# Isotope-Coded Dimethyl Tagging for Differential Quantification of Posttranslational Protein Carbonylation by 4-Hydroxy-2-nonenal, an End-Product of Lipid Peroxidation

Navin Rauniyar and Laszlo Prokai*

*Department of Molecular Biology & Immunology, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107*

**Supplementary Figures:**

**Figure S1.** Linearity of relative quantitation of the peptide FVNQHLC$^{\#}$GSHLVE (where # represents cysteine residue oxidized to cysteine-sulfonic acid, Cys-$SO_3H$), Glu–C fragment of the oxidized insulin β-chain, by dimethyl tagging strategy using isotopic variants of formaldehyde. Two identical solutions of the peptide were dimethylated using light ($H^{12}CHO$) or heavy ($D^{13}CDO$) isotopes of formaldehyde, respectively. As summarized in chart **a**, the differentially tagged peptides were combined in various molar ratios (1:1, 1:5, 5:1, 1:10 and 10:1), desalted and analyzed by LC–MS for relative quantification of the isotopic pairs from XICs of the doublet ions at *m/z* 779.87 (2+) and 782.89 (2+), respectively, which corresponded to the light and heavy dimethyl-labeled FVNQHLC$^{\#}$GSHLVE. The peptide was tagged only at its N-terminus as it contains a single amine group and, hence, Δ was 6 Da (3 Th for the doubly-charged ions). Averaged over the elution of the differentially labeled peptides, chart **b** displays the $[M+2H]^{2+}$ molecular-ion region of the recorded high resolution ESI mass spectra (full scan: from *m/z* 350 to *m/z* 1500).

**Figure S2.** CID-MS/MS spectra of the doubly charged (**a**) unlabeled (*m/z* 904.02), (**b**) light (*m/z* 918.03), and (**c**) heavy (*m/z* 921.04) dimethyl-labeled HNE-modified peptide fragment of ATP synthase subunit beta peptide, LVLEVAQH*LGESTVR (21-34).

* Corresponding author. Phone: 817-735-2206. Fax: 817-735-2118. E-mail: lprokai@hsc.unt.edu.

**Figure S3.** LC–ESI-MS analysis of plasma protein tryptic digest spiked with differentially dimethyl-labled HNE-modified angiotensin I, DRVYIHPFHL, in a ratio of 3:1 (without prior ennrichment of HNE-modified peptides) (a) Base-peak chromatogram and (b) averaged full-scan mass spectrum from acquisitions in the 0 to 90-min retention time window. The inset shows mass spectrum of ions mostly corresponding to peptides from plasma protein and the relative abundance of angiotensin I, that is ~2% in the mixture.

**Figure S4.** LC–ESI-MS analysis of the 'eluate' fraction obtained after acid-catalyzed hydrolysis of the captured hydrazones during enrichment of spiked dimethylated HNE-modified angiotensin I from plasma protein tryptic digest using hydrazide-coated glass beads for chemoprecipitation. (a) Base-peak chromatogram and (b) averaged full-scan mass spectrum of the 'eluate' fraction from acquisitions in the 0 to 90-min retention time window. Expanded view of full-scan mass spectrum of isotopomeric pair corresponding to $(M+3H)^{3+}$ ions of differentially dimethylated HNE-modified DRVYIHPFH*L (* indicate HNE moiety attached to the corresponding histidine) present in a ratio of 3:1 is shown in the inset.

**Figure S5. (a)** XICs and full-scan MS spectra of differentially dimethyl-labeled, triply-charged precursor ions of HNE-modified angiotensin I, $^{@}$DRVYPHIFH*L (where, $^{@}$ = dimethylated residue and * = HNE-modified residue), at *m/z* 494.28 and 496.29 spiked in a ratio of 1:3, 1:1 or 3:1 into plasma protein tryptic digest and reisolated from the complex matrix by hydrazide-based chemoprecipitation strategy. The observed ratios (i) 1:3, (ii) 1:1 and (iii) 3:1 of the $[M+3H]^{3+}$ isotopic peptide pairs after enrichment were in close agreement to the expected ratios.
**(b)** XICs and full-scan MS spectra of differentially dimethyl-labeled, triply-charged precursor ions of doubly HNE-modified angiotensin I, $^{@}$DRVYPH*IFH*L (where, $^{@}$ = dimethyl label and * = HNE-modified residues), at *m/z* 546.32 and 548.33 obtained after enrichment and depicting the observed light/heavy ratios (i) 1:3, (ii) 1:1 and (iii) 3:1 of the isotopic peptide pairs were in close agreement to the spiked ratios.

**Figure S6.** XICs and full-scan MS spectra of differentially dimethyl-labeled, doubly-charged precursor ions of HNE-modified vimentin tryptic peptide, $^{@}$QVQSLTC*EVDALK$^{@}$ (where, $^{@}$ = dimethylated residue and * = HNE-modified residue) at *m/z* 823.46 and 829.49, mixed in a ratio of 1:3, 1:1 or 3:1 and subsequently isolated by hydrazide-based chemoprecipitation strategy for

LC–MS analyses. The observed ratios (i) 1:3, (ii) 1:1 and (iii) 3:1 of the $[M+2H]^{2+}$ isotopic peptide pairs after enrichment were in close agreement to the expected ratios.

**Figure S7.** XICs of mono-dimethyl labeled, doubly charged precursor ions of HNE-modified ATP synthase subunit beta peptide fragment, $^{@}$LVLEVAQH*LGESTVR (where, $^{@}$ = dimethylated and * = HNE-modified residue).

**Figure S8.** XICs and CID-MS/MS spectra of dimethyl-tagged, doubly-charged precursor ions of Glu–C fragment of HNE-modified oxidized insulin beta chain, $^{@}$FVNQH*LC$^{\#}$GSHLVE (where, $^{@}$ = dimethylated, * = HNE-modified residue and $^{\#}$ = cysteine-sulfonic acid, Cys-$SO_3H$).

**Figure S9.** XICs and CID-MS/MS spectra of dimethyl-tagged, triply-charged precursor ions of HNE-modified peptide [$^{@}$LGFLGSNTPHVNHHMPPH]* (where, $^{@}$ = dimethylated). The neutral loss of HNE from the fragment ions during collision induced dissociation tandem mass spectrometry precluded the localization of the site of HNE modification (*).

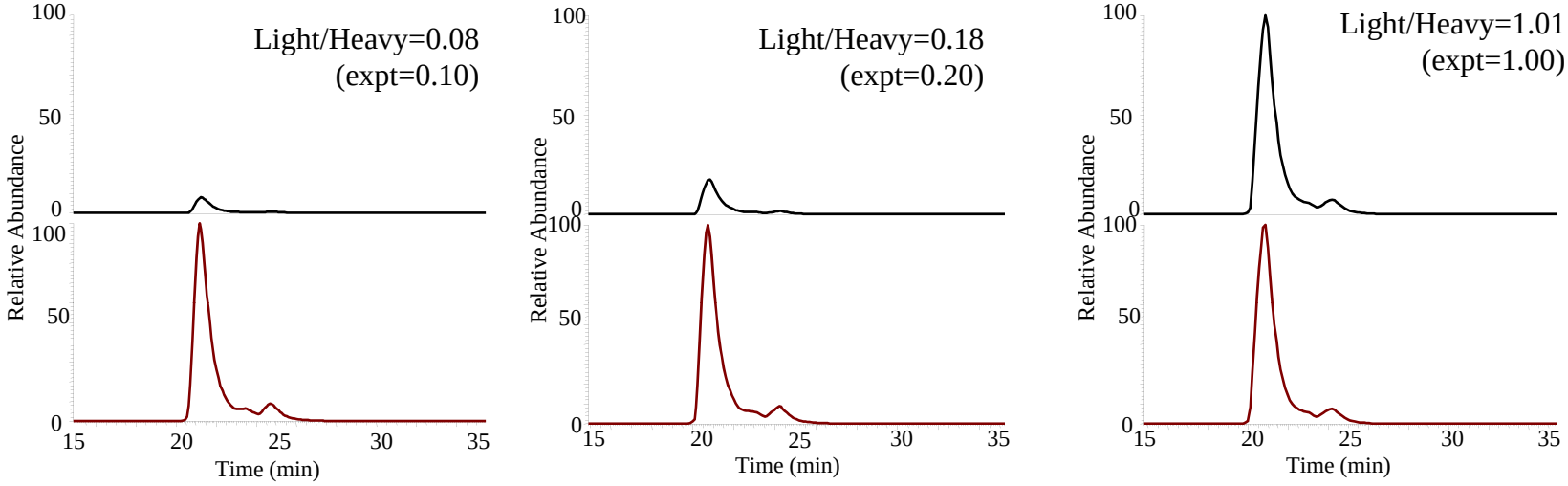
**Figure S10.** XICs and CID-MS/MS spectra of bis-dimethyl-tagged, doubly-charged precursor ions of HNE-modified apomyoglobin tryptic fragment, $^{@}$H*PGDFGADAQGAMTK$^{@}$ (where, $^{@}$ = dimethylated and * = HNE-modified residue).

