Improved identification of O-linked glycopeptides from ETD data with optimized scoring for different charge states and cleavage specificities

Amino Acids

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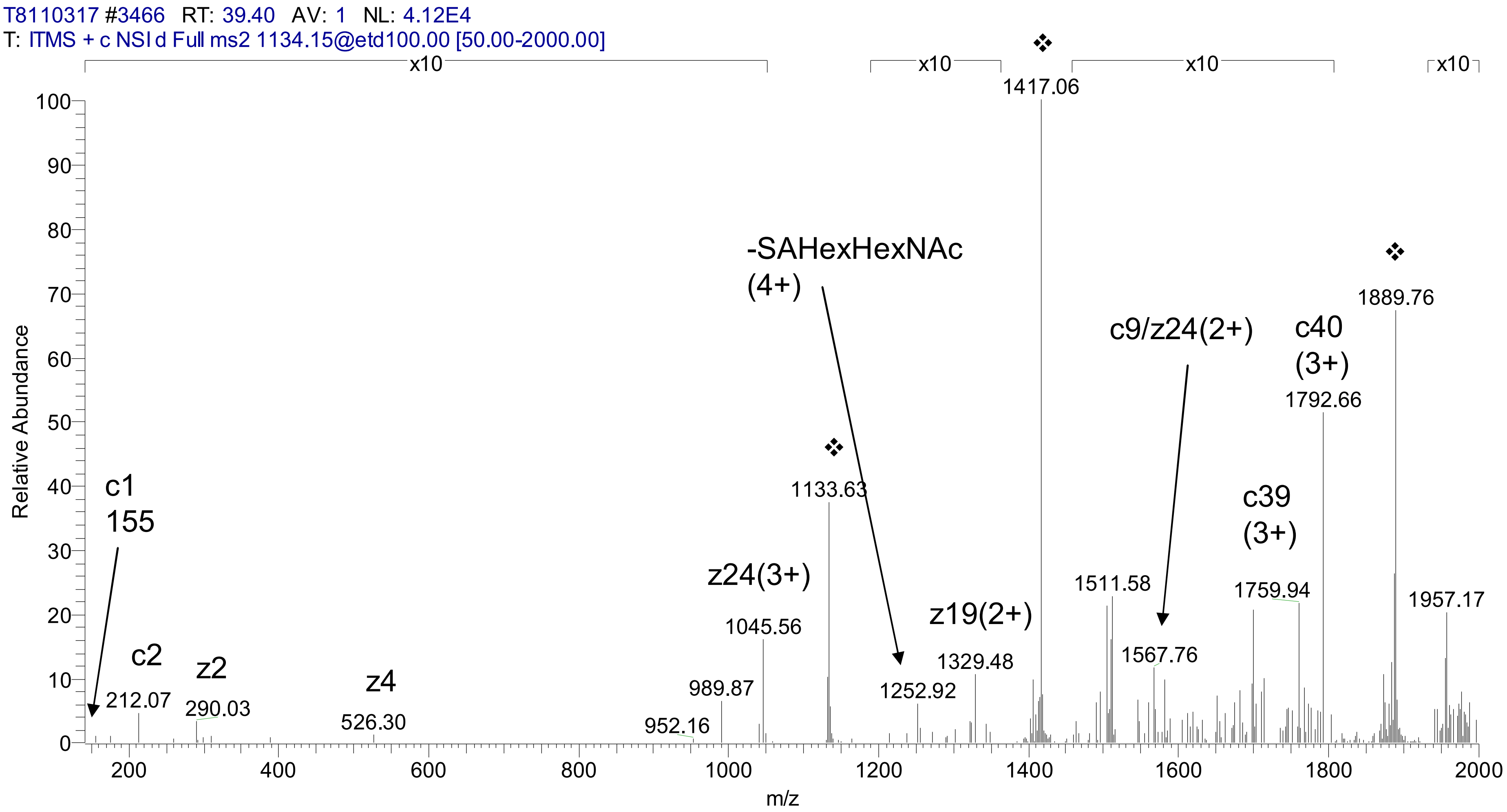
Legends

For ETD data

- z* = z+1;
- c* = c-1;
- ❖ = charge reduced ions
- * = 2x precursor ion;
- bold = z ion detected;
- underlined = c ion detected;
- italic = b or y ion detected

A5D7R6, ITIH2 protein

1133.5541(5+)



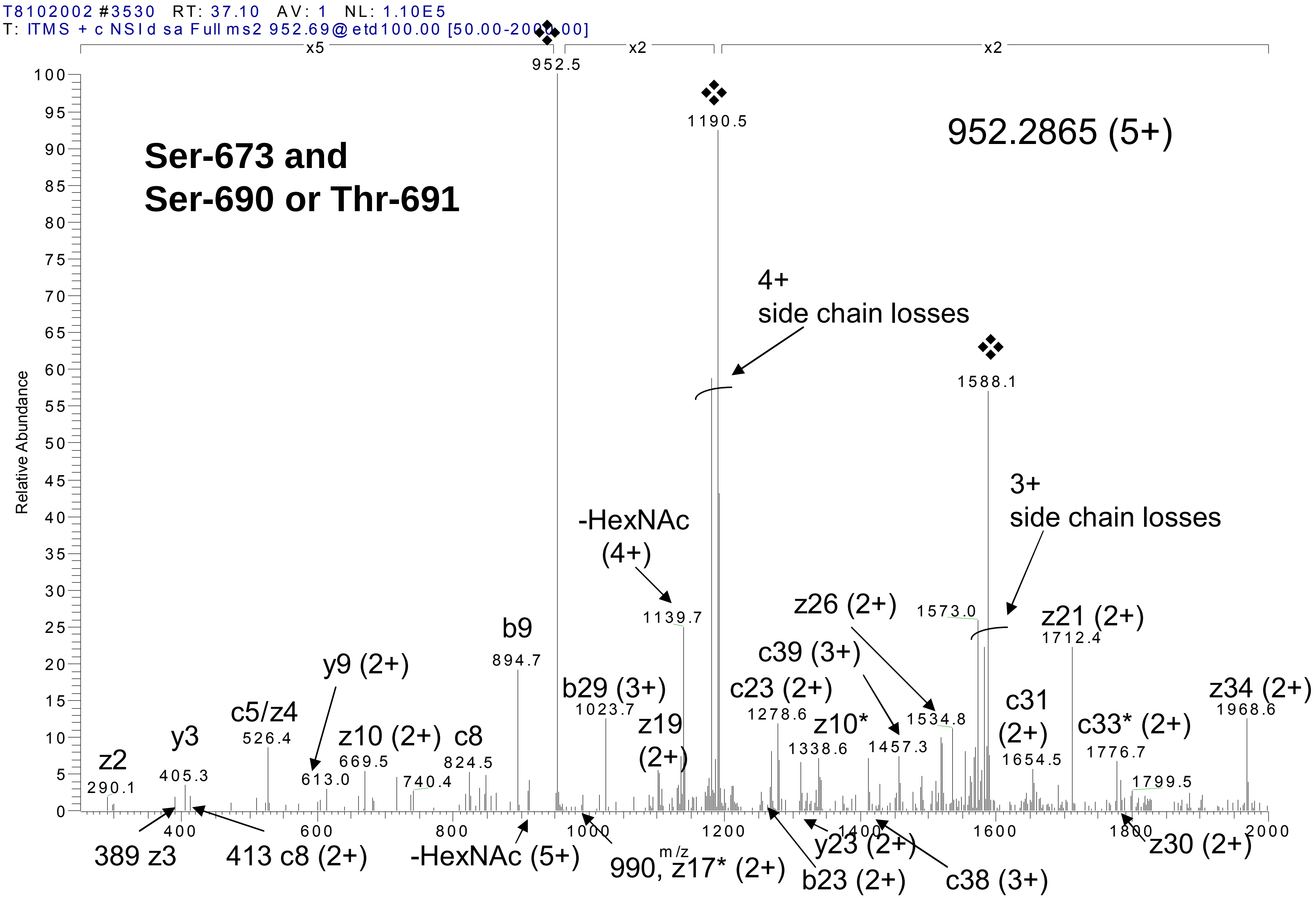
^{657}HGSKVS(GalNAcGalSA)PNSVPSWVNPSP**A**PVLPMP**A**VGAQVLES(GalNAcGalSA)-TPPP**H**V**M**R^{698}

