Post-Translational Modification (PTM) Analysis

Phosphorylation Sites

- Ser/Thr/Tyr phosphorylation
- Enrichment with TiO_2/IMAC
- Site localization algorithms

Glycosylation Patterns

- N-linked and O-linked glycans
- Heterogeneous modifications
- Deglycosylation strategies

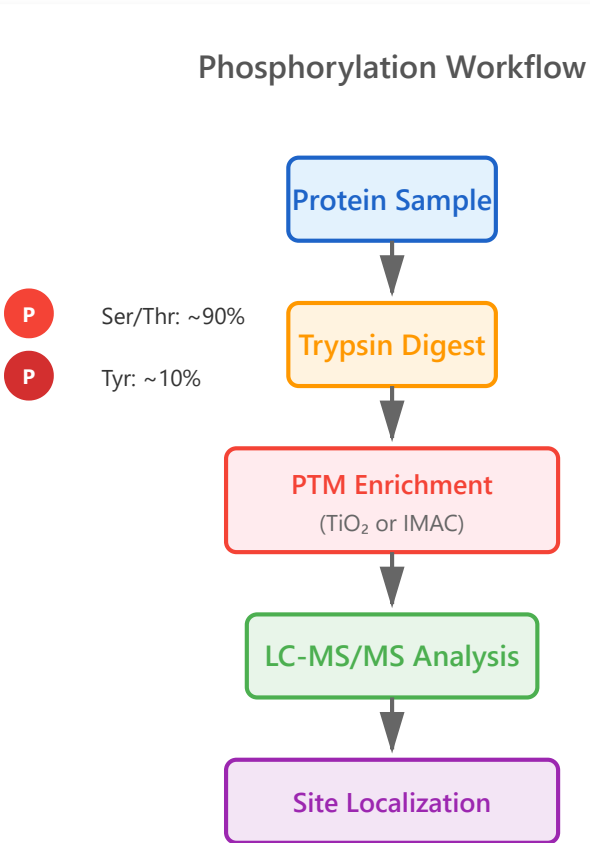
Acetylation/Methylation

- Lysine modifications
- Histone PTMs
- Epigenetic regulation

Enrichment Methods

- Immunoprecipitation
- Affinity chromatography
- Chemical derivatization

P Phosphorylation Analysis



Overview

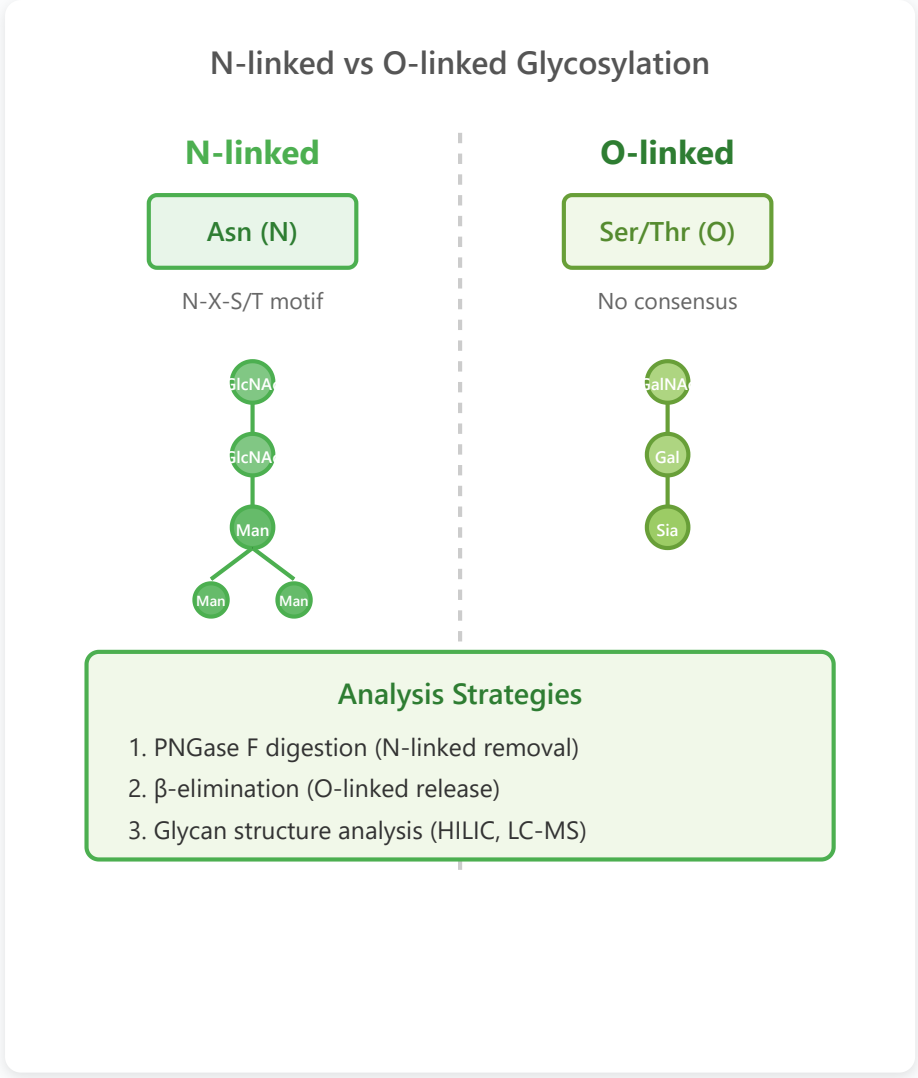
Phosphorylation is one of the most abundant and well-studied PTMs, playing crucial roles in cell signaling, protein regulation, and cellular processes. The addition of phosphate groups (PO_4^{3-}) primarily occurs on serine, threonine, and tyrosine residues.

