

CHEM 4540 ENZYMOLOGY
Winter 2005

Objectives: To integrate the practical aspects of enzymology with the kinetic theories to provide a mechanistic overview of enzyme activity and regulation in cells.

Lectures: Tuesday and Thursday @ 11:30 - 12:50 in AXEL 309

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Office hours: Tuesday and Thursday @ 12:50 – 13:50 or by appointment.

Textbook: No single textbook is sufficient for the material but the best overall 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 this text 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). A copy of the lecture notes, including illustrations, will be handed out in class for each lecture topic.

Laboratory: Wed, Thu, and Fri from 14:30 – 17:20 in SCIE 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 from Leona Varga SCIE Room 1255, Mol. & Cell. Biol. Department (hours of operation: 9:00 – 12:00; 13:00 – 16:00).

Demonstrators: Please see the Enzymology Lab Coordinator, Paula Russell, for details (Room 3115 SCIE, prussell@uoguelph.ca, ext. 58220).

Evaluation:	Quizzes (2)	30%
	Laboratory NSC 3101	35%
	Final Examination	35%

100%

There will be two in-class quizzes (75 min) involving short answer and problem questions (Quiz dates: Feb. 17 and Mar. 24). There are no alternate quizzes offered since they will be given **in class time**. If you are unable to write a quiz (legitimate excuse required) then the weighting will be 30% for the other quiz. The final exam is cumulative and will cover all lectures and is scheduled for Thu, Apr 14 from 8:30a – 10:30a. Biweekly 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 the questions on the quizzes and the final examination.

Chem 4540 Enzymology Winter 2005
Course Subject Outline

I. ENZYMES AS CATALYSTS (P&S Ch 1 & 2; Fer Ch 2; Palm Ch 1 & 16)

A. Overview--proteins as catalysts (historical background)

B. Enzyme characteristics and properties

C. Enzyme nomenclature/classification

D. Enzyme Purification and Assay

- (1) activity measurements
- (2) enzyme units
- (3) turnover number and properties
- (4) purification and purity
- (5) initial velocity measurements
- (6) assay conditions
- (7) methods for measurement
- (8) choice of assay method
- (9) practical considerations

II. ENZYME KINETICS (P&S Ch 4; Fer Ch 2, 3, 4, 6; Palm Ch 6-8)

A. Kinetics of single substrate reactions

- (1) kinetic concepts
- (2) enzyme kinetics
 - (a) Briggs-Haldane steady-state treatment
 - (b) Michaelis constant (K_m)
 - (c) specificity constant
- (3) single enzyme kinetics
- (4) graphical analysis

B. Enzyme inhibition

- (1) Classification
 - (a) competitive
 - (b) noncompetitive
 - (c) uncompetitive
 - (d) substrate

C. Multi-substrate reactions

- (1) convention
- (2) mechanisms

D. Substrate binding analysis

- (1) derivation
- (2) methodology

III. MECHANISM OF ENZYME CATALYSIS (P&S Ch 5; Fer Ch 2, 9; Palm Ch 10,11; Zub Ch 9)

A. Reaction Mechanisms and Catalysis

- (1) proximity effect
- (2) acid-base catalysts
- (3) electrostatic
- (4) functional groups
- (5) structural flexibility

B. Active Site Investigations

- (1) kinetic studies
- (2) detection of intermediates
- (3) x-ray crystallographic studies
- (4) chemical modification of amino acid side chains
- (5) site-directed mutagenesis studies
- (6) enzyme engineering

C. Specific enzymes

- (1) alcohol dehydrogenase
- (2) ribonuclease A
- (3) triose phosphate isomerase
- (4) amino acyl tRNA synthetases
- (5) carbonic anhydrase

IV. ENZYME REGULATION (P&S Ch 6; Zub Ch 10)

A. Partial Proteolysis

B. Phosphorylation, adenylation, disulphide reduction

C. Allosteric regulation

- (1) sigmoidal kinetics
- (2) symmetry model
- (3) concerted model
- (4) kinetics and functions of allosteric enzymes
 - (a) phosphofructokinase
 - (b) glycogen phosphorylase

Fer = Ferst; Palm = Palmer, P&S = Price and Stevens; Zub = Zubay.

SUPPLEMENTARY TEXTS

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

Nicolas Price & Lewis Stevens (1995) Fundamentals of Enzymology, 2nd edition, Oxford Univ. Press, New York, NY., publ 1995.

Trevor Palmer (1985) Understanding Enzymes, Second Edition, J. Wiley & Sons, New York.

Donald Voet & Judith Voet (1995) Biochemistry, J. Wiley & Sons, New York (Chapters 12 through 15).

Geoffrey Zubay (1993) Biochemistry, 3rd edition, Wm. C. Brown, Oxford (1993).

Robert K. Scopes (1988) Protein Purification, Second Edition, Springer-Verlag, Berlin.

Athel Cornish-Bowden (1979) Fundamentals of Enzyme Kinetics, Butterworths, London.

Methods in Enzymology, Vol. **182** (1990) Protein Purification.

Pharmacia LKB Biotechnology Press: Gel Filtration, Theory & Practice and Affinity Chromatography, Principles & Methods.

Laboratory References, a selection of papers cited in the lab manual and collected in a binder for student use.

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