Electronic Supplementary Material (ESI) for Molecular Omics.

Development of a 96-well plate sample preparation method for integrated *N*- and *O*-glycomics using porous graphitized carbon liquid chromatography-mass spectrometry

Supplementary Information

Table S1. Relative quantification of *N*-glycans released from fetuin standard using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 2B*)

Table S2. Relative quantification of *O*-glycans released from fetuin standard using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 2D*)

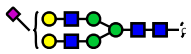
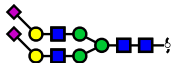
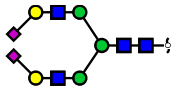
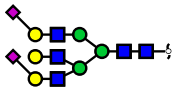
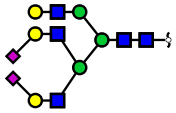
Table S3. *N*-glycans released from NMuMG cell line using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS.

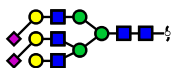
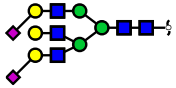
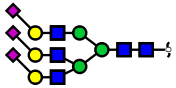
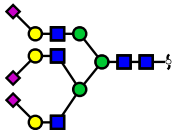
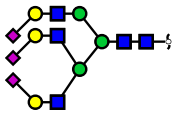
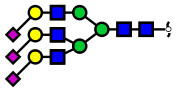
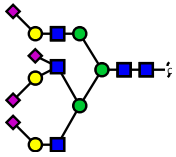
Table S4. Relative quantification of *N*-glycans released from NMuMG cells using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 3C*)

Table S5. Relative quantification of *O*-glycans released from NMuMG cells using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure3D*)

Figure S1. Elution pattern of *N*-glycans with and without phosphate on PGC.

Table S1. Relative quantification of *N*-glycans released from fetuin standard using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 2B*) Proposed structures were assigned based on MS/MS fragmentation and glycobiological pathway constraints. Structures are depicted according to the CFG (Consortium of Functional Glycomics). (intraday $n = 3$, interday $n = 6$, independent technical replicates on two different plates). Blue square: *N*-acetylglucosamine, green circle: mannose, yellow circle: galactose, red triangle: fucose, up pointing pink diamond: α2,6-linked *N*-acetylneuraminic acid, down pointing pink diamond: α2,3-linked *N*-acetylneuraminic acid. Composition: H: hexose; N: *N*-acetylhexosamines; S: *N*-acetylneuraminic acid; a, b, c, d: isomer number. SD: standard variation; CV: coefficient of variation/relative standard deviation.

Composition	$[M-H]^{1-}$ ($[M-2H]^{2-}$) [$[M-3H]^{3-}$]	Proposed structure	Average relative abundance %. (SD %) and *CV*		
			Intraday-1	Intraday-2	Interday
H5N4S1	**1932.66 (965.83)**		**1.9 (±0.1); *4.1%***	**2.0 (±0.2); *12.2%***	**1.9 (±0.2); *9.8%***
H5N4S2a	**2223.73 (1111.36)**		**10.6 (±0.8); *7.9%***	**9.7 (±0.8); *8.0%***	**10.1 (±0.9); *8.9%***
H5N4S2b	**2223.73 (1111.37)**		**12.8 (±0.9); *7.4%***	**12.1 (±0.4); *5.3%***	**12.5 (±0.9); *7.0%***
H6N5S2a	**2588.84 (1293.92)**		**2.3 (±0.2); *10.9%***	**2.9 (±0.2); *8.1%***	**2.6 (±0.4); *14.8%***
H6N5S2b	**2588.86 (1293.93)**		**4.7 (±0.4); *9.5%***	**5.9 (±0.6); *11.0%***	**5.3 (±0.8); *15.2%***

