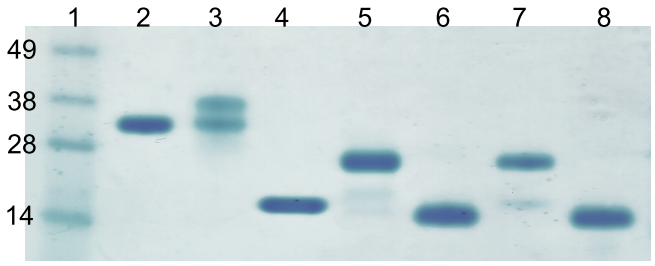
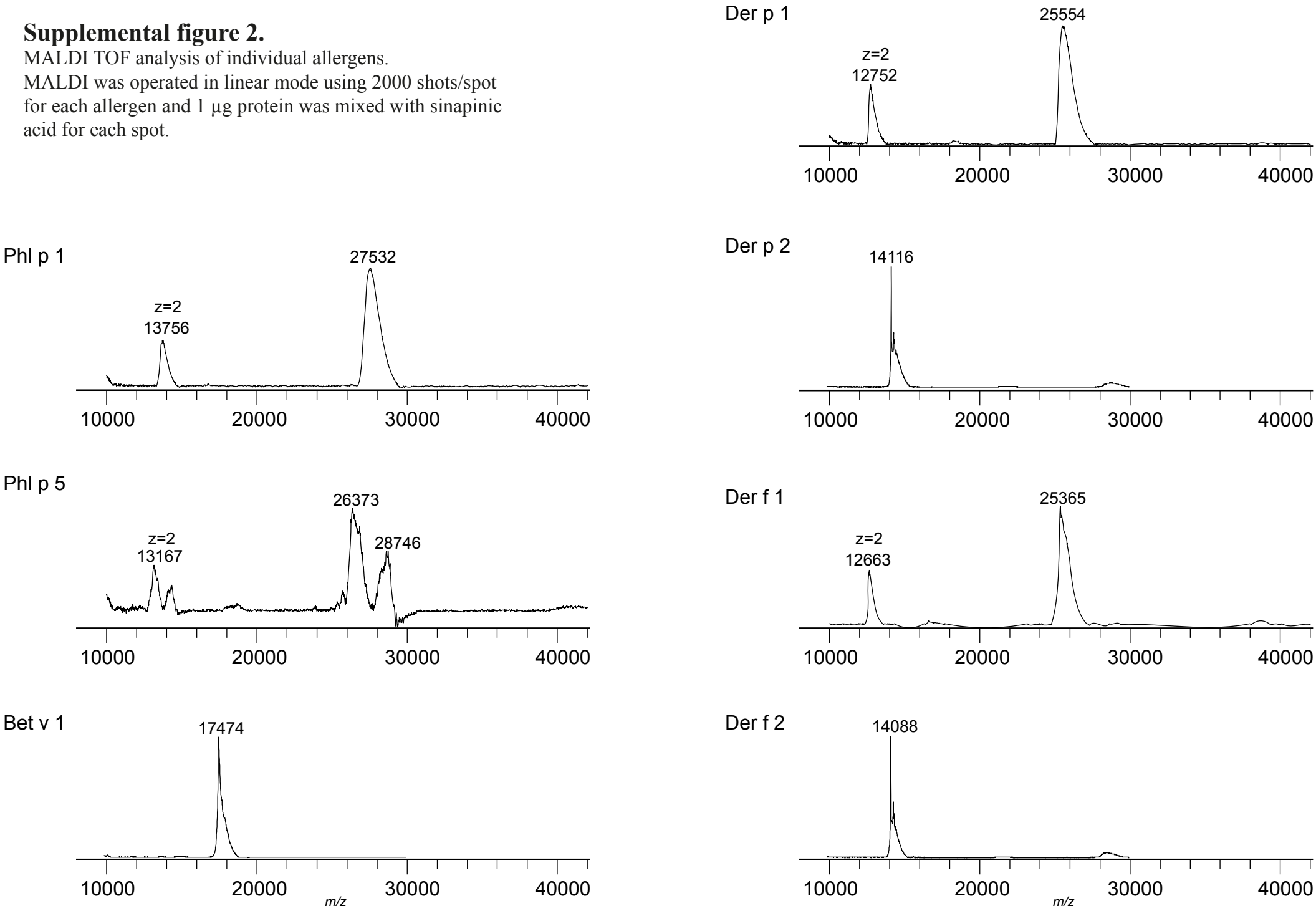


Supplemental figure 1.
SDS-PAGE analysis of allergens used in this study. Lane 1: molecular standard, lane 2: Phl p 1, lane 3: Phl p 5, lane 4: Bet v 1, lane 5: Der p 1, lane 6: Der p 2, lane 7: Der f 1, lane 8: Der f 2.

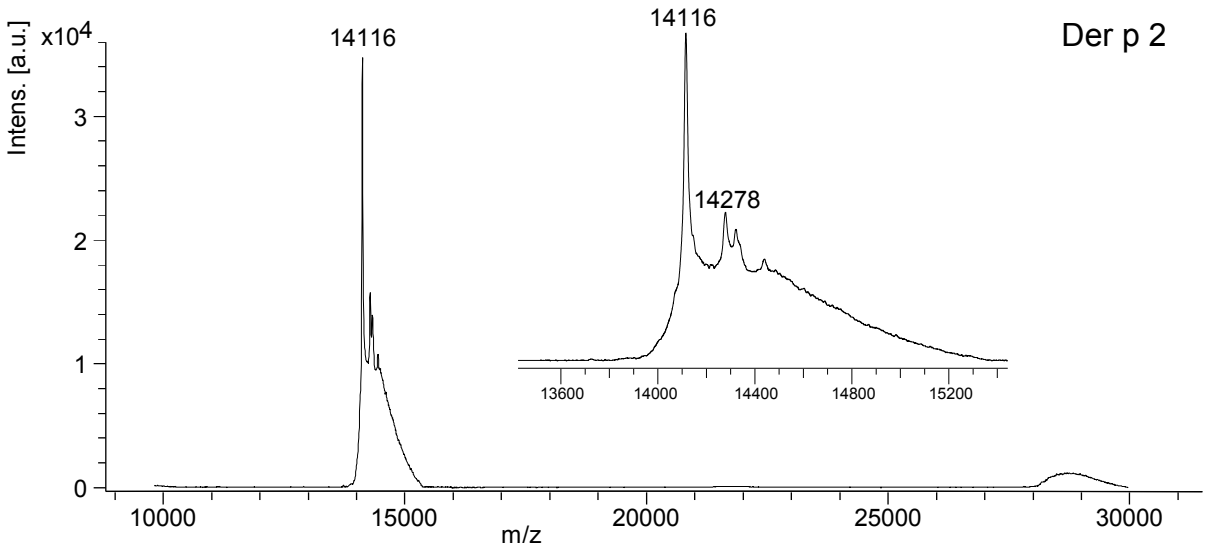
Supplemental figure 2.

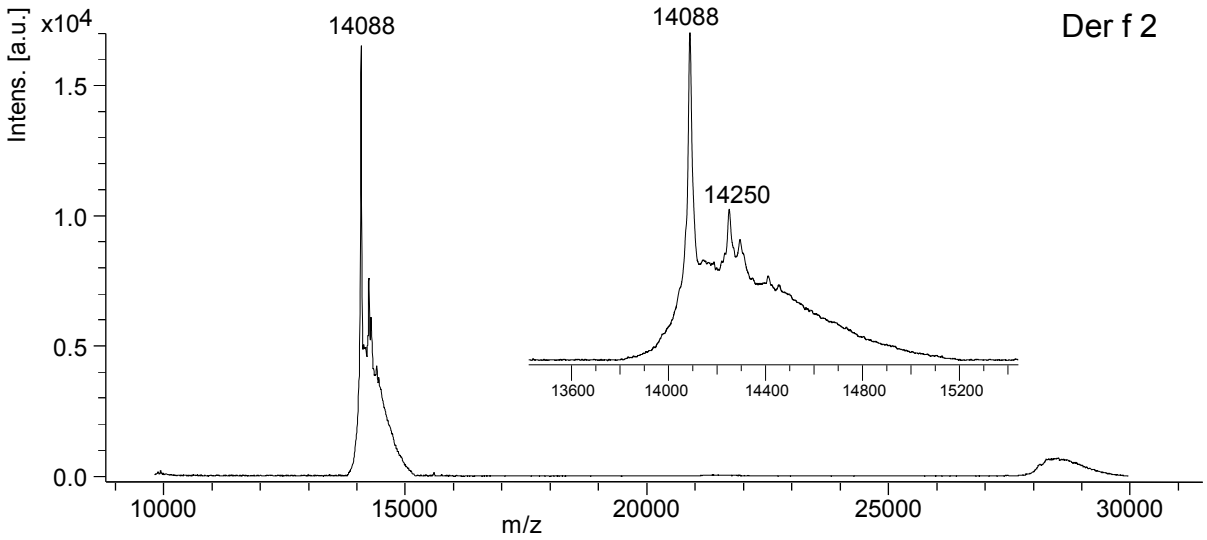
MALDI TOF analysis of individual allergens.
MALDI was operated in linear mode using 2000 shots/spot for each allergen and 1 µg protein was mixed with sinapinic acid for each spot.



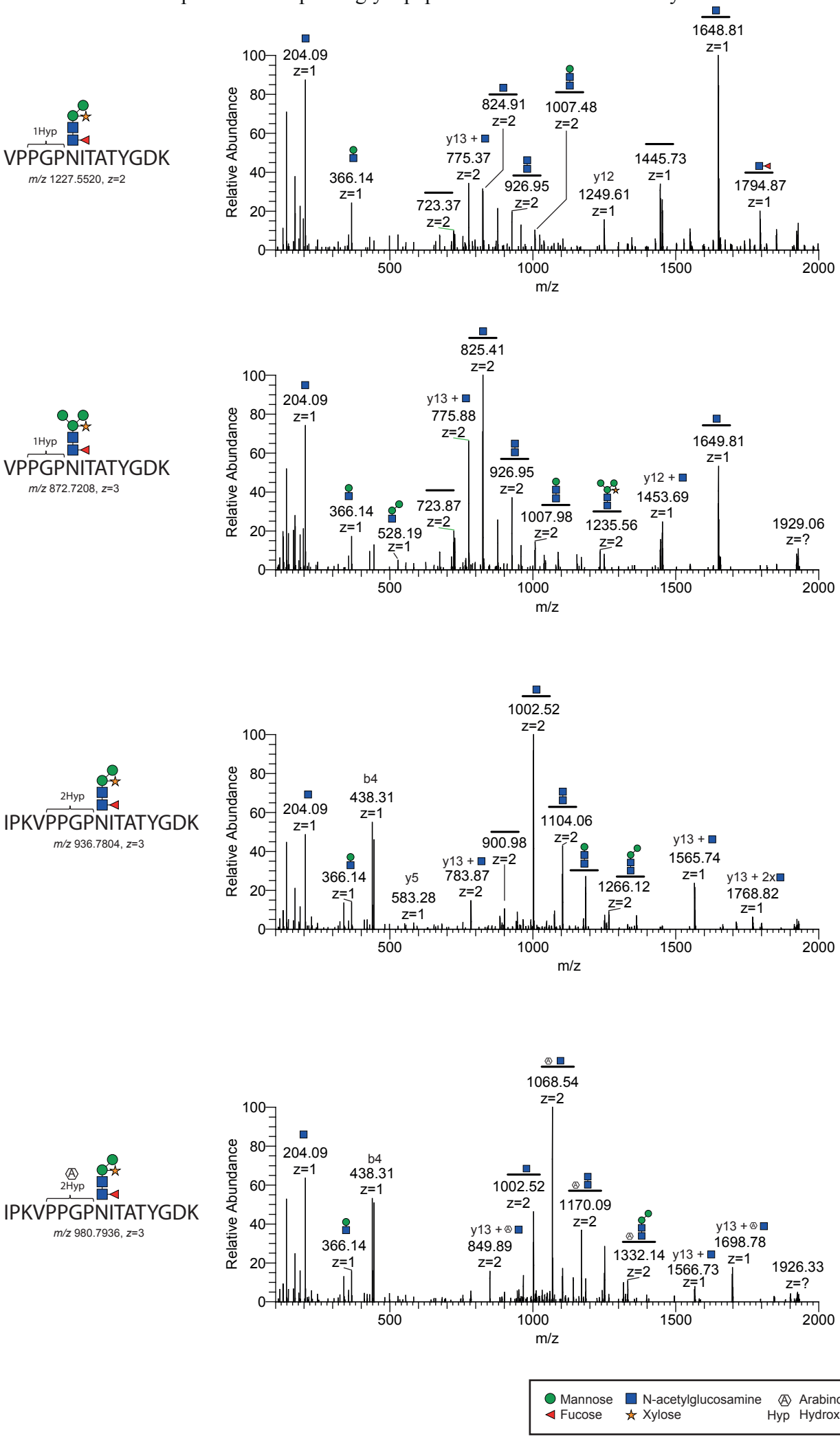
Supplemental figure 3.

MALDI TOF analysis of Der p2 and Der f 2.
Insert shows m/z 13600-15200 mass range. A second minor peak representing 162 amu mass increment can be seen for both allergens.

