

# Subsarcolemal Mitochondrial Proteome and Quantification Protein Expression

Jing Wang, Claudia S. Maier

Department of Chemistry, Oregon State University, Corvallis, OR 97331

## OVERVIEW

### Purpose

- Identification and quantification of free, exposed mitochondrial thiol proteins that are potential targets of oxidative damage using the isotope-coded affinity tags (ICAT) approach

### Methods

- ICAT labeling
- SCX, C18, Avidin affinity separation
- ESI-qTOF-MS/MS
- Mascot searching engine

### Results

- Identification of ICAT-labeled peptides/proteins from three different detergents
- Quantification of Heavy/Light ICAT-labeled peptide ratios from 36 mitochondrial proteins
- Several ICAT-labeled peptide pair ratios are different than 1

## INTRODUCTION

Mitochondrial thiol proteins play key roles in regulating mitochondrial functions. They are putative targets of oxidative stress-mediated modifications in the aging heart. During stages of increased oxidative stress, such as aging, redox imbalance causes accumulation of oxidatively modified proteins leading potentially to mitochondrial dysfunction. In this study we attempt to identify and quantify the potential protein thiols which are linked to aging and age-related disorders in cardiac mitochondria from young and old rats.

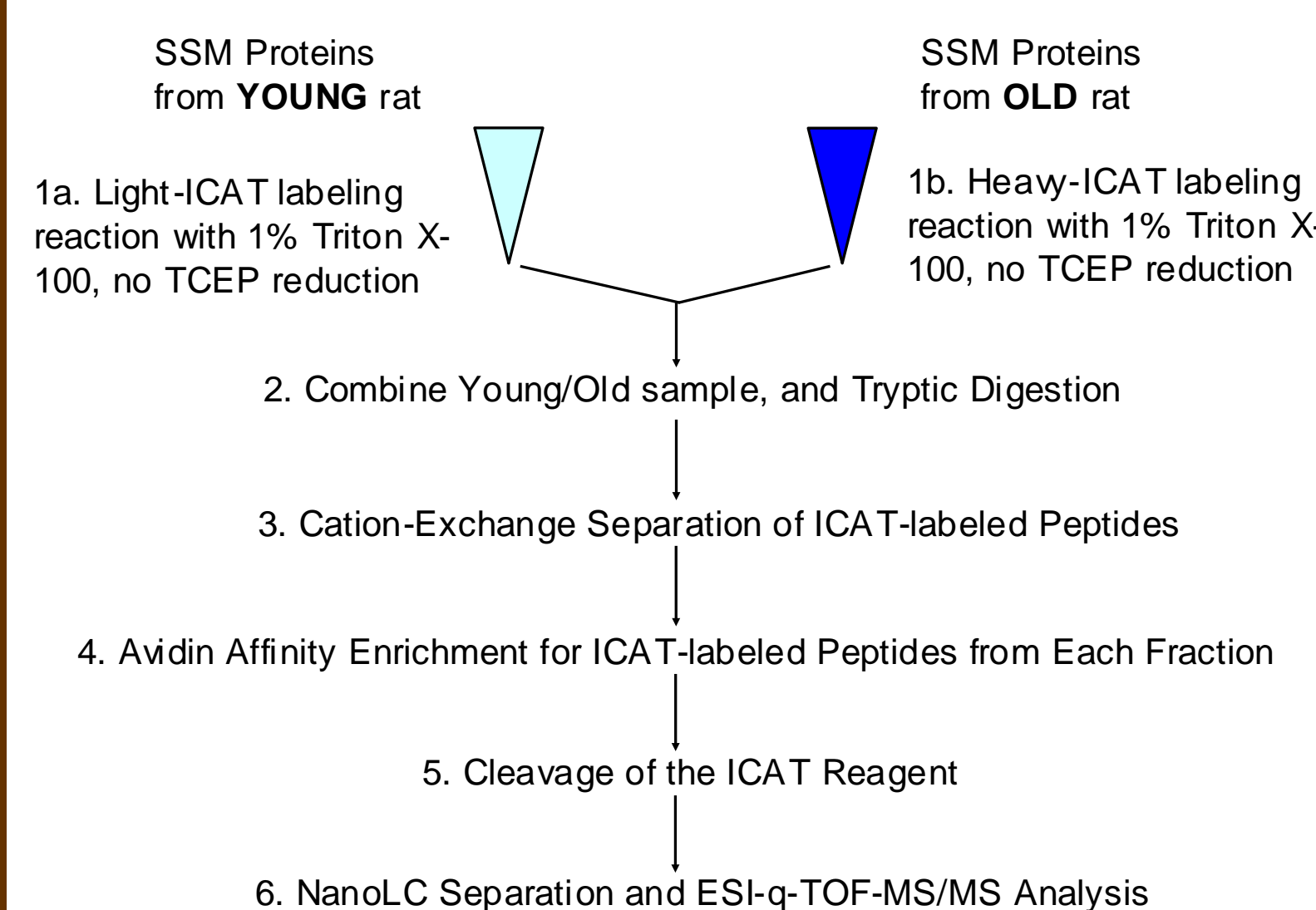
Commercial available sulfhydryl-specific reagents, cleavable isotope-coded affinity tags (cICAT),<sup>1,2</sup> were chosen as the proteomic tools to identify the thiol proteins in complex mixtures to obtain the relative quantification of peptides/proteins between young and old rats.

