Semester: 5	
Course: Major 1	
Paper Title: Enzyme Structure & Kinetics	
Paper code: C3BT230512T/P	**Credits: 3+1**
Hours/week: 3+3	
Category: Core/MDC/SEC/VAC: Core	
Theory / Practical / Composite: Composite	
No of Modules: Theory - 2	

Course Overview:

This course provides a comprehensive understanding of enzymes' structure-function relationship and steady-state kinetics of enzyme-catalysed reactions.

Structural studies will encompass enzymes like proteases, metalloenzymes, restriction endonucleases and AAA+ ATPases. Concepts of conversion of inactive to active enzymes like, apoenzymes to holoenzymes and inactive zymogens to functional enzymes, will be discussed. Various regulatory mechanisms of enzyme activity, including allosteric modulation exhibited by ATCase will be dealt with elaborately. Significance of transition state analogues in efficient competitive inhibition of enzymes will be stated.

Enzyme kinetics will be initiated with a discussion on a reaction co-ordinate diagram that depicts Gibb's free energy transitions in a biochemical reaction. From structural and thermodynamic viewpoints students will learn how enzymes, under *in vivo* mild conditions, help substrates to overcome activation energy barrier to form products. In depth discussion of steady-state enzyme kinetics, including derivation of Michaelis-Menten equation and its modification by Lineweaver-Burke, will be done. This knowledge will help students to distinguish amongst different types of reversible enzyme inhibitors that have immense application in drug industry. Mechanism of action of Penicillin, a suicide inhibitor, will be discussed in the light of rational drug design. Lysozyme will be used as a model enzyme to help students understand how different techniques can be used to study mechanism of catalysis. Concept of 'directed evolution of enzymes' will be touched upon. Finally, two amazing enzymes, pyruvate dehydrogenase and FoF_1 ATP Synthase, both multi-subunit complex enzymes, will be discussed to understand the underlying structure-function relationship.

Course Outcome:

Module A and B

1. Remember enzyme classification, cofactors, protein structure, and bioenergetics, all necessary for a complete understanding of enzyme function, the definitions of Apo and holoenzyme; Cofactor and prosthetic groups; Zymogens.
2. Understand the molecular mechanisms of different enzymes such as proteases, kinases, ATPases and Restriction endonucleases, Metalloenzymes from structural point of view. Importance of different Transition-state analogs, feedback control, covalent modification, Abzymes. Understand the molecular and thermodynamic principles governing enzyme catalysis; the concept of steady-state kinetics; postulations and derivation of Michaelis Menten equation (M-M); necessity of the Lineweaver-Burk equation (L-B).
3. Apply knowledge of enzyme kinetics to perform enzyme inhibition kinetics that help us to distinguish amongst different types of reversible inhibitors, many of which have been developed into therapeutic drugs. Enzyme kinetics can be applied to differentiate between single- and double- displacement reactions and also to determine turnover number (K_{cat}) of an enzyme.
4. Analyse the M-M equation to see whether it fits experimental observation under different conditions of [S], and $v_{o;}$ to analyse catalytic efficiency (K_{cat}/K_M) of an

enzyme; and to test whether an enzyme has achieved catalytic perfection. Analyse Cooperativity and allosteric effects from the structural point of view, Substrate-induced subunit remodeling in oligomeric proteins.

5. Evaluate particular enzymes' concentration and/or activity in medical diagnosis and prognosis (SGOT, SGPT in LFT). Gel electrophoretic patterns of certain isoenzymes obtained from patient's sample also help in diagnosis of certain diseases (CK, LDH, ALP, HK).
6. Create a flow chart of methodology to study mechanism of enzyme catalysis, using several biochemical and biophysical techniques. Study of lysozyme's catalytic mechanism may be considered as a model in this direction. Also, a student should be capable of creating enzymes with better efficiency, superior thermal or pH stability, or altered substrate specificity, as is achieved by directed evolution of enzymes.

Prerequisites: Basic knowledge of protein structure, thermodynamics, chemical kinetics, and bioenergetics.

SYLLABUS

UNIT/Module	CONTENT	HOURS or NUMBER OF CLASSES	CO Mapping	COGNITIVE LEVEL
Module A	**Unit-I Enzymes from a structural perspective:** Apo and holoenzyme; Cofactor and prosthetic groups; Zymogens and their activation (Proteases and Prothrombin); Structures and mechanisms of Serine proteases, Cystine Proteases, and Aspartic Proteases Restriction endonucleases Metalloenzyme (Carbonic anhydrase, MMPs), sensory kinases; Cooperativity and allosteric effects from the structural point of view - Aspartate transcarbamoylase (ATCase), Substrate-induced subunit remodelling in oligomeric proteins, AAA+ ATPases; Enzyme regulations - Regulations by competitive and non-competitive inhibitors, Importance of different Transition-state analogs, feedback control, covalent modification, Abzymes.	1 class per week	CO1-CO6	K1-K6
Module B	**Unit II: Enzyme Kinetics** Reaction co-ordinate diagrams of uncatalyzed vs. enzyme-catalysed reaction; Binding Energy - its	2 classes per week	CO1-CO6	K1-K6

