Supporting Information For:

"Analytical Performance of Immobilized Pronase for Glycopeptide Footprinting and Implications for Surpassing Reductionist Glycoproteomics"

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Supplementary Table 1. N-linked glycopeptides derived from immobilized pronase digestion of CEA. The root mean square (RMS) mass error of the assignments was 1.8 parts per million (ppm). *HexNAc: N-acetylhexosamine; Hex: hexose. [‡]All mass errors are absolute values (*i.e.*, represented as non-negative).

GP *m/z*	**Peptide**	**Site**	**Glycan***	**Ion Type**	**$\Delta m/z$[‡]**
1462.5683	^{293}NL^{294}	^{292}N	HexNAc$_2$ Hex$_5$	[M+H]$^+$	0.0009
1484.5484	^{293}NL^{294}	^{292}N	HexNAc$_2$ Hex$_5$	[M+Na]$^+$	0.0007
1503.5981	^{293}NL^{294}	^{292}N	HexNAc$_3$ Hex$_4$	[M+H]$^+$	0.0020
1563.6129	^{293}NLT^{295}	^{292}N	HexNAc$_2$ Hex$_5$	[M+H]$^+$	0.0010
1585.5947	^{293}NLT^{295}	^{292}N	HexNAc$_2$ Hex$_5$	[M+Na]$^+$	0.0013
1604.6395	^{293}NLT^{295}	^{292}N	HexNAc$_3$ Hex$_4$	[M+H]$^+$	0.0001
1624.6205	^{293}NL^{294}	^{292}N	HexNAc$_2$ Hex$_6$	[M+H]$^+$	0.0007
1626.6215	^{293}NLT^{295}	^{292}N	HexNAc$_3$ Hex$_4$	[M+Na]$^+$	0.0049
1646.6064	^{293}NL^{294}	^{292}N	HexNAc$_2$ Hex$_6$	[M+Na]$^+$	0.0026
1665.6471	^{293}NL^{294}	^{292}N	HexNAc$_3$ Hex$_5$	[M+H]$^+$	0.0015
1687.6284	^{293}NL^{294}	^{292}N	HexNAc$_3$ Hex$_5$	[M+Na]$^+$	0.0029
1725.6650	^{293}NLT^{295}	^{292}N	HexNAc$_2$ Hex$_6$	[M+H]$^+$	0.0027
1747.6476	^{293}NLT^{295}	^{292}N	HexNAc$_2$ Hex$_6$	[M+Na]$^+$	0.0013
1766.6908	^{293}NLT^{295}	^{292}N	HexNAc$_3$ Hex$_5$	[M+H]$^+$	0.0013
1788.6740	^{293}NLT^{295}	^{292}N	HexNAc$_3$ Hex$_5$	[M+Na]$^+$	0.0024
1807.7187	^{293}NLT^{295}	^{292}N	HexNAc$_4$ Hex$_4$	[M+H]$^+$	0.0011
1829.7006	^{293}NLT^{295}	^{292}N	HexNAc$_4$ Hex$_4$	[M+Na]$^+$	0.0009
1868.7284	^{293}NL^{294}	^{292}N	HexNAc$_4$ Hex$_5$	[M+H]$^+$	0.0019
1890.7068	^{293}NL^{294}	^{292}N	HexNAc$_4$ Hex$_5$	[M+Na]$^+$	0.0016
1969.7709	^{293}NLT^{295}	^{292}N	HexNAc$_4$ Hex$_5$	[M+H]$^+$	0.0038
1991.7547	^{293}NLT^{295}	^{292}N	HexNAc$_4$ Hex$_5$	[M+Na]$^+$	0.0033
2010.7942	^{293}NLT^{295}	^{292}N	HexNAc$_5$ Hex$_4$	[M+H]$^+$	0.0008
2032.7803	^{293}NLT^{295}	^{292}N	HexNAc$_5$ Hex$_4$	[M+Na]$^+$	0.0050
2172.8624	^{293}NLT^{295}	^{292}N	HexNAc$_5$ Hex$_5$	[M+H]$^+$	0.0122
2194.8402	^{293}NLT^{295}	^{292}N	HexNAc$_5$ Hex$_5$	[M+Na]$^+$	0.0075

Supplementary Table 2. N-linked glycopeptides derived from immobilized pronase digestion of HAT. The RMS mass error of the assignments was 4.6 ppm. *HexNAc: N-acetylhexosamine; Hex: hexose; NeuAc: N-acetylneuraminic acid. ‡All mass errors are absolute values (*i.e.*, represented as non-negative).

