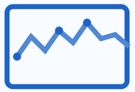
Quantitative Proteomics

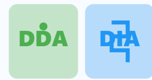
Comprehensive Guide to Modern Protein Quantification Methods



Label-Free Quantification

SILAC Labeling

TMT/iTRAQ Tags



Data Acquisition Strategies

Label-Free Quantification

- Spectral counting approaches
- Peak intensity measurement
- No chemical labeling required
- Cost-effective for large studies

SILAC Labeling

- Metabolic incorporation in vivo
- Heavy amino acids (^{13}C, ^{15}N)
- Cell culture applications
- High quantitative accuracy

TMT/iTRAQ Tags

- Isobaric mass tags
- Multiplexing 6-18 samples
- Reporter ion quantification

DIA vs DDA

- DIA: comprehensive ion fragmentation
- DDA: selective targeted approach
- Coverage vs reproducibility trade-offs

- Ideal for clinical samples

- Complementary methodologies

1 Label-Free Quantification (LFQ)

Label-Free Workflow

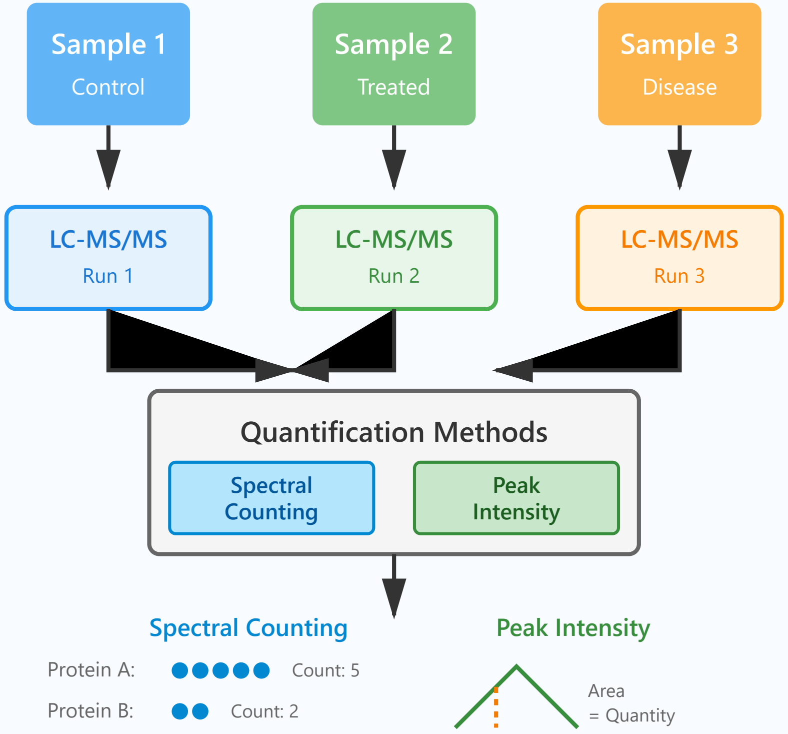
Overview

Label-free quantification determines protein abundance by analyzing MS signal intensities or spectral counts without introducing stable isotope labels. This approach offers flexibility and cost-effectiveness for large-scale proteomic studies.

Quantification Strategies

- ✓ **Spectral Counting:** Measures the number of MS/MS spectra identified for each protein. More abundant proteins generate more spectra.
- ✓ **Peak Intensity:** Quantifies the integrated area under chromatographic peaks (extracted ion chromatograms, XICs) for peptide ions.
- ✓ **iBAQ (intensity-Based Absolute Quantification):** Divides protein intensity by the number of theoretically observable

peptides.



