

## Questions – Additional Support

### Separations techniques in proteomics

1- What force(s) do(es) apply in electrophoresis?

- Gravitational force     Electrostatic force     Friction force     Retardation forces

2- For polyacrylamide gel polymerization, what do you need?

- Acrylamide     Ammonium persulfate and TEMED     Bisacrylamide     Styrene

3- What percentage of acrylamide would you recommend to separate proteins of 4-40 kDa?

- 10%     12.5%     20%     30%

4- In PAGE, what is the effect on the pore size when the percentage of acrylamide increases?

- The pore size increases     The pore size decreases     The pore size is not affected     None of the those

5- What is the effect of SDS on proteins?

- Protein conformation is affected     Protein charge is affected     Proteins become positively charges     Disulfide bridges are broken

6- What does PAGE stand for?

- Polymer aggregated gel electrophoresis     Polyacrylamide gel electrophoresis     Polyamine gel electrophoresis     None of those

7- In proteomics, how 1D SDS PAGE can be use with mass spectrometry?

- After in-solution digestion     With in-gel digestion     Both are not compatible     In the procedure, bands are cut after staining

8- What amino-acid(s) do(es) present positively charged lateral chains?

- Arginine     Glycine     Lysine     Aspartic acid

9- Glutamic acid has  $pK_1$  (-COOH) = 2.1,  $pK_2$  (-NH<sub>2</sub>) = 9.47 and  $pK_R$  (-R) = 4.07. What is the net electric charge of Glu at pH = 3?

- Positive     Negative     Zero

10- What is the approximate pI of Glu?

- 3.1                       5.8                       6.8                       2.1

11- During isoelectric focusing, in which direction do positively charged ions move?

- Toward the anode       Toward the cathode       They do not move

12- What property(ies) do(es) present carrier ampholytes?

- Amphoteric               Acidic                       "Carrier" of the current               Buffering

13- In 2D gel electrophoresis, what is the principle of the first dimension of the separation?

- PAGE                       IEF                       Size-based                       Liquid chromatography

14- What advantage(s) do(es) off-gel electrophoresis present with respect to classical IEF?

- Diffusion is absent       Focusing is much faster       Separated analytes are recovered in solution       Both proteins and peptides can be separated

15- What can explain an analyte did not efficiently focus during IEF?

- The slope of the titration curve at pI for this analyte is steep       Too many salts were present in the sample       The analyte is a protein       Voltage was stopped for 30 minutes before sample recovery

16- For what reason(s) can information on the pI be valuable?

- MS data validating/filtering       Optimizing protein digestion conditions       Phosphopeptide selection       Mass of peptide/protein is not anymore needed for their identification

17- What is necessary involved in chromatography?

- An analyte               A mobile phase               A stationary phase               A liquid phase

18- What was M.S. Tswett able to separate using chromatography?

- The components of serum       Some chlorophylls       Some carotenoids       Caffeine

19- In chromatography, what can explain band broadening?

- Multiple path of the analyte
- Perpendicular diffusion
- Mass transfer between phases
- High plate number

20- What factor(s) can affect the chromatographic resolution?

- The column length
- The flow rate
- The pore size of the packing material
- None of those

21- What type of chromatography(ies) would you recommend to separate proteins?

- Size exclusion
- Reversed-phase
- Affinity
- Strong-anion exchange

22- For what type of chromatography is hydrophobicity of the analytes relevant?

- Size exclusion
- Strong-cation exchange
- Partition
- Reversed-phase

23- Why HPLC was developed?

- To speed up the separation process
- To cope with smaller particle sizes
- To separate more samples
- To accommodate nano-flow rate

24- What type of chromatography is usually coupled to mass spectrometry?

- Size exclusion
- Strong-cation exchange
- Affinity
- Reversed-phase

25- What separation techniques may be complementary to RP-LC to separate peptide mixtures?

- IEF
- SCX
- SEC
- RP