Electronic Supplementary Material (ESI) for Molecular Omics.

N-glycosylation in isolated rat nerve terminals

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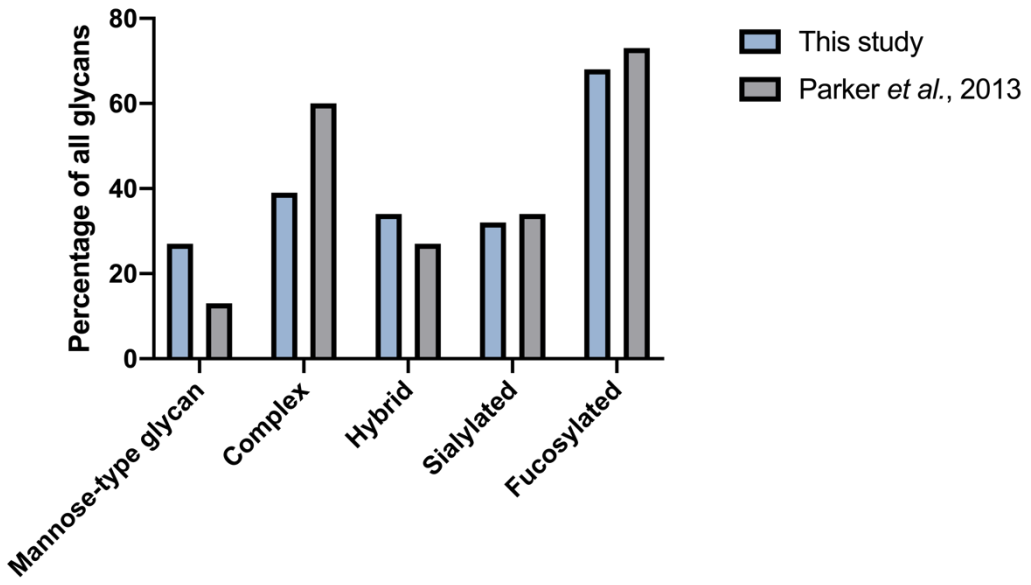
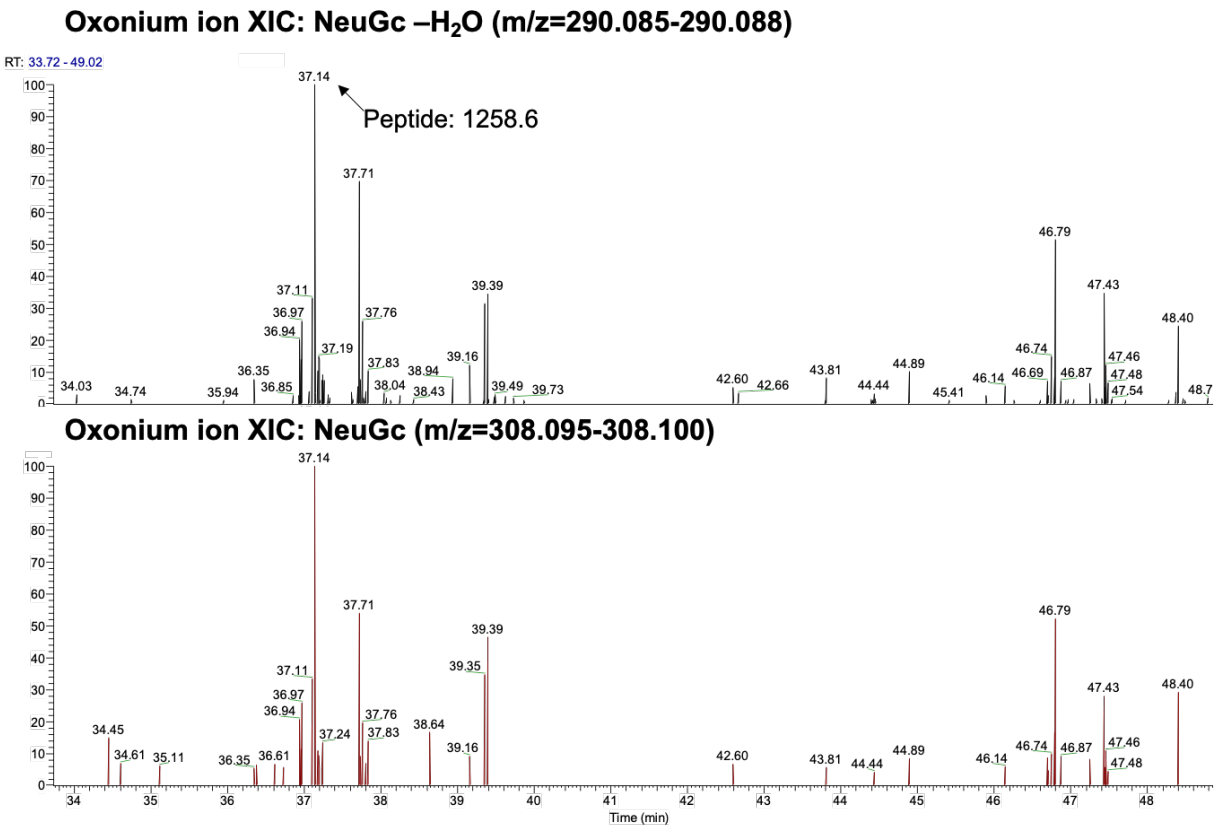
