Published in final edited form as:

J Proteomics. 2012 August 3; 75(15): 4602–4609. doi:10.1016/j.jprot.2012.02.014.

An MRM-based Workflow for Quantifying Cardiac Mitochondrial Protein Phosphorylation in Murine and Human Tissue

Maggie P.Y. Lam^{*,a}, Sarah B. Scruggs^{*,a}, Tae-Young Kim^a, Chenggong Zong^a, Edward Lau^a, Ding Wang^a, Christopher M. Ryan^b, Kym F. Faull^b, and Peipei Ping^a

^aDepartments of Physiology and Medicine, Division of Cardiology, David Geffen School of Medicine at UCLA, Los Angeles, California 90095, USA

^bThe Pasarow Mass Spectrometry Laboratory, Department of Psychiatry & Biobehavioral Sciences and the Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine at UCLA, Los Angeles, California 90095, USA

Abstract

The regulation of mitochondrial function is essential for cardiomyocyte adaptation to cellular stress. While it has long been understood that phosphorylation regulates flux through metabolic pathways, novel phosphorylation sites are continually being discovered in all functionally distinct areas of the mitochondrial proteome. Extracting biologically meaningful information from these phosphorylation sites requires an adaptable, sensitive, specific and robust method for their quantification. Here we report a multiple reaction monitoring-based mass spectrometric workflow for quantifying site-specific phosphorylation of mitochondrial proteins. Specifically, chromatographic and mass spectrometric conditions for 68 transitions derived from 23 murine and human phosphopeptides, and their corresponding unmodified peptides, were optimized. These methods enabled the quantification of endogenous phosphopeptides from the outer mitochondrial membrane protein VDAC, and the inner membrane proteins ANT and ETC complexes I, III and V. The development of this quantitative workflow is a pivotal step for advancing our knowledge and understanding of the regulatory effects of mitochondrial protein phosphorylation in cardiac physiology and pathophysiology.

Keywords

Phosphorylation; Mitochondria; Cardiovascular Disease; Protein Quantitation; MRM

Mitochondrial electron transport chain (ETC) complexes provide energy in the form of adenosine triphosphate (ATP) to sustain an array of biological processes. Cardiac cells consume substantial amounts of ATP to fuel contraction, and thus are susceptible to deterioration of mitochondrial function. This pathological remodeling of cardiac mitochondria has been linked to the development of several cardiovascular diseases [1-4].

© 2012 Elsevier B.V. All rights reserved.

Correspondence to Peipei Ping, PhD, FAHA, FISHR, David Geffen School of Medicine, University of California at Los Angeles, 675 Charles E. Young Dr, MRL Bldg, Suite 1-619, Los Angeles, CA 90095. pping@mednet.ucla.edu.

*These authors contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

As mitochondria must constantly adapt to fluctuating energy demands in health and in disease, the dynamic regulation of mitochondrial function is of vital importance.

Mounting evidence supports the concept of reversible phosphorylation as an important regulator of mitochondrial function, corroborated by the rapidly accumulating number of phosphorylation events identified across various types of mitochondria [5-7]. Nevertheless, our understanding of how mitochondrial phosphorylation alters protein function remains fragmented. Protein phosphorylation is a transient occurrence, and typically presents in sub-stoichiometric amounts, therefore determining the site-specific occupancy is technically challenging. An adaptable, sensitive, specific, and robust method for quantifying site-specific phosphorylation in mitochondria would aid in overcoming these challenges, and thus would be a substantial contribution to the field of mitochondrial biology.

We report the development of a multiple reaction monitoring (MRM)-based quantitative workflow for the characterization of site-specific protein phosphorylation in murine and human cardiac mitochondria. MRM quantification of phosphorylation was performed by measuring the ion current generated from analyte-specific transitions; this provides specificity and sensitivity as a result of utilizing liquid chromatographic retention time in combination with two selective mass filters of a triple quadrupole mass spectrometer [8, 9]. The incorporation of heavy-labeled synthetic peptides as internal standards excludes the need for *in vivo* or *in vitro* labeling of the samples. Accordingly, the same set of synthetic peptides can be employed across multiple experiments. As many of the selected peptide sequences are conserved among organisms and are localized in multiple tissues (e.g. liver, kidney, heart, etc.), deducing appropriate MRM transitions would be useful for obtaining insights into the effects of phosphorylation on protein function across various tissue types and organisms.

