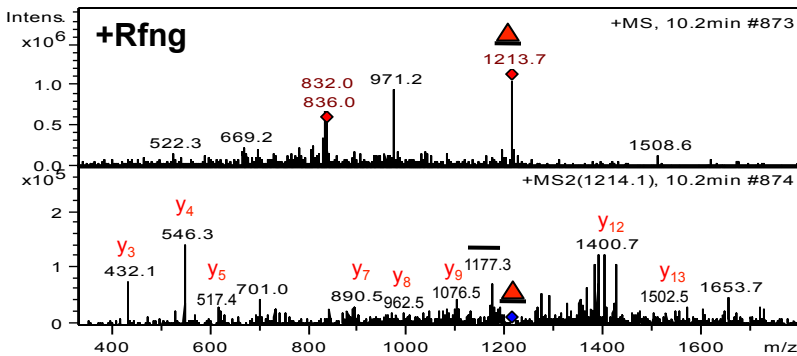
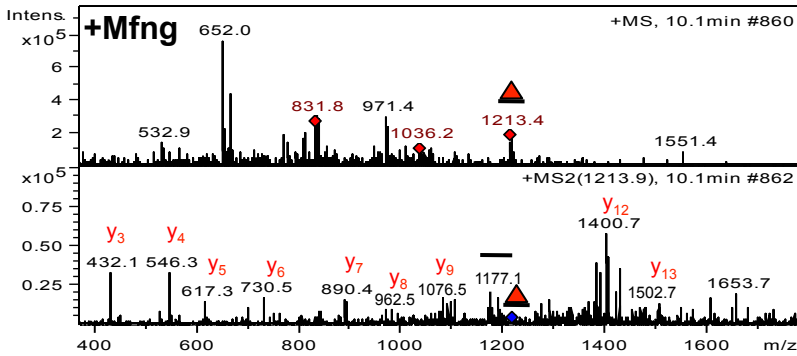
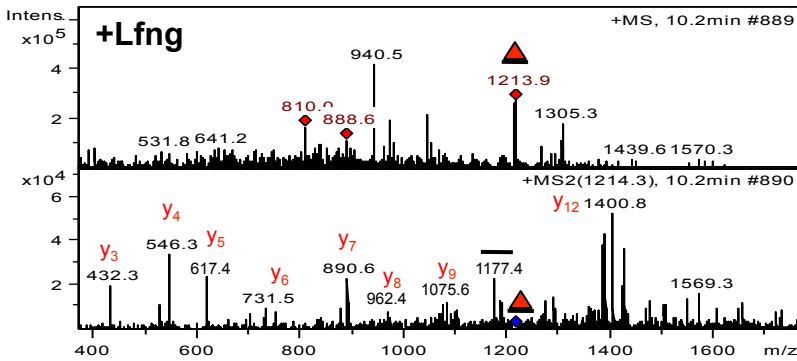
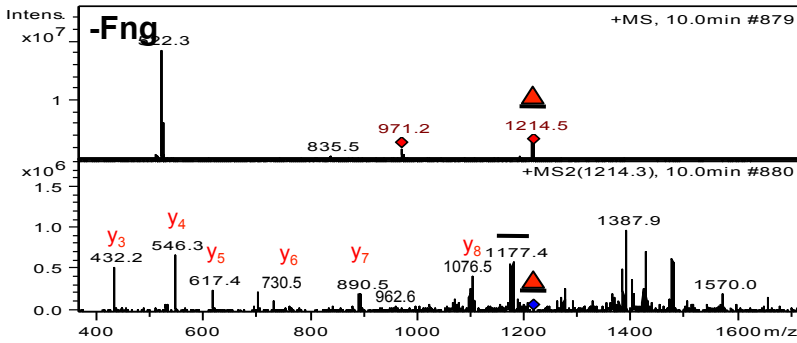


**Data S1: Mass spectra for *O*-fucose glycoforms of peptides from the 20 EGF repeats containing an *O*-fucose consensus sequence in mouse N1 EGF1-36 (Fig. 1C), related to Figure 3.** Samples were generated in HEK293T cells co-transfected with plasmid encoding EGF1-36 and EV (-Fng) or any of the Fringes (+Lfng, +Mfng or +Rfng)) as described in Experimental Procedures. Upper panels show an MS spectrum at a specific time, with the ion corresponding to the  $m/z$  of the peptide (red triangle above black bar) containing an *O*-fucose consensus sequence indicated. In the absence of any Fringe, this peptide is modified with *O*-fucose monosaccharide (red triangle). In the presence of Fringe samples (+Lfng, +Mfng or +Rfng), the indicated ions in the MS scan correspond to the  $m/z$  for *O*-fucose monosaccharide or Fringe elongated glycoforms of the same peptide from the EGF repeat as in the absence of Fringe. Lower panels show the MS/MS spectra confirming the identity of corresponding glycopeptides based on the presence of peptide-specific b- and/or y-ions. Tables (right) show the  $m/z$  used for the EIC searches for glycoforms of the peptide as in Fig. 2 (unmodified, mono-, di-, tri- and tetra-saccharide glycoforms). Key: black bar, unmodified peptide; red triangle, fucose; blue square, GlcNAc; yellow circle, galactose; purple diamond, sialic acid. Summaries of results are shown in Fig. 4.

# EGF 2

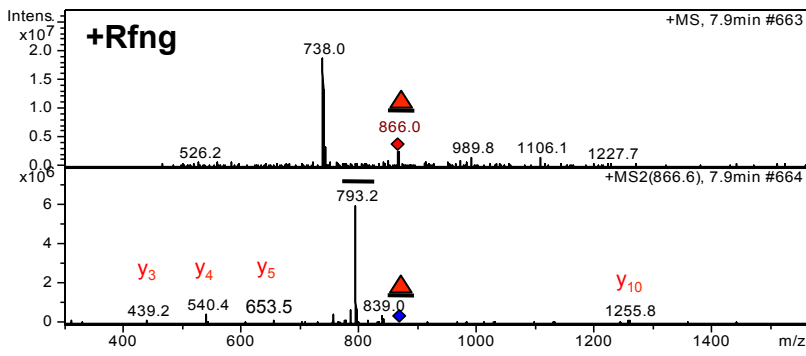
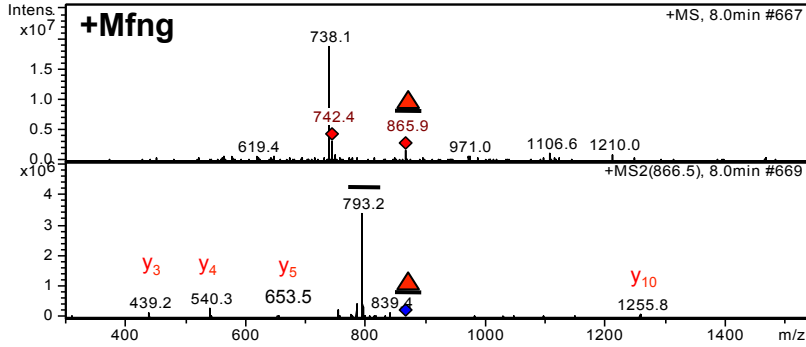
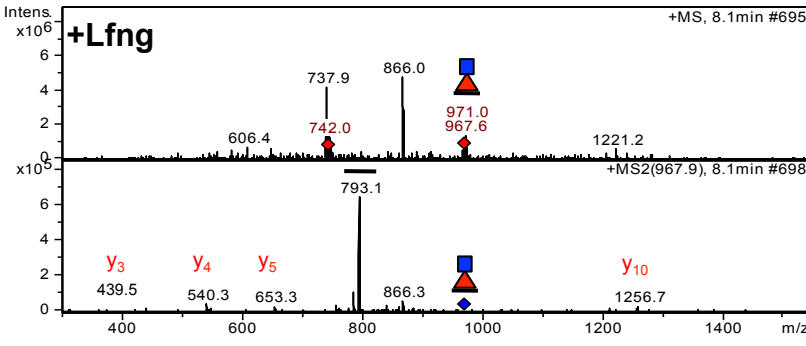
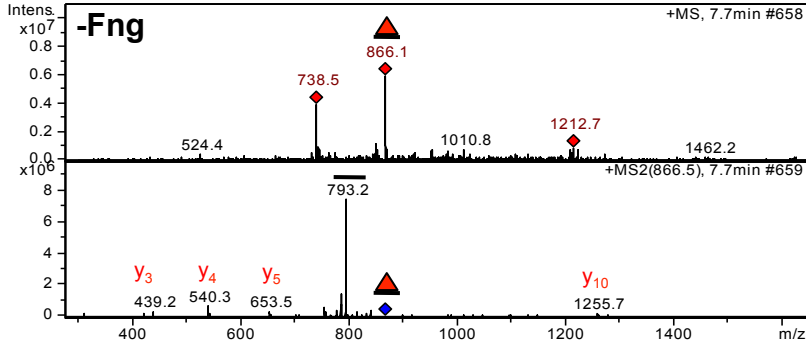
<sup>70</sup>NAGTCHVVDHGGTVDYACSCPL  
GFSGPLCLTPLDNA LANPCR<sup>112</sup>



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	1177.4
	1213.9
	1264.7
	1305.2
	1377.7
	1305.2
	1377.7

