Group – D

6. (a) Use a labelled flowchart to represent the protein engineering design cycle used in the rational redesign of proteins and enzymes.

   (b) "The use of design cycles and systems of rational design have been well characterized by the study and alterations of subtilisin". Explain this statement by answering the following questions what sort of an enzyme is subtilisin and how is its specificity defined? What establishes its industrial importance? What are the uses of engineered subtilisins? What properties of subtilisin have been targeted by protein engineering?

   (c) What are the unique structural and biochemical characteristics of the green fluorescent protein (GFP)? Specify how these properties have led to the widespread applications of GFP in biological and medical research. What are the protein engineering methods that have been applied to GFP to improve its properties e.g. its folding profile?

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

7. (a) Write the names of three expression vector, where you can express your desire protein in large amount. Explain the control of over expression in any one of the vector you mentioned.

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

   (c) 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?

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

Group – E

8. (a) Define the process of protein folding as a reversible reaction with its underlying thermodynamics and kinetics. "You cannot unscramble an egg" discuss this statement in the context of protein denaturation, refolding, modification and the known presence of chaperones.

   (b) In considering models of protein folding, explain succinctly the roles of helix formation, the molten globule intermediate and hydrophobic interactions. Give three examples of neurodegenerative diseases that arise incorrect folding of a key causative protein or precursor. Name the diseases and the protein involved.

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

9. (a) Explain Anffinsen dogma and Levinthol paradox?

   (b) Write the names of three different mechanism of protein folding. Explain one mechanism of protein folding with diagram.

   (c) Define non-ribosomal peptide (NRP) with example? Explain the mechanism of synthesis of NRP.

   (d) Write two names of human disease due to protein aggregation? Explain the mechanism of disease pathway any one of them as you mentioned.

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

B.TECH/BT/7TH SEM/BIOT 4164/2019

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) During an experiment; a student found increased activity of a protein, for which there were three possible explanations, viz., increased expression of protein, increased phosphorylation, or increased interaction with other effector proteins. After conducting several experiments, the student concluded that increased activity was due to increased phosphorylation. Which one of the following experiments will not support/provide the correct expiation drawn by the student?
   (a) Westren blotting hybridization analysis (b) Analysis of transcription rate
   (c) Mass spectrometry (d) Phospho amino acid analysis.

   (ii) In a protein array, the spot density of the immobilized protein spots is of the order of
   (a) 150-200 (b) 1500 (c) 20-60 (d) 15000.

   (iii) During sample preparation for a proteomic experiment, protease inhibitor cocktail (PIC) is generally added. What is the purpose of adding PIC?
   (a) It ensures the protection of proteins against proteolytic enzymes
   (b) It helps to reduce the proteins
   (c) It helps to denature the intact proteins
   (d) It increases the solubility of proteins in buffer.

   (iv) You have transiently expressed a new protein (for which no antibody is available) in a cell line to establish its structure-function relationship. Which one of the following strategies is the most straightforward way to examine the expression profile of a new protein?
   (a) By metabolic labelling using ^{35}S labelled amino acid
   (b) Making a GFP fusion protein with the new protein
   (c) Immunoprecipitating this protein with the help of another protein for which antibody is available
   (d) Running SDS-PAGE and subsequently identifying the protein.

   (v) Which one of the following activities is not involved in protein folding
   (a) Peptidylprolylisomerase (b) Protein disulphide isomerase
   (c) Protein glycosylation (d) Protein ubiquitination.

(vi) A multimeric protein when run on an SD-PAGE, showed two bands at 20 KDa and 40 KDa. However, when the protein was run on a native PAGE, it showed a single band at 120 KDa. The native form of the protein would be
(a) homotrimer (b) heterotetramer (c) heterodimer (d) heterotrimer.