Ser-659or 662 or 665 or 668 or 673
and Ser-690 or Thr-691

Supplemental Figure 1

A5D7R6, ITIH2 protein

Supplemental Figure 2

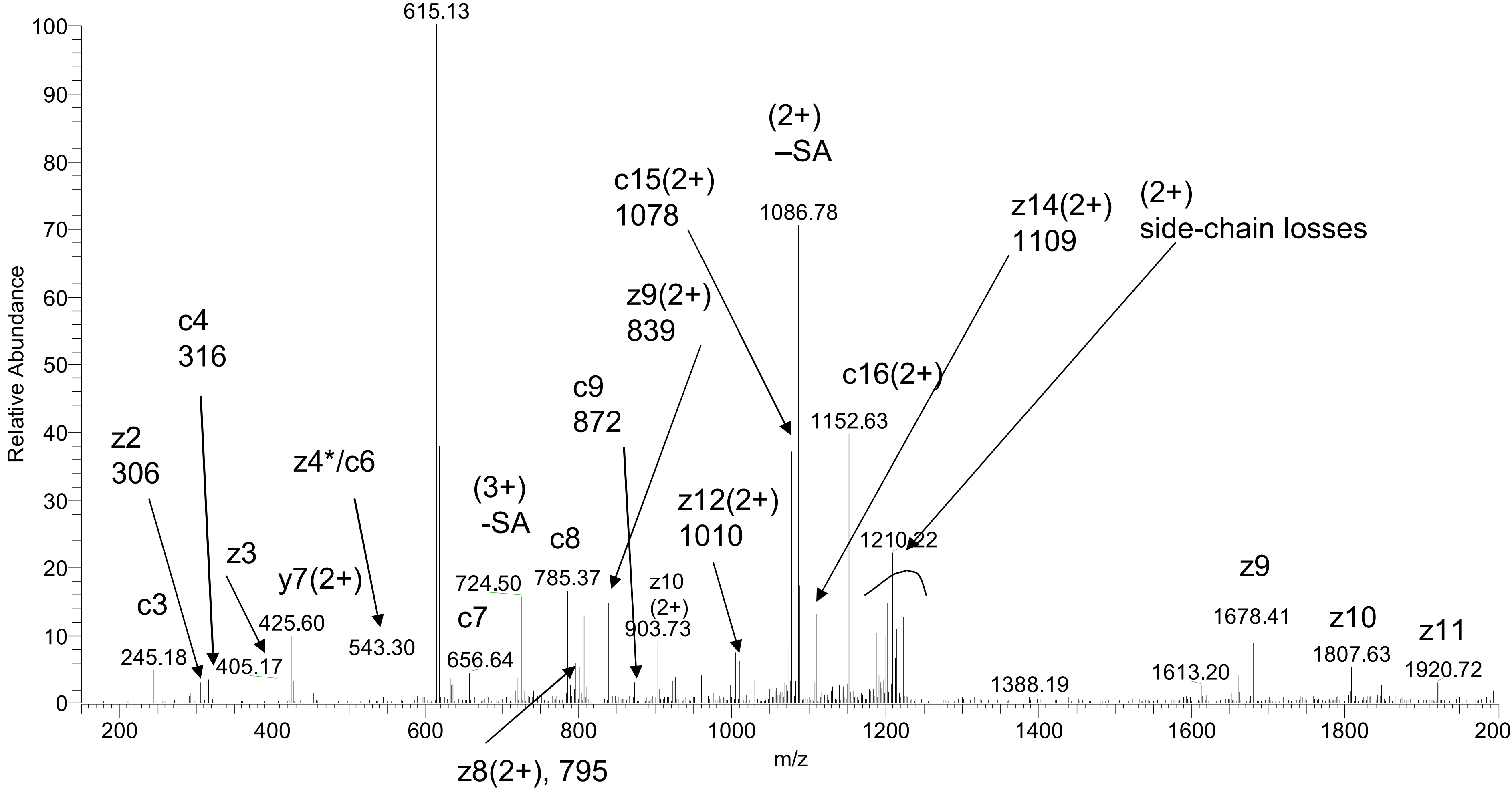


HGSKVSPN***S***VPS**W**VNP**S(HexNAc)**PA***P***V**L**P**M**P**A**VG*A*___Q___*VL***E*___S___T(HexNAc)PPPH___V___**M**R

A5D7R6, ITIH2 protein **Thr-691** 616.0485 (4+)

Supplemental Figure 3

Katalin_Szuszanna_53 #2537 RT: 28.86 AV: 1 NL: 1.54E3
T: ITMS + c NSI d sa Full ms2 616.30@etd90.00 [150.00-2000.00]



