Supporting Information for

"Mapping of Lysine Methylation and Acetylation in Core Histones in *Neurospora crassa*"

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by

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List of Supporting Figures:

Figure S1. The HPLC chromatogram for the separation of *Neurospora* core histones. Histones were eluted in the order of H2B, H4, H2A and H3.

Figure S2. Sequence coverage for the *Neurospora* core histones based on MS/MS analyses (Histones H2B, H2A, H3 and H4 are shown in panels A, B, C, and D, respectively). The sequences for *Neurospora* core histones were obtained from Swissprot, and the identified peptides produced by different proteases were underlined with different colors. The identified modification sites are shown in bold.

Figure S3. The MS/MS of the tri-methylated, mono-acetylated (A) and penta-methylated, mono-acetylated (B) tryptic peptide containing residues 1-12 of *Neurospora* H2B. The data were obtained on the Agilent Q-TOF mass spectrometer.

Figure S4. The MS/MS of the Asp-N produced peptide containing residues 25-34 of *Neurospora* histone H2B (A) and the Arg-C-produced peptide containing residues 6-19 of *Neurospora* histone H2A (B). The data were acquired on LTQ.

Figure S5. The MS of the Arg-C-produced *Neurospora* H3 peptides with residues 3-8 (A) and 73-83 (B). The MS/MS of the H3 peptides containing residues 3-8 with K4 being tri-methylated (C), residues 73-83 with K79 being di-methylated (D), residues 54-63 with K56 being acetylated (E), and residues 9-26 with K9, K14, K18 and K23 being acetylated (F). All data were acquired on the Q-TOF instrument.

Figure S6. The selected-ion chromatograms (SICs) of the triply-charged ions of the tri-methylated (A) and acetylated (B) histone H3 peptide with residues 9-17. These two peptides displayed different retention times during LC-MS/MS analysis on the Q-TOF mass spectrometer.

Figure S7. The MS of the Arg-C-produced histone H3 peptide bearing residues 27-42 with K36 being tri-methylated and with K27 being tri-methylated (A) or acetylated (B). The MS/MS of the hexa-methylated (C) or tri-methylated, acetylated (D) peptides 27-41 obtained on the Q-TOF.

Figure S8. (A) The MS of Arg-C-produced phosphorylated histone H3 peptide with residues 9-17. (B) The MS/MS of the peptide containing residues 9-17 with K9 being acetylated and S10 being phosphorylated. The spectra were obtained on the Q-TOF instrument.

Figure S9. (A) The MALDI-MS of the Asp-N-produced *Neurospora* H4 peptide with residues 1-23. (B) The ETD-MS/MS of the same peptide with the N-terminus and K16 being acetylated and K20 being tri-methylated.

Figure S10. The QTOF-produced MS/MS of the trypsin-produced *Neurospora* H4 peptides with residues 4-12 (A) and 9-17 (B) supporting the K5, K8, K12 and K16 acetylation.

Table S1. The fragment ion mass comparison of 3 example peptides to differentiate tri-methylation from acetylation by using MS/MS data acquired on the Agilent 6510 Q-TOF mass spectrometer. For each modification site, two b or y ions flanking the target lysine were chosen. The experimental mass difference (Measured ΔMass) of these two neighboring b or y ions was calculated, so were the corresponding theoretical mass differences with the lysine being tri-methylated or acetylated (Calcd. ΔMass, ac/me3). The two mass deviations (M.D.) between the measured and calculated mass differences were further calculated, with one being markedly smaller than the other. The modification type at the target lysine could be determined as the one with smaller deviation.

Peptide	Modification	Chosen ions	Measured mass 1	Measured mass 2	Measured ΔMass	Calcd. ΔMass,ac	M.D. /ppm	Calcd. ΔMass,me3	M.D. /ppm
H2B: 1-24	K7 Ac	b6, b7	648.3682	818.4735	170.1053	170.1056	**1.8**	170.1420	215.8
	K12 Ac	b10, b13	1114.6640	1442.8390	328.1749	328.1747	**-0.7**	328.2111	110.2
	K19 Ac	y3, y6	373.2076	701.3801	328.1725	328.1747	**6.7**	328.2111	117.6
H3: 9-26	K9 Ac	b2	0	258.1442	258.1442	258.1449	**2.7**	258.1813	143.7
	K14 Ac	b7, b5	473.2375	714.3794	241.1419	241.1427	**3.4**	241.1791	154.3
	K18 Ac	y5, y11	574.3299	1309.7710	735.4409	735.4392	**-2.3**	735.4756	47.2
	K23 Ac	y1, y5	175.1182	574.3299	399.2117	399.2118	**0.2**	399.2482	91.4
H4: 1-23	K20 3Me	b17, b22	1786.0630	2475.5320	689.4683	689.4337	-50.1	689.4701	**2.7**
	K16 Ac	y2, y11	288.1968	1375.866	1087.6700	1087.6727	**2.9**	1087.7090	36.3

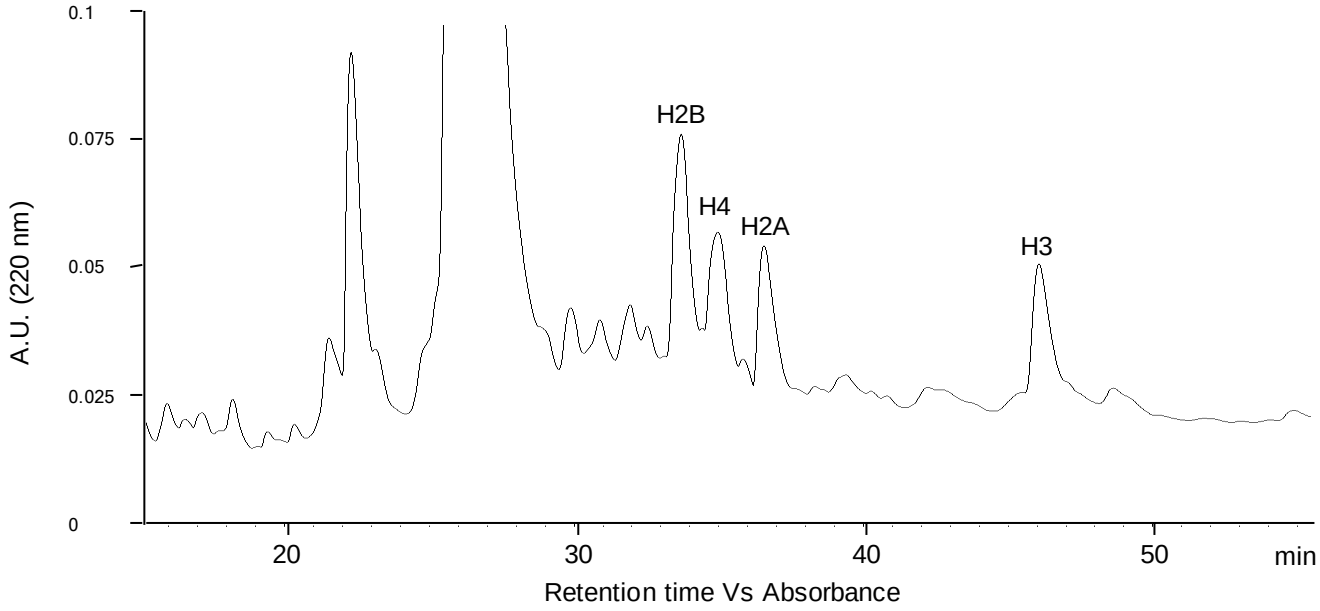
0.1
0.075
0.05
0.025
0
A.U. (220 nm)
H2B
H4
H2A
H3
20
30
40
50
min
Retention time Vs Absorbance

