Supplementary Information for:

Selective Enrichment of N-linked Glycopeptides by Using a Highly Hydrophilic Matrix Synthesized via Click Chemistry

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Materials and reagents. Spherical silica (5 μm particle size, 10 nm pore size, 300 m^2 g^{-1} surface area) was obtained from Fuji Silysia Chemical (Aichi, Japan). 3-Chloropropyltriethoxysilane was purchased from ABCR (Karlsruhe, Germany). Dimethylformamide (DMF), dichloromethane, acetone, sodium azide and sodium iodide were obtained from Tianjin Chemical Reagents (Tianjin, China). Propiolic acid was purchased from Acros (Fair Lawn, NJ). Human serum immunoglobulin G (IgG), bovine ribonuclease B (RNase B), human serum α_1-acid glycoprotein (AGP), chicken ovalbumin, Sepharose CL-6B, dithiothreitol (DTT), iodoacetic acid (IAA) and ammonium acetate (NH_4Ac) were purchased from Sigma (St. Louis, MO). Sequencing-grade-modified trypsin was obtained from Promega (Madison, WI). GELoader tips were from Eppendorf (Hamburg, Germany). The weak-cationic-exchange (WCX) matrix was purchased from Sipore (Dalian, China). C18 material for desalting was obtained from Sunchrom (Friedrichsdorf, Germany). Formic acid (FA) and methanol were from Acros (Geel, Belgium). Acetonitrile (CH_3CN) was purchased from Merck (Darmstadt, Germany). Water was purified by the Milli-Q system (Millipore, Bedford, MA).

Synthesis of azide-silica. Sodium azide (7.8 g) was added to the mixture of 3-chloropropyltriethoxysilane (24.0 mL) and dehydrated DMF (500.0 mL). Then, by the catalysis of sodium iodide (2.0 g), the reaction was carried out at 90-100 °C for 24 hours. Spherical silica (25 g) was added to the resulting mixture cooled to ambient temperature, followed by the reaction at 110 °C for another 24 hours. The resulting solids were washed in turn with dichloromethane, methanol, water and acetone (twice each), followed by the dryness at 60 °C for 12 hours.

Synthesis of Click CA. Propiolic acid (2.8 g) was dissolved in 60 mL of water/methanol (1:1, v/v) before the addition of azide-silica (10.0 g). The mixture was stirring at 75 °C for 48 hours. After filtration, the resulting solids (Click CA) were washed in turn with methanol, water and methanol (twice each), followed by the dryness at 60 °C for 12 hours.

Re-purification of commercial ovalbumin by using reversed phase liquid chromatography (RPLC). Purification experiments were performed on an Alliance HPLC system (Waters, Milford, MA). Commercial ovalbumin samples (1

mg mL^{-1}) were separated on a customized C8 column (150 mm×4.6 mm, 5 μm, 300 Å) at the flowrate of 1 mL min^{-1} and detected with a photodiode array detector. Solvent A was H_2O/0.05%TFA and solvent B was CH_3CN/0.05%TFA.The following gradient was used: 0-5 min, 10% B; 5-15 min, 10-45%B; 15-30 min, 45-55%B and 30-45 min, 55-80%B. The fraction from 20 to 28 min was collected manually and dried for tryptic digestion (See Fig. S1).

Glycoprotein digestion. Glycoproteins (500 μg) were denatured in urea (8 M) in NH_4HCO_3 solution (50 mM, 100 μL). After incubation for 3 h, the resulting samples were reduced with DTT (50 mM, 4 μL) for another 2 h at 37 °C, followed by the addition of IAA (50 mM, 5 μL) for alkylation. The solution was incubated in dark for 30 min at ambient temperature and then diluted tenfold with NH_4HCO_3 (50 mM) buffer. Trypsin was added to the solution at an enzyme/substrate ratio of 1:30 (w/w) and incubated for 16 h at 37 °C.

HILIC SPE of glycopeptides by using Click CA. All the enrichment procedures were performed under the optimized conditions. Click CA (about 1 mg) was slurried in CH_3CN (45 μL) and packed into the GELoader tip with an inert sieve plate placed in the tail. The resulting microcolumn was washed with CH_3CN/H_2O/FA (10:90:0.1 (v/v), 45 μL). For the enrichment of IgG and RNase B glycopeptides, tryptic digests were dried, redissolved in CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) and loaded onto the column, which has been equilibrated with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 90 μL). The column was then rinsed four times with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) to remove non-glycosylated peptides, followed by elution with CH_3CN/H_2O/FA (60:40:0.1 (v/v), 20 μL) for another three times. For the enrichment of ovalbumin glycopeptides, dried tryptic digest redissolved in CH_3CN/H_2O/FA (70:30:0.1 (v/v), 10 μL) was loaded onto the column equilibrated with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 90 μL), followed by rinsing with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 35 μL). Then, the column was eluted three times with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL). The resulting three fractions were combined, dried, redissolved in CH_3CN/H_2O/FA (50:50:0.1 (v/v), 20 μL) for the MS characterization. For the enrichment of AGP glycopeptides, dried tryptic digest was redissolved in CH_3CN/H_2O/FA (70:30:0.1 (v/v)) and loaded on the microcolumn that has been equilibrated with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 90 μL), followed by rinsing twice with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL). Then, the column was eluted with CH_3CN/H_2O/FA (50:50:0.1 (v/v), 90 μL). The glycopeptide fractions were dried and redissolved in H_2O/FA (100:0.1 (v/v), 20 μL) for nano-RPLC-MS analysis.

Glycopeptide elution window experiment by using the WCX matrix. The slurry (45 μL) of WCX matrix (about 1 mg) was pushed into the tail of the GELoader tip. The microcolumn was washed with CH_3CN/H_2O/FA (10:90:0.1 (v/v), 45 μL) and equilibrated with CH_3CN/H_2O/FA (80:20:0.1 (v/v), 90 μL). The tryptic digest of RNase B was dried, dissolved in CH_3CN/H_2O/FA (80:20:0.1 (v/v)) and loaded onto the column. The column was rinsed or eluted three times with CH_3CN/H_2O/FA (80:20:0.1 (v/v), 20 μL), CH_3CN/H_2O/FA (60:40:0.1 (v/v), 20 μL) and CH_3CN/H_2O/FA (50:50:0.1 (v/v), 20 μL), respectively. Each fraction was directly infused to MS and characterized.

HILIC SPE of glycopeptides by using Sepharose. The conditions for the enrichment of IgG and RNase B glycopeptides have also been optimized. Briefly, packed Sepharose CL-6B (10 μL) was pushed into the tail of the GELoader tip. The microcolumn was washed with CH_3CN/H_2O/FA (10:90:0.1 (v/v), 45 μL) and equilibrated with CH_3CN/H_2O/FA (80:20:0.1 (v/v), 90 μL). The tryptic digest of IgG or RNase B was dried, dissolved in CH_3CN/H_2O/FA (80:20:0.1 (v/v)) and loaded onto the column. The column was rinsed with CH_3CN/H_2O/FA (80:20:0.1 (v/v), 45 μL) to remove the non-glycosylated peptides, followed by the elution of glycopeptides with CH_3CN/H_2O/FA (50:50:0.1 (v/v), 90 μL). The glycopeptide fractions were dried and redissolved in CH_3CN/H_2O/FA (50:50:0.1 (v/v), 20 μL) for MS analysis.

Desalting. C18 material (about 1 mg) slurried in CH_3CN (45 μL) was packed into the GELoader tip. The microcolumn was washed with CH_3CN/H_2O/FA (50:50:0.1 (v/v), 45 μL) and then equilibrated with H_2O/FA (100:0.1 (v/v), 90 μL). After peptide mixture was loaded, the column was rinsed with H_2O/FA (100:0.1 (v/v), 45 μL), followed by the elution of peptide fraction with CH_3CN/H_2O/FA (50:50:0.1 (v/v), 20 μL).

