

Supporting information:

Section S-1

Methods

Glycoproteins were subjected to reduction (dithiothreitol), alkylation (iodoacetamide) and overnight tryptic digestion in the presence of 2,2,2-trifluoroethanol (5%) as denaturant, and ammonium bicarbonate (100 mM) as pH buffer. Reactions were stopped by heating at 95° C for 5 min. The masses of resulting peptides were analyzed using a Bruker Ultraflexxtreme MALDI MS (Bruker Daltonics, Billerica, MA) using 2,5-dihydroxybenzoic acid (DHB) as matrix. The samples were then dried down in a centrifugal evaporator.

Dry tryptic digests were reconstituted in a 1:1 mixture of water and acetonitrile with 0.1% formic acid to a 1 mg/mL concentration. The samples were then diluted in the appropriate loading conditions for C18 (98% water, 2% acetonitrile, 0.1% formic acid) or HILIC-C18 (80% acetonitrile, 20% water, 0.1% trifluoroacetic acid) analysis to approximately 100 fmol/μL and injected into the LC/MS system using the Agilent 1200 series autosampler. All analyses were performed on an Agilent 1200 series LC system capable of delivering nanoliter flow rates coupled to an Agilent 6550 ion-funnel quadrupole time-of-flight (Q-TOF) mass spectrometer, equipped with a chip-cube ionization source (Agilent Technologies, Santa Clara, CA), in the positive ion mode.

Approximately, 1 μg and 0.1 μg of the total proteolytic digests were injected without any cleanup for analyses on HILIC-C18 or C18 chip-LC/MS, respectively. Lower amounts of total material were loaded onto the C18 chip because in the absence of enrichment, the non-glycosylated peptides led to signal saturation. The Agilent HPLC chip consisted of laser ablated channels that were packed with appropriate stationary phases. The C18 chip used was a commercially available Polaris-HR-Chip-3C18 chip (Agilent Technologies, Santa Clara, CA) with a 75μm X 150mm separation/analytical column and a 360nL enrichment column, both packed with 3μm particle size and 180Å pore size, Agilent Polaris C18-A stationary phase. The HILIC-C18 hybrid chip consisted of a 75μm X 150mm analytical column packed with 5μm particle size and Polaris C18-A stationary phase and a 40nL enrichment column, packed with Amide-80 stationary phase (Tosoh Biosciences, Montgomeryville, PA), 5μm particle size and 80Å pore size. This chip is available from the vendor as a custom order. A binary pump with microliter flow rates was used as the “loading pump” to deliver sample and solvent to the trapping/enrichment column and a nanoscale pump was used as the “gradient pump” to deliver the gradient once the trapping and analytical columns were inline.

The HPLC mobile phases for the C18 chip were as follows: Solvent A was 99% water, 1% acetonitrile modified with 0.1% formic acid. Solvent B was 99% acetonitrile, 1% water modified with 0.1% formic acid. The enrichment column was equilibrated using the loading pump which was constantly pumping 98% solvent A and 2% solvent B as loading conditions. The sample was washed on the enrichment column with 4μL of the loading condition solvent and then the 6 port valve was switched to bring the enrichment column in-line with the analytical column. The analytical pump was used to deliver the solvent after the valve switched. 98% solvent A was pumped for the first 10 minutes after a gradient was

started from 98% solvent A to 15% solvent A over 30 min. The solvent conditions were then changed to 5% solvent A in the next 2 min and kept constant for 5 min thereafter to wash the columns. The columns were brought back to the starting condition, 98% solvent A, in 3 min and then equilibrated for 5 min.

For the HILIC-C18 chip, the HPLC conditions were as follows. Solvent A was 99% water, 1% acetonitrile with 0.1% formic acid. Solvent B was 99% acetonitrile, 1% water with 0.1% formic acid. A premixed special loading solvent, 80% acetonitrile, 20% water, modified with 0.1% trifluoroacetic acid, was delivered using the loading pump in this case as the loading condition. The samples were loaded and washed on the trapping column using the loading pump with 4 μ L of the special loading solvent. These high organic conditions were used to allow binding of the glycosylated peptides to the HILIC trapping column which the non-glycosylated peptides were washed away to waste. The analytical column was equilibrated with the regular mobile phases at 98% solvent A. After the washing step was complete, the trapping column was brought in-line with the analytical column and the setup received flow from the nanoscale analytical pump, which delivered 98% solvent A for the first 10 min. These high-aqueous conditions allowed elution of the glycosylated peptides from the HILIC trapping column and binding to the C18 reversed phase analytical column. After this a gradient was delivered by the analytical pump to resolve and elute the glycopeptides from the C18 analytical column. The gradient was similar to that used in the C18 chip setup. Mobile phase was changed from 98% solvent A to 15% solvent A over 30 min. The solvent conditions were then changed to 5% solvent A in the next 2 min and kept constant for 5 min thereafter to wash the columns. The columns were brought back to the starting condition, 98% solvent A, in 3 min and then equilibrated for 5 min.

Both HPLC chips (C18 and HILIC-C18) consisted of an integrated nano-ESI source, to allow sample introduction into the mass spectrometer. The MS and tandem MS conditions used for both C18 and HILIC-C18 runs were the same. Data dependent tandem mass spectra were acquired for the first 40 minutes of the run. Top 5 most abundant, multiply charged precursor ions were selected for fragmentation with dynamic exclusion enabled after 10 spectra for each precursor. MS spectra were acquired in the 200-2000 *m/z* range and MS/MS spectra in the 50-3000 *m/z* range. MS and MS/MS spectra were acquired at rates of 4 and 1 spectra/s, respectively. An isolation width of 4 *m/z* was used. Nitrogen was used as the collision gas and collision energy varied with precursor *m/z*. The collision energy was determined dynamically as a linear function of precursor *m/z*, with a slope of 5 and offset of -4.8 to yield peptide backbone fragments. For only glycan dissociation, a lower collision energy can be used, also calculated using the precursor *m/z*, with a slope of 0.9 and offset of 2. Data were acquired in the positive ion mode with source capillary voltage set at 2150 V for the first 40 minutes of the run. Drying gas flow was 13 l/min for the first 25 minutes and 11 l/min, thereafter with gas temperature set at 225°C throughout the run. Data files were acquired using Agilent MassHunter Workstation Data Acquisition software and analyzed using Agilent MassHunter Qualitative Analysis software package.

Online HILIC enrichment setup on 2.1mm column scale

To demonstrate that the use of online HILIC enrichment setup prior to reversed phase separation is not limited to the chip platform, we carried our glycopeptide enrichment and separation on an LC system consisting of a 4.6mm ID guard column driven by an LC pump, an external valve to interface the guard

column to the separation column, and a 2.1 mm ID separation column driven by a second LC pump. Specifically, glycopeptides were enriched using a 4.6 mm x 1.0 cm Amide-80 guard column (5 μm Amide-80, Tosoh) flowing at 500 $\mu\text{L min}^{-1}$ with a solvent composition of 80% ACN 0.1% TFA. After enrichment, the external valve was triggered and glycopeptides were separated using two 2.1 mm x 10 cm 300 SB-C18 columns (1.8 μm , Agilent Technologies) connected in series. The separation gradient was supplied by a 1290 binary LC (Agilent Technologies) at a flow rate of 200 $\mu\text{L min}^{-1}$. Solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid in acetonitrile. Glycopeptides were separated by gradient that reached 42% B in 20 minutes. The separation was performed on-line with a 6550 QTOF (Agilent Technologies). Five μL of a 2 pmol μL^{-1} tryptic digest of transferrin was injected onto the LC/MS system. The resulting LC-MS chromatogram has been presented in figure S-4.

Section S-2: Supplementary Figures

Figure S-1: Oxonium ion distributions. Base peak chromatograms from LC-MS/MS runs using HILIC-C18 and C18 chips, A and B, respectively, overlaid with respective extracted ion chromatograms for m/z 204.0864 (HexNAc).

