

Supplemental Material

Title

Changes in the Expression of N- and O-Glycopeptides in Patients with Colorectal Cancer and Hepatocellular Carcinoma Quantified by Full-MS Scan FT-ICR and Multiple Reaction Monitoring.

Authors

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Supplemental Table 1: Clinical characteristics of the study population. Each group includes 10 participants. CTRL, control; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus. TNM (tumor-node-metastasis), MELD (Model for End-Stage Liver Disease).

	CTRL	Colorectal	Colorectal + Liver metastases	HCC	HCC/HCV
Male gender	7	6	6	10	8
Age (mean)	57	69	63	68	56
Race					
Caucasian	6	10	10	9	6
African-American	4				4
Asian				1	
Etiology					
HBV				1	
HCV					10
Cirrhosis					
				4	10
TNM					
T1N0M0					5
T2N0M0					3
MELD					
			10.8±1.4	8.8±2.4	11.2±3

Supplemental Table 2: List of proteins identified in albumin-depleted human serum by data dependent analysis performed by FT-ICR. Glycopeptides of the highlighted proteins were further quantified by MRM and full-MS scan FT-ICR analysis.

Row	Accession	Protein	MW [kDa]	#Peptides	SC [%]	RMS90 [ppm]
1	IGKC_HUMAN	Ig kappa chain C region	11.6	9	85.8	0.77
2	LAC2_HUMAN	Ig lambda-2 chain C regions	11.3	4	74.5	0.44
3	ALBU_HUMAN	Serum albumin	69.3	51	73.7	0.74
4	FIBB_HUMAN	Fibrinogen beta chain	55.9	20	62.5	0.62
5	TRFE_HUMAN	Serotransferrin	77.0	34	59.6	0.63
6	LAC1_HUMAN	Ig lambda-1 chain C regions	11.3	4	58.5	0.24
7	IGHG1_HUMAN	Ig gamma-1 chain C region	36.1	12	53.6	0.59
8	TTHY_HUMAN	Transthyretin	15.9	5	53.1	1.49
9	HPT_HUMAN	Haptoglobin	45.2	14	51.0	0.56
10	IGHA1_HUMAN	Ig alpha-1 chain C region	37.6	12	47.3	0.49
11	IGHG2_HUMAN	Ig gamma-2 chain C region	35.9	10	38.0	0.56
12	IGHG3_HUMAN	Ig gamma-3 chain C region	41.3	10	37.1	0.52
13	IGHG4_HUMAN	Ig gamma-4 chain C region	35.9	7	35.8	0.79
14	FIBA_HUMAN	Fibrinogen alpha chain	94.9	20	33.8	0.74
15	IGHA2_HUMAN	Ig alpha-2 chain C region	36.5	8	33.5	0.39
16	A1AT_HUMAN	Alpha-1-antitrypsin	46.7	11	32.8	0.57
17	FIBG_HUMAN	Fibrinogen gamma chain	51.5	9	32.5	0.88
18	APOA1_HUMAN	Apolipoprotein A-I	30.8	7	27.3	0.34
19	CO3_HUMAN	Complement C3	187.0	29	27.1	0.55
20	IGHM_HUMAN	Ig mu chain C region	49.3	9	26.1	0.33
21	IGJ_HUMAN	Immunoglobulin J chain	18.1	3	23.3	0.50
22	KV204_HUMAN	Ig kappa chain V-II region TEW	12.3	1	21.2	0.56
23	ANT3_HUMAN	Antithrombin-III	52.6	5	21.1	0.56
24	A2MG_HUMAN	Alpha-2-macroglobulin	163.2	17	21.0	0.53
25	CLUS_HUMAN	Clusterin	52.5	5	20.3	0.47
26	AMBP_HUMAN	Protein AMBP	39.0	3	18.8	0.69
27	CFAH_HUMAN	Complement factor H	139.0	13	18.7	0.64
28	LV403_HUMAN	Ig lambda chain V-IV region Hil	11.5	1	17.8	0.20
29	APOH_HUMAN	Beta-2-glycoprotein 1	38.3	3	17.4	0.76
30	VTNC_HUMAN	Vitronectin	54.3	5	16.9	0.82
31	KV104_HUMAN	Ig kappa chain V-I region CAR	11.7	1	16.8	0.15
32	KV101_HUMAN	Ig kappa chain V-I region AG	12.0	1	16.7	0.58
33	KV302_HUMAN	Ig kappa chain V-III region SIE	11.8	1	16.5	0.46
34	HV305_HUMAN	Ig heavy chain V-III region BRO	13.2	1	15.8	0.05
35	VTDB_HUMAN	Vitamin D-binding protein	52.9	4	15.8	0.65
36	KV402_HUMAN	Ig kappa chain V-IV region Len	12.6	1	15.8	0.11
37	HEMO_HUMAN	Hemopexin	51.6	6	15.4	0.65
38	CO4A_HUMAN	Complement C4-A	192.7	12	14.5	0.97
39	LV302_HUMAN	Ig lambda chain V-III region LOI	11.9	1	14.4	0.05
40	PLMN_HUMAN	Plasminogen	90.5	7	13.5	0.32
41	HBB_HUMAN	Hemoglobin subunit beta	16.0	1	12.9	2.20
42	A1BG_HUMAN	Alpha-1B-glycoprotein	54.2	3	11.7	0.23
43	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2	106.4	4	9.6	1.19