# EGF 3

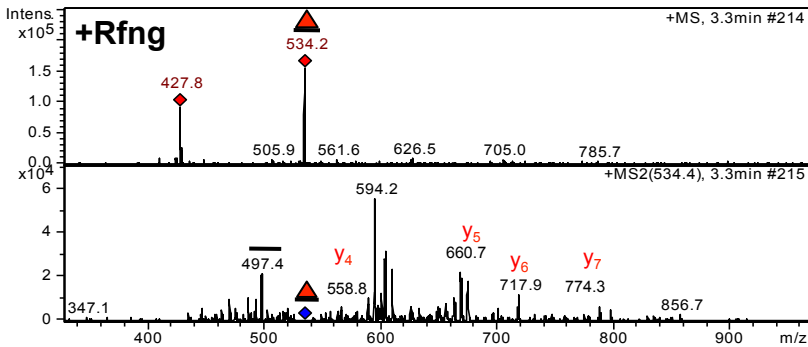
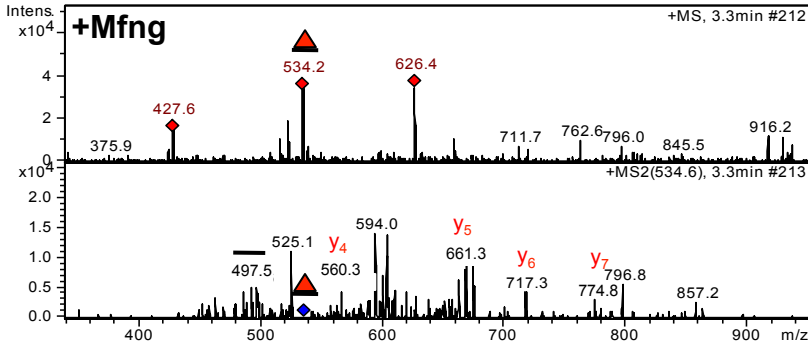
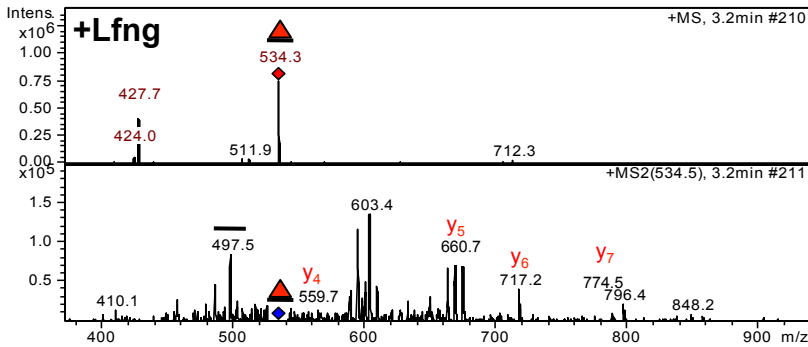
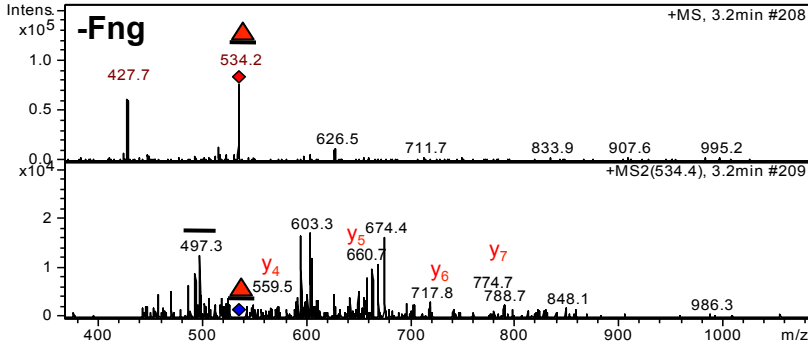
$^{113}\text{NNGTCDLLLTLEYK}^{126}$



Glycoform	Predicted Mass (M+2H) <sup>2+</sup>
	793.2
	866.2
	967.7
	1048.7
	1193.7

# EGF 5

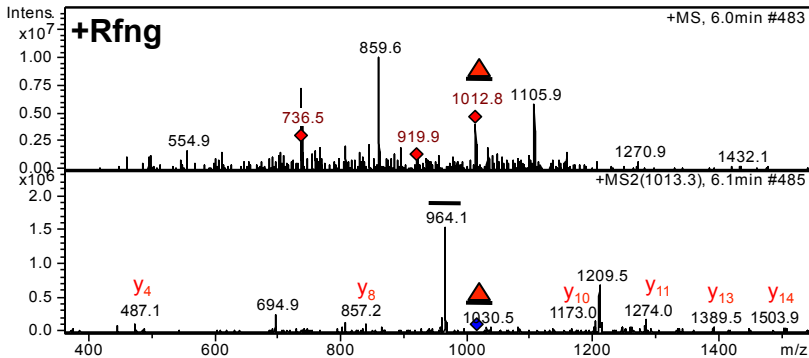
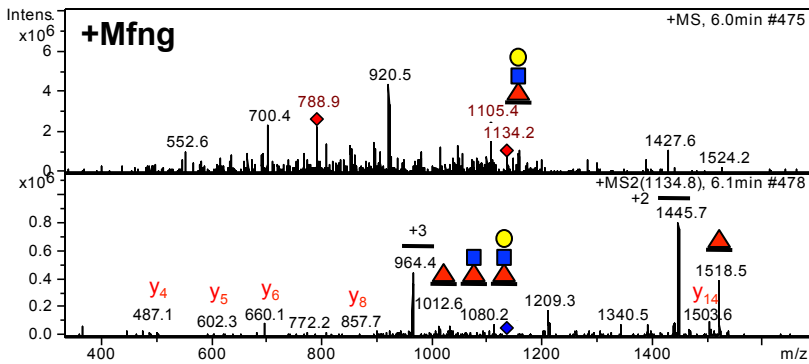
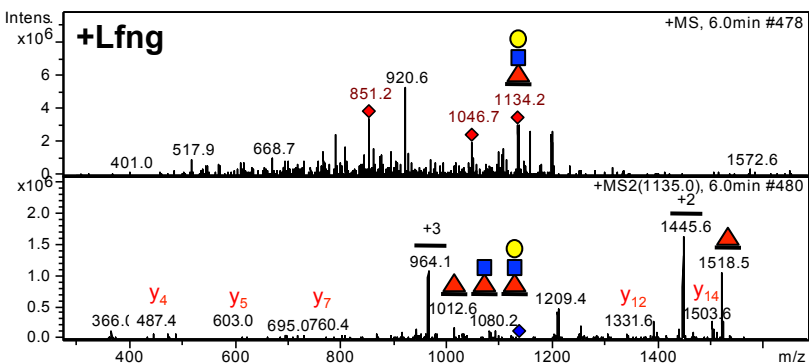
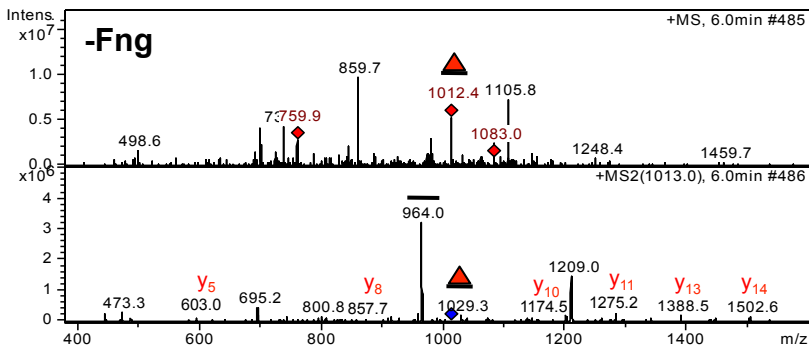
182CSQNPGLCRHGGTCHNE198



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	497.3
	533.8
	584.6
	625.1
	697.6

# EGF 6

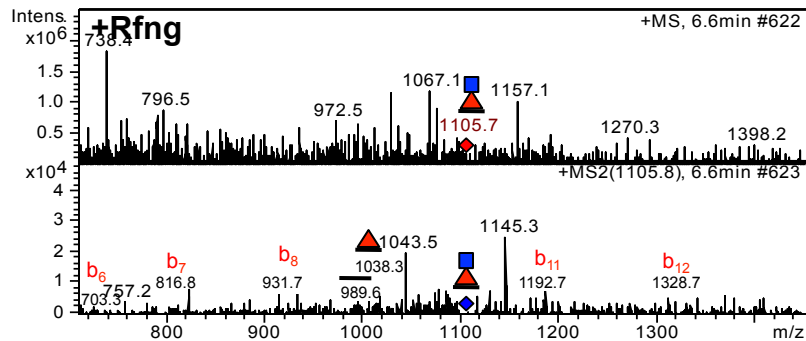
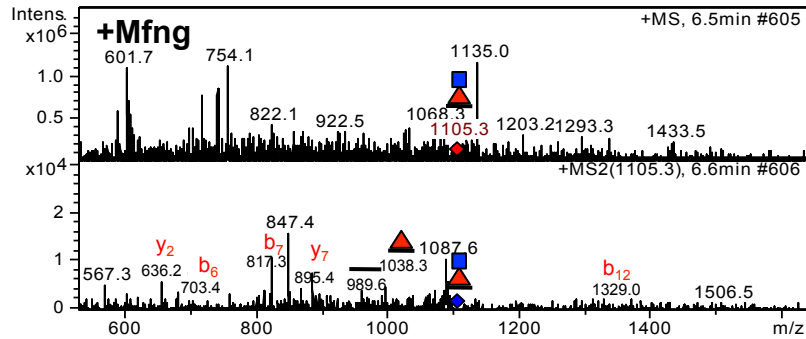
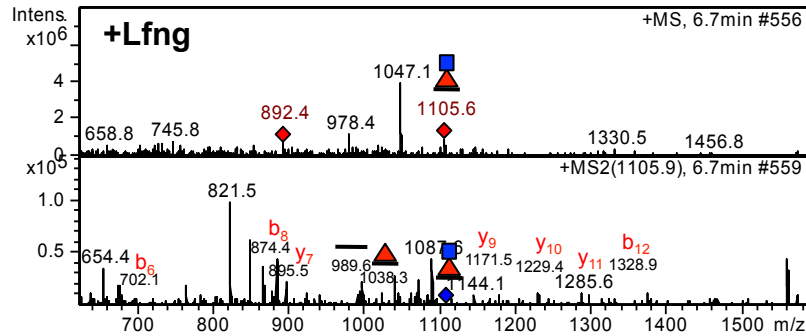
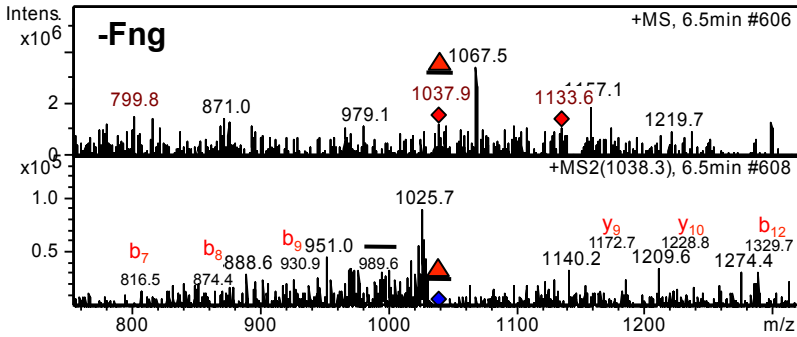
<sup>217</sup>LPYVPCSPSPCQNGGTCRPTGDTT<sup>242</sup>



Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	964.0
	1012.7
	1080.4
	1134.4
	1231.1

# EGF8

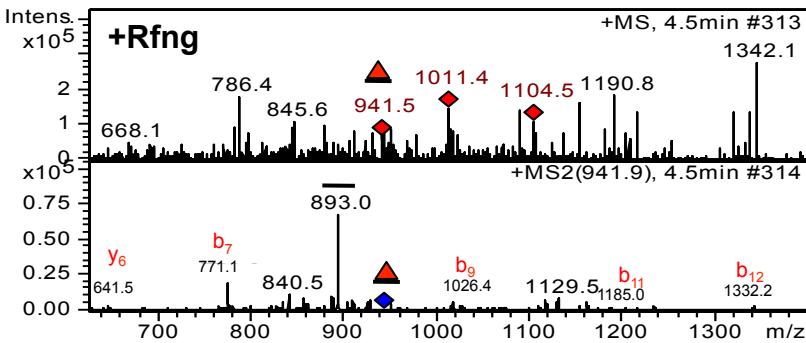
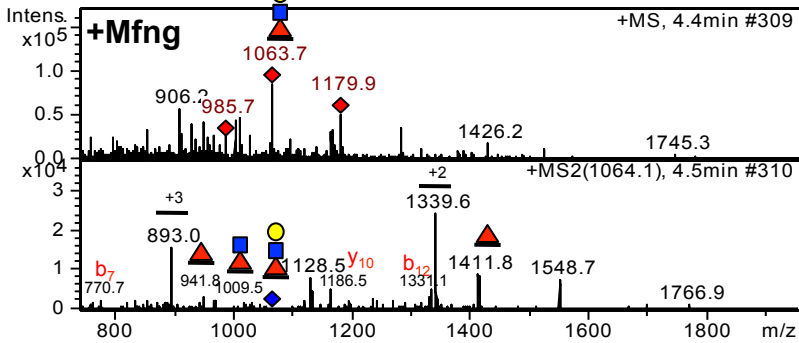
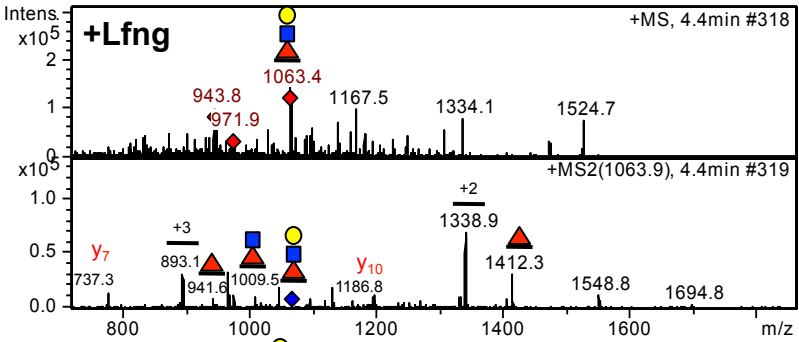
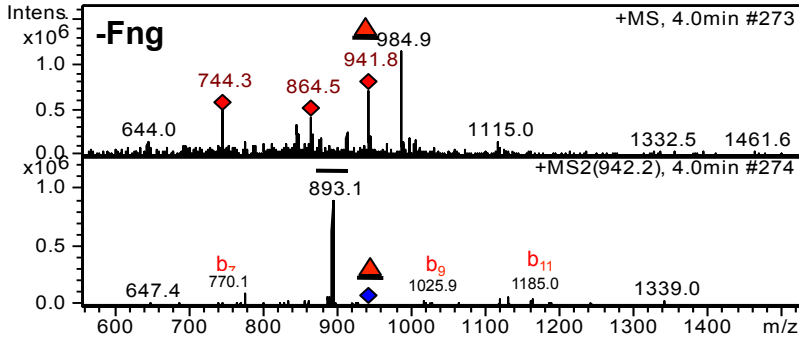
302MPNACQNGG**T**CHNTHGGYNCVCVNGW<sup>327</sup>



Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	989.6
	1038.3
	1106.0
	1160.0
	1257.1

# EGF9

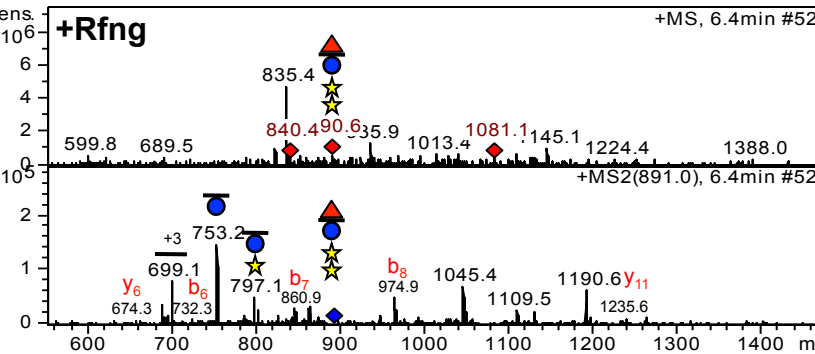
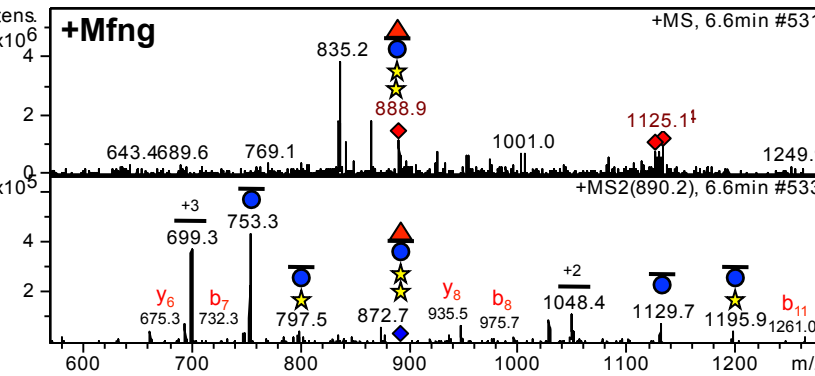
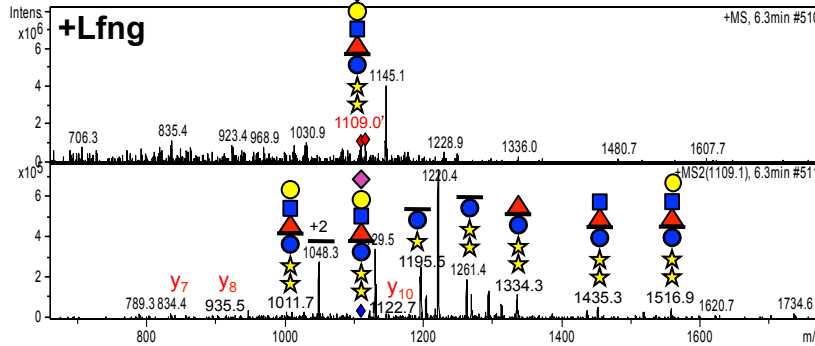
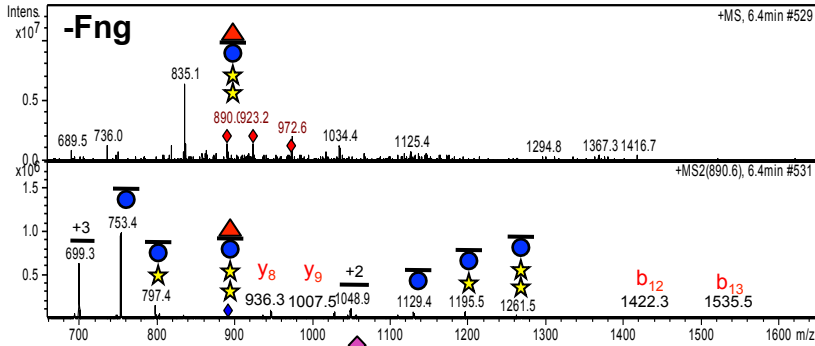
<sup>346</sup>QGATCHDRVASFYCECPHGRTGL<sup>368</sup>



Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	893.1
	941.8
	1009.5
	1063.5
	1160.2