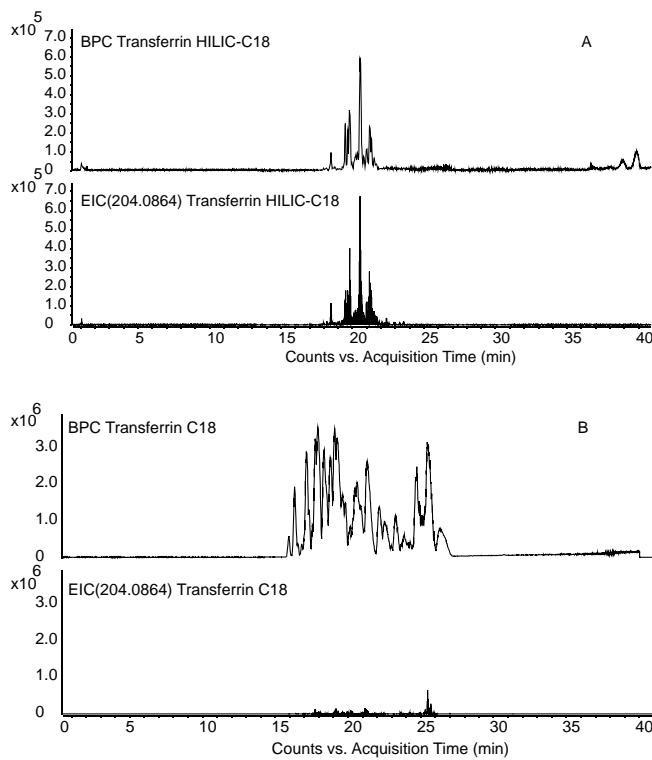


Figure S-1 (a) Transferrin HILIC-C18 (A) vs. C18 (B)

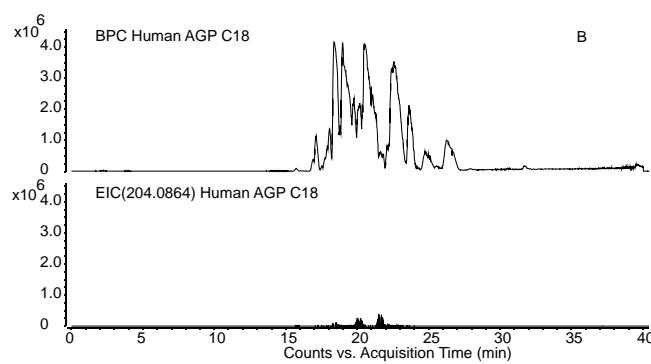
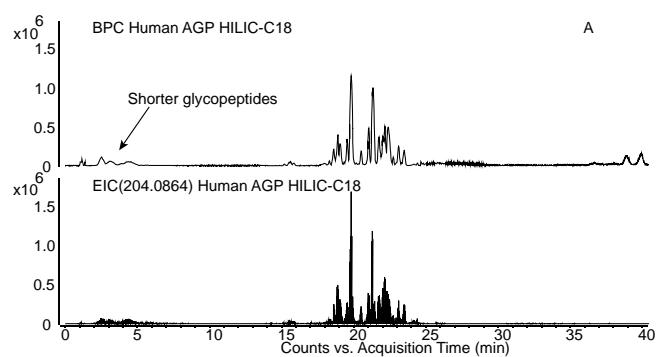


Figure S-1 (b) Alpha-1-acid glycoprotein HILIC-C18 (A) vs. C18 (B)

Figure S-2 Tandem MS of glycopeptides (See tables 2 and S-3 for details of product ions assigned)

Glycopeptide identifier is listed as Peptide-[a,b,c,d,e], where:

a = number of Hexoses

b = number of N-acetylhexosamines

c = number of Deoxyhexoses

d = number of N-acetylneuraminic acids

e = number of N-glycolylneuraminic acids

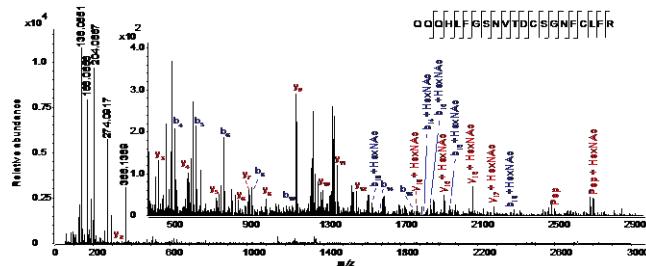


Figure S-2 (a) Tandem MS of Transferrin glycopeptide precursor ion 1180.7328 [M+4H]⁴⁺, identified as QQQHLFGSNVTDCSGNFCLFR-[5,4,0,2,0]

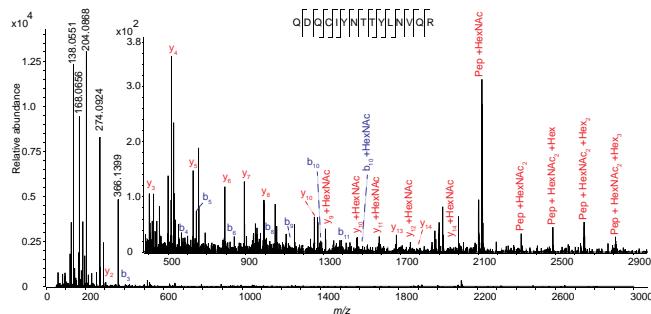


Figure S-2 (b) Tandem mass spectrum of AGP glycopeptide precursor ion 1194.9870 [M+4H]⁴⁺, identified as QDQCIYNTTYLNVQR-[6,5,0,3,0]

