DALHOUSIE UNIVERSITY

Enzymes Syllabus

Department of Biochemistry & Molecular Biology

BIOC 4701/5701 Winter 2024

Chemistry 226

Course Instructor

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* Please contact to set up an appointment

Course Description

What gives the cell its life and personality are enzymes. They govern all body processes; malfunction of even one enzyme can be fatal. Nothing in nature is so tangible and vital to our lives as enzymes, and yet so poorly understood and appreciated by all but a few scientists.

Arthur Kornberg
***For the Love of Enzymes*, 1989**

Fundamental principles of enzyme catalysis and its regulation will be examined. Use of tools such as steady-state and pre-steady-state kinetics, isotope effect measurements, site-directed mutagenesis, spectroscopy, x-ray crystallography, and mechanism-based inhibitors to study the architecture and mechanism of action of enzyme active sites will be presented. The catalytic mechanism and transition state stabilization will be considered in detail for selected enzymes that have been well-characterized structurally. Classic and current papers in the literature will be reviewed so that the experimental and conceptual approaches used may be critically appraised.

The lectures are designed to present the theory of selected topics in enzymology. Problem sets based on articles from the biochemical literature will serve to provide specific examples of the concepts introduced in the lectures. The problem sets will be assigned every two weeks. Since there is not a laboratory component associated with this course, the problem sets will be quite detailed and students are expected to work on them over the two-week period (i.e., not the night before they are due!).

Each graduate student will write an essay and present an oral presentation (30 min) based on their essay topic. Essay topics may be chosen from the list provided or in consultation with the course instructor. Attendance at presentations is mandatory. **All students will be responsible for lecture material, assigned readings, problem sets, and material presented during the graduate student seminars**.

COURSE PREREQUISITES

BIOC 3700.03; or (CHEM 3601.03 and CHEM 2301.03), all with a grades of B or higher or instructor's consent

COURSE OBJECTIVES/LEARNING OUTCOMES

1. Account for why a given ligand may be bound tightly by an enzyme or covalently modify an enzyme (transition state analogues).
2. Explain how given techniques (spectroscopy, radioactivity, HPLC) may be used to measure enzyme activity in direct or indirect assays.
3. Given a pH-dependent kinetic mechanism, derive an initial velocity equation and sketch the plot of kinetic constants as a function of pH.
4. Given the kinetic mechanism (with or without modifiers, e.g. inhibitors, activators, viscosogens), derive an initial velocity equation using either the steady-state assumption or the rapid equilibrium approach.
5. Show how entropic contributions lead to huge intramolecular rate enhancements.
6. Given the kinetic parameters for an enzyme-catalyzed reaction and the corresponding nonenzymatic reaction, calculate the efficiency, rate enhancement, proficiency, and extent of transition state stabilization.
7. Given the steady-state velocity expression for a multisubstrate enzyme, predict the product inhibition pattern and binding order in the presence of fixed and variable substrate concentrations.
8. Derive the steady-state velocity equation for a given kinetic mechanism for a multisubstrate enzyme using the King-Altman method.
9. Identify uniform and differential binding from site-directed mutagenesis studies or substrate mutilation studies to discern the role of residues in transition state and ground state binding.
10. Given an enzyme mechanism, design a reversible or irreversible inhibitor.
11. Given an irreversible inhibitor, design an experiment to determine the efficiency of inactivation and the binding affinity.

COURSE MATERIALS

Textbooks: Segel, I. (1993) *Enzyme Kinetics*, John Wiley & Sons, Inc., New York.
Course website: BrightSpace (https://dal.brightspace.com)

ADDITIONAL REFERENCES IN ENZYMOLOGY:

GENERAL

1. Copeland, R.A. (2000) *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*, 2nd ed., Wiley-VCH, Inc., New York. **(QU 135 c782e 2000)**
2. Fersht, A. (1999) *Structure and Mechanism in Protein Science*, W.H. Freeman and Co., New York. **(QU 55 F399s 1999)**
3. Fersht, A. (1985) *Enzyme Structure and Mechanism*, W.H. Freeman and Company, New York. **(QU 135 F411 1985)**
4. Dixon, M. & Webb, E.C. (1979) *Enzymes,* Academic Press, New York. **(QU135 D621 1979)**
5. *The Enzymes* (1970-) Volumes issued since 1970; an excellent resource. (Volumes XIX **(QU 135 B79 1990)** and XX **(QU 135 B79 1992)**, *Mechanisms of Catalysis* are particularly good.)
6. Price, N.C. & Stevens, L. (1989 & 1999) *Fundamentals of Enzymology*, Oxford University Press, Oxford. **(QU135 P946f 1989 & 1999)**
7. Meister, A. (Ed.) (1941-) *Advances in Enzymology* - contains comprehensive reviews.

