BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI, DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2013 – 2014
BIOT C344/ BIOT F345 PROTEOMICS
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 3hours **Date: 29.12.2013**
Weightage: 40% **Max. Marks: 40**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. Explain the Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption Ionization (MALDI) with suitable diagrams. [4.0]

2. What are the different variants of mass analyzers available? How Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) is different? Explain with suitable diagram. [4.0]

3. Why the pH gradients are used for 2D PAGE? Mention the applications of pH gradients. [4.0]

4. Why isotope-coded affinity tags (ICAT) are used in quantitative proteomics for protein identification? Explain with a suitable diagram. [4.0]

5. What is post translational modifications and list different PTM that occur in an eukaryotic cell and mention their functions? Explain in detail any one PTM and its role in cellular functions in eukaryotes. [4.0]

6. Explain the principle and operation of MudPIT with a diagram? Briefly explain any one applications of MudPIT in protein complexes. [4.0]

7. Explain the following:
   a. What is calmodulin (Cmd1)? [1.0]
   b. Mention at least any two functions of Cmd1 and protein partners involved in each category. [2.0]
   c. How the Cmd1 can be identified with respect to its specific functions in the cell? Explain with a suitable method for Cmd1 localization. [2.0]

8. What are the basic differences between protein and protein complex identification? If you want to identify protein complexes what are the requirements with respect to your mass analyzer? Give any one example of such protein complex and their composition and functions. [4.0]

9. Differentiate NMR and X-ray crystallography with any one example for each. Mention advantages and disadvantages of each method. [3.0]

10. Write a short note on Protein microarrays and compare with DNA microarrays. Mention advantages and disadvantages of each application in proteome and genome analysis. [4.0]

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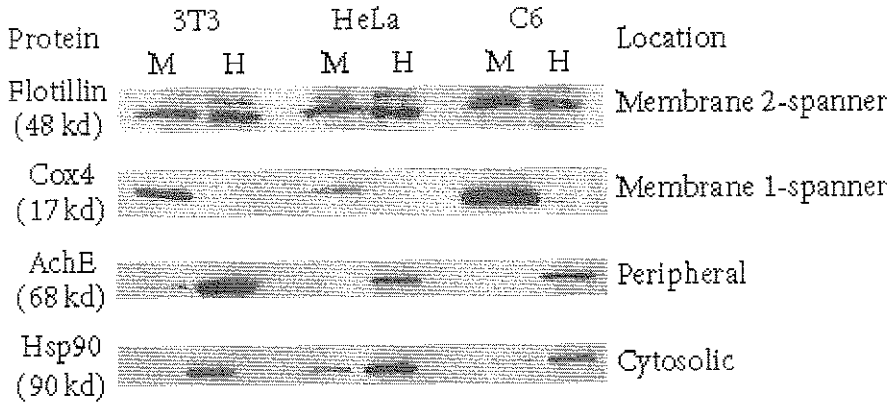
TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 10.11.2013**
Weightage: 20% **Max. Marks: 20**

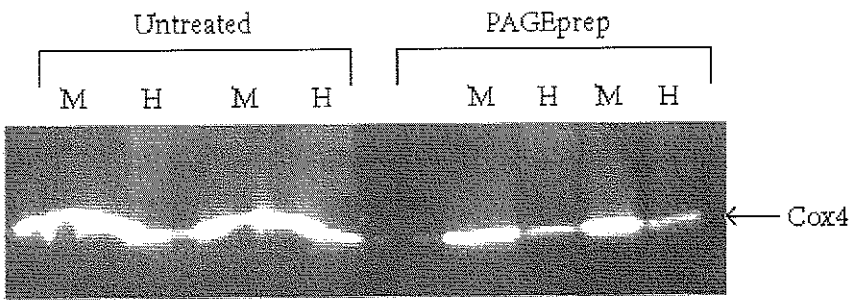
Note: *a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.*