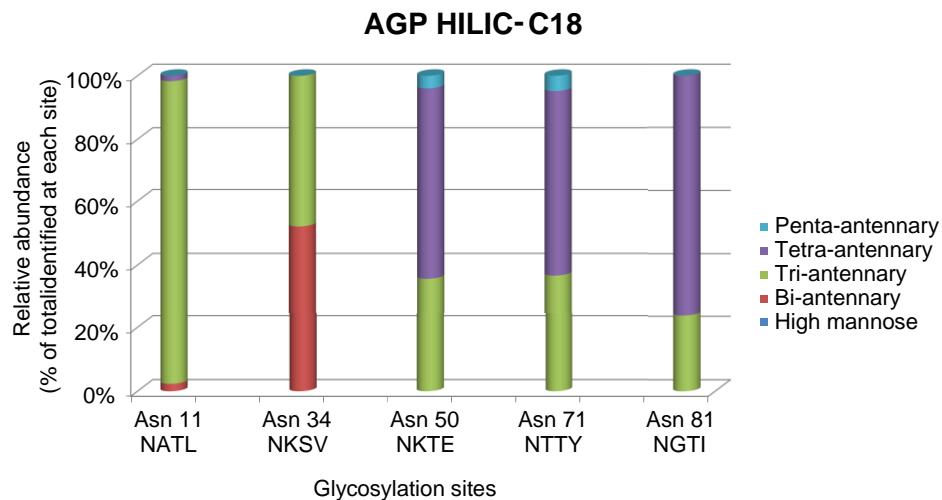


Figure S-3: Site-specific glycan profile of AGP

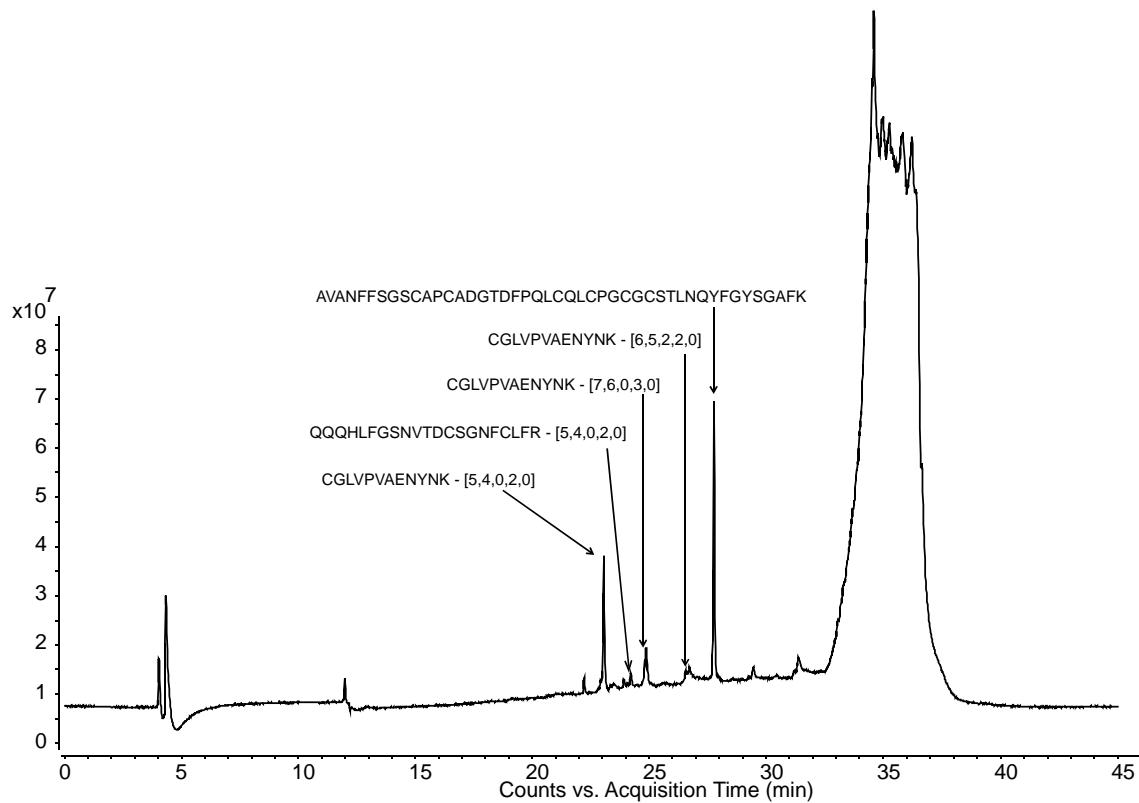


Figure S-4 HILIC-C18-MS of Transferrin tryptic digest on 2.1mm column scale. Glycan identifiers are described as [Hex, HexNAc, dHex, NeuAc, NeuGc]. Note: The large late-eluting peak corresponds to a singly charged column wash artifact (m/z 436.4366).

Section S-3

Theoretical mass lists for manual data analysis and for use with GlycReSoft:

The following standard glycoprotein sequences from UniProt database(<http://www.uniprot.org>) were subjected to theoretical digestion using Protein Prospector's MS-Digest utility (Baker, P.R. and Clauser, K.R. <http://prospector.ucsf.edu>):

1. Serotransferrin precursor - Homo sapiens (Human) - P02787
2. Alpha-1-acid glycoprotein 1 precursor - Homo sapiens (Human) – P02763
3. Recombinant hemagglutinin from A/USSR/90/1977 (H1N1) (Genebank #: ABD60933), amino acid 18-345 with sequence:
DTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLED SHNGKLCKLKGIA PLQLGKCSIAGWILGNPECESLVSKKS
WSYIAETPNSENGTCYPGYFADYEELREQLSSFERFEIFPKERSWP KHN VTRGV TASC SHKGKSSFYRNLL
WLTEKNGSYPNLSKSYVNNKEKEVLVLWGVHHPSNIEDQKTIYRKENAYVVSSNYNRRTPEIAERPKVRG
QAGRINYYWTLLEPGDTIIFEANGNLIAPWHAFALNRGFGSGIITSNASMDEC DTKCQT P QGAINSSL PFQNIH
PVTIGECPKYVRSTKLRMVTGLRNIPSIQSRG

Transferrin, AGP and hemagglutinin were subjected to theoretical digestion using trypsin with up to 2 missed cleavages and carbamidomethyl as the constant modification. Asparagine deamidation was used as the variable modification for hemagglutinin only. For transferrin and AGP, known glycopeptides were combined with a list of glycans to generate theoretical glycopeptides. For HA, the NetNGlyc 1.0 server (<http://www.cbs.dtu.dk/services/NetNGlyc>) was used to predict glycosylation sites. The *m/z*'s from data files were matched to the theoretical neutral mass lists within an error tolerance of +/- 10 ppm.

The lists of glycopeptides/peptides for each glycoprotein studied have been provided, as Table S-1 with the number of glycosylation sites/sequons considered as occupied on the glycopeptides. The masses of peptides listed below are for neutral peptides. The possible glycans considered occupying each sequon are listed in Table S-2, with their neutral masses. (**Both tables are downloadable as separate Excel spreadsheets with the supporting information.**)

To the glycopeptides listed in table S-1, glycan masses (table S-2) were added to generate glycopeptides. Glycan compositions used for the glycoproteins consisted possible glycan structures ranging from *N*-glycan core (Hex₃, HexNAc₂) to complex type penta-antennary structures containing N-acetylneuraminic acid and the high mannose type *N*-glycans. Glycan identifiers are listed as [a,b,c,d,e], where [a,b,c,d,e] describe the glycan compositions as follows:

- a = number of Hexoses
- b = number of N-acetylhexosamines
- c = number of Deoxyhexoses
- d = number of N-acetylneuraminic acids
- e = number of N-glycolylneuraminic acids

Section S-4

Peptide fragment ion mass tables for HILIC-C18 LC-MS/MS of glycopeptides identified and presented in Table 2.

Table S-3 (a) Transferrin glycopeptides tandem MS

	HILIC-C18		m/z	z
Glycopeptide	QQQHLFGSNVTDCSGNFCLFR-[6,5,1,3,0]		1381.3025	4+
Peptide	QQQHLFGSNVTDCSGNFCLFR			
		Observed m/z	Theoretical m/z	ppm error
y 1		175.1174	175.118952	-8.86
y 2		322.1828	322.187366	-14.17
y 5		742.3584	742.370428	-16.20
y 6		856.4059	856.413356	-8.71
y 7		913.4311	913.434819	-4.07
y 8		1000.4518	1000.46685	-15.04
y 9		1160.4855	1160.49743	-10.28
y 10		1275.5098	1275.52438	-11.43
y 11		1376.5634	1376.57205	-6.29
y 12		1475.6080	1475.64047	-22.00
y 15		1733.7168	1733.73689	-11.59
y 16		1880.8397	1880.8053	18.29
y 17		1993.8799	1993.88937	-4.75
y 18		2130.9503	2130.94828	0.95
y 13 + HexNAc		1792.7768	1792.76277	7.83
y 14 + HexNAc		1879.7452	1879.79479	-26.38
y 15 + HexNAc		1936.8295	1936.81626	6.84
y 17 + HexNAc		2196.9882	2196.96874	8.86
y 18 + HexNAc		2333.9954	2334.02765	-13.82
b 2		257.1224	257.124431	-7.90
b 3		385.1798	385.183009	-8.33
b 4		522.2357	522.241921	-11.91
b 5		635.3218	635.325985	-6.59
b 6		782.3807	782.394398	-17.51
b 8		926.4407	926.447891	-7.76
b 9		1040.4698	1040.49082	-20.20
b 13		1515.6835	1515.66444	12.58
b 14 + HexNAc		1805.7502	1805.77584	-14.20
b 15 + HexNAc		1862.8244	1862.7973	14.55
Peptide + HexNAc		2718.1394	2718.20338	-23.54

	possible backbone product ions	20.0000	% found	
	y ions	16.00	80%	
	b ions	10.00	50%	