44	HV306_HUMAN	Ig heavy chain V-III region BUT	12.4	1	9.6	0.58
45	KNG1_HUMAN	Kininogen-1	71.9	5	9.5	0.31

Supplemental Table 3: List of glycopeptides selected for quantification by MRM. Com. – Complement C3, Fib. – Fibronectin, Hpt. – Haptoglobin, Hpx. – Hemopexin, Mac. – Alpha-2-macroglobulin, Ser. – Serotransferin. Q1 – precursor ion, Q3 – transition.

Protein/sequence/glycopeptide	Q1 (m/z)	Q3 (m/z)	Protein/sequence/glycopeptide	Q1 (m/z)	Q3 (m/z)
Com.1601-1624-A2G2.204	1116.8	204	Hpx.181-193-A2G2.204	1009.8	204
Com.1601-1624-A2G2.366	1116.8	366.1	Hpx.181-193-A2G2.366	1009.8	366.1
Com.1601-1624-A2G2.pepHexNAc	1116.8	1015.5	Hpx.181-193-A2G2.pepHexNAc	1009.8	804.4
Com.74-94-A2G2.204	970.2	204	Hpx.181-193-A2G2F1.204	1058.4	204
Com.74-94-A2G2.366	970.2	366.1	Hpx.181-193-A2G2F1.366	1058.4	366.1
Com.74-94-A2G2.pepHexNAc	970.2	1229.6	Hpx.181-193-A2G2F1.pepHexNAc	1058.4	804.4
Com.74-94-A2G2F1.204	1006.7	204	Hpx.181-193-A3G3.204	848.9	204
Com.74-94-A2G2F1.366	1006.7	366.1	Hpx.181-193-A3G3.366	848.9	366.1
Com.74-94-A2G2F1.pepHexNAc	1006.7	1229.6	Hpx.181-193-A3G3.pepHexNAc	848.9	804.4
Com.74-94-A2G3.204	1061.5	204	Hpx.181-193-A3G3F1.204	885.4	204
Com.74-94-A2G3.366	1061.5	366.1	Hpx.181-193-A3G3F1.366	885.4	366.1
Com.74-94-A2G3.pepHexNAc	1061.5	1229.6	Hpx.181-193-A3G3F1.512	885.4	512.1
Com.74-94-A2G3F1.204	1098	204	Hpx.181-193-A3G3F1.pepHexNAc	885.4	804.4
Com.74-94-A2G3F1.366	1098	366.1	Hpx.181-193-A3G3F2.204	921.9	204
Com.74-94-A2G3F1.pepHexNAc	1098	1229.6	Hpx.181-193-A3G3F2.366	921.9	366.1
			Hpx.181-193-A3G3F2.512	921.9	512.1
Fib.377-395-A2G2.204	879.6	204	Hpx.181-193-A3G3F2.pepHexNAc	921.9	804.4
Fib.377-395-A2G2.366	879.6	366.1	Hpx.447-462-A2G2.204	1120.2	204
Fib.377-395-A2G2.pepHexNAc	879.6	1048.5	Hpx.447-462-A2G2.366	1120.2	366.1
Fib.377-395-A2G2F1.204	916.1	204	Hpx.447-462-A2G2.pepHexNAc	1120.2	970
Fib.377-395-A2G2F1.366	916.1	366.1	Hpx.447-462-A2G2F1.204	876.9	204
Fib.377-395-A2G2F1.pepHexNAc	916.1	1048.5	Hpx.447-462-A2G2F1.366	876.9	366.1
Fib.377-395-A3G3.204	970.9	204	Hpx.447-462-A2G2F1.pepHexNAc	876.9	970
Fib.377-395-A3G3.366	970.9	366.1	Hpx.447-462-A3G3.204	931.7	204
Fib.377-395-A3G3.pepHexNAc	970.9	1048.5	Hpx.447-462-A3G3.366	931.7	366.1
Fib.377-395-A3G3F1.204	1007.4	204	Hpx.447-462-A3G3.pepHexNAc	931.7	970
Fib.377-395-A3G3F1.366	1007.4	366.1	Hpx.447-462-A3G3F1.204	968.2	204
Fib.377-395-A3G3F1.pepHexNAc	1007.4	1048.5	Hpx.447-462-A3G3F1.366	968.2	366.1
Fib.427-436-A2G2.204	716.3	204	Hpx.447-462-A3G3F1.512	968.2	512.1
Fib.427-436-A2G2.366	716.3	366.1	Hpx.447-462-A3G3F1.pepHexNAc	968.2	970
Fib.427-436-A2G2.pepHexNAc	716.3	721.8	Hpx.447-462-A3G3F2.204	1004.7	204
Fib.427-436-A2G2F1.204	752.8	204	Hpx.447-462-A3G3F2.366	1004.7	366.1
Fib.427-436-A2G2F1.366	752.8	366.1	Hpx.447-462-A3G3F2.512	1004.7	512.1
Fib.427-436-A2G2F1.pepHexNAc	752.8	721.8	Hpx.447-462-A3G3F2.pepHexNAc	1004.7	970
Fib.427-436-A3G3.204	807.6	204	Hpx.O-glycans-1N1H1NA.204	843.6	204

