SUPPLEMENTAL FIGURES S1-S6:

Fig. S1.

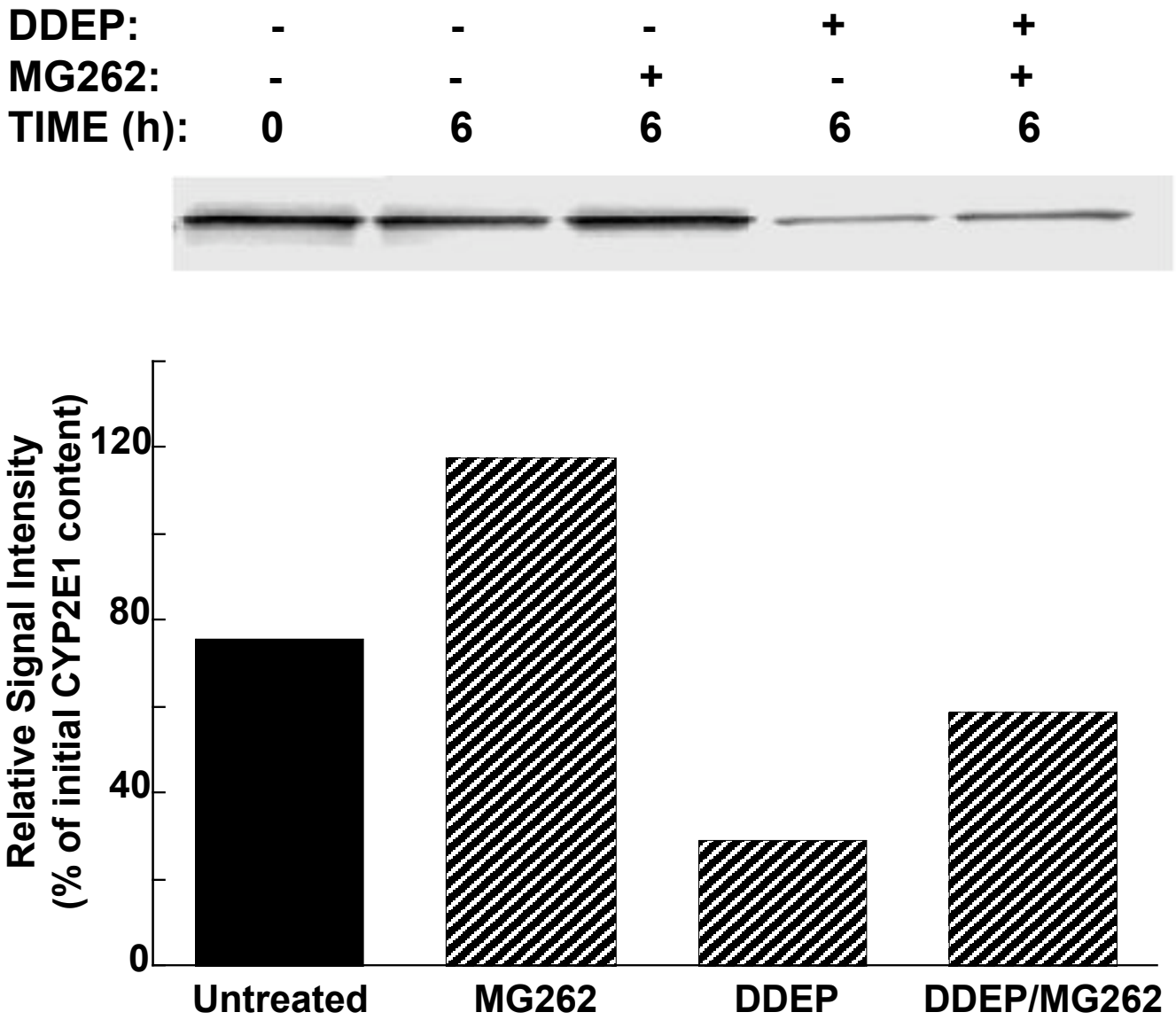
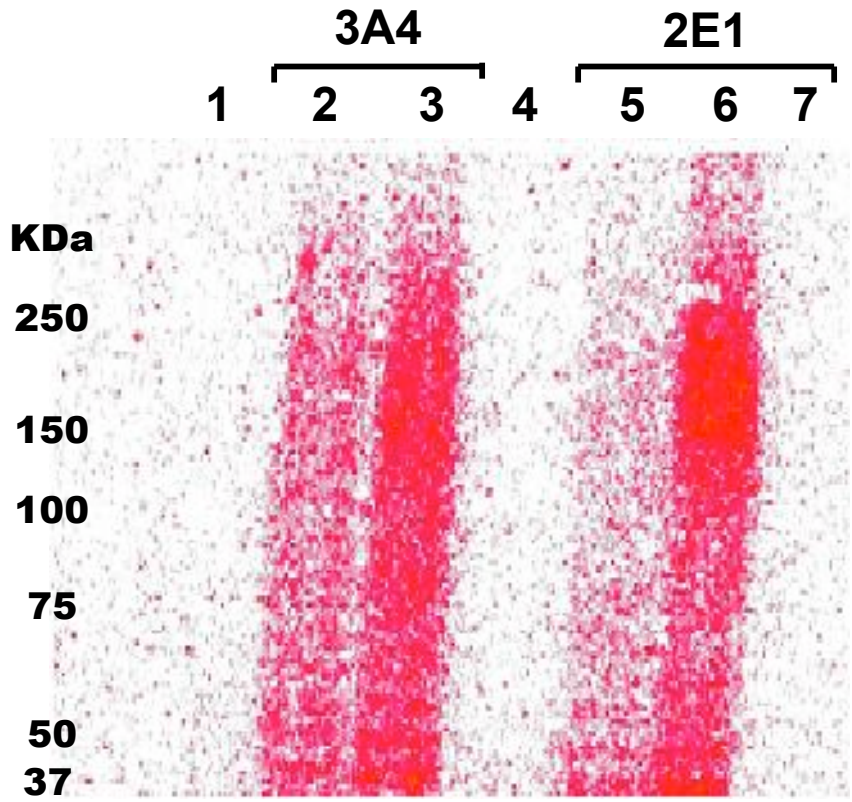

DDEP: - - - + +
MG262: - - + - +
TIME (h): 0 6 6 6 6
Relative Signal Intensity
(% of initial CYP2E1 content)
120
80
40
0
Untreated
MG262
DDEP
DDEP/MG262

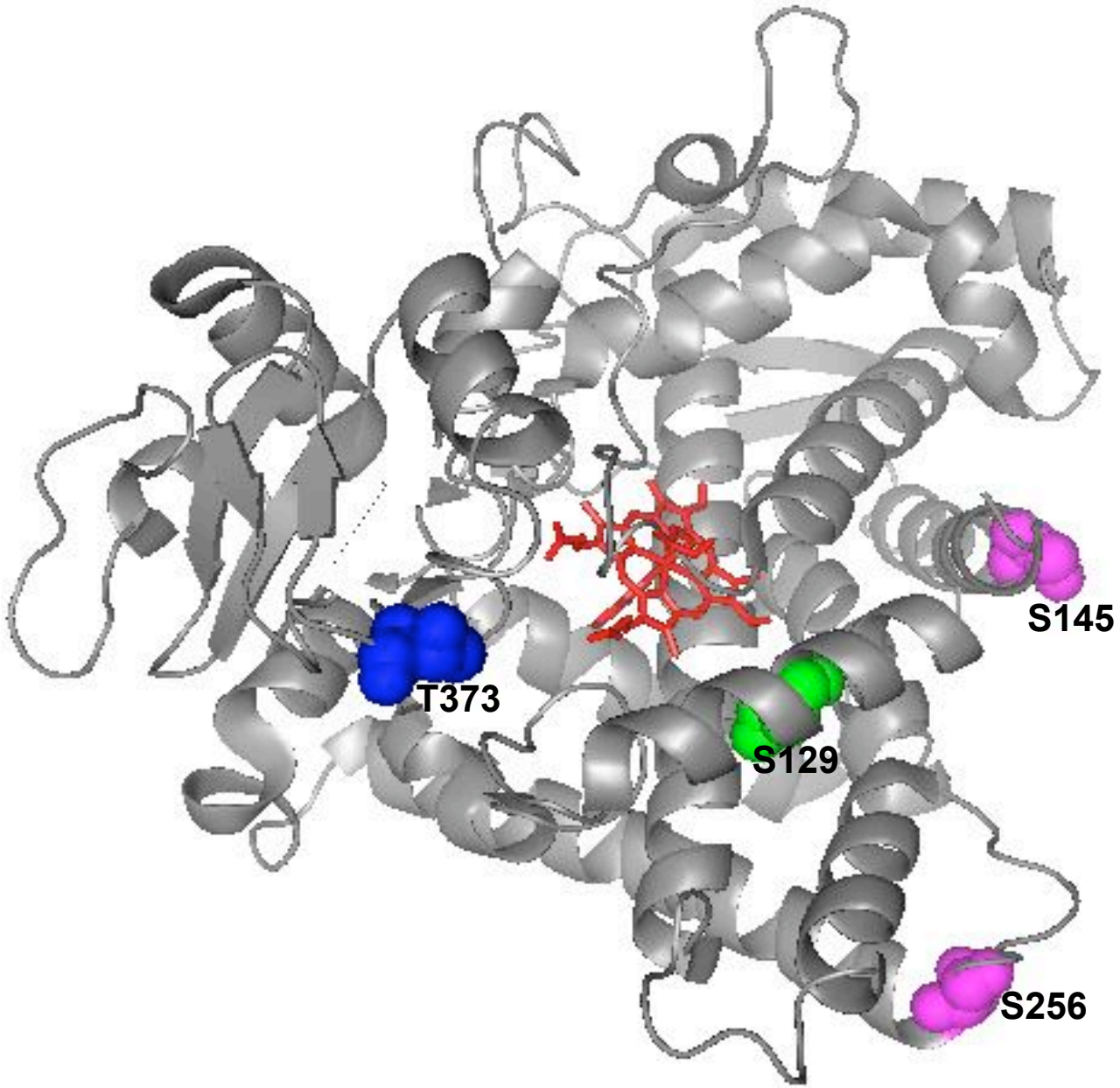
Fig. S2

	1	2	3	4	5	6	7
PKA/PKC:	-	-	+	+	-	+	+
^{32}P-Ub:	+	+	+	+	+	+	+
ATP:	+	+	+	+	+	+	-
TIME (min):	90	90	90	90	90	90	90