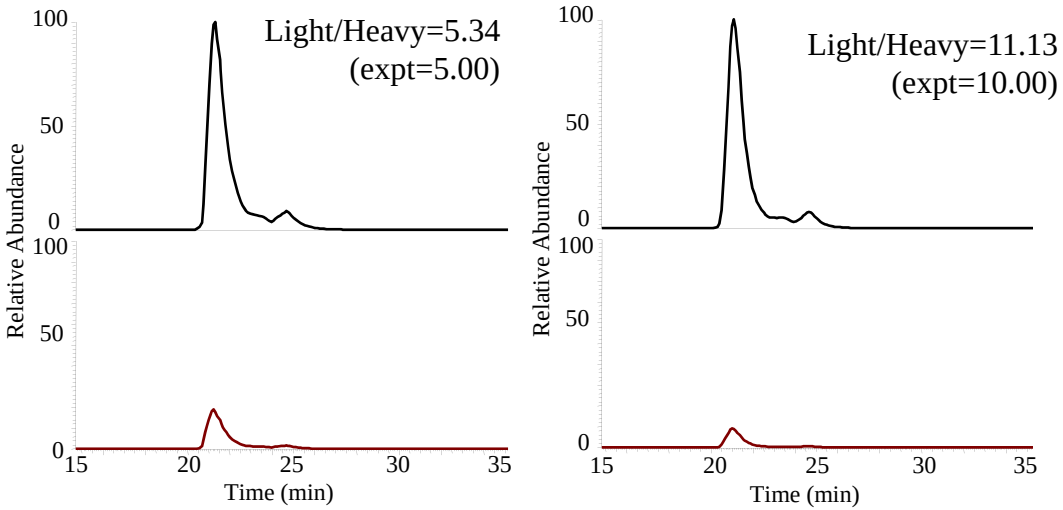
**Figure S11.** XICs and CID-MS/MS spectra of bis-dimethyl-tagged, doubly-charged precursor ions of HNE-modified apomyoglobin tryptic fragment, $^{@}$LFTGH*PETLEK$^{@}$ (where, $^{@}$ = dimethylated and * = HNE-modified residue).

# Figure S1a

$$\frac{(CH3)_2\text{-FVNQHLC}^{\#}\text{GSHLVE}}{(^{13}CD_2H)_2\text{-FVNQHLC}^{\#}\text{GSHLVE}} = \frac{\text{Light}}{\text{Heavy}}$$

| Expt ratios | Obs ratios |
|---|---|
| 0.1 | 0.08 |
| 0.2 | 0.18 |
| 1 | 1.005 |
| 5 | 5.36 |
| 10 | 11.16 |



