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PROTOCOL

A protocol for detection of mitochondrial protein malonylation and succinylation

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Abstract Recent advances in mass spectrometry and proteomics have revealed numerous novel lysine acylation modifications, which increase proteome functional diversity via covalent modification of lysine residues. Protein malonylation and succinylation are highly enriched in mitochondrial proteins. They use malonyl-CoA and succinyl-CoA, which are primarily synthesized in mitochondria, as acyl donors and can modulate protein structure and function. However, conventional mass spectrometry-based workflows often suffer from limited coverage and may fail to detect low-abundance modification sites. Therefore, we present an organelle-centered proteomic protocol. By purifying mitochondria before enriching modified peptides, the protocol minimizes interference from non-mitochondrial proteins, thereby offering a valuable reference for investigating diverse protein acylation events in metabolic-associated fatty liver disease (MASLD) and other physiological and pathological contexts.

Keywords Protein post-translational modification, Malonylation, Succinylation, Mitochondria, Metabolism, Metabolic-associated fatty liver disease (MASLD)

INTRODUCTION

Protein post-translational modifications (PTMs) increase the proteome functional diversity through the covalent attachment of functional groups to proteins, which directly modulate localization, stability, activity, and protein-nucleic acid interactions of proteins (Shang *et al.* 2022; Wang *et al.* 2022b). Protein acylation at lysine residues represents a crucial and diverse class of PTM. These modifications are mediated by the enzymatic or nonenzymatic transfer of various acyl groups from their corresponding activated acyl-CoA donors onto the specific lysine residues within target proteins. Acetylation in histones represents the first identified protein acylation (Phillips 1963) and is involved in the regulation of gene expression. Recent advances in mass spectrometry and proteomics have revealed multiple new lysine acyl modifications, including formylation (Kfo) (Jiang *et al.* 2007), propionylation (Kpr) (Chen *et al.* 2007), butyrylation (Kbu) (Chen *et al.* 2007), succinylation (Ksuc) (Zhang *et al.* 2011), malonylation (Kmal) (Peng *et al.* 2011), crotonylation (Kcr) (Tan *et al.* 2011), glutarylation (Kglu) (Tan *et al.* 2014), 2-hydroxyisobutyrylation (Khib) (Dai *et al.* 2014), β-hydroxybutyrylation (Kbhb) (Xie *et al.* 2016), benzoylation (Kbz) (Huang *et al.* 2018), lactylation (Kla)

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(Zhang *et al.* 2019) and acetoacetylation (Kacac) (Gao *et al.* 2023). Based on acyl-group structure, protein lysine acylations are generally grouped into three categories: hydrophobic acyl groups, negatively charged acidic acyl groups, and polar acyl groups (Shang *et al.* 2022). Hydrophobic acyl groups — including short-chain modifications like acetyl and crotonyl, and long-chain lipid modifications such as palmitoyl and myristoyl — increase protein hydrophobicity and enhance membrane binding. Negatively charged acidic acyl groups — including malonyl, succinyl, and glutaryl modifications — convert the lysine side-chain charge from +1 to −1. Polar acyl groups, such as β-hydroxybutyryl and lactyl modifications, introduce hydroxyl moieties that enable additional hydrogen bonding interactions (Sabari *et al.* 2017). Chemical structures of common lysine acylation modifications are shown in Fig. 1.