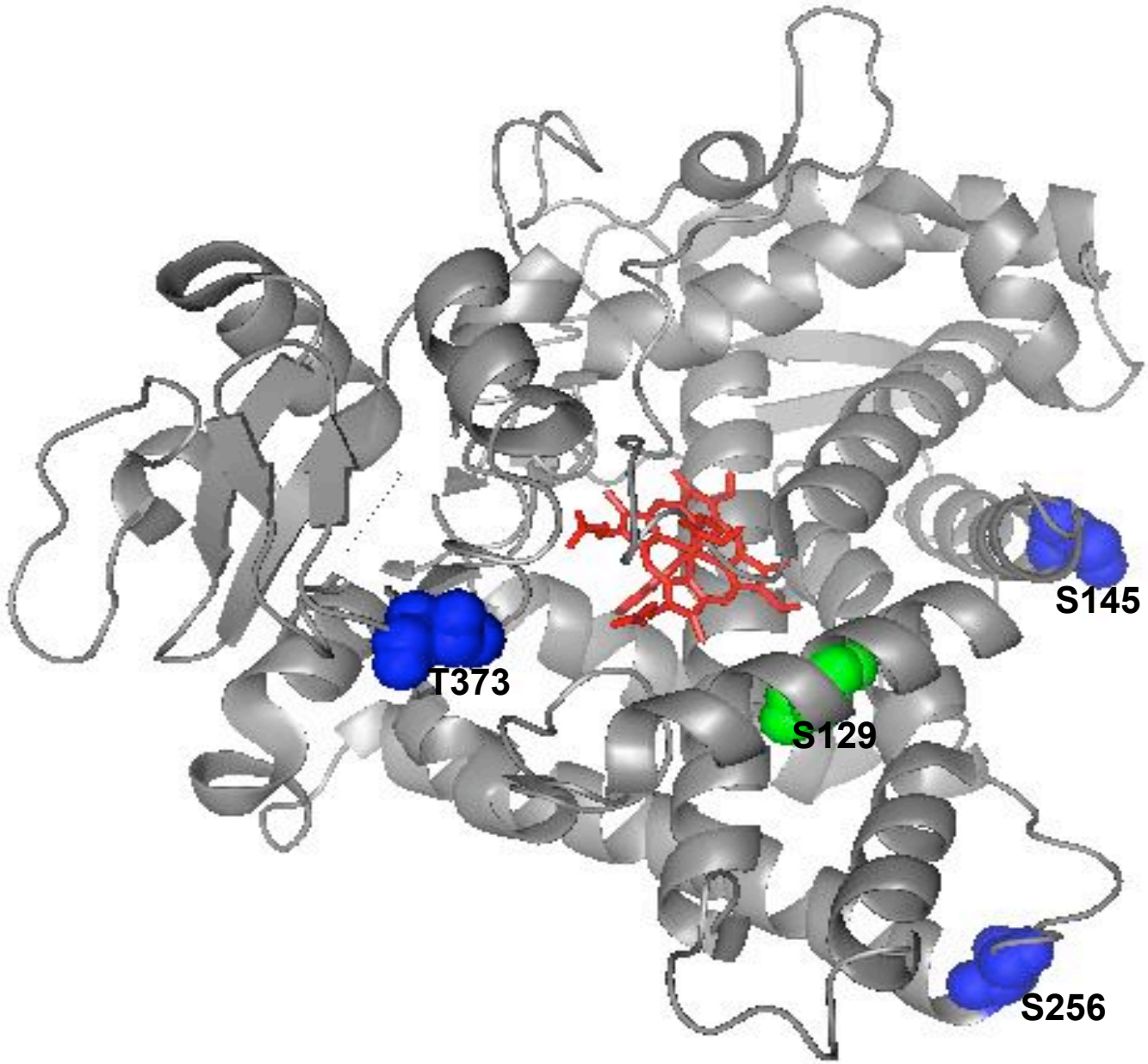
UBC7/gp78C

Fig. S3. Native CYP2E1 phosphorylation by PKA



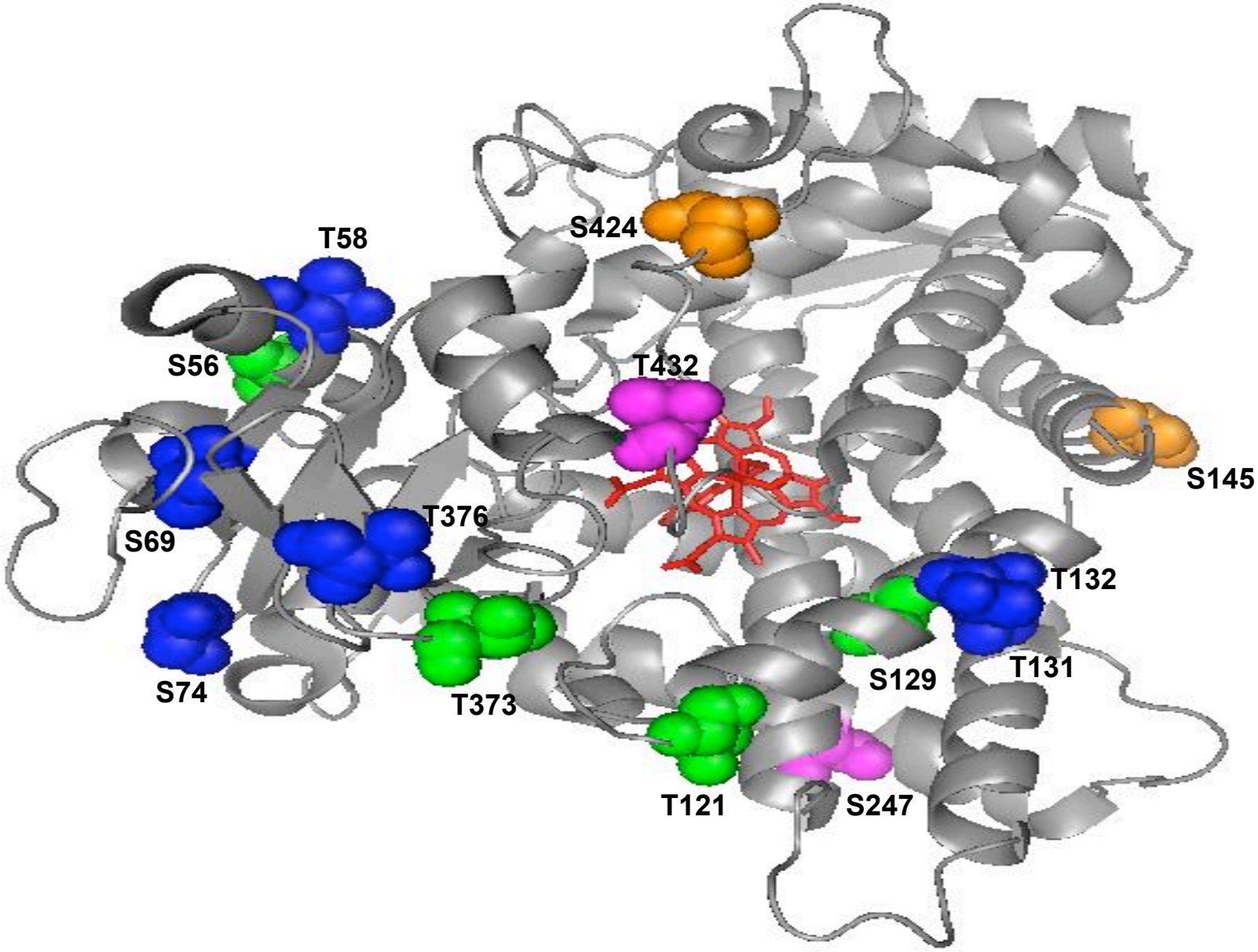
PKA-catalyzed native CYP2E1 phosphorylation
Phosphorylation intensity: <0.1% (orange), <1% (magenta), <10% (blue), >10% (green)

Fig. S4. CuOOH-inactivated CYP2E1 phosphorylation by PKA



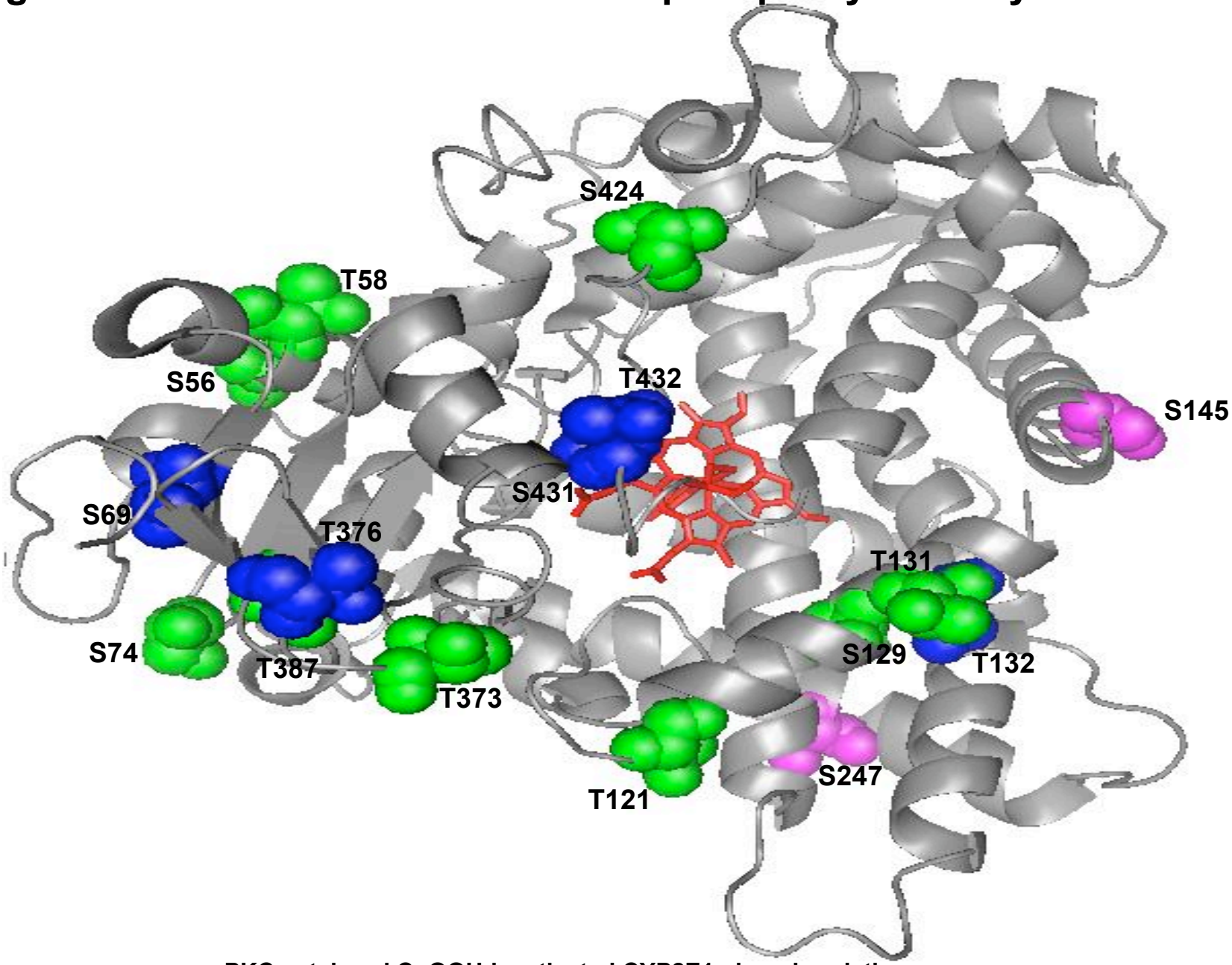
PKA-catalyzed CuOOH-inactivated CYP2E1 phosphorylation
Phosphorylation intensity: <0.1% (orange), <1% (magenta), <10% (blue), >10% (green)

Fig. S5. Native CYP2E1 phosphorylation by PKC



PKC-catalyzed native CYP2E1 phosphorylation
Phosphorylation intensity: <0.1% (orange), <1% (magenta), <10% (blue), >10% (green)

Fig. S6. CuOOH-inactivated CYP2E1 phosphorylation by PKC

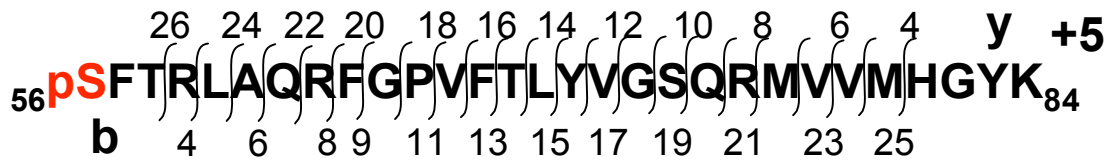


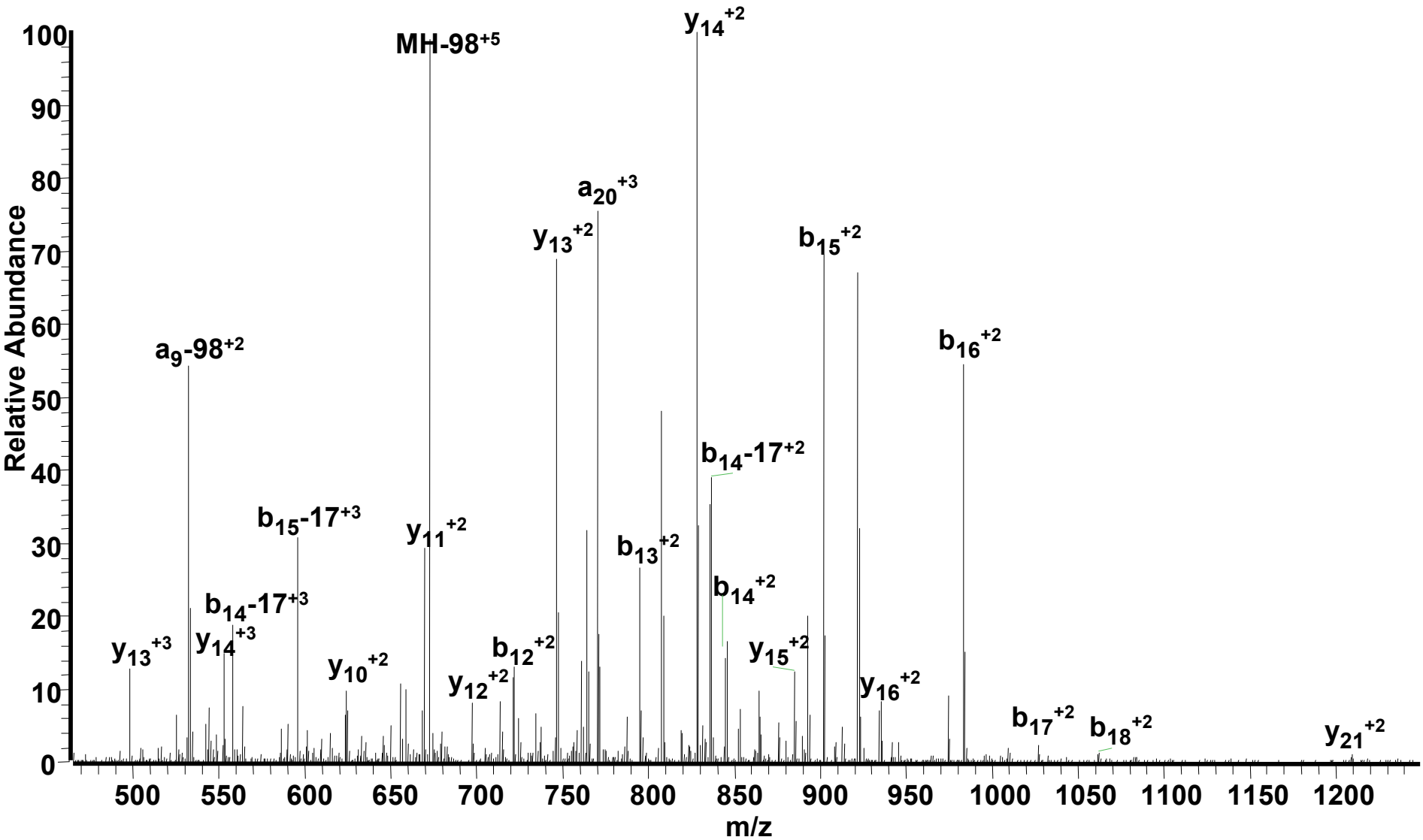
PKC-catalyzed CuOOH-inactivated CYP2E1 phosphorylation
Phosphorylation intensity: <0.1% (orange), <1% (magenta), <10% (blue), >10% (green)

Supplemental Data.