(vii) A small fraction of clear cellular lysate was run on an isoelctric focusing gel (IEF) to purify a particular protein, which showed a number of sharp bands corresponding to different pI value. The protein of interest has a pI of 5.2. Therefore, the band corresponding to pI 5.2 was cut, eluted with appropiriate buffer and subjected to SDS-PAGE, which showed 3 distinct bands. One of the following inferences can not be drawn from above observations?
(a) Several different proteins having same pI may be at the single band on IEF gel
(b) SD-PAGE showed 3 distinct bands which may represent molecular mass of a different protein
(c) The protein of interest may be composed of 3 subunits
(d) AS the IEF gel showed a distinct band corresponding to pI 5.2, which is the pI of protein of interest, the protein is composed of a single subunit.

(viii) Which of the following is/are the advantages of 2D-DIGE over conventional 2D-GE?
(a) The fluorescent Cy dyes used in 2D-DIGE are more sensitive than the dyes used to stain a 2D-GE gel and hence in case of 2D-DIGE, small amount of proteins in the sample can give better spot resolution
(b) 2D-DIGE technique has reduced the reproducibility issue that is inevitable in conventional 2D-GE
(c) Unlike 2D-GE, two different samples can be compared at a time in 2D-DIGE from a single gel run
(d) All of the above.

(ix) A protein undergoes post translational modification. In an experiment to identify the nature of modifications. In an experiment to identify the nature of modifications, the following experimental results were obtained.
P. Protein moved more slowly in the SDS-PAGE.
Q. IEF showed that there was no change in the pI.
R. Mass spectrometric analysis showed that the modification was on serine.
The modification that the protein undergoes is likely to be
(a) phosrylation (b) glycisylation
(c) ubiquitinization (d) ADP-ribosylation.

(x) One of the diseases that arise from "folding defects" is cystic fibrosis. The protein or precursor involved in the disease is
(a) LDL receptor (b) Prion protein
(c) CFTR (d) superoxide dismutase.

Group – B

2. (a) Define post translational modification of a protein (PTM). Give two common examples of PTM. What key purposes are served by PTMs? Use a flowchart to explain the general scheme for analysis of modified proteomics. How is PTM enrichment generally done here?

(b) How has high performance liquid chromatography been used for protein separation? Explain its operational principle using a diagram of an isocratic HPLC system.

(c) Use a diagram to illustrate the principle of TOF. What are the specific advantages of using TOF as a mass analyser? Calculate the m/z value for a TOF-MS experiment, where, TOF is 25 μs, voltage used 6500 V and length of the drift tube was 1 m.

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

3. (a) Write the names of pathway present inside a cell to degrade proteins. Explain the pathway by which cell degrade miss folded proteins with a labelled diagram.

(b) Write the names of four ionization techniques and four mass analyser name used in MS. Explain the principle of one ionizer and one analyser that you mentioned.

(c) A protein was isolated from human tissue and subjected to a variety of investigations. Relative molecular mass determinations gave values of approximately 12000 by size exclusion chromatography and 13000 by gel electrophoresis. After purification, a sample was subjected to electrospray ionisation mass spectrometry and the following data obtained.

m/z	773.9	825.5	884.3	952.3	1031.3
Abundance (%)	59	88	100	66	37

Assuming that the only ions in the mixture arise by protonation, calculate the average molecular mass for the protein by this method.

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

Group – C

4. (a) Define a biomarker explaining the difference between a disease and toxicity biomarkers. Use a suitable diagrammatic representation to explain how comparison of tumor and non-tumor tissue can lead to identify novel biomarkers. Give two examples of biomarkers that have been used for rheumatoid arthritis.

(b) How can proteomics be utilized for target validation? Use examples of function-specific affinity reagents to illustrate chemical proteomics based screening.

(c) Use a diagram to distinguish the processes of forward and reverse genetics. Explain the method of RNAi (gene silencing) using the example in *C.elegans.*

(d) In the crystallography of large proteins, calculation of correct electron density is often hampered by the phase problem. Itemize five different methods by which overcoming of the phase problem has been attempted?

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

5. (a) Draw a schematic of the process of reverse genetics.

(b) Based on genome configuration RNA viruses can be of six groups. Diagrammatically explain their mode of both transcription and translation.

(c) Outline three experimental (indirect) methods that have been extensively adopted to overcome the phase problem in x-ray crystallography.

2 + (3 × 2) + 4 = 12