Annexure No.	**42 K**
SCAA Dated	**29.02.2008**

BHARATHIAR UNIVERSITY, COIMBATORE – 641046

PG DIPLOMA IN MOLECULAR ENGINEERING (Colleges)

For the students who are admitted from the academic year 2007-08 batch and onwards.

DURATION: ONE YEAR (TWO SEMESTERS)

SUBJECT	THEORY PAPERS	PRACTICAL PAPERS
I semester	4x75=300	1x100=100
II semester	4x75=300	1x100=100
Total marks	600	200

SCHEME OF EXAMINATIONS:

SEMESTER	SUBJECT	INSTR HRS	UNIVERSITY EXAMS	
			Duration	Max .Marks *
FIRST				
Paper I	PROTEINS	75	3	75
Paper II	NUCLEIC ACIDS	75	3	75
Paper III	BASICS OF ENZYMOLOGY	75	3	75
Paper IV	INTRODUCTION TO BIOINFORMATICS	75	3	75
PRACTICAL I	PROTEIN BIOCHEMISTRY	60	-	-
SECOND				
Paper V	ENGINEERING OF PROTEINS	75	3	75
Paper VI	METABOLIC ENGINEERING	75	3	75
Paper VII	GENOMICS AND PROTEOMICS	75	3	75
Paper VIII	BIOETHICS, BIOSAFETY AND IPR	75	3	75
PRACTICAL I	PROTEIN BIOCHEMISTRY		3	100
PRACTICAL II	APPLIED MOLECULAR BIOLOGY	60	3	100

∗ Includes 25% continuous internal assessment marks.

Paper I - PROTEINS

Scope:

This course studies about the basics of proteins, their classification, structure, physical and chemical properties. Students are expected to understand about the various bonding patterns involved in maintaining the structural integrity of the proteins and thereby their functional specificity. They will also understand as to how the foreign molecules invading the cell are effectively recognized and is degraded by the immune system

Unit – I

Structure of atoms, molecules and chemical bonds; Principles of physical chemistry: Thermodynamics, kinetics, dissociation and association constants - pH and buffers.
Classification of amino acids – physical properties: solubility, optical isomerism, acid-base behavior, isoelectric point – chemical properties: Reactions of the carboxyl group, amino group, R group – peptides: physical properties.

Unit II

Proteins: Classification of proteins – Classification based on solubility, specific function, composition and molecular shape
Protein structure: Covalent and Noncovalent interactions - Van der Waals, Electrostatic, Hydrogen bonding and hydrophobic interactions, peptide bond – Protein configuration - Primary structure of proteins: amino acid sequence, Ramachandran plot

Unit III

Secondary structure: the alpha- helix, β-sheets and random coils – Tertiary structure: Folding of the chain (Myoglobin , Ribonuclease) - Quaternary and domain structure. Protein in solution and in membranes: properties of soluble and membrane protein; flexibility of protein structure; stability; mechanism of protein folding

Unit IV

Structural motifs of transcription factors (zinc finger; Leucine zipper) DNA polymerase as a multifunctional domain; Enzymes that bind to nucleotide (FMN/FAD/NAD – binding domain); Structure of spherical viruses (picorne virus; Bacteriophage MS2) Foreign molecular recognition by immune system – antigen binding site and hyper variable regions; Recognition of antigen in MHC

Unit V

Autocatalytic protein splicing; Specific signal for protein localization; Mechanism of protein degradation

References:

1. Proteins: Structure and Molecular properties-Thomas.E.Creighton,W.H.Freeman.
2. Principles of Biochemistry – Smith et al., McGraw – Hill International book Company, 8th Edition.
3. Biochemistry – Garrett and Grisham, Saunders College Publishing, 1995
4. Biochemistry – Mathews and Van Holde.
5. Introduction to protein structure by C. Branden and J. Tooze, Gerland Publishinig, 1991.

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Paper II - NUCLEIC ACIDS

Scope:

To extend knowledge and understanding of the nucleic acids, their structure, physical properties and significance has been discussed. Emphasis will be given to Geometry of bases and of Watson – Crick and Hogsteen Base pairs. Students are expected to understand about Ribonucleic acids, their types and their biological significance. We will also discuss about DNA-Protein interaction and the various techniques used to identify the interaction

Unit I

Nucleic acids: Structure of double stranded DNA (B, A, C, D, T and Z DNA). The biological significance of double strandedness, sequence dependent variation in the shape of DNA. Physical properties of double stranded DNA - DNA bending, DNA supercoiling.