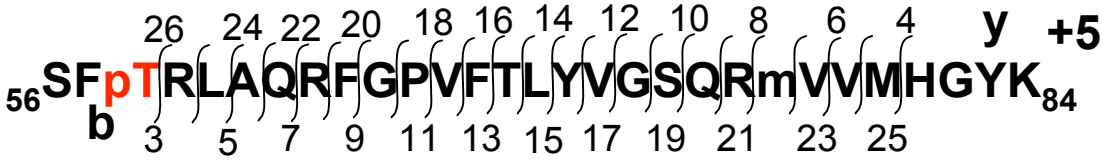
Annotated MS/MS spectra of CYP2E1 phosphorylation by PKA/PKC

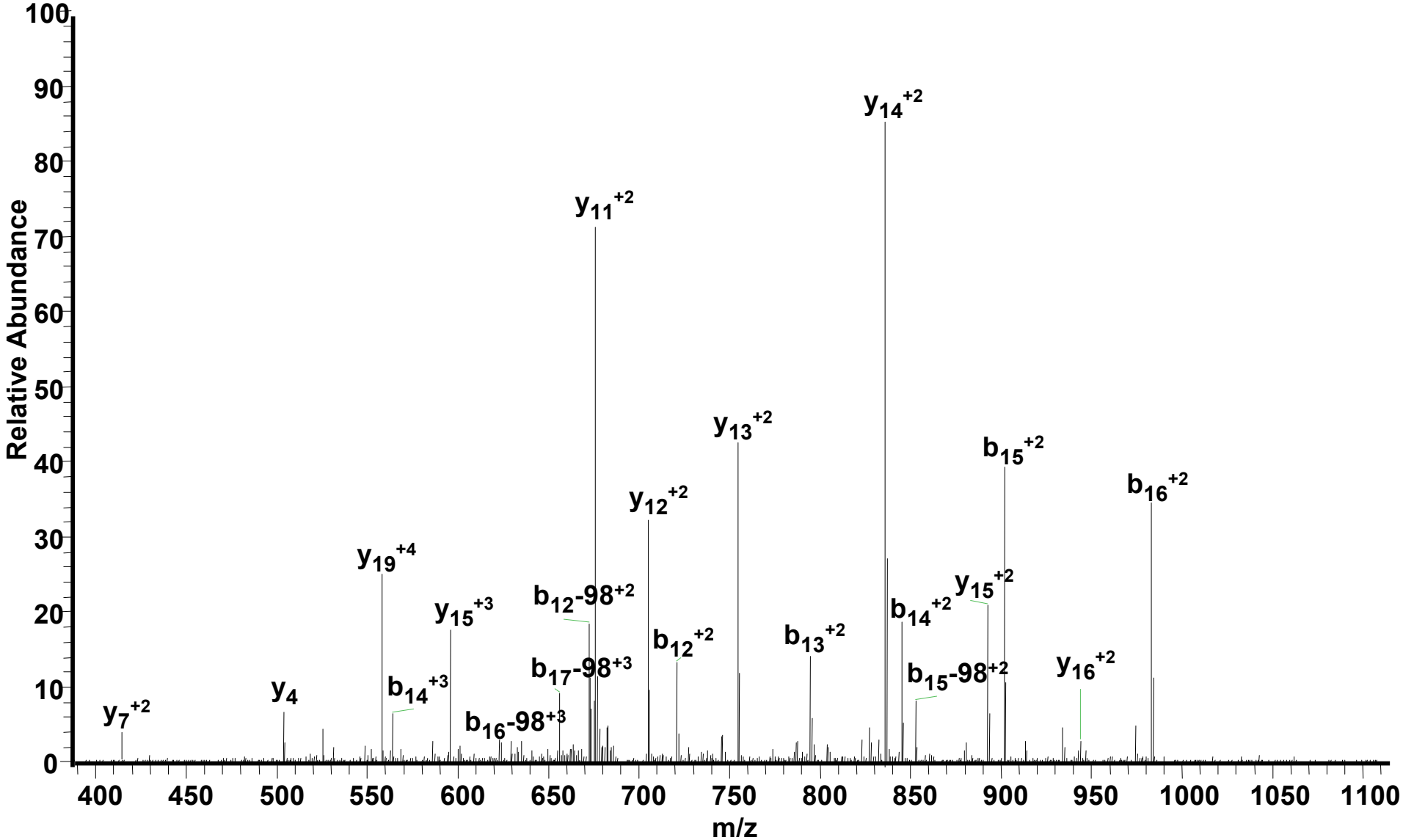
1. S56:


26 24 22 20 18 16 14 12 10 8 6 4 y +5
56pSFTRLAQRFGPVFTLYVGSQRMVVMHGYK84
b 4 6 8 9 11 13 15 17 19 21 23 25

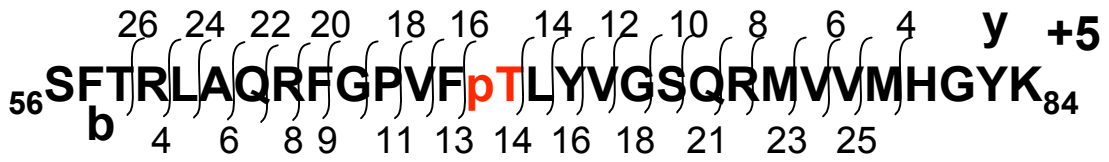

Relative Abundance
m/z
MH-98+5
y14+2
a20+3
y13+2
b15+2
b16+2
a9-98+2
b14-17+2
b15-17+3
y11+2
b13+2
b14+2
b14-17+3
y14+3
y13+3
y10+2
b12+2
y12+2
y15+2
y16+2
b17+2
b18+2
y21+2

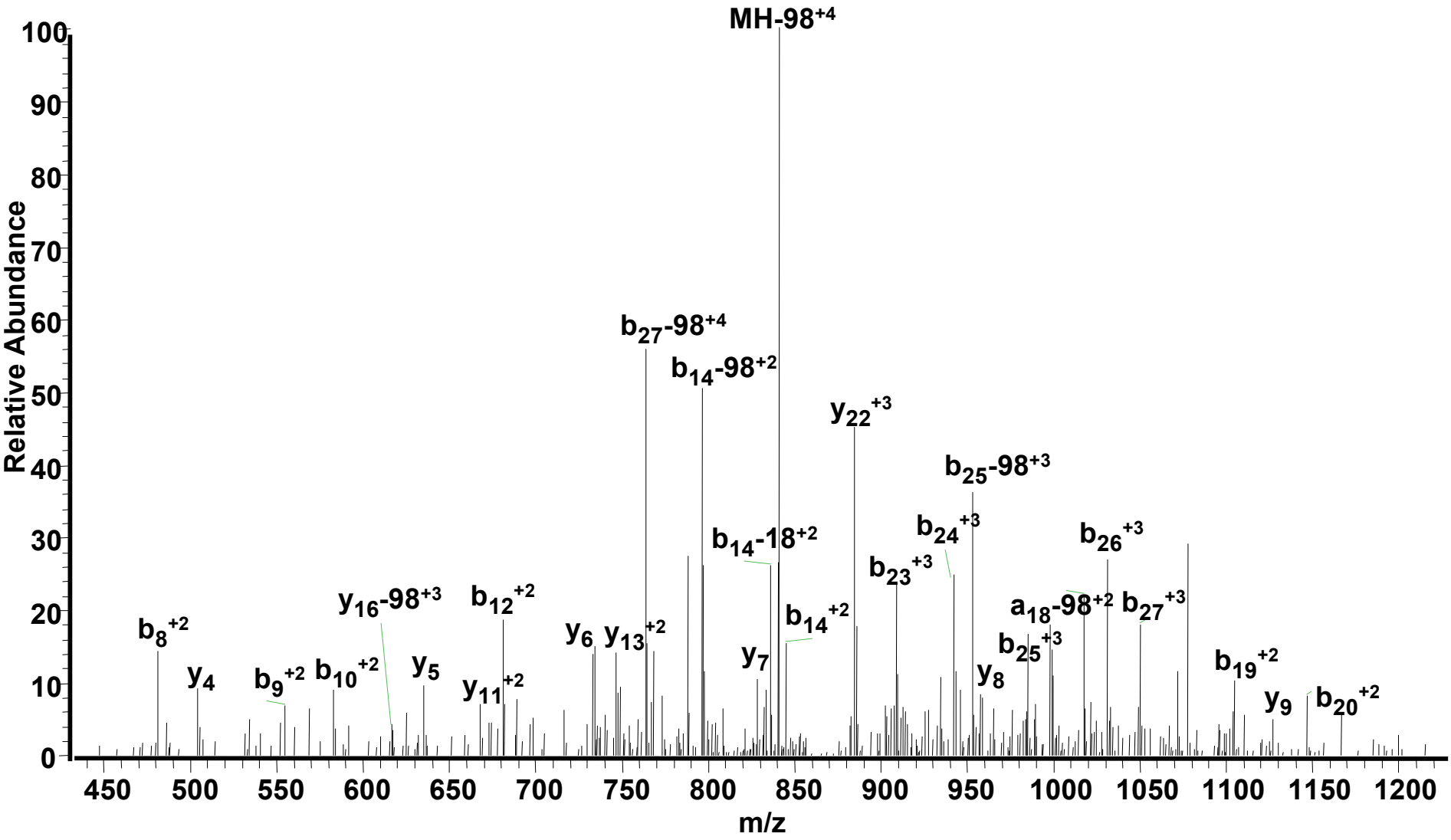
2. T58:


26 24 22 20 18 16 14 12 10 8 6 4 y +5
56SFpTRLAQRFGPVFTLYVGSQRmVVMHGYK84
b 3 5 7 9 11 13 15 17 19 21 23 25

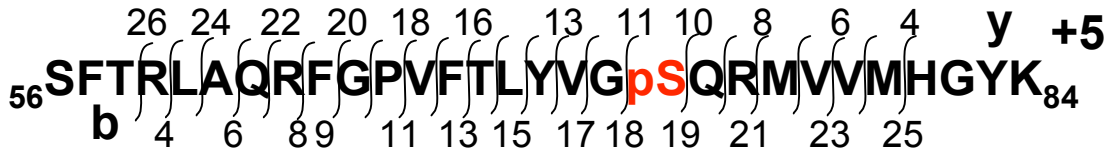

Relative Abundance
m/z
y7+2
y4
y19+4
b14+3
y15+3
b16-98+3
b17-98+3
b12-98+2
y11+2
y12+2
b12+2
y13+2
b13+2
y14+2
b14+2
b15-98+2
y15+2
b15+2
y16+2
b16+2

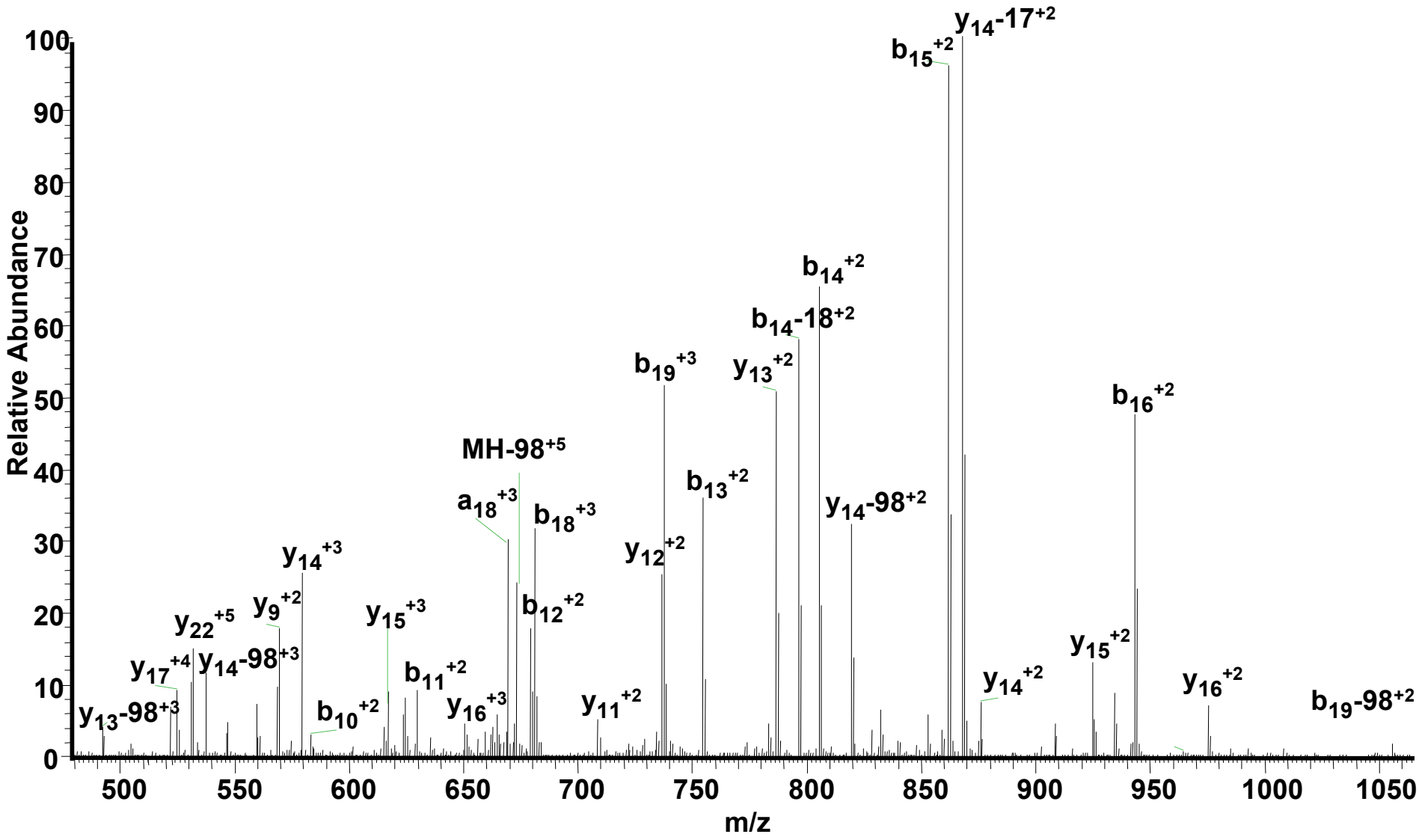
3. T69:


26 24 22 20 18 16 14 12 10 8 6 4 y +5
56SFTRLAQRFGPVFpTLYVGSQRMVVMHGYK84
b 4 6 8 9 11 13 14 16 18 21 23 25


Relative Abundance
m/z
MH-98+4
b27-98+4
b14-98+2
y22+3
b25-98+3
b24+3
b14-18+2
b26+3
b23+3
a18-98+2
b27+3
b14+2
b25+3
y16-98+3
b12+2
b8+2
y6
y13+2
y7
y8
b19+2
y4
b9+2
b10+2
y5
y11+2
y9
b20+2