Fib.427-436-A3G3.366	807.6	366.1	Hpx.O-glycans-1N1H1NA.366	843.6	366.1
Fib.427-436-A3G3.pepHexNAc	807.6	721.8	Hpx.O-glycans-1N1H1NA.pepHexNAc	843.6	973.5
Fib.427-436-A3G3F1.204	844.1	204	Hpx.O-glycans-1N1H2NA.204	916.4	204
Fib.427-436-A3G3F1.366	844.1	366.1	Hpx.O-glycans-1N1H2NA.366	916.4	366.1
Fib.427-436-A3G3F1.pepHexNAc	844.1	721.8	Hpx.O-glycans-1N1H2NA.pepHexNAc	916.4	973.5
Fib.427-445-A2G2.204	960.6	204			
Fib.427-445-A2G2.366	960.6	366.1	Mac.1422-1440-A2G2.204	947.2	204
Fib.427-445-A2G2.pepHexNAc	960.6	1210.5	Mac.1422-1440-A2G2.366	947.2	366.1
Fib.427-445-A2G2F1.204	997.2	204	Mac.1422-1440-A2G2.pepHexNAc	947.2	1183.6
Fib.427-445-A2G2F1.366	997.2	366.1	Mac.1422-1440-A2G2F1.204	983.7	204
Fib.427-445-A2G2F1.pepHexNAc	997.2	1210.5	Mac.1422-1440-A2G2F1.366	983.7	366.1
Fib.427-445-A3G3.204	1051.9	204	Mac.1422-1440-A2G2F1.pepHexNAc	983.7	1183.6
Fib.427-445-A3G3.366	1051.9	366.1	Mac.1422-1440-A3G3.204	1038.5	204
Fib.427-445-A3G3.pepHexNAc	1051.9	1048.5	Mac.1422-1440-A3G3.366	1038.5	366.1
Fib.427-445-A3G3F1.204	1088.4	204	Mac.1422-1440-A3G3.pepHexNAc	1038.5	1183.6
Fib.427-445-A3G3F1.366	1088.4	366.1	Mac.1422-1440-A3G3F1.204	1075	204
Fib.427-445-A3G3F1.pepHexNAc	1088.4	1048.5	Mac.1422-1440-A3G3F1.366	1075	366.1
			Mac.1422-1440-A3G3F1.512	1075	512.1
Hpt.197-202-A2G2.204	1076.2	204	Mac.1422-1440-A3G3F1.pepHexNAc	1075	1183.6
Hpt.197-202-A2G2.366	1076.2	366.1	Mac.1422-1440-A3G3F2.204	1111.5	204
Hpt.197-202-A2G2.pepHexNAc	1076.2	961.5	Mac.1422-1440-A3G3F2.366	1111.5	366.1