# EGF 12

456CISNPCQNDA**T**CLDQIGE473

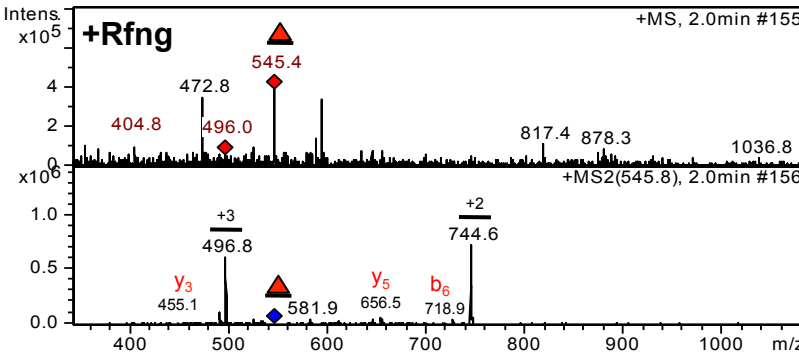
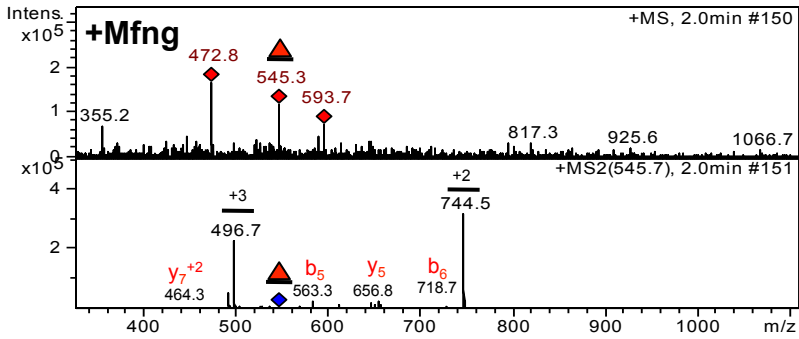
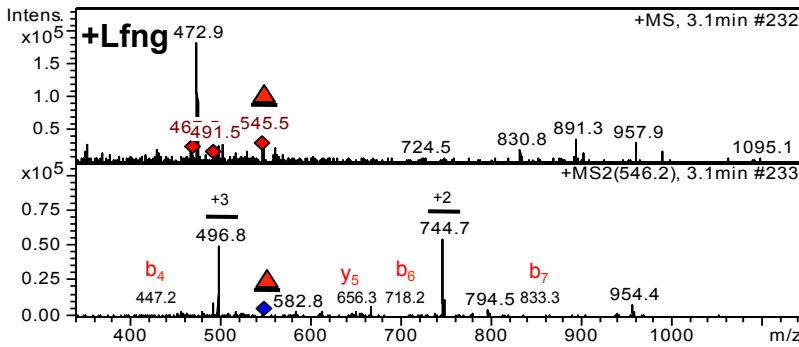
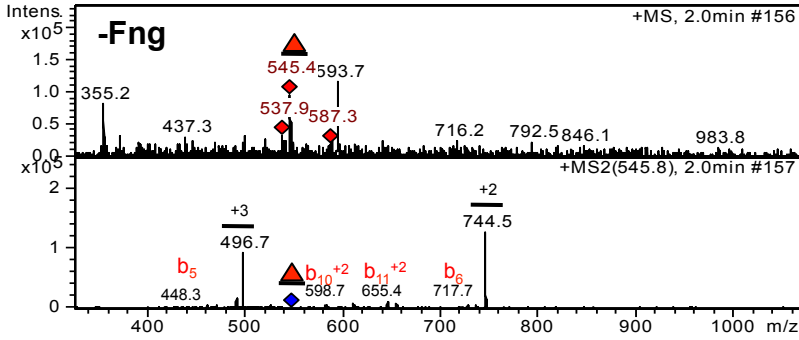


Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	699.3
	890.0
	957.7
	1011.7
	1108.8



# EGF16

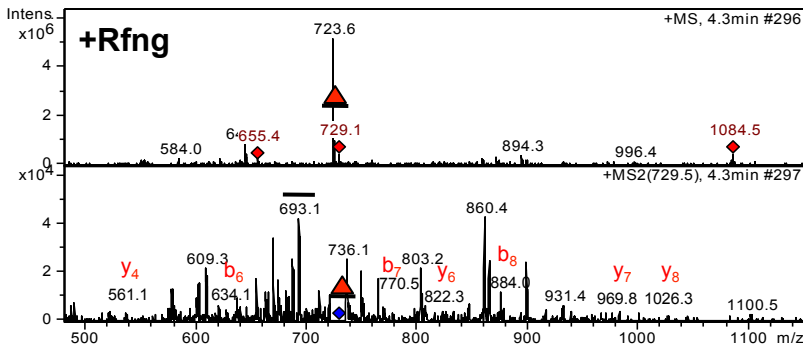
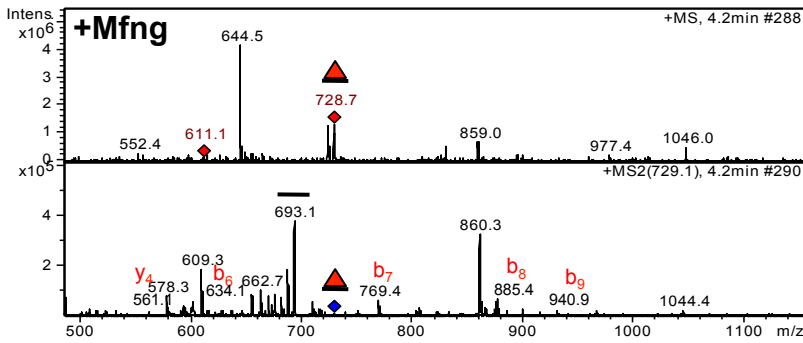
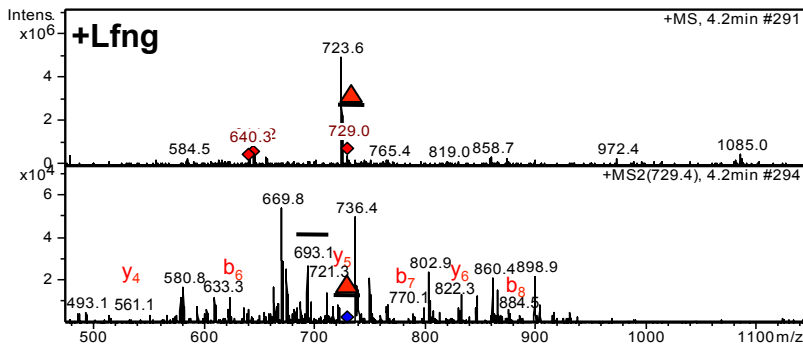
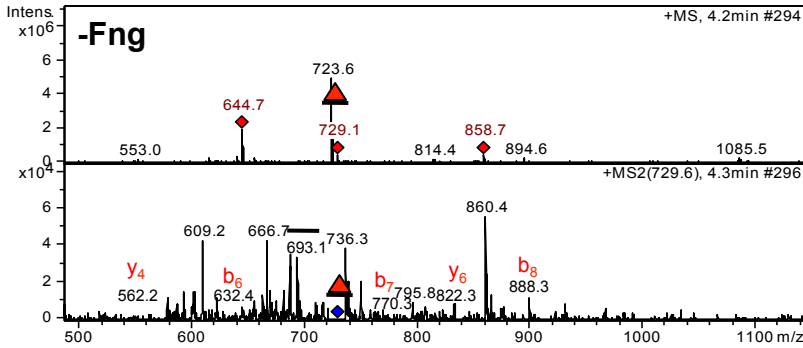
616GTCQDRDNSYLC627



Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	496.7
	545.4
	613.1
	667.1
	764.2

# EGF 18

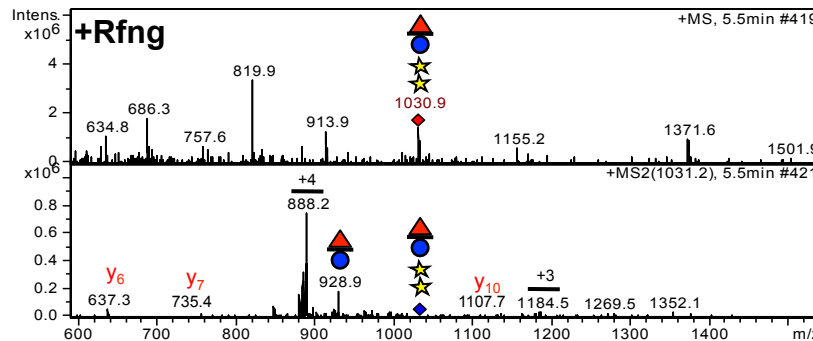
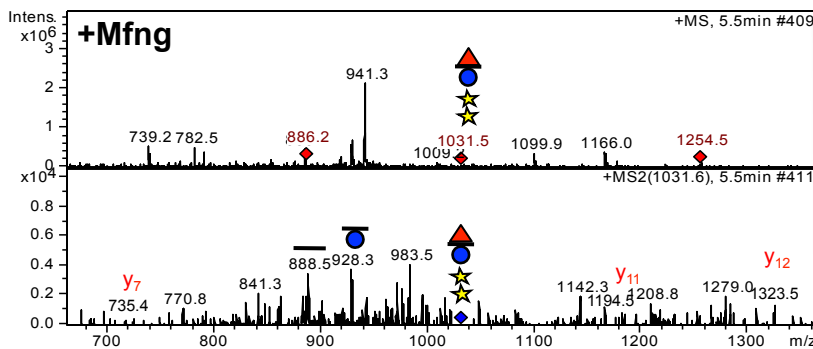
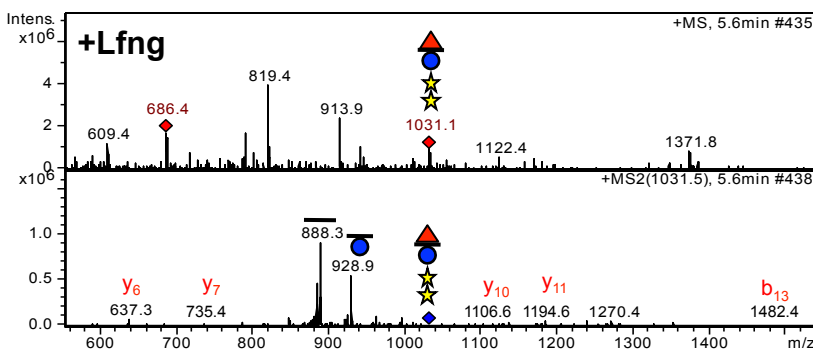
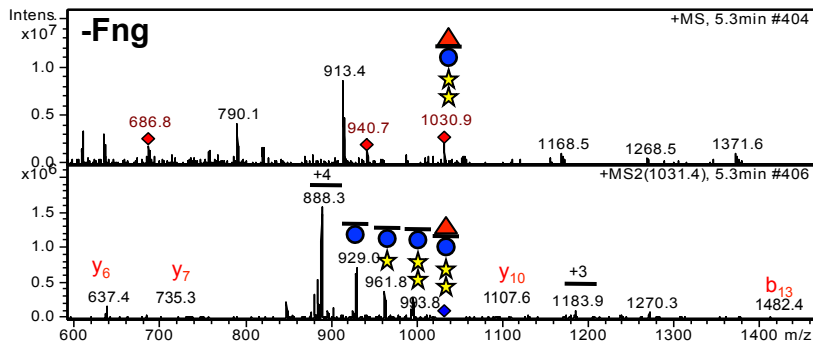
682CAGSPCHNGG**T**CEDGIAGFTCRCPE706



