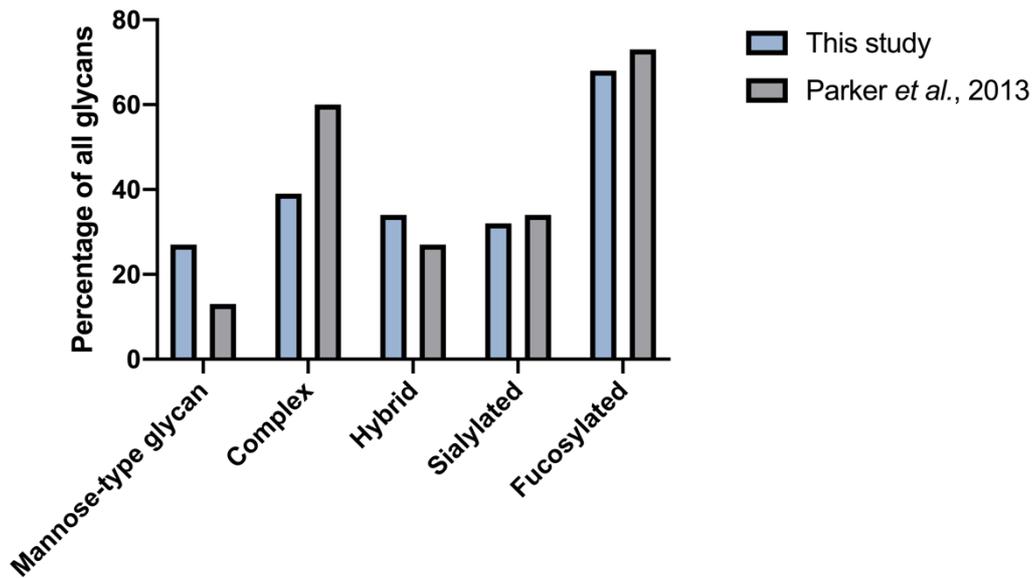


## N-glycosylation in isolated rat nerve terminals

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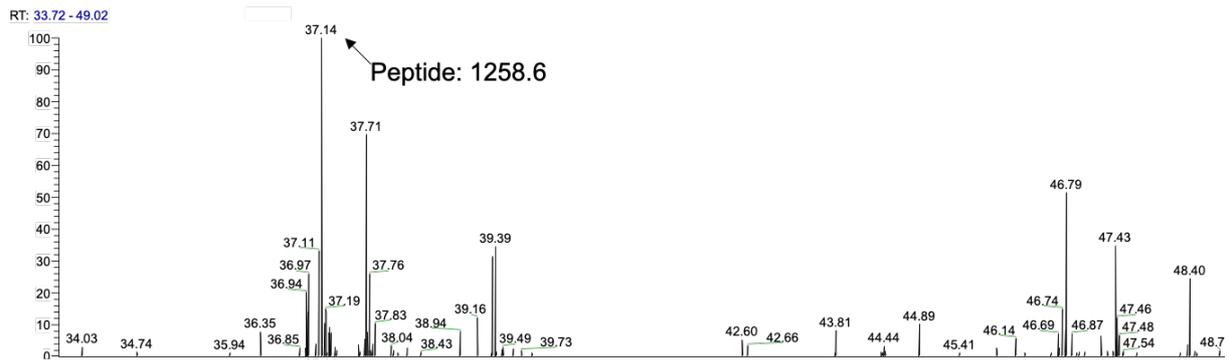
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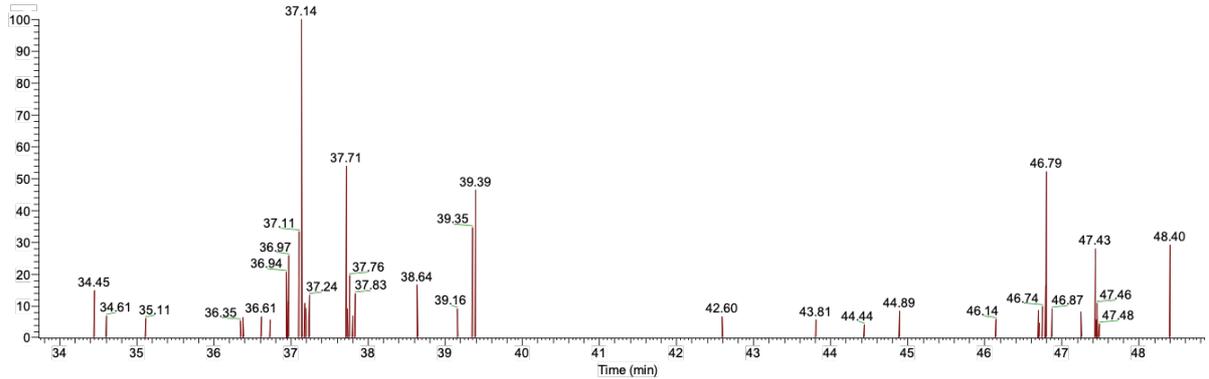


