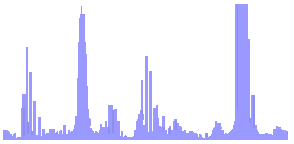
La Spettrometria di Massa nello Studio di Proteine e Peptidi



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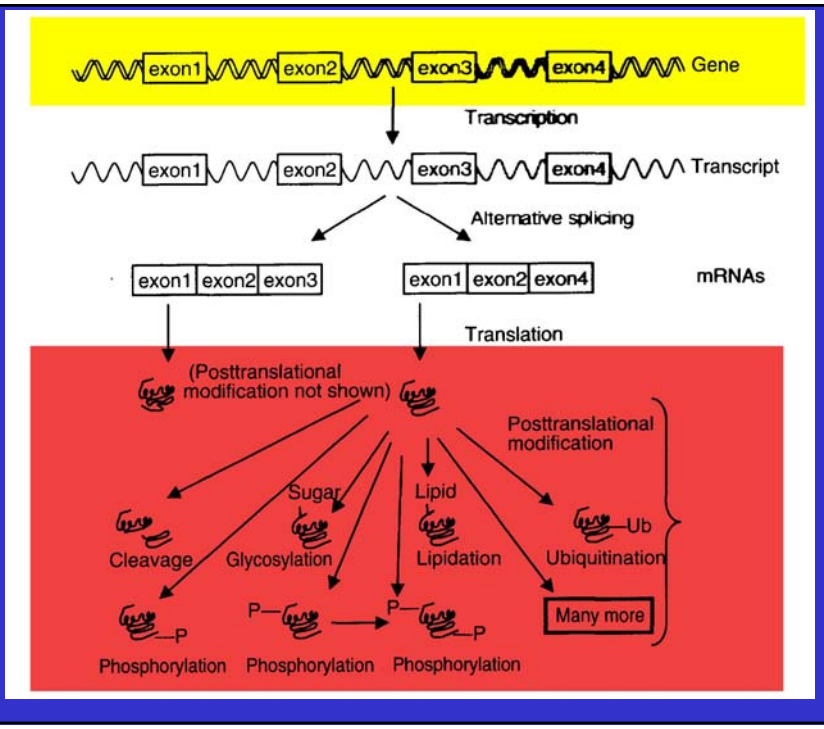


Challenges for mass spectrometry in proteome and genome analysis

Challenges	Requirements
mass spectrometry can handle only simple analyte mixtures, adduction complicates mass spectra	• sample preparation and purification using high-resolution separation methods • high-resolution mass analyzers
low amouts of sample available	• low detection limits • miniaturization
many samples to be screened	• fast scanning rate • multiplexing
identification of compounds	• high mass accuracy • MS/MS capability • Spectral libraries • database search

La Spettrometria di Massa nello studio di Proteine e Peptidi

1. Studio di Proteine

i) Estrazione

ii) Separazione

iii) Determinazione del PM

i) MALDI-TOF: spettro, risoluzione
ii) ESI: ioni multicarica, risoluzione
iii) Quale massa?
iv) Ricerca in banca dati

iv) Determinazione della struttura (Sequenza AA): MS^n

i) Proteina *in toto:* ECD, ETD

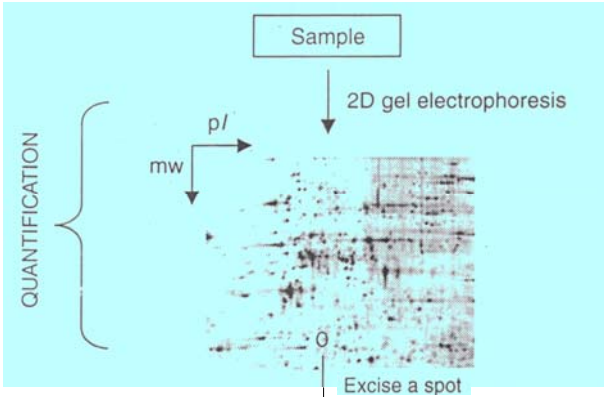
ii) Digestione enzimatica → **Peptidi**
   i) MALDI: peptide mass fingerprinting
   ii) HPLC-ESI-MS e MS^n

iii) Ricerca in banca dati

1. Studio di Proteine

i) **Estrazione**

ii) **Separazione**

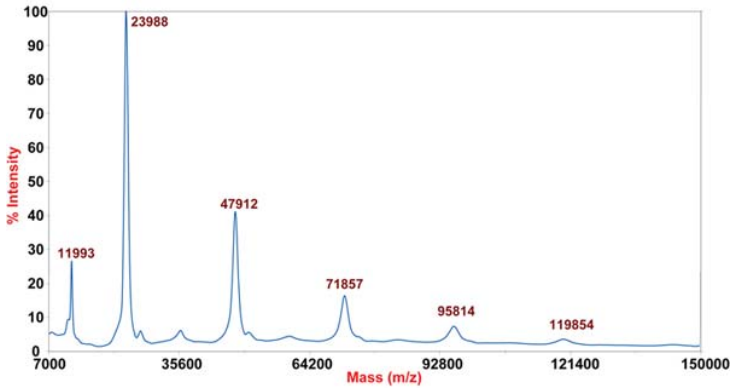


iii) Determinazione del peso molecolare

MALDI **ESI**

Proteine - Determinazione del PM

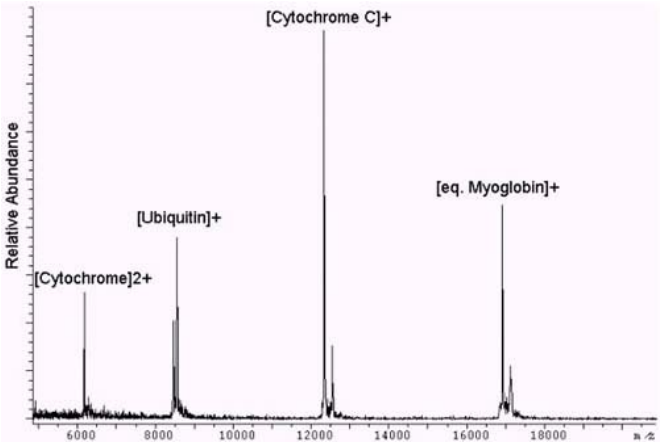
MALDI-TOF: spettro, risoluzione



MALDI-TOF linear mass spectrum of intact protein: Hg-Papain oligomerization

Proteine - Determinazione del PM

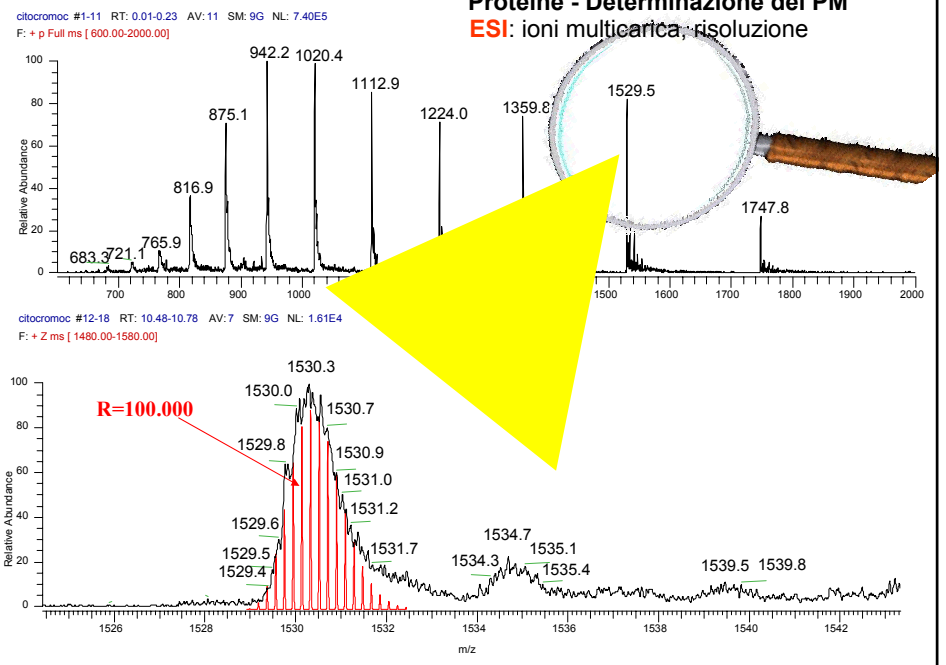
MALDI-TOF: spettro, risoluzione



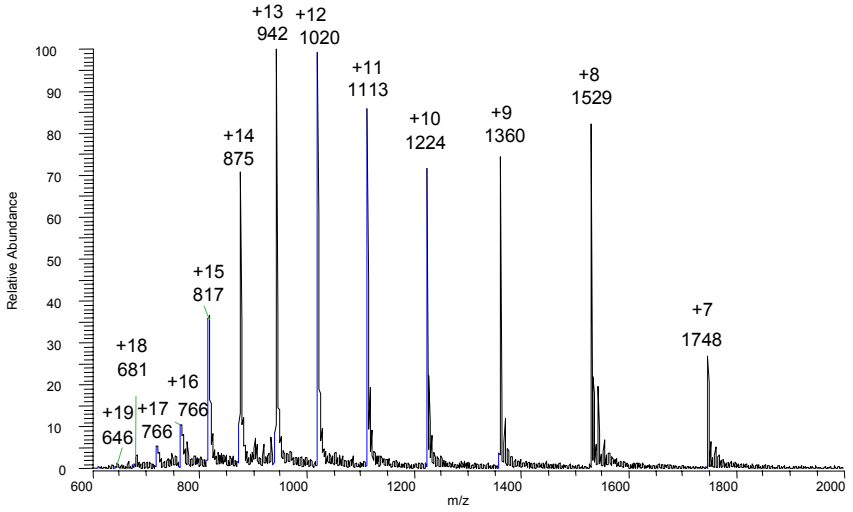
MALDI TOF mass spectrum of a mixture of ubiquitin, cytochrome C and equine myoglobin using 2,5-dihydroxybenzoic acid (DHB) as the matrix.

Proteine - Determinazione del PM

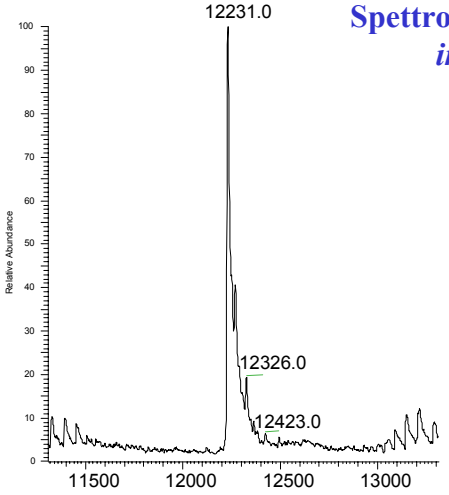
ESI: ioni multicarica, risoluzione



Assegnamento delle cariche



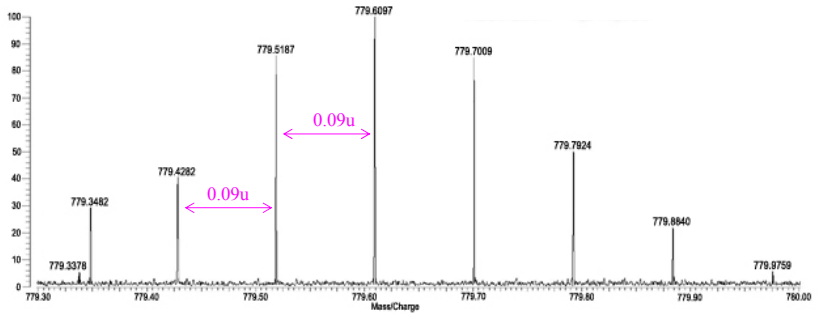
Spettro deconvoluto *in silico*



FT-ICR

ESI (+) of Ubiquitin

1,040,000 Resolution at *m/z* 779



Proteine & Peptidi- Determinazione del PM

Spettrometria di **massa** ……..

Quale massa?

L'accuratezza di massa diminuisce all'aumentare della grandezza dello ione

Poiché una proteina è una molecola grande, occorre

elevatissima risoluzione e notevole accuratezza di massa (**FT-ICR**)

per poter avere informazioni univoche sulla sua formula bruta

La Spettrometria di Massa nello studio di Proteine e Peptidi

1. Studio di Proteine

i) Estrazione

ii) Separazione

iii) Determinazione del PM

i) MALDI-TOF: spettro, risoluzione
ii) ESI: ioni multicarica, risoluzione
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iv) Determinazione della struttura (Sequenza AA): MS^n

i) Proteina *in toto*: ECD, ETD

ii) Digestione enzimatica → Peptidi

i) MALDI: peptide mass fingerprinting
ii) HPLC-ESI-MS e MS^n

iii) Ricerca in banca dati

Determinazione della struttura: MS^n

i) Proteina *in toto*:

CID non riesce a frammentare ioni prodotti da molecole grandi (PM > 3000 ca)

La decomposizione di ioni prodotti da molecole grandi può avvenire solo attraverso l'interazione con elettroni:

ECD = electron capture dissociation (FT-ICR)

ETD = electron transfer dissociation (2D e 3D IT)

Determinazione della struttura: MS^n

i) Proteina *in toto*:

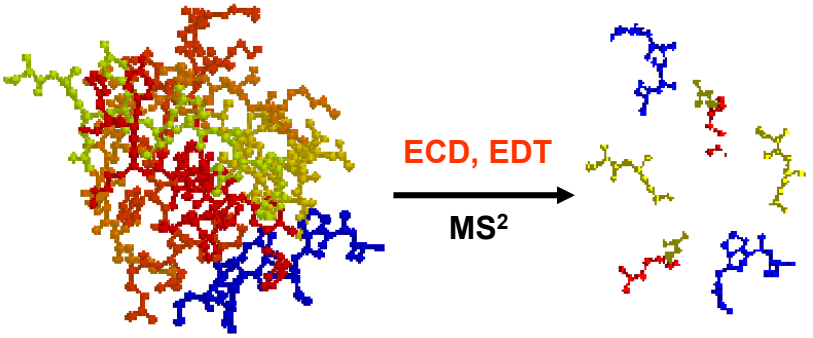
ECD = electron capture dissociation (FT-ICR)

ETD = electron transfer dissociation (Linear IT)