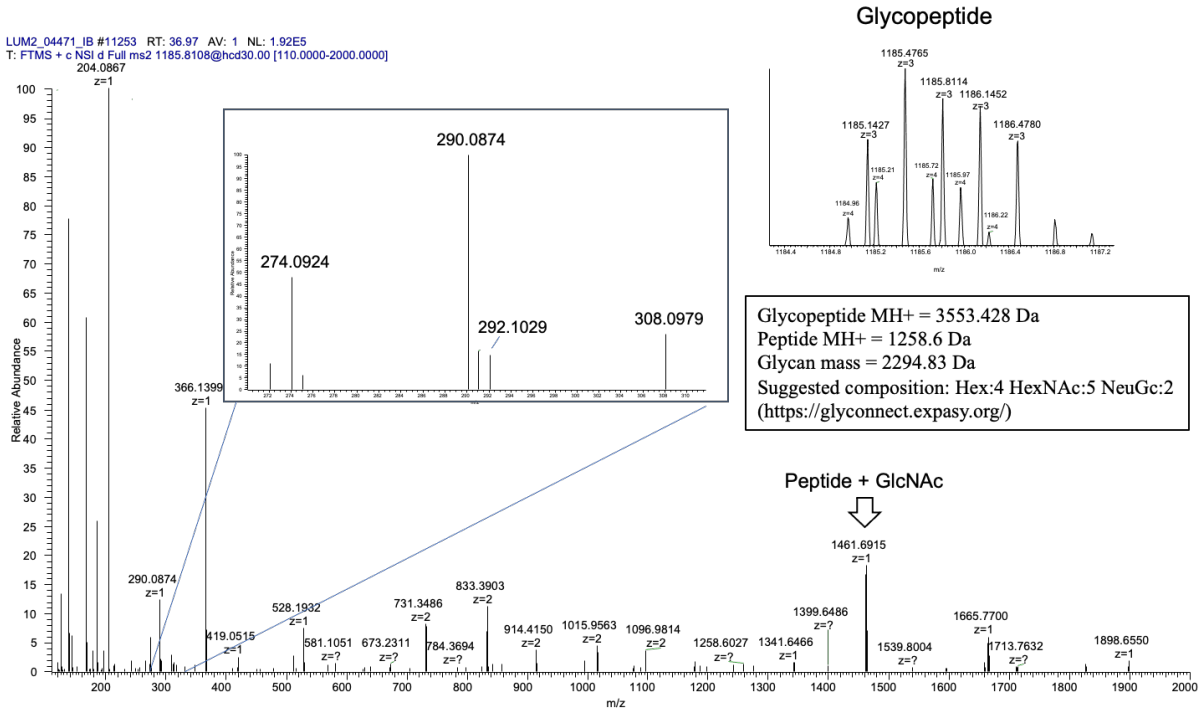
Supplementary Figures



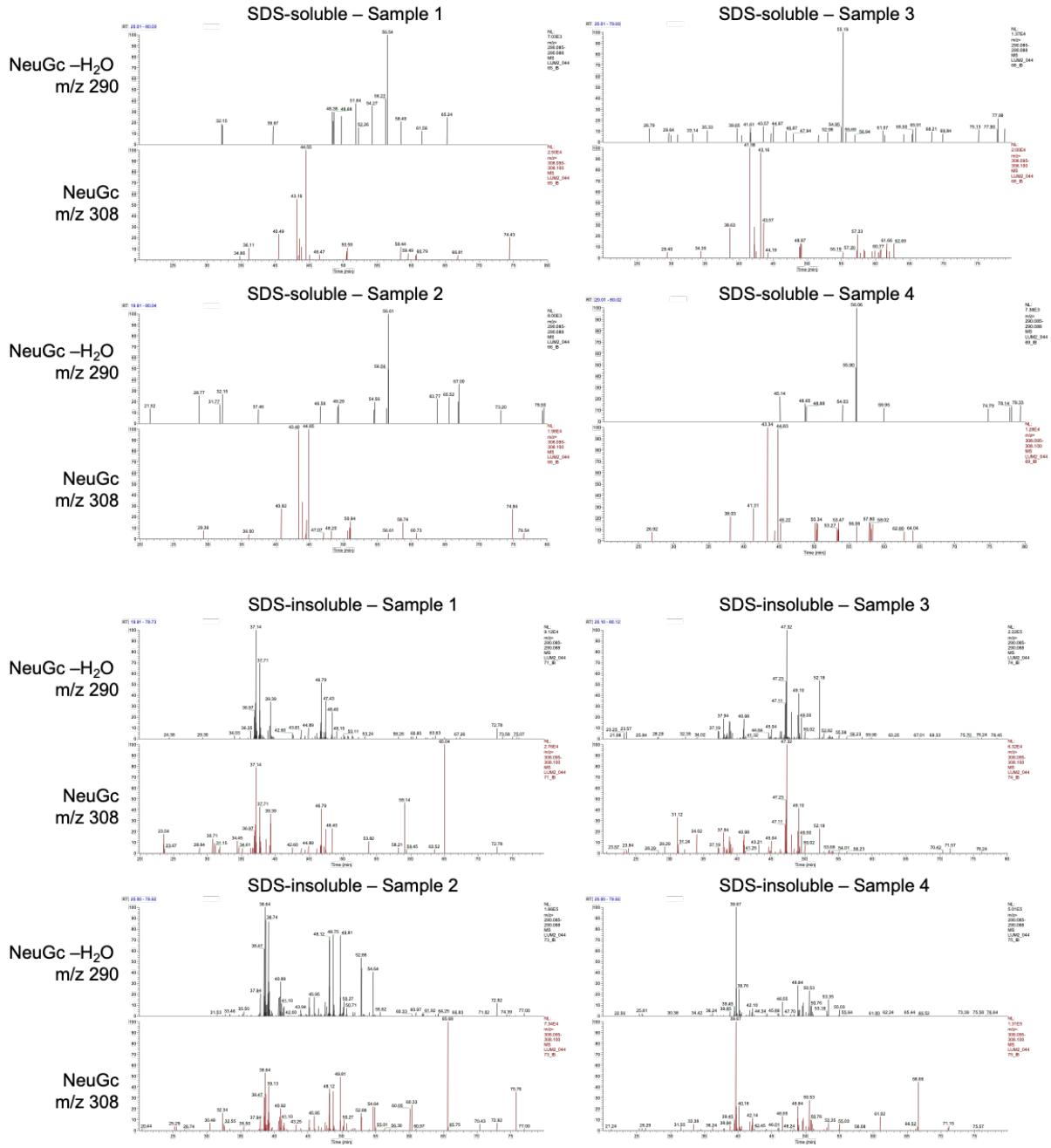
Supplementary Figure 1: **Comparison of N-glycans from this study to Parker *et al*.**. The identified synaptic N-glycans were grouped regarding their glycan characteristics and compared to the rat brain membrane protein glycome from Parker *et al*. [20]. Mannose-type glycans refers to paucimannose and oligomannose N-glycans.



