Mapping Sites of *O*-Glycosylation and Fringe Elongation on *Drosophila* Notch

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Running title: Mapping *O*-Glycosylation on *Drosophila* Notch

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Supplemental Figures

Supplemental Figure 1. ***O*-Fucosylation sites are elongated by Fringe to varying degrees**.
MRM analyses showing the relative levels of unmodified (black line), *O*-fucose monosaccharide (red line) and *O*-fucose disaccharide (blue line) of several peptides containing predicted *O*-fucose sites in the absence of Fringe (*A-H*) and presence of Fringe (*A'-H'*). List of peptides searched in each EIC are in Table 1, and corresponding spectra are in Fig. S3. *A, A'* (EGF3); *B, B'* (EGF5); *C, C'* (EGF7); *D, D'* (EGF8); *E, E'* (EGF9); *F, F'* (EGF21); *G, G'* (EGF28); *H, H'* (EGF31). Red triangle represents fucose (dHexose). Blue square is GlcNAc (HexNAc).

Supplemental Figure 2. **Sequence alignments of *O*-glycosylated EGF repeats**
Alignments of the amino acid sequences within and surrounding the current consensus sequences of *O*-glycosylation of EGF repeats containing *O*-fucose (*A-B*), *O*-glucose (*C*) and *O*-GlcNAc (*D*) sites shown using WebLogo (1). Sequences of the 22 EGF repeats containing *O*-fucose sites were organized into either four categories of approximate amounts of *O*-fucosylation based on EICs generated in the absence of Fringe (*A*) or four categories of fucose elongation from EICs generated in the presence of Fringe (*B*) (see Fig. 4). *C*, Sequences of the 18 EGF repeats containing *O*-glucose sites were organized into three categories of approximate amounts of glucose elongation based on EICs (see Fig. 5). *D*, Sequences of the 18 EGF repeats containing *O*-GlcNAc sites were organized into two categories of either having or not having apparent *O*-GlcNAc modification based on EICs (see Fig. 6).

Supplemental Figure 3**. Mass spectra of *O*-glycosylated peptides from *Drosophila* Notch**
Drosophila Notch was purified from S2 cells and prepared for mass spectral analyses. For each peptide, top panels show MS spectra at a specific retention time, and red diamonds indicate ions chosen for fragmentation. Parent ions corresponding to specified peptides are fragmented to produce MS2 spectra, shown in the bottom panels. Parent ions of the represented peptide are labeled with the charge state of the peptide in that specific spectrum. Other ions in the MS spectra are from co-eluting material. Blue diamonds in MS2 spectra indicate the position of the parent ion. Spectra representing the most glycosylated form of each peptide found in the MS/MS data are shown. For analyzing *O*-fucosylation, S2 cells were co-transfected with or without Fringe, and peptides containing an *O*-fucose consensus sequence were inspected in both conditions (-Fringe or +Fringe). Spectra analyzing *O*-glucosylation and *O*-

GlcNAcylation were taken from Notch generated in the absence of Fringe, except the EGF26 *O*-GlcNAc site. Tables to the right of spectra show the ions searched in EICs analyzing *O*-fucose, *O*-glucose and *O*-GlcNAc glycoforms in Figs. 3, 4*A*, 5*A*, 6*A* and Fig. 8 and MRM in Figs. 3 and S1. Red triangle represents fucose (dHexose). Blue circle is glucose (Hexose). Orange star is xylose (Pentose). Blue square is GlcNAc (HexNAc).

References

1. Crooks, G. E., Hon, G., Chandonia, J. M., and Brenner, S. E. (2004) WebLogo: a sequence logo generator. *Genome Res* **14**, 1188-1190

Fig. S1

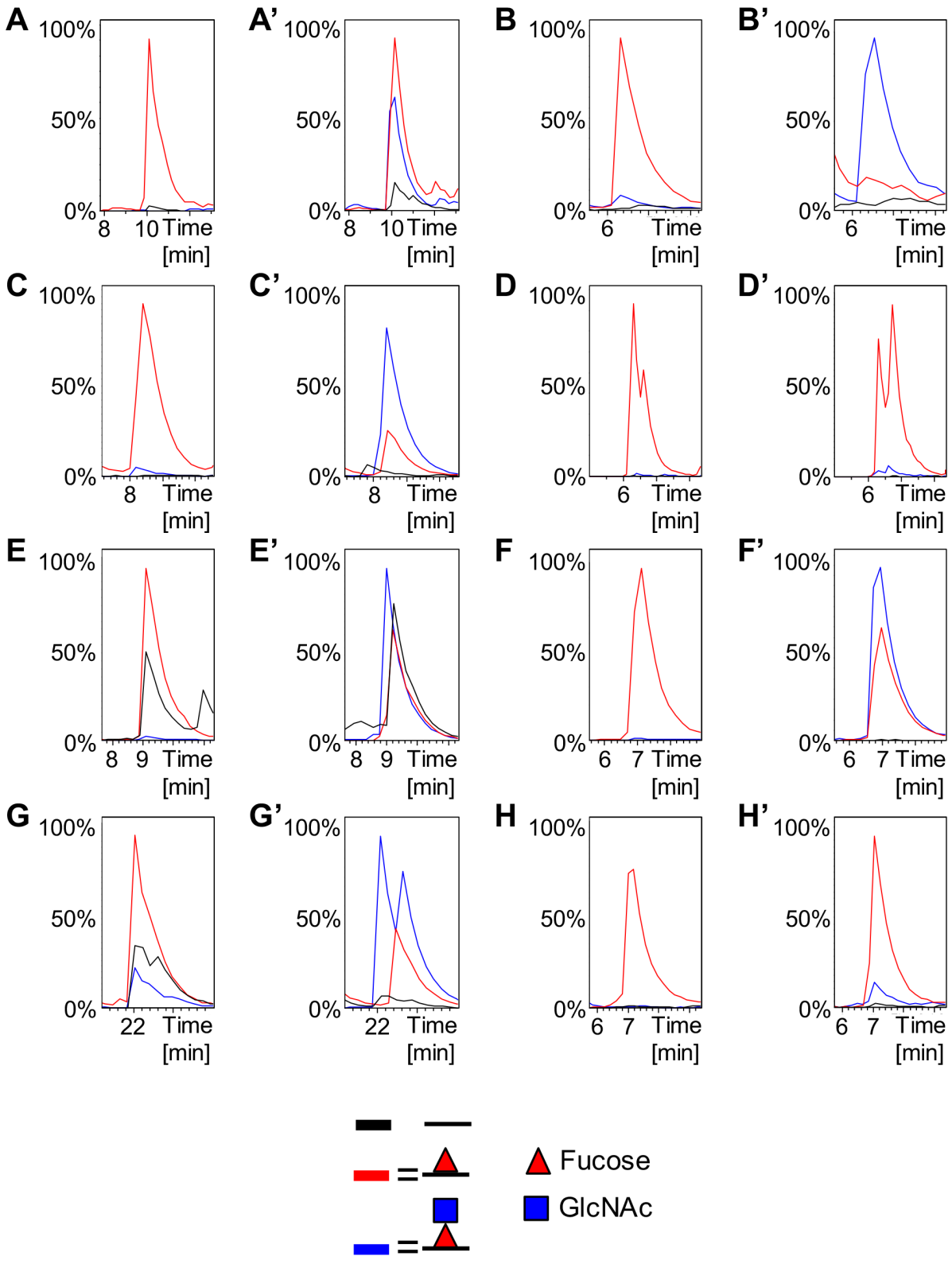


Fig. S2, A

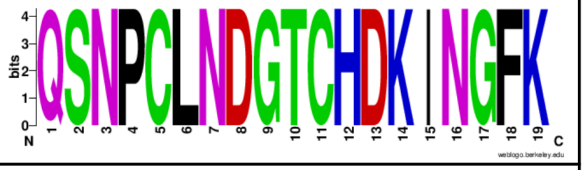
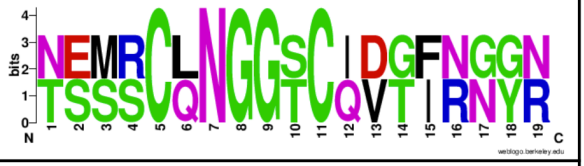
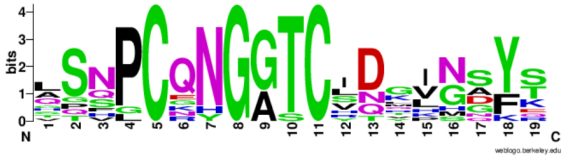
O-Fucose Modification	EGF Repeat	EGF Sequence	Alignment
<30%	13	DIDECQSNPCLNDGTCHDKINGFKCSCALGFTGARCQ	
30%-50%	2	HRNPCNSMRCQNGGTCQVTFRNGRPGISCKCPLGFDESLCE	
	26	NDEDCTESSCLNGGSCIDGINGYNCSCLAGYSGANCQ	
60-80%	3	VPNACDHVTCLNGGTCQLKTLEEYTCACANGYTGERCE	
	4	TKNLCASSPCRNGATCTALAGSSSFTCSCPPGFTGDTCS	
	8	DVDECAQRDHPVCQNGATCTNTHGSYSCICVNGWAGLDCS	
	9	NTDDCKQAACFYGATCIDGVGSFYCQCTKGKTGLLCH	
	12	NINECESHPCQNEGSCLDDPGTFRCVCMPGFTGTQCE	
	27	KLNKCDSNPCLNGATCHEQNNEYTCHCPSGFTGKQCS	
	32	NKDDCKPGACHNNGSCIDRVGGFECVCQPGFVGARCE	
≥80%	1	VAASCTSVGCQNGGTCVTQLNGKTYCACDSHYVGDYCE	
	5	DIEECQSNPCKYGGTCVNTHGSYQCMCPTGYTGKDCD	
	7	NYDDCLGHLCQNGGTCIDGISDYTCRCPPNFTGRFCQ	
	17	NVNECHSNPCNNGATCIDGINSYKCQCVPGFTGQHCE	
	20	DIDECSSNPCQHGGTCYDKLNAFSCQCMPGYTGQKCE	
	21	NIDDCVTNPCGNGGTCIDKVNGYKCVCKVPFTGRDCE	
	23	DIDECSLSSPCRNGASCLNVPGSYRCLCTKGYEGRDCA	
	24	NTDDCASFPCQNGGTCLDGIGDYSCLCVDGFDGKHCE	
	25	DINECLSQPCQNGATCSQYVNSYTCTCPLGFSGINCQ	
	28	YVDWCGQSPCENGATCSQMKHQFSCKCSAGWTGKLCD	
	30	EIDECQSQPCQNGGTCRDLIGAYECQCRQGFQGQNCE	
	31	NIDDCAPNPCQNGGTCHDRVMNFSCSCPPGTMGIICE	

Fig. S2, B

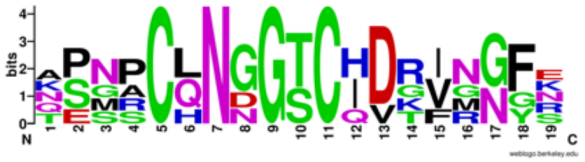
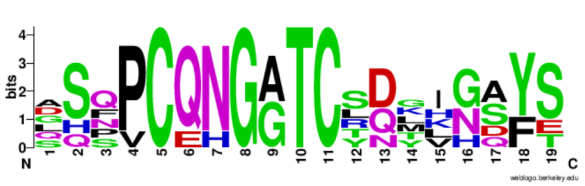
O-Fucose Elongation	EGF Repeat	EGF Sequence	Alignment
<25%	2	HRNPCNSMRCQNGGTCQVTFRNGRPGISCKCPLGFDESLCE	
	13	DIDECQSNPCLNDGTCHDKINGFKCSCALGFTGARCQ	
	26	NDEDCTESSCLNGGSCIDGINGYNCSCLAGYSGANCQ	
	31	NIDDCAPNPCQNGGTCHDRVMNFSCSCPPGTMGIICE	
	32	NKDDCKPGACHNNGSCIDRVGGFECVCQPGFVGARCE	
25%-50%	3	VPNACDHVTCLNGGTCQLKTLEEYTCACANGYTGERCE	
	4	TKNLCASSPCRNGATCTALAGSSSFTCSCPPGFTGDTCS	
	9	NTDDCKQAACFYGATCIDGVGSFYCQCTKGKTGLLCH	
	12	NINECESHPCQNEGSCLDDPGTFRCVCMPGFTGTQCE	
	17	NVNECHSNPCNNGATCIDGINSYKCQCVPGFTGQHCE	
	27	KLNKCDSNPCLNGATCHEQNNEYTCHCPSGFTGKQCS	
50%-75%	8	DVDECAQRDHPVCQNGATCTNTHGSYSCICVNGWAGLDCS	
	20	DIDECSSNPCQHGGTCYDKLNAFSCQCMPGYTGQKCE	
	24	NTDDCASFPCQNGGTCLDGIGDYSCLCVDGFDGKHCE	
	25	DINECLSQPCQNGATCSQYVNSYTCTCPLGFSGINCQ	
	28	YVDWCGQSPCENGATCSQMKHQFSCKCSAGWTGKLCD	
	30	EIDECQSQPCQNGGTCRDLIGAYECQCRQGFQGQNCE	
≥75%	1	VAASCTSVGCQNGGTCVTQLNGKTYCACDSHYVGDYCE	
	5	DIEECQSNPCKYGGTCVNTHGSYQCMCPTGYTGKDCD	
	7	NYDDCLGHLCQNGGTCIDGISDYTCRCPPNFTGRFCQ	
	21	NIDDCVTNPCGNGGTCIDKVNGYKCVCKVPFTGRDCE	
	23	DIDECSLSSPCRNGASCLNVPGSYRCLCTKGYEGRDCA	

Fig. S2, C

O-Glucose Elongation	EGF Repeat	EGF Sequence	Alignment
≤10%	4	TKNLCASSPCRNGATCTALAGSSSFTCSCPPGFTGDTCS	
	5	DIEECQSNPCKYGGTCVNTHGSYQCMCPTGYTGKDCD	
	14	NIDDCQSQPCRNRGICHDSIAGYSCECPPGYTGTSCE	
	24	NTDDCASFPCQNGGTCLDGIGDYSCLCVDGFDGKHCE	
	27	KLNKCDSNPCLNGATCHEQNNEYTCHCPSGFTGKQCS	
	30	EIDECQSQPCQNGGTCRDLIGAYECQCRQGFQGQNCE	
	33	DINECLSNPCSNAGTLDCVQLVNNYHCNCRPGHMGRHCE	
10—50%	35	SGQDCDSNPCRVGNCVVADEGFGYRCECPRGTLGEHCE	
	10	LDDACTSNPCHADAICDTSPINGSYACSCATGYKGVDCS	
	12	NINECESHPCQNEGSCLDDPGTFRCVCMPGFTGTQCE	
	13	DIDECQSNPCLNDGTCHDKINGFKCSCALGFTGARCQ	
	16	QINECESNPCQFDGHCQDRVGSYYCQCQAGTSGKNCE	
	17	NVNECHSNPCNNGATCIDGINSYKCQCVPGFTGQHCE	
	25	DINECLSQPCQNGATCSQYVNSYTCTCPLGFSGINCQ	
>50%	15	NINDCDSNPCHRGKCIDDVNSFKCLCDPGYTGYICQ	
	18	NVDECISSPCANNGVCIDQVNGYKCECPRGFYDAHCL	
	19	DVDECASNPCVNEGRCEDGINEFICHCPPGYTGKRCE	
	20	DIDECSSNPCQHGGTCYDKLNAFSCQCMPGYTGQKCE	

Fig. S2, D

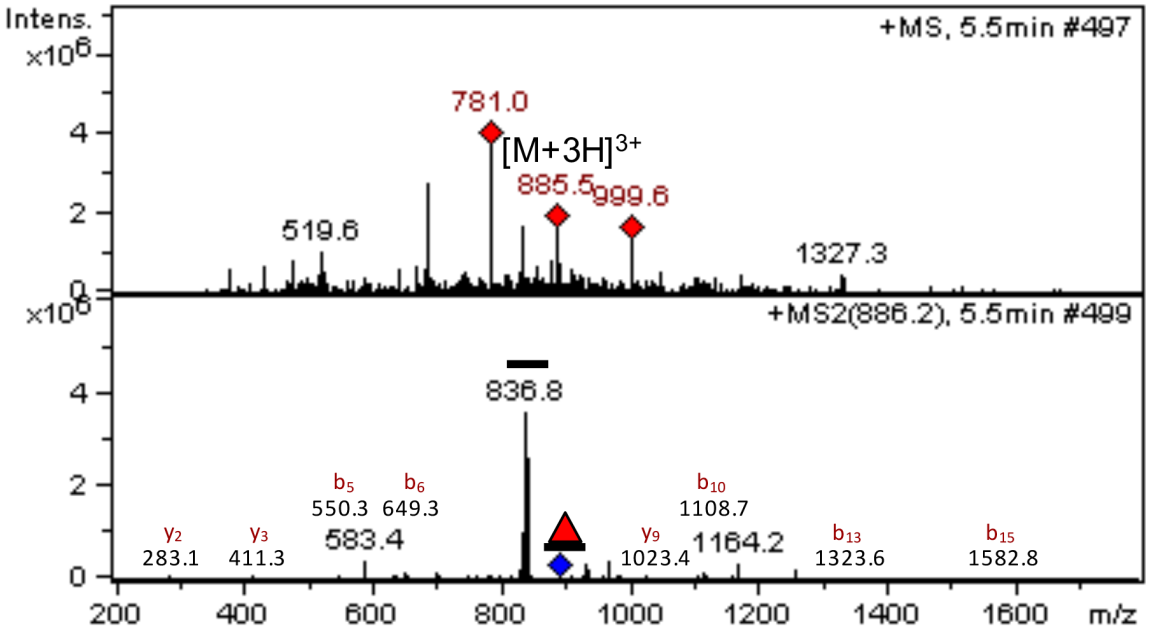
O-GlcNAc Modification	EGF Repeat	EGF Sequence	Alignment
No	3	VPNACDHVTCLNGGTCQLKTLEEYTCACANGYTGERCE	
	4	TKNLCASSPCRNGATCTALAGSSSFTCSCPPGFTGDTCS	
	5	DIEECQSNPCKYGGTCVNTHGSYQCMCPTGYTGKDCD	
	9	NTDDCKQAACFYGATCIDGVGSFYCQCTKGKTGLLCH	
	13	DIDECQSNPCLNDGTCHDKINGFKCSCALGFTGARCQ	
	15	NINDCDSNPCHRGKCIDDVNSFKCLCDPGYTGYICQ	
	16	QINECESNPCQFDGHCQDRVGSYYCQCQAGTSGKNCE	
	17	NVNECHSNPCNNGATCIDGINSYKCQCVPGFTGQHCE	
	19	DVDECASNPCVNEGRCEDGINEFICHCPPGYTGKRCE	
	22	KMDPCASNRCKNEAKCTPSSNFLDFSCTCKLGYTGRYCD	
	25	DINECLSQPCQNGATCSQYVNSYTCTCPLGFSGINCQ	
	27	KLNKCDSNPCLNGATCHEQNNEYTCHCPSGFTGKQCS	
	28	YVDWCGQSPCENGATCSQMKHQFSCKCSAGWTGKLCD	
Yes	4	TKNLCASSPCRNGATCTALAGSSSFTCSCPPGFTGDTCS	
	11	DIDECDQGSPCEHNGICVNTPGSYRCNCSQGFTGPRCE	
	12	NINECESHPCQNEGSCLDDPGTFRCVCMPGFTGTQCE	
	14	NIDDCQSQPCRNRGICHDSIAGYSCECPPGYTGTSCE	
	20	DIDECSSNPCQHGGTCYDKLNAFSCQCMPGYTGQKCE	

Fig. S3, A

EGF 1

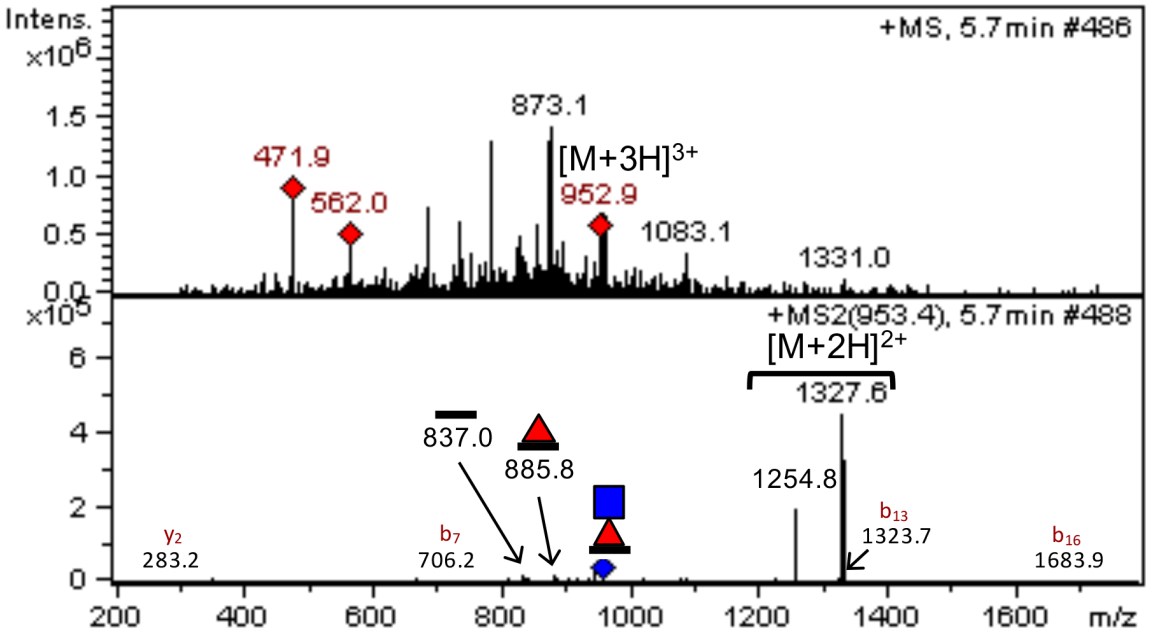
N[61]SCTSVGCQNGGTCVTQLNGKTY[82]

-Fringe



—	836.8;1254.7
▲	885.5;1327.7
■▲	953.2;1429.2

+Fringe



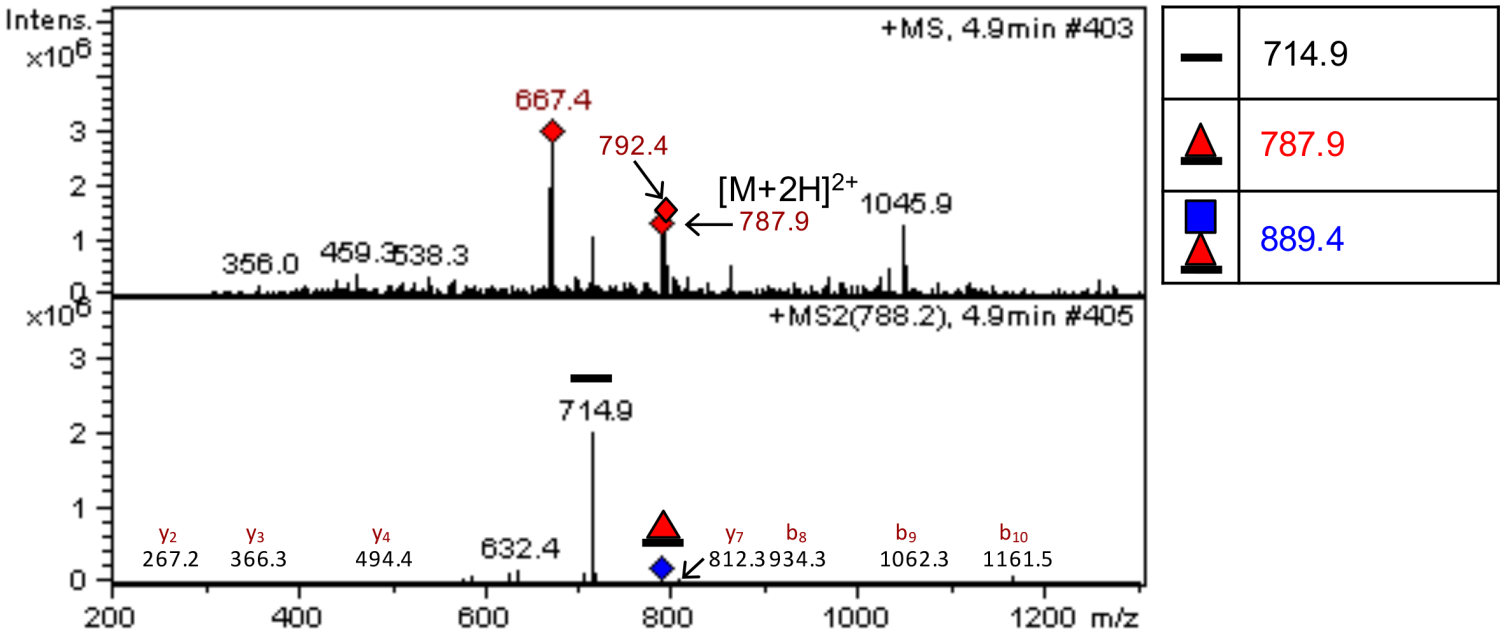
—	837.0;1254.8
▲	885.7;1327.8
■▲	954.4;1429.3

Fig. S3, B

EGF 2

^{104}RCQNGGTCQVTF^{115}

-Fringe



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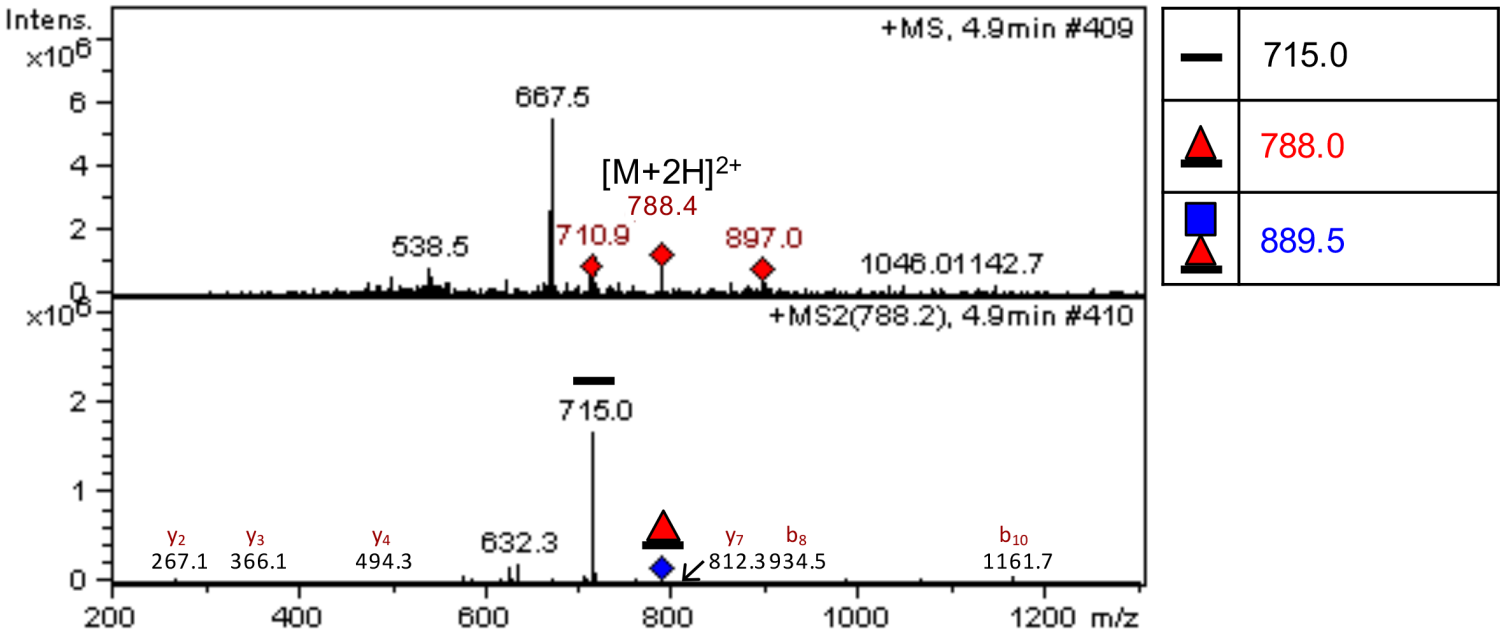
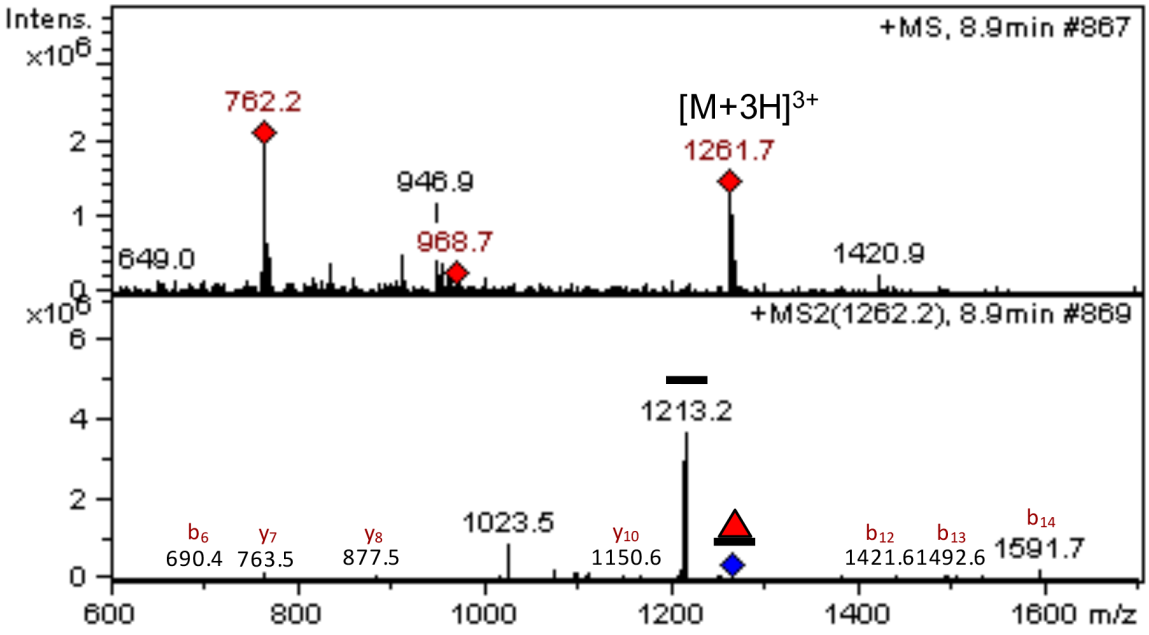