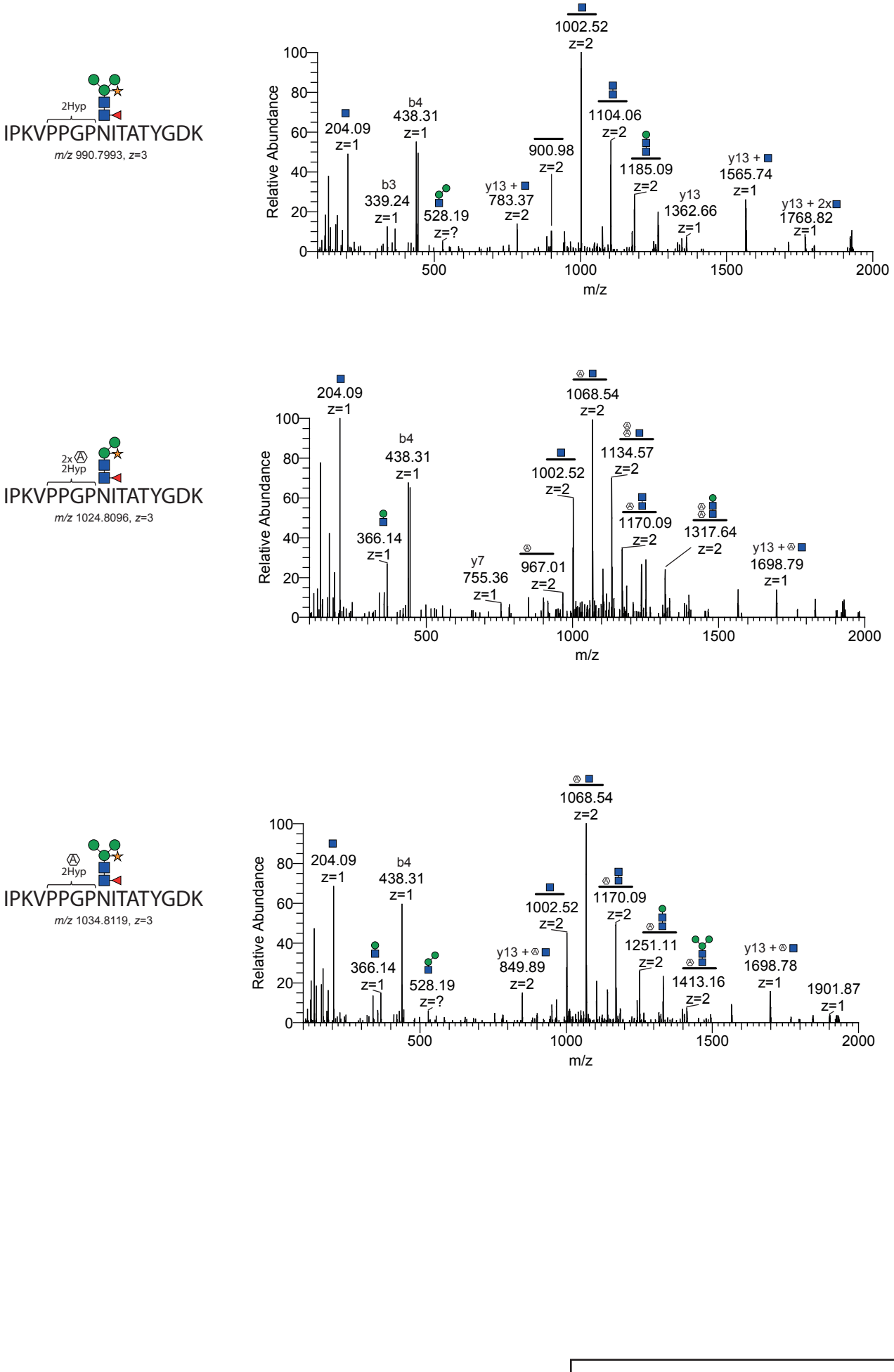




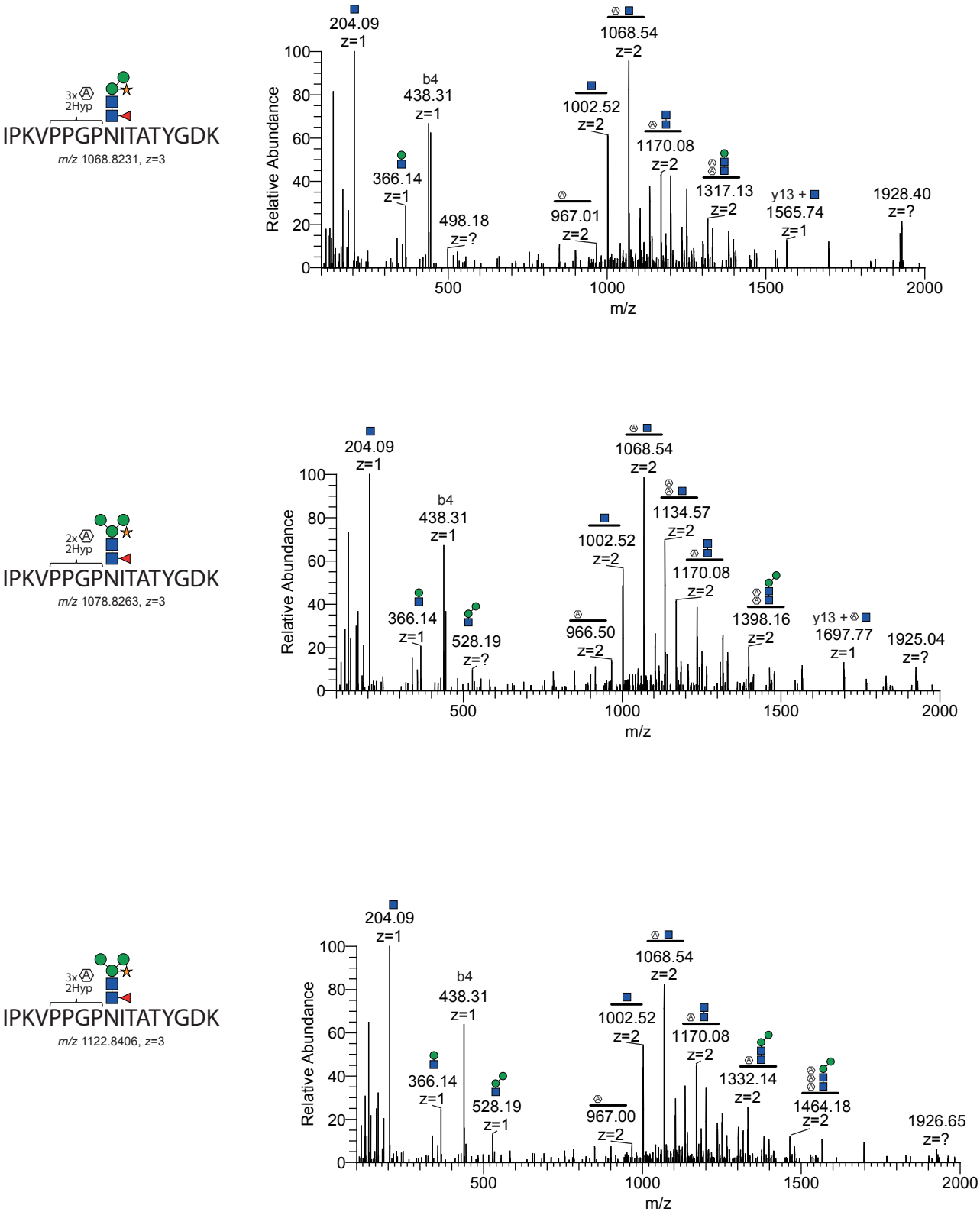
Supplemental figure 4.
Annotated HCD-MS2 spectra of Phl p 1 N-glycopeptides identified in this study.



Supplemental figure 4.
Annotated HCD-MS2 spectra of Phl p 1 N-glycopeptides identified in this study.

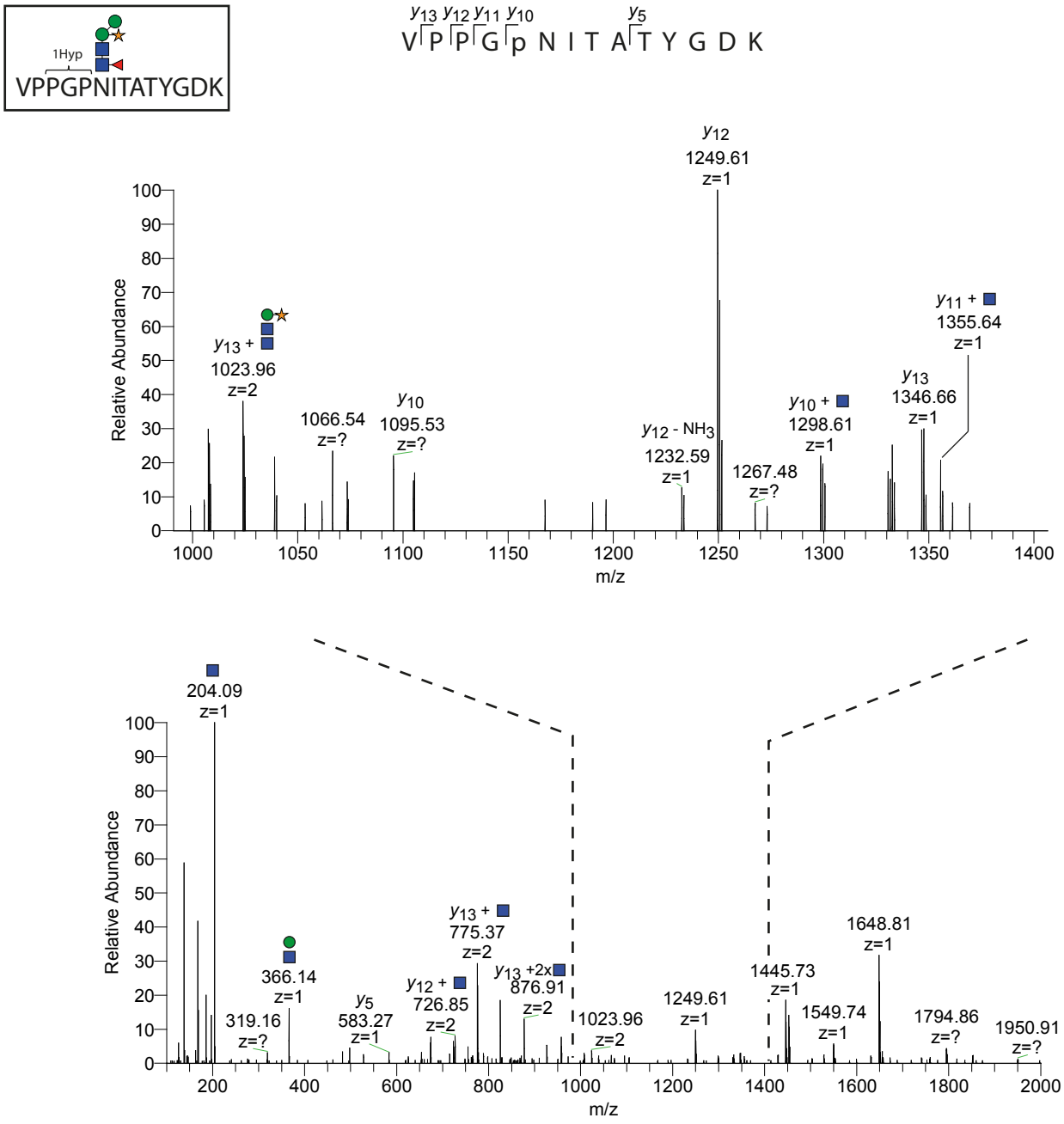


Supplemental figure 4.
Annotated HCD-MS2 spectra of Phl p 1 N-glycopeptides identified in this study.



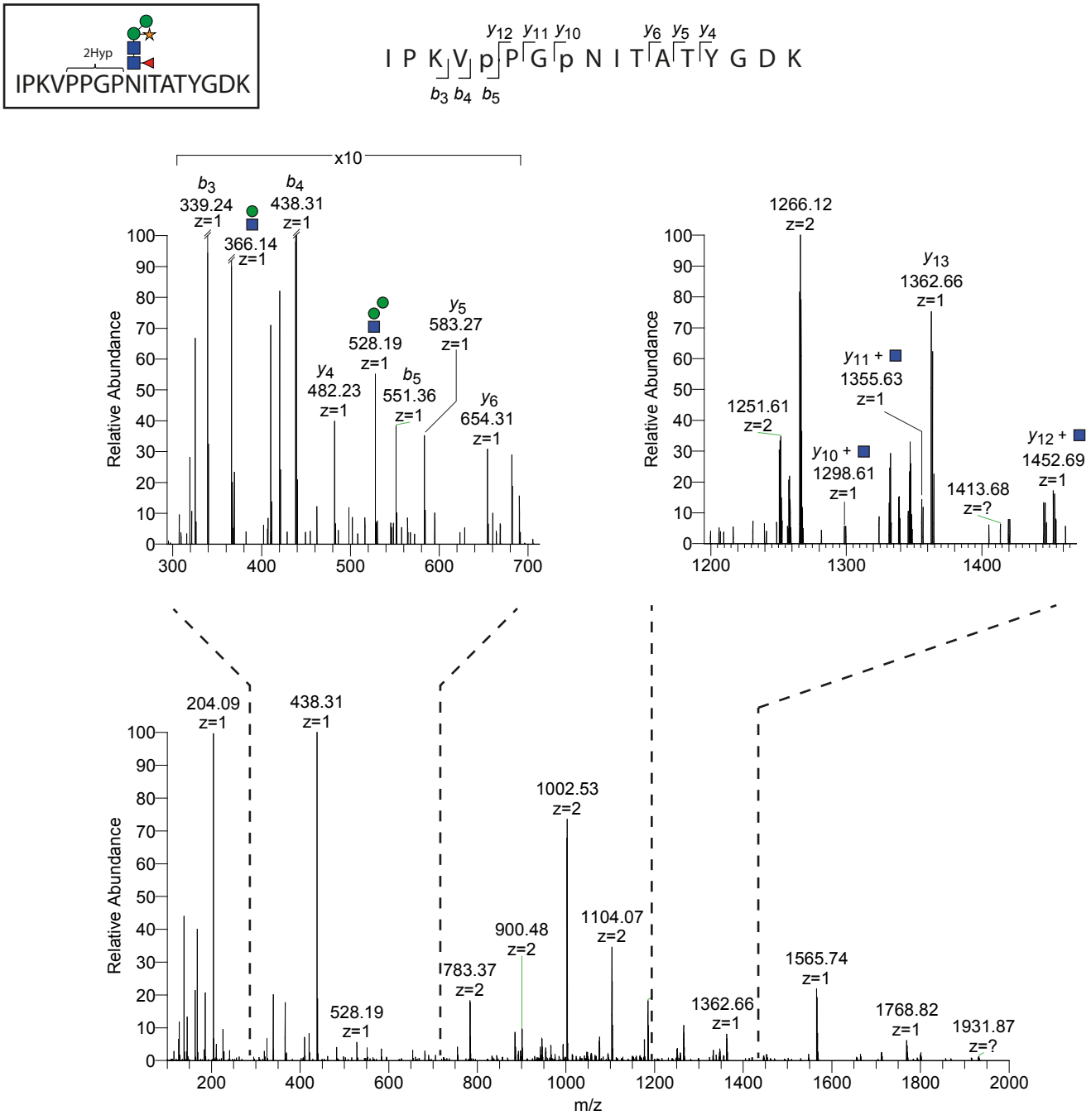
Supplemental figure 4B.

Annotated HCD-MS2 spectra of Phl p 1 N-glycopeptide V[27]PPGPNITATYGDK[40] (boxed) modified by a single Hyp. The y10+HexNAc fragment at m/z 1289.61 demonstrates that P[31] is the hydroxylated residue (indicated as p).



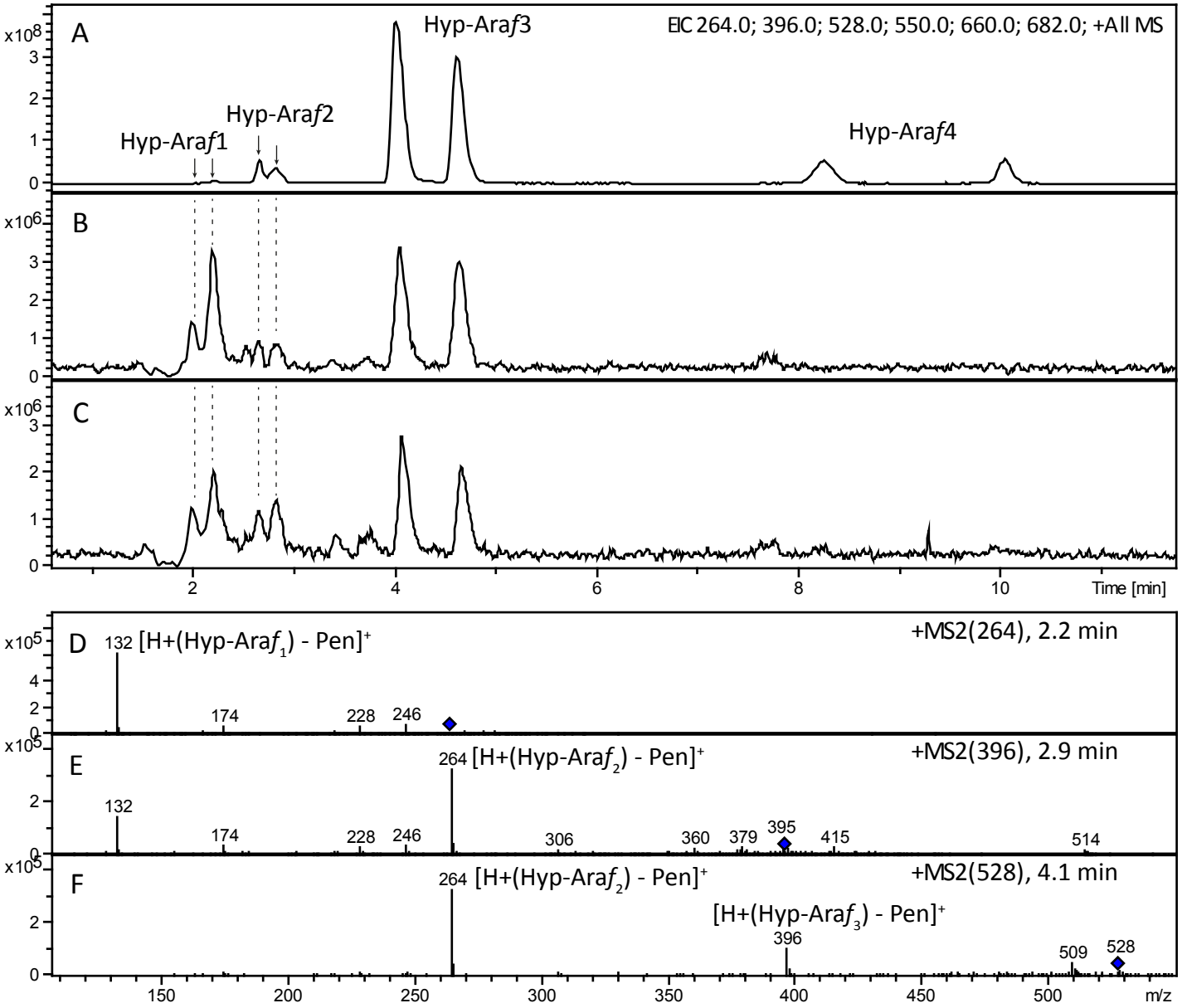
Supplemental figure 4C.

Annotated HCD-MS2 spectra of Phl p 1 N-glycopeptide I[24]PKVPPGPNITATYGDK[40] (boxed) modified by two Hyp residues. The y10+HexNAc fragment at m/z 1289.61 demonstrates that P[31] is hydroxylated. The b3 (m/z 339.24) and b5 (m/z 551.36) fragmets demonstrate that the second hydroxylation is on P[28] residue (indicated as p).



Supplemental figure 5.

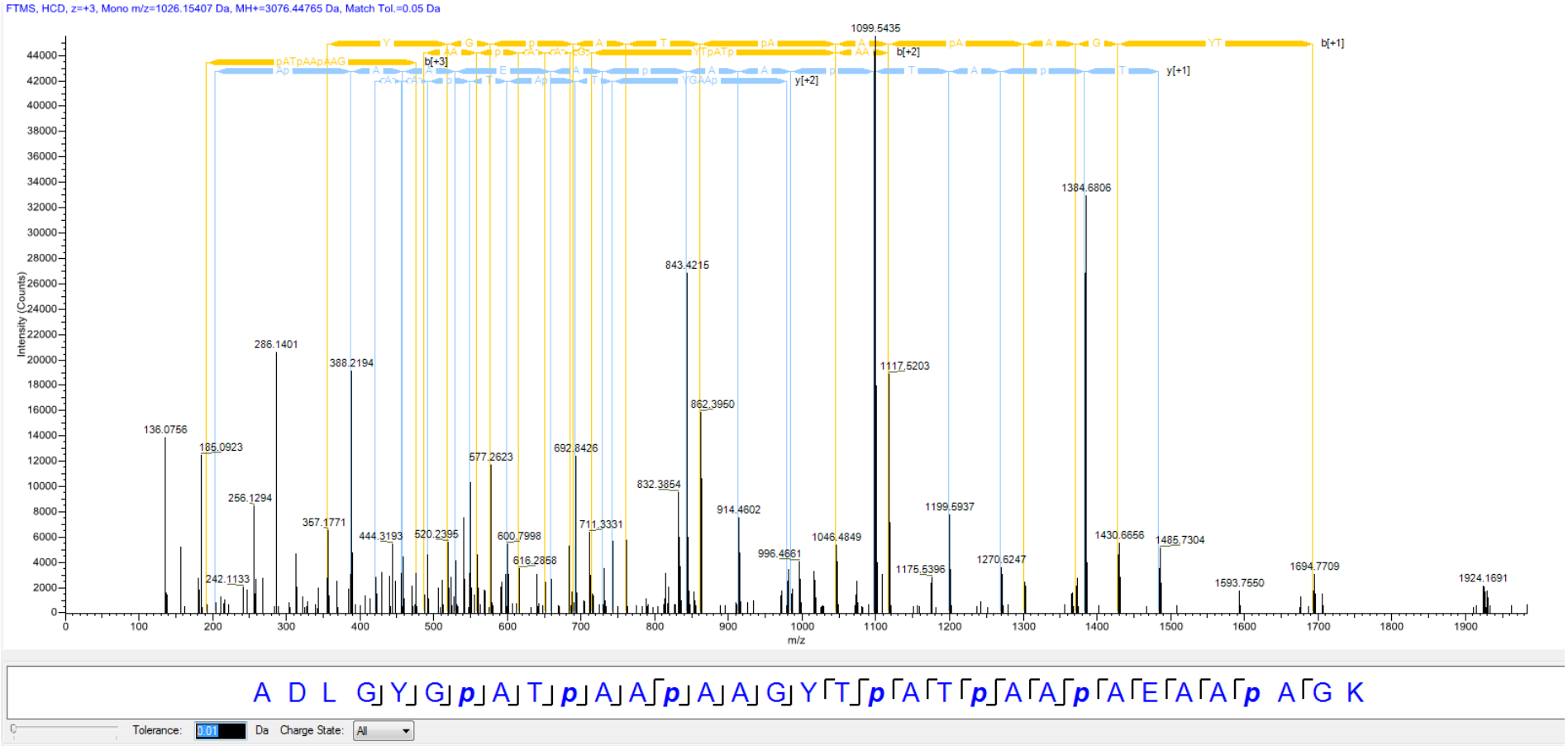
LC-ESI-MS of bariumhydroxide mediated hydrolysates of Phl p 1, Phl p 5 and an extensin enriched fraction of wild type Arabidopsis thaliana roots. A-C: Extracted ion traces of [M+H/Na]+ (m/z 264 (Hyp-Araf1), 396 (Hyp-Araf2), 528/550 (Hyp-Araf3) and 660/682 (Hyp-Araf4)) of an extensin enriched fraction derived from roots of A. thaliana (A), Phl p 5 (B) and Phl p 1 (C). Each of the Hyp-Arafn species elutes as two peaks due to the C-4 R/S stereo chemistry of Hyps. D-F: MS2 spectra of Phl p 1 derived Hyp-Araf1 (D), Hyp-Araf2 (E) and Hyp-Araf3 (F).



