Mass Spectrometry I: Fundamentals and Electron Impact

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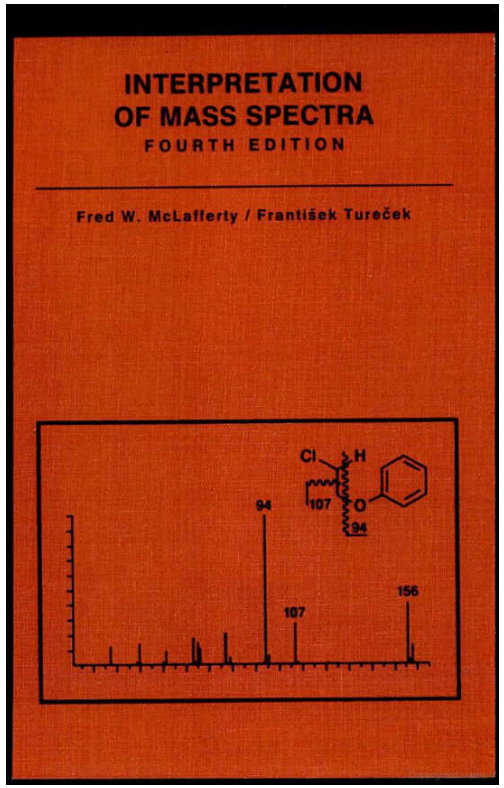
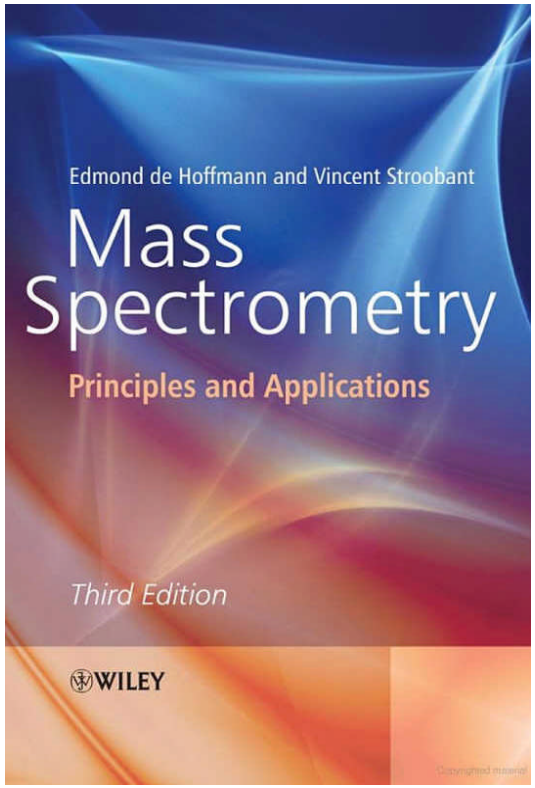
October 14th, 2009

The basics of mass spectrometry

American Society for Mass Spectrometry:
http://www.asms.org/whatisms/p1.html

Wikipedia
http://en.wikipedia.org/wiki/Mass_spectrometry





Also, special thanks to:

Prof. Roman Zubarev
(Karolinska
Institute, Stockholm, Sweden)

and

Dr. Ann Dixon
(Warwick!)

for slides.

Mass ***Spectrometry***

- Mass spec. is *not* a method based on absorption of electromagnetic radiation
  - … but it complements these methods (UV, IR, NMR)
- In Mass spectrometry, molecules are ionized, and then their mass is measured by sorting them out in magnetic and electric fields.
- Thus, the function of mass spectrometers is all about how ions move in electric and magnetic fields, and this all starts with the Maxwell equations.

What information is in Mass Spectra?

1. **Masses**
   Useful for testing your theory of your chemical structure
2. **Elemental compositions**
   Or at least estimates thereof...
3. Mixture Compositions
4. Abundances (quantitation)
5. Charge states
6. Mass differences (MS/MS)
   - Sequences (Proteins, peptides, polymers, DNA, etc)
   - Linkages (carbohydrates, hydrogen bonding, etc.
7. Fragment stabilities
   Breakdown curves yield relative (Net) transition state energies
8. Higher order structure
   - Hydrogen/Deuterium Exchange (HDX) experiments
   - Electron Capture/Transfer Dissociation

Mass Spectrometry and Isotopic Distributions

Why so many peaks?

What is Molecular Mass?

Mass: $M = \Sigma m_e \cdot n_e$,

m_e – mass of an element

n_e – number of atoms of this element in the molecule

Isotope	*Mass*	*Abundance*	*Chemical mass*	*Deviation from the whole number*
^{1}H	**1.00782510**	**99.9852%**	**1.00794**	**+0.0079**
^{2}H (D)	**2.01410222**	**0.0148%**		
^{12}C	**12.0(0)**	**98.892%**	**12.011**	**+0.011**
^{13}C	**13.0033544**	**1.108%**		
^{14}N	**14.00307439**	**99.635%**	**14.00674**	**+0.007**
^{15}N	**15.0001077**	**0.365%**		
^{16}O	**15.99491502**	**99.759%**	**15.9994**	**-0.0006**
^{17}O	**16.9991329**	**0.037%**		
^{18}O	**17.99916002**	**0.204%**		
^{31}P	**30.9737647**	**100%**	**30.9737647**	**-0.0262**
^{32}S	**31.9720737**	**95.0%**	**32.066**	**+0.066**
^{33}S	**32.9714619**	**0.76%**		
^{34}S	**33.9678646**	**4.22%**		
^{36}S	**35.967090**	**0.014%**		

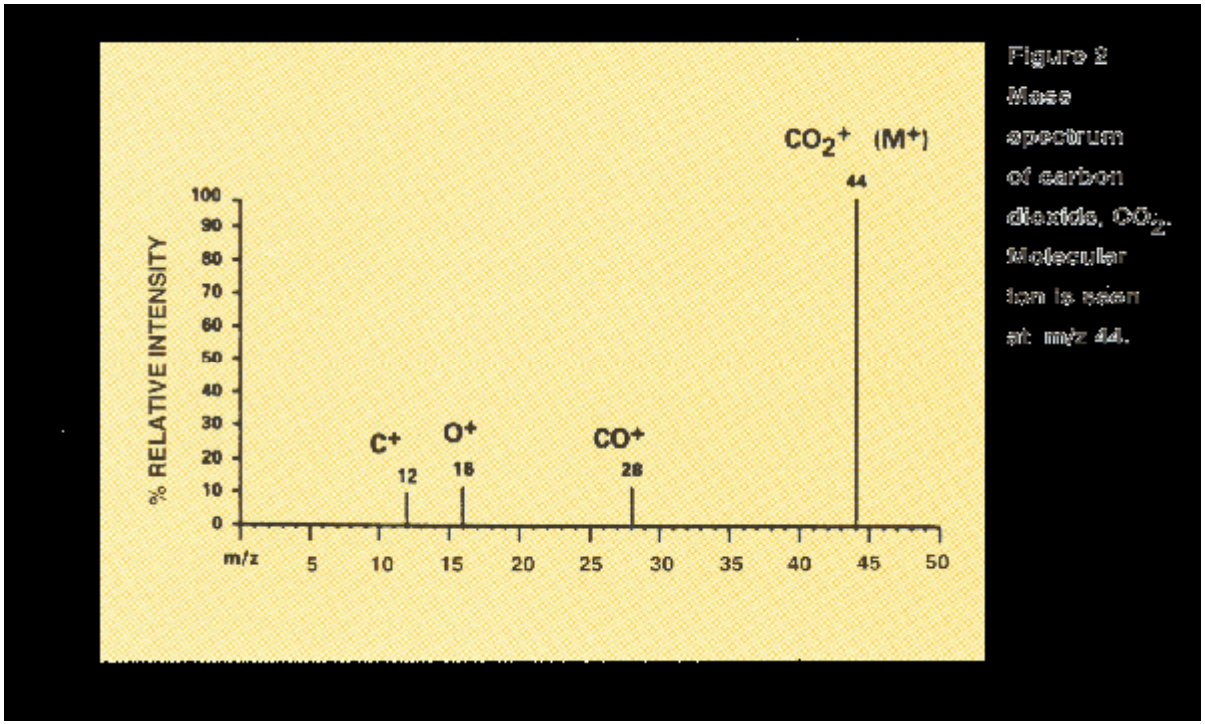
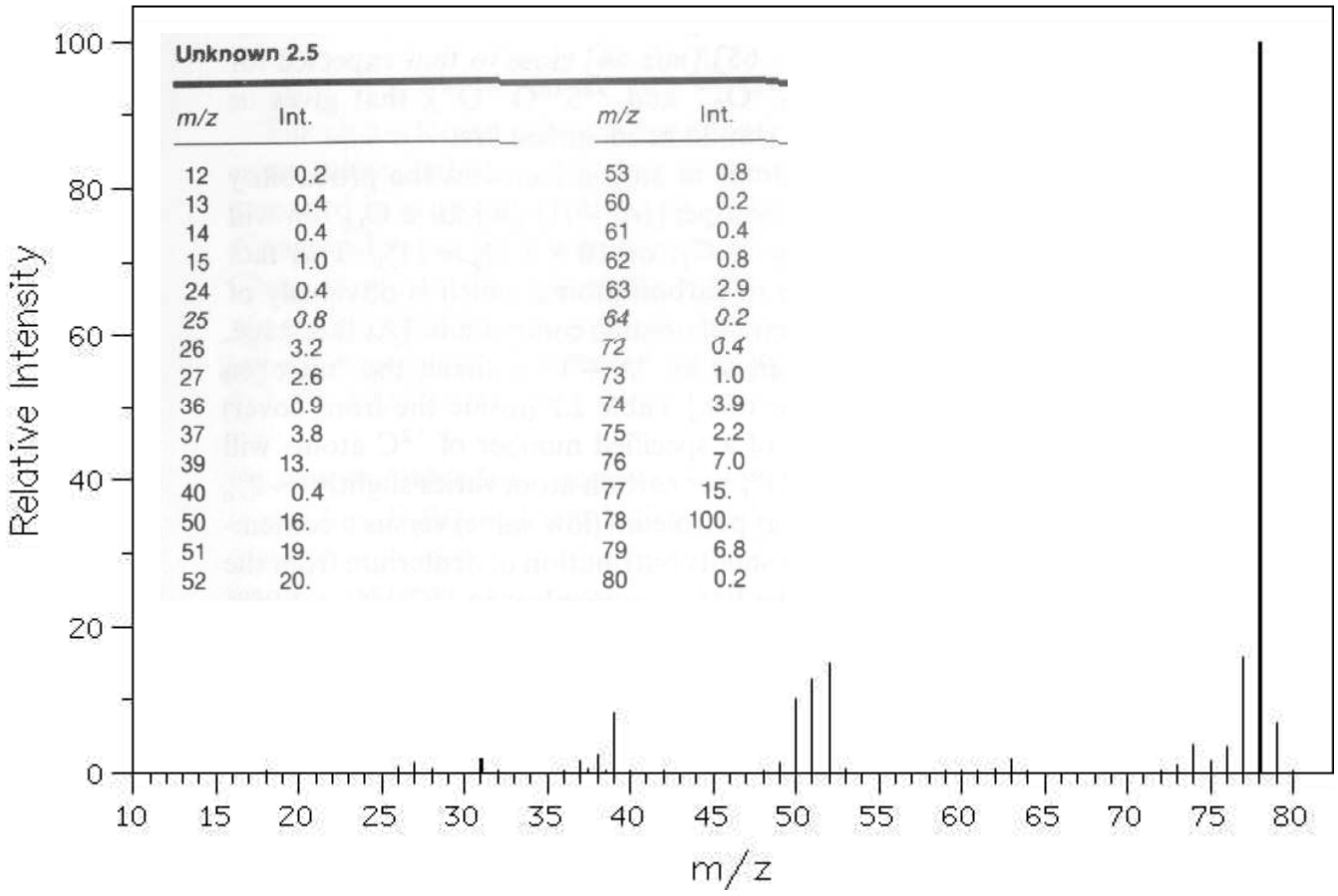


Figure 2
Mass spectrum of carbon dioxide, CO_2. Molecular ion is seen at m/z 44.

The mass spectrum of benzene (C_6H_6)

Mass spectrum:



1. Monoisotopic “A” elements
(fluorine, phosphorus, cesium, sodium, iodine)

2. “A+1” elements
(carbon, nitrogen, hydrogen)

3. “A+2” elements

(oxygen, chlorine, bromine, silicon, sulfur)

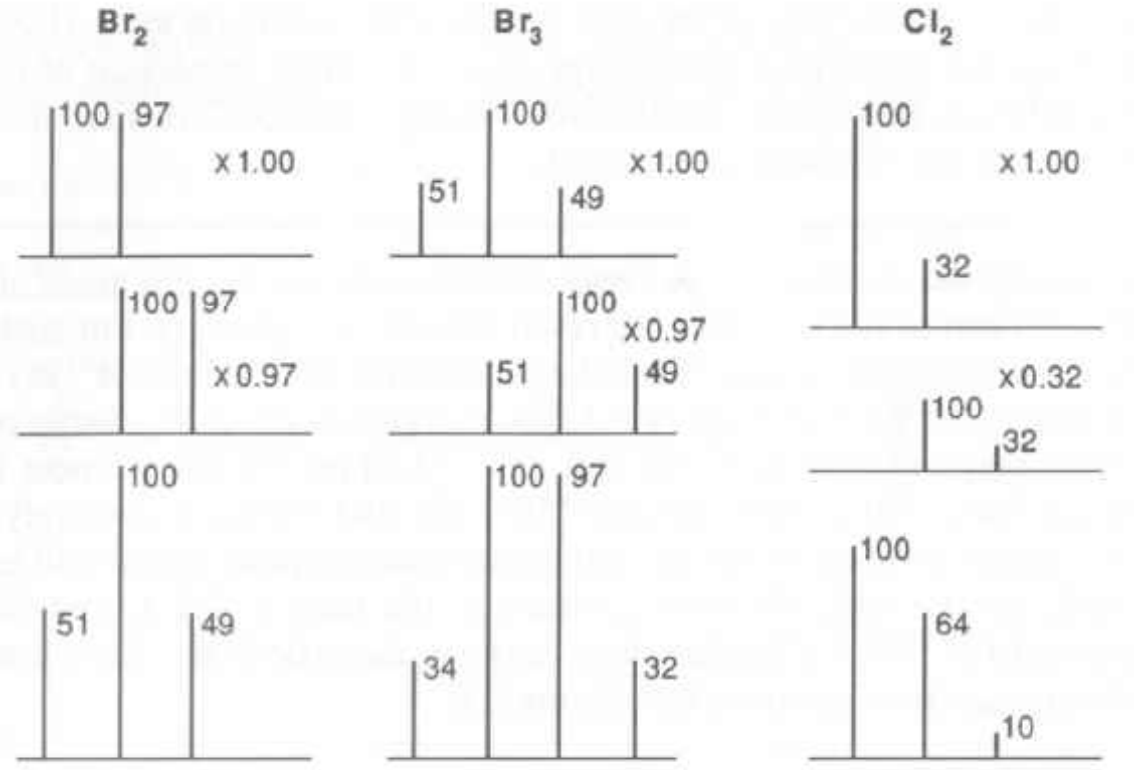


Figure 2.1. Linear superposition of bromine and chlorine peak patterns.

Elemental Compositions of Metals

Magnesium	
23.98504	78.7
24.98584	10
25.98259	11.3

Aluminum	
26.98153	100

Zinc	
63.9291	49
65.926	28
66.9271	4
67.9249	18.6

silver	
106.9041	52
108.9047	48

Titanium	
45.95263	8
46.9518	7
47.94795	74
48.94787	5.5
49.9448	5

Gold	
196.9666	100

Lead	
203.973	1.5
205.9745	23
206.9759	22.6
207.9766	52.3

Iron	
53.9396	5.8
55.9349	92

Mercury	
197.9668	10
198.9683	17
199.9683	23
200.9703	13
201.9706	30
203.9735	7

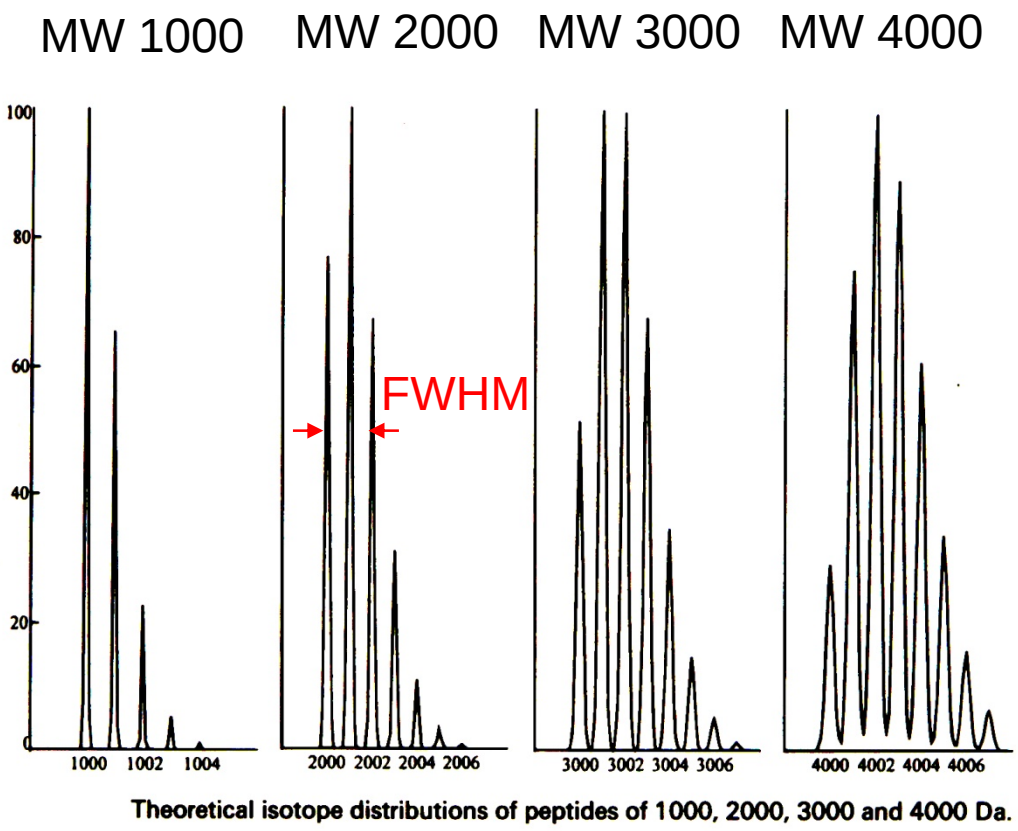
Uranium	
235.0439	0.7
238.0508	99.3

Selenium	
75.9192	9
76.9199	7.6
77.9173	23.5
79.9165	49.8
81.9167	9

Palladium	
101.9049	1
103.9036	11
104.9046	22
105.9032	27
107.903	28
109.9045	12

CRC Handbook of Chemistry and Physics, 48th Edition, 1967

How do isotopic distributions change with mass?



Theoretical isotope distributions of peptides of 1000, 2000, 3000 and 4000 Da.

Yergey J, Heller D, Hansen G, Cotter RJ, Fenselau C.
Anal. Chem. 1983, *55,* 353-356.

- monoisotopic mass dominates up to MW ~1100
- above MW ~7000, the monoisotopic peak is vanishingly small
- becomes more symmetric
- the width grows sublinearly. For <3 kDa, MW/FWHM ~1100, for 10 kDa, MW/FWHM ~2000
- the most abundant mass is 0..1 Da *below* the average mass
- *fine structure* for all peaks but monoisotopic.

Molecular mass *is* the isotopic distribution!

Mass quantities:

Nominal mass: m_e is the **integer** mass value for the most abundant isotope (H=1, etc.).

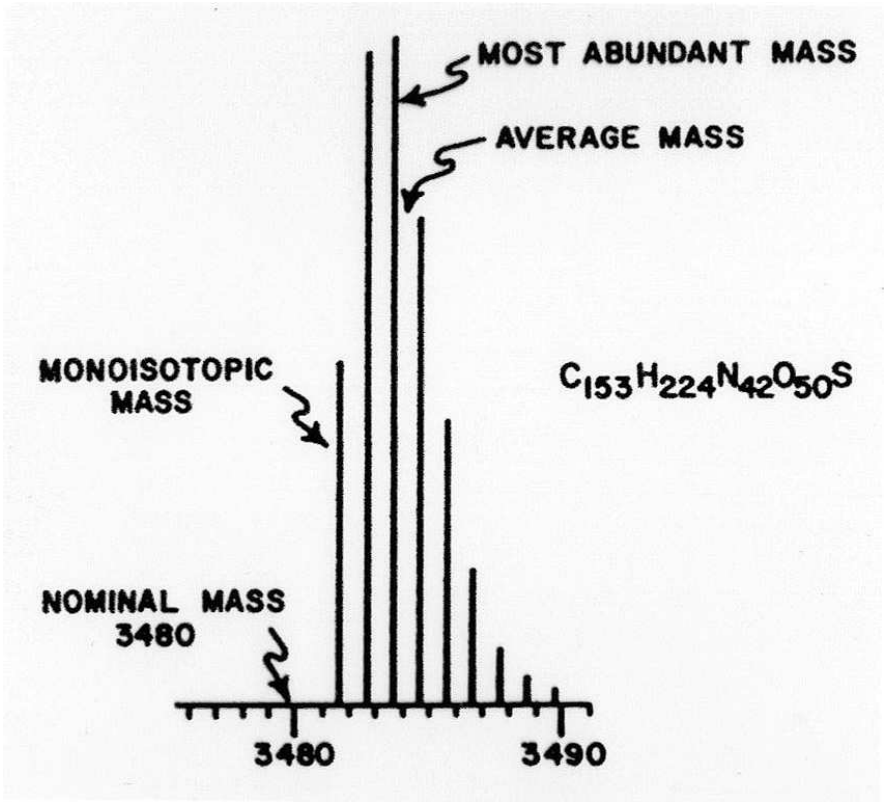
Monoisotopic mass: m_e is the **exact** mass value for the **most abundant** isotope (H=1.00782510, etc.).