Fig. S3, C

EGF 3

^{126}CPLGFDESLCEIAVPNACDHVTCLNGGTCQLK^{157}

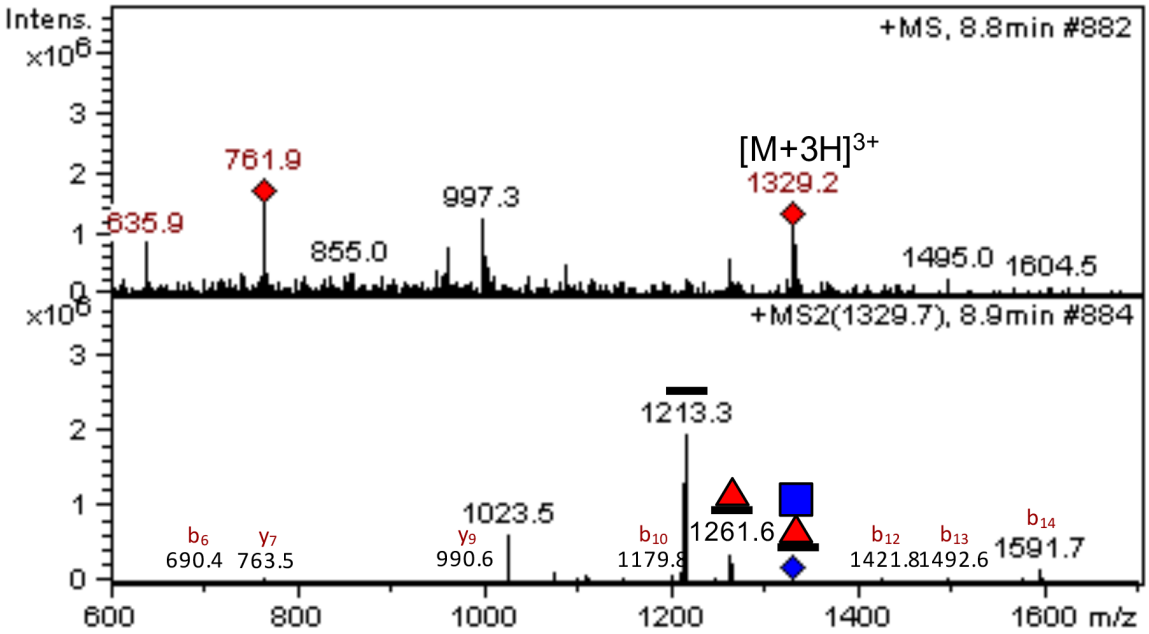
-Fringe



For EICs	
—	1213.2;910.2
▲	1261.9;946.7
■▲	1329.6;997.4

For MRM	
—	1214.0→i764.1
▲	1262.5→i1214.0
■▲	1330.3→i1214.0

+Fringe



For EICs	
—	1213.3;910.3
▲	1262.0;946.8
■▲	1329.7;997.5

For MRM	
—	1214.0→i764.1
▲	1262.5→i1214.0
■▲	1330.3→i1214.0

Fig. S3, D

EGF 3

^{158}TLEEYTCACANGYTGER^{174}

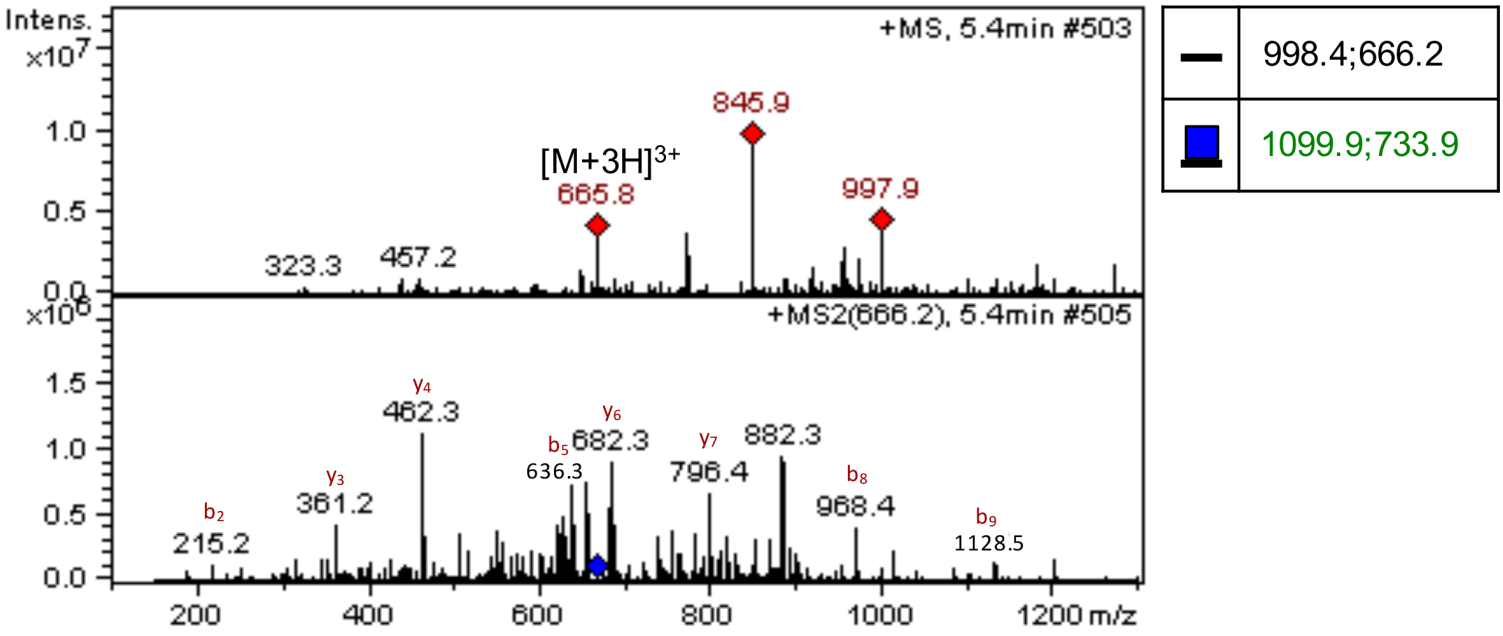


Fig. S3, E

EGF 4

^{179}NLCASSPCR^{187}

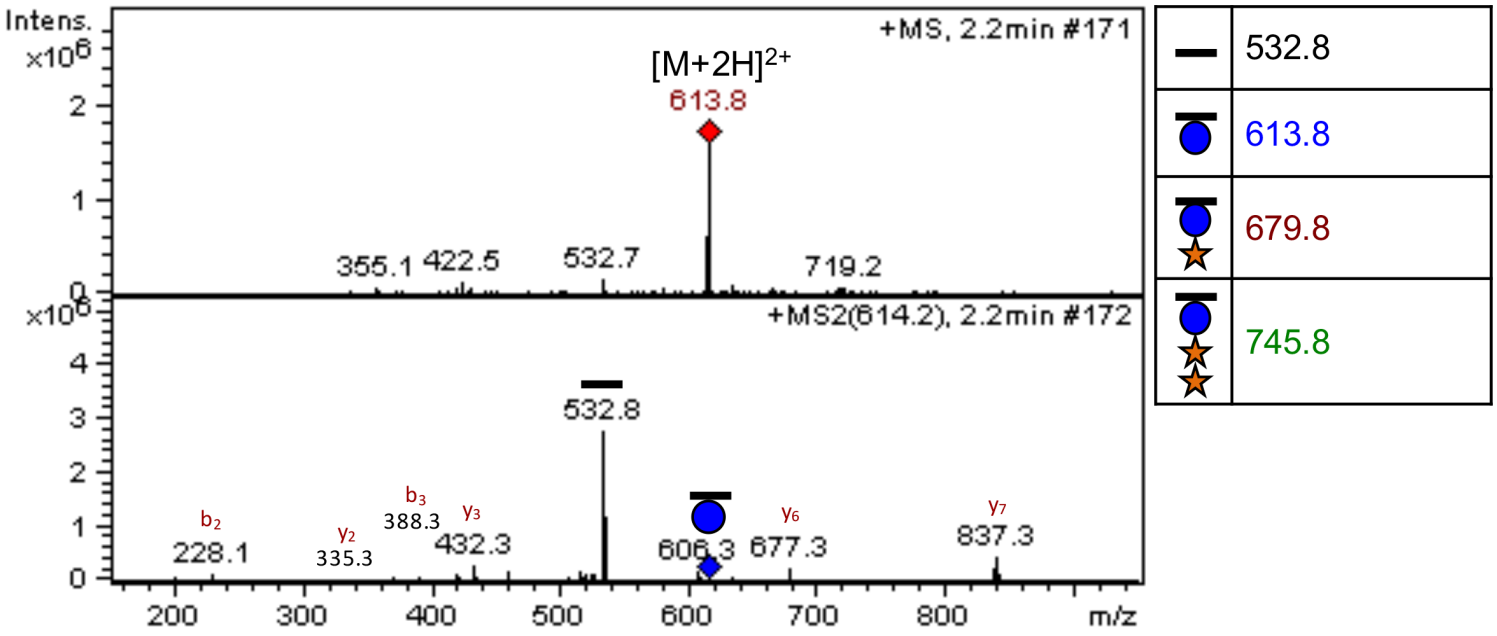
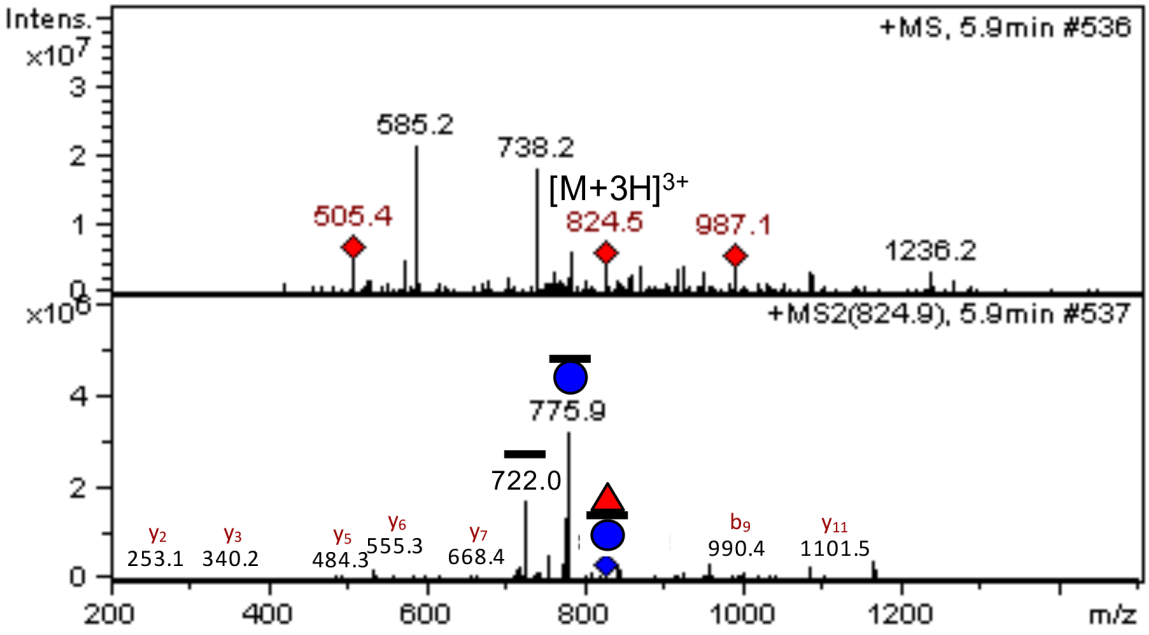


Fig. S3, F

EGF 4

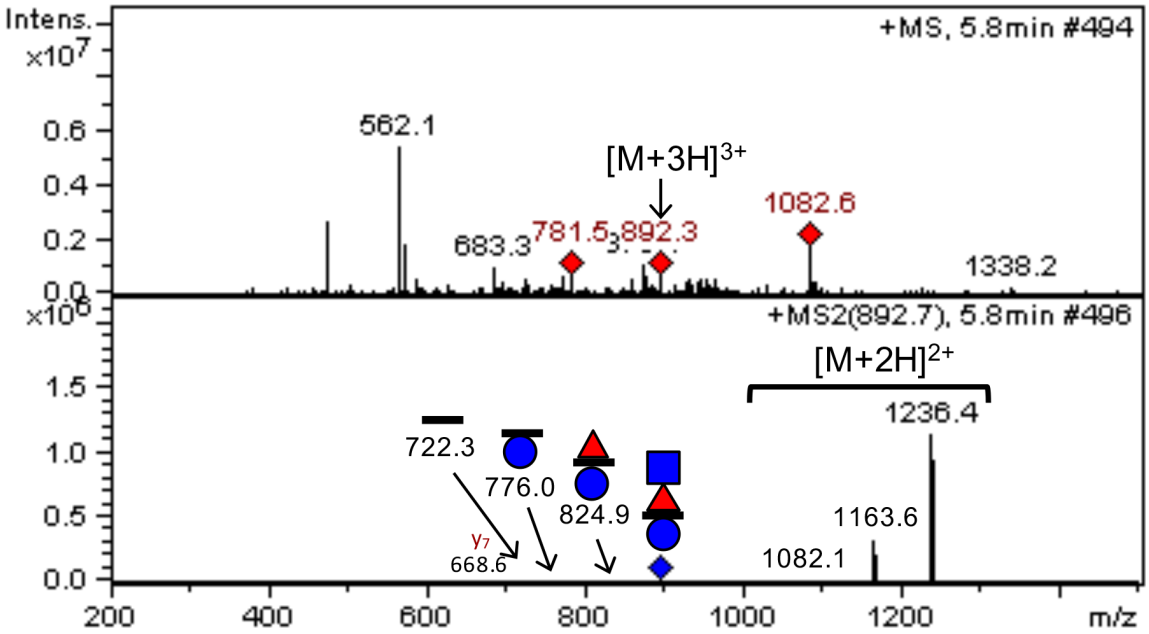
^{181}CASSPCRNGATCTALAGSSSF^{201}

-Fringe



	1082.5;722.0; 1163.5;776.0
	1155.5;770.7; 1236.5;824.7
	1257.0;838.4; 1338.0;892.4

+Fringe



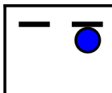
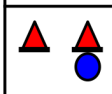
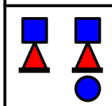
	1082.1;722.3 1163.1;776.3
	1155.1;771.0 1236.1;825.0
	1256.6;838.7; 1337.6;892.7

Fig. S3, G

EGF 4

^{202}TCSCPPGFTGDTCSY^{216}

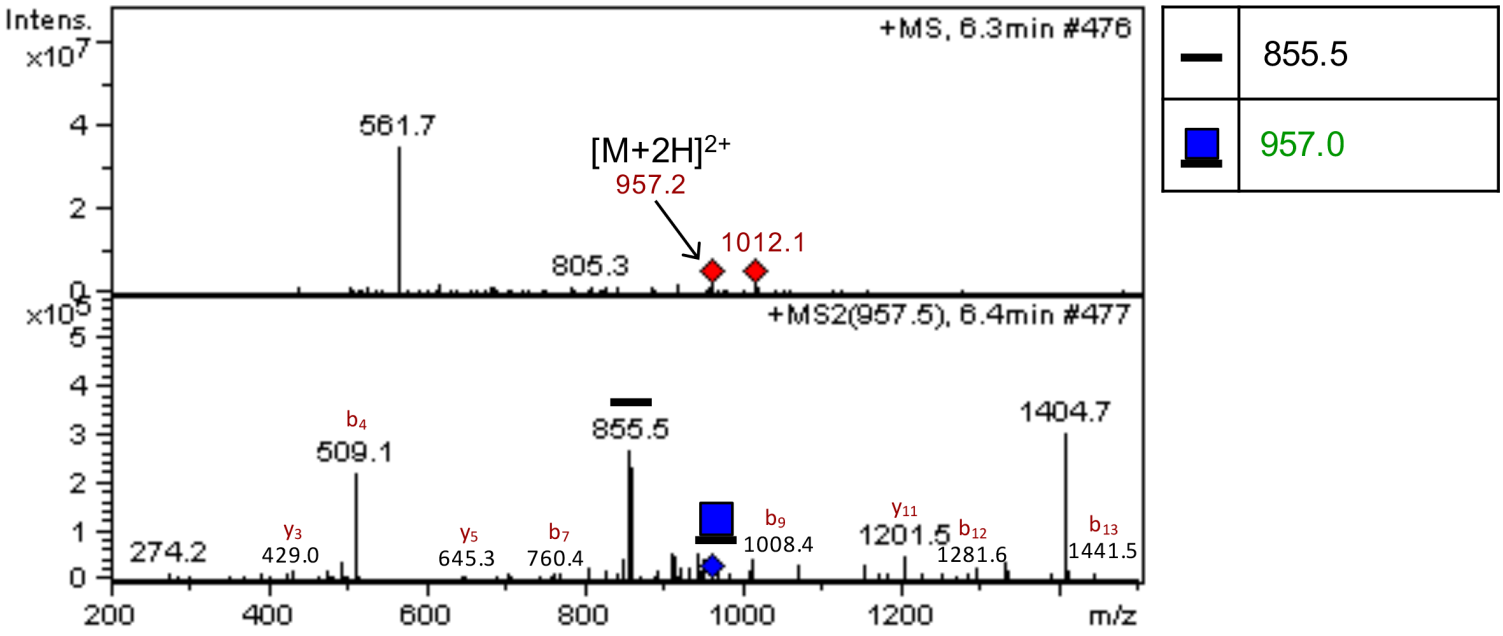


Fig. S3, H

EGF 5

^{217}DIEECQ**S**NPCKY^{228}

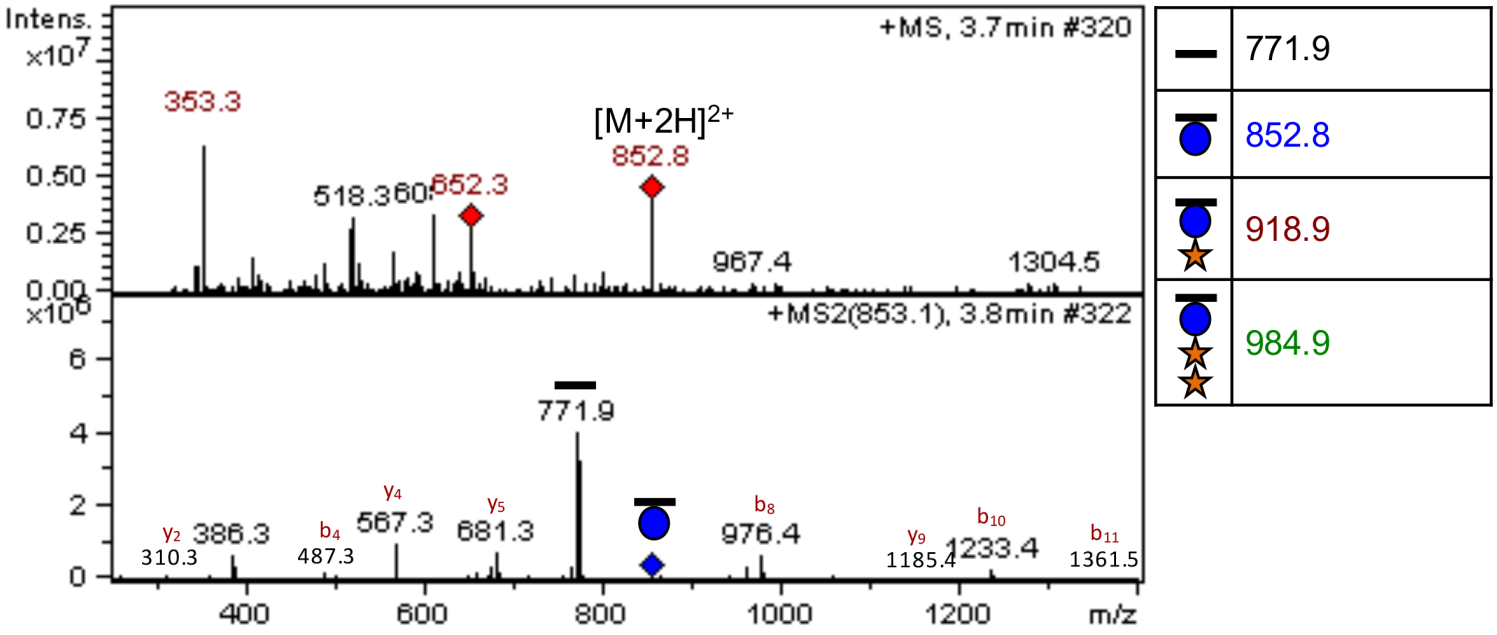
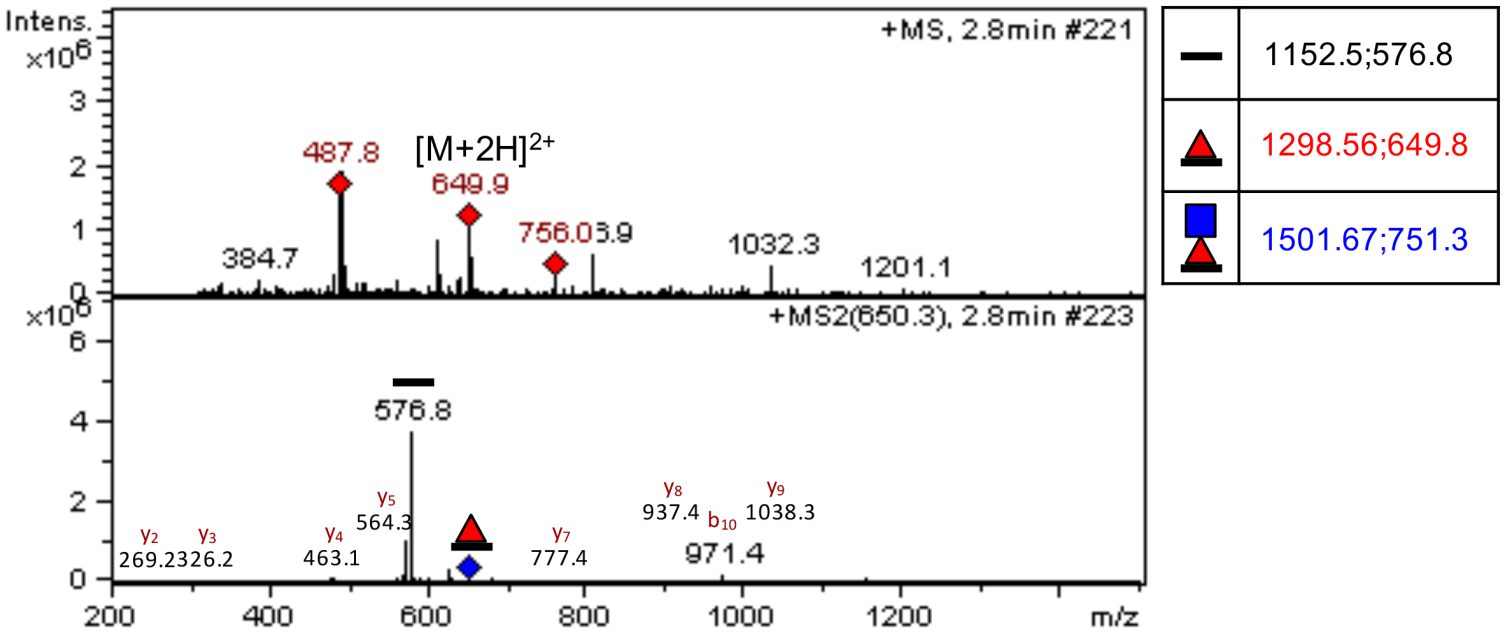


Fig. S3, I

EGF 5

^{229}GG**T**CVNTHGSY^{239}

-Fringe



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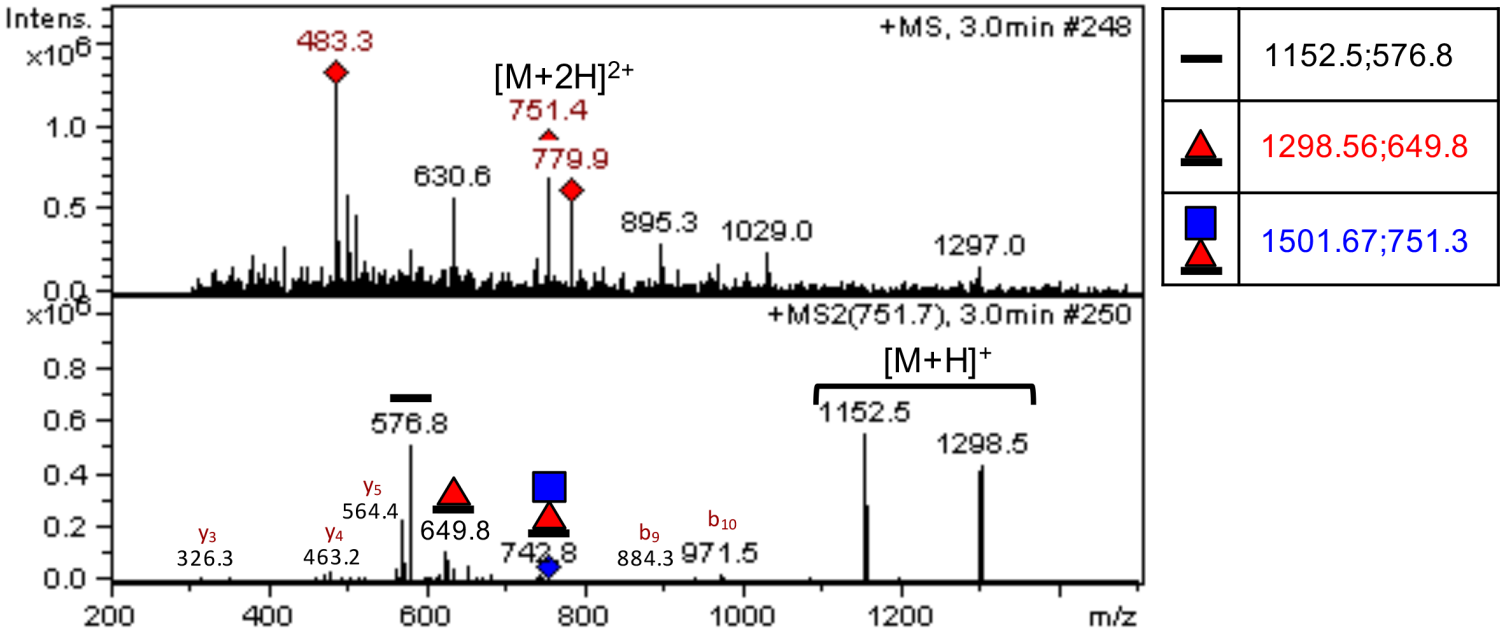
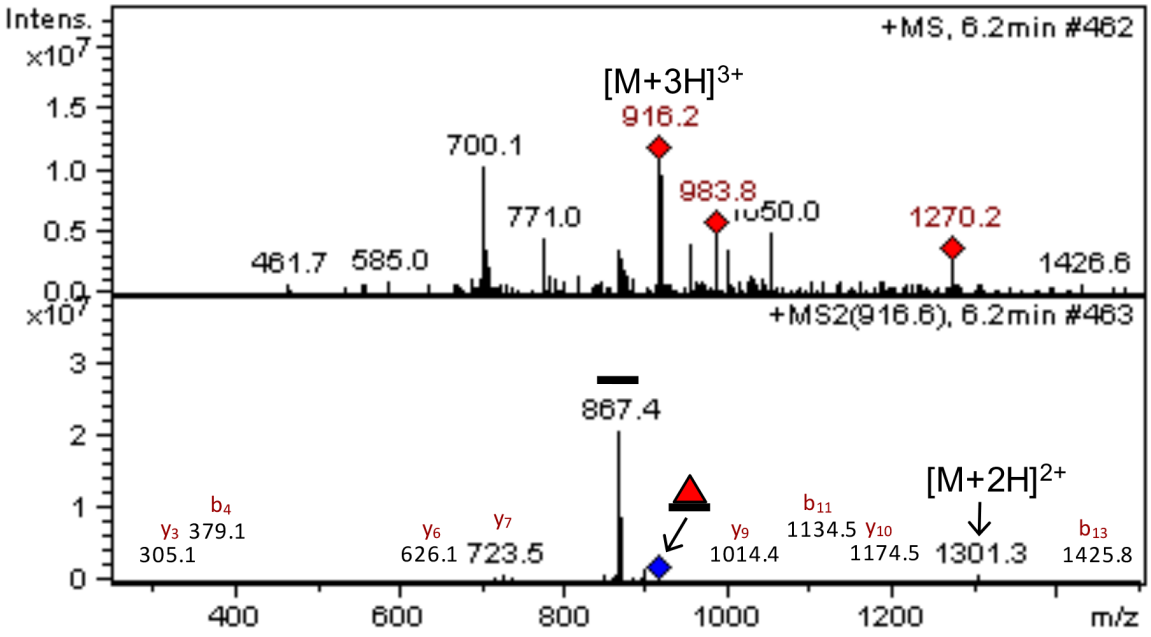


