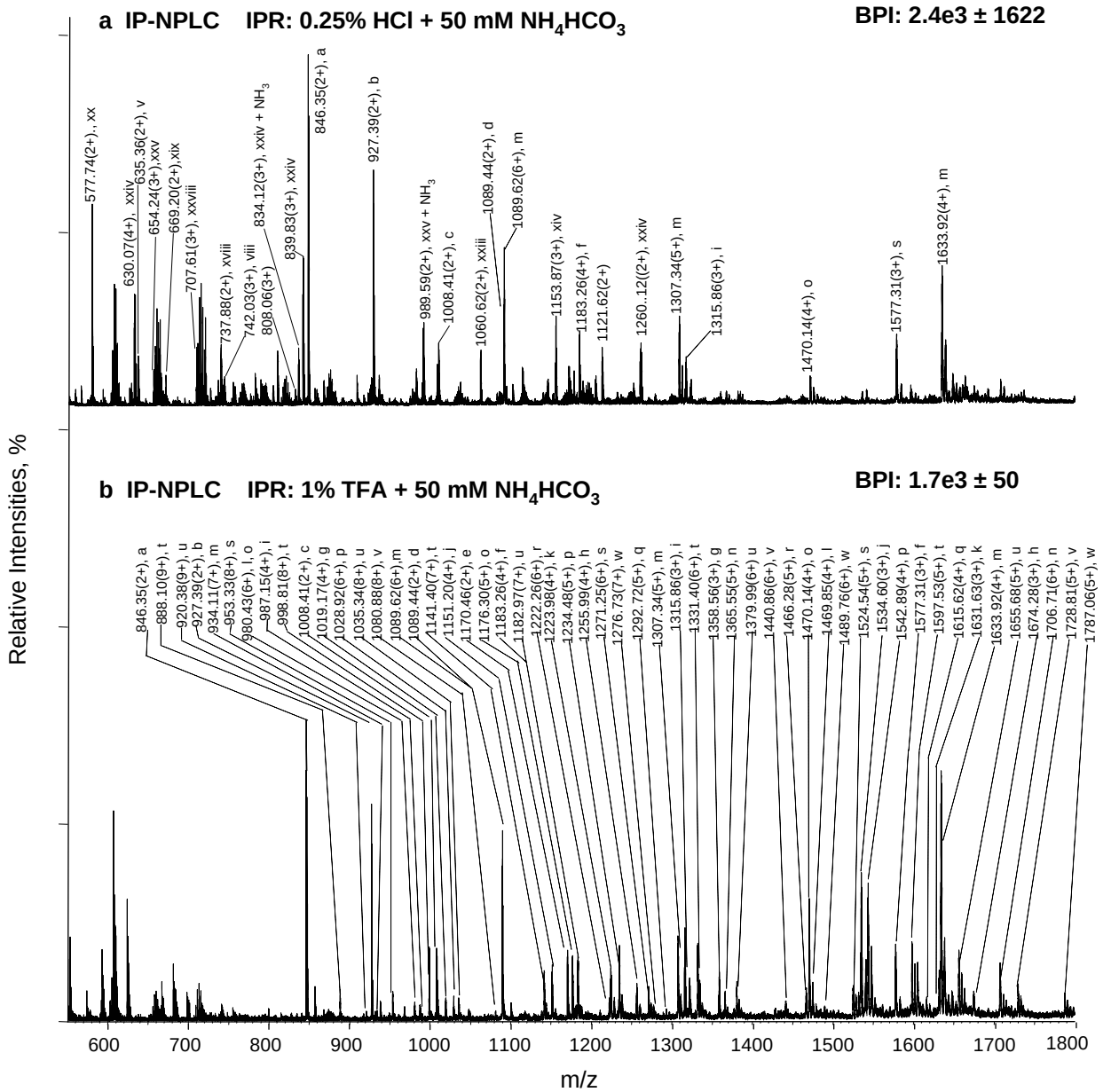
Supplemental Data

Supplemental Figure S1

Supplemental Figure S1



Supplemental Fig. S1: Total ion mass spectrum from IP-NPLC-ESI-MS of peptides in the glycopeptide fraction from a RNase B and fetuin tryptic digest injected with the following ion-pairing reagents (IPR): (a) 0.25% HCl + 50 mM NH_4HCO_3 in 80% ACN/20% H_2O and (b) 1% TFA + 50 mM NH_4HCO_3 in 85% ACN/20% H_2O. The tryptic digest contains: 14.2 pmol RNase B tryptic digest + 13.8 pmol fetuin tryptic digest; BPI: Base peak intensity; All other abundant ions not labeled are singly charged contaminants. Ammonium adducts and minor peptide peaks are not labeled.

