Paucimannose-Rich *N*-glycosylation of Spatiotemporally Regulated Human Neutrophil Elastase Modulates Its Immune Functions

Supplementary Data

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Supplementary figure legends

Supplementary Figure S1: Canonical amino acid sequence of human neutrophil elastase (HNE) (UniProtKB identifier: ELNE_HUMAN, P08246). The position and numbering (based on the preproprotein amino acid sequence) of the four putative *N*-glycosylation sites (sequons), the identified signal peptide and the N- and C-terminal propeptides are indicated e.g. the four putative *N*-glycosylation sites are Asn88: NLS, Asn124: NGS, Asn173: NVT, Asn185: NVC (see legend for colour coding).

Supplementary Figure S2: Overview of the identified HNE glycopeptide "families" and their C_{18}-reversed phase (RP) LC-MS/MS elution profile after trypsin and HNE auto-digestion and ZIC-HILIC-SPE enrichment. The top panel shows the MS1-level base peak chromatogram (*m/z* 300-2,200) and bottom panels show the fragment (MS/MS) XICs of *m/z* 366, 528 and 657 signals that are diagnostic for MS/MS spectra containing the HexHexNAc, Hex_2HexNAc and HexHexNAcNeuAc saccharide oxonium ions, respectively. The glycopeptide clusters covering the individual *N*-glycosylation sites of HNE are indicated i.e. Asn88 (green), Asn124 (red) and Asn173 (blue). See also Supplementary Table S1.

Supplementary Figure S3: (**A**) PGC-LC-MS/MS based *N*-glycome profiling of HNE revealed 21 *N*-glycans comprising chitobiose core, paucimannosidic and complex type *N*-glycans. The structures, linkages and relative abundances of the individual *N*-glycans are illustrated with their short hand nomenclature. *N*-glycans observed at the *N*-glycopeptide level are underlined and in bold. Data points are plotted as mean ± S.D, n = 3 technical replicates. All glycans were observed in their reduced anionic form. (**B**) Annotated PGC-LC-ESI-CID-MS/MS spectra of selected *N*-glycans released from HNE. Only *N*-glycans observed on both the *N*-glycome and at the *N*-glycopeptide level are shown. The *N*-glycans were analysed in their reduced (alditol), but otherwise native form in negative ion polarity mode. The resonance activation (ion trap) CID fragments of the HNE glycans were manually annotated according to the established Domon-Costello nomenclature (**2**). The glycans were further validated based on their molecular masses and their relative and absolute PGC-LC retention. Few structures were elucidated by spectral and retention time matching to *N*-glycan reference compounds (see inserts). Some *N*-glycan glycosidic linkages, topologies and glycan sequence features were inferred based on the established knowledge of the human *N*-glycosylation machinery and the biosynthetic relatedness between the observed structures (6,7). The presence of previously established MS/MS diagnostic ions was used to determine the *N*-glycan topologies e.g. the α1,3/6-mannose arm position of the antennas (3-5). The *N*-glycans were visualised according to the established symbol nomenclature (8). See Supplementary Table S2 for the structural overview of the observed HNE *N*-glycans.

Supplementary Figure S4: (**A**) Annotated RP-LC-ESI-CID/ETD-MS/MS spectra of all observed glycosylated and non-glycosylated peptides identified from the unenriched and enriched peptide mixture of HNE. The resonance activation (ion trap) CID- and ETD-MS/MS fragments of the HNE (glyco)peptides were manually annotated according to the established fragment nomenclature. The (glyco)peptides were further validated using their molecular mass and their LC retention. The CID- and ETD-MS/MS spectra of the HNE C- and N-terminal peptides are also shown. Carbamidomethylated cysteine residues are underlined. See Supplementary Table S1 for a structural overview of the observed HNE *N*-glycopeptides. (**B**) Peptide sequence coverage of HNE in the unenriched peptide mixture.

Supplementary Figure S5: (**A**) Annotated RP-LC-ESI-Q-TOF-MS of all identified intact (native) HNE glycoforms (labelled A-O from highest to lowest abundance). The full list of the experimental and theoretical average masses (neutral M, in Da) after spectral deconvolution, mass differences (in ppm) and the proposed corresponding monosaccharide compositions in a site-unspecific manner are shown. * denotes adduct formation of HNE. (**B**) CID-MS/MS fragmentation of intact HNE revealed that Arg248 forms the C-terminal of mature HNE.

Supplementary Figure S6: Immunoblotting and SDS-PAGE analysis of human neutrophil cell surface-bound and intracellular proteins. (**A**) Immunoblotting with anti-HNE antibody. (**B**) Immunoblotting with anti-paucimannose antibody (Mannitou). (**C**) CMB stained SDS-PAGE gel (HNE region, 25-27 kDa indicated with a broken red box).

Supplementary Figure S7: (**A**) Annotated RP-LC-ESI-CID-MS/MS spectra of tryptic HNE peptides observed in the 25-27 kDa gel region identified to originate from cell surface HNE derived from stimulated neutrophils. XICs of the identified peptides are shown in inserts. Carbamidomethylated cysteine residues are underlined. (**B**) The observed HNE peptides from this gel region are highlighted in red and bold in the full-length polypeptide sequence of HNE (UniProtKB: P08246).

Supplementary Figure S8: Annotated RP-LC-ESI-CID-MS/MS spectra of ZIC-HILIC-SPE enriched *N*-glycopeptides covering site Asn88 and Asn124 of HNE identified in the cell surface captured fraction of activated neutrophils.

Supplementary Figure S9: Annotated RP-LC-ESI-Q-TOF-MS spectra of the identified glycoforms of the HNE:A1AT complex formed at a 1:3 ratio. The deconvoluted experimental and theoretical average masses (neutral M, Da), mass differences (in ppm) and the proposed monosaccharide compositions of HNE and human A1AT presented in a site-unspecific manner are shown.

Supplementary Figure S10: Bacterial growth profile of PASS1 cultured alone (control, blue trace) and with HNE, nCG and their released *N*-glycans in the same micromolar concentration range. Enzymatically active (**A**) HNE and (**B**) nCG at concentrations of 1.8 μM (red trace) and 3.6 μM (green trace) and *N*-glycosidase F released *N*-glycans from HNE and nCG (3.6 μM) (black trace) (n = 3 technical replicates). Data points are plotted as mean ± S.D. * $p < 0.05$ comparing control and released *N*-glycans, unpaired two-tailed type 2 Student's t-test.

Supplementary Table S1: Site-specific distribution of HNE *N*-glycopeptides of neutrophil lysates (provided as a separate Excel file).

Supplementary Table S2: Overview of the observed HNE *N*-glycans including their assigned numbers, experimental and theoretical masses, charge states, monosaccharide compositions, their short-hand nomenclature and their structure including their *N*-glycan monosaccharide linkages and topologies partially based on experimental data and partially inferred from the general knowledge of the human *N*-glycosylation machinery and the relatedness between the observed *N*-glycans.

Supplementary Table S3: PDB-derived X-ray crystal structures of HNE. Site-specific solvent accessibilities were assessed for each of the three putative *N*-glycosylation sites from each HNE 3D structure (n = 19). The PDB entries, and the structural resolution, polypeptide chain sequence covered and mono-/dimeric status and the (often partially) assigned *N*-glycans on the individual HNE structures are indicated (provided as a separate Excel file).

Supplementary Table S4: Granule- and site-specific *N*-glycosylation of HNE derived from human neutrophils. The relative abundance of non-glycosylated and *N*-glycosylated tryptic peptides covering Asn88 and Asn173 were manually profiled from proteomics data acquired from the individual neutrophil compartments i.e. azurophilic, specific, gelatinase, secretory, ficolin and plasma membrane (**1**). The relative abundances of the individual glycoforms (to the resolution of monosaccharide compositions) and of their corresponding *N*-glycan types (i.e. chitobiose, paucimannose and complex type *N*-glycans) observed in the respective granule compartments are shown (provided as a separate Excel file).

Supplementary Table S5: Assessing the binding affinity of HNE glycoforms to immobilised MBL. The relative abundances of the glyoforms of total, MBL-unbound and MBL-bound HNE assessed by RP-LC-ESI-Q-TOF-MS. Quantitation was based on the relative height of the relevant mass signals of the deconvoluted spectra (provided as a separate Excel file).

Supplementary Table S6: The interaction of HNE glycoforms with A1AT at 1:3 ratio. Relative abundance of HNE:A1AT complex, A1AT complexed, HNE complexed, native A1AT and total HNE assessed by RP-LC-ESI-Q-TOF-MS were quantitatively profiled using the relative signal height of the relevant mass signals of the deconvoluted spectra (provided as a separate Excel file).

References

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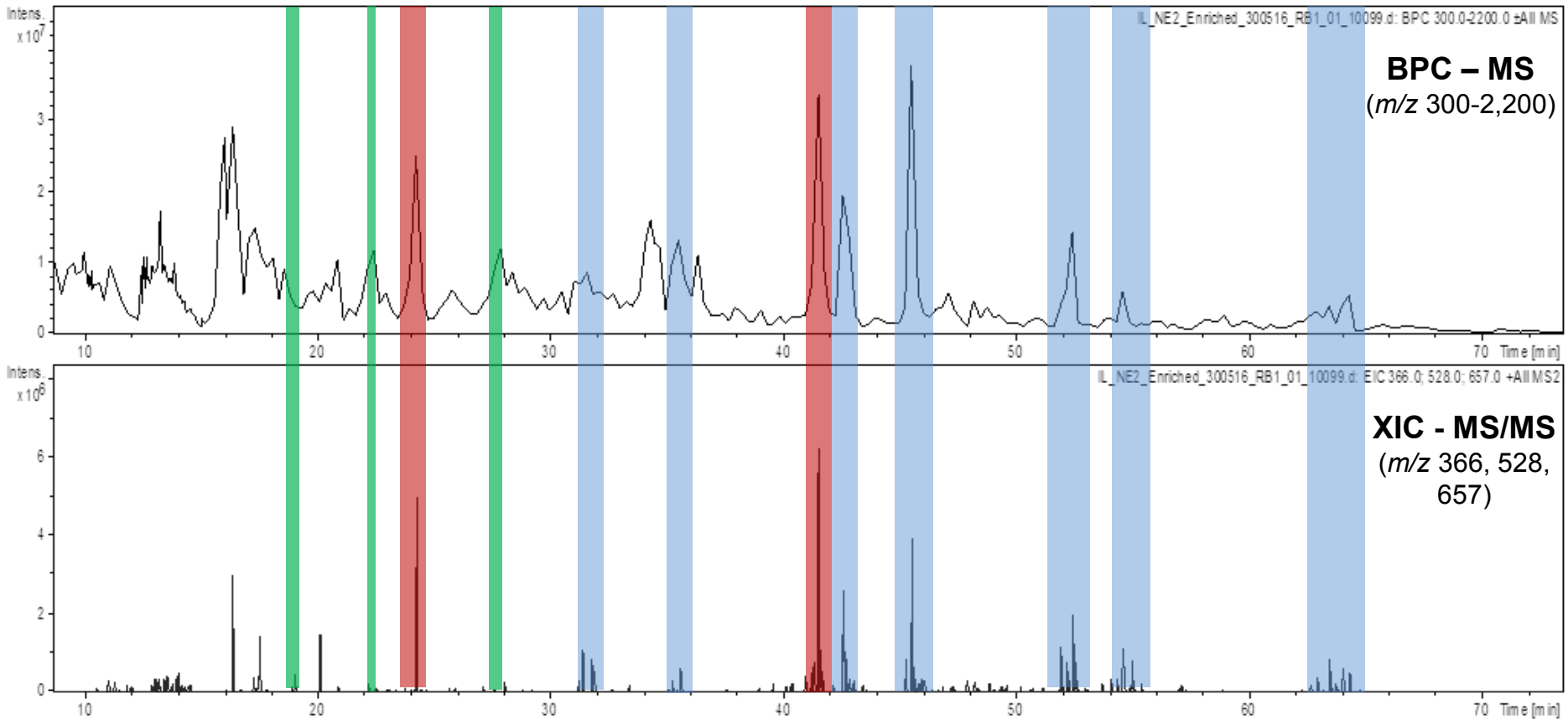
Supplementary Figure S1

[1]MTLGRRLACLFLACVLPALLLGGTALA[27]SE[29]IVGGRRARPHAWPFMVS
LQLRGGHFCGATLIAPNFVMSAAHCVANVNVRAVRVVLGAH**N**[88]LSRREP
TRQVFAVQRIFENGYDPVNLLNDIVILQL**N**[124]GSATINANVQVAQLPAQGR
RLGNGVQCLAMGWGLLGRNRGIASVLQEL**N**[173]VTVVTSLCRRS**N**[185]VCT
LVRGRQAGVCFGDSGSPLVCNGLIHGIASFVRGGCASGLYPDAFAPVAQ
FVNWIDSIIQ[247]R[248]SEDNPCPHPRDPDPASRTH[267]

Signal peptide
N-terminal propeptide
C-terminal propeptide
Putative *N*-glycosylation site

Supplementary Figure S2

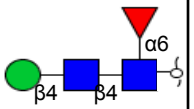
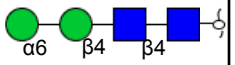
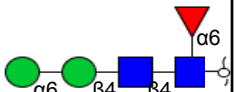
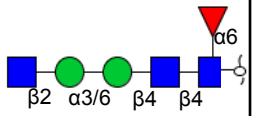
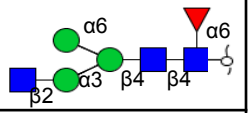
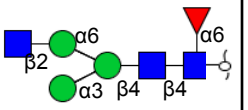
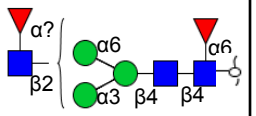
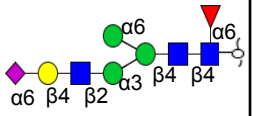
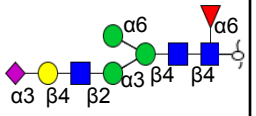


Asn88 glycopeptides:
VLGAHNLSR
LGAHNLSR
VVLGAHNLSR

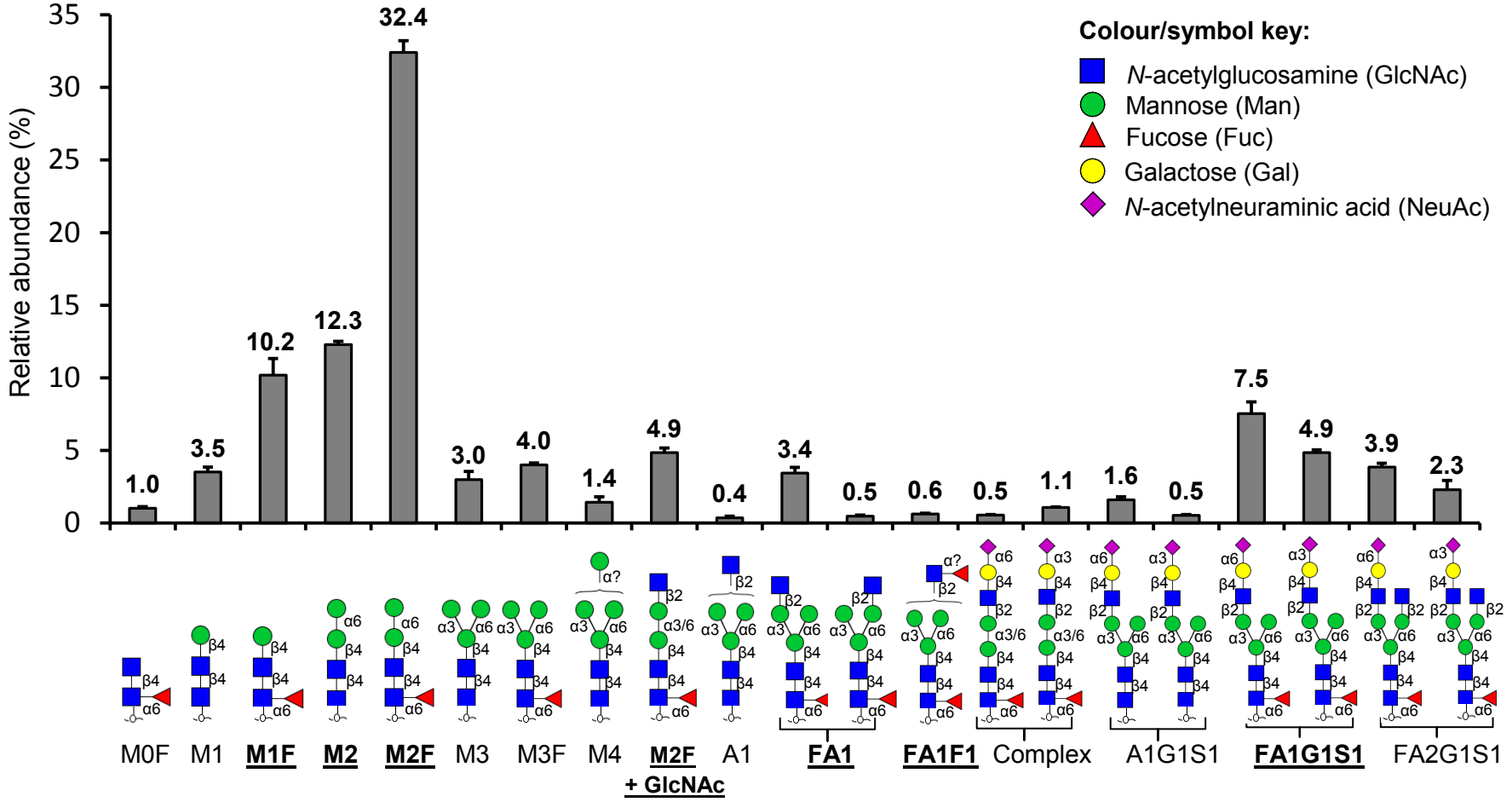
Asn124 glycopeptides:
ILQLNGSATI
ILQLNGS
VILQLNGSAT
QLNGSATI

Asn173 glycopeptides:
LQELNV, LQELNVTV
QELNVTVVT, LQELNVTVV, QELNVTVV, VLQELNVTVV
SVLQELNVTVV, VLQELNV
ASVLQELNV, ASVLQELNVTVV
GIASVLQELNVTVV, GIASVLQELNVTVVTSLCR

Supplementary Table S2

Glycan #	RT (min)	*m/z*	Charge (Z)	Obs. mass (M, Da)	Theo. mass (M, Da)	Hex	HexNAc	Fuc	NeuAc	Glycan features and short-hand nomenclature	*N*-glycan structure
1	38.6	733.3	1-	734.3	734.2	1	2	1		Monomannosylchitobiose core fucosylated (M1F)	
2	34.8	749.3	1-	750.3	750.2	2	2			Bimannosylchitobiose (M2)	
3	41.7	895.4	1-	896.4	896.3	2	2	1		Bimannosylchitobiose core fucosylated (M2F)	
4	44.3	1098.5	1-	1099.5	1099.4	2	3	1		Bimannosylchitobiose core fucosylated with terminal β1,2-linked GlcNAc (M2F + GlcNAc)	
5a	46.2	1260.4	1-	1261.4	1261.4	3	3	1		Trimannosylchitobiose core fucosylated with β1,2-linked terminal GlcNAc (FA1)	
5b	50.5	1260.4	1-	1261.4	1261.4	3	3	1		Trimannosylchitobiose core fucosylated with terminal β1,2-linked GlcNAc (FA1)	
6	44.5	1406.7	1-	1407.7	1407.7	3	3	2		Trimannosylchitobiose core fucosylated with terminal β1,2-linked GlcNAc and fucose (FA1F1)	
7a	43.6	856.3	2-	1714.6	1714.6	4	3	1	1	Trimannosylchitobiose core monoantennary core fucosylated α2,6-monosialylated (FA1G1S1)	
7b	50.0	856.3	2-	1714.6	1714.6	4	3	1	1	Trimannosylchitobiose core monoantennary core fucosylated α2,3-monosialylated (FA1G1S1)	

Supplementary Figure S3A



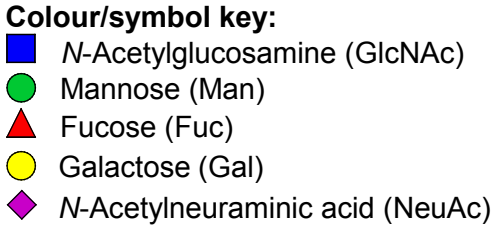
(Mean ± S.D, n = 3 technical replicates)

Supplementary Figure S3B

Annotated PGC-LC-ESI-CID-MS/MS spectra of the observed *N*-glycans released from HNE (see **Supplementary Table S2** for overview of structures). Only HNE *N*-glycans observed in both the *N*-glycan and *N*-glycopeptide analyses are shown. Glycan fragments were annotated according to the Domon-Costello nomenclature based on established fragmentation rules (2-5). Please note that multiple glycan fragments may be assigned to the same fragment ion; the most likely glycan fragment based on GlycoWorkBench *in silico* fragmentation was used in these cases. Identified structures with key fragments and diagnostic D ions are shown. All glycans were observed in their reduced (alditol) anionic form.

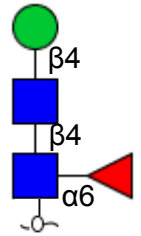
The relative and absolute PGC-LC retention of the *N*-glycans, the molecular precursor masses and the presence of MS/MS fragments and diagnostic ions were used to characterise the glycans. Please note that some *N*-glycan glycosidic linkages, topologies and glycan sequence features were inferred based on established knowledge of human *N*-glycosylation and the biosynthetic relatedness between the observed structures (6,7).

N-glycans are visualised according to the established symbol nomenclature (8).
Key of the monosaccharide symbols is shown below.



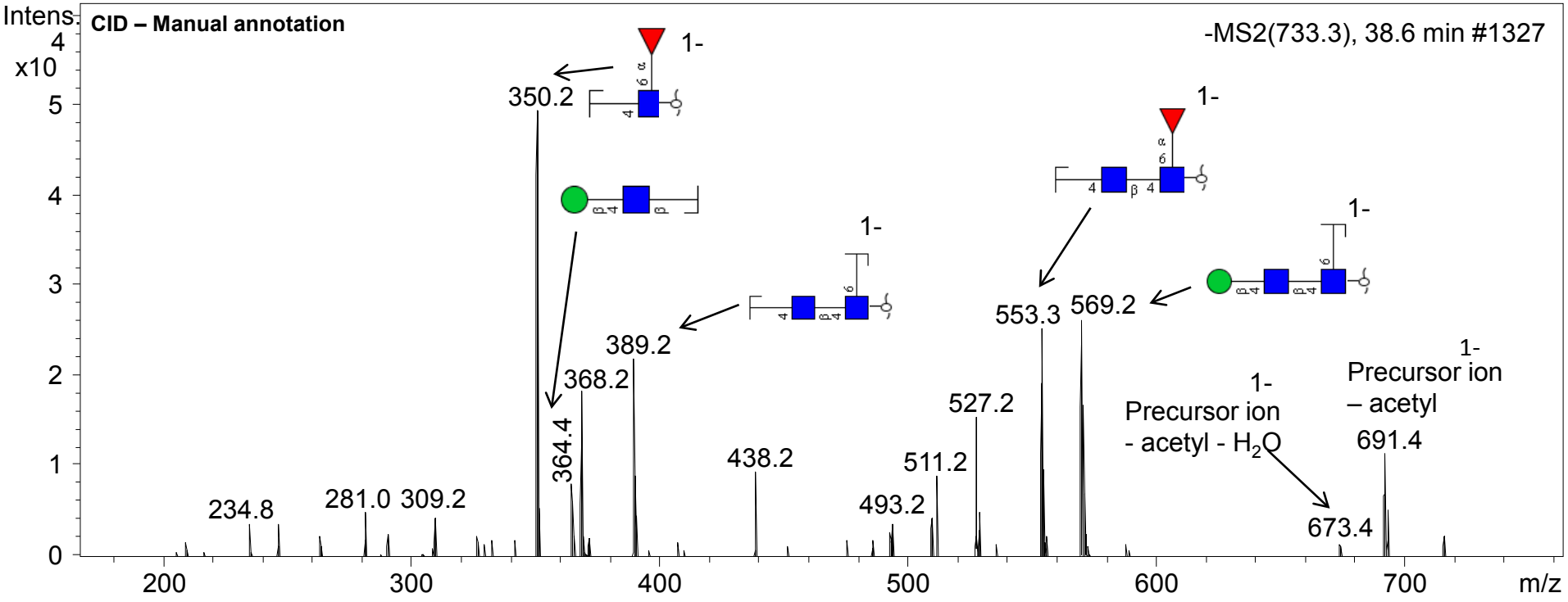
Supplementary Figure S3B

Glycan # 1



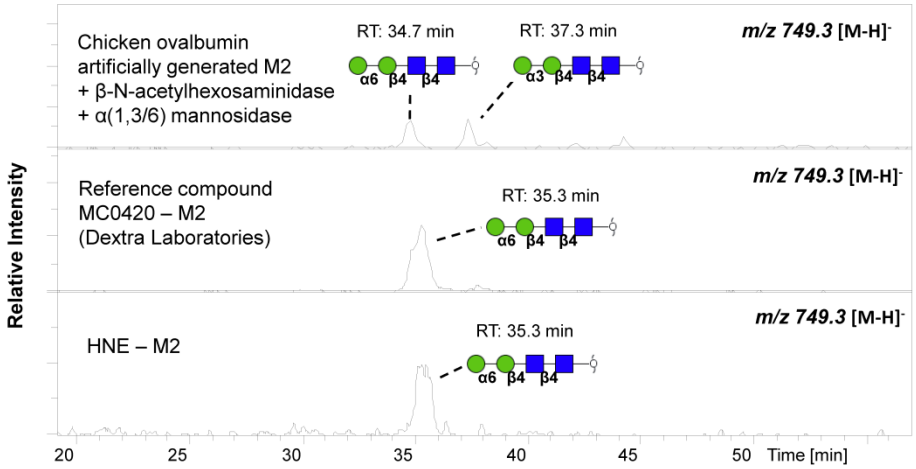
M1F

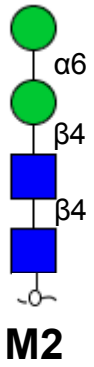
Observed *m/z* 733.3 (1-), RT: 38.6 min
$[M-H]^-$ 733.3 Da



Supplementary Figure S3B
Glycan # 2

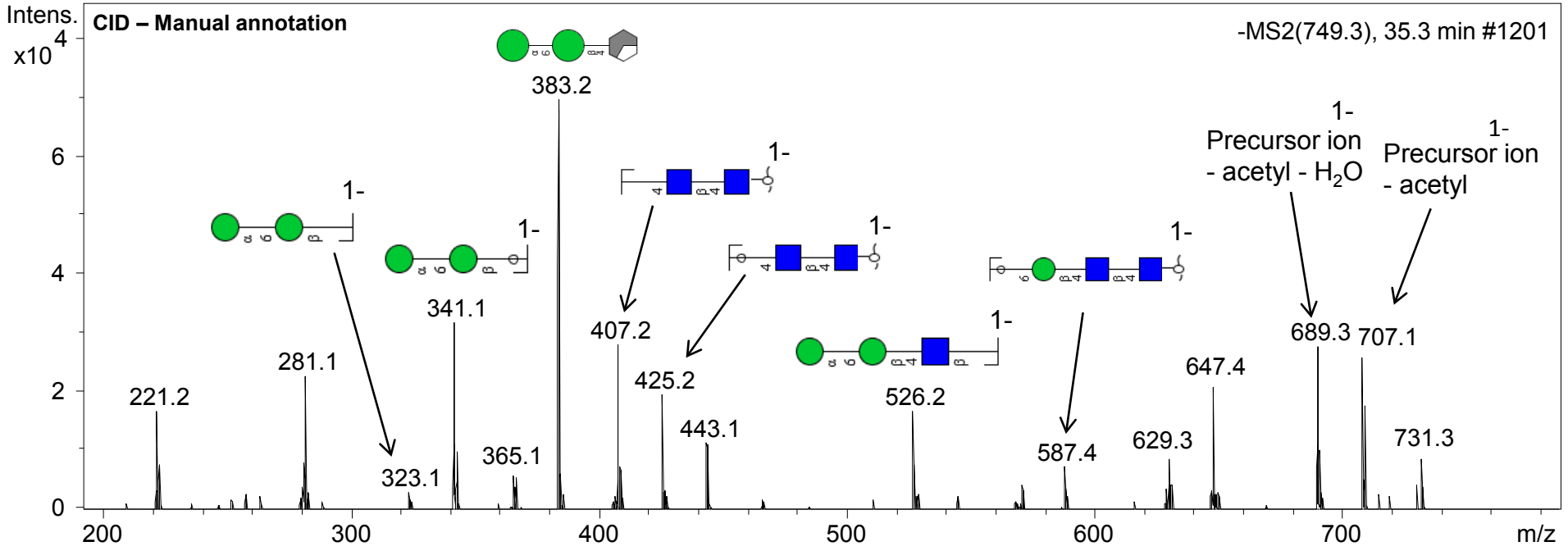
PGC-LC retention of HNE M2 (α1,6-Man) isomer (bottom chromatogram) relative to reference compound (MC0420, α1,6-Man M2) (middle chromatogram) and artificially generated mixtures of α1,3-Man and α1,6-Man M2 isomers from chicken ovalbumin using exoglycosidases (top chromatogram).





M2

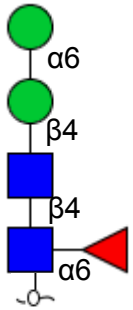
Observed *m/z* 749.3 (1-), RT: 35.3 min
$[M-H]^-$ 749.3 Da



Supplementary Figure S3B

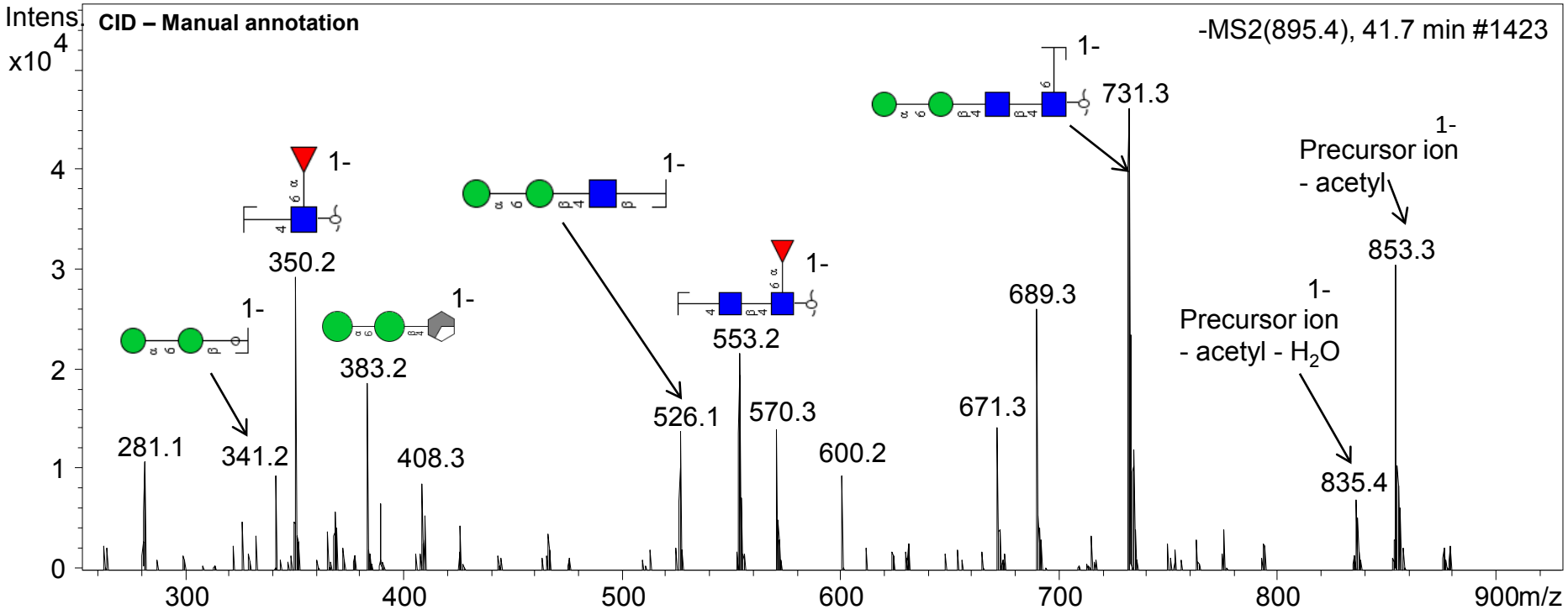
Glycan # 3

Similar to Glycan #2 (M2) only one isomer was observed for M2F. The non-reducing end Man is predicted to be present in an α1,6-linkage since M2F is biosynthetically similar to M2 (see previous spectrum).



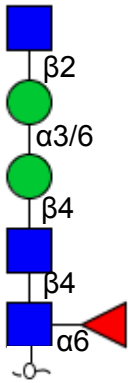
M2F

Observed *m/z* 895.4 (1-), RT: 41.7 min
$[M-H]^-$ 895.4 Da



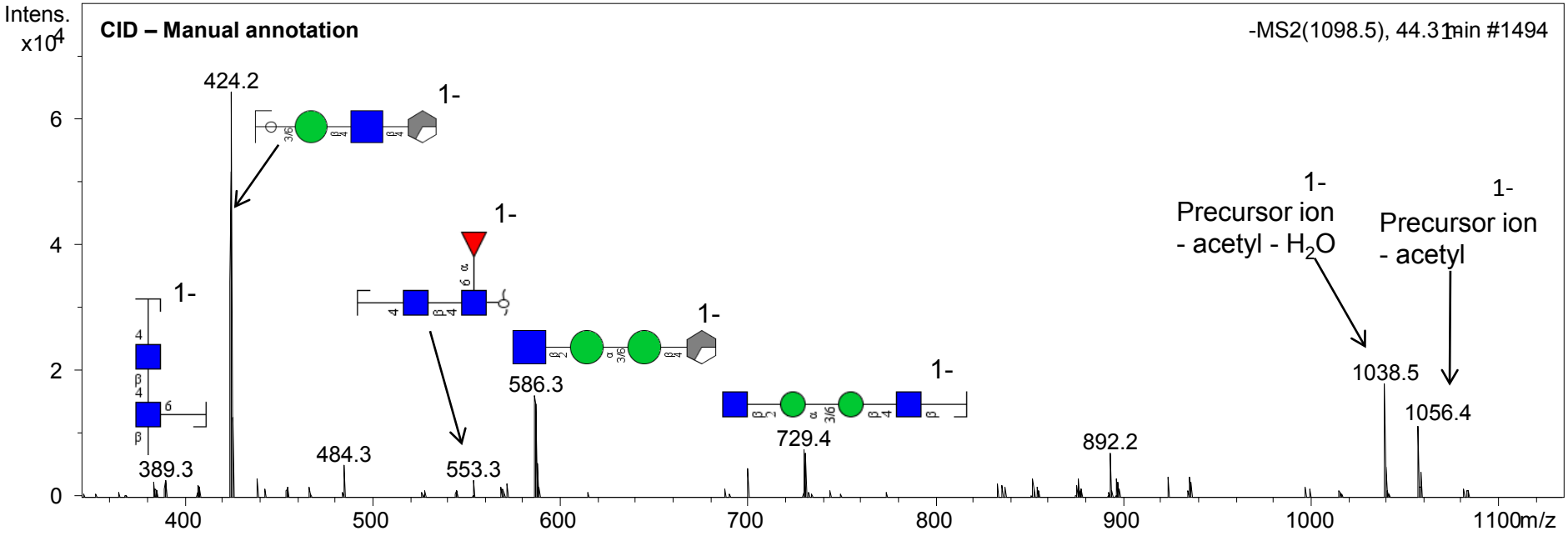
Supplementary Figure S3B

Glycan # 4



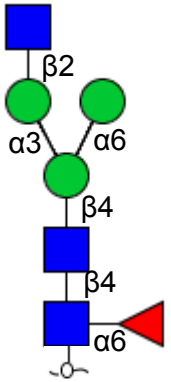
M2F + GlcNAc

Observed *m/z* 1098.5 (1-), RT: 44.3 min
$[M-H]^-$ 1098.5 Da



Supplementary Figure S3B

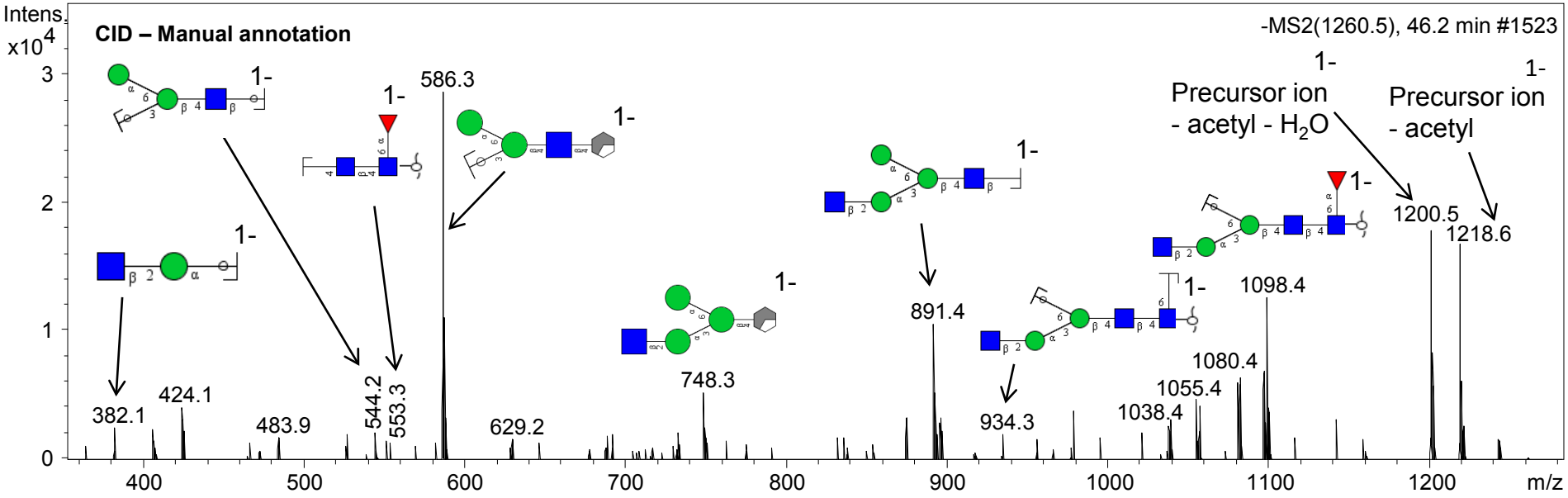
Glycan # 5a



FA1

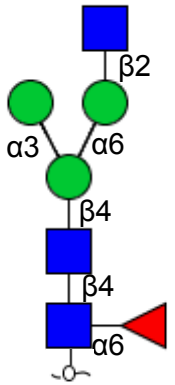
No D ion was observed suggesting a Man 3' arm position of the non-reducing end β2-GlcNAc residue.

