BIOC*4540 Enzymology

Winter 2018
Section(s): C01

Department of Molecular and Cellular Biology
Credit Weight: 0.75
Version 3.00 - January 04, 2018

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1 Course Details

1.1 Calendar Description

This is a laboratory-intensive course where the topics studied include enzyme active sites and the mechanisms of enzyme action; enzyme kinetics and regulation; recombinant proteins and site-directed mutagenesis as tools for understanding enzymes.

Pre-Requisite(s): BIOC*3560 (may be taken concurrently), BIOC*3570

1.2 Course Description

This is a required Biochemistry fourth-year course on the subject of Enzyme Structure, Function and Mechanism. It features a laboratory component (5 laboratory modules) and an Independent Study where the students research an enzyme of choice and present a Powerpoint seminar with a partner.

1.3 Timetable

- Lectures: Tuesday and Thursday @ 10:00 – 11:20 in MCKN 223
- Laboratory sections will be held on Mon, Tue, Wed, and Thu from 14:30 – 17:20 in SSC 3101.

1.4 Final Exam

Exam time and location is subject to change. Please see WebAdvisor for the latest information.

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2 Instructional Support

2.1 Instructor(s)

Rod Merrill
Email: rmerrill@uoguelph.ca
Telephone: +1-519-824-4120 x53806
Office: SC1 2204
Office Hours: Tuesday and Thursday @ 1:00 – 2:30 pm or by appointment

2.2 Demonstrators

Please see the Enzymology Lab Coordinator, Dr. Colin Cooper for details (Room 3502 SSC, x58763). The lab TAs are Madison Turner, and Liam Doyle.

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3 Learning Resources

3.1 Required Resources(s)

Lab Manual (Lab Manual)

Laboratory manuals must be purchased before the first lab and you should come to the lab prepared to conduct the first experiment. The manuals may be purchased in SSC, Room 2302 in the Mol. & Cell. Biol. Dept. starting on Jan 8-10, 2018 (hours of operation: 9:30 – 11:30; 13:00 – 15:00).

3.2 Recommended Resources(s)

Principles in Biochemistry (Readings)

- No single textbook is sufficient for the lecture material but Lehninger: Principles in Biochemistry (5^{th} or 6^{th} editions) Chapter 6 serves as the basis for basic enzyme understanding and theory and this chapter should be read and carefully studied.
- On reserve

3.3 Additional Resources(s)

Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding (Textbook)

- Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, Series in Structural Biology – vol. 9 (2017), Alan Fersht.
- On reserve

Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, Series in Structural Biology (Textbook)

Alan Fersht (2017) Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, Series in Structural Biology – vol. 9 (2017), World Scientific Co. Pte. Ltd, Hakensack, New Jersey.

Lehninger: Principles of Biochemistry (Textbook)

David Nelson and Michael Cox (2013) Lehninger: Principles of Biochemistry, 6^{th} edition, W.H. Freeman & Co., New York.

Enzymes: Biochemistry, Biotechnology and Clinical Chemistry (Textbook)

Trevor Palmer (2007) Enzymes: Biochemistry, Biotechnology and Clinical Chemistry, 2^{nd} edition, Albion Press.

Introduction to Protein Structure (Textbook)

Carl Branden & John Tooze (1999) Introduction to Protein Structure, 2^{nd} edition, Garland Publ., New York.

3.4 Note

- A number of related texts have been placed on reserve as resources and to provide background information on the various topics discussed in the course (see Course Subject Outline).
- The Adobe Acrobat (*.pdf) files for each Powerpoint lecture will be available for download from the Courselink website and each lecture will be made available at least 2 weeks before the specified lecture date.

3.4 Additional Texts

- All indicated additional texts, papers and treatises are available at the Reserve Desk at the library on two hour loan.

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4 Learning Outcomes

Objectives: To integrate the practical aspects of enzymology with the kinetic theories to provide a mechanistic overview of enzyme activity and regulation in cells; and (ii) to prepare students to confidently and competently work with enzyme systems in both Academia and Industry.