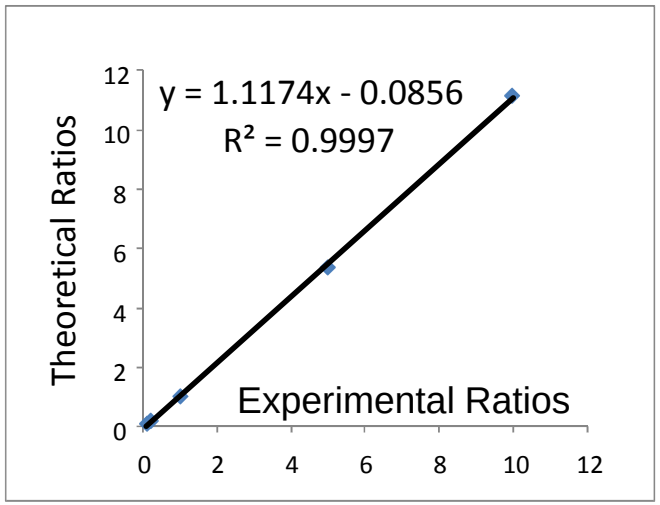



**Figure S1b**

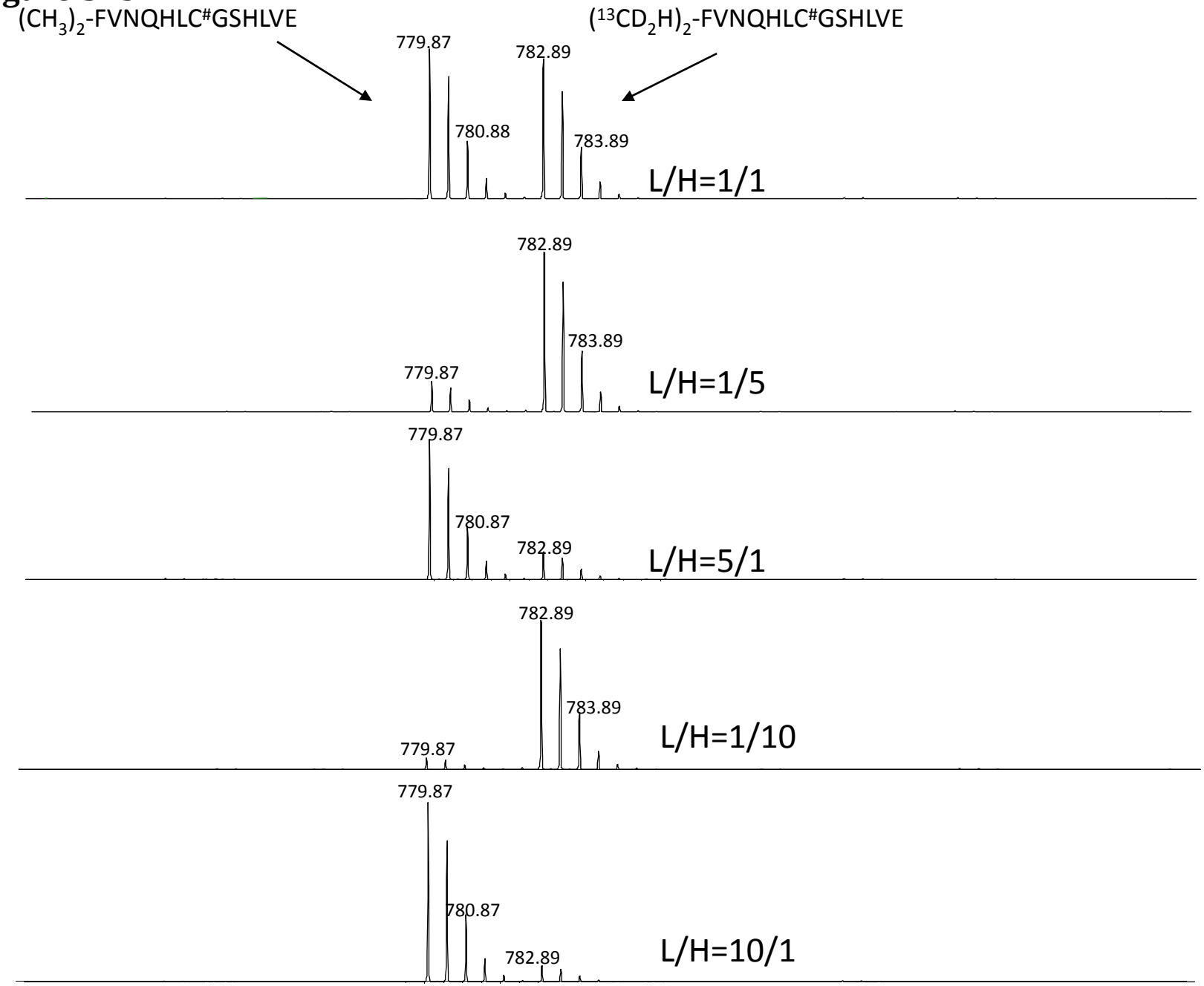

(CH3)2-FVNQHLC#GSHLVE
(13CD2H)2-FVNQHLC#GSHLVE
779.87
782.89
780.88
783.89
L/H=1/1
782.89
783.89
779.87
L/H=1/5
779.87
780.87
782.89
L/H=5/1
782.89
783.89
779.87
L/H=1/10
779.87
780.87
782.89
L/H=10/1

**Figure S2**

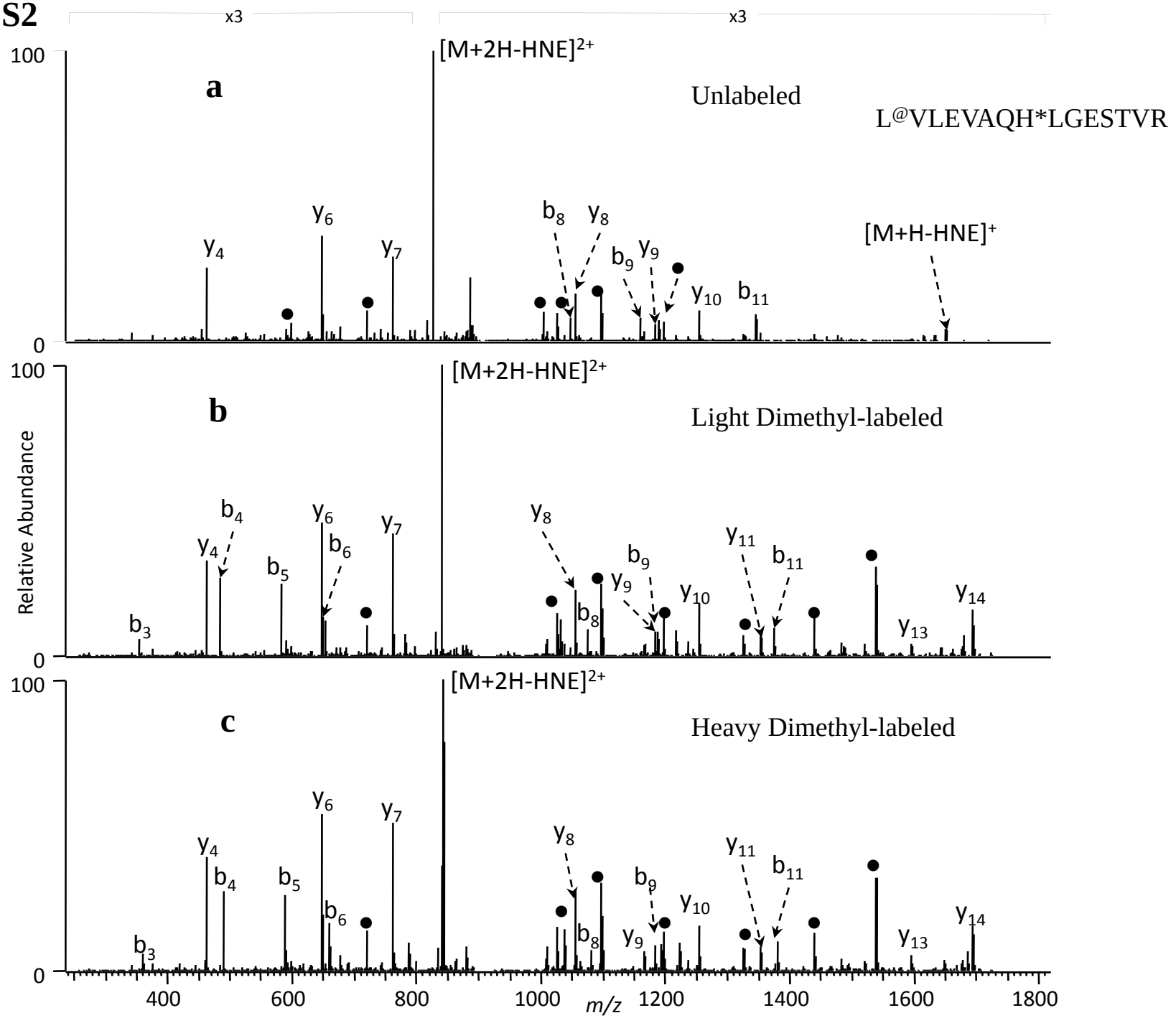

x3
x3
a
Unlabeled
L@VLEVAQH*LGESTVR
[M+2H-HNE]2+
[M+H-HNE]+
b
Light Dimethyl-labeled
c
Heavy Dimethyl-labeled
Relative Abundance
m/z
100
0
400
600
800
1000
1200
1400
1600
1800