Nano-ESI-MS and nano-RPLC-ESI-MS analysis. MS experiments were performed on an X'TremeSimple nano-LC system (Micro-Tech Scientific, Vista, CA) coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Manchester, UK). The nano-ESI source was operated under positive ion mode and the capillary voltage was set at 2.0 kV. For RNase B, IgG and ovalbumin, each fraction from Click CA or C18 was directly infused into the mass spectrometer. MS data were acquired at m/z 500-2000. For AGP, glycopeptide fractions were dried and redissolved in H_2O/FA (100:0.1 (v/v)) prior to RPLC separation. The resulting samples were first loaded onto a C18 trap column (10 mm×150 μm, 3 mm, Micro-Tech Scientific, Vista, CA) at the flowrate of 3 μL min^{-1}. Then, peptides on the trap column were transferred to the C18 analytical column (150 mm×150 μm, 3 μm, Micro-Tech Scientific, Vista, CA), on which they were separated at the flowrate of 1 μL min^{-1}. Mobile phase A for RPLC separation is H_2O/0.1%FA and mobile phase B is CH_3CN/0.1%FA. The following gradient was used: 5% B for the first 5 min, 5-35% B in 45 min and 35% B for the last 10 min. MS data were acquired at m/z 500-2000 for MS analysis and m/z 100-2000 for MS/MS analysis. To confirm the deduced glycopeptide structure, MS/MS analysis was performed with the collision energy varied from 20 to 40 eV depending on the size and charge of the glycopeptides.

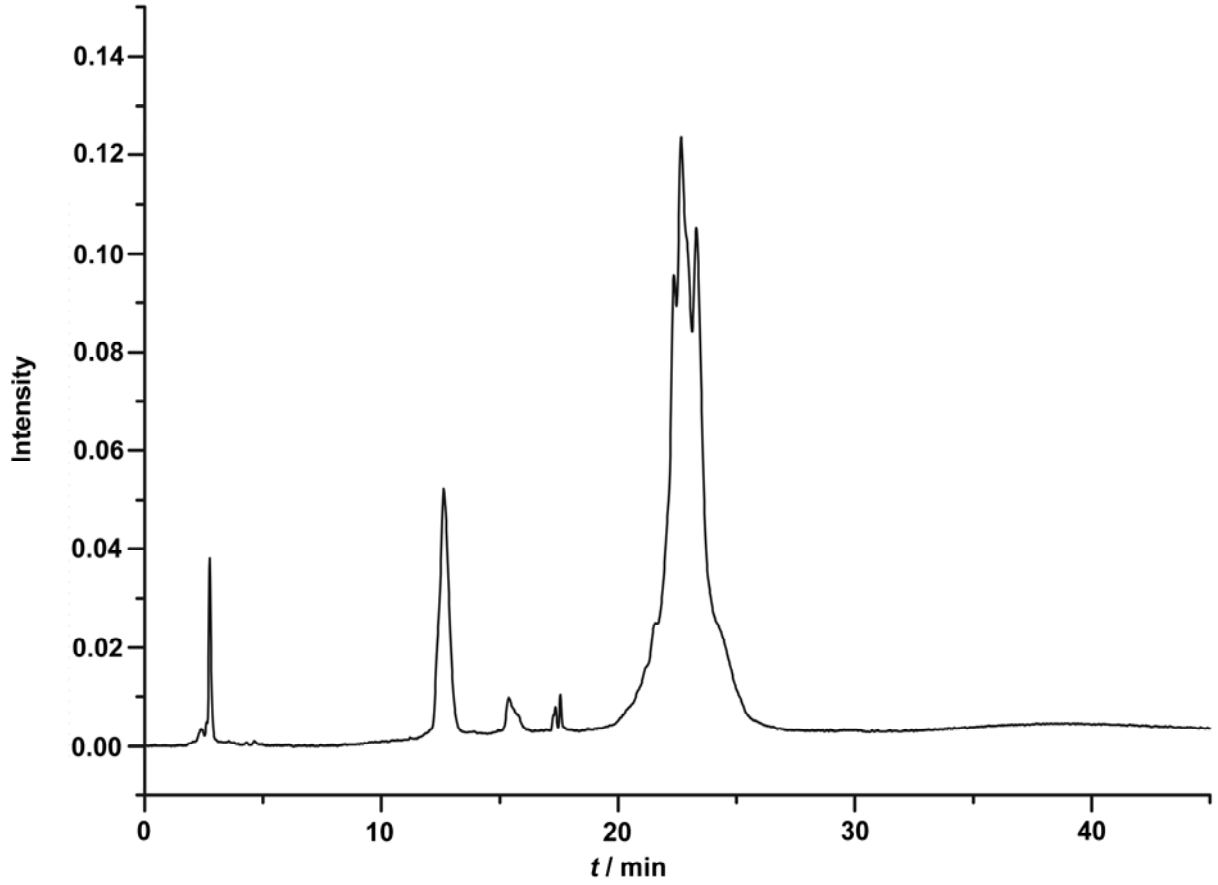

Fig. S1 Re-purification of commercially available ovalbumin by using RPLC.

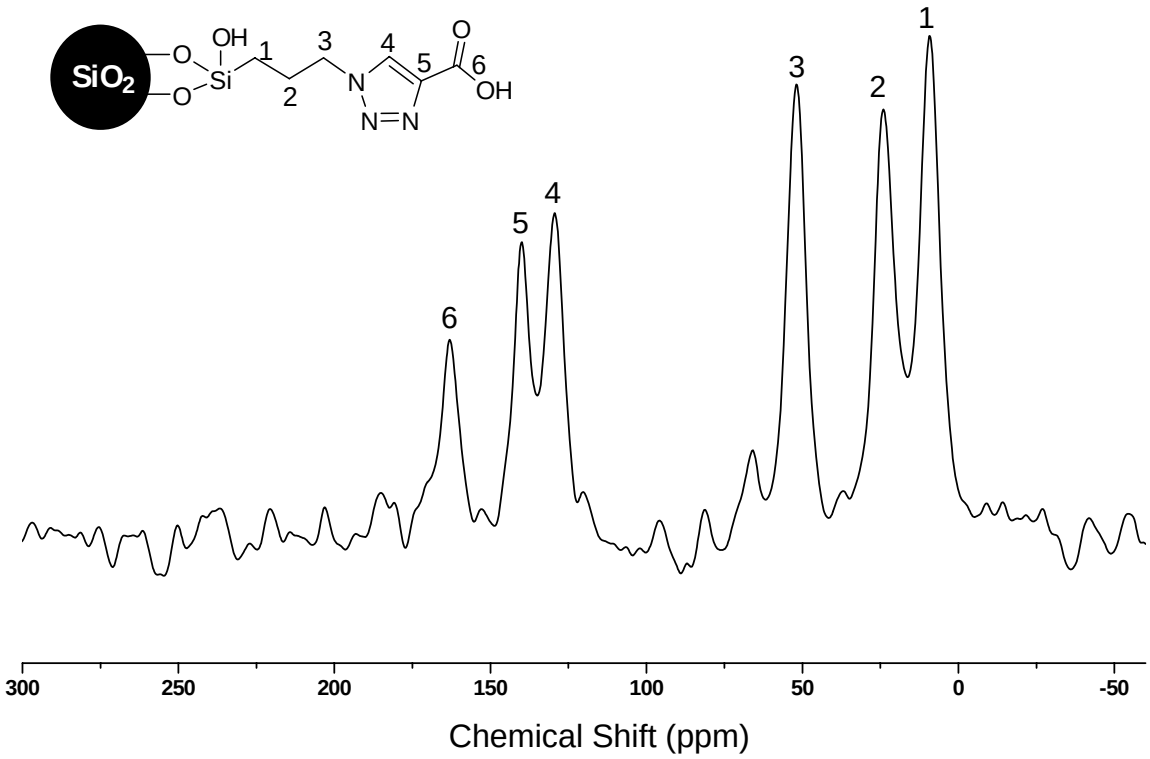

Fig. S2 Solid state ^{13}C NMR spectra of Click CA. Peak 6 at 163.1 ppm is assigned to the carbon atoms of the carboxyl group. Peak 5 and 4 at 140.1 ppm and 129.3 ppm are assigned to the two carbon atoms in the triazole ring. The three peaks 1, 2 and 3 at 9.6 ppm, 24.6 ppm and 52.1 ppm are assigned to the rest three carbon atoms.