Hpt.197-202-A2G2F1.204	1112.8	204	Mac.1422-1440-A3G3F2.512	1111.5	512.1
Hpt.197-202-A2G2F1.366	1112.8	366.1	Mac.1422-1440-A3G3F2.pepHexNAc	1111.5	1183.6
Hpt.197-202-A2G2F1.pepHexNAc	1112.8	961.5	Mac.47-68-A2G2.204	1005.7	204
Hpt.197-202-A3G3.204	1167.5	204	Mac.47-68-A2G2.366	1005.7	366.1
Hpt.197-202-A3G3.366	1167.5	366.1	Mac.47-68-A2G2.pepHexNAc	1005.7	867.1
Hpt.197-202-A3G3.pepHexNAc	1167.5	961.5	Mac.47-68-A2G2F1.204	1042.2	204
Hpt.197-202-A3G3F1.204	1204	204	Mac.47-68-A2G2F1.366	1042.2	366.1
Hpt.197-202-A3G3F1.366	1204	366.1	Mac.47-68-A2G2F1.pepHexNAc	1042.2	867.1
Hpt.197-202-A3G3F1.512	1204	512.1	Mac.47-68-A3G3.204	1097	204
Hpt.197-202-A3G3F1.pepHexNAc	1204	961.5	Mac.47-68-A3G3.366	1097	366.1
Hpt.197-202-A3G3F2.204	992.7	204	Mac.47-68-A3G3.pepHexNAc	1097	867.1
Hpt.197-202-A3G3F2.366	992.7	366.1	Mac.47-68-A3G3F1.204	1133.5	204
Hpt.197-202-A3G3F2.512	992.7	512.1	Mac.47-68-A3G3F1.366	1133.5	366.1
Hpt.197-202-A3G3F2.pepHexNAc	992.7	961.5	Mac.47-68-A3G3F1.512	1133.5	512.1
Hpt.197-202-A4G4.204	1007.3	204	Mac.47-68-A3G3F1.pepHexNAc	1133.5	867.1
Hpt.197-202-A4G4.366	1007.3	366.1	Mac.47-68-A3G3F2.204	1170	204
Hpt.197-202-A4G4.pepHexNAc	1007.3	961.5	Mac.47-68-A3G3F2.366	1170	366.1
Hpt.197-202-A4G4F1.204	1036.5	204	Mac.47-68-A3G3F2.512	1170	512.1
Hpt.197-202-A4G4F1.366	1036.5	366.1	Mac.47-68-A3G3F2.pepHexNAc	1170	867.1
Hpt.197-202-A4G4F1.512	1036.5	512.1			
Hpt.197-202-A4G4F1.pepHexNAc	1036.5	961.5	Ser.421-433-A2G2.204	775.6	204
Hpt.197-202-A4G4F2.204	1065.7	204	Ser.421-433-A2G2.366	775.6	366.1