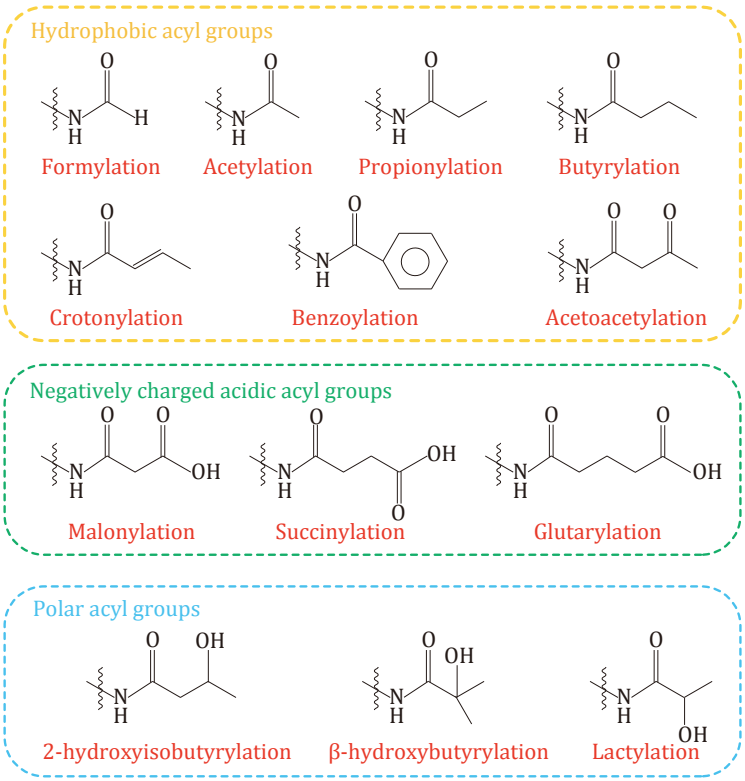

Fig. 1 Chemical structures of common lysine acylation modifications

Most acyl group donors for protein acylation modifications are intermediate metabolites generated during cellular metabolism (Fu *et al.* 2022). For example, malonyl-CoA, generated through the carboxylation of acetyl-CoA, acts as an essential intermediate in *de novo* fatty acid biosynthesis and chain elongation in mammals (Saggerson 2008). Succinyl-CoA, in addition to its central role in the TCA cycle, is additionally produced through the oxidative catabolism of branched-chain amino acids (isoleucine and valine) and odd-chain fatty acids (Zhang *et al.* 2011). Both malonyl-CoA and succinyl-CoA can modify the lysine residues of proteins, through enzyme-dependent or non-enzymatic mechanisms (Wang *et al.* 2017). Protein acylation is now recognized as a crucial regulator of both physiological and pathophysiological processes. Diverse acyl modifications on histones enhance the precision of histone epigenetic regulation, whereas modifications on non-histone proteins, particularly metabolic enzymes, modulate enzymatic activity, protein stability, and subcellular localization. Compared with acetylation, the addition of a malonyl or succinyl group to a lysine residue reverses the side-chain charge, typically shifting lysine residues from +1 to −1. Owing to their larger molecular mass (~100 Da), greater structural complexity, and greater steric hindrance, both malonylation and

succinylation have stronger structural and functional impacts (Hirschey and Zhao 2015). It has been reported that malonyl-CoA can modulate energy metabolism (Wolfgang and Lane 2008) and is closely associated with metabolic diseases (Du *et al.* 2016), inflammatory responses (Galván-Peña *et al.* 2019) and cancer (Wang *et al.* 2022a). Succinyl-CoA has been implicated in physiological and pathological processes, including carcinoma tumorigenesis (Ma *et al.* 2024), metabolic syndrome (Wu *et al.* 2024), neurological disorders (Deng *et al.* 2024), cardiovascular diseases (Zhou *et al.* 2021), immune system diseases (Wang *et al.* 2025), and mitochondrial dysfunction (Hu *et al.* 2023). These effects are considered to be mediated, at least in part, by protein acylation (Shang *et al.* 2022).

Given the importance of malonylation and succinylation modifications, omics-scale investigations are needed to explore these modifications and validate their biological functions. Altered levels of these modifications have been frequently observed in patients with metabolic disorders as well as in corresponding animal models. However, tissue heterogeneity, together with the influence of multiple confounding factors, poses substantial challenges for the accurate identification of protein modifications. Current major mass spectrometry-based identification methods include chemical derivatization (Maile *et al.* 2015), metabolic isotope labeling (Noberini *et al.* 2021), data-independent acquisition (DIA) mode (Yang *et al.* 2020), antibody-based enrichment (Zhang *et al.* 2019), and targeted mass spectrometry (Simithy *et al.* 2017). However, a major limitation of these methods is that they generate exceedingly complex peptide mixtures from heterogeneous tissues, leading to significant information loss. We and other laboratories have detected that a large portion of proteins modified by Kmal and Ksuc are localized in mitochondria. Nevertheless, many mitochondrial proteins that exhibit these modifications remain undetected with conventional one-pot approaches. For instance, during the detection of protein modification sites in the liver of ob/ob mice, excessive lipid droplets and glycogen can obscure mitochondrial protein signals, resulting in underrepresentation or loss in mass spectrometry analysis.

As the central hub of cellular metabolism, mitochondria integrate glucose, lipid, amino acid, and nucleotide pathways by hosting fundamental biochemical processes, including the citric acid cycle, oxidative phosphorylation, and fatty acid β-oxidation (Feng *et al.* 2025). Mitochondria are also involved in the regulation of cellular apoptosis, redox homeostasis (Willems *et al.* 2015), calcium homeostasis (Bravo-Sagua *et al.* 2017), and immune function (Weinberg *et al.* 2015). Given the pivotal role of mitochondria in cellular energy and biosynthetic metabolism, a tightly regulated bidirectional crosstalk exists between protein acylation modifications and mitochondrial function. During metabolic syndrome and tumor-associated metabolic reprogramming, alterations in intermediate metabolites directly modulate mitochondrial protein acylation modifications. Conversely, mitochondrial protein acylation modifications regulate global energy metabolism by modulating the activity of metabolic enzymes or transcription factors (Shang *et al.* 2022). Since malonyl-CoA and succinyl-CoA are primarily synthesized within mitochondria, their corresponding acylation events are enriched in mitochondrial proteins. This spatial confinement poses a challenge for conventional proteomic workflows, where signals from acylated mitochondrial proteins are often masked or diluted by the abundant background of the total cellular proteome. As a result, these approaches frequently yield limited coverage and may fail to detect low-abundance or dynamically regulated modification sites. To address these limitations, we implemented an organelle-centric proteomic strategy. In this workflow, mitochondria are first isolated with high purity through subcellular fractionation. Mitochondrial proteins are subjected to affinity enrichment for specific acylation, and the resulting peptides are analyzed by high-resolution mass spectrometry.

