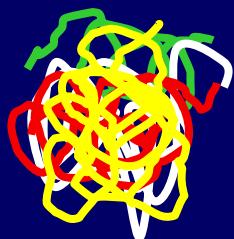


De Novo Sequencing of MS Spectra

Only a manually confirmed spectrum
is a correct spectrum

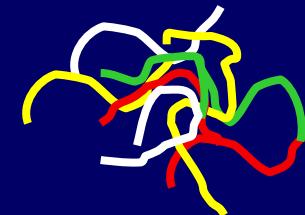
Beatrix Ueberheide
February 25th 2014

Biological Mass Spectrometry



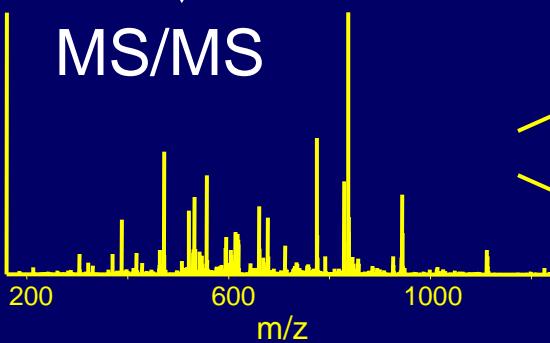
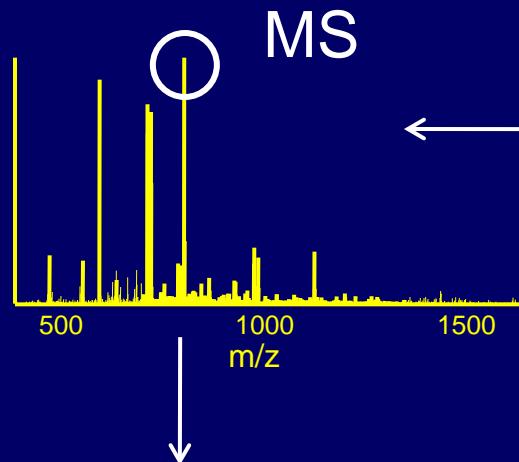
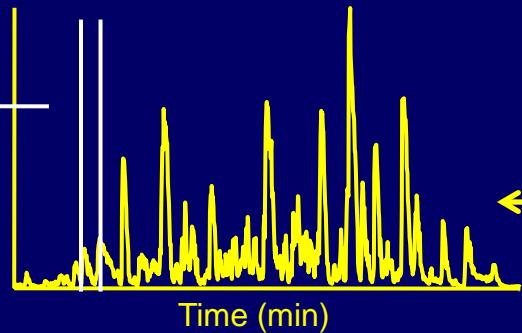
Protein(s)

Proteolytic digestion



Peptides

Base Peak Chromatogram



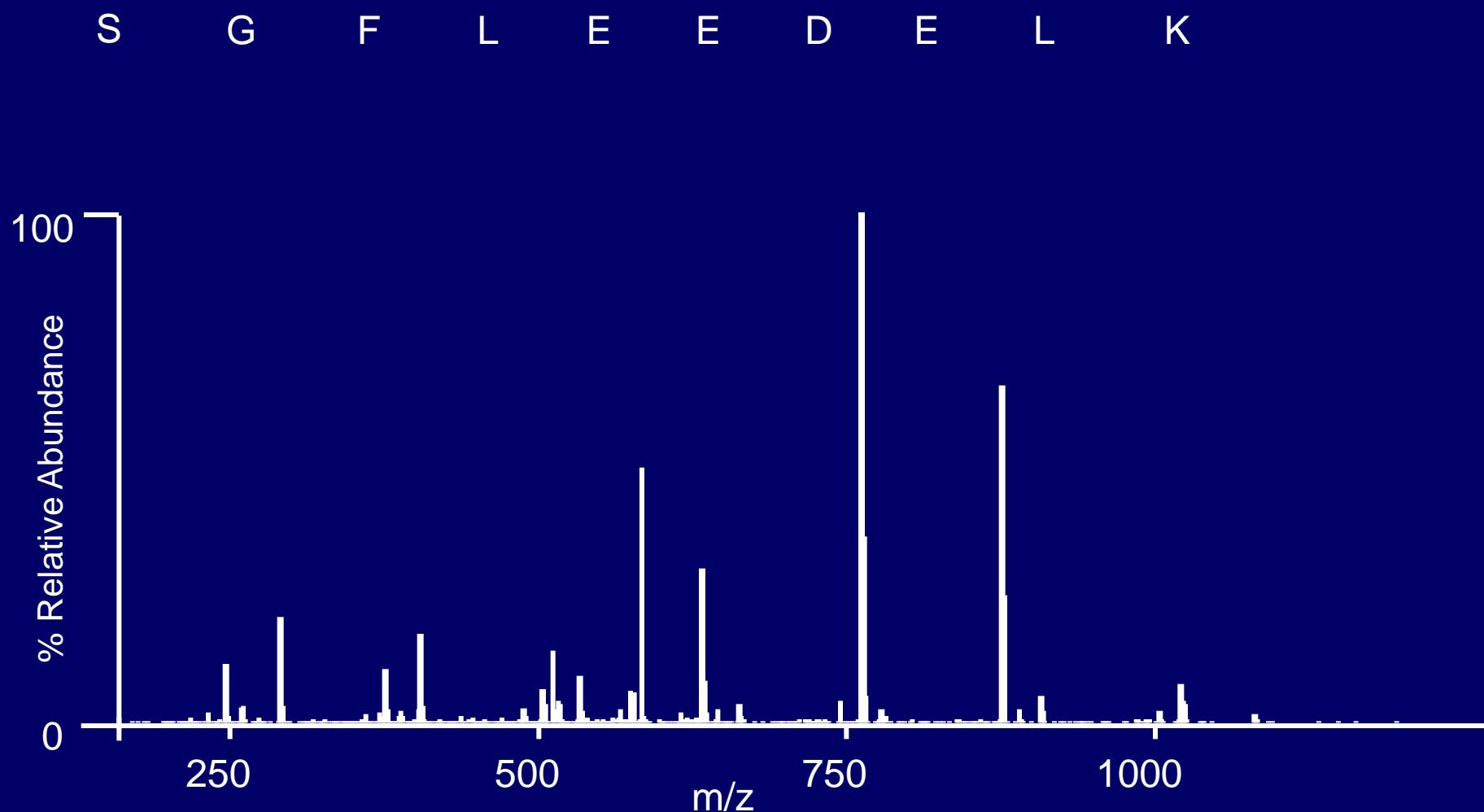
Mass Spectrometer

Database Search

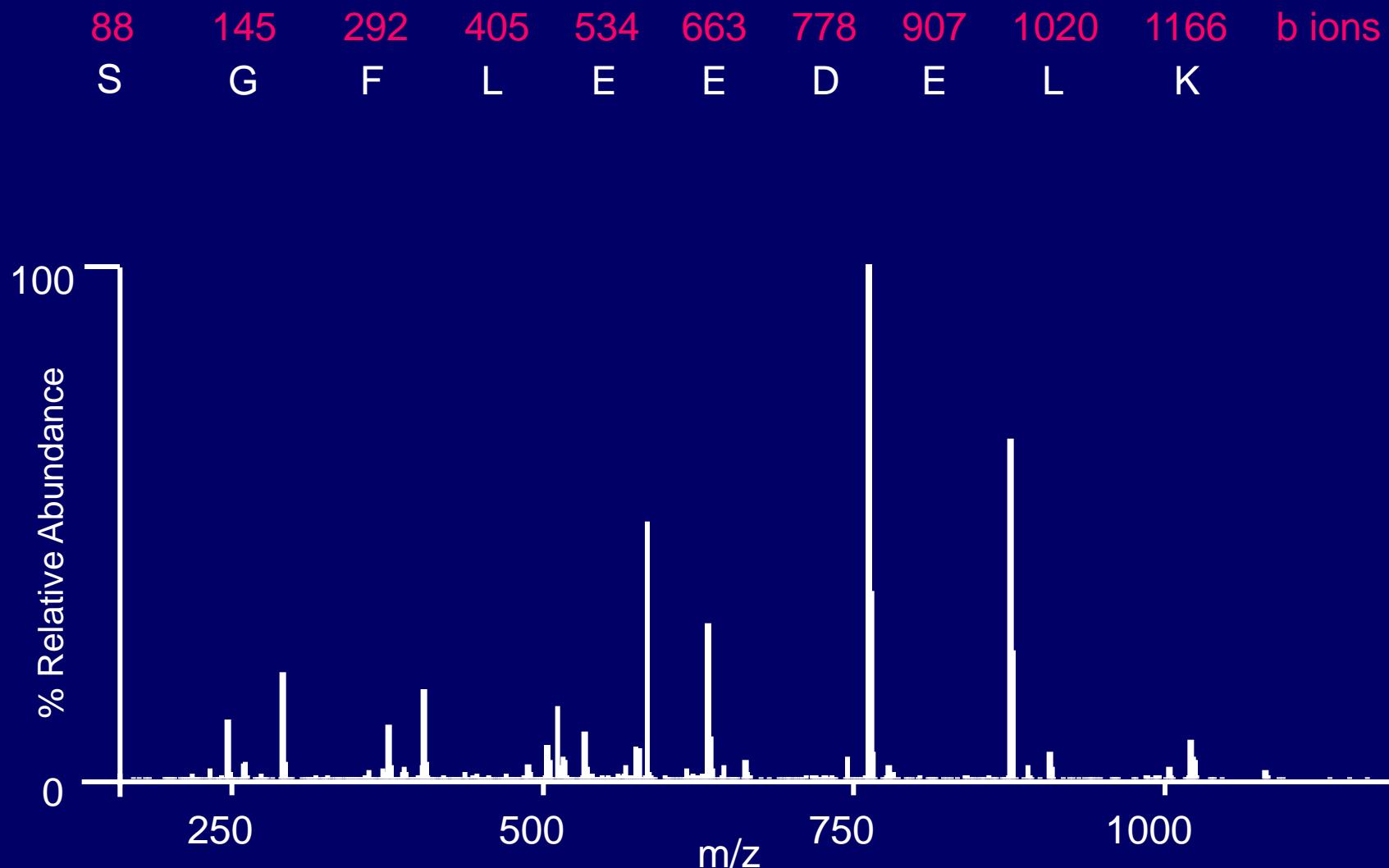


Manual Interpretation

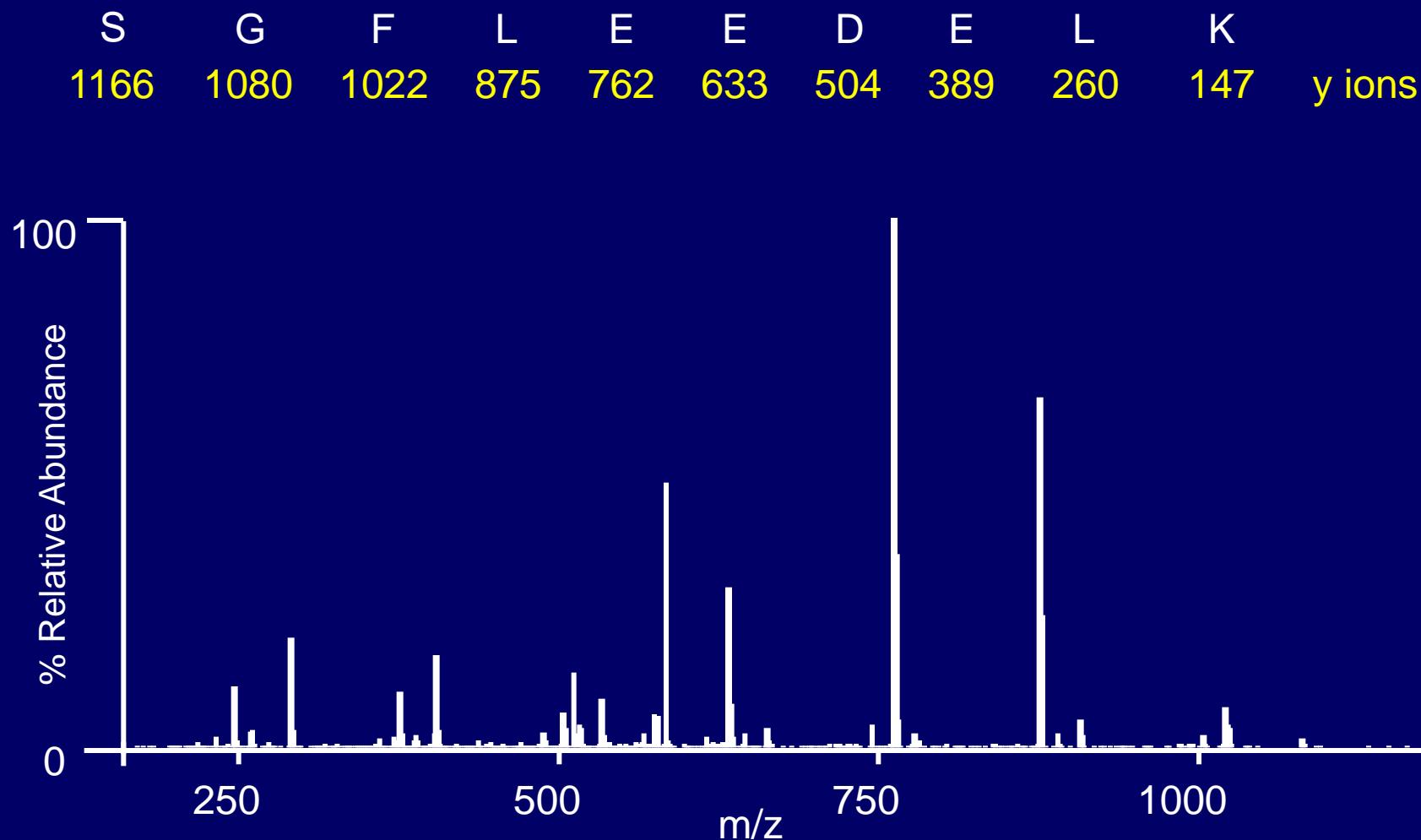
Peptide Sequencing using Mass Spectrometry



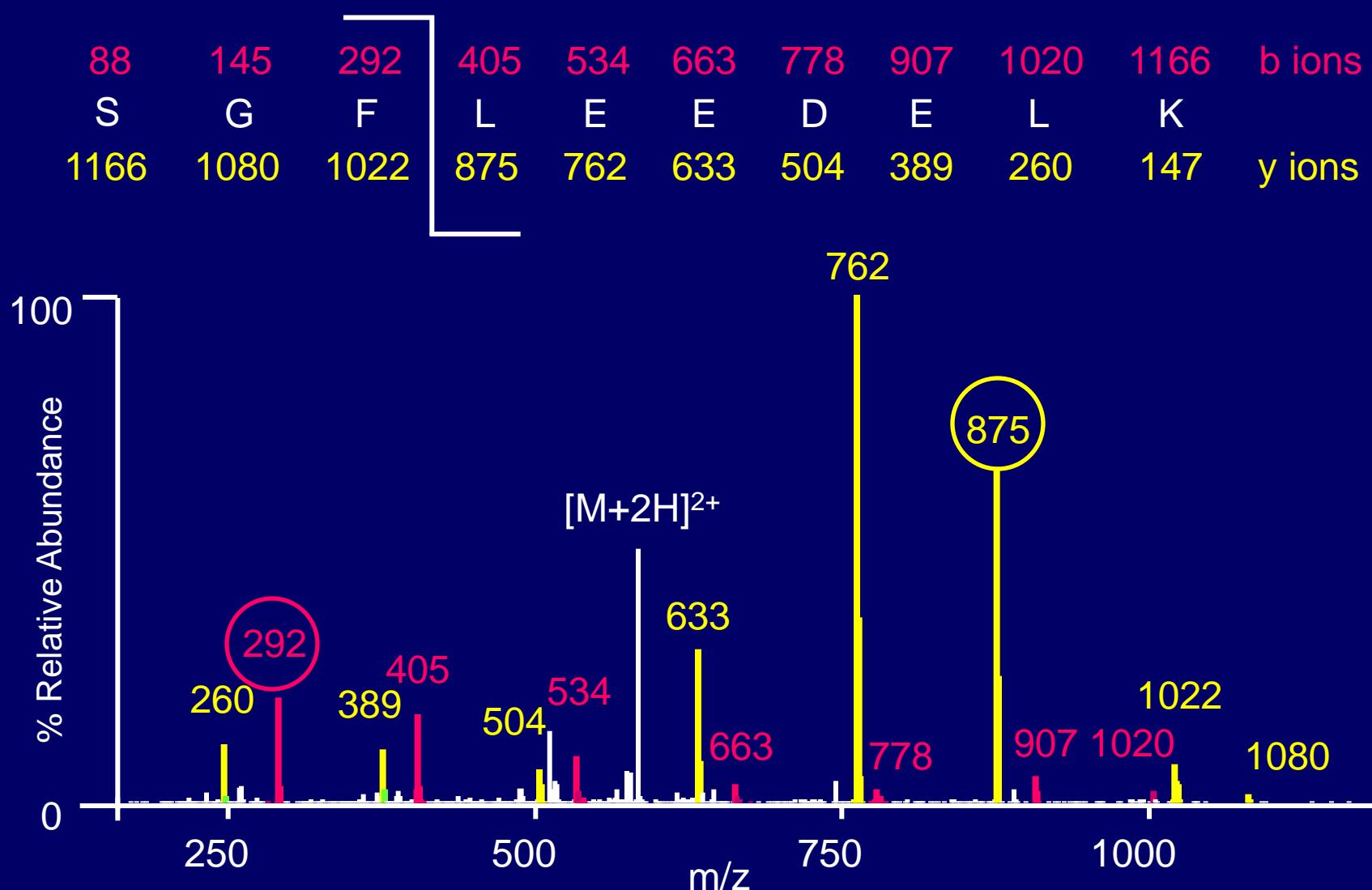
Peptide Sequencing using Mass Spectrometry



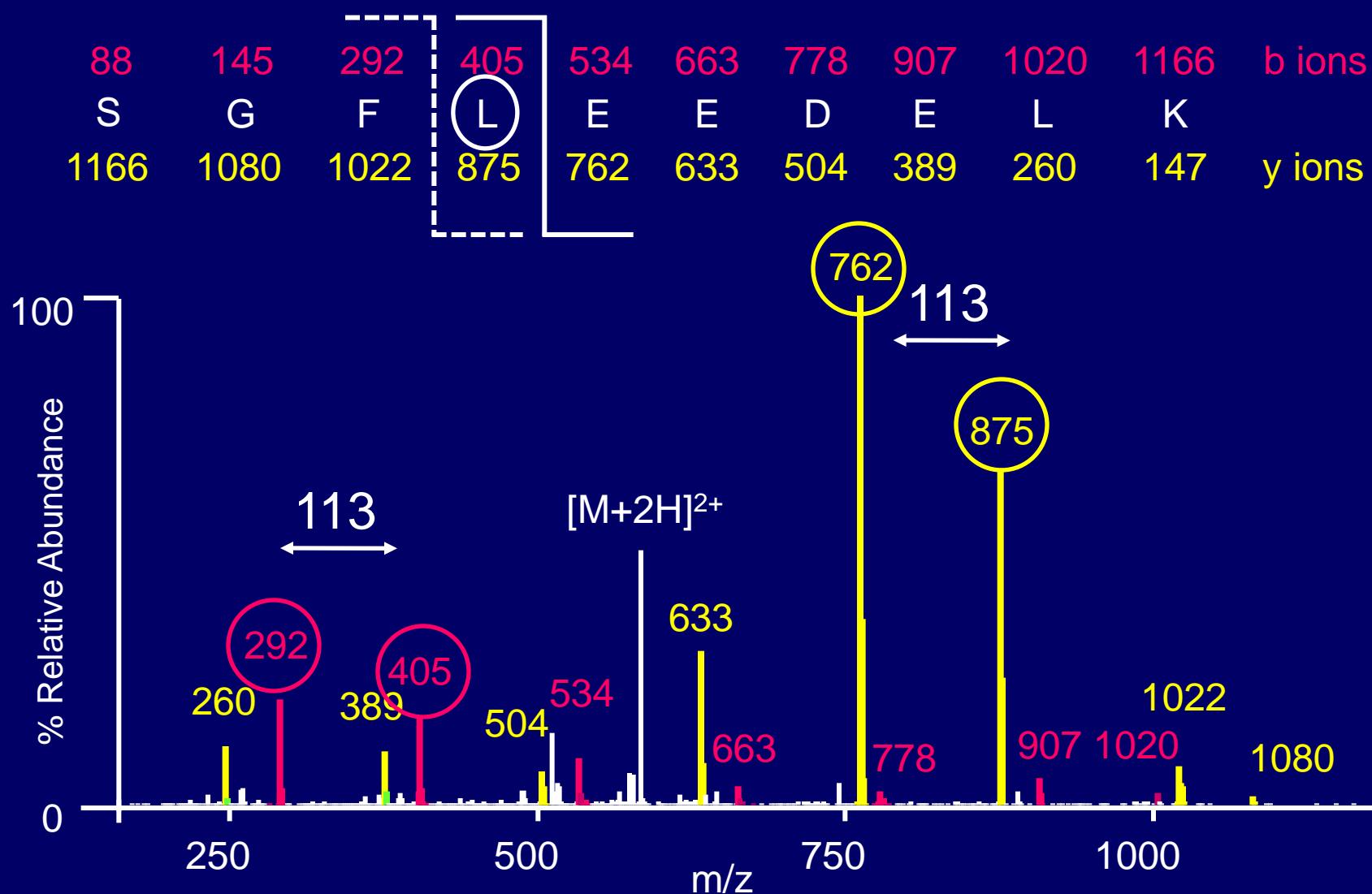
Peptide Sequencing using Mass Spectrometry



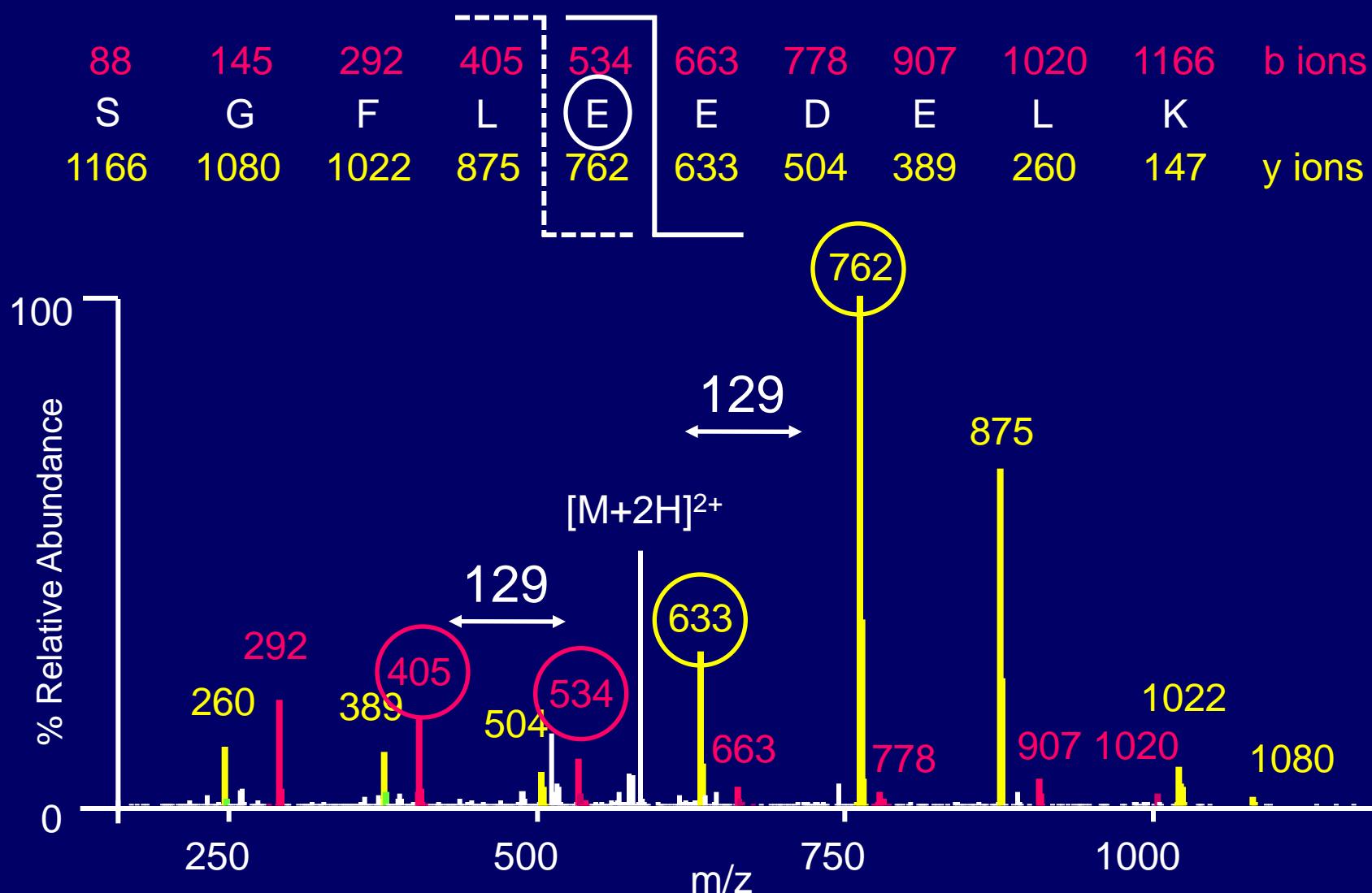
Peptide Sequencing using Mass Spectrometry



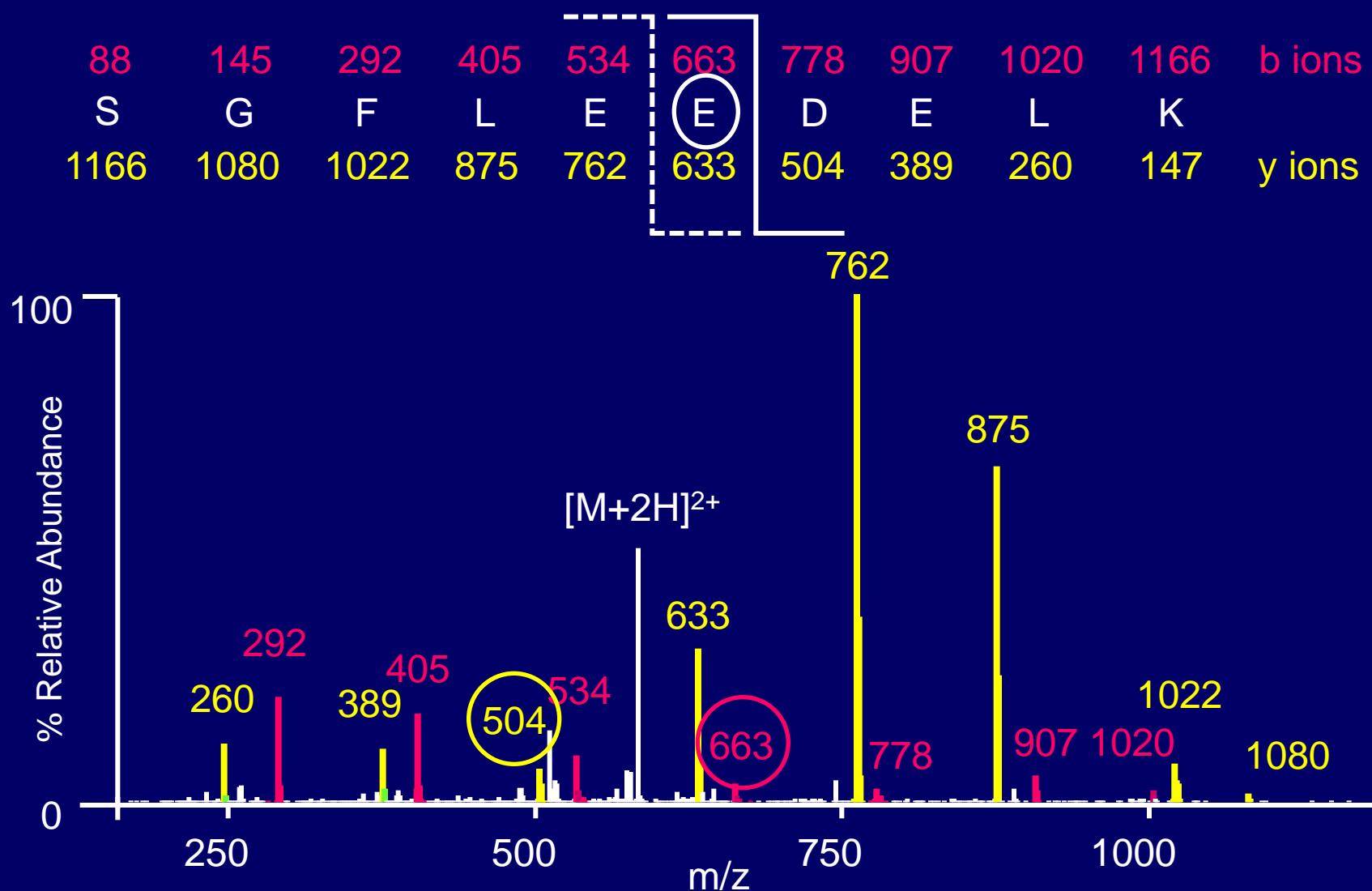
Peptide Sequencing using Mass Spectrometry



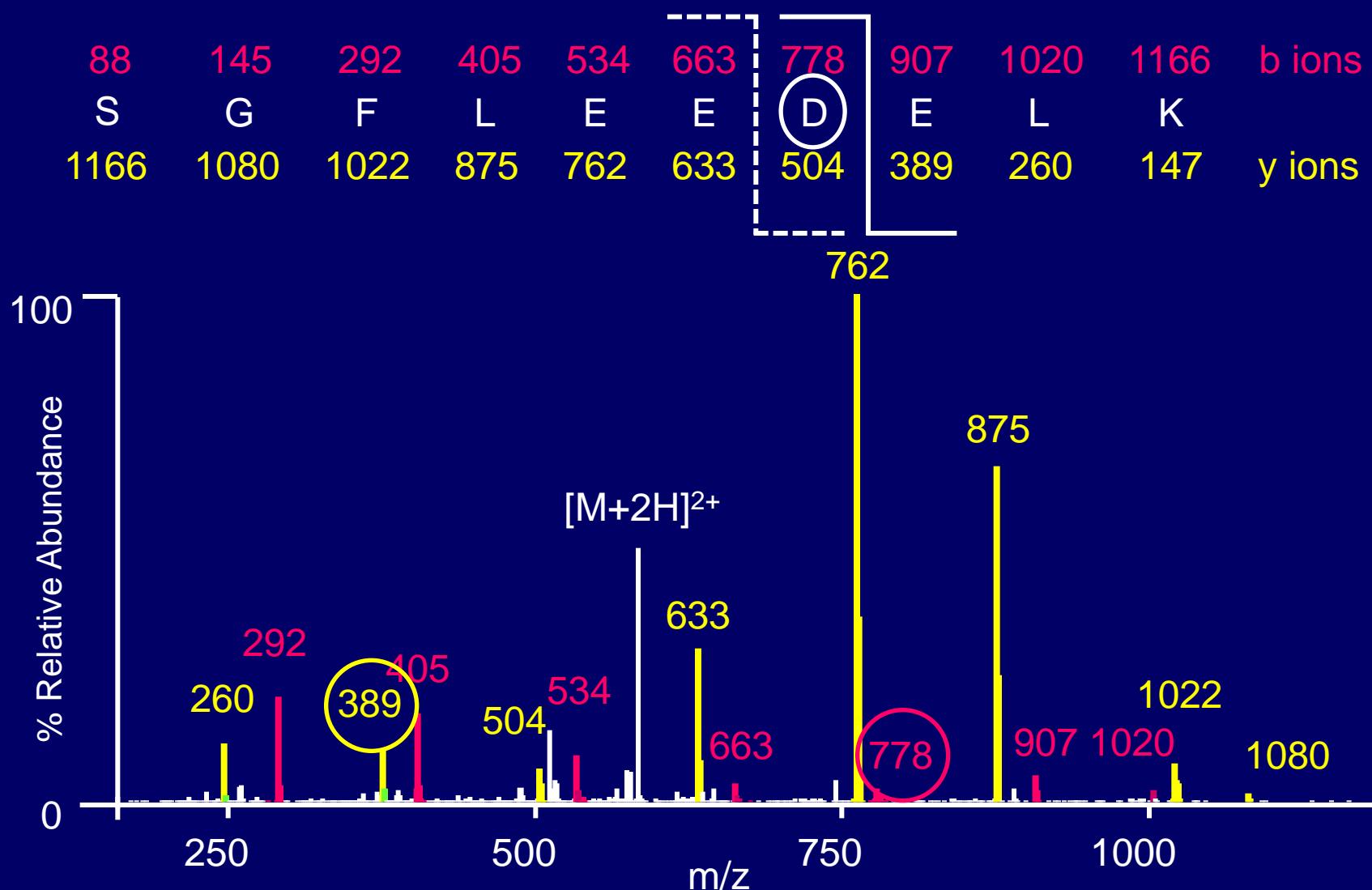
Peptide Sequencing using Mass Spectrometry



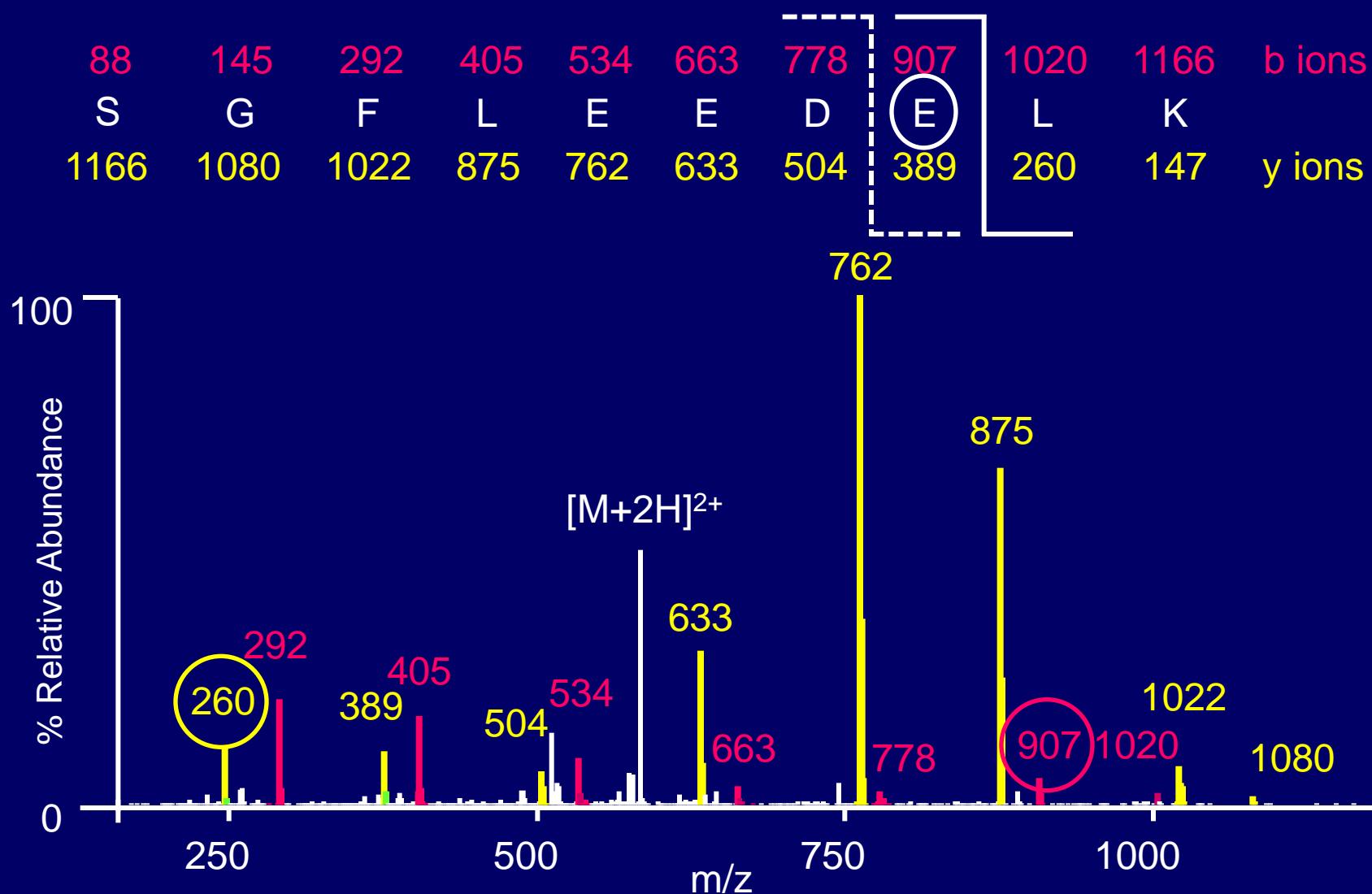
Peptide Sequencing using Mass Spectrometry



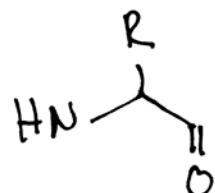
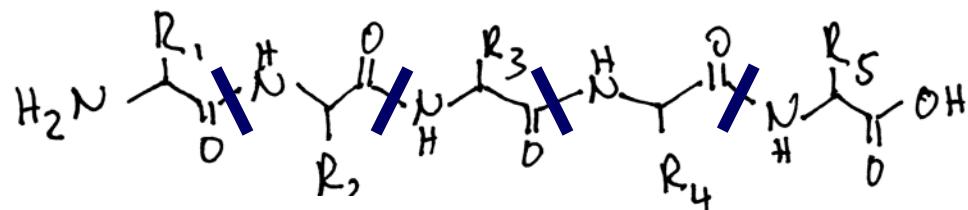
Peptide Sequencing using Mass Spectrometry



Peptide Sequencing using Mass Spectrometry

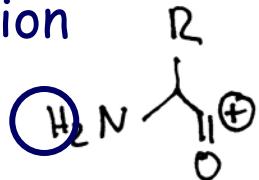


How to Sequence

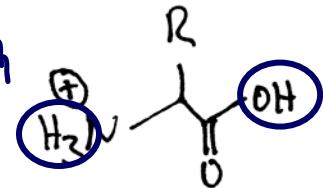


Residue Mass (RM)

b ion



How to calculate the very first γ ion?
How to calculate the very first b ion?