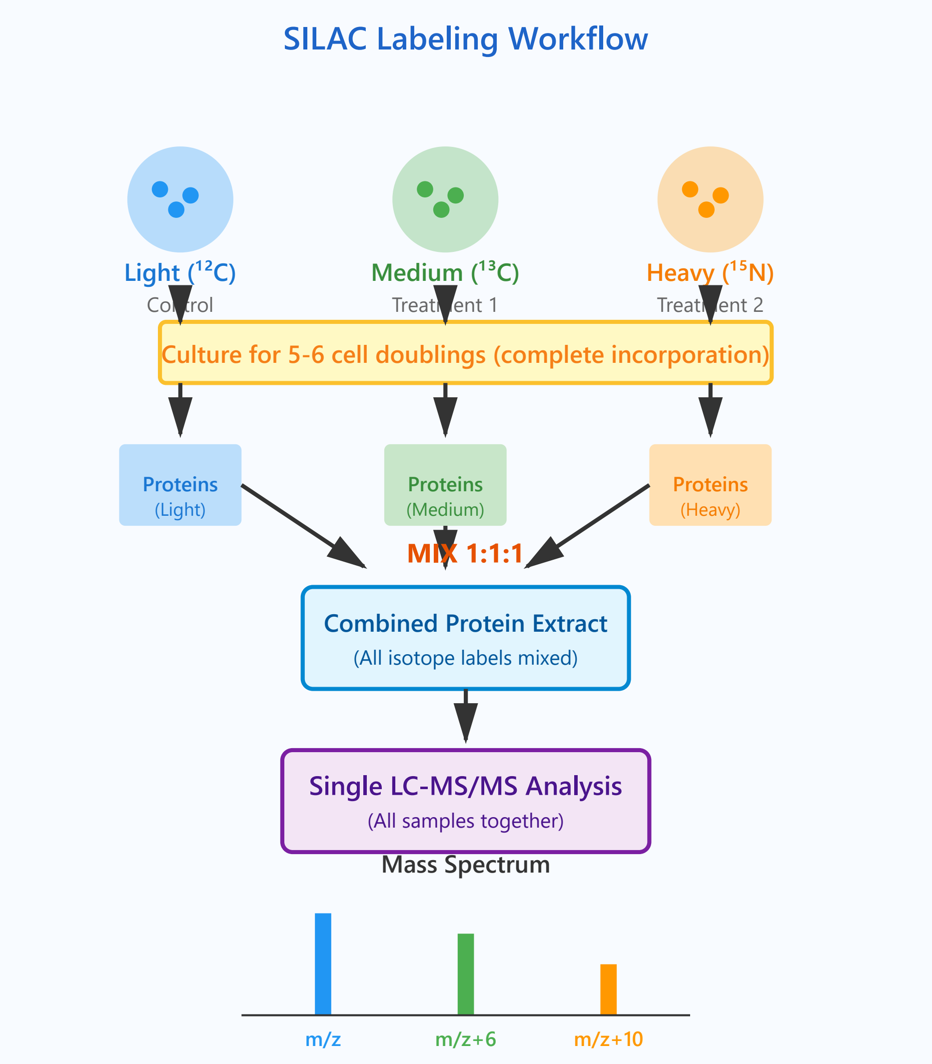
Advantages

- ✓ No limit on number of samples compared
- ✓ Cost-effective (no expensive reagents)
- ✓ Suitable for any sample type
- ✓ Large dynamic range achievable

Limitations

- ✓ Requires highly reproducible LC-MS conditions
- ✓ Run-to-run variation affects accuracy
- ✓ More complex data analysis and normalization
- ✓ Lower precision compared to labeled methods

2 SILAC (Stable Isotope Labeling by Amino acids in Cell culture)



Metabolic Labeling Principle

SILAC incorporates heavy isotope-labeled amino acids (typically lysine and arginine with ^{13}C or ^{15}N) into cellular proteins during cell growth. This creates distinct mass differences between samples that can be distinguished by mass spectrometry.

Workflow Steps

1. Culture cells in media containing light (^{12}C), medium (^{13}C), or heavy (^{13}C + ^{15}N) amino acids
2. Allow 5-6 cell doublings for complete isotope incorporation (>95%)
3. Apply different treatments to each SILAC population
4. Mix cell lysates at 1:1:1 ratio (or other defined ratios)
5. Perform single LC-MS/MS analysis on combined sample

