Supporting Information

In-Depth Compositional and Structural Characterization of N-Glycans Derived from Human Urinary Exosomes

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Supporting Information includes:

Experimental Section

Materials. The following materials were used: acetic acid, acetone, ammonium persulfate, citric acid, dimethyl sulfoxide (DMSO), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2-mercaptoethanol, (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluoro-phosphate (PyAOP), 4-methylmorpholine, Protease Inhibitor Cocktail tablets (PIC), potassium phosphate monobasic, sodium cyanoborohydride, γ-methacryloxypropyltrimethoxysilane (MAPTOS), methanol, tetramethylethylenediamine (TEMED), methyl iodide, hydroxybenzotriazole, methylamine hydrochloride, sodium hydroxide beads, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, and triethanolamine from Sigma-Aldrich; ammonium hydroxide from J.T. Baker (Phillipsburg, NJ); acrylamide and sodium dodecyl sulfate (SDS) from Bio-Rad Laboratories, Inc. (Hercules, CA); 8-aminopyrene-1,3,6-trisulfonic acid (APTS), Pierce BCA protein assay kit, albumin standard, and sodium hydroxide from Fisher Scientific (Waltham, MA); hydrogen peroxide from Macron Fine Chemicals (Radnor, PA); sodium azide from Mallinckrodt; isopropylamine hydrochloride from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); sucrose from IBI Scientific; chloroform and N,N-dimethylformamide (DMF) from Mallinckrodt Baker (Phillipsburg, NJ); trifluoroacetic acid (TFA), 2,5-dihydroxybenzoic acid (DHB), and sodium acetate from EMD Chemicals, Inc. (Gibbstown, NJ); Microposit MF-319 developer from MicroChem Corp. (Westborough, MA); peptide-N-glycosidase F (PNGase F) and α1-2,3,4,6 fucosidase from ProZyme; α2-3,6,8 sialidase and α2-3 sialidase S from New England BioLabs, Inc. (Ipswich, MA); nonidet P40 substitute (NP-40) from Roche Diagnostics Corp. (Indianapolis, IN); pooled normal human urine from BioreclamationIVT (Westbury, NY); chromium etchant 8002-A, chromium etchant 1020 and buffered oxide etchant (BOE) from Transene Co., Inc. (Danvers, MA); B270 glass substrates and cover plates from Telic

Co. (Valencia, CA); activated charcoal Micro SpinColumns, hydrophilic interaction chromatography (Amino) Micro SpinColumns, C-18 Micro SpinColumns, empty Micro SpinColumns, and 1000 Da cut off Micro DispoDIALYZER from Harvard Apparatus (Holliston, MA); and Oasis MAX extraction cartridges from Waters Corp. (Milford, MA).

LC-MS Characterization of Urinary Exosomes. Tryptic digests of exosomes were analyzed with LC-MS.[1] MS/MS spectra were submitted to the search engine MASCOT, and 93 proteins with a MudPIT score of ≥ 50 were identified. These proteins are similar to previous reports of proteins identified in human urinary exosomes.[2-3]

Exosome Size Measurements by Dynamic Light Scattering. Urinary exosomes isolated from 20 mL of commercial normal human urine or urinary samples from healthy male volunteers were reconstituted in 3 mL of PBS buffer. An aliquot of 1 mL dissolved urinary exosomes was transferred into a disposable cuvette and characterized by a Zetasizer Nano S instrument (Malvern Instruments, Malvern, UK).

Total Protein Determination by Bicinchoninic Acid Assay (BCA Assay). The Pierce™ BCA Protein Assay Kit was used to determine the protein concentration of the isolated exosomes. A set of protein standards was prepared from concentrated albumin standard. A working solution was prepared by mixing 0.5 mL BCA Reagent B and 25 mL BCA Reagent A. Afterwards, each 200 µL aliquot of the working solution was added into each 20 µL aliquot of protein standard solution and unknown exosome solution. Mixed solutions were then incubated at 37 ºC for 30 min, followed by an absorbance measurement at 562 nm.

Reduction of N-Glycans. N-Glycans were reduced to their alditol form according to the previously published method.[1] The N-glycans were incubated with 10 µL of 10 mg/mL borane-ammonia complex for 60 min at 60 °C. Afterwards, a 1 µL aliquot of glacial acetic acid was added

to the reaction system three times. The leftover volatile salt was removed through repeated evaporation in the presence of methanol.

Permethylation of N-Glycans. N-Glycans were permethylated through a solid-phase procedure.[4] Briefly, samples were dissolved in 65 μL of dimethylformamide, 5 μL of water, and 35 μL of methyl iodide. The samples were loaded on sodium hydroxide beads packed in Micro SpinColumns, incubated for 20 min, and centrifuged down to the microcentrifuge tubes. With the addition of another 35 μL of methyl iodide, the sample solutions were loaded onto the Micro SpinColumns, incubated for 20 min, and collected through centrifugation. Permethylated N-glycans were recovered from the reaction mixture through chloroform extraction.

Extraction of Permethylated Sulfated N-Glycans. Permethylated sulfated N-glycans were recovered from the permethylation reaction mixture through a modified chloroform extraction method.[5] Briefly, permethylated samples were dissolved in 400 μL of chloroform, and the chloroform phase was washed with an equivalent volume of 0.5 M NaCl once and HPLC-grade water twice to extract leftover NaOH and charged glycans into the aqueous phase. Neutral, non-sulfated glycans were recovered from the chloroform phase through Centrivap drying. Solid-phase extraction on a C-18 Micro SpinColumn was used to remove leftover NaOH from the aqueous phase, and the retained sulfated glycans were eluted and recovered after Centrivap drying.

Fractionation of Permethylated N-Glycans through C-18 Micro SpinColumn. Permethylated N-glycans were fractionated through C-18 Micro SpinColumn as previously described.[1] Briefly, reduced and permethylated N-glycans were dissolved in 200 μL of 50% methanol and loaded on a pre-conditioned C-18 Micro SpinColumn three times. N-Glycans were eluted stepwise with 600 μL of 5%, 15%, 35%, 50%, and 75% acetonitrile containing 0.1% TFA. In the end, N-glycan fractions were dried in a Centrivap concentrator.

Digestion of N-Glycans with Sialidase. Fractionated native N-glycans were treated with 1 μL of α2-3,6,8 sialidase at 37 ºC for 18 h or 1 μL of α2-3 sialidase at 37 ºC for 4 h. The reaction mixture was then purified by solid-phase extraction on active charcoal columns.

Digestion of N-Glycans with α1-2,3,4,6 Fucosidase. Neutralized N-glycans fractions were treated with 8 μL of α1-2,3,4,6 fucosidase at 37 ºC for 48 h. The reaction mixture was then purified by solid phase extraction on active charcoal columns.

Fabrication of Microfluidic CE Devices. The schematic of the microfluidic device is shown in **Figure S2**,[6] which includes a cross intersection for the modified pinched injection[7] and a 22 cm long serpentine channel for separation. As shown in the inset of **Figure S2**, two asymmetrically tapered 180° turns with a taper ratio of 3 were designed to eliminate the "racetrack" effect and improve resolution.[7] Standard photolithography was used to generate the microfluidic design on the glass substrate.[8] Briefly, a B270 glass substrate coated with 120 nm of Cr and 530 nm of AZ1518 photoresist was exposed to a 200 mJ/cm^2 UV radiation through a photomask (HTA Photomask) with the designed feature. The exposed substrate was immersed in MF-319 developer for 2 min and immersed in chromium etchant 8002-A for 8 min to generate the pattern in the photoresist layer and the Cr layer, respectively. The microfluidic pattern was then transferred into B270 glass substrate through etching the substrate in buffered oxide etchant (BOE). A stylus-based profiler (KLA-Tencor, Milpitas, CA) was used to measure the dimensions of channels, which were ~87 μm in width and ~15 μm in depth.

Holes were sandblasted at the end of each channel to allow fluidic and electrical access to the microchannels. Afterwards, leftover photoresist was removed by rinsing with methanol and acetone. The remaining Cr layer was removed after being immersed in chromium etchant 1020 for 10 min. Then, the surface of the cover plate and substrate were immersed in 200 mL of hydrolysis

solution (40% ammonium hydroxide and 20% hydrogen peroxide in water) at 70 °C for 20 min. The hydrolyzed cover plate and substrate were bonded together and placed in an oven at 90 ºC overnight to dry, followed by annealing in a furnace at 545 °C for 10 h. Finally, small sections of glass tubes were attached over the sandblasted holes as reservoirs.

Poly(acrylamide) Coating of Microchannels. The microchannel was coated with linear polyacrylamide to minimize both sample adsorption and electroosmotic flow.[8] Before coating, the microchannel was pre-conditioned stepwise with 1.0 M sodium hydroxide, water, and methanol. MAPTOS was then covalently bound to the microchannel wall by flushing the microchannel with 3% MAPTOS and 0.02 M acetic acid in methanol and letting stand at room temperature for 1 h. Excess MAPTOS was then removed by sequential rinsing with methanol and water. To conjugate linear polyacrylamide with the MAPTOS, an aqueous solution with 2.4% (w/w) acrylamide, 1.0 μL/mL TEMED, and 1.0 mg/mL ammonium persulfate was flushed into the microchannel and let stand for 48 h.

Microfluidic Capillary Electrophoresis (CE) Analysis. APTS-labeled glycans were reconstituted in 15-30 μL of 10 mM HEPES buffer. A 4 μL aliquot of sample was placed into the sample reservoir, and the sample was loaded into the analysis channel with a modified pinched injection.[7] For the electrophoretic separation, potentials were applied to the waste, buffer, and sample reservoirs with a fast-slewing high-voltage supply (0-10 kV). A commercial high-voltage supply (0-30 kV; CZE 1000R, Spellman High Voltage Electronics Corp., Hauppauge, NY) applied the potential to the analysis reservoir. The voltage outputs were controlled by an analog output board (PCI-6713, National Instruments Corp., Austin, TX) and a LabVIEW program (National Instruments Corp.).

Separated glycans were detected by laser-induced fluorescence (LIF). Samples were excited with the 457-nm line of an argon ion laser (Melles Griot, Inc., Carlsbad, CA) at an attenuated power of 1.1 mW. The emitted fluorescence signal was monitored on an inverted optical microscope (TE-2000U, Nikon, Inc., Tokyo, Japan) with a 20x objective and a GFP filter cube (Chroma Technology Corp., Bellows Falls, VT). The fluorescence signal was spatially filtered with a 600 μm pinhole, detected by a photomultiplier tube (H5783-01, Hamamatsu Corp., Bridgewater, NJ,), amplified by a low-noise current preamplifier (SR570, Stanford Research Systems, Inc., Sunnyvale, CA), and recorded at 100 Hz by the LabVIEW program with a multifunction data acquisition board (PCI-6032E, National Instruments Corp., Austin, TX).

MALDI-MS Analysis. Methylamidated, permethylated, and SALSA-derivatized glycans from 25-50 μL aliquots of urinary exosomes were reconstituted in 5-20 μL of 50% methanol. Aliquots of 0.5 μL glycan samples were loaded onto the MALDI target plate and dried in air for 10 min. Aliquots of 0.5 μL matrix solution (10 mg/mL DHB and 1 mM sodium acetate in 50% methanol) were subsequently loaded onto the sample spots and dried in a desiccator for 2 min. With native dextrin as a calibration standard, the samples were analyzed with an Applied Biosystems 4800 MALDI-TOF/TOF mass spectrometer (SCIEX, Framingham, MA) in the positive reflector mode.

LC-MS and MS/MS Analysis. Reduced native N-glycans, reduced permethylated N-glycans, and reduced methylamidated N-glycans equivalent to 16 μg of glycoprotein were dissolved in water and separated on a 900 × 0.075 mm capillary packed in-house with 3 μm porous graphitized carbon (Bruker-Michrom, Inc., Auburn, CA). The column efficiencies typically featured around 100,000 theoretical plates. Buffer A was 0.15% formic acid (FA), adjusted to pH 3 with ammonia in water, whereas buffer B was acetonitrile with 0.15% FA. Using an Acquity M class UPLC instrument (Waters Corporation, Milford, MA), we set the elution gradient at 0-10 min 5% B, 40

min 15% B, 140 min 40% B, 200 min 80% B, and 230 min 80% B with a flow-rate of 0.3 µL/min. The glycans were analyzed with an Agilent LC/MSD ion trap XCT Plus mass spectrometer (Agilent Technologies, Alpharetta, GA) with an on-line nanosource (G1982B), and the scan range was set to m/z 600-2200 with a MS^n threshold of 2000 counts. The flow rate of the drying gas was 5 L/min at a temperature of 160 °C. The capillary current was kept between 100-200 nA, and the capillary plate offset was –500 V.

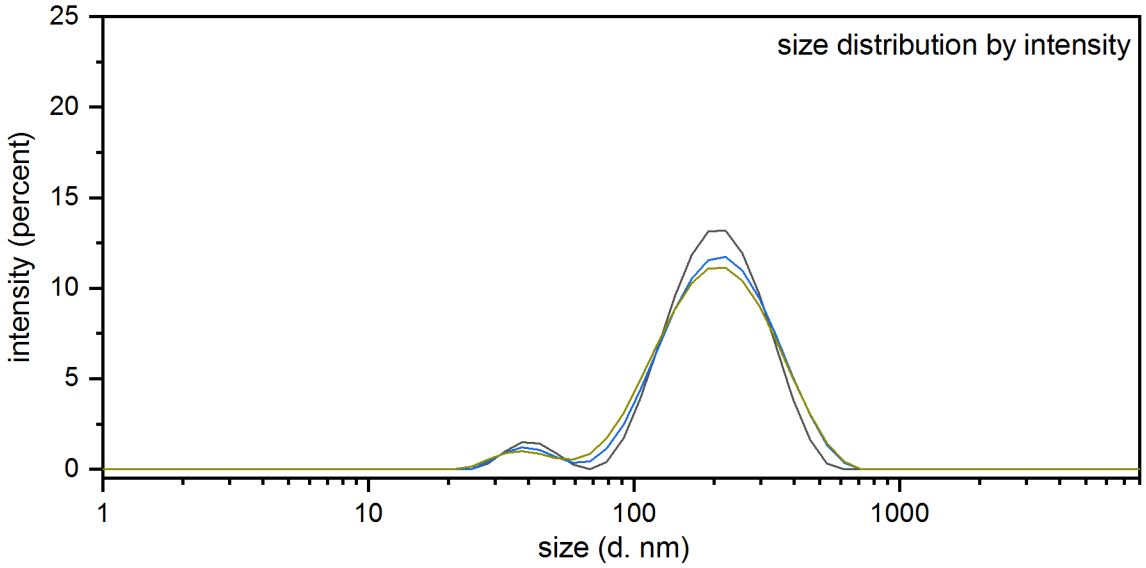


Figure S1. Size distribution of exosomes isolated from commercial human urine and measured by dynamic light scattering (DLS). Exosomes isolated from 20 mL of human urine were dissolved in 3 mL of PBS buffer for DLS analysis.

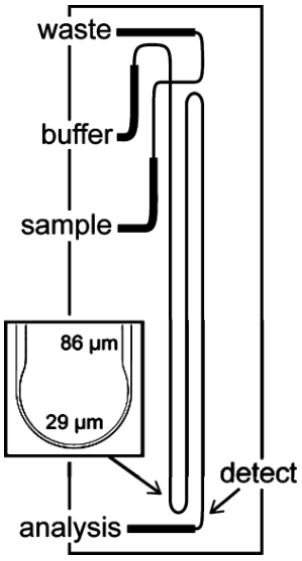


Figure S2. Schematic of the microfluidic device with a 22 cm separation channel. The channel has two asymmetrically tapered 180° turns with a taper ratio of 3.

