



PTM BIO Proteomics Services Guide

PTM BIO

COMPANY PROFILE



14,000 m² (2021)

45,000 m² (2026)

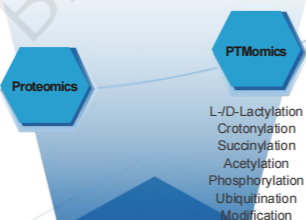


Company Profile

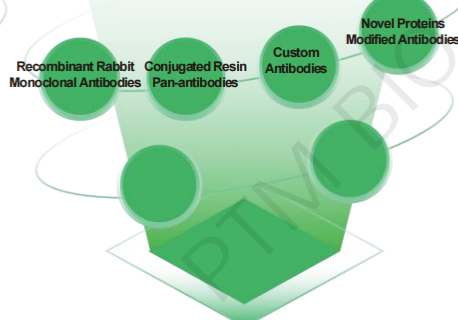
PTM BIO is a biotechnology company specializing in mass spectrometry-based proteomics, PTM profiling, antibody development, and integrated research services. By combining advanced mass spectrometry platforms, proprietary enrichment reagents, automated sample preparation, and bioinformatics expertise, PTM BIO supports research workflows from protein discovery and quantitative profiling to target validation and mechanistic investigation. Our service portfolio spans global proteomics, post-translational modification profiling, blood proteomics, spatial proteomics, single-cell proteomics, and chromatin profiling technologies.

An Innovative Platform-driven High-tech Enterprise Integrating R&D, Production, and Technical Services

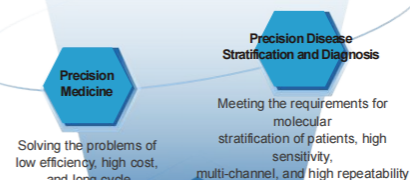
Proteomics Technology Services



PTMab[®] Antibody Reagents



Biomarker Discovery



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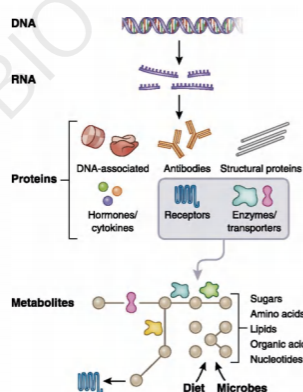
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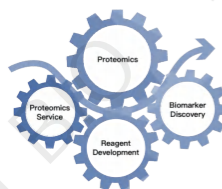
General Proteomics Services

Overview

Proteomics: Proteins are active biological macromolecules that carry out biological functions. Proteomics is a systems biology technology that comprehensively analyzes the entire state of proteins. Compared to genomics and transcriptomics, proteomics directly and comprehensively resolves the protein abundance or post-translational modification levels in samples, uncovering protein-specific regulatory mechanisms that cannot be determined at the gene and transcriptional levels. It is the core content of the 'post-genome' era and holds great significance for deepening our understanding of the essence of life phenomena, disease mechanisms, disease diagnosis, and even drug development.



◀ The basic flow of information in biological systems, from DNA (genome) to RNA (transcriptome) to proteins (proteome) to metabolites (metabolome).



Proteomics-Driven Precision Medicine

Proteomic studies can reveal the physiological mechanisms of animals and plants, such as growth and development, neural signal transduction. It also provides theoretical foundations and solutions for elucidating the mechanisms of various diseases, such as tumor metastasis, cardiovascular diseases, etc. Furthermore, screening protein biomarkers can provide molecular markers for the early diagnosis and treatment of diseases.

Research Methods

Quantitative Proteomics Analysis: 10X Proteomics, 4D-FastDIA/4D-LFQ, TMT/iTRAQ, label-Free, etc.

	Method	Technical Principle	Advantages	Limitations	Recommended Applications
Label-Free	10X Proteomics	Ultra-fast acquisition at >300 Hz, enabling near-complete ion utilization	Higher throughput, deeper proteome coverage, and improved sensitivity	Requires specialized MS instrumentation	Large-scale proteome and PTM profiling across diverse sample types
	4D-FastDIA	Ion mobility-assisted DIA acquisition for near-complete ion utilization	High reproducibility, deeper coverage, no need for library pre-building	Requires specialized MS instrumentation	Large-scale proteomics analysis
	Label-Free Quantification	MS1-based intensity quantification	Flexible workflow and broad sample compatibility	More missing values and relatively lower reproducibility	Proteome and PTMomics analysis across diverse sample types
	4D-LFQ	MS1-based intensity quantification with 4D feature alignment	Improved identification depth, quantification accuracy, and reproducibility	Requires specialized MS instrumentation	Proteome and PTMomics analysis across diverse sample types
Label-Based	TMT/iTRAQ	Isobaric labeling-based quantification	High reproducibility and well-established workflow	Subject to ratio compression	Comparative quantification of 2–18 samples per batch; commonly used for paired designs and accurate relative quantification
Targeted	4D-PRM/PRM	Targeted MS2-based quantification of selected peptides	High sensitivity and accuracy, enables precise absolute quantification	Limited to predefined proteins or peptides; not suitable for global proteome profiling	Follow-up validation of candidate proteins or peptides

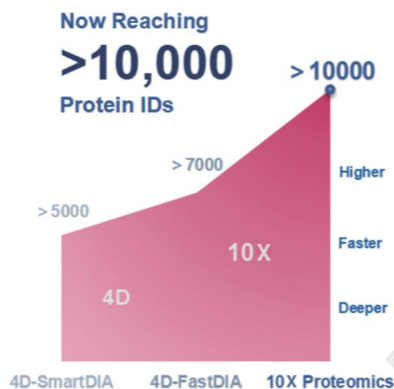
10X Deep Proteomics Profiling

Service Overview

Proteomics and PTMomics studies are core drivers of the post-genomic era and precision medicine, providing endless momentum for the development of life sciences and basic medical research.

10X Proteomics is a significant advancement based on the next-generation Orbitrap Astral and timsTOF HT high-resolution mass spectrometers, integrating front-end sample processing automation, modification antibody development and modification peptide enrichment technology, and deep learning-based mass spectrometry data search libraries. It brings significant improvements in identification depth, accuracy, and throughput, redefining new standards for proteomics analysis.

Technical Advantages



- **Excellent technology**(Breaking Limits in Sensitivity, Accuracy, Throughput):

Detection depth exceeding 10K+ proteins, 10-fold hardware performance enhancement, 10-fold sensitivity improvement, high throughput exceeding 100 samples per day.

- **Most Comprehensive Product**(Full Coverage of Analysis Types, Multi-Domain Applications):

10X high-depth proteomics, 10X high-depth blood proteomics, 10X high-depth modification profiling (5 classic post-translational modifications and 8 novel modifications).

- **Proven Project Experience**(High-level Scientific Outcomes):

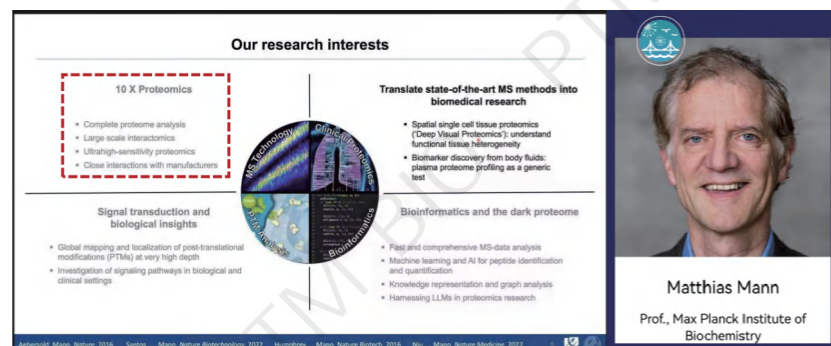
Completed quantitative analysis of over 10,000 various samples; Created multiple records for proteomics identification depth.

- **Largest Scale**(Industry-Leading Mass Spectrometry Cluster):

Five X Orbitrap Astral mass spectrometers, three X timsTOF HT high-resolution mass spectrometers.

Application Scenarios

- 10X Proteomics
- Classic PTMs/
Novel Acylation Modification
- Single-cell Proteomics
- Spatial Proteomics/
Spatial Phosphorylation
- Plasma Proteomics



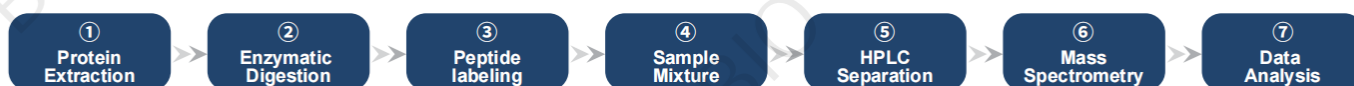
Prominent proteomics leader Matthias Mann primarily focuses on applying advanced mass spectrometry techniques to biomedical research, biological functions, and bioinformatics research. Recent advances in high-speed, high-sensitivity mass spectrometry have substantially expanded proteome depth and throughput, enabling deep proteome profiling across large sample cohorts.

TMT/iTRAQ labeled Quantification

Service Overview

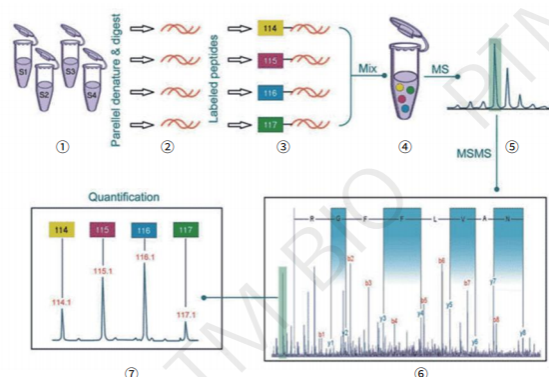
TMT (Tandem Mass Tags) and iTRAQ (Isobaric Tags for Relative Absolute Quantitation) are multiplex peptide labeling and quantitation technologies developed by Thermo (USA) and AB Sciex (USA), respectively. TMT includes up to 16 sets of stable isotope labeling reagents, while iTRAQ includes up to 8 sets, with each set composed of reporter group, equilibrium group, and reactive group. TMT/iTRAQ labeling and quantitation can compare proteins from up to 16/8 different samples in a single run, offering high labeling efficiency, enabling nearly all proteins in a sample to be labeled. The mass spectrometry detection is highly sensitive, capable of detecting low-abundance proteins, with broad applicability, making it widely used in research fields.

Technical Principle



► TMT/iTRAQ labeling Quantification Technology Principles and Procedures

- ① TMT/iTRAQ reagents label the tryptic peptides, which react with the Primary amine groups at the N-terminus and side chains of amino acids.
- ② In the MS(1) spectrum, the same peptide from different samples, after labeling, appears at the same mass-to-charge ratio. In the MS/MS spectrum, the bonds between the reporter group, equilibrium group, and reactive group are cleaved, and the same peptide with different isotopic labels produces reporter ions with different masses.
- ③ Quantitative information for the identical peptide segments across samples is obtained based on the abundance of reporter ions, which is then processed by software to yield protein quantitative data.



Technical Advantages

- ① Parallel comparison of 2-16 samples.
- ② High protein throughput with extensive coverage.
- ③ Suitable for various types of biological samples.



Applications

- ① High-throughput protein expression profiling in cellular and tissue samples under various stress, physiological, and pathological conditions.
- ② Construction of proteomic maps across diverse species.

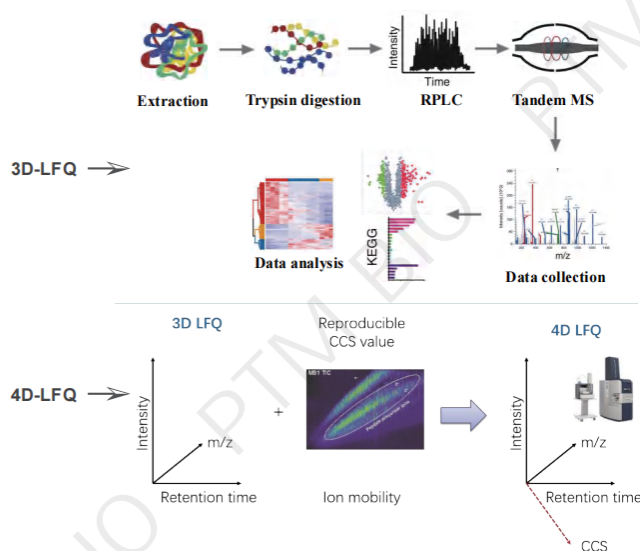
4D LFQ Quantitative Proteomics

Service Overview

Label-free protein quantification technology is a new protein quantification method that does not rely on isotope labeling. This technology analyzes protein enzymatic peptide segments using liquid chromatography-mass spectrometry, without the need for expensive stable isotope labels as internal standards. It only requires analyzing the mass spectrometry data generated during large-scale protein identification, comparing the signal intensities of corresponding peptide segments in different samples, and can then perform relative quantification of the corresponding proteins.

4D-LFQ proteomics adds a fourth dimension to 3D separation, based on ion mobility (mobility) separation. Ion mobility primarily separates ions according to their shape and cross-section, bringing more possibilities for the analysis of complex sample systems.

Technical Principle



Mass spectrometry-based proteomics research fundamentally analyses samples by measuring the physicochemical properties of ions. Qualitative and quantitative results are derived from the mass spectra and associated information of the samples. In 3D proteomics, mass spectrometry identifies and quantifies peptide ions based on three dimensions: retention time (retention time), m/z (mass-to-charge ratio), and ion intensity (intensity). 4D proteomics adds a fourth dimension to the 3D separation, which is the separation of ion mobility. Ion mobility is mainly separated based on the shape and cross-section of the ions, bringing more possibilities for the determination of complex system samples.



Applications

4D proteomics greatly improves the detection of proteomics based on its enhanced speed, sensitivity, and throughput.

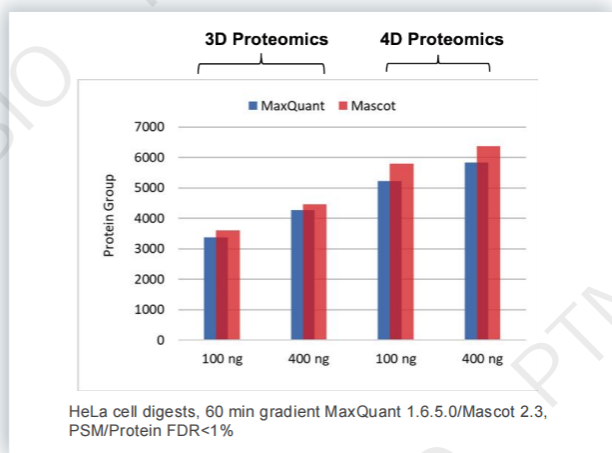
- ① Routine proteomics analysis (plant, animal tissue, cells, microorganisms, etc.)
- ② Clinical proteomics analysis (clinical samples, such as clinical tissues, FFPE, urine, etc.)
- ③ Ultra-microanalysis of rare samples (limited-number samples for analysis, such as stem cells, immune cells, etc.)



Technical Advantages

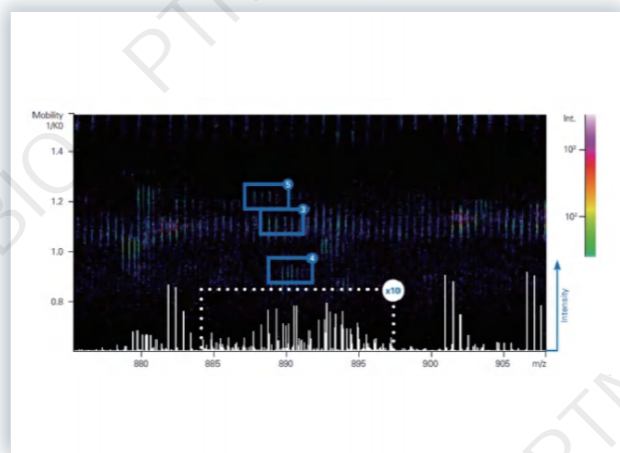
Superior Identification Depth and Sensitivity

4D proteomics distinguishes and identifies low-abundance signals, significantly reducing sample volume requirements while enhancing identification depth by 50%-100%.



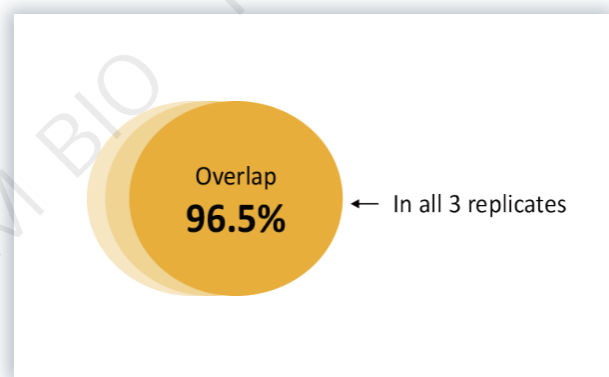
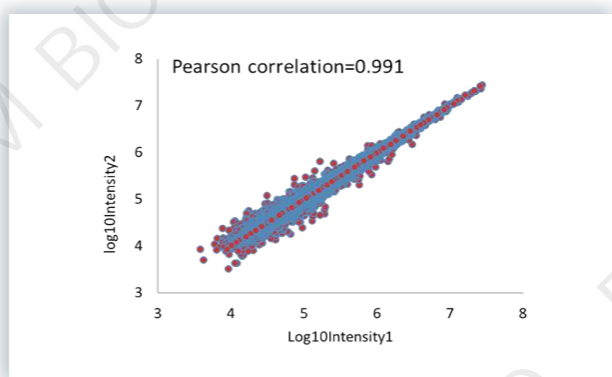
Enhanced Detection Sensitivity

The newly incorporated ion mobility separation distinguishes co-eluting peptides with minute m/z differences, thereby generating more specific MS/MS spectra. This enables the identification of low-abundance protein signals obscured under 3D conditions, substantially enhancing detection sensitivity.



Higher Accuracy and Better Reproducibility

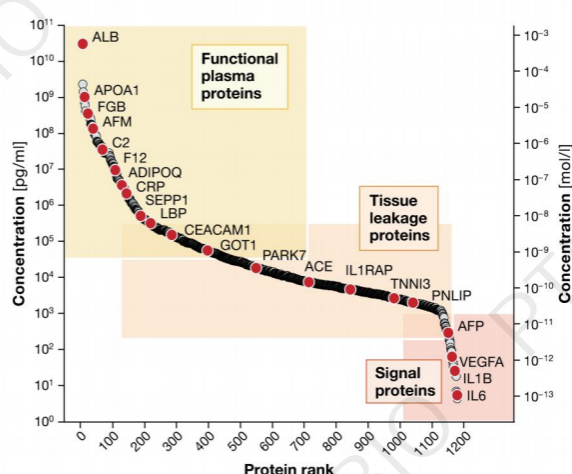
4D Proteomics, with its high-speed data acquisition and four-dimensional alignment features, fundamentally and significantly reduces the randomness of shotgun proteomics, greatly improving quantitative accuracy and reproducibility. The literature reports and our test data demonstrate that 4D Proteomics achieves the following without labelling reagents or spectral libraries :



Blood+DIA Deep Blood Proteomics

Service Overview

Blood is the most commonly used specimen in clinical diagnostics, and changes in its proteins serve as biomarkers that can indicate the occurrence and progression of various diseases. From alanine aminotransferase (ALT) in routine health examinations to protein markers such as AFP and CA125 in cancer diagnosis, these provide indispensable important information for clinical diagnosis. In recent years, with the advancement of mass spectrometry technology, using omics methods to screen for new blood biomarkers has become an increasingly important direction for research and translational applications.

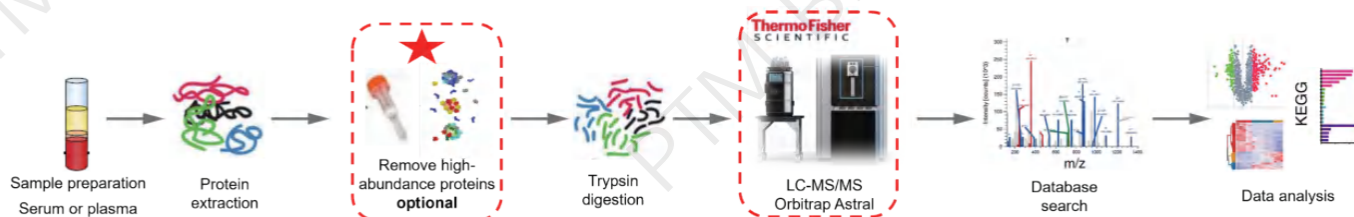


◀ Blood Analysis Challenges

- ① The plasma proteome spans an exceptionally wide concentration range, exceeding ten orders of magnitude. Highly abundant proteins can mask the detection of low-abundance proteins with potential biomarker value.
- ② Rich in high-abundance proteins, distributed very unevenly
- ③ Low detection depth, traditional proteomics identification depth of 500-600

Technical Principle

PTM BIO introduces the technical routes of Blood+DIA (antibody-based) and Blood+DIA (magnetic beads-based): The technical advantage of Blood+DIA (antibody-based) lies in its ability to specifically remove high-abundance proteins, thereby increasing the number of medium- and low-abundance proteins identified. The technical advantage of Blood+DIA (magnetic beads-based) lies in its ability to enrich low-abundance proteins while removing high-abundance proteins, achieving an 'equalization' effect, with a significant increase in protein depth, and it is also particularly suitable for the analysis of large-scale cohort samples.



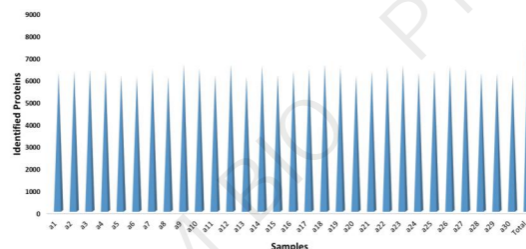
10X technology improves identification of mid- and low-abundance proteins



Technical Advantages

8000+ High-depth Plasma Protein Identification

Our 10X High-Depth Blood+DIA technology, successfully overcomes challenges such as the high dynamic range of proteins and low-abundance proteins in plasma samples, elevating the detection depth of plasma proteome to 8000+.



10X High-depth Blood+DIA Reveals 8000+ Plasma Proteins

High-quality Blood Proteome Data

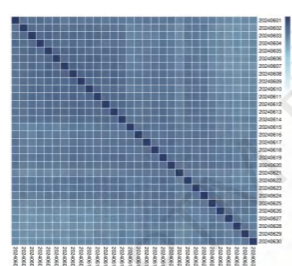
Interference from platelets and red blood cells in plasma severely affects protein data quality, impacting subsequent validation. We perform strict quality control on blood samples (platelet protein ratio all below 3%) and combine it with a rigorous protein database (2 million+ protein sequences) to ensure data reliability, reduce various IgG variants and protein fragments, and guarantee high reliability of results.

Number	Technological Type	Depth of Protein Identification	Platelet Protein Content
1	10X High-depth Blood+DIA	6402	1.2%
2	10X High-depth Blood+DIA	6340	1.7%
3	10X High-depth Blood+DIA	6024	2.8%
4	10X High-depth Blood+DIA	6544	2.0%
5	10X High-depth Blood+DIA	6014	1.5%
6	10X High-depth Blood+DIA	6283	2.6%

Low Platelet Percentage (Less than 3%) Ensures True Proteomics Data

High-stability Data Supports Large-scale Research

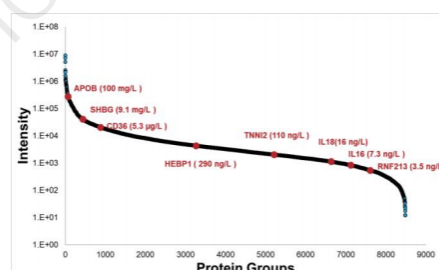
Large-scale proteomics studies require high stability in mass spectrometry technology and experimental systems. PTM BIO establishes a high-stability technical system by integrating automated quantification technology and process optimization. After continuous operation for one month, the correlation between all samples reached ≥ 0.98 , ensuring the overall quality and credibility of large-scale studies.



After Continuous Operation for a Month, Correlation ≥ 0.98 , Mass Spectrometry Quantitative System Highly Stable

Proteomics Data Fully Covers the Dynamic Range

Our plasma protein quantification results cover a series of important functional proteins, with identification results spanning the entire dynamic range of the HPPP database: such as the high-abundance protein APOB involved in lipoprotein metabolism (100 mg/L) and the extremely low-abundance inflammatory factor IL16 (7.3 ng/L), as well as the lowest concentration RNF213 (3.5 ng/L).