Figure S1

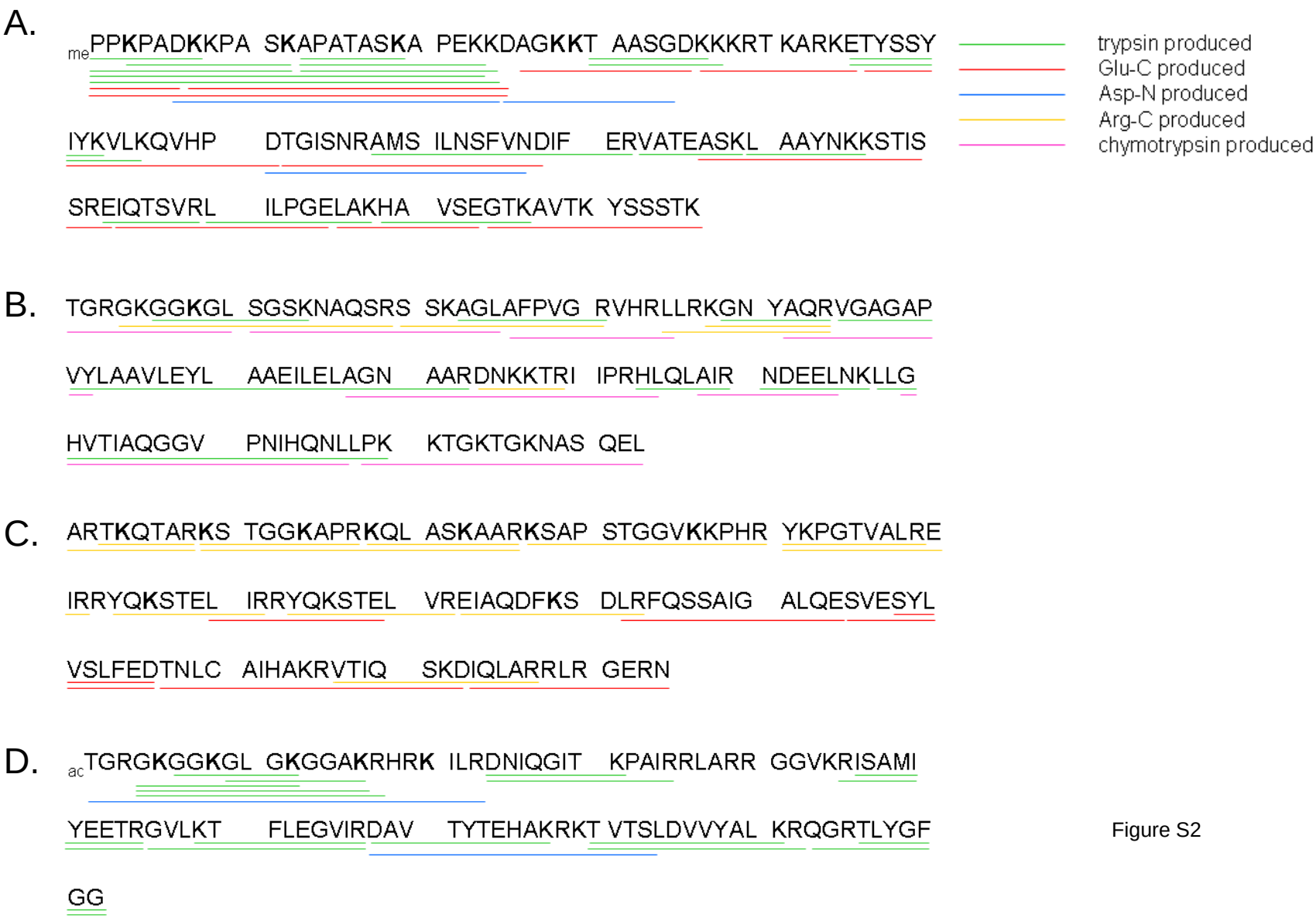
A.
mePPKPADKKPA SKAPATASKA PEKKDAGKKT AASGDKKKRT KARKETYSSY
IYKVLKQVHP DTGISNRAMS ILNSFVNDIF ERVATEASKL AAYNKKSTIS
SREIQTSVRL ILPGELAKHA VSEGTKAVTK YSSSTK
B.
TGRGKGGKGL SGSKNAQSRS SKAGLAFPVG RVHRLLRKGN YAQRVGAGAP
VYLAAVLEYL AAEILELAGN AARDNKKTRI IPRHLQLAIR NDEELNKLLG
HVTIAQGGV PNIHQNLLPK KTGKTGKNAS QEL
C.
ARTKQTARKS TGGKAPRKQL ASKAARKSAP STGGVKKPHR YKPGTVALRE
IRRYQKSTEL IRRYQKSTEL VREIAQDFKS DLRFQSSAIG ALQESVESYL
VSLFEDTNLC AIHAKRVTIQ SKDIQLARRLR GERN
D.
acTGRGKGGKGL GKGGAKRHRK ILRDNIQGIT KPAIRRLARR GGVKRISAMI
YEETRGVLKT FLEGVIRDAV TYTEHAKRKT VTSLDVVYAL KRQGRTLYGF
GG
trypsin produced
Glu-C produced
Asp-N produced
Arg-C produced
chymotrypsin produced