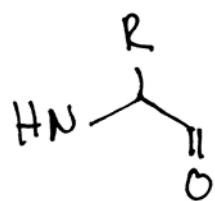


$$\gamma_1 = \text{RM} + 19$$

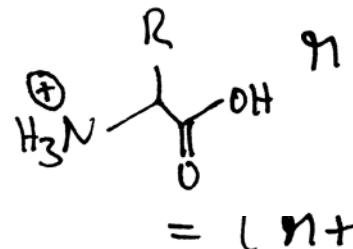
$$b_1 = \text{RM} + 1$$

Example of how to calculate theoretical fragment ions

88	159	290	387	500	629	803
S	A	M	P	L	E	R
803	716	645	514	417	304	175

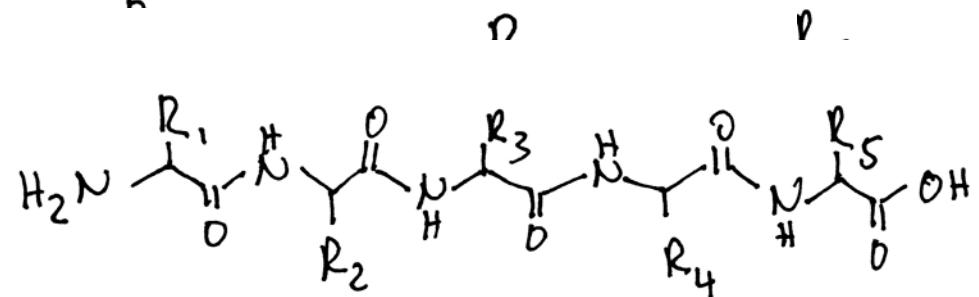


first



Residue Mass

The first b ion



The first y ion

Table 1. Mass Values for Peptide Fragment Ions

One Let Code Amino Acid	Three Let Code	Mono Isotopic			Mono Isotopic																																																																		
		Integer Mass	Neutral Residue Mass Change	Da Lost	Methyl Ester Mass	Accurate Residue Mass	Average Residue Mass																																																																
G	Glycine	Gly	57	Da 0	Da		57.02																																																																
A	Alanine	Ala	71	Da 14	Da	71.04	71.08																																																																
S	Serine	Ser	87	Da 16	Da	87.03	87.08																																																																
P	Proline	Pro	97	Da 10	Da	97.05	97.12																																																																
V	Valine	Val	99	Da 2	Da	99.07	99.13																																																																
T	Threonine	Thr	101	Da 2	Da	101.05	101.11																																																																
C	Cysteine	Cys	103	Da 2	Da	103.01	103.14																																																																
L	Leucine	Leu	113	Da 10	Da	113.08	113.16																																																																
I	Isoleucine	Ile	113	Da		113.08	113.16																																																																
N	Asparagine	Asn	114	Da 1	Da	114.04	114.1																																																																
D	Aspartic Acid	Asp	115	Da 1	Da	115.03	115.09																																																																
K	Lysine	Lys	128	Da 13	Da	128.09	128.17																																																																
Q	Glutamine	Gln	128	Da		128.06	128.13																																																																
E	Glutamic Acid	Glu	129	Da 1	Da	129.04	129.12																																																																
M	Methionine	Met	131	Da 2	Da	131.04	131.19																																																																
H	Histidine	His	137	Da 6	Da	137.06	137.14																																																																
F	Phenylalanine	Phe	147	Da 10	Da	147.07	147.18																																																																
R	Arginine	Arg	156	Da 9	Da	156.1	156.19																																																																
Y	Tyrosine	Tyr	163	Da 7	Da	163.06	163.18																																																																
W	Tryptophan	Trp	186	Da 23	Da	186.08	186.21																																																																
<hr/>																																																																							
<table border="1"> <tr> <td>Hydrogen</td> <td>H</td> <td>1</td> <td>1.008</td> <td>1.008</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Carbon</td> <td>C</td> <td>12</td> <td>12</td> <td>12.011</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Nitrogen</td> <td>N</td> <td>14</td> <td>14.003</td> <td>14.007</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Oxygen</td> <td>O</td> <td>16</td> <td>16.006</td> <td>16.009</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Sulfur</td> <td>S</td> <td>32</td> <td>31.972</td> <td>32.06</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Methyl</td> <td></td> <td>15</td> <td>15.02</td> <td>15.04</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Water</td> <td></td> <td>18</td> <td>18.01</td> <td>18.02</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Acetyl</td> <td></td> <td>43</td> <td>43</td> <td>43.05</td> <td></td> <td></td> <td></td> </tr> </table>								Hydrogen	H	1	1.008	1.008				Carbon	C	12	12	12.011				Nitrogen	N	14	14.003	14.007				Oxygen	O	16	16.006	16.009				Sulfur	S	32	31.972	32.06				Methyl		15	15.02	15.04				Water		18	18.01	18.02				Acetyl		43	43	43.05			
Hydrogen	H	1	1.008	1.008																																																																			
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Acetyl		43	43	43.05																																																																			
<p>Residue Mass (RM) = $\text{NH} \begin{array}{c} \text{R} \\ \\ \text{O} \end{array}$</p>																																																																							

Observed neutral losses and mass additions:

- Ammonia

- Water

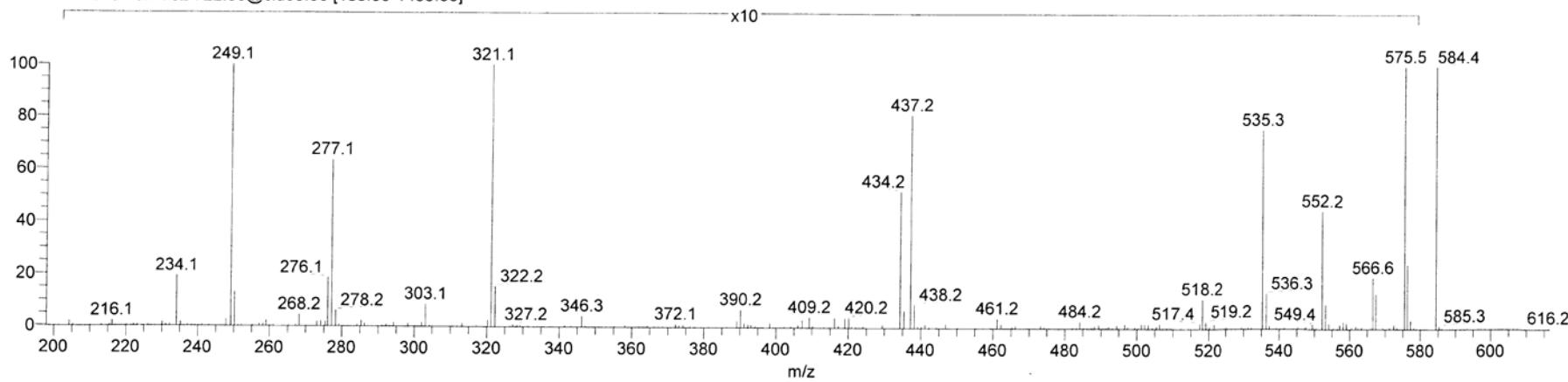
- Carbon Monoxide from b ions

- Phosphoric acid from phosphorylated serine and threonine

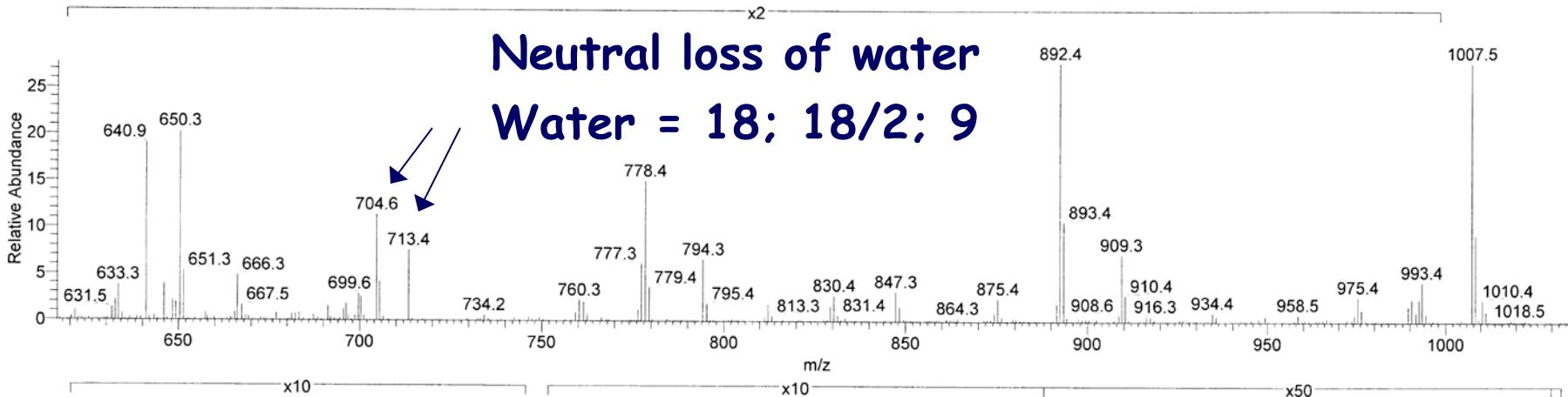
- Carbamidomethyl modification on cysteines upon alkylation with iodoacetamide

First Example

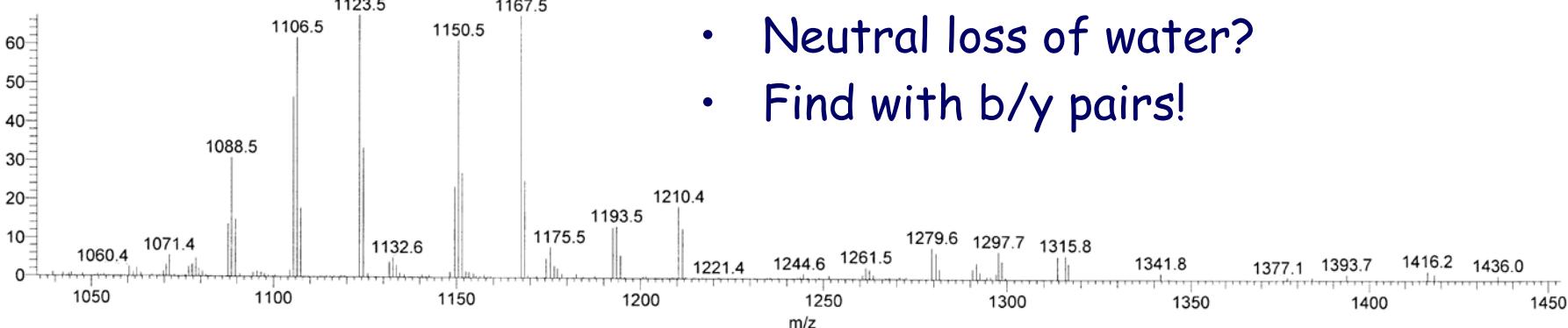
What is the charge state?



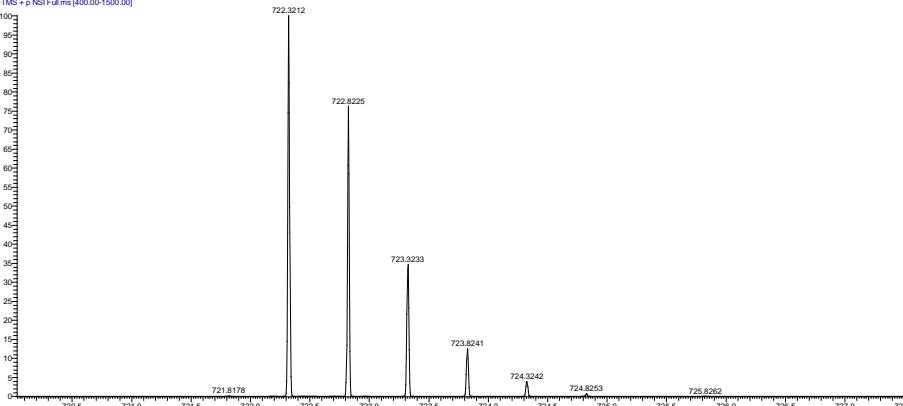
Neutral loss of water
Water = 18; 18/2; 9



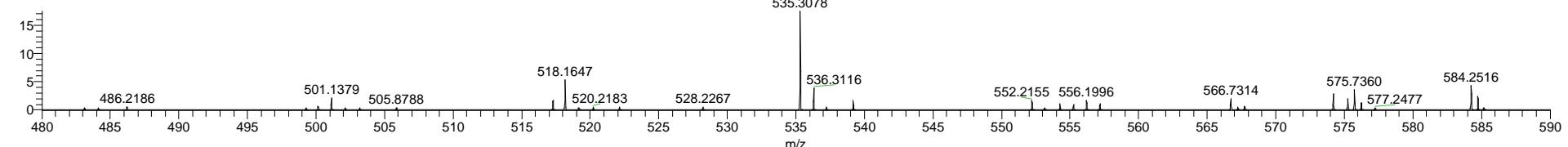
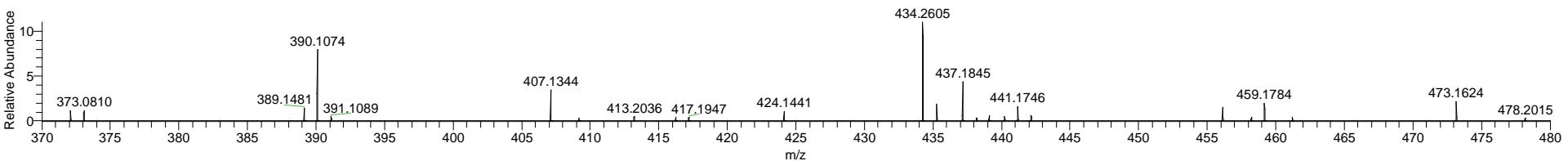
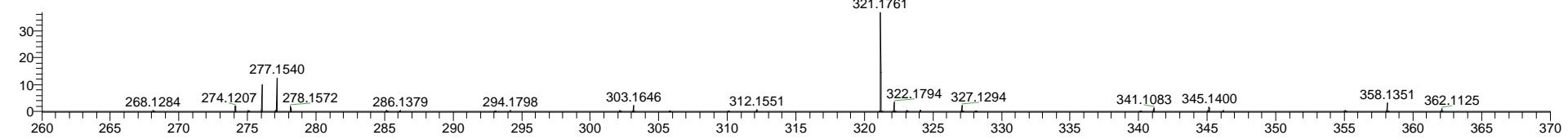
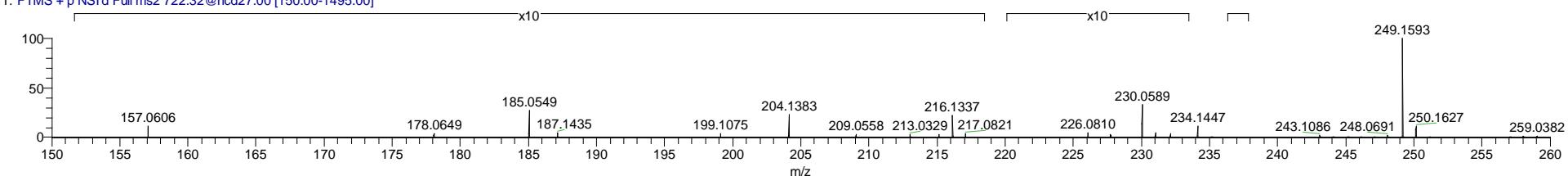
- Neutral loss of water?
- Find with b/y pairs!



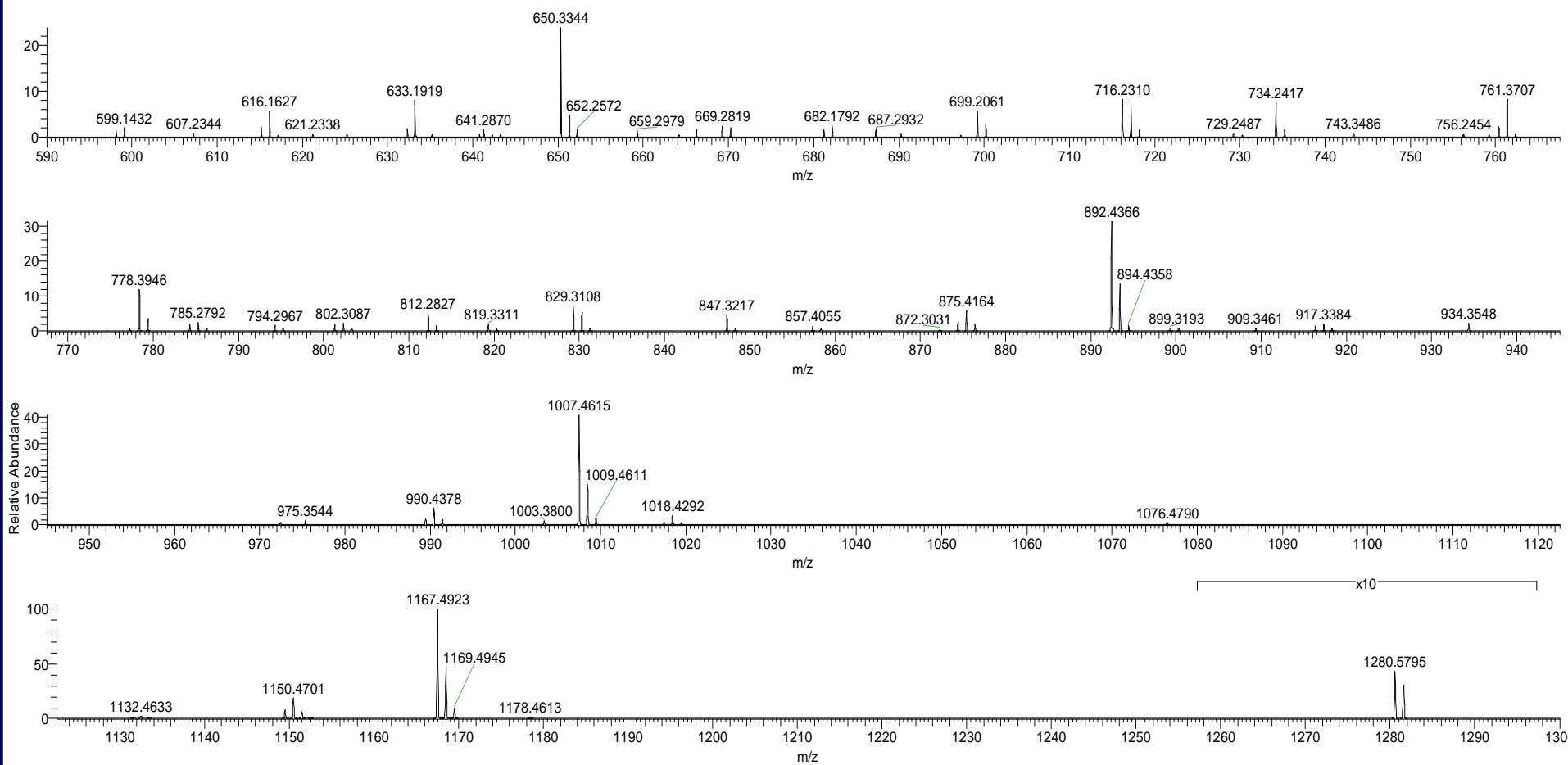
022014_SN_std3 #6058 RT: 26.59 AV: 1 NL: 9.72E7
T: FTMS + p NSI Full ms [400.00-1500.00]



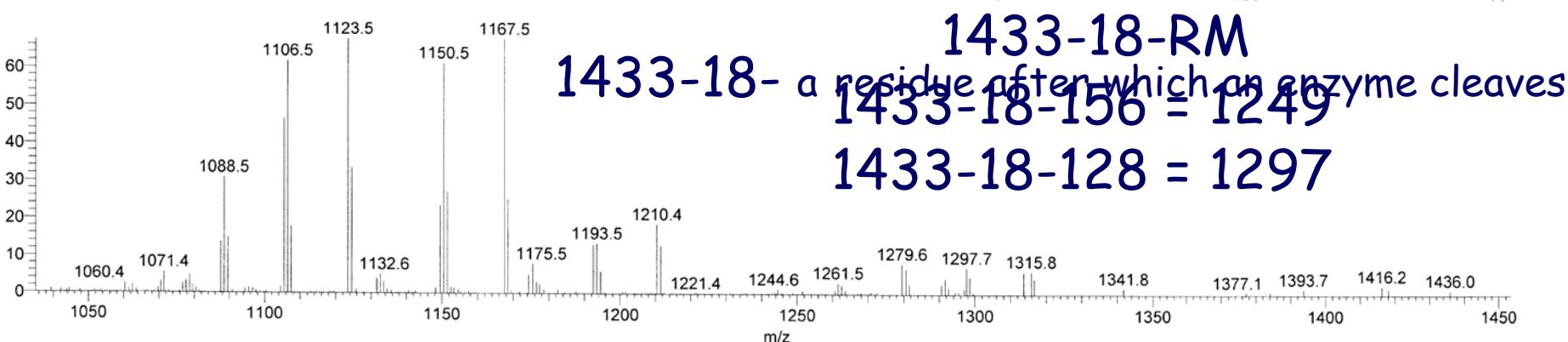
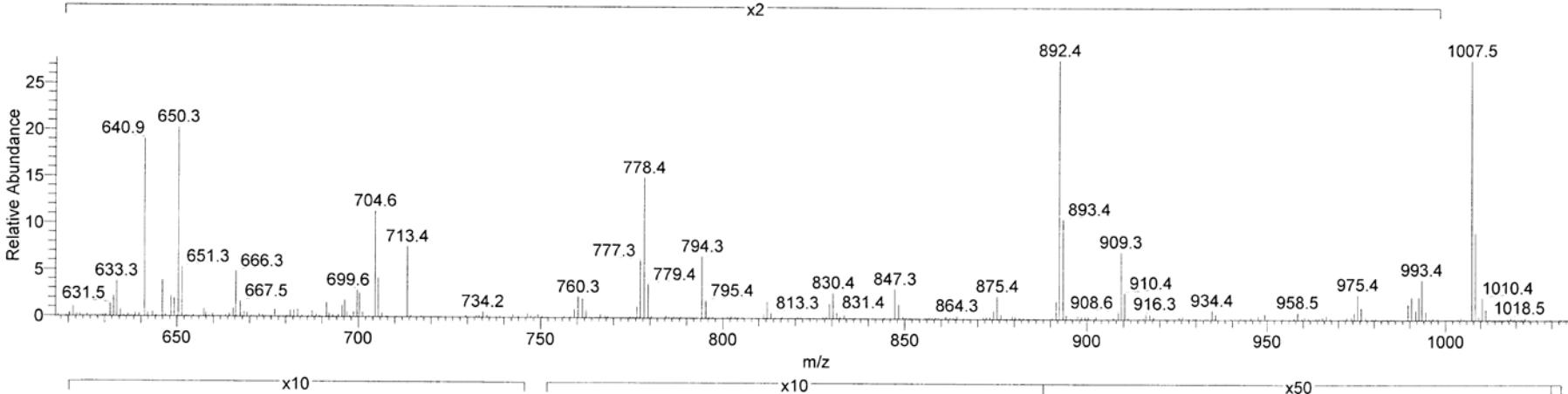
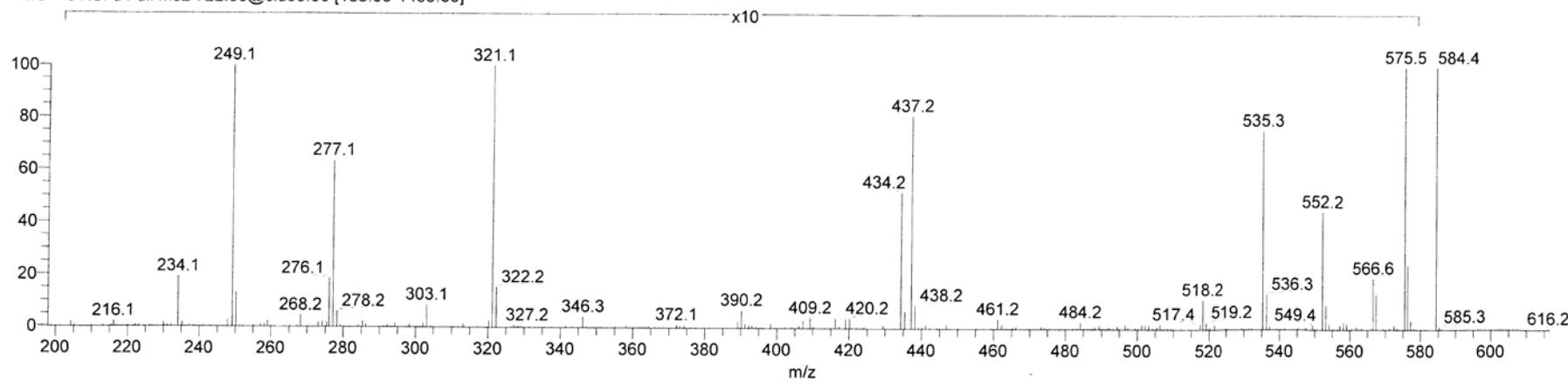
022014_SN_std3 #6055 RT: 26.58 AV: 1 NL: 1.25E7
T: FTMS + p NSI Full ms2 722.32@hcd27.00 [150.00-1495.00]



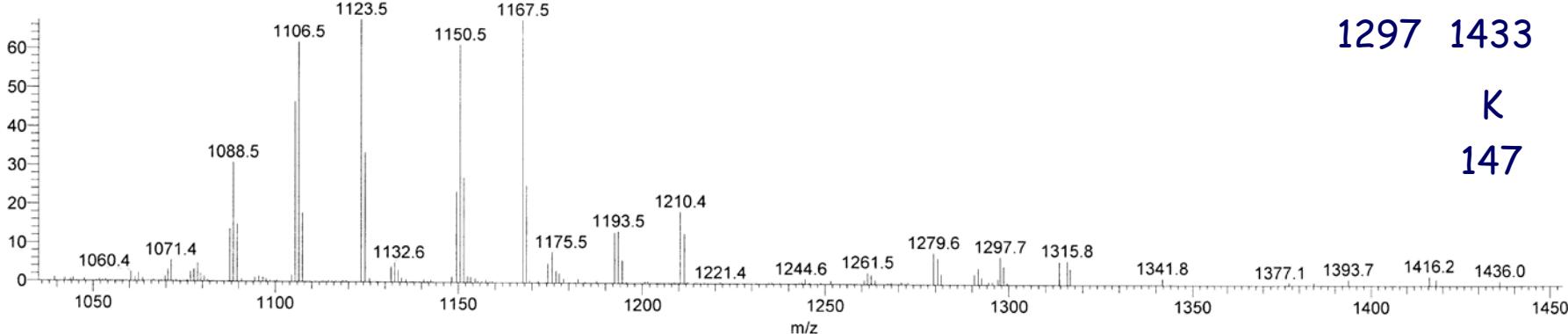
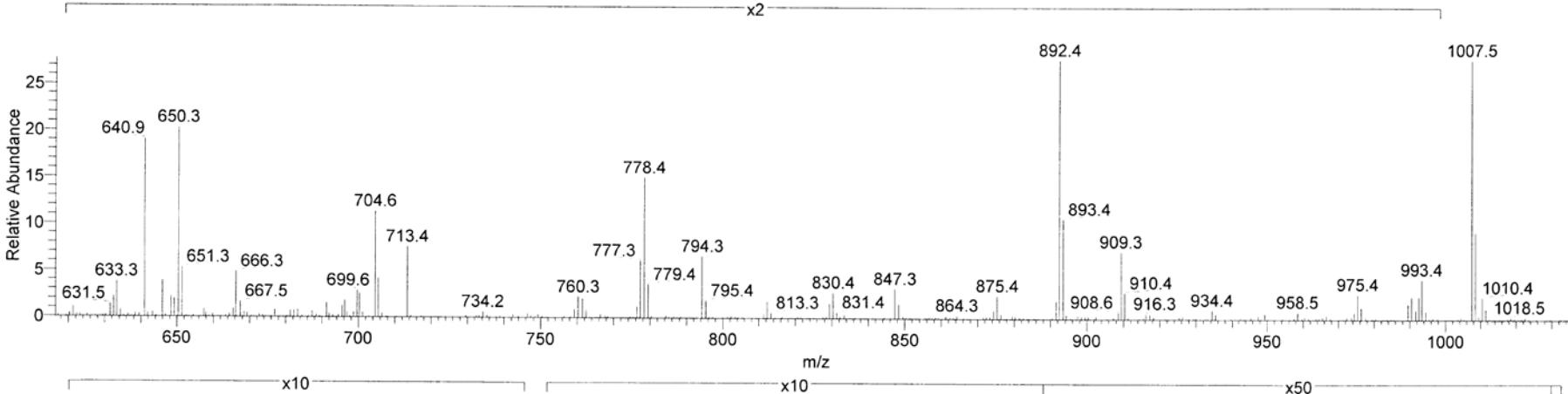
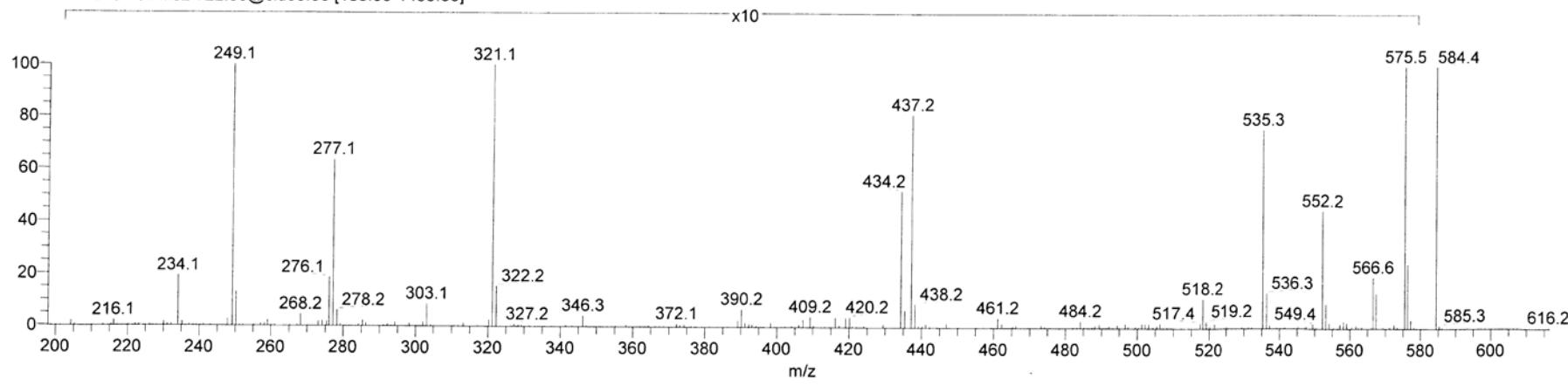
022014_SN_std3 #6055 RT: 26.58 AV: 1 NL: 8.82E6
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Search for 'biggest ion'