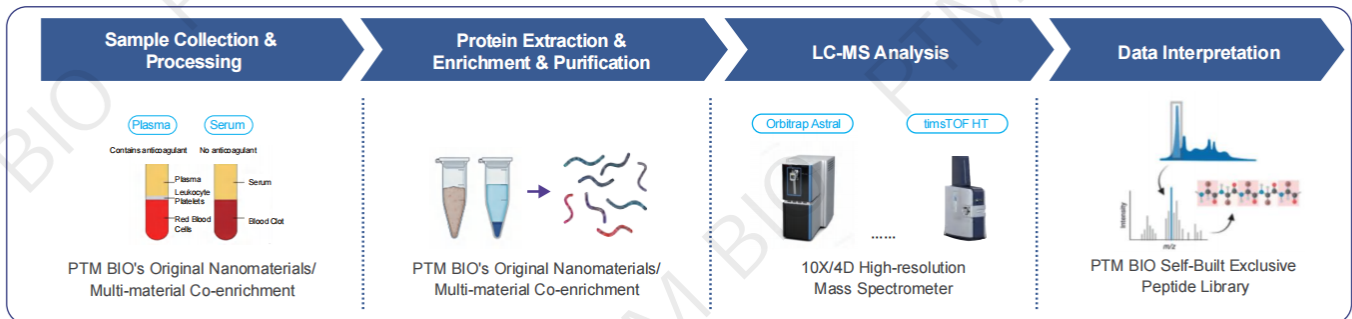


8487 identified plasma proteins cover a series of important functional proteins

Blood Peptidomics

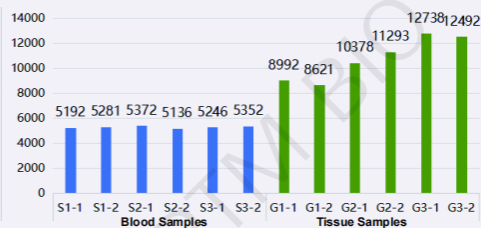
Service Overview

Peptides are short-chain amino acid molecules with specific functions in living organisms. As non-classical proteins, peptides are encoded by non-coding RNA or produced by protease hydrolysis. They are important signal regulatory molecules in living organisms, playing a crucial role in disease prevention, diagnosis, and treatment. PTM BIO unique peptidomics technology solves challenges such as low peptide content, slow library searches, difficulty in discovering new peptides, and high verification difficulty, providing a powerful tool for peptide discovery, identification, and functional research.



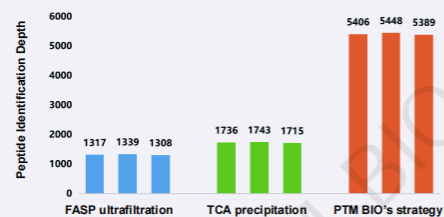
High Quality Data

High-depth Peptide Identification Provides Highly Reproducible Data



Peptidomics detection depth

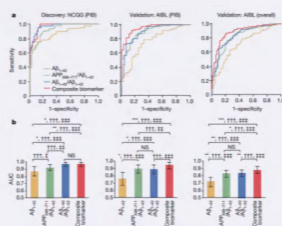
High-depth Peptide Identification is 4 Times More Efficient Than Traditional Methods



Comparison of plasma peptidomics results with different enrichment strategies

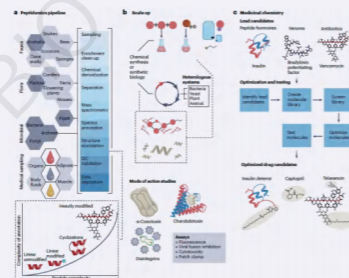
Typical Applications

Disease Diagnosis and Treatment Targets



Plasma Aβ peptides for the Diagnosis of Alzheimer's disease

Peptide Drug Development



Peptide structure analysis→Large-scale production of peptides→Optimization of lead peptides

4D-FastDIA Quantitative Proteomics

Service Overview

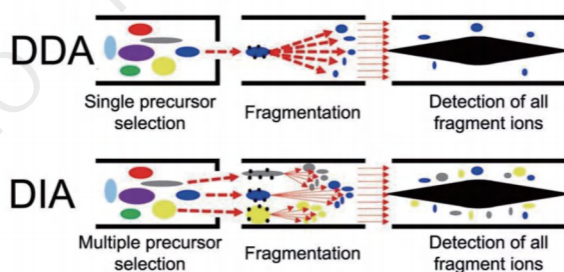
Building upon industry insights and experience from over ten thousand proteomic analyses, PTM BIO has upgraded to launch the next-generation 4D-FastDIA proteomic quantification service. This technology retains 4D proteomics' signature features of peptide fractionation-free analysis and high depth, while integrating the 4D-DIA strategy to reduce missing values and enhance quantification stability and reproducibility. It is particularly suited for large-scale proteomic quantitative analysis.

Technical Principle

Proteomics techniques can be divided into data-dependent (Data-dependent Acquisition, DDA) strategies and data-independent acquisition (Data-independent Acquisition, DIA) strategies. Compared to DDA, which collects signals from a limited number of peptide ions with stronger signals in each time window for fragmentation analysis in tandem mass spectrometry (MS/MS), DIA technology divides the entire full-scan range of the mass spectrometry into several windows and

cyclically selects, fragments, and detects all ions in each window, allowing for the complete fragmentation information of all ions in the sample to be obtained without omission. However, the data acquisition strategy of traditional DIA makes the chromatogram highly complex, posing significant challenges for data analysis and raising some controversy about its quantitative reliability.

4D-FastDIA builds upon the 4D proteomics platform's significant improvements in acquisition speed and sensitivity, achieving deep protein coverage. By adopting the 4D-DIA data acquisition strategy, it enhances parallelism and stability, demonstrating exceptional robustness in large-scale sample analysis. Based on a deep neural network library search strategy, it achieves high reliability and high resolution in peptide information extraction. Particularly suited for large-scale proteomics analysis.



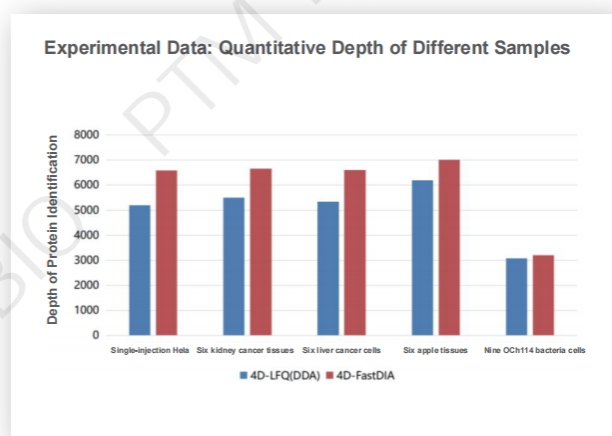
	Advantages	Disadvantages
DDA	1. Methodology is mature. 2. Highly versatile, applicable to various high-resolution mass spectrometry techniques.	1. Certain randomness, with many missing values. 2. Poor quantitative reproducibility.
Traditional DIA	1. Good reproducibility, few missing values. 2. Highly versatile, applicable to various high-resolution mass spectrometry techniques.	1. Spectrograms are highly complex, posing significant challenges to data parsing. 2. The reliability of low-abundance protein detection is controversial.
4D-FastDIA	1. High scanning speed, high identification depth, more cost-effective. 2. Significantly reduces missing values, improves quantitative reproducibility and data integrity. 3. DIA-PASEF technology, 4D alignment enhances DIA data quality. 4. No additional database construction required, reliably acquires protein information with high resolution.	Requires instrument equipment and specialized software that support 4D.



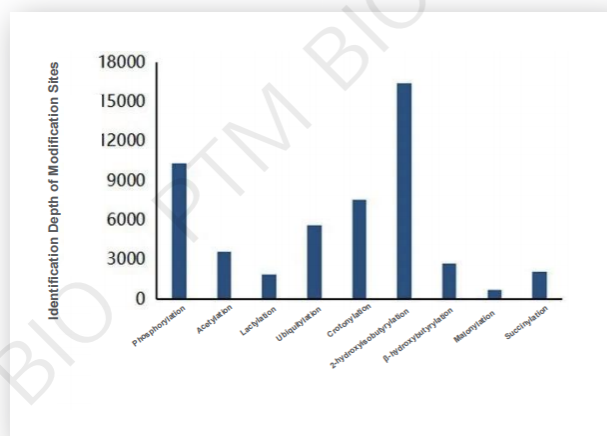
Technical Advantages

Superior Identification Depth and Sensitivity

4D-FastDIA integrates numerous authoritative technologies from the 4D proteomics, dia-PASEF and deep neural network library search, further enhancing the depth, reproducibility, and stability of proteomics analysis.



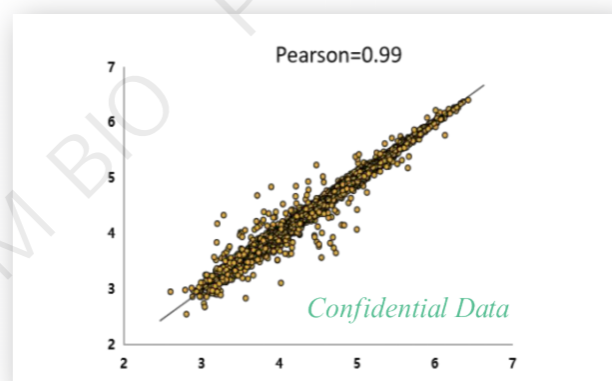
The detection depth of 4D-FastDIA is further improved compared to 4D-LFQ(DDA).



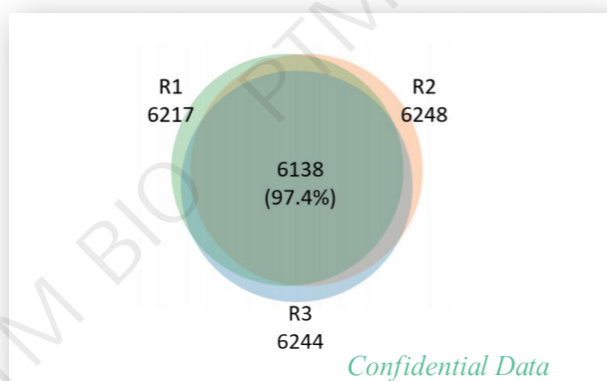
4D-FastDIA modification group quantitative depth for human liver cancer samples. 4D-FastDIA further enhances the depth, reproducibility, and stability of modification group quantification.

Greater Accuracy and Reproducibility

4D-FastDIA technology retains the features of 4D proteomics requiring no peptide fractionation and high depth, further combining the 4D-DIA strategy to reduce missing values and enhance the stability and parallelism of quantification, particularly suitable for large-scale proteomics quantitative analysis.



Technical reproducibility as high as Pearson $r=0.99$, compared to DDA data (typically 85-90%), data randomness decreases, and missing values significantly decline.



A total of 6,299 proteins were identified through three technical replicates, with overlap proteins accounting for 97.4%, demonstrating high data reproducibility.

SILAC-Based Quantitative Proteomics

Service Overview

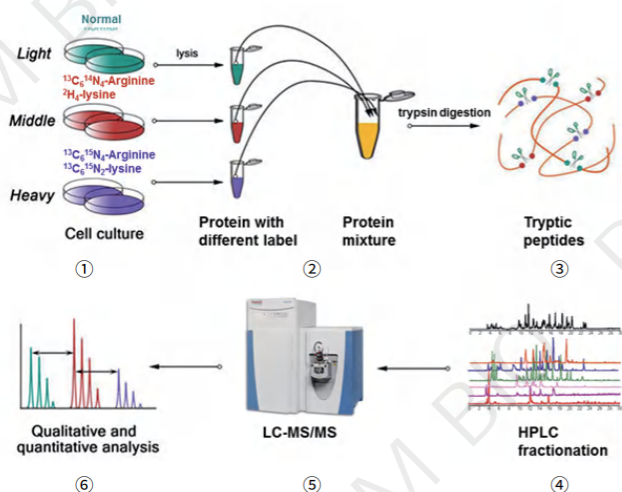
SILAC (Stable Isotope labeling with Amino acids in Cell culture) refers to stable isotope labeling technology in cell culture. This method was first developed by the Danish Mann laboratory in 2002, which further improved the AACT technology, providing an effective approach for comprehensive and systematic qualitative and quantitative analysis of complex cellular proteomes.

Technical Principle



► SILAC Quantification Principles and Procedures

The basic principle of SILAC is to replace corresponding amino acids in the cell culture medium with natural isotope (light) or stable isotope (heavy) labeled essential amino acids. After >6 cell doubling cycles, the stable isotope labeled amino acids are fully incorporated into the newly synthesized proteins of the cells, replacing the original amino acids. The lysed proteins of differently labeled cells are mixed at a ratio of cell number or protein amount, then separated, purified, and identified by mass spectrometry.



Technical Advantages

- ① Multiple samples are pooled for simultaneous separation, digestion, and identification, ensuring consistent experimental impact on subsequent analyses and minimizing quantitative errors introduced by manual handling or instrumentation.
- ② Capable of labelling diverse cell types cultured in DMEM, DMEM-F12, and RPMI 1640 media.



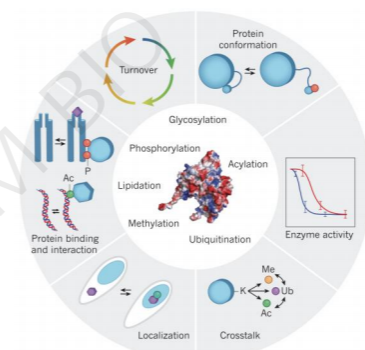
Application Area

- ① Disease biomarker screening
- ② Mechanism research
- ③ Drug target research
- ④ Screening of special functional proteins

Profiling of Post-translational Modification

Overview

Protein post-translational modification (PTM) refers to the addition of chemical modifications to specific amino acids after translation. Post-translational modifications can alter the structure of proteins, contributing to the diversity of protein structure and function. Increasingly, research findings show that many important life processes and disease occurrences are not only related to the abundance of proteins but are more importantly regulated by various types of post-translational modifications. In-depth study of protein post-translational modifications is of great significance for revealing the mechanisms of life processes, screening clinical biomarkers for diseases, and identifying drug targets. PTM BIO provides comprehensive research solutions for modified protein groups, enabling quantitative comparison of biological samples at the post-translational modification level under different physiological and pathological conditions, and deeply revealing the close relationship between PTM changes and life activities.

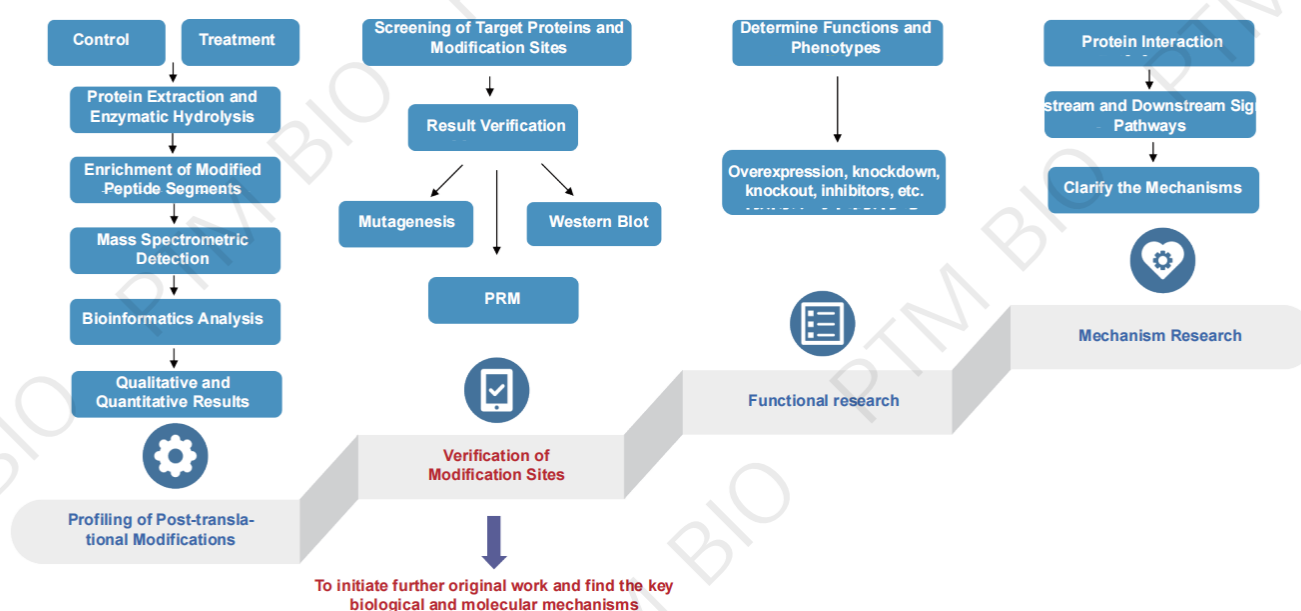


Phosphorylation, acetylation, lactylation, ubiquitylation, butyrylation, 2-hydroxyisobutyrylation, β -hydroxybutyrylation, malonylation, succinylation.

Modification Types	Biological Functions	Common Application Directions
Phosphorylation	The most widespread type of post-translational modification, participates in enzymatic reactions, mediates protein activity and receptors to regulate and control protein activity and function.	Widely applied, especially in signal transduction, development and differentiation, cancer mechanisms, stress responses, etc.
Acetylation	The most studied acetylation modification, participates in epigenetics, signal transduction, metabolic regulation, apoptosis, and other biological processes.	Immune responses, tumor formation, metabolic diseases, apoptosis, etc.
L- lactylation	First discovered in 2019, it is an important way for lactate, a carbon-containing metabolite in the cellular glycolysis pathway, to function, regulating diseases such as cancer and inflammation.	Tumor formation and progression, inflammation, metabolic regulation, etc.
D- lactylation	Three isomers of lactoylation modification successfully identified in 2024.	Intestinal diseases, brain diseases, tumors, etc.
Succinylation	Affecting metabolic processes in mitochondria, it holds significant value for studying mitochondrial dysfunction-related diseases, participating in cellular differentiation, metabolic regulation, epigenetic regulation, signal transduction, and other physiological activities.	Inflammation, metabolic diseases, tumors, etc.
Crotonylation	One of <i>Cells</i> 'Top Five Research Highlights' in 2011, regulating gene expression by combining with transcriptionally active chromatin regions.	Reproductive regulation, epigenetics, tumor metabolism, DNA damage repair, etc.
β -hydroxybutyrylation	First reported 16 years ago, closely related to cellular core metabolism.	Energy metabolism, tumor research, DNA damage repair, liver metabolism, liver cancer, etc.
2-hydroxyisobutyrylation	Chromatin function regulation.	Epigenetics, glycolytic metabolic pathway.
Malonylation	Energy metabolism, biosynthesis of antibiotics, metabolic regulation, and photosynthesis.	Metabolic disorder diseases, immune regulation.
Benzoylation	First discovered in 2018, it regulates glycerophospholipid metabolism, ovarian steroid biosynthesis, the signaling pathway of phospholipase D, and serotonin, compared to acetylation Synapses and the secretion process of insulin.	Epigenetics, metabolic regulation
N-glycosylation	A widely existing type of post-translational protein modification, primarily affecting protein proper folding, active state, intracellular transport, etc.	Cell-to-cell recognition, cell adhesion, signal transduction, immune response, cell transformation, etc.
O-glycosylation	Involved in regulating multiple signaling pathways, participating in processes such as growth, proliferation, and hormone response.	Individual development, cellular stress, transcriptional regulation, signal transduction, Metabolic diseases, tumor occurrence and development.
Intact N-glycopeptide	Listed as one of the focus technologies by Nature Methods, primarily affecting proteins spatial conformation, activity, transport, and localization, etc.	Physiological mechanisms, disease pathology, discovery of disease biomarkers. Pathogens interact with hosts, plant growth and development.
Ubiquitylation	A key type of modification that downregulates protein expression, mediates protein degradation, and alters cellular localization, activity, and other factors affecting substrate function and activity.	Cell cycle, apoptosis, aging, neurodegenerative diseases, signal transduction.
SUMOylation	A common ubiquitin-like modification that participates in various important physiological and biochemical reactions in cells, such as protein-protein and protein-DNA interactions regulating their functions, playing an indispensable role.	Cardiovascular diseases, cancer, neurodegenerative diseases, and immune diseases, etc. The occurrence and development of various diseases.
Methylation	Involved in the regulation of gene expression, the modulation of protein function, and the processing of ribonucleic acid (RNA).	Epigenetics, cancer mechanisms, aging, neurodegenerative diseases.
Cysteine-Redoxome Proteomics	Capable of reversibly regulating protein activity, interaction, and localization, thereby achieving precise control over many life processes.	The occurrence of major diseases, fat metabolism regulation, plant stress responses, etc.

Research Approach

Modification proteomics is an effective tool for studying post-translational modifications and has attracted widespread attention from scientists. A classic approach to proteomics research primarily includes:



Research Methods

Methods		Applications
Modification Identification Analysis	Modification Site Identification	Target Protein Function Mining ^[1]
Qualitative Analysis	Modification Site Identification	Drawing Species Modification Spectra ^[2]
Quantitative Analysis	Quantification of Modified Proteome Analysis Classic Modifications: Phosphorylation, Acetylation, Ubiquitylation, Methylation, Glycosylation	In-depth Molecular Mechanism Research ^[3]
Absolute Quantification of Modifications	Absolute Quantification of Modifications	Protein Modification Validation ^[4]

Reference Applications and Cases



[1] Identification Analysis: Protein Acetylation Plays a Key Role in Antiviral Innate Immunity. KAT8 selectively inhibits antiviral immunity by acetylating IRF3. *Journal of Experimental Medicine*.



[2] Modification spectrum analysis: Mapping the Lysine Succinylation Modification Expression Profile of *Aspergillus flavus*. Lysine succinylation contributes to aflatoxin production and pathogenicity in *Aspergillus flavus*. *Molecular & Cellular Proteomics*.



[3] Quantitative Analysis: Crotonylation Reveals Its Important Role in DNA Damage Repair. Global crotonylome reveals CDYL-regulated RPA1 crotonylation in homologous recombination-mediated DNA repair. *Science Advances*.



[4] PTMomics Quantification +PRM: Phosphorylation Modification Proteomics Reveals New Mechanisms of Platelet Activation Regulation. Temporal quantitative phosphoproteomics of ADP stimulation reveals novel central nodes in platelet activation and inhibition. *Blood*.

Phosphorylation

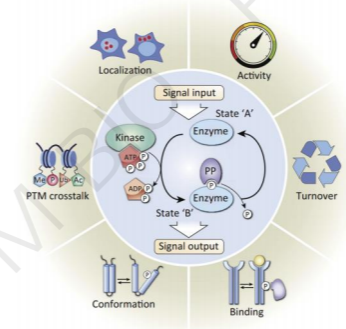
Service Overview

Protein phosphorylation (Phosphorylation) refers to the process in which a kinase catalyzes the transfer of a phosphate group from ATP or GTP to a specific site on a protein (amino acid residues Ser, Tyr, Thr).

Phosphorylation modification is a widely existing type of post-translational modification, with over 30% of proteins in cells undergoing phosphorylation modification. Therefore, phosphorylation modification is one of the most fundamental, universal, and most important mechanisms for regulating and controlling protein activity and function.

Phosphorylation is involved in various physiological and pathological processes, regulating cellular activities such as proliferation, development, differentiation, and apoptosis. It is widely used in research fields including cellular signal transduction, apoptosis, development, differentiation, and cancer mechanisms.

PTM BIO high-depth phosphorylation modification quantitative analysis, using proprietary antibodies for targeted enrichment of phosphorylated modification peptides to reduce sample complexity. Combined with strict dual quality control and bioinformatics analysis, they deliver a powerful solution widely applied in phosphorylation mechanism research under physiological and pathological conditions.



Reference Applications and Cases

Signal Transduction

Science: Phosphoproteomics Reveals Opioid Receptor Signaling In Vivo.

In vivo brain GPCR signaling elucidated by phosphoproteomics. *Science*.

Cancer Mechanisms

Nature: Phosphoproteomics Reveals Molecular Subtyping and Novel Targets for Precision Therapy in Hepatocellular Carcinoma.

