Mass Spectrometric Analysis of Proteins and Peptides

Outline

1 The fundamentals-- Brief overview of hardwares and data acquisition.

2 Protein characterization, i.e. sequencing.

3 Protein identification.

4 Protein quantification.

5 Other applications.

The oldie and goldie:

Methods in Enzymology 1990

Volume 193, 47 articles and 862 pages.

The new and improved:

Methods in Enzymology 2005

Volume 402, 14 articles and 440 pages.

The most fundamental concept:

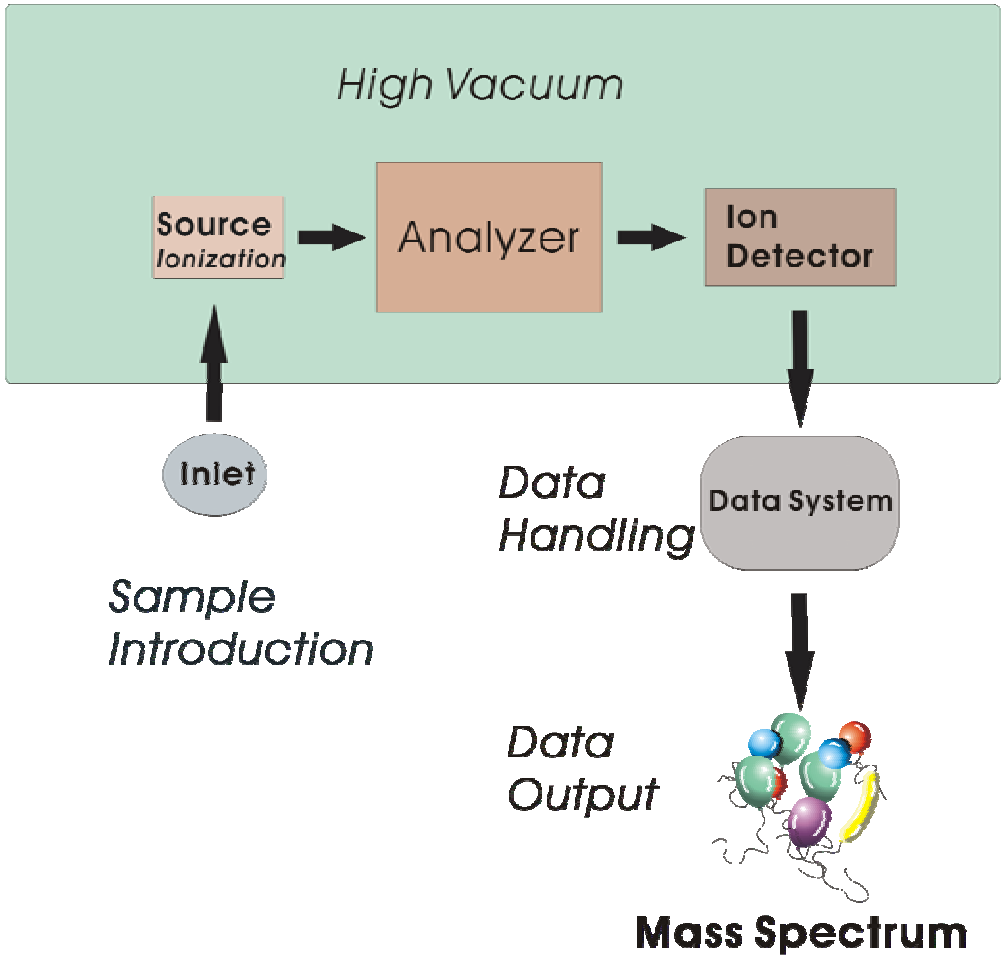
Mass Spectrometry Is Only a Tool to Give Accurate Masses of gaseous samples: Proteins, Peptides, DNA Fragments, sugars, metabolites, chemical compounds……..

A mass spectrometer is an instrument that measures the masses of individual molecules that have been converted into ions, i.e., molecules that have been electrically charged.

The process is called 'Ionization' meaning:

The sample has to be in gaseous phase and carries a charge before it can fly!

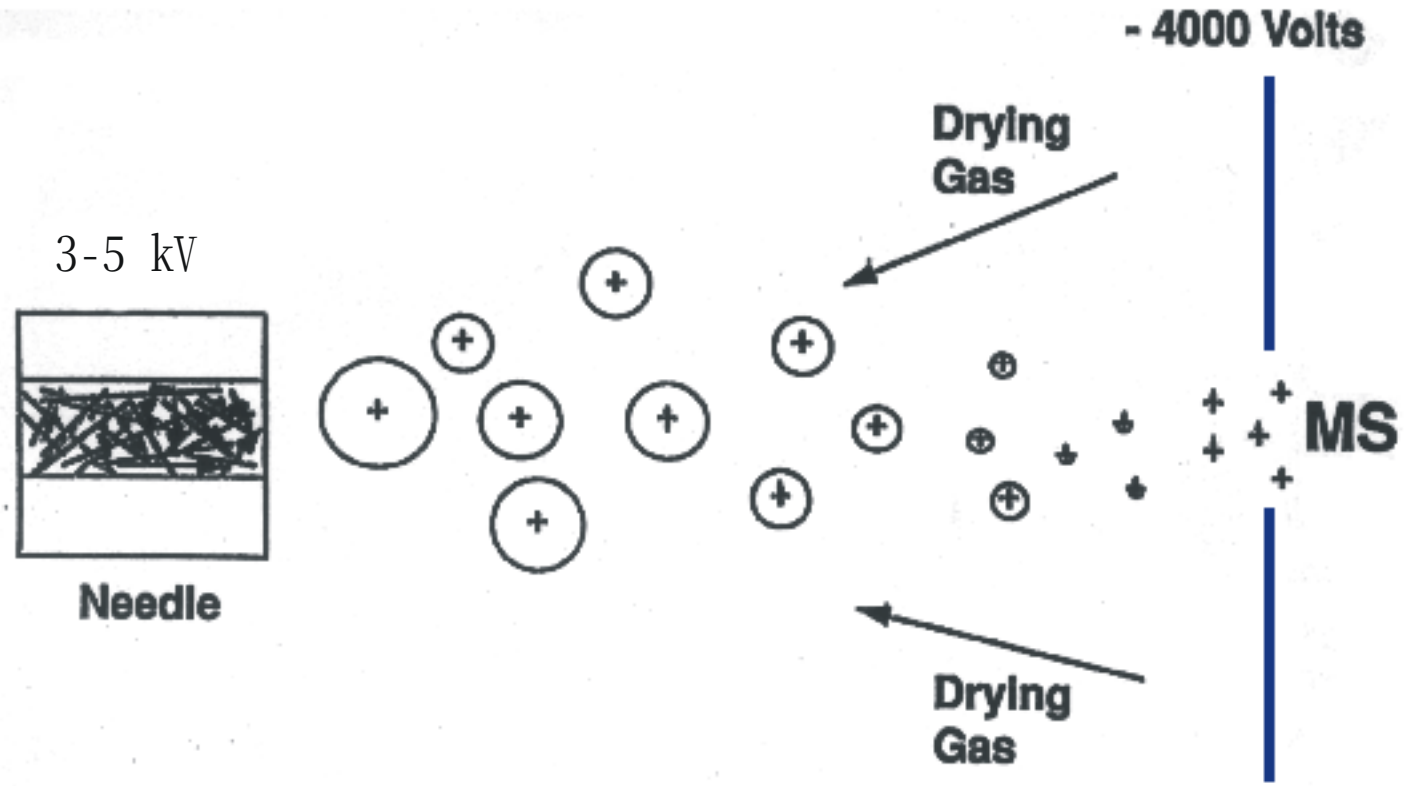
Components of a mass spectrometer



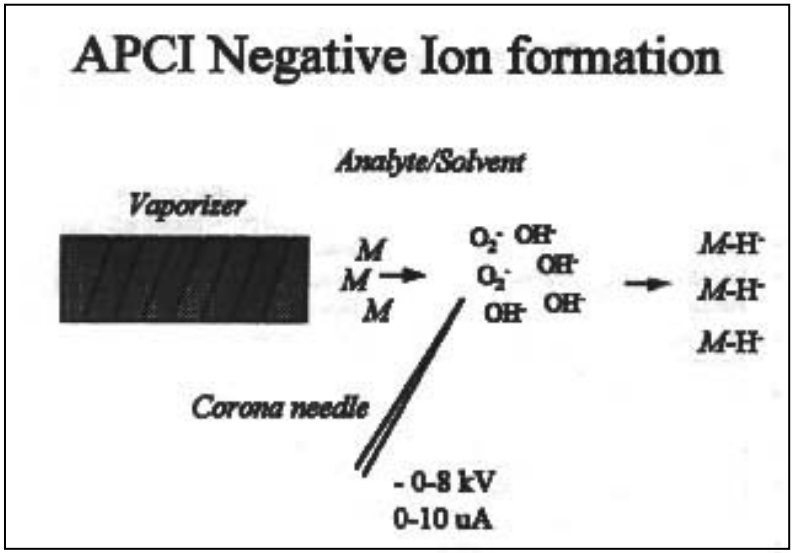
Common ionization methods for biological samples

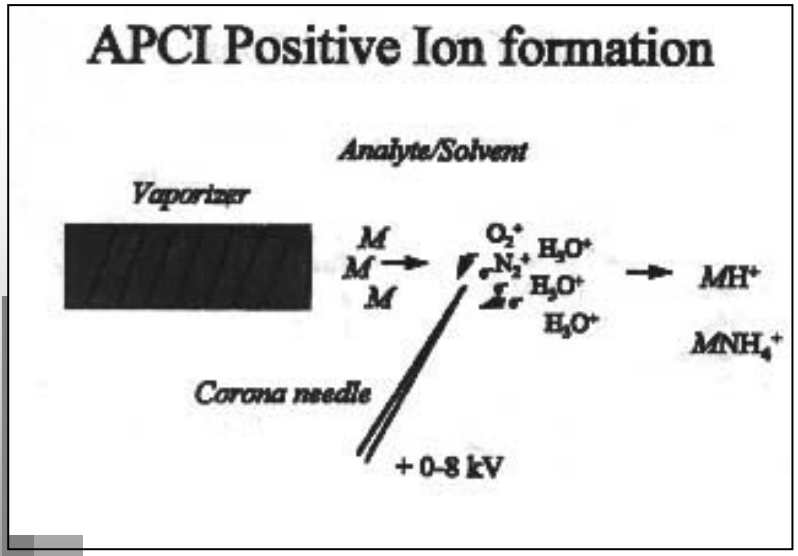
- Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI)
- Matrix Assisted Laser Desoption Ionization (MALDI)

The ESI Theory



APCI


APCI Negative Ion formation
Analyte/Solvent
Vaporizer
M
M
M
O_2^- OH^-
O_2^- OH^-
OH^- OH^-
M-H⁻
M-H⁻
M-H⁻
Corona needle
- 0-8 kV
0-10 uA

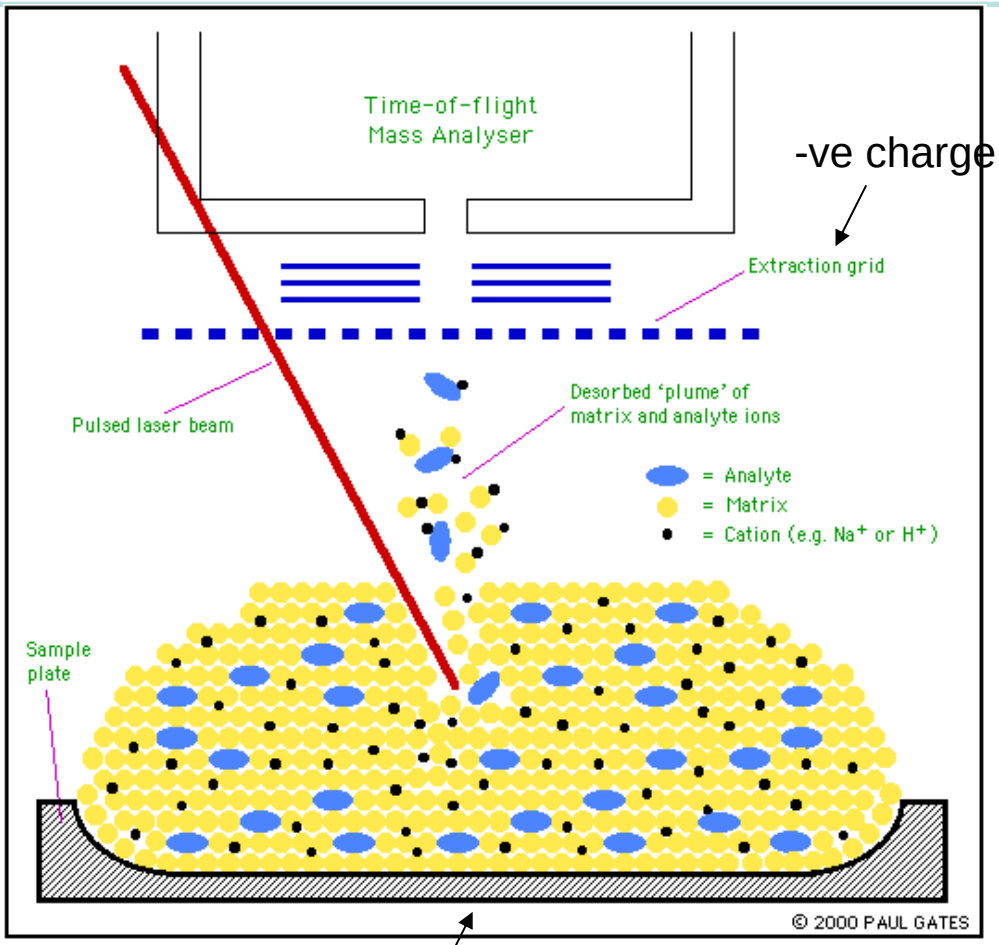

APCI Positive Ion formation
Analyte/Solvent
Vaporizer
M
M
M
O_2^+
N_2^+
H_3O^+
H_3O^+
H_3O^+
MH⁺
MNH_4^+
Corona needle
+ 0-8 kV

MALDI

- Sample mixed with organic compounds, co-crystallized, then subjected to laser excitation

a-cyano-4-hydroxycinnamic acid	CCA
Sinapinic acid	SA
2,5-dihydroxybenzoic acid	DHB

Etc, etc, etc……


Time-of-flight
Mass Analyser
-ve charge
Extraction grid
Pulsed laser beam
Desorbed 'plume' of
matrix and analyte ions
= Analyte
= Matrix
= Cation (e.g. Na+ or H+)
Sample
plate
© 2000 PAUL GATES

+ 20-25 kV

- Note that for the ionization methods discussed, they all utilize high voltages to accelerate the macromolecules.
- Depending on the acquired voltage, the macromolecule will attain a translational energy of:

$$V_1 e = m_1 v_1^2/2 = (m_1 v_1)^2/2m_1$$

$$V_1 e = m_1 v_1^2/2 = (m_1 v_1)^2/2m_1$$

or

$$m_1 v_1 = (2V_1 e m_1)^{1/2}$$

- It means the velocity of the ion is proportional to the reciprocal of the square root of its mass. Or momentum increases with the square root of its mass.
- At fixed accelerating voltage, separation of ions based on their momentum or their velocity is equivalent to a separation based on mass.

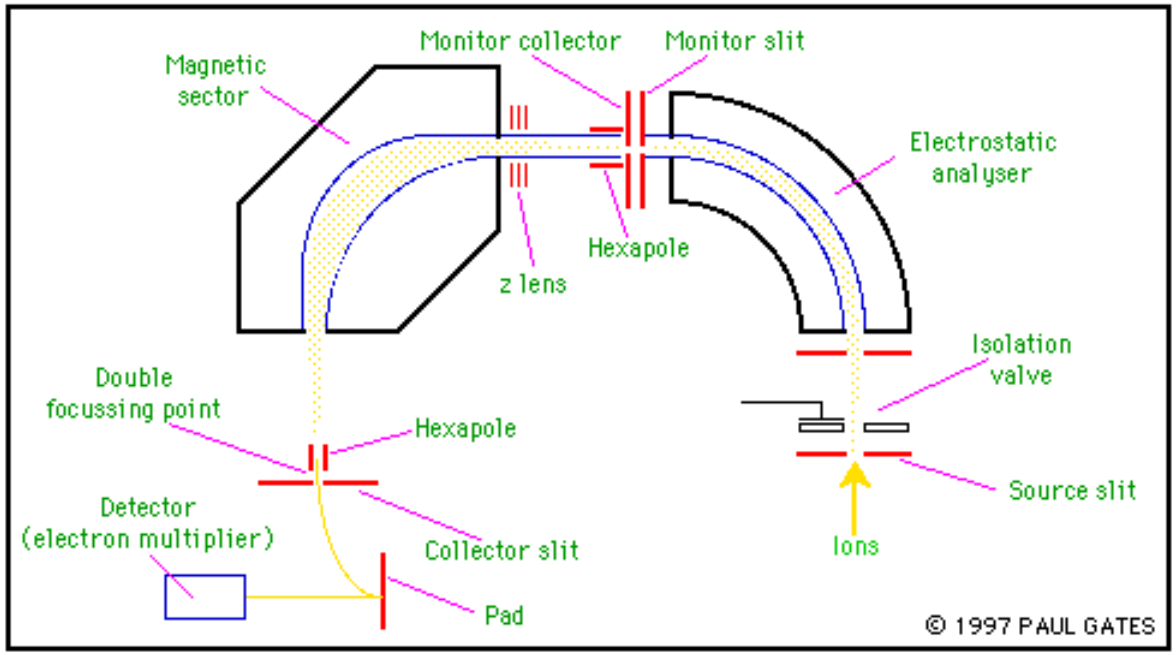
Analyzers

- Magnetic sectors
- Quadrupoles
- Ion trap
- Time-of-flight
- Fourier transform ion cyclotron resonance FT-ICR

Choice of mass analyzer:

- Mass range
- Resolving power (separation of ions of close m/z)
- Mass accuracy
- Ion transmission—sensitivity
- Scanning speed
- Each of use with ancillary equipment

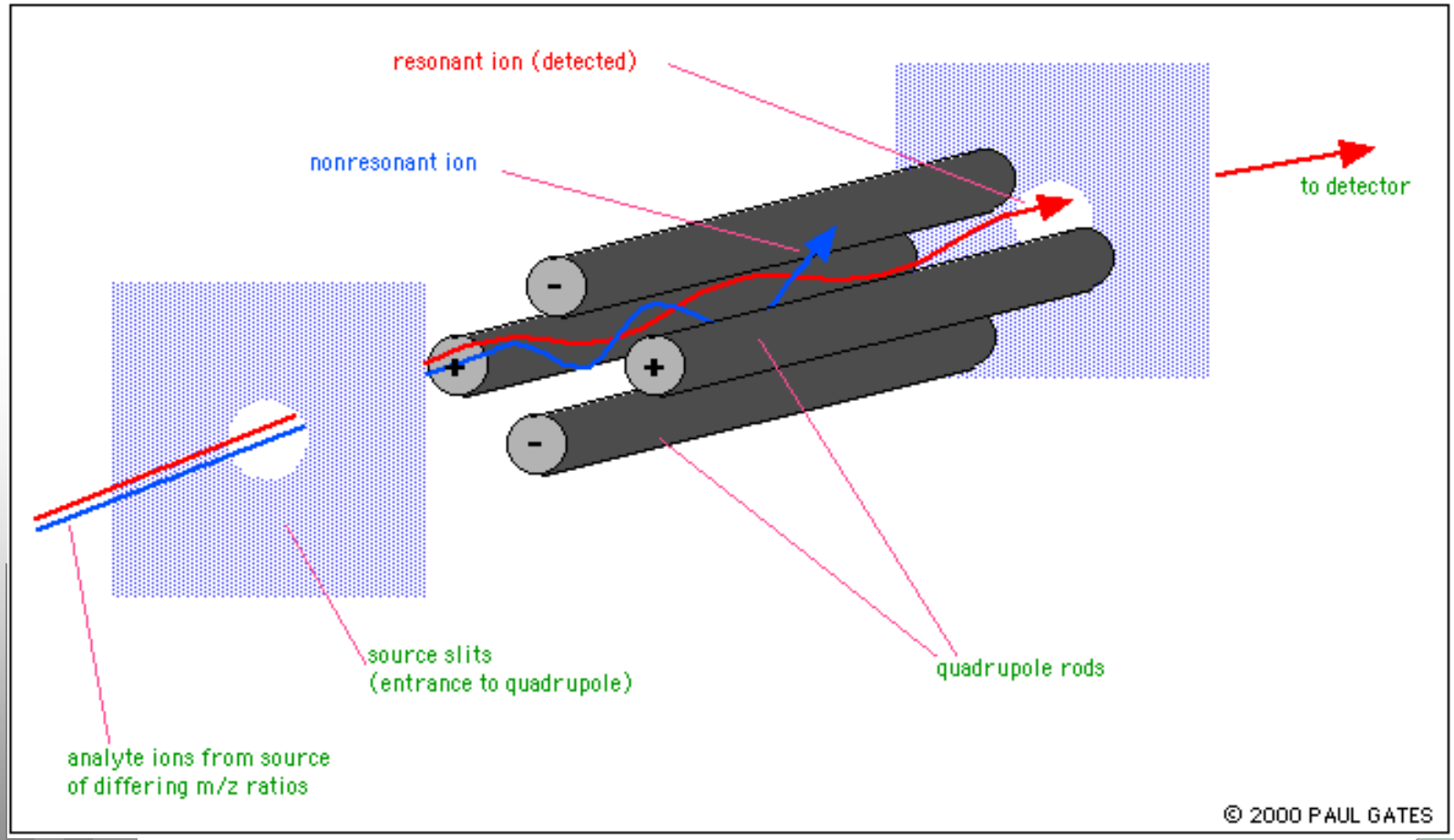
Magnetic sectors



$$\frac{m_i}{z_i} = \frac{B^2 r^2}{2V_a}$$

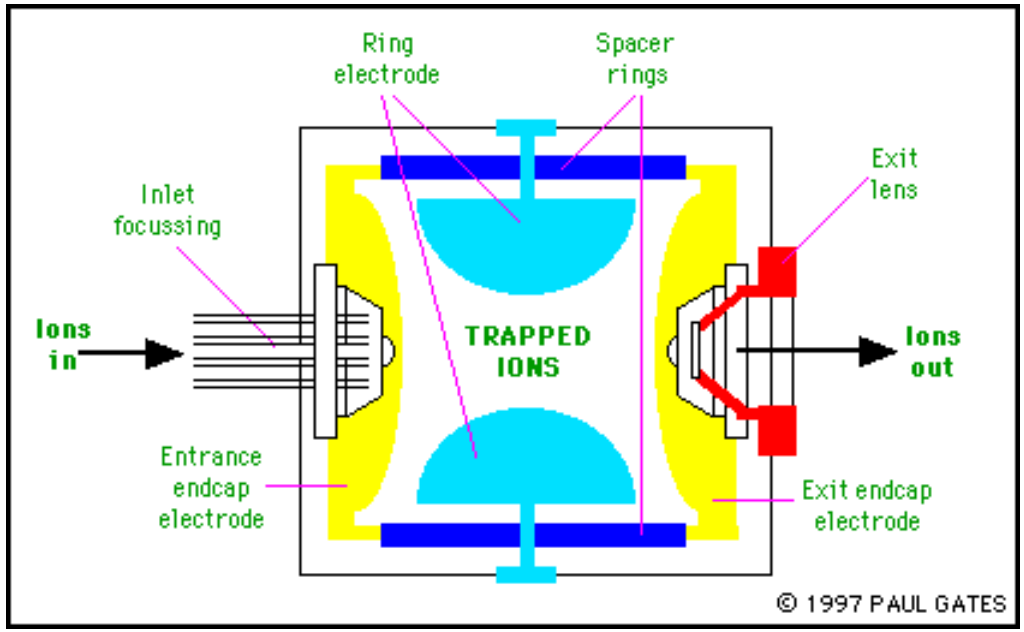
- Magnetic sectors bend the trajectories of ions into circular paths of radii that depend on the momentum-to-charge ratios of the ions.
- Ions of larger m/z follow larger radius paths than ions of smaller m/z values so ions of differing m/z values are dispersed in space.
- By changing the ion trajectories through variations of the magnetic field strength, ions of different nominal mass-to-charge ratios can be focused on a detector

Quadrupoles



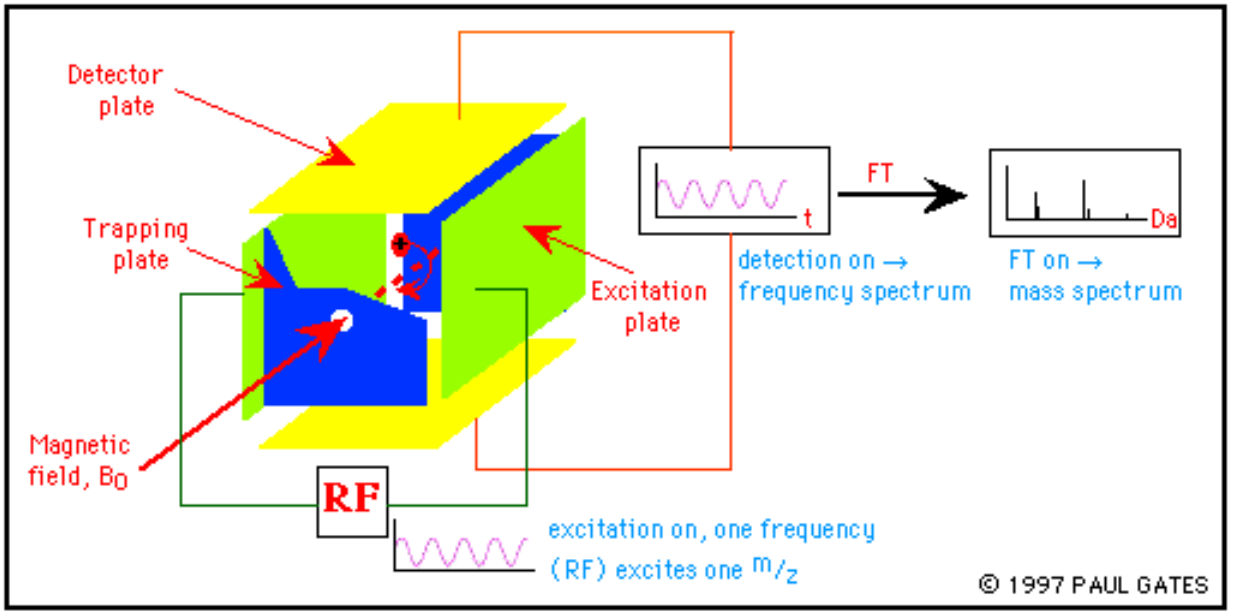
- A quadrupole mass filter consists of four parallel poles or rods.
- Mass sorting depends on ion motion resulting from simultaneously applied constant (dc) and radio frequency electric (rf) electric fields.
- Scanning is accomplished by systematically changing the field strengths, thereby changing the m/z value that can be transmitted through the analyzer.
- Quadrupole mass spectrometers provide lower resolution than double focusing instruments but tend to be more easily interfaced to various inlet systems and to be less costly.

Ion trap


Ring electrode
Spacer rings
Exit lens
Inlet focussing
Ions in
TRAPPED IONS
Ions out
Entrance endcap electrode
Exit endcap electrode
© 1997 PAUL GATES

- The quadrupole ion trap mass spectrometer (Figure 6) operates on a principle similar to a quadrupole mass filter. However, it does not operate as a filter. Rather, the ion trap stores ions for subsequent experiments and analysis.
- It uses fields generated by rf (and sometimes dc) voltages applied to electrodes arranged in a sandwich geometry: a ring electrode in the middle with cap electrodes on each end. Within a selected range of mass-to-charge ratios determined by the applied voltages, the device traps ions in the space bounded by the electrodes.
- Typically, a mass spectrum is produced by scanning the applied rf voltages to eject ions sequentially of increasing mass-to-charge ratio through an end cap opening for detection.

FT-ICR



$$\frac{m_i}{z_i} = \frac{\mathbf{B}_0}{2\pi\omega_c}$$

- For FT-ICR spectrometer ions are trapped electrostatically within a cubic cell in a constant magnetic field.
- A covalent orbital ("cyclotron") motion is induced by the application of a radio-frequency pulse between the excite plates. The orbiting ions generate a faint signal in the detect plates of the cell.
- The frequency of the signal from each ion is equal to its orbital frequency, which in turn is inversely related to its m/z value. The signal intensity of each frequency is proportional to the number of ions having that m/z value.
- The signal is amplified and all the frequency components are determined, yielding the mass spectrum.
- If the pressure in the cell is very low, the ion orbital motion can be maintained over many cycles and the frequency can be measured with very high precision. The FT-ICR instrument can therefore be used to generate very high resolution spectra.