6. Quantify by comparing peak intensities of isotopic peptide pairs

Advantages

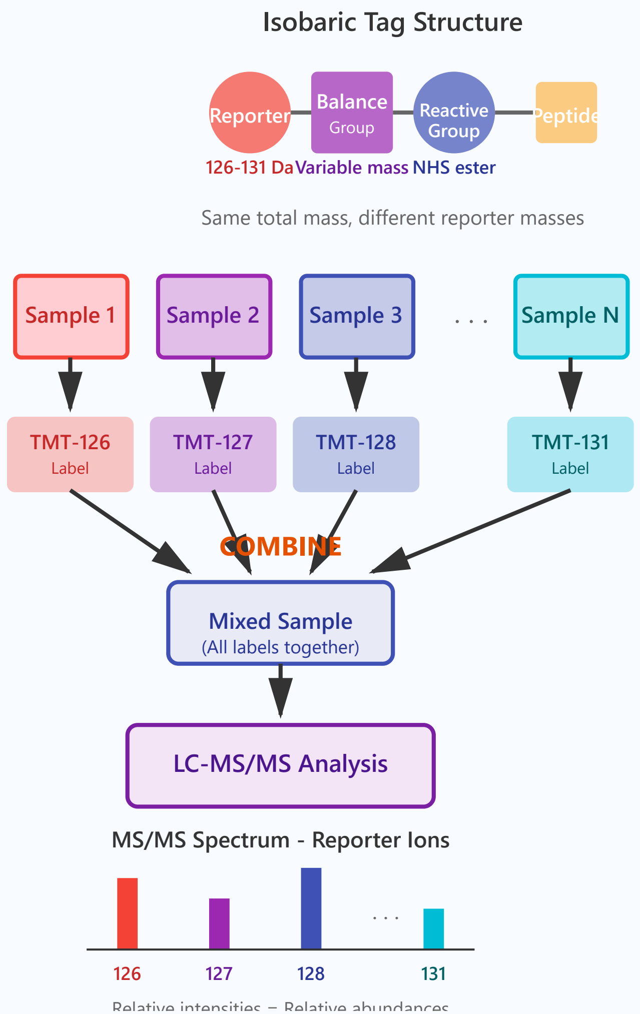
- ✓ High quantitative accuracy and precision
- ✓ Early sample mixing eliminates handling errors
- ✓ Multiplexing up to 3 conditions (light/medium/heavy)
- ✓ Direct comparison in single MS run

Limitations

- ✓ Limited to cell culture systems (not for tissues)
- ✓ Requires extended culture time (expensive)
- ✓ Maximum of 3-5 samples compared
- ✓ Arginine-to-proline conversion in some cell types

3 TMT/iTRAQ Isobaric Labeling

Isobaric Tag Structure & Workflow



Isobaric Labeling Principle

TMT (Tandem Mass Tags) and iTRAQ (Isobaric Tags for Relative and Absolute Quantification) use chemical labels with the same total mass but different reporter ion masses. This allows multiplexing of 6-18 samples in a single LC-MS/MS run.

Tag Chemistry

- ✓ **Reporter Group:** Low-mass fragment (126-131 Da for TMT) released upon MS/MS fragmentation. Different isotope compositions create distinct masses.
- ✓ **Balance Group:** Complementary mass that ensures all tags have identical total mass in MS1 spectra.
- ✓ **Reactive Group:** NHS-ester chemistry that reacts with primary amines (N-terminus and lysine side chains).

Multiplexing Capacity

- ✓ iTRAQ: 4-plex or 8-plex
- ✓ TMT: 6-plex, 10-plex, 11-plex, 16-plex, or 18-plex
- ✓ TMTpro: Latest generation with improved quantification accuracy

Advantages

- ✓ High multiplexing capacity (up to 18 samples)
- ✓ Applicable to any sample type (cells, tissues, fluids)
- ✓ Early sample pooling reduces variability
- ✓ Efficient use of MS instrument time