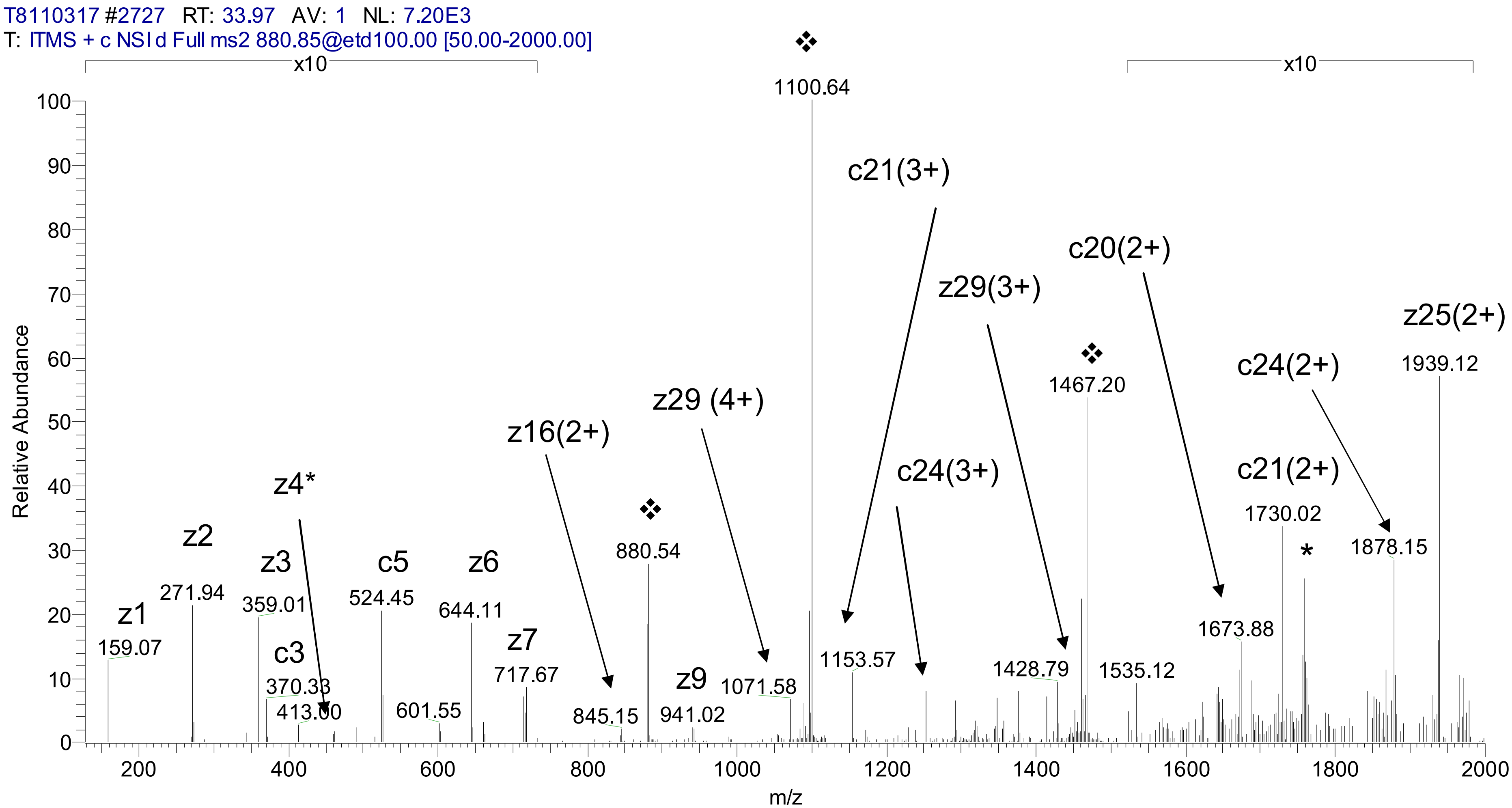
AVG**A**Q**VLEST**(HexNAcHexSA)***P***PP**HVM(Oxidation)**R

A4IFA5, VASN protein

Ser-455 & Thr-460

Supplemental Figure 4

880.2454 (5+)

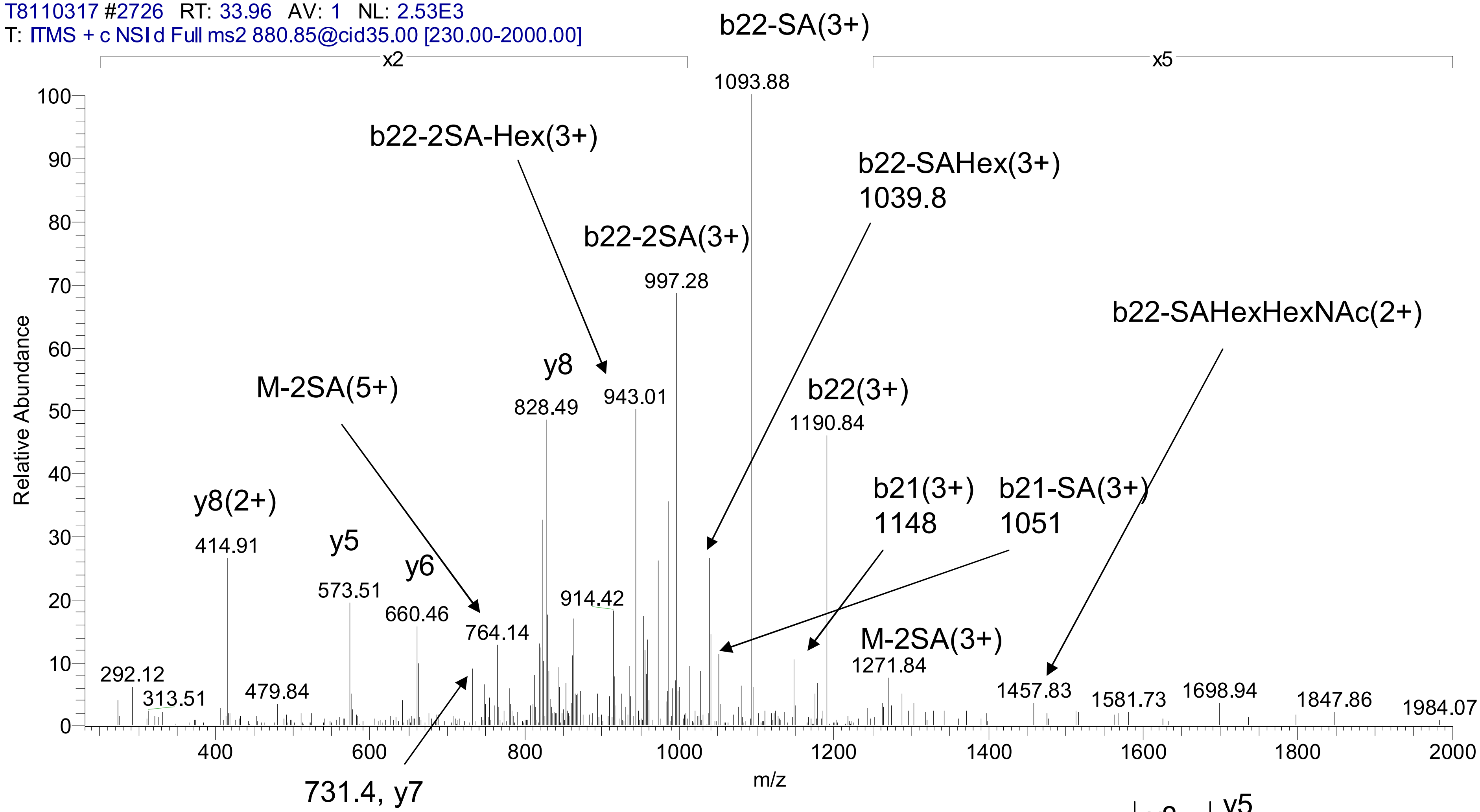


V**R**PGP**R**PS(HexNAcHexSA)PAPAT(HexNAcHexSA)P**R**PLPLGI**E**P**AS**P**TSLR**

A4IFA5, VASN protein

880.2454 (5+)