	HILIC-C18		<i>m/z</i>	z
Glycopeptide	QQQHLFGSNVTDCSGNFCLFR -[5,4,0,2,0]		1180.7328	4+
Peptide	QQQHLFGSNVTDCSGNFCLFR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	175.1185	175.1190	-2.58
	y 2	322.1872	322.1874	-0.51
	y 3	435.2695	435.2714	-4.43
	y 4	595.3050	595.3020	5.02
	y 5	742.3654	742.3704	-6.77
	y 6	856.3990	856.4134	-16.76
	y 7	913.4225	913.4348	-13.49
	y 8	1000.4547	1000.4668	-12.14
	y 9	1160.4902	1160.4974	-6.23
	y 10	1275.5185	1275.5244	-4.61
	y 11	1376.5675	1376.5721	-3.31
	y 12	1475.6279	1475.6405	-8.52
	y 13	1589.6846	1589.6834	0.76
	y 14	1676.6916	1676.7154	-14.21
	y 15	1733.7374	1733.7369	0.30
	y 16	1880.7859	1880.8053	-10.32
	y 17	1993.8961	1993.8894	3.38
	y 18	2130.9452	2130.9483	-1.44
	y 19	2258.9746	2259.0069	-14.28
	y 13 + HexNAc	1792.7565	1792.7628	-3.49
	y 15 + HexNAc	1936.8053	1936.8163	-5.66
	y 16 + HexNAc	2083.8727	2083.8847	-5.74
	y 17 + HexNAc	2196.9742	2196.9687	2.49
	b 2	257.1238	257.1244	-2.45
	b 3	385.1794	385.1830	-9.37
	b 4	522.2386	522.2419	-6.36
	b 5	635.3246	635.3260	-2.18
	b 6	782.3917	782.3944	-3.45
	b 8	926.4447	926.4479	-3.44
	b 9	1040.5048	1040.4908	13.44
	b 10	1139.5450	1139.5592	-12.49
	b 14	1602.6972	1602.6965	0.46

	b 16	1773.7319	1773.7609	-16.33
	b 9 + HexNAc	1243.5625	1243.5702	-6.18
	b 11 + HexNAc	1443.6720	1443.6863	-9.89
	b 12 + HexNAc	1558.7207	1558.7132	4.80
	b 14 + HexNAc	1805.7800	1805.7758	2.31
	b 15 + HexNAc	1862.7935	1862.7973	-2.04
	b 16 + HexNAc	1976.8756	1976.8402	17.89
	b 18 + HexNAc	2283.9704	2283.9392	13.65
	Peptide	2515.1287	2515.1240	1.86
	Peptide + HexNAc	2718.1905	2718.2034	-4.74
	Peptide + HexNAc - Water	2700.1619	2700.1930	-11.52
	possible backbone product ions	20.0000	% found	
	y ions	19.00	95%	
	b ions	14.00	70%	

	HILIC-C18		m/z	z
Glycopeptide	CGLVPVLAENYNK -[5,4,0,2,0]		921.1389	4+
Peptide	CGLVPVLAENYNK			
		Observed m/z	Theoretical m/z	ppm error
	y 1	147.1107	147.1128	-14.30
	y 2	261.1531	261.1557	-10.08
	y 6	738.3419	738.3417	0.28
	y 9	1047.5368	1047.5469	-9.68
	y 10	1146.6207	1146.6154	4.67
	y 4 + HexNAc	741.3462	741.3414	6.53
	y 5 + HexNAc	870.3922	870.3840	9.48
	y 7 + HexNAc	1054.4910	1054.5051	-13.40
	y 9 + HexNAc	1250.6159	1250.6263	-8.32
	y 10 + HexNAc	1349.6940	1349.6947	-0.53
	b 2	218.0588	218.05932	-2.40
	b 3	331.1433	331.14339	-0.27
	b 4	430.2125	430.2118	1.62
	b 5	527.2667	527.26457	4.05
	b 6	626.3438	626.33298	17.27
	b 8	810.4497	810.45416	-5.50
	b 10	1053.5576	1053.5397	17.01
	b 11	1216.5864	1216.603	-13.65
	Peptide	1476.7515	1476.7515	0.03
	Peptide + HexNAc	1679.8191	1679.8308	-6.98
	peptide + HexNAc2 + Hex3	2369.0745	2369.0687	2.46

	possible backbone product ions	12.0000	% found	
	y ions	8.00	67%	
	b ions	8.00	67%	

	HILIC-C18		<i>m/z</i>	z
Glycopeptide	QQQHLFGSNVTDCSGNFCLFR -[5,4,1,2,0]		1217.2468	4+
Peptide	QQQHLFGSNVTDCSGNFCLFR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	175.1183	175.1189518	-3.72
	y 2	322.1848	322.1873657	-7.96
	y 3	435.2672	435.2714297	-9.72
	y 4	595.3004	595.3020143	-2.71
	y 5	742.3685	742.3704282	-2.60
	y 6	856.4138	856.4133557	0.52
	y 7	913.4274	913.4348194	-8.12
	y 8	1000.459	1000.466848	-8.14
	y 9	1160.49	1160.497432	-6.75
	y 10	1275.508	1275.524375	-13.15
	y 11	1376.591	1376.572054	13.62
	y 12	1475.626	1475.640468	-10.01
	y 15	1733.746	1733.736887	5.49
	y 16	1880.805	1880.805301	-0.21
	y 17	1993.915	1993.889365	12.71
	y 13 + HexNAc	1792.753	1792.762765	-5.56
	y 14 + HexNAc	1879.779	1879.794794	-8.56
	y 16 + HexNAc	2083.894	2083.884671	4.38
	y 17 + HexNAc	2196.941	2196.968735	-12.76
	b 2	257.1221	257.1244311	-9.07
	b 3	385.1815	385.1830087	-3.92
	b 4	522.2393	522.2419205	-5.02
	b 5	635.3301	635.3259845	6.48
	b 6	782.4043	782.3943985	12.66
	b 8	926.4465	926.4478907	-1.50
	b 9	1040.483	1040.490818	-7.61
	b 14	1602.698	1602.696467	0.64
	b 16	1773.742	1773.760858	-10.58
	b 11 + HexNAc	1443.673	1443.686281	-9.48
	b 15 + HexNAc	1862.779	1862.7973	-9.99
	b 16 + HexNAc	1976.806	1976.840228	-17.42
	Peptide	2515.115	2515.12401	-3.62

	Peptide + HexNAc	2718.178	2718.20338	-9.30
	Peptide + HexNAc - Water	2700.192	2700.193	-0.30
	possible backbone product ions	20.0000	% found	
	y ions	16.00	80%	
	b ions	11.00	55%	

	C18		<i>m/z</i>	z
Glycopeptide	CGLVPVLAENYNK -[5,4,0,2,0]		921.1389	4+
Peptide	CGLVPVLAENYNK			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	147.1117	147.1128	-7.50
	y 2	261.1555	261.1557	-0.89
	y 4	538.2579	538.262	-7.59
	y 6	738.3365	738.3417	-7.04
	y 9	1047.5342	1047.547	-12.16
	y 10	1146.6154	1146.615	0.04
	y 2 + HexNAc	464.2347	464.2351	-0.86
	y 4 + HexNAc	741.3468	741.3414	7.34
	y 5 + HexNAc	870.3922	870.384	9.48
	y 6 + HexNAc	941.4106	941.4211	-11.12
	y 7 + HexNAc	1054.5059	1054.505	0.73
	y 9 + HexNAc	1250.6247	1250.626	-1.28
	b 2	218.0601	218.0593	3.56
	b 3	331.1428	331.1434	-1.78
	b 4	430.2102	430.2118	-3.72
	b 8	810.4549	810.4542	0.92
	b 9	939.4873	939.4968	-10.06
	b 10	1053.5326	1053.54	-6.72
	b 11	1216.6069	1216.603	3.20
	b 12	1330.6311	1330.646	-11.15
	b 12 + HexNAc	1533.7095	1533.725	-10.30
	Peptide	1476.7521	1476.751	0.43
	Peptide + HexNAc	1679.8193	1679.831	-6.87
	Peptide + HexNAc -Water	1661.8405	1661.82	12.17
	Peptide + 2 HexNAc	1882.88	1882.91	-16.04
	Peptide + 2 HexNAc + 1 Hexose	2044.9542	2044.963	-4.31
	peptide + HexNAc2 + Hex2	2207.0612	2207.016	20.55
	peptide + HexNAc2 + Hex3	2369.1084	2369.069	16.77
	possible backbone product ions	12.0000	% found	