Time of flight

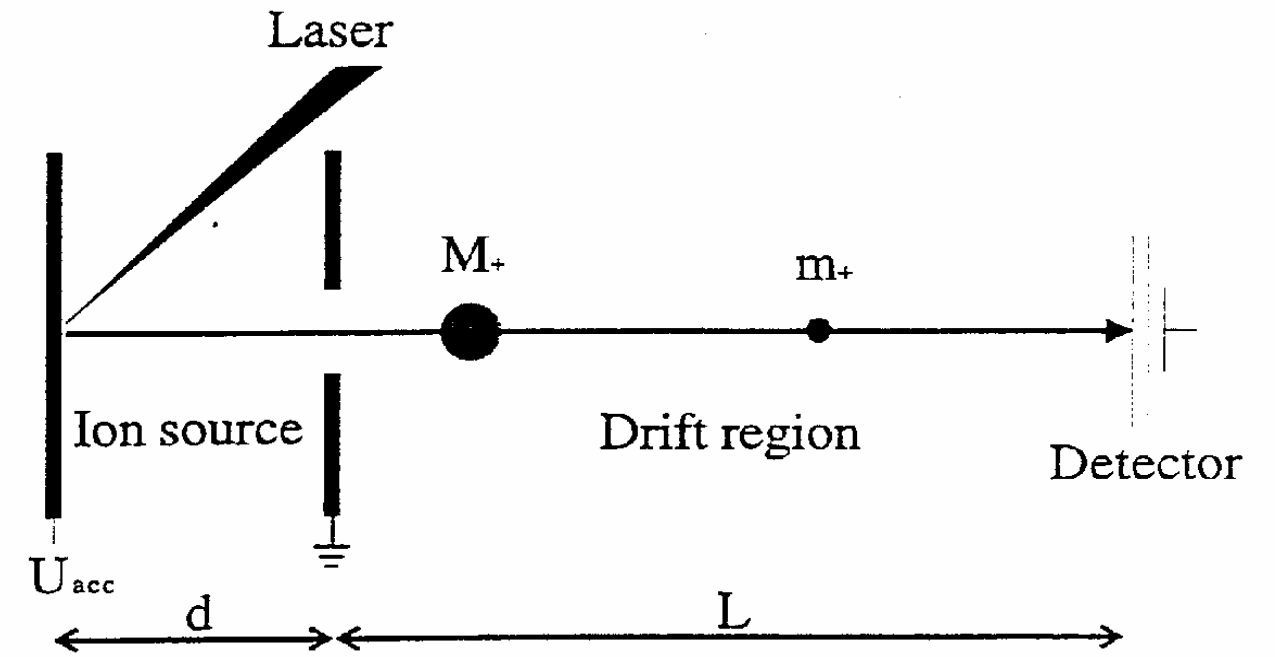


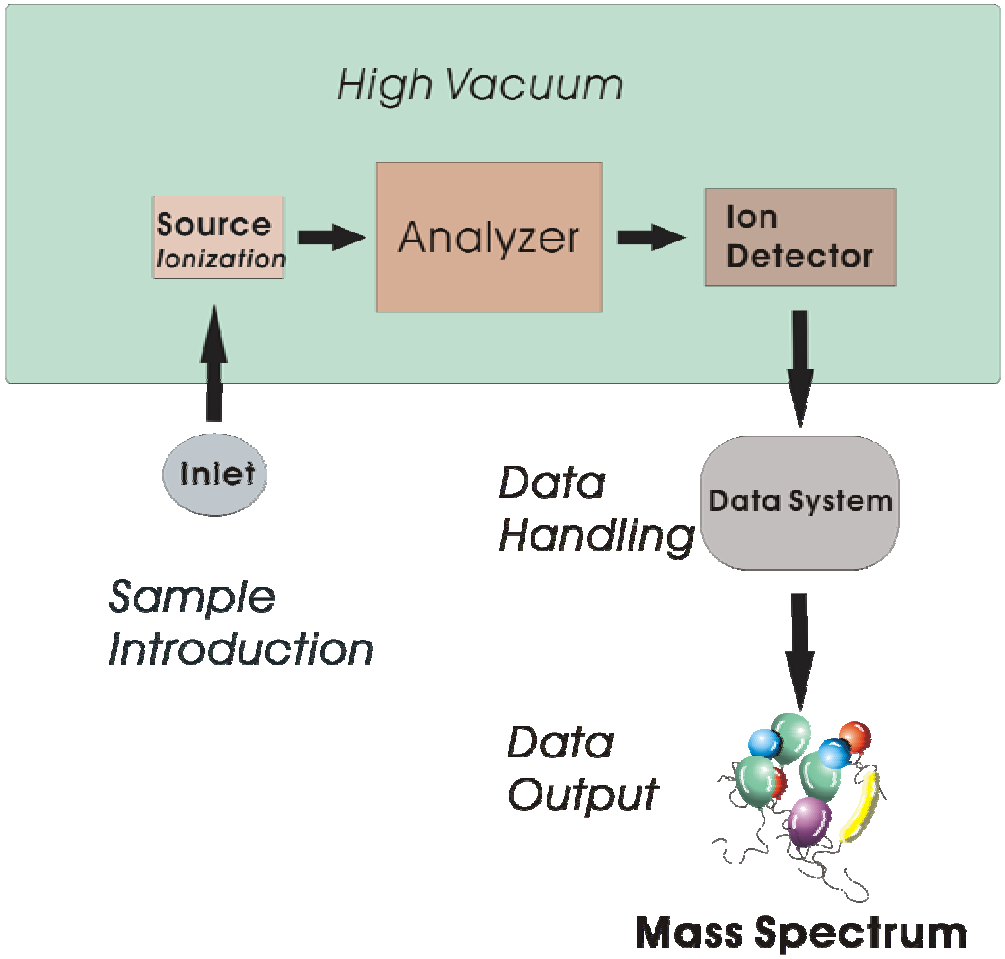
Figure 1.1. Schematic diagram of a linear TOF mass spectrometer

- Time-of-flight mass analyzers separate ions by virtue of their different flight times over a known distance.
- A brief burst of ions is emitted from a source. These ions are accelerated so that ions of like charge have equal kinetic energy are directed into a flight tube.
- Kinetic energy is equal to $1/2\ mv^2$, the lower the ion's mass, the greater the velocity and shorter its flight time. The travel time from the ion source through the flight tube to the detector, can be transformed to the m/z value.
- Because all ion masses are measured for each ion burst, TOF mass spectrometers offer high sensitivity as well as rapid scanning. They can provide mass data for very high-mass biomolecules.

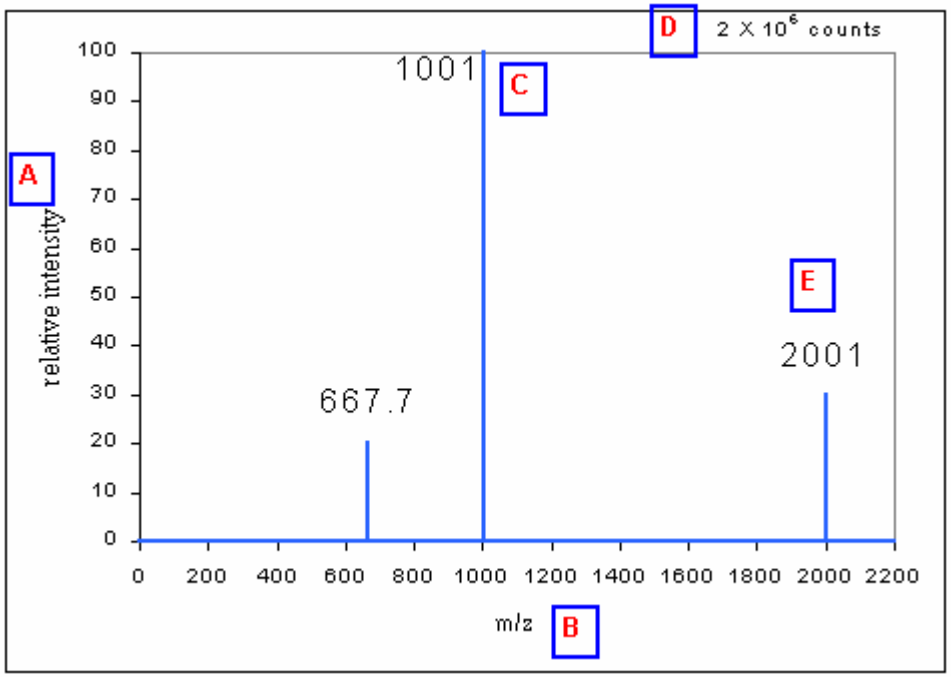
Analyzers

- ~~Magnetic sectors~~
- Quadrupoles
- Ion trap
- Time-of-flight
- Fourier transform ion cyclotron resonance FT-ICR

Components of a mass spectrometer

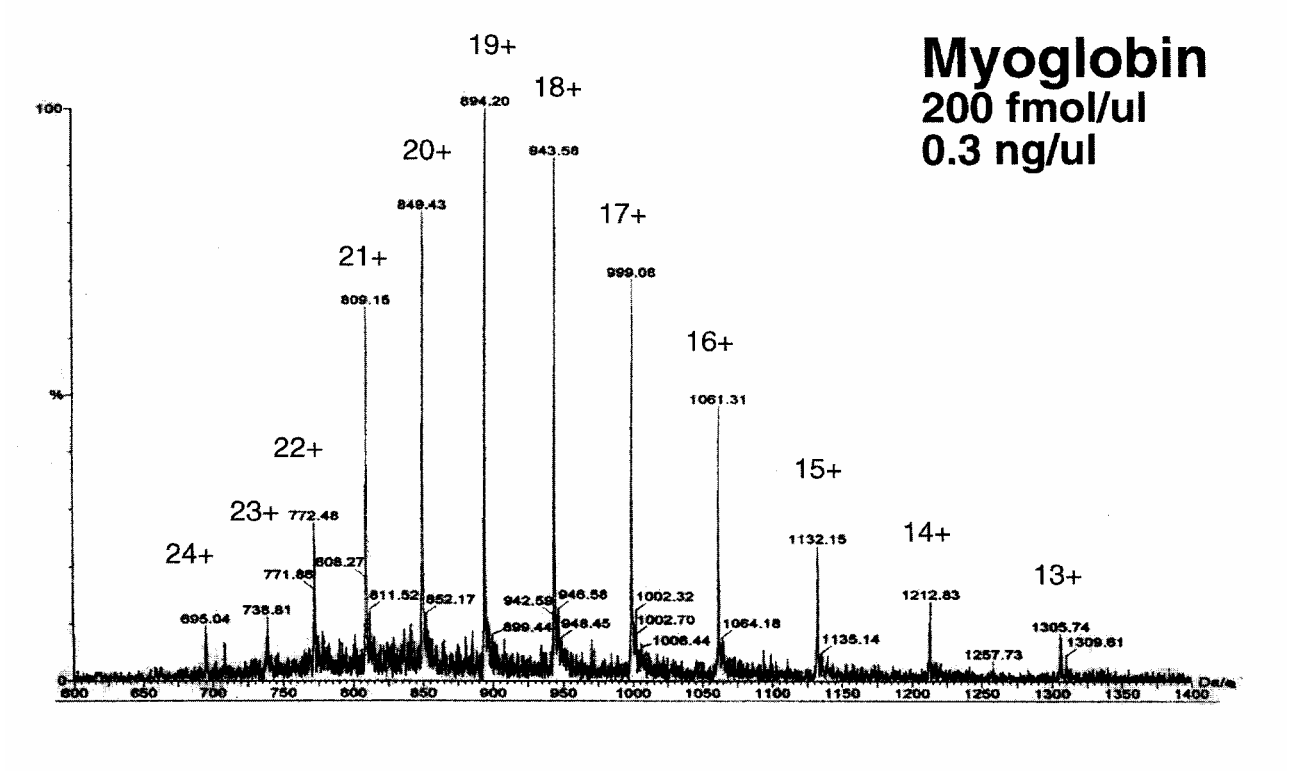


What do you see in a spectrum?



- A = relative intensity, related to the base peak.
- C is the base peak.
- D is counts related to the base peak.
- B is the m/z, all three peaks are from the same compound.

ESI spectrum from quadrupole machine



$M_1Z_1 = Mr + 1.0079\ Z_1$

$M_2Z_2 = Mr + 1.0079\ Z_2$

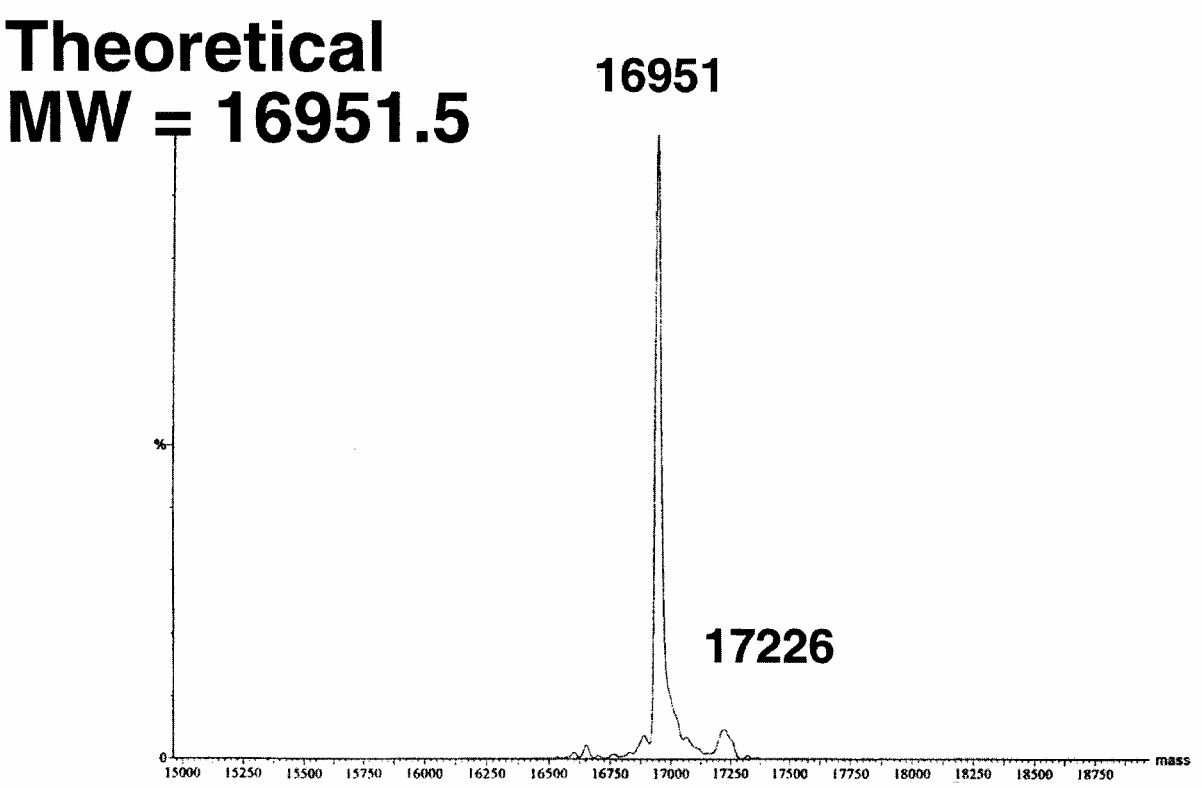
Assume adjacent peaks represent species differing by only one charge, and charge is due to protonation.

At $M_2 > M_1$

$Z_2 = Z_1 - 1$

Then $Z_1 = (M_2 - 1.0079)/(M_2 - M_1)$

Average of data pairs gives high accuracy.



Average molecular weight!

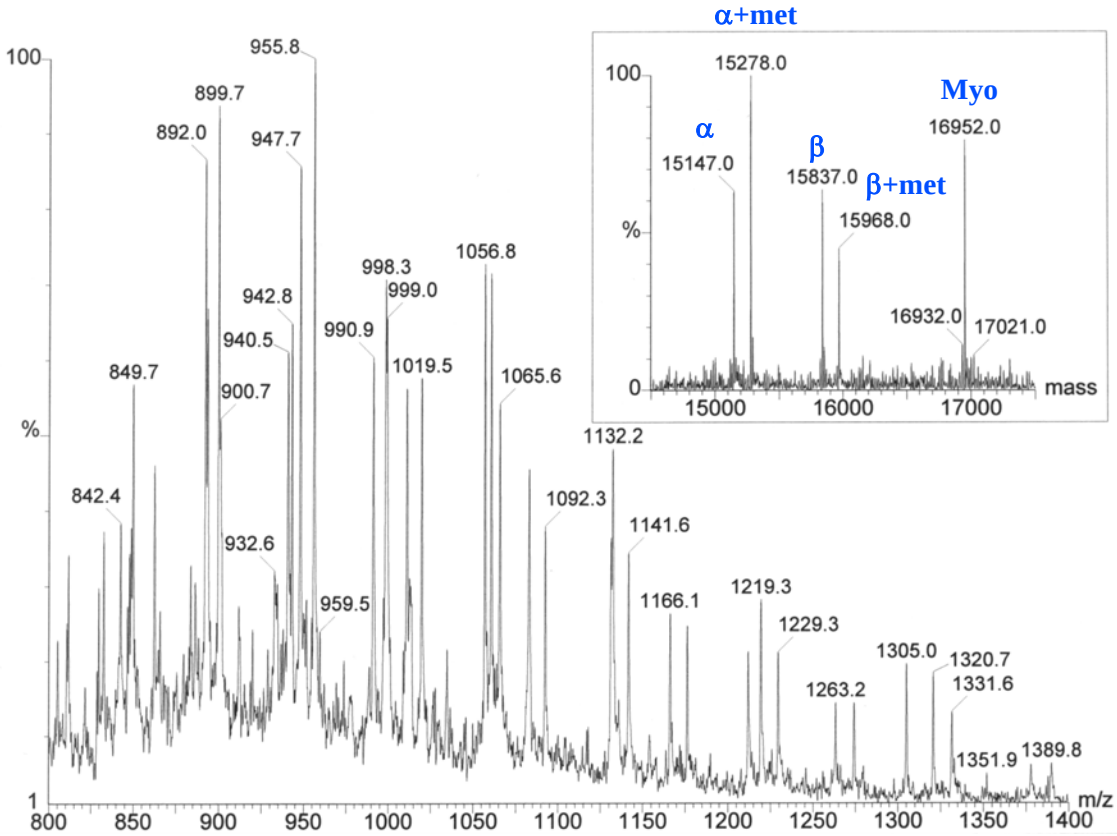
Expect 1 Da deviation per 10,000 Da (0.01% error or 100 ppm)

We talk about accuracy here only!

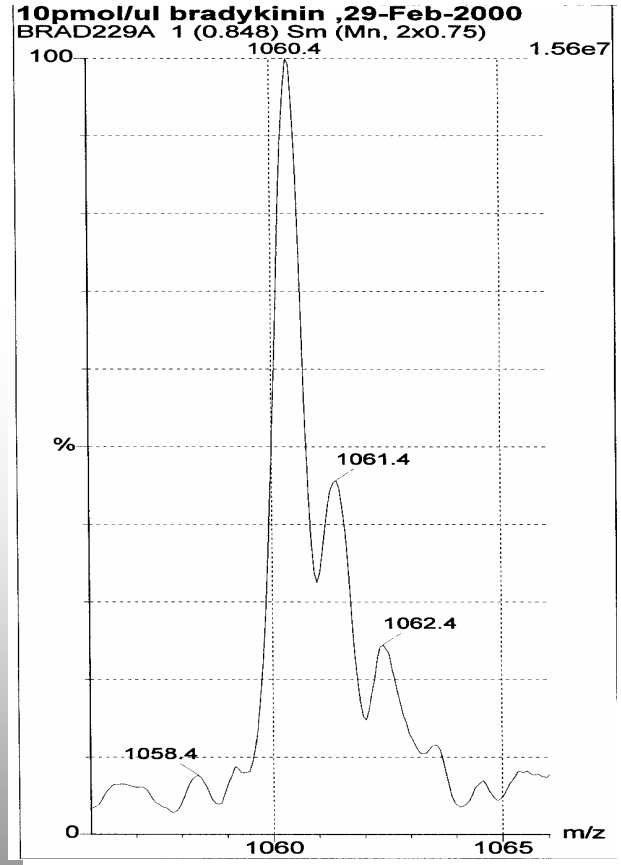
MS 101: you don't need 100% pure sample for measurement.

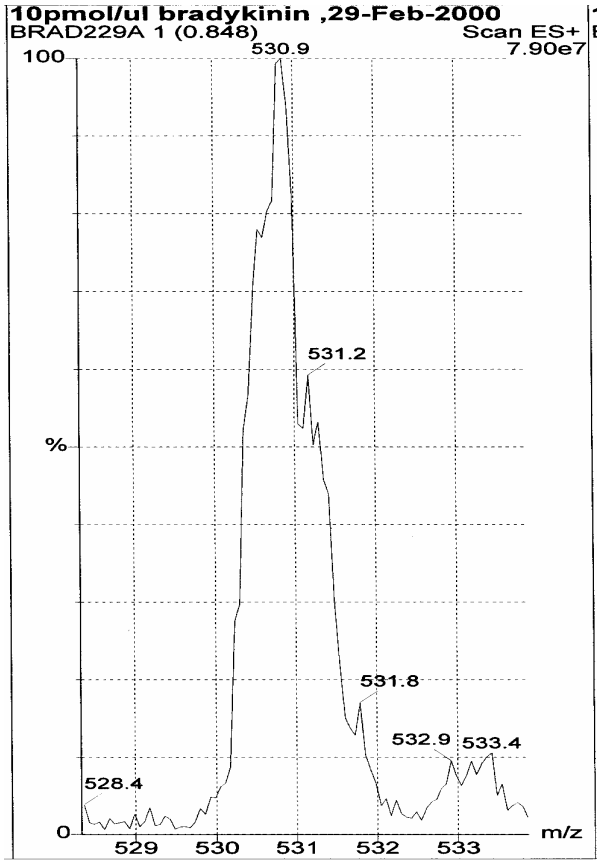
Recombinant Human Hemoglobin αH20R/βE6V, E22Q

α=15145 Da β=15836 Da

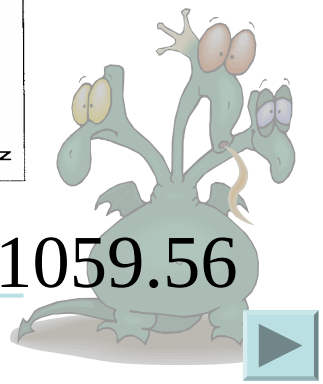


The sharp peaks are not sharp on a quadrupole machine! Bradykinin sample

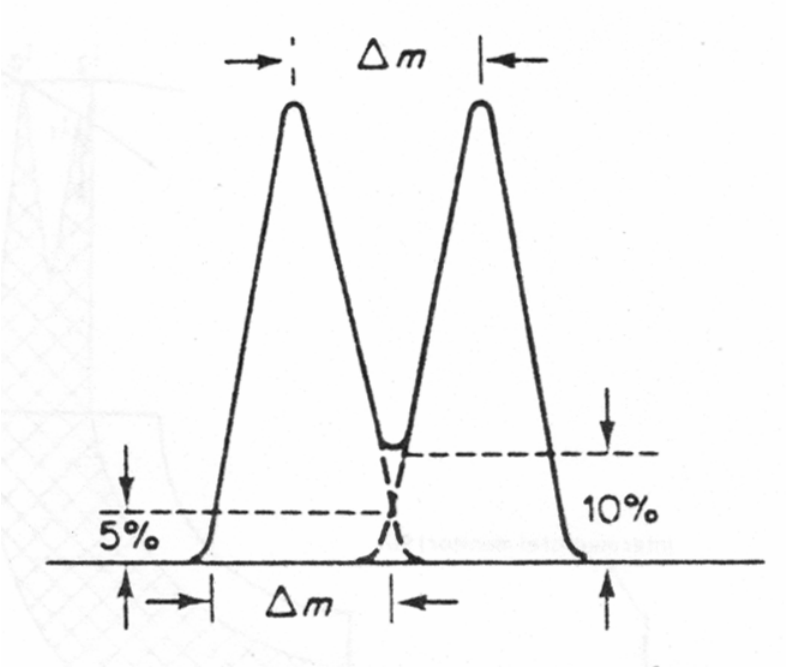




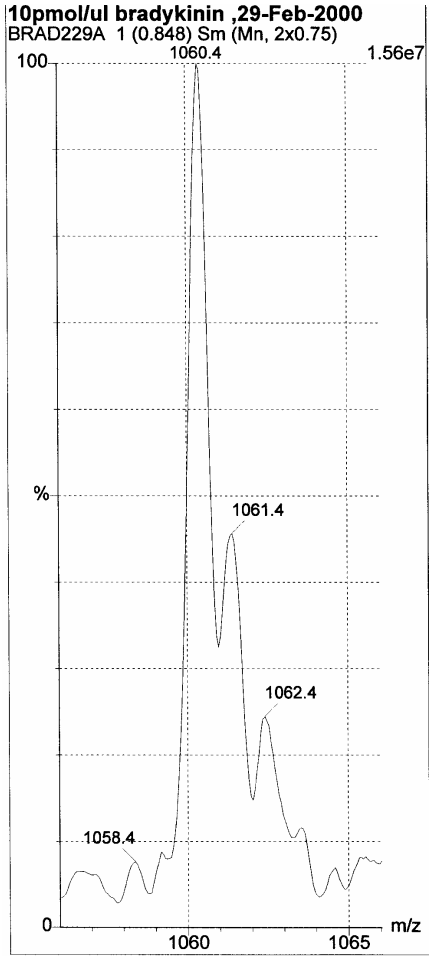
Ave MW 1060.22 Monoisotopic MW 1059.56



That brings to the question of resolution



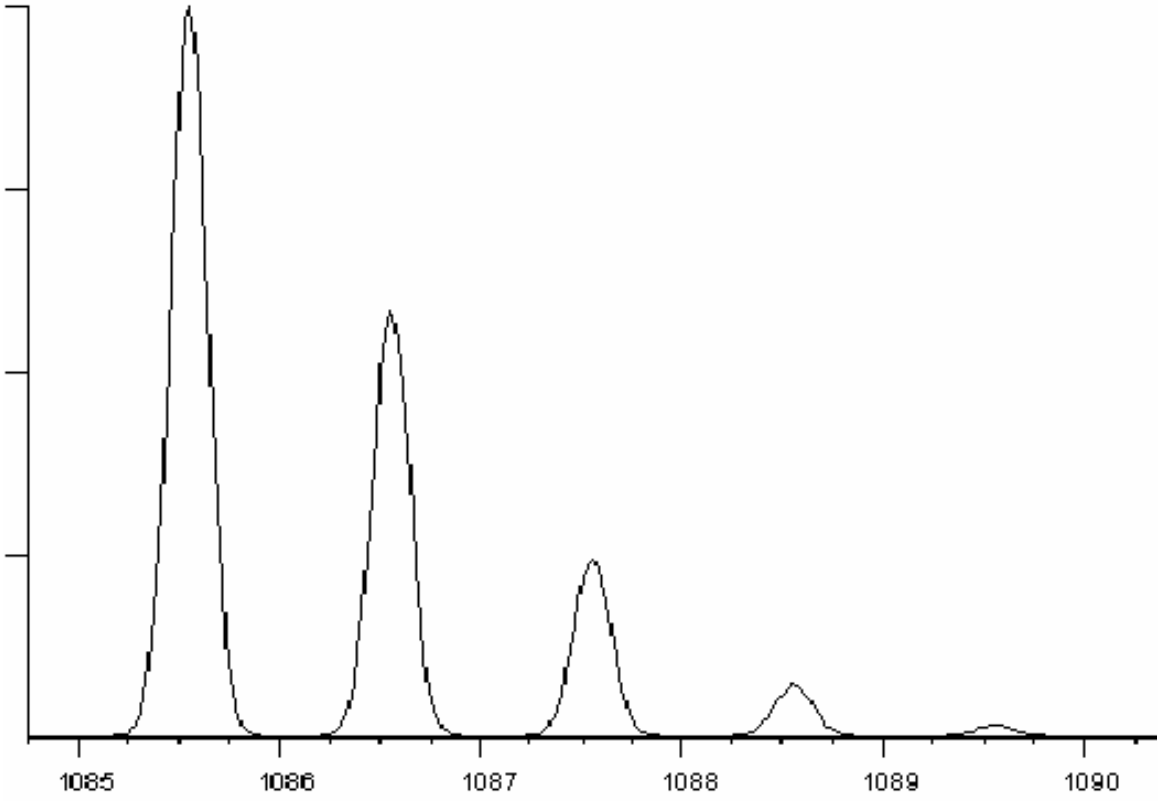
$m/\Delta m$



Note that for quadrupole machine, according to definition, the peaks are not resolved.

