Sample Preparation for Mass Spectrometric Analysis of Human Serum N-glycans by Using Hydrophilic Interaction Chromatography-Based Solid Phase Extraction

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Table S-1. The *m/z* list of the N-linked glycans identified in Figure 3B.

Table S-2. The intrabatch coefficients of variation for forty seven human serum N-linked glycans identified in the present study.

Table S-3. The interbatch coefficients of variation for forty seven human serum N-linked glycans identified in the present study.

Figure S-1. MALDI mass spectra of the N-glycans released from human serum proteins after enrichment with Click TE-Cys. The contaminants were washed out five times with 200 µl of ACN/H_2O/FA (80/19/1). ■: N-acetylglucosamine; ●: Mannose or Galactose; ▲: Fucose; ◆: Sialic acid.

Figure S-2. MS/MS spectra of the N-glycans released from IgG: (A) with *m/z* 1485.410 (1+); (B) with *m/z* 1647.442 (1+); (C) with *m/z* 2012.539 (1+). ■: N-acetylglucosamine; ●: Mannose; ●: Galactose; ▲: Fucose.

Figure S-3. MALDI mass spectra of a mixture of the N-glycans released from RNase B and the tryptic digest of HSA (1:1, w/w): (A) by direct analysis; (B) after enrichment with Click TE-Cys; N-linked glycans are labeled with their structures. ■: N-acetylglucosamine; ●: Mannose.

Figure S-4. MALDI mass spectra of the N-glycans released from human serum proteins by direct analysis.

Figure S-5. MS/MS spectra of the N-glycans released from human serum proteins: (A) with *m/z* 933.140 (1+); (B) with *m/z* 1095.163 (1+); (C) with *m/z* 1136.188 (1+); (D) with *m/z* 1257.187 (1+); (E) with *m/z* 1298.202 (1+); (F) with *m/z* 1501.248 (1+); (G) with *m/z* 1663.277 (1+); (H) with *m/z* 1809.303 (1+); (I) with *m/z* 1976.296 (1+). ■: N-acetylglucosamine; ●: Mannose; ●: Galactose; ◆: Sialic acid.

Table S-1.

Observed *m/z*	Calculated *m/z*	Delta molecular weight (Da) [a]
1136.285 (1+)	1136.396 (1+)	-0.111
1282.344 (1+)	1282.454 (1+)	-0.110
1298.324 (1+)	1298.448 (1+)	-0.124
1339.368 (1+)	1339.475 (1+)	-0.107
1444.378 (1+)	1444.506 (1+)	-0.128
1485.410 (1+)	1485.533 (1+)	-0.123
1501.384 (1+)	1501.528 (1+)	-0.144
1647.442 (1+)	1647.586 (1+)	-0.144
1663.426 (1+)	1663.581 (1+)	-0.155
1688.468 (1+)	1688.612 (1+)	-0.144
1809.484 (1+)	1809.639 (1+)	-0.155
1850.505 (1+)	1850.665 (1+)	-0.160
1866.484 (1+)	1866.660 (1+)	-0.176
2012.539 (1+)	2012.718 (1+)	-0.179
2122.539 (1+)	2122.730 (1+)	-0.191
2325.618 (1+)	2325.809 (1+)	-0.191

[a]: Delta molecular weight = Observed *m/z*—Calculated *m/z*

Table S-2.

Observed *m/z*	Intrabatch Experiment 1 Normalized Intensity	Intrabatch Experiment 2 Normalized Intensity	Intrabatch Experiment 3 Normalized Intensity	Coefficient of Variation
933.140	0.0228	0.0230	0.0237	0.0157
1095.163	0.0332	0.0355	0.0366	0.0408
1136.188	0.0112	0.0119	0.0126	0.0459
1257.187	0.0281	0.0268	0.0353	0.1252
1282.209	0.0114	0.0115	0.0129	0.0570
1298.202	0.0638	0.0630	0.0662	0.0211
1339.220	0.0057	0.0054	0.0056	0.0273
1419.207	0.0128	0.0110	0.0138	0.0919
1444.232	0.0288	0.0302	0.0279	0.0327
1460.230	0.0376	0.0383	0.0386	0.0111
1485.258	0.0159	0.0155	0.0169	0.0381
1501.248	0.0097	0.0102	0.0156	0.2280
1542.264	0.0175	0.0165	0.0161	0.0342
1581.231	0.0128	0.0151	0.0153	0.0781
1606.265	0.0028	0.0022	0.0030	0.1335
1622.244	0.0045	0.0043	0.0023	0.2647
1647.274	0.0333	0.0360	0.0336	0.0350
1663.277	0.1843	0.1873	0.1846	0.0072
1688.271	0.0018	0.0022	0.0020	0.0686

