SUPPLEMENTARY DATA

Cell Type-Specific and Site Directed *N*-Glycosylation Pattern of FcγRIIIa

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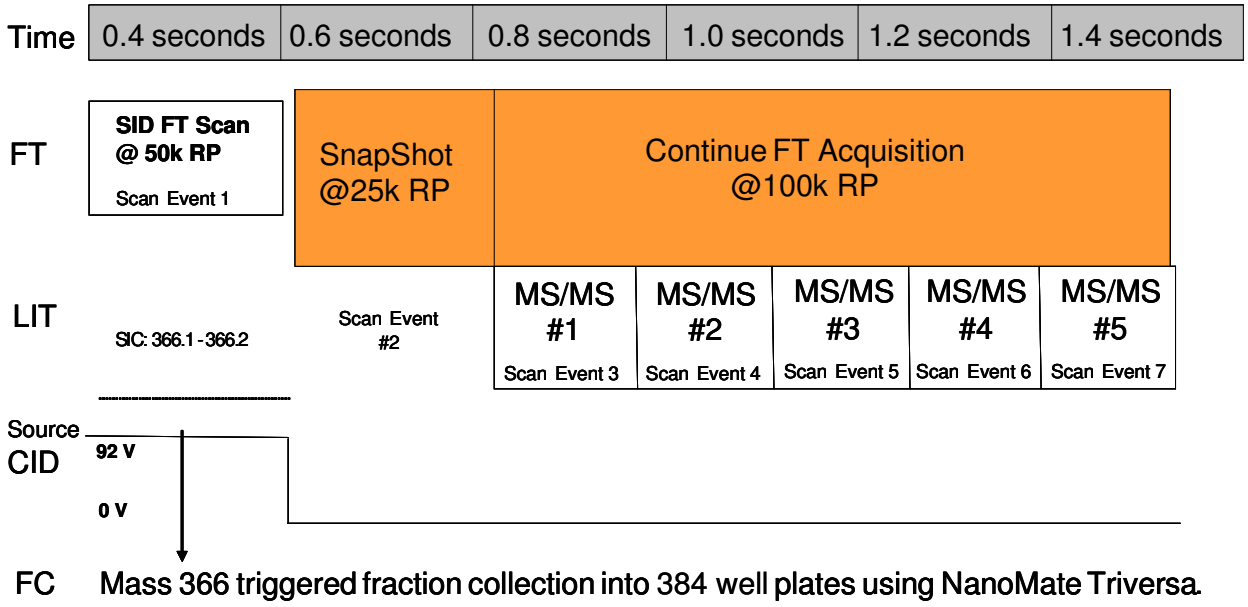
Supporting Materials and Methods

Parameters used for on-line LC-MS/MS analysis and data processing

The NanoMate spray voltage was 1.4 kV, the capillary temperature was 175 °C, the ion trap collision energy for generation of MS/MS spectra was set to 40% and the tube lens voltage was 100 V. The dynamic exclusion feature was enabled (Repeat Count: 1, Exclusion Duration: 8 sec, Exclusion mass width: 3 ppm). A temporal representation of the scan event cycle employed is shown in

Supplemental Figure S 1. For data processing the combined mass spectra of glycopeptides coeluting in a chromatographic peak were deconvoluted and monoisotopic masses were obtained by using the Xtract ™ Software provided with the mass spectrometer. Relative quantification of the glycostructures was performed at

glycopeptide level using the peak intensities of the deconvoluted monoisotopic mass peaks.

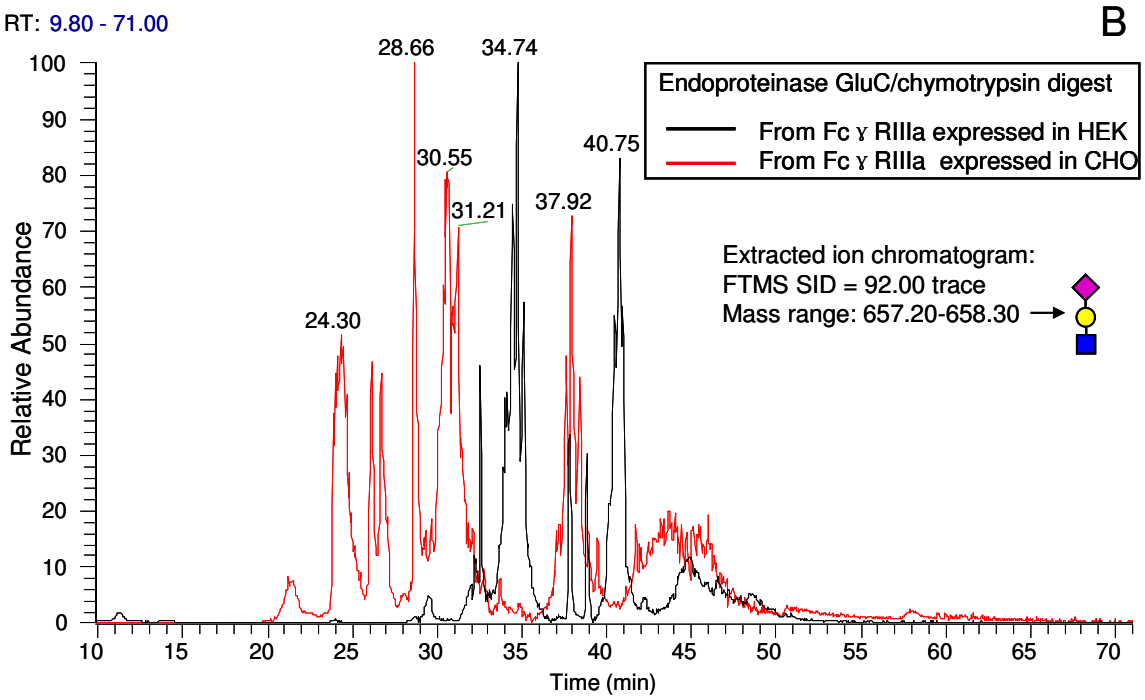


Supplemental Figure S 1: Temporal representation of the scan event cycle employed for the on-line characterization of enzymatic peptides and for mass triggered fraction collection for off-line measurement of glycopeptides.

Identification of FcγRIIIa cell-type specific glycosylation fingerprints by LC-SID-MS/MS

The glycan predictive oxonium ions were detected using the high mass accuracy of the FT ICR cell To reduce the false positive identification rate.

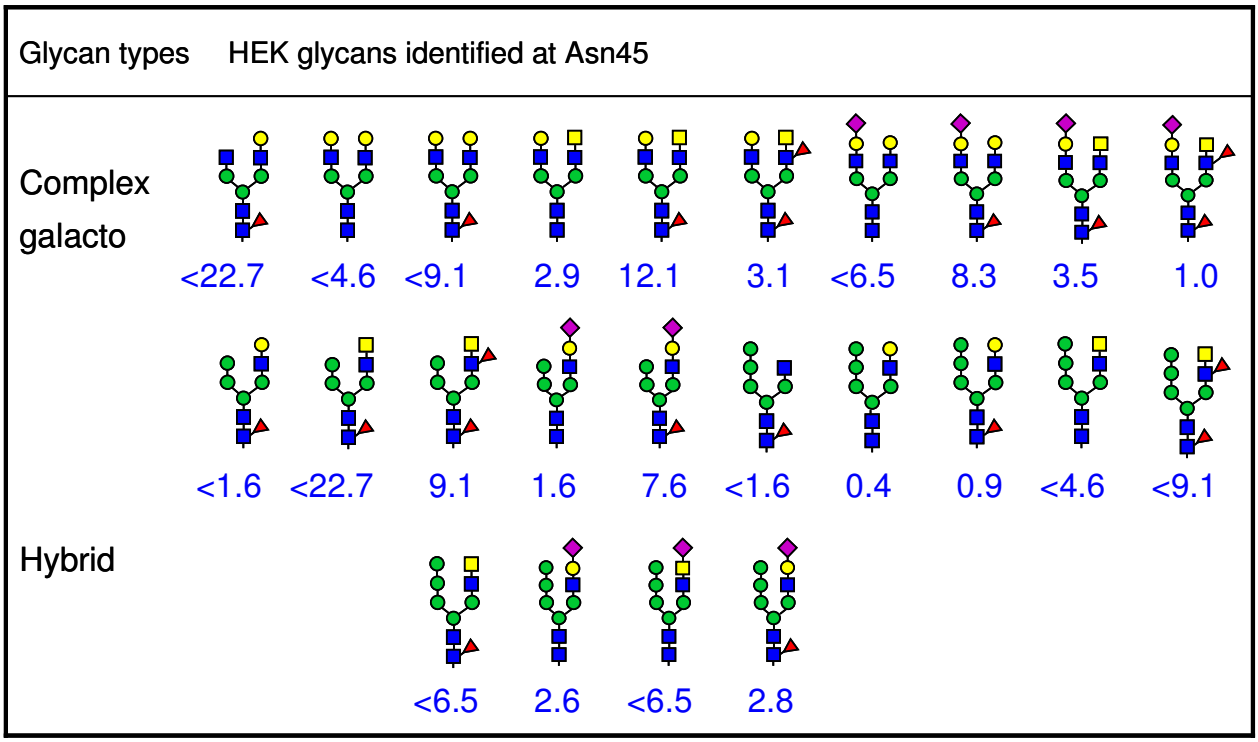
As supplemental example, the source SID trace of m/z range 657.20-657.30 is shown in Supplemental Figure S 2. It demonstrates clearly that glycans containing Neu5Ac are prevalent in FcγRIIIa from CHO cells but not in the HEK derived receptor.



Supplemental Figure S 2: Extracted ion chromatograms of typical glycan predictive oxonium ions obtained from source induced dissociation (SID) of a combined digest of FcγRIIIa by endoproteinase Glu-C and chymotrypsin. B: EIC of HexNAcHexNeuAc+ (m/z 657.2).

Site-directed shFcγRIIIa glycosylation

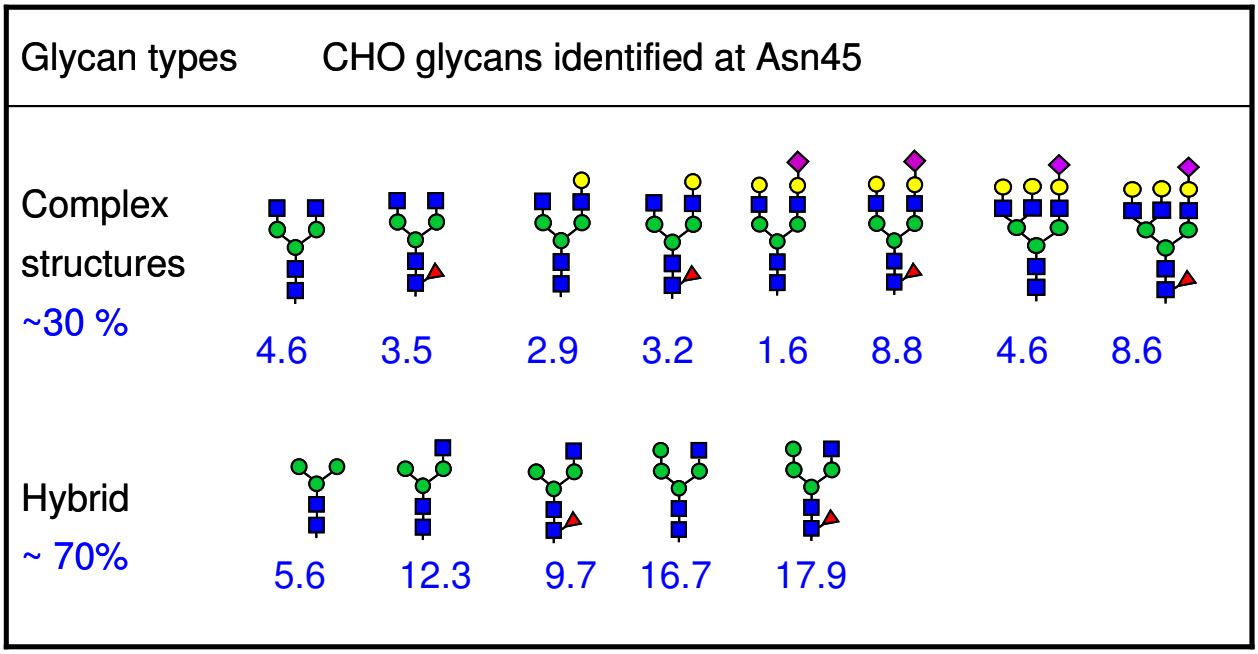
Supplemental Figures S 3 to S 6 list the glycan identified for the N-glycosylation Sites Asn-45 and Asn-74. Additional MS and MS/MS data for Asn-162 from HEK and CHO is contained in Supplemental Figures S 7 to S 11



Supplemental Figure S 3:

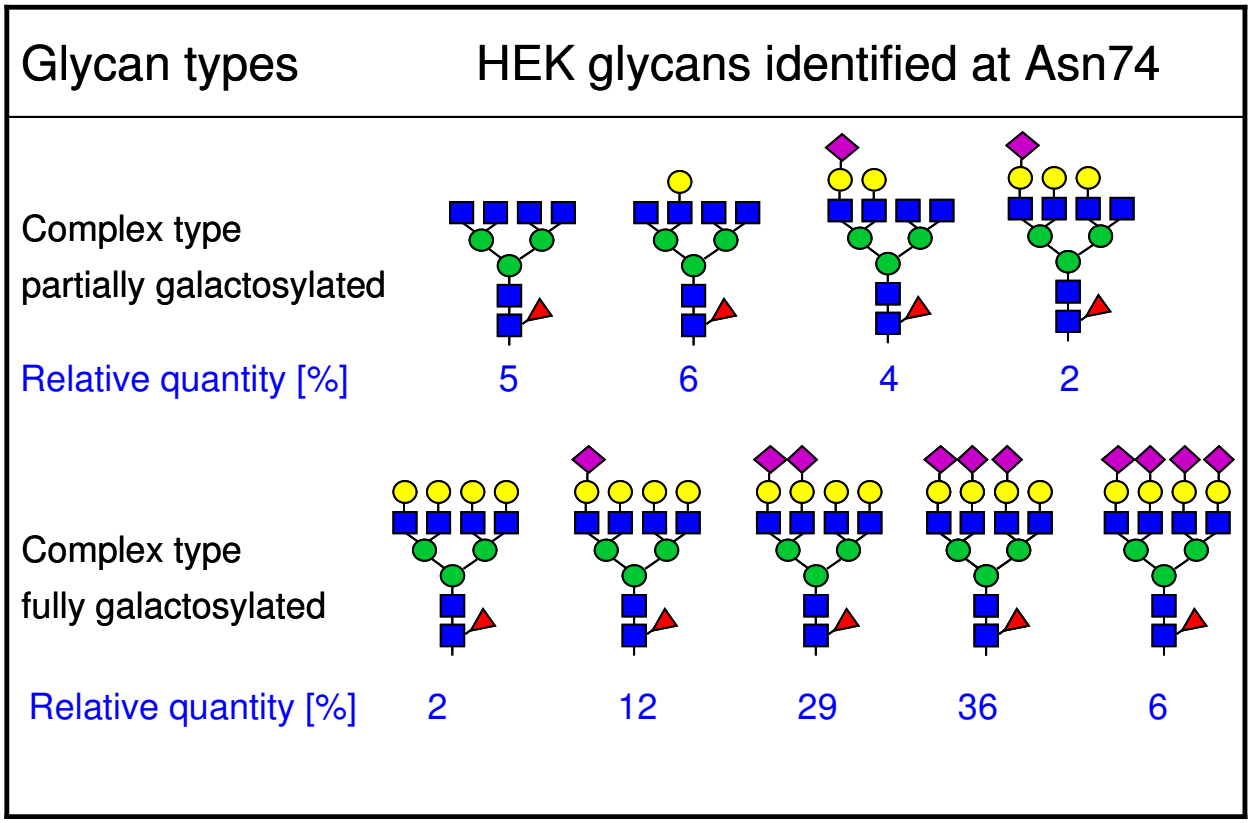
Glycans attached to Asn-45 found in shFcγRIIIa expressed by HEK cells. Approx. 18 % of the attached glycans are not core-fucosylated; approx. 30 % of the glycans are sialylated.

The relative quantitative amount is given underneath each structure. Isobaric structures have identical relative quantity and are marked with "<".

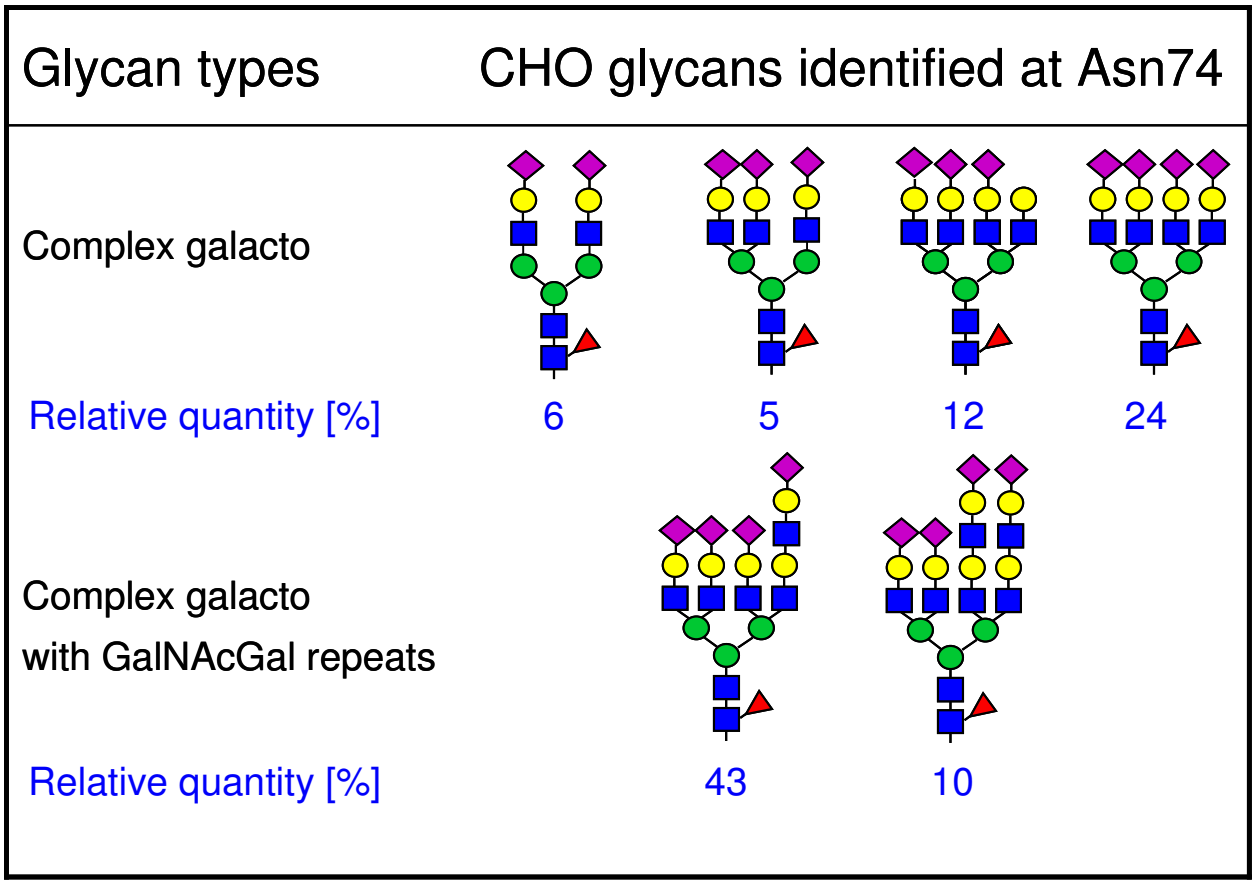


Supplemental Figure S 4:

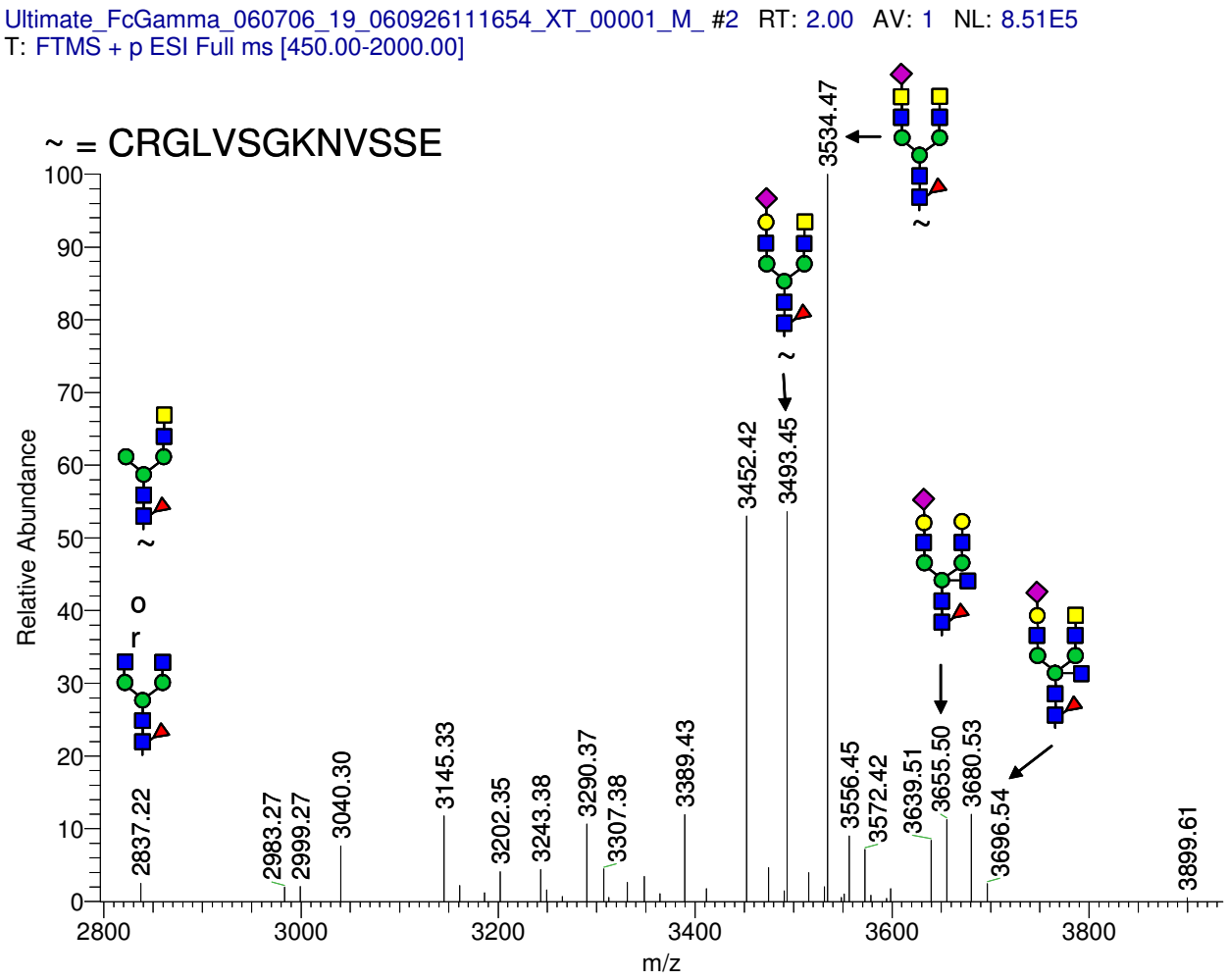
Glycans attached to Asn-45 found in shFcγRIIIa expressed by CHO cells. Approx. 50 % of the attached glycans are not core-fucosylated; approx. 25 % of the glycans are sialylated.
Green circle, Man; yellow circle, Gal; yellow square, GalNAc, red triangle, Fuc; blue square, GlcNAc; purple diamond, Neu5Ac.



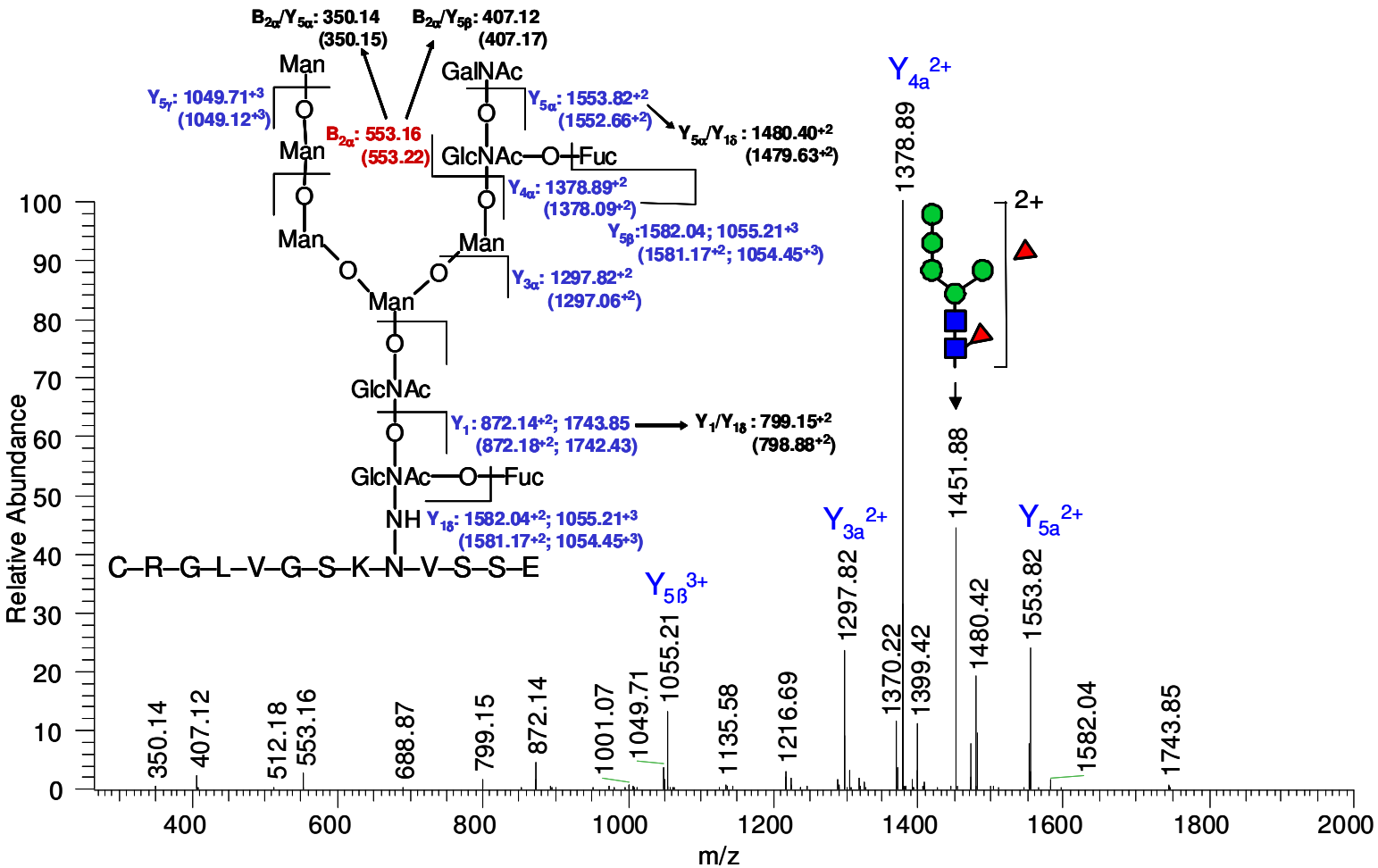
Supplemental Figure S 5:
Glycans attached to Asn-74 found in shFcγRIIIa expressed by HEK cells. All glycans identified are core-fucosylated and sialylated. Tetraantennary structures are predominant.