Observed *m/z* 1260.4 (1-), RT: 46.2 min
$[M-H]^-$ 1260.4 Da



Supplementary Figure S3B

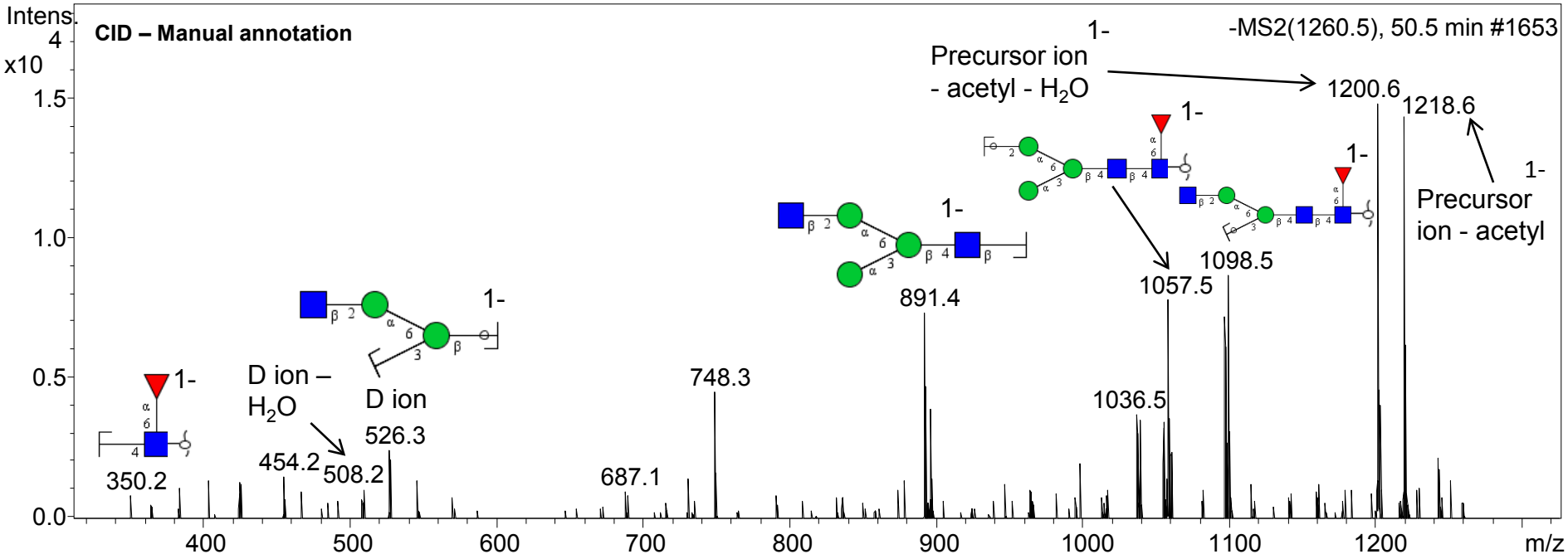
Glycan # 5b



FA1

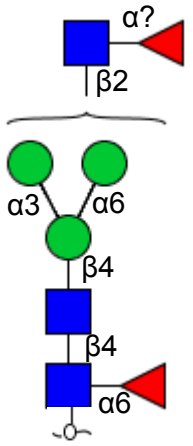
The D ion was observed suggesting a Man 6' arm position of the non-reducing end β2-GlcNAc residue.

Observed *m/z* 1260.4 (1-), RT: 50.5 min
$[M-H]^-$ 1260.4 Da



Supplementary Figure S3B

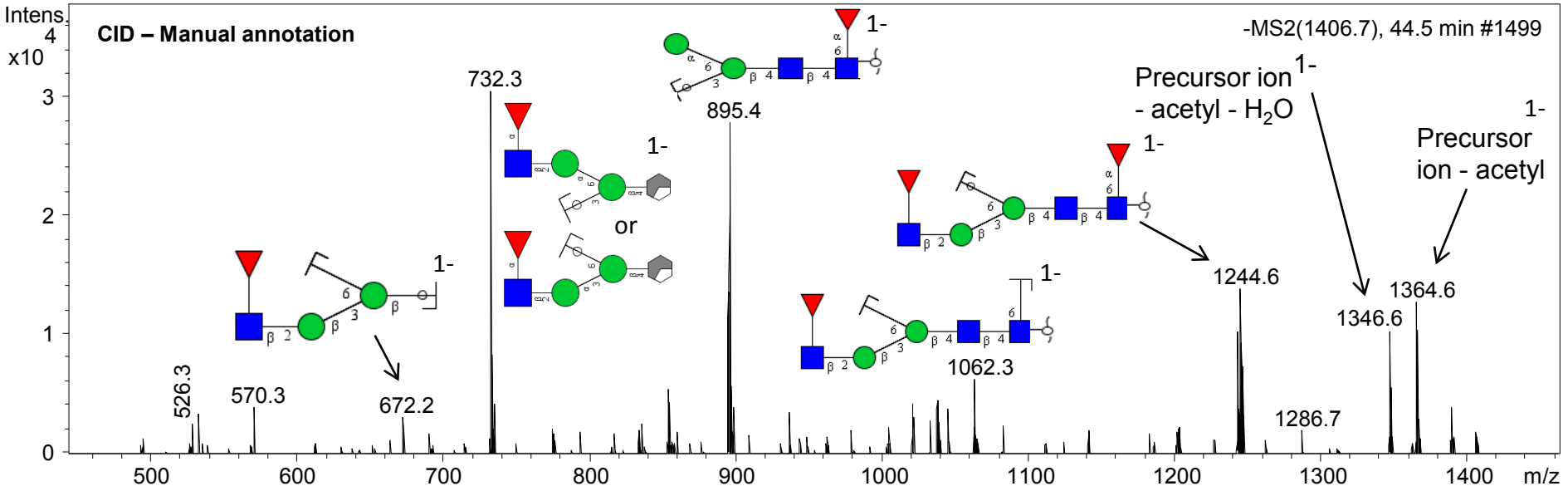
Glycan # 6



FA1F1

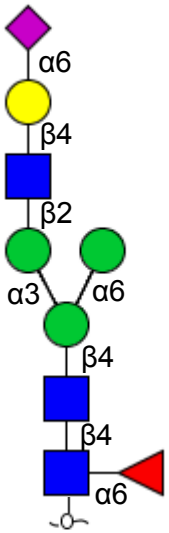
D ion (*m/z* 526.3) was observed, but was not prominent. Thus, neither a Man 3' or Man 6' arm position can be assigned based on the present spectrum and this structural feature is left unassigned for this glycan (may be an unresolved mixture of the two isoforms).

Observed *m/z* 1406.7 (1-), RT: 44.5 min
$[M-H]^-$ 1406.7 Da



Supplementary Figure S3B

Glycan # 7a

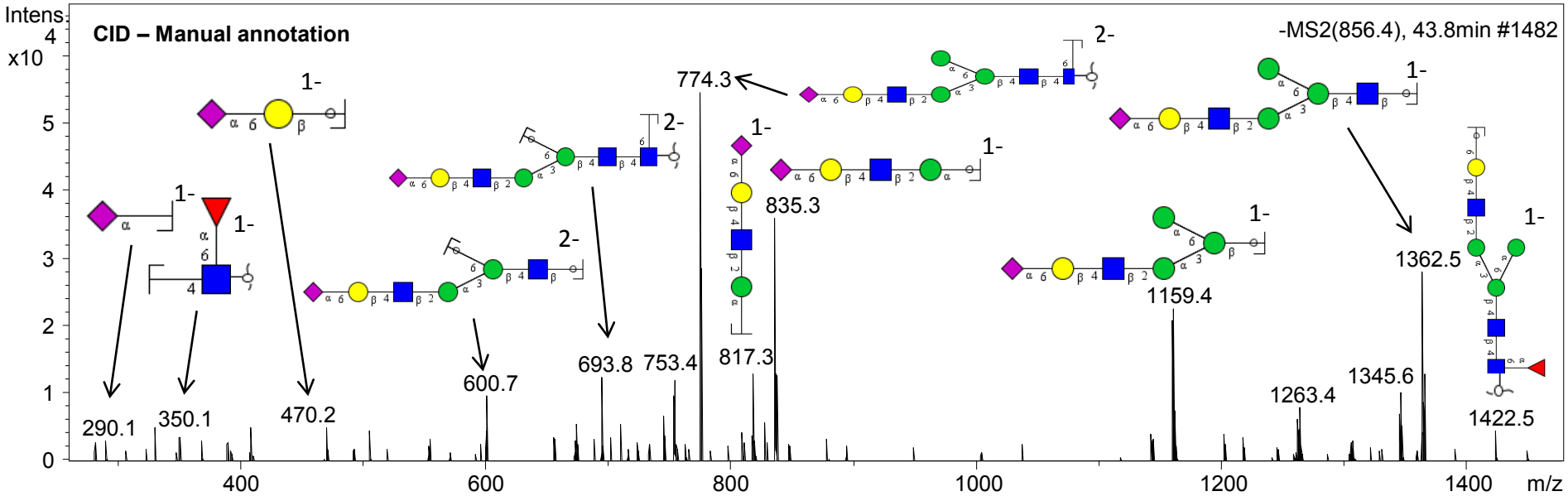


FA1G1S1

Observed *m/z* 856.3 (2-), RT: 43.8 min

$[M-H]^-$ 1713.6 Da

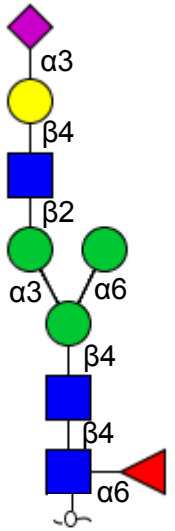
No D ions were observed suggesting a Man 3' arm position of the sialyl LacNAc antennae. Relative PGC-LC retention time was used to determine sialyl linkage.



Supplementary Figure S3B

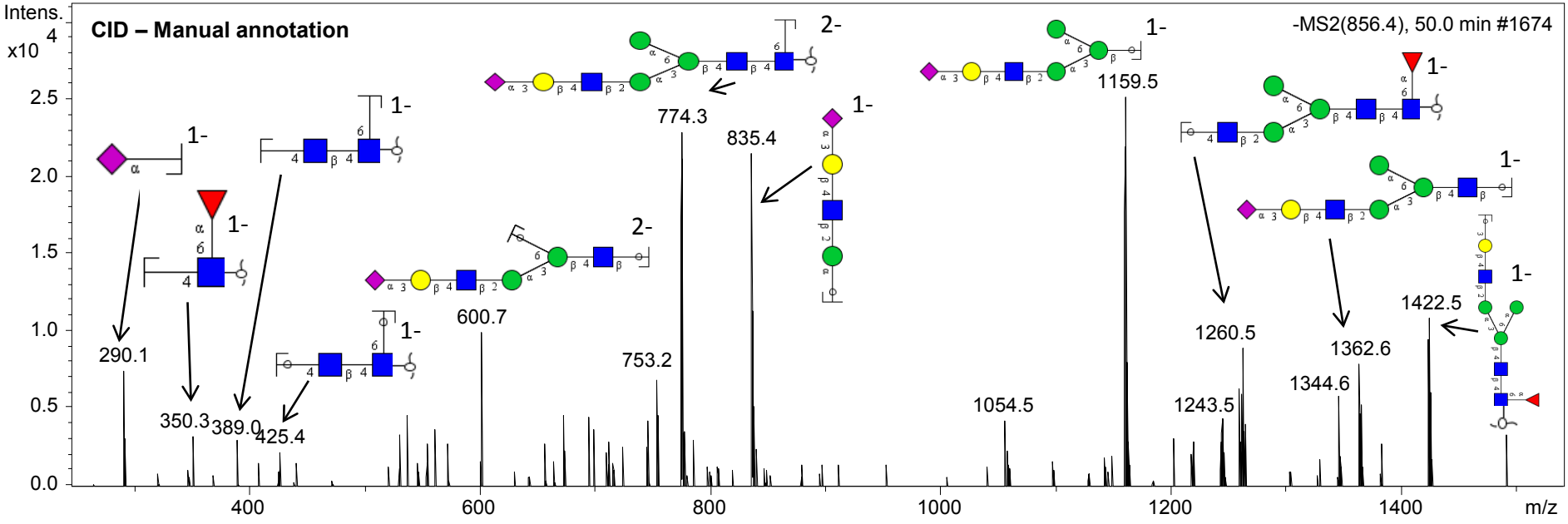
Glycan # 7b

No D ions observed suggesting a Man 3' arm position of the sialyl LacNAc antennae. Relative PGC-LC retention time was used to determine sialyl linkage.



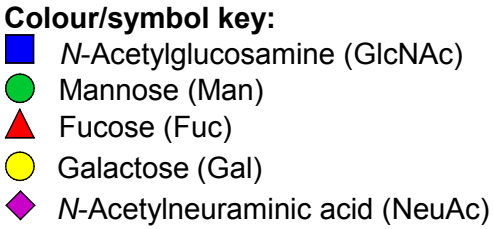
FA1G1S1

Observed *m/z* 856.3 (2-), RT: 50.0 min
$[M-H]^-$ 1713.6 Da



Supplementary Figure S4A

Annotated reversed phase-LC-ESI-CID/ETD-MS/MS spectra of all glycosylated and non-glycosylated peptides identified from the unenriched and enriched peptide mixture of the HNE protein preparation. Annotated CID/ETD-MS/MS spectra of the N-terminal and C-terminal of HNE are also shown.



Human neutrophil elastase – P08246

IVGGR (**N-terminal peptide, Ile30**)

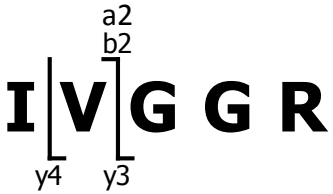
Obs. *m/z* 251.3 (2+)

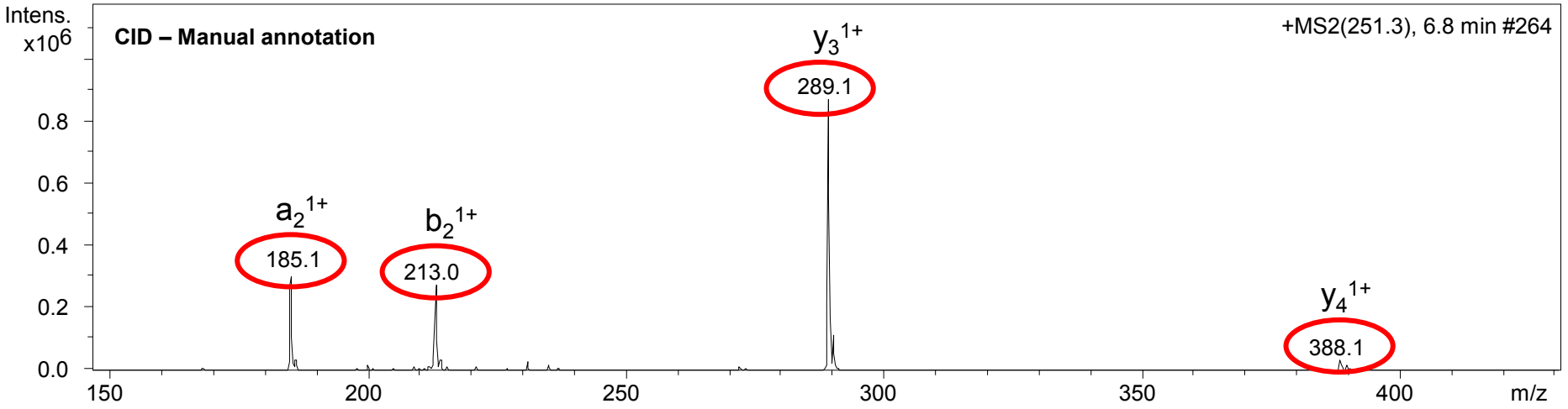
Obs. $[M+H]^+$ = 501.6 Da

Calc. $[M+H]^+$ = 501.3 Da

Retention time: 7 min

Supplementary Figure S4A





Human neutrophil elastase – P08246

NWIDSIIQR (**C-terminal peptide, Arg248**)

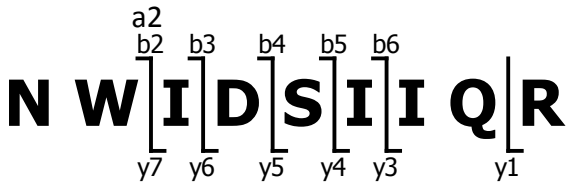
Obs. *m/z* 572.9 (2+)

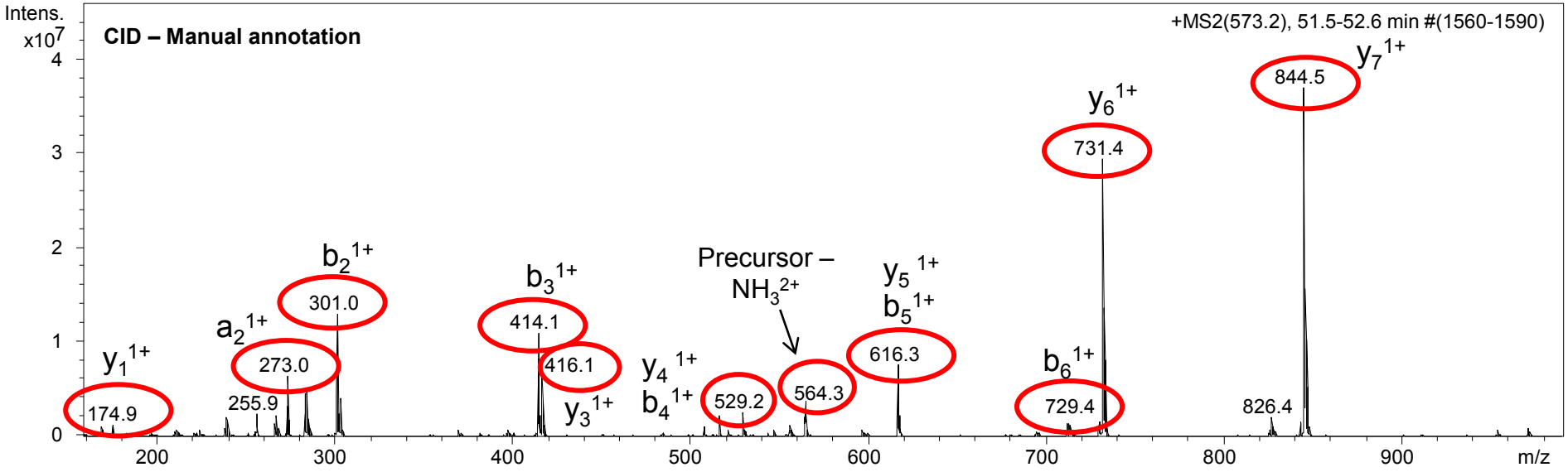
Obs. $[M+H]^+$ = 1144.8 Da

Calc. $[M+H]^+$ = 1144.6 Da

Retention time: 53 min

Supplementary Figure S4A





Human neutrophil elastase – P08246

VLGAHNLSR (Asn88 peptide)

Obs. *m/z* 483.8 (2+)

Obs. $[M+H]^+$ = 966.6 Da

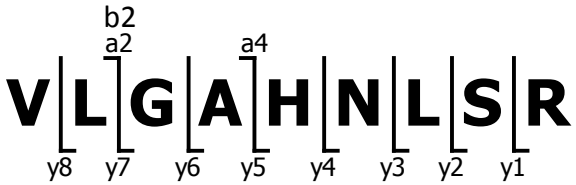
Non-glycosylated peptide

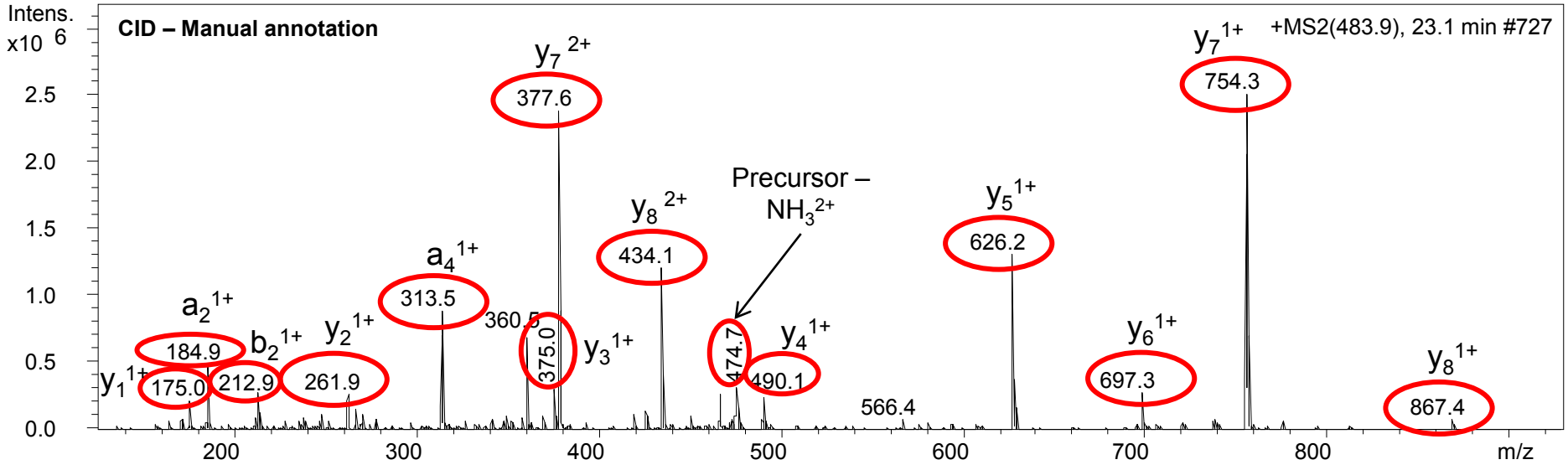
Calc. $[M+H]^+$ = 966.6 Da

Retention time: 23 min

Supplementary Figure S4A

Non-glycosylated peptide





Human neutrophil elastase – P08246

Supplementary Figure S4A

VLGAH**N**LSR (Asn88 glycopeptide)

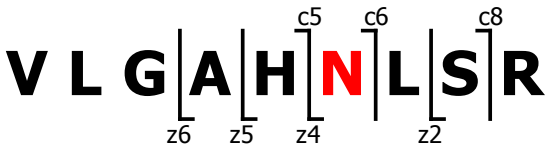
Obs. *m/z* 566.4 (3+)

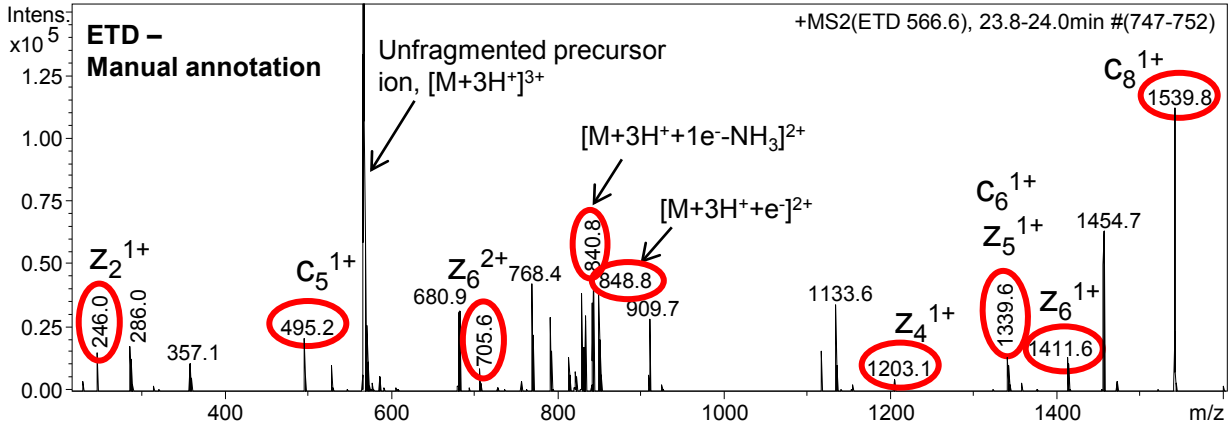
Obs. $[M+H]^+$ = 1697.2 Da

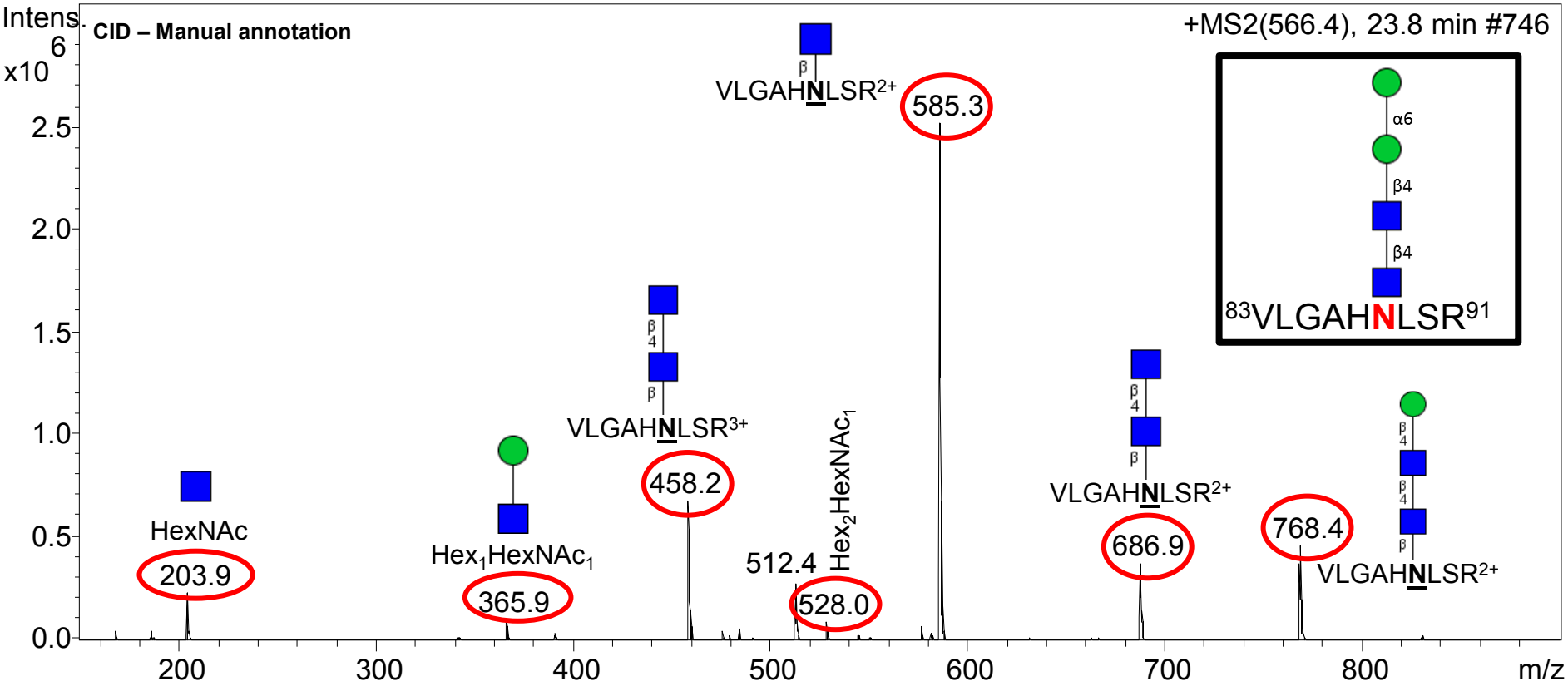
Glycan: 730.3 Da ($Hex_2HexNAc_2$)

Calc. $[M+H]^+$ = 1696.8 Da

Retention time: 24 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

LGAHNLSR (Asn88 peptide)

Obs. *m/z* 434.2 (2+)

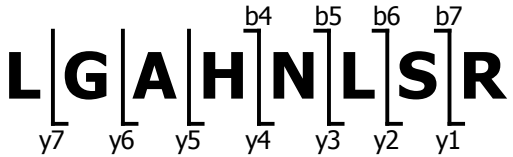
Obs. $[M+H]^+$ = 867.4 Da

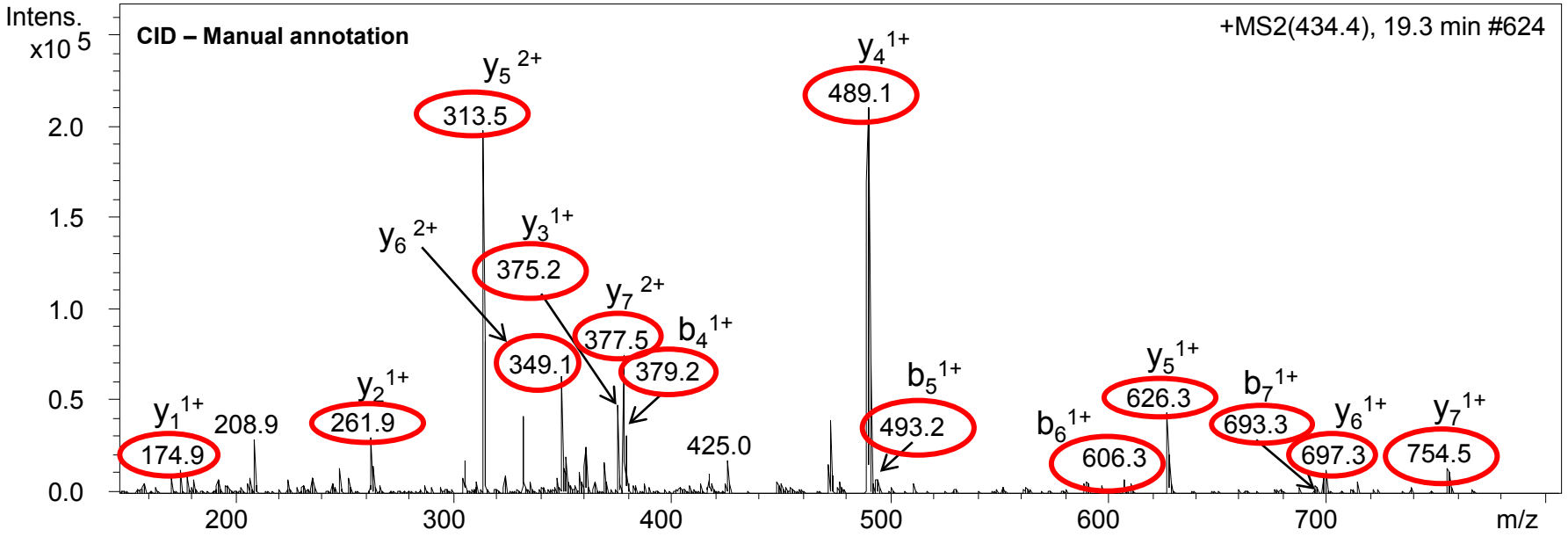
Non-glycosylated peptide

Calc. $[M+H]^+$ = 867.4 Da

Retention time: 19 min

Non-glycosylated peptide

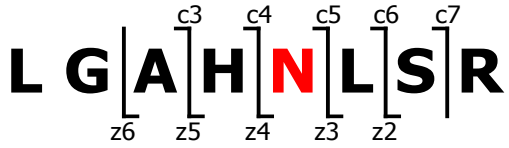


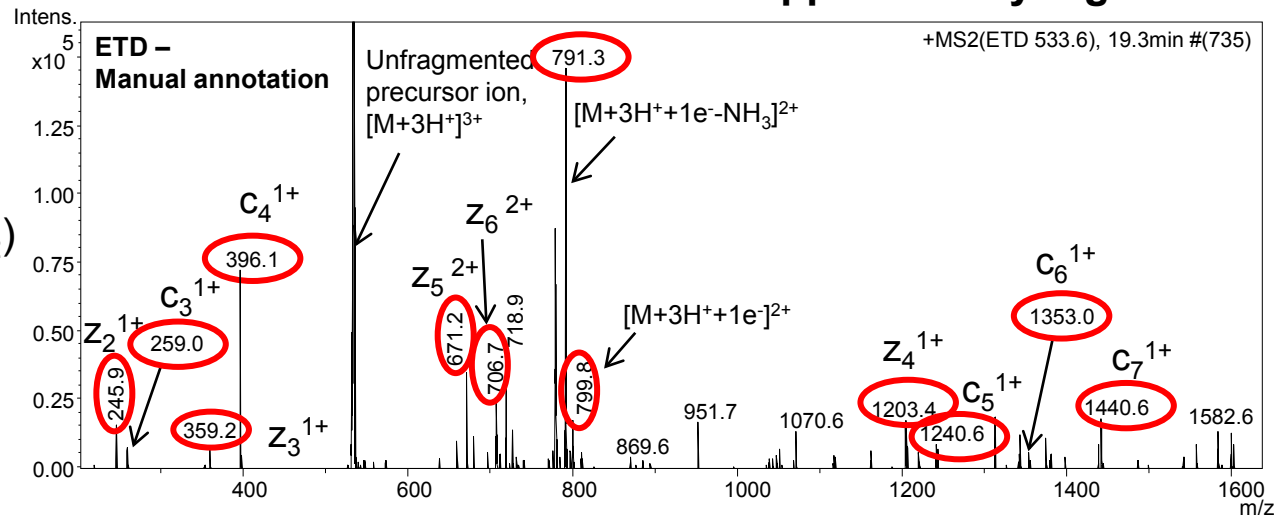


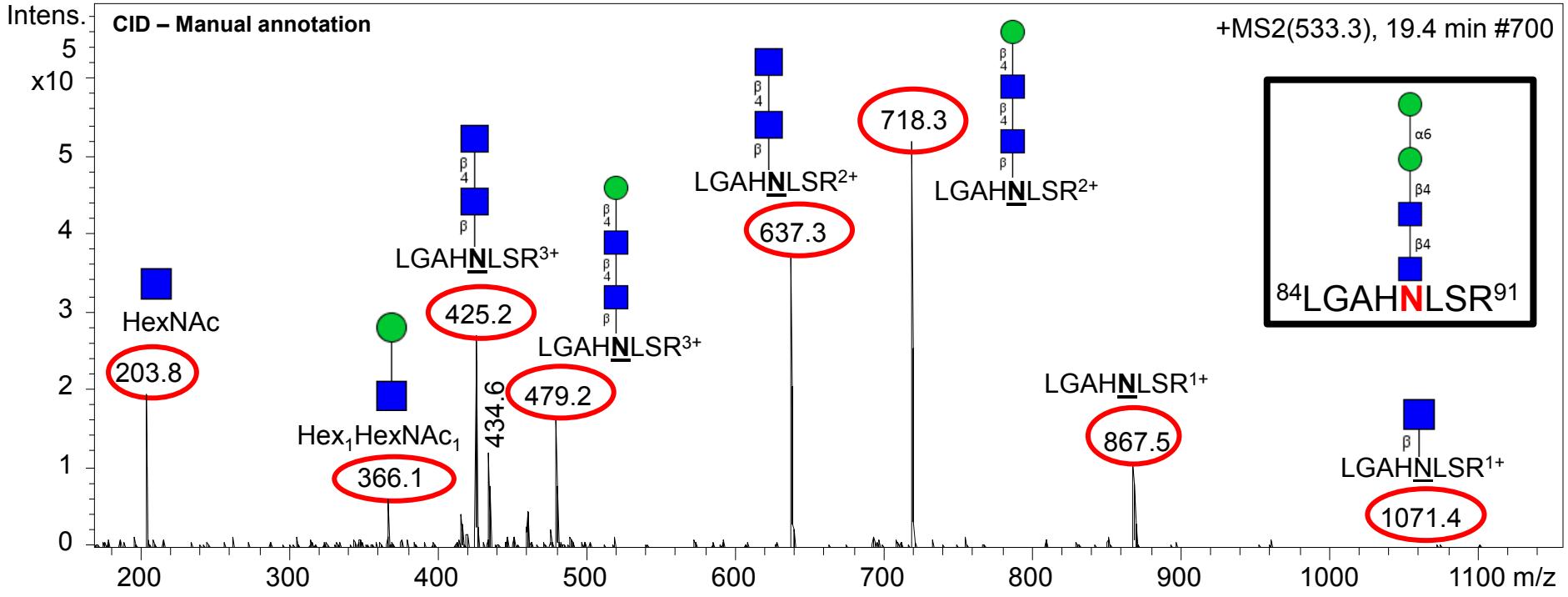
Human neutrophil elastase – P08246

Supplementary Figure S4A

LGAH**N**LSR
(Asn88 glycopeptide)
Obs. *m/z* 533.3 (3+)
Obs. $[M+H]^+$ = 1597.9 Da
Glycan: 730.2 Da ($Hex_2HexNAc_2$)
Calc. $[M+H]^+$ = 1597.7 Da
Retention time: 19 min







