Supplementary Material for:

A Dual Modifications Strategy to Quantify Neutral and Sialylated N-Glycans Simultaneously by MALDI-MS

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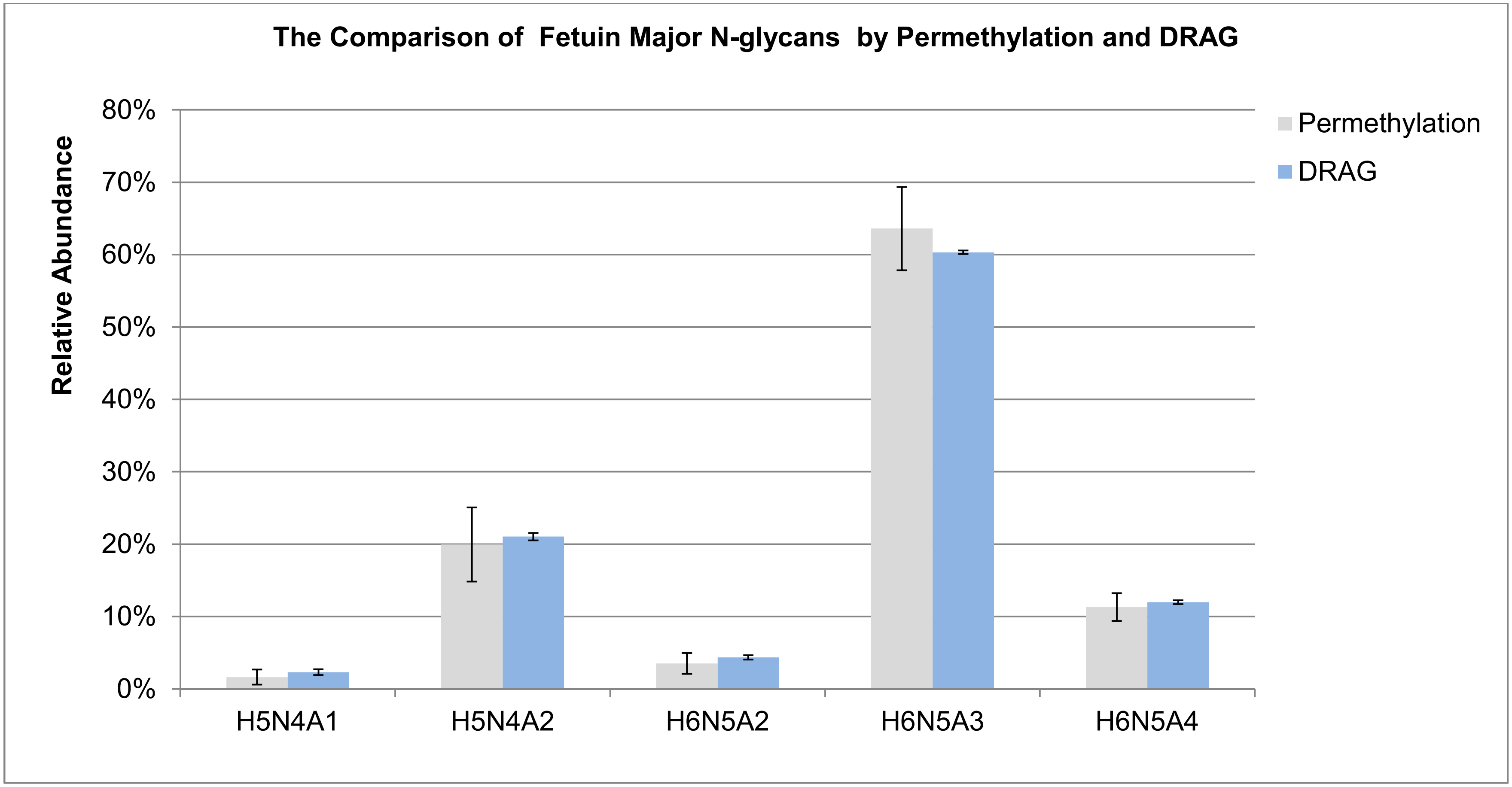
Supplementary Table S1. The application of DRAG strategy to compare two urinary N-glycomes (**AM *vs.* PM**). The N-glycomes were labeled with 2-AA (**AM**) and 2-^{13}[C_6]-AA (**PM**), respectively. The sum of first three peak heights of each composition was listed and used for the quantitative comparisons between **AM** and **PM** samples. The relative distribution of each composition within **AM** or **PM** was calculated using the height of individual divided by the total heights of every molecule within the same sample. (H: Hexose, N: HexNAc, A: Neu5Ac, F: Fucose).