OVERVIEW OF THE PROTOCOL

As shown in Fig. 2, the experimental workflow consists of the following steps: First, mitochondria are initially isolated from mouse liver tissue, followed by the extraction of mitochondrial proteins. The proteins are then being enzymatically digested into peptides, and malonylated and succinylated peptides are enriched by immunoprecipitation using the anti-Kmal antibody and anti-Ksu antibody conjugated agarose beads, respectively. The enriched modified peptides are subsequently subjected to quantitative analysis of modification levels using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Following MS analysis, database searching and quantification of the identified peptides are carried out. Finally, comprehensive bioinformatic analyses are conducted to interpret the biological significance of the results.

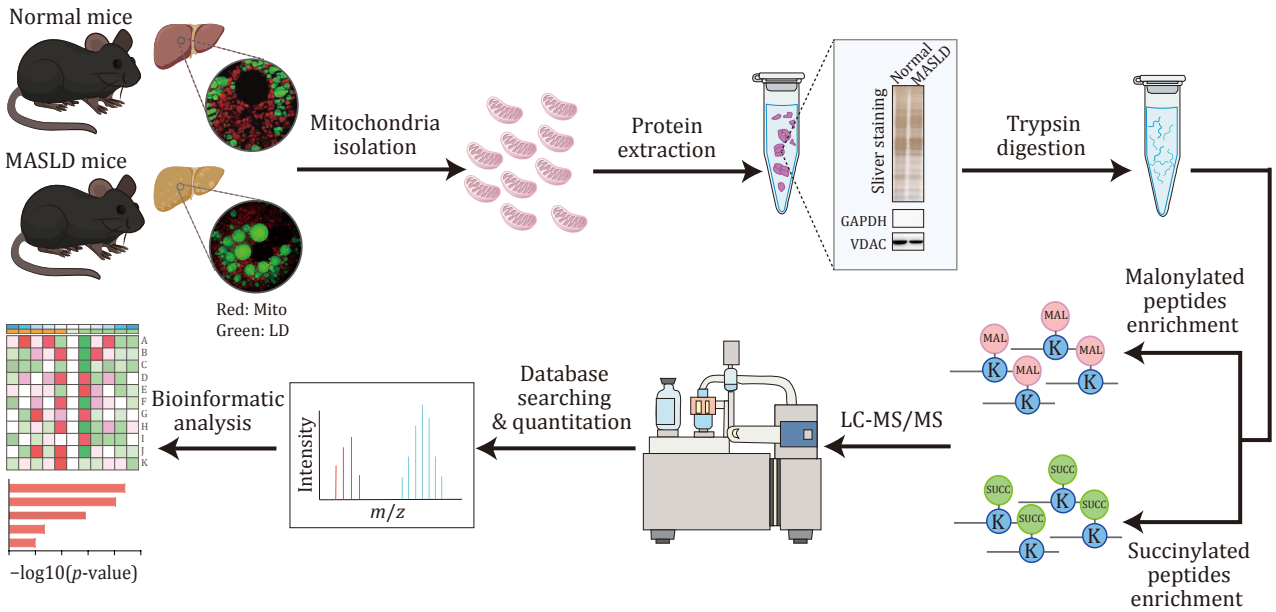

Fig. 2 Theschematic of mitochondrial protein acylation modification omics

STEP-BY-STEP PROCEDURE

Step 1: Isolation of mitochondria and extraction of mitochondrial proteins

Step 1.1: According to the experimental requirements, select the mice of the appropriate strain, age and sex. Euthanize mice, then dissect and rapidly harvest the liver.

Step 1.2: Rinse the liver twice with pre-chilled (4°C) saline to remove residual blood from the surface.

[TIP] To prevent protein degradation, all subsequent steps for liver mitochondrial isolation must be performed on ice.

Step 1.3: Add 10 mL of mitochondrial homogenization buffer A (250 mmol/L sucrose, 20 mmol/L Tricine, pH 7.8) containing protease inhibitors per gram of murine liver tissue, and mince the tissue into fine fragments (< 1 mm^3) using dissecting scissors.

Step 1.4: Transfer the homogenate to a glass Dounce homogenizer and homogenize thoroughly.

Step 1.5: Transfer the homogenate to a 15 mL centrifuge tube and centrifuge at 800*g* for 10 min at 4°C.

Step 1.6: Collect the supernatant and centrifuge at 8000*g* for 10 min at 4°C.

Step 1.7: Discard the supernatant and wash twice with Buffer B (20 mmol/L HEPES, pH 7.4, 100 mmol/L KCl, 2 mmol/L $MgCl_2$), followed by centrifugation at 8000*g* for 10 min at 4°C after each wash.

Step 1.8: Resuspend the pellet with 1.5 mL of Buffer B and carefully layer onto a pre-formed Percoll density gradient (2 mL 80% Percoll, 4.5 mL 52% Percoll, 4.5 mL 26% Percoll).

Step 1.9: Centrifuge at 18,000*g* for 45 min at 4°C and collect the mitochondrial fraction at the 26%–52% interface, then dilute and resuspend in Buffer B.

Step 1.10: Centrifuge at 2000*g* for 10 min at 4°C. The resulting pellet represents the purified mitochondrial fraction, which should be kept on ice for subsequent use.

