

Supporting Information

Structural Characterization of Serum N-Glycans by Methylamidation, Fluorescent Labeling, and Analysis by Microchip Electrophoresis

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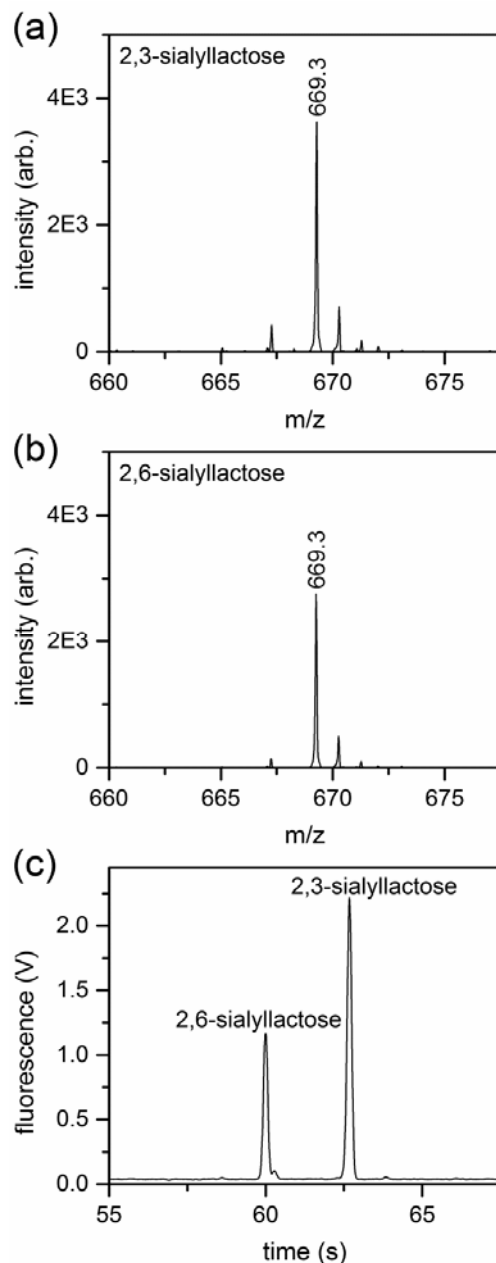


Figure S1. MALDI-MS spectra, acquired in positive-ion mode, of (a) 2,3-sialyllactose and (b) 2,6-sialyllactose neutralized by covalent derivatization with methylamine. (c) Electropherogram of a mixed sample of these linkage isomers. Reported MALDI-MS masses correspond to monosodium adducts $[M+Na]^+$ of methylamidated 2,3-sialyllactose and 2,6-sialyllactose. Underivatized sialyllactose compounds, which were detected at m/z 656.3, are not present in the spectra of the derivatized samples. For the electrophoretic separation, the electric field strength was 1250 V/cm, the separation length was 22 cm, and the samples were labeled with APTS. A few minor peaks were observed by both MS-based and electrophoretic-based methods and are most likely due to the presence of impurities in the sialyllactose samples.

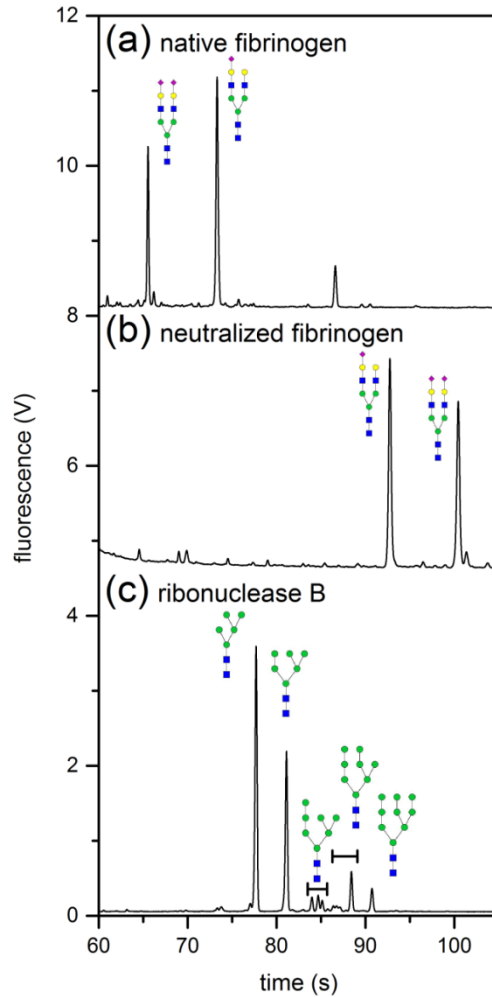
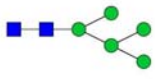
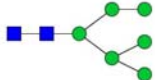
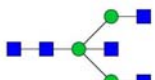
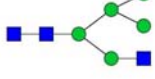
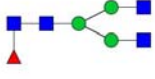
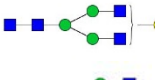



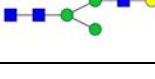
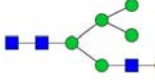
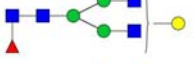


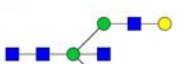
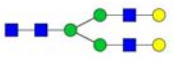
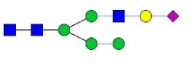
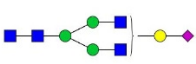
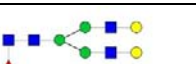