The first step in the development of the workflow was to identify candidate phosphopeptides from a discovery LC-MS/MS dataset. All selected phosphopeptides were originally identified using comprehensive, high resolution LC-MS/MS of purified murine cardiac mitochondria [5], therefore all phosphorylation sites exist endogenously. To make the study translational, we used murine peptide sequences to obtain a list of human homolog sequences through BLAST analyses. In total, 23 peptides comprising both phosphorylated and unmodified counterparts were analyzed by the workflow outlined in Figure 1. Six phosphopeptides were exclusively from human and four were exclusively from murine. Nineteen of the peptides originated from the ETC complexes: 2 belonged to the NADH dehydrogenase subunit 5 (complex I), 4 from NADH dehydrogenase 1 alpha subcomplex subunit 10 (complex I), 4 from cytochrome b-c 1 complex subunit 2 (complex III), 5 from ATP synthase subunit alpha (complex V), and 4 from ATP synthase subunit beta (complex V). Additionally, 2 peptides from the ADP/ATP translocase 1 (ANT1) and 2 peptides from the voltage-dependent anion-selective channel protein 1 (VDAC) were targeted. All peptides were synthesized by Thermo Scientific Open Biosystems with ^{13}C or ^{15}N incorporation into the carboxyl terminal residue, giving rise to a mass shift of 6 to 10 Da. Phospho-MRM is more restrictive than traditional MRM because the choice of target peptides must include the phosphorylation sites of interest. Thus, the selected peptides may have challenging chemical properties known to complicate mass spectrometric analysis. These include but are not limited to peptide length, missed/partial tryptic cleavages and inclusion of methionine (Met) residues. For example, although peptide P4/N4 from the Complex V beta subunit (Table 1) contains a missed tryptic cleavage site, it was the only form of the phosphopeptide detected endogenously [5]. But the fully tryptic form (VLDsGAPIK) will henceforth be included since multiple forms may exist under different cellular conditions. In addition, the majority of selected phosphopeptides contain Met residues. In order to determine which forms (oxidized or non-oxidized) to target via MRM, we thoroughly searched high-resolution LC-

MS/MS spectra for endogenous Met oxidation. While significant Met oxidation was not detected, this remains an important consideration as Met oxidation can occur via sample processing. Deliberate Met oxidation to quantitatively convert all residues to their fully oxidized forms is ill-advised because of the likely introduction of multiple side reactions in the endogenous mitochondrial sample.

The second step in the workflow was to determine target MRM transitions. All transitions were chosen from LC-MS/MS spectra collected on an Agilent 6520A quadrupole time-of-flight (QTOF) instrument coupled to a ChipCube ion source. Samples were injected (5 pmol in 2 μ L) onto a ProtID-Chip-150 II HPLC-Chip (packed with reversed-phase (RP) Zorbax 300SB-C18 5 μ m resin) equilibrated in solvent A (water/formic acid, 100/0.1, v/v) and eluted with an increasing concentration of solvent B (acetonitrile (ACN)/formic acid, 100/0.1, v/v; min/%B, 0/15, 10/55) at 0.3 μ L/min. Mass spectra and LC chromatograms were visualized by Mass Hunter Qualitative Analysis software. It was advantageous to use the Agilent QTOF as the discovery-based instrument, because the ion source and collision cell are identical in the QTOF and the triple quadrupole. This results in similar fragmentation spectra. Two or more transitions were chosen for each phosphopeptide from the most abundant precursors (typically $z=2$ or 3) combined with intense fragment ions. In addition to signal intensity, transitions with higher m/z ratio were preferred to avoid the low-mass region with higher background. Transitions were selected manually, due to special consideration of the gas phase chemistry unique to phosphopeptides. The loss of H_3PO_4 from phospho-Thr or phospho-Ser peptides is energetically favorable during collision-induced dissociation (CID), and the resulting neutral loss ion may be the most abundant in the MS/MS spectra. This phenomenon was examined, and in cases where the neutral loss fragment ions were dominant, the corresponding transitions were included for MRM analysis. An additional consideration unique to phosphopeptide transitions is that the choice of fragment ions is not only restricted to those that provide high sensitivity, but also those that unequivocally demarcate the location of the phosphorylation site. The latter point is critical for candidates that contain more than one potential phosphorylation site.