**Supplementary Figure 1: Comparison of N-glycans from this study to Parker *et al.*** The identified synaptic N-glycans were grouped regarding their glycan characteristics and compared to the rat brain membrane protein glycome from Parker *et al.* [20]. Mannose-type glycans refers to paucimannose and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

### Oxonium ion XIC: NeuGc –H<sub>2</sub>O (m/z=290.085-290.088)

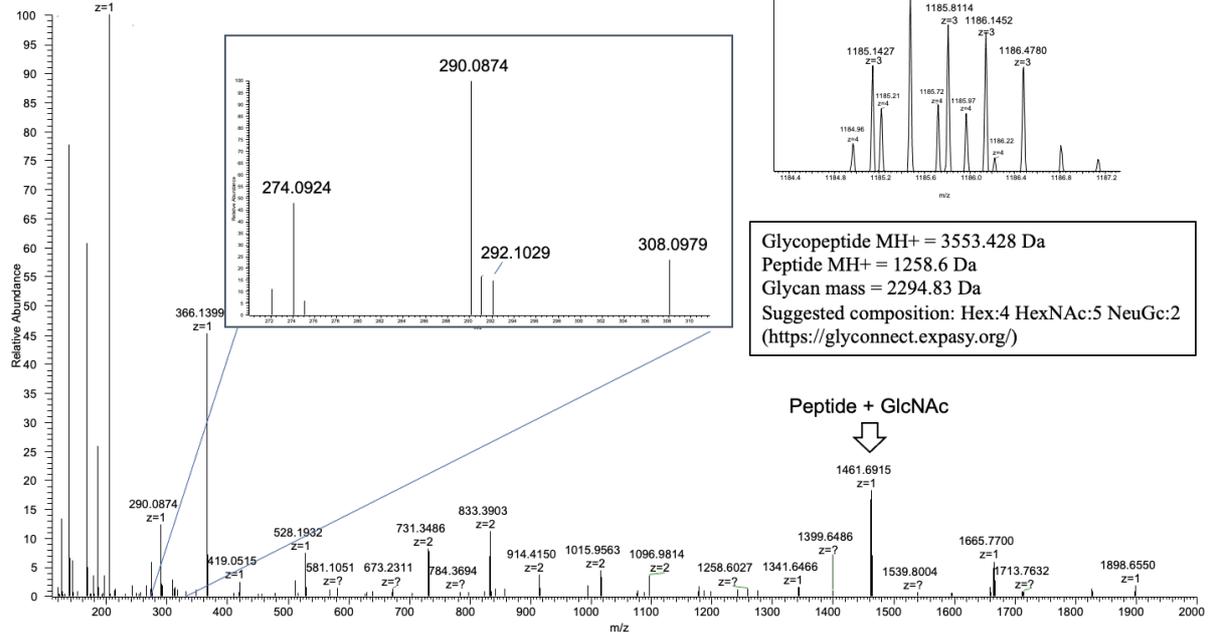


### Oxonium ion XIC: NeuGc (m/z=308.095-308.100)

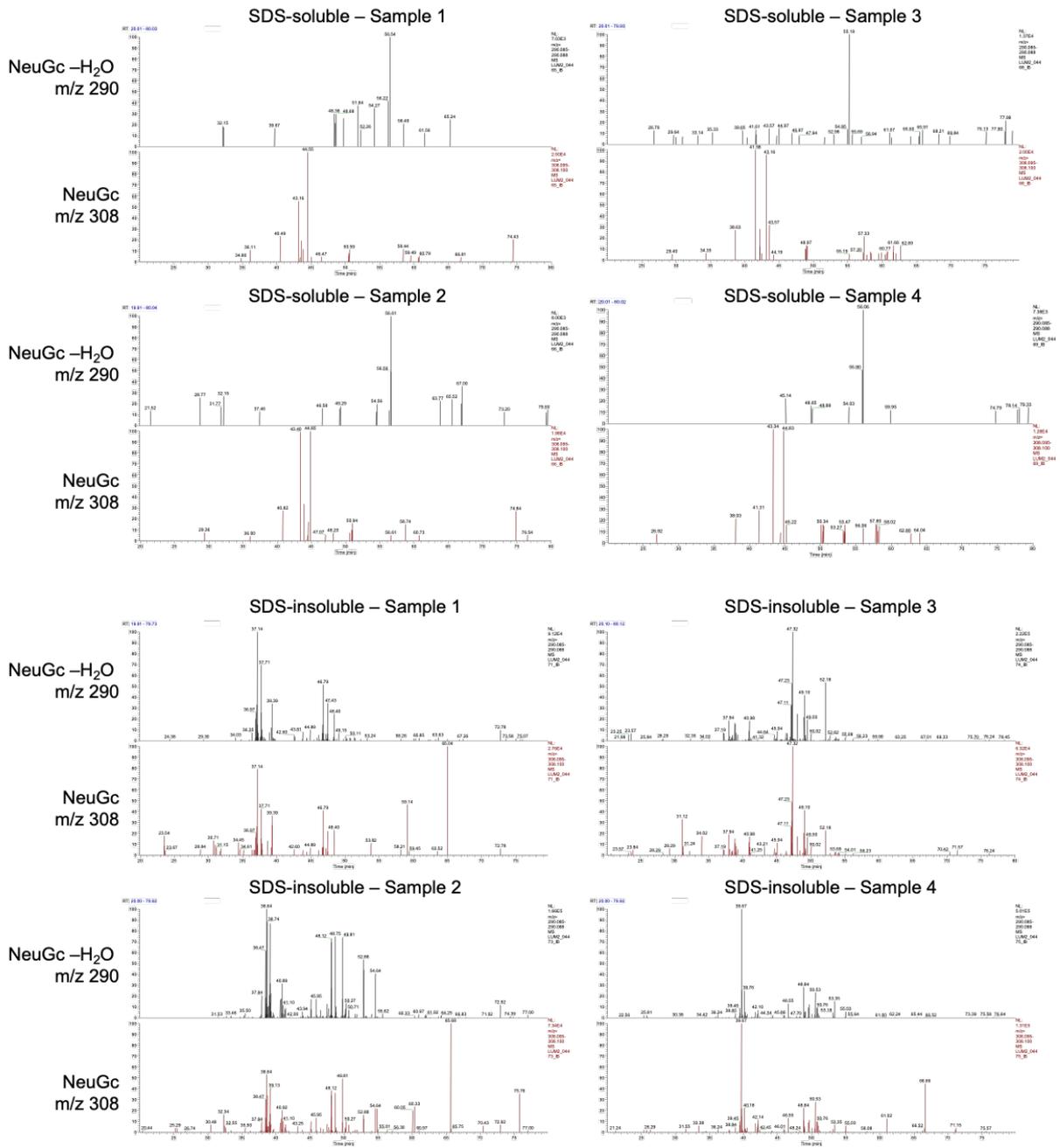


**Supplementary Figure 2: Extracted ion chromatograms for oxonium ions from NeuGc and water loss.** The presence of NeuGc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the NeuGc-H<sub>2</sub>O (290) and the NeuGc (308) oxonium ions. The elution profile of glycopeptides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

LUM2\_04471\_IB #11253 RT: 36.97 AV: 1 NL: 1.92E5  
T: FTMS + c NSI d Full ms2 1185.8108@hcd30.00 [110.0000-2000.0000]  
204.0867

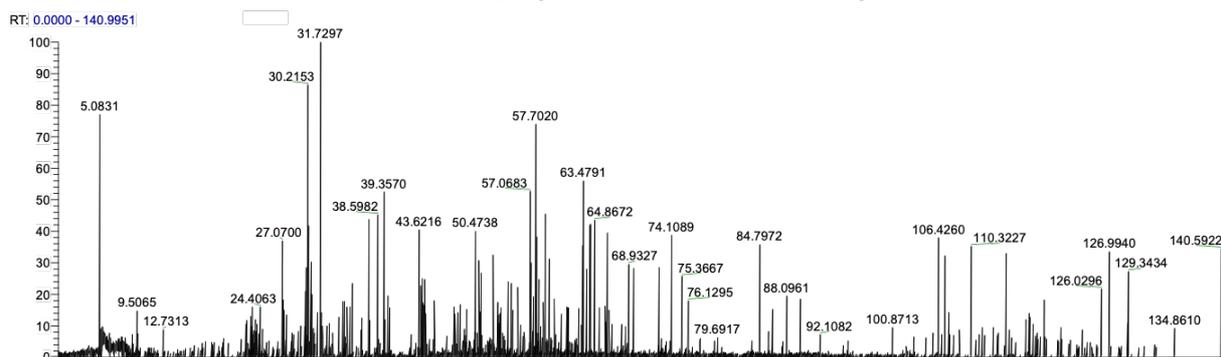


**Supplementary Figure 3: Example of a NeuGc containing N-linked glycopeptide.** MS/MS spectrum of a glycopeptide with intense diagnostic oxonium ions for NeuGc. The peptide mass was determined but the backbone fragmentation is limited.

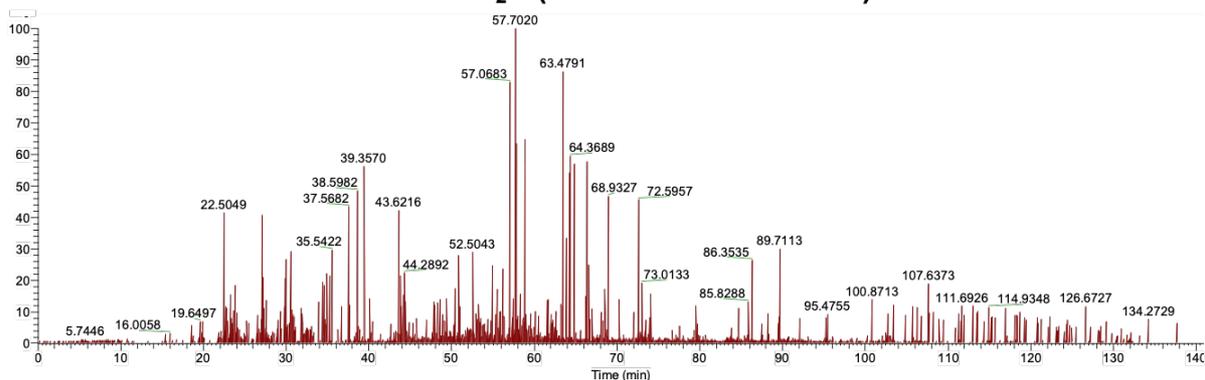


**Supplementary Figure 4: Differences in NeuGc oxonium ion signatures in the SDS-insoluble and SDS-soluble fraction.** The presence of NeuGc containing glycopeptides in synaptosome fractions is illustrated by extracted ion chromatograms (XIC) of the NeuGc-H<sub>2</sub>O (290) and the NeuGc (308) oxonium ions.