Average mass: m_e is the **chemical** (average) atomic mass value (H=1.00794, etc.).

Isotopic cluster (distribution): a group of isotopic peaks representing the same molecule.

Most abundant mass: such in the isotopic cluster.

Yergey J, Heller D, Hansen G, Cotter RJ, Fenselau C. Anal. Chem. 1983, *55,* 353-356.



How to calculate the isotopic distribution?

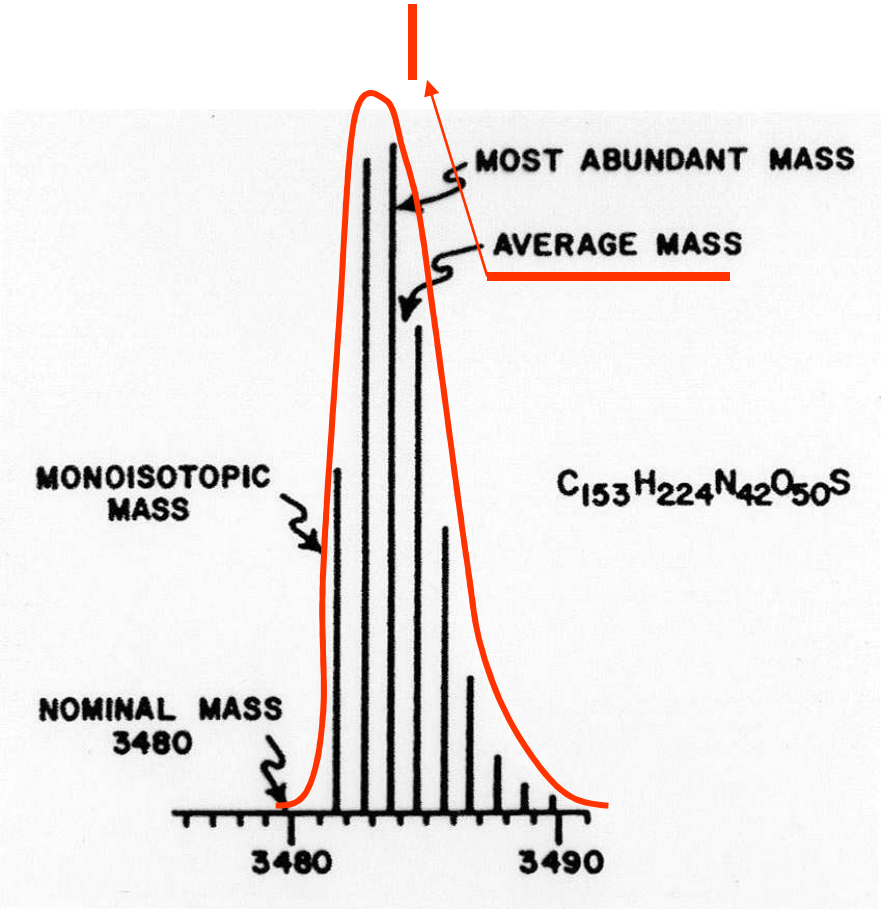
Use the binomial distribution.

$$P(i) = \frac{N!}{i!(N - i!)} p^i (1 - p)^{N-i}$$

N= number of atoms
I = ith isotope
p = probability of being heavy isotope (e.g. ^{13}C)

Note: the total isotopic distribution is the sum (actually the mathematical convolution) of the individual isotopic distributions for each possible isotope.

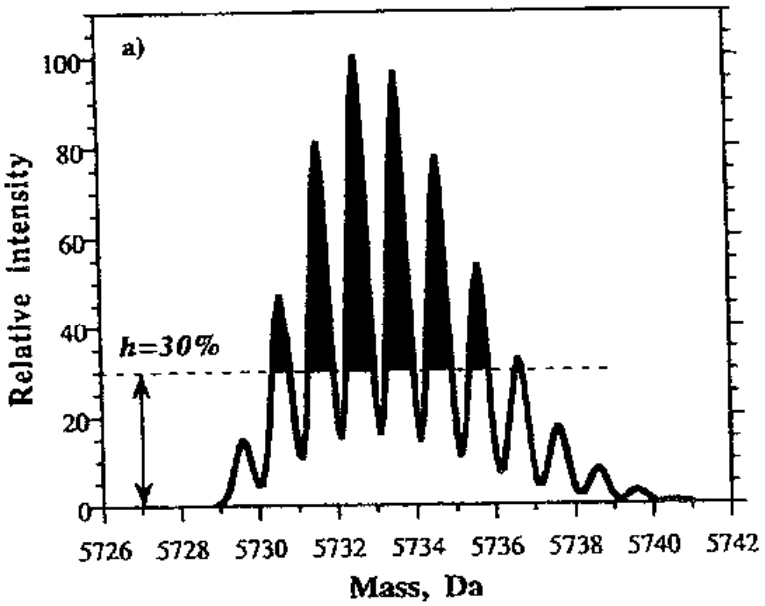
Is the Average Mass Reliable?

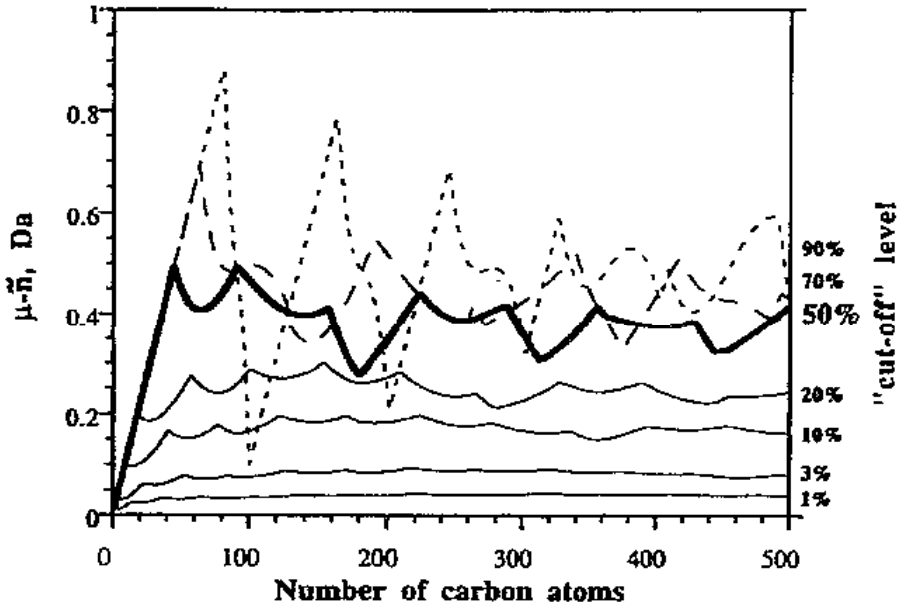


Inherent uncertainty of average mass is ca. **10 ppm**.

Is average mass reliable?

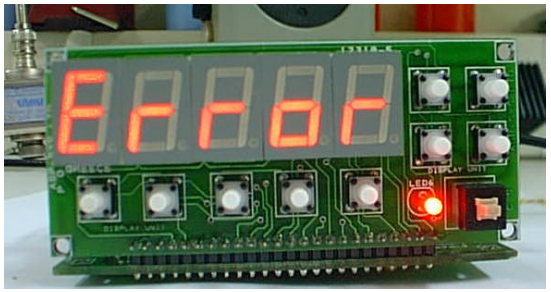
Zubarev RA, Demirev PA, Håkansson P, Sundqvist BUR. Anal. Chem. 1995, *67,* 3793-3798.





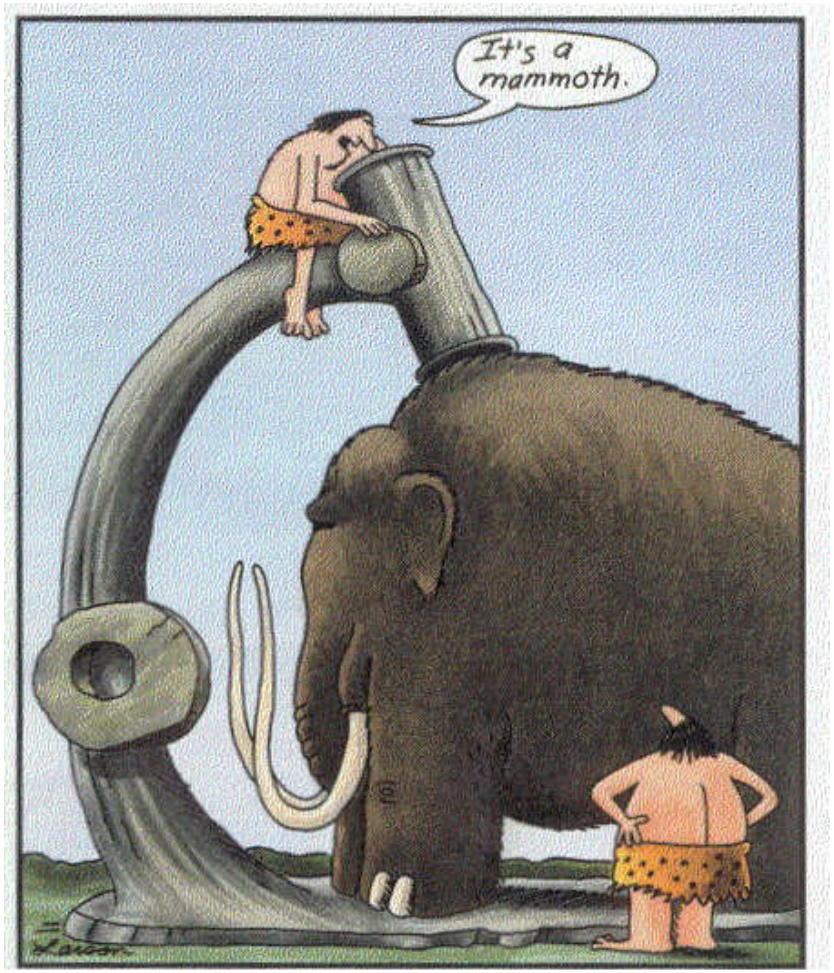
Underestimation by 0.45±0.10 Da.

Minimal

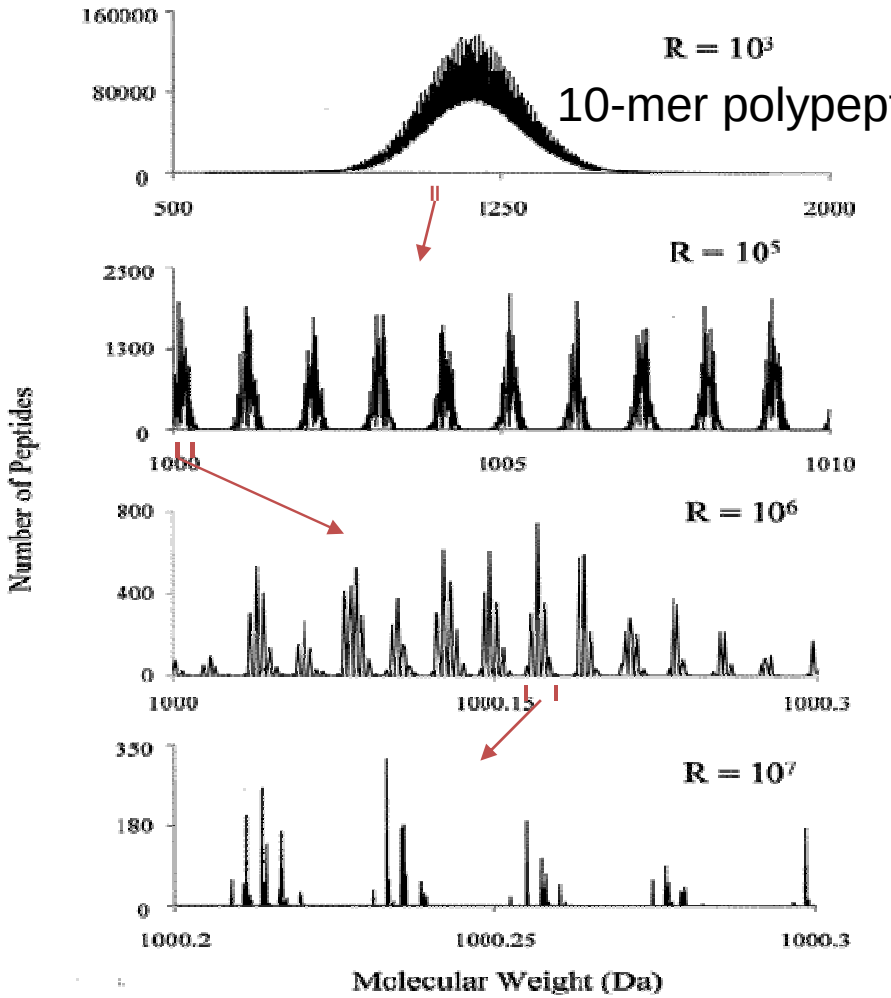


0.1 Da!

What is Molecular Mass?

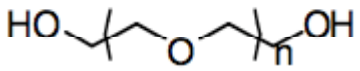


Conrads TP, Anderson GA, Veenstra TD, Pasa-Tolic L, Smith RD, Anal. Chem. 2000, *72,* 3349-3354.



Example #1

What would the electrospray mass spectrum of poly-ethylene glycol (PEG : $HO\text{-}[CH_2CH_2O]_nH$) look like? Assume n = 100-120.

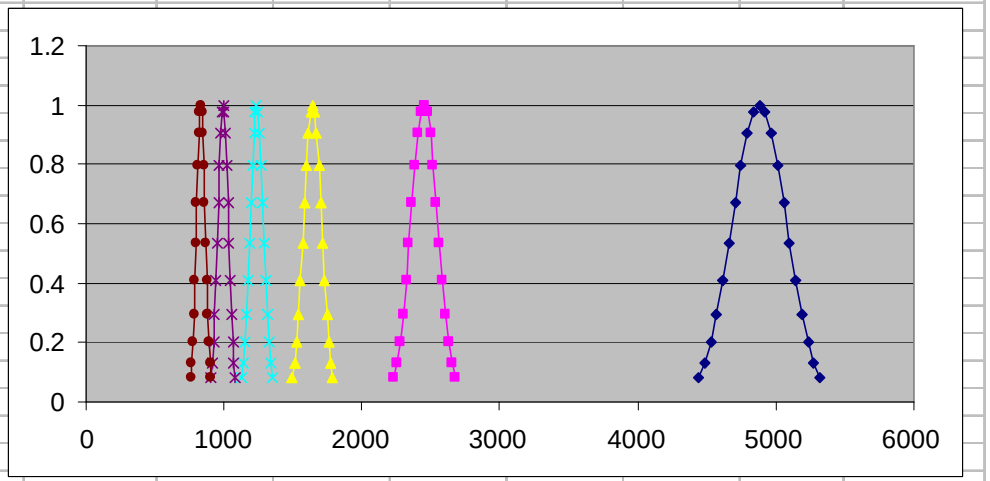


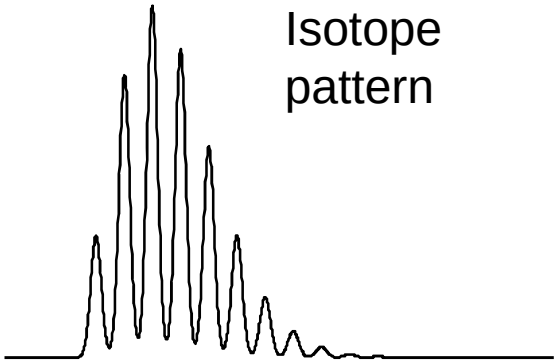
http://en.wikipedia.org/wiki/Polyethylene_glycol

#1. What would the electrospray mass spectrum of poly-ethylene glycol (PEG : $HO\text{-}[CH_2CH_2O]_nH$) look like? Assume n = 100-120.

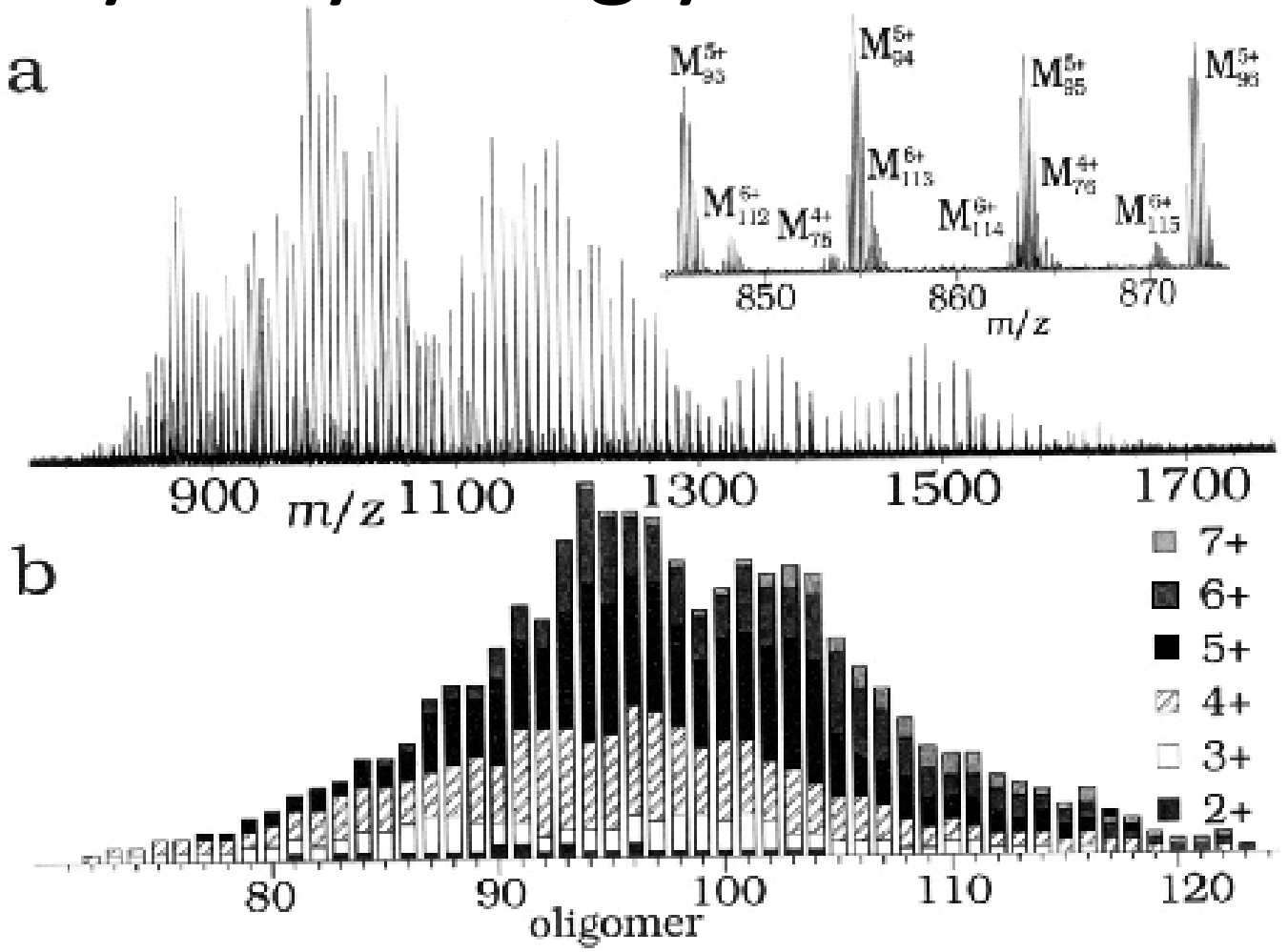
masses

n	M+Na+	(M+2Na)2+	(M+3Na)3+	(M+4Na)4+	(M+5Na)5+	(M+6Na)6+		abundance
100	4440.9898	2231.9898	1495.6565	1127.4898	906.5898	759.32313		0.082084999
101	4484.9898	2253.9898	1510.3231	1138.4898	915.3898	766.65647		0.131993843
102	4528.9898	2275.9898	1524.9898	1149.4898	924.1898	773.9898		0.201896518
103	4572.9898	2297.9898	1539.6565	1160.4898	932.9898	781.32313		0.2937577
104	4616.9898	2319.9898	1554.3231	1171.4898	941.7898	788.65647		0.40656966
105	4660.9898	2341.9898	1568.9898	1182.4898	950.5898	795.9898		0.535261429
106	4704.9898	2363.9898	1583.6565	1193.4898	959.3898	803.32313		0.670320046
107	4748.9898	2385.9898	1598.3231	1204.4898	968.1898	810.65647		0.798516219
108	4792.9898	2407.9898	1612.9898	1215.4898	976.9898	817.9898		0.904837418
109	4836.9898	2429.9898	1627.6565	1226.4898	985.7898	825.32313		0.975309912
110	4880.9898	2451.9898	1642.3231	1237.4898	994.5898	832.65647		1
111	4924.9898	2473.9898	1656.9898	1248.4898	1003.3898	839.9898		0.975309912
112	4968.9898	2495.9898	1671.6565	1259.4898	1012.1898	847.32313		0.904837418
113	5012.9898	2517.9898	1686.3231	1270.4898	1020.9898	854.65647		0.798516219
114	5056.9898	2539.9898	1700.9898	1281.4898	1029.7898	861.9898		0.670320046
115	5100.9898	2561.9898	1715.6565	1292.4898	1038.5898	869.32313		0.535261429
116	5144.9898	2583.9898	1730.3231	1303.4898	1047.3898	876.65647		0.40656966
117	5188.9898	2605.9898	1744.9898	1314.4898	1056.1898	883.9898		0.2937577
118	5232.9898	2627.9898	1759.6565	1325.4898	1064.9898	891.32313		0.201896518
119	5276.9898	2649.9898	1774.3231	1336.4898	1073.7898	898.65647		0.131993843
120	5320.9898	2671.9898	1788.9898	1347.4898	1082.5898	905.9898		0.082084999





Poly ethylene glycol distribution



1. O'Connor, P. B.; McLafferty, F. W. Oligomer characterizationof 4-22 kda polymers by electrospray fourier-transform mass spectrometry *J. Am. Chem. Soc.* **1996**, *117*, 12826-12831.

