Supplemental Figure

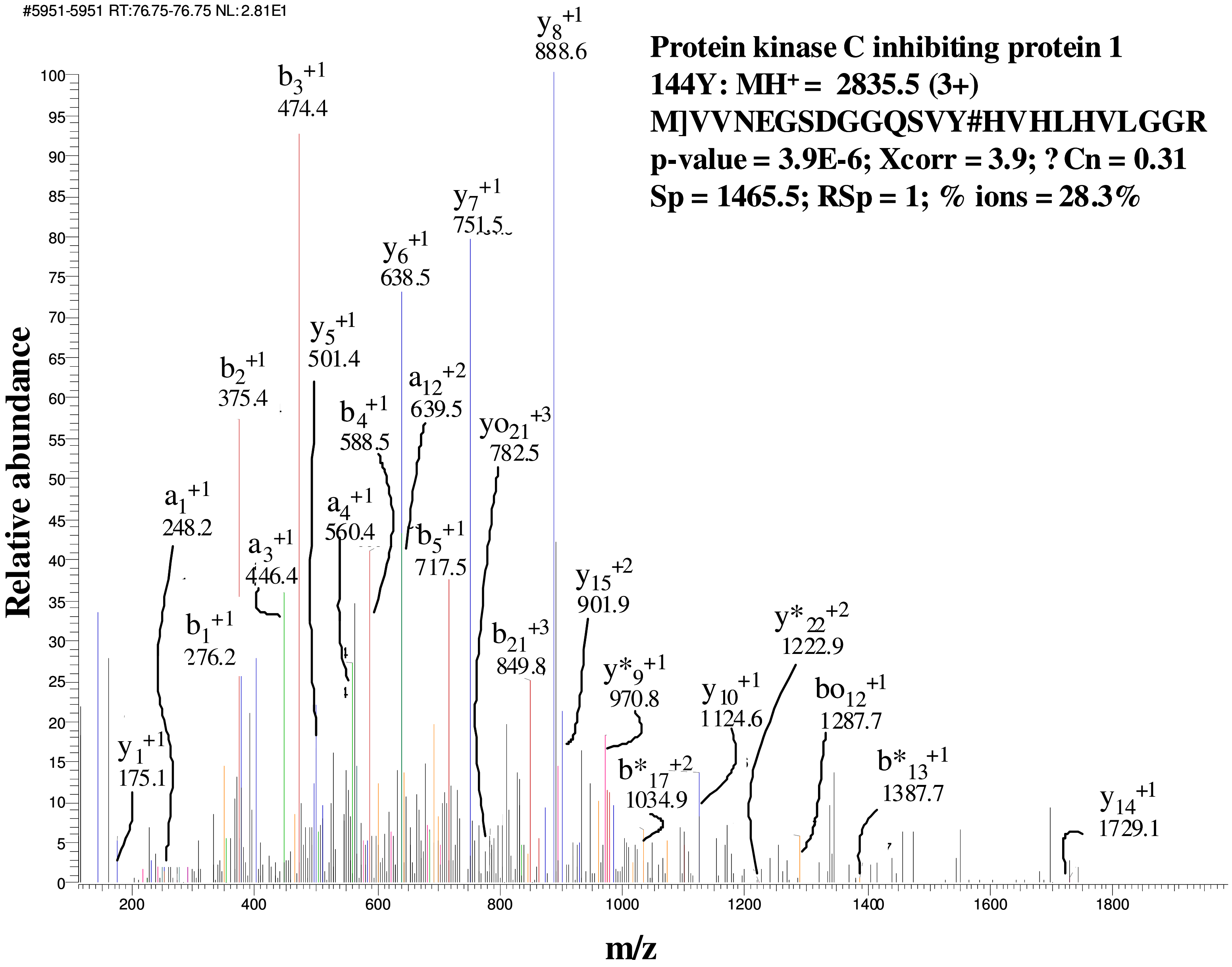
Selected tandem mass spectra for labeled peptides. (**A**) P49773 Protein kinase C inhibiting protein 1 (**144Y**): M]VVNEGSDGGQSVY#HVHLHVLGGR; (**B**) P06733 α-Enolase (**144C**): S]GETEDTFIADLVVGLC#TGQIK*; (**C**) P52948 Nup 98-Nuclear pore complex protein (**42K**): LYQTPLELK#LK*; (**D**) P63313 Thymosin beta-10 (**43K**): A]DK#PDMGEIASFDK*AK*; (**E**) P05783 Cytokeratin-18 (**16M**): Y]ALQM#EQLNGILLHLESELAQTR.

