B.TECH/BT/7TH SEM/BIOT 4121/2021

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) The active site of a protein is at most 10% of the protein's volume. The rest of the protein is necessary partially for which ONE of the following choices?
(a) It is required to bring the active site residues into their correct spatial conformation
(b) It provides the space for the levers and fulcra for conformational change in the protein to occur
(c) (a) +(b)
(d) None of the above.

(ii) The following part of the structure of a regulatory network, A→B, represents which one of the following interactions?
(a) Reciprocal interaction (b) Auto regulatory interaction
(c) Stimulating interaction (d) Inhibitory interaction

(iii) Which one of the following DOES NOT represent a protein engineering method?
(a) Traceless Staudinger ligation (b) Cofactor binding spatial change
(c) Designed divergent evolution (d) mRNA display

(iv) In 1944 the physicist Erwin Schrodinger posed the question "What is life?" in an attempt to understand the physical properties of a living cell by techniques familiar to chemistry and physics. Which one of the following discoveries to application choices exemplifies the above statement?
(a) Development of NMR spectroscopy as a method for determination of the structure of biological macromolecules in solution
(b) Signal transduction in the nervous system
(c) Both (a) and (b)
(d) Principles of elucidation of the structure of nucleic acid-protein complexes.

(v) Which one of the following enzyme properties are typically improved in food industry applications?
(a) Thermostability (b) Specificity
(c) Catalytic efficiency (d) All of the above

(vi) SDS is generally used in SDS-PAGE. What is the role of SDS?
(a) Gel can't be run without SDS because it helps in polymerization of gel
(b) It being an anionic detergent provides similar charge to mass ratio to all the proteins
(c) It helps to break the disulfide bonds of the proteins
(d) It provides an overall positive charge to the proteins so that they can move from anode to cathode

(vii) Which of the following mathematical expressions is critically important for measuring the concentration C of a protein in determining its secondary structure using circular dichroism (CD)
(a) $\Delta\varepsilon = S/(32980Cl)$ (b) $\nu=1/2\pi$ sqrt k/μ
(c) $\Delta E= h\nu=g\beta H$ (d) $NOE_{ij}=1/r^6$

(viii) Ishaan is working on a cell line. To identify how a Drug X affects the cells, he treated the cells with the drug and pelleted down the treated cells. He has extracted the proteins from treated cell and wishes to proceed for mass spectrometry. What is the correct order of the steps he should follow before submitting the protein sample to the central mass spectrometry facility?
(a) SDS-PAGE for the quality check > Reduction and alkylation > Tryptic digestion > Desalting of the peptides
(b) Tryptic digestion > Reduction and alkylation > Desalting of the peptides > SDS-PAGE for the quality check
(c) Desalting of the peptides > Tryptic digestion > Reduction and alkylation > SDS-PAGE for the quality check
(d) SDS-PAGE for the quality check > Tryptic digestion > Reduction and alkylation > Desalting of the peptides

(ix) In order to attain a native 3-dimensional confirmation, polypeptide chains in protein undergo protein folding. Which of the following statements is correct for the process of protein folding?
(a) The protein folding process is governed by an overall decrease in entropy
(b) Folding of proteins favours the position of hydrophilic amino acids towards the outer side
(c) ProteinFolding is a nonspontaneous process
(d) Protein folding is majorly driven by various covalent interactions

(x) Which of the following does not hold true in terms of advantages of 2D-DIGE over conventional 2-D-PAGE?
(a) The Cy dyes used in 2D-DIGE are more sensitive than the dyes used to stain a 2-D-PAGE.
(b) 2-D DIGE technique has reduced the reproducibility issue that is faced in conventional 2D-PAGE.

(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 2D-PAGE.

Group- B

2. (a) Write the names three protein visualization techniques in 2D-GE. Explain the principle of anyone protein visualization techniques you have mentioned, with diagram. [(CO1) (Remember/LOCQ)]

(b) Explain the principle and steps of the SEC techniques for the study protein-protein interaction (PPI) with diagram. [(CO2) (Understand/IOCQ)]

(c) Derive the equation for the determination of average mass using two consecutive peaks from the mass spectrum of a protein.[(CO3)(Evaluate/HOCQ)]

(1 + 3) + 4 + 4 = 12

3. (a) Write the names of pathways present inside a eukaryotic cell that are used to degrade proteins. Explain the pathway by which a cell degrades misfolded proteins using a labelled diagram.[(CO2) (Remember/LOCQ)]

(b) Name three ionization and three mass analysing techniques that are used in mass spectrometry (MS). Using a diagrammatic representation, explain the principles behind anyone of the ionizing and analysing techniques that you cited. [(CO1) (Understand/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 from protonation, deduce an average molecular mass for the protein by this method. [(CO6) (Analyze/HOCQ)]

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

Group - C

4. (a) "Analysis of the chemical shifts (δ) and the couplings (J) from the 2D NMR spectrum of a protein of MW 35kDa yields three different sets of restraints ." Elucidate this statement by referencing the types, numbers and specifications of these restraints. What ultimate improvement occurs in the protein NMR spectrum by having accurate restraints analysis? [(CO3) (Crticize/HOCQ)]

(b) With one example in each case, briefly explain how proteomics provides methods for (i) identification of *drug targets* based on human disease specific

proteins and (ii) identification of *drug targets*in the pathogen proteome. Identify one specific type of protein implicated in the pathogen proteome and one computational method that is common to both (i) and (ii).