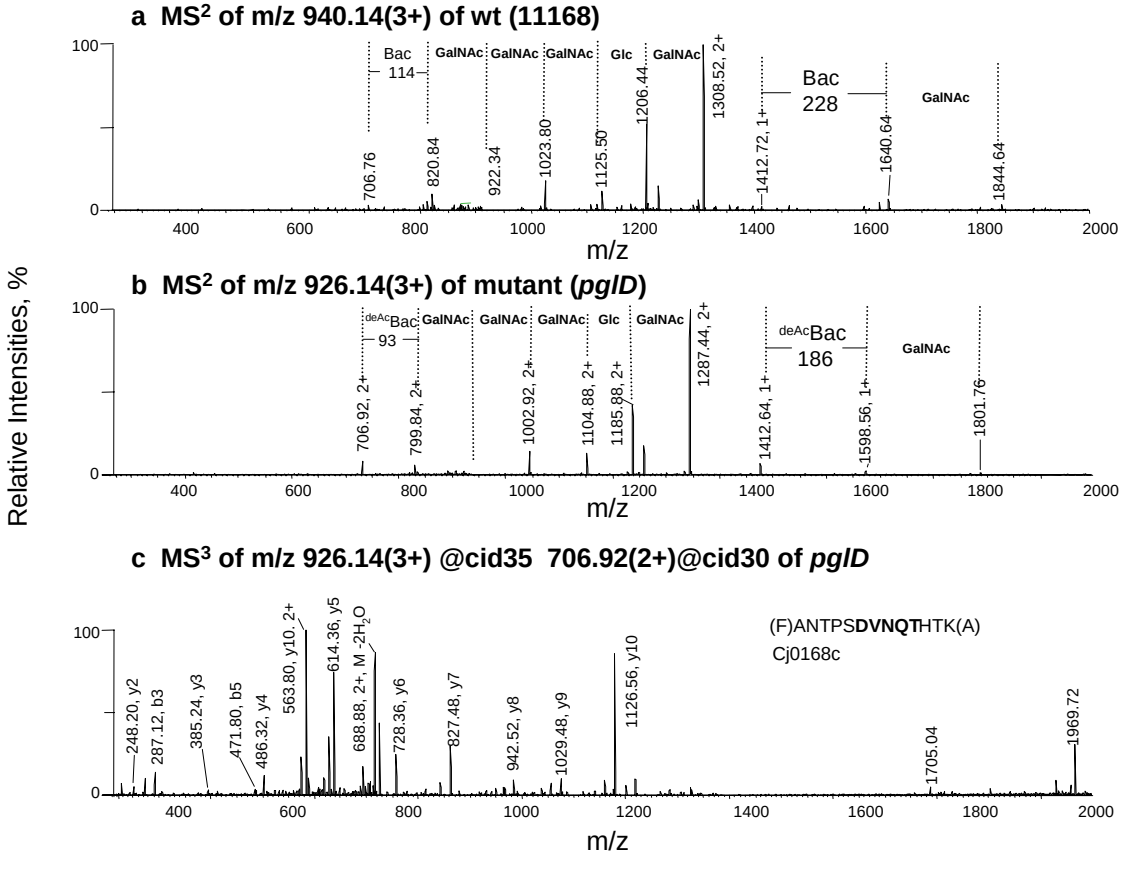
Table S1. Effect of acids as ion-pairing reagents on peptide retention and ion intensities in IP-NPLC-MS of a tryptic digest of RNase B and fetuin mixture.