The third step in our workflow was to determine optimal MS conditions for selected transitions. All MRM data were acquired at unit mass resolution by an Agilent 6460 triple quadrupole MS equipped with a ChipCube ion source. Potential transitions for each peptide were tuned by the *Optimizer for Peptides* software (Agilent) to obtain the optimal collision energy (CE) and fragmentor voltage (FV). FV is the voltage applied between the end of the capillary and skimmer and affects the transmission and fragmentation of ions between the ion source and mass analyzer. Optimization was performed by injection of an aliquot (5 pmol) of each synthetic peptide onto a C18 Chip equilibrated in solvent C (water/ACN/formic acid, 100/3/0.1, v/v/v), and eluted with an increasing concentration of solvent D (water/ACN/formic acid, 3/100/0.1, v/v/v; min/%D, 0/0, 9/70) at a flow rate of 0.6 μ L/min. MS acquisition was carried out in the positive ion selected ion monitoring (SIM) mode. To optimize the FV, the *Optimizer* software applied increasing FV over a user-entered range of 50–200V. The software then further refined the FV over a narrow voltage range in SIM mode, and automatically selected the optimal voltage for each transition. To optimize CE, *Optimizer* acquired MRMs for each transition over a range of increasing CE (0–50V, at an interval of 4V). Peak areas were manually integrated to determine the optimal CE for each transition. Figures 2a and 2b present a representative example of the CE optimization process for the peptide ITsAYLQDIENAY[K($^{13}C_6$; $^{15}N_2$)] [lowercase letter(s) in amino acid sequences indicate phosphorylated residue(s)] from a human sequence. Optimized CE and FV results are summarized in Table 1. Following optimization, triplicate LC-MRM analyses were used to verify chromatographic alignment of all transitions (Figure 2c and Supplementary Figure S1). Dwell times were varied based upon sample complexity and

signal intensity. A typical dwell time was 50 ms per transition that was increased to 300 ms for weak signals, when possible.

Since phosphopeptides typically exist in sub-stoichiometric amounts, the next step in the workflow was to develop enrichment conditions for these low-abundance peptides [10, 11]. Online enrichment with a TiO₂ Phosphochip (Agilent Phosphochip II) proved to be convenient and effective [12], and eliminated extra sample handling steps, which in turn may reduce sample losses as well as minimize Met oxidation. The Phosphochip consists of an RP-TiO₂-RP sandwich trapping column in line with an analytical RP column. With this system, all sufficiently hydrophobic peptides are retained by the first RP layer of the trap sandwich. Application of the RP gradient then resolves these unmodified peptides, while phosphopeptides are retained by the TiO₂ layer of the trap sandwich. The phosphopeptides are then eluted from the TiO₂ trap by injection of 16 µL of a manufacturer-supplied elution buffer, and resolved by a second round of RP gradient. In this mode of operation, the Phosphochip produces one data set of unmodified peptides and a sequential separate data set of phosphopeptides that are trapped by TiO₂. This is in contrast to off-line phosphopeptide enrichment schemes in which the fraction containing unmodified peptides is usually discarded.

