A facile method for cellular N-glycomic profiling by matrix-assisted laser desorption/ionization mass spectrometry †

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Permethylation

The dried glycans was redissolved in 50 μ L of dimethyl sulfoxide (DMSO) and 100 μ L of DMSO-NaOH slurry and agitated manually. 50 μ L of methyl iodide was added to the mixture and shaken the reaction mixture for 15min at room temperature. 500 μ L water was added to the sample and vortexed to quench the reaction. Immediately 250 μ L chloroform was added and the tube was vortexed. After separation of the phases, the upper layer was removed carefully and the lower organic layer containing the permethylated glycans was washed with water for 3 times. The organic phase was removed to a new tube and dried by a rotary concentrator.



Fig. S1 Representative MALDI-MS mass spectra of N-glycans obtained from HeLa cells with different protein extraction method. (a) extraction of the cellular proteins with chloroform-methanol-water (CMW); (b) extraction of the cellular proteins with ethanol.



Fig. S2 Typical MALDI-MS mass spectra of N-glycans obtained from HeLa cells with different matrix used (a and b) and photographs of a DHB dried-droplet preparation and a CHCA dried-droplet preparation (c) on a stainless steel target. For the Dried-droplet preparation, a 0.5 μ L portion of DHB or CHCA solution was mixed with 0.5 μ L of sample solution and dried on the target. The amounts of the loaded was same for (a) and (b). The capital letter "R" in (a) and (b) was the abbreviation of resolution.



Fig. S3 Repeatability of HeLa cell N-glycan profiling by MALDI-TOF-MS using the new developed method. The experiment was performed three times on separate days, indicated as experiments 1-3. The graph shows the average relative intensities observed for the 27 major glycan species (normalized to the overall sum of intensities), with error bars for standard deviation (n=3). Abbreviations used are hexose (H), fucose (F), N-acetylhexosamine (N) and N-acetylneuraminic acid (S).

m/z	Resolution			S/N		
	DHB	CHCA	CHCA/DHB	DHB	CHCA	CHCA/DHB
1482.66	9043.95	17575.9	1.943393	12.84	108.65	8.461838
1628.70	9503.12	16112.8	1.695534	16.85	47.24	2.803561
1644.74	5506.24	16134.7	2.930272	27.06	163.88	6.056171
1685.70	6317.17	15445.9	2.445074	10.25	54.3	5.297561
1806.79	8794.58	17598.2	2.001028	250.73	777.93	3.10266
1831.79	12546.9	18411.2	1.467394	12.59	79.86	6.343129
1847.86	5602.42	16762.1	2.991943	12.79	85.78	6.706802
1968.86	7720.91	17473.1	2.263092	759.21	2022.01	2.663308
1993.89	11327.3	19845.3	1.751993	19.42	99.31	5.1138
2009.89	9182.95	17552.0	1.911373	20.94	81.45	3.889685
2034.85	15293.15	17620.3	1.15217	11.03	42.3	3.834995
2130.93	7609.7	17351.1	2.280139	761.27	2007.79	2.637422
2151.98	7482.62	17734.1	2.370042	101.66	281.79	2.771887
2213.00	6277.33	17396.8	2.771369	15.53	63.76	4.105602
2293.00	6855.59	15600.4	2.275575	1091.7	2502.23	2.292049
2454.06	8259.52	15565.3	1.884537	13.58	1201.96	88.50957
2476.11	6287.77	14405.4	2.291033	197.38	451.38	2.286858
2517.14	6290.55	14855.2	2.361512	175.53	497.66	2.835185
2617.12	6225.17	14784.9	2.375021	29.58	84.42	2.853955
2663.22	7405.67	14314.7	1.932939	325.87	880.54	2.70212
2809.28	7540.51	12884.7	1.70874	161.31	361.07	2.238361
2821.29	7128.94	13405.2	1.880395	354.28	712.82	2.012024
2882.27	6688.84	12120.2	1.812005	21.62	47.71	2.206753
2967.34	7907.78	12313.6	1.55716	504.03	985.47	1.955181
2983.28	10123.87	11610.9	1.146885	19.94	16.23	0.813942
3028.31	6647.36	10353.5	1.557537	19.77	77.66	3.928174
3186.42	10407.96	12050.7	1.15784	18.92	116.7	6.168076
3332.57	6366.71	10403.4	1.634043	46.99	162.7	3.462439
3393.49	6998.87	10220.0	1.460237	12	45.83	3.819167
3478.49	8058.78	9562.17	1.186553	23.69	49.36	2.08358
3490.55	8913.53	9418.22	1.056621	73.55	156.29	2.124949
3636.66	6365.72	8685.27	1.364381	92.02	164.73	1.790154
3697.68	24546.79	9262.89	0.377356	14.42	37.46	2.597781
4001.83	9699.13	7481.01	0.771307	19.58	59.04	3.015322
4307.94	7665.22	10560.9	1.377773	13.75	32.07	2.332364

Table. S1 The resolution and S/N by using CHCA and DHB as the matrix forMALDI-MS analysis of derivatized N-glycan.