## METHODS

Cardiac subsarcolemal mitochondria (SSM) were isolated by differential centrifugation.<sup>3</sup> Three different types of detergents were chosen to dissolve the heart mitochondrial proteins. 6M urea and 0.1% SDS, 1% Triton X-100 (v/v) and 0.5% CHAPS (w/v) were added to same amount SSM for 1 hour. Half of the detergent-treated proteins were reduced by TCEP later, and followed by ICAT-labeling reaction for 2 hours at 37 °C. The other half the protein were directly reacted with ICAT reagents. Proteolytic digests of ICAT-labeled protein mixtures were separated by strong cation exchange (SCX) cartridge, then followed by mono-avidin beads affinity enrichment. Each enriched SCX fraction was analyzed by nanoLC-ESI-Q-TOF.

SSM from Young (3 months) and old (24 months) rat hearts were treated with 1% Triton X-100 (v/v), then labeled with light and heavy ICAT reagents separately. The mixtures of light and heavy ICAT-labeled proteins were digested by trypsin, followed by the SCX, avidin-affinity, and C18 separation before mass spectrometric analysis as described previously. Mascot searching was used for protein identification.

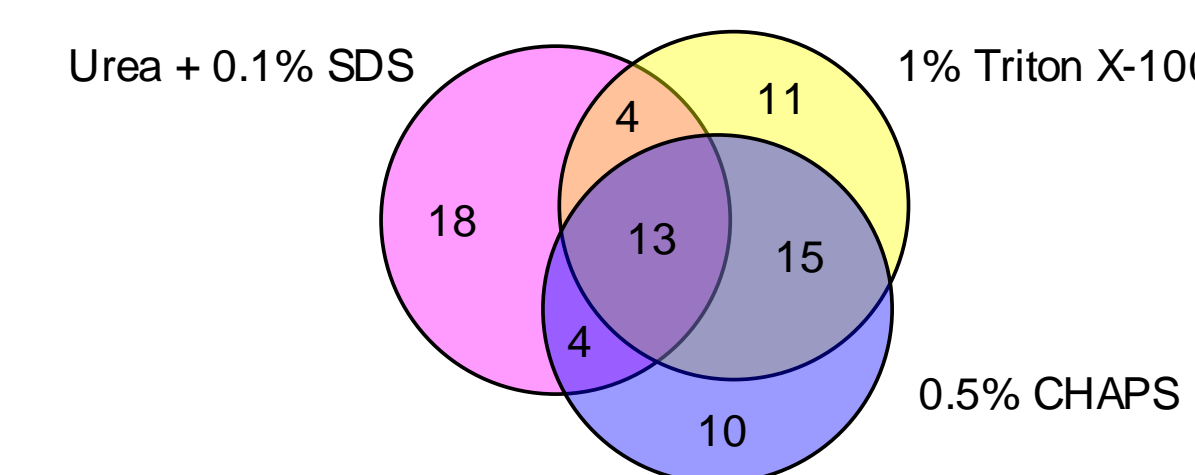
### Flow Chart of Experimental Procedure