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	693.3
	729.8
	780.6
	821.1
	893.9

# EGF 20

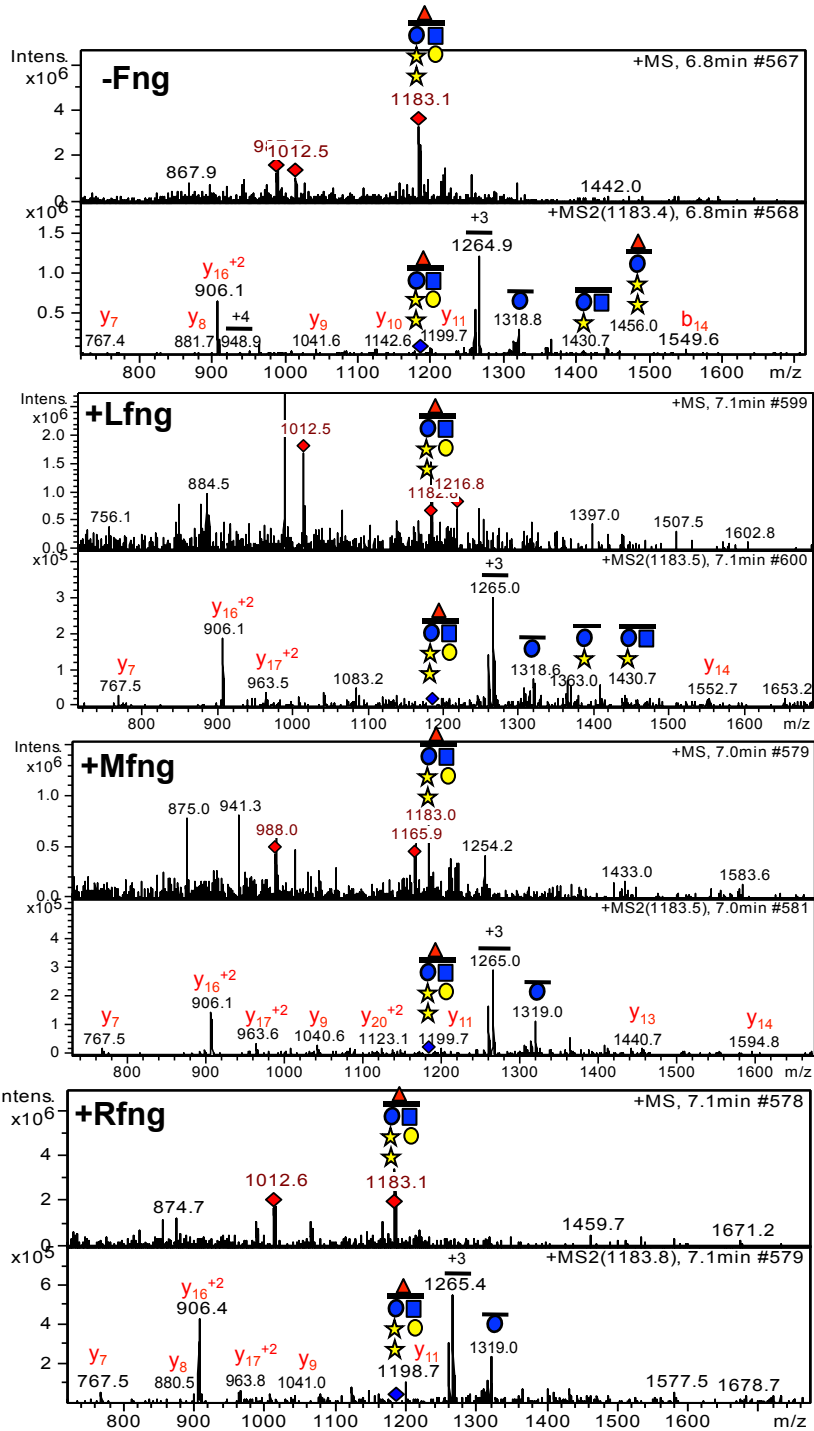
739CDCAPGWSGTNCDINNNECESN**PCV**NGG**T**CK769



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	888.3
	994.8
	1031.3
	1082.1
	1122.6
	1195.1

# EGF 21

781EGFSGPNCQTNINECASNPCLNQGT**C**IDDVAGYK814

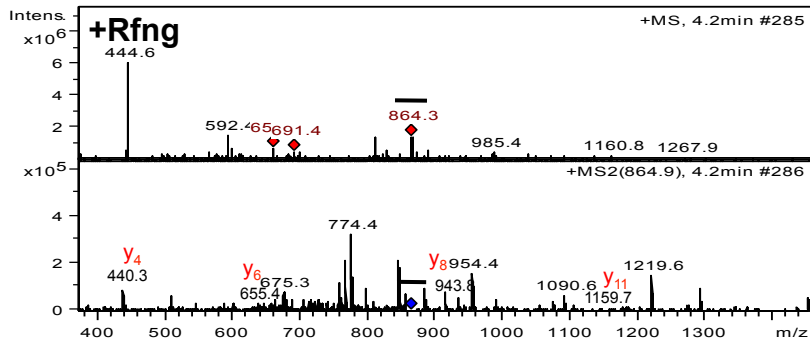
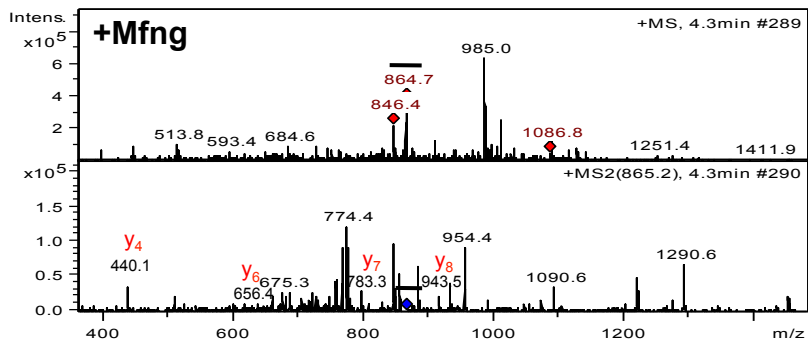
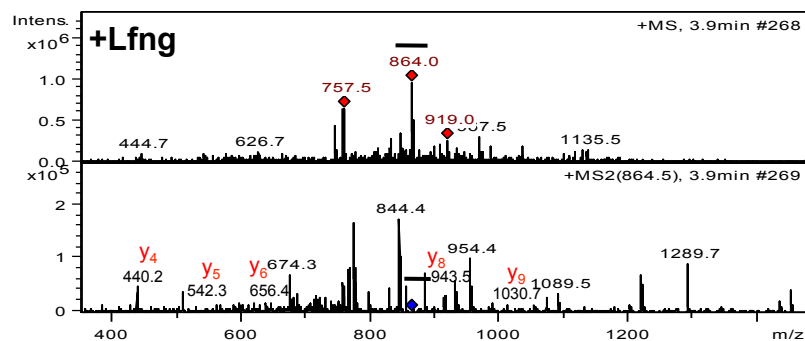
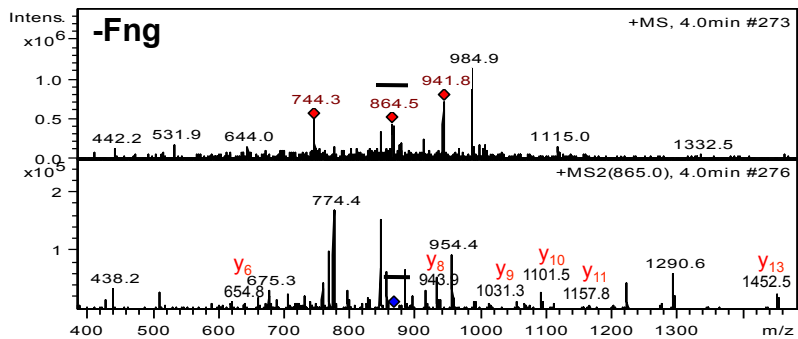


Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	948.9
	1091.9
	1150.2
	1183.2

No Fringe-mediated elongation of these three glycoforms of *O*-fucosylated peptide have been detected. Thus, the masses Fringe-modified peptides are omitted from the key here.

