Supplementary Information

Comprehensive *N*- and *O*-glycosylation Mapping of Human Coagulation Factor V

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Running Title: Glycosylation of human factor V

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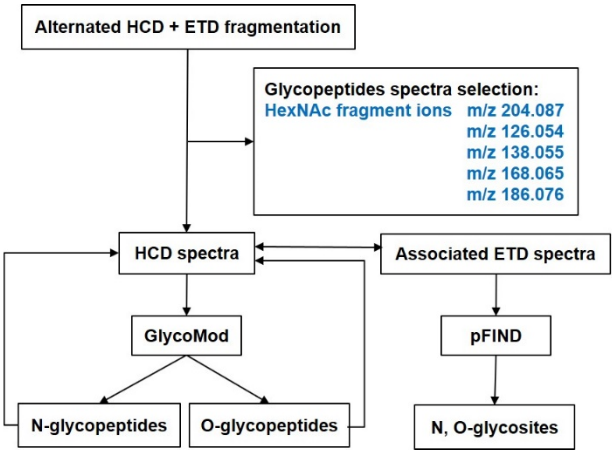
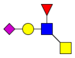
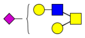
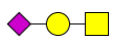
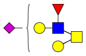
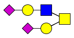
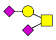
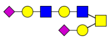
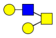
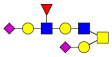
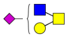
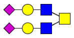
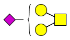
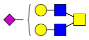
I. Scheme S1



Scheme S1. The workflow of HCD- and ETD-MS/MS analysis.

II. Table S1

No.	Glycoform	Glycosite		No.	Glycoform	Glycosite
41		T805, S1044, S1150		**50**		S720
42		T805, S985/T986, S1044, T1047, S1053, S1150, S1926, T2053		**51**		T805, S1018
43		T805, T898, S917 S985/T986, S1044, T1047, S1150, T1211, T1238, T1283, T1302, S1481, T1520, S1594, S1868, S1926		**52**		T805, T1029
44		S917, S1053		**53**		T805, S985/T986
45		S985/T986, S1053, S1150, T1211, T1238, T1283, T1302, S1594		**54**		S985/T986, S2090
46		S1150, T1247		**55**		S985/T986
47		T805		**56**		S2090
48		T1990/T1991		**57**		S1781
49		S310				

List of 17 identified O-glycoforms and 26 O-glycosites from FV. ■ N-acetylglucosamine (GlcNAc), □ N-acetylgalactosamine (GalNAc), ○ Galactose (Gal), ◆ N-acetylneuraminic acid (Neu5Ac), ▼ L-Fucose

III. Tandem MS annotation of N- and O-glycopeptide

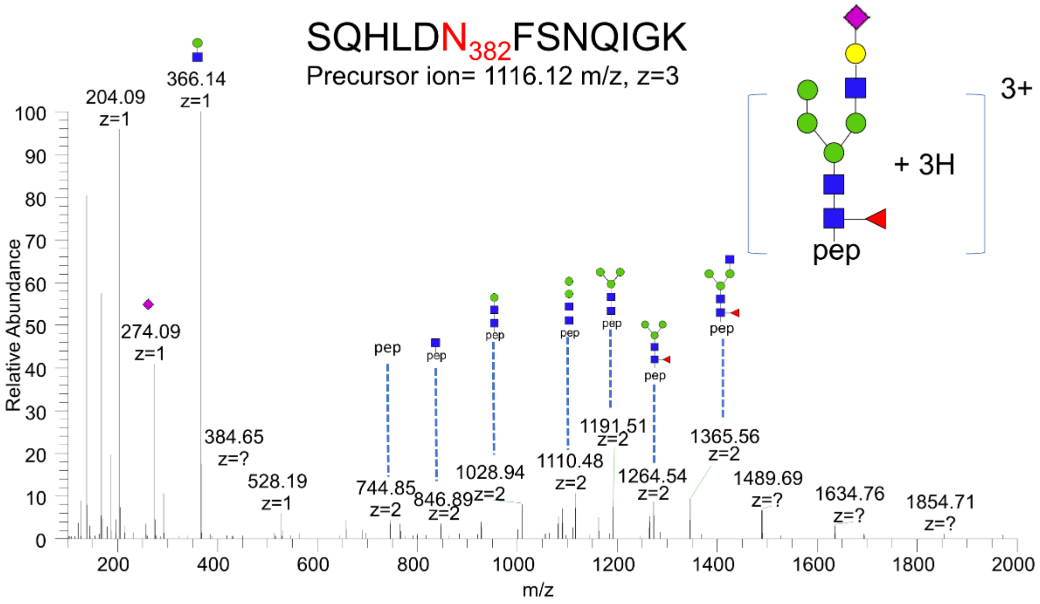


Figure S1. Tandem MS annotation of N-glycopeptide SQHLDN382FSNQIGK with hybrid type glycoform.

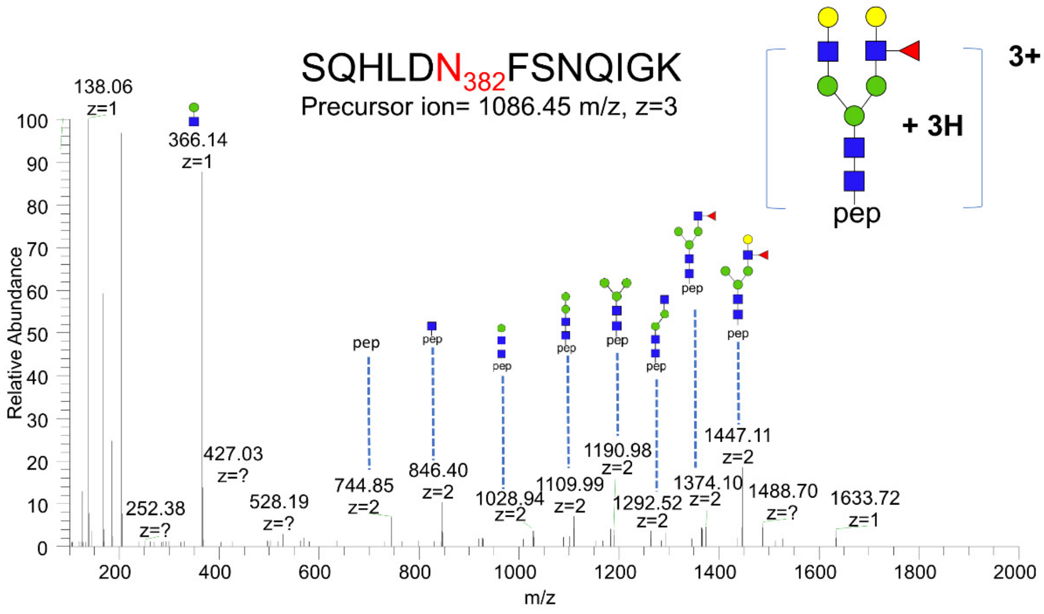


Figure S2. Tandem MS annotation of N-glycopeptide SQHLDN382FSNQIGK with complex type glycoform.