Proteomics identifies therapeutic targets of early-stage hepatocellular carcinoma. *Nature*.

Immune Inflammation

Immunity: Phosphoproteomics Reveals Dynamic Signaling Pathways After T Cell Activation.

Integrative proteomics and phosphoproteomics profiling reveals dynamic signaling networks and bioenergetics pathways underlying T cell activation. *Immunity*.

Metabolic Regulation

Cell Metab: Phosphoproteomics Reveals Key Pathogenic Signals in Diabetes.

Phosphoproteomics reveals the GSK3-PDX1 Axis as a key pathogenic signaling node in diabetic islets. *Cell Metabolism*.

Apoptosis

Autophagy: Phosphoproteomics Reveals the Important Role in Neuronal Autophagy.

Phosphoproteome-based kinase activity profiling reveals the critical role of MAP2K2 and PLK1 in neuronal autophagy. *Autophagy*.

Stress

Mol Cell: Phosphoproteomics Reveals the Plant Abiotic Stress Molecules.

Reciprocal regulation of the TOR Kinase and ABA receptor balances plant growth and stress response. *Molecular Cell*.

Food Science

J Agric Food Chem: Phosphoproteomics Reveals Its Role in Biological Functions of Poultry Egg Proteins and Food Characteristics.

Comparative quantitative phosphoproteomic analysis of the Chicken Egg during Incubation based on tandem mass tag labeling. *Journal of Agricultural and Food Chemistry*.

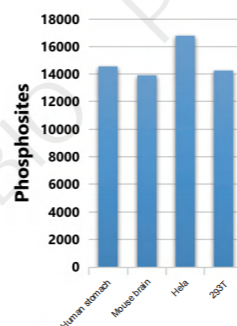


Technical Advantages

Technology Upgrade

*Identification of Phosphorylation Sites with Deeper Coverage

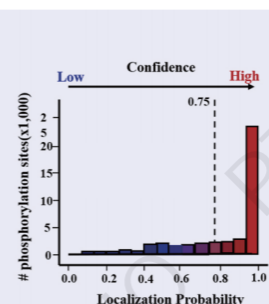
Based on the IMAC enrichment strategy combined with 4D proteomics technology, it brings a significant increase in detection sensitivity, greatly improving the number of phosphorylation modification sites identified.



Quality Control Upgrade

*Dual Quality Control Standards

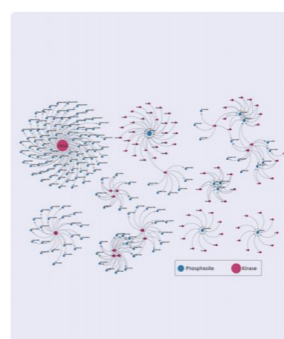
For PTM peptide identification, quality control should extend beyond false discovery rate (FDR) control to include modification-site localization confidence. Sites with a localization probability below 0.75 are excluded to ensure reliable site assignment.



Bioinformatic Analysis Upgrade

*Kinase Prediction, Signal Pathway Analysis, Deeply Mining Data

Based on the Phosphoproteomics identification results, bioinformatics analysis packages can be further upgraded, including kinase prediction, signaling pathway analysis, and in-depth data mining, to provide a foundation for investigating mechanisms under physiological and pathological conditions.

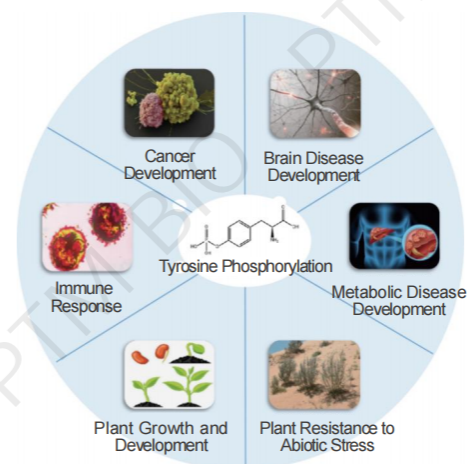


Tyrosine Phosphorylation / Full-Spectrum Phosphorylation

Service Overview

Tyrosine phosphorylation (Tyrosine Phosphorylation, pY) refers to phosphorylation modification occurring on tyrosine residues. It can regulate multi-level signaling networks by controlling protease activity and protein interactions. It plays a crucial role in various physiological and pathological processes such as cellular signal transduction, cell proliferation, differentiation, and disease development. Simultaneously, it holds a unique advantage in clinical drug applications and has garnered increasing attention and importance from researchers.

Post-translational modification proteomics technologies based on mass spectrometry are important methods for revealing the function and mechanism of tyrosine phosphorylation. To further meet the needs of tyrosine phosphorylation research, PTM BIO has launched a series of new products:



1. Tyrosine Phosphoproteomics 2.0: Through major technological innovations, it enables deep and sensitive profiling of tyrosine phosphorylation sites.

2. Full-spectrum phosphorylation modification: It covers both conventional serine/threonine phosphorylation detection while ensuring high-depth detection of tyrosine phosphorylation, which has great research potential, achieving comprehensive coverage of phosphorylation modification sites for serine, threonine, and tyrosine phosphorylation sites and opening a new paradigm for phosphorylation research.

Technical Advantages

1.Enrichment Method- Exclusive Monoclonal Antibody:

Higher consistency and reproducibility, significantly improving the specificity and sensitivity of modification peptide enrichment.

2.Detection Platform-10X Proteomics Platform:

The industry's highest specification high-resolution mass spectrometry cluster, ensuring data detection depth and accuracy from higher, faster, and deeper dimensions.

3.In-House Spectra Library- Greatly Enhanced Depth:

Our self-built, ultra-large-scale spectral library (containing over 50,000 real tyrosine phosphorylation sites) significantly improves data resolution. It fully covers 58 known receptor tyrosine kinases across 20 major classes, including numerous prominent proteins and novel sites.

4.Dedicated Bioinformatics Analysis:

Developed specifically for tyrosine phosphorylation proteins, enabling full-process analysis from protein and site information to regulatory kinases, upstream small molecules, DrugBank and other compound and drug-target databases, bridging the gap between basic research and clinical translation.

5.One-Stop Service for the Entire Process:

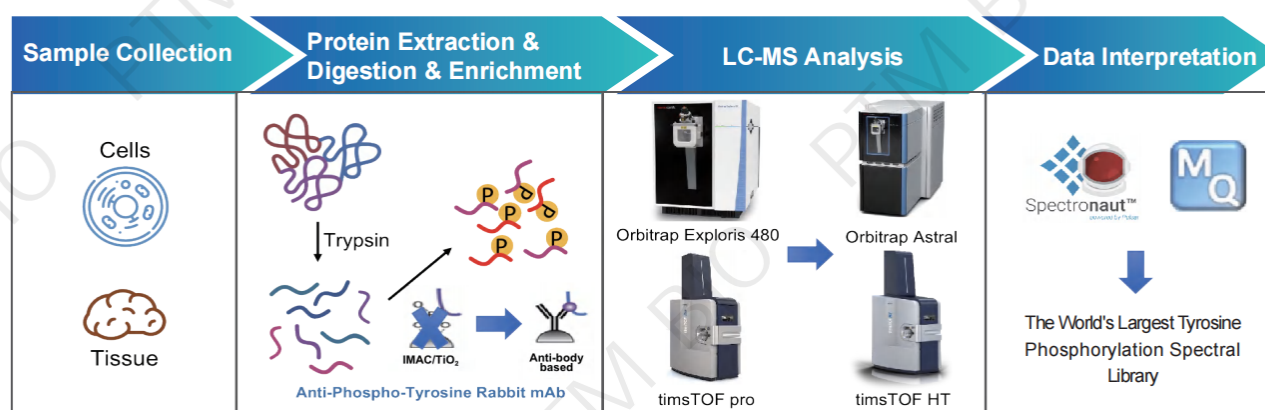
PTM BIO independently develops high-quality antibodies and industry-leading analytical platforms, offering a full-process service for tyrosine phosphorylation pan-antibody WB modification screening → high-depth tyrosine phosphorylation proteomics detection → dedicated bioinformatics analysis → site-specific antibody customization → and CUT&Tag analysis, making it more convenient.

4. Dedicated Bioinformatics Analysis:

Developed specifically for tyrosine phosphorylation proteins, enabling full-process analysis from protein and site information to regulatory kinases, upstream small molecules, DrugBank, and other drug libraries, bridging the gap between basic research and clinical translation.

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PTM BIO independently develops high-quality antibodies and industry-leading analytical platforms, offering a full-process service for tyrosine phosphorylation pan-antibody WB modification screening → high-depth tyrosine phosphorylation proteomics detection → dedicated bioinformatics analysis → site-specific antibody customization → CUT&Tag analysis, making it more convenient.



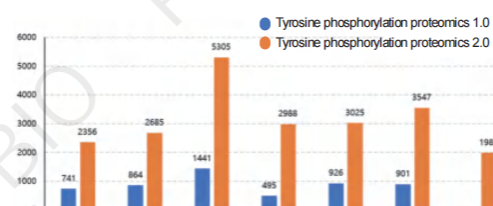
Tyrosine phosphorylation proteomics technical route

Internal Data

*Tyrosine Phosphorylation Identification Depth:

An average of more than **3,000** phosphotyrosine sites was identified per LC-MS/MS run, with selected samples yielding more than **5,000** sites, it has a 3- to 6-fold increase compared to version 1.0. Whether it is endogenous tyrosine phosphorylation or exogenous stimulus, the identification depth of PTM BIO has been improved compared to the literature under the condition of significantly reduced sample size **3~10** times! Under exogenous stimulation, a single injection can detect up to **13,663** phosphorylation sites!

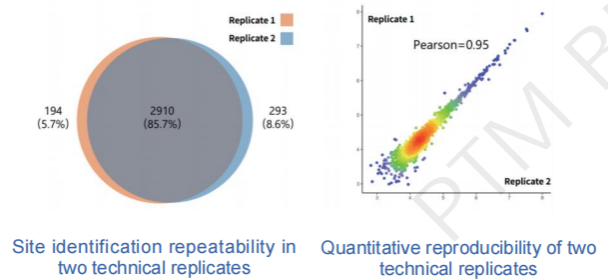
Comparison of tyrosine phosphorylation proteomics depths 1.0 and 2.0



*Tyrosine Phosphorylation Detection Repeatability and Stability:

The overlap rate of sites between the two technical replicates was as high as **85.7%** !

The quantitative correlation coefficient of two technical replicates reached **0.95** !



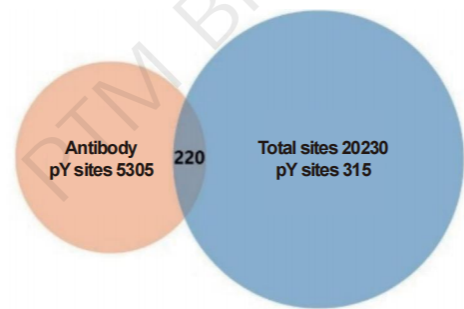
*10X Full- spectrum Phosphorylation:

10X Full- spectrum Phosphorylation:

The pY sites identified by antibody enrichment are 16.8 times deeper than those identified by IMAC enrichment.

More Comprehensive Research:

Full-spectrum phosphorylation identified 5,400 pY sites, with the proportion of pY sites increasing from 1.56% to 21.3% compared to IMAC.



Total number of phosphorylation sites in mouse brain samples: 25315

Reference Applications and Cases

nature

Nature: Tyrosine Phosphoproteomics Uncovers Pancreatic Cancer Therapeutic Targets and Biomarkers.
Targeting LIF-mediated paracrine interaction for pancreatic cancer therapy and monitoring

This study employs tyrosine phosphoproteomics and other methods to analyze the secreted factors of astrocytes and their impact on cancer cell signaling pathways, revealing that the phosphorylation activation of transcription factor STAT3 is a significant molecular change. The LIF secreted by astrocytes is a key paracrine factor that mediates unidirectional intercellular signaling through cancer cell surface receptors, and has been proven effective as a therapeutic target and biomarker in mouse models and clinical samples.

Cancer Cell

Cancer Cell: Tyrosine Phosphoproteomics Reveals the Molecular Mechanisms of Corticosteroid Therapy for Lymphoma.
Molecular targets of glucocorticoids that elucidate their therapeutic efficacy in aggressive lymphomas

This study used techniques such as tyrosine phosphoproteomics to investigate the molecular mechanism of glucocorticoid combination therapy for lymphoma in detail. It found that glucocorticoids can inhibit the progression of lymphoma by inhibiting the CSK-regulated BCR signaling pathway.

Cell

Cell: Full-spectrum Phosphoproteomics Reveals the Mechanism by Which EGFR Mutations in Lung Cancer Trigger Kinase Network Signal Remodeling.
Oncogenic Mutations Rewire Signaling Pathways by Switching Protein Recruitment to Phosphotyrosine Sites

This study measured the in vivo EGF-dependent signaling network in lung tissue, quantified tyrosine phosphorylation sites, and identified protein signaling complexes recruited by tyrosine phosphorylation sites in lung tissue through interaction proteomics, revealing the specific molecular mechanism by which EGFR oncogenic mutations lead to downstream phosphorylation signaling remodeling.

Science Translational Medicine

Sci Transl Med: Full-spectrum Phosphoproteomics Reveals the Sensitivity and Resistance Targets of Colorectal Cancer to EGFR Blockade.
Phosphoproteomics of patient-derived xenografts identifies targets and markers associated with sensitivity and resistance to EGFR blockade in colorectal cancer

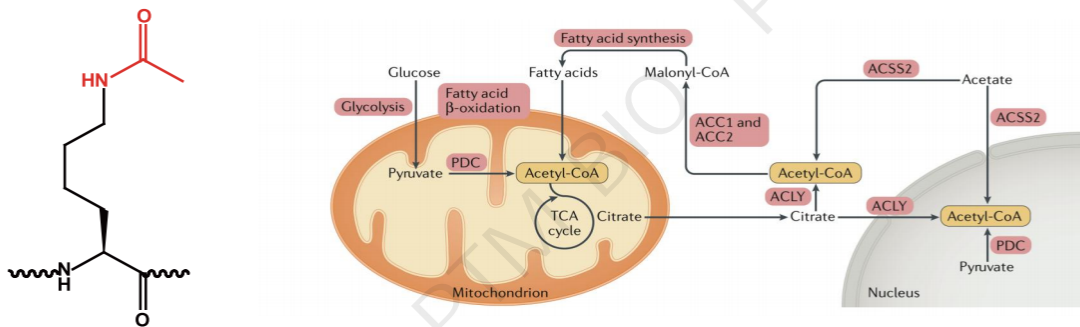
This study conducted proteomics and global phosphoproteomics on 30 patient-derived xenografts (PDX) of metastatic colorectal cancer (mCRC) with genomic and pharmacological characteristics to investigate the molecular basis of response to EGFR blockade and identified alternative drug targets to overcome resistance.

Acetylation

Service Overview

Acetylation is the most common type of acylation modification, referring to the process of transferring and adding the acetyl group of acetyl-CoA to the lysine residues of proteins under the catalysis of acetyltransferases.

Acetylation modifications are mainly divided into two categories: acetylation modifications at the protein N-terminus and acetylation modifications on protein lysine. N-terminal acetylation modifications mostly occur on proteins in eukaryotes and are catalyzed by N-acetyltransferases (NATs). Lysine acetylation modification is a reversible process, mainly catalyzed by lysine acetyltransferases (KATs) and lysine deacetylases (KDACs).



Schematic Diagram of Reversible Acetylation and Its Involvement in Metabolic Regulation

Potential Application Areas

① Functions of Acetylation:

Gene transcription, cell cycle, DNA damage repair, cell signaling, cytoskeletal remodeling, protein aggregation

② Acetylation Regulation of Enzymes:

Regulating enzyme activity, altering enzyme-substrate specificity

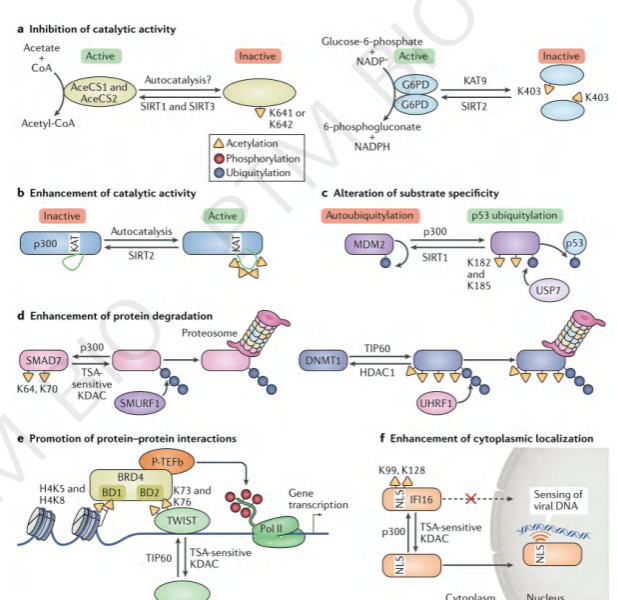
③ Protein Degradation Regulation:

It can regulate both proteasome-dependent and proteasome-independent protein degradation systems

④ Protein Interactions:

Promoting or inhibiting protein-protein interactions

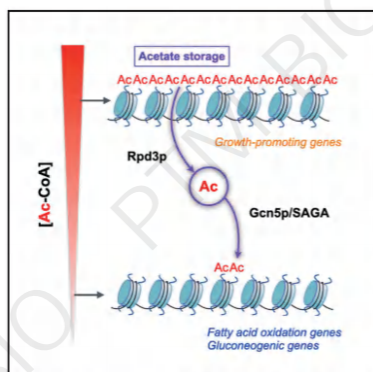
⑤ Adjust Subcellular Localization.



Reference Applications and Cases

Acetylation modification proteomics research primarily focuses on three aspects: epigenetics, signal transduction, and metabolic regulation.

- **Epigenetics:** Histone acetylation modification is an important epigenetic mechanism that plays a significant role in biological functions such as transcriptional regulation, chromatin dynamics, development and lifespan regulation, and cell fate determination.

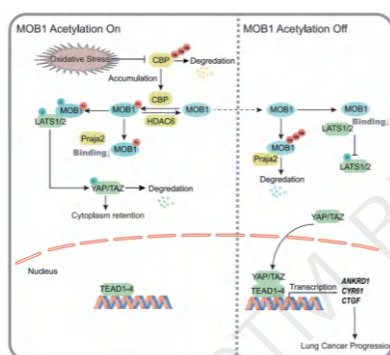


Molecular cell: Starvation Stress Induces Histone Acetylation Remodeling, Activating Gluconeogenesis and Fat Metabolism.

Glucose starvation induces a switch in the histone acetylome for activation of gluconeogenic and fat metabolism genes. *Molecular Cell*.

Using the yeast model, the transcriptional regulatory role of acetyl-CoA under starvation stress (glucose deprivation) was studied. It was found that starvation stress leads to a decrease in intracellular acetyl-CoA levels, thereby inducing a global restructuring of histone acetylation. Through the redistribution of acetyl groups, the acetylation and transcription of genes involved in gluconeogenesis and lipid metabolism were promoted. This study reveals the dynamic restructuring of histone acetylation under starvation stress and its novel regulatory mechanism on transcriptional networks.

- **Signal Transduction:** Histone acetylation modification is involved in various cellular processes and is also a major regulator of gene transcription, participating in gene transcriptional regulation, signal transduction, apoptosis, DNA damage repair, protein aggregation, and other processes.

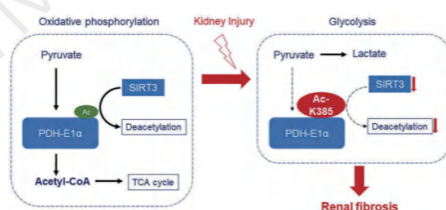


Nucleic Acids Res: Oxidative Stress Regulation Promotes MOB1 Acetylation, Activating the Hippo Signaling Pathway and Inhibiting Lung Cancer Cell Growth.

Oxidative stress-CBP axis modulates MOB1 acetylation and activates the Hippo signaling pathway. *Nucleic Acids Research*.

Oxidative stress stimulation, as a potential influencing factor in physiology and pathology, can promote the acetylation of MOB1 through upregulating acetyltransferase CBP, a process that does not depend on the activity of MST1/2 or the phosphorylation modification of MOB1. Thus, MOB1 acts as a signal strength sensor, undergoing varying degrees of acetylation in response to the intensity of upstream oxidative stress stimulation. It regulates downstream effectors YAP by controlling the activation level of LATS1/2, thereby modulating the Hippo signaling pathway.

- **Metabolic Regulation:** Metabolic regulation is the most important function of acetylation modification, as well as the most basic and important function of life. Tumor metabolism, growth and development, disease occurrence and progression, plant stress resistance, immune regulation, and other important biological processes are all closely related to acetylation modification.



Cell Death Dis: Sirt3 Regulates Mitochondrial Protein Deacetylation and Improves Renal Fibrosis.

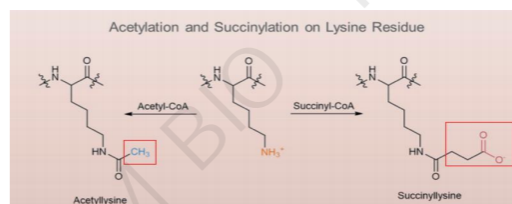
Sirtuin 3 regulates mitochondrial protein acetylation and metabolism in tubular epithelial cells during renal fibrosis. *Cell Death & Disease*.

This study is the first to analyze acetylation. As a key regulator of mitochondrial protein acetylation and metabolic homeostasis, Sirt3 participates in the regulation of mitochondrial energy metabolism during the progression of renal fibrosis through its deacetylation modification of PDH-E1 α , thereby inhibiting renal fibrosis. This study also reemphasizes the important role of acetylation modification in renal diseases and provides new directions for the development of targeted drugs to inhibit renal fibrosis.

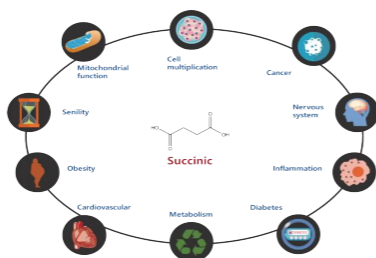
Succinylation

Service Overview

Succinylation refers to the process by which a succinyl group is covalently attached to a lysine residue through enzymatic or non-enzymatic methods. Compared to methylation and acetylation, succinylation modification can induce more changes in protein properties. This is because the lysine residue undergoing succinylation is given 2 negative charges, changing its valence from +1 to -1, which is higher than the charge change caused by acetylation (+1 to 0) and monomethylation (no change); Moreover, succinylation introduces a larger structural group, leading to greater alterations in protein structure and function.

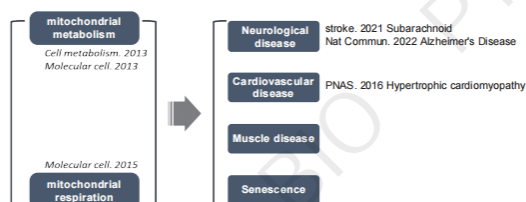


Potential Application Areas



Substrates for succinylation modification- succinic acid is a metabolite involved in mitochondrial respiration; apart from participating in energy metabolism, it has also been reported to be involved in various physiological processes, such as stress, inflammation, neuroregulation, and immunoregulation. Thereby affecting various diseases such as aging, obesity, tumor occurrence, cardiovascular diseases, and neurodegenerative diseases.

Succinylation modification is widely involved in biological processes and disease occurrence related to mitochondrial function, such as mitochondrial metabolism and mitochondrial respiration:



Beyond the biological processes involving succinate and those related to mitochondrial function, succinylation modification is also associated with the nervous system, inflammation, tumors, metabolism, nucleosome regulation, and more.

Succinylation Modification Regulatory Mechanisms

The regulation of succinylation modification is divided into non-enzymatic regulation and enzymatic regulation. The modifier succinyl-CoA primarily comes from the TCA cycle, amino acid metabolism, and the downregulation of SDH enzymes.

Non-enzymatic regulation of Ksucc

Modified substrate concentration (succinic acid, succinyl-CoA), mutagenesis of IDH and pH

Enzymatic regulation of Ksucc

Succinyltransferase KGDHC, KAT2A, CPT1A,

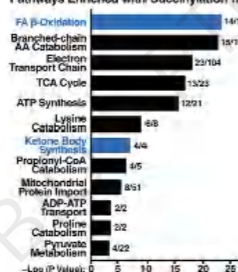
Desuccinylase CobB (Prokaryotes), SIRT5, SIRT7(Histone)

Reference Applications and Cases

Succinylation modification is widely involved in metabolic regulation, epigenetic control, signal transduction, and numerous other biological processes. Many metabolic enzymes in central and intermediate metabolism exhibit succinylation modification and are closely related to diseases such as tumors, cardiovascular system disorders, and inflammation.

