PROTEIN POST-TRANSLATIONAL MODIFICATION

INTRODUCTION OF POST-TRANSLATIONAL MODIFICATIONS

Living systems are inundated with diverse intracellular and environmental signals. Therefore, cells must be able to rapidly respond to programmed and unprogrammed cues.

Post-translational modification (PTM) refers to the covalent, usually enzymatic modification of proteins, and protein process during or after protein biosynthesis. PTM provides an energy-saving mechanism that can reversibly regulate protein functions in a rapid time frame. Protein PTM increases the functional diversity of the proteome by modifying proteins with functional groups such as phosphate, acetate, amide or methyl, and affects almost all aspects of normal cell biology and pathogenesis. It plays a key role in many cellular processes, such as cell differentiation, protein degradation, signal transduction and regulation processes, gene expression regulation, and protein-protein interactions.

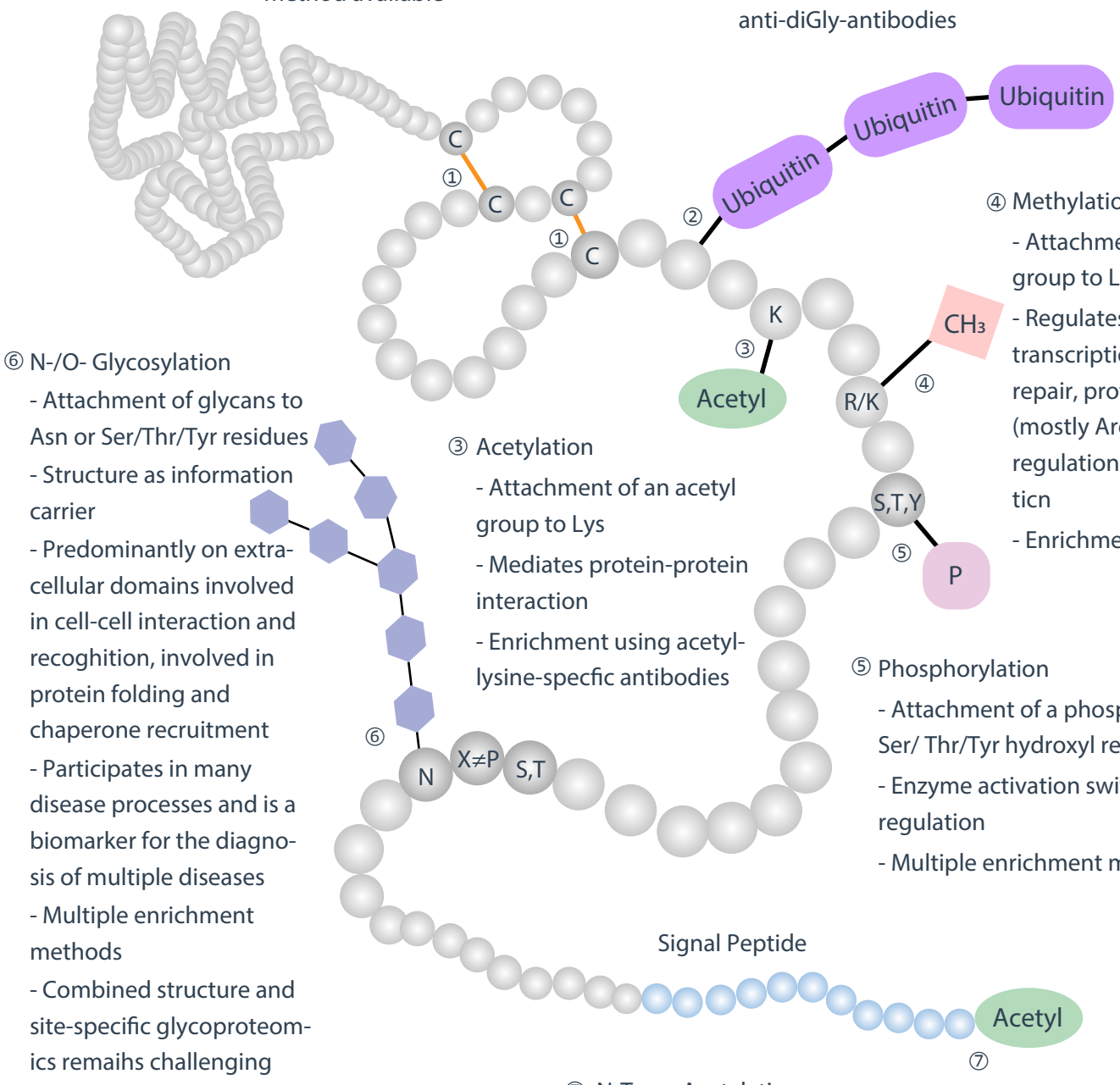
Common types of protein post-translational modifications