**Figure S3**

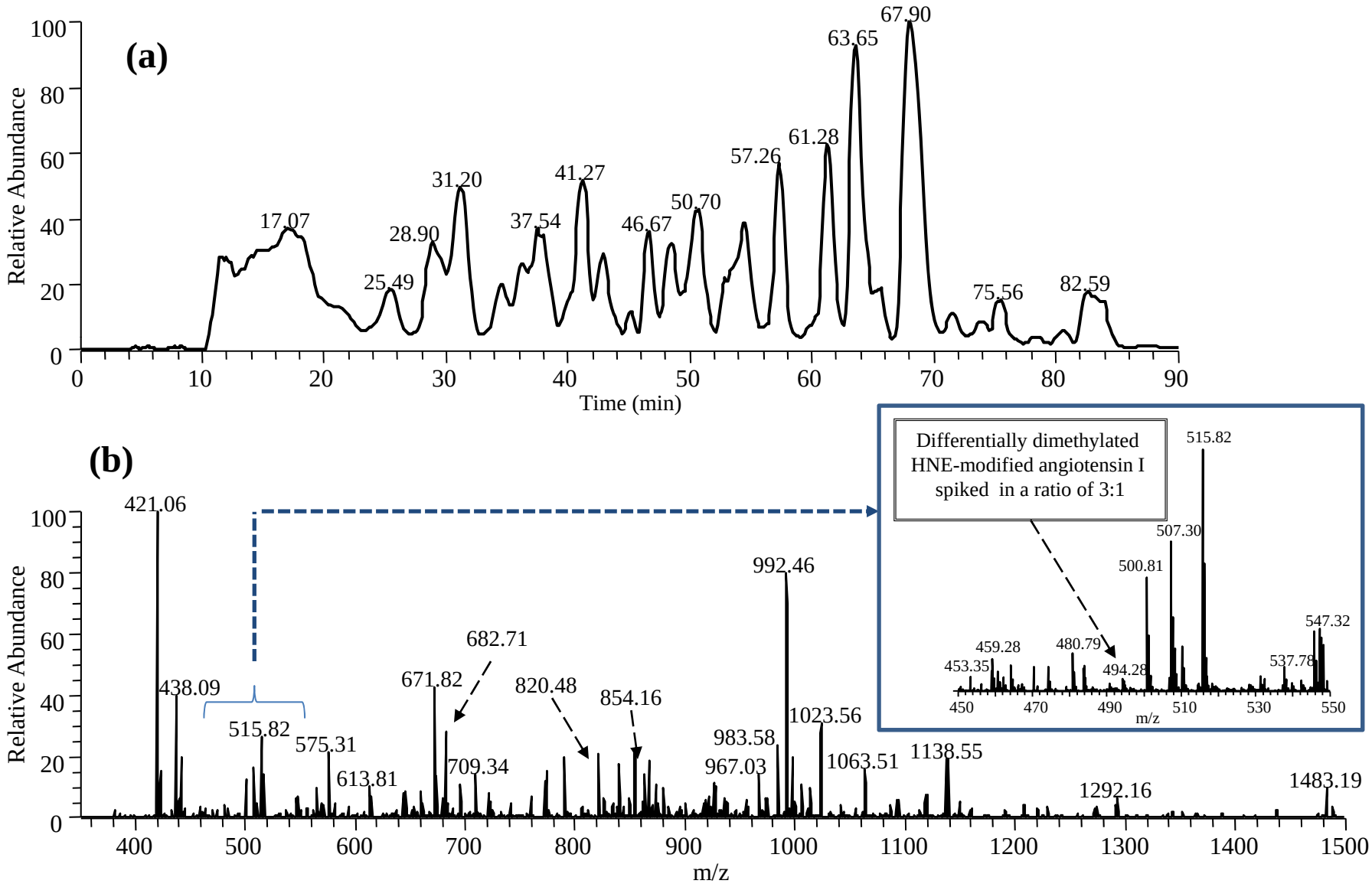

(a)
Relative Abundance
Time (min)
17.07
25.49
28.90
31.20
37.54
41.27
46.67
50.70
57.26
61.28
63.65
67.90
75.56
82.59
(b)
Relative Abundance
m/z
421.06
438.09
515.82
575.31
613.81
671.82
682.71
709.34
820.48
854.16
967.03
983.58
992.46
1023.56
1063.51
1138.55
1292.16
1483.19
Differentially dimethylated
HNE-modified angiotensin I
spiked in a ratio of 3:1
515.82
507.30
500.81
547.32
459.28
480.79
453.35
494.28
537.78
m/z

**Figure S4** $(C^{12}H3)_2$ – DRVYIHPFH*L & $(C^{13}HD_2)_2$ – DRVYIHPFH*L

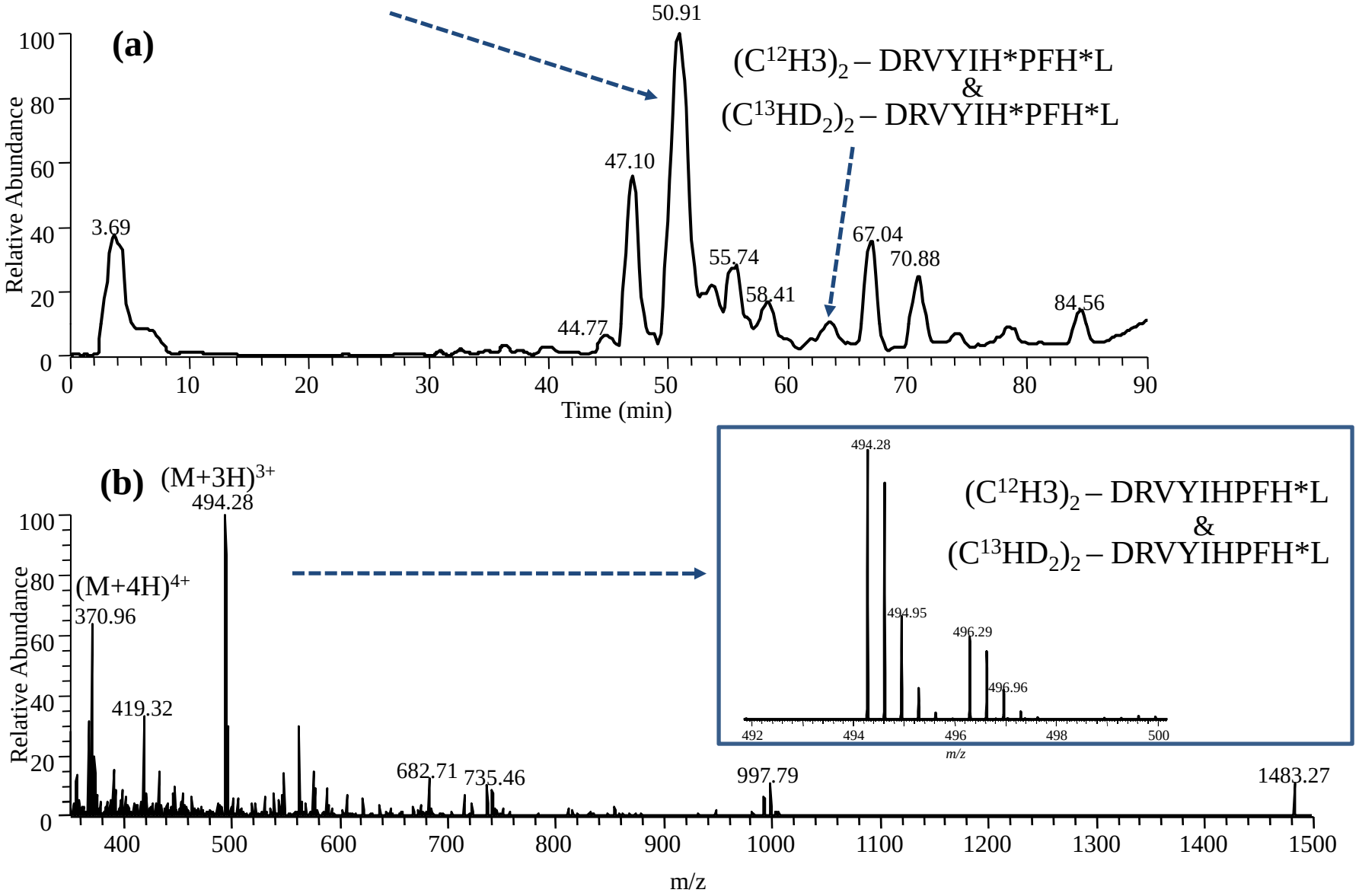
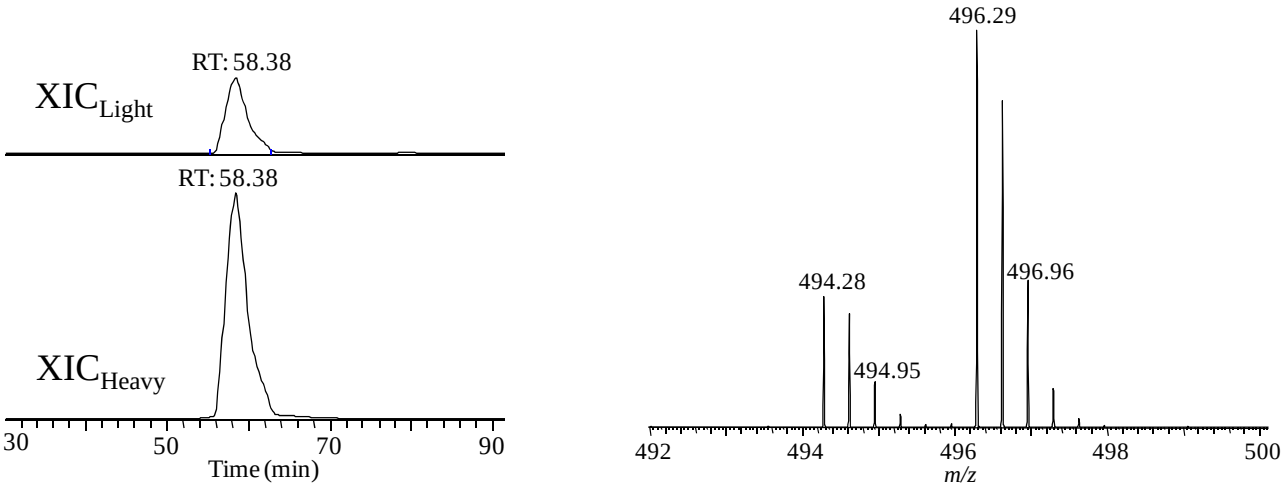
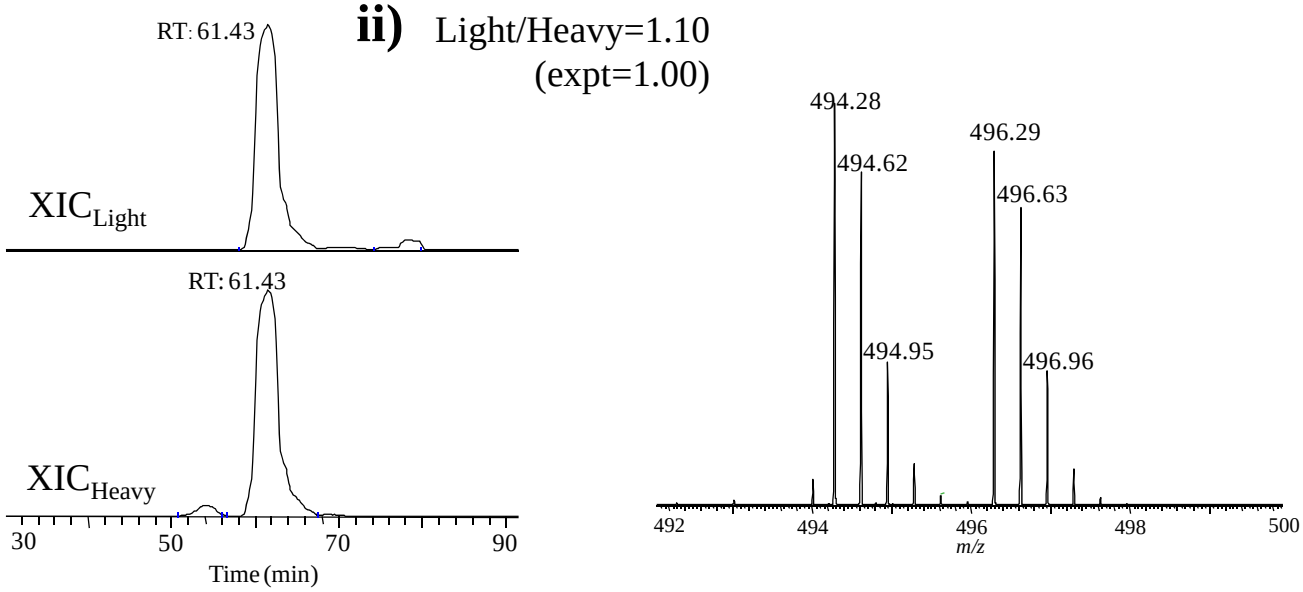