Supplemental figure 6A

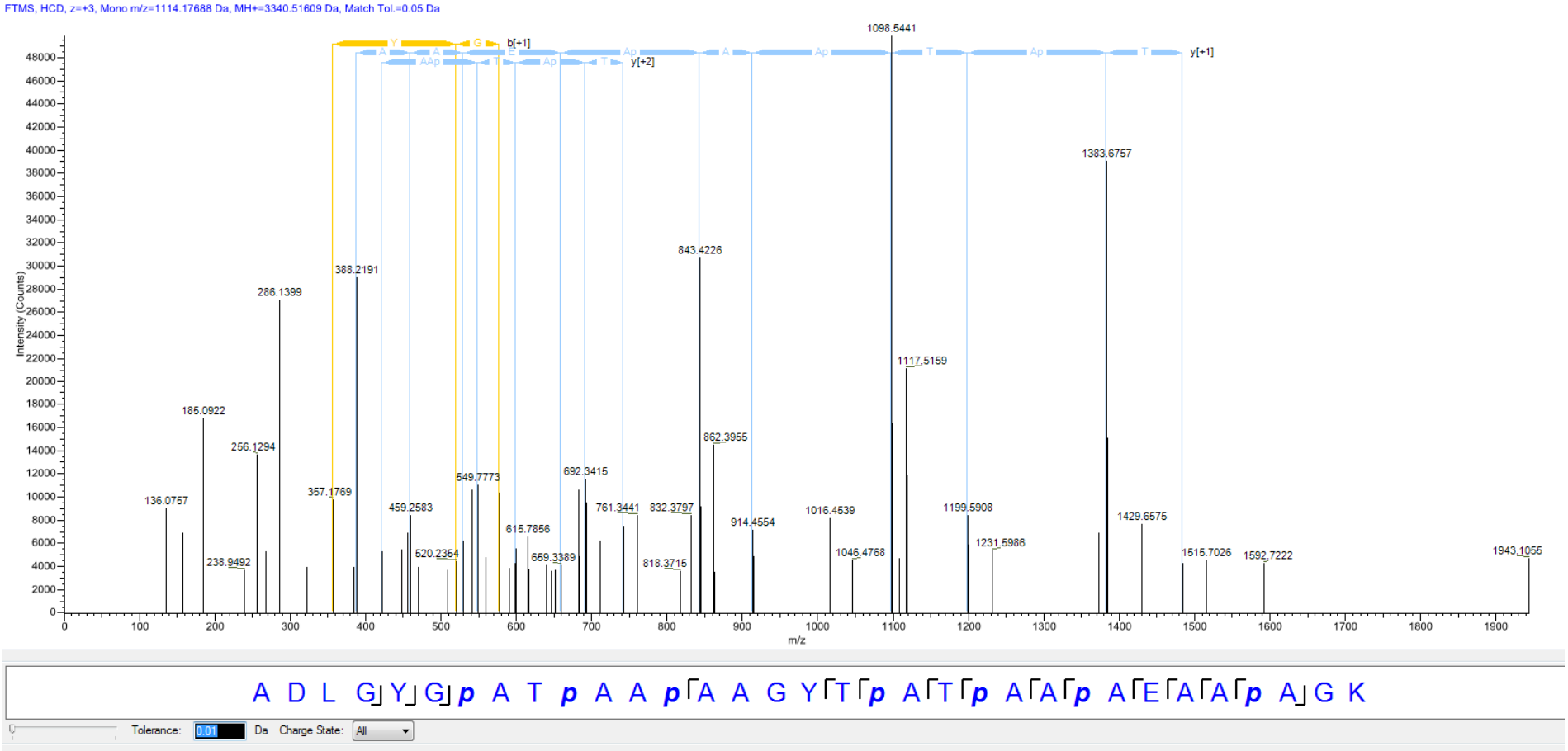
Phl p 5

HCD-MS2 of the A[26]DLGYGPATPAAPAAGYTPATPAAPAEAAPAGK[58] peptide modified by 7 Hyp residues. Hyp residues are indicated as *p*.



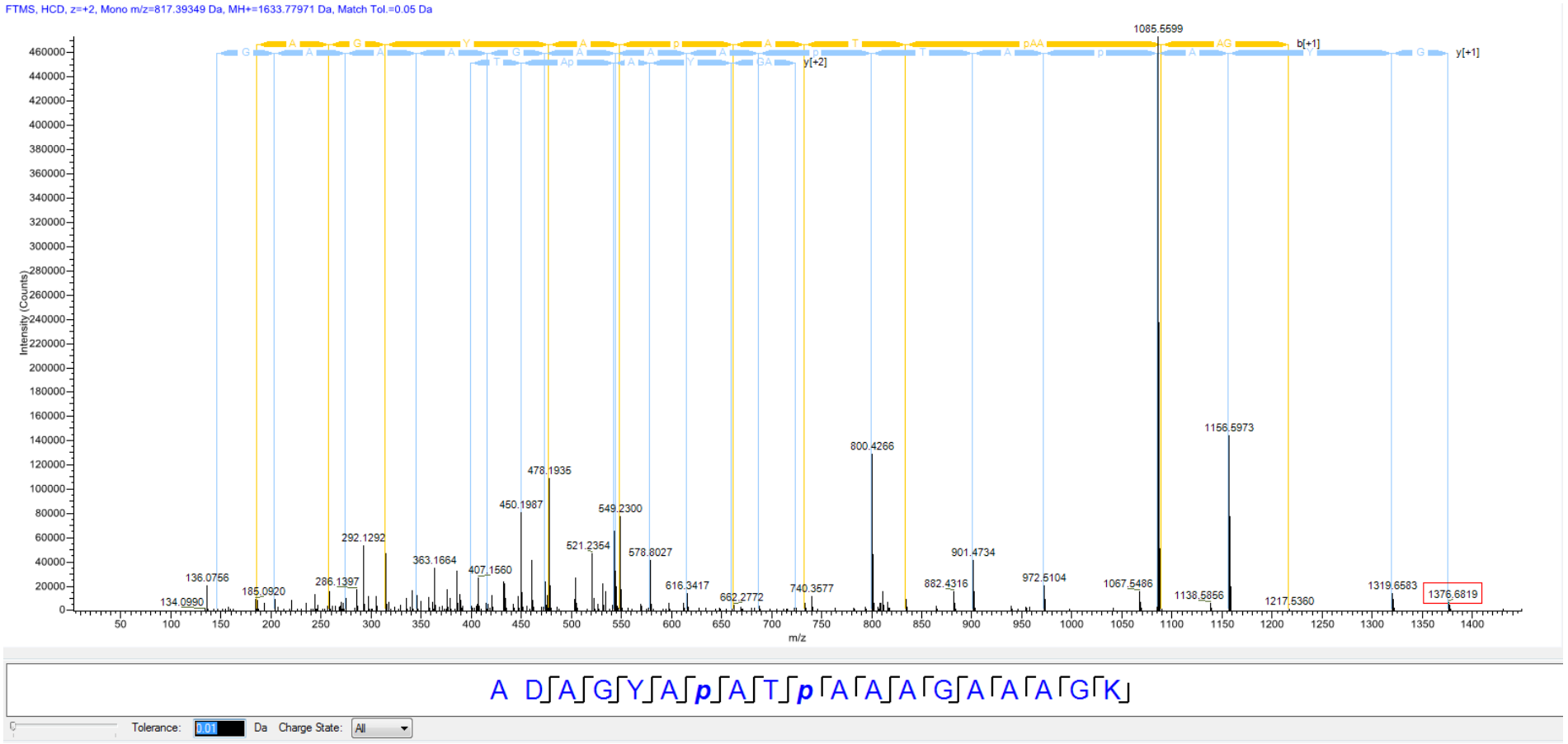
Supplemental figure 6B
Phl p 5

HCD-MS2 of the A[26]DLGYGPATPAAPAAGYTPATPAAPAEAAPAGK[58] peptide modified by 7 Hyp residues and 2 Pen sugars. The Pen modified Hyp residues have not been determined. Hyp residues are indicated as *p*.



Supplemental figure 6C
Phl p 5

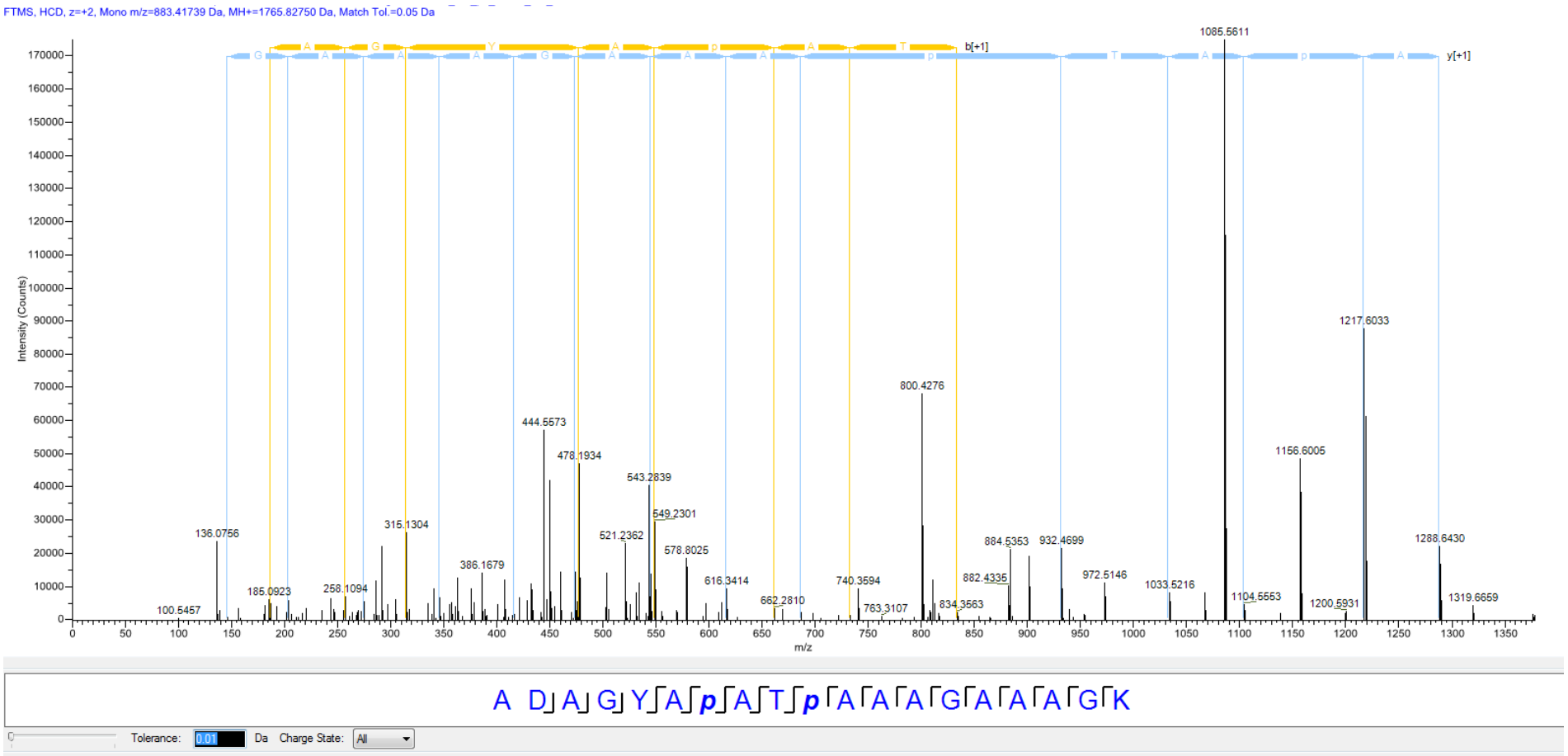
HCD-MS2 of the A[20]DAGYAPATPAAAGAAAGK[38] peptide modified by 2 Hyp residues (indicated as *p*).



Supplemental figure 6D

Phl p 5

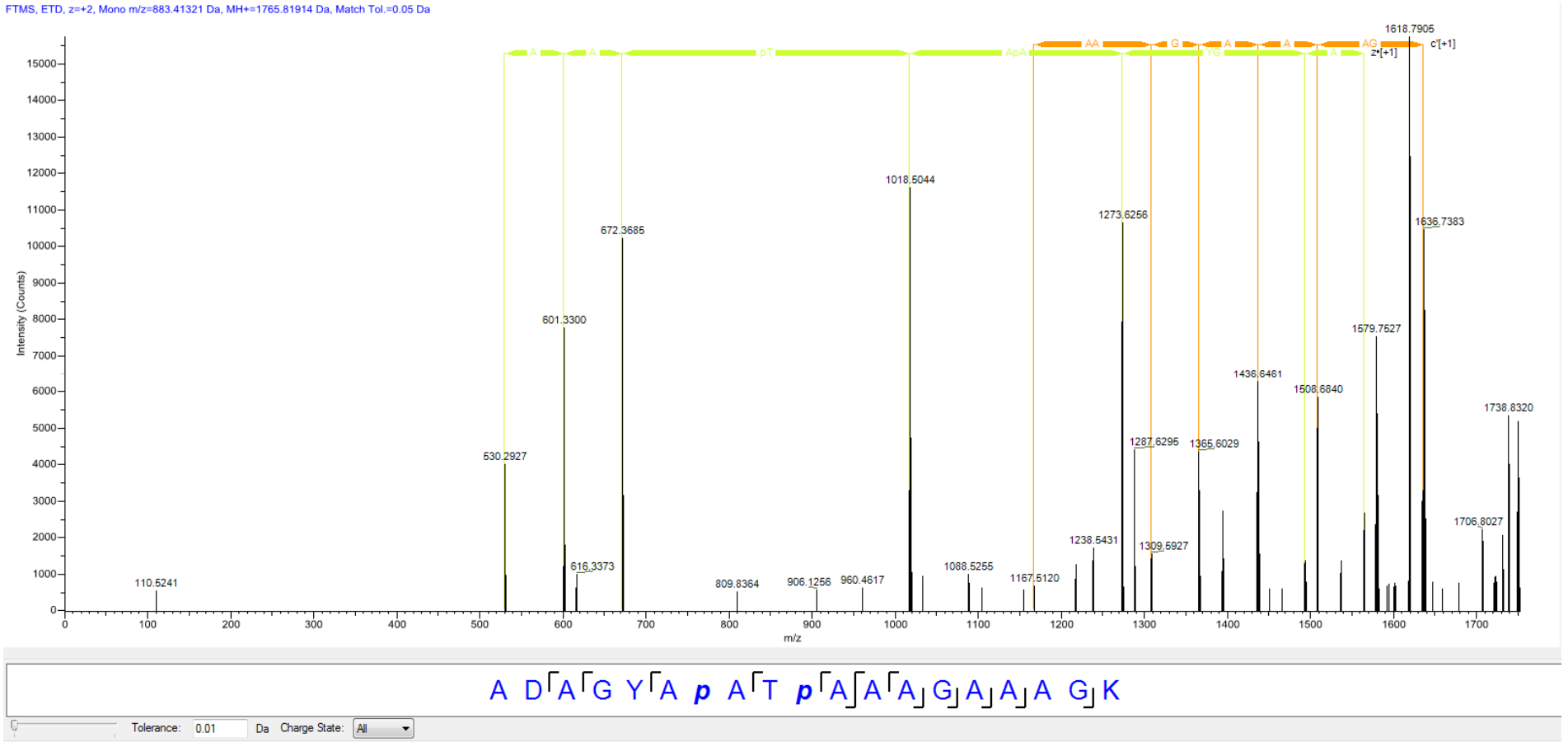
HCD-MS2 of the A[20]DAGYAPATPAAAGAAAGK[38] peptide modified by 2 Hyp residues (indicated as *p*) and one Pen sugar (site not determined).