Supplemental Figure 5A



y8 | y5

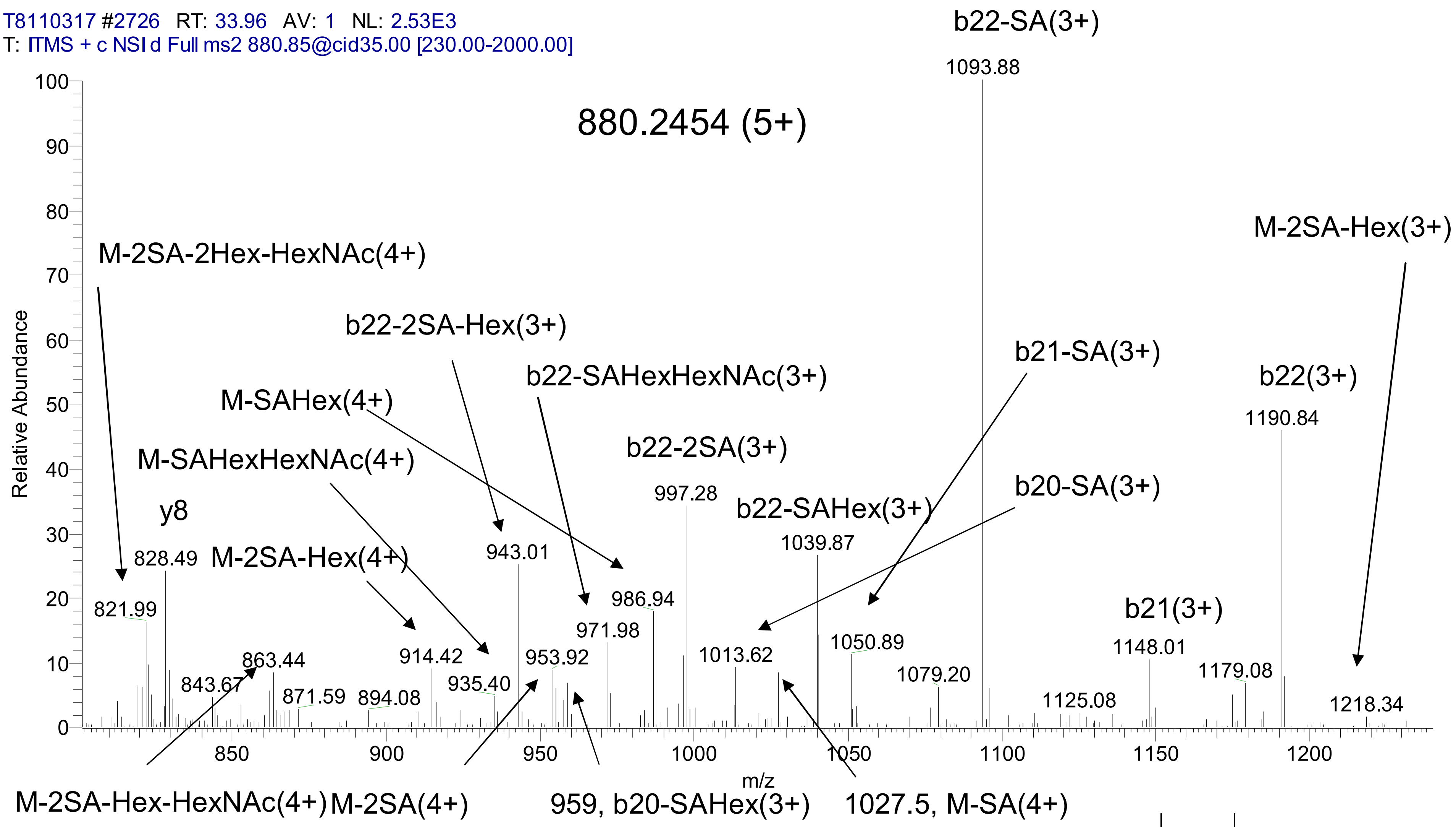
VRPGPRPS(HexNAcHexSA)PAPAT(HexNAcHexSA)PRPLPLGIE|PAS|PTSLR

b22

CID !