Note 1: In the peptide amino acid sequence: "]" and "*" indicate the iTRAQ tag at the Nt and Lys residues, respectively; "#" indicates the additional amino acid modification.

Note 2: Fragment **b/y** ions that carry the modification and immediately adjacent to the modification are provided in boxes; the modification site is provided in ***bold***.

Note 3: In tandem mass spectra: "o" represents ions that lost one molecule of H_2O; "*" represents ions that lost one molecule of NH_3; "I" represents internal fragments.

Note 4: In tandem mass spectrum **A,** the site of modification ($\mathbf{y_{11}}$) could not be assigned. However, the presence of Nt sequence ions ($\mathbf{b_1}$, $\mathbf{b_2}$, $\mathbf{b_3}$, $\mathbf{b_4}$, $\mathbf{b_5}$), of $\mathbf{y_5}$, $\mathbf{y_6}$, $\mathbf{y_7}$, $\mathbf{y_8}$, $\mathbf{y_9}$, and $\mathbf{y_{10}}$ ion series before the modification, and of good Sequest scores, confirmed the iTRAQ labeling of the Tyr residue. In tandem mass spectra **2B/C/D/E**, the precise site of modification was assigned. The **b/y** ions adjacent and at the modification site are provided in boxes, the modification site being provided in ***bold***.



Protein kinase C inhibiting protein 1
144Y: MH⁺ = 2835.5 (3+)
M]VVNEGSDGGQSVY#HVHLHVLGGR
p-value = 3.9E-6; Xcorr = 3.9; ? Cn = 0.31
Sp = 1465.5; RSp = 1; % ions = 28.3%

