Supplementary Data

Figure legend

Supplementary Figure 1: MALDI-TOF spectra of permethylated *O*-glycans derived from control/PyMT and ST3Gal-I/PyMT mice tumours. A, B and C *O*-glycan profiles are from individual tumours arising in three control/PyMT mice; D and E *O*-glycan profiles are from individual tumours arising in two ST3Gal-I/PyMT mice. Profiles of *O*-glycans are from the 35% MeCN fraction from a C_{18} Sep-Pak ("Materials and Methods"). All molecular ions are $[M+Na]^+$. Putative structures based on composition, tandem mass spectrometry and the literature shown. Cartoon structures are according to the Consortium for Functional Glycomics (http://www.functionalglycomics.org) guidelines. Structures that show sugars outside a bracket have not been unequivocally defined. Structures indicated with "M" and "m" suggest major and minor abundances respectively.

O-glycan profiles from control/PyMT and ST3Gal-I/PyMT mice tumours exhibited core 1, core 2 as well as extended core 1 and core 2 structures partially decorated with sialic acids and fucose residues. Despite the fact that ST3Gal-I/PyMT mice tumours showed an increase in sialyltrasferase activity (see "Results"), no substantial differences in % relative abundances were detected between the control/PyMT and ST3Gal-I/PyMT mice tumours

Materials and Methods

Analysis of O-glycans of mice tumours

All mice tumours were treated as described previously (Sutton-Smith M, Dell A and Jang-Lee J, North SJ, et al. 2006) Briefly, each tumour sample was subjected to reduction in 4M guanidine-HCl

(Pierce, Cramlington, Northumberland, UK), carboxymethylation and trypsin digestion. The digested glycoproteins were purified by C_{18}-Sep-Pak (Waters Corp, Hertfordshire, UK). *O*-glycans were released by reductive elimination and then permethylated using the sodium hydroxide procedure. Finally, the permethylated *O*-glycans were purified by C_{18}-Sep-Pak.

MS and MS/MS data were acquired using a 4800 MALDI-TOF/TOF (Applied Biosystems, Darmstadt, Germany) mass spectrometer. Permethylated samples were dissolved in 10 μL of methanol and 1 μL of dissolved sample was premixed with 1 μL of matrix (20 mg/mL 2,5-dihydroxybenzoic acid (DHB) in 70% (v/v) aqueous methanol), spotted onto a target plate (2×0.5 μL) and dried under vacuum. The collision energy was set to 1 kV, and argon was used as collision gas. The 4700 Calibration standard kit, calmix (Applied Biosystems), was used as the external calibrant for the MS mode and [Glu1] fibrinopeptide B human (Sigma-Aldrich) was used as an external calibrant for the MS/MS mode.

The MS and MS/MS data were processed using Data Explorer 4.9 Software (Applied Biosystems). The spectra were subjected to manual assignment and annotation with the aid of the glycobioinformatics tool GlycoWorkBench (Ceroni A, Maass K, Geyer H, et al. 2008). The proposed assignments for the selected peaks were based on ^{12}C isotopic composition together with knowledge of the biosynthetic pathways. The proposed structures were then confirmed by data obtained from MS/MS experiments.

References

Sutton-Smith M, Dell A. Analysis of carbohydrates/glycoproteins by mass spectrometry. In: Cell Biology: A Laboratory Handbook. Celis JE, editor. Amsterdam: Elsevier Academic. p. 415–425.

Jang-Lee J, North SJ, et al. (2006) Profiling of Cells and Tissues by Mass Spectrometry: Fingerprinting and Sequencing Technologies. *Methods Enzymol*, 415, 59-86.

Ceroni A, Maass K, Geyer H, et al. (2008) A tool for the computer-assisted annotation of mass spectra of glycans. *J Proteome Res*, **7**, 1650–1659.