A4IFA5, VASN protein

Supplemental Figure 5B



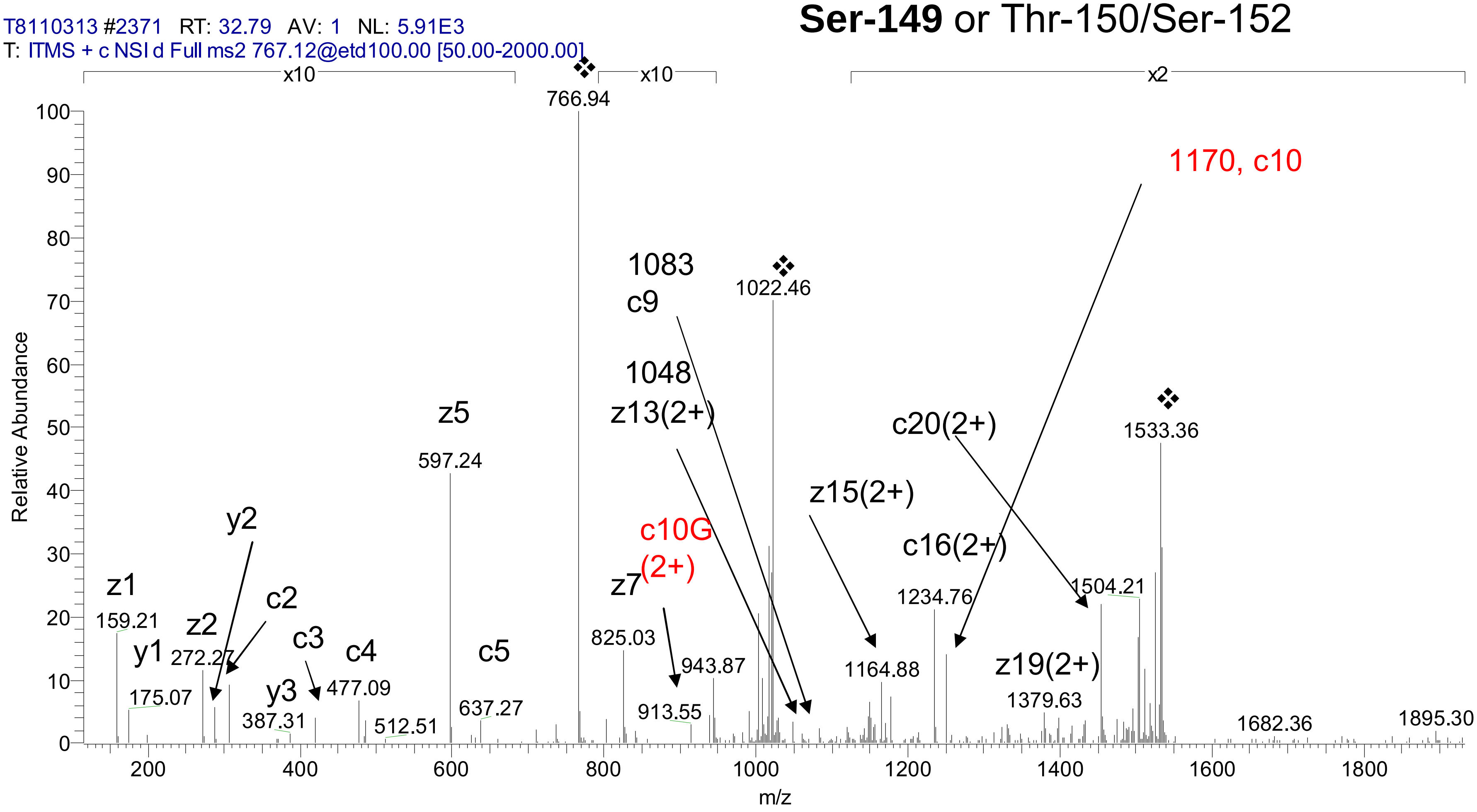
VRPGPRPS(HexNAcHexSA)PAPAT(HexNAcHexSA)PRPLPLGIE|PAS|PTSLR

b22

CID!

P01044, Kininogen-1 766.6159 (4+) **Supplemental Figure 6**

Ser-149 or Thr-150/Ser-152

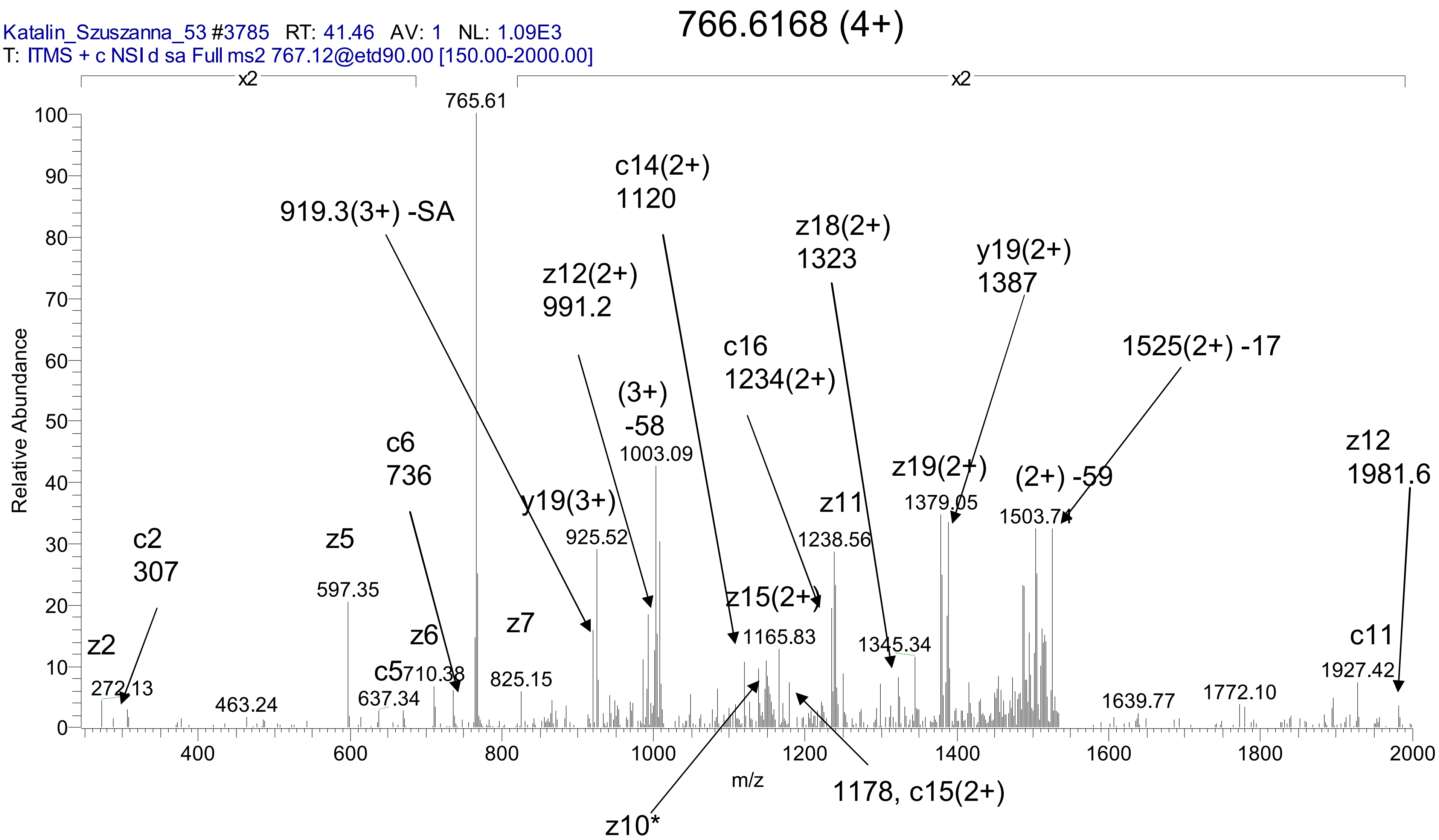


EC(Carbamidomethyl)**L**GC(Carbamidomethyl)V**H**P**I**S(HexNAcHexSA)TKSP**D**L**E**P**VLR**$^{+4}$