**Figure S5 (a)**

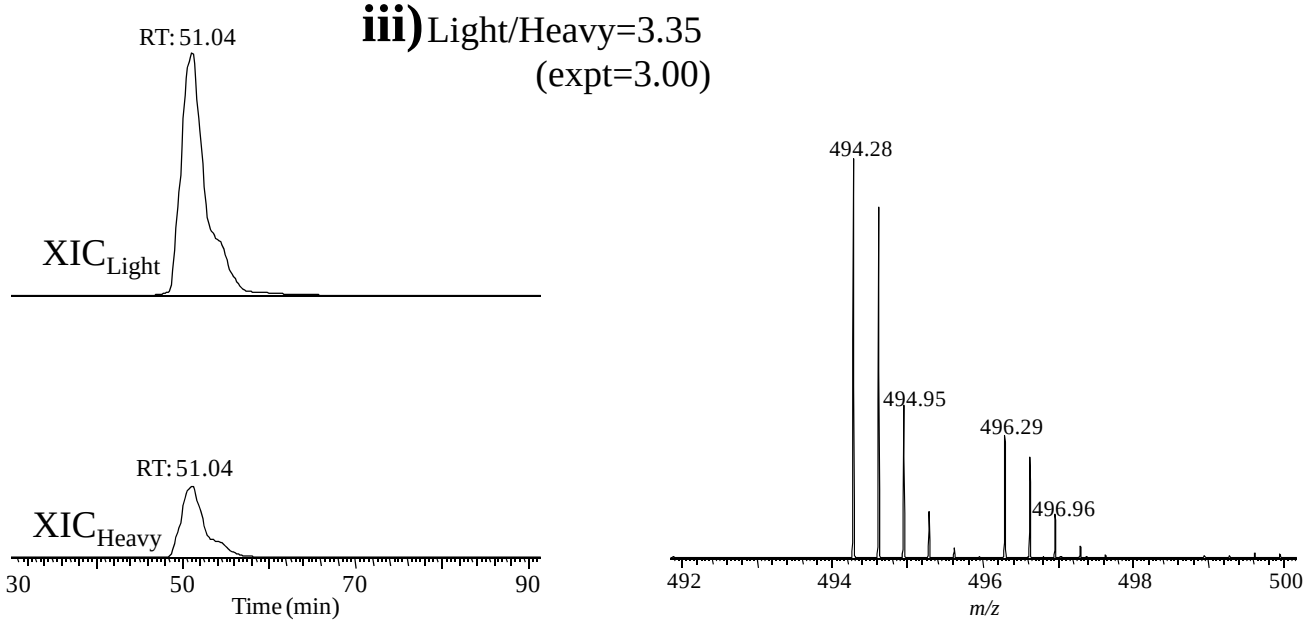
**i)** Light/Heavy=0.33
(expt=0.33)