The Phosphochip was tested with bovine beta-casein derived FQsEEQQQTEDELQDK (100 fmol) mixed with tryptic digested bovine serum albumin (100 fmol), dissolved and injected (1 µL) in water onto the trap column, which was equilibrated in solvent F (water/acetic acid/formic acid/ACN 100/0.6/2/2, all by v) at 3 µL/min. The unmodified peptides were eluted (0.3 µL/min) from the analytical column equilibrated in solvent G (water/acetic acid/formic acid, 100/0.6/0.5, all by v) with an increasing concentration of solvent H (ACN/acetic acid/formic acid, 100/0.6/0.5, all by v; min/%H, 0/3, 7/45). The phosphopeptides were displaced from the TiO₂ trap as described in the above paragraph, and eluted from the analytical column in the same way as the unmodified peptides. The peak areas of the extracted ion chromatograms (XIC) for FQsEEQQQTEDELQDK (m/z 688 [M+3H]³⁺, 1032 [M+2H]²⁺) from the first elution (unmodified peptides) and the second elution (phosphopeptides) were about 1:100, respectively, demonstrating a trapping efficiency for this phosphopeptide in a complex matrix of about 99% (Supplementary Figure S3).

The next step in the workflow was to prepare the endogenous sample for analysis and quantification. Murine hearts were homogenized in 250 mM sucrose containing 1 mM EGTA, 20 mM HEPES, protease inhibitor mixture (Roche, Product # 05892970001), and phosphatase inhibitors (Sigma Aldrich, Product # P5726 and P0044 which included sodium orthovanadate, sodium molybdate, sodium tartrate, imidazole, cantharidin, (-)-p-bromotetramisole, and calyculin A) [5]. The nuclear and sarcomeric fractions along with tissue debris were removed via low speed centrifugation (800 × *g*, 10 min, 4 °C). Crude mitochondria were then collected via a second higher speed centrifugation (4,000 × *g*, 20 min, 4 °C). The resulting pellet was further purified using Percoll gradient density separation (19%, 30% and 60%) in the isolation buffer. Purified mitochondria were retrieved from the bottom layer after centrifugation (10,000 × *g*, 15 min, 4 °C). Proteins were extracted in 10 mM Tris (pH 7.4) with brief sonication and were either tryptically digested in-solution or subjected to Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) for enrichment of ETC complexes (Supplementary Figure S2). BN-PAGE separates assembled ETC complexes from free subunits. This is necessary to measure phosphorylation status in functional complexes. For trypsinization, purified mitochondria (100 µg) were solubilized in 100 mM ammonium bicarbonate (100 µL), disulfide bonds were reduced with DTT (6 µL, 50 mM, 60 °C, 30 min), and cysteine residues were alkylated with iodoacetamide (6 µL, 100 mM, 60 min, ambient temperature). Proteins were digested using sequencing-grade trypsin

(1:25, w/w, Promega, 16 hr, 37 °C). The digested sample was purified using C18 spin columns (Thermo Scientific, Product # 89870) according to the manufacturer's protocol.