4.1 Course Learning Outcomes

By the end of this course, you should be able to:

1. Plan and execute an enzyme assay
2. Analyse enzyme kinetic data
3. Analyse kinetic inhibition data and to determine the mechanism of inhibition
4. Perform library research on a specific enzyme topic
5. Prepare and deliver a Powerpoint seminar to their peers

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5 Teaching and Learning Activities

5.1 Course Subject Outline

I. ENZYMES AS CATALYSTS (Lehninger Ch 6; Fer Ch 2, 3, 4, 6; Palm Ch 1, 6, 8, 16)

- Lect#1: Introduction and History of Enzymes
  - Historical Aspects
  - Discovery of enzymes
  - Chemistry of enzymes
  - Function and importance
  - Enzymes in Biotechnology
- Lect#2: Enzyme Purification and Assay
  - Initial velocity measurements
  - Assay types

    - Enzyme units of activity
    - Turnover number and properties
    - Purification and assessment
    - Methods for measurements
- Lect#3: Michaelis-Menten Kinetics
    - Introduction
    - Assumptions
    - Derivation
    - Description of v_0 versus $[S_{\mathbf{o}}]$
    - Michaelis constant (K_M)
    - Specificity/Substrate constant (SpC)
- Lect#4: Graphical Analysis of Kinetic Data, pH and Temp Dependence
    - Graphical Analysis
        - Lineweaver-Burk Analysis
        - Hanes-Woolf Analysis
        - Eadie-Hofstee Analysis
        - Direct Linear Plot (Eisenthal/Cornish-Bowden Plot)
        - Nonlinear Curve Fitting
    - pH-dependence of Michaelis-Menten Enzymes
    - Temperature-Dependence of Enzyme Reaction
- Lect#5: Characteristics and Properties
    - Catalytic power and specificity
    - Enzymes as catalysts
    - Enzyme - substrate interactions
        - lock & key model
        - induced fit model
        - transition state model
        - quantum tunnelling model
    - Enzymes as proteins
    - Non-protein cofactors
        - metal ions
        - organic cofactors
- Lect#6: Enzyme Inhibition and Kinetics
    - Classification of Inhibitors
        - Reversible
        - Irreversible
            - Iodoacetamide
            - DIFP
            - Additional examples
    - Classification of Reversible Inhibitors
        - Competitive
        - Uncompetitive
        - Noncompetitive
        - Substrate
- Lect#7: Nomenclature / Classification
    - Nomenclature/Classification

        - Oxidoreductase-dehydrogenase
        - Transferase
        - Hydrolase
        - Lyase
        - Isomerase
        - Ligase
    - Regulatory Enzymes
        - Mechanism
        - Kinetics
        - Examples
- Lect#8: Single Molecule Enzymology
    - Movies of Single Enzymes
    - Advantages of Single Molecule Studies
    - Applications of Single Molecule Studies
    - Following Enzymes in Real Time
    - ATP Synthase
    - ATP Synthase with Tethered Actin
    - Myosin-V
    - Kinesin motor attached to a fluorescent bead
    - Single Molecule Studies of Cholesterol Oxidase
    - ß-galactosidase: a model Michaelis-Menten enzyme?
- Lect#9: Multi-substrate Reactions and Substrate Binding Analysis
    - Multi-substrate Reactions
        - Cleland Convention
        - Ordered and Random Mechanisms
        - Sequential and Nonsequential Mechanisms
            - Sequential
            - Nonsequential
    - Substrate Binding Analysis
        - Single Binding Site Model
        - Binding Data Plots
        - Direct Plot
        - Reciprocal Plot
        - Scatchard Plot
    - Determination of Enzyme-Substrate Dissociation Constants
        - Kinetics
        - Equilibrium Dialysis
        - Equilibrium Gel Filtration
        - Ultracentrifugation
        - Spectroscopic Methods

II. MECHANISM OF ENZYME CATALYSIS (Lehninger Ch 6; Fer Ch 2, 9; Palm Ch 10, 11)

- Lect#10: Enzyme Mechanisms-I
    - Reaction Mechanisms and Catalysis
        - Enzyme-transition state complementarity

        - Structure-activity correlations
        - Transition state analogues
        - Catalytic antibodies
        - Summary
    - Preferential transition state binding
        - Transition state theory
    - Proximity effect
    - Acid-base catalysts
- Lect#11: Enzyme Mechanisms II
    - Covalent catalysis
    - Metal ion catalysis
    - Electrostatic catalysis
    - Low barrier H-bonds
    - Structural flexibility
- Lect#12: Enzyme Mechanisms-III: Techniques for Drug Discovery
    - Drug Design
    - Techniques of Drug Discovery
        - Complexity of Drug Discovery
        - SARS and QSARS
        - Structure-based Drug Design
        - Combinatorial Chemistry and High-Throughput Screening
    - Introduction to Pharmacology
        - Pharmocokinetics
        - Toxicity and Adverse Reactions Eliminate Most Drug Candidates
            - Phase I
            - Phase II
            - Phase III
        - Drug Candidate Statistics
        - Cytochrome P450 Metabolizes Drugs
        - Many Drugs are Enzyme Inhibitors
            - Sulfadrugs
            - Viagara
- Lect#13: Active Site Investigations I
    - Kinetic Studies
    - Variation of substrate concentration
    - Variation of substrate structure
    - Reversible inhibition
    - Variation of pH
    - Pre-steady state kinetics
    - Detection of Intermediates
    - X-ray Crystallographic Studies
    - NMR for Protein Structure Determination
- Lect#14: MID-TERM EXAMINATION (during class time) Thu, Mar 1, 2018
- Lect#15: Active Site Investigations II
    - Chemical Modifications
        - Applications

