Supporting Information — Suppression of O-linked Glycosylation of the SARS-CoV-2 Spike by Quaternary Structural Restraints

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>HexaPro_SARS-CoV_2

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTK
RFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWM
ESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIG
INITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKG
IYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLND
LCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFER
DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNF
NGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEV
PVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQSIIAYTM
SLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK
NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSPIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKF
NGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGPALQIPFPMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI
GKIQDSLSSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVT
QQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHD
GKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHT
SPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQAYVRKDGEWVLLSTFLG
RSLEVLFQGPGHHHHHHHHSAWSHPQFEK

Figure S1 Sequence for HexaPro construct

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTK
RFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWM
ESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIG
INITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKG
IYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLND
LCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFER
DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNF
NGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEV
PVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQSIIAYTM
SLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK
NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKF
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GKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVT
QQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHD
GKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHT
SPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQAYVRKDGEWVLLSTFLG
RSLEVLFQGPGHHHHHHHHSAWSHPQFEK

Figure S2 Sequence for 2P construct

>SARS-CoV-2_RBD

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRIS
NCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAW
NSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVV
LSFELLHAPATVCGPKKSTNLVKNKCVNGSGSGGSGHHHHHH

Figure S3 Sequence for RBD construct

Site	N17	N61	N74	N122	N149	N165	N234	N282	N331	N343	N603	N616	N657	N709	N717	N801	N1074	N1098	N1134	N1158	N1173	N1994	
M9Glc	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
M9	0.00%	97.71%	72.21%	96.81%	82.77%	89.17%	99.32%	95.18%	67.96%	94.15%	93.33%	98.05%	99.86%	87.90%	93.41%	93.88%	88.89%	76.06%	97.93%	87.23%	100.00%	59.53%	
M8	0.00%	0.00%	15.81%	0.00%	11.60%	6.10%	0.57%	2.36%	8.68%	0.00%	0.00%	0.00%	0.00%	12.10%	0.00%	2.44%	0.00%	0.00%	0.00%	8.92%	0.00%	23.82%	
M7	0.00%	0.00%	3.89%	0.00%	0.00%	0.29%	0.04%	0.00%	4.26%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.56%	0.00%	0.35%	0.00%	0.00%	0.00%	16.63%	
M6	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.48%	0.00%	0.75%	0.00%	0.34%	0.00%	0.00%	0.00%	
M5	0.00%	0.00%	0.00%	0.00%	0.00%	3.00%	0.00%	0.48%	0.00%	0.00%	2.85%	0.00%	0.00%	0.00%	4.11%	2.52%	0.85%	0.00%	0.00%	0.00%	0.00%	0.00%	
M4	0.00%	0.25%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.79%	0.00%	0.00%	0.00%	0.00%	0.00%	
M3	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.22%	0.00%	0.00%	0.00%	0.00%	0.00%	
FM	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
HYBRID	0.00%	0.00%	0.00%	0.87%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.28%	0.19%	0.00%	0.00%	0.00%	0.00%	
FHYBRID	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
A1	0.00%	0.19%	0.00%	0.79%	0.00%	0.00%	0.00%	0.27%	0.00%	0.00%	0.00%	0.86%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
FA1	0.00%	0.00%	0.00%	0.78%	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.32%	0.00%	0.62%	0.00%	0.00%	0.00%	
A2/A1B	0.00%	1.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.08%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
FA2/FA1B	0.00%	0.00%	0.00%	0.50%	4.83%	0.00%	0.00%	0.00%	8.23%	4.97%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.06%	0.00%	0.00%	0.00%	0.00%	0.00%	
A3/A2B	0.00%	0.31%	0.00%	0.00%	0.08%	0.22%	0.00%	1.71%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
FA3/FA2B	0.00%	0.00%	0.00%	0.00%	0.72%	0.00%	0.00%	0.00%	1.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.27%	0.00%	0.53%	0.00%	0.00%	0.00%	
A4/A3B	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
FA4/FA3B	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	
UNOCC	0.00%	0.00%	1.67%	0.25%	0.00%	0.00%	0.00%	0.00%	9.85%	0.00%	0.00%	0.00%	0.14%	0.00%	0.00%	0.00%	3.56%	23.40%	0.00%	2.47%	0.00%	0.02%	
Core	0.00%	0.00%	6.41%	0.00%	0.00%	1.23%	0.07%	0.00%	0.00%	0.34%	3.82%	0.00%	0.00%	0.00%	0.00%	0.60%	0.00%	0.00%	0.59%	1.38%	0.00%	0.00%	
																							SUM
Hybrid	0.00%	0.00%	0.00%	0.87%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.28%	0.19%	0.00%	0.00%	0.00%	0.00%	0.06%
Complex	0.00%	2.04%	0.00%	2.07%	5.63%	0.22%	0.00%	1.98%	9.25%	5.51%	0.00%	1.95%	0.00%	0.00%	0.00%	0.00%	4.66%	0.00%	1.15%	0.00%	0.00%	0.00%	1.64%
Unoccupied	0.00%	0.00%	1.67%	0.25%	0.00%	0.00%	0.00%	0.00%	9.85%	0.00%	0.00%	0.00%	0.14%	0.00%	0.00%	0.00%	3.56%	23.40%	0.00%	2.47%	0.00%	0.02%	1.97%
Man9	0.00%	97.71%	72.21%	96.81%	82.77%	89.17%	99.32%	95.18%	67.96%	94.15%	93.33%	98.05%	99.86%	87.90%	93.41%	93.88%	88.89%	76.06%	97.93%	87.23%	100.00%	59.53%	89.11%
Man8-5	0.00%	0.00%	19.70%	0.00%	11.60%	9.39%	0.61%	2.84%	12.94%	0.00%	2.85%	0.00%	0.00%	12.10%	6.59%	5.52%	1.60%	0.35%	0.34%	8.92%	0.00%	40.45%	6.47%
Core	0.00%	0.00%	6.41%	0.00%	0.00%	1.23%	0.07%	0.00%	0.00%	0.34%	3.82%	0.00%	0.00%	0.00%	0.00%	0.60%	0.00%	0.00%	0.59%	1.38%	0.00%	0.00%	0.69%

Figure S4: Full occupancy data for N-linked glycan sites, broken down by composition

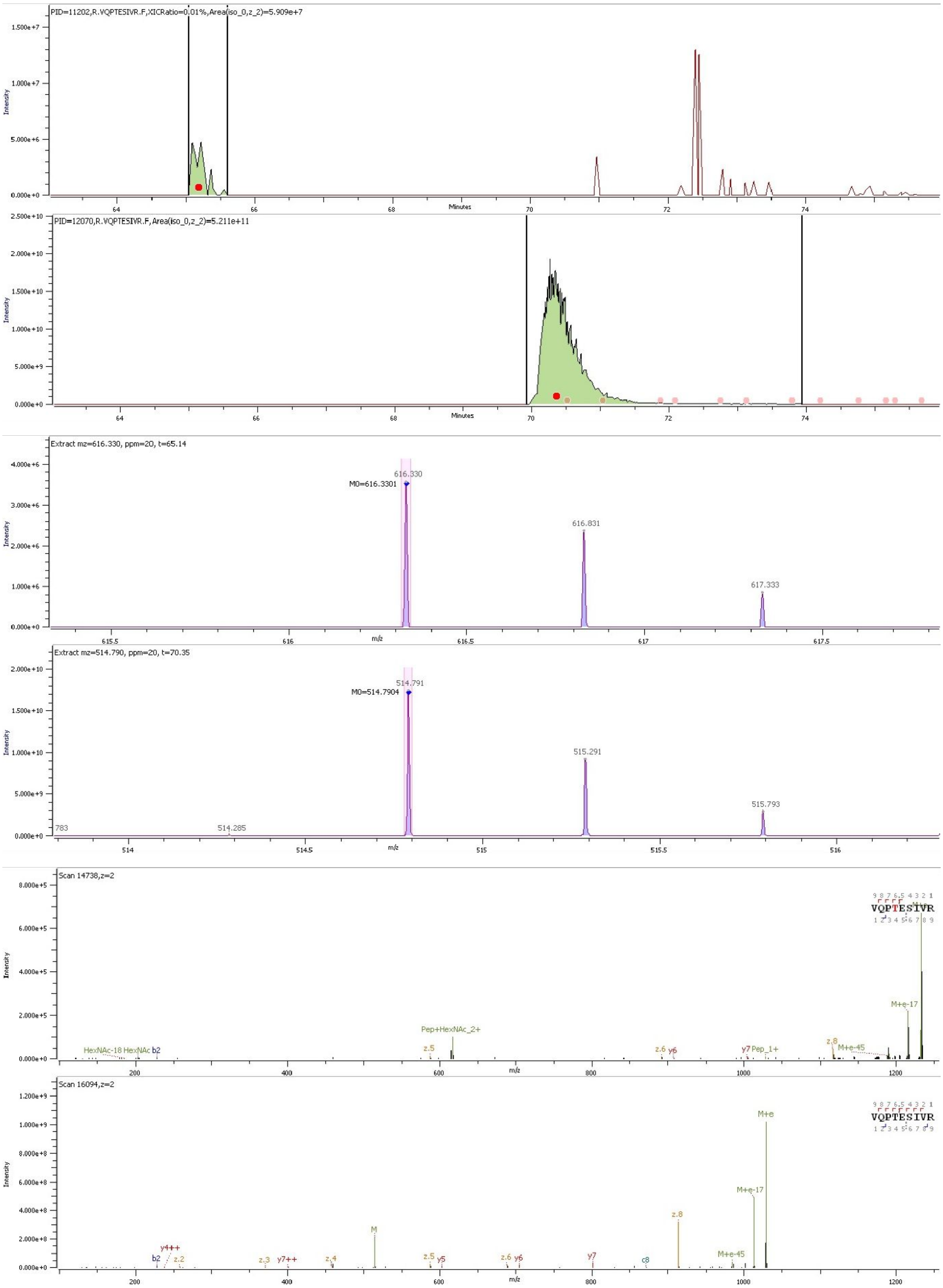
Site	Construct	Sequence	Glycans	Score	XIC Occupied	XIC Unoccupied	occupancy
T323	HexaPro	R.VQPtESIVR.F	HexNAc(1)	176.05	5.91E+07	5.21E+11	0.01%
T323	HexaPro	S.NFRVQPtESIV.R	HexNAc(1)	191.31	1.42E+08	3.00E+11	0.05%
T323	HexaPro	R.VQPtESIVR.F	HexNAc(1)	176.14	5.32E+07	6.25E+11	0.01%
S1261	HexaPro	H.SAWsHPQFEK.-	HexNAc(1)	201.3	2.65E+08	4.62E+09	5.42%
S1261	HexaPro	HHHSAWSHPQFEK	HexNAc(2)Hex(2)NeuAc(2)	349.16	1.15E+07	1.63E+08	6.59%

Table S1: Unambiguously verified O-linked glycans in the Hexapro construct

Figure S5: XIC, precursor spectra, and fragmentation spectra of manually verified true positive O-linked glycans for the kifunensine treated spike HexaPro construct

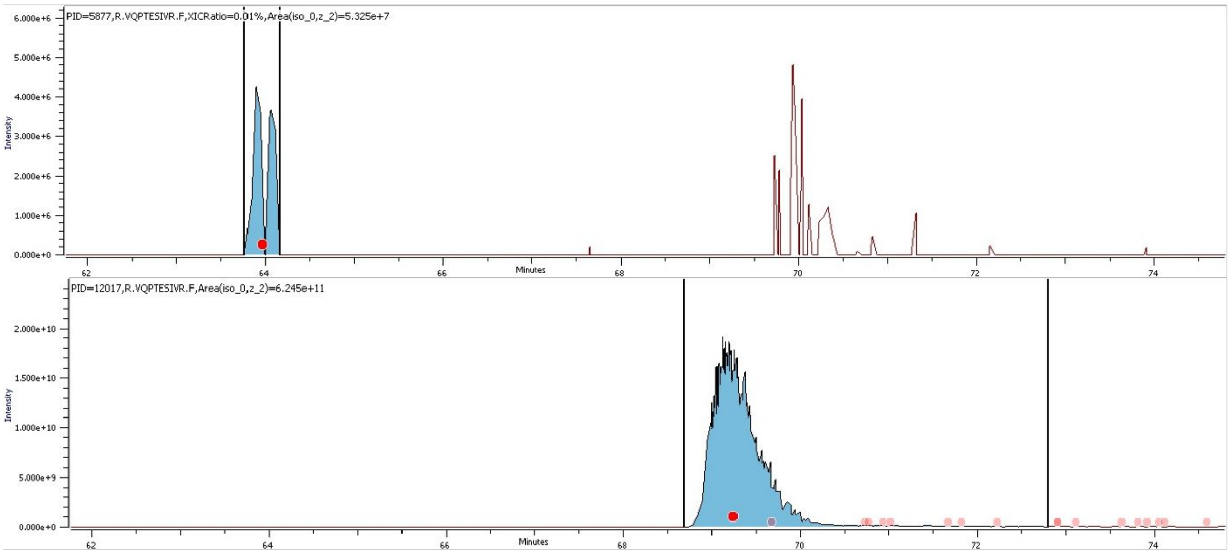
HexaPro - T323

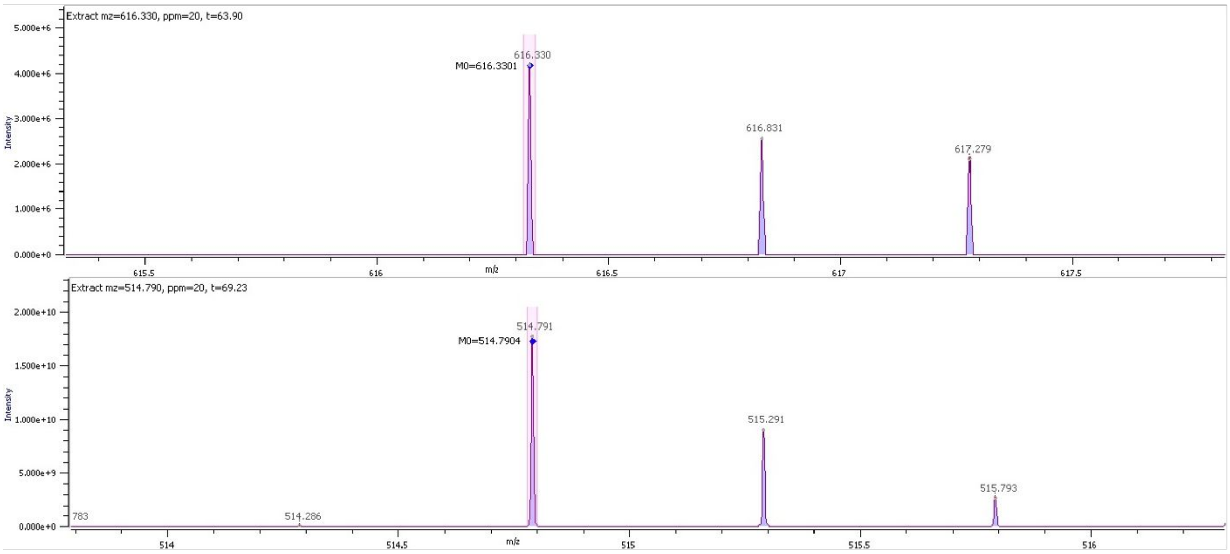
T323
Sequence = VQPTESIVR; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 176.05
XIC Area = 5.91e+7; XIC % Mod = 0.011
Expected m/z = 616.3301; Obs m/z = 616.3304
Error (ppm) = 0.61
Retention Time (min) = 65.21
InSilico status:

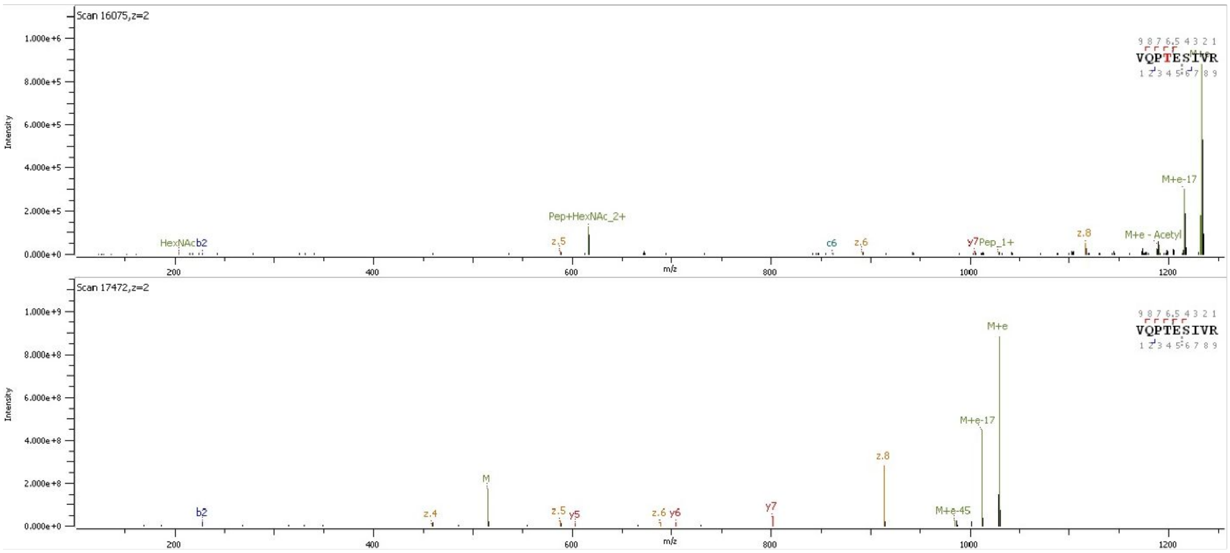


HexaPro - T323

T323
Sequence = VQPTESIVR; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 176.14
XIC Area = 5.32e+7; XIC % Mod = 0.009
Expected m/z = 616.3301; Obs m/z = 616.3301
Error (ppm) = 0.02
Retention Time (min) = 63.90
InSilico status:

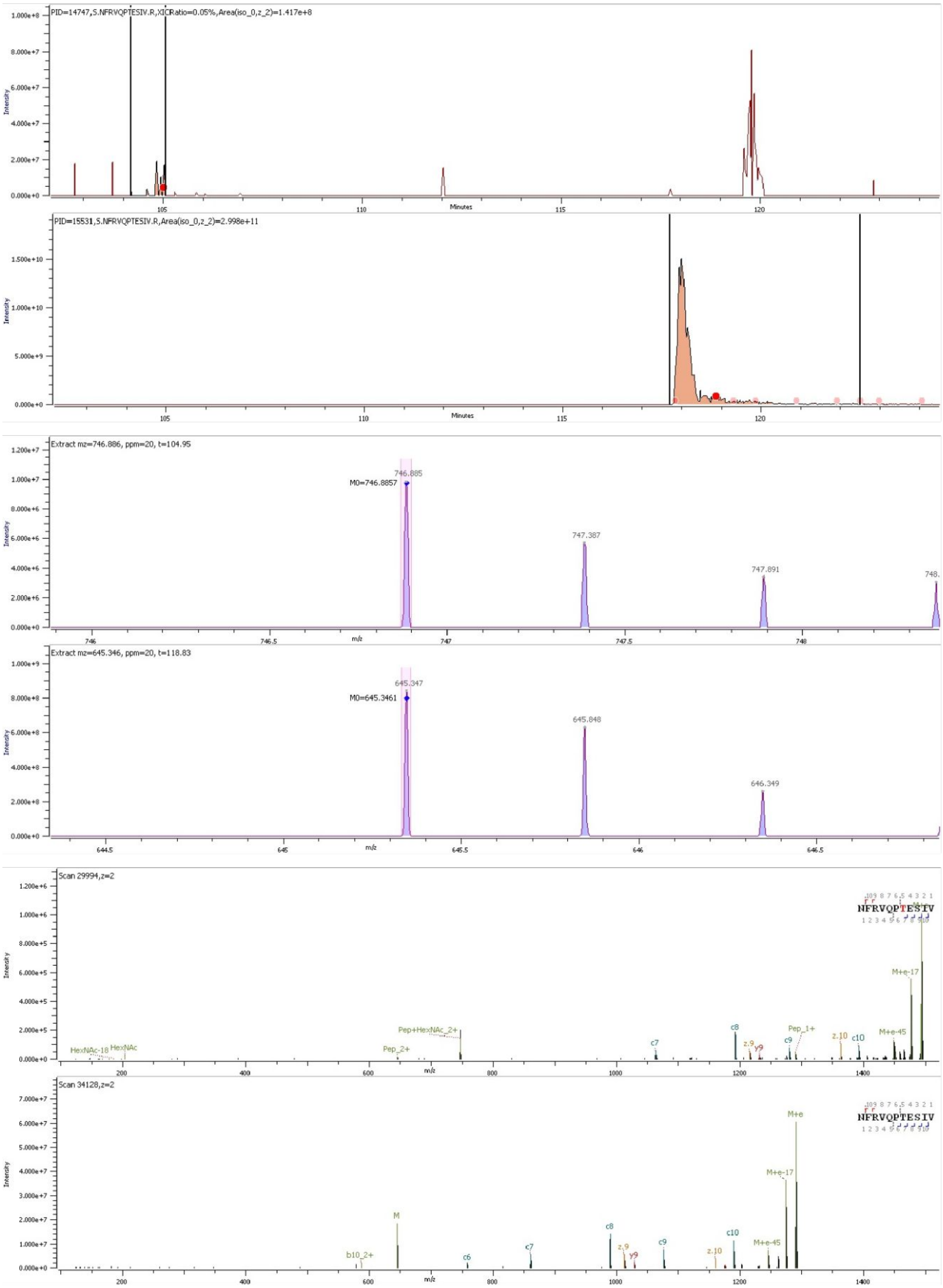






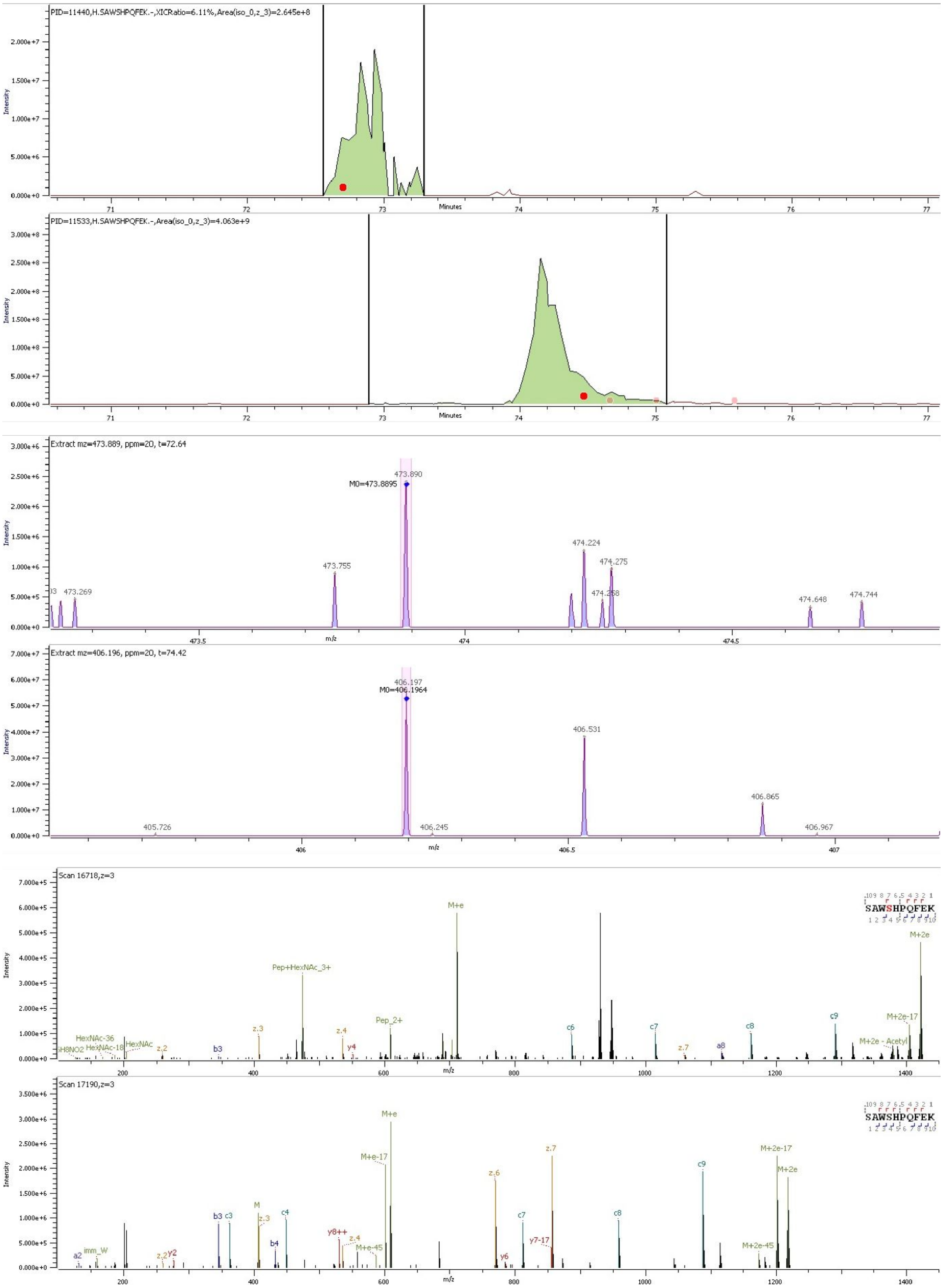
HexaPro - T323

T323
Sequence = NFRVQPTESIV; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 191.31
XIC Area = 1.42e+8; XIC % Mod = 0.047
Expected m/z = 746.8857; Obs m/z = 746.8862
Error (ppm) = 0.62
Retention Time (min) = 104.83
InSilico status:



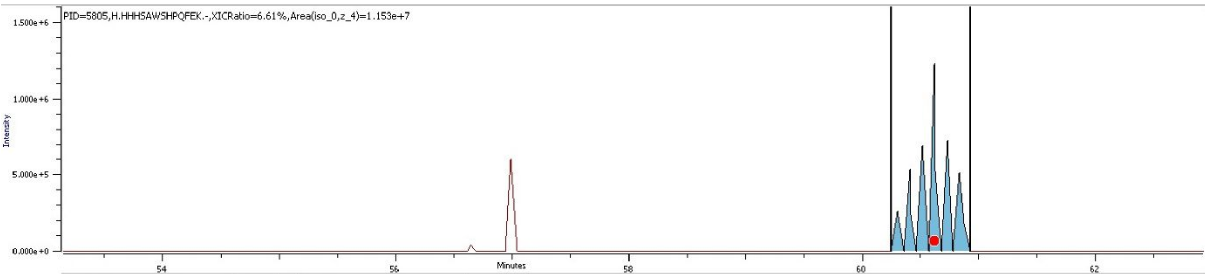
HexaPro - S1261

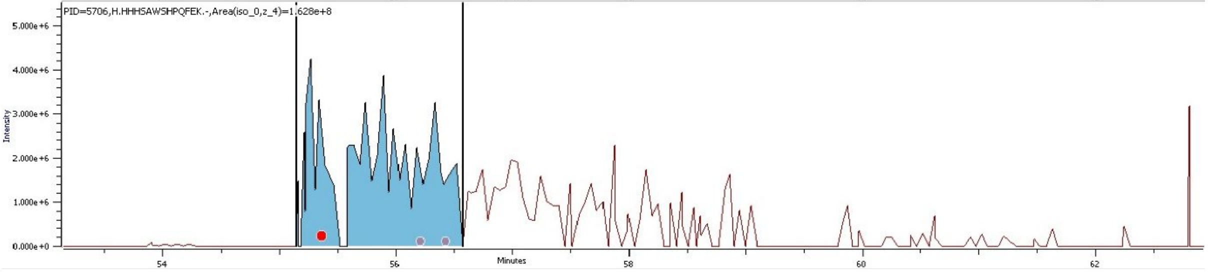
S1261
Sequence = SAWSHPQFEK; Mods = OGlycan/203.0794 z=3
Glycan = HexNAc(1)
Score = 201.30
XIC Area = 2.65e+8; XIC % Mod = 6.112
Expected m/z = 473.8895; Obs m/z = 473.8896
Error (ppm) = 0.14
Retention Time (min) = 72.94
InSilico status:

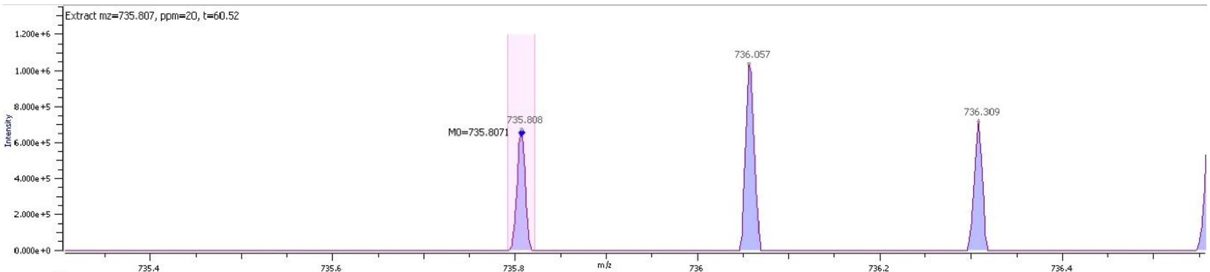


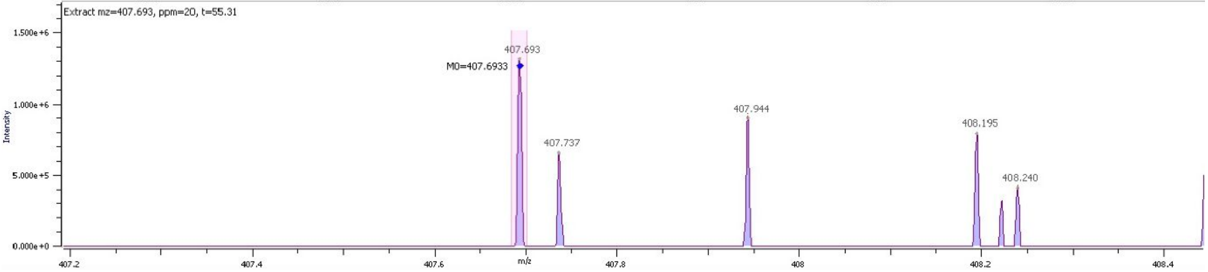
HexaPro - S1261

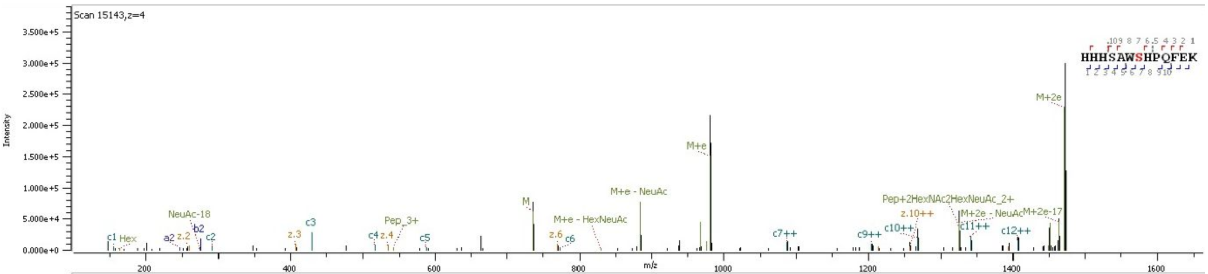
S1261
Sequence = HHHSAWSHPQFEK; Mods = OGlycan/1312.4552 z=4
Glycan = HexNAc(2)Hex(2)NeuAc(2)
Score = 349.16
XIC Area = 1.15e+7; XIC % Mod = 6.613
Expected m/z = 735.8071; Obs m/z = 735.8071
Error (ppm) = 0.02
Retention Time (min) = 60.62
InSilico status:

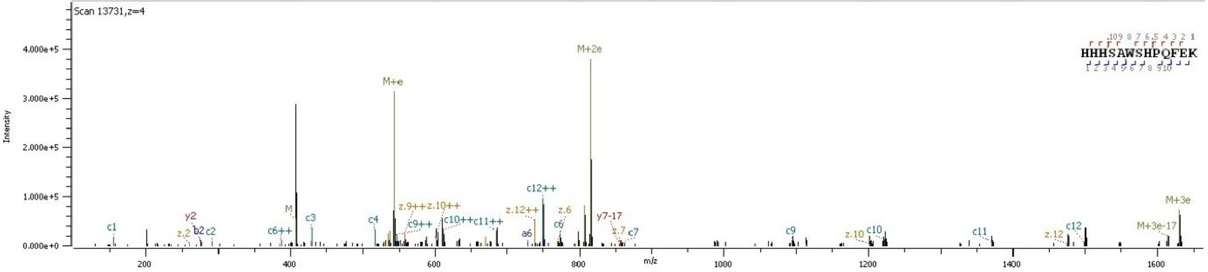












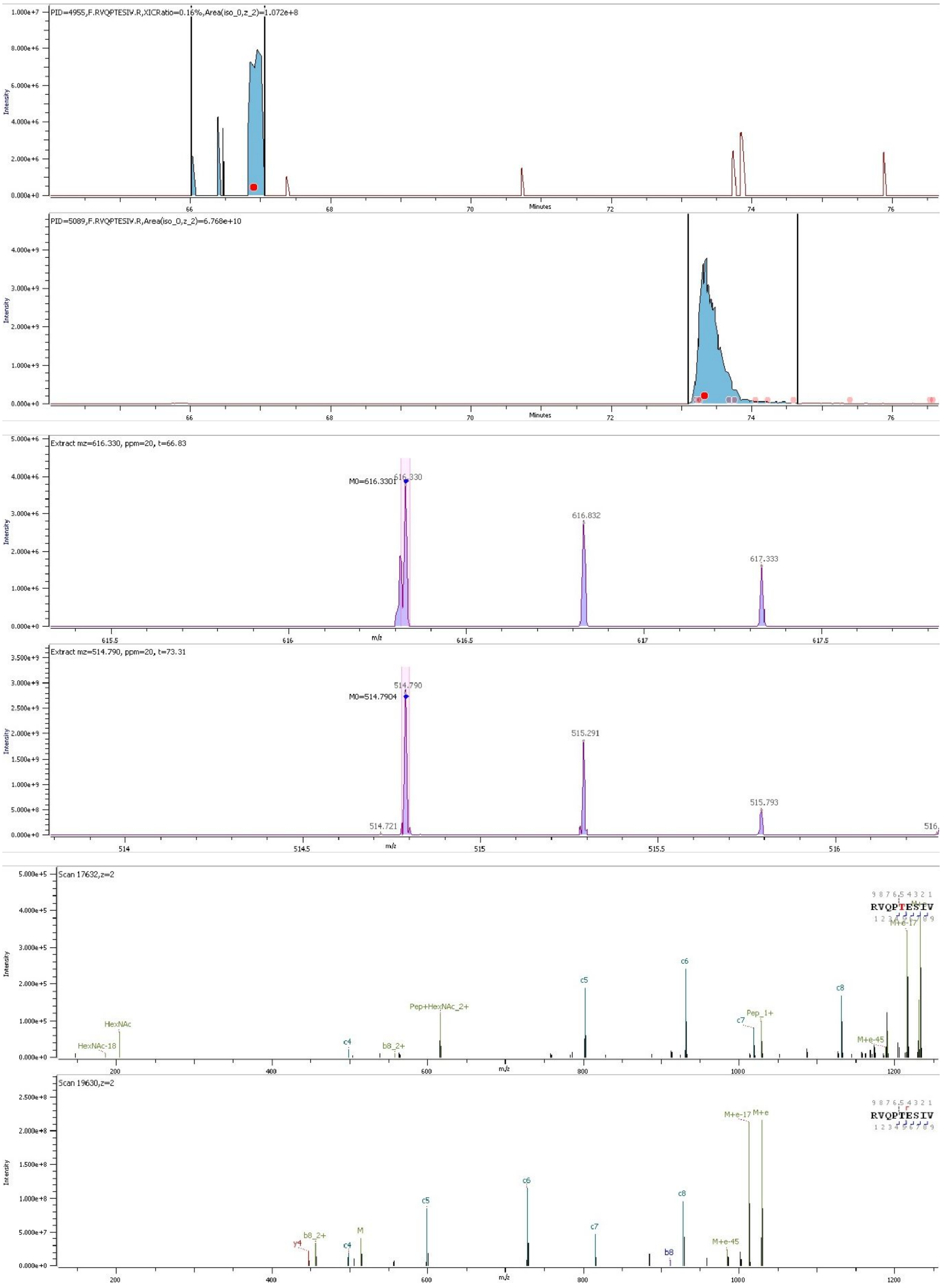
Site	Construct	Sequence	Glycans	Score	XIC Occupied	XIC Unoccupied	occupancy
T323	2P	S.NFRVQPtESIV.R	HexNAc(1)	170.59	3.22E+08	6.77E+10	0.47%
T323	2P	R.VQPtESIVR.F	HexNAc(1)	197.33	5.36E+08	3.01E+11	0.18%
T323	2P	F.RVQPtESIV.R	HexNAc(1)	192.53	1.07E+08	6.77E+10	0.16%
T323	2P	F.RVQPtESIV.R	HexNAc(1)	171.6	3.40E+07	6.55E+10	0.05%
S1261	2P	H.HHHSAWsHPQFEK.-	HexNAc(1)	234.20 - 531.98	5.25E+08	5.55E+09	8.64%
S1261	2P	H.HHHSAWsHPQFEK.-	HexNAc(1)Hex(1)NeuAc(2)	82.57 - 142.57	9.41E+07	5.55E+09	2.00%
S1261	2P	H.HHHHSAWsHPQFEK.-	HexNAc(1)Hex(1)NeuAc(2)	307.88 - 480.37	1.23E+09	8.14E+08	56.89%
S1261	2P	H.SAWsHPQFEK.-	HexNAc(1)	196.52	1.98E+09	2.04E+10	7.23%
S1261	2P	S.AWsHPQFEK.-	HexNAc(1)	279.7	1.12E+07	5.14E+08	2.13%

Table S2: Unambiguously verified O-linked glycans in the 2P construct

Figure S6: XIC, precursor spectra, and fragmentation spectra of manually verified true positive O-linked glycans for the kifunensine treated spike 2P construct