H6N5S2c	**2588.87 (1293.94)**		**2.6 (±0.3);** ***10.0%***	**2.7 (±0.3);** ***11.9%***	**2.7 (±0.3);** ***11.2%***
H6N5S2d	**2588.86 (1293.93)**		**2.1 (±0.2);** ***12.1%***	**2.6 (±0.4);** ***15.7%***	**2.3 (±0.4);** ***19.1%***
H6N5S3a	**2879.95 (1439.47) [959.28]**		**3.0 (±0.4);** ***14.4%***	**2.6 (±0.1);** ***3.6%***	**2.8 (±0.3);** ***12.6%***
H6N5S3b	**2879.94 (1439.47) [959.31]**		**16.4 (±0.5);** ***3.1%***	**15.9 (±1.0);** ***6.1%***	**16.2 (±0.8);** ***5.1%***
H6N5S3c	**2879.94 (1439.47) [959.29]**		**26.8 (±0.4);** ***4.7%***	**25.1 (±0.9);** ***3.5%***	**25.9 (±1.1);** ***4.2%***
H6N5S3d	**2879.94 (1439.47) [959.29]**		**3.1 (±0.1);** ***3.9%***	**3.1 (±0.5);** ***15.1%***	**3.1 (±0.3);** ***11.0%***
H6N5S4a	**3170.98 (1584.99) [1056.32]**		**5.2 (±0.6);** ***12.3%***	**5.3 (±0.1);** ***2.6%***	**5.3 (±0.5);** ***8.8%***

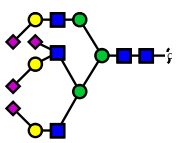
	3170.99				
	(158.00)		**8.5 (±0.3);**	**10.0 (±0.3);**	**9.3 (±0.8);**
H6N5S4b	**[1056.31]**		***4.0%***	***3.3%***	***8.6%***

Table S2. Relative quantification of *O*-glycans released from fetuin standard using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 2D*) Proposed structures were assigned based on MS/MS fragmentation and glycobiological pathway constraints. Structures are depicted according to the CFG (Consortium of Functional Glycomics). (intraday $n = 3$, interday $n = 6$, independent technical replicates on two different plates). Blue square: *N*-acetylglucosamine, green circle: mannose, yellow circle: galactose, red triangle: fucose, pink diamond: *N*-acetylneuraminic acid. Composition: H: hexose; N: *N*-acetylhexosamines; S: *N*-acetylneuraminic acid.

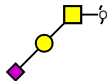
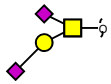
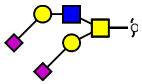
Composition	$[M-H]^{1-}$ ($[M-2H]^{2-}$)	Proposed structure	Average relative abundance %. SD % and *CV*		
			Intraday-1	Intraday-2	Interday
H1N1S1	675.27		33.8 (±0.4); *1.3%*	39.9 (±1.7); *4.3%*	36.9 (±3.3); *8.9%*
H1N1S2	966.35		47.9 (±0.8); *1.6%*	45.1 (±1.1); *2.3%*	46.5 (±1.7); *3.6%*
H2N2S2	1331.49 (665.24)		18.2 (±0.5); *2.7%*	14.9 (±0.6); *4.3%*	16.6 (±1.8); *10.6%*

Table S3. *N*-glycans released from NMuMG cell line using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS. The composition of *N*-glycans have been assigned on the basis of mass and in the relevant extracted ion chromatograms for six *m/z* ratios corresponding to the major monosaccharide compositions for the reported *N*-glycans in mouse. H: hexose; N: *N*-acetylhexosamines; F: fucose; S: *N*-acetylneuraminic acid; P: phosphate. a, b, c, d: isomer number. n.d.: not determined.

Number	Composition	Type	Retention Time	Observed ions				Experimental $[M-H]^-$	Theoretical $[M-H]^-$	Deviation $\Delta[M-H]^-$
				$[M-H]^-$	$[M-2H]^{2-}$	$[M-3H]^{3-}$	$[M-4H]^{4-}$			
1	H2N2F1	Paucimannose	41.8 min	895.331	n.d.	n.d.	n.d.	895.331	895.341	0.010
2	H3N2	Paucimannose	39.0 min	911.313	n.d.	n.d.	n.d.	911.313	911.336	0.023
3	H3N2F1	Paucimannose	50.9 min	1057.359	n.d.	n.d.	n.d.	1057.359	1057.394	0.035
4	H5N2	Oligomannose	33.8 min	1235.400	n.d.	n.d.	n.d.	1235.400	1235.442	0.042
5	H6N2a	Oligomannose	33.7 min	1397.449	698.201	n.d.	n.d.	1397.449	1397.495	0.046
6	H6N2b	Oligomannose	35.5 min	1397.452	698.229	n.d.	n.d.	1397.452	1397.495	0.043
7	H6N2c	Oligomannose	36.8 min	1397.439	698.189	n.d.	n.d.	1397.439	1397.495	0.056
8	H6N2P1a	Oligomannose	20.5 min	1477.391	738.192	n.d.	n.d.	1477.391	1477.461	0.070
9	H6N2P1b	Oligomannose	23.9 min	1477.391	738.192	n.d.	n.d.	1477.391	1477.461	0.070
10	H7N2	Oligomannose	33.1 min	1559.498	779.253	n.d.	n.d.	1559.498	1559.547	0.047
11	H7N2P1a	Oligomannose	21.0 min	1639.423	819.207	n.d.	n.d.	1639.423	1639.514	0.091
12	H7N2P1b	Oligomannose	21.8 min	1639.434	819.213	n.d.	n.d.	1639.434	1639.514	0.080
13	H7N2P2	Oligomannose	14.9 min	1719.371	859.182	n.d.	n.d.	1719.371	1719.480	0.109
14	H8N2	Oligomannose	33.3 min	1721.534	860.263	n.d.	n.d.	1721.534	1721.600	0.064
15	H8N2P1	Oligomannose	20.0 min	1801.474	900.252	n.d.	n.d.	1801.474	1801.567	0.093
16	H9N2	Oligomannose	33.8 min	1883.586	941.267	n.d.	n.d.	1883.534	1883.653	0.119

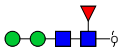
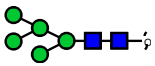
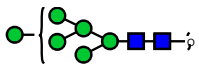
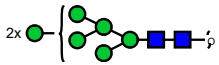
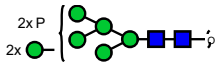
17	H9N2P1	Oligomannose	22.2 min	n.d.	981.228	n.d.	n.d.	1963.456	1963.619	0.163
18	H10N2	Oligomannose	36.2 min	n.d.	1022.287	n.d.	n.d.	2045.574	2045.706	0.132
19	H10N2P1	Oligomannose	22.1 min	n.d.	1062.247	n.d.	n.d.	2125.494	2125.672	0.178
20	H6N3	Hybrid	38.3 min	1600.569	799.767	n.d.	n.d.	1600.569	1600.574	0.015
21	H6N3P1	Hybrid	16.6 min	1680.417	839.735	n.d.	n.d.	1680.417	1680.540	0.123
22	H6N3P1	Hybrid	24.5 min	1680.453	839.735	n.d.	n.d.	1680.453	1680.540	0.087
23	H7N3	Hybrid	45.9 min	1762.442	880.781	n.d.	n.d.	1762.442	1762.627	0.085
24	H6N3F1P1	Hybrid	24.9 min	1826.462	912.764	n.d.	n.d.	1826.462	1826.598	0.136
25	H7N3P1	Hybrid	26.5 min	n.d.	920.745	n.d.	n.d.	1842.490	1842.593	0.103
26	H5N3F1S1a	Hybrid	37.7 min	1875.552	937.286	n.d.	n.d.	1875.552	1875.674	0.122
27	H5N3F1S1b	Hybrid	48.4 min	1875.578.	937.313	n.d.	n.d.	1875.578	1875.674	0.096
28	H6N3S1	Hybrid	34.9 min	1891.586	945.268	n.d.	n.d.	1891.586	1891.669	0.083
29	H6N3S1	Hybrid	45.1 min	1891.596	945.291	n.d.	n.d.	1891.596	1891.669	0.073
30	H7N3S1	Hybrid	35.8 min	n.d.	1026.288	n.d.	n.d.	2053.576	2053.722	0.146
31	H3N3F1a	Complex	39.5 min	1260.291	n.d.	n.d.	n.d.	1260.291	1260.473	0.182
32	H3N3F1b	Complex	54.0 min	1260.423	n.d.	n.d.	n.d.	1260.423	1260.473	0.050
33	H3N4F1a	Complex	41.2 min	1463.496	731.244	n.d.	n.d.	1463.496	1463.553	0.054
34	H3N4F1a	Complex	42.7 min	1463.482	731.257	n.d.	n.d.	1463.482	1463.553	0.071
35	H3N5	Complex	21.5 min	1520.430	759.755	n.d.	n.d.	1520.430	1520.574	0.144
36	H5N4a	Complex	24.2 min	1641.568	820.242	n.d.	n.d.	1641.568	1641.601	0.033
37	H5N4b	Complex	28.6 min	1641.548	820.268	n.d.	n.d.	1641.548	1641.601	0.053

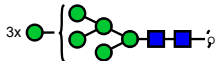
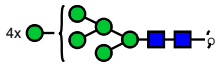
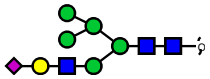
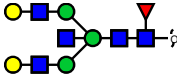
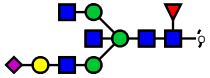
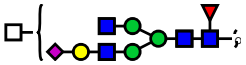
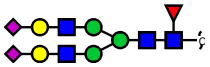
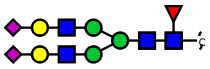
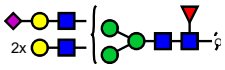
38	H3N5F1	Complex	31.3 min	1666.548	832.782	n.d.	n.d.	1666.548	1666.632	0.084
39	H4N5a	Complex	24.6 min	1682.582	840.756	n.d.	n.d.	1682.582	1682.627	0.045
40	H4N5b	Complex	25.8 min	1682.544	840.777	n.d.	n.d.	1682.544	1682.627	0.063
41	H4N5F1a	Complex	33.2 min	n.d.	913.808	n.d.	n.d.	1828.616	1828.685	0.069
42	H4N5F1b	Complex	34.2 min	1828.660	913.798	n.d.	n.d.	1828.596	1828.685	0.089
43	H5N6a	Complex	28.8 min	n.d.	921.791	n.d.	n.d.	1844.582	1844.680	0.098
44	H5N6b	Complex	31.6 min	1844.626	921.766	n.d.	n.d.	1844.532	1844.680	0.148
45	H5N5F1a	Complex	36.6 min	n.d.	994.791	n.d.	n.d.	1990.582	1990.738	0.156
46	H5N5F1b	Complex	39.4 min	n.d.	994.806	n.d.	n.d.	1989.612	1990.738	0.126
47	H4N3F1S1	Complex	60.0 min	1713.639	856.311	n.d.	n.d.	1713.639	1713.622	-0.017
48	H5N4F1a	Complex	31.7 min	1787.711	893.266	n.d.	n.d.	1787.711	1787.659	-0.052
49	H5N4F1b	Complex	36.3 min	1787.572	893.281	n.d.	n.d.	1787.572	1787.659	0.087
50	H5N5a	Complex	28.8 min	n.d.	921.791	n.d.	n.d.	1844.582	1844.680	0.098
51	H5N5b	Complex	31.8 min	1844.626	921.766	n.d.	n.d.	1844.532	1844.680	0.148
52	H4N5S1a	Complex	23.8 min	1973.754	986.281	n.d.	n.d.	1973.562	1973.723	0.161
53	H4N5S1b	Complex	29.9 min	1973.610	986.288	n.d.	n.d.	1973.576	1973.723	0.147
54	H5N4F1S1a	Complex	39.4 min	n.d.	1038.814	n.d.	n.d.	2078.628	2078.754	0.126
55	H5N4F1S1b	Complex	44.3 min	n.d.	1038.823	n.d.	n.d.	2078.646	2078.754	0.108
56	H5N4F1S1c	Complex	53.7 min	n.d.	1038.834	n.d.	n.d.	2078.668	2078.754	0.084
57	H4N5F1S1a	Complex	30.5 min	n.d.	1059.321	n.d.	n.d.	2119.642	2119.780	0.138
58	H4N5F1S1b	Complex	36.5 min	n.d.	1059.326	n.d.	n.d.	2119.652	2119.780	0.128

59	H4N5F1S1c	Complex	37.4 min	n.d.	1059.320	n.d.	n.d.	2119.640	2119.780	0.140
60	H5N4S2a	Complex	36.3 min	n.d.	1111.299	740.586	n.d.	2223.598	2223.791	0.193
61	H5N4S2b	Complex	46.5 min	n.d.	1111.349	740.577	n.d.	2333.698	2223.791	0.093
62	H5N4S2c	Complex	53.7 min	n.d.	1111.344	n.d.	n.d.	2223.688	2223.791	0.103
63	H6N4F1S1a	Complex	29.8 min	n.d.	1119.846	n.d.	n.d.	2239.692	2240.807	0.115
64	H6N4F1S1b	Complex	35.5 min	n.d.	1119.832	n.d.	n.d.	2239.664	2240.807	0.143
65	H6N4F1S1c	Complex	49.0 min	n.d.	1119.866	n.d.	n.d.	2239.732	2240.807	0.075
66	H6N4F1S1d	Complex	57.1 min	n.d.	1119.839	n.d.	n.d.	2239.678	2240.807	0.129
67	H5N4F1S2a	Complex	42.1 min	n.d.	1184.362	789.300	n.d.	2369.724	2369.849	0.125
68	H5N4F1S2b	Complex	50.5 min	n.d.	1184.373	n.d.	n.d.	2369.746	2369.849	0.103
69	H5N4F1S2c	Complex	51.6 min	n.d.	1184.380	789.315	n.d.	2369.760	2369.849	0.089
70	H5N4F1S2d	Complex	58.9 min	n.d.	1184.377	789.221	n.d.	2369.754	2369.849	0.095
71	H6N5F1S1a	Complex	38.4 min	n.d.	1221.388	813.925	n.d.	2443.776	2443.886	0.110
72	H6N5F1S1b	Complex	46.7 min	n.d.	1221.375	813.944	n.d.	2443.760	2443.886	0.126
73	H5N5F1S2a	Complex	32.4 min	n.d.	1285.917	856.898	n.d.	2572.834	2572.929	0.095
74	H5N5F1S2b	Complex	39.8 min	n.d.	1285.910	856.875	n.d.	2572.820	2572.929	0.109
75	H5N5F1S2c	Complex	48.8 min	n.d.	1285.918	856.960	n.d.	2572.836	2572.929	0.093
76	H6N5S2a	Complex	32.1 min	n.d.	1293.896	862.307	n.d.	2588.792	2588.924	0.132
77	H6N5S2b	Complex	39.3 min	n.d.	1293.897	862.222	n.d.	2588.794	2588.924	0.130
78	H7N5S2	Complex	52.8 min	n.d.	1374.938	n.d.	n.d.	2750.876	2750.976	0.100
79	H6N5S3a	Complex	48.6 min	n.d.	1439.444	959.328	n.d.	2879.876	2880.019	0.143