Step 1.11: Add RIPA lysis buffer containing protease inhibitor cocktail to the pellet, and mix thoroughly by pipetting. After complete lysis, centrifuge at 12,000*g* for 10 min at 4°C.

Step 1.12: Collect the supernatant and measure protein concentration using the BCA assay kit and adjust to a final concentration of 10 μg/mL.

Step 1.13: Perform silver staining and Western blot analysis on the extracted mitochondrial proteins to verify purity and conduct quality control.

[TIP] Select appropriate proteins as markers, such as GAPDH (a cytoplasmic marker) and VDAC (a mitochondrial marker), to enable verification of the relative purity of extracted mitochondrial proteins. A pure mitochondrial fraction will show high levels of VDAC and low or undetectable levels of GAPDH, indicating minimal contamination from the cytoplasm.

Step 2: Trypsin digestion

Step 2.1: Transfer 100 μL of mitochondrial protein lysate to a new tube, add 100 μL of 10× trichloroacetic acid (TCA) and 800 μL acetone. After vigorous vortex mixing, centrifuge at 14,000*g* for 10 min at 4°C to precipitate proteins.

Step 2.2: Wash the precipitate twice with pre-chilled acetone to ensure complete removal of residual TCA.

Step 2.3: Resuspend the pellet in 100 μL of 8 mol/L urea.

[TIP] Urea can be replaced with 100 mmol/L ammonium bicarbonate (pH 8.0). If the protein pellet is not fully dissolved, sonication can be used to facilitate solubilization.

Step 2.4: Add 1 mol/L dithiothreitol (DTT) solution to a final concentration of 10 mmol/L, then incubate in a water bath at 37°C for 30 min.

Step 2.5: After cooling to room temperature (RT), add 1 mol/L iodoacetamide (IAA) solution to a final concentration of 20 mmol/L, then incubate in the dark at RT for 30 min.

[TIP] IAA is light-sensitive and should be stored at 2–8°C in amber vials protected from light exposure. To ensure stability, it should be prepared immediately before use, and light-protective measures must be taken during experimental handling.

Step 2.6: Add 1 mol/L cysteine to a final concentration of 15 mmol/L and incubate at RT for 30 min to quench excess IAA.

Step 2.7: Add 50 mmol/L ammonium bicarbonate solution (pH 8.0) to reduce the urea concentration to <1 mol/L.

Step 2.8: Add trypsin at an enzyme/protein ratio of 1:50 (*w*/*w*) according to the amount of protein and incubate in a 37°C water bath for 14 h, followed by additional supplement of trypsin at an enzyme/protein ratio of 1:100 (*w*/*w*) and incubate at 37°C for 3 h.

Step 2.9: Place the tryptic peptide solution in a 10 kDa ultrafiltration centrifuge tube and centrifuge at 3000*g* for 20 min to remove trypsin. Collect the filtrate for later use.

Step 3: Enrichment of malonylated and succinylated peptides

Step 3.1: Wash the 20 μL anti-malonyllysine antibody conjugated agarose beads or anti-succinyllysine antibody conjugated agarose beads twice with ETN buffer (50 mmol/L Tris-HCl, pH 8.0, 100 mmol/L NaCl, 1 mmol/L EDTA), centrifuge at 1000*g* at 4°C for 30 s, and resuspend the resin in 20 μL ETN buffer.

[TIP] Prior to the formal experiment, confirm the antibody's specific recognition of the target modification through dot blot experiments.

Step 3.2: Add anti-malonyllysine antibody conjugated agarose beads or anti-succinyllysine antibody conjugated agarose beads to the digested peptide solution and incubate overnight at 4°C with slow rotation.

Step 3.3: Centrifuge at 2000*g* at 4°C for 30 s, and then wash twice with ETN buffer.

Step 3.4: Wash twice with deionized water.

Step 3.5: Elute peptides with 0.1% trifluoroacetic acid (TFA) solution, centrifuge at 12,000*g* at 4°C for 1 min, and collect the supernatant.

Step 3.6: Dry the samples using a SpeedVac vacuum concentrator.

Step 3.7: Reconstitute the dried peptides in 0.1% TFA solution, desalt with C18 ZipTips, and dissolve in 0.1% formic acid (FA) for LC-MS/MS analysis.

Step 4: High pH reversed-phase HPLC fractionation

Step 4.1: Prepare the mobile phases. Solvent A is 0.1% ammonium hydroxide in water, pH 10 and Solvent B is 0.1% ammonium hydroxide in acetonitrile.

Step 4.2: Set up the HPLC system. Install a Waters XBridge BEH130 C18 column (5 μm, 4.6 × 250 mm) on the L-3000 HPLC System (Rigol).

Step 4.3: Elute the peptides with a 76-minute linear gradient as follows: 5%–9% B (3 min); 9%–18% B (27 min); 18%–34% B (32 min); 34%–95% B (2 min); 95% B (4 min); 95%–5% B (1 min); 5% B (7 min), at a flow rate of 0.7 mL/min.

Step 4.4: The eluted peptides were monitored at 214 nm. All fractions were collected at 90-s intervals and concatenated into 12 post-fractions and lyophilized.