At high resolution machines, the peaks can be resolved on the mass spectrum

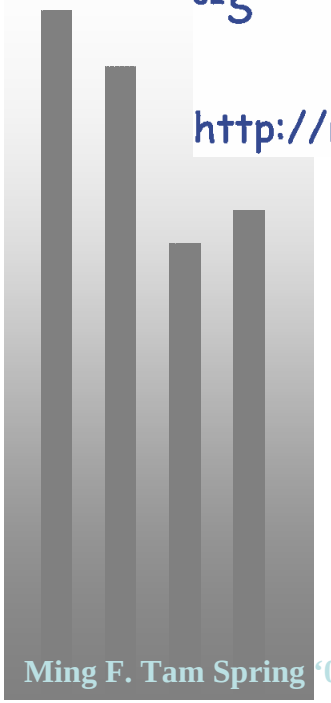
Peptide HLKTEAEMK



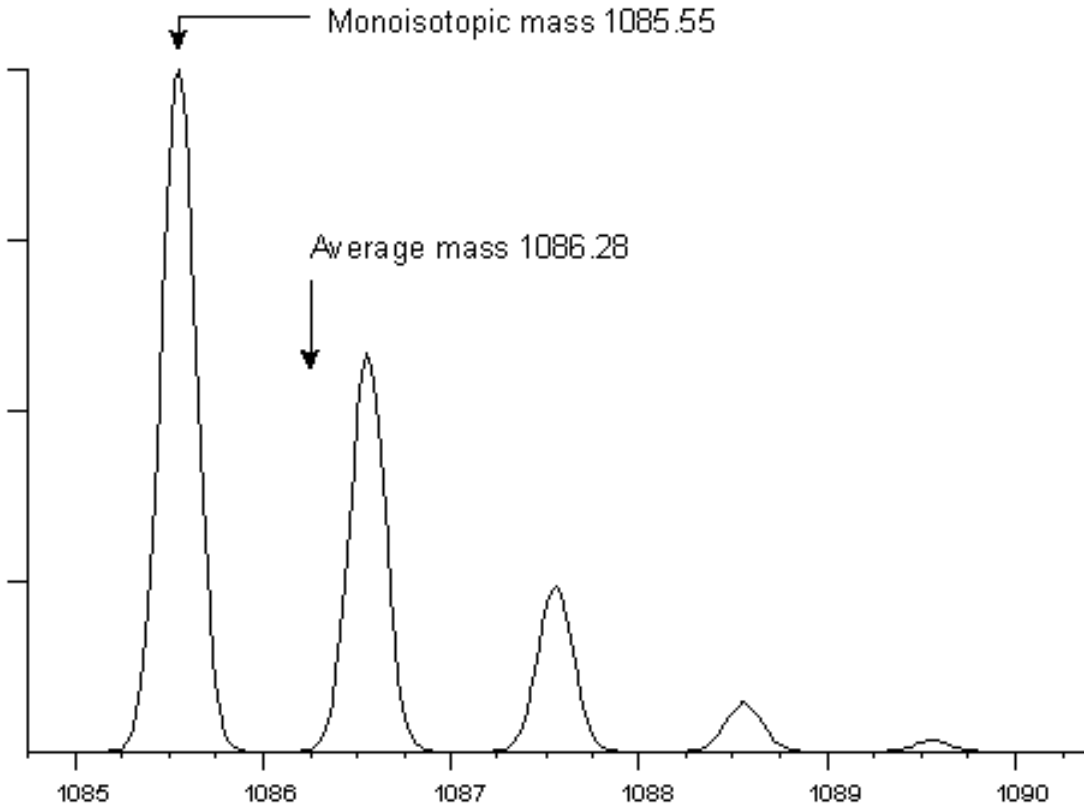
Atomic Masses and Abundances

Isotope	%	Isotope	%	Isotope	%	Isotope	%
^{12}C	98.93	^{13}C	1.07	^{14}C			
^{1}H	99.9885	^{2}H	0.0115	^{3}H			
^{14}N	99.632	^{15}N	0.368				
^{16}O	99.757	^{17}O	0.038	^{18}O	0.205		
^{32}S	94.93	^{33}S	0.76	^{34}S	4.29	^{36}S	0.02

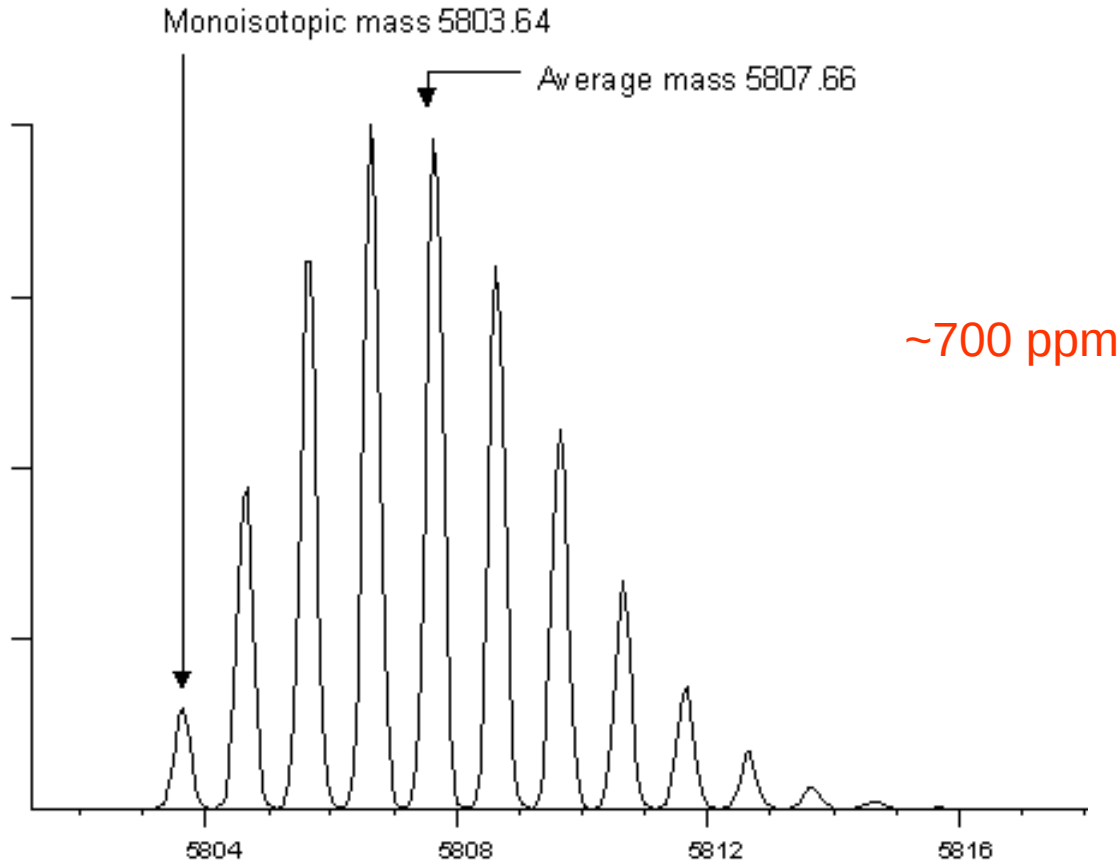
http://ionsource.com/Card/Mass/mass.htm



Peptide HLKTEAEMK

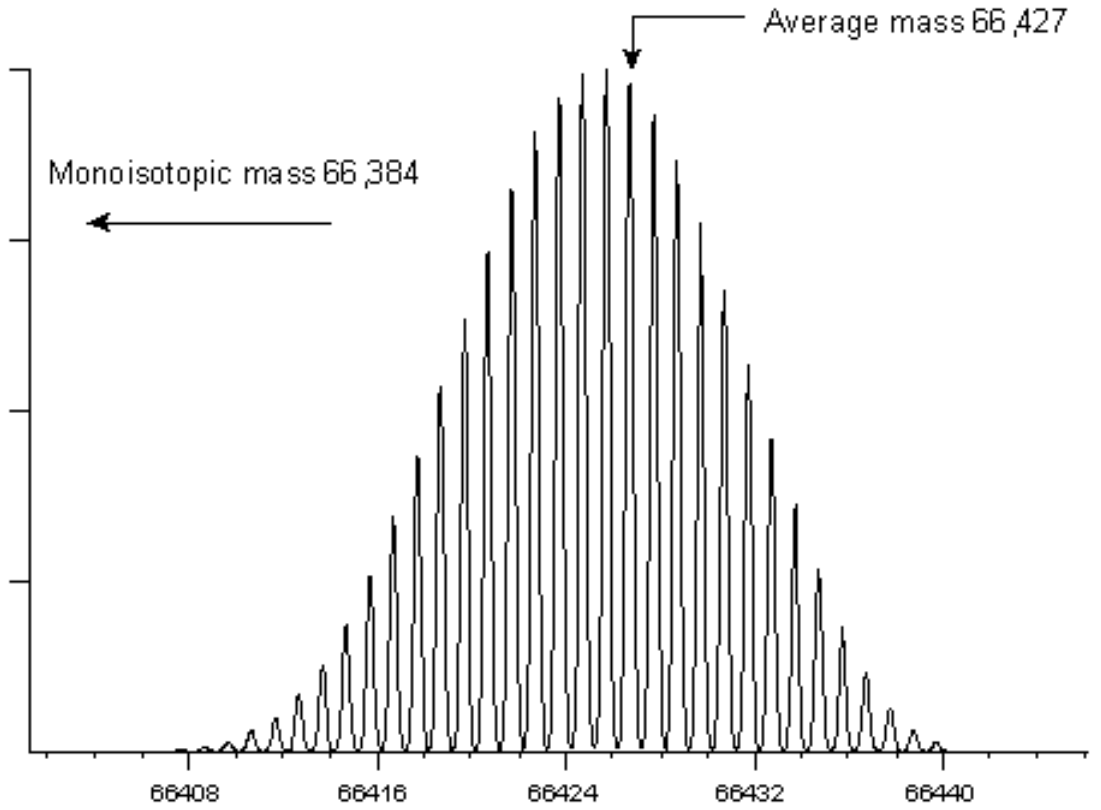


Insulin/small protein TOF spectrum



~700 ppm

BSA/average mass only!



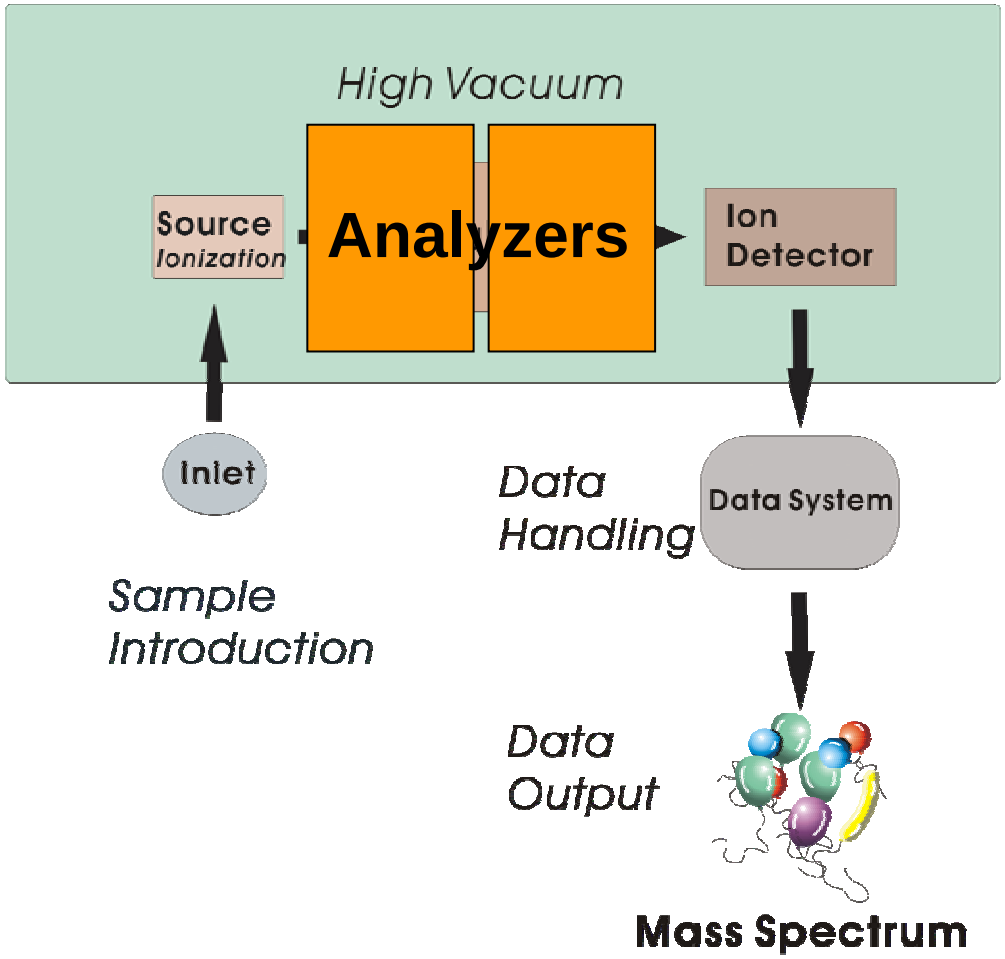
Read the tutorial:

http://ionsource.com/tutorial/isotopes/slide1.htm

Still available on 6/5/2006

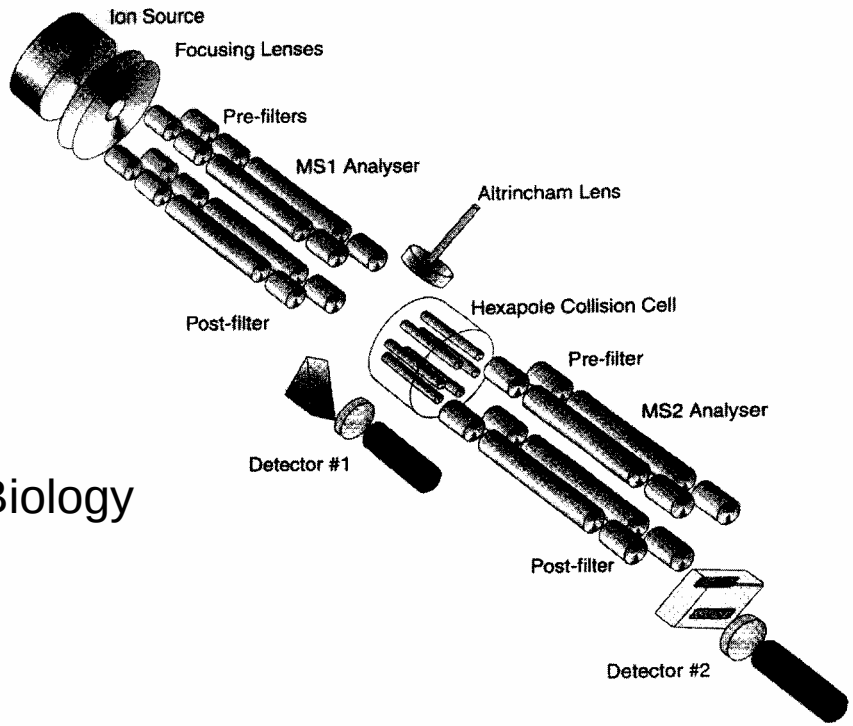
Basic system

Use a combination of analyzers for more versatile applications



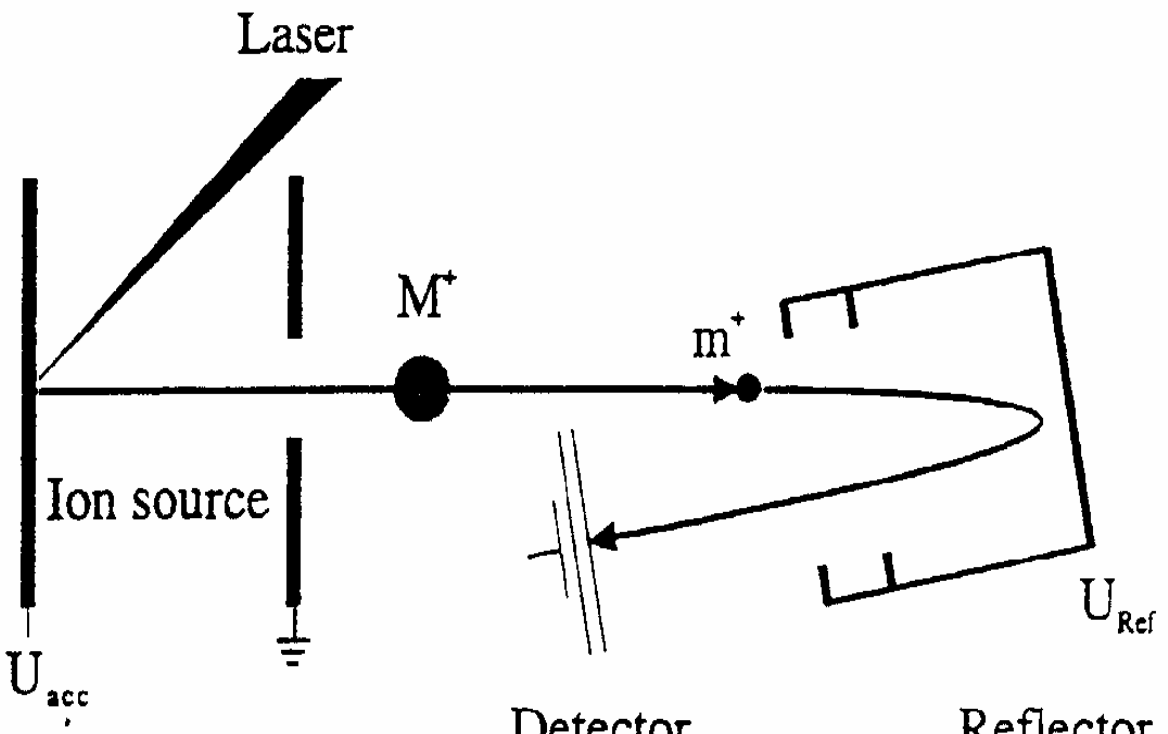
Triple quadrupoles

- Precursor scan
- Sequencing via collison induced dissociation (CID)

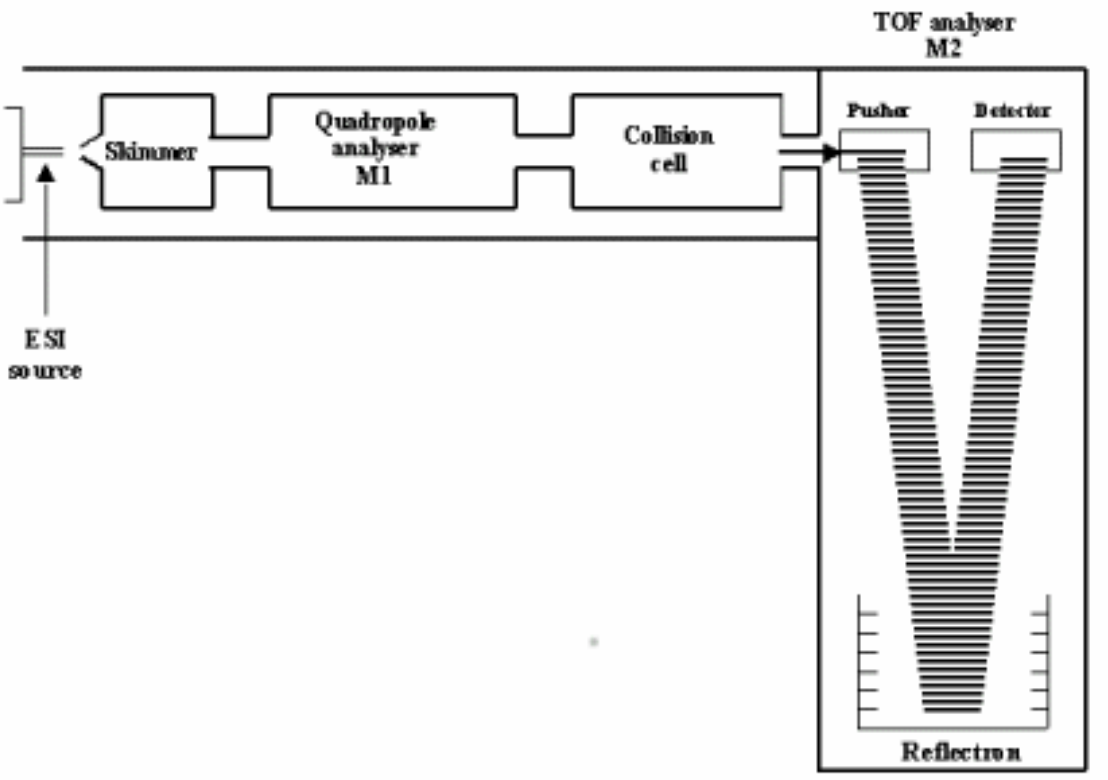


Standing, KG (2003)
Current Opinion Structural Biology
13, 595-601.

Reflectron can do PSD and give partial sequence

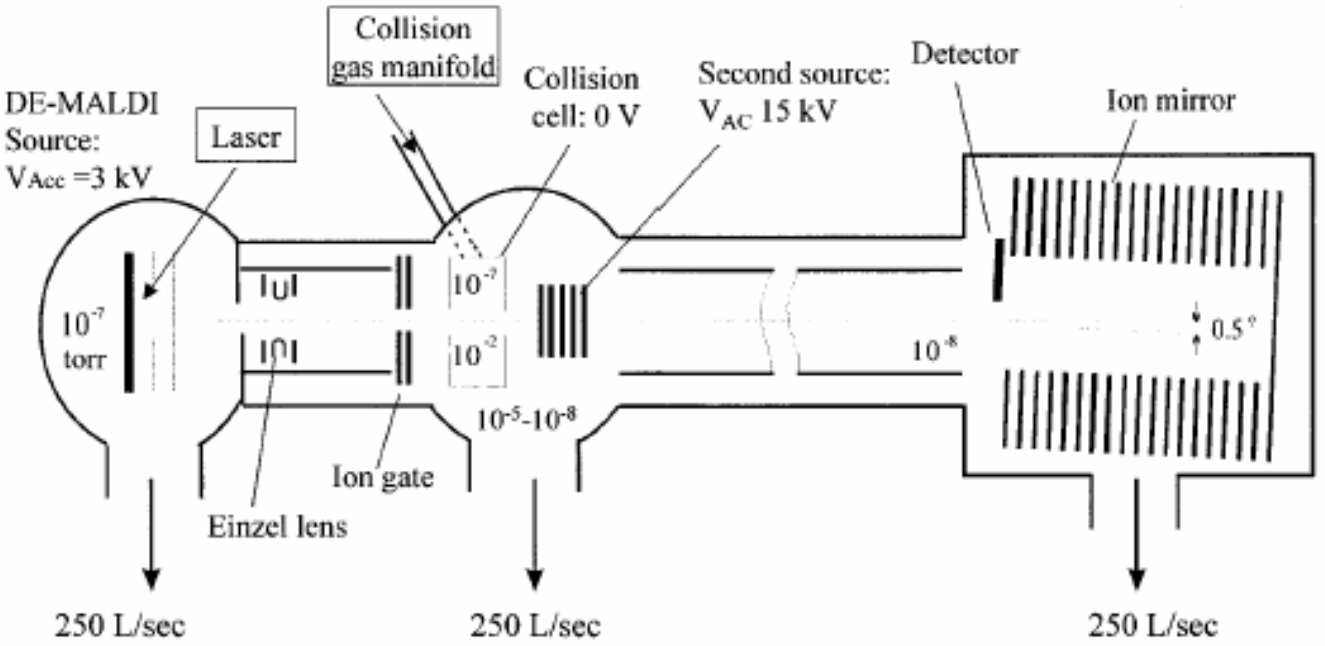


Q-TOF machine

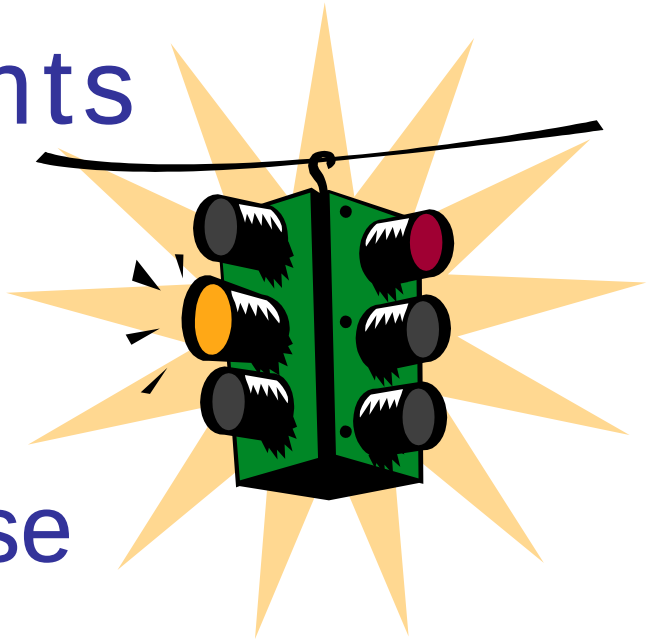


TOF-TOF mass spectrometer

– Anal. Chem. 2000, 72, 552-558



ESI/APCI Instruments



- LC-MS to partially purify the sample. Use mostly RP column

No more big columns

Regular ESI at 1-4 ul/min

Regular bore (4.6 mm) at 0.6 ml/min

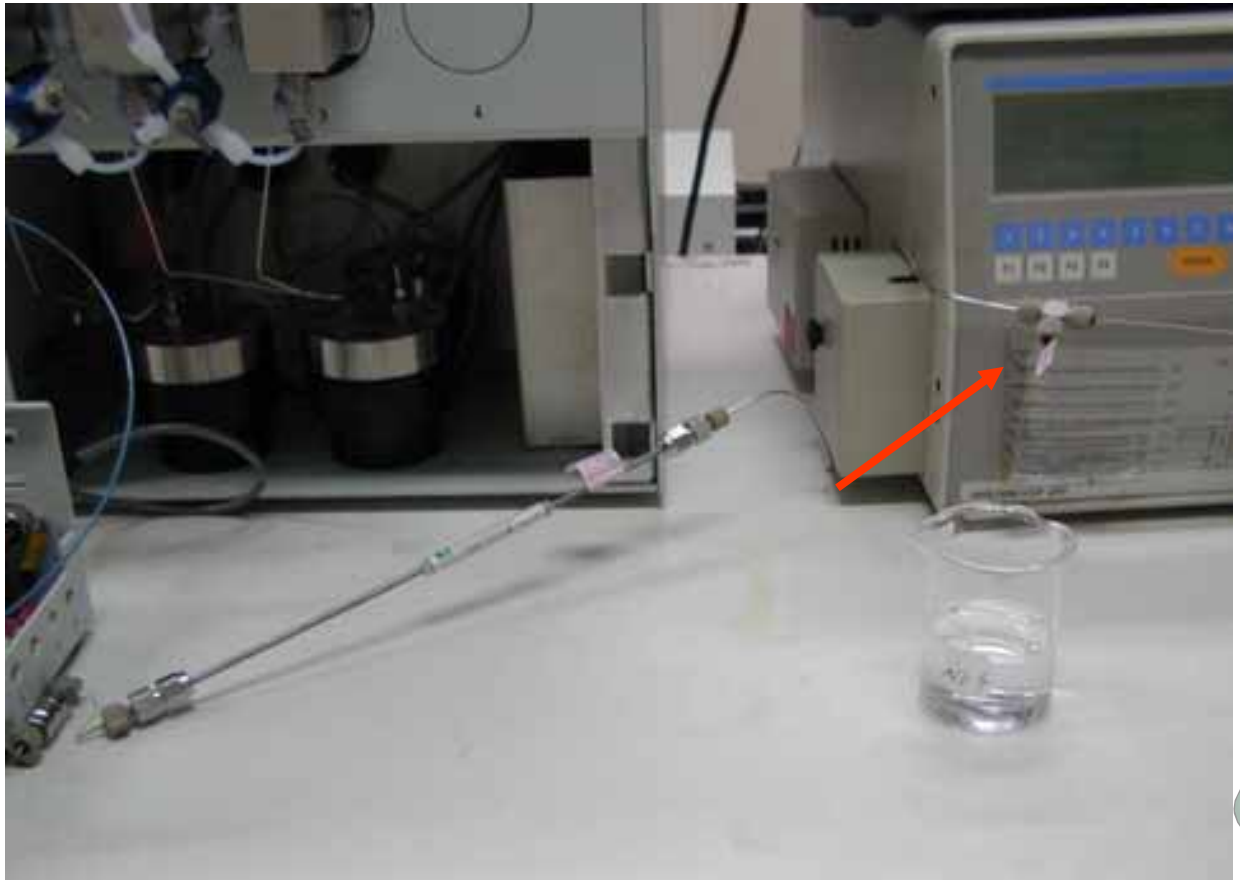
Narrow bore (2.1 mm) at 0.125 ml/min

Micro bore (1 mm) at 28 ul/min

Stone et al. *Methods Enzymol* 1990, 193: 389-412

Most of the sample is wasted

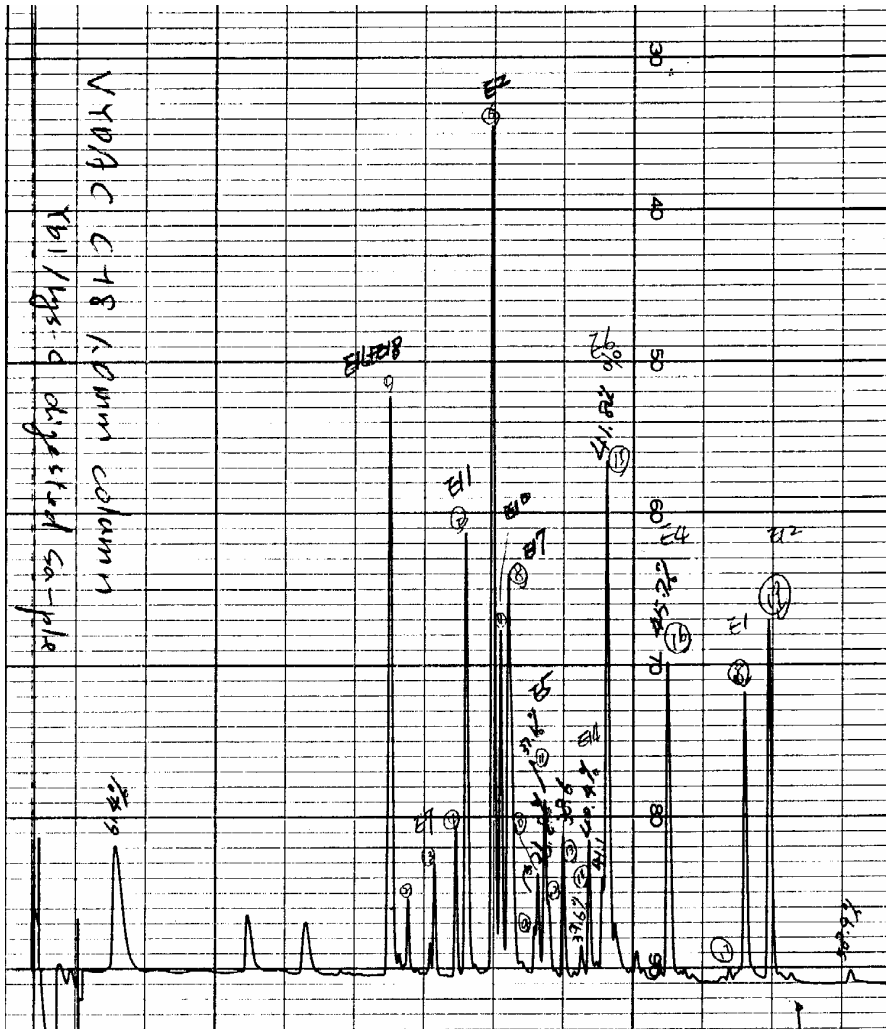
Homemade splitter with capillary tubing—after column splitting