80	H6N5S3b	Complex	57.4 min	n.d.	1439.464	959.326	n.d.	2879.928	2880.019	0.091
81	H7N5F1S2a	Complex	54.3 min	n.d.	1447.931	n.d.	n.d.	2896.862	2897.034	0.172
82	H7N5F1S2b	Complex	57.1 min	n.d.	1447.944	n.d.	n.d.	2896.888	2897.034	0.166
83	H7N5F1S2c	Complex	58.3 min	n.d.	1447.936	n.d.	n.d.	2896.872	2897.034	0.162
84	H7N5F1S2d	Complex	62.7 min	n.d.	1447.978	n.d.	n.d.	2896.956	2897.034	0.078
85	H6N5F1S3a	Complex	52.7 min	n.d.	1512.477	1007.968	n.d.	3025.954	3026.077	0.123
86	H6N5F1S3b	Complex	54.7 min	n.d.	1512.475	1007.998	n.d.	3025.950	3026.077	0.127
87	H6N5F1S3c	Complex	60.9 min	n.d.	1512.479	1007.943	n.d.	3025.968	3026.077	0.109
88	H6N5F1S3d	Complex	62.6 min	n.d.	1512.469	1007.932	n.d.	3025.938	3026.077	0.139
89	H7N6S4a	Complex	59.4 min	n.d.	1767.548	1178.012	883.296	3536.096	3536.247	0.151
90	H7N6S4b	Complex	62.2 min	n.d.	1767.480	1178.033	883.238	3535.960	3536.247	0.187
91	H7N6F1S4a	Complex	62.6 min	n.d.	1840.582	1226.729	919.792	3682.164	3682.305	0.141
92	H7N6F1S4b	Complex	65.3 min	n.d.	1840.485	1226.710	919.826	3681.970	3682.305	0.335
93	H7N7F1S4a	Complex	52.8 min	n.d.	n.d.	1294.095	970.534	3885.285	3885.384	0.099
94	H7N7F1S4b	Complex	53.9 min	n.d.	1942.075	n.d.	970.558	3885.150	3885.384	0.234

Table S4. Relative quantification of *N*-glycans released from NMuMG cell line using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 3C*) Proposed structures were assigned based on MS/MS fragmentation and glycobiological pathway constraints. Structures are depicted according to the CFG (Consortium of Functional Glycomics). Blue square: *N*-acetylglucosamine, green circle: mannose, yellow circle: galactose, red triangle: fucose, pink diamond: *N*-acetylneuraminic acid, H: hexose, N: *N*-acetylhexosamines, P: phosphate, a, b, c, d: isomer number. n=3. SD: standard variation; CV: coefficient of variation/relative standard deviation.

				Average relative abundance %. SD % and *CV*		
	Composition	**$[M-H]^{1-}$ ($[M-2H]^{2-}$) $[[M-3H]^{3-}]$**	**Proposed structure**	**Average relative abundance %**	**SD %**	***CV***
1	H2N2F1	**895.33**		**2.3**	**±0.4**	***15.9%***
4	H5N2	**1235.40**		**4.5**	**±0.7**	***15.1%***
5	H6N2a	**1397.45 (698.20)**		**8.7**	**±0.5**	***5.8%***
10	H7N2	**1559.50 (779.25)**		**5.1**	**±0.4**	***8.2%***
13	H7N2P2	**1719.37 (859.18)**		**2.4**	**±0.3**	***11.1%***

14	H8N2	**1721.53 (860.26)**		**7.0**	**±0.1**	***1.6%***
16	H9N2	**1883.59 (941.27)**		**25.0**	**±0.7**	***3.0%***
28	H6N3S1	**1891.59 (945.27)**		**5.3**	**±0.1**	***1.9%***
46	H5N5F1b	**1990.60 (994.81)**		**2.7**	**±0.2**	***8.7%***
57	H4N5F1S1a	**2119.64 (1059.32)**		**3.1**	**±0.2**	***7.2%***
58	H4N5F1S1b	**2119.65 (1059.33)**		**4.3**	**±0.2**	***4.3%***
67	H5N4F1S2a	**2369.72 (1184.36)**		**4.4**	**±0.4**	***8.2%***
68	H5N4F1S2c	**2369.75 (1184.37)**		**2.7**	**±0.4**	***16.2%***
71	H6N5F1S1a	**2443.78 (1221.39)**		**2.3**	**±0.4**	***15.8%***

