

Course title: Proteomics and Protein Engineering				
Course code: BBP 166		No. of credits: 3	L-T-P: 45-0-0	
Learning hours: 45				
Pre-requisite course code and title (if any): Principles of Biochemistry and Biophysics (BBP161)				
Department: Department of Biotechnology				
Course coordinator: Dr. Chaithanya Madhurantakam			Course instructor : Dr. Chaithanya Madhurantakam	
Contact details: chaithanya.madhurantakam@terisas.ac.in				
Course type: Core			Course offered in: Semester 3	
Course description: Protein engineering has revolutionized the field of biosciences with varied applications and this course will provide students with the concepts along with knowledge of methods and tools used to engineer proteins. Further, the topics in proteomics will deal with outcomes of functional genomics and its applications in the health sector. The myriad of techniques that have evolved in mass spectrometry aiding advanced proteomics will be dealt with in the course.				
Course objectives:				
<ol style="list-style-type: none"> 1. Students will be acquainted with methods and tools for protein synthesis and separation. 2. Familiarizing students with various domains and platforms used in Mass Spectrometry and concepts related to advanced MS techniques. 3. Students will be provided with the concept of designing proteins, artificial macromolecular scaffolds, and its applications. 4. Providing students with information on techniques involved in deciphering the structure function relationship in proteins. 5. Familiarizing students with varied applications of engineered proteins. 				
Course contents				
Module	Topic	L	T	P
Module 1: Protein Synthesis and Separation				
	Protein expression, purification and separation, generation of pH gradients, IEF, 2-D PAGE, Cell-free translation systems, Random mutagenesis and selection, saturation mutagenesis (impact of mutagenesis on protein structure, solubility, and function), designed divergent evolution, receptor based QSAR methods, phage display, yeast surface display and ribosomal display mechanisms	10	0	0
Module 2: Proteomics				
	Mass Spectrometry (MS): principles, quadrupole, mass analyzers, timescale of events in MS, quasi equilibrium theory (QET), unimolecular dissociations in MS, mass spectrum and isotopes, data dependent MS/MS, targeted MS/MS (SID-MRM-MS), protein identification: N-terminal sequencing, MALDI –TOF, LC-MS/MS, Tandem-MS/MS. SELDI-TOF, ICAT, I TRAQ (4-plex, 8-plex), MUDPIT, Protein interaction maps, analysis of cellular constituents, metabolomics, functional proteomics; Clinical and biomedical application of proteomics; Proteome database	12	0	0
Module 3: Protein design and engineering- components, methods, and tools				
	Molecular scaffolds: Repeat proteins like tetratricopeptide repeats (TPRs), ankyrin repeats (ANKs), leucine-rich repeats (LRRs), armadillo repeats (ARMs), and hexapeptide repeats (HPRs) for diagnostics and PPI studies, artificial protein design, <i>de novo</i> protein design (ROSETTA), Antibody engineering, Protein chips	8	0	0
Module 4: Structure function data on proteins				
	Structural and functional information through X-ray crystallography, NMR, Electron Microscopy, computational techniques	8	0	0

Module 5: Engineered protein applications			
Peptidomimetics (common intermediate, solid phase synthesis and combinatorial approaches), stimulus responsive peptide systems, biosensors, drug delivery, nanodevices and tissue engineering (biopolymers), microbial proteins (dairy, pharmaceutical, industrial, and environmental units)	7	0	0
Total	45	0	0
Evaluation criteria:			
1. Minor test 1	30%		
2. Minor test 2	30%		
3. Major test (end semester)	40%		
Learning outcomes:			
1. Able to gain understanding of protein separation and synthesis methods. (Minor test 1)			
2. Acquire a deep insight into the tools and techniques of proteomics with special emphasis on mass spectrometric methods. (Minor test 1 and Minor test 2)			
3. An understanding of various design strategies of proteins. (Minor test 2)			
4. Able to apprehend the structure function relationship of proteins through three-dimensional structural analysis. (Minor test 2 and Major test)			
5. Able to grasp applications of engineered proteins. (Major test)			
Pedagogical Approach:			
1. Lectures and tutorials, demonstration of online resources.			
2. Providing case studies to support the concepts.			
3. Peer-reviewed research articles to discuss various modules in the course.			
Skill Set:			
1. Knowledge of protein separation techniques.			
2. Knowledge of tools employed in mass spectrometry, applications and, how to characterize a proteome.			
3. Knowledge of methods to design artificial protein scaffolds and applications.			
Employability:			
The course will provide skillsets and knowledge that may play a key role to get employed in Universities, R & D industries, Medical centres/Colleges, Research Institutes and Diagnostic centres apart from specialized units like pharma, breweries, dairy and agri sectors.			
Materials:			
Suggested Readings			
1. Radziwon K, Weeks AM. Protein engineering for selective proteomics. <i>Curr Opin Chem Biol</i> . 2021 Feb;60:10-19. doi: 10.1016/j.cbpa.2020.07.003. Epub 2020 Aug 5. PMID: 32768891.			
2. Cattaneo A, Chirichella M. Targeting the Post-translational Proteome with Intrabodies. <i>Trends Biotechnol</i> . 2019 Jun;37(6):578-591. doi: 10.1016/j.tibtech.2018.11.009. Epub 2018 Dec 18. PMID: 30577991.			
3. Černý M, Skalák J, Cerna H, Brzobohatý B. Advances in purification and separation of posttranslationally modified proteins. <i>J Proteomics</i> . 2013 Oct 30;92:2-27. doi: 10.1016/j.jprot.2013.05.040. Epub 2013 Jun 15. PMID: 23777897.			
4. Yakubu RR, Nieves E, Weiss LM. The Methods Employed in Mass Spectrometric Analysis of Posttranslational Modifications (PTMs) and Protein-Protein Interactions (PPIs). <i>Adv Exp Med Biol</i> . 2019;1140:169-198. doi: 10.1007/978-3-030-15950-4_10. PMID: 31347048; PMCID: PMC7059822.			
5. Mirza SP, Olivier M. Methods and approaches for the comprehensive characterization and quantification of cellular proteomes using mass spectrometry. <i>Physiol Genomics</i> . 2008 Mar 14;33(1):3-11. doi: 10.1152/physiolgenomics.00292.2007. Epub 2007 Dec 27. PMID: 18162499;			