Figure S2

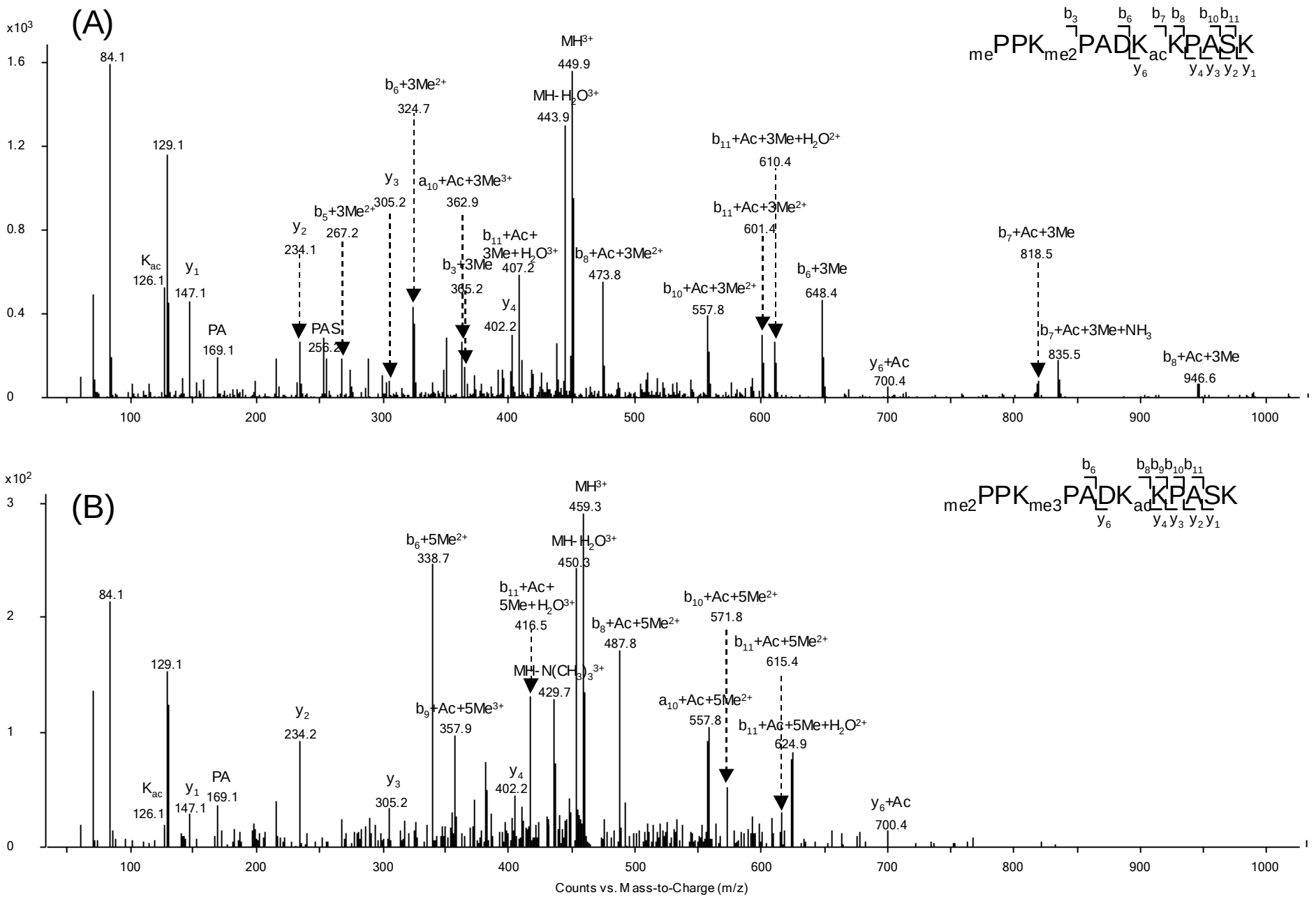
(A)
x10^3
1.6
1.2
0.8
0.4
0
84.1
129.1
K_ac 126.1
y_1 147.1
PA 169.1
y_2 234.1
b_5+3Me^2+ 267.2
PAS 256.2
y_3 305.2
b_6+3Me^2+ 324.7
a_10+Ac+3Me^3+ 362.9
b_3+3Me 365.2
b_11+Ac+ 3Me+H_2O^3+ 407.2
y_4 402.2
MH^3+ 449.9
MH-H_2O^3+ 443.9
b_8+Ac+3Me^2+ 473.8
b_10+Ac+3Me^2+ 557.8
b_11+Ac+3Me+H_2O^2+ 610.4
b_11+Ac+3Me^2+ 601.4
b_6+3Me 648.4
y_6+Ac 700.4
b_7+Ac+3Me 818.5
b_7+Ac+3Me+NH_3 835.5
b_8+Ac+3Me 946.6
b_3 b_6 b_7 b_8 b_10 b_11
mePPK_me2PADK_ac KPASK
y_6 y_4 y_3 y_2 y_1
100 200 300 400 500 600 700 800 900 1000
(B)
x10^2
3
2
1
0
84.1
129.1
K_ac 126.1
y_1 147.1
PA 169.1
y_2 234.2
y_3 305.2
b_9+Ac+5Me^3+ 357.9
b_6+5Me^2+ 338.7
b_11+Ac+ 5Me+H_2O^3+ 416.5
y_4 402.2
MH-N(CH_3)_3^3+ 429.7
MH^3+ 459.3
MH-H_2O^3+ 450.3
b_8+Ac+5Me^2+ 487.8
a_10+Ac+5Me^2+ 557.8
b_10+Ac+5Me^2+ 571.8
b_11+Ac+5Me^2+ 615.4
b_11+Ac+5Me+H_2O^2+ 624.9
y_6+Ac 700.4
b_6 b_8 b_9 b_10 b_11
me2PPK_me3PADK_ac KPASK
y_6 y_4 y_3 y_2 y_1
100 200 300 400 500 600 700 800 900 1000
Counts vs. Mass-to-Charge (m/z)

Figure S3

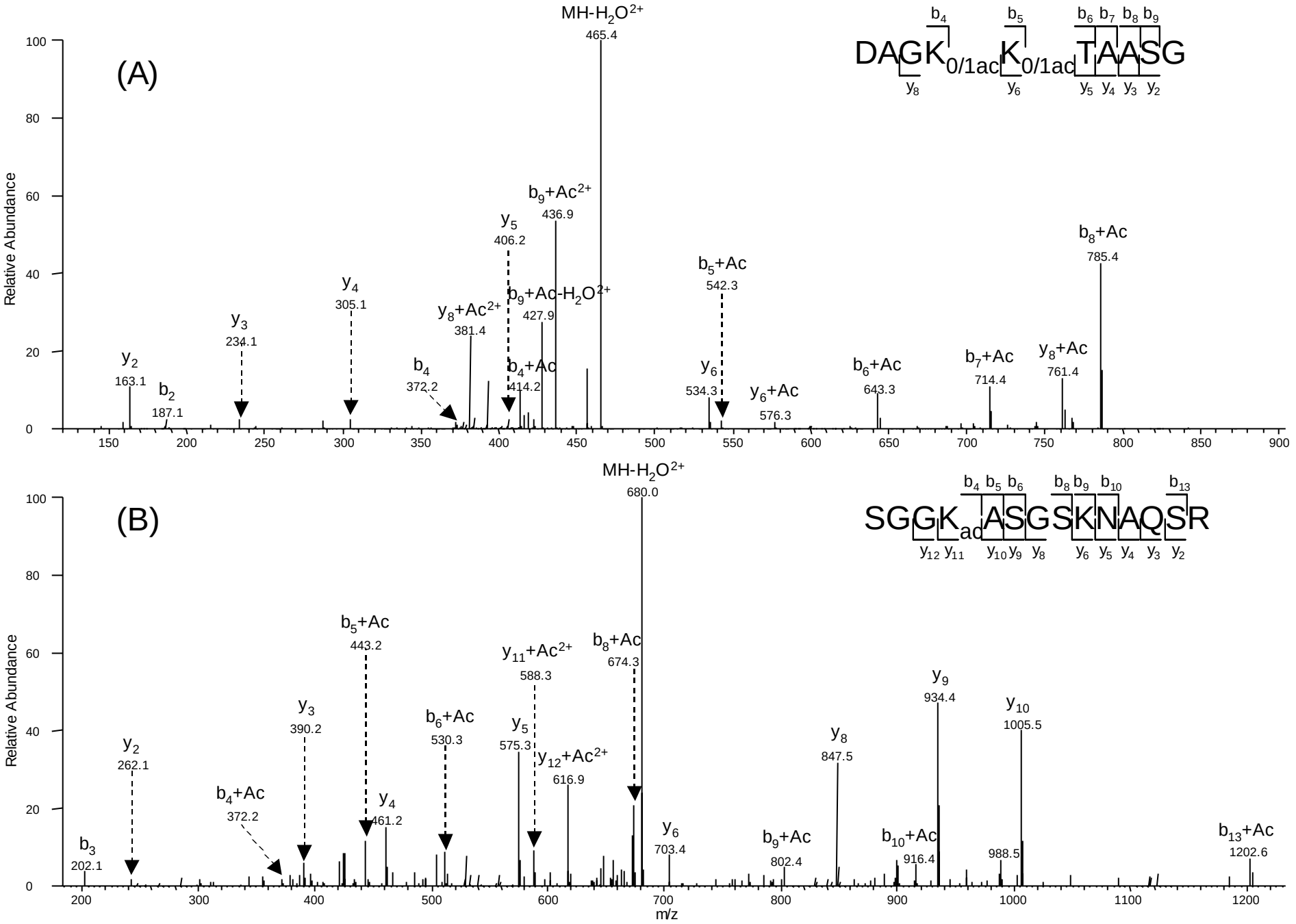
(A)
DAGK0/1acK0/1acTAASG
b4 b5 b6 b7 b8 b9
y8 y6 y5 y4 y3 y2
MH-H2O2+
465.4
b9+Ac2+
436.9
y5
406.2
b8+Ac
785.4
b5+Ac
542.3
y4
305.1
b9+Ac-H2O2+
427.9
y8+Ac2+
381.4
y3
234.1
y2
163.1
b4
372.2
b4+Ac
414.2
y6
534.3
b6+Ac
643.3
b7+Ac
714.4
y8+Ac
761.4
b2
187.1
y6+Ac
576.3
Relative Abundance
(B)
SGGKacASGSKNAQSR
b4 b5 b6 b8 b9 b10 b13
y12 y11 y10 y9 y8 y6 y5 y4 y3 y2
MH-H2O2+
680.0
b5+Ac
443.2
y11+Ac2+
588.3
b8+Ac
674.3
y9
934.4
y10
1005.5
y3
390.2
b6+Ac
530.3
y5
575.3
y8
847.5
y2
262.1
y12+Ac2+
616.9
b4+Ac
372.2
y4
461.2
b3
202.1
y6
703.4
b9+Ac
802.4
b10+Ac
916.4
988.5
b13+Ac
1202.6
Relative Abundance
m/z