Human neutrophil elastase – P08246

VVLGAHNLSR (Asn88 peptide)

Obs. *m/z* 356.0 (3+)

Obs. $[M+H]^+$ = 1066.0 Da

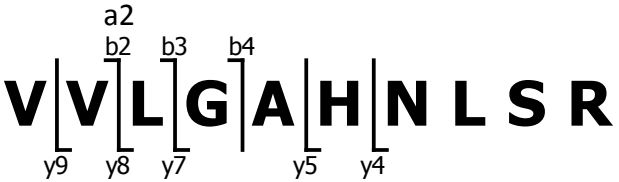
Non-glycosylated peptide

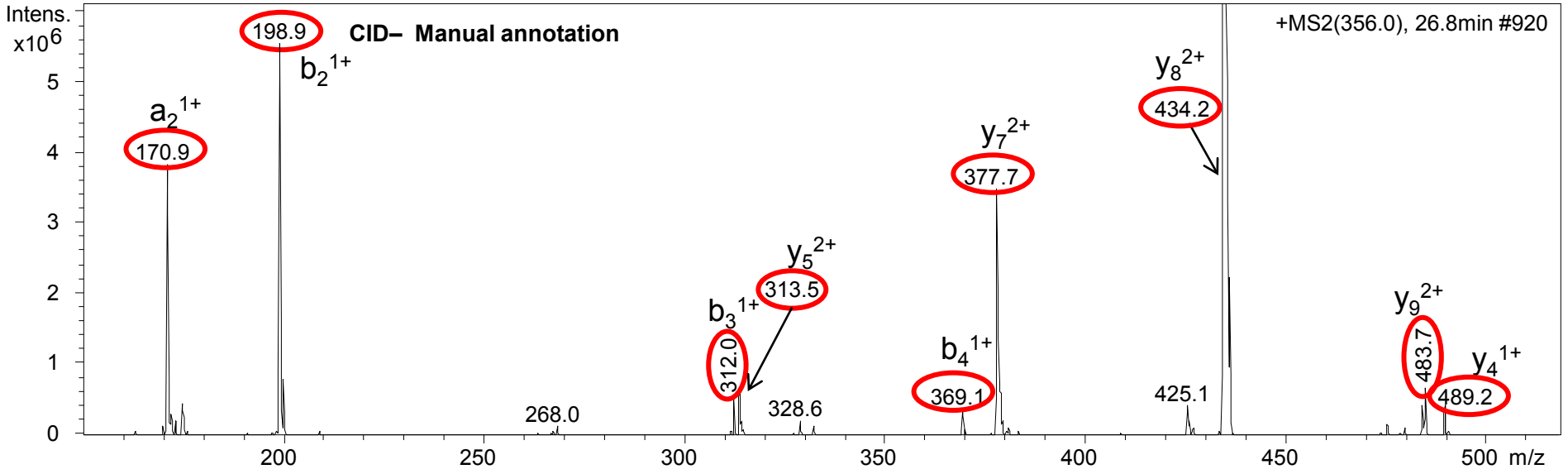
Calc. $[M+H]^+$ = 1065.8 Da

Retention time: 26 min

Supplementary Figure S4A

Non-glycosylated peptide





Human neutrophil elastase – P08246

Supplementary Figure S4A

VVLGAH**N**LSR (Asn88 glycopeptide)

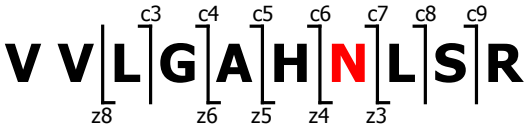
Obs. *m/z* 599.3 (3+)

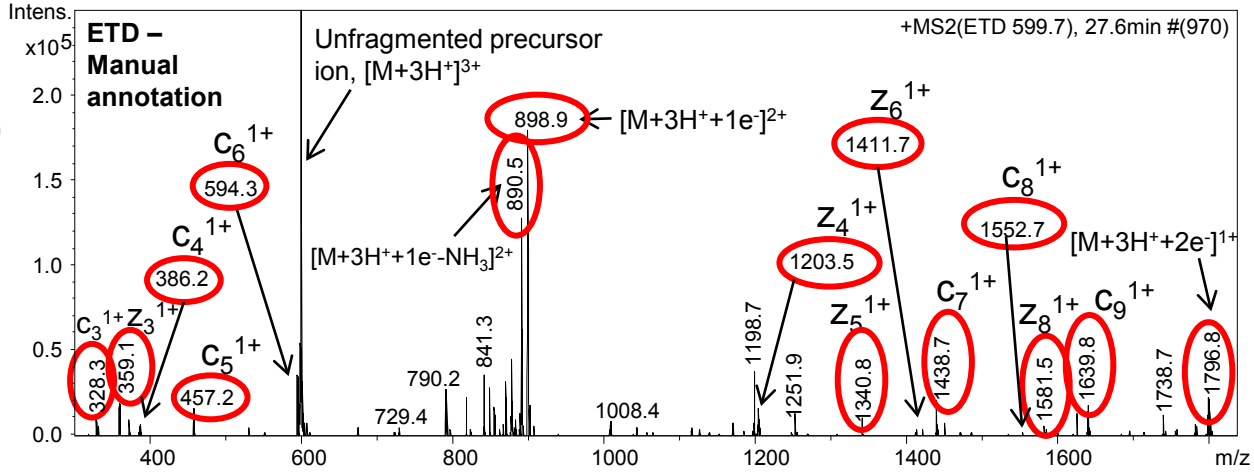
Obs. $[M+H]^+$ = 1795.9 Da

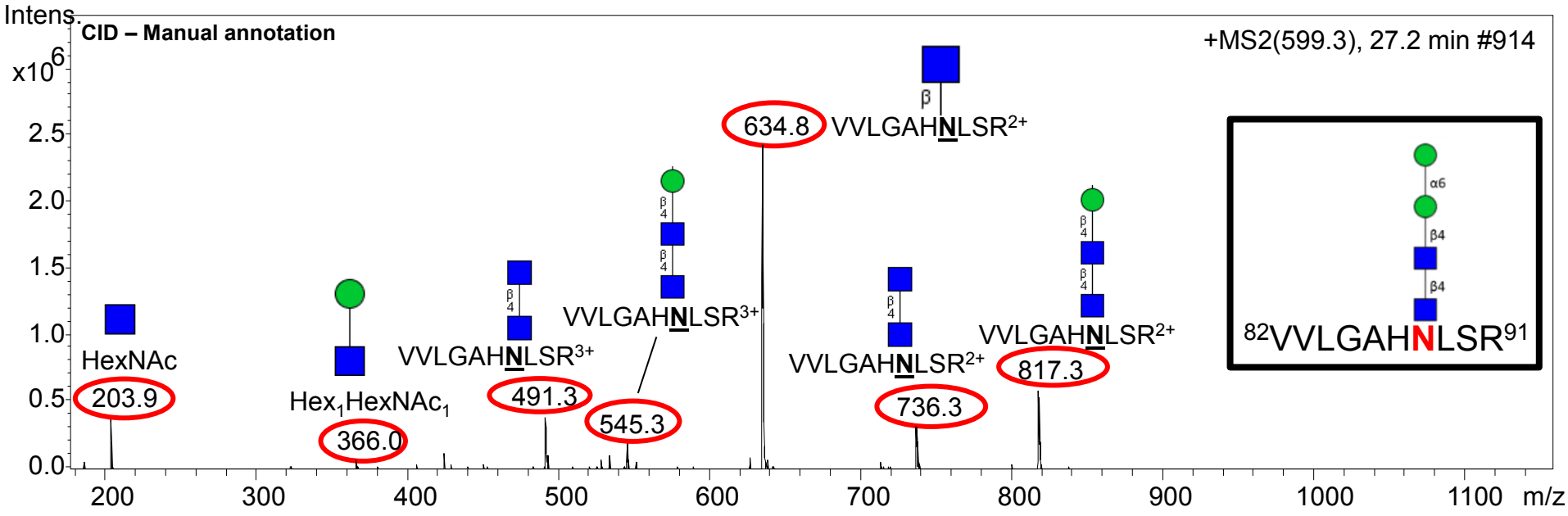
Glycan: 730.3 Da ($Hex_2HexNAc_2$)

Calc. $[M+H]^+$ = 1795.8 Da

Retention time: 27 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

ILQL**N**GSATI (Asn124 glycopeptide)

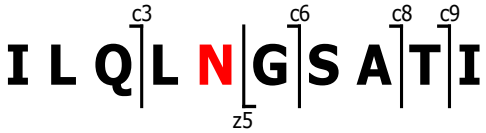
Obs. *m/z* 806.4 (3+)

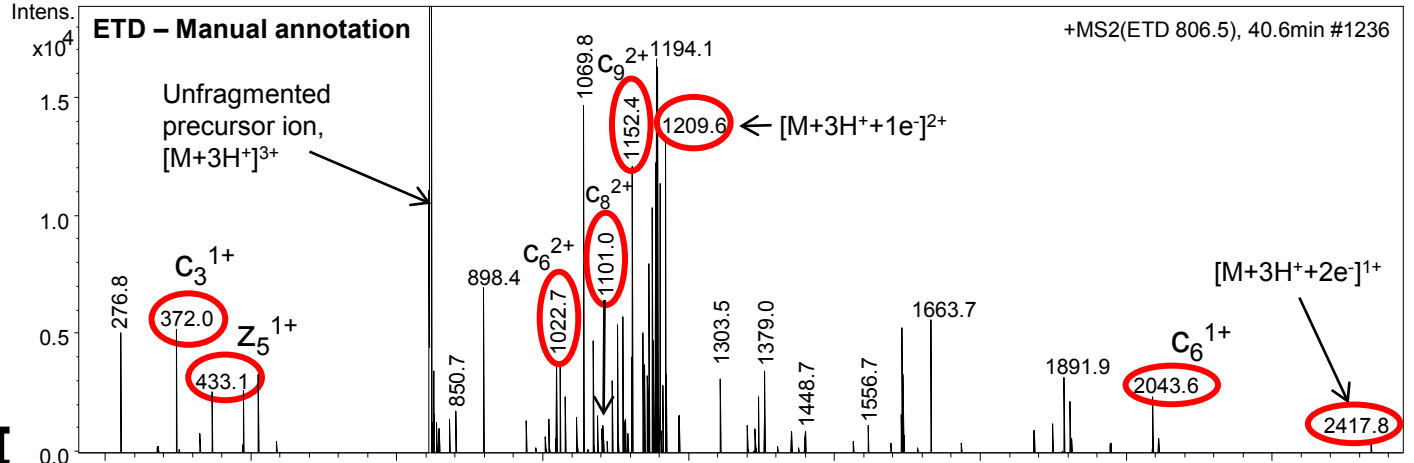
Obs. $[M+H]^+$ = 2417.2 Da

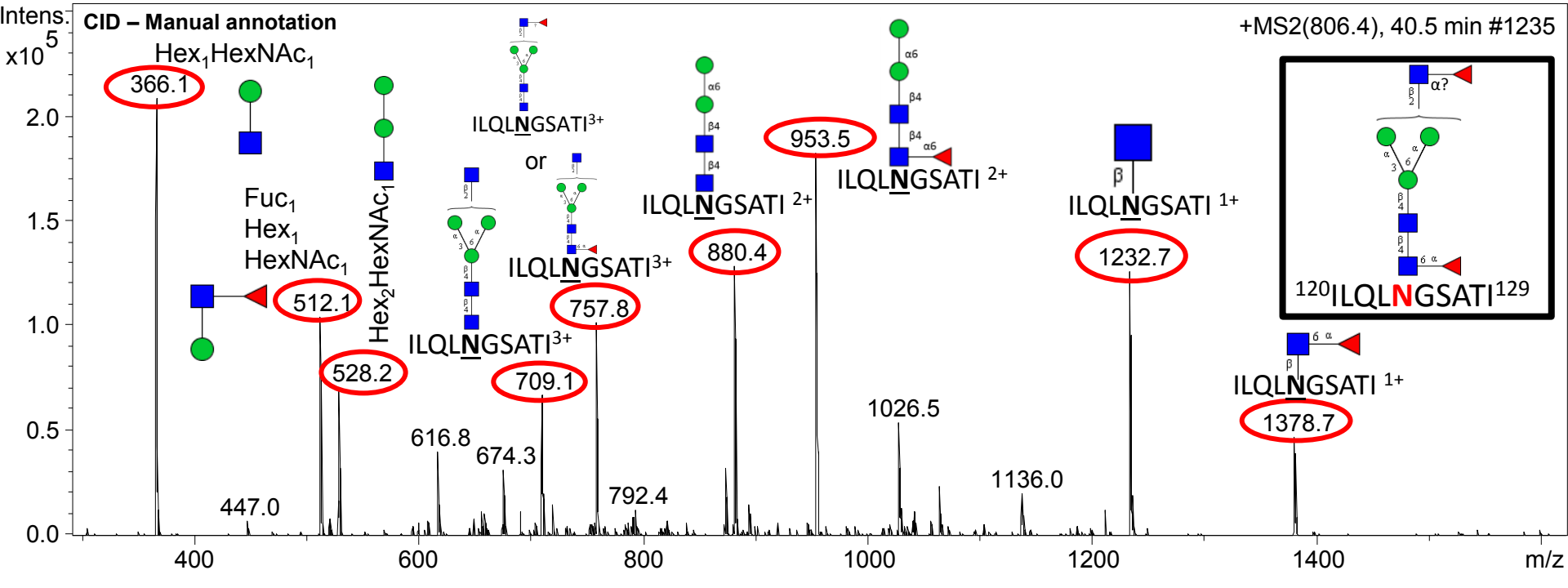
Glycan: 1387.5 Da ($Hex_3HexNAc_3Fuc_2$)

Calc. $[M+H]^+$ = 2417.3 Da

Retention time: 41 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

ILQL**N**GSATI (Asn124 glycopeptide)

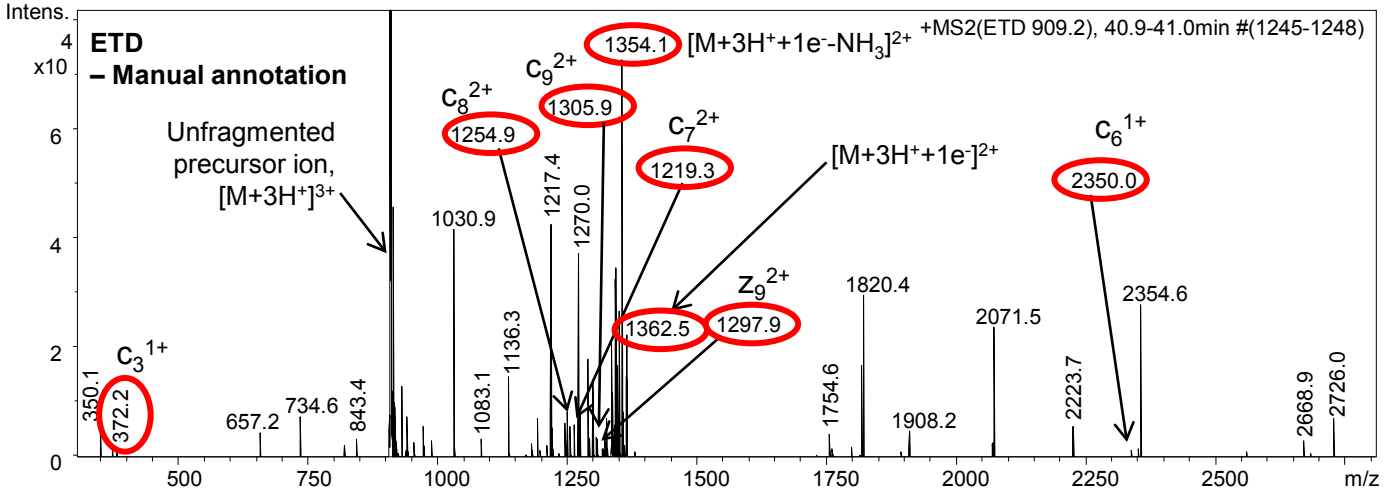
Obs. *m/z* 908.9 (3+)

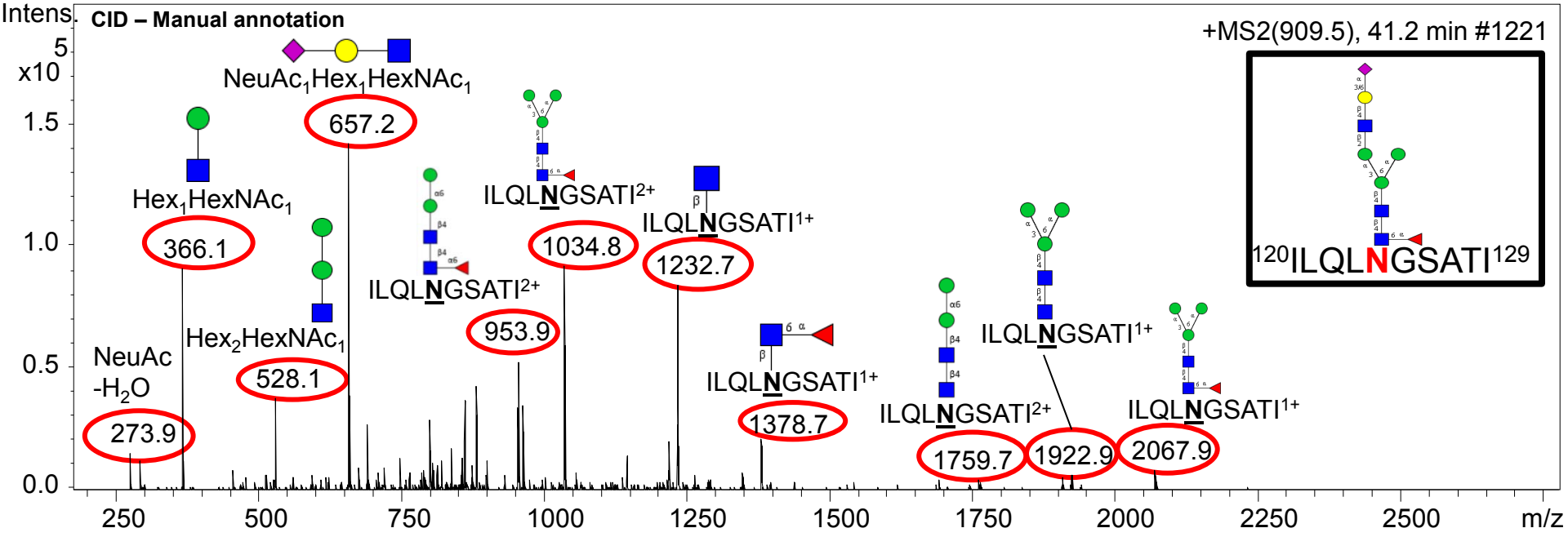
Obs. $[M+H]^+$ = 2724.7 Da

Glycan: 1694.6 Da ($Hex_4HexNAc_3Fuc_1NeuAc_1$)

Calc. $[M+H]^+$ = 2724.4 Da

Retention time: 41 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

ILQL**N**GSATI (Asn124 glycopeptide)

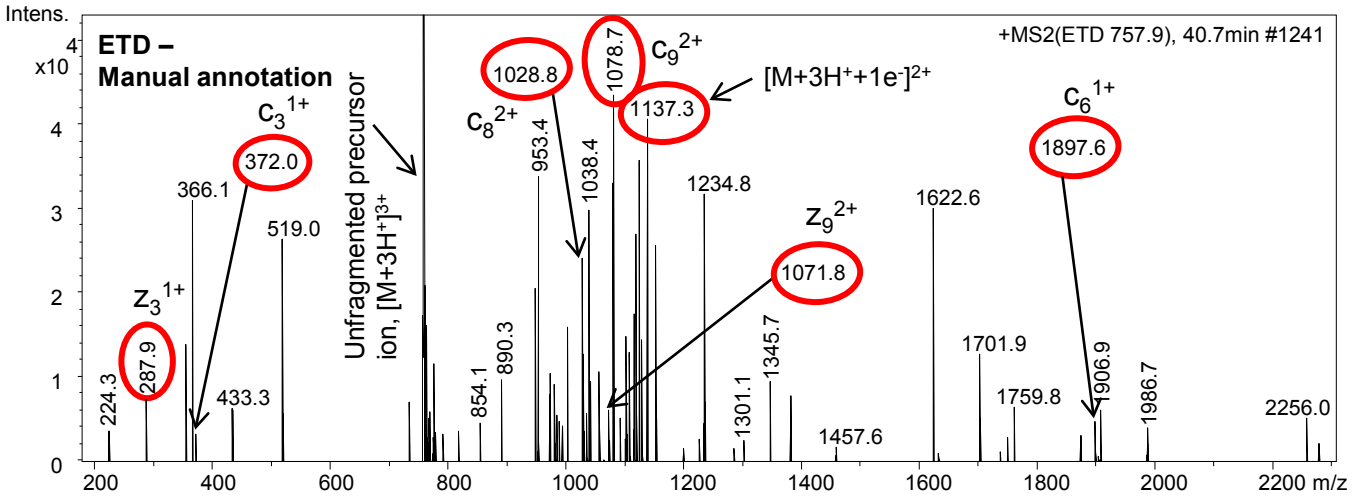
Obs. *m/z* 757.9 (3+)

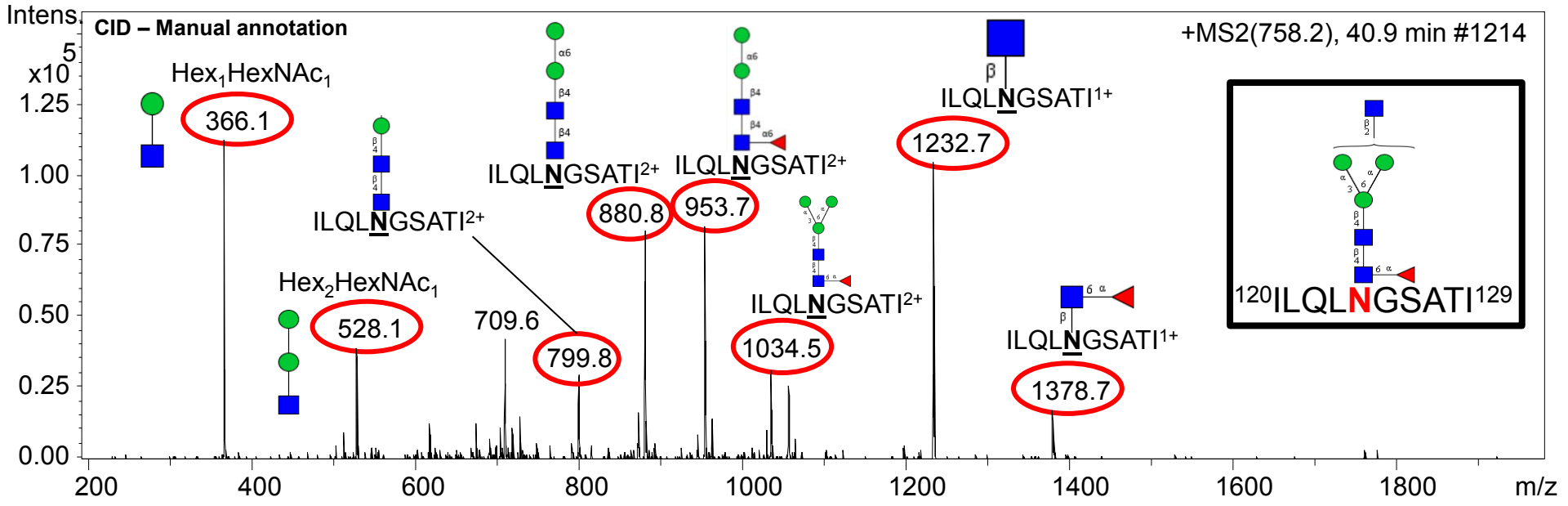
Obs. $[M+H]^+$ = 2271.7 Da

Glycan: 1241.5 Da ($Hex_3HexNAc_3Fuc_1$)

Calc. $[M+H]^+$ = 2271.0 Da

Retention time: 41 min





Human neutrophil elastase– P08246

Supplementary Figure S4A

ILQL**N**GSATI (Asn124 glycopeptide)

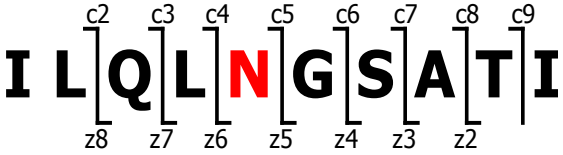
Obs. *m/z* 636.1 (3+)

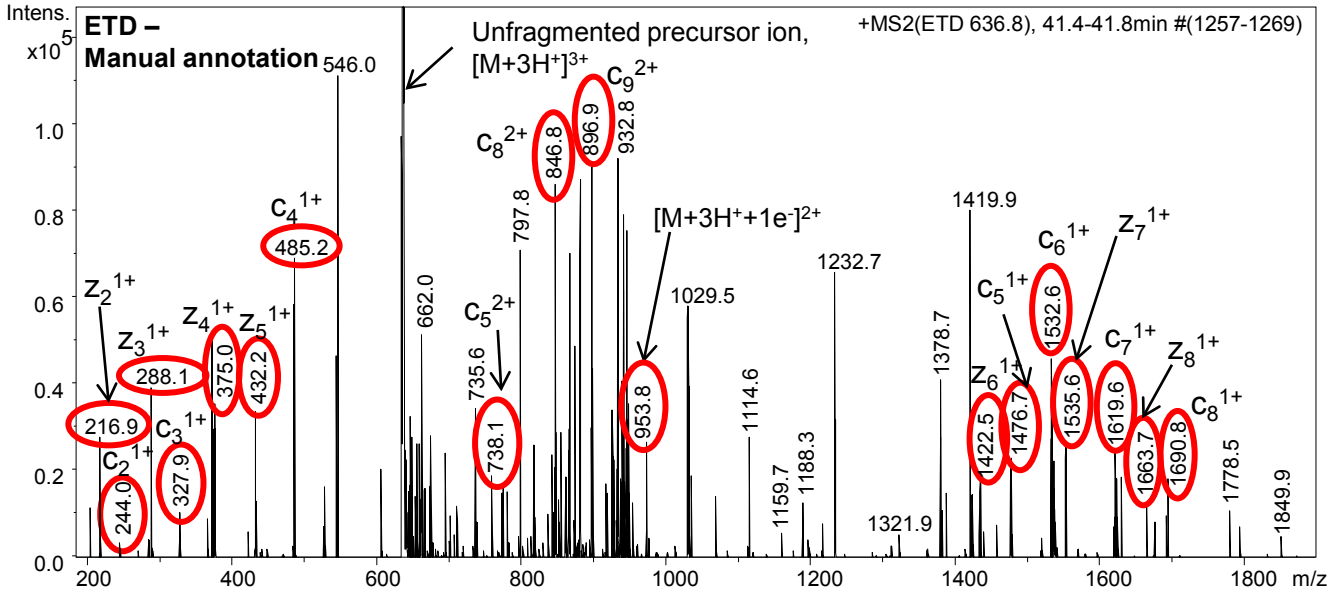
Obs. $[M+H]^+$ = 1906.3 Da

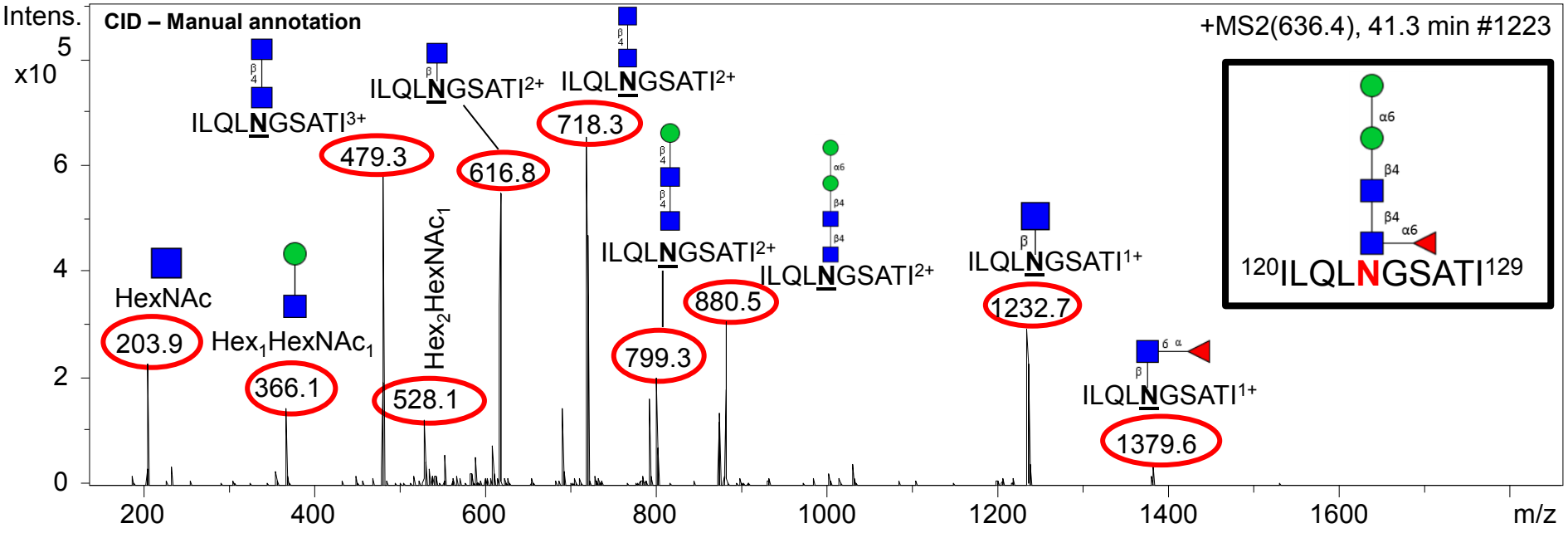
Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1905.9 Da

Retention time: 41 min







Human neutrophil elastase– P08246

Supplementary Figure S4A

ILQL**N**GSATI (Asn124 glycopeptide)

Obs. *m/z* 880.5 (2+)

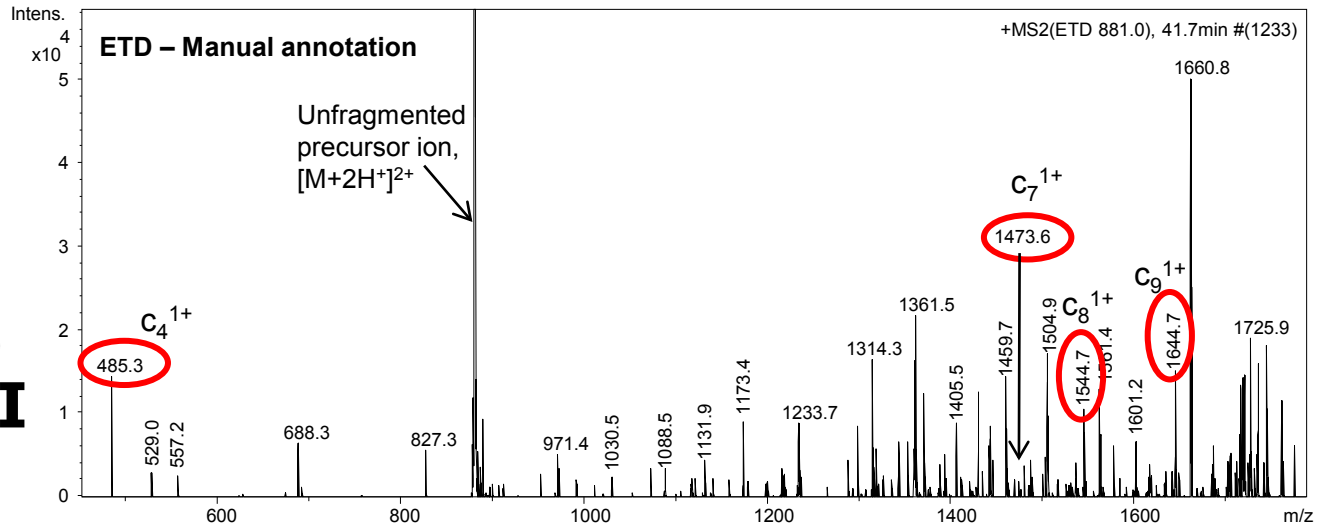
Obs. $[M+H]^+$ = 1760.0 Da

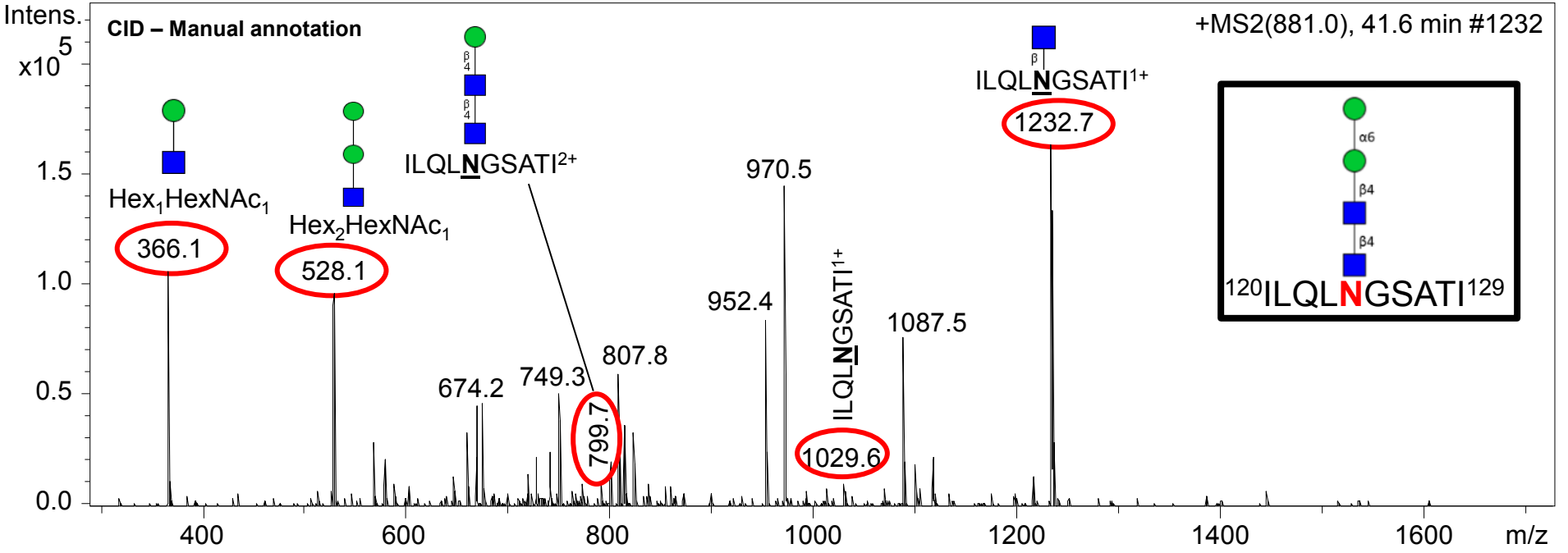
Glycan: 730.3 Da

($Hex_2HexNAc_2$)