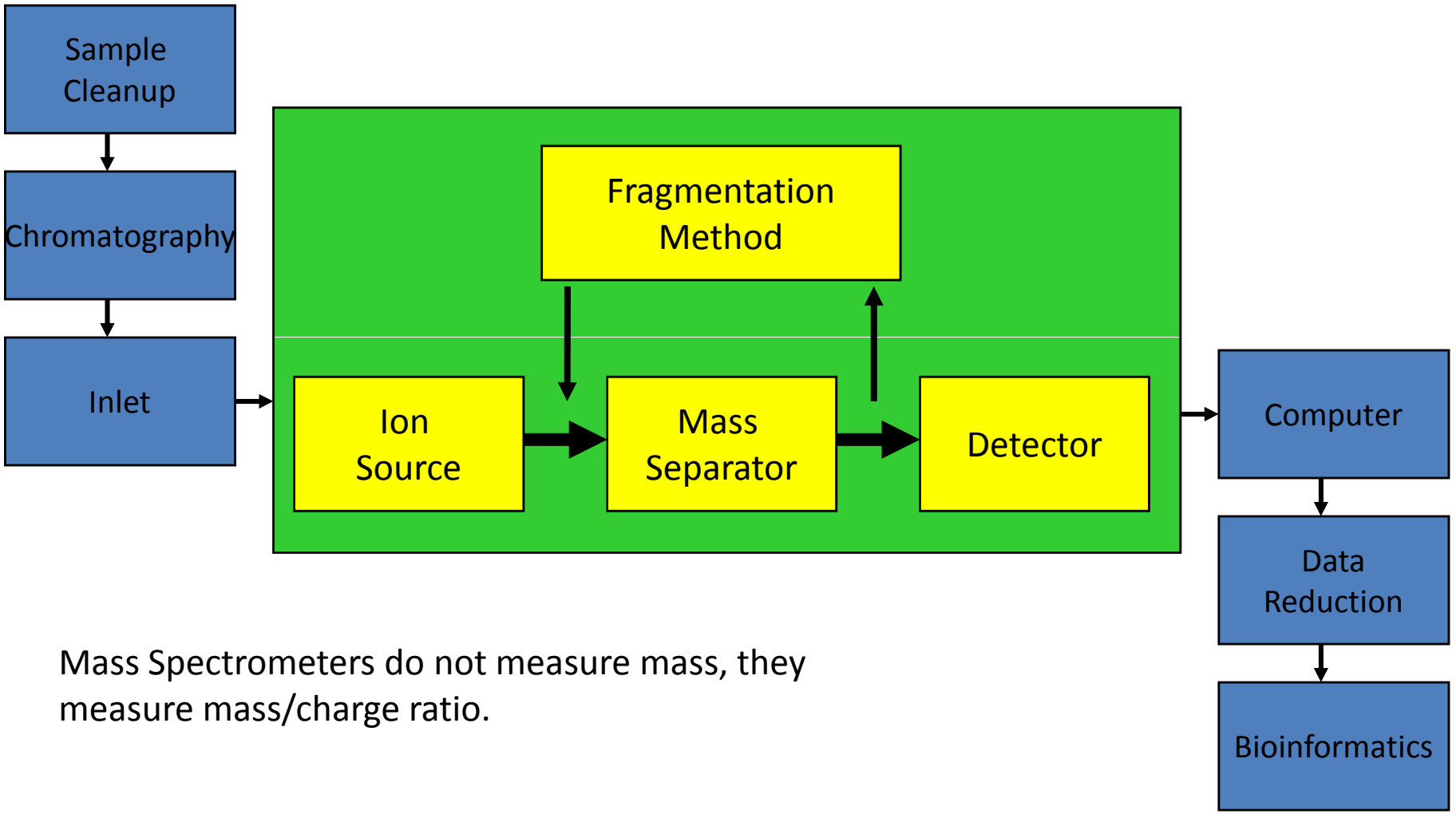
Mass Spectrometry Instruments

So many choices, so little time....

Mass ***Spectrometry***

- Mass spec. is *not* a method based on absorption of electromagnetic radiation
  - … but it complements these methods (UV, IR, NMR)
- In Mass spectrometry, molecules are ionized, and then their mass is measured by sorting them out in magnetic and electric fields.
- Thus, the function of mass spectrometers is all about how ions move in electric and magnetic fields, and this all starts with the Maxwell equations.

MS Block Diagram

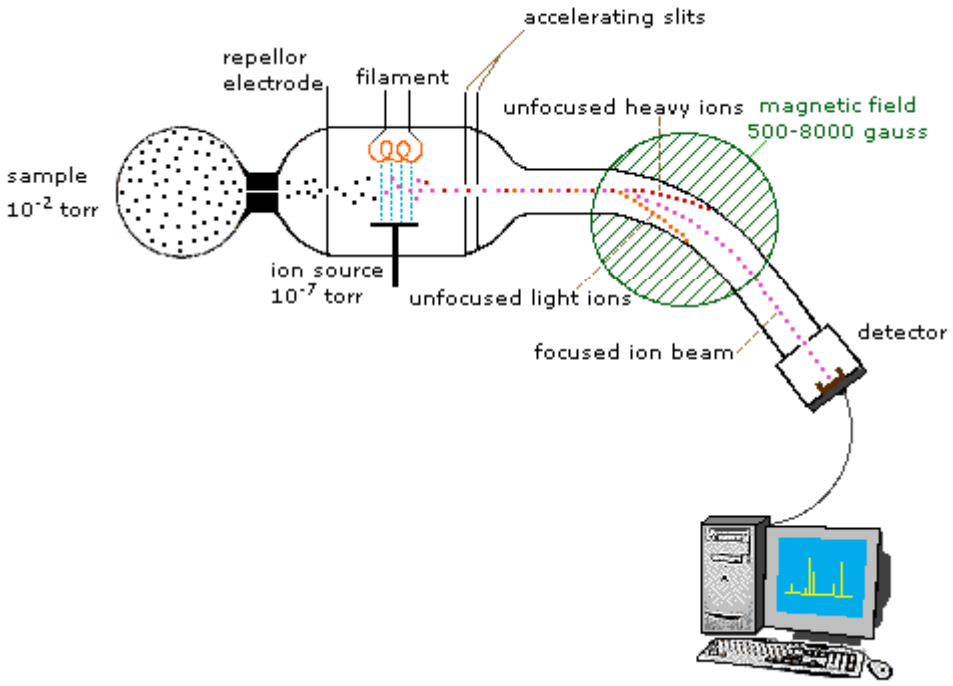


Mass Spectrometers do not measure mass, they measure mass/charge ratio.

Source

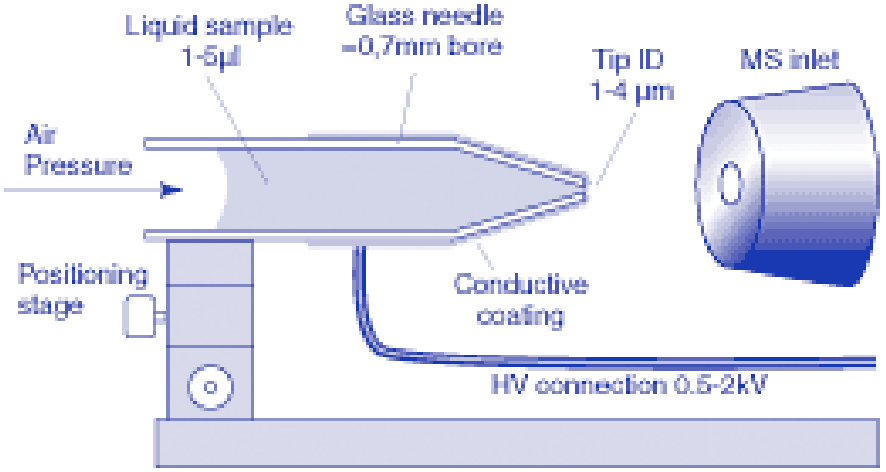
- One of the most important differences between mass spectrometers is the source
- In the source, create gas phase ions:
  - degree of ionization can vary, as can the degree of fragmentation
  - Some methods induce no fragmentation (M^+ only),
  - Others can lead to fragmentation (provides vital structural information).
- Commonly used ionization methods are EI, CI, MALDI and ESI.
- EI, CI, and MALDI generate 1+ or 1- ions (almost always)
- ESI produces multiply charged ions (5+ - 13+ for example)

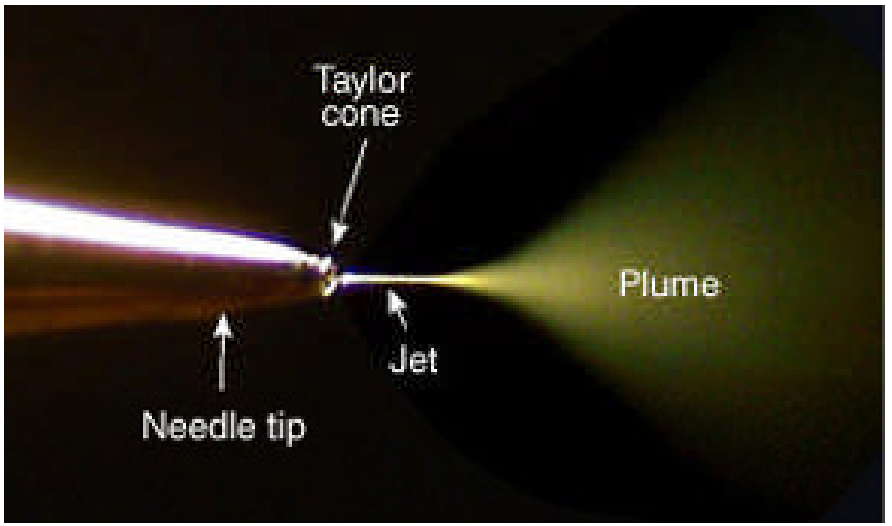
Electron impact



http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/MassSpec/masspec1.htm

Taylor cone

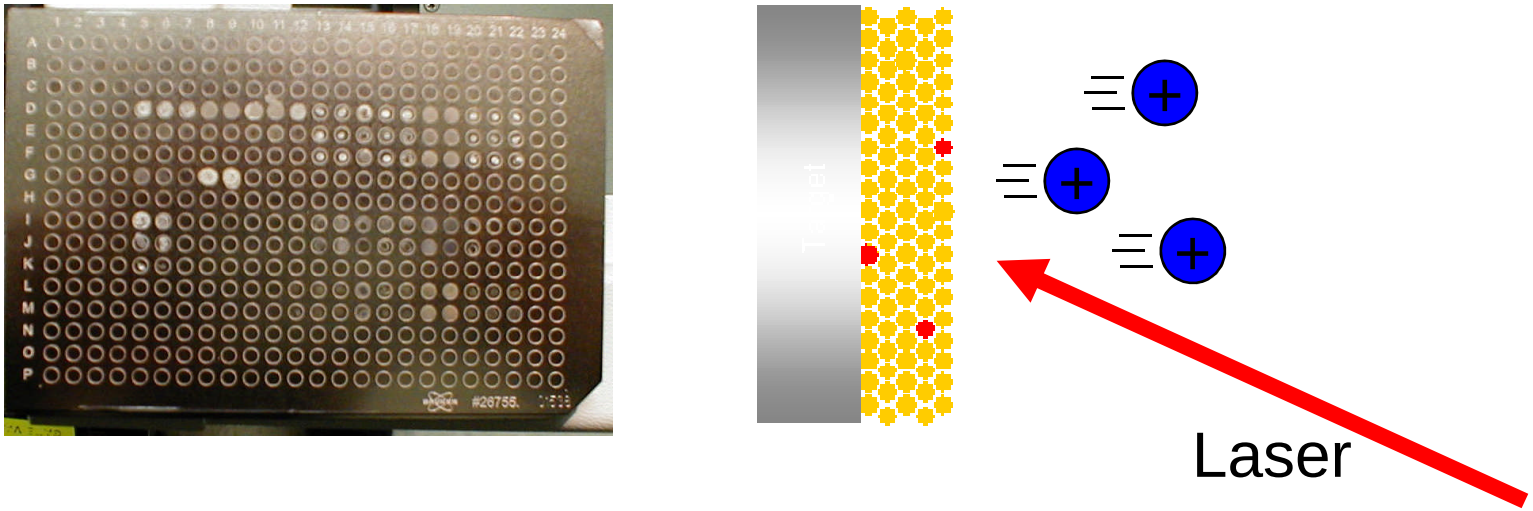




Spray ~1 microliter/min

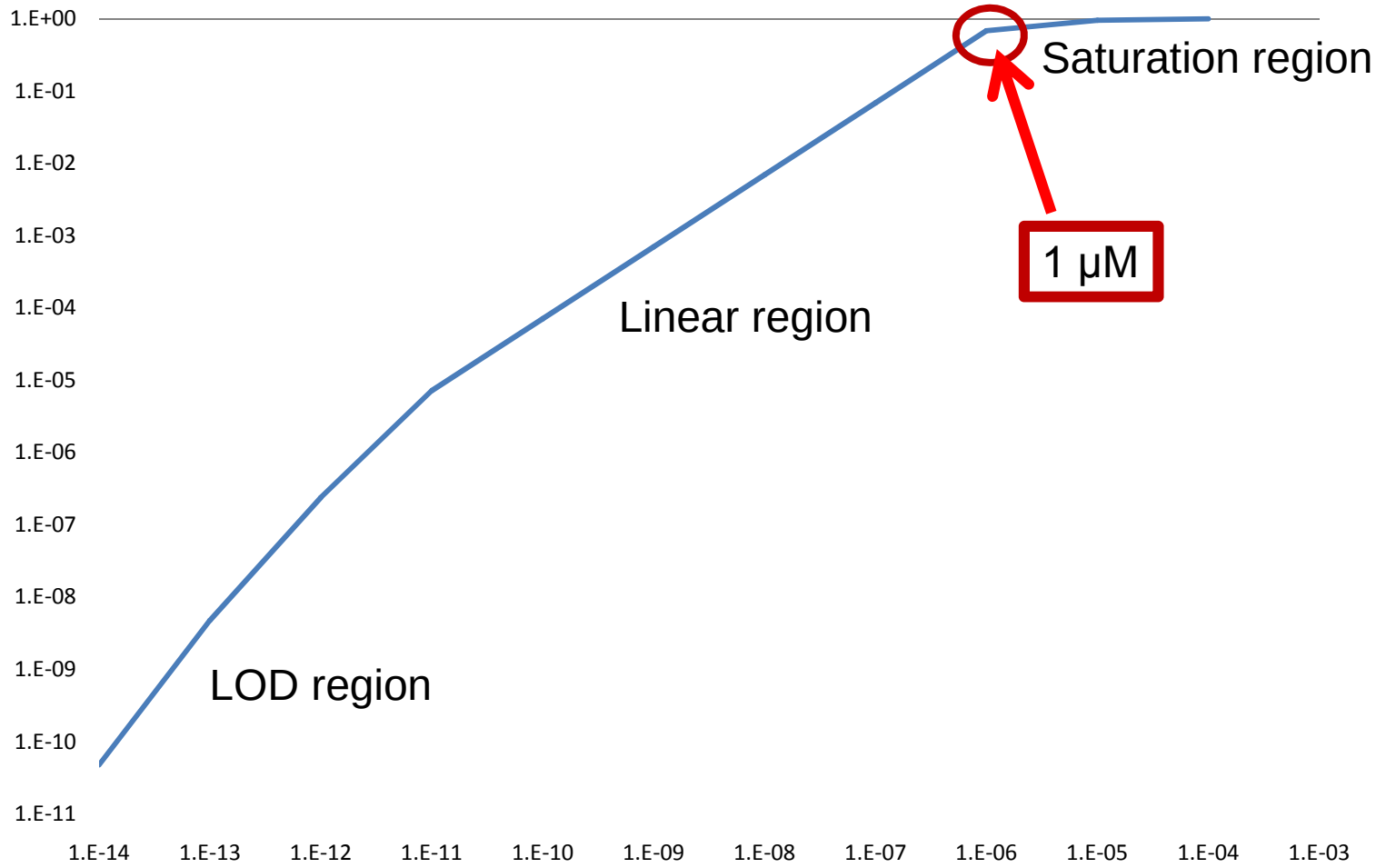
In “nanospray”, flow rates of ~1 nl/min are used. The taylor cone and plume become invisible because the droplets are in the 100 nm diameter range, and sensitivity goes up due to greatly reduced space charge and improved capture efficiency.

MALDI mass spectrometry



Most commonly, Laser is a <10 nsec pulse at 337 nm (N_2 laser) or 355 (frequency tripled Nd:YAG). 50 nsec pulse at 2.94 (Er:YAG) is also used.

Sensitivity plot



Sensitivity plot

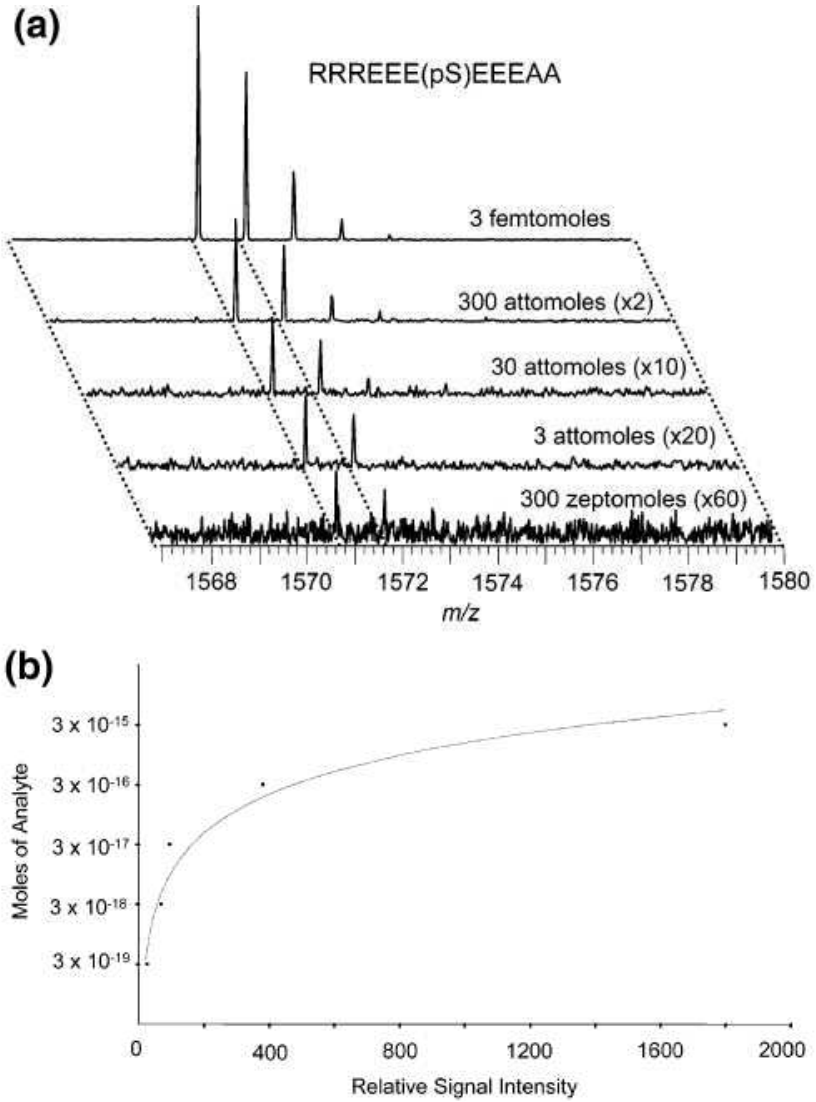


Figure 5. (a) High-pressure MALDI FTMS analysis of a serial dilution series of the phosphopeptide RRREEE(pS)EEEAA by multi-shot accumulation of the ions from 15 laser shots and one scan per spectrum. (b) Decrease in ion signal for this peptide does not follow a linear regression.