Hpt.197-202-A4G4F2.366	1065.7	366.1	Ser.421-433-A2G2.pepHexNAc	775.6	840.4
Hpt.197-202-A4G4F2.512	1065.7	512.1	Ser.421-433-A2G2F1.204	812.1	204
Hpt.197-202-A4G4F2.pepHexNAc	1065.7	961.5	Ser.421-433-A2G2F1.366	812.1	366.1
Hpt.197-202-A4G4F3.204	1094.9	204	Ser.421-433-A2G2F1.pepHexNAc	812.1	840.4
Hpt.197-202-A4G4F3.366	1094.9	366.1	Ser.421-433-A3G3.204	866.9	204
Hpt.197-202-A4G4F3.512	1094.9	512.1	Ser.421-433-A3G3.366	866.9	366.1
Hpt.197-202-A4G4F3.pepHexNAc	1094.9	961.5	Ser.421-433-A3G3.pepHexNAc	866.9	840.4
Hpt.236-251-A2G2.204	855.2	204	Ser.421-433-A3G3F1.204	903.4	204
Hpt.236-251-A2G2.366	855.2	366.1	Ser.421-433-A3G3F1.366	903.4	366.1
Hpt.236-251-A2G2.pepHexNAc	855.2	999.5	Ser.421-433-A3G3F1.pepHexNAc	903.4	840.4
Hpt.236-251-A2G2F1.204	891.7	204	Ser.622-646-A2G2.204	1035.2	204
Hpt.236-251-A2G2F1.366	891.7	366.1	Ser.622-646-A2G2.366	1035.2	366.1
Hpt.236-251-A2G2F1.pepHexNAc	891.7	999.5	Ser.622-646-A2G2.pepHexNAc	1035.2	906.7
Hpt.236-251-A3G3.204	946.4	204	Ser.622-646-A2G2F1.204	1071.7	204
Hpt.236-251-A3G3.366	946.4	366.1	Ser.622-646-A2G2F1.366	1071.7	366.1
Hpt.236-251-A3G3.pepHexNAc	946.4	999.5	Ser.622-646-A2G2F1.pepHexNAc	1071.7	906.7
Hpt.236-251-A3G3F1.204	983	204	Ser.622-646-A3G3.204	1126.5	204
Hpt.236-251-A3G3F1.366	983	366.1	Ser.622-646-A3G3.366	1126.5	366.1
Hpt.236-251-A3G3F1.512	983	512.1	Ser.622-646-A3G3.pepHexNAc	1126.5	906.7
Hpt.236-251-A3G3F1.pepHexNAc	983	999.5	Ser.622-646-A3G3F1.204	1163	204
Hpt.236-251-A3G3F2.204	1019.5	204	Ser.622-646-A3G3F1.366	1163	366.1
Hpt.236-251-A3G3F2.366	1019.5	366.1	Ser.622-646-A3G3F1.pepHexNAc	1163	906.7
Hpt.236-251-A3G3F2.512	1019.5	512.1			
Hpt.236-251-A3G3F2.pepHexNAc	1019.5	999.5			
Hpt.236-251-A4G4.204	1037.7	204			
Hpt.236-251-A4G4.366	1037.7	366.1			
Hpt.236-251-A4G4.pepHexNAc	1037.7	999.5			
Hpt.236-251-A4G4F1.204	1074.2	204			
Hpt.236-251-A4G4F1.366	1074.2	366.1			
Hpt.236-251-A4G4F1.512	1074.2	512.1			
Hpt.236-251-A4G4F1.pepHexNAc	1074.2	999.5			
Hpt.236-251-A4G4F2.204	1110.7	204			
Hpt.236-251-A4G4F2.366	1110.7	366.1			
Hpt.236-251-A4G4F2.512	1110.7	512.1			
Hpt.236-251-A4G4F2.pepHexNAc	1110.7	999.5			
Hpt.236-251-A4G4F3.204	1147.3	204			
Hpt.236-251-A4G4F3.366	1147.3	366.1			
Hpt.236-251-A4G4F3.512	1147.3	512.1			
Hpt.236-251-A4G4F3.pepHexNAc	1147.3	999.5			

