

**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) 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.
- (ii) In quantitative mass spectrometry methods which of the following methods has a linear dynamic range of 1-2 logs?
(a) Chemical protein labelling (MS) (b) Chemical peptide labelling (MS/MS)
(c) Label-free (spectrum counting) (d) Label-free(ion intensity)
- (iii) The glycosylation of the proteins occur in_____.
(a) Glyoxysomes (b) Lysosomes
(c) Golgi apparatus (d) Plasma membrane.
- (iv) Which of the following enzymes have a common structure and hence functionally related closely to the TIM barrel fold?
(a) mandelate racemase (b) 2-oxoglutarate dehydrogenase
(c) lactate dehydrogenase (d) transaldolase
- (v) 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.

- (vi) Which one of the following statements is NOT TRUE about sequencing peptides with mass spectroscopy?
- the entire protein can be sequenced simultaneously
 - two rounds of the technique are used to determine sequence
 - in order to determine the sequence, a pure sample of the protein is obtained through 2D PAGE or HPLC
 - undesirable characteristics like hydrophobicity and solubility are eliminated by digesting purified protein with proteases
- (vii) Among the reagents given below which one of the combination of reagents will not break the disulphide bonds in the immunoglobulin molecules?
- (P) Reduced glutathione
(Q) Dithiothritol
(R) Sodium dodecyl sulphate
(S) Methionine
- (a) R & S (b) P & R (c) P & S (d) Q & R.
- (viii) Which of the following expressions represents the important vector, F_{hkl} , known as structure factor in x-ray diffraction of both small molecules and proteins?
- $F_{hkl} = k |F_{obs}|$
 - $F_{hkl} = \sum f \cos \psi + \sum if \sin \psi$
 - $F_{hkl} = F_P + F_H$
 - $F_{hkl} = F_{obs} - F_{cal}$
- (ix) 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-Sepharose |
| (S) Decreasing concentration of H^+ | (iv) Ni-NTA |
- Choose the combination of Group-I that correctly list with Group-II.
- P-iii; Q-iv; R-iii; S-ii
 - P-ii; Q-iv; R-i; S-iii
 - P-i; Q-ii; R-iii; S-iv
 - P- iv; Q-ii; R-iii; S-1.
- (x) Trypsin cleaves the peptide bond containing
- Ala or Val
 - Glu or Asp
 - Met or Trp
 - Arg or Lys.

Group – B

- What 2-DIGE? Describe the detailed steps of 2-DIGE.
 - Explain the principle and steps of MALDI-TOF with a labelled diagram.
 - Write the principle of separation of protein by reverse phase chromatography.
 - 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, determine an average molecular mass for the protein by this method.

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

3. (a) What are the functional definitions of a protein and proteome?
- (b) Use a labelled diagram to describe the life cycle of a protein.
- (c) In a tabular fashion, list the differences between proteomics and protein chemistry.
- (d) Elucidate the basic principles behind the yeast 2 hybrid (Y2H) protein complementation assay. Use a labelled diagram to highlight both the principles and steps. What are the large scale applications of the Y2H method?

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

Group – C

4. (a) Use a flow chart to represent how RNA silencing is used in the genomic analysis of *C.elegans*.
- (b) What is the physiological definition of a biomarker in a medical context? What are the relevant characteristics of a toxicity biomarker? Highlight with an example.
- (c) Protein-protein interactions form the modern basis of understanding the progression of disease. If a typical protein-protein interface that is serving as a model for disease analysis has an area of $1700 \text{ \AA}^2$ (i) how many intermolecular hydrogen bonds would you expect to be formed? and (ii) how many fixed water molecules would you expect to find in the interface? If the entire buried area of the interface is hydrophobic, what contribution to the free energy of stabilization would you expect it to make?

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

5. (a) Based on your knowledge of cryo EM spectroscopy as applied to structure determination of viruses, answer the following questions (i) why are the following steps important in sample preparation for this technique? protein fixation; immobilization/dehydration, sample staining; (ii) how have the technical advantages of cryo EM ultimately helped in the design of a vaccine?
- (b) Explain the maximal response equation $\text{Response}_{\text{max}} = \text{Response}_{\text{ligand}} \times \frac{M_{\text{r analyte}}}{M_{\text{r ligand}}} \times \frac{(\delta n / \delta c)_{\text{analyte}}}{(\delta n / \delta c)_{\text{ligand}}}$ utilized in SPR data analysis. What stoichiometry of binding is this response equation applied to? Name the *instrumentation* parameters that are relevant in a SPR response.

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

Group – D

6. (a) If one considers protein engineering in the context of a broader genetic engineering discipline, what are the distinguishing features of protein engineering?

- (b) How has protein interaction analysis (essentially protein-protein interaction studies) been used to predict functions for plant proteins? Highlight your answer with two examples.
- (c) What characteristics of oxygenases make them ideal for protein engineering modifications? Use an example of oxygenases used for bioremediation applications through improvement of microbial processes to highlight your answer.
- (d) Use two different therapeutic categories to explain how protein engineering principles and techniques have been used for developing treatments for cancer.

3 + 3 + 3 + 3 = 12

7. (a) Using examples show how standard computer aided drug design methods have been used for antibody modelling? How can they be classified as protein engineering applications?
- (b) Green fluorescent proteins (GFPs) are useful molecular probes. What are the unique biophysical and biochemical characteristics of GFP that have made it useful for biological and medical applications?
- (c) How have protein engineering principles been applied to the selection of targets for nanoscale protein-based drug delivery?

(2 + 2) + 4 + 4 = 12**Group – E**

8. (a) What is Anfinsen dogma for protein folding? Describe the experiment based on which this was known.
- (b) Draw and explain the curve for protein denaturation with diagram. Show, how you can calculate ΔG^0 from that protein denaturation curve.
- (c) Write the four properties by which MGstate of proteins can be characterized. Write the steps of ubiquitination of protein with labelled diagram.

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

9. (a) Define protein folding with the help of a thermodynamic diagram and parameters.
- (b) Itemize and explain the forces that participate in the self assembly of protein molecules.
- (c) Discuss the principles of protein folding by discussing the protein folding in the GroEL-ES chaperone system.
- (d) Name two diseases that are currently thought of as being caused by protein misfolding.

1 + 4 + 5 + 2 = 12

Department & Section	Submission Link
BT	https://classroom.google.com/w/MTE5MzI0MTM4NDM0/tc/Mjc0ODMzMzExNjAw