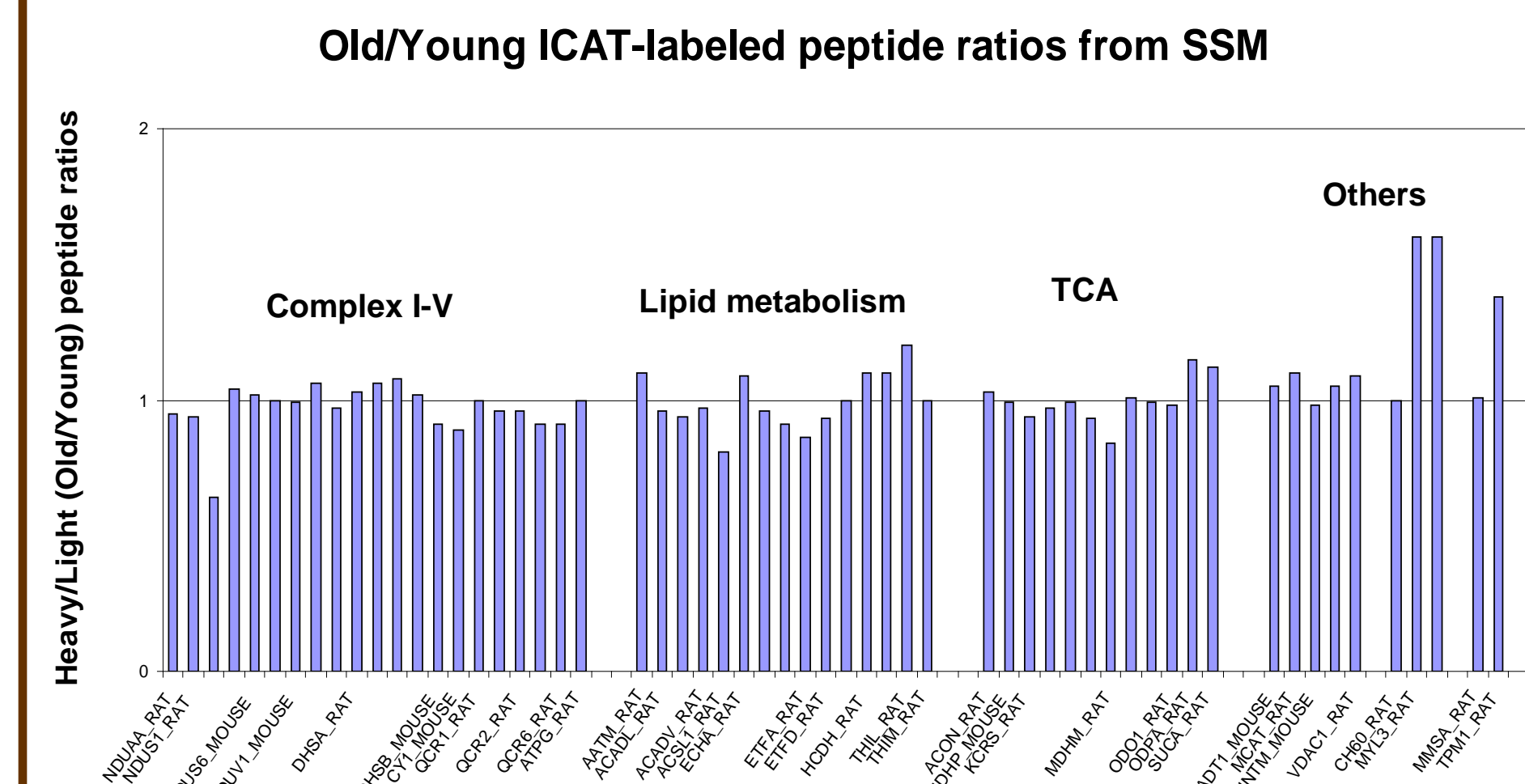
## RESULTS

**Table 1.** Summary of the number of protein identification across the three detergents

	6 M Urea + 0.1% SDS (anionic)	Triton X-100 (nonionic)	CHAPS (zwitterionic)
TCEP Reduction	40	45	42