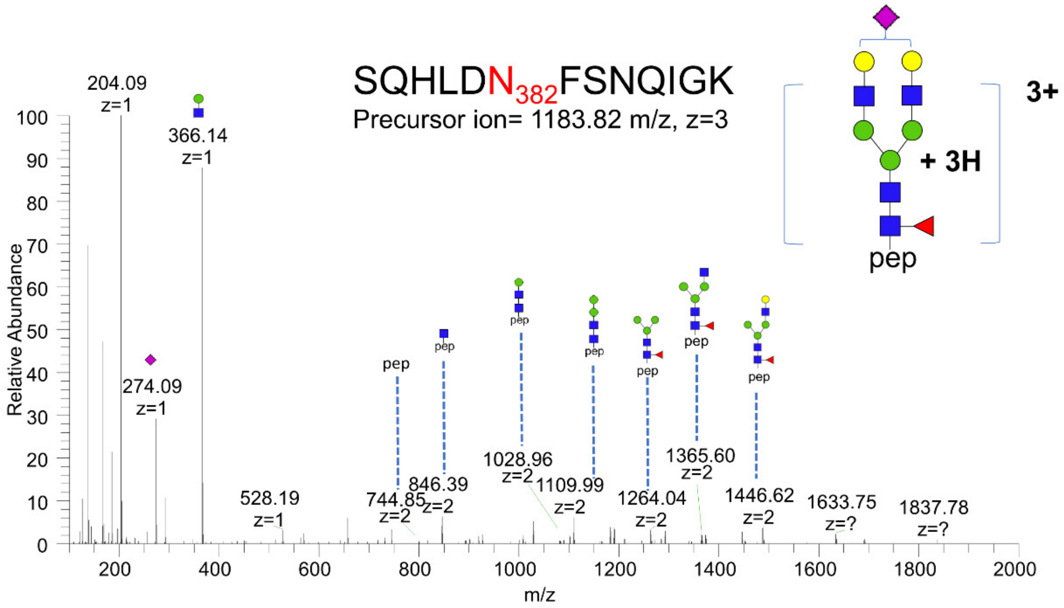


Figure S3. Tandem MS annotation of N-glycopeptide SQHLDN382FSNQIGK with sialylated complex type glycoform.

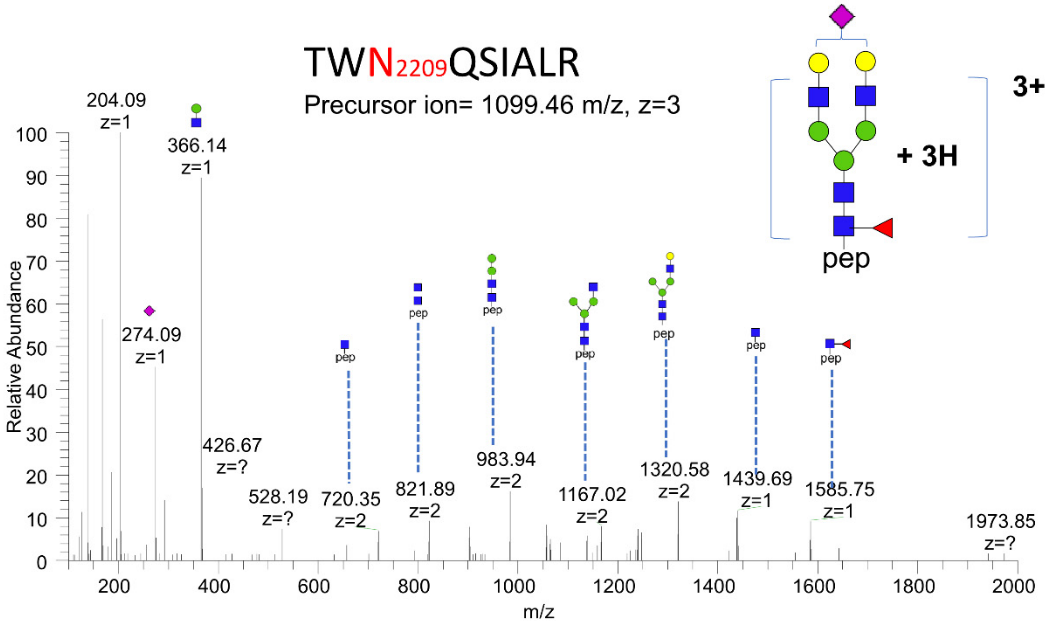


Figure S4. Tandem MS annotation of N-glycopeptide TWN382QSIALR with sialylated complex type glycoform.

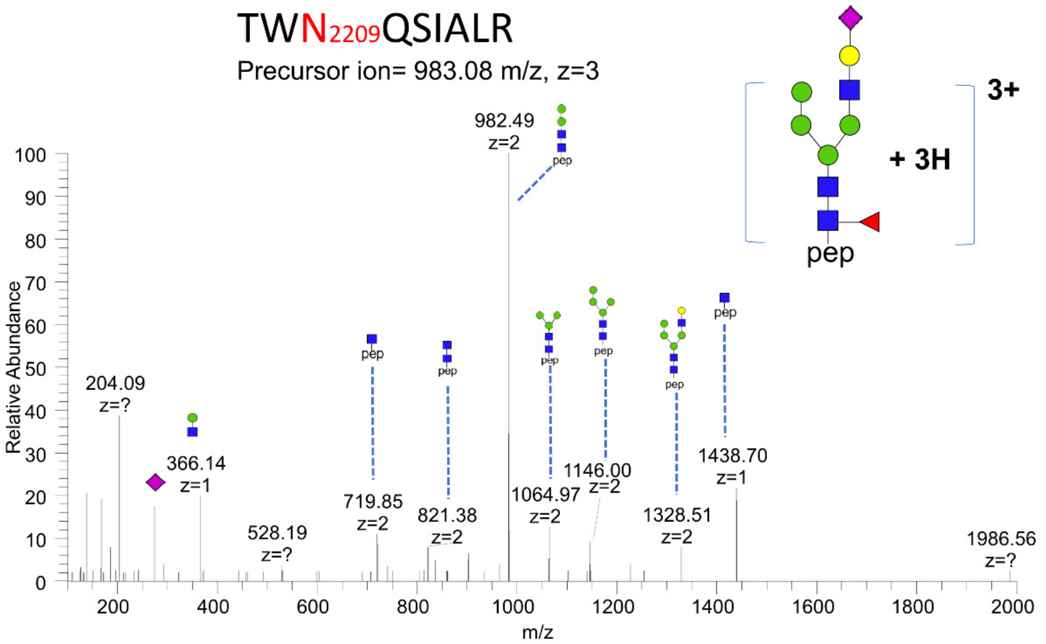


Figure S5. Tandem MS annotation of N-glycopeptide TWN$_{382}$QSIALR with sialylated hybrid type glycoform.

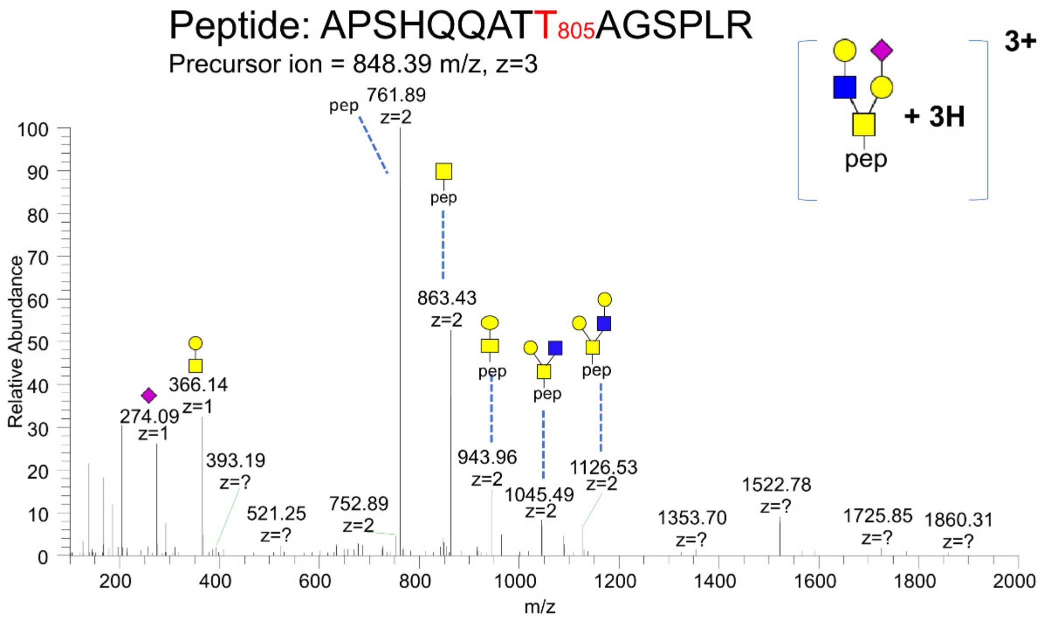


Figure S6. Tandem MS annotation of O-glycopeptide APSHQQATT$_{805}$AGSPLR.

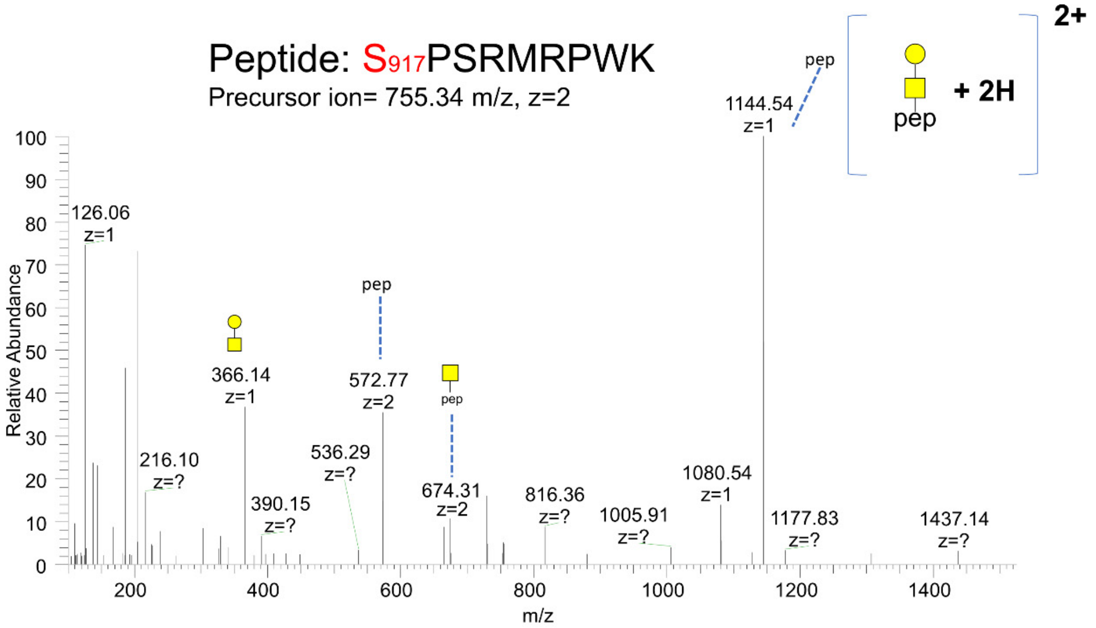


Figure S7. Tandem MS annotation of O-glycopeptide S_{917}PSRMRPWK.

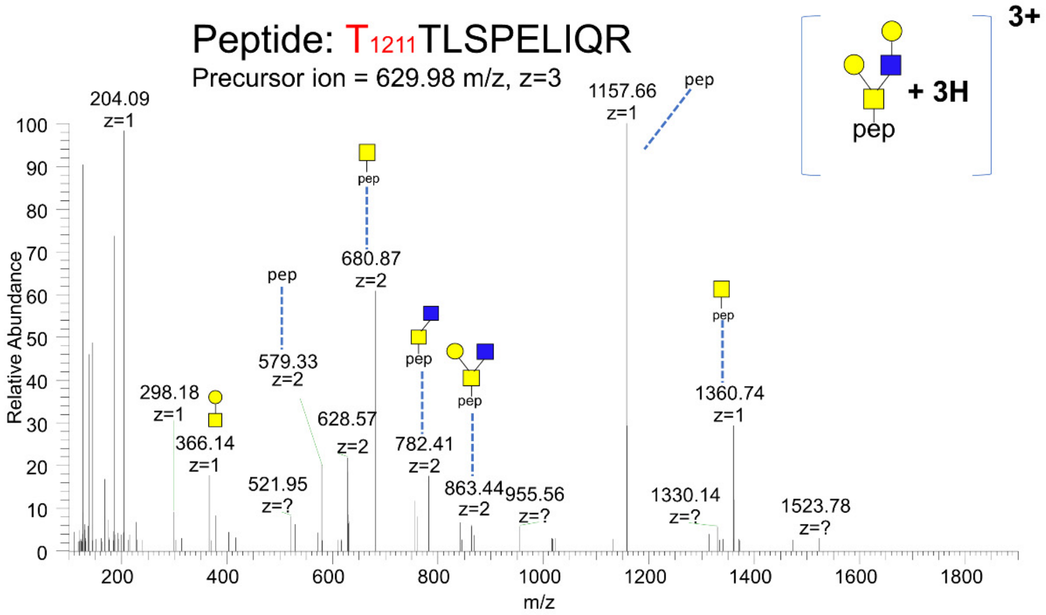


Figure S8. Tandem MS annotation of O-glycopeptide T_{1211}TLSPELIQR.

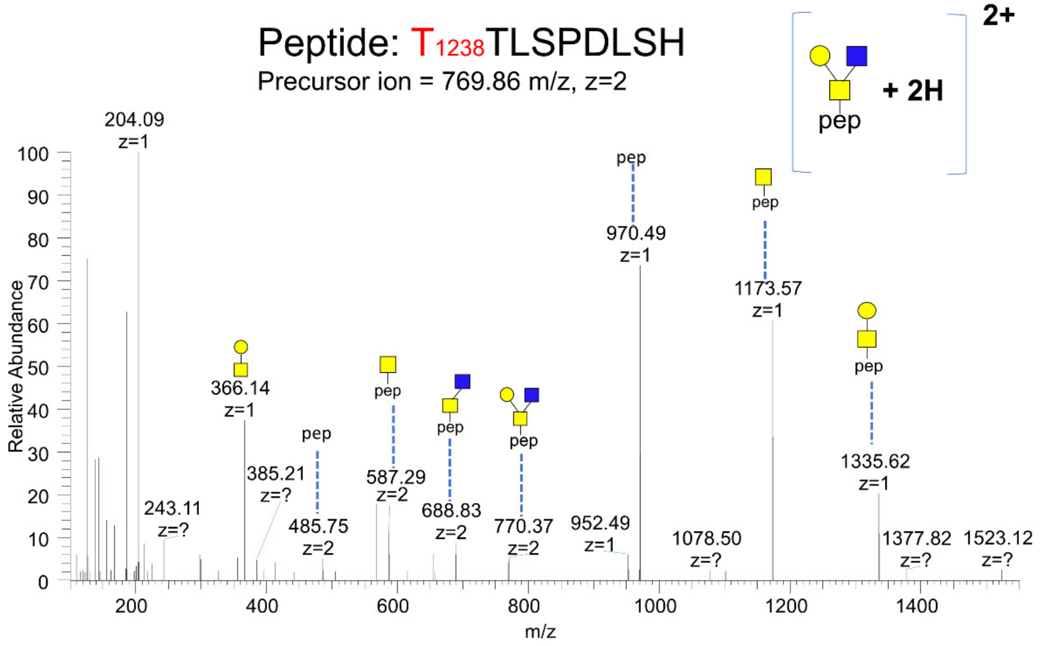


Figure S9. Tandem MS annotation of O-glycopeptide T1238TLSPDLSH.

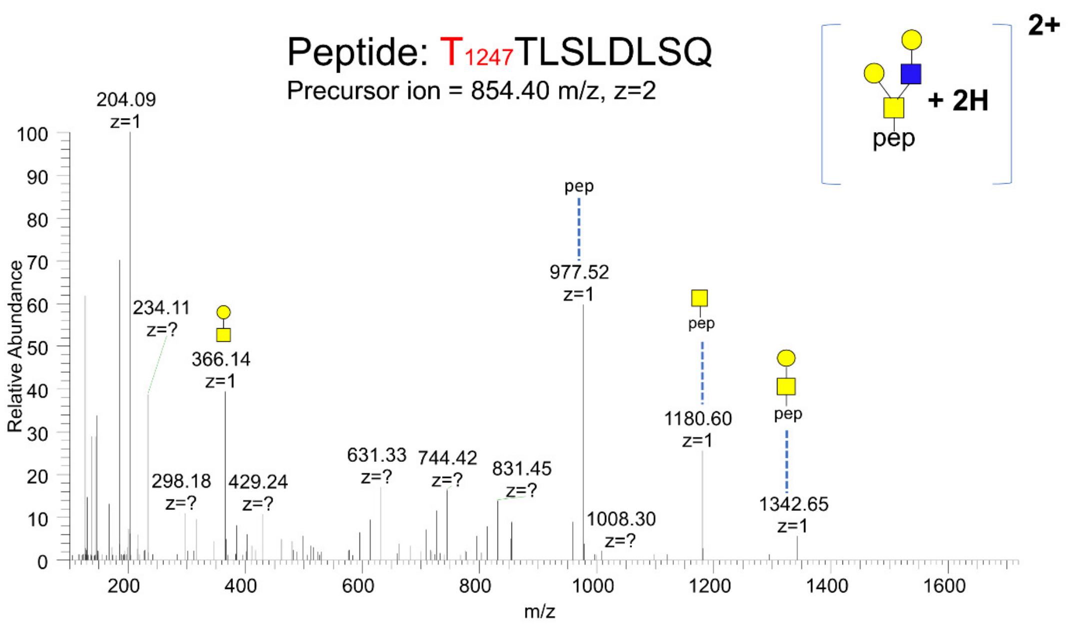


Figure S10. Tandem MS annotation of O-glycopeptide T1247TLSLDLSQ.

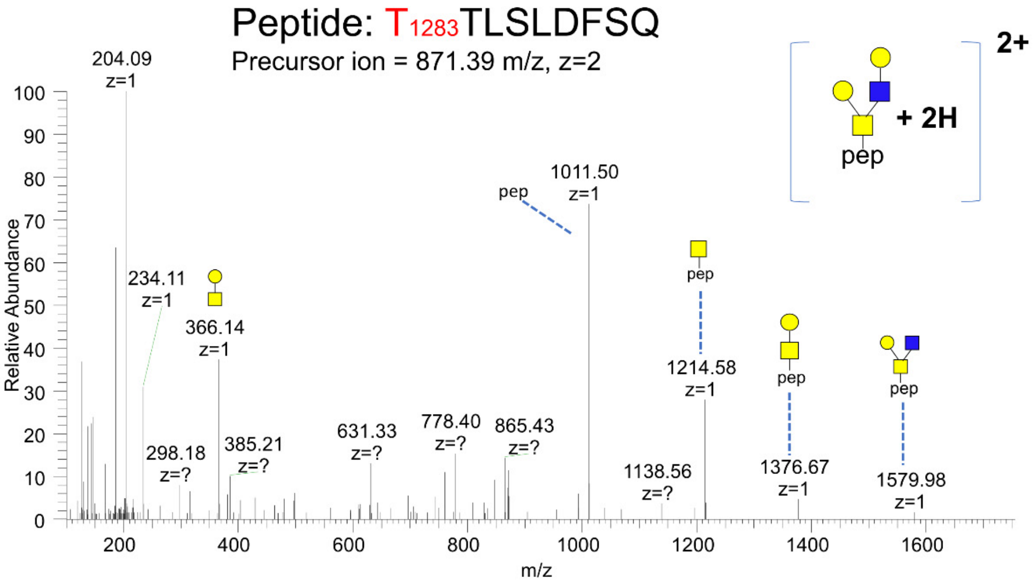
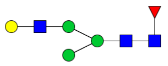
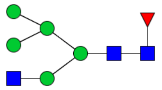
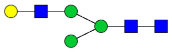
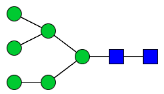
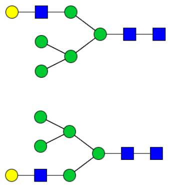
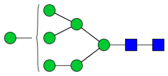
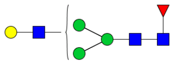
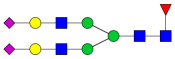
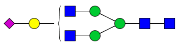
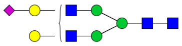


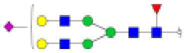
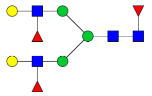
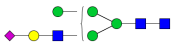
Figure S11. Tandem MS annotation of O-glycopeptide T_{1247}TLSLDFSQ.

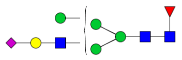
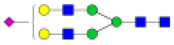
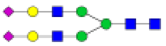
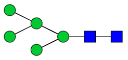
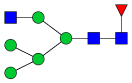
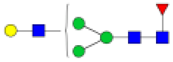
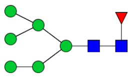
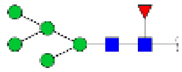
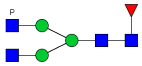
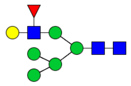
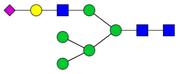
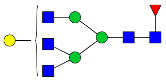
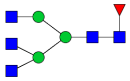
IV. Identified N-glycopeptides in human FV

1. N554: N$_{554}$KSWYLE and N$_{554}$KSWYLED

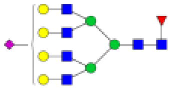
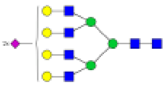
Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass	Glycopeptide mass	CFG structure
1403.507	8.067	(Hex)1(HexNAc)1(Deoxyhexose)1+ (Man)3(GlcNAc)2	hybrid/complex	1053.477	2457.991	
1565.56	3.714	(Hex)2(HexNAc)1(Deoxyhexose)1+ (Man)3(GlcNAc)2	hybrid/complex	1053.477	2620.044	
1257.449	7.366	(Hex)1(HexNAc)1+ (Man)3(GlcNAc)2	hybrid/complex	1053.477	2311.933	
1378.476	7.575	(Hex)3+(Man)3(GlcNAc)2	high_man	1053.477	2432.960	
1581.555	6.878	(Hex)3(HexNAc)1+ (Man)3(GlcNAc)2	hybrid/complex	1053.477	2636.039	
1540.528	7.95	(Hex)4+(Man)3(GlcNAc)2	high_man	1053.477	2595.012	
1403.507	8.067	(Hex)1(HexNAc)1(Deoxyhexose)1+ (Man)3(GlcNAc)2	hybrid/complex	1053.477	2457.991	
2432.884	-1.829	(HexNAc)4(Deoxyhexose)1(NeuAc)2+(Man)3(GlcNAc)2	hybrid/complex	938.45	3372.341	
1751.624	16.463	(Hex)1(HexNAc)2(NeuAc)1 + (Man)3(GlcNAc)2	hybrid/complex	2315.111	4067.742	
1954.704	16.061	(Hex)1(HexNAc)3(NeuAc)1 + (Man)3(GlcNAc)2	hybrid/complex	2331.106	4286.817	

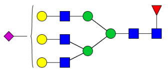
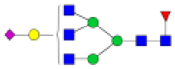
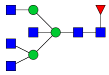
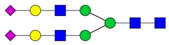
2. N382: SQHLDN$_{382}$FSNQIGK and SQHLDN$_{382}$FSNQIGKHYK

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass	Glycopeptide mass	CFG structure
2059.735	-6.032	(Hex)2(HexNAc)2(Deoxyhexose)1 (NeuAc)1+(Man)3(GlcNAc)2	hybrid/complex	1486.716	3547.459	
2060.755	-17.238	(Hex)2(HexNAc)2(Deoxyhexose)3+ (Man)3(GlcNAc)2	hybrid/complex	1486.716	3548.478	
1710.598	-8.994	(Hex)2(HexNAc)1(NeuAc)1+ (Man)3(GlcNAc)2	hybrid/complex	1486.716	3198.321	

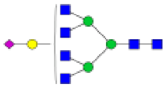
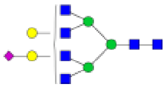
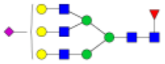
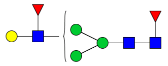
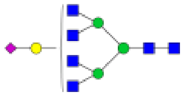
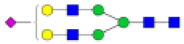
1856.656	-5.462	(Hex)2 (HexNAc)1(Deoxyhexose)1 (NeuAc)1+(Man)3(GlcNAc)2	hybrid/complex	1486.716	3344.379	
1913.677	-6.252	(Hex)2(HexNAc)2(NeuAc)1+(Man)3(GlcNAc)2	hybrid/complex	1486.716	3401.400	
2204.772	2.473	(Hex)2(HexNAc)2(NeuAc)2+ (Man)3(GlcNAc)2	hybrid/complex	1486.716	3692.495	
1216.423	-9.289	(Hex)2+(Man)3(GlcNAc)2	high_man	1914.934	3132.364	
1565.560	-3.963	(Hex)2(HexNAc)1(Deoxyhexose)1+ (Man)3(GlcNAc)2	hybrid/complex	1914.934	3481.501	
1403.507	-4.668	(Hex)1(HexNAc)1(Deoxyhexose)1+ (Man)3(GlcNAc)2	hybrid/complex	1914.934	3319.448	
1524.534	-5.027	(Hex)3(Deoxyhexose)1+ (Man)3(GlcNAc)2	high_man	1914.934	3440.475	
1362.481	-8.012	(Hex)2(Deoxyhexose)1+(Man)3(GlcNAc)2	high_man	1914.934	3278.422	
1524.500	4.398	(HexNAc)2(Deoxyhexose)1(Phos)1+(Man)3(GlcNAc)2	hybrid/complex	1914.934	3440.441	
1727.613	-4.251	(Hex)3 (HexNAc)1(Deoxyhexose)1+ (Man)3(GlcNAc)2	hybrid/complex	1486.716	3215.336	
2018.708	-8.746	(Hex)3 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	hybrid/complex	1486.716	3506.431	
1809.666	5.905	(Hex)1(HexNAc)3(Deoxyhexose)1 +(Man)3(GlcNAc)2	hybrid/complex	1914.934	3725.607	
1647.613	5.556	(HexNAc)3(Deoxyhexose)1+(Man)3(GlcNAc)2	hybrid/complex	1914.934	3563.554	

3. N938: QSN$_{938}$SSK, QSN$_{938}$SSKILVGRWHLASEK, and LLLLKQSN$_{938}$SSKILVGRWHLA SE

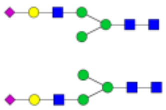
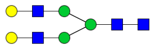
Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass	Glycopeptide mass	CFG structure
2789.999	26.023	(Hex)4(HexNAc)4(Deoxyhexose)1 (NeuAc)1+(Man)3(GlcNAc)2	hybrid/complex	649.303	3440.309	
2935.037	9.129	(Hex)4(HexNAc)4(NeuAc)2 +(Man)3(GlcNAc)2	hybrid/complex	649.303	3585.347	

2715.963	15.337	(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2+(Man)3(GlcNAc)2	hybrid/complex	649.303	3366.273	
1938.709	11.046	(Hex)1(HexNAc)3(Deoxyhexose)1(NeuAc)1+(Man)3(GlcNAc)2	hybrid/complex	2039.091	3978.807	
1850.693	10.006	(HexNAc)4(Deoxyhexose)1 +(Man)3(GlcNAc)2	hybrid/complex	2039.091	3890.791	
2204.772	3.178	(Hex)2(HexNAc)2(NeuAc)2 + (Man)3(GlcNAc)2	hybrid/complex	2491.427 (Glu-C)	4697.206	

4. N2209: TWN$_{2209}$QSIALR or FIRVIPKTWN$_{2209}$QSIALR

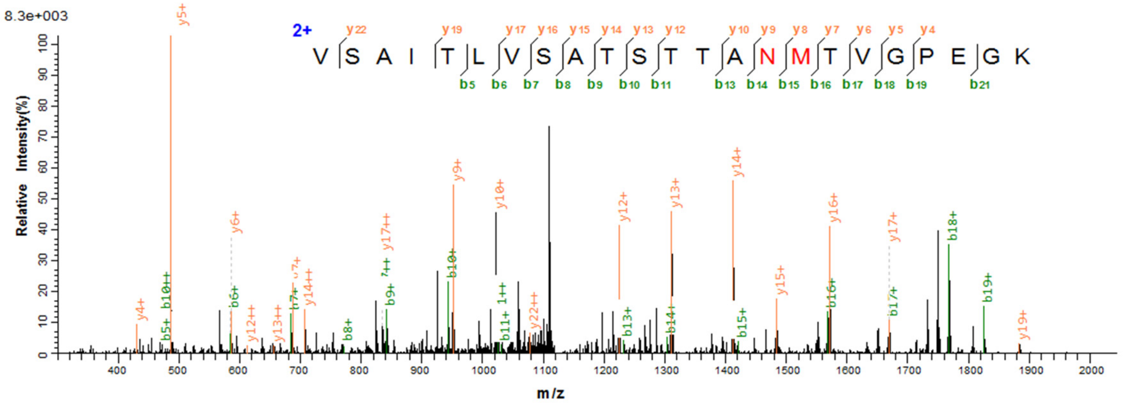
Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass	Glycopeptide mass	CFG structure
2157.783	14.764	(Hex)1(HexNAc)4(NeuAc)1+ (Man)3(GlcNAc)2	hybrid/complex	1087.577	3246.367	
2319.836	15.852	(Hex)2(HexNAc)4(NeuAc)1+ (Man)3(GlcNAc)2	hybrid/complex	1087.577	3408.42	
2715.963	26.642	(Hex)3(HexNAc)3(Deoxyhexose)1 (NeuAc)2+(Man)3(GlcNAc)2	hybrid/complex	1957.126	4674.096	
1590.592	-40.97	(HexNAc)2(Deoxyhexose)2+(M an)3(GlcNAc)2	hybrid/complex	1524.878	3116.477	
2157.783	-27.589	(Hex)1(HexNAc)4(NeuAc)1+(Man)3(GlcNAc)2	hybrid/complex	1540.873	3699.663	
1913.677	1.852	(Hex)2(HexNAc)2(NeuAc)1+ (Man)3(GlcNAc)2	hybrid/complex	1524.878	3439.562	

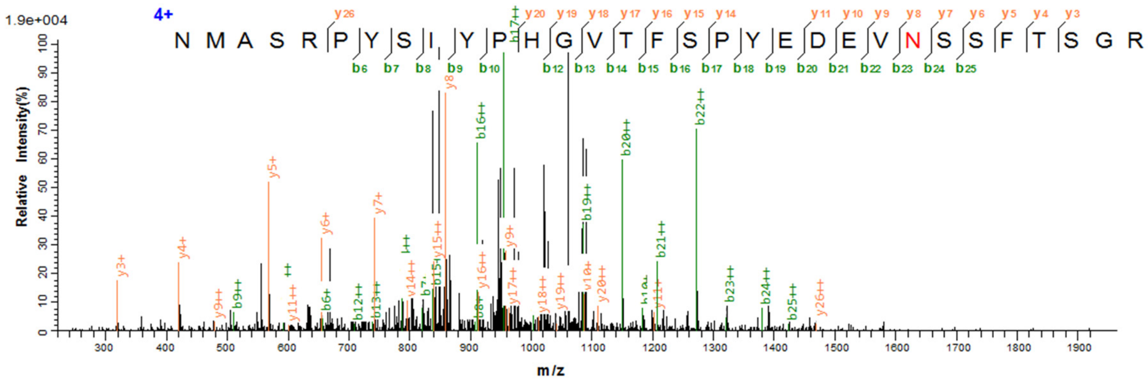
5. N1074: RRLKHSLVLHKSN$_{1074}$E

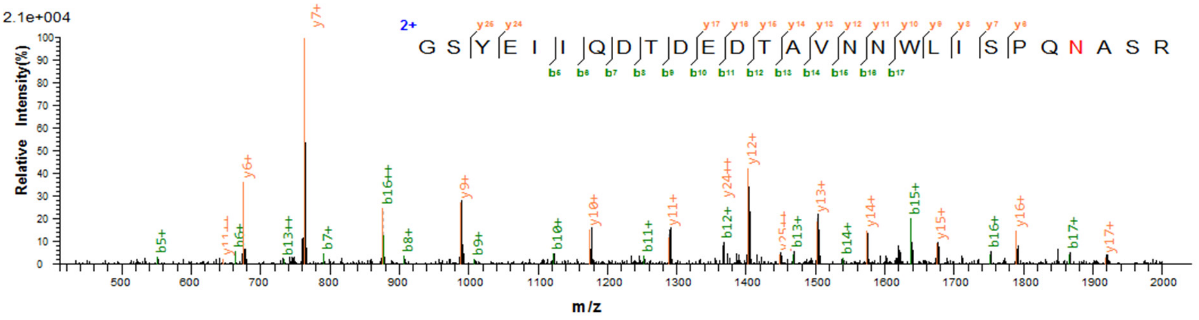
Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass	Glycopeptide mass	CFG structure
1548.545	-3.083	(Hex)1(HexNAc)1(NeuAc)1+ (Hex)3(HexNAc)2	hybrid/complex	1715.991	3265.543	
1622.582	0.210	(Hex)2(HexNAc)2+ (Hex)3(HexNAc)2	hybrid/complex	1715.991	3339.58	