Supplemental Table 4: List of glycopeptides with observed CID peptide or peptide+HexNAc fragments.

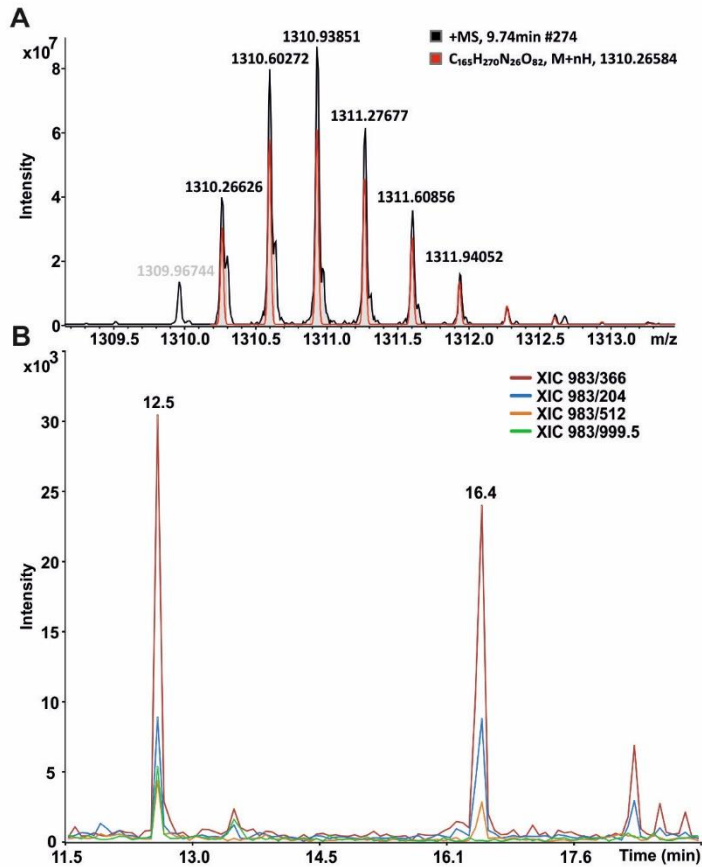
Protein	Peptide	Glycan	Precursor mass	Peptide Fragments
Serotransferrin	CGLVPVLAENYNK	A2G2	1033.7833 (3+)	Pep+GlcNAc-840.42 (2+)
	QQQHLFGSNVTDCSGNFCLFR	A2G2	1379.9010 (3+)	Pep+GlcNAc-1359.61 (2+)
Hemopexin	SWPAVGNCSALR	A2G2	1514.1293 (2+)	Pep+GlcNAc-804.38 (2+)
	TPLPPTSAHGNAEGETKDPDPVTER	1N1H1NA	1124.5225 (3+)	y4-504.24 (1+), y6-716.36 (1+), y8-928.44 (1+)
Haptoglobin	VVLHPNYSQVDIGLIK	A2G2	1139.8693 (3+)	Pep+GlcNAc-999.55 (2+), b3-449.29 (1+)
	MVSHHNLTTGATLINEQWLLTTAK	A2G2	1434.6615 (3+)	Pep+GlcNAc-1441.73 (2+)
Complement factor H	IPCSQPPQIEHGTINSSR	A2G2	1215.1938 (3+)	Pep+GlcNAc-1112.53 (2+)
Clusterin 1	HNSTGCLR	A2G2	1284.0117 (2+)	Pep+GlcNAc-1148.52 (1+)