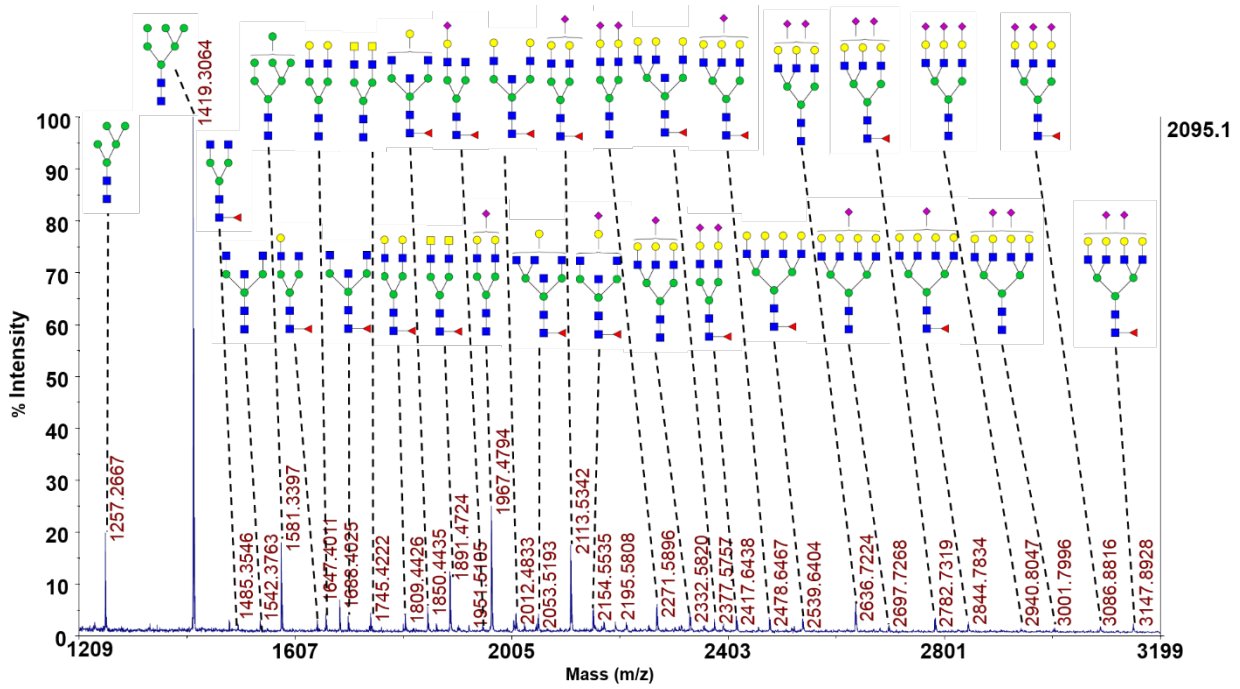
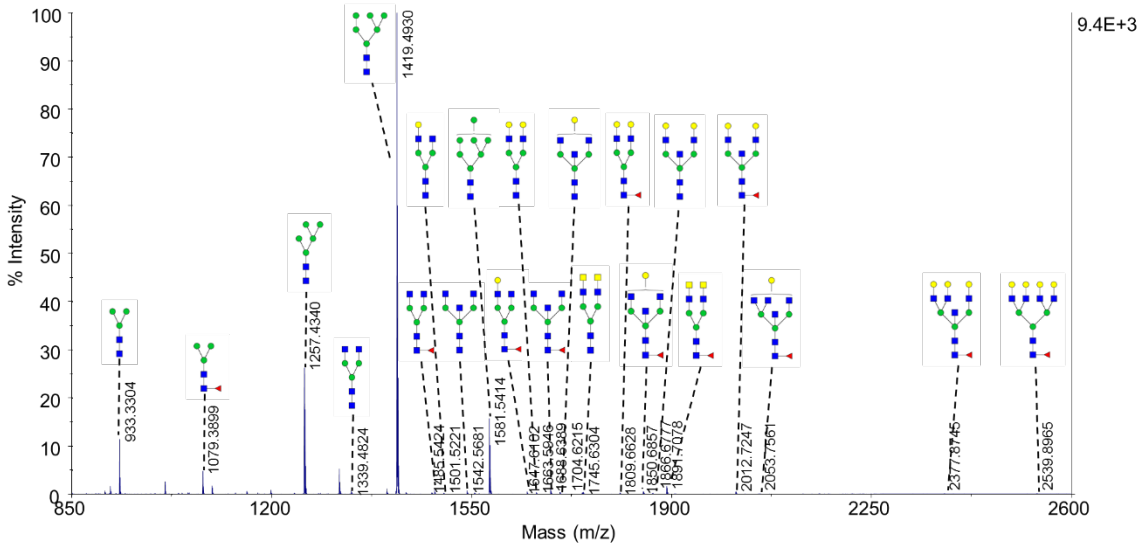
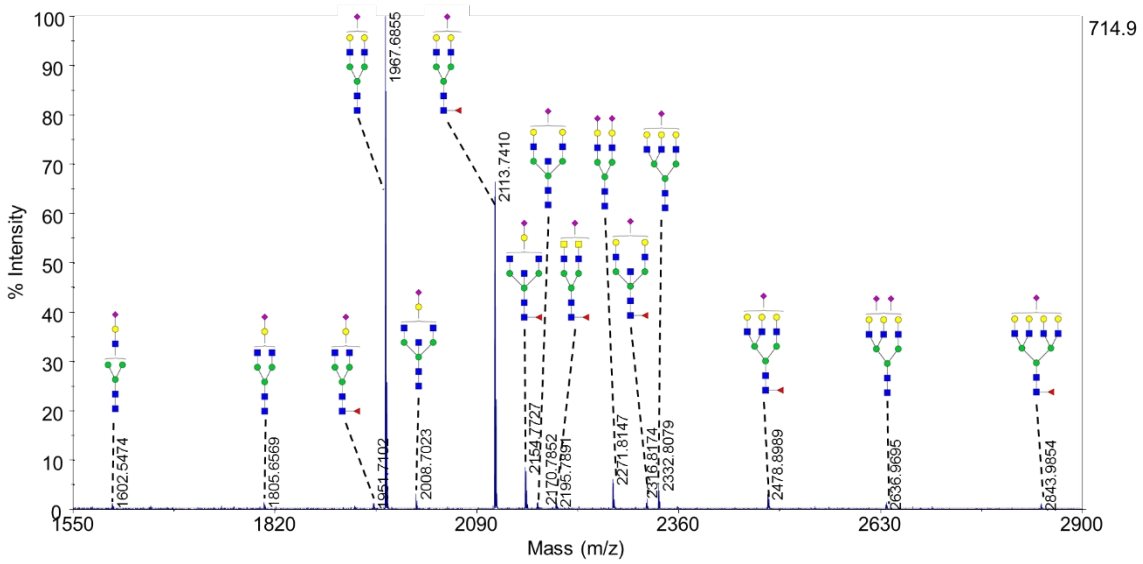


Figure S3. MALDI-MS spectrum of methylamidated N-glycans derived from urinary exosomes. Most of the previously reported N-glycan compositions of urinary exosomes observed a range of tetra-antennary N-glycans.[1] Absence of some large tetra-antennary N-glycans structures can be attributed to the following two reasons. First, as opposed to the permethylation reaction used in the previous publication, methylamidation was used here to neutralize sialic acid groups and retain the reducing ends. Consequently, methylamidated glycans ionized less efficiently than permethylated glycans and produced reduced signal intensity. Second, the relative hydrophilic nature of larger N-glycan structures can result in lower ionization efficiency[9] and further reduces the signal intensities of large N-glycans.

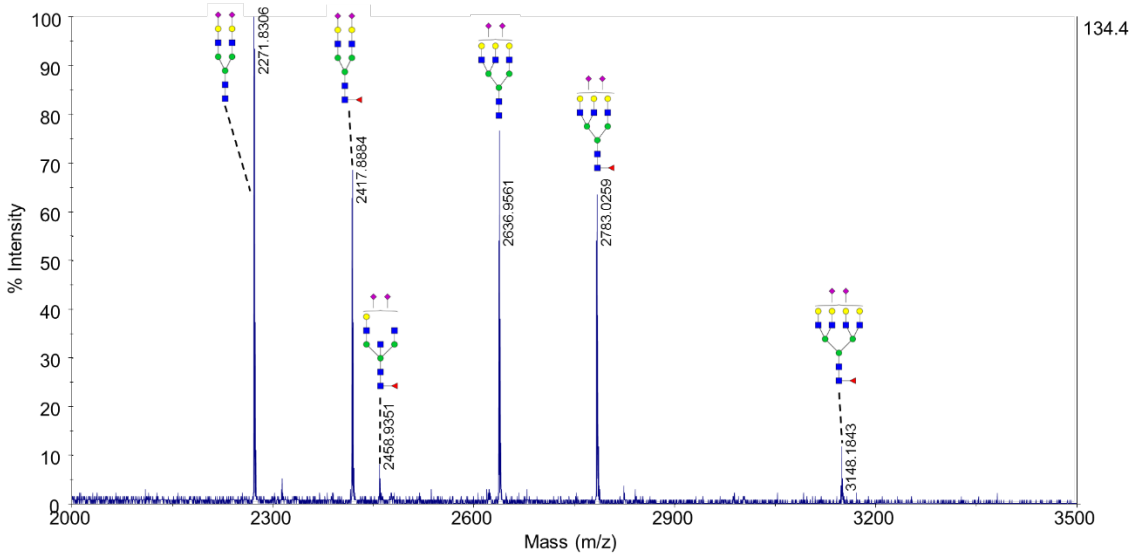
(a)


9.4E+3
% Intensity
Mass (m/z)
933.3304
1079.3899
1257.4340
1339.4824
1419.4930
1581.5414
1704.6215
1745.6304
1809.6628
2012.7247
2053.7561
2539.8965

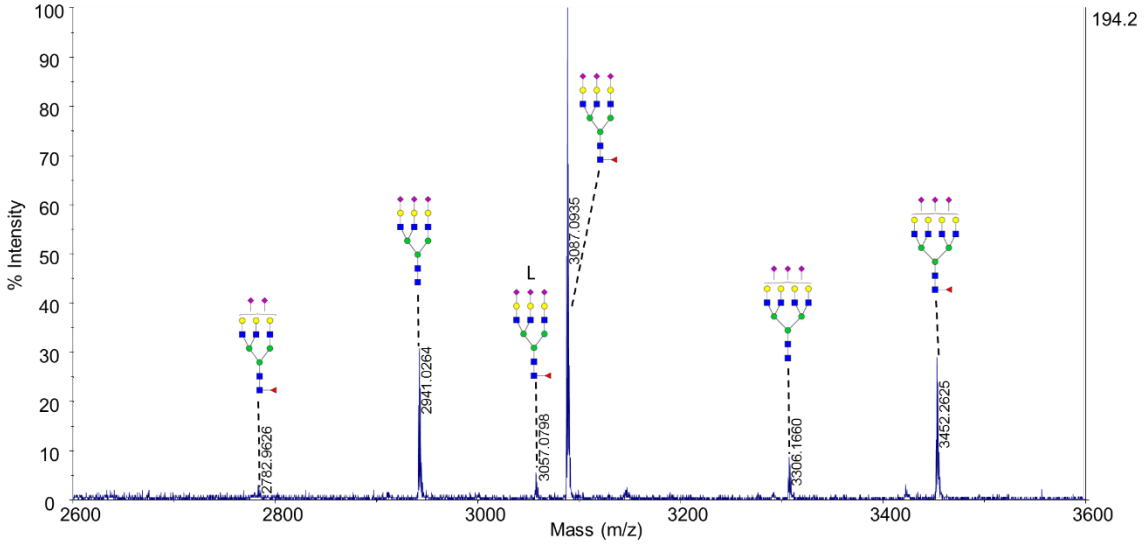
(b)


714.9
% Intensity
Mass (m/z)
1602.5474
1805.6569
1967.6855
2008.7023
2113.7410
2154.7727
2170.7852
2195.7891
2271.8147
2315.8174
2332.8079
2478.8989
2636.9695
2843.9854

(c)


134.4
% Intensity
Mass (m/z)
2271.8306
2417.8884
2458.9351
2636.9561
2783.0259
3148.1843

(d)


194.2
% Intensity
Mass (m/z)
L
2782.9626
2941.0264
3057.0798
3087.0935
3306.1660
3452.2625

(e)

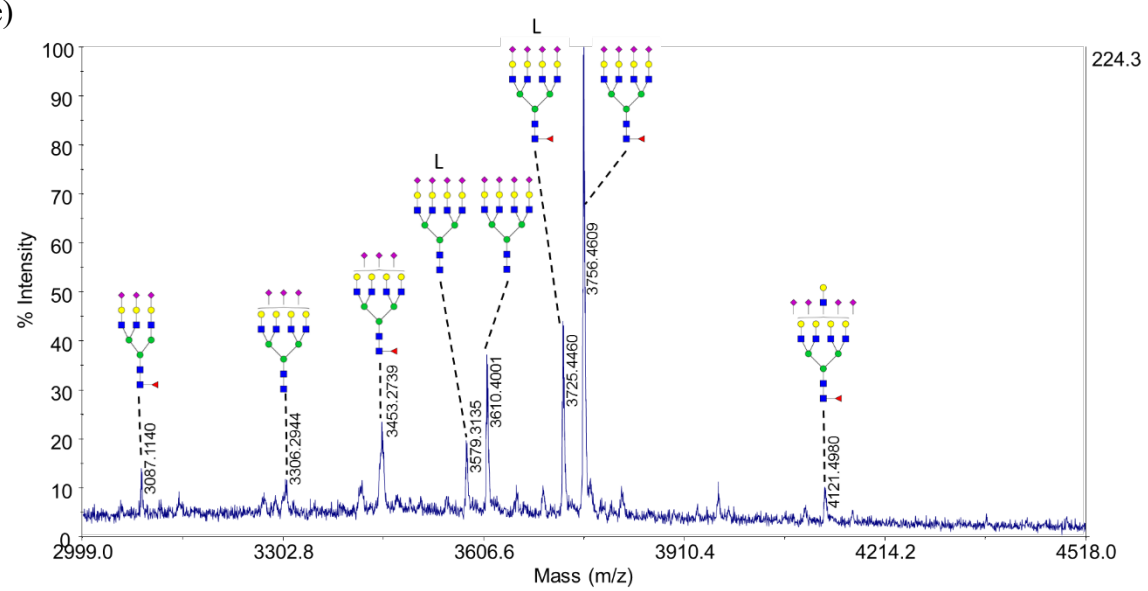
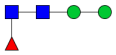
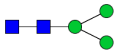
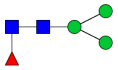
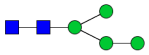
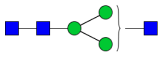
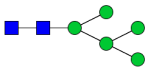
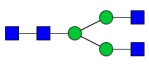
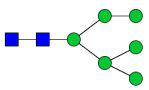
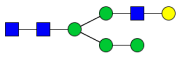
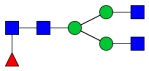
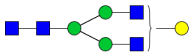
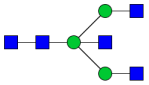
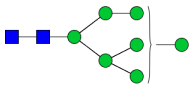
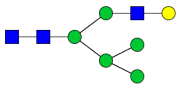
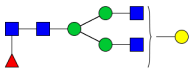
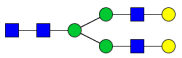
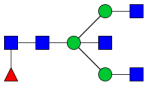
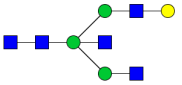
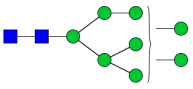
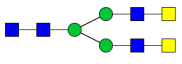
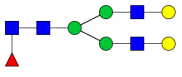
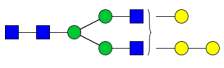
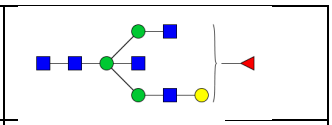
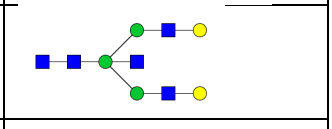
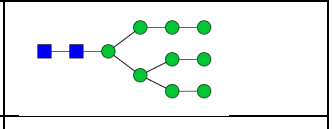
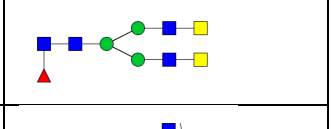
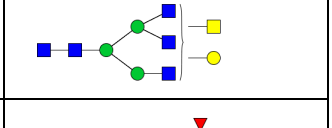
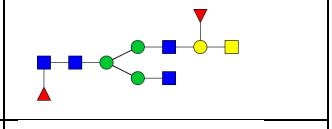
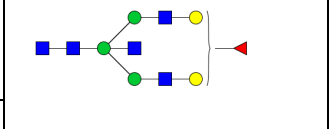
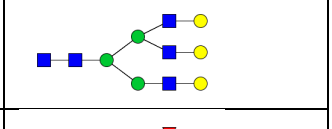
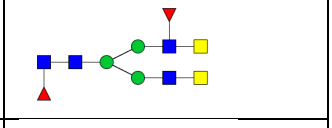
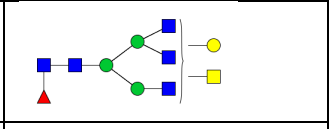
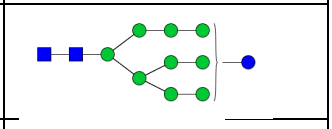
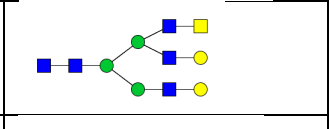
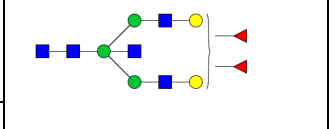


Figure S4. MALDI-MS spectra of methylamidated N-glycans fractions derived from urinary exosomes: (a) neutral fraction, (b) mono-sialylated fraction, (c) di-sialylated fraction, (d) tri-sialylated fraction, and (e) tetra-sialylated fraction. Structures annotated by L represent lactonized glycans. During the methylamidation reaction, a few α2-3 linked sialic acids formed lactones by intramolecular dehydration and were not successfully converted to the methylamide form. Because of the existence of highly α2-3 sialylated N-glycans in the tri- and tetra-sialylated fractions, some lactonized N-glycan structures were detected. These lactonized species were not identified in the electropherograms, indicating that the incomplete methylamidation reaction does not influence the structural assignment process.