Unit – II

Nucleic acids: Transitional angles and their ranges. Sugar puckering models; the pseudorotation cycle, syn-anti orientation about glycosyl bond. Geometry of bases preferred sugar nucleoring models – bond distances and angles in furanoses orientation about the C- O and P – O ester bonds.

Unit – III

Details of geometries of Watson – Crick and Hogsteen Base pairs – thermodynamic description of stacking interactions – forces stabilizing, base stacking, hydrophobic bond of first order description. Base pair tautomerism and wobbling polymorphisms of DNA versus structural conservations of RNA. Ribonucleic acids - Types of RNA: and their biological significance – messenger RNA – ribosomal RNA – transfer RNA – structures; catalytic RNA – various catalytic activities; Group I and II introns and their function; RNAase P; viroids catalytic activity

Unit IV

Multifunctional oncogenes – quantitative or qualitative changes, Ras proto oncogene, Src type; oncoprotein regulation of gene expression p53 as DNA binding protein; immortalization phenomena genetic instability in cancer; Nucleotide binding ligands

Unit V

DNA-Protein interaction: leucine zipper; zinc binding domain; transcription interaction; Fluorescence Resonance energy transfer (FRET) and Nucleic acid biochemistry; Use of DNA micro circles; electrophoretic mobility; microcalorimetry in protein-DNA interactions; hydroxyl radical foot printing site-specific protein-DNA photo crosslinking

References:

1. Polymers in Nature – MacGregar
2. DNA structure and function-Richard.R.Sinden, Academic press – 1994.
3. Principles of Biochemistry – Smith et al., McGraw – Hill International book Company, 8th Edition.
4. Gene VIII by Lewin, International Edition, 2004
5. DNA-Protein Interaction by A. Travers and M. Buckle, Indian Edition, 2000 Osford University Press

Paper III - BASICS OF ENZYMOLOGY

Scope: This course extends knowledge and understanding of the enzymes, its classification and structure. Emphasis will be given to the enzyme kinetics and the various type of mechanisms underlying the inhibition of enzymes. It also provide an introduction to the active site structure of an enzyme and the mechanism of catalysis of a substrate. On completion, the student will have a clear understanding of enzymes and appreciate its importance in the living system.

Unit I:
History of enzymes; Classification of enzymes; Three dimensional structure of proteins, Function Domains of protein. Interaction of protein with other molecules – protein conformation and binding; Allostery; Hemoglobin and other allosteric O_2 binding proteins

Unit II:
Enzymes kinetics: Characterization of enzyme activity; Progress curve; Reaction velocity; Equilibrium and steady state model; Plot of v versus [s]; Determination of Km and Vmax, MM kinetics ; LB plot modification, Advantages of them in the determination of catalytic parameters.

Unit III:
Enzyme Inhibitors: Reversible inhibitors- competitive, uncompetitive, non-competitive, mixed, partial, substrate and allosteric types; Irreversible inhibitors.

Unit IV:
Investigation of active site structure: Binding sites, catalytic sites; Use of substrate analogues; Modification of enzyme- chemical, protease and site directed mutagenesis.

Unit V:
Chemical behavior of enzyme catalysis: Mechanism of catalysis, reaction mediated by cofactors; Metalloenzymes; Coenzyme mediated catalysis: Allosteric enzymes. Examples of enzyme mechanism: proteases, lysozyme, tyrosyl tRNA synthethase

Reference:

1. Understanding Enzymes by T.Palmer, Prentice Hall Ellis Horwood Publication
2. Enzyme kinetics (A modern Approach) by A.G. Marangoni John Wiley & Sons, Inc. Publication

Paper IV - INTRODUCTION TO BIOINFORMATICS

Scope: The paper provides an insight into the various operating systems used in the emerging field of bioinformatics, the methods to acquire data and its management. The course also helps to analyse the sequence with the data acquired and to further predict their structure and phylogenetic relationships. After completion of the course, the student will be have a through knowledge on the use of microaaray to study expression of genes and the use of 2-dimensional gel electrophoresis as an efficient tool in the field of proteomics.

Unit – I

Principles of computing: Operating systems, application and advantages of Unix/Linux in bioinformatics. Basic word processing and database management soft wares.

Unit – II

Data acquisition and management: Types of data-DNA, RNA and protein sequences, protein structure data, gene and protein expression data. Databases: the public biological databases (Ex. Genbank, SWISSPROT, PDB, etc)- searching and retrieving data form databases- FASTA and BLAST and PHI-BLAST).

Unit – III

Sequence analysis with acquired data: Sequence comparison with pair wise and multiple sequence alignment. Deducing phylogenetic relationships from multiple sequence alignment. Building phylogenetic trees. Profiles and motifs.

Unit – IV

Bioinformatics in structure analysis: Format of a protein structure data. Retrieving protein structures from PDB. Using molecular visualization tools to study protein structure. Predicting protein structure from sequence by comparative modelling and other methods.

Unit – V

Genomics and proteomics: Use of micro arrays to study gene expression. Genome sequencing projects, 2D-PAGE as a tool in proteomics. Bioinformatics and drug discovery.

References

1. Bioinformatics-A beginner's guide by Jean – Michel Claverie and Cedric Notredame, Wiley- Dream Tech India Pvt. Ltd.
2. Developing bioinformatics computer skills by Cynthia Gibas and Per Jambeck, O' Reilly publications.
3. Introduction to bioinformatics by T.K. Attwood and D.J. Parry –smith, Pearson Education Asia.
4. Bioinformatics by David.W.Mount, CBS publishers and distributers.
5. Instant notes in bioinformatics by D.R. Westhead, J.H.Parish and R.M.Twyman.

Paper V - ENGINEERING OF PROTEINS

Scope: This paper deals with the basics of protein engineering, the conformation of proteins, the relationship between the protein sequence and structure. On completion, the student will have a clear understanding of basis of protein engineering, appreciate the potential use of recombinant proteins and their use as vaccines.

Unit I

Introduction to protein engineering conformation of proteins; structure prediction –protein anatomy, secondary and super secondary structure; sequence,structure relationship; structure –function of enzyme and its active site inhibitor complex structure; genetically fused protein for the novel functions; potential applications of recombinant proteins as substitutive enzyme therapy.

Unit II

Recombinant proteins using fusion protein strategies for enhanced recovery; engineering protein for the affinity purification (engineering of streptavidin); solvent engineering modulation of lipases; enzymes in organic solvent and supercritical fluids;

Unit III

Stabilization of enzymes by protein engineering (eg.*Pseudomanas isoamylase, Agrobacterium radiobacter*); mutational effect on substrate specificity (Yeast carboxypeptidase Y, glucoamylase)

Unit IV

Engineering catalytic domains for thermal stability increasing catalytic activity, specificity changes; engineering substrate –binding domain Mutational analysis of residues at the site of catalysis, substrate binding and allosteric site; homologue loop replacement; protein engineering for the biosensors; chemically engineered electronic proteins

Unit V

Engineering proteins for the degradation of recalcitrant compounds; environmentally hazardous compounds; protein engineering in vaccine development. minibody; HCV protease; expression and tagged cell surface receptors for the drug discovery.

Reference:

1. Protein engineering in industrial biotechnology, Ed.Lila Albergina (2000) Harwood academic publishers.

Paper VI - METABOLIC ENGINEERING

Scope: The focus is to give an understanding of the principles of metabolism and the disorders in which cause various diseases. The paper aims to give an understanding of the tools for metabolic engineering of *E.coli* and the role of antibiotics. On successful completion the students will be able to appreciate the various down stream processes of commercial importance, metabolic engineering of secondary metabolites and biodegradation of pollutants.

Unit I:
Principles of metabolism, Resolution of metabolism, compartalization; Enzyme regulation of metabolism; Metabolic disorders and its consequences. Metabolic flux balance analysis; challenges and promise of metabolic engineering; Engineering of central metabolism of *E.coli* an over view.

Unit II:
Tools for metabolic engineering of *E.coli*. Metabolic engineering of *E.coli* for the production of aromatic compounds. Strategies for the production of Polyhydroxy alkanoates, A family of biodegradable polymers. Amino acid production: Principles of metabolic engineering. Metabolic engineering for process improvement of Antibiotics; Biosynthesis. The Generation of organic molecule diversity; Regulation of metabolism solvent production by *Clostridium acetobutylicum*.

Unit III:
Role of down stream processing: Biomass separation – intracellular/extracellular product – membrane separation techniques – precipitation method – solevent extraction – two phase separation – Affinity based enrichment for microbial product recovery.

Unit IV:
Metabolic Engineering of yeast for substrate utilization and metabolic production. Metabolic Engineering of Mammalian cells molecular Bioremediation: Metabolic engineering for Biodegradation of recalcitrant pollutants. Engineering heterologous secretion in yeast

Unit V:
Metabolic engineering for the production of plant secondary metabolites. Molecular Bioremediation: Metabolic engineering for Biodegradation of recalcitrant pollutants. Online metabolic flux analysis for fermentation operation.

Reference:

1. Metabolic Engineering By Sang Yup Lee, Eleftherios T. Papoutsakis Marcel Dekker, Inc USA.
2. Product recovery in Bioprocess Technology. Biotol Series, VCH, Oxford. 1998

Paper VII - GENOMICS AND PROTEOMICS

Scope: The paper aims in providing a through understanding of the genomics and proteomics, gene finding, genome mapping and protein characterization. The students also gains an understanding about the high- throughput techniques used in the analysis of proteins

Unit – I

Overview : Genomes of bacteria . Archaea and Eucarya ; genome shape and topology ; chromatin , supercoiling and packaging ;study of genomes – mapping, genetic and physical mapping, single nucleotide polymorphisms and RFLPs.

Unit – II

Genome sequencing; chain termination and automated DNA sequencing; shotgun and gene cloning strategies; library construction; sequence assembly and gap closure; genome resources – NCBI Map viewer, ORF finder, Locuslink, etc.

Unit – III

Gene finding and functional annotation: sequence annotation and bioinformatics tools for genomics and genome comparison; Analyzing gene expression – DNA microarrays – design, analysis and visualization of data. RNA data handling/manipulation; using gene expression arrays for disease profiling.

Unit-IV

Protein structure and function – methods to quantitate proteins; densitometry and classical methods: proteomics methods; two dimensional gel electrophoresis. Mass spectrometry. MALDI-TOF, MALDI-TOF-TOF. Protein expression profiling, protein-protein interactions; RNA interference; Cy Dye; DIGE method of protein identification; Preparing for data base search

Unit – V

Genome mapping projects – human genome projects – methods and insights obtained; transcriptome analysis of *E. coli* and *S. cerevisiae*. Biochemical pathway databases – WIT and KEGG.

References

1. Genomes – T. A. Brown, Wiley – Liss Publications, 2002
2. Mount, David W. Bioinformatics sequence and genome analysis. Cold Spring Harbor Laboratories press, CSH New York, 2001
3. Liebler, Introduction to proteomics. Tools for the new biology. Humana Press 1st Ed. December, 2001
4. Proteomics: From protein sequence to function S. Pennington, M. J. Dunn Bios Scientific Publications Ltd. 2nd Ed. (2001)
5. Proteomics in Practice by R. Westermeier and T. Naveen, Wiley-VCH Verlag-GmbH, 2002.

Paper VIII – BIOETHICS, BIOSAFETY AND IPR

Scope: The course aims to impart knowledge on the bioethics, biosafety and Intellectual property rights. The papers deals with the ethical aspects of biotechnology especially in gene cloning and the genetically modified crops and also gives an introduction to biosafety measures to be followed in the laboratory. Also the students gains an understanding of the Intellectual property rights and the legal protection of their innovative inventions by patenting.

Unit I

Introduction to ethics/bioethics – framework for ethical decision making; biotechnology and ethics – benefits and risks of genetic engineering – ethical aspects of genetic testing – ethical aspects relating to use of genetic information – genetic engineering and biowarfare

Unit II

Ethical implications of cloning: Reproductive cloning , therapeutic cloning ; Ethical, legal and socio-economic aspects of gene therapy, germ line, somatic, embryonic and adult stem cell research- GM crops and GMO's – biotechnology and biopiracy – ELSI of human genome project

Unit III

Introduction to biosafety – biosafety issues in biotechnology – risk assessment and risk management – safety protocols: risk groups – biosafety levels – biosafety guidelines and regulations (National and International) – operation of biosafety guidelines and regulations – types of biosafety containment

Unit IV

Introduction to intellectual property and intellectual property rights – types: patents, copy rights, trade marks, design rights, geographical indications – importance of IPR - world intellectual property rights organization (WIPO)

Unit V

what can and what cannot be patented? – patenting life – legal protection of biotechnological inventions – Patenting in India: Indian patent act.

References:

1. Principles of cloning, Jose Cibelli, Robert P. lanza, Keith H. S . Campbell, Michael D.West, Academic Press,2002
2. http://books.cambridge.org/0521384737.htm
3. http://online.sfsu.edu/%7Erone/GEessays/gedanger.htm
4. http://www.actahort.org/members/showpdf?booknrarnr=447_125
5. http://www.cordis.lu/elsa/src/about.htm
6. http://www.csmt.ewu.edu/csmt/chem/jcorkill/bioch480/bioLN98.html
7. http://www.accessexcellence.org/AE/AEPC/BE02/ethics/ethintro.html
8. http://lawlib.samford.edu/cio/ciofeb03.pdf
9. http://www.biomedcentral.com/content/pdf/1472-6939-2-2.pdf
10. http://lifesciences.cornell.edu/vision/accelerating_focus05.php
11. http://thompson.com/libraries/fooddrug/
12. http://assets.cambridge.org/0521792495/sample/0521792495WS.PDF
13. http://europa.eu.int/eurlex/pri/en/oj/dat/1998/I_213/I_21319980730en00130021.pdf
    http://www.clubofamsterdam.com/content.asp?contentid=281
14. Biosafety issues related to transgenic crops,DBT guidelines, Biotech Consortium India Limited, New Delhi

SEMESTER I
PRACTICAL I – PROTEIN BIOCHEMISTRY

S.No.	Title of the practical
1	Protein estimation: Biurette method/ Lowry method/Dye binding method/ Bardford assay/BAC assay
2	Nucleic acid determination by Spectrophotometric assay, Nucleic acid determination by Fluorescence method; bisbenzimidazole; Ethidium bromide
3	Enzyme assay and specific activity of an enzyme
4	a)Determination of pKa value of amino acids b)Determination of PI value of protein
5	Determining the Coupling of ligand/protein to sepharose by Epoxy activation
6	Standardization of Ion exchange column chromatography for protein purification (Selection of ion exchange; Choice of the buffer; Preparation of ion exchanger; Use of ion exchanger)
7	Enzyme kinetics: determination of Turn over number; Catalytic efficiency –Kcat, Km
8	a) Effect of hydrophobic molecule and temperature on enzyme activity using Arrhenius plot. b) Identification of inhibitor by kinetic study
9	Protein profile at different stages of purification by SDS-PAGE
10	Cholinesterase active site aminoacid identification by chemical modification
11	Protein- DNA interaction using mobility shift assay; Poly amine stability Gel filtration to study ligand-protein interactions eg: phenol red-BSA mixture (Preparation of sephadex G-25 column, 1.5X15 cm; Scatchard plot of V/L versus V)
12	a)Sequence comparison through Bioinformatics (BLAST & FASTA) b)Analysis of Protein structure in Silico (Swiss – PDB veiwer
13	Subcelluar organelle isolation and marker enzyme assay - SDH
14	Green Fluorescent protein transformation
15	Production of His-tag protein and purification

SEMESTER II
PRACTICAL II – APPLIED MOLECULAR BIOLOGY

S.No.	Title of the practical
1	Isolation of DNA from animal tissue by non enzymatic method
2	Cloning in E.coli: ligation and transformation
3	Gene expression and analysis of gene (cry/Taq polymerase) product
4	Polymerase chain reaction and RAPD
5	cDNA synthesis by RT-PCR
6	Electroporation method of gene transfer in plants using *GUS* gene.
7	*GUS* assay using histochemical method.
8	Production of amylase by immobilized cells
9	Isolation of membrane receptor and analysis of cAMP production
10	Production of His-tag protein and purification
11	Isolation of RNA from blood
12	Liposome mediated transformation in animal cells
13	HRP conjugation of Ag or Ab
14	Western blotting
15	Southern blotting (Colony hybridization/Dot blotting)
16	Site-directed mutagenesis