Fig. S3, J

EGF 5

^{228}YGG**T**CVNTHGSYQCMCPTGY**T**GK^{250}

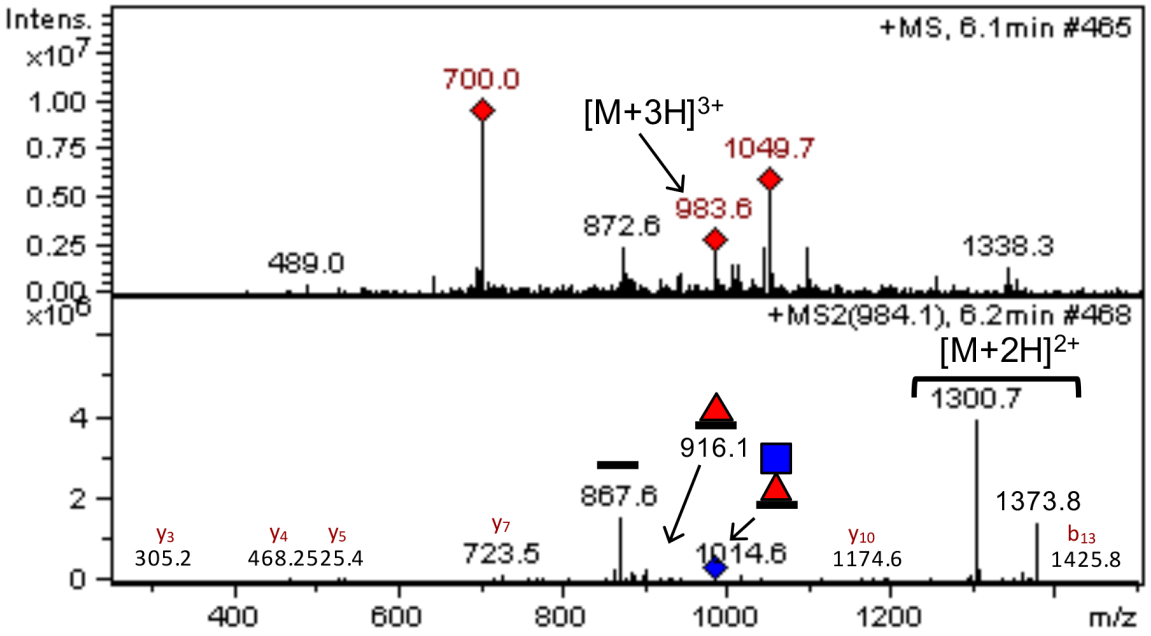
-Fringe



For EICs	
—	867.4;1301.3
▲	916.1;1374.3
■▲	983.8;1475.8

For MRM	
—	867.6→i787.6
▲	916.4→i867.6
■▲	984.1→i867.6

+Fringe



For EICs	
—	867.6;1300.7
▲	1373.7;916.3
■▲	1475.2;984.0

For MRM	
—	867.6→i787.6
▲	916.4→i867.6
■▲	984.1→i867.6

Fig. S3, K

EGF 5

^{248}TGKDCDTKYKPCSPSPCQNGGICRSNGLSY^{277}

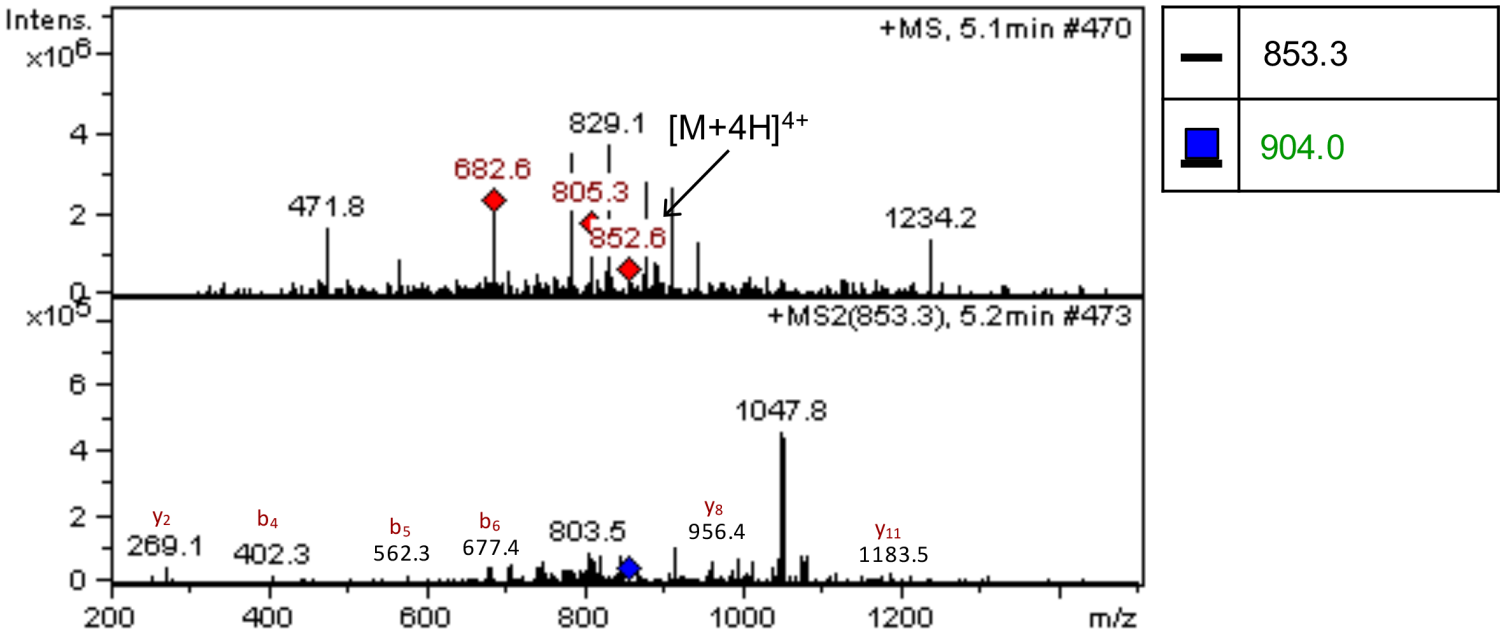
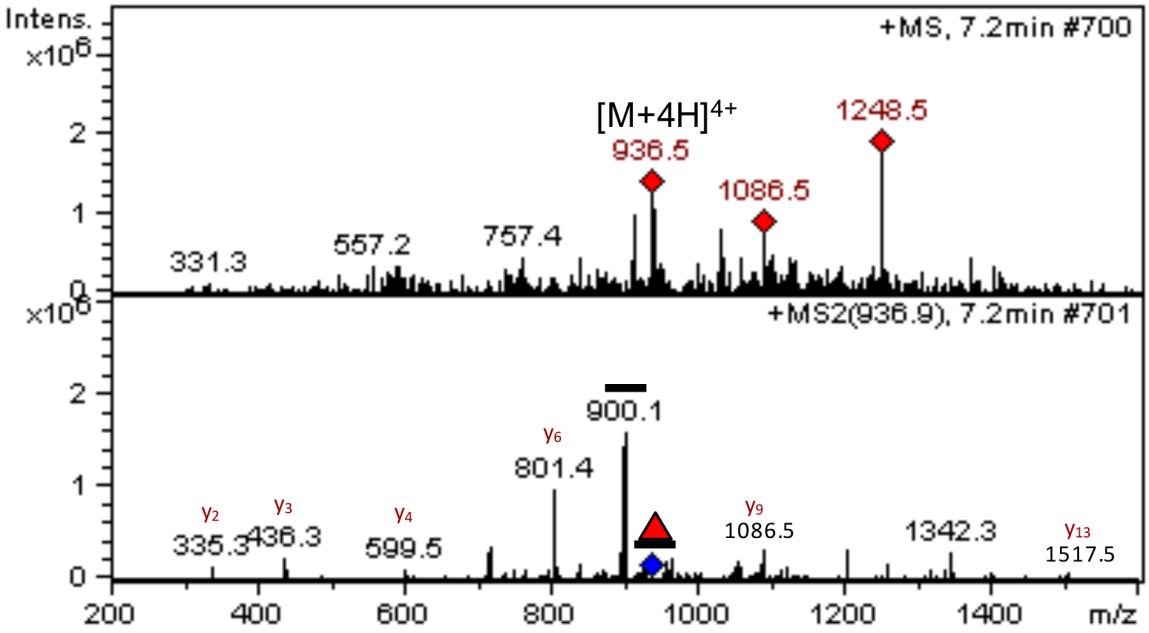


Fig. S3, L

EGF 7

^{289}NCEQNYDDCLGHLCQNGG**T**CIDGISDYTCR^{318}

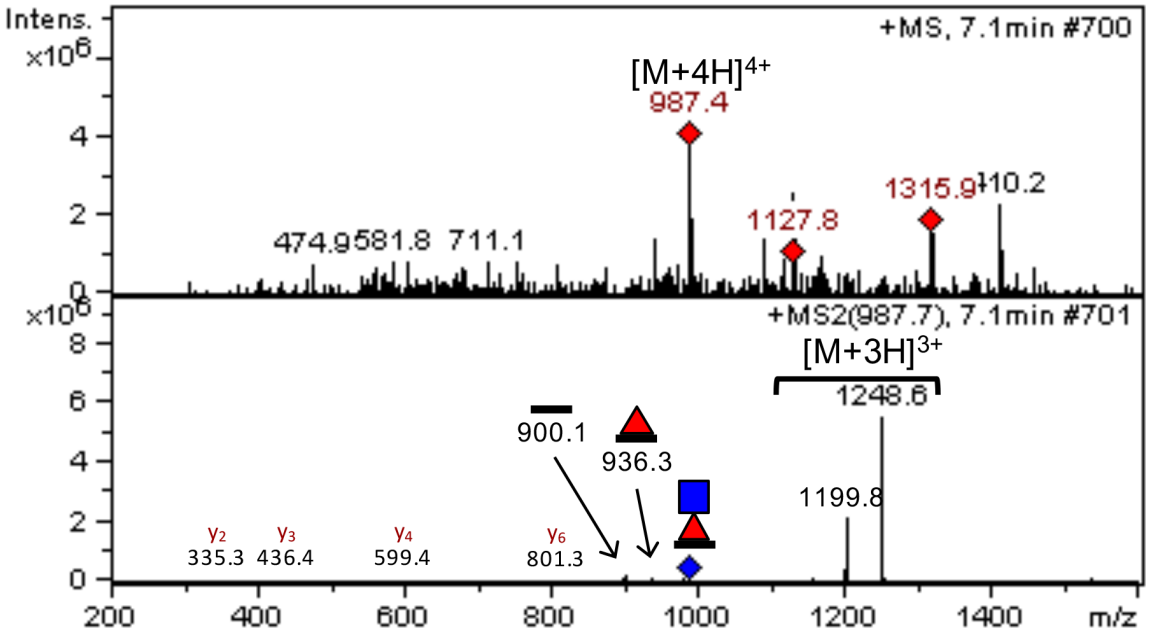
-Fringe



For EICs	
—	1199.8;900.1
▲	1248.5;936.6
■▲	1316.2;987.3

For MRM	
—	1199.9→i1313.7
▲	1248.4→i1199.9
■▲	1316.4→i1199.9

+Fringe



For EICs	
—	1199.8;900.1
▲	1248.5;936.6
■▲	1316.2;987.3

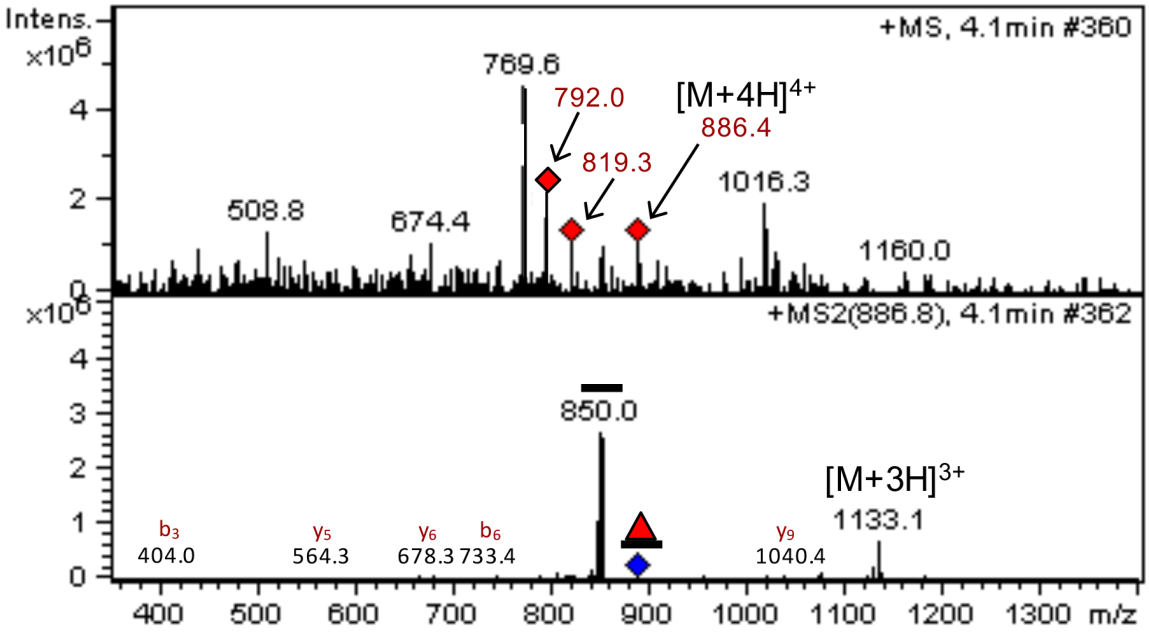
For MRM	
—	1199.9→i1313.7
▲	1248.4→i1199.9
■▲	1316.4→i1199.9

Fig. S3, M

EGF 8

^{328}CQDDVDECAQRDHPVCQNGA**T**CTNTHGSY^{356}

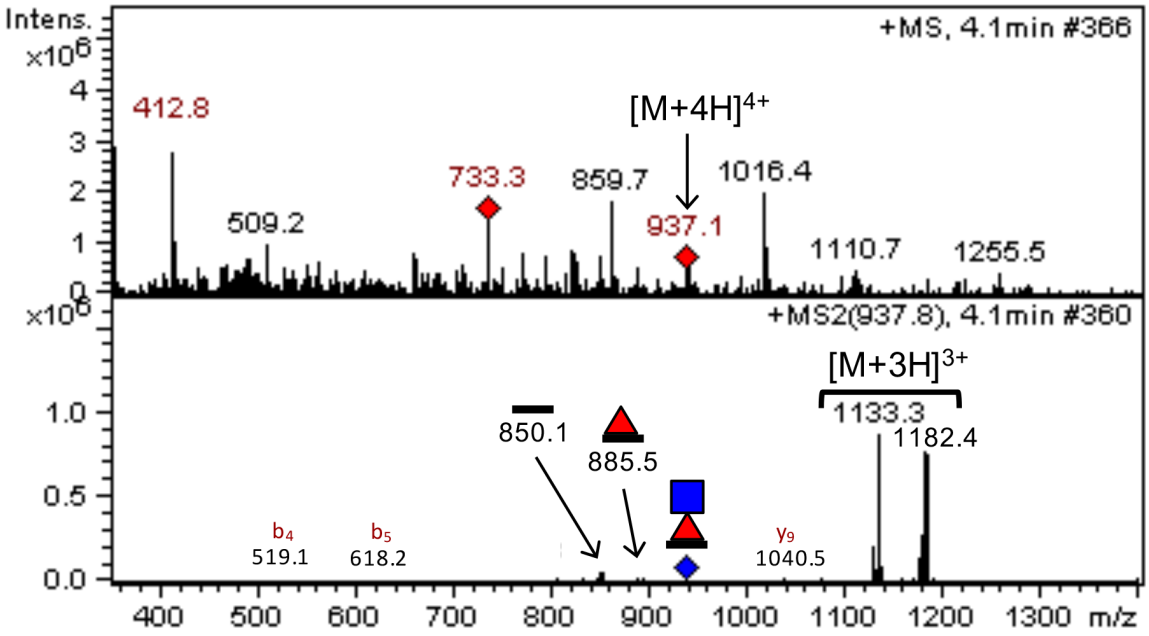
-Fringe



For EICs	
—	1133.1;850.0
▲	1181.8;886.5
■▲	1249.5;937.2

For MRM	
—	1133.0→i1534.7
▲	1181.8→i1133.0
■▲	1250.5→i1133.0

+Fringe



For EICs	
—	1133.3;850.1
▲	1182.0;886.6
■▲	1249.7;937.3

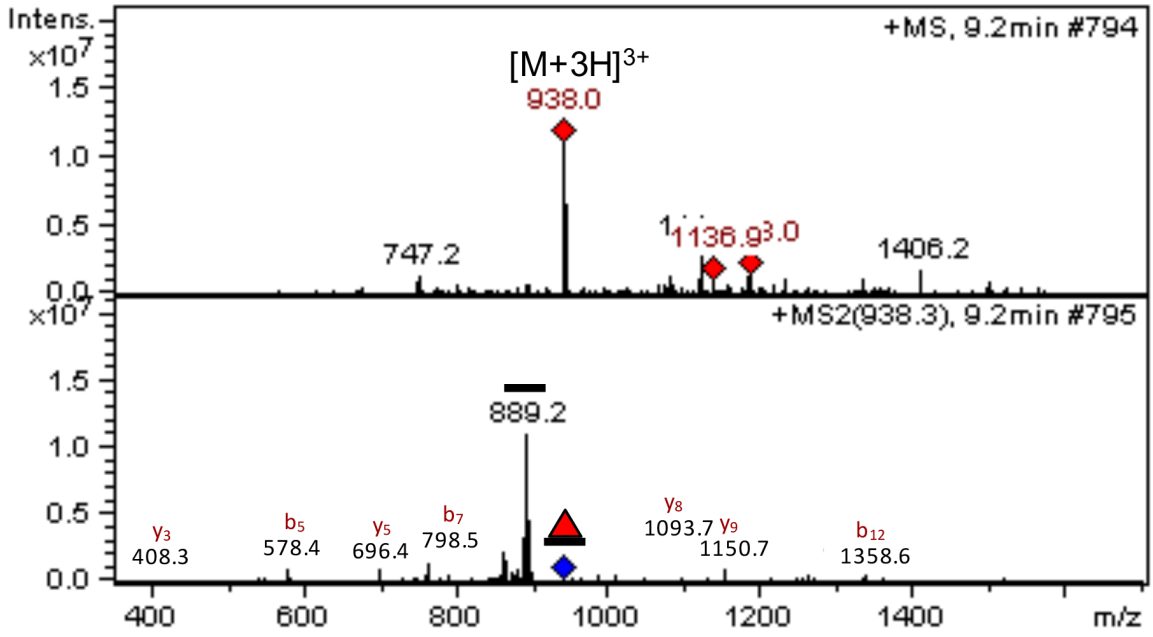
For MRM	
—	1133.0→i1534.7
▲	1181.8→i1133.0
■▲	1250.5→i1133.0