Figure S4

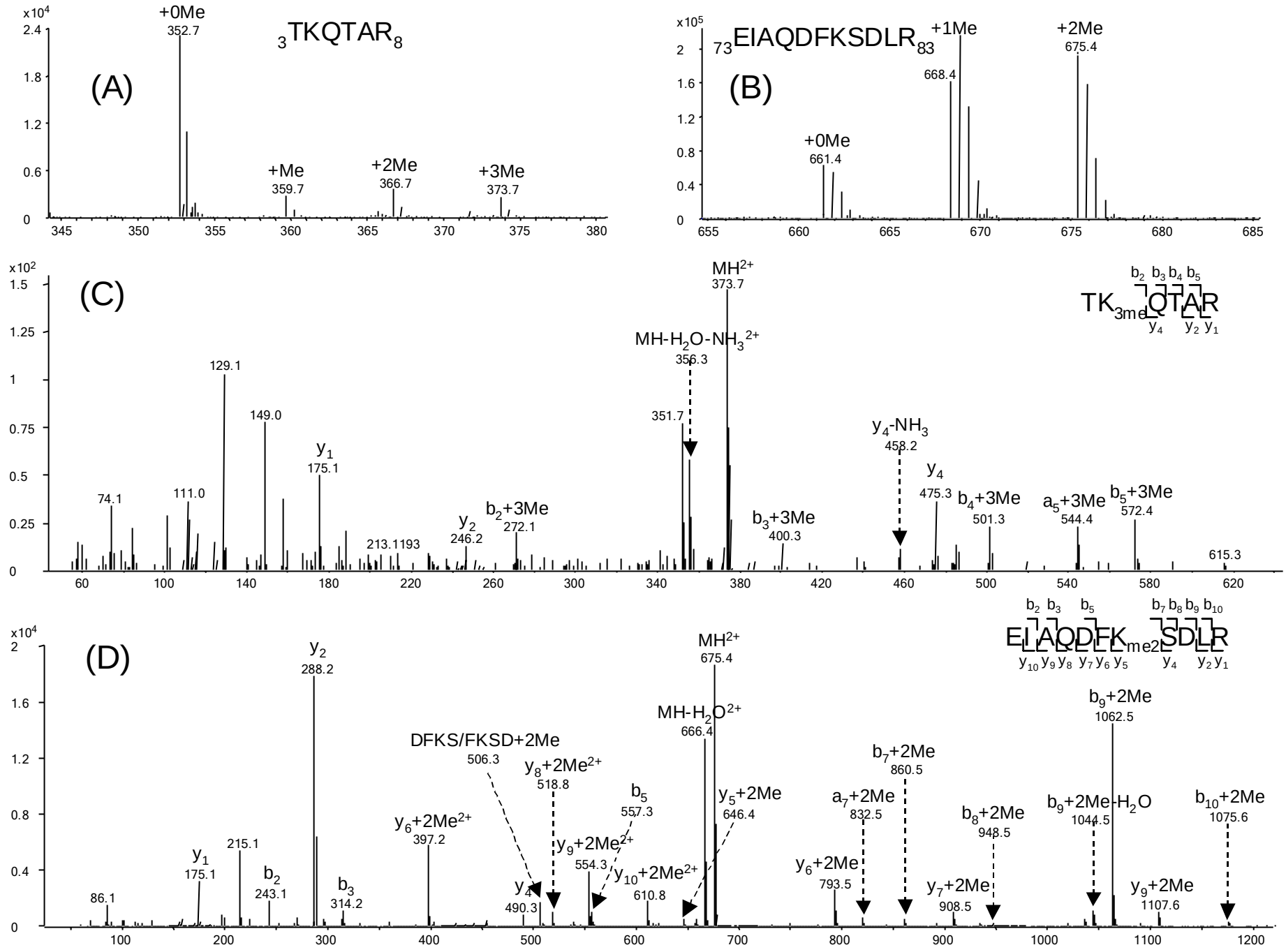
(A)
3TKQTAR8
+0Me
352.7
+Me
359.7
+2Me
366.7
+3Me
373.7
(B)
73EIAQDFKSDLR83
+0Me
661.4
+1Me
668.4
+2Me
675.4
(C)
TK3meQTAR
MH2+
373.7
MH-H2O-NH3 2+
356.3
351.7
129.1
149.0
y1
175.1
74.1
111.0
213.1193
y2
246.2
b2+3Me
272.1
b3+3Me
400.3
y4-NH3
458.2
y4
475.3
b4+3Me
501.3
a5+3Me
544.4
b5+3Me
572.4
615.3
(D)
EIAQDFKme2SDLR
y2
288.2
MH2+
675.4
MH-H2O2+
666.4
DFKS/FKSD+2Me
506.3
y8+2Me2+
518.8
b5
557.3
y5+2Me
646.4
y6+2Me2+
397.2
y9+2Me2+
554.3
y10+2Me2+
610.8
y1
175.1
215.1
b2
243.1
b3
314.2
86.1
y4
490.3
b7+2Me
860.5
a7+2Me
832.5
b9+2Me
1062.5
b9+2Me-H2O
1044.5
b10+2Me
1075.6
b8+2Me
948.5
y6+2Me
793.5
y7+2Me
908.5
y9+2Me
1107.6

