B.TECH / BT /7TH SEM/ BIOT 4164/2017

PROTEOMICS & 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) Which of the following statements is not true about sequencing peptides with mass spectroscopy?
- (a) The entire protein can be sequenced all at once using mass spectroscopy.
- (b) Two rounds of mass spectroscopy are used to determine sequence.
- (c) some purified protein must be digested with proteases to eliminate undesirable characteriscts such as hydrophobicity and solubility.
- (d) In order to determine the sequence, a pure sample of protein is obtained through 2D-PAGE or HPLC.

(ii) Co-immunoprecipitation is used
- (a) to determine if a protein-of-interest binds to a specific DNA sequence.
- (b) to examine protein-protein interaction in the nucleus instead of in the cytoplasm.
- (c) to examine protein-protein interactions in the cytoplasm instead of in the nucleus.
- (d) to allow protein to be expressed in mammalian cell culture.

(iii) Which one of the following statements about prion proteins is incorrect?
- (a) Prion proteins form cross-beta filaments
- (b) Prion proteins are heat resistant
- (c) Prion proteins are protease sensitive
- (d) Prion proteins can convert the normally folded prion protein to pathological form.

(iv) Which of the following are label free methods for proteomics studies?
(a) surface Plasmon resonance (b) static light scattering
(c) photon correlation spectroscopy (d) all of the above.

(v) Trypsin cleave the peptide bond containing
(a) Ala or Val (b) Glu or Asp (c) Met or Trp (d)Arg or Lys.

(vi) Reverse genetics deals with
- (a) taking an uncharacterized gene sequence and modifying it
- (b) taking a characterized gene sequence and modifying it
- (c) moving from phenotype to gene
- (d) annotating an unknown gene.

(vii) Which of the following techniques involves isolation of cell surface proteins
(a) labelling with dyes (b) labelling with 18O
(c) cell surface shaving (d) all of the above.

(viii) Which of the following conditions absolutely requires accurate X-ray diffraction?
- (a) well ordered protein crystals
- (b) a high throughput automated crystallization workstation
- (c) molecular replacement protocol
- (d) multiple isomorphous replacement technology.

(ix) Functional protein microarrays can be used to study
(a) enzyme-substrate interactions (b) protein-protein interactions
(c) protein-nucleic acid interactions (d) all of the above.

(x) Which of the following is a chromatographic surface on Protein chip technology
(a) IMAC (b) DNA-protein (c) receptor-ligand (d) antibody antigen.

Group - B

2.(a) What is the definition of proteomics? Explain the concept of a proteome. Why is the latter considered to be a complex dynamic entity?

(b) Use a flowchart to depict/explain some current technological challenges facing proteomics.

(c) What are the basic differences between proteomics and protein chemistry?

(d) Write the basic principles, steps with labeled diagram and application about Yeast two hybrid.

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

3.(a) Write the different steps of 2-D PAGE and describe the basic principles of these with labeled diagram.

(b) An unknown peptide was analyzed by mass spectrometric and chromatographic methods as follows:

(i) MALDI-TOF mass spectrometry of the peptide gave two signal at m/z = 3569 and 1785;

(ii) The data obtained from analysis of the peptide using coupled HPLC-MS operating through an ESI source were m/z =510.7, 595.7, 714.6, 893.0 and 1190.3.

Determine the molecular mass of the peptide.

(c) Draw a table incorporating three types of covalent protein PTMs and their respective sub-categories. Give one example for each sub-category of covalent modifications.

4 + 4 + 4 = 12

Group - C

4.(a) Explain the technique of cryo-electron microscopy and its use in structural proteomics. Mention two examples in structural proteomics where cryo-EM was utilized.

(b) What are the technical complications that are typically associated with x-ray diffraction and NMR spectroscopy for high throughput structural analysis in proteomics?

(c) Elaborate on the applications of surface plasmon resonance in proteomics research.

(3 + 1)+ 4 + 4 = 12

5.(a) What characteristics of proteins make them suitable as disease biomarkers? Explain the parameter of bioavailability in the context of protein based drugs. Wherever applicable use suitable examples to explain your answer.

(b) Explain the reverse genetics approaches of homologous recombination and gene silencing, highlighting each of the techniques by examples.

(c) Use a flowchart to depict the stages of drug development. What is the value of proteomics in (i) target identification and (ii) identification of potential protein therapeutics and targets in the pathogen proteome?

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

Group - D

6. (a) What is the role of proteomics in the detergents industry? Which category (ies) of protein engineering principles do such applications fall in?

(b) How can site directed mutagenesis be applied for protein stability measurements? How is measurement of protein stability typically done? Name two globular proteins that served as models for protein stability measurements.

(c) How has protein stability measurements been applied to the food processing and environmental biotechnology industries. Cite two examples in each industry category.

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

7. (a) How have principles of rational design been applied to the purification of proteins and design of hybrid enzymes? Use examples to highlight the commercial applications of such rational design experiments.

(b) Name 4 expression systems for the generation of recombinant proteins. What are the disadvantages associated with the most common of them?

(c) Draw a labelled flowchart for a general solid phase peptide synthesis cycle. Give a simple definition of selective deprotection.

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

Group - E

8. (a) What do you mean by protein folding?

(b) Discuss the forces that help in self-assembly of protein molecules.

(c) What are the factors that govern protein stability? Use a free energy of folding plot for soluble proteins to illustrate your answer. What important observations about the protein folding process were made when bacteriorhodopsin's folding mechanism was compared to that of ribonuclease?

(1 + 3) + 4 + 4=12

9.(a) Briefly outline the non-lysosomal ATP dependent pathway of intracellular proteolysis.

(b) Circular dichroism is sometimes used for protein folding studies. What aspects of protein folding can be suitably studies by utilizing circular dichroism as a measurement technique?

(c) Name two diseases that are cased by misfolded proteins. Discuss the mechanism of cystic fibrosis in relation to prion.

4 + 4 + (2 + 2) = 12