Table S1. List of N-glycans structures derived from glycoproteins in human urinary exosomes and analyzed by CE-MS and LC-MS. Released native N-glycans were fractionated, methylamidated, labeled by GT reagent, and analyzed by CE-MS. Reduced native N-glycan fractions, reduced methylamidated N-glycan fractions, and reduced permethylated N-glycans fractionated through C-18 column were analyzed through LC-MS to confirm the presence of listed N-glycan compositions. Moreover, MS/MS was used to confirm the structural information of some listed N-glycans. The observed *m/z* was compared with theoretical *m/z*, and the mass measurement errors were calculated to be less than 85 ppm. H = hexose, N = N-acetylhexosamine, F = deoxyhexose, and S = sialic acid.

observed *m/z* CE-MS	theoretical *m/z* CE-MS	observed *m/z* LC-MS	theoretical *m/z* LC-MS	possible composition	diagnostic fragment ions, LC-MS	tentative structure
504.72	504.76	897.3	897.4	$H_2N_2F_1$	365.7, 527.9, 674.1, 751.1	
512.72	512.76	913.3	913.3	H_3N_2	527.9, 690.1, 751.1	
585.75	585.79	1059.4	1059.4	$H_3N_2F_1$	690.1	
593.75	593.78	707.8**	707.4	H_4N_2		
614.26	614.30			H_3N_3		
674.77	674.81	619.0	619.2	H_5N_2	294.5, 375.6, 456.7, 537.8, 751.1, 913.2	
715.80	715.84			H_3N_4		
755.80	755.84	708.7 (NH_4^+)	700.3	H_6N_2	294.6, 375.7, 456.8, 537.9, 700.1 751.1, 913.2, 1075.3	

776.31	776.35			H_5N_3		
788.83	788.87			$H_3N_4F_1$		
796.83	796.86			H_4N_4		
817.34	817.36	761.8	761.8	H_3N_5	365.7, 1116.4, 1319.5	
836.83	836.86	789.8 (NH_4^+)	781.3	H_7N_2	375.6, 456.8, 537.9, 619.0, 700.1, 781.1, 913.2, 1075.3	
857.34	857.38			H_6N_3		
869.85	869.89			$H_4N_4F_1$		
877.85	877.89	822.3	822.3	H_5N_4	365.6, 527.8, 1055.4, 1278.4	
890.37*	890.41			$H_3N_5F_1$		
898.36	898.40	842.9	842.8	H_4N_5	365.7, 1116.4, 1481.6	
917.85	917.89	870.9 (NH_4^+)	862.3	H_8N_2	456.7, 537.9, 619.0, 700.1, 781.1, 862.1, 913.2, 1075.3, 1237.4	
918.88	918.92	863.3	863.3	H_3N_6	406.7, 751.3, 913.3, 1096.4, 1319.5	
950.88	950.92	1142.2**	1141.6	$H_5N_4F_1$		
958.88	958.92			H_6N_4		
971.39	971.43	916.0*	915.85	$H_4N_5F_1$	365.6, 511.9, 1262.5, 1627.5	

		915.9*			365.6, 1262.5, 1461.5 1627.5	
979.39	979.43	924.0	923.85	H_5N_5	365.6, 1481.7	
998.88	998.91			H_9N_2		
991.91	991.95	1182.7**	1182.6	$H_3N_6F_1$		
999.91	999.94			H_4N_6		
1044.42	1044.46			$H_4N_5F_2$		
1052.42	1052.46	997.2*	996.88	$H_5N_5F_1$	365.7, 511.9, 1481.5, 1627.5	
		997.1*			365.7, 1627.7	
1060.42	1060.46			H_6N_5		
1064.94	1064.98			$H_3N_6F_2$		
1072.93	1072.97			$H_4N_6F_1$		
1079.90	1079.94			$H_{10}N_2$		
1080.93	1080.97			H_5N_6		
1125.45	1125.49	1070.2*	1069.91	$H_5N_5F_2$	365.6, 511.9, 1627.5, 1773.4	
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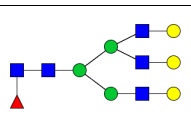
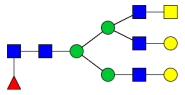
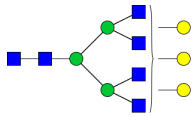
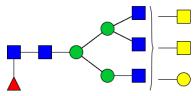
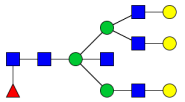
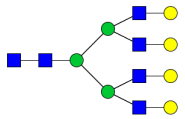
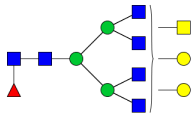
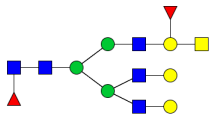
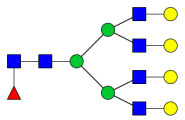
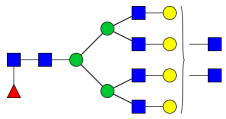
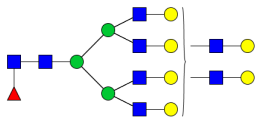
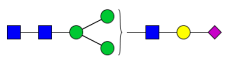
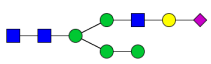
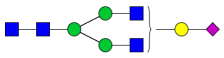
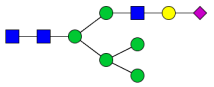
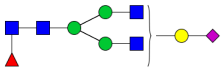
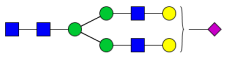
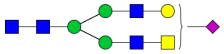
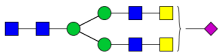
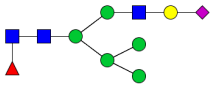
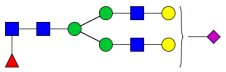
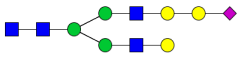
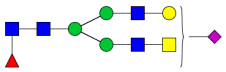
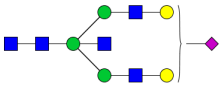
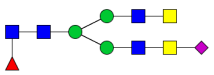
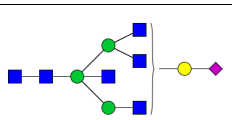
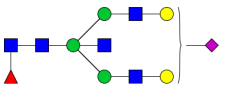
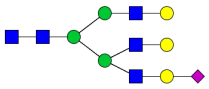
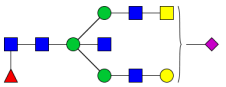
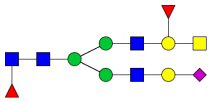
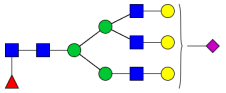
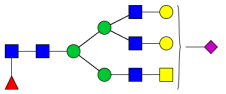
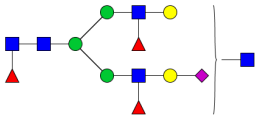
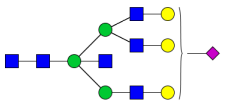
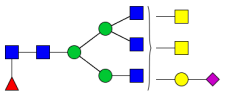
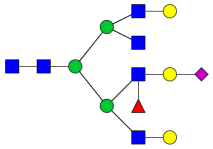
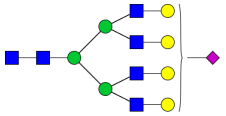
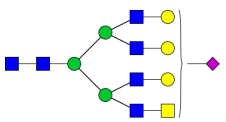
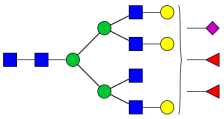
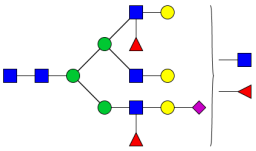
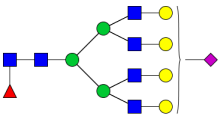
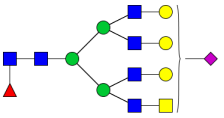
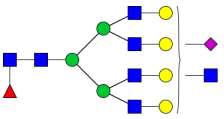
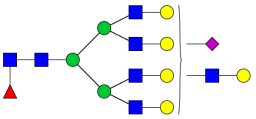
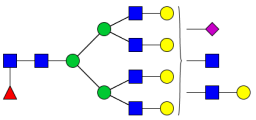
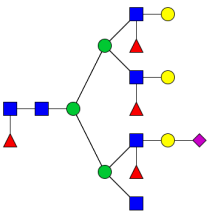
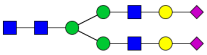
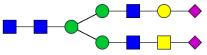
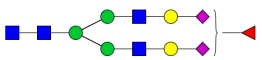
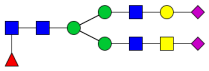
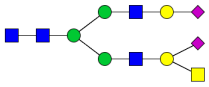
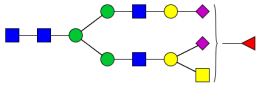
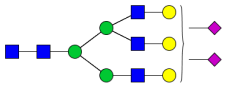
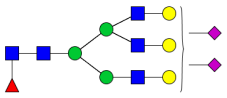
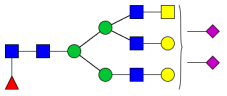
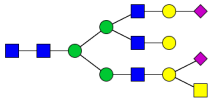
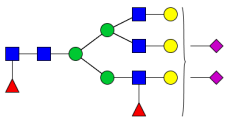
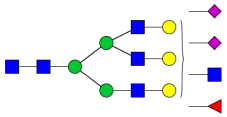
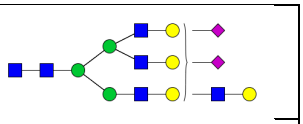
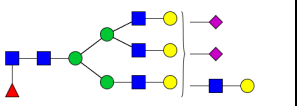
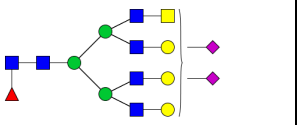
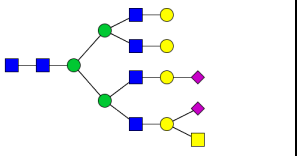
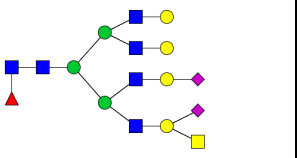
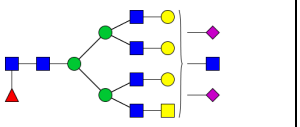
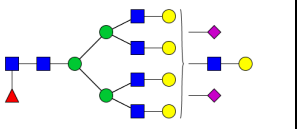
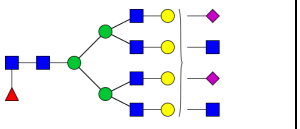
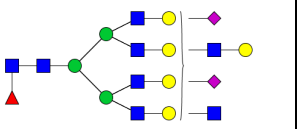
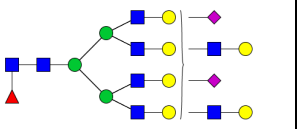
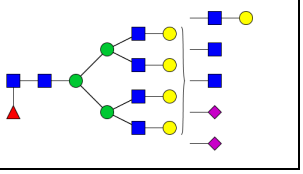
1133.45	1133.49			$H_6N_5F_1$		
1153.96	1154.00			$H_5N_6F_1$		
1161.96	1162.00			H_6N_6		
1174.47	1174.51	1408.6**	1407.2	$H_4N_7F_1$		
1234.99	1235.03			$H_6N_6F_1$		
1242.98	1243.02			H_7N_6		
1255.5	1255.54			$H_5N_7F_1$		
1308.02	1308.05			$H_6N_6F_2$		
1316.01	1316.05			$H_7N_6F_1$		
1013.07	1013.09			$H_7N_8F_1$		
1121.10	1121.12			$H_9N_8F_1$		
847.35	847.38			$H_4N_3S_1$		
928.38	928.40	866.7	866.3	$H_5N_3S_1$	365.6, 657.0, 1075.4	

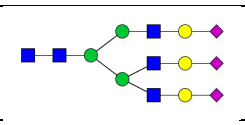
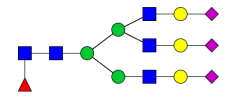
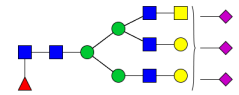
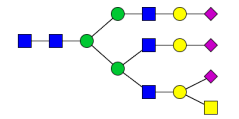
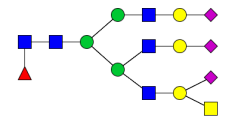
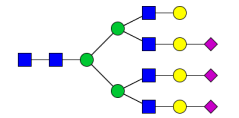
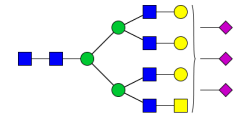
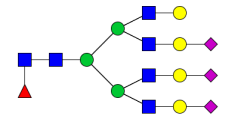
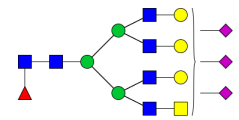
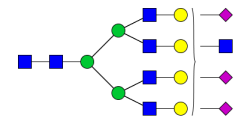
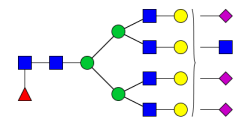
948.89	948.92			$H_4N_4S_1$		
1009.40	1009.43			$H_6N_3S_1$		
1021.92	1021.94			$H_4N_4F_1S_1$		
1029.92	1029.94	968.0* 968.1*	967.9	$H_5N_4S_1$	365.6, 657.0, 1278.5 365.6, 657.0, 1278.4	
1050.43	1050.46			$H_4N_5S_1$		
1070.94	1070.97			$H_3N_6S_1$		
1082.43	1082.46			$H_6N_3F_1S_1$		
1102.94	1102.97	1041.2* 1041.1*	1040.9	$H_5N_4F_1S_1$	365.6, 657.0, 819.3, 968.0, 1424.5, 1711.5 365.6, 657.0, 1424.5, 1711.5	
1110.94	1110.97			$H_6N_4S_1$		
1123.46	1123.46	1067.9*** 1068.0***	1067.9	$H_4N_5F_1S_1$	365.6, 406.6, 1765.4	
1131.46	1131.48			$H_5N_5S_1$		
1143.97	1144.00	1088.6***	1088.4	$H_3N_6F_1S_1$	406.6, 711.1, 936.3, 1059.3, 1465.4, 1668.4, 1769.4	