Fig. S3, N

EGF 9

^{378}QAACFYGA**T**CIDGVGSFYCQCTK^{400}

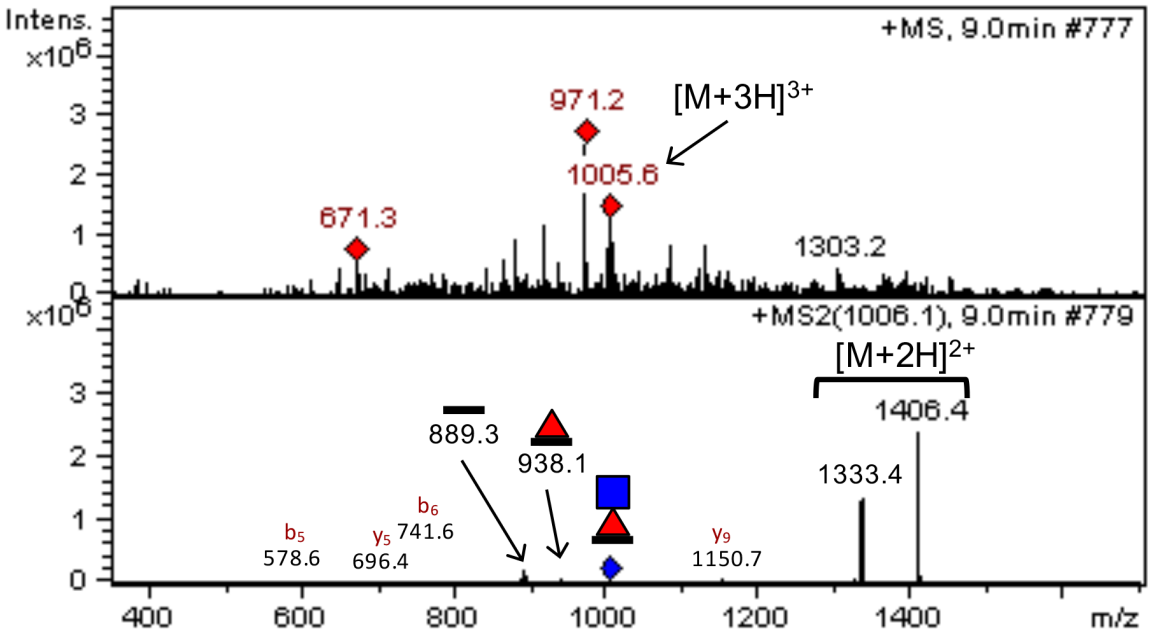
-Fringe



For EICs	
—	889.2;1333.4
▲	937.9;1406.4
■▲	1005.6;1507.9

For MRM	
—	1333.3→i1307.0
▲	1406.7→i1333.3
■▲	1508.2→i1333.3

+Fringe



For EICs	
—	889.3;1333.4
▲	938.0;1406.4
■▲	1005.7;1507.9

For MRM	
—	1333.3→i1307.0
▲	1406.7→i1333.3
■▲	1508.2→i1333.3

Fig. S3, O

EGF 9

^{396}CQCTKGK**T**GLL^{406}

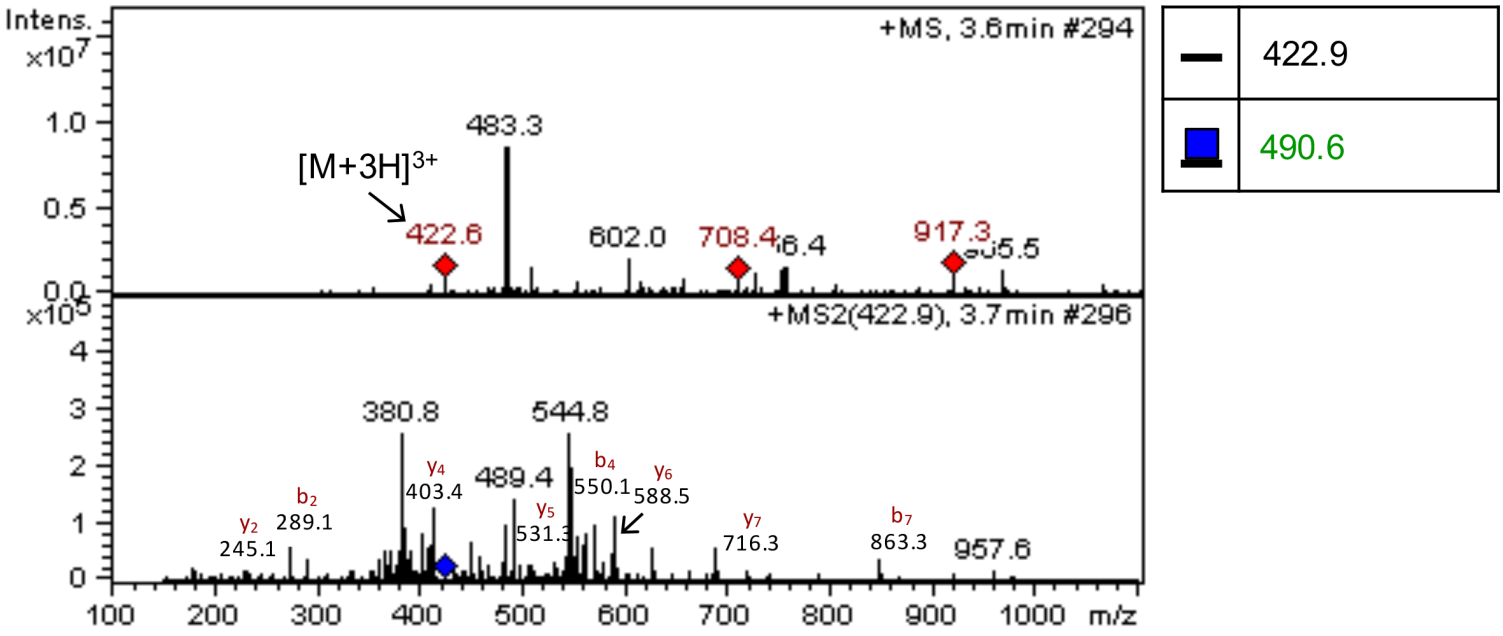


Fig. S3, P

EGF 9 and EGF 10

^{403}**T**GLLCHLDDACTSNPCHADAICDT**S**PINGSYACSCATGYK^{442}

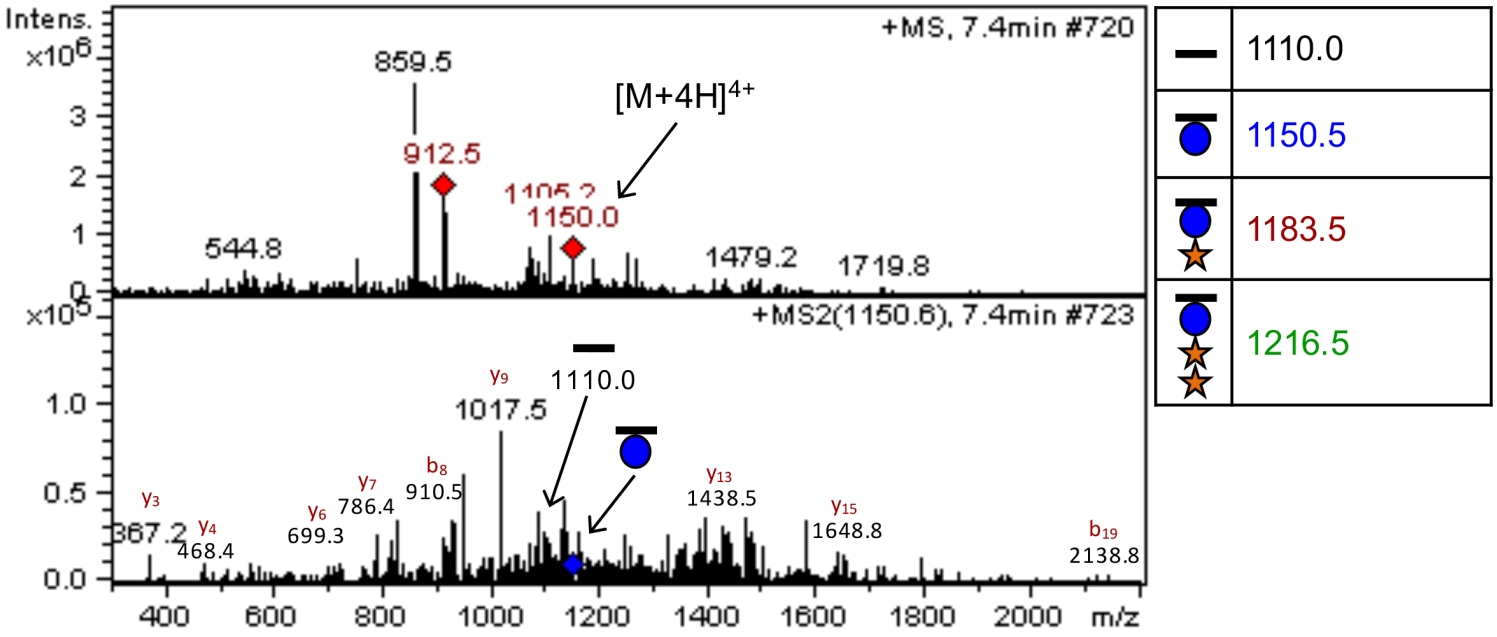


Fig. S3, Q

EGF 11

^{474}CNCSQGF**T**GPR^{484}

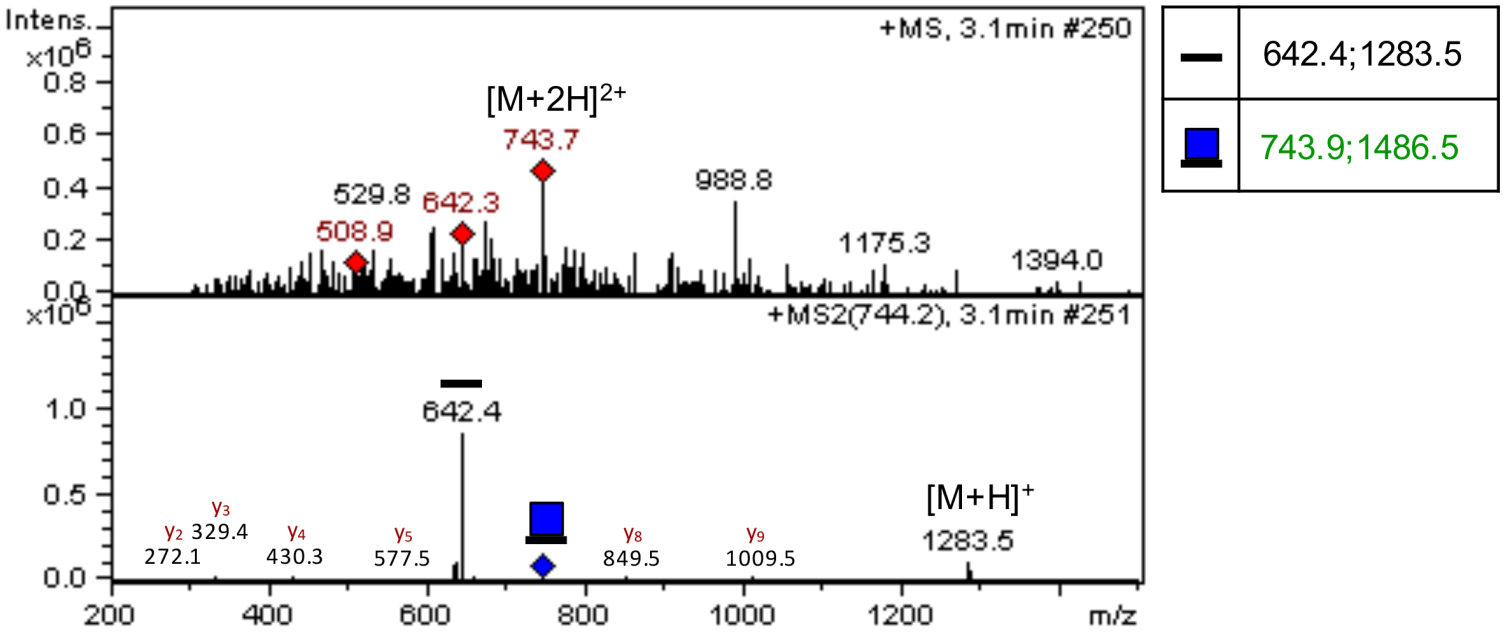
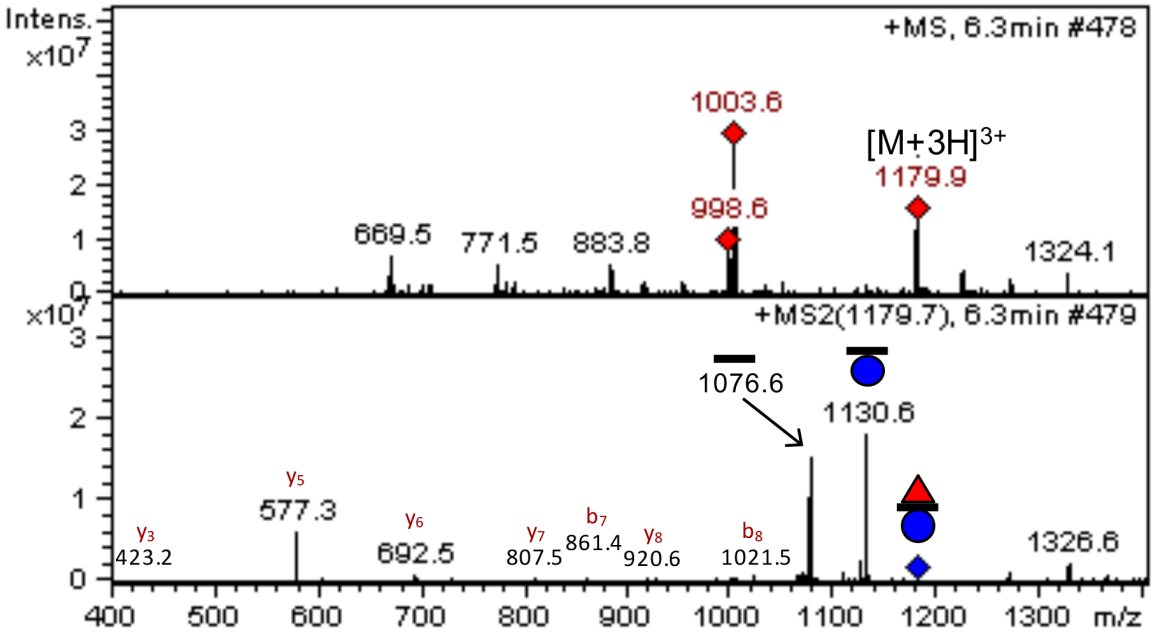


Fig. S3, R

EGF 12

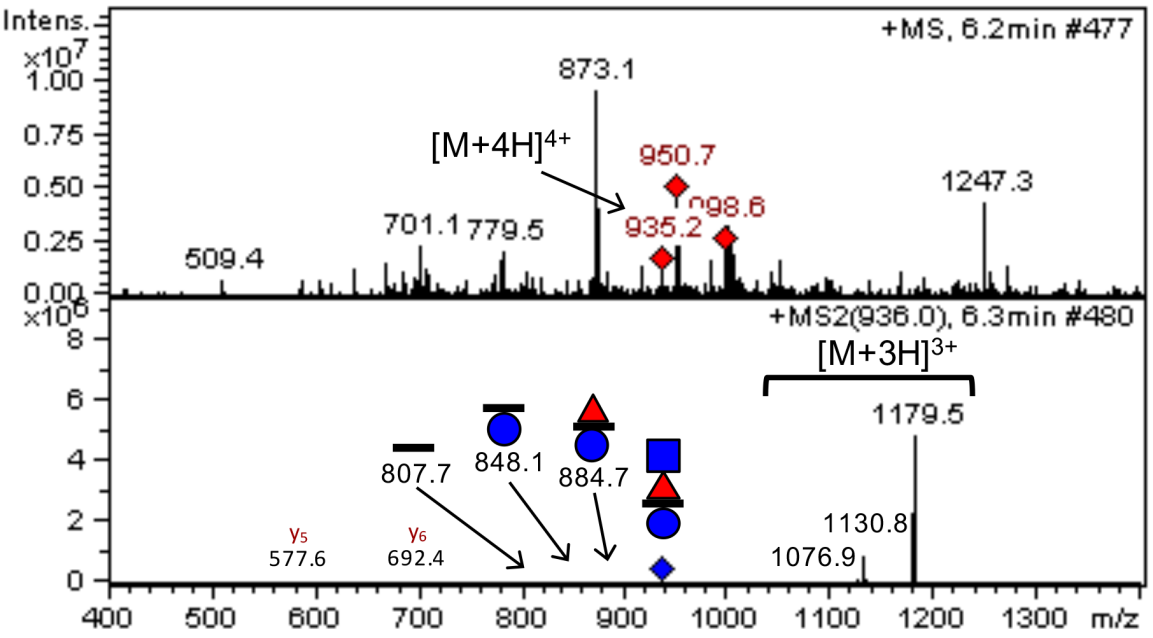
^{485}CETNINECE**S**HPCQNEG**S**CLDDPGTFR^{511}

-Fringe



	1076.6;807.7; 1130.6;848.2
	1125.3;844.2; 1179.3;884.7
	1193.0;894.9; 1247.0;935.4
	1076.6;807.7; 1125.3;844.2
	1130.6;848.2 1179.3;884.2
	1174.6;881.2 1223.3;917.7
	1218.6;914.2; 1267.3;950.7

+Fringe



	1076.9;807.7; 1130.9;848.2
	1125.6;844.2 1179.6;884.7
	1193.3;894.9; 1247.3;935.4

Fig. S3, S

EGF 12

^{501}G**S**CLDDPGTFRCVCMPGF**T**GTQCE^{524}

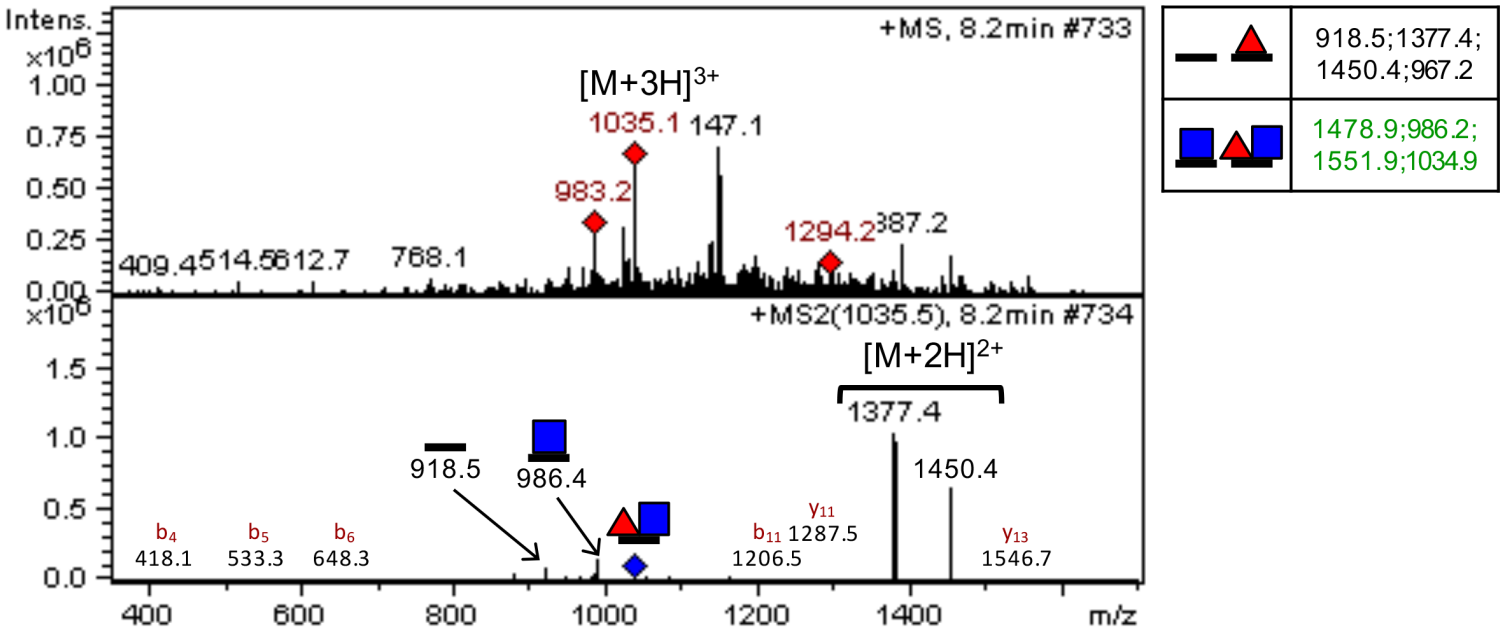
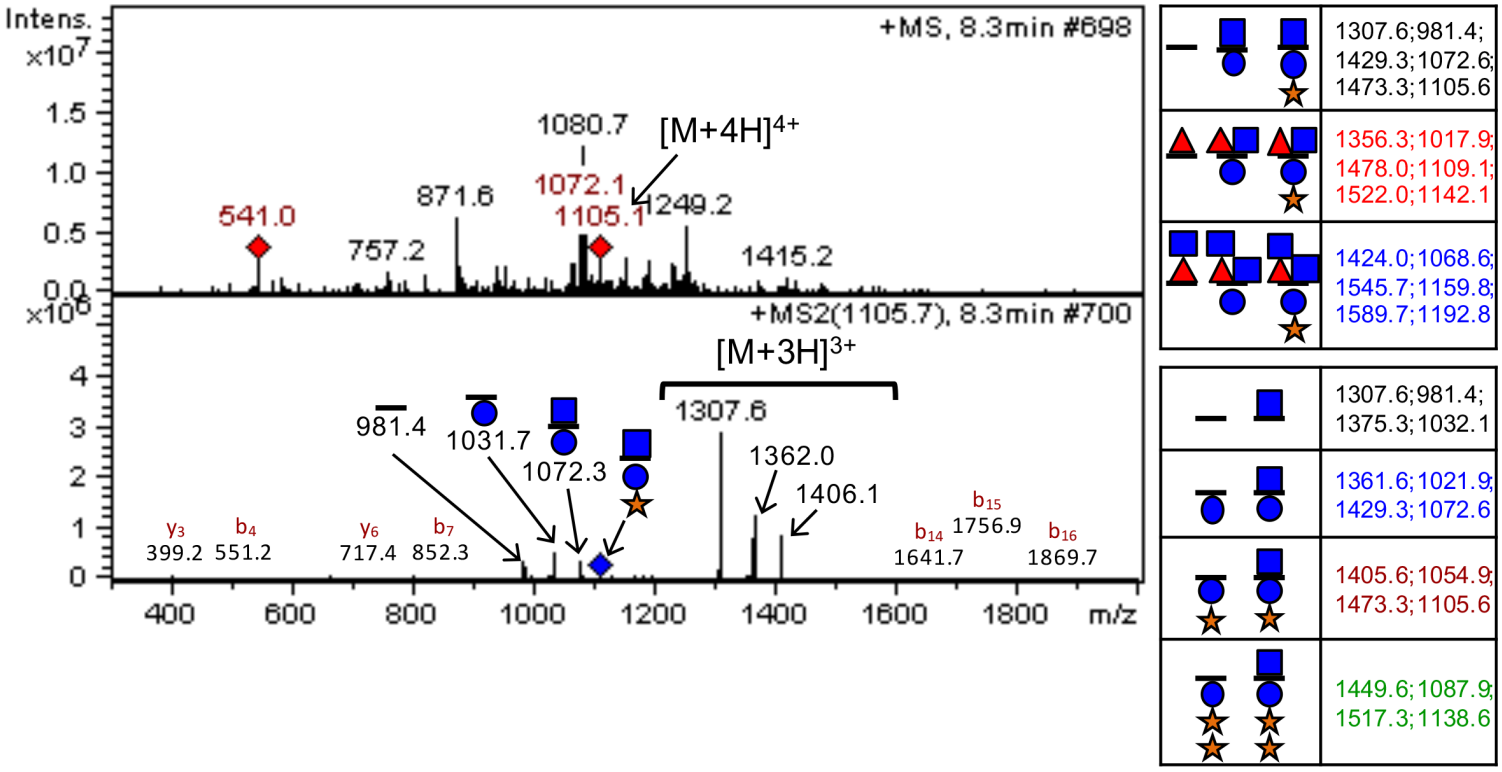


Fig. S3, T

EGF 12 and EGF 13

^{512}CVCMPGFTGTQCEIDIDECQSNPCLNDGTCHDK^{544}

-Fringe



+Fringe

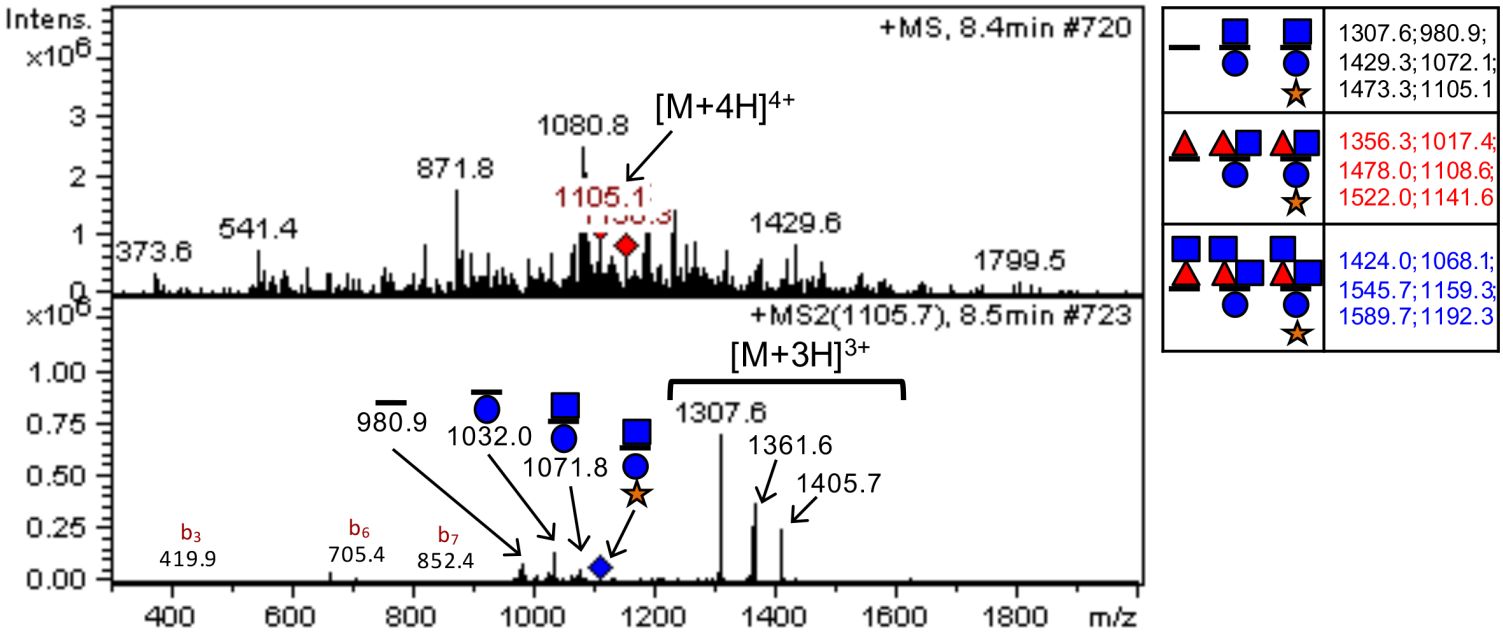


Fig. S3, U

EGF 13

^{550}CSCALGF**T**GAR^{560}

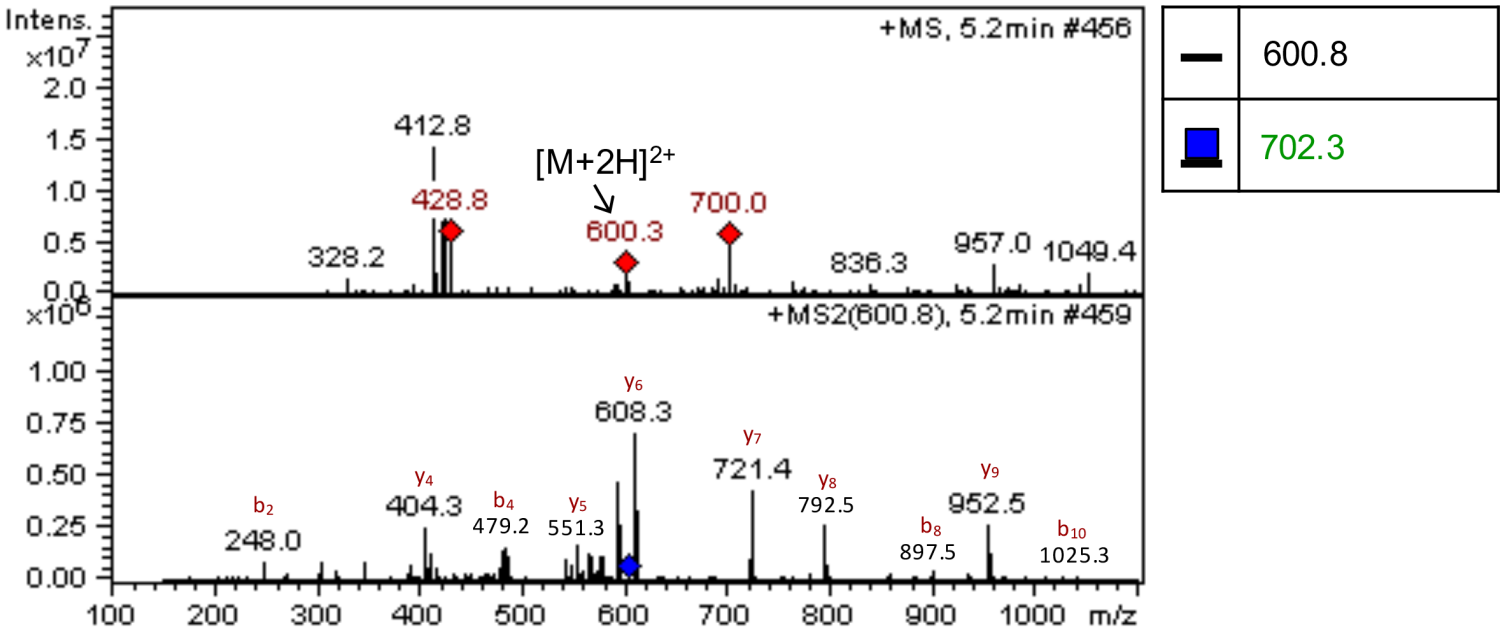


Fig. S3, V

EGF 13 and EGF 14

^{557}TGARCQINIDDCQSQPCRNRGICHDSIAGY^{586}

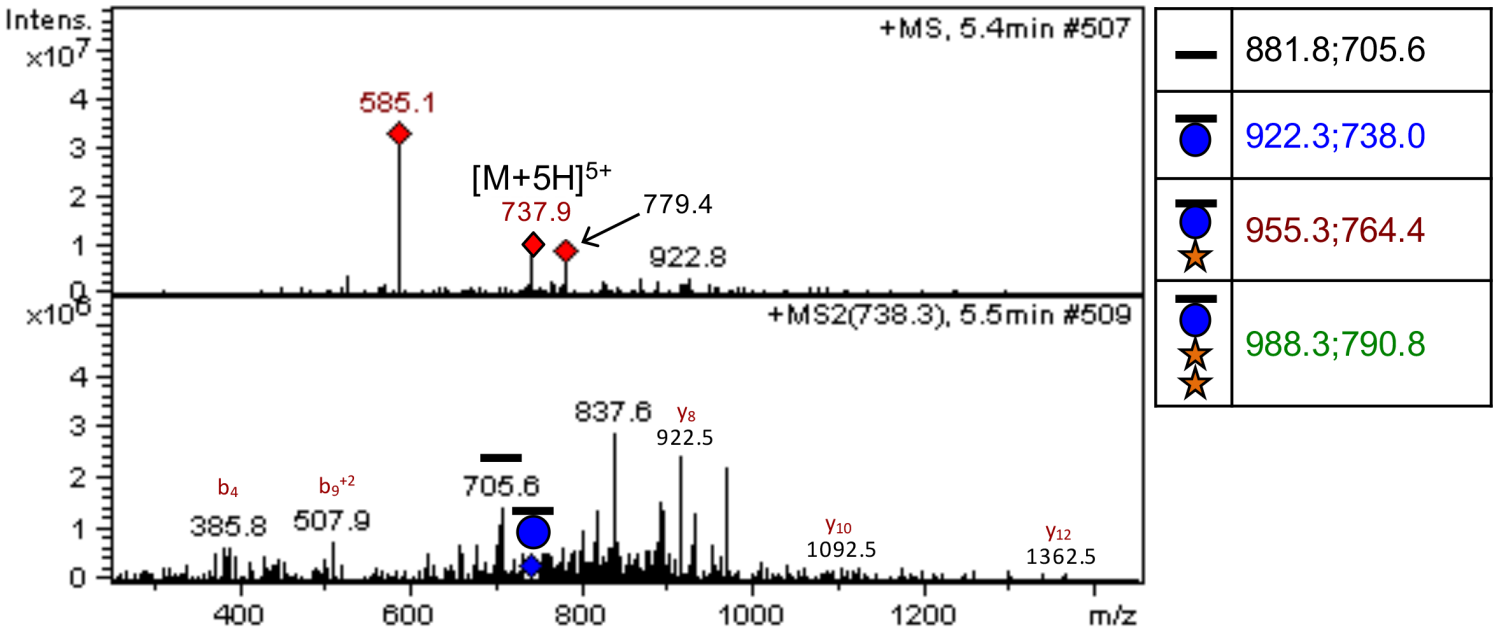
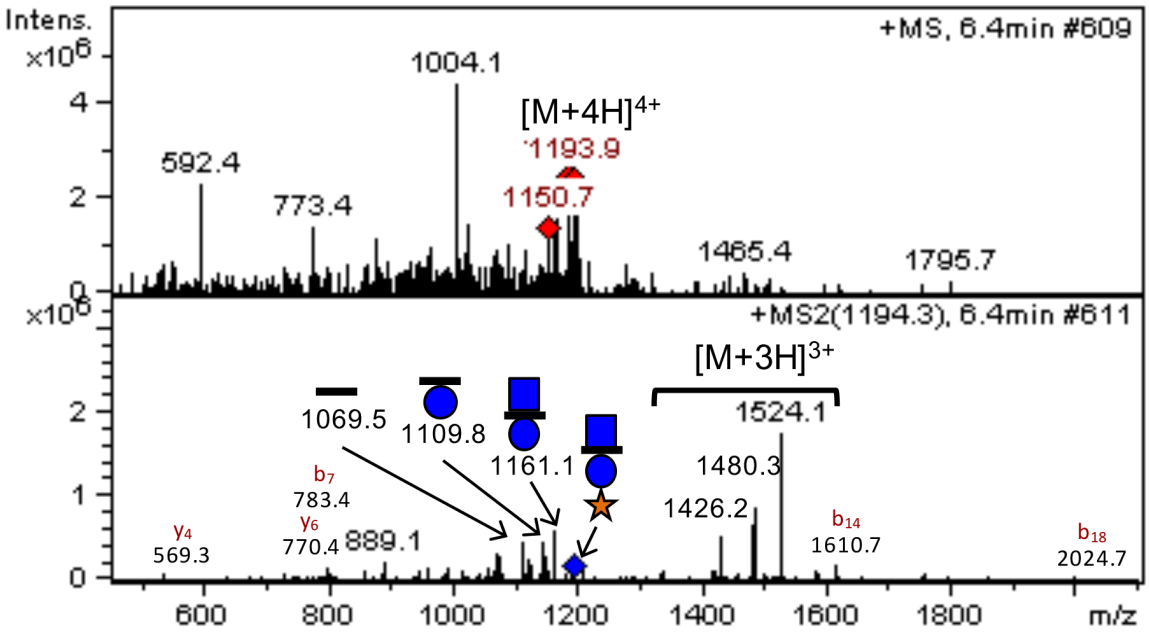
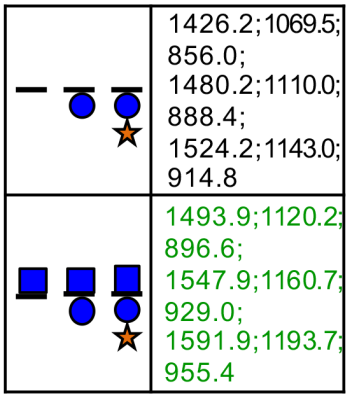