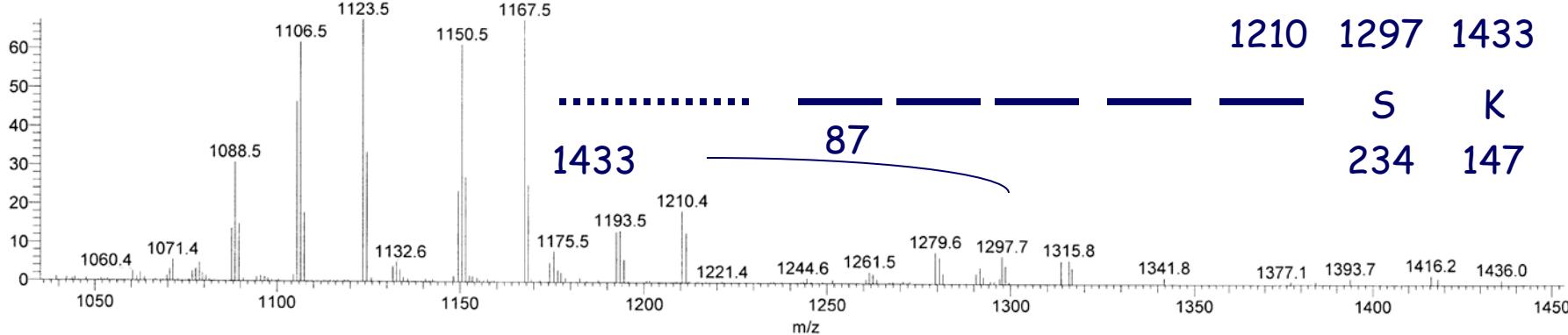
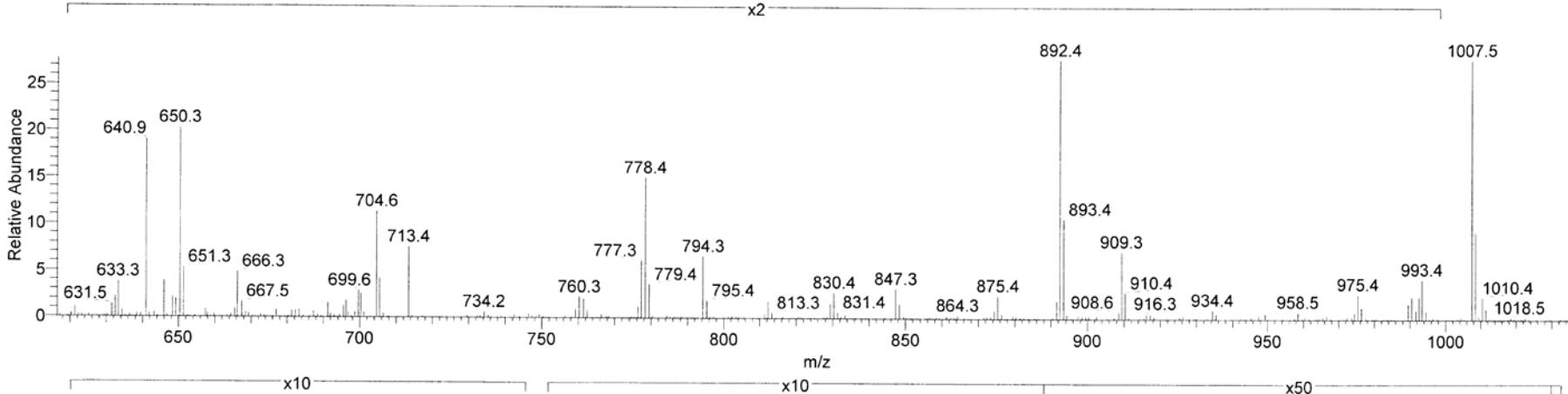
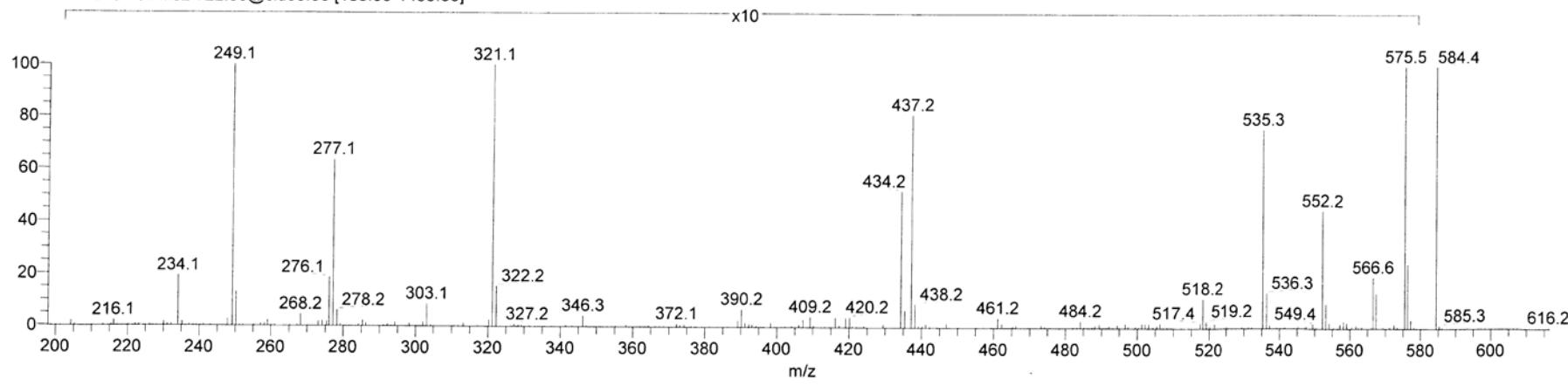
1433-18-RM
1433-18- a residue often which an enzyme cleaves
1433-18-156 = 1249
1433-18-128 = 1297



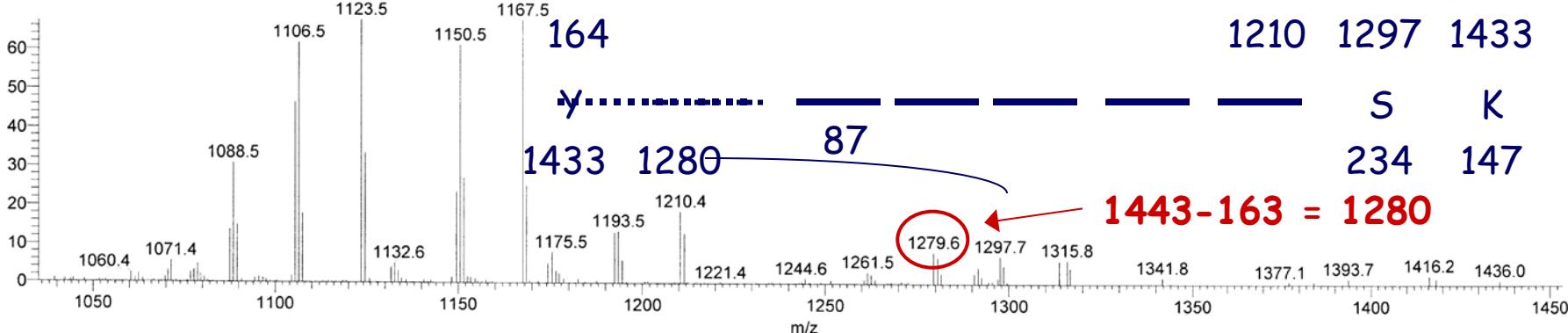
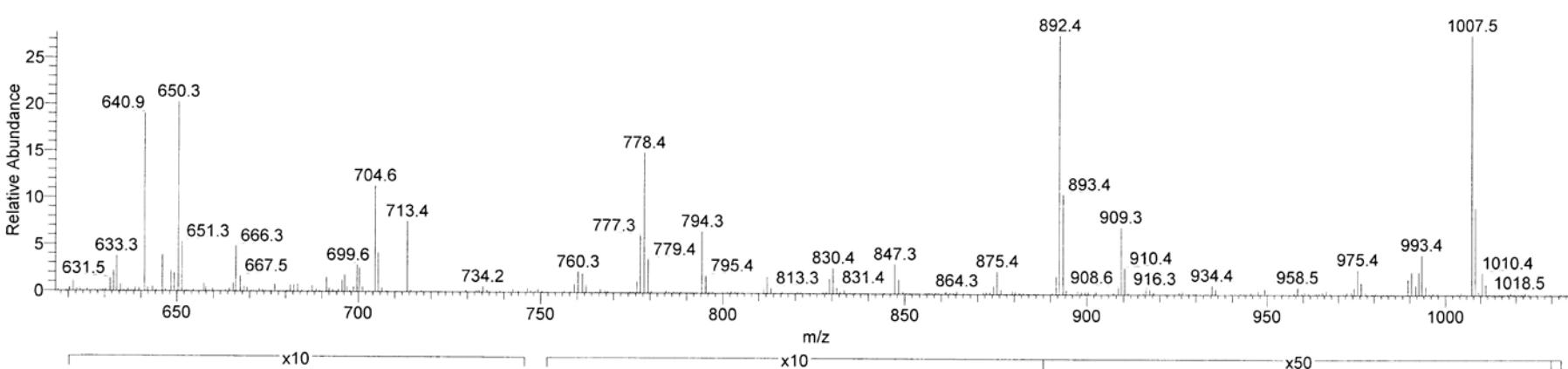
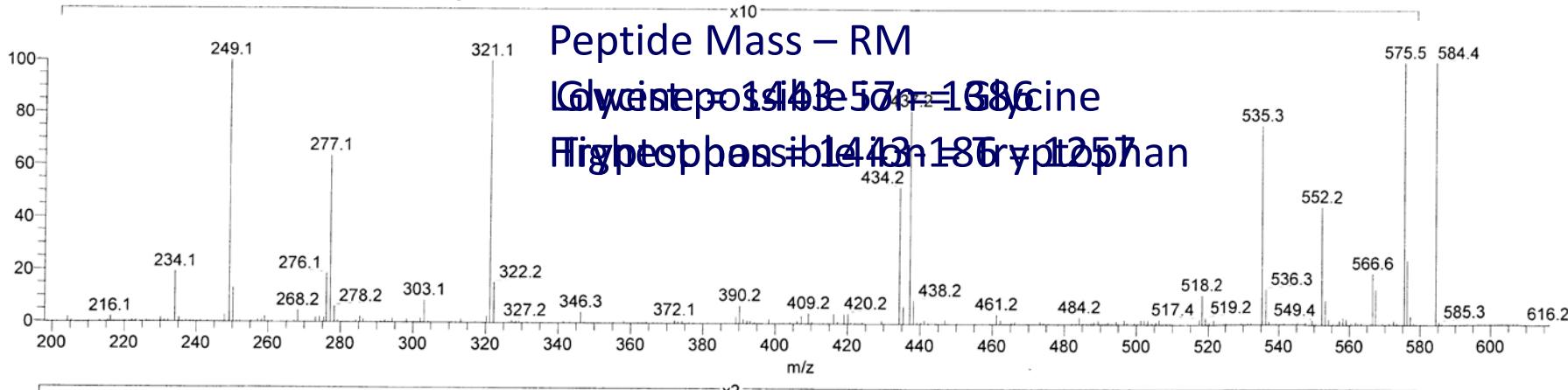
1297 1433

K

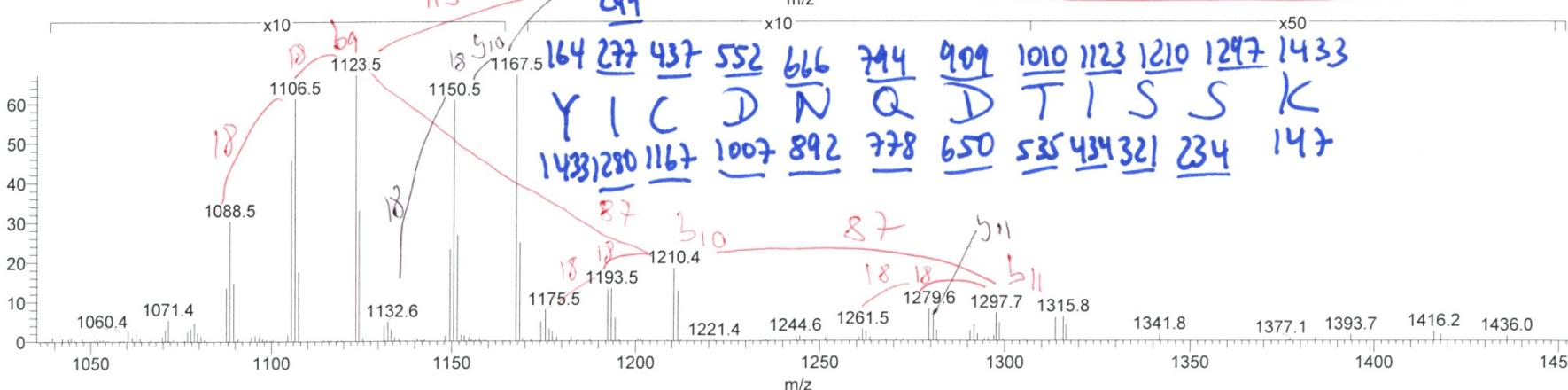
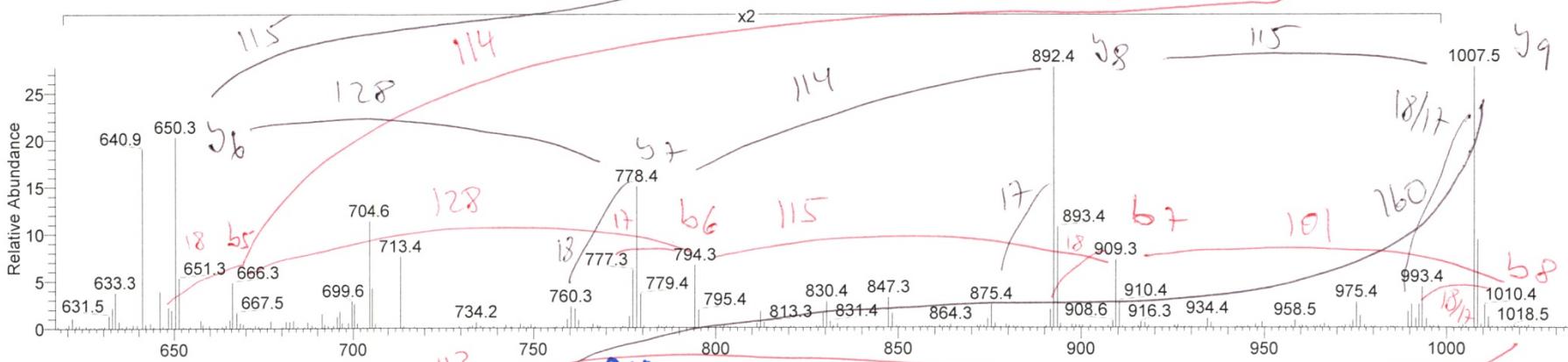
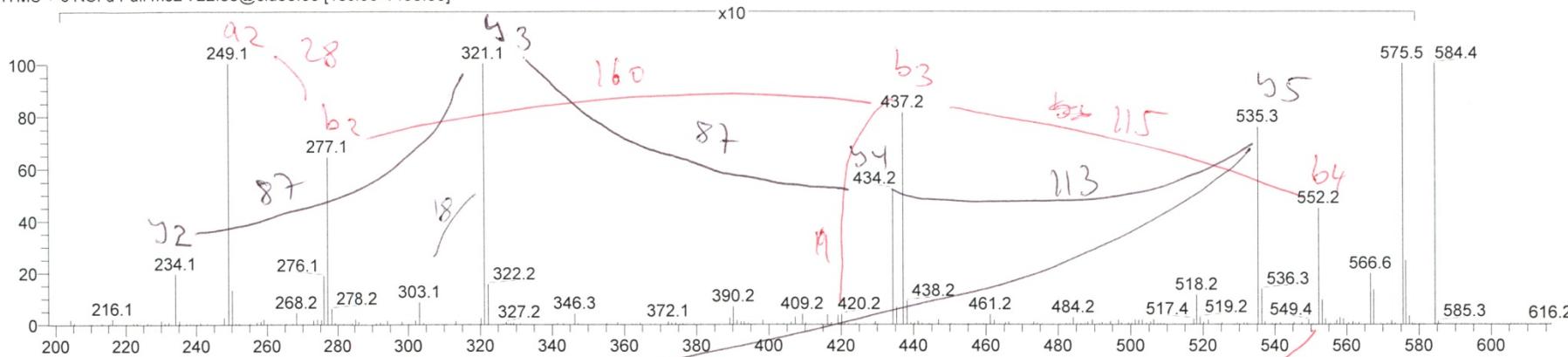
147



Find the biggest y ion!



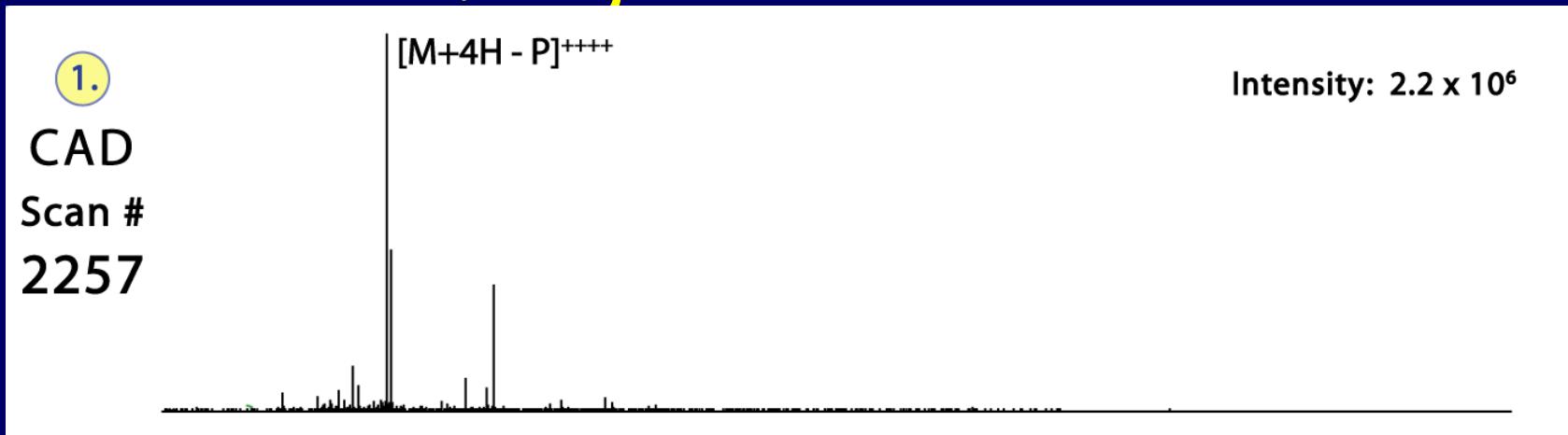
And the sequence is.....

030713_jrc_std4 #1725 RT: 18.01 AV: 1 NL: 7.45E4
T: ITMS + c NSI d Full ms2 722.33@cid35.00 [185.00-1455.00]

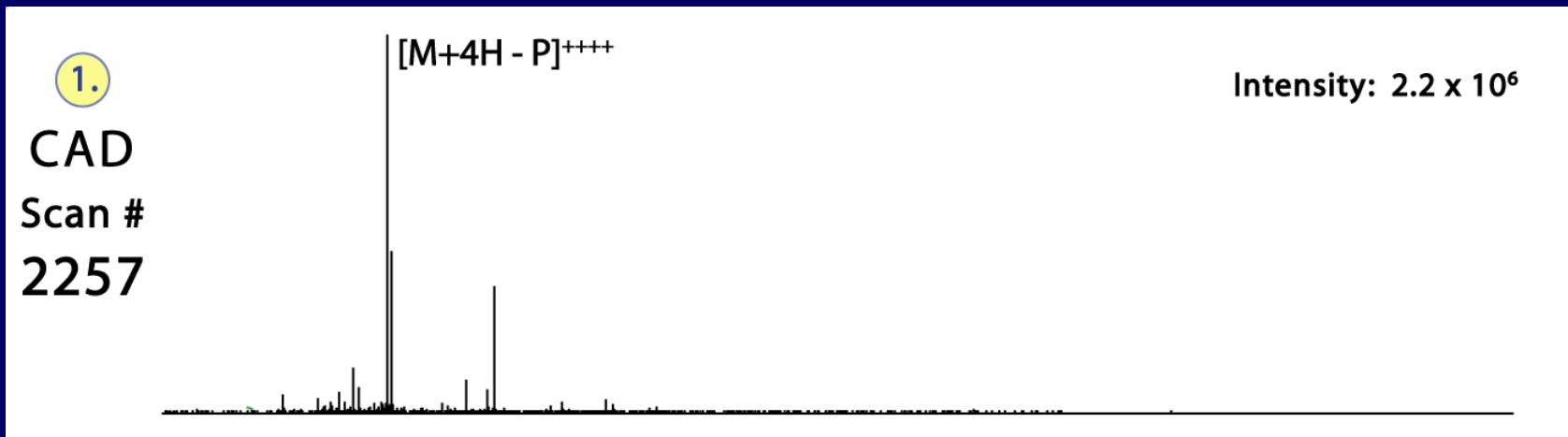
ETD

Electron Transfer Dissociation

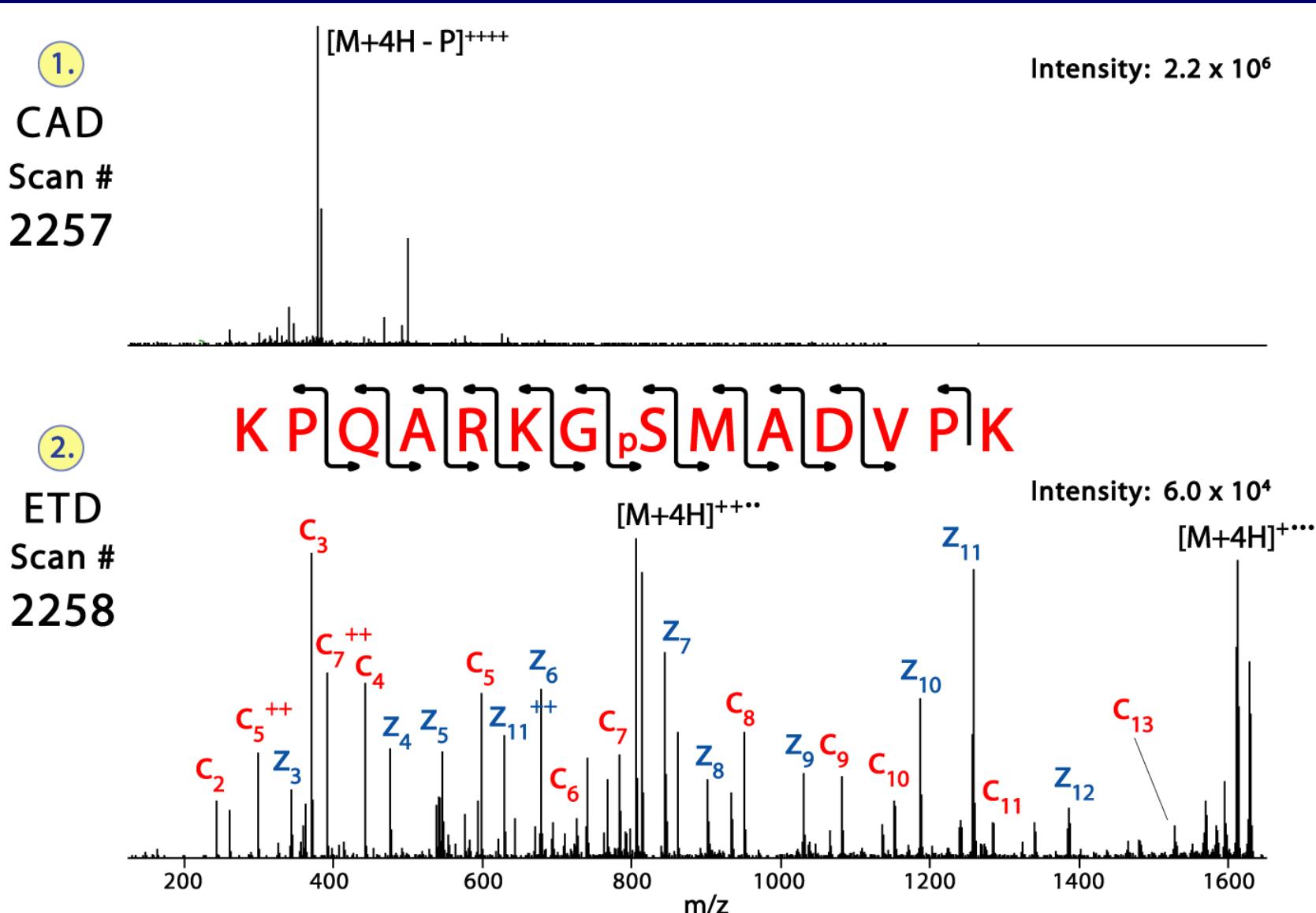
What if we do not get good fragmentation?



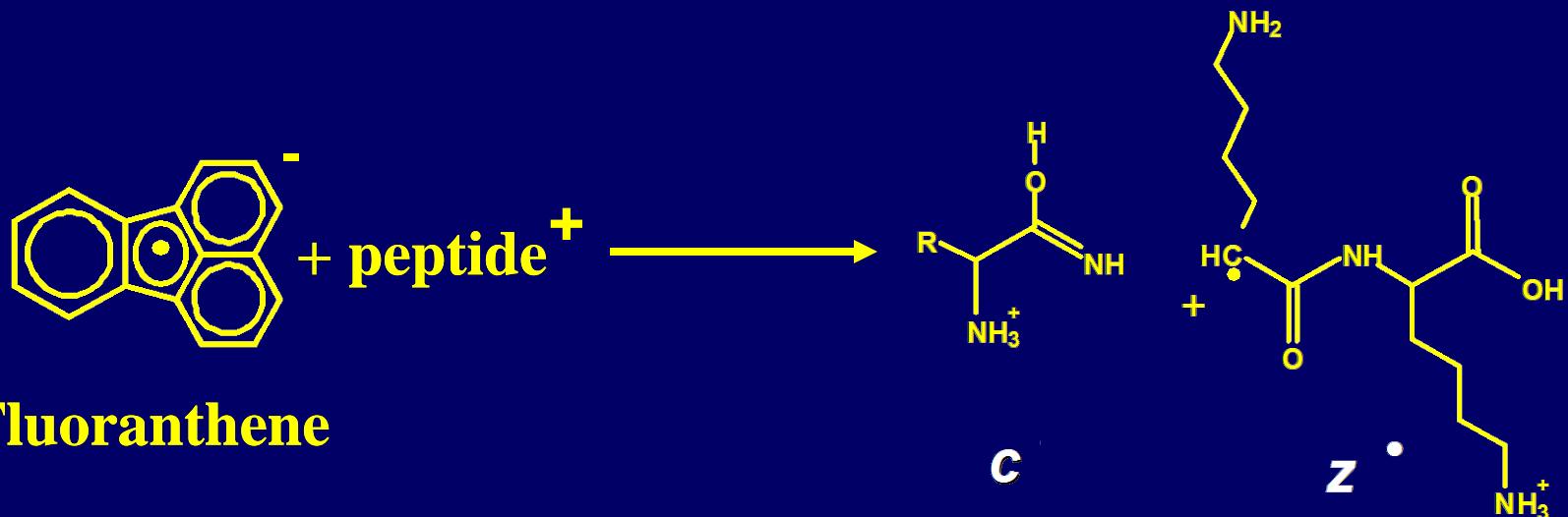
Try a different mode of dissociation



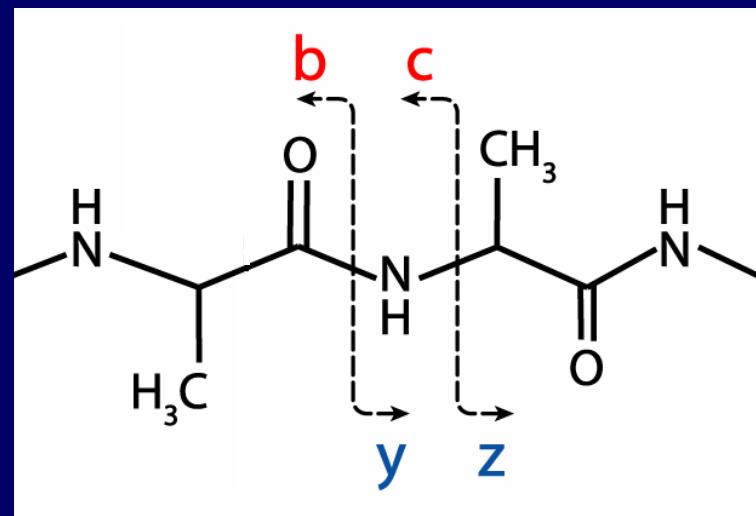
ETD



Electron Transfer Dissociation



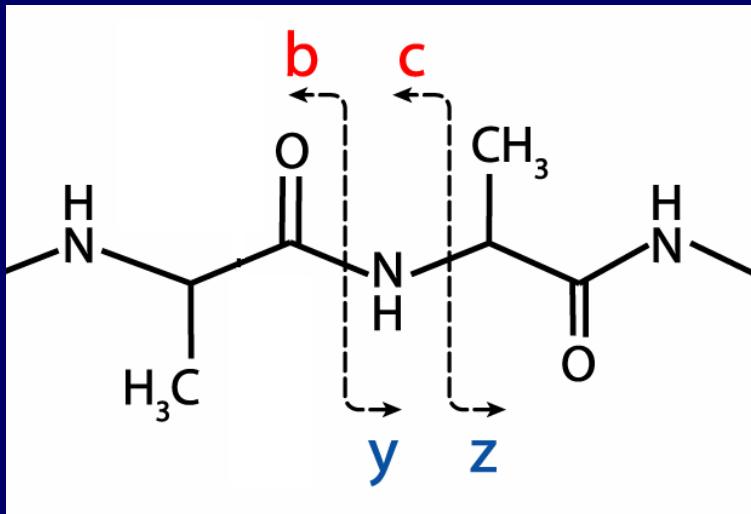
Fluoranthene



Tandem MS - Dissociation Techniques

CAD: Collision Activated Dissociation (b, y ions)

⇒ increase of internal energy through collisions



ETD: Electron Transfer Dissociation (c, z ions)

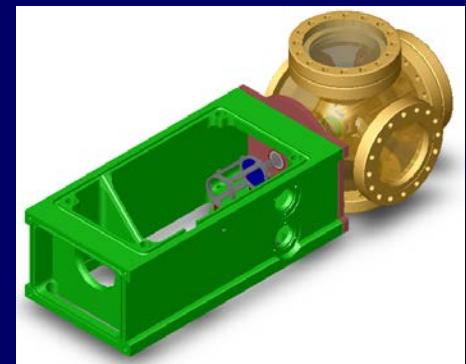
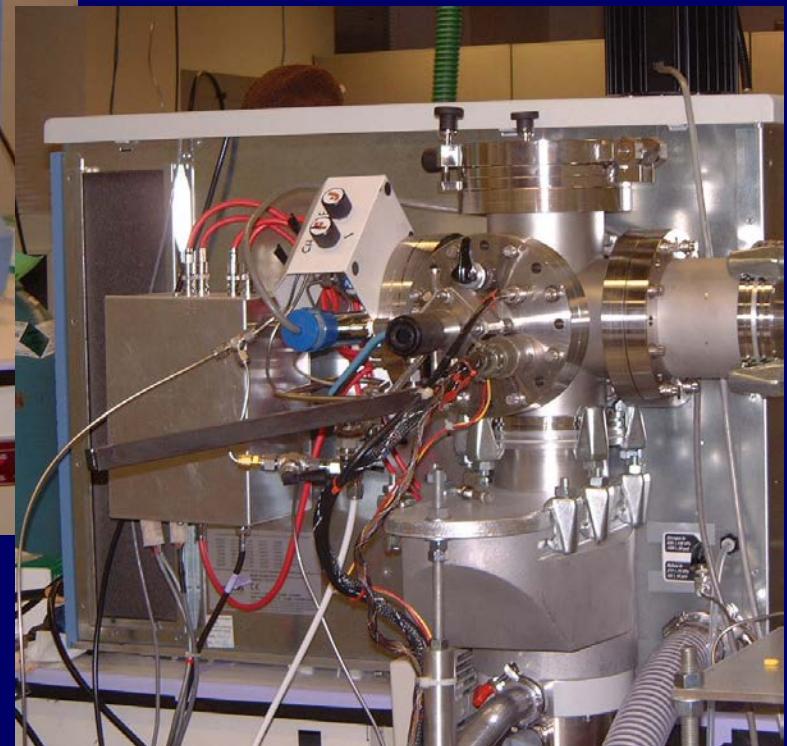
⇒ bombardment of peptides with electrons
(radical driven fragmentation)