	y ions	8.00	67%	
	b ions	8.00	67%	

	C18		<i>m/z</i>	z
Glycopeptide	QQQHLFGSNVTDCSGNFCLFR -[5,4,0,2,0]		1180.7328	4+
Peptide	QQQHLFGSNVTDCSGNFCLFR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	175.1192	175.1190	1.42
	y 2	322.1851	322.1874	-7.03
	y 3	435.2732	435.2714	4.07
	y 4	595.2989	595.3020	-5.23
	y 5	742.3674	742.3704	-4.08
	y 6	856.4046	856.4134	-10.22
	y 7	913.4277	913.4348	-7.79
	y 8	1000.4646	1000.4668	-2.25
	y 9	1160.4898	1160.4974	-6.58
	y 11	1376.5686	1376.5721	-2.51
	y 12	1475.6413	1475.6405	0.56
	y 13	1589.6920	1589.6834	5.41
	y 14	1676.7348	1676.7154	11.56
	y 15	1733.7229	1733.7369	-8.07
	y 16	1880.7819	1880.8053	-12.44
	y 17	1993.8903	1993.8894	0.47
	y 18	2130.9328	2130.9483	-7.26
	y 13 + HexNAc	1792.7394	1792.7628	-13.03
	y 15 + HexNAc	1936.7923	1936.8163	-12.37
	y 16 + HexNAc	2083.8646	2083.8847	-9.63
	y 17 + HexNAc	2196.9391	2196.9687	-13.49
	b 2	257.1243	257.1244	-0.51
	b 3	385.1803	385.1830	-7.03
	b 4	522.2407	522.2419	-2.34
	b 5	635.3231	635.3260	-4.54
	b 6	782.3877	782.3944	-8.56
	b 7	839.4083	839.4159	-9.01
	b 8	926.4462	926.4479	-1.82
	b 10	1139.5543	1139.5592	-4.33
	b 14	1602.6965	1602.6965	0.02
	b 15	1659.6991	1659.7179	-11.35
	b 16	1773.7555	1773.7609	-3.02

	b 19	2193.9091	2193.9439	-15.87
	b 14 + HexNAc	1805.8077	1805.7758	17.64
	b 16 + HexNAc	1976.8615	1976.8402	10.76
	b 17 + HexNAc	2123.8766	2123.9086	-15.09
	b 20 + HexNAc	2544.0722	2544.0917	-7.67
	Peptide	2515.1150	2515.1240	-3.58
	Peptide + HexNAc	2718.1838	2718.2034	-7.20
	possible backbone product ions	20.0000	% found	
	γ ions	17.00	85%	
	b ions	14.00	70%	

Table S-3 (b) AGP glycopeptides tandem MS

	HILIC-C18		<i>m/z</i>	z
Glycopeptide	ENGTISR -[6,5,1,3,0]		1261.8232	3+
Peptide	ENGTISR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y1	175.1171	175.1190	-10.57
	Peptide	776.3774	776.3897	-15.85
	Peptide + HexNAc	979.4687	979.4691	-0.38
	Peptide + 2 HexNAc	1182.531	1182.5484	-15.01
	Peptide + 2 HexNAc + 1 Hexose	1344.595	1344.6013	-4.74
	Peptide + 2 HexNAc + 2 Hexose	1506.629	1506.6541	-16.72
	Peptide + 3 HexNAc + 2 Hexose	1709.725	1709.7335	-4.89
	Peptide + 3 HexNAc + 3 Hexose	1871.819	1871.7863	17.64
	Peptide + 3 HexNAc + 4 Hexose	2033.806	2033.8391	-16.32
	possible backbone product ions	6	% found	
	y ions	1	17%	
	b ions	0	0%	

	HILIC-C18		<i>m/z</i>	z
Glycopeptide	ENGTVSR -[7,6,0,4,0]		1070.6586	4+
Peptide	ENGTVSR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y1	175.1156	175.1190	-19.14
	y2	262.1463	262.1510	-17.85
	y3	361.2136	361.2194	-16.04
	y4	462.2702	462.2671	6.77
	y5	519.2827	519.2885	-11.24
	y6 + HexNAc	836.4216	836.4108	12.87
	b 2	244.0891	244.0928	-15.14
	b 6	588.2632	588.2624	1.39
	b 6 + HexNAc	791.3356	791.3418	-7.77
	Peptide + HexNAc	965.4510	965.4534	-2.51
	Peptide + 2 HexNAc	1168.5273	1168.5328	-4.70
	Peptide + 2 HexNAc + 1 Hexose	1330.5748	1330.5856	-8.13
	Peptide + 2 HexNAc + 2 Hexose	1492.6228	1492.6384	-10.48
	Peptide + 3 HexNAc + 2 Hexose	1695.7292	1695.7178	6.72
	Peptide + 3 HexNAc + 3 Hexose	1857.7716	1857.7706	0.52

	Peptide + 3 HexNAc + 4 Hexose	2019.8270	2019.8234	1.76
	Peptide + 4 HexNAc + 4 Hexose	2222.9048	2222.9028	0.89
	Peptide + 4 HexNAc + 5 Hexose	2384.9677	2384.9556	5.06
	Peptide + 5 HexNAc + 6 Hexose	2750.0792	2750.0878	-3.14
	possible backbone product ions	6.0000	% found	
	y ions	6.00	100%	
	b ions	2.00	33%	

	HILIC-C18		m/z	z
Glycopeptide	SVQEIQATFFYFTPNKTEDTIFLR -[7,6,0,4,0]		1283.342	5+
Peptide	SVQEIQATFFYFTPNKTEDTIFLR			
		Observed m/z	Theoretical m/z	ppm error
	y1	175.1179	175.1190	-6.01
	y3	435.2684	435.2714	-6.96
	y4	548.3481	548.3555	-13.48
	y5	649.4017	649.4032	-2.27
	y6	764.4272	764.4301	-3.81
	y7	893.4661	893.4727	-7.40
	y8	994.5176	994.5204	-2.80
	y9	1122.6098	1122.6154	-4.94
	y 12	1434.737	1434.759	-15.21
	y 15	1891.949	1891.959	-5.43
	y11 + HexNAc	1536.7857	1536.7904	-3.07
	y12 + HexNAc	1637.8186	1637.8381	-11.90
	y13 + HexNAc	1784.9000	1784.9065	-3.64
	y14 + HexNAc	1947.9796	1947.9698	5.01
	y15 + HexNAc	2095.0210	2095.0382	-8.23
	y16 + HexNAc	2242.0876	2242.1067	-8.50
	y17 + HexNAc	2343.1460	2343.1543	-3.56
	y18 + HexNAc	2414.1482	2414.1915	-17.92
	y19 + HexNAc	2542.2279	2542.2500	-8.71
	b 3	315.1638	315.1663	-7.92
	b 4	444.2078	444.2089	-2.45
	b 5	557.2905	557.293	-4.40
	b 6	685.3449	685.3515	-9.67
	b 7	756.3854	756.3886	-4.29
	b 8	857.4299	857.4363	-7.49
	b 9	1004.5	1004.505	-4.52
	b 10	1151.569	1151.573	-3.87
	b 11	1314.627	1314.636	-7.36

	b 12	1461.693	1461.705	-8.41
	b 13	1562.771	1562.753	11.92
	b 16	1901.937	1901.943	-3.38
	b 17	2002.998	2002.991	3.49
	b 17 + HexNAc	2206.073	2206.07	1.37
	b 19	2247.057	2247.06	-1.35
	b 22	2608.314	2608.261	20.28
	b 21 + HexNAc	2664.25	2664.272	-8.24