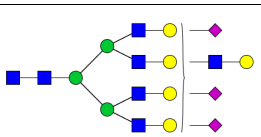
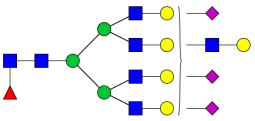
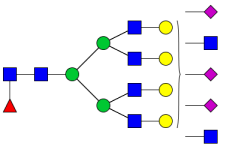
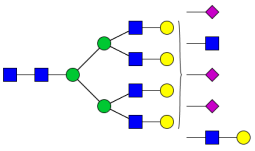
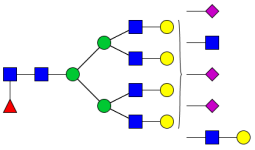
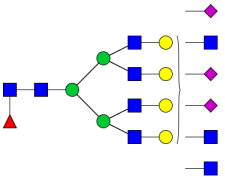
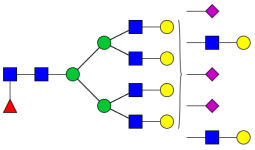
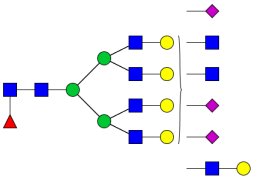
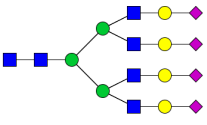
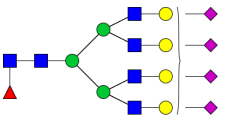
1151.97	1151.99			$H_4N_6S_1$		
1204.48	1204.51			$H_5N_5F_1S_1$		
1212.48	1212.51	1050.8	1150.4	$H_6N_5S_1$	365.6, 657.0, 1116.4, 1184.3	
1225.00	1225.02			$H_4N_6F_1S_1$		
1277.51	1277.54			$H_5N_5F_2S_1$		
1285.51	1285.54	820.5***	820.3	$H_6N_5F_1S_1$	365.6, 670.0, 893.1, 1047.7, 1078.1, 1262.3, 1789.3	
1306.02	1306.05			$H_5N_6F_1S_1$		
900.71	900.70	863.9***	863.7	$H_5N_5F_3S_1$	365.5, 511.8, 670.0, 816.0, 1143.0, 1408.3, 1773.3, 1919.3	
1314.02	1314.05			$H_6N_6S_1$		
1326.54	1326.56			$H_4N_7F_1S_1$		
925.04	925.05	884.1	883.66	$H_6N_6F_1S_1$	365.8, 657.0, 803.2, 1319.5, 1918.4	
930.37	930.39			$H_7N_6S_1$		
944.04	944.06			$H_6N_7S_1$		

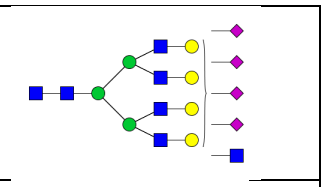
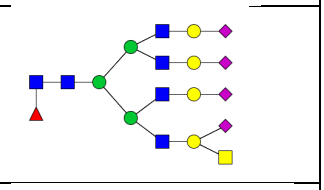
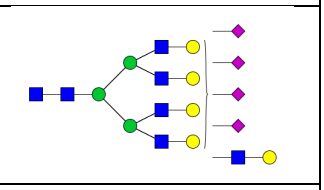
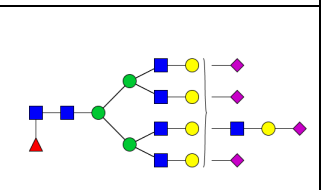
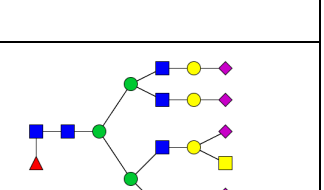
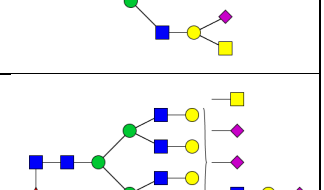
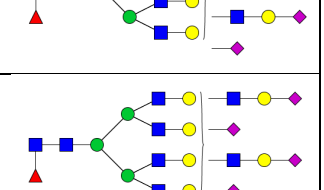
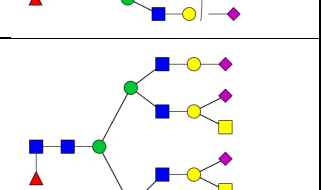
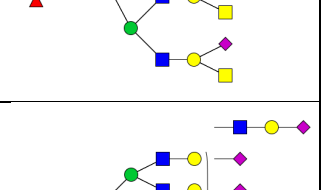
973.72	973.74	932.7* 932.6*	932.4	$H_6N_6F_2S_1$	365.6, 657.0, 803.2, 1182.8, 1627.5 365.6, 657.0, 803.2, 1627.5	
1022.41	1022.42	985.6***	985.4	$H_6N_6F_3S_1$	365.5, 511.8, 670.0, 816.1, 1070.2, 1252.8, 1325.8, 1627.4, 1773.4, 2139.3	
979.05	979.07	942.4*** 942.2***	942.0	$H_7N_6F_1S_1$	365.6, 670.0, 2154.3	
992.73	992.75			$H_6N_7F_1S_1$		
1046.75	1046.76			$H_7N_7F_1S_1$		
1100.77	1100.78			$H_8N_7F_1S_1$		
1168.46	1168.47			$H_8N_8F_1S_1$		
1071.11	1071.09	1034.3***	1034.1	$H_6N_6F_3S_1$	365.6, 511.8, 670.0, 816.1, 1143.3, 1773.4, 1920.3, 2139.3	
788.32	788.33	1113.7*	1113.4	$H_5N_4S_2$	365.6, 657.0, 1569.4	
802.00	802.01	1134.3	1133.9	$H_4N_5S_2$	365.6, 406.8, 657.0, 698.1, 1569.4, 1610.6	

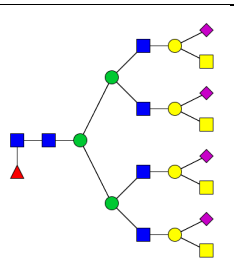
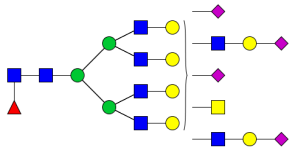
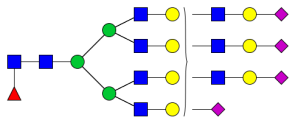
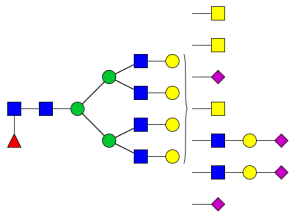
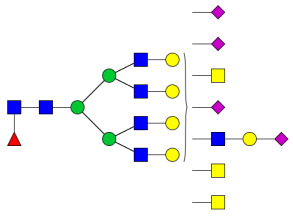
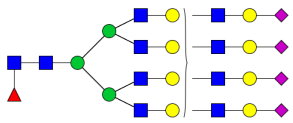
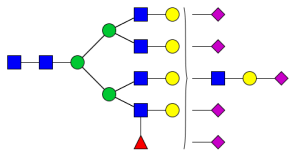
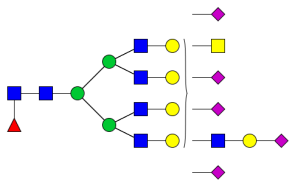
837.01	837.02	1186.8*	1186.4	$H_5N_4F_1S_2$	365.6, 657.0, 1715.4	
850.68	850.69	1207.3	1206.9	$H_4N_5F_1S_2$	365.6, 406.8, 657.0, 698.1, 1715.5, 2043.4	
		1215.3	1214.9	$H_5N_5S_2$	365.6, 657.0, 860.2, 1569.4, 1772.4	
904.70	904.71	1288.4	1288.0	$H_5N_5F_1S_2$	365.6, 657.0, 860.2, 1715.4, 1918.4	
910.03	910.04	864.8*	864.3	$H_6N_5S_2$	365.6, 657.0, 1475.4	
		864.6*			365.6, 657.0	
		1304.9 (NH_4^+)*	1296.0		365.6, 657.0, 1475.3	
958.72	958.73	913.4*	913.0	$H_6N_5F_1S_2$	365.6, 657.0, 1262.2	
		913.3*			365.6, 657.0, 1262.2, 1475.5	
972.39	972.40			$H_4N_5F_1S_2$		
		932.3	932.0	$H_6N_6S_2$	365.6, 657.0, 860.2	
1007.40	1007.42			$H_6N_5F_2S_2$		
1026.41	1026.42	981.3*	980.7	$H_6N_6F_1S_2$	365.6, 657.0, 803.2, 1919.3	
		981.1*			365.6, 657.0, 860.2	
1031.74	1031.75	986.4 *	986.0	$H_7N_6S_2$	365.6, 657.0, 1296.3, 1475.3	

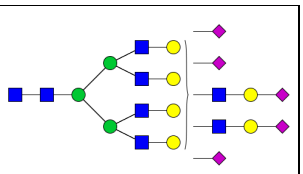
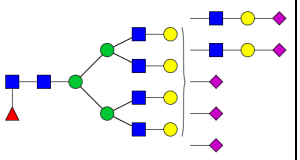
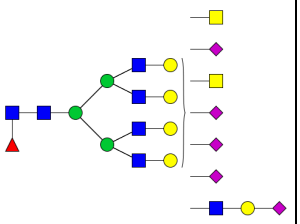
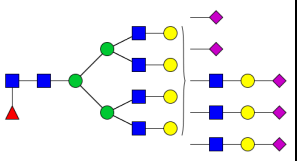
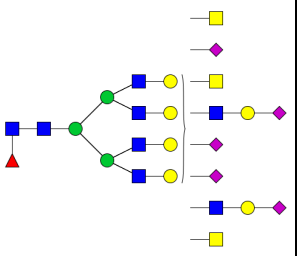
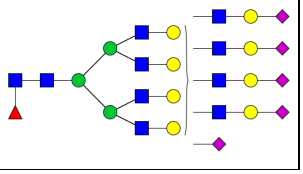
					365.6, 657.0, 1022.2	
1080.43	1080.44	1035.1*	1034.7	$H_7N_6F_1S_2$	365.5, 657.0, 1022.3, 1262.3	
1094.11	1094.11			$H_6N_7F_1S_2$		
1099.44	1099.45	1054.1	1053.7	$H_7N_7S_2$	365.6, 657.0, 860.2, 1397.6	
1148.12	1148.13	1102.9* 1102.8*	1102.4	$H_7N_7F_1S_2$	365.6, 657.0, 860.2, 1678.4	
1161.80	1161.81			$H_6N_8F_1S_2$		
1202.14	1202.15			$H_8N_7F_1S_2$		
1215.82	1215.82			$H_7N_8F_1S_2$		
1269.83	1269.84			$H_8N_8F_1S_2$		
1323.85	1323.86			$H_9N_8F_1S_2$		
1337.53	1337.54			$H_8N_9F_1S_2$		

1011.41	1011.41	961.3*	961.7	$H_6N_5S_3$	365.6, 657.0	
1060.09	1060.10	1010.4*	1010.0	$H_6N_5F_1S_3$	365.6, 657.0, 2002.3	
1073.77	1073.77			$H_5N_6F_1S_3$		
1079.10	1079.10	1029.5*	1029.0	$H_6N_6S_3$	365.5, 657.0, 860.1, 1407.3	
1127.79	1127.79	1077.7*	1078.6	$H_6N_6F_1S_3$	365.6, 657.0,860.1	
1133.12	1133.12	1083.5* 1083.4*	1083.1	$H_7N_6S_3$	365.6, 657.0, 1295.9, 1935.3	
1146.80	1146.78			$H_6N_7S_3$		
1181.80	1181.81	1132.1*	1131.7	$H_7N_6F_1S_3$	365.6, 657.0, 1369.3, 2081.3	
1195.48	1195.48			$H_6N_7F_1S_3$		
1200.81	1200.81			$H_7N_7S_3$		
1249.50	1249.50			$H_7N_7F_1S_3$		

1254.83	1254.83			$H_8N_7S_3$		
1303.51	1303.52			$H_8N_7F_1S_3$		
1317.19	1317.19			$H_7N_8F_1S_3$		
1322.52	1322.52			$H_8N_8S_3$		
1371.21	1371.21			$H_8N_8F_1S_3$		
1384.88	1384.89			$H_7N_9F_1S_3$		
1425.23	1425.23			$H_9N_8F_1S_3$		
1438.90	1438.90			$H_8N_9F_1S_3$		
1234.49	1234.49	1180.5	1180.1	$H_7N_6S_4$	365.6, 657.0, 1442.0	
1283.18	1283.17	1229.3	1228.8	$H_7N_6F_1S_4$	365.6, 657.0, 1515.2	

1302.19	1302.18			$H_7N_7S_4$		
1350.87	1350.87	1632.8**	1631.5	$H_7N_7F_1S_4$		
1356.20	1356.20			$H_8N_7S_4$		
1404.89	1404.88	1026.3*** 1026.4***	1205.6	$H_8N_7F_1S_4$		
1418.56	1418.56	1714.4**	1713.2	$H_7N_8F_1S_4$		
1472.58	1472.58	1782.0**	1781.2	$H_8N_8F_1S_4$		
1145.20	1145.20	1850.5	1849.2	$H_9N_8F_1S_4$		
1486.26	1486.25	1795.9**	1794.9	$H_7N_9F_1S_4$		
1155.46	1155.47			$H_8N_9F_1S_4$		

1165.71	1165.71	1877.2**	1876.6	$H_7N_{10}F_1S_4$		
1195.97	1195.99			$H_9N_9F_1S_4$		
1236.48	1236.50			$H_{10}N_9F_1S_4$		
1297.51	1297.53			$H_9N_{11}F_1S_4$		
1206.23	1206.24			$H_8N_{10}F_1S_4$		
1327.78	1327.78			$H_{11}N_{10}F_1S_4$		
1129.95	1129.96			$H_8N_7F_1S_5$		
1180.72	1180.73			$H_8N_8F_1S_5$		

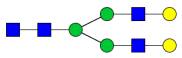
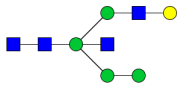
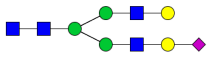
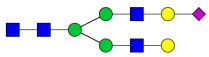
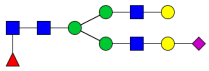
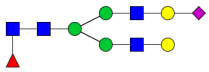
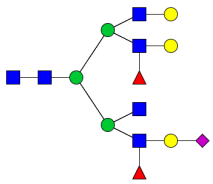
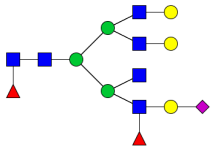
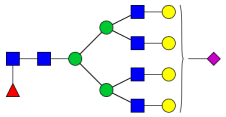
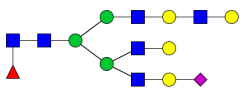
1184.72	1184.73			$H_9N_8S_5$		
1221.23	1221.25			$H_9N_8F_1S_5$		
1231.49	1231.50			$H_8N_9F_1S_5$		
1312.52	1312.53			$H_{10}N_9F_1S_5$		
1373.53	1373.56			$H_9N_{11}F_1S_5$		
1403.80	1403.81			$H_{11}N_{10}F_1S_5$		

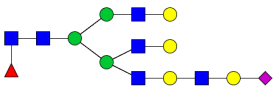
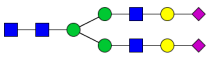
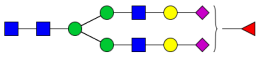
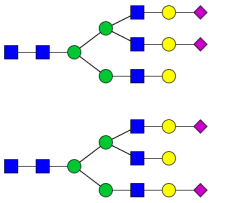
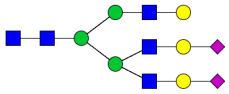
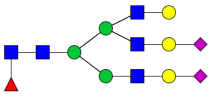
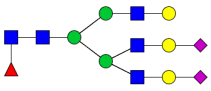
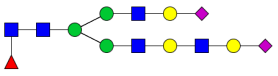
*Multiple peaks of the same possible composition were detected in the LC runs

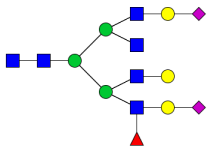
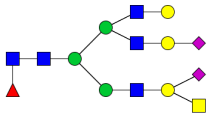
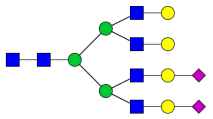
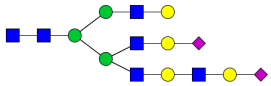
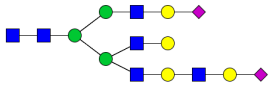
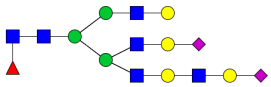
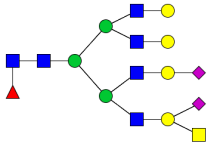
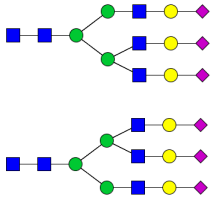
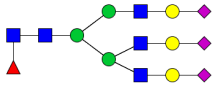
**Possible compositions that were only detected in the permethylated N-glycan sample