► **Metabolic Regulation:** Succinylation modification plays a broad role in regulating cellular metabolism, being widely present in mitochondrial energy metabolism regulatory enzymes. It participates in regulating processes including the tricarboxylic acid cycle, multiple metabolic signaling pathways, including amino acid metabolism and fatty acid metabolism.

Pathways Enriched with Succinylation Targets

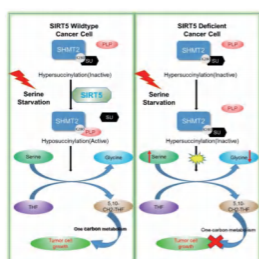


Cell Metabolism: SIRT5 Broadly Regulates Metabolic-Related Proteins.

SIRT5 regulates the mitochondrial lysine succinylome and metabolic networks. *Cell Metabolism*.

Sirtuins represent a highly class of deacetylase families from bacteria to humans. The recognized members of the human Sirtuin family include 7: SIRT1-SIRT7. Researchers have discovered through high-throughput quantitative proteomics that SIRT5 selectively removes succinylation at specific sites on multiple different proteins, which is associated with some basic metabolic signal pathways, including fatty acid oxidation, oxidative phosphorylation, and ketone body generation. In mitochondria, many proteins and signal pathways exhibit extensive succinylation. Lysine succinylation can have a significant impact on enzyme activity.

► **Tumor proliferation:** Rate-limiting enzymes in metabolic pathways such as glycolysis-TCA cycle-glutamate metabolism are subject to succinylation modification by proteins, thereby affecting the occurrence of diseases such as tumors and inflammation.

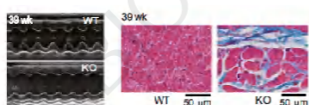


Cancer Research: SIRT5 Regulates the Succinylase levels of SHMT2 to Promote Tumor.

SHMT2 Desuccinylation by SIRT5 Drives Cancer Cell Proliferation. *Cancer Research*.

SHMT2 acts as a regulator of serine catabolism and one-carbon metabolism, with certain small-molecule compounds in serine catabolism and one-carbon metabolism regulation processes controlling cellular proliferation and intracellular redox metabolic balance. Under serine-starvation conditions, SIRT5 desuccinylates SHMT2, promoting the process where SHMT2 catalyzes serine cleavage into glycine while producing 5,10-CH₂-THF, thereby enhancing tumor cell proliferation.

► **Cardiovascular diseases:** Succinylation modification plays an important role in regulating energy metabolism and oxidative stress. Research has found that it is closely related to cardiovascular system diseases such as myocardial hypertrophy and atrial fibrillation.

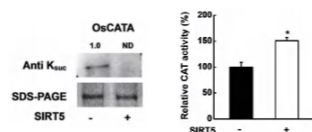


PNAS: Succinylation Reveals the Important Role of SIRT5 in Cardiac Physiological Function.

Metabolomics-assisted proteomics identifies succinylation and SIRT5 as important regulators of cardiac function. *PNAS*.

After Sirt5 knockout, the succinylation levels of fatty acid metabolism-related proteins on ECHA will be significantly increased, and this increase will lead to a decrease in ECHA activity. The decrease in ECHA activity will then affect the ATP levels in the heart. Ultimately, animal experiments demonstrated that the loss of Sirt5 can lead to dilated cardiomyopathy by affecting succinylation.

► **Plant Stress Responses:** Acetylation and succinylation modifications are involved in glycolysis, TCA cycle, and pentose phosphate pathway, thereby regulating plants' responses to stress.



Plant Cell Environ: Rice Responds to Abiotic Oxidative Stress by Regulating Acetylation and Succinylation Modifications.

Oxidative stress-triggered interactions between the succinyl- and acetyl-proteomes of rice leaves. *Plant Cell Environ*.

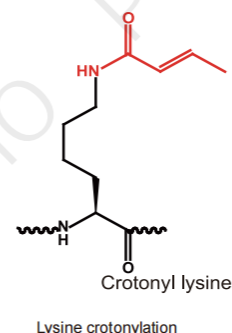
Under oxidative stress conditions, changes in the level of lysine succinylation modification affect the activity of rice metabolic enzymes.

Crotonylation

Service Overview

Crotonylation modification refers to a type of modification in which a crotonyl group is transferred to a lysine residue by histone crotonyltransferase (HCT) using crotonyl-CoA (Cr-CoA) as the donor. Crotonylation modification was first discovered by the research team of Professor Yingming Zhao at the University of Chicago in 2011, and the related research was honored as one of the top five research highlights of *Cell* in 2011.

Crotonylation is evolutionarily conserved and typically associated with transcriptionally active chromatin regions, playing a significant role in regulating gene expression. Crotonylation is also found to be closely related to cancer metabolism, DNA damage repair, stem cell differentiation, reproductive regulation, and other processes.



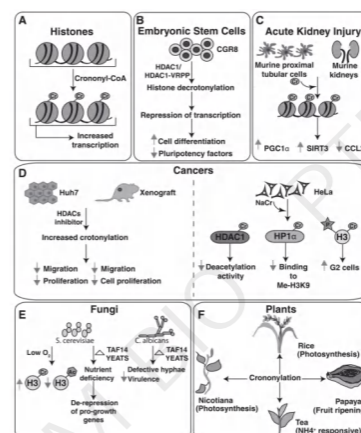
Potential Application Areas

Crotonylation Involves Biological Functions:

- Gene Expression and Transcription
- Reproductive Regulation
- Cell Cycle
- DNA Damage Response
- Telomere Maintenance and Aging
- Stem Cell Related

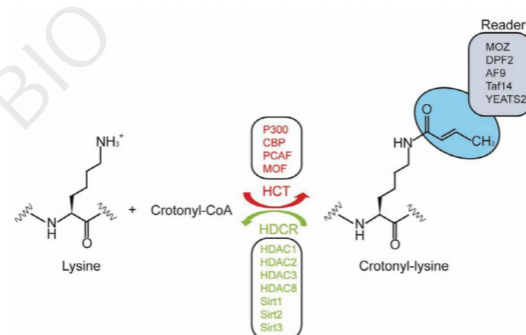
Crotonylation-associated diseases:

- Neurological disorders
- HIV
- Kidney-related diseases
- Hypertrophic cardiomyopathy
- Cancer-related



Regulatory Mechanisms Related to Crotonylation Modification

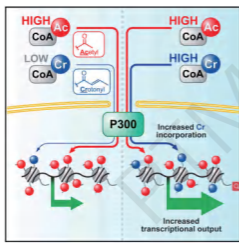
	Enzyme family	Members
Writers	p300/CBP	p300/CBP
	GNAT	Gcn5, PCAF
	MYST	MOF, HBO1, TIP60
Erasers	HDACs	Class I HDACs (1-3, 8)
	Sirtuins	SIRT1-3
Readers	bromodomain	
	YEATS domain	
	DPF domain(double PHD finger)	



Reference Applications and Cases

Proteomics of crotonylation modification is widely applied in research on transcriptional regulation, cancer metabolism, DNA damage repair, stem cell differentiation, and reproductive regulation, becoming a research hotspot in the life sciences field.

► **Epigenetics:** Histone crotonylation modification has been confirmed to maintain the activity of genes associated with meiosis metaphase sex chromosomes, among other functions.

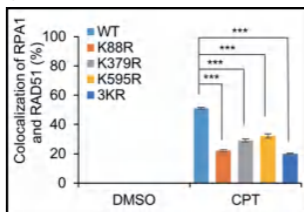


Molecular Cell: P300 Catalyzes Histone Crotonylation to Stimulate Transcription.

Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. *Molecular Cell.*

The coactivator p300 possesses both crotonyltransferase and acetyltransferase activities, and the histone crotonylation catalyzed by P300 directly stimulates transcription more effectively than histone acetylation. The level of histone crotonylation regulated by the intracellular concentration of crotonyl-CoA, and this state can change due to genetic and environmental variations. Local changes in histone acylation differences (i.e., between acetylation and crotonylation) can affect gene expression.

► **DNA Damage Repair:** Recent studies have found that crotonylated proteins widely participate in various biological processes, including RNA splicing, protein synthesis and degradation, DNA replication and repair, endocytosis, and intercellular junctions.

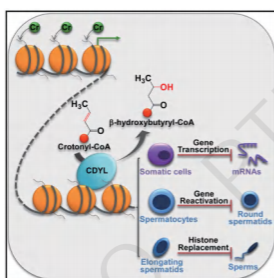


Science Advances: Crotonylation Analysis Reveals its Important Role in DNA Damage Repair.

Global crotonylome reveals CDYL-regulated RPA1 crotonylation in homologous recombination-mediated DNA repair. *Science Advances.*

Crotonylation data show that three sites on RPA1—K88, K397, and K595—are crotonylated; crotonylation of RPA1 does not affect its recruitment to DNA damage sites but can influence its binding with other HR factors (homologous recombination factors) like RAD51, thereby affecting RPA1's DNA damage repair function.

► **Reproductive Development:** Crotonylation modification is closely related to reproductive regulation, and it is highly abundant in post-meiotic sperm cells, concentrated on testis-specific genes on sex chromosomes.

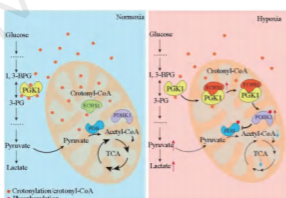


Molecular Cell: CDYL Regulates Histone Crotonylation, Affecting Spermatogenesis.

Chromodomain protein CDYL acts as a crotonyl-CoA hydratase to regulate histone crotonylation and spermatogenesis. *Molecular Cell.*

CDYL can regulate the level of histone crotonylation modification by reducing substrate concentration. In mouse assays, CDYL and histone crotonylation exhibit repulsive distribution, which aligns with their negative regulatory relationship. Mice overexpressing CDYL show a decrease in histone crotonylation levels, functionally participating in the reactivation and prolongation of round spermatid chromatin-related genes during spermatogenesis, and leading to a reduction in sperm quantity and decreased sperm vitality.

► **Tumor Metabolism:** Crotonylation modification is also closely related to the tumor formation process, participating in various signaling pathways and nuclear-related processes.



Nat Commun: Crotonylation Regulates Tumor Progression Mechanisms Under Hypoxia Conditions.

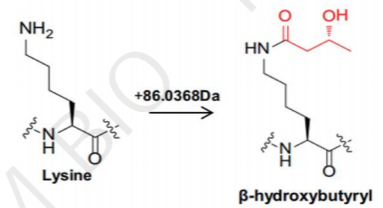
Hypoxia-induced downregulation of PGK1 crotonylation promotes tumorigenesis by coordinating glycolysis and the TCA cycle. *Nat Commun.*

This study, using crotonylation technology, revealed the promoting effect of decreased crotonylation levels of PGK1 (phosphoglycerate kinase 1) under hypoxic conditions on tumor occurrence and its molecular mechanism: hypoxia-induced upregulation of ECHS1 expression leads to reduced crotonyl-CoA levels in cells, simultaneously, PGK1 translocates to the mitochondria and interacts with ECHS1 to inhibit its own crotonylation levels, promoting interaction between PGK1 and PDHK1, resulting in upregulated phosphorylation of PDHK1, inhibiting pyruvate metabolism, promoting glycolysis, and thereby promoting the growth of breast cancer tumors.

β -hydroxybutyrylation

Service Overview

β -hydroxybutyrylation (Kbhb) is a novel protein acylation modification mediated by β -hydroxybutyric acid, first reported by the research team of Professor Yingming Zhao at the University of Chicago in 2016, and found to be closely related to fatty acid oxidation metabolism and energy metabolism regulation. Recent studies have shown its broad functions, participating in various biological processes such as tumorigenesis and DNA damage repair.



Potential Application Areas

Energy metabolism: Kbhb proteins are enriched in many core metabolic pathways, including the metabolism of the three major nutrients, glutathione metabolism, fatty acid oxidation metabolism, and others. Additionally, Kbhb proteins exhibit differences under conditions of nutrient deficiency or fasting in the body. Kbhb is closely related to energy metabolism and can be applied to research fields such as metabolic diseases and biological rhythm regulation.

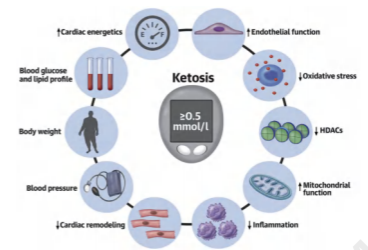
Cancer research: Kbhb plays important functions in metabolism, chromatin remodeling, and gene expression regulation. Recent studies have demonstrated that tumor suppressor proteins such as p53 are regulated by Kbhb.

DNA Damage Repair: Research has found that Kbhb is involved in the DNA repair process and can be applied in various DNA damage repair model studies (cancer radiotherapy/chemotherapy treatment, radiation damage, etc.).

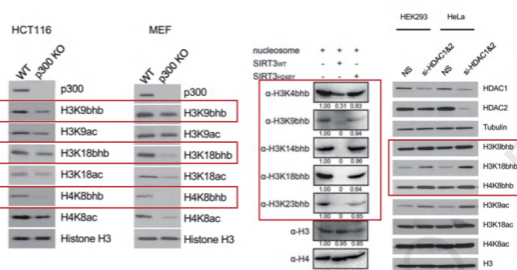
Other Molecular Level Studies: Studies show that multiple proteins can undergo Kbhb modification during processes such as chromatin remodeling, spliceosome activity, RNA metabolism, and transport.

Other Diseases: It has been reported that β -hydroxybutyrate can be one of the drugs used to treat epilepsy, and β -hydroxybutyrate is an indirect substrate of Kbhb modification, therefore Kbhb may also be involved in the pathogenesis, development, and treatment of epilepsy.

Crosstalk with Other Modifications: Research reports that some important regulatory enzymes involved in other modification processes (acetylation, SUMOylation, ubiquitylation, etc.) can undergo Kbhb modification, and the crosstalk between modifications can also serve as a new area for exploration.



β -hydroxybutyrylation modification regulatory mechanisms



Kbhb writer

p300

Kbhb eraser

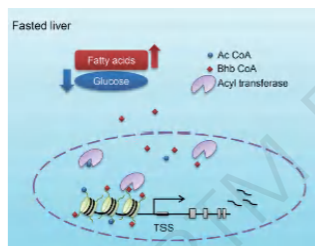
SIRT3
HDAC1/2

Kbhb reader

Specific reader has not been reported

Reference Applications and Cases

- **Epigenetics:** The body can regulate gene transcription through histone β -hydroxybutyrylation modifications, helping it quickly adapt to changes brought by the environment.



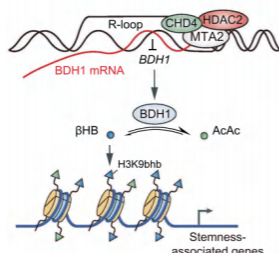
Molecular Cell: Prolonged Fasting Significantly Induces Kbhb Modification of Histones, Thereby Upregulating Genes Related to Starvation-responsive Metabolic Pathways.

Metabolic Regulation of Gene Expression by Histone Lysine β -hydroxybutyrylation. *Molecular Cell*.

Under starvation conditions, the proportion of carbohydrates as energy sources gradually decreases, while the level of ketone bodies produced by fat gradually increases. Meanwhile, the level of histone lysine 3-hydroxybutyrate modification in liver cells significantly increases, while the acetylation modification level, primarily derived from carbohydrate metabolism, does not change significantly.

Further chromatin immunoprecipitation experiments and gene expression sequencing revealed that the upregulation of histone β -hydroxybutyrylation modifications was accompanied by the expression of some genes related to the physiological response to starvation, such as amino acid metabolism, fatty acid metabolism, redox homeostasis, and circadian regulation.

- **Tumor proliferation:** Ketones play a role in cancer biology, β -hydroxybutyrate (B-OHB) is involved in the development of melanoma, androgen-dependent prostate cancer, breast, cervical, and liver cancers.



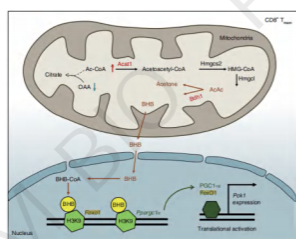
Signal Transduct Target Ther: β -hydroxybutyrylation Modification Promotes the Proliferation of Liver Cancer Stem Cells.

MTA2 triggered R-loop trans-regulates BDH1-mediated β -hydroxybutyrylation and potentiates propagation of hepatocellular carcinoma stem cells. *STTT*.

A complex composed of apart of the NuRD complex, specifically MTA2-HDAC2-CHD4, was discovered, revealing that MTA2 can interact with HDAC2/CHD4 and transcriptionally repress BDH1 through R-loops, leading to the accumulation of β HB and an increase in H3K9bhb, thereby producing a cascade effect that promotes HCC formation and progression.

It further confirms that abnormal metabolism and the microenvironment are also important conditions for tumor formation and development, highlighting the significant research value of post-translational modifications, especially novel acylations like β -hydroxybutyrylation, lactylation, and crotonylation, which are closely related to metabolism, in tumor metabolism.

- **Immune system:** The main product of ketone body metabolism, β -hydroxybutyrate, acts as an epigenetic regulatory factor in the development, maintenance, and long-term survival of memory T cells.



Nature Cell Biology: β -hydroxybutyrate Modification Promotes Memory T Cell Development.

Ketogenesis-generated β -hydroxybutyrate is an epigenetic regulator of CD8⁺ T cell memory development. *Nature Cell Biology*.

In CD8⁺ memory T cells, there exists ketone body-derived β -hydroxybutyrate, and the accumulation of β -hydroxybutyrate leads to the upregulation of FoxO1 and PGC-1 α gene histone H3 undergoes bhb modification at K9, thereby upregulating the expression of FoxO1 and PGC-1 α .

FoxO1 and PGC-1 α synergistically upregulate Pck1 expression, thereby guiding carbon along the gluconeogenesis pathway towards glycogen and the pentose phosphate pathway.

- **Tumor Development:** β -hydroxybutyrylation modification, in addition to modifying histones to participate in epigenetic regulation, can also regulate protein activity in processes such as metabolism, tumor development, and progression by modifying non-histone proteins.

Cell Death & Disease : P53 β -hydroxybutyrylation Attenuates Its Tumor Suppressor Function.

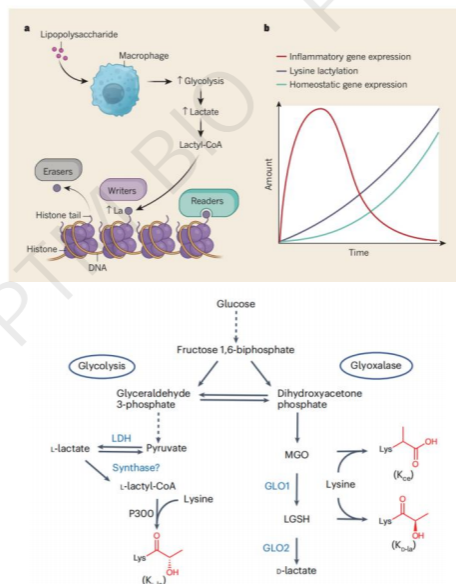
β -hydroxybutyrylation attenuates p53 activity. *Cell Death & Disease*.

L-/D- lactylation

Service Overview

Lactylation modification (Lactylation, K1a) is a newly discovered type of post-translational modification led by Professor Yingming Zhao of the University of Chicago in 2019. The study found that lactate accumulated during metabolic processes can serve as a precursor to cause histone lysine lactylation modification and participate in the homeostatic regulation of M1 macrophages in bacterial infections, regulating diseases such as inflammation and cancer. Related research results were published in *Nature*. This groundbreaking research has broadened the boundaries of metabolic regulation and sparked a surge of research into lactylation.

In 2024, the research team collaborated with PTM BIO, successfully distinguishing and identifying three types of lactylation isomers, namely: L-lactylation modification (KL-1a), D-lactylation modification (KD-1a), and N-ε-carboxyethylated modification (Kce). Among them, L-lactylation modification is the earliest studied and most extensively researched type of lactylated modification. It is a major responder to glycolysis and the Warburg effect in eukaryotic organisms, widely present in various tissues and organs of the body, playing a crucial role and exerting significant effects in fields such as tumors, cardiovascular diseases, brain diseases, and digestive system disorders.

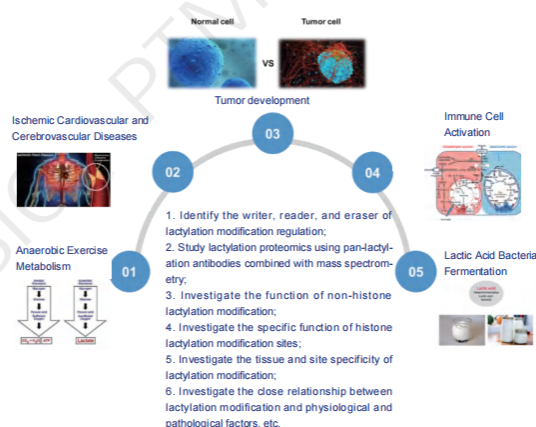


Potential Application Areas

Given that lactic acid metabolism is a metabolic pathway widely regulated and playing an important role in numerous physiological and pathological processes, the newly discovered acylation modification can provide a new idea and regulatory mechanism for further in-depth and extensive research.

PTM BIO, as the world's first company to originally develop lactylation pan-antibodies and modification detection services, can assist in the following research directions:

- ① Mechanism research: Revealing the enzymes that catalyze the production of lactyl-CoA, the site-specific functions of histone lactylation modification, and the 'writer', 'eraser', and 'reader' of histone lactylation modification.
- ② Physiological and pathological functions: Lactate is a widely existing metabolite, and the novel histone modifications mediated by lactate will play important roles in both biological processes such as DNA damage repair and muscle movement, as well as pathological processes like inflammation and cancer.

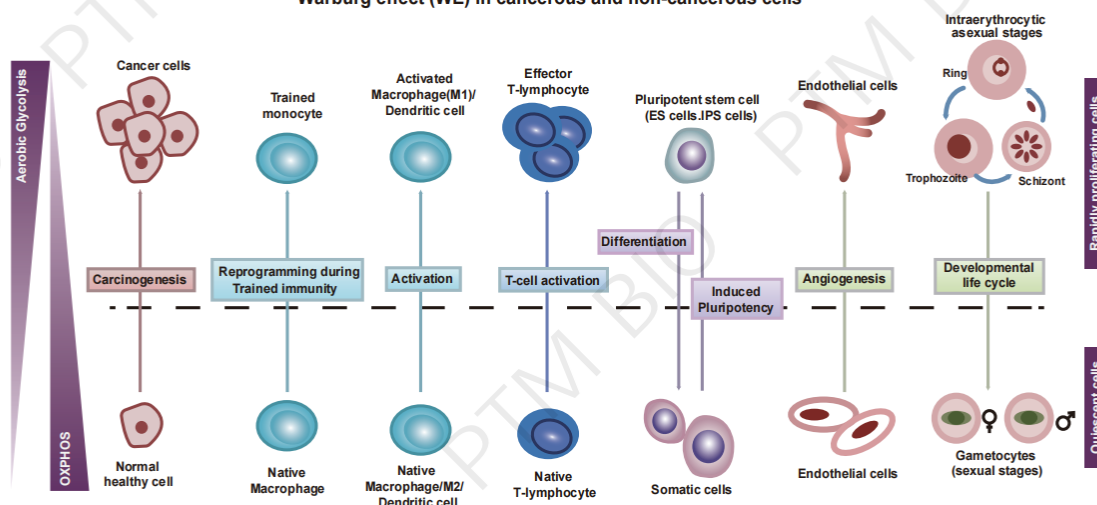


The Important Role of Lactic Acid Metabolism in Physiology

Lactic acid was once considered a 'waste' product of anaerobic metabolism, but it is now proven to serve as a major recyclable carbohydrate fuel in mammals, playing a role in metabolic regulation.