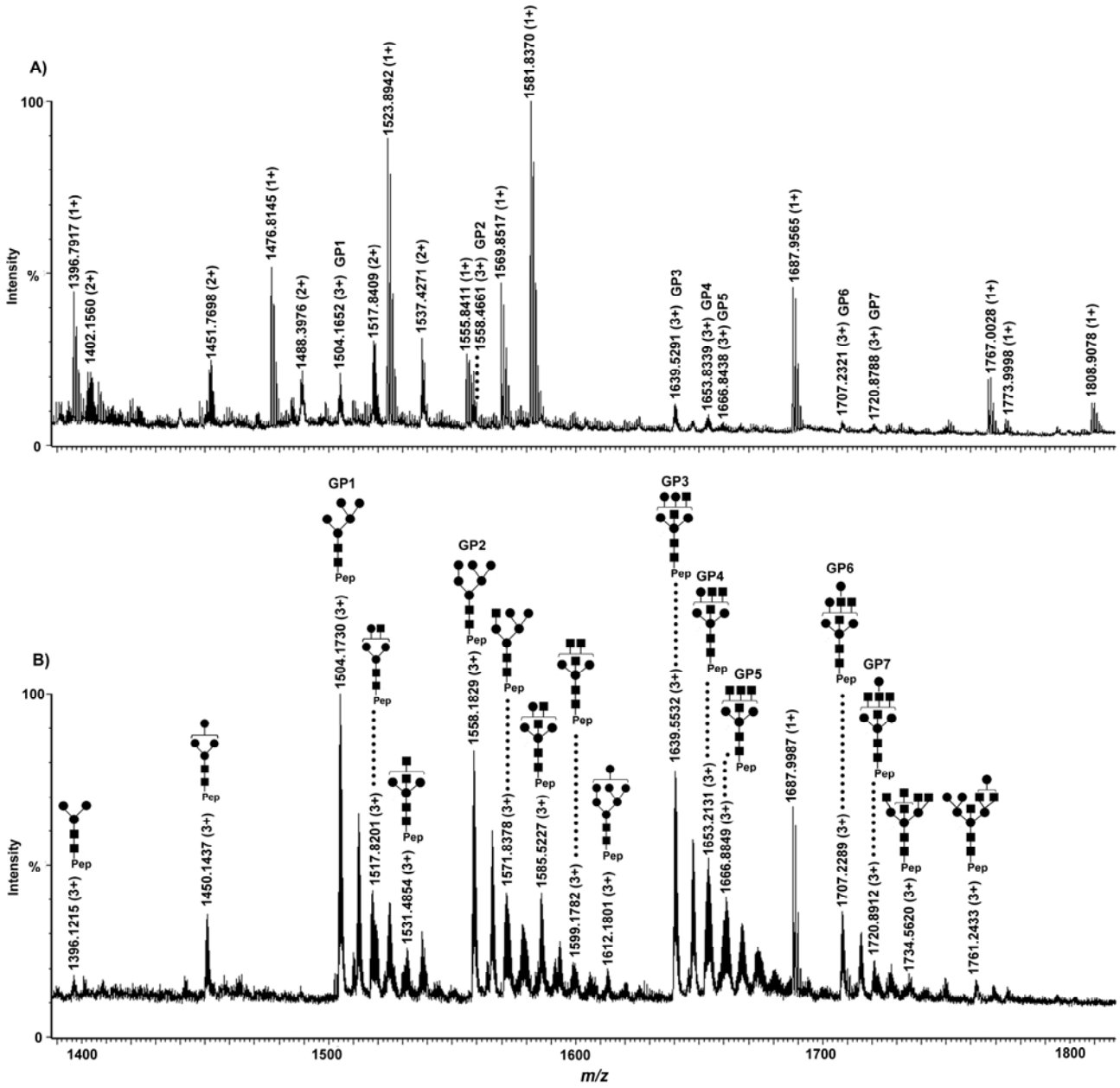

Fig. S3 Mass spectrum of desalted ovalbumin digest (A) and the ovalbumin glycopeptide fraction enriched with Click CA (B). Glycopeptides are labelled with their structures. ■ N-acetylglucosamine; ● Mannose or Galactose. Peptide sequence YNLTSVLMAMGITDVFSSSANLSGISSAESLK. Note that the structures of seven glycopeptides that both appeared in the two fractions are only labelled in Fig. S3B. In Fig. S3A, they are marked with the symbols GP1-7.

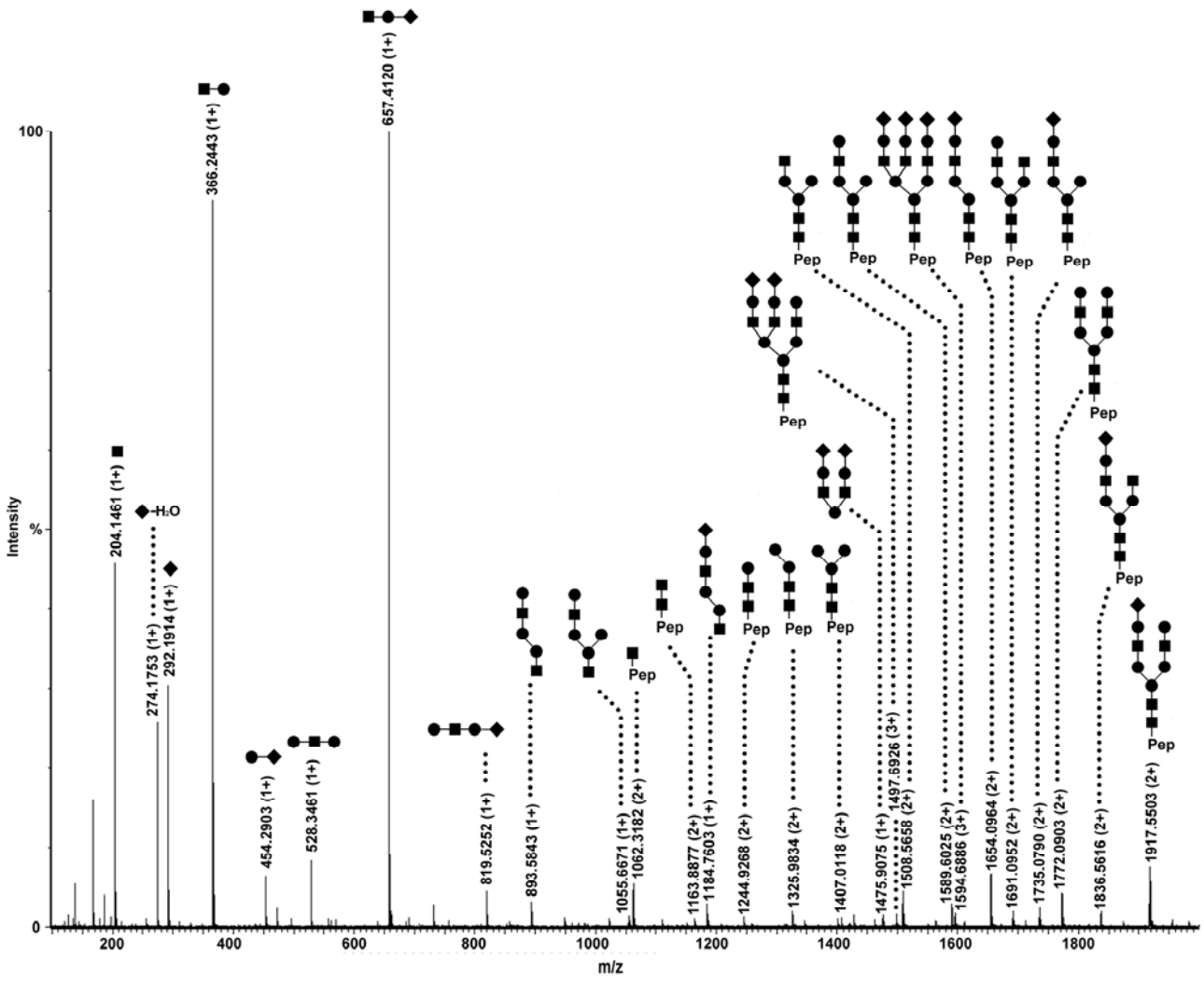

Fig. S4 Nano-RPLC-MS/MS characterization of glycopeptide bearing 1594.7 (3+) from site III of AGP. ■ N-acetylglucosamine; ● Mannose or Galactose ◆ Sialic acid.