Since c10 is present with and without the sugar, this is most likely a mixture

Supplemental Figure 7

P01044, Kininogen-1 Ser-149



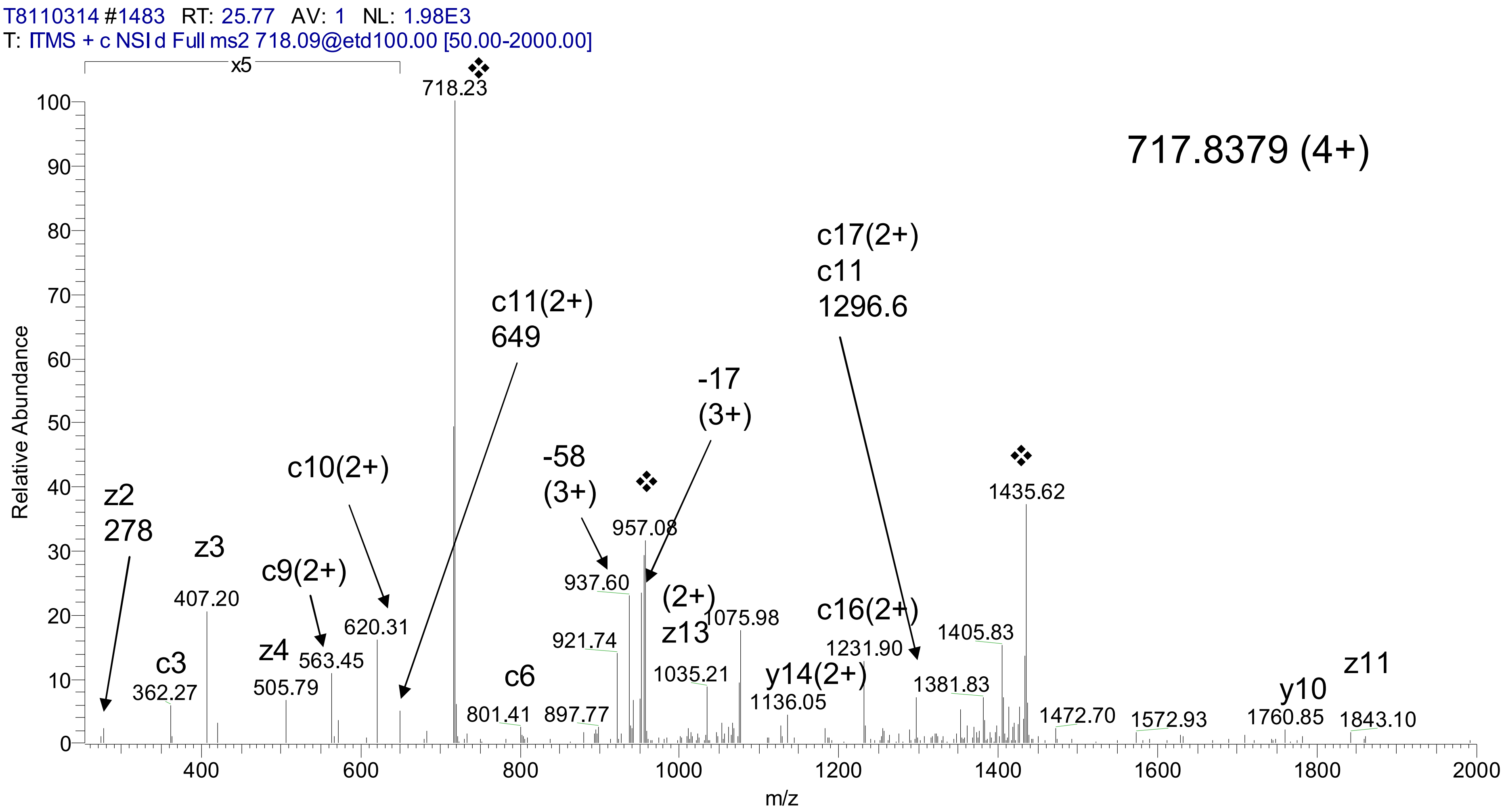
EC(Carbamidomethyl)**LG**C(Carbamidomethyl)V**H**PI**S**(HexNAcHexSA)**TK**SP**DLE**PV**L**R^{+4}

There is a weak m/z 1170, that may correspond to c10 without the sugar! + a weak m/z 1896, z11 if Thr-150/Ser-152 is glycosylated)