				Ion-pairing reagents																	
				None				20% acetic acid				0.25% HCl				1% HCl + 50 mM NH_4HCO_3				0.25% TFA			
Peptide no.	peptide m/z measured	z	Calculated hydrophobicity values, (ΔG_{octw}, kcal/mol)*	RT	% error	Intensities	% error	RT	% error	Intensities	% error	RT	% error	Intensities	% error	RT	% error	Intensities	% error	RT	% error	Intensities	% error
i	557.25	1	-8.0	5.1	10.9	1388	102.6	—	—	—	—	3.8	42.7	3290	45.3	2.1	0.9	216	10.1	3.5	0.8	702	1.5
ii	1260.16	2	-8.5	5.4	2.0	5587	58.6	—	—	—	—	4.2	36.4	4150	13.5	1.6	2.4	1360	13.1	1.7	0.0	1170	3.4
iii	451.20	1	-8.8	2.0	2.7	4420	20.9	—	—	—	—	5.0	0.6	2010	42.7	—	—	—	—	1.7	0.0	808	34.9
iv	457.21	1	-8.9	—	—	—	—	—	—	—	—	4.3	3.5	1215	45.3	—	—	—	—	3.5	0.7	395	4.3
v	635.30	2	-10.1	9.0	30.4	5713	81.7	—	—	—	—	5.1	5.8	4840	11.7	2.1	0.5	98	5.4	3.5	0.2	1083	6.5
vi	847.38	1	-10.3	—	—	—	—	8.2	2.2	1480	29.0	5.2	8.1	1214	38.3	—	—	—	—	—	—	—	—
vii	602.30	1	-10.4	—	—	—	—	—	—	—	—	5.5	5.2	1920	39.4	—	—	—	—	3.4	0.9	715	4.1
viii	742.03	3	-10.7	6.3	15.0	1715	57.3	9.2	3.7	470	21.7	5.8	2.9	3660	10.0	5.8	0.5	148	12.4	3.4	0.7	612	19.0
viv	1006.49	3	-10.7	—	—	—	—	—	—	—	—	5.4	6.9	1228	39.6	2.1	1.4	49	11.8	3.5	0.2	527	6.1
x	802.60	1	-10.9	—	—	—	—	—	—	—	—	5.5	1.7	1263	47.1	—	—	—	—	2.5	2.3	112	6.1
xi	915.41	1	-11.4	6.9	12.4	509	80.5	11.8	0.5	149	11.9	5.7	4.6	955	42.2	—	—	—	—	3.4	1.4	413	3.0
xii	522.22	1	-11.6	—	—	—	—	—	—	—	—	6.2	11.5	591	52.7	6.5	1.5	143	2.1	3.5	1.0	558	5.2
xiii	774.38	1	-11.7	—	—	—	—	—	—	—	—	6.2	11.5	854	21.7	6.6	1.2	149	19.4	3.5	0.7	358	3.5
xiv	1153.90.5	3	-11.9	9.8	4.5	3033	33.8	7.7	15.3	2273	6.3	6.0	0.6	8043	10.6	6.2	0.5	831	5.0	3.5	0.2	1550	3.0
xv	816.42	1	-12.0	10.4	3.4	825	60.7	—	—	—	—	5.3	1.7	2837	31.2	5.4	1.2	118	12.6	3.5	0.4	692	2.2
xvi	631.35	1	-12.2	—	—	—	—	—	—	—	—	5.9	3.5	2393	35.1	6.1	1.9	276	13.7	3.5	0.8	865	0.5
xvii	591.24	1	-12.2	—	—	—	—	—	—	—	—	6.2	0.6	1109	18.2	6.5	1.3	180	7.5	3.5	0.3	673	2.4
xviii	737.88	2	-12.7	6.2	14.0	4550	88.4	11.7	1.3	8637	3.3	5.3	1.7	11200	12.0	5.4	2.4	836	3.8	3.4	0.6	2137	4.0
xix	669.20	2	-13.3	9.7	3.2	403	31.5	—	—	—	—	6.7	3.5	1383	18.1	6.9	0.6	181	1.3	3.6	0.3	430	1.4
xx	577.74	2	-13.6	11.8	12.5	782	92.5	11.9	1.0	3863	26.6	6.6	0.6	11767	7.9	6.9	0.3	1643	2.4	3.5	0.3	2520	0.8
xxi	608.22	1	-13.8	13.2	5.9	565	94.6	11.0	12.7	1223	15.3	6.9	0.6	695	8.0	—	—	—	—	3.6	1.1	300	3.7
xxii	662.32	1	-14.0	—	—	—	—	—	—	—	—	7.0	1.7	576	5.0	7.2	1.1	78	6.7	3.6	0.3	402	0.4
xxiii	1143.44	2	-14.1	8.5	11.4	293	78.3	—	—	—	—	7.0	0.0	2440	4.6	7.2	0.3	283	15.6	3.6	0.4	635	2.6
xxiv	834.12	3	-14.9	12.7	2.5	385	136.1	11.7	1.6	3486	22.0	6.5	0.6	7803	7.4	6.8	0.5	790	3.1	3.5	2.4	2577	2.1
xxv	654.24	3	-15.9	12.4	11.7	528	107.7	13.3	0.4	1560	55.6	6.9	2.9	2710	5.9	7.2	0.2	289	0.4	3.5	0.3	833	2.6
xxvi	733.94	2	-16.1	—	—	—	—	—	—	—	—	7.4	0.6	1617	8.2	7.4	0.1	191	8.2	3.6	0.6	451	4.6
xxvii	659.99	3	-17.9	—	—	—	—	—	—	—	—	7.5	0.6	5203	6.4	7.8	0.2	544	9.4	3.5	0.7	1093	1.4
xxviii	707.32	3	-18.4	—	—	—	—	11.9	0.7	3530	118.7	7.1	0.6	5357	7.0	7.4	0.4	651	3.2	3.5	0.3	1177	2.1
xxix	789.31	3	-18.6	—	—	—	—	11.9	0.4	335	4.5	7.7	0.6	1963	11.8	7.9	1.4	137	21.5	4.7	0.6	204	4.4
a	846.35	2	*-20.0*	—	—	—	—	11.9	0.6	721	27.8	8.9	0.1	3390	3.7	9.0	0.2	392	3.2	8.7	0.1	3476	1.0
b	927.39	2	*-20.6*	—	—	—	—	11.8	0.5	446	21.3	9.1	0.2	2243	2.5	9.2	0.4	244	4.1	8.9	0.3	2593	4.7
c	1008.41	2	*-21.1*	—	—	—	—	—	—	—	—	9.3	0.1	699	6.1	9.6	0.2	149	5.4	9.1	0.8	759	9.4
d	1089.44	2	*-21.6*	—	—	—	—	11.8	0.5	234	12.0	9.5	0.2	1183	12.1	9.4	0.2	73	2.1	9.2	0.3	826	21.9
e	1170.46	2	*-21.9*	—	—	—	—	—	—	—	—	9.6	0.1	503	4.4	9.7	0.3	69	2.6	9.3	0.1	301	16.7
f	1183.26	4	*-26.2*	9.4	3.3	139	61.6	—	—	—	—	11.3	0.6	2037	20.7	10.9	2.2	116	2.3	11.4	0.4	549	3.3
g	1256.01	4	*-28.8*	—	—	—	—	—	—	—	—	12.3	1.3	84	8.3	11.4	0.1	56	7.5	10.5	0.1	178	5.0
h	1358.56	3	*-21.8*	—	—	—	—	—	—	—	—	9.6	0.2	571	1.1	9.6	0.5	89	9.4	10.4	0.6	194	20.4
i	1315.86	3	*-22.5*	6.0	5.8	79	94.1	—	—	—	—	9.8	0.3	1217	10.7	9.6	0	167	5.7	10.4	0.4	572	8.2
j	1534.60	3	*-25.2*	8.8	5.2	102	83.1	—	—	—	—	10.9	6.7	2187	23.8	12.4	4.6	382	7.0	12.0	0.1	1047	4.0
k	1631.64	3	—	—	—	—	—	—	—	—	—	—	—	—	—	10.7	0.1	61	16.5	10.6	0.2	508	6.5
l	1469.85	4	*-18.4*	—	—	—	—	—	—	—	—	8.2	0.3	183	11.0	8.3	0.1	45	12.5	8.5	0.4	74	13.1
m	1633.92	4	*-22.9*	9.8	2.0	280	60.5	—	—	—	—	10.0	0.1	5047	9.5	9.9	0.1	587	5.3	11.1	0.1	2036	0.3
n	1706.71	6	*-27.3*	—	—	—	—	—	—	—	—	11.7	0.2	712	14.7	11.1	1.07	157	5.7	11.8	0.4	543	1.9
o	1470.14	4	*-20.7*	9.3	2.9	105	107.6	11.8	2.1	264	45.7	9.1	0.1	605	6.4	9.2	0.1	92	3.2	11.4	1.4	796	2.0
p	1542.89	4	*-22.2*	9.3	3.5	202	119.5	12.0	0.4	271	42.0	9.7	0.5	524	35.7	10.5	10.7	85	10.4	12.2	1.1	833	5.9
q	1292.72	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10.5	0.3	67	41.2
r	1466.28	5	*-18.3*	—	—	—	—	—	—	—	—	8.2	0.2	834	8.8	8.3	0.4	82	14.2	8.5	0.1	143	9.5
s	1271.25	6	*-19.2*	—	—	—	—	—	—	—	—	8.6	0.2	646	17.4	8.6	0.1	57	16.4	9.6	0.1	172	12.9
t	1597.53	5	*-20.4*	—	—	—	—	—	—	—	—	9.0	0.2	1467	13.3	9.0	0.1	125	7.0	9.8	0.1	341	6.8
u	1655.68	5	*-22.6*	—	—	—	—	—	—	—	—	9.9	9.4	1377	17.0	9.6	1.6	124	16.1	10.6	0.3	600	3.9
v	1728.81	5	*-24.4*	—	—	—	—	—	—	—	—	10.5	10.8	609	5.0	10.4	2.2	79	17.0	10.9	0.1	228	4.6
w	1787.06	5	*-25.6*	—	—	—	—	—	—	—	—	11.0	4.4	44	12.0	10.6	4.8	44	3.2	12.5	0.4	201	6.5

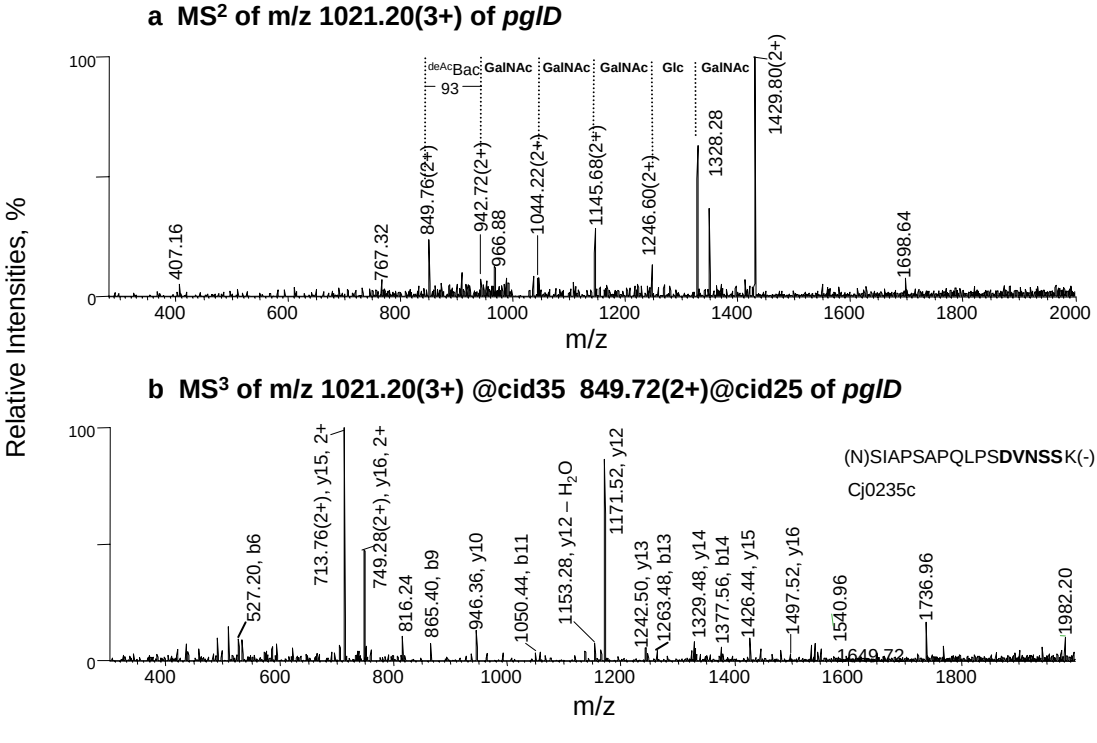
*: The hydrophobicity values of glycopeptides (a-w) (ΔG_{octw}) which can not be calculated using MPExTotalizer due to the presence of glycans, were projected based on their IP-NPLC retention times (RT) and the linear regression equation for non-glycopeptides (i-xxix) (ΔG_{octw}=2.6RT - 3.1) using 0.25% HCl as an ion-pairing reagent as described in Fig. 4. The IP-NPLC peak width at the base is < 2 min for > 90% of the glycopeptides detected.

—: Not detected; Intensities: Peak area; % error is based on triplicate IP-NPLC-MS analyses of the standard tryptic digest.