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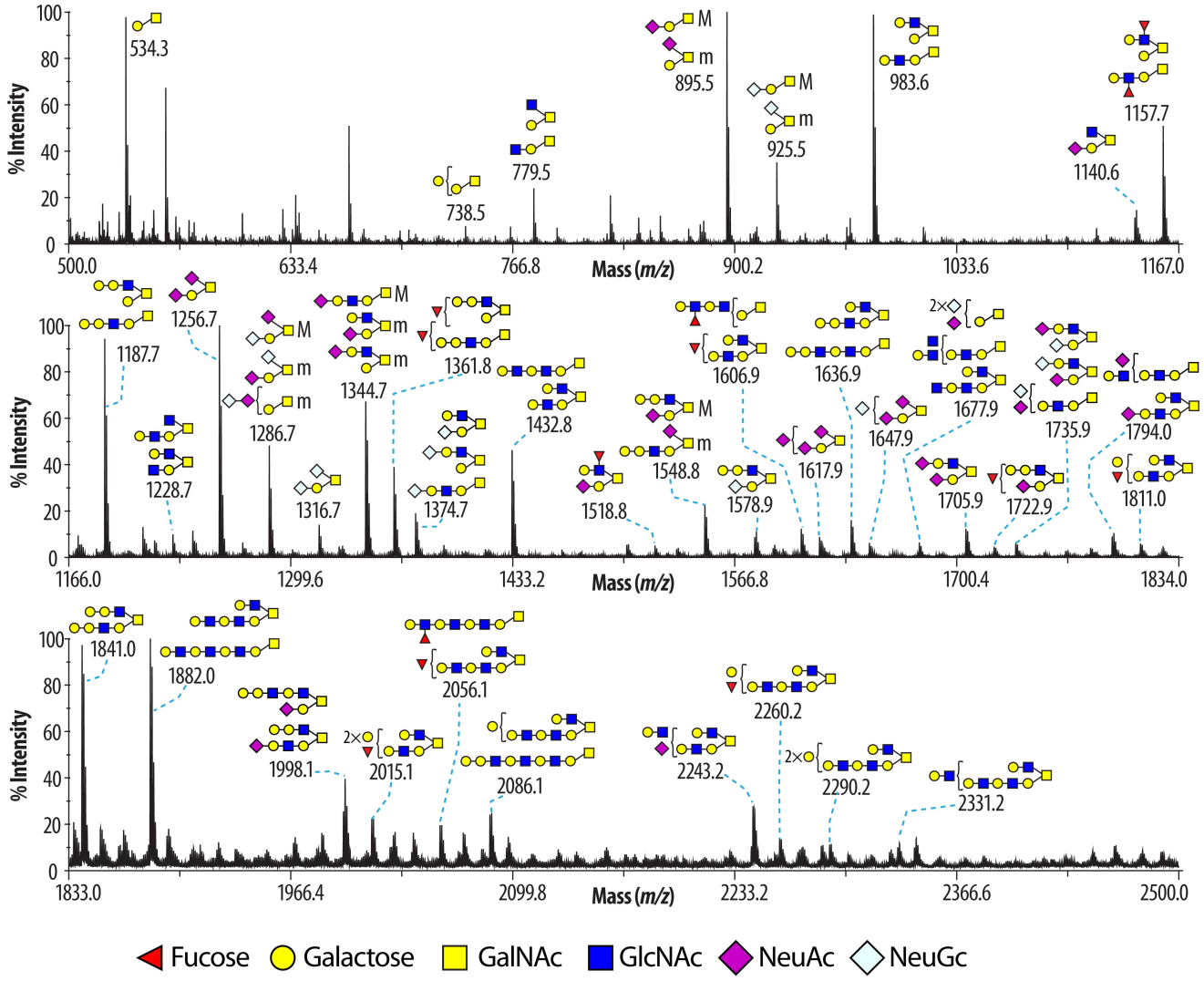