1704.290	0.0034	0.0038	0.0033	0.0577
1727.243	0.0051	0.0057	0.0061	0.0747
1743.247	0.0038	0.0032	0.0043	0.1259
1777.228	0.0036	0.0045	0.0037	0.1068
1809.303	0.0640	0.0661	0.0598	0.0417
1814.265	0.0517	0.0511	0.0518	0.0057
1825.284	0.0032	0.0038	0.0022	0.2129
1850.324	0.0071	0.0072	0.0063	0.0549
1866.321	0.0069	0.0065	0.0075	0.0561
1889.268	0.0014	0.0017	0.0012	0.1574
1905.258	0.0023	0.0017	0.0021	0.1223
1976.296	0.1178	0.1102	0.1109	0.0305
2012.346	0.0281	0.0288	0.0247	0.0658
2017.276	0.0112	0.0133	0.0130	0.0756
2028.334	0.0138	0.0132	0.0134	0.0200
2122.322	0.0319	0.0291	0.0274	0.0632
2110.283	0.0020	0.0018	0.0013	0.1754
2174.316	0.0051	0.0066	0.0065	0.1099
2240.320	0.0013	0.0016	0.0016	0.1028
2289.311	0.0545	0.0503	0.0484	0.0495
2325.356	0.0150	0.0145	0.0134	0.0466
2341.349	0.0086	0.0103	0.0096	0.0753

2393.358	0.0020	0.0024	0.0025	0.0971
2435.344	0.0057	0.0065	0.0045	0.1490
2487.383	0.0027	0.0020	0.0029	0.1486
2638.389	0.0043	0.0031	0.0032	0.1571
2654.360	0.0046	0.0044	0.0043	0.0270
2800.468	0.0007	0.0008	0.0013	0.2605

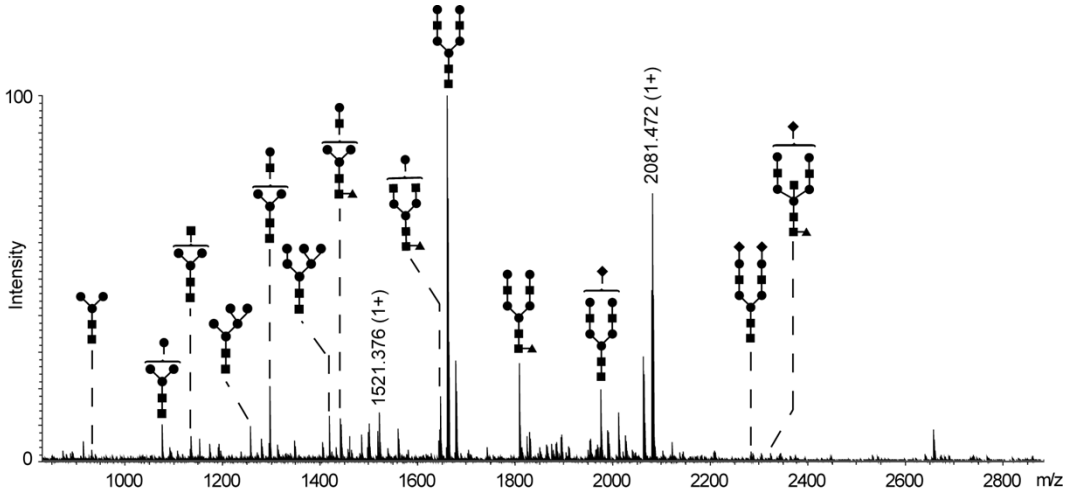
Table S-3.

Observed *m/z*	Interbatch Experiment 1 Normalized Intensity	Interbatch Experiment 2 Normalized Intensity	Interbatch Experiment 3 Normalized Intensity	Coefficient of Variation
933.140	0.0228	0.0201	0.0224	0.0551
1095.163	0.0332	0.0363	0.0373	0.0488
1136.188	0.0112	0.0111	0.0110	0.0089
1257.187	0.0281	0.0252	0.0345	0.1329
1282.209	0.0114	0.0108	0.0053	0.2985
1298.202	0.0638	0.0613	0.0682	0.0442
1339.220	0.0057	0.0064	0.0058	0.0535
1419.207	0.0128	0.0115	0.0149	0.1096
1444.232	0.0288	0.0285	0.0278	0.0133
1460.230	0.0376	0.0406	0.0408	0.0367
1485.258	0.0159	0.0120	0.0120	0.1378
1501.248	0.0097	0.0101	0.0101	0.0175
1542.264	0.0175	0.0186	0.0180	0.0259
1581.231	0.0128	0.0156	0.0157	0.0911
1606.265	0.0028	0.0024	0.0031	0.0935
1622.244	0.0045	0.0038	0.0033	0.1262
1647.274	0.0333	0.0320	0.0325	0.0162
1663.277	0.1843	0.1607	0.1795	0.0583
1688.271	0.0018	0.0017	0.0016	0.0569