① Disulfde Bonds

- Linkage between two Cys residues, mostly intramolecular
- Stabilizes protein structure
- Currently no large-scale enrichment method available

② Ubiquitination (Ubq)

- Ubiquitin, a 8.5 kDa protein, is attached to Lys via an isopeptide bond
- Mediates protein degradation, signaling, trafficking
- Upon tryptic digestion, a Gly-Gly residue remains at the Lys and modified peptides can be enriched using anti-diGly-antibodies



⑥ N-/O- Glycosylation

- Attachment of glycans to Asn or Ser/Thr/Tyr residues
- Structure as information carrier
- Predominantly on extracellular domains involved in cell-cell interaction and recoghition, involved in protein folding and chaperone recruitment
- Participates in many disease processes and is a biomarker for the diagnosis of multiple diseases
- Multiple enrichment methods
- Combined structure and site-specific glycoproteomics remaihs challenging

③ Acetylation

- Attachment of an acetyl group to Lys
- Mediates protein-protein interaction
- Enrichment using acetyl-lysine-specfic antibodies

④ Methylation

- Attachment of a methyl group to Lys/Arg
- Regulates RNA processing, transcription, DNA damage repair, protein translocation (mostly Arg), and epigenetic regulation of gene transcripticn
- Enrichment using antibodies

⑤ Phosphorylation

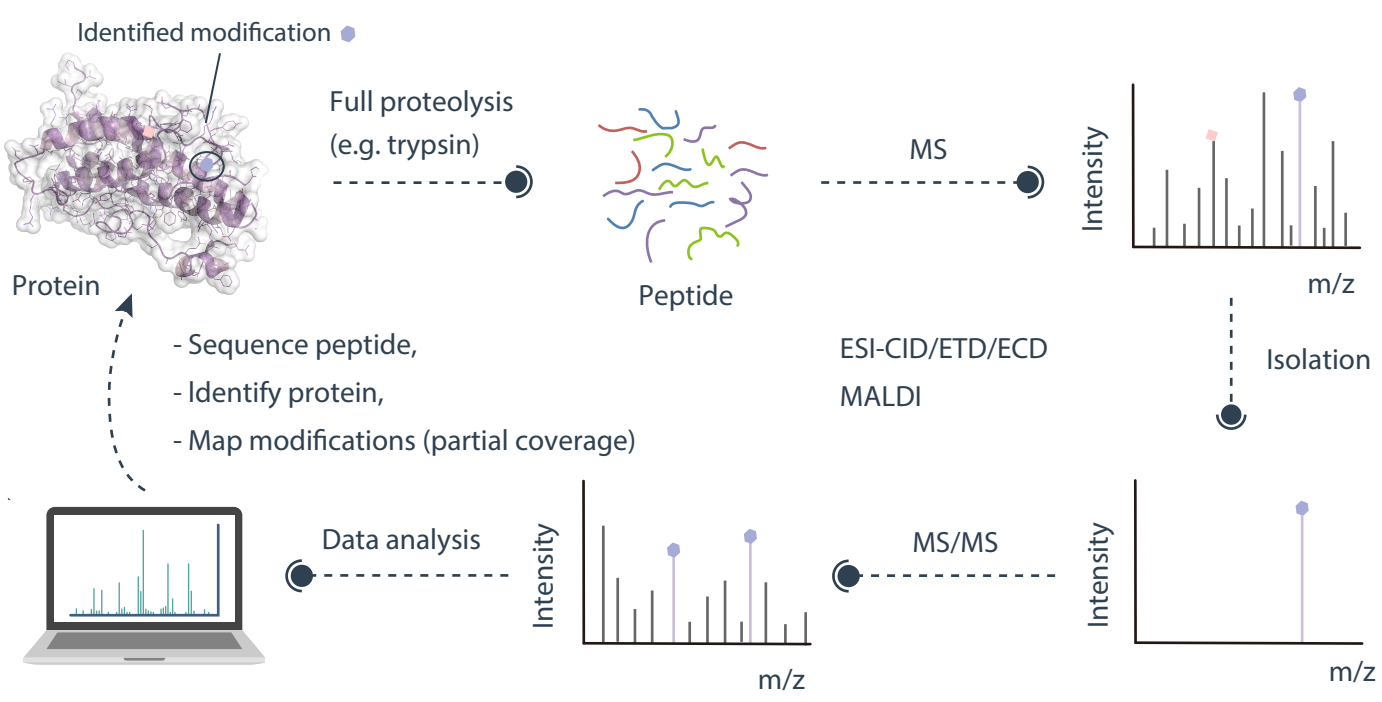
- Attachment of a phosphate group to Ser/ Thr/Tyr hydroxyl residues
- Enzyme activation switch, activity regulation
- Multiple enrichment methods