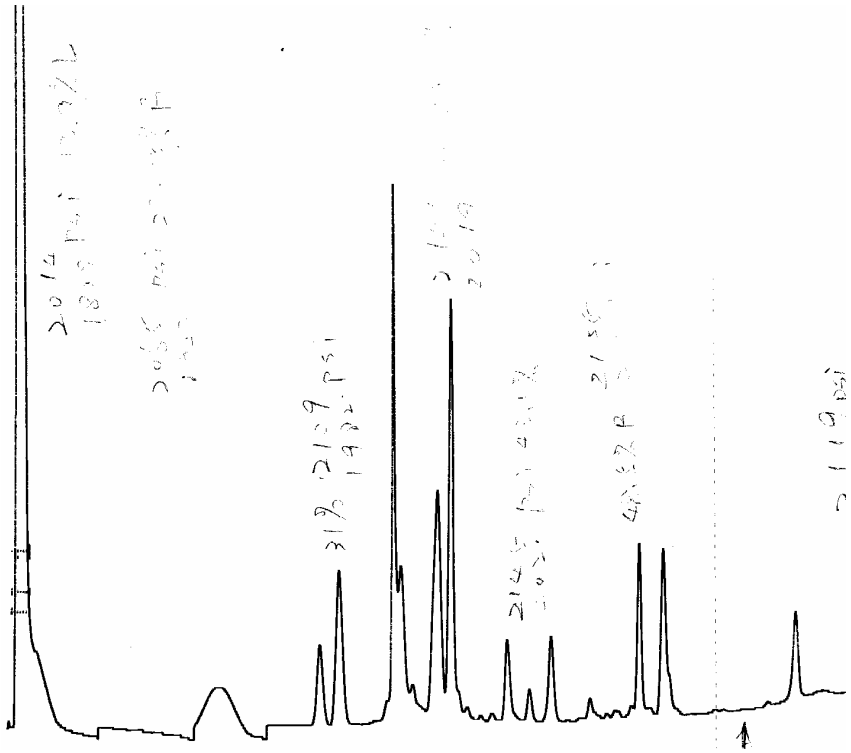
- Capillary column

  0.254 mm id
  2 ul/min

Vydac C18, 1 X 250 mm



SPT C18 , 0.254 X 110 mm



Southan et al. (1999) Anal. Biochem. 271, 152-158

Nano Sprayer + Nano LC

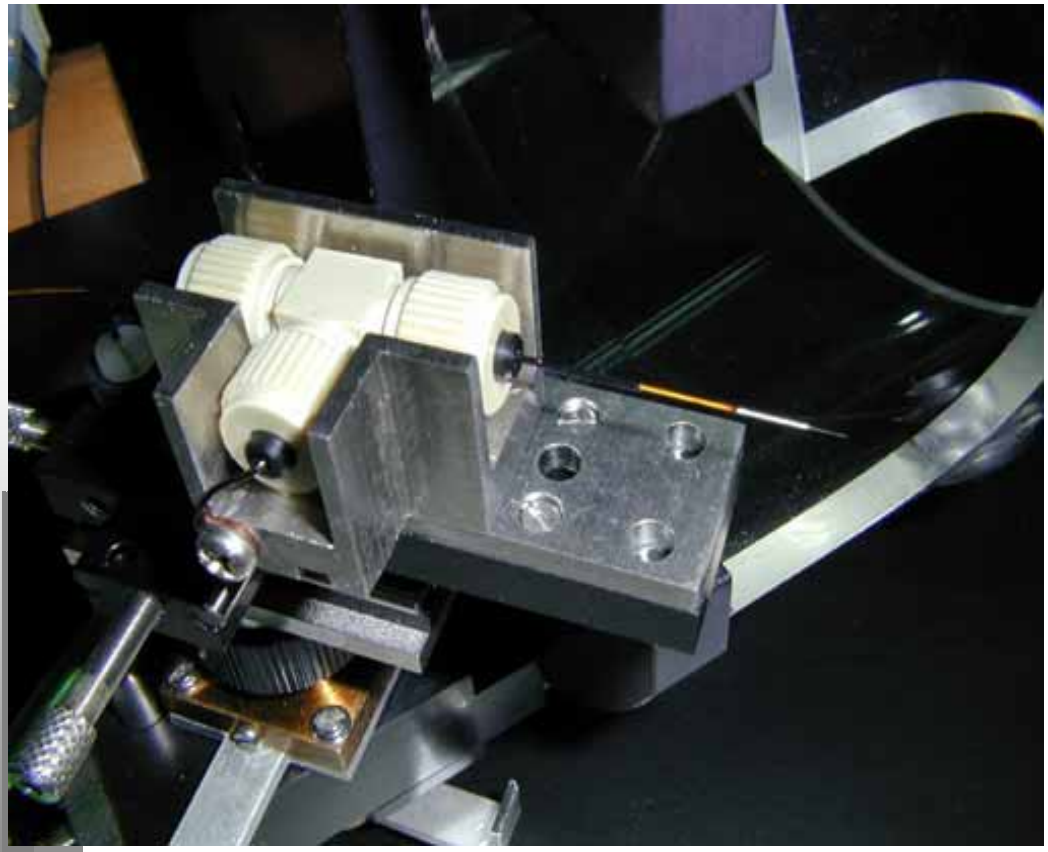
0.15 mm ID	300 nl/min
0.05 mm ID	50-70 nl/min

Monolithic column.
Column in the sprayer tip.

Nanoflow—continuous flow

Modification + Monolithic column

0.15 mm ID	300 nl/min
0.05 mm ID	50-70 nl/min



Moore et al. (1998)
Anal. Chem.
70, 4879-4884

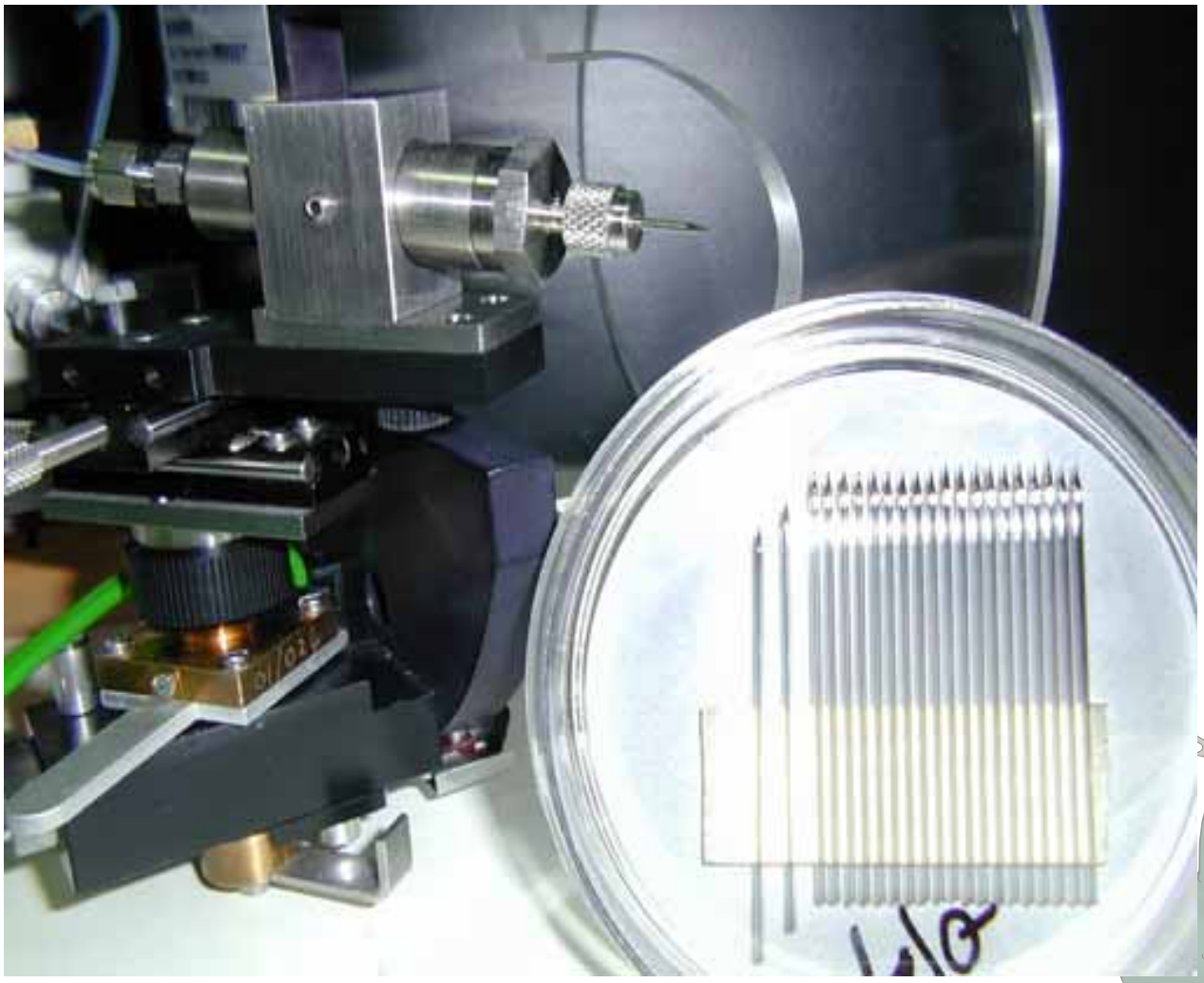
Technical problems:

Delays, sample and void volumns, after column diffusion.

Hydrophilic peptides, hydrophobic peptides.

Buffer problems.

- Nanoflow—single shot
  20-50 nl/min



Read:

"Rapid protein identification using direct infusion nanoelectrospray ionization mass spectrometry"

Sixue Chan (2006) Proteomics, 6: 16-25.

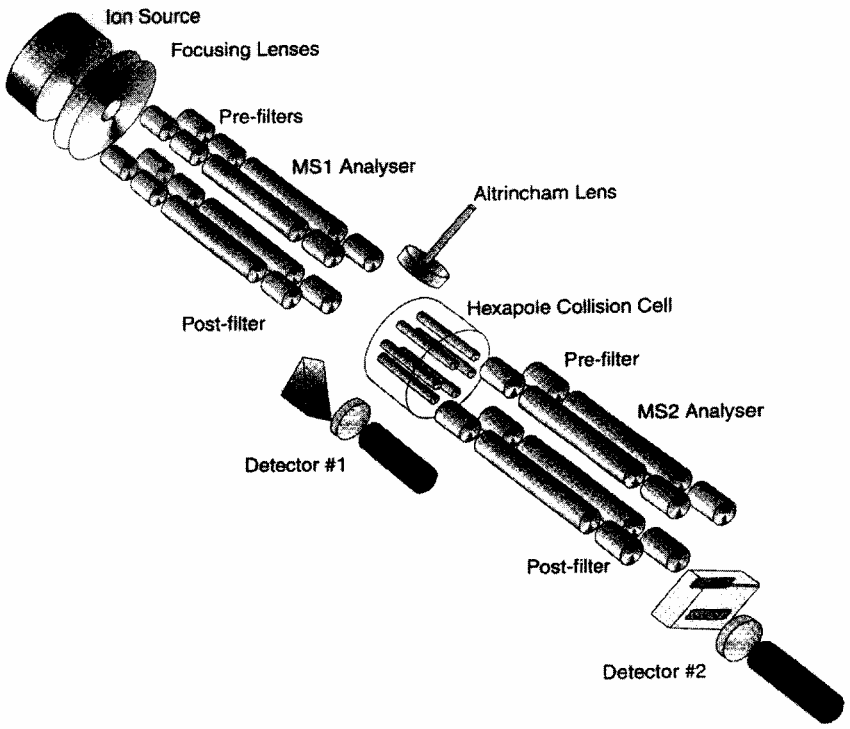
Summary

- Brief introduction of machines

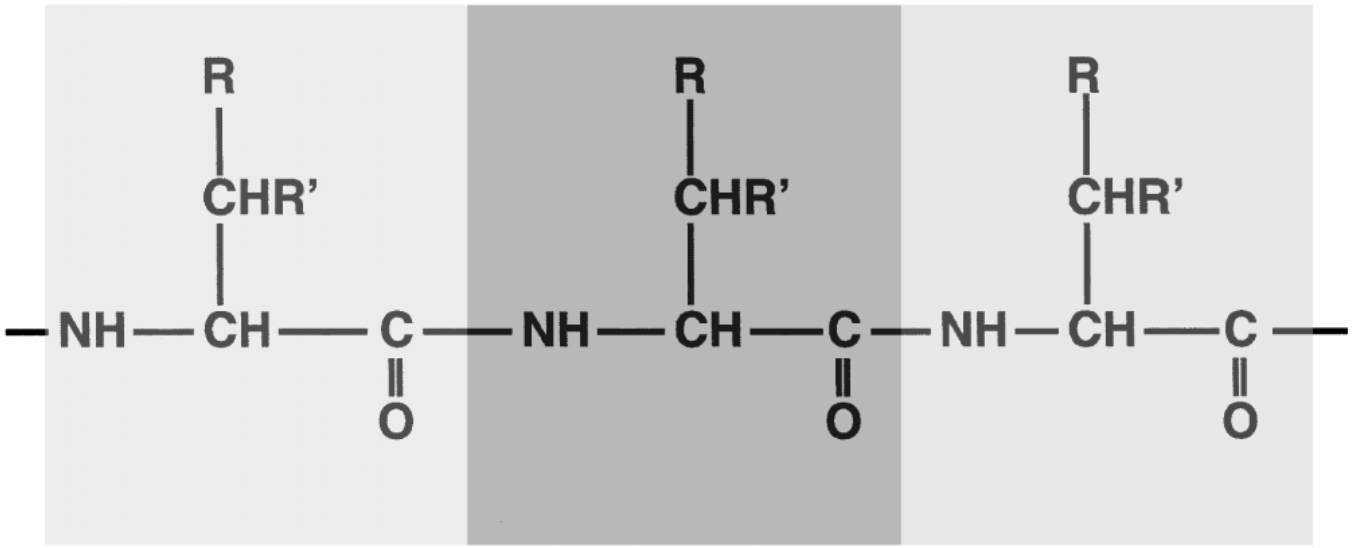
  Ionization, Analyzers
- Brief introduction to spectrum interpretation, calculation of mass.
- Brief introduction of isotopic peaks.
- Introduction to MS/MS instruments.
- Introduction to LC/MS and nanoflow.


Intermission

Peptide sequencing on triple quadrupoles



This is a peptide:



Residue$_{n-1}$ Residue$_{n}$ Residue$_{n+1}$

Residue mass = mass of a.a. – H_2O

Gly	57	Gln	128
Ala	71	Lys	128
Ser	87	Glu	129
Pro	97	Met	131
Val	99	His	137
Thr	101	Phe	147
Cys	103	Arg	156
Lxx	113	Tyr	163
Asn	114	Trp	186
Asp	115		

Before fragmentation, the ion of the peptide is called the molecular ion or parent ion.

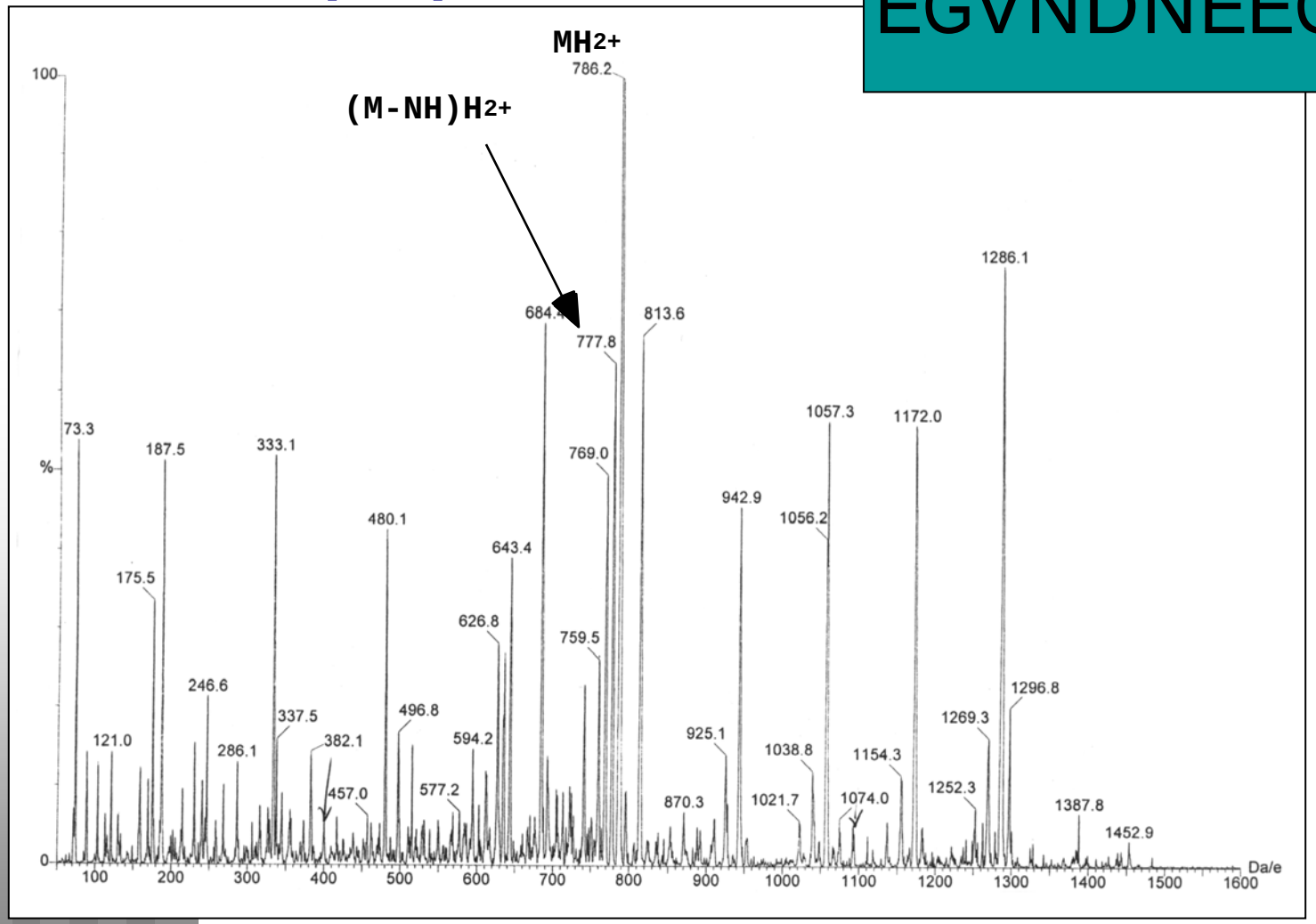
The ions generated after fragmentation are called daughter ions.

*For a singly charged molecular ion (MH^+), where is the additional proton?

** If we have a doubly charged molecular ion ($M+2H^+$), where are the protons?

Fibrinopeptide B

EGVNDNEEGFFSAR



EGVNDNEEGFFSAR

Molecular ion $(M+H)^+$ is the parent ion.

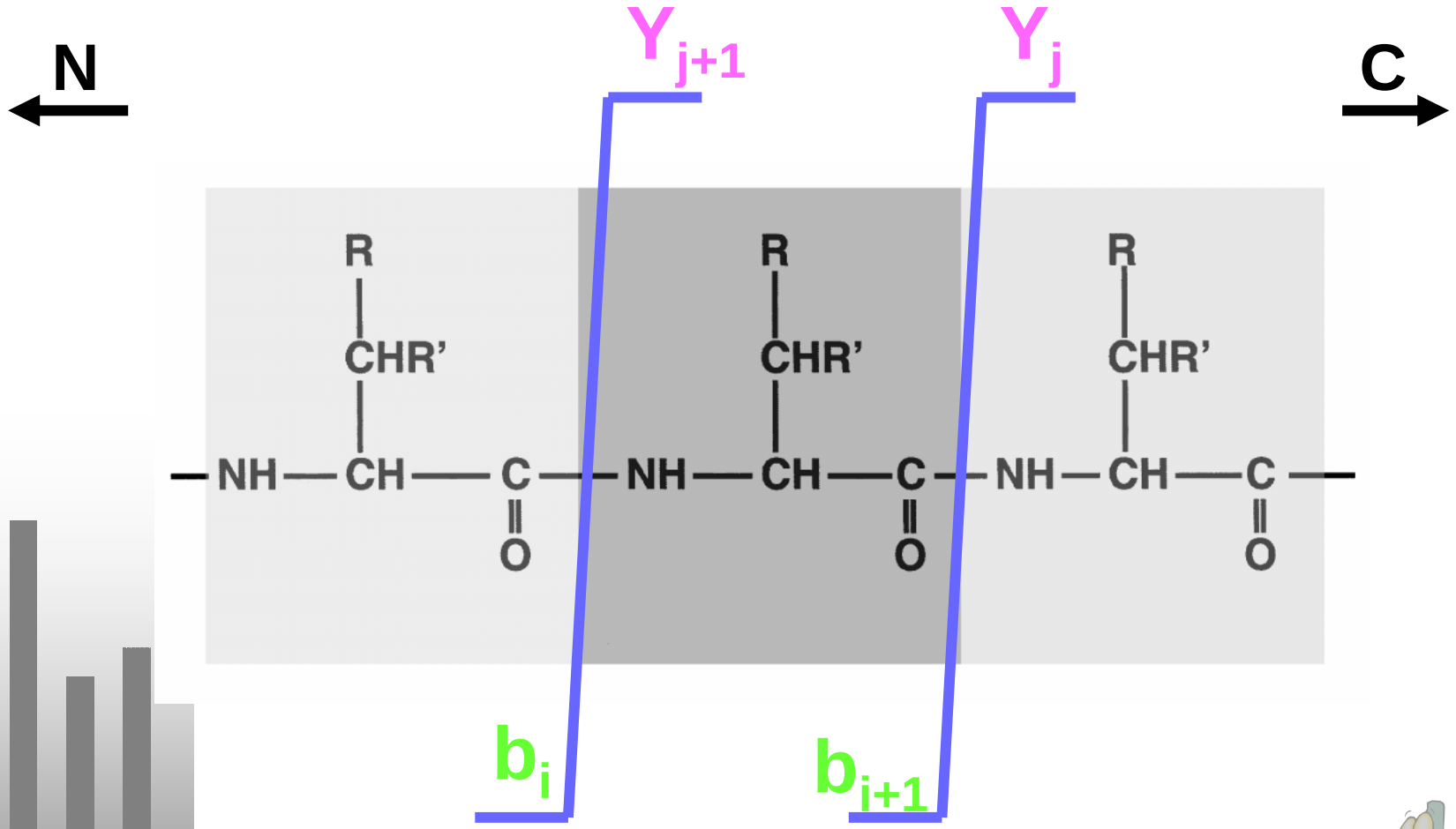
Theoretical	1571.6 (average)
	1570.7 (monoisotopic)
Observed $(M+2H)^{2+}$	786.2
Calculated	786.3 (average)
	785.9 (monoisotopic)

Should know that quadrupoles cannot give monoisotopic ions.

For tyrptic fragments, doubly charged ions are common.

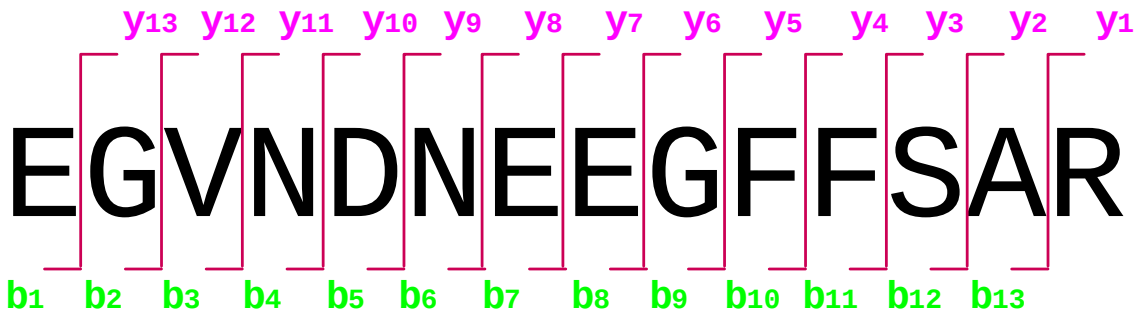
Gives the expected # of residues

For soft ionization:



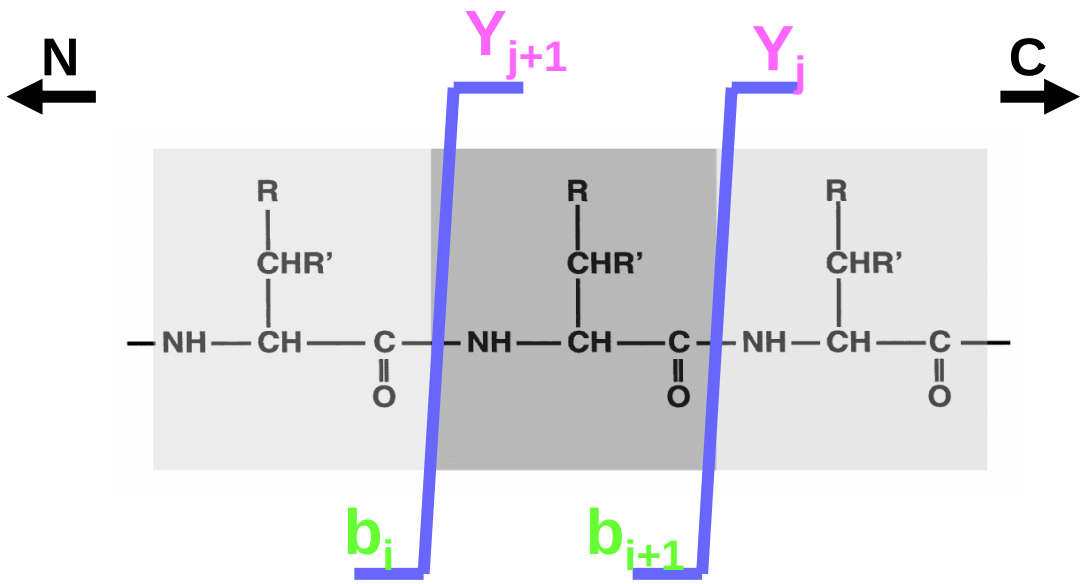
For every b ion, there should be a corresponding y ion.