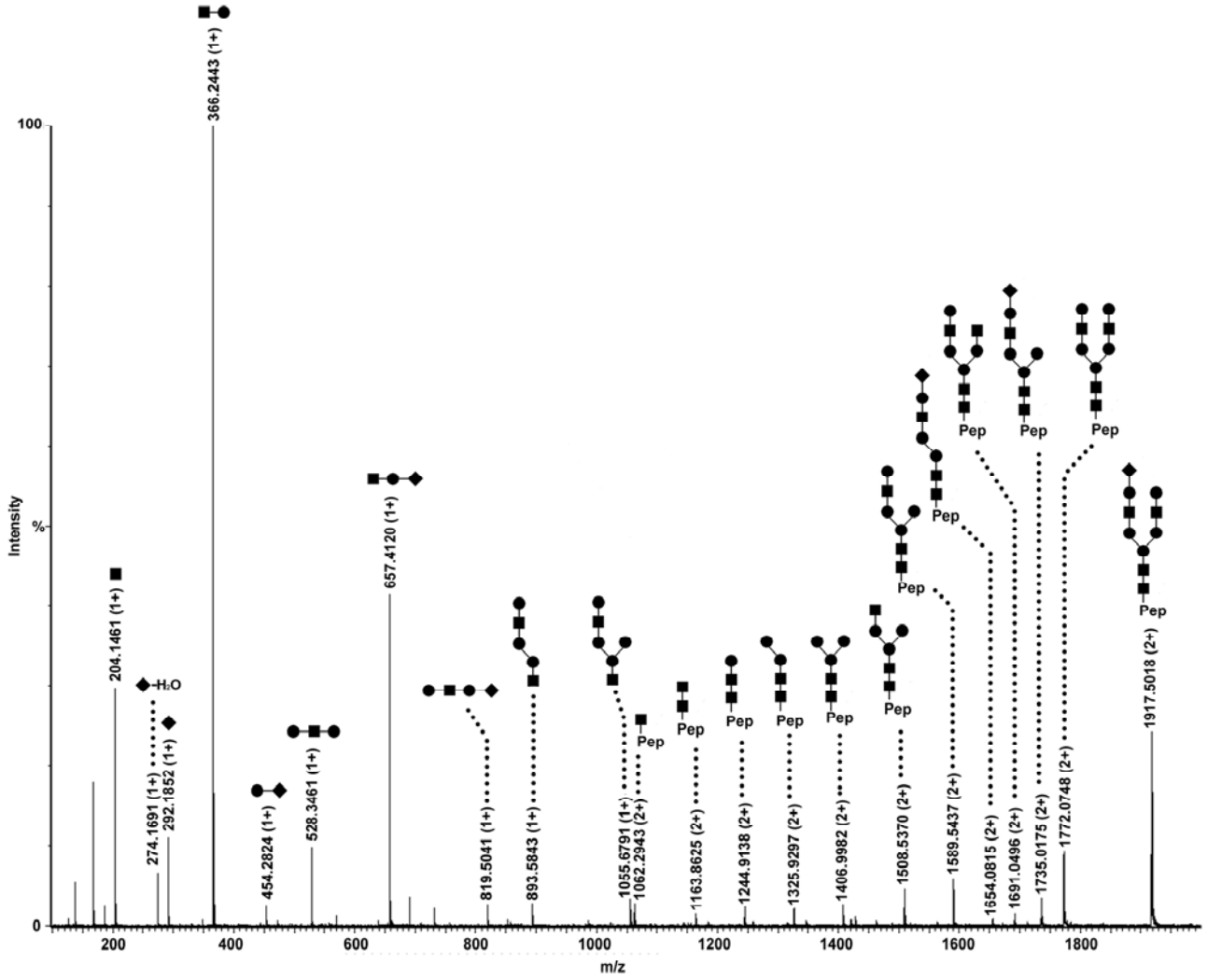

Fig. S5 Nano-RPLC-MS/MS characterization of glycopeptide bearing 1917.5 (2+) from site III of AGP. ■ N-acetylglucosamine; ● Mannose or Galactose ◆ Sialic acid.

Table S1 Glycopeptides from Site I of AGP-1 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [1-24] ^{pyro}QIPLCANLVPVPITNATLDQITGK. Retention time 50.5 min.

Glycans	Theoretical m/z	Observed m/z
[Hex][a]5[HexNAc][b]4[NeuAc][c]1	1492.0207 (3+)/1119.2656 (4+)	1492.2715 (3+)/1119.7015 (4+)
[Hex]5[HexNAc]4[NeuAc]2	1589.0525 (3+)/1192.0394 (4+)	1589.3068 (3+)/1192.2471 (4+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc1][d]	1637.7385 (3+)/1228.5539 (4+)	1637.9823 (3+)/1228.7257 (4+)
[Hex]6[HexNAc]5[NeuAc]1	1613.7314 (3+)/1210.5486 (4+)	1613.9611 (3+)/1210.7456 (4+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc1]	1662.4174 (3+)/1247.0631 (4+)	1662.6630 (3+)/1247.4910 (4+)
[Hex]6[HexNAc]5[NeuAc]2	1710.7632 (3+)/1283.3224 (4+)	1711.0331 (3+)/1283.5404 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1759.4492 (3+)/1319.8369 (4+)	1759.7393 (3+)/1320.1896 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1807.7950 (3+)/1356.0963 (4+)	1808.0594 (3+)/1356.3217 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1856.4810 (3+)/1392.6108 (4+)	1856.7625 (3+)/1392.8507 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1735.4421 (3+)	1735.7010 (3+)
[Hex]7[HexNAc]6[NeuAc]1[Fuc]1	1784.1281 (3+)	1784.3796 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1832.4739 (3+)/1374.6055 (4+)	1832.7179 (3+)/1374.8268 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1881.1599 (3+)/1411.1199 (4+)	1881.4125 (3+)/1411.3248 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1929.5057 (3+)/1447.3793 (4+)	1929.7543 (3+)/1447.6116 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1978.1917 (3+)/1483.8938 (4+)	1978.6661 (3+)/1484.1593 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1520.1532 (3+)	1520.6290 (3+)

[a] Hex, Hexose. [b] HexNAc, N-acetylhexosamine. [c] NeuAc, Sialic acid. [d] Fuc, Fucose.

