Electronic Supplementary Material (ESI) for Analytical Methods.

ELECTRONIC SUPPLEMENTARY MATERIAL

Glycosylation profiling of selected proteins in cerebrospinal fluid from Alzheimer´s disease and healthy subjects

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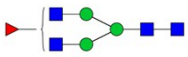
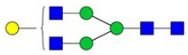
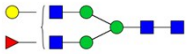
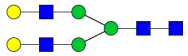
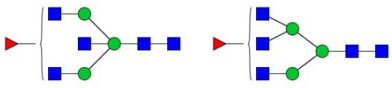
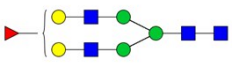
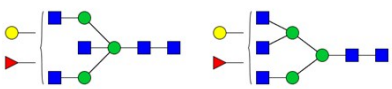
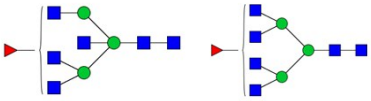
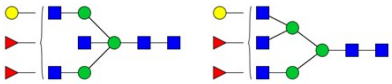
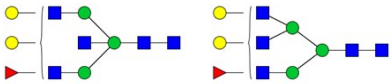
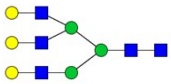
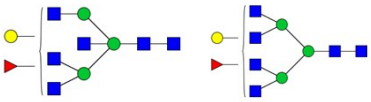
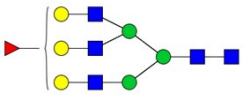
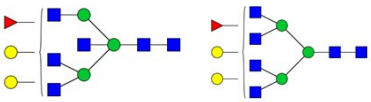
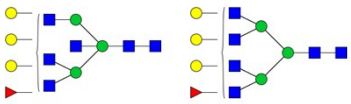
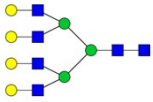
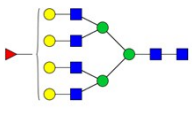
Contents

Possible structures of the detected glycans

Table S1. Identification, m/z values (measured as sodium adducts), possible structure, and sugar composition of the identified desialylated glycan species. Monosaccharide symbols follow the SNFG (Symbol Nomenclature for Glycans) system, N-acetylglucosamine (GlcNAc): blue square; Mannose (Man): green circle; Galactose (Gal); yellow circle; Fucose (Fuc): red triangle. Identity of the abbreviations: H, Hexose; N, N-Acetyl Hexose; F, Fucose. Possible structures were obtained without MS/MS or exoglycosidase treatment, by searching the CFG, Carbbank, GlycomeDB, and Glycosciences databases, and drawn by Glycoworkbench software.

m/z [M+Na]$^+$	Sugar Composition	Possible Structure
1485	H3N4F$^{1-4}$	
1502	H4N4$^{1, 2, 5}$	
1648	H4N4F$^{1-4}$	
1664	H5N4$^{1, 6, 7}$	
1688	H3N5F$^{1, 2, 4}$	
1810	H5N4F$^{1-4}$	
1851	H4N5F$^{1-4, 8}$	

1891	H3N6F[1, 9]	
1996	H4N5F2[1]	
2013	H5N5F[1, 2, 4]	
2028	H6N5[1, 10]	
2053	H4N6F[1, 11]	
2175	H6N5F[1, 12]	
2216	H5N6F[1, 11, 12]	
2378	H6N6F[1, 13]	
2394	H7N6[1, 14]	
2540	H7N6F[1, 14]	

Effectivity of the desialylation process

Experimental

To estimate the desialylation efficiency, standard transferrin (TFN) was used as a model source of glycans. To mimic the immunoaffinity extraction conditions used for the cerebrospinal fluid (CSF), two sample sets, consisting of triplicates of 10 µg of standard TFN (purified from human serum, purchased from Sigma-Aldrich, St. Louis, MO, USA) desolved in PBS (corresponding to the content of 50 µl of CSF with a TFN level of 0.2 mg/mL) were subjected to the whole experimental procedure as described in the main text, *Immunoaffinity extraction of the selected proteins* paragraph. The deglycosylation was performed as well in the same conditions but, to maintain the sialic acids, the first sample set was not treated with the α-2-3,6,8 Neuraminidase. Same conditions were used during the analysis, with the exception of mass range (m/z 1200 – 5000) which was extended to account for the potential presence of large heavily sialylated glycans. MALDI-MS analyses were carried out in triplicate.

Results

Figure S1 shows exemplificative spectra for TFN glycans without (a) and with treatment with neuraminidase (b). Sialylated glycans showed an overall lower intensity, probably due to difficulties in ionization in positive mode, as well as the presence of multiple peaks belonging to the same species, but coordinating a different number of alkali cations. Ionization of sialylated glycans in positive mode in fact, often results in the production of $[M+nNa-(n-1)H]^+$ ions, where n is the number of sialic acids.