At the end of the fragmentation reactions, there should be a mixture of b_i to b_{i+n} and y_j to y_{j+m} ions, in addition to others.



Note the way of counting the ions.

Higher the number, heavier the ion.



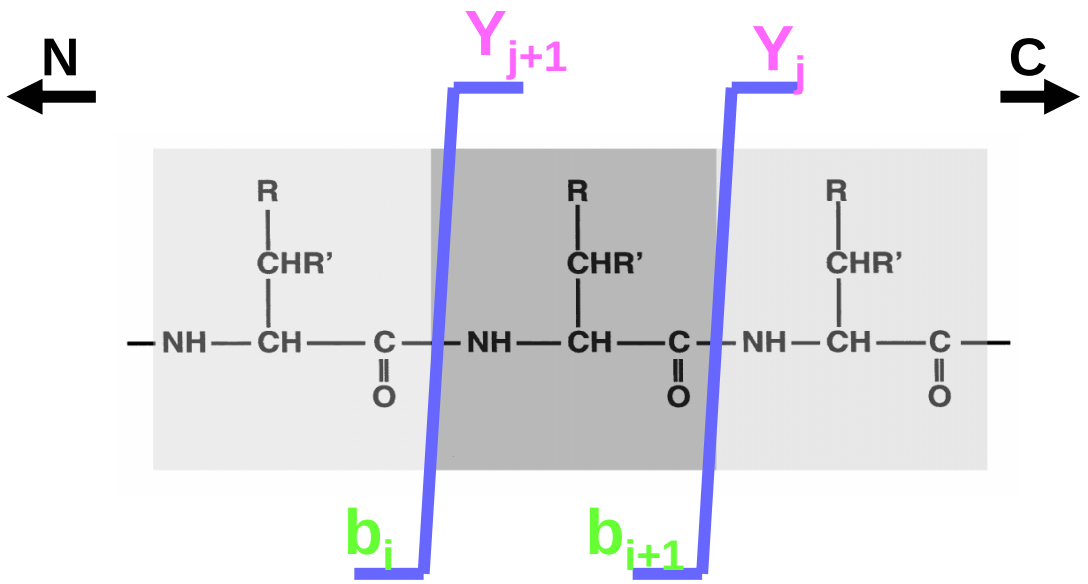
For any peptide,

Molecular ion = ☝residue ions + H_2O +H^+

$b_{i+1} - b_i$ = residue mass of aa_{i+1}

For a peptide of n residues,

Mass of b_{n-1} ion = $(M+H)^+$ - H_2O – residue mass$_n$



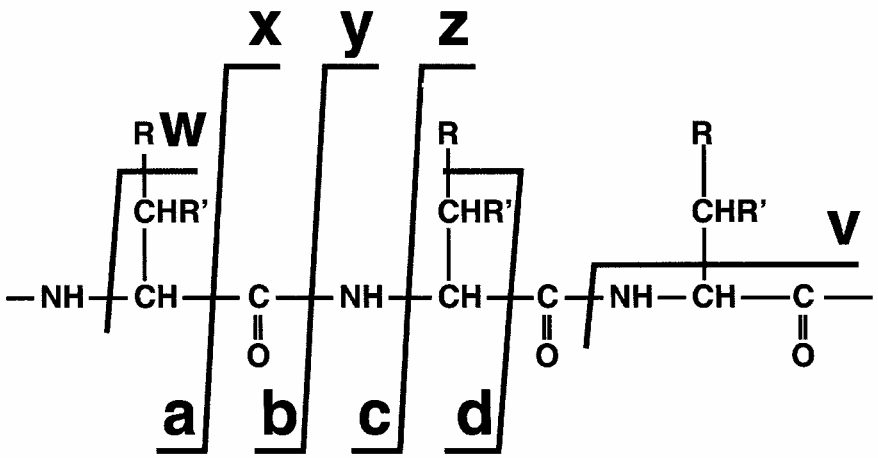
y_1 ion = residue mass + H_2O $+H^+$

y ion = $(M+H)^+$ - b ion + 1 or

b ion = $(M+H)^+$ - y ion + 1 or

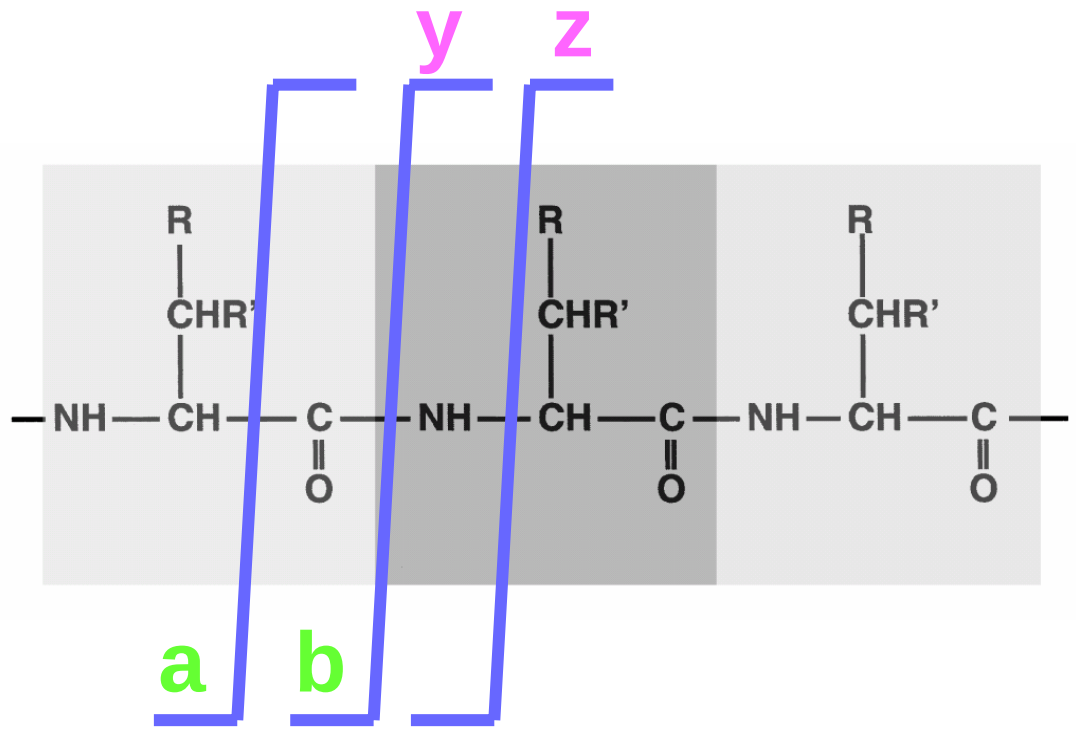
$(M+H)^+$ = b ion + y ion - 1

Besides b and y, there are other fragmentation ions



a, b, c are N-terminal fragments.

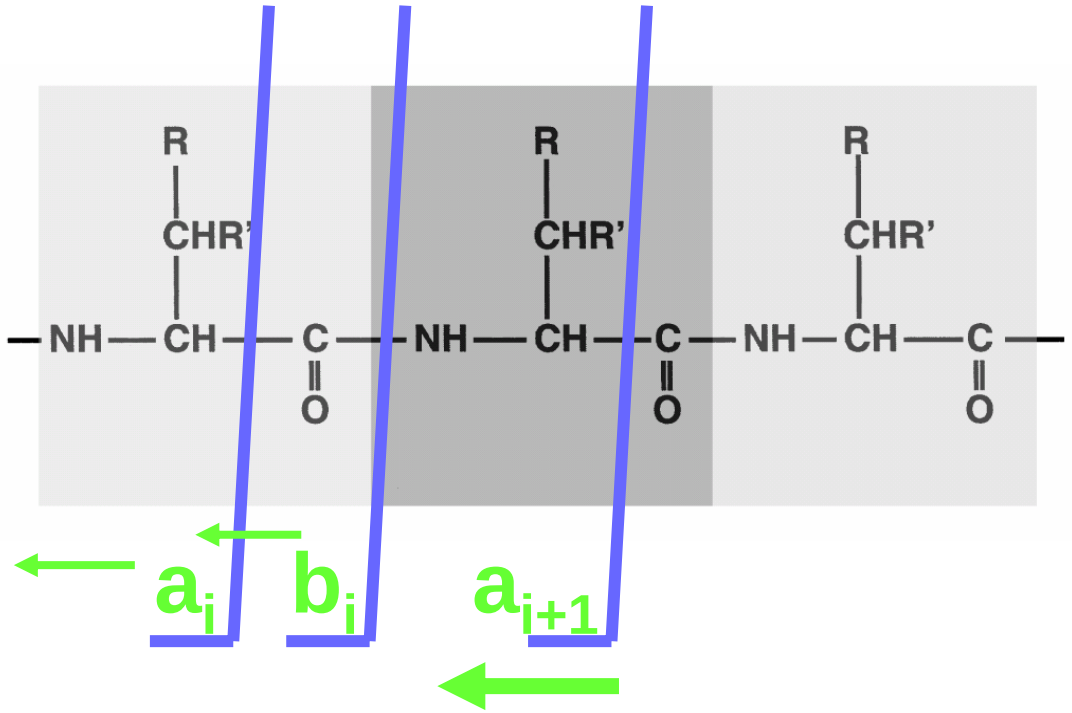
x, y, z are C-terminal fragments.



a ion = b ion – CO

z ion = y ion - NH

This is an a ion



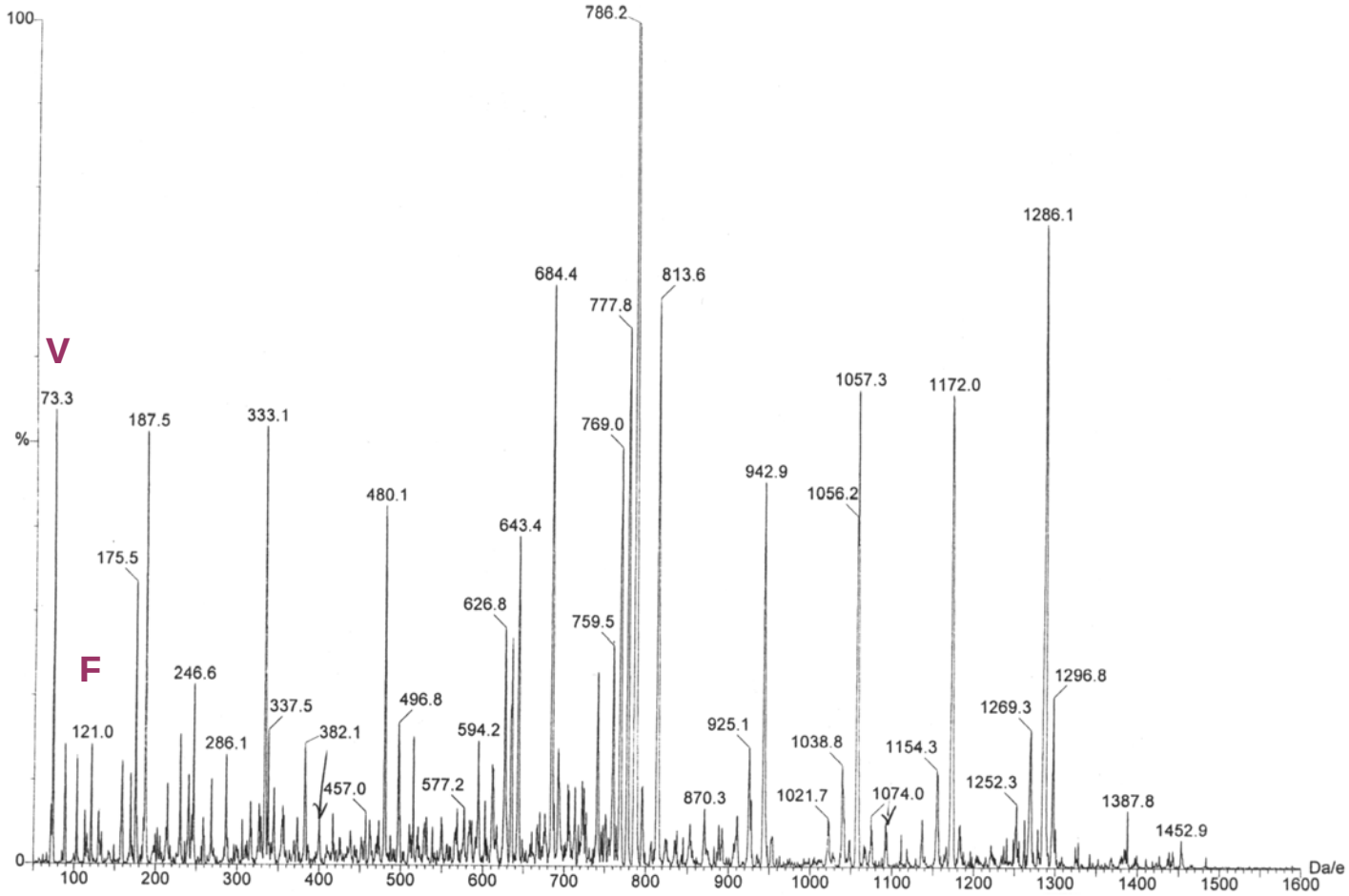
Generate $^{+}NH_2$=CHR ions at low mass end of the spectrum.

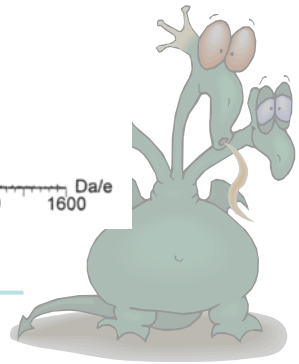
$^{+}NH_2$=CHR ions

Pro	70	Phe	120
Val	72	Cmc	134
Lxx	86	Tyr	136
Met	104	Trp	159
His	110		

Give the a.a. composition of the peptide

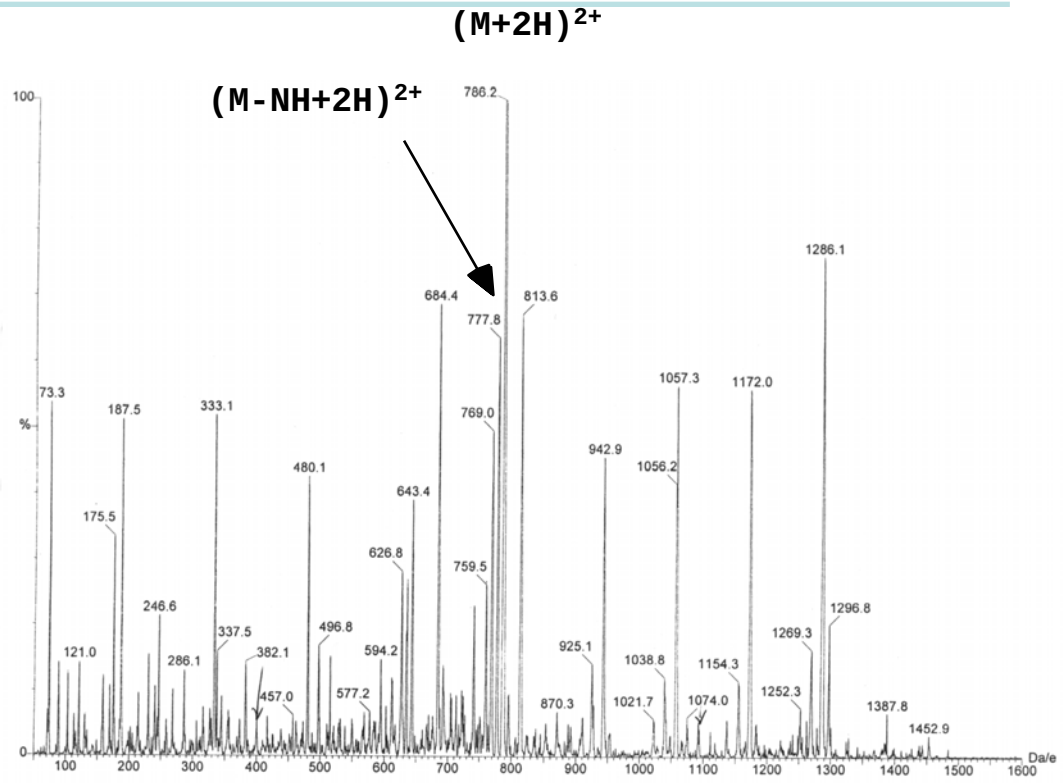
From the low mass end , identify possible amino acids in the peptide.





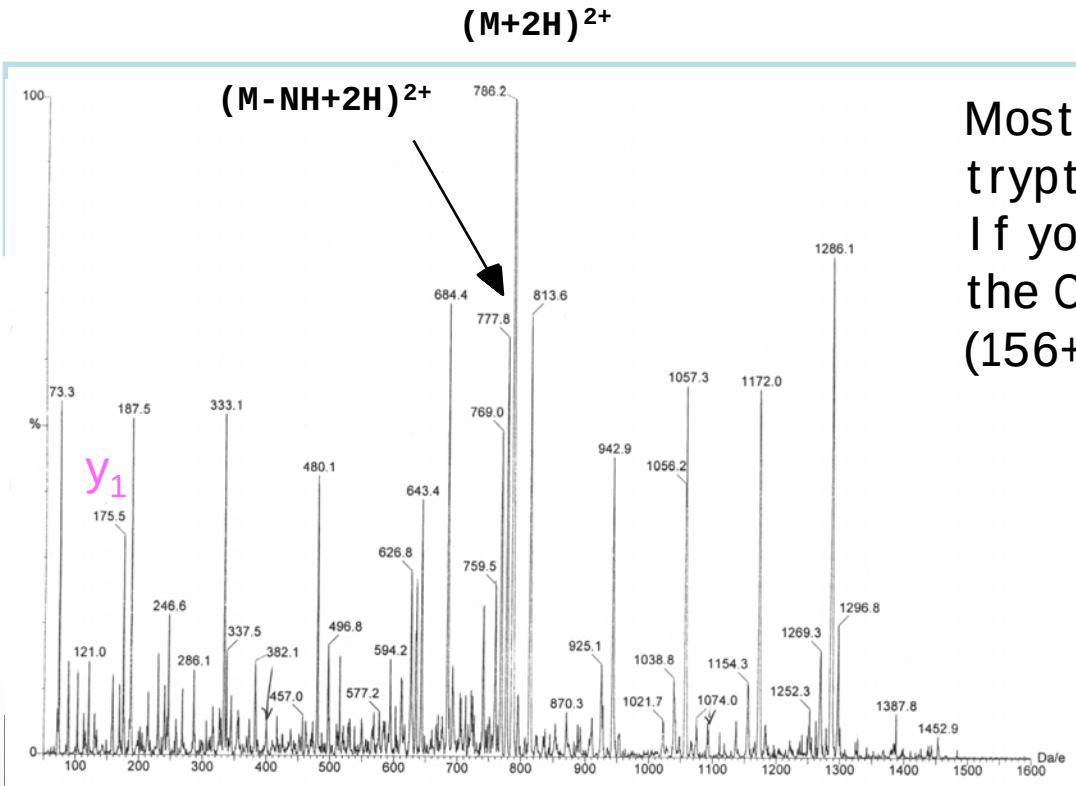
Pick out the largest peak, usually represents the molecular ion.

Look at the isotopic peaks and determine the number of charges on the ion.



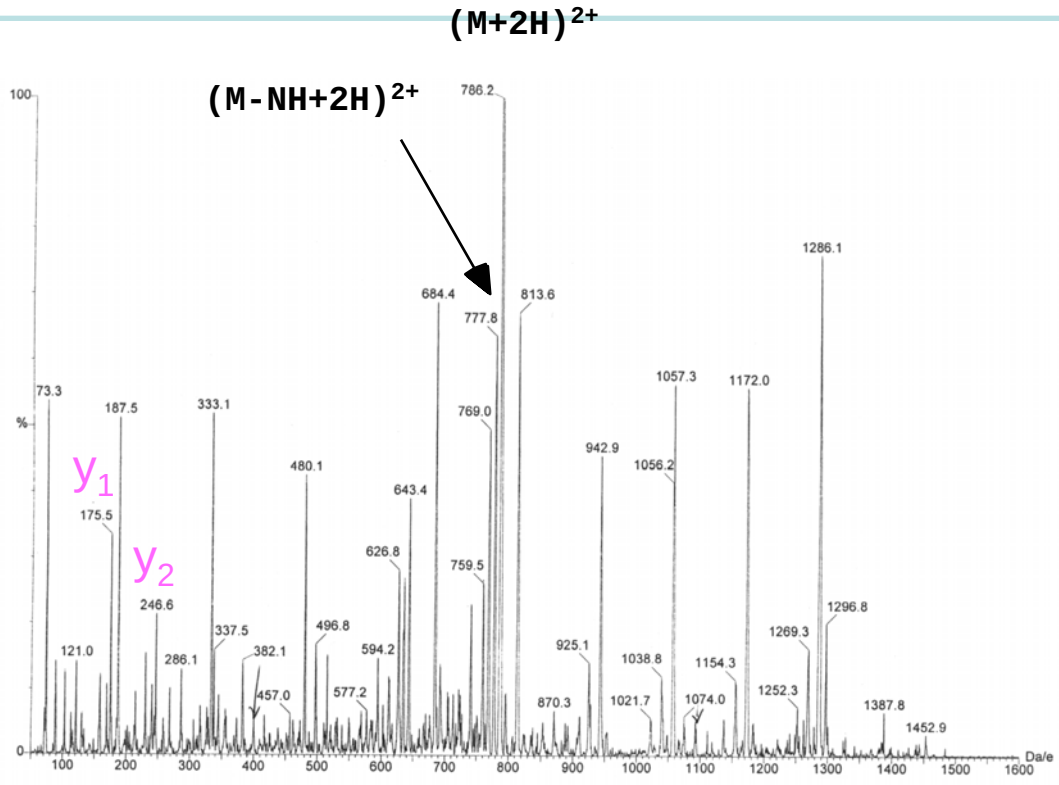
If 786 is the doubly charged ion, then the molecular ion (MH^+) should be (786 x 2) – 1 → 1571.

Give a rough estimate that there are 13-15 amino acids.



Most likely you are dealing with a tryptic peptide.
If you remove an arginine or lysine from the C-terminal, the y_1 ion should be: (156+18+1 = 175) or (128+18+1 = 147).

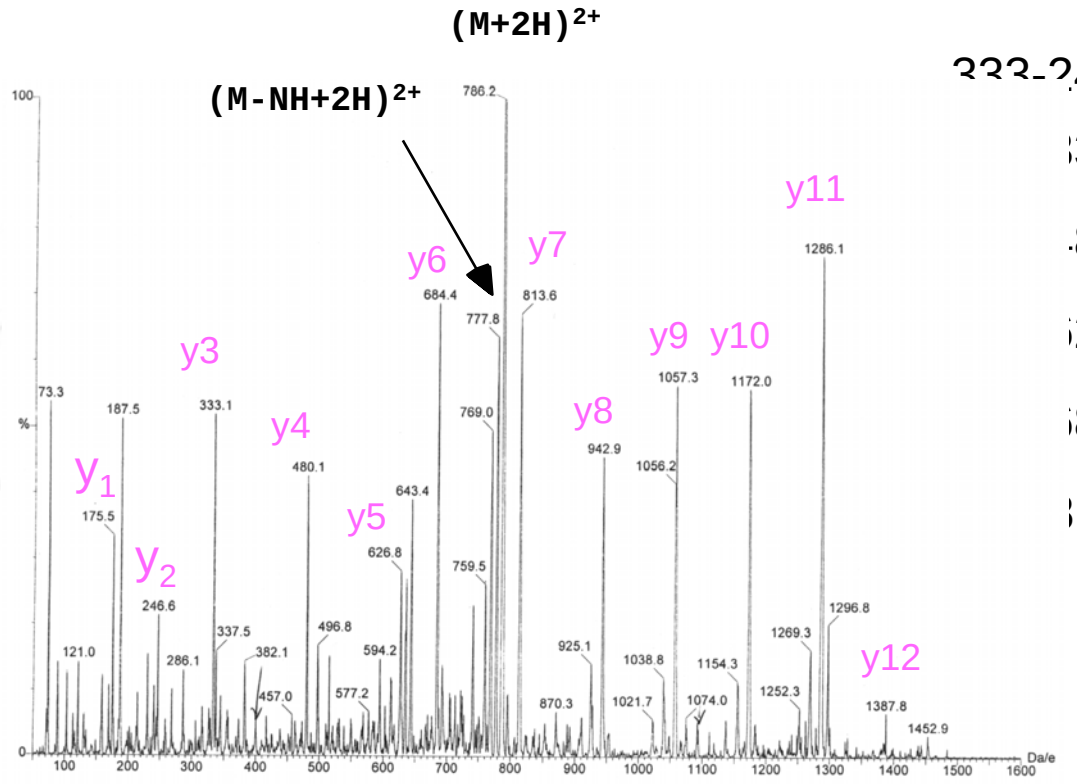
Since b ion = MH^+ - y ion + 1
= 1571 – 175 + 1 → 1397
or = 1571 – 147 +1 → 1425
Therefore, the b_{n-1} ion is missing



Next, from Y_1, look for a higher mass ion peak that equals to (Y_1 + residue mass).

246-175 = 71 → Ala → Y_2

The b_{n-2} (1571 – 246 + 1 → 1326) ion is still missing.