DOI:10.1021/ac034938x

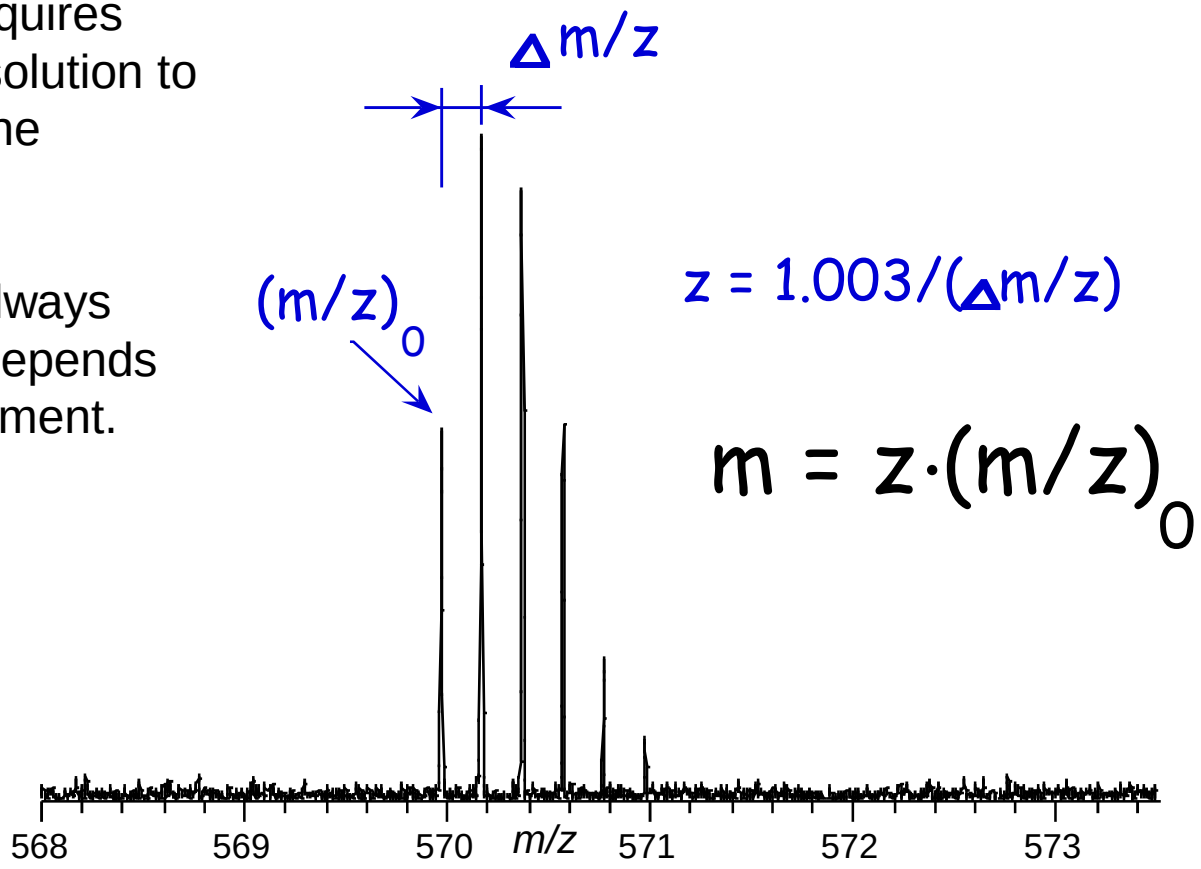
Mass Analyser

- Ion formed in source are sent to mass analyzer for sorting according to m/z and focussing onto detector
- Various types of mass analyser available:
  - ***time-of-flight mass analyzers***
  - magnetic sector
  - ***quadrupole mass filters***
  - quadrupole ion traps
  - Fourier transform ion cyclotron resonance spectrometers

Since we measure m/z, not mass, we need to know z. How do we do that?

Note: this requires sufficient resolution to distinguish the isotopes!

This is not always possible, it depends on the instrument.



Mass Spectrometers

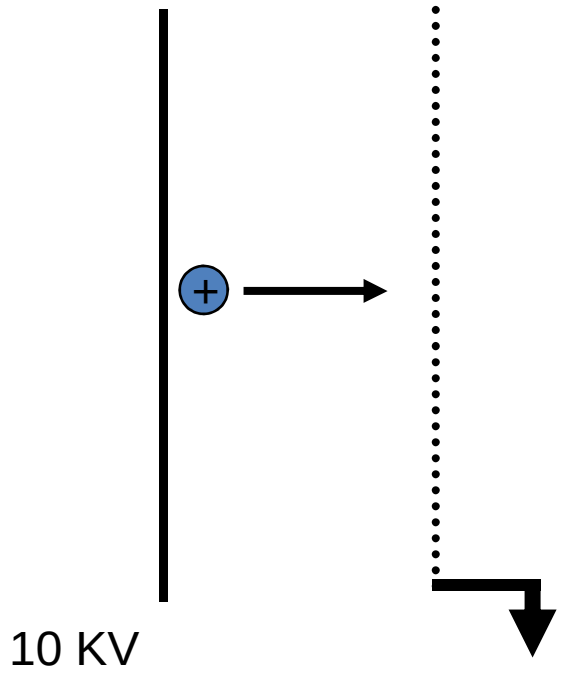
Mass Spectrometers DO NOT measure
mass. They measure mass/charge ratio.

Understanding how mass spectrometers work is understanding how ions move in electric and magnetic fields.

- Time of Flight
- Magnetic Sector
- Quadrupole
- Triple Quad
- Ion Trap
- FTICRMS

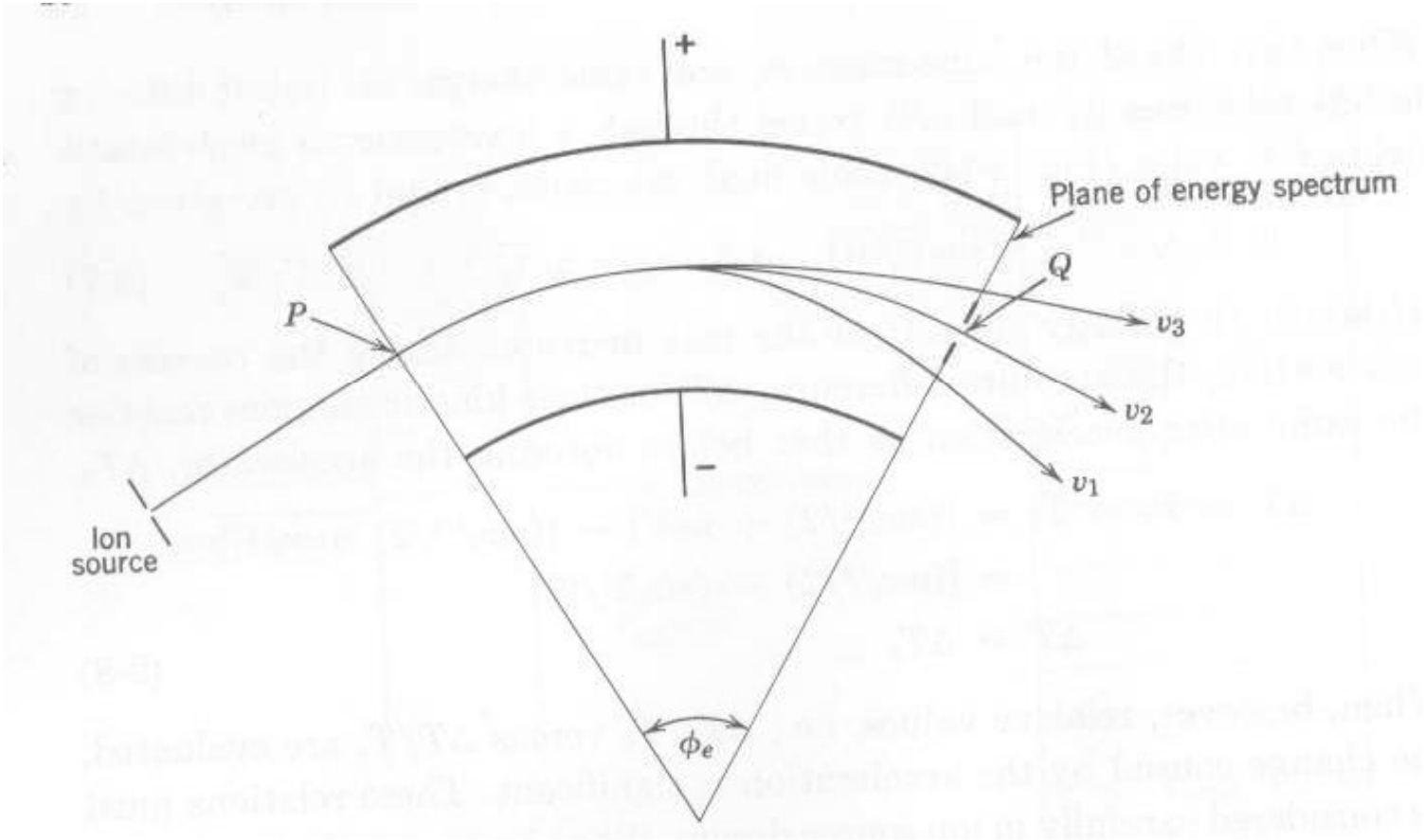
Ions in a DC Electric Field

$F = qE = m\, d^2x/dt^2$

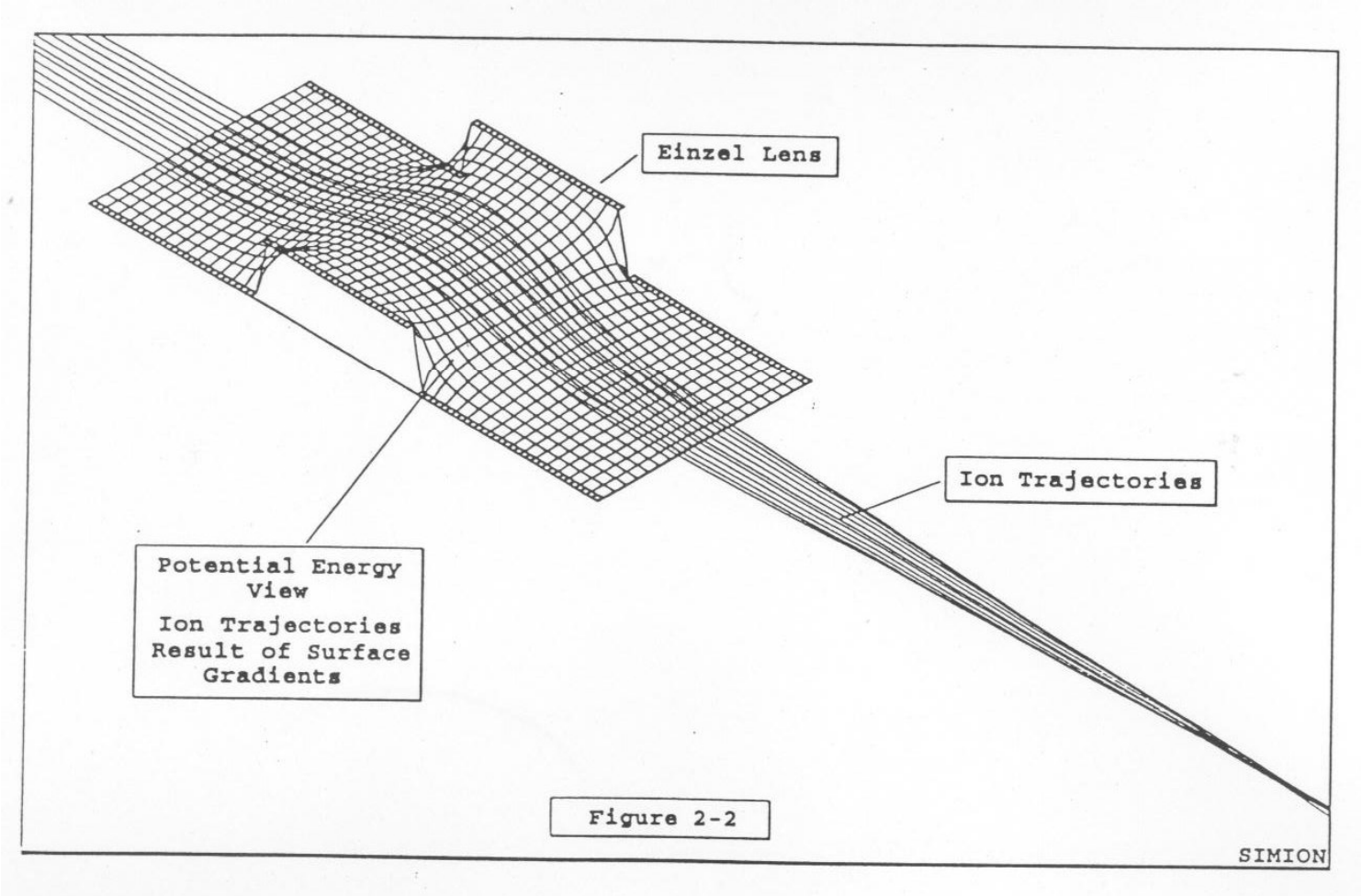


Note. Electrostatic analyzer and Einzel lens

Electrostatic analyzer



Einzel lens


Einzel Lens
Ion Trajectories
Potential Energy View
Ion Trajectories Result of Surface Gradients
Figure 2-2
SIMION

Time of Flight Mass Spectrometry

The most simple of all mass spectrometers, at least conceptually.

Linear versus reflectron

Delayed extraction (time lag focusing)

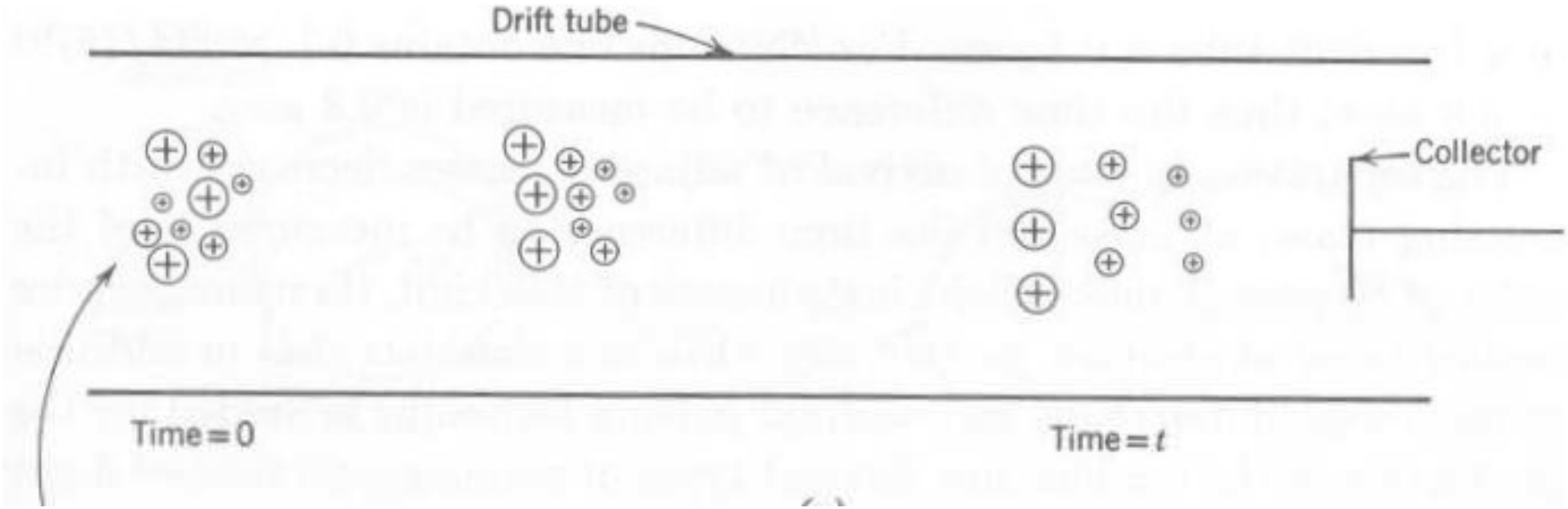
Detection electronics

PSD scan

Orthogonal injection

- MALDI-TOF
- EI-TOF
- ESI-TOF

Basic TOF mass spectrometer



Typical “Proteomics” experiment using a MALDI-TOF

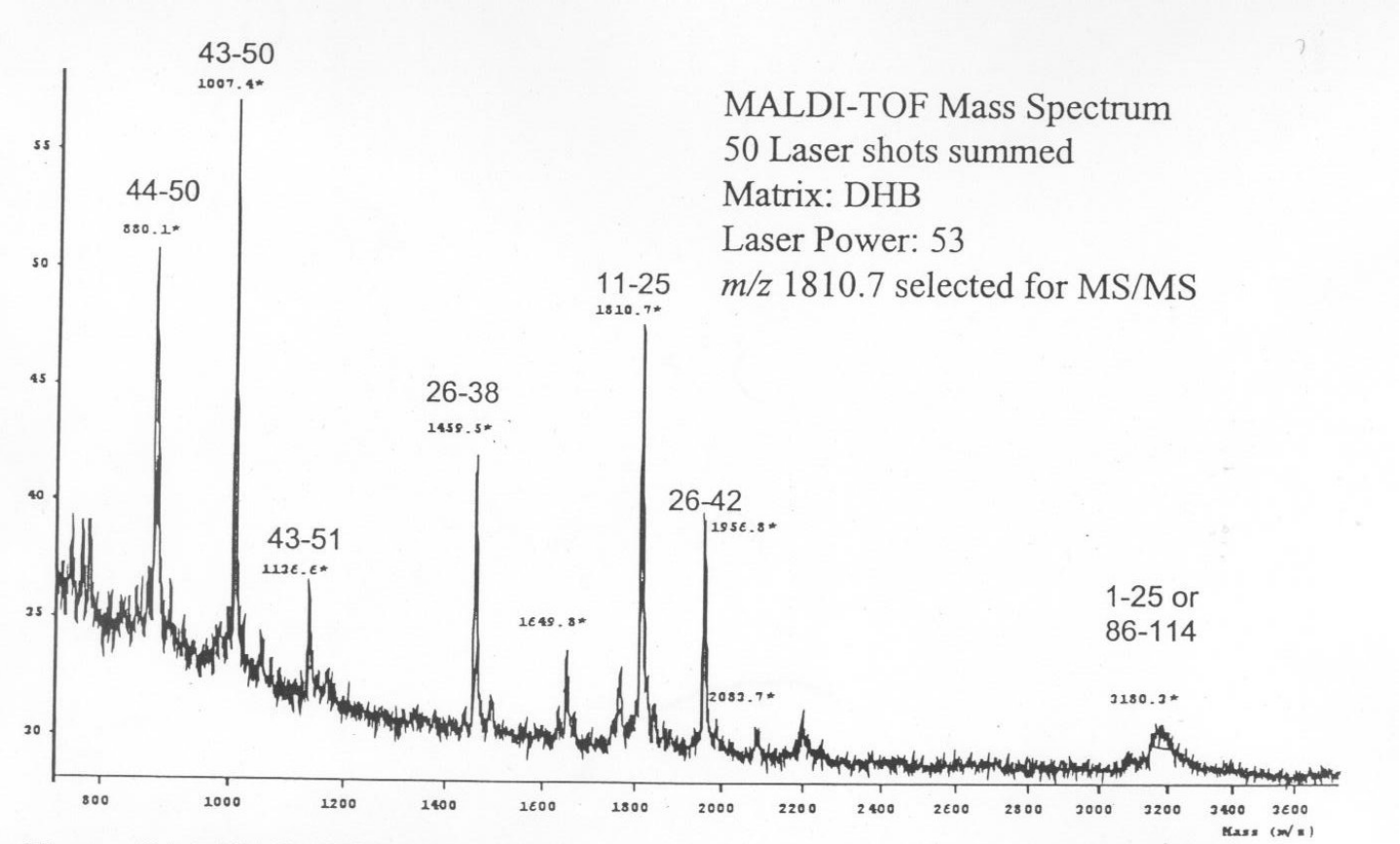


Figure 6A MALDI-TOF MS spectrum of tryptic peptides from Spot 4, pellicle 5-8

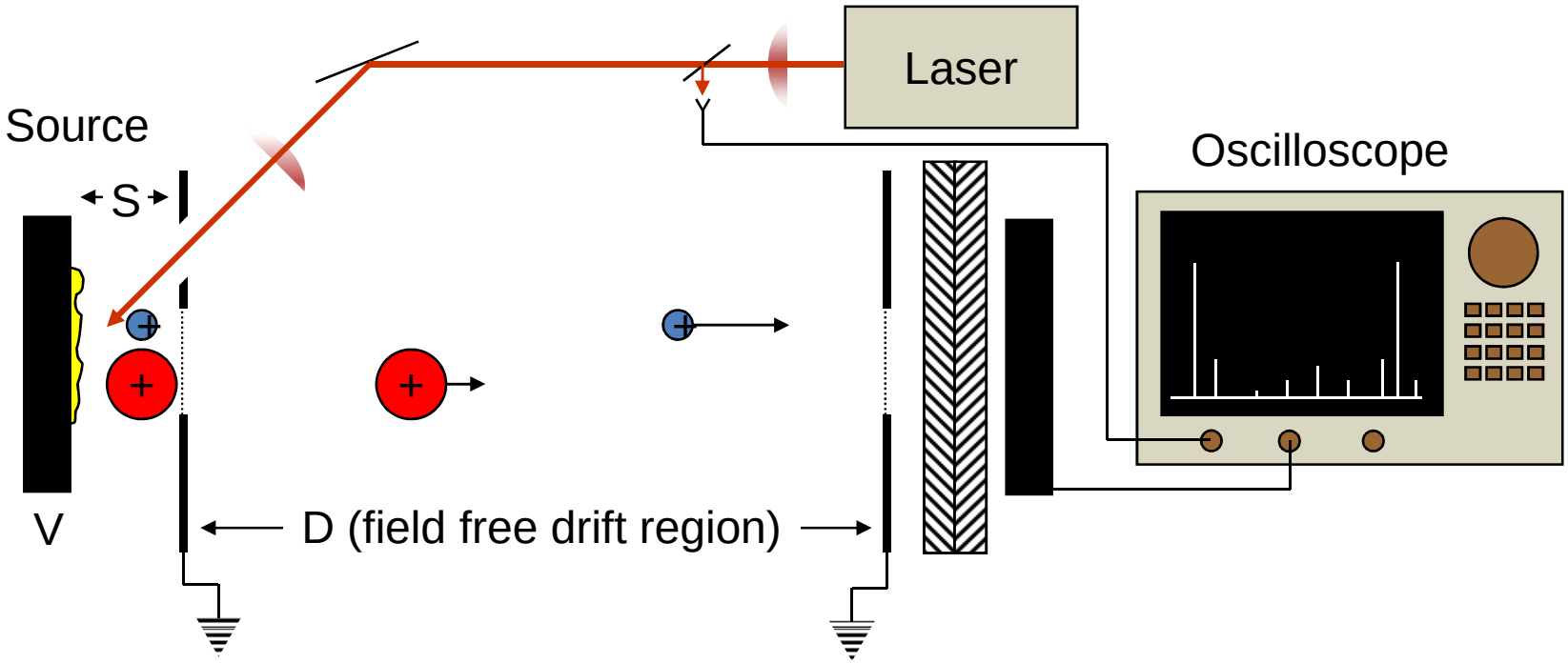


Figure 3. The principle of MALDI time-of-flight mass spectrometry.