⑦ N-Term-Acetylation

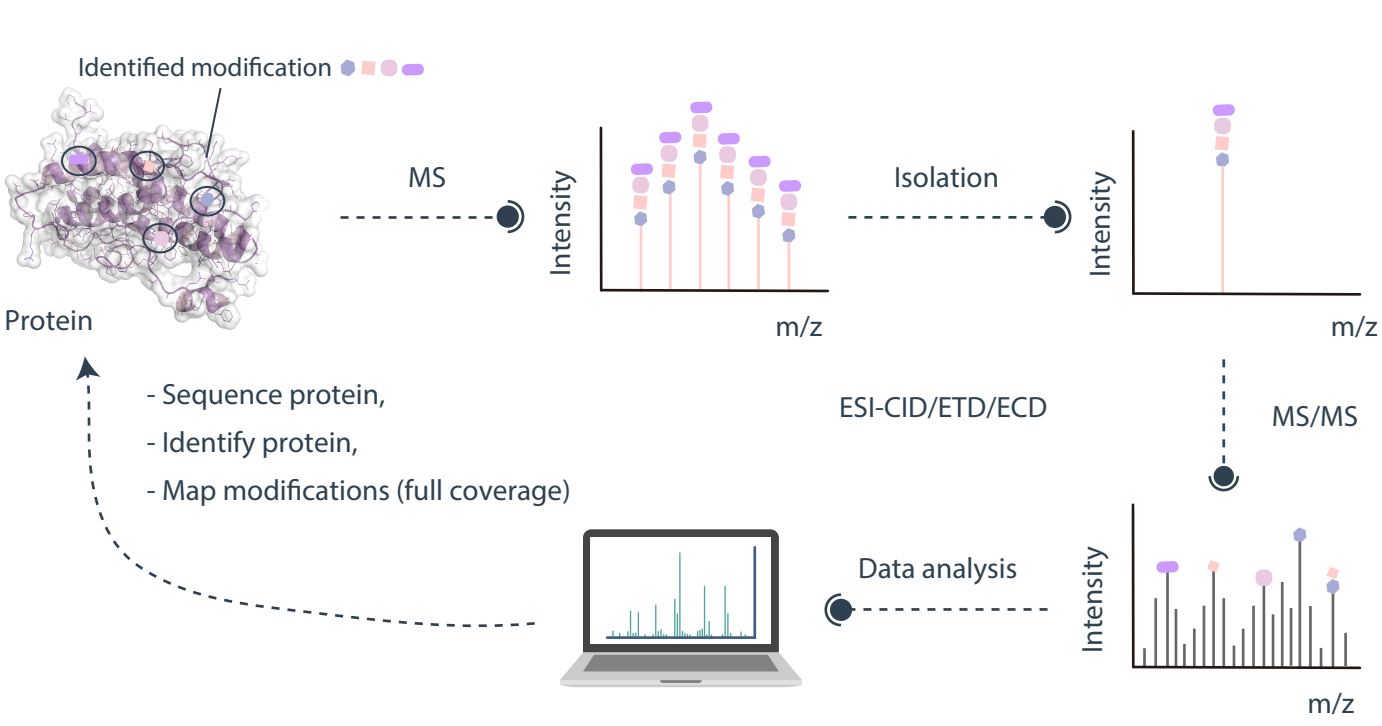
- Acetylation on mature protein N-termini, often after removal of initial Met
- Linked to protein stability, localization and interaction
- Enrichment using SCX, COFRADIC, TAILS, ChaFRADIC

STRATEGIES TO ANALYZE PROTEIN PTM

Bottom-up MS approach



Top-down MS approach



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