Calc. $[M+H]^+$ = 1759.8 Da

Retention time: 42 min

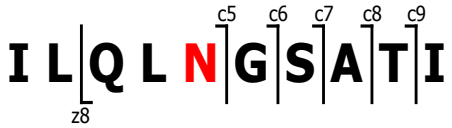


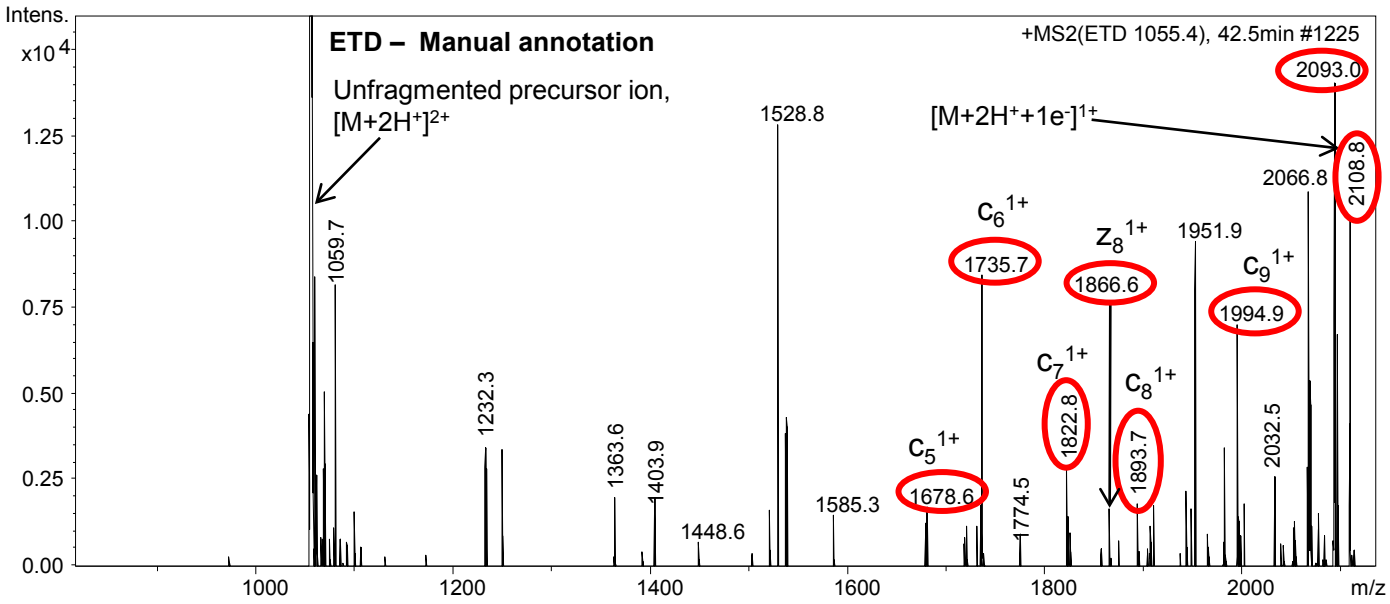


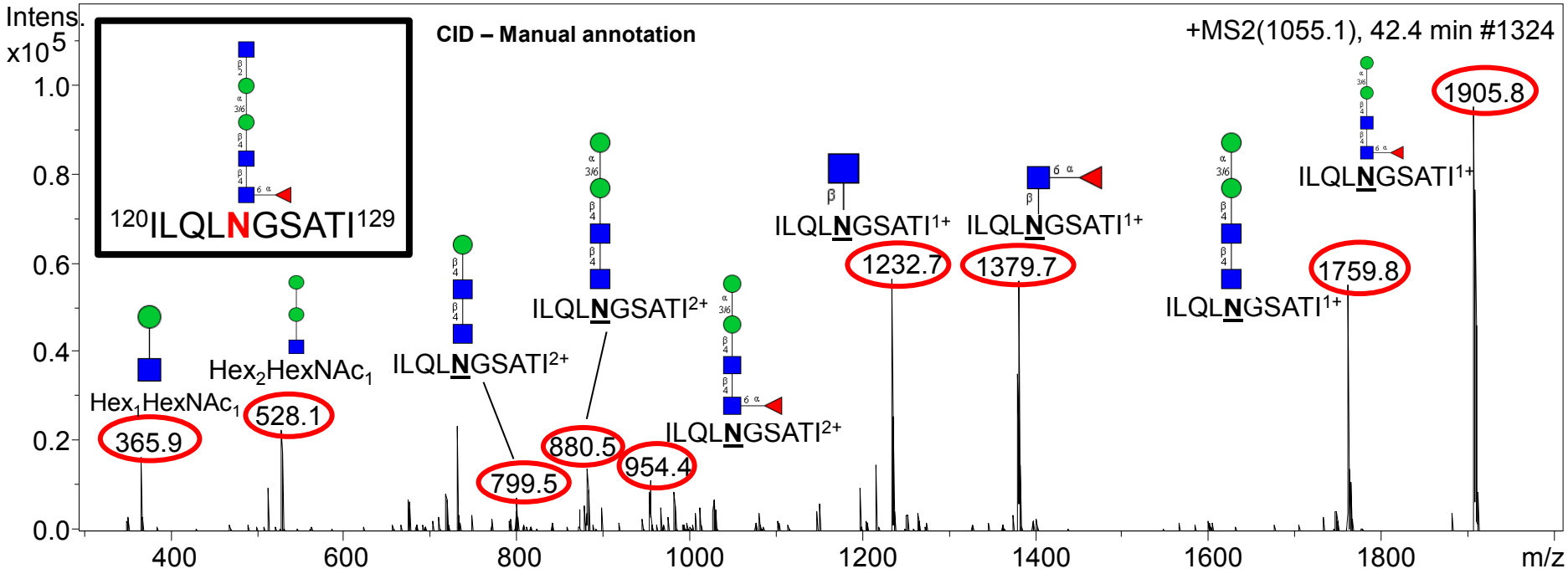
Human neutrophil elastase – P08246

Supplementary Figure S4A

ILQL**N**GSATI
(Asn124 glycopeptide)
Obs. *m/z* 1055.1 (2+)
Obs. $[M+H]^+$ = 2109.2 Da
Glycan: 1079.4 Da
($Hex_2HexNAc_3Fuc_1$)
Calc. $[M+H]^+$ = 2108.9 Da
Retention time: 42 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

ILQL**N**GS (Asn124 glycopeptide)

Obs. *m/z* 811.0 (2+)

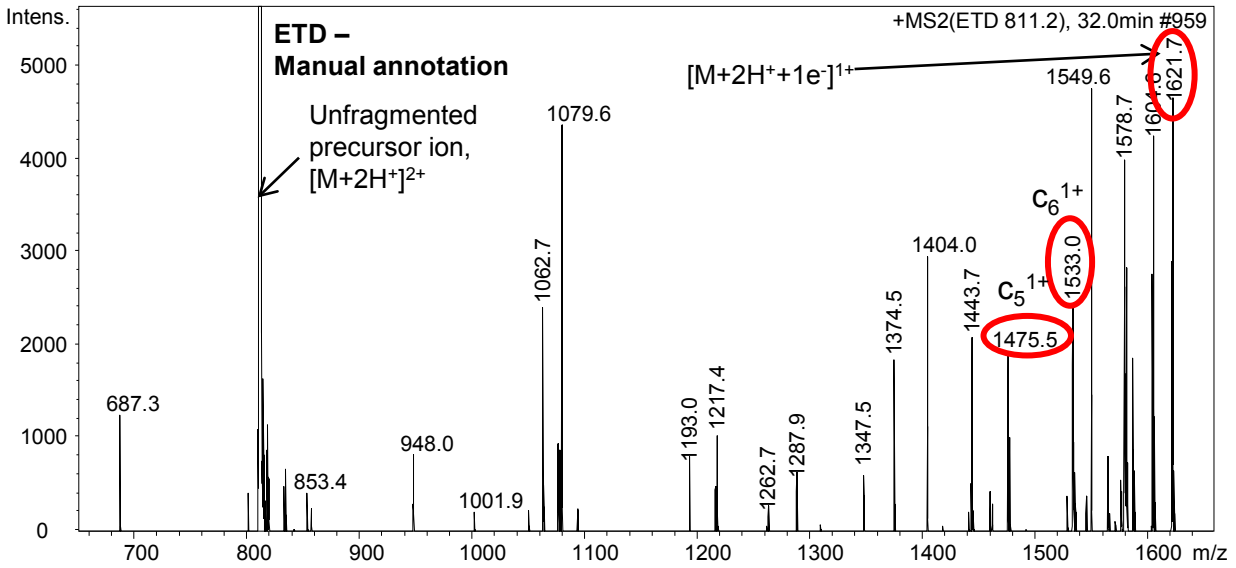
Obs. $[M+H]^+$ = 1621.0 Da

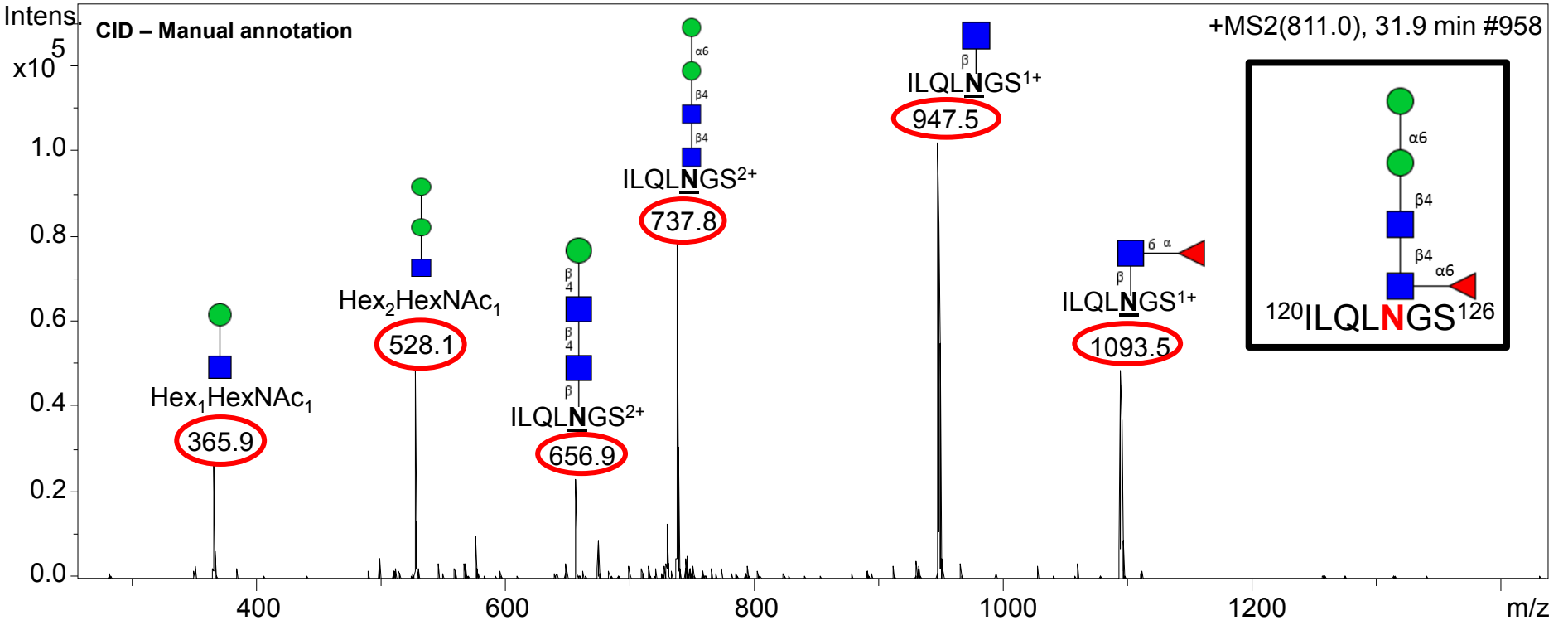
Glycan: 876.3 Da

($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1620.7 Da

Retention time: 32 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

VILQL**N**GSAT (Asn124 glycopeptide)

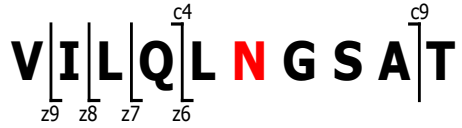
Obs. *m/z* 752.7 (3+)

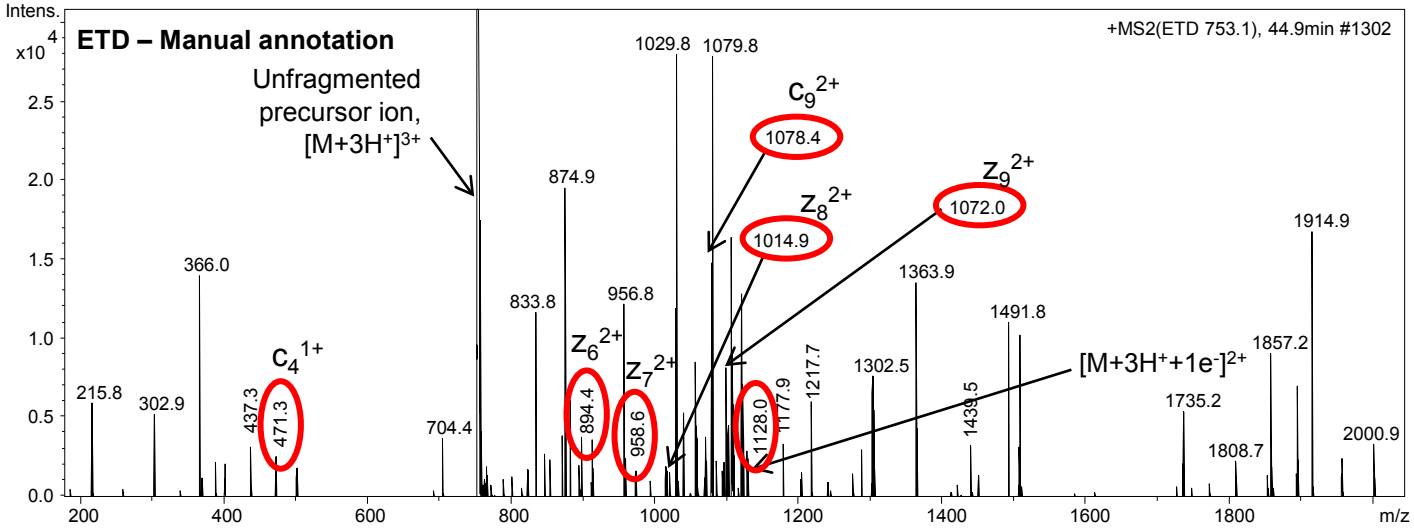
Obs. $[M+H]^+$ = 2256.1 Da

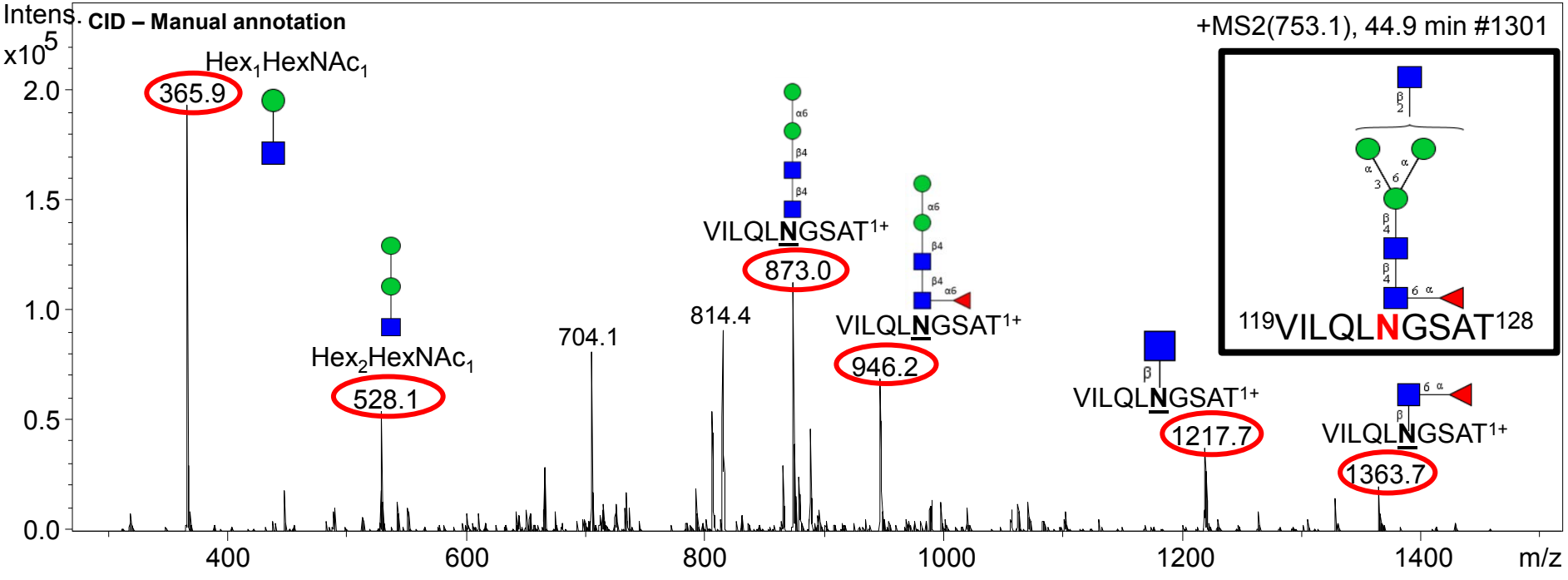
Glycan: 1241.5 Da ($Hex_3HexNAc_3Fuc_1$)

Calc. $[M+H]^+$ = 2257.0 Da

Retention time: 45 min





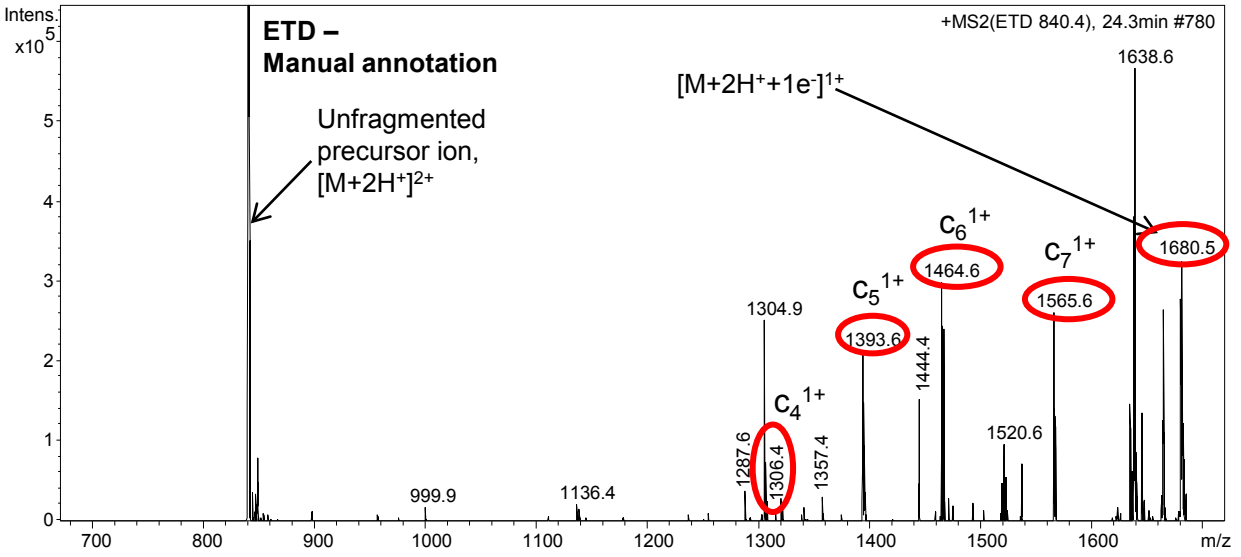


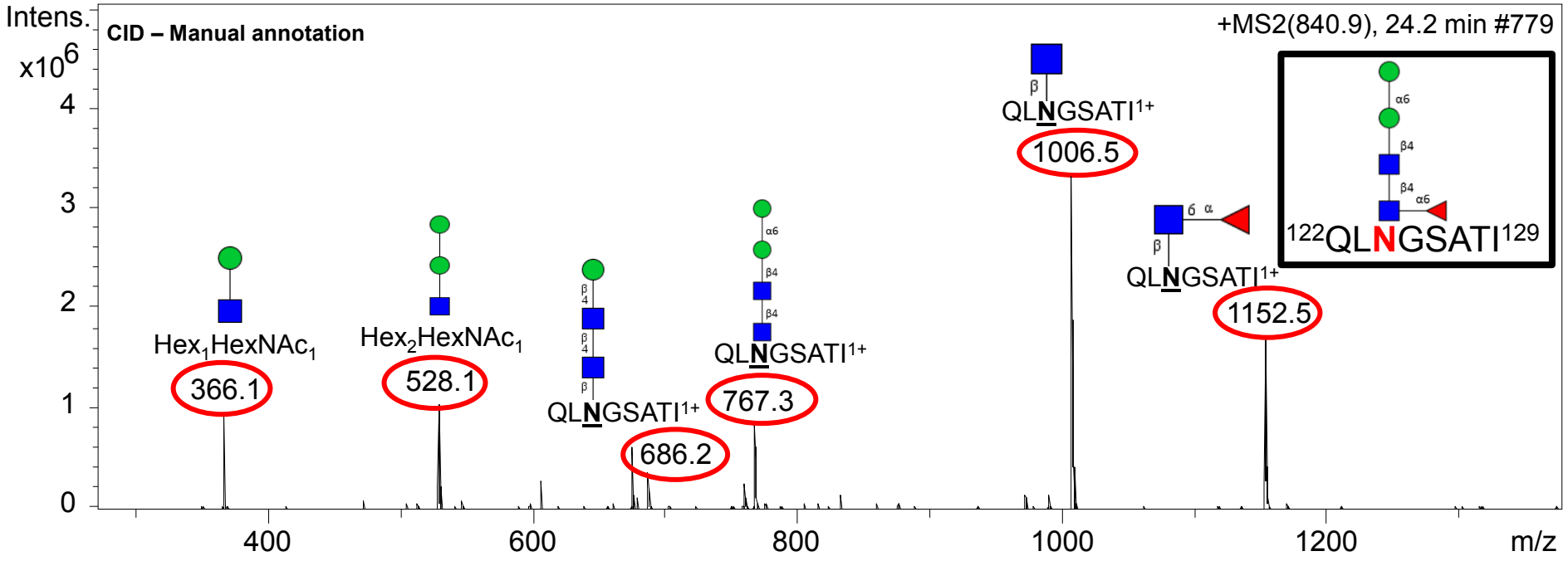
Human neutrophil elastase – P08246

Supplementary Figure S4A

QL**N**GSATI (Asn124 glycopeptide)
Obs. *m/z* 840.5 (2+)
Obs. $[M+H]^+$ = 1680.0 Da
Glycan: 876.3 Da
($Hex_2HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 1679.9 Da
Retention time: 24 min

c4 c5 c6 c7
Q L N G S A T I





Human neutrophil elastase – P08246

Supplementary Figure S4A

LQEL**N**V (Asn173 glycopeptide)

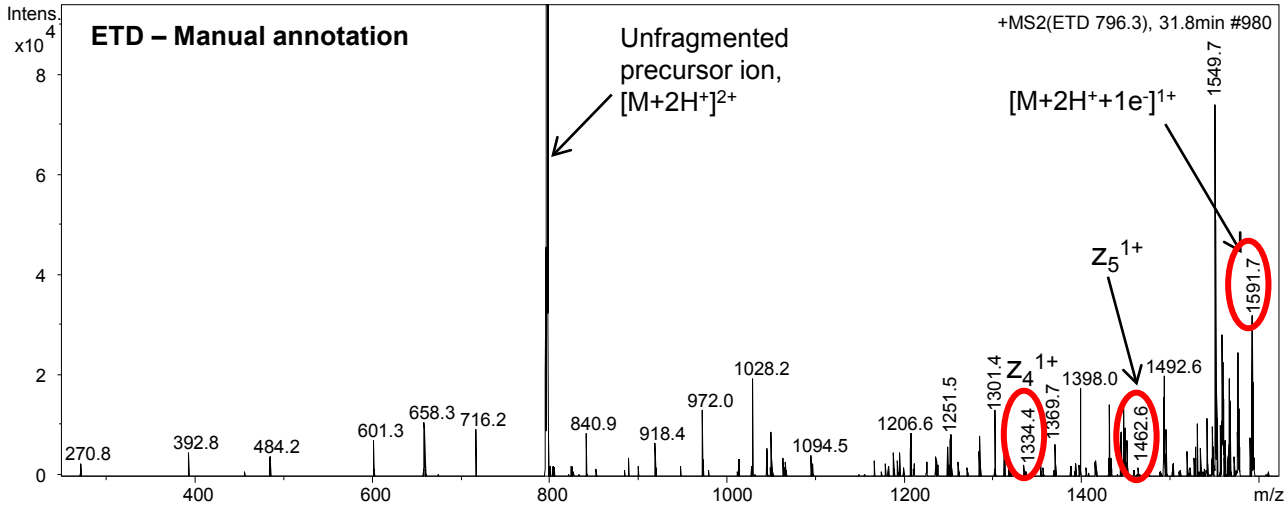
Obs. *m/z* 796.4 (2+)

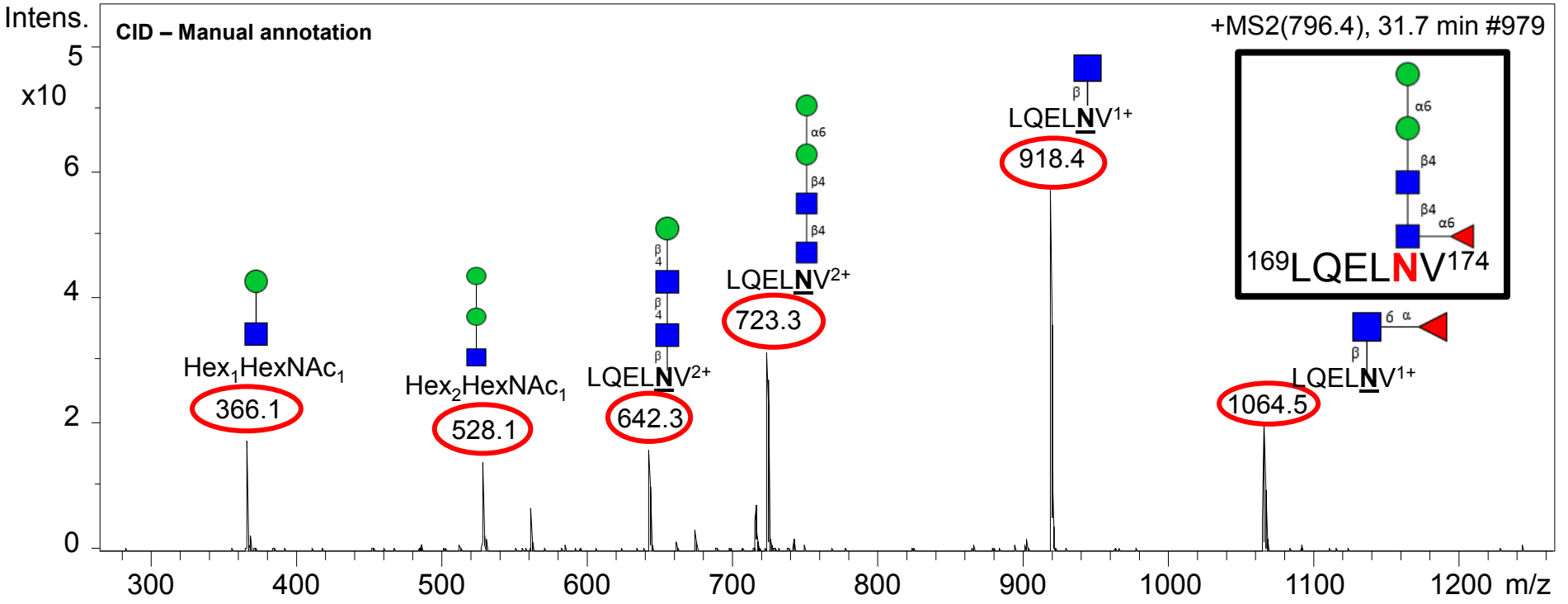
Obs. $[M+H]^+$ = 1591.8 Da

Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1591.7 Da

Retention time: 32 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

LQELNV (Asn173 glycopeptide)

Obs. *m/z* 804.1 (3+)

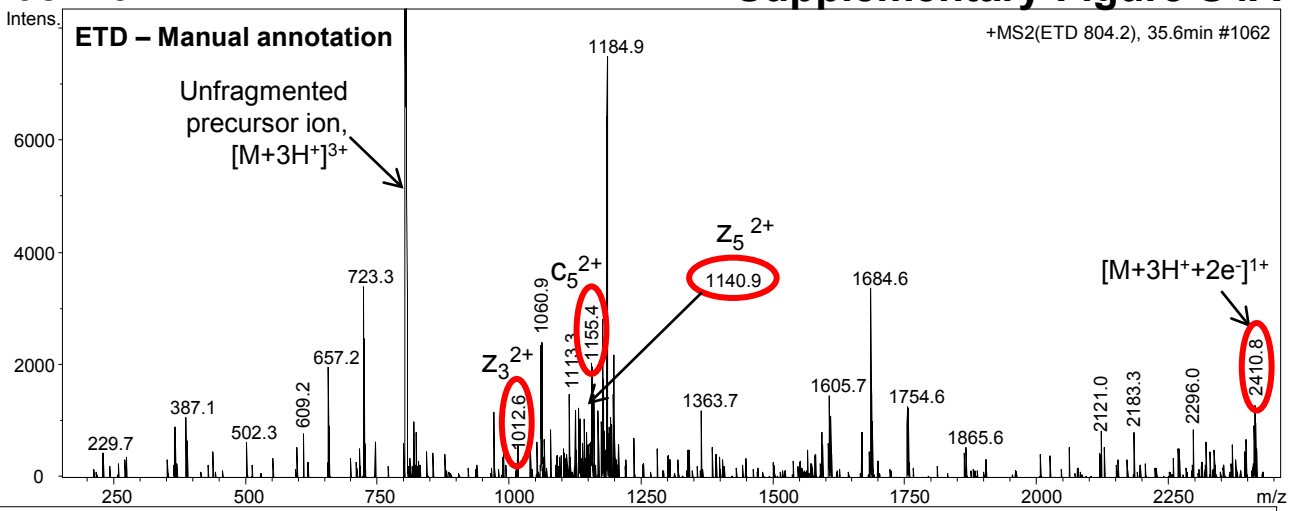
Obs. $[M+H]^+$ = 2410.3 Da

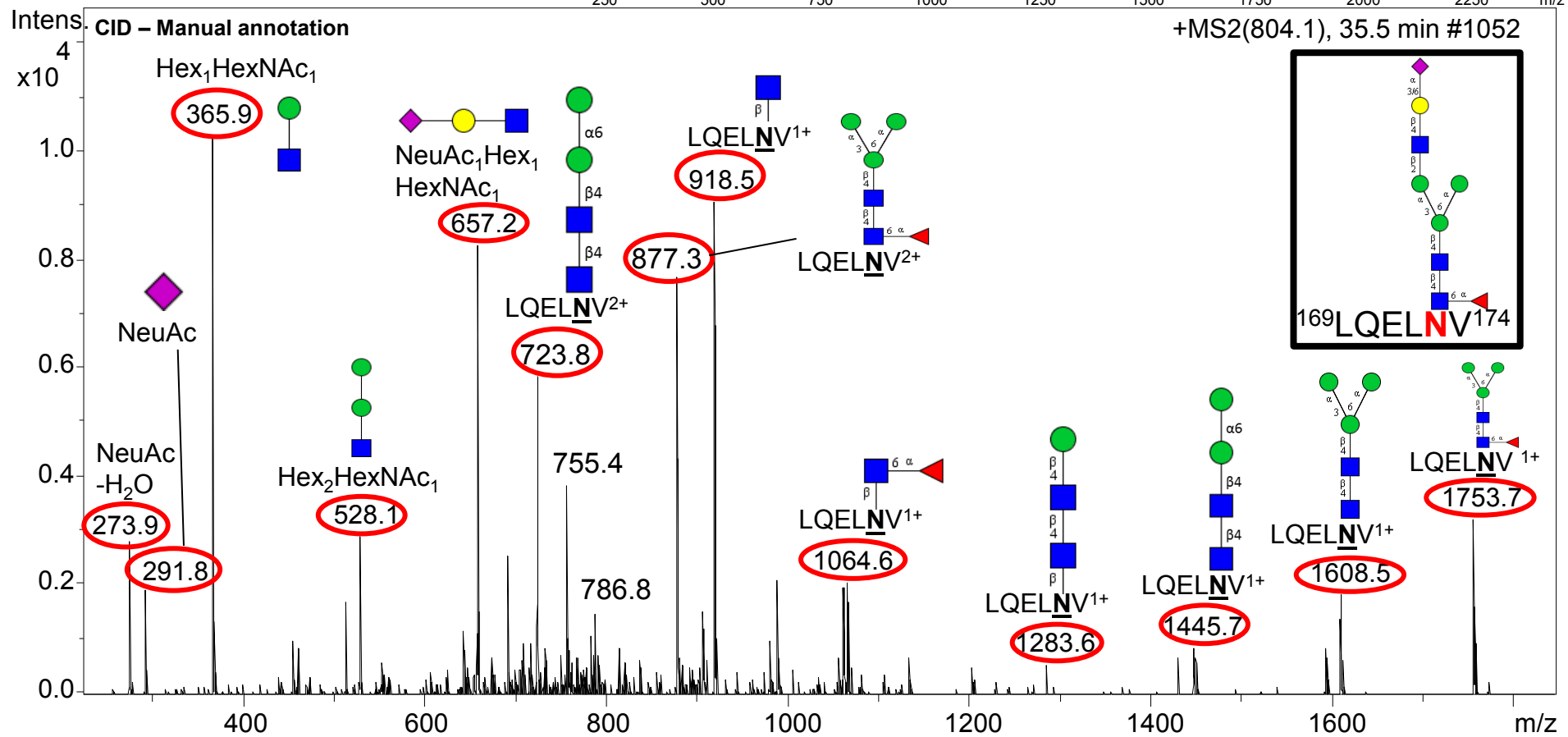
Glycan: 1694.6 Da

($Hex_4HexNAc_3Fuc_1NeuAc_1$)