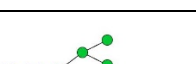
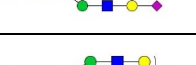

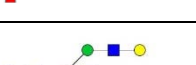
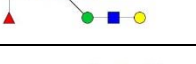
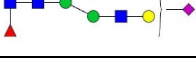
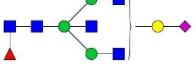
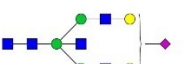
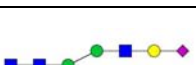
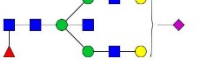
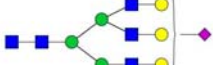
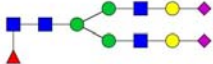
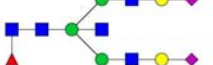
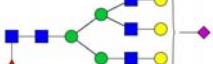
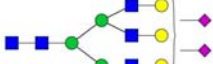

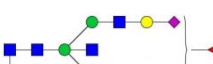
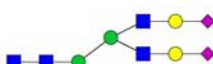


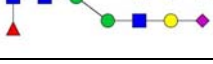



Figure S2. Electropherograms of (a) native and (b) methylamidated N-glycans derived from fibrinogen and (c) N-glycans derived from ribonuclease B that do not contain sialic acids. Symbols represent N-acetylglucosamine (blue square), mannose (green circle), galactose (yellow circle), and N-acetylneuraminic acid (purple diamond). The electric field strength was 1250 V/cm, the separation length was 22 cm, and the samples were labeled with APTS.

Table S1. N-Glycan structures, their amidated masses, relative intensities from MALDI-TOF-MS analysis, and relative peak areas from microchip electrophoresis (ME) for identified N-glycans. Peak areas from co-migrating structures in the electropherograms are reported as a single area. For comparison, MALDI intensities are reported for each individual mass and as a sum when structures co-migrate in the electropherograms. Rows marked in red correspond to N-glycans not identified in the electropherograms.

structure	amidated mass (Da)	MALDI-MS relative intensity (%) (individual)	MALDI-MS relative intensity (%) (summed)	ME relative peak area (%) (individual)	ME relative peak area (%) (summed)
	1234.4287	1.7952		1.6996	
	1396.4824	0.6828		1.3305	
	1437.5128	0.0180	0.0574	--	0.5145
	1519.5575	0.0394		--	
	1462.5121	2.8075		3.6424	
	1478.5394	0.1256	0.3407	--	0.4875
	1665.6042	0.2152		--	
	1558.5391	0.0288	0.1077	--	0.3228
	1681.6187	0.0789		--	
	1579.5654	0.5864		N/A	
	1599.5656	0.0152		N/A	

	1624.5791	4.474		--	
	1720.5919	0.0429	5.2268	--	5.2115
	1827.6766	0.5503		--	
	1843.6715	0.1598		--	
	1640.5922	0.2474		2.0406	
	1741.6875	0.1023		0.5058	
	1782.7179	0.2858		0.7217	
	1786.6501	1.7893	1.8270	--	3.5238
	1882.6447	0.0378		--	
	1903.6710	0.0529		N/A	
	1928.7027	0.2402	11.7076	--	7.8300
	1944.6976	11.4673		--	
	1989.7295	0.2101		1.0219	
	2090.7644	3.7959		4.5428	
	2131.7820	0.0676		N/A	
	2147.7770	1.4416		0.9003	
	2248.7964	56.0153	56.0704	--	33.8128
	2451.9680	0.0551		--	

	2293.7832	1.6307		3.0739	
	2309.8297	0.3143		0.5288	
	2394.8918	3.8949	4.9940	--	8.1444
	2597.9403	1.0991		--	
	2455.8877	0.0494		N/A	
	2613.9392	0.7909		2.9976	
	2759.9602	0.0174		N/A (insufficient intensity)	
	2743.9982	0.0917		N/A	
	2918.0605	3.9507		6.7570	
	2979.0674	0.0425		N/A	
	3064.0583	0.5014		1.6419	
	3125.1253	0.0244		N/A	
	3210.1564	0.0246		N/A	

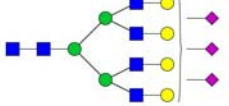
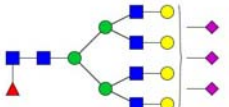
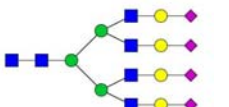
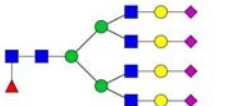
	3283.1728	0.0380		N/A (insufficient intensity)	
	3429.2307	0.0287		N/A (insufficient intensity)	
	3587.2822	0.0390		N/A (insufficient intensity)	
	3733.3496	0.0335		N/A (insufficient intensity)	

Table S2. Equations fitted to the data in **Figure 3** and their R^2 values. Fits were used to estimate electrophoretic mobilities of unknown N-glycans that did not have a corresponding standard N-glycan.

category of structures	equation (x = molar mass, y = mobility)
bisected, bi-antennary	$y = -4.84E-08x + 2.79E-04, R^2 = 0.9859$
non-bisected, bi-antennary	$y = -5.04E-08x + 2.75E-04, R^2 = 0.9888$
non-bisected, tri- and tetra-antennary	$y = -2.58E-08x + 2.18E-04, R^2 = 0.9883$