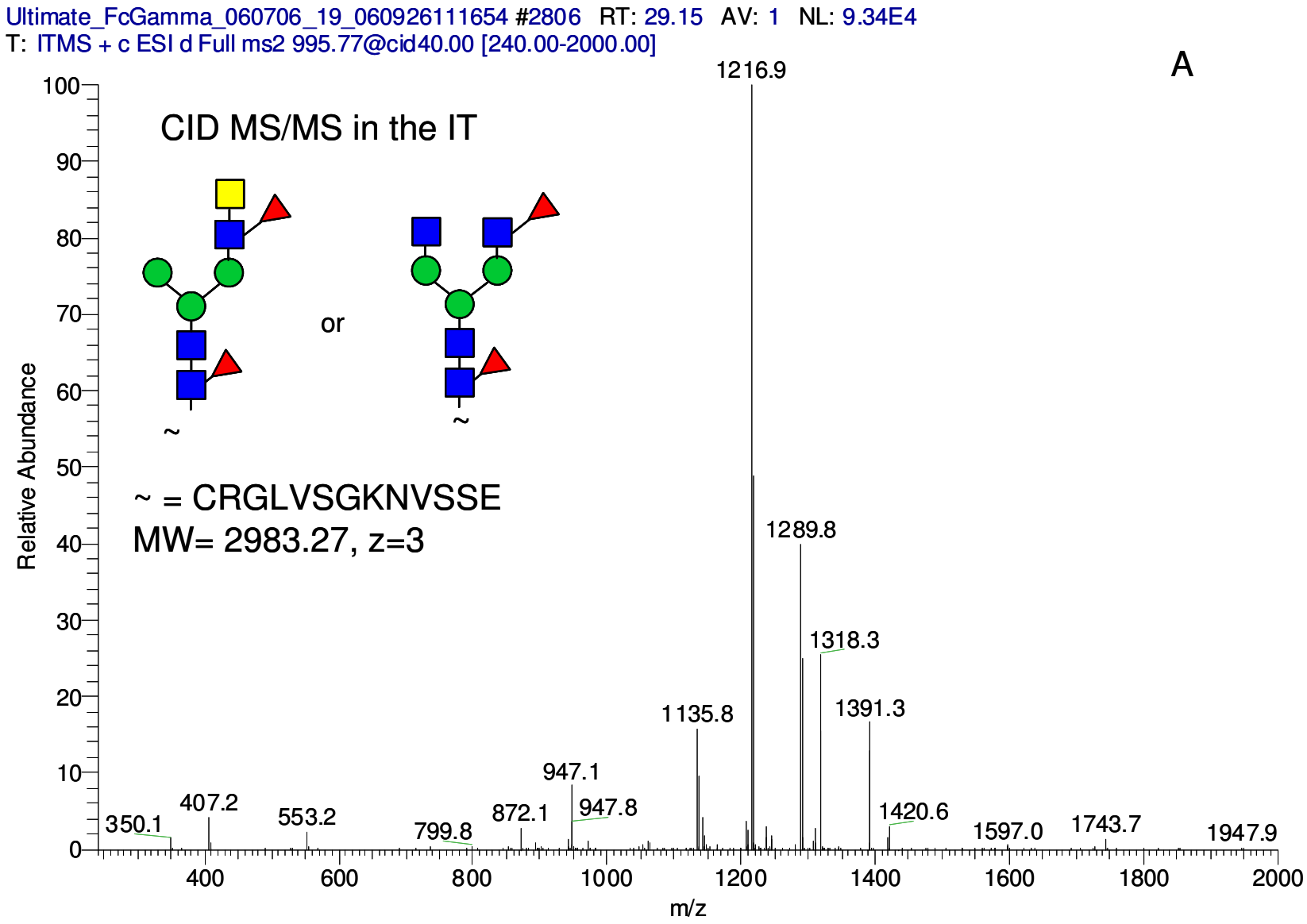
Supplemental Figure S 6:
Glycans attached to Asn-74 found in shFcγRIIIa expressed by CHO cells. All glycans identified are core-fucosylated and sialylated. Complex tetraantennary structures are predominant.



Supplemental Figure S 7: Deconvoluted mass spectrum from 29.4-29.6. Asn-162 FcGammaRIIIa expressed in HEK; labelled are only glcyoforms not labelled in the article.



Supplemental Figure S 8: Fragment ion spectrum of a hybrid type glycan found at Asn-162 expressed by HEK cells. Green circle, Man; blue square, GlcNAc; red triangle, Fuc.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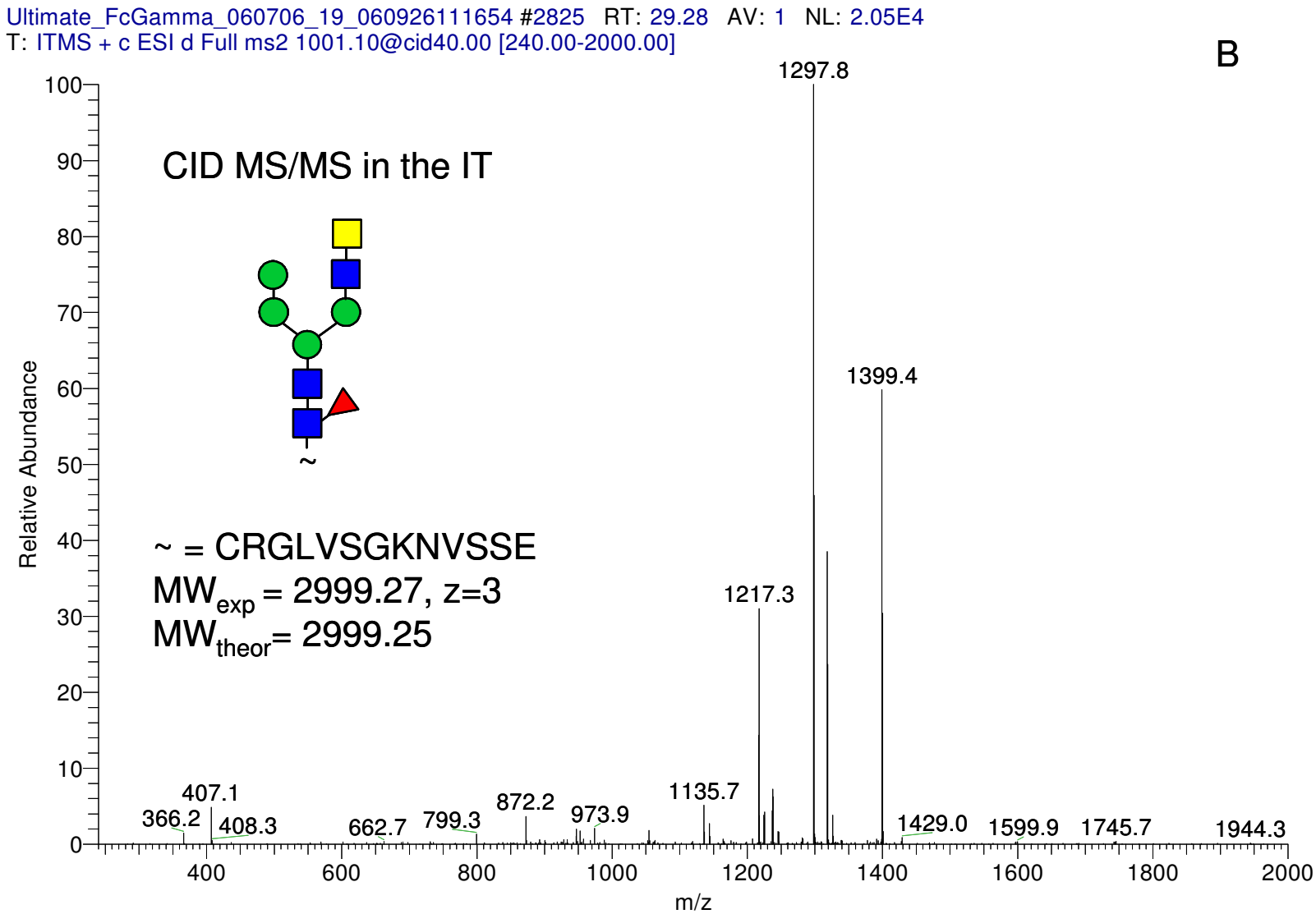
A
CID MS/MS in the IT
or
~
~ = CRGLVSGKNVSSE
MW= 2983.27, z=3
Relative Abundance
m/z
1216.9
1289.8
1318.3
1391.3
1135.8
947.1
947.8
872.1
799.8
553.2
407.2
350.1
1420.6
1597.0
1743.7
1947.9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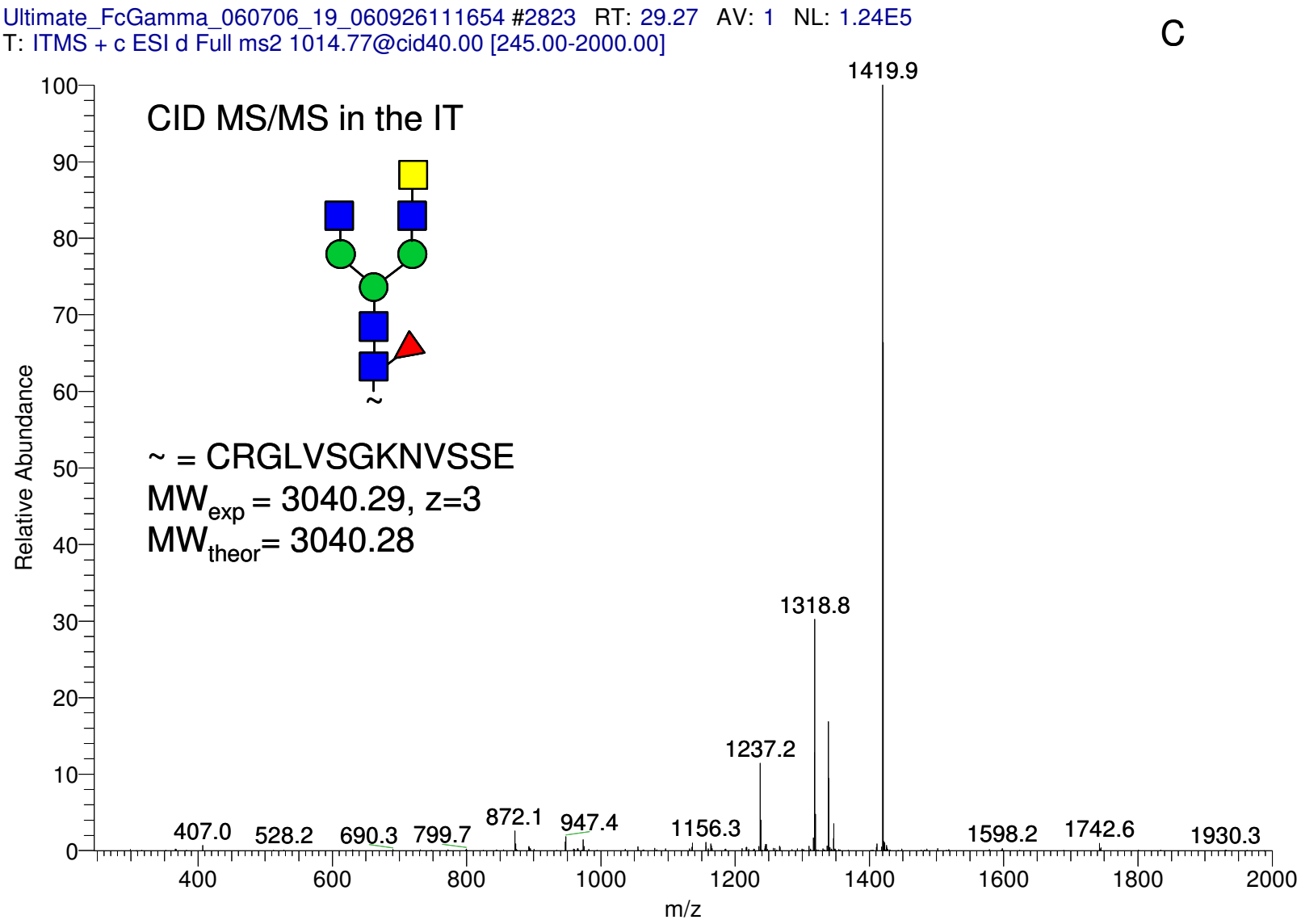
B
CID MS/MS in the IT
~
~ = CRGLVSGKNVSSE
MWexp = 2999.27, z=3
MWtheor= 2999.25
Relative Abundance
m/z
1297.8
1399.4
1217.3
407.1
366.2
408.3
662.7
799.3
872.2
973.9
1135.7
1429.0
1599.9
1745.7
1944.3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C
CID MS/MS in the IT
~ = CRGLVSGKNVSSE
MW exp = 3040.29, z=3
MW theor= 3040.28
Relative Abundance
m/z
1419.9
1318.8
1237.2
407.0
528.2
690.3
799.7
872.1
947.4
1156.3
1598.2
1742.6
1930.3

