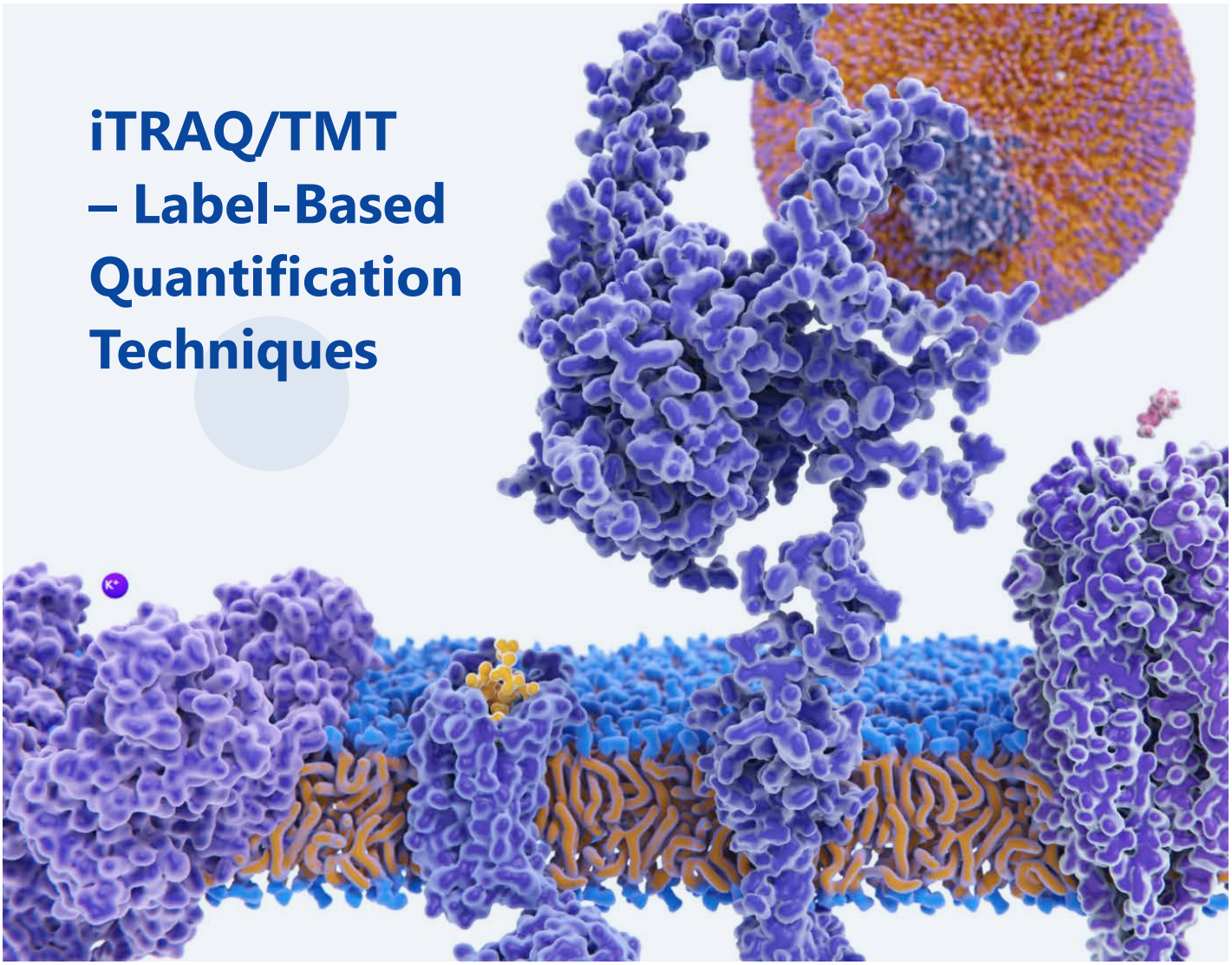
iTRAQ/TMT – Label-Based Quantification Techniques



Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) and Tandem Mass Tag (TMT) technologies are *in vitro* peptide labeling and quantification techniques developed by AB Sciex and Thermo Fisher Scientific, respectively. These techniques involved the use of isobaric labeling tags, which can be further linked to amino acid groups (including N-terminal amino acid residues and lysine side chains) through chemical reactions, thus enabling the simultaneous characterization and relative quantification of multiple proteomes by high-resolution mass spectrometry analysis.