Figure S5

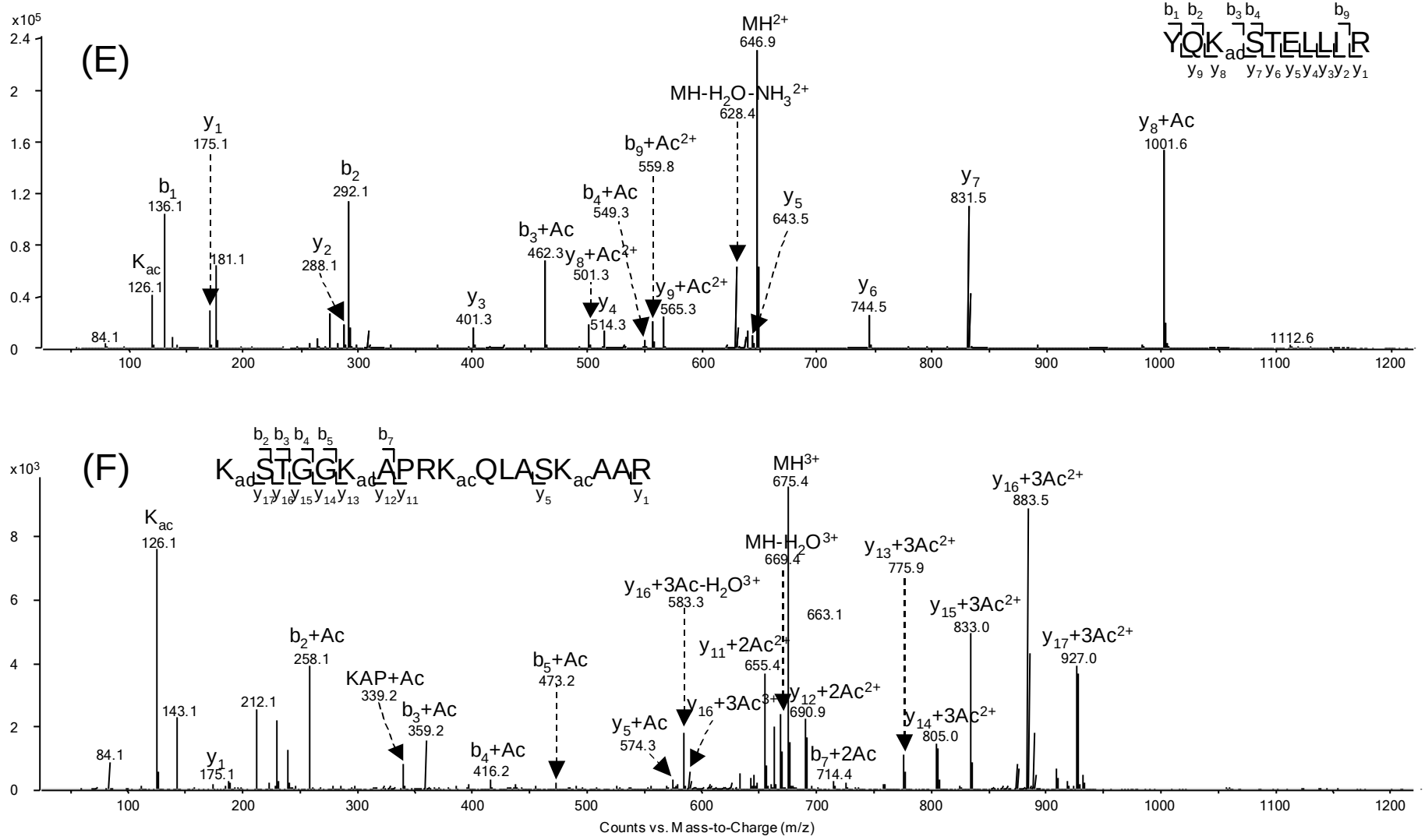
(E)
x10^5
MH^2+ 646.9
MH-H2O-NH3^2+ 628.4
y1 175.1
b1 136.1
b2 292.1
b9+Ac^2+ 559.8
b4+Ac 549.3
y5 643.5
y7 831.5
y8+Ac 1001.6
b3+Ac 462.3
y2 288.1
Kac 126.1
181.1
y8+Ac^2+ 501.3
y9+Ac^2+ 565.3
y3 401.3
y4 514.3
y6 744.5
84.1
1112.6
b1 b2 b3 b4 b9
YQKadSTELLLR
y9 y8 y7 y6 y5 y4 y3 y2 y1
(F)
x10^3
b2 b3 b4 b5 b7
Kad STGGKad APRKac QLASKac AAR
y17 y16 y15 y14 y13 y12 y11 y5 y1
MH^3+ 675.4
y16+3Ac^2+ 883.5
Kac 126.1
MH-H2O^3+ 669.4
y13+3Ac^2+ 775.9
y16+3Ac-H2O^3+ 583.3
663.1
y15+3Ac^2+ 833.0
b2+Ac 258.1
y11+2Ac^2+ 655.4
y17+3Ac^2+ 927.0
b5+Ac 473.2
KAP+Ac 339.2
y12+2Ac^2+ 690.9
143.1
212.1
b3+Ac 359.2
y16+3Ac^3+
y5+Ac 574.3
y14+3Ac^2+ 805.0
84.1
y1 175.1
b4+Ac 416.2
b7+2Ac 714.4
Counts vs. Mass-to-Charge (m/z)

Figure S5

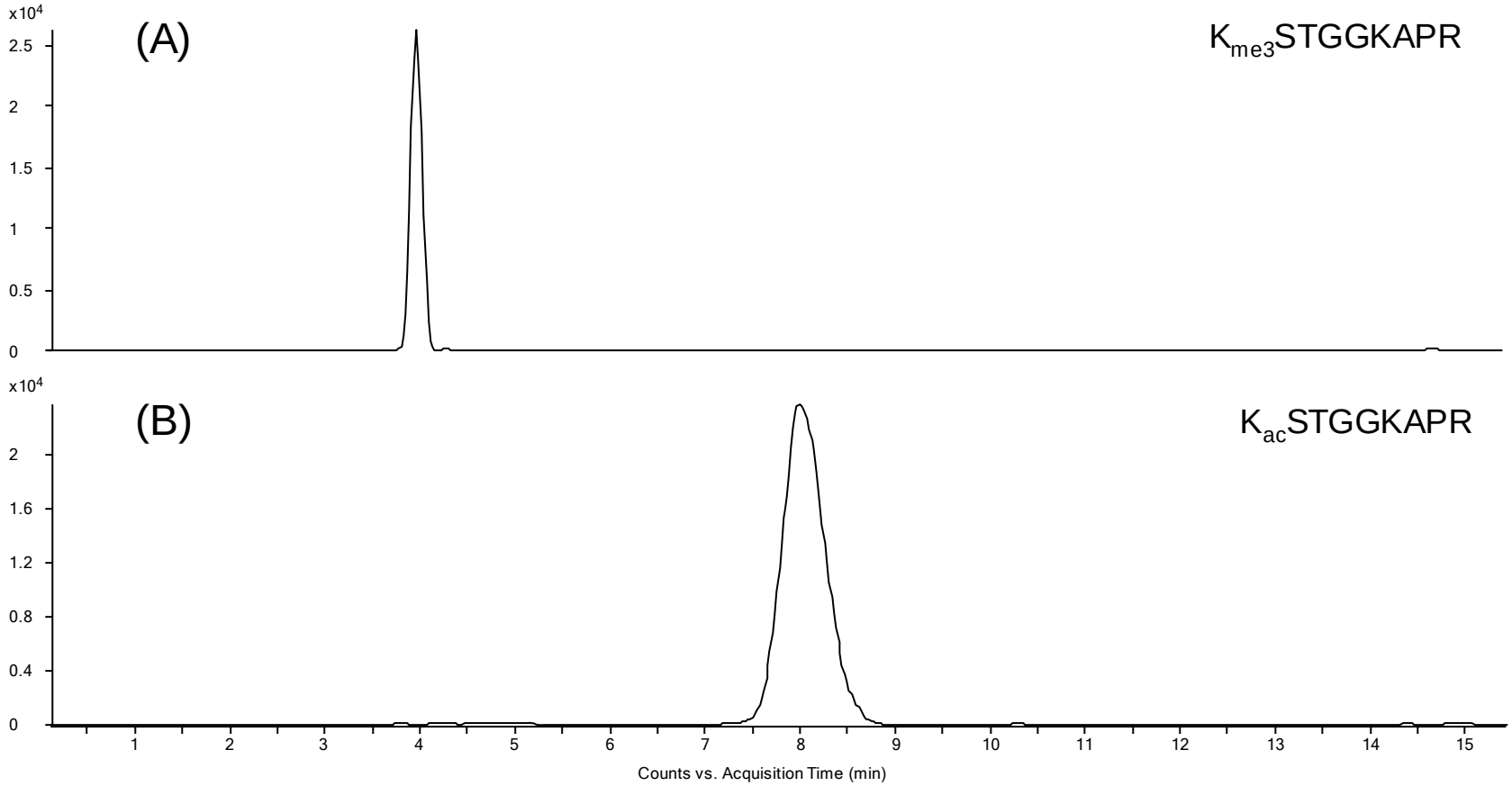
x10^4
(A)
K_me3STGGKAPR
2.5
2
1.5
1
0.5
0
x10^4
(B)
K_acSTGGKAPR
2
1.6
1.2
0.8
0.4
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Counts vs. Acquisition Time (min)

