End-Semester Examination (50 marks; 3 hours)

Handout

Question 1. Explain the following (pointed and short answers) (0.5 X 10 = 5 marks)

1.1. Genomics *vs*. Proteomics

1.2. Cell-free protein expression *vs.* Cell-based protein expression

1.3. Label-free *vs.* Label-based detection techniques

1.4. SPR *vs.* SPRi

1.5. PMF *vs.* Intact protein analysis

1.6. 3D structure modeling *vs.* Molecular Docking

1.7. Carbon nanotubes *vs.* Quantum dots

1.8. Serum proteomics *vs.* Urine proteomics

1.9. Nucleic acid programmable protein array (NAPPA) *vs.* DNA Array to Protein Arrays (DAPA)

1.10. Direct labeling *vs.* Sandwich labeling in antibody microarrays

PAGE BREAK

Question 2. Write "True" or "False" for the following statements and give proper justifications for your answer (**1** X **10 = 10 marks**)

2.1. Proteomic analysis is much more challenging than genomic analysis.

2.2. Yeast-two hybrid system is more effective than SPR for high-throughput screening of protein-protein interactions.

2.3. BS3 linker helps in attaching the anti-GST antibodies to the protein array surface.

2.4. Amylase is the most abundant protein in saliva and albumin in serum.

2.5. GOLD/ Glide are not suitable for docking of the protein-ligand molecules.

2.6. Reverse phase protein arrays depend on laser capture microdissection (LCM) and microfabrication.

2.7. Coupling of microarrays with surface plasmon resonance imaging is advantageous because it increases sensitivity and throughput.

2.8. Multi-protein drug targeting is better than single protein targeting.

2.9. Epitope tagging is a label-free and ellipsometry is a label-based detection technique.

2.10. Phosphorylation and dephosphorylation have no significant effect on the cell cycle, apoptosis and signal transduction pathways.

PAGE BREAK

Question 3. Please write the answer of following questions (1X5 = 5 marks)

3.1. What are the major challenges associated with gel-based and MS-based proteomics?

3.2. What are the foremost initiatives taken by HUPO for development of organ specific proteome projects during the last decade?

3.3. What are the major advantages of cell-free expression-based protein microarrays? Mention two conventional approaches for *in vitro* translation and two advanced technologies for cell-free expression-based protein microarrays.

3.4. What is the basic principle behind the SPR-based label-free biosensing? Briefly, illustrate the different components of a conventional SPR set-up.

3.5. Describe how proteomics based approaches can be used for (a) Identification of drug targets, and (b) screening of potential drug molecules?

PAGE BREAK

Question 4. Please write short description of followings (pointed and short answers) (1X10 = 10 marks).

4.1. Influence of glycosylation on protein folding.

4.2. Advantages of SPR and SPRi in biomarker discovery.

4.3. Applications of Surface Enhanced Raman Scattering (SERS) in signal amplification.

4.4. Pre-fractionation techniques in serum proteomics.

4.5. Multiple Spotting Technique (MIST) in preparation of high-density arrays.

4.6. Challenges associated with the extraction and analysis of membrane proteins.

4.7. KEGG for pathway analysis of wide range of organisms.

4.8. Capillary Electrophoresis coupled to Mass Spectrometry (CE-MS) for analysis of urine proteomics.

4.9. Initiatives of Human Proteome Organization (HUPO) for development of plasma proteome map.

4.10. Multiplexing in quantitative proteomics.

PAGE BREAK

Question 5.

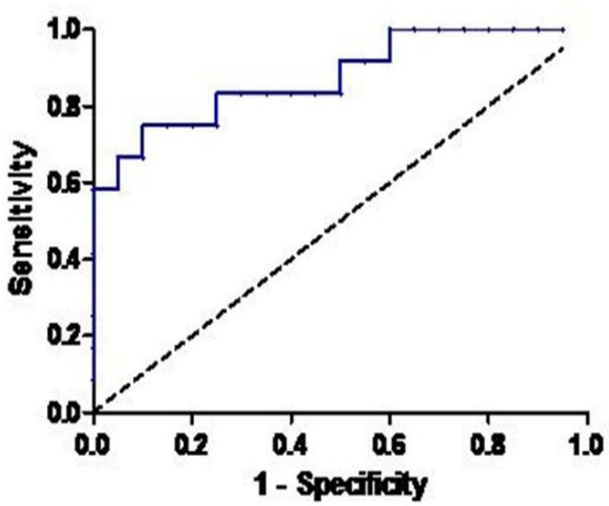
Please match the contents of "Column A" with "Column B" (0.5 X 10 = 5 marks)