PMCID: PMC2771641.

6. Sawyer N, Gassaway BM, Haimovich AD, Isaacs FJ, Rinehart J, Regan L. Designed phosphoprotein recognition in Escherichia coli. ACS Chem Biol. 2014 Nov 21;9(11):2502-7. doi: 10.1021/cb500658w. Epub 2014 Oct 6. PMID: 25272187; PMCID: PMC4245168.
7. Hansen S, Kiefer JD, Madhurantakam C, Mittl PRE, Plückthun A. Structures of designed armadillo repeat proteins binding to peptides fused to globular domains. Protein Sci. 2017 Oct;26(10):1942-1952. doi: 10.1002/pro.3229. Epub 2017 Jul 25. PMID: 28691351; PMCID: PMC5606530.
8. Hansen S, Tremmel D, Madhurantakam C, Reichen C, Mittl PR, Plückthun A. Structure and Energetic Contributions of a Designed Modular Peptide-Binding Protein with Picomolar Affinity. J Am Chem Soc. 2016 Mar 16;138(10):3526-32. doi: 10.1021/jacs.6b00099. Epub 2016 Mar 2. PMID: 26878586.
9. Ernst P, Plückthun A. Advances in the design and engineering of peptide-binding repeat proteins. Biol Chem. 2017 Jan 1;398(1):23-29. doi: 10.1515/hsz-2016-0233. PMID: 27636831.
10. Parmeggiani F, Huang PS. Designing repeat proteins: a modular approach to protein design. Curr Opin Struct Biol. 2017 Aug;45:116-123. doi: 10.1016/j.sbi.2017.02.001. Epub 2017 Mar 3. PMID: 28267654.
11. Javadi Y, Itzhaki LS. Tandem-repeat proteins: regularity plus modularity equals design-ability. Curr Opin Struct Biol. 2013 Aug;23(4):622-31. doi: 10.1016/j.sbi.2013.06.011. Epub 2013 Jul 4. PMID: 23831287.
12. Wei R, von Haugwitz G, Pfaff L, Mican J, Badenhorst CPS, Liu W, Weber G, Austin HP, Bednar D, Damborsky J, Bornscheuer UT. Mechanism-Based Design of Efficient PET Hydrolases. ACS Catal. 2022 Mar 18;12(6):3382-3396. doi: 10.1021/acscatal.1c05856. Epub 2022 Feb 28. PMID: 35368328; PMCID: PMC8939324.
13. Samak NA, Jia Y, Sharshar MM, Mu T, Yang M, Peh S, Xing J. Recent advances in biocatalysts engineering for polyethylene terephthalate plastic waste green recycling. Environ Int. 2020 Dec;145:106144. doi: 10.1016/j.envint.2020.106144. Epub 2020 Sep 25. PMID: 32987219.
14. Jisna VA, Jayaraj PB. Protein Structure Prediction: Conventional and Deep Learning Perspectives. Protein J. 2021 Aug;40(4):522-544. doi: 10.1007/s10930-021-10003-y. Epub 2021 May 28. PMID: 34050498.
15. Zhang Y. Protein structure prediction: when is it useful? Curr Opin Struct Biol. 2009 Apr;19(2):145-55. doi: 10.1016/j.sbi.2009.02.005. Epub 2009 Mar 25. PMID: 19327982; PMCID: PMC2673339.

Additional information (if any): The course framework and modules were designed and conceptualized by Dr. Chaithanya Madhurantakam. Further inputs were received from Dr. Pooja Anjali Mazumdar (PhD, IIT KGP), Independent Investigator, New Delhi.

Student responsibilities:

1. Study of course material as specified by the instructor.

Course Reviewers:

1. Dr. Peer Mittl, Senior Scientist, Department of Biochemistry, University of Zurich, Switzerland
2. Dr. Nidhi Pareek, Associate Professor, Deptt. of Sports Bioscience, School of Sports Sciences, Central University of Rajasthan, Bandarsindri, N.H. 8, Kishangarh, Ajmer - 305 817, Rajasthan, India