GP *m/z*	Peptide	Site	Glycan*	Ion Type	Δ*m/z*‡
1680.6334	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_4$	$[M+H]^+$	0.0118
1702.6147	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_4$	$[M+Na]^+$	0.0112
1721.6953	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_4$	$[M+H]^+$	0.0108
1743.6711	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_4$	$[M+Na]^+$	0.0047
1777.6276	^{432}N or ^{630}N	^{432}N or ^{630}N	HexNAc$_4$ Hex$_5$	$[M+Na]^+$	0.0033
1842.6846	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$	$[M+H]^+$	0.0102
1864.6545	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$	$[M+Na]^+$	0.0018
1876.7006	^{630}NV^{631}	^{630}N	HexNAc$_4$ Hex$_5$	$[M+Na]^+$	0.0079
1883.7271	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$	$[M+H]^+$	0.0102
1905.7168	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$	$[M+Na]^+$	0.0025
1921.6847	^{628}GSN^{630}	^{630}N	HexNAc$_4$ Hex$_5$	$[M+Na]^+$	0.0069
1927.6949	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$	$[M-H+2Na]^+$	0.0063
2068.7150	^{432}N or ^{630}N	^{432}N or ^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+Na]^+$	0.0047
2090.7025	^{432}N or ^{630}N	^{432}N or ^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M-H+2Na]^+$	0.0009
2133.7746	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+H]^+$	0.0048
2155.7493	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+Na]^+$	0.0025
2167.7854	^{630}NV^{631}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+Na]^+$	0.0027
2174.8295	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+H]^+$	0.0032
2177.7042	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M-H+2Na]^+$	0.0294
2189.7620	^{630}NV^{631}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M-H+2Na]^+$	0.0080
2196.8188	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+Na]^+$	0.0041
2212.7812	^{628}GSN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M+Na]^+$	0.0080
2218.7943	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M-H+2Na]^+$	0.0023
2234.7563	^{628}GSN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_1$	$[M-H+2Na]^+$	0.0012
2359.7959	^{432}N or ^{630}N	^{432}N or ^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	$[M+Na]^+$	0.0192
2424.8563	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	$[M+H]^+$	0.0089
2446.8393	^{629}SN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	$[M+Na]^+$	0.0078
2465.9176	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	$[M+H]^+$	0.0105
2487.9121	^{432}NK^{433}	^{432}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	$[M+Na]^+$	0.0021
2525.8304	^{628}GSN^{630}	^{630}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	$[M-H+2Na]^+$	0.0201

Supplementary Table 3. N-linked and O-linked glycopeptides derived from immobilized pronase digestion of BF. The RMS mass error of the assignments was 3.2 ppm. †Parentheses are used to indicate ambiguous assignment of terminating amino acid residues. *HexNAc: N-acetylhexosamine; Hex: hexose; NeuAc: N-acetylneuraminic acid. ‡All mass errors are absolute values (*i.e.*, represented as non-negative).

GP *m/z*	**Peptide†**	**Site**	**Glycan***	**Ion Type**	**Δ*m/z*‡**
1114.4518	^{278}GPTPS^{282}	^{280}T or ^{282}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+H]$^+$	0.0004
1136.4339	^{278}GPTPS^{282}	^{280}T or ^{282}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+Na]$^+$	0.0002
1185.4900	^{277}(A)GPTPS(A)^{283}	^{280}T or ^{282}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+H]$^+$	0.0008
1280.4387	^{156}NDS^{158}	^{156}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	[M+Na+H]$^{2+}$	0.0053
1291.4247	^{156}NDS^{158}	^{156}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	[M+2Na]$^{2+}$	0.0008
1312.5526	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+H]$^+$	0.0001
1334.5359	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+Na]$^+$	0.0014
1388.5667	^{277}(A)GPTPS(A)^{283}	^{280}T or ^{282}S	HexNAc$_2$ Hex$_1$ NeuAc$_1$	[M+H]$^+$	0.0019
1405.5458	^{278}GPTPS^{282}	^{280}T or ^{282}S	HexNAc$_1$ Hex$_1$ NeuAc$_2$	[M+H]$^+$	0.0017
1463.0001	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_2$	[M+Na+H]$^{2+}$	0.0006
1473.9844	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_2$	[M+2Na]$^{2+}$	0.0060
1550.6179	^{277}(A)GPTPS(A)^{283}	^{280}T or ^{282}S	HexNAc$_2$ Hex$_2$ NeuAc$_1$	[M+H]$^+$	0.0036
1591.5374	^{99}NCS^{101}	^{99}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+2H]$^{2+}$	0.0080
1597.5662	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+2H]$^{2+}$	0.0100
1603.6419	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_2$	[M+H]$^+$	0.0060
1608.5495	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+Na+H]$^{2+}$	0.0023
1619.5393	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+2Na]$^{2+}$	0.0012
1625.6274	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_2$	[M+Na]$^+$	0.0025
1630.5211	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+3Na-H]$^{2+}$	0.0080
1737.1004	^{99}NCS^{101}	^{99}N	HexNAc$_5$ Hex$_6$ NeuAc$_4$	[M+2H]$^{2+}$	0.0053
1754.1062	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_4$	[M+Na+H]$^{2+}$	0.0113
1765.0970	^{156}NDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_4$	[M+2Na]$^{2+}$	0.0112
1841.7190	^{277}(A)GPTPS(A)^{283}	^{280}T or ^{282}S	HexNAc$_2$ Hex$_2$ NeuAc$_2$	[M+H]$^+$	0.0022
1863.7017	^{277}(A)GPTPS(A)^{283}	^{280}T or ^{282}S	HexNAc$_2$ Hex$_2$ NeuAc$_2$	[M+Na]$^+$	0.0030
2539.9022	^{156}NDS^{158}	^{156}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	[M+H]$^+$	0.0001
2561.8847	^{156}NDS^{158}	^{156}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	[M+Na]$^+$	0.0107