1704.290	0.0034	0.0024	0.0026	0.1624
1727.243	0.0051	0.0065	0.0059	0.1006
1743.247	0.0038	0.0026	0.0033	0.1476
1777.228	0.0036	0.0050	0.0043	0.1368
1809.303	0.0640	0.0597	0.0613	0.0292
1814.265	0.0517	0.0640	0.0550	0.0910
1825.284	0.0032	0.0044	0.0043	0.1317
1850.324	0.0071	0.0071	0.0064	0.0493
1866.321	0.0069	0.0071	0.0061	0.0635
1889.268	0.0014	0.0019	0.0016	0.1103
1905.258	0.0023	0.0019	0.0017	0.1361
1976.296	0.1178	0.1149	0.1153	0.0110
2012.346	0.0281	0.0323	0.0277	0.0706
2017.276	0.0112	0.0142	0.0120	0.1001
2028.334	0.0138	0.0135	0.0134	0.0152
2122.322	0.0319	0.0333	0.0301	0.0402
2110.283	0.0020	0.0022	0.0020	0.0532
2174.316	0.0051	0.0051	0.0051	0.0018
2240.320	0.0013	0.0023	0.0021	0.2302
2289.311	0.0545	0.0596	0.0529	0.0519
2325.356	0.0150	0.0193	0.0167	0.1056
2341.349	0.0086	0.0112	0.0094	0.1116

2393.358	0.0020	0.0024	0.0018	0.1110
2435.344	0.0057	0.0063	0.0055	0.0591
2487.383	0.0027	0.0020	0.0016	0.2270
2638.389	0.0043	0.0037	0.0031	0.1339
2654.360	0.0046	0.0051	0.0039	0.1082
2800.468	0.0007	0.0015	0.0009	0.3188

Figure S-1.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1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
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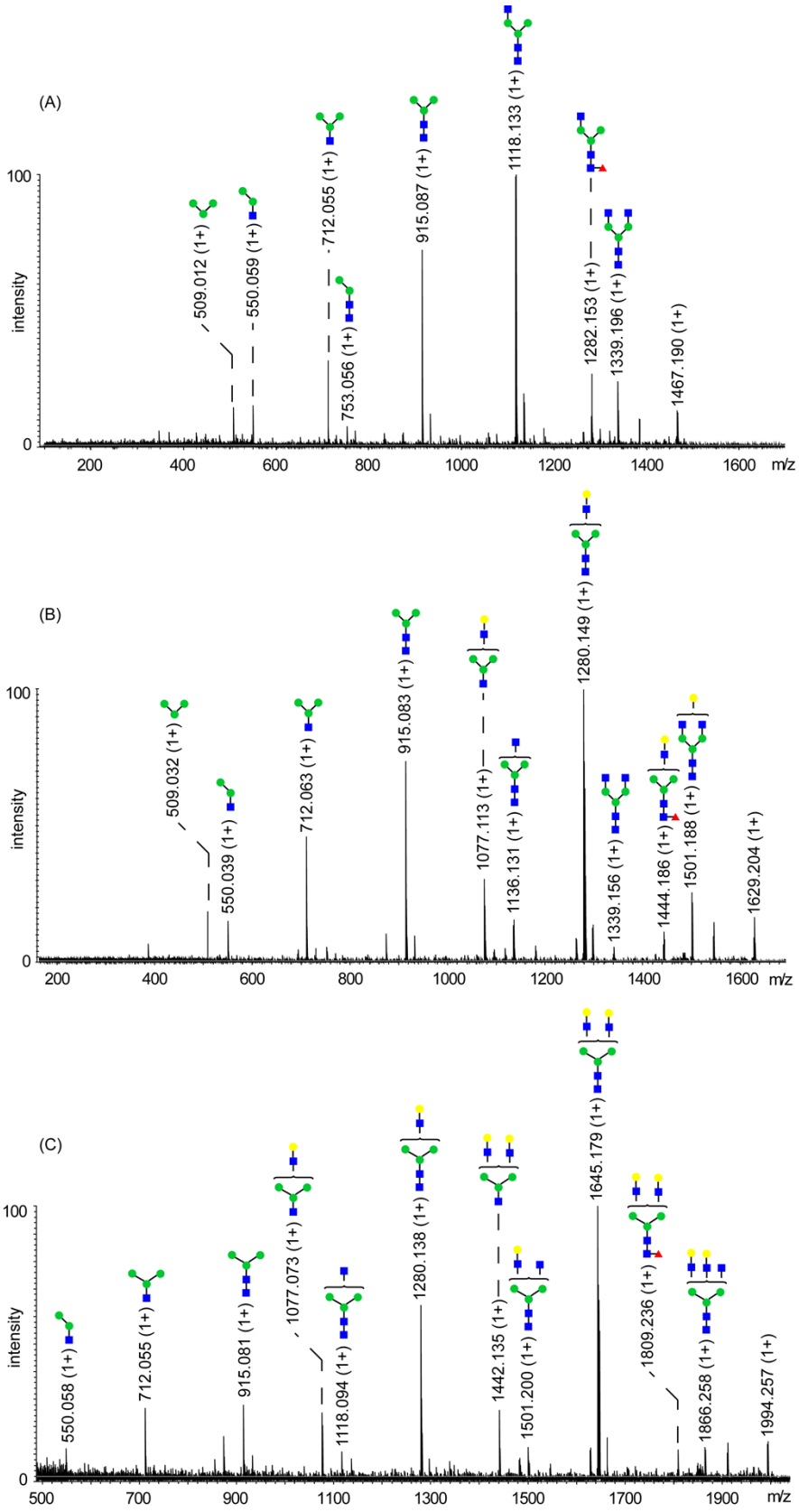
2081.472 (1+)