1. TOF requires a pulsed ion source
2. TOF requires a small kinetic energy distribution in the ions
3. Radial dispersion causes signal loss
4. TOF requires a detector/oscilloscope/digitizer that's MUCH faster than the ion flight time.

Magnetic Sector Mass Spectrometry

Large, expensive, obsolete.

Swept beam instrument

The first “High Resolution” mass spectrometer (> 10k RP)

Lousy sensitivity (~1 nmol)

High energy collisional fragmentation

Extremely linear detector response (isotope ratio mass spectrometry)

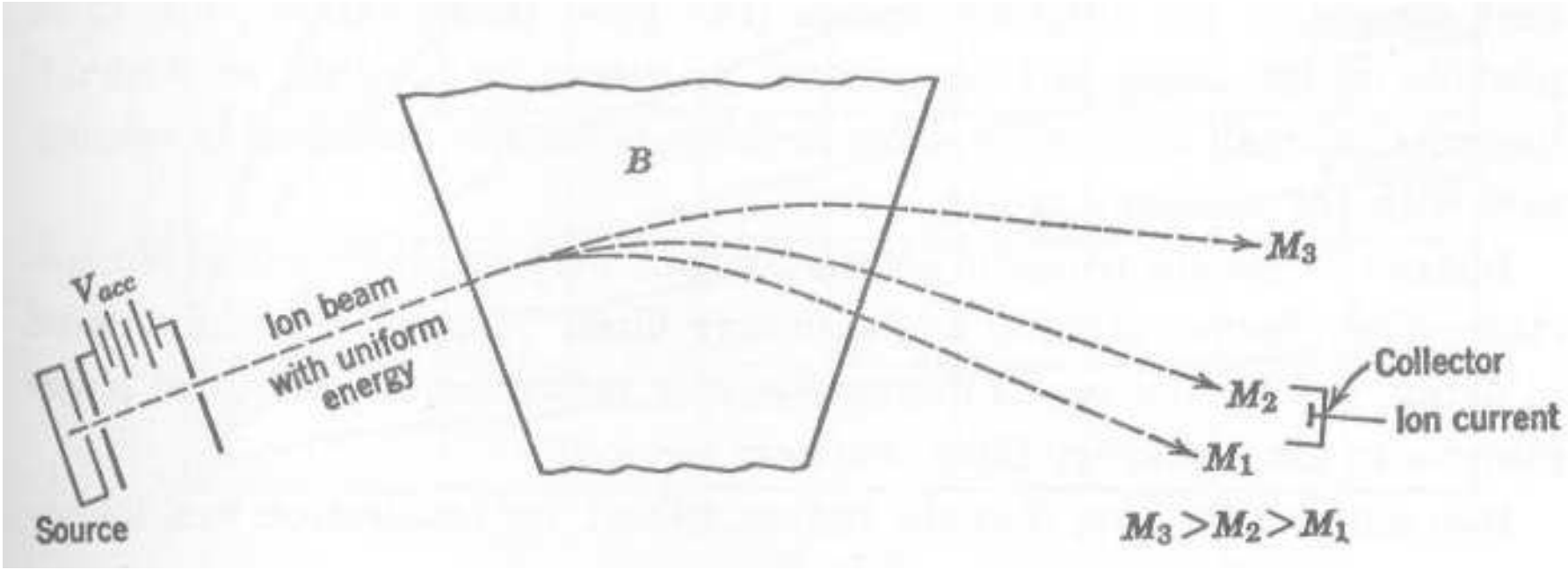
Jeol and Thermo-Finnigan MAT

- MALDI
- EI
- ESI

Sector Calibration Equation

$m = AB_0^2r^2/V$

Ions in a magnetic field



Ions in a magnetic field

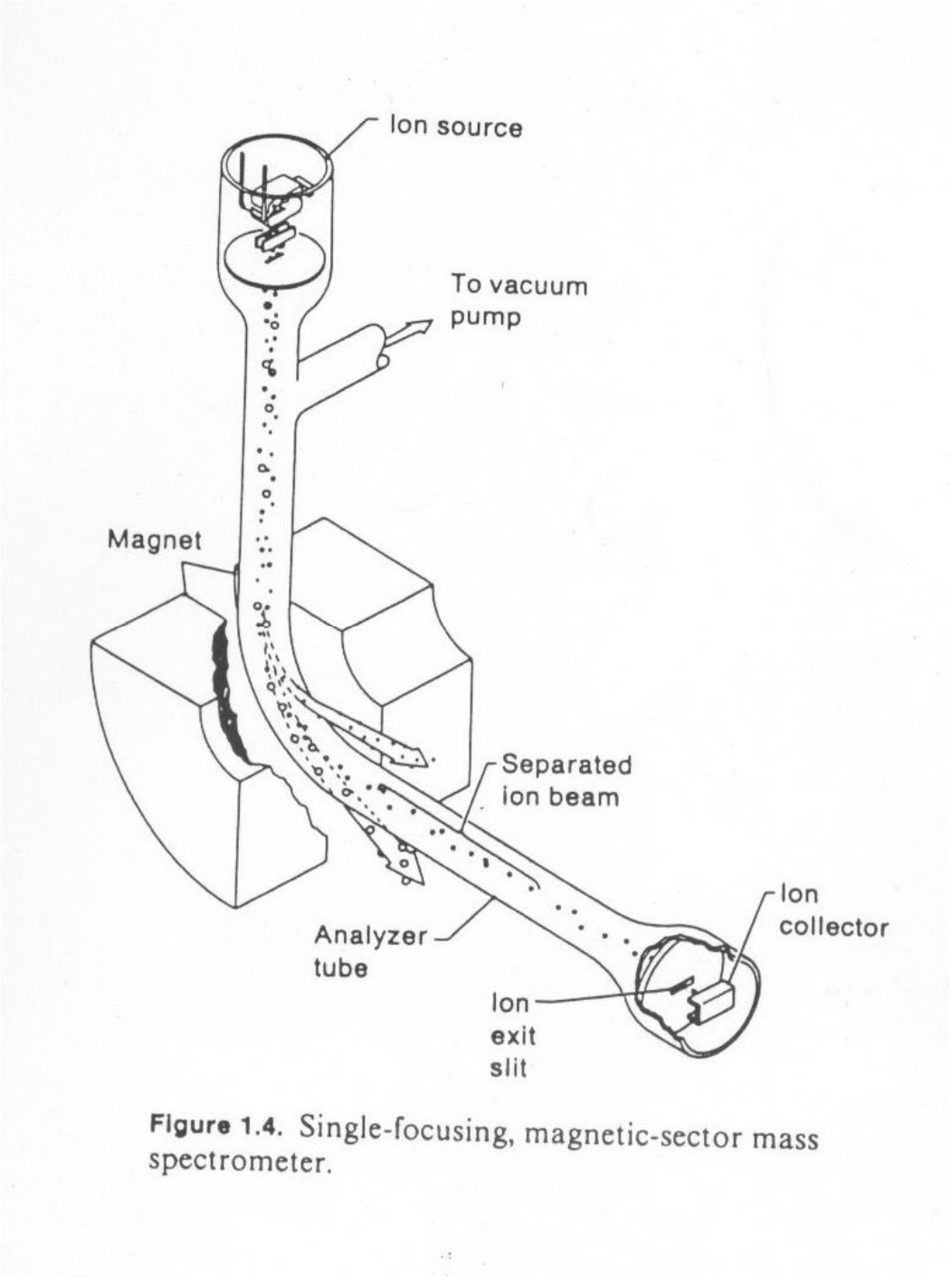


Figure 1.4. Single-focusing, magnetic-sector mass spectrometer.

Typical Sector mass spectrometer

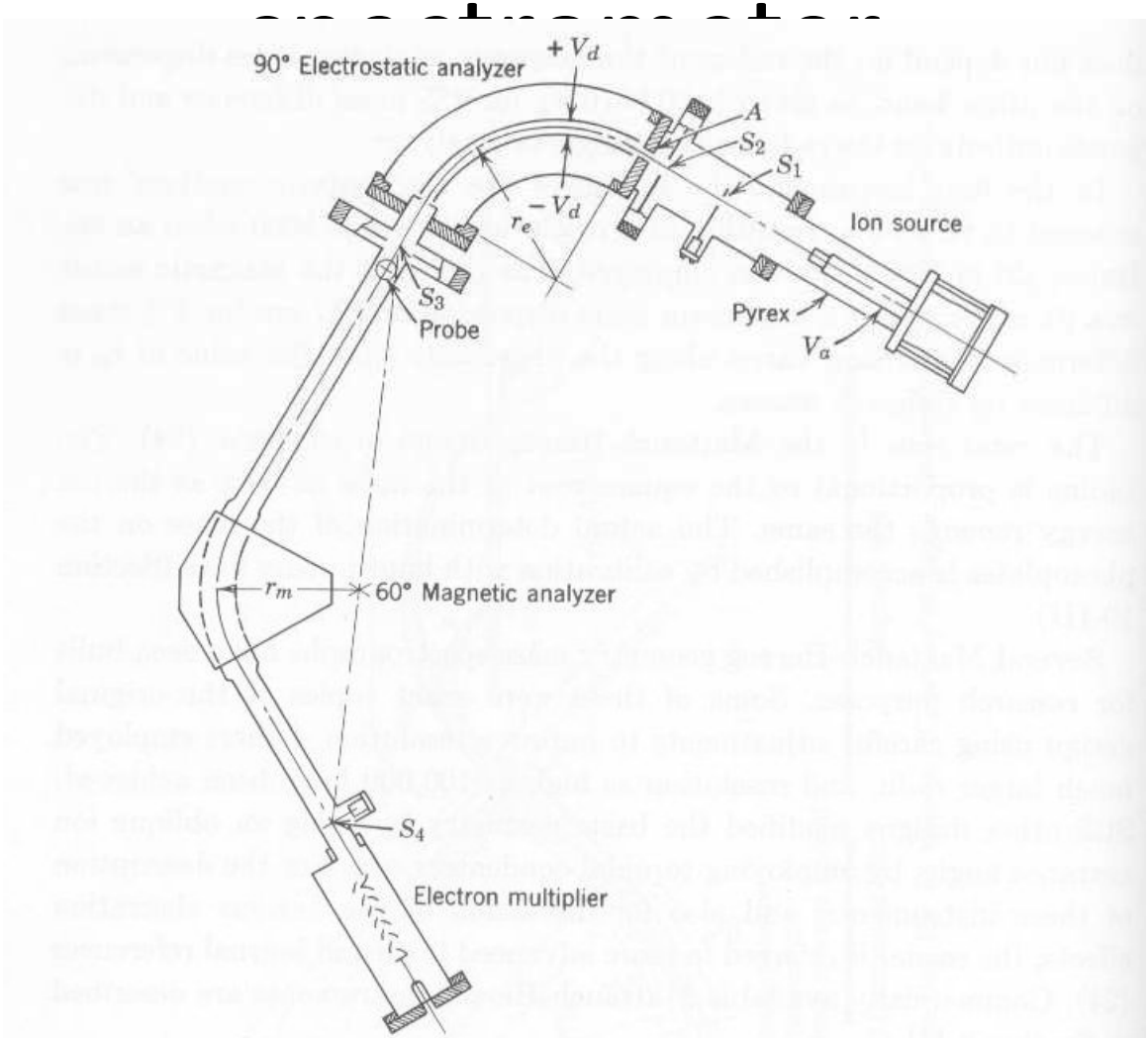
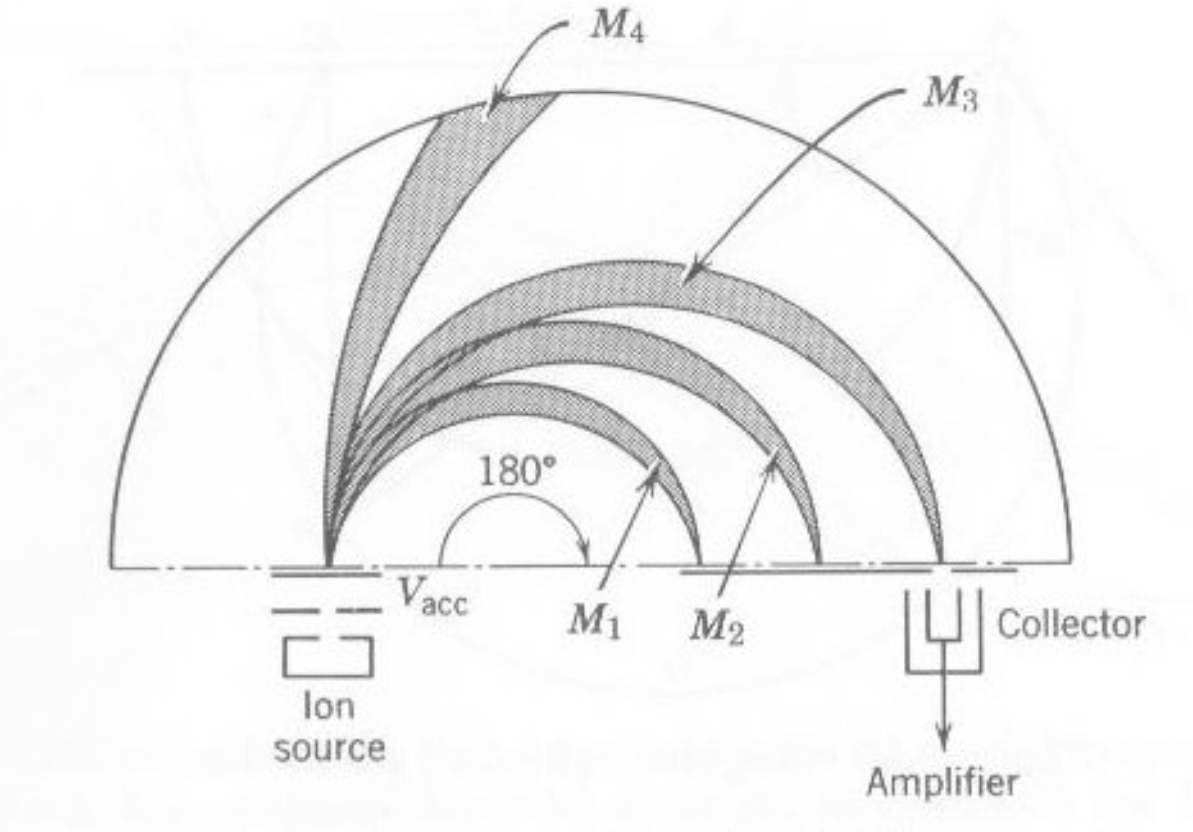


Figure 3-14. **Nier-Johnson double-focusing mass spectrometer; direction focusing of the second order (27).**

Isotope Ratio Mass Spectrometer



Quadrupoles

Small, cheap, ubiquitous.

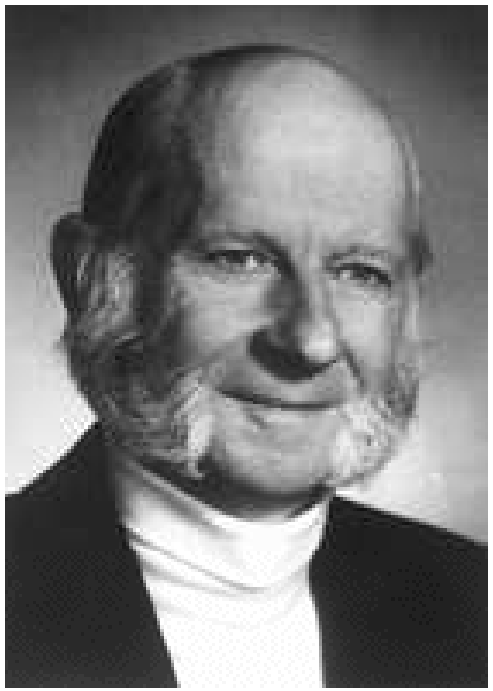
Swept beam instrument

Resolution typically 1000, mass accuracy typically 0.1%

Sensitivity depends on the source. Typically in the 100 fmol range.

- MALDI
- EI
- ESI

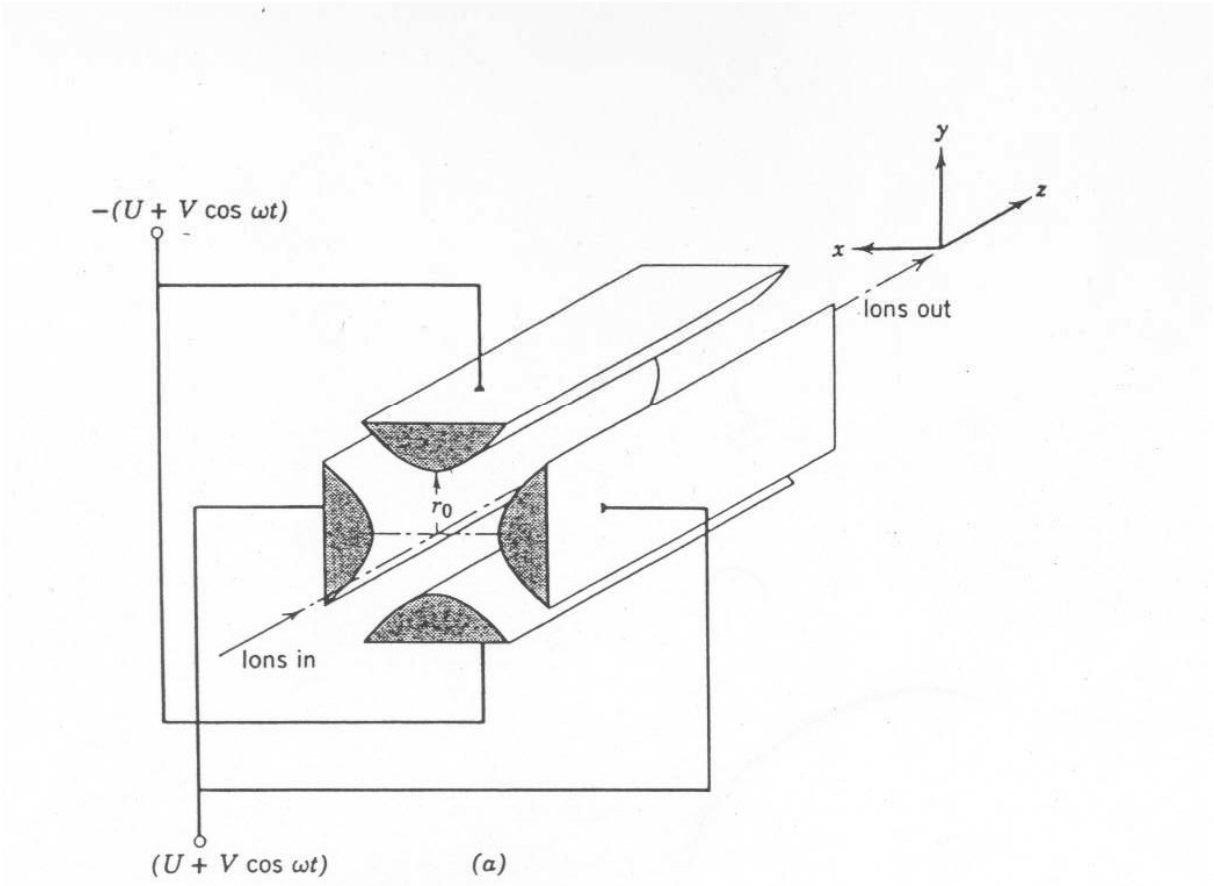
Wolfgang Paul
(quadrupole ion traps)

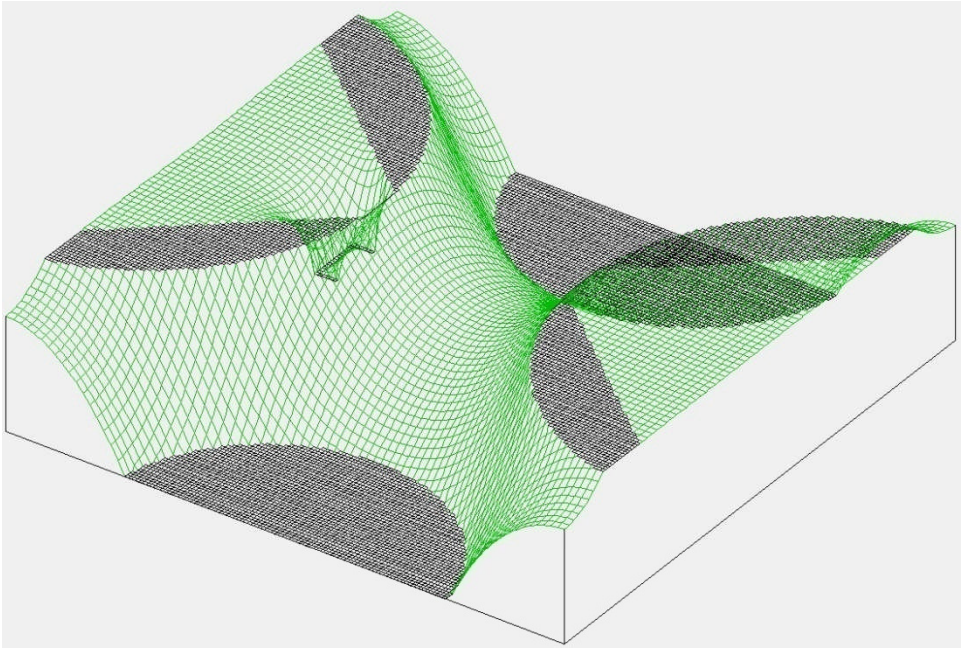


Hans Dehmelt
(Penning ion traps)

1989 Nobel Prize in Physics for development of ion trapping techniques

Wiring of a quadrupole

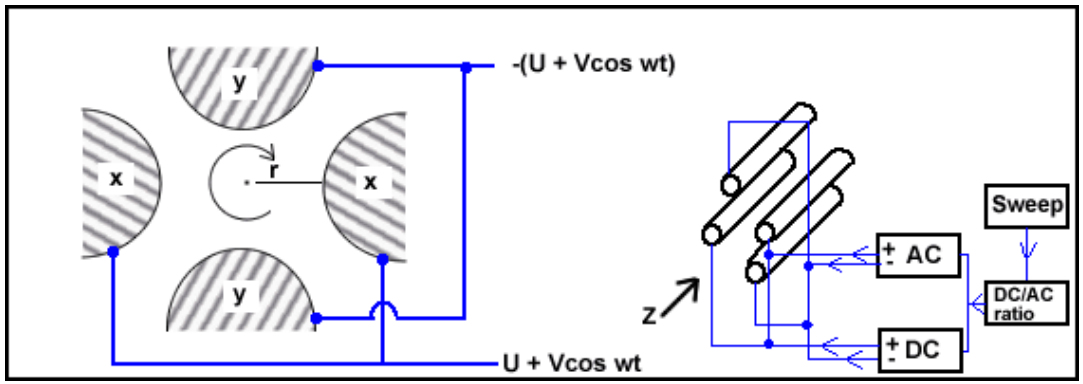




The potential energy diagram of a quadrupole showing the saddlepoint in the electric field (generated using Simion 7.0)

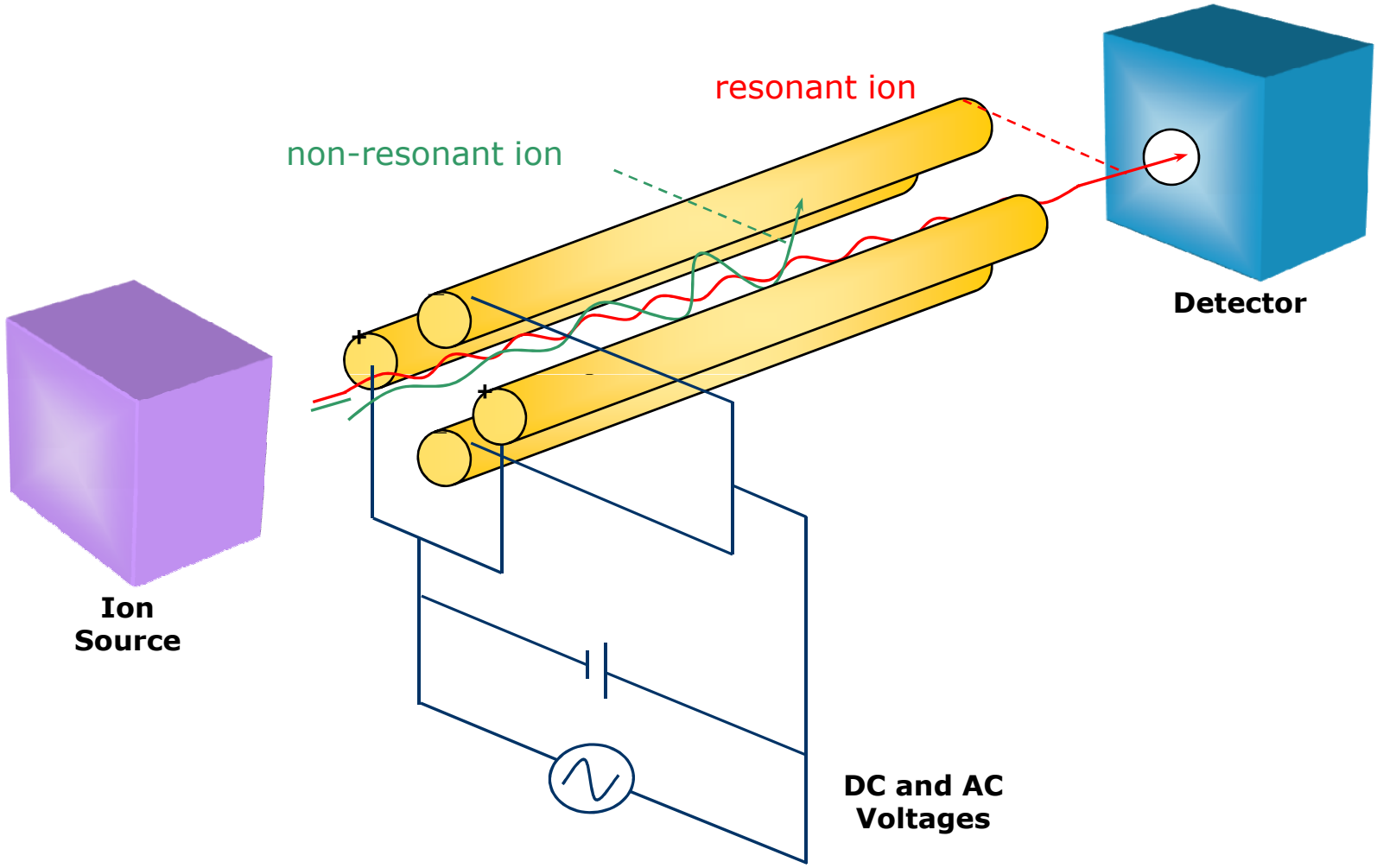
Quadrupole Analyser

- Cheaper, but lower performance and resolution
- Advantage: easy to interface with GC and LC (on-line)
  - don't use high potentials in the ion source; faster scanning
- DC and 180° out of phase RF AC potentials applied across opposite pairs of cylindrical rods



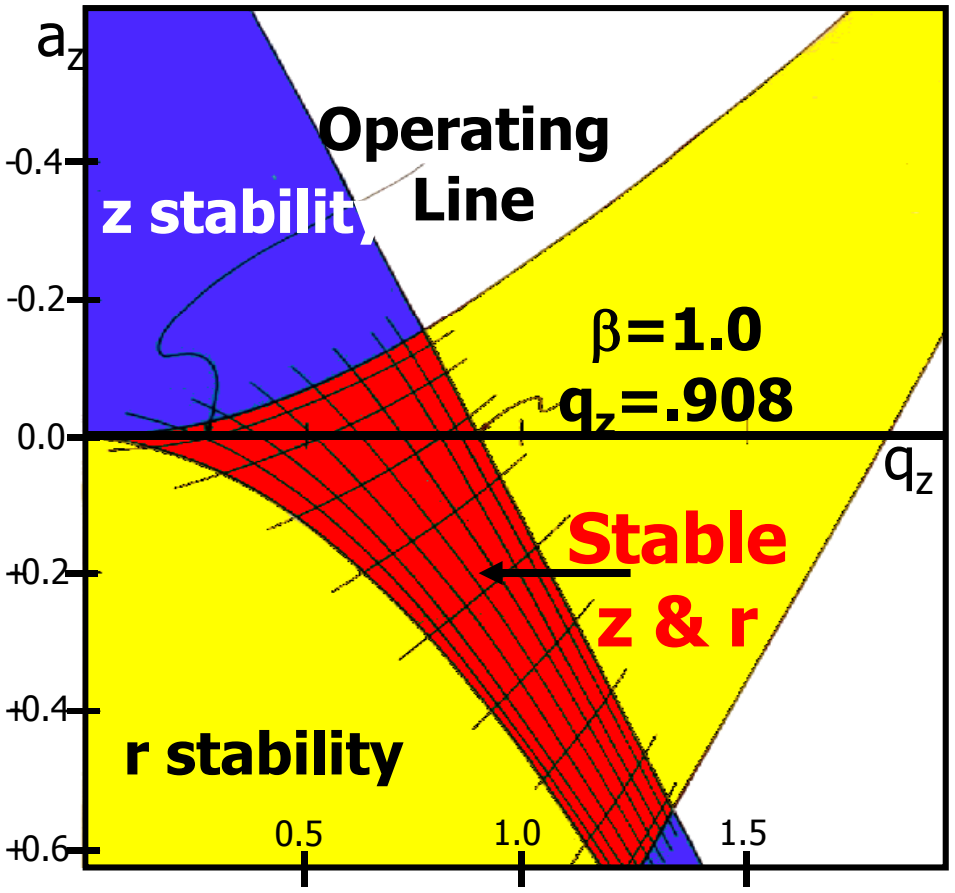
- Ions injected along z; follow a spiral path through the analyser due to the oscillating field.
- Under given set of conditions, ions of only a single m/z focussed to detector (others collide with rods)
- Vary DC and RF to successively bring all ions to detector
  - range m/z = 1000 - 4000 Da

Quadrupole Analyser



Ions in an Oscillating Electric Field

$$\frac{d^2u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0$$



$A_{\pm} = U \pm V\sin(\omega t)$

"Matthieu eqn"

$a_z = 8eU/m\omega^2r^2$

$q_z = 4eV/m\omega^2r^2$

- $q_z \alpha V/m$
- $q_z \alpha f_{ion}$
- $a_z \alpha U/m$

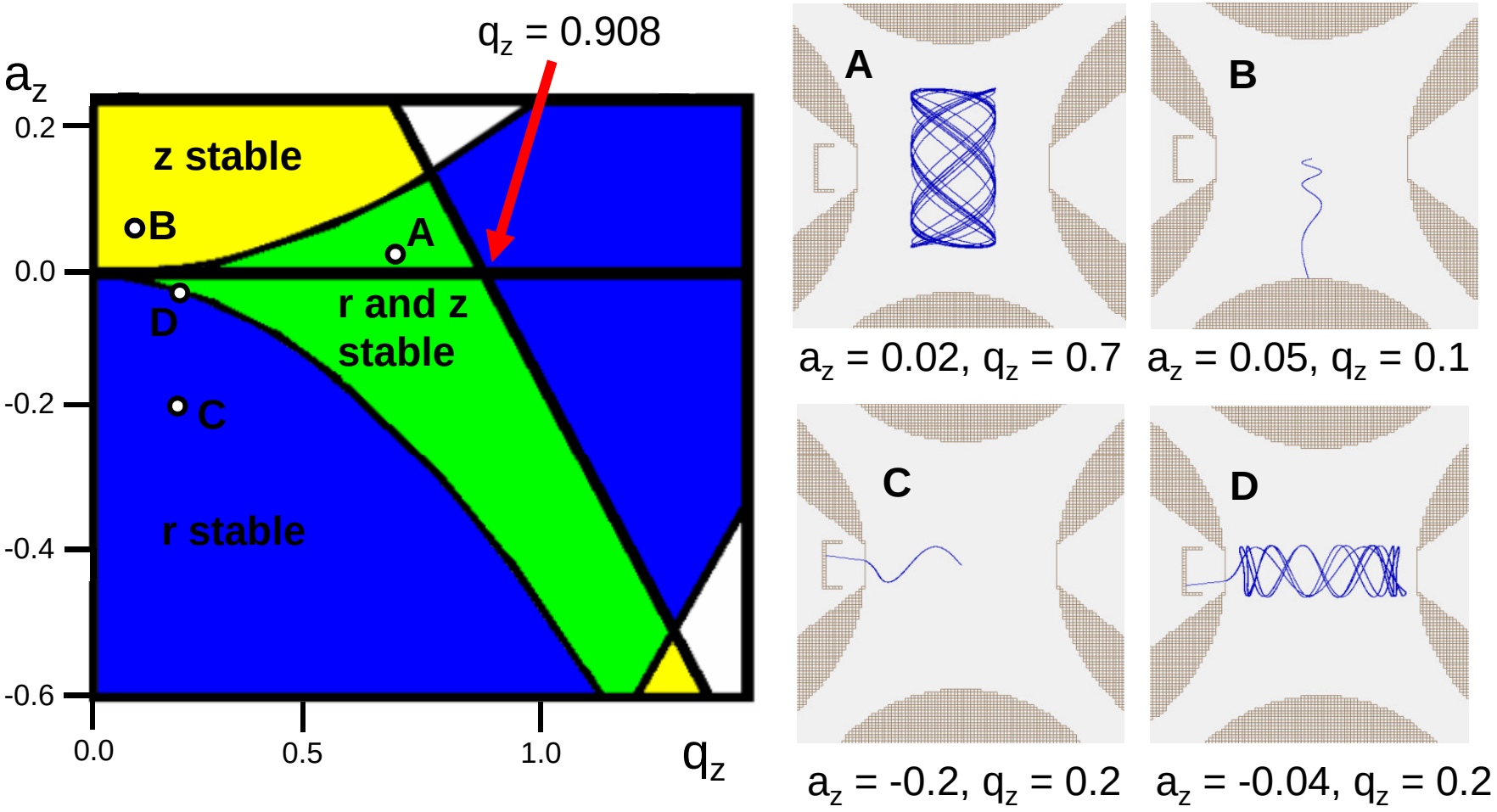
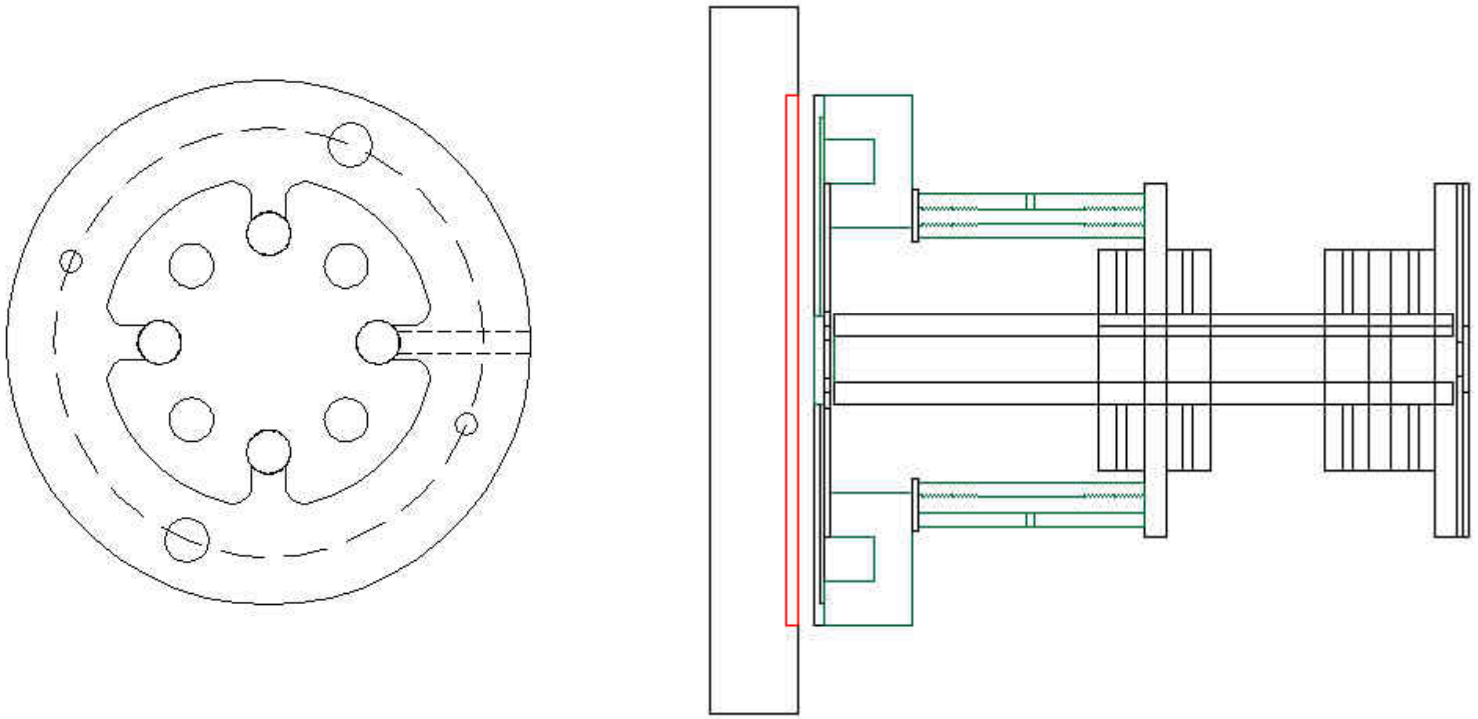


Figure 12. Mathieu stability diagram with four stability points marked. Typical corresponding ion trajectories are shown on the right.

Octopole/hexapole linear ion trap



Octopole ion guide/trap

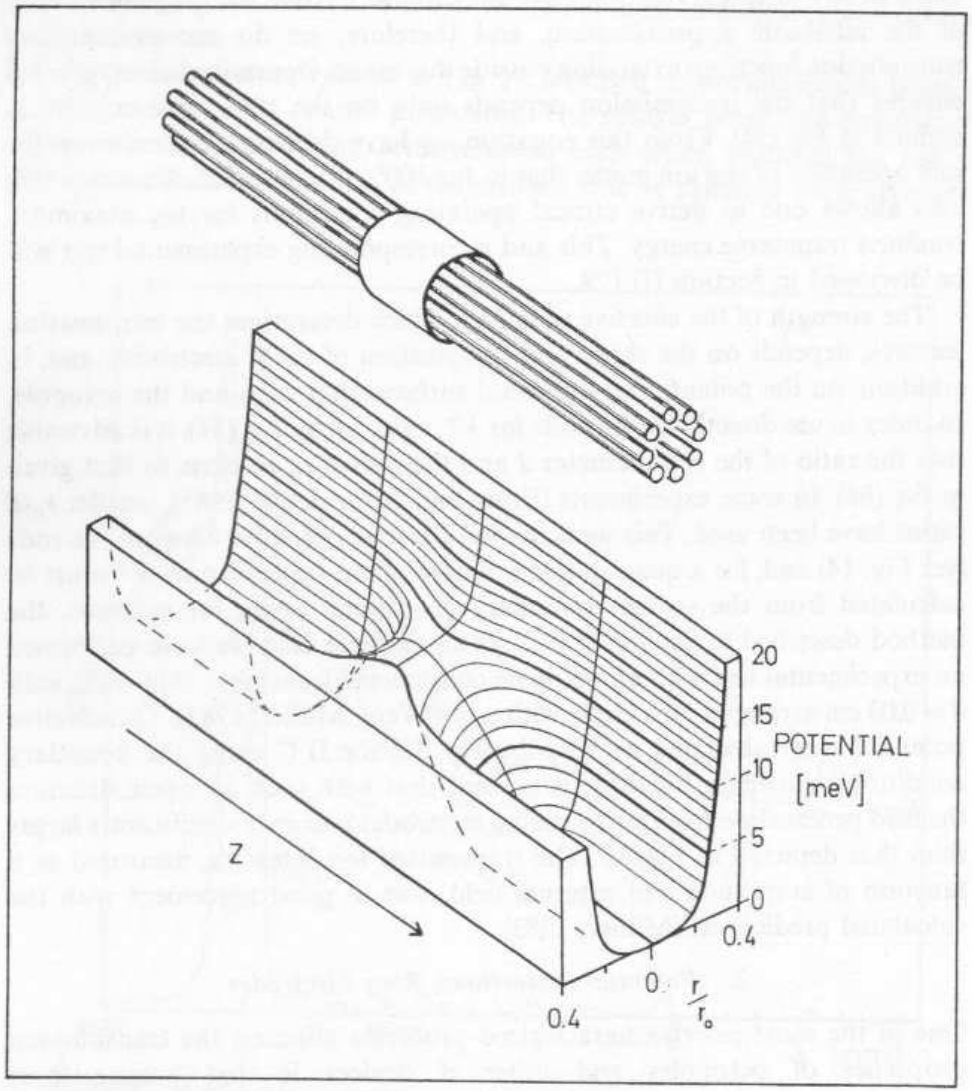


Figure 29. Perspective view of the sum of the effective potential and a dc potential distortion caused by a cylindrical ring electrode in an octopole. The penetrating field creates a local barrier of a few millivolts per volt applied to the ring.

Octopole ion guide/trap

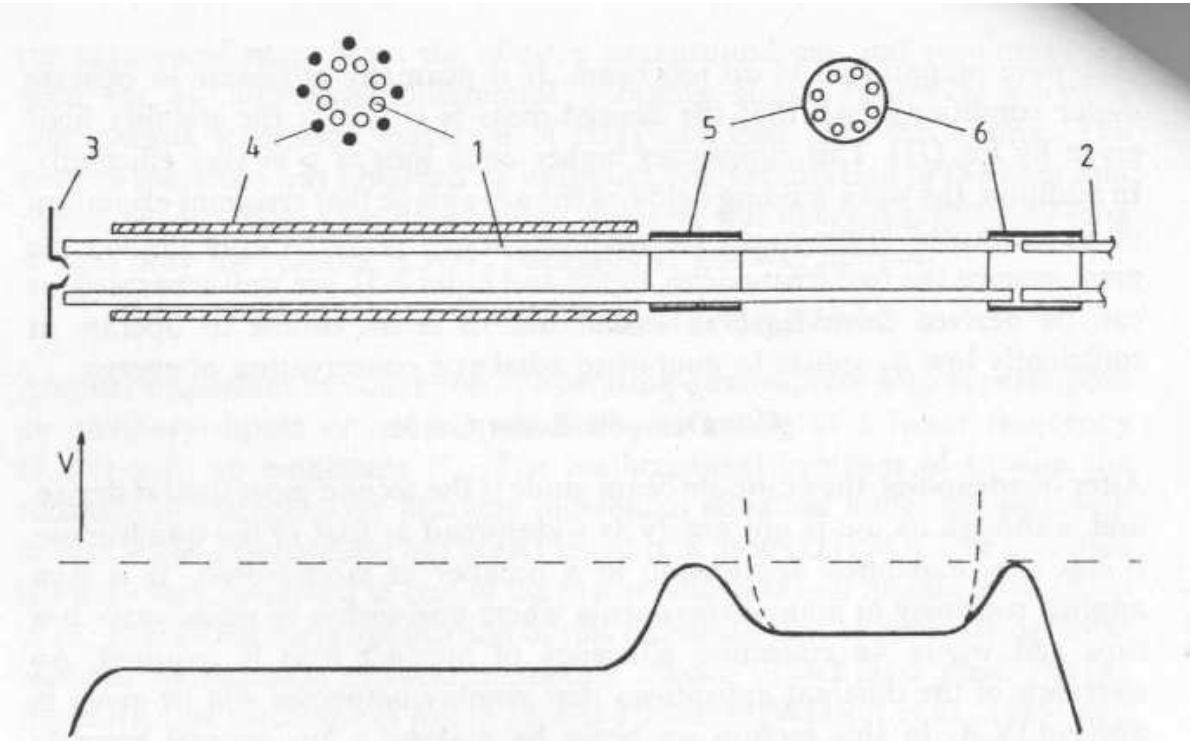


Figure 30. System of two octopoles (1, 2) with electrodes for ion injection (3) and for field correction (4–6). Correction electrode (4) consists of eight rods, staggered as indicated, (5) and (6) are cylinders. The lower panel shows a schematic representation of the dc potential along the axis of the system. In this example, the electrode (4) was used to lower the potential slightly, while (5) and (6) were used to create barriers.

Octopole ion guide/trap

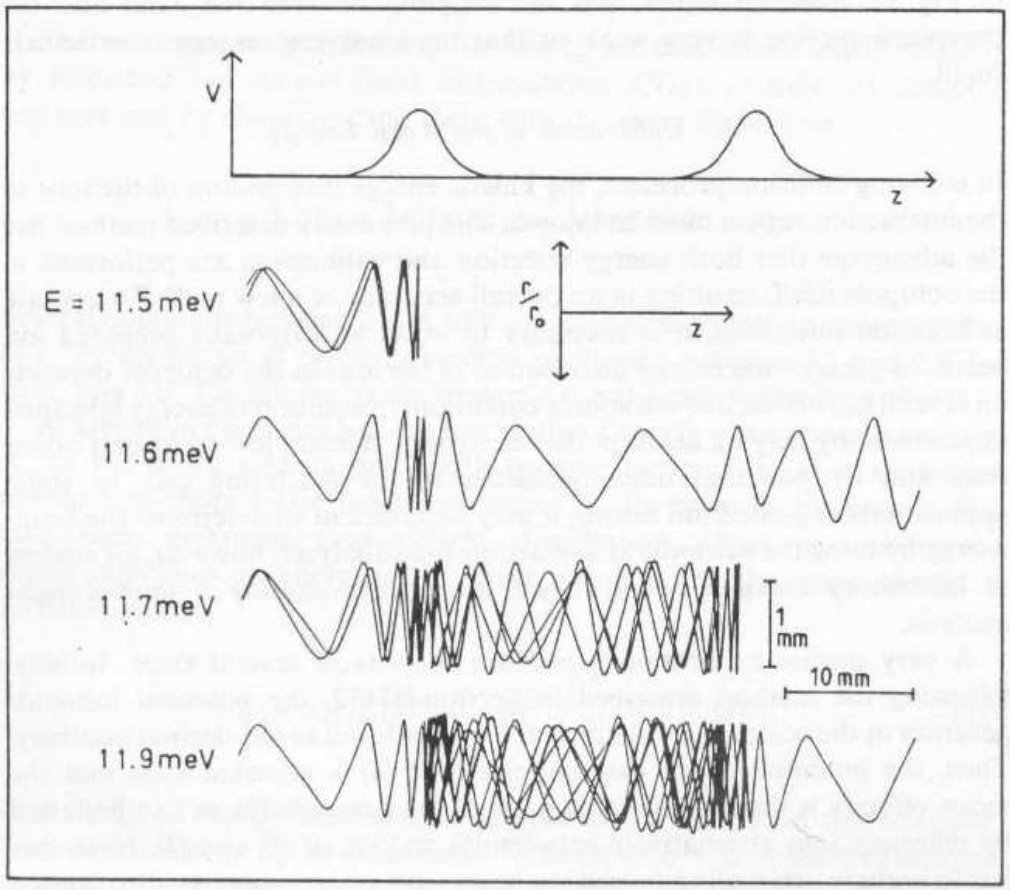
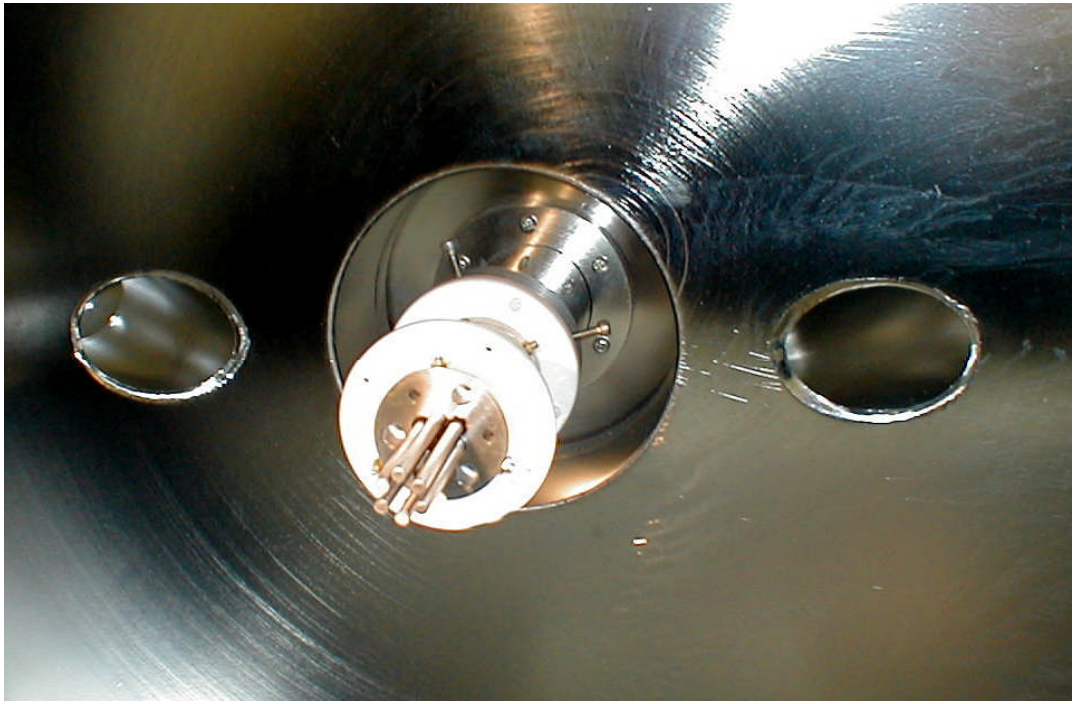


Figure 31. Model calculations of ion trajectories in an octopole with two equally high potential barriers. Several sequences of reflections at the barriers are obtained depending on the kinetic energy and the angle of the incoming ion.

Hexapole ion trap



A couple of examples

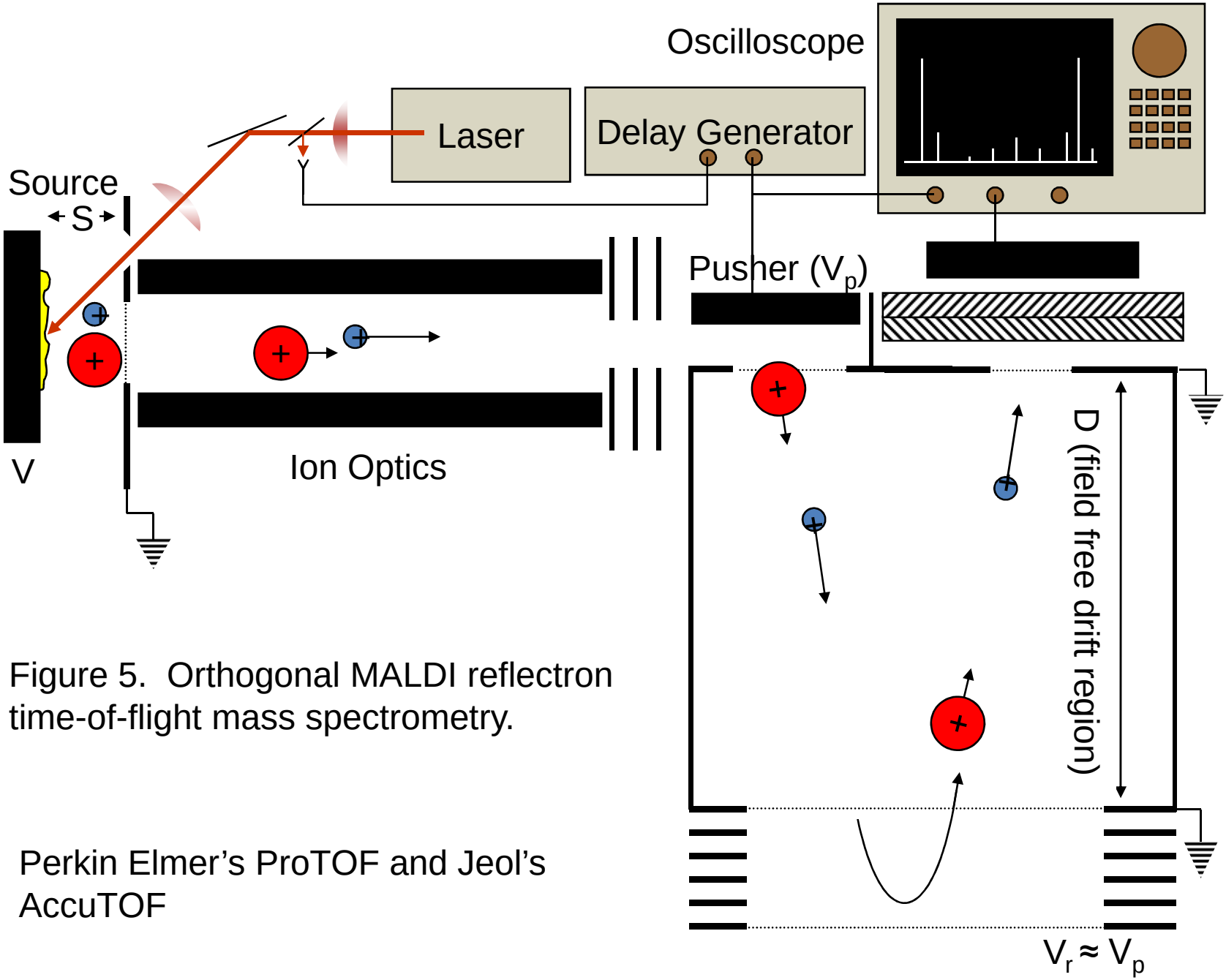


Figure 5. Orthogonal MALDI reflectron time-of-flight mass spectrometry.

Perkin Elmer's ProTOF and Jeol's AccuTOF

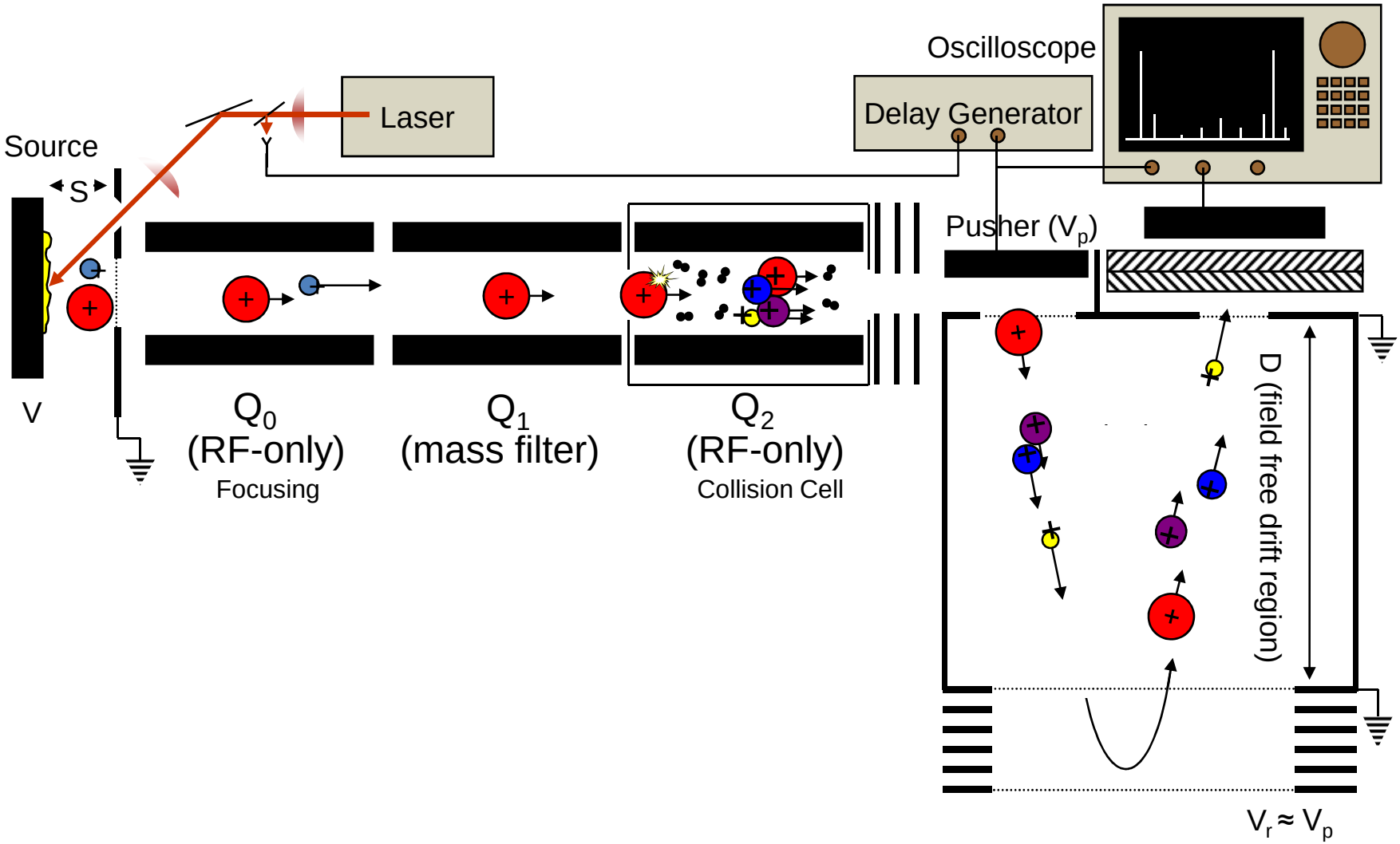


Figure 14. Quadrupole Time-of-Flight Hybrid

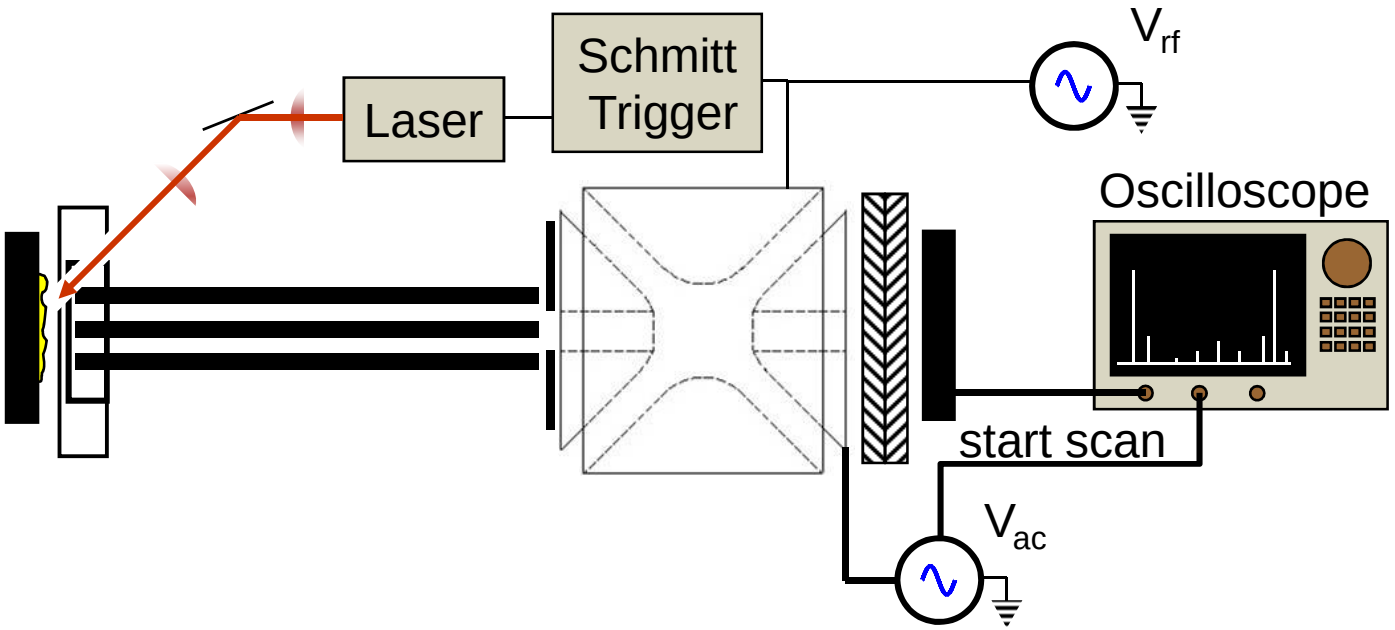


Figure 13. MALDI ion trap mass spectrometry.