Fig. S3, W

EGF 14 and15

^{577}GICHDSIAGYSCECPPGY**T**GTSCEININDCD**S**NPCHR^{613}





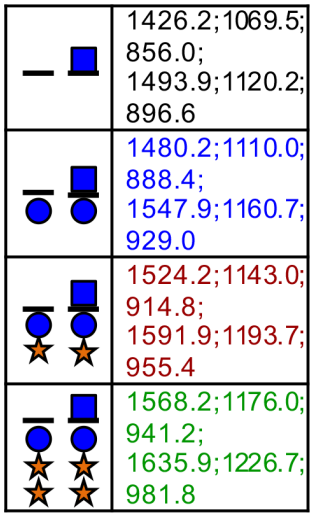


Fig. S3, X

EGF 15

^{625}CLCDPGY**T**GYICQK^{638}

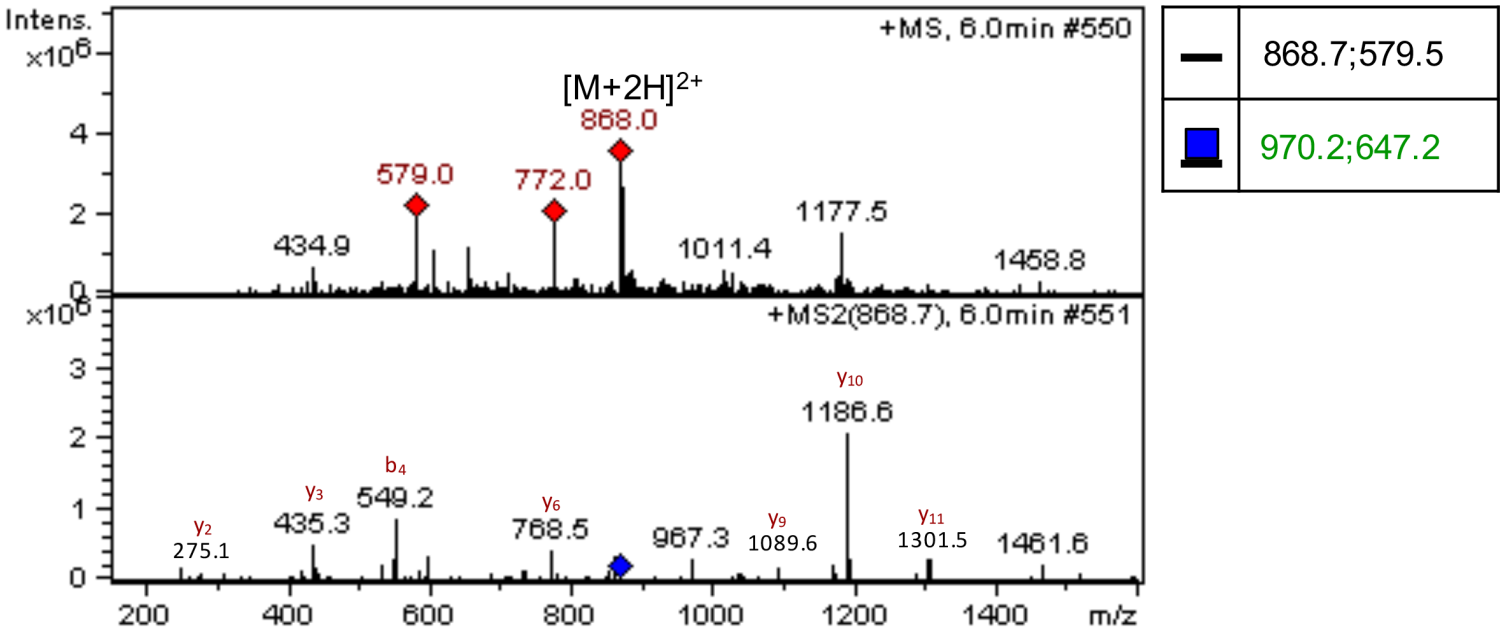


Fig. S3, Y

EGF 16

^{635}ICQKQINECE**S**NPCQF^{650}

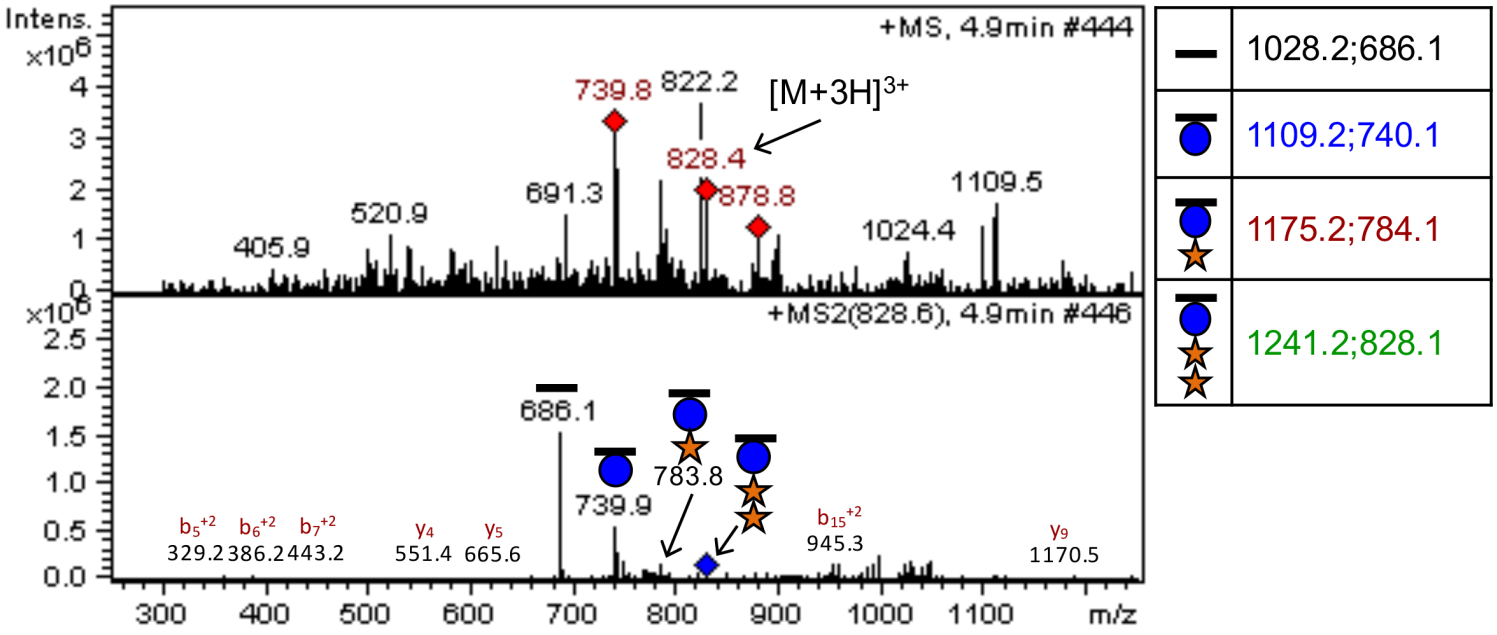


Fig. S3, Z

EGF 16

^{658}VGSYYCQCQAGT**S**GK^{672}

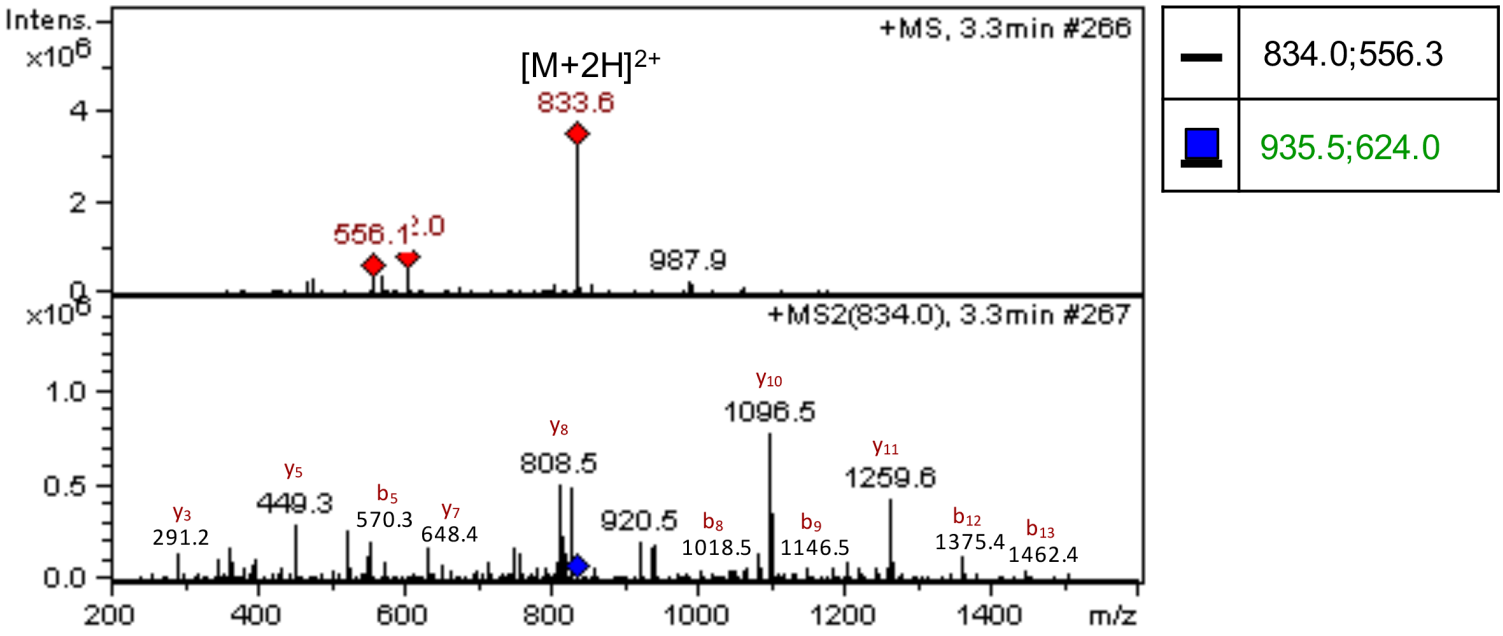
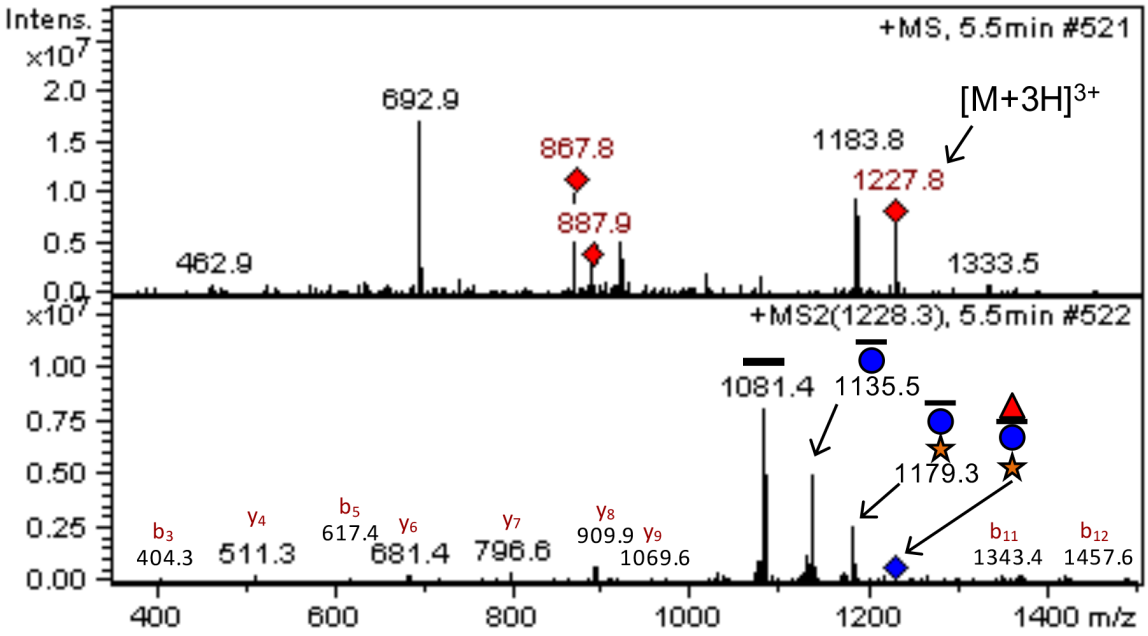


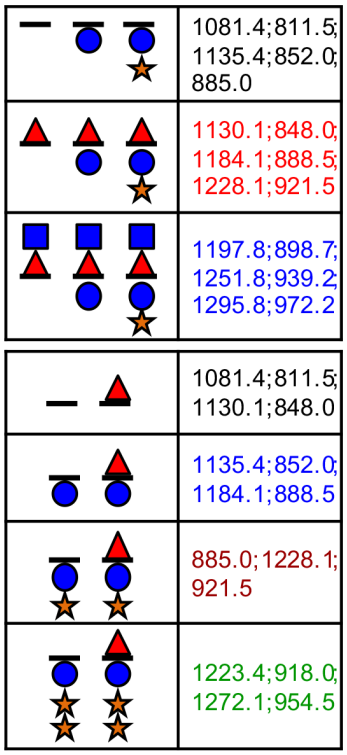
Fig. S3, A’

EGF 17

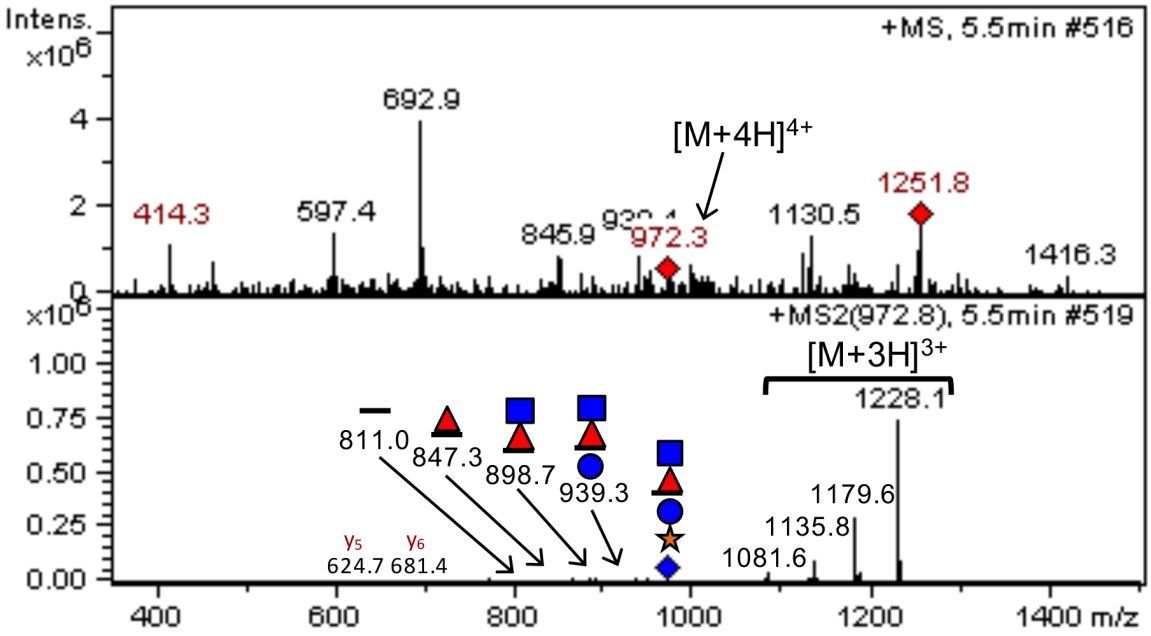
^{673}NCEVNVNECH**S**NPCNNGA**T**CIDGINSYK^{700}

-Fringe





+Fringe



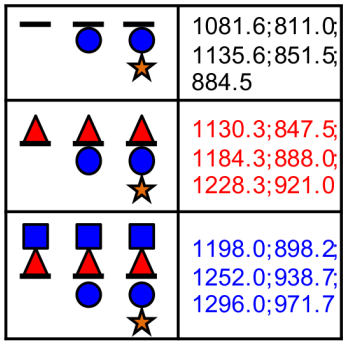


Fig. S3, B’

EGF 17

^{701}CQCVPGF**T**GQHCEK^{714}

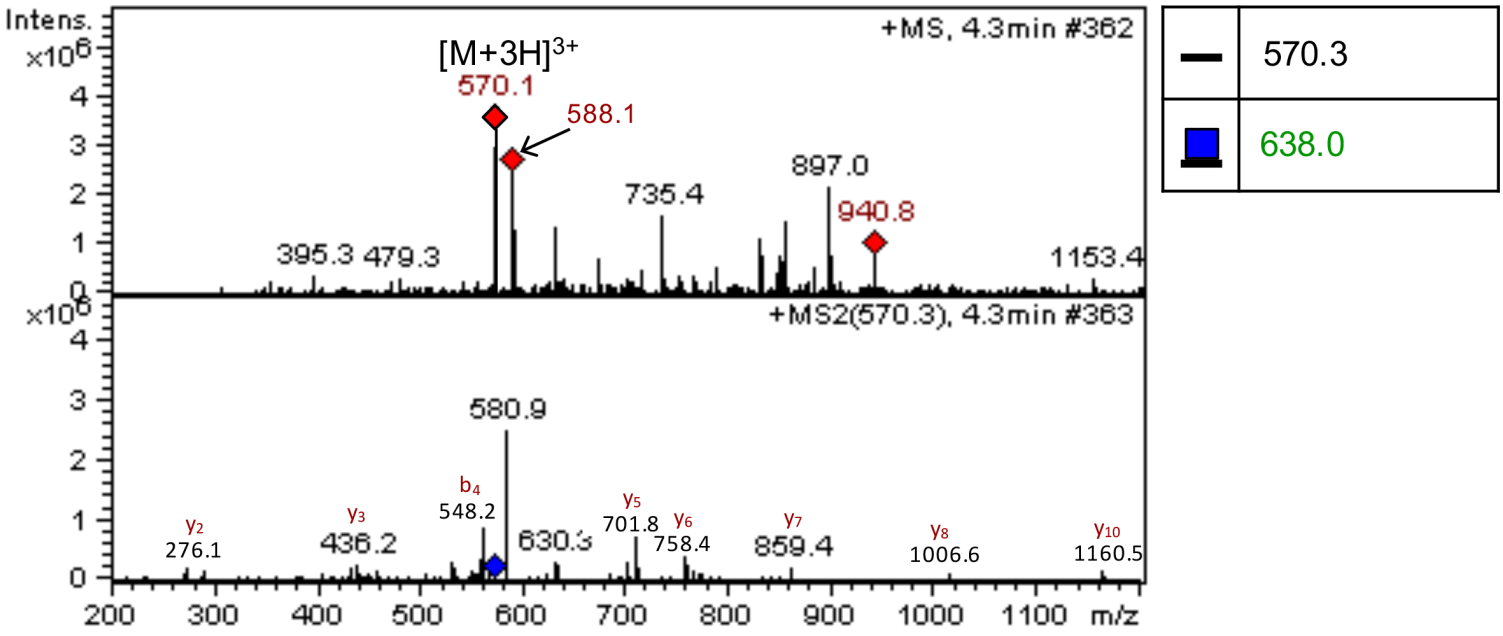


Fig. S3, C’

EGF 18

^{715}NVDECI**S**SPCANNGVCIDQVNGYK^{738}

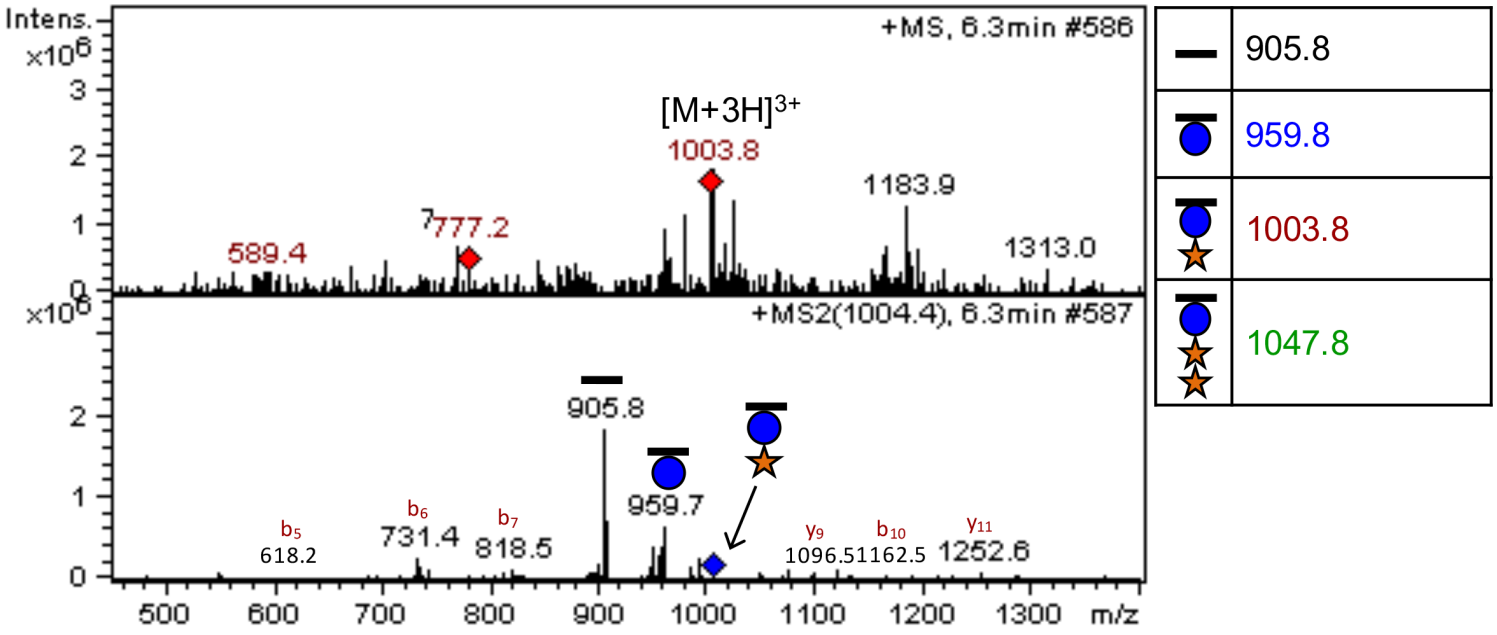


Fig. S3, D’

EGF 19

^{744}GFYDAHCLSDVDECA**S**NPCVNEGR^{767}

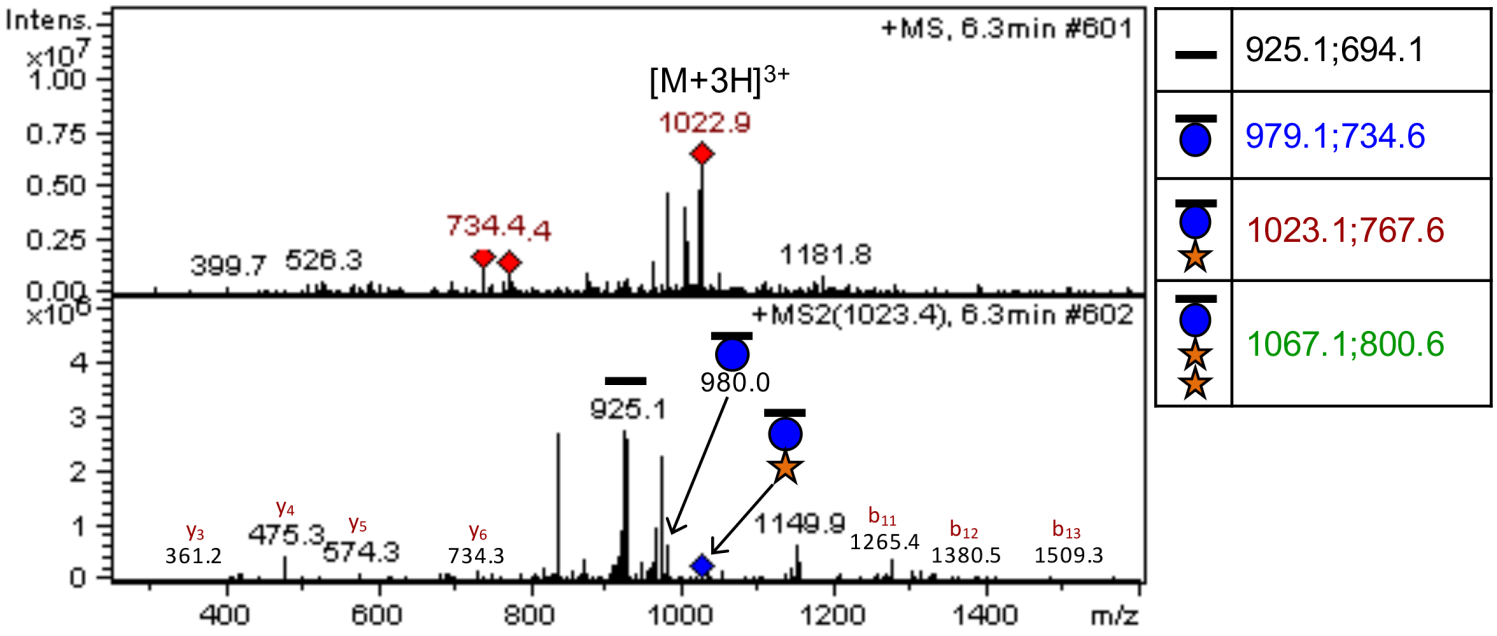


Fig. S3, E’

