

ENZYMOLGY

CHEM 6520/8520

The University of Toledo
Department of Chemistry & Biochemistry
College of Natural Sciences and Mathematics

Instructor:	Dr. John Bellizzi (he/him)	Class Location:	BO 2059
E-Mail:	john.bellizzi@utoledo.edu	Class Day/Time:	Mon/Wed 2:00-3:50 pm
Office Hours:	Wed, Fri 1-1:45 Tue 2:00-3:15 and by appointment	Credit Hours:	4
Office:	Wolfe Hall 4203A	Term:	Spring 2022
Office Phone:	419-530-5926	CRN:	26942/26943

Catalog Description: Survey of current methods to study enzyme-catalyzed reactions, and application to examples from major enzyme groups.

Course Overview: This course is intended to provide you with a foundation in the theory and practice of enzymology, with an emphasis on kinetics and mechanism. Enzymes are remarkable biomolecular machines! If their central role in all processes of life at the molecular level isn't enough to make you want to understand them better, enzymes also have many important applications, including biotechnology and fermentation, and they are the targets of many widely used and commercially important products like pharmaceuticals, herbicides, and pesticides.

My approach to this course is inspired by two courses I took as a graduate student – one on enzyme kinetics (which was very math-intensive and exceptionally challenging), and a course called “chemical aspects of biological processes” which approached enzyme function from a mechanistic organic chemistry (curved arrow electron-pushing) perspective. These two courses (along with a few courses in X-ray crystallography) are responsible for steering me towards my chosen field of research. It is my hope that you come away from this course with at least some of my appreciation and enthusiasm for the world of enzyme chemistry! Due to the staggeringly large number of diverse enzymes in nature, it's not possible for the course to comprehensively cover all types of enzyme-catalyzed reactions, but I have chosen topics to reflect a broad and representative selection, and the course will give you the skills you will need to generalize from the examples we discuss to learn about other enzymes in your future studies or work.

Prerequisite: Permission of instructor (students enrolled in a graduate program). The material in this course builds on concepts and principles from undergraduate organic chemistry and biochemistry, including the structures of amino acids and proteins, hydrogen bonds and other inter- and intramolecular forces, and organic reaction mechanisms. If you are uncertain about whether you have mastered these concepts, contact me and I can recommend resources that can help you learn or review the material, which should prepare you for the course.

Student Learning Outcomes: Upon completion of the course, students should be able to do the following:

- Use transition state theory to describe principles of catalysis by enzymes
- Be fluent in the concepts and equations used in steady state and transient enzyme kinetic analysis
- Understand different types of enzyme inhibitors, how they affect enzyme function, and how they can be differentiated through kinetic analysis
- Understand the theory and application of methods to determine the kinetic mechanism and chemical mechanism of an enzyme-catalyzed reaction.
- Present detailed chemical mechanisms for representative enzyme-catalyzed reactions and propose reasonable mechanisms for newly encountered enzymes.
- Find relevant experimental information about enzymes of interest in research databases, and
- Critically read, analyze, and summarize the biochemical literature.

Course Materials: Students are not required to purchase a textbook or any other specific course materials, since we will be taking advantage of several excellent electronic texts available through the library, papers from the literature which will be selected and posted on Blackboard, and online databases. The only requirement is that students have access to a computer with reliable internet access.

- Readings will be assigned from the following textbooks, which are available electronically through Carlson Library and/or OhioLink (login with UToledo credentials required). Links to the books can also be found on Blackboard.
 - Bisswanger, H. (2017). *Enzyme Kinetics: Principles and Methods*, Third Edition, Wiley-VCH.
<https://olc1.ohiolink.edu/record=b38737298~S0>
 - Bugg, T. D. H. (2012). *Introduction to Enzyme and Coenzyme Chemistry*, Third Edition, John Wiley & Sons.
<https://olc1.ohiolink.edu/record=b33036799~S0>
 - Frey, P.A. and Hegeman, A. D. (2007). *Enzymatic Reaction Mechanisms*, Oxford University Press.
<https://ebookcentral.proquest.com/lib/univtoledo-ebooks/detail.action?docID=415278>
- Articles from the primary and review literature will be periodically assigned and posted on the course Blackboard site, along with lecture slides and occasional handouts.
- An undergraduate biochemistry textbook can be useful for review and reference (if you don't have one and there is not one that you can borrow, I have put one on library reserve).
- In addition, the following electronic book is available through Carlson Library and is an accessible survey/overview of enzymology, though I will not be assigning any specific readings from it:
 - Engel, Paul (2020). *Enzymes: A Very Short Introduction* Oxford University Press.
<https://www.veryshortintroductions.com/view/10.1093/actrade/9780198824985.001.0001/actrade-9780198824985>