Approccio **Top Down**

Determinazione della struttura

Proteina *in toto*

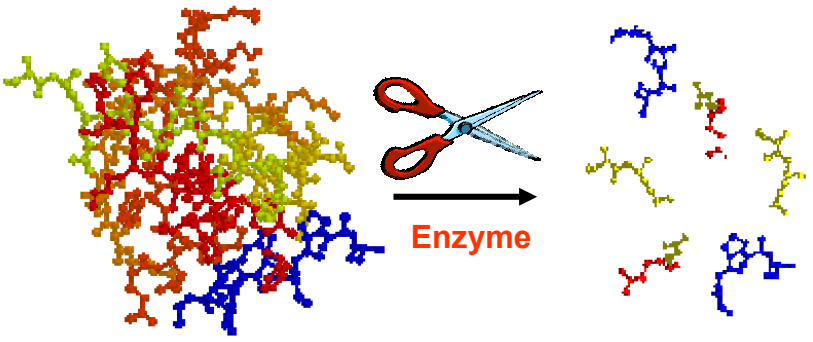


Proteina
$[M+nH]^{n+}$

Frammenti

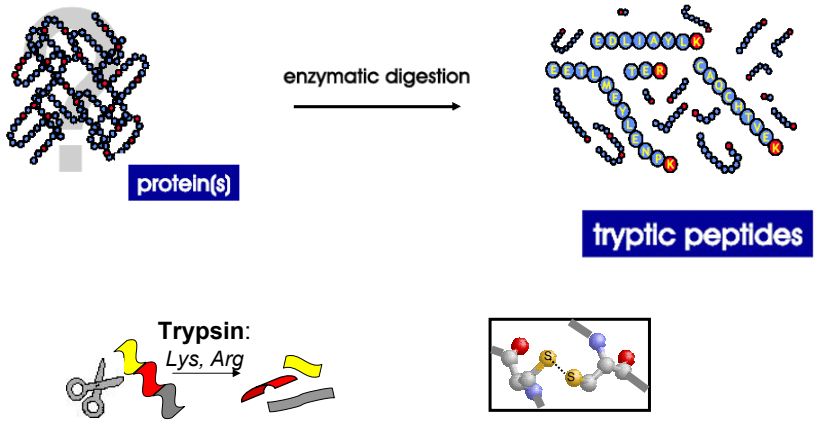
Determinazione della struttura

ii) Digestione enzimatica → Peptidi

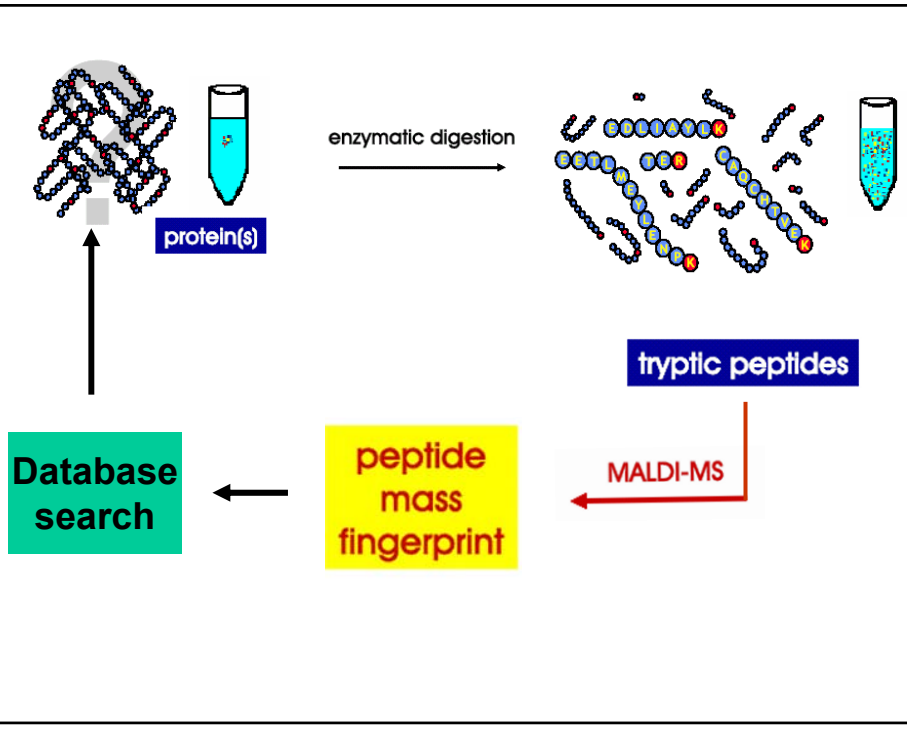


1 Protein
(in solution)

Many Peptides
(in solution)

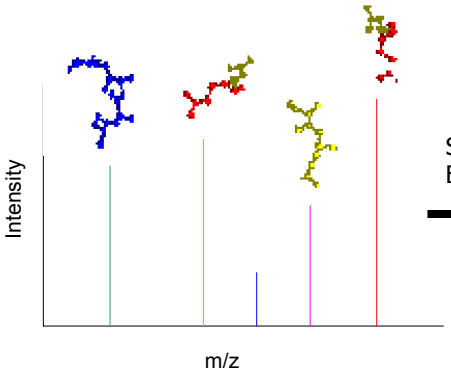


Approccio **Bottom Up**



Identification of a Protein via Peptide Mass Fingerprinting

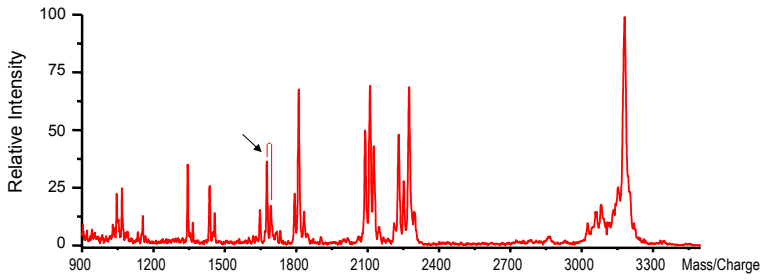
Direct analysis of the **entire peptide mixture**



Search Engine

MQRVNMIMAESPSLITICLLGYLLSAECT
VFLDHENANKILNRPKRYNSGKLEEFVQ
GNLERECMEEKCSFEEAREVFENTEK
TTEFWKQYVDGDQCESNPCLNGGSCK
DDINSYECWCPFGFEGKNCELDVTCNI
KNGRCEQFCKNSADNKVVCSCTEGYR
LAENQKSCEPAVPFPCGRVSVSQTSK
LTRAEAVFPDVDYVNPTEAETILDNITQ
GTQSFNDFTRVVGGEDAKPGQFPWQ
VVLNGKVDAFCGGSIVNEKWIVTAAHC
VETGVKITVVAGEHNIEETEHTEQKRN
VIRAIIPHHNYNAAINKYNHDIALLELDE
PLVLNSYVTPICIADKEYTNIFLKFGSG
YVSGWGRVFHKGRSALVLQYLRVPLV
DRATCLRSTKFTIYNNMFCAGFHEGG
RDSCQGDSGGPHVTEVEGTSFLTGII
SWGEECAMKGKYGIYTKVSRYVNWIK
EKTKLT

Protein identification by peptide mapping

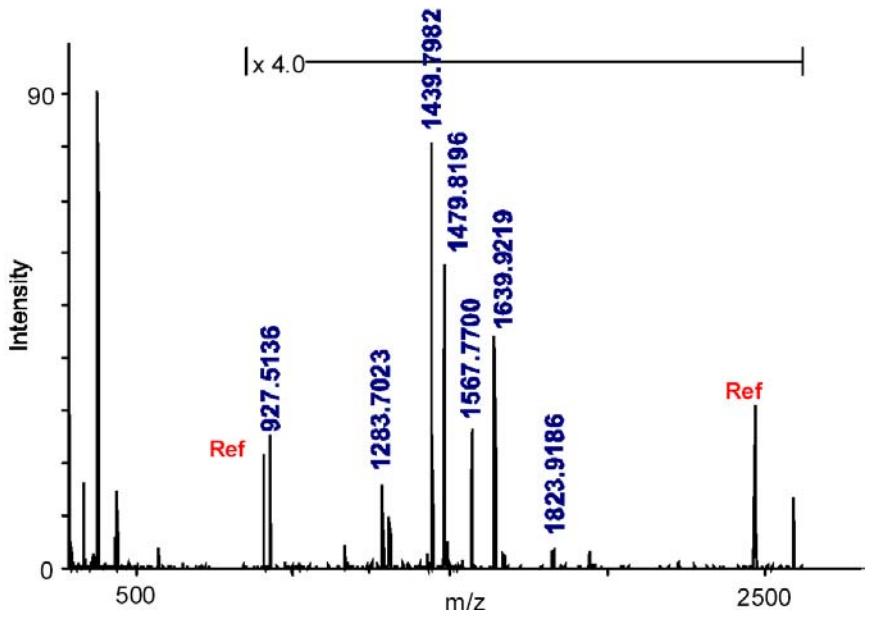


Database search (www.expasy.ch)

Aldolase A

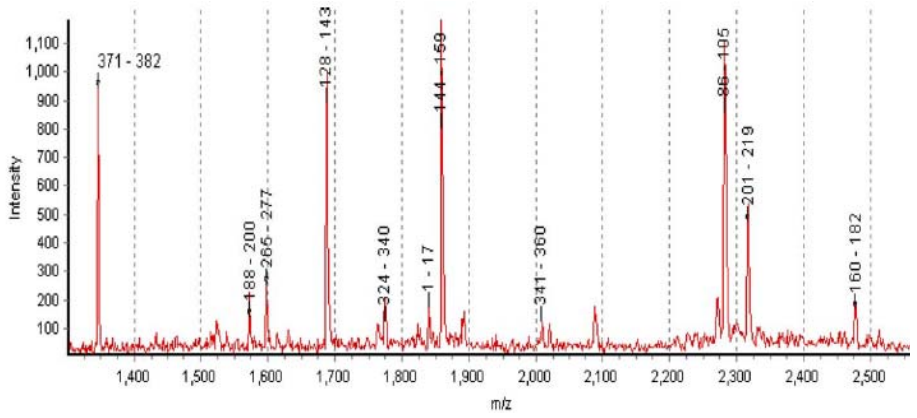
```
  1 PYQYPALTPEQKKELSDIAHRIVAPGKGILAADESTGSIAKRLQSIGTEN
 51 TEENRRFYRQLLLTADDRVNPCIGGVILFHETLYQKADDGRPFPQVIKSK
101 GGVVGIKVDKGVVPLAGTNGETTTQGLDGLSERCAQYKKDGADFAKWRCV
151 LKIGEHTPSALAIMENANVLARYASICQQNGIVPIVEPEILPDGDHDLKR
201 CQYVTEKVLAAVYKALSDHHIYLEGTLLKPNMVTPGHACTQKFSHEEIAM
251 ATVTALRRTVPPAVTGITFLSGGQSEEEASINLNAINKCPLLKPWALTFS
301 YGRALQASALKAWGGKKENLKAAQEEYVKRALANSLACQGKYTPSGQAGA
351 AASESLFVSNHAY
```

MS of a peptide mixture by MALDI-TOF



Peptide mass fingerprinting

MALDI-TOF



Identification of a spot based on peptide mapping

Selected protein

Rank: 1 Expectation: 0.000 Coverage: 48.2 pI: 5.2 Mass: 42.86 kDa Matched peptides: 11 Measured peptides: 18

Protein information: gi|808974|emb|CAA23716.1 - ovalbumin [Gallus gallus]

Matched peptides

Meas. mass	Ave/Isotopic	Comp. mass	Error (ppm)	Res. start	Res. end	M. cut	Peptide sequence
1344.706	Mono	1344.730	-18.1465	371	382	0	HIATNAVLFFGR
1570.734	Mono	1570.709	16.1735	188	200	1	AFKDEDTQAMPFR[III](1) +O@M
1596.663	Mono	1596.709	-28.5483	265	277	0	LTEWTSSNVMEER[III](1) +O@M
1686.774	Mono	1686.833	-34.9025	128	143	0	GGLEPINFQTAADQAR
1772.802	Mono	1772.892	-50.8825	324	340	0	ISQAVHAAHAEINEAGR
1838.630	Mono	1838.804	-94.6184	1	17	0	MGSIGAASMEFCFDVFK
1857.842	Mono	1857.958	-62.5625	144	159	0	ELINSWVESQTNGIIR
2007.771	Mono	2007.939	-83.3058	341	360	0	EVVGSAEAGVDAASVSEEFR

Protein identifications were made by peptide mass mapping (peptide mass fingerprinting) using an unspecified search program and an unspecified database.

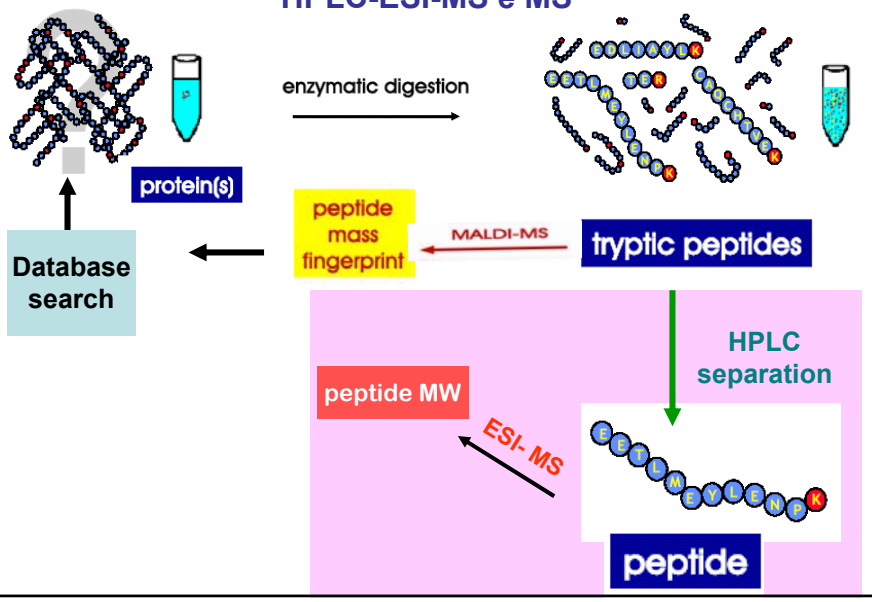
Se il TOF ha alta risoluzione, è possibile determinare la massa accurata e la formula bruta di ciascun peptide

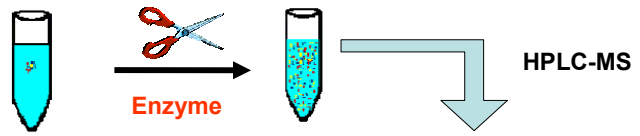
Se proteine diverse, digerite con lo stesso enzima nelle stesse condizioni sperimentali, producono lo stesso peptide mass fingerprint, le due proteine sono uguali

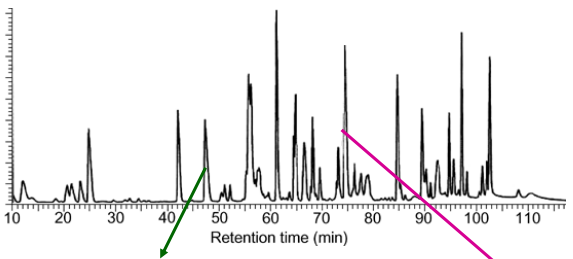
Proteine: Determinazione della struttura