The final step in the workflow was to quantify site-specific phosphorylation in murine cardiac mitochondrial proteins on the Phosphochip using the preselected transitions. Thirteen peptide sequences from ETC complexes, 2 peptides from VDAC, and 2 peptides from ANT1 were monitored from 4 µg of digested mitochondrial samples spiked with synthetic peptides as internal standards (1 pmol for ETC peptides, 5 pmol for VDAC and ANT1 peptides). The peptides were eluted from the column with solvent H (min/% H, 0/5, 90/40). Dwell times were chosen to obtain at least 8 data points per LC peak [13]. Alternatively, a dynamic or scheduled MRM method utilizing predetermined retention times may be employed if a higher number of transitions are to be monitored. Figure 2d shows light and heavy transition pairs for the phosphopeptides VLDsGAPIKIPVGPETLGR (Complex V), LTFDSSFsPNTGK (VDAC), and AAYFGVYDTAK (ANT1) detected in endogenous murine mitochondrial lysates. Three transitions each for Complex V and VDAC, and two transitions for ANT1 were detected at retention times identical to that of the heavy peptide standards. The MRM traces for the corresponding unmodified peptides are shown in Figure 2e. From these data, light peptides were quantified from $[(\text{area of light peptide} / \text{area of heavy peptide}) \times \text{quantity of heavy peptide standard}]$, and phosphorylation site occupancy was determined from $[\text{quantity of phosphopeptide} / (\text{quantity of phosphopeptide} + \text{quantity of unmodified peptide})]$. The estimated proportions of phosphopeptides were calculated as 1.17% (Complex V), 1.04% (VDAC) and <1% (ANT1). These are approximate values because the crude standard peptides used for development of the assay exhibited a large range of purity and could not be relied upon for accurate stoichiometric measurements. Studies using high-purity phosphopeptides to obtain accurate measurements are ongoing in our laboratory. There were 6 phosphopeptides not detected in our assay. This is likely due to low stoichiometric abundance below the limit of detection, rather than technical issues since we confidently identified all transitions in the heavy-labeled standard phosphopeptides. It is also conceivable that these sites may not be phosphorylated under basal conditions, or that pertinent phosphatases were active during tryptic digestion at 37°C. To address the latter, a known quantity of heavy-labeled standard phosphopeptide was added to samples prior to digestion and negligible dephosphorylation was found. This however remains an important consideration for future analysis, as certain phosphorylation sites are more labile than others and are therefore more susceptible to phosphatase activity.

In conclusion, an MRM-based workflow for quantifying important site-specific phosphorylation in cardiac mitochondrial proteins has been described. Sixty-eight transitions from 23 phosphorylated and unmodified peptides have been determined and optimized. An online phosphopeptide enrichment technology was utilized and proven to be effective. Eight phosphopeptide transitions and 24 unmodified peptide transitions were quantified in 4 µg of murine mitochondrial protein digests. Stoichiometric phosphorylation was quantified for three unique phosphorylation sites in mitochondrial outer (VDAC) and inner (ANT1 and ETC complexes) membrane proteins. These proteins constitute major signaling and metabolic hubs in cardiac mitochondria, and thus understanding their regulation via phosphorylation is of critical importance to cardiovascular biology. Our study also integrates an organelle proteomics-based approach, which has several advantages over traditional whole-cell proteome-wide approaches. First, the set of phosphopeptides under investigation is focused, therefore quantitative information obtained is immediately translational to understanding protein function. Simply stated, we know a significant amount regarding the mitochondrial biology of the protein set of interest, (e.g., locations of phosphorylation sites within protein crystal structures), therefore extracting meaningful information and developing novel hypotheses regarding the regulation of these proteins by phosphorylation

is an immediate next step following site-specific quantification. Second, an organelle-based approach effectively enriches the sample for proteins of interest. This is a critical factor in phosphorylation studies due to low stoichiometric abundances of phosphorylation site occupancies. Third, the simultaneous quantification of all phosphorylation sites within a given organelle allows for the identification of changes in phosphorylation patterns or trends. From this line of information, biologists can model how alterations in the mitochondrial phosphoproteome change with the function of the organelle in the diseased heart. A substantial amount of information can be obtained when organelle proteomic approaches are integrated with a sensitive, specific, and high-throughput quantitative technique like MRM. Our investigation offers unique utilities and beneficial information for both mitochondria-specific and cardiovascular focused research communities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Andy Gieschen of Agilent Technologies for his contributions. This work is sponsored in part by NIH awards HL 63901, HL 101228, and HHSN268201000035C to Dr. Ping; and by F32 HL 099029 to Dr. Scruggs.