Calc. $[M+H]^+$ = 2410.0 Da

Retention time: 36 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

LQEL**N**VTV (Asn173 glycopeptide)

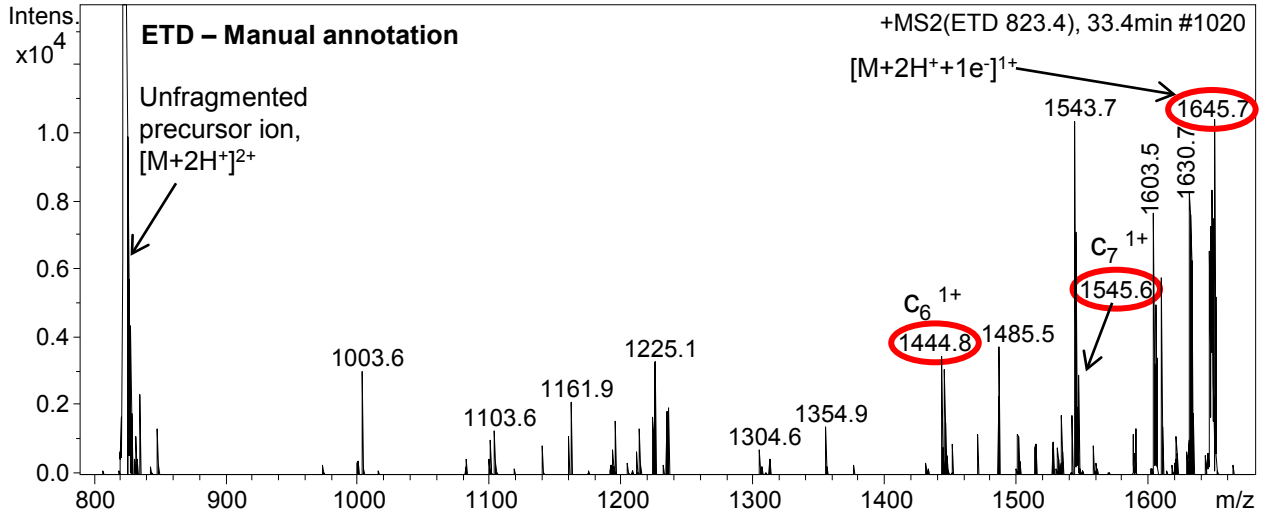
Obs. *m/z* 823.4 (2+)

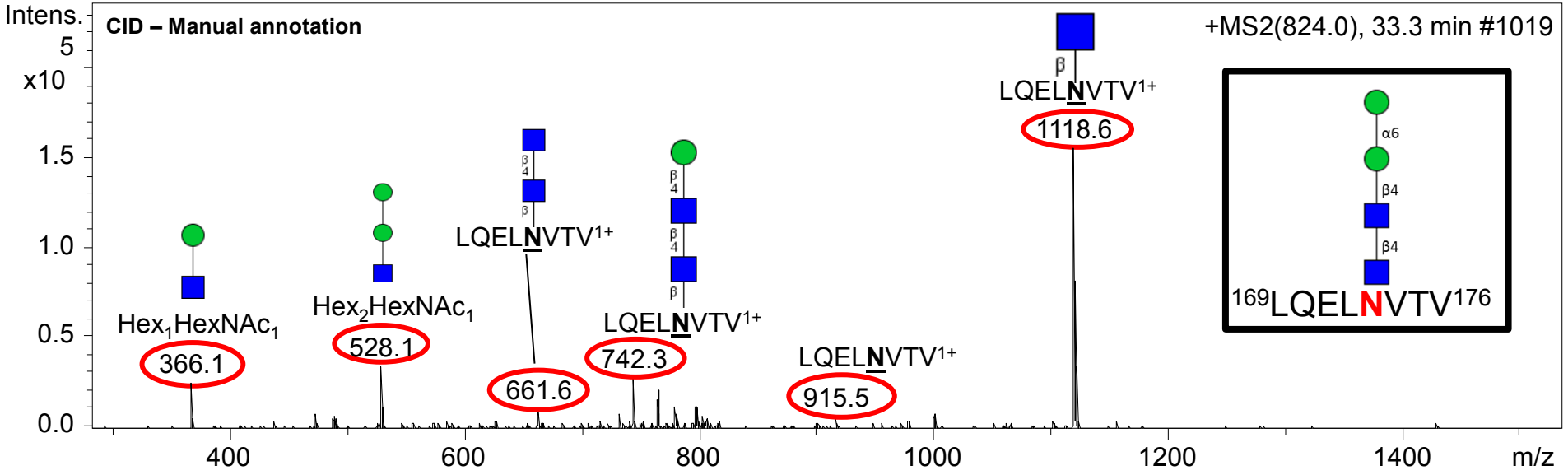
Obs. $[M+H]^+$ = 1645.8 Da

Glycan: 730.3 Da ($Hex_2HexNAc_2$)

Calc. $[M+H]^+$ = 1645.7 Da

Retention time: 33 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

LQEL**N**VTV (Asn173 glycopeptide)

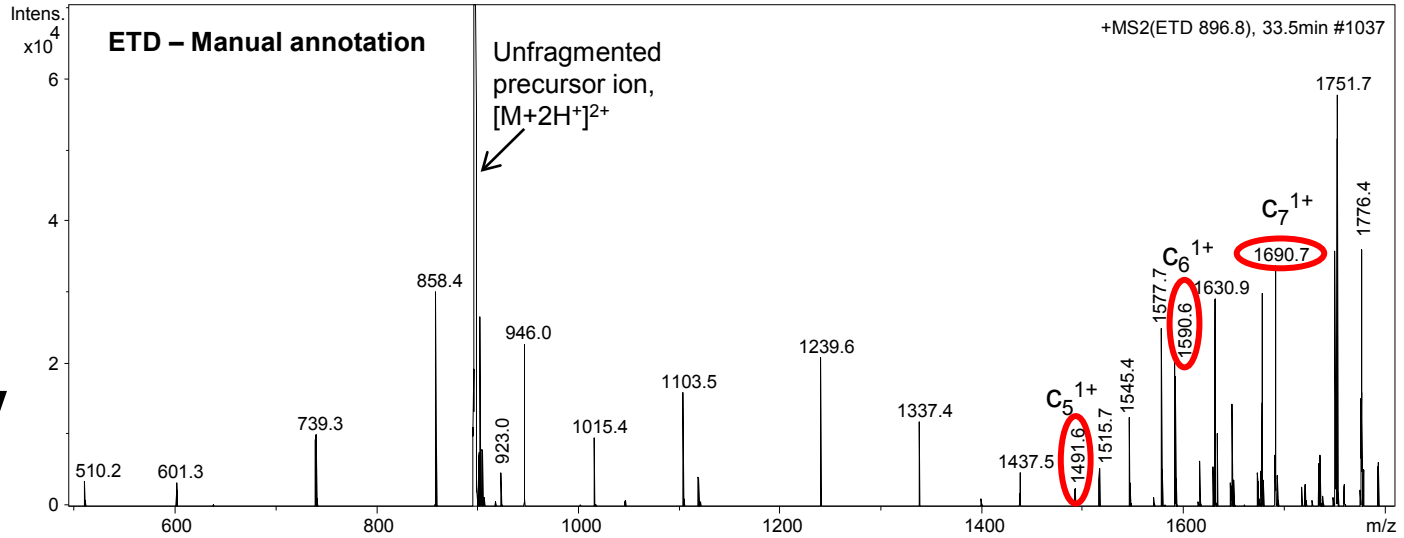
Obs. *m/z* 896.5 (2+)

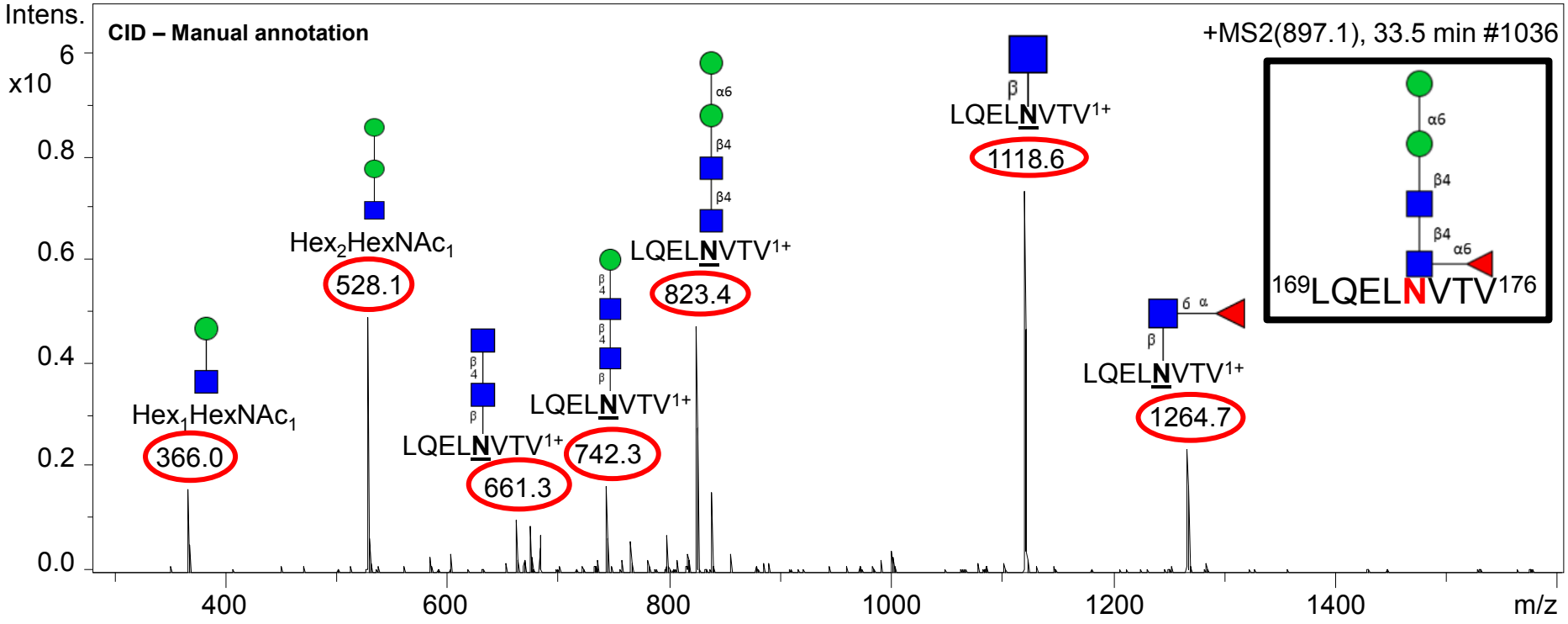
Obs. $[M+H]^+$ = 1792.0 Da

Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1791.8 Da

Retention time: 34 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

QEL**N**VTVVT (Asn173 glycopeptide)

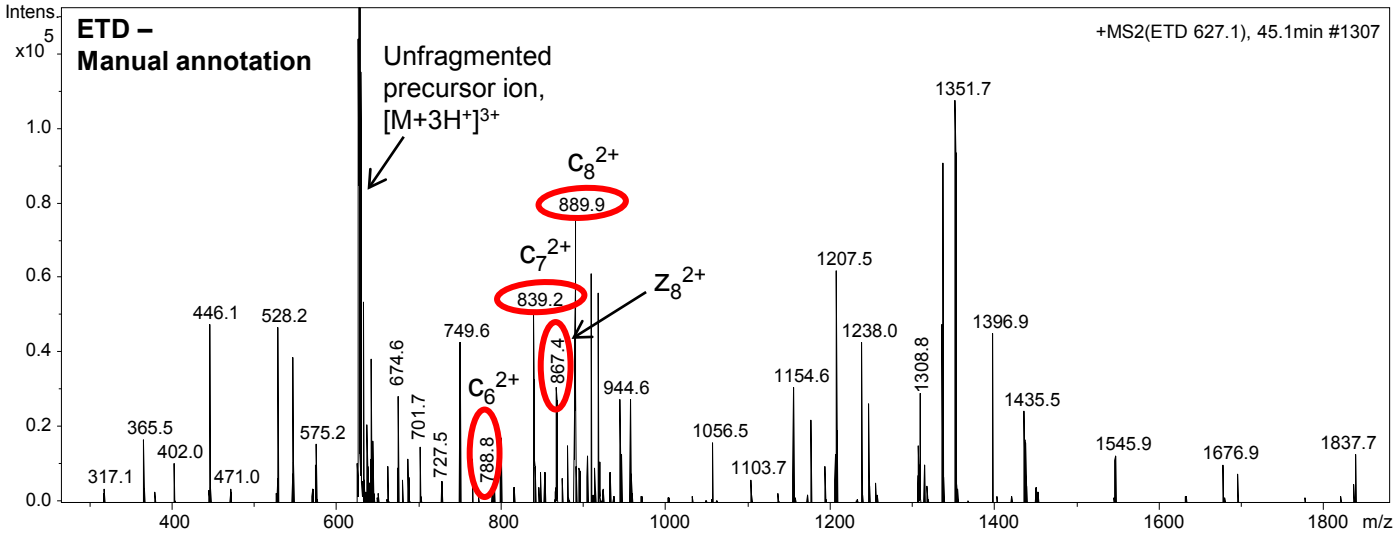
Obs. *m/z* 627.0 (3+)

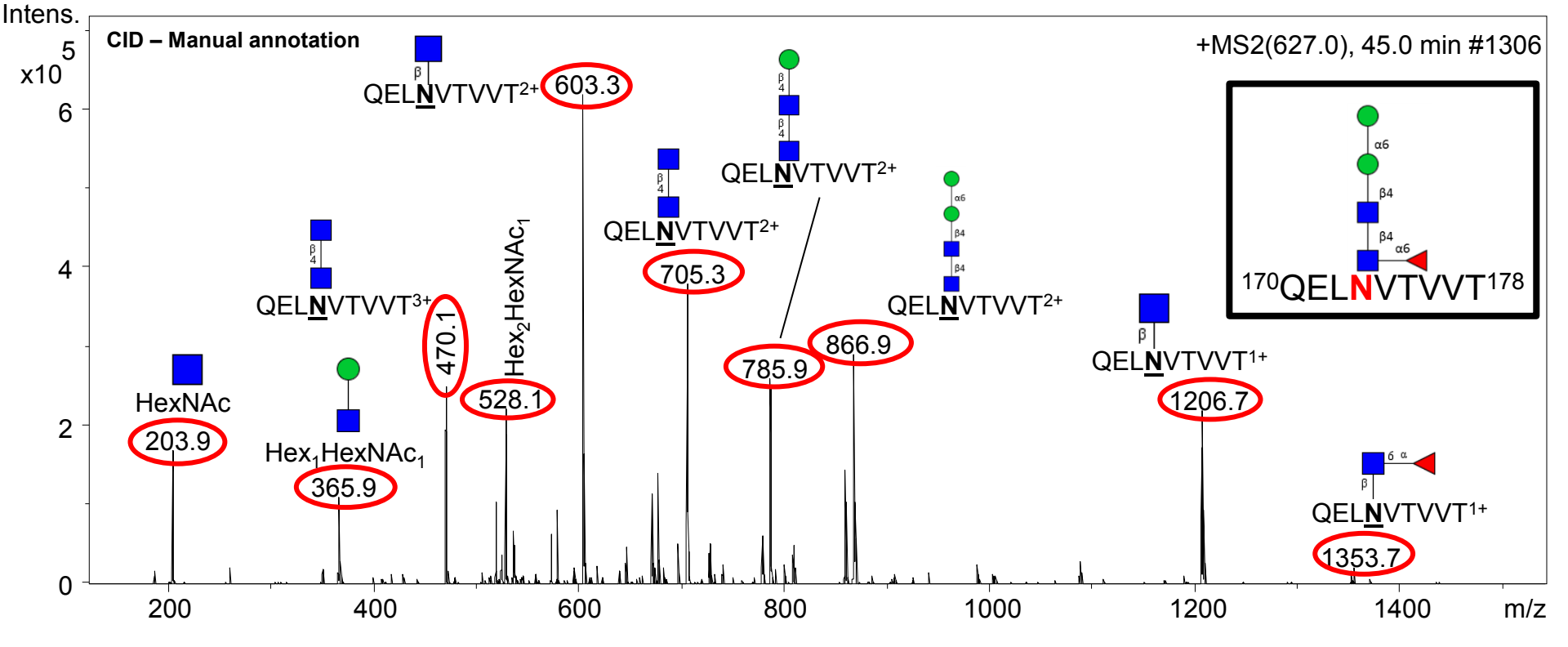
Obs. $[M+H]^+$ = 1879.0 Da

Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1878.8 Da

Retention time: 45 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

LQEL**N**VTVV (Asn173 glycopeptide)

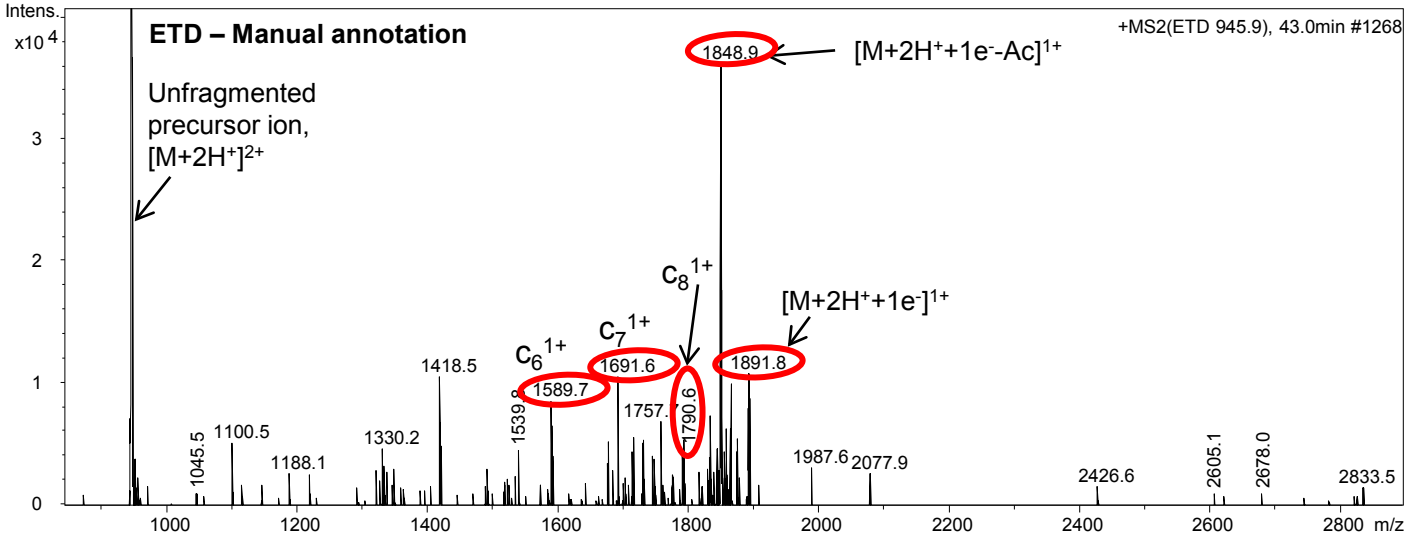
Obs. *m/z* 946.2 (2+)

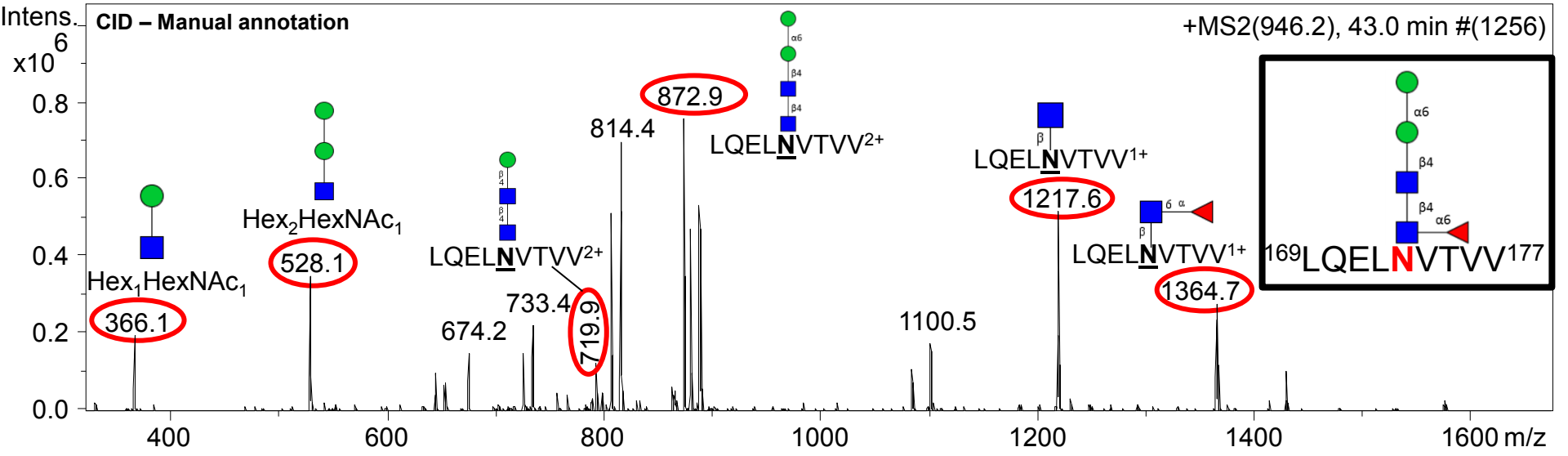
Obs. $[M+H]^+$ = 1891.4 Da

Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1890.9 Da

Retention time: 43 min





Human neutrophil elastase – P08246 **Supplementary Figure S4A**

LQEL**N**VTVV (Asn173 glycopeptide)

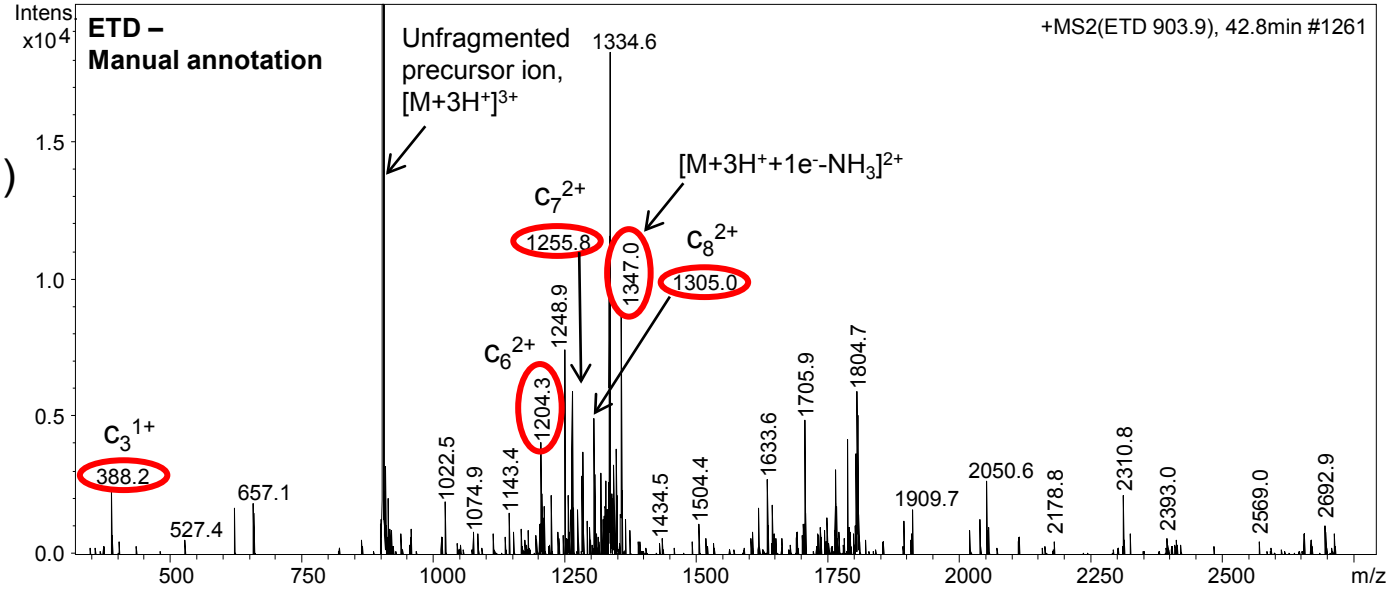
Obs. *m/z* 903.9 (3+)

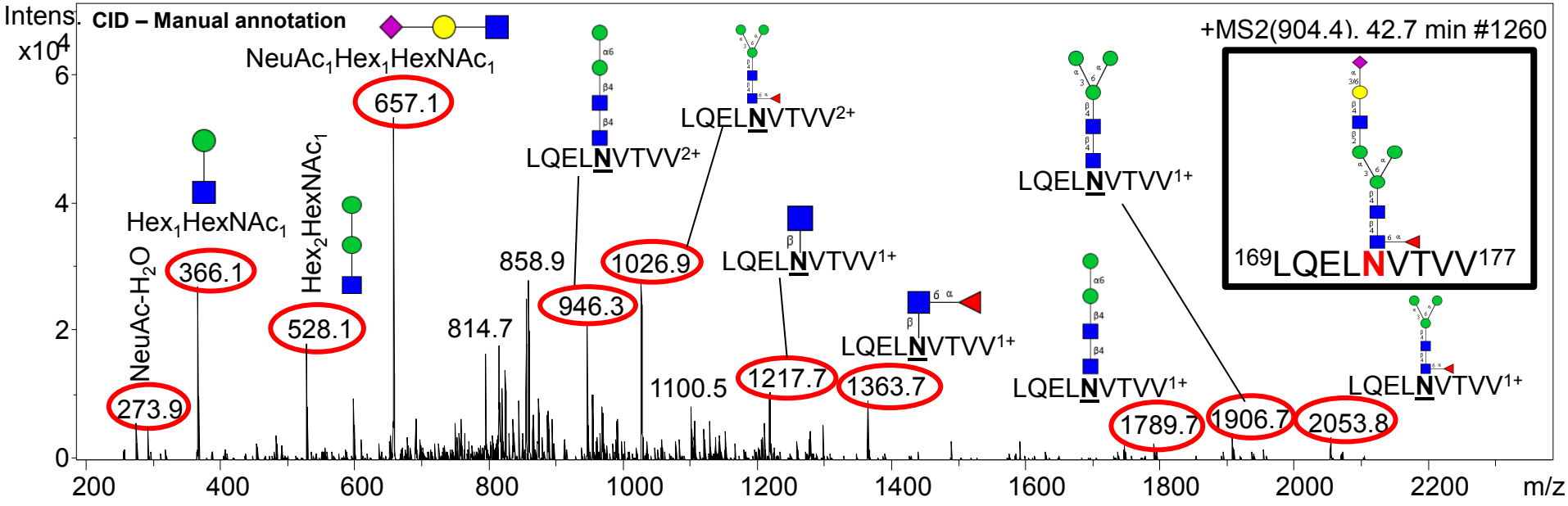
Obs. $[M+H]^+$ = 2709.7 Da

Glycan: 1694.6 Da ($Hex_4HexNAc_3Fuc_1NeuAc_1$)

Calc. $[M+H]^+$ = 2709.3 Da

Retention time: 43 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

LQEL**N**VTVV (Asn173 glycopeptide)

Obs. *m/z* 576.7 (3+)

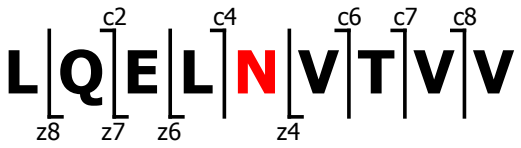
Obs. $[M+H]^+$ = 1728.1 Da

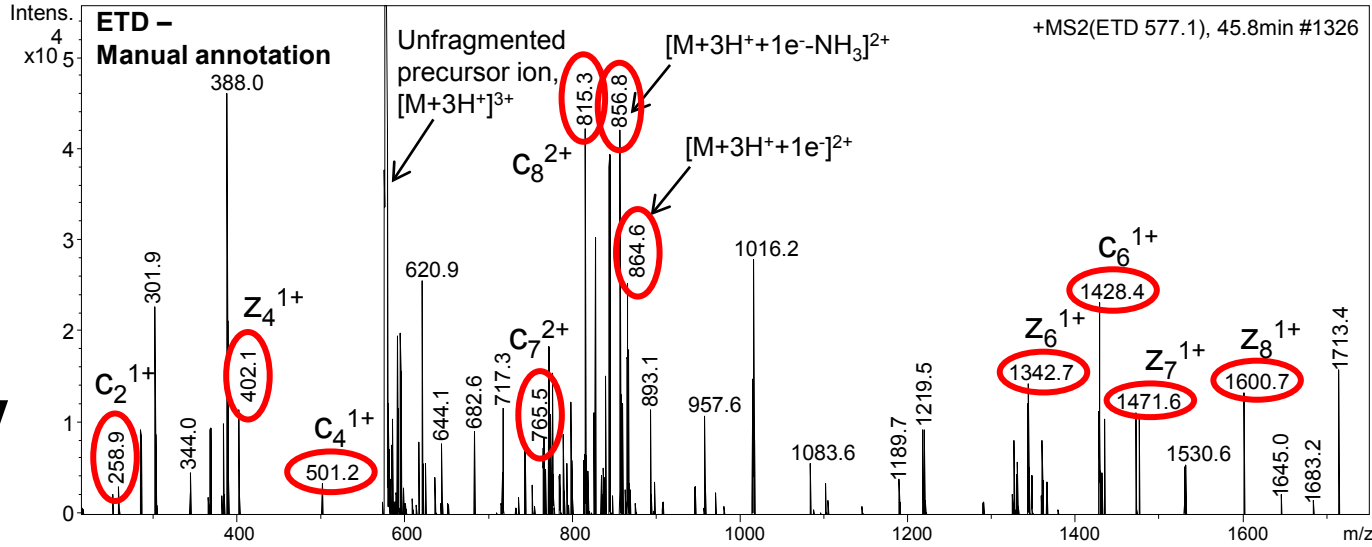
Glycan: 714.3 Da

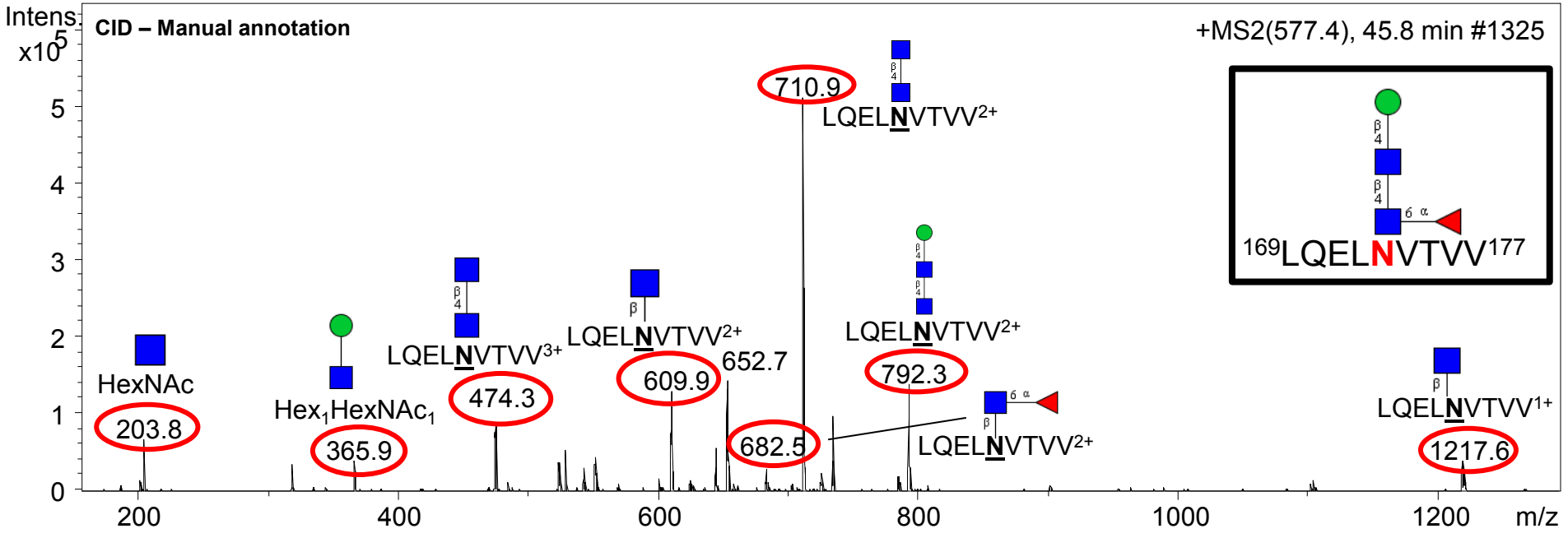
($Hex_1HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1728.9 Da

Retention time: 46 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

QEL**N**VTVV (Asn173 glycopeptide)

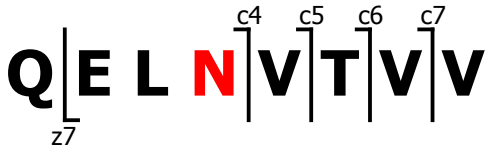
Obs. *m/z* 889.4 (2+)