Supplemental figure 6E

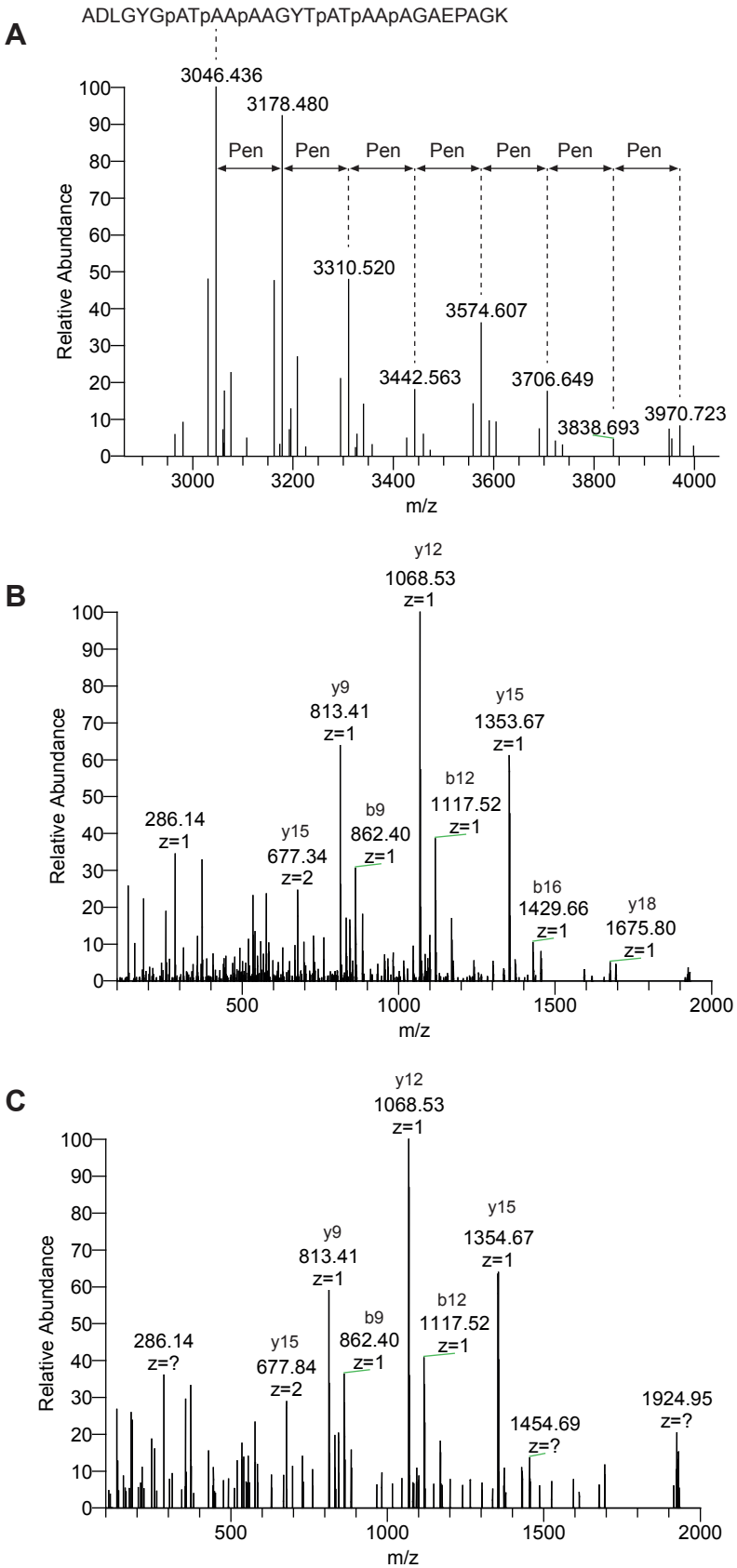
Phl p 5

ETD-MS2 of the A[20]DAGYAPATPAAAGAAAGK[38] peptide modified by 2 Hyp residues (indicated as *p*) and one Pen sugar (site mapped to Hyp[10] on the peptide sequence).



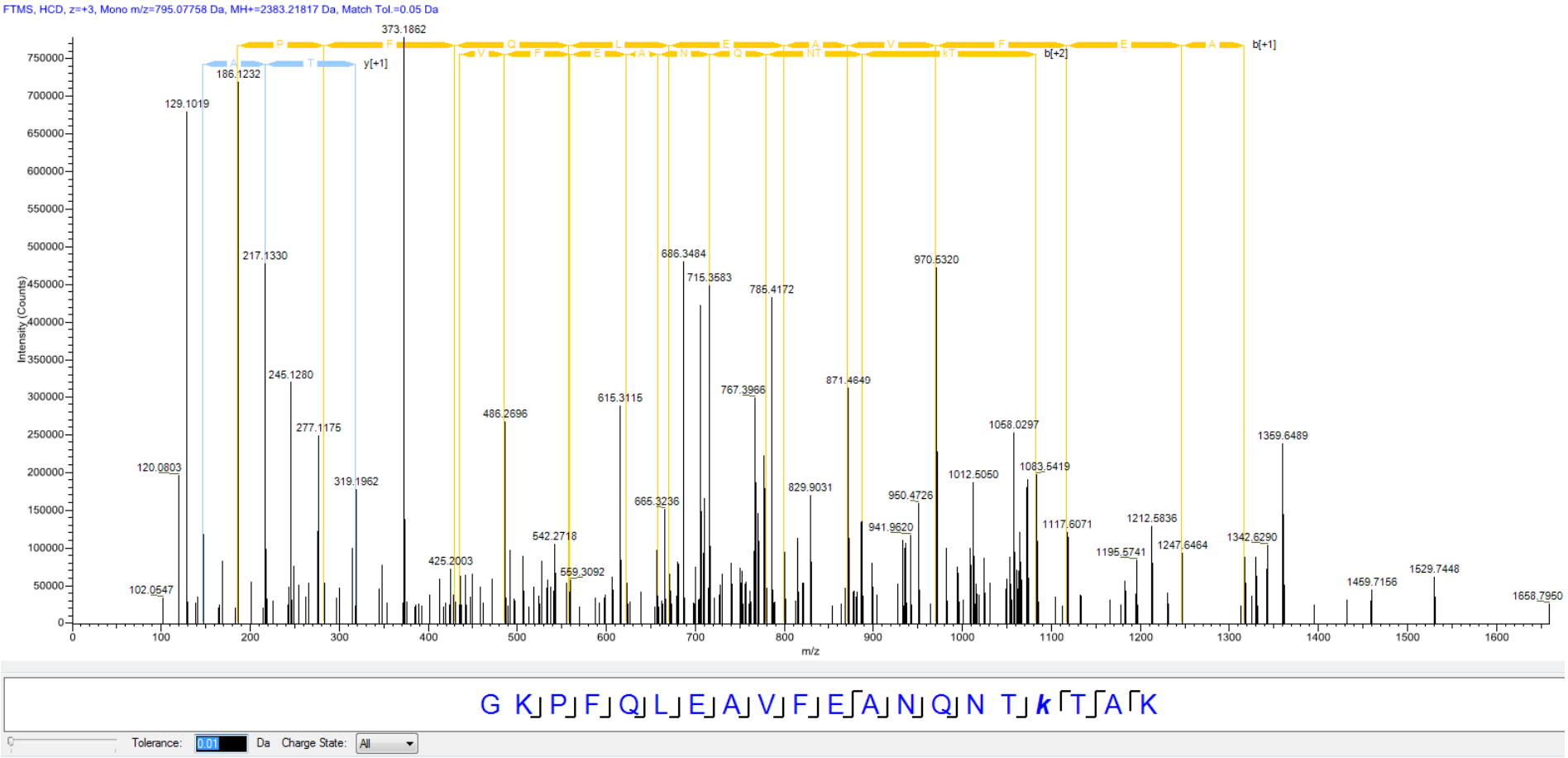
Supplemental figure 7.

(A) N-terminal peptide of Phl p 5 identified with 6 Hyp and up to 7 pentose residues.
Full MS1 spectra have been deconvoluted into their monoisotopic [M+H]+ masses by the Xtract tool.
HCD-MS2 fragmentation of the precursor ion at m/z 3442.56 and m/z 3046.43, corresponding to the A^{26}DLGYGPATPAAPAAGYTPATPAAPAGAEPAGK^{58} peptide modified by 7Hyp;Pen3 (B) and 7Hyp (C).
Hyp residues are indicated as p. The b- and y-ion fragments verify the peptide identity.



Supplemental figure 8A
Der p 2

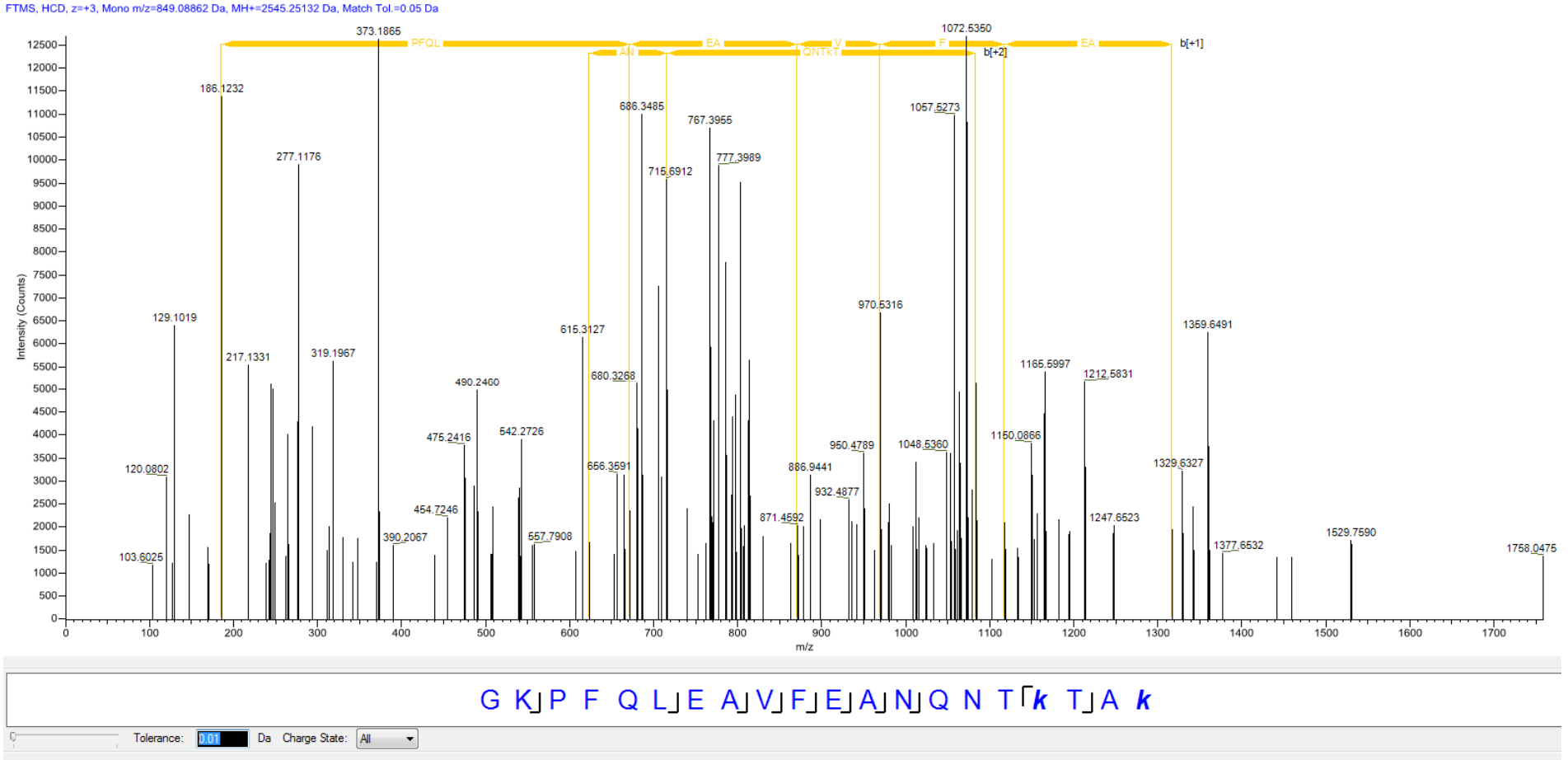
HCD-MS2 of the G[49]KPFQLEAVFEANQNTKTAK[68] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 8B

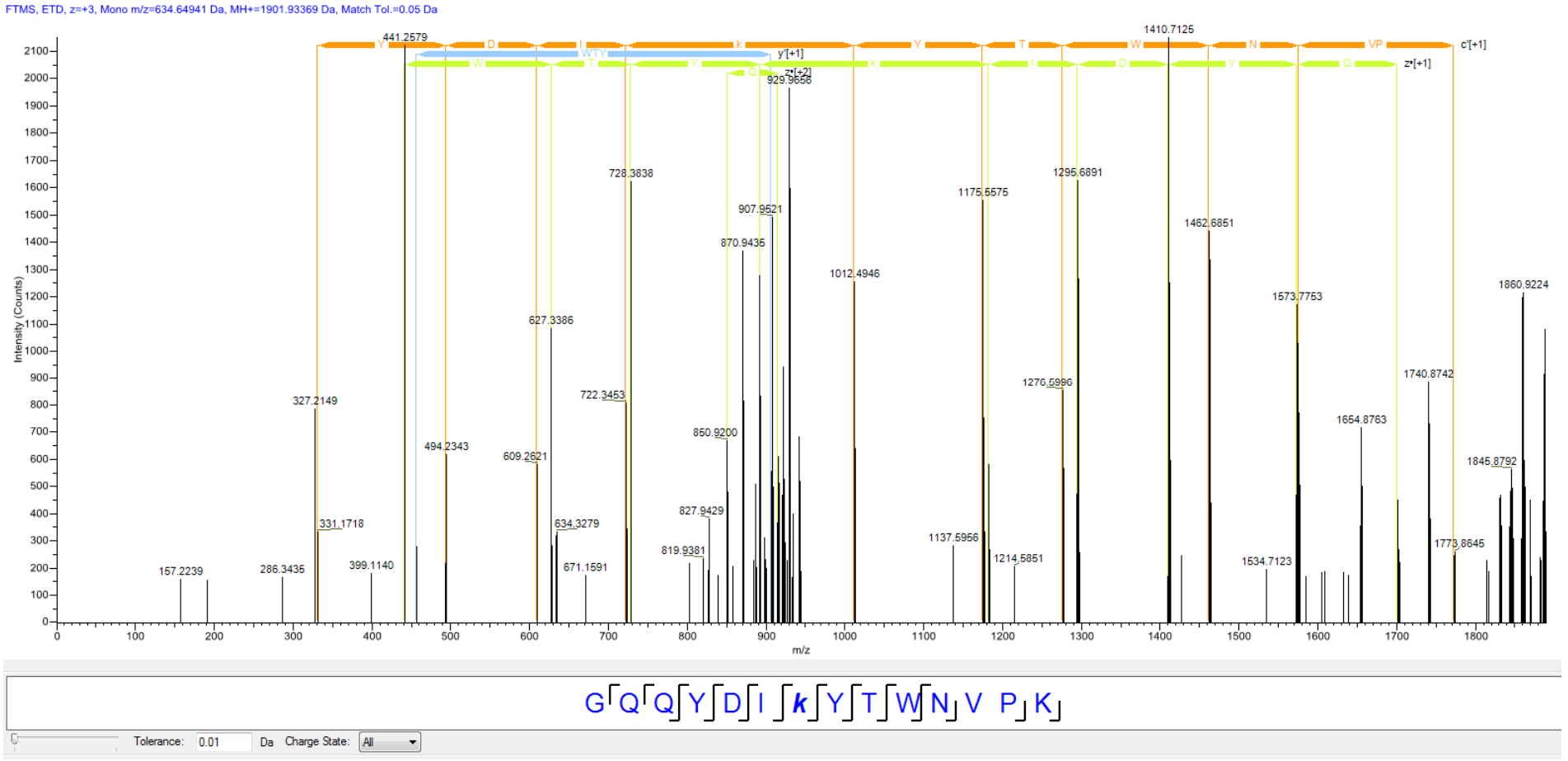
Der p 2

HCD-MS2 of the G[49]KPFQLEAVFEANQNTKTAK[68] peptide modified by a two Hex on Lys. The site or glycan heterogeneity has not been determined.



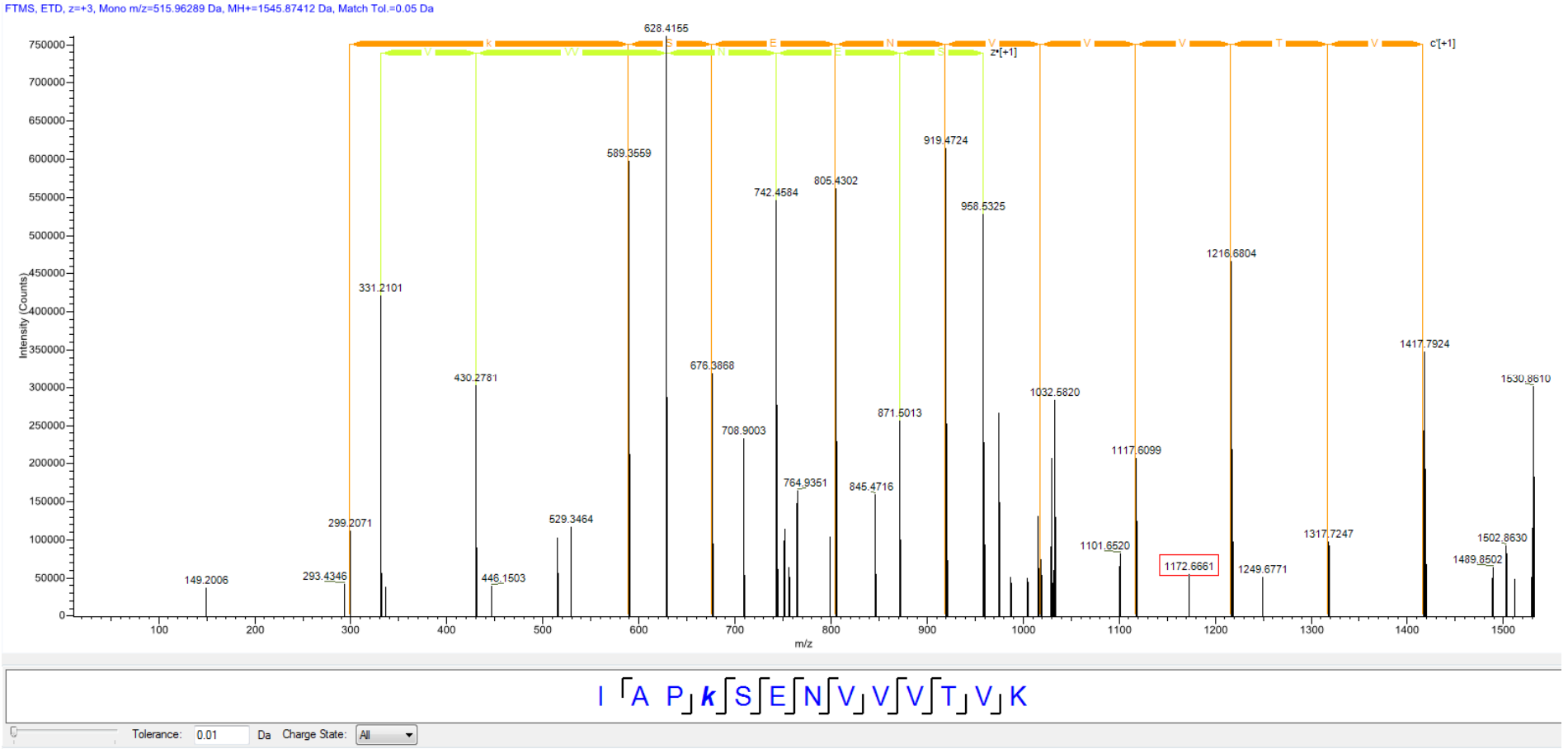
Supplemental figure 8C
Der p 2

ETD-MS2 of the G[100]QQYDIKYTWNVPK[113] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 8D
Der p 2

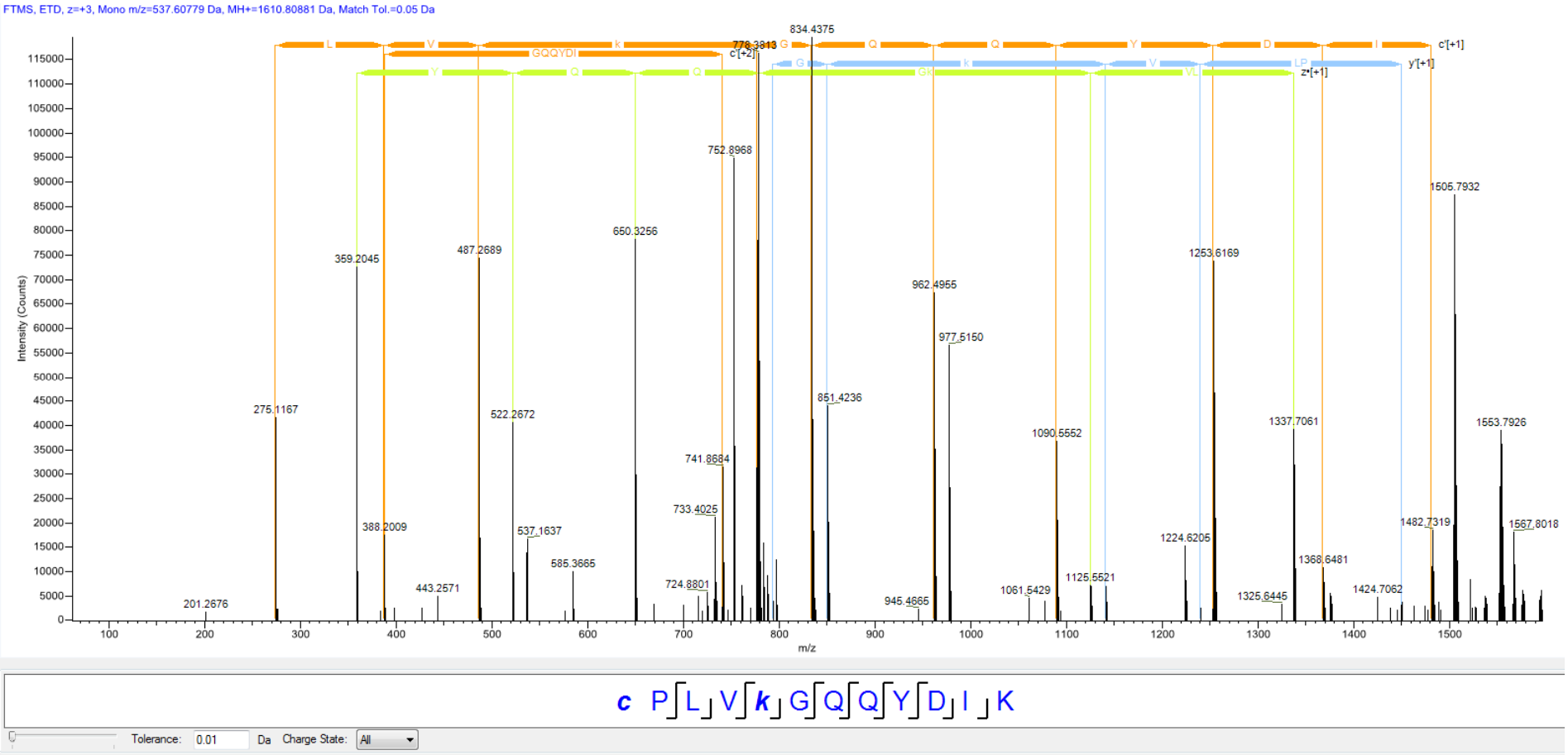
ETD-MS2 of the I[114]APKSENVVVTVK[126] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 9A

Der f 2

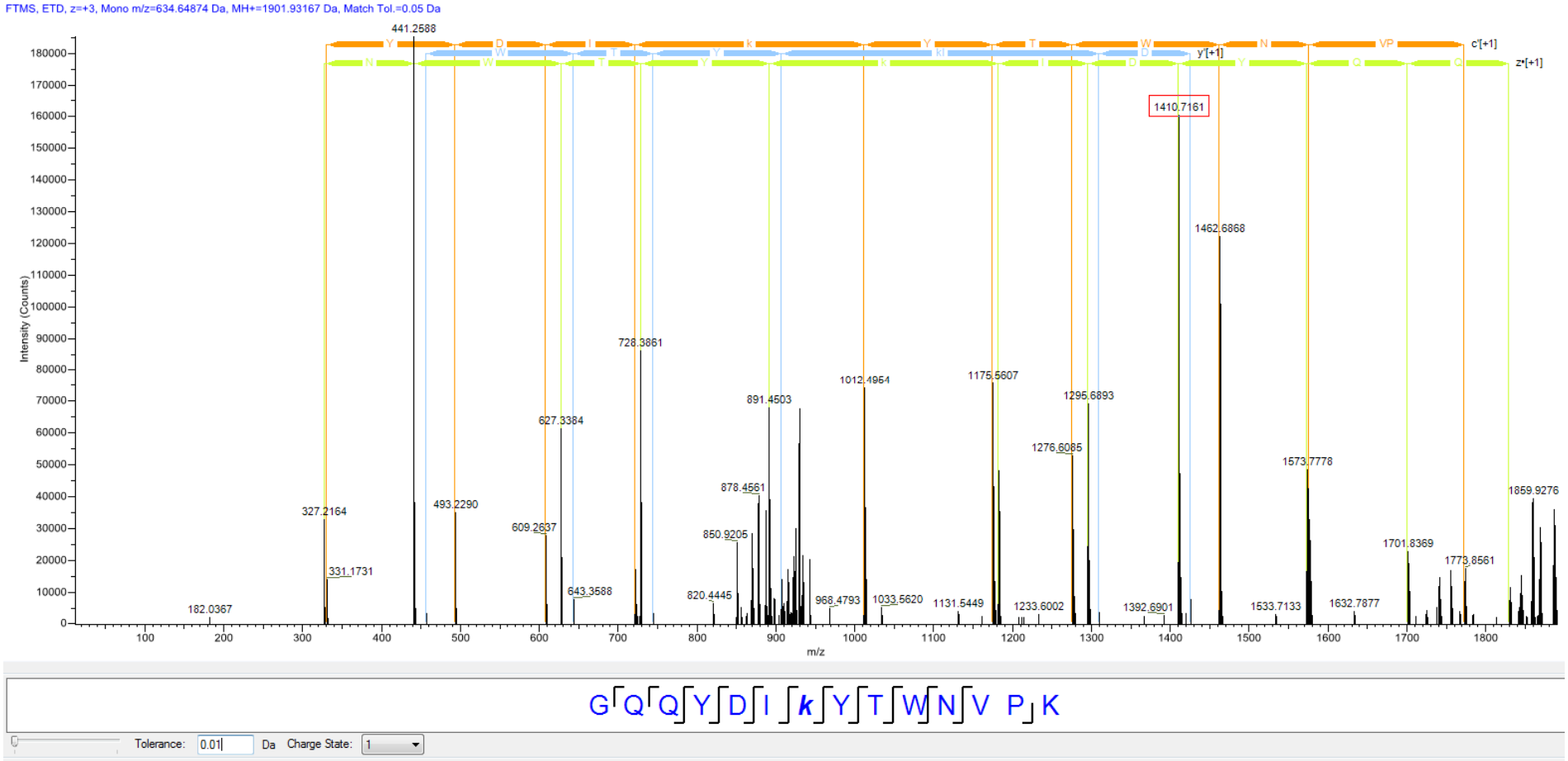
ETD-MS2 of the C[95]PLVKGQQYDIK[106] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 9B

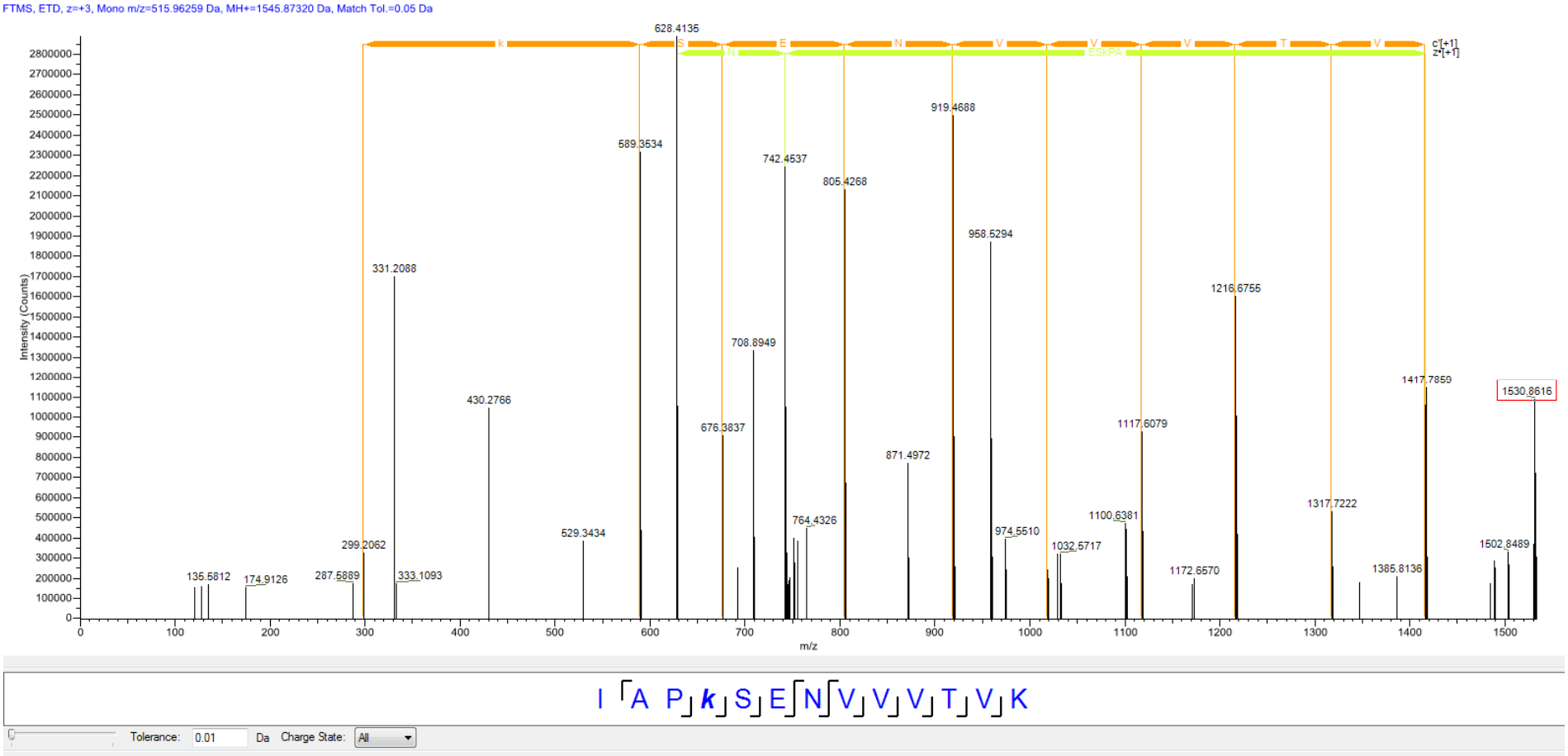
Der f 2

ETD-MS2 of the G[100]QQYDIKYTWNVPK[113] peptide modified by a single Hex on Lys (indicated as *k*).



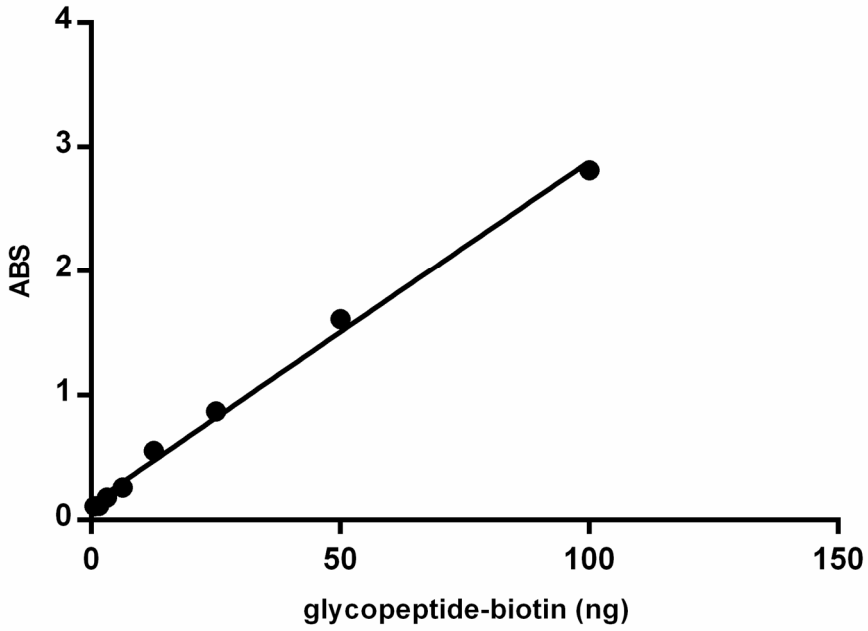
Supplemental figure 9C
Der f 2

ETD-MS2 of the I[114]APKSENVVVTVK[126] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 10.

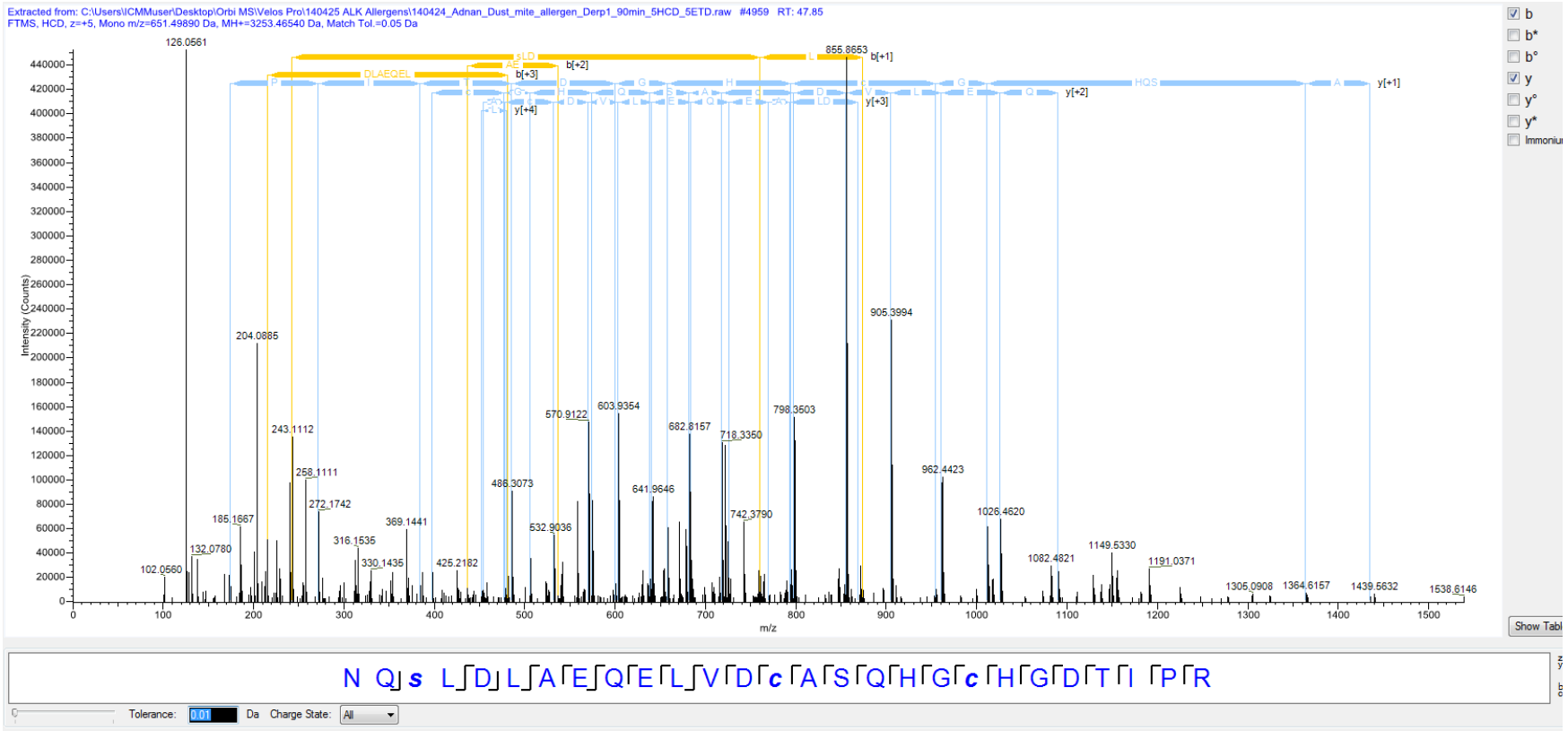
ELISA standard curve of biotinylated O-mannosylated peptide used to determine the carbohydrate content of Der p 2 and Der f 2. O-mannosylated peptide standard, Der p 2 and Der f 2 were specifically biotinylated on the hexose residue through periodate oxidation followed by aldehyde-hydrazide chemistry to incorporate the biotin. Amount (molar ratio) of Der p2 and Der f2 containing glycans was subsequently determined by ELISA.



Der p2	34%
Der f2	42%
Neg control	1%

Supplemental figure 11.

Annotated HCD-MS2 spectrum of the tryptic NQSLDLAEQELVDCASQHGCHGDTIPR peptide of Der p 1 modified by 1 HexNAc. The b2 fragment annotation is incorrect. (B) ETD-MS2 of the same peptide mapping the HexNAc modification to N150. (C) HCD-MS2 spectrum of the same peptide modified by 2 HexNAc residues. The b2 fragment annotation is incorrect.



Supplemental figure 11A

Der p 1

N^{150}QSLDLAEQELVDCASQHGCHGDTIPR^{176} peptide + 1HexNAc

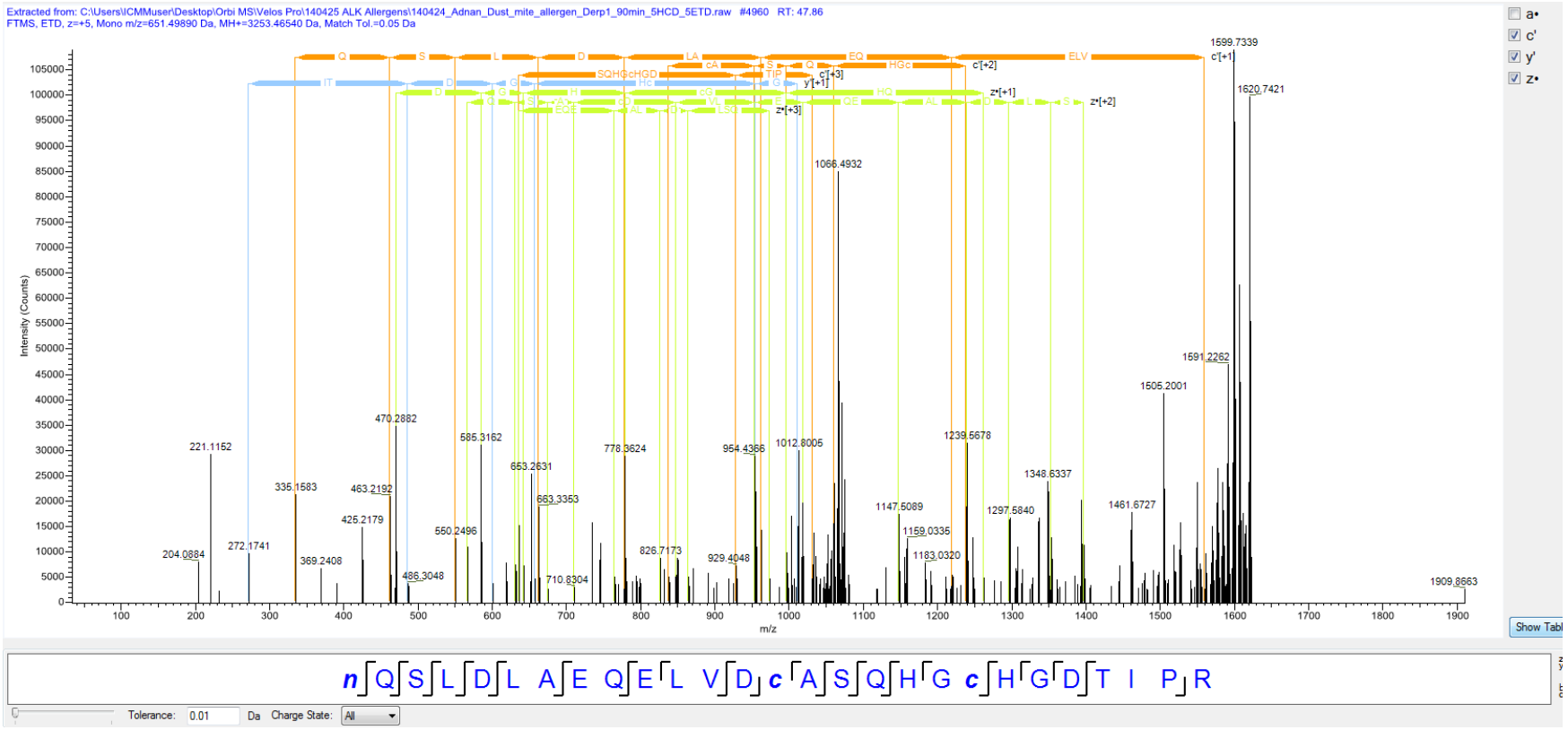
HCD-MS2

Supplemental figure 11B

Der p 1

N[150]QSLDLAEQELVDCASQHGCHGDTIPR[176] peptide + 1HexNAc (Asn150)

ETD-MS2

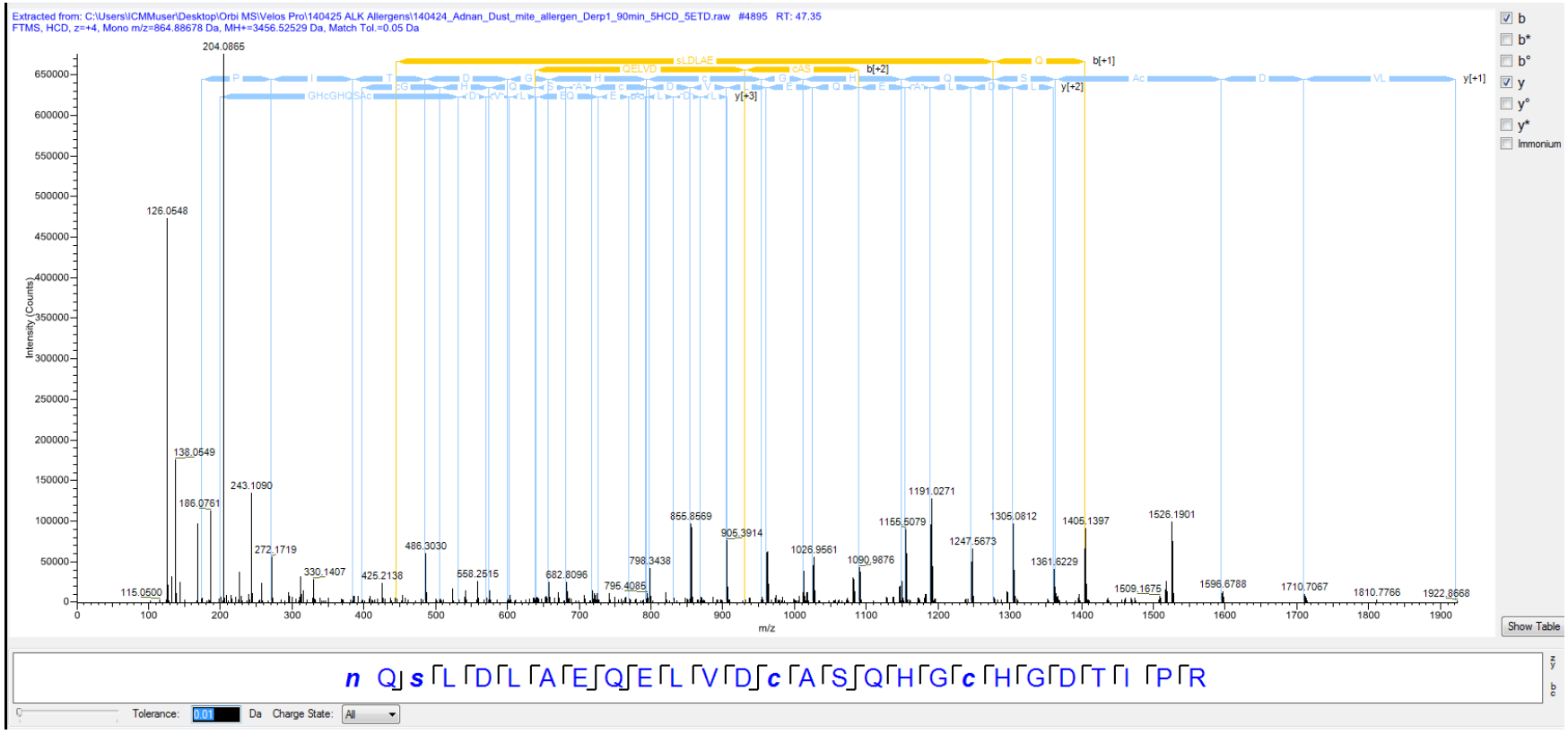


Supplemental figure 11C

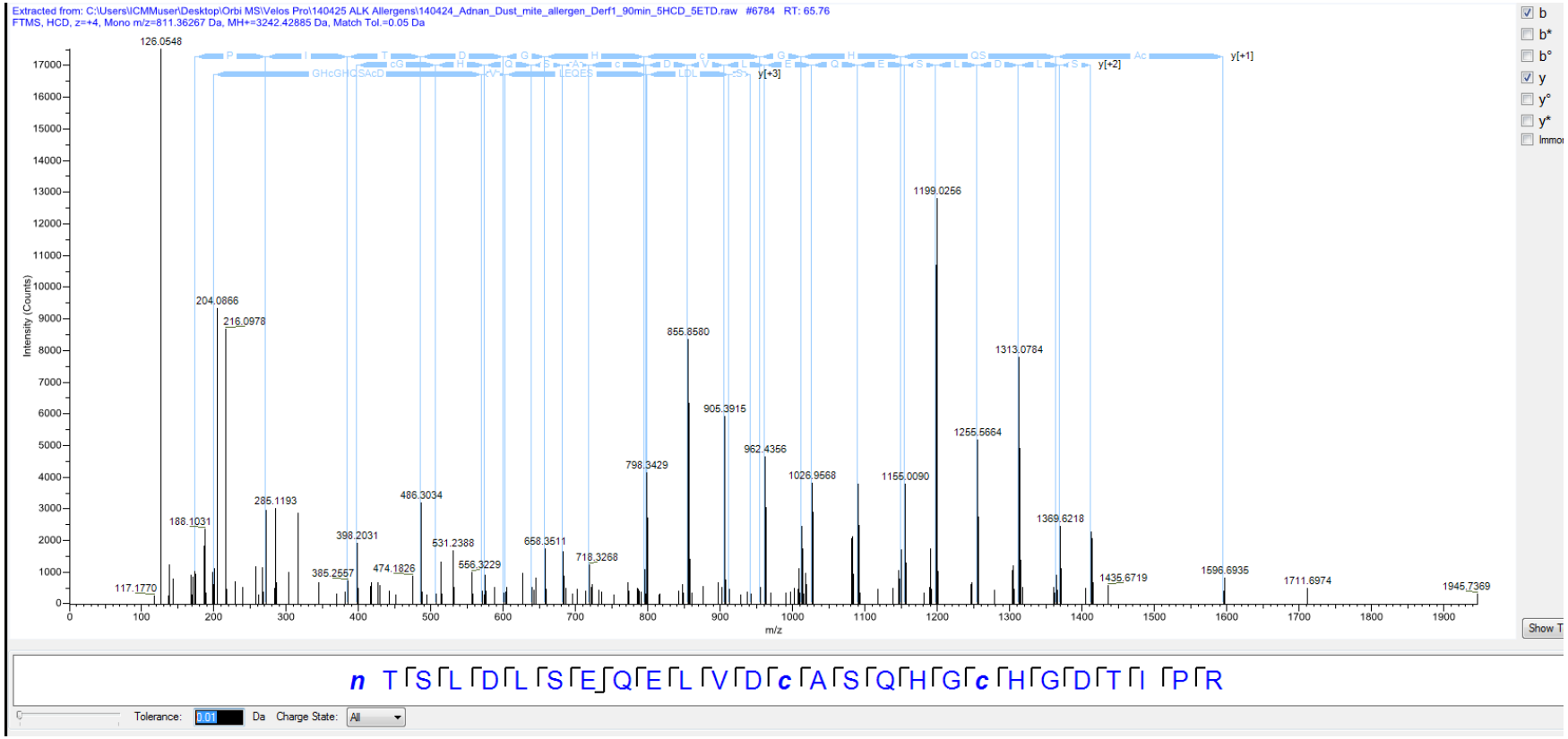
Der p 1

N[150]QSLDLAEQELVDCASQHGCHGDTIPR[176] peptide + 2HexNAc

HCD-MS2



Supplemental figure 12.
Annotated HCD-MS2 spectra of the tryptic NTSLDLSEQELVDCASQHGCHGDTIPR peptide of Der f 1 modified by (A) 1 HexNAc and (B) 2 HexNAc residues. The b2 and y25 fragment annotations are incorrect.



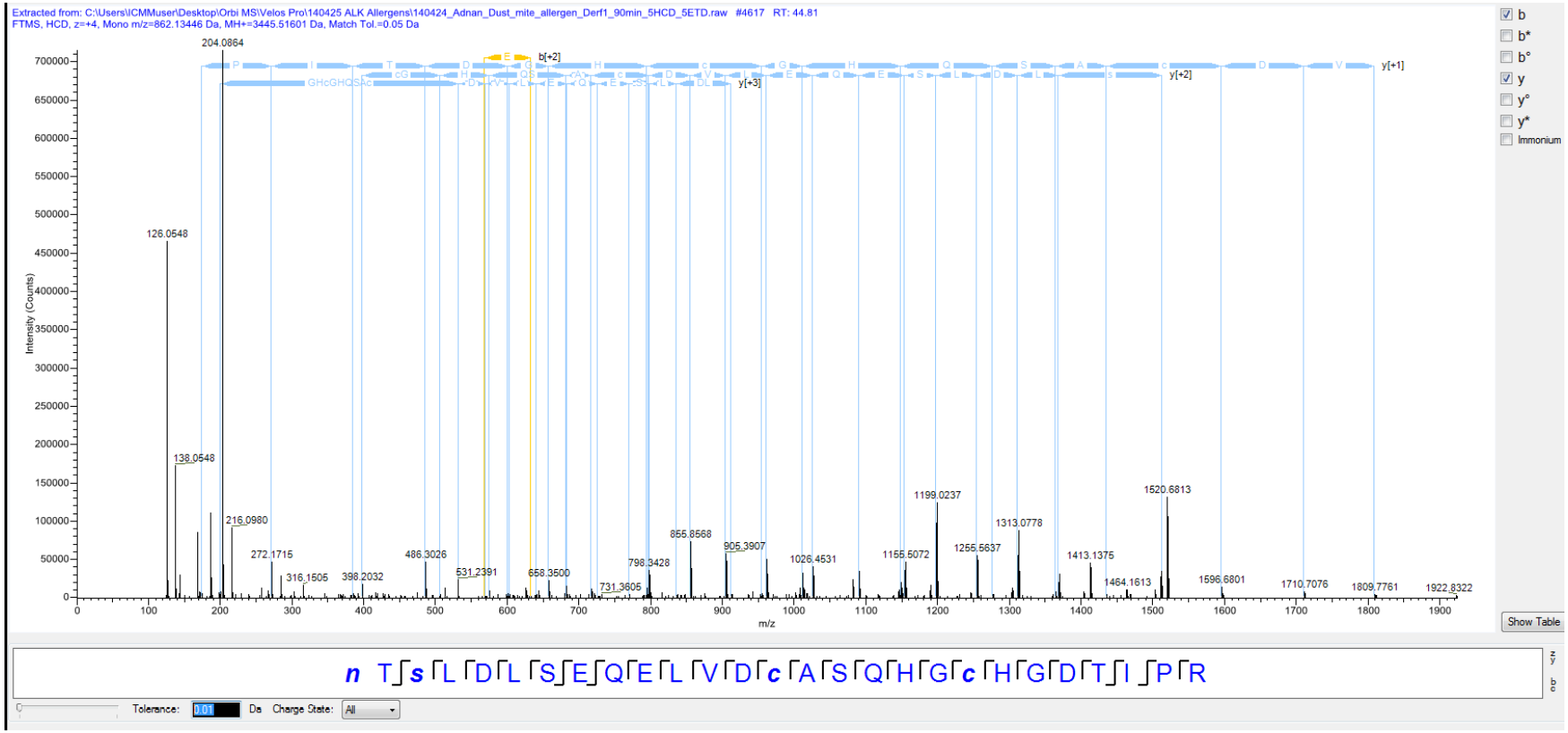
Supplemental figure 12A
Der f 1
N^{151}TSLDLSEQELVDCASQHGCHGDTIPR^{177} peptide + 1HexNAc
HCD-MS2

Supplemental figure 12B

Der f 1

N[151]TSLDLSEQELVDCASQHGCHGDTIPR[177] peptide + 2HexNAc

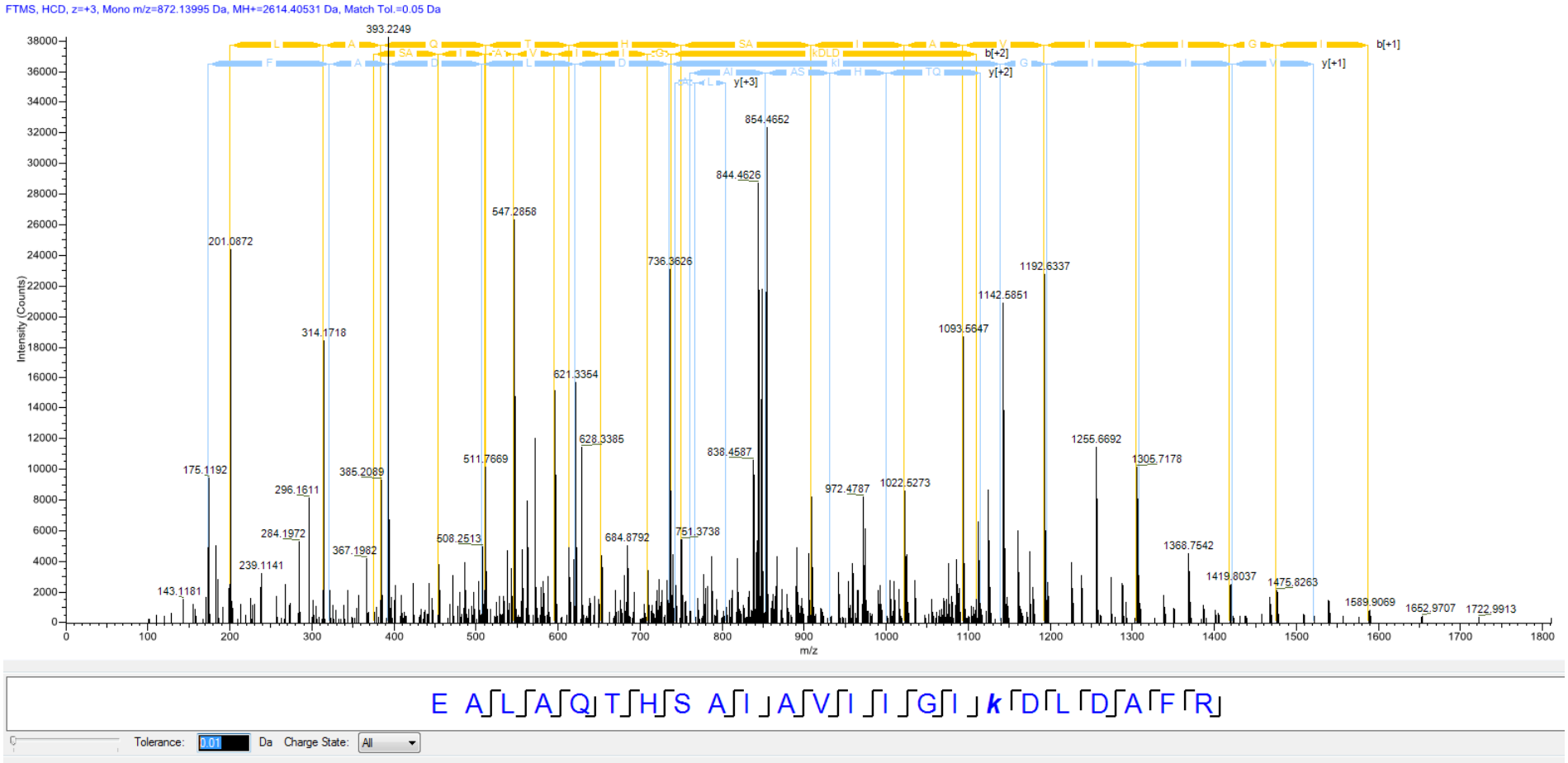
HCD-MS2



Supplemental figure 13A

Der p 1

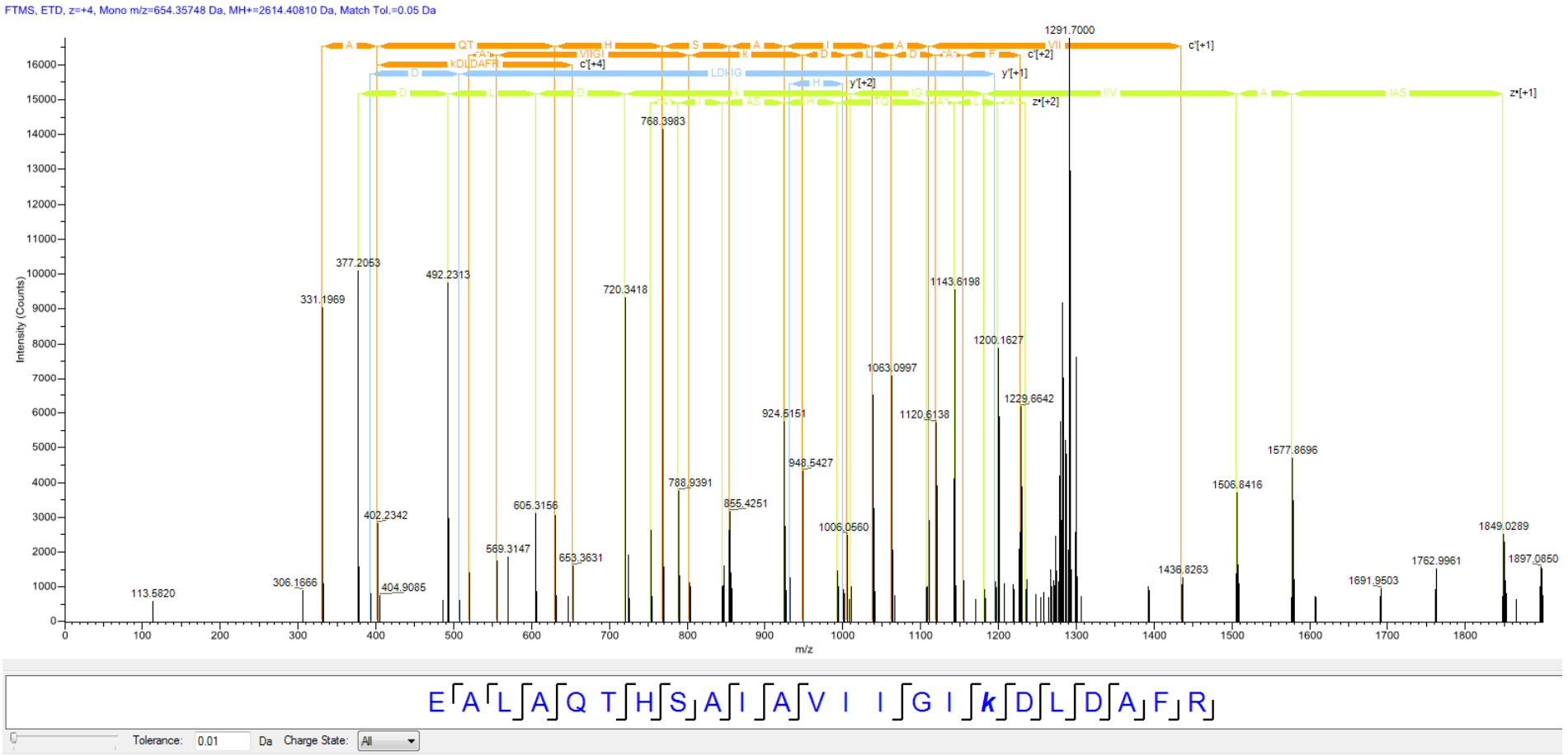
HCD-MS2 of the E[227]ALAQTHSAIAVIIGIKDLDAFR[249] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 13B

Der p 1

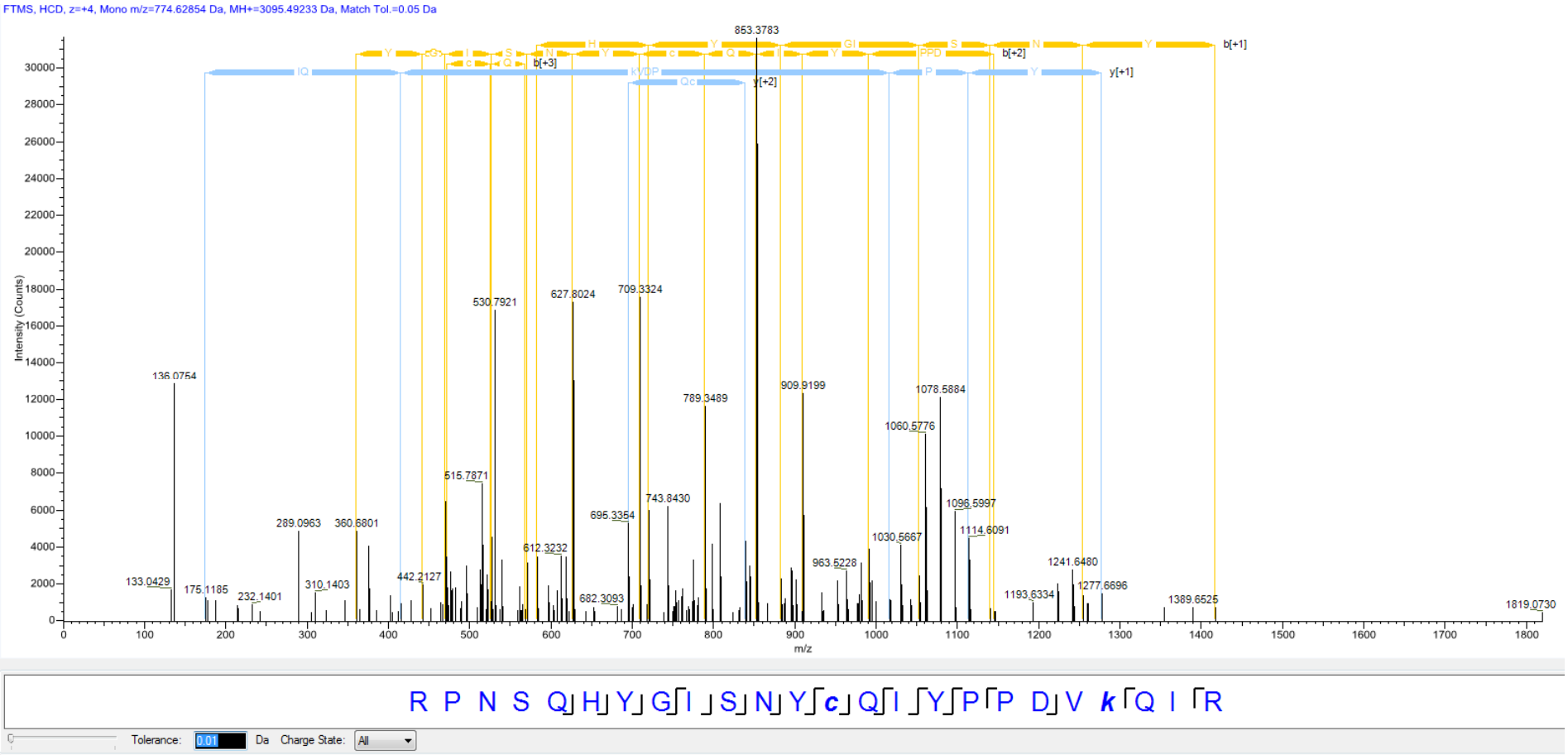
ETD-MS2 of the E[227]ALAQTHSAIAVIIGIKDLDAFR[249] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 13C

Der f 1

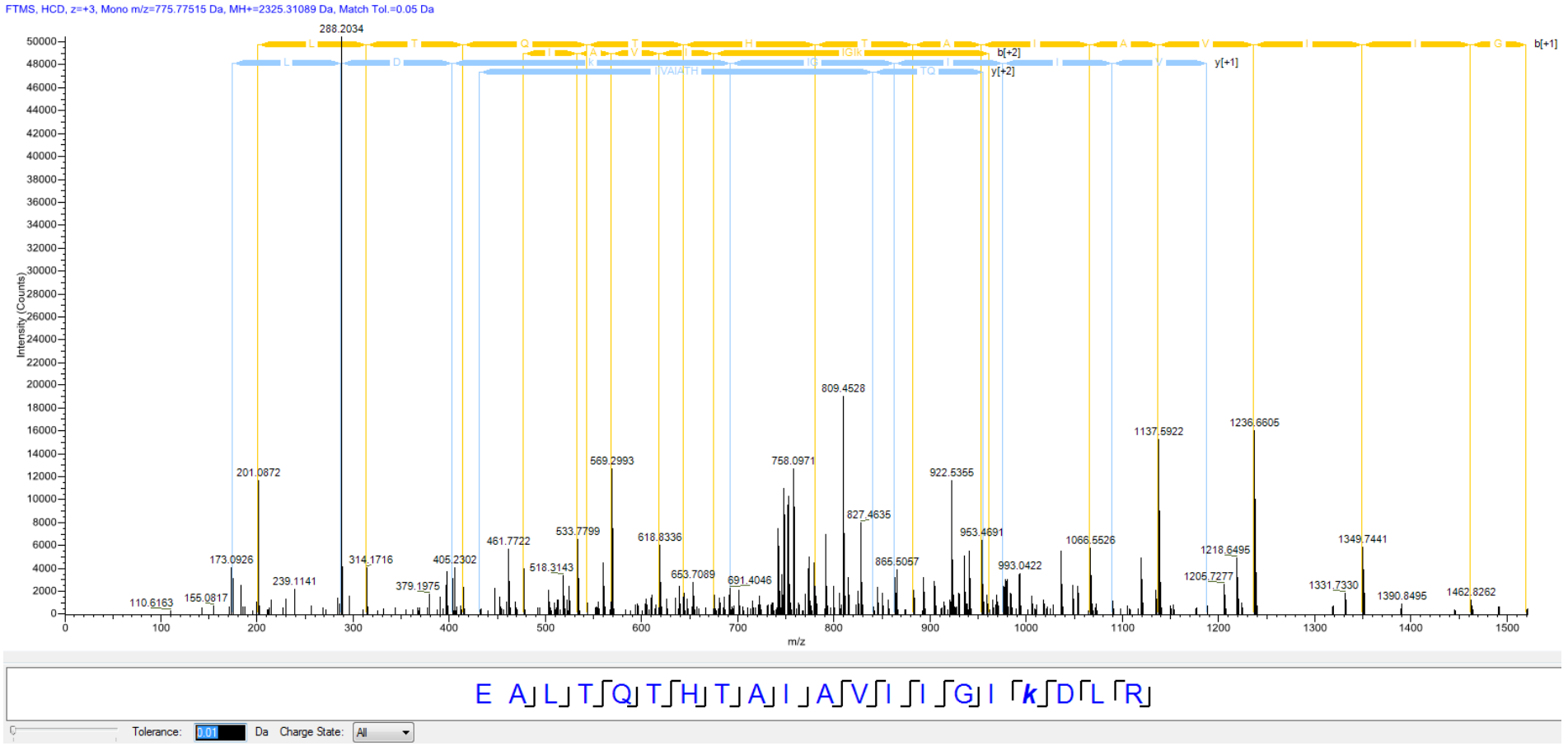
HCD-MS2 of the R[204]PNSQHYGISNYCQIYPPDVKQIR[227] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 13D

Der f 1

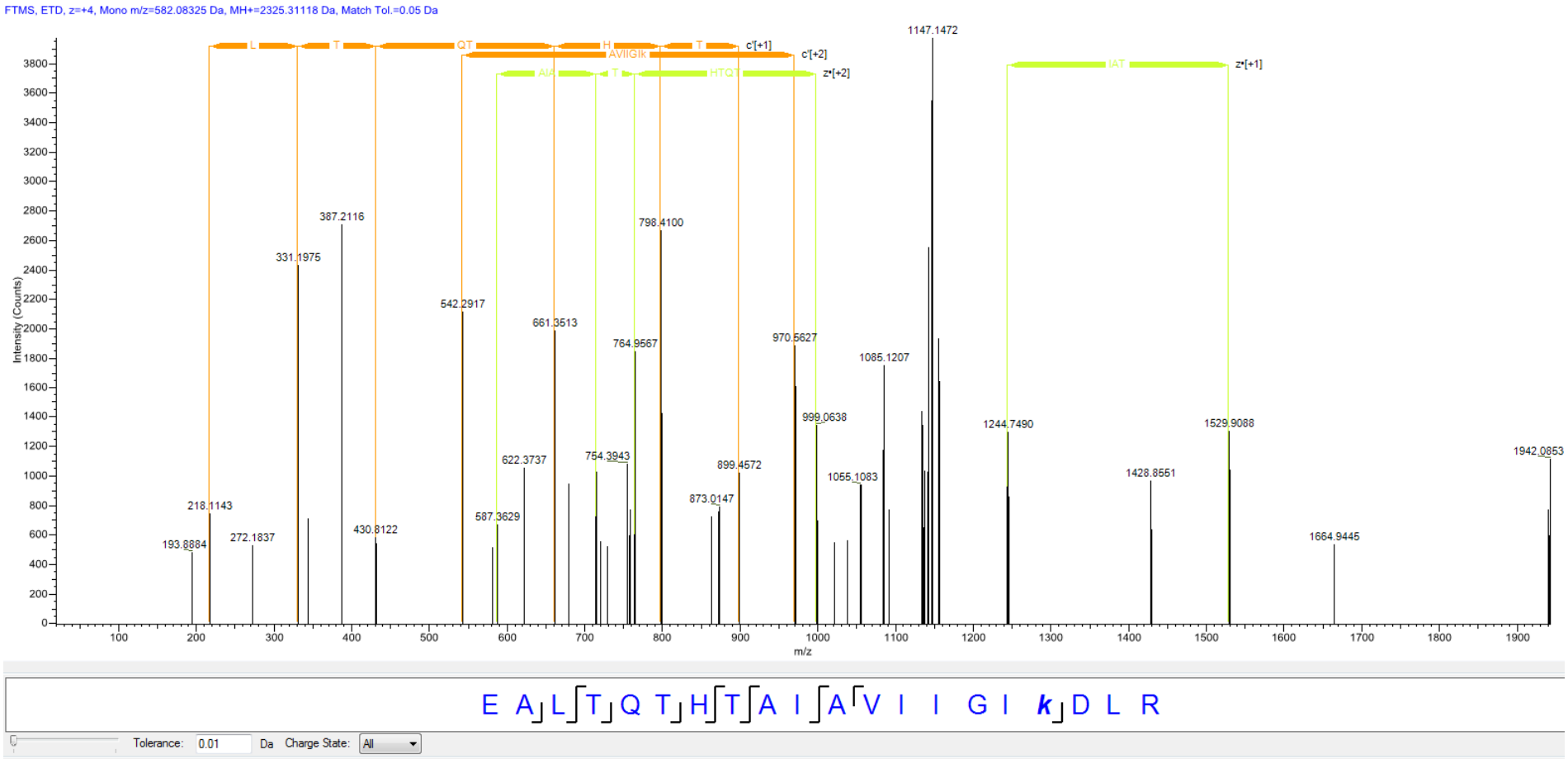
HCD-MS2 of the E[228]ALTQTHTAIAVIIGIKDLR[247] peptide modified by a single Hex on Lys (indicated as *k*).



