

**PROTEOMICS AND PROTEIN ENGINEERING
(BIOT 4121)**

Time Allotted : 2½ hrs

Full Marks : 60

Figures out of the right margin indicate full marks.

*Candidates are required to answer Group A and
any 4 (four) from Group B to E, taking one from each group.*

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

Group - A

1. Answer any twelve:

12 × 1 = 12

Choose the correct alternative for the following

(i) What is the fold-purification?

Purification data for an enzyme is given below:

Steps	Purification step	Volume (ml)	Total protein (mg)	Total activity (micromoles per min)
I	Cell-free extract	20	100	150
II	Ni-NTA chromatography	4	10	120

(a) 8

(b) 13.5

(c) 10.5

(d) 18

(ii) Which one of the following techniques cannot be used to remove salt from a protein solution

(a) Ultrafiltration

(b) Ion exchange chromatography

(c) Gel filtration chromatography

(d) Dialysis

(iii) For performing 2-Dimensional gel electrophoresis several steps are involved. Which of the following is the correct order of the steps involved?

(a) Sample solubilisation > Equilibration > Isoelectric focusing > SDS-PAGE > Staining and Image analysis > Spot picking

(b) Sample solubilisation > Isoelectric focusing > Equilibration > SDS-PAGE > Staining and Image analysis > Spot picking

(c) Equilibration > Sample solubilisation > SDS-PAGE > Isoelectric focusing > Staining and Image analysis > Spot picking

(d) Sample solubilisation > Isoelectric focusing > Spot picking > Equilibration > SDS-PAGE > Staining and Image analysis

(iv) A small fraction of clear cellular lysate was run on an isoelectric 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 appropriate buffer and subjected to SDS-PAGE, which showed 3 distinct bands. One of the following inferences cannot 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.

(v) Match the stationary phase (Column I) with its corresponding chromatography technique (Column II)

	Column-I		Column-II
P	Protein A	1.	Size exclusion chromatography
Q	Sephadex	2.	Ion-exchange chromatography
R	Phenylsepharose	3.	Affinity chromatography
S	Diethylaminoethyl cellulose	4.	Hydrophobic interaction chromatography

(a) P-1, Q-4, R-2, S-3

(b) P-3, Q-1, R-4, S-2

(c) P-3, Q-4, R-2, S-1

(d) P-4, Q-1, R-3, S-2

(vi) Subir is a cancer researcher and wants to do deep proteome-based shotgun analysis of one of his patient samples. Which of the following instruments would be most preferred for her experiment?

(a) Triple Quadrupole mass spectrometer

(b) Q-TOF

(c) Orbitrap Fusion

(d) MALDI-TOF

(vii) 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.

(viii) Intracellular proteins are targeted for proteolytic degradation in proteasomes upon conjugation with:

(a) ubiquitin

(b) integrin

(c) peptidase

(d) calreticulin

- (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) Which of the following diseases is not caused by protein misfolding in humans?
 (a) Type 2 Diabetes (b) Cystic fibrosis
 (c) Neurofibromatosis type I (d) Parkinson's disease

Fill in the blanks with the correct word

- (xi) The maximum size of the protein can be identified by ESI-TOF is _____.
- (xii) Example of one homo-oligomeric protein is _____.
- (xiii) Co-translational translocation of proteins is observed in _____.
- (xiv) _____ are class of protein that facilitate the proper folding of newly synthesized proteins.
- (xv) Unlike 1 D-SDS-PAGE, _____ gel is not required for 2 D-PAGE.

Group - B

2. (a) Write the names of three techniques for determination of molecular mass of a protein. Explain the principle of determination of mass of a pure protein by the technique you mentioned which does not use either electrophoresis or chromatography, with diagram. [[CO1](Remember-Explain/IOCQ)]
- (b) Write different types of molecular chaperon present in cytoplasm of cells. Explain the features and functions of all molecular chaperons in QC for protein. [[CO2](Remember/LOCQ)]
- (c) A protein was isolated from mouse tissue and subjected to a variety of investigations. After purification, a sample was subjected to MALDI-TOF mass spectrometry and the following data obtained.

m/z	673.9	725.5	784.3	852.3	991.3
Abundance	55	82	95	61	32

assuming that the only ions in the mixture arise by protonation, determine the average molecular mass for the protein by this method. [[CO6](Analyse/HOCQ)]