1. The membrane proteins have been predicted to comprise approximately 30% of eukaryotic proteomes. Membrane proteins are the most elusive and the most sought after proteins in drug discovery. They play a key role in signal transduction, cell adhesion, and ion transport and are important pharmacological targets. Yet, because of their hydrophobic and basic nature, and frequently large size, their isolation is not easy. Traditional methods for membrane isolation are often cumbersome and protein yields are poor. Although proteomics technologies have made rapid progress in the characterization and investigation of soluble proteins in recent years, MPs have lagged behind. The major challenge of membrane proteome study is the low solubility due to the complex structure and hydrophobic nature of the membrane proteins and their low abundance. Therefore, new strategies for the identification and characterization of these special kinds of proteins are of great interest in modern proteomic research.

   a. With the help of the following diagram what information you derive out in terms of protein expression and localization. Briefly explain. [4.0]



   b. How will you enhance the separation and detection of membrane proteins and list advantages and disadvantages of the method of your choice including the one above given as an example? How the PAGEprep shown in picture is advantageous over other method? Justify with your answer. [4.0+3=6]

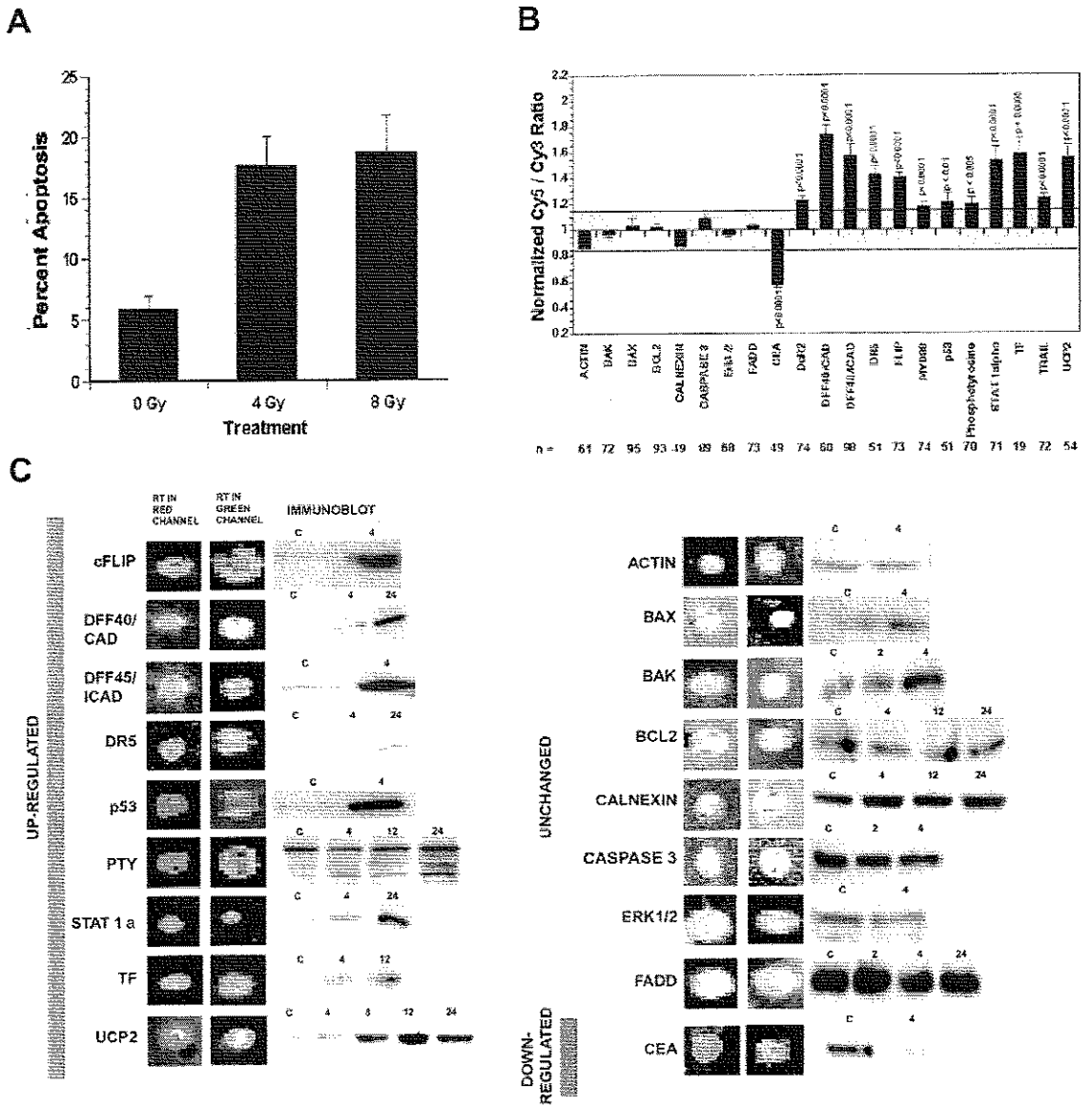


2. The radiation-induced alterations of protein levels and activity in LoVo cells as monitored using protein profiling of cancer cells using protein detection methods and discovery of novel radiation-regulated proteins is shown below. A) LoVo cells treated with 4 or 8 Gy radiation begin to undergo apoptosis within 4 h after irradiation. B) Differential expression of selected proteins in response to radiation treatment in LoVo cells. C) Validation of protein data with microarrays by fluorescent dye-reversal and WesternImmunoblot analysis.

   a. Why do you need to validate protein microarray data with immunoblot assays? Briefly outline what type of candidate protein which you will be selecting for such an analysis? Mention the pre-requirements for such an analysis of your chosen protein by Western immunoblot analysis. [3.0]

   b. i) Can you use isotope labelling and quantitative protein analysis in this sample? Briefly outline the method and expected outcomes of your experiment from the data. [3.0]

   ii) Will you be getting new type of data from such an experiements, if so outline the novel results of your experiments? [2.5]



3. Give an example for the following: [1.5]
    a. Isolation method for subcellular organelles
    b. Charged proteins
    c. Protein isolation method based on isoelectric point

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 13.10.2013**
Weightage: 25% **Max. Marks: 25**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. Explain any two peptide ionization methods with suitable diagrams and mention advantages of each method. [5.0]

2. What are the major different types of biological samples used and mention the role of proteomics in protein identification? Explain. [3.0]

3. What is peptide mass fingerprinting in proteomics and how this is used in protein identification methods? Mention the importance of in-silico protein sequence data and theorical tryptic peptides mass values in protein databases. Give schematic representations. [4.0]

4. What is the principle of Fourier Transform Ion Cyclotron Resonance mass spectrometry and how it is different from qTOF in protein identification technology? Explain with a schematic diagram. [5.0]

5. What are the disadvantages of proteins separated using single dimensional protein gel over the two dimensional polyacrylamide gel for protein identification? Explain. [5.0]

6. What are the different reagents used in sample preparation in 2D PAGE and mention the functions of each component in the sample buffer. [3.0]

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QUIZ-II (CLOSED BOOK)

Duration: 20 min. **Date: 5.12.2013**
Weightage: 7% **Max. Marks: 7**

Note: *a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.*

1. Give any two protein interactions databases for the protein networking and protein network visualization. [1.0]

2. Compare GFP with DsRed as protein localization tag (any two points for each). [1.0]

3. What are the functions of Cmd1 and mention any four protein partners? [1.5]

4. Write the principle of FRET based sensors and mention any two examples. [1.5]

5. What is collisional cooling and why it is required in protein complex identification process? [1.0]

6. Mention any two protein complexes and their approximate molecular mass, composition and their functions. [1.0]

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 27.10.2013**
Weightage: 8% **Max. Marks: 8**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. How the high sensitivity and accurate quantitative data on detection of proteins is achieved by using radioisotope labelled proteins? Mention any four limitations of using radiolabing as a method for protein identification. [1.5]

2. What is ligand blot overlay technique and mention any three types of proteins which can be identified using this method? [1.5]

3. How the glycoproteins and phosphoproteins are identified in 2D PAGE? Mention what types of aminoacids which may get phosphorylated/ glycosylated for detection. [2.0]

4. Why 2D PAGE image analysis software tools are required and mention any four software for image analysis? [1.5]

5. What is pre-gel fractionation? How it is achieved and why? [1.5]