	contribution to reaction specificity and catalysis; Enzyme Kinetics – derivation of Michaelis-Menten (MM) Equation, transformation of MM-equation to Lineweaver-Burk (LB) Equation, advantage of LB plot over MM-plot in determining V_{max} and K_M, Eadie-Hofstee Plot; Enzyme Inhibition – reversible inhibition kinetics (Competitive, Uncompetitive, Mixed) including modified LB equations and respective double reciprocal plots, irreversible inhibition and how irreversibly modified enzymes can help in active-site mapping by mass spectrometry, suicide or Mechanism-based inhibition; Bi-Bi reactions – single-displacement (ordered or random) and double-displacement reactions and their identification by kinetics or isotope exchange method; Lysozyme as a model enzyme to learn how structural and kinetic studies can help to decipher enzyme's mechanism of action; Directed Evolution of Enzymes. **Unit-III:** Structural and functional relationship in complex enzymes like Pyruvate Dehydrogenase Complex – substrate tunnelling; FoF1 ATPase – how proton-motive force drives the synthesis of ATP.			

Practical:

1. Malachite green assays for ATPase/GTPase
2. Calculation of dissociation constant (K_d) value from intrinsic fluorescence quenching data obtained upon binding of the substrate analog with enzyme (demonstration experiment followed by calculations).
3. Determination of activity and specific activity of an enzyme (alkaline phosphatase from calf-intestinal mucosa, CIAP) under optimum conditions.
4. Determination of Km and Vmax of CIAP, in the absence and presence of inhibitor.
5. Determination of K_{cat} of CIAP.
6. Determination of pH optima.

Text Books

1. Biochemistry - Lubert Stryer
2. Lehninger Principles of Biochemistry - David L. Nelson and Michael Cox

Suggested readings

1. Biochemistry – Voet & Voet

Evaluation

Theory:

CIA - 10
Assignment - 02
Attendance - 03
Semester Exam - 45

Evaluation

Practical:

CA – 30
Attendance – 2
Semester Exam – 8

Paper Structure for Theory Semester Exam:

Module A (20 marks)

Q1. Compulsory – 6 marks
Any two from three questions (Q2 - Q4) – each 7 marks
[No sub-part will be less than 1 mark and more than 4 marks]

Module B (25 marks)

Q5. Compulsory – 10 marks
Any one from two questions (Q6 - Q7) – each 15 marks
[No sub-part will be less than 1 mark and more than 5 marks]

COURSE OUTCOMES (COS) AND COGNITIVE LEVEL MAPPING

COs	CO Description	Cognitive levels
CO1	**Remember** Apo and holoenzyme; Cofactor and prosthetic groups; Zymogens and their activation (Proteases and Prothrombin);	K1 to K4
CO2	**Understand** Structures and mechanisms of Serine proteases, Cystine Proteases, and Aspartic Proteases Restriction endonucleases Metalloenzyme (Carbonic anhydrase, MMPs), sensory kinases; Cooperativity and allosteric effects from the structural point of view - Aspartate transcarbamoylase (ATCase), Substrate-induced subunit remodelling in oligomeric proteins, AAA+ ATPases; Enzyme Inhibition – reversible inhibition kinetics (Competitive, Uncompetitive, Mixed) including modified LB equations and respective double reciprocal plots, irreversible inhibition and how irreversibly modified enzymes can help in active-site mapping by mass spectrometry, suicide or Mechanism-based inhibition; Enzyme regulations - Regulations by competitive and non-competitive inhibitors, Importance of different Transition-state analogs, feedback control, covalent modification, Abzymes.	K1 to K4

CO3	**Apply** Concepts associated with Bi-Bi reactions – single-displacement (ordered or random) and double-displacement reactions and their identification by kinetics or isotope exchange method; Lysozyme as a model enzyme to learn how structural and kinetic studies can help to decipher enzyme's mechanism of action; Directed Evolution of Enzymes.	K1 to K4
CO4	**Analyze** Reaction co-ordinate diagrams of uncatalyzed vs. enzyme-catalysed reaction; Binding Energy - its contribution to reaction specificity and catalysis; Enzyme Kinetics – derivation of Michaelis-Menten (MM) Equation, transformation of MM-equation to Lineweaver-Burk (LB) Equation, advantage of LB plot over MM-plot in determining V_{max} and K_M, Eadie-Hofstee Plot;	K3 to K6
CO5	**Evaluate** Structural and functional relationship in complex enzymes like Pyruvate Dehydrogenase Complex – substrate tunnelling; FoF1 ATPase – how proton-motive force drives the synthesis of ATP.	K3 to K6
CO6	**Create** innovative approaches in sustainable agriculture and biotechnology by integrating concepts from physiology, signaling, and developmental anatomy to conceptualize real world scenarios associated with climate change.	K3 to K6