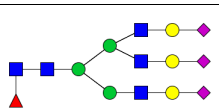
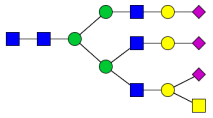
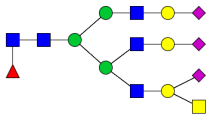
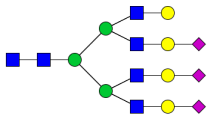
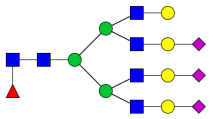
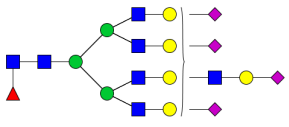
***Possible compositions that were only detected in the methylamidated N-glycan sample

Table S2. List of isomeric N-glycan structures derived from glycoproteins in human urinary exosomes and detected by LC-MS. Isomers were resolved through LC-MS and potential structures were proposed with the assistance of MS/MS analysis. H = hexose, N = N-acetylhexosamine, F = deoxyhexose, and S = sialic acid.

observed *m/z* in LC-MS	theoretical *m/z* in LC-MS	retention time (min)	possible composition	diagnostic fragment ions, LC-MS	tentative structures
822.3	822.3	87.4	H_5N_4	365.6, 527.8, 1055.4, 1278.4	
822.3		79.5		365.6, 1116.4, 1278.5, 1440.5	
968.1	967.9	121.9, 98.4	$H_5N_4S_1$	365.6, 657.0, 1278.5	
968.0		126.6, 108.8		365.6, 657.0, 1278.4	
1041.2	1040.9	124.3, 104.4	$H_5N_4F_1S_1$	365.6, 657.0, 819.3, 968.0, 1424.5, 1711.5	
1041.1		128.9		365.6, 657.0, 1424.5, 1711.5	
932.7	932.4	117.9	$H_6N_6F_2S_1$	365.6, 657.0, 803.2, 1182.8, 1627.5	
932.6		120.9		365.6, 657.0, 803.2, 1627.5	
942.4	942.0	101.8	$H_7N_6F_1S_1$	365.6, 670.0, 893.1, 1077.9, 1230.3, 1260.8, 1789.8, 2154.3	
942.2		107.8		365.6, 670.0, 731.0, 1078.1, 1258.3, 1424.3, 1789.3, 2154.3	

942.2		119.1		365.5, 670.0, 731.0, 1035.3, 1260.7, 1424.3, 1790.3, 2154.3	
1113.7	1113.4	131.5, 109.3	$H_5N_4S_2$	365.6, 657.0, 1569.4	
		137.6, 120.1			
		128.4			
1186.8	1186.4	139.9, 114.8	$H_5N_4F_1S_2$	365.6, 657.0, 1715.4	
		124.8			
		132.7			
864.8	864.3	141.1, 125.6	$H_6N_5S_2$	365.6, 657.0, 1475.4	
864.6		136.8,120.3		365.6, 657.0	
1304.9 (NH_4^+)	1296.0	120.6		365.6, 657.0, 1475.3	
		125.4			
913.4	913.0	123.9	$H_6N_5F_1S_2$	365.6, 657.0, 1262.2	
913.3		138.6, 129.1		365.6, 657.0, 1262.2, 1475.5	
921.9	921.7	120.4		365.5, 670.0, 1145.2, 1382.4, 1565.2, 1789.2	

981.3	980.7	120.2	$H_6N_6F_1S_2$	365.6, 657.0, 803.2, 1919.3	
981.1		135.5, 118.6		365.6, 657.0, 860.2	
986.4	986.0	134.7, 117.9	$H_7N_6S_2$	365.6, 657.0, 1296.3, 1475.3	
		141.1, 123.5			
		145.6, 135.8		365.6, 657.0, 1022.2	
		141.5			
1035.1*	1034.7	138.4	$H_7N_6F_1S_2$	365.5, 657.0, 1022.3, 1262.3	
		143.2			
1102.9	1102.4	116.5	$H_7N_7F_1S_2$	365.6, 657.0, 860.2, 1678.4	
1102.8		122.1			
961.3	961.7	185.8	$H_6N_5S_3$	365.6, 657.0	
		187.6			
1010.4	1010.0	175.1	$H_6N_5F_1S_3$	365.6, 657.0, 2002.3	
		181.3			

		189.1			
1029.5	1029.0	173.3 177.5	$H_6N_6S_3$	365.5, 657.0, 860.1, 1407.3	
1077.7	1078.6	174.0 177.5	$H_6N_6F_1S_3$	365.6, 657.0,860.1	
1083.5 1083.4	1083.1	175.8, 161.1 154.5 156.2	$H_7N_6S_3$	365.6, 657.0, 1295.9, 1935.3	
1132.1	1131.7	156.0 157.8 176.3, 160.3 185.8, 162.8 187.1, 164.6	$H_7N_6F_1S_3$	365.6, 657.0, 1369.3, 2081.3	
1026.3 1026.4	1025.61	123.1 130.2	$H_8N_7F_1S_4$		

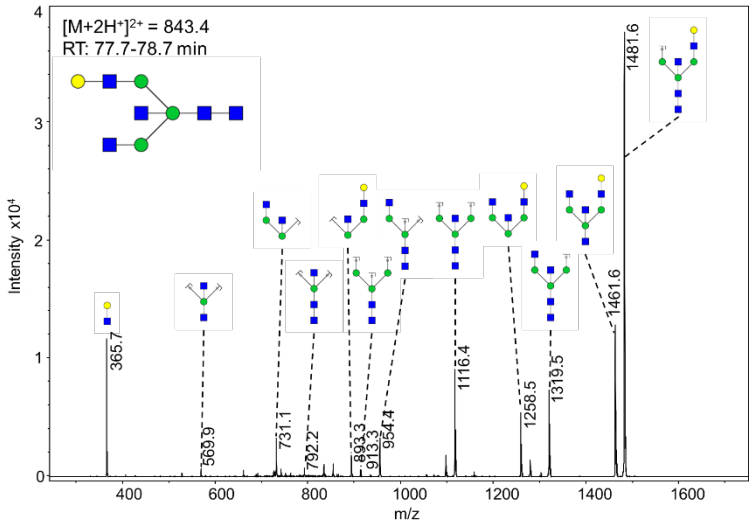


Figure S5. MS/MS spectrum of the molecular ion *m/z* 843.4 $[M+2H]^{2+}$ eluting 77.7–78.7 min from the PGC column and representing a bisected N-glycan. Several diagnostic ions indicate a bisecting GlcNAc, including *m/z* 792.2.

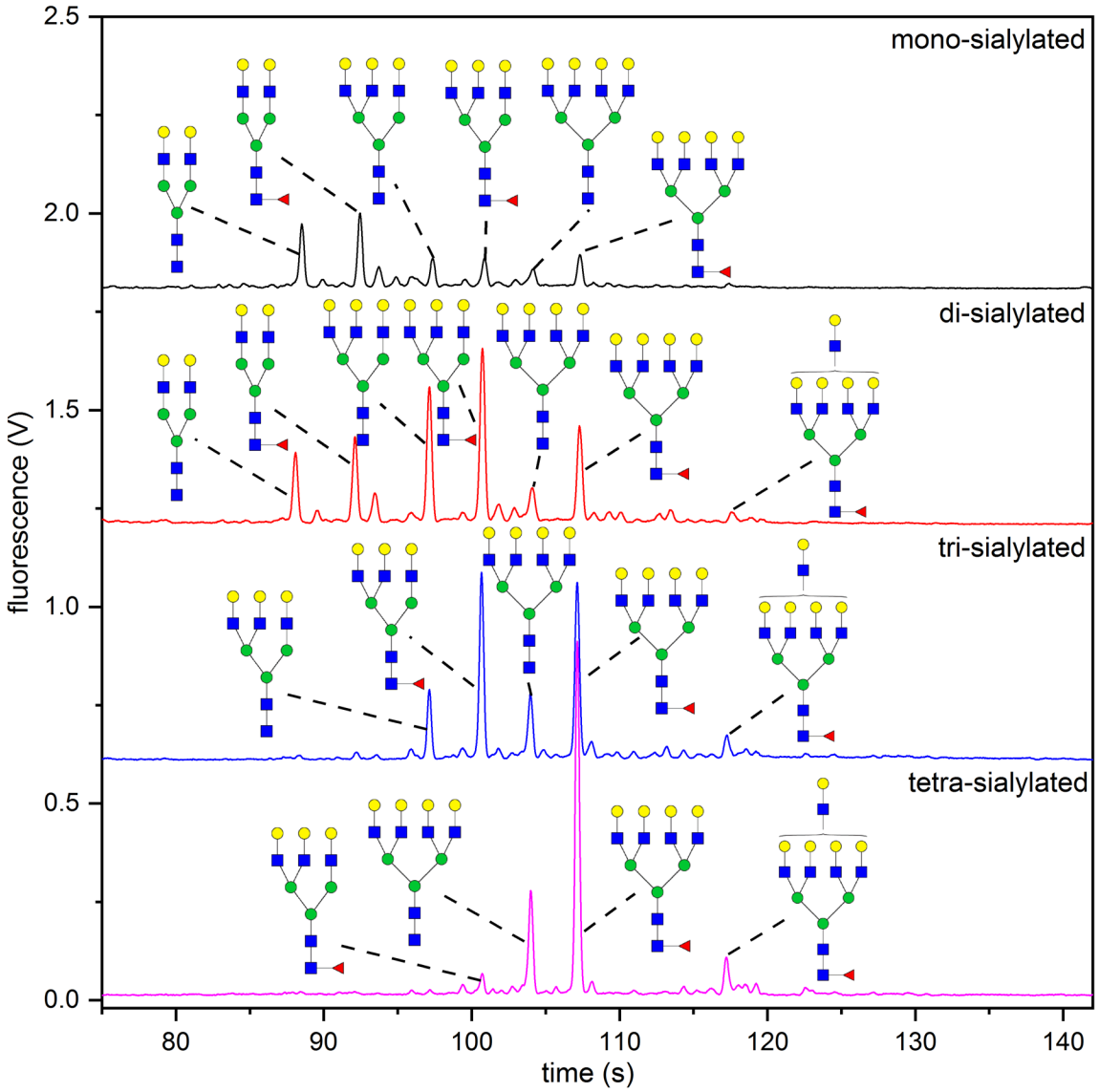


Figure S6. Electropherograms of α2-3,6,8 sialidase-treated N-glycans fractions of urinary exosomes. Structures were assigned with the assistance of MALDI-TOF-MS. By releasing all the terminal sialic acid groups, the complexity of profiles was reduced significantly.

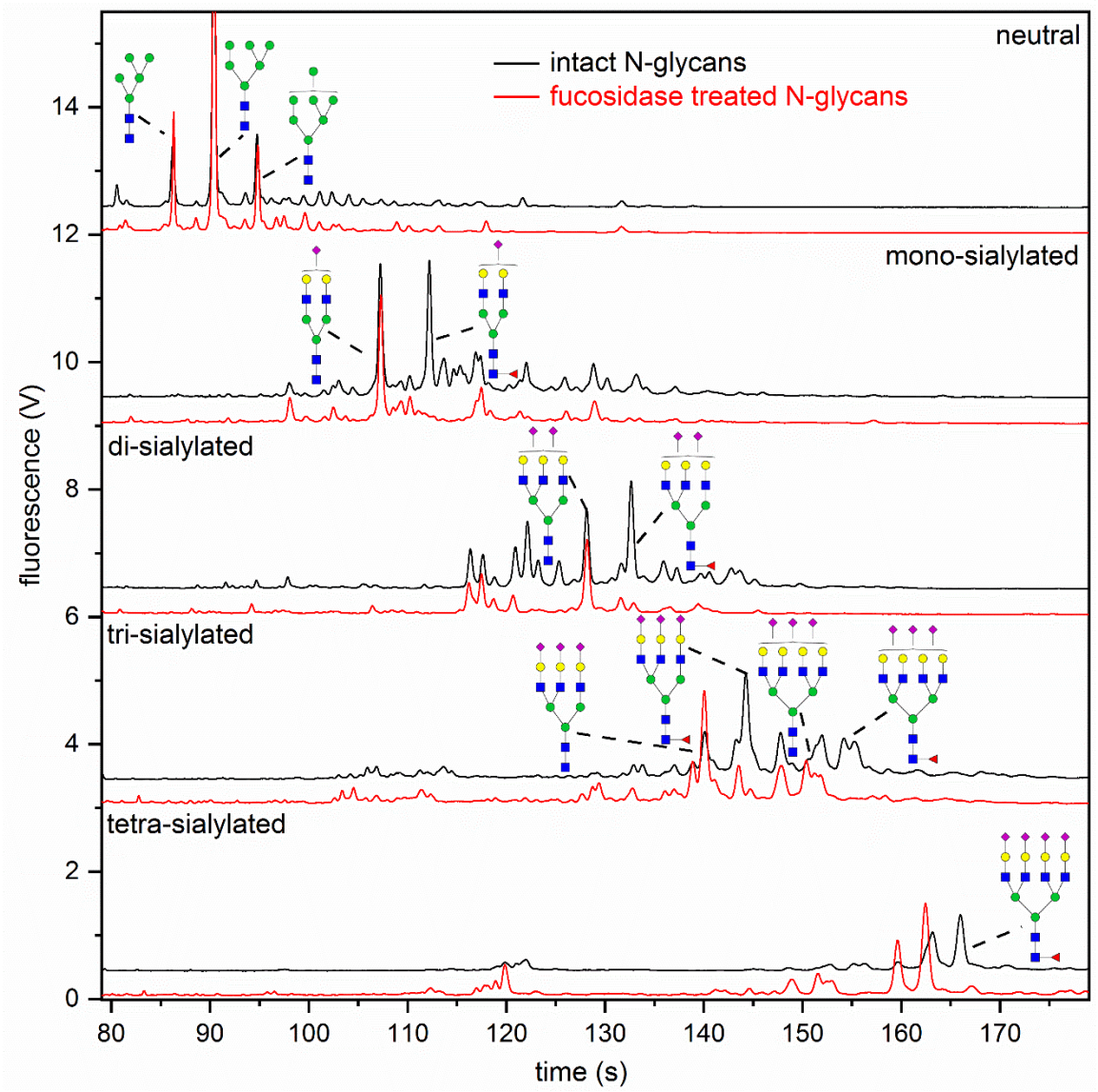
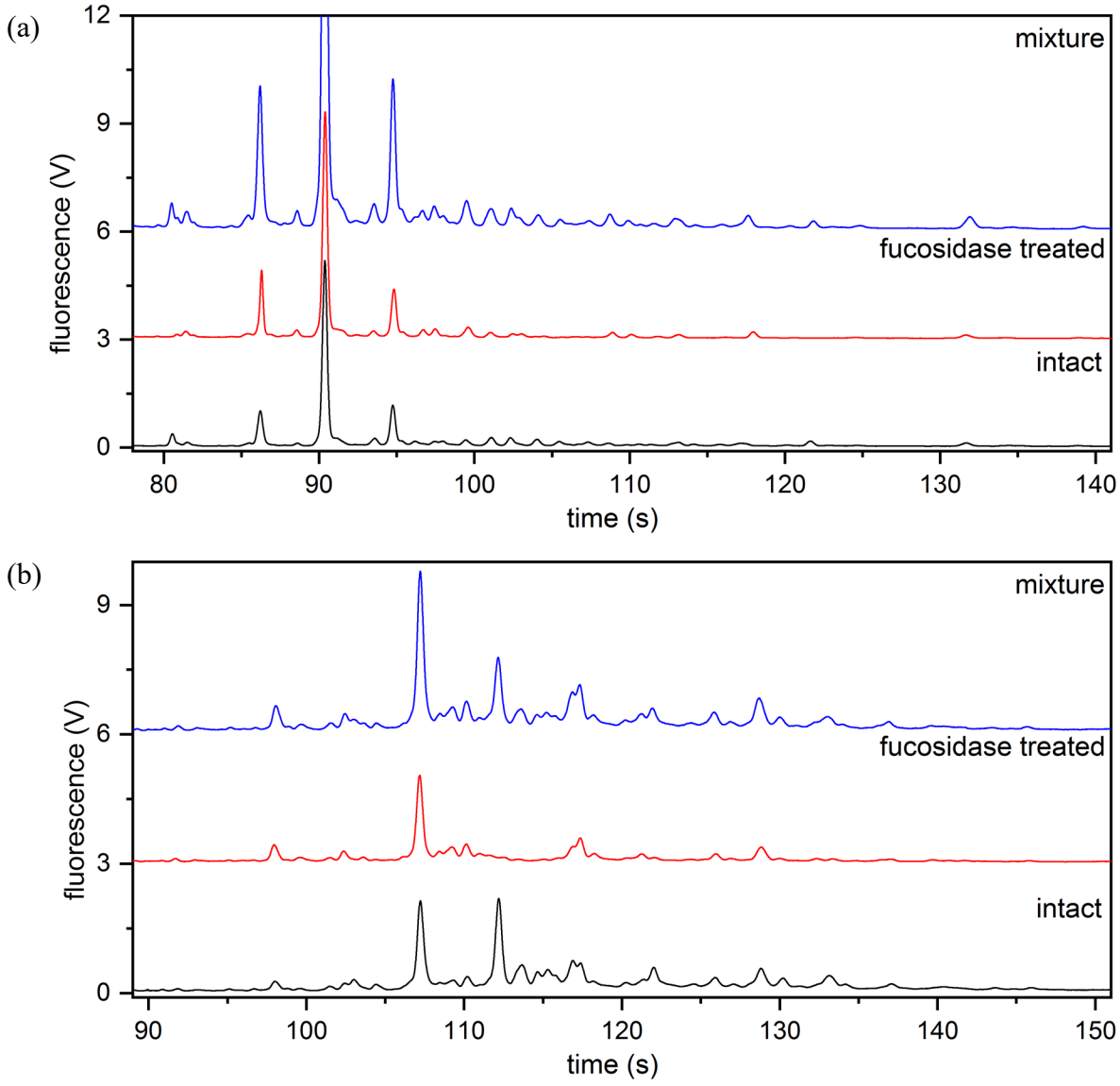
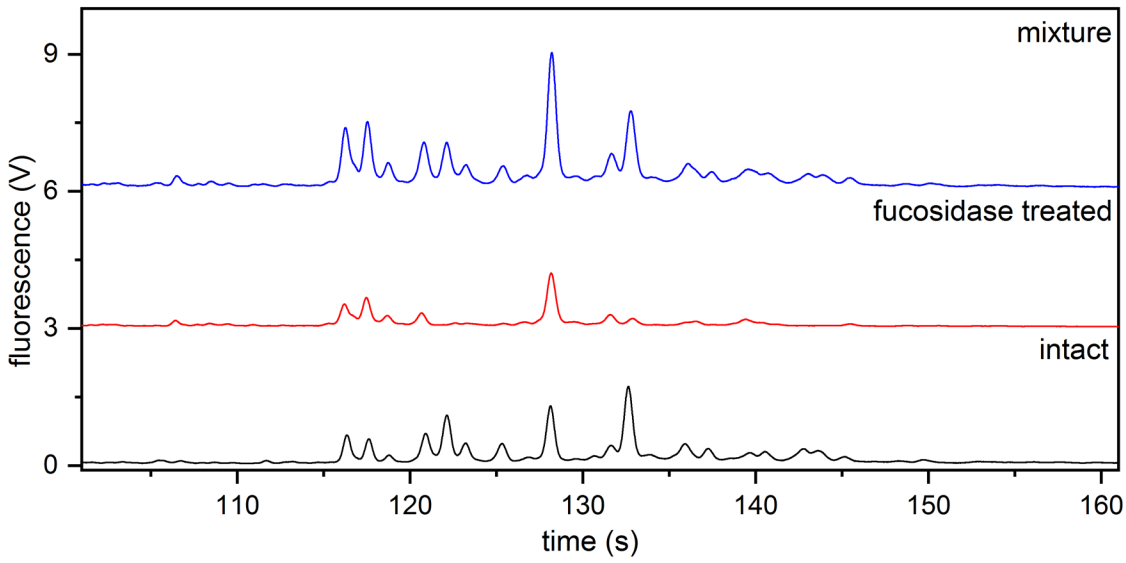