The Prototype Instrument



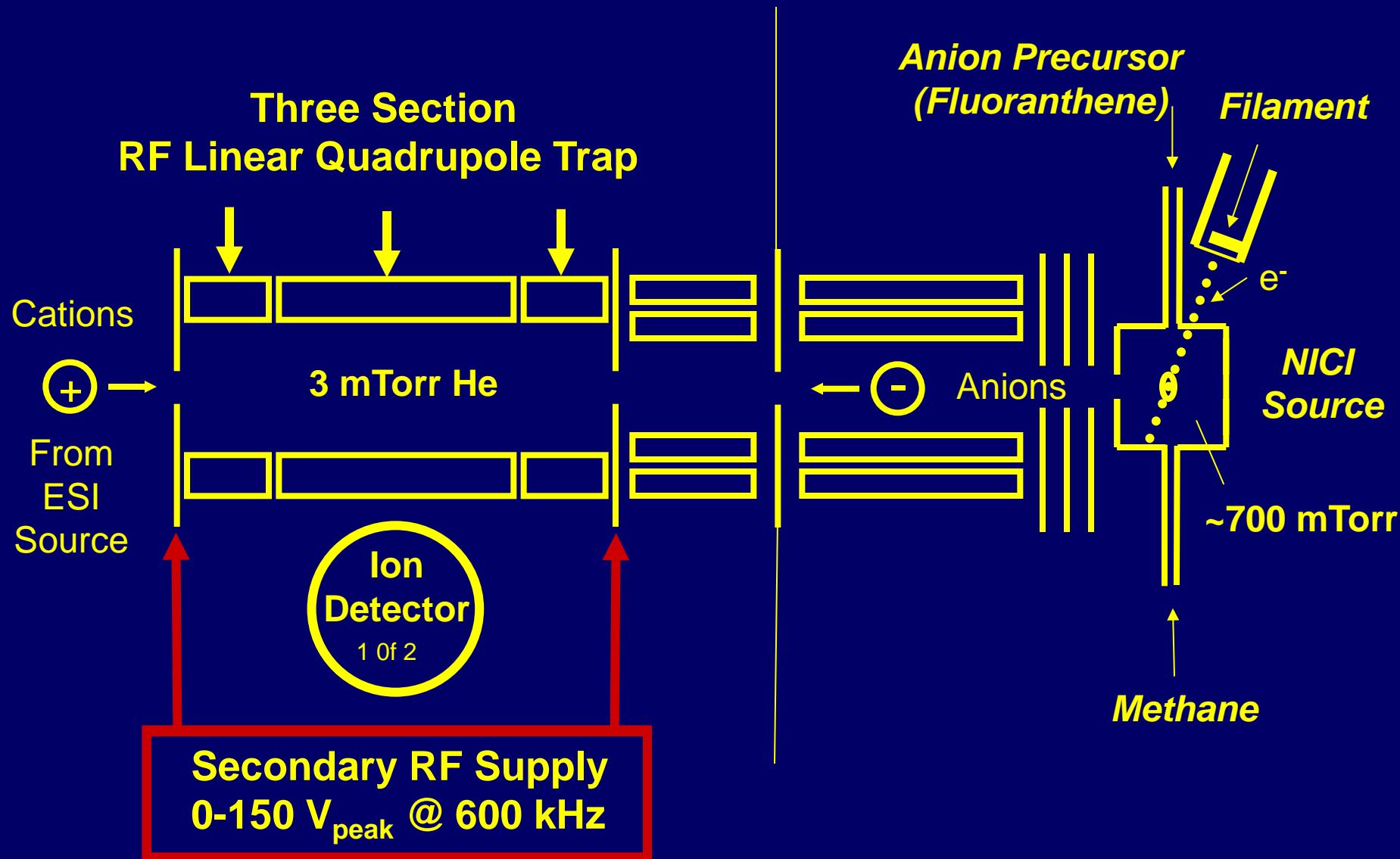
HPLC

LTQ front

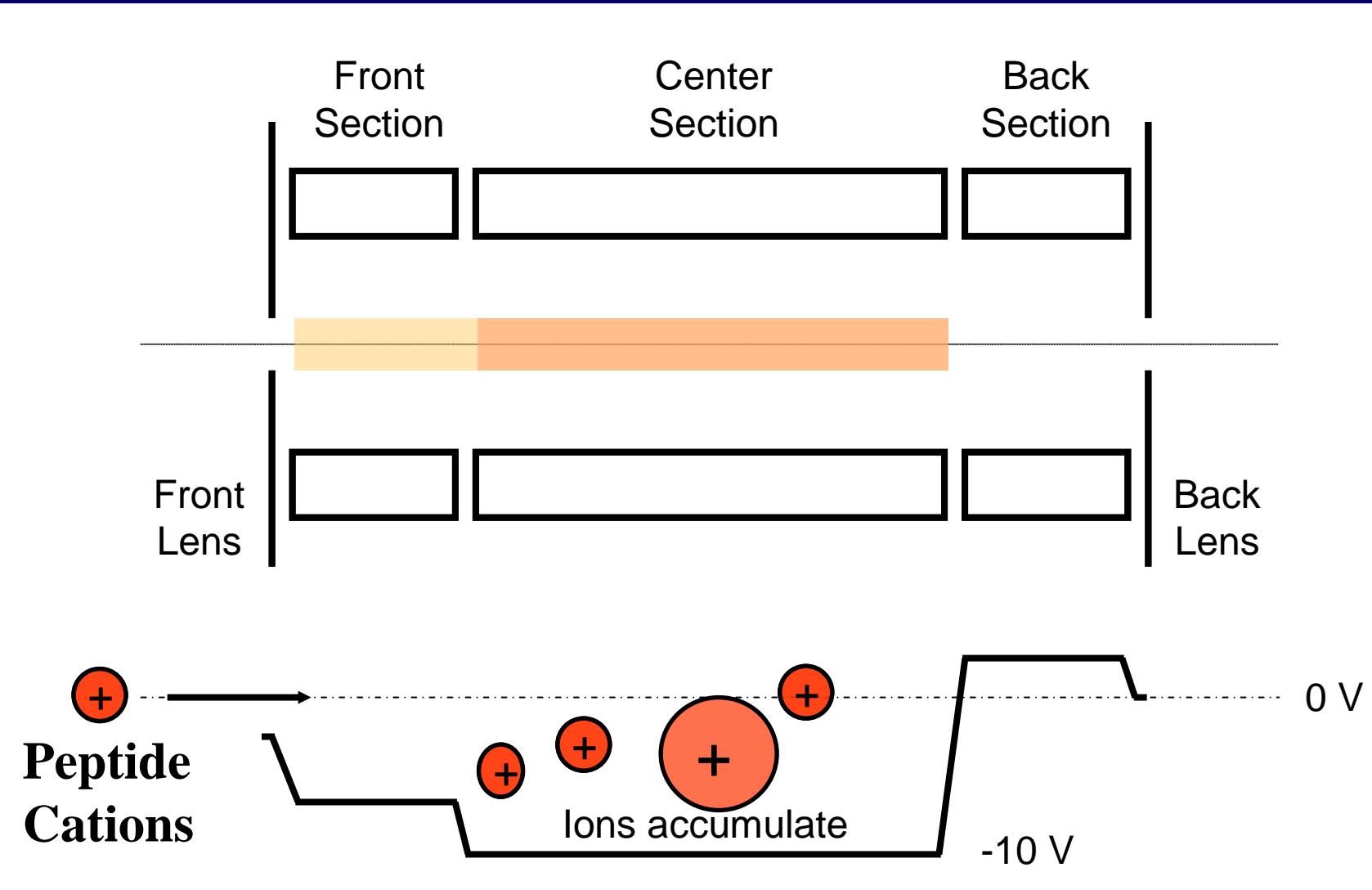


Modified rear / CI source

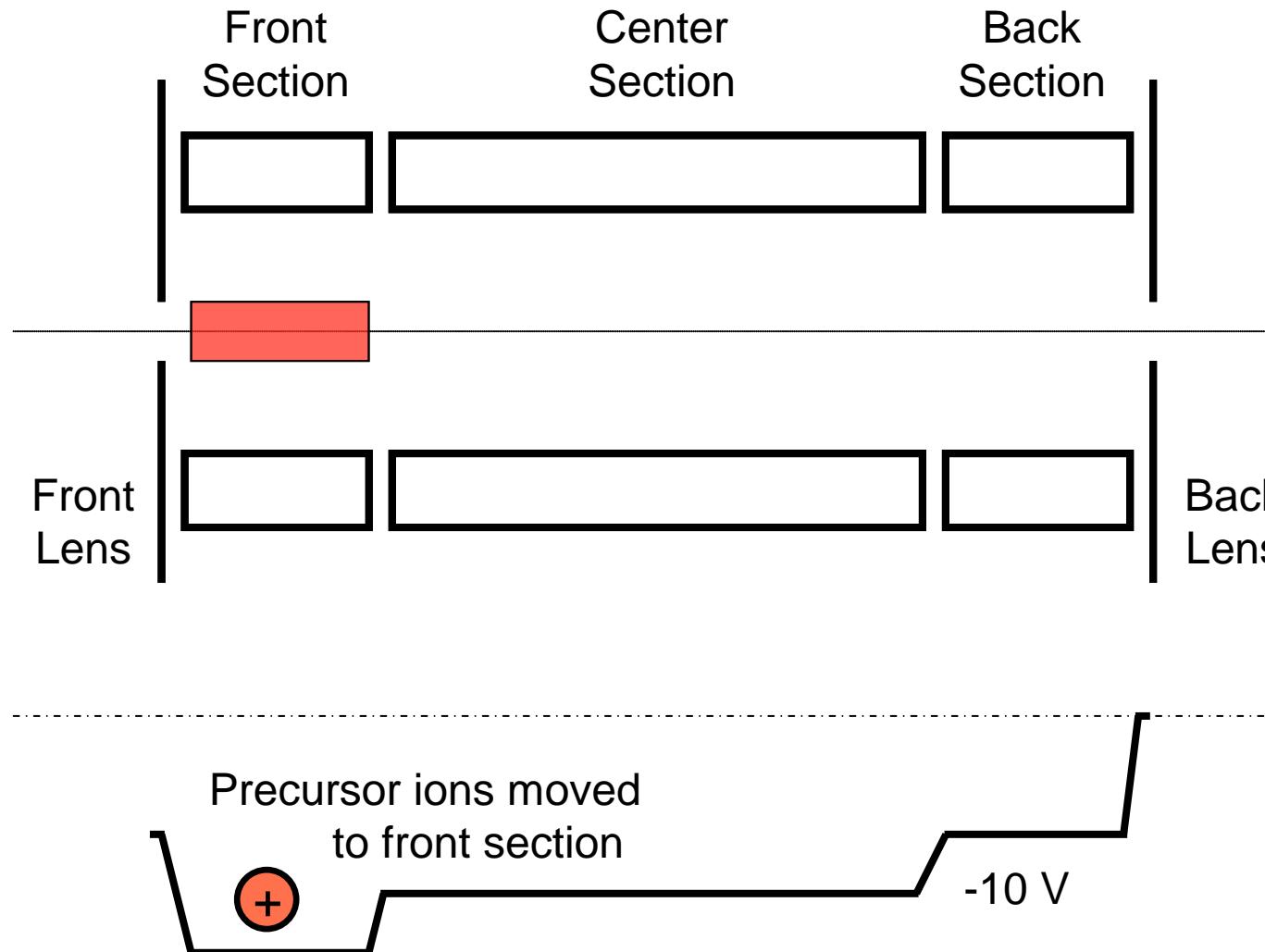
Modifications For Ion/Ion Experiments



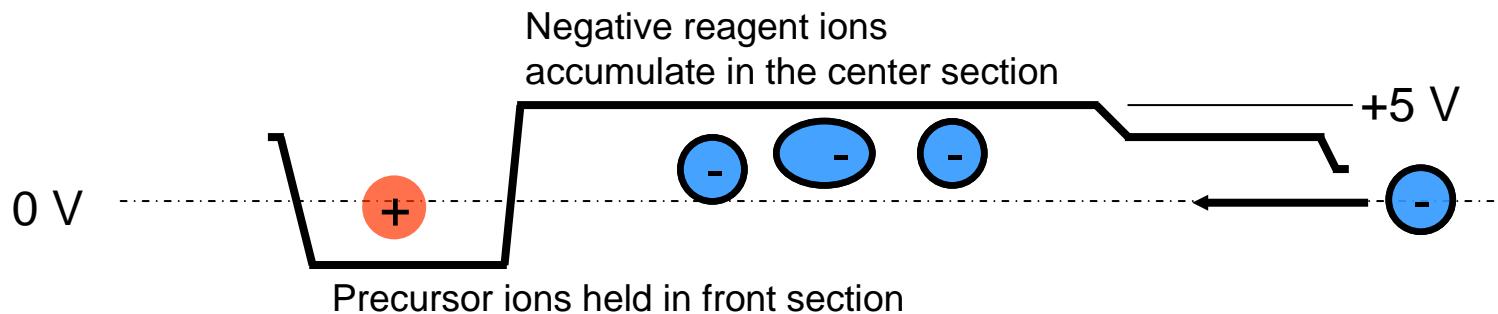
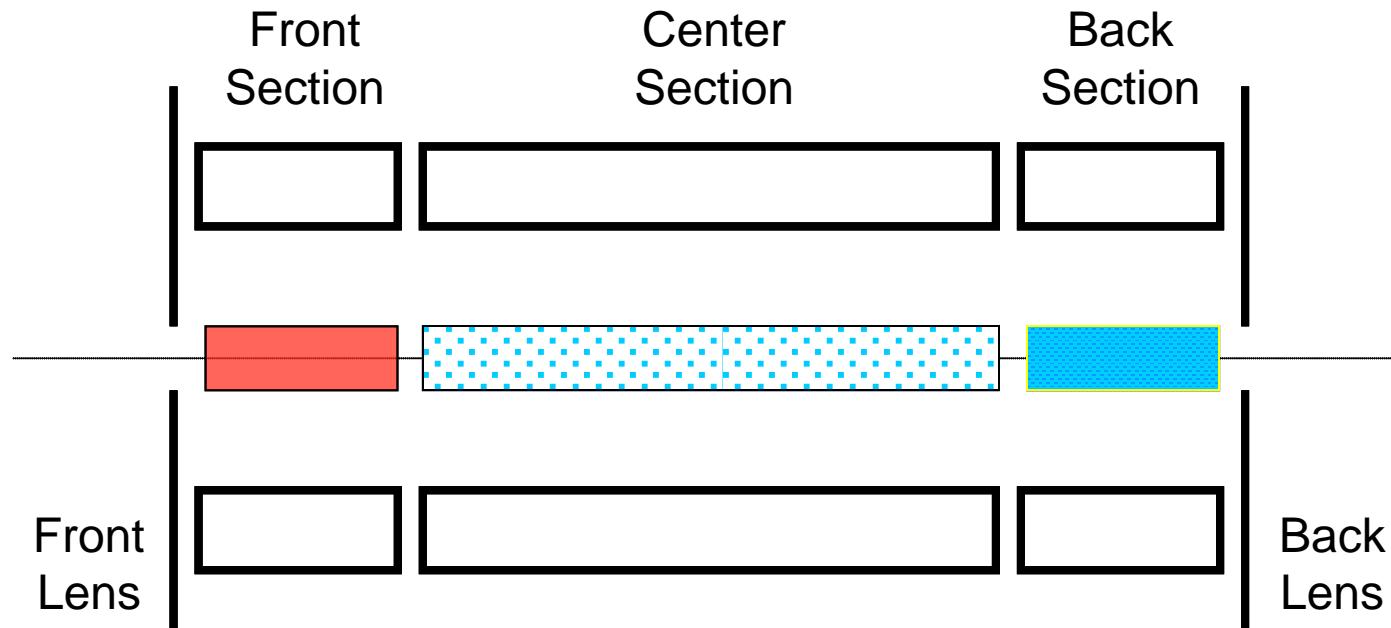
Injection of Positive Ions (ESI)



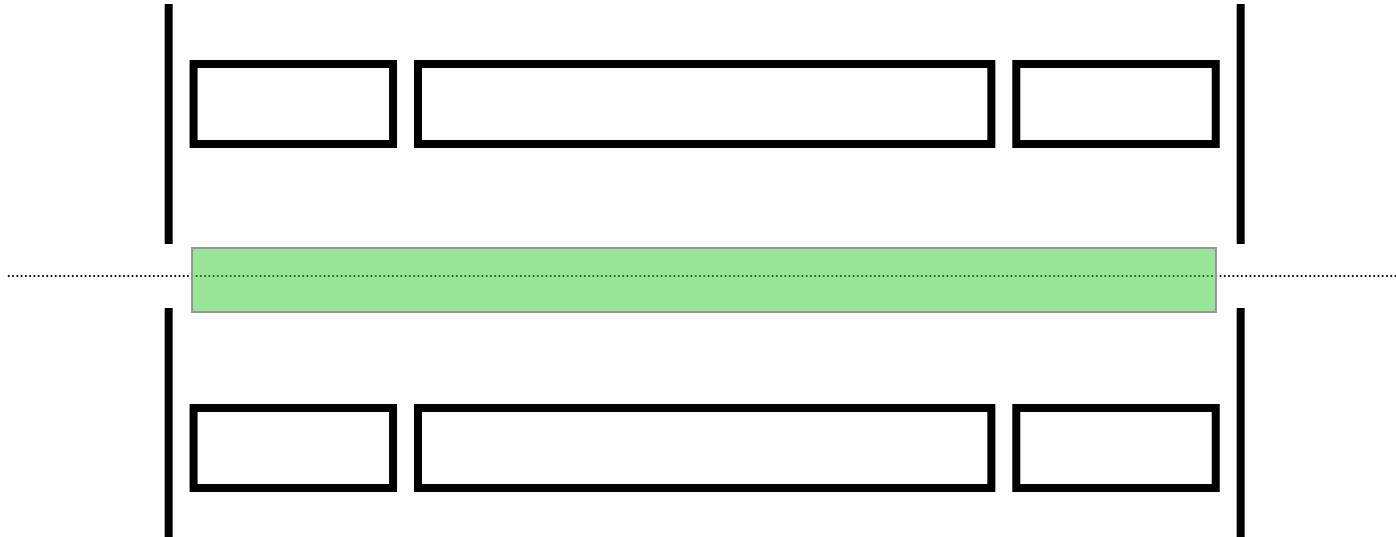
Precursor Storage in Front Section



Injection of Negative Ions (CI)

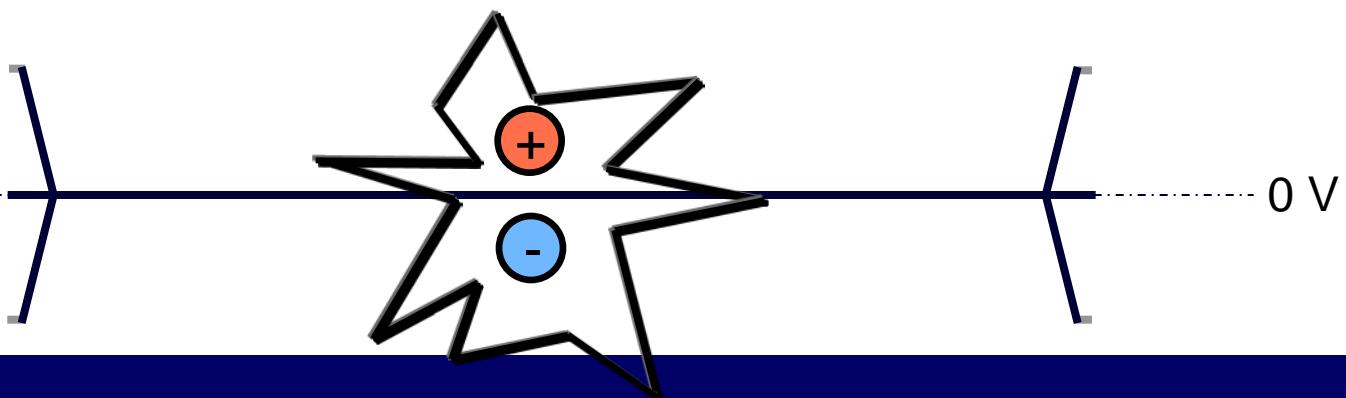


Charge-Sign Independent Trapping

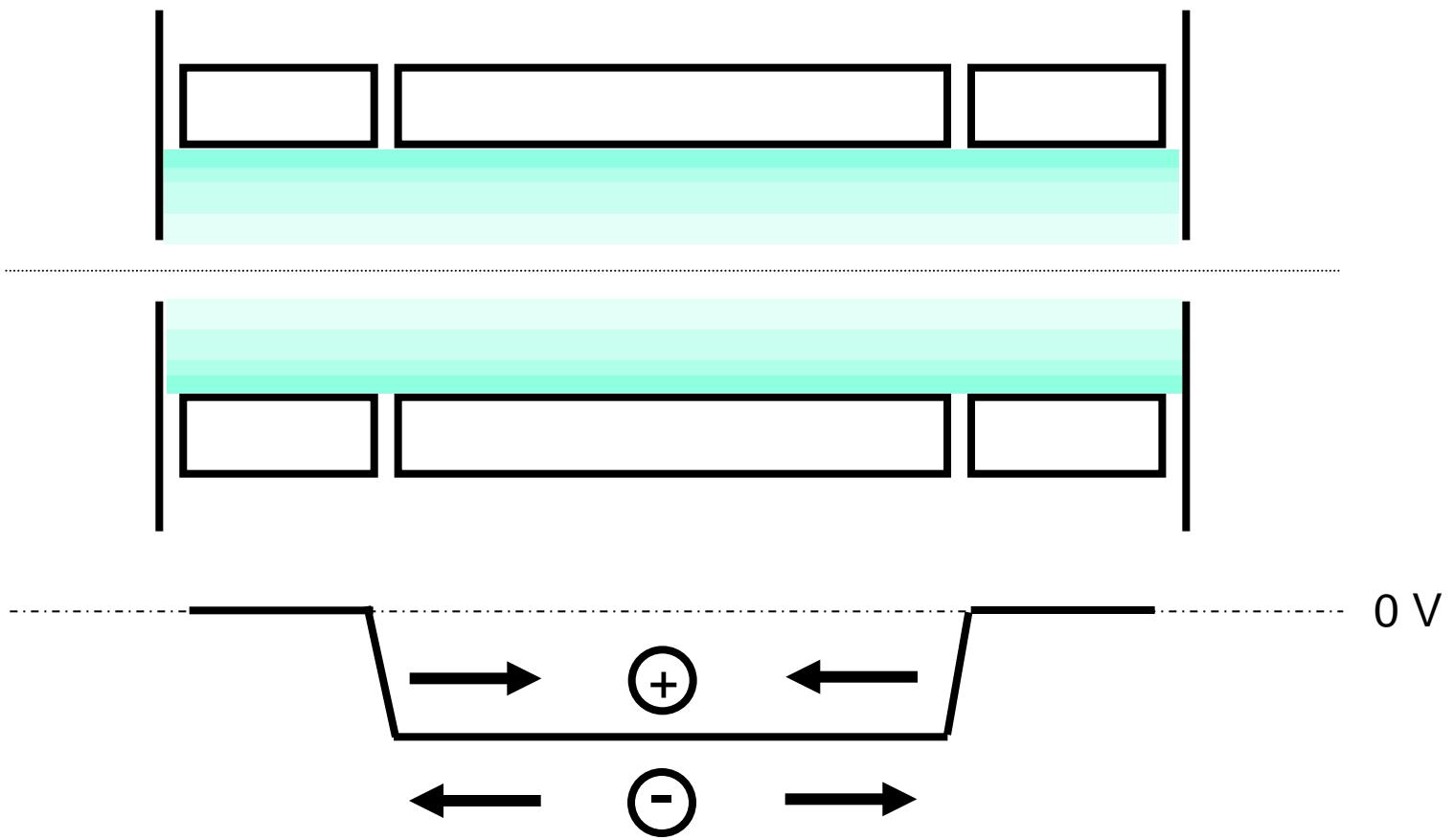


Positive and negative ions react while trapped in axial pseudo-potential

Pseudo-potential created by
+150 V_p
600 kHz applied to lenses

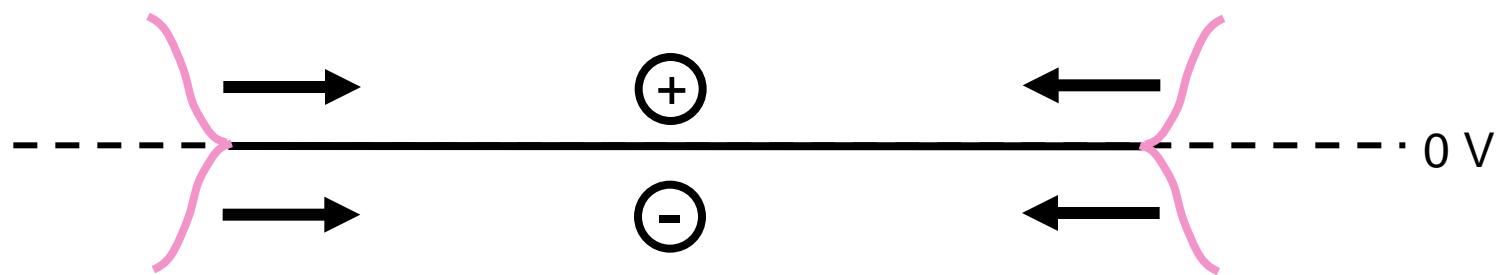
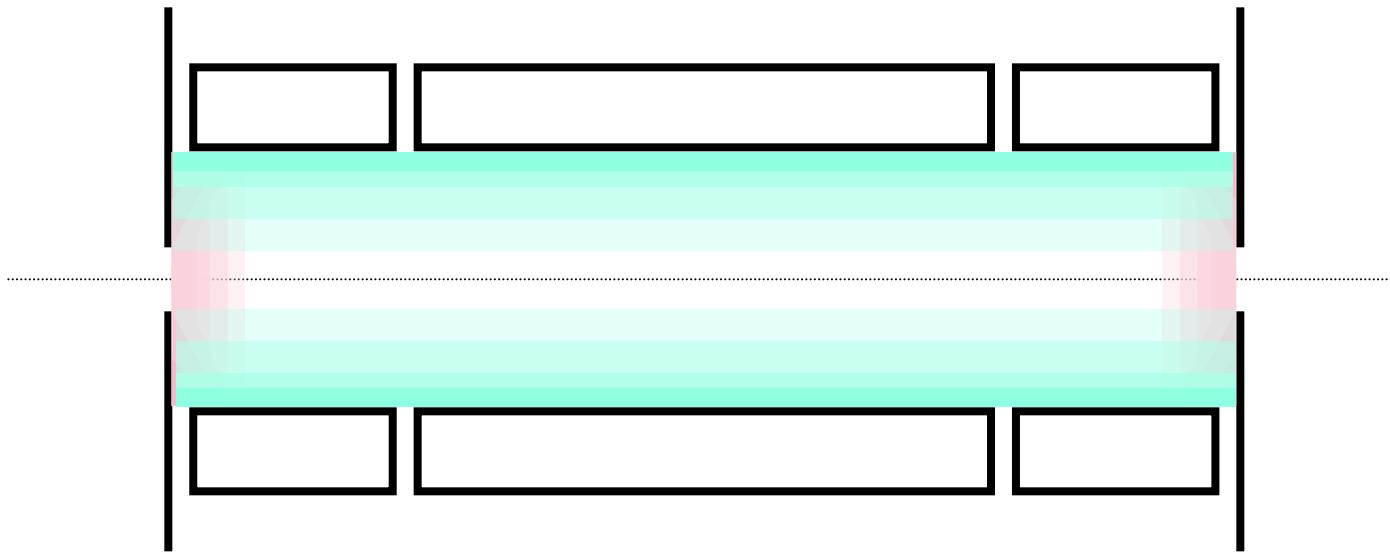


Charge sign independent radial confinement

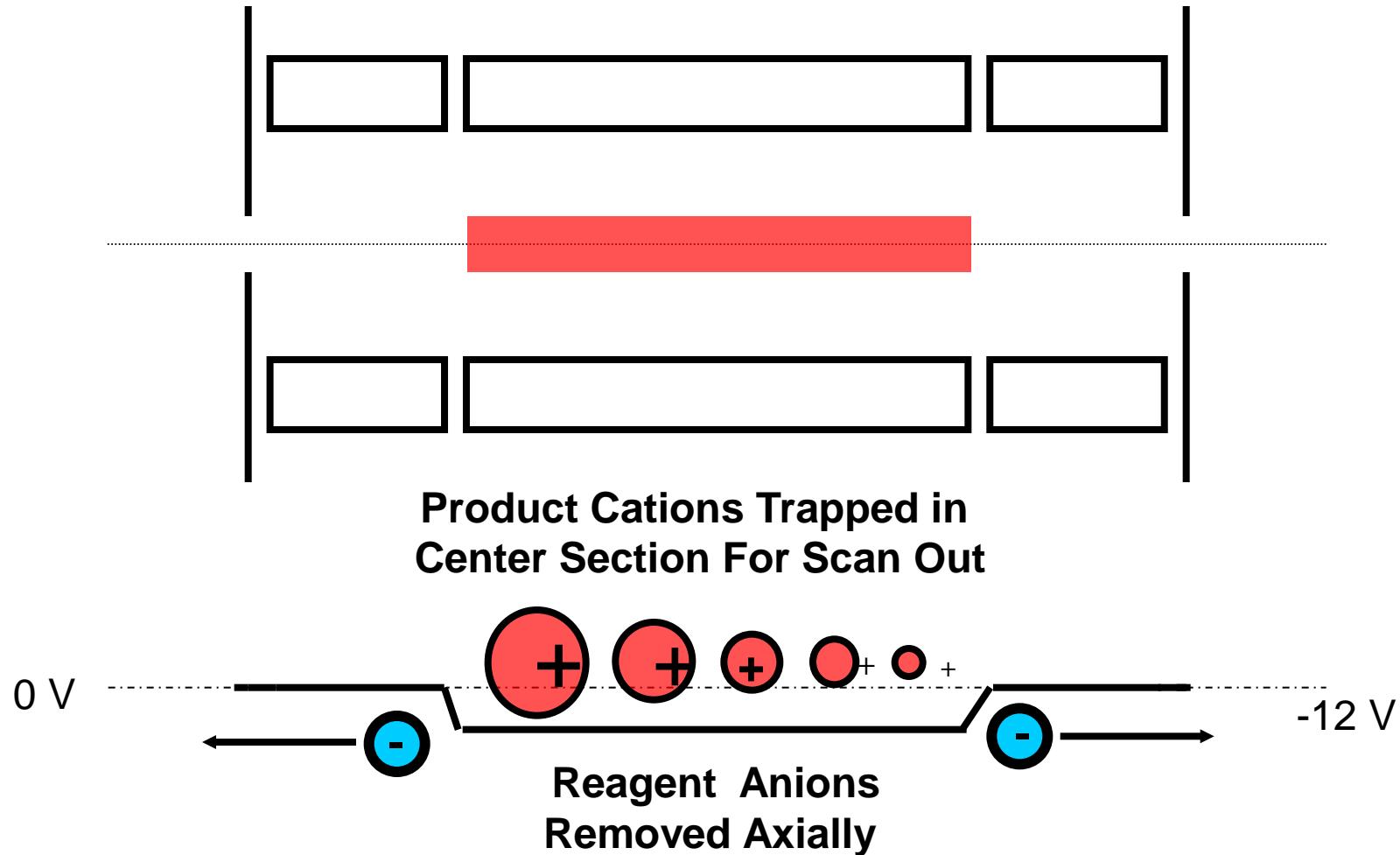


Axial Confinement With DC Potentials
Trapping is Charge Sign Dependent

Charge sign independent axial confinement with combined RF Quadrupole and end lens RF pseudo-potentials

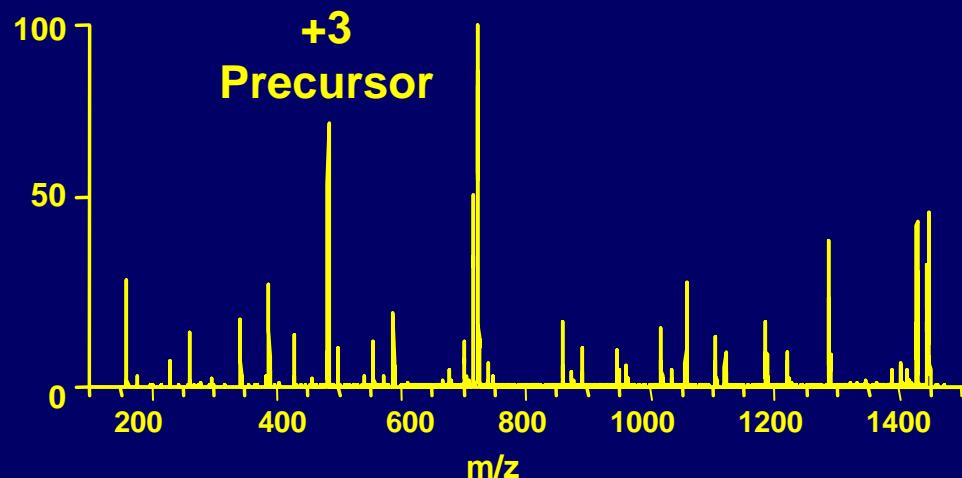
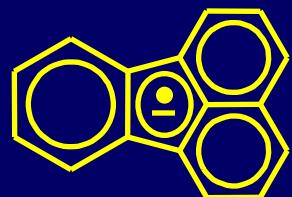


End ion/ion reactions prepare for product ion analysis



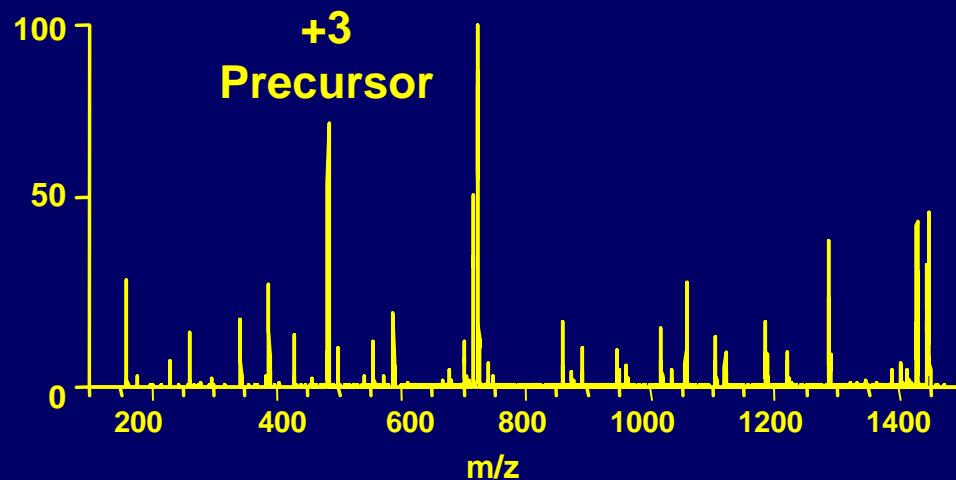
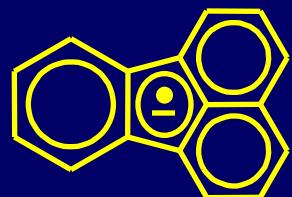
Electron Transfer - Proton Transfer

Fragmentation (ETD)

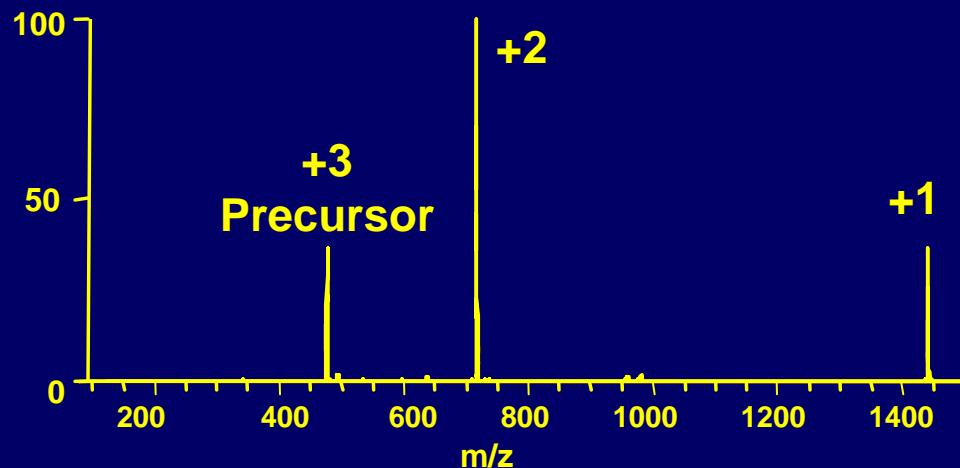
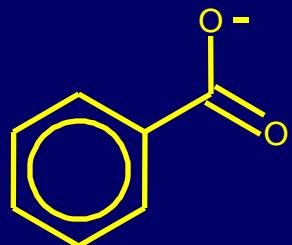


Electron Transfer - Proton Transfer

Fragmentation (ETD)



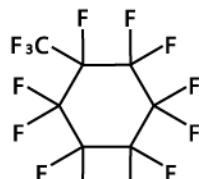
Charge Reduction (PTR)



The two types of ion reactions

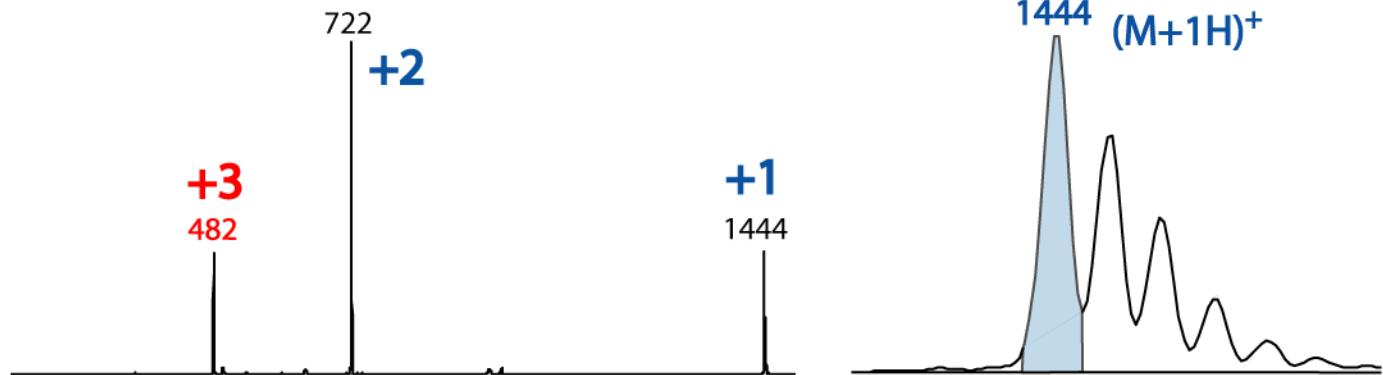
1.