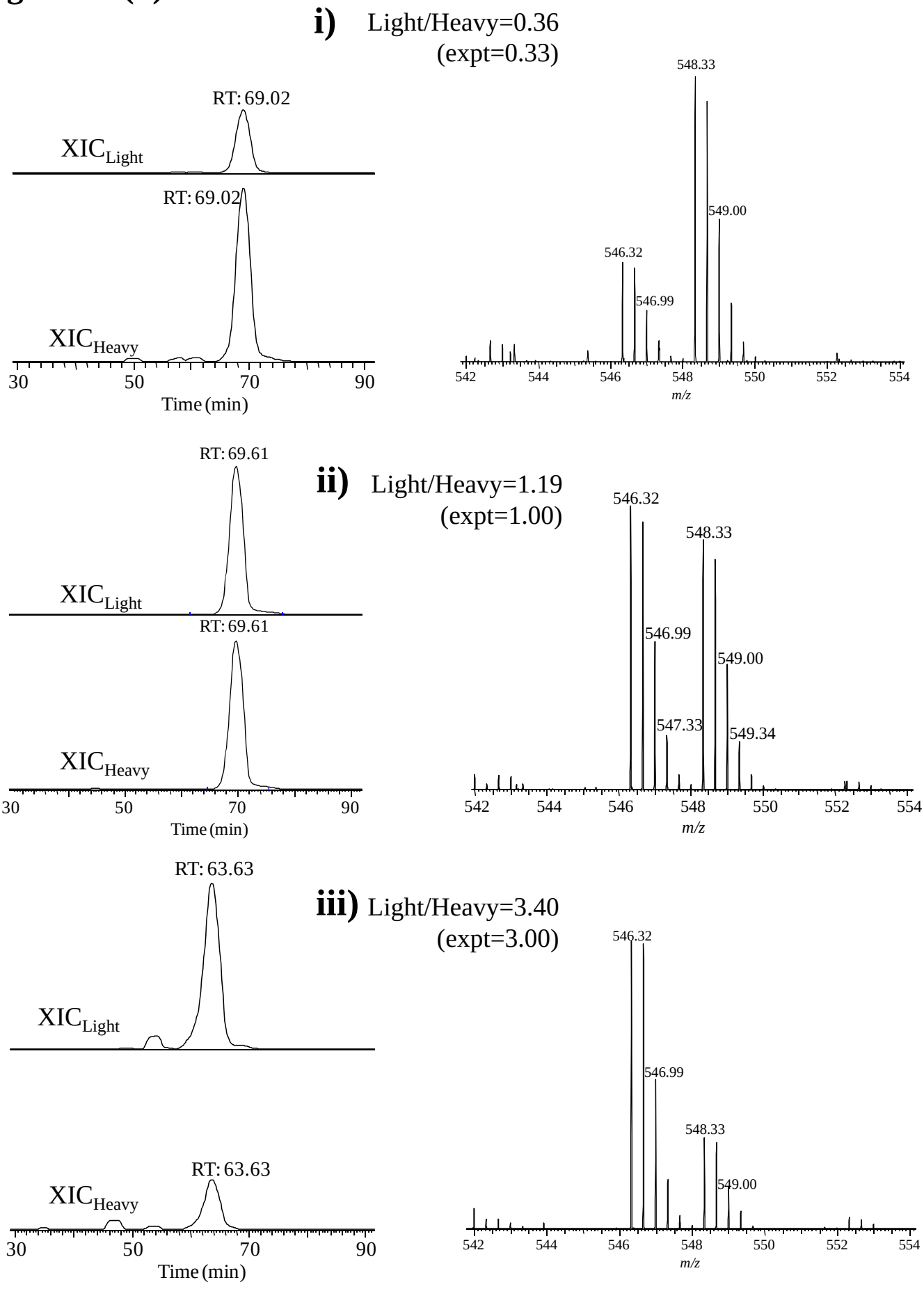

**ii)** Light/Heavy=1.10
(expt=1.00)



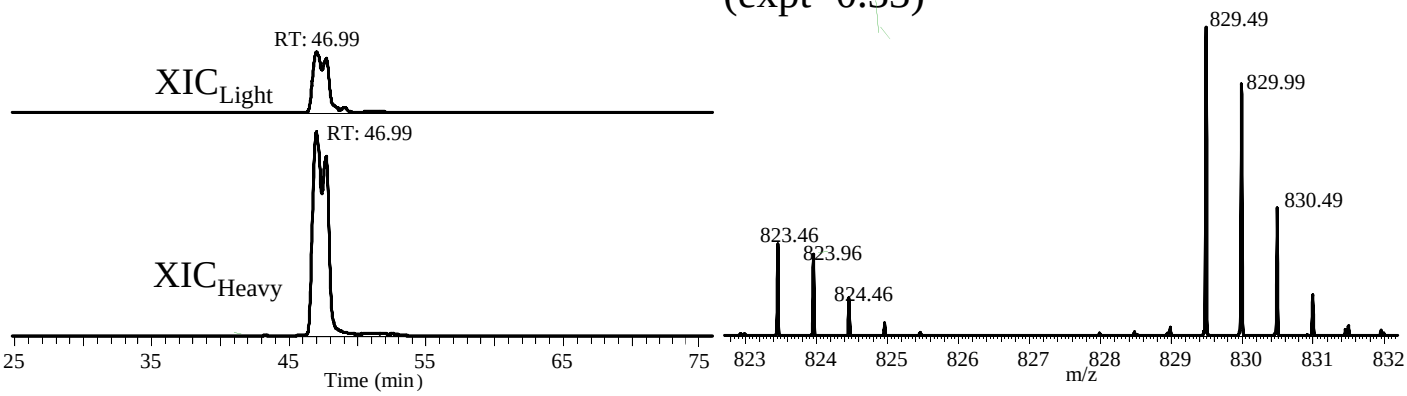

**iii)** Light/Heavy=3.35
(expt=3.00)



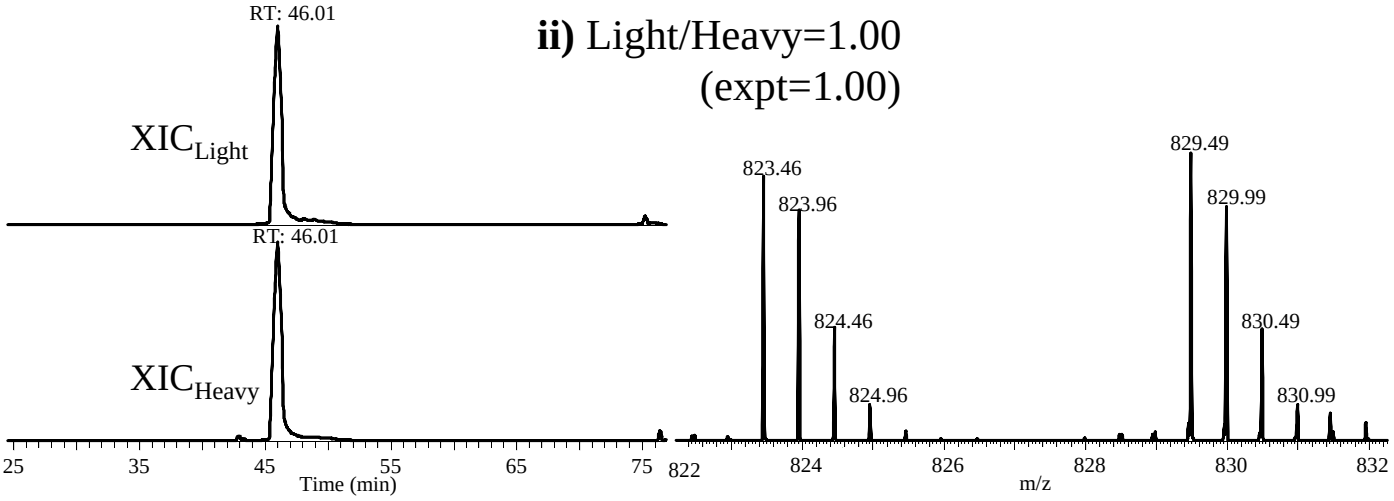
# Figure S5 (b)


i) Light/Heavy=0.36
(expt=0.33)
RT: 69.02
XIC Light
RT: 69.02
XIC Heavy
30
50
70
90
Time (min)
548.33
549.00
546.32
546.99
542
544
546
548
550
552
554
m/z
ii) Light/Heavy=1.19
(expt=1.00)
RT: 69.61
XIC Light
RT: 69.61
XIC Heavy
30
50
70
90
Time (min)
546.32
548.33
546.99
549.00
547.33
549.34
542
544
546
548
550
552
554
m/z
iii) Light/Heavy=3.40
(expt=3.00)
RT: 63.63
XIC Light
RT: 63.63
XIC Heavy
30
50
70
90
Time (min)
546.32
546.99
548.33
549.00
542
544
546
548
550
552
554
m/z