Table S2 Glycopeptides from Site I of AGP-1 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [5-24] CANLVPVPITNATLDQITGK. Retention time 43.4 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1837.8267 (2+)	1838.1003 (2+)
[Hex]5[HexNAc]4[NeuAc]1	1347.2618 (3+)	1347.5026 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1444.2936 (3+)/1083.4702 (4+)	1444.5479 (3+)/1083.6738 (4+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1492.9796 (3+)	1493.2281 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1468.9725 (3+)	1469.2050 (3+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1517.6585 (3+)	1517.8777 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1566.0043 (3+)/1174.7533 (4+)	1566.2750 (3+)/1174.9550 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1614.6903 (3+)/1211.2677 (4+)	1614.9561 (3+)/1211.4789 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1663.0361 (3+)/1247.5271 (4+)	1663.3110 (3+)/1247.7521 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1711.7221 (3+)/1284.0416 (4+)	1711.9961 (3+)/1284.2688 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1590.6832 (3+)/1193.2624 (4+)	1591.2374 (3+)/1193.4342 (4+)
[Hex]7[HexNAc]6[NeuAc]1[Fuc]1	1229.7769 (4+)	1229.9438 (4+)
[Hex]7[HexNAc]6[NeuAc]2	1687.7150 (3+)/1266.0363 (4+)	1687.9681 (3+)/1266.4902 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1736.4010 (3+)/1302.5508 (4+)	1736.6095 (3+)/1302.7312 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1784.7468 (3+)/1338.8101 (4+)	1785.0195 (3+)/1339.0367 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1833.7786 (3+)/1375.3246 (4+)	1833.6195 (3+)/1375.5122 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1881.7786 (3+)/1411.5840 (4+)	1882.0216 (3+)/1411.7970 (4+)

Table S3 Glycopeptides from Site I of AGP-1 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [8-24] LVPVPITNATLDQITGK. Retention time 41.6 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1664.7794 (2+)/1110.1862 (3+)	1665.0446 (2+)/1110.3839 (3+)
[Hex]5[HexNAc]4[NeuAc]1	1847.3454 (2+)/1231.8969 (3+)	1847.6342 (2+)/1232.1223 (3+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1920.3744 (2+)/1280.5829 (3+)	1920.6750 (2+)/1280.4969 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1992.8931 (2+)/1328.9287 (3+)	1993.1658 (2+)/1329.1692 (3+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1377.6147 (3+)	1377.8434 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1015.4557 (4+)	1015.6345 (4+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1402.2936 (3+)	1402.5085 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1450.6394 (3+)/1088.2296 (4+)	1450.8896 (3+)/1088.4241 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1499.3254 (3+)/1124.7441 (4+)	1499.5898 (3+)/1124.9388 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1547.6712 (3+)/1161.0034 (4+)	1547.9298 (3+)/1161.2119 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1596.3572 (3+)/1197.5179 (4+)	1596.4225 (3+)/1197.7280 (4+)
[Hex]7[HexNAc]6[NeuAc]2	1572.3501 (3+)/1179.5126 (4+)	1572.5997 (3+)/1179.7102 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1621.0361 (3+)/1216.0271 (4+)	1621.2440 (3+)/1216.1787 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1669.3819 (3+)/1252.2865 (4+)	1669.6470 (3+)/1252.4954 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1718.0679 (3+)/1288.8009 (4+)	1718.3166 (3+)/1289.0148 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1766.4137 (3+)/1325.0603 (4+)	1766.6442 (3+)/1325.2646 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1361.5748 (4+)	1361.5125 (4+)

Table S4. Glycopeptides from Site I of AGP-2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [1-20] ^{pyro}QIPLCANLVPVPITNATLDR. Retention time 48.1 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1869.3623 (2+)/1246.5748 (3+)	1869.6344 (2+)/1246.7469 (3+)
[Hex]5[HexNAc]4[NeuAc]1	1368.2855 (3+)	1368.5306 (3+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1416.9715 (3+)	1417.2178 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1465.3173 (3+)/1099.2380 (4+)	1465.5668 (3+)/1099.4379 (4+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1514.0033 (3+)	1514.2515 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1489.9962 (3+)/1117.7472 (4+)	1490.2449 (3+)/1117.9336 (4+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1538.6822 (3+)	1538.9282 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1587.0280 (3+)/1190.5210 (4+)	1587.2889 (3+)/1190.7289 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1635.7140 (3+)/1227.0355 (4+)	1635.9785 (3+)/1227.2493 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1684.0598 (3+)/1263.2949 (4+)	1684.3262 (3+)/1263.5195 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1732.7458 (3+)/1299.8094 (4+)	1733.0233 (3+)/1300.0382 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1611.7069 (3+)/1209.0302 (4+)	1611.9425 (3+)/1209.4473 (4+)
[Hex]7[HexNAc]6[NeuAc]2	1708.7387 (3+)/1281.8041 (4+)	1709.0005 (3+)/1282.0182 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1757.4247 (3+)/1318.3185 (4+)	1757.6781 (3+)/1318.7740 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1805.7705 (3+)/1354.5779 (4+)	1806.0330 (3+)/1354.8248 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1854.4565 (3+)/1391.0924 (4+)	1855.7410 (3+)/1391.3339 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1427.3518 (4+)	1427.5599 (4+)

Table S5 Glycopeptides from Site I of AGP-2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [5-20] CANLVPVPITNATLDR. Retention time 38.5 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1652.2239 (2+)	1652.5071 (2+)
[Hex]5[HexNAc]4[NeuAc]1	1834.7899 (2+)/1223.5266 (3+)	1835.0597 (2+)/1223.7045 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1980.3376 (2+)/1320.5584 (3+)	1980.6154 (2+)/1320.8015 (3+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1369.2444 (3+)/1027.1833 (4+)	1369.4878 (3+)/1027.6129 (4+)
[Hex]6[HexNAc]5[NeuAc]1	1345.2373 (3+)	1345.4819 (3+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1393.9233 (3+)	1394.1888 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1442.2691 (3+)/1081.9518 (4+)	1442.5259 (3+)/1082.1416 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1490.9551 (3+)	1491.2152 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1539.3009 (3+)/1154.7257 (4+)	1539.5663 (3+)/1154.9360 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1587.9869 (3+)/1191.2402 (4+)	1588.2461 (3+)/1191.4434 (4+)
[Hex]7[HexNAc]6[NeuAc]1[Fuc]1	1515.6340 (3+)	1515.5604 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1563.9798 (3+)	1564.2281 (3+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1612.6658 (3+)	1612.8923 (3+)
[Hex]7[HexNAc]6[NeuAc]3	1661.0116 (3+)	1661.3613 (3+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1709.6976 (3+)/1282.5232 (4+)	1709.6116 (3+)/1282.4681 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1806.7294 (3+)/1355.2970 (4+)	1806.9755 (3+)/1355.2465 (4+)

Table S6 Glycopeptides from Site I of AGP-2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [8-20] LVPVPITNATLDR. Retention time 34.4 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]5[HexNAc]4[NeuAc]1	1108.1617 (3+)	1108.2911 (3+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1156.8477 (3+)	1157.0974 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1807.2903 (2+)/1205.1935 (3+)	1807.5880 (2+)/1205.4017 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1229.8724 (3+)/922.6543 (4+)	1230.0215 (3+)/922.7393 (4+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1917.3376 (2+)/1278.5584 (3+)	1917.5831 (2+)/1278.7385 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1989.8563 (2+)/1326.9042 (3+)	1990.1151 (2+)/1327.1354 (3+)
[Hex]6[HexNAc]5[NeuAc]3	1423.9360 (3+)/1068.2020 (4+)	1424.1688 (3+)/1068.3984 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1472.6220 (3+)/1104.7165 (4+)	1472.8762 (3+)/1104.9102 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1013.9373 (4+)	1014.0569 (4+)
[Hex]7[HexNAc]6[NeuAc]1[Fuc]1	1400.2691 (3+)	1400.4606 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1448.6149 (3+)/1086.7112 (4+)	1448.8070 (3+)/1086.8395 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1123.2257 (4+)	1123.3776 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1545.6467 (3+)/1159.4850 (4+)	1545.8805 (3+)/1159.6508 (4+)