# EGF 23

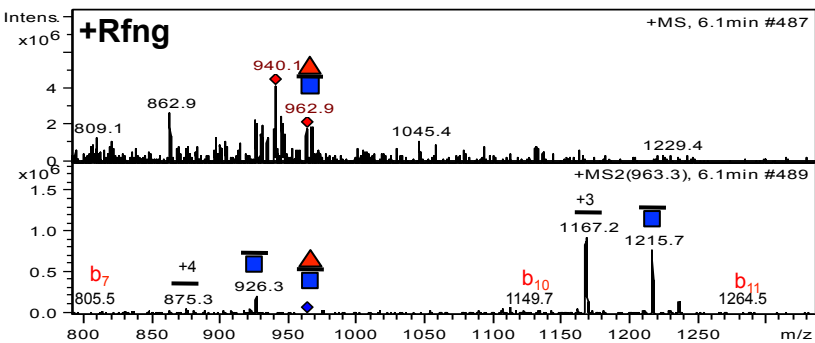
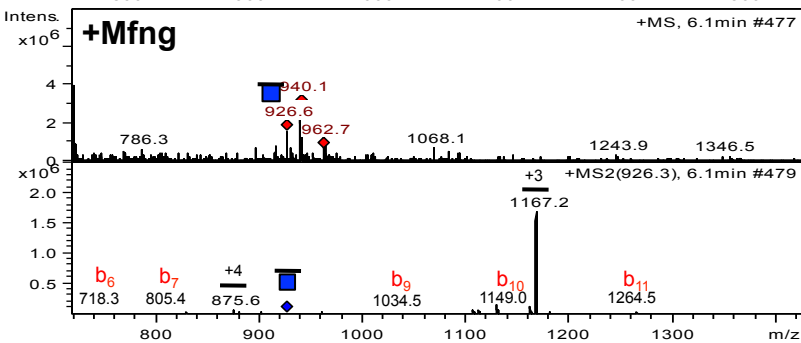
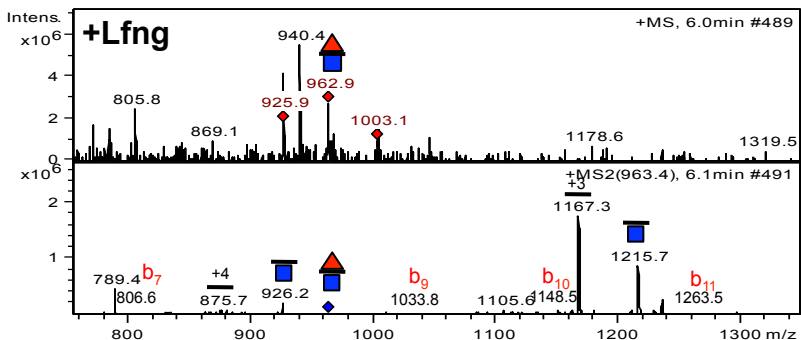
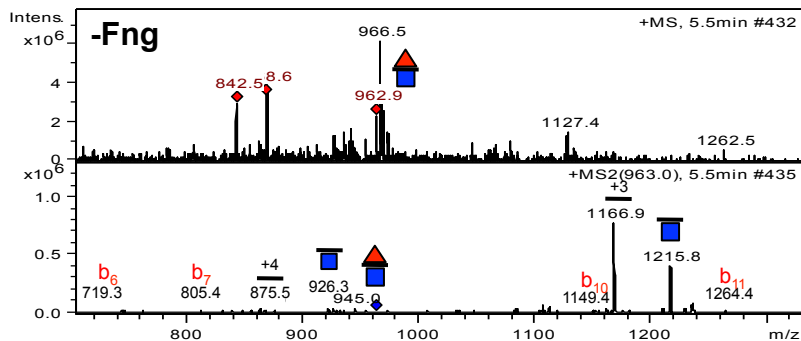
862QGQTCEVDINECVKSPCRHGAS**C**QNTNGSY891



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	865.4
	901.9
	952.7
	993.1
	1065.9

# EGF 24

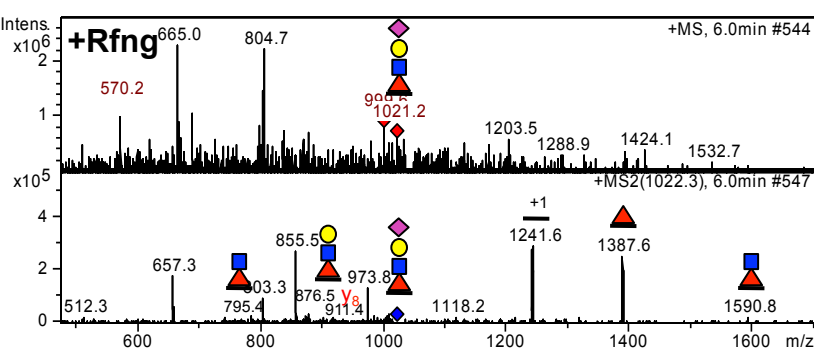
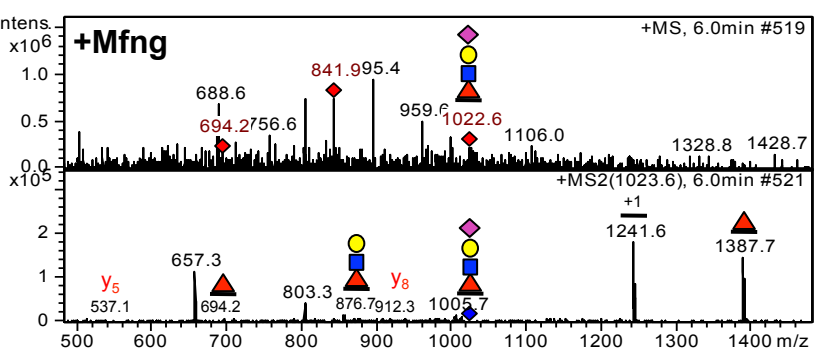
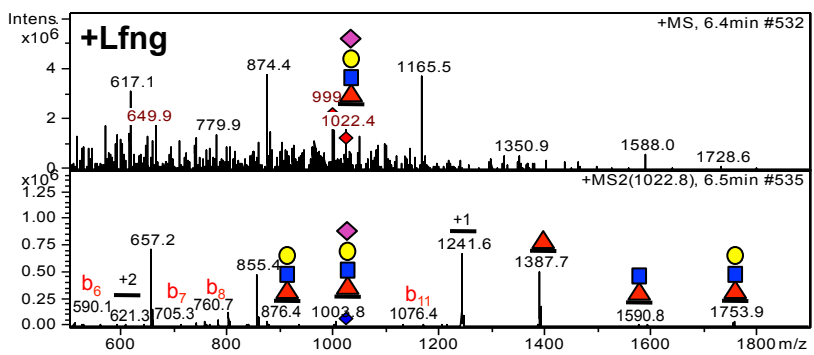
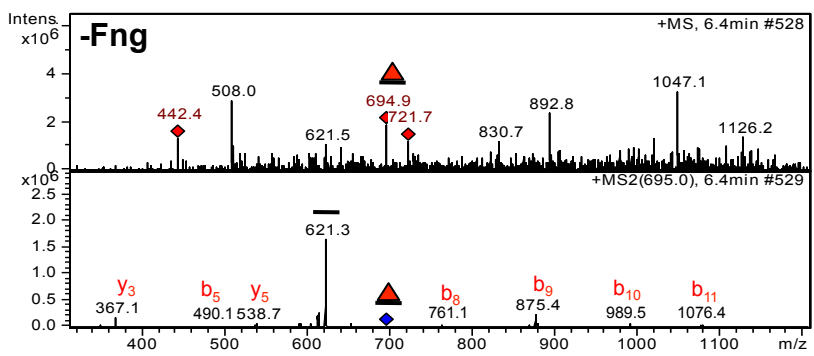
900TGRNCESDIDDCRPNPCHNGGSCTDGINTAF930



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	875.5
	926.3
	962.8
	1013.6
	1054.1
	1126.6

# EGF 26

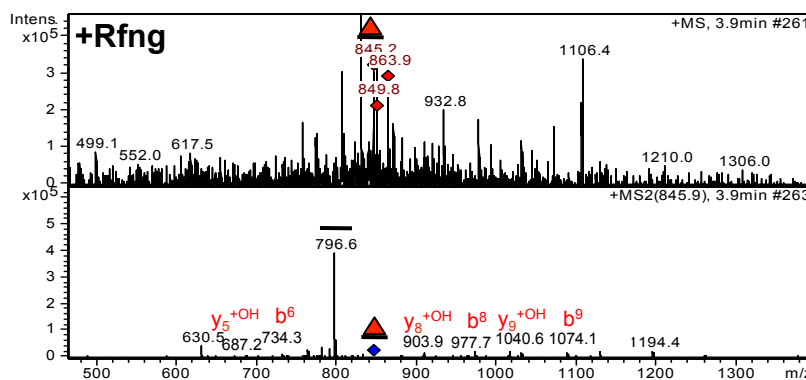
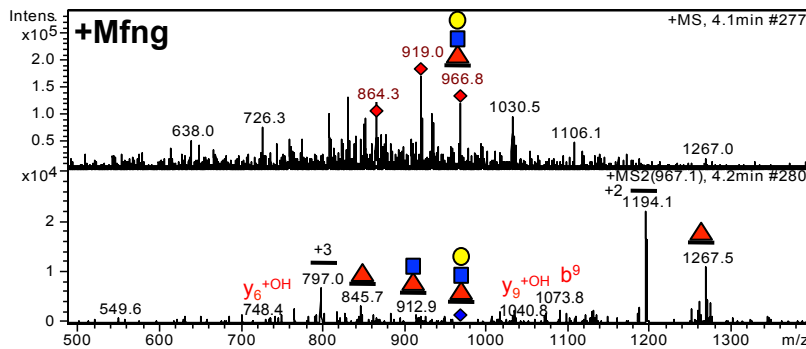
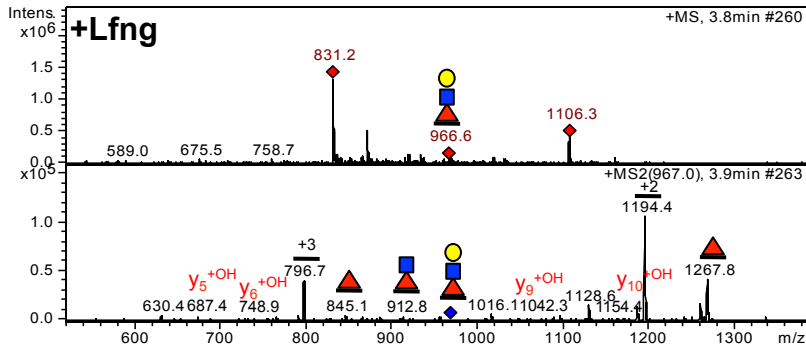
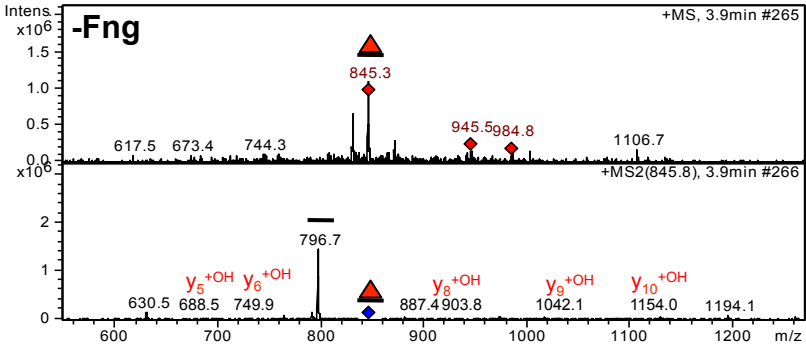
994NGGTCVDGINSF1005



Glycoform	Predicted Mass (M+2H) <sup>2+</sup>
	621.3
	694.3
	795.8
	876.8
	1022.4