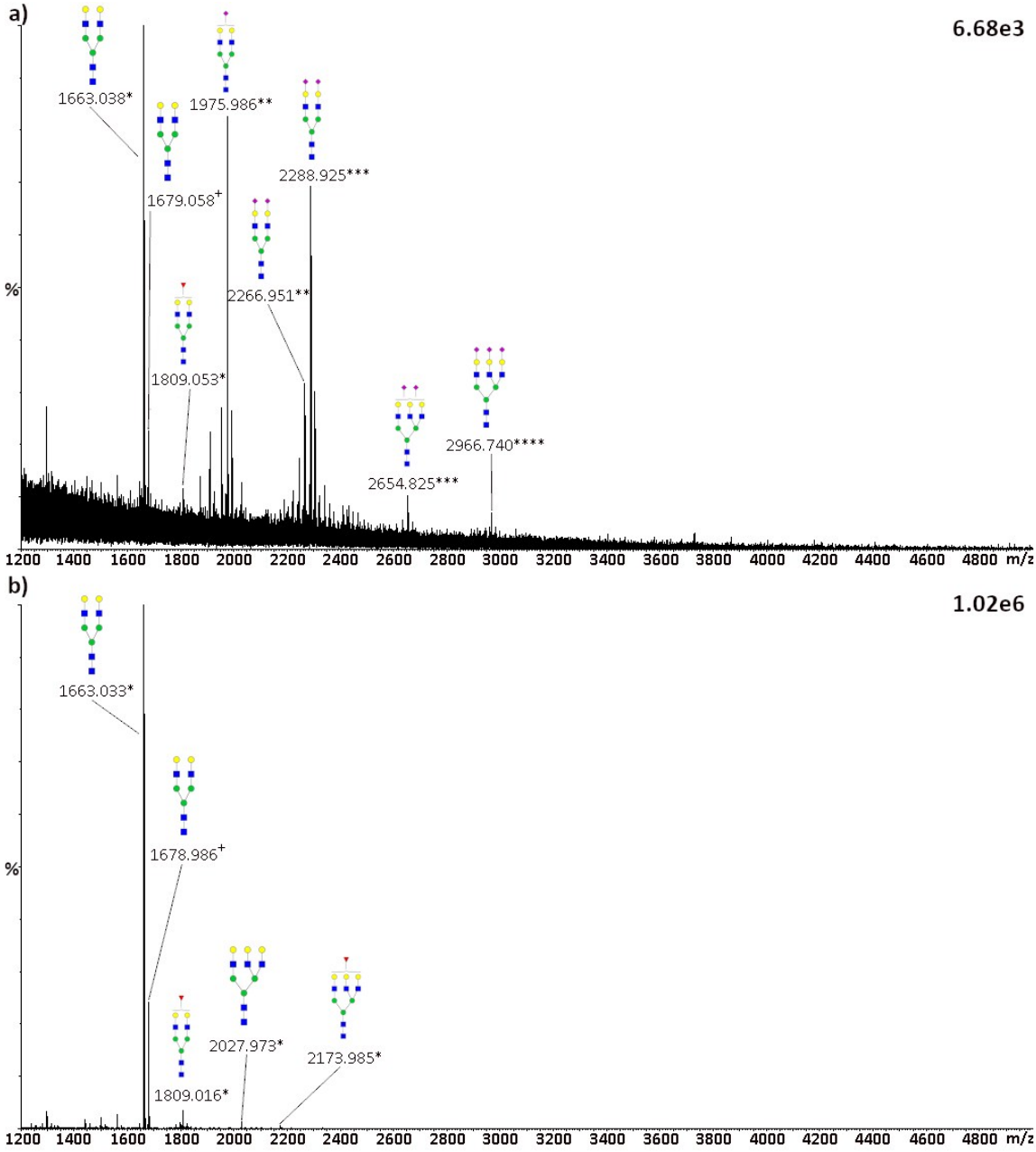


Figure S1. Comparison of enzymatically released TFN glycans (**a**) without treatment of Neuraminidase (i.e. sialic acids have not been cleaved off) and (**b**) with Neuraminidase treatment (i.e. the glycans have been desialylated). The * indicates coordination of Na ions and the + indicates K ions. As it is in positive mode, sialylated species are often seen as $[M+nNa-(n-1)H]^+$, where n is the number of sialic acids.

Glycosylation of serum TFN consists mostly in two biantennary di-sialylated glycans (H5N4S2), accounting for 80% of the total.[15] Peaks related to this glycan were detected at 5 different m/z values, related to the fully sialylated species coordinating 2 or 3 Na^+ cations, to the monosialylated species with 2 Na^+ cations, and to the

non-sialylated species coordinating a Na^+ cation or a K^+ cation. The presence of the non-sialylated species can be explained with the labile nature of the α-glycosidic bond, prone to dissociation under MALDI ionization conditions, while the monosialylated species is often present on TFN.[15] In a similar way, only two minor peaks could be detected for the tri-antennary glycan, corresponding to H6N5S2, coordinating 3 Na^+ cations, and H6N5S3, coordinating 4 Na^+ cations, while no signal was detected for the related fucosylated species. Analysis of enzymatically de-sialylated glycans showed the presence of 4 core structure, with 3 order of magnitude higher intensities. All the core structures observed for the sialylated glycans were detected, with the addition of the tri-antennary fucosylated species (H6N5F). No residual sialylated glycans could be detected when the spectra were inspected for their expected masses, indicating that treatment with neuraminidase in the described conditions is able to reduce the presence of acidic glycans to below the limits of detection of the used technique.

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