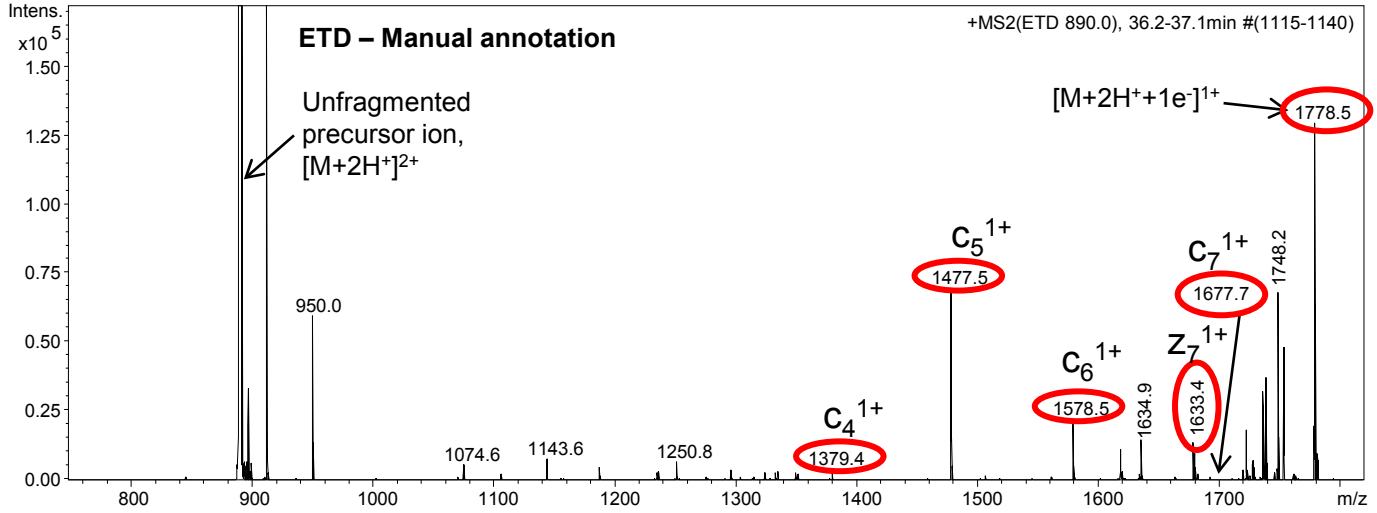
Obs. $[M+H]^+$ = 1777.8 Da

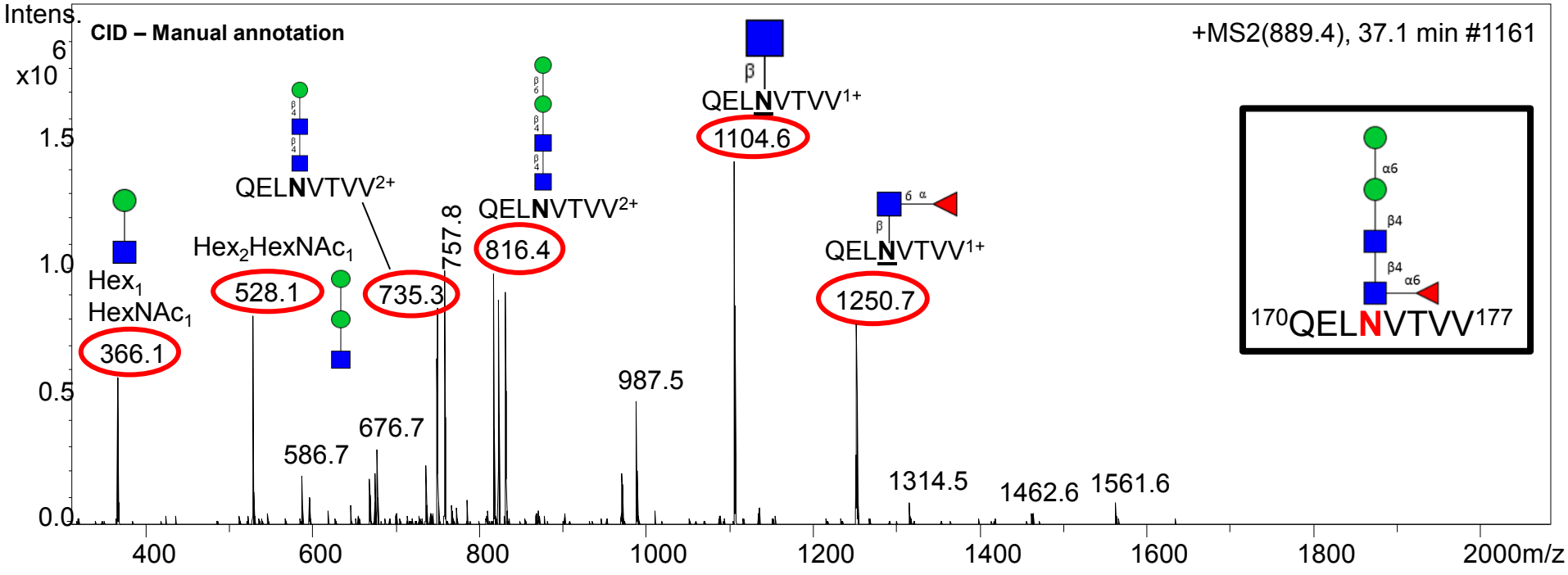
Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1777.8 Da

Retention time: 37 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

VLQEL**N**VTVV (Asn173 glycopeptide)

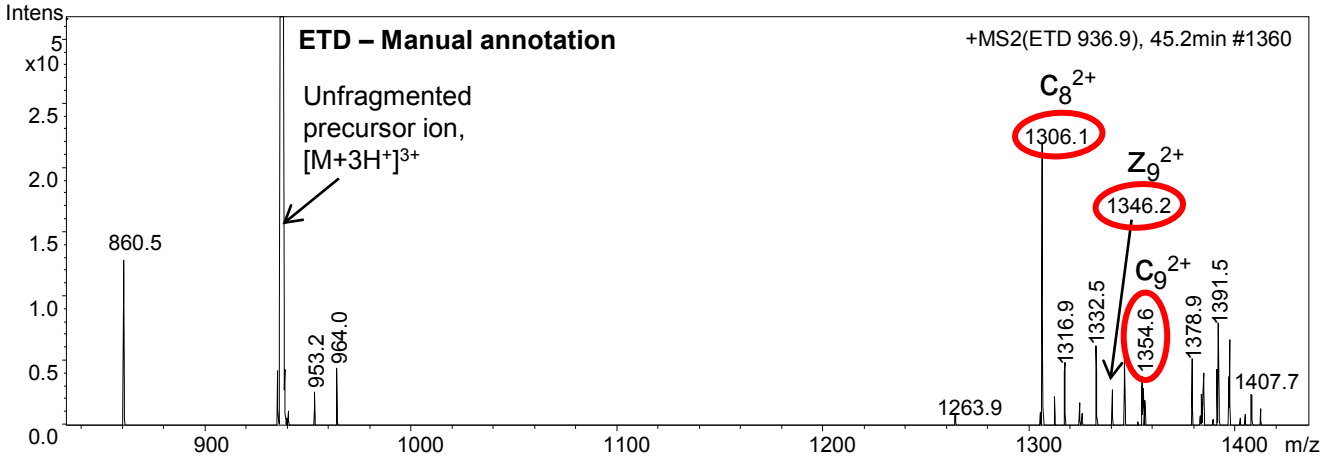
Obs. *m/z* 936.9 (3+)

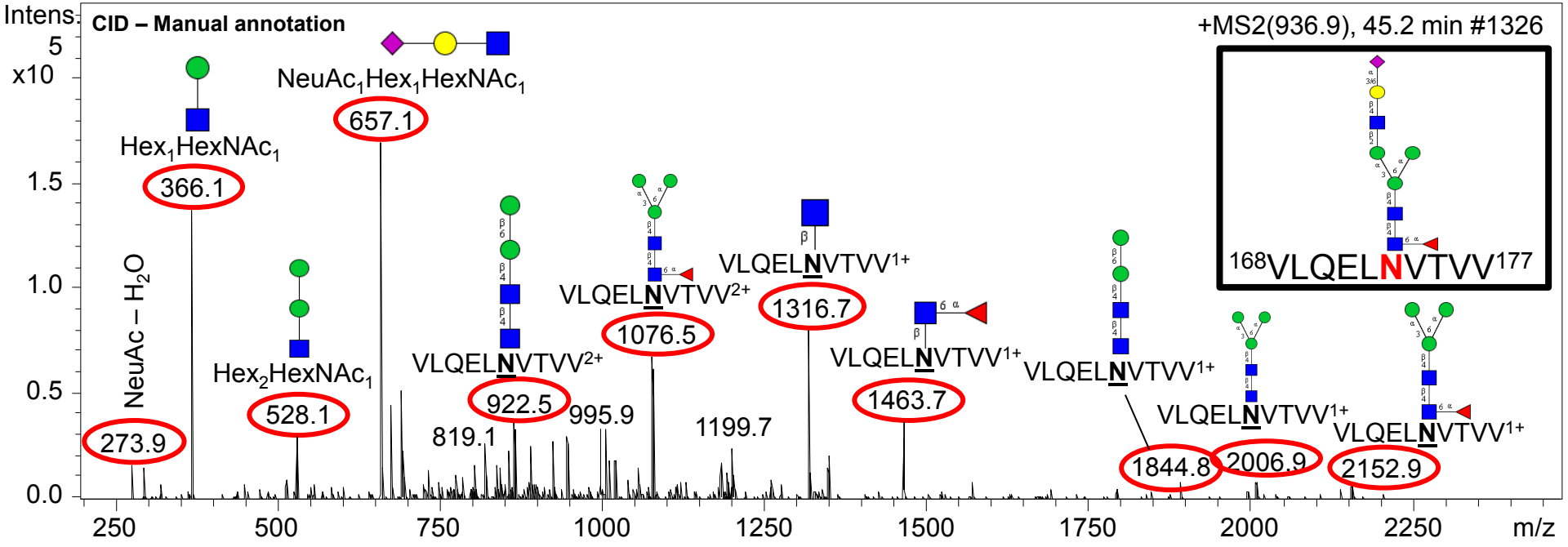
Obs. $[M+H]^+$ = 2808.7 Da

Glycan: 1694.6 Da ($Hex_4HexNAc_3Fuc_1NeuAc_1$)

Calc. $[M+H]^+$ = 2808.3 Da

Retention time: 45 min





Human neutrophil elastase – P08246

Supplementary Figure S4A

VLQEL**N**VTVV (Asn173 glycopeptide)

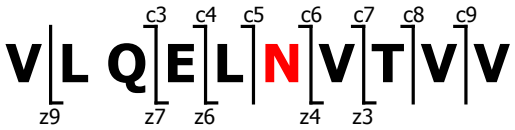
Obs. *m/z* 664.1 (3+)

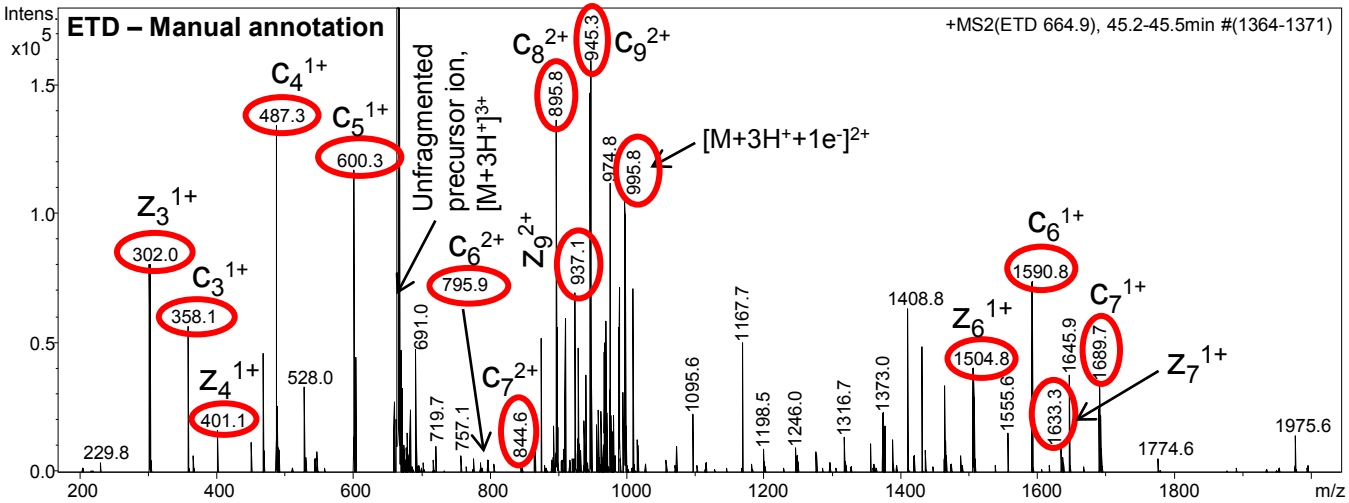
Obs. $[M+H]^+$ = 1990.3 Da

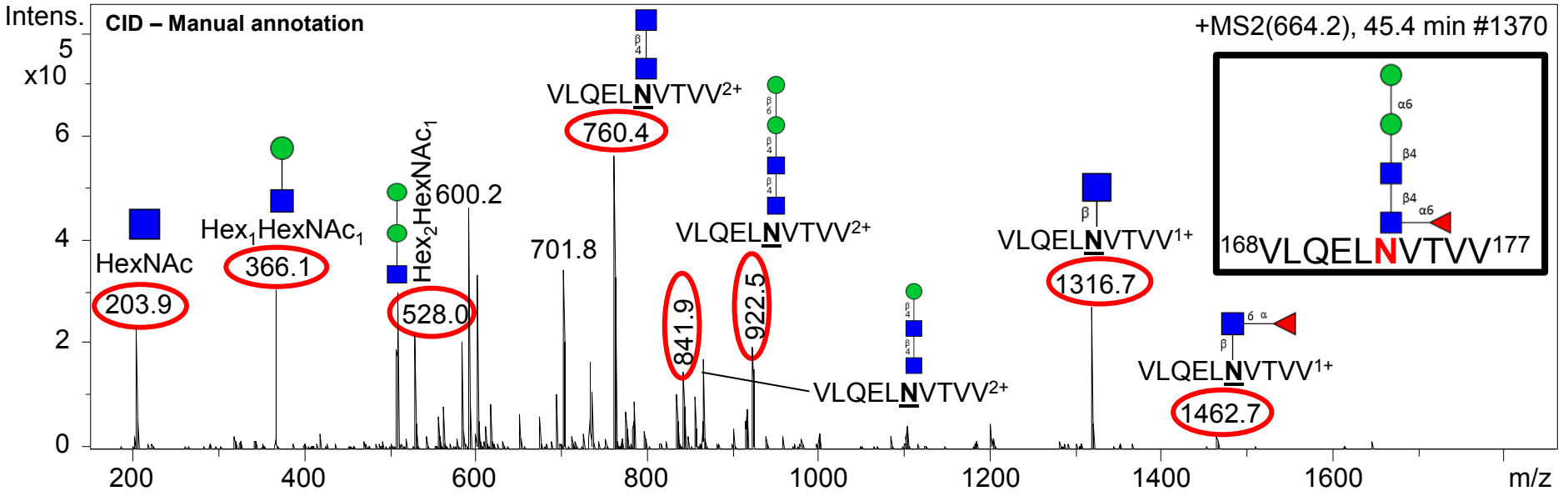
Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1990.0 Da

Retention time: 45 min







Human neutrophil elastase – P08246

Supplementary Figure S4A

SVLQELNVTVV (Asn173 glycopeptide)

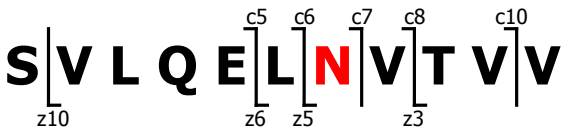
Obs. *m/z* 965.9 (3+)

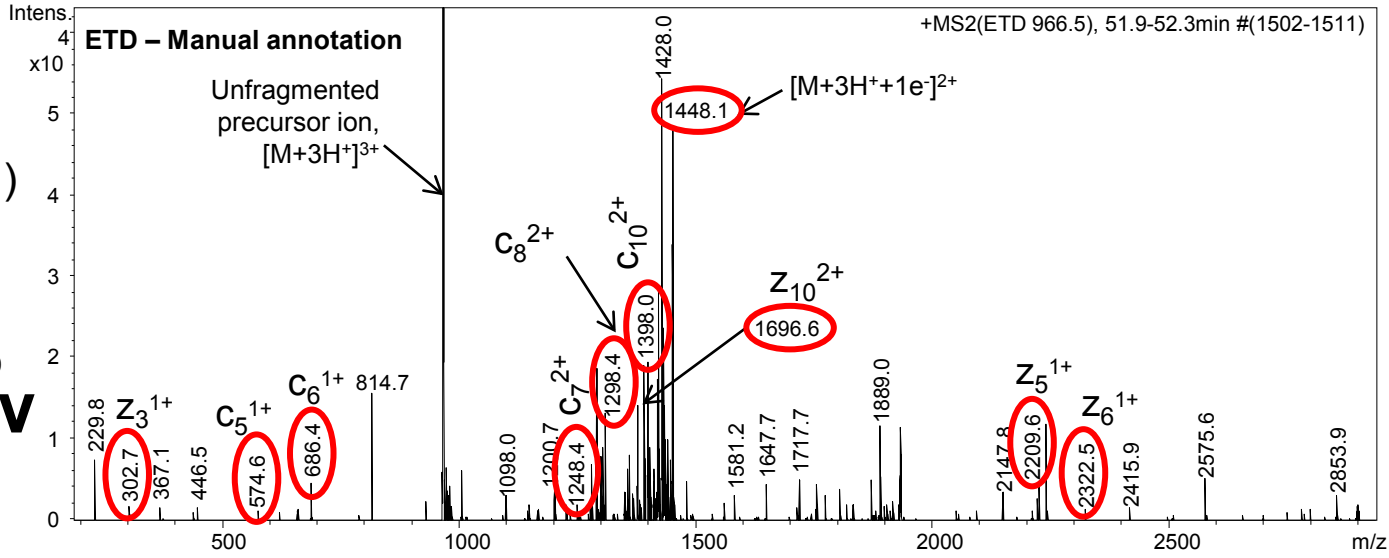
Obs. $[M+H]^+$ = 2895.7 Da

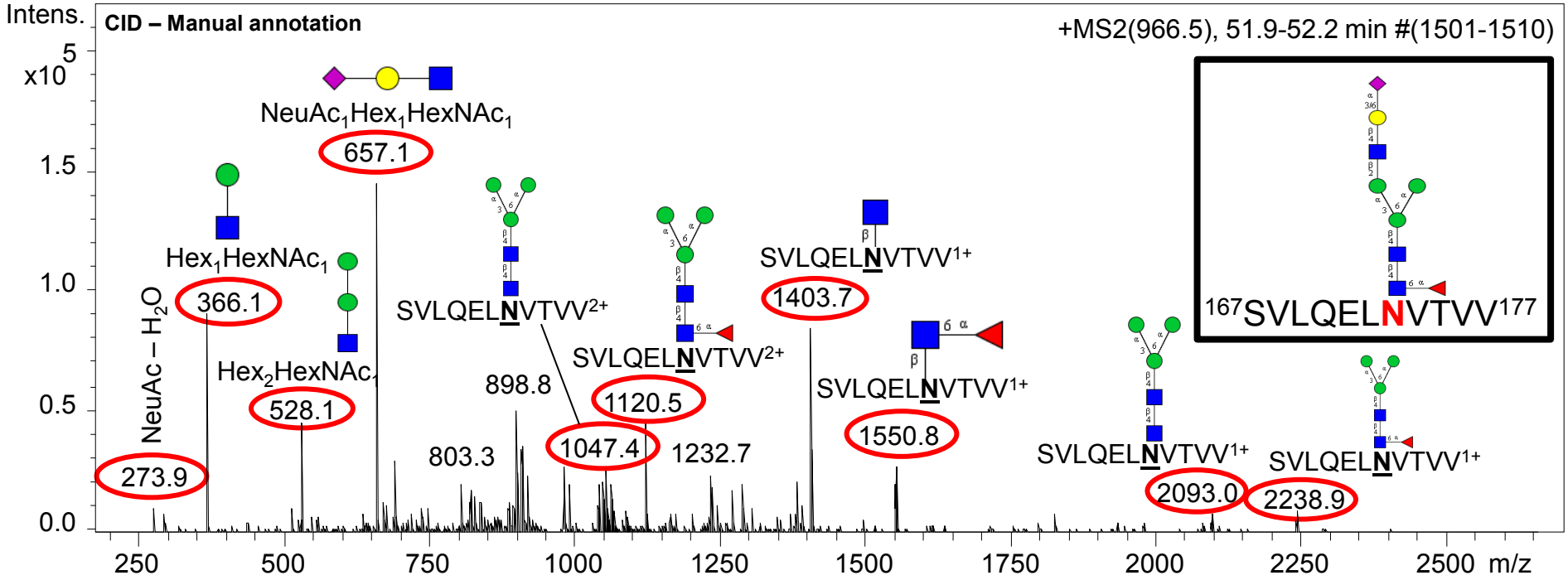
Glycan: 1694.6 Da ($Hex_4HexNAc_3Fuc_1NeuAc_1$)

Calc. $[M+H]^+$ = 2895.3 Da

Retention time: 52 min



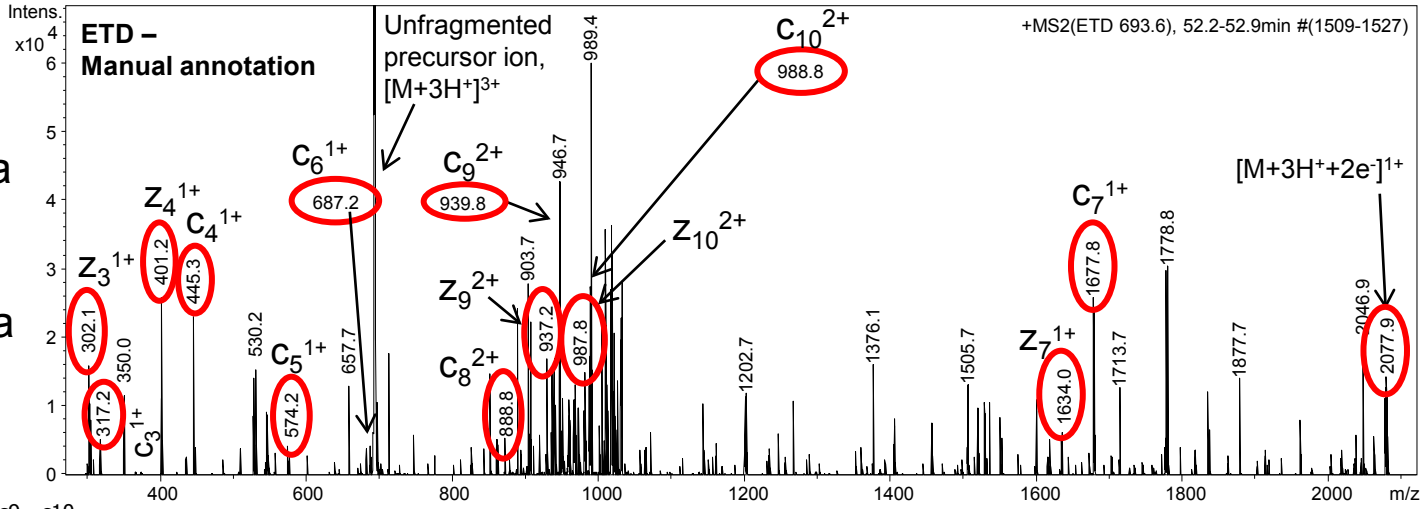


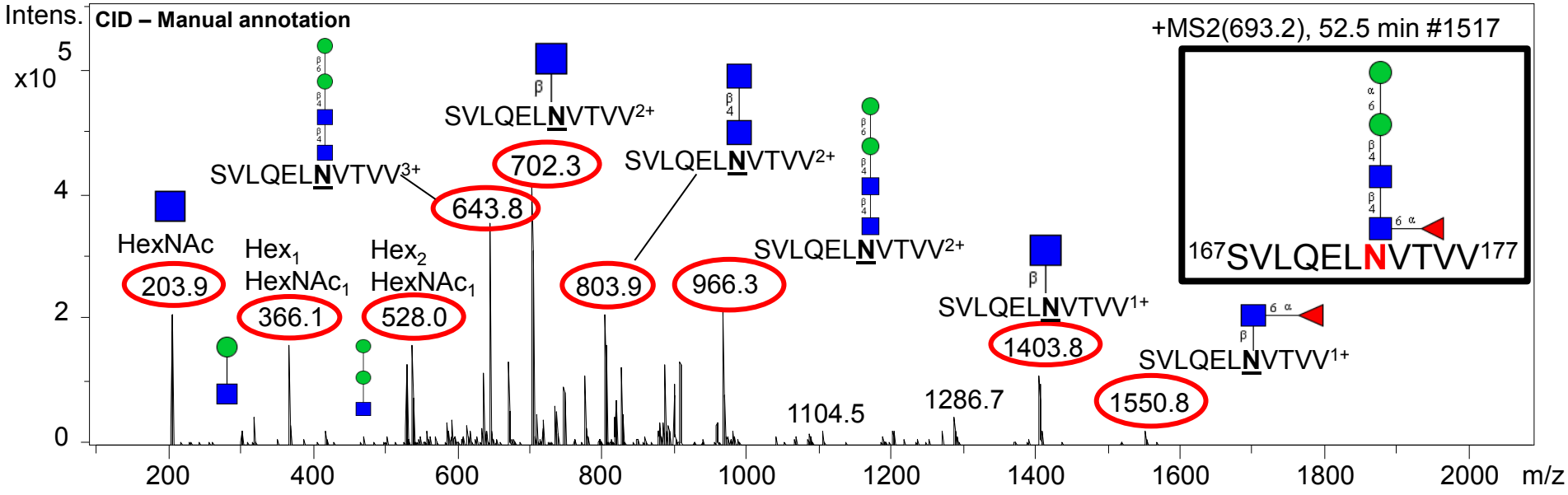


Human neutrophil elastase – P08246

Supplementary Figure S4A

SVLQELNVTVV
(Asn173 glycopeptide)
Obs. *m/z* 693.2 (3+)
Obs. $[M+H]^+$ = 2077.6 Da
Glycan: 876.3 Da
($Hex_2HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 2077.2 Da
Retention time: 53 min



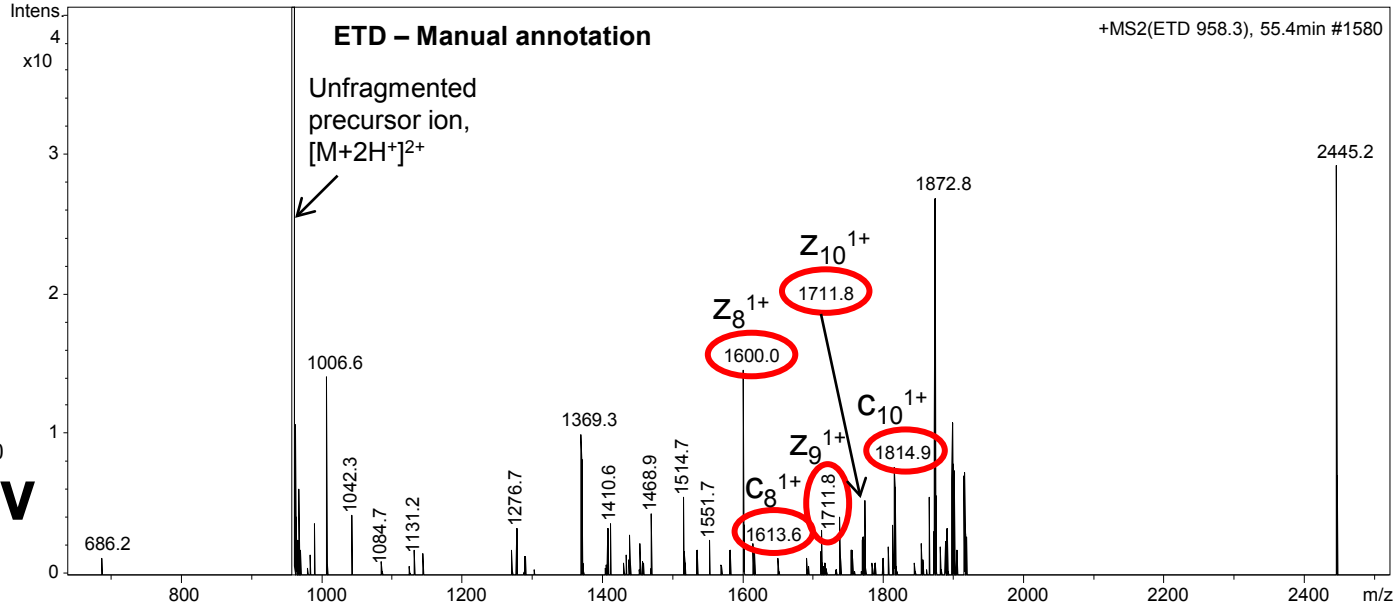


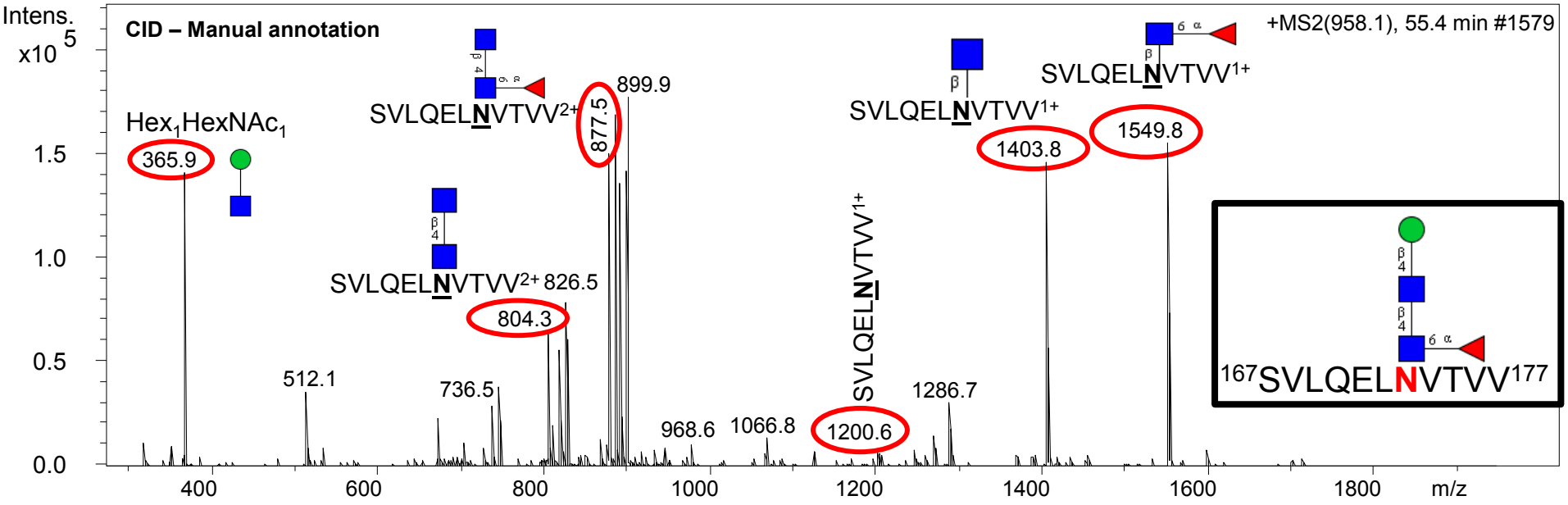
Human neutrophil elastase – P08246

Supplementary Figure S4A

SVLQELNVTVV
(Asn173 glycopeptide)
Obs. *m/z* 958.1 (2+)
Obs. $[M+H]^+$ = 1915.2 Da
Glycan: 714.3 Da
($Hex_1HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 1914.9 Da
Retention time: 55 min

S V L Q E L N V T V V
c8 c10
z10 z9 z8





Human neutrophil elastase – P08246

Supplementary Figure S4A

VLQEL**N**V (Asn173 glycopeptide)

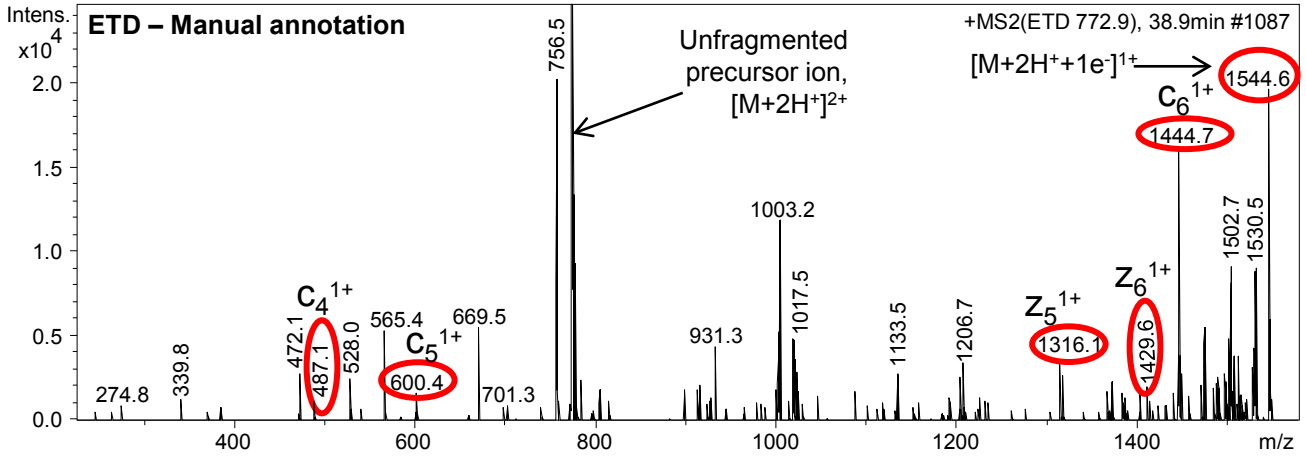
Obs. *m/z* 772.7 (2+)

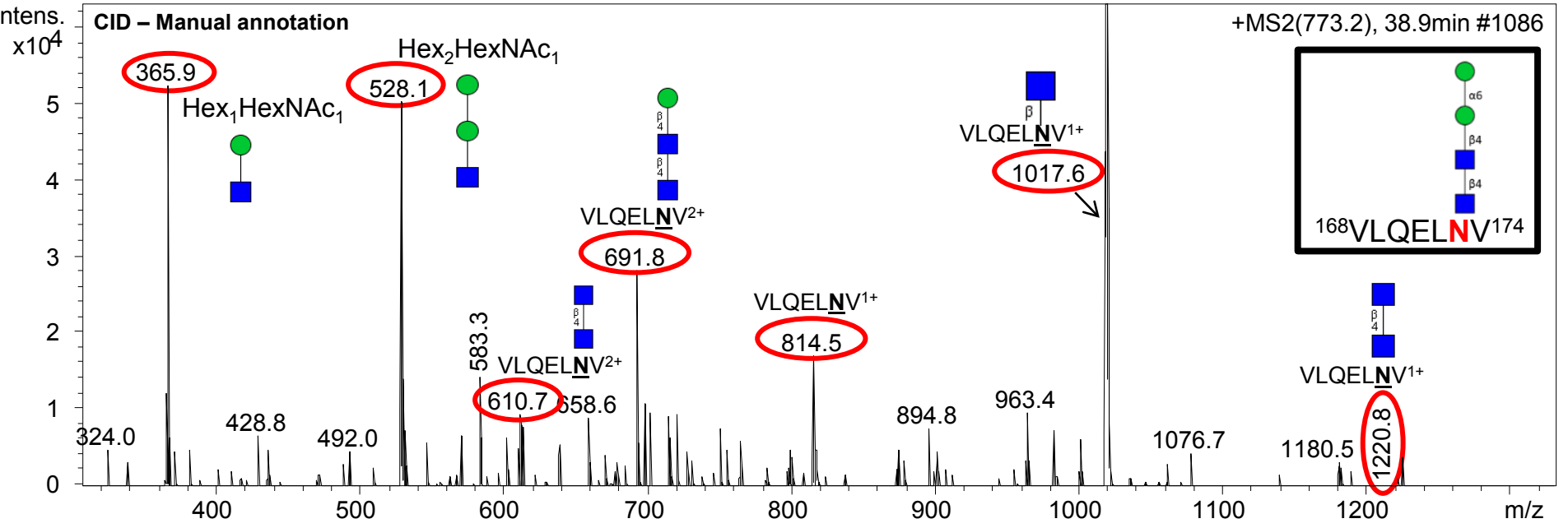
Obs. $[M+H]^+$ = 1544.4 Da

Glycan: 730.3 Da ($Hex_2HexNAc_2$)

Calc. $[M+H]^+$ = 1544.7 Da

Retention time: 39 min





Human neutrophil elastase – P08246 **Supplementary Figure S4A**

ASVLQEL**N**V (Asn173 glycopeptide)

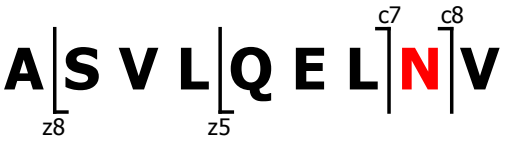
Obs. *m/z* 925.0 (2+)