# EGF 27

1021DVNECDSRPCLHGGT**C**QDSY1040  
OH

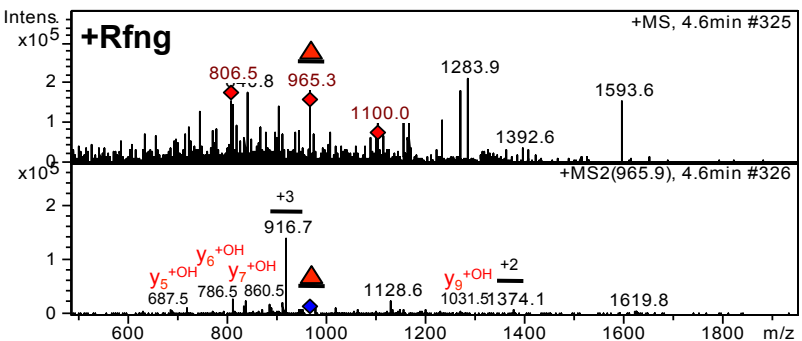
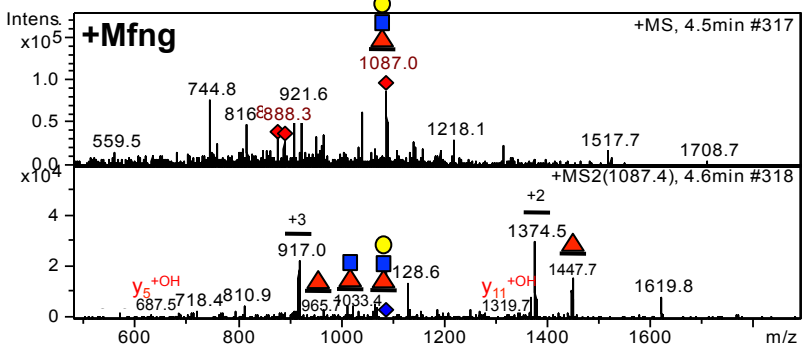
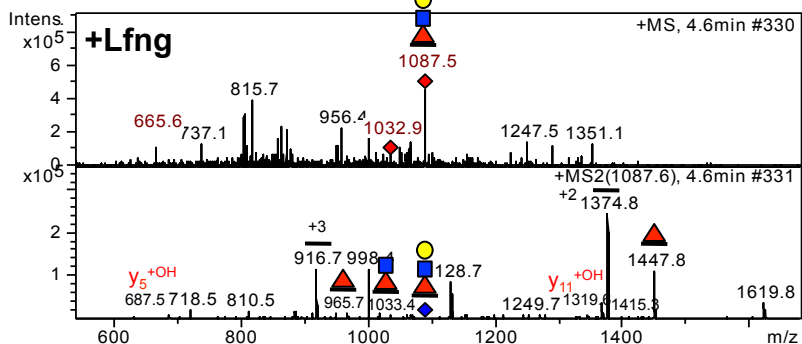
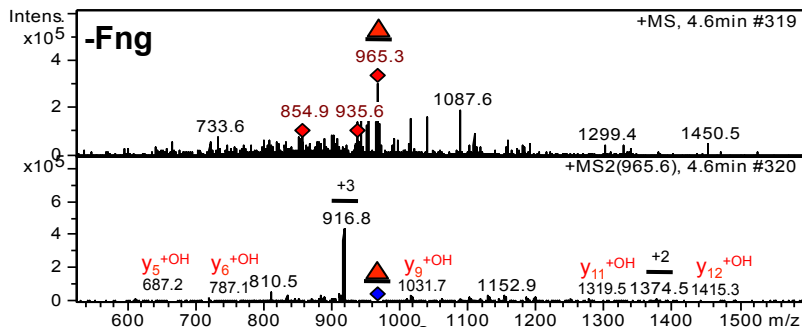


Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	796.4
	845.1
	912.8
	966.8
	1063.5



# EGF 30

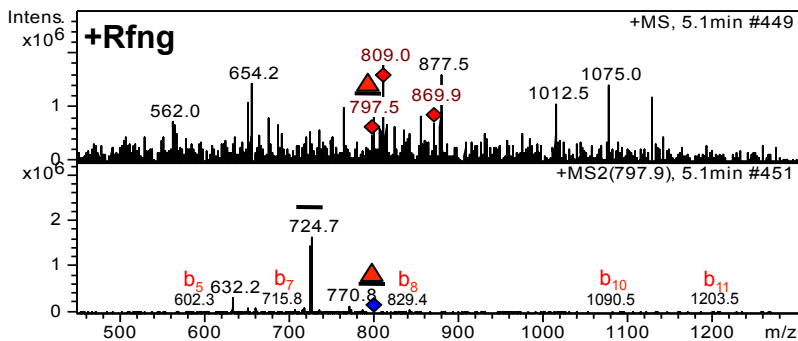
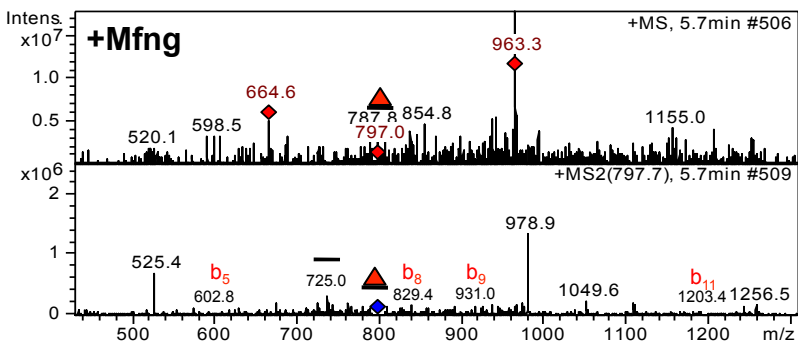
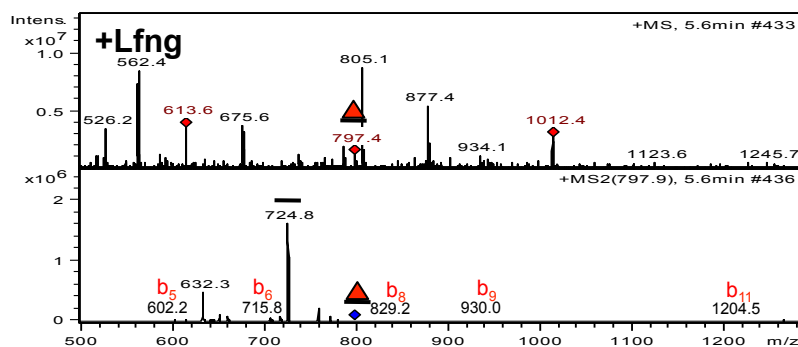
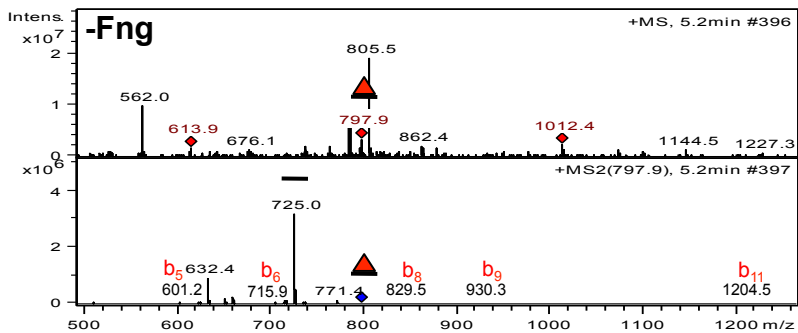
1142CEDEVDECSPNPCQNGATCTDYL1164  
OH



Glycoform	Predicted Mass (M+3H) <sup>3+</sup>
	917.0
	965.7
	1033.4
	1087.4
	1184.5

# EGF 31

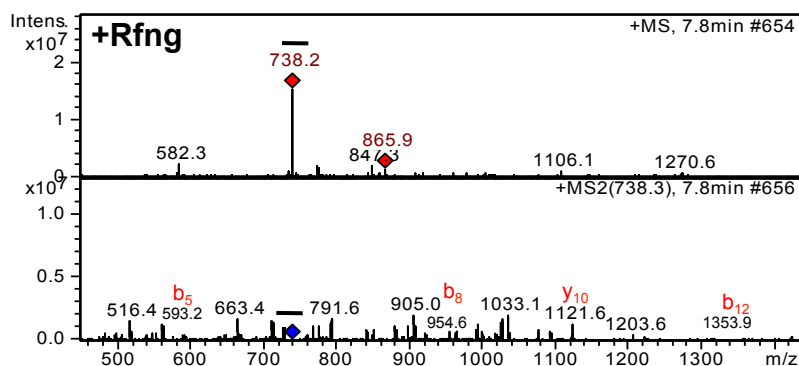
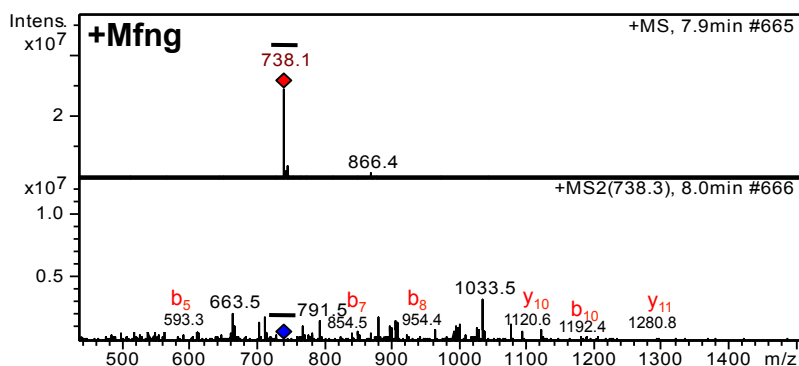
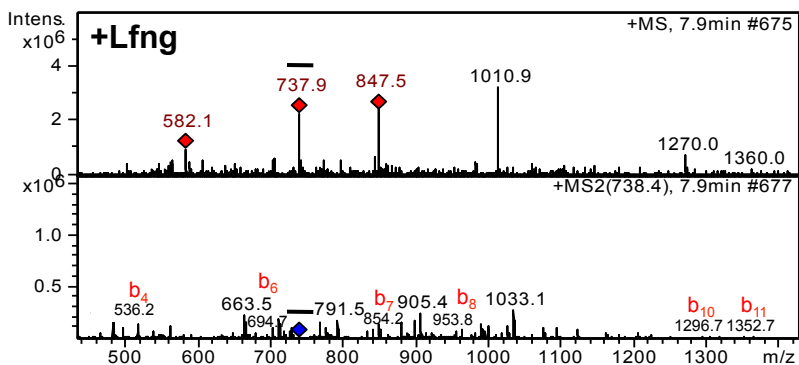
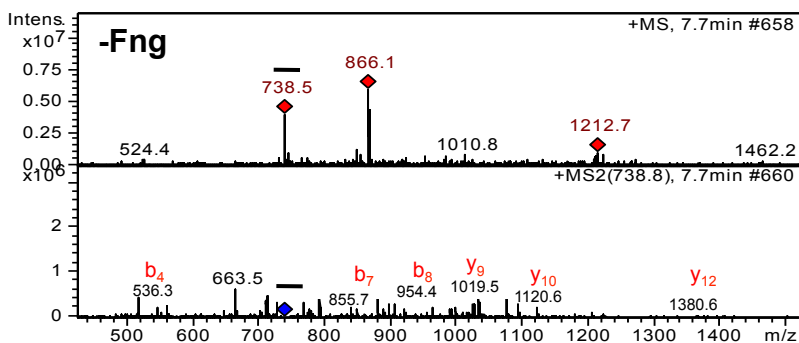
1189SQPCQNGGTCIDL1201