PDCH



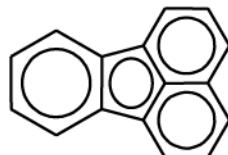
65 ms

Proton transfer (PTR)



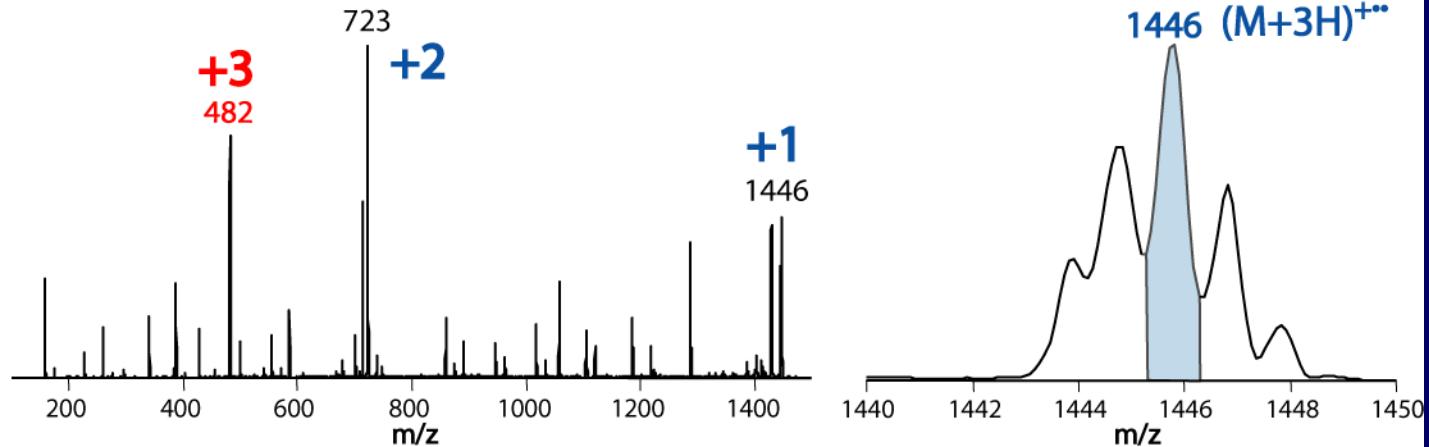
2.

fluoranthene



65 ms

Electron transfer (ETD)



ET or ETD



Intact
Charge-Reduced
Products



Mass ?
m/z ?
Charge (z)
Sequence ?
Temperature ?
Anion ?
He Pressure?

Fragmentation
Products
c, z, etc.

ET or ETD



Intact
Charge-Reduced
Products

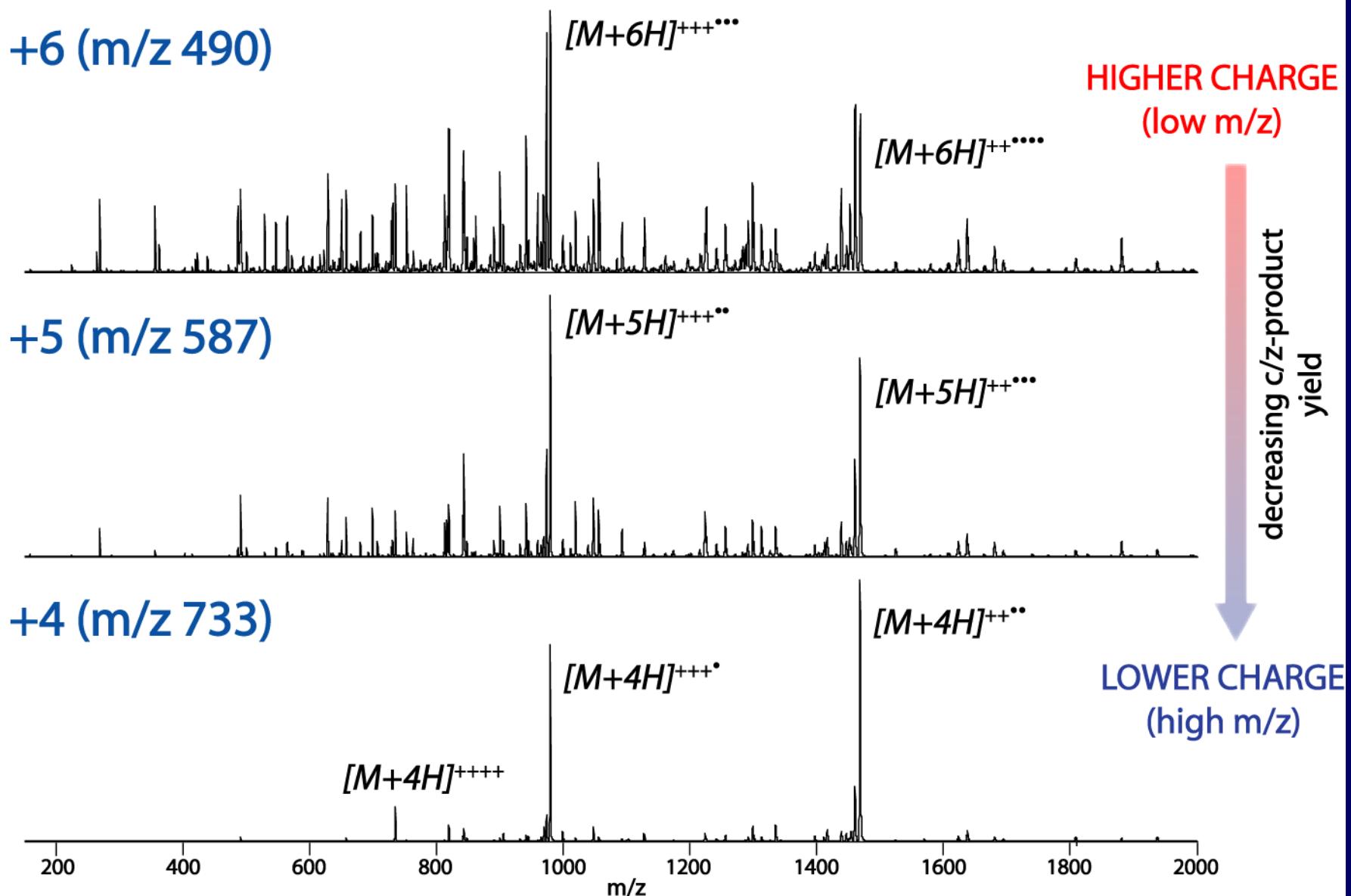


Mass ?
m/z ?
Charge (z)
Sequence ?
Temperature ?
Anion ?
He Pressure?

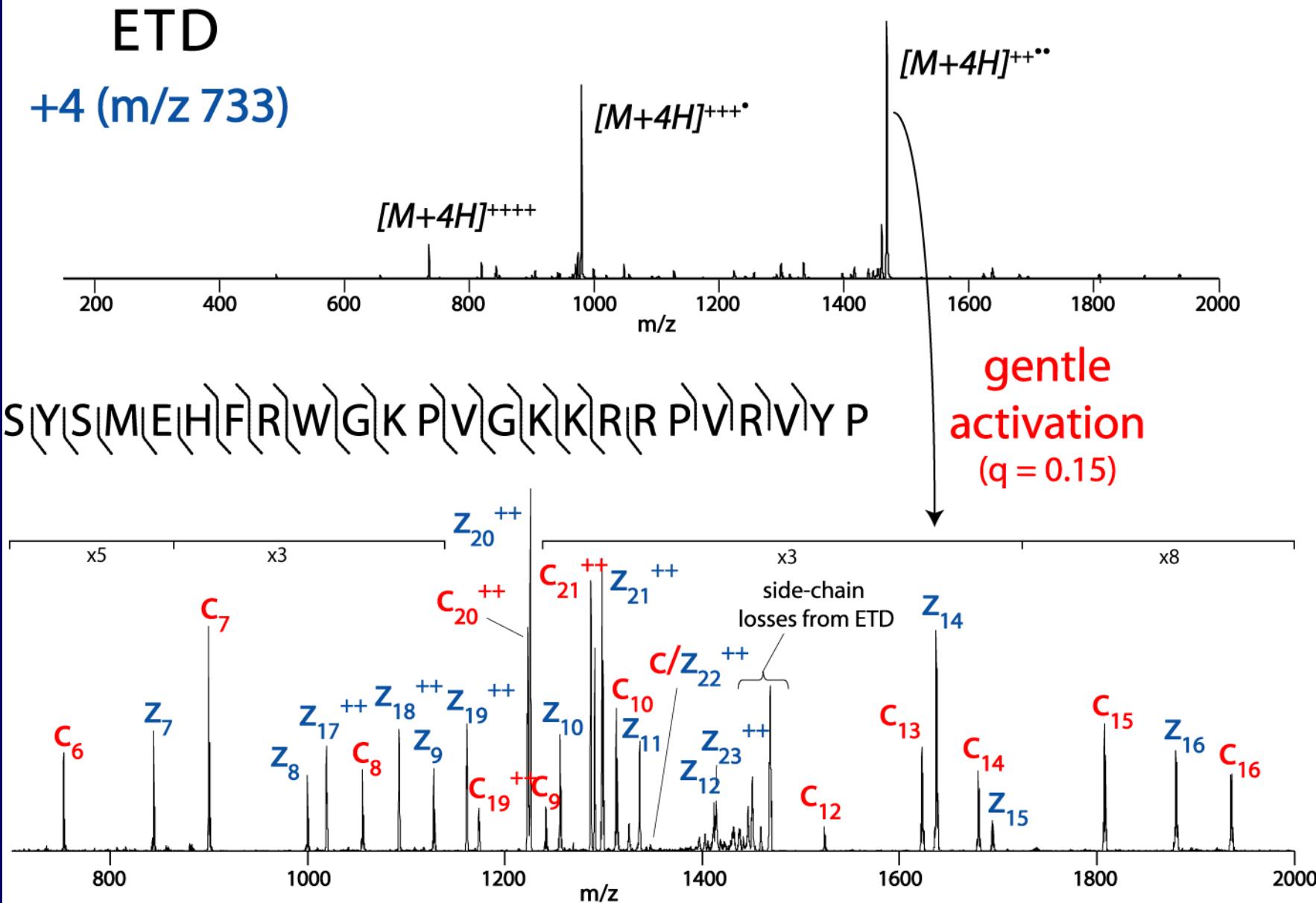
Fragmentation
Products
c, z, etc.

CAD

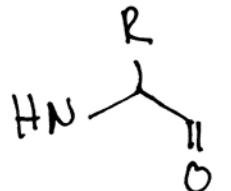
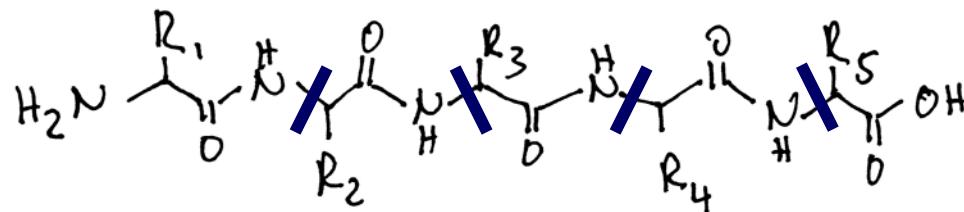
Charge dependence in fragmentation



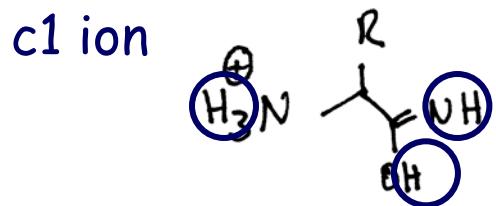
Gentle off resonance activation



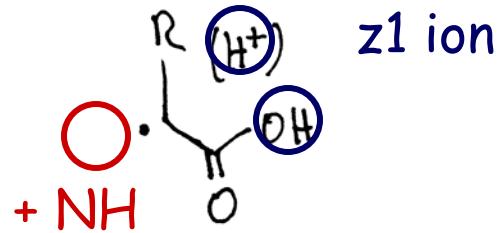
How to Sequence ETD



Residue Mass (RM)



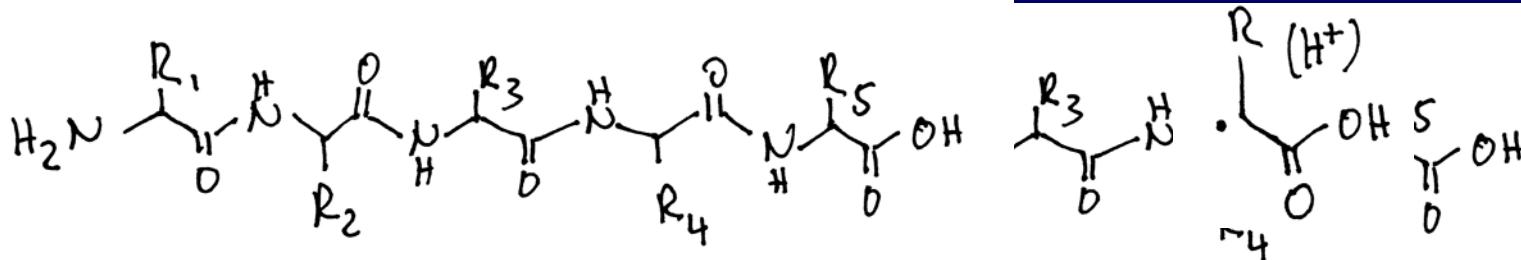
$$\text{c1} = \text{RM} + 18$$



$$\text{z1} = \text{RM} + 3$$

Example of how to calculate theoretical fragment ions

105	176	307	404	517	646	803
S	A	M	P	L	E	R
803	700	629	498	401	288	159

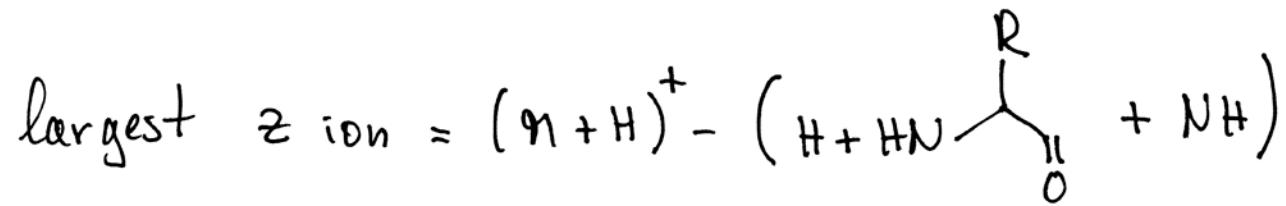


The first c ion

Residue Mass

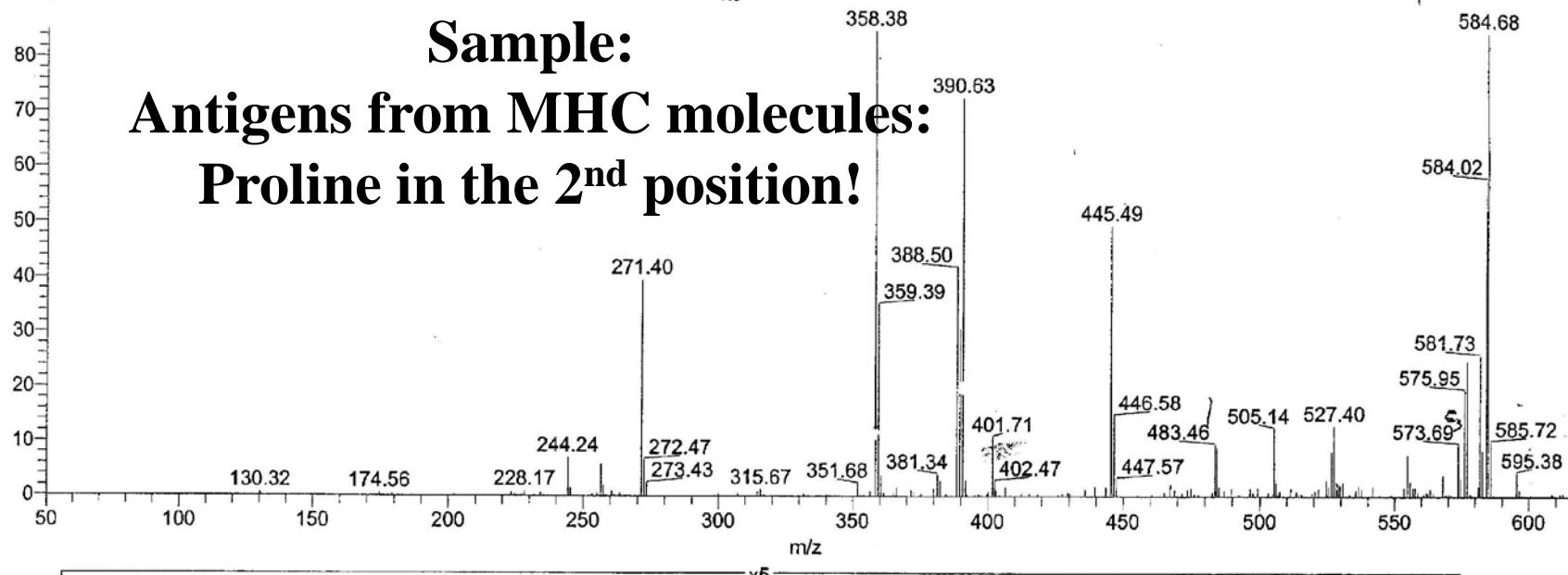
The first z ion

Largest c and z ions

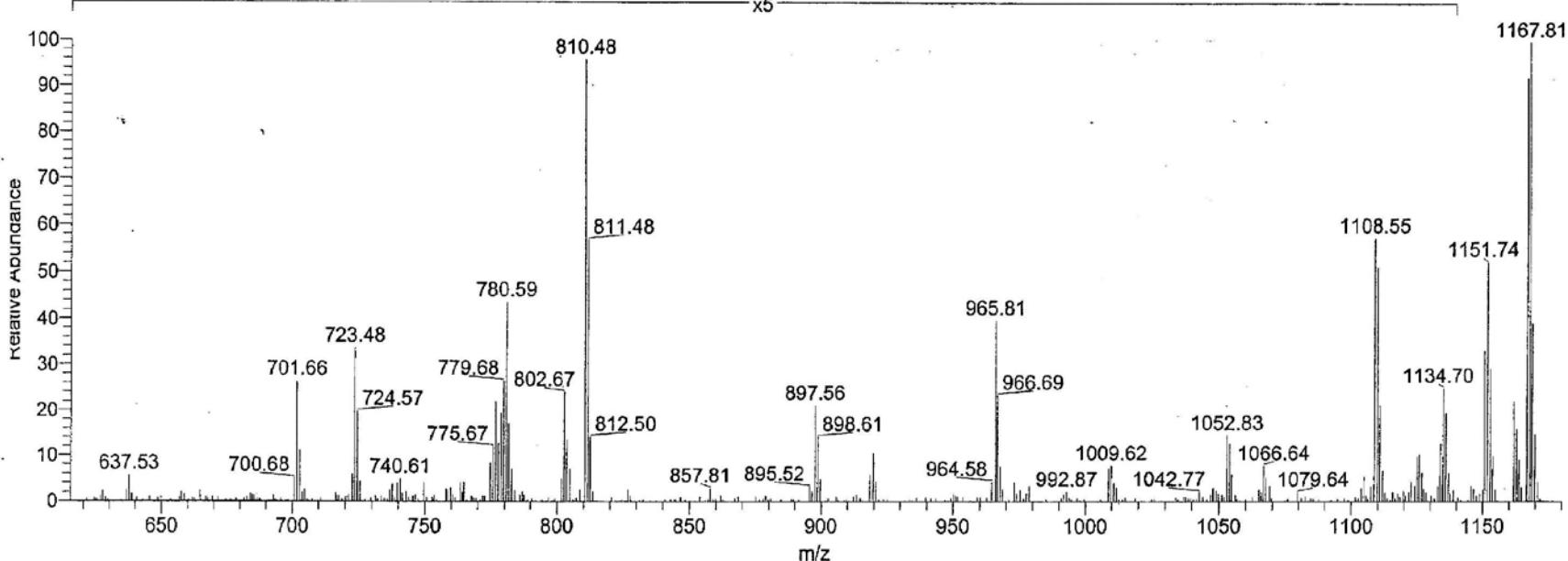


YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

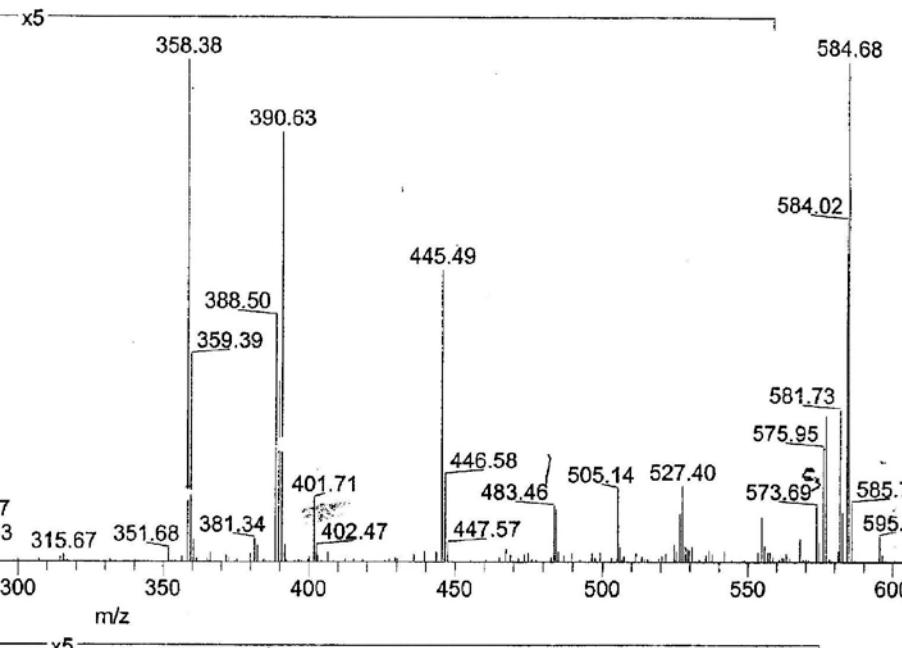
x5



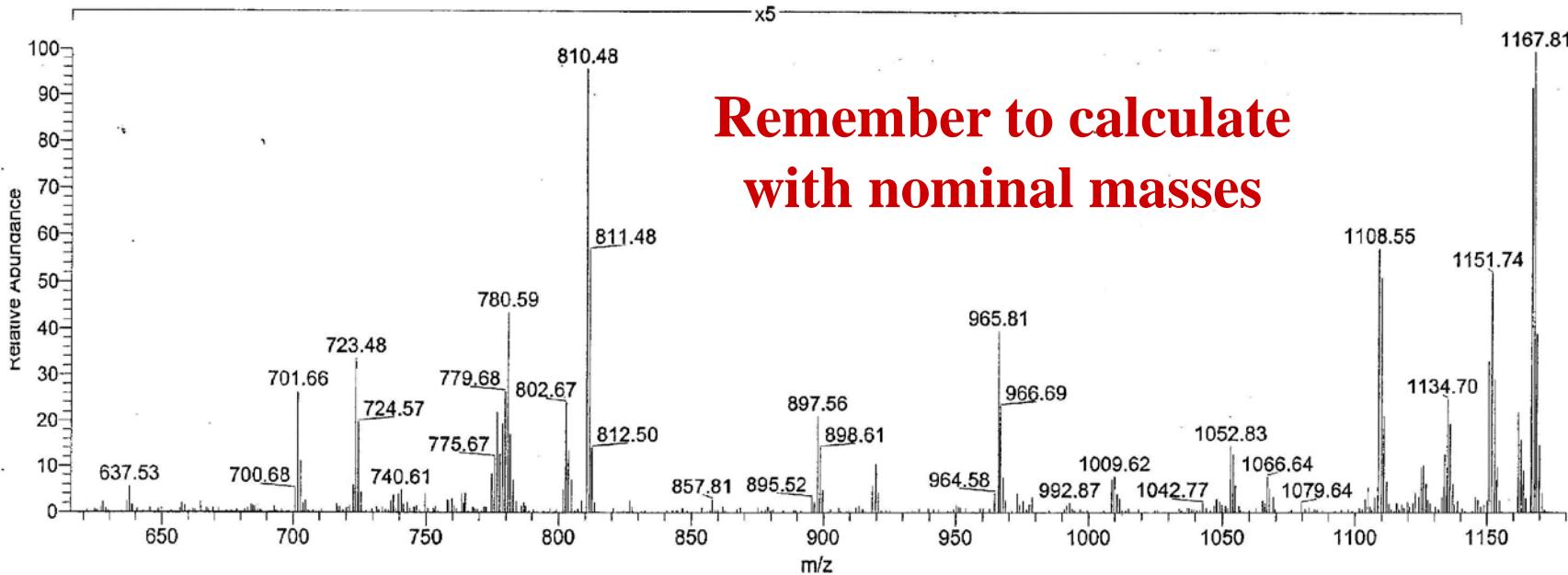
x5



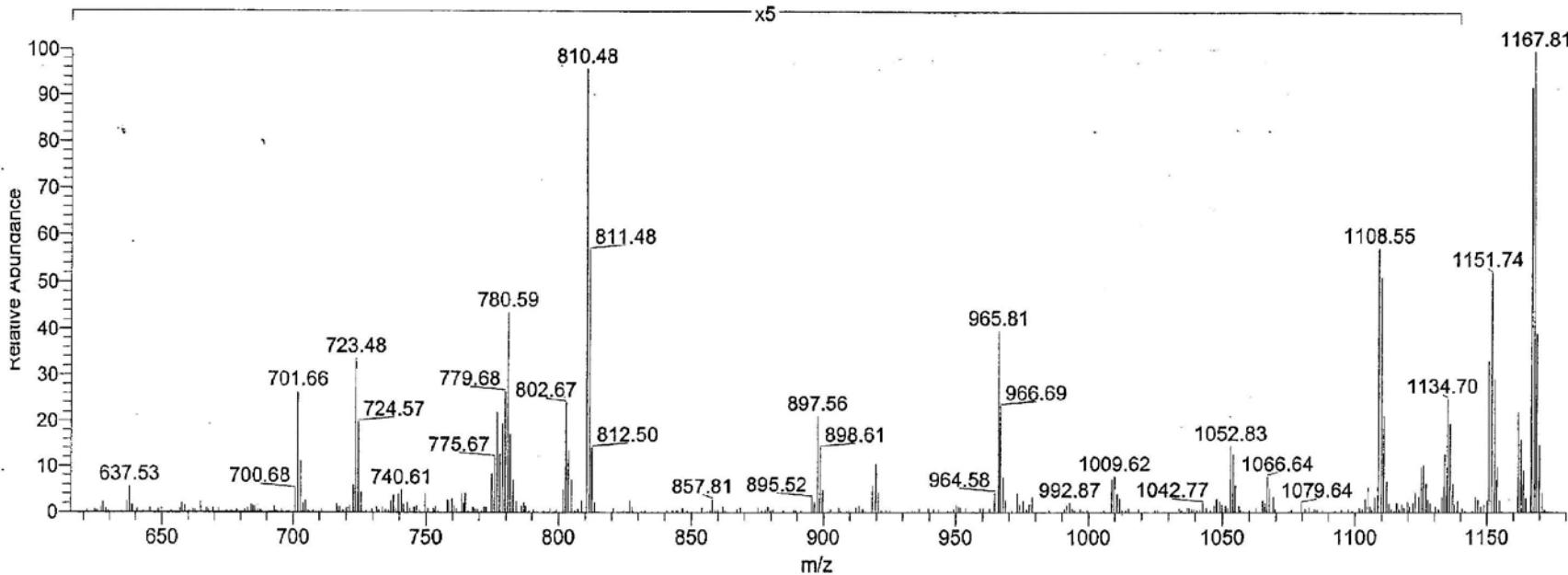
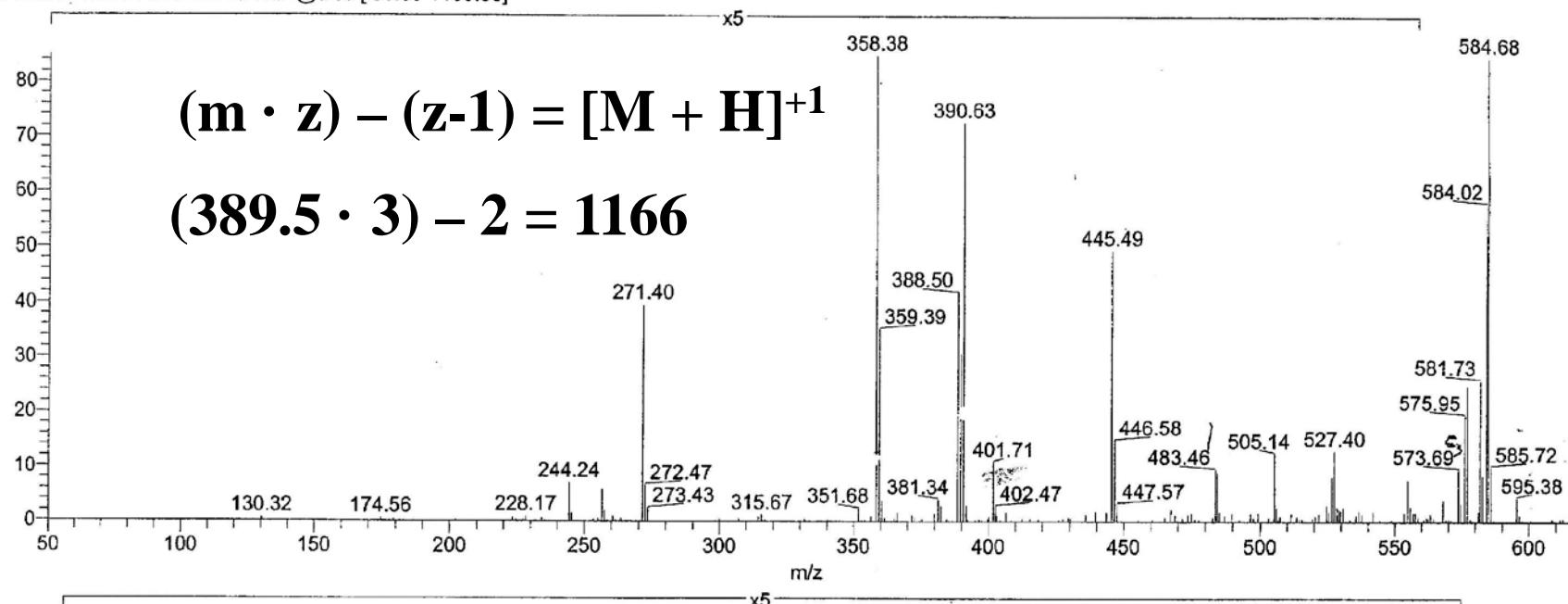
YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

