iTRAQ-based proteomics approaches

Introduced By :

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❑ Definition:

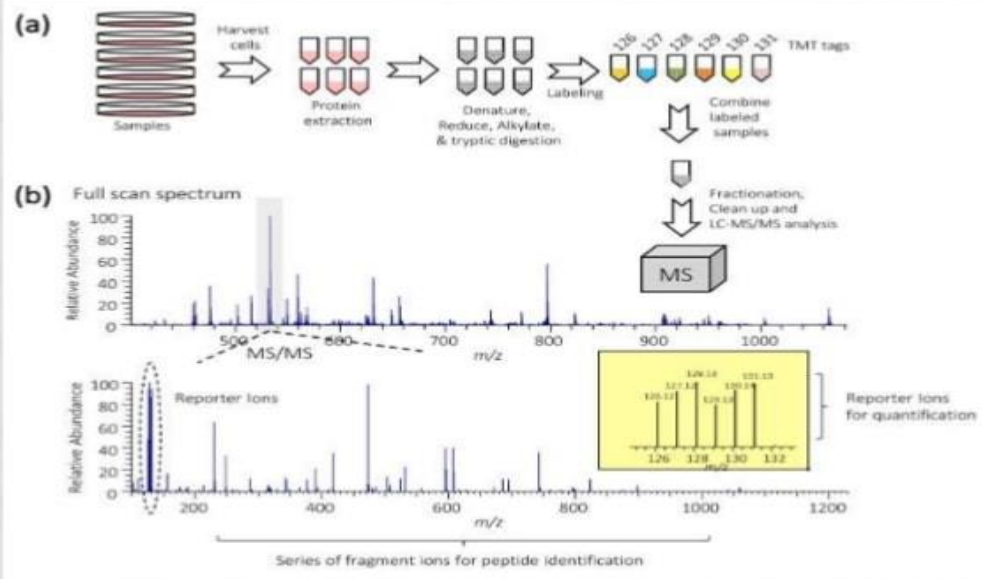
- **Isobaric tags for relative and absolute quantitation** (iTRAQ): is an isobaric labeling method used in quantitative proteomics by tandem mass spectrometry to determine the amount of proteins from different sources in **a single experiment** .

- It uses stable isotope labeled molecules that can be covalent bonded to the N-terminus and side chain amines of proteins.
- Use multiplexed isobaric mass tags to lable peptide digest mixtures.
- Available always are set of **4-plex and 8-plex** mass tages that can be used to lable and get quentitive information on up to 4 and 8 different samples in on experiment.

  - Current identification software is ProQuant software.
  - Tracker software has been developed to extract reporter ion peak ratios from non-centroided tandem MS peak lists.
  - Developed by Applided Biosystem in corporation in 2004.

❑ Objective of developing iTRAQ :