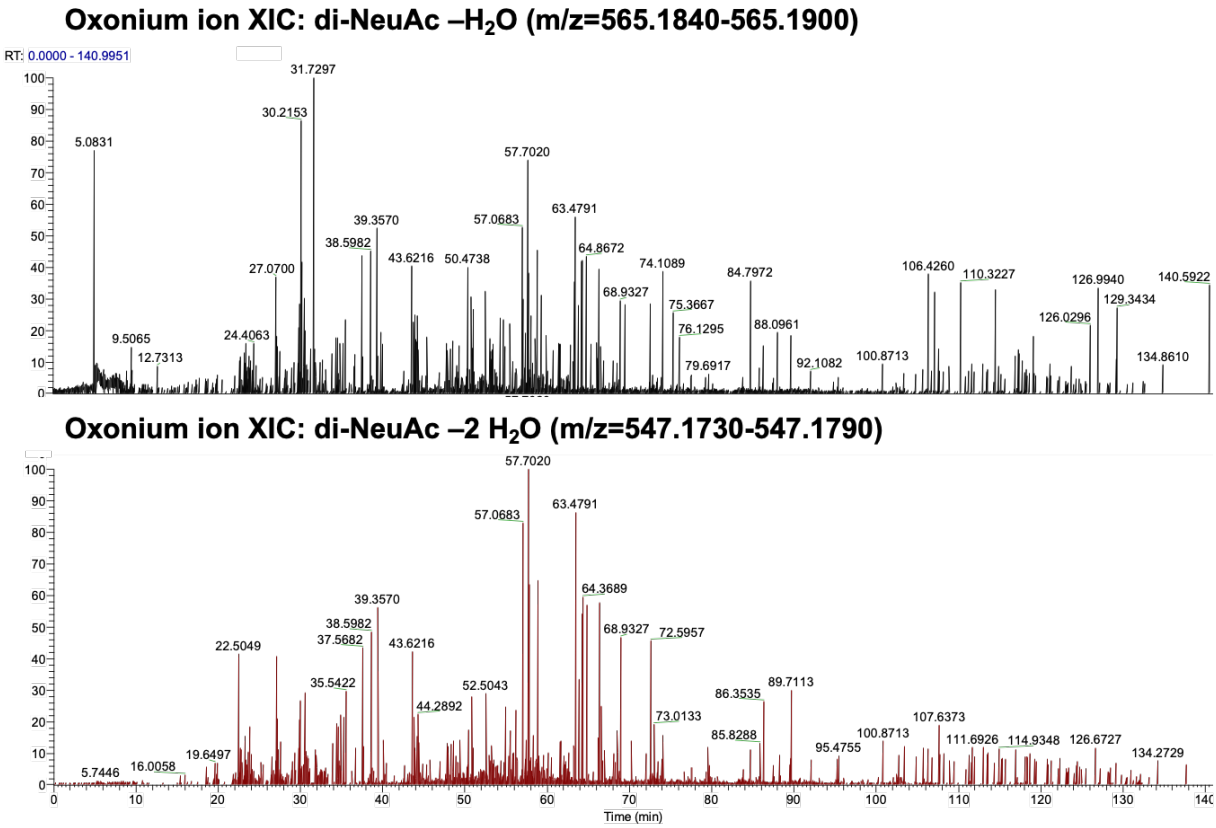
Supplementary Figure 2: **Extracted ion chromatograms for oxonium ions from NeuGc and water loss.** The presence of NeuGc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the NeuGc-H_2O (290) and the NeuGc (308) oxonium ions. The elution profile of glycopeptides from the SDS-insoluble fraction after TiO_2 enrichment is exemplified.



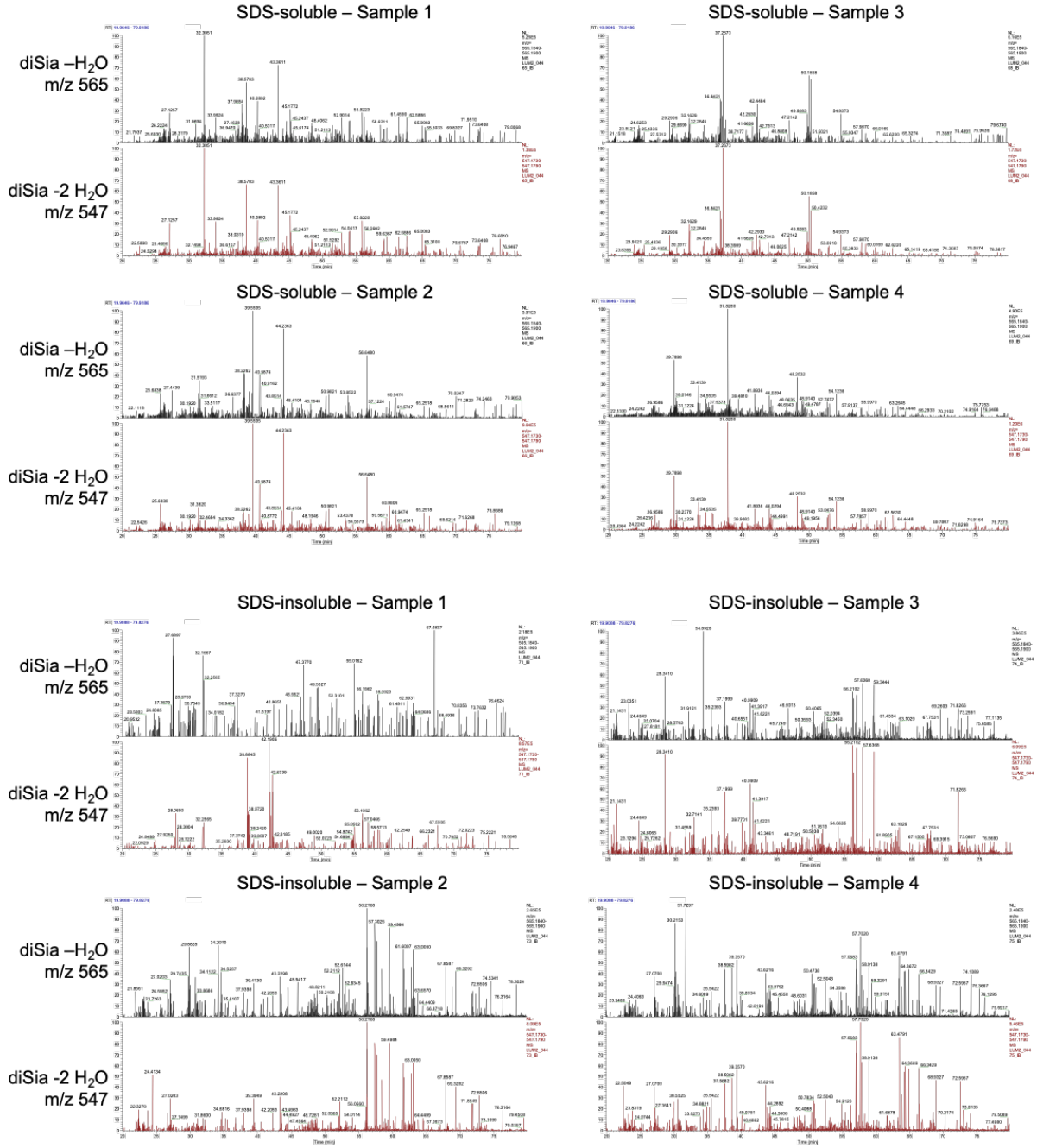