Under aerobic conditions, tumor cells tend to obtain energy through glycolysis, resulting in a large accumulation of lactic acid, a phenomenon known as the Warburg effect. This effect also plays a central role in the development of rapidly proliferating parasites (such as *Plasmodium* and *Toxoplasma*). Furthermore, the Warburg effect can be observed in large number of rapidly proliferating cells under physiological and pathological conditions, such as in T cell activation, macrophage polarization, malaria life cycle development, angiogenesis, embryonic stem cell differentiation, and induced pluripotent stem cell generation.

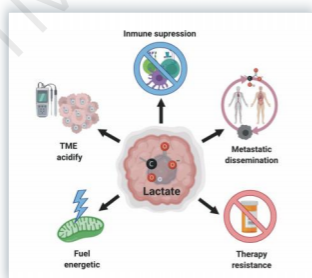
Warburg effect (WE) in cancerous and non-cancerous cells



Hotspots of Lactylation Modification

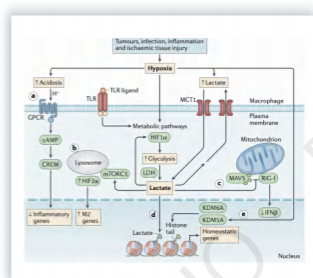
Tumor Microenvironment

Lactic acid can promote tumor progression: high concentrations of lactic acid are transported into cells as fuel substrates and metabolized. Lactic acid promotes tumor invasion and metastasis. Lactic acid is essential for tumor angiogenesis. The immunosuppressive function of lactic acid, etc.



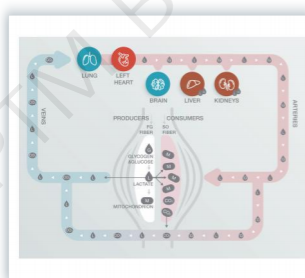
Inflammation and Immune Regulation Research

Lactic acid regulates tumor progression, tumor immunity, suppresses hypoxia-induced macrophage activation, and the progression of various diseases through lactylation modification. Its synergistic mechanisms with inflammatory pathways will be a key research direction.



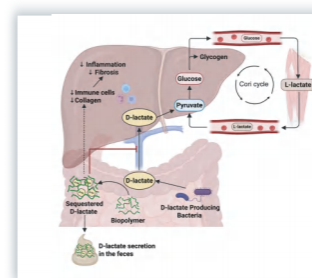
Mechanism Research of Cardiovascular Disease

Elevated lactic acid in the blood indicates insufficient oxidation and is associated with the diagnosis and prognosis of hypertension and acute heart disease, whether lactate accumulation during ischemia triggers cardiocerebral injury and its mechanisms remain to be elucidated.



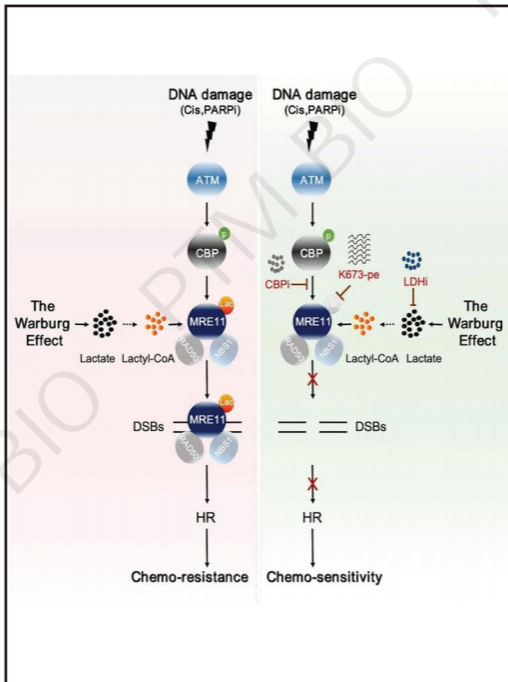
D-lactylation

L-lactic acid is a product of metabolism in certain bacteria. Abnormal L-lactic acid metabolism is closely related to the occurrence and development of diseases such as immune-suppressive tumor microenvironments in short bowel syndrome, acute neural injury, and hepatocellular carcinoma, playing an important role in intestinal diseases, brain diseases, and tumors.



Featured Collaboration Cases

► **Keywords:** Lactylation Modification Proteomics, DNA Damage Repair, Chemoresistance



Cell: Metabolic Regulation of Homologous Recombination Repair by MRE11 Lactylation

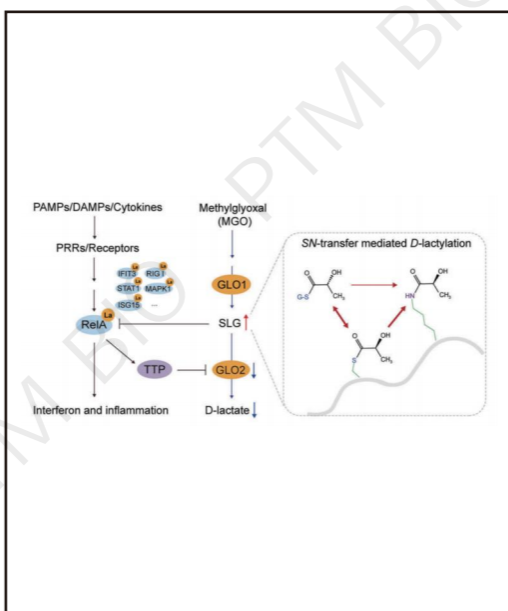
Main Conclusions of the Study:

- ① TCGA analysis showed that low LDHA expression was associated with higher homologous recombination deficiency (HRD) scores. Cellular studies further demonstrated that increased protein lactylation enhanced homologous recombination repair.
- ② L-lactylation modification proteomics profiling identified the homologous recombination repair protein MRE11 and its lactylation site. Further validation demonstrated that CBP mediates MRE11 K673 lactylation.
- ③ Mechanistically, MRE11 lactylation enhances its DNA binding, promoting DNA end resection and facilitates homologous recombination repair.
- ④ Inhibiting MRE11 lactylation increased tumor cell sensitivity to chemotherapy, highlighting a potential mechanism of chemoresistance.

References

Yuping Chen, et al. 2024. Metabolic Regulation of Homologous Recombination Repair by MRE11 Lactylation. *Cell*.

► **Keywords:** D-lactylation, immune response



Cell Research: D-lactylation Regulates the Mechanism of Immune-inflammatory Response.

Nonenzymatic lysine D-lactylation induced by glyoxalase II substrate SLG dampens inflammatory immune responses. *Cell Research*.

Main conclusions of the study:

- ① The study found that GLO2 downregulation is most significant in macrophages, confirming that GLO2 may play a role in immune homeostasis and immune responses.
- ② The study, based on the pan-antibodies for L-/D-lactylation and carboxyethyl modification provided by PTM BIO, confirmed that GLO2 downregulation induces SLG accumulation and D-lactylation modifications.
- ③ Lactylation proteomics and L-/D-lactylation and carboxyethyl modification pan-antibodies confirmed that RelA undergoes D-lactylation modification, which inhibits NF-κB activation, thereby suppressing immune activation and inflammatory signal transduction.
- ④ Mouse model experiments show that the GLO2 feedback axis coordinates immune activation and inflammation both *in vitro* and *in vivo*.

References

Qihang Zhao, et al. 2025. Nonenzymatic lysine D-lactylation induced by glyoxalase II substrate SLG dampens inflammatory immune responses. *Cell Res*.

Pyruvylation

Service Overview

Pyruvate is the end product of glycolysis and a key entry point into multiple downstream metabolic pathways. As a central metabolic intermediate, pyruvate plays critical roles in cellular energy metabolism, tumor metabolic reprogramming, and the coordination of carbohydrate, lipid, and amino acid metabolism.

A Metabolic Switch for Cellular Energy Production

The fate of pyruvate is largely determined by oxygen availability in the cellular microenvironment. Under aerobic conditions, pyruvate enters mitochondria and fuels the tricarboxylic acid cycle and oxidative phosphorylation, supporting efficient ATP production in differentiated cells. Under hypoxic or anaerobic conditions, pyruvate remains in the cytosol and is reduced to lactate through anaerobic glycolysis. Although this pathway is less energy efficient, it allows cells to rapidly generate energy and maintain survival under oxygen-limited conditions.

A Central Node in Tumor Metabolic Reprogramming

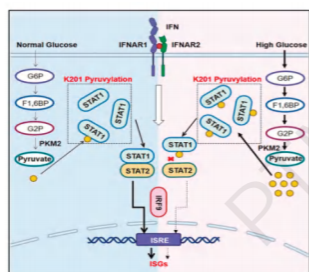
Pyruvate is also a key metabolic node in the Warburg effect. Even in the presence of sufficient oxygen, tumor cells preferentially convert a large proportion of pyruvate into lactate through aerobic glycolysis, while only a limited fraction enters mitochondrial metabolism.

A Bridge Connecting Major Metabolic Pathways

Beyond glycolysis, pyruvate connects multiple branches of cellular metabolism. It serves as the starting point for gluconeogenesis, can be converted into alanine through transamination to participate in amino acid metabolism, and can be converted into acetyl-CoA to support lipid biosynthesis.

Reference Applications and Case Studies

► Immune Regulation: Pyruvylation Links Metabolism to Antiviral Immunity



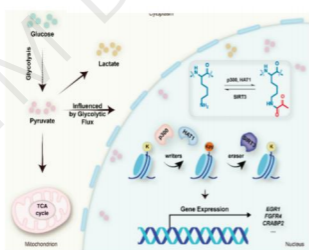
Cell : Pyruvylation Reveals a New Metabolism–Immunity Regulatory Mechanism

Pyruvate is a natural suppressor of interferon signaling by inducing STAT1 protein pyruvylation. *Cell*.

This study identified lysine pyruvylation as a novel protein post-translational modification. It showed that high-glucose conditions enhance glycolytic activity and promote pyruvylation of STAT1 at lysine 201 (**STAT1-K201Pyr**), disrupting the STAT1–STAT2 interaction and suppressing type I interferon signaling and antiviral immunity.

These findings reveal a new mechanism by which cellular metabolism directly regulates immune responses.

► Epigenetic Regulation: Pyruvylation Connects Glycolysis with gene expression



Nature Metabolism : Lysine Pyruvylation and Its Regulatory Mechanism

Lysine pyruvylation couples glycolytic flux to epigenetic regulation. *Nature Metabolism*.

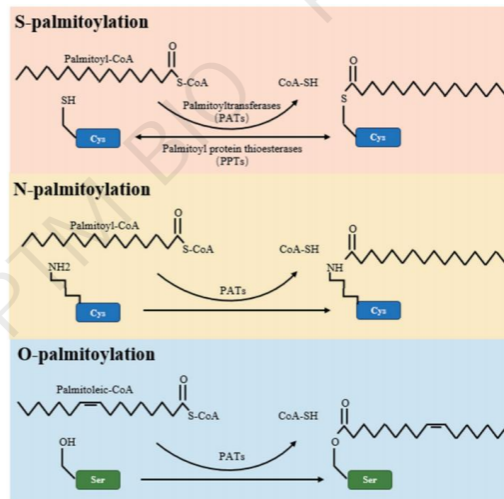
This study systematically identified and characterized lysine pyruvylation (Kpy), a pyruvate-driven protein post-translational modification derived from glycolytic metabolism. The work revealed its regulatory mechanism and demonstrated that Kpy is involved in transcriptional activation.

These findings provide a new perspective on how metabolic flux can directly influence epigenetic regulation and gene expression.

Palmitoylation

Service Overview

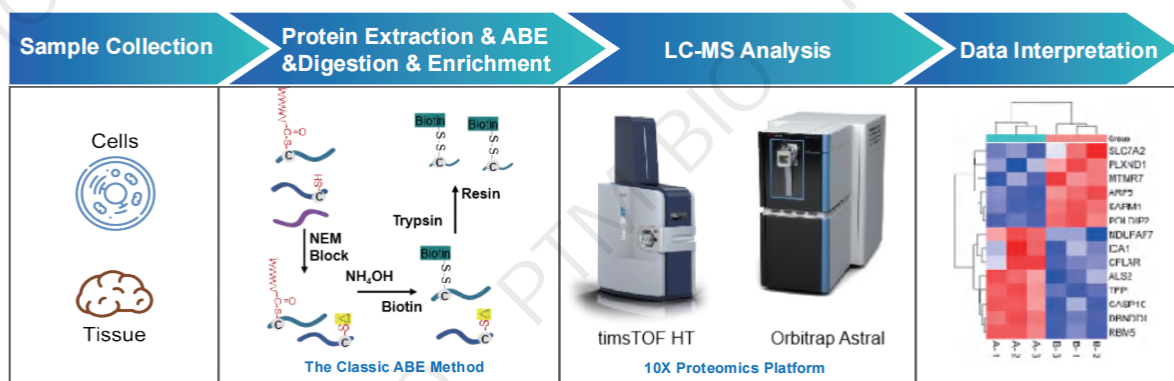
Palmitoylation modification is a common fatty acid modification, mainly consisting of three types: palmitoyl groups connected to glycine or cysteine via amide bonds, known as N-palmitoylation. Palmitoyl groups connected to serine via ester bonds, known as O-palmitoylation, and palmitoyl groups covalently linked to cysteine via thioester bonds, known as S-palmitoylation. Among these, S-palmitoylation is the most common, and due to its covalent bonding, it exhibits reversibility, allowing dynamic regulation of protein function and influencing life activities. Under normal physiological conditions, S-palmitoylation maintains a dynamic balance, whereas in disease conditions (such as cancer, immune disorders, and neurodegenerative diseases), the disruption of this dynamic balance is often closely associated with disease onset and progression.



PTM BIO palmitoylation proteomics products, employing the classic ABE (Acyl-Biotin Exchange) method and combining with the 10X high-depth mass spectrometry platform, enable high-depth detection and analysis of palmitoylation in various biological samples such as tissues and cells, assisting researchers in deciphering the regulatory mechanisms of palmitoylation in life activities.

Technical Principles and Procedures

PTM BIO's S-palmitoylation profiling workflow is based on the classical acyl-biotin exchange (ABE) method. Samples are first treated with N-ethylmaleimide (NEM) to block free thiol groups. Hydroxylamine (NH_2OH) is then used to selectively cleave S-palmitoyl thioester bonds, exposing newly available cysteine thiols. These thiols are labeled with HPDP-biotin, followed by protein digestion and streptavidin-based enrichment of biotinylated peptides. The enriched peptides are subsequently analyzed by high-resolution LC-MS/MS on the 10X Proteomics platform to identify S-palmitoylated proteins and modification sites.

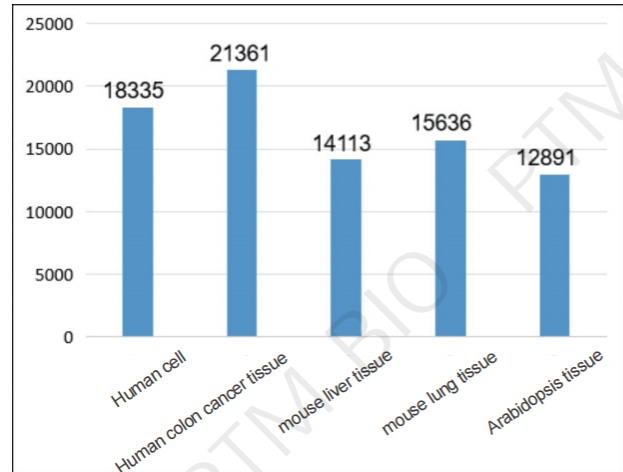


Palmitoylation Technical Route



Technical Advantages

1. Number of human cell/tissue palmitoylation modification sites: 18,000-22,000
2. Number of mouse-derived palmitoylation modification sites: 14,000-16,000
3. Number of plant-derived palmitoylation modification sites: 13,000



Reference Applications and Cases

nature

Nature: A New Mechanism of STAT3 Palmitoylation in T_H17 Cell Differentiation.

A STAT3 palmitoylation cycle promotes T_H17 differentiation and colitis

The balance of STAT3 palmitoylation (DHHC) and depalmitoylation (APT2) promotes T_H17 cell differentiation. In mouse models, knockout of DHHC7 or APT2 can both alleviate colitis development.

nature

Nature: GSDMD Palmitoylation in Pyroptosis: A New Mechanism.

ROS-dependent S-palmitoylation activates cleaved and intact gasdermin D

GSDMD undergoes S-palmitoylation at Cys191, and palmitoylation is essential for the transmembrane pore, as even uncleaved GSDMD can form pores on the cell membrane to induce pyroptosis after S-palmitoylation.

Science

Science: New Mechanism of NOD1 and NOD2 Palmitoylation in Bacteria-Mediated Inflammation.

Palmitoylation of NOD1 and NOD2 is required for bacterial sensing

The two important receptor proteins in the NLR family, NOD1 and NOD2, can undergo palmitoylation modification under the action of palmitoyltransferase ZDHHC5, thereby mediating the activation of bacterial inflammatory signaling pathways.

Molecular Cell

Molecular Cell: Mitochondrial Protein CPT1A Responds to Different Stimulus Signals to Regulate the Molecular Mechanism and Function of MAVS Protein S-palmitoylation Modification.

CPT1A induction following epigenetic perturbation promotes MAVS palmitoylation and activation to potentiate antitumor immunity

CPT1A promotes S-palmitoylation of MAVS at Cys79, subsequently affecting the ubiquitination modification of K48 and K63, promoting MAVS stability and activation, thereby maintaining the IFN-I response and enhancing the antitumor immune effects of epigenetic intervention.

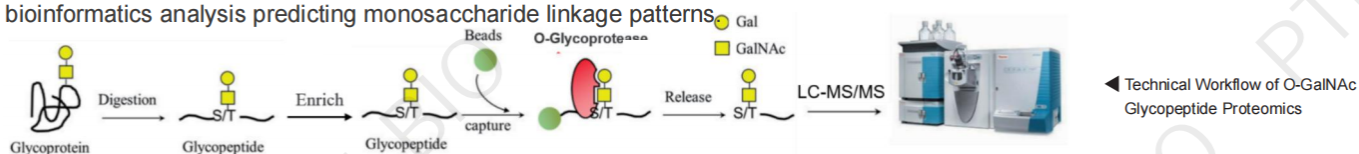
Intact O-GalNAc Glycopeptide

Service Overview

The main forms of protein glycosylation are N-glycosylation and O-glycosylation, among which the O-GalNAc modification linked to oxygen is the most abundant type of O-glycosylation on membrane and secretory proteins. O-GalNAc modification affects protein folding, stability, transport, and protein interactions, participating in physiological processes such as inflammation, pathogen immune evasion, cell adhesion, migration, and apoptosis. Current research has shown that abnormal protein O-GalNAc modification is closely related to the occurrence and development of pathogen infections, tumors, autoimmune diseases, metabolic diseases, cardiovascular diseases, and neurodegenerative diseases.

Indeed, the numerous biological functions of O-GalNAc modification and its close association with various diseases have gradually made it one of the forefront hotspots in glycomics research. However, due to the complexity of O-GalNAc modification sites and glycan structures, as well as the lack of effective purification strategies for these modified peptides, O-GalNAc detection and analysis based on mass spectrometry have always presented significant challenges. This has greatly limited related research on O-GalNAc functions. Therefore, there is an urgent need to identify O-GalNAc proteins and sites, and their important biological significance and regulatory functions also urgently require further exploration.

In recent years, PTM BIO has successively launched three glycosylation detection services: N-glycosylation profiling, O-GlcNAc modification profiling, and intact N-glycopeptide profiling. Building on this foundation, we have continuously overcome technical challenges and optimized experimental methods, now proudly launching intact O-GalNAc glycopeptide profiling. Without enzymatic deglycosylation, it enables simultaneous qualitative and quantitative analysis of both sites and glycan types, with bioinformatics analysis predicting monosaccharide linkage patterns.



Reference Applications and Cases

nature chemical biology

Nat Chem Biol: O-GlcNAc Modification Regulates a New Mechanism of Nucleotide de novo Synthesis in Tumor Cells.
Direct stimulation of de novo nucleotide synthesis by O-GlcNAcylation

This study reports a novel mechanism by which abnormal glucose metabolism in tumor cells causes increased O-GlcNAc modification. This enhances PRPS1 enzyme activity by promoting the formation of PRPS1 hexamers and reducing the feedback inhibition of PRPS1 by ADP/GDP, thereby promoting nucleotide synthesis, tumor growth, and radiochemotherapy resistance in tumor cells.

JMCC
JOURNAL OF MOLECULAR
CELLULAR CARDIOLOGY

J Mol Cell Cardiol: GALNT4 Promotes Atherosclerosis Development by Regulating PSGL-1 O-GalNAc Glycosylation.
GALNT4 primes monocytes adhesion and transmigration by regulating O-Glycosylation of PSGL-1 in atherosclerosis

This study demonstrates that during the development of atherosclerosis, upregulation of GALNT4 expression in monocytes promotes PSGL-1 O-GalNAc modification, further enhancing monocyte adhesion to the vascular wall, leading to vessel stenosis and accelerating the development of atherosclerotic cardiovascular disease.

Biochim Biophys Acta Gen Subj

Biochim Biophys Acta Gen Subj: O-GalNAc Modification of Amyloid Precursor Protein APP Promotes A β Production.
Comprehensive analysis of O-Glycosylation of amyloid precursor protein (APP) using targeted and multi-fragmentation MS strategy

This study developed a method for analyzing targeted intact O-glycopeptides based on a combination of mass spectrometry multi-fragmentation. Researchers used this method to precisely map the O-glycosylation modification sites and glycan structures of the APP protein, providing new insights for understanding the molecular function of APP and the pathogenesis of AD, identifying AD treatment targets, and developing early diagnostic strategies for AD at the protein glycosylation modification level.

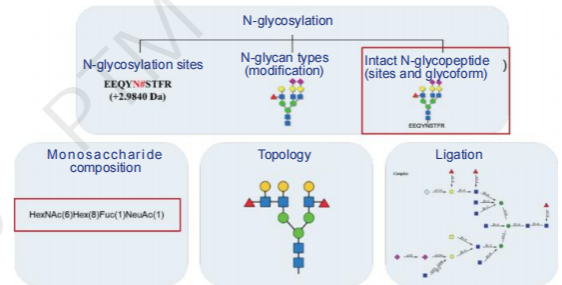
Intact N-glycopeptide Analysis

Service Overview

Glycosylation modification is one of the most common post-translational modifications of proteins, playing a pivotal role in maintaining normal physiological functions in the human body, and has always been a key focus of modification proteomics research. In practical applications, biomarkers for diagnosing various diseases and protein drugs are glycoproteins, with a large number of glycoproteins of significant and special functions and their correlations with diseases urgently awaiting in-depth analysis. Due to its high value, glycoproteomics was listed as a ***Nature Methods*** 'Method to Watch' in 2021.

However, the identification of glycoproteins remains the most challenging aspect of proteomics to date. The diversity of glycan types and the complexity of glycan structures are the main obstacles hindering glycoproteomics research. Traditional N-glycoproteomics studies commonly employ methods that remove the glycans, separating the glycan chains from the modified peptides. While this strategy reduces analytical complexity, it also loses important corresponding relationship information between the glycan chains and the glycosylation sites of the proteins.

Our intact N-glycopeptide proteomics products provide comprehensive information on glycoproteins, glycosylation sites, and glycan types, helping to obtain corresponding relationship information between glycan chains and glycosylation sites. This offers a more powerful research tool for biomarker discovery and disease mechanism studies.