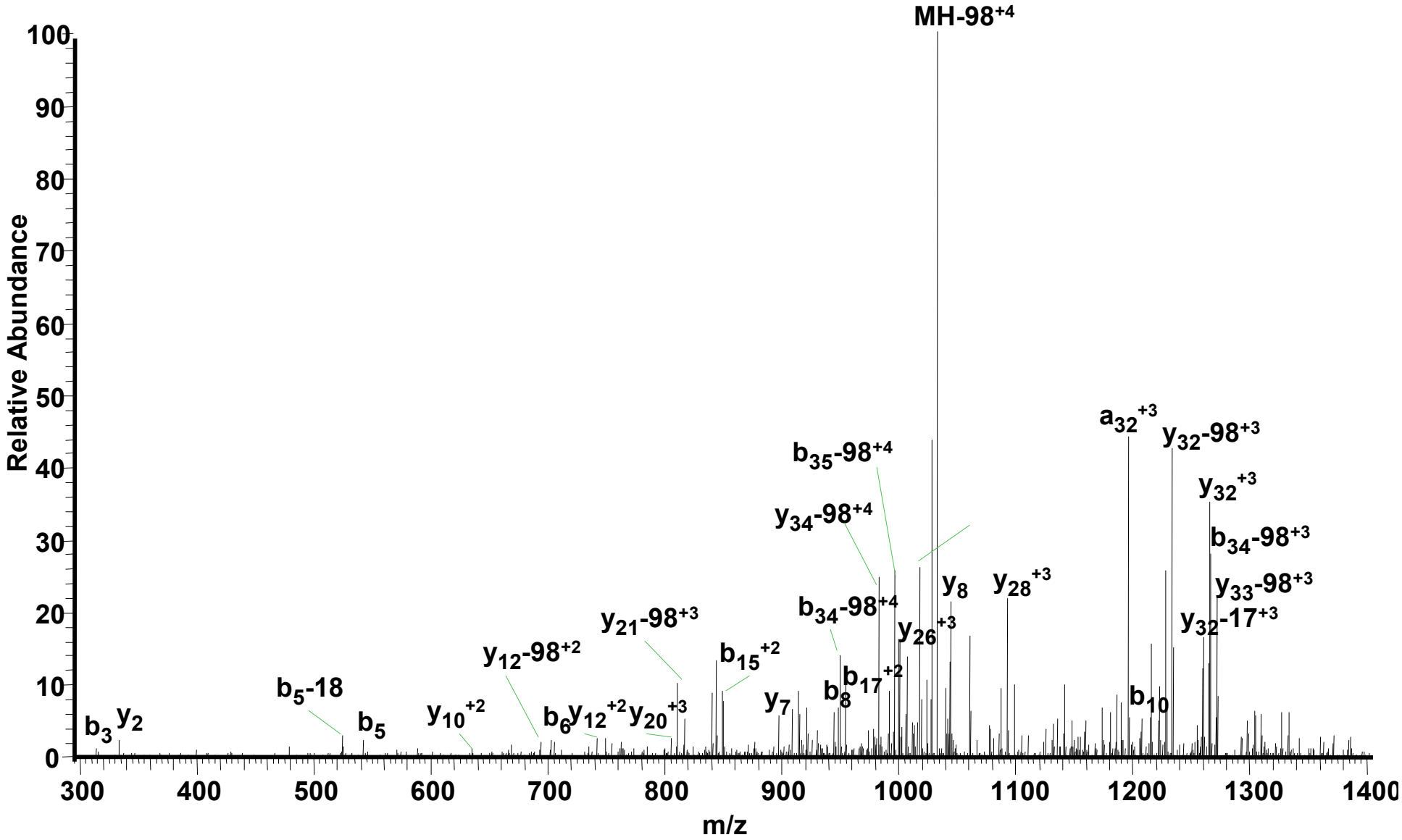
4. S74:


26 24 22 20 18 16 13 11 10 8 6 4 y +5
56SFTRLAQRFGPVFTLYVGpSQRMVVMHGYK84
b 4 6 8 9 11 13 15 17 18 19 21 23 25

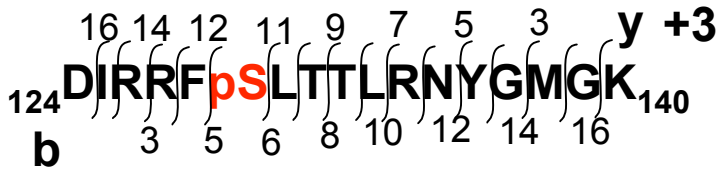

Relative Abundance
m/z
y14-17+2
b15+2
b14+2
b14-18+2
b19+3
y13+2
b16+2
MH-98+5
b13+2
a18+3
b18+3
y14-98+2
y12+2
y14+3
y9+2
y15+3
b12+2
y22+5
y17+4
y14-98+3
b11+2
y15+2
y14+2
y16+2
y16+3
y11+2
b10+2
y13-98+3
b19-98+2

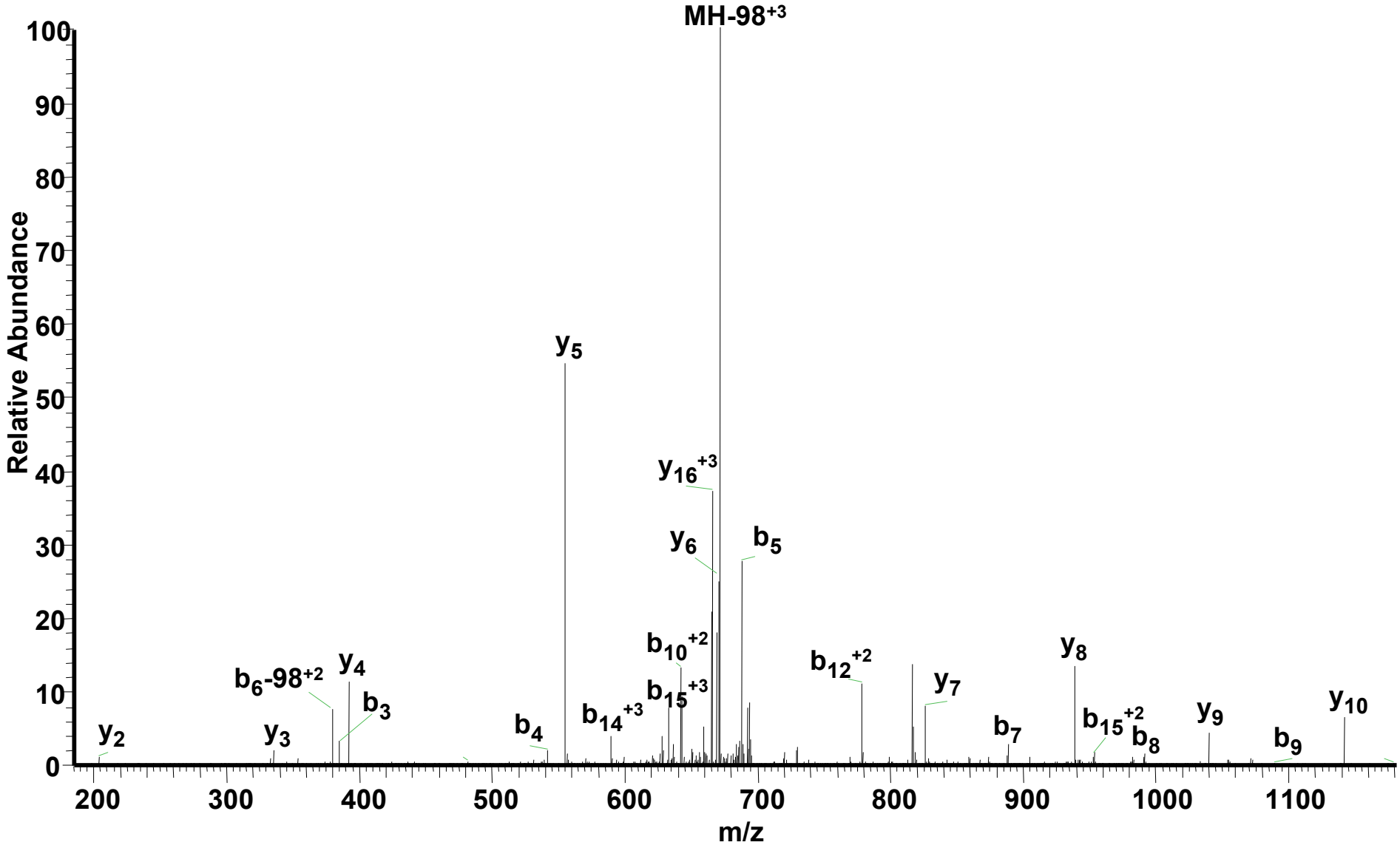
5. T121:

33 31 29 27 25 23 21 19 17 15 13 11 8 6 4
y +4
88EALLDYKDEFSGRGDLPAFHAHRDRGIIFNNGPpTWK123
b
8 9 11 13 15 17 19 21 23 25 28 30 32 34

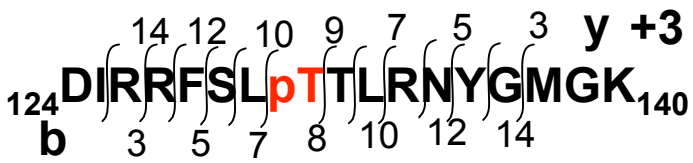

Relative Abundance
m/z
MH-98+4
a32+3
y32-98+3
y32+3
b34-98+3
y33-98+3
y32-17+3
b35-98+4
y34-98+4
y8
y28+3
b34-98+4
y26+3
y21-98+3
b15+2
y12-98+2
b17+2
b8
y7
b10
b5-18
b5
y10+2
b6
y12+2
y20+3
b3
y2

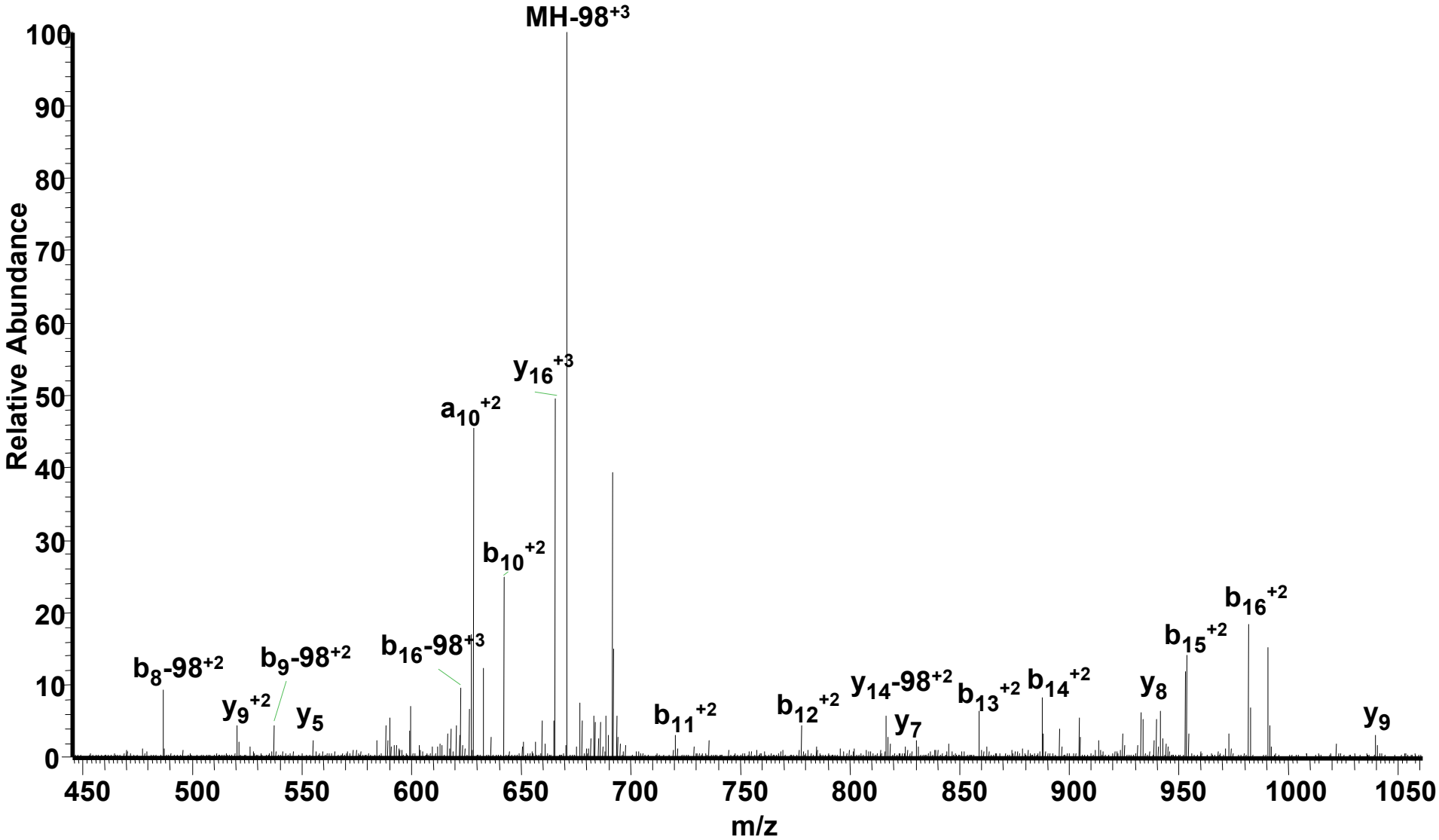
6. S129:


16 14 12 11 9 7 5 3 y +3
124DIRRFpSLTTLRNYGMGK140
b 3 5 6 8 10 12 14 16

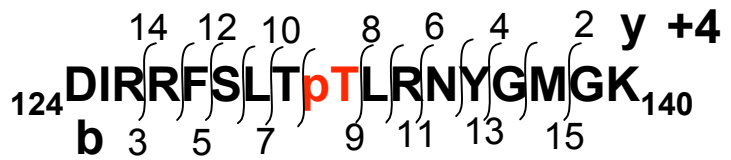

MH-98+3
y5
y16+3
y6
b5
b10+2
b15+3
b12+2
y7
y8
b14+3
b6-98+2
y4
b3
y2
y3
b4
b7
b15+2
b8
y9
b9
y10
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
300
400
500
600
700
800
900
1000
1100