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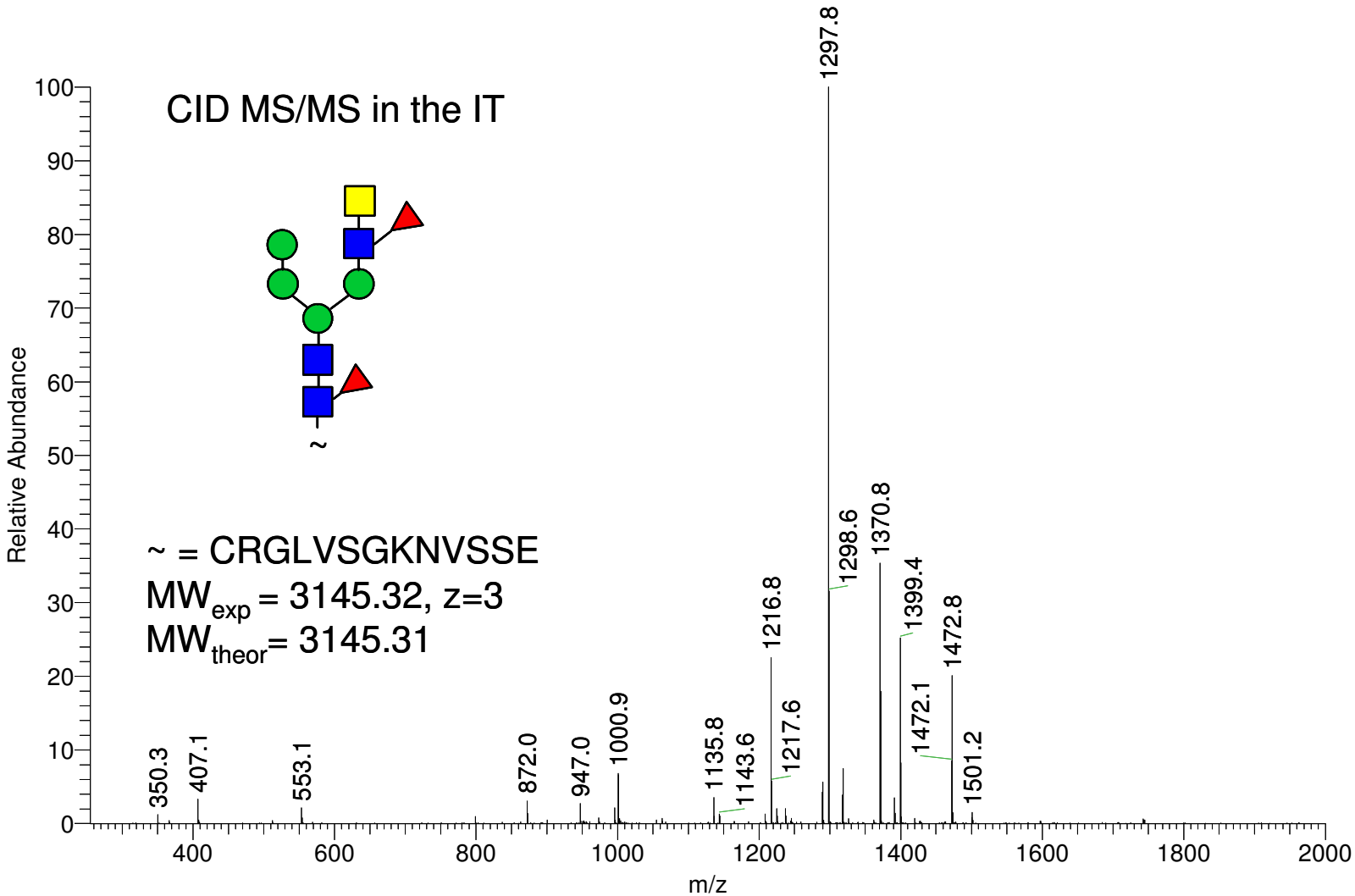
D



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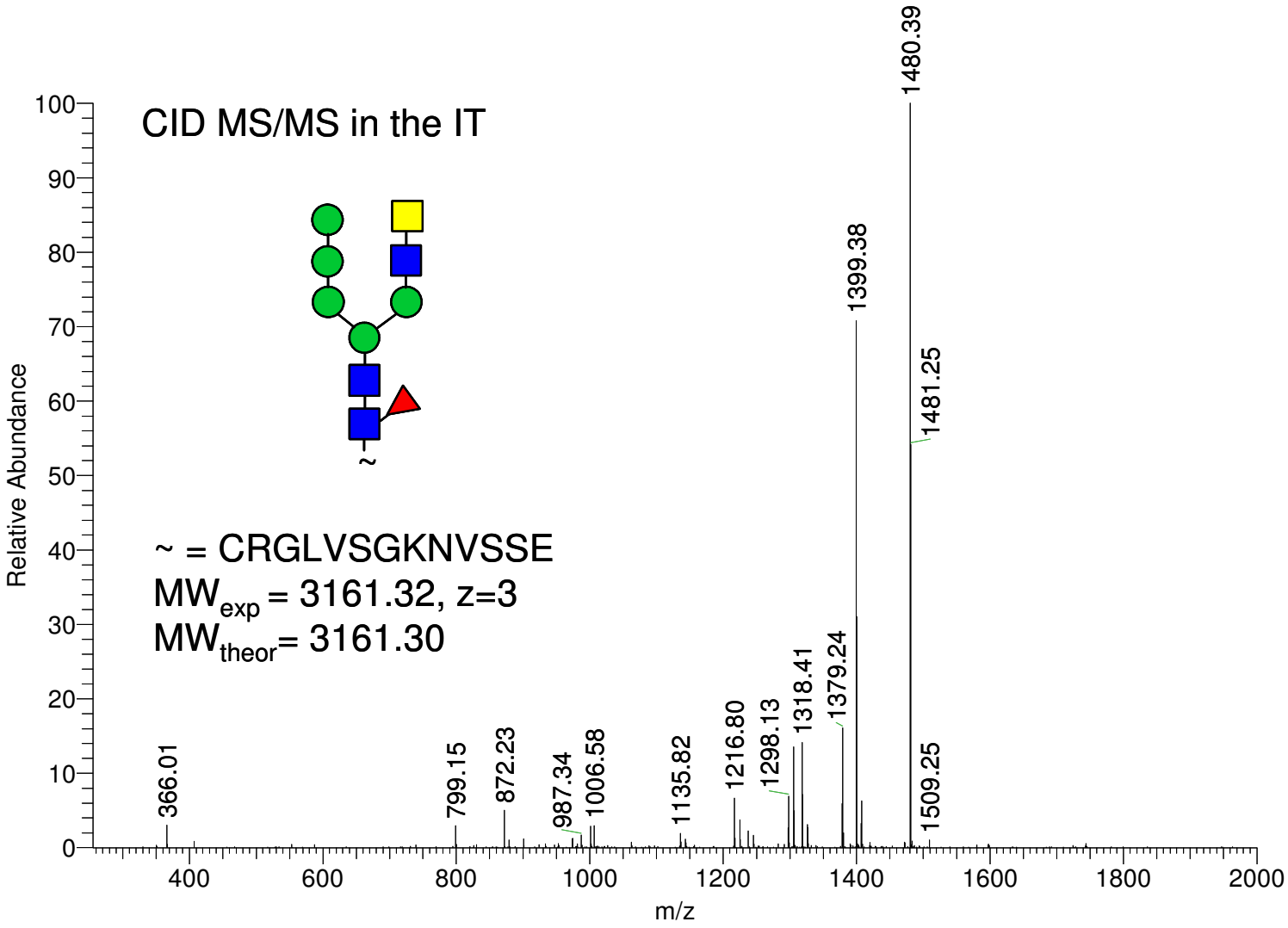

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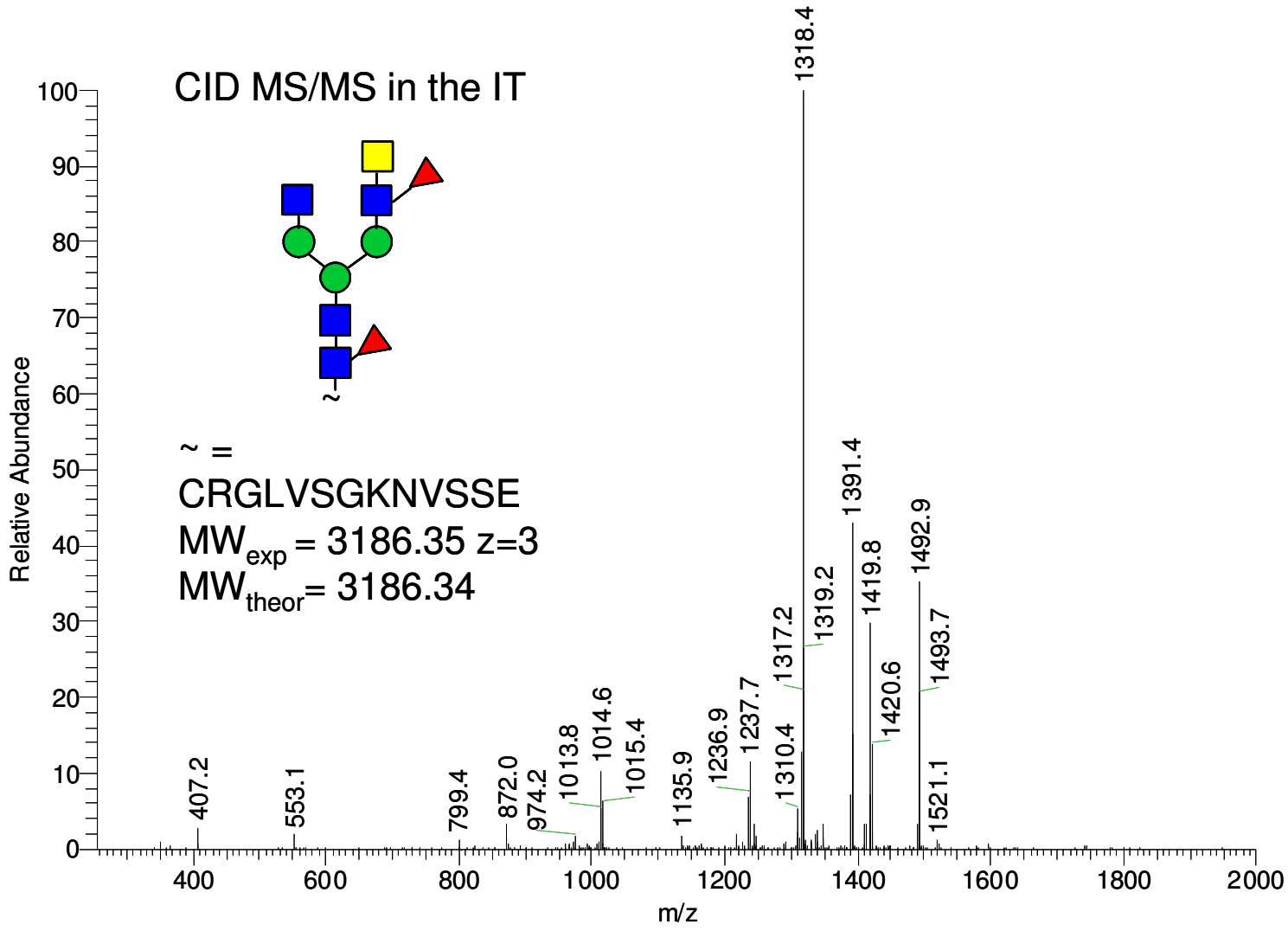
F



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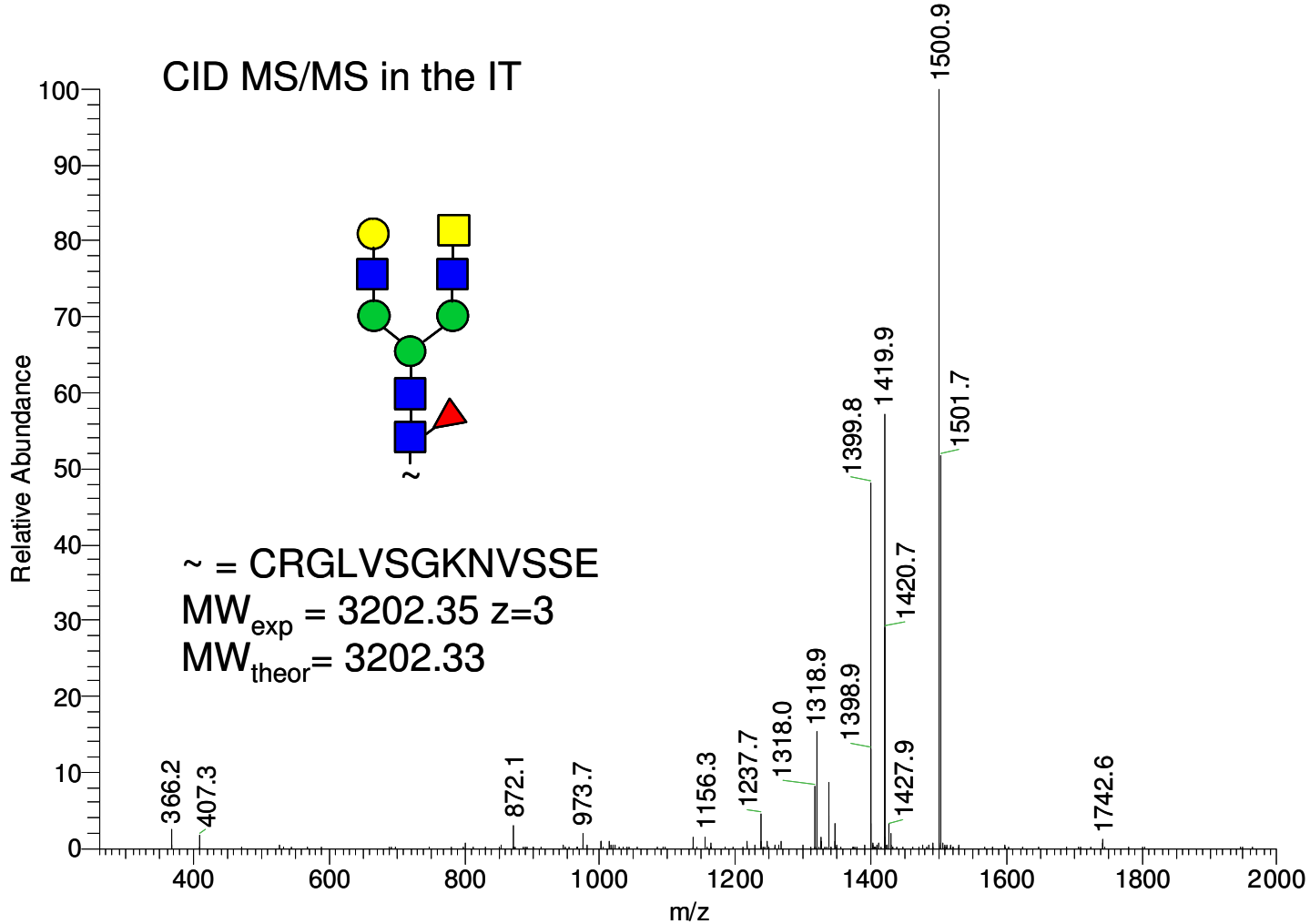
G