No.	Glycans* (<i>m/z</i>)	Chemical composition	Proposed structure
1	1320.51	Hex ₂ HexNAc ₂	••••••••••••••••••••••••••••••••••••••
2	1466.54	Hex ₂ HexNAc ₂ Fuc ₁	
3	1482.53	Hex ₃ HexNAc ₂	1.679
4	1628.60	Hex ₃ HexNAc ₂ Fuc ₁	- tore
5	1644.58	Hex ₄ HexNAc ₂	0 -0-0-0-307
6	1685.61	Hex ₃ HexNAc ₃	
7	1790.68	Hex ₄ HexNAc ₂ Fuc ₁	
8	1806.64	Hex5HexNAc2	
9	1831.68	Hex ₃ HexNAc ₃ Fuc ₁	
10	1847.66	Hex ₄ HexNAc ₃	•
11	1888.68	Hex ₃ HexNAc ₄	
12	1968.70	Hex ₆ HexNAc ₂	
13	1993.72	Hex ₄ HexNAc ₃ Fuc ₁	•-=-
14	2009.74	Hex5HexNAc3	
15	2034.75	Hex ₃ HexNAc ₄ Fuc ₁	
16	2050.76	Hex4HexNAc4	
17	2130.75	Hex ₇ HexNAc ₂	•
18	2151.80	Hex ₄ HexNAc ₃ Neu5Ac ₁	+•=-[>===
19	2167.81	Hex ₄ HexNAc ₃ Neu5Gc ₁	<
20	2171.78	Hex ₆ HexNAc ₃	

Table. S2 Detected N-glycans from HeLa cells by MALDI-MS.

21	2196.81	$Hex_4HexNAc_4Fuc_1$
22	2212.81	Hex5HexNAc4
23	2237.84	Hex ₃ HexNAc ₅ Fuc ₁
24	2292.82	Hex ₈ HexNAc ₂
25	2297.87	$Hex_4HexNAc_3Neu5Ac_1Fuc_1$
26	2313.86	Hex ₅ HexNAc ₃ Neu5Ac ₁
27	2354.88	Hex ₄ HexNAc ₄ Neu5Ac ₁
28	2358.88	$Hex_5HexNAc_4Fuc_1$
29	2454.87	Hex ₉ HexNAc ₂
30	2475.93	Hex ₆ HexNAc ₃ Neu5Ac ₁
31	2504.94	$Hex_5HexNAc_4Fuc_2$
32	2516.95	$Hex_5HexNAc_4Neu5Ac_1$
33	2532.95	Hex ₅ HexNAc ₄ Neu5Gc ₁
34	2577.96	Hex ₆ HexNAc ₅
35	2616.94	$Hex_{10}HexNAc_2$
36	2663.02	$Hex_5HexNAc_4Neu5Ac_1Fuc_1$
37	2679.04	Hex ₆ HexNAc ₄ Neu5Ac ₁
38	2724.03	Hex ₆ HexNAc ₅ Fuc ₁
39	2809.09	$Hex_5HexNAc_4Neu5Ac_1Fuc_2$
40	2821.10	Hex5HexNAc4Neu5Ac2
41	2882.08	Hex ₆ HexNAc ₅ Neu5Ac ₁



42	2967.17	$Hex_5HexNAc_4Neu5Ac_2Fuc_1$
43	2982.18	$Hex_5HexNAc_4Neu5Gc_1Neu5Ac_1Fuc_1$
44	3024.21	Hex ₅ HexNAc ₅ Neu5Ac ₂
45	3028.19	$Hex_6HexNAc_5Neu5Ac_2Fuc_1$
46	3089.22	Hex ₇ HexNAc ₆ Fuc ₁
47	3125.21	Hex ₅ HexNAc ₄ Neu5Ac ₃
48	3186.26	Hex ₆ HexNAc ₅ Neu5Ac ₂
49	3202.24	Hex ₆ HexNAc ₅ Neu5Ac ₁ Neu5Gc ₁
50	3332.35	$Hex_6HexNAc_5Neu5Ac_2Fuc_1$
51	3348.32	$Hex_6HexNAc_5Neu5Ac_1Neu5Gc_1Fuc_1$
52	3393.34	$Hex_7HexNAc_6Neu5Ac_1Fuc_1$
53	3478.41	$Hex_6HexNAc_5Neu5Ac_2Fuc_2$
54	3490.43	Hex ₆ HexNAc ₅ Neu5Ac ₃
55	3506.47	$Hex_6HexNAc_5Neu5Ac_2Neu5Gc_1$
56	3551.42	Hex ₇ HexNAc ₆ Neu5Ac ₂
57	3636.52	$Hex_6HexNAc_5Neu5Ac_3Fuc_1$
58	3653.55	$Hex_6HexNAc_5Neu5Ac_2Neu5Gc_1Fuc_1$
59	3697.44	$Hex_7HexNAc_6Neu5Ac_2Fuc_1$
60	3856.68	Hex ₇ HexNAc ₆ Neu5Ac ₃
61	4001.66	Hex ₇ HexNAc ₆ Neu5Ac ₃ Fuc ₁
62	4305.82	Hex ₇ HexNAc ₆ Neu5Ac ₄ Fuc ₁



* monoisotopic mass