Intact N-glycopeptide proteomics analysis provides comprehensive glycan information at glycosylation modification sites

Product and Sample Types

- ① **Complete Information** | Provides information on glycoproteins, glycosylation modification sites, and complete glycan information
- ② **High Sensitivity** | Utilizes QE Orbitrap Astral ultra-high resolution mass spectrometer
- ③ **Quality Assurance** | Validated in large-cohort tissue studies
- ④ **In-depth Analysis** | Innovative multi-omics analysis for in-depth information mining

Intact N-Glycopeptide

Intact Glycan Information

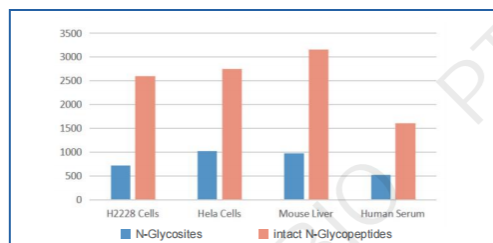
Glycoprotein Information

Glycosylation Modification Site Information

Applicable Samples

Human Mammal	Cell Samples	Fresh Tissue Sample	Blood (serum/plasma)

Internal Data

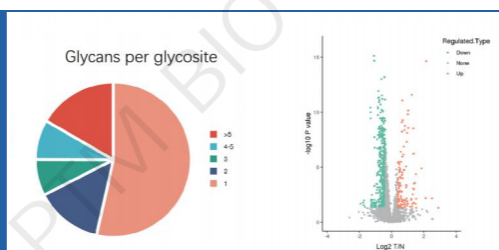


Routine Cells/ Tissues:

- 1 | Identified **800-1000** N-glycosylation Sites
- 2 | Identified **2400-2600** Intact N-glycans

Large-Cohort Project Results:

- 1 | Identified N-glycosylation Sites **3400+**
- 2 | Identified Intact N-glycopeptides **12000+**
- 3 | Quantifiable Proportion Exceeds **80%**

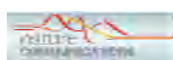


Reference Applications and Cases

Cell

Cell: Potential Therapeutic Targets and Early Diagnostic Markers for Pancreatic Cancer.
Proteogenomic characterization of pancreatic ductal adenocarcinoma

This study employed comprehensive N-glycoproteomics to compare the molecular characteristics of PDAC, NATs, and normal ductal tissues. The findings revealed that 75 N-linked glycoproteins were upregulated in PDAC compared to NATs, with the upregulated N-linked glycoproteins in tumors primarily modified by sialylated and/or fucosylated complex glycans, while the downregulated N-linked glycoproteins were mainly modified by low-mannose glycans. Sialylation and/or fucosylation of IGP showed a positive correlation with the expression of glycosyltransferases, suggesting that inhibiting the activity of related glycosyltransferases may represent a potential therapeutic strategy for PDAC.



Nat Commun: Intact N-glycopeptide Proteomics Reveals Subtypes and Clinical Prognosis of High-Grade Serous Ovarian Cancer.

Glycoproteomics-based signatures for tumor subtyping and clinical outcome prediction of high-grade serous ovarian cancer

This study conducted a comprehensive N-glycopeptide modification proteomics analysis on 119 HGSC tissue samples. Clustering analysis divided all samples into three tumor subtypes, and N-glycosylation structures showed a high correlation with high-grade serous ovarian cancer molecular subtypes, such as the correlation between fucosylation and mesenchymal subtypes. Further survival analysis revealed that glycoprotein glycan type information (e.g., sialylated glycan types, high-mannose types) was associated with poor clinical prognosis in HGSC patients.



Sci Adv: Intact N-glycopeptide Proteomics Reveals Mechanisms and Biomarker Research for Alzheimer's Disease.

Integrative glycoproteomics reveals protein N-glycosylation aberrations and glycoproteomic network alterations in Alzheimer's Disease

This study conducted the first large-scale, locus-based comprehensive N-glycosylation modification genomics mapping on the brains of AD patients and healthy individuals, depicting a systematic panorama of human brain N-glycoproteins and N-glycosylation sites, and identifying disease-specific changes in N-glycopeptides, N-glycoproteins, and N-glycosylation sites in AD. The study revealed the role of abnormal N-glycosylation in the pathogenesis of AD, providing new insights for understanding and treating AD.



Theranostics: Intact N-glycopeptide Proteomics Reveals TSTA3 Promotes Esophageal Cancer Development by Inducing Glycosylation.

TSTA3 facilitates esophageal squamous cell carcinoma progression through regulating fucosylation of LAMP2 and ERBB2

This study employs complete N-glycopeptide proteomics from PTM BIO to discover that high expression of TSTA3 is typically associated with the progression of esophageal squamous cell carcinoma and poor clinical prognosis, while its knockdown in cell lines can inhibit tumor cell invasion and metastasis by overall reducing fucosylation levels. Additionally, fucosylation of LAMP2 and ERBB2 proteins plays an important regulatory role in the process of TSTA3 controlling tumor progression.

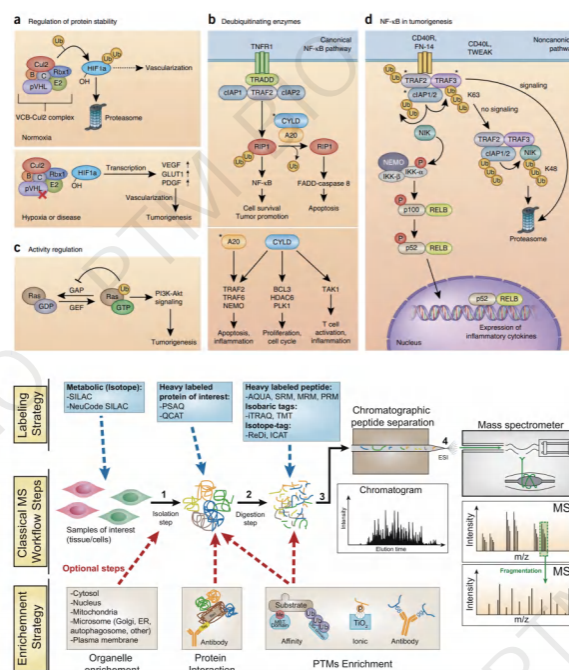
Ubiquitylation

Service Overview

Ubiquitylation refers to the process by which one or more ubiquitin molecules (a polypeptide composed of 76 amino acids) act under the action of a series of specific enzymes to classify proteins within the cell, select target protein molecules, and perform specific modifications on the target proteins.

Ubiquitylation modification is an important post-translational modification of proteins, playing a crucial role in protein regulation. First, the ubiquitin-proteasome degradation pathway is the most important protein degradation pathway in eukaryotic cells, involved in various physiological processes, including signal transduction, transcriptional regulation, cell cycle, proliferation-apoptosis, DNA damage repair, inflammation and immunity, and is closely related to the onset of many diseases such as tumors and cardiovascular diseases.

Modified proteomics is a new tool for studying ubiquitylation. Based on proprietary highly specific protein modification antibodies and leading mass spectrometry clusters, PTM BIO provides you with high-quality technical services.



Reference Applications and Cases

► **Physiological Mechanism Research:** Ubiquitylation modification is involved in the regulation of almost all life activities, including signal transduction, transcriptional regulation, cell cycle, proliferation-apoptosis, DNA damage repair, inflammation and immunity.



Nat Immunol: Ubiquitinomics Reveals Non-degradative Ubiquitination Modifications in T cell Activation. Integrative proteomics reveals an increase in non-degradative ubiquitylation in activated CD4(+) T cells. *Nature Immunology*.

► **Pathology Research:** Ubiquitylation modifications regulating abnormalities are often associated with disease occurrence, such as cancer, cardiovascular diseases, neurodegenerative diseases, liver diseases, and more.



Nat Commun: Ubiquitinomics Reveals a New Mechanism of Deubiquitinase USP14 in Non-alcoholic Fatty Liver. Proteome-wide analysis of USP14 substrates revealed its role in hepatosteatosis via stabilization of FASN. *Nature Communications*.

► **Agriculture and Forestry:** Ubiquitylation modifications are closely related to plant physiology. Numerous studies have demonstrated that Ubiquitylation modifications are involved in plant growth and development, abiotic stress, plant metabolism, biotic stress, and other aspects.

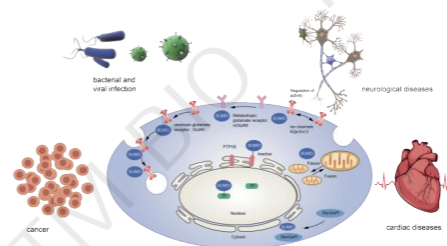


Plant Journal: Ubiquitinomics Reveals Dynamic Regulatory Mechanisms of Rice Seed Germination. Quantitative ubiquitylomics approach for characterizing the dynamic change and extensive modulation of ubiquitylation in rice seed germination. *Plant Journal*.

SUMOylation

Service Overview

Small ubiquitin-like modifier (SUMO) is a small ubiquitin-like modifier protein with a molecular weight of approximately 12kDa, and the family consists of 5 members. SUMOylation is a common post-translational modification, referring to the covalent connection of the SUMO C-terminal di-Gly to the Lys side chain ϵ -NH₂ on the target protein through an isopeptide bond. It participates in various important physiological and biochemical reactions in cells and plays an indispensable role. Studies have found that SUMOylation modification is involved in the regulation of protein-protein and protein-DNA interactions, and is also related to the occurrence and development of various diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, and immune diseases.



Reference Applications and Cases

Gut: SUMOylation Activates Antitumor Immunity in PDAC.

Targeting pancreatic cancer by TAK-981: a SUMOylation inhibitor that activates the immune system and blocks cancer cell cycle progression in a preclinical model. *Gut*.

Evaluated the potential role of SUMOylation modification in pancreatic cancer tissues, finding that the vast majority of cancer samples showed high levels of SUMOylation modification. Based on this, researchers selected a small molecule inhibitor to study the correlation between SUMOylation modification and pancreatic cancer cell proliferation. The results showed that the inhibitor selectively reduced SUMOylation modification levels, thereby blocking cell proliferation, leading to pancreatic cancer cells. Failure of mitosis and chromosomal segregation defects.

Sci Adv: SUMOylation Modification Responds to New Cellular Stress Mechanisms.

Epac1 activation by cAMP regulates cellular SUMOylation and promotes the formation of biomolecular condensates. *Sci Adv*.

Epac1 activation promotes cellular SUMOylation, and cAMP activation of Epac1 triggers phase separation and the formation of nuclear condensates containing Epac1 and SUMOylated components to promote cellular SUMOylation. Additionally, Epac1 gene knockout eliminated cellular SUMOylation induced by oxidized low-density lipoprotein in macrophages, thereby inhibiting foam cell formation.

MCP: SUMOylation and Stem Cell Development.

Identification of SUMO Targets Associated With the Pluripotent State in Human Stem Cells. *MCP*.

After treating cells with SUMO-activating enzyme inhibitors, the inhibited SUMOylation leads to reduced expression of pluripotency markers and morphological changes in cells. Moreover, the cell proteome undergoes almost no large-scale changes, suggesting that the absence of SUMOylation modification in cells may affect changes in important factors related to nuclear structure and function, thereby influencing protein abundance and ultimately affecting the phenotype.

EMBO Reports: SUMOylation and EGFR Signaling Regulation.

Transient deSUMOylation of IRF2BP proteins controls early transcription in EGFR signaling. *EMBO Reports*.

SUMOylation acts as a molecular switch in epidermal growth factor receptor (EGFR) signaling.

Cell Death Dis: SUMOylation and Neurogenesis

The Sumo proteome of proliferating and neuronal-differentiating cells reveals Ulf1 among key Sumo targets involved in neurogenesis. *Cell Death Dis*.

Analyzed how SUMOylation regulates the function of Ulf1 (regulating chromatin affinity; promoting binding to decapping enzyme Dcp1a), revealing its specific functional mechanism as a SUMOylation target protein associated with neurogenesis.

Product Features

PTM BIO, combining its leading 4D proteomics technology platform, has launched the 4D-SUMOylation proteomics analysis product. It enables high-depth and high-accuracy qualitative and quantitative analysis of SUMOylated peptides and sites in various biological samples such as tissues and cells, helping researchers unlock the unknown regulatory mechanisms of protein SUMOylation.

★ **Application of *Nature Methods**, even more authoritative and professional.**

WaLP enzyme digestion + K-ε-GG antibody enrichment, higher site coverage.

★ **Systematic Bioinformatics Analysis, In-Depth Data Analysis.**

Information! Automated analysis and personalized customization, in-depth mining of new SUMOylation regulatory mechanisms.

★ **Next-generation 4D Technology.**

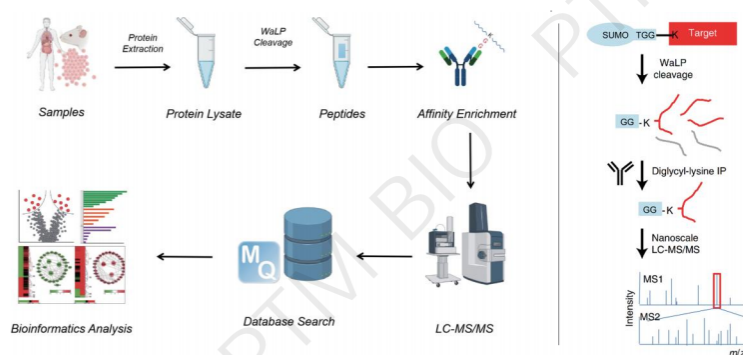
Higher depth of modification identification. Based on timsTOF Pro/timsTOF Pro2, significantly enhances the identification depth of SUMOylated peptides and sites.

Internal Data (Human Cell Samples)

700+ Average
Identification Depth for a
Single Sample

900+ Total Identification
Depth for Three Technical Replicates

Technical Route



Figure, (Left) Flowchart of 4D-SUMOylation proteomics technology. (Right) Schematic diagram of WaLP enzymatic digestion and high-specificity K-ε-GG antibody enrichment. Cell samples are lysed and proteins are extracted, then digested using specific WaLP protease. WaLP is a serine endopeptidase that specifically cleaves.

The carboxyl terminal side of amino acids such as alanine, serine, threonine, and valine, produces double glycine residues. Subsequently, SUMOylated peptides were enriched using a high-specificity K-ε-GG antibody.

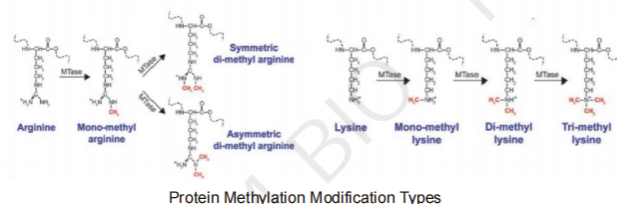
Common Sample Types and Sample Quantities

Sample Types	Sample Quantities
Common animal tissues (such as liver, brain, heart, etc.)	150 mg
Cells	4×10^7 >130 μ L
Fungi, bacteria, protein solution	/
Conventional plant tissues (such as leaves)	/

Methylation

Service Overview

Methylation is an important modification of proteins and nucleic acids, regulating gene expression and silencing, and is closely related to many diseases such as cancer, aging, and Alzheimer's Disease, making it one of the key research topics in epigenetics. The most common methylation modifications include DNA methylation, RNA methylation, and protein methylation. Protein methylation refers to the process of transferring a methyl group to the ϵ -amino group of lysine or the guanidino group of arginine on proteins under the catalytic action of methyltransferases, using S-adenosylmethionine (SAM) as the donor. Among these, histone methylation is the most extensively studied type. The functions of histone methylation are primarily manifested in heterochromatin formation, gene imprinting, X-chromosome inactivation, and transcriptional regulation.



Methyltransferases can be divided into lysine methyltransferases (KMTs) and arginine methyltransferases (PRMTs). Based on the number of methyl groups on the amino acid, methylation can be classified into monomethylation, dimethylation, and trimethylation. Arginine can undergo monomethylation or dimethylation, while lysine residues can undergo monomethylation, dimethylation, or trimethylation, depending on the type of methyltransferase.

Our protein methylation proteomics products provide site identification and quantitative analysis of monomethylated, dimethylated, and trimethylated modifications. The principle involves enriching methylated modification peptides using high-quality methylation pan-antibodies, followed by loading onto liquid chromatography-tandem mass spectrometry for analysis. By searching and matching relevant databases, hundreds or even thousands of modification sites can be identified at once.

Reference Applications and Cases

Cell

Cell: Methylation Modification Reveals the Mechanism by Which METTL13 Promotes Tumorigenesis.

METTL13 methylation of eEF1A increases translational output to promote tumorigenesis

This study, combining high-precision methylation proteomics, found that METTL13 can act as a lysine methyltransferase. By catalyzing the K55me2 modification of eEF1A, it enhances the GTPase activity of eEF1A, increases protein translation efficiency, and promotes the role of cancer progression. Knocking out METTL13 significantly inhibits tumor development. The research reveals METTL13 as a potential drug target, providing new insights for the treatment of prostate cancer and lung cancer.



Nat Cell Biol: P53 Methylation Regulates Its Tumor-suppressive Function.

Arginine methylation regulates the p53 response

This study identified, through methylation modification proteomics, that p53 residues R333, R335, and R337 can undergo methylation modification. Further research found that protein arginine methyltransferase (PRMT)5, as a cofactor of the DNA damage response co-activation complex, interacts with p53, is responsible for p53 methylation, and affects the specificity of p53 target genes. Therefore, p53 methylation is a potential regulatory mechanism for its tumor-suppressive function.



Cell Death Differ: RUNX3 Methylation Drives Hypoxia-induced Cell Proliferation and Anti-apoptotic Processes.

RUNX3 methylation drives hypoxia-induced cell proliferation and antiapoptosis in early tumorigenesis

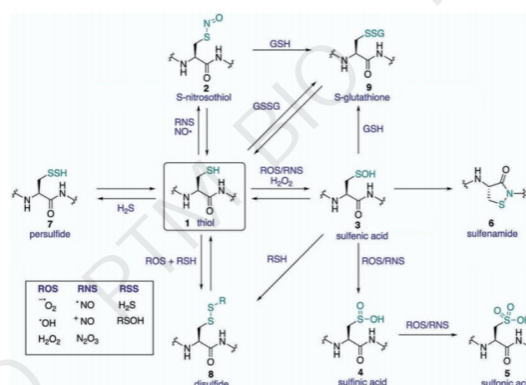
In this study, researchers investigated through methylation modification proteomics, ChIP-seq, and various biochemical experiments, and found that methyltransferase G9a interacts with RUNX3 in hypoxic tumor cells, increasing the methylation levels of RUNX3 at K129 and K171 sites, thereby reducing p300's acetylation of RUNX3, promoting cell proliferation and anti-apoptotic processes. This study suggests that RUNX3 may be a therapeutic or preventive target for controlling tumor growth in the early stages of tumor development.

Cysteine Redox Proteomics

Service Overview

Intracellularly, reactive oxygen species and nitrogen species can perform various chemical modifications on specific amino acids, regulating the redox balance. Redox, as the dominant role of biochemical reactions, is associated with the most abundant proteins related to oxidation and reduction processes in cells (Nature.2020Jun;582(7813):592-596.). Cysteine, due to its inherent nucleophilicity and redox sensitivity, can undergo extensive redox modifications, including S-nitrosylation, S-sulfonation, S-thiolation, and S-glutathionylation.

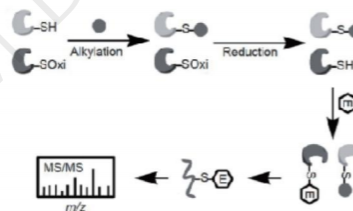
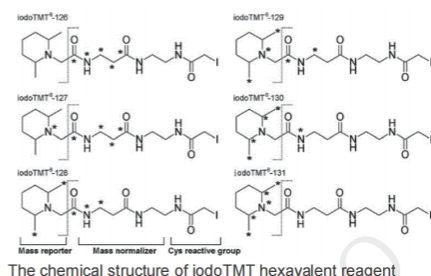
We offer a variety of redox modification proteomics services, helping researchers decipher the regulatory mechanisms of redox modification on physiological and pathological processes in the body.



Modification Types	Overview
Overall Redox Modifications	Qualitative and quantitative analysis of all cysteine residues undergoing redox modification in cell or tissue samples (Overall characterization, cannot achieve site-specific identification and quantitative analysis of a particular modification)
S-nitrosylation modifications	Also known as S-nitrosation, nitrosonium ion NO ⁺ covalently binds with the thiol group-SH of cysteine to form S-nitroso (-SNO), a reversible process
S-glutathionylation modifications	Reduced glutathione (GSH) forms a reversible mixed disulfide bond with cysteine, generating protein S-glutathionylation
S-sulfonation modifications	After oxidation by ROS, the thiol group -SH of cysteine generates the sulfenic acid group-SOH, which can be reduced back to-SH, or further undergo sulfenation and sulfonation modifications
S-Thiolation Modification	Hydrogen sulfide (H ₂ S) covalently binds with the thiol group of cysteine (-SH) to form the persulfide group (-SSH)

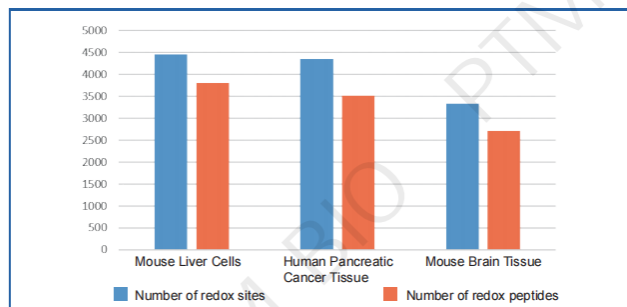
Technical Process

PTM BIO's cysteine redox proteomics workflow enables site-specific identification and quantitative profiling of reversible cysteine modifications, including S-nitrosylation and S-glutathionylation. The workflow uses a selective reduction strategy. Free cysteine thiols are first blocked, after which reversibly oxidized cysteine residues are selectively reduced using chemical or enzymatic reagents. The newly exposed thiols are then labeled with thiol-reactive reagents, such as iodoacetyl tandem mass tags (iodoTMT), or enriched using solid-phase capture methods, such as resin-assisted capture. The resulting peptides are analyzed by LC-MS/MS, followed by database searching and bioinformatics analysis to identify modification sites and obtain quantitative measurements.



Analysis strategy for detecting and analyzing cysteine-specific types of oxidative modifications based on iodoTMT labeling

Internal Data

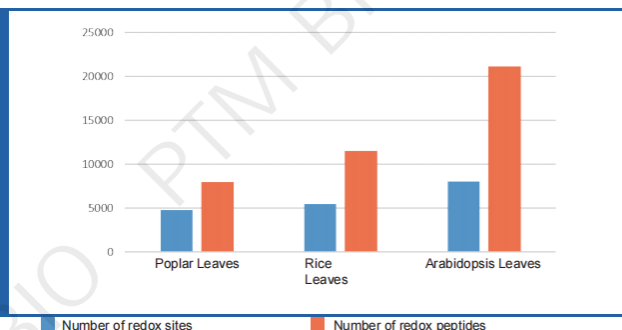


Conventional Animal Cells/Tissues:

- 1 | Identifies **3000-5000** Redox Sites
- 2 | Identifies **2500-4000** Redox Peptides

Conventional Plant Cells/Tissues:

- 1 | Detected **5000-8000** Redox Sites
- 1 | Detected **8000-20000** Redox Peptides



Reference Applications and Cases

Cell

Cell: Redox Modification Maps Provide New Insights for Aging Research.

A Quantitative Tissue-Specific Landscape of Protein Redox Regulation during Aging

Dysregulation of ROS and redox signals is one of the potential causes of physiological decline in tissues with age, but the tissue-specific physiological mechanisms mediated by ROS-modified targets in vivo remain unclear. In this study, 10 organs were collected from 16-week-old and 80-week-old male mice, and a comprehensive mouse redox aging map was drawn based on global redox modification proteomics, revealing that the cysteine redox network within tissues is tissue-selective. A disease network of redox modifications was comprehensively identified, establishing a systematic molecular basis for the long-standing link between redox dysregulation and tissue aging.

**Nat Commun:** S-Nitrosylation Modification Proteomics Reveals Vancomycin Resistance Mechanism of Staphylococcus Aureus.

Transcription tuned by S-nitrosylation underlies a mechanism for Staphylococcus aureus to circumvent vancomycin killing

Staphylococcus aureus infections are severely threatening public health safety. The vancomycin resistance of Staphylococcus aureus poses a significant challenge to clinical treatment. This study, using the VISA strain -XN108 isolated clinically, based on S-nitrosylation modification proteomics detection from PTM BIO, found that nitric oxide (NO) derived from nitric oxide synthase (NOS) mediates S-nitrosylation modification of transcription factors MgrA or WalR, leading to decreased cellular autolysis activity, mediated by increased cell wall thickness, thereby promoting vancomycin resistance in Staphylococcus aureus.

**Nat Commun:** New Mechanism of Protein Glutathionylation Modification Regulating Acute Lung Injury.

Oxidative stress-induced FABP5 S-glutathionylation protects against acute lung injury by suppressing inflammation in macrophages

In this study, researchers performed localization and quantitative analysis of S-glutathionylated proteins based on the iodoTMT redoxome proteomics of PTM BIO, elucidating the molecular mechanism by which Cys127-glutathionylated FABP5 in macrophages alleviates acute lung injury through its anti-inflammatory function. The findings provide a new theoretical basis for the regulatory mechanisms of fatty acid metabolism in acute lung injury.