Digestione enzimatica → **Peptidi**

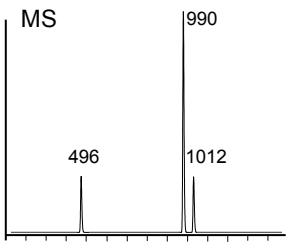
HPLC-ESI-MS e MS^n

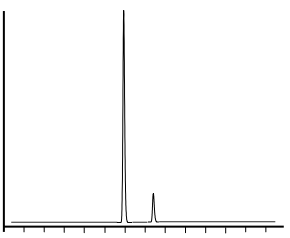


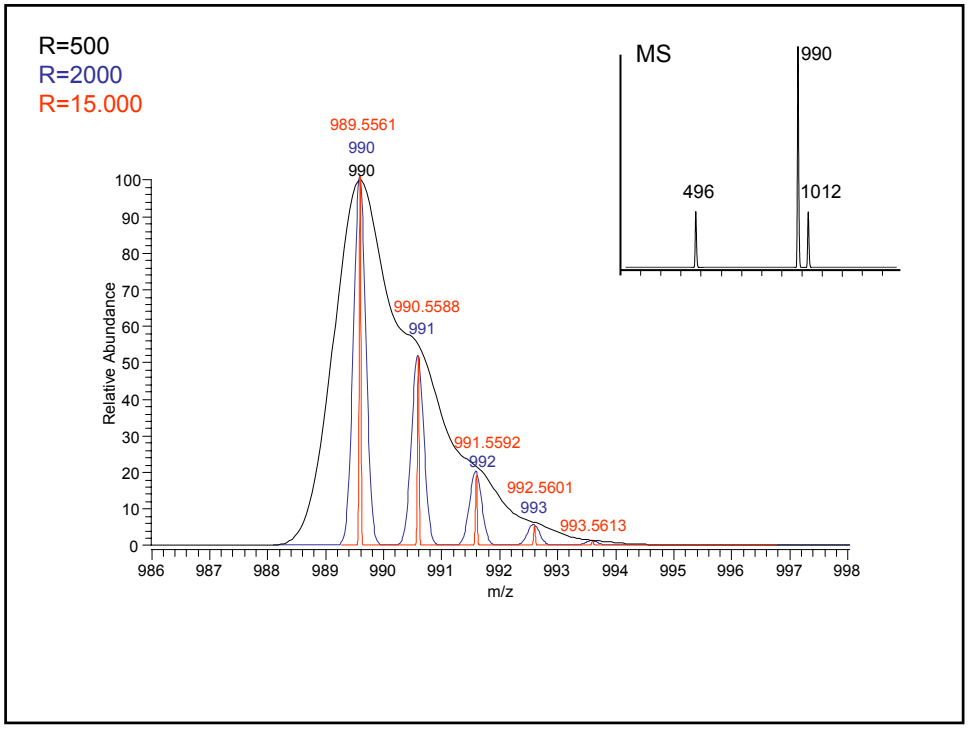


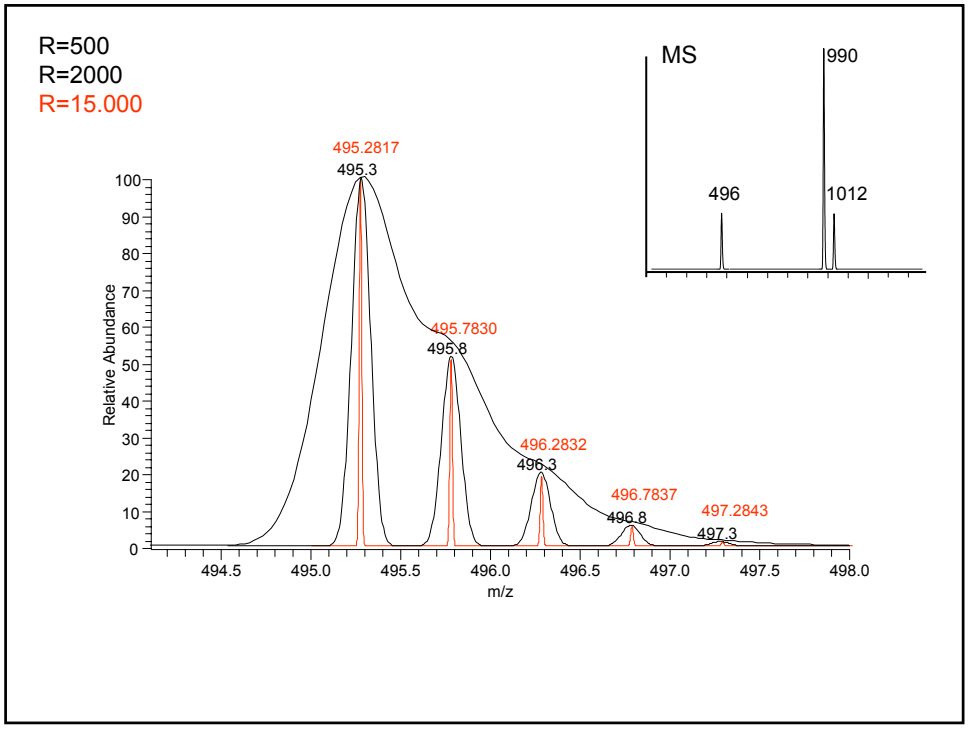


Tempo di ritenzione di ciascun peptide






R=500
R=2000
R=15.000
MS
990
496
1012
989.5561
990
990
990.5588
991
991.5592
992
992.5601
993
993.5613
Relative Abundance
m/z


R=500
R=2000
R=15.000
MS
990
496
1012
495.2817
495.3
495.7830
495.8
496.2832
496.3
496.7837
496.8
497.2843
497.3
Relative Abundance
m/z

Se si dispone di un analizzatore con alta risoluzione e massa accurata

settori, ToF, Orbitrap, FT-ICR

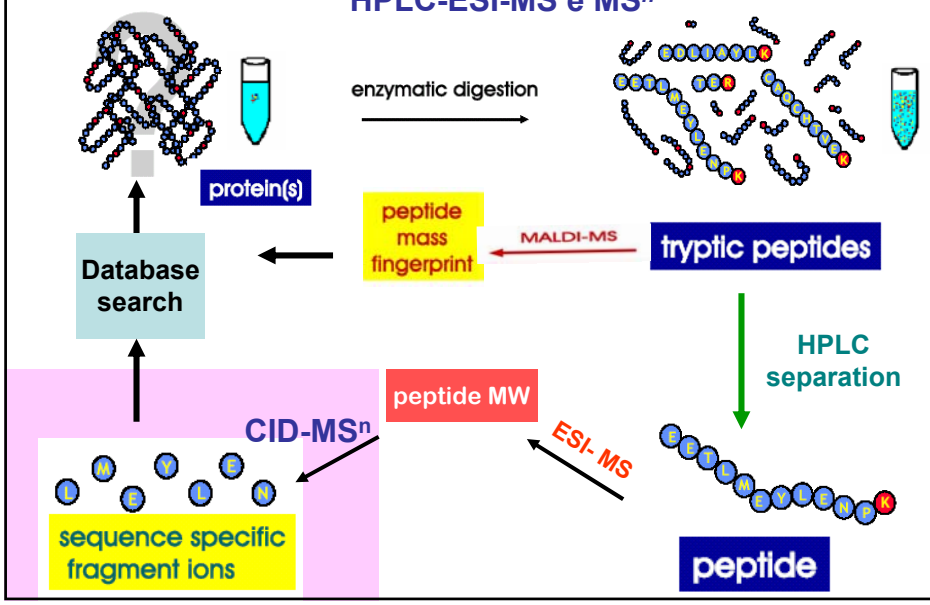
è possibile misurare la massa accurata di ciascun peptide e quindi la formula bruta

Poiché le tecniche di ionizzazione utilizzate sono *soft*
non si ha frammentazione in sorgente
e quindi non si hanno informazioni strutturali, ovvero non è possibile determinare la sequenza AA

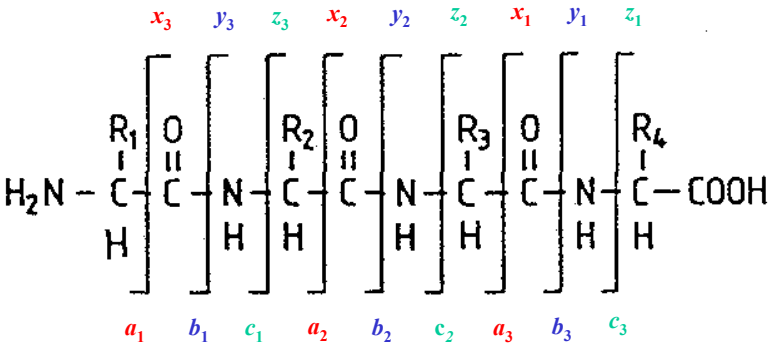
Proteine: Determinazione della struttura

Digestione enzimatica → **Peptidi**

HPLC-ESI-MS e MS^n



CID-MS^n di peptidi

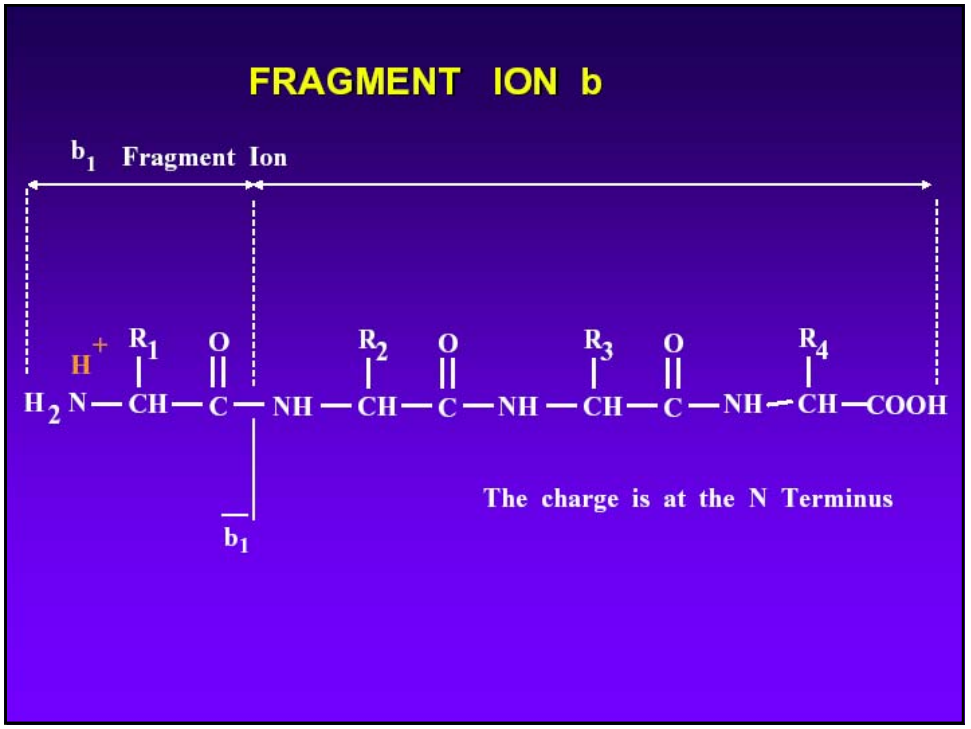


Ioni C-terminali:

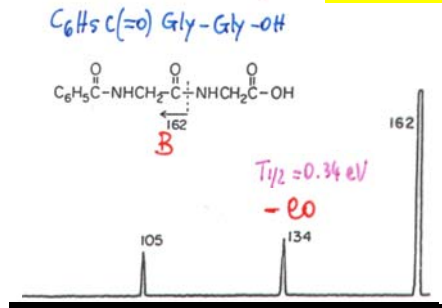
y, x, z

Ioni N-terminali:

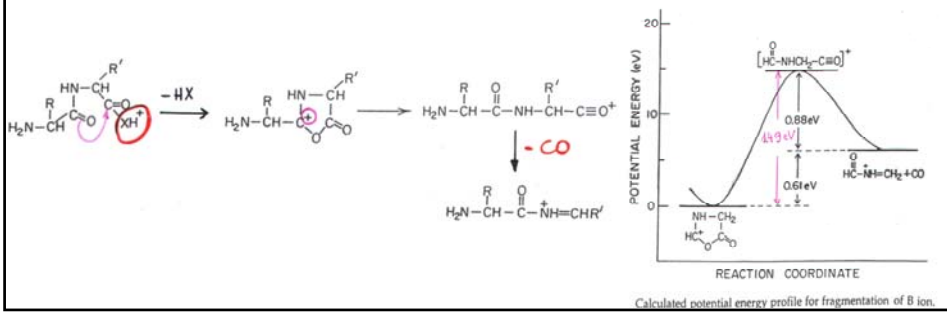
b, a, c



Why *b* ions are very stable species?

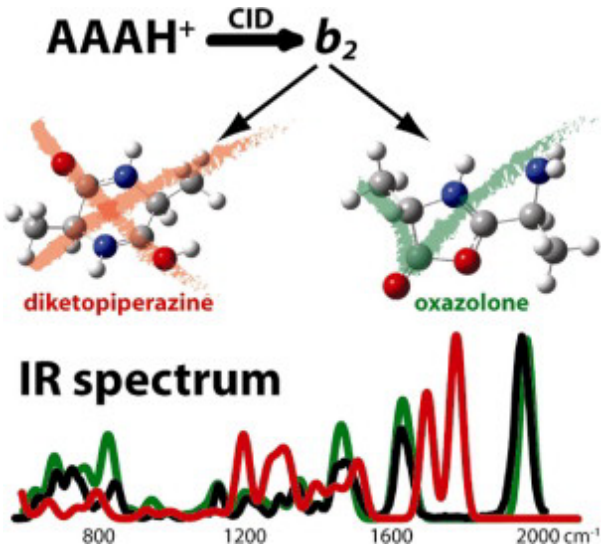


CID spectra (45 eV) of B ion derived from *N*-benzoyl- Gly-Gly-OH



Calculated potential energy profile for fragmentation of B ion.

Spectroscopic Evidence for an Oxazolone Structure of the b_2 Fragment Ion from Protonated Tri-Alanine

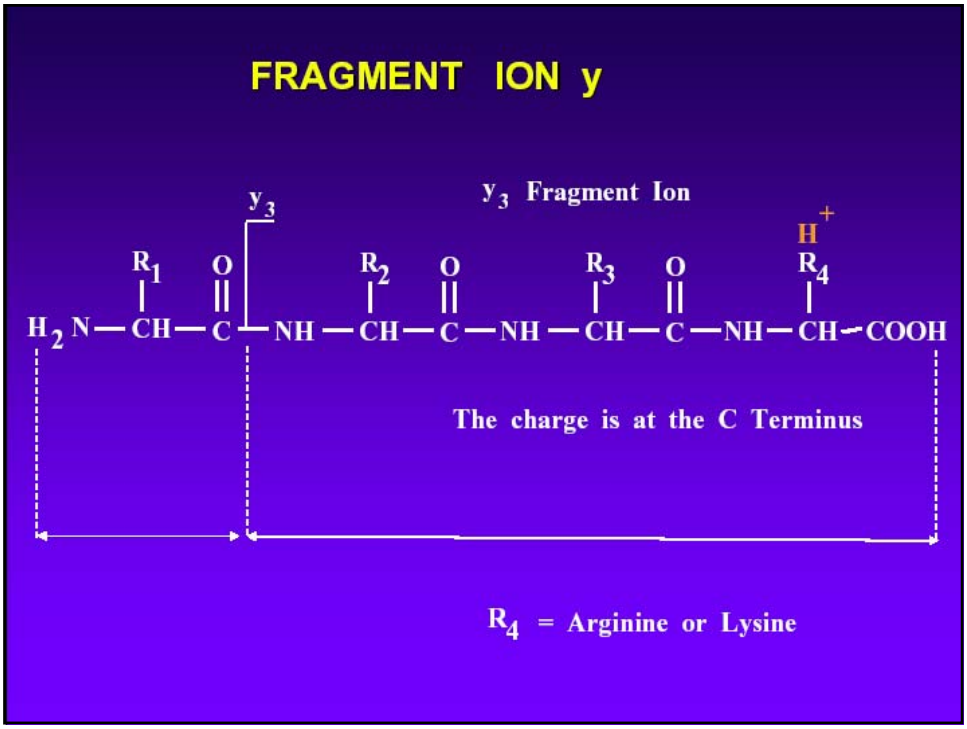


Oomens J et al, *J. Am. Soc. Mass. Spectrom.* **20**, 334 (2009)

Whenever you identify a ***b*** ion look for an ***a*** ion at -28u.