Table S7 Glycopeptides from Site II of AGP-1 and 2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [34-39] NEEYNK. Retention time 12.8 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1172.9422 (2+)	1173.1565 (2+)
[Hex]4[HexNAc]3[NeuAc]1[Fuc]1	1245.9712 (2+)	1246.2120 (2+)
[Hex]5[HexNAc]4	1209.9606 (2+)	1210.1927 (2+)
[Hex]5[HexNAc]4[NeuAc]1	1355.5083 (2+)/904.0055 (3+)	1355.7500 (2+)/904.1634 (3+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	952.6915 (3+)	952.8672 (2+)
[Hex]5[HexNAc]4[NeuAc]2	1501.0560 (2+)/1001.0373 (3+)	1501.3076 (2+)/1001.2104 (3+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1574.0849 (2+)/1049.7233 (3+)	1574.3588 (2+)/1049.9115 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1538.0743 (2+)/1025.7162 (3+)	1538.3339 (2+)/1025.8958 (3+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1611.1033 (2+)	1611.3641 (2+)
[Hex]6[HexNAc]5[NeuAc]2	1683.6220 (2+)	1683.9016 (2+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1756.6510 (2+)/1171.4340 (3+)	1756.9344 (2+)/1171.6506 (3+)
[Hex]6[HexNAc]5[NeuAc]3	1829.1697 (2+)/1219.7798 (3+)	1829.4446 (2+)/1219.9967 (3+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1902.1987 (2+)/1268.4658 (3+)	1902.4836 (2+)/1268.6877 (3+)
[Hex]7[HexNAc]6[NeuAc]1	1147.4269 (3+)	1147.3748 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1244.4587 (3+)	1244.6729 (3+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1293.1447 (3+)	1293.4105 (3+)
[Hex]7[HexNAc]6[NeuAc]3	1341.4905 (3+)	1341.7294 (3+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1390.1765 (3+)	1390.4241 (3+)
[Hex]7[HexNAc]6[NeuAc]4	1438.5223 (3+)	1438.7946 (3+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1487.2083 (3+)	1487.4783 (3+)

Table S8 Glycopeptides from Site III of AGP-1 and 2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [40-55] SVQEIQATFFYFTPNK. Retention time 45.5 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1156.8303 (3+)	1157.0470 (3+)
[Hex]4[HexNAc]4	1127.4916 (3+)	1127.6924 (3+)
[Hex]5[HexNAc]4	886.3819 (4+)	886.5686 (4+)
[Hex]5[HexNAc]4[NeuAc]1	1917.3116 (2+)/1278.5410 (3+)	1917.5995 (2+)/1278.7649 (3+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1990.3405 (2+)	1990.6425 (2+)
[Hex]5[HexNAc]4[NeuAc]2	1375.5728 (3+)/1031.9296 (4+)	1375.8138 (3+)/1032.1199 (4+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1424.2588 (3+)/1068.4441 (4+)	1424.5175 (3+)/1068.6754 (4+)
[Hex]6[HexNAc]5[NeuAc]1	1400.2517 (3+)/1050.4388 (4+)	1400.5023 (3+)/1050.6541 (4+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1448.9377 (3+)	1449.2009 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1497.2835 (3+)/1123.2127 (4+)	1497.5439 (3+)/1123.4147 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1545.9695 (3+)/1159.7271 (4+)	1546.2292 (3+)/1159.9277 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1594.3153 (3+)/1195.9865 (4+)	1594.5708 (3+)/1196.1936 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1643.0013 (3+)/1232.5010 (4+)	1643.2811 (3+)/1232.7191 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1521.9624 (3+)/1141.7218 (4+)	1522.2291 (3+)/1141.9229 (4+)
[Hex]7[HexNAc]6[NeuAc]1[Fuc]1	1570.6484 (3+)	1570.5635 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1618.9942 (3+)/1214.4857 (4+)	1619.2655 (3+)/1214.7097 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1667.6802 (3+)/1251.0102 (4+)	1667.9562 (3+)/1251.2227 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1716.0260 (3+)/1287.2695 (4+)	1716.3108 (3+)/1287.5026 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1764.7120 (3+)/1323.7840 (4+)	1764.9983 (3+)/1324.0271 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1813.0578 (3+)/1360.0434 (4+)	1813.3428 (3+)/1360.2852 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc1]	1861.7438 (3+)/1396.5579 (4+)	1862.0208 (3+)/1396.7981 (4+)

Table S9 Glycopeptides from Site III of AGP-1 and 2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [40-63] SVQEIQATFFYFTPNKTEDTIFLR. Retention time 49.6 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]5[HexNAc]4[NeuAc]1	1604.0443 (3+)/1203.2832 (4+)	1603.9849 (3+)/1203.2600 (4+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1652.7303 (3+)/1239.7977 (4+)	1652.6423 (3+)/1239.7101 (4+)
[Hex]5[HexNAc]4[NeuAc]2	1701.0761 (3+)/1276.0571 (4+)	1701.0201 (3+)/1276.0308 (4+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1749.7621 (3+)	1749.7084 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1725.7550 (3+)/1294.5663 (4+)	1725.6930 (3+)/1294.5257 (4+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1774.4410 (3+)/1331.0807 (4+)	1774.3721 (3+)/1331.2717 (4+)
[Hex]6[HexNAc]5[NeuAc]2	1822.7868 (3+)/1367.3401 (4+)	1822.0715 (3+)/1367.7009 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1871.4728 (3+)/1403.8546 (4+)	1871.4244 (3+)/1403.8512 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1919.8186 (3+)/1440.1140 (4+)	1919.7681 (3+)/1440.1127 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1968.5046 (3+)/1476.6284 (4+)	1968.4487 (3+)/1476.8787 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1847.4657 (3+)/1385.8493 (4+)	1847.4276 (3+)/1385.8252 (4+)
[Hex]7[HexNAc]6[NeuAc]2	1944.4975 (3+)/1458.6231 (4+)	1944.4398 (3+)/1458.6283 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1993.1835 (3+)/1495.1376 (4+)	1993.4626 (3+)/1495.1422 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1531.3970 (4+)	1531.4131 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1567.9115 (4+)	1567.9281 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1604.1708 (4+)	1604.4437 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1641.2904 (4+)	1640.6906 (4+)

Table S10 Glycopeptides from Site IV of AGP-1 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [69-83] QDQCIYNTTYLNVQR. Retention time 34.4 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]5[HexNAc]4[NeuAc]1	1915.7750 (2+)/1277.5167 (3+)	1916.0463 (2+)/1277.7343 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1374.5485 (3+)	1374.7721 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1496.2592 (3+)/1122.4444 (4+)	1496.5145 (3+)/1122.6467 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1544.9451 (3+)/1158.9589 (4+)	1545.1976 (3+)/1159.3990 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1593.2910 (3+)/1195.2182 (4+)	1593.5526 (3+)/1195.4268 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1641.9769 (3+)/1231.7327 (4+)	1642.2476 (3+)/1231.9537 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1520.9381 (3+)	1521.1912 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1617.9699 (3+)/1213.7274 (4+)	1618.2397 (3+)/1213.9626 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1666.6558 (3+)/1250.2419 (4+)	1666.9299 (3+)/1250.4559 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1715.0017 (3+)/1286.5013 (4+)	1715.2698 (3+)/1286.7334 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1763.6876 (3+)/1323.0157 (4+)	1763.9739 (3+)/1323.4893 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1812.0335 (3+)/1359.2751 (4+)	1812.3201 (3+)/1359.4948 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1860.7194 (3+)/1395.7896 (4+)	1861.0002 (3+)/1396.0663 (4+)