Supplementary Figure 3: **Example of a NeuGc containing N-linked glycopeptide.** MS/MS spectrum of a glycopeptide with intense diagnostic oxonium ions for NeuGc. The peptide mass was determined but the backbone fragmentation is limited.



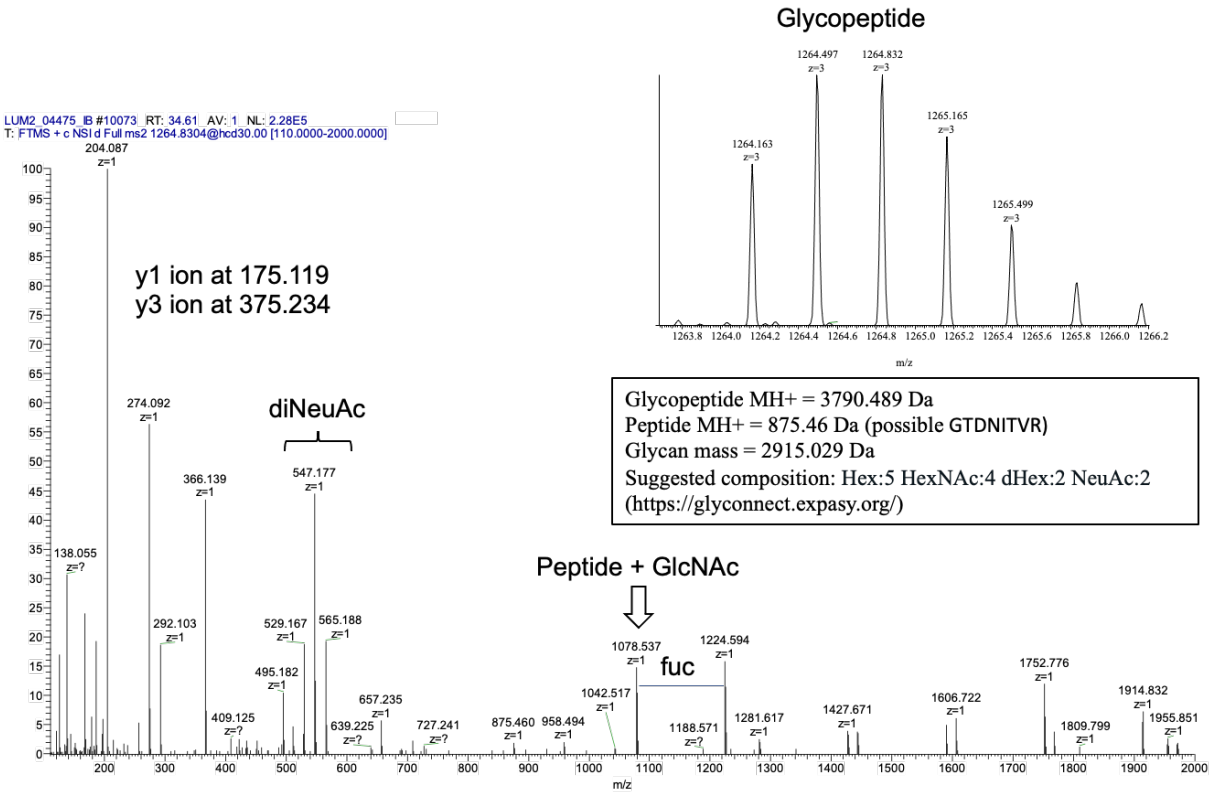
Supplementary Figure 4: Differences in NeuGc oxonium ion signatures in the SDS-insoluble and SDS-soluble fraction. The presence of NeuGc containing glycopeptides in synaptosome fractions is illustrated by extracted ion chromatograms (XIC) of the NeuGc-H_2O (290) and the NeuGc (308) oxonium ions.



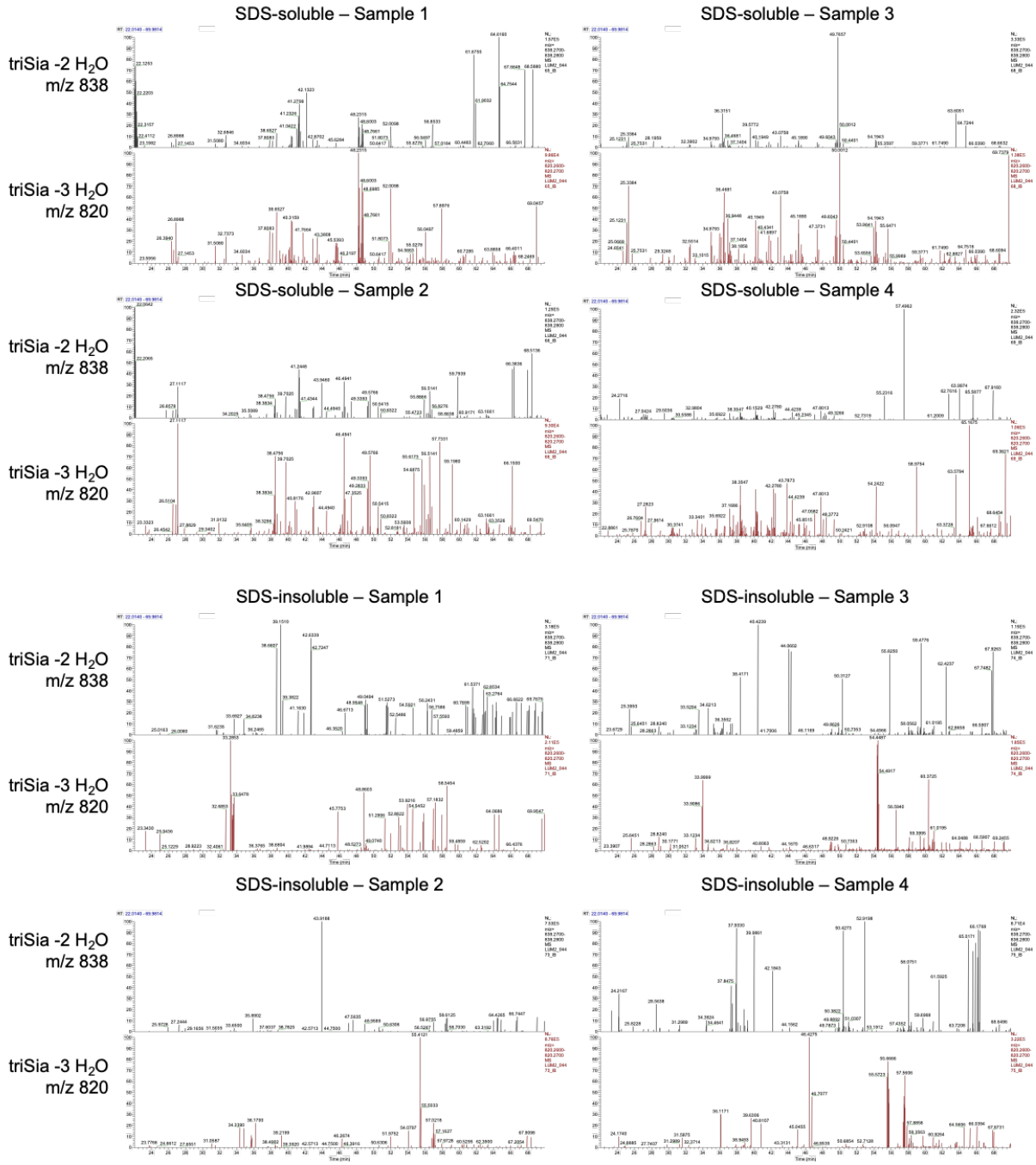
Supplementary Figure 5: **Extracted ion chromatograms for oxonium ions from di-sialic acid (NeuAc).** The presence of di-NeuAc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the di-NeuAc-H_2O (565) and the di-NeuAc-2 H_2O (547) oxonium ions. The elution profile of glycopeptides from the SDS-insoluble fraction after TiO_2 enrichment is exemplified.