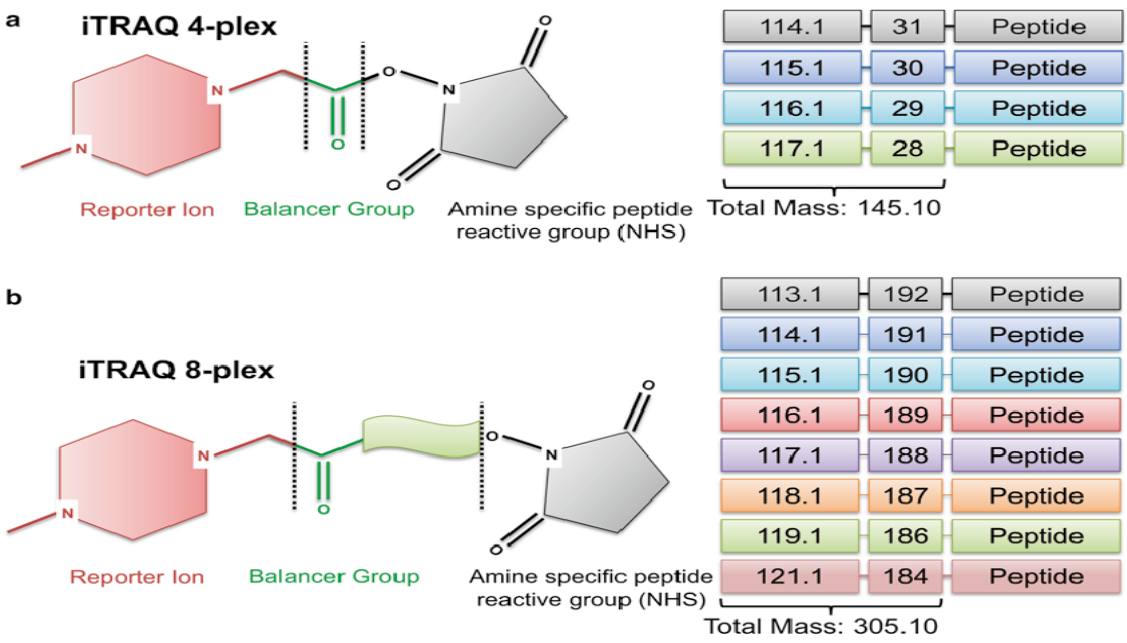
- Many differential effects on proteins themselves come from post- translational modifications.
- Protein expression cannot be measured or identified by looking at the mRNA levels.
- By studying effector molecules will contribute to better understanding of disease & in developing new treatment.



(Journal of proteome research, Rauniyar N *et al* (2014))

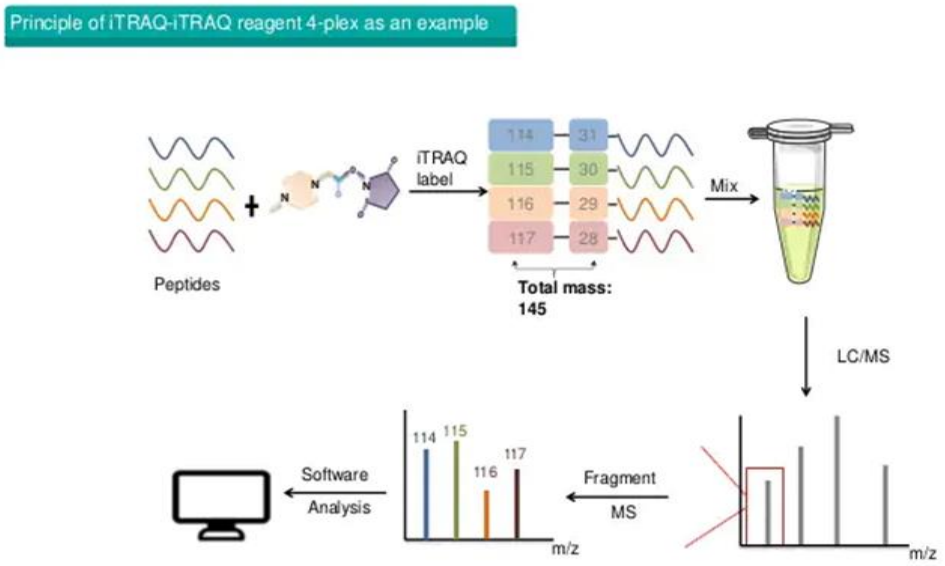
❑ The structure of iTRAQ reagents:

- unique charged reporter group.
- Peptide reactive group.
- Neutral balance group.
- The peptide reactive group links an iTRAQ with each lysine side chain and N-terminal group.



❑ The principle of iTRAQ -iTRAQ reagent 4-plex :

- Mixture of peptides is obtained by hydrolyzing the protein sample.
- All peptide in the sample are labeled with different iTRAQ reagents.
- All labeled protein samples are mixed.
- The peptides are subjected to mass spectrometry to obtain the mass spectrum.
- The same peptide from different sources is completely identical in molecular weight and they appear as the same peak samples,



This is the first stage of mass spectrometry.