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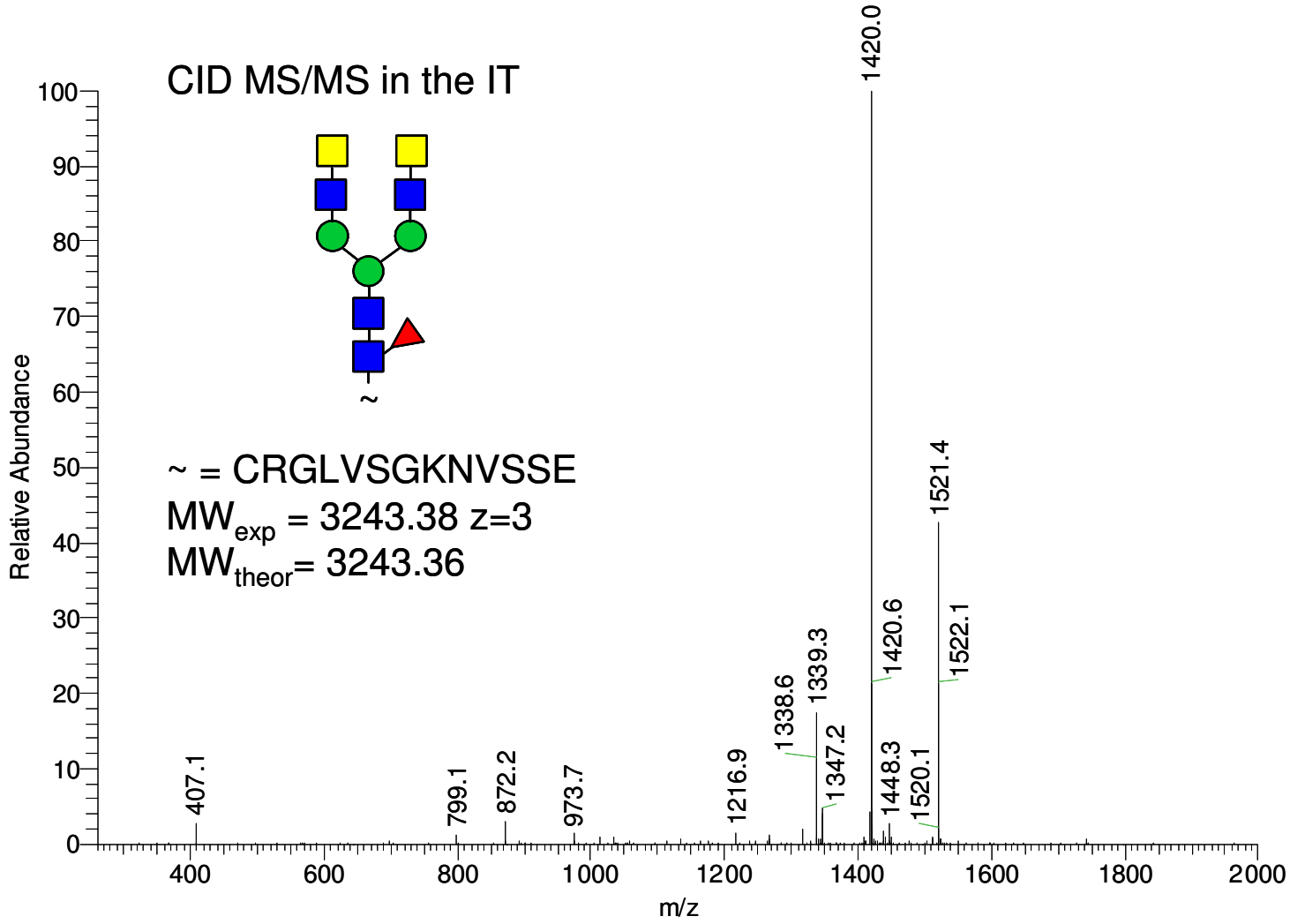
H



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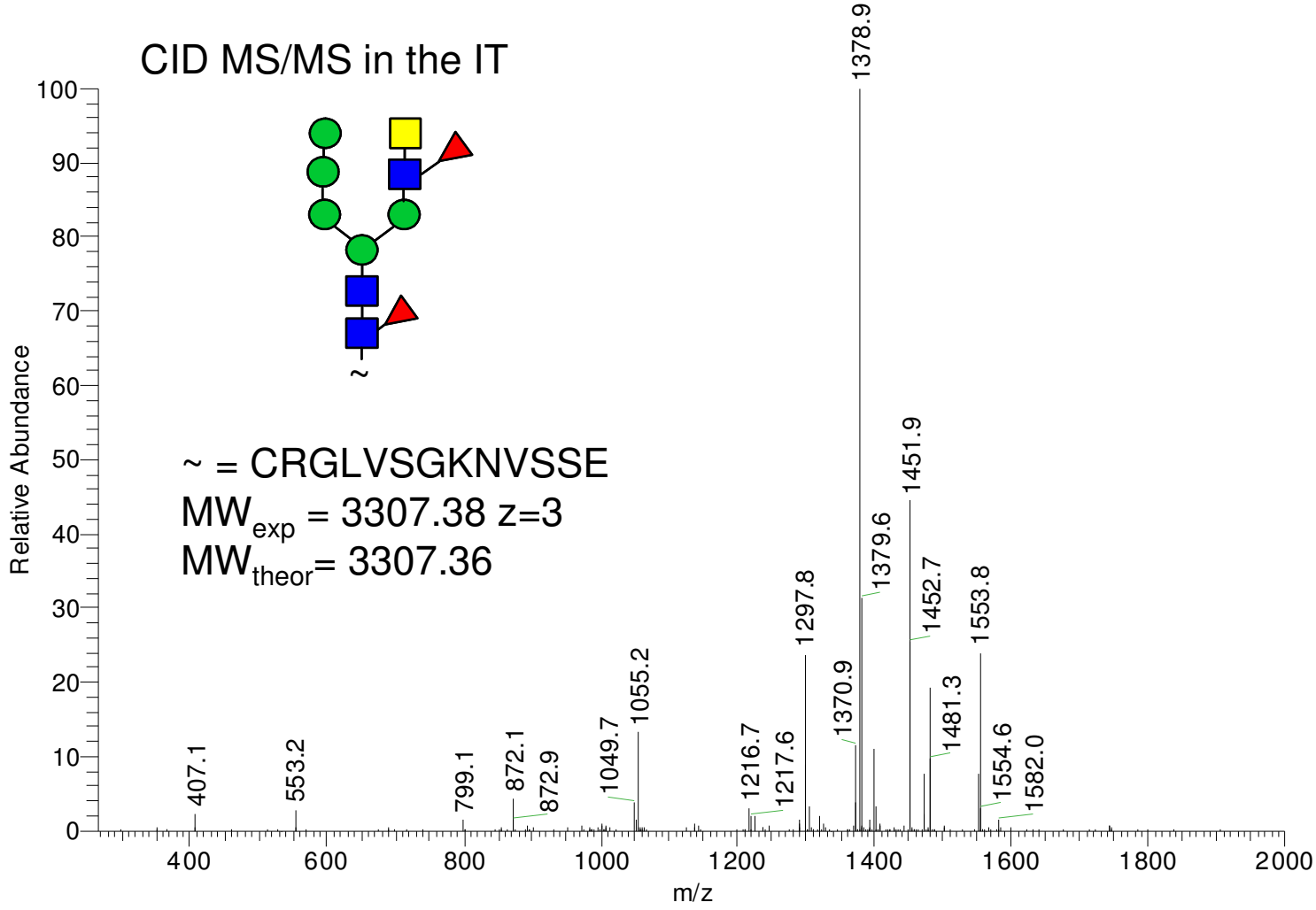

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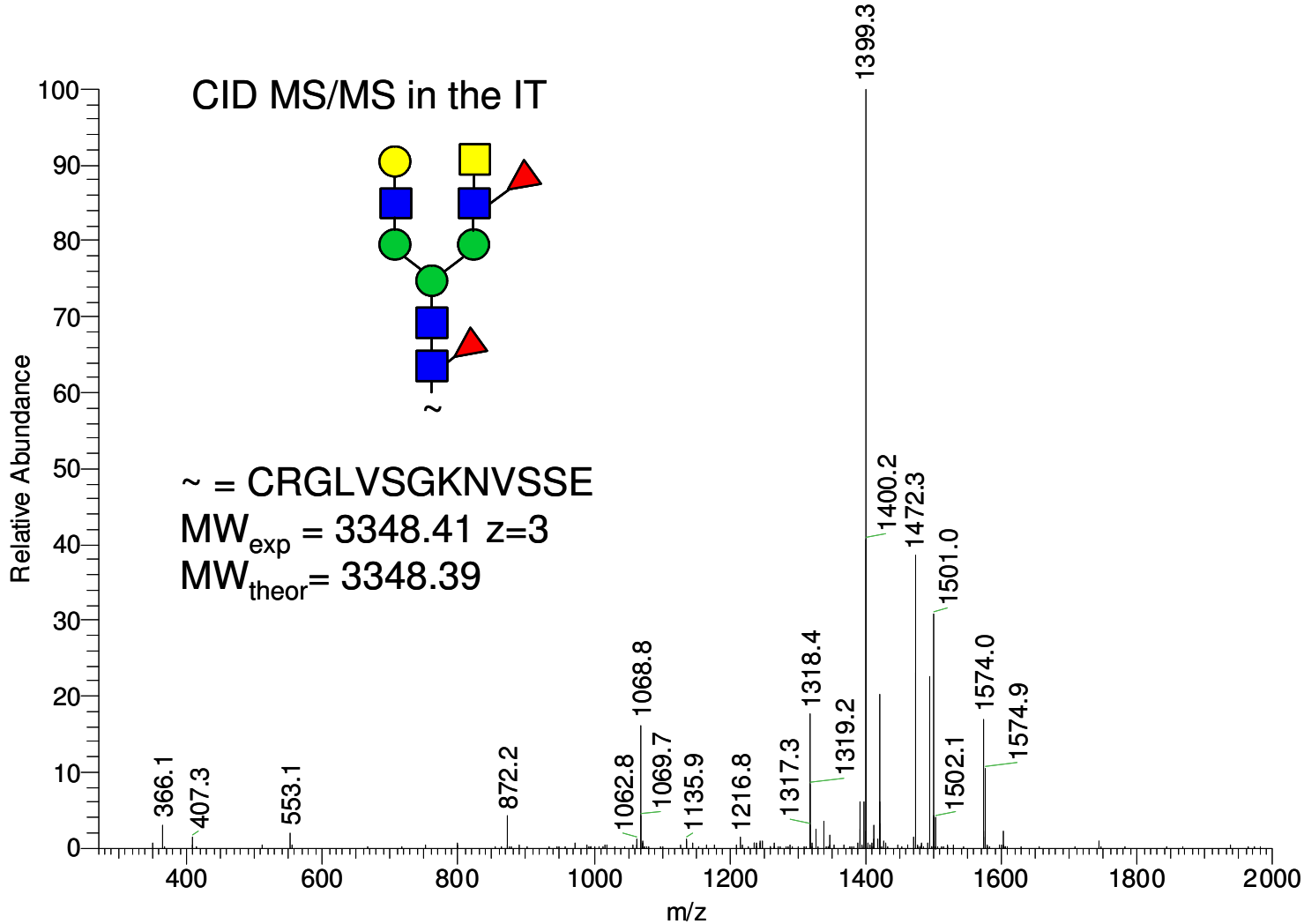
J