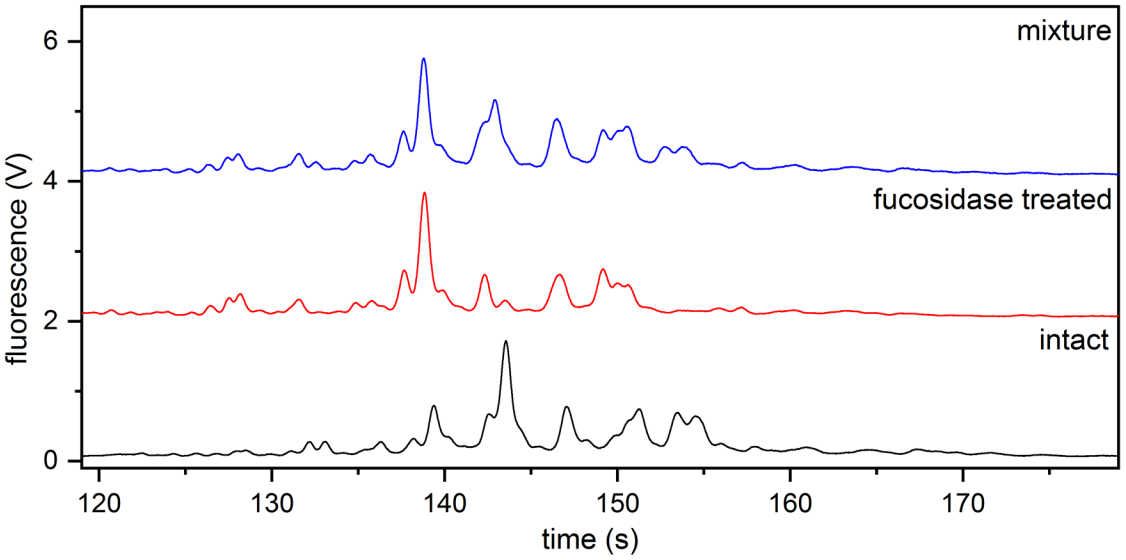
Figure S7. Electropherograms of intact and fucosidase-treated N-glycan fractions. For the alignment of electropherograms, each intact N-glycan fraction was mixed with corresponding fucosidase-treated N-glycan fraction for the electrophoretic mobility matching. Structures were assigned with the assistance of MALDI-MS.


(a)
mixture
fucosidase treated
intact
fluorescence (V)
time (s)
(b)
mixture
fucosidase treated
intact
fluorescence (V)
time (s)

(c)


mixture
fucosidase treated
intact
fluorescence (V)
time (s)
0
3
6
9
110
120
130
140
150
160

(d)


mixture
fucosidase treated
intact
fluorescence (V)
time (s)
0
2
4
6
120
130
140
150
160
170

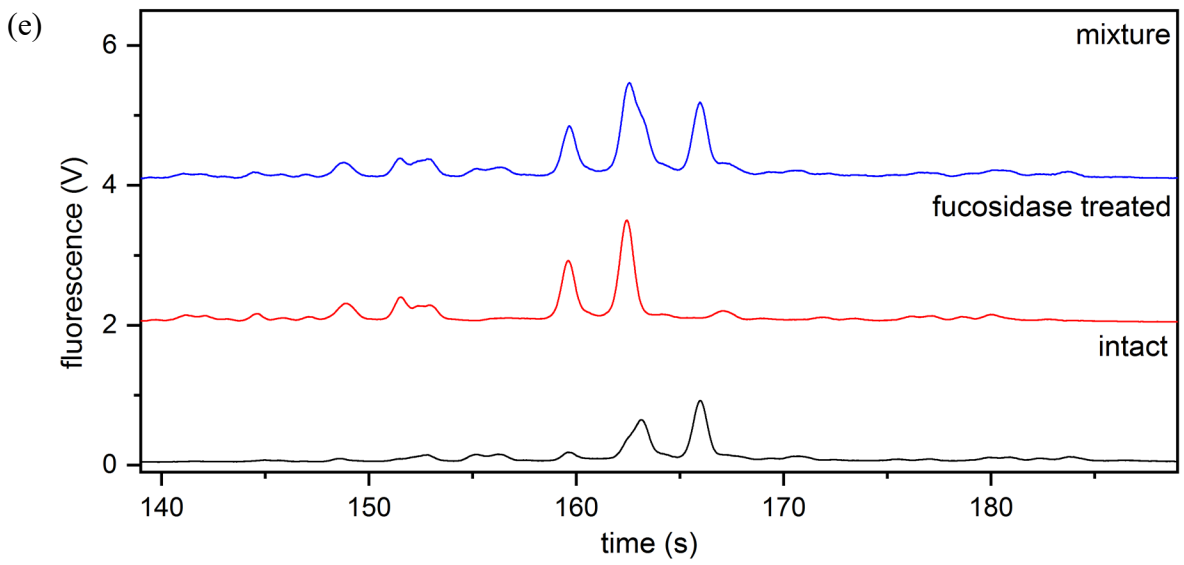


Figure S8. Electropherograms of intact and fucosidase-treated N-glycan fractions of urinary exosomes: (a) mono-sialylated, (b) di-sialylated, (c) tri-sialylated, and (d) tetra-sialylated fractions. Intact and fucosidase-treated N-glycan samples were mixed together for the alignment of electropherograms.

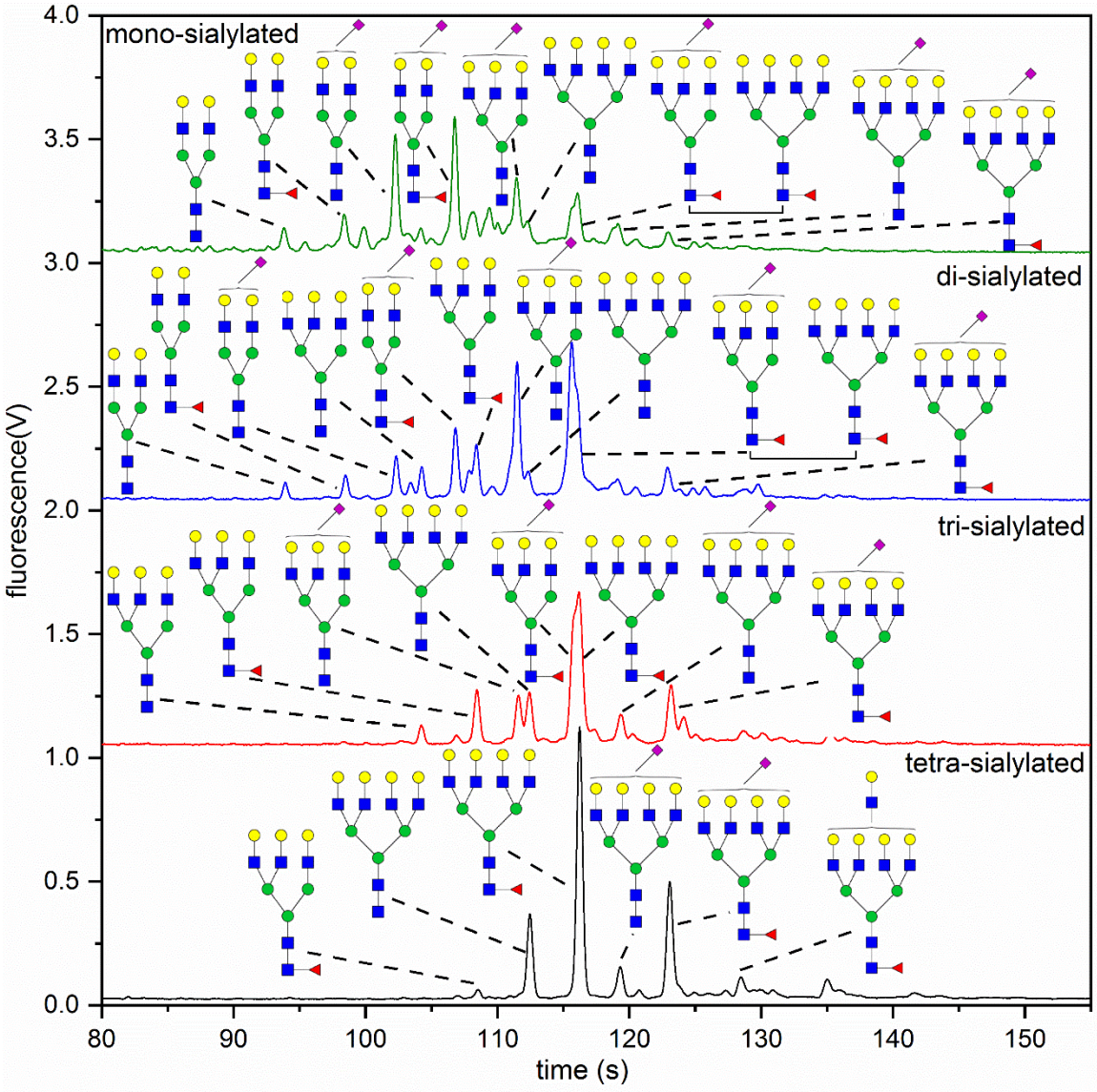
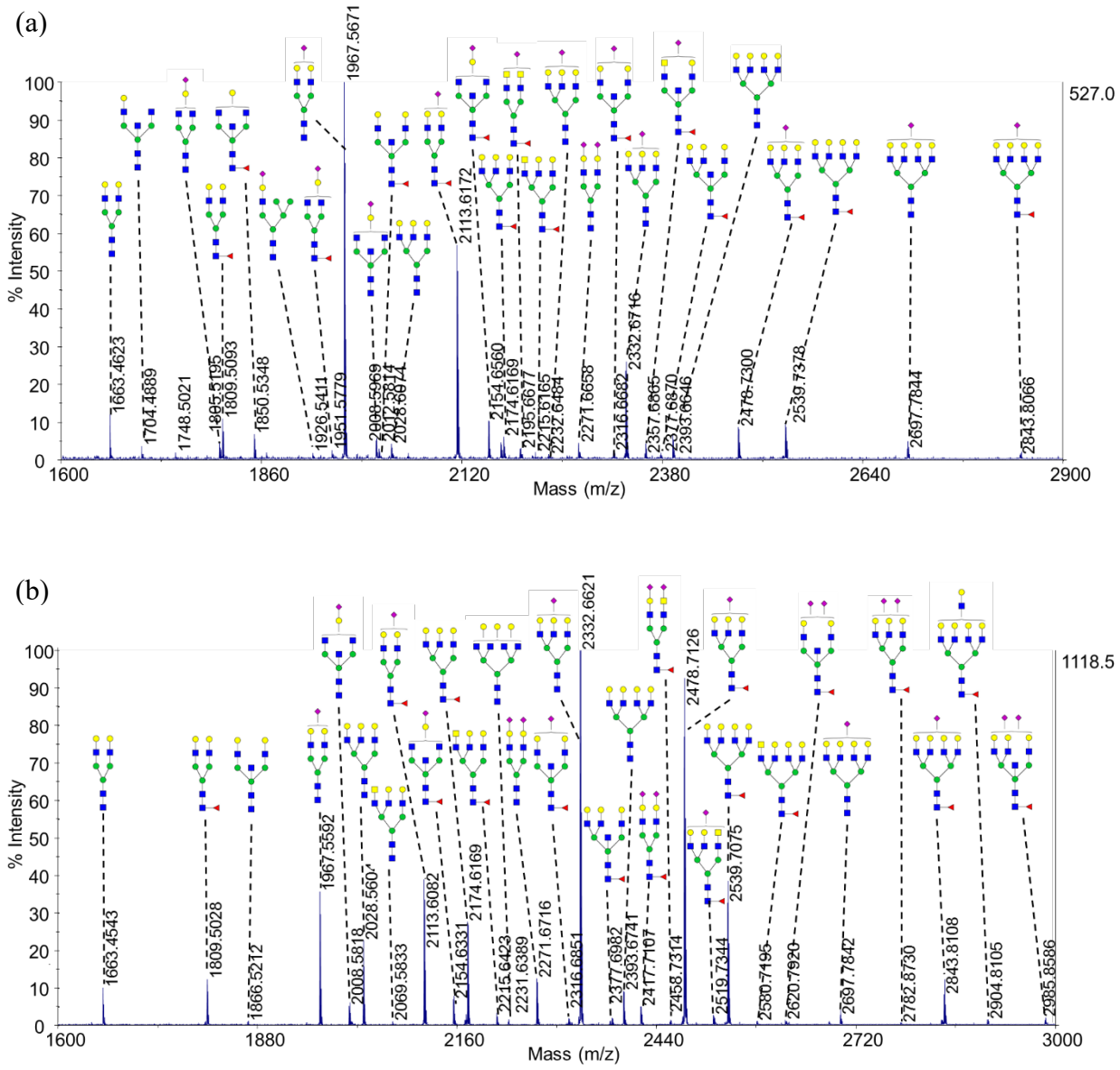


Figure S9. Electropherograms of α2-3 sialidase treated N-glycans fractions of urinary exosomes. Structures were assigned with the assistance of MALDI-MS. For all fractions, most structures were identified to be either neutral or mono-sialylated structures, indicating the existence of less than two α2-6 linked sialic acids for the majority of N-glycan structures derived from urinary exosomes.