Key Technical Points

- **Target Residues:** Serine (~86%), Threonine (~12%), Tyrosine (~2%)
- **Mass Shift:** +79.966 Da (HPO_3) or +97.977 Da (H_3PO_4)
- **Enrichment:** TiO_2 beads or IMAC (Fe^{3+}/Ga^{3+}) columns
- **Challenges:** Low stoichiometry, neutral loss during fragmentation
- **Localization:** Ascore, ptmRS, or MaxQuant site probability

Biological Significance: Regulates enzyme activity, protein-protein interactions, subcellular localization, and signal transduction cascades. Dysregulation is implicated in cancer, diabetes, and neurodegenerative diseases.

G Glycosylation Patterns



Overview

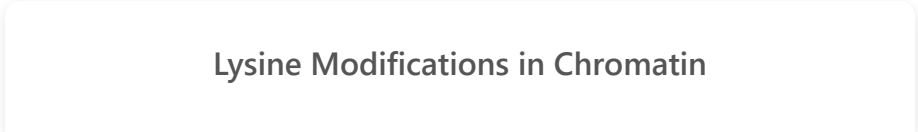
Glycosylation involves the attachment of oligosaccharide chains to proteins, creating significant structural and functional diversity. It's one of the most complex PTMs due to heterogeneity in glycan composition and branching patterns.

Key Technical Points

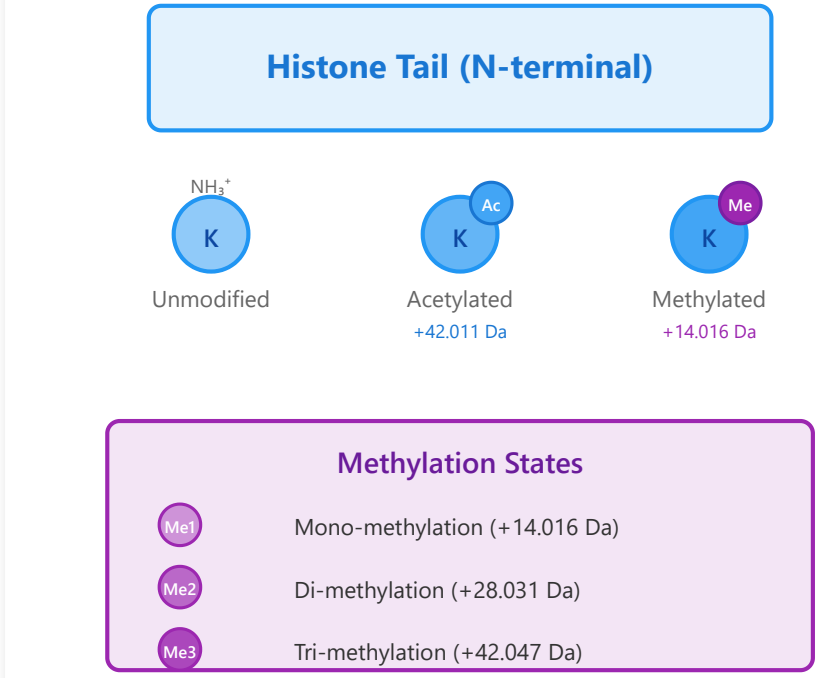
- **N-glycosylation:** Occurs at Asn in N-X-S/T sequons (X ≠ Pro)
- **O-glycosylation:** Occurs at Ser/Thr, no strict consensus sequence
- **Core Structures:** N: $GlcNAc_2Man_3$; O: GalNAc-Ser/Thr
- **Deglycosylation:** PNGase F for N-linked, chemical β-elimination for O-linked
- **Detection:** Mass shift analysis, glycopeptide enrichment (HILIC, lectin)

Biological Significance: Critical for protein folding, stability, cell-cell recognition, immune response, and cell signaling. Aberrant glycosylation is a hallmark of cancer and inflammatory diseases.