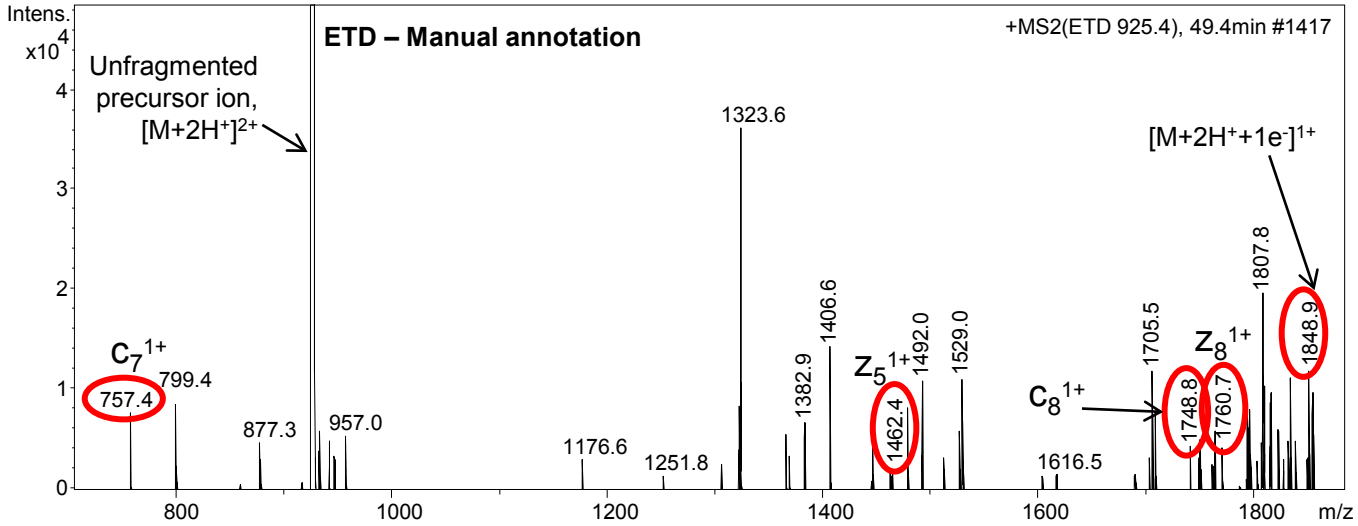
Obs. $[M+H]^+$ = 1849.0 Da

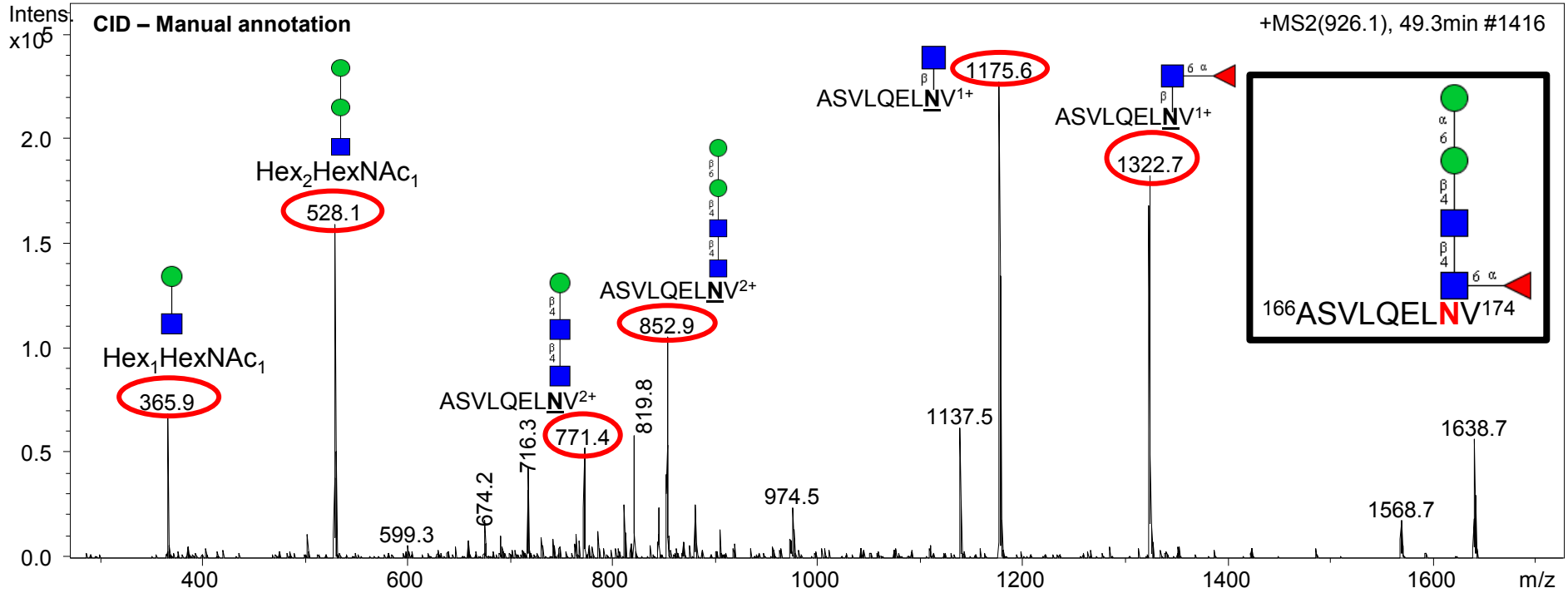
Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)

Calc. $[M+H]^+$ = 1848.8 Da

Retention time: 49 min







Human neutrophil elastase – P08246 **Supplementary Figure S4A**

ASVLQEL**N**VTVV (Asn173 glycopeptide)

Obs. *m/z* 989.6 (3+)

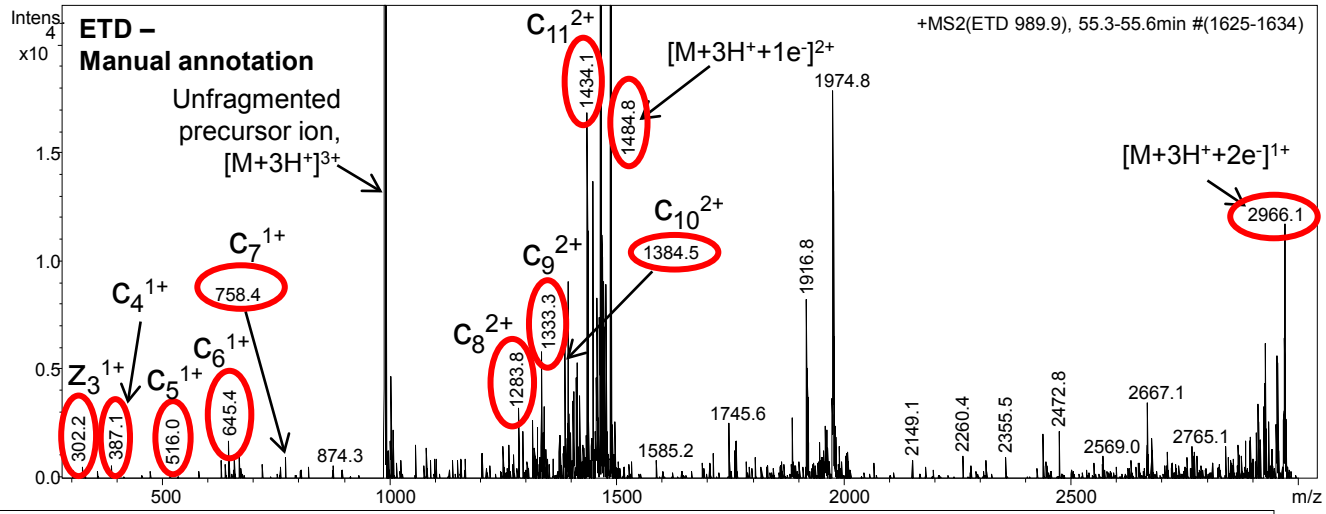
Obs. $[M+H]^+$ = 2966.8 Da

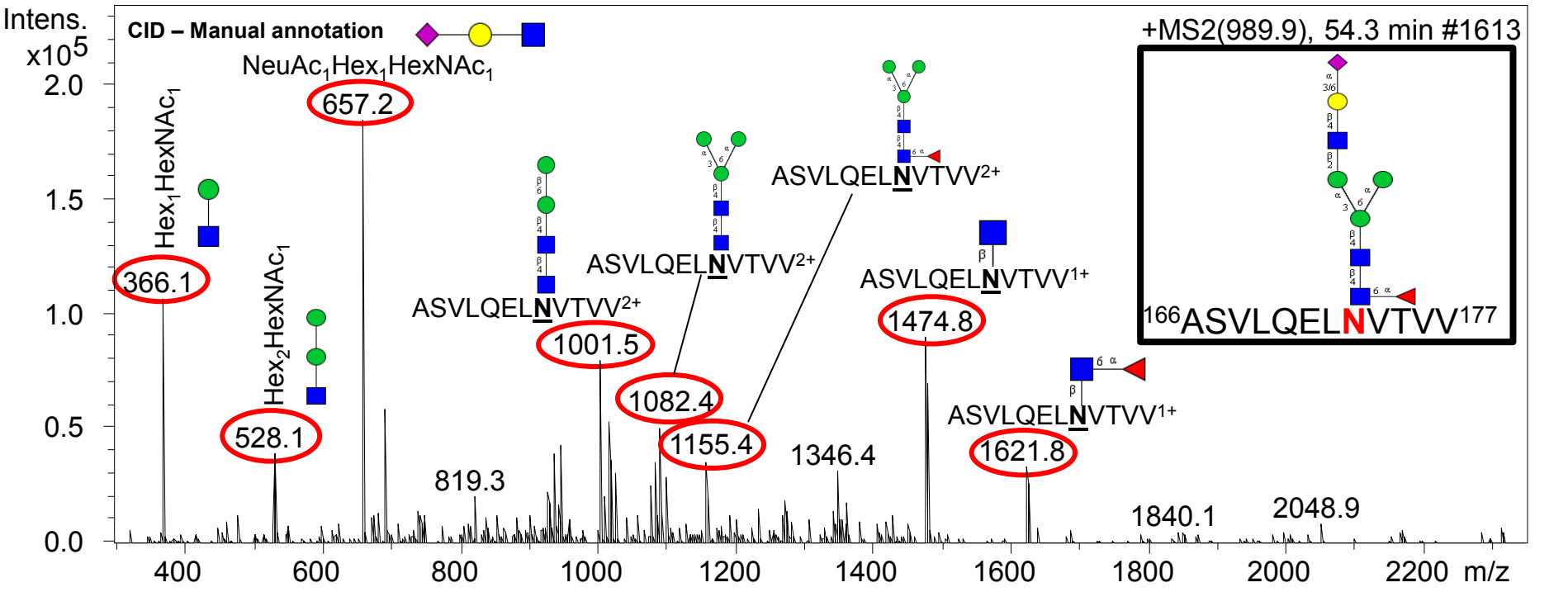
Glycan: 1694.6 Da

($Hex_4HexNAc_3Fuc_1NeuAc_1$)

Calc. $[M+H]^+$ = 2966.3 Da

Retention time: 54 min

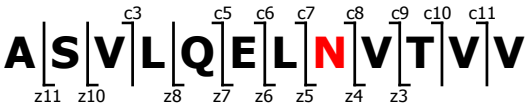


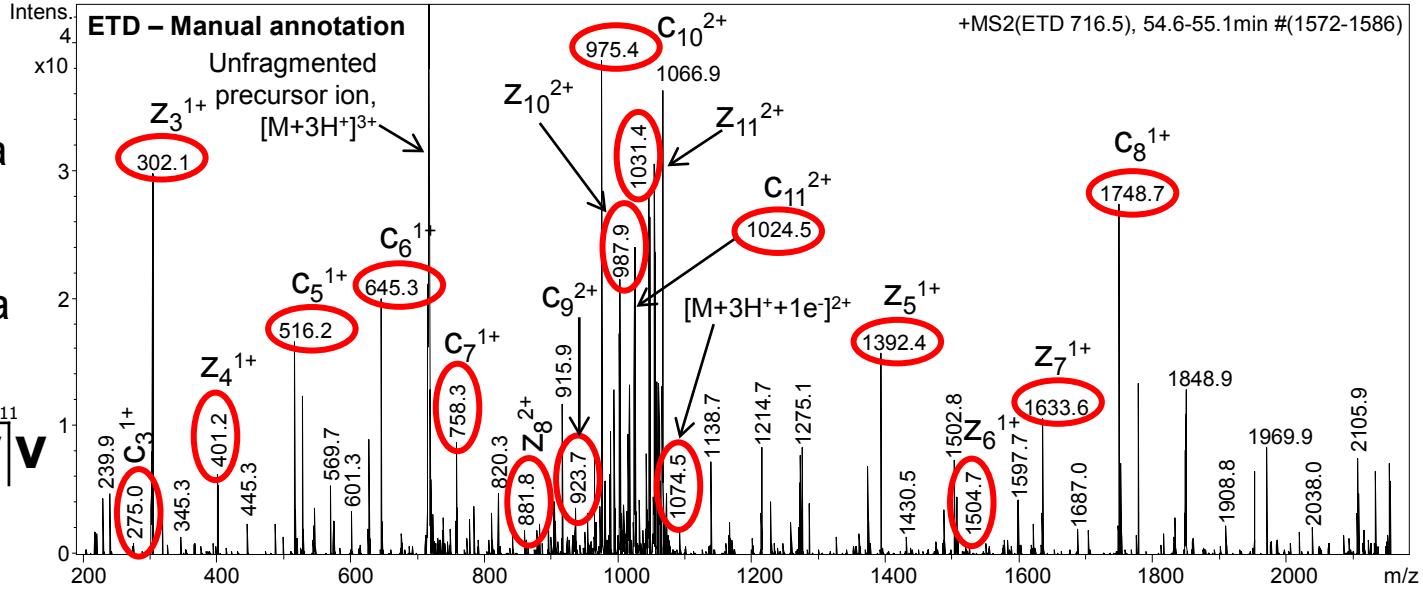


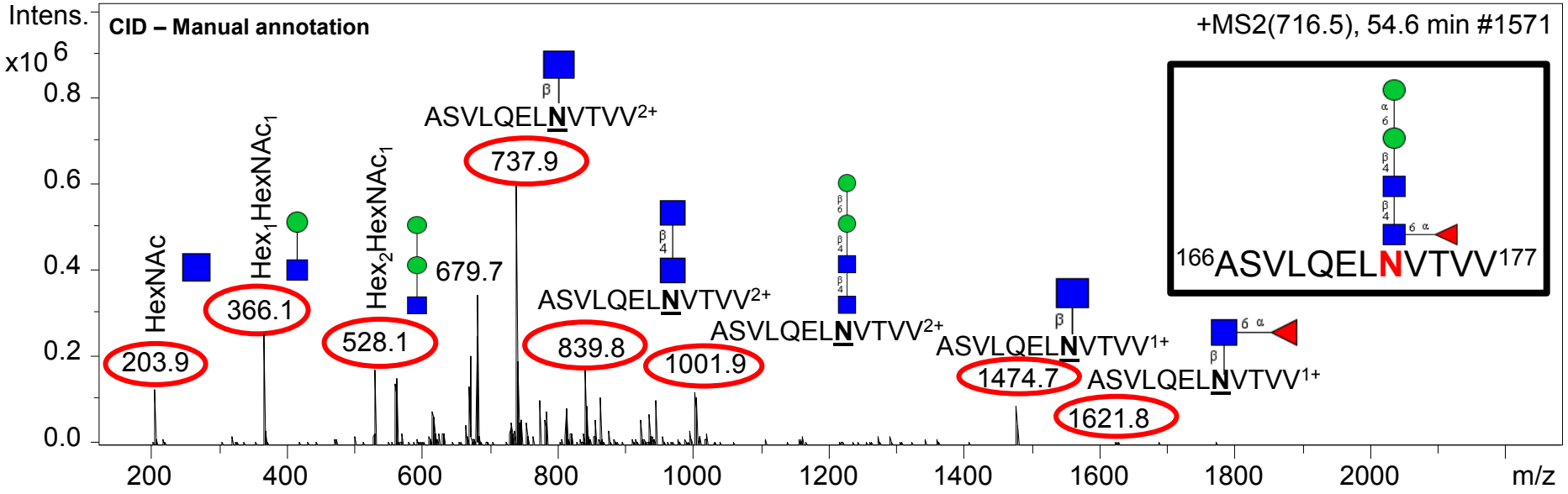
Human neutrophil elastase – P08246

Supplementary Figure S4A

ASVLQELNVTVV
(Asn173 glycopeptide)
Obs. *m/z* 716.5 (3+)
Obs. $[M+H]^+$ = 2147.5 Da
Glycan: 876 Da
($Hex_2HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 2147.7 Da
Retention time: 55 min



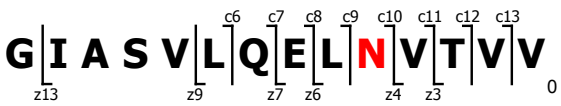


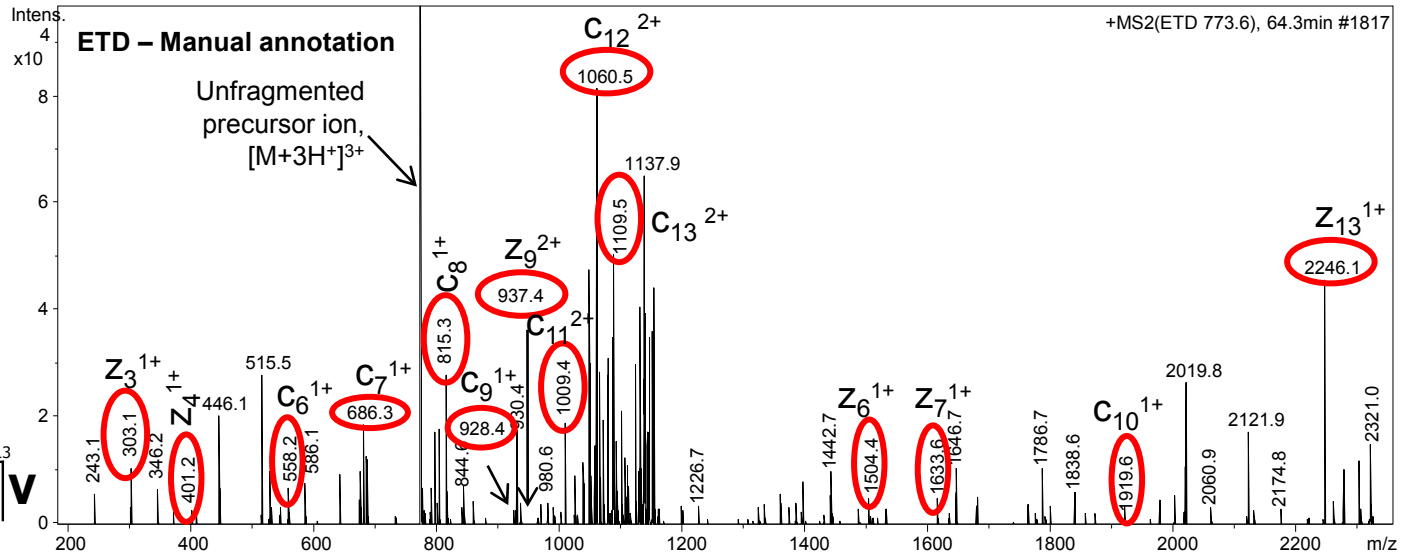


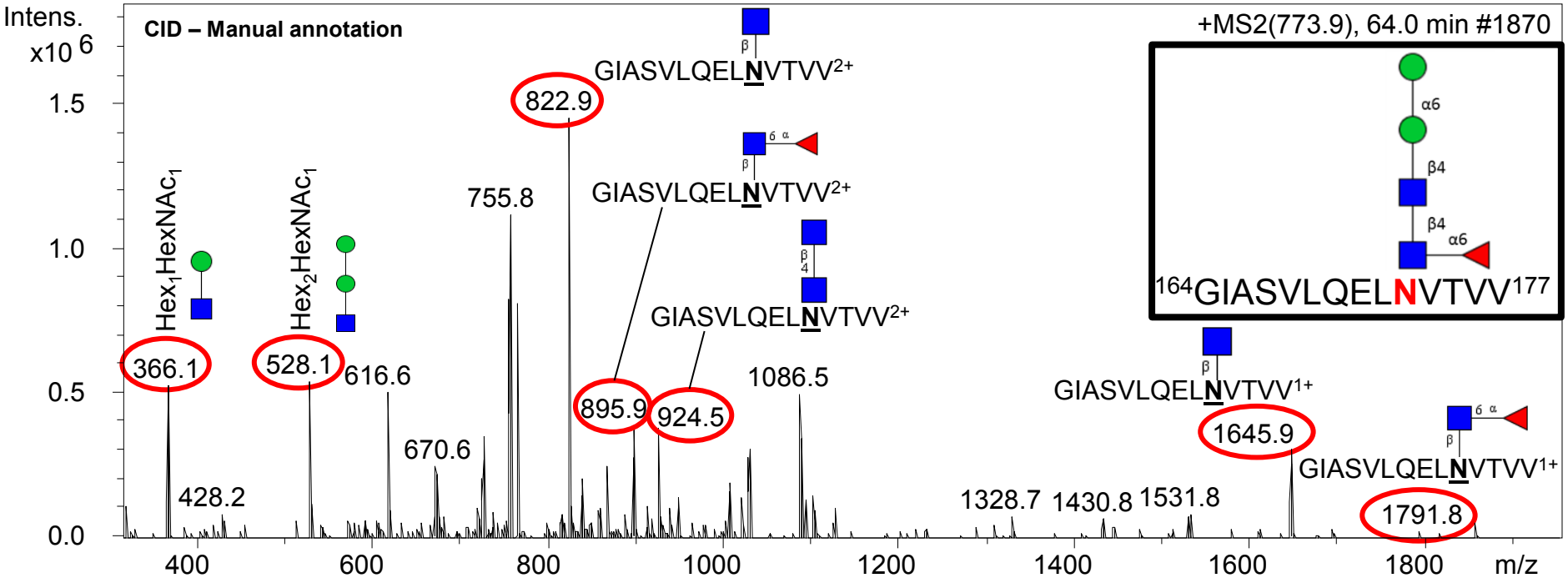
Human neutrophil elastase – P08246

Supplementary Figure S4A

GIASVLQELNVTVV
(Asn173 glycopeptide)
Obs. *m/z* 773.3 (3+)
Obs. $[M+H]^+$ = 2317.9 Da
Glycan: 876.3 Da
($Hex_2HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 2318.1 Da
Retention time: 64 min



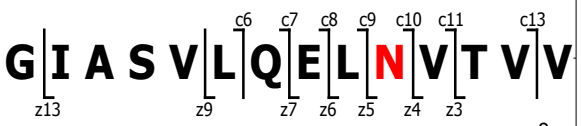


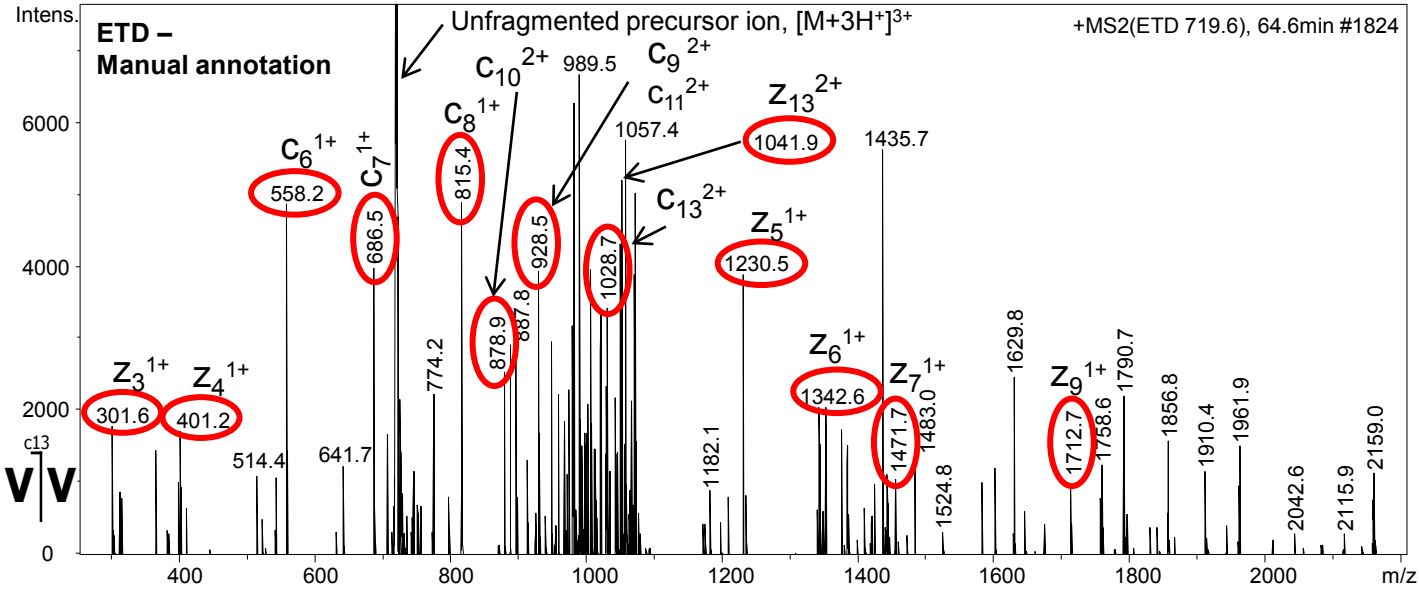


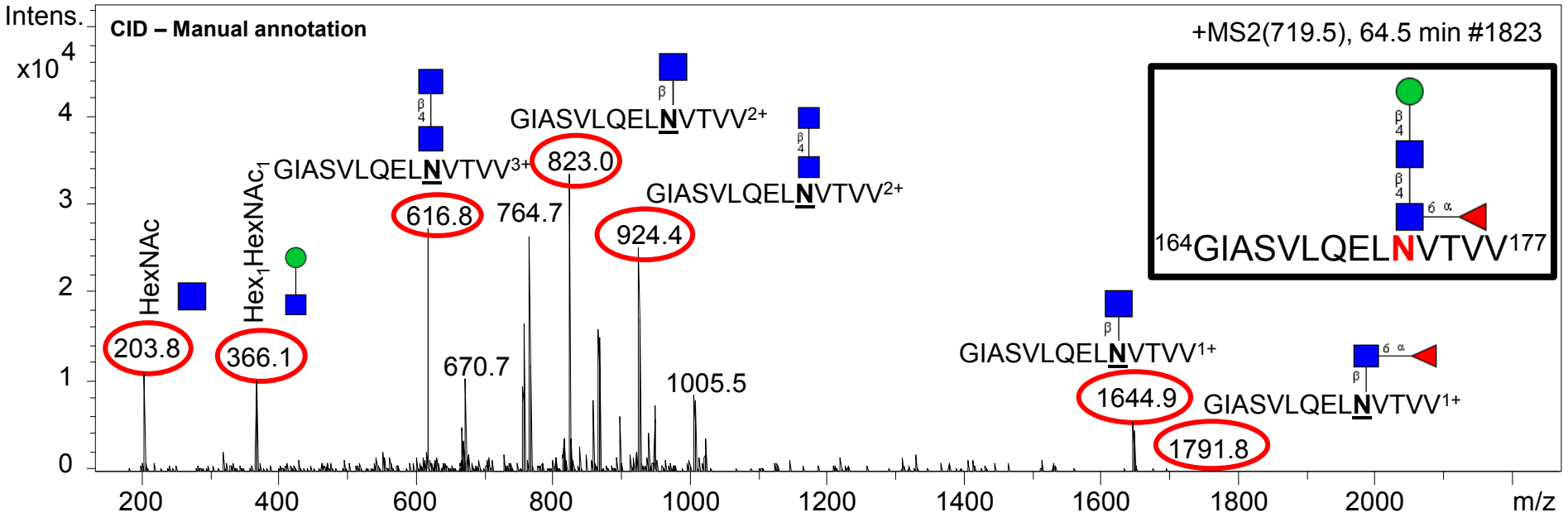
Human neutrophil elastase – P08246

Supplementary Figure S4A

GIASVLQEL**N**VTVV
(Asn173 glycopeptide)
Obs. *m/z* 719.3 (3+)
Obs. $[M+H]^+$ = 2155.9 Da
Glycan: 714.3 Da
($Hex_1HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 2156.0 Da
Retention time: 65 min



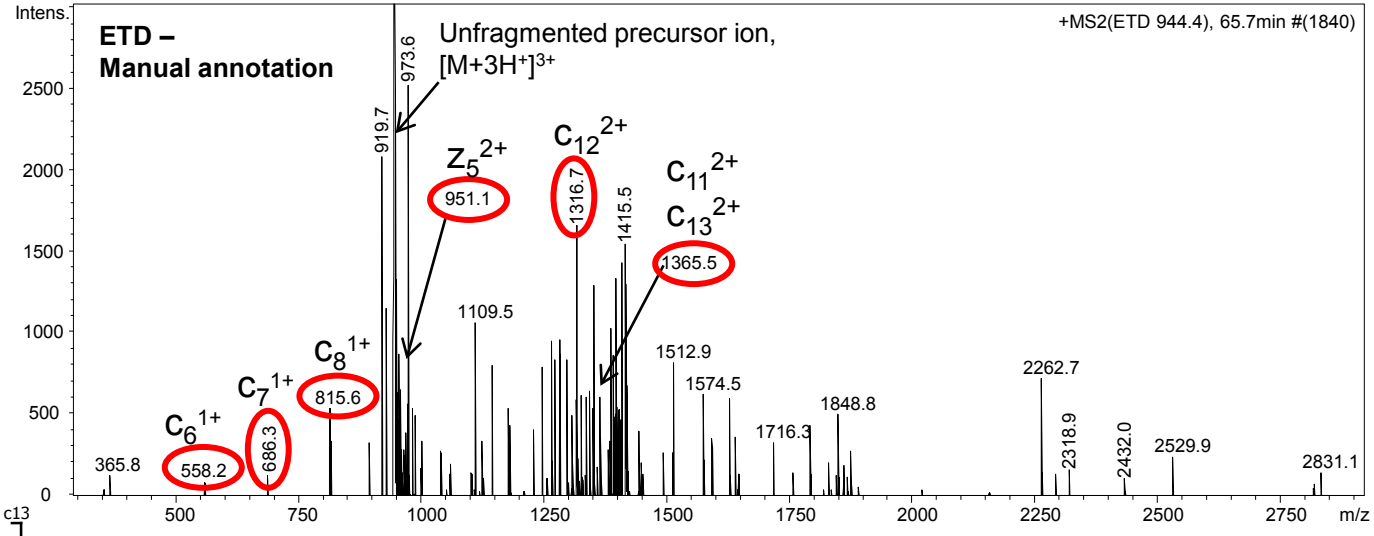


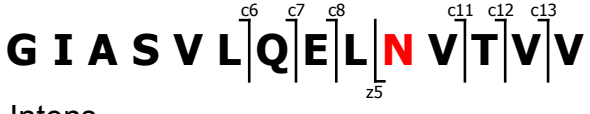


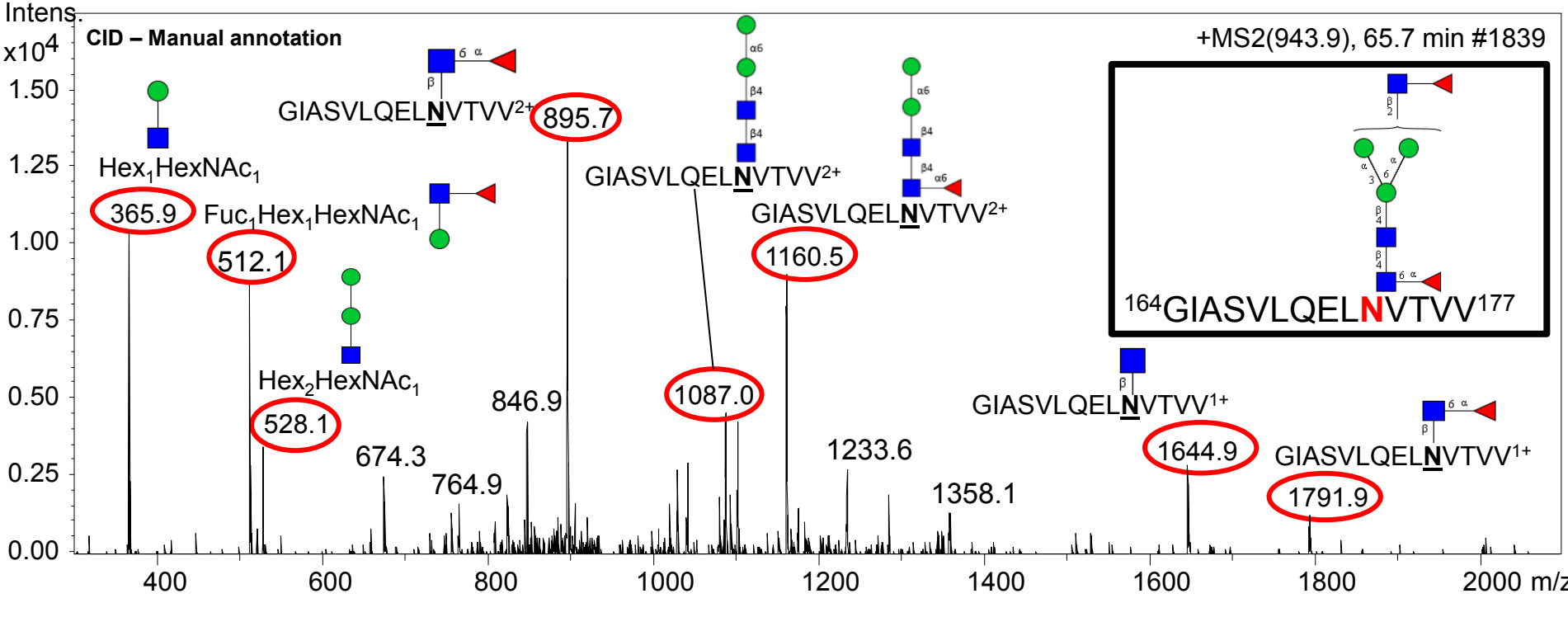
Human neutrophil elastase –P08246

Supplementary Figure S4A

GIASVLQEL**N**VTVV
(Asn173 glycopeptide)
Obs. *m/z* 943.9 (3+)
Obs. $[M+H]^+$ = 2829.7 Da
Glycan: 1387.5 Da
($Hex_3HexNAc_3Fuc_2$)
Calc. $[M+H]^+$ = 2829.3 Da
Retention time: 66 min