The iTRAQ/TMT reagent consists of a reporter group, a balance group and a reactive group, which can form 2-18 different isobaric mass tags that label the N-terminal amino acid residues and lysine side chains of enzymatically cleaved peptides. During MS/MS analysis, each isotopic label is capable of generating a unique ion profile and can therefore be utilized for protein quantification. Labeled peptides are indistinguishable within the MS mode (MS1). However, due to bond breakage between the reporter group, balance group, and reactive within the MS/MS mode (MS2), the same peptide of different isotopic labels reveals different reporter ion intensities, hence suggesting different molecular masses. Nevertheless, proteins can be quantified by comparing the intensity of different reporter groups in MS2.

iTRAQ VS TMT

Reagents	Similarities	Differences		
		Specification	Specification Molecular mass of reporting group	Characteristic
iTRAQ	*In vitro* labeling reagents	2-8	113-121; Minimum molecular mass difference of 1 Da between labeling reagents	Suitable for Orbitrap mass spectrometry
TMT		2-18	126-135; Minimum molecular mass difference between labeling reagents 6/1000 Da	Mass spectrometer resolution must be higher than 50,000 for label distinguishing

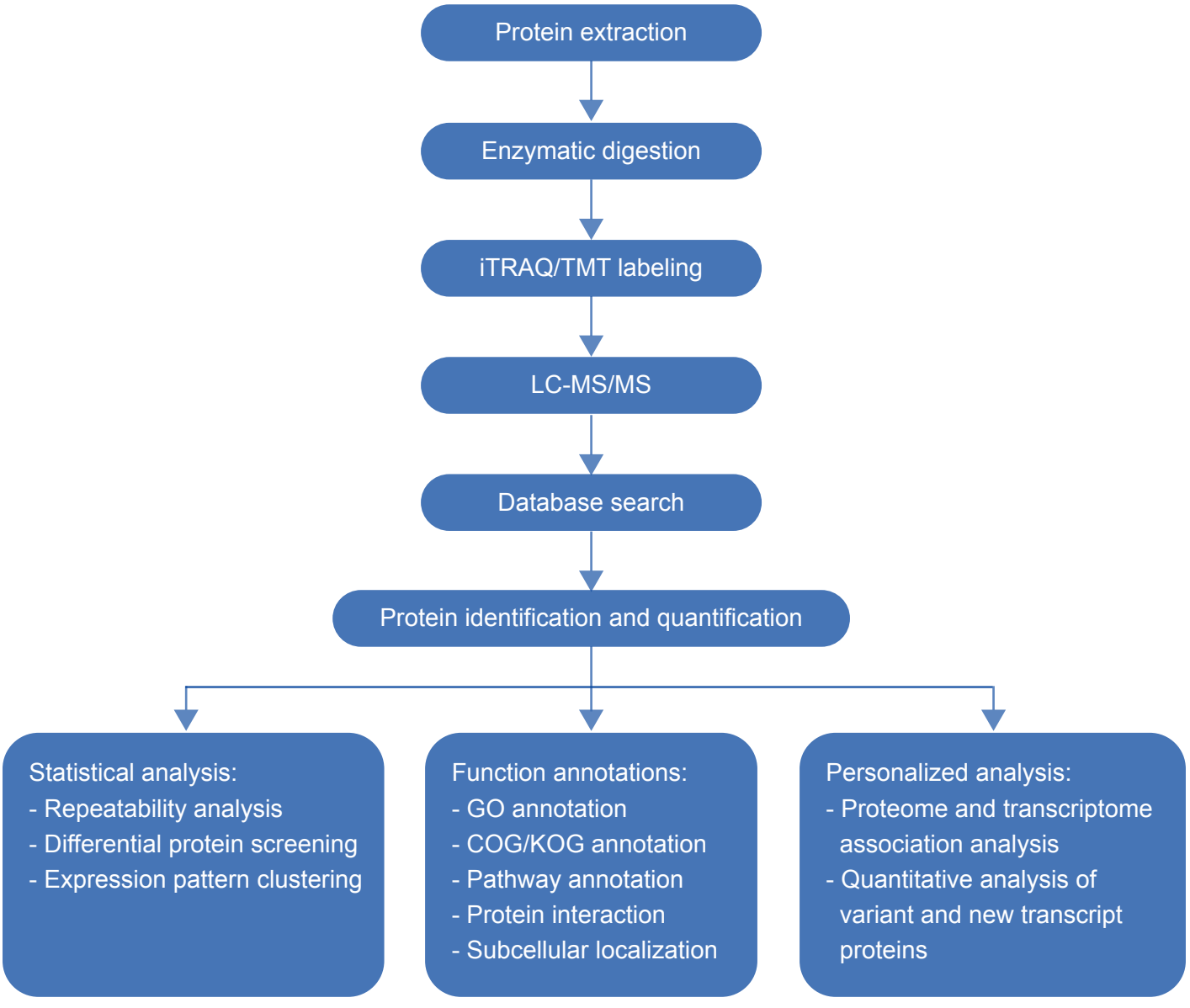


Fig 1. Workflow of iTRAQ/TMT mass spectrometry for quantitative proteomics

Technical Features

- Wide range of applications: *In vitro* labeling technique for protein analysis of most biological samples, including cells, tissues, plasma, serum, CSF, plant samples, etc.
- High sensitivity: Low detection limit for low abundance proteins
- High throughput: Qualitative and quantitative analysis of multiple samples simultaneously, suitable for differential protein analysis of samples with multiple treatments or from multiple processing times
- Reliable results: Simultaneous quantification and characterization, direct quantification according to the signal intensity of reporter groups
- High degree of automation: Liquid-phase and mass spectrometry are used in conjunction, supporting good separation performance and fast analytical efficiency
- High repeatability: Samples are mixed on the machine, eliminating systematic errors