This gives some assurance that your assignment is correct.

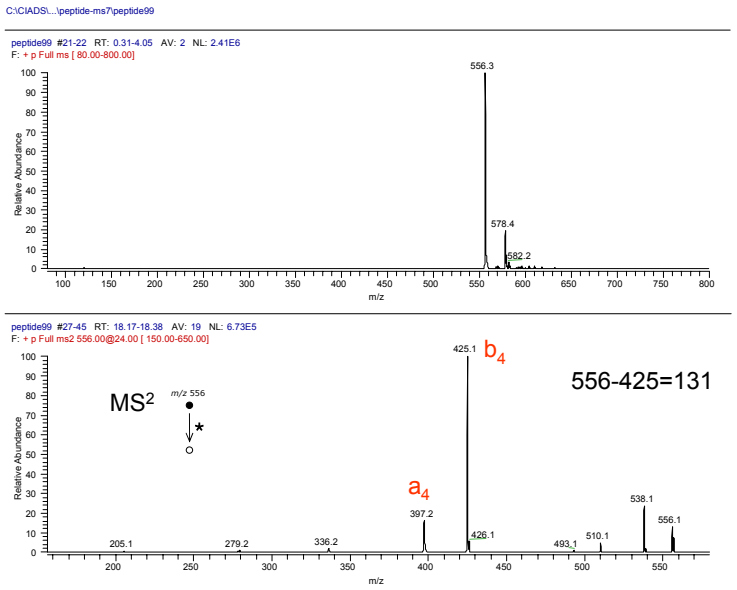
Also look for ammonia and water losses, -17 and -18u respectively.



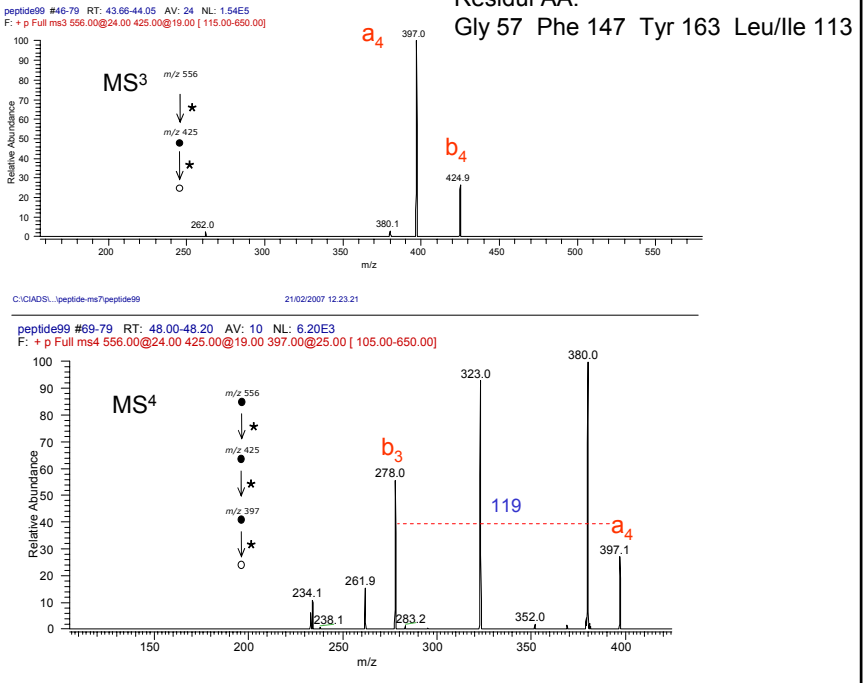
Attribuire la giusta sequenza al peptide

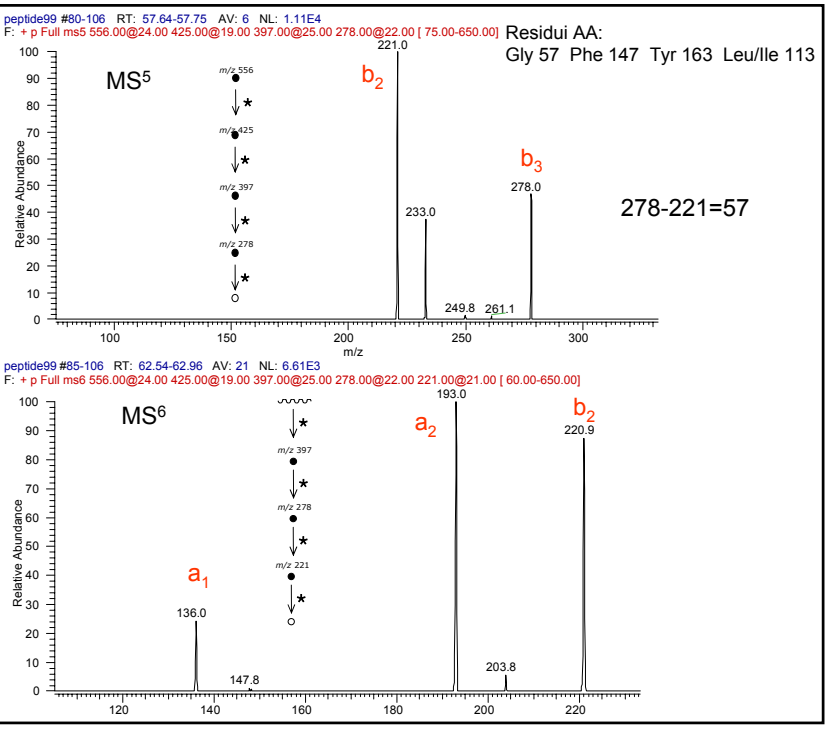
Gly Phe Tyr Gly Leu/Ile

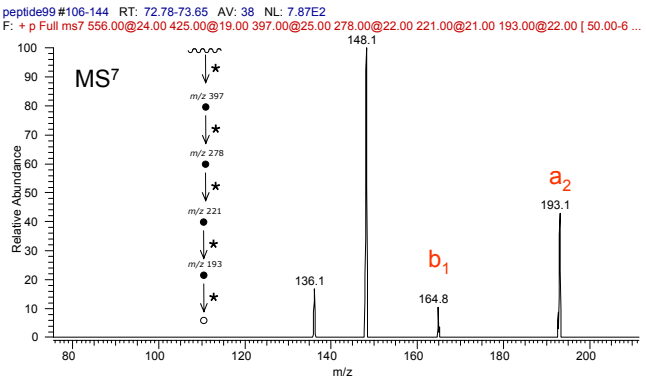
Residui AA:
Gly 57 Phe 147 Tyr 163 Leu/Ile 113



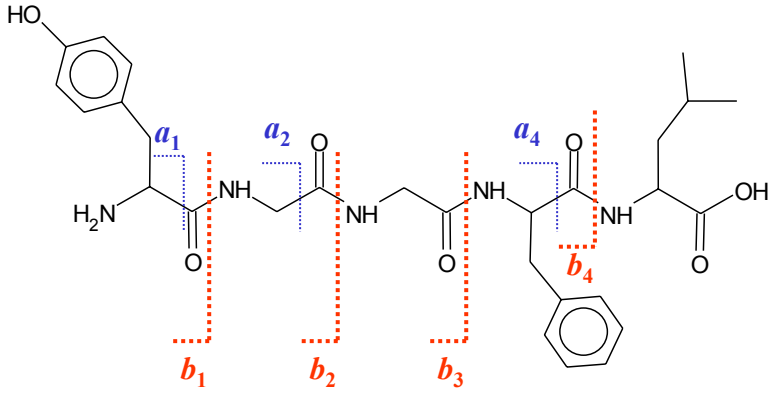
Residui AA:
Gly 57 Phe 147 Tyr 163 Leu/Ile 113



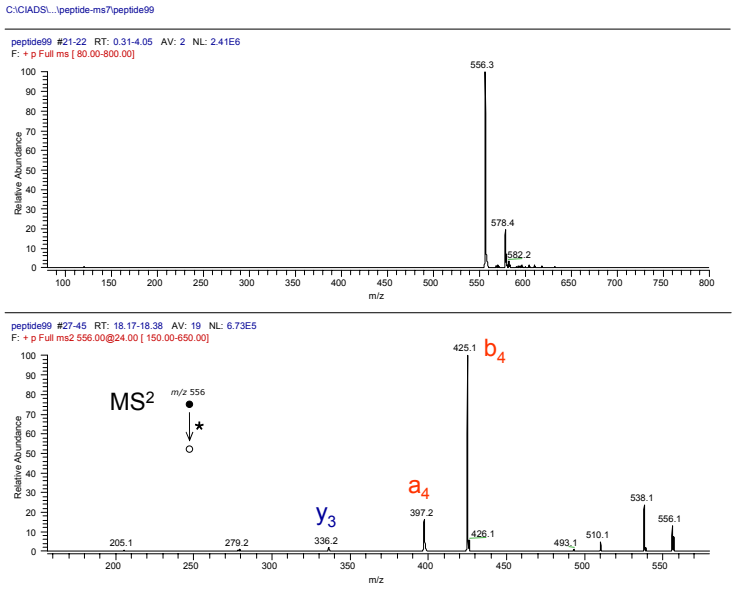

peptide99 #80-106 RT: 57.64-57.75 AV: 6 NL: 1.11E4
F: + p Full ms5 556.00@24.00 425.00@19.00 397.00@25.00 278.00@22.00 [75.00-650.00]
MS5
m/z 556
m/z 425
m/z 397
m/z 278
b2
221.0
b3
278.0
233.0
249.8
261.1
Relative Abundance
m/z
Residui AA:
Gly 57 Phe 147 Tyr 163 Leu/Ile 113
278-221=57
peptide99 #85-106 RT: 62.54-62.96 AV: 21 NL: 6.61E3
F: + p Full ms6 556.00@24.00 425.00@19.00 397.00@25.00 278.00@22.00 221.00@21.00 [60.00-650.00]
MS6
m/z 397
m/z 278
m/z 221
a1
136.0
147.8
a2
193.0
203.8
b2
220.9
Relative Abundance


peptide99 #106-144 RT: 72.78-73.65 AV: 38 NL: 7.87E2
F: + p Full ms7 556.00@24.00 425.00@19.00 397.00@25.00 278.00@22.00 221.00@21.00 193.00@22.00 [50.00-6 ...
MS7
m/z 397
m/z 278
m/z 221
m/z 193
148.1
a2
193.1
b1
136.1
164.8
Relative Abundance
m/z

Tyr-Gly-Gly-Phe-Leu



Attribuire la sequenza al peptide



Cyclic peptides:

Naturally occurring in bacteria, fungi and plants

Synthetic compounds

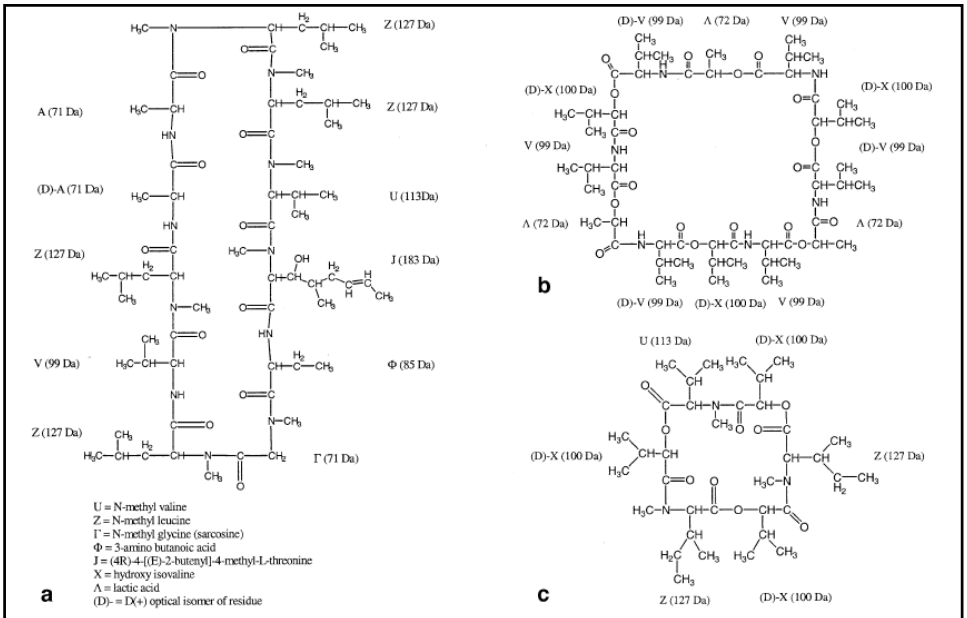
They can have a lot of biological and pharmaceutical properties:

enzyme inhibitors, antifungal and antibacterial agents, immunosuppressant, antibiotics and anticancer drugs

Better bioavailability and higher resistance to proteolytic degradation than their linear analogues

Highly selective metal ion chelators:

carriers of metal ions inside the cells. Use in radiotherapy



Fi ructures of (**a**) cyclosporin A and depsipeptides (**b**) valinomycin and (**c**) enniatin A_1.

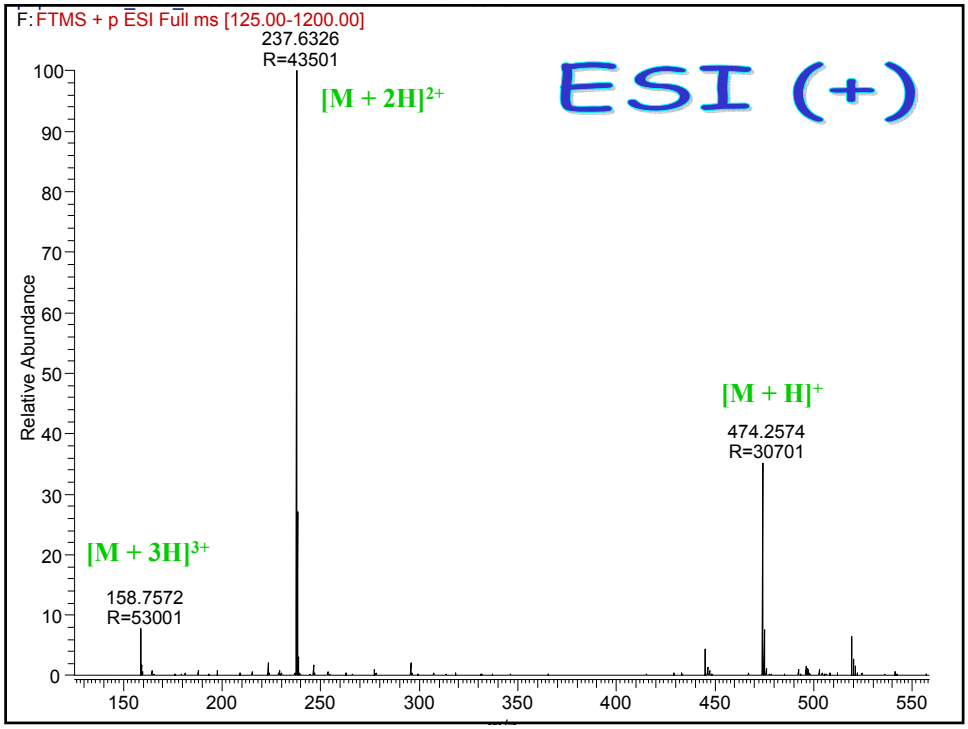
Brodbelt et al. *J Am Soc Mass Spectrom* **2004**, *15*, 1039–1054

Characterization and sequencing of cyclic peptides represent an important challenge for mass spectrometry.

