

Questions – Chapter 04

1- Why is MS-based proteomics not inherently quantitative?

- There are losses of peptides during analysis and differences in the ionization efficiency of peptides
- The intensity of a peak in a mass spectrum is not a good indicator of the amount of the analyte in the sample, although differences in peak intensity of the same analyte between multiple samples accurately reflect relative differences in its abundance
- Detection efficiencies for ions with different m/z values are unequal
- The relationship between the amount of analyte present and measured signal intensity is complex and incompletely understood

2- What technique(s) can provide comparison of each individual peptide between experiments/samples?

- Label-free techniques
- SILAC
- Isobaric labelling
- MS gives directly the concentrations of analytes, which can be compared between the samples

3- What does the spectral counting approach compare in different analyzed samples?

- The number of all spectra associated with a specific protein
- The sum of all precursor intensities of peptides associated with a specific protein
- The precursor intensities of the 3 most intense detected peptides
- The count of observed peptides *versus* all possible peptides

4- What are some advantages of the label-based techniques?

- As the labeling occurs during sample preparation, quantitative artifacts are minimized
- The techniques are usually very cheap
- Multiplexing of samples is possible
- They can be performed at protein or peptide level

5- What does SILAC stand for?

- Selective *In-vivo* Labeling After Chemical reaction
- Nothing in particular
- Static Isoforms Labels for Affinity Capture
- Stable Isotope Labeling with Amino Acids in Cell Culture

6- At what MS level does SILAC quantification occur?

MS¹ MS² MS³ MSⁿ

7- At what MS level does TMT or iTRAQ quantification occur?

MS¹ MS² MS³ MSⁿ

8- How is it possible to compare the liver proteome of two mice?

Using *in-vivo* SILAC Using SILAC Using isobaric labeling Using a label-free approach

9- In general, how many generations of animals are needed at least to completely label all organs in a SILAC mouse?

1 2 10 5

10- What amino acids are generally available to perform a SILAC experiment?

Lysine and arginine Glycine and Leucine Lysine and proline Leucine and isoleucine

11- What is called a super-SILAC mix?

A mixture of samples labelled with TMT A mixture of cell lines labelled by SILAC A mixture of non-labeled healthy tissues

12- What multiplexing capabilities are available with TMT?

2-plex 4-plex 6-plex 10-plex

13- What amino acid(s) is/are labeled with TMT or iTRAQ?

Lysine Arginine N-terminus Cysteine

14- At what level is TMT or iTRAQ labeling generally performed?

Amino acid level Peptide level Protein level Cell culture level

15- In order to decipher subtle changes in phosphorylation regulation in two cell lines, what quantitative approach(es) would you recommend?

SILAC *In-vivo* SILAC Isobaric labeling Spectral counting

16- What mass spectrometer is commonly used for targeted protein quantification with stable isotope dilution?

FT-ICR

QqQ

Ion trap

MADLI-TOF

17- How are peptides selected for their use as heavy AQUA standards?

They need to be proteotypic

They need to contain more than 25 amino acids

They need to be heavily modified post-translationally

They need to fragment efficiently

18- What does PSAQ stand for?

Protein Standard
Absolute
Quantification

Protein for Stable
Accurate
Quantification

Peptide Standard for
Absolute
Quantification

None of these

19- How is abbreviated multiple selected reaction monitoring?

SRM

MRM

mSRM

MSM

20- What type of mass spectrometer is used for parallel reaction monitoring?

QqQ

FT-ICR

Orbitrap

Ion trap

21- What does PRM required for its development?

Selection of
transitions

Optimization of
collision energies

Selection of peptides
to be used as heavy
standards

An LC system