Step 5: LC-MS/MS analysis

Separate the samples and perform LC-MS/MS analysis using a Q Exactive mass spectrometer coupled with an Easy n-LC 1000 ultra-high performance liquid chromatography (UHPLC) system.

Step 5.1: Prepare the mobile phases. Solvent A is 0.1% formic acid in water solution and Solvent B is 0.1% formic acid in acetonitrile solution.

Step 5.2: Load the peptides onto a 100 μm × 2 cm fused silica trap column packed in-house with reversed phase silica (Reprosil-Pur C18 AQ, 5 μm, Dr. Maisch GmbH) and then separate peptides on a 75 μm × 20 cm C18 column packed with reversed phase silica (Reprosil-Pur C18 AQ, 3 μm, Dr. Maisch GmbH).

Step 5.3: Elute the peptides bound to the column using the following 78-minute linear gradient: 5%–8% B (8 min); 8%–22% B (50 min); 22%–32% B (12 min);

32%–95% B (1 min); 95% B (7 min), at a flow rate of 300 nL/min.

Step 5.4: MS analysis was performed on a Q Exactive mass spectrometer in data-dependent acquisition mode.

[TIP] The parameters for mass spectrometry detection are set as follows. Primary mass spectrometry resolution is 70,000 (m/z 200), the parent ion scan range is 300–1600 m/z, the target value is 3.00×10^6, and the maximum ion injection time is 60 ms.

Step 5.5: Select the top 20 most intense ions from MS1 for MS2 analysis using higher-energy collisional dissociation (HCD) with 27% normalized collision energy.

[TIP] Secondary mass spectrometry resolution is 17,500 (m/z 200), the target value is 5.00×10^4, the maximum ion implantation time is 80 ms, and the dynamic exclusion time is 40 s. For the nano electrospray ion source setting, the spray voltage is 2.0 kV, no sheath gas flow, and the heated capillary temperature is 320°C.

Step 6: Database searching and quantitation

Step 6.1: Search the MS/MS data against the UniProt Mouse Proteome database (release 03/2025) using the SEQUEST HT search engine of Thermo Proteome Discoverer (v2.2.0.388) to identify the protein.

[TIP] Retrieval parameters: select full tryptic digestion with up to two missed cleavages; set precursor ion mass error less than 10 mg/L and fragment ion mass error less than 20 mDa. Set iodoacetamide alkylation of cysteine as a fixed modification, oxidation of methionine as a variable modification, and succinylation of lysine as a variable modification. Search result filtering parameters: use Percolator for spectral filtering with a Delta Cn threshold of <0.1 and 1% FDR; select peptide confidence as High, and control FDR at the protein level to 1%. And set LFQ analysis using the Consensus node with parameter settings: unique + razor for quantitative peptide segments, intensity for precursor abundance based on, none or total peptide amount for normalization mode, pairwise ratio based for ratio calculation, and 100 for maximum allowable fold change.

Step 6.2: Compare the identified mitochondrial proteome to the MitoCarta3.0 database.

[TIP] MitoCarta3.0 is an updated mitochondrial proteome now with sub-organelle localization and pathway annotations (Rath *et al.* 2021). Confirm that the identified proteins exhibit high overlap with the MitoCarta3.0 database to validate mitochondrial enrichment.

Step 7: Bioinformatic analysis

Step 7.1: Cluster analysis. First, normalize the quantitative information of the target protein acylation. Perform a Z-transform on the normalized data matrix for functional classification to convert the data into a standard normal distribution. Use the hierarchical clustering method to perform unilateral clustering analysis. Choose Euclidean distance as the distance metric and average linkage clustering as the clustering method. Use the Heatmap R package (v3.4 version) to draw the heat map for visual display. The heatmap provides a clear visualization of the distribution patterns of protein acylation levels and their correlations.

Step 7.2: Enrichment analysis. Perform Gene Ontology (GO) enrichment analysis to classify the target proteins based on their involvement in key biological processes, their localization within cellular components, and their specific molecular functions. The statistical significance of enrichment for each GO term was determined using a two-tailed Fisher's exact test, with a p-value < 0.05 considered the threshold for significance.

ADVANTAGES AND LIMITATIONS OF THIS PROTOCOL

This protocol details a novel, organelle-centric proteomics approach for analyzing acylation modifications. By beginning with the purification of liver mitochondria and subsequent enrichment of modified peptides, this method minimizes interference from non-mitochondrial proteins, substantially increases the abundance of target peptides, and significantly enhances the depth of the proteomic analysis. However, this protocol is focused on acylation modifications in liver mitochondrial proteins. Acylation within other tissues, such as muscle and adipose, is also highly relevant for understanding the mechanisms underlying metabolic disorders. Moreover, we primarily focus on establishing the global modification profile of mitochondria, as the separation process of mitochondrial substructures may lead to the loss of some low-abundance and unstable modifications. The current focus of research is to provide a panoramic overview of the modification of liver mitochondria as a basis for further in-depth research. It should be noted that substructure-level analyses are essential for a deeper understanding of modification functions in future research. Furthermore, the method depends heavily on

antibody-based enrichment. Due to its antibody dependence, each assay is generally limited to a single modification type. In summary, this protocol outlines an organelle-specific purification strategy that increases the relative abundance of target modified peptides in experimental samples and significantly enhances the depth of mass spectrometry-based detection. Consequently, it serves as a valuable methodological framework for exploring diverse protein acylation events in MASLD and other diseases.