No Reduction	39	43	42



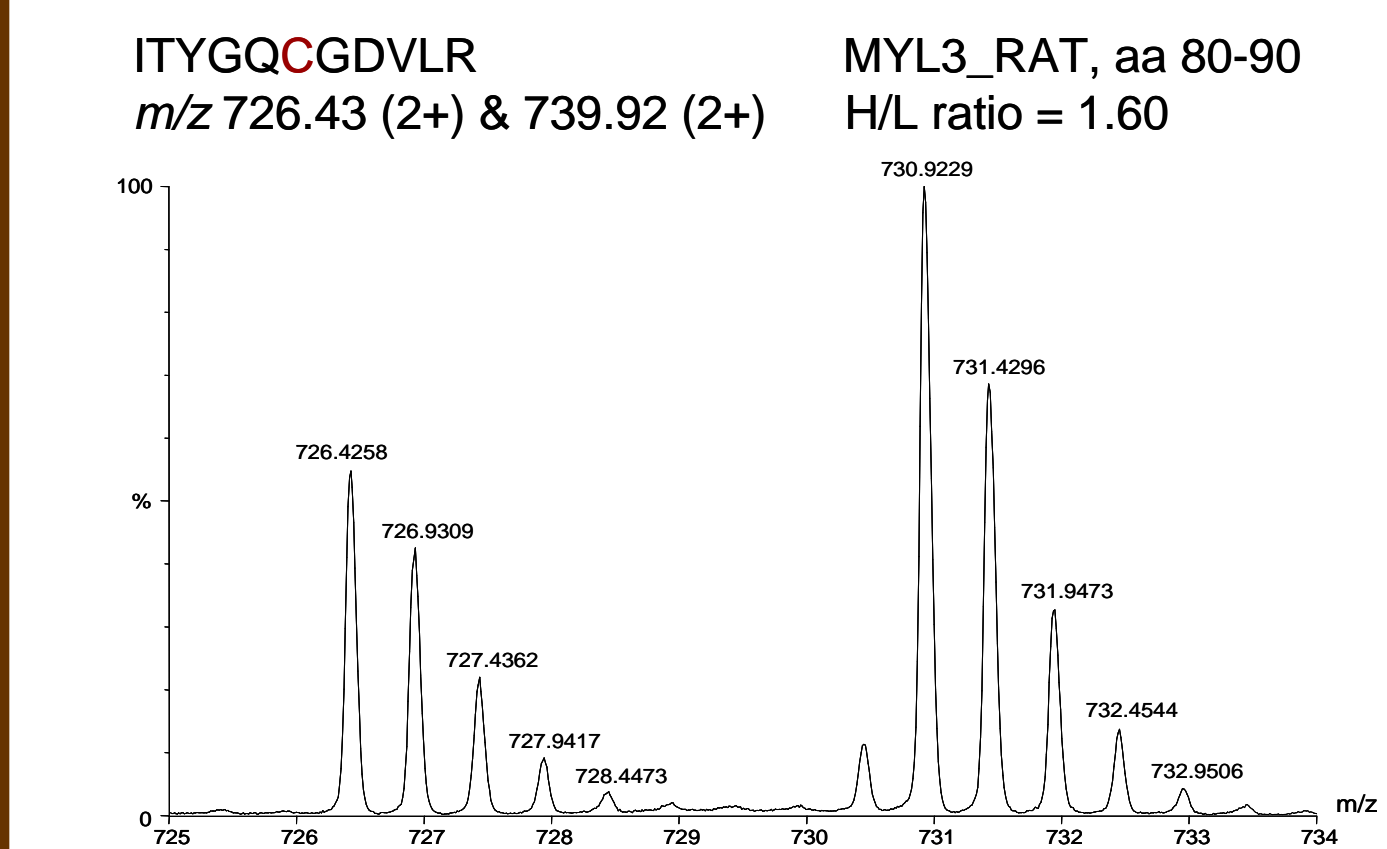
**Figure 1.** Number of Non-reduced Proteins identified from 3 different detergents, anionic, nonionic and zwitterionic. Total 58 proteins were identified from all three methods.