Figure A

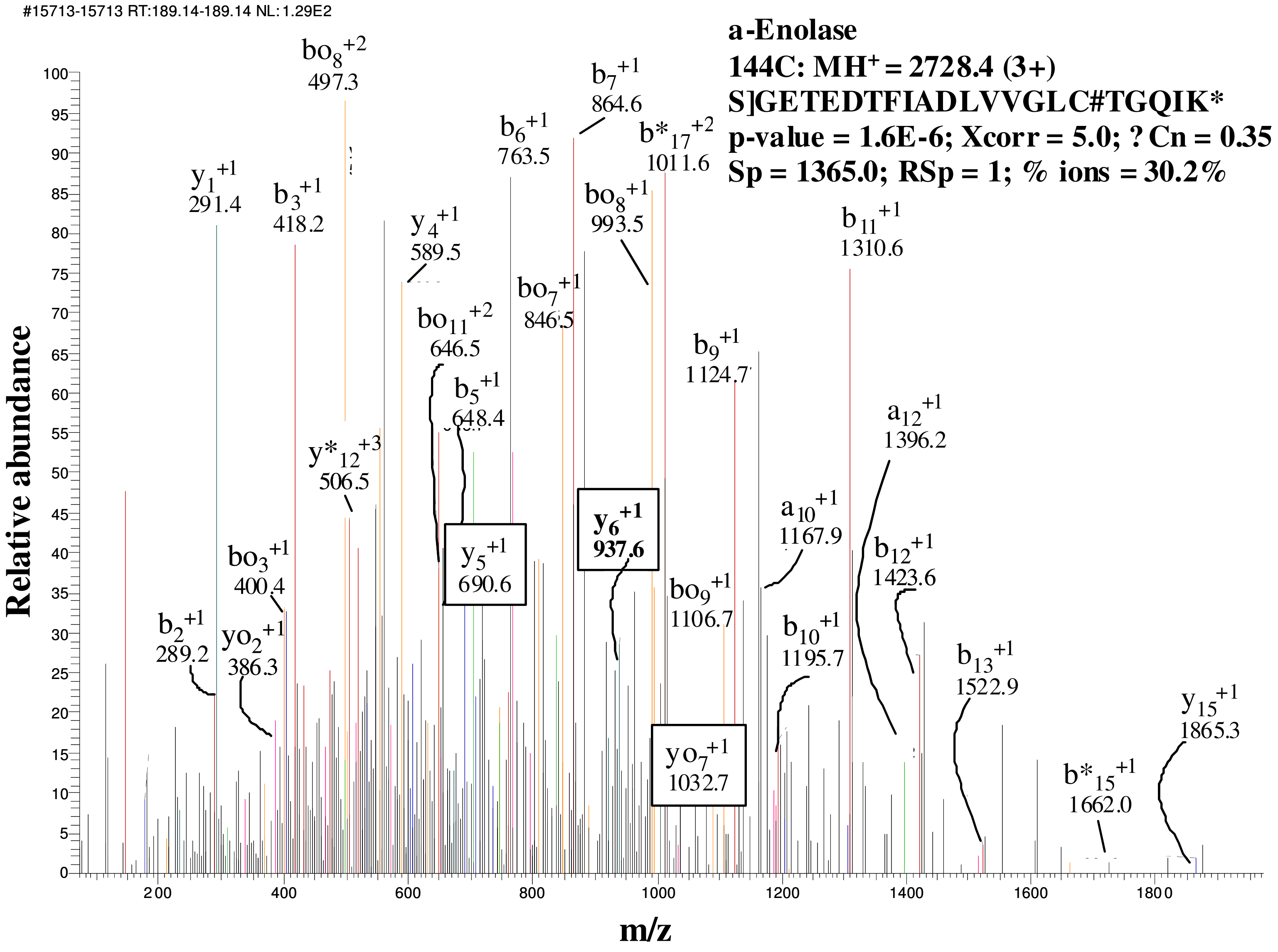

#15713-15713 RT:189.14-189.14 NL:1.29E2
a-Enolase
144C: MH+ = 2728.4 (3+)
S]GETEDTFIADLVVGLC#TGQIK*
p-value = 1.6E-6; Xcorr = 5.0; ? Cn = 0.35
Sp = 1365.0; RSp = 1; % ions = 30.2%
Relative abundance
m/z
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
200
400
600
800
1000
1200
1400
1600
1800
bo8+2 497.3
b7+1 864.6
b6+1 763.5
b*17+2 1011.6
y1+1 291.4
b3+1 418.2
y4+1 589.5
bo8+1 993.5
b11+1 1310.6
bo7+1 846.5
bo11+2 646.5
b9+1 1124.7
b5+1 648.4
a12+1 1396.2
y*12+3 506.5
y5+1 690.6
y6+1 937.6
a10+1 1167.9
b12+1 1423.6
bo3+1 400.4
bo9+1 1106.7
b2+1 289.2
yo2+1 386.3
b10+1 1195.7
b13+1 1522.9
yo7+1 1032.7
y15+1 1865.3
b*15+1 1662.0

Figure B

.