MECHANISMS

8. Page, M.I. & Williams, A. (1993) *Enzyme Mechanisms*, The Royal Society of Chemistry, Cambridge. **(QU135 E6105 1987)**
9. Walsh, C. (1979) *Enzymatic Reaction Mechanisms*, W.H. Freeman and Company, San Francisco.
10. Kyte, J. (1995) *Mechanism in Protein Chemistry*, Garland Publishing, Inc. New York. **(QU 55 K97 1995)**
11. Jencks, W.P. (1969) *Catalysis in Chemistry and Enzymology*, McGraw Hill, Inc., New York.
12. Dugas, H. (1996) *Bioorganic Chemistry. A Chemical Approach to Enzyme Action*, Spriger-Verlag, New York. **(QU 135 D866b 1996)**

ASSAYS AND PURIFICATION

13. Eisenthal, R. & Danson, M.J. (1993) *Enzyme Assays*, IRL Press at Oxford University Press, New York.
14. *Methods in Enzymology* - a series which reviews biochemical methods including the preparation and assay of various enzymes (1955-). **(QU 135 M592)**
15. Deutscher, M.P. (Ed.) (1990) *Guide to Protein Purification*, *Methods Enzymol.*, **182**.

KINETICS

16. Purich, D.L. (Ed.) (1983) *Contemporary Enzyme Kinetics*, Academic Press, New York. (This is a collection of articles that appeared in the series *Methods In Enzymology*.) **(QU135 C761 1983)**
17. Segel, I.H. (1975) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, John Wiley & Sons, Inc., New York. **(QU 135 S454e 1993)**
18. Schulz, A.R. (1994) *Enzyme Kinetics: From Diastase to Multi-enzyme Systems*, Cambridge University Press, New York. **(QU135 S3883e 1994)**
19. Walter, C. (1965) *Steady-State Applications in Enzyme Kinetics*, The Ronald Press Company, New York. **(QU135 W23 1965)**
20. Cornish-Bowden, A. (1995) *Fundamentals of Enzyme Kinetics*, Portland Press, Boston. **(QU135 C818f 1995)**
21. Roberts, D.V. (1977) *Enzyme Kinetics,* Cambridge University Press, New York. **(QU135 R45 1977)**
22. Kuby, S. (1990) *A Study of Enzymes* (Volume I), CRC Press, Boca Raton. **(QU135 S9335 1990)**
23. Cook, P.F., & Cleland, W.W. (2007) *Enzyme Kinetics and Mechanism*, Garland Science, New York. **(QU 135 C771e 2007)**

COURSE ASSESSMENT

Component	Weight (% of final grade)‡		Date‡
	BIOC 4701	BIOC 5701	
1st Midterm	15%	12.5%	February 13, 2024
2nd Midterm	15%	12.5%	March 19, 2024
Problem Sets	30%	25%	(~ every 2 weeks)
Graduate Student Essay	n/a	15%	February 15, 2024
Graduate Student Presentation	n/a		February 29, 2024
Final exam (3 h)*	40%	35%	(Scheduled by Registrar)

‡ Alterations may be necessary in the event of a flu pandemic.

*** You must pass the final to pass the course.**

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CONVERSION OF NUMERICAL GRADES TO FINAL LETTER GRADES FOLLOWS THE DALHOUSIE COMMON GRADE SCALE

A+	(90-100)	**B+**	(77-79)	**C+**	(65-69)	**D**	(50-54)
A	(85-89)	**B**	(73-76)	**C**	(60-64)	**F**	(<50)
A–	(80-84)	**B–**	(70-72)	**C–**	(55-59)		

COURSE POLICIES ON MISSED EXAMINATIONS/MID-TERMS AND ASSIGNMENTS

Missed evaluations:

A student who misses a midterm test or final examination due to illness should, if possible, notify the instructor, course coordinator, or department office either prior to, or within 48 h of the scheduled time or due date for that component. The student must also submit a Student Declaration of Absence (SDA) Form (through the course Brightspace page or to their instructor via e-mail) within three (3) calendar days following the last day of absence. Special 'make-up' tests (**if offered**) will normally be written within 7 calendar days after the missed test. Absence for non-medical reasons is not ordinarily acceptable unless prearranged with the instructor for exceptional circumstances. A missed evaluation component for which no satisfactory arrangement has been made will be given a mark of zero. The SDA form can only be submitted up to two (2) separate times per course during a term. Students who exceed this limit must inform their course instructor(s) and will be required to register with an Advisor at Student Academic Success (SAS). If students have recurring short-term absences and do not register with SAS, it is at the instructor's discretion to disallow any further Student Declarations and deny alternate coursework arrangements. **SDA forms will not be accepted for the problem sets. Problem sets submitted after the due date and time will not be accepted and will be given a grade of zero.**

Please refer to the link below for further information on the University policy regarding Long-term absence:

https://www.dal.ca/dept/university_secretariat/policies/academic/missed-or-late-academic-requirements-due-to-student-absence.html

Missed Final exam:

A student who misses the final examination due to illness must notify the course coordinator or department office within 48 h to provide a medical certificate (see Dalhousie Calendar, section 16.8). Absence for non-medical reasons is not acceptable. If warranted, a make-up final examination will be held at or shortly after the end of the official exam period, and typically before May 1. Students who need to write a make-up exam for medical or other reasons are expected to be available during this period.

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COURSE CONTENT

*LECTURE OUTLINE 2024**

SECTION I: ENZYME KINETICS

Jan. 9 Fundamental Concepts of Kinetics.
Jan. 11 Kinetics of Single Substrate Systems.
Jan. 16 Altering Enzyme Activity: Inhibition Kinetics.
Jan. 18 Altering Enzyme Activity: Tight Binding Inhibitors and Time-Dependent Inhibition 1.
Jan. 23 Altering Enzyme Activity: Tight Binding Inhibitors and Time-Dependent Inhibition 2.
Presentation of Graduate Student Essay Topics
Jan. 25 Altering Enzyme Activity: Active Site-directed Irreversible Inhibitors.
Assignment of Graduate Student Essay Topics
Jan. 30 Altering Enzyme Activity: pH Effects 1.
Feb. 1 Altering Enzyme Activity: pH Effects 2.
Feb. 6 Altering Enzyme Activity: Viscosity Effects
Feb. 8 Multisubstrate Systems 1: Steady-state Equations / King-Altman Method.
Feb. 13 MID-TERM TEST I (material up to and including viscosity effects)
Feb. 15 Multisubstrate Systems 2: Steady-state Equations / King-Altman Method.
(**GRADUATE STUDENT ESSAYS DUE**)
Feb. 20 Study Break
Feb. 22 Study Break
Feb. 27 Multisubstrate Systems 3: Inhibition Studies and Binding Order.
Feb. 29 GRADUATE STUDENT PRESENTATIONS & Kinetic Isotope Effects

SECTION II: CATALYSIS, MECHANISM, AND ENZYME ARCHITECTURE

Mar. 5 Kinetic Isotope Effects
Mar. 7 Enzyme Efficiency, Rate Enhancements, and Transition State Stabilization.
Mar. 12 Principles of Catalysis: Effective Molarity and Entropy.
Mar. 14 Principles of Catalysis: Mechanisms.
Mar. 19 MID-TERM TEST II (from multisubstrate systems kinetics to principles of catalysis)
Mar. 21 Transition State Stabilization: Transition State Analogues.
Mar. 26 Transition State Stabilization: Structural Alterations to the Protein and Transition State Analogue Inhibitors.
Mar. 28 Transition State Stabilization: Catalytic Antibodies.
Apr. 2 Architecture of Enzymes and Their Active Sites: Three-dimensional Structure of Triosephosphate Isomerase.
Apr. 4 Binding Energy and Catalysis: Evolution of Enzyme Efficiency.
Apr. 9 Binding Energy and Catalysis: Evolution of Enzyme Efficiency.
Apr. 10 no classes
Apr. 11 **EXAMINATIONS BEGIN**

*** LECTURE SCHEDULE MAY BE ALTERED DEPENDING ON TIME CONSTRAINTS AND THE TIME REQUIRED TO COVER SPECIFIC TOPICS**

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University Policies and Statements