A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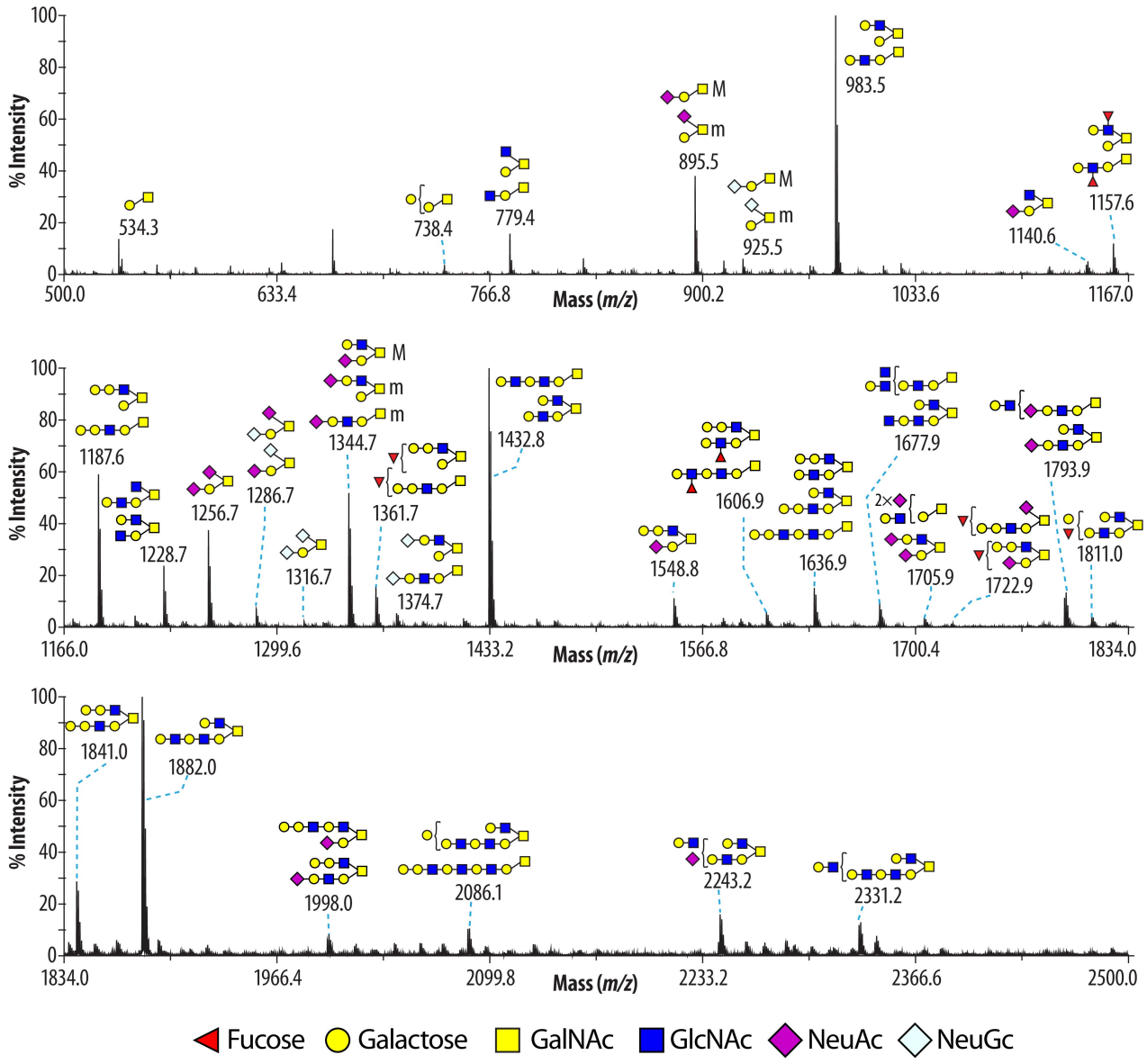