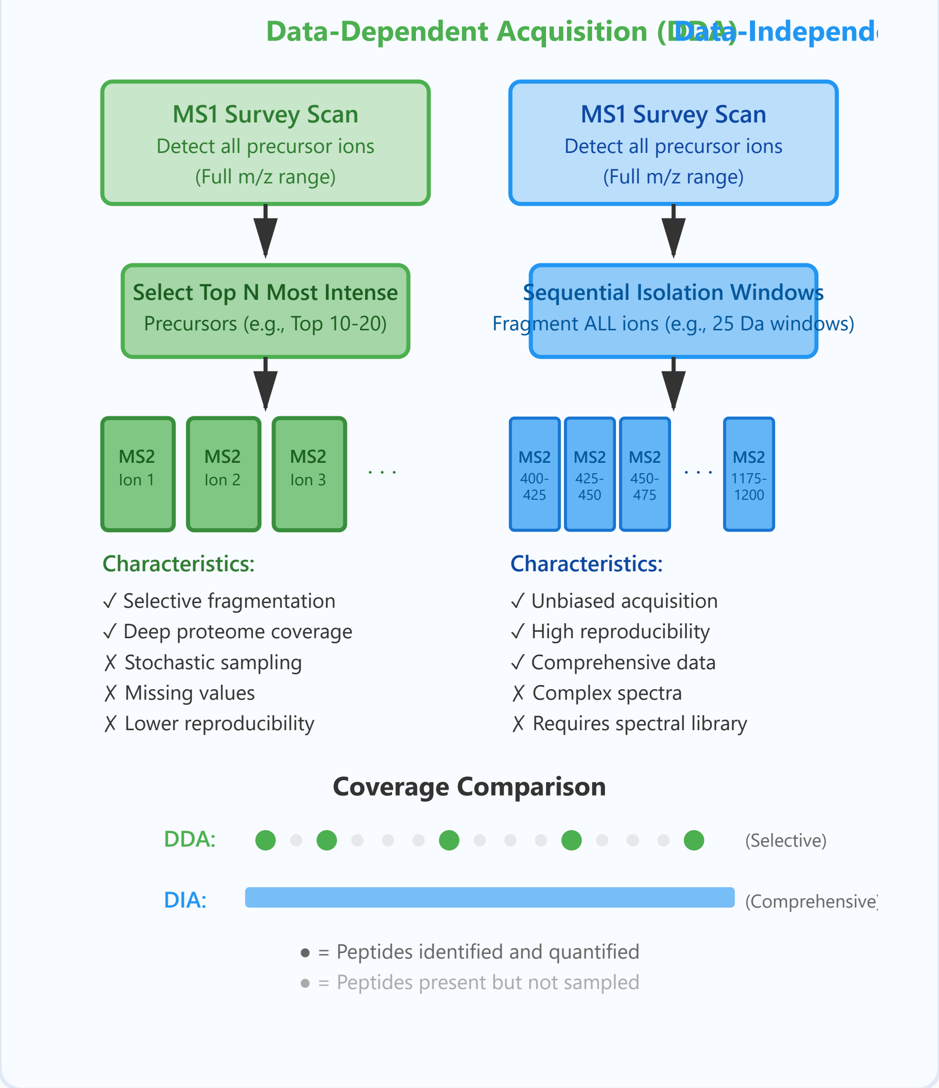
Limitations

- ✓ Ratio compression due to co-isolated ions
- ✓ Expensive reagents for large studies
- ✓ Requires MS3 or SPS-MS3 for accurate quantification
- ✓ Lower sensitivity compared to label-free methods

4 Data Acquisition Strategies: DDA vs DIA

DDA vs DIA Comparison

DDA (Data-Dependent Acquisition)



DDA performs an MS1 survey scan, then selects the most intense precursor ions for targeted MS/MS fragmentation. This "traditional" approach has been the standard for discovery proteomics for two decades.

DDA Workflow

1. MS1 survey scan: measure all precursor ions (full m/z range)
2. Rank precursors by intensity and select top N (typically 10-20)
3. Fragment selected precursors individually (MS/MS)
4. Exclude fragmented ions (dynamic exclusion) and repeat

DIA (Data-Independent Acquisition)

DIA systematically fragments all detectable precursor ions by cycling through predefined m/z isolation windows. This ensures comprehensive and reproducible data collection, often called SWATH-MS (Sequential Window Acquisition of all THeoretical spectra).

DIA Workflow

1. MS1 survey scan: measure all precursor ions
2. Divide m/z range into windows (e.g., 25 Da wide)
3. Fragment ALL ions in each window sequentially
4. Deconvolute complex spectra using spectral libraries

When to Use DDA

- ✓ Discovery proteomics and deep coverage needed
- ✓ Exploratory studies without prior knowledge
- ✓ Building spectral libraries for DIA
- ✓ Post-translational modification studies

When to Use DIA

- ✓ Targeted quantification of known proteins
- ✓ Large cohort studies requiring reproducibility
- ✓ Biomarker validation studies

✓ Retrospective data mining (reanalyze old data)