Creative Proteomics

LABEL-BASED PROTEIN QUANTFICATION

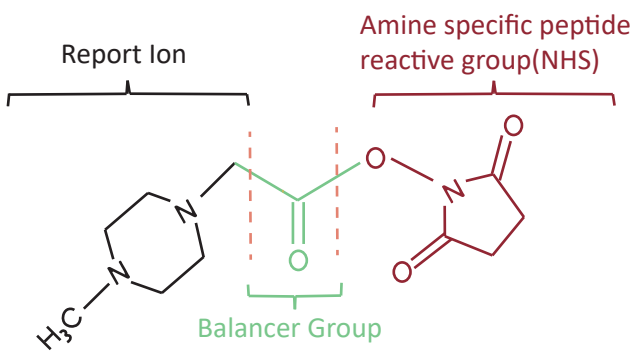
iTRAQ, TMT, SILAC

INTRODUCTION OF ITRAQ, TMT AND SILAC

iTRAQ

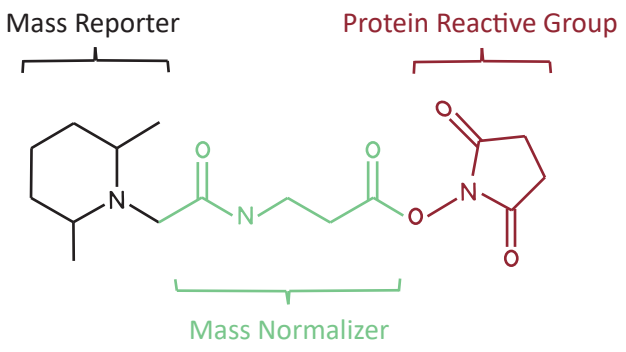
Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) technology utilizes isobaric reagents to label the primary amines of peptides and proteins.

The iTRAQ reagents usually consist of an N-methyl piperazine reporter group, a balance group, and an N-hydroxy succinimide ester group that is reactive with the primary amines of peptides. The balance groups present in each of the iTRAQ reagents function to make the labeled peptides from each sample isobaric and the quantification is facilitated through analysis of reporter groups that are generated upon fragmentation in the mass spectrometer.



There are currently two mainly used reagents: 4-plex and 8-plex, which can be used to label all peptides from different samples/treatments.

TMT



Tandem Mass Tags (TMT) can be used to identify and quantify proteins in different kinds of samples. Each TMT tagging reagent is composed of an amine-reactive NHS-ester group, a spacer arm and an MS/MS reporter. And the isobaric tagging reagent within a set has the same nominal parent (precursor) mass.

Eleven tags are available in TMT to label almost any peptide or protein samples. Using TMT, multiplex analysis, time efficiency, and control over technical variations can all be achieved.

SILAC

Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) technique performs protein labeling by adding light, medium, or heavy stable isotope-labeled essential amino acids (lysine and arginine) to cell culture media.

Through the normal metabolism of the cell, after 5-6 multiplication cycles, the stable isotope-labeled amino acids are completely incorporated into the newly synthesized proteins of the cell, so that the newly synthesized proteins are labeled with stable isotopes.