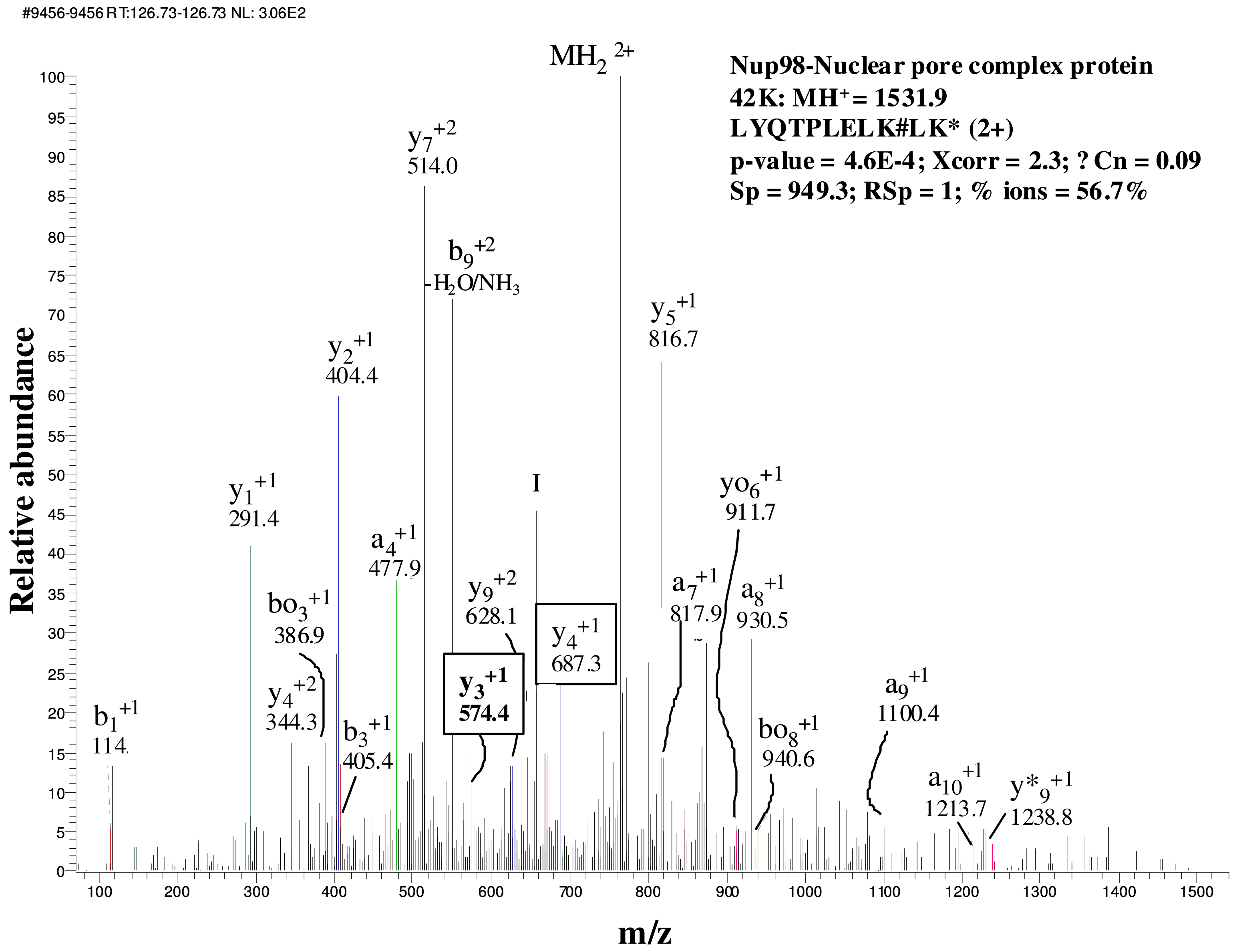
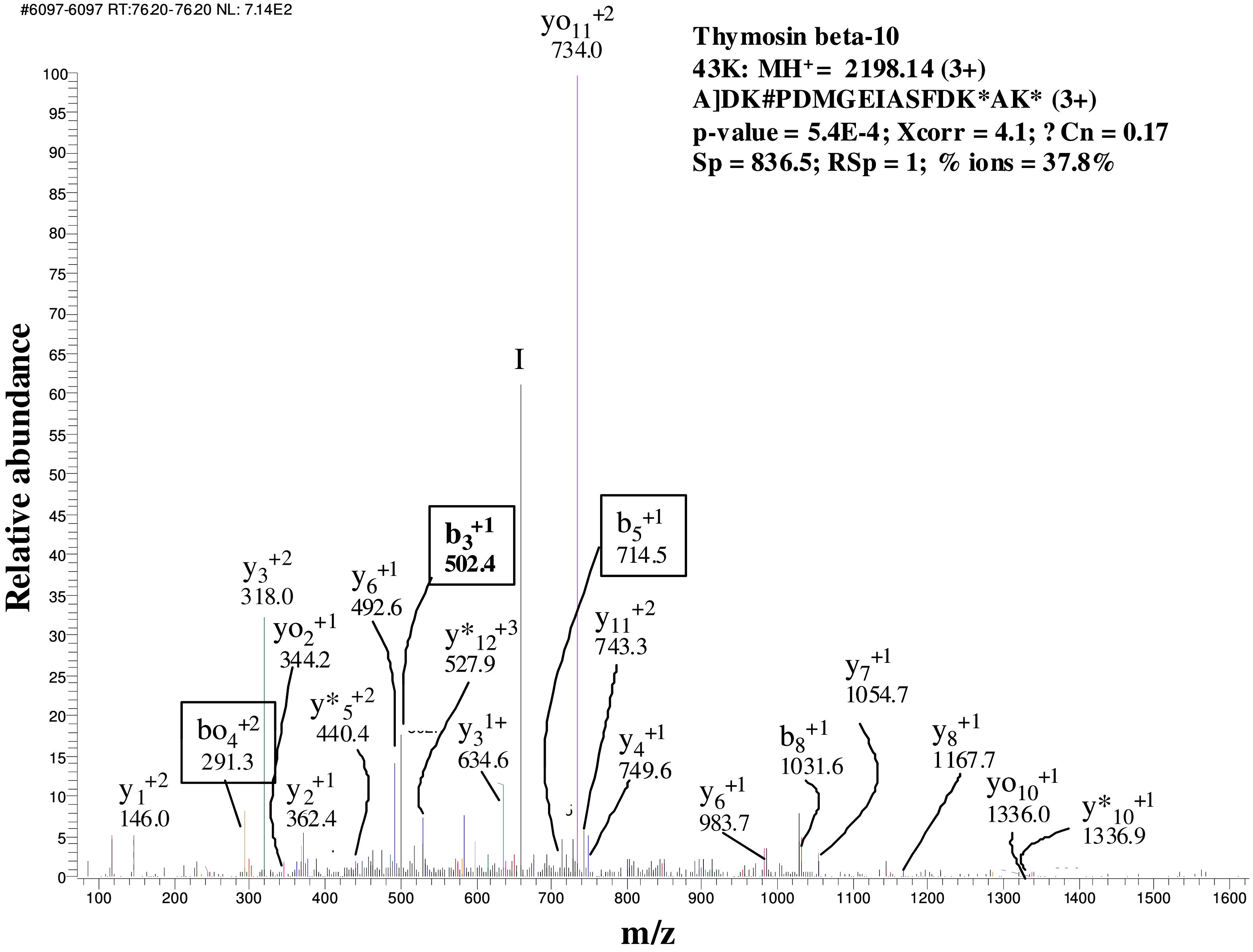