Databases: There are several useful on-line databases in which different kinds of useful information about enzymes and other proteins are archived and curated (coordinate files for three-dimensional structures, amino acid sequences, enzyme kinetic data, and functional annotation). A few of these that are of particular importance for the study of enzymes are listed below. Students are encouraged to visit these databases and familiarize themselves with their contents and how to use them.

Protein Data Bank (PDB)	http://www.rscb.org/pdb
BRENDA Comprehensive Enzyme Information System	http://www.brenda-enzymes.org
ENZYME nomenclature database	http://enzyme.expasy.org
UniProt KnowledgeBase	http://www.uniprot.org
National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov

Course Structure

Class Meetings (Mon/Wed 2:00-3:50) will be a mixture of lecture and discussion. There will also be student presentations during the second half of the term. Due to the length of the class period, we will take a short (~5 minute) break approximately halfway through the class.

Exams: There will be two mid-term exams (in class on Wednesday February 23 and Wednesday April 6) and a comprehensive final examination (during final exams week on Monday May 2 from 2:45 – 4:45 pm). These exams will test your ability to apply concepts and ideas from lecture and from the readings. You will be graded based on your reasoning and explanation as well as your final answer.

Homework

1. For most lectures, there will be chapters/sections assigned from one or more of the electronic textbooks and/or papers from the literature to read.
2. There will be five papers from the literature that will be assigned for you to read for in-class literature discussions on the dates noted on the schedule. For three of these papers, you will also have to turn in a one-page summary of the paper.
3. Periodically I will assign a question or topic in class for you to consider and come prepared to discuss in the next class period.
4. I will post four problem sets on Blackboard (two before spring break, and two after spring break). These will not be collected or graded, but they will challenge you to think about and apply the material from the readings, lectures, and class discussions. They will also give you practice in solving problems of the type you can be expected to see on exams. Students may work together on problem sets (in fact, it is encouraged). Detailed solution keys will be posted about a week after the problem sets are released.

Presentations

- **Small group paper chalk talks:** Groups of 3-4 students will each be assigned a paper from the literature and will have to prepare a 10-minute presentation (8-minute explanation + 2 minutes for questions/answers) in which they describe the enzyme being studied, discuss the experiments done and the data presented in the paper, and summarize the major conclusions. These presentations will be in class on Wednesday March 30.
- **Graduate student presentations:** M.S. and Ph.D. students registered for CHEM 6520 or 8520 will be assigned a specific enzyme, search the literature and databases for information about that enzyme, and present an oral research presentation (with slides and references) on the structure, function, and mechanism of that enzyme. Each presentation should be 20 minutes long with 5 minutes reserved for questions and answers. The presentations will be during class the weeks of April 11 and April 18.
- All students (graduate and undergraduate) are expected to attend all graduate student presentations and ask questions as well as provide written comments/critiques that will be compiled and provided to the presenters. The feedback you provide will count towards your in-class participation grade.

Course Grading:

In-class activities

Lecture attendance, preparation, and participation (up to 2 pts/lecture)	30 points
Survey feedback (3 x 5 points)	15 points
Literature discussions (preparation and participation 5 x 10 pts)	50 points
Small group literature mini-presentation	40 points
20-minute enzyme oral presentation	90 points
Questions/critiques for graduate student presentations	15 points
Literature written summaries (3 x 20 pts)	60 points
Mid-term exams (2 x 100 points)	200 points
<u>Comprehensive final examination</u>	<u>100 points</u>
TOTAL	600 points

Course grades will be assigned based on the percentage of points earned out of 500 according to the scale below.

Cutoffs for each letter grade may be adjusted downward but will not increase. Grades will not be curved.