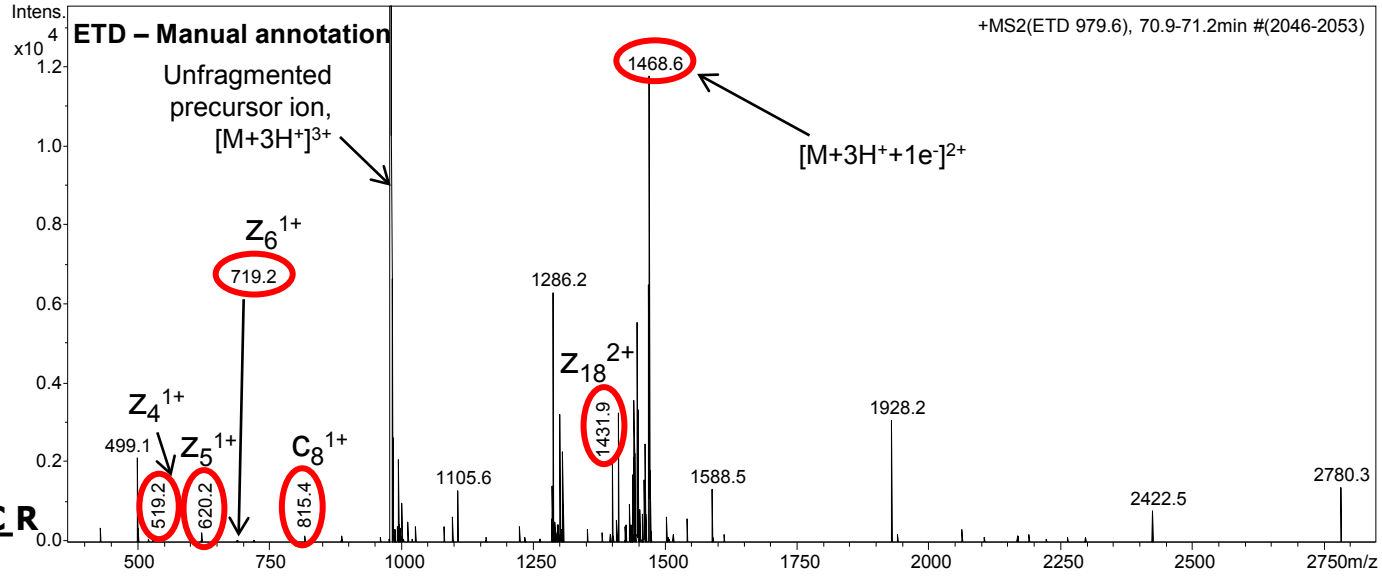


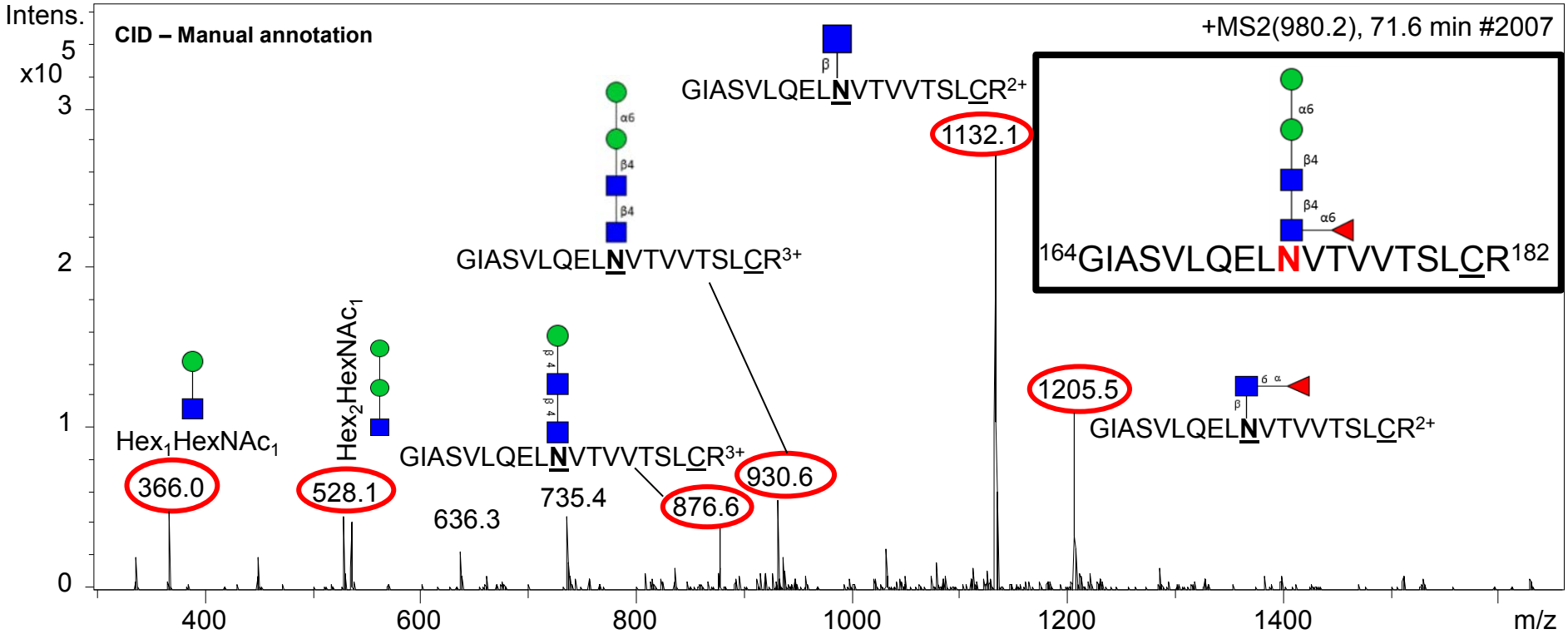


Human neutrophil elastase – P08246

Supplementary Figure S4A

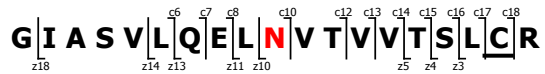
GIASVLQELNVTVVTSLCR
(Asn173 glycopeptide)
Obs. *m/z* 979.2 (3+)
Obs. $[M+H]^+$ = 2935.6 Da
Glycan: 876.3 Da
($Hex_2HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 2935.4 Da
Retention time: 72 min

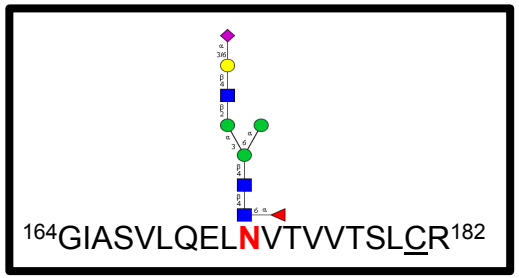




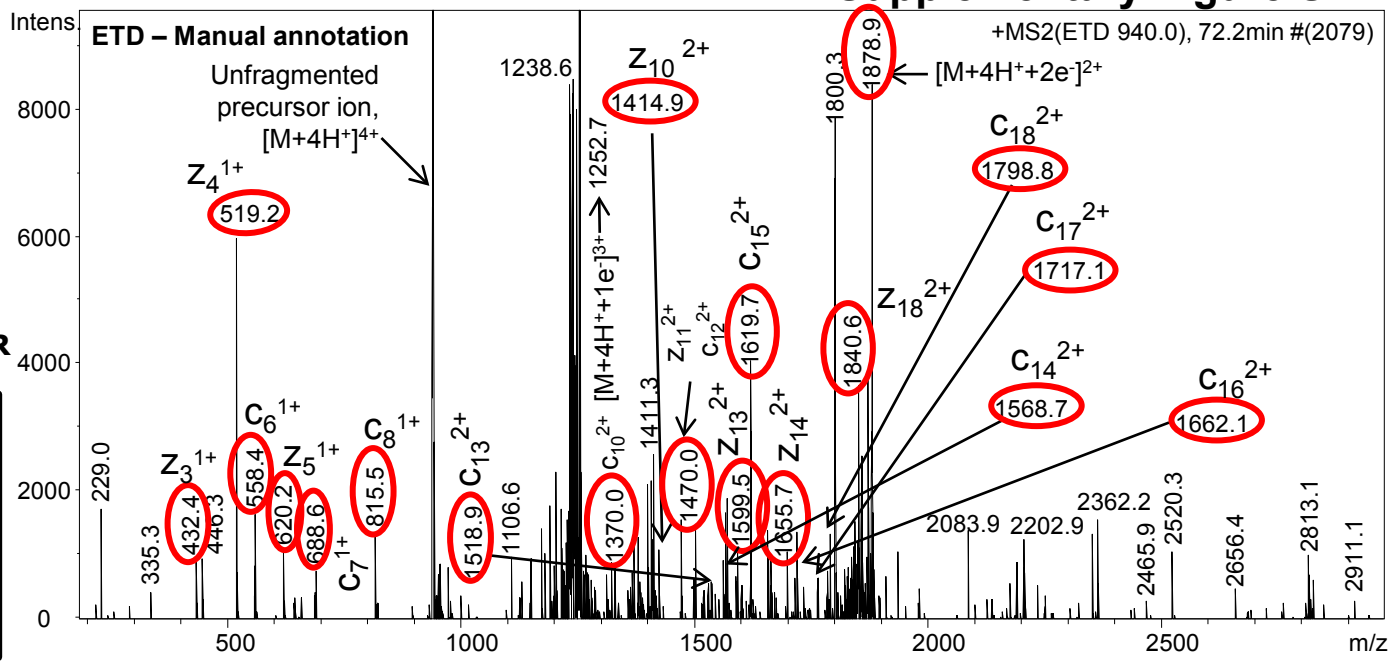
Human neutrophil elastase–P08246

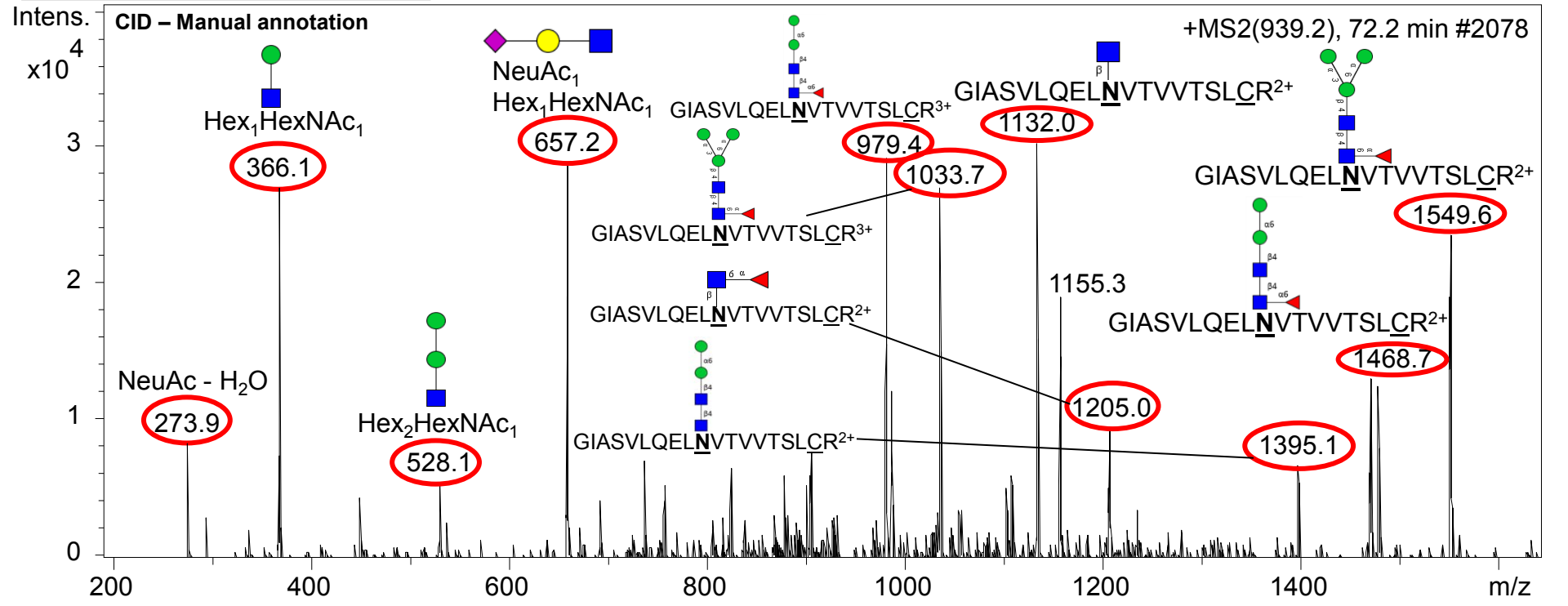
GIASVLQELNVTVVTSLCR
(Asn173 glycopeptide)
Obs. *m/z* 939.2 (4+)
Obs. $[M+H]^+$ = 3753.8 Da
Glycan: 1694.6 Da
($Hex_4HexNAc_3Fuc_1NeuAc_1$)
Calc. $[M+H]^+$ = 3753.7 Da
Retention time: 72 min





Supplementary Figure S4A



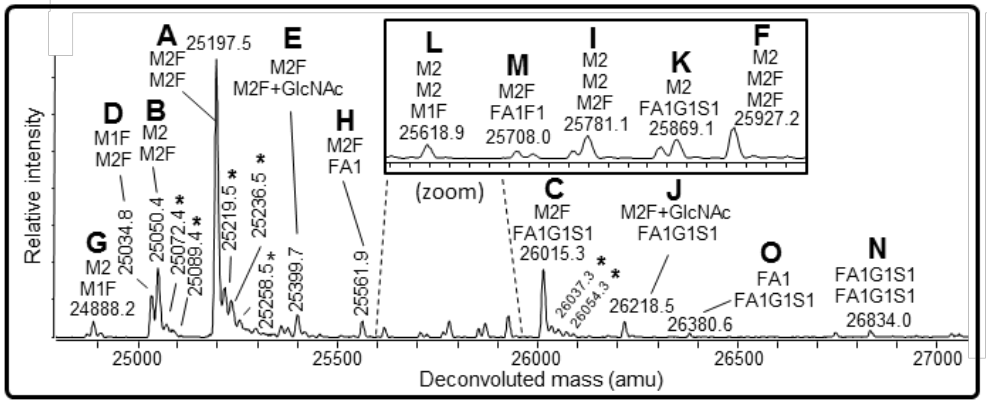


Supplementary Figure S4B

Tryptic, semi-tryptic and non-tryptic peptide sequence coverage of HNE (red, underlined sequences indicate observed peptides).

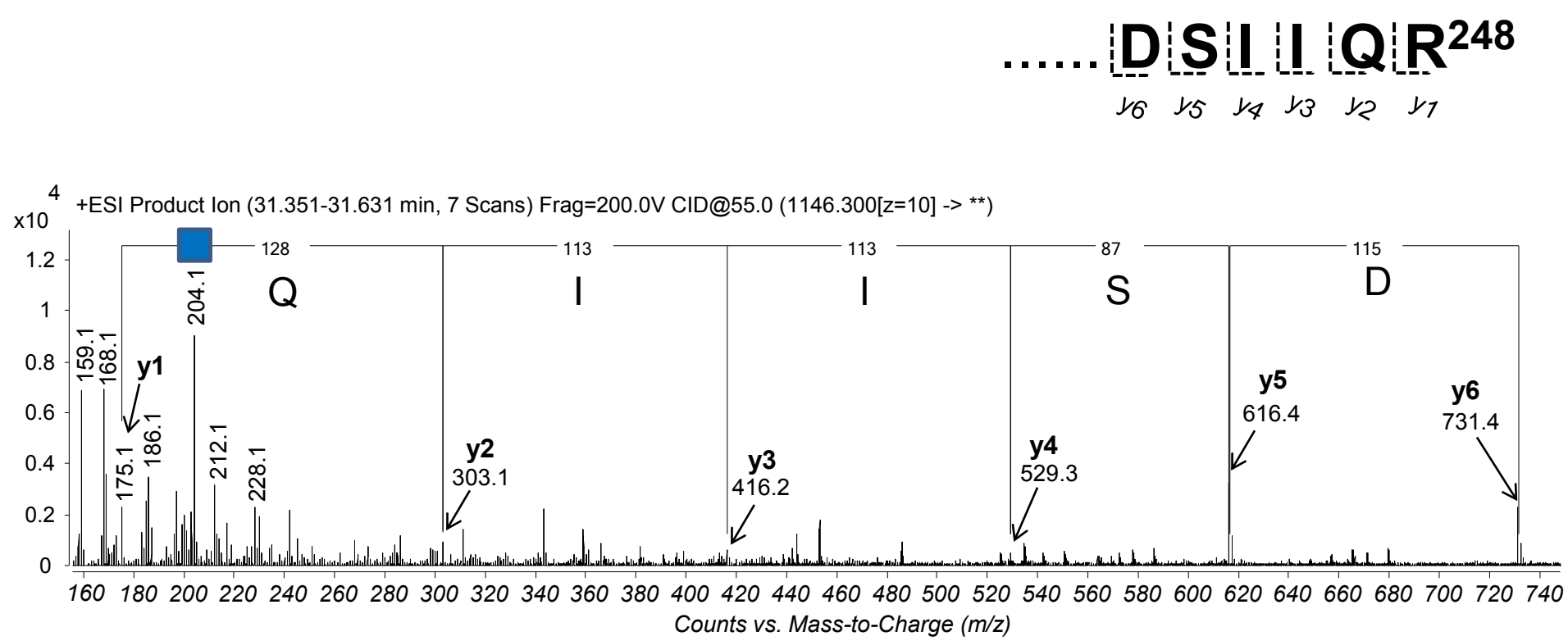
^{1}MTLGRRLACLFLACVLPALLLGGTALA^{27}SE^{29}IVGGRRA
RPHAWPFMVSLQLRGGHFCGATLIAPNFVMSAAHCVA
NVNVRAVRVVLGAHN^{88}LSRREPTRQVFAVQRIFENGYD
PVNLLNDIVILQLN^{124}GSATINANVQVAQLPAQGRRLGN
GVQCLAMGWGLLGRNRGIASVLQELN^{173}VTVVTSLCRR
SN^{185}VCTLVRGRQAGVCFGDSGSPLVCNGLIHGIASFV
RGGCASGLYPDAFAPVAQFVNWIDSIIQR^{248}SEDNPCPH
PRDPDPASRTH^{267}

Supplementary Figure S5A

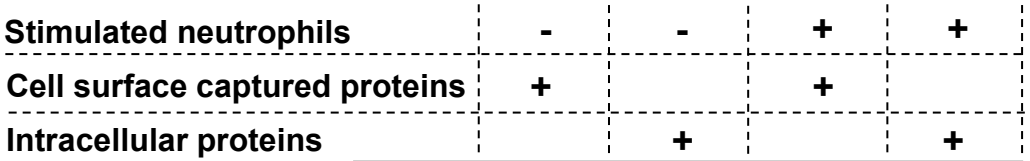


HNE glycoforms	$M_{experimental}$ (Da)	$M_{theoretical}$ (Da)	Δppm	**Proposed total monosaccharide compositions** (site-unspecific annotation)
A	25197.5	25196.5	-38.7	$HexNAc_4Hex_4Fuc_2$
B	25050.4	25050.4	-0.7	$HexNAc_4Hex_4Fuc_1$
C	26015.3	26015.3	-1.4	$HexNAc_5Hex_6Fuc_2NeuAc_1$
D	25034.8	25034.3	-16.7	$HexNAc_4Hex_3Fuc_2$
E	25399.7	25399.7	0.8	$HexNAc_5Hex_4Fuc_2$
F	25927.2	25927.8	0.0	$HexNAc_6Hex_6Fuc_2$
G	24888.2	24888.2	1.6	$HexNAc_4Hex_3Fuc_1$
H	25561.9	25561.9	-1.5	$HexNAc_5Hex_5Fuc_2$
I	25781.1	25781.1	-1.7	$HexNAc_6Hex_6Fuc_1$
J	26218.5	26218.5	-1.6	$HexNAc_6Hex_6Fuc_2NeuAc_1$
K	25869.1	25869.1	0.8	$HexNAc_5Hex_6Fuc_1NeuAc_1$
L	25618.9	25618.9	0.6	$HexNAc_6Hex_5Fuc_1$
M	25708.0	25708.0	-0.2	$HexNAc_5Hex_5Fuc_3$
N	26834.0	26834.0	0.0	$HexNAc_6Hex_8Fuc_2NeuAc_2$
O	26380.6	26380.6	0.0	$HexNAc_6Hex_7Fuc_2NeuAc_1$

Supplementary Figure S5B

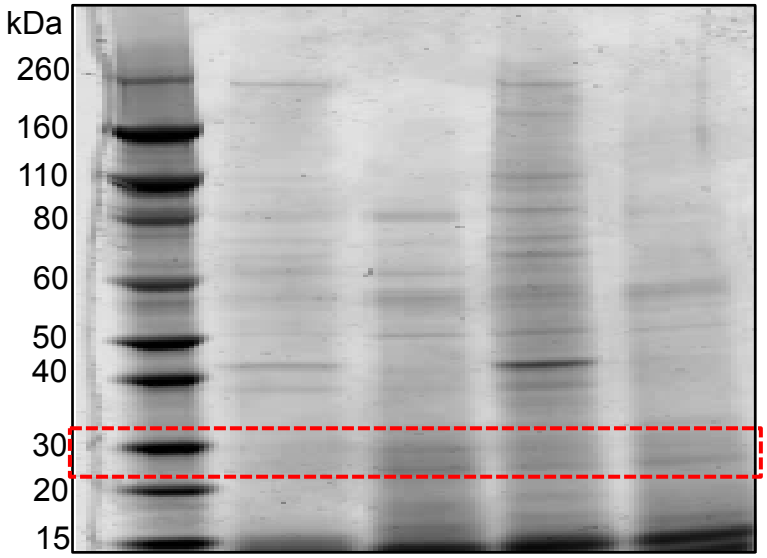


Supplementary Figure S6



(A) Anti-HNE antibody

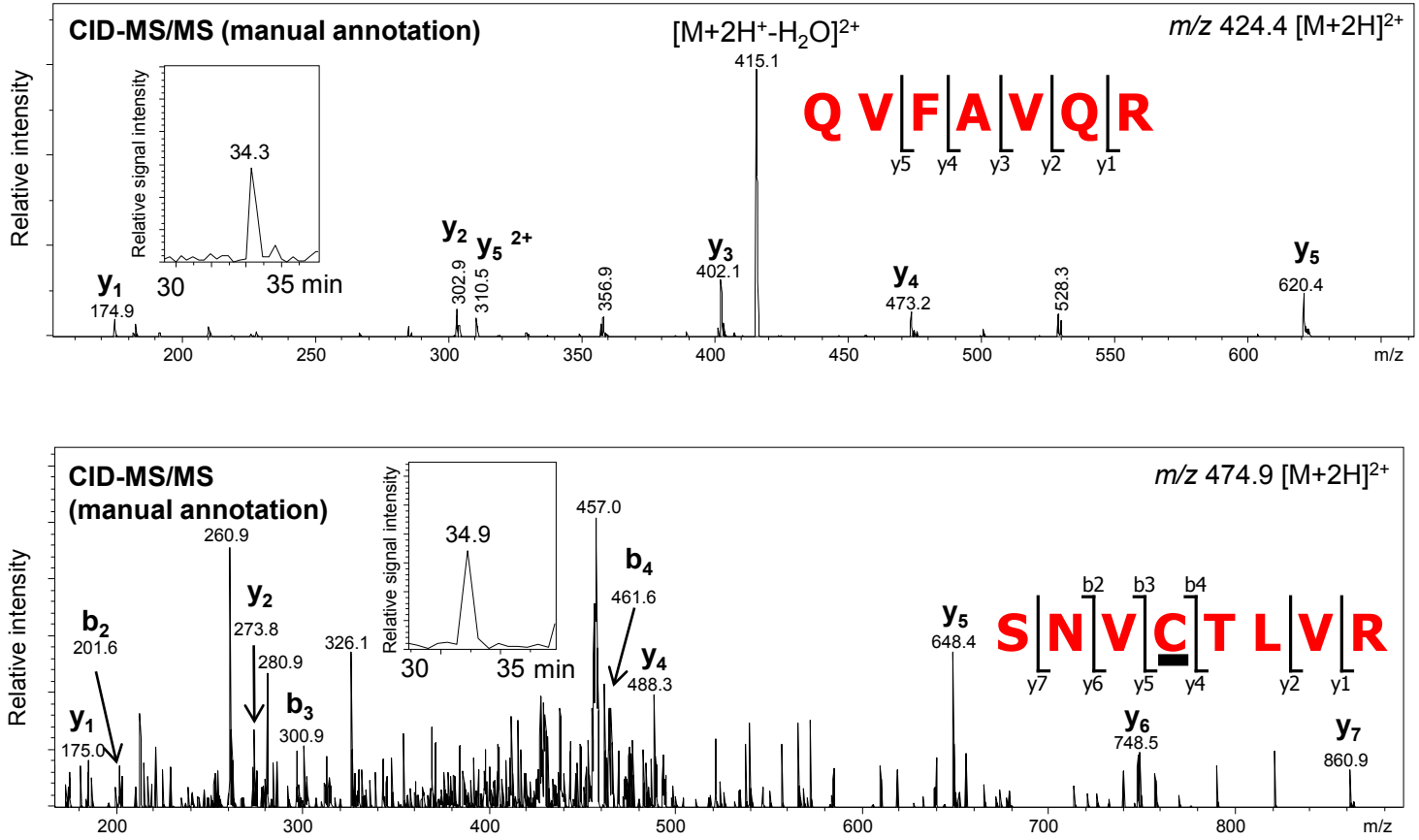
(B) Anti-paucimannose antibody (Mannitou)



(C) CMB-stained SDS-PAGE gel

Supplementary Figure S7

A

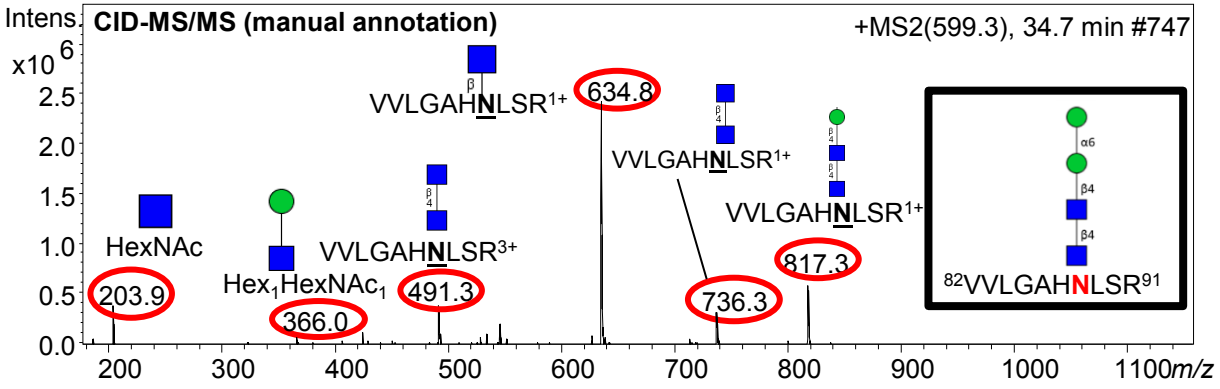


B

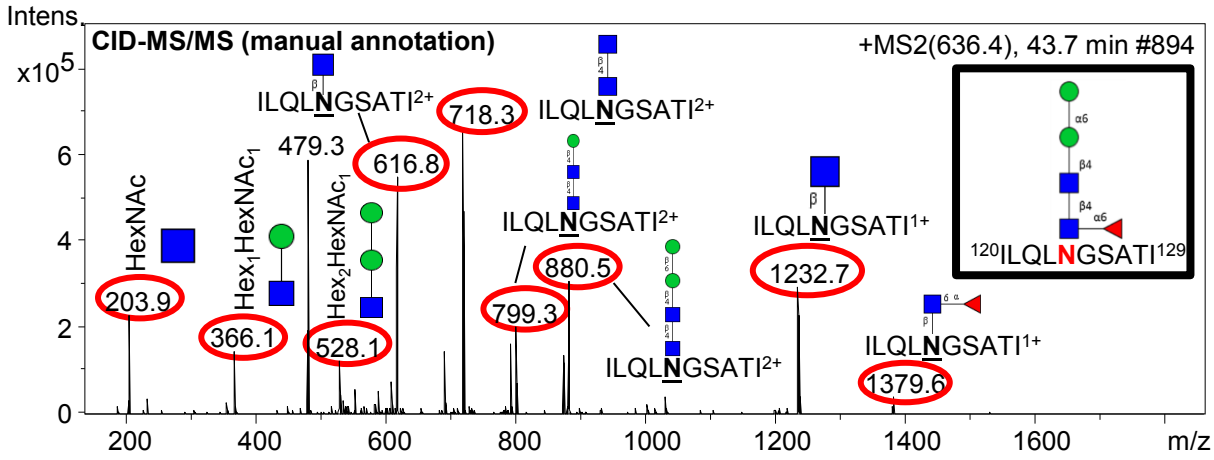
[1]MTLGRRLACLFLACVLPALLLGGTALA[27]SE[29]IVGGRRARPHAWPFMVSLQLRGGHFC
GATLIAPNFVMSAAHCVANVNVRAVRVVLGAHN[88]LSRREPTR**QVFAVQR**IFENGYDPV
NLLNDIVILQLN[124]GSATINANVQVAQLPAQGRRLGNGVQCLAMGWGLLGRNRGIASV
LQELN[173]VTVVTSLCRR**SN[185]VCTLVR**GRQAGVCFGDSGSPLVCNGLIHGIASFVRGG
CASGLYPDAFAPVAQFVNWIDSIIQR[248]SEDNPCPHPRDPDPASRTH[267]

Supplementary Figure S8

Human neutrophil elastase – P08246
VVLGAH**N**LSR (Asn88)
Obs. *m/z* 599.3 (3+)
Obs. $[M+H]^+$ = 1795.9 Da
Glycan: 730.3 Da ($Hex_2HexNAc_2$)
Calc. $[M+H]^+$ = 1795.8 Da
Retention time: 35 min

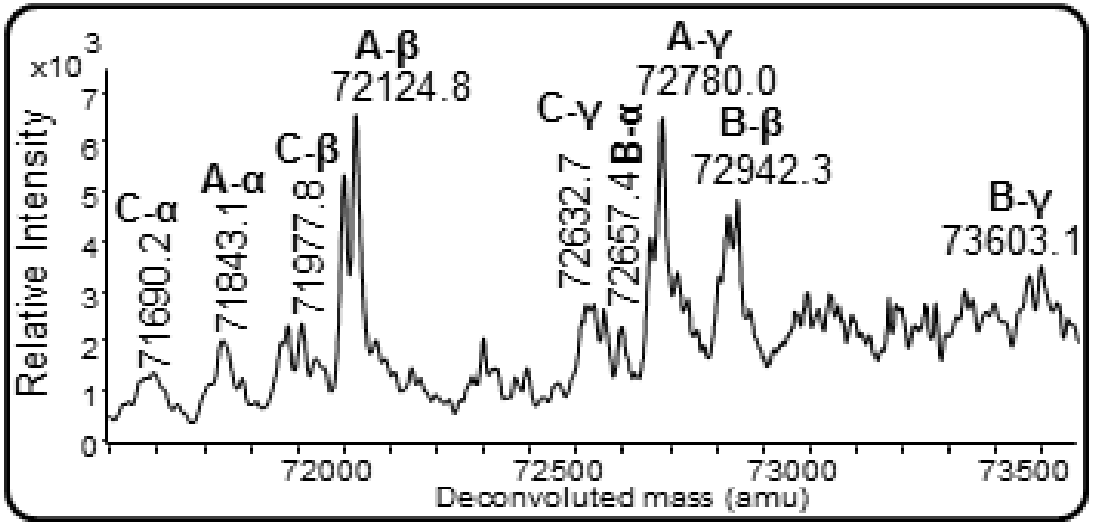


Human neutrophil elastase– P08246
ILQL**N**GSATI (Asn124)
Obs. *m/z* 636.1 (3+)
Obs. $[M+H]^+$ = 1906.3 Da
Glycan: 876.3 Da ($Hex_2HexNAc_2Fuc_1$)
Calc. $[M+H]^+$ = 1906.1 Da
Retention time: 44 min



Colour/symbol key:
- *N*-Acetylglucosamine (GlcNAc)
- Mannose (Man)
- Fucose (Fuc)
- Galactose (Gal)
- *N*-Acetylneuraminic acid (NeuAc)

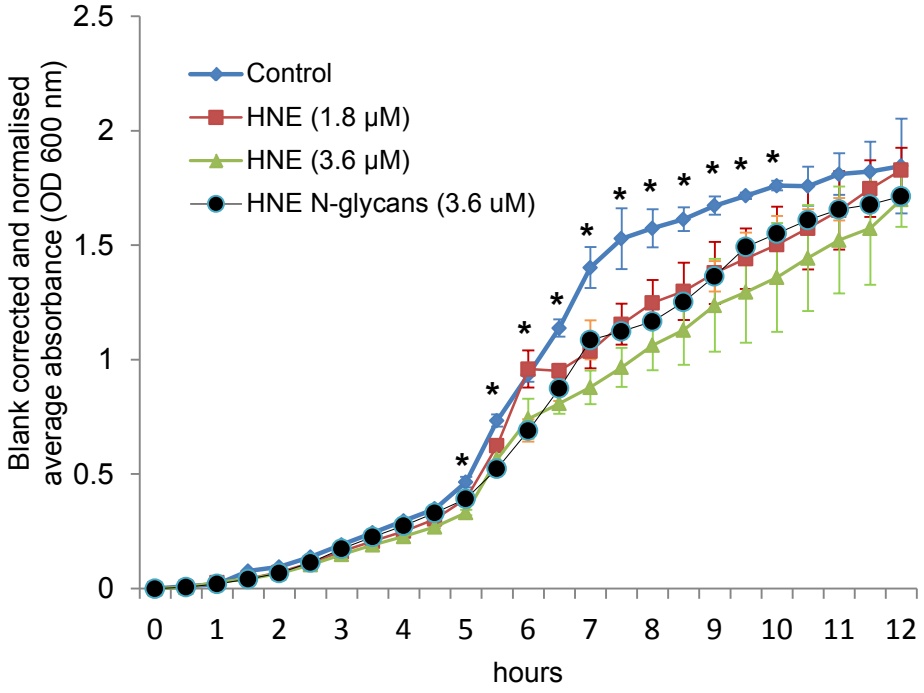
Supplementary Figure S9



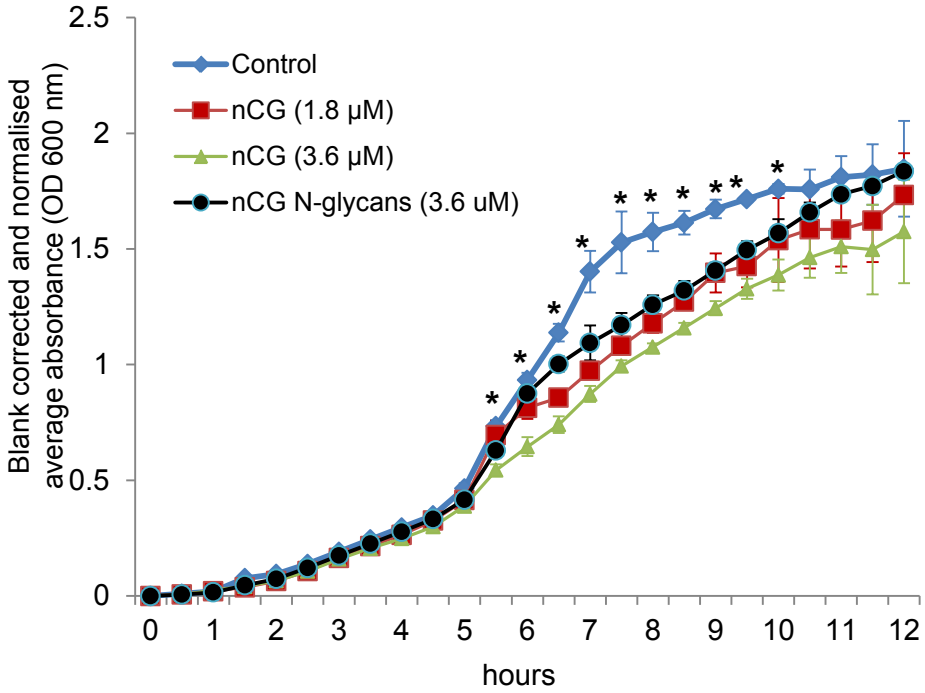
HNE:A1AT glycoforms	$M_{experimental}$, (Da)	$M_{theoretical}$, (Da)	Δppm	Proposed monosaccharide compositions of HNE (site-unspecific annotation)	Proposed monosaccharide compositions of human A1AT (site-unspecific annotation)
A-α	71843.1	71834.3	-123.0	$HexNAc_4Hex_4Fuc_2$	$HexNAc_{12}Hex_{15}NeuAc_5$
B-α	72657.4	72653.0	-60.5	$HexNAc_5Hex_6Fuc_2NeuAc_1$	$HexNAc_{12}Hex_{15}NeuAc_5$
C-α	71690.2	71688.1	-29.8	$HexNAc_4Hex_4Fuc_1$	$HexNAc_{12}Hex_{15}NeuAc_5$
A-β	72124.8	72123.0	-24.3	$HexNAc_4Hex_4Fuc_2$	$HexNAc_{12}Hex_{15}NeuAc_6$
B-β	72942.3	72941.8	-7.1	$HexNAc_5Hex_6Fuc_2NeuAc_1$	$HexNAc_{12}Hex_{15}NeuAc_6$
C-β	71977.8	71976.9	-12.5	$HexNAc_4Hex_4Fuc_1$	$HexNAc_{12}Hex_{15}NeuAc_6$
A-γ	72780.0	72779.8	-2.7	$HexNAc_4Hex_4Fuc_2$	$HexNAc_{13}Hex_{16}NeuAc_7$
B-γ	73603.1	73598.5	-61.9	$HexNAc_5Hex_6Fuc_2NeuAc_1$	$HexNAc_{13}Hex_{16}NeuAc_7$
C-γ	72632.7	72633.7	13.3	$HexNAc_4Hex_4Fuc_1$	$HexNAc_{13}Hex_{16}NeuAc_7$

Supplementary Figure S10

A HNE-based growth inhibition of PASS1



B nCG-based growth inhibition of PASS1



* $p < 0.05$ comparing control (untreated PASS1) and PASS1 grown with released (free, native) *N*-glycans

(Mean ± S.D, n = 3 technical replicates)