Table S11 Glycopeptides from Site IV of AGP-2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [69-83] QNQCFYNSSYLNVQR. Retention time 34.4 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]5[HexNAc]4[NeuAc]1	1918.2596 (2+)/1279.1730 (3+)	1918.5864 (2+)/1279.4259 (3+)
[Hex]5[HexNAc]4[NeuAc]2	1376.2048 (3+)	1376.4856 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1497.9155 (3+)/1123.6867 (4+)	1497.1875 (3+)/1123.8855 (4+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1546.6015 (3+)/1160.2011 (4+)	1546.8831 (3+)/1160.3937 (4+)
[Hex]6[HexNAc]5[NeuAc]3	1594.9473 (3+)/1196.4605 (4+)	1595.2200 (3+)/1196.6794 (4+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1643.6333 (3+)/1232.9750 (4+)	1643.9254 (3+)/1233.1993 (4+)
[Hex]7[HexNAc]6[NeuAc]1	1522.5944 (3+)	1522.8778 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1619.6262 (3+)/1214.9697 (4+)	1619.9053 (3+)/1215.1863 (4+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1668.3122 (3+)/1251.4842 (4+)	1668.6052 (3+)/1251.7108 (4+)
[Hex]7[HexNAc]6[NeuAc]3	1716.6580 (3+)/1287.7435 (4+)	1716.9385 (3+)/1287.9799 (4+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1765.3440 (3+)/1324.2580 (4+)	1765.6348 (3+)/1324.4977 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1813.6898 (3+)/1360.5174 (4+)	1813.9882 (3+)/1360.7623 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1862.3758 (3+)/1397.0319 (4+)	1862.6747 (3+)/1397.2816 (4+)

Table S12 Glycopeptides from Site V of AGP-1 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [84-90] ENGTISR. Retention time 12.8 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1162.9635 (2+)	1163.1776 (2+)
[Hex]5[HexNAc]4	1199.9818(2+)/800.3212 (3+)	1199.6217 (2+)/800.0793 (3+)
[Hex]5[HexNAc]4[NeuAc]1	1345.5295(2+)/897.3530 (3+)	1345.7666 (2+)/897.5188 (3+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1418.5585 (2+)	1418.7903 (2+)
[Hex]5[HexNAc]4[NeuAc]2	1491.0772 (2+)/994.3848 (3+)	1491.3436 (2+)/994.5674 (3+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1564.1062 (2+)/1043.0708 (3+)	1564.3743 (2+)/1043.2517 (3+)
[Hex]6[HexNAc]5	1382.5479 (2+)/990.0319 (3+)	1382.2091 (2+)/922.1443 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1528.0956 (2+)/1019.0637 (3+)	1528.2909 (2+)/1019.1946 (3+)
[Hex]6[HexNAc]5[NeuAc]1[Fuc]1	1601.1245 (2+)	1601.3662 (2+)
[Hex]6[HexNAc]5[NeuAc]2	1673.6433 (2+)/1116.0955 (3+)	1673.9230 (2+)/1116.3032 (3+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1746.6722 (2+)/1164.7815 (3+)	1746.9580 (2+)/1164.9934 (3+)
[Hex]6[HexNAc]5[NeuAc]3	1819.1910 (2+)/1213.1273 (3+)	1819.4692 (2+)/1213.3446 (3+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1892.2199 (2+)/1261.8133 (3+)	1892.5040 (2+)/1262.0356 (3+)
[Hex]7[HexNAc]6[NeuAc]1	1710.6616 (2+)/1140.7744 (3+)	1710.8954 (2+)/1140.5996 (3+)
[Hex]7[HexNAc]6[NeuAc]1[Fuc]1	1189.4604 (3+)	1189.6326 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1856.4758 (2+)/1237.8062 (3+)	1856.4758 (2+)/1237.9880 (3+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1929.2383 (2+)/1286.4922 (3+)	1929.4948 (2+)/1286.6804 (3+)
[Hex]7[HexNAc]6[NeuAc]3	1334.8380 (3+)	1335.0774 (3+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1383.5240 (3+)/1037.8930 (4+)	1383.7623 (3+)/1038.1606 (4+)
[Hex]7[HexNAc]6[NeuAc]4	1431.8698 (3+)/1074.1524 (4+)	1432.1156 (3+)/1074.3381 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1480.5558 (3+)/1110.6668 (4+)	1480.8153 (3+)/1110.8641 (4+)

Table S13 Glycopeptides from Site V of AGP-2 identified with Click CA enrichment combined to nano-RPLC-MS. Peptide sequence [84-90] ENGTVSR. Retention time 11.0 min.

Glycans	Theoretical m/z	Observed m/z
[Hex]4[HexNAc]3[NeuAc]1	1155.9557 (2+)	1156.1797 (2+)
[Hex]5[HexNAc]4[NeuAc]1	1338.5217 (2+)	1338.7528 (2+)
[Hex]5[HexNAc]4[NeuAc]1[Fuc]1	1411.5507 (2+)	1411.8246 (2+)
[Hex]5[HexNAc]4[NeuAc]2	1484.0694 (2+)/989.7129 (3+)	1484.3302 (2+)/989.8873 (3+)
[Hex]5[HexNAc]4[NeuAc]2[Fuc]1	1557.0984 (2+)/1038.3989 (3+)	1557.3807 (2+)/1038.5895 (3+)
[Hex]6[HexNAc]5[NeuAc]1	1521.0878 (2+)/1014.3918 (3+)	1521.3640 (2+)/1014.5864 (3+)
[Hex]6[HexNAc]5[NeuAc]2	1666.6355 (2+)/1111.4236 (3+)	1666.9150 (2+)/1111.6281 (3+)
[Hex]6[HexNAc]5[NeuAc]2[Fuc]1	1739.6644 (2+)/1160.1096 (3+)	1739.9684 (2+)/1160.3810 (3+)
[Hex]6[HexNAc]5[NeuAc]3	1812.1832 (2+)/1208.4554 (3+)	1812.4617 (2+)/1208.6760 (3+)
[Hex]6[HexNAc]5[NeuAc]3[Fuc]1	1885.2121 (2+)/1257.1414 (3+)	1885.5018 (2+)/1257.3658 (3+)
[Hex]7[HexNAc]6[NeuAc]2	1849.2015 (2+)/1233.3940 (3+)	1849.4769 (2+)/1233.3940 (3+)
[Hex]7[HexNAc]6[NeuAc]2[Fuc]1	1922.2305 (2+)	1922.5054 (2+)
[Hex]7[HexNAc]6[NeuAc]3	1994.7492 (2+)/1330.1661 (3+)	1994.0138 (2+)/1330.4091 (3+)
[Hex]7[HexNAc]6[NeuAc]3[Fuc]1	1378.8521 (3+)/1034.3891 (3+)	1379.0919 (2+)/1034.5674 (3+)
[Hex]7[HexNAc]6[NeuAc]4	1427.1979 (3+)/1070.6485 (4+)	1427.4481 (3+)/1070.8401 (4+)
[Hex]7[HexNAc]6[NeuAc]4[Fuc]1	1475.8839 (3+)/1107.1629 (4+)	1476.1400 (3+)/1107.3562 (4+)