Plant Communications

Plant Commun: Oxidative-Reductive Modification Proteomics Reveals New Mechanisms of Rice Disease Resistance.

Comparative oxidation proteomics analyses suggest redox regulation of cytosolic translation in rice leaves upon Magnaporthe oryzae infection

This study, based on the global oxidative modification proteomics of rice leaves before and after infection with Magnaporthe oryzae, found that oxidatively modified proteins are enriched in translation, peptide biosynthesis, and peptide metabolism processes, indicating that oxidative modification may be involved in regulating rice translation during Magnaporthe oryzae infection, increasing the translation efficiency of cytosolic ribosomes to enhance rice resistance.

Spatiotemporal Proteomics

Comprehensive Spatial Proteomics Solutions

Spatial Proteomics: A Key Direction for Precision Medicine

With the rapid advancement of life science research and biotechnology, spatial omics has become one of the key driving forces in modern biomedical research.

Since spatial omics was recognized as the Method of the Year by Nature Methods in 2020, it has continued to gain global attention. It was further highlighted among the Seven Technologies to Watch in 2022, included in the Top 10

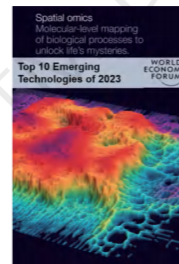
Emerging Technologies of 2023, and recognized again by Nature Methods in 2024. Spatial omics is widely regarded as one of the next major frontiers in life science.

Since launching its spatial proteomics platform in June 2021, PTM BIO has continuously optimized its technologies and workflows, achieving multiple breakthroughs in spatial proteomics. PTM BIO has now established an integrated spatial omics platform that combines spatial proteomics, spatial targeted PTM analysis, and holographic spatial proteomics, providing comprehensive solutions for diverse protein research needs.

SEVEN TECHNOLOGIES TO WATCH IN 2022

Nature 2022 601(7894):658-661

Spatial proteomics has thereby provided unprecedented insight into biological processes not only from a fundamental cell biology perspective, but also from a clinical perspective, to study how protein localization changes in pathogenic cells, which could prove useful for finding biomarkers and developing new therapies.



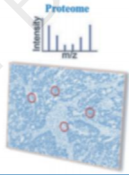
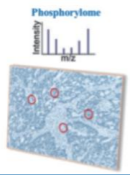
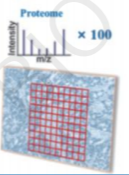
SPATIAL OMICS The dawn of spatial omics

MOSAIC is the world's largest spatial multimodal dataset in oncology

Multi-Omic Spatial Atlas in Cancer

nature methods
Spatial single-cell mass spectrometry defines zonation of the hepatocyte proteome

Integrated Solution Portfolio

Analysis Solution			
What It Enables	Select multiple regions or spots from a single tissue section for spatial proteome profiling	Select multiple regions or spots from a single tissue section for spatial phosphoproteome profiling	Select multiple regions from a single tissue section to generate large-scale spatial proteome datasets
Compatible Sample Types	FFPE samples; fresh-frozen samples; fixed-frozen samples	fresh-frozen samples; fixed-frozen samples	FFPE samples; fresh-frozen samples; fixed-frozen samples
Data Output	Proteomics data from each selected spot or region	Phosphorylation proteomics data from each selected spot or region	Proteomics data from 100+ spatial regions in a single project

Technology Advantages

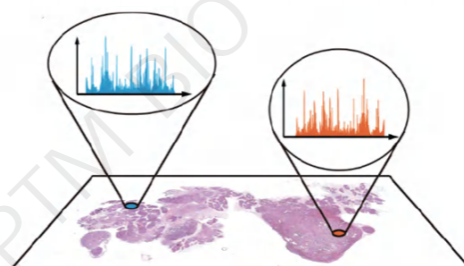
1. Built on PTM BIO's innovative 10X Proteomics platform
2. Fully integrated one-stop spatial omics operation platform
3. Cell-level customized microdissection
4. Deep detection depth
5. Extensive project experience
6. High-impact collaborative publications

Spatial Proteomics / Spatial Phosphoproteomics

Service Overview

Spatial proteomics profiles protein abundance from defined regions within tissue sections, preserving spatial context and revealing region-specific protein expression patterns. Compared with bulk tissue proteomics, it captures molecular heterogeneity across different cellular and functional regions. Compared with single-cell proteomics, it retains tissue architecture and spatial localization information.

PTM BIO offers an integrated spatial omics solution combining **spatial proteomics** and **spatial phosphoproteomics**, enabling high-sensitivity analysis of spatial molecular features and potential regulatory targets.



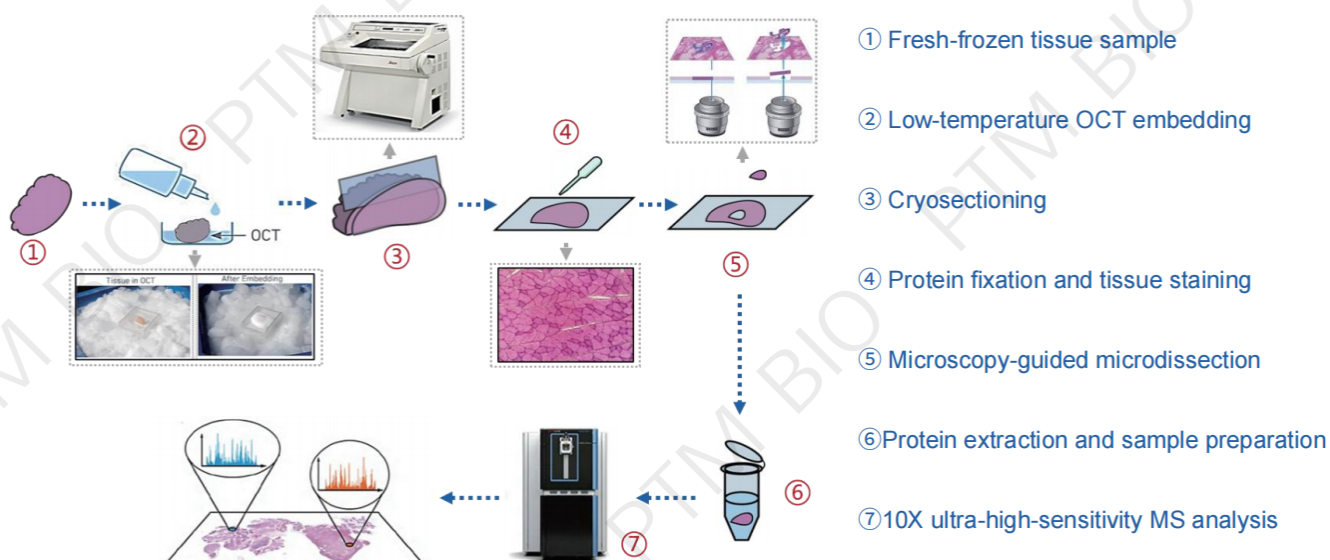
Technical Workflow

「Key Technology Features」

Precise Microdissection of
Small Spatial Regions

Loss-minimized processing of
ultra-low-input proteins

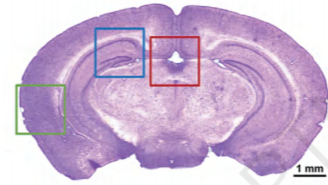
Ultra-High-Sensitivity Mass
Spectrometry Analysis



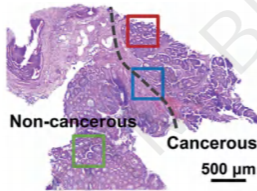
Application Scenarios

Spatial Proteomic Profiling of Functional Regions in Complex Tissues

Region-specific microdissection and proteomic analysis enable protein-level characterization of functional brain regions, including the cerebral cortex, hippocampus, and third ventricle.



□ Cerebral cortex □ Hippocampus □ Third ventricle

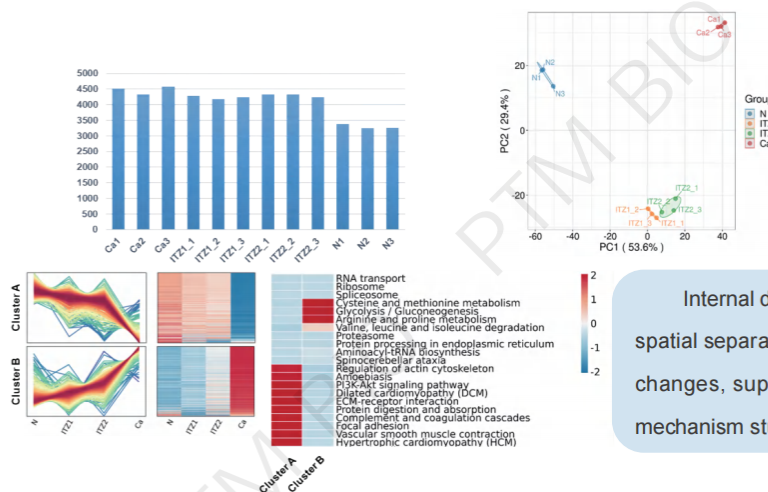


□ Normal tissue □ Boundary region □ Tumor tissue

Revealing Tumor Heterogeneity Through Spatial Proteomics

Spatial proteomics profiles tumor, boundary, and adjacent normal regions on the same tissue section, revealing tumor heterogeneity, aberrantly activated pathways, and potential therapeutic targets.

Internal Validation Data



Internal data demonstrate robust protein identification, clear spatial separation of tissue regions, and region-specific pathway changes, supporting spatial biomarker discovery and disease mechanism studies.

Representative Applications and Case Studies

nature
International journal of science

Nature: Proteomics reveals NNMT as a master metabolic regulator of cancer-associated fibroblasts.

Science

Science: Immobility-associated thromboprotection is conserved across mammalian species from bear to human.

Cell

Cell: Spatial proteomics in three-dimensional intact specimens.

nature genetics

Nat Genet: Multiomic analysis of cervical squamous cell carcinoma identifies cellular ecosystems with biological and clinical relevance.

nature genetics

Nat Genet: Spatially resolved multi-omics of human metabolic dysfunction-associated steatotic liver disease.

Signal Transduction and Targeted Therapy

STTT: Robust neurogenesis in chronic stroke monkeys following mesenchymal stem cell transplantation plus intermittent theta-burst stimulation.

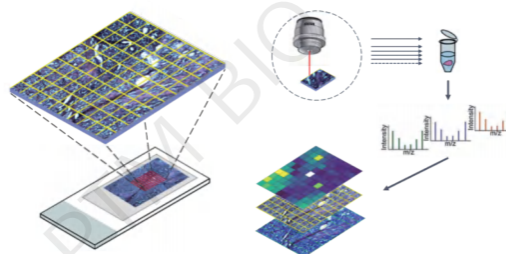
Cell Stem Cell

Cell Stem Cell: 3D post-implantation co-culture of human embryo and endometrium.

Holographic Spatial Proteomics

Service Overview

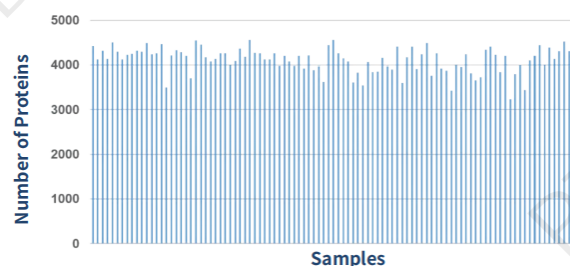
Holographic Spatial Proteomics combines **high-precision LCM** with **LC-MS/MS-based proteomic analysis** to generate spatially resolved proteome maps across defined tissue microregions. By covering multiple regions within the tissue microenvironment, the workflow enables comprehensive characterization of spatial molecular heterogeneity.



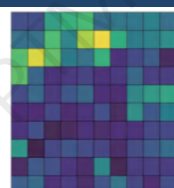
Technical Advantages

10X In-Depth Proteome Quantification

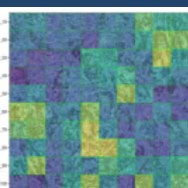
The workflow generates **100 spatially annotated proteome datasets**, identifying **5,500+ proteins** in total and **4,100+ proteins per sample on average**, providing one of the most comprehensive holographic spatial proteomics datasets currently available.



Advanced Holographic Spatial Bioinformatics



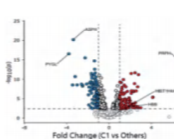
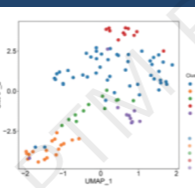
Visualization of Individual Differential Proteins



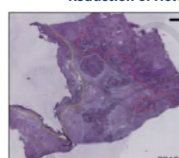
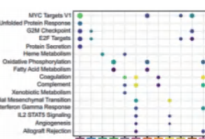
Protein Expression Overlay on H&E-Stained Images



Unsupervised Clustering and Dimensionality Reduction of Holographic Spatial Sampling



Between-Group Differential Analysis & Functional Annotation of Differential Proteins



Cell-Type Score and Proportion Annotation Using Deconvolution-Based Algorithms



Comprehensive spatial bioinformatics analysis, including unsupervised clustering, dimensionality reduction, differential protein analysis, functional annotation, single-protein spatial visualization, pathology-guided regional comparison, and deconvolution-based cell-type scoring and proportion estimation.

Spatial X Holographic Proteomics

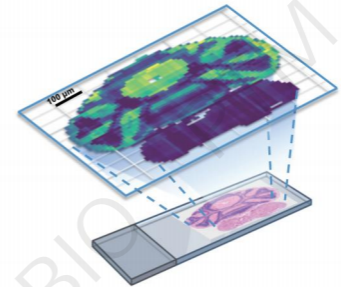
Service Overview

Powered by advanced **AI-based image reconstruction**, PTM BIO introduces the **Spatial X Holographic Proteomics Solution**.

This platform overcomes the limitations of conventional spatial proteomics in both **spatial coverage** and **data depth**, enabling:

centimeter-scale tissue coverage, micron-level precise microdissection, and proprietary PTM decoding.

By integrating whole-tissue spatial profiling with deep proteome and PTM analysis, Spatial X helps researchers generate a panoramic molecular map of protein regulation associated with **drug resistance, relapse, and disease progression**.



Technology Workflow

Centimeter-Scale Spatial Coverage

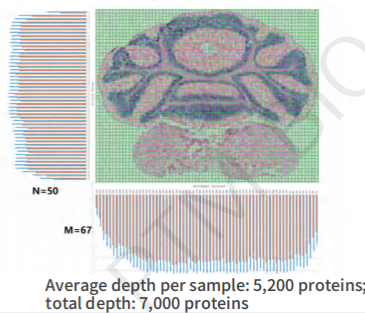
Whole-Tissue Spatial Coverage

Centimeter-Scale
Whole-Tissue Sectioning

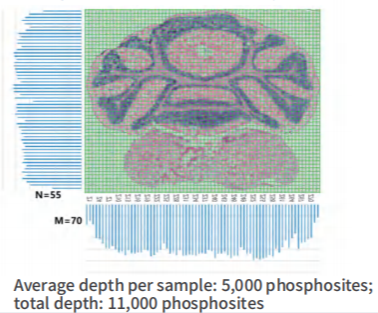
Deep Spatial Proteome Coverage

7,000+ Total Protein
Identifications
11,000+ Total Phosphosite
Identifications

Spatial X Holographic Spatial Proteomics



Spatial X Holographic Spatial Phosphorylation Proteomics



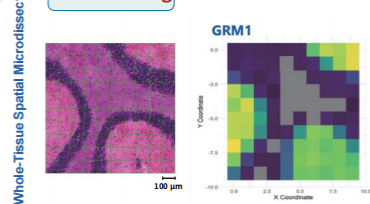
Micron-Level Precision Microdissection

100 μm or Smaller Spatial
Resolution

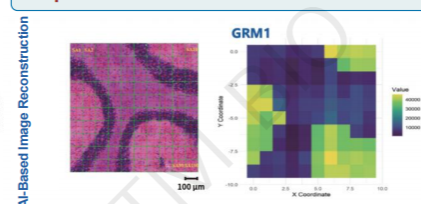
Precise Spatial Interpretation

AI-based whole-tissue
reconstruction shows high
consistency with real

H&E Staining



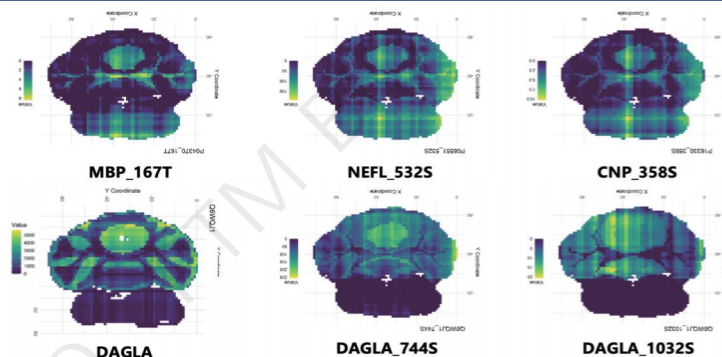
Spatial Distribution of Marker Proteins



Proprietary PTM Decoding

Deep PTM Exploration

In situ mapping of protein
modification sites to
decode spatial regulatory
mechanisms.



Single-Cell Proteomics

Service Overview

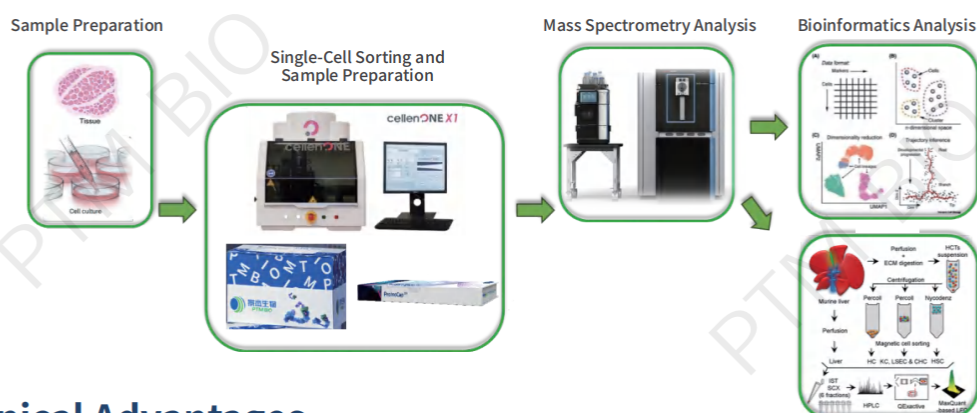
Proteins are the primary drivers of cellular function. Mass spectrometry-based single-cell proteomics enables protein-level analysis of individual cells, offering powerful research and translational value. By directly measuring proteins at single-cell resolution, single-cell proteomics fills critical gaps left by single-cell genomics and transcriptomics, especially in areas such as **protein abundance, post-transcriptional regulation, and post-translational modifications**. It provides precise, functional-level information for studying **cellular heterogeneity**, constructing **high-resolution protein molecular atlases**, and analyzing spatial proteome networks. This makes it a valuable approach for research areas such as **tumor heterogeneity, stem cell differentiation, and germ cell development**.

Technology Workflow

PTM BIO integrates its proprietary **single-cell sample preparation system** with an industry-leading **single-cell sorting platform**, high-resolution **10X Proteomics mass spectrometry**, and a dedicated **single-cell bioinformatics analysis platform**.

This end-to-end workflow covers:

Cell suspension → **Single-cell sorting and sample preparation** → **Accurate single-cell protein identification** → **Single-cell proteomics bioinformatics analysis**



Technical Advantages

Advanced Single-Cell Sorting

PTM BIO uses the fully integrated and automated CellenONE single-cell sorting platform, enabling gentle cell sorting, real-time optical monitoring, high single-cell capture efficiency, low sample loss, and automated workflow control.

High-Performance Proteomics Platform

Powered by the industry-leading 10X Proteomics high-resolution mass spectrometry platform, PTM BIO delivers higher sensitivity, higher throughput, and deeper proteome coverage for single-cell proteomics research.

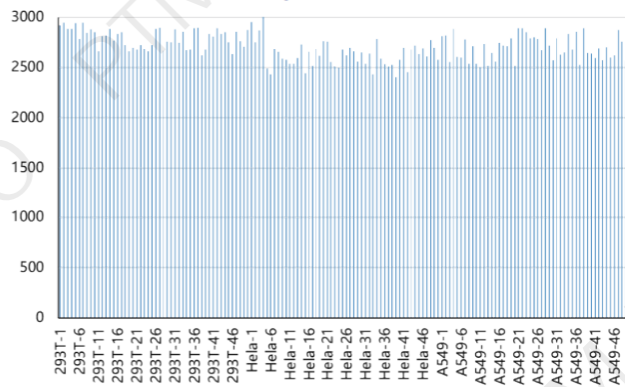
Sample Preparation Platform Advantages

PTM BIO's proprietary single-cell sample preparation system improves protein extraction efficiency from individual cells while minimizing sample loss. This optimized workflow enables more reliable and efficient single-cell proteomics sample processing.

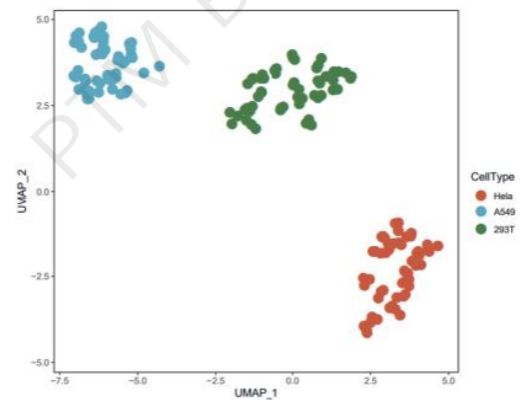
Bioinformatics Platform Advantages

PTM BIO has developed a dedicated single-cell proteomics bioinformatics workflow for single-cell-level data interpretation. This platform provides high-quality, protein-level insights for cellular heterogeneity studies and high-resolution cellular protein atlas construction.

Higher Identification Depth, Greater Stability, and Stronger Consistency



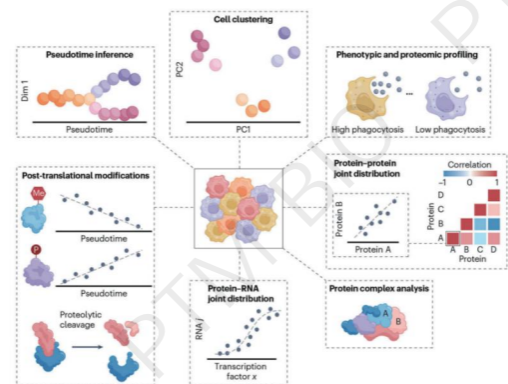
150 single cells analyzed | Average depth: 2,728 protein groups | Total depth: >3,600 protein groups



High data stability and consistency

Application Areas

- Cellular and tissue heterogeneity
- Cell growth, differentiation, and developmental biology
- Cellular transformation and disease mechanism studies
- Tissue microenvironment research
- Therapy sensitivity and resistance studies
- Subcellular protein atlas construction



Reference Applications and Case Studies

Cell Stem Cell

Cell Stem Cell: Cell Stem Cell | Single-Cell Proteomics Reveals Stem Cell Heterogeneity

Deciphering lineage specification during early embryogenesis in mouse gastruloids using multilayered proteomics. **Cell Stem Cell**.

This study used single-cell proteomics to characterize lineage specification and cellular heterogeneity during early embryogenesis in mouse gastruloids. The results revealed distinct protein expression profiles across cell populations and provided protein-level insights into developmental trajectories and regulatory programs.

Science

Science: Single-Cell Proteomics Identifies Key Molecular Features of Human Blood Cell Differentiation

Mapping early human blood cell differentiation using single-cell proteomics and transcriptomics. **Science**.

This study integrated single-cell proteomics with transcriptomics to map early human blood cell differentiation. The results identified key protein-level features associated with lineage commitment and provided new insights into hematopoietic development.

