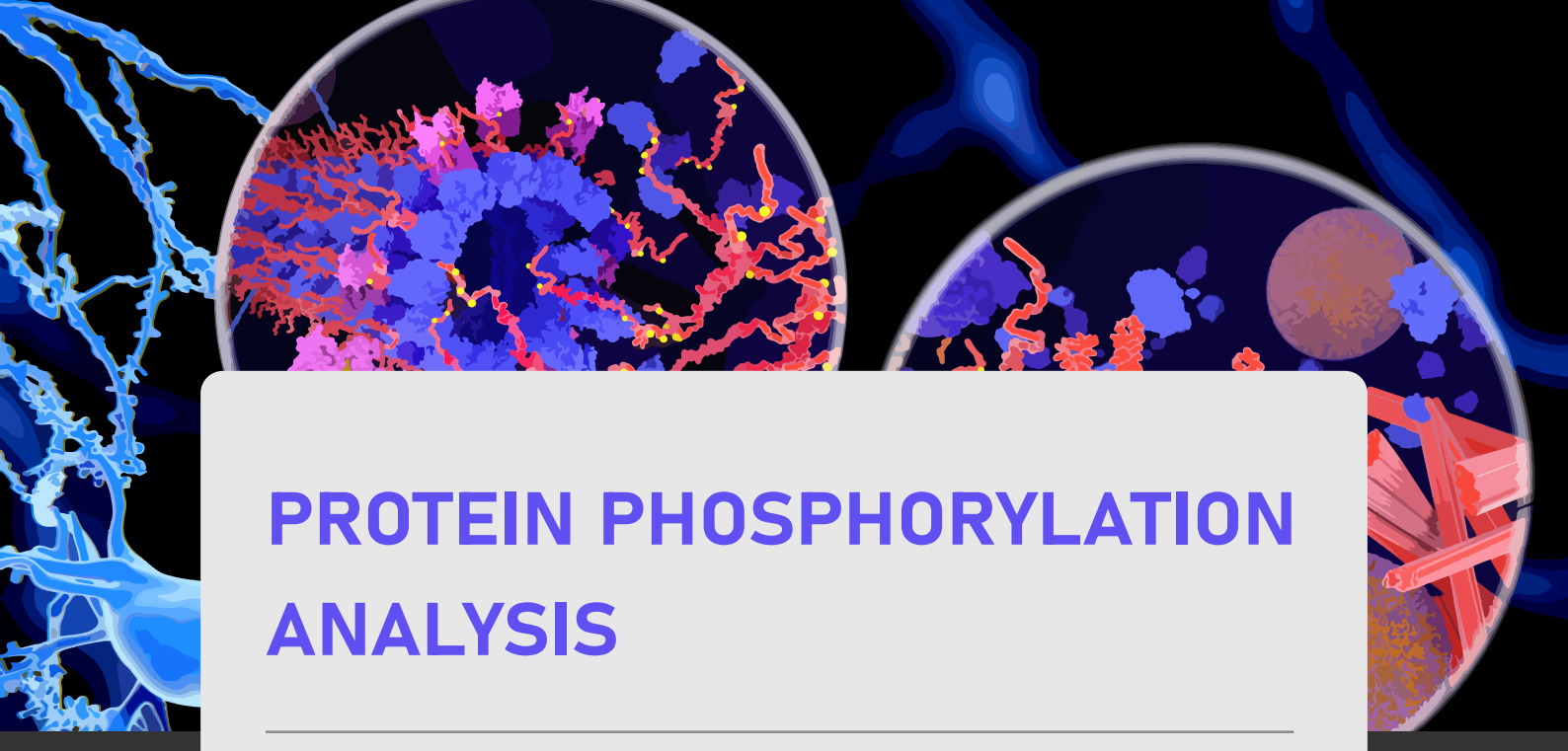


PROTEIN PHOSPHORYLATION ANALYSIS

Phosphorylated Peptide Enrichment

Mass Spectrometry-based Phosphoproteomics

In organisms, phosphorylation is the most common but important form of covalent modification for protein post-translational modifications that regulate both prokaryotes and eukaryotes. Phosphorylation plays an important regulatory role in the proper functioning of proteins. In order to catalyze protein phosphorylation, protein kinase transfers a phosphate group from the γ-position of ATP or GTP to specific amino acid residues of the substrate protein, such as Serine, Threonine, and Tyrosine. In contrast, its reverse process involves the removal of the corresponding phosphate group by protein phosphatases. The conflicting functions of these two enzymes, as well as the engagement of energy expenditure and generation, make phosphorylation the favored regulation for many physiological activities in the body. Furthermore, over one-third of proteins in eukaryotic organisms can undergo phosphorylation modifications. Therefore, phosphorylation plays a critical role in a variety of biological processes, including normal physiology, immune response, disease development, and plant response to stress and hormone expression.

Research Objectives

Mass spectrometry is a critical research tool for the study of phosphoproteomics in identifying protein phosphorylation sites and quantifying phosphorylation. Thus, mass spectrometry-based phosphoproteomics analysis includes:

(i) Identifying the presence of phosphorylated peptides

(ii) Determining the type of phosphorylation based on amino acid sequences

(iii) Identifying the site of phosphorylation

(iv) Determining the phosphorylation sites among samples under different experimental conditions

Phosphorylation Enrichment

Mass spectrometry-based phosphoproteomics was advanced by its high resolution and comprehensiveness. However, due to the low content and wide dynamic range of phosphorylation-modified proteins in biological samples, enrichment of phosphorylated peptides is required prior to detection analysis. Current enrichment strategies include TiO_2 enrichment, anti-phosphotyrosine antibodies, immobilized metal affinity chromatography (IMAC), chemical modifications, and strong cation exchange chromatography (SCX).