EGF 19

^{768}CEDGINEFICHCPPGY**T**GK^{786}

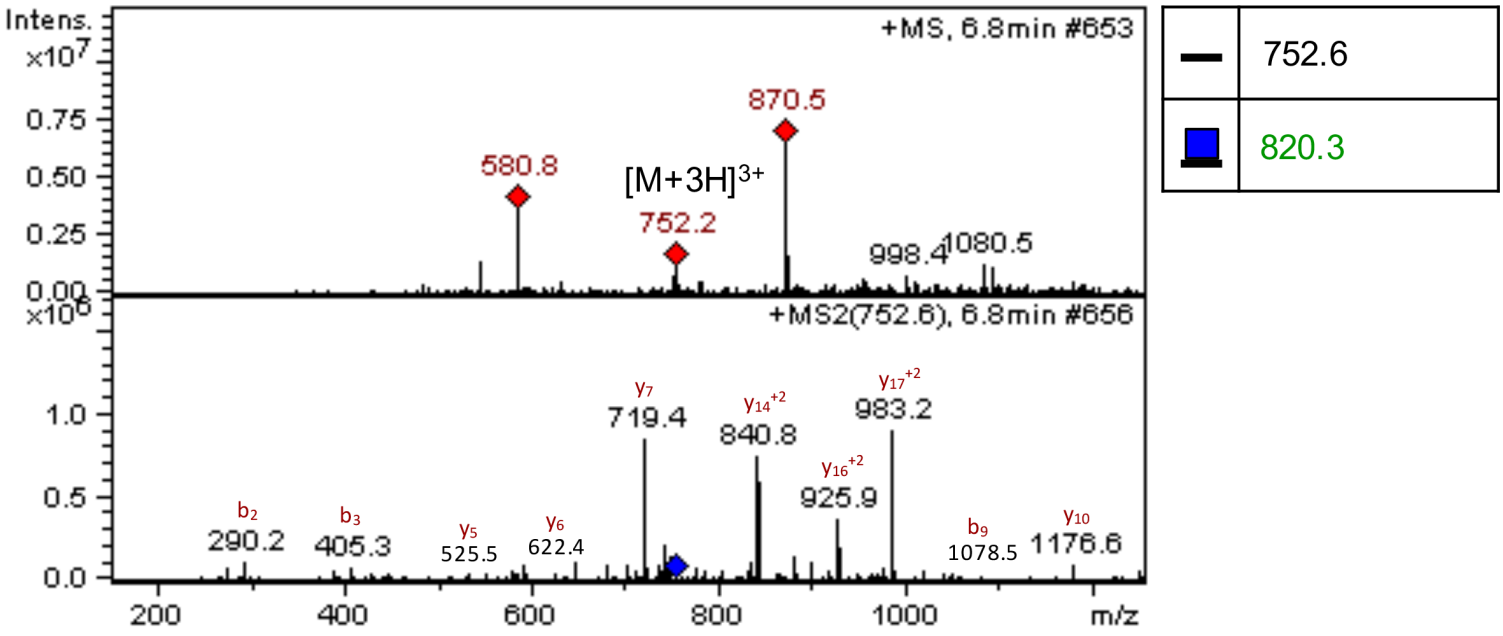


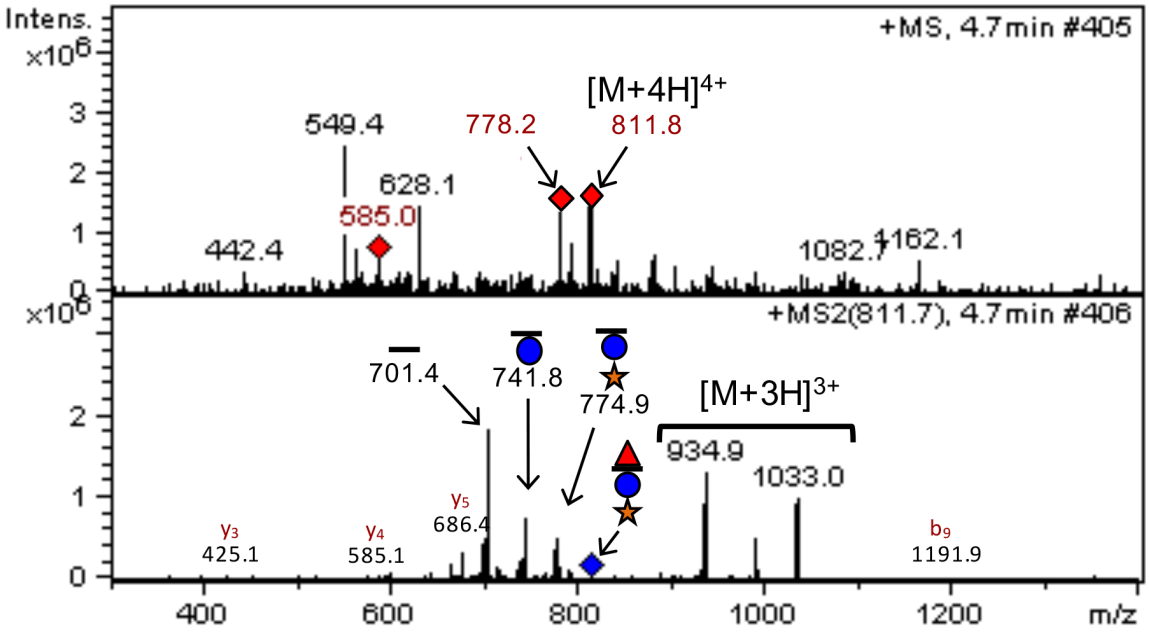
Fig. S3, F’

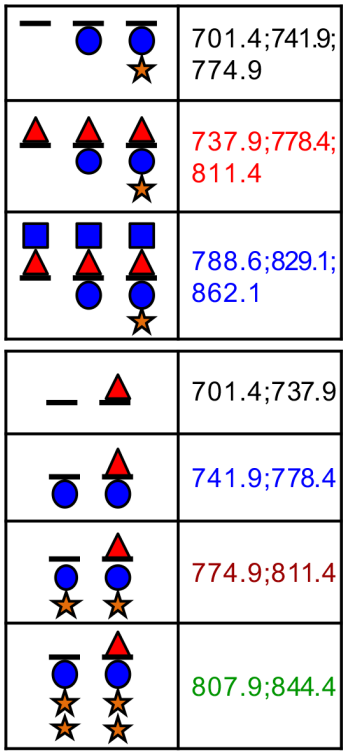
EGF 20

^{787}RCELDIDECS**S**NPCQHGG**T**CYDK^{809}

Note: $[M+H]^{3+}$ is observed in spectra, but is not in EICs

-Fringe





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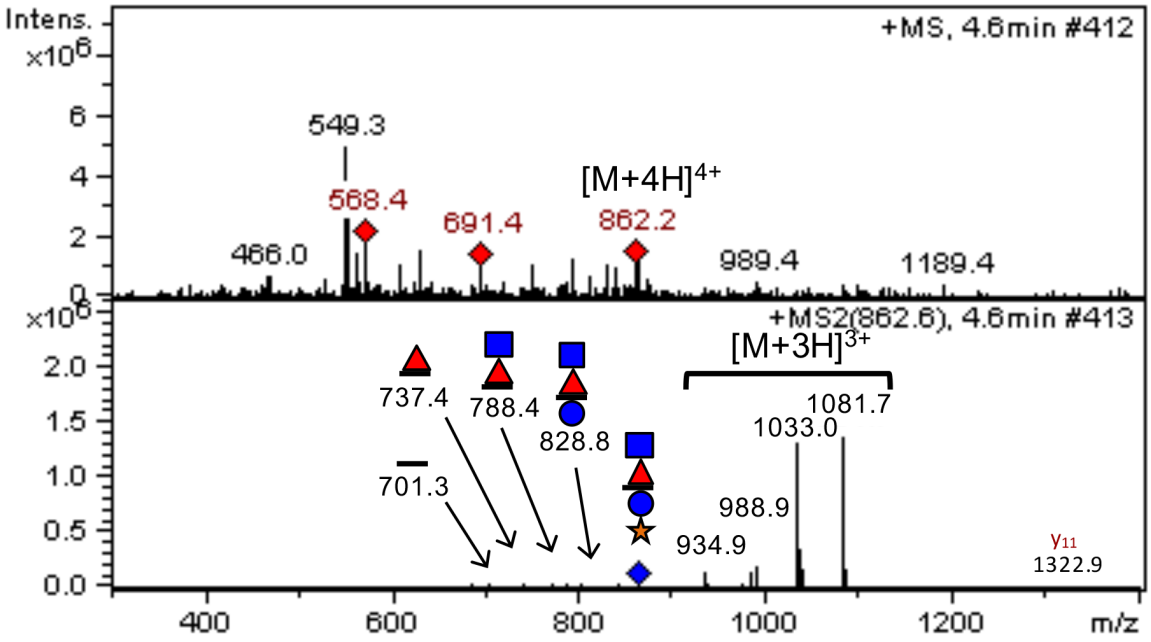


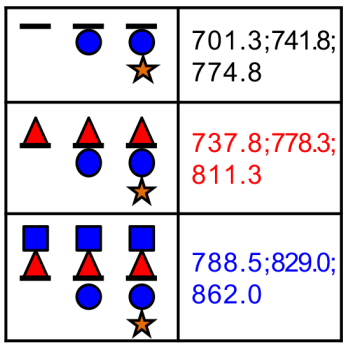


Fig. S3, G’

EGF 20

^{810}LNAFSCQCMPGYTGQK^{825}

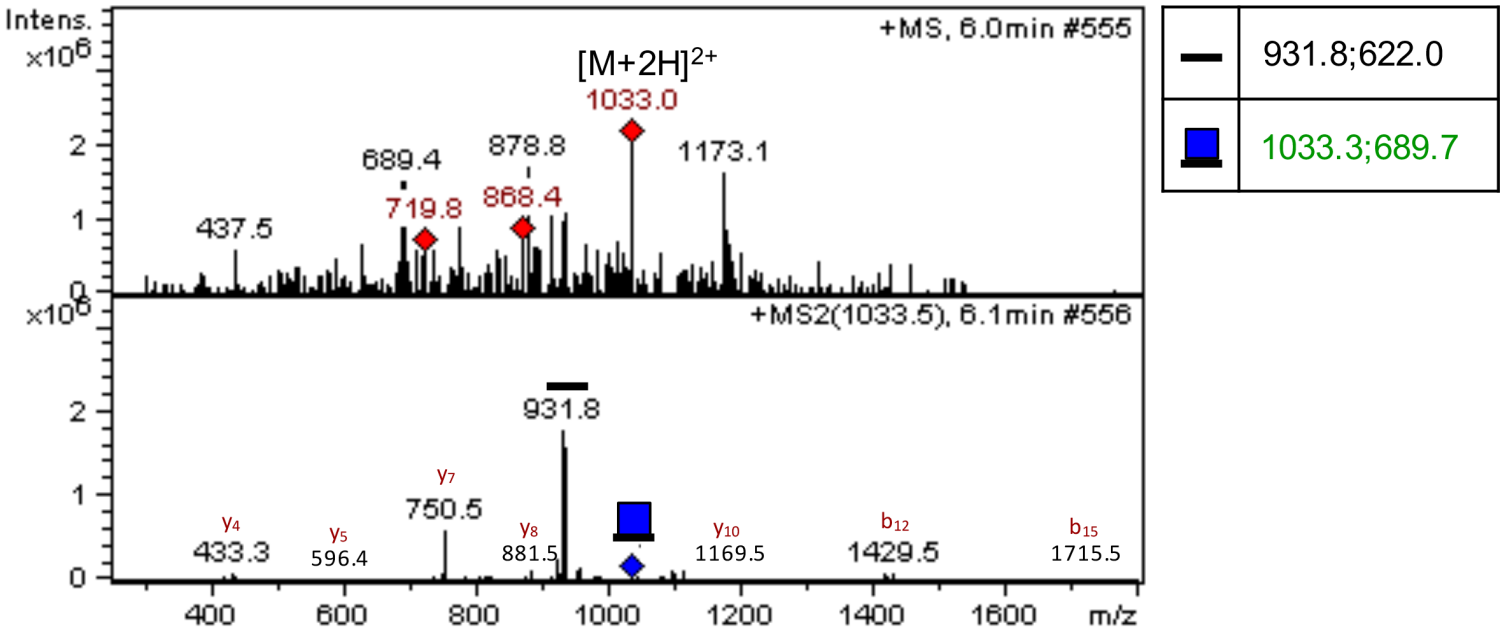
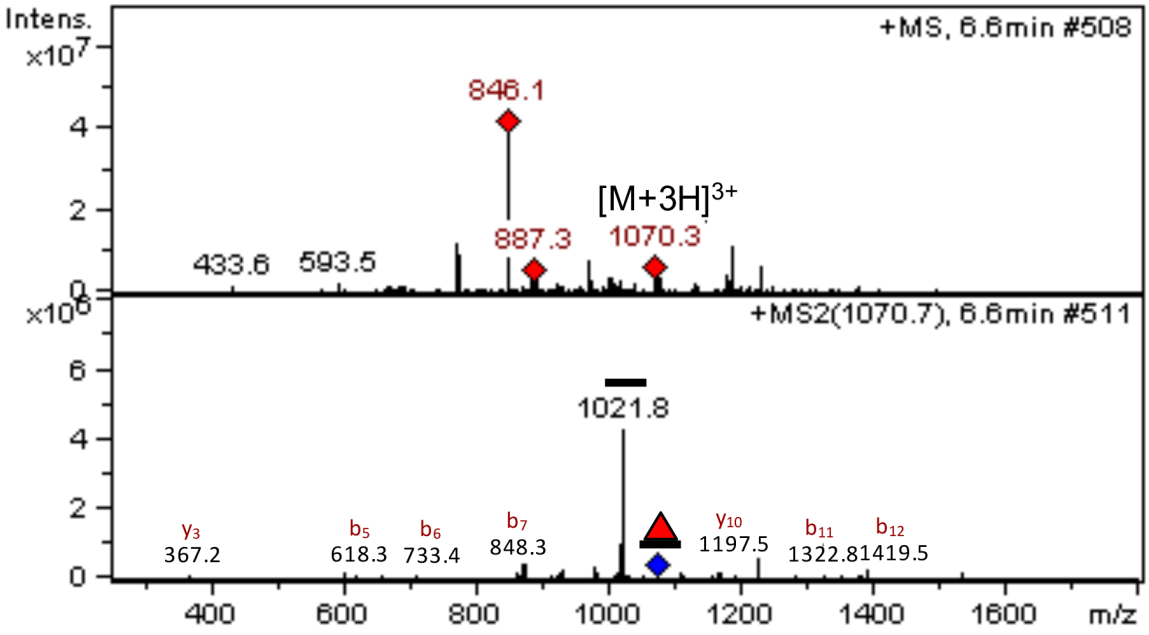


Fig. S3, H’

EGF 21

^{826}CETNIDDCVTNPCGNGG**T**CIDKVNGYK^{852}

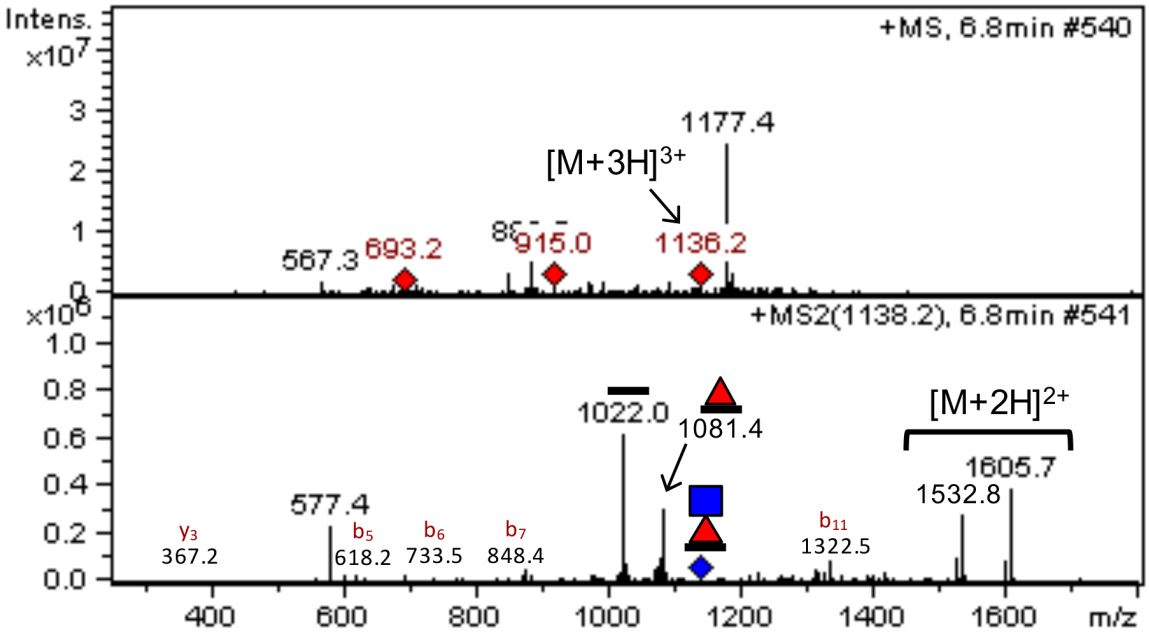
-Fringe



For EICs	
—	1532.8;1021.8
▲	1605.8;1070.5
■▲	1707.3;1138.2

For MRM	
—	1021.9→i708.8
▲	1070.8→i1021.9
■▲	1138.6→i1021.9

+Fringe



For EICs	
—	1532.8;1022.0
▲	1605.8;1070.7
■▲	1707.3;1138.4

For MRM	
—	1021.9→i708.8
▲	1070.8→i1021.9
■▲	1138.6→i1021.9

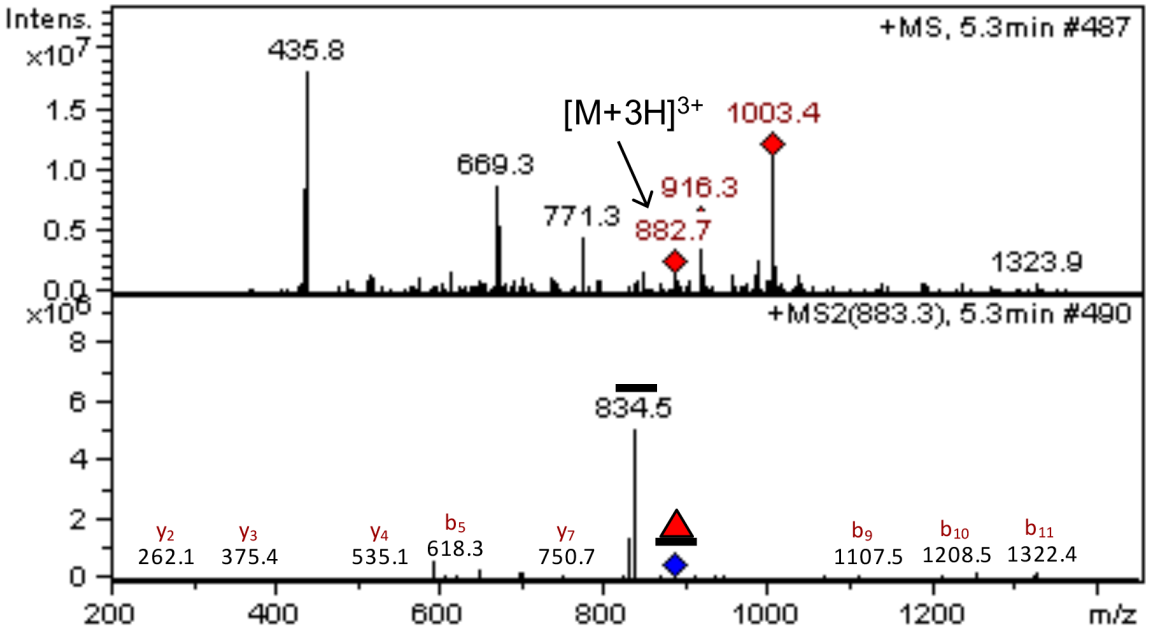
Fig. S3, I’

EGF 21

^{826}CETNIDDCVTNPCGNGG**T**CIDK^{847}

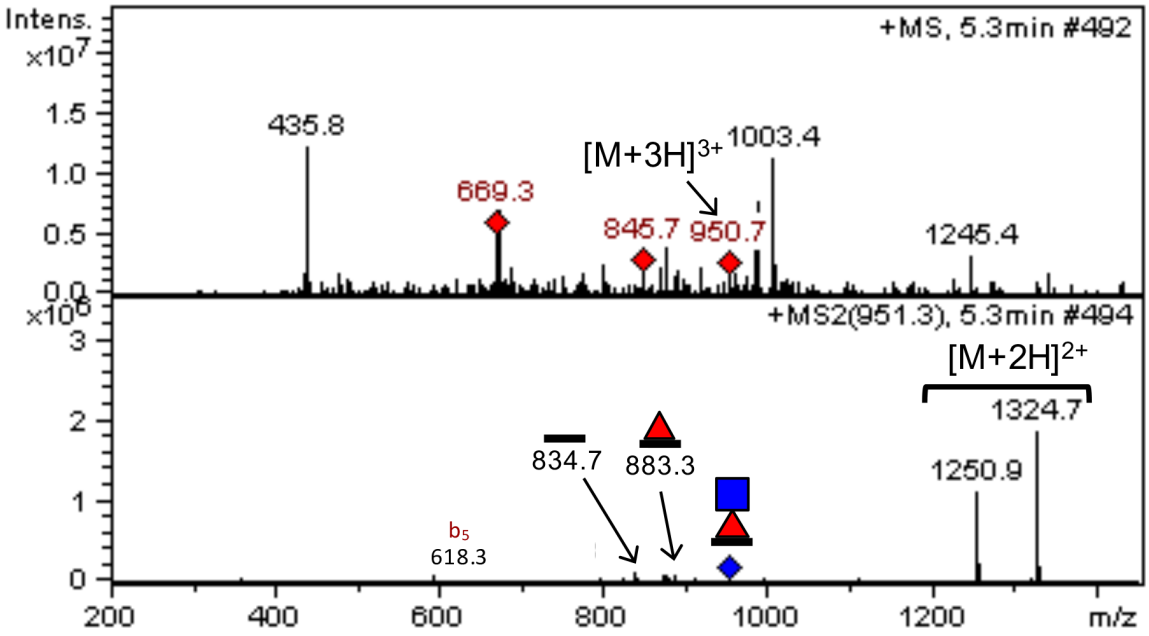
Used for Fig. 8*A;* Note: Shows same results as previous peptide

-Fringe



—	1250.9;834.5
▲	1323.9;883.2
■▲	1425.4;950.9

+Fringe



—	1250.9;834.7
▲	1323.9;883.4
■▲	1425.4;951.1

Fig. S3, J’

EGF 22

^{900}TGRYCDEDIDECSL^{913}

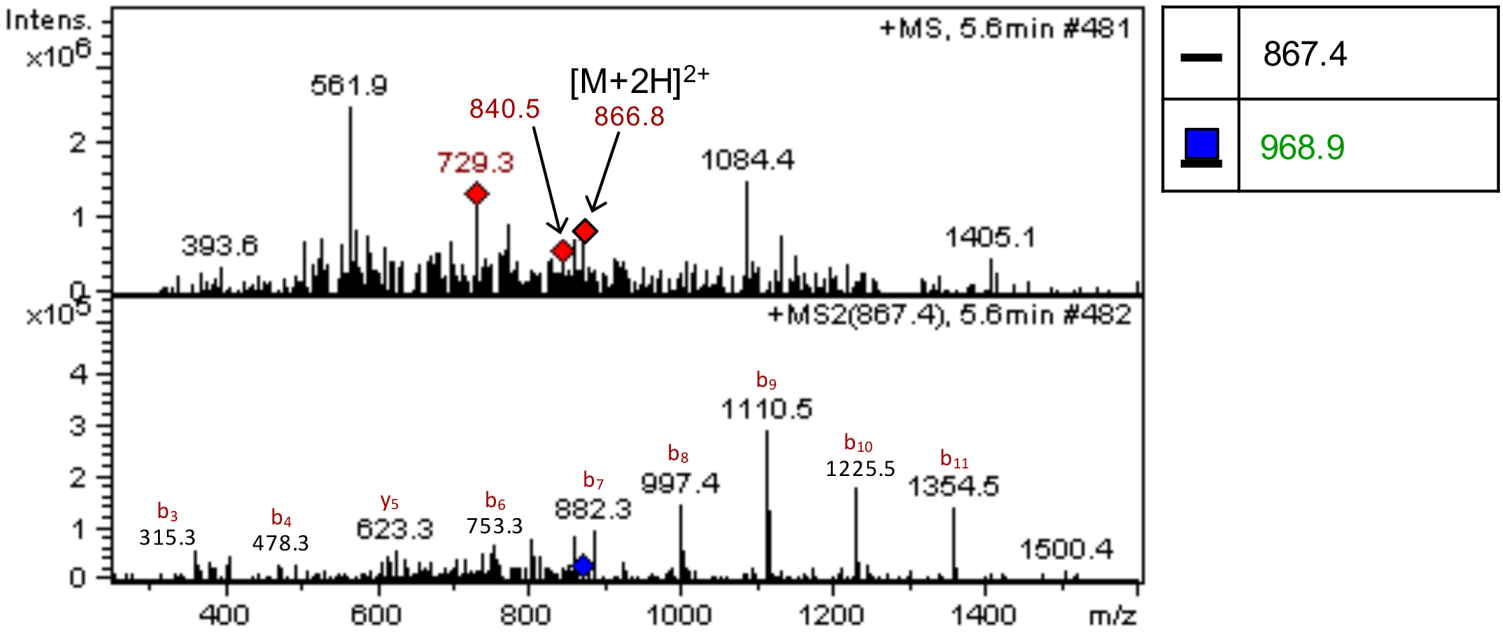
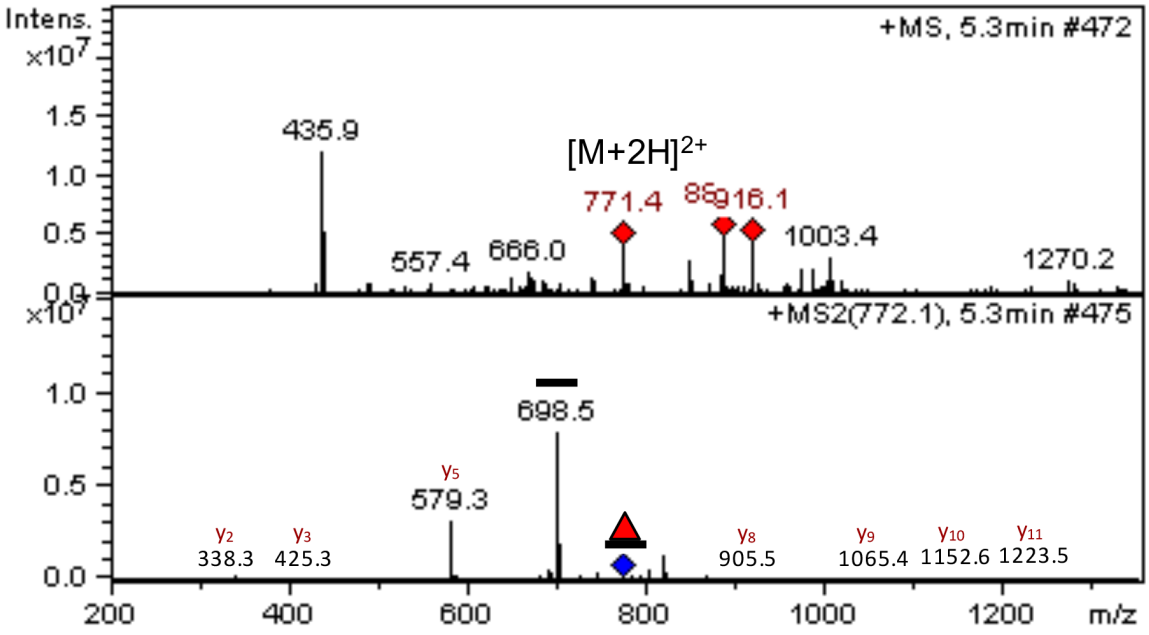


Fig. S3, K’

EGF 23

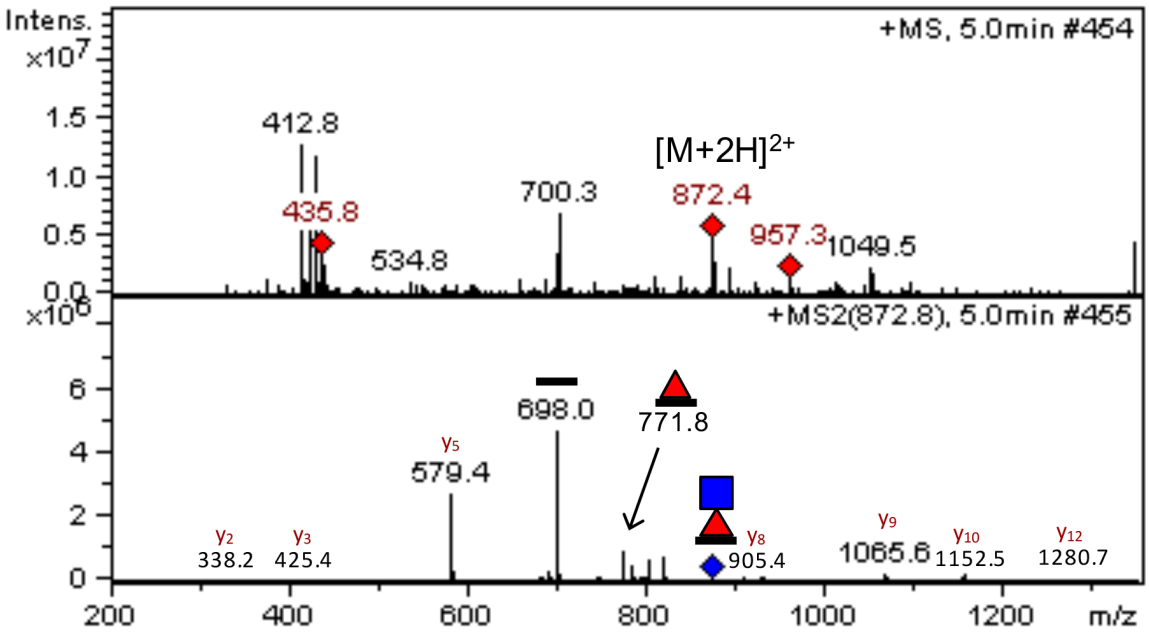
^{919}NGA**S**CLNVPGSYR^{931}

-Fringe



—	698.5
▲	771.5
■▲	873.0

+Fringe



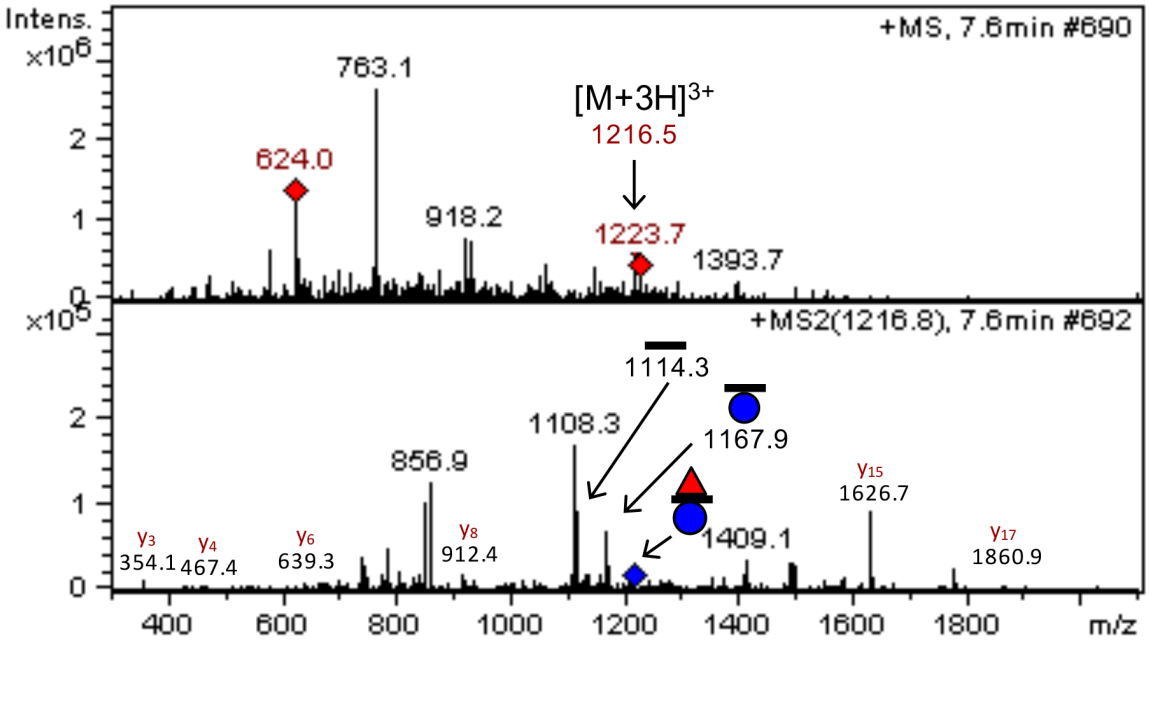
—	698.0
▲	771.0
■▲	872.5

Fig. S3, L’