No definite terminations, as it occurs in their linear analogues.

The elimination of an aminoacidic residue must involve the fission of two bonds of the cycle.

The ring-opening may occur at several backbone positions, meaning that all the fragment ions are not referenced to a single “terminal” position.



Resolving powerS:

Orbitrap:

at constant detection time:

resolving power inversely proportional to $\sqrt{m/z}$

Thus if

R=**100,000** at *m/z* 100,

at *m/z* 1000 it will be R=100,000(100/1000)$^{1/2}$=**31,646**

Elemental composition search on mass 474.26

m/z= 469.26-479.26

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
474.2573	474.2572	0.18	5.0	$C_{22}H_{38}O_9N_2$
	474.2572	0.20	10.5	$C_{21}H_{32}O_4N_9$
	474.2585	-2.64	10.0	$C_{23}H_{34}O_5N_6$
	474.2558	3.02	5.5	$C_{20}H_{36}O_8N_5$
	474.2599	-5.46	15.0	$C_{24}H_{30}O_1N_{10}$
	474.2599	-5.47	9.5	$C_{25}H_{36}O_6N_3$

Elemental composition search on mass 475.26

m/z= 470.26-480.26

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
475.2605	475.2605	-0.03	10.5	$C_{20}{}^{13}C_1H_{32}O_4N_9$
	475.2605	-0.04	5.0	$C_{21}{}^{13}C_1H_{38}O_9N_2$
	475.2605	0.10	18.5	$C_{30}H_{31}N_6$
	475.2610	-0.98	0.5	$C_{17}H_{39}O_{11}N_4$
	475.2596	1.85	1.0	$C_{15}H_{37}O_{10}N_7$
	475.2592	2.79	5.5	$C_{19}{}^{13}C_1H_{36}O_8N_5$

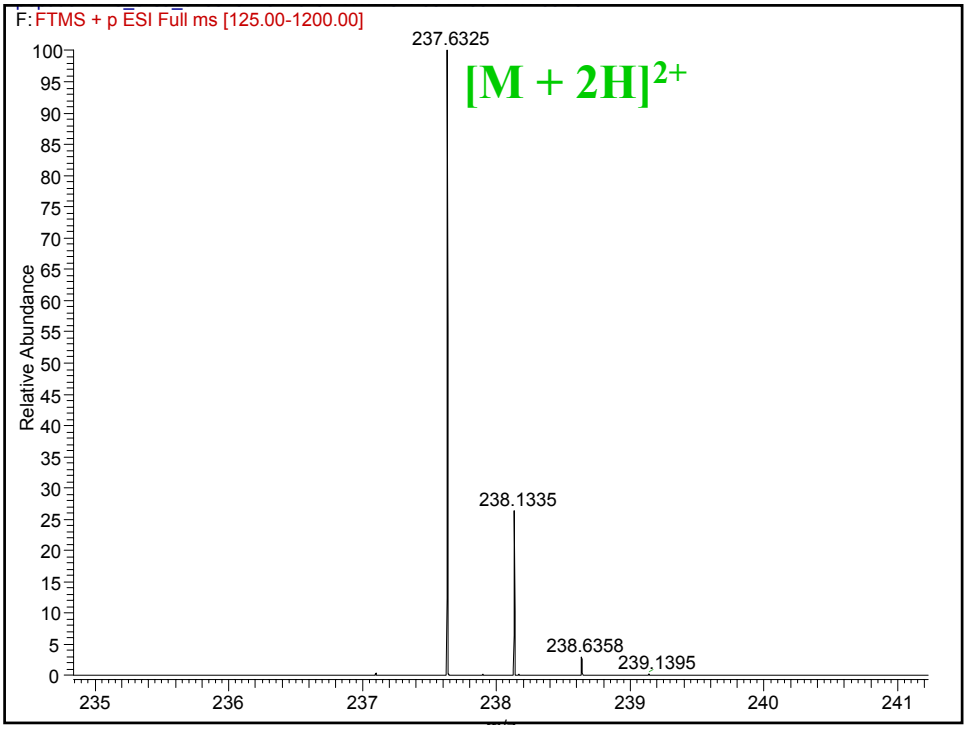
RDB=double bond/ring equivalents

$$D = 1 + \frac{\left[\sum_i^{imax} Ni(Vi - 2)\right]}{2}$$

For ions with odd charge:

Odd-electron ions = RDB integer
Even-electron ions= RDB x.5

^{12}C	0÷30
^{13}C	0÷1
^{14}N	0÷10
^{16}O	0÷15
^{1}H	0÷60



Elemental composition search on mass 237.63

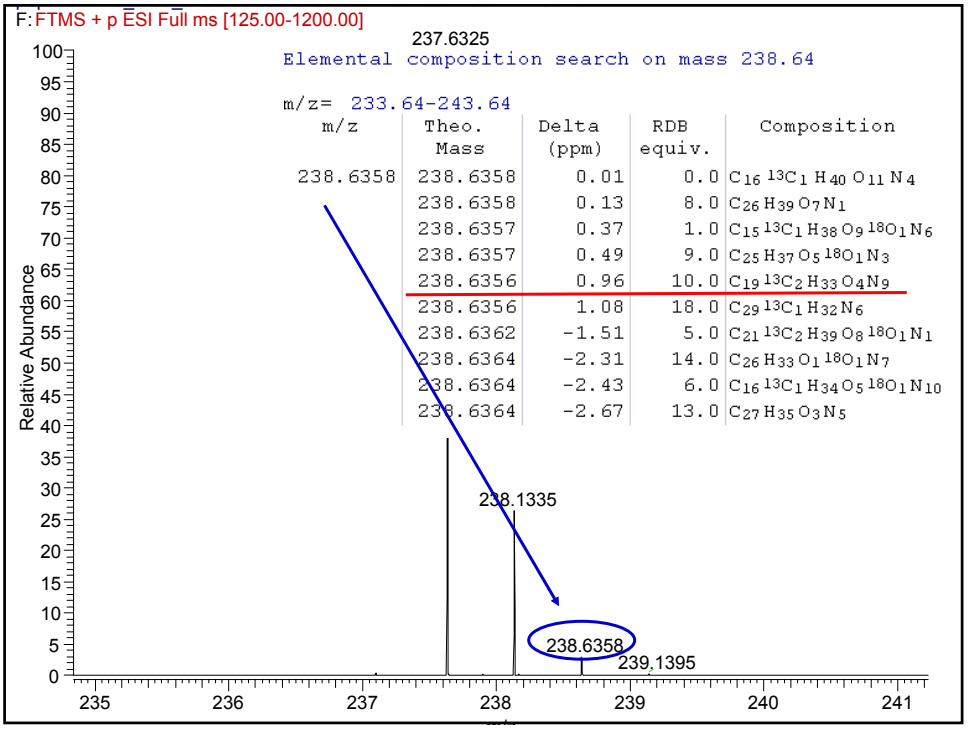
m/z= 232.63-242.63

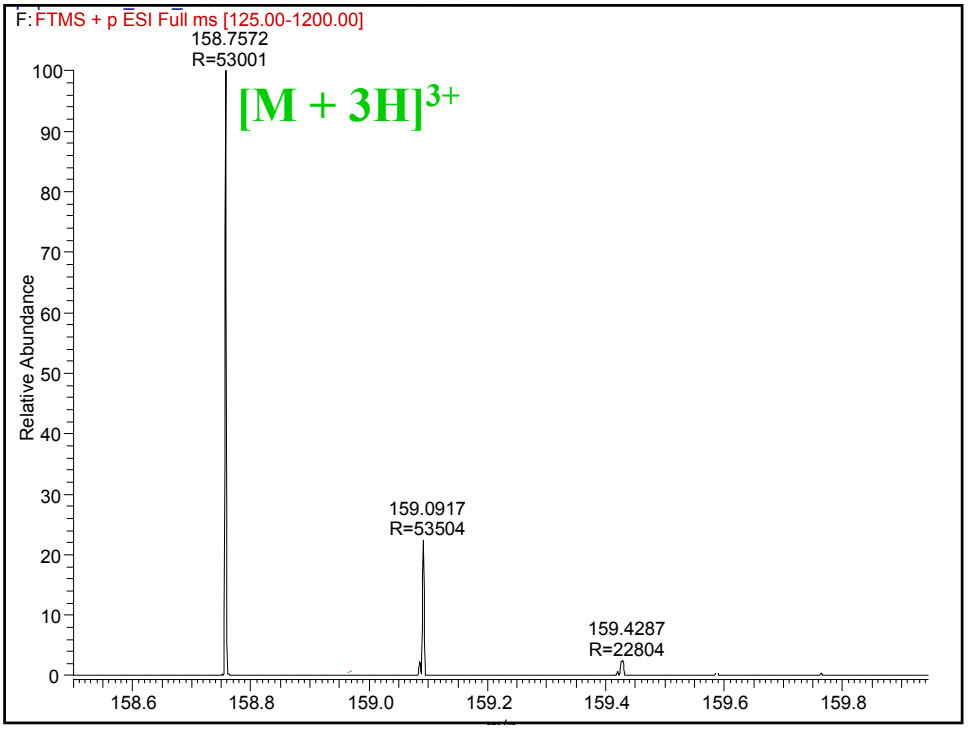
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
237.6325	237.6327	-0.78	14.0	$C_{27}{}^{13}C_1H_{34}O_3N_4$
	237.6322	1.11	10.0	$C_{21}H_{33}O_4N_9$
	237.6320	2.04	9.0	$C_{26}{}^{13}C_1H_{38}O_7$
	237.6316	3.92	5.0	$C_{20}H_{37}O_8N_5$
	237.6336	-4.54	9.0	$C_{25}H_{37}O_6N_3$
	237.6336	-4.67	1.0	$C_{15}{}^{13}C_1H_{38}O_{10}N_6$

Elemental composition search on mass 238.13

m/z= 233.13-243.13

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
238.1335	238.1332	1.11	5.0	$C_{19}{}^{13}C_1H_{37}O_8N_5$
	238.1332	1.24	13.0	$C_{29}H_{36}O_4N_2$
	238.1339	-1.57	18.0	$C_{30}H_{32}N_6$
	238.1339	-1.70	10.0	$C_{20}{}^{13}C_1H_{33}O_4N_9$
	238.1341	-2.65	0.0	$C_{17}H_{40}O_{11}N_4$
	238.1328	2.99	1.0	$C_{13}H_{36}O_9N_{10}$


F: FTMS + p ESI Full ms [125.00-1200.00]
237.6325
Elemental composition search on mass 238.64
m/z= 233.64-243.64
m/z | Theo. Mass | Delta (ppm) | RDB equiv. | Composition
238.6358 | 238.6358 | 0.01 | 0.0 | C16 13C1 H40 O11 N4
238.6358 | 0.13 | 8.0 | C26 H39 O7 N1
238.6357 | 0.37 | 1.0 | C15 13C1 H38 O9 18O1 N6
238.6357 | 0.49 | 9.0 | C25 H37 O5 18O1 N3
238.6356 | 0.96 | 10.0 | C19 13C2 H33 O4 N9
238.6356 | 1.08 | 18.0 | C29 13C1 H32 N6
238.6362 | -1.51 | 5.0 | C21 13C2 H39 O8 18O1 N1
238.6364 | -2.31 | 14.0 | C26 H33 O1 18O1 N7
238.6364 | -2.43 | 6.0 | C16 13C1 H34 O5 18O1 N10
238.6364 | -2.67 | 13.0 | C27 H35 O3 N5
Relative Abundance
238.1335
238.6358
239.1395


F: FTMS + p ESI Full ms [125.00-1200.00]
158.7572
R=53001
[M + 3H]3+
Relative Abundance
159.0917
R=53504
159.4287
R=22804

Elemental composition search on mass 158.76

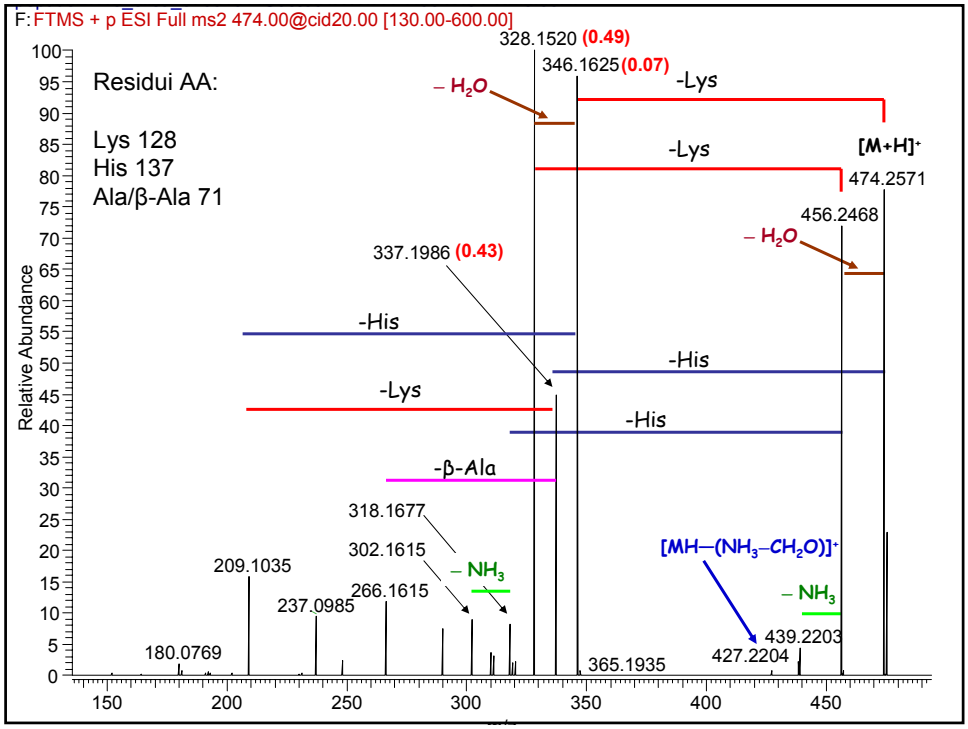
m/z= 153.76-163.76

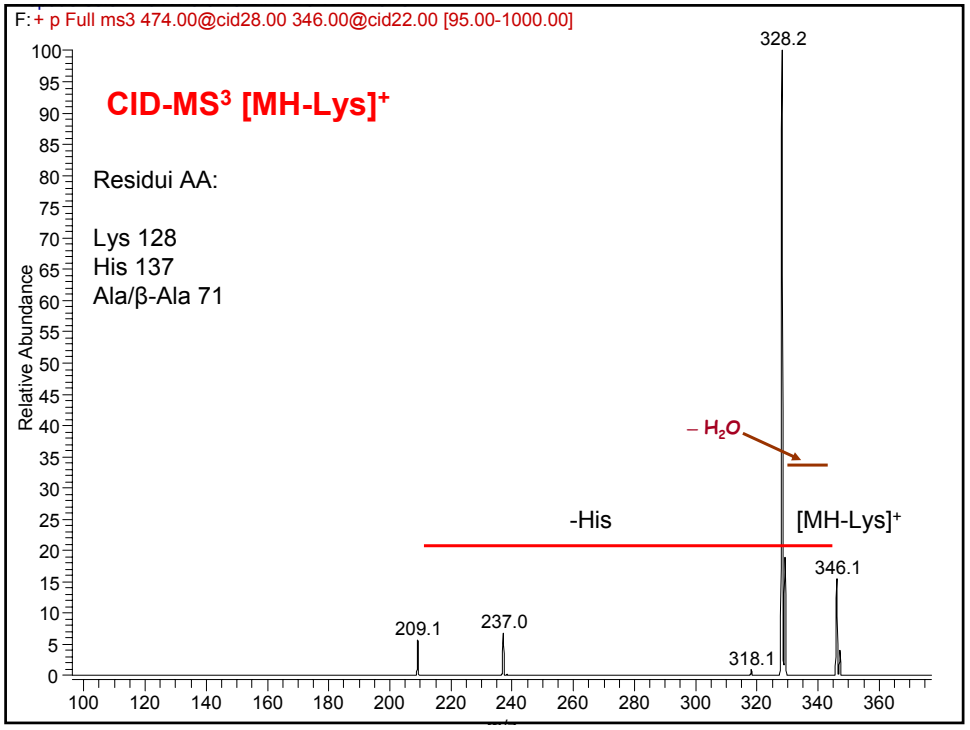
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
158.7572	158.7572	-0.34	9.5	$C_{21}H_{34}O_4N_9$
	158.7572	-0.35	4.0	$C_{22}H_{40}O_9N_2$
	158.7571	0.59	8.5	$C_{26}{}^{13}C_1H_{39}O_7$
	158.7571	0.60	14.0	$C_{25}{}^{13}C_1H_{33}O_2N_7$
	158.7575	-2.22	13.5	$C_{27}{}^{13}C_1H_{35}O_3N_4$
	158.7568	2.47	4.5	$C_{20}H_{38}O_8N_5$