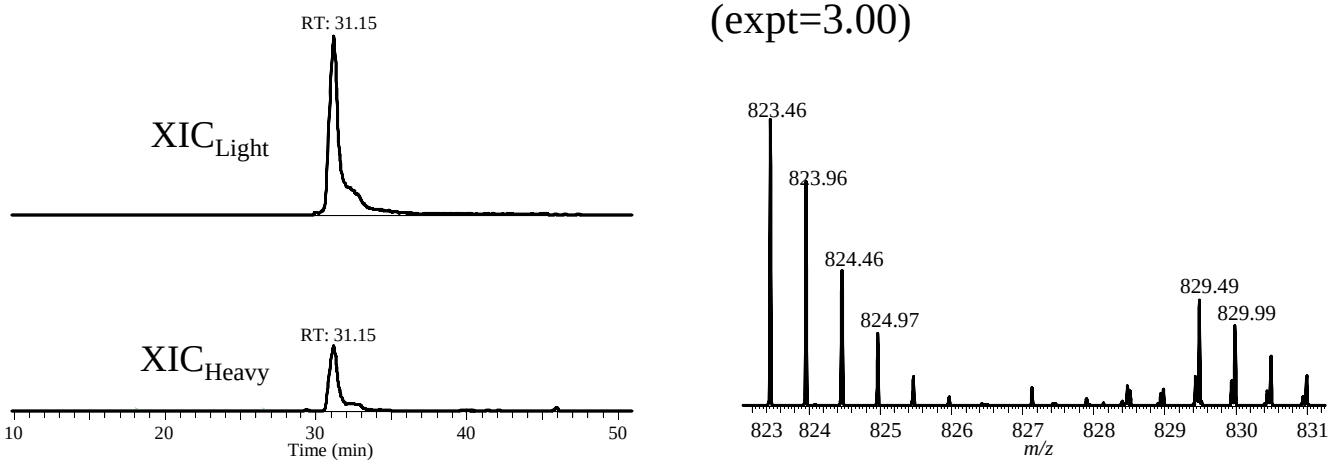
**i)** Light/Heavy=0.31
(expt=0.33)