EGF 24

^{939}EGRDCAINTDDCA**S**FPCQNGG**T**CLDGIGDY^{968}

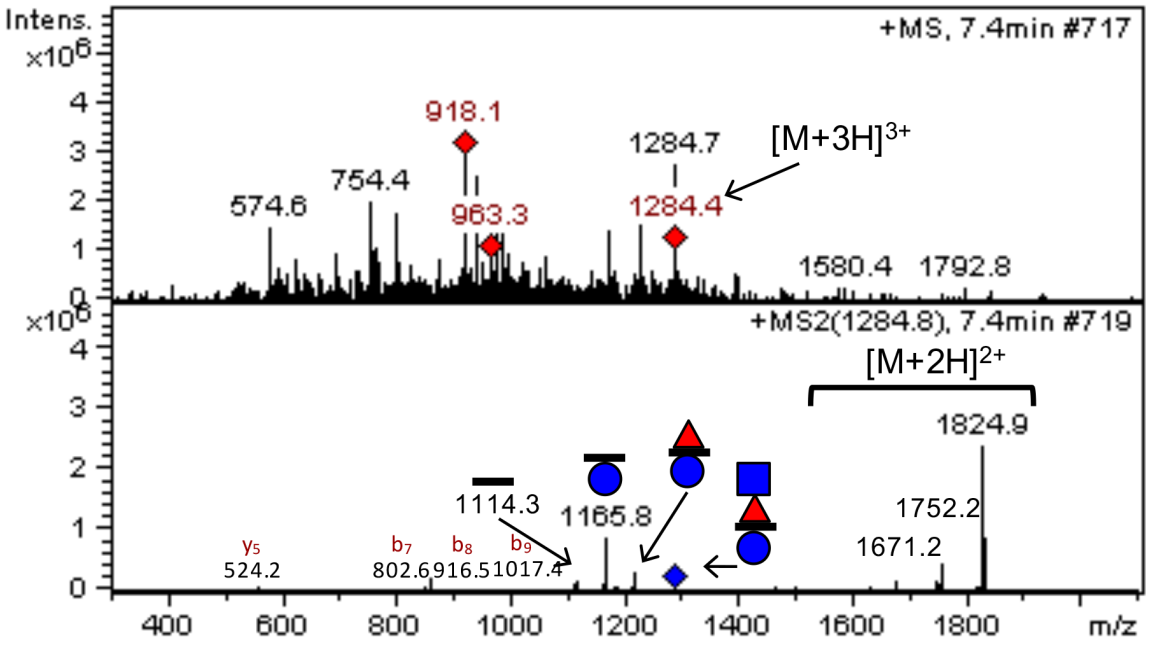
-Fringe



	1670.8;1114.3; 1751.8;1168.3
	1743.8;1163.0; 1824.8;1217.0
	1845.3;1230.7; 1926.3;1284.7

	1670.8;1114.3; 1743.8;1163.0
	1751.8;1168.3; 1824.8;1217.0
	1817.8;1212.3; 1890.8;1261.0
	1883.8;1256.3; 1956.8;1305.0

+Fringe



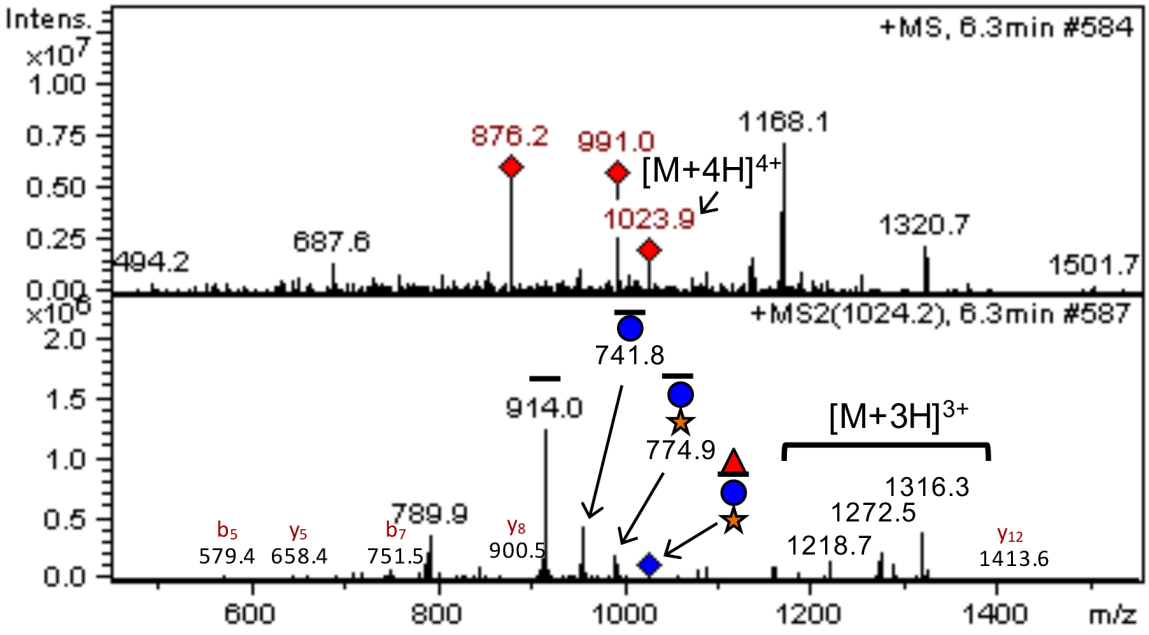
	1670.8;1114.3; 1751.8;1168.3
	1743.8;1163.0; 1824.8;1217.0
	1845.3;1230.7; 1926.3;1284.7

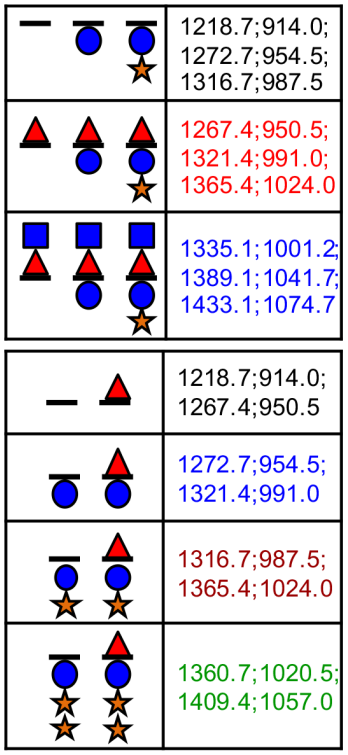
Fig. S3, M’

EGF 25

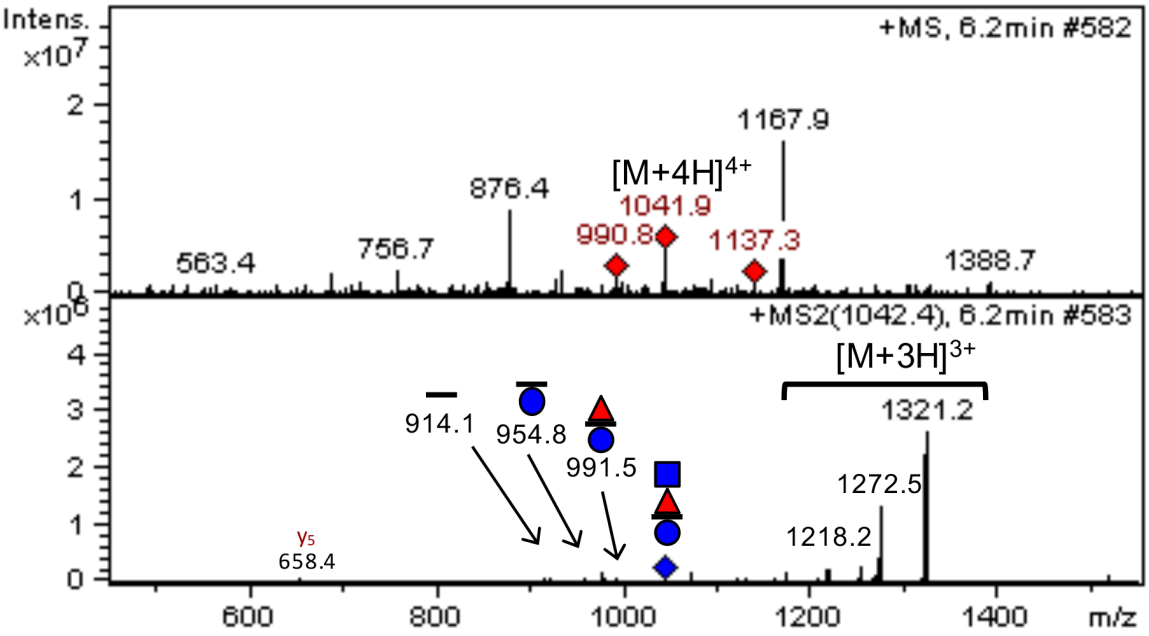
^{972}CVDGFDGKHCETDINECL**S**QPCQNGA**T**CSQY^{1002}

-Fringe





+Fringe



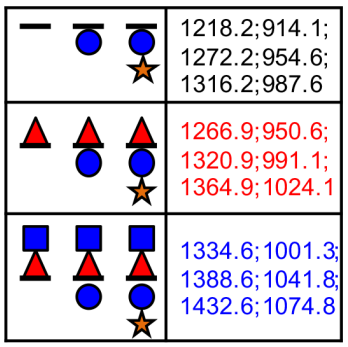
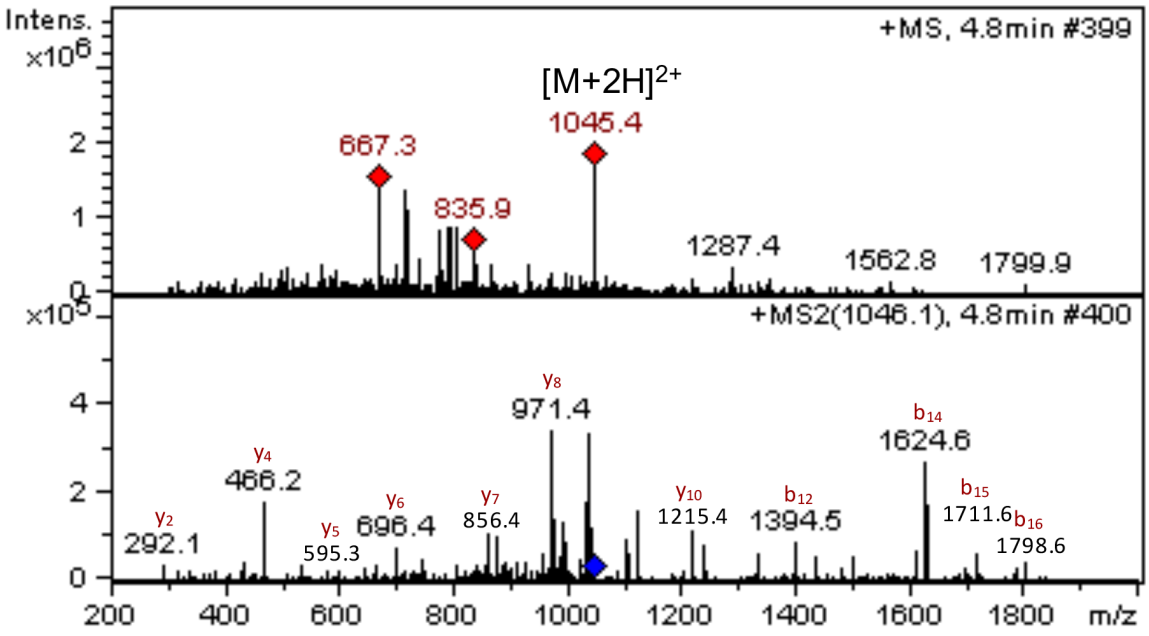


Fig. S3, N’

EGF 25

^{1015}**S**GINCQTNDEDCTESSCL^{1032}



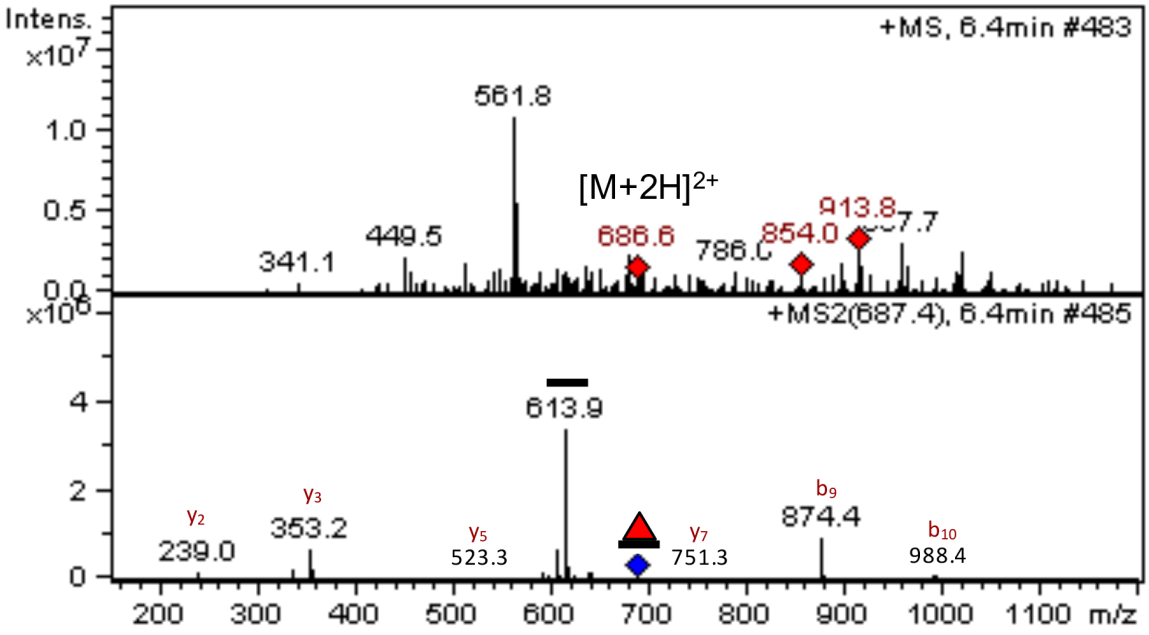
—	1046.1;698.2
■	1147.6;765.9

Fig. S3, O’

EGF 26

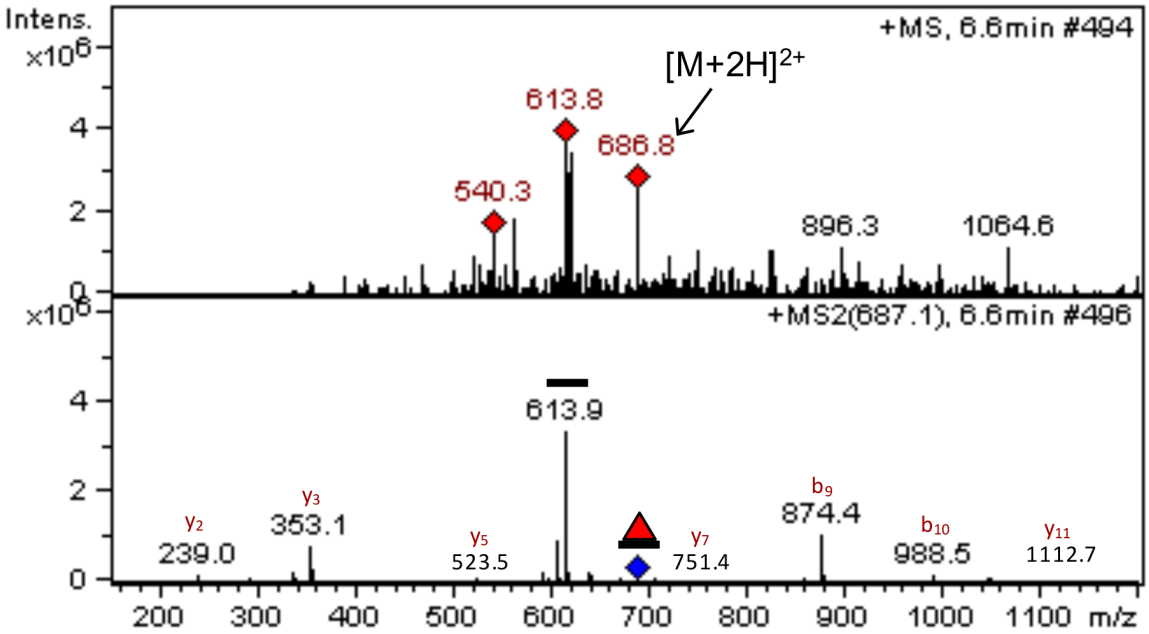
^{1033}NGG**S**CIDGINGY^{1044}

-Fringe



—	613.9
▲	686.9
■▲	788.4

+Fringe

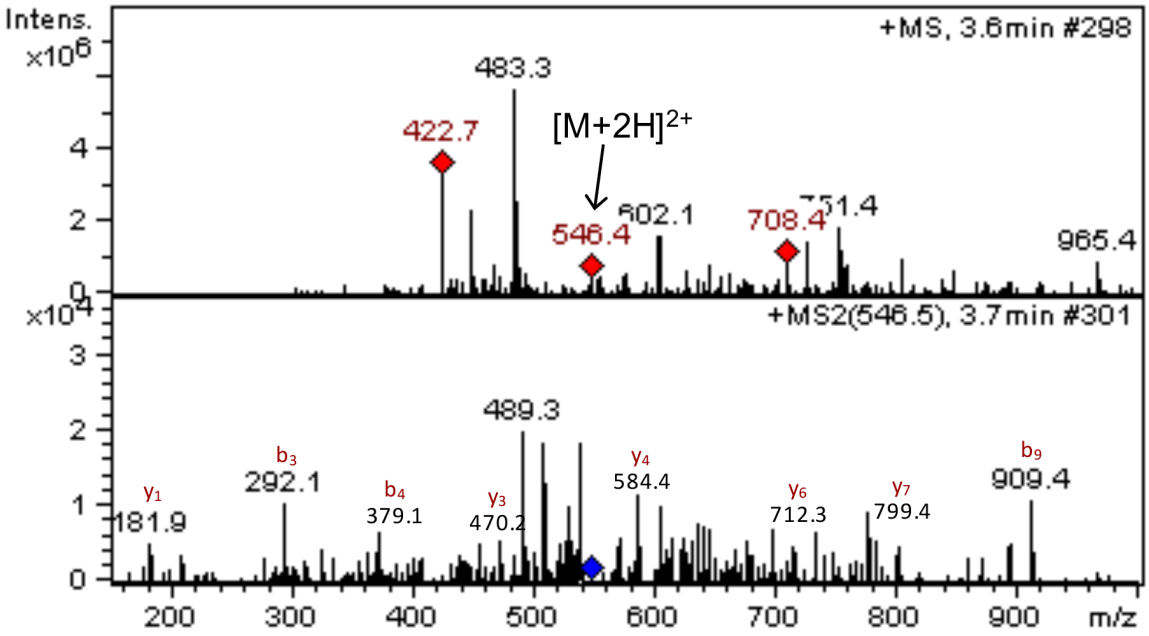


—	613.9
▲	686.9
■▲	788.4

Fig. S3, P’

EGF 26

^{1050}AGY**S**GANCQY^{1059}



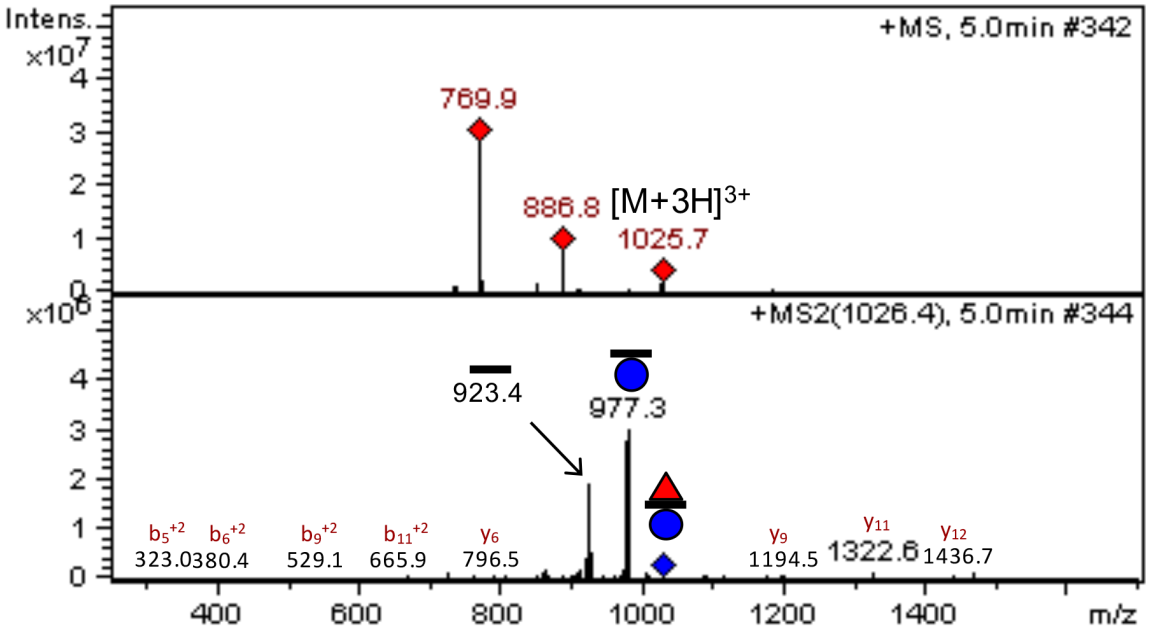
—	546.5
■	648.0

Fig. S3, Q’

EGF 27

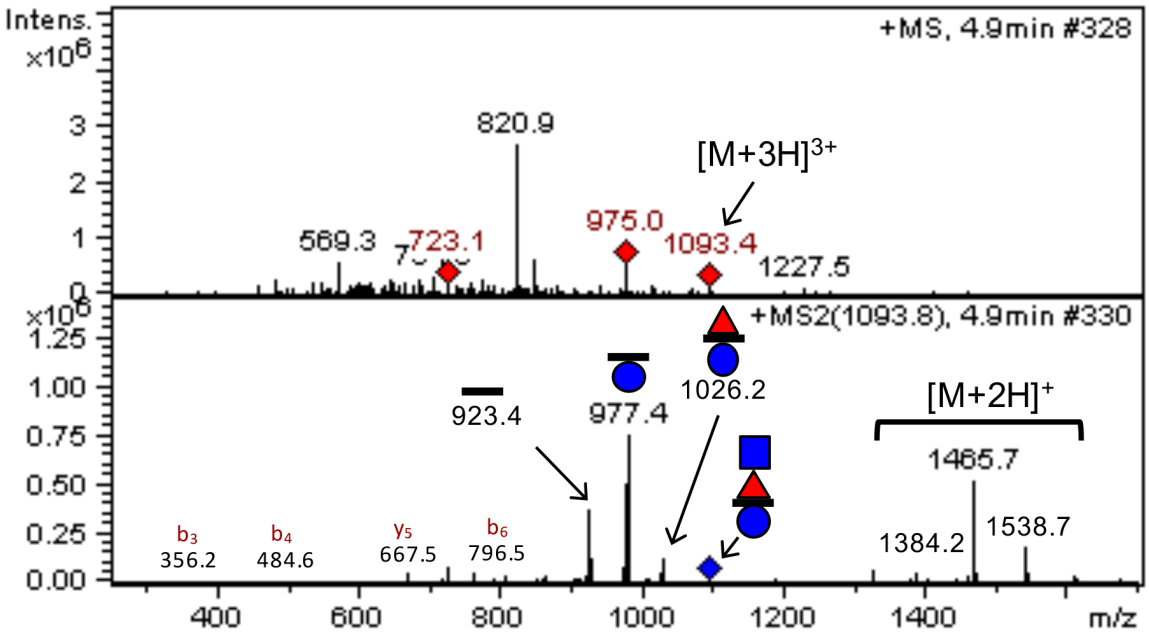
^{1060}KLNKCD**S**NPCLNGA**T**CHEQNNEY^{1082}

-Fringe



● —	1384.6;923.4; 1465.6;977.4
▲ ▲ ●	1457.6;972.1; 1538.6;1026.1
■ ▲ ●	1559.1;1039.8; 1640.1;1093.8
— ▲	1384.6;923.4; 1457.6;972.1
● ▲	1465.6;977.4; 1538.6;1026.1
● ▲ ★	1531.6;1021.4; 1604.6;1070.1
● ▲ ★ ★	1597.6;1065.4; 1670.6;1114.1

+Fringe



● —	1384.2;923.4; 1465.2;977.4
▲ ▲ ●	1457.2;972.1; 1538.2;1026.1
■ ▲ ●	1558.7;1039.8; 1639.7;1093.8

Fig. S3, R’