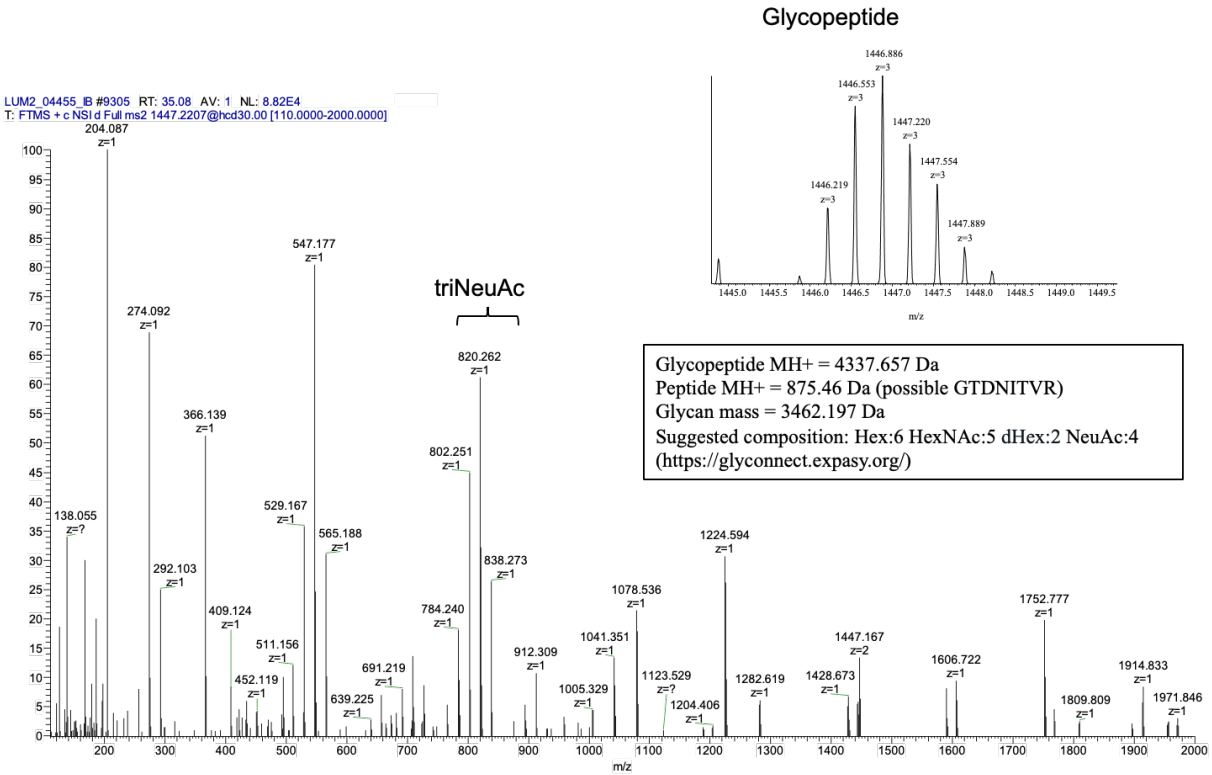
Supplementary Figure 6: Comparison of di-sialic acid (NeuAc) oxonium ion signatures in synaptosome fractions. The presence of di-NeuAc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the di-NeuAc-H_2O (565) and the di-NeuAc-2 H_2O (547) oxonium ions. The elution profiles of glycopeptides from the SDS-insoluble and SDS-soluble fraction are compared.