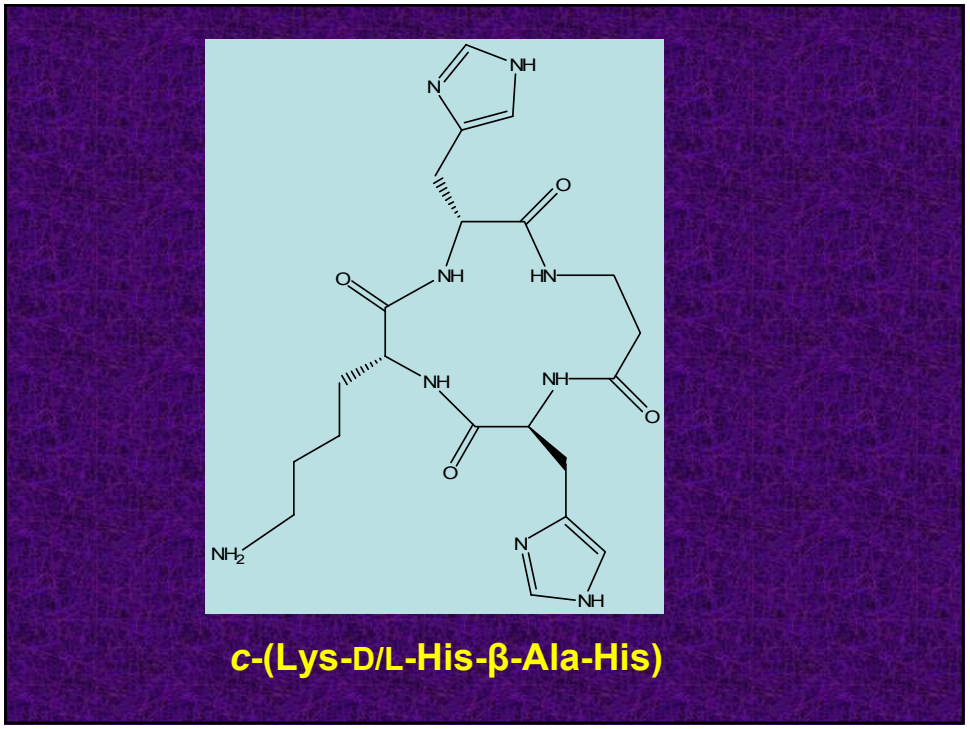
Elemental composition search on mass 159.09

m/z= 154.09-164.09

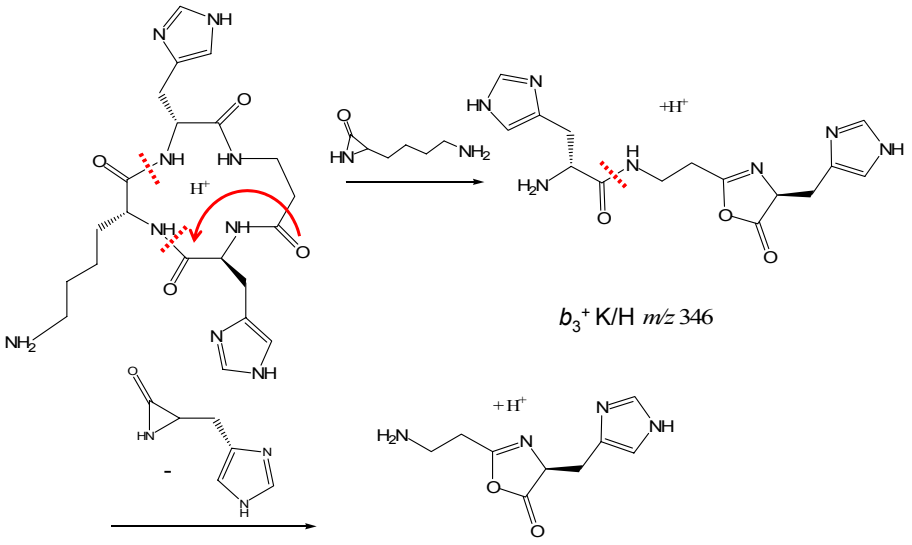
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
159.0916	159.0917	-0.28	17.5	$C_{30}H_{33}N_6$
	159.0917	-0.41	9.5	$C_{20}{}^{13}C_1H_{34}O_4N_9$
	159.0917	-0.42	4.0	$C_{21}{}^{13}C_1H_{40}O_9N_2$
	159.0918	-1.36	-0.5	$C_{17}H_{41}O_{11}N_4$
	159.0914	1.45	0.0	$C_{15}H_{39}O_{10}N_7$
	159.0912	2.39	4.5	$C_{19}{}^{13}C_1H_{38}O_8N_5$





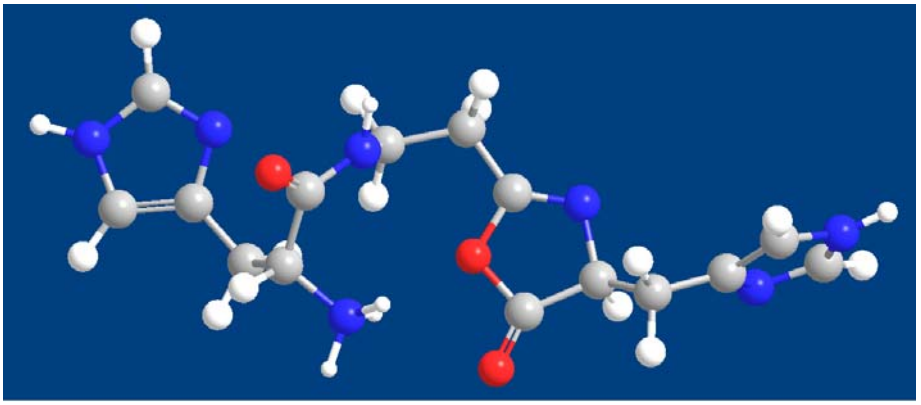


c-(Lys-D/L-His-β-Ala-His)

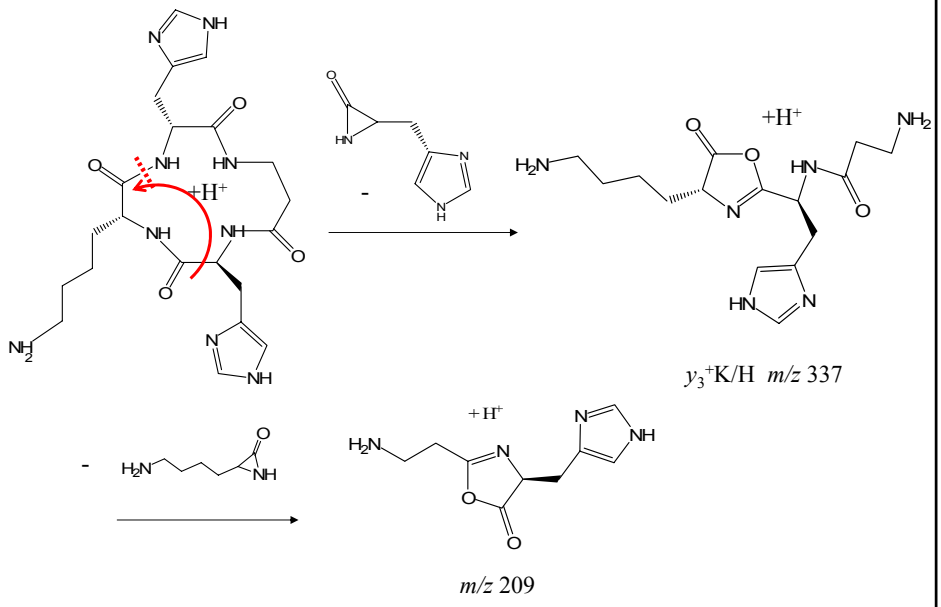


b_3^+ K/H *m/z* 346

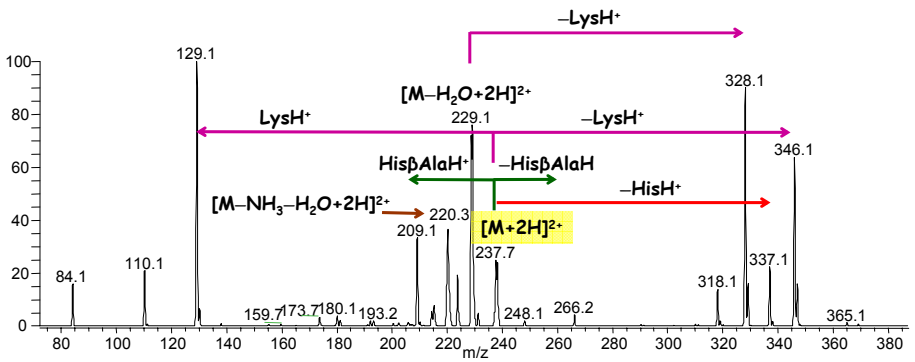
m/z 209



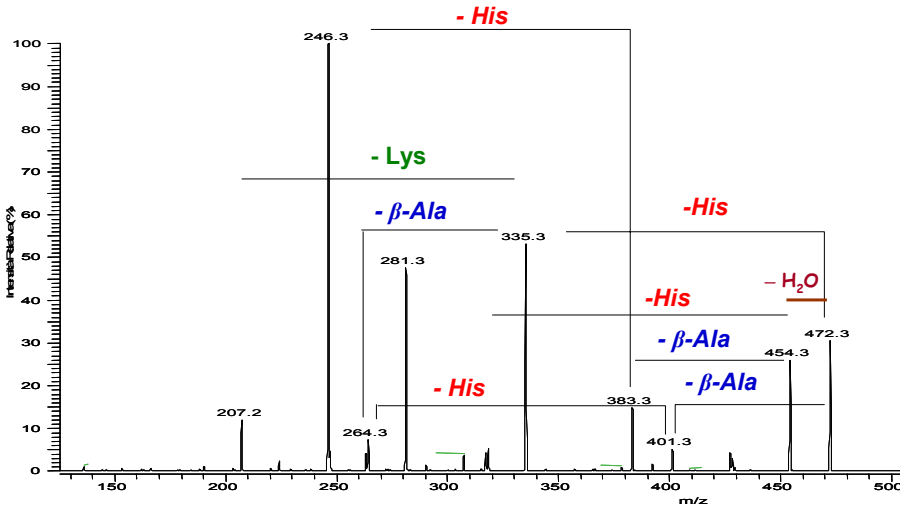
b_3^+ K/H *m/z* 346

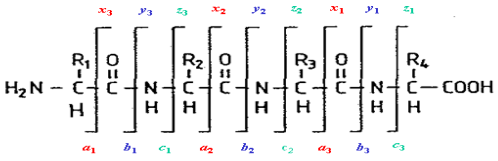

+H+
-
+H+
y_3^+K/H *m/z* 337
-
+H+
m/z 209

CID-MS^2 [M+2H]$^{2+}$


–LysH+
129.1
[M–H2O+2H]2+
229.1
328.1
LysH+
–LysH+
346.1
HisβAlaH+
–HisβAlaH
–HisH+
[M–NH3–H2O+2H]2+
220.3
209.1
[M+2H]2+
237.7
337.1
110.1
84.1
318.1
159.7
173.7
180.1
193.2
248.1
266.2
365.1
m/z

CID-MS^2 [M-H]$^-$





If you are working with a **tryptic peptide**, look for the arginine or lysine **y_1 ion** at the low end of the spectrum:

$[M+H]^+$ -156 - 18 = **penultimate b ion** Arg is the carboxy AA

$[M+H]^+$ -128 -18 = **penultimate b ion** Lys is the carboxy AA

This may give you a clue to the **C-terminal residue** of your tryptic peptide.

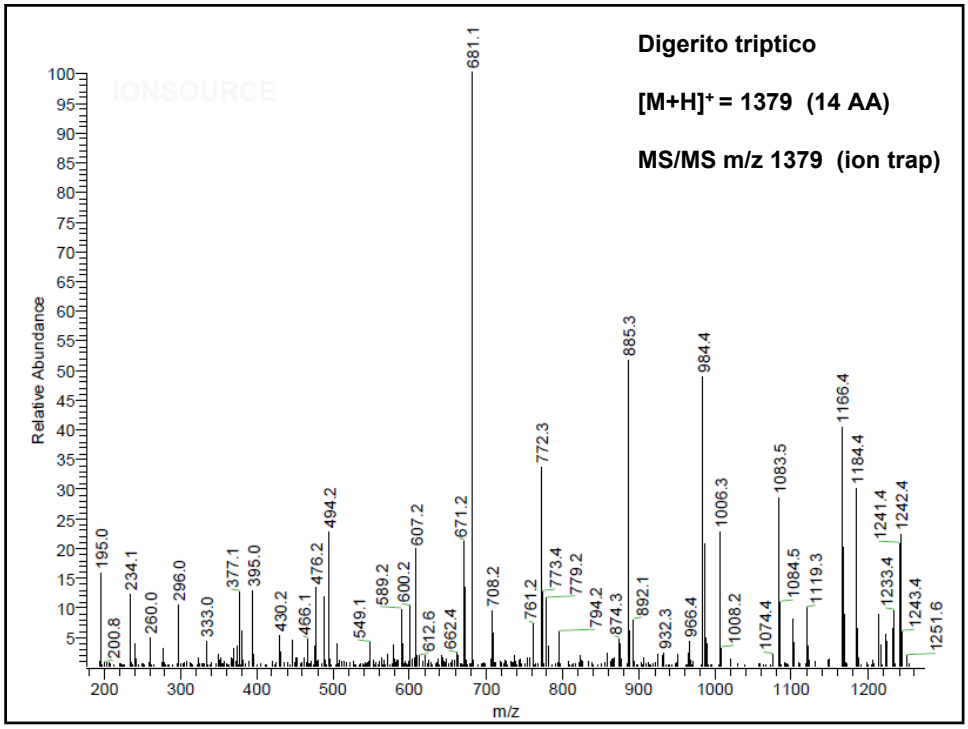
In general: **protonated peptide - AA residue mass - 18 = penultimate b ion**

or you could use the standard formula for calculating the corresponding **b** ion once the **y** ion is known.