Figure S-2.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550.059 (1+)
712.055 (1+)
753.056 (1+)
915.087 (1+)
1118.133 (1+)
1282.153 (1+)
1339.196 (1+)
1467.190 (1+)
200
400
600
800
1000
1200
1400
1600
m/z
(B)
100
intensity
0
509.032 (1+)
550.039 (1+)
712.063 (1+)
915.083 (1+)
1077.113 (1+)
1136.131 (1+)
1280.149 (1+)
1339.156 (1+)
1444.186 (1+)
1501.188 (1+)
1629.204 (1+)
200
400
600
800
1000
1200
1400
1600
m/z
(C)
100
intensity
0
550.058 (1+)
712.055 (1+)
915.081 (1+)
1077.073 (1+)
1118.094 (1+)
1280.138 (1+)
1442.135 (1+)
1501.200 (1+)
1645.179 (1+)
1809.236 (1+)
1866.258 (1+)
1994.257 (1+)
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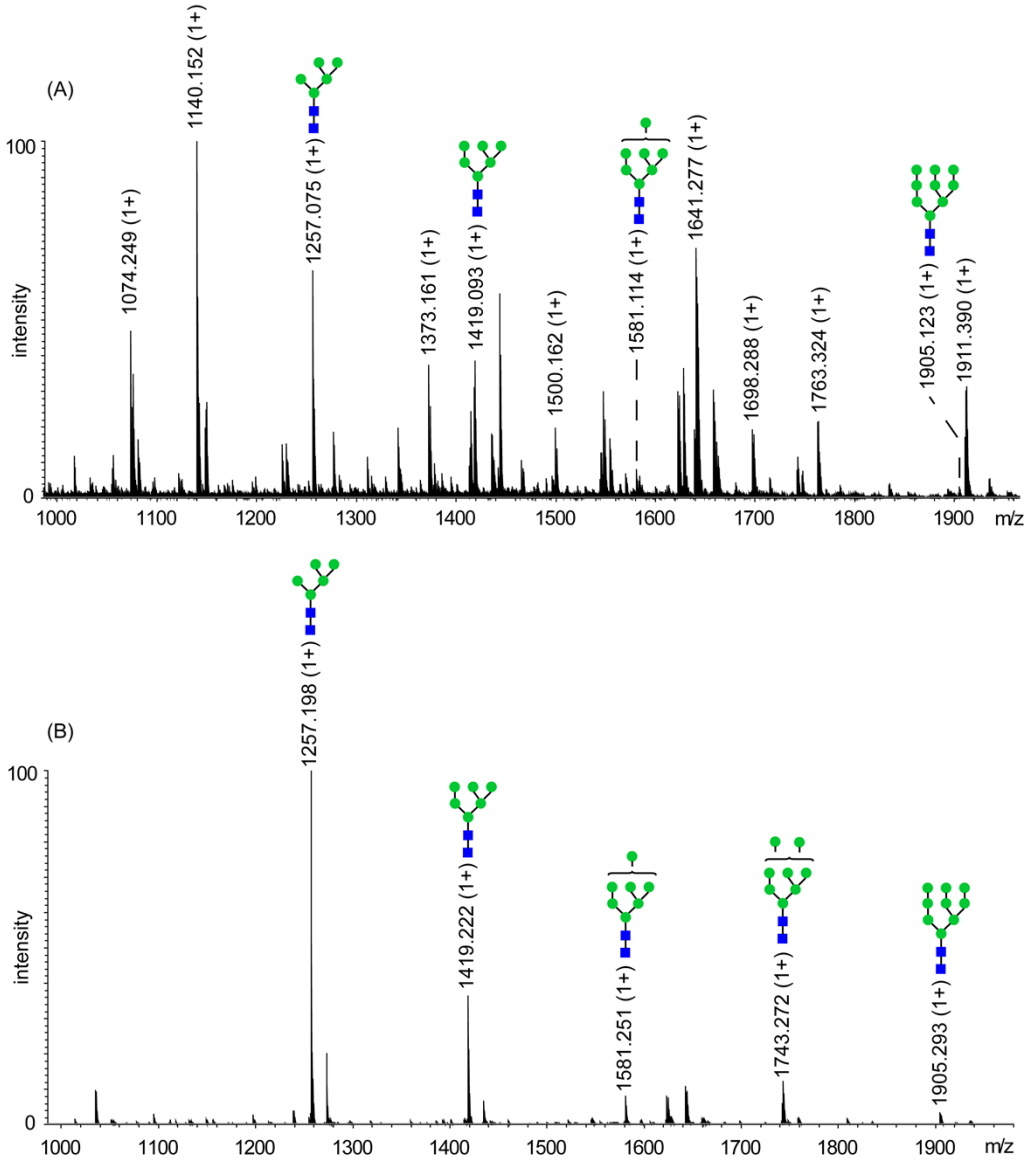
900
1100
1300
1500
1700
1900
m/z