EGF 27

^{1091}TGKQCSEYVDW^{1101}

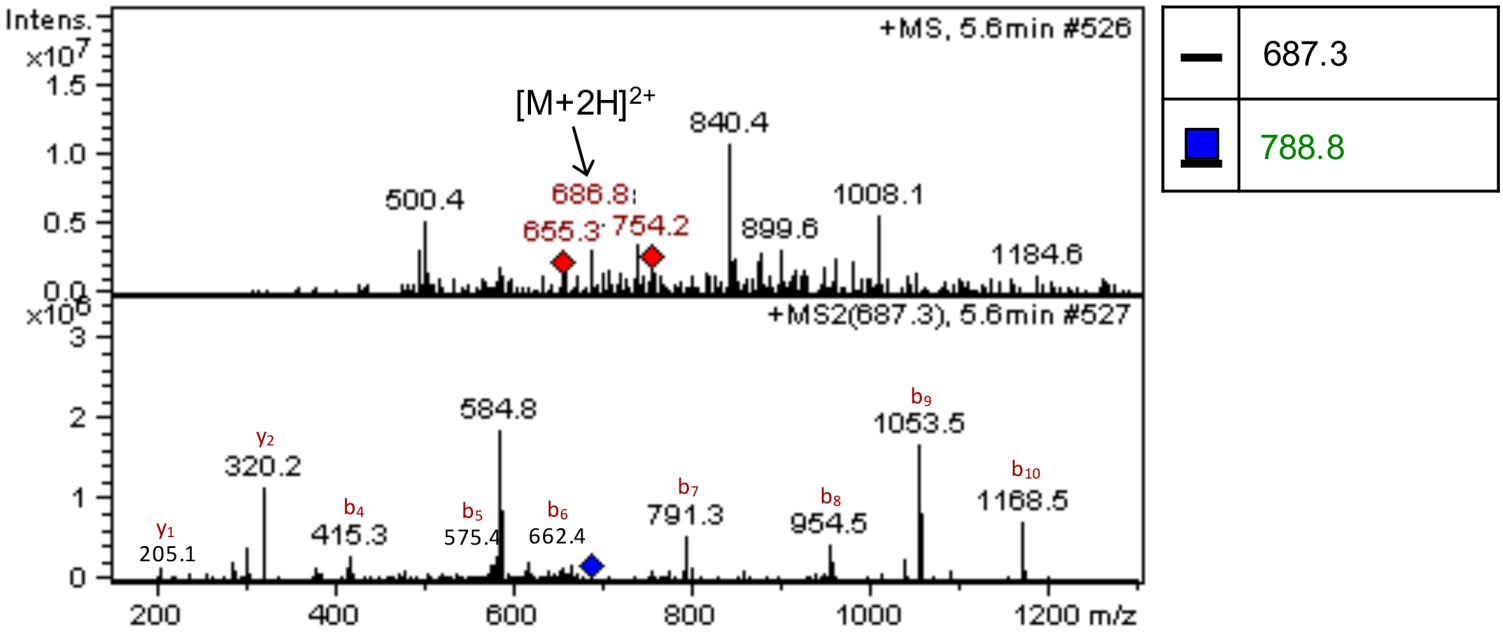
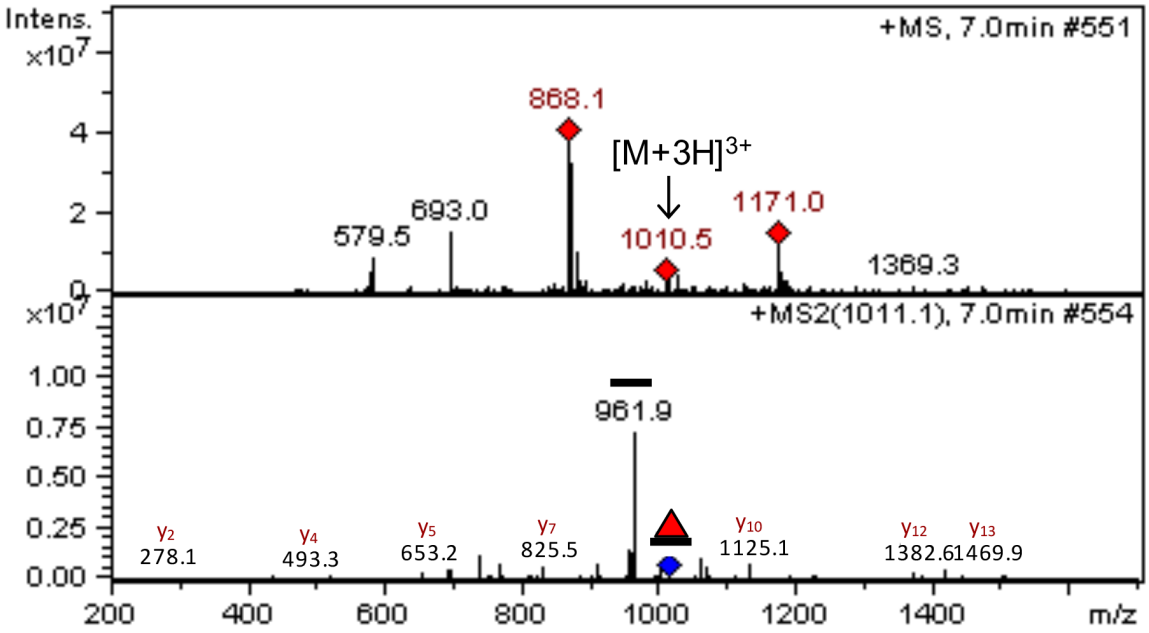


Fig. S3, S’

EGF 28

^{1094}QCSEYVDWCGQSPCENGA**T**CSQMK^{1117}

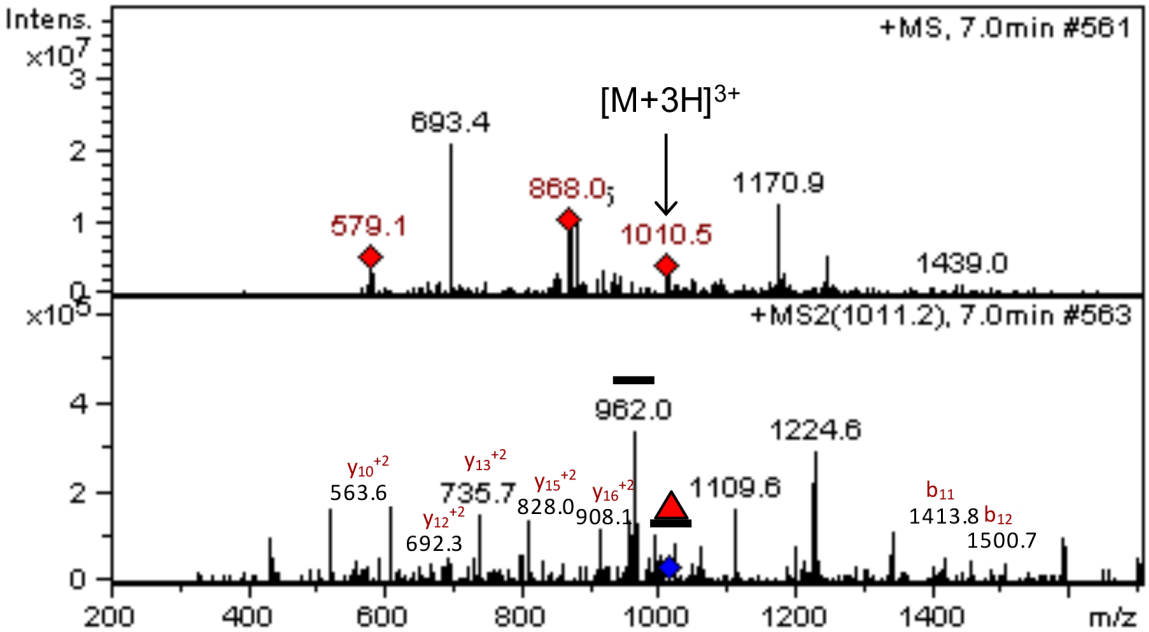
-Fringe



For EICs	
—	961.9
▲	1010.6
■▲	1078.3

For MRM	
—	962.0→i1124.9
▲	1011.3→i962.0
■▲	1079.0→i962.0

+Fringe



For EICs	
—	962.0
▲	1010.7
■▲	1078.4

For MRM	
—	962.0→i1124.9
▲	1011.3→i962.0
■▲	1079.0→i962.0

Fig. S3, T’

EGF 28

^{1129}**T**GKLCDVQTISCQDAADRKGL^{1149}

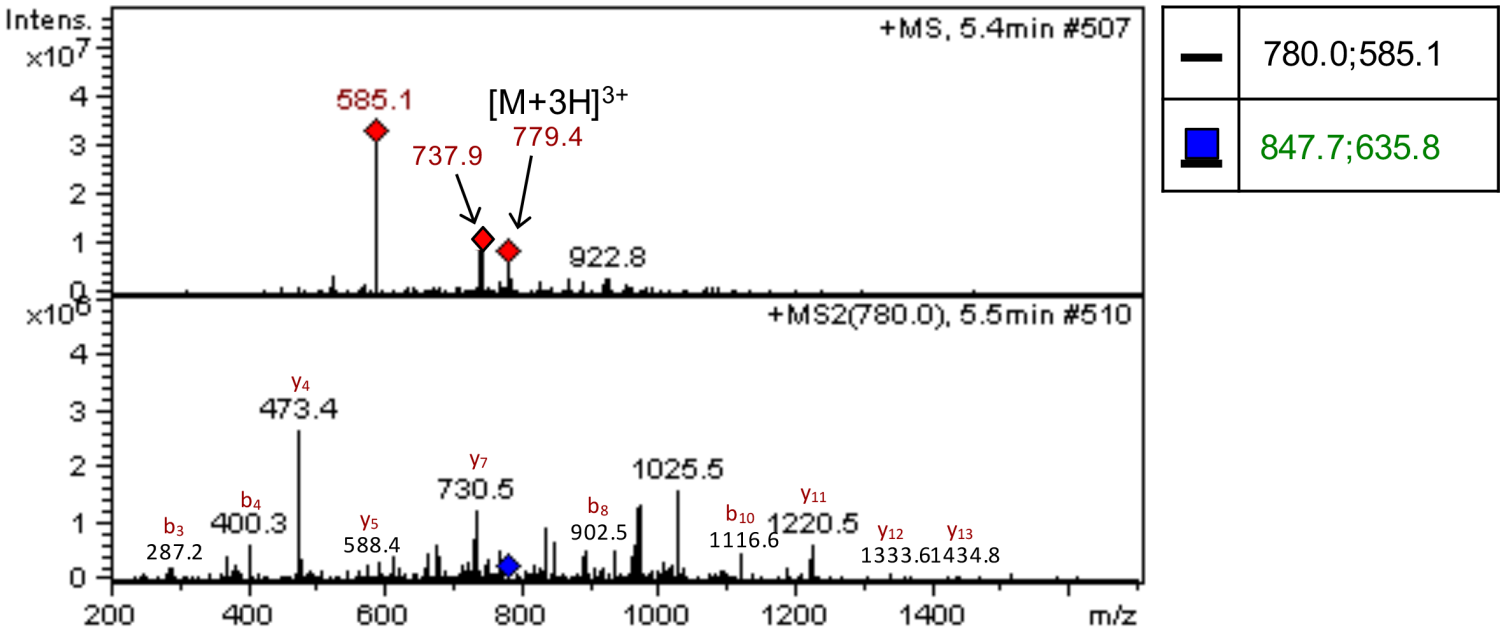
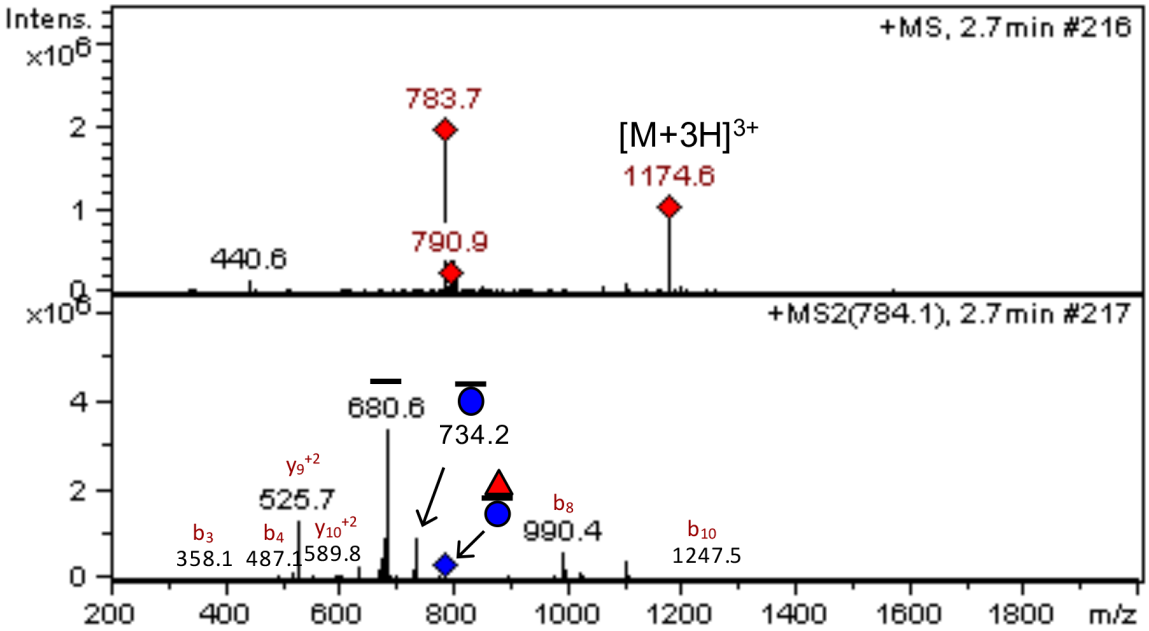


Fig. S3, U’

EGF 30

^{1183}EIDECQ**S**QPCQNGG**T**CR^{1199}

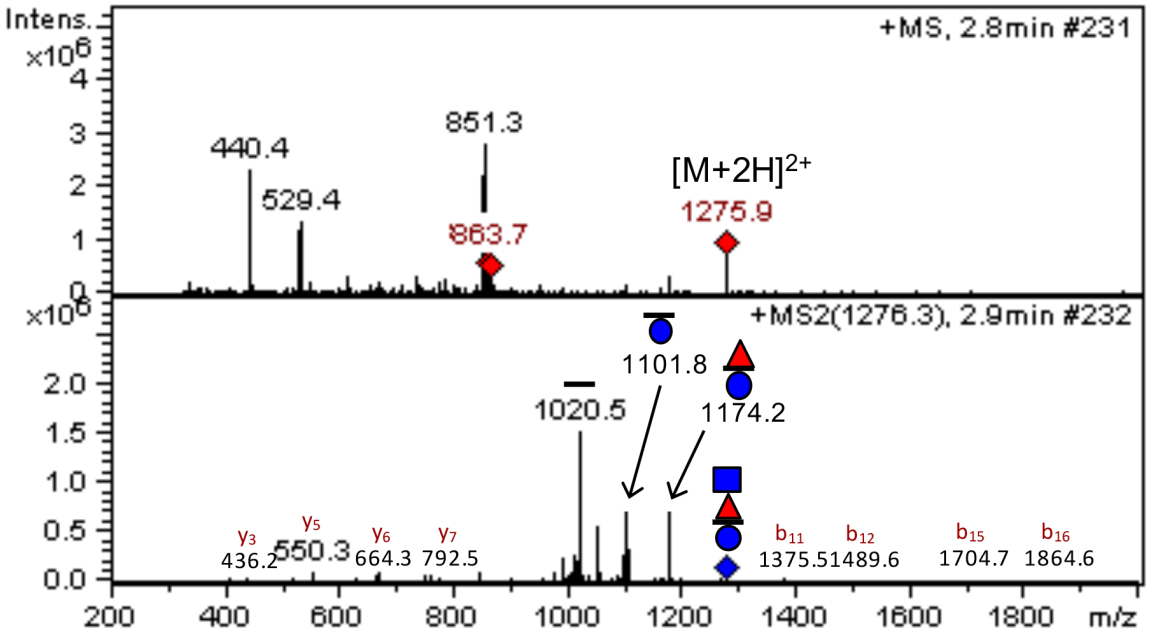
-Fringe



	1020.5;680.6; 1101.5;734.6
	1093.5;729.3; 1174.5;783.3
	1195.0;797.0; 1276.0;851.0

	1020.5;680.6; 1093.5;729.3
	1101.5;734.6; 1174.5;783.3
	1167.5;778.6; 1240.5;827.3
	1233.5;822.6; 1306.5;871.3

+Fringe



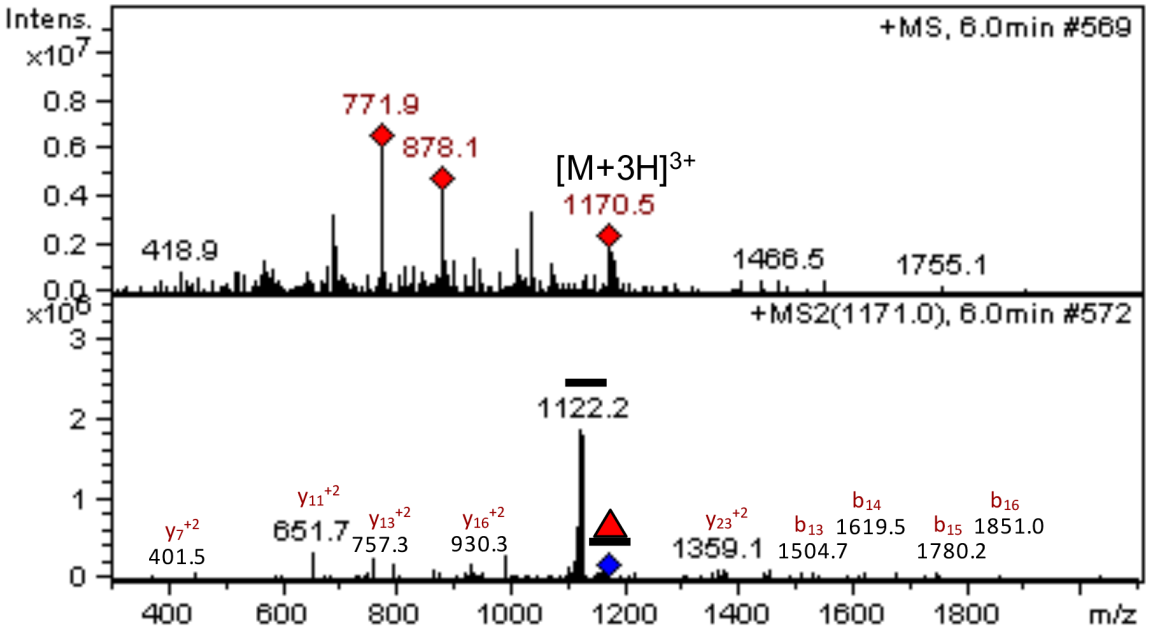
	1020.5;680.6; 1101.5;734.6
	1093.5;729.3; 1174.5;783.3
	1195.0;797.0; 1276.0;851.0

Fig. S3, V’

EGF 31

^{1211}QGFQGQNCELNIDDCAPNPCQNGG**T**CHDR^{1239}

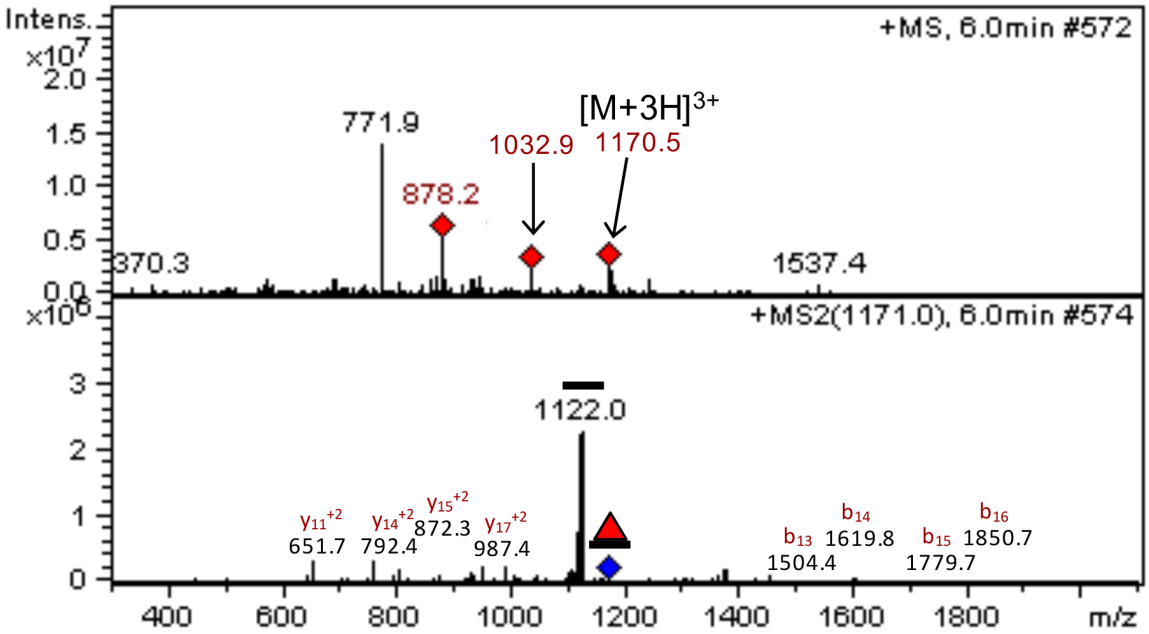
-Fringe



For EICs	
—	1122.2;841.9
▲	1170.9;878.4
■▲	1238.6;929.1

For MRM	
—	1122.1→i916.6
▲	1171.1→i1122.1
■▲	1238.8→i1122.1

+Fringe



For EICs	
—	1122.0;841.9
▲	1170.7;878.4
■▲	1238.4;929.1

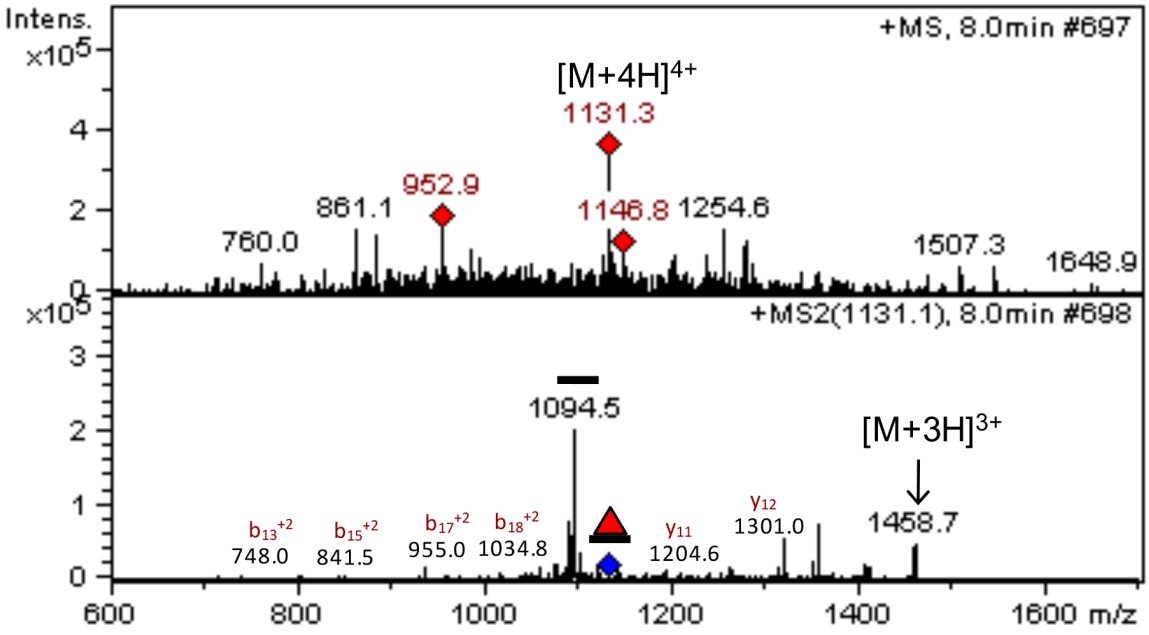
For MRM	
—	1122.1→i916.6
▲	1171.1→i1122.1
■▲	1238.8→i1122.1

Fig. S3, W’

EGF 32

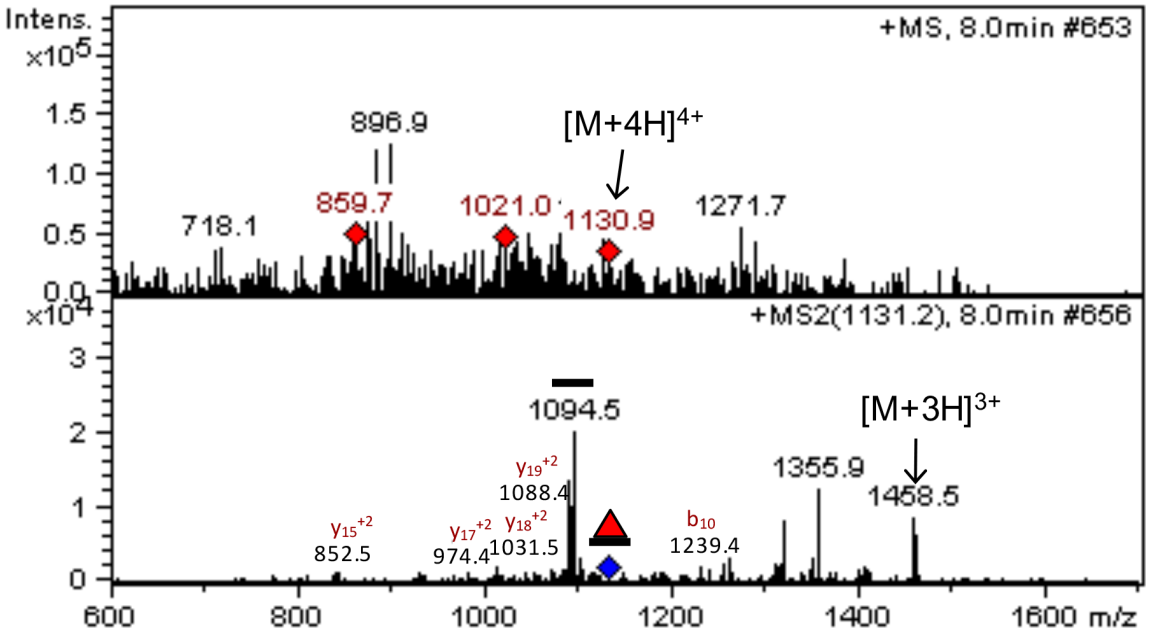
^{1239}RVMNFSCSCPPGTMGIICEINKDDCKPGACHNNG**S**CID^{1276}

-Fringe



▬	1458.7;1094.5
▲	1507.4;1131.0
■▲	1575.1;1181.7

+Fringe



▬	1458.5;1094.5
▲	1507.2;1131.0
■▲	1574.9;1181.7

Fig. S3, X’

EGF 33

^{1303}**S**NPCSNAGTLDCVQL^{1317}

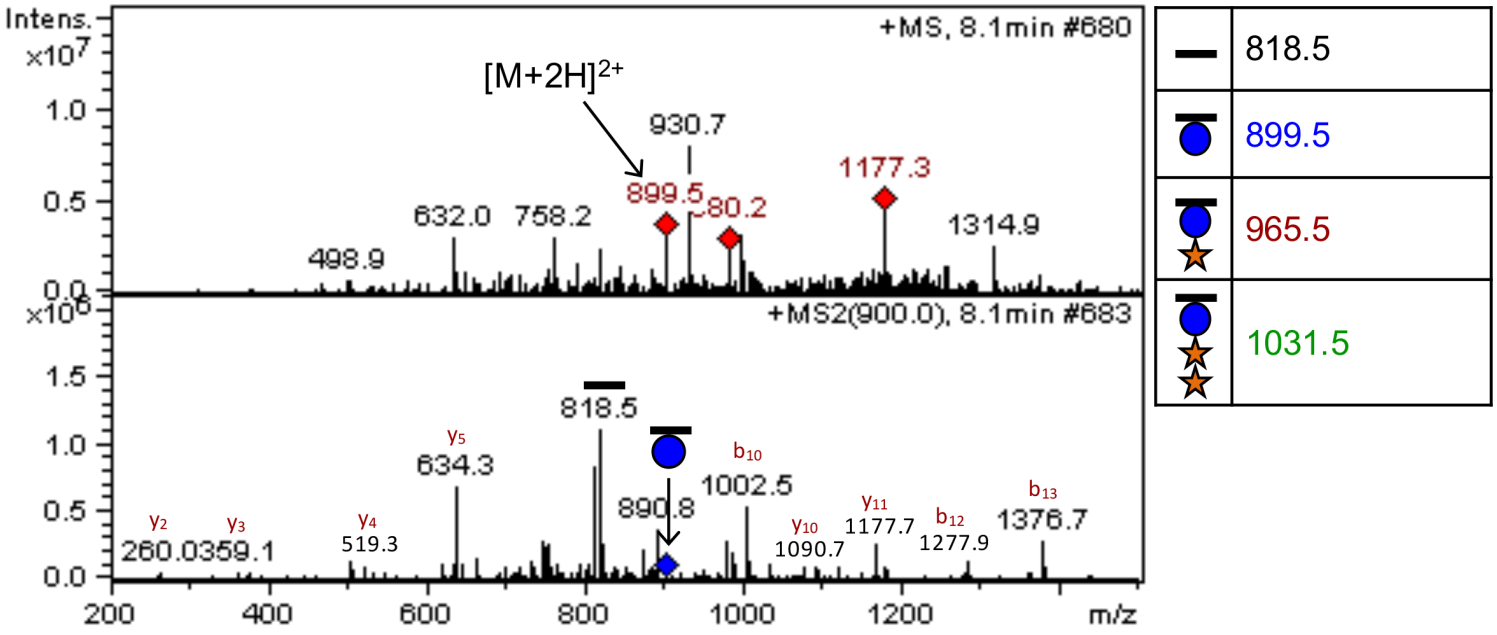


Fig. S3, Y’

EGF 35

^{1371}NCELSGQDCD**S**NPCR^{1385}