PROTEOMICS AND PROTEIN ENGINEERING
(BIOT 4164)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

(i) Which of the following is an orthomyoxovirus?
(a) Vesicular stomatitis virus (b) Rabies virus
(c) Sendai virus (d) Influenza virus C.

(ii) Match the protein elution condition given in Group I with the appropriate chromatography matrices from Group II.

Group I	Group II
P) Increasing concentration of sodium chloride.	i) Phenyl-Sepharose
Q) Increasing concentration of histidine.	ii) Chromatofocusing
R) Decreasing concentration of ammonium sulphate.	iii) DEAE-Sephacryl
S) Decreasing concentration of H+	iv) Ni-NTA

(a) P-iii; Q-iv; R-i; S-ii (b) P-ii; Q-iv; R-i; S-iii
(c) P-i; Q-ii; R-iii; S-iv (d) P- iv; Q-ii; R-iii; S-i.

(iii) The following polypeptide chain was sequentially treated with dithiothreitol, cyanogen bromide, and trypsin. Phe-Trp-Lys-Tyr-Met-Gly-Ala-Cys-Cys-Pro-Met-Asp-Gly-Arg-Phe-Ala-Gly-Trp. The total number of fragments expected at the end of complete digestion of the polypeptide are _____.
(a) 6 (b) 15 (c) 7 (d) 5.

(iv) NOESY in 2D-NMR takes advantage of
(a) interaction of two protons that are bonded to the C atom
(b) detection of a group of photons interacting through a coupled network
(c) the nuclear overhauser effect
(d) degree of perturbation of adjoining nucleus

(v) Secretory proteins synthesized by ER-associated ribosomes traverse through
(a) mitochondria (b) peroxisomes
(c) the Golgi apparatus (d) the nucleus.

(vi) Which one of the following microscopes would you use to visualize a protein fused to an appropriate reporter in a living cell?
(a) Fluorescence microscope
(b) Scanning electron microscope
(c) Differential interference contrast microscope
(d) Phase contrast microscope.

(vii) Top-down proteomics refers to
(a) microarray proteomics
(b) fragmentation of intact proteins
(c) dedicated proteomics resource within INSDC sequence database
(d) holistic analysis of complex protein interactions.

(viii) Transcriptomics led to the development of which of the following technologies?
(a) Serial analysis of gene expression (SAGE)
(b) Massively parallel signature sequencing (MPSS)
(c) Espressed sequence tags
(d) All of the above.

(ix) Which of the following technique(s) can be used to study conformational changes in myoglobin?
P. Mass spectrometry,
Q. Fluorescence spectroscopy
R. Circular dichroism spectroscopy,
S. Light microscopy
(a) P only (b) P and S only
(c) Q and R only (d) S only.

(x) A mixture contains three similarly sized peptides P, Q and R. The peptide P is positively charged, Q is weakly negative and R is strongly negative. If this mixture is passed through an ion-exchange chromatography column containing an anionic resin, their order of elution will be
(a) P, Q, R (b) R, Q, P
(c) Q, R, P (d) P, Q and R elute together.

Group – B

2. (a) What are the different branches of proteomics? Among its branches, what is the relevance of expression proteomics? Cite five factors that pre-eminently established the need for proteomics.

(b) Use a flowchart ONLY to represent the current technological challenges facing proteomics in sample preparation, separation / identification and data analysis.

(c) Write names of four different techniques which can be used to study protein-protein interaction study. Describe principle of one technique in brief with a labelled diagram.

(2 + 1 + 2) + 3 + (2 + 2) = 12

3. (a) Write the names of pathway present inside a cell to degrade proteins.

(b) Miss folded proteins are degraded in which pathway? Describe mechanism of that pathway with a labelled diagram.

(c) Draw a labelled diagram of the MALDI ionisation mechanism and steps of the sample plate preparation. What are the technical reasons behind coupling a time-of-flight analyzer with MALDI? Why is MALDI-TOF the default proteomics separation / identification mass spectrometric method?

(d) What problems of the *in vitro* assay format does the Y2H system address? Briefly explain its operating principle.

2 + (1 + 4) + 3 + 2 = 12

Group – C

4. (a) Draw a schematic diagram of a SPR spectrometer and its corresponding sensorgram plot. What are the specific proteomics-based applications of SPR? Why is it suited for measurement of binary interactions?

(b) What new strategy of antibiotics production is in development through the use of the protein peptide deformylase? Explain the essential mechanism.

(c) Use a generalized flowchart to represent protein patterns in disease diagnosis (e.g. diagnosis of rheumatoid arthritis by biomarkers using MS/MS detection).

(3 + 2 + 1) + (2 + 1) + 3 = 12

5. (a) Draw a schematic of the process of reverse genetics.
(b) Outline three experimental (indirect) methods that have been extensively adopted to overcome the phase problem in X-ray crystallography.

(c) How has solid state NMR become a high throughput method for protein structure determination? Give two examples of such applications. How can the CP-MAS technique enhance the structural information obtained from a protein?

2 + (3 × 2) + (2 + 1 + 1) = 12

Group – D

6. (a) What is protein engineering? Write the steps of protein engineering.

(b) Describe a strategy for oligonucleotide directed mutagenesis of a gene 'X' with PCR.

(c) Explain principle of solid phase peptide synthesis.

(d) What are the food industry uses of (i) wheat gluten proteins and (ii) proteases with associated tools of protein engineering? What two specific characteristics of proteins has made proteomics technologies an effective tool in food safety assessment? What are the techniques utilized?

(1 + 1) + 3 + 2 + (2 + 2 + 1) = 12

7. (a) (i) What properties of green fluorescent protein (GFP) has made possible its wide usage in protein engineering? Name four such applications.
(ii) How has a computational modelling technique like receptor based QSAR extended the range of applications in protein engineering?

(b) (i) Using examples from hydroxylases and oxygenases, explain how protein engineering has been utilized for developing environmental bioremediation applications.
(ii) Using two examples illustrate how properties of redox proteins / enzymes have been modified to make them suitable for use as biosensors.

(3 + 3) + (3 + 3) = 12

Group – E

8. (a) What is Affinsen dogma for protein folding? Describe the experiment based on which this was known.

(b) Describe Levinthol paradox.

(c) Write names of four different techniques for study of protein folding. Describe techniques with labelled diagram.

(1 + 3) + 2 + (2 + 4) = 12

9. (a) Describe the kinetics of protein folding.

(b) Describe the thermodynamics of protein folding.

(c) Write four names of human disease due to protein misfolding? Describe the mechanism of disease pathway on one of them.

4 + 4 + (2 + 2) = 12