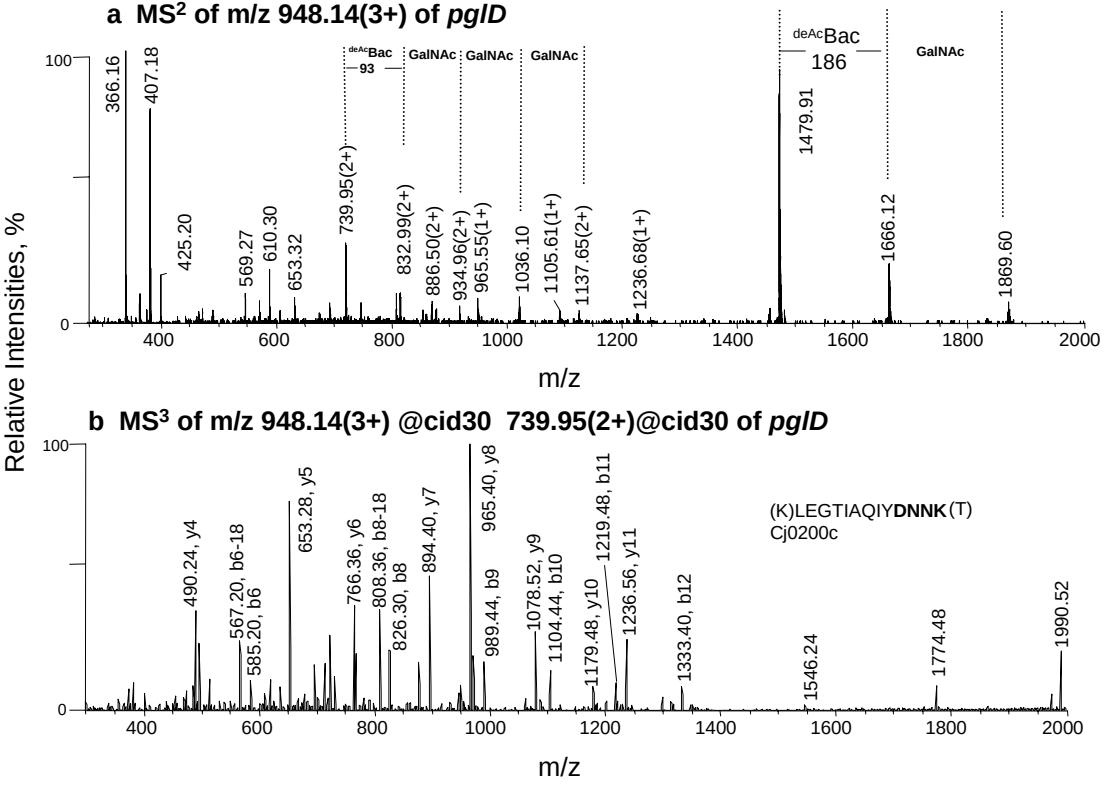
Supplemental Figure S2 Suggested location: Page 3 – 7