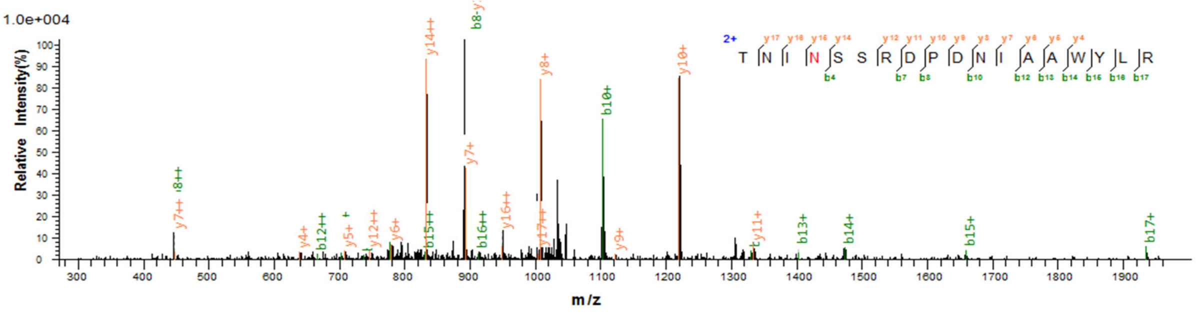
V. N-glycosylation sites of human FV (Trypsin and Glu-C)

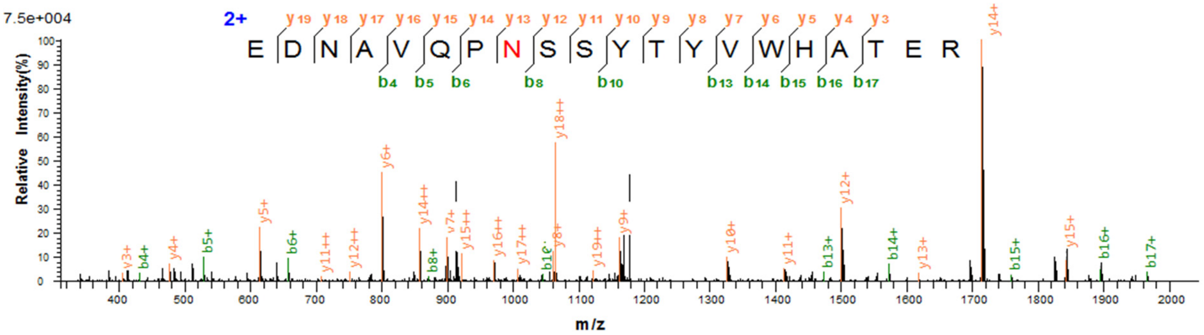
Peptides	Modification	Score	MW	Sites
VSAITLVSATSTTAnMTVGPEGK	15,N(18O_deamidated); 16,M(Oxidation[M]);	2.18E-26	2253.12955	297
NMASRPYSIYPHGVTFSPYEDEVnSSFTSGR	24,N(18O_deamidated);	3.20E-11	3497.57192	468
PYSIYPHGVTFSPYEDEVnSSFTSGR	19,N(18O_deamidated);	7.74E-27	2938.31826	468
GSYEIIQDTDEDTAVNNWLISPQnASR	24,N(18O_deamidated);	7.23E-26	3038.39905	977
TNInSSRDPDNIAAWYLR	4,N(18O_deamidated);	1.63E-11	2108.01724	1559
TYEDDSPEWFKEDNAVQPnSSYTYVWHATER	14,N(18O_deamidated); 19,N(18O_deamidated);	2.47E-15	3769.61036	1703
EDNAVQPnSSYTYVWHATER	8,N(18O_deamidated);	1.99E-23	2369.04457	1703
TWnQSIALRLELFGCDIY	15,C(Carbamidomethyl[C]);	4.99E-19	2198.08299	2209
TWnQSIALR	3,N(18O_deamidated);	2.49E-15	1090.56569	2209






2.1e+004
2+
GSYEIIQDTDEDTAVNNWLISPQNASR
Relative Intensity(%)
m/z
y7+
y6+
y9+
y10+
y11+
y12+
y24++
y13+
y14+
y15+
y16+
y17+
b5+
b6+
b7+
b8+
b9+
b10+
b11+
b12+
b13+
b13++
b14+
b15+
b16+
b16++
b17+


1.0e+004
2+
TNINSSRDPDNIAAWYLR
Relative Intensity(%)
m/z
y14++
y8+
y7+
y10+
b10+
b8+
y7++
y4+
y5+
y6+
y9+
y11+
y12++
y16++
y17++
b12++
b13+
b14+
b15+
b15++
b16++
b17+


7.5e+004
2+
EDNAVQPNSSYTYVWHATER
Relative Intensity(%)
m/z
y14+
y18++
y6+
y12+
y3+
y4+
y5+
y7+
y8+
y9+
y10+
y11+
y11++
y12++
y13+
y14++
y15+
y15++
y16++
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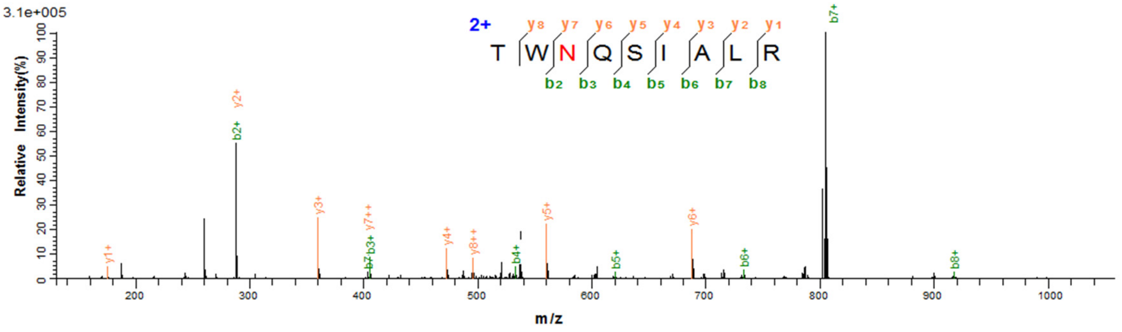
b4+
b5+
b6+
b8+
b13+
b14+
b15+
b16+
b17+

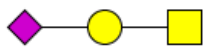
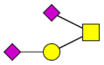

3.1e+005
2+
TWNQSIALR
Relative Intensity(%)
m/z
b7+
y2+
b2+
y1+
y3+
y4+
y5+
y6+
y7++
y8++
b3+
b4+
b5+
b6+
b8+

VI. Identified O-glycopeptides in human FV

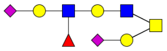
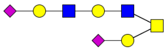
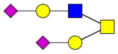
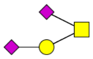
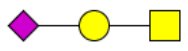
1. S1053: S$_{1053}$EAYNTFSER