Supplemental Figure 8

P01044, Kininogen-1

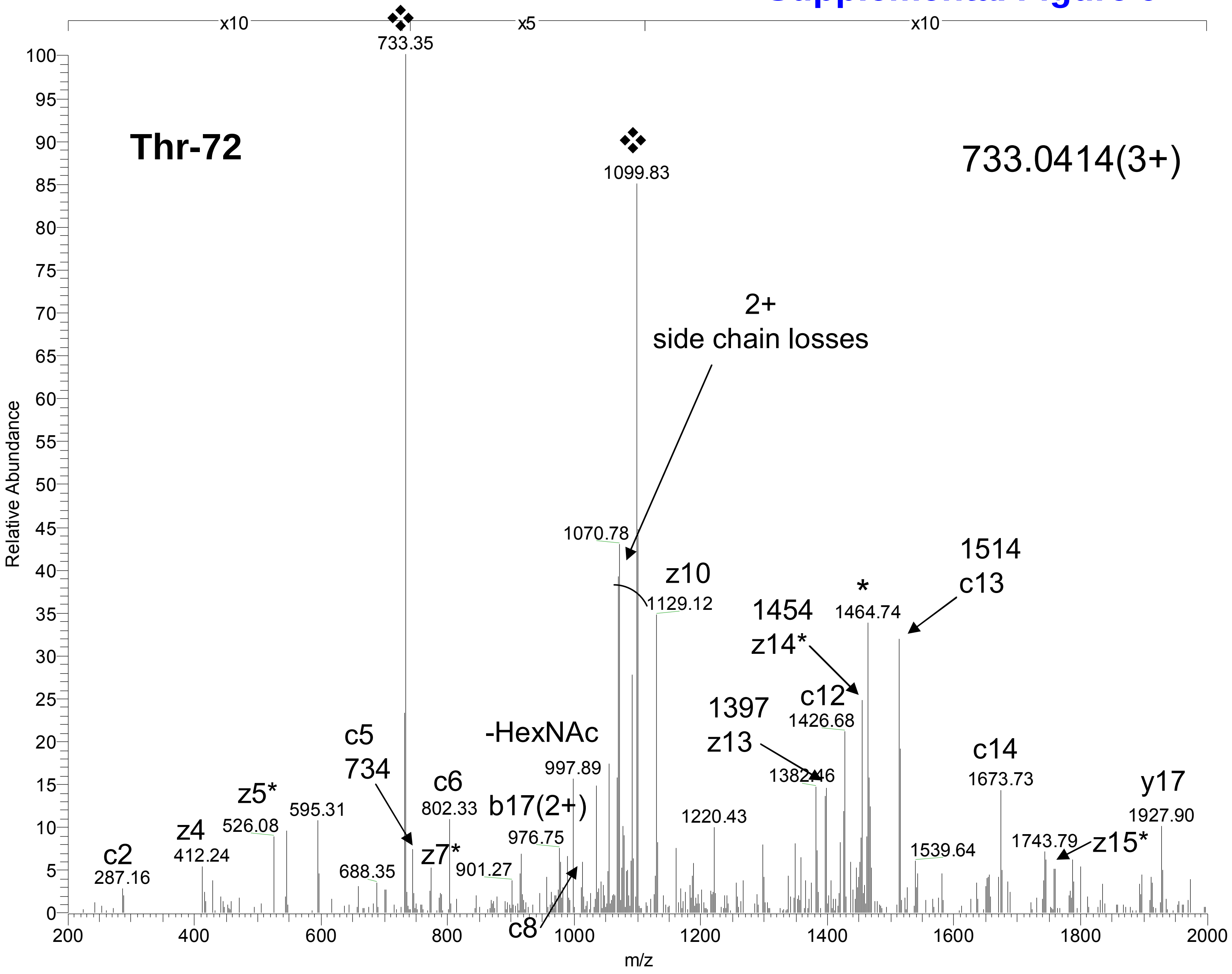
Thr-605



C(Carbamidomethyl)PSRP***W*K**P**V*N***GVNPT(HexNAcHexSA)**VEM**(Oxidation)K

Q2KIU3, Putative uncharacterized protein

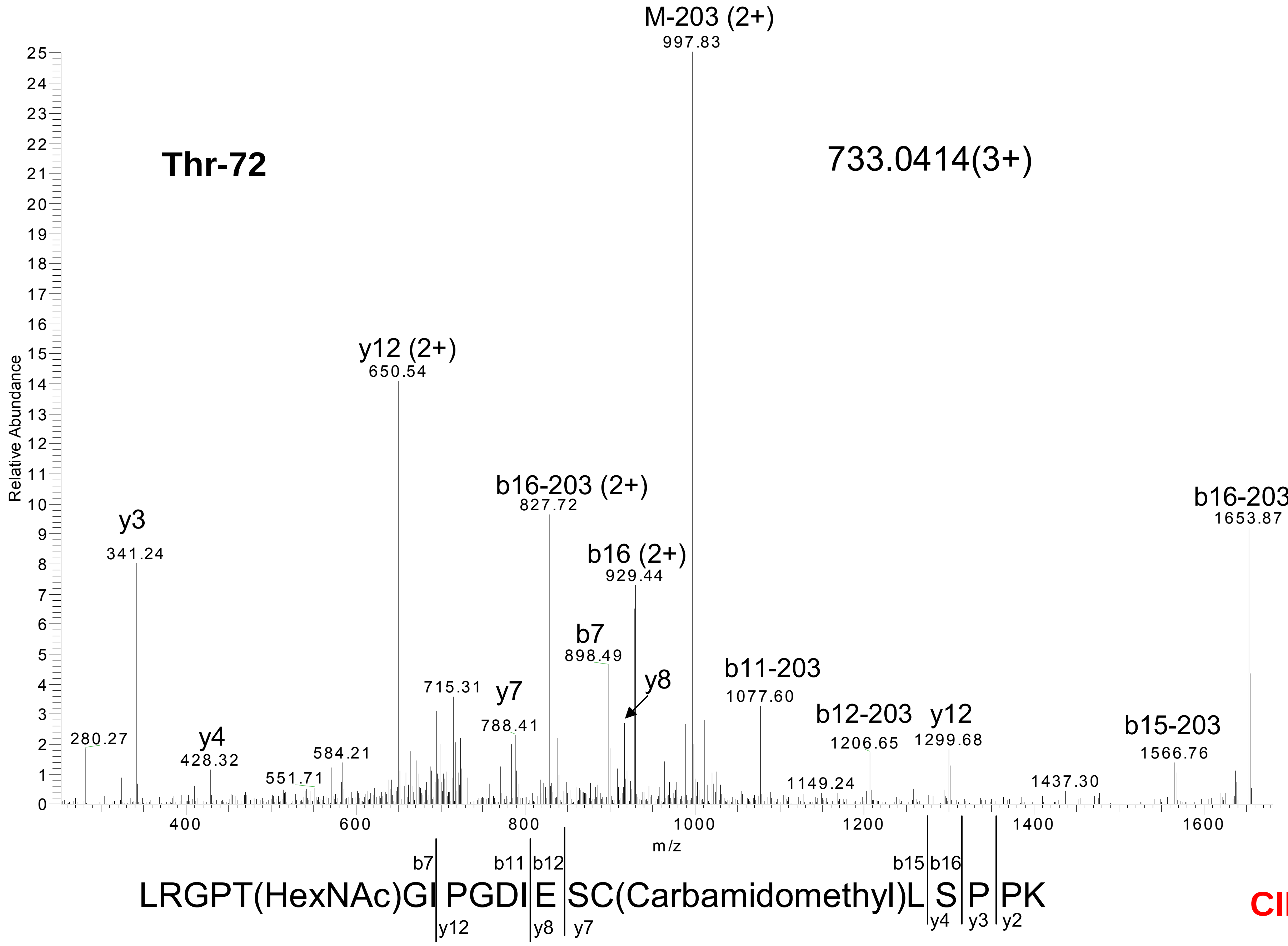
Supplemental Figure 9



LRGP**T(HexNAc)G**IP**GD**IESC(Carbamidomethyl)**LS**PPK

Q2KIU3, Putative uncharacterized protein

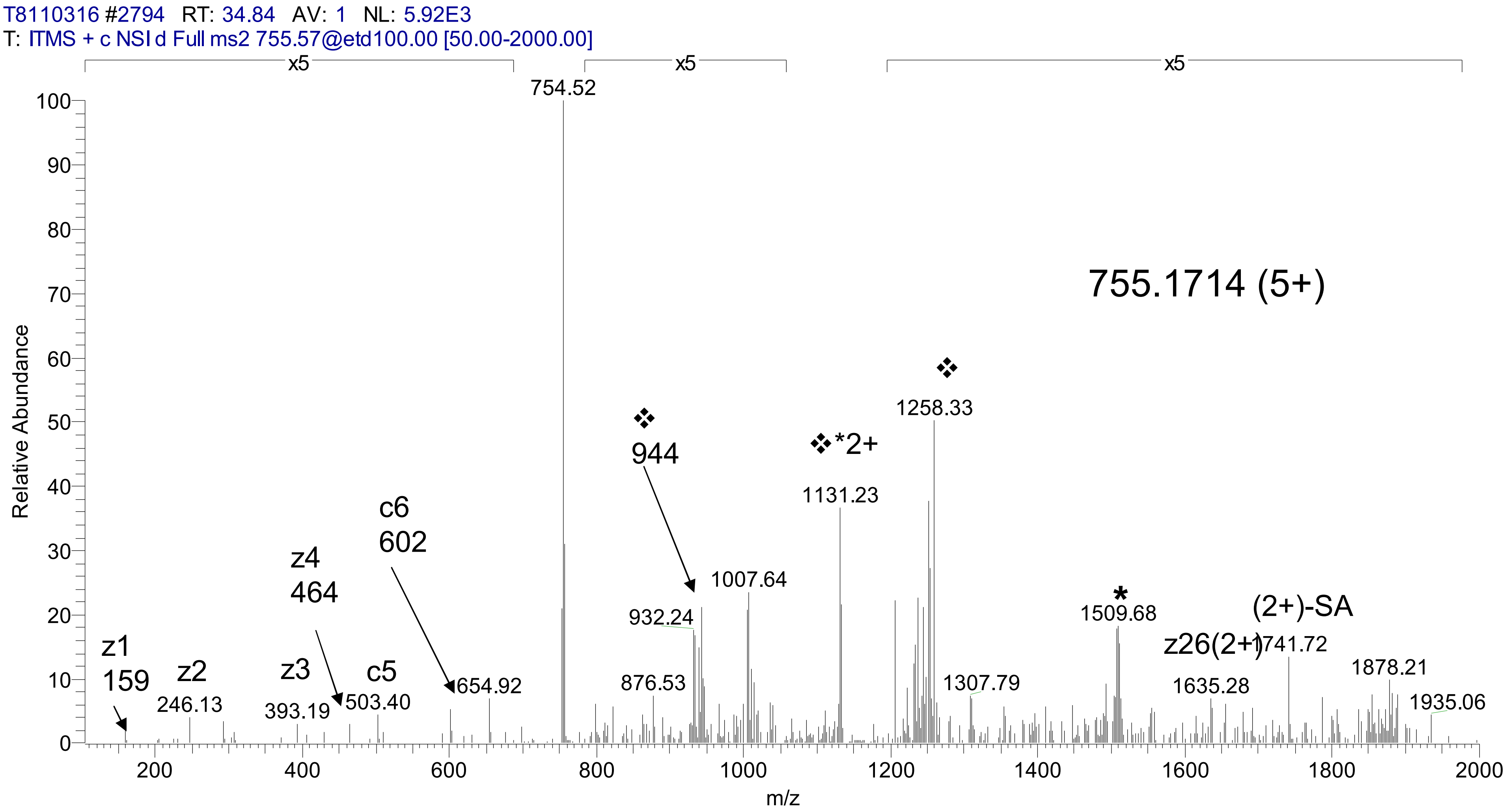