A/M Acetylation & Methylation



Overview



Lysine acetylation and methylation are reversible PTMs that play fundamental roles in epigenetic regulation, particularly in chromatin structure and gene transcription. These modifications neutralize positive charges and alter protein-DNA interactions.

Key Technical Points

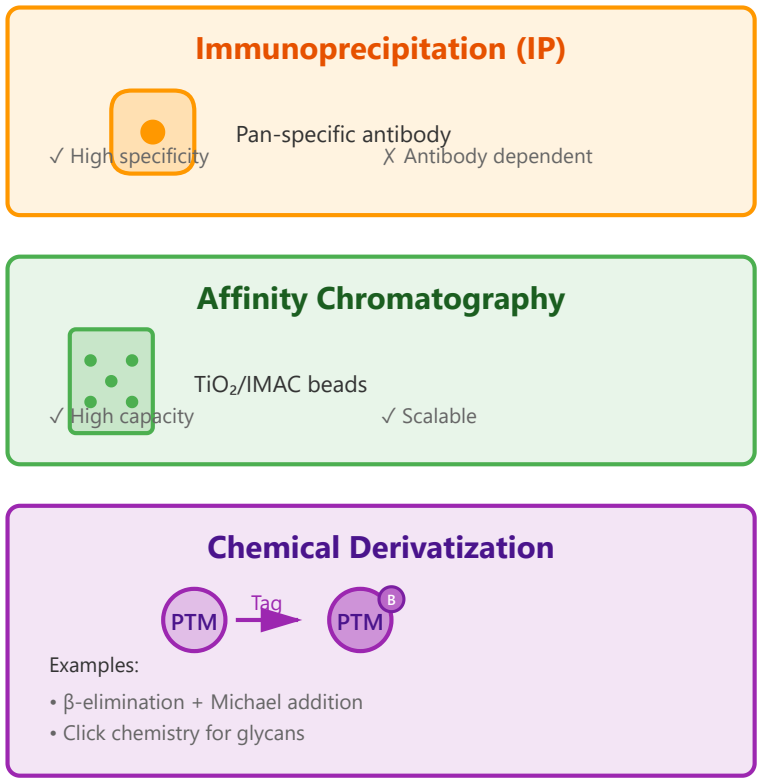
- **Acetylation:** Adds acetyl group ($COCH_3$), mass +42.011 Da
- **Methylation:** Adds methyl groups (CH_3), +14.016 Da per methyl
- **Target Sites:** Primarily lysine, also arginine for methylation
- **Enrichment:** Pan-acetyl-lysine or pan-methyl-lysine antibodies
- **Histone Code:** H3K4me3, H3K9ac, H3K27me3, H4K16ac, etc.
- **Writers/Erasers:** HATs/HDACs for acetylation, KMTs/KDMs for methylation

Biological Significance: Acetylation generally activates transcription by opening chromatin, while methylation effects depend on specific sites (activation or repression). Critical in cancer, aging, and metabolic diseases.

E PTM Enrichment Methods

Enrichment Strategy Comparison

Overview



PTM enrichment is critical because modified peptides are typically present at low stoichiometry (often <1% of total protein). Effective enrichment strategies can increase PTM detection by 10-1000 fold, enabling comprehensive PTM characterization.

Key Technical Points

- **Immunoprecipitation:** Uses antibodies against specific PTMs (e.g., pan-acetyl-K, pTyr)
- **Affinity Chromatography:** TiO_2/IMAC for phosphopeptides, lectin for glycopeptides
- **Chemical Methods:** β-elimination for O-glycans, biotin tagging for click chemistry
- **Enrichment Factor:** Typically 10-100× for IP, up to 1000× for IMAC/TiO_2
- **Considerations:** Sample loss, bias toward abundant proteins, batch effects

Strategy Selection: Choose based on PTM type, sample amount, desired specificity, and downstream analysis. Often combine multiple methods for comprehensive coverage (e.g., IMAC + TiO_2 for phosphoproteomics).