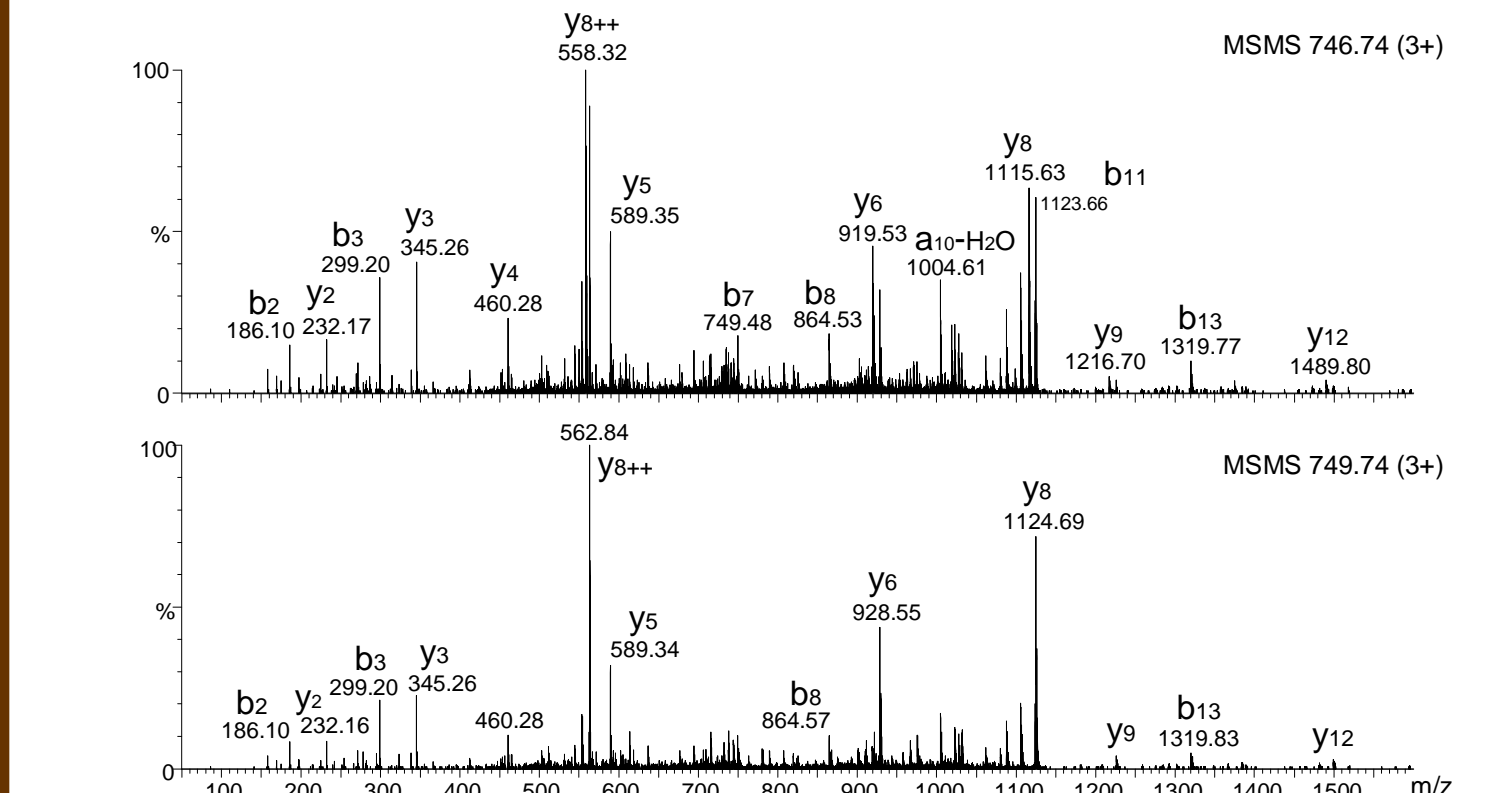
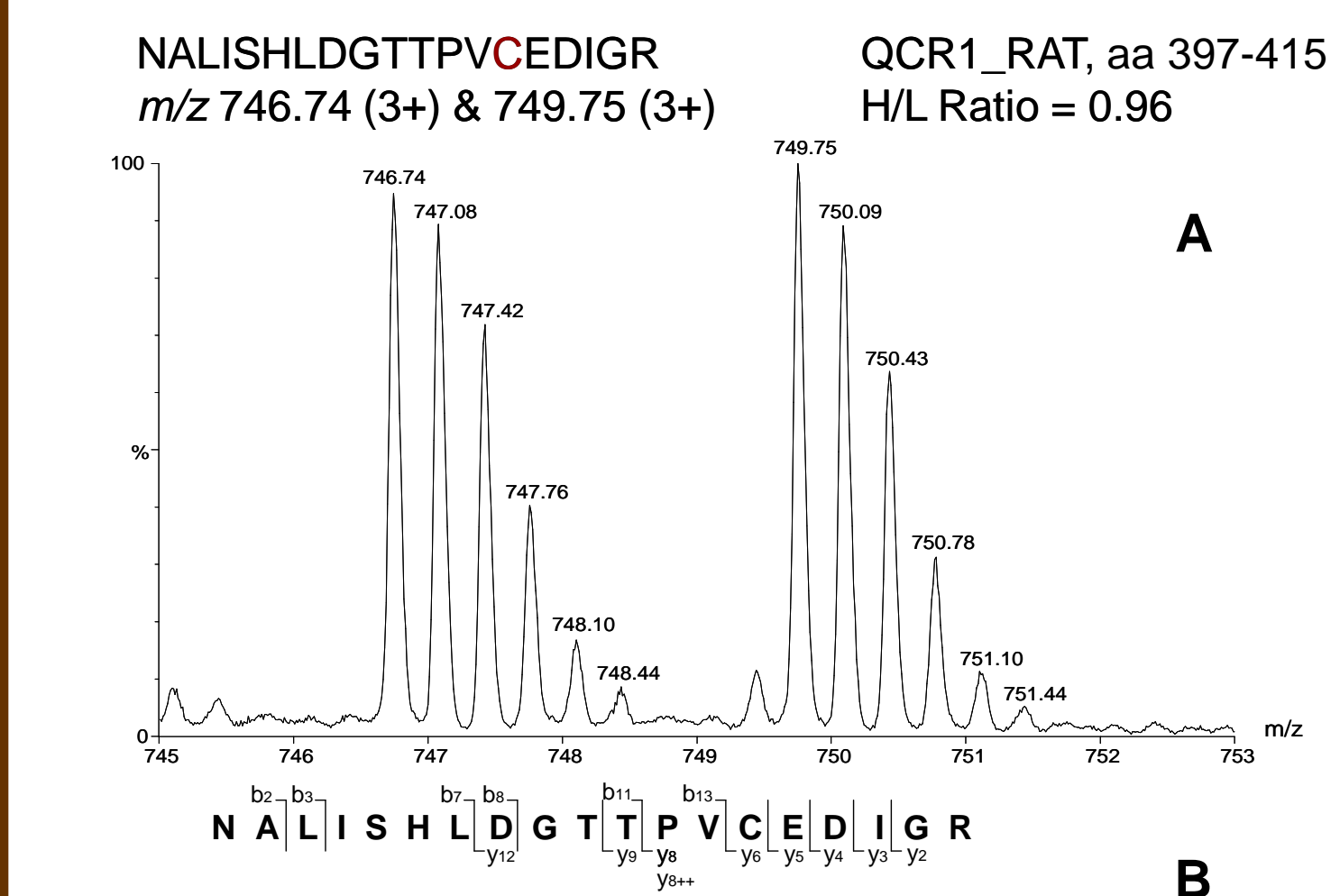
**Figure 2.** Heavy/Light ICAT-labeled peptide peak area ratios observed in ESI-qTOF survey scan from Old/Young rat heart SSM