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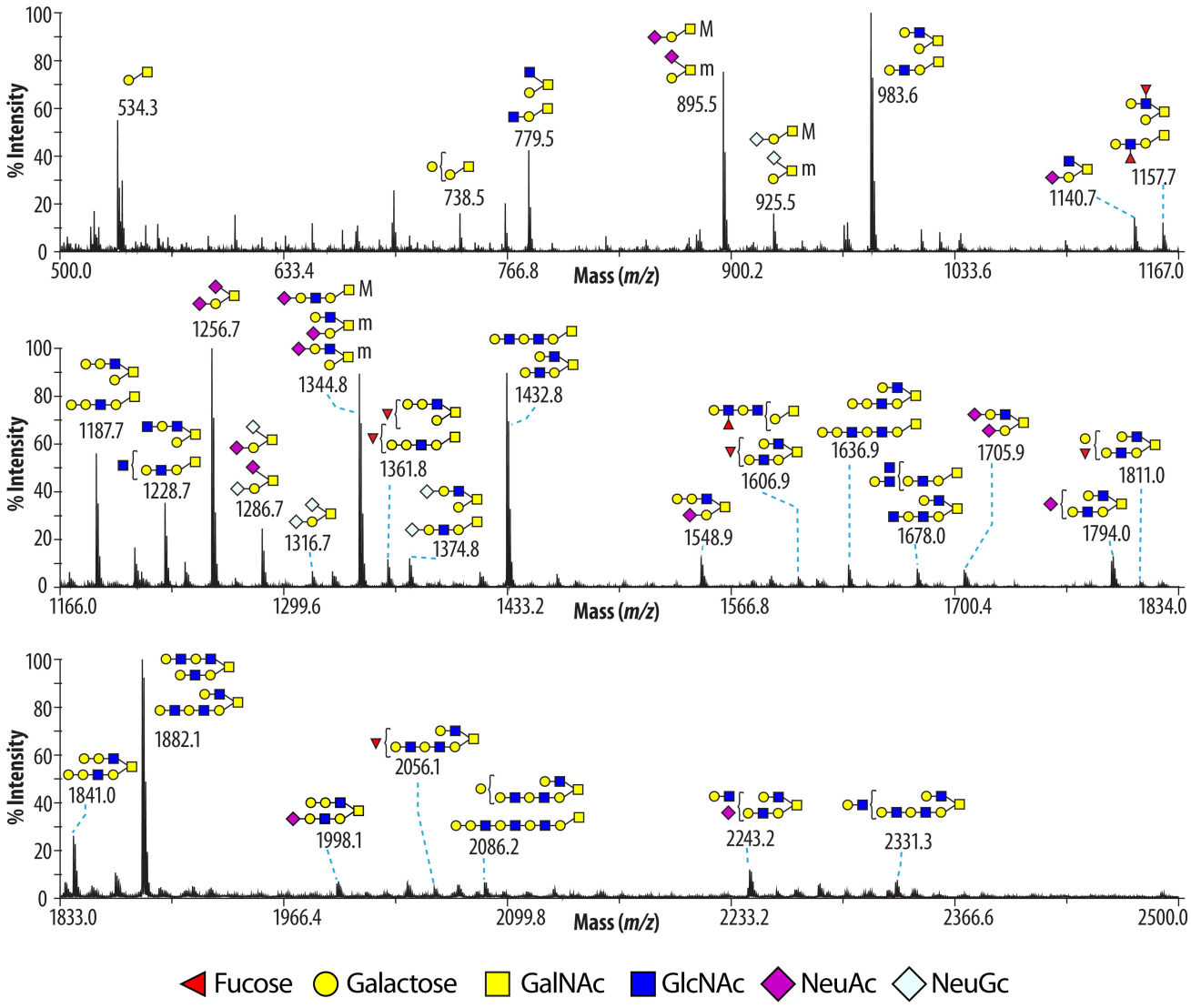
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