Glycoform	Predicted Mass (M+2H) <sup>2+</sup>
	725.0
	798.0
	899.6
	980.6
	1126.2

# EGF 32

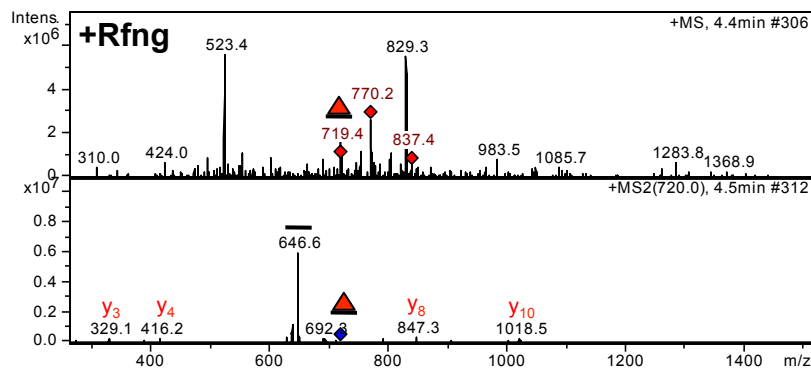
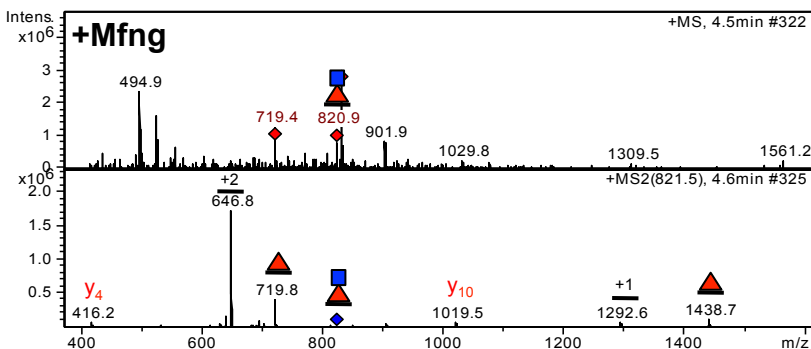
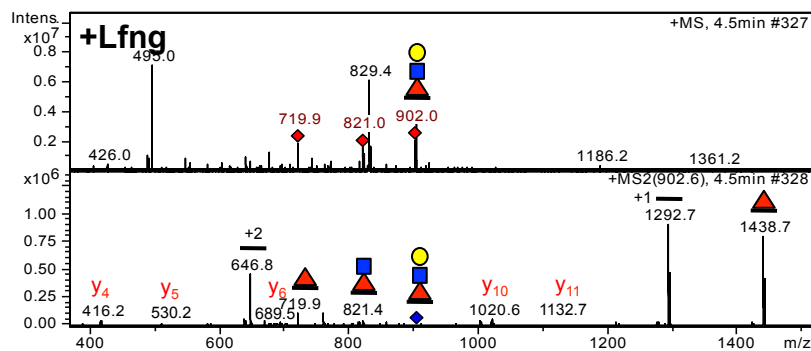
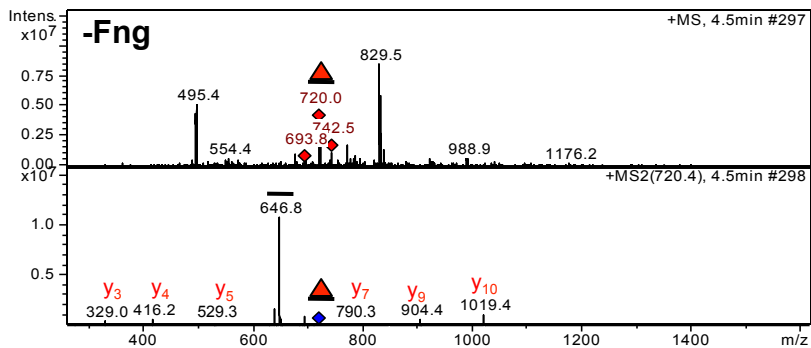
1238CFNNGT**T**CVDQVGGYTCTCPPGFVGER1263



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	738.5
	775.0
	825.8
	866.2
	939.0

# EGF 35

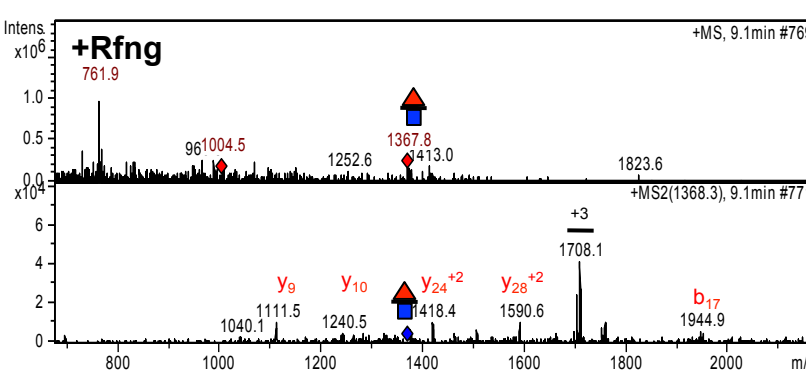
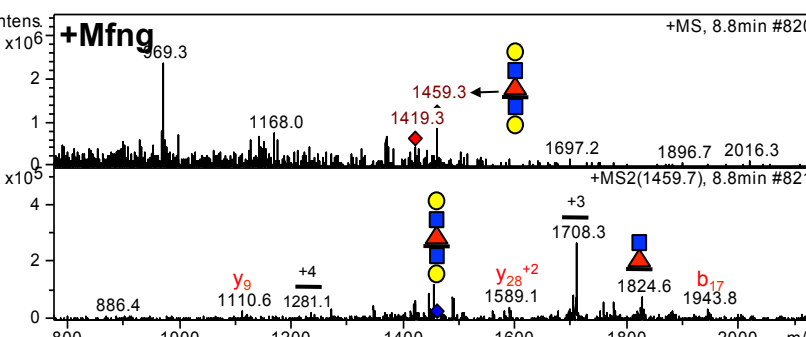
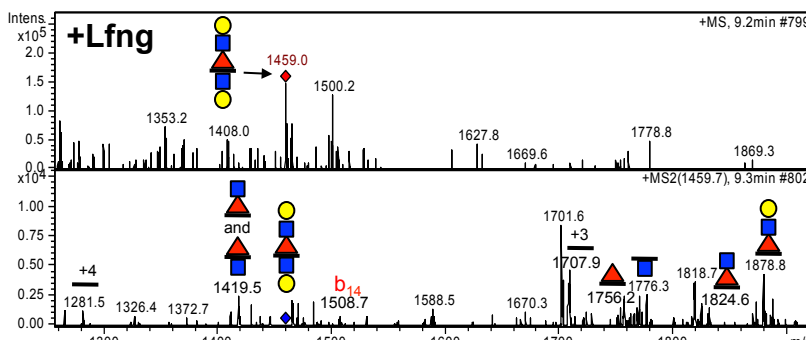
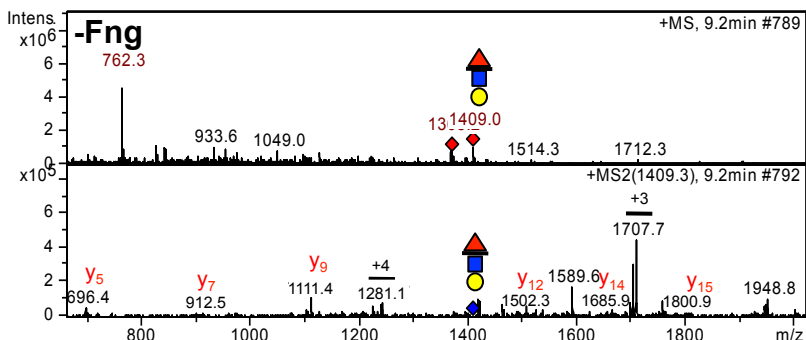
1357CLNGGTCISGPR1368



Glycoform	Predicted Mass (M+2H) <sup>2+</sup>
	646.8
	719.8
	821.4
	902.4
	1048.0

# EGF36

<sup>1369</sup>SPTCLCLGSFTGPEQCQFPASSPCVGSNP  
CYNQGTCEPTSENPFYR<sup>1413</sup>



Glycoform	Predicted Mass (M+4H) <sup>4+</sup>
	1281.1
	1368.4
	1408.9
	1419.2
	1459.7
	1500.2
	1573.0