**Table 2.** Heavy/Light ICAT-labeled peptide peak area ratios and protein classification

protein number	Classification	Protein accession numbers	Peptide sequence	H/L Ratio
1	Complex I	NDUAA_RAT	VITVDGNICSGK	0.95
2		NDUS1_RAT	LLFLLGADGGCITR	0.94
			MCLVEIEKAPK	0.64
			NPPKLLFLLGADGGCITR	1.04
3	Complex II	NDUS6_MOUSE	IACDGGGGALGHPK	1.02
			TGTCGYGGLQFK	1
4	Complex II	NDUV1_MOUSE	HDPHKLVEGCLVGGRR	0.99
			LVEGCLVGGRR	1.06
		YLVVNADEGEPGTCOK	0.97	
		AAFLGSEAGFNCLTK	1.03	
5	Complex II	DHSA_RAT	HVNGDQIVPGLYACGEAACASVHG	1.06
			ANR	1.08
		TLNEADCATVPPAIR	VGSVLQEGCEK	1.02
6	Complex III	DHSB_MOUSE	CGPMVLDALIK	0.91
		CY1_MOUSE	HGGEDYVFSLLTGYCEPTGVSLR	0.89
7	Complex III		HQQLDLAQDFHSSVQVYEEAVP	1
			SITPCR	1
8	Complex III	QCR1_RAT	NALISHLDGTTPVCEIDGR	0.96
			IENLHDVAYKNALANPLYCPDYR	0.96
9	Complex III	QCR2_RAT	NALANPLYCPDYR	0.91
			SQTEEDCTEELDFLHAR	0.91
10	Complex III	QCR6_RAT	GLCGAIHSSVAK	1
		ATPG_RAT		1
11	Complex III			1
				1
12	Lipid	AATM_RAT	NLDKEYLPIGGLADFCCK	1.1
		ACADL_RAT	AFVDSCLQLHETKR	0.96
13	Lipid		CIGAIAMTEPGAGSDLGVR	0.94
			VASGQALAAFCLTEPSSGSDVASIR	0.97
14	Lipid	ACADV_RAT	GIQVSNDDGCLGSR	0.81
		ACSL1_RAT	ALMGLYNGQVLCKK	1.09
15	Lipid	ECHA_RAT	EVESVTPHECFASNTSALPNQIAAVS	0.96
			QRPEK	0.91
		KYESAYGTQFTPCQLLR	0.86	
17	Lipid	ETFA_RAT	TIYAGNALCTVK	0.93
		ETFD_RAT	FCPAGVYEFVPLEQDGFGR	1
18	Lipid		LQINANCVHCK	1.1
			HPVSCDKDTPGFVNR	1.1
19	Lipid	HCDH_RAT	TFESLVDFOK	1.2
			IHMGNCAENTAK	1
20	Lipid	THIL_RAT	VVGYPVSGDPAIMGIPVPAITGALK	1
			K	1
21	Lipid	THIM_RAT		1.03
			VGLIGSCTNSSYEDMGR	0.99
22	TCA cycle	ACON_RAT	DLAGCIHGLSNVK	0.94
		IDHP_MOUSE	HNNCMAECLTPTIYAK	0.97
23	TCA cycle	KCRS_RAT	LGYLTCPSNLGTGLR	0.99
			LIDHFLFDKVPSPLLTCAGMAR	0.93
24	TCA cycle		MTPSGYTLQCIQIGVDNPGHPFK	0.84
			EGVIECSFVQSK	1.01
25	TCA cycle	MDHM_RAT	GYLGPQLPDCLK	0.99
			TIPLISQCTPK	0.98
26	TCA cycle	ODO1_RAT	VVNAPIFHVNSDDPEAVMYVCK	1.15
		ODPA_RAT	EATKFAAAYCR	1.12
27	TCA cycle	SUCA_RAT	TRLIGPNCPIIMPGECK	1.05
				1.09
28	TCA cycle			1.05