Figure S6

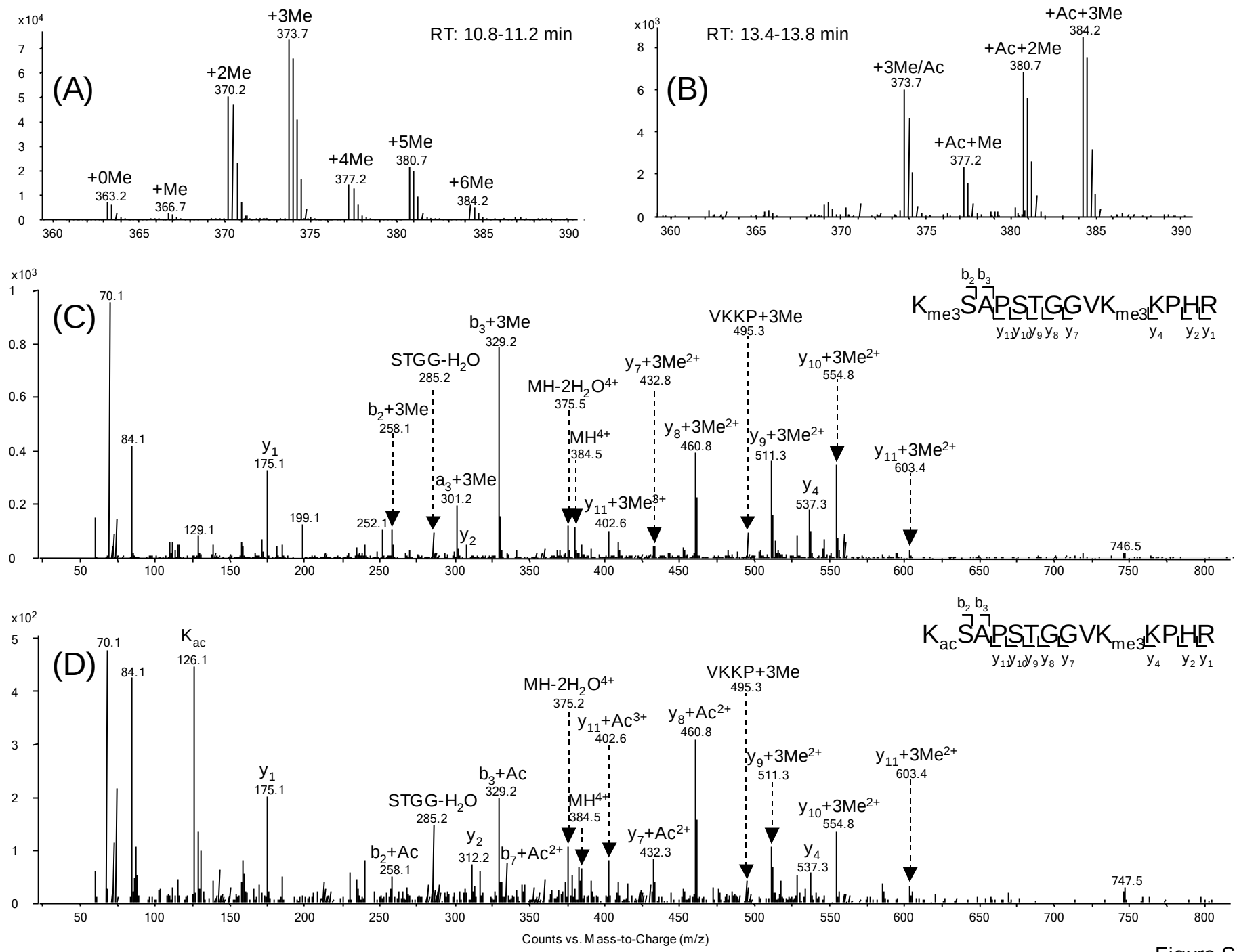
(A)
RT: 10.8-11.2 min
+0Me 363.2
+Me 366.7
+2Me 370.2
+3Me 373.7
+4Me 377.2
+5Me 380.7
+6Me 384.2
(B)
RT: 13.4-13.8 min
+3Me/Ac 373.7
+Ac+Me 377.2
+Ac+2Me 380.7
+Ac+3Me 384.2
(C)
K me3 S A P S T G G V K me3 L K P H R
b2 b3
y11 y10 y9 y8 y7 y4 y2 y1
70.1
84.1
129.1
y1 175.1
199.1
252.1
b2+3Me 258.1
STGG-H2O 285.2
a3+3Me 301.2
y2
b3+3Me 329.2
MH-2H2O4+ 375.5
MH4+ 384.5
y11+3Me3+ 402.6
y7+3Me2+ 432.8
y8+3Me2+ 460.8
VKKP+3Me 495.3
y9+3Me2+ 511.3
y4 537.3
y10+3Me2+ 554.8
y11+3Me2+ 603.4
746.5
(D)
K ac S A P S T G G V K me3 L K P H R
b2 b3
y11 y10 y9 y8 y7 y4 y2 y1
70.1
84.1
Kac 126.1
y1 175.1
b2+Ac 258.1
STGG-H2O 285.2
y2 312.2
b3+Ac 329.2
b7+Ac2+
MH-2H2O4+ 375.2
y11+Ac3+ 402.6
MH4+ 384.5
y7+Ac2+ 432.3
y8+Ac2+ 460.8
VKKP+3Me 495.3
y9+3Me2+ 511.3
y10+3Me2+ 554.8
y4 537.3
y11+3Me2+ 603.4
747.5
Counts vs. Mass-to-Charge (m/z)