A > 85% **B** 70-84 % **C** 55-69% **D** 40-54% **F** < 40%

Course Expectations and Policies

Attendance: Attendance in class and participation in discussions is expected and will contribute to your grade. In general, excused absences will be granted for circumstances missing the criteria of the UT Missed Class policy (including COVID-19 related illness, quarantine, and other effects). I will work with affected students to make up missed major assignments such as presentations and exams. If you have a conflict that you know about ahead of time, please discuss it with me as early as possible so that appropriate accommodations can be made.

Grading Policies: The class is designed to provide a variety of opportunities for students to demonstrate their understanding of the material and receive feedback on their progress so that they can identify areas in which they can apply more effort or new strategies. My hope is that all students will succeed. Grades in the course will not be curved, because your grade should be based on your performance alone and should not be tied to the performance of other students. It is my intention to make the grading of assignments and exams as transparent as possible and provide partial credit and feedback to help you improve. If you feel that a mistake has been made in grading, please make an appointment with me and I will be happy to go over the assignment or exam with you.

Academic Integrity: You are capable of doing well in this course, and if you become concerned about how well you are doing in this course, please come speak with me. Integrity and honesty are a core component of science, and I expect all students to comply fully with the University of Toledo policy on Academic Honesty. Students are permitted (and encouraged) to study with one another, discuss course material with each other. However, all graded assignments and exams must be the work of the named student only. Cheating in any way or facilitating the cheating of others on an exam will result in a grade of 0 for that exam. A second violation will result in an automatic F in the course and referral to the Dean's Office. This includes but is not limited to talking during an exam, using unauthorized material (other than your single page of notes), looking at another student's paper, allowing someone to copy your answers, or discussing exam questions with others before the exams have been turned in. In addition, plagiarism of the work of others (peers or published sources) is also considered academic misconduct.

Communication: E-mail sent from your rockets.utoledo.edu e-mail account is the best way to communicate with me outside of class. I intend to answer student emails within one business day of receiving them, but in the event you don't hear from me in that time frame, feel free to send a reminder message if you are waiting on an urgent response. Course announcements will be made on Blackboard and distributed by e-mail.

For questions about class material, I encourage you to use the discussion board on Blackboard so that other students (who may have the same question that you do) can see your question and the responses, and if you know the answer to a question that is posted, I encourage you to share that knowledge with your classmates.

Preferred Name: If your preferred name is not the same as the name that appears on the course roster for this class, please let me know what you would like to be called, as well as the pronouns you use (if applicable).

Office Hours: My regularly scheduled open office hours for this course (drop-in, no appointment necessary) are Tuesday from 2:00-3:15 and Wednesday and Friday from 1:00-1:45. Occasionally conflicts may arise that may require the cancellation or rescheduling of those hours, and any such changes will be announced in class and/or by e-mail/Blackboard notice. Additional office hours will be scheduled before exams. If you wish to meet outside of office hours, please contact me by e-mail for an appointment request and I will do my best to accommodate you. Anyone who is uncomfortable attending in person office hours due to COVID-19 concerns may contact me and we can meet virtually in Blackboard Collaborate or WebEx during scheduled office hours or by appointment.

Inclement Weather Policy: If the University cancels classes on the day of a literature discussion, presentation, or exam, it will be pushed to the next regularly scheduled class day.

Course Conduct: I am committed to creating a learning environment where diverse perspectives are recognized and valued as a source of strength. I request that all students work with me to create a class culture based on open communication, mutual respect, and inclusion. As a class we will approach all discussions with respect and civility. Disagreements and debates in academic discourse are expected and welcome, but personal attacks are never OK, and will not be tolerated. I

strive to ensure an open and welcoming classroom for all students. If you have concerns, please don't hesitate to come and talk to me.

School-Life Balance: Many students face obstacles to their education as a result of work or family obligations or unforeseen personal, financial, or health difficulties. I have great respect for students who are balancing their studies with the responsibilities of caring for children or other family members or working to pay for their education and/or support their families. If you are experiencing challenges throughout the term that are impacting your ability to succeed in this course, or in your academic career more broadly, please reach out to me so that we can work together to form a plan for your academic success.

Drop and Withdrawal: The last day to drop the course is **February 1, 2022**, and you may withdraw from the course (with a grade of W) until **March 25, 2022**

Support: All of us need a support system, and UToledo has a number of resources to help students achieve the success they are capable of. Most students make use of some kind of support at some point during their studies. Please follow these links to view a comprehensive list of [Student Academic and Support Services](#) and [Campus Health and Safety Services](#) available to you as a student.