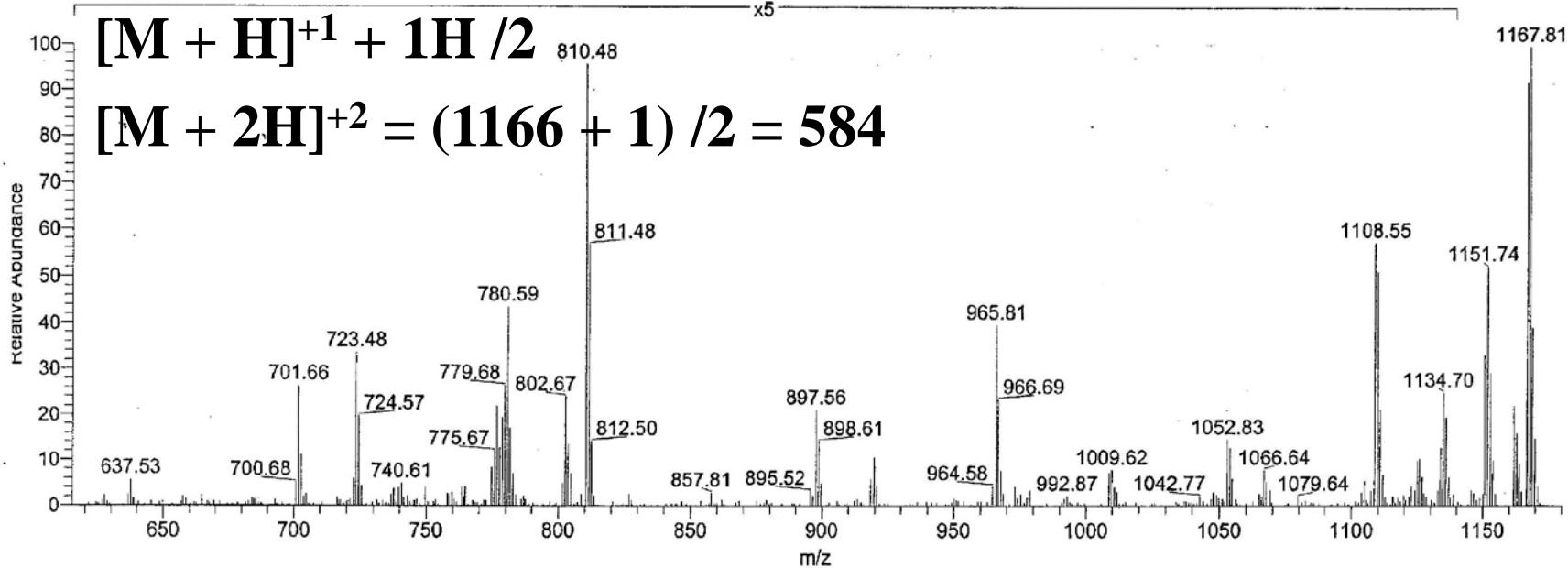
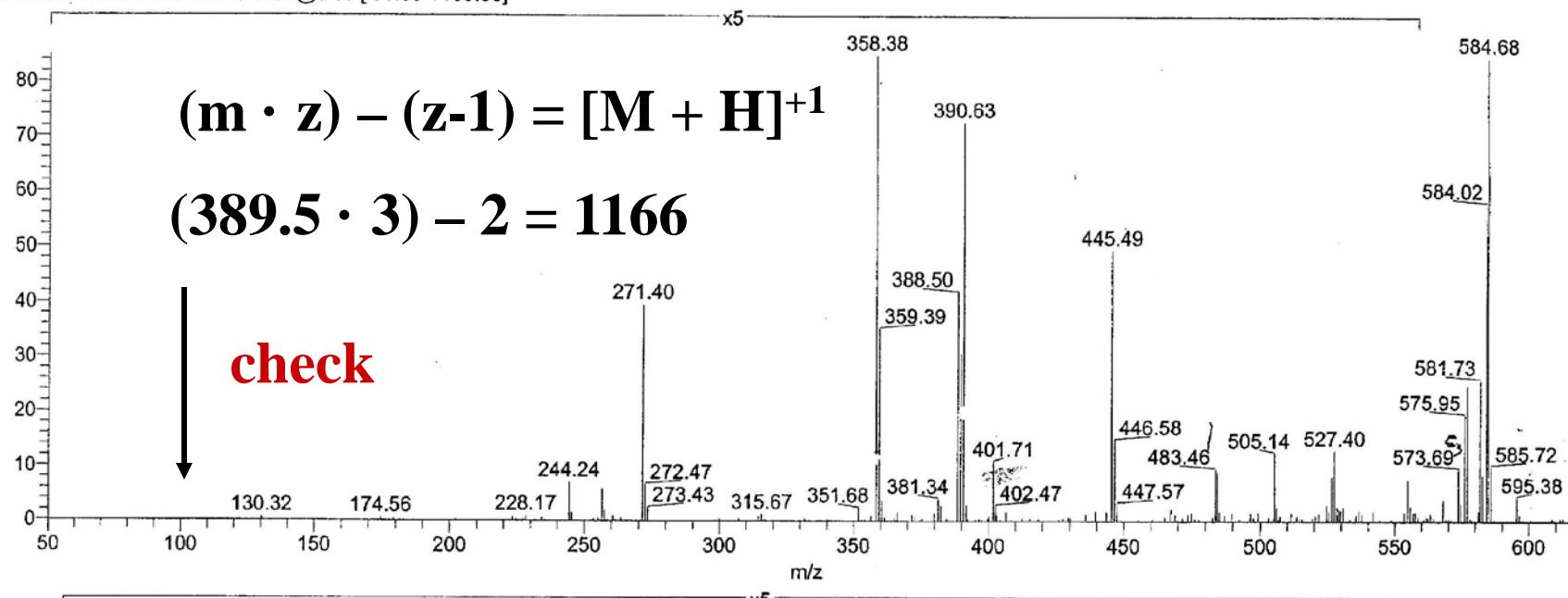


Remember to calculate
with nominal masses

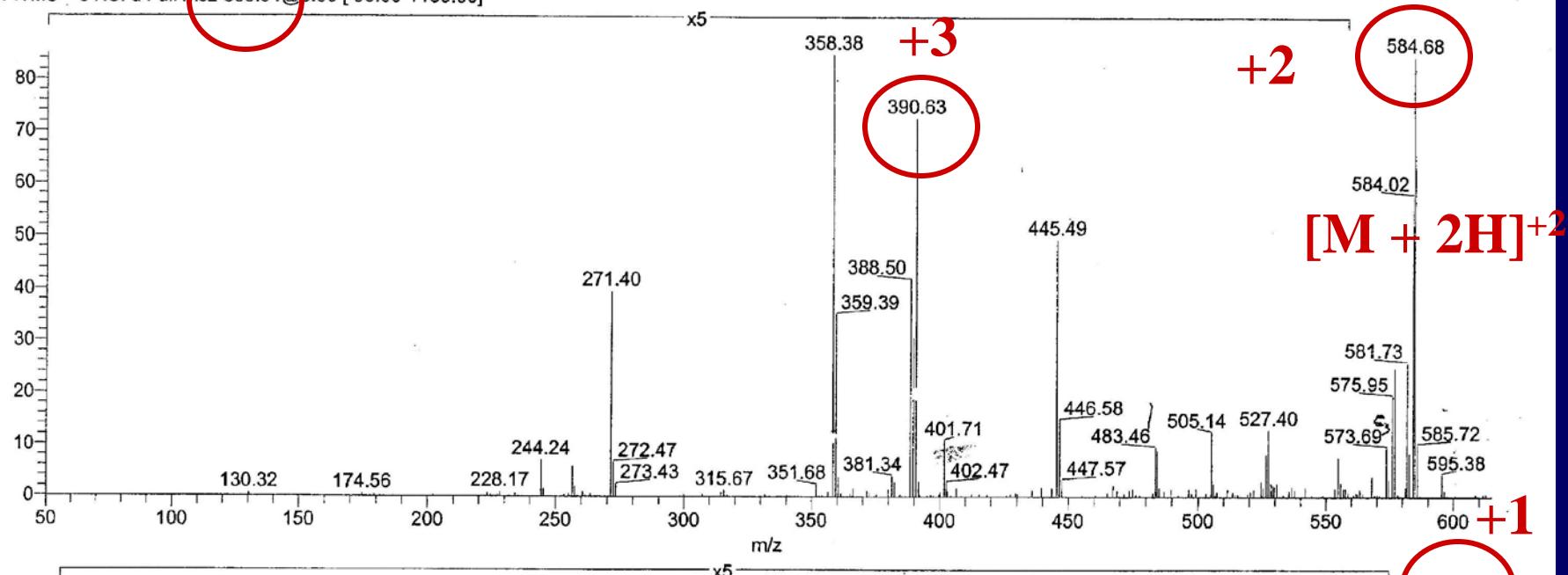
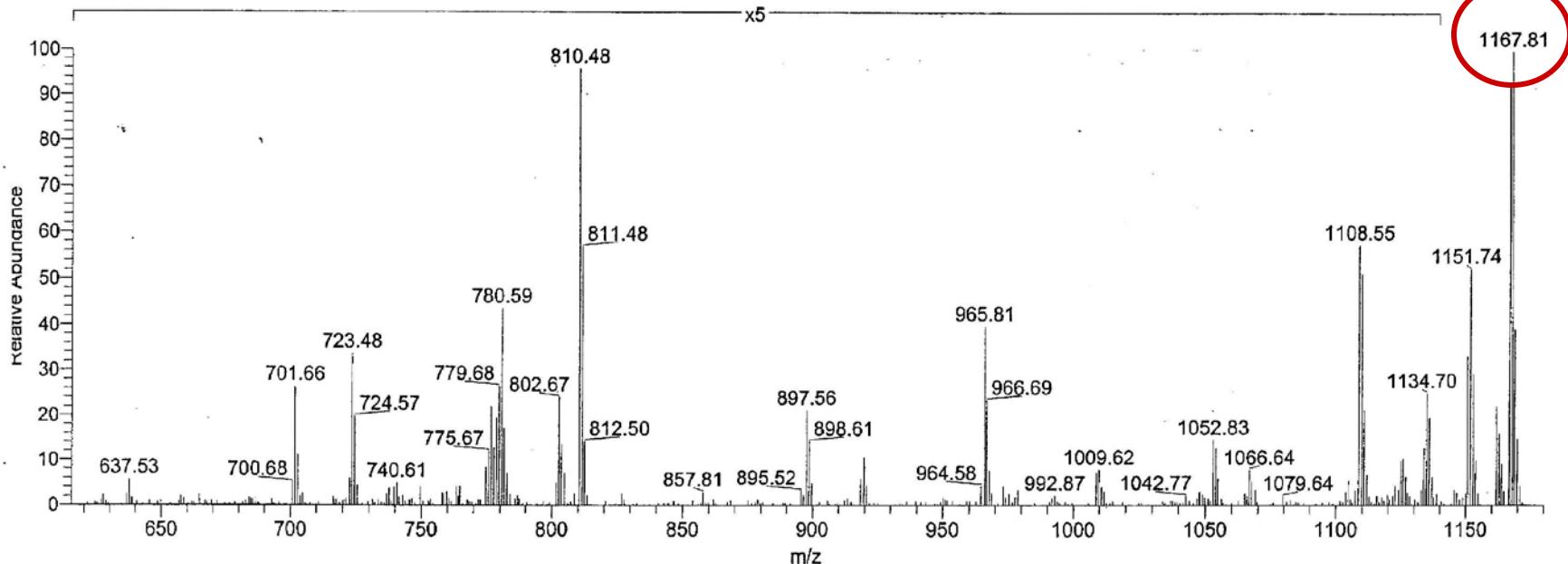


YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

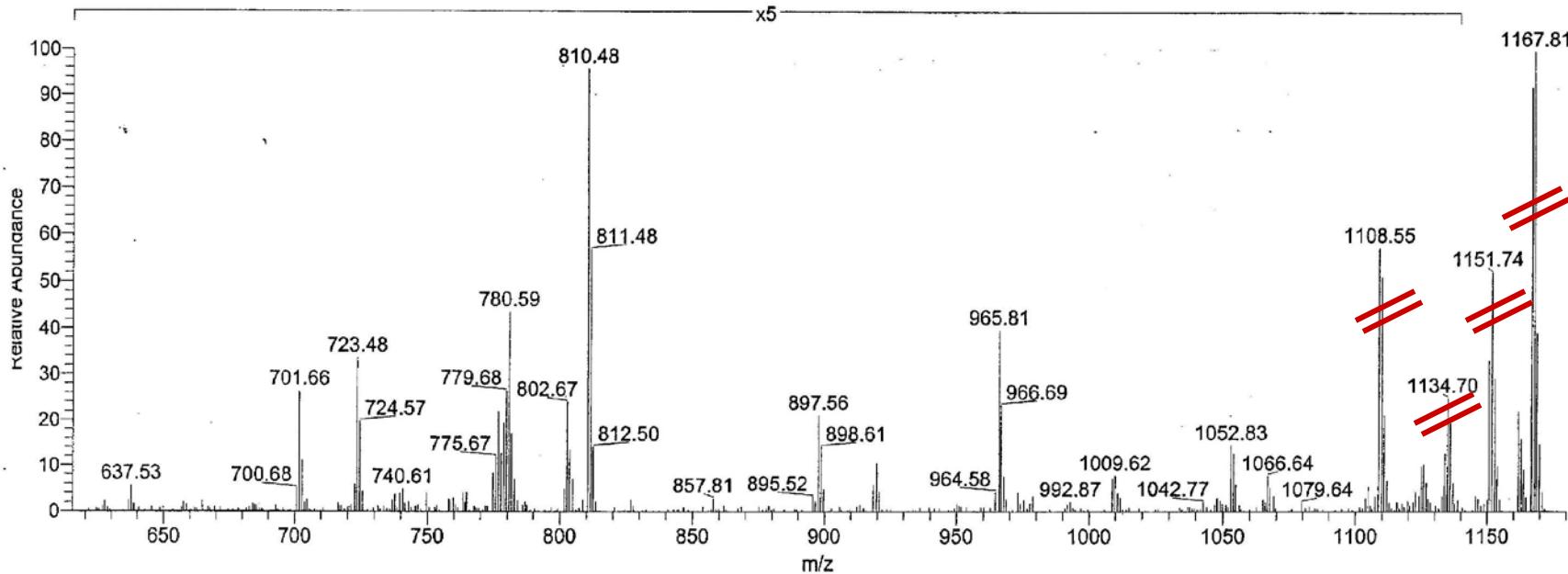
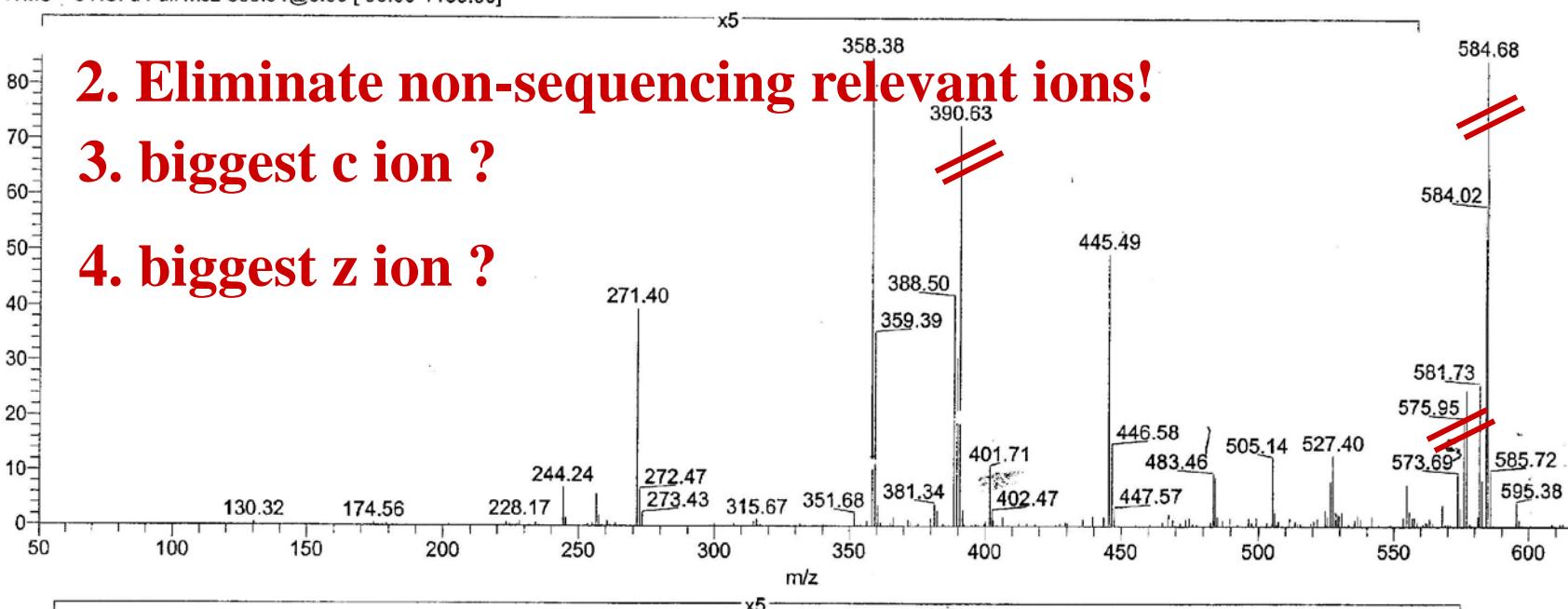


YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

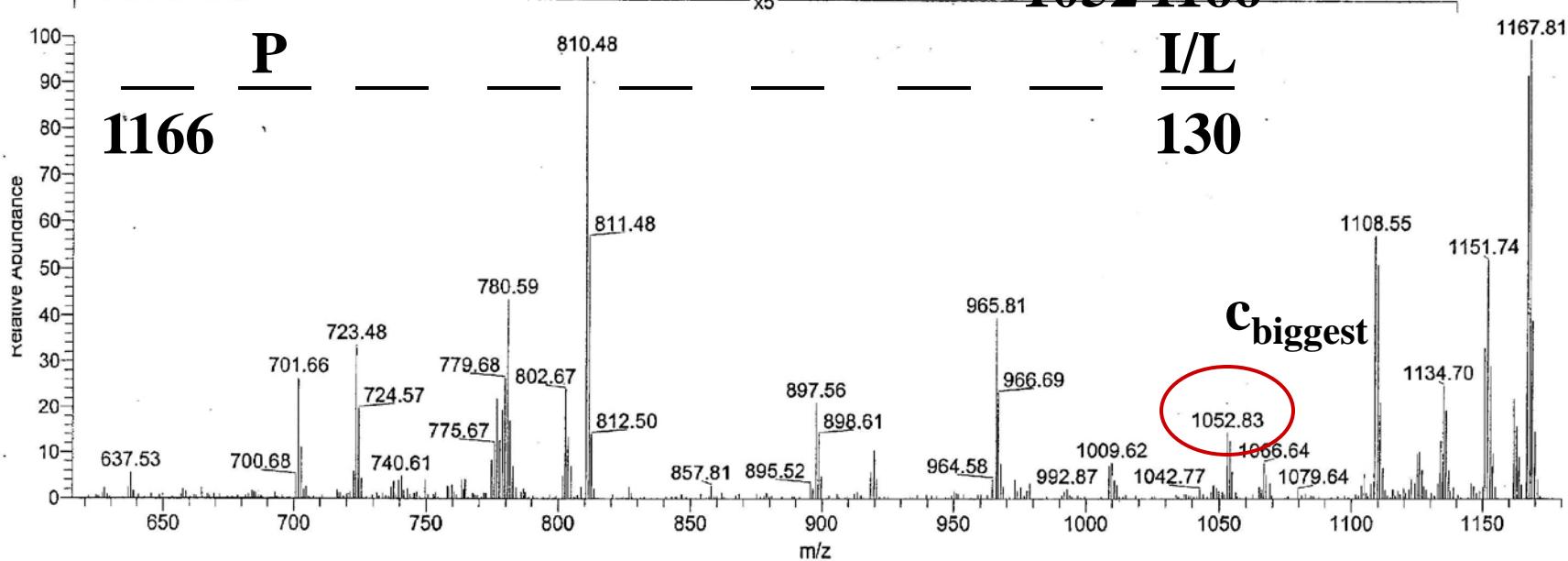
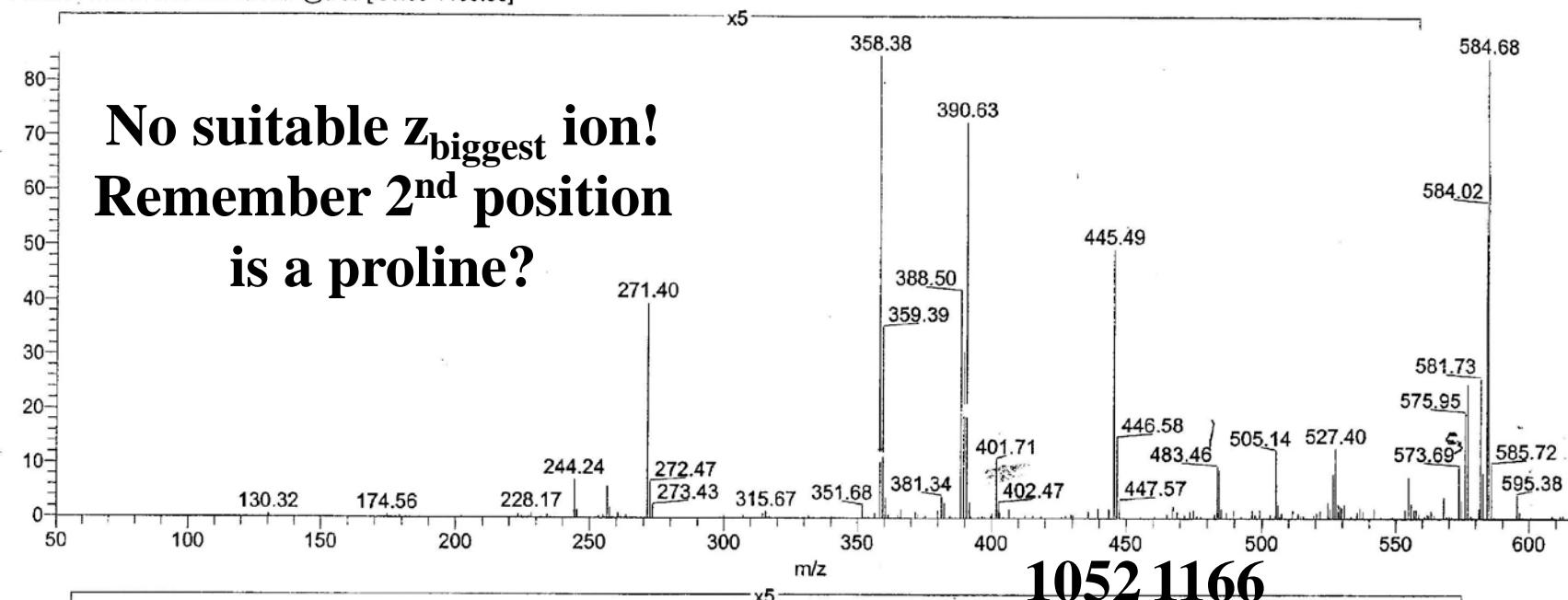
10 microliters, ~ 8 E 7 cell equivalents

YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00] $[M + 3H]^{+3}$  $[M + 2H]^{+2}$ 

YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

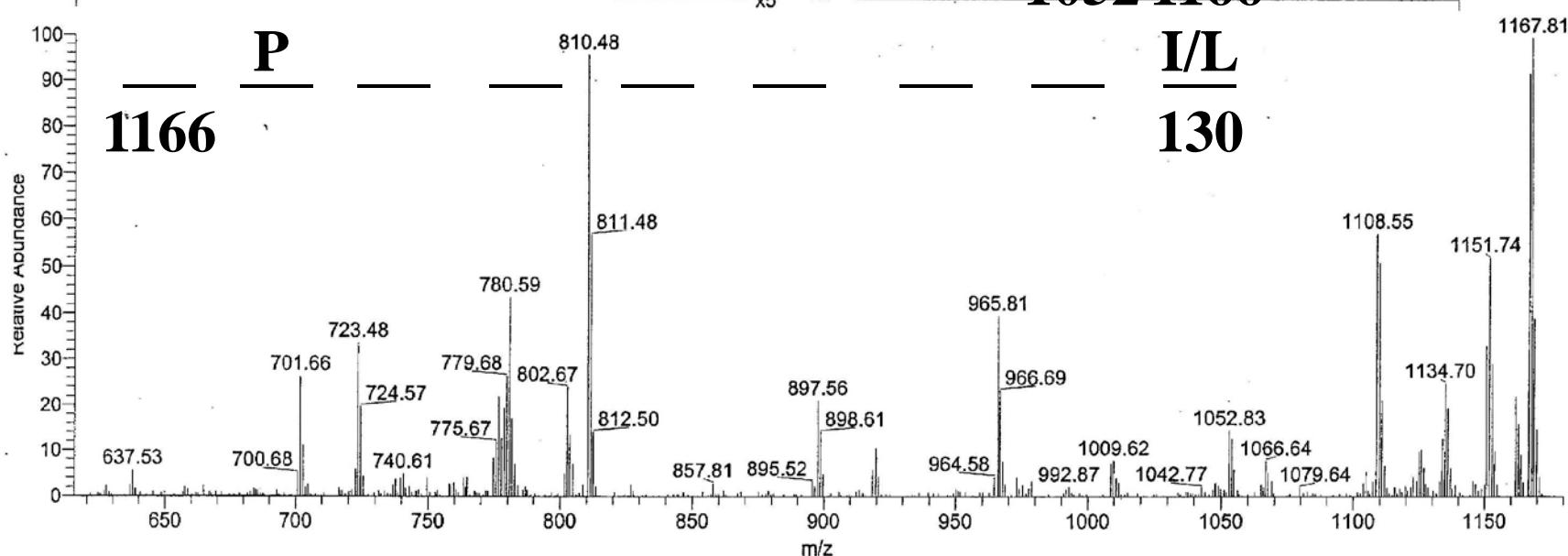
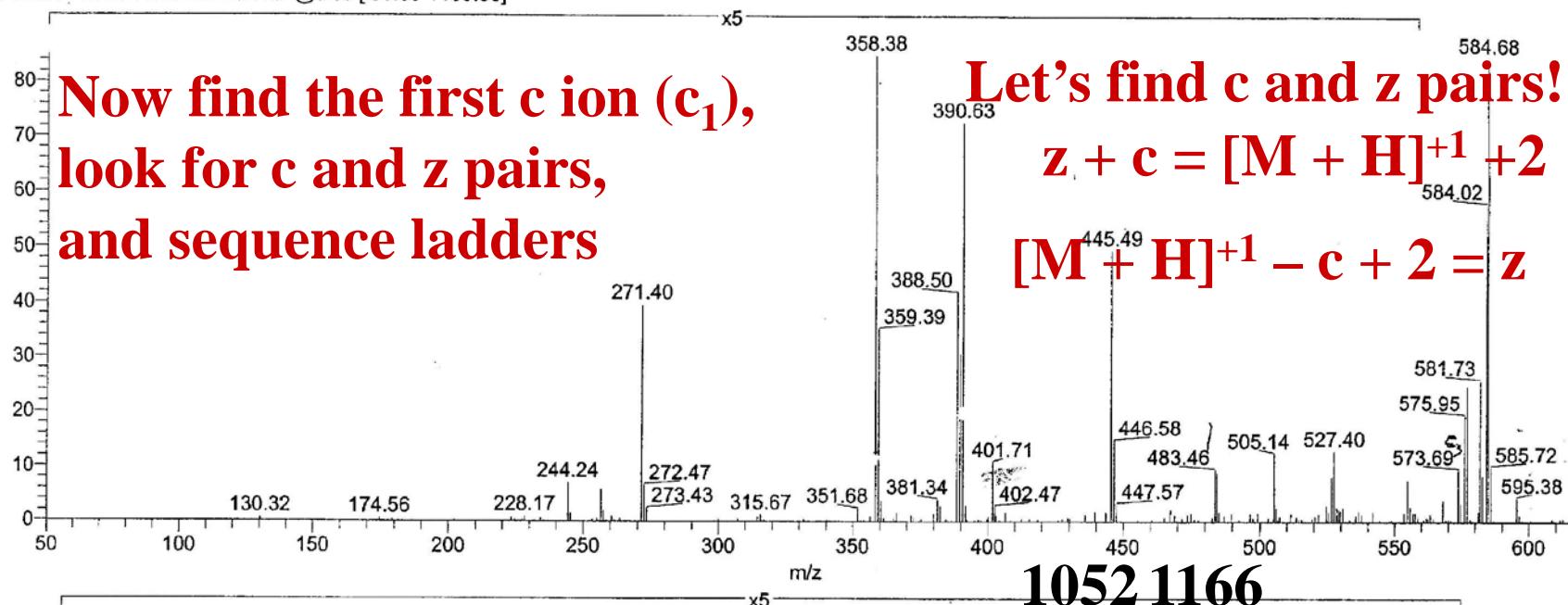


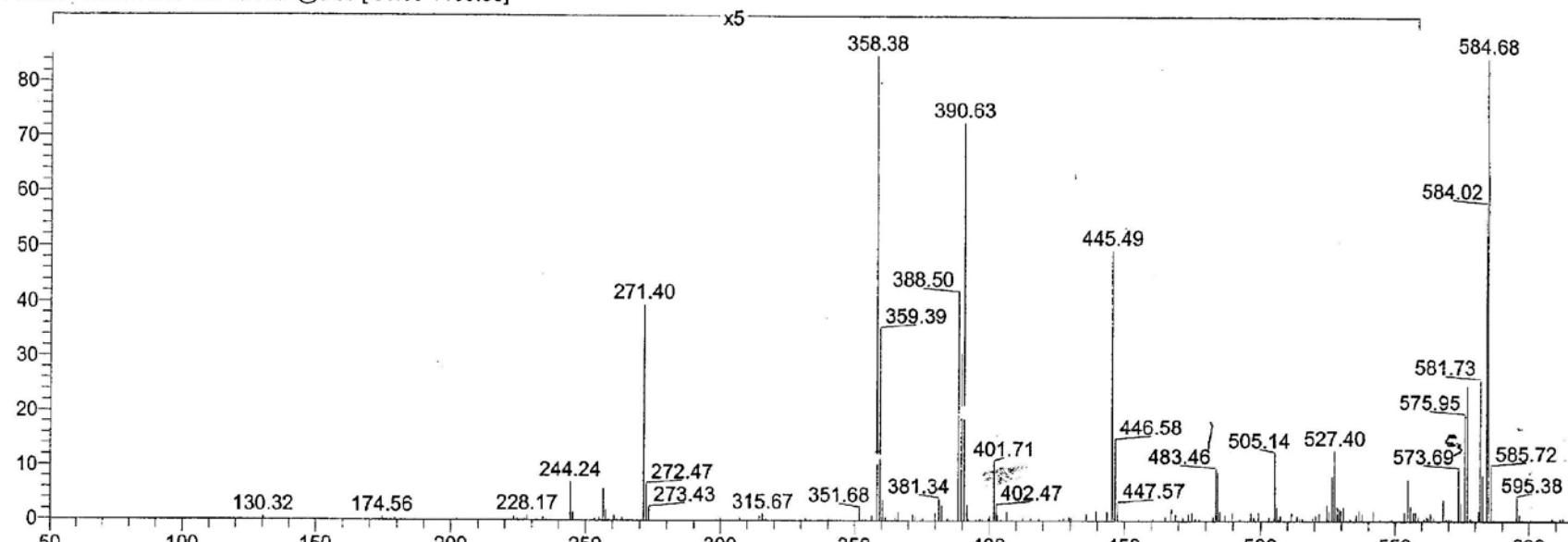
YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]



YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
: ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

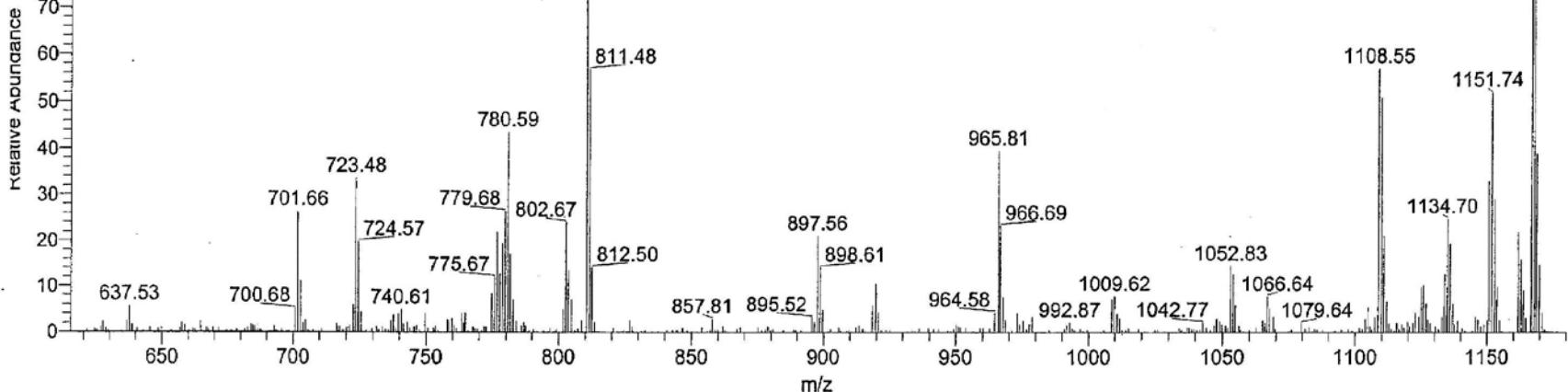
Now find the first c ion (c_1),
look for c and z pairs,
and sequence ladders



YB7_031104_040311132712 #1172 RT: 15.11 AV: 1 NL: 3.38E4
ITMS + c NSI d Full ms2 389.94@0.00 [50.00-1180.00]

174 271 358 445 573 701 802 965 1052 1166

R P S S Q/K Q/K S T I/L I/L
1166 994 897 810 723 595 467 366 203 116



2nd Example

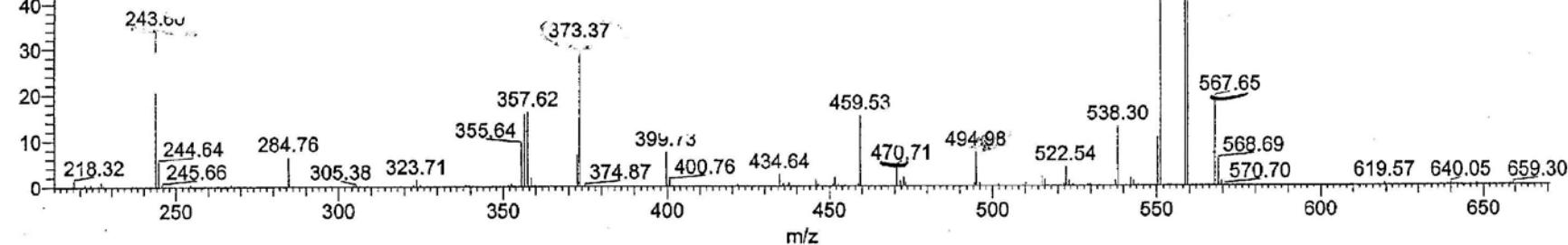
Methylesters

: JYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]

x5

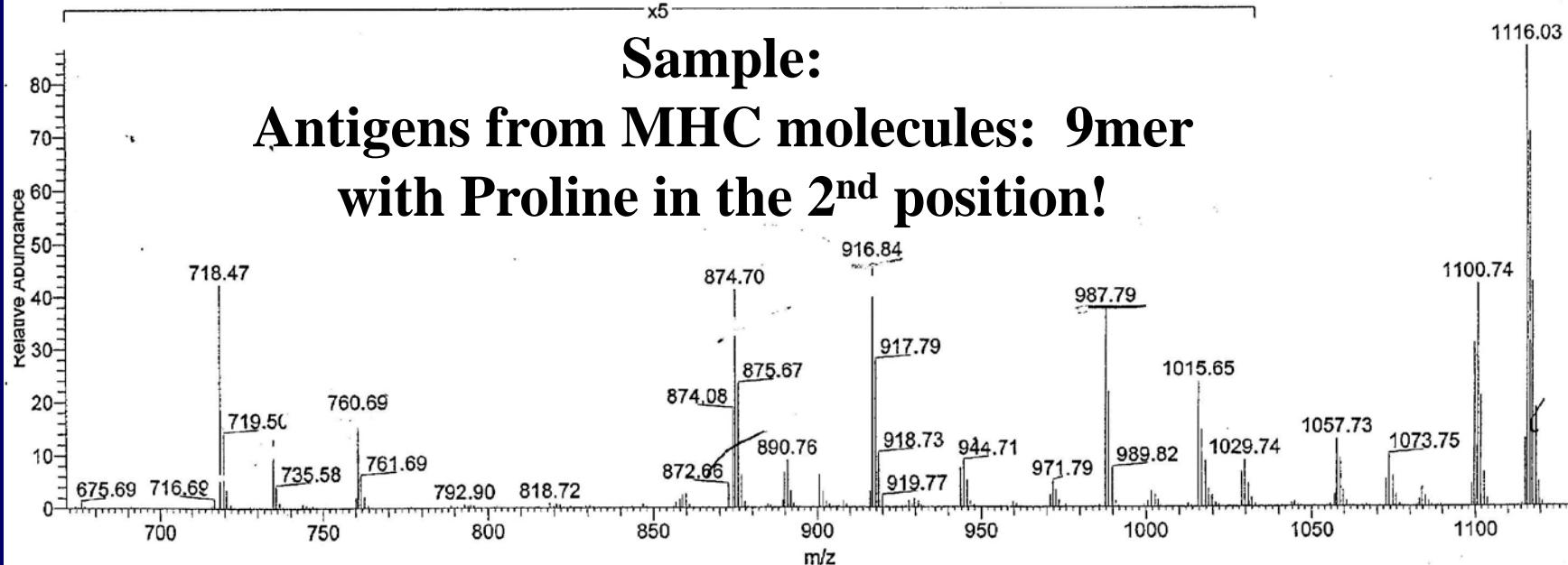
Important to know:

This sample was converted to Methylesters
(+14 on C-term and D/E side chains)
and analyzed after IMAC!