(a)
% Intensity
Mass (m/z)
1600
1860
2120
2380
2640
2900
1967.5671
527.0
(b)
% Intensity
Mass (m/z)
1600
1880
2160
2440
2720
3000
2332.6621
2478.7126
1118.5

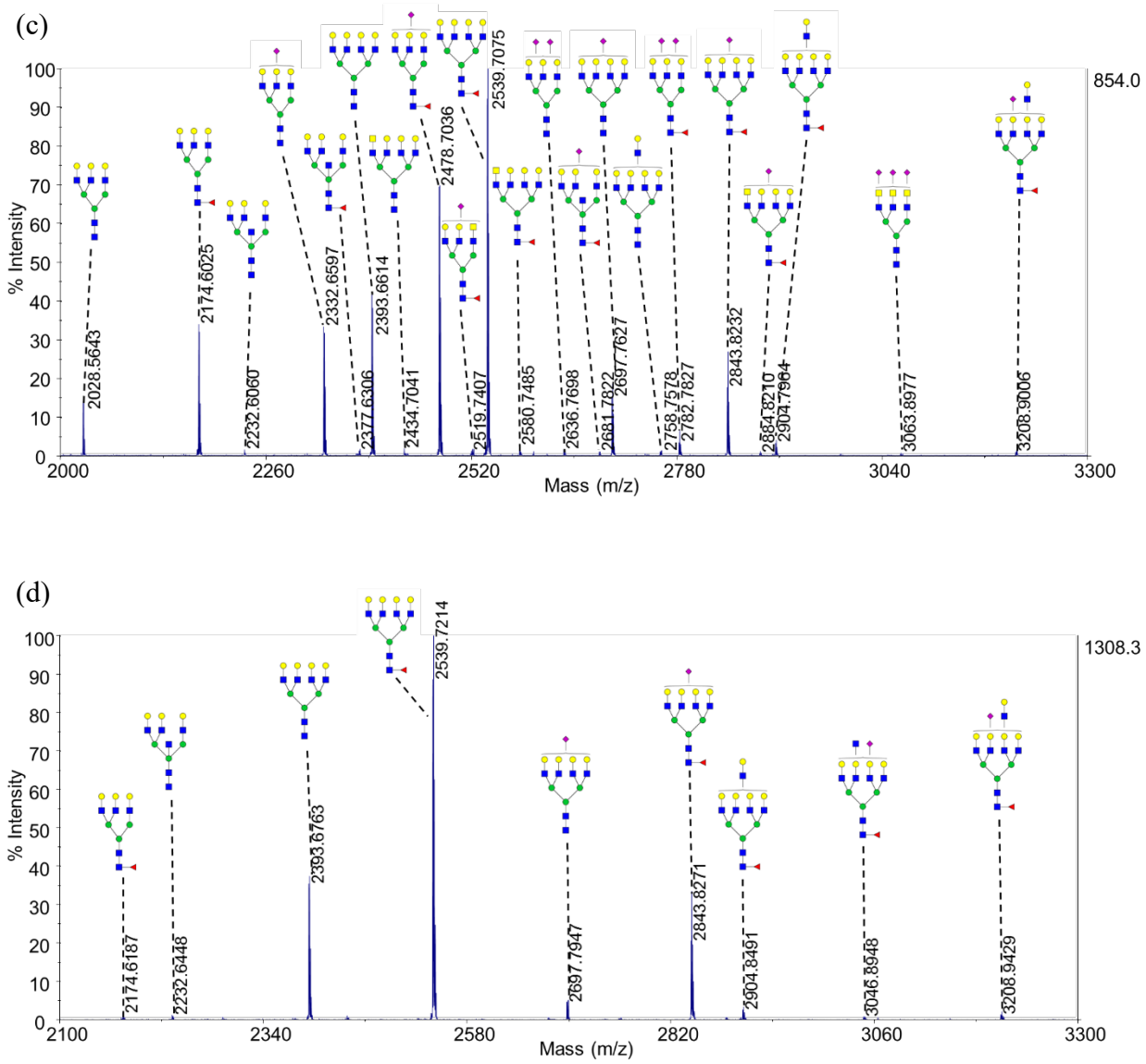
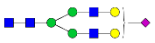
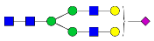
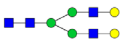
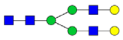
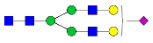
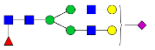
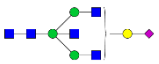
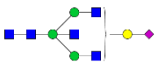
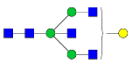
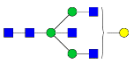
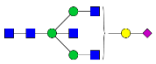
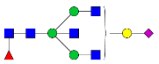
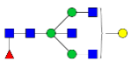
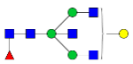
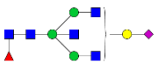
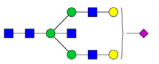
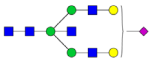
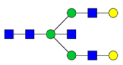
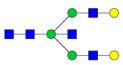
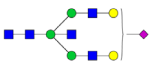
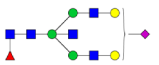
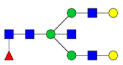
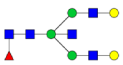
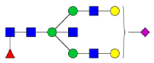
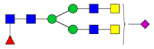
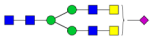
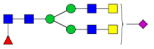
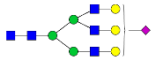
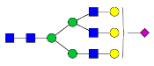
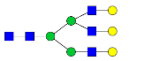
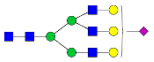
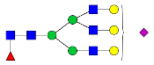
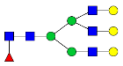
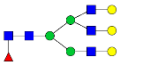
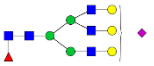
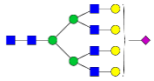
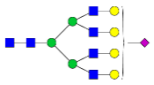
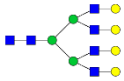
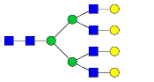
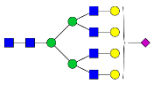
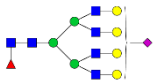
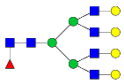
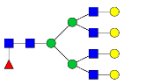
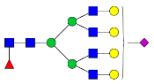
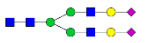
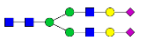
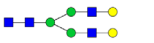
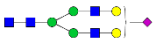
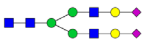
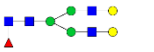
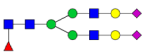
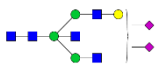
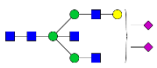
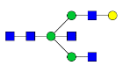
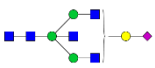
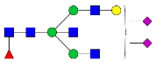
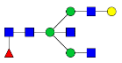
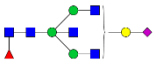
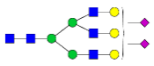
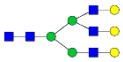
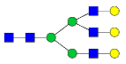
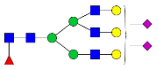
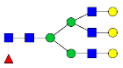
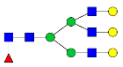
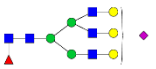
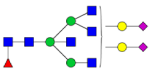
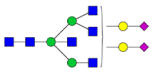
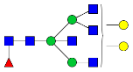
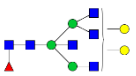
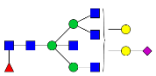
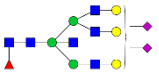
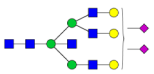
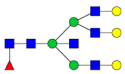
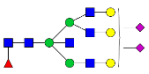
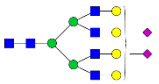
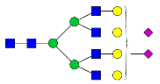
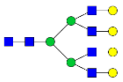
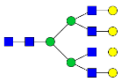
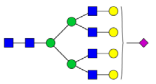
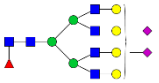
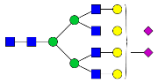
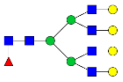
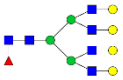
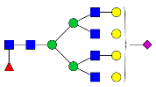
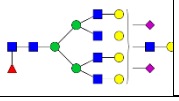
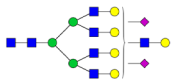
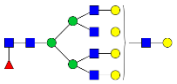
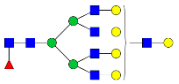
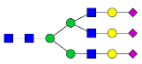
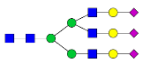
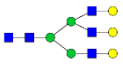
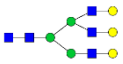


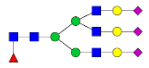
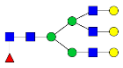
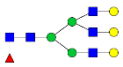
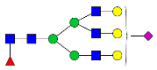
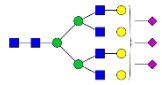
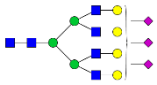
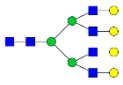
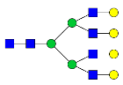
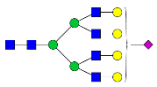
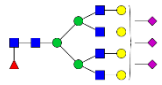
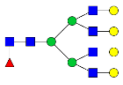
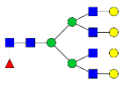
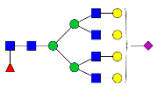
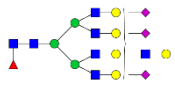
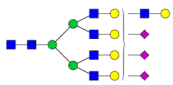
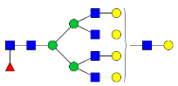
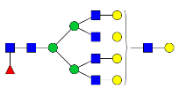
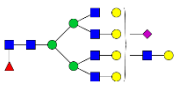
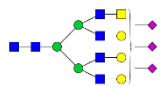
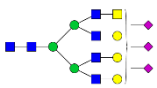
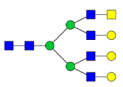
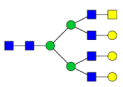
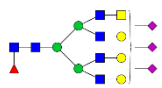
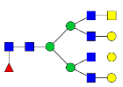
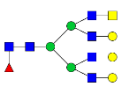
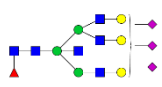
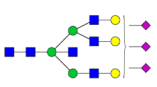
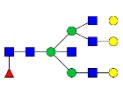
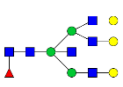
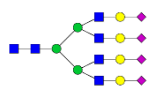
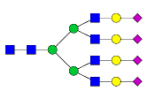
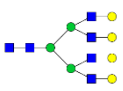
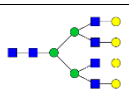
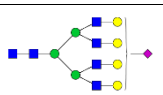
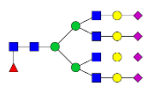
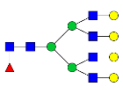
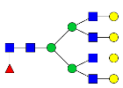
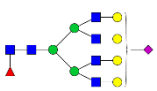
Figure S10. MALDI-MS spectra of α2-3 sialidase-treated N-glycans fractions derived from urinary exosomes: (a) mono-sialylated, (b) di-sialylated, (c) tri-sialylated, and (d) tetra-sialylated fractions. In all fractions, neutral and mono-sialylated N-glycans were predominantly observed.

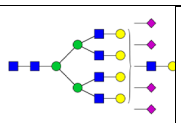
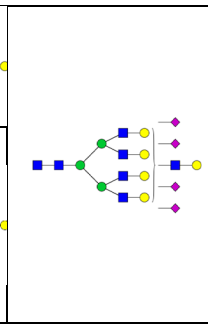
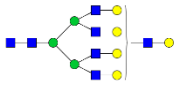
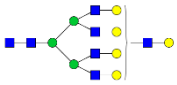
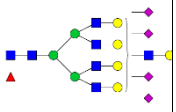
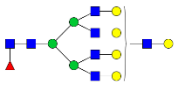
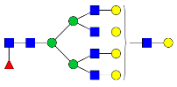
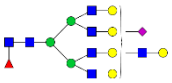
Table S3. List of N-glycan structures and their normalized apparent peak areas analyzed by MALDI-MS and microfluidic CE. The N-glycans were derived from human urinary exosomes and treated with exoglycosidases (α1-2,3,4,6 fucosidase, α2-3,6,8 sialidase, and α2-3 sialidase). Normalized apparent peak areas of sialylated N-glycans were calculated relative to the mass spectra and electropherograms of the α2-3 sialidase-treated N-glycan fractions. N/A represents N-glycan structures that were not assigned in the electropherogram.

intact structure	post α1-2,3,4,6 fucosidase treatment	post α2-3,6,8 sialidase treatment	post α2-3 sialidase treatment	normalized apparent peak area (MALDI)	normalized apparent peak area (MCE)
mono-sialylated					
				7.58%	15.44%
				100.00%	89.31%
				7.94%	25.44%
				62.85%	100.00%
				2.48%	N/A
				3.98%	N/A
				6.30%	N/A
				8.83%	N/A
				1.23%	N/A
				2.45%	N/A
				2.05%	N/A

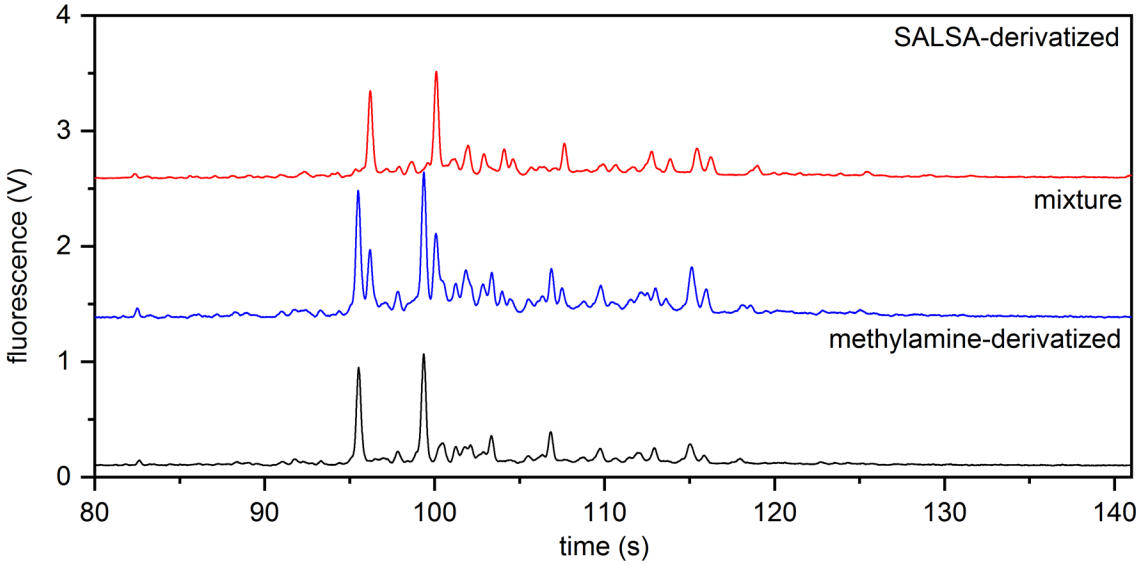
				2.97%	N/A
				2.24%	N/A
				3.60%	N/A
				22.24%	56.00%
				4.82%	N/A
				7.99%	29.86%
				5.36%	16.09%
				3.72%	23.24%
				7.63%	34.40%
				1.50%	11.90%
di-sialylated					
				7.46%	8.43%
				30.56%	23.42%
				10.08%	N/A
				9.00%	12.61%
				31.40%	39.79%
				7.64%	N/A
				3.62%	N/A

				6.25%	N/A
				16.89%	15.18%
				100.00%	92.00%
				24.28%	27.48%
				79.88%	100.00%
				0.44%	N/A
				2.96%	N/A
				2.45%	N/A
				0.87%	N/A
				7.57%	10.48%
				6.73%	N/A
				31.36%	56.80%
				6.73%	14.41%
				0.96%	N/A
tri-sialylated					
				15.23%	9.49%
				33.76%	28.18%
				1.41%	N/A