References

- [1]. Bindoff L. Mitochondria and the heart. *Eur Heart J*. 2003; 24:221–4. [PubMed: 12590900]
- [2]. Rosca MG, Hoppel CL. Mitochondria in heart failure. *Cardiovasc Res*. 2010; 88:40–50. [PubMed: 20668004]
- [3]. Krzywanski DM, Moellering DR, Fetterman JL, Dunham-Snary KJ, Sammy MJ, Ballinger SW. The mitochondrial paradigm for cardiovascular disease susceptibility and cellular function: A complementary concept to mendelian genetics. *Lab Invest*. 2011; 91:1122–35. [PubMed: 21647091]
- [4]. Zimmerman MC, Zucker IH. Mitochondrial dysfunction and mitochondrial-produced reactive oxygen species. *Hypertension*. 2009; 53:112–4. [PubMed: 19114641]
- [5]. Deng N, Zhang J, Zong C, Wang Y, Lu H, Yang P, et al. Phosphoproteome analysis reveals regulatory sites in major pathways of cardiac mitochondria. *Mol Cell Proteomics*. 2011; 10:1–14.
- [6]. Kane LA, Youngman MJ, Jensen RE, Van Eyk JE. Phosphorylation of the F1Fo ATP synthase beta subunit. *Circul Res*. 2010; 106:504–13.
- [7]. Feng J, Zhu M, Schaub MC, Gehrig P, Roschitzki B, Lucchinetti E, et al. Phosphoproteome analysis of isoflurane-protected heart mitochondria: Phosphorylation of adenine nucleotide translocator-1 on tyr194 regulates mitochondrial function. *Cardiovasc Res*. 2008; 80:20–9. [PubMed: 18558627]
- [8]. Kettenbach AN, Rush J, Gerber SA. Absolute quantification of protein and post-translational modification abundance with stable isotope-labeled synthetic peptides. *Nat Protocols*. 2011; 6:175–86.
- [9]. Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *PNAS*. 2003; 100:6940–5. [PubMed: 12771378]
- [10]. Mazanek M, Mituloviae G, Herzog F, Stingl C, Hutchins JRA, Peters J-M, et al. Titanium dioxide as a chemo-affinity solid phase in offline phosphopeptide chromatography prior to HPLC-MS/MS analysis. *Nat Protocols*. 2007; 2:1059–69.
- [11]. Pocsfalvi G, Cuccurullo M, Schlosser G, Scacco S, Papa S, Malorni A. Phosphorylation of b14.5a subunit from bovine heart complex I identified by titanium dioxide selective enrichment and shotgun proteomics. *Mol Cell Proteomics*. 2007; 6:231–7. [PubMed: 17114648]

- [12]. Mohammed S, Kraiczek K, Pinkse MWH, Lemeer S, Benshop JJ, Heck AJR. Chip-based enrichment and NanoLC-MS/MS analysis of phosphopeptides from whole lysates. *J Proteome Res.* 2008; 7:1565–71. [PubMed: 18307298]
- [13]. Lange V, Picotti P, Domon B, Aebersold R. Selected reaction monitoring for quantitative proteomics: A tutorial. *Mol Syst Biol.* 2008; 4

Highlights

- > Studying mitochondrial phosphorylation requires sensitive, reliable quantitation methods.
- > A workflow combining peptide separation and multiple reaction monitoring is described.
- > Optimization of 68 transitions from phosphorylation sites in mouse and human.
- > Detection of 11 endogenous peptides using the optimized MRM method.

Figure 1. General Workflow for the MRM-based Quantification of Cardiac Mitochondrial Protein Phosphorylation

1) Synthetic peptides labeled with heavy isotopes are subjected to LC-MS/MS to determine potential transitions. A, Choosing MRM transitions from the MS/MS spectrum of the heavy-labeled VLDsGAPIKIPVGPETLGR phosphopeptide standard. B, Traces for three transitions from the same peptide during MRM optimization of fragmentor voltage and collision energy. C, LC/MS/MS-MRM (21 total transitions) total ion current display following injection of a mitochondrial digest showing quality of LC separation, with the arrow indicating the peak for the VLDsGAPIKIPVGPETLGR peptides. 2) Murine cardiac mitochondria are isolated. 3) Coomassie stained BN-PAGE of the mitochondrial protein preparation shows the separation of Complexes I, III and V. 4) MRM traces for quantification of the heavy standard and light endogenous forms of the VLDsGAPIKIPVGPETLGR peptide.