MATERIALS AND EQUIPMENT

Reagents and materials, and the equipment used in this protocol are listed in Tables 1 and 2.

Table 1 Key reagents used in this protocol

Reagents	Source	Identifier
Saline	Sinopharm	010303001
Sucrose	Sinopharm	10021418
Tricine	Lablead	GAS10-1
Protease inhibitors	Selleck	B14001
HEPES	Alfa Aesar	7365-45-9
KCl	Sinopharm	10016308
$MgCl_2$	Sinopharm	TM3688500G
Percoll	Solarbio	P8370
RIPA lysis buffer	Solarbio	R0020
BCA assay kit	Thermo Scientific	23225
Trichloroacetic acid (TCA)	Sinopharm	80132618
Acetone	Sinopharm	40064460
Urea	Sinopharm	10023218
Ammonium bicarbonate	Sinopharm	20002760
Dithiothreitol (DTT)	Sinopharm	63002632
Iodoacetamide (IAA)	Sinopharm	XW0114448901
Cysteine	Sinopharm	62007234
Trypsin	Promega	V5280
10 kDa ultrafiltration centrifuge tube	Millipore	UFC5010BK
Anti-malonyllysine antibody conjugated agarose beads	PTMBio	PTM-904
Anti-succinyllysine antibody conjugated agarose beads	PTMBio	PTM-402
Anti-GAPDH antibody	ProteinTech	60004-1-Ig
Anti-VDAC antibody	ProteinTech	55259-1-AP
Tris	Lablead	0497-1
HCl	Sinopharm	10011008
NaCl	Sinopharm	10019318
EDTA	Thermo Scientific	AM9260G
Trifluoroacetic acid (TFA)	Sinopharm	80134716
C18 ZipTips	Millipore	ZTC18S960
Formic acid (FA)	Sinopharm	40023774
Acetonitrile	Sinopharm	40064184

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Table 2 Key equipment used in this protocol

Equipments	Source	Identifier
Dissecting scissors	Sigma Aldrich	Z265977
Micro-dissecting forceps	Sigma Aldrich	Straight F4017, Curve F4142
Dounce homogenizer	Sigma Aldrich	D9938
Centrifuge	Eppendorf	5810R,5430R
Ultrasonic cell disintegrator	Scientz	JY98-IIIDN
Vortex	Scientific Industries	Genie 2
Water bath	JINDIAN	XMTD-6000
Rotary mixer	Scientz	DH-II
Vacuum concentrator	Thermo Scientific	SpeedVac
Mass spectrometer	Thermo Scientific	Q Exactive
HPLC system	Thermo Scientific	Easy n-LC 1000

Compliance with Ethical Standards

Conflict of interest Juncheng Zhao, Xiaoqing Cao, Jifeng Wang, Sunyuntao Xu, Hao Hu, Xiaoxia Pei, Taotao Wei and Min Xiao declare that they have no conflict of interest.

Human and animal rights and informed consent All institutional and national guidelines for the care and use of laboratory animals were followed. The animal study was reviewed and approved by the Ethics Committee of Institute of Biophysics, Chinese Academy of Sciences and the Ethics Committee of University of Chinese Academy of Sciences.

References

Bravo-Sagua R, Parra V, López-Crisosto C, Díaz P, Quest AFG, Lavandero S (2017) Calcium transport and signaling in mitochondria. Compr Physiol 7(2): 623–634

Chen Y, Sprung R, Tang Y, Ball H, Sangras B, Kim SC, Falck JR, Peng J, Gu W, Zhao Y (2007) Lysine propionylation and butyrylation are novel post-translational modifications in histones. Mol Cell Proteomics 6(5): 812–819

Dai L, Peng C, Montellier E, Lu Z, Chen Y, Ishii H, Debernardi A, Buchou T, Rousseaux S, Jin F, Sabari BR, Deng Z, Allis CD, Ren B, Khochbin S, Zhao Y (2014) Lysine 2-hydroxyisobutyrylation is a widely distributed active histone mark. Nat Chem Biol 10(5): 365–370

Deng P, Fan T, Gao P, Peng Y, Li M, Li J, Qin M, Hao R, Wang L, Li M, Zhang L, Chen C, He M, Lu Y, Ma Q, Luo Y, Tian L, Xie J, Chen M, Xu S, Zhou Z, Yu Z, Pi H (2024) SIRT5-mediated desuccinylation of RAB7A protects against cadmium-induced Alzheimer's disease-like pathology by restoring autophagic flux. Adv Sci 11(30): e2402030. https://doi.org/10.1002/advs.202402030

Du Y, Zhai Z, Li Y, Lu M, Cai T, Zhou B, Huang L, Wei T, Li T (2016) Prediction of protein lysine acylation by integrating primary sequence information with multiple functional features. J Proteome Res 15(12): 4234–4244