333-246 = 87 Ser y3

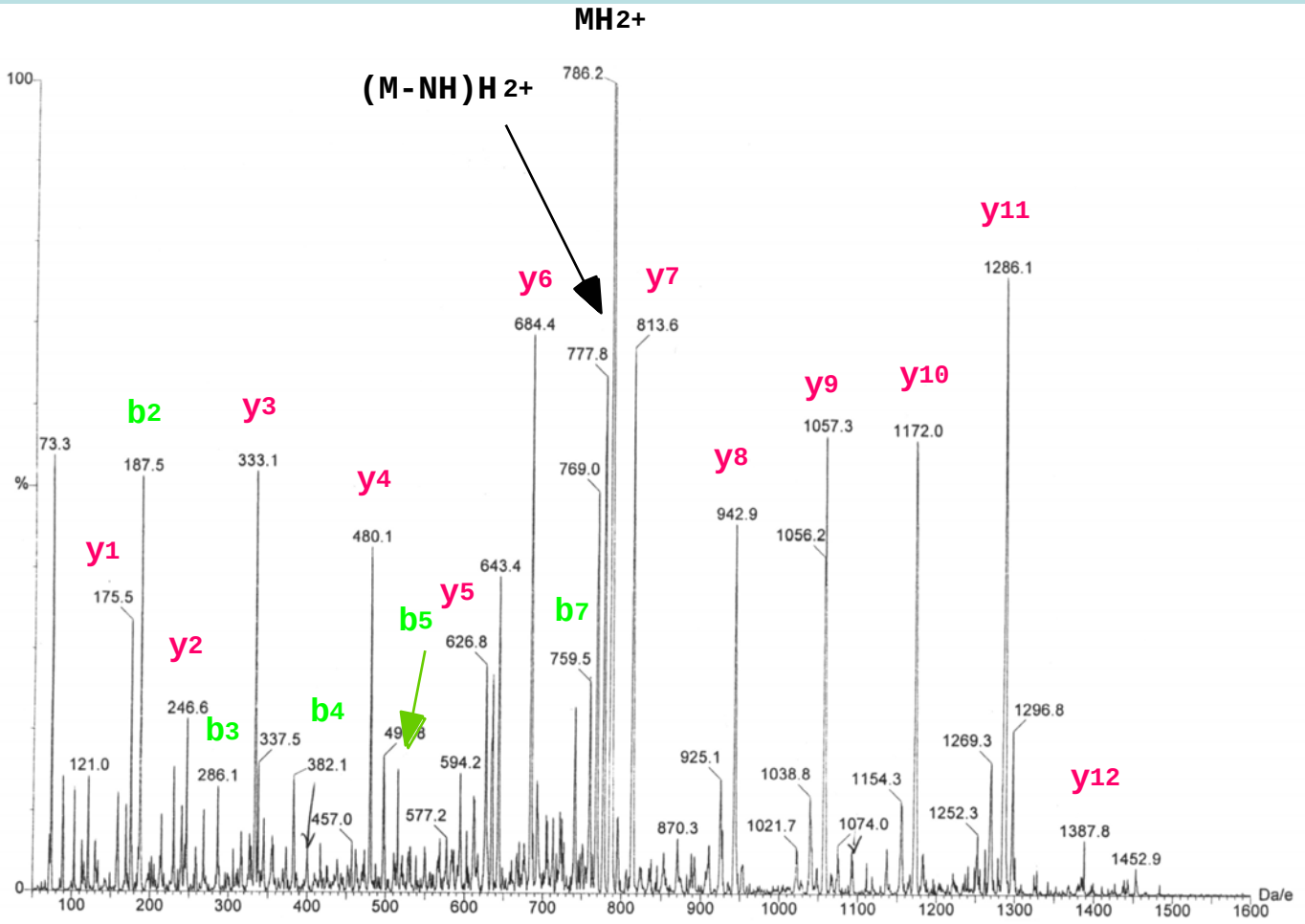
333 = 147 Phe y4

480 = 147 Phe y5

527 = 57 Gly y6

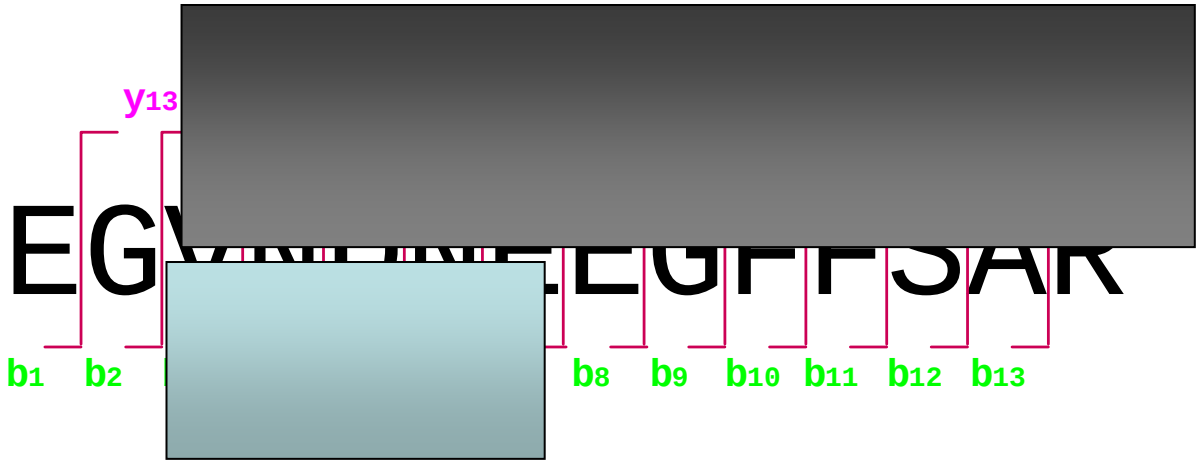
684 = 129 Glu y7

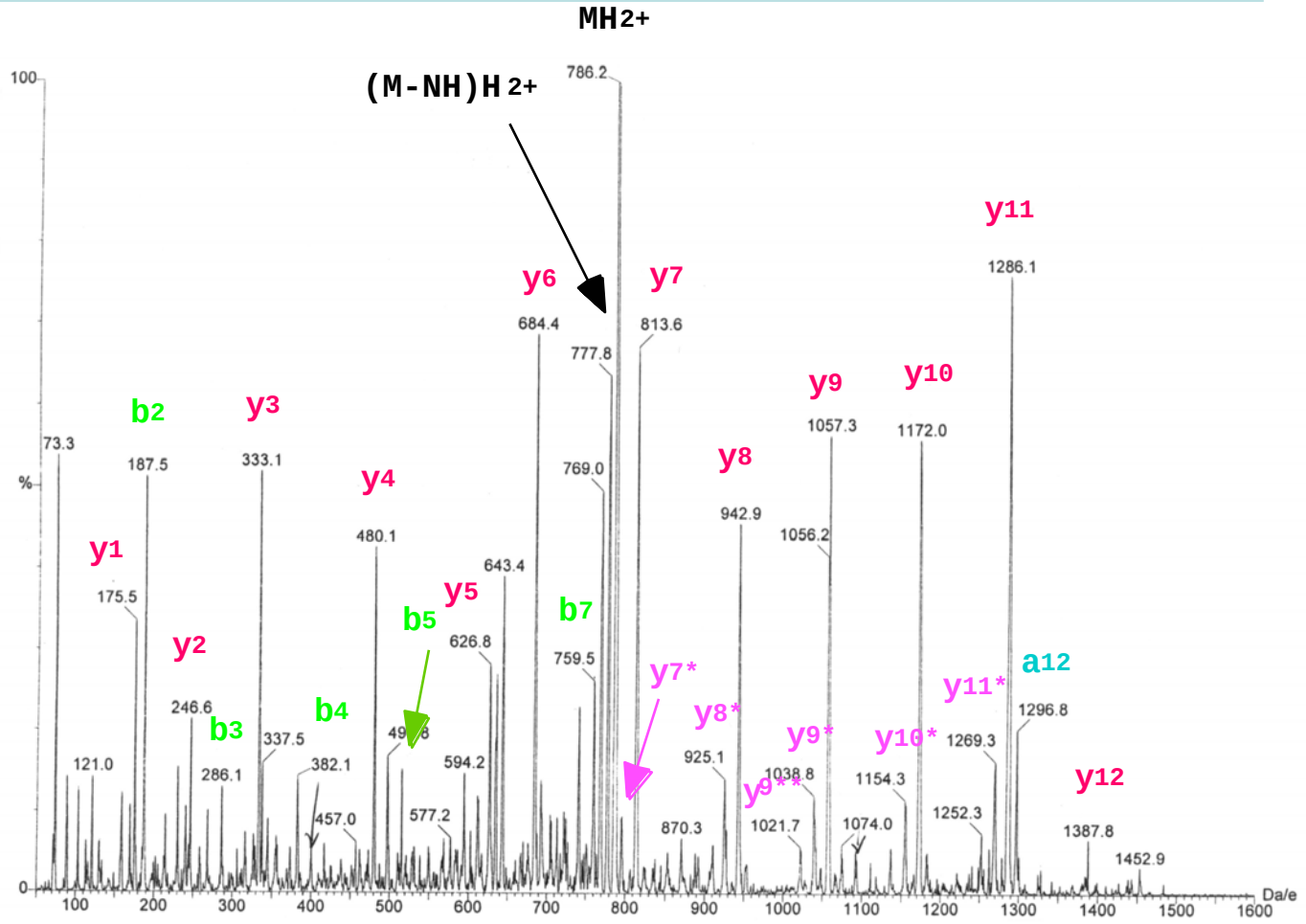
813 = 130? Glu y8



From the y ions, calculate the corresponding b ions.

$b = MH^+ - y + 1$


y13
b1
b2
b8
b9
b10
b11
b12
b13



Other losses:

-water from Ser, Thr and N-terminal Glu

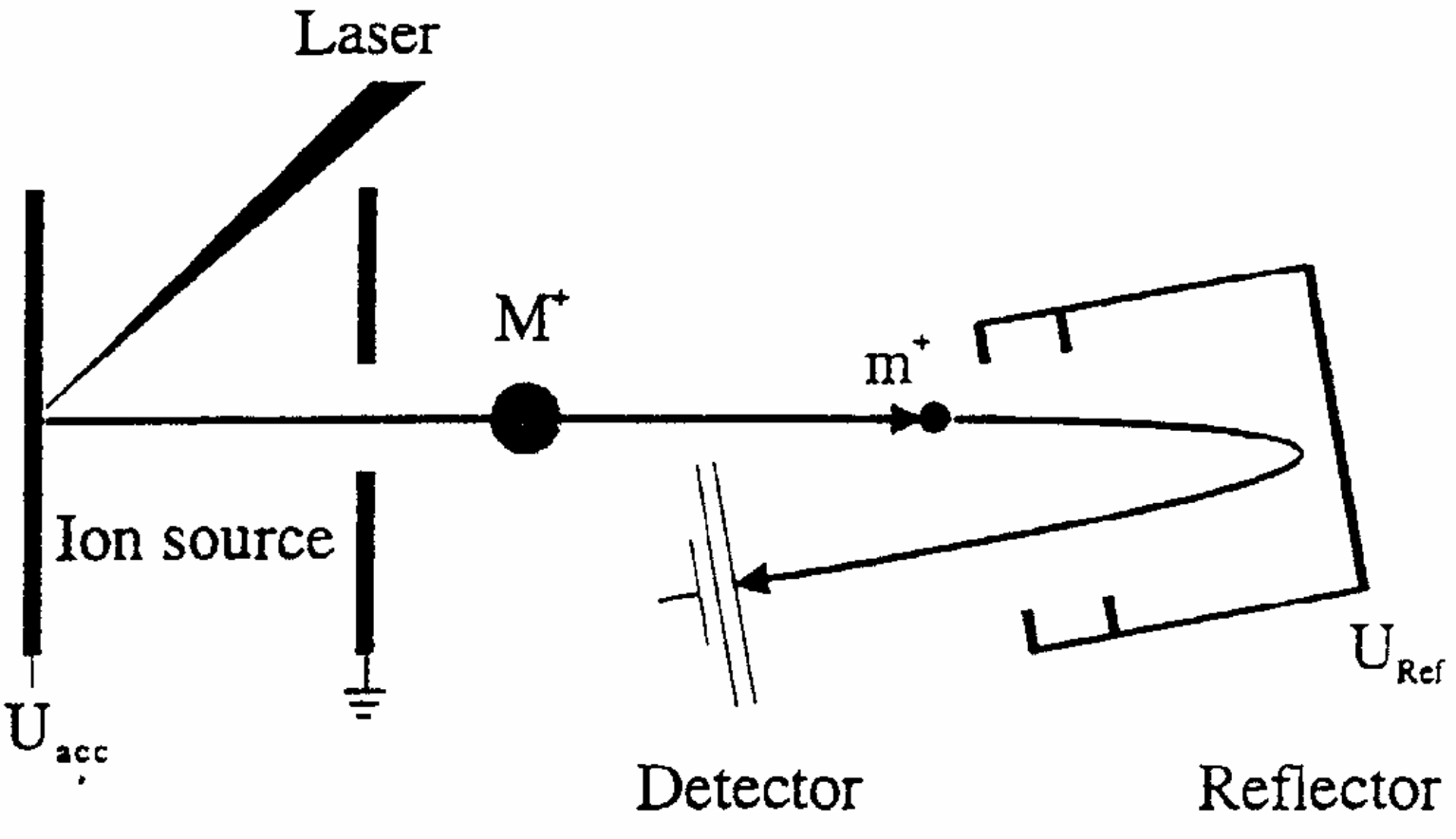
-ammonia from Arg, Lys, Gln and Asn

Note that b_1 and y_{13} are missing.

Dipeptides:

Gly-Gly	114	=	Asn
Gly-Ala	128	=	Gln or Lys
Val-Gly	156	=	Arg
Gly-Glu	186	=	Trp
Ala-Asp	186	=	Trp
Ser-Val	186	=	Trp
AcGly	99	=	Val
AcAla	113	=	Lxx
AcSer	129	=	Glu
AcAsn	156	=	Arg

Sequencing can also be done with MALDI-TOF in PSD mode

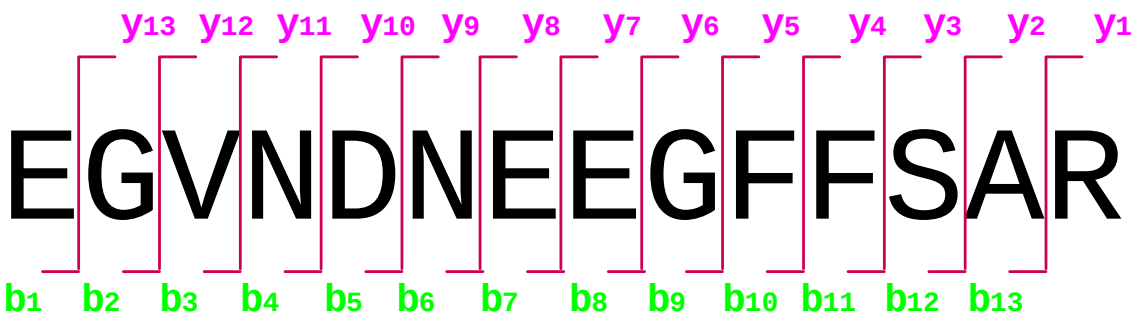


Life is easier as the spectrum become more simple

Convert –COOH to Methyl esters

-COOH → $-COOCH_3$ +14 daltons

Affect C-terminal fragments, tell # acidic aa

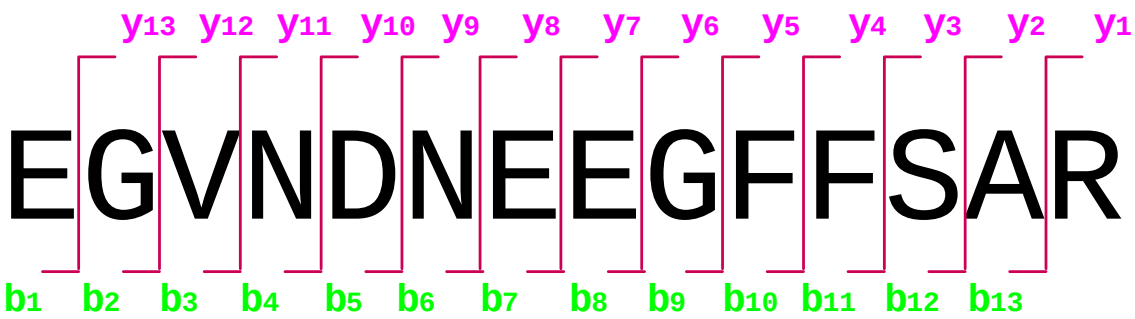


- Can tell right away how many carboxyl groups on the peptide.
- Only ions carrying carboxyl group(s) will shift (compare to unmodified peptide).
- y1 to y6 ions shift by 14 Da.
- y7 ion shifts by 28 Da.
- y8 and y9 by 42 Da.
- y10 to y12 by 56 Da.

Convert $-NH_2$ to $-NH\text{-}COCH_3$ +42daltons

Affect N-terminal and lysine containing fragments.

Can distinguish K from Q, both have residual mass of 128 Da



All the b ions will shift by 42.

All the y ions stay put—if they don't have lysine

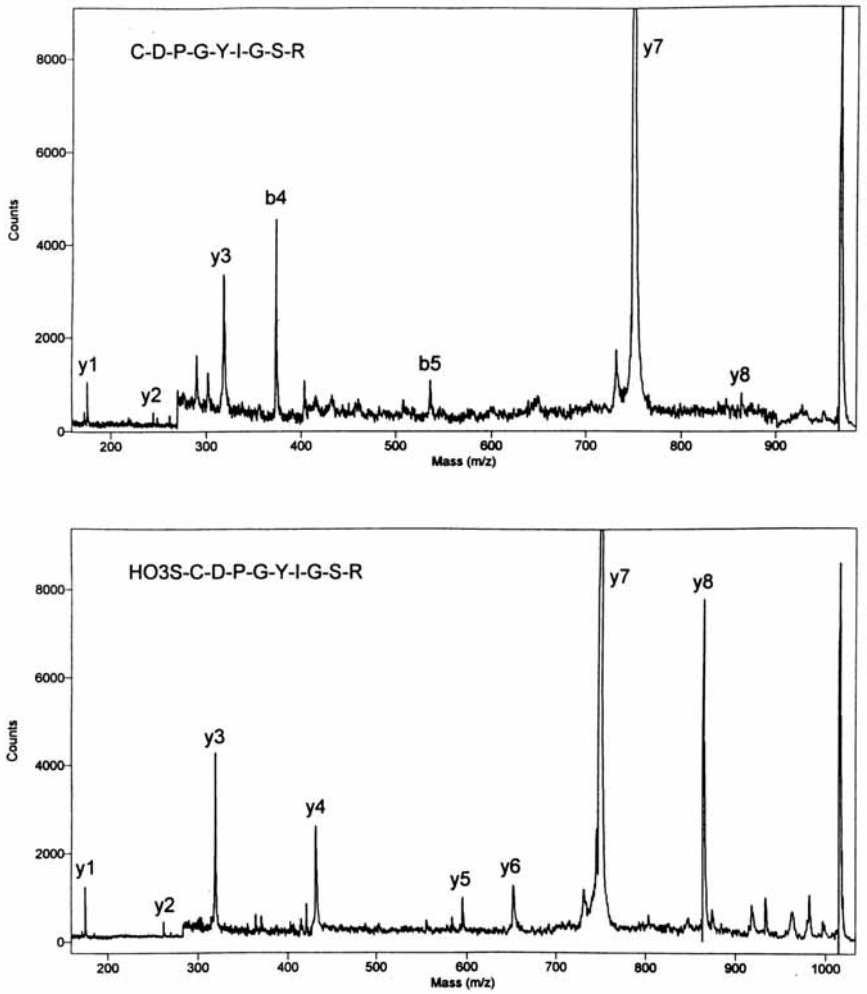
But you have to run the experiment twice!

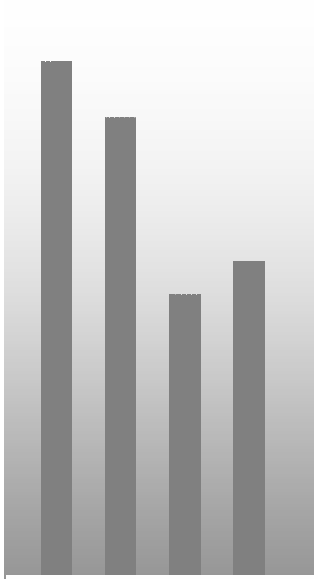
Derivatize peptides to observe only N- or C-terminal ions—Run the experiment only once!

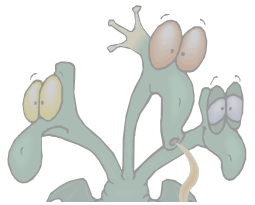
A method for high-sensitivity peptide sequencing using postsource decay matrix-assisted laser desorption ionization mass spectrometry
Keough et al. Proc. Natl. Acad. Sci. USA (1999) 96, 7131-7136.

Sulfonic acid derivatives for peptide sequencing by MALDI MS
Keough et al. Anal. Chem. (2003) 75, 156A-165A.

Fig. 1. PSD fragment ion mass spectra of CDPGYIGSR before (Upper) and after (Lower) oxidation with performic acid



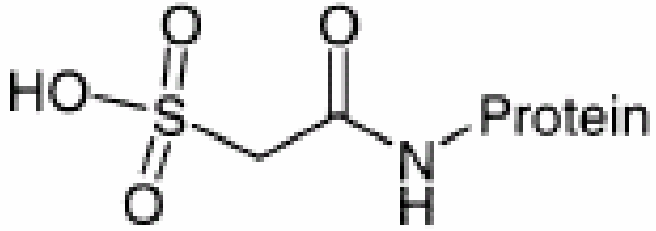




Keough, T. et al. (1999) Proc. Natl. Acad. Sci. USA 96, 7131-7136

React peptides with chlorosulfonylacetyl chloride. $ClO_2S\text{-}CH_2\text{-}COCl$

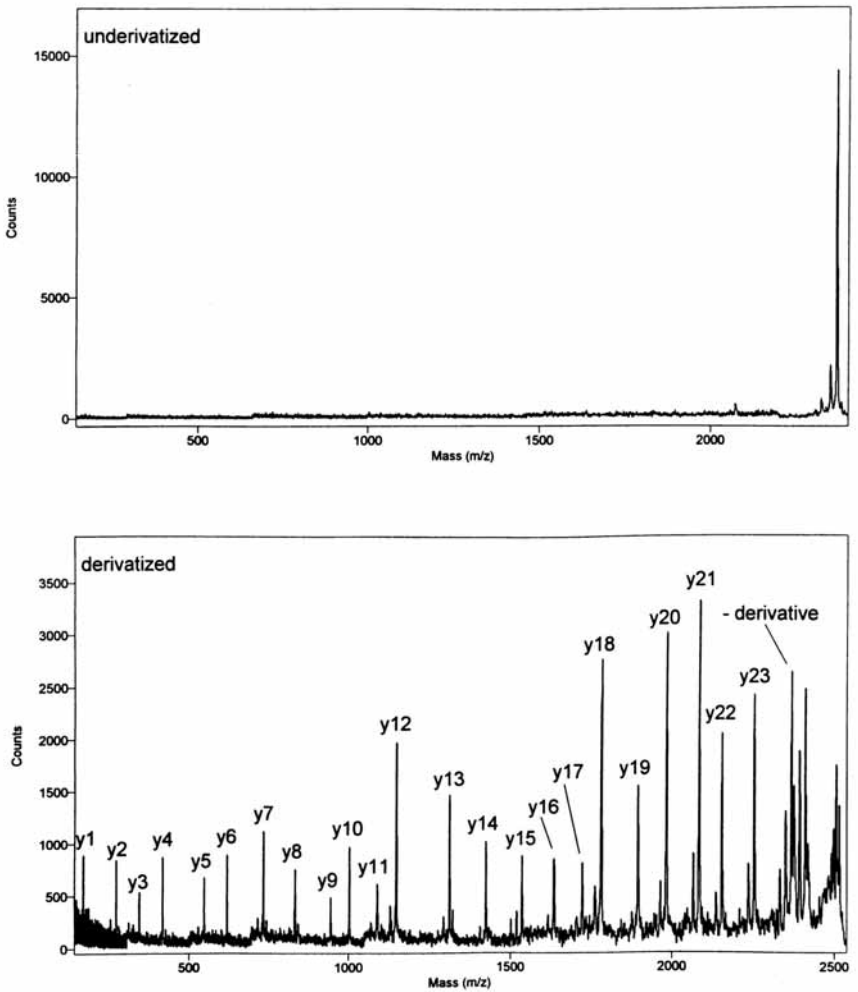
$-O_3S\text{-}CH_2\text{-}CO\text{-}NH\text{-peptide-}COOH$



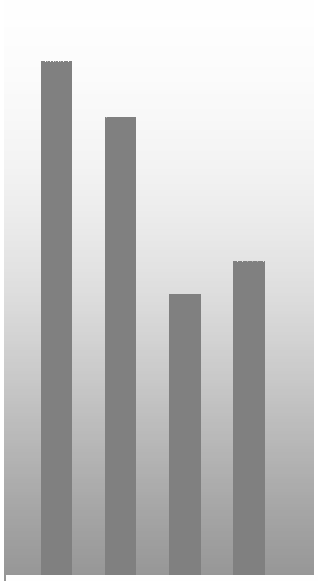
Reaction carries out in non-aqueous condition.

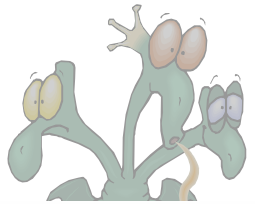
Introduce –ve charge to eliminate the N-terminal ions.

Fig. 2. PSD MALDI mass spectra of a tryptic peptide before (Upper) and after (Lower) derivatization



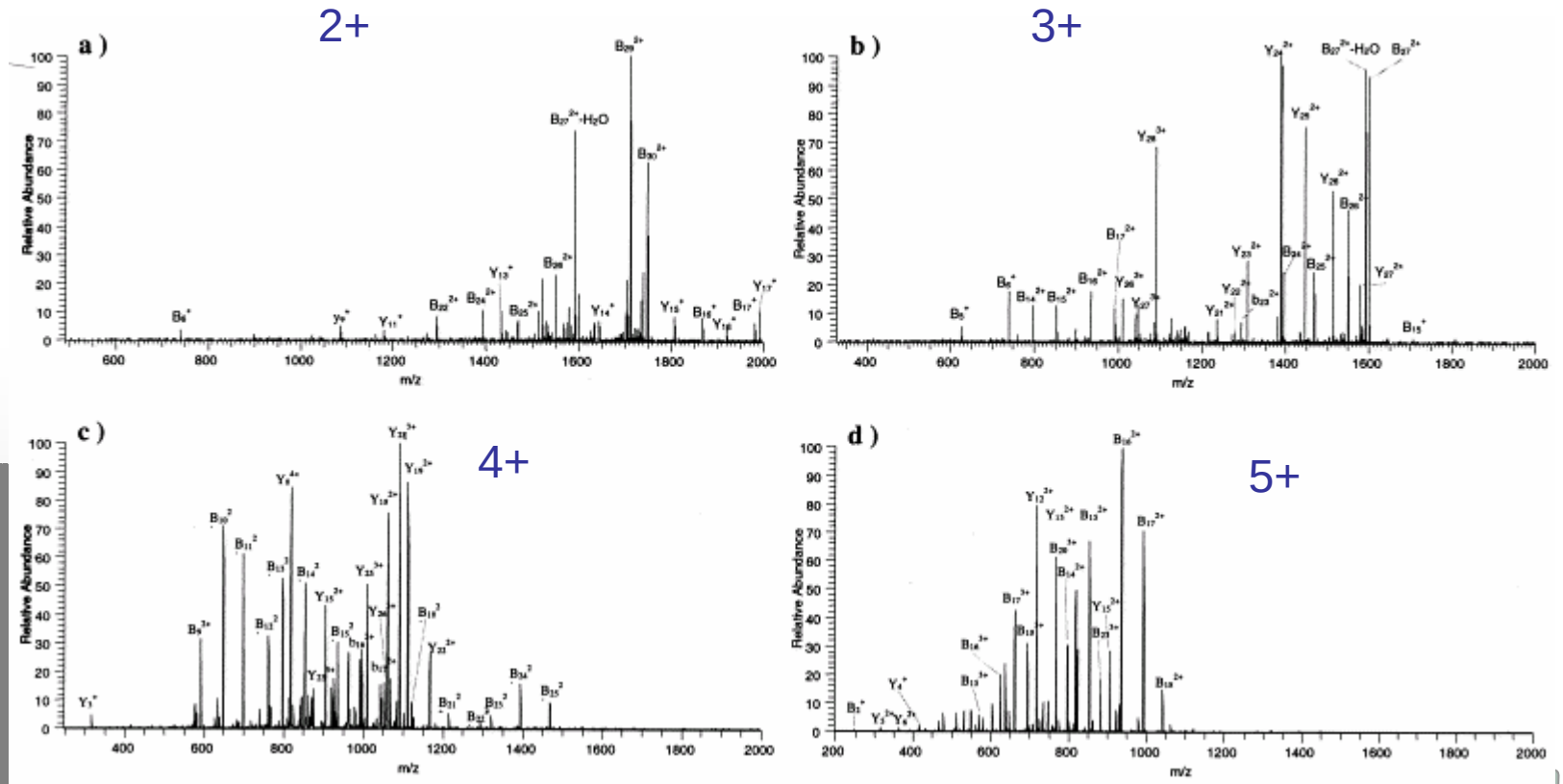
Note the y scale



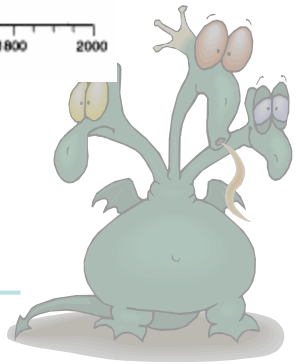


Keough, T. et al. (1999) Proc. Natl. Acad. Sci. USA 96, 7131-7136

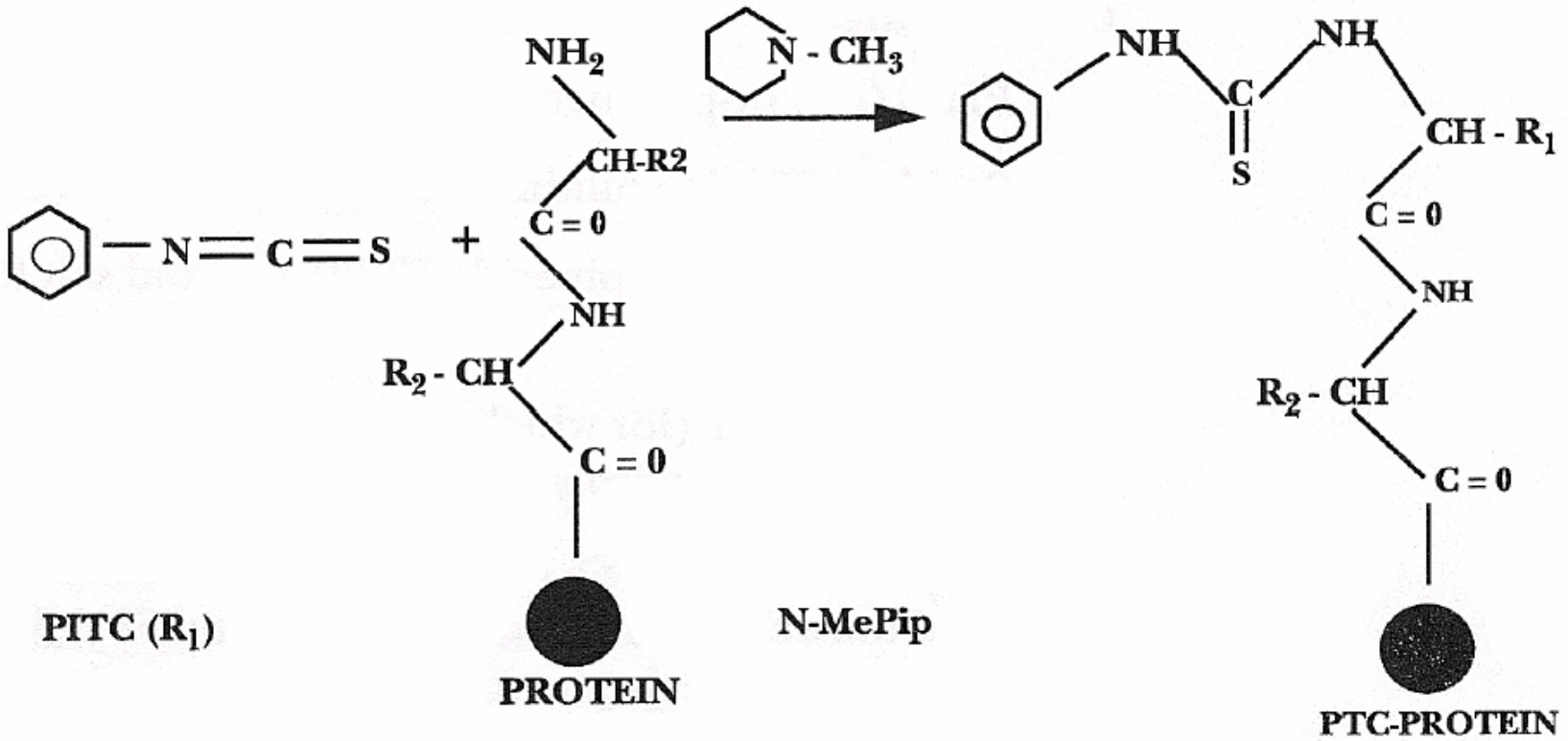
Read Sonsmann et al. “Investigation of the influence of charge derivatization on the fragmentation of multiply protonated peptides“ JASMS (2002) 13, 47-58.