Sl No.	Column A	Column B
1.	SPR and SPRi	Measurement of changes in conductivity or refractive index
2.	2011	Initiation of the Human Plasma Proteome Project (PPP)
3.	APTES (aminopropyltriethoxysilane)	Official launching of the HUPO Brain Proteome Project (HUPO BPP)
4.	Personalized proteomics and medicine	Selective enrichment of low-abundance proteins
5.	Label-free detection	Individualized health care
6.	2002	*In vitro* translation
7.	Rabbit reticulocyte lysate	High-throughput screening of protein-ligand interactions
8.	Hydrogel particles	Release of the Human Protein Atlas portal
9.	QDs	Influences attachment of DNA and proteins to glass slides
10.	August 2005	Size-tunable narrow and symmetric emission spectra

PAGE BREAK

Question 6. Receiver operating characteristic (ROC) curve analysis is carried out to evaluate the accuracy of a serum protein for cancer prediction. The area under the ROC curve (AUC) indicates the accuracy of different classifier proteins to distinguish cancer patients from healthy subjects. ROC curve and specificity and sensitivity values obtained in different cut off values of the potential marker protein are provided below.

Cut off (µg/mL)	Sensitivity	Specificity
< 27.35	0.5833	0.95
< 27.85	0.6667	0.95
< 28.11	0.6667	0.9
< 28.61	0.75	0.9
< 29.29	0.75	0.85
< 30.68	0.75	0.8
< 32.25	0.75	0.75
< 33.21	0.8333	0.75
< 33.72	0.8333	0.7
< 34.47	0.8333	0.65
< 36.40	0.8333	0.6
< 37.81	0.8333	0.55
< 38.10	0.8333	0.5
< 38.53	0.9167	0.5
< 38.84	0.9167	0.45
< 38.96	0.9167	0.4



Please answer the following questions (1X5 = 5 marks):

6.1. At what concentration (cut off) of the protein best sensitivity will be obtained?

6.2. At what concentration (cut off) of the protein best specificity will be obtained?

6.3. At what concentration (cut off) of the protein optimum combination of specificity will be obtained?

6.4. If the AUC for this protein is 0.83; then how many times more effective it will be than the identity line, which represents 50% accuracy?

6.5. Comment on cancer prediction potential of this candidate biomarker. PAGE BREAK

Question 7. Protein microarrays are very useful platform for sensitive, multiplexed protein detection in an arrayed format. However, due to the large dynamic range of protein concentrations and extremely low-abundance of the potential marker proteins, often it requires the use of nanoparticles for signal amplification for improving the sensitivity of such assays. In a recent study macromolecular, single-walled carbon nanotube (SWNTs) has been used to protein arrays to enhance sensitivity. Two potential cancer biomarkers Protein X and Protein Y are measured in diluted human serum and PBS buffer. Limit of detection (LOD) for both the proteins in presence and absence of the secondary amplifier is summarized below.

	Regular detection (Without signal amplifier)		**With SWNTs as signal amplifiers**	
	Diluted serum	**Buffer**	**Diluted serum**	**Buffer**
1. Protein X	10 nM	100 pM	10 pM	100 fM
2. Protein Y	250 nM	1.75 nM	155 pM	0.50 pM

Please answer the following questions (2 X 1.5 + 2 = 5 marks):

7.1. What magnitude of higher sensitivity is obtained for Protein X and Protein Y in buffer solution compared to the diluted serum in regular detection method? Give possible reason and write an explanation.

7.2. What magnitude of improvement of sensitivity obtained after using SWNTs as signal amplifiers?

(a) For Protein X in buffer and diluted serum.

(b) For Protein Y in buffer and diluted serum.

7.3. If the normal serum concentration range of Protein X is 100-350 pg/mL (~200-500nM) and it is around 50 to 75-fold down regulated in cancer patients; whether it will be possible to measure Protein X concentrations in cancer patients using regular detection method? What will be the possibility of measuring Protein X concentrations in cancer patients if SWNTs is employed as signal amplifiers? Give explanation for your answer.

PAGE BREAK

Question 8. Answer the following questions based on label-free techniques.

8.1. Calculate the K_{on}, K_{off} and K_d for the following SPR data (**2 marks**)?

Substrate (nM)	1.25	2.5	5	10	20	40	80
K_{obs} (1/min)	0.0295	0.0392	0.0662	0.0925	0.1258	0.2013	0.3224

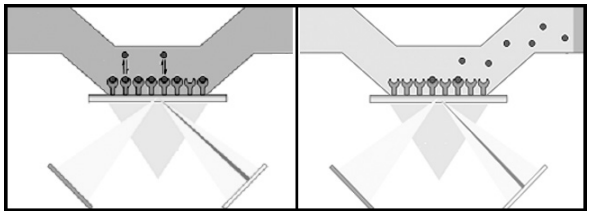
8.2. Write-down the principle based on which label-free techniques measure the bio-molecular interactions? (0.25 X 4 = **1 marks**)
(i) Spectral reflectance imaging biosensor _____

(ii) Ellipsometry_____

(iii) Nanowire sensor arrays_____

(iv) Surface Plasmon resonance_____

8.3. Please draw the sensorgram for the binding events shown in figure (**2 marks**).



PAGE BREAK