### Oxonium ion XIC: di-NeuAc -H<sub>2</sub>O (m/z=565.1840-565.1900)



### Oxonium ion XIC: di-NeuAc -2 H<sub>2</sub>O (m/z=547.1730-547.1790)

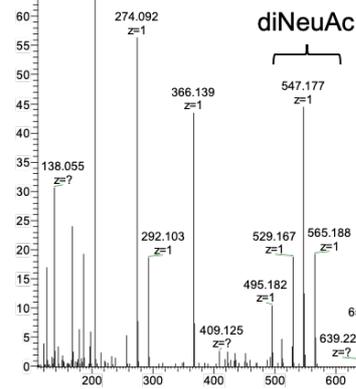


**Supplementary Figure 5: Extracted ion chromatograms for oxonium ions from di-sialic acid (NeuAc).** The presence of di-NeuAc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the di-NeuAc-H<sub>2</sub>O (565) and the di-NeuAc-2 H<sub>2</sub>O (547) oxonium ions. The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

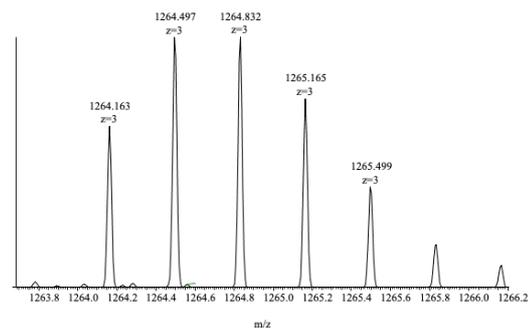


LUM2\_04475\_B#10073 RT: 34.61 AV: 1 NL: 2.28E5  
T: FTMS + c NSI d Full ms2 1264.8304@hcd30.00 [110.0000-2000.0000]