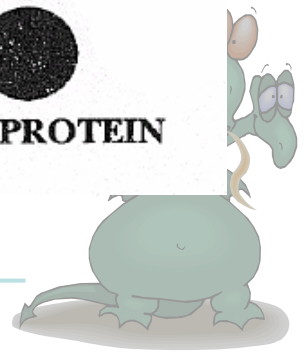


Insulin β-chain on an ESI/ion-trap instrument.

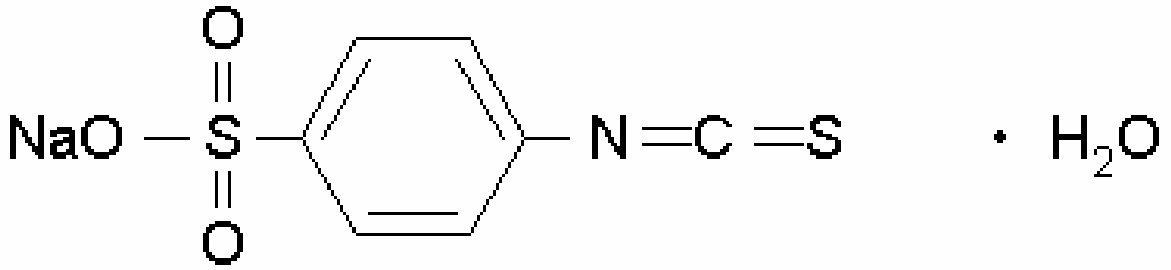


Step back in time!
Edman sequencing





Use 4-sulfophenyl isothiocyanate (SPITC) as modification reagent

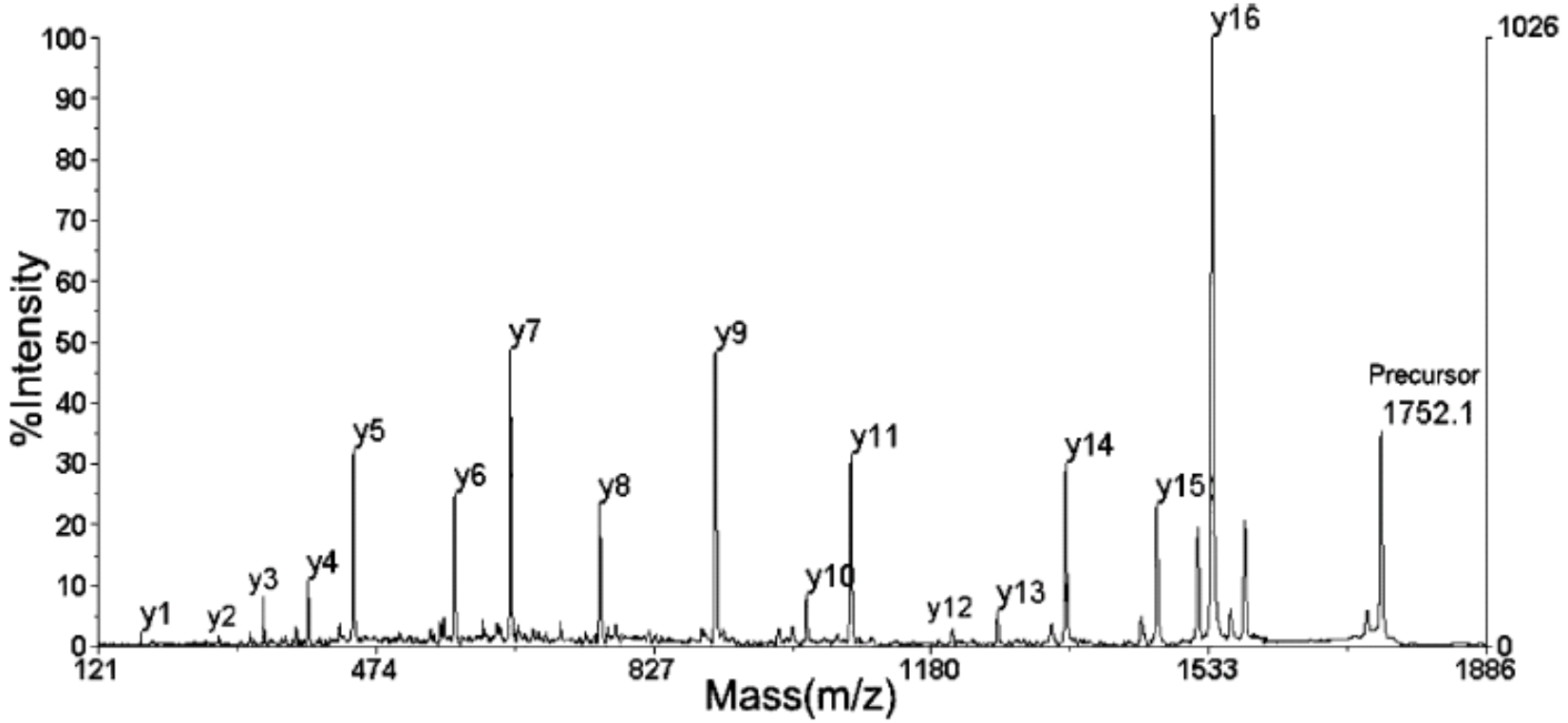


Protein identification based on matrix assisted laser desorption/ionization-post source decay-mass spectrometry

Gevaert et al. (2001) Electrophoresis 22, 1645-1651

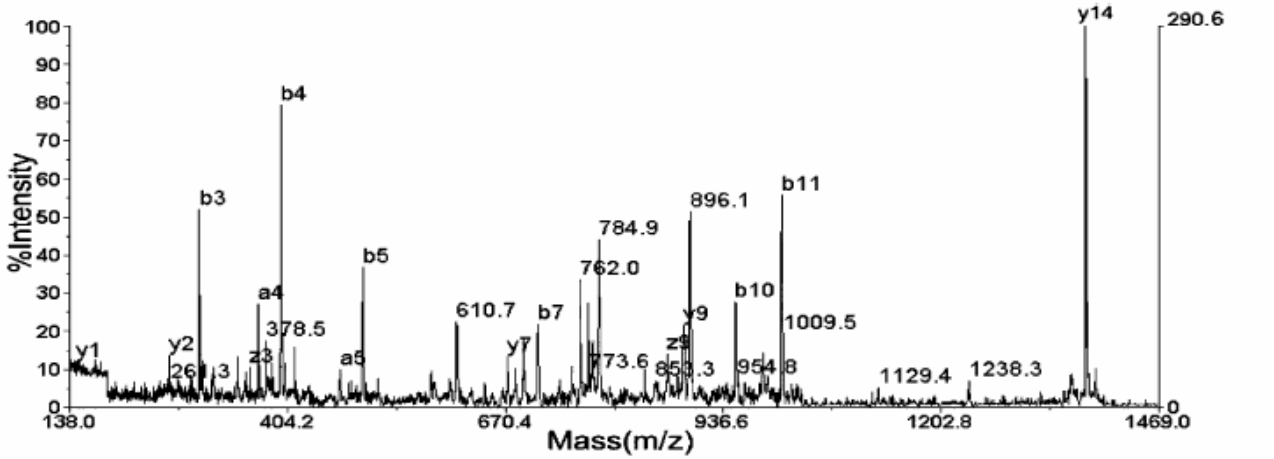
Chen et al. RCM (2004) 18, 191-198

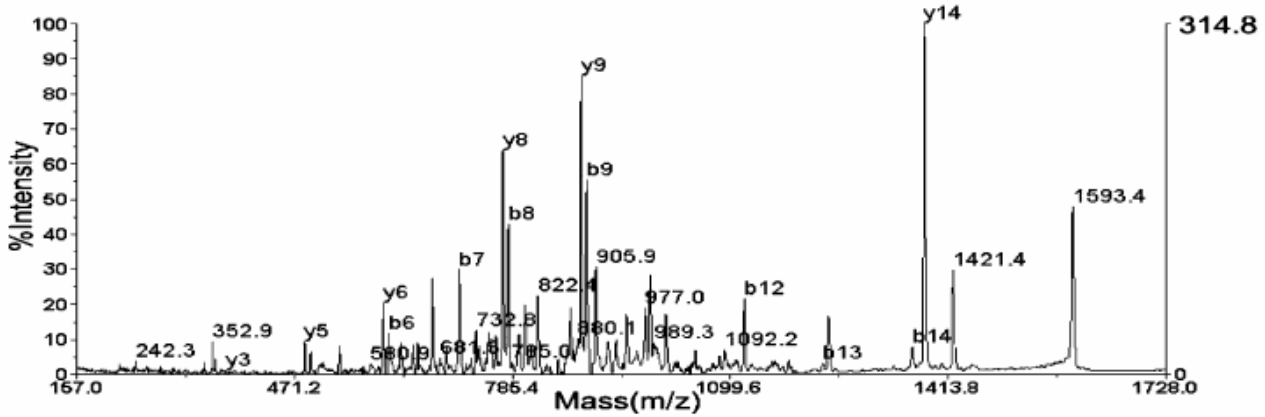
De novo sequencing of tryptic peptides sulfonated by 4-sulfophenyl isothiocyanate for unambiguous protein identification using PSD MALDI MS



PSD MALDI spectrum of sulfonated arginine-terminated peptide ADSGEGDFLAEGGGVR

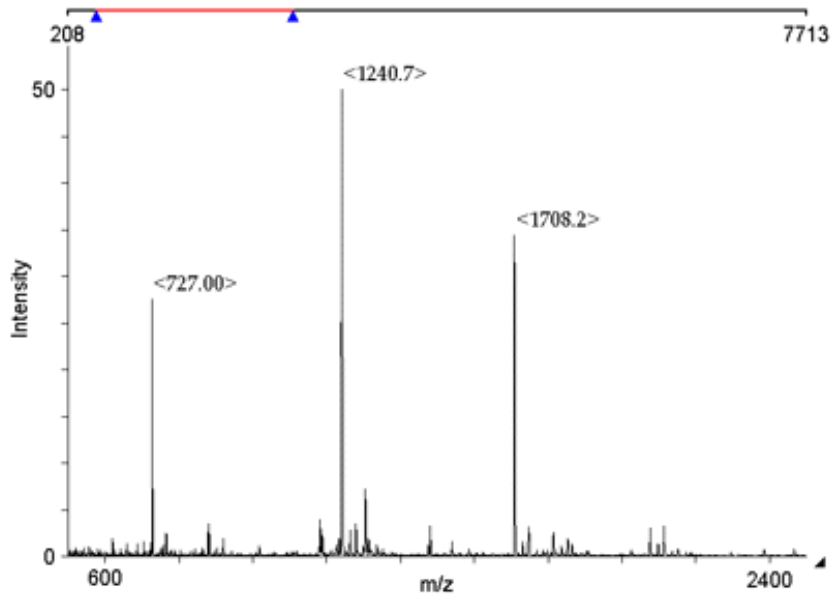
PSD MALDI spectra of HGTVVLTALGGILK without and with sulfonylation. Why the difference???





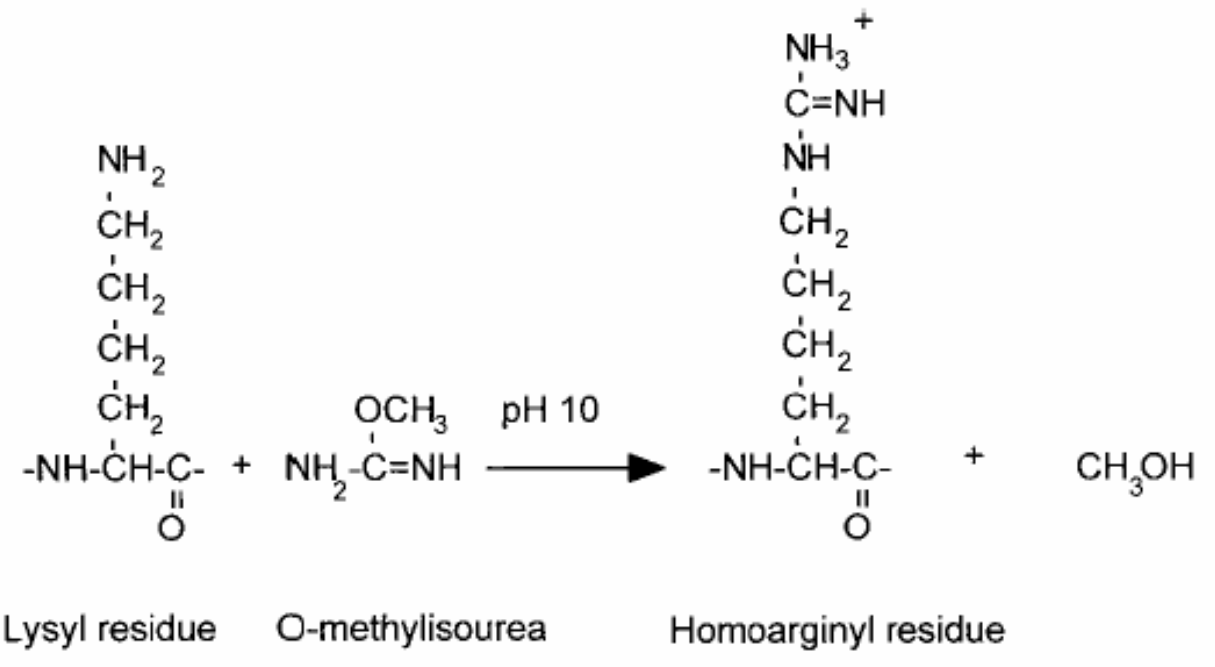
HGTVVLTALGGILK.

Difference in ionization efficiency of Arg- and Lys-terminating peptides

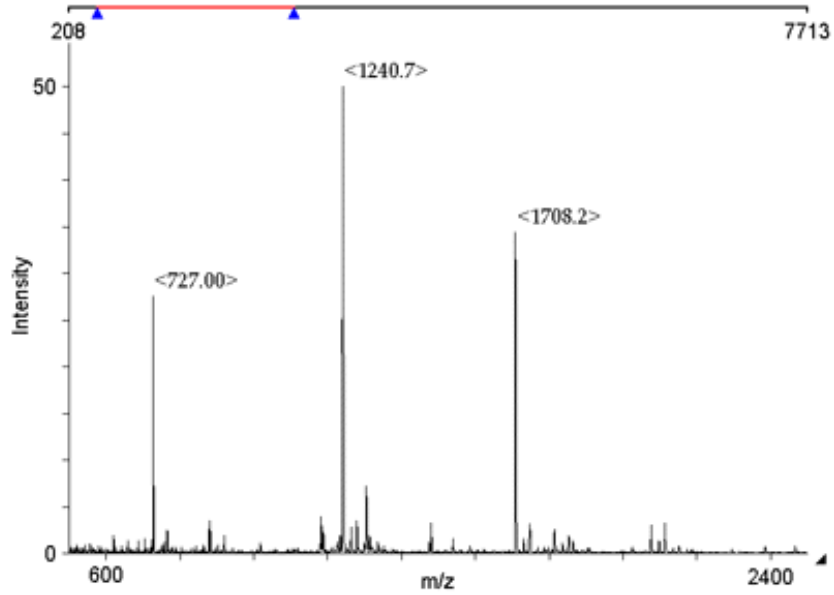


Tryptic digests of yeast L12

Hale et al. (2000) Anal Biochem 287, 110-117

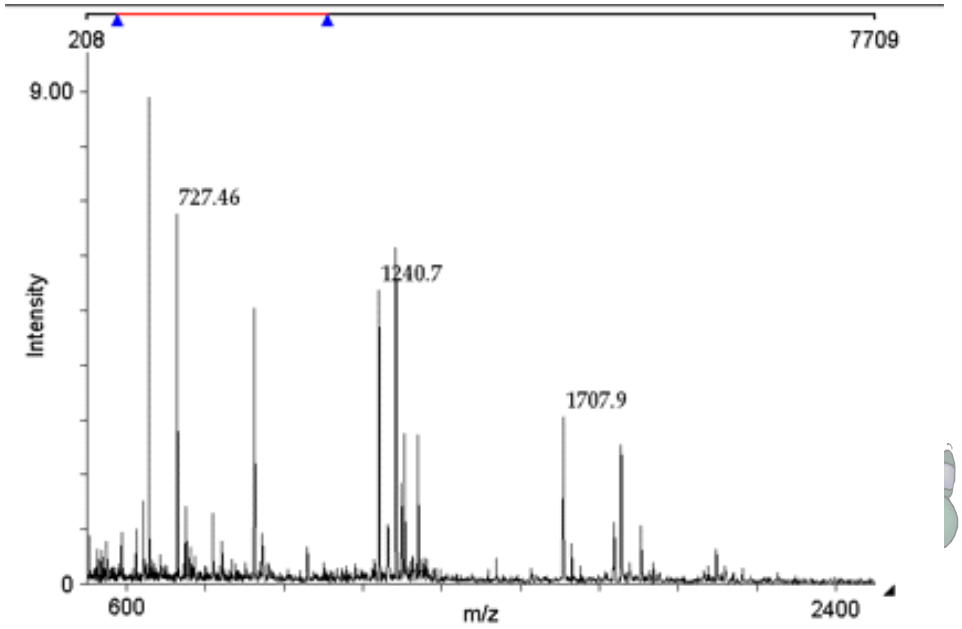


Yeast L12 tryptic digest

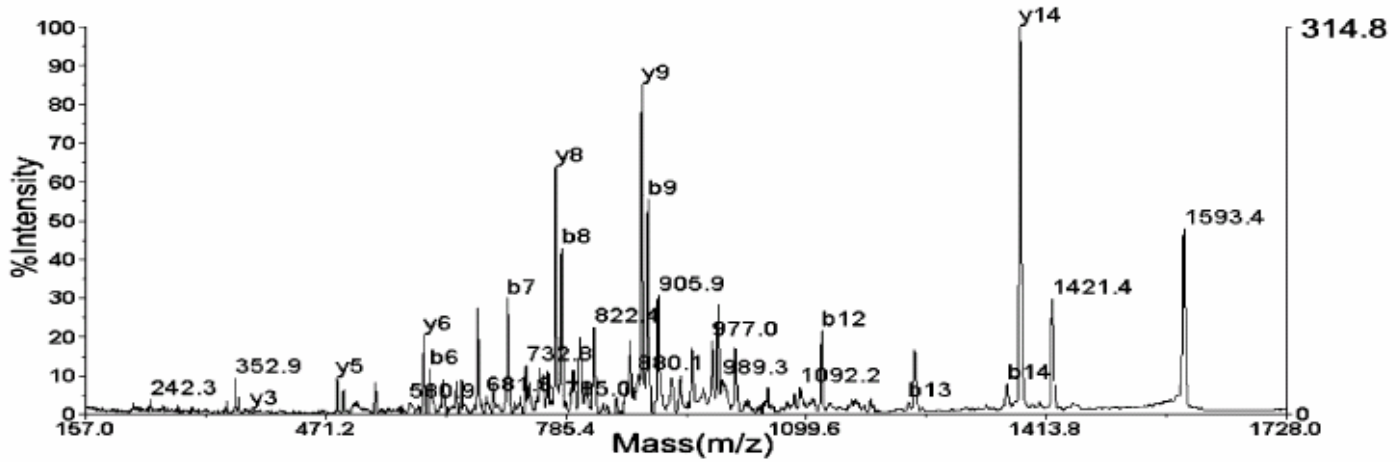


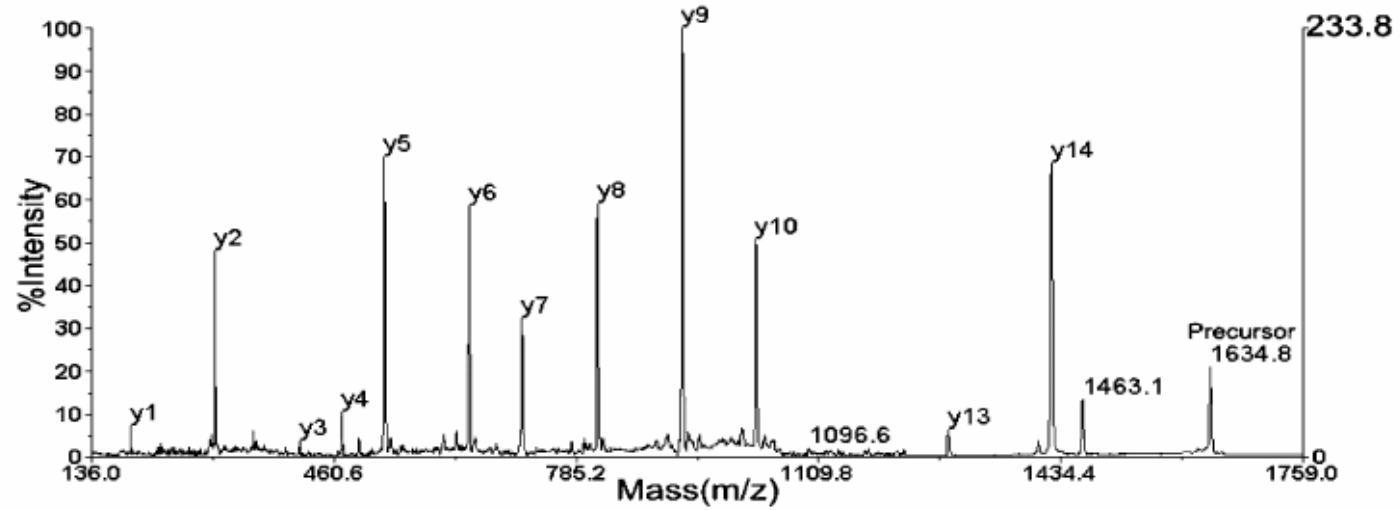
Before

After

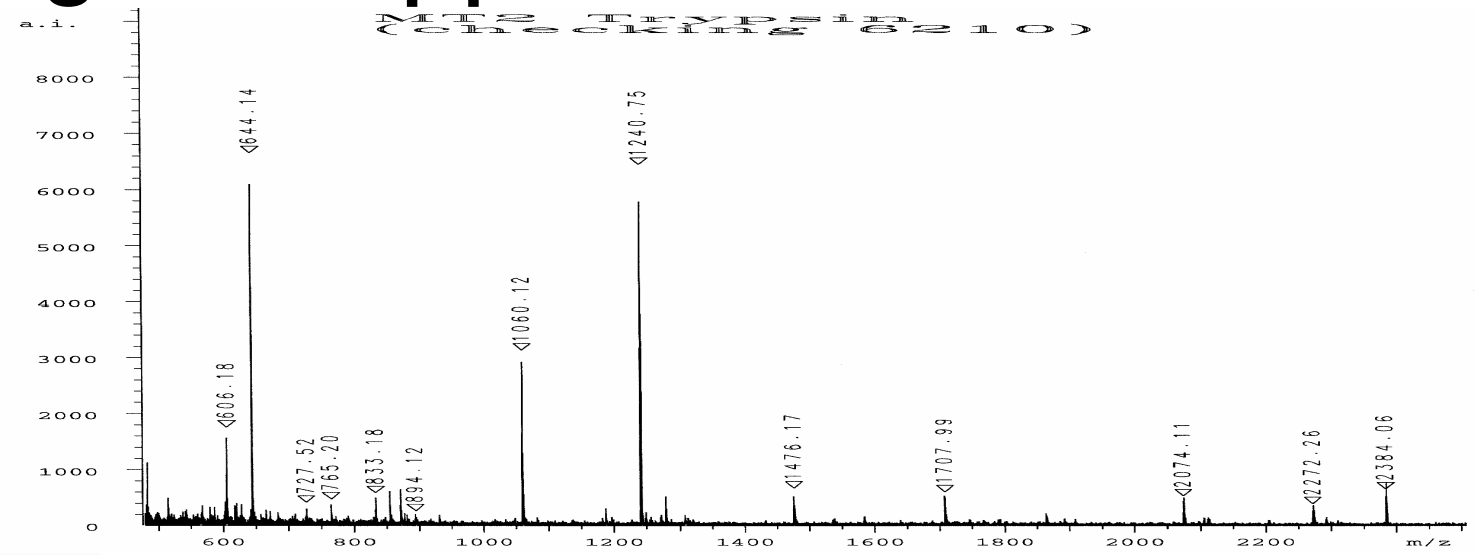


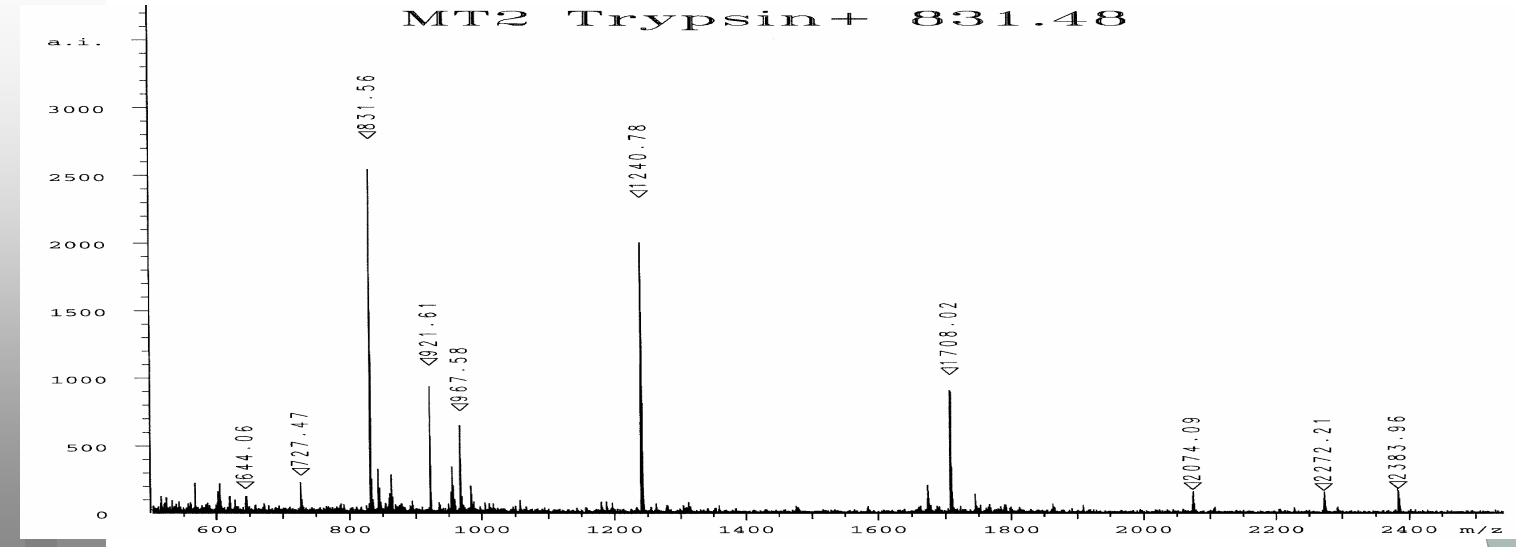
Go back to Chen's RCM 2004 data

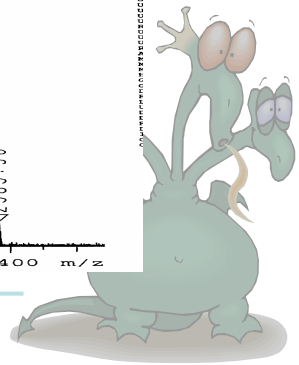




Signal suppression!!!