Supplemental Table 5: List of all quantified glycopeptides by FT-ICR/MRM (upper part) and by FT-ICR only (lower part) with calculated median intensities (Me Intensity), median ratios (Me Ratio), standard deviations (SD) and p-values.

Protein/Peptide		Glycoform		FT-ICR					MRM				
Haptoglobin				Colorectal	HCC	Colorectal+met	HCC/HCV	Control	Colorectal	HCC	Colorectal+met	HCC/HCV	Control
MVSHHNLTTGATLINEQWLLTTAK	A3G3F1	Me Intensity		4.6E+06	1.1E+07	2.4E+07	3.4E+06	4.8E+06	2.0E+06	9.9E+05	2.5E+06	4.0E+05	3.8E+05
	A3G3	Me Intensity		9.3E+07	8.3E+07	1.1E+08	1.1E+07	2.3E+07	5.0E+06	3.7E+06	8.7E+06	3.8E+05	2.4E+06
	A2G2	Me Intensity		9.7E+08	5.1E+08	7.5E+08	9.4E+07	2.0E+08	3.8E+07	1.8E+07	4.7E+07	3.2E+06	1.7E+07
	A3G3F1/A3G3	Me Ratio		7.1	13.5	17.0	36.0	27.3	26.8	28.6	23.4	89.4	15.6
		SD		15.1	13.7	13.2	41.2	14.3	14.3	12.3	14.3	57.0	10.2
		p-value		0.1	0.3	0.3	0.2		0.2	0.09	0.3	0.0008	
	A3G3/A2G2	Me Ratio		9.5	14.1	14.7	10.4	8.6	17.2	19.8	18.4	13.7	15.1
		SD		4.2	6.3	7.5	9.5	13.7	7.8	6.5	5.0	4.3	5.6
p-value			0.3	0.003	0.01	0.4		0.5	0.009	0.07	0.9		
VVLHPNYSQVDIGLIK	A3G3F1	Me Intensity		4.7E+07	1.2E+08	7.2E+07	1.0E+07	7.9E+06	8.1E+05	5.0E+05	1.1E+06	1.4E+05	6.2E+04
	A3G3	Me Intensity		5.3E+08	6.1E+08	7.9E+08	4.2E+07	1.8E+08	6.7E+06	3.0E+06	9.3E+06	3.5E+05	1.6E+06
	A2G2	Me Intensity		4.1E+09	3.3E+09	4.6E+09	5.4E+08	1.1E+09	3.2E+07	1.9E+07	5.6E+07	2.1E+06	1.4E+07
	A3G3F1/A3G3	Me Ratio		12.0	17.3	9.2	23.8	4.0	9.5	15.8	8.2	35.2	4.9
		SD		6.9	9.8	7.6	34.4	4.2	6.2	10.0	8.4	23.3	3.2
		p-value		0.08	0.001	0.06	0.0009		0.03	0.002	0.04	0.0009	
	A3G3/A2G2	Me Ratio		14.5	18.1	16.8	8.5	9.5	21.5	16.5	17.3	19.2	15.2
		SD		7.8	2.8	4.9	3.2	1.7	5.2	3.3	3.4	6.9	5.9
p-value			0.2	0.001	0.002	0.9		0.2	0.8	0.9	0.7		
Hemopexin TPLPPTSAHGNVAEGETKPPDPVTER	1N1H2NA	Me Intensity		5.1E+07	8.2E+07	6.1E+07	7.2E+07	3.0E+07	6.7E+05	5.0E+05	5.8E+05	6.2E+05	2.7E+05
	1N1H1NA	Me Intensity		2.1E+09	1.8E+09	2.3E+09	6.8E+08	1.9E+09	5.6E+07	4.0E+07	3.8E+07	9.5E+06	3.4E+07
	1N1H2NA/1N1H1NA	Me Ratio		3.1	4.2	2.6	13.0	1.6	1.1	1.7	1.5	5.8	0.9
		SD		5.2	2.3	1.3	12.3	0.8	0.6	0.8	0.6	6.4	0.3
		p-value		0.01	0.004	0.03	0.0002		0.08	0.004	0.007	0.0003	
Protein/Peptide	Glycoform		FT-ICR										
Hemopexin SWPAVGNCSSALR	A3G3F1	Me Intensity		2.7E+07	1.7E+07	2.1E+07	1.4E+07	8.7E+06					
	A3G3	Me Intensity		4.9E+07	3.1E+07	3.4E+07	9.9E+06	1.7E+07					
	A2G2F1	Me Intensity		6.1E+07	7.2E+07	1.2E+08	7.1E+07	4.9E+07					
	A2G2	Me Intensity		1.0E+09	1.0E+09	1.3E+09	5.1E+08	7.9E+08					
	A3G3F1/A3G3	Me Ratio		59.2	60.5	49.3	148.2	49.2					
		SD		28.2	41.0	34.9	98.7	43.4					
		p-value		0.6	0.3	1.0	0.007						
	A2G2F1/A2G2	Me Ratio		8.3	7.8	9.4	13.4	7.1					

