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) Match the techniques in Group I with applications given in Group II.

	Group I		Group II
1	Salting out	P	pI determination
2	Ultracentrifugation	Q	Protein precipitation
3	Dialysis	R	Sedimentation coefficient
4	Isoelectric focusing	S	Removal of low molecular weight impurities

(a) 1-Q; 2-R; 3-S; 4-P (b) 1-P; 2-R; 3-Q; 4-S
(c) 1-S; 2-P; 3R-; 4-Q (d) 1-Q; 2-S; 3-R; 4-P

(ii) What would be the number of protein molecules present in 1.0 mg of protein having a molecular weight of 25 KDa?
(a) 2.4×10^{15} (b) 2.4×10^{16} (c) 2.4×10^{17} (d) 2.4×10^{18}.

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

(v) A protein undergoes post translational modification. 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) glycosylation (c) ubiquitinization (d) ADP-ribosylation

(vi) Which of the following techniques are capable of accurately measuring protein-protein interactions?
(a) Microarray methods (b) Surface Plasmon resonance
(c) Resonance energy transfer (d) All of the above.

(vii) A recombinant protein is expressed in E. coli under T7 promoter at 37°C. However no biological activity is obtained in the cell lysate. If the same experiment is carried out at 25°C, the cell lysate shows a reasonable biological activity. The most probable explanation for this is
(a) lower temperature increases recombinant protein stability
(b) lower temperature increases rate of production of recombinant protein
(c) IPTG used for induction does not get degraded
(d) recombinant protein is properly folded at low temperature.

(viii) 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.

(ix) The main difference between domain and motif in protein structure is
(a) domain can remain stable, independent of the rest of the protein while motif cannot
(b) domain cannot remain stable, independent of the rest of the protein while motif can
(c) domain can be predicted but motif cannot be predicted
(d) both are synonyms and there is no difference.

(x) Which one of the following methods cannot be used to determine the secondary structure content of a protein?
(a) Circular dichroism spectroscopy (b) Fourier transform infrared spectroscopy
(c) Mass spectrometry (d) X-ray crystallography.

Fill in the blanks with the correct word

(xi) The maximum size of the protein can be identified by MALDI is _____.

(xii) Intracellular protein degradation system of cell is _____.

(xiii) The number of different possible ways of forming five intramolecular disulfide bonds with ten cysteine residues of a protein are _____.

(xiv) β-mercaptoethanol breaks the _____ covalent bond between light and heavy chains of an immunoglobulin molecule.

(xv) If a denatured protein of human origin is injected into a rabbit, antibodies generated will recognize the _____ structure of the protein.

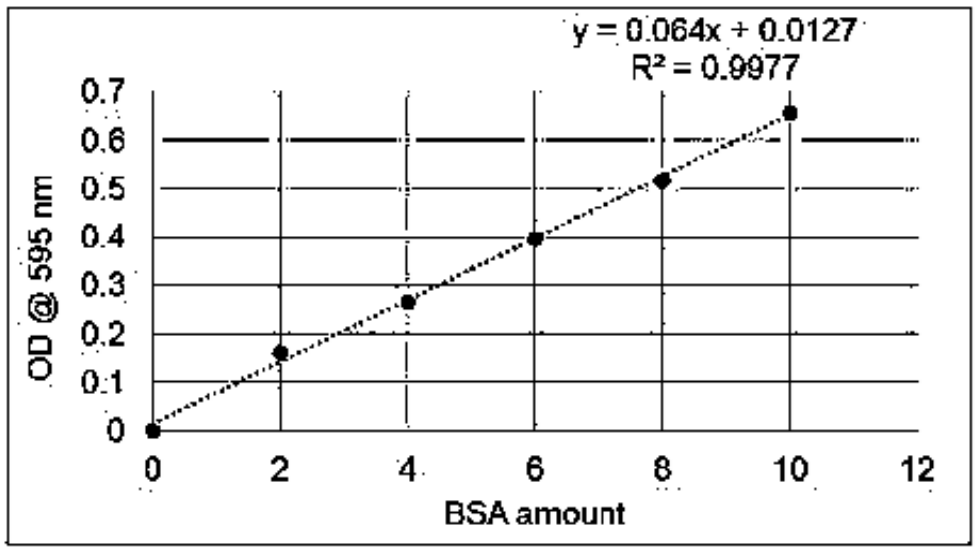
Group - B

2. (a) Explain how affinity chromatography can used to study protein-protein interaction with a labeled diagram.
*[(CO1)(*Explain-Remember */IOCQ)]*

(b) Write the names of five common types of HPLC detectors. Explain operation of any two detectors that you have mentioned.
[(CO2)(Remember-understand/LOCQ)]

(c) Soham wants to perform the SDS-PAGE for four protein samples A, B, C and D, for which he first quantified the protein concentration using Bradford method of protein quantification. He had dissolved his protein samples in 50 µl of buffer and for each sample, then 2 µl of each protein sample was taken in duplicates to perform the quantification.
Standard protein BSA was used to plot the standard curve taking different amount of BSA as 2 µg, 4 µg, 6 µg, 8 µg and 10 µg. Given below is the standard plot obtained for BSA along with the respective absorbance.