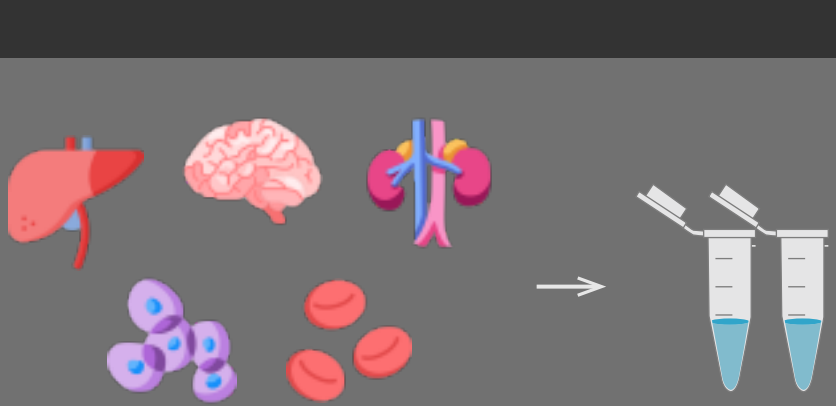
Detection Methods

Once the enrichment of the phosphorylated peptide is completed, it is ready for mass spectrometry analysis. Various detection methods are available, including:

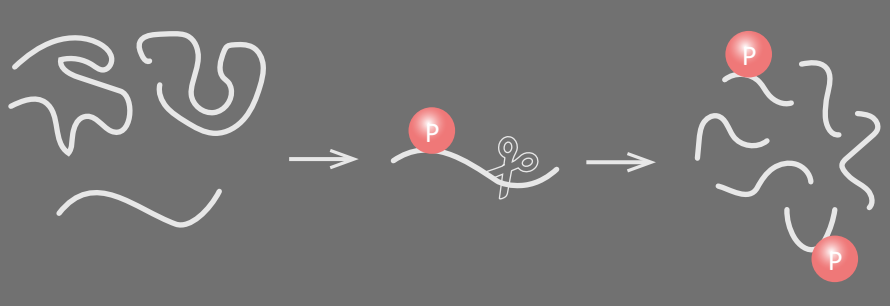
Label-free method: Parent-ion peak intensity quantification; No labeling required; Unlimited sample size

iTRAQ or TMT method: MS2 quantification with high accuracy; Two-dimensional reversed-phase liquid chromatography system for in-depth phosphoproteomics profiling

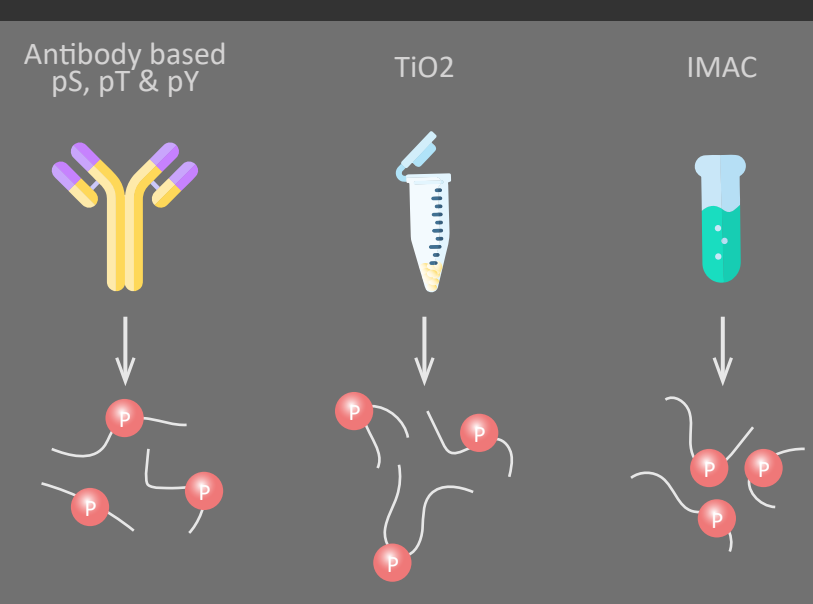
DIA method: MS2 quantification with high accuracy and effectively improve repeatability; Fewer missing values



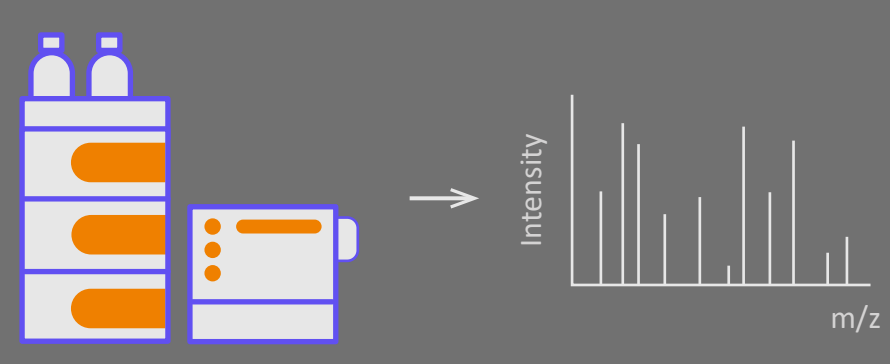
EXTRACTION



DIGESTION



ENRICHMENT



MS ANALYSIS

PTM motif analysis

PTM abundance cluster analysis

BIOINFORMATIC ANALYSIS

Contact Us

Creative Proteomics