BIOC*4540 ENZYMOLOGY
Winter 2016

Objectives:

(i) 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.

(ii) The Learning objectives for this course are the following: the students will learn (a) to plan and execute an enzyme assay; (b) to analyse enzyme kinetic data; (c) to analyse kinetic inhibition data and to determine the mechanism of inhibition; (d) to perform library research on a specific enzyme topic; and (e) to prepare and deliver a Powerpoint seminar to their peers.

Lectures:

Tuesday and Thursday @ 10:00 – 11:20 in MCKN 230

Lecturer:

Rod Merrill Ext. 53806 Office: SSC 2250
Email: rmerrill@uoguelph.ca

Office hours:

Tuesday and Thursday @ 1:00 – 2:30 pm or by appointment.

Prerequisites:

BIOC*3560 (may be taken concurrently); BIOC*3570

Instructor and 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.

Textbook:

No single textbook is **sufficient** for the lecture material but Lehninger: Principles in Biochemistry (4^{th}, 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. Another useful reference text is Structure

and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, 2nd edition (1999), Alan Fersht, W.H. Freeman and Co. New York, NY 1999. A copy of each of these texts has been placed on reserve for reference purposes. Also, 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.

Laboratory:
Laboratory sections will be held on Mon, Tue, Wed, and Thu from 14:30 – 17:20 in SSC 3101. 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 11-13, 2016 (hours of operation: 10:00 – 12:00; 13:00 – 15:00).

Demonstrators:
Please see the Enzymology Lab Coordinator, Paula Russell, for details (Room 3502 SCIE, prussell@uoguelph.ca, ext. 58763). The lab TAs are: Paula Russell, Alison Berezuk, and Sean Liston.

Course Drop and Add:

Notification is **not** needed for dropping the course before the **DROP** deadline (40th class day, Mar 11, 2016). Program approval is only needed for drops and adds if your category is "Special" or "Provisional".

Evaluation:

Form of Assessment	**Weight (% grade)**
Midterm	20%
Laboratory SCIE 3101	30%
Independent Study/Seminar	20%
Final Examination	30%
	100%

There will be a Mid-term Examination (80 min, in class-time) involving short answer and problem questions (**Midterm date: Tue, Mar 1, 2016**). There are no alternate exams offered since the Midterm 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! The Final Exam is **cumulative** and will cover all lectures

and is scheduled for **Sat, April 16, 2016 from 14:30 – 16:30 (location, TBA).** Students who score a significantly higher grade on the Final Exam, compared with the Midterm Exam, may receive a higher weighting of the Final Exam (Midterm: 10%, Final: 40%), at my discretion. A significantly higher grade is one that is 25 percentage points higher.

Problem Sets: Five 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 Midterm and the Final Examinations.

Independent Study and Seminars: Commencing with Lecture#17 (March 15, 2016), 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). 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. Russell and Merrill along with Lab TA's, Alison Berezuk and Sean Liston, will complete evaluation forms on each presentation, comments/feedback will be given, but not the marks until all of 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 19th, 2016 and the team must decide upon a case-study enzyme for their presentation and clear the topic with Drs. Russell or Merrill by Jan 26th, 2016 (4 pm). **The lecture/seminar dates are: Mar 15, 17, 22, 24, 29, 31, Apr 5, and 7.** Dr. Russell will coordinate the scheduling of the presentation dates for all the teams. Some research and preparation time will be given during the Enzymology lab sessions (see Dr. Russell for details).

BIOC*4540 Enzymology Winter 2016
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

(1) Historical Aspects
(2) Discovery of enzymes
(3) Chemistry of enzymes
(4) Function and importance
(5) Enzymes in Biotechnology

Lect#2: Characteristics and Properties

(1) Catalytic power and specificity
(2) Enzymes as catalysts
(3) Enzyme - substrate interactions
    (a) lock & key model
    (b) induced fit model
    (c) transition state model
    (d) quantum tunnelling model
(4) Enzymes as proteins
(5) Non-protein cofactors
    (a) metal ions
    (b) organic cofactors

Lect#3: Enzyme Purification and Assay

(1) Initial velocity measurements
(2) Assay types
(3) Enzyme units of activity
(4) Turnover number and properties
(5) Purification and assessment
(6) Methods for measurements

Lect#4: Michaelis-Menten Kinetics

(1) Introduction
(2) Assumptions
(3) Derivation
(4) Description of v_o versus [S]
(5) Michaelis constant (K_M)
(6) Specificity/Substrate constant (SpC)

Lect#5: Graphical Analysis of Kinetic Data, pH and Temp Dependence

- **(1) Graphical Analysis**
  - **(a) Lineweaver-Burk Analysis**
  - **(b) Hanes-Woolf Analysis**
  - **(c) Eadie-Hofstee Analysis**
  - **(d) Direct Linear Plot (Eisenthal/Cornish-Bowden Plot)**
  - **(e) Nonlinear Curve Fitting**
- **(2) pH-dependence of Michaelis-Menten Enzymes**
- **(3) Temperature-Dependence of Enzyme Reactions**

Lect#6: Enzyme Inhibition and Kinetics

- **A. Classification of Inhibitors**
  - **(1) Reversible**
  - **(2) Irreversible**
    - **(a) Iodoacetamide**
    - **(b) DIFP**
    - **(c) Additional examples**
  - **(3) Classification of Reversible Inhibitors**
    - **(a) Competitive**
    - **(b) Uncompetitive**
    - **(c) Noncompetitive**
    - **(d) Substrate**

Lect#7: Nomenclature / Classification

- **A. Nomenclature/Classification**
  - **(1) Oxidoreductase-dehydrogenase**
  - **(2) Transferase**
  - **(3) Hydrolase**
  - **(4) Lyase**
  - **(5) Isomerase**
  - **(6) Ligase**
- **B. Regulatory Enzymes**
  - **(1) Mechanism**
  - **(2) Kinetics**
  - **(3) Examples**

Lect#8: Single Molecule Enzymology

- **(1) Movies of Single Enzymes**
- **(2) Advantages of Single Molecule Studies**
- **(3) Applications of Single Molecule Studies**
- **(4) Following Enzymes in Real Time**
  - **(a) ATP Synthase**
  - **(b) ATP Synthase with Tethered Actin**

(c) Myosin-V
(d) Kinesin motor attached to a fluorescent bead
(e) Single Molecule Studies of Cholesterol Oxidase
(f) β-galactosidase: a model Michaelis-Menten enzyme?

Lect#9: Multi-substrate Reactions and Substrate Binding Analysis

A. Multi-substrate Reactions
  (1) Cleland Convention
  (2) Ordered and Random Mechanisms
  (3) Sequential and Nonsequential Mechanisms
    (a) Sequential
    (b) Nonsequential

B. Substrate Binding Analysis
  (1) Single Binding Site Model
  (2) Binding Data Plots
    (a) Direct Plot
    (b) Reciprocal Plot
    (c) Scatchard Plot

C. Determination of Enzyme-Substrate Dissociation Constants
  (1) Kinetics
  (2) Equilibrium Dialysis
  (3) Equilibrium Gel Filtration
  (4) Ultracentrifugation
  (5) Spectroscopic Methods

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

Lect#10: Enzyme Mechanisms-I

A. Reaction Mechanisms and Catalysis
  (1) Enzyme-transition state complementarity
    (a) Structure-activity correlations
    (b) Transition state analogues
    (c) Catalytic antibodies
    (d) Summary
  (2) Preferential transition state binding
    (a) Transition state theory
  (3) proximity effect
  (4) Acid-base catalysts

Lect#11: Enzyme Mechanisms II

- **(5) Covalent catalysis**
- **(6) Metal ion catalysis**
- **(7) Electrostatic catalysis**
- **(8) Low barrier H-bonds**
- **(9) Structural flexibility**

Lect#12: Enzyme Mechanisms-III: Techniques for Drug Discovery

- **A. Drug Design**
- **B. Techniques of Drug Discovery**
  - **(1) Complexity of Drug Discovery**
  - **(2) SARS and QSARS**
  - **(3) Structure-based Drug Design**
  - **(4) Combinatorial Chemistry and High-Throughput Screening**
- **C. Introduction to Pharmacology**
  - **(1) Pharmocokinetics**
  - **(2) Toxicity and Adverse Reactions Eliminate Most Drug Candidates**
    - **(a) Phase I**
    - **(b) Phase II**
    - **(c) Phase III**
  - **(3) Drug Candidate Statistics**
  - **(4) Cytochrome P450 Metabolizes Drugs**
  - **(5) Many Drugs are Enzyme Inhibitors**
    - **(a) Sulfadrugs**
    - **(b) Viagara**

Lect#13: Midterm Examination (during class time) Tue, Mar 1, 2016

Lect#14: Active Site Investigations I

- **(1) Kinetic Studies**
  - **(a) Variation of substrate concentration**
  - **(b) Variation of substrate structure**
  - **(c) Reversible inhibition**
  - **(d) Variation of pH**
  - **(e) Pre-steady state kinetics**
- **(2) Detection of Intermediates**
- **(3) X-ray Crystallographic Studies**
- **(4) NMR for Protein Structure Determination**

Lect#15: Active Site Investigations II

- **(5) Chemical Modifications**
  - **(a) Applications**
- **(6) Super-reactive Sidechains**
- **(7) Suicide Substrates**
- **(8) Interpretation of Chemical Modification Experiments**
- **(9) Criteria for establishment of side chain involvement in catalysis**

Lect#16: Enzyme Engineering and Design

- **(1) Substitution**
- **(2) Insertion**
- **(3) Hybrid Proteins**
- **(4) Genes for Novel Enzymes**
  - **(a) Aequorin**
  - **(b) Enviropig**
- **(5) Engineering More Stable Enzymes**
- **(6) Incorporation of Non-natural Amino Acids into Enzymes**
- **(7) Protein Engineering by Combinatorial Methods**
- **(8) 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) if necessary, and/or Course Review.

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

SUPPLEMENTARY TEXTS

Alan Fersht (1999) Structure and Mechanism in Protein Science, 2nd edition, W.H. Freeman & Co.

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

Trevor Palmer (1985) Understanding Enzymes, 2nd edition, J. Wiley & Sons, New York.

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

All indicated supplementary texts, papers and treatises are available at the Reserve Desk at the library on two hour loan. Dr. Paula Russell also has a collection of references that specifically pertain to the laboratory.