PROTEOMICS AND PROTEIN ENGINEERING
(BIOT 4121)

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 does NOT hold true in terms of advantages of 2D-DIGE over conventional 2-DE?
(a) The Cy dyes used in 2D-DIGE are more sensitive than the dyes used to stain a 2-DE gel
(b) 2-D DIGE technique has reduced the reproducibility issue that is faced in conventional 2DE
(c) Absence of the equilibration steps in 2D-DIGE reduces the overall time of experiment
(d) Two different samples can be compared at a time in 2D-DIGE which cannot be done in 2DE.

(ii) Which of the following statements are correct about molecular chaperones?
(a) The pattern of protein folding depends upon the sequence of amino acids in chaperone protein
(b) They prevent the aggregation of proteins
(c) Chaperones always work passively without the use of ATP as energy source
(d) Chaperones not involved in protein folding.

(iii) A protein contains six cysteine. How many ways can six cysteine residues form disulphide bonds?
(a) 360 (b) 72 (c) 60 (d) 90.

(iv) An important aspect of proteome analysis deals with post translational modifications (PTMs). Accurate prediction of PTMs can be done by which of the following methods?
(a) Pure sequence motif based bioinformatics tool
(b) A combined wet and dry lab consensus protein motif method
(c) Use of a bioinformatics tool that uses a statistical learning process
(d) None of the above.

(v) What marks the difference between alpha helix and beta sheets in protein structure?
(a) Beta helix is a secondary structure whereas alpha helix is not
(b) Hydrogen bonding is intramolecular in alpha helix whereas it is intermolecular in beta sheets
(c) Alpha helix is found in fibrous proteins whereas beta sheets are found in globular proteins
(d) Alpha helix is rich in amino acid proline whereas beta sheets lack proline.

(vi) Which of the following technique is NOT used for removing salt from a proteomic sample?
(a) Dialysis (b) Gas chromatography
(c) Spin dialysis (d) Gel filtration.

(vii) An individual is a cancer researcher and wants to perform deep proteome-based shotgun analysis of one of his/her patient samples. Which of the following instruments would be most preferred for the experiment?
(a) Triple Quadrupole mass spectrometer (b) Q-TOF
(c) Orbitrap Fusion (d) MALDI-TOF.

(viii) A mixture of monomeric proteins (W,X,Y,Z) eluted from Sephadex-G-200 column in the order (from 1st to last) of W, X, Y, Z. The mixer of proteins was subjected to standard SDS-PAGE. What will be the order of their appearance of the proteins from top to bottom in SDS-PAGE?
(a) Z, Y, X, W (b) X, Y, Z, W (c) Y, Z, X, W (d) W, X, Y, Z.

(ix) Which of the following holds true for 1-D SDS-PAGE and 2D-PAGE?
(a) Unlike 1 D -SDS-PAGE, stacking gel is not required for 2 D-PAGE
(b) Like 1D SDS-PAGE, isoelectric focusing is performed in 2D-PAGE also
(c) In 1D-SDS-PAGE, proteins are separated on the basis of molecular weight whereas in 2DE-SDS-PAGE, proteins are separated on the basis of mass to density ratio
(d) TEMED is not required for casting gel for 2DE which is required for 1D SDS-PAGE.

(x) Cystic fibrosis is a genetic disorder that affects lungs in most of the cases. What is the molecular basis of this disorder?
(a) Nucleotide polymorphism from UUC to UUG that changes Phenylalanine to Leucine
(b) Nucleotide polymorphism that changes a normal codon to stop codon that leads to truncated protein
(c) It involves the expansion of CAG repeats also known as a trinucleotide repeat expansion
(d) Deletion of three nucleotides that leads to loss of the amino acid phenylalanine.

Group–B

2. (a) Explain the principle and steps of 2D-DIGE with labelled diagram. [(CO1)(Remember/LOCQ)]
(b) Write names of three techniques to study protein-protein interaction (PPI). Explain any one technique you mentioned with diagram for the study of PPI. [(CO2)(Remember/IOCQ)]
(c) Explain the principle and steps of protein identification by LC-MS/MS using coded affinity tagging. [(CO1)(Understand/HOCQ)]
4 + (1 + 3) + 4 = 12

3. (a) Write names of three protein separation techniques for proteomics. Explain the major steps of anyone protein separation technique you have mentioned, with labelled diagram. [(CO1)(Remember/LOCQ)]
(b) Explain the principle and steps of ESI-Q MS for determination of mass of protein with labelled diagram. [(CO2)(Understand/IOCQ)]
(c) (i) Derive the equation for the determination of average mass using two consecutive peaks from the mass spectrum of a protein.