    - Super-reactive Sidechains
    - Suicide Substrates
    - Interpretation of Chemical Modification Experiments
    - Criteria for establishment of side chain involvement in catalysis
- Lect#16: Enzyme Engineering and Design
    - Substitution
    - Insertion
    - Hybrid Proteins
    - Genes for Novel Enzymes
        - Aequorin
        - Enviropig
    - Engineering More Stable Enzymes
    - Incorporation of Non-natural Amino Acids into Enzymes
    - Protein Engineering by Combinatorial Methods
    - DNA Shuffling

III. CASE STUDY ENZYMES/INDEPENDENT PROJECT/SEMINAR

- Lect#17: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#18: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#19: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#20: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#21: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#22: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#23: Student Presentations (n = 3 presentations, groups of 2 students)
- Lect#24: Student Presentations (n = 3 presentations, groups of 2 students)

Fer = Ferst; Lehninger = Lehninger 5th ed., Palm = Palmer

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6 Assessments

6.1 Marking Schemes & Distributions

Name	Scheme A (%)
Mid-term	20.00
Laboratory SCIE 3101	30.00
Independent Study/Seminar	20.00
Final Examination	30.00
Total	100.00

6.2 Assessment Details

Midterm (20.00%)
Date: Thu, Mar 1, In class

- There will be a Mid-term Examination (80 min, in class-time) involving short answer and problem questions.
- There are no alternate exams offered since the Mid-term will be given in class time.
- The final grade can be based entirely on the Final Exam (and other components) only if reasons for missing the Mid-term exam are adequately documented.
- Both exams are required!
- Students who score a significantly higher grade on the Final Exam, compared with the Mid-term Exam, may receive a higher weighting of the Final Exam (Mid-term: 10%, Final: 40%), at my discretion. A significantly higher grade is one that is 25 percentage points higher.
- Four problem sets will be assigned, which will assist you in understanding and learning the lecture material (quantitative aspects) and which will serve as prototypes for some of the questions on the Mid-term and the Final Examination.

Laboratory SCIE 3101 (30.00%)

Independent Study/Seminar (20.00%)

- Commencing with Lecture#17 (March 13, 2018), we will have three Powerpoint presentations per lecture period with each seminar being a group effort (two students per team). The presentations will be 15 min in length followed by a 5-min question period. Each team member will receive an identical mark for the presentation, including the ability to answer questions (independent study/seminar is worth 20% of the course grade). Therefore, it is important to help each other and to work as a team! It is important to remember that anything that you include or say during your presentation is open to questions from the audience and, so you should ensure that you fully understand it. A seminar rubric will be used for evaluation of the seminars. Drs. Cooper and Merrill along with Lab TA's, Madison Turner and Liam Doyle, will complete evaluation forms on each presentation, comments/feedback will be given, but not the marks until all the presentations have been completed. A grade (5% of your seminar mark) will also be given for seminar attendance and participation in the question period (details to follow later).
- Students must form a two-person team by Jan 23th, 2018 and the team must decide upon a case-study enzyme for their presentation and clear the topic with Drs. Cooper or Merrill by Jan 26th, 2018 (4 pm). The lecture/seminar dates are: Mar 13, 15, 20, 22, 27, 29, Apr 3, and 5. Dr. Cooper will schedule the presentation dates for all the teams. Some research and preparation time will be given during the Enzymology lab sessions (see Dr. Cooper for details). If you are unable to present your seminar on the scheduled date, a separate project/assignment will be given.

Final Examination (30.00%)

Date: Thu, Apr 12, 2:30 PM - 4:30 PM, TBA

- The final grade can be based entirely on the Final Exam (and other components) only if reasons for missing the Mid-term exam are adequately documented.
- Both exams are required!
- The Final Exam is cumulative and will cover all lectures

- Students who score a significantly higher grade on the Final Exam, compared with the Mid-term Exam, may receive a higher weighting of the Final Exam (Mid-term: 10%, Final: 40%), at my discretion. A significantly higher grade is one that is 25 percentage points higher.
- Four problem sets will be assigned, which will assist you in understanding and learning the lecture material (quantitative aspects) and which will serve as prototypes for some of the questions on the Mid-term and the Final Examination.

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7 Course Statements

7.1 Instructor & Course Evaluation

As part of the evaluation process in the Department of Molecular and Cellular Biology, written comments on the Course and/or the Instructors' teaching performance may be sent to the Chair, Department of Molecular and Cellular Biology, at any time. Such letters must be signed. Departmental Evaluations will also be conducted near the end of the semester. Copies of evaluations will be made available to the Instructor after submission of the final grade.

7.2 Course Add and Drop

Notification is not needed for dropping the course before the DROP deadline (40th class day, Mar 9, 2018). Program approval is only needed for drops and adds if your category is "Special" or "Provisional".

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8 Department of Molecular and Cellular Biology Statements

8.1 Academic Advisors

If you are concerned about any aspect of your academic program:

- Make an appointment with a program counsellor in your degree program. B.Sc. Academic Advising or Program Counsellors

8.2 Academic Support

If you are struggling to succeed academically:

- Learning Commons: There are numerous academic resources offered by the Learning Commons including, Supported Learning Groups for a variety of courses, workshops related to time management, taking multiple choice exams, and general study skills. You can also set up individualized appointments with a learning specialist. http://www.learningcommons.uoguelph.ca/
- Science Commons: Located in the library, the Science Commons provides support for physics, mathematic/statistics, and chemistry. Details on their hours of operations can be

found at: http://www.lib.uoguelph.ca/get-assistance/studying/chemistry-physics-help and http://www.lib.uoguelph.ca/get-assistance/studying/math-stats-help

8.3 Wellness

If you are struggling with personal or health issues:

- Counselling services offers individualized appointments to help students work through personal struggles that may be impacting their academic performance. https://www.uoguelph.ca/counselling/
- Student Health Services is located on campus and is available to provide medical attention. https://www.uoguelph.ca/studenthealthservices/clinic
- For support related to stress and anxiety, besides Health Services and Counselling Services, Kathy Somers runs training workshops and one-on-one sessions related to stress management and high performance situations. http://www.uoguelph.ca/~ksomers/

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9 University Statements

9.1 Email Communication

As per university regulations, all students are required to check their e-mail account regularly: e-mail is the official route of communication between the University and its students.

9.2 When You Cannot Meet a Course Requirement

When you find yourself unable to meet an in-course requirement because of illness or compassionate reasons please advise the course instructor (or designated person, such as a teaching assistant) in writing, with your name, id#, and e-mail contact. The regulations and procedures for Academic Consideration are detailed in the Undergraduate Calendar.

9.3 Drop Date

Courses that are one semester long must be dropped by the end of the fortieth class day; two-semester courses must be dropped by the last day of the add period in the second semester. The regulations and procedures for Dropping Courses are available in the Undergraduate Calendar.

9.4 Copies of Out-of-class Assignments

Keep paper and/or other reliable back-up copies of all out-of-class assignments: you may be asked to resubmit work at any time.

9.5 Accessibility

The University promotes the full participation of students who experience disabilities in their academic programs. To that end, the provision of academic accommodation is a shared responsibility between the University and the student.

When accommodations are needed, the student is required to first register with Student Accessibility Services (SAS). Documentation to substantiate the existence of a disability is required, however, interim accommodations may be possible while that process is underway.

Accommodations are available for both permanent and temporary disabilities. It should be noted that common illnesses such as a cold or the flu do not constitute a disability.

Use of the SAS Exam Centre requires students to book their exams at least 7 days in advance, and not later than the 40th Class Day.

More information: www.uoguelph.ca/sas

9.6 Academic Misconduct

The University of Guelph is committed to upholding the highest standards of academic integrity and it is the responsibility of all members of the University community – faculty, staff, and students – to be aware of what constitutes academic misconduct and to do as much as possible to prevent academic offences from occurring. University of Guelph students have the responsibility of abiding by the University's policy on academic misconduct regardless of their location of study; faculty, staff and students have the responsibility of supporting an environment that discourages misconduct. Students need to remain aware that instructors have access to and the right to use electronic and other means of detection.

Please note: Whether or not a student intended to commit academic misconduct is not relevant for a finding of guilt. Hurried or careless submission of assignments does not excuse students from responsibility for verifying the academic integrity of their work before submitting it. Students who are in any doubt as to whether an action on their part could be construed as an academic offence should consult with a faculty member or faculty advisor.

The Academic Misconduct Policy is detailed in the Undergraduate Calendar.

9.7 Recording of Materials

Presentations which are made in relation to course work—including lectures—cannot be recorded or copied without the permission of the presenter, whether the instructor, a classmate or guest lecturer. Material recorded with permission is restricted to use for that course unless further permission is granted.

9.8 Resources

The Academic Calendars are the source of information about the University of Guelph's procedures, policies and regulations which apply to undergraduate, graduate and diploma programs.

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