Figure S-3.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1581.114 (1+)
1641.277 (1+)
1698.288 (1+)
1763.324 (1+)
1905.123 (1+)
1911.390 (1+)
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(B)
100
intensity
0
1257.198 (1+)
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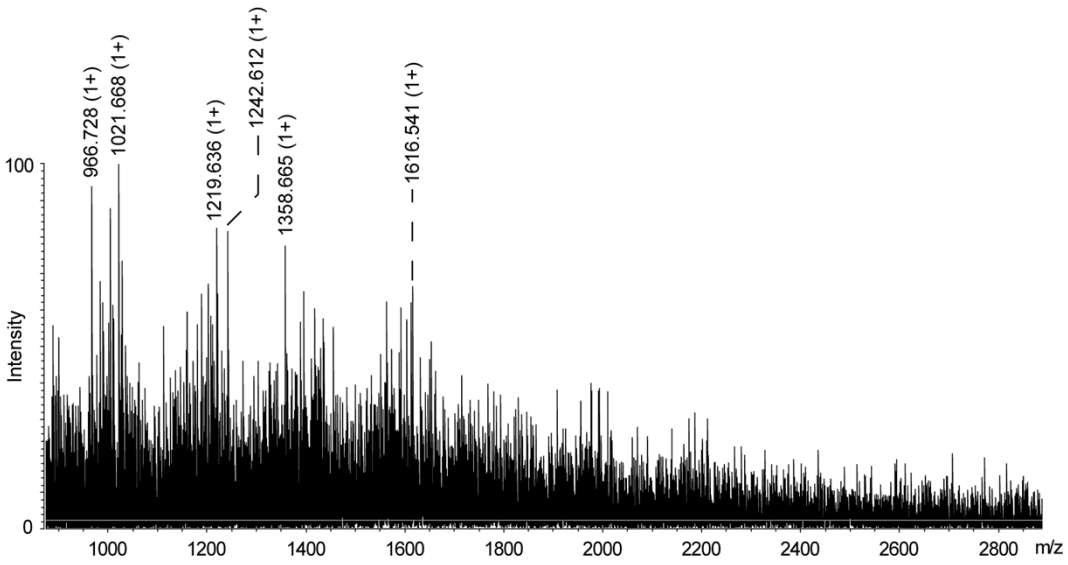
Figure S-4.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1021.668 (1+)
1219.636 (1+)
1242.612 (1+)
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1616.541 (1+)
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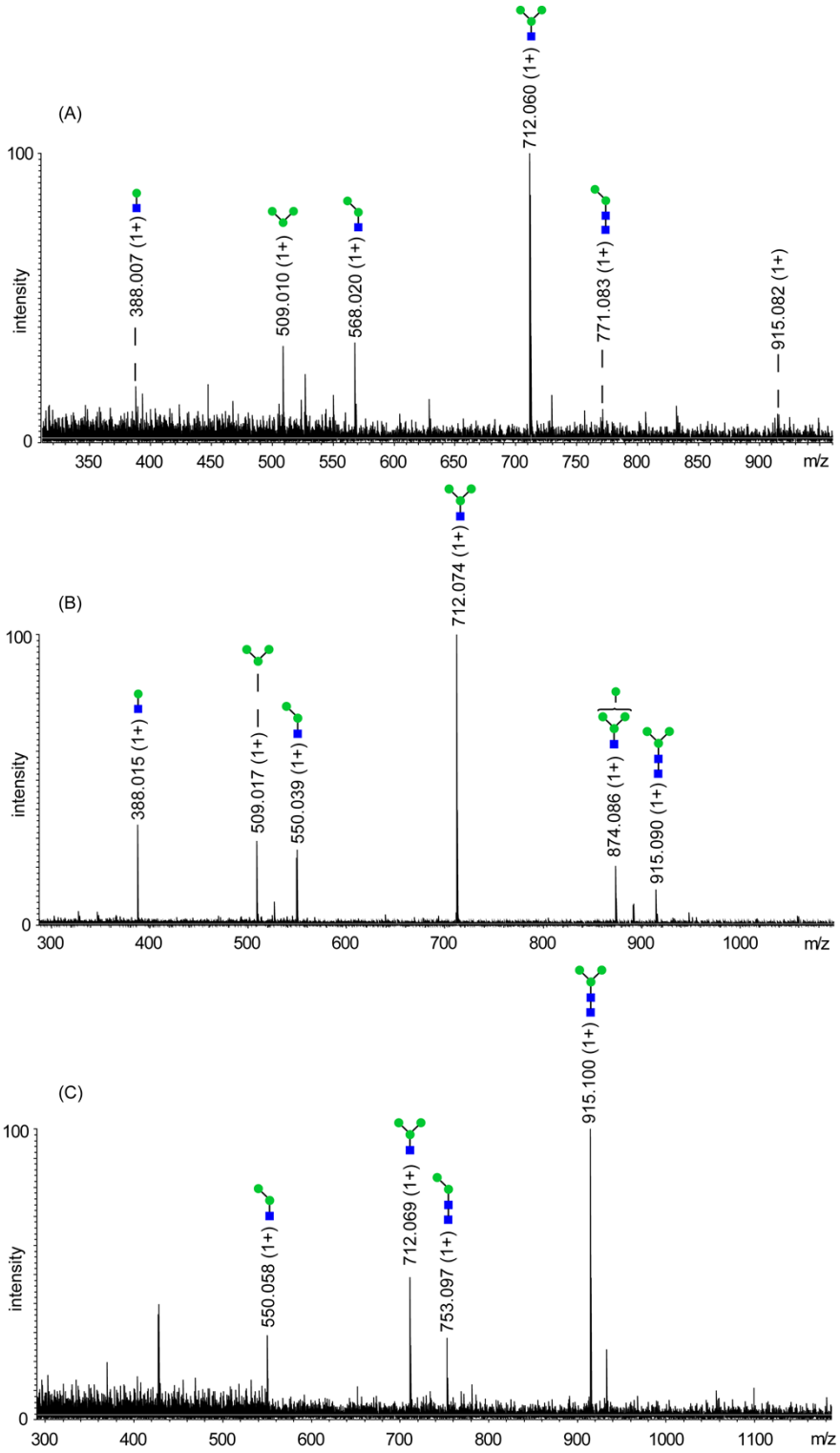
Intensity
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z

