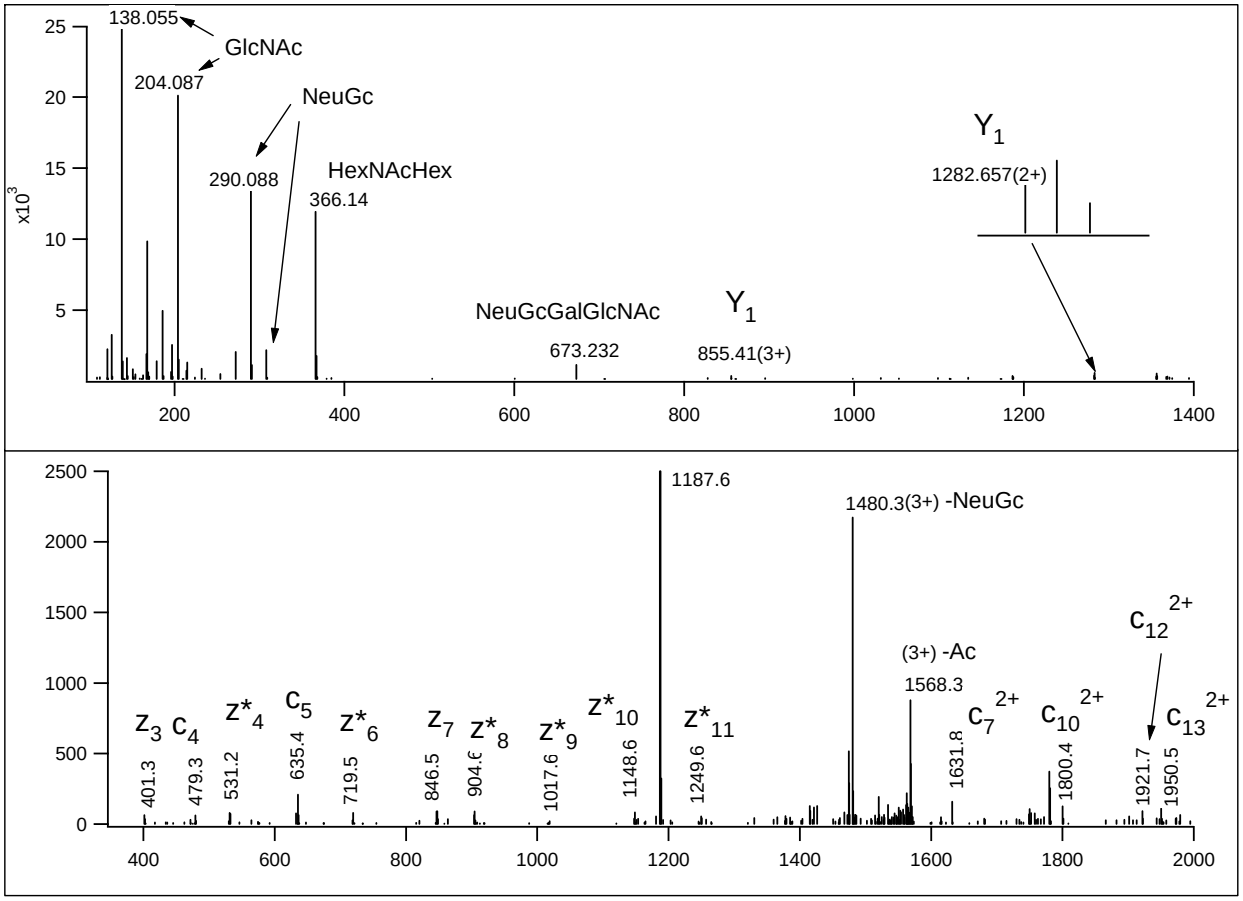
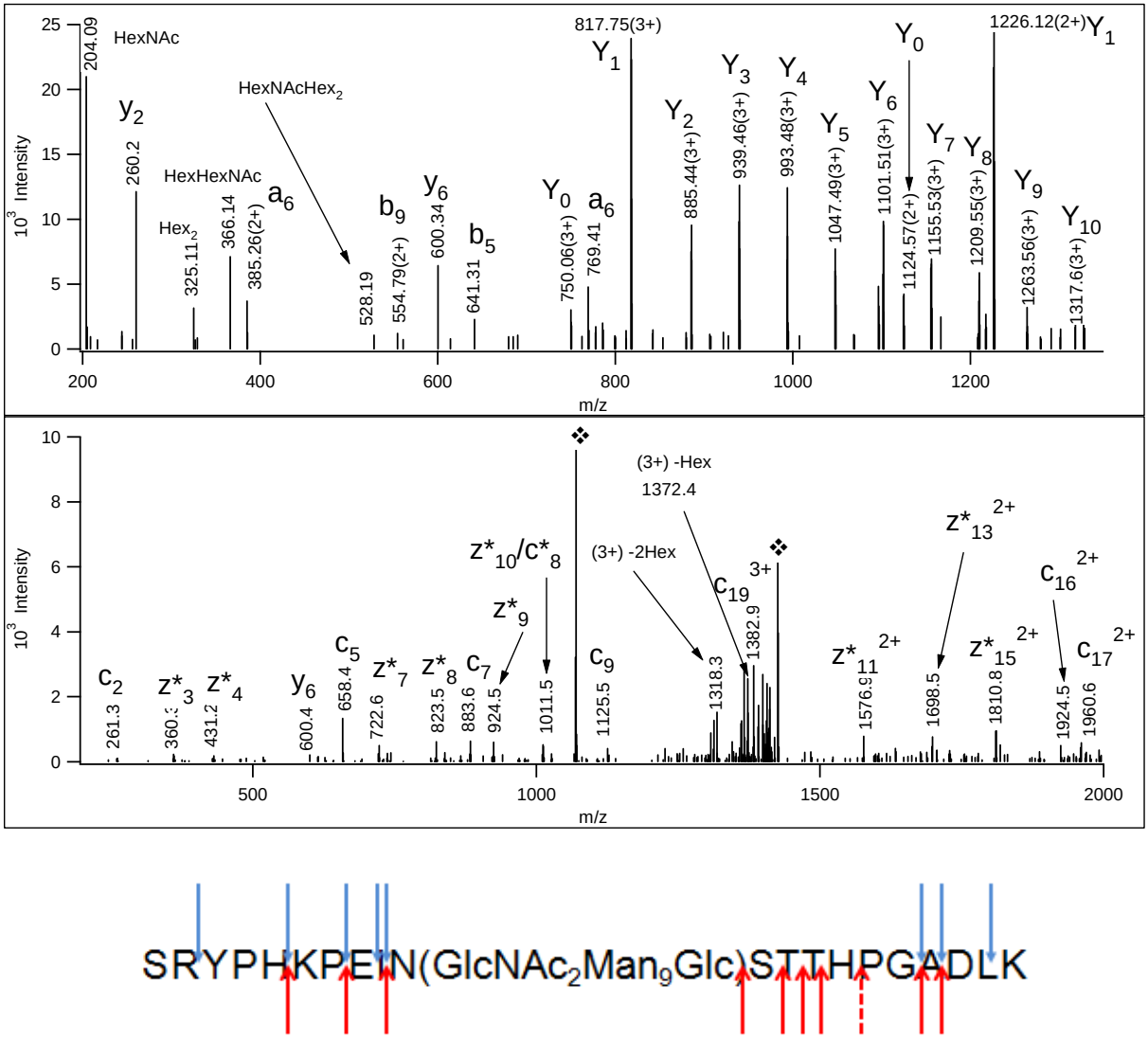
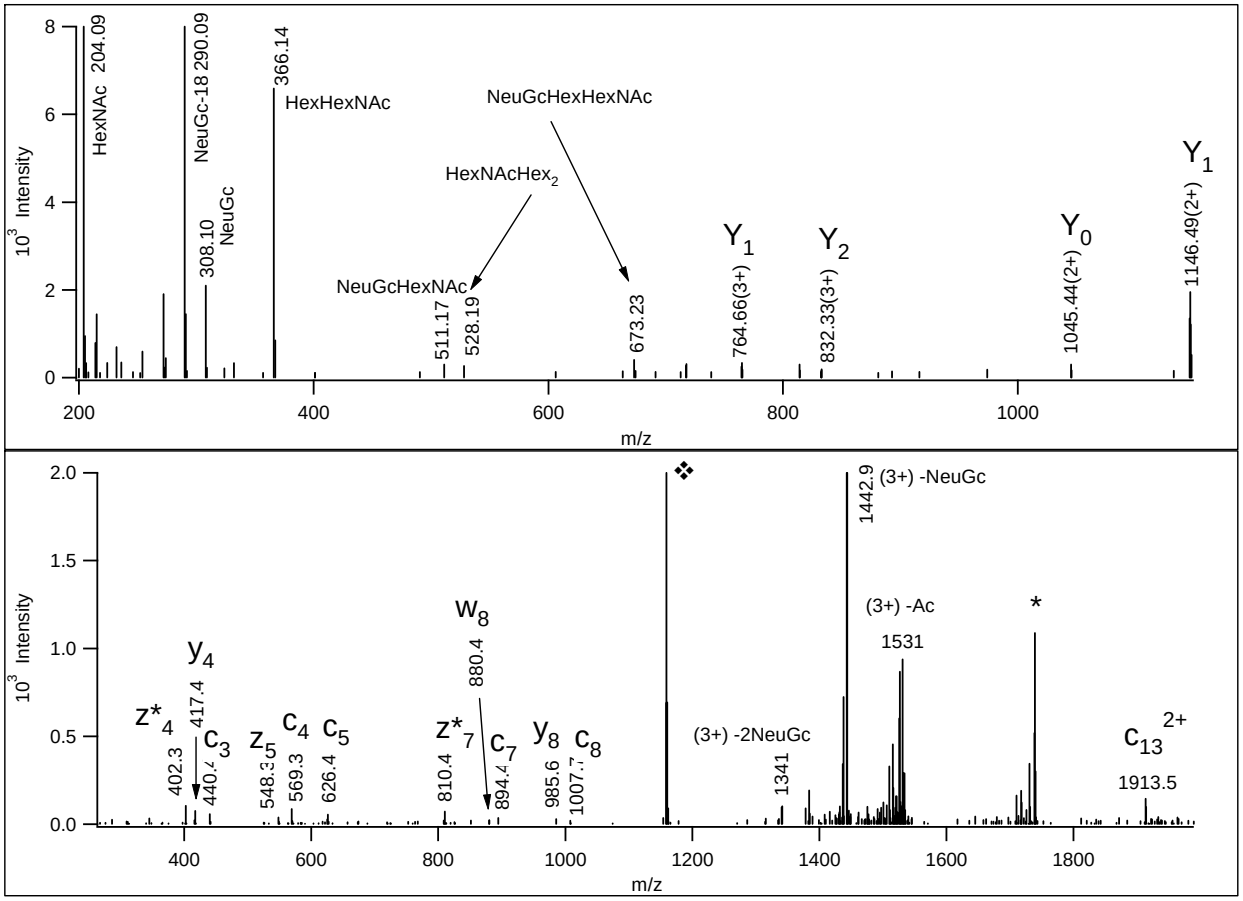
Supplemental Figures for the article "Tissue-specific glycosylation at the glycopeptide level" written by K.F. Medzihradszky, K. Kaasik, and R.J. Chalkley.



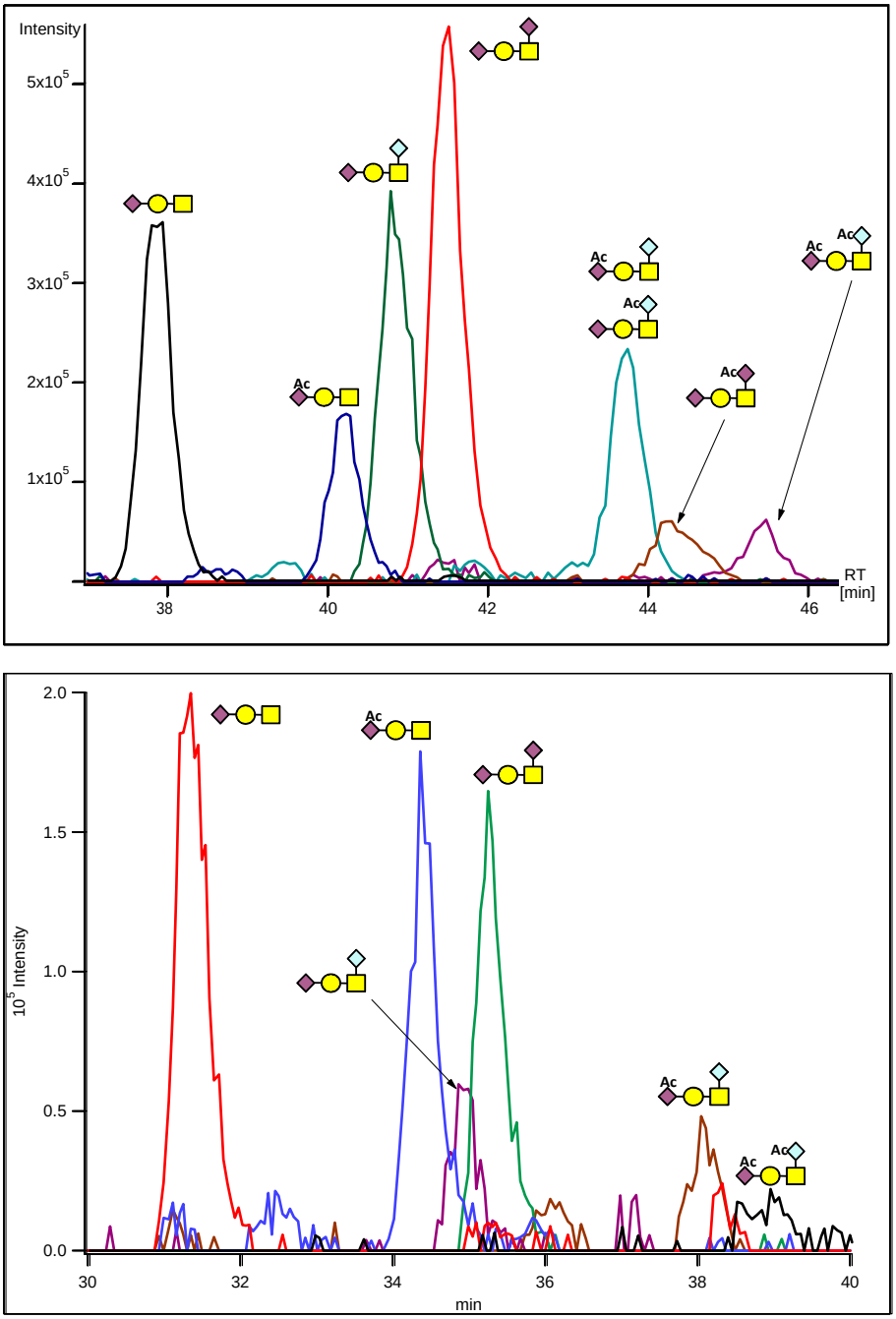
Supplemental Figure 1. The HCD and ETD data acquired from a precursor ion at m/z 1186.7532(4+). ^{125}LGQYRN(HexNAc4Hex5FucSAOx2)EVHTMLGQSTEEIR^{144}, a non-consensus N-glycopeptide from Apoliprotein E was identified from these data. Y_1 stands for the peptide retaining the core GlcNAc, and the inset shows its accurate mass measured. [Carbohydrate fragmentation nomenclature: Domon, B., Costello, C.E. (1988) A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates *Glycoconjugate J* **5,** 397-409.]. SAOx stands for NeuGc in Protein Prospector.