Supplementary Table 4. N-linked and O-linked glycopeptides derived from immobilized pronase digestion of a mixture of RNase B, κ-CN, BF, and the nonglycosylated protein BSA. The RMS mass error of the assignments was 3.0 ppm. $^{\dagger * \ddagger}$See caption to Supplementary Table 3.

Source	**GP *m/z***	**Peptide**†	**Site**	**Glycan***	**Ion Type**	**Δ*m/z***‡
κ-CN	1312.5545	^{182}(T)VQVTS(T)^{188}	^{186}T	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+Na]$^+$	0.0043
BF	1312.5545	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+H]$^+$	0.0019
κ -CN	1334.5389	^{182}(T)VQVTS(T)^{188}	^{186}T	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M-H+2Na]$^+$	0.0068
BF	1334.5389	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_1$	[M+Na]$^+$	0.0044
RNase B	1349.4875	^{60}N	^{60}N	HexNAc$_2$ Hex$_5$	[M+H]$^+$	0.0039
RNase B	1371.4683	^{60}N	^{60}N	HexNAc$_2$ Hex$_5$	[M+Na]$^+$	0.0028
RNase B	1462.5684	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_5$	[M+H]$^+$	0.0007
RNase B	1484.5548	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_5$	[M+Na]$^+$	0.0053
RNase B	1505.5855	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_5$	[M+H]$^+$	0.0008
RNase B	1511.5354	^{60}N	^{60}N	HexNAc$_2$ Hex$_6$	[M+H]$^+$	0.0010
RNase B	1527.5670	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_5$	[M+Na]$^+$	0.0004
RNase B	1533.5210	^{60}N	^{60}N	HexNAc$_2$ Hex$_6$	[M+Na]$^+$	0.0027
κ -CN	1603.6481	^{182}(T)VQVTS(T)^{188}	^{186}T	HexNAc$_1$ Hex$_1$ NeuAc$_2$	[M+Na]$^+$	0.0025
BF	1603.6481	^{269}(A)PSAVPD(A)^{276}	^{271}S	HexNAc$_1$ Hex$_1$ NeuAc$_2$	[M+H]$^+$	0.0001
RNase B	1624.6180	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_6$	[M+H]$^+$	0.0024
RNase B	1646.6041	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_6$	[M+Na]$^+$	0.0017
RNase B	1667.6400	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_6$	[M+H]$^+$	0.0025
RNase B	1673.5883	^{60}N	^{60}N	HexNAc$_2$ Hex$_7$	[M+H]$^+$	0.0009
RNase B	1689.6057	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_6$	[M+Na]$^+$	0.0137
RNase B	1695.5737	^{60}N	^{60}N	HexNAc$_2$ Hex$_7$	[M+Na]$^+$	0.0026
BF	1739.1566	^{153}APLNDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+2H]$^{+2}$	0.0061
BF	1750.1479	^{153}APLNDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_3$	[M+H+Na]$^{+2}$	0.0065
RNase B	1786.6767	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_7$	[M+H]$^+$	0.0034
RNase B	1808.6496	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_7$	[M+Na]$^+$	0.0056
RNase B	1829.6918	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_7$	[M+H]$^+$	0.0015
RNase B	1835.6380	^{60}N	^{60}N	HexNAc$_2$ Hex$_8$	[M+H]$^+$	0.0040
BF	1895.6805	^{153}APLNDS^{158}	^{156}N	HexNAc$_5$ Hex$_6$ NeuAc$_4$	[M+H+Na]$^{+2}$	0.0086
RNase B	1948.7255	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_8$	[M+H]$^+$	0.0006
RNase B	1970.7082	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_8$	[M+Na]$^+$	0.0003
RNase B	1991.7420	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_8$	[M+H]$^+$	0.0011
RNase B	2110.7758	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_9$	[M+H]$^+$	0.0031
RNase B	2132.7605	^{60}NL^{61}	^{60}N	HexNAc$_2$ Hex$_9$	[M+Na]$^+$	0.0003
RNase B	2153.7781	^{59}RN^{60}	^{60}N	HexNAc$_2$ Hex$_9$	[M+H]$^+$	0.0178
BF	2821.0821	^{153}APLNDS^{158}	^{156}N	HexNAc$_4$ Hex$_5$ NeuAc$_2$	[M+H]$^+$	0.0160