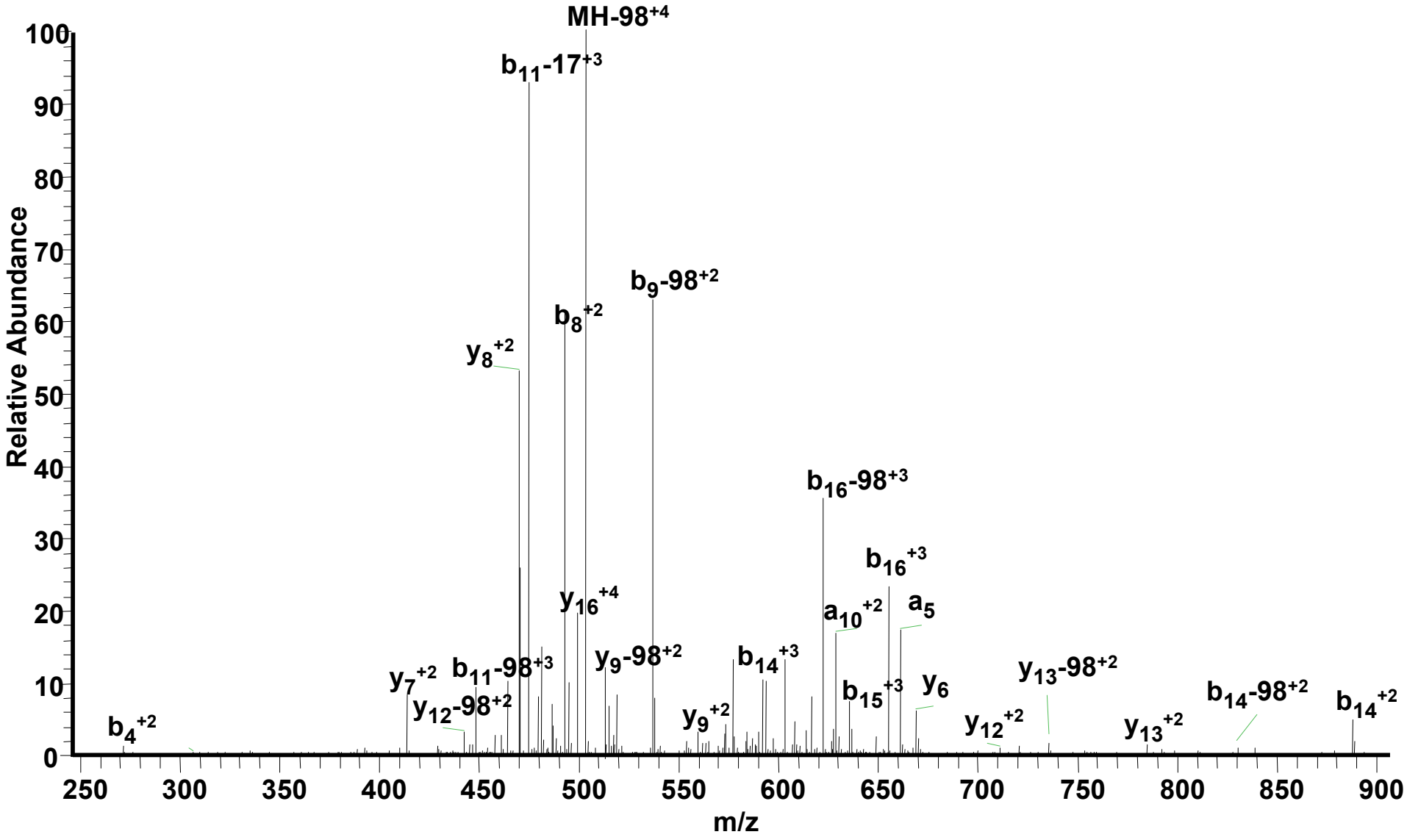
7. T131:


14 12 10 9 7 5 3 y +3
124DIRRFSLpTTLRNYGMGK140
b 3 5 7 8 10 12 14

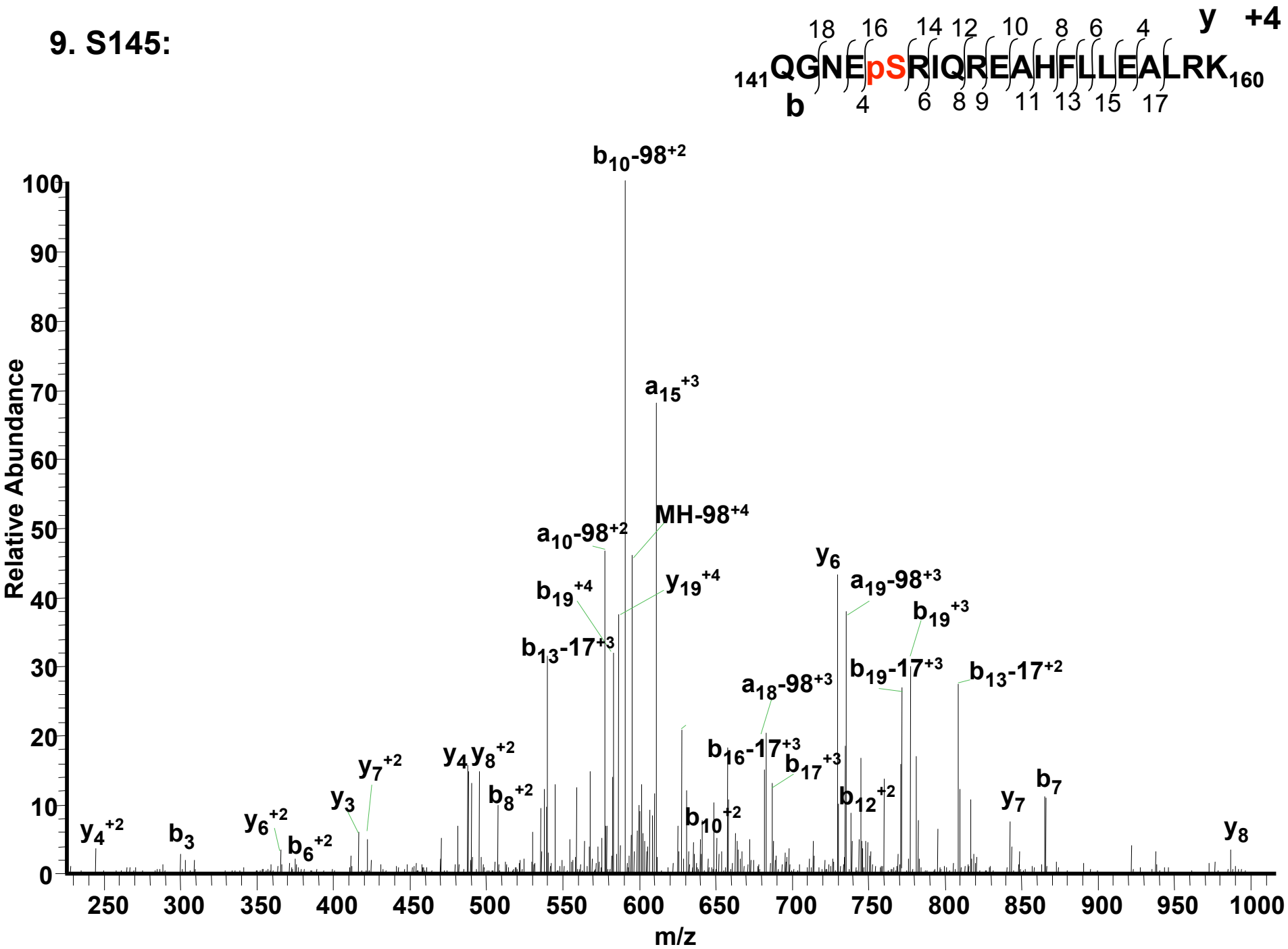

MH-98+3
y16+3
a10+2
b10+2
b16+2
b15+2
b16-98+3
b8-98+2
b9-98+2
y9+2
y5
b11+2
b12+2
y14-98+2
y7
b13+2
b14+2
y8
y9
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
450
500
550
600
650
700
750
800
850
900
950
1000
1050

8. T132:

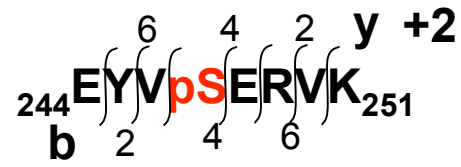

14 12 10 8 6 4 2 y +4
124DIRRFSLTpTLRNYGMGK140
b 3 5 7 9 11 13 15


Relative Abundance
m/z
MH-98+4
b11-17+3
b9-98+2
b8+2
y8+2
b16-98+3
b16+3
a5
a10+2
y16+4
y9-98+2
b14+3
y7+2
b11-98+3
y12-98+2
y9+2
b15+3
y6
y13-98+2
b14-98+2
b14+2
y12+2
y13+2
b4+2

9. S145:


y +4
18 16 14 12 10 8 6 4
141QGNEpSRIQREAHFLLEALRK160
b 4 6 8 9 11 13 15 17
Relative Abundance
m/z
b10-98+2
a15+3
MH-98+4
a10-98+2
y19+4
b19+4
b13-17+3
y6
a19-98+3
b19+3
b19-17+3
b13-17+2
a18-98+3
b16-17+3
b17+3
b12+2
b10+2
y7+2
y4
y8+2
b8+2
y3
y6+2
b6+2
y4+2
b3
y7
b7
y8

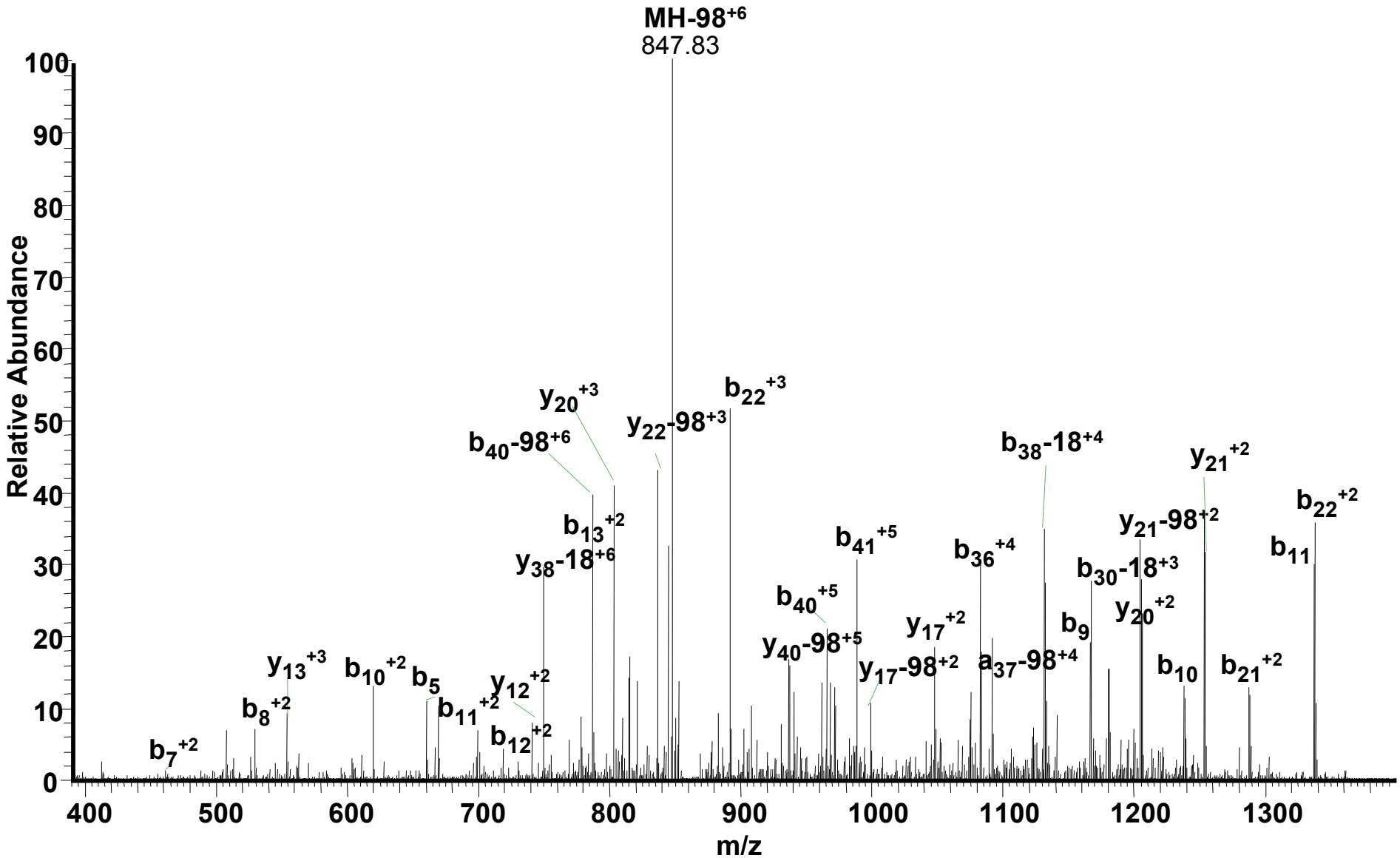
10. S247:


6 4 2 y +2
244EYVpSERVK251
b 2 4 6

MH-18+2
MH-98+2
y5
y6
y3
y6+2
y5+2
y7+2
y2
b2
y6-18
b7-18
Relative Abundance
m/z
0
5
10
15
20
25
30
35
40
45
50
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900