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
656.228	2.672	(Hex)1(HexNAc)1(NeuAc)1	Trypsin	1202.52	1859.756	
365.132	2.531	(Hex)1(HexNAc)1	Trypsin	1202.52	1568.66	

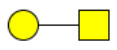
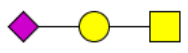
2. S1594: YS$_{1594}$EFVQRE

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
656.228	1.943	(Hex)1(HexNAc)1(NeuAc)1	Glu-C	1056.488	1713.723	
947.323	-2.029	(Hex)1(HexNAc)1(NeuAc)2	Glu-C	1056.488	2004.818	

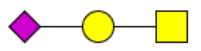
3. 985,986 AWGESTPLANKPGK

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
1823.645	-11.764	(Hex)3(HexNAc)3(Deoxyhexose)1 (NeuAc)2	Trypsin	1470.747	3295.399	
1677.587	-12.691	(Hex)3(HexNAc)3(NeuAc)2	Trypsin	1470.747	3149.341	
1312.455	-17.947	(Hex)2(HexNAc)2(NeuAc)2	Trypsin	1470.747	2784.209	
947.323	-3.286	(Hex)1(HexNAc)1(NeuAc)2	Trypsin	1454.752	2403.082	
656.228	-3.929	(Hex)1(HexNAc)1(NeuAc)1	Trypsin	1454.752	2111.987	
365.132	-1.592	(Hex)1(HexNAc)1	Trypsin	1454.752	1820.891	

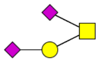
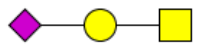
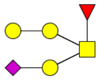
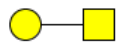
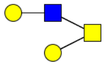
4. T1047: T$_{1047}$FHPLR

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
365.132	0.396	(Hex)1(HexNAc)1	Trypsin	769.423	1135.563	
656.228	3.035	(Hex)1(HexNAc)1(NeuAc)1	Trypsin	769.423	1426.658	

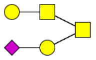
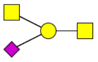
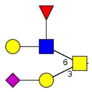
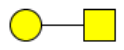
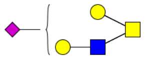
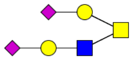
5. S1926: AS$_{1926}$EFLGYWEPR

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
656.228	-0.951	(Hex)1 (HexNAc)1 (NeuAc)1	Trypsin	1353.635	2010.871	
365.132	-0.951	(Hex)1(HexNAc)1	Trypsin	1353.635	1719.775	

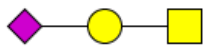
6. S1150 SSS$_{1150}$PELSEMLEYDR

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
947.323	0.131	(Hex)1(HexNAc)1(NeuAc)2	Trypsin	1641.719	2590.049	
656.228	2.497	(Hex)1(HexNAc)1(NeuAc)1	Trypsin	1641.719	2298.954	
203.079	1.484	HexNAc	Trypsin	1641.719	1845.805	
1126.391	15.326	(Hex)3(HexNAc)1(Deoxyhexose)1 (NeuAc)1	Trypsin	1641.719	2769.117	
365.132	1.663	(Hex)1(HexNAc)1	Trypsin	1641.719	2007.858	
730.264	3.692	(Hex)2(HexNAc)2	Trypsin	1657.714	2388.985	

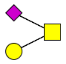
7. T805 APSHQQATT$_{805}$AGSPLR

Glycan mass	Δmass (ppm)	Structure	Type	Peptide mass [M]	Glycopeptide mass	CFG structure
1021.36	-6.011	(Hex)2(HexNAc)2(NeuAc)1	Trypsin	1520.77	2543.137	
859.307	-3.145	(Hex)1(HexNAc)2(NeuAc)1	Trypsin	1520.77	2381.084	
1167.418	-0.248	(Hex)2(HexNAc)2 (Deoxyhexose)1(NeuAc)1	Trypsin	1520.77	2689.195	
203.079	-2.196	(HexNAc)1	Trypsin	1520.77	1724.856	
365.132	1.436	(Hex)1(HexNAc)1	Trypsin	1520.77	1886.909	
656.228	-3.300	(Hex)1(HexNAc)1(NeuAc)1	Trypsin	1520.77	2178.005	
1021.36	-1.017	(Hex)2(HexNAc)2(NeuAc)1	Trypsin	1520.77	2543.137	
1312.455	2.657	(Hex)2(HexNAc)2(NeuAc)2	Trypsin	1520.77	2834.232	

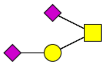
8. S1044 HTHHAPLS$_{1044}$PR

Glycan mass	**Δmass (ppm)**	**Structure**	**Type**	**Peptide mass [M]**	**Glycopeptide mass**	**CFG structure**
656.228	-0.751	(Hex)1(HexNAc)1(NeuAc)1	Trypsin	1151.595	1808.83	
365.132	-1.686	(Hex)1(HexNAc)1	Trypsin	1151.595	1517.727	
203.079	1.088	(HexNAc)1	Trypsin	1151.595	1354.674	

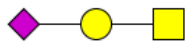
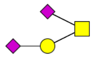
9. S1053 KHTHHAPLSPRTFHPLRS$_{1053}$E

Glycan mass	**Δmass (ppm)**	**Structure**	**Type**	**Peptide mass [M]**	**Glycopeptide mass**	**CFG structure**
656.228	-0.712	(Hex)1(HexNAc)1(NeuAc)1	Glu-C	2247.177	2904.413	

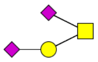
10. T1211 LSHT$_{1211}$TLSPE

Glycan mass	**Δmass (ppm)**	**Structure**	**Type**	**Peptide mass [M]**	**Glycopeptide mass**	**CFG structure**
656.228	0.004	(Hex)1(HexNAc)1(NeuAc)1	Glu-C	983.492	1640.727	
947.323	2.707	(Hex)1(HexNAc)1(NeuAc)2	Glu-C	983.492	1931.822	

11. 1298-1306 LSHMTLSPE

Glycan mass	**Δmass (ppm)**	**Structure**	**Type**	**Peptide mass [M]**	**Glycopeptide mass**	**CFG structure**
656.228	1.897	(Hex)1(HexNAc)1(NeuAc)1	Glu-C	1013.485	1670.72	
947.323	2.737	(Hex)1(HexNAc)1(NeuAc)2	Glu-C	1013.485	1961.815	

12. T1238 LSHT$_{1238}$TLSPD

Glycan mass	**Δmass (ppm)**	**Structure**	**Type**	**Peptide mass [M]**	**Glycopeptide mass**	**CFG structure**
947.323	1.788	(Hex)1(HexNAc)1(NeuAc)2	Glu-C	969.477	1917.807	

13. T1283 LSHT$_{1283}$TLSLD

Glycan mass	**Δmass (ppm)**	**Structure**	**Type**	**Peptide mass [M]**	**Glycopeptide mass**	**CFG structure**
947.323	1.619	(Hex)1(HexNAc)1(NeuAc)2	Glu-C	985.508	1933.838