Semester	**V**
Course	**Major 1**
Paper Title	Enzyme Structure and Kinetics
Paper Code	C3BT230512T/P
No of Credits	4 (3+1)
Theory /Practical /Composite	Composite
Minimum No. of preparatory hours per week a student has to devote	4
Number of Modules	2
Syllabus	**MODULE A [20 Marks]** **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 remodeling 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)* **MODULE B [25 Marks]** **Unit-II: Enzyme Kinetics** 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; 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: Structure-Function Relationships in Complex Enzymes** Pyruvate Dehydrogenase Complex – substrate tunnelling; F_oF_1 ATPase – how proton-motive force drives the synthesis of ATP. *(2 classes per week)* **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 K_m and V_{max} of CIAP, in the absence and presence of inhibitor. 5. Determination of K_{cat} of CIAP. 6. Determination of pH optima.
Learning Outcomes	1. Realization of the significance of enzymes in living systems. 2. Importance of thermodynamics in enzyme catalysis. 3. Structure-function relationship of the enzymes. 4. Mechanism of actions of a few model enzymes. 5. Kinetics of enzyme catalysis with special emphasis on enzyme inhibitors. 6. Enzyme regulations and their physiological importance. 7. Significance of enzymes in industry and medicines. 8. In practical module the students will be given hands on training in enzyme activity/specific activity measurements.
Reading / Reference List	1. Lehninger Principles of Biochemistry - Cox & Nelson 2. Biochemistry - Voet and Voet 3. Biochemistry – Berg, Tymoczko & Stryer
Evaluation	**Theory** CIA- 10 Assignment – 02 Attendance - 03 Semester Exam- 45 **Practical** CA- 30 Attendance - 02 Semester Exam- 08
Paper Structure for Theory Semester Exam	**Module A (20 marks)** • 1 Compulsory objective-type Question of 6 marks • Any 2 out of 3 questions; each of 7 marks, with subparts (not less than 1, not more than 4)

	Module B (25 Marks) • 1 Compulsory Question of 10 marks with sub-parts (not less than 1, not more than 5) • Any 1 out of 2 questions; each of 15 marks, with subparts (not less than 1, not more than 5)