Feng J-J, Guo M, Ouyang Z, Lü B (2025) The role of mitochondrial quality control in glycolipid metabolism and metabolic diseases. Prog Biochem Biophys 52(7): 1673–1686

Fu Y, Yu J, Li F, Ge S (2022) Oncometabolites drive tumorigenesis by enhancing protein acylation: from chromosomal remodelling to nonhistone modification. J Exp Clin Cancer Res 41(1): 144. https://doi.org/10.1186/s13046-022-02338-w

Galván-Peña S, Carroll RG, Newman C, Hinchy EC, Palsson-McDermott E, Robinson EK, Covarrubias S, Nadin A, James AM, Haneklaus M, Carpenter S, Kelly VP, Murphy MP, Modis LK, O'Neill LA (2019) Malonylation of GAPDH is an inflammatory signal in macrophages. Nat Commun 10(1): 338. https://doi.org/10.1038/s41467-018-08187-6

Gao Y, Sheng X, Tan D, Kim S, Choi S, Paudel S, Lee T, Yan C, Tan M, Kim KM, Cho SS, Ki SH, Huang H, Zhao Y, Lee S (2023) Identification of histone lysine acetoacetylation as a dynamic post-translational modification regulated by HBO1. Adv Sci 10(25): e2300032. https://doi.org/10.1002/advs.202300032

Hirschey MD, Zhao Y (2015) Metabolic regulation by lysine malonylation, succinylation, and glutarylation. Mol Cell Proteomics 14(9): 2308–2315

Hu Q, Xu J, Wang L, Yuan Y, Luo R, Gan M, Wang K, Zhao T, Wang Y, Han T, Wang JB (2023) SUCLG2 regulates mitochondrial dysfunction through succinylation in lung adenocarcinoma. Adv Sci 10(35): e2303535. https://doi.org/10.1002/advs.202303535

Huang H, Zhang D, Wang Y, Perez-Neut M, Han Z, Zheng YG, Hao Q, Zhao Y (2018) Lysine benzoylation is a histone mark regulated by SIRT2. Nat Commun 9(1): 3374. https://doi.org/10.1038/s41467-018-05567-w

Jiang T, Zhou X, Taghizadeh K, Dong M, Dedon PC (2007) N-formylation of lysine in histone proteins as a secondary modification arising from oxidative DNA damage. Proc Natl Acad Sci USA 104(1): 60–65

Ma W, Sun Y, Yan R, Zhang P, Shen S, Lu H, Zhou Z, Jiang Z, Ye L, Mao Q, Xiong N, Jia W, Sun L, Gao P, Zhang H (2024) OXCT1

functions as a succinyltransferase, contributing to hepatocellular carcinoma via succinylating LACTB. Mol Cell 84(3): 538–551. e7

Maile TM, Izrael-Tomasevic A, Cheung T, Guler GD, Tindell C, Masselot A, Liang J, Zhao F, Trojer P, Classon M, Arnott D (2015) Mass spectrometric quantification of histone post-translational modifications by a hybrid chemical labeling method. Mol Cell Proteomics 14(4): 1148–1158

Noberini R, Savoia EO, Brandini S, Greco F, Marra F, Bertalot G, Pruneri G, McDonnell LA, Bonaldi T (2021) Spatial epiproteomics enabled by histone post-translational modification analysis from low-abundance clinical samples. Clin Epigenetics 13(1): 145. https://doi.org/10.1186/s13148-021-01120-7

Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, Tan M, Luo H, Zhang Y, He W, Yang K, Zwaans BMM, Tishkoff D, Ho L, Lombard D, He TC, Dai J, Verdin E, Ye Y, Zhao Y (2011) The first identification of lysine malonylation substrates and its regulatory enzyme. Mol Cell Proteomics 10(12): M111.012658. https://doi.org/10.1074/mcp.M111.012658

Phillips DMP (1963) The presence of acetyl groups in histones. Biochem J 87(2): 258–263

Rath S, Sharma R, Gupta R, Ast T, Chan C, Durham TJ, Goodman RP, Grabarek Z, Haas ME, Hung WHW, Joshi PR, Jourdain AA, Kim SH, Kotrys AV, Lam SS, McCoy JG, Meisel JD, Miranda M, Panda A, Patgiri A, Rogers R, Sadre S, Shah H, Skinner OS, To TL, Walker MA, Wang H, Ward PS, Wengrod J, Yuan CC, Calvo SE, Mootha VK (2021) MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. Nucleic Acids Res 49(D1): D1541–D1547

Sabari BR, Zhang D, Allis CD, Zhao Y (2017) Metabolic regulation of gene expression through histone acylations. Nat Rev Mol Cell Biol 18(2): 90–101

Saggerson D (2008) Malonyl-CoA, a key signaling molecule in mammalian cells. Annu Rev Nutr 28: 253–272

Shang S, Liu J, Hua F (2022) Protein acylation: mechanisms, biological functions and therapeutic targets. Signal Transduct Target Ther 7(1): 396. https://doi.org/10.1038/s41392-022-01245-y