204.087  
z=1  
y1 ion at 175.119  
y3 ion at 375.234

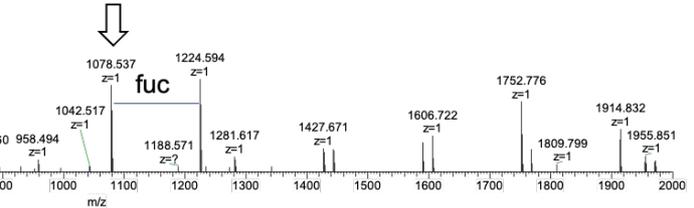


### Glycopeptide

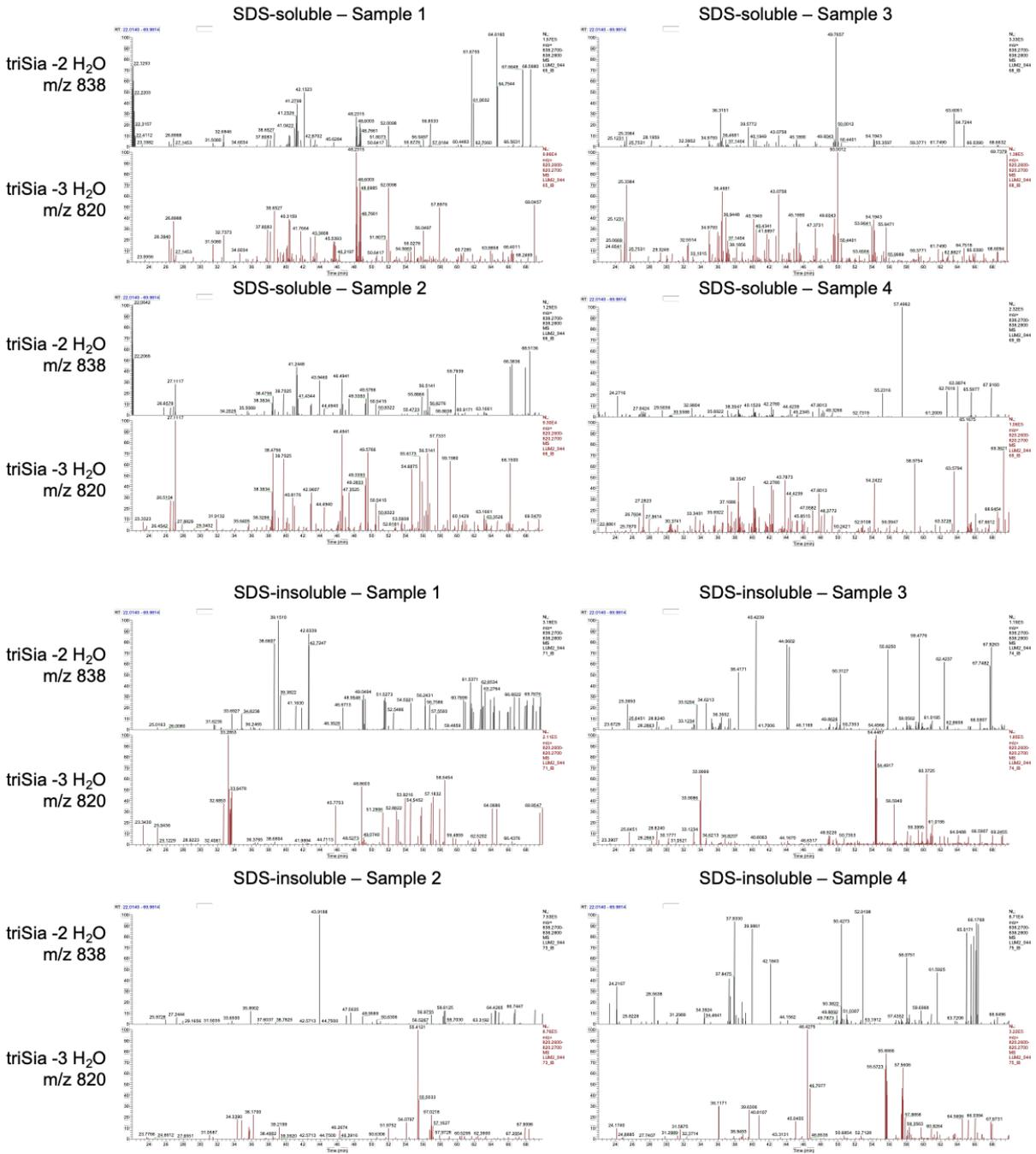


Glycopeptide MH+ = 3790.489 Da  
Peptide MH+ = 875.46 Da (possible GTDNITVR)  
Glycan mass = 2915.029 Da  
Suggested composition: Hex:5 HexNAc:4 dHex:2 NeuAc:2  
(<https://glyconnect.expasy.org/>)