$[M+H]^+ - y_1+1$ = **penultimate b ion**

If you are using an ion trap you may not be able to observe the low end of the spectrum, and in this case, you will need to do both of these calculations

[M+H]+ - observed **b** ion + 1 = corresponding **y** ion.



Sequence: **HGTVVLTALGGILK**, pI: **8.75725**

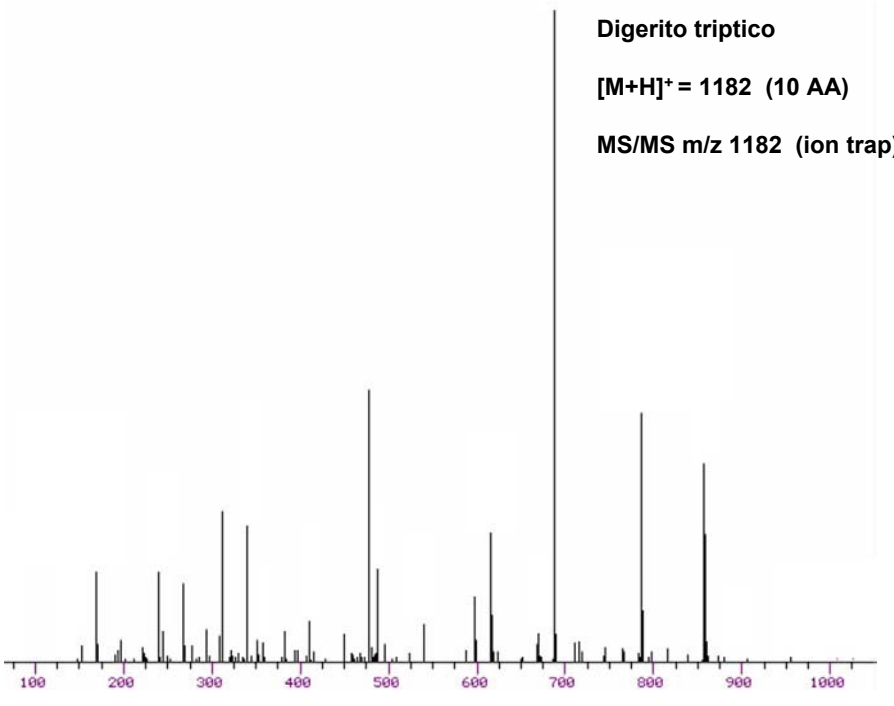
Fragment Ion Table, monoisotopic masses

Seq	#	A	B	C	X	Y	Z	# (+1)
H	1	110.07187	138.06678	155.09333	-	1378.84225	1361.81570	14
G	2	167.09333	195.08825	212.11479	1267.76260	1241.78334	1224.75679	13
T	3	268.14101	296.13592	313.16247	1210.74114	1184.76188	1167.73533	12
V	4	367.20942	395.20434	412.23089	1109.69346	1083.71420	1066.68765	11
V	5	466.27784	494.27275	511.29930	1010.62505	984.64578	967.61924	10
L	6	579.36190	607.35681	624.38336	911.55664	885.57737	868.55082	9
T	7	680.40958	708.40449	725.43104	798.47257	772.49331	755.46676	8
A	8	751.44669	779.44161	796.46815	697.42489	671.44563	654.41908	7
L	9	864.53076	892.52567	909.55222	626.38778	600.40852	583.38197	6
G	10	921.55222	949.54713	966.57368	513.30372	487.32445	470.29790	5
G	11	978.57368	1006.56860	1023.59515	456.28225	430.30299	413.27644	4
I	12	1091.65775	1119.65266	1136.67921	399.26079	373.28152	356.25498	3
L	13	1204.74181	1232.73672	1249.76327	286.17673	260.19746	243.17091	2
K	14	1332.83677	1360.83169	-	173.09266	147.11340	130.08685	1

Digerito triptico

$[M+H]^+$ = 1182 (10 AA)

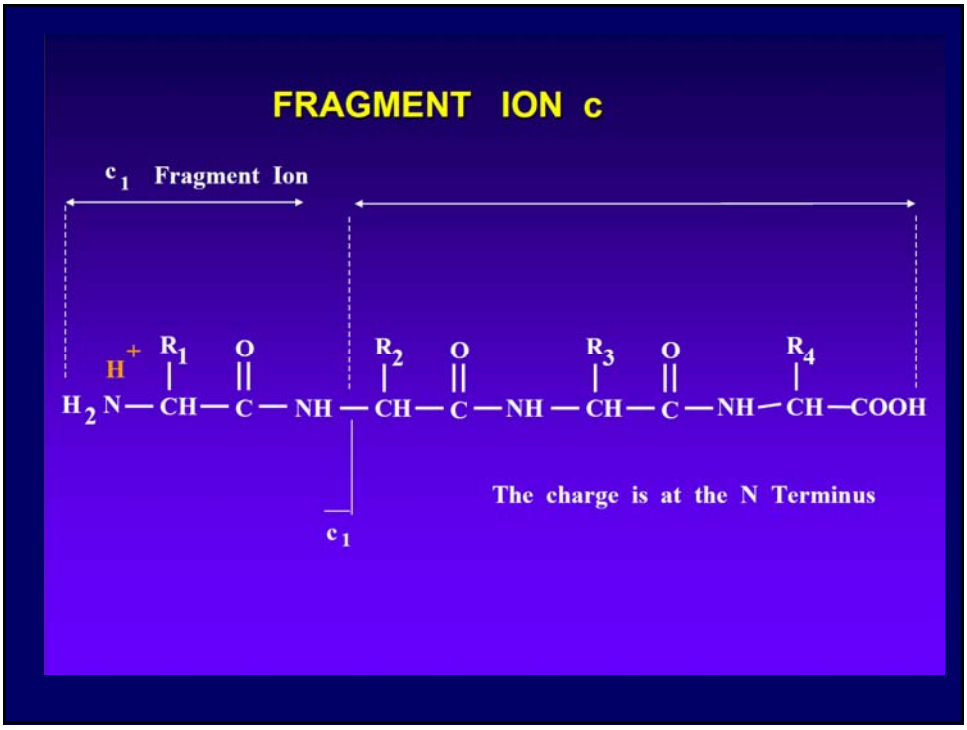
MS/MS m/z 1182 (ion trap)

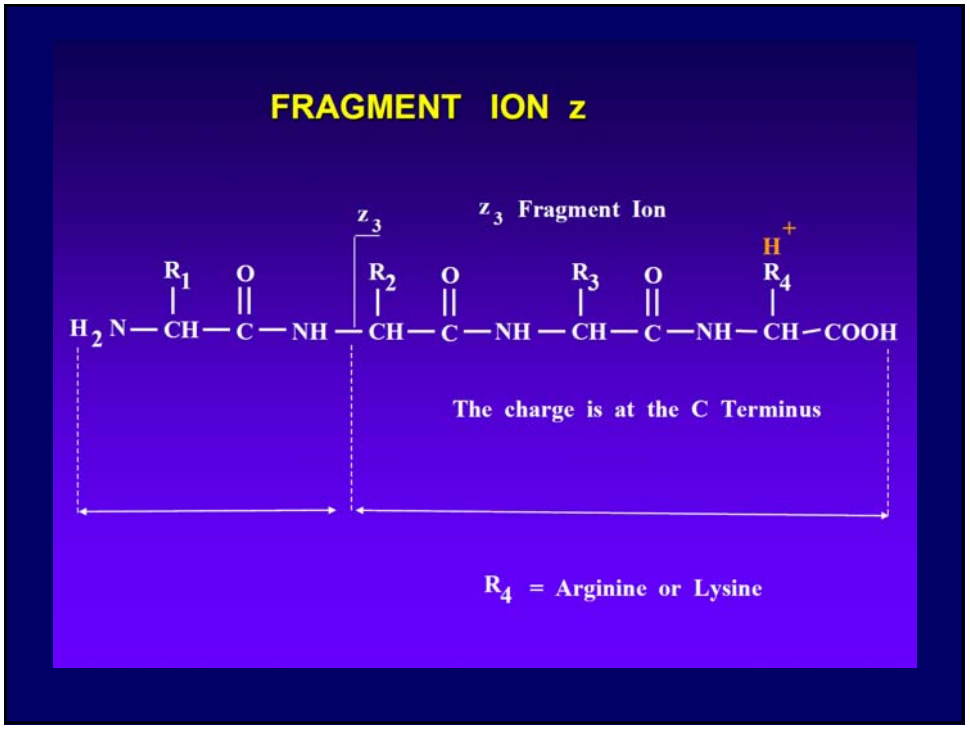


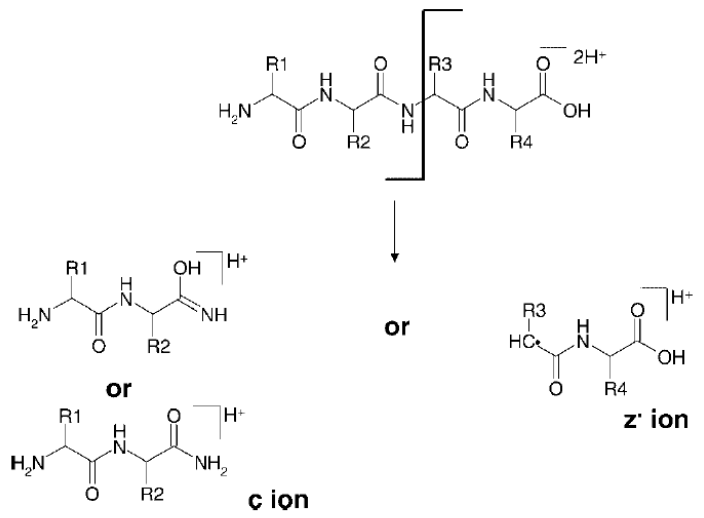
ETD, ECD

High Collision CID

Ions *c*, *z*







Fragmentation of peptide amine bonds to produce *N*-terminal *c* and *C*-terminal $z^{\bullet}$ ions. This type of fragmentation is unique to ECD.

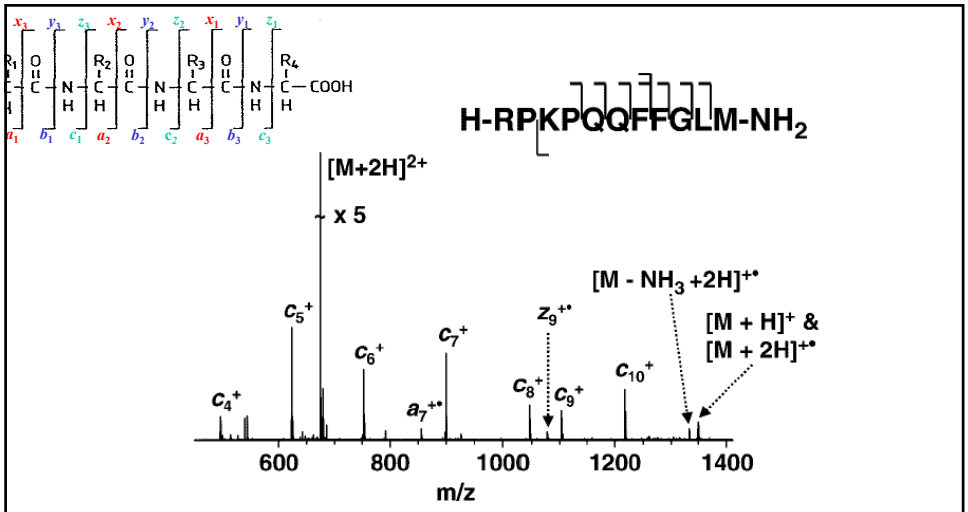
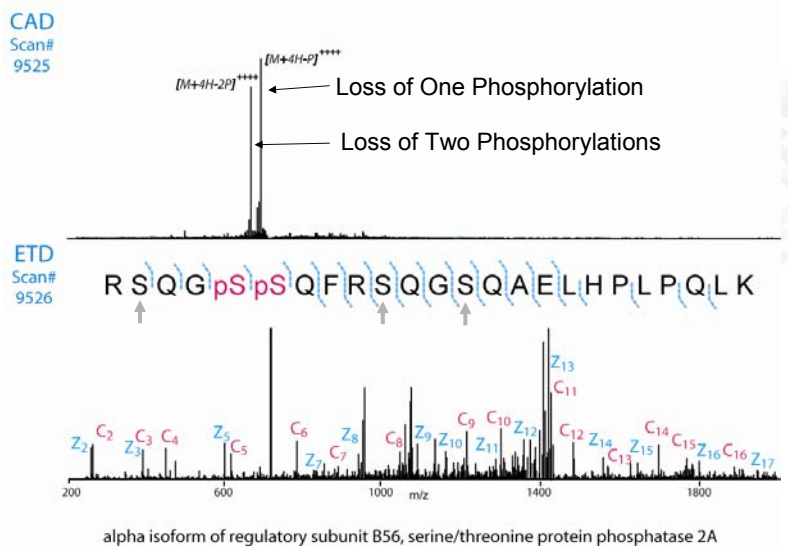


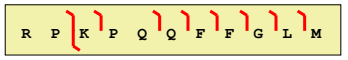
FIGURE 1. Electron capture dissociation (ECD) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrum obtained from the peptide, substance P $[M+2H]^{2+}$ ions. Spectrum obtained from the 9.4 T homebuilt instrument at the National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL. (Figure reproduced from Håkansson et al., 2003a, with permission from American Chemical Society, copyright 2003.)