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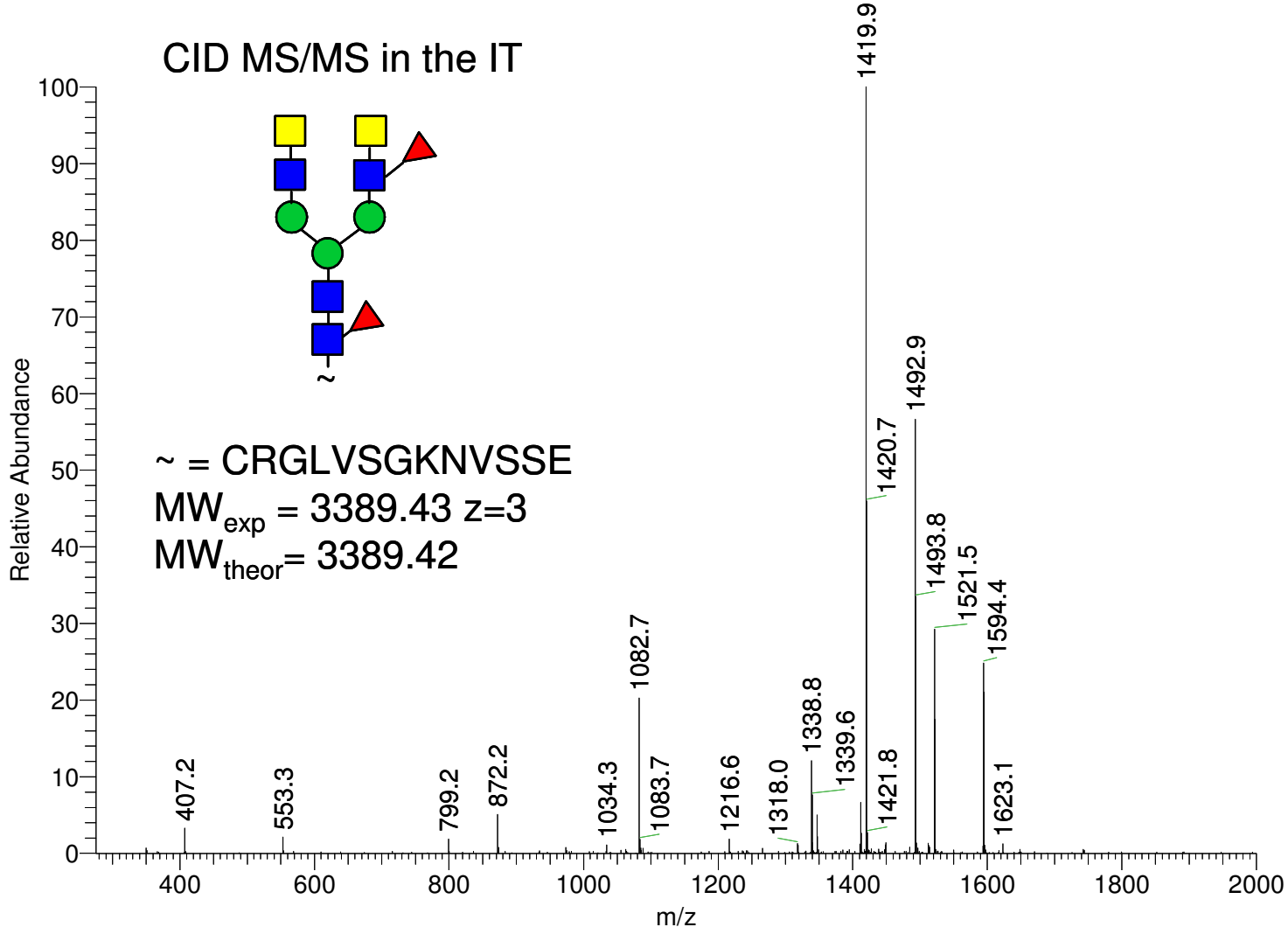
K



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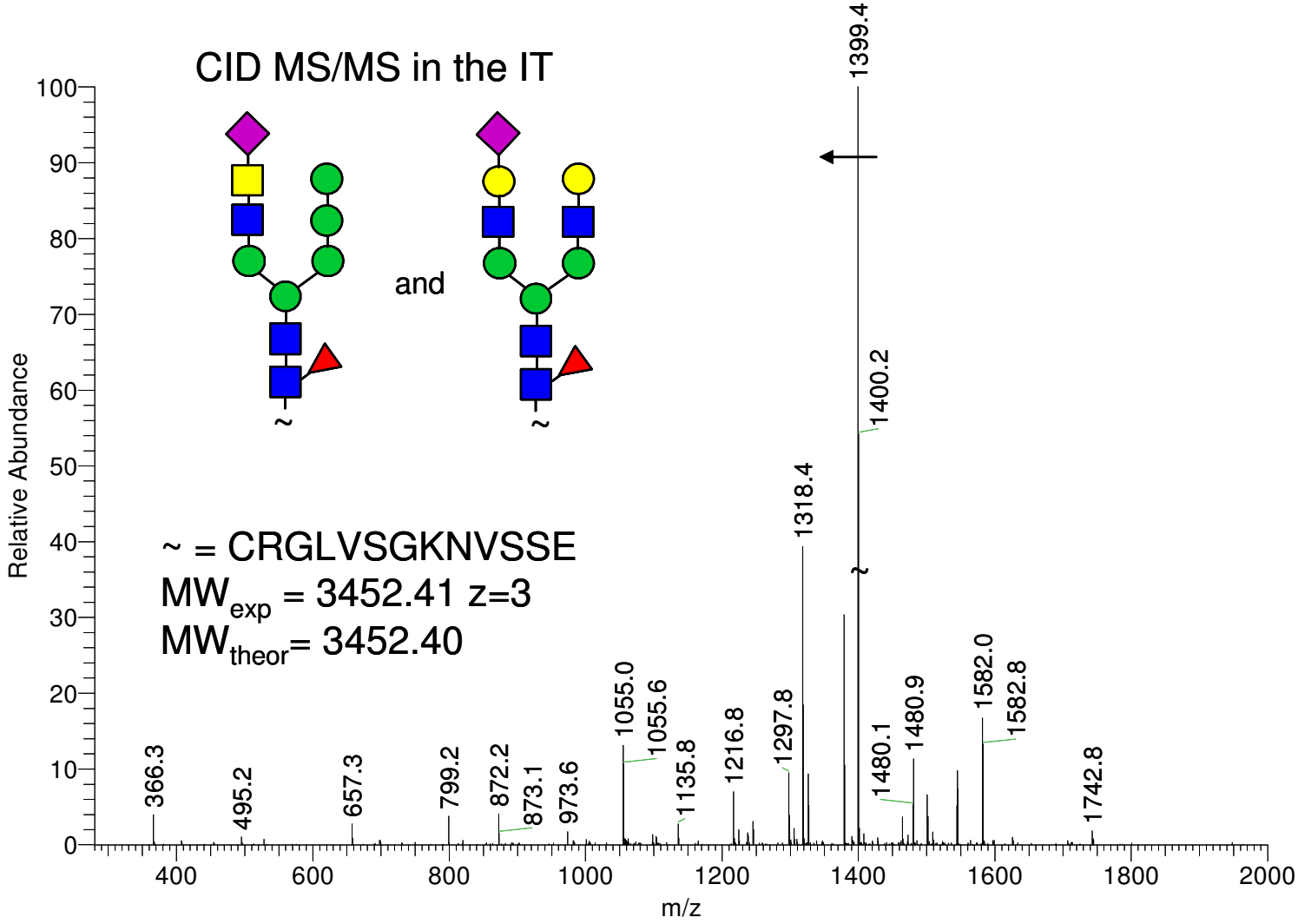
L



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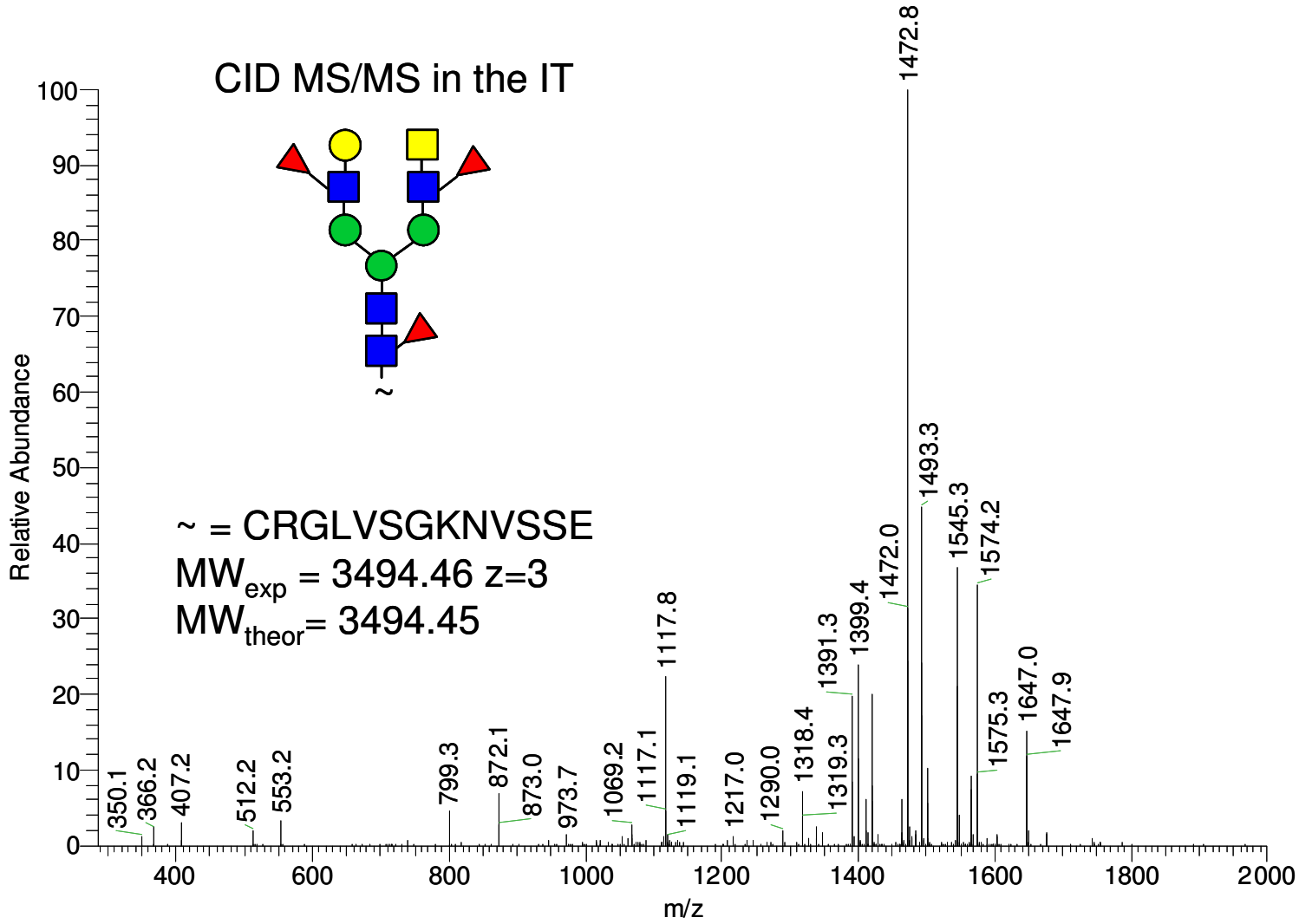
M



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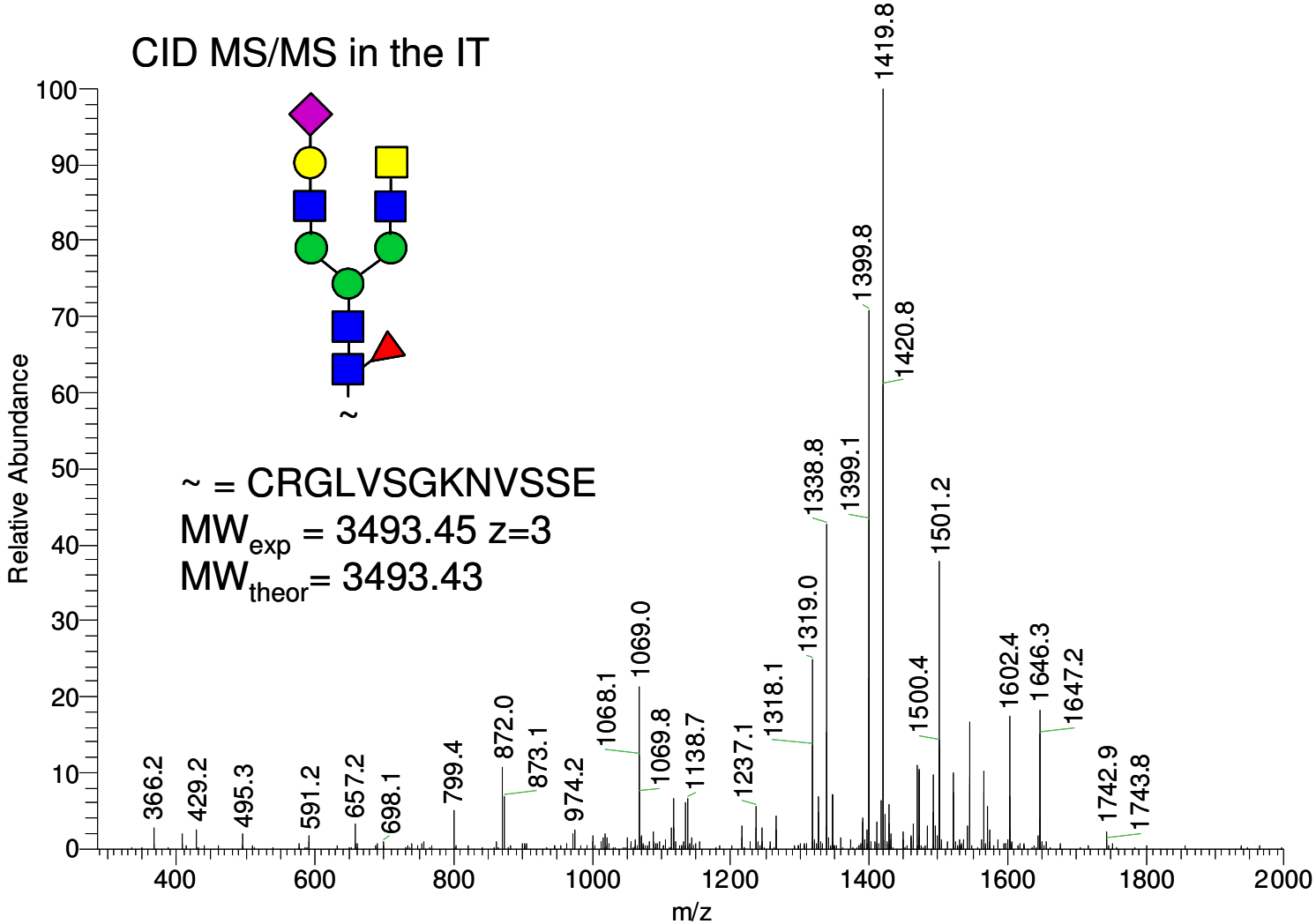

