus Pharmacy. Once you

receive the COVID vaccination, please register on the COVID Vaccine Registry site at: https://utvaccinereg.utoledo.edu/.

SPECIAL NOTES

It's important to note, that based on the unpredictability of the COVID-19 virus, things can change at any time. So please be patient and understanding as we move through the semester. I also ask that you keep me informed of concerns you may have about class, completing course work/assignments timely and/or health concerns related to COVID.