Scope of Application

- Suitable for any sample types
- Label up to 18 samples simultaneously
- Require protein reference database, EST sequence (transcriptome) or genome annotation

Application Areas

Agriculture

- Resistance mechanisms
- Developmental mechanisms
- Plant breeding
- Plant protection

Animal Husbandry

- Meat and dairy products
- Disease treatment mechanism
- Drug development
- Drug toxicology

Medicine

- Marker screening
- Pathogenesis
- Drug targets
- Personalized therapy

Food Industry

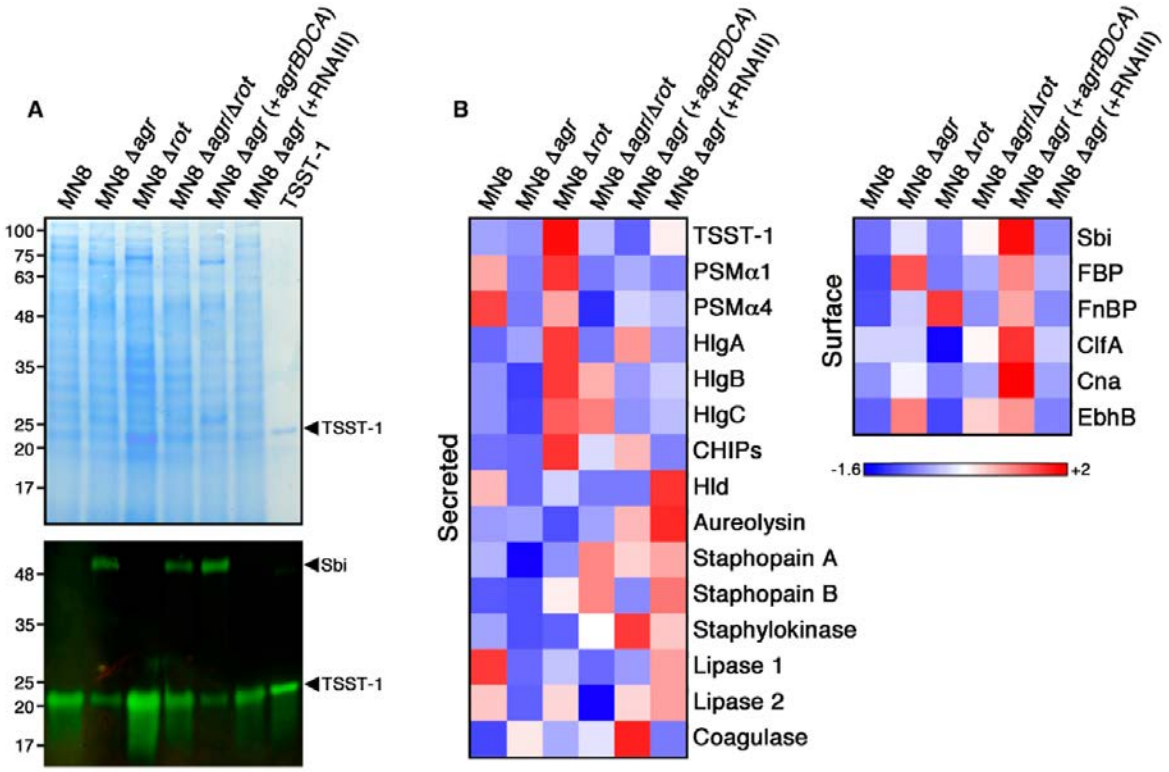
- Optimization of food storage and processing conditions
- Food component and quality identification
- Functional food development
- Food safety testing

Microorganisms

- Pathogenesis
- Drug resistance mechanisms
- Pathogen-host interactions

Comparative proteomic analysis of *Staphylococcus aureus*

To help further understand the specificity of the agr/rot axis beyond TSST-1, researchers performed the comparative proteomics analysis to gain insight into the frequency of this system used to regulate other secreted factors. In this study, proteins from the extracellular components of wild-type *S. aureus* MN8, Δagr, Δrot, and Δagr/Δrot null mutants, as well as Δagr mutants complemented with agrBDCA or RNAIII, were subjected to iTRAQ proteomics analysis to compare relative protein expression levels. Results revealed that in the absence of RNAIII, the agr system might function directly or indirectly as an early positive regulator of multiple surface-expressed proteins in *S. aureus* MN8.



Regulation of secreted and surface proteins by the agr/rot axis. A. 12% SDS-PAGE (top panel) and anti-TSST-1 Western blot analysis (bottom panel) of exoprotein profiles from the indicated strains at 4 h post-inoculation used for the iTRAQ analysis. B. Heat map of secreted (left panel) and surface (right panel) expressed *S. aureus* virulence factors.

Reference

Tuffs, S. W., Herfst, C. A., *et al*. (2019). Regulation of toxic shock syndrome toxin-1 by the accessory gene regulator in Staphylococcus aureus is mediated by the repressor of toxins. Molecular Microbiology, 112(4), 1163-1177.