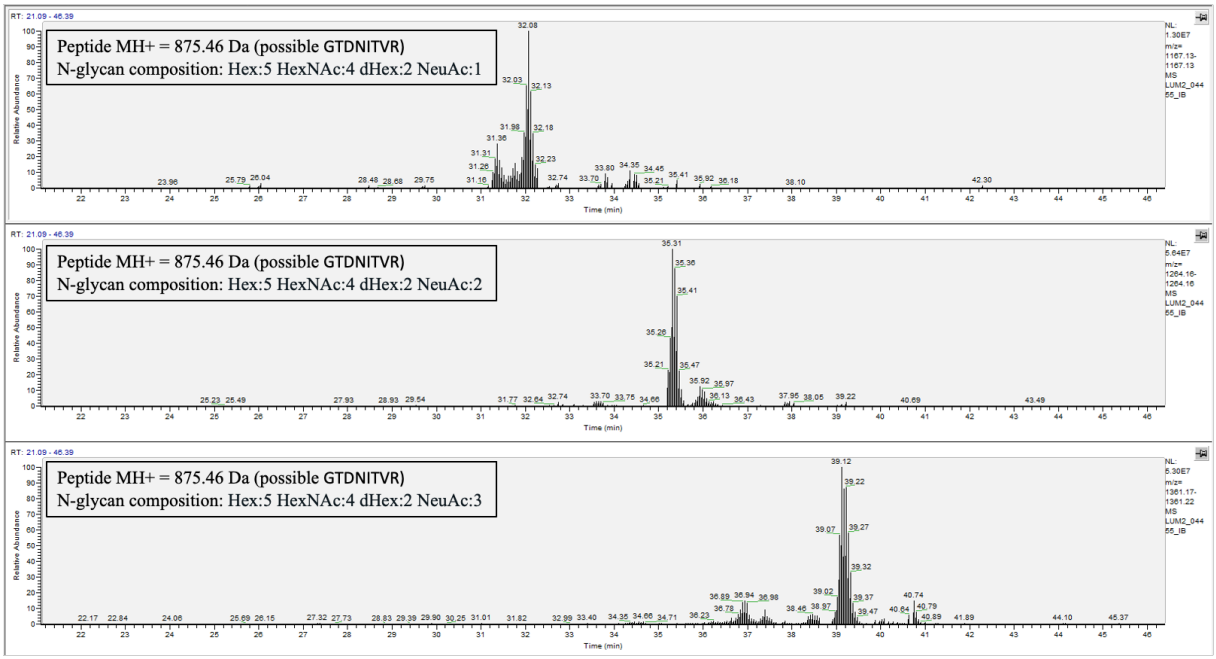
Supplementary Figure 7: **Example of a di-Sia containing N-linked glycopeptide.** MS/MS spectrum of a glycopeptide with intense diagnostic oxonium ions for di-NeuAc. The peptide mass indicates a peptide sequence of GTDNITVR associated with the Limbic system-associated membrane protein but the assignment is ambiguous as only two sequence ions support the peptide assignment.



Supplementary Figure 8: **Tri-sialic acid (NeuAc) oxonium ion signatures in synaptosome fractions.** The presence of tri-NeuAc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the tri-NeuAc -2xH_2O (838) and the tri-NeuAc -3xH_2O (820) oxonium ions. The elution profiles of glycopeptides from the SDS-insoluble and SDS-soluble fraction are compared.



Supplementary Figure 9: **Example of a tri-Sia containing N-linked glycopeptide.** MS/MS spectrum of a glycopeptide with diagnostic oxonium ions for tri-NeuAc (820). The peptide mass indicates a peptide sequence of GTDNITVR associated with the Limbic system-associated membrane protein but the assignment is ambiguous as only the Y1 ion of the peptide with the GlcNAc was detected.



Supplementary Figure 10: **Retention time differences of sialylated N-glycopeptides.** Extracted ion chromatograms of three glycopeptides with the same peptide backbone (possibly GTDNITVR) but with a different sialic acid content of the N-glycan are compared.