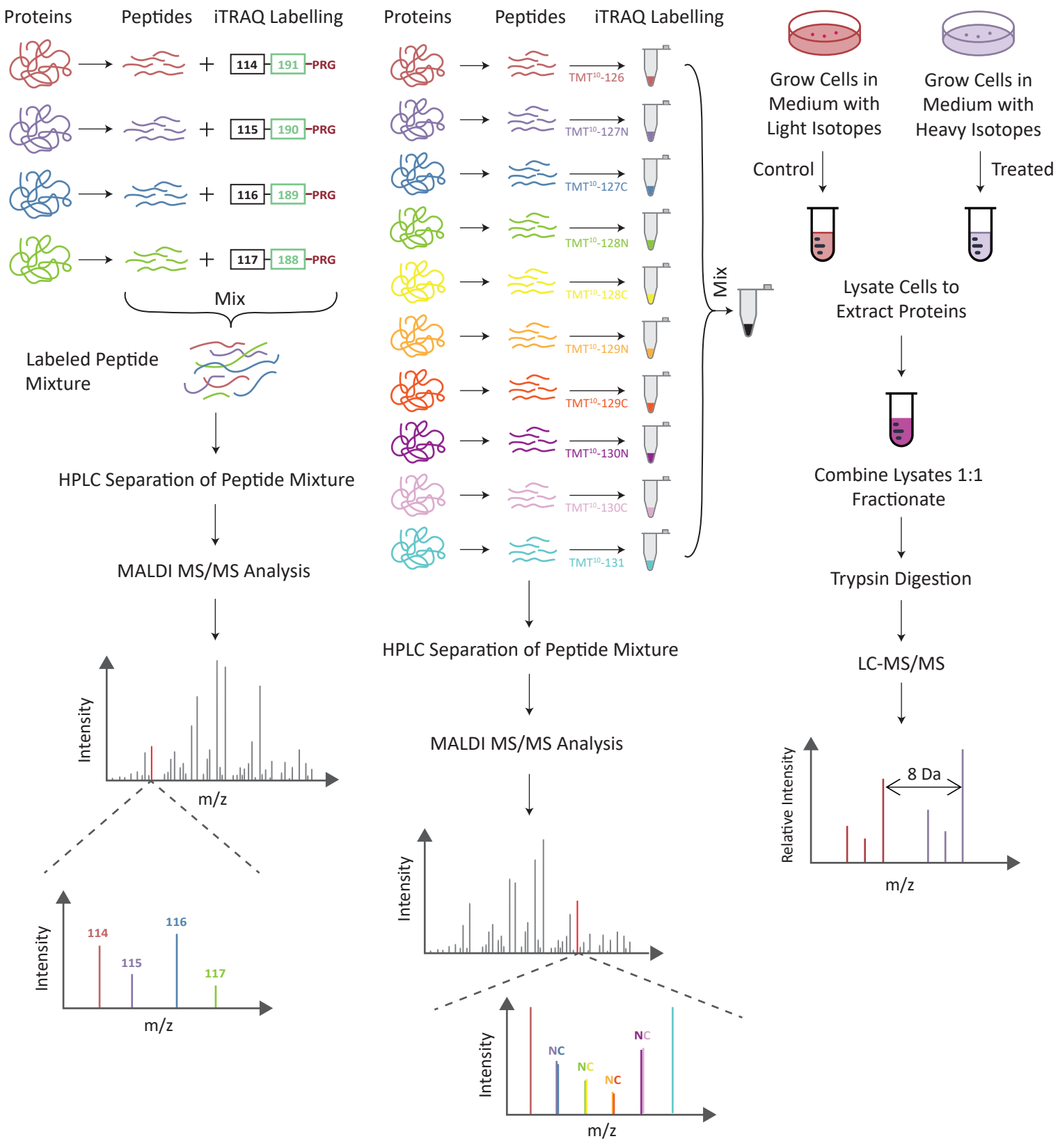
In this way, the area size of isotope peak type in mass spectrogram was compared for relative quantification, and the sequence of peptide was determined by secondary harmonic diagram for protein identification.

WORKFLOW OF ITRAQ, TMT AND SILAC

iTRAQ

TMT

SILAC



CHARACTERISTICS OF ITRAQ, TMT AND SILAC

Characteristics	iTRAQ	TMT	SILAC
Labeling Method	*In vitro*	*In vitro*	*In vivo*
Label Specifications	4plex/8plex	Duplex/6plex/10plex	-
Reporting group molecular mass	113-121; The minimum molecular mass difference between labeled reagents was 1 Da	126-131; The minimum molecular mass difference between labeled reagents was 6/1000 Da	-
Suitable mass spectrometer	TOF and Orbitrap mass spectrometry	The mass spectrometer has a resolution of at least 50,000 to distinguish different tags. Older versions of obtrap instruments can only distinguish 6plex	TOF and Orbitrap mass spectrometry
Advantages	High throughput High protein coverage Quantitative accuracy High credibility Data-rich	High throughput High protein coverage Quantitative accuracy High credibility Data-rich	A. Labeling efficiency can be up to 100% B. Good quantitative repeatability, low protein consumption C. Suitable for the identification and quantification of membrane proteins D. Closer to the real state of the sample
Disadvantages	A. Sample preparation and enzymatic processes may cause discrepancies between parallel samples, resulting in biased results. B. Occurrence of interference between co-screening and co-fragmentation of the precursor ion for complex samples.		It is mainly suitable for passageable cells or bacteria. Other experimental materials are not applicable. Long turnaround time

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