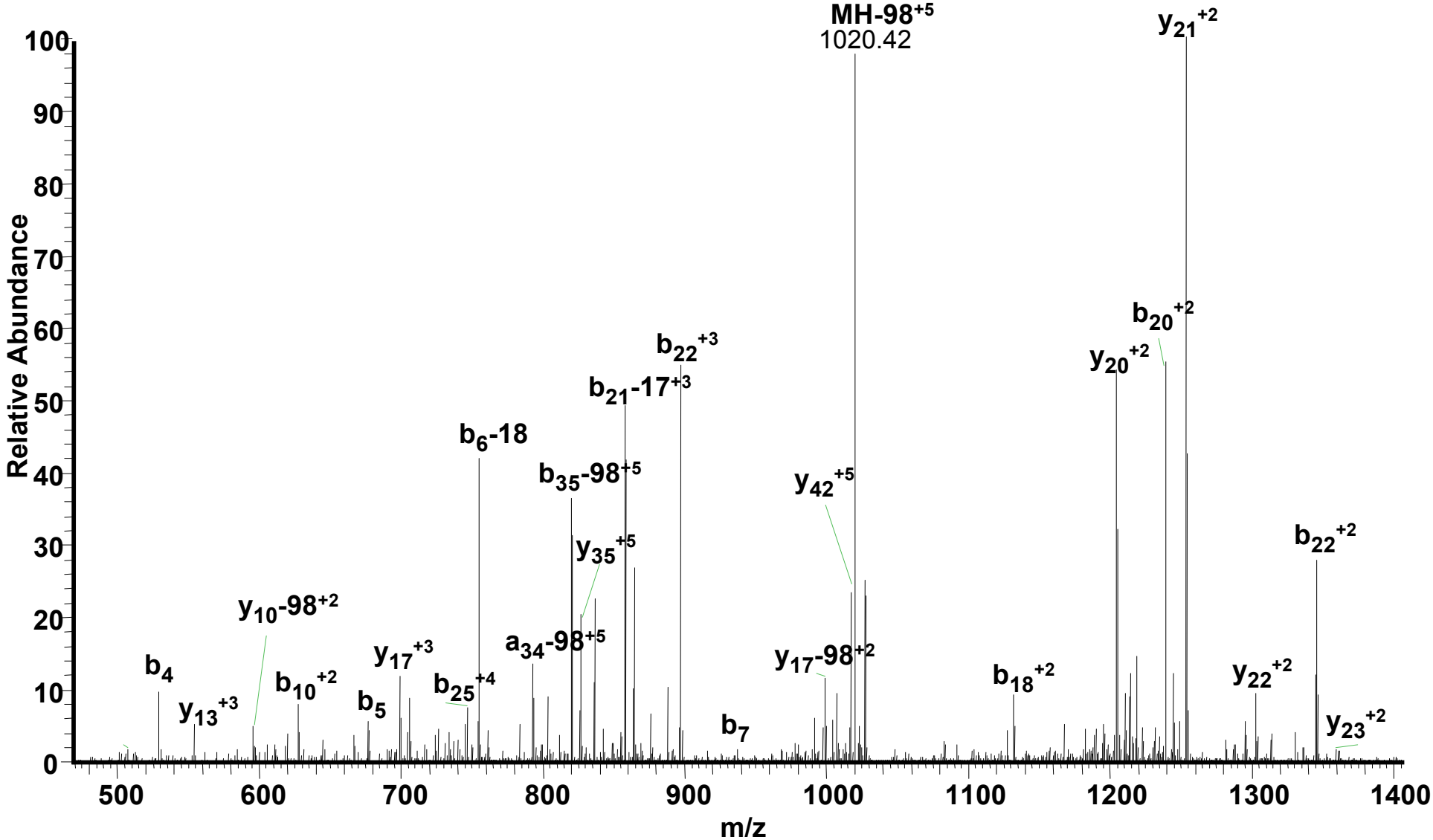
11. T373:

41 39 37 35 33 31 29 27 25 22 20 18 16 14 12 10 8 6 4
y
+6
346DRQEMPYMDAVVHEIQRFITLVPSNLPHEApTRDTIFRGYLIPK388
b
4 6 8 9 11 13 16 18 21 23 25 27 29 31 33 35 37 39 41

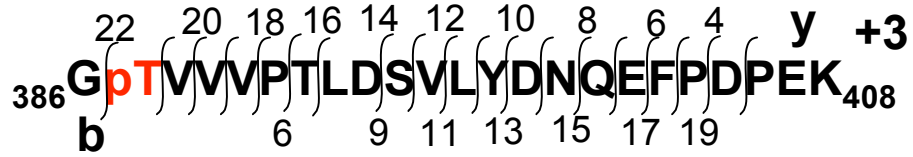

MH-98+6
847.83
Relative Abundance
m/z
y20+3
b22+3
y22-98+3
b40-98+6
b38-18+4
y21+2
b22+2
b13+2
y38-18+6
b41+5
b36+4
y21-98+2
b11
b30-18+3
b40+5
y20+2
y17+2
b9
y40-98+5
y17-98+2
a37-98+4
b10
b21+2
y13+3
b10+2
b5
y12+2
b11+2
b8+2
b12+2
b7+2

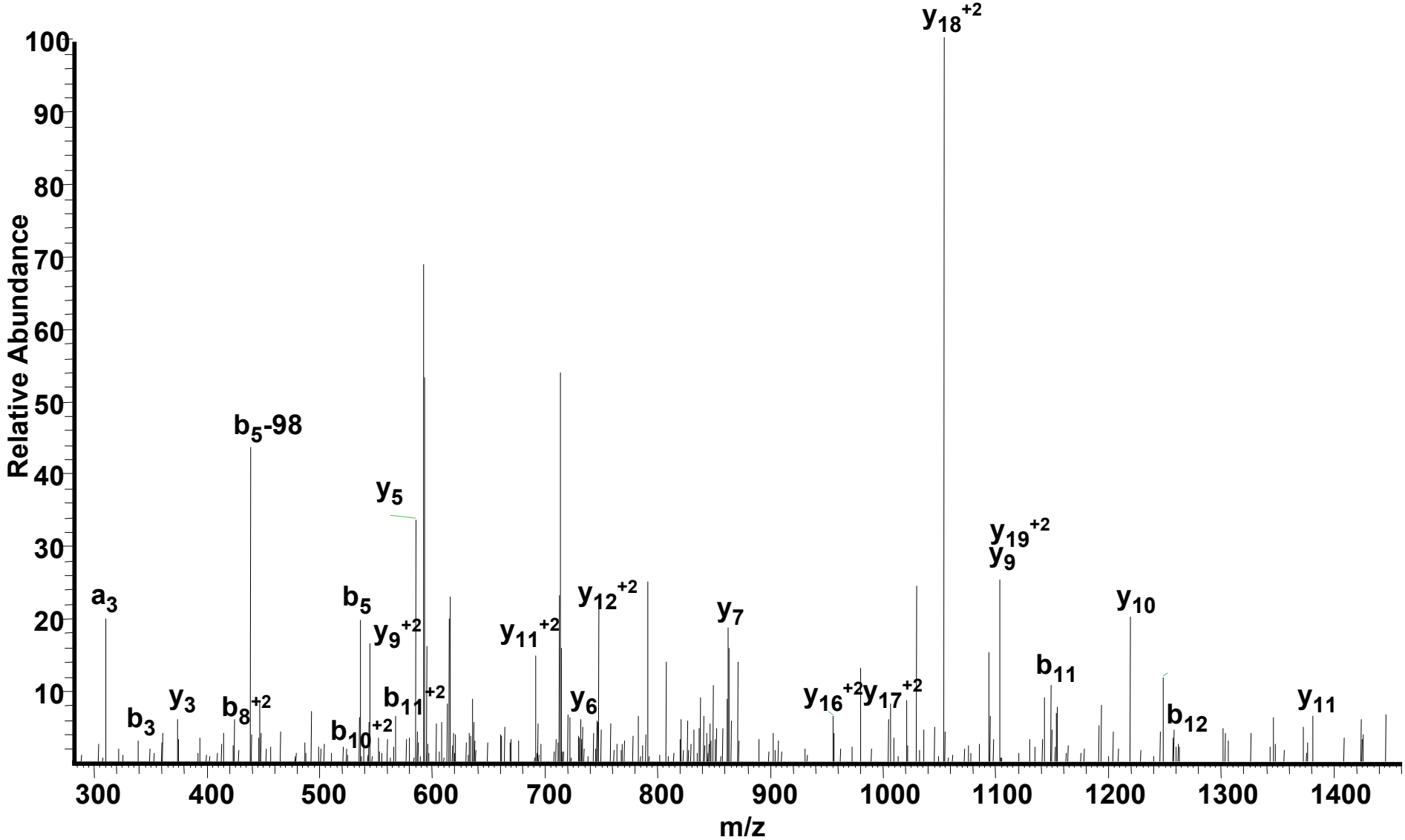
12. T376:

41 39 37 35 33 31 29 27 25 22 20 18 16 14 12 10 8 6 4 y +5
346DRQEmPYMDAVVHEIQRFITLVPSNLPHEATRDpTIFRGYLIPK388
b 4 6 8 9 11 13 16 18 21 23 25 27 29 31 33 35 37 39 41

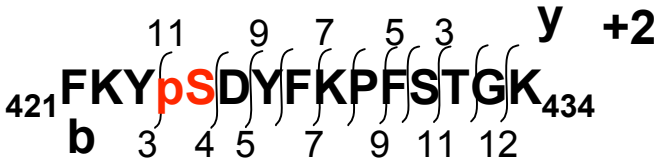

Relative Abundance
m/z
MH-98+5
1020.42
y21+2
b20+2
y20+2
b22+3
b21-17+3
b6-18
b35-98+5
y42+5
y35+5
b22+2
y10-98+2
a34-98+5
y17+3
y17-98+2
b4
b10+2
b25+4
b18+2
y22+2
y13+3
b5
b7
y23+2

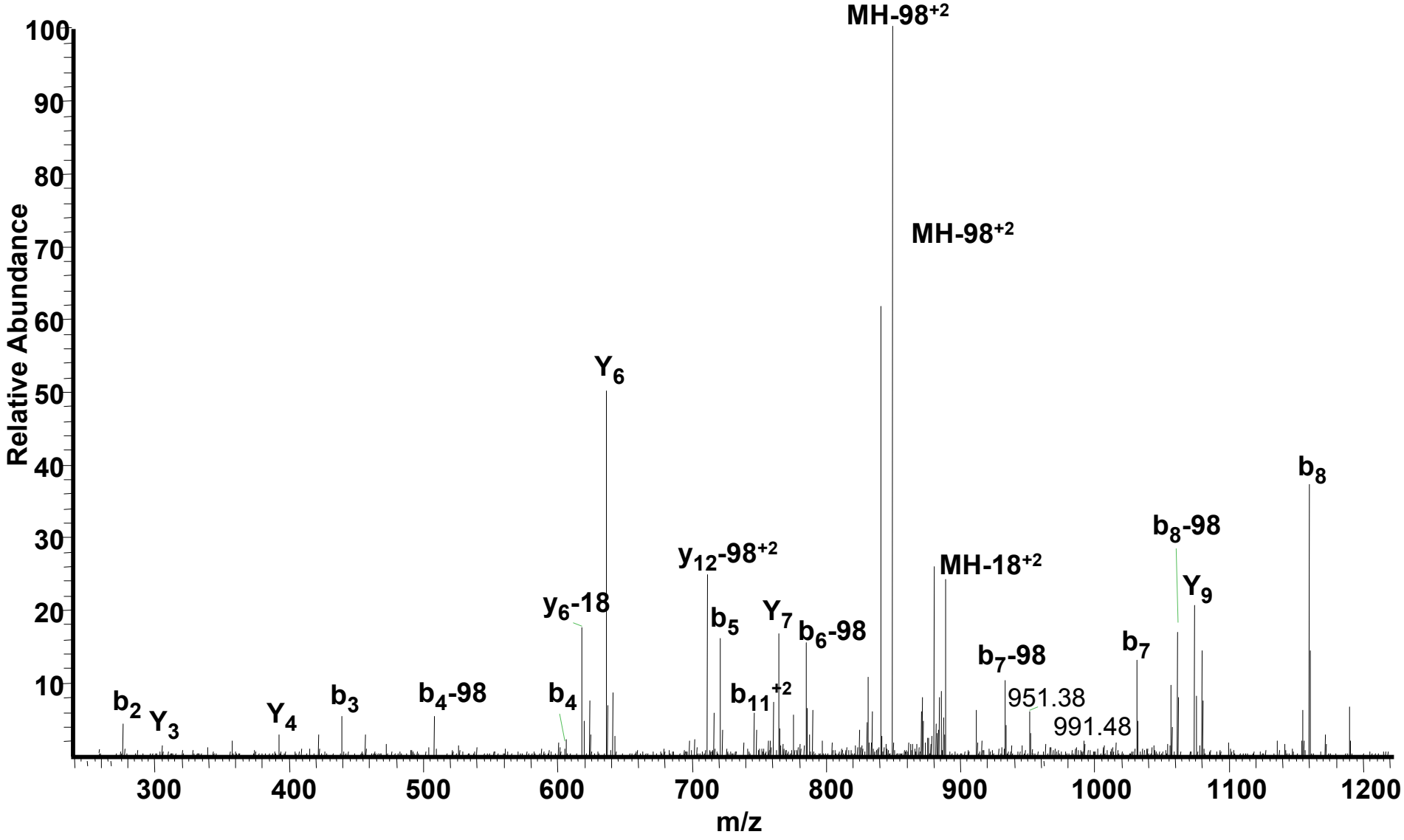
13. T387:


22 20 18 16 14 12 10 8 6 4 y +3
386GpTVVVPTLDSVLYDNQEFPDPEK408
b
6 9 11 13 15 17 19

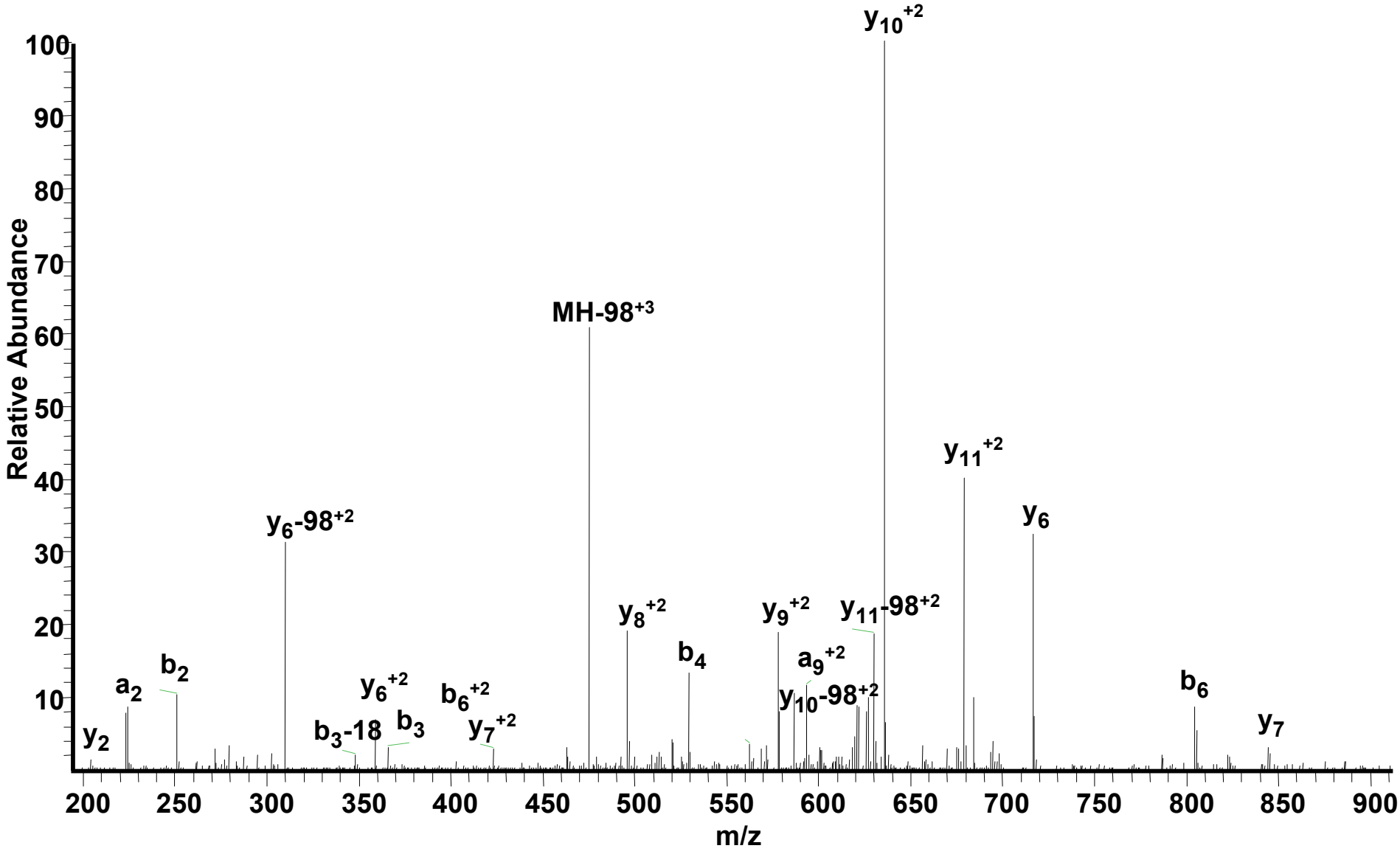

Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
y18+2
b5-98
y5
a3
b5
y9+2
y11+2
y12+2
y7
y19+2
y9
y10
b11
b3
y3
b8+2
b11+2
b10+2
y6
y16+2
y17+2
b12
y11

14. S424:


11 9 7 5 3
y +2
421FKYpSDYFKPFSTGK434
b 3 4 5 7 9 11 12

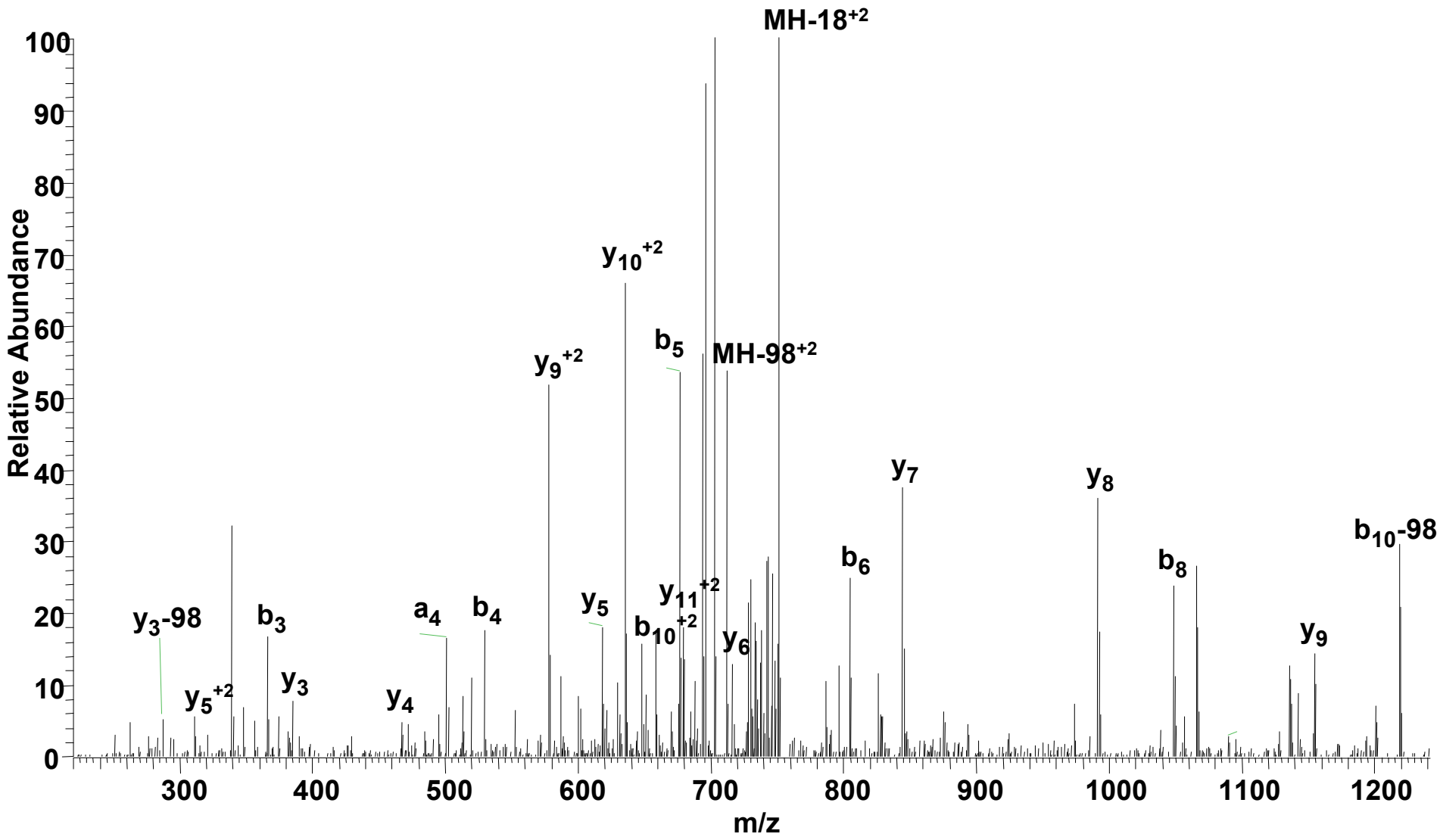

Relative Abundance
m/z
MH-98+2
MH-98+2
Y6
y12-98+2
y6-18
b5
Y7
b6-98
MH-18+2
b8
b8-98
Y9
b7
b7-98
951.38
991.48
b11+2
b2
Y3
Y4
b3
b4-98
b4

15. S431:


y+3
11 9 7 5 3
423YSDYFKPFpSTGK434
b 3 5 7 9 10
Relative Abundance
m/z
MH-98+3
y10+2
y11+2
y6
y6-98+2
y8+2
y9+2
y11-98+2
b4
a9+2
y10-98+2
b6
y7
y6+2
b6+2
b3-18
b3
y7+2
a2
b2
y2

16. T432:

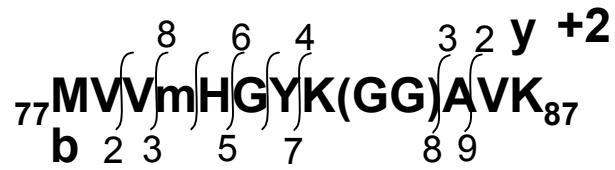
y+2
11 9 7 5 3
423 YSDYFKPFSpTGK 434
b 3 5 7 9 10

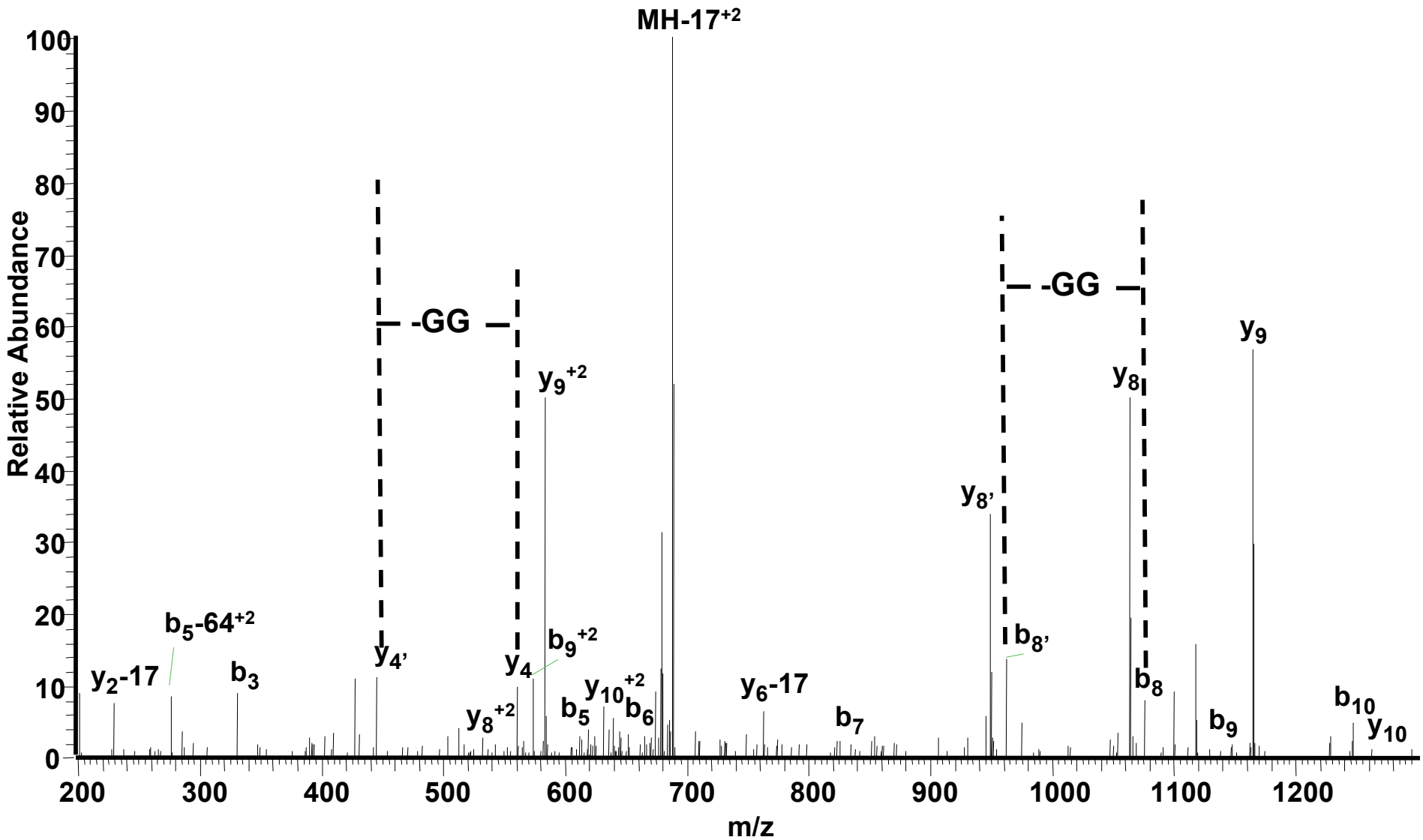

Relative Abundance
m/z
MH-18+2
y10+2
b5
MH-98+2
y9+2
y7
y8
b10-98
b6
b8
y11+2
y5
b4
a4
b3
y3-98
b10+2
y6
y9
y5+2
y3
y4

Data-2

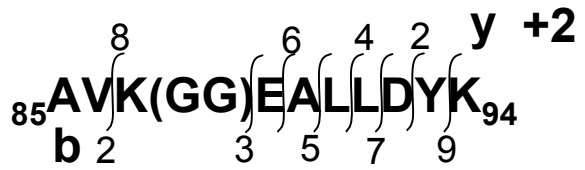
Annotated MS/MS spectra of Ubc7/gp78-catalyzed CYP2E1 ubiquitination

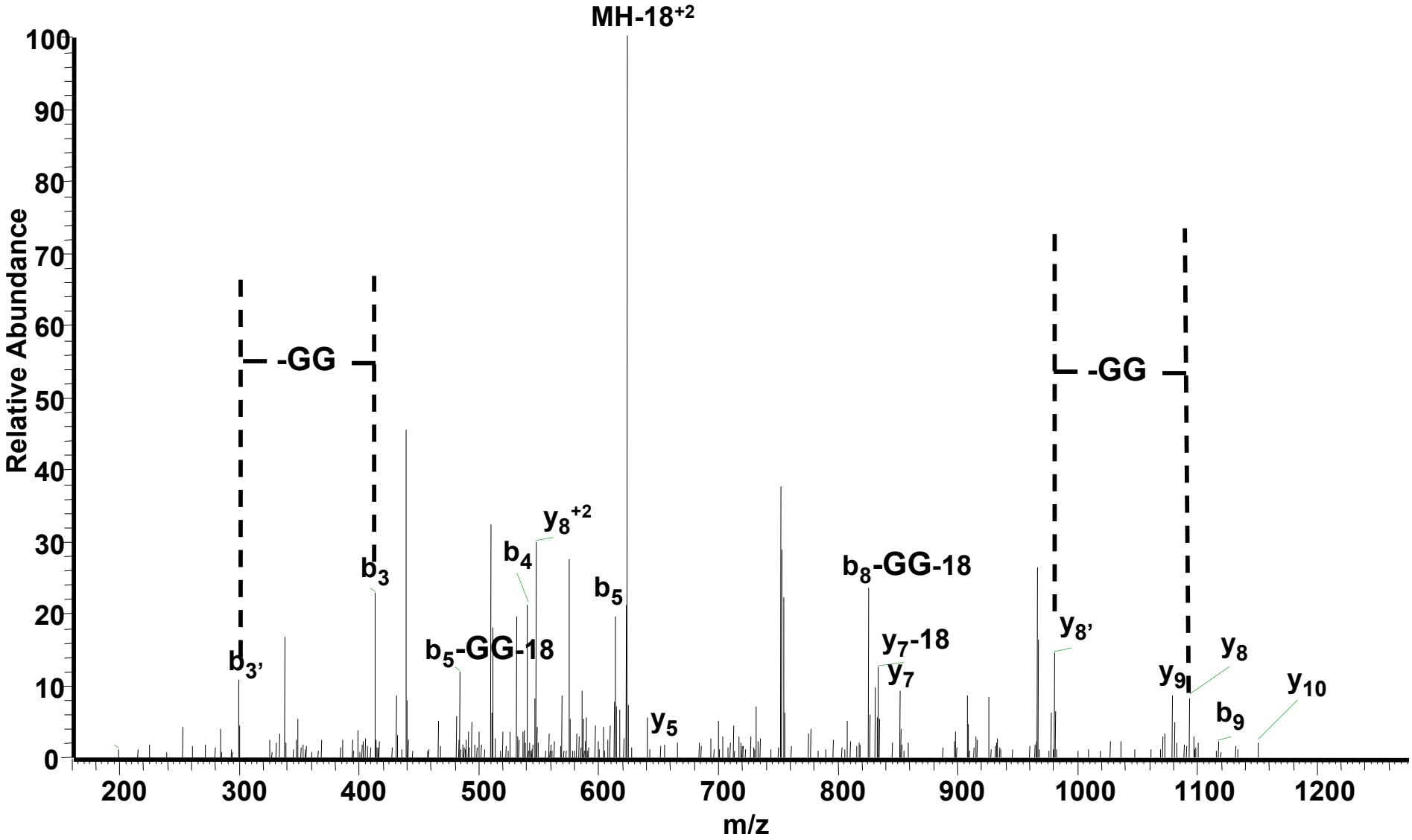
1. K84: 696.826, +2, 17ppm



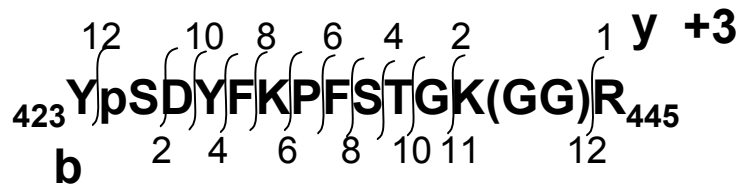


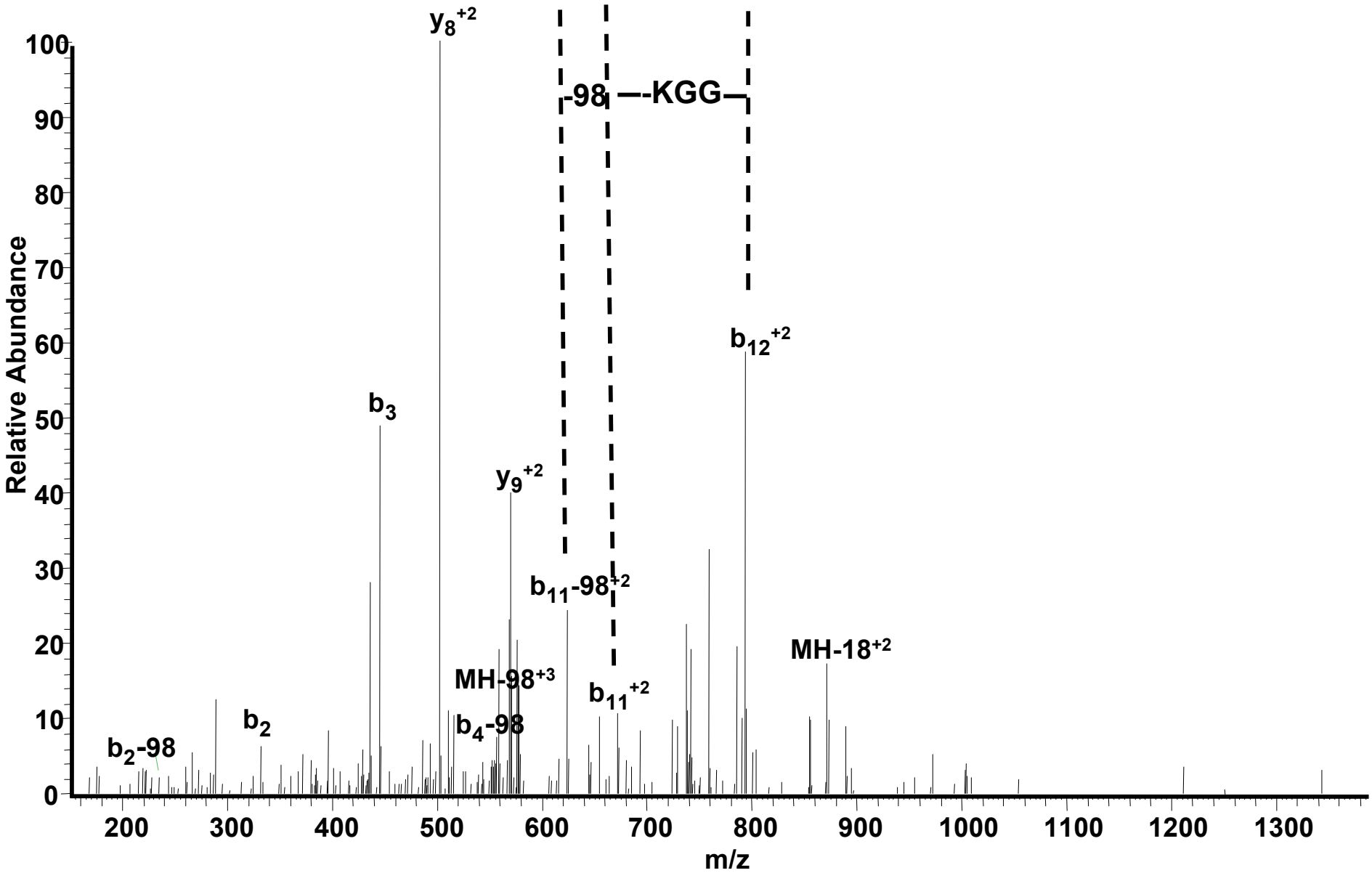
2. K87: 632.351, +2, 0.47ppm





3. K434: 587.320, +3, 27ppm

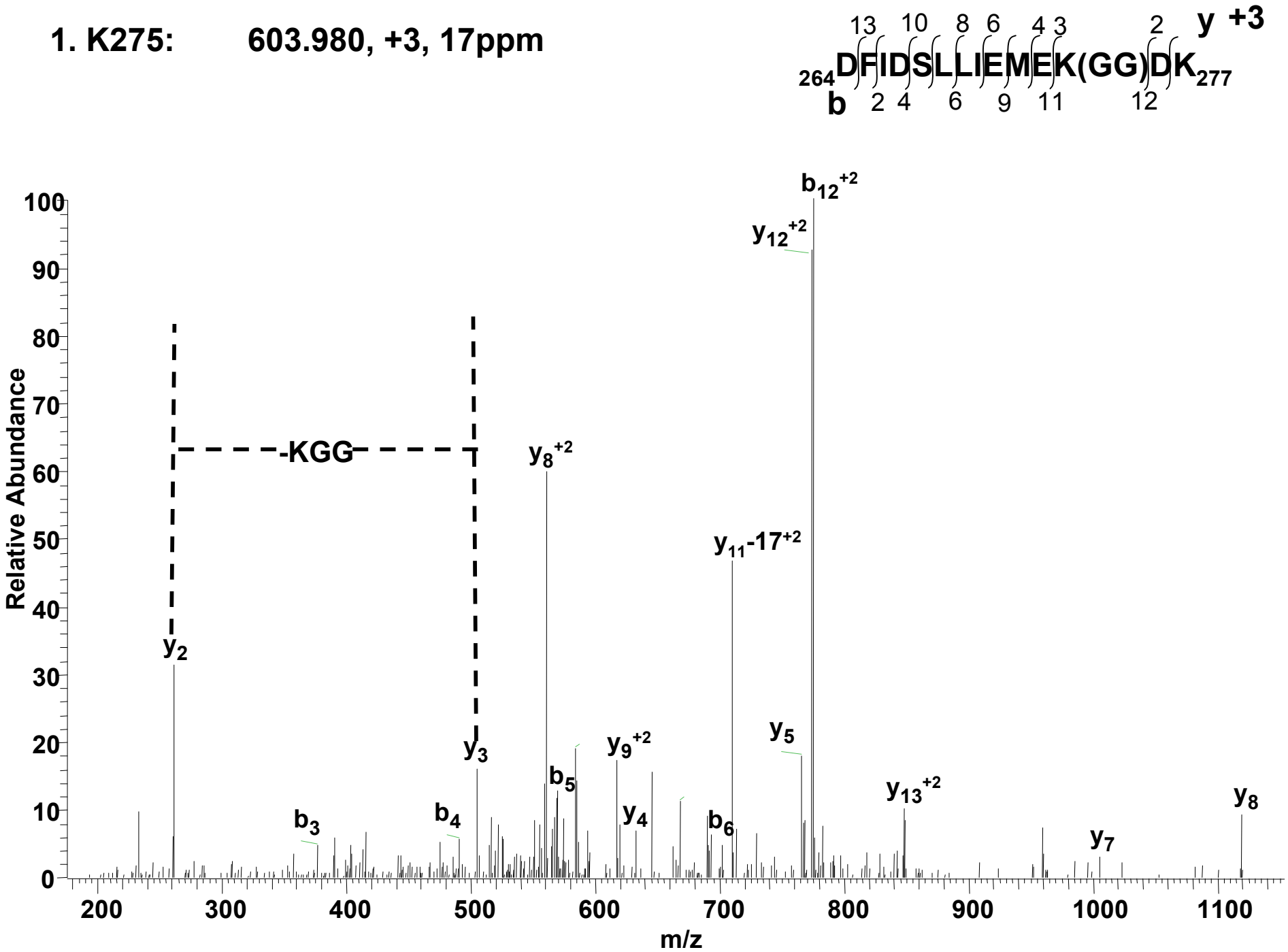
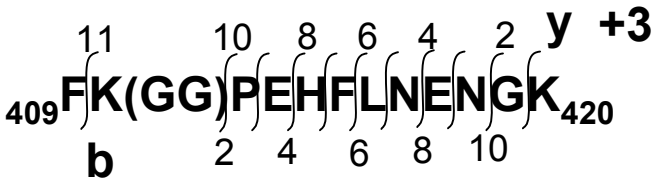
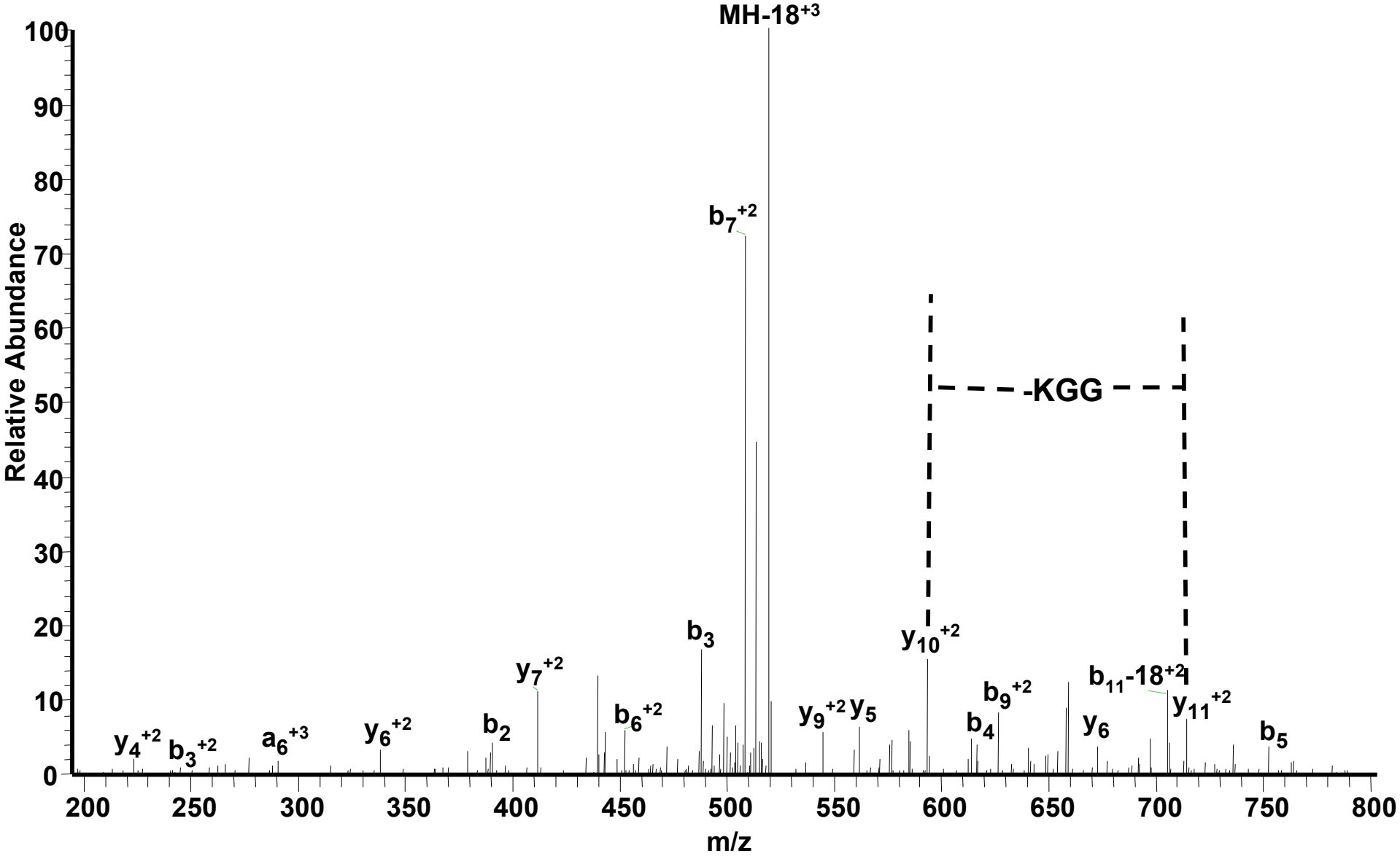




Data-3

Annotated MS/MS spectra of Ubc5a/CHIP-catalyzed CYP2E1 ubiquitination

1. K275: 603.980, +3, 17ppm



2. K410: 525.263, +3, -15ppm