	HILIC-C18		m/z	z
Glycopeptide	ENGTISR -[7,6,0,4,0]		1074.163	4+
Peptide	ENGTISR			
		Observed m/z	Theoretical m/z	ppm error
	y1	175.1166	175.1190	-13.43
	y2	262.1497	262.1510	-4.88
	y3	375.2352	375.2350	0.42
	y4	476.2769	476.2827	-12.23
	y5	533.3041	533.3042	-0.16
	y6 + HexNAc	850.4158	850.4265	-12.56
	b 3	301.1078	301.1143	-21.46
	b 4	402.1574	402.1619	-11.29
	b 5	515.2479	515.246	3.68
	b 6	602.2772	602.278	-1.38
	b 6 + HexNAc	805.366	805.3574	10.68
	Peptide	776.3906	776.3897	1.15
	Peptide + HexNAc	979.4676	979.4691	-1.51
	Peptide + 2 HexNAc	1182.5475	1182.5484	-0.80
	Peptide + 2 HexNAc + 1 Hexose	1344.5997	1344.6013	-1.17
	Peptide + 2 HexNAc + 2 Hexose	1506.6562	1506.6541	1.40
	Peptide + 2 HexNAc + 3 Hexose	1668.7097	1668.7069	1.67
	Peptide + 3 HexNAc + 2 Hexose	1709.7472	1709.7335	8.04
	Peptide + 3 HexNAc + 3 Hexose	1871.7838	1871.7863	-1.32
	Peptide + 3 HexNAc + 4 Hexose	2033.8299	2033.8391	-4.52
	Peptide + 4 HexNAc + 3 Hexose	2074.8703	2074.8656	2.24
	Peptide + 4 HexNAc + 4 Hexose	2236.9040	2236.9185	-6.47
	Peptide + 4 HexNAc + 5 Hexose	2398.9753	2398.9713	1.67
	possible backbone product ions	6.0000	% found	
	y ions	6.00	100%	

	b ions	5.00	80%	

	HILIC-C18		<i>m/z</i>	z
Glycopeptide	NEEYNK-[5,4,0,2,0]		751.0409	4+
Peptide	NEEYNK			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	147.1106	147.1128	-14.98
	y 2	261.1545	261.1557	-4.71
	y 4	553.2545	553.2617	-12.93
	y 5	682.2981	682.3042	-9.01
	y2 + HexNAc	464.2350	464.2351	-0.22
	y3 + HexNAc	627.2983	627.2984	-0.21
	y4 + HexNAc	756.3386	756.3410	-3.20
	y5 + HexNAc	885.4005	885.3836	19.07
	b 2	244.0916	244.0928	-4.90
	b 3	373.1330	373.1354	-6.40
	b 4	536.1947	536.1987	-7.49
	b 5	650.2396	650.2416	-3.15
	Peptide	796.3435	796.3472	-4.61
	Peptide + HexNAc	999.4290	999.4265	2.46
	Peptide + 2 HexNAc	1202.5052	1202.5059	-0.59
	Peptide + 2 HexNAc + 1 Hexose	1364.5526	1364.5587	-4.49
	Peptide + 2 HexNAc + 2 Hexose	1526.6189	1526.612	4.81
	Peptide + 2 HexNAc + 3 Hexose	1688.6578	1688.664	-3.89
	possible backbone product ions	5.0000	% found	
	y ions	5.00	100%	
	b ions	4.00	80%	

	HILIC-C18		<i>m/z</i>	z
Glycopeptide	QDQCIYNTTYLNVQR-[6,5,0,3,0]		1194.9868	4+
Peptide	QDQCIYNTTYLNVQR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y1	175.1181	175.1190	-4.86
	y2	303.1769	303.1775	-2.08
	y3	402.2440	402.2459	-4.83
	y4	516.2902	516.2889	2.57
	y5	629.3707	629.3729	-3.55
	y6	792.4355	792.4363	-0.96
	y7	893.4851	893.4839	1.30
	y8	994.5241	994.5316	-7.56
	y 10	1271.6305	1271.6379	-5.80
	y 13	1672.8007	1672.8111	-6.22
	y 14	1787.8318	1787.8380	-3.49
	y9 + HexNAc	1311.6511	1311.6539	-2.15
	y10 + HexNAc	1474.7142	1474.7172	-2.07
	y11 + HexNAc	1587.8195	1587.8013	11.46
	y12 + HexNAc	1747.8614	1747.8319	16.88
	y14 + HexNAc	1990.8979	1990.9174	-9.80
	b 3	372.1489	372.1514	-6.65
	b 4	532.1782	532.1820	-7.06
	b 5	645.2666	645.2660	0.89
	b 6	808.3357	808.3294	7.85
	b 8	1023.4065	1023.4200	-13.15
	b 9	1124.4473	1124.4676	-18.09
	b 10	1287.5057	1287.5310	-19.62
	b 11	1400.5907	1400.6150	-17.37
	b 10 + HexNAc	1490.5931	1490.6103	-11.56
	Peptide + HexNAc	2118.9728	2118.9760	-1.51
	Peptide + 2 HexNAc	2322.0571	2322.0554	0.75
	Peptide + 2 HexNAc + 1 Hexose	2484.0972	2484.1082	-4.42
	Peptide + 2 HexNAc + 2 Hexose	2646.1560	2646.1610	-1.89
	Peptide + 2 HexNAc + 3 Hexose	2808.1819	2808.2138	-11.37
	possible backbone product ions	14.0000	% found	
	y ions	14.00	100%	
	b ions	8.00	57%	

	C18		<i>m/z</i>	z
Glycopeptide	QDQCIYNTTYLNVQR -[6,5,0,3,0]		1194.9868	4+
Peptide	QDQCIYNTTYLNVQR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y1	175.1194	175.1190	2.56
	y4	516.2955	516.2889	12.84
	y6	792.4377	792.4363	1.81
	y7	893.4122	893.4839	-80.30
	y9 + HexNAc	1311.6352	1311.6539	-14.27
	y10 + HexNAc	1474.0000	1474.7172	-486.60
	b 3	372.1529	372.1514	4.10
	b 5	645.2644	645.2660	-2.51
	Peptide	1915.9015	1915.8966	2.55
	Peptide + HexNAc	2118.9607	2118.9760	-7.22
	possible backbone product ions	14.0000	% found	
	y ions	6.00	43%	
	b ions	2.00	14%	