Epigenetic Regulation & Chromatin Interaction Technologies

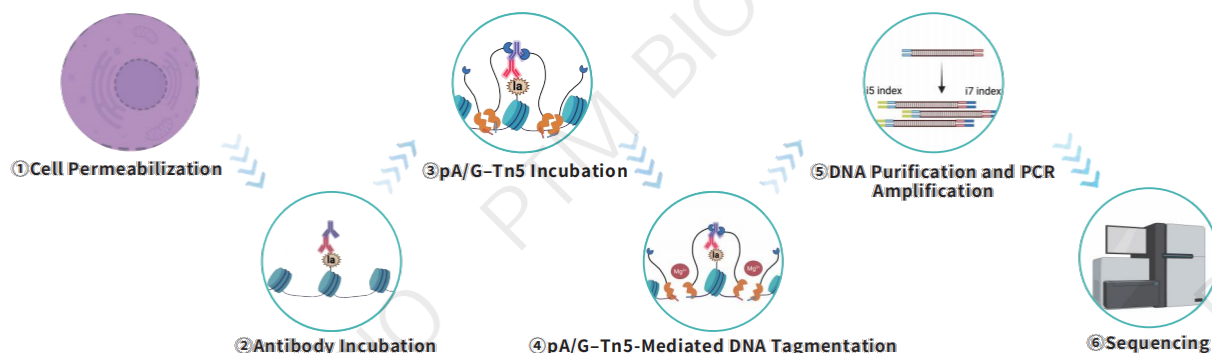
CUT&Tag

Service Overview

CUT&Tag — Cleavage Under Targets and Tagmentation — is a highly efficient, high-resolution method for building DNA sequencing libraries using hyperactive **Tn5 transposase-based tagmentation**. It is widely used to study **protein–DNA interactions**, including chromatin-associated proteins, transcription factors, and histone modifications.

Technology Workflow

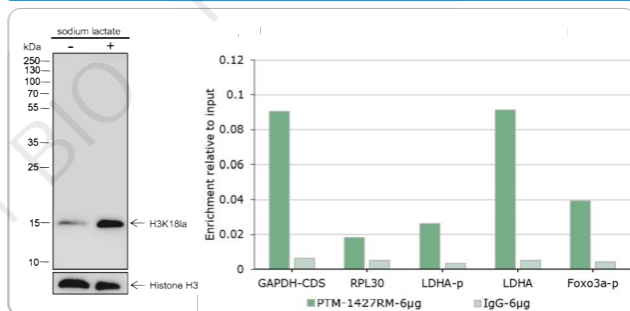
CUT&Tag uses a Protein A/G–Tn5 transposase fusion system. Guided by a specific antibody, the enzyme is recruited to the target protein and performs DNA fragmentation near the binding site, enabling precise profiling of target-associated genomic regions.



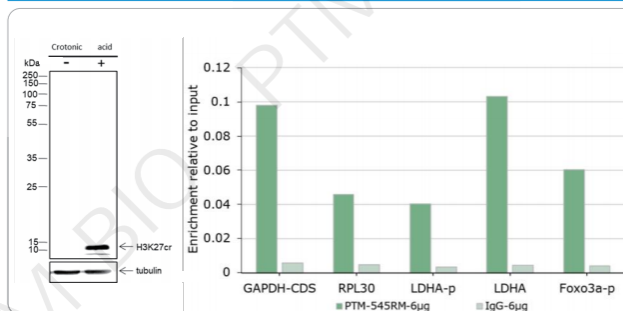
PTM BIO CUT&Tag Service Highlights

All CUT&Tag services use in-house ChIP-grade antibodies, ensuring reliable performance at the most critical step of the workflow.

Anti-Lactyl-Histone H3 (Lys18) Rabbit mAb-ChIP Grade (PTM-1427RM)



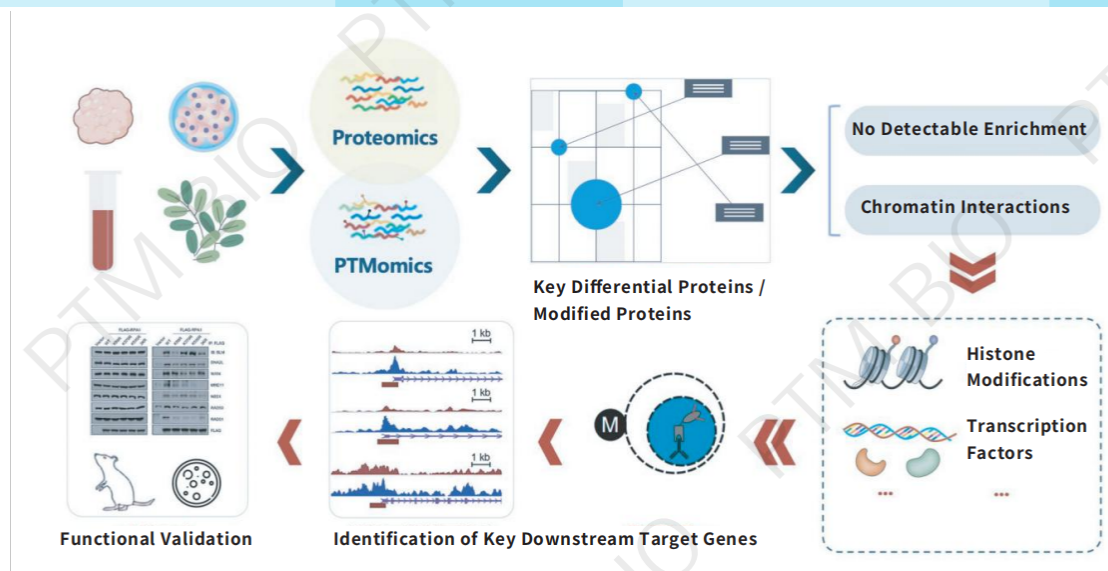
Anti-Crotonyl-Histone H3 (Lys27) Rabbit mAb (PTM-545RM)



PTM BIO supports CUT&Tag profiling for a broad range of emerging histone acylation marks. This service helps bridge **metabolism and epigenetic regulation**, empowering researchers to explore new frontiers in chromatin biology and disease mechanisms.

PTM BIO offers specialized histone modification antibody panels to help researchers quickly screen key modification types and candidate regulatory sites.

CUT&Tag Strategy for Functional Validation

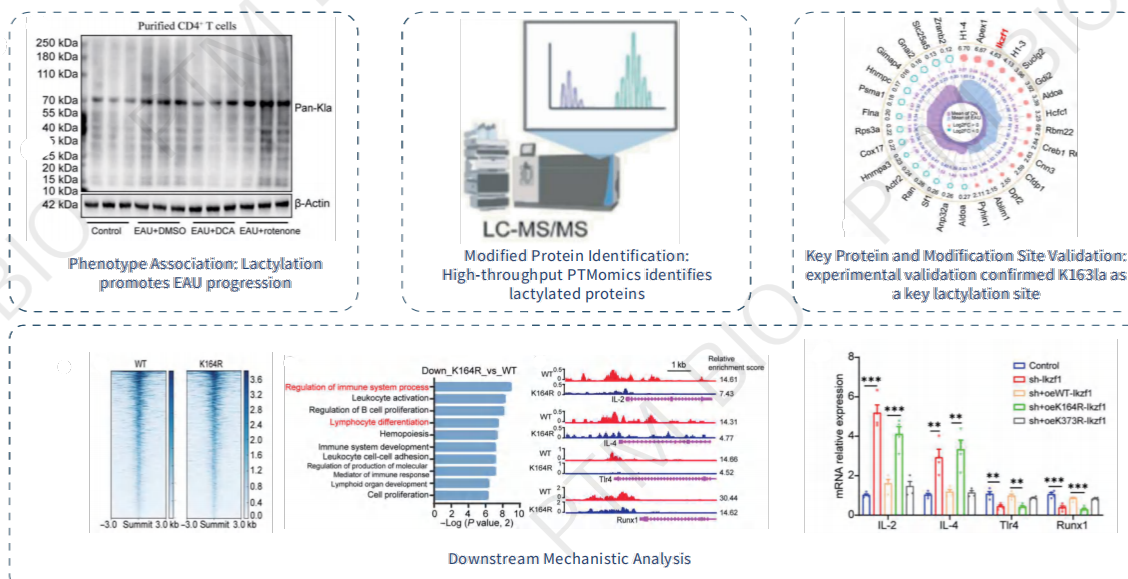


CUT&Tag links proteomics and PTMomics discoveries to chromatin-level mechanisms. By mapping genome-wide binding profiles of candidate proteins or modified proteins, CUT&Tag helps identify downstream target genes and build a complete research loop from proteomics discovery to mechanistic validation.

Case Study | Transcription Factor Lactylation in Experimental Autoimmune Uveitis (EAU)

SCIENCE ADVANCES | RESEARCH ARTICLE

Global lactylome reveals lactylation-dependent mechanisms underlying T_H17 differentiation in experimental autoimmune uveitis



Hi-Plex CUT&Tag

Service Overview

Hi-Plex CUT&Tag is PTM BIO's multiplex CUT&Tag technology designed for dissecting epigenetic regulatory networks.

Using antibodies carrying distinct barcodes, Hi-Plex CUT&Tag enables simultaneous profiling of multiple chromatin-associated proteins or histone modifications **within the same sample**. This allows researchers to directly examine target localization, co-localization patterns, and regulatory relationships across multiple epigenetic marks.

Compared with conventional CUT&Tag or ChIP-seq, Hi-Plex CUT&Tag supports multiplex detection of **3, 5, 10, 20, or 30 targets in a single experiment**, with as few as **~100,000 cells** required for multi-target profiling.

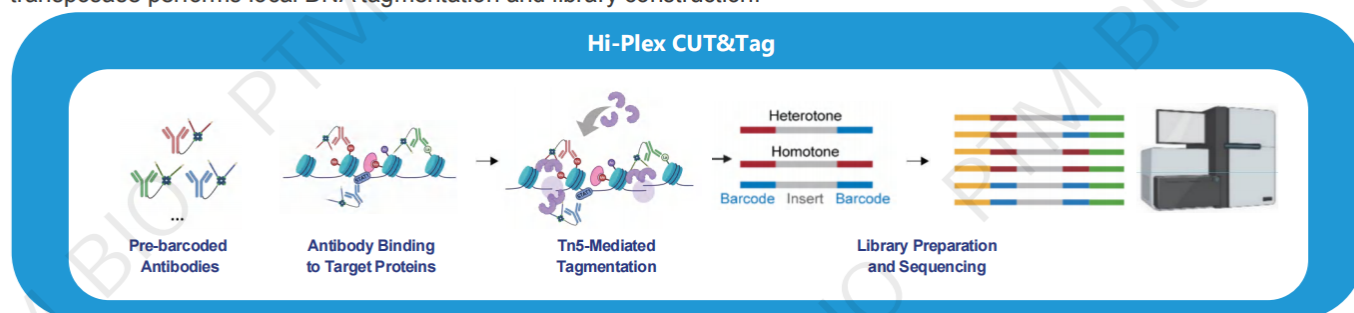
This makes it especially powerful for studies involving **precious samples, histone modification crosstalk, cis-regulatory element mapping, and mechanism validation**.

Technology Advantages

- Capture multiple targets in one experiment to map protein–DNA interactions and combinatorial histone modification landscapes.
- Screen up to 30 targets simultaneously, significantly shortening the target-by-target validation cycle.
- Provides multiplexed, in situ information on the genomic co-localization patterns of multiple targets and reduces bias introduced by conventional antibody-pooling strategies.
- Enables multi-target profiling from approximately 100,000 cells, making it suitable for precious clinical or primary samples.

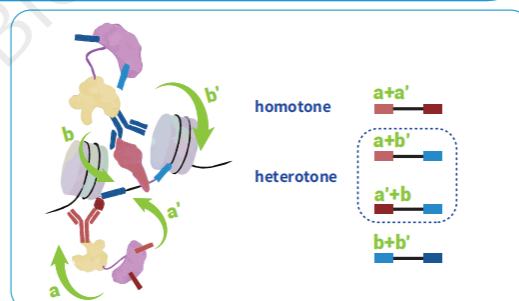
Technology Principle and Workflow

Core concept: Each antibody is pre-labeled with a unique barcode. After the antibody binds to its target protein, Tn5 transposase performs local DNA fragmentation and library construction.



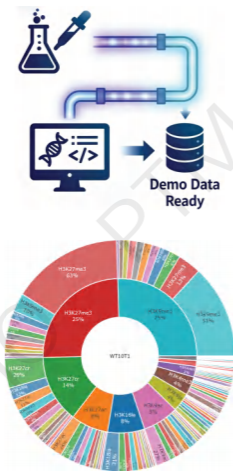
When multiple targets co-localize within the same chromatin region, sequencing reads can carry adapters from different antibody barcodes, generating **heterotone signals**. This enables **single-molecule-level co-localization analysis** and helps reveal combinatorial chromatin regulatory patterns.

Schematic diagram of the heterotone principle ►

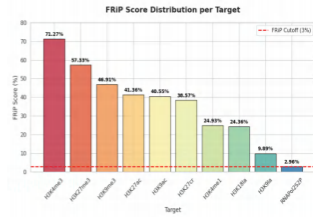


Internal Validation Results

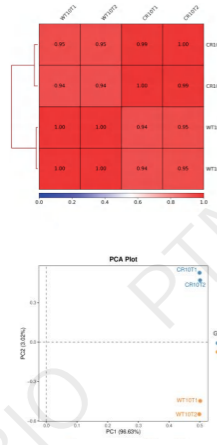
End-to-End Workflow



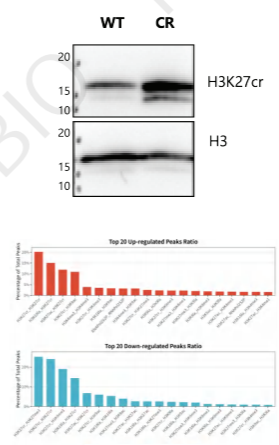
10-Targets Multiplex Panel



High Quality & Credibility



Biological Validation



Wet-lab experiment, library preparation, sequencing, and demo data analysis were successfully completed.

Peak enrichment and target-specific signal profiles met analysis requirements across targets.

Replicate correlations were >0.8 , with clear sample separation by PCA.

Hi-Plex CUT&Tag results were consistent with WB validation and public reference datasets.

10-Target Internal Validation Project

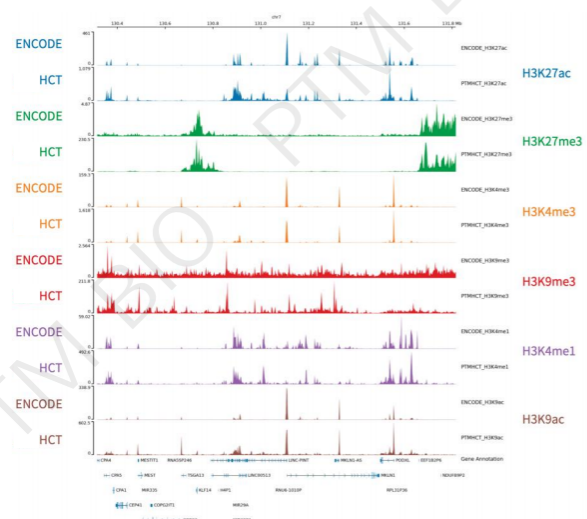
PTM BIO has completed end-to-end internal validation for a 10-target Hi-Plex CUT&Tag panel, demonstrating reliable performance across the full workflow.

IGV Visualization Validation

PTM BIO compared 10-target Hi-Plex CUT&Tag homotone signals with public ENCODE ChIP-seq datasets using IGV visualization. For canonical histone marks, including H3K27ac, H3K27me3, H3K4me3, H3K9me3, H3K4me1, and H3K9ac, the Hi-Plex CUT&Tag profiles showed strong agreement with ENCODE ChIP-seq data in terms of peak distribution, signal location, signal intensity, and genome-wide trend.

These results demonstrate that single-target signals generated by Hi-Plex CUT&Tag are reliable and biologically meaningful.

In addition, heterotone signals can directly reveal combinatorial multi-target signals at co-localized genomic regions, helping researchers uncover co-regulatory patterns that are difficult to capture using conventional single-target workflows.



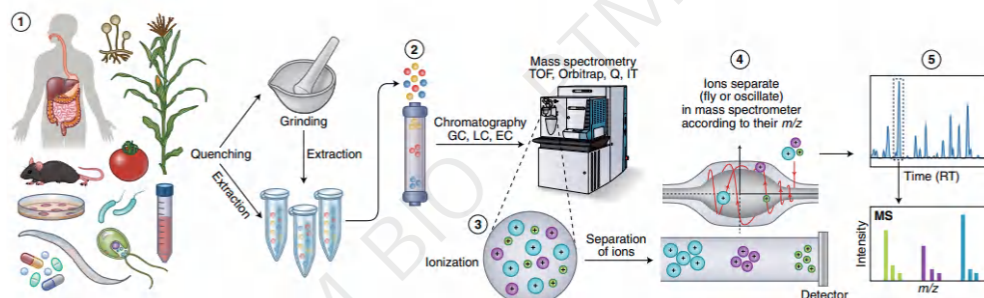
Others

4D Untargeted Metabolomics

Service Overview

Metabolites from cells, organs, tissues, or biological fluids serve as signaling molecules, immunomodulators, endogenous toxins, and environmental sensors, playing diverse and important roles in many biological processes. Metabolomics, by high-throughput characterization of metabolite information, is enhancing our understanding of normal physiology and the pathophysiology of many diseases. These insights and discoveries have generated new perspectives on how metabolites affect organ function, immune function, nutrient sensing, and gut physiology.

Known metabolites number in the tens of thousands, with diverse properties (wide chemical structures and abundance ranges), thus requiring more powerful techniques for detection and quantitative analysis. PTM BIO combines 4D proteomics' unique capture ion mobility (TIMS) for the fourth-dimensional separation, enhancing quantitative selectivity and accuracy, significantly increasing the number of metabolites analyzed and improving the accuracy of quantitative information.



Metabolomics Brief Workflow
Nature Methods
Nature Methods vol. 18,7 (2021): 747-756.



Technical Advantages

- ① Fully based on experimentally acquired MS/MS information for identification, more reliable results.
- ② Based on timsTOF Pro Ultra-High Performance Platform, maintaining high resolution while achieving deeper depth.
- ③ Based on mobility (CCS) separation, multi-dimensional differentiation of metabolite isomers, richer information.

Sample Delivery Tips

- (1) Cell samples must be accurately counted before submission to ensure consistent sample quantity.
- (2) If proteomics and 4D untargeted metabolomics projects need to be conducted simultaneously on the same batch of cell samples, proper aliquoting must be done in advance.
- (3) All sample collection operations should be performed as quickly as possible. After collection, samples should be placed on dry ice or a -80°C freezer as soon as possible, and repeated freeze-thaw cycles should be avoided before experiments to prevent changes in metabolites.

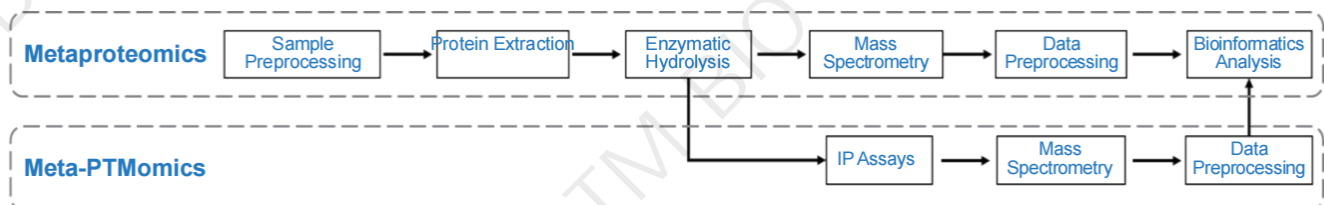
Metaproteomics

Service Overview

Metaproteomics is a new technology that applies proteomics techniques to study microbial communities, conducting large-scale identification of all proteins in microbial communities at specific times.

Metaproteomics can provide functional information about microorganisms in ecosystems. It holds the potential to link the genetic and functional diversity of microbial communities. Metaproteomic analysis across different environments can capture new functional genes and metabolic pathways, identify proteins associated with specific stresses, and combining proteomics with metagenomics data can better reveal the taxonomic, functional diversity, and biological processes of environmental communities.

Technical Principle



Technical Advantages

- ① Breaking the Barrier of Culturable Microorganisms.
- ② Analyzing Microbiome Composition and Protein Function Analysis.
- ③ Integrated Analysis of Metagenomics, Metaproteomics, and Meta-PTMomics.



Application Areas

- ① Gut Microbes and Medical Research.
- ② Discovery of New Environmental Biomarkers.

Reference Applications and Cases

Case: Proteomic Analysis Reveals New Mechanisms of Pediatric Inflammatory Bowel Disease.

Metaproteomics reveals associations between microbiome and intestinal extracellular vesicle proteins in pediatric inflammatory bowel disease. *Nature Communications*.

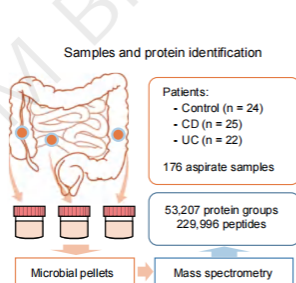


Figure 1. Experimental flowchart

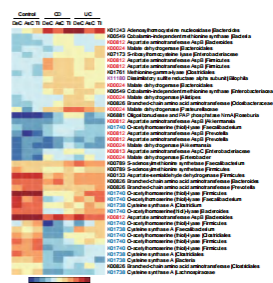


Figure 2. Significantly altered sulfur or cysteine metabolism-related functions in IBD samples

The research team from the University of Ottawa, Canada, Daniel Figeys, used metaproteomics technology, combining gut microbiota and exosome studies, to conduct a large-scale intestinal microbiota metaproteomics study on pediatric onset IBD, providing in-depth insights into the associations between the mechanisms of IBD onset and development and host-microbiota group interactions.

Meta-PTMomics

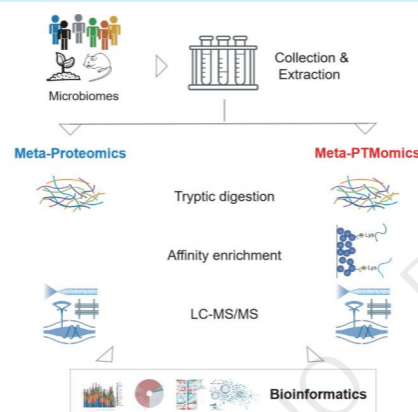
Service Overview

The broad application of Meta-PTMomics provides data support for better understanding the functional characteristics of the microbiome, enhancing our knowledge of various diseases such as tumors, immunity, and neurology. Post-translational modifications (PTMs) are crucial for regulating protein activity. However, existing research has primarily focused on individual microorganisms or model organisms, with limited studies on the PTMomics in the entire microbiome. This makes the regulatory role of PTMs on the gut microbiome still unclear.

Our Meta-PTMomics, covers over ten post-translational modification types such as acetylation, crotonylation, and succinylation, precisely mapping the protein expression and modification profiles of complex microbial communities, and proactively helping to decipher the intricate interaction mechanisms between 'microbiome-host-environment'.

Technical Route and Research Highlights

- ★ 10+ different modification types, meta-lysine acylation proteomics
- ★ Whole proteome and modification profiling of complex microbiome
- ★ Mining the intrinsic mechanisms and causal relationships between microbiome-host-environment
- ★ Revealing microbial modification distribution and protein function
- ★ Integrative analysis of omics (genomics, proteomics, PTMomics)



Product Introduction

Metaproteomics	Simultaneously measure the origin proteins of the host and gut microbiota, providing an in-depth understanding of the molecular and cellular mechanisms by which the human microbiota influences the host.
Meta-PTMomics acetylation	The most thoroughly studied type of acylation modification, which participates in almost all major biological processes.
Meta-PTMomics succinylation	Widely present in eukaryotes and prokaryotes, especially many metabolic enzymes involved in central metabolism and intermediate metabolism exhibit succinylation modification.
Meta-PTMomics crotonylation	One of <i>Cell</i> 's 'Top Five Research Highlights' of 2011, it is involved in metabolic regulation, tumor development, and DNA damage repair.
Meta-PTMomics 2-hydroxyisobutyrylation	High abundance, widely present in both prokaryotes and eukaryotes. Closely related to biological processes such as glycometabolism, amino acid synthesis, and glycolysis.
Meta-PTMomics β-hydroxybutyrylation	Kbhb-modified protein are enriched in many core metabolic pathways, including the metabolism of the three major nutrients, glutathione metabolism, fatty acid oxidation metabolism, and others. It shows differences under conditions of nutrient deficiency or dietary restriction in the body.

400-100-1145

<https://ptmbio.com>

Building 8, Phase II, Biopharmaceutical Town, 291 Fucheng Road, Qiantang New District,
Hangzhou, Zhejiang Province