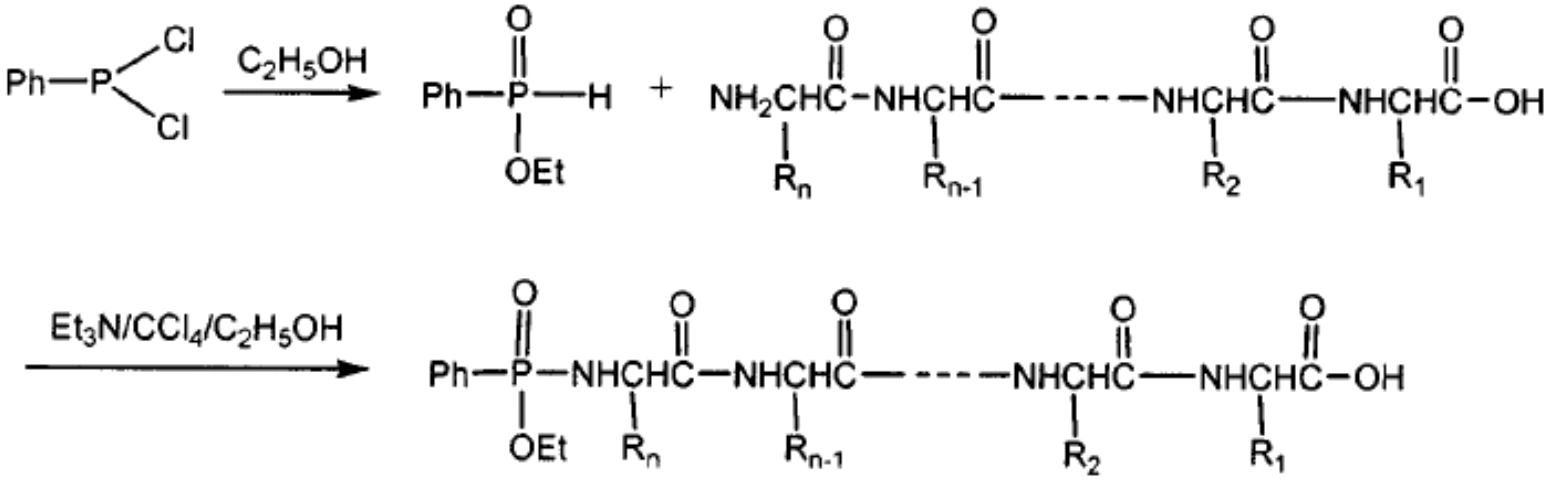
The method can be used with ESI/Ion trap

Lee et al. Proteomics 2004, 4, 1684-1694.

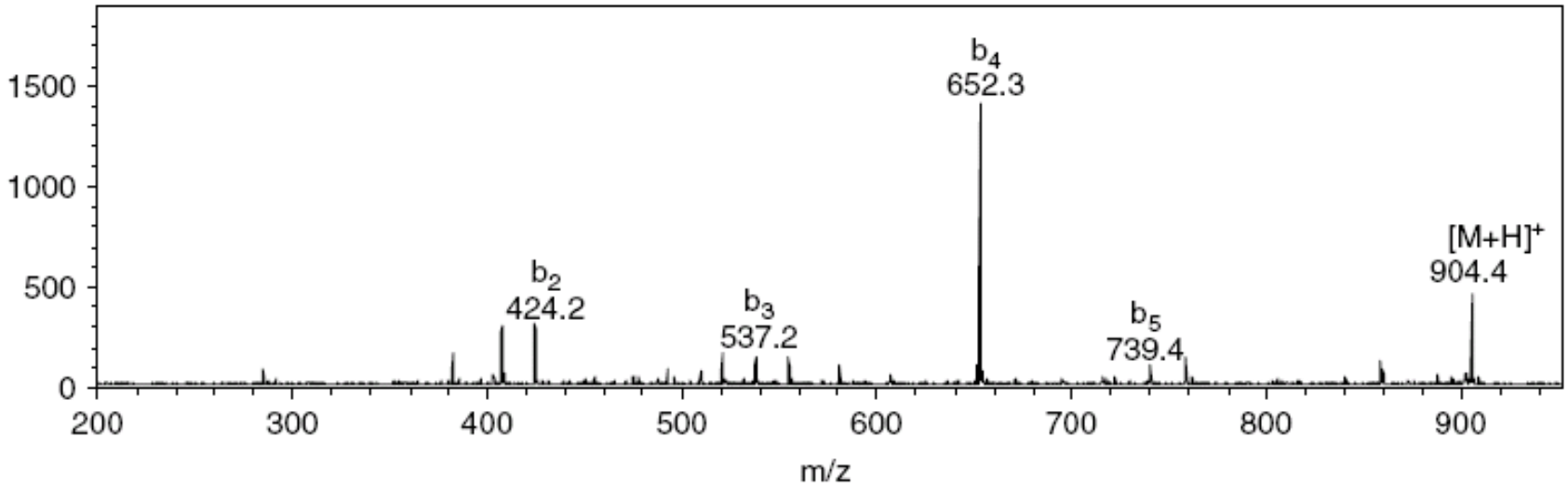
"Highly informative proteome analysis by combining improved N-terminal sulfonation for de novo peptide sequencing and online capillary reverse-phase liquid chromotography/tandem mass spectrometry"

Other alternatives

Bao et al. JMS (2005) 40, 772-776.



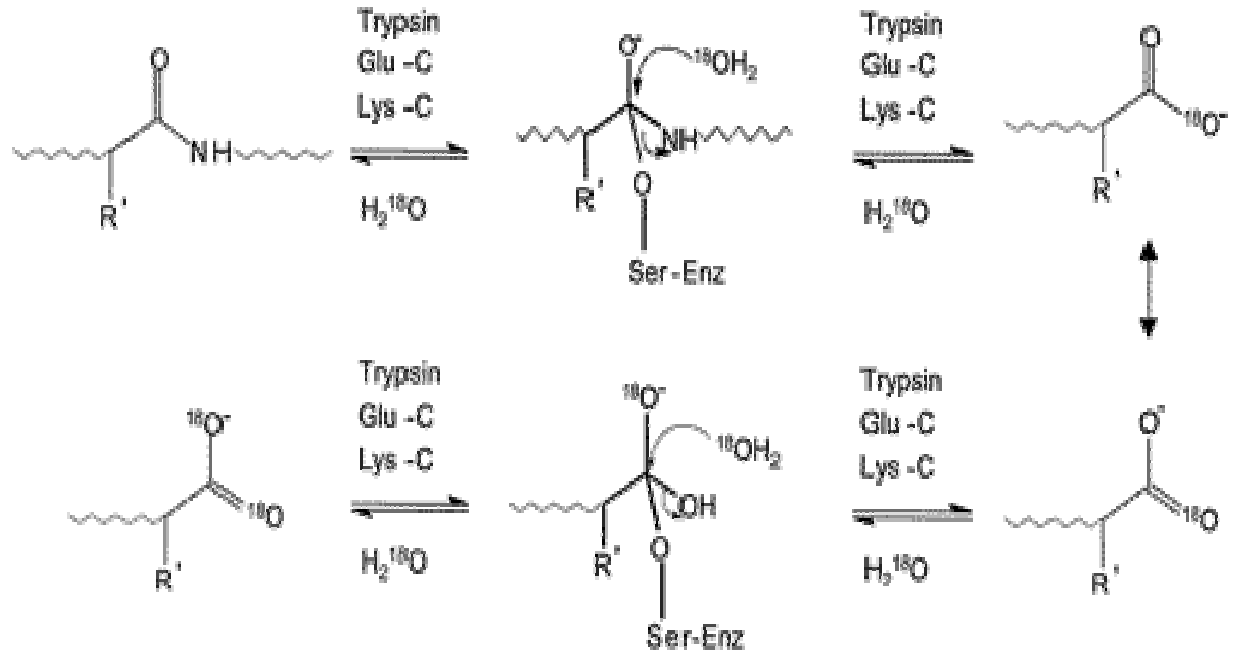
Scheme 1. Synthetic route of phosphonamidate peptides.



ESI-MS^2 of the protonated molecule at *m/z* 904 of EPP-Val-Arg-Leu-Asp-Ser-PheOH.

Method not vigorously tested.

Enzymatic digestion in H_2O^{18}



Schnolzer et al. *Electrophoresis* 1996, 17:945-953
"Protease-catalyzed incorporation of O-18 into peptide fragments and its application
For protein sequencing by electrospray and matrix-assisted laser desorption ionization
Mass spectrometry"

Read:

J Proteome Res. (2005) 4, 101-108.

J Proteome Res. (2005) 4, 2099-2108.

Anal. Chem. (2005) 77, 7783-7795.

For pitfalls and other sequencing applications.

Are proteases absolutely necessary for protein sequencing with mass spectrometric analysis?

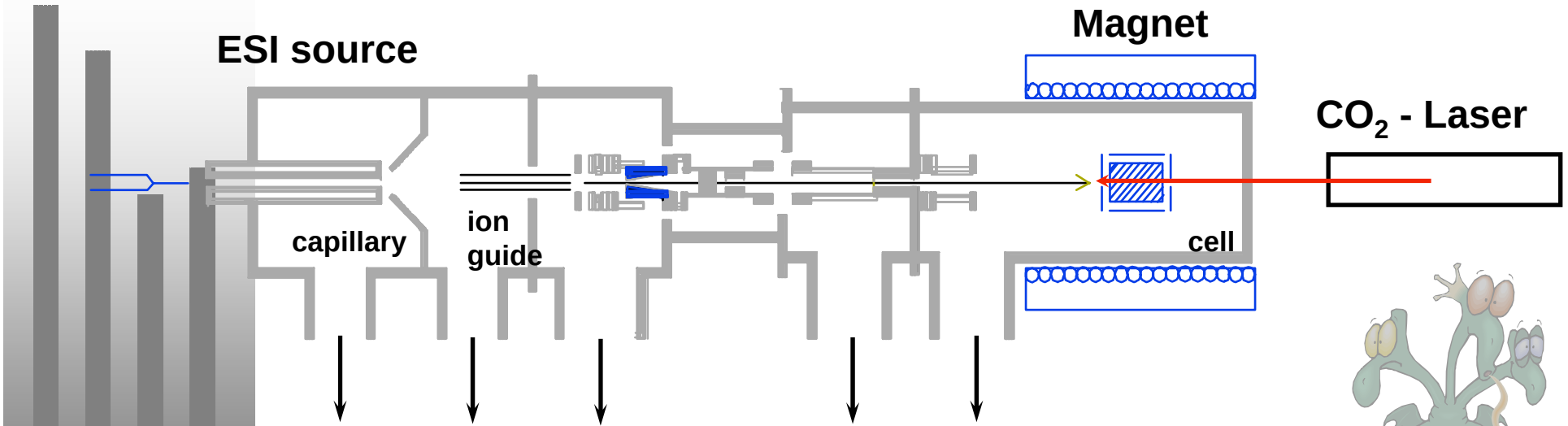
IRMPD FT-ICR

Anal. Chem. **2000,** *72,* 4266−4274

Subfemtomole MS and MS/MS Peptide Sequence Analysis Using Nano-HPLC Micro-ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Susan E. Martin,† Jeffrey Shabanowitz, Donald F. Hunt,*,‡ and Jarrod A. Marto

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904-4319



ECD + FT-ICR

Anal. Chem. **2000,** *72,* 563−573

Electron Capture Dissociation for Structural Characterization of Multiply Charged Protein Cations

Roman A. Zubarev, David M. Horn, Einar K. Fridriksson, Neil L. Kelleher, Nathan A. Kruger, Mark A. Lewis, Barry K. Carpenter, and Fred W. McLafferty*

Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, New York 14853-1301

Automated *de novo* sequencing of proteins by tandem high-resolution mass spectrometry

David M. Horn, Roman A. Zubarev, and Fred W. McLafferty*

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853-1301

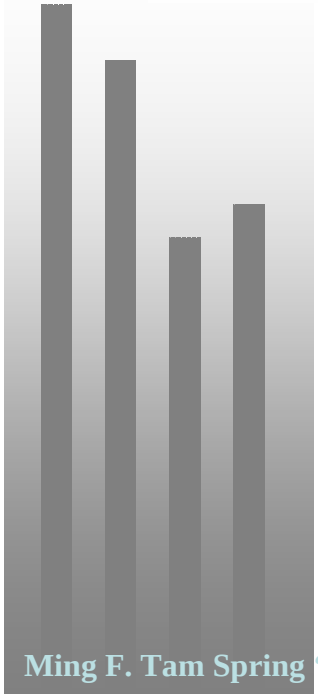
Contributed by Fred W. McLafferty, June 16, 2000 PNAS (2000) 97, 10313-10317

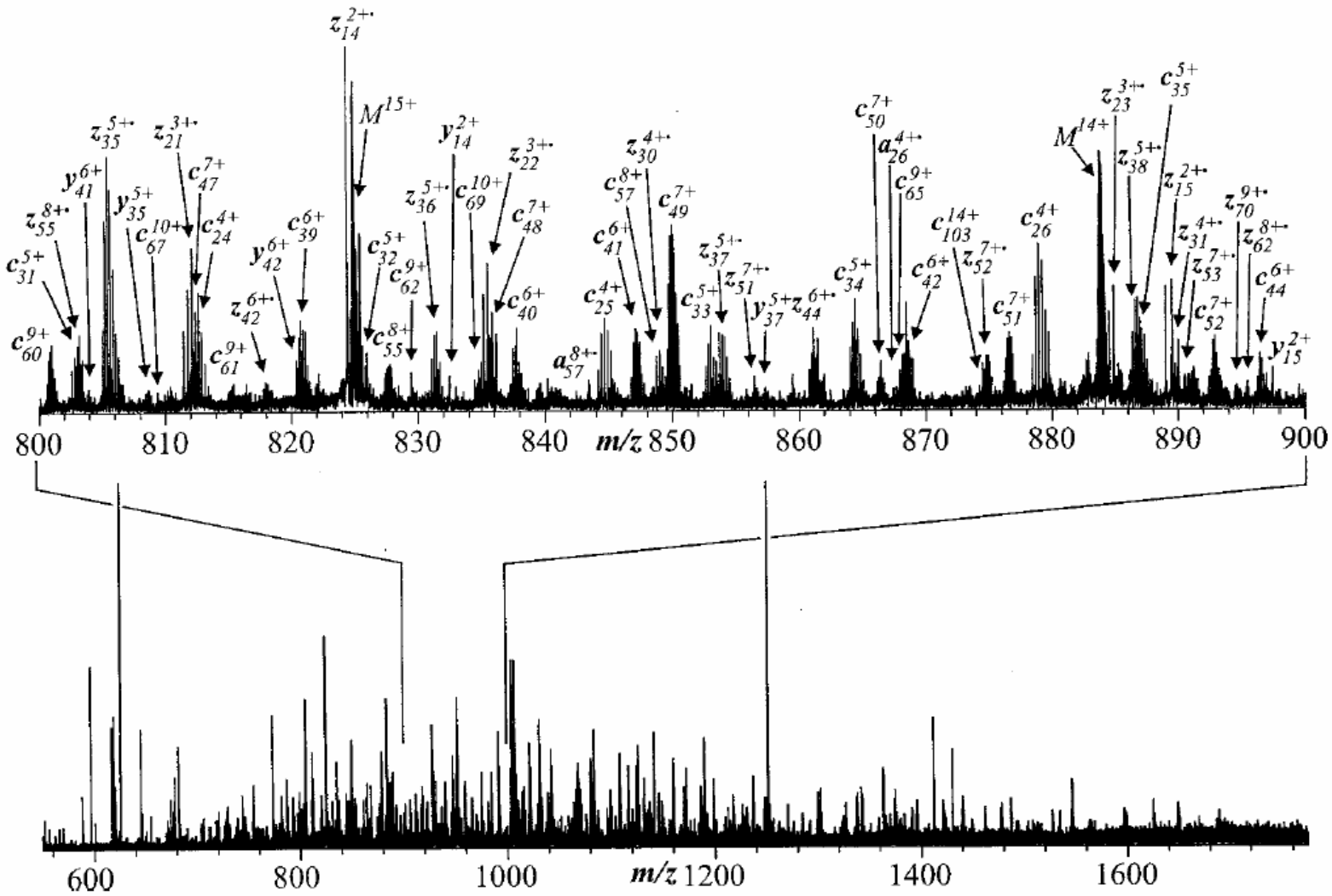
Anal. Chem. **2000,** *72,* 4778−4784

Activated Ion Electron Capture Dissociation for Mass Spectral Sequencing of Larger (42 kDa) Proteins

David M. Horn, Ying Ge, and Fred W. McLafferty*

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301





In-beam AI ECD spectrum (50 scans) of equine cytochrome *c*.

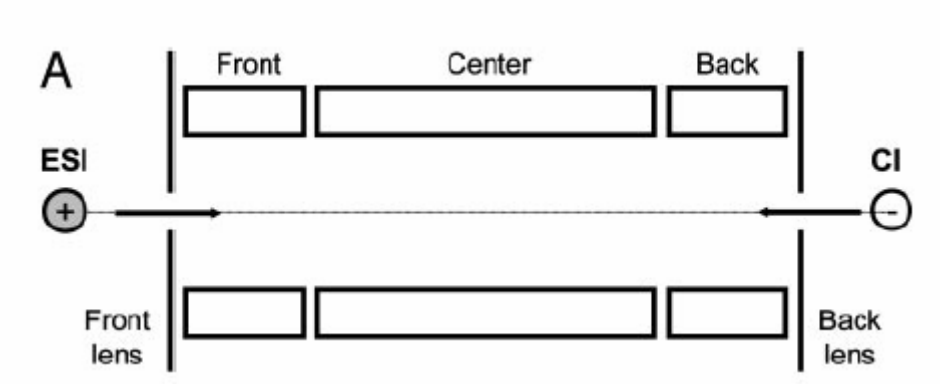
Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry

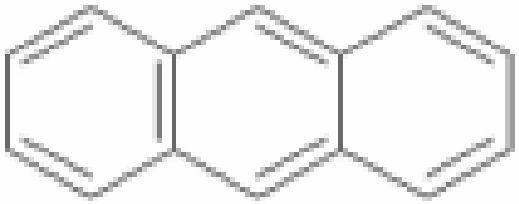
John E. P. Syka*†‡, Joshua J. Coon‡§, Melanie J. Schroeder§, Jeffrey Shabanowitz§, and Donald F. Hunt§¶‖

*Engineering Physics Program and §Department of Chemistry, University of Virginia, Charlottesville, VA 22901; †Thermo Electron, San Jose, CA 95134; and ¶Department of Pathology, Health Sciences Center, University of Virginia, Charlottesville, VA 22908

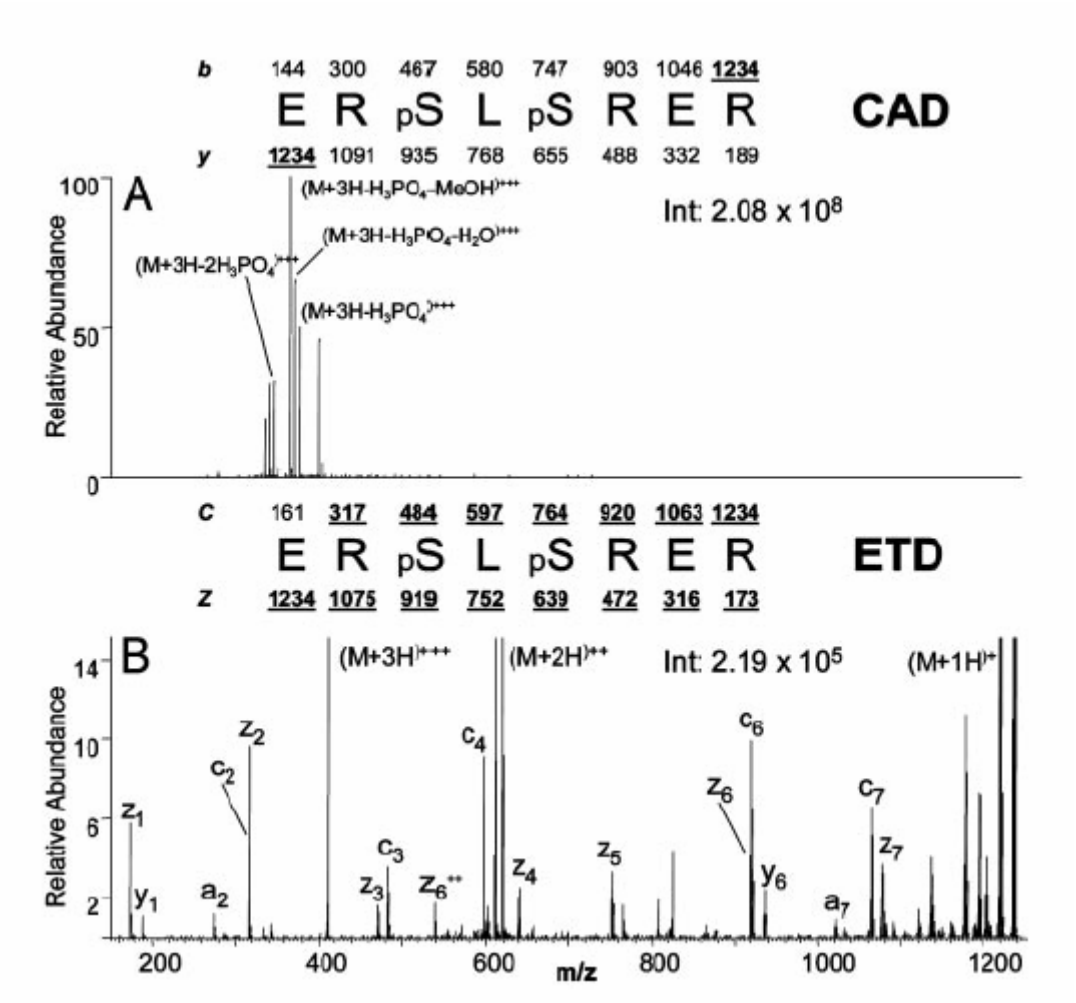
PNAS 204, 101, 9528-9533.

Use a quadrupole linear ion-trap instead of FT-ICR





Introduce –ve ions of anthracene thru CI.
Transfer of electron to peptide backbones for fragmentation.

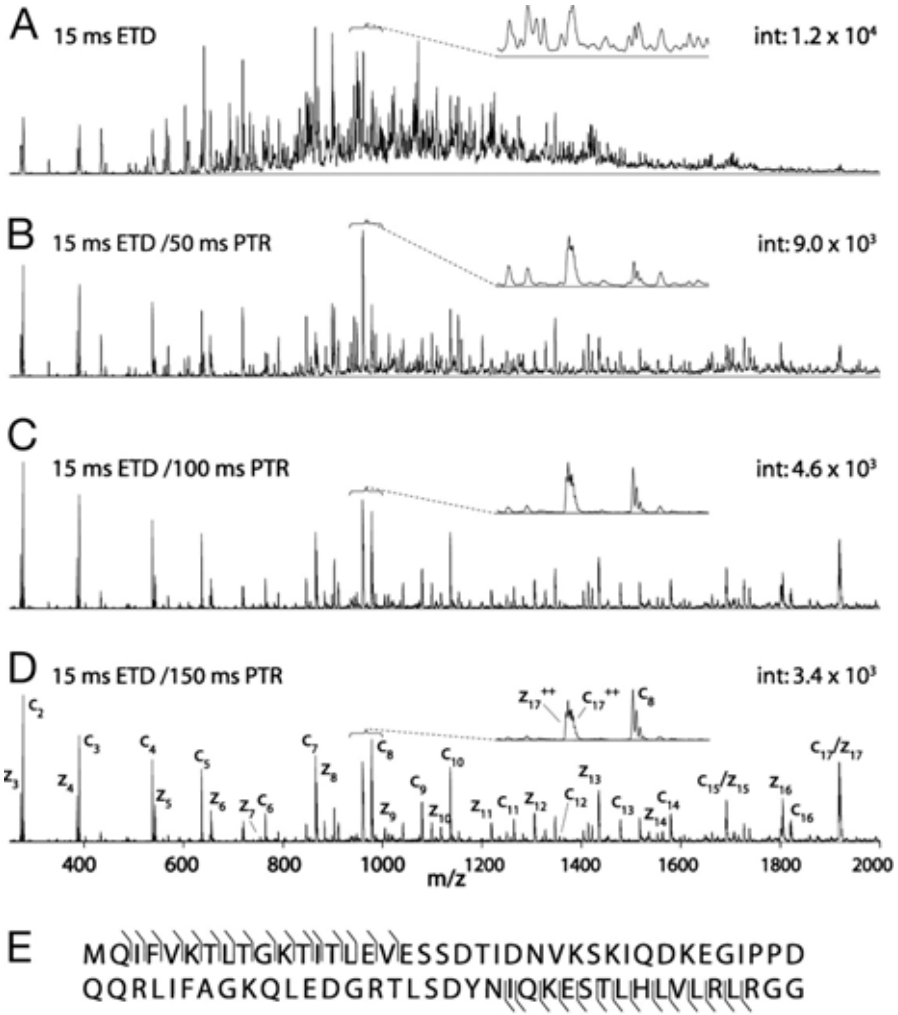

b 144 300 467 580 747 903 1046 1234
E R pS L pS R E R
CAD
y 1234 1091 935 768 655 488 332 189
A
Relative Abundance
100
50
0
(M+3H-H3PO4-MeOH)+++
(M+3H-H3PO4-H2O)+++
(M+3H-2H3PO4)+++
(M+3H-H3PO4)+++
Int: 2.08 x 10^8
C 161 317 484 597 764 920 1063 1234
E R pS L pS R E R
ETD
Z 1234 1075 919 752 639 472 316 173
B
Relative Abundance
14
10
6
2
(M+3H)+++
(M+2H)++
Int: 2.19 x 10^5
(M+1H)+
z1
y1
z2
c2
a2
c3
z3
z6++
c4
z4
z5
c6
z6
y6
a7
c7
z7
200
400
600
800
1000
1200
m/z

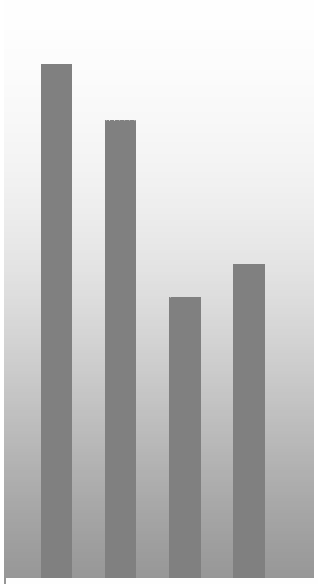
Protein identification using sequential ion/ion reactions and tandem mass spectrometry

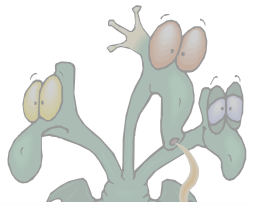
Coon et al. PNAS 2005, 102, 9463-9468.

React with deprotonated benzoic acid in proton transfer charge reduction AFTER ETD on a LTQ.

Fig. 1. Tandem mass spectrum of ubiquitin generated by sequential ion/ion reactions







Coon, Joshua J. et al. (2005) Proc. Natl. Acad. Sci. USA 102, 9463-9468

Electrochemical Oxidation and Cleavage of Proteins with On-line Mass Spectrometric Detection: Development of an Instrumental Alternative to Enzymatic Protein Digestion

Hjalmar P. Permentier and Andries P. Bruins
Center for Pharmacy, University of Groningen, Groningen, The Netherlands

J. Am Soc Mass Spectrom 2004, 15, 1707-1716

Summary

- Intrepretation of MS/MS spectra.
- Chemical derivatization of peptide for sequence analyses:
  - Methyl esterification of COOH
  - Acetylation of amino groups
  - Sulfonylation of N-termini
  - Phosphonylation of N-termini
  - O^{18} exchange at C-termini
- Whole protein sequence determination with FT-ICR.
- Protein cleavage with electrochemical oxidation.