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

3. (a) Write the names of pathway present inside a eukaryotic cell to degrade proteins. Explain the pathway by which cell degrade misfolded proteins with a labelled diagram. [[CO2](Remember/HOCQ)]
- (b) Write the names of three ionization and three mass analysing techniques name, which are used in MS. Explain the principle of anyone ionizing and analysing techniques that you mentioned, with diagram. [[CO1](Remember/IOCQ)]
- (c) A protein was isolated from human tissue and subjected to a variety of investigations. Relative molecular mass determinations gave values of approximately 12,000 by size exclusion chromatography and 13,000 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, deduce an average molecular mass for the protein by this method. [[CO6](Apply/HOCQ)]

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

Group - C

4. (a) (i) Why has cancer as a disease been a primary target for proteomic analysis? (ii) Use a labelled diagram/flowchart *only* to explain how cancer proteomics has led to the development of novel biomarkers, technologies and diagnostic patterns for treatment. (iii) The protein strathmin has been used as a reliable biomarker for leukemia. What is the distinguishing feature of this protein biomarker? If strathmin were to be developed as target for leukemia, what would be its preferred mode of delivery? Explain your answer. [[CO3](Understand-analyse /IOCQ)]
- (b) Itemize the applications of proteomics in drug development using a flowchart to represent the major stages in proteomics based drug development. [[CO3](Understand-analyse /IOCQ)]
- $$(4 + 2) + 6 = 12$$
5. (a) In x-ray diffraction pattern based model of a protein, the process of refinement of a model in an iterative fashion progressively improves the agreement with experimental data. Explain how the crystallographic R-factor defined as $R = \frac{\sum (F_{obs} - F_{cal})}{\sum F_{obs}}$ can be used to assess the quality of "good crystal structures". Define all the terms in the equation and explain the relationship of R to the resolution of a crystal structure. [[CO3](Analyse/HOCQ)]
- (b) (ii) How can the temperature factor in each atom of the structural model of a protein built from a x ray diffraction pattern be used as a measure of the "dynamics of a protein crystal"? Explain your answer. [[CO3](Remember/LOCQ)]

- (c) α_1 -antitrypsin is a serpin biomarker in the inflammation cascade and is specifically identified as such with emphysema/Chronic pulmonary obstructive disorder (COPD). As a serum biomarker itemize pointwise how this protein is suited for this purpose including two techniques that would be suitable for its detection.

[[CO3](Apply/IOCQ)]

4 + 3 + 5 = 12

Group - D

6. (a) What is protein engineering? What are the purposes of protein engineering? [[CO4](Analyse/IOCQ)]
 (b) Explain the protein engineering strategy for increasing the stability of a protein that has (i) no cysteine residues, (ii) an odd number of cysteine residues. [[CO4](Explain/IOCQ)]
 (c) Explain principle and steps of solid phase peptide synthesis with diagram. [[CO4](Remember/IOCQ)]

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

7. (a) Define directed evolution (DE). What are some of the other terms used to describe DE? Use a diagram to explain four possible directed evolution (DE) formats that operate on DNA. [[CO4](Understand-analyse/IOCQ)]
 (b) What are the principles that have made Green Fluorescent Protein (GFP) widely used for biomedical purposes? Cite the specific protein engineering methods that have been utilized for improvement of its protein properties. [[CO4](Understand-analyse/LOCQ)]

(2 + 4) + (2 + 4) = 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. [[CO5](Remember-Explain/LOCQ)]
 (b) Explain the three protein folding models with the help of Free energy funnel? [[CO5](Explain/IOCQ)]
 (c) Write the names of different classes of molecular chaperones. Explain, how Hsp70 family of molecular chaperones facilitate protein folding, with labelled diagram. [[CO5](Remember-explain/IOCQ)]

4 + 4 + 4 = 12

9. (a) Explain the molecular pathway of amyloid plaque and neurofibrillary tangles (NFT) formation in Alzheimer's disease, with diagram. [[CO5](Analyse/IOCQ)]
 (b) Explain through molecular pathway, why Huntington Disease (HD) is called polyglutamine disease, with diagram. [[CO5](Remember/IOCQ)]
 (c) Explain the molecular mechanism of Creutzfeldt-Jakob disease (CJD) in humans, with diagram. [[CO5](Apply/IOCQ)]

(3 + 3) + 3 + 3 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	18.75	65.6	15.6

