## BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI, HYDERABAD CAMPUS INSTRUCTION DIVISION, FIRST SEMESTER 2021-2022

**Course Handout (Part-II)**

**03/08/2021**

In addition to part I (General Handout for all courses appended to the time table this portion gives further specific details regarding the course.

***Course No.* : BIO G651**

***Course Title* : PROTEIN AND ENZYME ENGINERING**

***Instructor-in-Charge* : RAMAKRISHNA VADREVU**

***Team of Instructors* I Shivakumar, Deepika K, Kodam Pradeep**

1. **Course Description**:

Sources, isolation, purification and storage of protein and/or enzymes; kinetics of enzyme catalyzed reactions; biocatalyst reaction engineering; techniques of production and recovery of enzymes; protein and enzyme modification; clinical and industrial applications of free and immobilized enzymes

The course will provide fundamental insights into the aspects of protein engineering principles, techniques and recent advances in manipulating proteins and enzymes, and their clinical and biotechnology applications. Enzyme production, kinetics and immobilization methods will be emphasized together with case studies to reinforce the impact of protein and enzyme engineering in basic biological research and biotechnology applications. The main objective of the course is to understand the principles and procedures to altering proteins and enzymes for applications in diverse areas.

# **Text Book (T):** Enzyme Technology, Noorlabettu Krishna Prasad, PHI Learning Private Ltd. New Delhi, 2011.

1. Reference Books

# **R1**. Sheldon Park and Jennifer Cochran. Protein Engineering and Design. CRC Press, 2010 **R2**. Carl Branden & John Tooze, Introduction to Protein Structure, Second Edition, Garland Publishing Inc.

In addition to the text and reference books, necessary and relevant material indicated as Articles Review(s) and will be followed.

**R3**. Protein analysis and purification: benchtop techniques/ Ian M Rosenberg (2nd Ed) Boston [Birkhauser](http://libraryopac.bits-hyderabad.ac.in/cgi-bin/koha/opac-search.pl?q=Provider%3ABirkha%EF%BE%A8user) 2005.

## 4 Course Plan :

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| **Lec. No.** | **Topic** | **Learning Objective** | **Chapter in**  **Text Book (Ref book)** |
| 1 | Protein engineering | Perspective | Class Notes |
| 2-11 | Protein structure  /function & Protein Folding/stability | Characteristics of individual amino acids and their effect on the solubility, structure and function Protein Structure Hierarchy, Classes  Protein folds and concept of active site structures in some protein folds. Protein structure, stability and folding, CD/FL spectrophotometric methods for structure and stability analysis | R2 (2-6) Class Notes/Published Research Articles |
| 11-14 | Protein/enzymes production | Sources Isolation  Purification methods Storage of proteins | R3 (5,10)  Class Notes |
| 15-23 | Overview of methods in Protein Engineering | Phage Display Systems  Cell Surface Display Systems Cell Free Display Systems Library construction | R1 (1-4)  Class Notes/Published  Research Articles |

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| 24-31 | Strategies of Protein/Enzyme Engineering | Rational Methods De novo design Directed Evolution  Knowledge-Based Protein Design | R1 (4,11)  Class Notes Research  Articles/Review s |
| 31-33 | Enzymes and Protein Modifications | Properties of Enzymes  Protein modifications (Mass spectrometry, Basic theory and applications in detection of proteins and protein modifications/proteomics) | T1 (1) Class Notes/Literature articles/reviews |
| 34-36 | Enzyme Kinetics | Free energy of activation  Factors affecting Enzyme kinetics  Michaelis-Menten Theory of Enzyme kinetics Linear equations  Enzyme inhibition | T1 (2)  Class notes |
| 37-40 | Enzyme Immobilization | Immobilization techniques, experimental procedures, properties of immobilized enzymes, enzyme stabilization | T1 (6), Class notes, Review articles |

**LIST OF EXPERIMENTS**

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| **Practical No.** | **Experiment Title** |
| 1-3 | Exploring primary and secondary databases on protein sequence and structural properties (EXPASY, PDB, PDBSUM) / Protein Visualization (Pymol) structure prediction software |
| 4 | Measurement of protein secondary structure by Circular Dichroism and estimation of secondary structure content using prediction algorithms |
| 5-6 | Measurement of fluorescence excitation and emission spectra of proteins and determination of thermal stability of proteins (protein unfolding and obtaining thermodynamic stability measurements from either CD/or FL spectroscopy) |
| 6-7 | Protein expression in bacteria by IPTG induction (bacterial growth and concentration of IPTG optimization): SDS Page electrophoresis |
| 8-11 | Protein Purification using AKTA FPLC (Bacterial protein expression, centrifugation, sonication, dialysis, column chromatography) |
| 12 | ESI-MS spectrum of purified protein. Determination of molecular weight of protein(s) |

1. **Evaluation Scheme:**

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| **Evaluation Component** | Duration | Weightage (%) | Date & Time | Remarks |
| Mid Semester Exam | 90 min | 25 |  | Closed Book |
| Assignments and or Quizzes  (Class/Take Home) Research Design/Presentation etc. |  | 20 |  | Open Book |
| Practical /Record Book/Class Interaction/viva/quiz) | Stipulated hours in the TT | 20 |  | Open Book |
| Comprehensive Exam | 2 hours | 35 |  | Closed Book |

1. **Chamber Consultation Hour:** Will be announced in the Class.
2. **Notices:** Notice, if any, concerning the course will be displayed only on CMS
3. **Make-up Policy:** For genuine cases only for Tests & Comprehensive exam. Prior permission of the Instructor-in-Charge is necessary. No make-up will be granted for other components. No make-up for lab experiments/components.
4. **Academic Honesty and Integrity Policy**: Academic honesty and integrity are to be maintained by all the students throughout the semester and no type of academic dishonesty is acceptable.

## INSTRUCTOR-IN-CHARGE