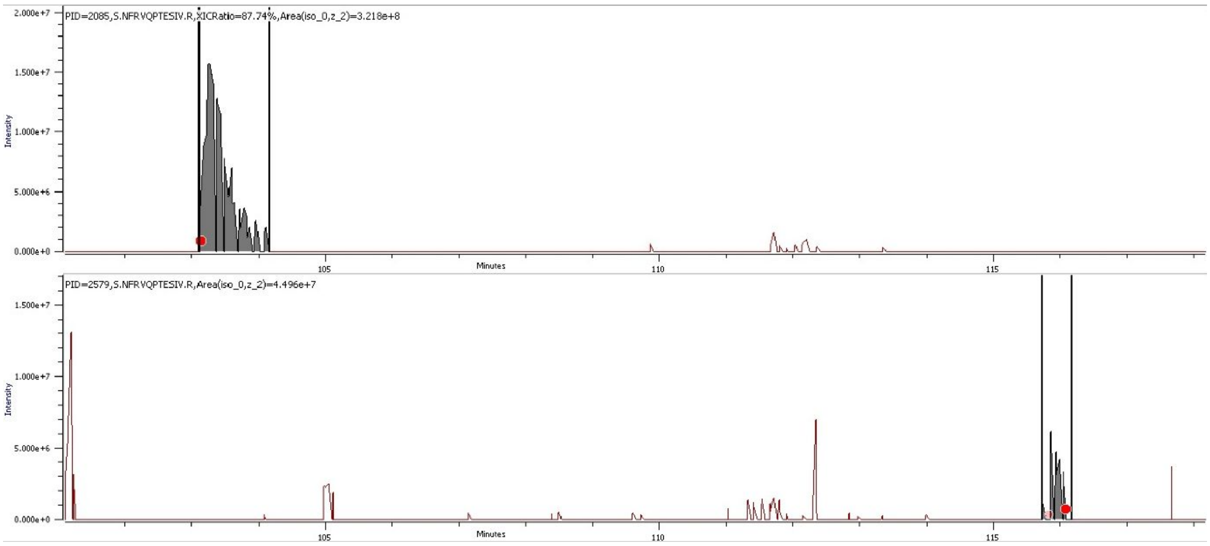
2P - T323

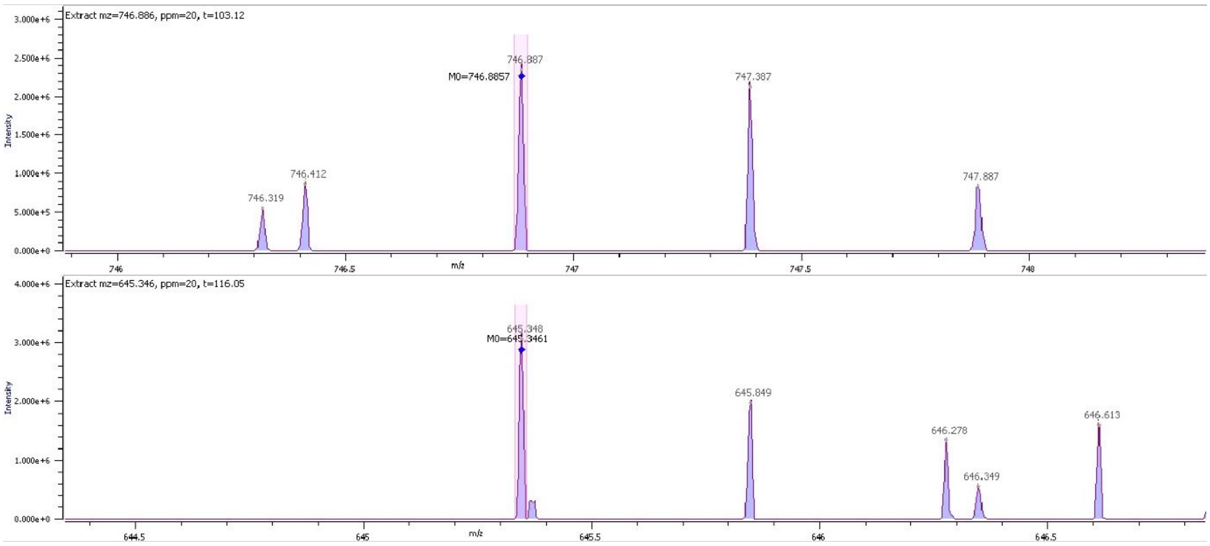
T323
Sequence = RVQPTESIV; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 192.53
XIC Area = 1.07e+8; XIC % Mod = 0.158
Expected m/z = 616.3301; Obs m/z = 616.3307
Error (ppm) = 1.01

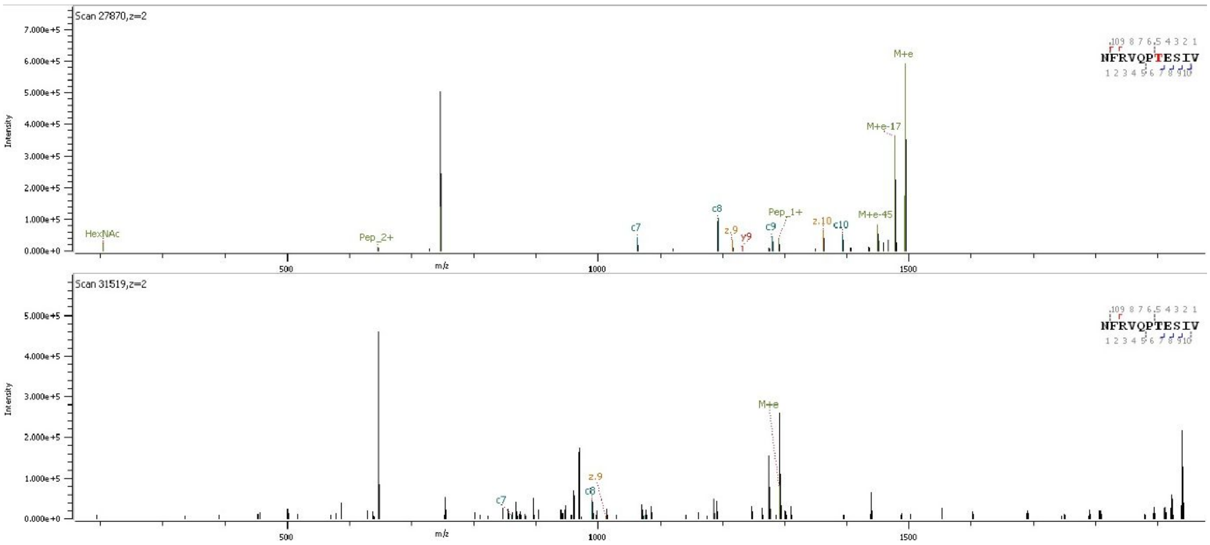


2P - T323

T323
Sequence = NFRVQPTESIV; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 170.59
XIC Area = 3.22e+8; XIC % Mod = 87.741
Expected m/z = 746.8857; Obs m/z = 746.8860
Error (ppm) = 0.41

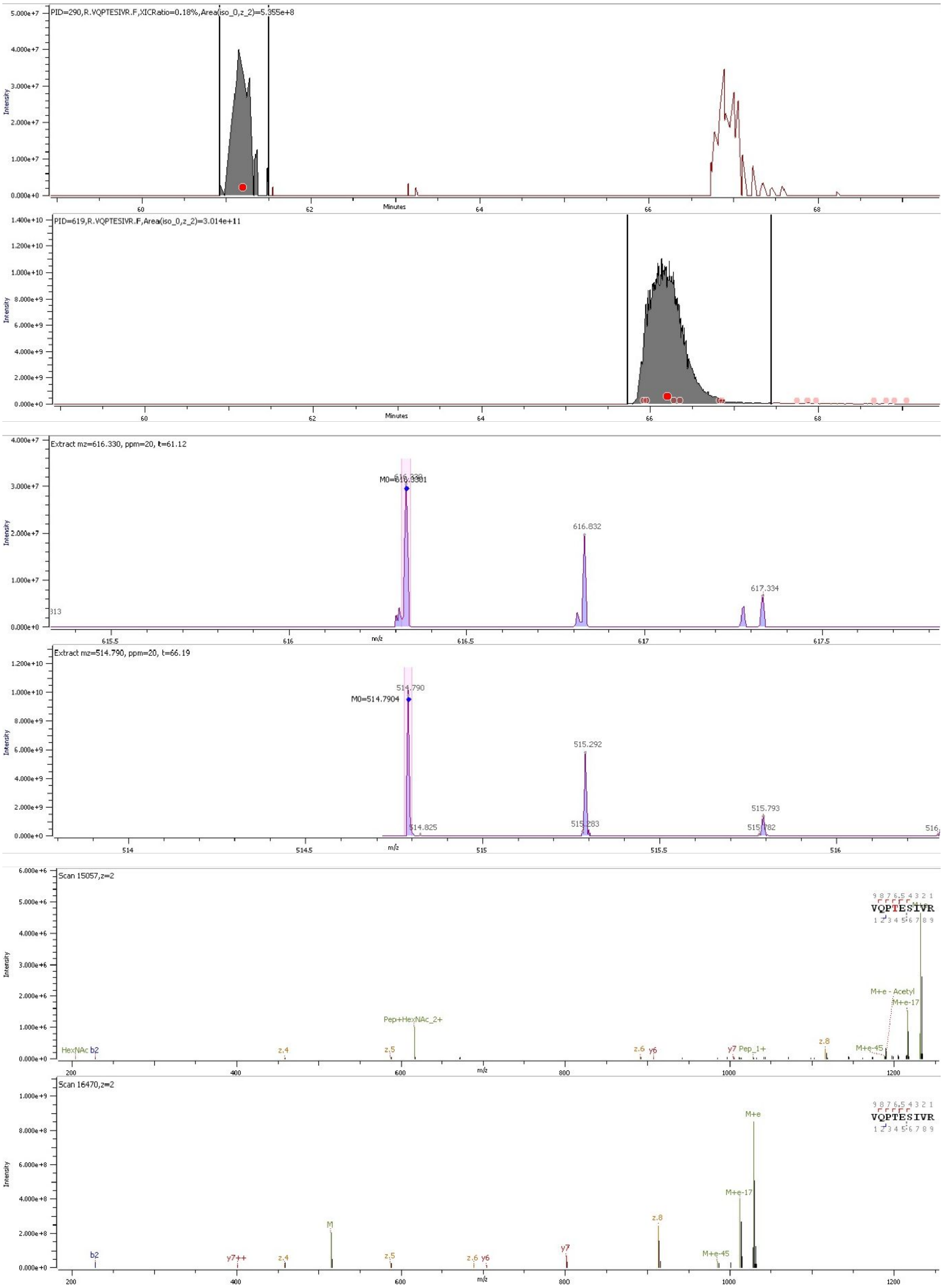






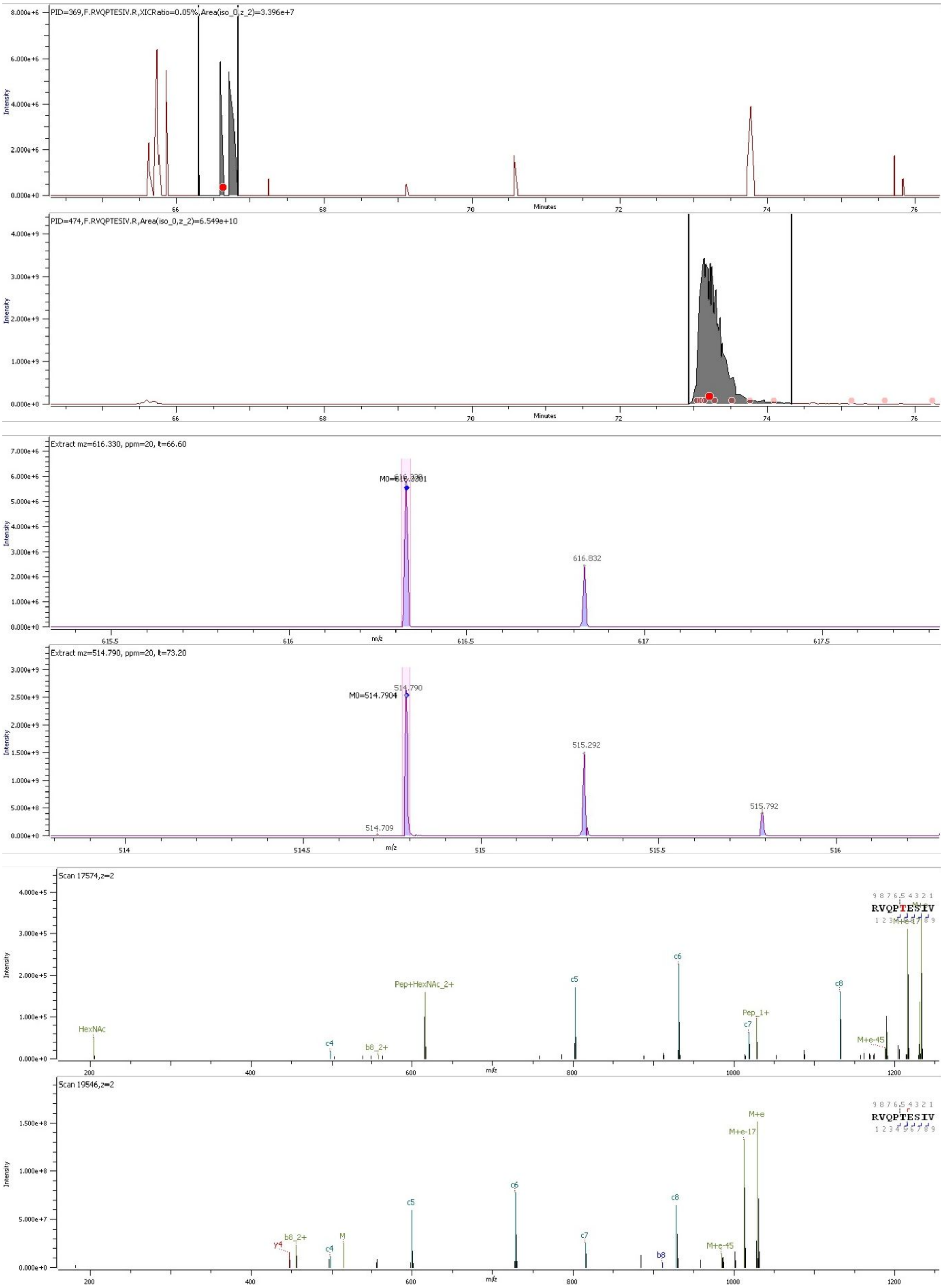
2P - T323

T323
Sequence = VQPTESIVR; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 197.33
XIC Area = 5.36e+8; XIC % Mod = 0.177
Expected m/z = 616.3301; Obs m/z = 616.3301
Error (ppm) = 0.12



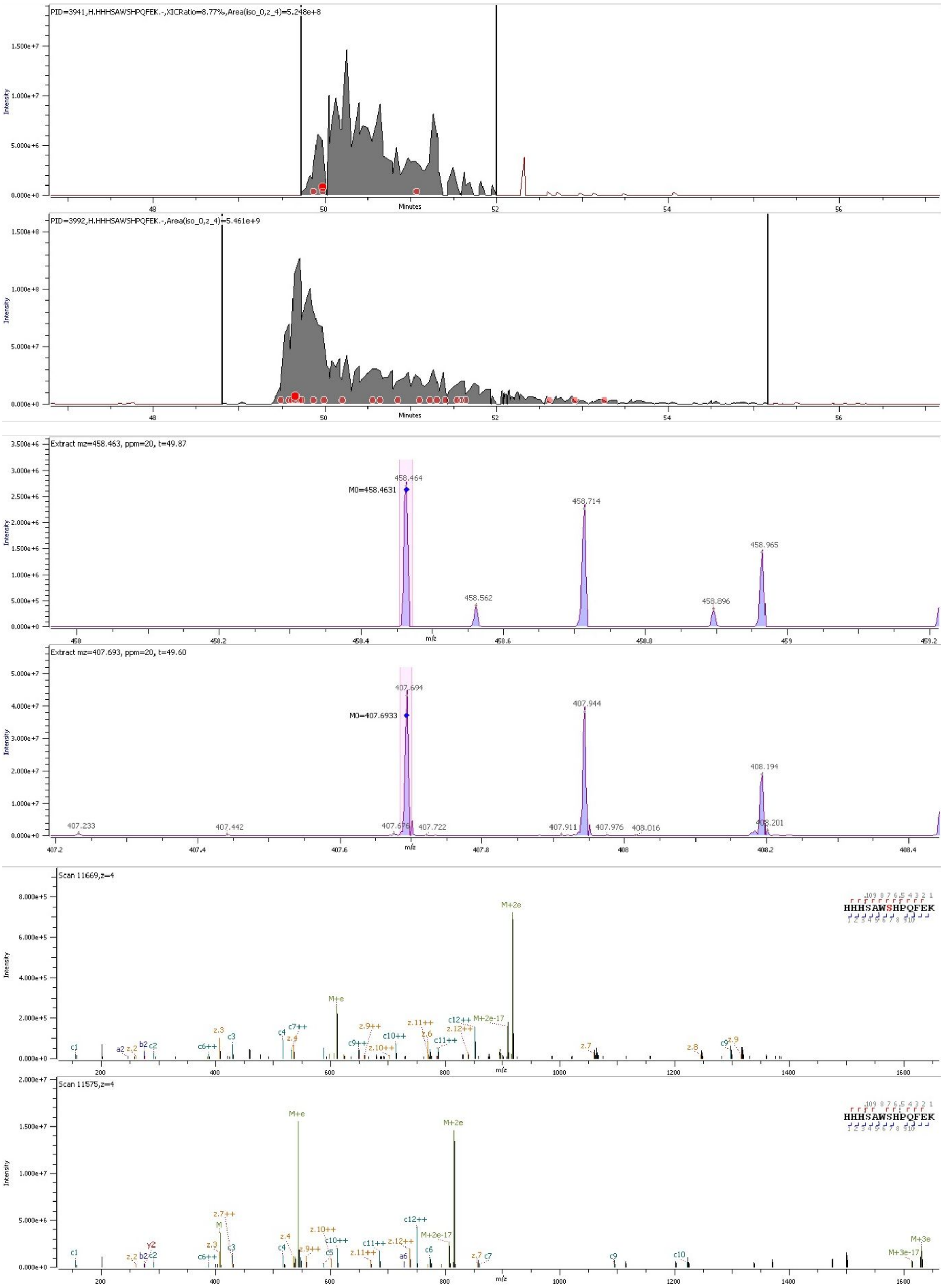
2P - T323

T323
Sequence = RVQPTESIV; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 171.60
XIC Area = 3.4e+7; XIC % Mod = 0.052
Expected m/z = 616.3301; Obs m/z = 616.3304
Error (ppm) = 0.51