	HILIC-C18			
		<i>m/z</i>	z	
Glycopeptide	SVQEIQATFFYFTPNK -[7,6,0,4,0]	1088.2441	5+	
Peptide	SVQEIQATFFYFTPNK			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y1	147.1125	147.1128	-2.06
	y2	261.1519	261.1557	-14.67
	y3	358.2063	358.2085	-6.13
	y4	459.2487	459.2562	-16.27
	y5	606.3179	606.3246	-11.03
	y6	769.3798	769.3879	-10.55
	y7	916.4518	916.4563	-4.94
	y8	1063.5147	1063.5247	-9.44
	y9	1164.5832	1164.5724	9.25
	y10	1235.5985	1235.6095	-8.93
	y11	1363.6637	1363.6681	-3.24
	y12	1476.7234	1476.7522	-19.49
	y13	1605.7993	1605.7948	2.82

	y3 + HexNAc	561.2845	561.28787	-6.00
	y4 + HexNAc	662.3326	662.33554	-4.44
	y5 + HexNAc	809.4062	809.40396	2.77
	y6 + HexNAc	972.4679	972.46729	0.63
	y7 + HexNAc	1119.5267	1119.5357	-8.04
	y8 + HexNAc	1266.6004	1266.6041	-2.93
	y9 + HexNAc	1367.6379	1367.6518	-10.16
	y10 + HexNAc	1438.6860	1438.6889	-2.02
	y11 + HexNAc	1566.7448	1566.7475	-1.71
	y12 + HexNAc	1679.8491	1679.8315	10.45
	y14 + HexNAc	1936.9112	1936.9327	-11.11
	b 3	315.1646	315.1663	-5.38
	b 4	444.2048	444.2089	-9.21
	b 5	557.2930	557.2930	0.08
	b 6	685.3473	685.3515	-6.17
	b 7	756.3911	756.3886	3.25
	b 8	857.4334	857.4363	-3.41
	b 9	1004.4931	1004.5047	-11.58
	b 10	1151.5640	1151.5732	-7.95
	b 11	1314.6423	1314.6365	4.43
	b 12	1461.6989	1461.7049	-4.10
	b 13	1562.7397	1562.7526	-8.24
	b 15	1773.8266	1773.8483	-12.21
	Peptide	1919.9542	1919.9538	0.21
	Peptide + HexNAc	2123.0217	2123.0332	-5.40
	Peptide + 2 HexNAc	2326.0847	2326.1125	-11.96
	Peptide + 2 HexNAc + Hexose	2488.1914	2488.1654	10.47
	Peptide + 2 HexNAc + 2 Hexose	2650.2004	2650.2182	-6.71
	Peptide + 2 HexNAc + 3 Hexose	2812.2723	2812.2710	0.47
	possible backbone product ions	15.0000	% found	
	y ions	14.00	93%	
	b ions	12.00	80%	

Table S-3 (c) Hemagglutinin glycopeptides tandem MS

	HILIC-C18			
			<i>m/z</i>	<i>z</i>
Glycopeptide	NGSYPNLSK-[5,4,1,1,0]		1013.7474	3
Peptide	NGSYPNLSK			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 2	234.144	234.145	-3.55
	b 3	259.102	259.104	-7.32
	y 3	347.228	347.229	-1.43
	b 4	422.162	422.167	-12.61
	y 4	461.268	461.272	-8.51
	b 5	519.216	519.220	-7.68
	y 5	558.324	558.325	-1.05
	y 6	721.386	721.388	-2.10
	b 7	746.345	746.347	-2.52
	y 5 + HexNAc	761.402	761.404	-3.23
	y 7	808.420	808.420	0.19
	b 8	833.380	833.379	0.95
	y 8	865.440	865.441	-1.97
	y 6 + HexNAc	924.461	924.467	-7.02
	b 7 + HexNAc	949.425	949.426	-1.21
	protonated peptide	979.483	979.484	-1.57
	y 7 + HexNAc	1011.500	1011.499	0.48
	b 8 + HexNAc	1036.468	1036.458	9.67
	y 8 + HexNAc	1068.521	1068.521	-0.17
	peptide + HexNAc	1182.563	1182.564	-0.35
	peptide + HexNAc2	1385.639	1385.643	-3.31
	peptide + HexNAc2 + Hex	1547.686	1547.696	-6.40
	peptide + HexNAc2 + Hex2	1709.747	1709.749	-1.19
	peptide + HexNAc2 + Hex3	1871.791	1871.802	-5.80
	possible backbone product ions	8	% found	
	y ions	7	88%	
	b ions	5	63%	

	HILIC-C18			
			<i>m/z</i>	<i>z</i>
Glycopeptide	NVTVTHSVNLLEDSHN(deamidated)GK-[7,6,1,3,0]		1068.2338	5+
Peptide	NVTVTHSVNLLEDSHN(deamidated)GK			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	147.1108	147.1128	-13.62
	b 2	214.1160	214.1186	-12.22
	b 3	315.1644	315.1663	-6.02
	y 3	319.1585	319.1612	-8.48
	b 4	414.2260	414.2347	-21.03
	b 2 + HexNAc	417.1999	417.1980	4.58
	y 4	456.2186	456.2201	-3.32
	b 5	515.2763	515.2824	-11.82
	b 3 + HexNAc	518.2476	518.2457	3.73
	y 5	543.2487	543.2521	-6.34
	b 4 + HexNAc	617.3090	617.3141	-8.23
	b 6	652.3321	652.3413	-14.10
	y 6	658.2612	658.2791	-27.18
	b 7	739.3668	739.3733	-8.83
	y 7	787.3161	787.3217	-7.09
	b 8	838.4354	838.4417	-7.56
	b 6 + HexNAc	855.4139	855.4207	-7.91
	y 8	900.4005	900.4057	-5.83
	b 7 + HexNAc	942.4532	942.4527	0.53
	b 9	952.4850	952.4847	0.35
	y 9	1013.4791	1013.4898	-10.57
	b 10	1065.5609	1065.5687	-7.35
	y 10	1127.5267	1127.5327	-5.35
	b 9 + HexNAc	1155.5530	1155.5640	-9.55
	y 11	1226.5983	1226.6012	-2.32
	b 12	1307.6857	1307.6954	-7.41
	y 12	1313.6227	1313.6332	-7.98
	b 11 + HexNAc	1381.7250	1381.7322	-5.19
	b 13	1422.7126	1422.7223	-6.84
	y 13	1450.6695	1450.6921	-15.57
	b 12 + HexNAc	1510.7657	1510.7748	-6.00
	y 14	1551.7295	1551.7398	-6.62
	b 13 + HexNAc	1625.7885	1625.8017	-8.12
	b 15	1646.8039	1646.8133	-5.69
	y 15	1650.7778	1650.8082	-18.41
	y 16	1751.8259	1751.8559	-17.10

	b 16	1761.8224	1761.8402	-10.11
	b 17	1818.8291	1818.8617	-17.91
	b 15 + HexNAc	1849.8810	1849.8926	-6.29
	protonated peptide	1964.9563	1964.9672	-5.55
	b 17 + HexNAc	2021.9255	2021.9410	-7.69
	peptide + HexNAc	2168.0326	2168.0466	-6.45
	peptide + HexNAc2	2371.0991	2371.1259	-11.32
	peptide + HexNAc2 + Hex	2533.1436	2533.1788	-13.88
	peptide + HexNAc2 + Hex2	2695.2083	2695.2316	-8.64
	possible backbone product ions	17	% found	
	y ions	15	88%	
	b ions	15	88%	

	HILIC-C18			
			m/z	z
Glycopeptide	GFGSGIITSNASMDECDTK-[7,6,1,3,0]		1341.2684	4+
Peptide	GFGSGIITSNASMDECDTK			
		Observed m/z	Theoretical m/z	ppm error
	y 1	147.1112	147.1128	-10.90
	y 2	248.1607	248.1605	0.88
	b 4	349.1444	349.1506	-17.89
	y 3	363.1841	363.1874	-9.16
	b 5	406.1701	406.1721	-4.95
	y 4	523.2146	523.2180	-6.52
	b 7	632.3387	632.3402	-2.43
	y 5	652.2580	652.2606	-3.99
	b 8	733.3841	733.3879	-5.20
	y 6	767.2845	767.2875	-3.97
	b 9	820.4112	820.4199	-10.66
	y 7	898.3257	898.3280	-2.59
	b 10	934.4526	934.4629	-10.99
	y 8	985.3553	985.3601	-4.83
	b 11	1005.4953	1005.5000	-4.66
	y 9	1056.3950	1056.3972	-2.06
	y 10	1170.4369	1170.4401	-2.73
	b 13	1223.5647	1223.5725	-6.37
	y 11	1257.4641	1257.4721	-6.38
	b 14	1338.5923	1338.5994	-5.34
	y 12	1358.5220	1358.5198	1.61
	y 10 + HexNAc	1373.5205	1373.5195	0.75