Recognition of Mi'kmaq Territory

Dalhousie University would like to acknowledge that the University is on Traditional Mi'kmaq Territory. The Elders in Residence program provides students with access to First Nations elders for guidance, counsel, and support. Visit or e-mail the Indigenous Student Centre at 1321 Edward St or elders@dal.ca. Additional information regarding the Indigenous Student Centre can be found at: https://www.dal.ca/campus_life/communities/indigenous.html

Internationalization

At Dalhousie, 'thinking and acting globally' enhances the quality and impact of education, supporting learning that is "interdisciplinary, cross-cultural, global in reach, and orientated toward solving problems that extend across national borders." Additional internationalization information can be found at: https://www.dal.ca/about-dal/internationalization.html

Academic Integrity

At Dalhousie University, we are guided in all our work by the values of academic integrity: honesty, trust, fairness, responsibility, and respect. As a student, you are required to demonstrate these values in all the work you do. The University provides policies and procedures that every member of the university community is required to follow to ensure academic integrity. Additional academic integrity information can be found at: https://www.dal.ca/dept/university_secretariat/academic-integrity.html

Accessibility

The Student Accessibility Centre is Dalhousie's centre of expertise for matters related to student accessibility and accommodation. If there are aspects of the design, instruction, and/or experiences within this course (online or in-person) that result in barriers to your inclusion, please contact the Student Accessibility Centre (https://www.dal.ca/campus_life/academic-support/accessibility.html) for all courses offered by Dalhousie with the exception of Truro. For courses offered by the Faculty of Agriculture, please contact the Student Success Centre in Truro (https://www.dal.ca/about-dal/agricultural-campus/student-success-centre.html)

Conduct in the Classroom – Culture of Respect

Substantial and constructive dialogue on challenging issues is an important part of academic inquiry and exchange. It requires willingness to listen and tolerance of opposing points of view. Consideration of individual differences and alternative viewpoints is required of all class members, towards each other, towards instructors, and towards guest speakers. While expressions of differing perspectives are welcome and encouraged, the words and language used should remain within acceptable bounds of civility and respect.

Diversity and Inclusion – Culture of Respect

Every person at Dalhousie has a right to be respected and safe. We believe inclusiveness is fundamental to education. We stand for equality. Dalhousie is strengthened in our diversity. We are a respectful and inclusive community. We are committed to being a place where everyone feels welcome and supported, which is why our Strategic Direction prioritizes fostering a culture of diversity and inclusiveness (Strategic Priority 5.2). Additional diversity and inclusion information can be found at: http://www.dal.ca/cultureofrespect.html

Student Code of Conduct

Everyone at Dalhousie is expected to treat others with dignity and respect. The Code of Student Conduct allows Dalhousie to take disciplinary action if students don't follow this community expectation. When appropriate,

violations of the code can be resolved in a reasonable and informal manner - perhaps through a restorative justice process. If an informal resolution can't be reached, or would be inappropriate, procedures exist for formal dispute resolution. The full Code of Student Conduct can be found at: https://www.dal.ca/dept/university_secretariat/policies/student-life/code-of-student-conduct.html

Fair Dealing Policy

The Dalhousie University Fair Dealing Policy provides guidance for the limited use of copyright protected material without the risk of infringement and without having to seek the permission of copyright owners. It is intended to provide a balance between the rights of creators and the rights of users at Dalhousie. Additional information regarding the Fair Dealing Policy can be found at: https://www.dal.ca/dept/university_secretariat/policies/academic/fair-dealing-policy-.html

Originality Checking Software

The course instructor may use Dalhousie's approved originality checking software and Google to check the originality of any work submitted for credit, in accordance with the Student Submission of Assignments and Use of Originality Checking Software Policy. Students are free, without penalty of grade, to choose an alternative method of attesting to the authenticity of their work and must inform the instructor no later than the last day to add/drop classes of their intent to choose an alternate method. Additional information regarding Originality Checking Software can be found at: https://www.dal.ca/dept/university_secretariat/policies/academic/student-submission-of-assignments-and-use-of-originality-checking-software-policy-.html

Student Use of Course Materials

Course materials are designed for use as part of this course at Dalhousie University and are the property of the instructor unless otherwise stated. Third party copyrighted materials (such as books, journal articles, music, videos, etc.) have either been licensed for use in this course or fall under an exception or limitation in Canadian Copyright law. Copying this course material for distribution (e.g. uploading to a commercial third-party website) may lead to a violation of Copyright law.