Simithy J, Sidoli S, Yuan ZF, Coradin M, Bhanu NV, Marchione DM, Klein BJ, Bazilevsky GA, McCullough CE, Magin RS, Kutateladze TG, Snyder NW, Marmorstein R, Garcia BA (2017) Characterization of histone acylations links chromatin modifications with metabolism. Nat Commun 8(1): 1141. https://doi.org/10.1038/s41467-017-01384-9

Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N, Lu Z, Ye Z, Zhu Q, Wysocka J, Ye Y, Khochbin S, Ren B, Zhao Y (2011) Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 146(6): 1016–1028

Tan M, Peng C, Anderson KA, Chhoy P, Xie Z, Dai L, Park J, Chen Y, Huang H, Zhang Y, Ro J, Wagner GR, Green MF, Madsen AS, Schmiesing J, Peterson BS, Xu G, Ilkayeva OR, Muehlbauer MJ, Braulke T, Mühlhausen C, Backos DS, Olsen CA, McGuire PJ, Pletcher SD, Lombard DB, Hirschey MD, Zhao Y (2014) Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. Cell Metab 19(4): 605–617

Wang H, Hu D, Cheng Y, Gao Q, Liu K, Mani NL, Tang AY, Iyer R, Gao B, Sun L, Zhou Q, Yu Q, Weinberg SE, Zhang X, Cong Y, Dulai PS, Zhang Y, Liu Z, Fang D (2025) Succinate drives gut inflammation by promoting FOXP3 degradation through a molecular switch. Nat Immunol 26(6): 866–880

Wang H-L, Chen Y, Wang Y-Q, Tao E-W, Tan J, Liu Q-Q, Li C-M, Tong X-M, Gao Q-Y, Hong J, Chen Y-X, Fang J-Y (2022a) Sirtuin5 protects colorectal cancer from DNA damage by keeping nucleotide availability. Nat Commun 13(1): 6121. https://doi.org/10.1038/s41467-022-33903-8

Wang S, Osgood AO, Chatterjee A (2022b) Uncovering post-translational modification-associated protein-protein interactions. Curr Opin Struct Biol 74: 102352. https://doi.org/10.1016/j.sbi.2022.102352

Wang Y, Guo YR, Liu K, Yin Z, Liu R, Xia Y, Tan L, Yang P, Lee JH, Li XJ, Hawke D, Zheng Y, Qian X, Lyu J, He J, Xing D, Tao YJ, Lu Z (2017) KAT2A coupled with the α-KGDH complex acts as a histone H3 succinyltransferase. Nature 552(7684): 273–277

Weinberg SE, Sena LA, Chandel NS (2015) Mitochondria in the regulation of innate and adaptive immunity. Immunity 42(3): 406–417

Willems PHGM, Rossignol R, Dieteren CEJ, Murphy MP, Koopman WJH (2015) Redox homeostasis and mitochondrial dynamics. Cell Metab 22(2): 207–218

Wolfgang MJ, Lane MD (2008) Hypothalamic malonyl-coenzyme A and the control of energy balance. Mol Endocrinol 22(9): 2012–2020

Wu M, Tan J, Cao Z, Cai Y, Huang Z, Chen Z, He W, Liu X, Jiang Y, Gao Q, Deng B, Wang J, Yuan W, Zhang H, Chen Y (2024) Sirt5 improves cardiomyocytes fatty acid metabolism and ameliorates cardiac lipotoxicity in diabetic cardiomyopathy via CPT2 de-succinylation. Redox Biol 73: 103184. https://doi.org/10.1016/j.redox.2024.103184

Xie Z, Zhang D, Chung D, Tang Z, Huang H, Dai L, Qi S, Li J, Colak G, Chen Y, Xia C, Peng C, Ruan H, Kirkey M, Wang D, Jensen LM, Kwon OK, Lee S, Pletcher SD, Tan M, Lombard DB, White KP, Zhao H, Li J, Roeder RG, Yang X, Zhao Y (2016) Metabolic regulation of gene expression by histone lysine β-hydroxybutyrylation. Mol Cell 62(2): 194–206

Yang Y, Liu X, Shen C, Lin Y, Yang P, Qiao L (2020) *In silico* spectral libraries by deep learning facilitate data-independent acquisition proteomics. Nat Commun 11(1): 146. https://doi.org/10.1038/s41467-019-13866-z

Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, Liu W, Kim S, Lee S, Perez-Neut M, Ding J, Czyz D, Hu R, Ye Z, He M, Zheng YG, Shuman HA, Dai L, Ren B, Roeder RG, Becker L, Zhao Y (2019) Metabolic regulation of gene expression by histone lactylation. Nature 574(7779): 575–580

Zhang Z, Tan M, Xie Z, Dai L, Chen Y, Zhao Y (2011) Identification of lysine succinylation as a new post-translational modification. Nat Chem Biol 7(1): 58–63

Zhou B, Xiao M, Hu H, Pei X, Xue Y, Miao G, Wang J, Li W, Du Y, Zhang P, Wei T (2021) Cardioprotective Role of SIRT5 in response to acute ischemia through a novel liver-cardiac crosstalk mechanism. Front Cell Dev Biol 9: 687559. https://doi.org/10.3389/fcell.2021.687559