[(CO6) (Analyze/IOCQ)]

(c) Define a transposon. Why is insertional mutagenesis suited as a reverse genetics technique? Cite an example of the application of this technique to identify genes/gene products that are responsible for infectivity in a pathogen.
[(CO6 and CO3)(Remember, understand/LOCQ)]

(4 + 1) + 4 + 3 = 12

5. (a) (i) 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 $R= \Sigma (F_{obs}-F_{cal})/ \Sigma F_{obs}$ can be used to assess the quality of "good crystal structures". Define all the terms in the equation and draw the relationship of R to the resolution of a crystal structure. [(CO3) (Derive/HOCQ)]

(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"? Justify your answer. [(CO3) (Analyze/IOCQ]

(b) 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. [(CO6) (Analyze/IOCQ)]

(4 + 3) + 5 = 12

Group - D

6. (a) Use a flowchart to explain the steps of a RPD (rational protein design) cycle. Explain how disulphide bonds affect protein stability. What would you expect to be the effect on protein stability of introducing a disulphide bond using mutagenesis? [(CO5) (Remember-understand/LOCQ)]

(b) How the following two proteomics based areas have impacted developments in environment related technologies? (i) in the case of proteins/peptides absorbed on cell exteriors and (ii) use of "omics" based technologies for biodegradation uses. [(CO4) (Understand-evaluate /HOCQ)]

(c) The average centrifugal force, g_{av} , is based on the RCF (relative centrifugal force) typically expressed in g used for centrifugation purposes. Explain stepwise how "cell-free translation systems" were developed as an important tool for protein engineering based on centrifugation. Cite two distinct advantages of this protein engineering tool. [(CO6) (Analyze/IOCQ)]

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

7. (a) One of the fundamental applications of computational protein engineering is prediction of complex loop formations in proteins. Explain how computational

techniques like receptor based structure activity have been proven to be useful for such applications extending to pharmacological information about therapeutic targets. [(CO4) (Remember-understand/LOCQ)]

(b) (i)Protein engineering methods have been used for the synthesis of higher generation function specific peptides for nanobiotechnology and biomedical applications. Give two examples of such applications and itemize the specific methods used.[(CO4) (Remember-understand/LOCQ)]

(ii)The half-life of a drug in plasma is given by $t_{1/2}=0.693XV_d/Cl_{int}$. Define the parameters in this equation. Typically $t_{1/2}$ varies from 1 to 24 hours. An important protein engineering method is *in vitro* glycoprotein synthesis that has been used in the development of new protein based drugs. Using one example illustrate in specific how this method has been used to develop and improve pharmacokinetic (PK) properties (like $t_{1/2}$) of a protein-based drug.

[(CO3) Illustrate/IOCQ)]

(c) How have techniques of protein engineering helped in advancing biomedical applications with antibodies? Your answer should include references to the following technologies- *recombinant DNA technology, recognition units and phage display libraries and specific applications therein*. [(CO4)(Analyze/IOCQ)]

4 + (2 + 2) + 4 = 12

Group - E

8. (a) How you will identify the intermediate molten globule structure in the pathway of protein folding? [(CO5) (Remember/LOCQ)]

(b) Write names of different proteins that help in protein folding. Explain the role of chaperons in protein folding in *E.coli* with labelled diagram. [(CO5) (Illustrate/IOCQ)]

(c) Explain the energy landscape theory for protein folding.

[(CO6) (Understand/IOCQ)]

(d) You have purified a recombinant protein and wonder whether it adopts a folded structure. How might you address this problem? [(CO6) (Criticize/HOCQ)]

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/HOCQ)]

(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) (Evaluate/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. [(CO3) (Understand/LOCQ)]

4 + 4 + (1 + 3) = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	30%	43%	27 %

Course Outcome (CO):

After the completion of the course students will be able to

1. Understand different large scale protein separation , estimation, identification and sequencing techniques ; application of the knowledge to solve and analyze problems linked to the proteome
2. Understand in-vivo and in-vitro protein-protein interactions techniques
3. Description of the techniques of structural proteomics and application of knowledge of proteomics to drug discovery
4. Description of the basics and significance of protein engineering; demonstrate the design and modification of proteins according to industrial needs and applications
5. Understand the stability of protein structure and mechanism of protein folding; ; application of this knowledge in the study of protein misfolding related diseases
6. Analysis and solution of problems related to proteomics and protein engineering technology

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

Department & Section	Submission Link
BT	https://classroom.google.com/c/NDA0NzExNTQ1NjYw/a/NDY0MjM0MzM4Nzcx/details