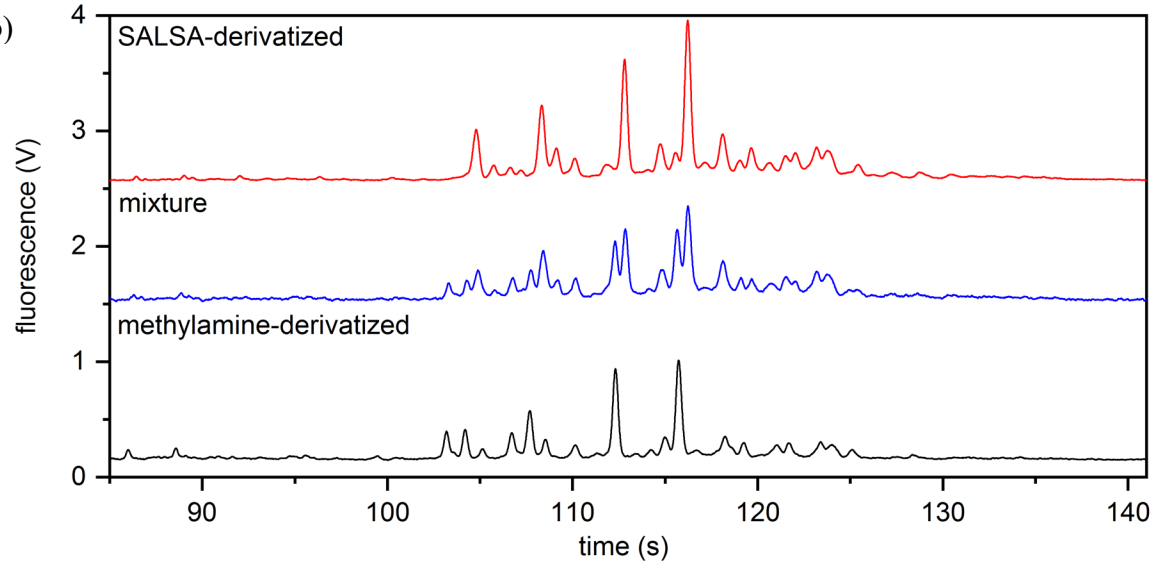
				37.80%	30.72%
				75.43%	64.44%
				3.86%	N/A
				41.75%	29.91%
				13.20%	16.47%
				100.00%	100.00%
				17.66%	35.43%
				2.90%	N/A
				1.04%	N/A
				1.52%	N/A
				1.97%	N/A
				1.69%	N/A
tetra-sialylated					
				35.52%	29.48%
				4.00%	11.29%
				100.00%	100.00%
				24.75%	46.20%

				0.37%	N/A
				2.46%	7.12%
				1.13%	N/A

(a)


SALSA-derivatized
mixture
methylamine-derivatized
fluorescence (V)
time (s)
0
1
2
3
4
80
90
100
110
120
130
140

(b)


SALSA-derivatized
mixture
methylamine-derivatized
fluorescence (V)
time (s)
0
1
2
3
4
90
100
110
120
130
140

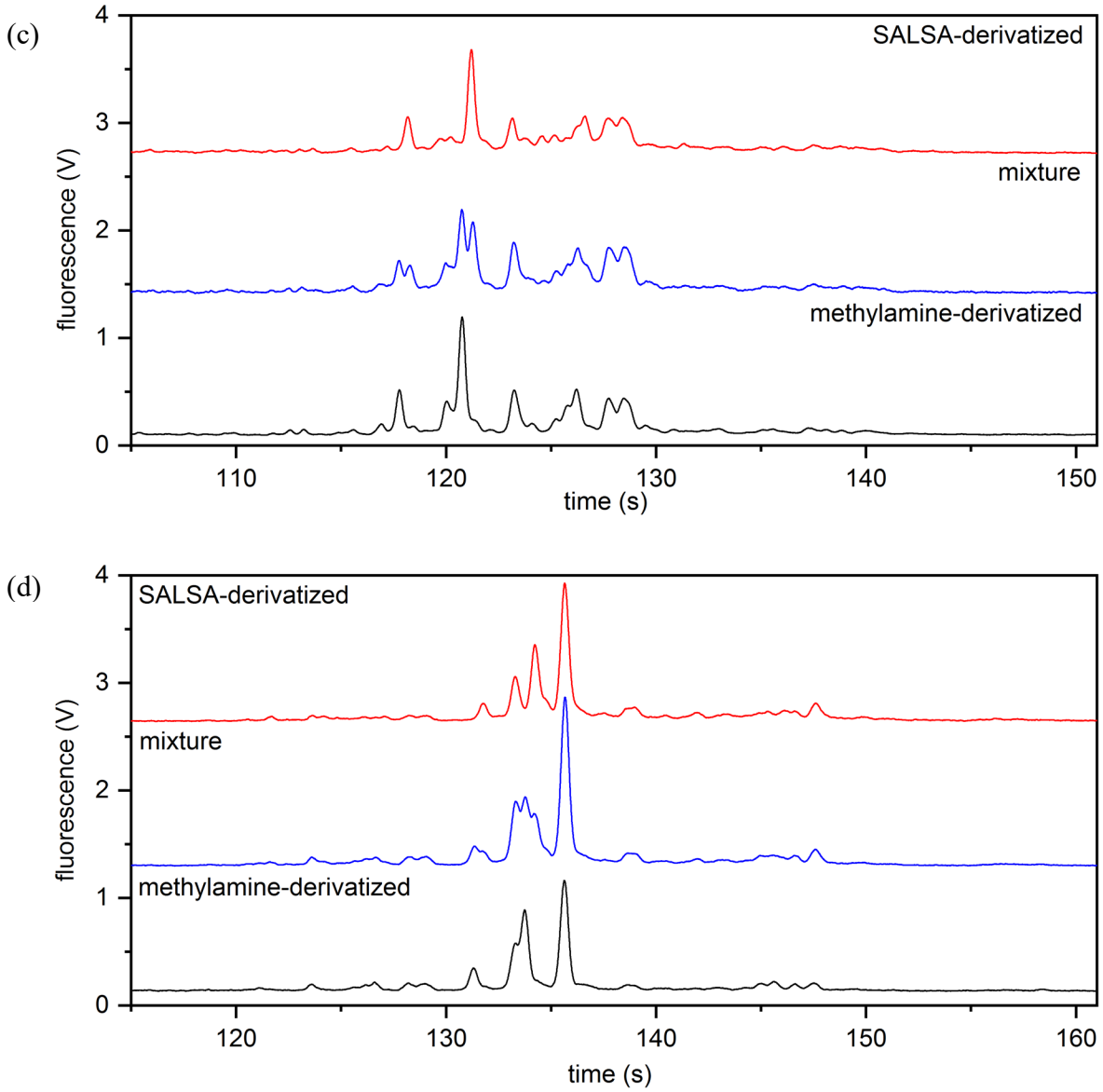
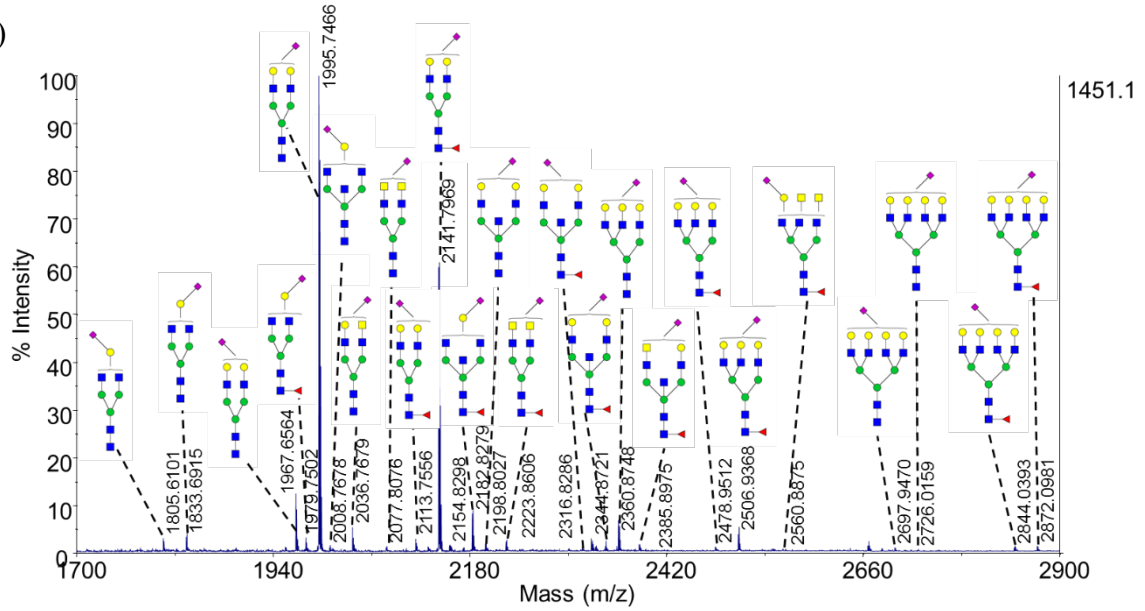
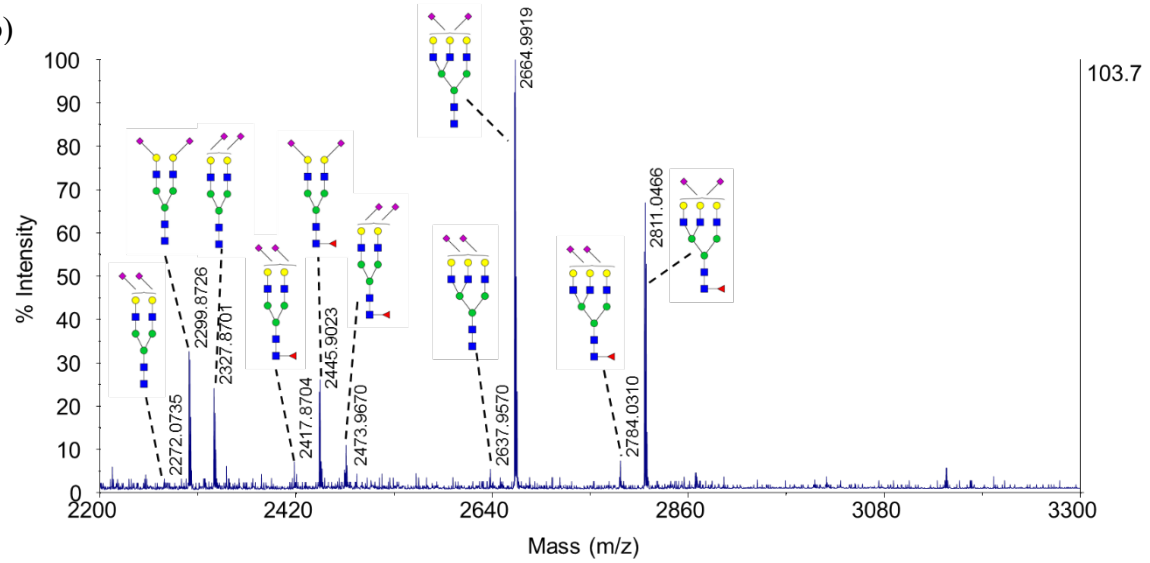


Figure S11. Electropherograms of N-glycan fractions from urinary exosomes derivatized with methylamine- and SALSA: (a) mono-sialylated, (b) di-sialylated, (c) tri-sialylated, and (d) tetra-sialylated fractions. Methylamine- and SALSA-derivatized N-glycan samples were mixed together for the alignment of electropherograms..

(a)


1451.1
% Intensity
Mass (m/z)
1995.7466
2141.7969
1805.6101
1833.6915
1967.6564
1979.7502
2008.7678
2036.7679
2077.8076
2113.7556
2154.8298
2182.8279
2198.8027
2223.8606
2316.8286
2344.8721
2360.8748
2385.8975
2478.9512
2506.9368
2560.8875
2697.9470
2726.0159
2844.0393
2872.0981

(b)


103.7
% Intensity
Mass (m/z)
2664.9919
2811.0466
2272.0735
2299.8726
2327.8701
2417.8704
2445.9023
2473.9670
2637.9570
2784.0310

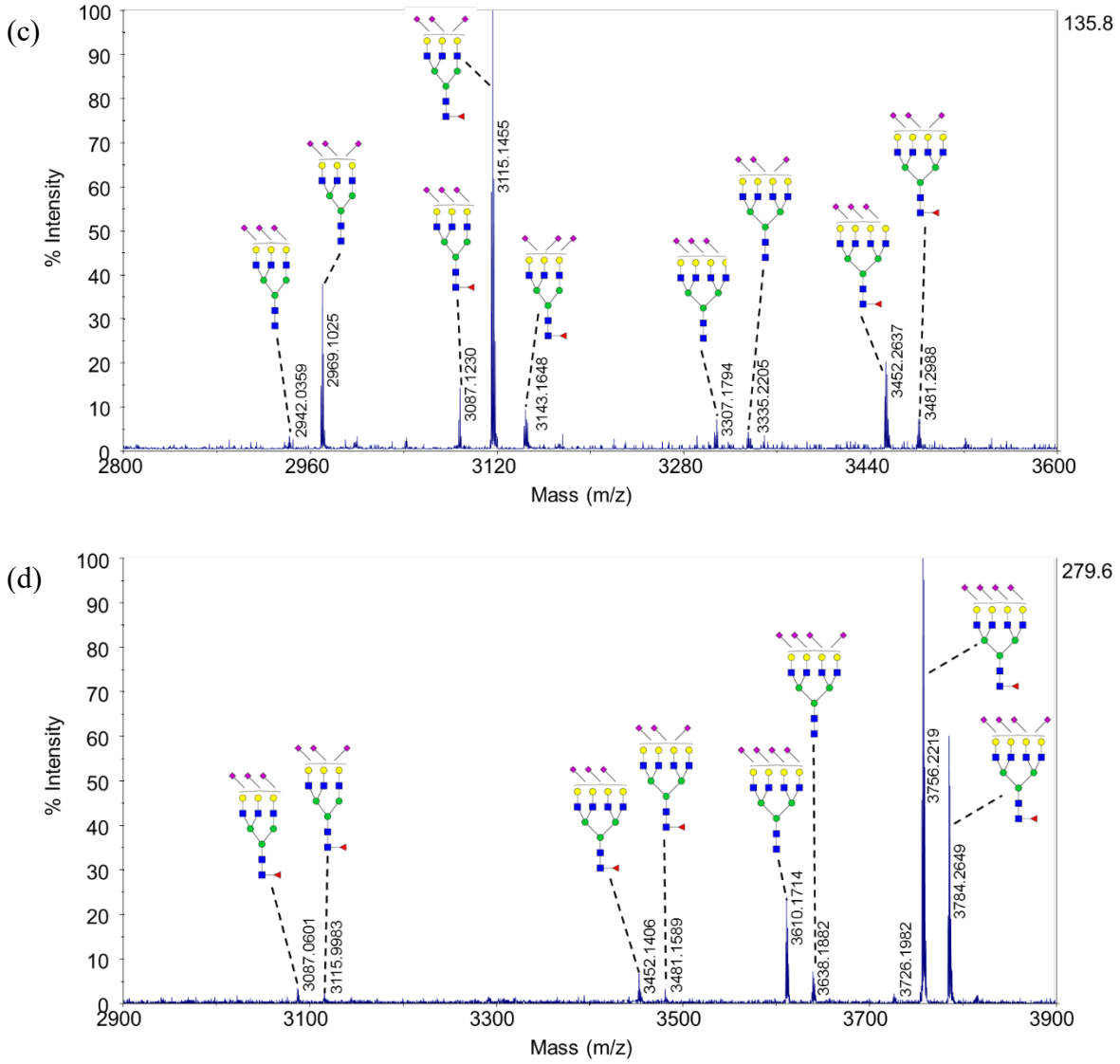


Figure S12. MALDI-MS spectra of SALSA-derivatized N-glycans fractions derived from urinary exosomes: (a) mono-sialylated, (b) di-sialylated, (c) tri-sialylated, and (d) tetra-sialylated fractions. Observed sialic acid linkage isomers are in good agreement with the α2-3 sialidase digestion results.

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