BSA amount	OD1	OD2	Avg OD
0	0	0	0
2	0.163	0.159	0.161
4	0.261	0.271	0.266
6	0.433	0.358	0.396
8	0.511	0.522	0.517
10	0.656	0.657	0.657



The absorbance obtained for samples A to D were 0.505, 0.353, 0.1845 and 0.2125 respectively. If Soham wishes to load 15 µg protein for each sample in SDS-PAGE, what would be the volume required for the samples A and D. *[(CO2)(Analyse/HOCQ)]*

4 + (1 + 4) + 3 = 12

3. (a) Explain the principle and steps of 2D-DIGE with labelled diagram. *[(CO1)(Analyse/HOCQ)]*

(b) Write names of three techniques to study protein-protein interaction (PPI). Explain anyone technique you mentioned with diagram for the study of PPI. *[(CO2)(Remember-explain/IOCQ)]*

(c) Explain the principle and steps of protein identification by LC-MS/MS using coded affinity tagging. *[(CO1)(Understand/IOCQ)]*

4 + (1 + 3) + 4 = 12

Group - C

4. (a) 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. *[(CO3)(Analyse/IOCQ)]*

(b) "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 target validation 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)]

4 + (6 + 2) = 12

5. (a) 2D-NMR based techniques for protein analysis have accelerated the rate at which large protein structures can be solved with accurate structure models". What were the technical problems associated with 2D NMR structure determination of

large proteins? How have TROSY and other technical improvements helped in solving those problems with respect to structure determination of large proteins. *[(CO3)(Analyse/IOCQ)]*

(b) (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. *[(CO4)(Remember-analyse/IOCQ)]*

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

Group - D

6. (a) Write the names of three expression vector, where you can express your desire protein in large amount. Explain the control of overexpression in any one of the vectors you mentioned. *[(CO4)(Remember-explain/IOCQ)]*

(b) Explain a strategy for oligonucleotide directed mutagenesis of a gene 'X' with PCR. *[(CO4)(Remember/LOCQ)]*

(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. *[(CO4)(Apply/HOCQ)]*

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

7. (a) How can unusual amino acids be incorporated into proteins, thereby producing an altered form of the target protein? *[(CO3)(Analyse/HOCQ)]*

(b) How would you engineer streptokinase so that it was less sensitive to proteolytic digestion? *[(CO4)(Remember/LOCQ)]*

(c) How can the gene(s) encoding a Fab fragment of a monoclonal antibody be modified so that the specificity of the antibody is altered? *[(CO2)(Apply/IOCQ)]*

4 + 4 + 4 = 12

Group - E

8. (a) What is molten globule? *[(CO5)(Analyse/HOCQ)]*

(b) Write names of different protein that help in protein folding. Explain role of chaperon in protein folding in *E. coli* with labelled diagram? *[(CO5)(Remember/LOCQ)]*

(c) Explain the energy landscape theory for protein folding. *[(CO5)(Apply/IOCQ)]*

(d) You have purified a recombinant protein and wonder whether it adopts a folded structure. How might you address this problem? *[(CO2)(Apply/IOCQ)]*

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

9. (a) How you will determine thermodynamic stability of protein, assuming that *in vitro* protein folding is a reversible reaction between native state (N) and unfolded state (U). *[(CO6)(Evaluate/IOCQ)]*

(b) For a certain solution of protein RNaseA, in which the total protein concentration is 2.0×10^{-5} M, the concentration of the native and denatured protein at 50 and 100°C are listed below.

Temperature	Protein (denatured)	Protein (native)
50°C	5.1×10^{-6} M	2.0×10^{-3} M
100°C	2.8×10^{-4} M	1.7×10^{-3} M

Determine ΔS^{o} and ΔH^{o} for the folding reaction. *[(CO6)(Analyse/HOCQ)]*

(c) Write names of three experimental techniques for the study of *in vitro* protein folding. Explain the principle and steps to study protein folding through anyone of those techniques you have mentioned. *[(CO5)(Understand/IOCQ)]*

4 + 4 + (1 + 3) = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	16.66	61.45	21.87