### Peptide + GlcNAc

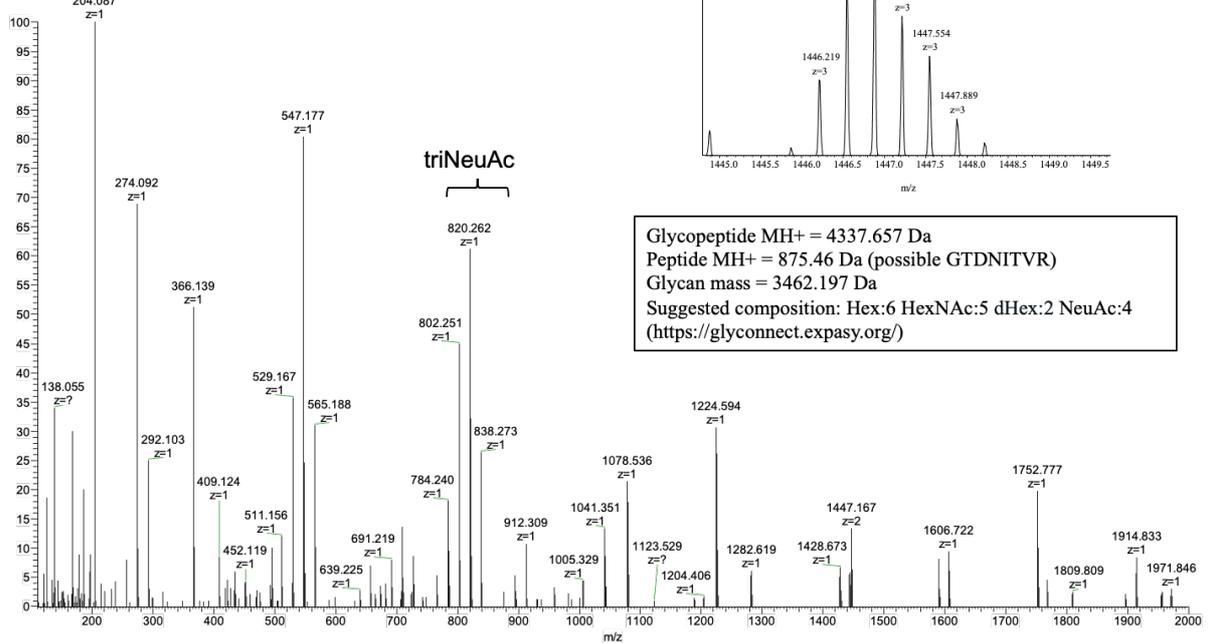


**Supplementary Figure 7: Example of a di-Sia containing N-linked glycopeptide.** MS/MS spectrum of a glycopeptide with intense diagnostic oxonium ions for di-NeuAc. The peptide mass indicates a peptide sequence of GTDNITVR associated with the Limbic system-associated membrane protein but the assignment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

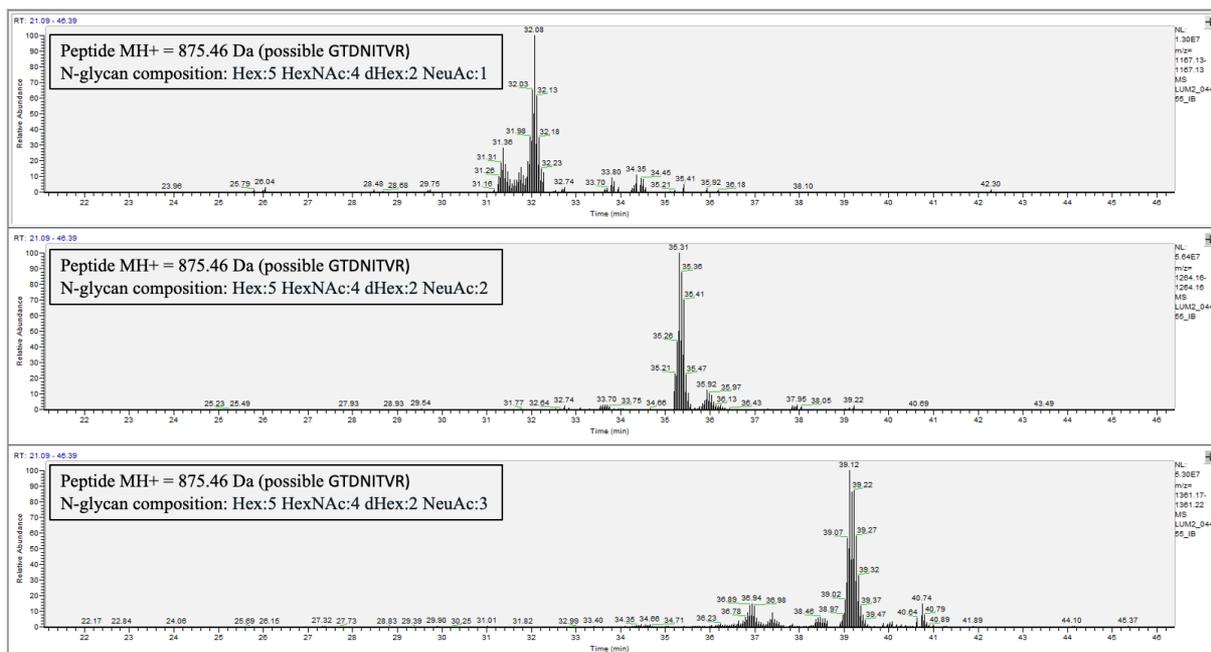


**Supplementary Figure 8: Tri-sialic acid (NeuAc) oxonium ion signatures in synaptosome fractions.** The presence of tri-NeuAc containing glycopeptides is illustrated by an extracted ion chromatogram (XIC) of the tri-NeuAc -2xH<sub>2</sub>O (838) and the tri-NeuAc -3xH<sub>2</sub>O (820) oxonium ions.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

LUM2\_04455\_B #9305 RT: 35.08 AV: 1 NL: 8.82E4  
T: FTMS + c NSI d Full ms2 1447.2207@hcd30.00 [110.0000-2000.0000]



**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.