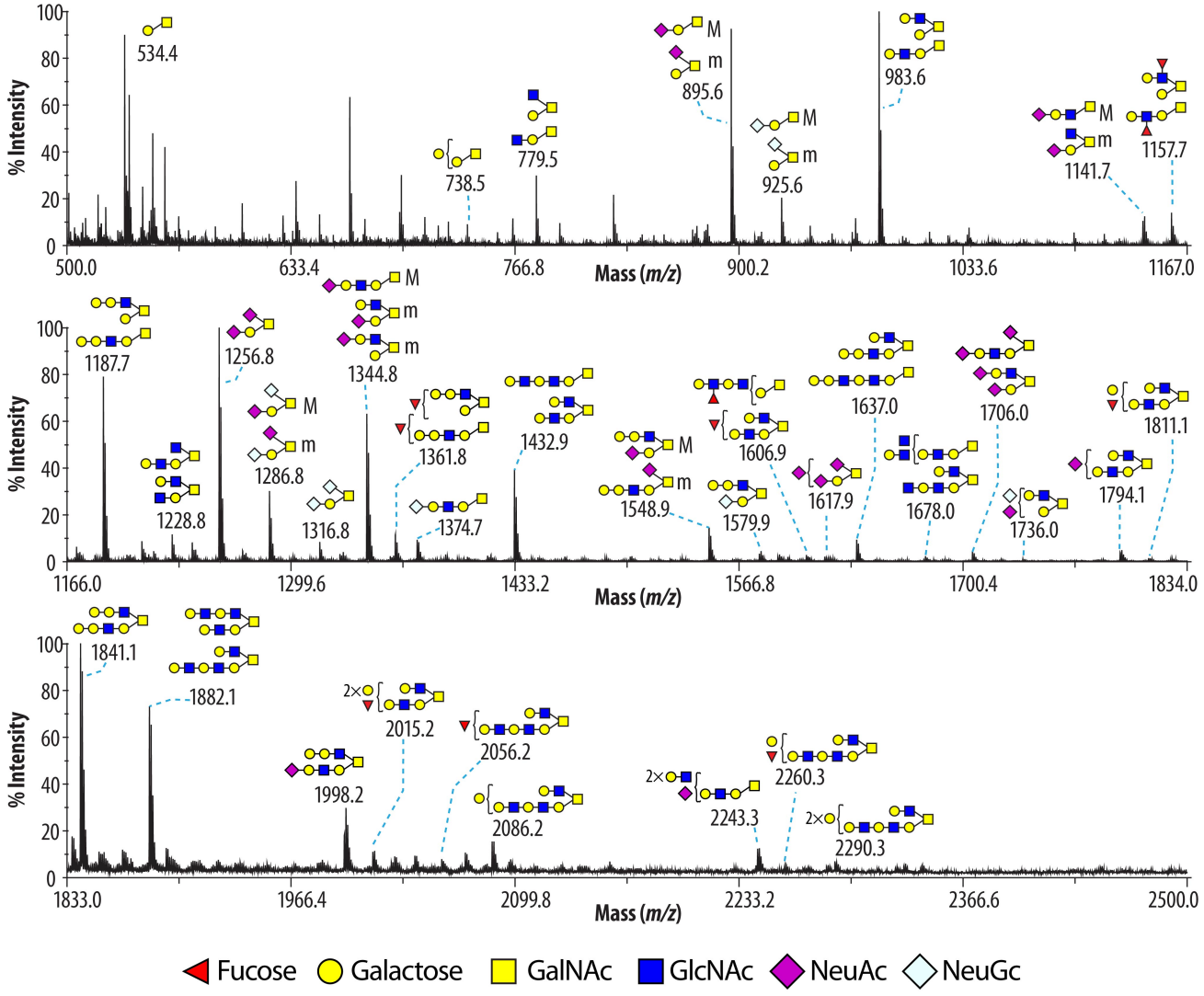
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