CID vs ETD for Phosphorylated Peptides



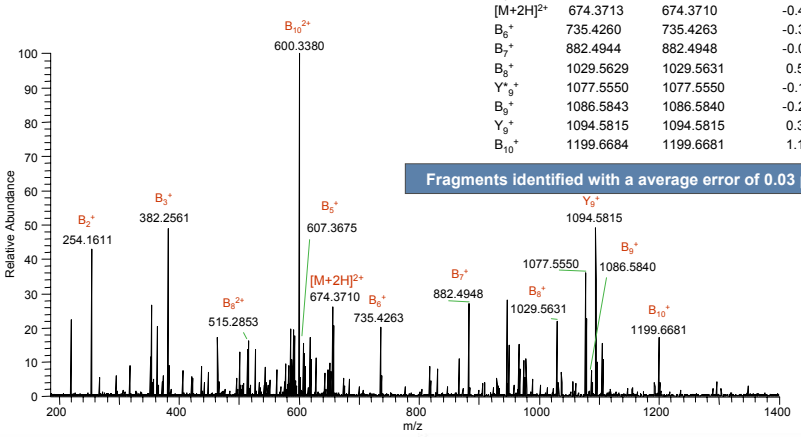
alpha isoform of regulatory subunit B56, serine/threonine protein phosphatase 2A

Single Scan Ion Trap CID with ICR Cell Detection

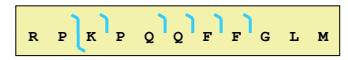


Ion	Theoretical	Measured	ppm
B_2^+	254.1612	254.1611	0.19
B_3^+	382.2561	382.2561	-0.04
B_8^{2+}	515.2851	515.2853	0.64
B_{10}^{2+}	600.3378	600.3380	-0.06
B_5^+	607.3675	607.3675	0.00
$[M+2H]^{2+}$	674.3713	674.3710	-0.44
B_6^+	735.4260	735.4263	-0.31
B_7^+	882.4944	882.4948	-0.00
B_8^+	1029.5629	1029.5631	0.52
$Y^*_9{}^+$	1077.5550	1077.5550	-0.18
B_9^+	1086.5843	1086.5840	-0.28
Y_9^+	1094.5815	1094.5815	0.32
B_{10}^+	1199.6684	1199.6681	1.17

Fragments identified with a average error of 0.03 ppm

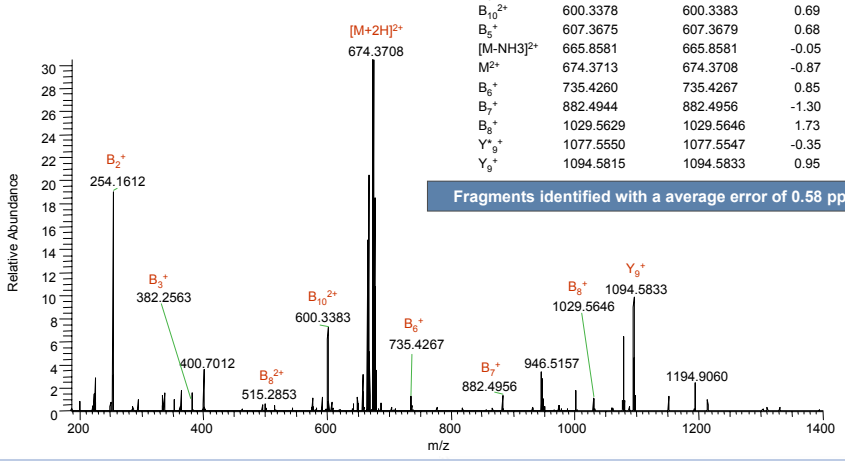


Single Scan IRMPD with ICR Cell Detection

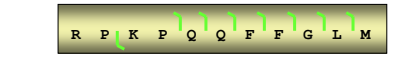


Ion	Theoretical	Measured	ppm
B_2^+	254.1612	254.1612	0.04
B_3^+	382.2561	382.2563	0.38
B_8^2	515.2851	515.2853	0.51
B_{10}^{2+}	600.3378	600.3383	0.69
B_5^+	607.3675	607.3679	0.68
$[M-NH3]^{2+}$	665.8581	665.8581	-0.05
M^{2+}	674.3713	674.3708	-0.87
B_6^+	735.4260	735.4267	0.85
B_7^+	882.4944	882.4956	-1.30
B_8^+	1029.5629	1029.5646	1.73
$Y^*_9{}^+$	1077.5550	1077.5547	-0.35
Y_9^+	1094.5815	1094.5833	0.95

Fragments identified with a average error of 0.58 ppm

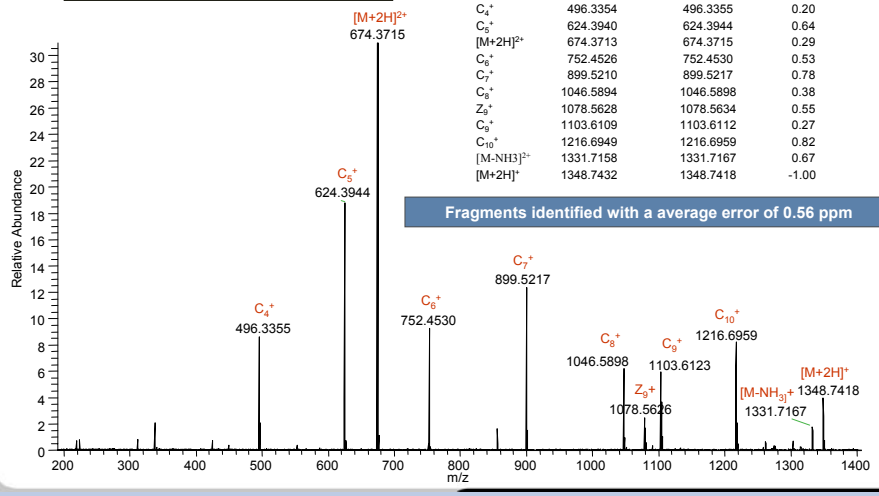


Single Scan ECD with ICR Cell Detection



Ion	Theoretical	Measured	ppm
C_4^+	496.3354	496.3355	0.20
C_5^+	624.3940	624.3944	0.64
$[M+2H]^{2+}$	674.3713	674.3715	0.29
C_6^+	752.4526	752.4530	0.53
C_7^+	899.5210	899.5217	0.78
C_8^+	1046.5894	1046.5898	0.38
Z_3^+	1078.5628	1078.5634	0.55
C_9^+	1103.6109	1103.6112	0.27
C_{10}^+	1216.6949	1216.6959	0.82
$[M-NH3]^{2+}$	1331.7158	1331.7167	0.67
$[M+2H]^+$	1348.7432	1348.7418	-1.00

Fragments identified with a average error of 0.56 ppm



Top down

vs approaches

Bottom up

for studying large molecules

Determinazione della struttura: MS^n

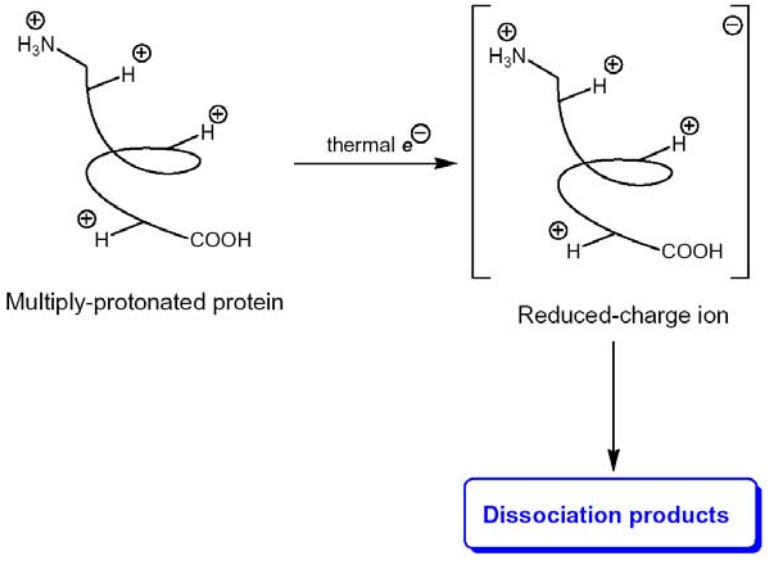
i) Proteina *in toto*:

ECD = electron capture dissociation (FT-ICR)

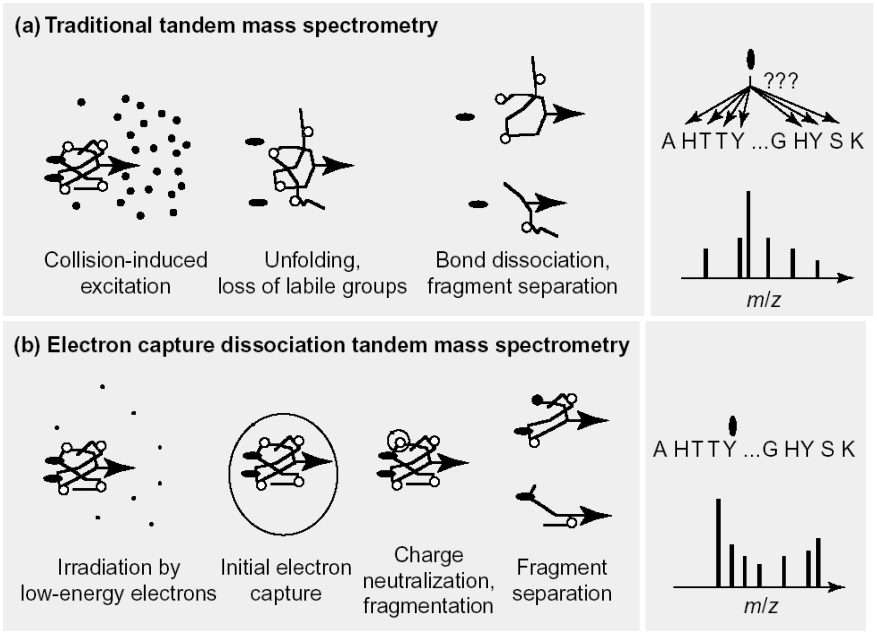
ETD = electron transfer dissociation (Linear IT)

Approccio Top Down

Electron Capture Dissociation



Zubarev, McLafferty, et al. *Anal. Chem.* **2000**, *72*, 563-573.



Comparison of **(a)** the traditional (collision-based) MS/MS and **(b)** ECD-based MS/MS techniques in the analysis of modified polypeptides. Whereas collisional excitation leads to polypeptide chain unfolding and losses of labile groups, ECD largely preserves the secondary structure and

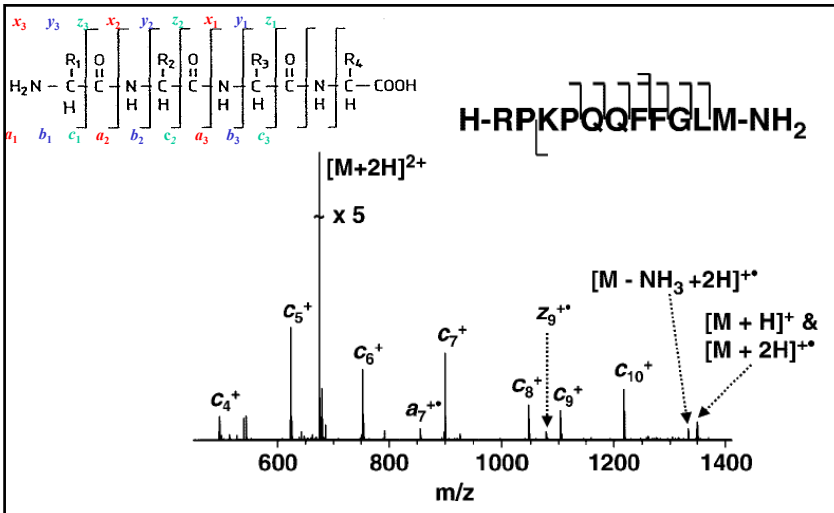
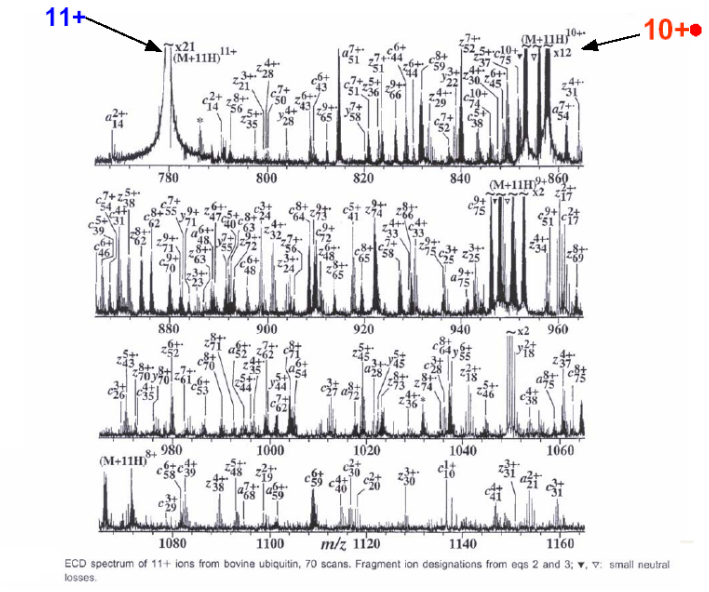


FIGURE 1. Electron capture dissociation (ECD) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrum obtained from the peptide, substance P $[M+2H]^{2+}$ ions. Spectrum obtained from the 9.4 T homebuilt instrument at the National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL. (Figure reproduced from Håkansson et al., 2003a, with permission from American Chemical Society, copyright 2003.)

ECD Spectrum of (Ubiquitin + 11H)$^{11+}$



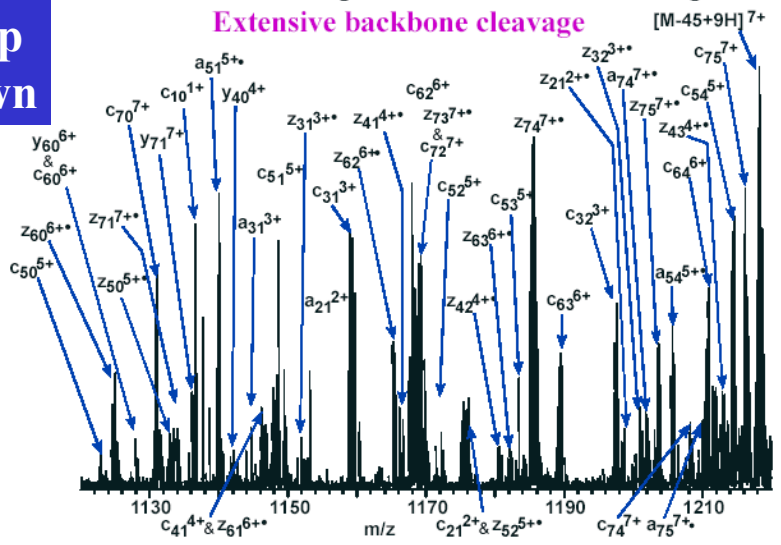
ECD spectrum of 11+ ions from bovine ubiquitin, 70 scans. Fragment ion designations from eqs 2 and 3; ▼, ▽: small neutral losses.

Zubarev, Kelleher, McLafferty, *JACS* **1998,** *120*, **3265-3266.**

Top down

ECD FT-ICR Mass Spectrum of Bovine Ubiquitin

Extensive backbone cleavage

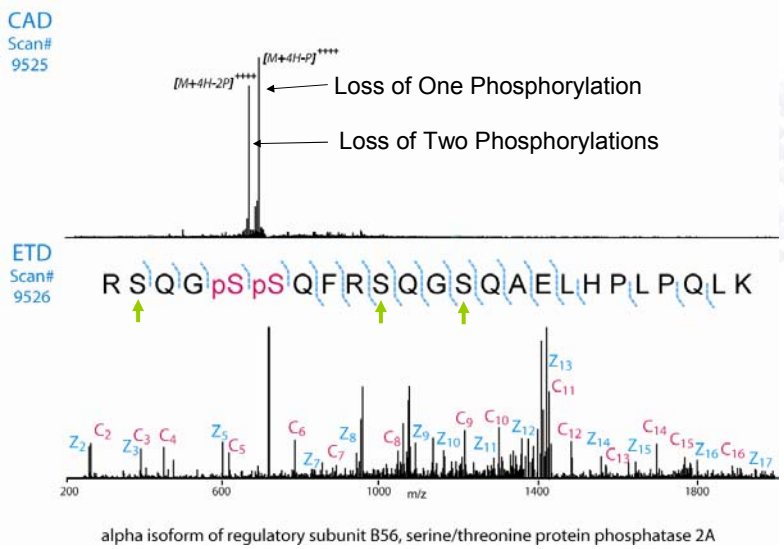


K Håkansson, MJ Chalmers, JP Quinn, MA McFarland, CL Hendrickson, AG Marshall. *Anal. Chem.* **2003,** *75*, 3256.

ETD

Electron Transfer Dissociation

CID vs ETD for Phosphorylated Peptides



Courtesy by G. Vago Thermo