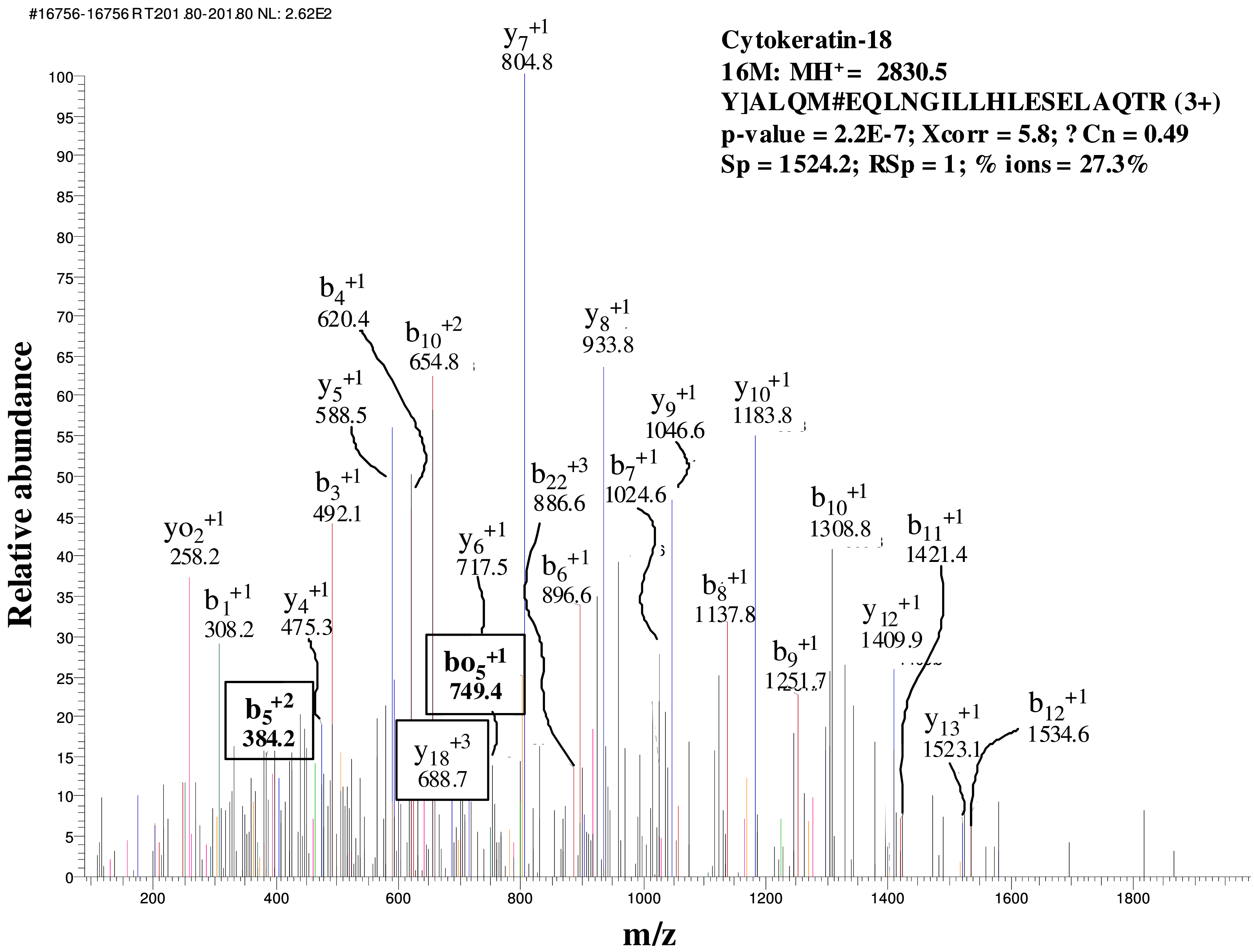