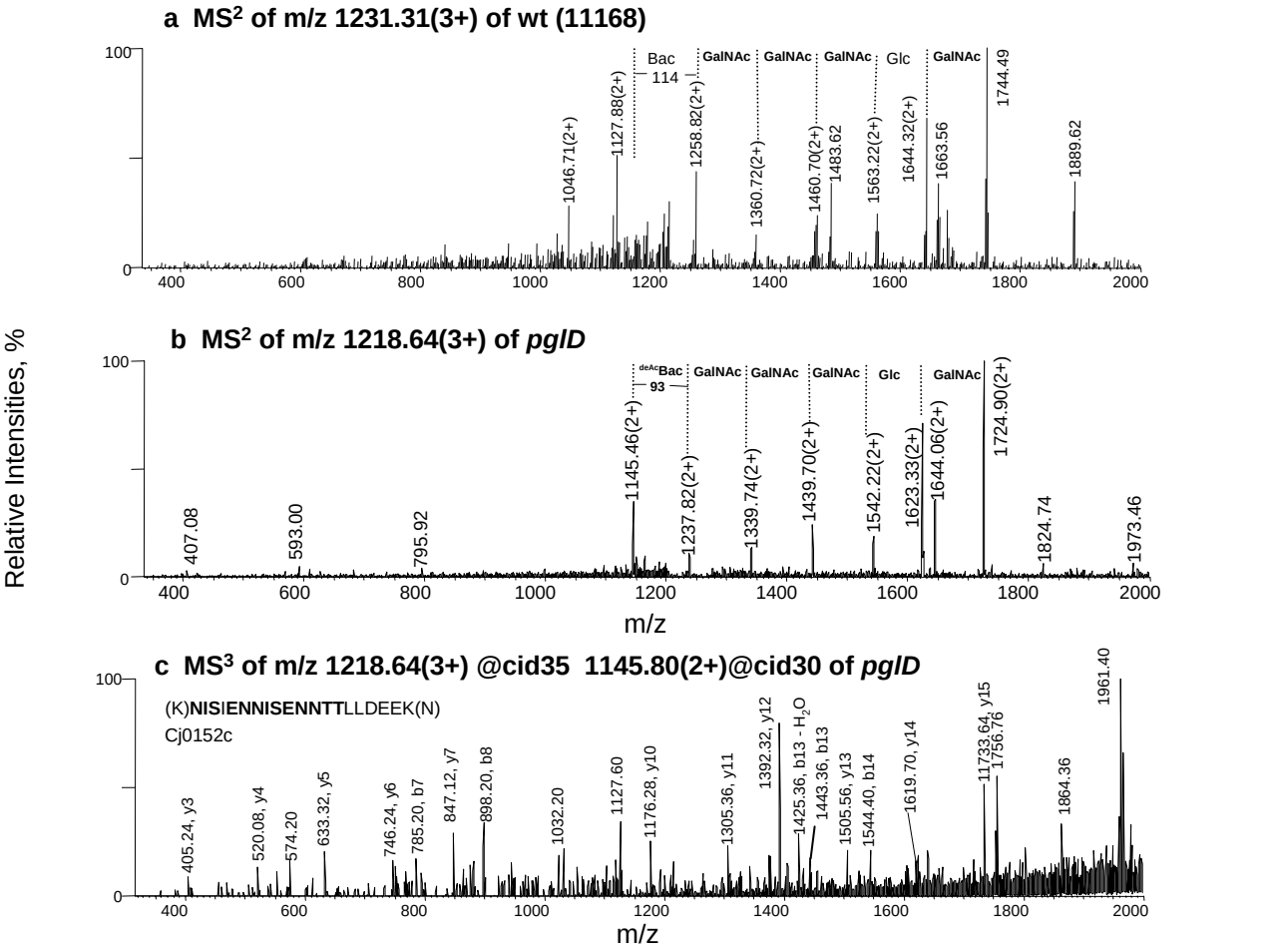
Supplemental Figure S2A



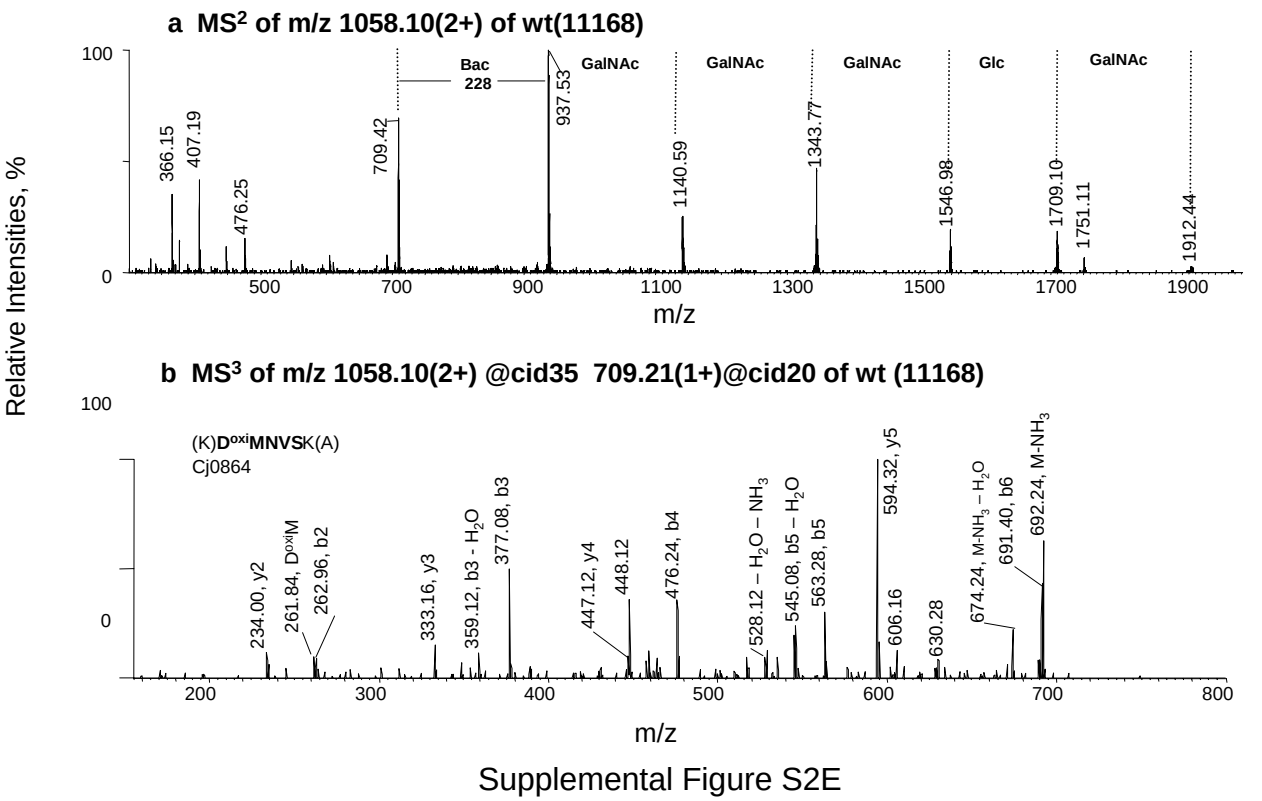
Supplemental Figure S2B



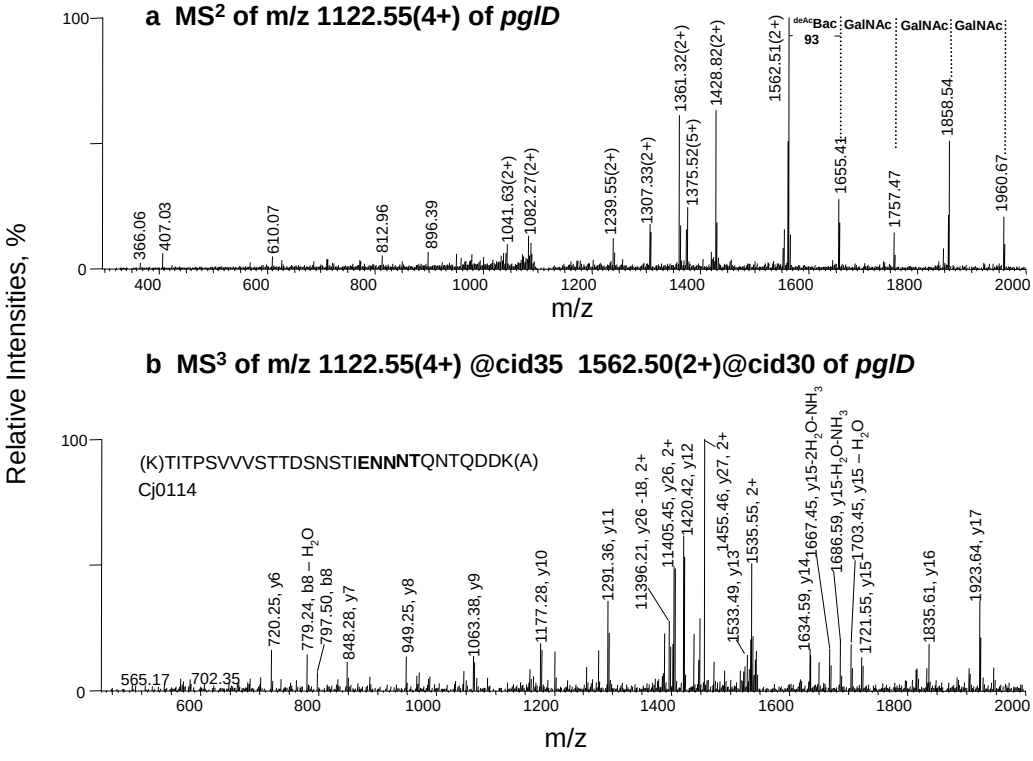
Supplemental Figure S2C



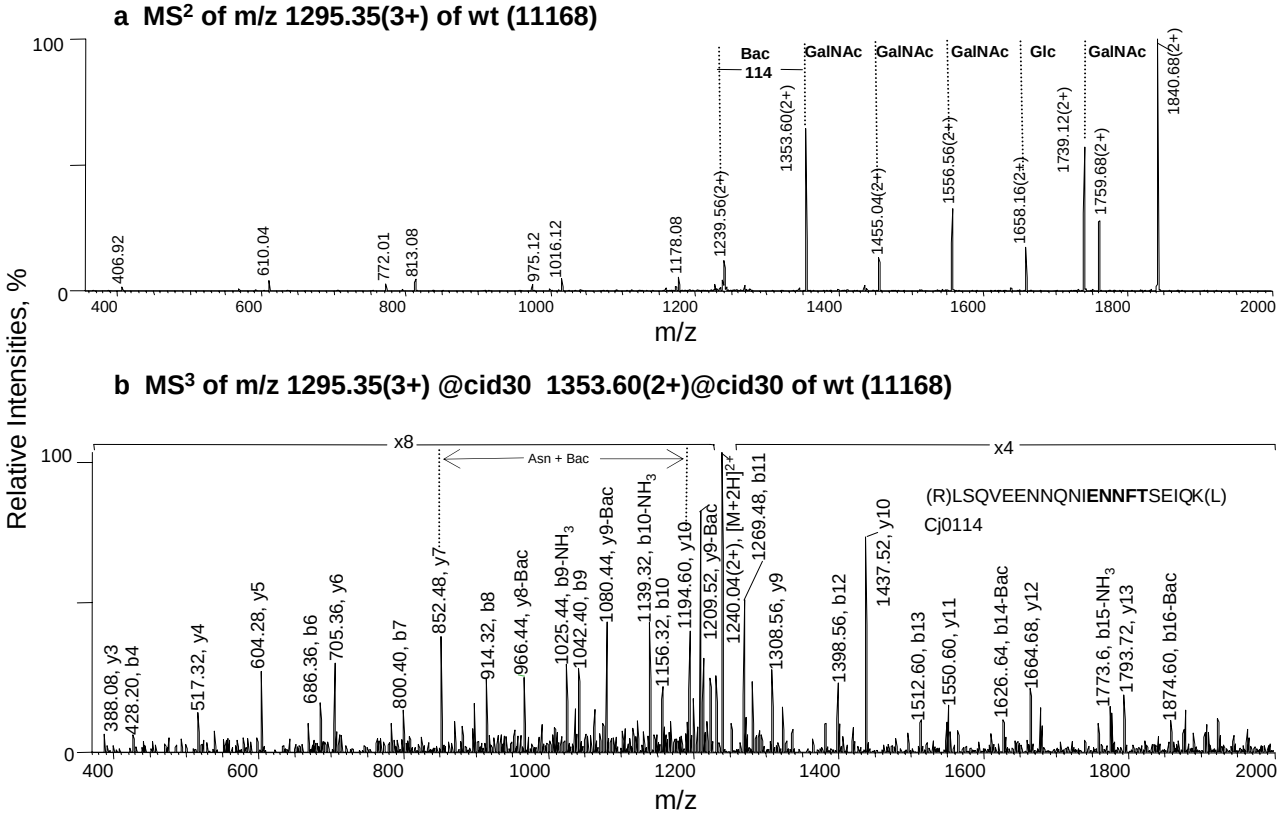
Supplemental Figure S2D



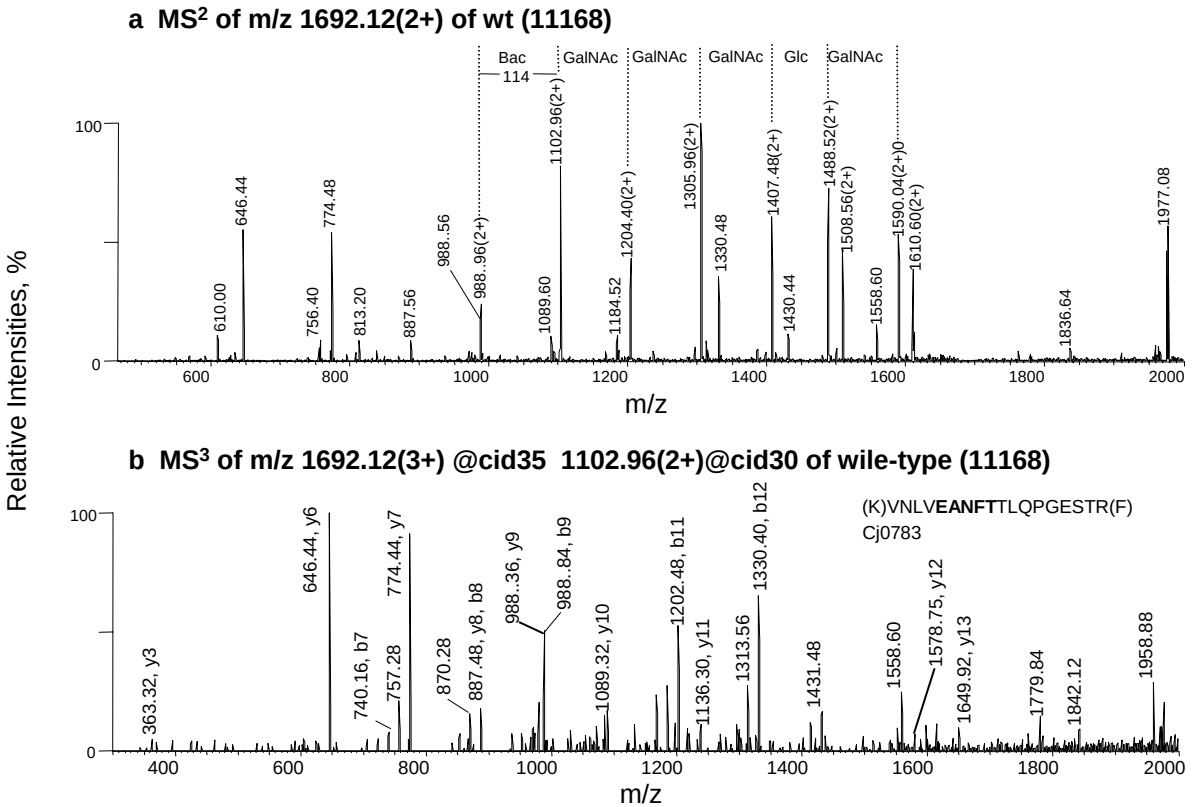
Supplemental Figure S2E



Supplemental Figure S2F



Supplemental Figure S2G



Supplemental Figure S2H

Supplemental Fig. S2: MS^2 and MS^3 spectra for the glycopeptides identified from the periplasmic protein extracts of *C. jejuni* 11168 wt and *pglD* mutant. The amino acid sequence and protein I.D. for each of the glycopeptides are provided in the insets in the MS^3 spectra. Further details about these glycopeptides can be found