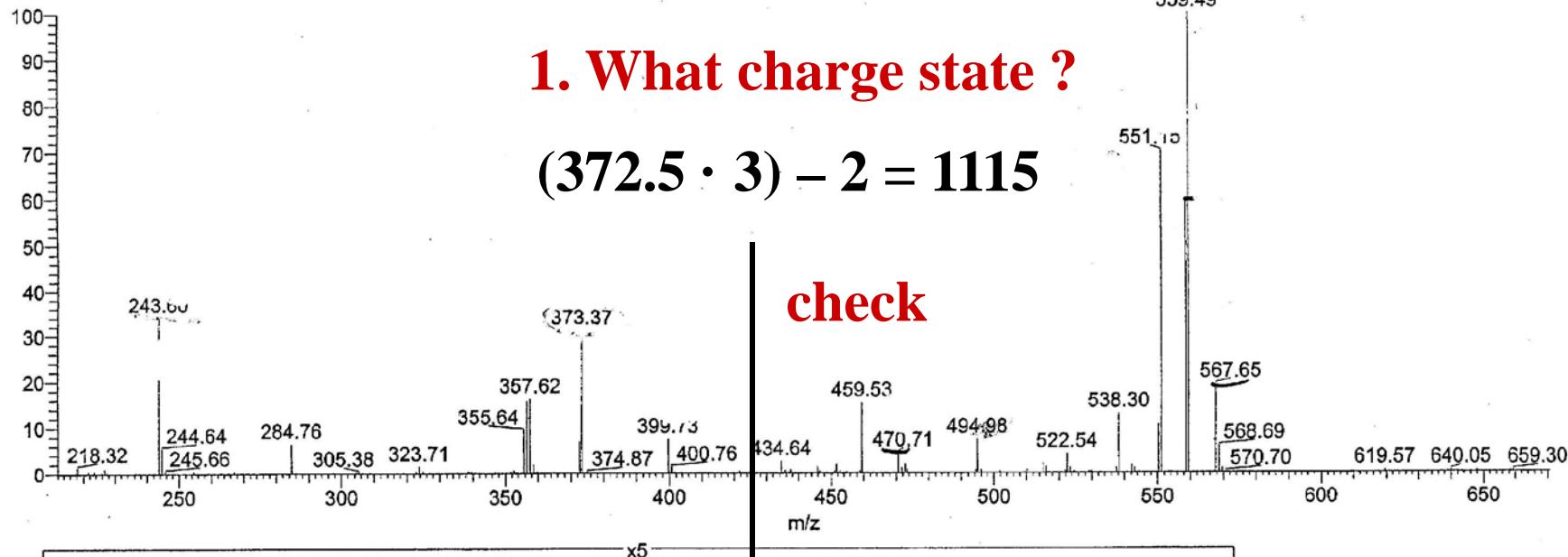
Sample:

Antigens from MHC molecules: 9mer
with Proline in the 2nd position!



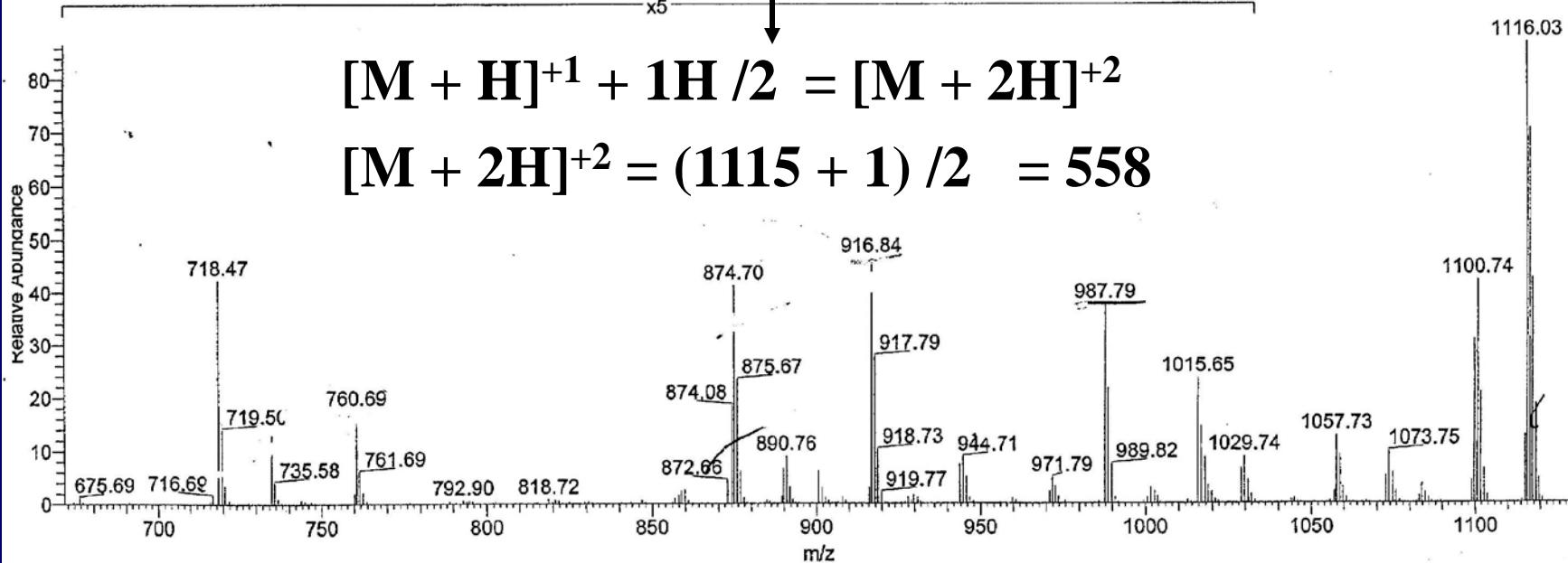
*metaphilates*mjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]

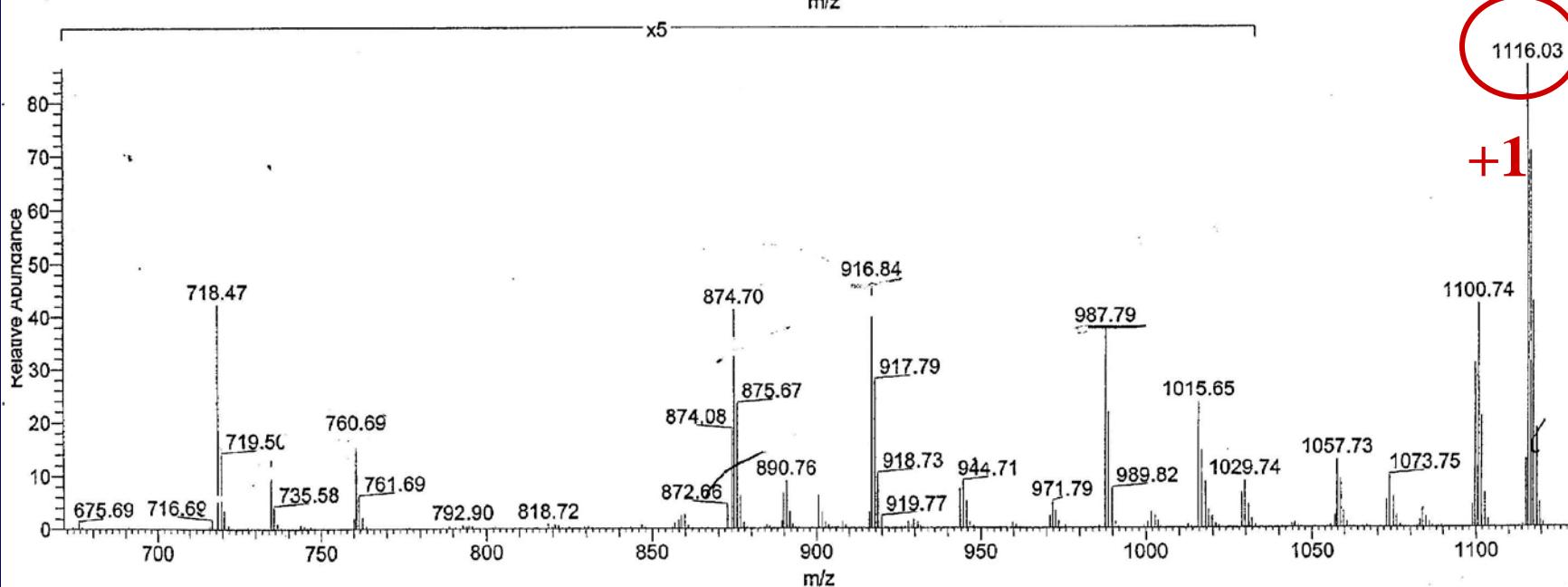
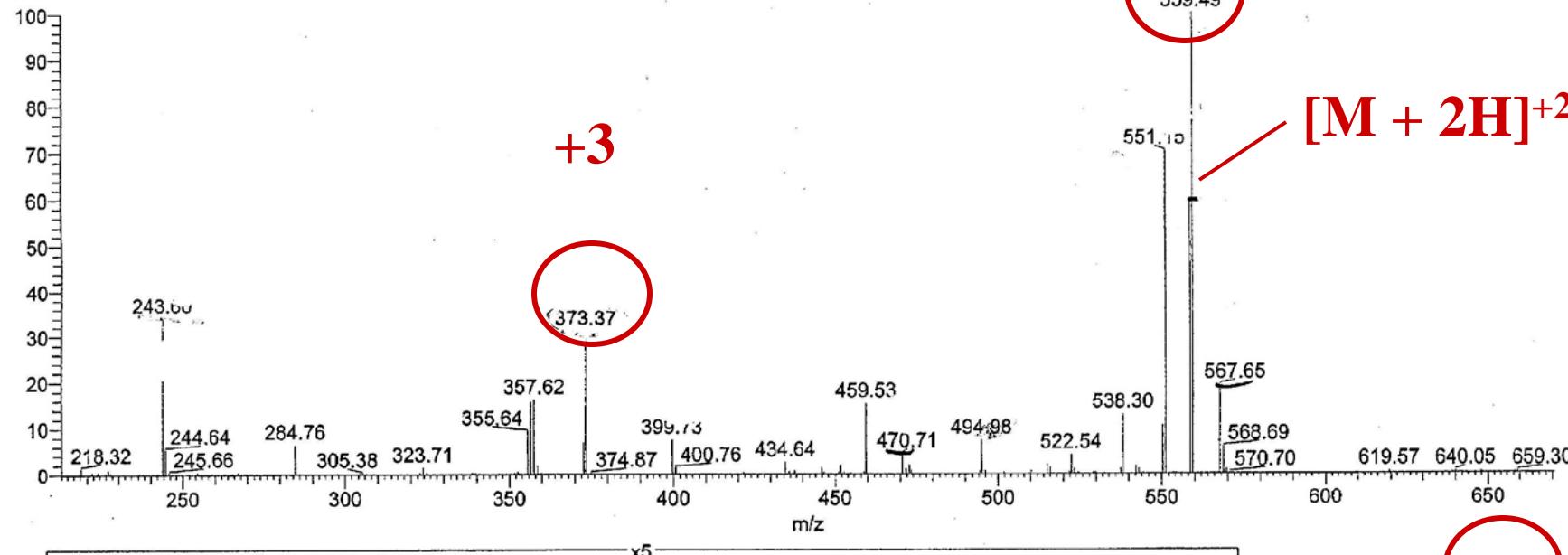
x5

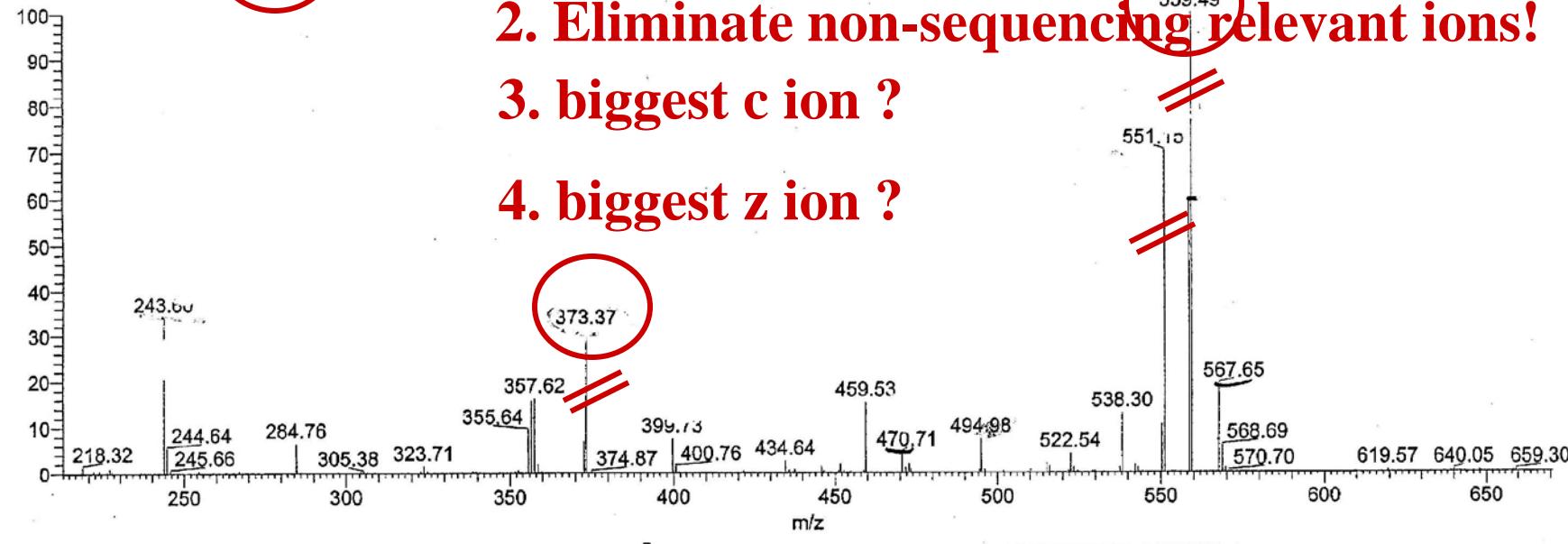


$$[M + H]^{+1} + 1H/2 = [M + 2H]^{+2}$$

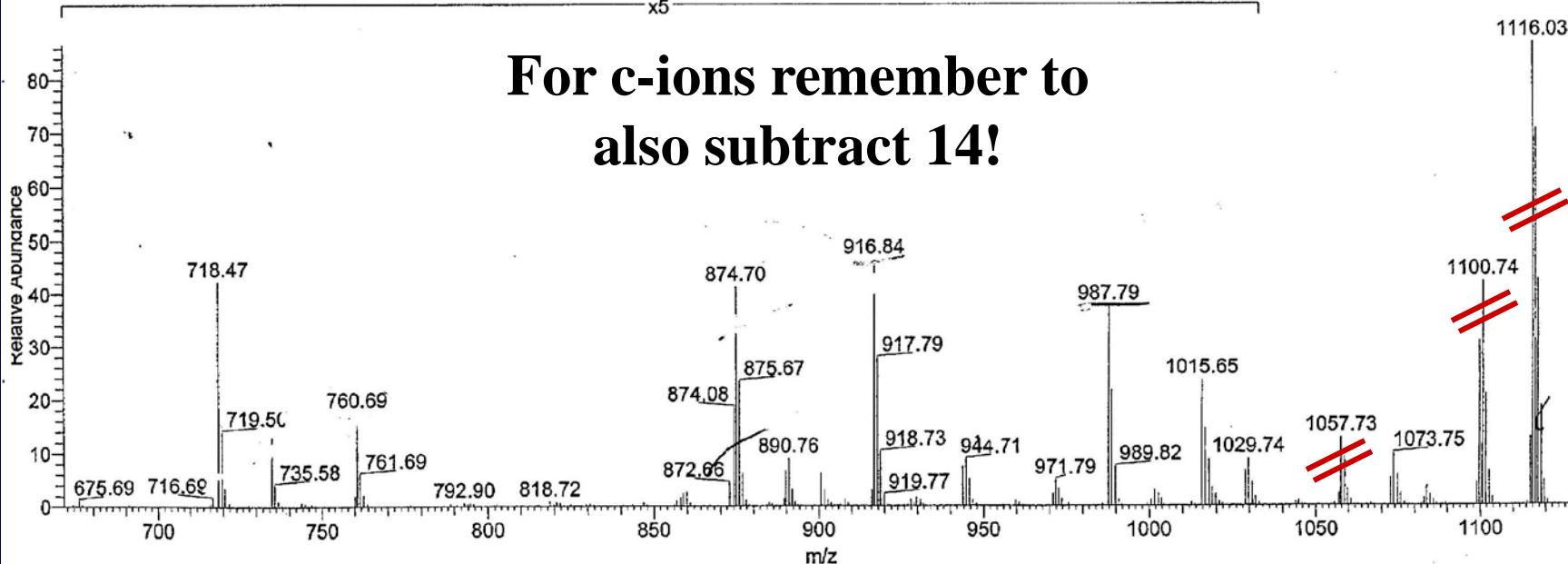
$$[M + 2H]^{+2} = (1115 + 1)/2 = 558$$

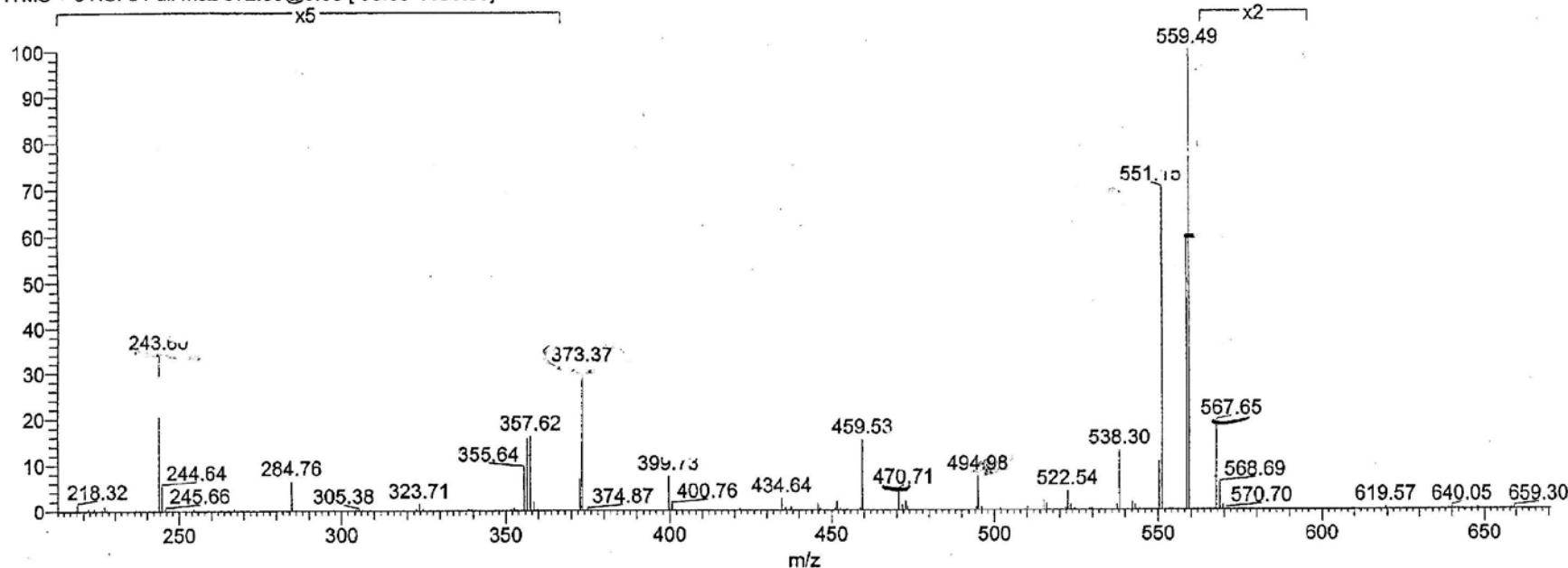


*Ruthenates*mjsJYB7\IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]

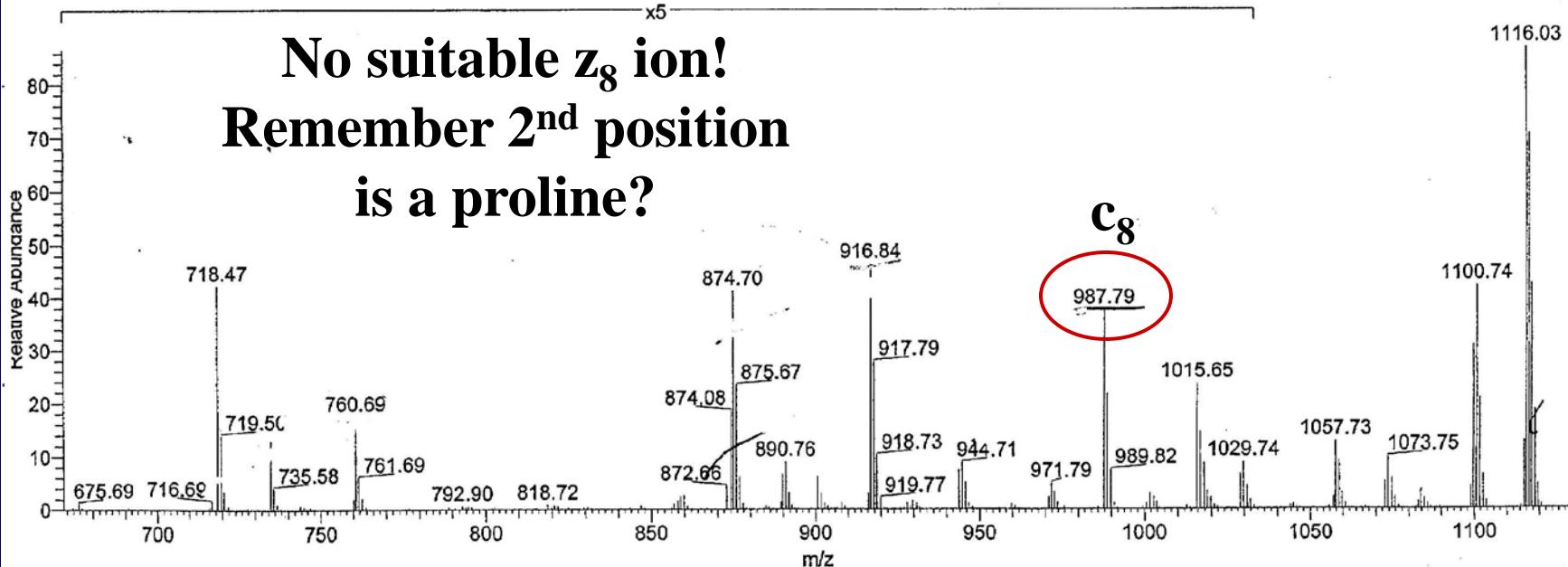
jjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]**2. Eliminate non-sequencing relevant ions!****3. biggest c ion ?****4. biggest z ion ?**

For c-ions remember to
also subtract 14!



jjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]

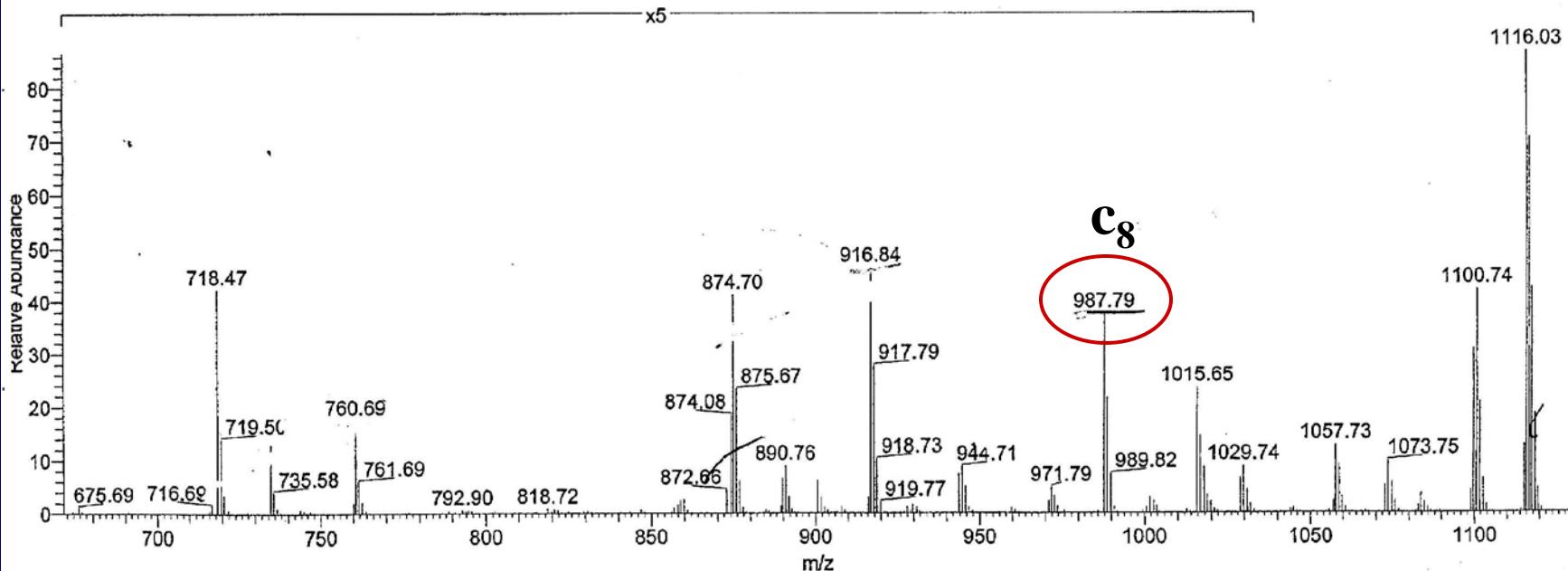
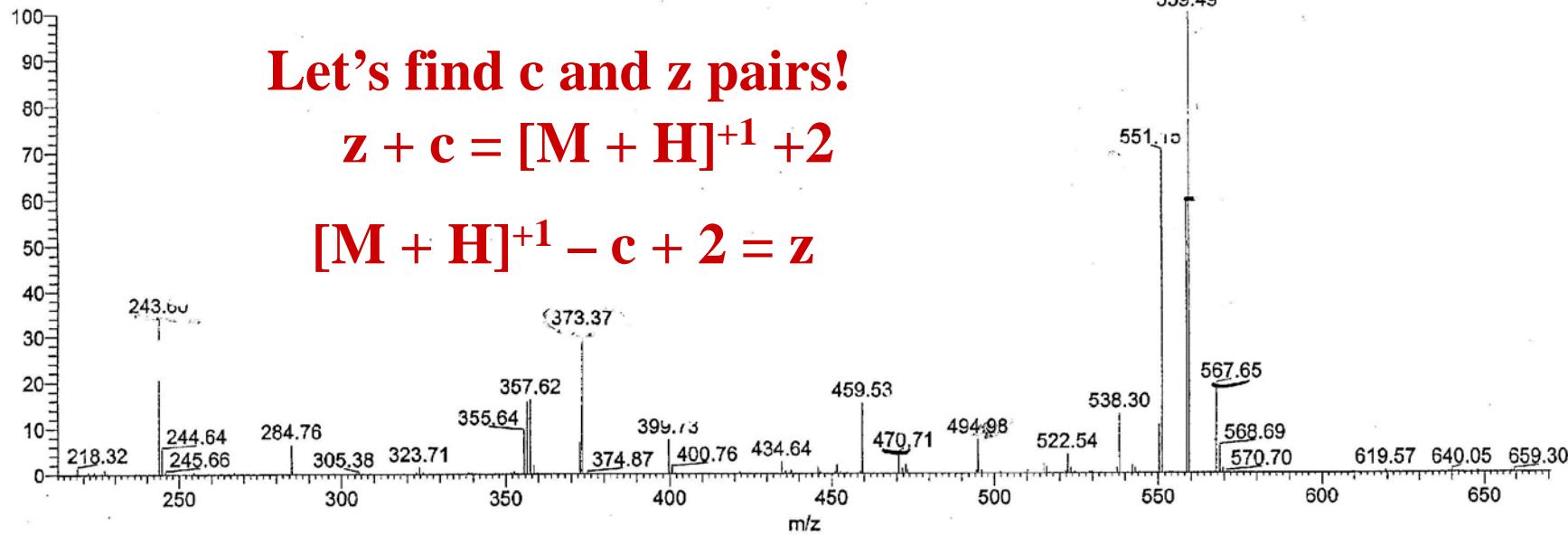
No suitable z_8 ion!
Remember 2nd position
is a proline?



jjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]**Let's find c and z pairs!**

$$z + c = [M + H]^{+1} + 2$$

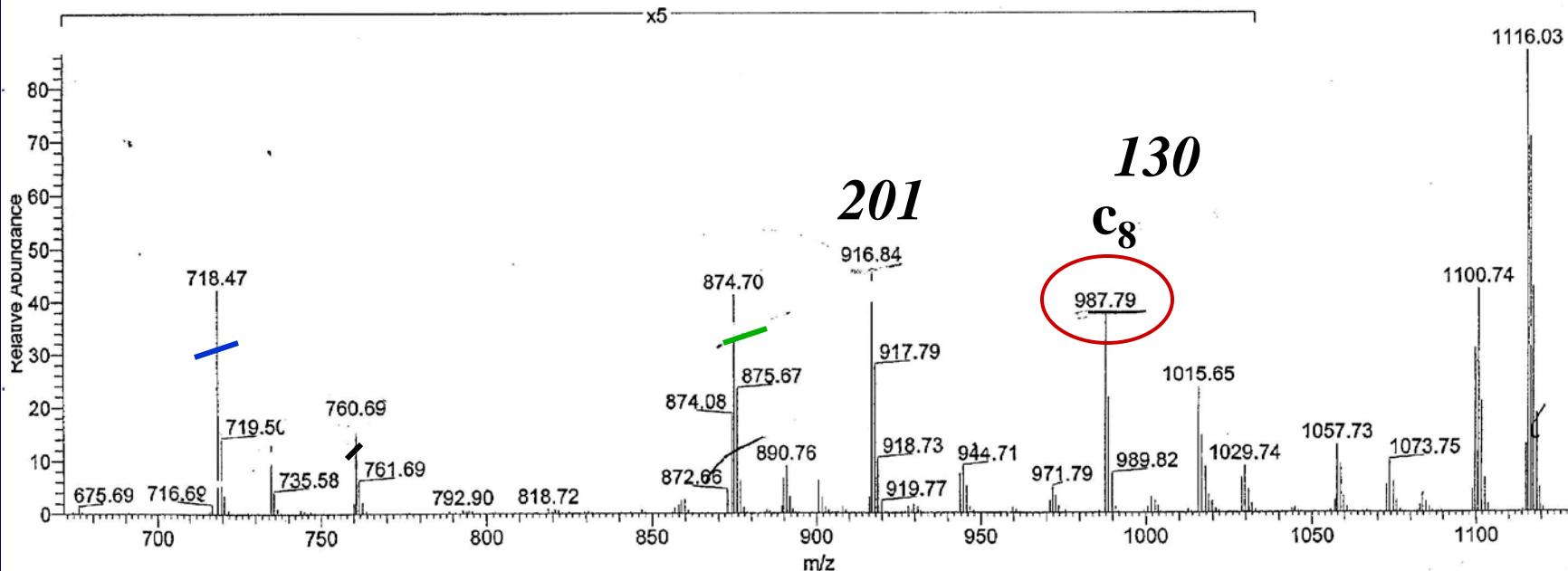
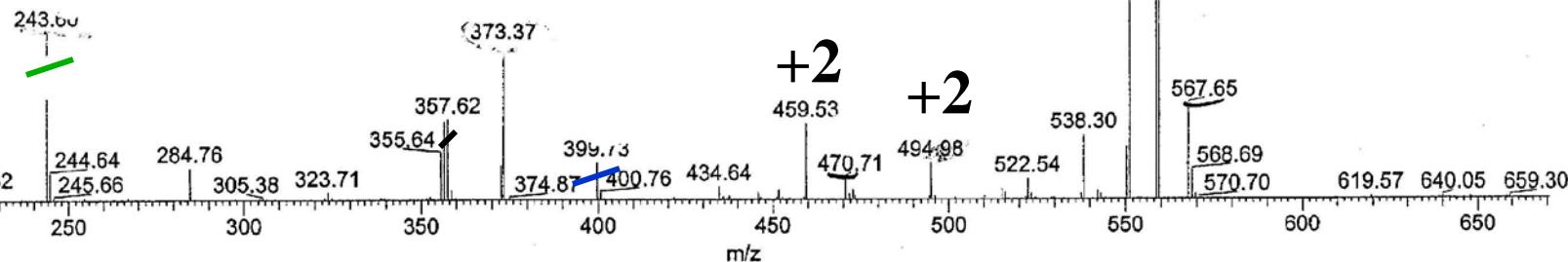
$$[M + H]^{+1} - c + 2 = z$$

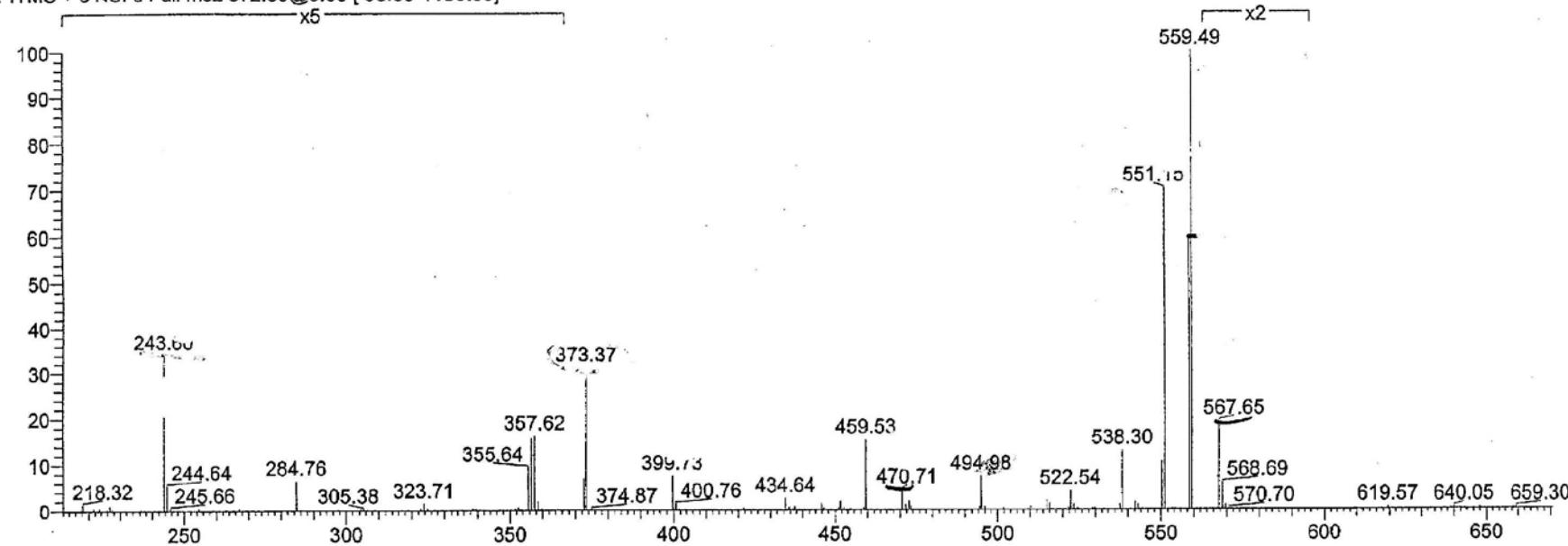


jjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]**Let's find c and z pairs!**

$$z + c = [M + H]^{+1} + 2$$

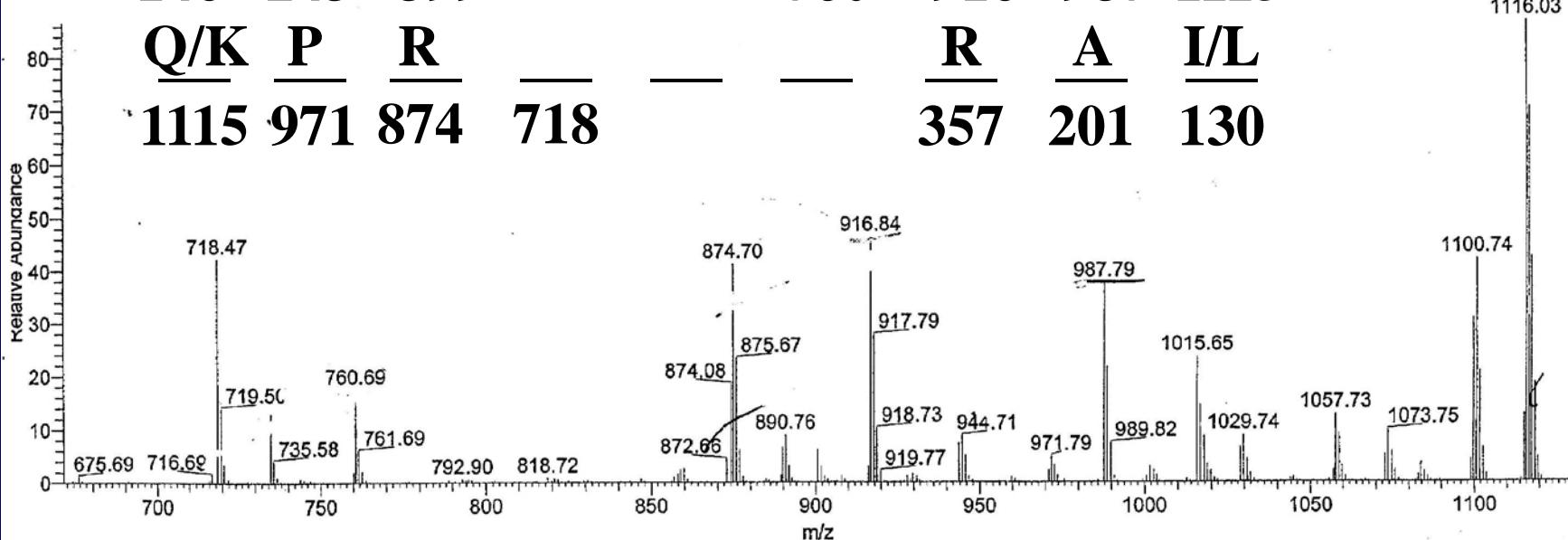
$$[M + H]^{+1} - c + 2 = z$$

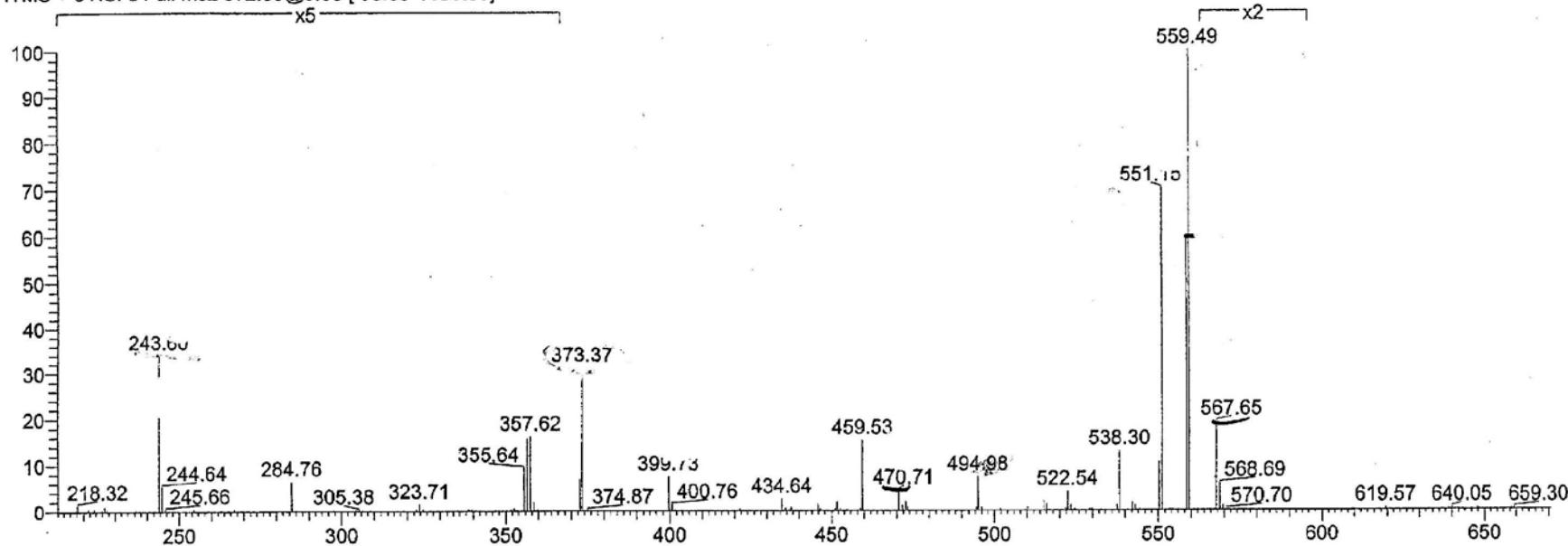


jjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]

146 243 399
Q/K P R
1115 971 874 **718**

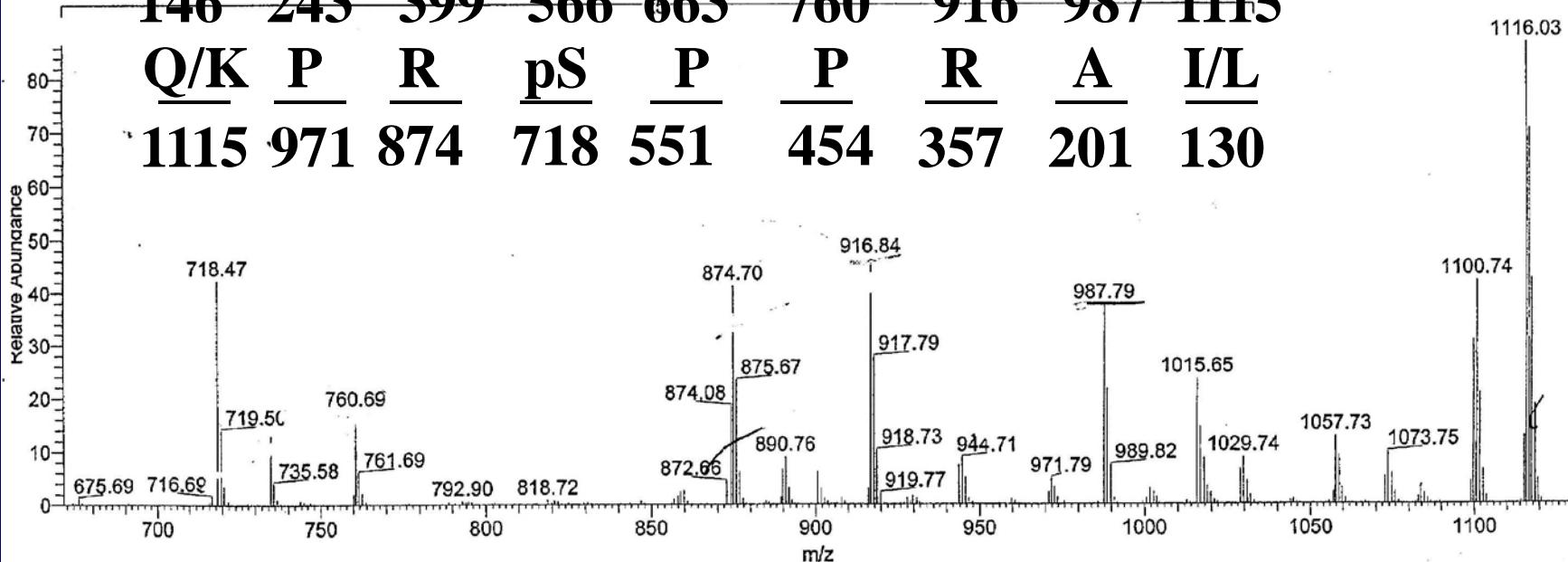
760 916 987 1115
— **R** **A** I/L
357 201 130



jjsJYB7IMAC_030304 #2144 RT: 26.21 AV: 1 NL: 2.12E6
: ITMS + c NSI d Full ms2 372.85@0.00 [90.00-1130.00]

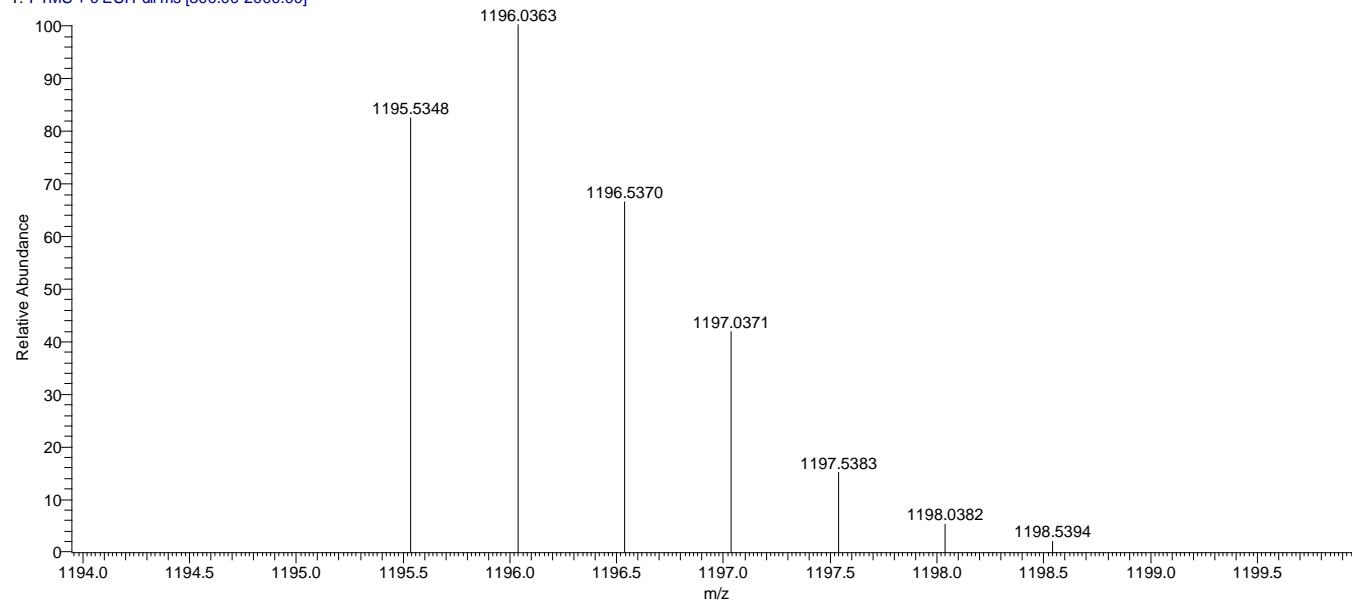
146 243 399 566 663 760 916 987 1115

Q/K P R pS P P R A I/L
1115 971 874 718 551 454 357 201 130

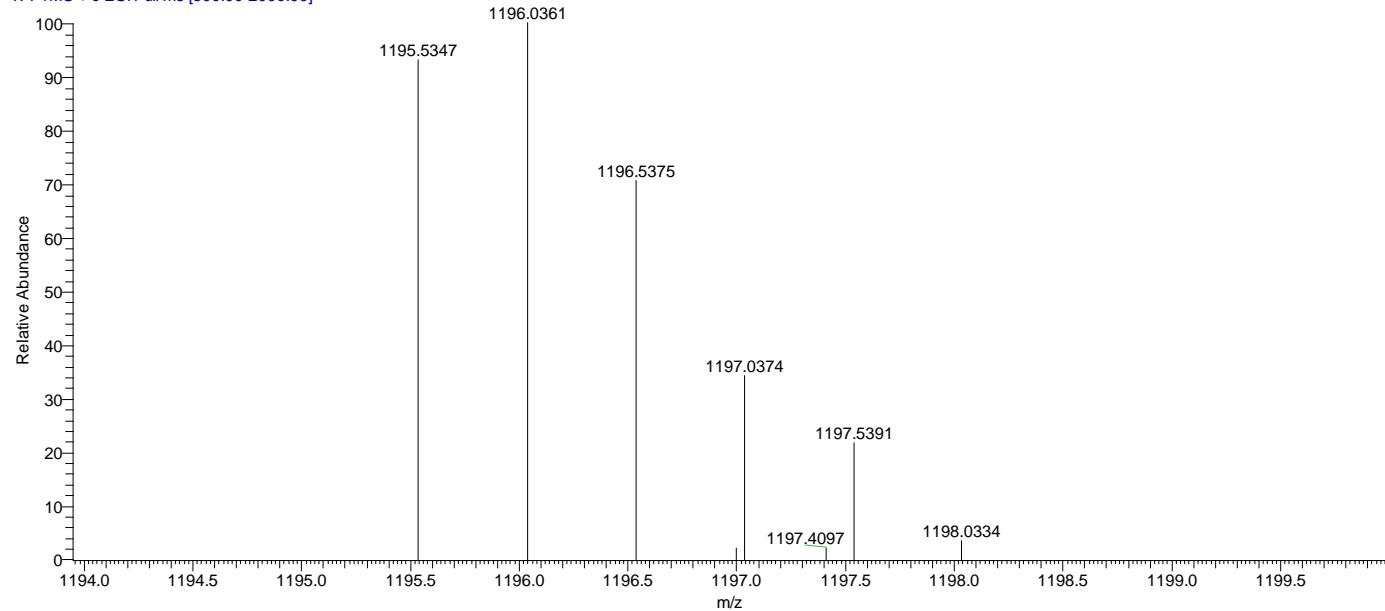


Second CAD Example

Stat3_cytosolic_a #7951 RT: 62.86 AV: 1 NL: 1.16E6
T: FTMS + c ESI Full ms [300.00-2000.00]



Stat3_cytosolic_a #7998 RT: 63.20 AV: 1 NL: 5.53E5
T: FTMS + c ESI Full ms [300.00-2000.00]



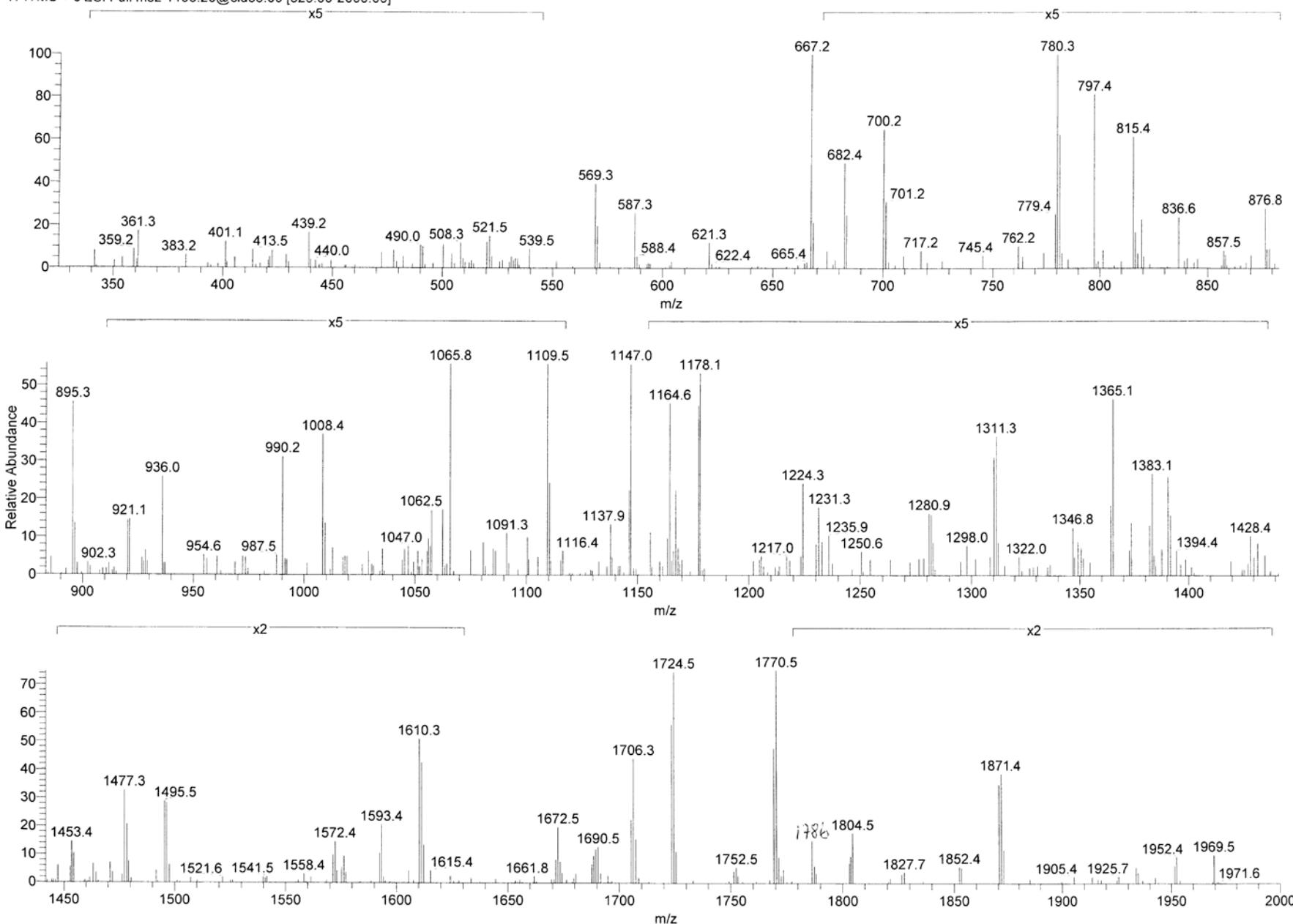
E:\for lecture\Stat3_cytosolic_a

loaded 3 ul (total ~12ul) of gel digested Stat3

Stat3_cytosolic_a #8004 RT: 63.24 AV: 1 NL: 1.15E3

T: ITMS + c ESI Full ms2 1196.20@cid35.00 [325.00-2000.00]

2/28/2011 12:57:52 PM



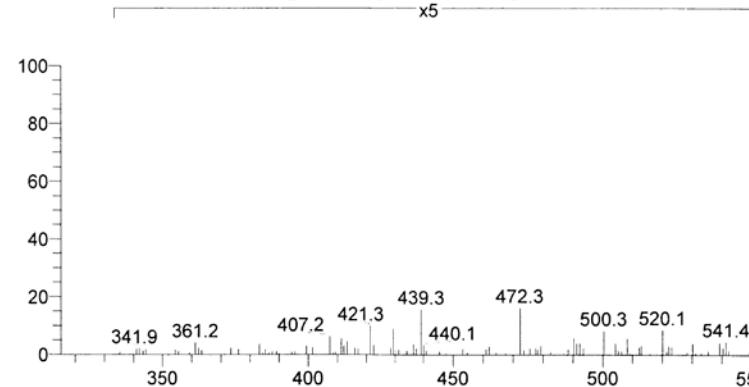
E:\for lecture\Stat3_cytosolic_a

loaded 3 ul (total ~12ul) of gel digested Stat3

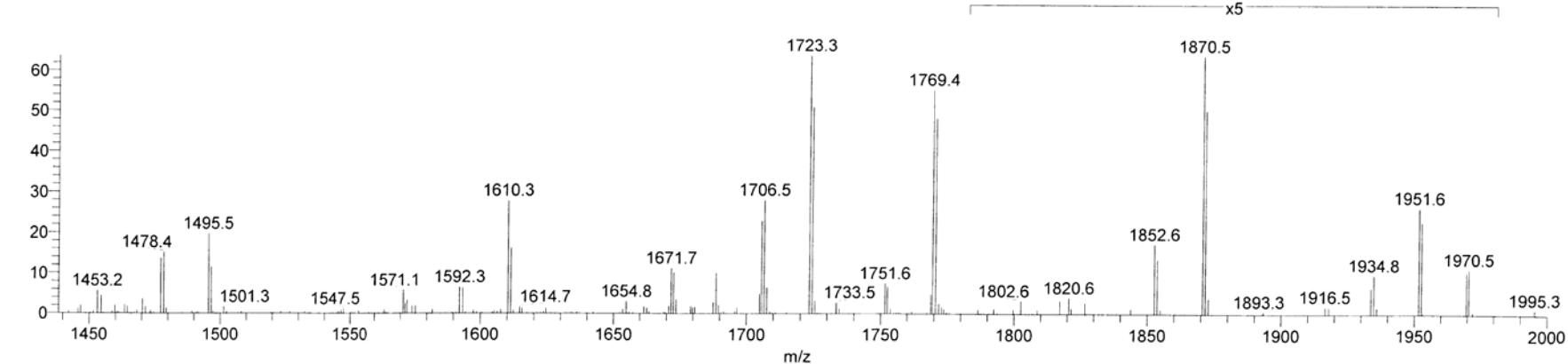
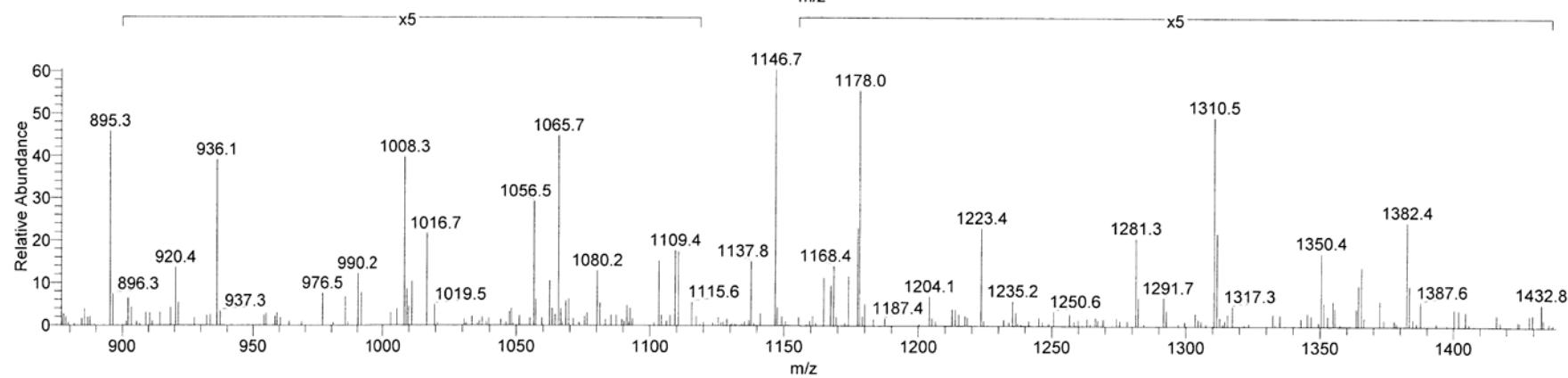
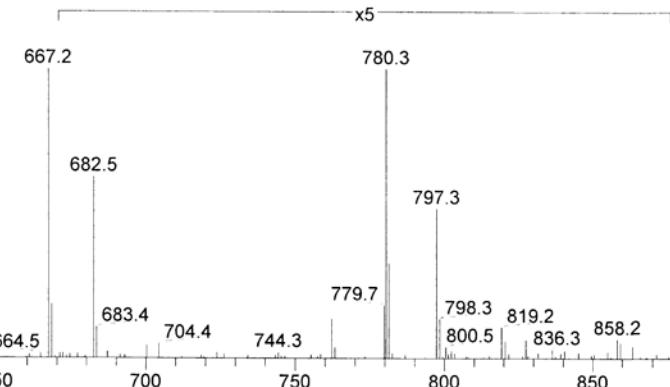
Stat3_cytosolic_a #7952 RT: 62.88 AV: 1 NL: 6.00E3

T: ITMS + c ESI d Full ms2 1196.04@cid35.00 [315.00-2000.00]

2/28/2011 12:57:52 PM



(2)



FIC(+57.0214)VTPTTC(+57.0214)SNTIDLPMSPRUser AA Formula 1: **C2 H3 N1 O1**

MH⁺¹(av)	MH⁺¹(mono)	MH⁺²(av)	MH⁺²(mono)	MH⁺³(av)	MH⁺³(mono)
2311.6583	2310.0926	1156.3329	1155.5499	771.2244	770.7024

[−] Main Sequence Ions

b		y		y⁺²
---	1	F	20	---
---			---	---
261.1598	2	I	19	2163.0241 1082.0157
421.1903	3	C(+57.0214)	18	2049.9401 1025.4737
520.2588	4	V	17	1889.9095 945.4584
621.3064	5	T	16	1790.8411 895.9242
718.3592	6	P	15	1689.7934 845.4003
819.4069	7	T	14	1592.7406 796.8740
920.4546	8	T	13	1491.6930 746.3501
1080.4851	9	C(+57.0214)	12	1390.6453 695.8263
1167.5172	10	S	11	1230.6147 615.8110
1281.5601	11	N	10	1143.5827 572.2950
1382.6078	12	T	9	1029.5397 515.2735
1495.6918	13	I	8	928.4921 464.7497
1610.7188	14	D	7	815.4080 408.2076
1723.8028	15	L	6	700.3811 350.6942
1820.8556	16	P	5	587.2970 294.1521
1951.8961	17	M	4	490.2442 245.6258
2038.9281	18	S	3	359.2037 180.1055
2135.9809	19	P	2	272.1717 136.5895
---	20	R	1	175.1190 88.0631

FIC(+57.0214)VTPTTC(+57.0214)SNTIDLPMsPRUser AA Formula 1: **C2 H3 N1 O1**

MH⁺¹(av)	MH⁺¹(mono)	MH⁺²(av)	MH⁺²(mono)	MH⁺³(av)	MH⁺³(mono)
2391.6382	2390.0589	1196.3228	1195.5331	797.8843	797.3578

[−] Main Sequence Ions

b			y		y⁺²
---	1	F	20	---	---
261.1598	2	I	19	2242.9905	1121.9989
421.1903	3	C(+57.0214)	18	2129.9064	1065.4568
520.2588	4	V	17	1969.8758	985.4416
621.3064	5	T	16	1870.8074	935.9073
718.3592	6	P	15	1769.7597	885.3835
819.4069	7	T	14	1672.7070	836.8571
920.4546	8	T	13	1571.6593	786.3333
1080.4851	9	C(+57.0214)	12	1470.6116	735.8094
1167.5172	10	S	11	1310.5810	655.7942
1281.5601	11	N	10	1223.5490	612.2781
1382.6078	12	T	9	1109.5061	555.2567
1495.6918	13	I	8	1008.4584	504.7328
1610.7188	14	D	7	895.3743	448.1908
1723.8028	15	L	6	780.3474	390.6773
1820.8556	16	P	5	667.2633	334.1353
1951.8961	17	M	4	570.2106	285.6089
2118.8944	18	s	3	439.1701	220.0887
2215.9472	19	P	2	272.1717	136.5895
---	20	R	1	175.1190	88.0631

FIC(+57.0214)VtPTTC(+57.0214)SNTIDLPMSPRUser AA Formula 1: **C2 H3 N1 O1**

MH⁺¹(av)	MH⁺¹(mono)	MH⁺²(av)	MH⁺²(mono)	MH⁺³(av)	MH⁺³(mono)
2391.6382	2390.0589	1196.3228	1195.5331	797.8843	797.3578

[–] Main Sequence Ions

b		y		y⁺²
---	1	F	20	---
261.1598	2	I	19	2242.9905 1121.9989
421.1903	3	C(+57.0214)	18	2129.9064 1065.4568
520.2588	4	V	17	1969.8758 985.4416
701.2728	5	t	16	1870.8074 935.9073
798.3255	6	P	15	1689.7934 845.4003
899.3732	7	T	14	1592.7406 796.8740
1000.4209	8	T	13	1491.6930 746.3501
1160.4515	9	C(+57.0214)	12	1390.6453 695.8263
1247.4835	10	S	11	1230.6147 615.8110
1361.5264	11	N	10	1143.5827 572.2950
1462.5741	12	T	9	1029.5397 515.2735
1575.6582	13	I	8	928.4921 464.7497
1690.6851	14	D	7	815.4080 408.2076
1803.7692	15	L	6	700.3811 350.6942
1900.8219	16	P	5	587.2970 294.1521
2031.8624	17	M	4	490.2442 245.6258
2118.8944	18	S	3	359.2037 180.1055
2215.9472	19	P	2	272.1717 136.5895
---	20	R	1	175.1190 88.0631