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) (<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.

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 should contact their primary care physician or the Main Campus Health Center at 419.530.3451 or Health Science Campus Student Health and Wellness Center at 419.383.5000. 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. Please refer to <https://www.utoledo.edu/coronavirus/> on a regular basis for updates to current requirements or mandates. 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.

Week	Day/Date	Lec	Assignment	Lecture Topic (Subject to change)	Textbook readings*
1	Mon Jan 17			<i>No Class (MLK Day)</i>	
	Wed Jan 19	1	Ascend survey (in class)	Part I: Catalysis and Kinetics Introduction to enzymology	
2	Mon Jan 24	2		Catalysis by enzymes	Bu Ch. 1-3, FH Ch. 1
	Wed Jan 26	3	Paper 1 discussion (in class)	Steady state kinetics - single substrate reactions	Bi Ch. 3, Bu Ch. 4 FH Ch. 2, Appendices A, B, D
3	Mon Jan 31	4	Paper 1 summary due	Steady state kinetics - single substrate reactions	
	Wed Feb 2	5		Kinetics of enzyme inhibition	
4	Mon Feb 7	6		Kinetics of multi-substrate reactions	
	Wed Feb 9	7	Paper 2 discussion (in class)	Transient kinetics	
5	Mon Feb 14	8		Part II: Practical Enzymology Enzyme assays	Bi Ch. 6, 9, 12-15 Bu Ch. 4
	Wed Feb 16	9	Paper 3 discussion (in class)	Enzyme purification	
6	Mon Feb 21	10	Paper 3 summary due	Tools and techniques for investigating structure and mechanism	
	Wed Feb 23		Exam 1	Mid-Term Exam 1 (Lectures 1-9)	
7	Mon Feb 28	11		Part III: Enzyme Structure and Mechanism Nicotinamide-dependent dehydrogenases	Bu Ch. 6 FH Ch. 16
	Wed Mar 2	12	Paper 4 discussion (in class)	Flavin-dependent oxidoreductases Metal-dependent oxidoreductases	Bu Ch. 6 FH Ch. 17
No Class (Spring Break) Week of March 7					
8	Mon Mar 14	13	Ascend survey (in class)	Enzyme-catalyzed C-C bond formation/cleavage; enzyme-catalyzed carboxylation/decarboxylation	Bu Ch. 7, FH Ch. 8, 14
	Wed Mar 16	14	Paper 5 discussion (in class)	Enzyme-catalyzed addition/elimination reactions	Bu Ch. 8 FH Ch. 9
9	Mon Mar 21	15	Paper 5 summary due	PLP-dependent enzymes; enzyme-catalyzed alkyl group transfers	Bu Ch. 95, 9 FH Ch. 3, 15
	Wed Mar 23	16	Group Lit discussion (in class)	Enzyme-catalyzed isomerizations/rearrangements	Bu Ch. 10, 11.2 FH Ch. 7
10	Mon Mar 28	17	Group Lit discussion (in class)	Enzyme-catalyzed glycosyl group transfer	Bu Ch. 5 FH Ch. 12
	Wed Mar 30		Chalk talks	Group literature chalk talks	
11	Mon Apr 4	18		Enzyme-catalyzed phosphoryl group transfer	Bu Ch. 5 FH Ch. 10
	Wed Apr 6		Exam 2	Mid-Term Exam 2 (Lectures 10-17)	
12	Mon Apr 11		Presentations	Graduate student enzyme presentations	
	Wed Apr 13		Presentations	Graduate student enzyme presentations	
13	Mon Apr 18		Presentations	Graduate student enzyme presentations	
	Wed Apr 20		Presentations	Graduate student enzyme presentations	
14	Mon Apr 25	19		Enzyme engineering; ribozymes; catalytic antibodies	Bu Ch. 12
	Wed Apr 27	20	Ascend survey (in class)	Enzyme case studies	
	Mon May 2 2:45-4:45 pm		Final Exam	Comprehensive Final Exam (Lectures 1-20)	

* FH = Frey and Hegeman, *Enzymatic Reaction Mechanisms*
Bi = Bisswanger, *Enzyme Kinetics: Principles and Methods*
Bu = Bugg, *Introduction to Enzyme and Coenzyme Chemistry*