Figure 2. Optimization of Collision Energy Using *Optimizer for Peptides* and Application of Optimized MRM Parameters to a Mitochondrial Sample

A) The collision energy (CE) for each transition was optimized by increasing the CE at an interval of 4V (from 0V to 50V) while acquiring in MRM mode. The CE giving rise to the most intense peak area was selected by manual inspection. B) Plot of peak area versus CE voltage for the m/z 858.5 → 988.5 transition. C) Representative overlaid LC-MRM chromatograms (3 transitions per peptide) illustrating chromatographic alignment and symmetry of the selected transitions (refer to Supp. Figure S1 for non-overlaid chromatograms). Peaks are labeled with peptide ID (refer to Table 1). P: phosphorylated peptide; N: unmodified peptide). D) LC-MRM signals from phosphopeptides, and E) unmodified peptides detected in the endogenous mitochondrial sample. Heavy standard peptide signals are shown above the corresponding light endogenous peptide signals. Transitions are displayed in overlaid mode for each peptide and m/z values are labeled.

Table 1
Optimized CE and Fragmentor Voltage of Heavy-Labeled Mitochondrial Peptides.

<i>a</i> Gene Name	UniProt ID	<i>c</i> Peptide ID	Peptide / Amino Acid Sequence	Species	Precursor m/z	Transition	Ion	<i>e</i> FV	<i>e</i> CE
Alp5a1	<i>b</i> Q03265 (M), P25705 (H)	P1	<i>d</i> GMSLNLEPDNVGVVFGND[K(¹³ C ₆ ; ¹⁵ N ₂)]	<i>b</i> M, H	731.3	356.1	b3	175	24
						588.3	y5	175	16
						1367.7	y13	175	16
Alp5a1	Q03265 (M), P25705 (H)	N1	GMSLNLEPDNVGVVFGND[K(¹³ C ₆ ; ¹⁵ N ₂)]	M, H	704.7	276.1	b3	155	28
						588.3	y5	155	16
						942.5	y9	155	16
Alp5a1	Q03265 (M), P25705 (H)	P2a	TGTAEMSSILEE[R(¹³ C ₆ ; ¹⁵ N ₄)]	M, H	757.3	669.4	y5	220	12
						974.5	y8	220	20
						1257.6	y11-H ₃ PO ₄	220	24
Alp5a1	Q03265 (M), P25705 (H)	P2b	IGTAEMSSILEE[R(¹³ C ₆ ; ¹⁵ N ₄)]	M, H	757.3	708.3	MH-H ₃ PO ₄	205	16
						974.5	y8	205	24
						1332.6	y12	205	28
Alp5a1	Q03265 (M), P25705 (H)	N2	TGTAEMSSILEE[R(¹³ C ₆ ; ¹⁵ N ₄)]	M, H	717.3	260.1	b3	195	24
						843.4	y7	195	24
						974.5	y8	195	24
Alp5b	P56480 (M), P06576 (H)	P3	FLSQPFQVAEVFGHMG[K(¹³ C ₆ ; ¹⁵ N ₂)]	M, H	704.3	769.8	y13+2	175	16
						818.4	y14+2	175	12
						1164.5	y10	175	20
Alp5b	P56480 (M), P06576 (H)	N3	FLSQPFQVAEVFGHMG[K(¹³ C ₆ ; ¹⁵ N ₂)]	M, H	677.7	638.3	y6	115	24
						778.4	y14+2	115	16
						884.5	y8	115	20
Alp5b	P56480 (M), P06576 (H)	P4	VLDsGAPIKIPVGPETLGR[¹³ C ₆ ; ¹⁵ N ₄)]	M, H	670.4	739.4	y7	170	20
						758.0	y15+2	170	12
						935.5	y9	170	24
Alp5b	P56480 (M), P06576 (H)	N4	VLDsGAPIKIPVGPETLGR[¹³ C ₆ ; ¹⁵ N ₄)]	M, H	643.7	758	y15+2	175	12