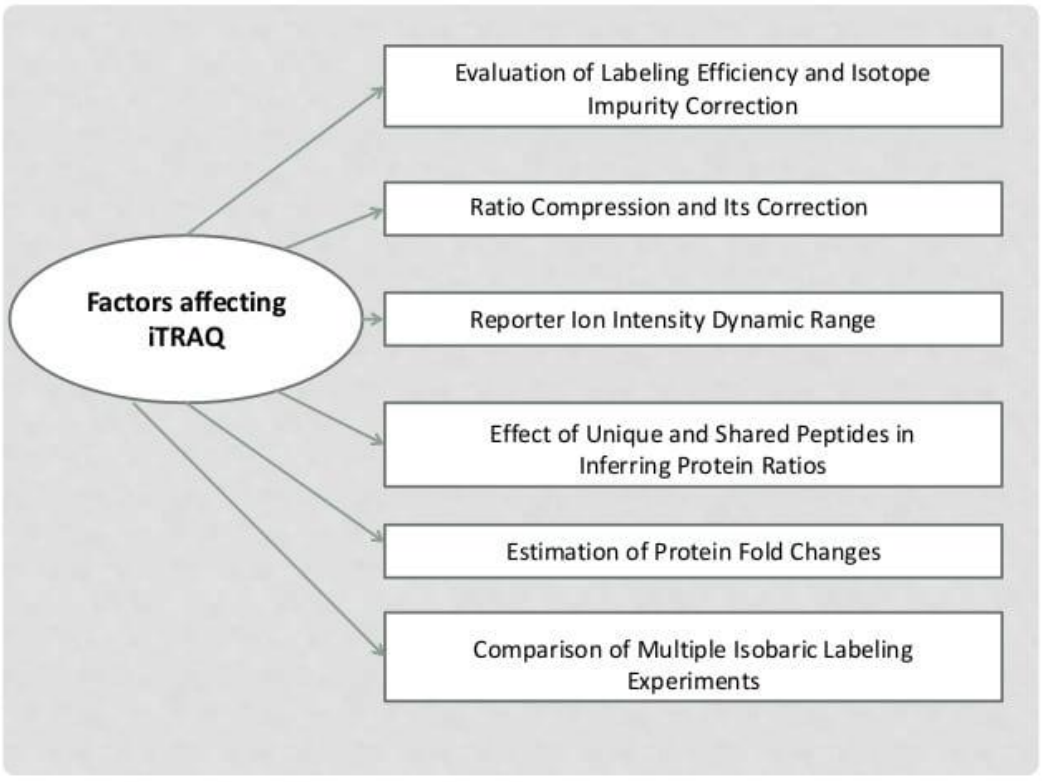
In the second stage of mass spectrometry ,

- The bond between the balance group and peptide reactive group is broken and balance group is lost .
- The same peptide with different isotopic labels produces different masses of reporter ions.
- Reporter ions exhibit different peaks quantitative information of the same peptides between different samples can be obtained by analyzing related data with software.
- Each sample in data bases in the general workflow is reduce system block.
- Each sample digested with trypsin.
- Each sample labeled with a different itraq tag in a single tube and then we can combine all labeled samples into one sample mixture for liquid chromatography tandem mass spectrometry analysis.

Workflow of iTRAQ

❑ Factors affecting results of iTRAQ :

- Evaluation of labeling efficiency and isotope impurity correction .
- Ratio compression and its correction.
- Reporter ion intensity dynamic range.
- Effect of unique and shared peptides in inferring protein ratios.
- Estimation of protein fold changes.



❑ Advantages of iTRAQ :

- Using mass spectrometry .
- Ability to analyze proteins from cell and tissues.
- Multiplexing ability.
- Reduce overall time and variation.
- Cutting-edge facilities & optimized protocols
- Untargeted approach for biomarker discovery

❑ Disadvantages of iTRAQ:

- Costly.
- Very sensitive to contamination form salts.
- A requirement of sophisticated software.
- Variability arising due to the inefficient enzymatic digestion.