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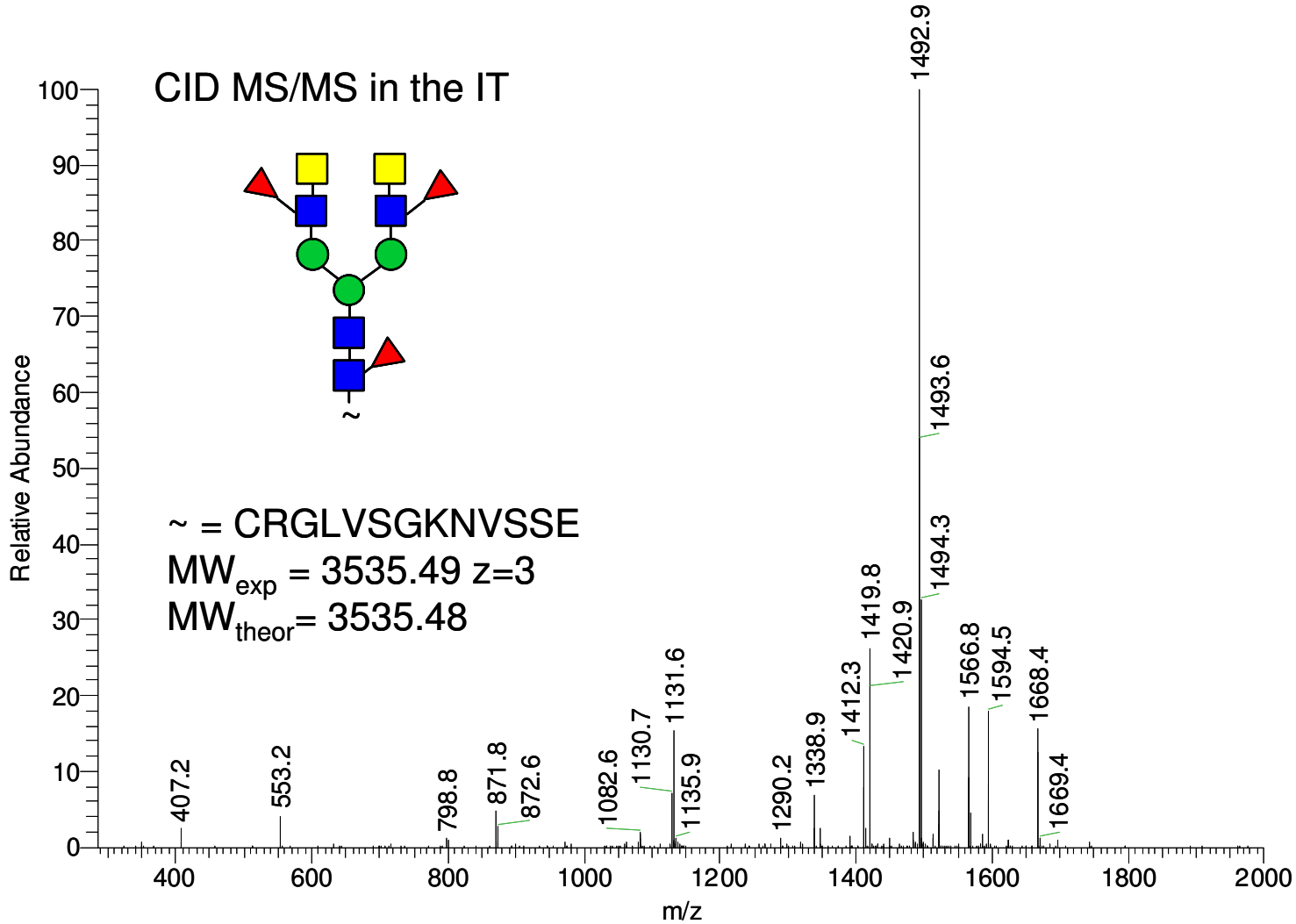
O



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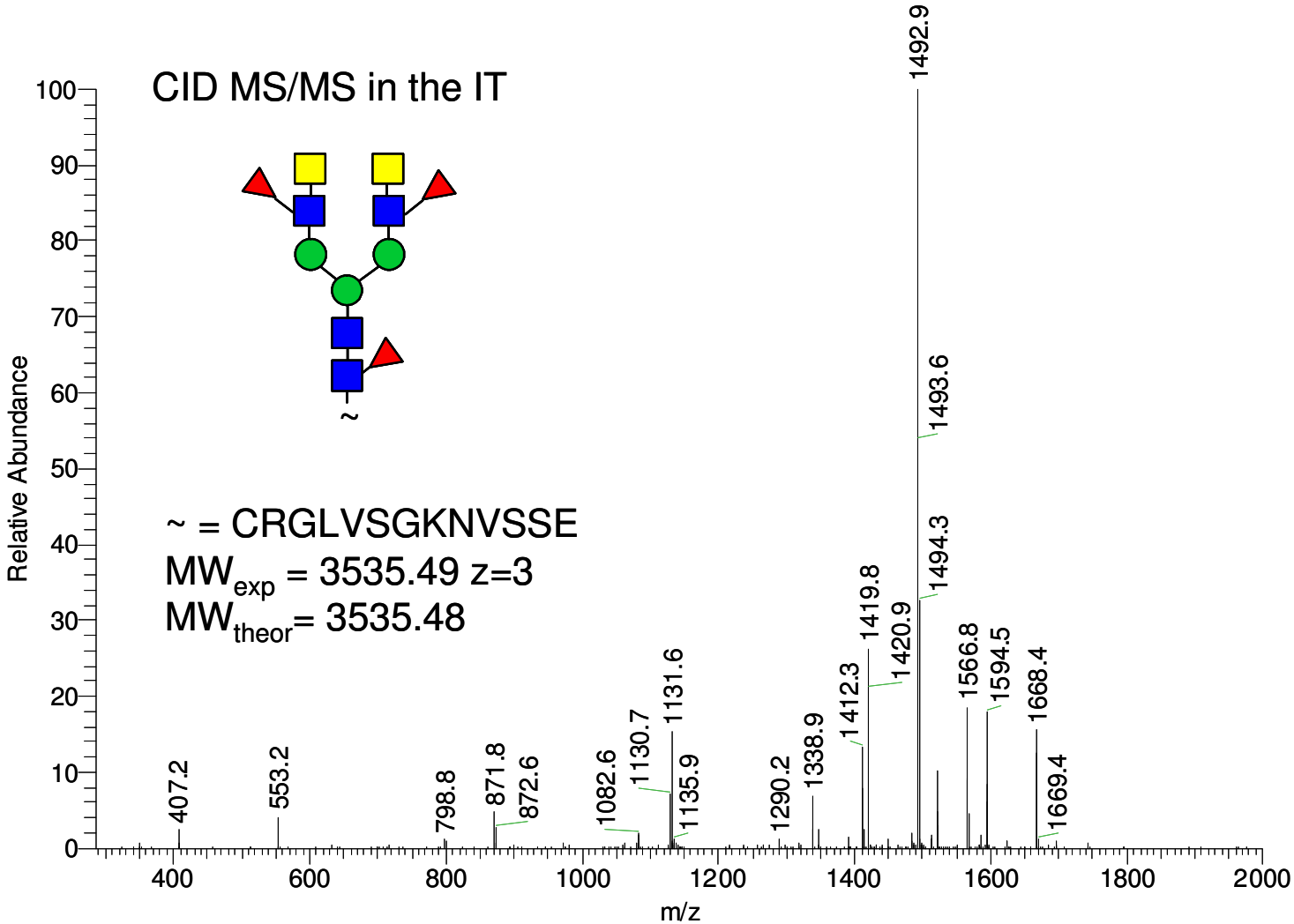
O



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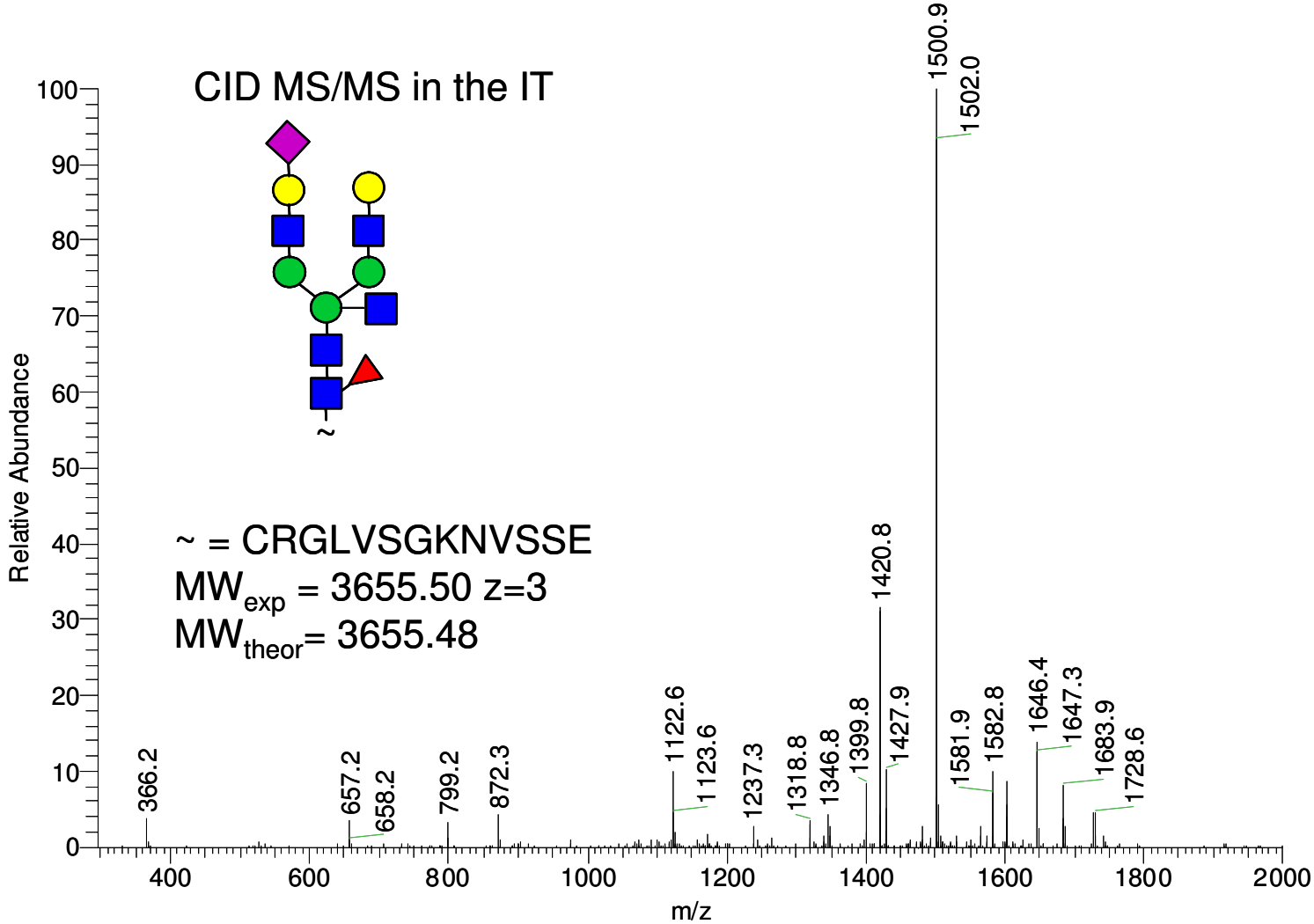
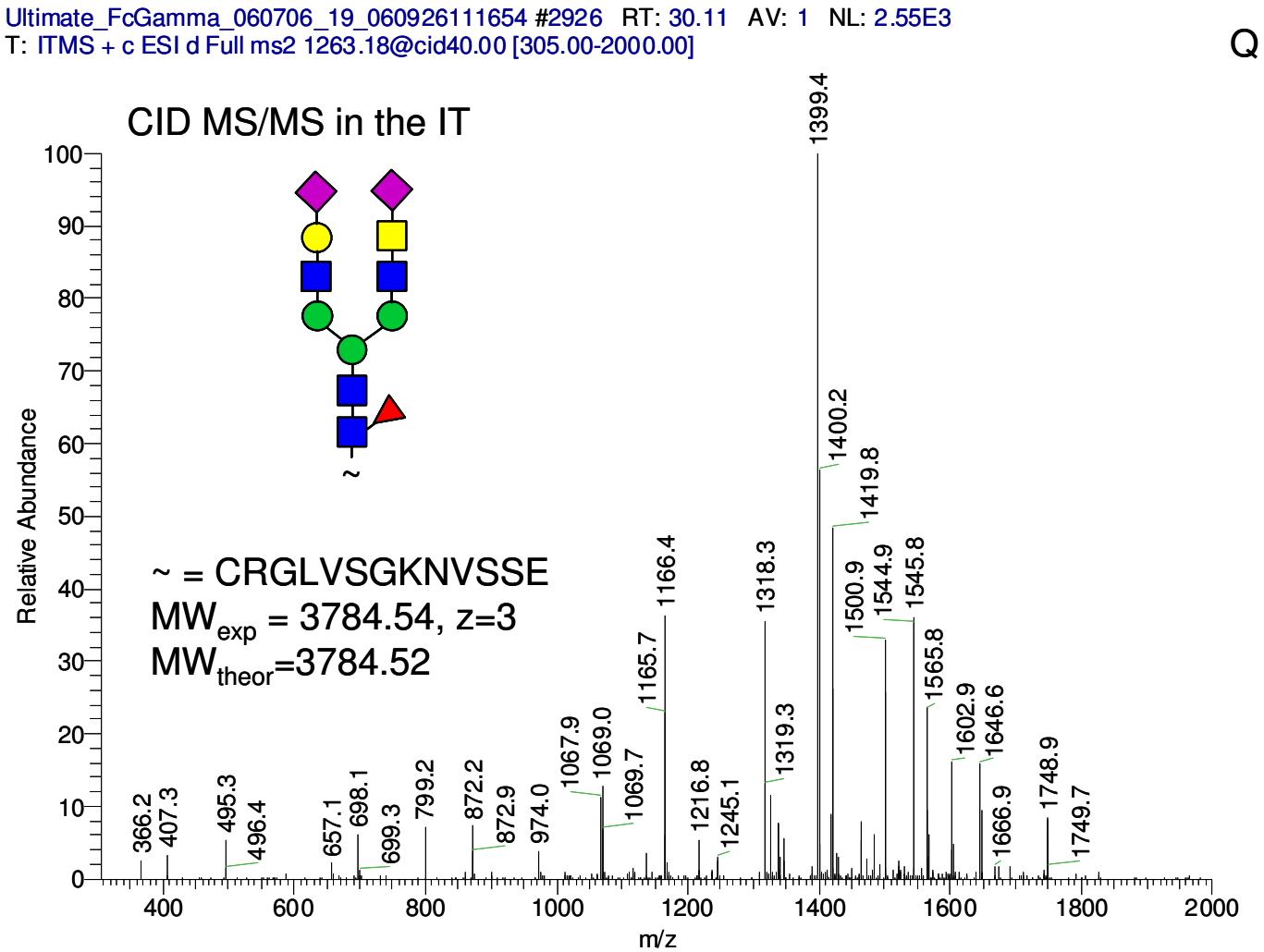
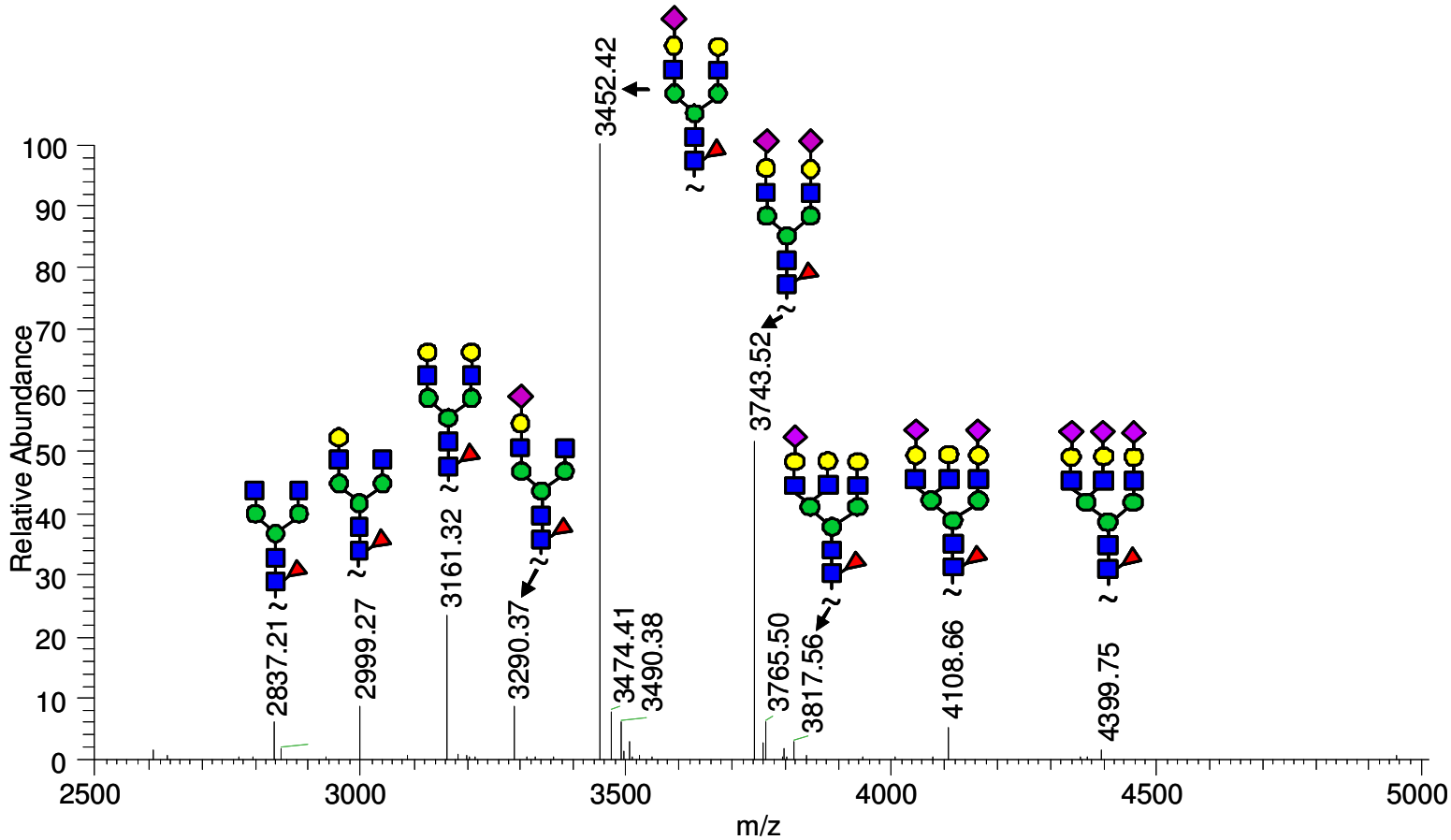
P