^a Gene Name	UniProt ID	^c Peptide ID	Peptide / Amino Acid Sequence	Species	Precursor m/z	Transition	Ion	^e FV	^e CE
						801.5	y16 ⁺²	175	16
						859.0	y17 ⁺²	175	16
Mtnd5	P03915 (H)	P5	MTMHHTMTTTLTSLPPILTLVNPN[K(¹³ C ₆ , ¹⁵ N ₂)]	H	1057.9	366.2	y3	225	24
						697.9	y12 ⁺²	225	24
						873.4	y7	225	36
Mtnd5	P03915 (H)	N5	MTMHHTMTTTLTSLPPILTLVNPN[K(¹³ C ₆ , ¹⁵ N ₂)]	H	1031.2	366.2	y3	210	40
						657.9	y12 ⁺²	210	20
						793.5	y7	210	44
Ndufa10	O95299 (H)	P6	ITSAYLQDIENAY[K(¹³ C ₆ , ¹⁵ N ₂)]	H	858.8	988.5	y8	245	24
						1101.6	y9	245	28
						1502.7	y12	245	24
Ndufa10	O95299 (H)	N6	ITSAYLQDIENAY[K(¹³ C ₆ , ¹⁵ N ₂)]	H	818.9	988.5	y8	220	20
						1101.6	y9	220	24
						1422.7	y12	220	24
Ndufa10	Q99LC3 (M)	P7	VTSAYLQDIENAY[K(¹³ C ₆ , ¹⁵ N ₂)]	M	851.9	860.4	y7	260	24
						1101.6	y9	240	28
						1502.7	y12	260	24
Ndufa10	Q99LC3 (M)	N7	VTSAYLQDIENAY[K(¹³ C ₆ , ¹⁵ N ₂)]	M	811.9	201.1	b2	215	24
						860.4	y7	215	24
						1101.6	y9	215	24
Uqere2	P22695 (H)	P8	sMAASGNLGHHTPFVDE[L(¹³ C ₆ , ¹⁵ N)]	H	916.9	201.1	b2-H ₃ PO ₄	240	48
						370.1	b3	240	36
						1021.5	y9	240	24
Uqere2	P22695 (H)	N8	sMAASGNLGHHTPFVDE[L(¹³ C ₆ , ¹⁵ N)]	H	876.9	219.1	b2	240	40
						290.1	b3	240	32
						1027.5	b11	240	24
Uqere2	Q9DB77 (M)	P9	sMAASGNLGHHTPFVDE[L(¹³ C ₆ , ¹⁵ N)]	M	923.9	370.1	b3	245	32
						740.4	y6	245	24

^a Gene Name	UniProt ID	^c Peptide ID	Peptide / Amino Acid Sequence	Species	Precursor m/z	Transition	Ion	^e FV	^e CE
Uqerc2	Q9DB77 (M)	N9	SMAASGNLGHTPFLDE[L(¹³ C ₆ , ¹⁵ N ₂)]	M	883.9	290.1	b3	230	32
Antl	P48962 (M), P12235 (H)	P10	AAyFGVYDTA[K(¹³ C ₆ , ¹⁵ N ₂)]	M, H	647.3	605.5	y5	180	20
Antl	P48962 (M), P12235 (H)	N10	AAyFGVYDTA[K(¹³ C ₆ , ¹⁵ N ₂)]	M, H	607.3	761.4	y7	135	16
Vdac1	Q60932 (M), P21796 (H)	P11	LTFDSSFsPNTG[K(¹³ C ₆ , ¹⁵ N ₂)]	M, H	744.8	215.2	b2	215	24
Vdac1	Q60932 (M), P21796 (H)	N11	LTFDSSFsPNTG[K(¹³ C ₆ , ¹⁵ N ₂)]	M, H	704.8	524.3	y5	180	32
						611.3	y6	180	20

^aGene name from murine

^bM: Murine; H: Human

^cCustom assigned peptide ID

^dLowercase letters in sequences denote phosphorylated residue

^eCE: Collision Energy; FV: Fragmentor Voltage