Figure S-5.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771.083 (1+)
915.082 (1+)
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(B)
100
intensity
0
388.015 (1+)
509.017 (1+)
550.039 (1+)
712.074 (1+)
874.086 (1+)
915.090 (1+)
300 400 500 600 700 800 900 1000 m/z
(C)
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intensity
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550.058 (1+)
712.069 (1+)
753.097 (1+)
915.100 (1+)
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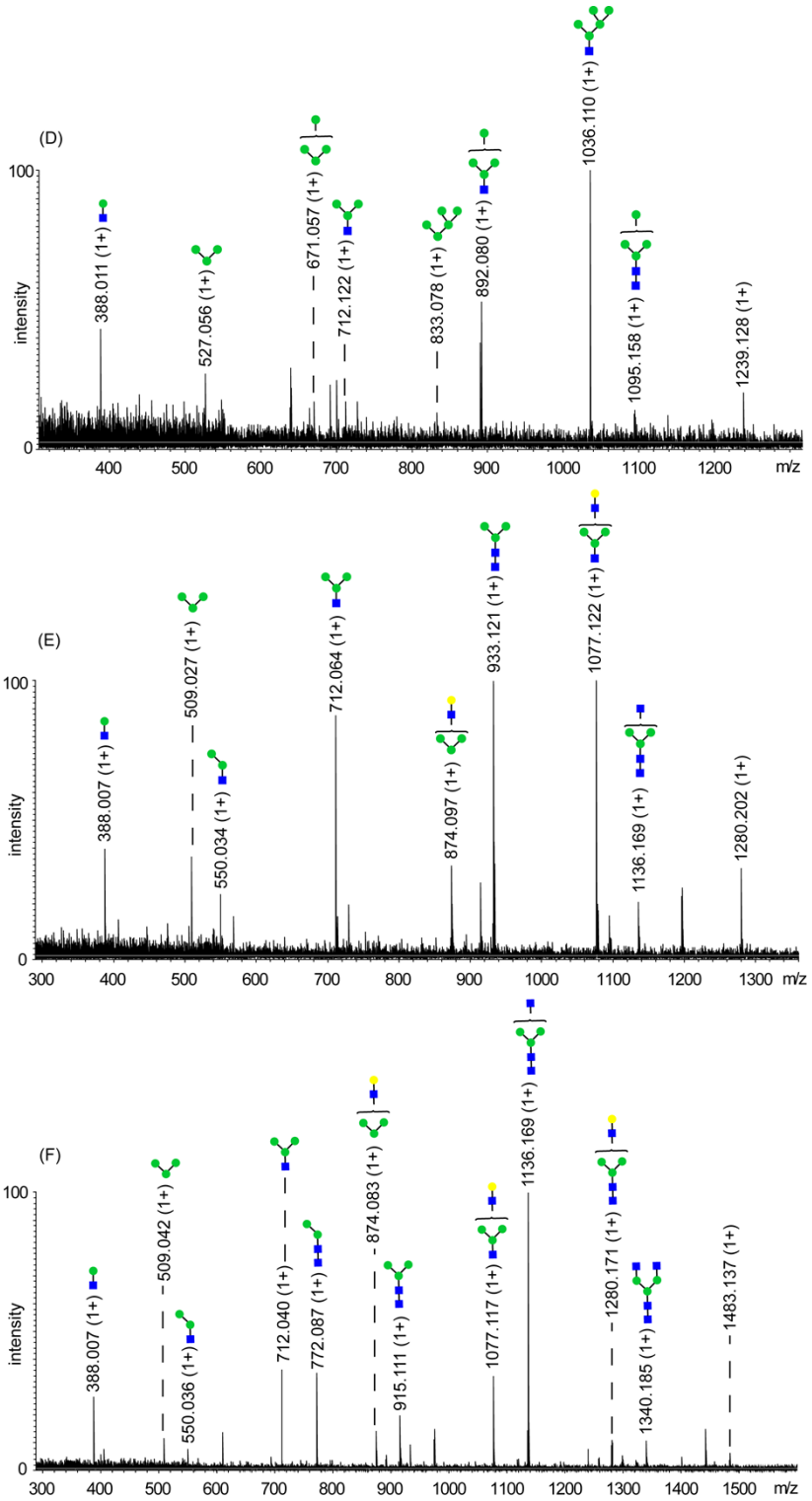
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1036.110 (1+)
1095.158 (1+)
1239.128 (1+)
400
500
600
700
800
900
1000
1100
1200
m/z
(E)
100
intensity
0
388.007 (1+)
509.027 (1+)
550.034 (1+)
712.064 (1+)
874.097 (1+)
933.121 (1+)
1077.122 (1+)
1136.169 (1+)
1280.202 (1+)
300
400
500
600
700
800
900
1000
1100
1200
1300
m/z
(F)
100
intensity
0
388.007 (1+)
509.042 (1+)
550.036 (1+)
712.040 (1+)
772.087 (1+)
874.083 (1+)