Figure S7

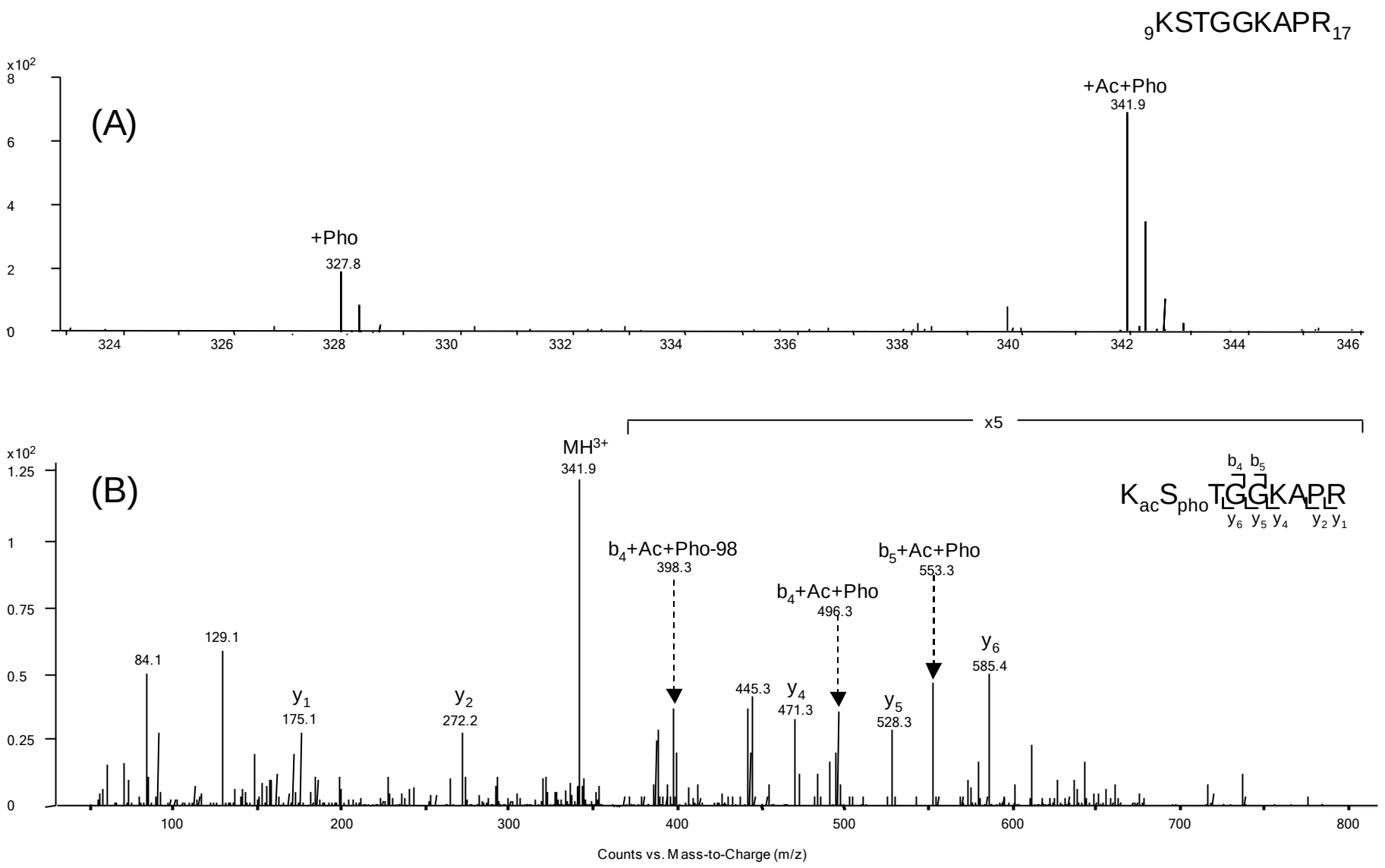
9KSTGGKAPR17
(A)
x10^2
+Ac+Pho
341.9
+Pho
327.8
(B)
x5
MH3+
341.9
b4 b5
KacSphoTGGKAPR
y6 y5 y4 y2 y1
b4+Ac+Pho-98
398.3
b5+Ac+Pho
553.3
b4+Ac+Pho
496.3
129.1
84.1
y1
175.1
y2
272.2
445.3
y4
471.3
y5
528.3
y6
585.4
Counts vs. Mass-to-Charge (m/z)

Figure S8

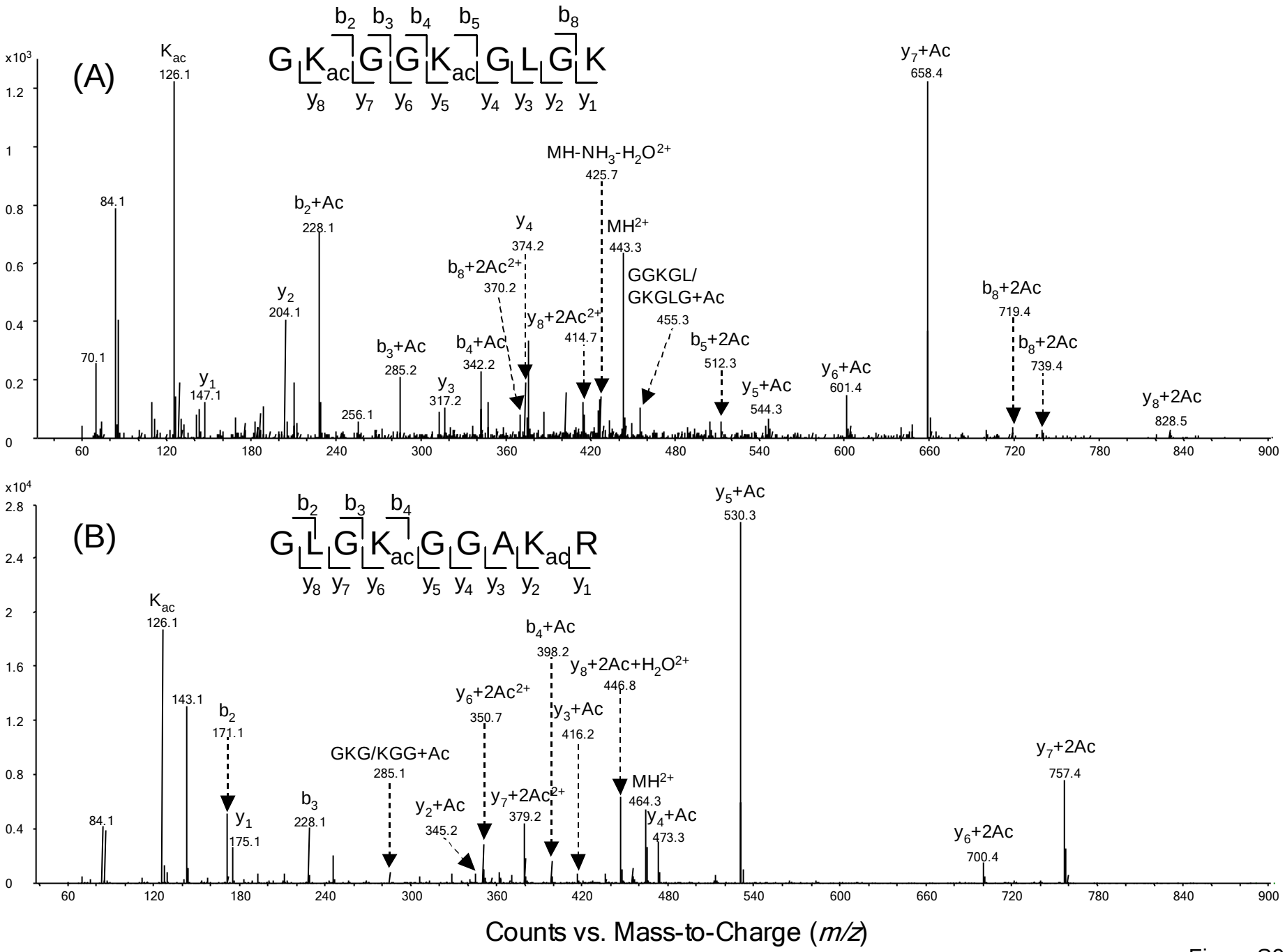
(A)
G K$_{ac}$ G G K$_{ac}$ G L G K
b$_2$ b$_3$ b$_4$ b$_5$ b$_8$
y$_8$ y$_7$ y$_6$ y$_5$ y$_4$ y$_3$ y$_2$ y$_1$
x10^3
K$_{ac}$ 126.1
y$_7$+Ac 658.4
MH-NH$_3$-H$_2$O^{2+} 425.7
84.1
b$_2$+Ac 228.1
y$_4$ 374.2
MH^{2+} 443.3
b$_8$+2Ac^{2+} 370.2
GGKGL/ GKGLG+Ac
y$_2$ 204.1
y$_8$+2Ac^{2+} 414.7
455.3
b$_8$+2Ac 719.4
b$_3$+Ac 285.2
b$_4$+Ac 342.2
b$_5$+2Ac 512.3
b$_8$+2Ac 739.4
70.1
y$_1$ 147.1
y$_3$ 317.2
y$_6$+Ac 601.4
y$_5$+Ac 544.3
y$_8$+2Ac 828.5
256.1
(B)
G L G K$_{ac}$ G G A K$_{ac}$ R
b$_2$ b$_3$ b$_4$
y$_8$ y$_7$ y$_6$ y$_5$ y$_4$ y$_3$ y$_2$ y$_1$
x10^4
y$_5$+Ac 530.3
K$_{ac}$ 126.1
b$_4$+Ac 398.2
y$_8$+2Ac+H$_2$O^{2+} 446.8
143.1
y$_6$+2Ac^{2+} 350.7
b$_2$ 171.1
y$_3$+Ac 416.2
y$_7$+2Ac 757.4
GKG/KGG+Ac 285.1
MH^{2+} 464.3
b$_3$ 228.1
y$_2$+Ac 345.2
y$_7$+2Ac^{2+} 379.2
y$_4$+Ac 473.3
84.1
y$_1$ 175.1
y$_6$+2Ac 700.4
Counts vs. Mass-to-Charge (*m/z*)