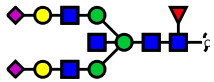
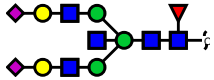
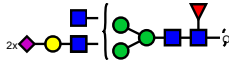
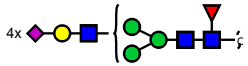
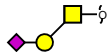
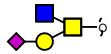
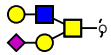
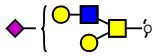
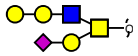
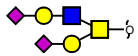
		[813.93]				
73	H5N5F1S2a	**2572.83 (1285.92) [856.90]**		**4.7**	**±0.4**	***7.9%***
74	H5N5F1S2b	**2572.82 (1285.91) [856.88]**		**8.5**	**±0.3**	***3.5%***
75	H5N5F1S2c	**2572.84 (1285.92) [856.96]**		**4.9**	**±0.3**	***6.4%***
91	H7N6F1S4a	**3682.16 (1840.58) [1226.73]**		**2.2**	**±0.2**	***7.9%***

Table S5. Relative quantification of *O*-glycans released from NMuMG using a 96-well plate sample preparation method and PGC nano-LC-ESI-MS/MS (*Relates to Figure 3D*) Proposed structures were assigned based on MS/MS fragmentation and glycobiological pathway constraints. Structures are depicted according to the CFG (Consortium of Functional Glycomics). Blue square: *N*-acetylglucosamine, green circle: mannose, yellow circle: galactose, red triangle: fucose, pink diamond: *N*-acetylneuraminic acid, a, b: isomer number. n=3; SD: standard variation; CV: coefficient of variation/relative standard deviation.

Composition	$[M-H]^{1-}$ ($[M-2H]^{2-}$)	Proposed structure	Average relative abundance %. SD % and *CV*		
			Average relative abundance %	SD %	*CV*
H1N1S1	**675.27**		**12.2**	**±0.6**	***4.5%***
H1N2S1	**878.29**		**1.7**	**±0.2**	***9.5%***
H2N2S1a	**1040.38**		**3.7**	**±0.2**	***5.3%***
H2N2S1b	**1040.38**		**6.1**	**±0.9**	***15.0%***
H3N2S1	**1202.41**		**6.6**	**±0.8**	***12.6%***
H2N2S2	**1331.48 (665.25)**		**29.4**	**±1.9**	***6.4%***

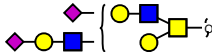
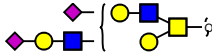
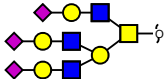
H3N3S2a	**1696.54 (847.77)**		**15.9**	**±1.9**	***11.9%***
H3N3S2b	**1696.54 (847.77)**		**15.5**	**±0.8**	***4.9%***
H4N4S3	**2352.80 (1175.90)**		**8.8**	**±0.8**	***8.6%***

Figure S1. Elution pattern of *N*-glycans with and without phosphate on PGC. Proposed structures were assigned based on MS/MS fragmentation and glycobiological pathway constraints. **A**. Combined extracted ion chromatograms of oligomannose H6N2 (Blue) and phosphorylated H6N2P1 (Red); **B**. Combined EIC of oligomannose H7N2 (Blue), phosphorylated H7N2P1 (Red) and diphosphorylated H7N2P2 (Green); **C**. Combined EIC of oligomannose H8N2 (Blue) and phosphorylated H8N2P1 (Red); **D**. Combined EIC of oligomannose H9N2 (Blue) and phosphorylated H9N2P1 (Red); **E**. Combined EIC of oligomannose H10N2 (Blue) and phosphorylated H10N2P1 (Red). Blue square, N: *N*-acetylglucosamine, green circle, H: mannose, P: phosphate.