#9456-9456 RT:126.73-126.73 NL: 3.06E2
Nup98-Nuclear pore complex protein
42K: MH+ = 1531.9
LYQTPLELK#LK* (2+)
p-value = 4.6E-4; Xcorr = 2.3; ? Cn = 0.09
Sp = 949.3; RSp = 1; % ions = 56.7%
Relative abundance
m/z
MH2 2+
y7 +2 514.0
b9 +2 -H2O/NH3
y5 +1 816.7
y2 +1 404.4
y1 +1 291.4
I
yo6 +1 911.7
a4 +1 477.9
bo3 +1 386.9
y9 +2 628.1
y4 +1 687.3
a7 +1 817.9
a8 +1 930.5
y4 +2 344.3
y3 +1 574.4
b3 +1 405.4
b1 +1 114.
bo8 +1 940.6
a9 +1 1100.4
a10 +1 1213.7
y*9 +1 1238.8
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500

Figure C



Thymosin beta-10
43K: MH⁺= 2198.14 (3+)
A]DK#PDMGEIASFDK*AK* (3+)
p-value = 5.4E-4; Xcorr = 4.1; ? Cn = 0.17
Sp = 836.5; RSp = 1; % ions = 37.8%

Figure D



Cytokeratin-18
16M: MH$^+$= 2830.5
Y]ALQM#EQLNGILLHLESELAQTR (3+)
p-value = 2.2E-7; Xcorr = 5.8; ? Cn = 0.49
Sp = 1524.2; RSp = 1; % ions = 27.3%

Figure E