(ii) The data obtained from MS analysis of a peptide using coupled HPLC-MS operating through an ESI-TOF sourcewerem/z=510.7, 595.7, 714.6, 893.0 and 1190.3. Determine a molecular mass of the peptide using the formula you derived. [(CO3)(Evaluate/HOCQ)]

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

Group - C

4. (a) State the essential steps for preparing a sample for a cryo-electron microscope. What are the low-dose and high-dose techniques in cryo-EM? How does this choice of techniques impact signal-to-noise of the EM signal? Explain in *two specific points* why this technique is suited for structure determination of large macromolecular assemblies. [(CO3)(Remember-Understand/IOCQ)]

(b) What are the specific *biochemical and biological* parameters in a protein that are generally indicative of the onset or progression of a disease? Define a biomarker. Name the *two dominant biomarker types* that are commonly used in a medical context. Cite one example of each. Use a diagram to explain how one of these biomarkers function. [(CO2)(Understand/LOCQ)]

(2 × 3) + (3 × 2) = 12

5. (a) "In the field of proteomics based drug discovery, the large scale high throughput technologies associated with proteomics have led to increased discovery of potential new targets BUT has caused a downstream bottle neck at the target validation stage". Elucidate this statement with a flowchart of the protein-based drug discovery process. Explain in quantitative terms why targetvalidation is a low throughput enterprise. Cite four specific methods of proteomics based target validation. Using a specific example explain how protein-protein interactions can be used for target validation. [(CO3)(Understand-analyze/IOCQ)]

(b) A novel protein is being investigated as a drug target. Determination of an accurate relative molecular mass M_r as part of protein purification is an important parameter to establish in the preclinical phase of drug development. Following are some relevant information and parameters for this process: (i) separation of the novel protein is being done by size exclusion (SE) chromatography on a Sephadex column (ii) the enzymes aldolase, catalase, ferritin, thyroglobulin and blue dextran are being used as standards (iii) M_rS and the retention volumes (V_rS) of the standards are known and (iv) the retention volume of the unknown protein is known.
Stepwise explain how you would deduce the relative molecular mass of this unknown protein. Show all calculations. [(CO6)(calculate-analyze/IOCQ)]

(2 + 1 + 3) + 6 = 12

Group - D

6. (a) How will you quantitatively parameterize whether proteins change conformation upon protein-ligand or protein-protein complex formation? Briefly define the features and parameters. [(CO6)(Understand-analyze/IOCQ)]

(b) "In protein engineering, manipulation of the gene of interest prior to expression of the protein product is advantageous". Elucidate this statement by citing four specific advantages of this process. How is intracellular degradation of the cloned protein prevented by this process? [(CO4)(Understand-analyze/IOCQ]

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

7. (a) Tabulate the theoretical principles behind the method of "designed divergent evolution". What is its primary application in enzyme analysis? How is this method particularly suited for this application? [(CO4)(Understand-analyze/IOCQ)]

   (b) "Genetically engineered proteins for inorganic materials (GEPIs) are important tools for the self-assembly of molecular systems in nanobio-technology". Using an example, explain how this has been achieved. For what purposes have such GEPIs been used for? Itemize the applications that higher generation function-specific peptides have been utilized for. [(CO4)(Analyze/HOCQ)]

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

Group – E

8. (a) Draw and explain the curve for protein denaturation with diagram. Show, how you can calculate ΔG^0 from that protein denaturation curve. [(CO6)(Remember-Explain/LOCQ)]

   (b) Explain the three protein folding models with the help of Free energy funnel. [(CO6)(Remember/LOCQ)]

   (c) Write the names of different classes of molecular chaperones. Explain, how Hsp70 family of molecular chaperones facilitate protein folding, with labelled diagram. [(CO6)(Remember/LOCQ)]

4 + 4 + 4 = 12

9. (a) State and briefly explain Anfinsen's dogma and Leventhal's paradox? [(CO5)(Explain/IOCQ)]

   (b) Write the names of three different mechanism of protein folding. Explain anyone mechanism of protein folding with diagram. [(CO5)(Understand/IOCQ)]

   (c) Name two human diseases that are theorized to be caused by protein aggregation. Explain the disease progression/mechanism pathway of any one of the two diseases. [(CO5) (Remember/HOCQ)]

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

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	27.08	54.17	18.75

Course Outcome (CO):

After the completion of the course students will be able to

CO1. Understand different large scale protein separation, estimation, identification and sequencing techniques. Apply the knowledge to solve and analysis of proteome.

CO2. Understand the in vivo and in vitro protein-protein interactions techniques.

CO3. Describe the techniques for structural proteomics and apply knowledge of proteomics in drug discovery.

CO4. Describe the basics and significance of protein engineering; demonstrate the modification and design of protein according to the demand of industry and application.

CO5. Understand the stability of protein structure and mechanism of protein folding; apply this knowledge in study of protein misfolding related diseases.

CO6. Analyze and solve problems related to proteomics and protein engineering technology.

*LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question