Supplemental Figure 2. MS/MS data from m/z 1069.4700(4+), ❖ labels the different charge states of the precursor ion. The ETD data (lower panel) identified prothrombin (P19221) peptide, ^{135}SRYPHKPEINSTTHPGADLK^{154} bearing a 2026.7 Da oligosaccharide at Asn-144. Based on the mass increase the peptide is modified with a $HexNAc_2Hex_{10}$ structure, that is most likely an immature N-linked sugar, the original Man_9 structure still featuring one of the capping Glc units. The blue and red arrows indicate the cleavages leading to the formation of the **c** and **z˙** fragments detected, respectively. The asterisks label c-1˙ and z+1 fragments. The HCD data confirmed the identity of the peptide primarily with Y_0 and Y_1 fragments [Domon & Costello] and a few sequence ions were also detected. However, the majority of the fragment ions are the results of glycosidic bond cleavages. The labels starting from Y_5 do not comply with the nomenclature quoted, just simply indicate the presence of one more Hex unit.



Supplemental Figure 3. HCD (upper panel) and ETD (lower panel) data of m/z 1158.9447(4+). Fibronectin peptide ^{534}RHEEGHMLNC*TC*FGQGR^{550}, carrying a trisialo biantennary oligosaccharide (added monoisotopic mass = 2543.8525) was identified from the ETD data. The measured precursor mass is within 3 ppm of the calculated value. The HCD data confirm the peptide's mass in form of Y_0-Y_2 fragments and also indicate that one of the sialic acids is linked to a HexNAc unit. That indicates a Gal beta 1-3 GlcNAc linkage in one of the antennae, since the sugars with more common Gal beta 1-4 linkage cannot be sialylated on the subterminal GlcNAc. C* stands for carbamidomethyl Cys.



Supplemental Figure 4. A) Superimposed extracted ion chromatograms of eight different glycoforms of ^{31}AAPPQEDSQAgTETPDTGLYYHR^{52} from Nucleobindin-1 listed in Supplementary Table 3. Data are normalized to the most abundant component, the glycoform bearing the tetrasaccharide containing 2 NeuAcs. This Figure was reprinted with permission from [Medzihradszky, K.F., Kaasik, K., Chalkley, R.J. (2015) Characterizing sialic acid variants at the glycopeptide level. *Anal Chem.* 87, 3064-3071.] Copyright (2015) American Chemical Society. **B)** Superimposed extracted ion chromatograms of the confidently identified glycoforms of tryptic peptide ^{399}KQQLQEQgSAPPSKPDGQLQFR ^{419} of Nucleobindin-1 listed in Supplementary Table 3, except the heaviest glycoform that was not selected for MS/MS analysis, and was indetified only from its accurate mass and chromatographic behavior. The values were normalized to the most abundant glycoform, the one bearing the NeuAcGalGalNAc trisaccharide.