915.111 (1+)
1077.117 (1+)
1136.169 (1+)
1280.171 (1+)
1340.185 (1+)
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400
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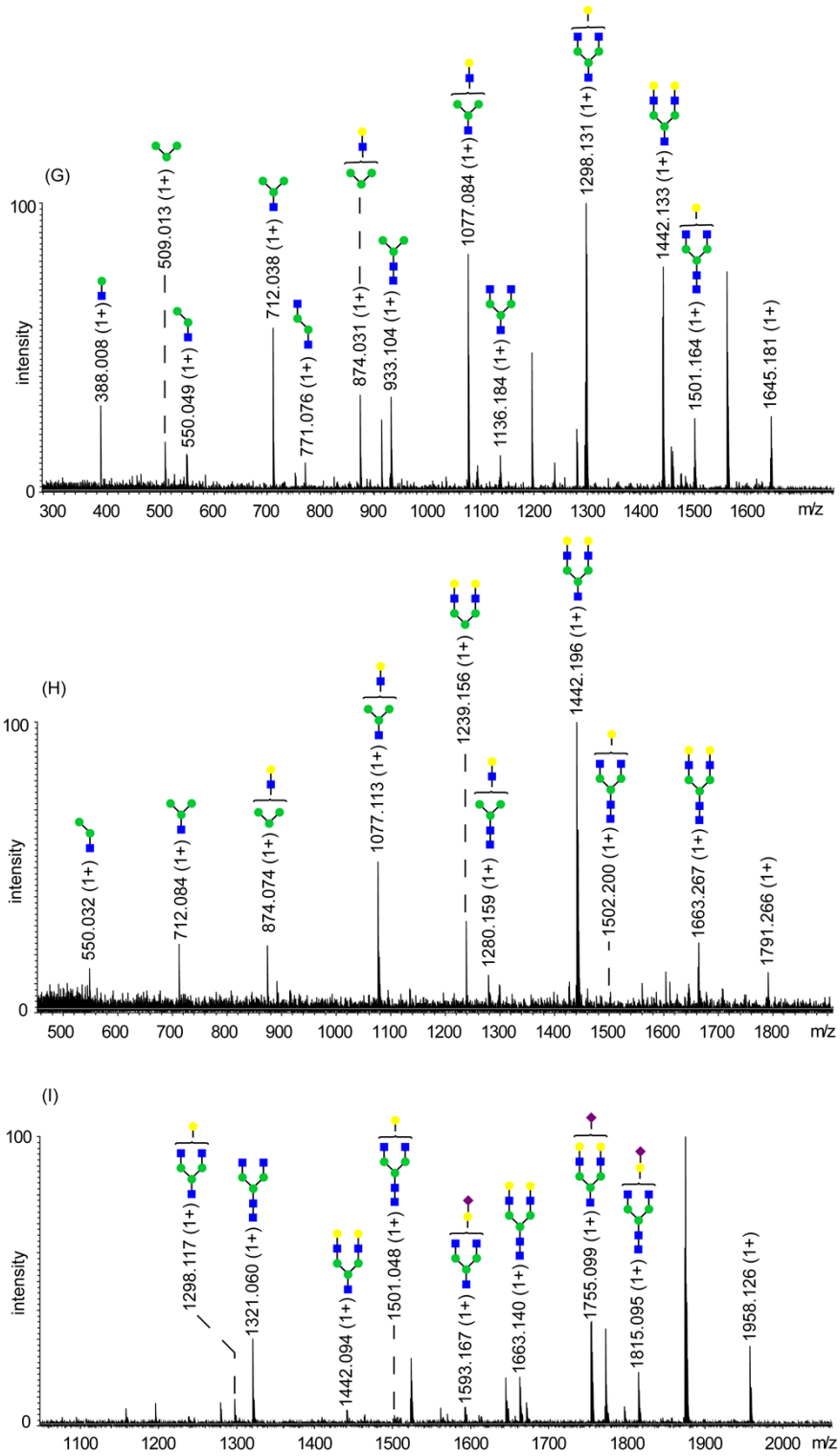
1100
1200
1300
1400
1500
m/z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1136.184 (1+)
1298.131 (1+)
1442.133 (1+)
1501.164 (1+)
1645.181 (1+)
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(H)
100
intensity
0
550.032 (1+)
712.084 (1+)
874.074 (1+)
1077.113 (1+)
1239.156 (1+)
1280.159 (1+)
1442.196 (1+)
1502.200 (1+)
1663.267 (1+)
1791.266 (1+)
500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 m/z
(I)
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intensity
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1321.060 (1+)
1442.094 (1+)
1501.048 (1+)
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1663.140 (1+)
1755.099 (1+)
1815.095 (1+)
1958.126 (1+)
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