**ii)** Light/Heavy=1.00
(expt=1.00)



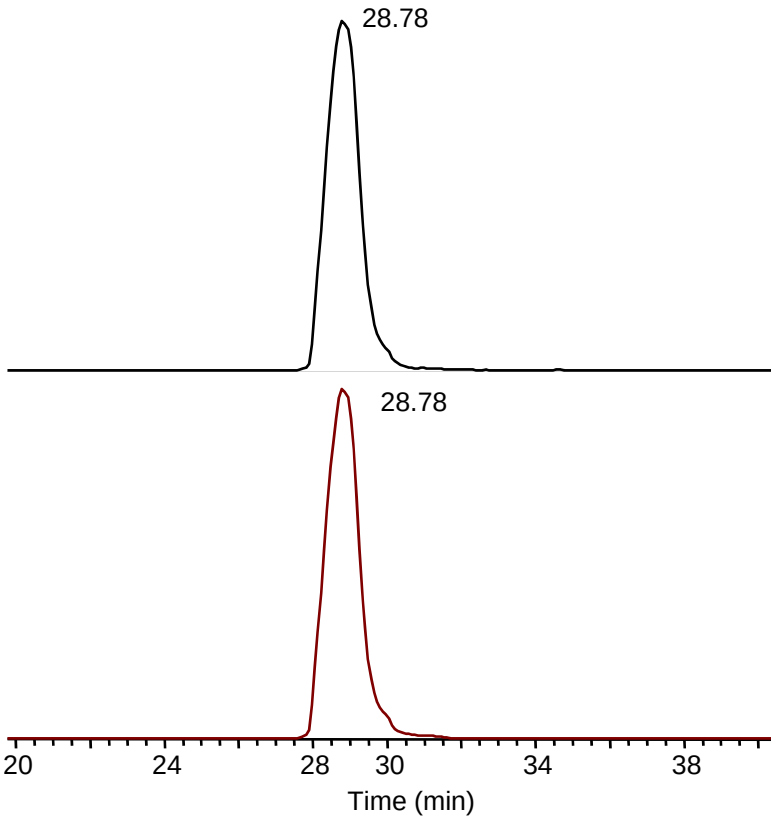

**iii)** Light/Heavy=2.86
(expt=3.00)



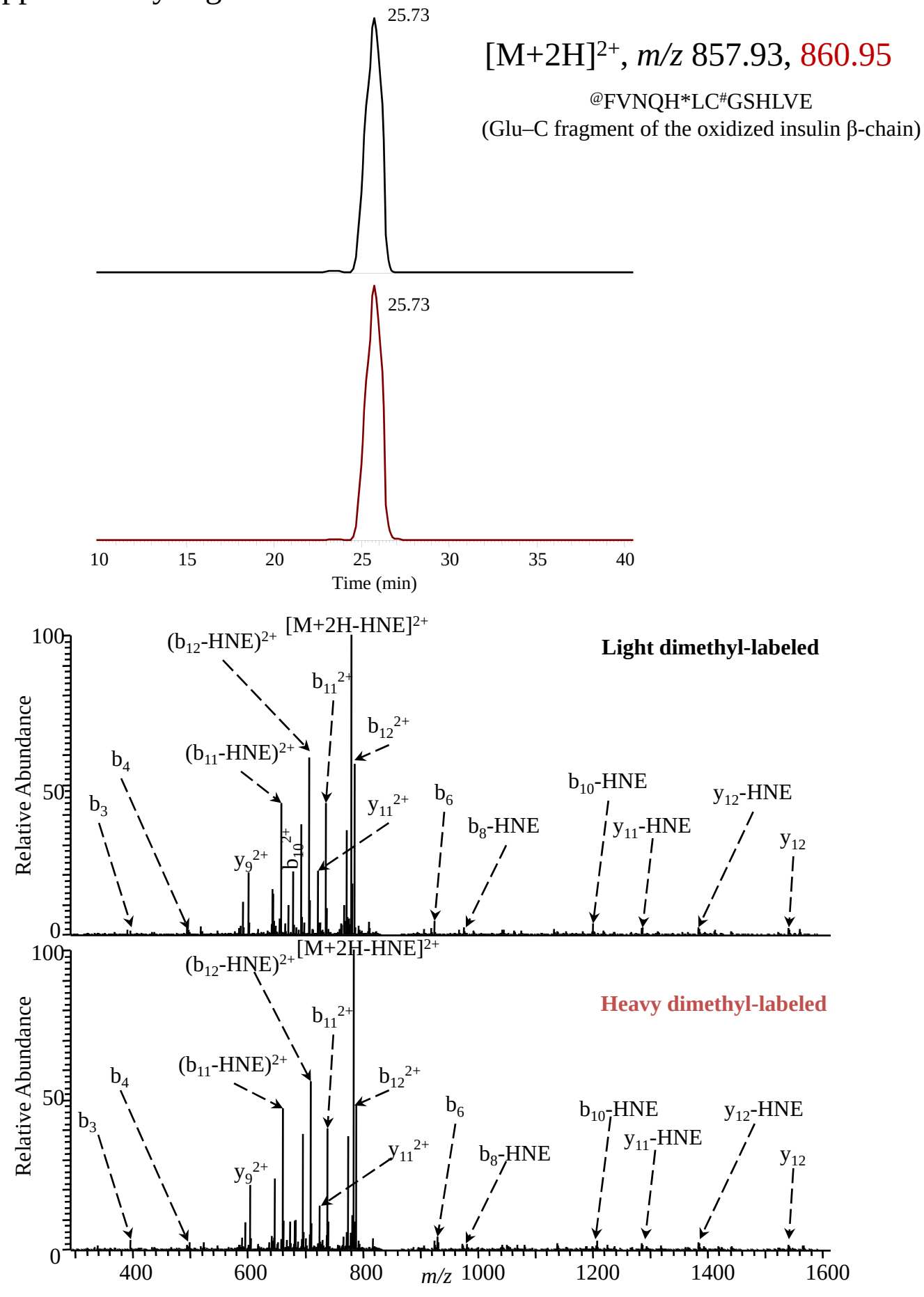
Supplementary Figure S7

$[M+2H]^{2+}$, *m/z* 918.03, 921.05

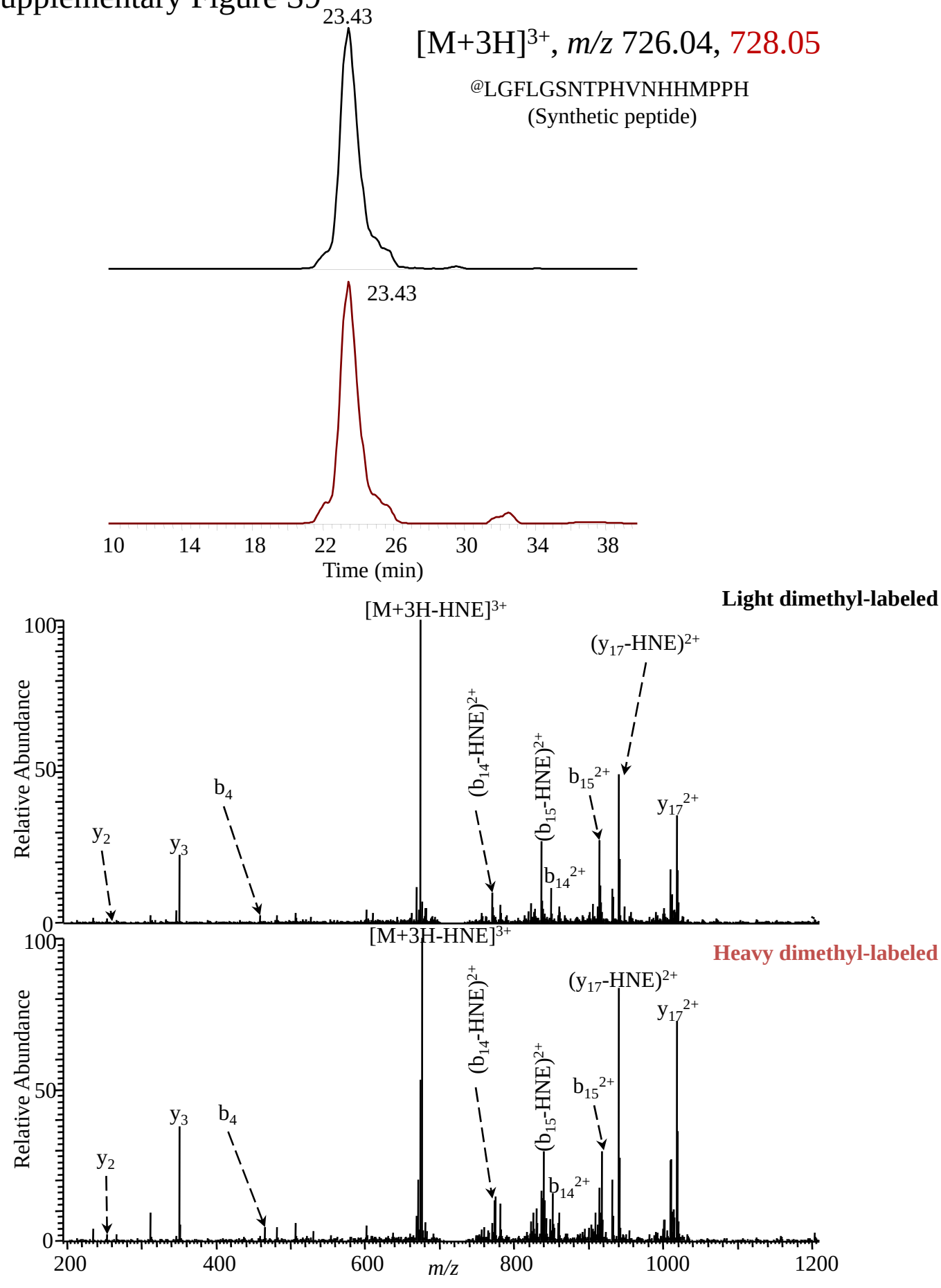
@LVLEVAQH*LGESTVR
(HNE-modified ATP synthase β-subunit peptide:
MS/MS spectra shown in Fig. S2)



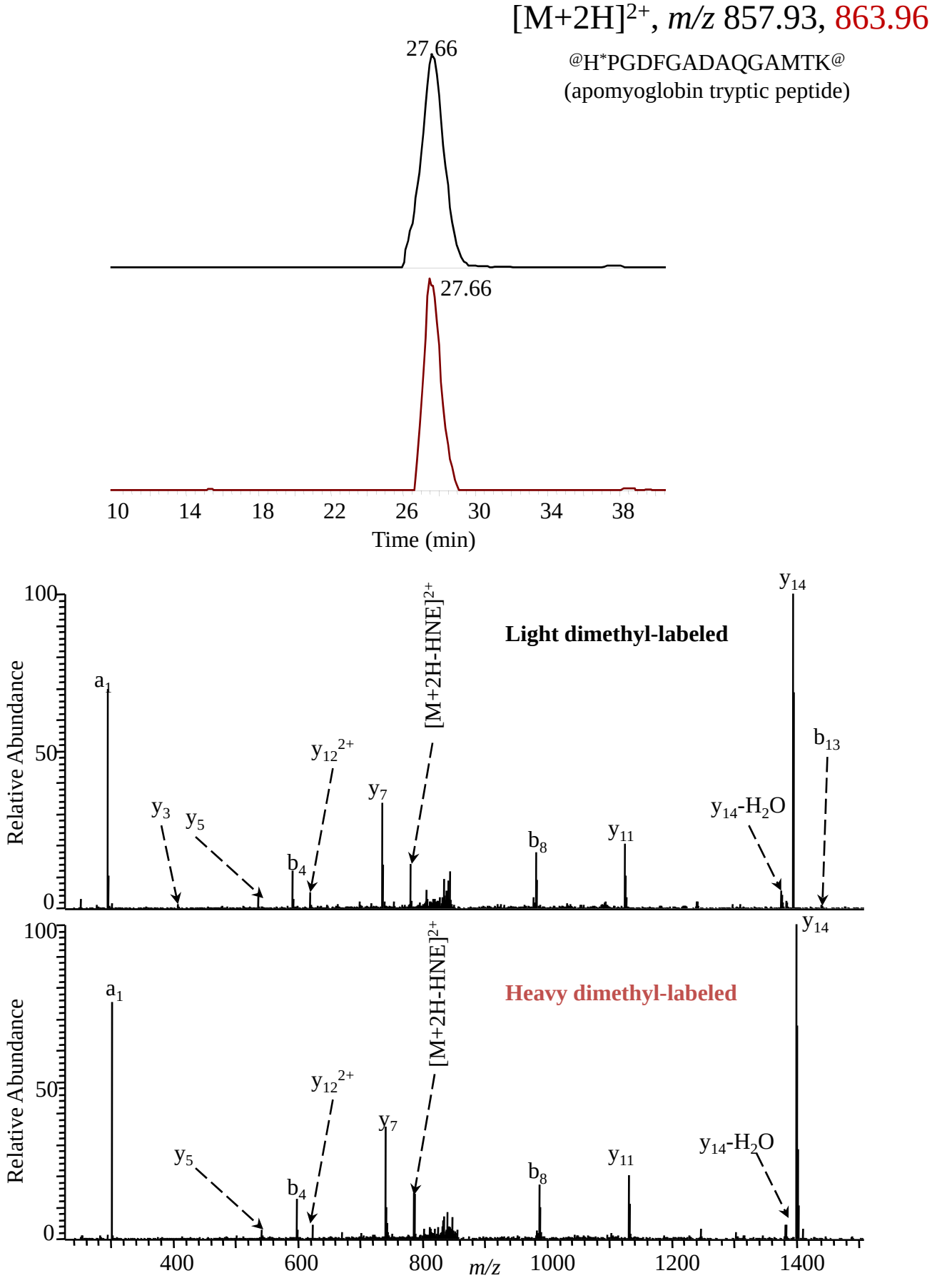
Supplementary Figure S8



Supplementary Figure S9



Supplementary Figure S10



Supplementary Figure S11