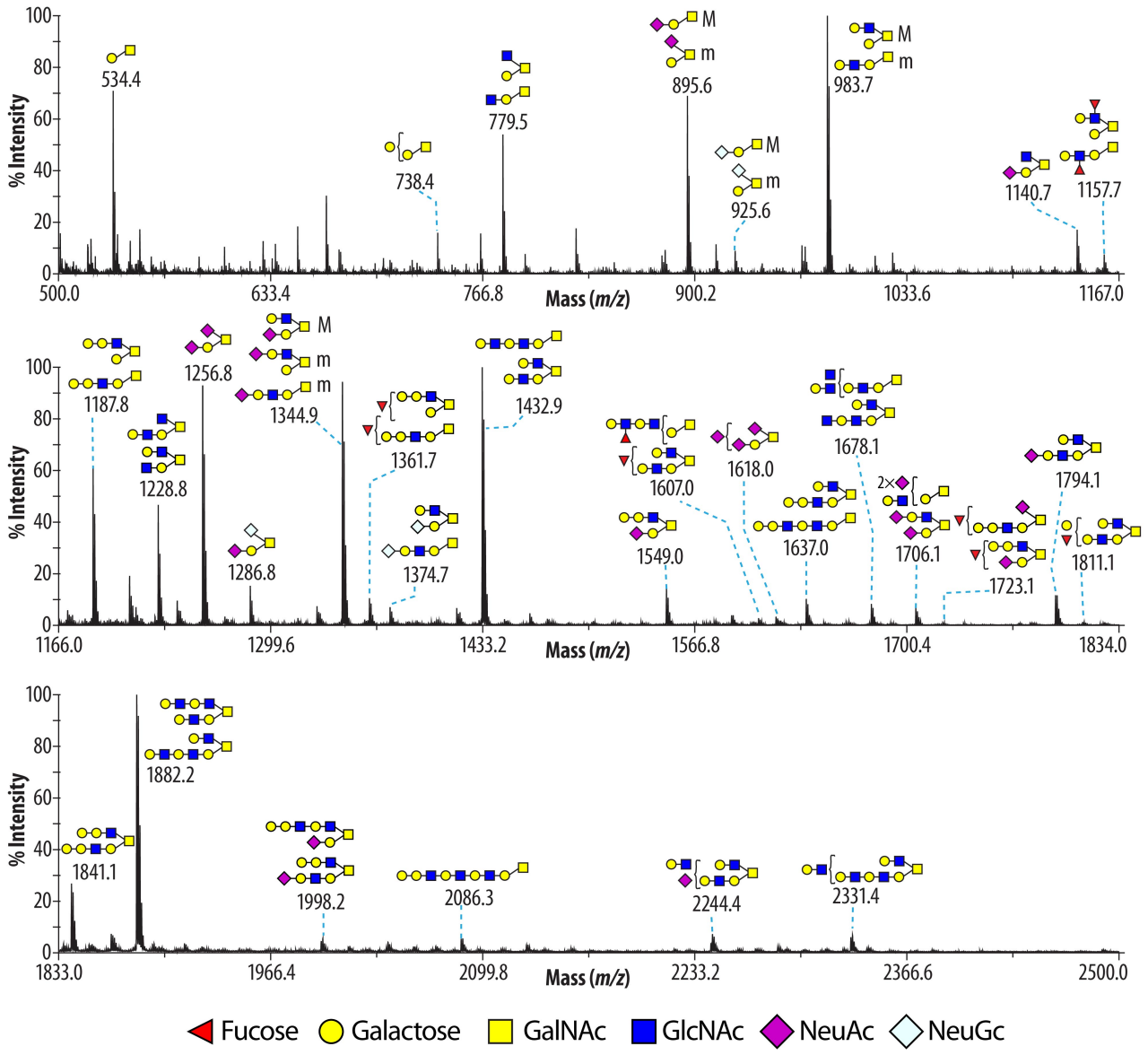
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