#	Monosaccharide Composition	AM (m/z)	PM (m/z)	SUM Height (AM)	SUM Height (PM)	Relative Dis. (AM, %)	Relative Dis. (PM, %)	Comparative Ratio (AM *vs.* PM)
1	H7N6A4F1	3890.5	3896.5	27	27	0.33%	0.33%	1.00
2	H7N6A3F1	3586.4	3592.4	22	23	0.27%	0.28%	0.96
3	H7N6A2F1	3282.2	3288.3	25	25	0.31%	0.31%	1.00
4	H6N5A3F1	3221.2	3227.3	48	55	0.59%	0.67%	0.87
5	H6N5A3	3075.2	3081.2	48	64	0.59%	0.78%	0.75
6	H6N5A2F1	2917.1	2923.1	77	76	0.94%	0.93%	1.01
7	H6N5A2	2771.1	2777.1	67	54	0.82%	0.66%	1.24
8	H5N5A2F1	2755.1	2761.1	45	55	0.55%	0.67%	0.82
9	H6N5A1F1	2613.0	2619.0	80	85	0.98%	1.04%	0.94
10	H4N5A2F1	2593.0	2599.0	43	63	0.53%	0.77%	0.68
11	H5N4A2F1	2552.0	2558.0	388	479	4.76%	5.87%	0.81
12	H6N5A1	2466.9	2472.9	57	44	0.70%	0.54%	1.30
13	H5N5A1F1	2450.9	2457.0	83	97	1.02%	1.19%	0.86
14	H5N4A2	2405.9	2411.9	1043	1163	12.79%	14.26%	0.90
15	H5N4A1F2	2393.9	2399.9	103	91	1.26%	1.12%	1.13
16	H3N6A1F1	2329.9	2335.9	46	50	0.56%	0.61%	0.92
17	H5N4A1F1	2247.9	2253.9	526	550	6.45%	6.75%	0.96
18	H5N5F1	2146.8	2152.8	111	126	1.36%	1.55%	0.88
19	H5N4A1	2101.8	2107.8	322	323	3.95%	3.96%	1.00
20	H4N4A1F1	2085.8	2091.8	54	105	0.66%	1.29%	0.51
21	H9N2	2039.7	2045.7	30	41	0.37%	0.50%	0.73

22	H3N6F1	2025.8	2031.8	66	68	0.81%	0.83%	0.97
23	H5N5	2000.7	2006.8	44	38	0.54%	0.47%	1.16
24	H4N5F1	1984.8	1990.8	91	117	1.12%	1.43%	0.78
25	H5N3A1	1898.7	1904.7	22	27	0.27%	0.33%	0.81
26	H4N5	1838.7	1844.7	52	52	0.64%	0.64%	1.00
27	H3N5F1	1822.7	1828.7	72	82	0.88%	1.01%	0.88
28	H5N4	1797.7	1803.7	68	78	0.83%	0.96%	0.87
29	H4N4F1	1781.7	1787.7	105	142	1.29%	1.74%	0.74
30	H7N2	1715.6	1721.6	548	574	6.72%	7.04%	0.95
31	H3N4F1	1619.6	1625.6	149	182	1.83%	2.23%	0.82
32	H6N2	1553.6	1559.6	2365	1850	29.00%	22.69%	1.28
33	H5N2	1391.5	1397.5	732	716	8.98%	8.78%	1.02
34	H3N2F1	1213.5	1219.5	595	632	7.30%	7.75%	0.94
Total				**8154**	**8154**	**100.0%**	**100.0%**	**1.00**

Supplementary Table S2. Three biological replicates were processed for urinary N-glycome comparison (**AM *vs.* PM**). The sum of first three peak heights of each composition was used to calculate the quantitative ratio between **AM** and **PM** samples. Several abundant compositions including high mannose types (H5N2, H6N2, and H7N2) and sialoglycans (H5N4A1, H5N4A1F1, H5N4A2, and H5N4A2F1) were compared side-by-side to evaluate the reproducibility of DRAG strategy. (H: Hexose, N: HexNAc, A: Neu5Ac, F: Fucose).

Urine Glycans	Rep. 1	Rep. 2	Rep. 3	Mean	Std Dev.	Relative Std Dev.
H5N2	1.03	0.99	1.02	1.01	0.021	2.08%
H6N2	1.23	1.25	1.28	1.25	0.025	2.00%
H7N2	0.93	0.94	0.95	0.94	0.012	1.24%
H5N4A1	0.97	0.98	1.00	0.98	0.014	1.47%
H5N4A1F1	0.97	0.94	0.96	0.96	0.018	1.90%
H5N4A2	0.92	0.92	0.90	0.91	0.009	1.00%
H5N4A2F1	0.78	0.86	0.81	0.82	0.037	4.50%



Supplementary Figure S1. N-glycans from bovine fetuin were repeatedly derivatized (five replicates) by permethylation and DRAG strategy (2-AA then methylamidation), respectively. Five abundant compositions (H5N4A1, H5N4A2, H6N5A2, H6N5A3, and H6N5A4) were selected to calculate their relative abundances based on their respective MALDI-MS signals (Height). The slight differences of each composition between two strategies were likely caused by the side reactions of permethylation and ionization efficiency differences between two derivatization strategies.