		SD	3.2	5.5	2.9	2.2	1.5
		p-value	0.3	0.1	0.05	0.001	
Complement factor H							
IPCSQPPQIEHGTINSSR	A3G3F1	Me Intensity	8.7E+06	9.0E+06	8.5E+06	1.5E+07	1.2E+07
	A3G3	Me Intensity	3.9E+07	2.8E+07	3.7E+07	8.8E+06	3.3E+07
	A2G2F1	Me Intensity	1.0E+08	6.4E+07	1.2E+08	7.0E+07	7.9E+07
	A2G2	Me Intensity	1.6E+09	9.3E+08	1.5E+09	3.3E+08	1.4E+09
	A3G3F1/A3G3	Me Ratio	30.0	33.5	26.3	139.8	40.3
		SD	9.5	9.2	18.2	169.9	28.3
		p-value	0.4	0.5	0.3	0.0006	
	A2G2F1/A2G2	Me Ratio	6.7	6.5	6.6	18.7	6.1
		SD	1.3	2.5	1.8	18.4	1.4
		p-value	1.0	0.8	0.7	0.0002	

Supplemental Figure 1: A) The simulated (red) and measured (black) isotopic pattern of haptoglobin glycopeptide VVLHPNYSQVDIGLIK bearing the tri-antennary fucosylated glycan observed as triply charged ion at m/z 1310.26626 by FT-ICR. One of the co-eluted ion at m/z 1309.96744 is shown in grey. B) XICs of the transitions of the same glycopeptide analyzed by MRM. All monitored transitions have maximum intensity at retention time 12.5 min.



Supplemental Figure 2: Extract ion chromatograms of bi-antennary (high abundant) and tri-antennary fucosylated (low abundant) haptoglobin glycopeptides VVLHPNYSQVDIGLIK measured in three technical replicates of the control sample. The XICs of the low abundant fucosylated glycopeptide are reproducible which enabled its quantification.

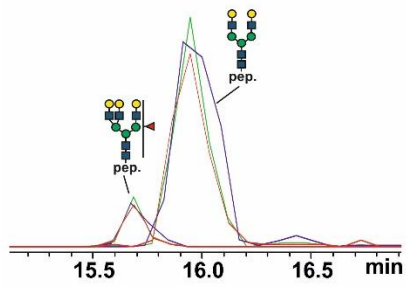


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