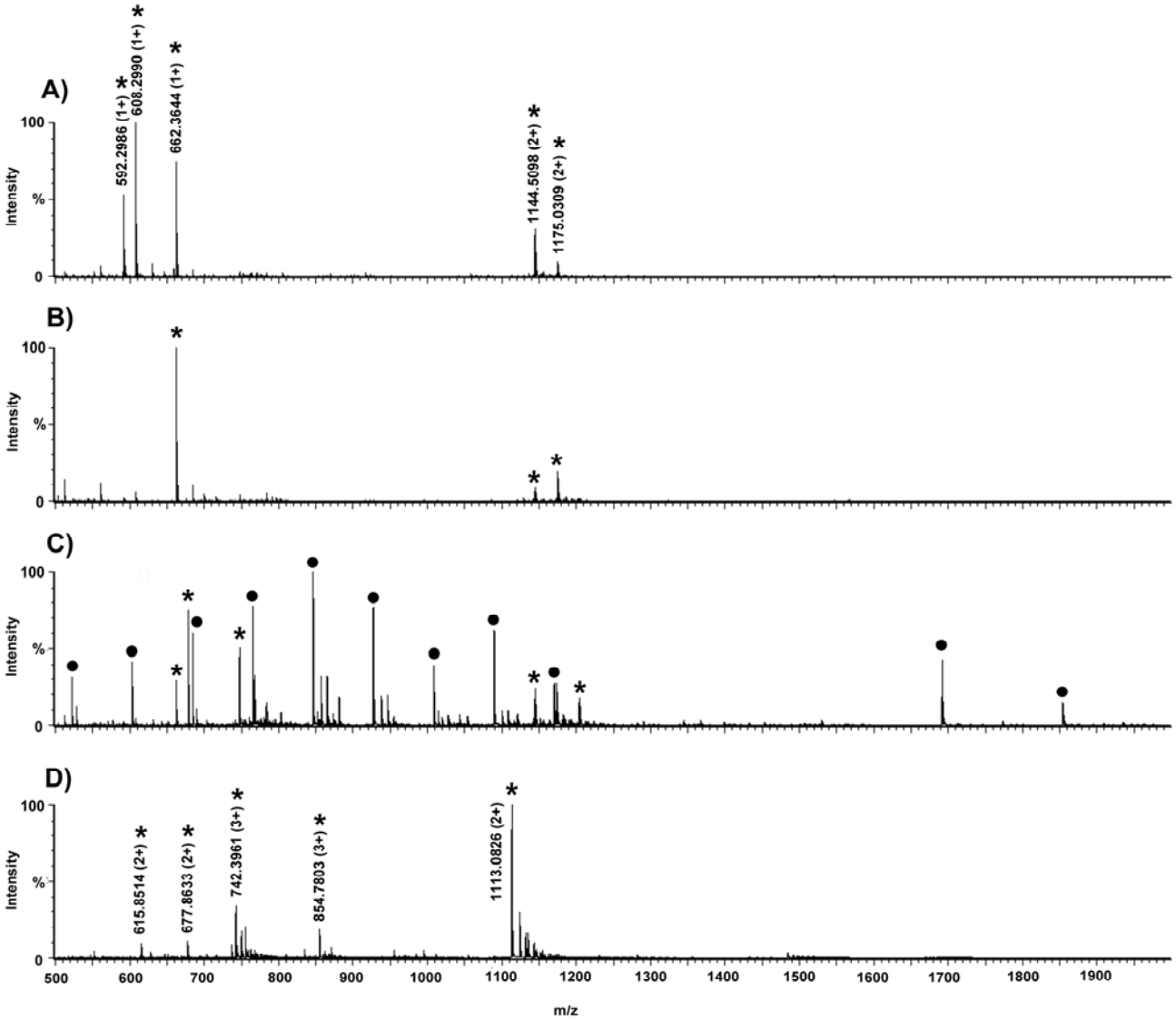

Fig. S6 Nano-ESI-MS characterization of RNase fractions from Click CA rinsed or eluted with the solvents containing different CH_3CN content (glycopeptide elution window experiment). A) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) for the third time. B) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) for the fourth time. C) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (60:40:0.1 (v/v), 20 μL) for the first time. D) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (60:40:0.1 (v/v), 20 μL) for the second time. Peptides were labelled with their m/z ratio only when appeared for the first time. Non-glycosylated peptides and glycopeptides were marked with asterisks and black circles, respectively.

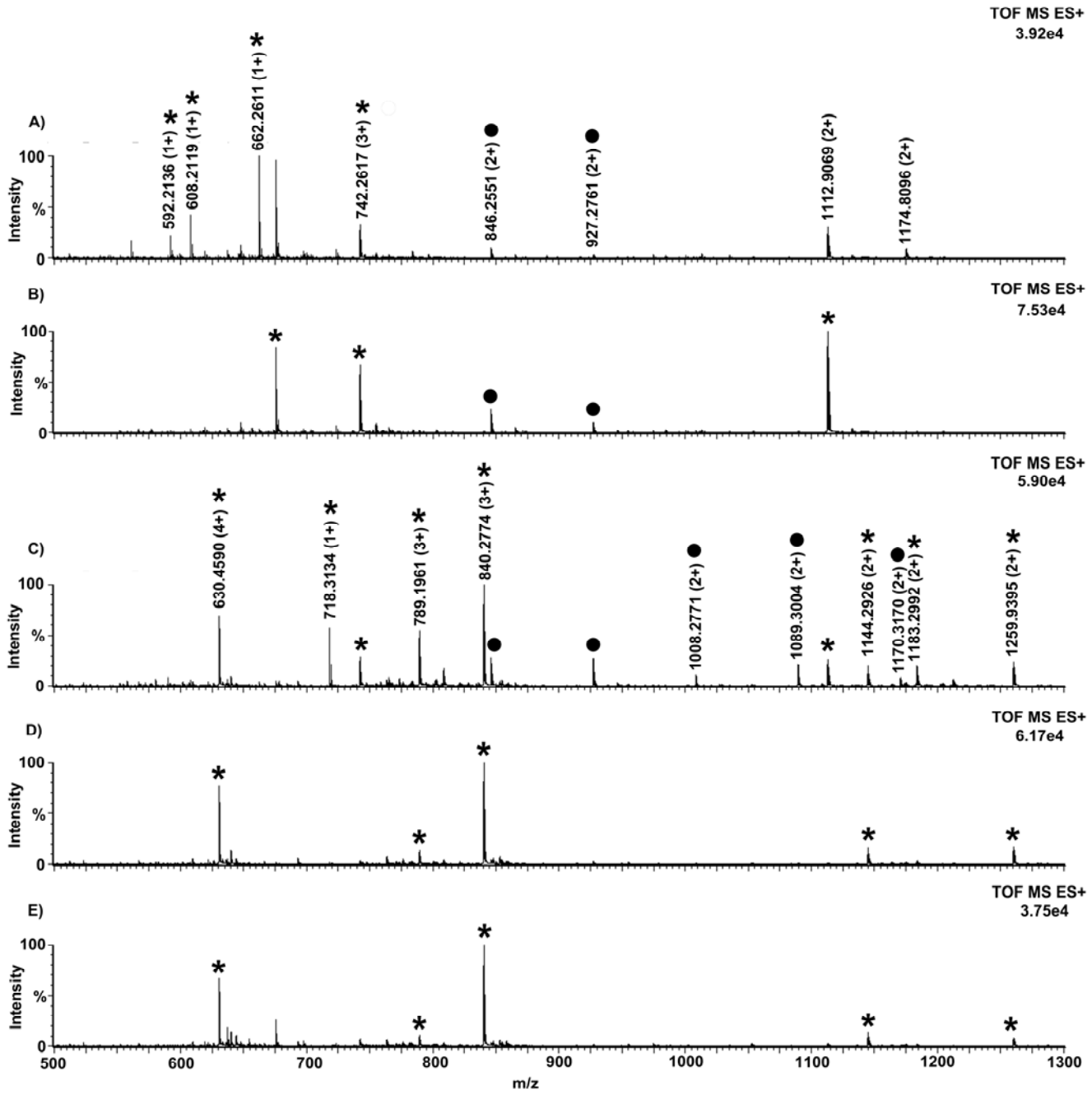

Fig. S7 Nano-ESI-MS characterization of RNase fractions from the WCX matrix rinsed or eluted with the solvents containing different CH_3CN content (glycopeptide elution window experiment). A) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (80:20:0.1 (v/v), 20 μL) for the second time. B) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (80:20:0.1 (v/v), 20 μL) for the third time. C) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) for the first time. D) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) for the second time. E) Mass spectrum of the fraction rinsed with CH_3CN/H_2O/FA (70:30:0.1 (v/v), 20 μL) for the third time. Peptides were labelled with their m/z ratio only when appeared for the first time. Non-glycosylated peptides and glycopeptides were marked with asterisks and black circles, respectively.