Supplemental figure 13E

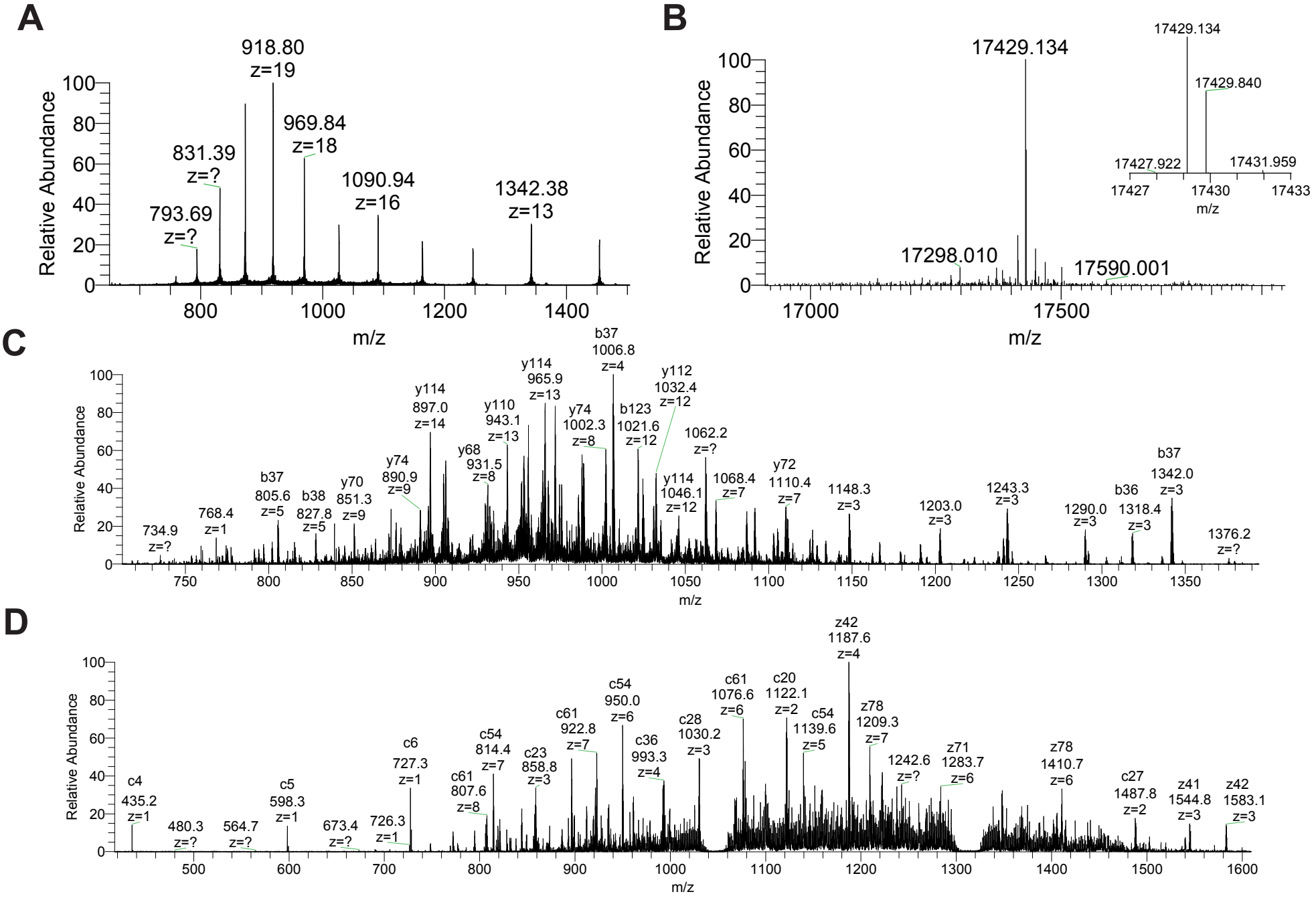
Der f 1

ETD-MS2 of the E[228]ALTQTHTAIAVIIGIKDLR[247] peptide modified by a single Hex on Lys (indicated as *k*).



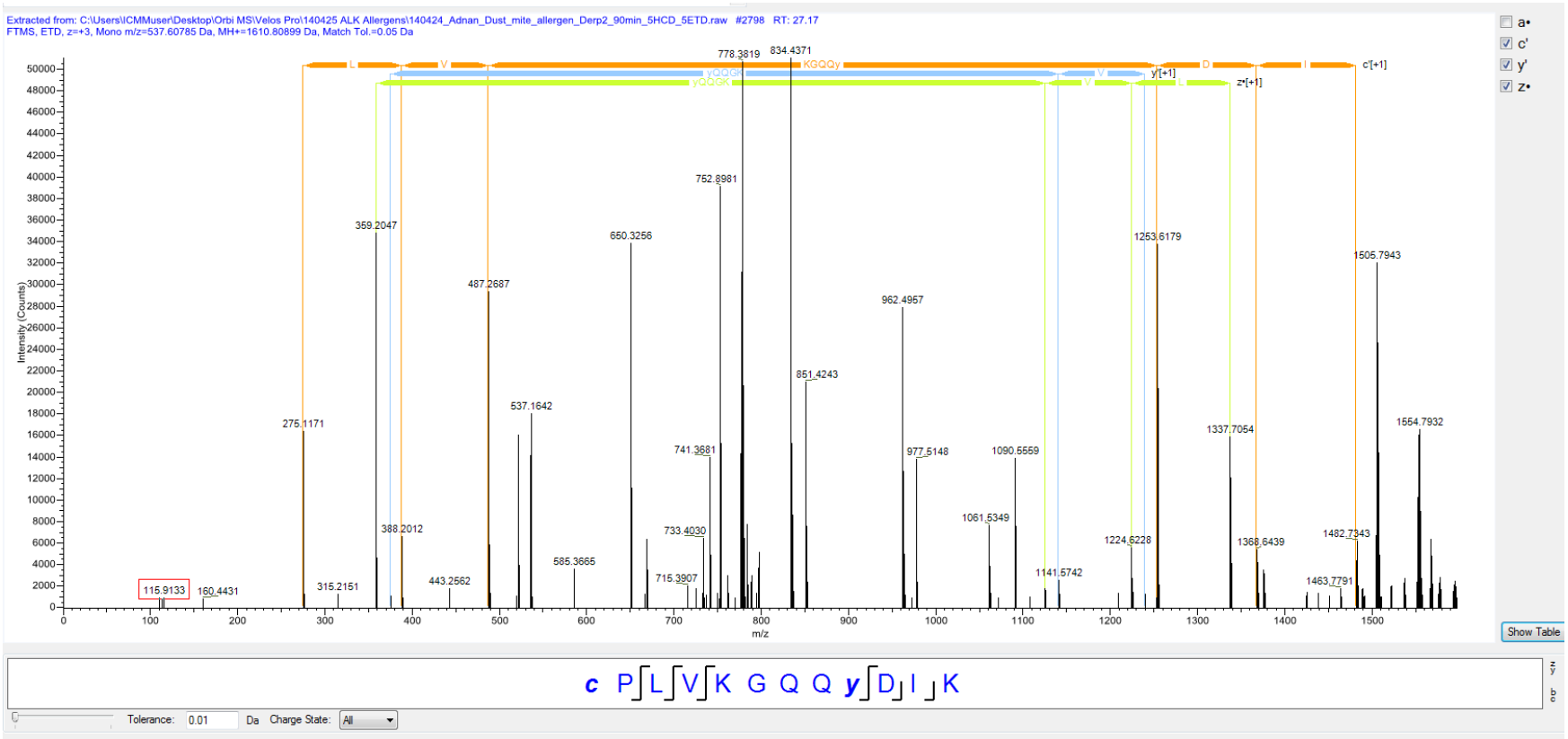
Supplemental figure 14.

Top-down FTMS of Bet v 1 allergen. (A) Charge-state distribution following direct infusion and full MS1 scan at 100000 resolving power at m/z 400. (B) Deconvoluted MS1 spectra showing the monoisotopic masses of the two most abundant precursor ions (insert). (C) HCD fragmentation of the intact Bet v 1 allergen. (D) ETD fragmentation of the intact Bet v 1 allergen.



Supplemental figure 15.

ETD-MS2 analysis of Der p 2 and Der f 2 (shared) peptide CPLVKGQQYDIK modified by 1 hexose.
(A) Annotation of the ETD spectra by Proteome Discoverer 1.4 using variable modification at Ser/Thr/Tyr residues.
(B) Annotation of the ETD spectra by Proteome Discoverer 1.4 using variable modification at Lys/Ser/Thr/Tyr residues.



Supplemental figure 15A
Der p 2 and Der f 2
C^{95}PLVKGQQYDIK^{106} peptide + 1Hex ETD-MS2
Variable Hex modification at Ser/Thr/Tyr

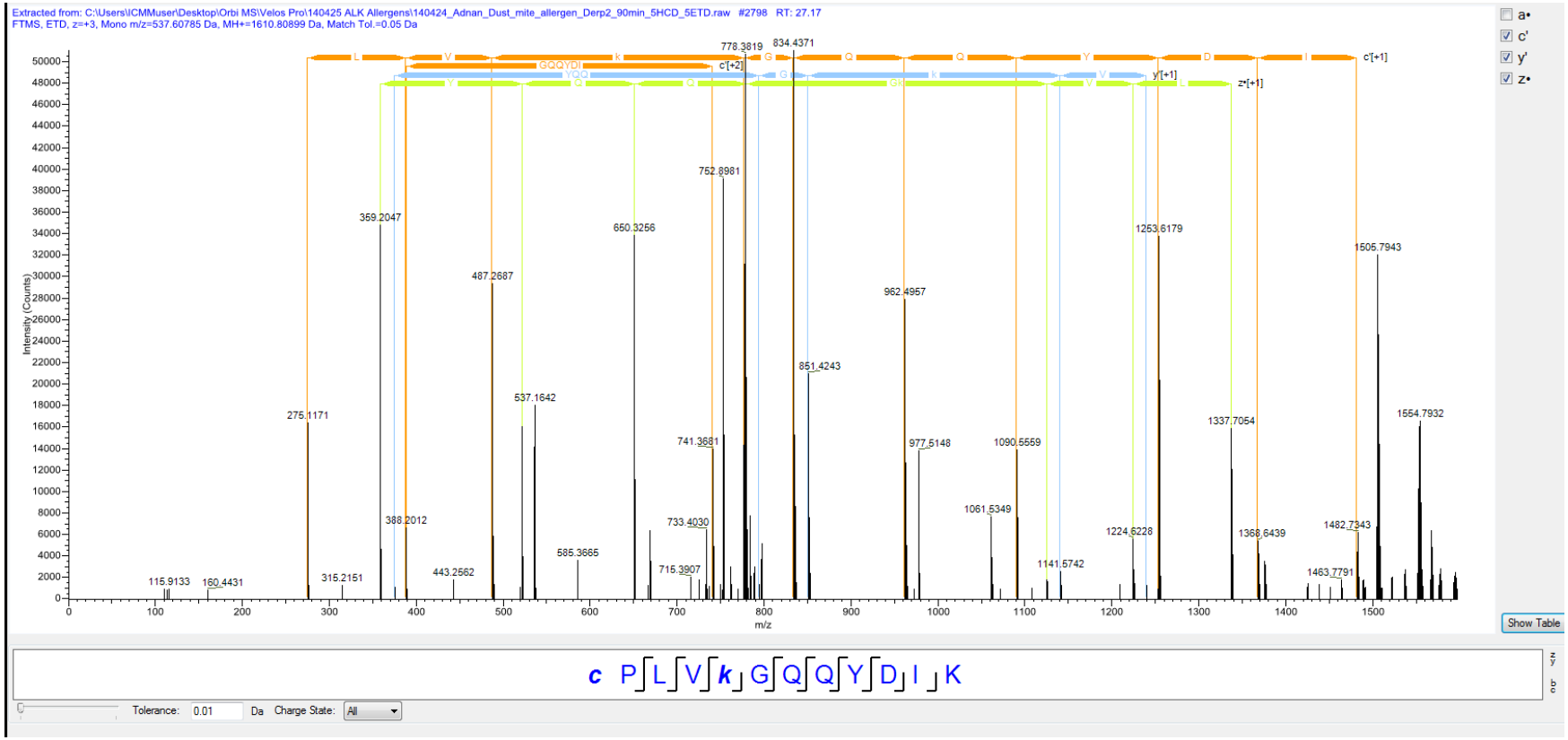
Supplemental figure 15B

Der p 2 and Der f 2

C^{95}PLVKGQQYDIK^{106} peptide + 1Hex

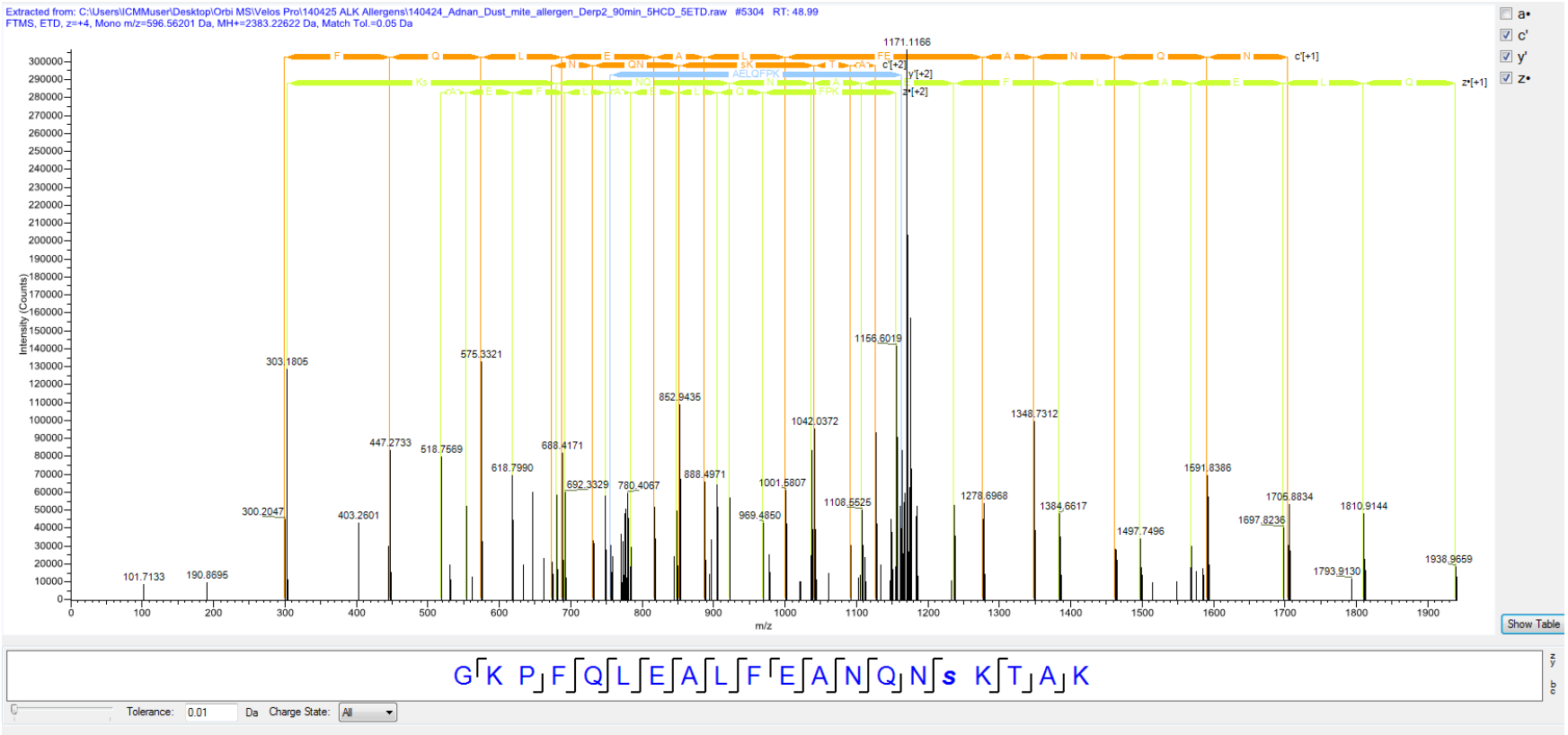
ETD-MS2

Variable Hex modification at Lys/Ser/Thr/Tyr



Supplemental figure 16.

ETD-MS2 analysis of Der p 2 (Uniprot accession I2CMD6) peptide GKPFQLEALFEANQNSKTAK modified by 1 hexose.
(A) Annotation of the ETD spectra by Proteome Discoverer 1.4 using variable modification at Ser/Thr/Tyr residues.
(B) Annotation of the ETD spectra by Proteome Discoverer 1.4 using variable modification at Lys/Ser/Thr/Tyr residues.



Supplemental figure 16A
Der p 2
G[49]KPFQLEALFEANQNSKTAK[68] peptide + 1Hex ETD-MS2
Variable Hex modification at Ser/Thr/Tyr

Supplemental figure 16B

Der p 2

G[49]KPFQLEALFEANQNSKTAK[68] peptide + 1Hex

ETD-MS2

Variable Hex modification at Lys/Ser/Thr/Tyr