				1.1
29	Transport	ADT1_MOUSE	GSSQRFENGLDCLTK	1.1
		MCAT_RAT	CLLQIQASSGK	0.98
30	Transport	NNTM_MOUSE	EANSVITPGYGLCAAK	1.05
			KTCDAQAK	1.09
31	Transport		EHINLCCDVFDFDIAGPSIR	1
				1.6
32	Transport	VDAC1_RAT		1.01
				1.38
33	Targeting Structural	CH60_RAT	AAVEEGIVLGGGCALLR	1
		MYL3_RAT	ITYGCGDVLRL	1.6
34	Targeting Structural		LTEDEVEKLMAGQEDSNGCINYEAFV	1.6
			K	1.01
35	Other	MMSA_RAT	VCNLIDSGAK	1.01
		TPM1_RAT	CAELEEELKTVTNNLK	1.38



**Figure 3.** Relative quantification of an ICAT-labeled peptide from Myosin light chain 3



**Figure 4.** (A) Relative quantification of an ICAT-labeled peptide from a complex III protein, cytochrome b-c1 complex subunit 1. (B) Tandem mass spectra of Light and Heavy ICAT-labeled peptides in (A)

## CONCLUSIONS

\* Protein recovery from three detergents are comparative. Total 75 peptides from 58 proteins were identified. Similar number of protein were identified from each treatment. There are 13 proteins found by all three methods, 23 found in two method, and remaining 22 found exclusively by one method.

\* There are total 58 light/heavy ICAT-labeled peptide pairs identified from 36 proteins. The relative quantification of these heavy/light pairs clearly show the difference among the peptides. Most of the ratios are close to 1, while several are clearly different than 1.

\* The largest ratio (old/young) observed in two peptide pairs is about 1.6. They both come from myosin light chain 3 (MYL3\_RAT), aa 80-90 and aa 173-199.

\* There are 6 peptides from 5 proteins were also identified by the HICAT-labeled oxylipid peptide conjugates from our group study<sup>4</sup>. For instance, succinate dehydrogenase [ubiquinone] flavoprotein subunit (DHSA\_RAT) aa 640-654 in complex II, cytochrome b-c1 complex subunit 1 & 2 (QCR1\_RAT, aa 397-415, and QCR2\_RAT, aa 183-195) in complex III.

## ACKNOWLEDGEMENT

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