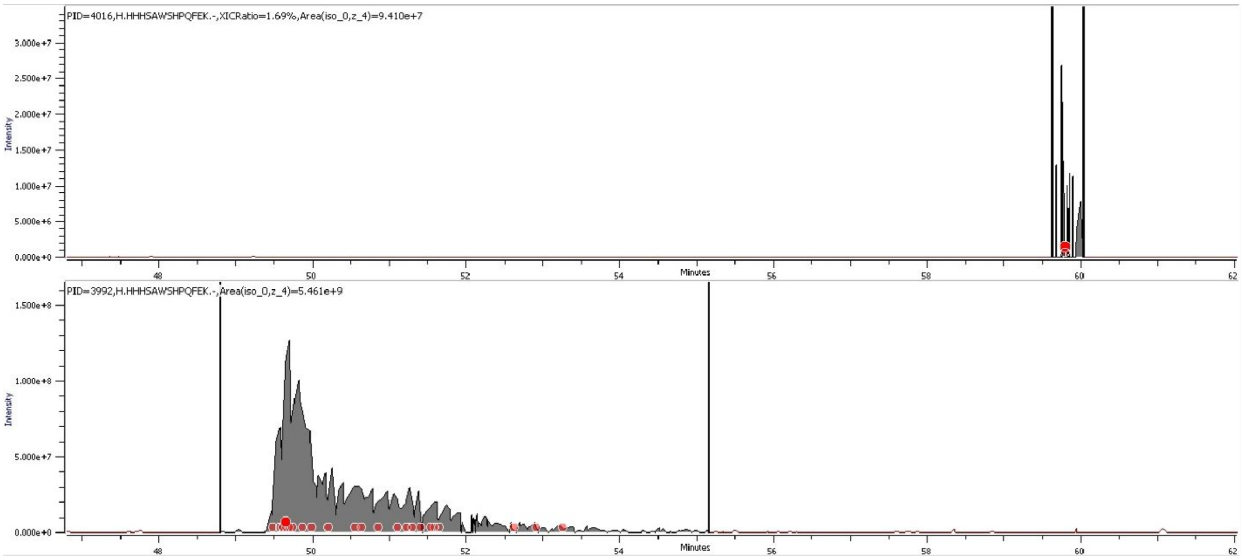
2P - S1261

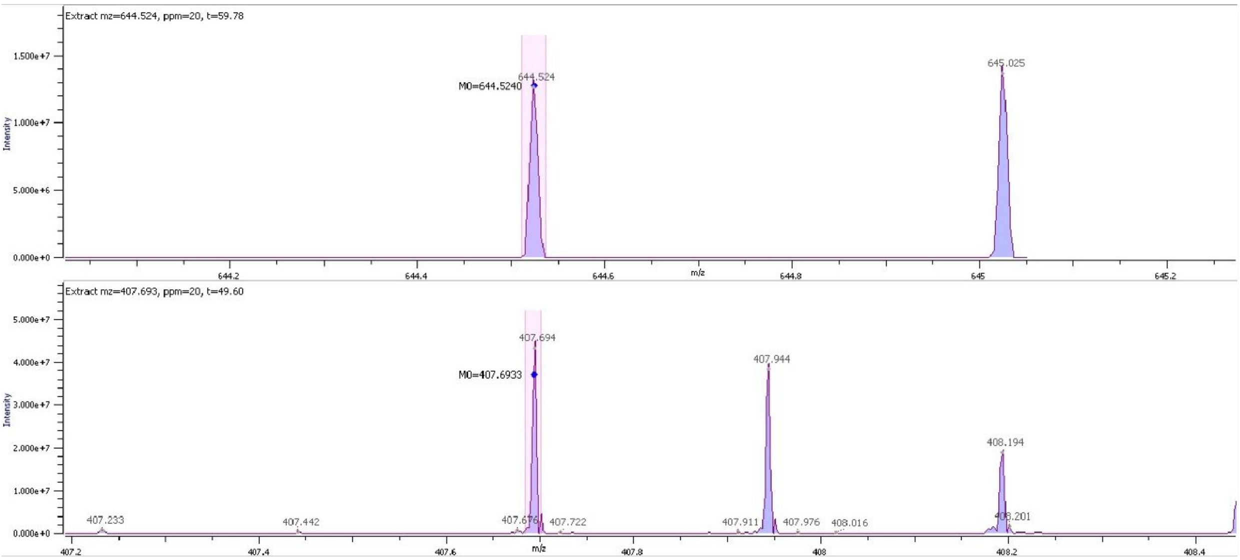
S1261
Sequence = HHHSAWSHPQFEK; Mods = OGlycan/203.0794 z=4
Glycan = HexNAc(1)
Score = 234.20 - 531.98
XIC Area = 5.25e+8; XIC % Mod = 8.767
Expected m/z = 458.4631; Obs m/z = 458.4631 - 458.4633
Error (ppm) = 0.43; 0.30; -0.04

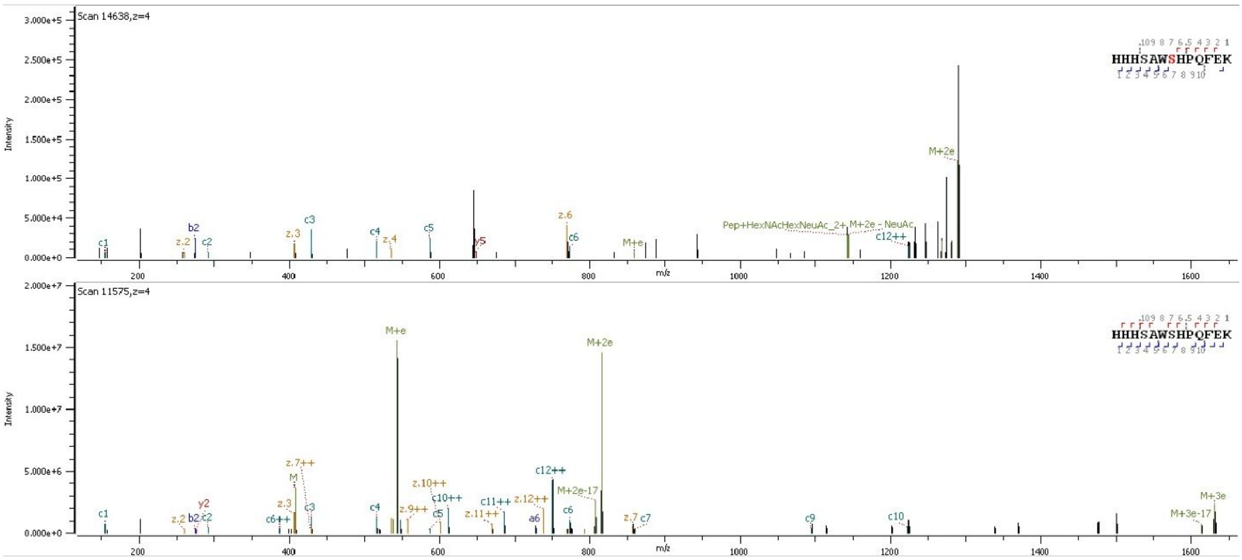


2P - S1261

S1261
Sequence = HHHSAWSHPQFEK; Mods = OGlycan/947.3230 z=4
Glycan = HexNAc(1)Hex(1)NeuAc(2)
Score = 57.17 - 117.17
XIC Area = 9.41e+7; XIC % Mod = 1.694
Expected m/z = 644.5240; Obs m/z = 644.5247
Error (ppm) = 1.10

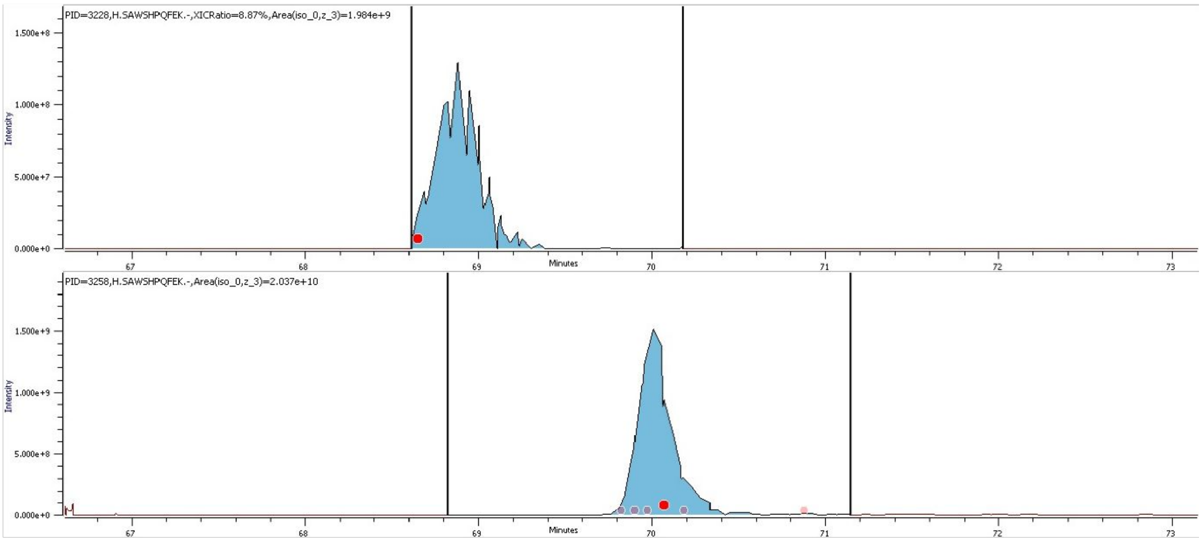


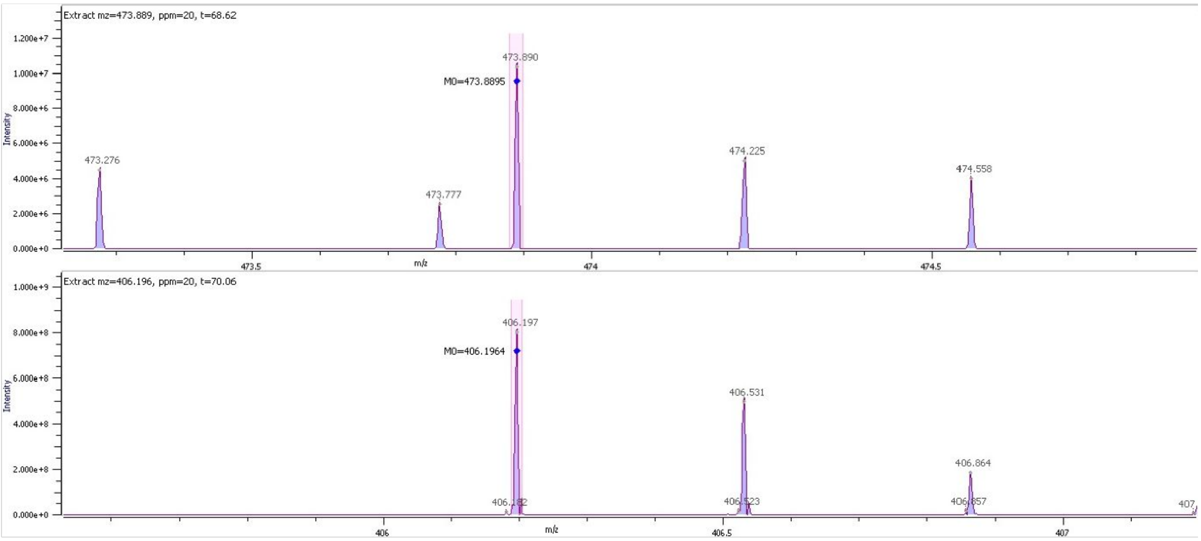


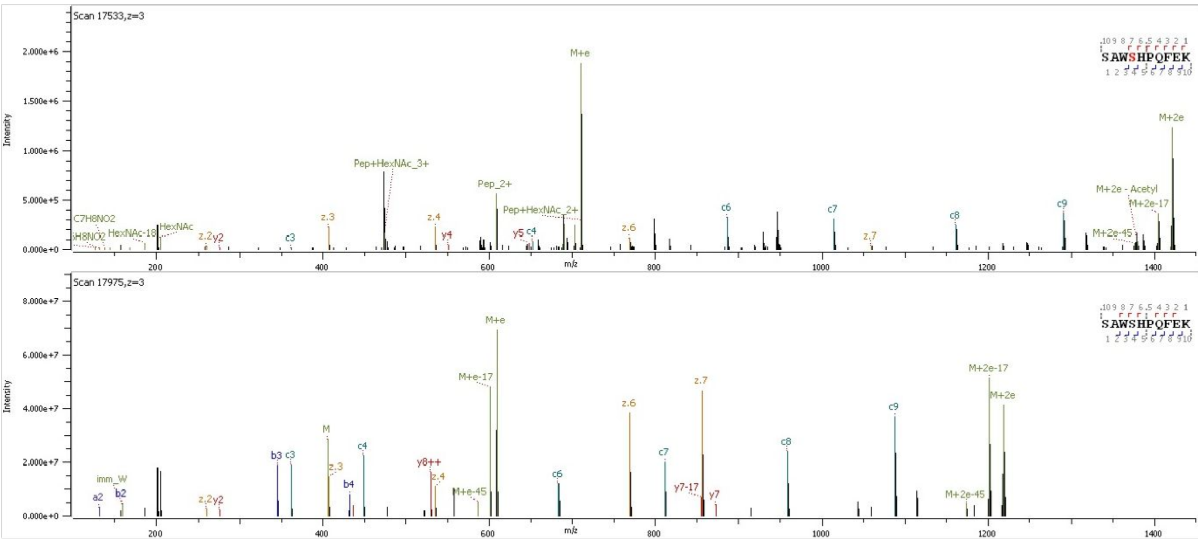


2P - S1261

S1261
Sequence = SAWSHPQFEK; Mods = OGlycan/203.0794 z=3
Glycan = HexNAc(1)
Score = 196.52
XIC Area = 1.98e+9; XIC % Mod = 8.874
Expected m/z = 473.8895; Obs m/z = 473.8899
Error (ppm) = 0.86
Retention Time (min) = 68.88
InSilico status:

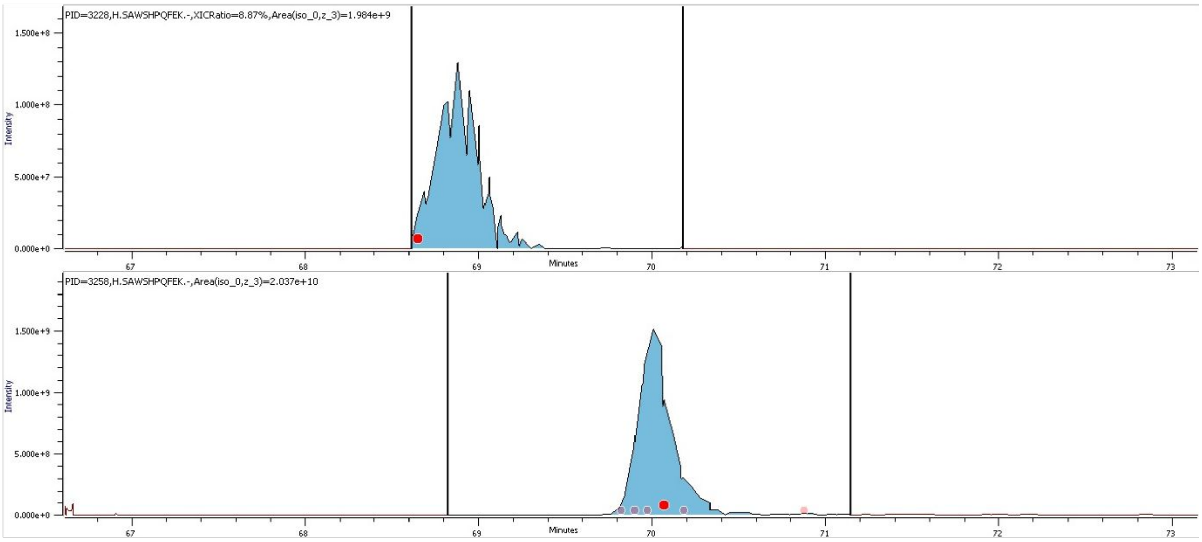


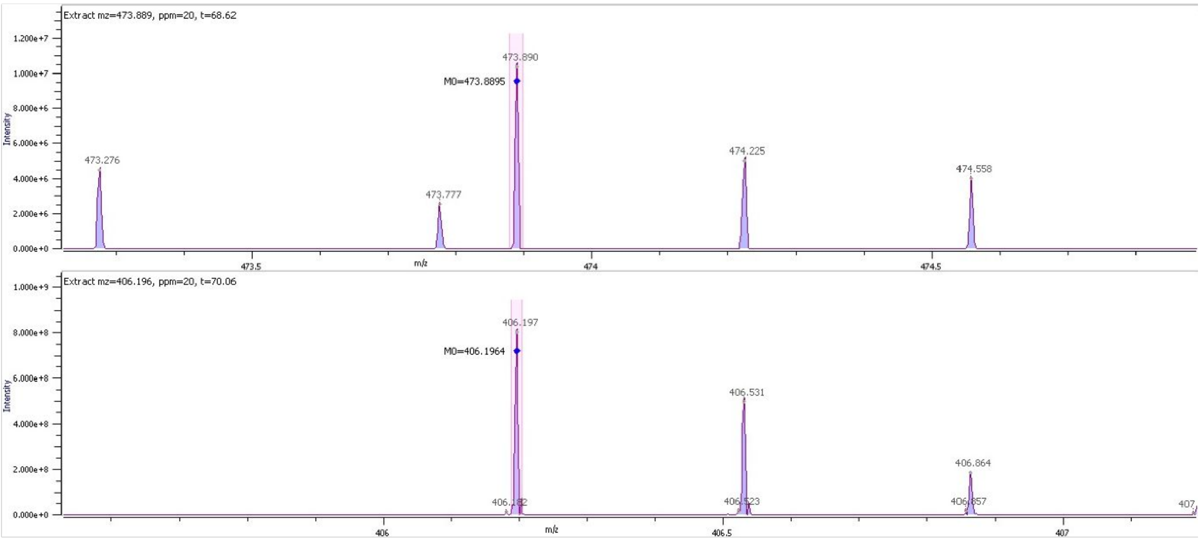


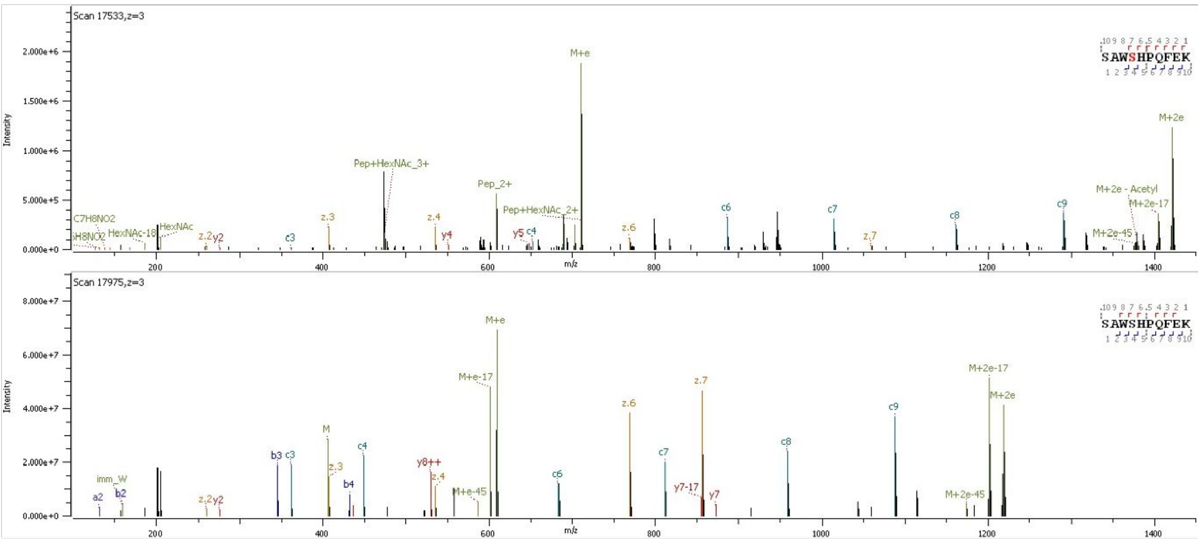


2P - S1261

S1261
Sequence = SAWSHPQFEK; Mods = OGlycan/203.0794 z=3
Glycan = HexNAc(1)
Score = 196.52
XIC Area = 1.98e+9; XIC % Mod = 8.874
Expected m/z = 473.8895; Obs m/z = 473.8899
Error (ppm) = 0.86
Retention Time (min) = 68.88
InSilico status:







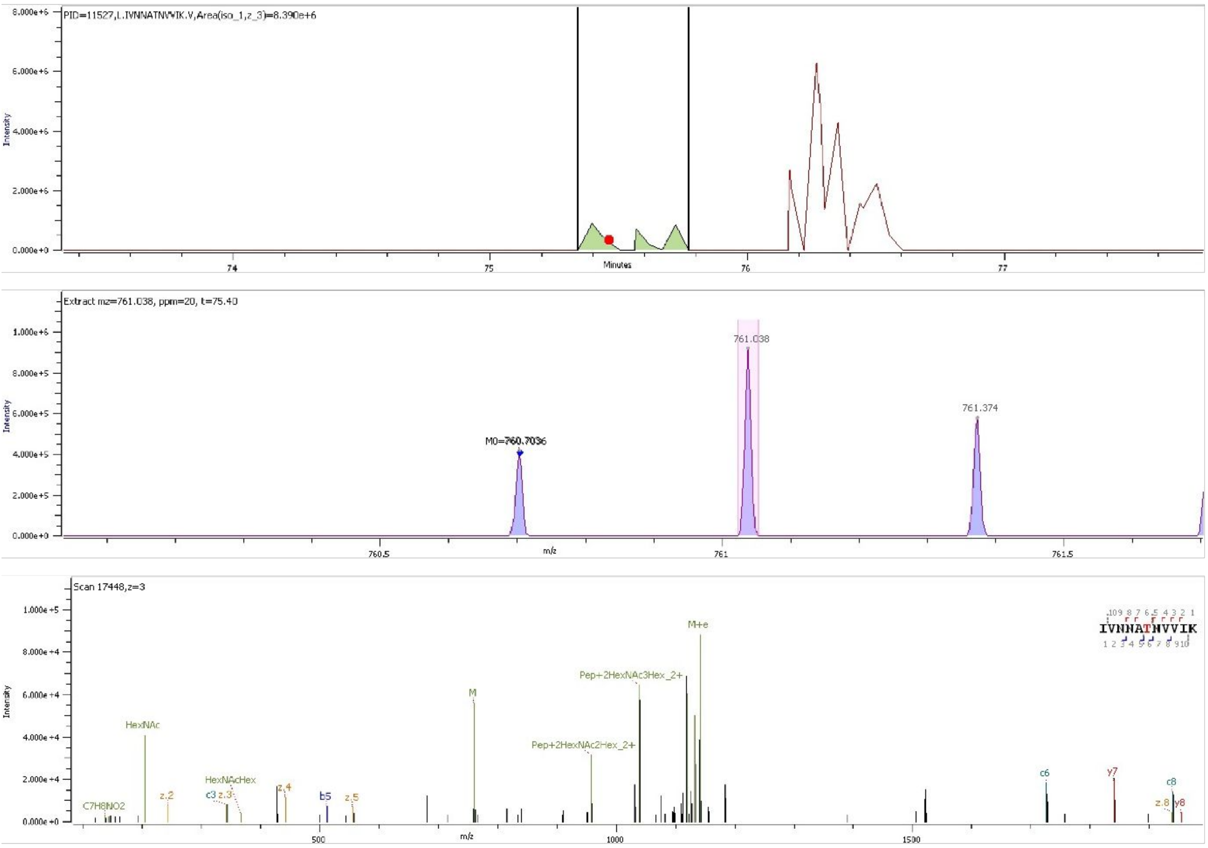
2P - S1261

S1261
Sequence = HHHHSAWSHPQFEK; Mods = OGlycan/947.3230 z=4
Glycan = HexNAc(1)Hex(1)NeuAc(2)
Score = 480.37
XIC Area = 4.27e+8; XIC % Mod = 34.400
Expected m/z = 678.7888; Obs m/z = 678.7898
Error (ppm) = 1.46
Retention Time (min) = 56.50
InSilico status:

Figure S 7: XIC, precursor spectra, and fragmentation spectra of ambiguous O-linked glycans form both HexaPro and 2P S protein constructs

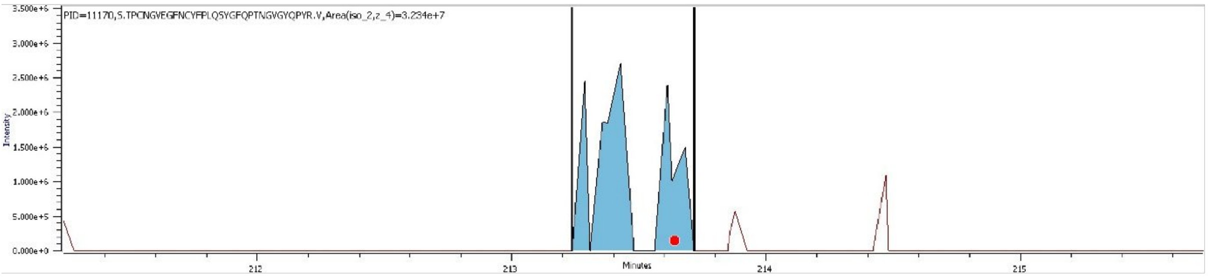
HexaPro - T124

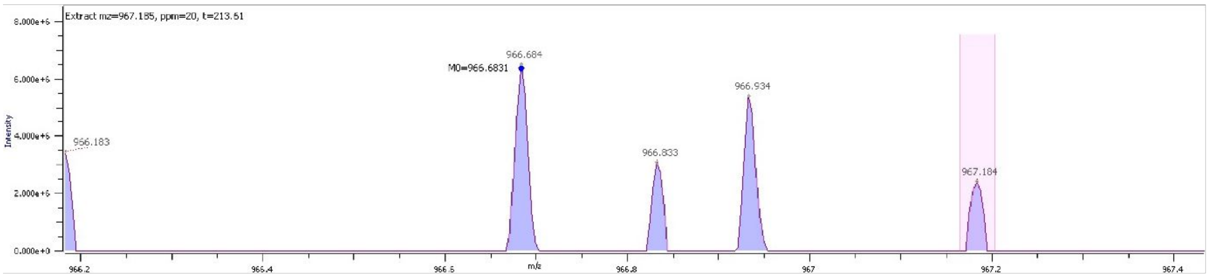
T124
Sequence = IVNNATNVVIK; Mods = OGlycan/1095.3966 z=3
Glycan = HexNAc(3)Hex(3)
Score = 144.15
XIC Area = 8.39e+6; XIC % Mod =
Expected m/z = 760.7036; Obs m/z = 760.7038
Error (ppm) = 0.26
Retention Time (min) = 75.40
InSilico status:

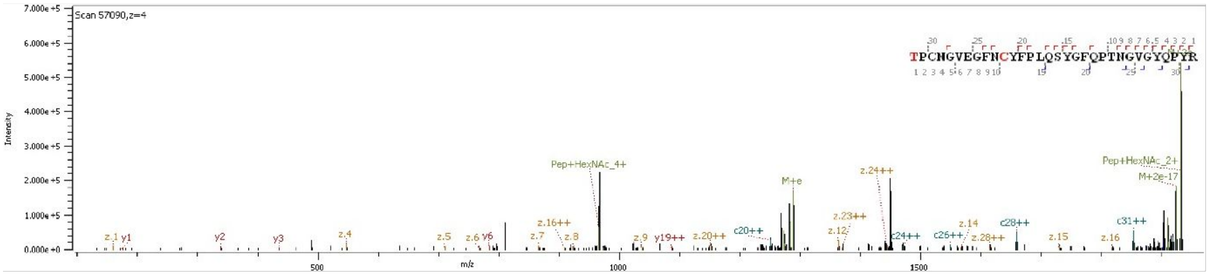


HexaPro - T478

T,C478,488
Sequence = TPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYR; Mods = OGlycan/203.0794; Carbamidomethyl/57.0215 z=4
Glycan = HexNAc(1)
Score = 159.60
XIC Area = 3.23e+7; XIC % Mod =
Expected m/z = 966.6831; Obs m/z = 966.6841
Error (ppm) = 1.06
Retention Time (min) = 213.43
InSilico status:

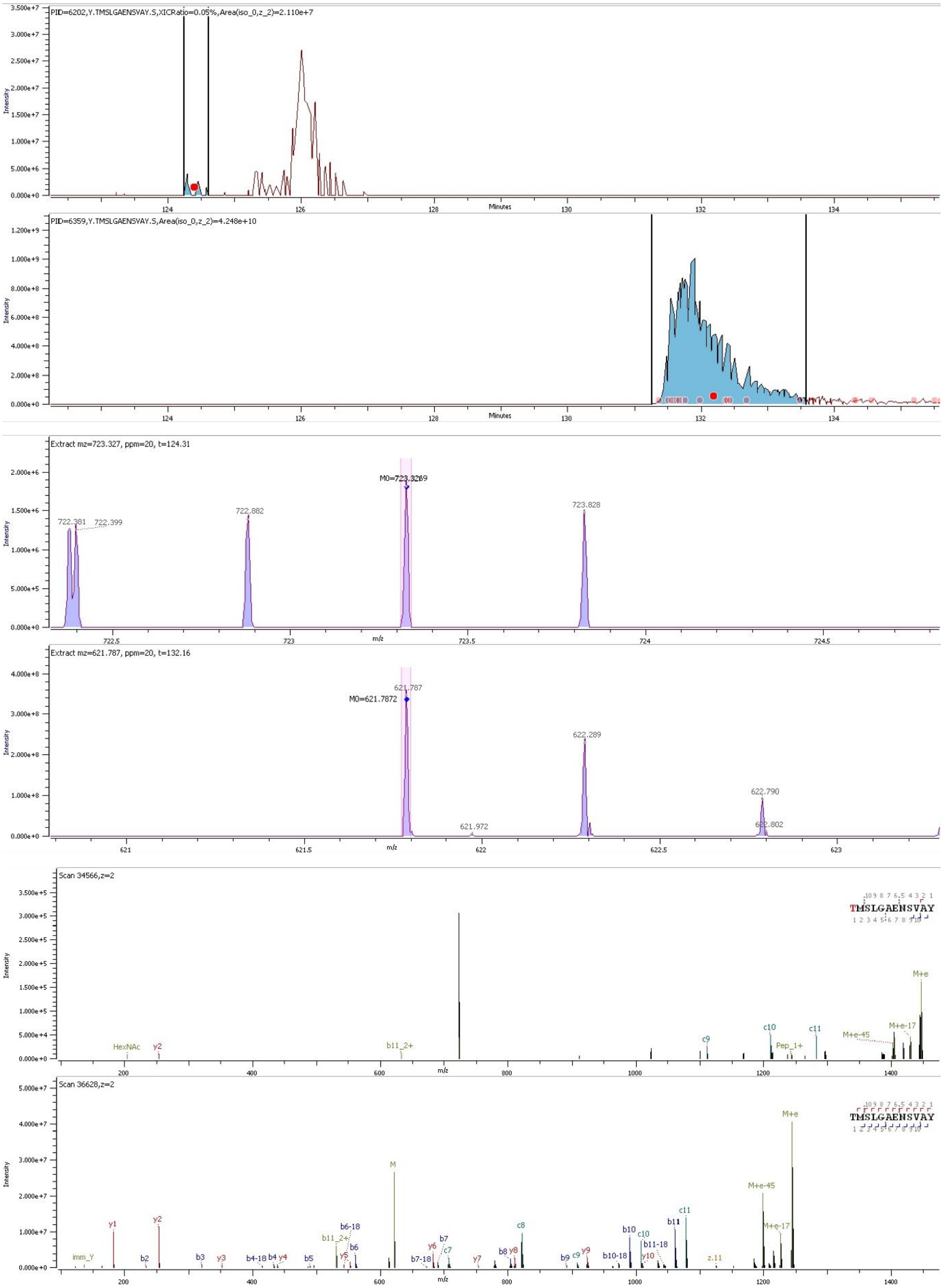






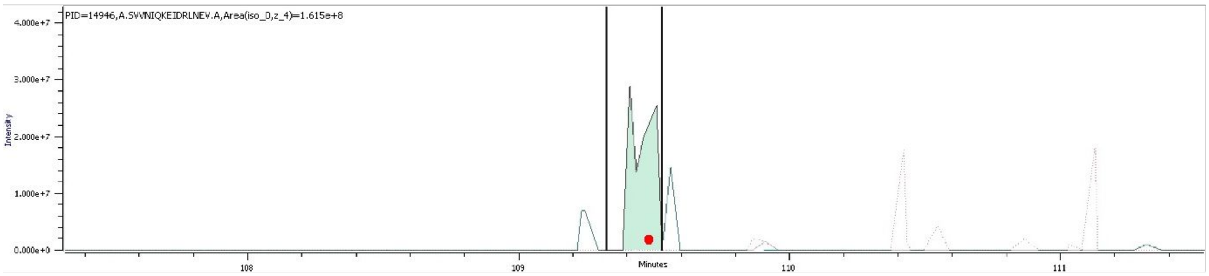
2P - T696/S698/S704

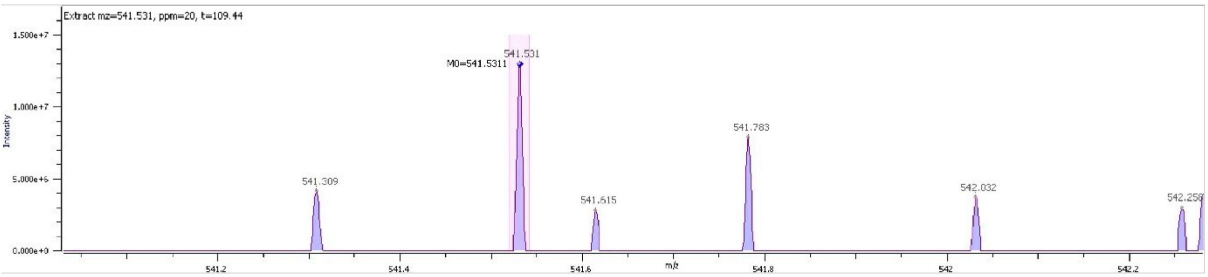
T696
Sequence = TMSLGAENSVAY; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 127.67
XIC Area = 2.11e+7; XIC % Mod = 0.050
Expected m/z = 723.3269; Obs m/z = 723.3271
Error (ppm) = 0.33
Retention Time (min) = 124.29
InSilico status:

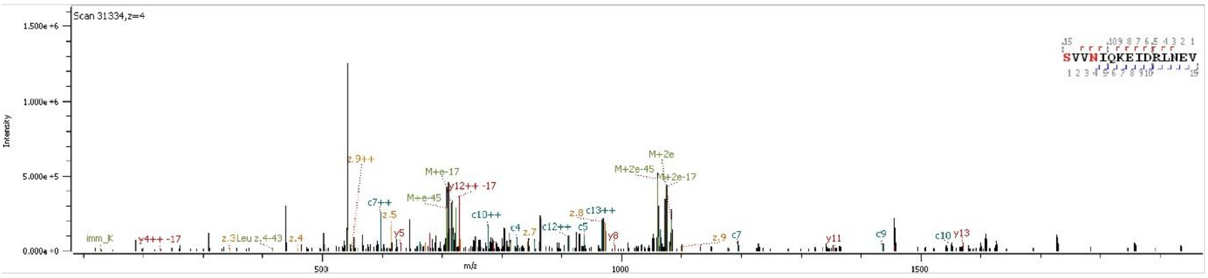


HexaPro - S1175

S,N1175,1178
Sequence = SVVNIQKEIDRLNEV; Mods = OGlycan/406.1587; Deamidated/0.9840 z=4
Glycan = HexNAc(2)
Score = 288.22
XIC Area = 1.62e+8; XIC % Mod =
Expected m/z = 541.5311; Obs m/z = 541.5321
Error (ppm) = 1.84
Retention Time (min) = 109.41
InSilico status:





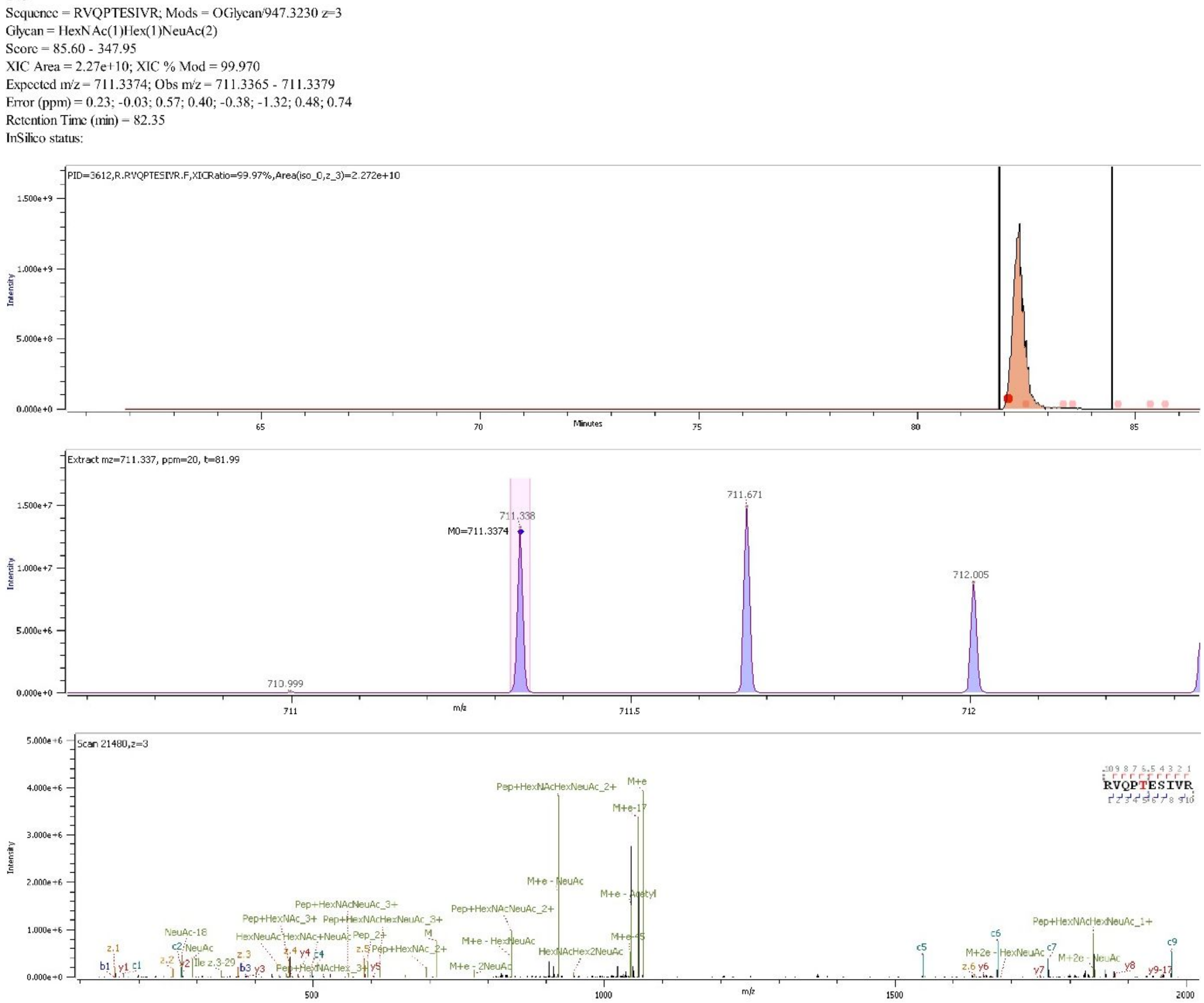


Site	Construct	Run	Sequence	Glycans	Score	XIC	occupancy
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(1)	186.76 - 461.02	6.22E+08	0.50%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(1)Hex(1)	141.58 - 540.85	1.53E+09	1.23%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(1)	178.17 - 520.06	5.07E+10	40.92%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(2)		8.83E+09	7.13%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(1)Fuc(1)	231.50 - 256.09	2.63E+07	0.02%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(1)NeuAc(1)	345.4	5.57E+08	0.45%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)	181.05 - 491.12	2.46E+10	19.85%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)Fuc(1)	102.95 - 457.45	7.36E+09	5.94%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)Fuc(1)NeuAc(1)	309.57 - 309.71	6.34E+08	0.51%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)Fuc(2)	312.32 - 452.71	1.83E+09	1.48%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)Fuc(2)NeuAc(1)	231.33	2.62E+08	0.21%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)NeuAc(1)	346.53	7.69E+08	0.62%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(3)	172.45 - 260.85	2.02E+07	0.02%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(3)Hex(1)	46.89 - 530.14	9.85E+09	7.95%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(3)Hex(1)Fuc(1)	96.80 - 417.07	8.37E+09	6.75%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(3)Hex(1)Fuc(2)	273.95 - 277.01	6.58E+07	0.05%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(3)Hex(2)Fuc(1)	239.49	1.22E+07	0.01%
T323	RBD WT	1	R.RVQPtESIVR.F	HexNAc(4)Hex(1)Fuc(1)	150.93	3.37E+06	0.00%
T323	RBD WT	1	R.RVQPtESIVR.F	Unoccupied	97.95 - 572.78	7.87E+09	6.35%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(1)Hex(1)	42.13 - 249.41	2.26E+07	0.06%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(1)	91.96 - 443.49	2.81E+10	76.53%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(2)	40.96 - 538.64	5.46E+09	14.87%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(2)Hex(2)Fuc(1)NeuAc(1)	328.00 - 332.19	3.58E+08	0.97%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(2)Hex(2)Fuc(2)NeuAc(1)	229.99	1.10E+08	0.30%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(2)Hex(2)NeuAc(1)	190.64 - 470.79	1.56E+09	4.25%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(3)Hex(1)	175.66	2.44E+07	0.07%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(3)Hex(1)Fuc(1)NeuAc(1)	109.9	3.04E+06	0.01%
T323	RBD WT	2	R.RVQPtESIVR.F	HexNAc(3)Hex(1)NeuAc(1)	105.02	1.58E+06	0.00%
T323	RBD WT	2	R.RVQPtESIVR.F	unoccupied	26.52	1.08E+09	2.94%
T323	RBD WT	3	R.RVQPtESIVR.F	HexNAc(1)Hex(1)	183.7	2.70E+08	1.58%
T323	RBD WT	3	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(1)	204.95 - 292.23	1.55E+10	90.80%
T323	RBD WT	3	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(2)	84.91 - 248.78	1.18E+09	6.91%
T323	RBD WT	3	R.RVQPtESIVR.F	HexNAc(2)Hex(1)NeuAc(1)	80.66 - 238.06	5.68E+07	0.33%
T323	RBD WT	3	R.RVQPtESIVR.F	HexNAc(3)Hex(1)NeuAc(1)	155.48	4.25E+07	0.25%
T323	RBD WT	3	R.RVQPtESIVR.F	unoccupied	132.39	2.07E+07	0.12%
T323	RBD Kif	1	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(1)	274.14 - 288.49	5.67E+07	0.25%
T323	RBD Kif	1	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(2)	85.60 - 347.95	2.28E+10	99.35%
T323	RBD Kif	1	R.RVQPtESIVR.F	HexNAc(2)Hex(2)NeuAc(2)	18.43 - 301.96	8.63E+07	0.38%
T323	RBD Kif	1	R.RVQPtESIVR.F	unoccupied	142.87	6.90E+06	0.03%
T323	RBD Kif	2	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(2)	3.04 - 306.48	2.26E+10	99.27%
T323	RBD Kif	2	R.RVQPtESIVR.F	HexNAc(2)Hex(2)NeuAc(2)	26.87 - 336.19	8.59E+07	0.38%
T323	RBD Kif	2	R.RVQPtESIVR.F	unoccupied	25.04 - 223.91	7.98E+07	0.35%
T323	RBD Kif	3	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(1)	24.47 - 133.08	2.14E+07	0.28%
T323	RBD Kif	3	R.RVQPtESIVR.F	HexNAc(1)Hex(1)NeuAc(2)	31.18 - 333.96	7.68E+09	99.69%
T323	RBD Kif	3	R.RVQPtESIVR.F	unoccupied	179.14	2.49E+06	0.03%

Table S3: Unambiguously assigned O-links on peptide ^{319}RVQPTESIVR^{328} from WT and KT RBD

Figure S8 MS2 spectra of the most abundant glycans from ^{319}RVQPTESIVR^{328} from WT and KT RBD

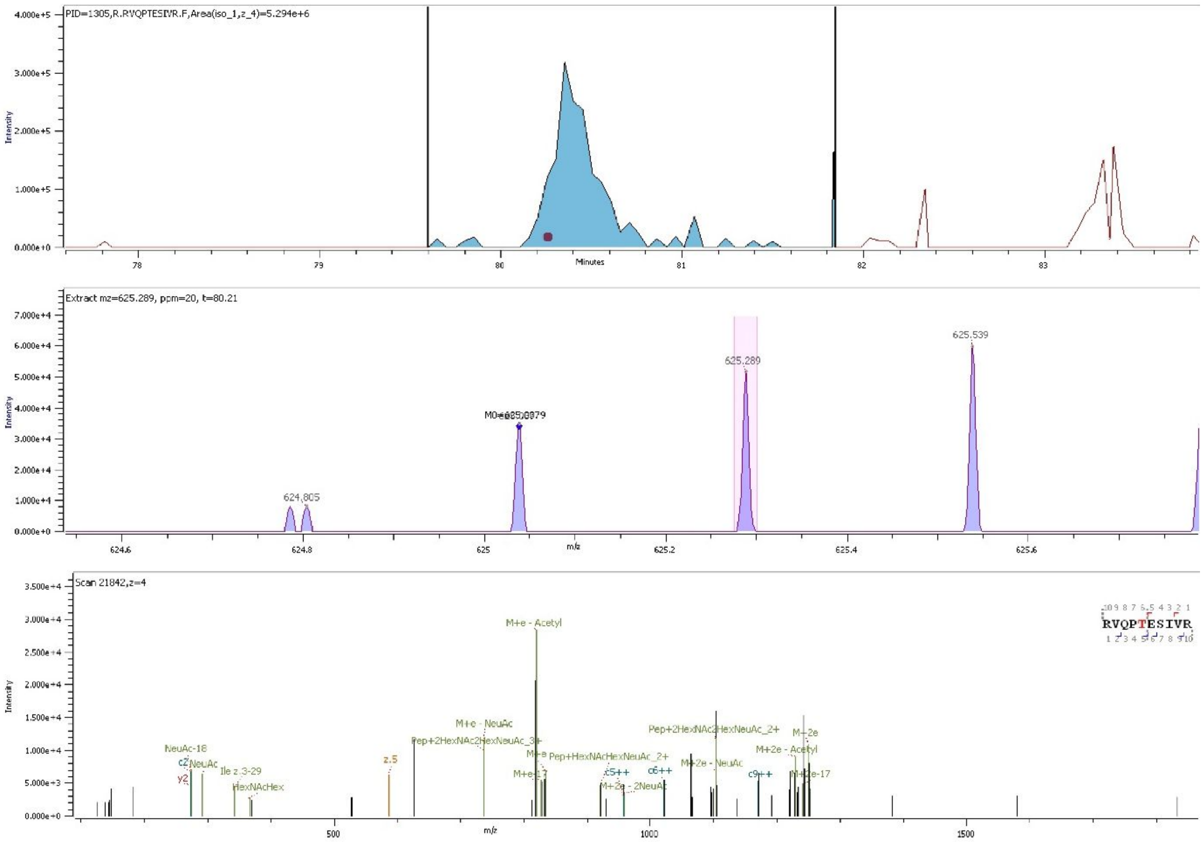
RBD KT - T323



T40
Sequence = RVQPTESIVR; Mods = OGlycan/947.3230 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(2)
Score = 85.60 - 347.95
XIC Area = 2.27e+10; XIC % Mod = 99.970
Expected m/z = 711.3374; Obs m/z = 711.3365 - 711.3379
Error (ppm) = 0.23; -0.03; 0.57; 0.40; -0.38; -1.32; 0.48; 0.74
Retention Time (min) = 82.35
InSilico status:

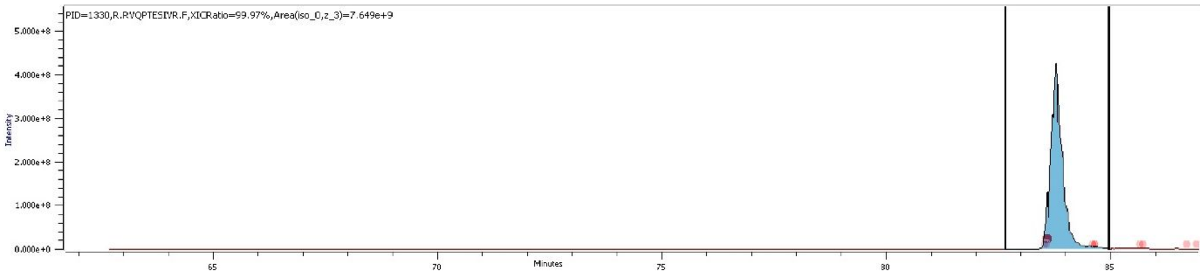
RBD KT - T323

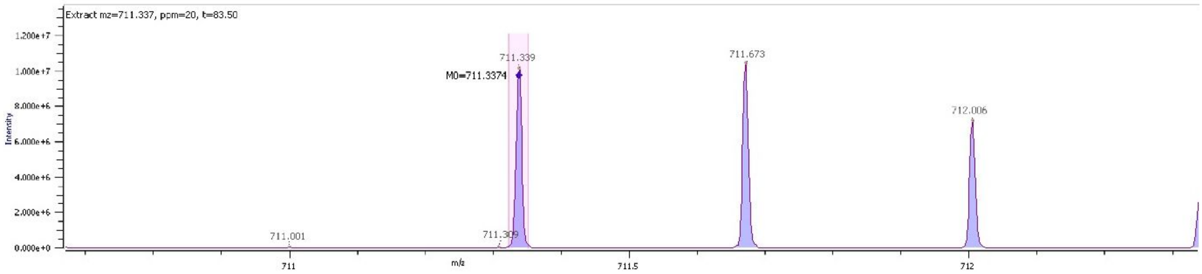
T40
Sequence = RVQPTESIVR; Mods = OGlycan/1312.4552 z=4
Glycan = HexNAc(2)Hex(2)NeuAc(2)
Score = 26.87
XIC Area = 5.29e+6; XIC % Mod =
Expected m/z = 625.0379; Obs m/z = 625.0381
Error (ppm) = 0.31
Retention Time (min) = 80.35
InSilico status:

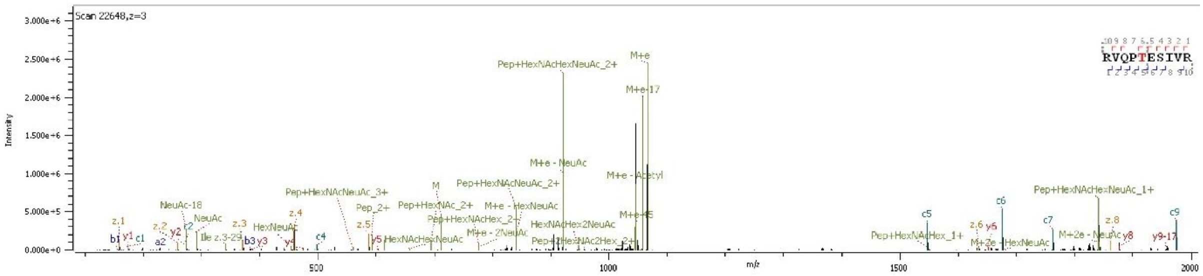


RBD KT - T323

T40
Sequence = RVQPTESIVR; Mods = OGlycan/947.3230 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(2)
Score = 31.18 - 333.96
XIC Area = 7.65e+9; XIC % Mod = 99.968
Expected m/z = 711.3374; Obs m/z = 711.3374 - 711.3382
Error (ppm) = 0.23; 0.65; 0.48; 0.14; 0.57; 1.08; -0.03
Retention Time (min) = 83.79
InSilico status:

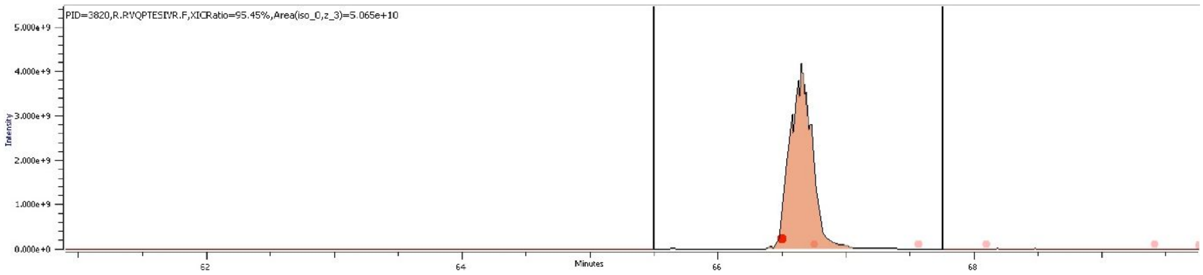


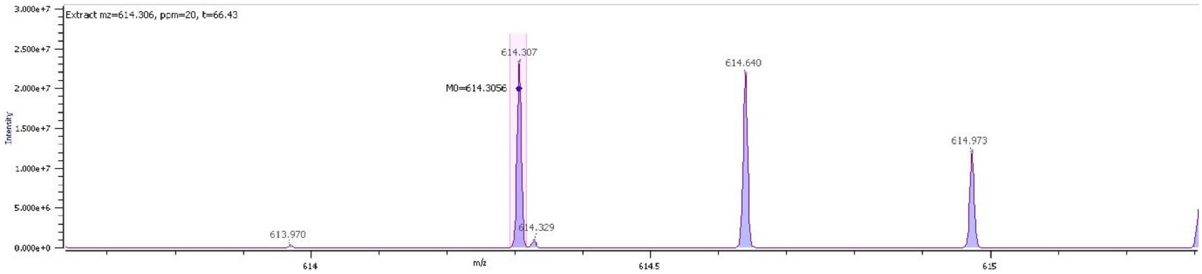


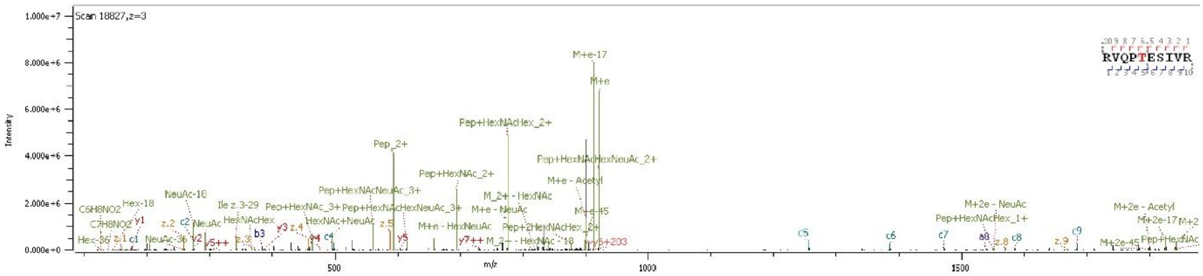


RBD WT - T323

T40
Sequence = RVQPTESIVR; Mods = OGlycan/656.2276 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(1)
Score = 178.17 - 520.06
XIC Area = 5.07e+10; XIC % Mod = 95.449
Expected m/z = 614.3056; Obs m/z = 614.3053 - 614.3079
Error (ppm) = 3.81; 1.82; 0.62; 0.12; 0.52; 0.03; 0.22; -0.47
Retention Time (min) = 66.65
InSilico status:

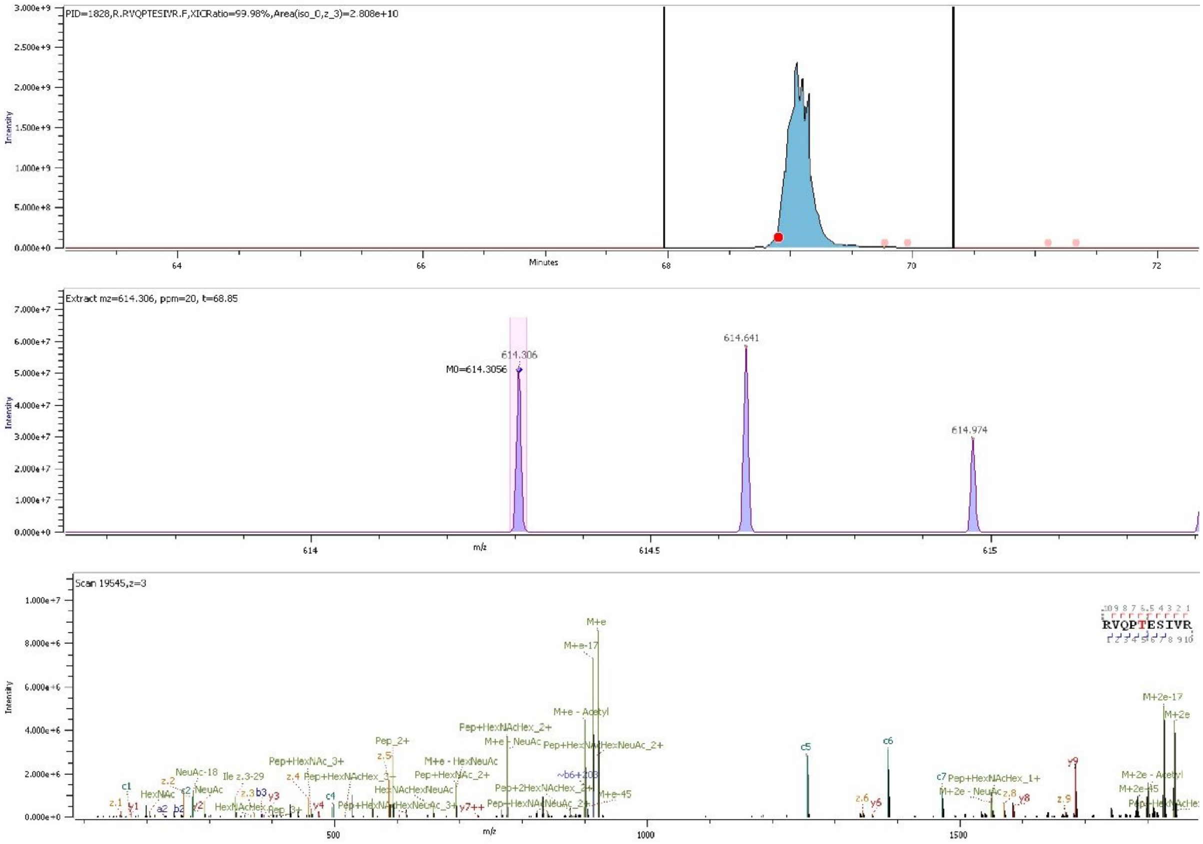






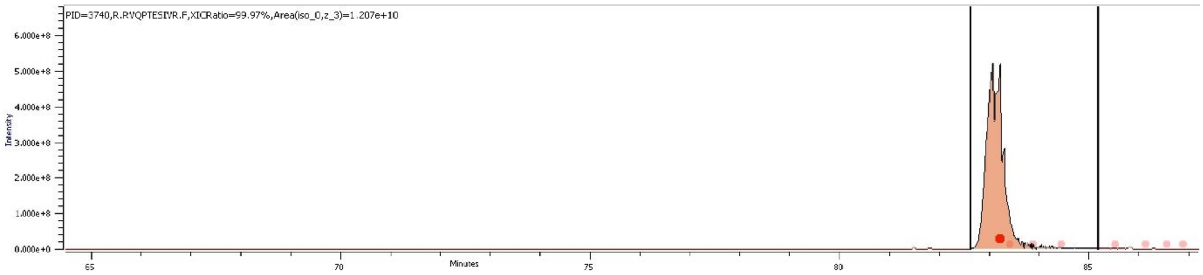
RBD WT - T323

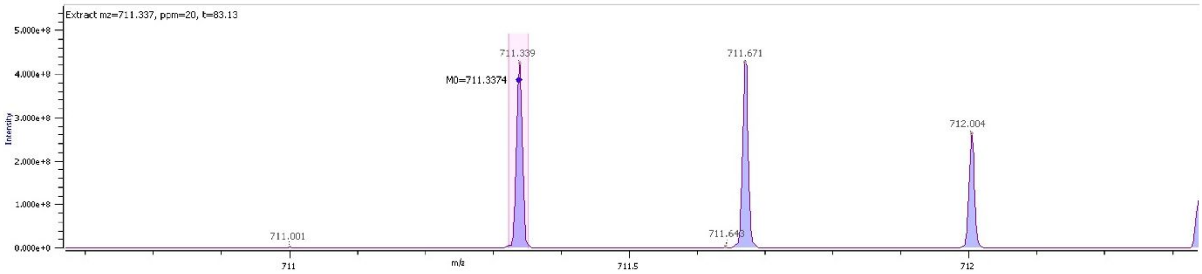
T40
Sequence = RVQPTESIVR; Mods = OGlycan/656.2276 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(1)
Score = 91.96 - 443.49
XIC Area = 2.81e+10; XIC % Mod = 99.984
Expected m/z = 614.3056; Obs m/z = 614.3050 - 614.3069
Error (ppm) = -0.57; 2.21; 0.42; 0.72; -0.37; -0.27; -0.07; -0.97
Retention Time (min) = 69.06
InSilico status:

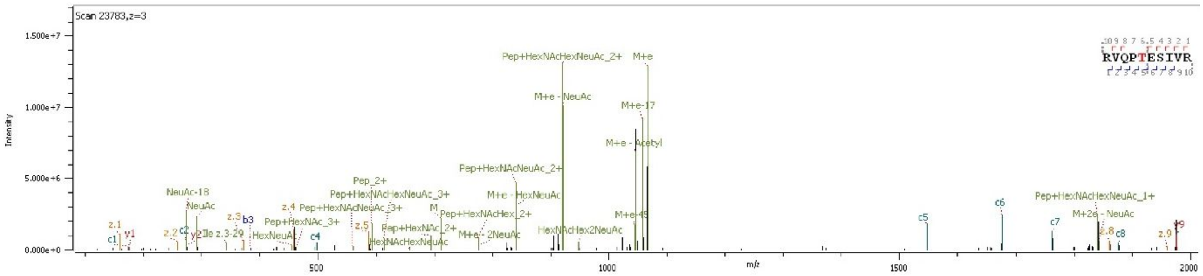


RBD WT - T323

T40
Sequence = RVQPTESIVR; Mods = OGlycan/947.3230 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(2)
Score = 13.94 - 274.44
XIC Area = 1.21e+10; XIC % Mod = 99.971
Expected m/z = 711.3374; Obs m/z = 711.3376 - 711.3379
Error (ppm) = 0.74; 0.31; 0.48; 0.23; 0.40
Retention Time (min) = 83.07
InSilico status:







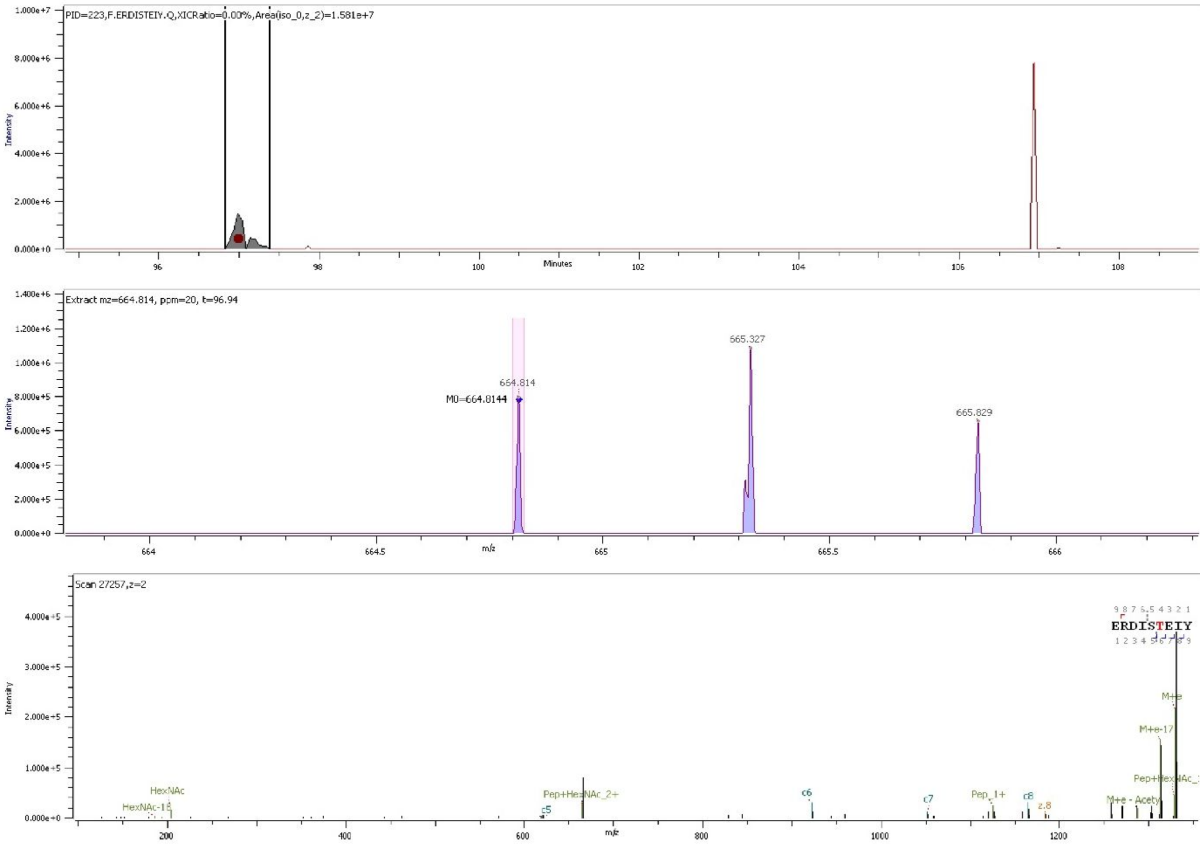
Site	Construct	Run	Sequence	Glycans	Score	XIC	occupancy
S469/T470	RBD WT	1	F.ERDIsTEIY.Q	HexNAc(1)Hex(1)	231.4	7.31E+07	0.0100%
S469/T470	RBD WT	2	F.ERDIsTEIY.Q	HexNAc(1)	148.82	1.64E+07	0.0012%
S469/T470	RBD WT	3	F.ERDIsTEIY.Q	HexNAc(1)Hex(1)	179.88	6.10E+07	0.0100%
T523	RBD WT	2	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)Hex(1)NeuAc(2)	248.28	2.28E+07	0.0040%
T523	RBD WT	2	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)Hex(1)NeuAc(1)	305.09 - 452.70	1.36E+08	0.0238%
T523	RBD WT	3	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)	242.09	3.27E+07	0.0028%
T523	RBD WT	3	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)Hex(1)NeuAc(1)	298.7	2.02E+08	0.0171%
T470	RBD KT	2	F.ERDIStEIY.Q	HexNAc(1)	172.27	1.58E+07	0.0021%
T470	RBD KT	2	F.ERDIsTEIY.Q	HexNAc(1)Hex(1)	182.28	1.80E+07	0.0024%
T470	RBD KT	3	F.ERDIsTEIY.Q	HexNAc(1)Hex(1)	157.85 - 193.39	1.68E+07	0.0020%
T470	RBD KT	3	F.ERDIStEIY.Q	HexNAc(1)	175.95 - 191.04	2.23E+07	0.0027%
T523	RBD KT	1	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)Hex(1)NeuAc(2)	296.11	4.65E+06	0.0039%
T523	RBD KT	2	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(2)Hex(2)Fuc(1)NeuAc(1)	138.84	1.38E+06	0.0010%
T523	RBD KT	2	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)	200.26 - 222.34	6.29E+07	0.0459%
T523	RBD KT	2	R.VVVLSFELLHAPAtVcGPK.K	HexNAc(1)Hex(1)NeuAc(2)	1.47 - 634.75	6.87E+07	0.0501%

Table S4: Unambiguously assigned O-links on peptides ^{465}ERDISTEIY^{473} and ^{510}VVVLSFELLHAPATVCGPK^{528} from WT and KT RBD

Figure S9 MS2 spectra of the most abundant glycans on peptides ^{465}ERDISTEIY^{473} and ^{510}VVVLSFELLHAPATVCGPK^{528} from WT and KT RBD

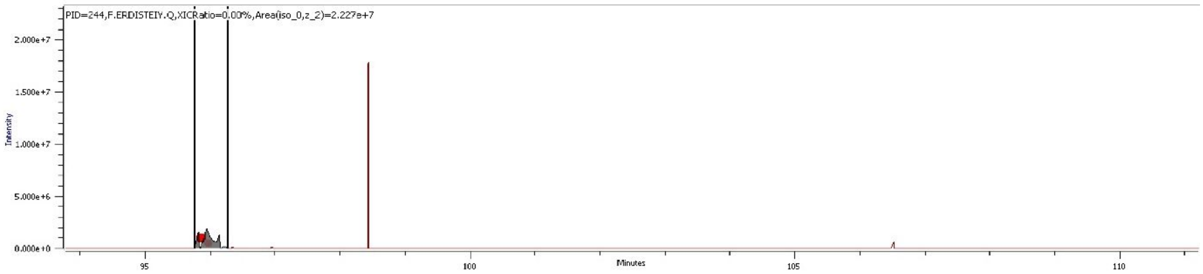
RBD KT - T470

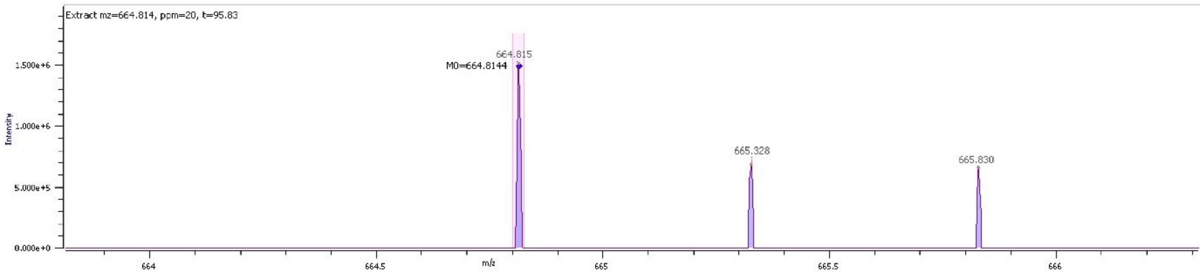
T187
Sequence = ERDISTEIY; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 172.27
XIC Area = 1.58e+7; XIC % Mod = 0.002
Expected m/z = 664.8144; Obs m/z = 664.8148
Error (ppm) = 0.56
Retention Time (min) = 96.99
InSilico status:

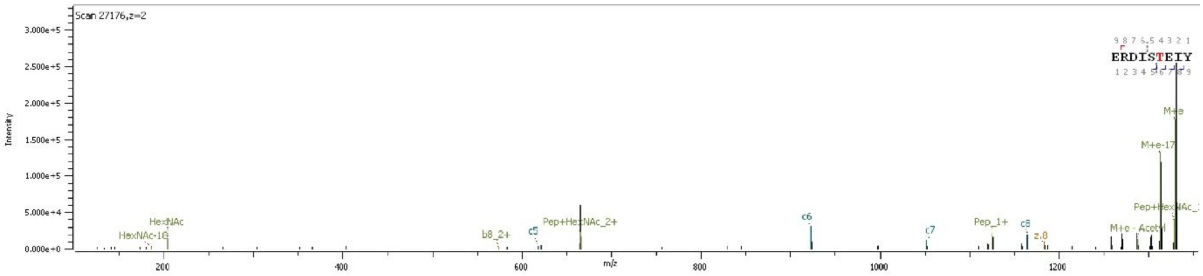


RBD KT - T470

T187
Sequence = ERDISTEIY; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 175.95 - 191.04
XIC Area = 2.23e+7; XIC % Mod = 0.003
Expected m/z = 664.8144; Obs m/z = 664.8145 - 664.8152
Error (ppm) = 0.10; 1.13
Retention Time (min) = 95.94
InSilico status:

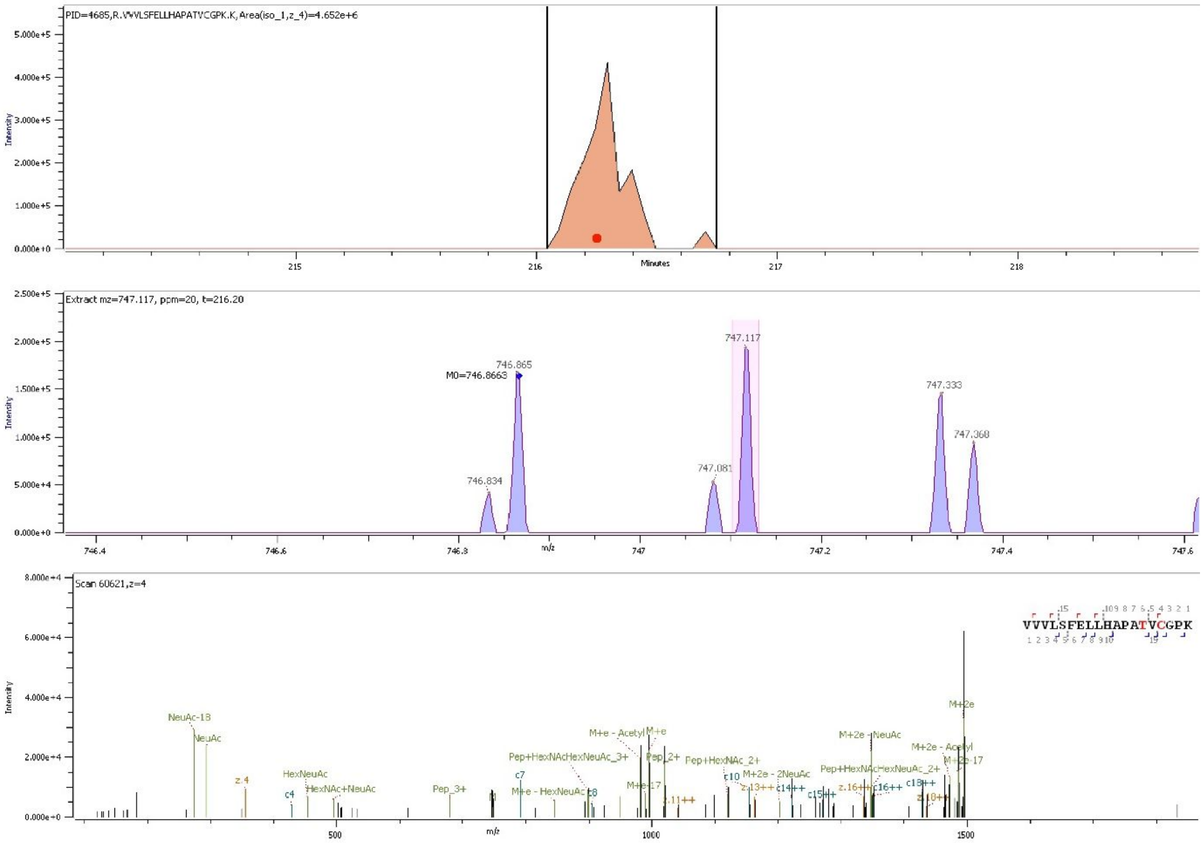






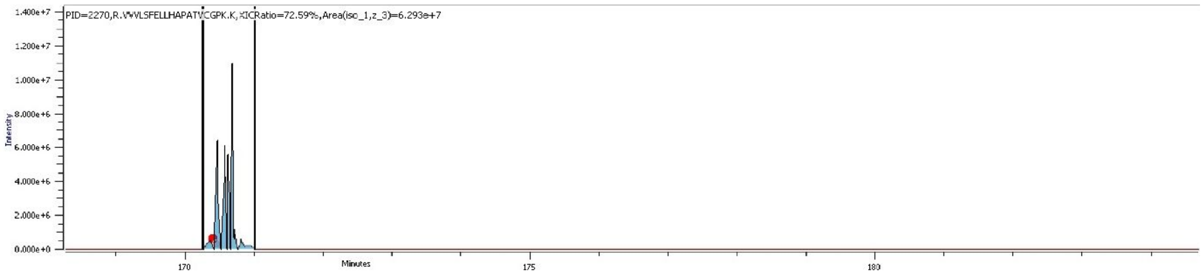
RBD KT - T523

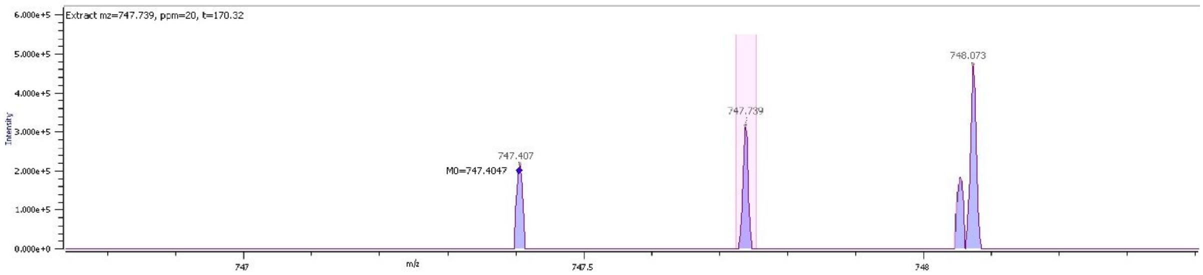
T,C240,242
Sequence = VVVLSFELLHAPATVCGPK; Mods = OGlycan/947.3230; Carbamidomethyl/57.0215 z=4
Glycan = HexNAc(1)Hex(1)NeuAc(2)
Score = 296.11
XIC Area = 4.65e+6; XIC % Mod =
Expected m/z = 746.8663; Obs m/z = 746.8661
Error (ppm) = -0.19
Retention Time (min) = 216.30
InSilico status:

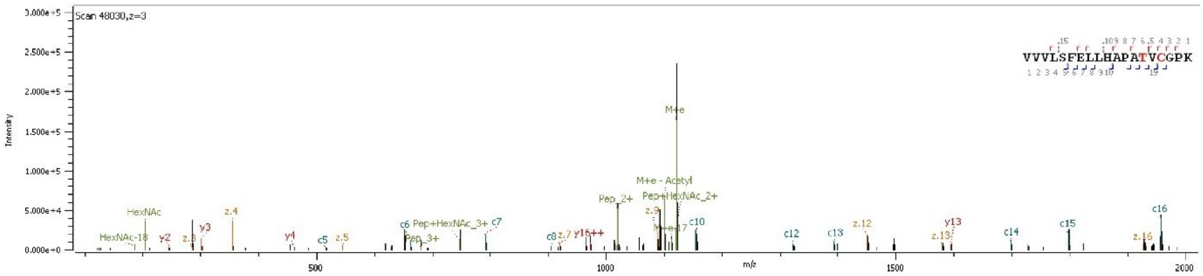


RBD KT - T523

T,C240,242
Sequence = VVVLSFELLHAPATVCGPK; Mods = OGlycan/203.0794; Carbamidomethyl/57.0215 z=3
Glycan = HexNAc(1)
Score = 200.26 - 222.34
XIC Area = 6.29e+7; XIC % Mod = 72.591
Expected m/z = 747.4047; Obs m/z = 747.4049 - 747.4050
Error (ppm) = 0.41; 0.25
Retention Time (min) = 170.70
InSilico status:

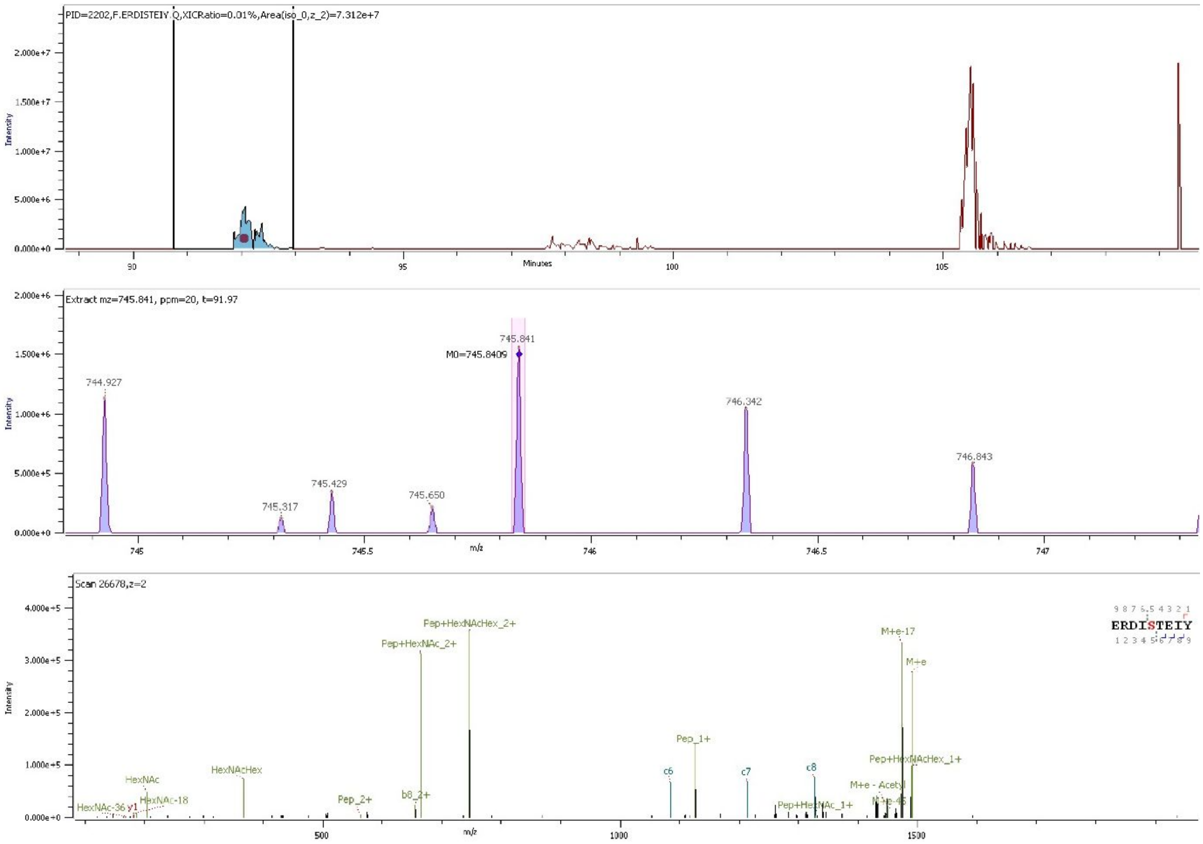






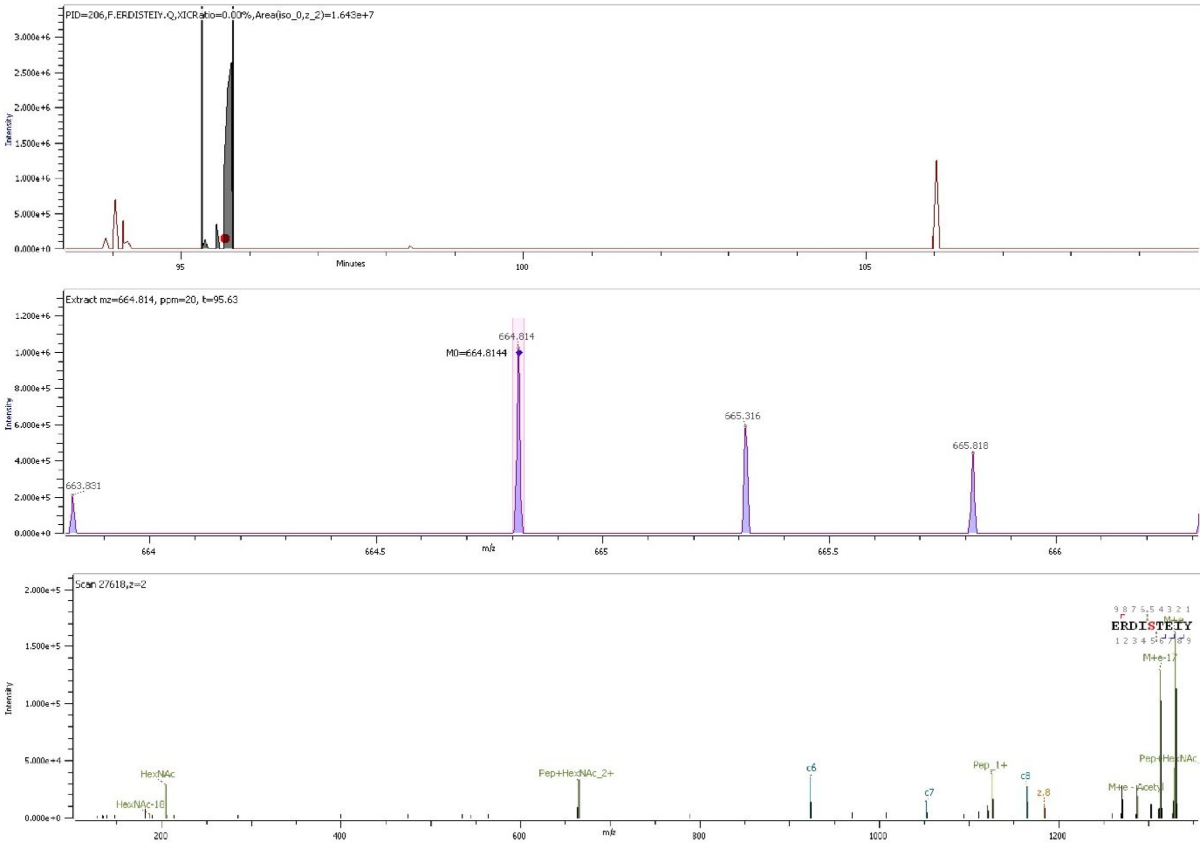
RBD WT - S469/T470

S186
Sequence = ERDISTEIY; Mods = OGlycan/365.1322 z=2
Glycan = HexNAc(1)Hex(1)
Score = 231.40
XIC Area = 7.31e+7; XIC % Mod = 0.006
Expected m/z = 745.8409; Obs m/z = 745.8414
Error (ppm) = 0.69
Retention Time (min) = 92.07
InSilico status:



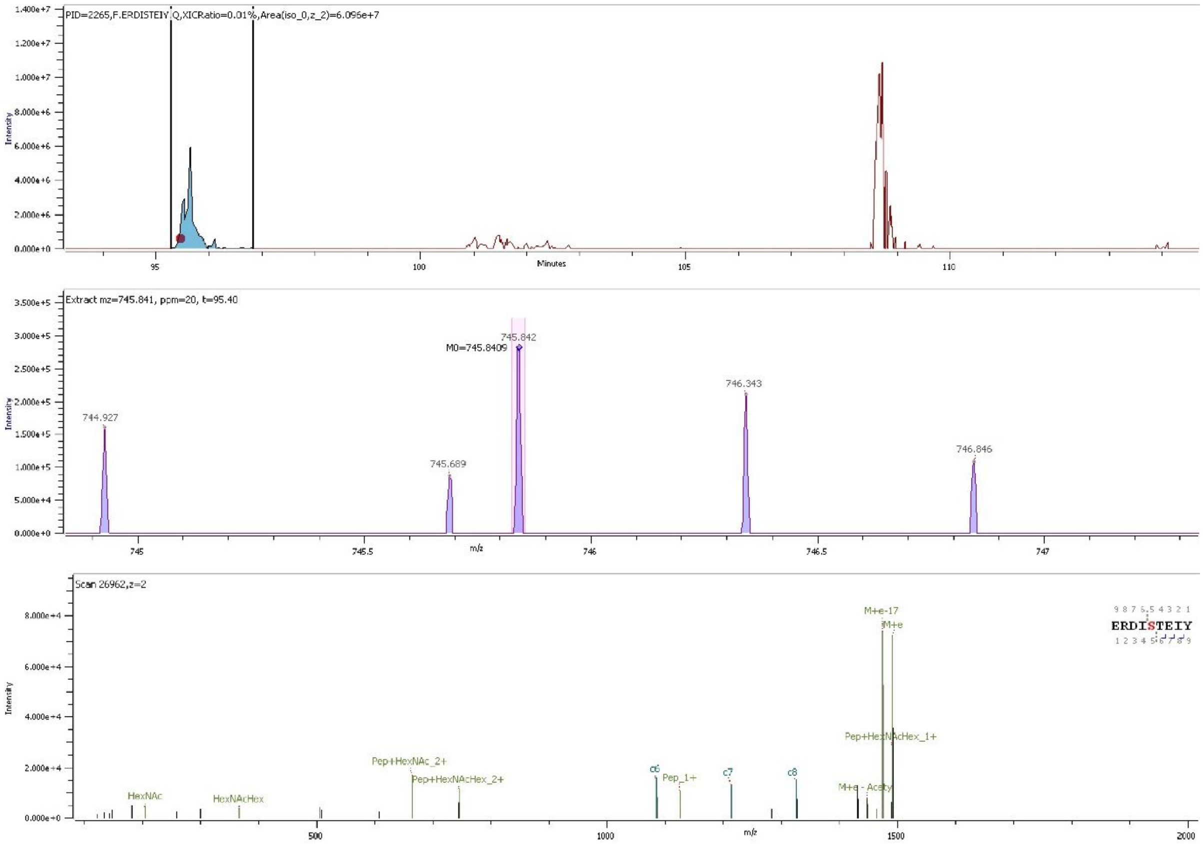
RBD WT - S469/T470

S186
Sequence = ERDISTEIY; Mods = OGlycan/203.0794 z=2
Glycan = HexNAc(1)
Score = 148.82
XIC Area = 1.64e+7; XIC % Mod = 0.001
Expected m/z = 664.8144; Obs m/z = 664.8148
Error (ppm) = 0.56
Retention Time (min) = 95.73
InSilico status:



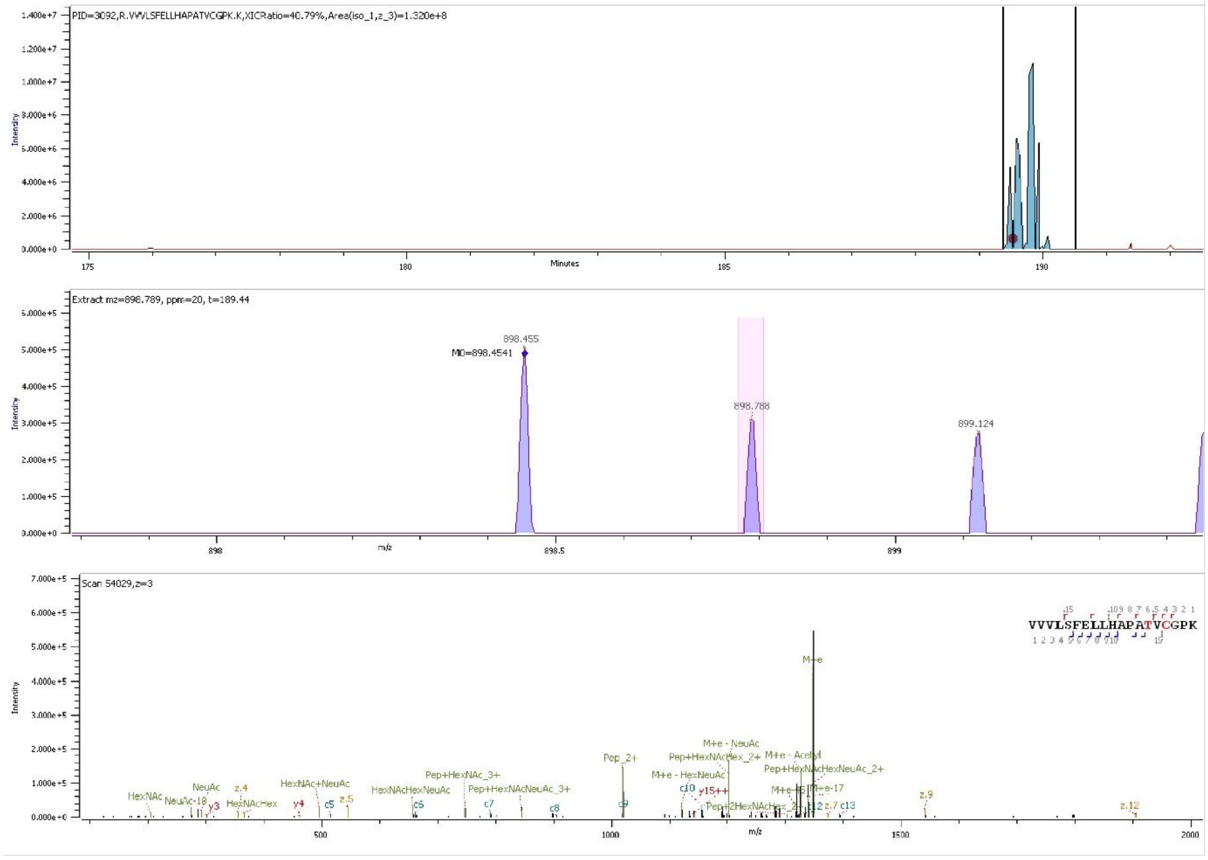
RBD WT - S469/T470

S186
Sequence = ERDISTEIY; Mods = OGlycan/365.1322 z=2
Glycan = HexNAc(1)Hex(1)
Score = 179.88
XIC Area = 6.1e+7; XIC % Mod = 0.007
Expected m/z = 745.8409; Obs m/z = 745.8410
Error (ppm) = 0.19
Retention Time (min) = 95.66
InSilico status:

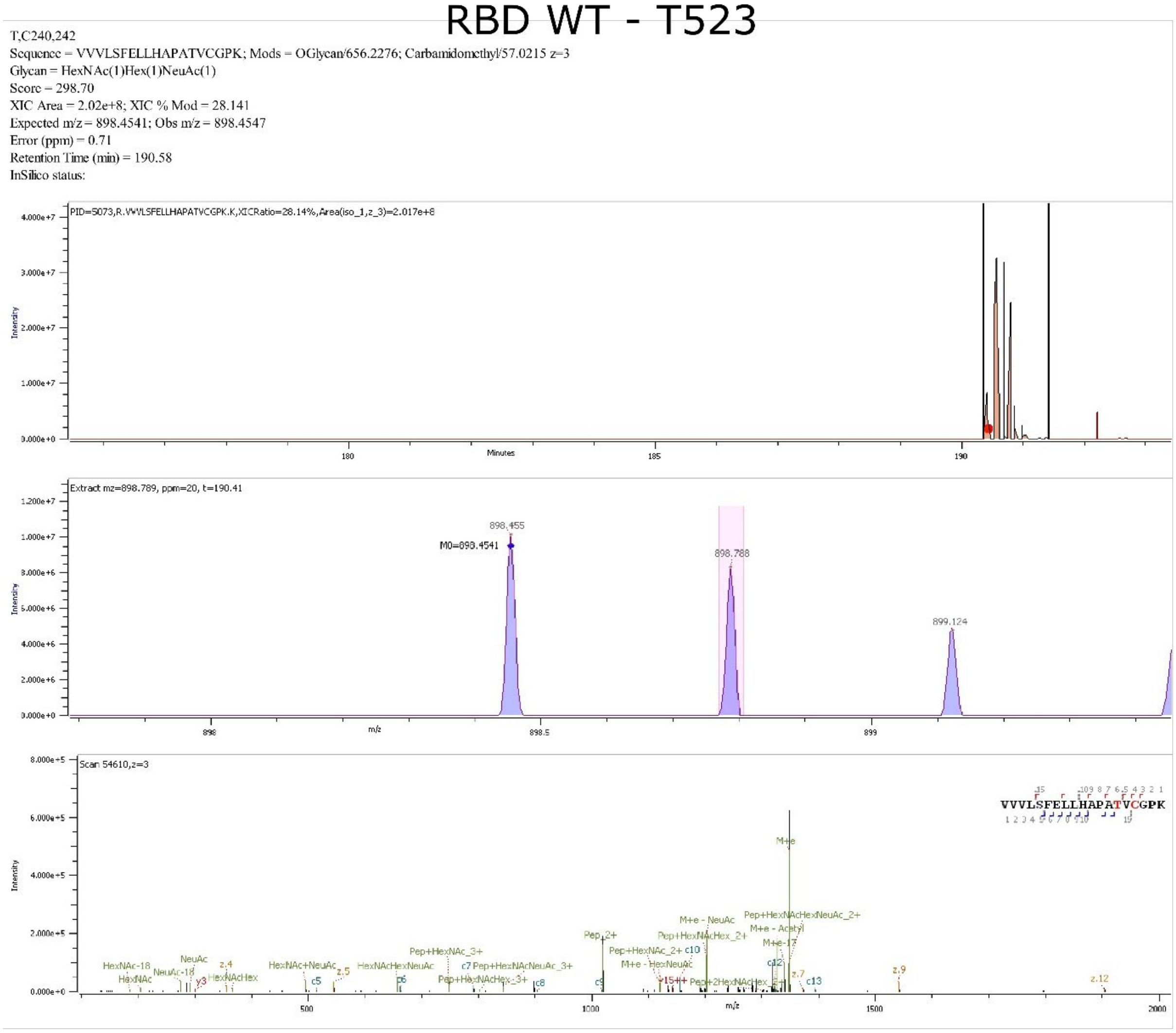


RBD WT - T523

T,C240,242
Sequence = VVVLSFELLHAPATVCGPK; Mods = OGlycan/656.2276; Carbamidomethyl/57.0215 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(1)
Score = 305.09
XIC Area = 1.32e+8; XIC % Mod = 40.791
Expected m/z = 898.4541; Obs m/z = 898.4549
Error (ppm) = 0.85
Retention Time (min) = 189.84
InSilico status:



RBD WT - T523



T,C240,242
Sequence = VVVLSFELLHAPATVCGPK; Mods = OGlycan/656.2276; Carbamidomethyl/57.0215 z=3
Glycan = HexNAc(1)Hex(1)NeuAc(1)
Score = 298.70
XIC Area = 2.02e+8; XIC % Mod = 28.141
Expected m/z = 898.4541; Obs m/z = 898.4547
Error (ppm) = 0.71
Retention Time (min) = 190.58
InSilico status: