

## Mid-semester examination (50 marks; 2 hours)

**Question 1.** Explain the following (pointed and short answers) (10 marks)

1.1. Minimum labeling vs. Saturation labeling

1.2. IPG strips vs. tube gels

1.3. Forward vs. reverse proteomics

1.4. On-gel electrophoresis vs. Off-gel electrophoresis

1.5. Ion exchange vs. gel exclusion chromatography

1.6. Soft vs. hard ionization

1.7. Equilibration I vs. II


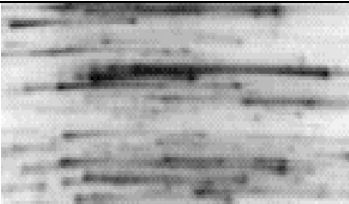
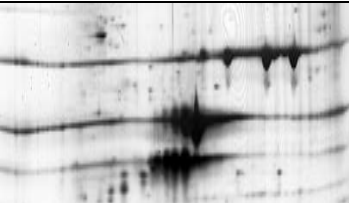
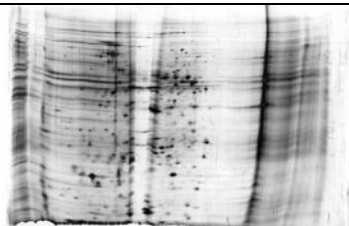
1.8. “b” vs. “y” ions

1.9. Proteomics vs. protein chemistry

1.10. Passive rehydration vs. Active rehydration

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**Question 2.** Identify the problem as demonstrated in these 2D gels and describe your suggestions to overcome the problem in each gel image (1.5+1 = 2.5 X 4 = **10 marks**)

	2D gel image	Problem (s)	Your recommendation (how to overcome the problem)
i			
ii			
iii			
iv			

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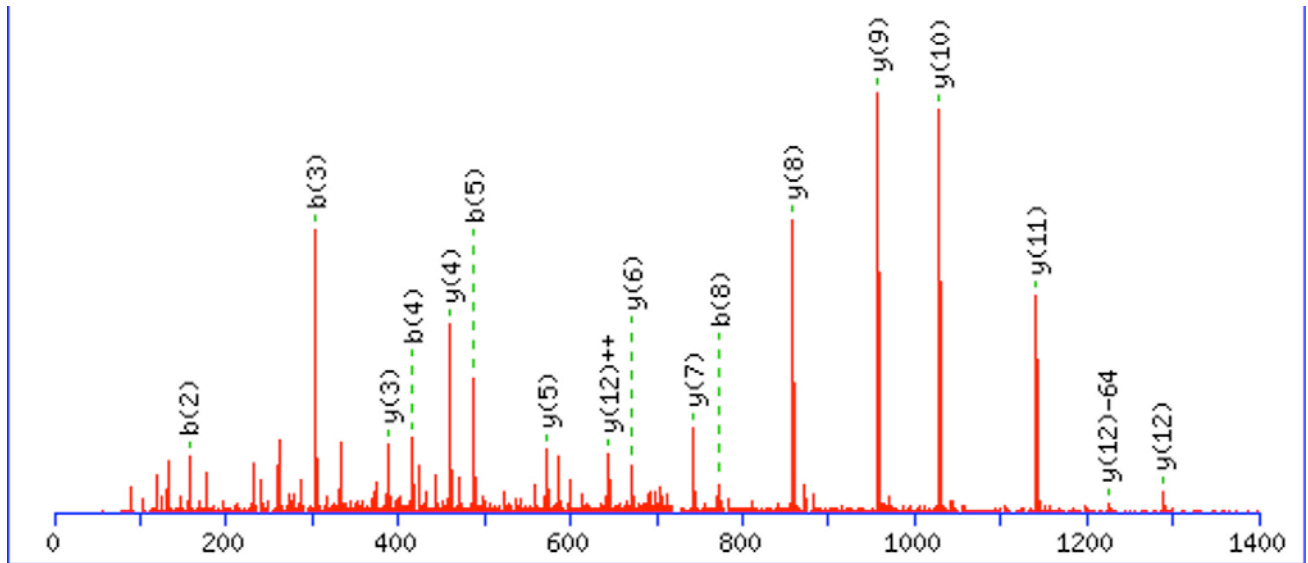
### Question 3. (2 X 6 = 12 marks)

Glutathione peroxidase 3 (Gpx3) is an important antioxidant enzyme in *Saccharomyces cerevisiae*. A study compared the wild type *S. cerevisiae* strain with its Gpx3 mutant strain by using two dimensional gel electrophoresis on 4-7 pI, 24 cm IPG strips. 2D gel images were analyzed using software and 6 spots were selected for further electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-Q-TOF MS/MS) analysis. Raw MS data was processed by MASCOT search program (Version 2.2, Matrix Science) and used for protein identification. Details of proteins identified by MS/MS is listed in table.

Analyse this data in Table and determine what will be your evaluation for data (proteins A-F) on a scale of 1-5 (1 – Best data; 2 – Good data; 3 – OK but acceptable; 4 – Doubtful, there is further need to look at raw data; 5 – Reject). Please fill in your option (only one option for each) in given space. Also, write-down your rationale for choosing which option is correct.

Protein	Species	pI	Accession No.	Peptide match	MOWSE score	Fold change	Your answer (1-5 scale)
A	<i>Saccharomyces pombe</i>	5.9	gi 6715442	1	13	-3.1	
B	<i>Saccharomyces cerevisiae</i>	6.3	gi 195650293	13	200	+2.3	
C	<i>Saccharomyces cerevisiae</i>	7.0	gi 195639750	7	25	+1.7	
D	<i>Bacillus subtilis</i>	5.5	gi 3036946	1	17	+2.6	
E	<i>Saccharomyces cerevisiae</i>	8.6	gi 13096169	2	39	-2.6	
F	<i>Saccharomyces cerevisiae</i>	5.1	gi 194701222	3	60	-1.9	

**Question 4.** In MS spectrum provided to you, please write-down the peptide sequence and also show your steps in answer book. You are free to use calculator (**8 marks**).



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**Question 5.** Please write the answer of following questions (2X5 = 10 marks)

- 5.1. In reverse phase chromatography what are mobile and stationary phases?
- 5.2. What is the extraction solution used for in-gel digestion procedure?
- 5.3. The processes by which an ion is selected for MS/MS analysis is known as.....?
- 5.4. In multidimensional separation, size exclusion chromatography followed by reversed phase is less used due to \_\_\_\_\_
- 5.5. The reflector in TOF analyzer is used for which purpose?