Supplemental Figure S 9: Fragment ion spectra (A-Q) of the glycopeptides containing Asn-162 in shFcγRIIIa expressed by HEK cells.



Supplemental Figure S 10: Deconvoluted monoisotopic mass spectrum of a series of glycopeptides containing the peptide "CRGLVGSKNVSSE" (abbreviated ~) obtained from shFcγRIIIa expressed by CHO cells and digested with endoproteinase GluC/chymotrypsin (RT 25.5-27.5).

Ultimate_FcGamma_060706_20 #2100 RT: 26.30 AV: 1 NL: 6.45E4
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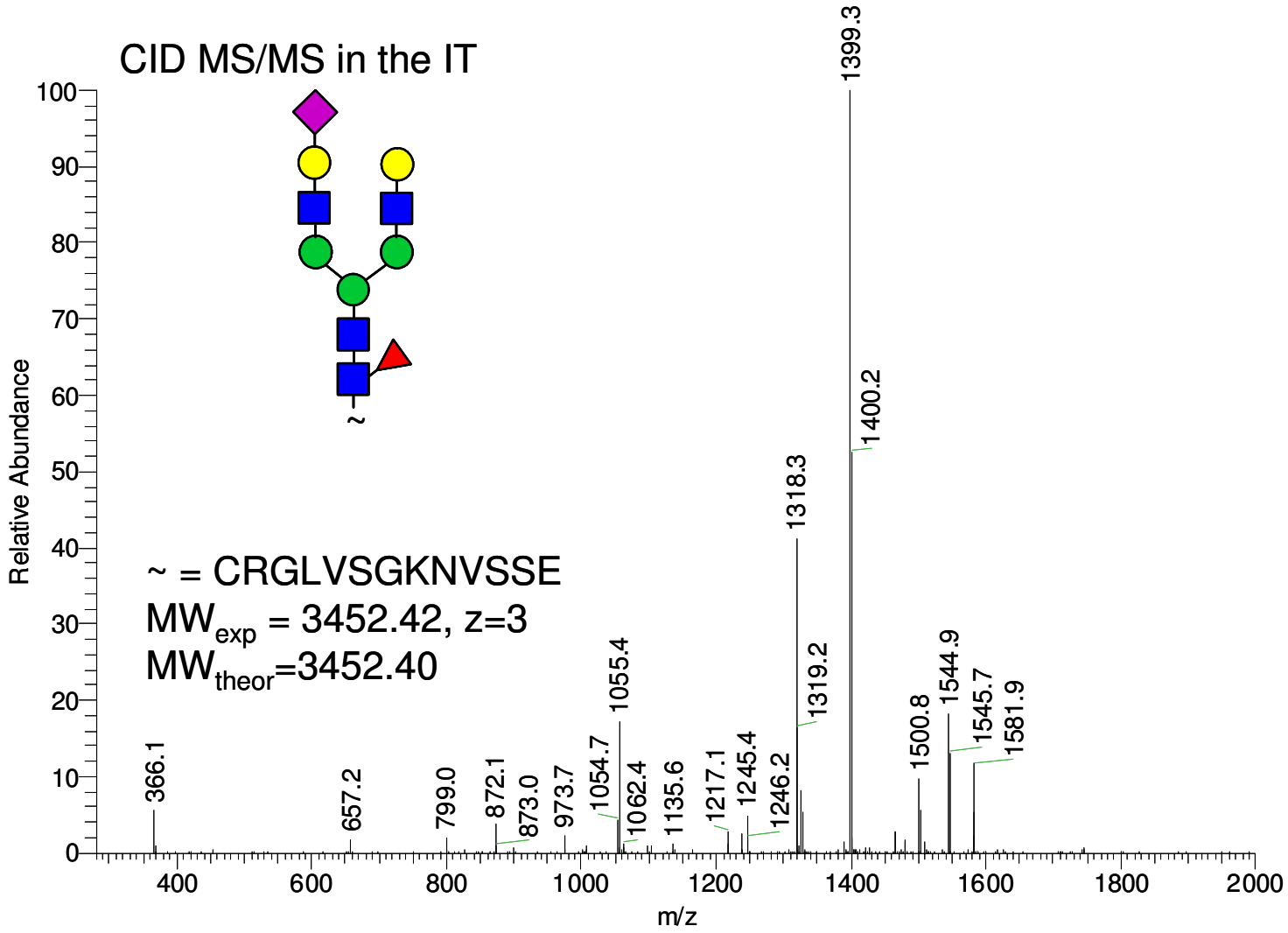
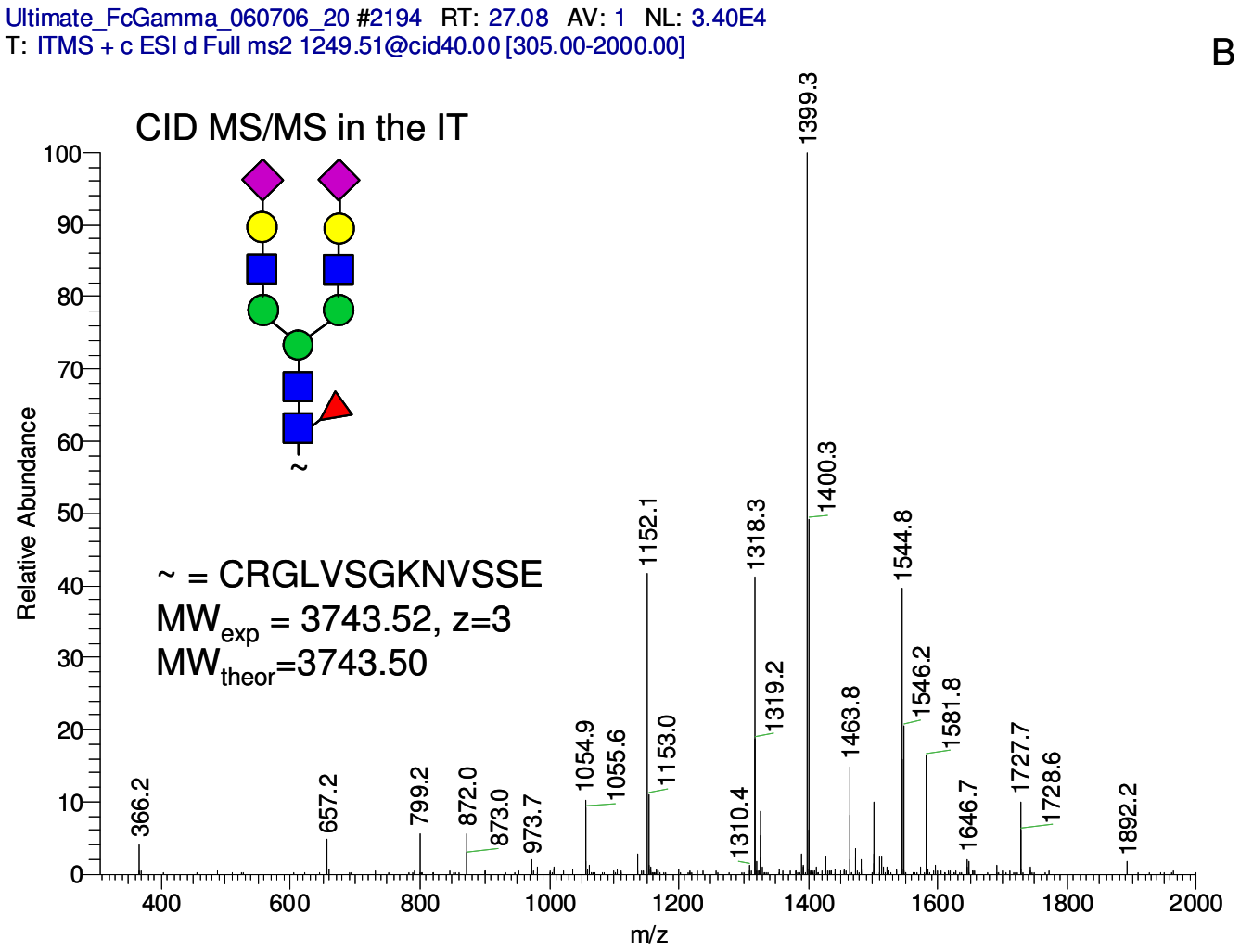
A





Supplemental Figure S 11: Fragment ion spectra (A and B) of the glycopeptides containing Asn-162 from shFcγRIIIa expressed by CHO cells.