Figure S9

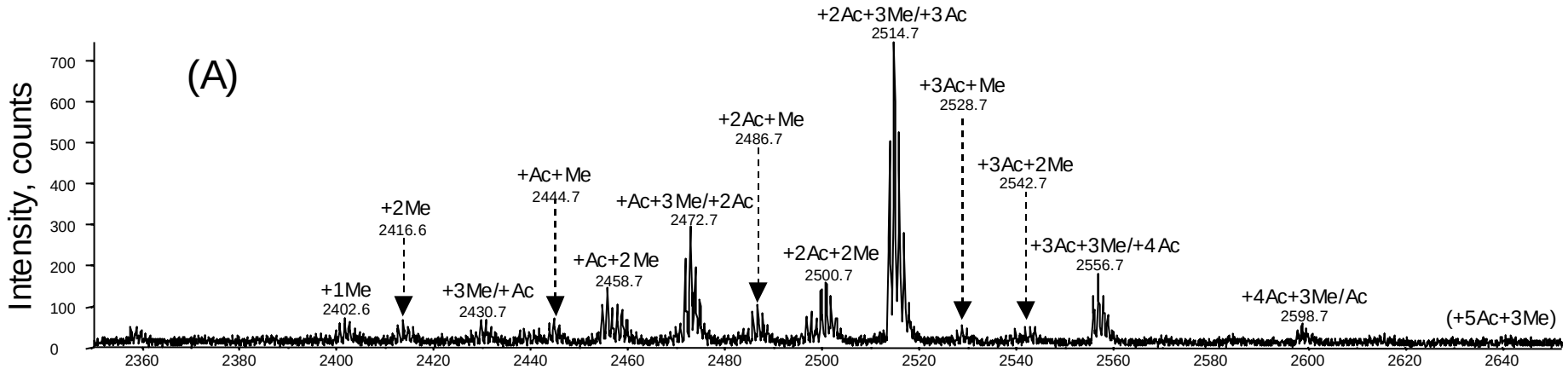
(A)
Intensity, counts
0
100
200
300
400
500
600
700
2360
2380
2400
2420
2440
2460
2480
2500
2520
2540
2560
2580
2600
2620
2640
+1Me
2402.6
+2Me
2416.6
+3Me/+Ac
2430.7
+Ac+Me
2444.7
+Ac+2Me
2458.7
+Ac+3Me/+2Ac
2472.7
+2Ac+Me
2486.7
+2Ac+2Me
2500.7
+2Ac+3Me/+3Ac
2514.7
+3Ac+Me
2528.7
+3Ac+2Me
2542.7
+3Ac+3Me/+4Ac
2556.7
+4Ac+3Me/Ac
2598.7
(+5Ac+3Me)

Figure S10

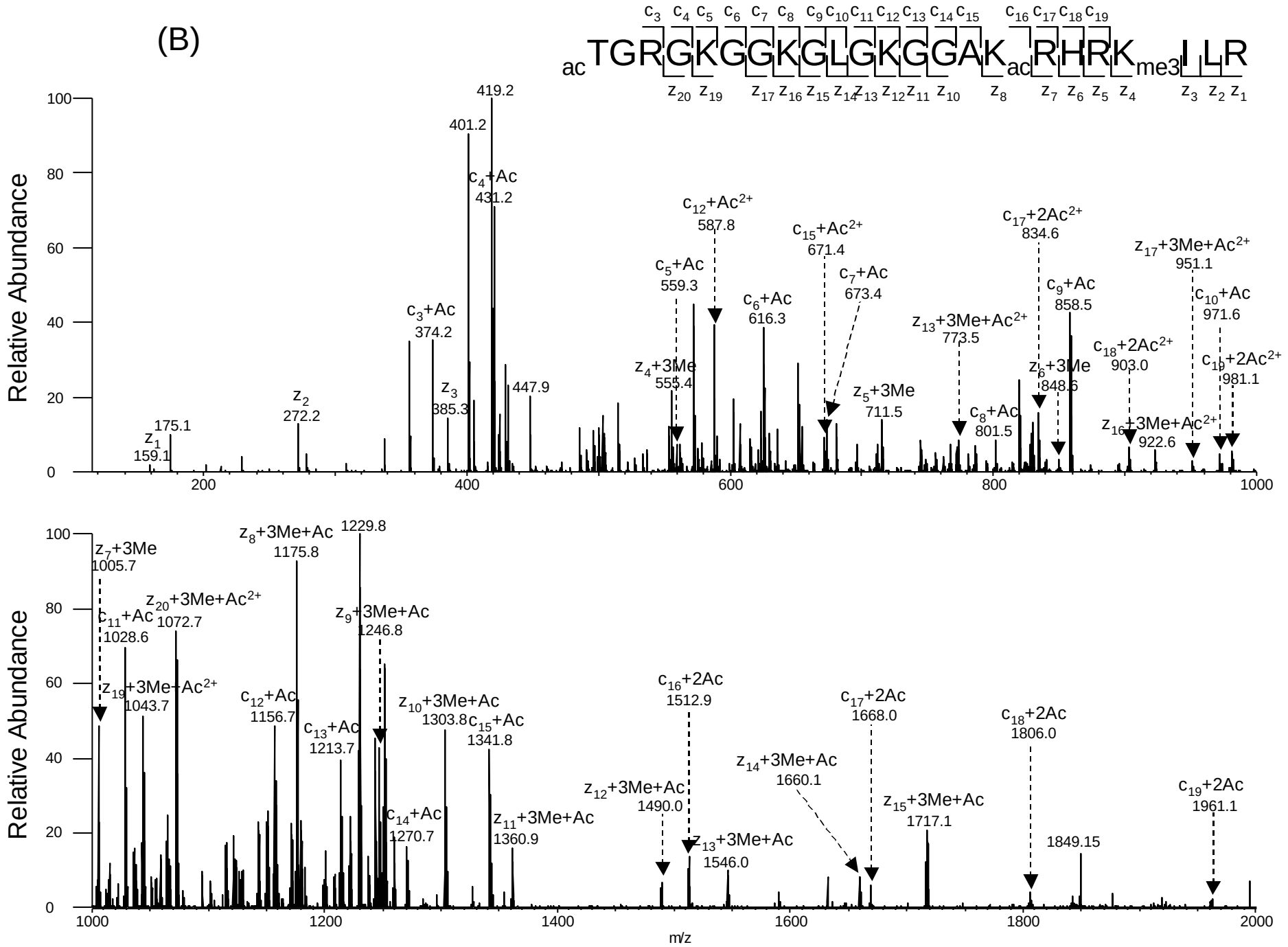
(B)
acTGRGKGGKGLGKGGAKacRHRKme3LLR
Relative Abundance
m/z

Figure S10