Supplemental Figure 10



CID !

Supplemental Figure 11

❖ *also overlapping (3+)

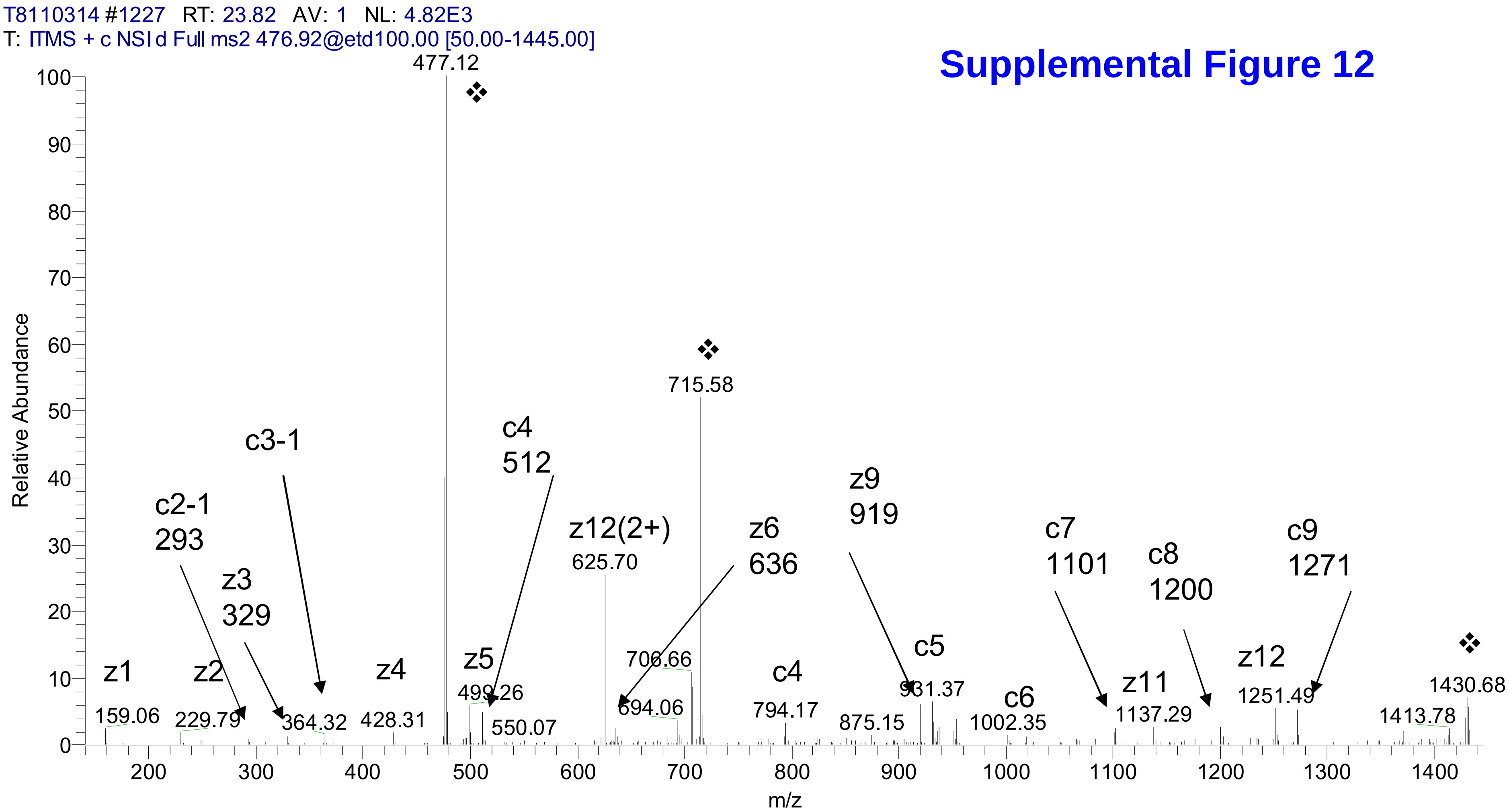


LVVS<u>S**V**</u>QHQSALELSEAGVQAAAATST(HexNAcHexSA)**AM(Oxidation)SR**

If the ID is accepted, still seven potential glycosylation sites

~~B3VTM3, Lactoferrin~~ **Q29443, serotransferrin** 476.9216 (3+)

Supplemental Figure 12



QVPS(HexNAcHex)**HAVVAR**

This is NOT a glycopeptide!

(C) YLAM(O)**VPSHAVVAR**