in Table 3. MS^3 analysis was performed on fragment ions composed of the peptide with or without the bacillosamine residue. Bac: bacillosamine (2, 4-diacetamido-2, 4, 6-trideoxyglucopyranose); ^{deAc}Bac: monoacetylated bacillosamine at the C2 position only (2-acetamido-4-amino-2, 4, 6-trideoxyglucopyranose). Wt: wild-type; ^{Oxi}M: oxidized methionine.

Table S2. List of identified peptides in the IP-NPLC non-glycopeptide fraction of the periplasmic protein extract of the isogenic pglD mutant of C. jejuni 11168 that generate a MS/MS fragment ion at m/z 204.09 *.

Accession number	Protein name	Precursor m/z	z	Sequences of identified non-glycopeptides†	Ion score	Sequence§ coverage %	R.T. (min)
NP_282485	Flagellin	949.49	2	(R)LMEELDNIANTTSFNGK(Q)	117	19	31.5
NP_281358	thioredoxin	816.97	2	(M)LAPVIDELSNDFDGK(A)	62	30	34.8
NP_281358	thioredoxin	890.46	2	(R)MLAPVIDELSNDFDGK(A)	85	30	35.4
NP_282368	chaperonin GroEL	882.49	2	(K)GEYVNMLESGIIDPVK(V)	75	22	37.0
NP_282264	membrane bound zinc metallopeptidase	851.51	2	(K)EAPAIVFIDEIDAIGK(S)	59	2	41.8
NP_282065	DNA-binding protein HU homolog	953.50	2	(K)DATTATDAVISTITDVLAK(G)	83	19	44.1

*: Variable modifications used for database searching: carbamidomethyl (C), deamidated (N), and oxidation (M) with no enzyme specified.
†: Only the peptide sequences whose ions yielded 204.09(1+) (HexNAc+) upon CID is provided.
§: The sequence coverage is based on all the peptides identified from a protein.

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