	y 11 + HexNAc	1460.5441	1460.5515	-5.07
	b 15	1467.6255	1467.6420	-11.27
	y 13	1471.6009	1471.6039	-2.02
	b 14 + HexNAc	1541.6727	1541.6788	-3.96
	y 12 + HexNAc	1561.5898	1561.5992	-6.01
	y 15	1641.6838	1641.7094	-15.59
	b 15 + HexNAc	1670.6899	1670.7214	-18.86
	y 13 + HexNAc	1674.6863	1674.6832	1.83
	b 17	1742.7083	1742.6996	5.01
	y 17	1785.7645	1785.7629	0.90
	b 17 + HexNAc	1945.7675	1945.7789	-5.88
	protonated peptide	1989.8460	1989.8528	-3.40
	peptide + HexNAc	2192.9244	2192.9321	-3.53
	peptide + HexNAc2	2395.9952	2396.0115	-6.81
	peptide + HexNAc2 + Hex	2558.0484	2558.0643	-6.23
	peptide + HexNAc2 + Hex2	2720.1064	2720.1172	-3.96
	peptide + HexNAc2 + Hex3	2882.1538	2882.1700	-5.62
	possible backbone product ions	18	% found	
	y ions	15	83%	
	b ions	11	61%	

	HILIC-C18			
			<i>m/z</i>	z
Glycopeptide	SWSYIAETPNSENGTCYPGYFADYEELR-[7,6,1,3,0]		1339.127	5+
Peptide	SWSYIAETPNSENGTCYPGYFADYEELR			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	b 10	1149.5089	1149.5211	-10.63
	b 12	1365.5856	1365.5957	-7.42
	b 13	1479.6185	1479.6387	-13.63
	b 15	1637.6754	1637.7078	-19.79
	b 16	1797.7078	1797.7384	-17.02
	b 17	1960.7803	1960.8017	-10.92
	b 17 + HexNAc	2163.8944	2163.8811	6.15
	b 18	2057.8461	2057.8545	-4.07
	b 18 + HexNAc	2260.8985	2260.9339	-15.64
	b 20	2277.9413	2277.9393	0.89
	b 20 + HexNAc	2481.0215	2481.0186	1.15
	b 23	2611.0396	2611.0717	-12.31

	b 3	361.1484	361.1506	-6.22
	b 4	524.2118	524.2140	-4.15
	b 5	637.2929	637.2980	-8.06
	b 6	708.3242	708.3352	-15.46
	b 7	837.3693	837.3777	-10.09
	b 8	938.4152	938.4254	-10.89
	b 9	1035.4623	1035.4782	-15.34
	y 1	175.1170	175.1190	-11.15
	y 10	1262.5528	1262.5688	-12.67
	y 11	1359.6107	1359.6216	-7.99
	y 12	1522.6688	1522.6849	-10.56
	y 13	1682.6928	1682.7155	-13.47
	y 14	1783.7462	1783.7631	-9.50
	y 15	1840.7661	1840.7846	-10.06
	y 16 + HexNAc	2157.8850	2157.9069	-10.15
	y 17	2083.8732	2083.8701	1.47
	y 17 + HexNAc	2286.9058	2286.9495	-19.11
	y 18	2170.8698	2170.9022	-14.91
	y 18 + HexNAc	2373.9678	2373.9815	-5.78
	y 2	288.1998	288.2030	-11.16
	y 20	2381.9667	2381.9979	-13.08
	y 20 + HexNAc	2585.0541	2585.0772	-8.95
	y 21 + HexNAc	2686.0805	2686.1249	-16.53
	y 22 + HexNAc	2815.1257	2815.1675	-14.85
	y 23 + HexNAc	2886.1594	2886.2046	-15.66
	y 3	417.2411	417.2456	-10.81
	y 4	546.2830	546.2882	-9.52
	y 5	709.3451	709.3515	-9.07
	y 6	824.3718	824.3785	-8.10
	y 7	895.4076	895.4156	-8.92
	y 8	1042.4747	1042.4840	-8.92
	y 9	1205.5335	1205.5473	-11.47
	possible backbone product ions	27	% found	
	y ions	22	81%	
	b ions	16	59%	

	HILIC-C18			
			m/z	z
Glycopeptide	GFGSGIITSNASMDECCTK-[7,6,1,4,0]		1131.439	5+
Peptide	GFGSGIITSNASMDECCTK			
		Observed m/z	Theoretical m/z	ppm error
	y 1	147.1114	147.1128	-9.54
	y 2	248.1593	248.1605	-4.76
	b 3	262.1139	262.1186	-18.00
	y 3	363.1923	363.1874	13.42
	b 5	406.1687	406.1721	-8.39
	b 6	519.2523	519.2562	-7.46
	y 4	523.2129	523.2180	-9.77
	b 7	632.3330	632.3402	-11.45
	y 5	652.2550	652.2606	-8.59
	b 8	733.3783	733.3879	-13.11
	y 6	767.2883	767.2875	0.98
	b 9	820.4057	820.4199	-17.36
	y 7	898.3219	898.3280	-6.82
	b 10	934.4490	934.4629	-14.85
	y 8	985.3582	985.3601	-1.89
	b 11	1005.4944	1005.5000	-5.56
	y 9	1056.3900	1056.3972	-6.79
	b 12	1092.5222	1092.5320	-8.98
	y 10	1170.4517	1170.4401	9.91
	b 13	1223.5887	1223.5725	13.24
	y 11	1257.4770	1257.4721	3.87
	y 12	1358.5045	1358.5198	-11.27
	y 11 + HexNAc	1460.5428	1460.5515	-5.96
	b 14 + HexNAc	1541.6670	1541.6788	-7.66
	y 12 + HexNAc	1561.6034	1561.5992	2.70
	b 17	1742.7077	1742.6996	4.67
	y 15 + HexNAc	1844.8194	1844.7888	16.60
	y 18	1932.8149	1932.8313	-8.49
	protonated peptide	1989.8438	1989.8528	-4.51
	y 18 + HexNAc	2135.8829	2135.9107	-13.00
	peptide + HexNAc	2192.9191	2192.9321	-5.95
	peptide + HexNAc2 + Hex3	2882.1268	2882.1700	-14.98
	possible backbone product ions	18	% found	
	y ions	14	78%	
	b ions	12	67%	

	C18			
			<i>m/z</i>	<i>z</i>
Glycopeptide	NGSYPNLSK-[5,4,1,1,0]		1013.7467	3
Peptide	NGSYPNLSK			
		Observed <i>m/z</i>	Theoretical <i>m/z</i>	ppm error
	y 1	147.1099	147.1128	-19.74
	y 2	234.1424	234.1448	-10.39
	b 3	259.1000	259.1037	-14.26
	y 3	347.2262	347.2289	-7.77
	b 4	422.1643	422.1670	-6.45
	y 4	461.2676	461.2718	-9.16
	y 5	558.3236	558.3246	-1.77
	b 6	633.2524	633.2627	-16.29
	y 6	721.3859	721.3879	-2.79
	b 7	746.3444	746.3468	-3.19
	y 7	808.4163	808.4199	-4.51
	b 8	833.3797	833.3788	1.07
	y 8	865.4392	865.4414	-2.55
	protonated peptide	979.4823	979.4843	-2.08
	peptide + HexNAc	1182.5622	1182.5637	-1.28
	peptide + HexNAc2	1385.6479	1385.6431	3.48
	peptide + HexNAc2 + Hex	1547.6915	1547.6959	-2.85
	peptide + HexNAc2 + Hex2	1709.7505	1709.7487	1.04
	peptide + HexNAc2 + Hex3	1871.7977	1871.8016	-2.06
	possible backbone product ions	8	% found	
	y ions	8	100%	
	b ions	5	63%	