Home built instrument from Brian Chait's group

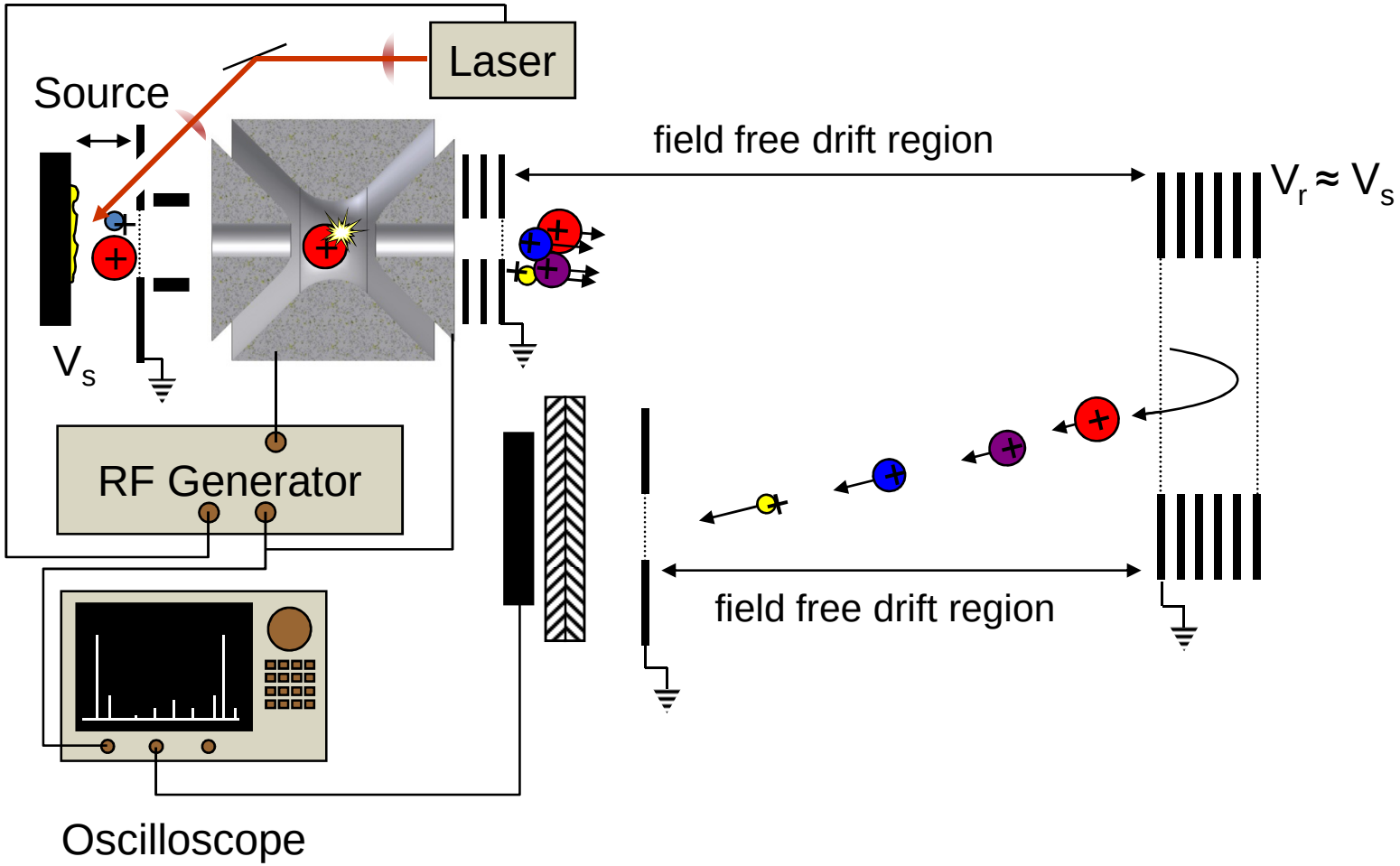
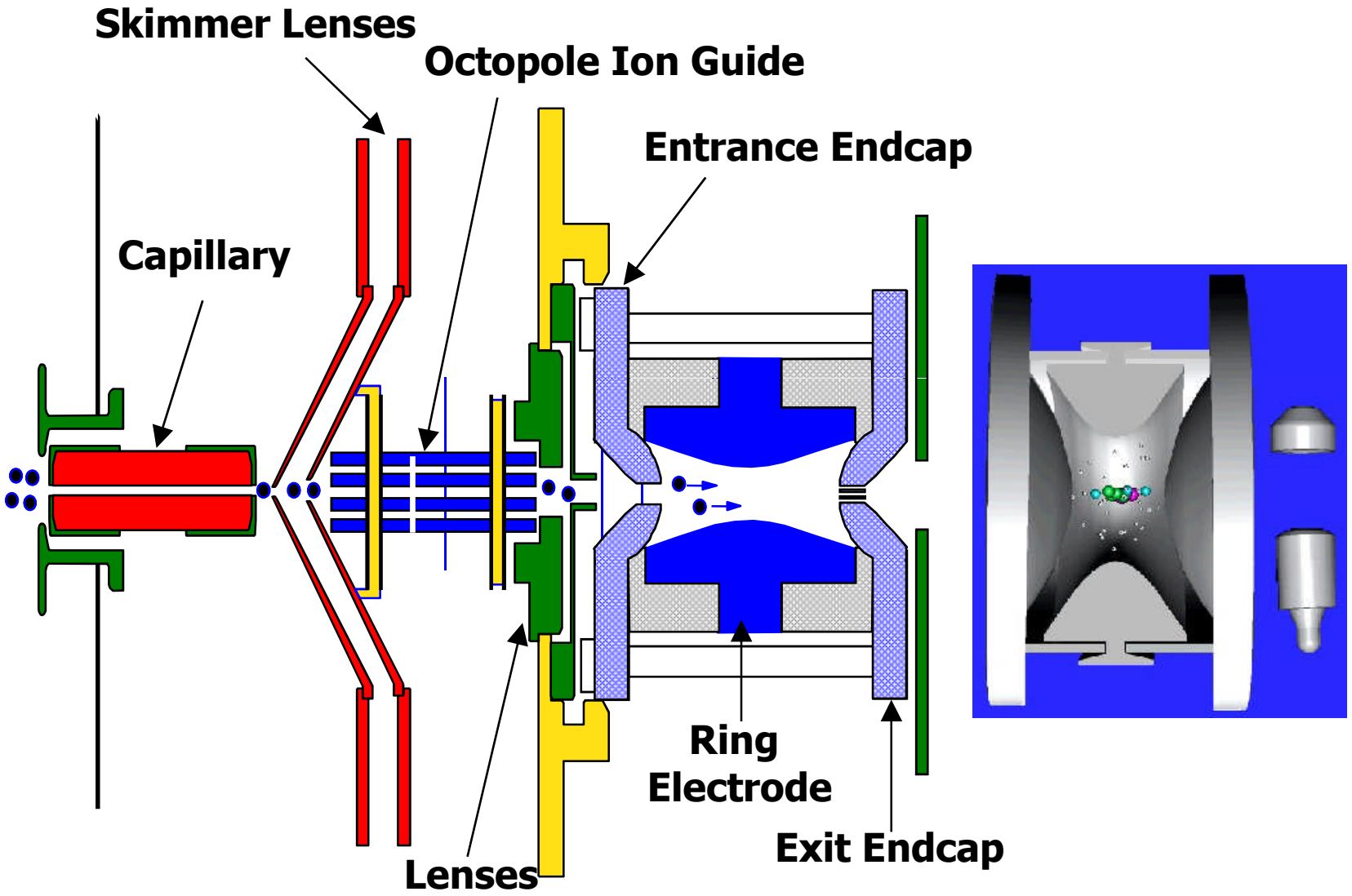


Figure 17. MALDI Ion-Trap time-of-flight mass spectrometer.

Kratos/Shimadzu instrument. Mediocre TOF performance, why?

Quadrupole Ion Traps



Fourier Transform Mass Spectrometer

Big, expensive, but superior performance.

Ion trap instrument

Resolution typically >50000 broadband, >1,000,000 narrowband

Mass accuracy typically 1 ppm internally calibrated 5-10 ppm externally calibrated

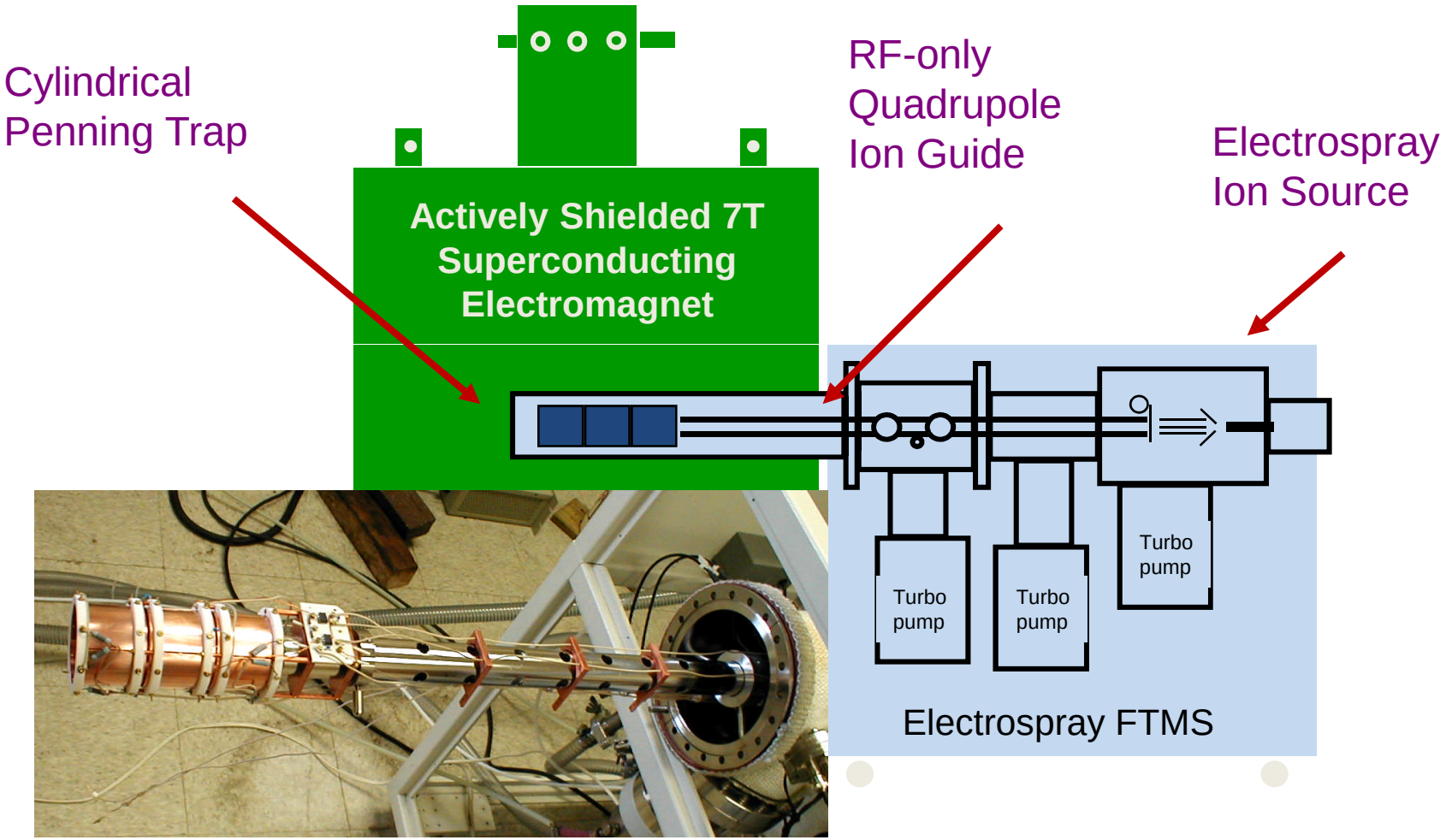
Sensitivity depends on the source. Typically in the 100 fmol range.

MS^n compatible

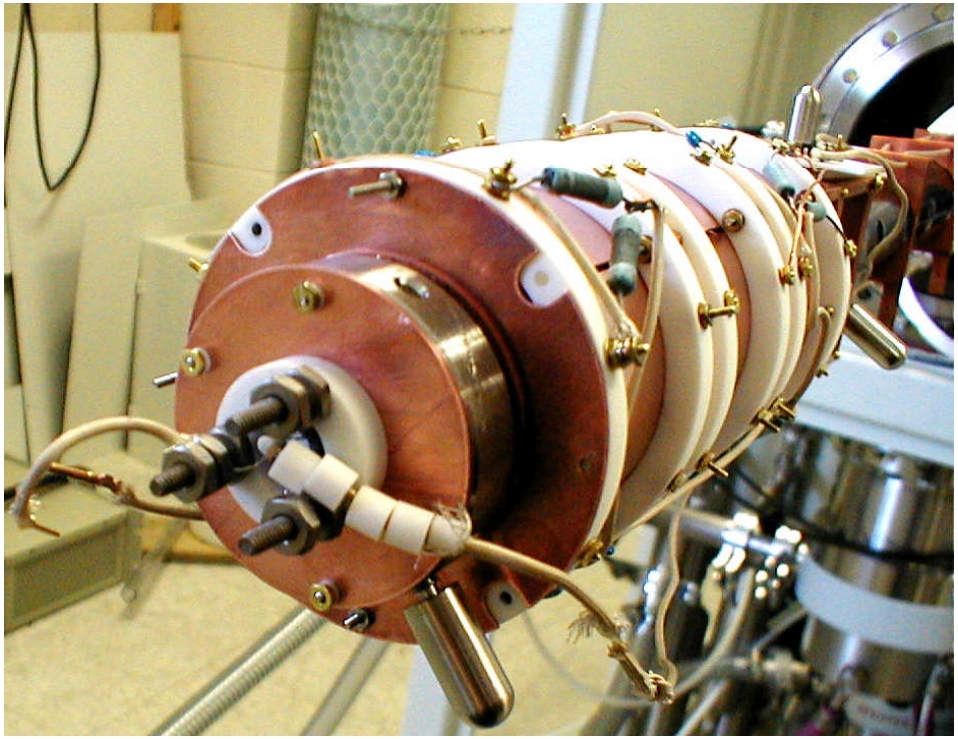
Ion Molecule Reactions (e.g. gas phase H/D Exchange)

- MALDI
- EI
- ESI

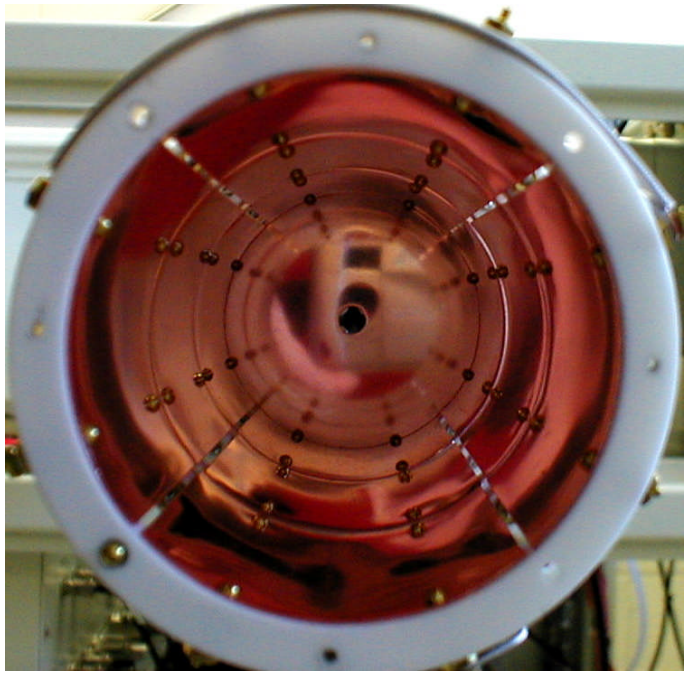
How Does FTMS Work?



How Does FTMS Work?

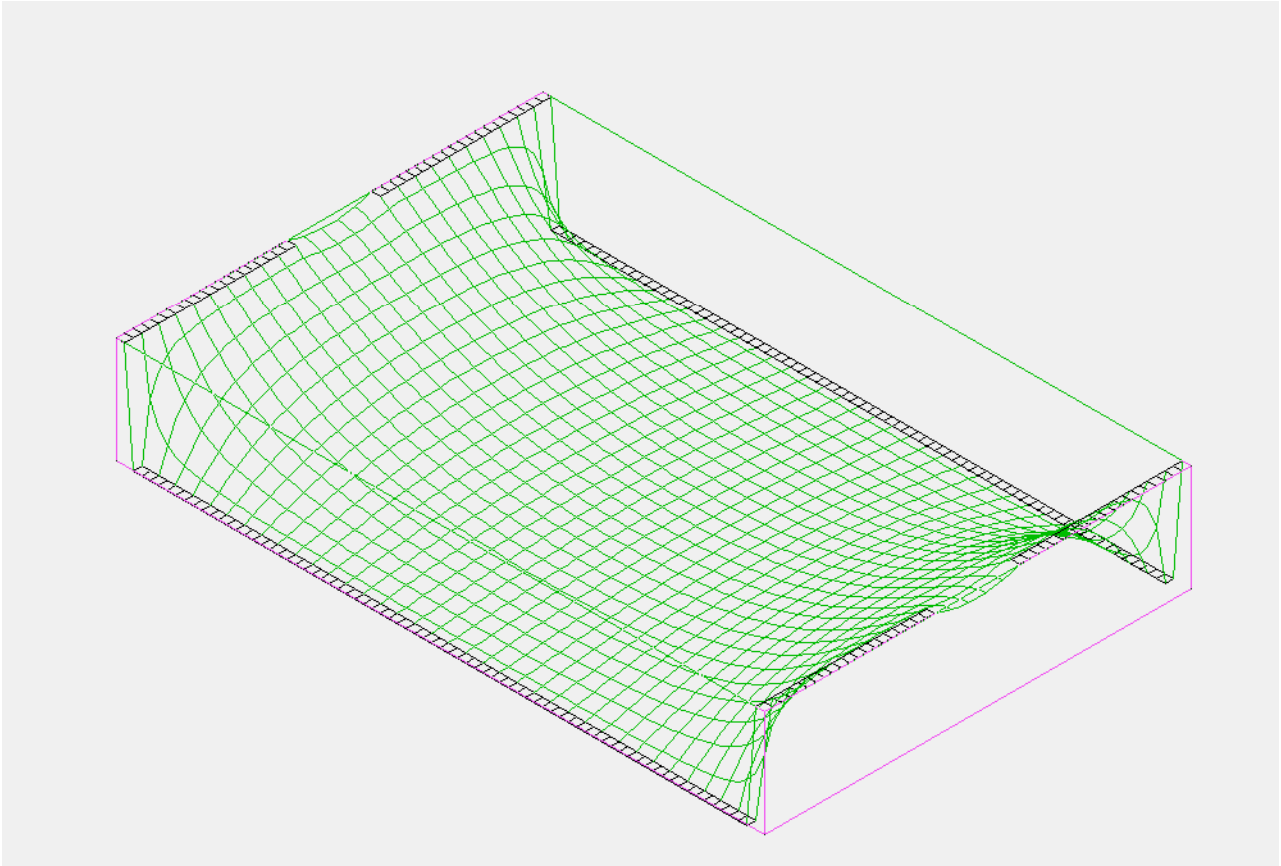


The Penning Trap

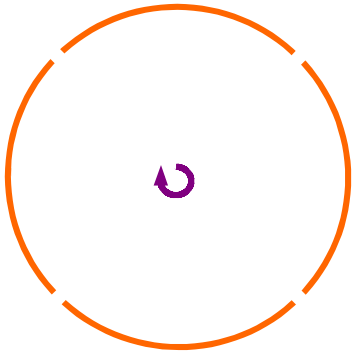


The ions' view of the cell

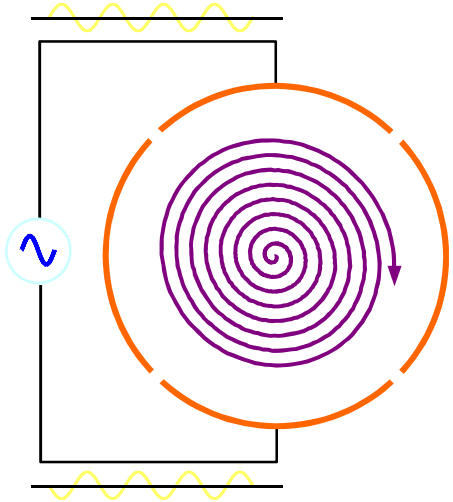
The Trapping Field of an Elongated Cubic ICR Cell.



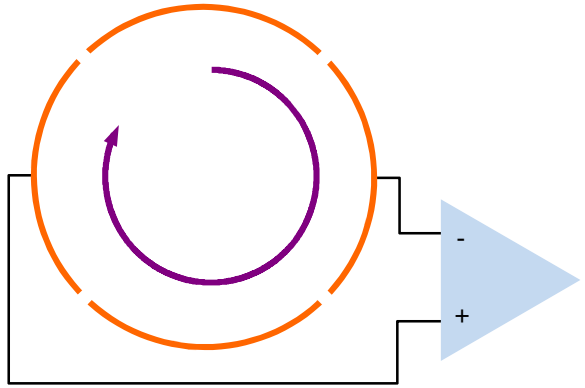
How Does FTMS Work?



Ions are trapped and oscillate with low, incoherent, th ermal amplitude

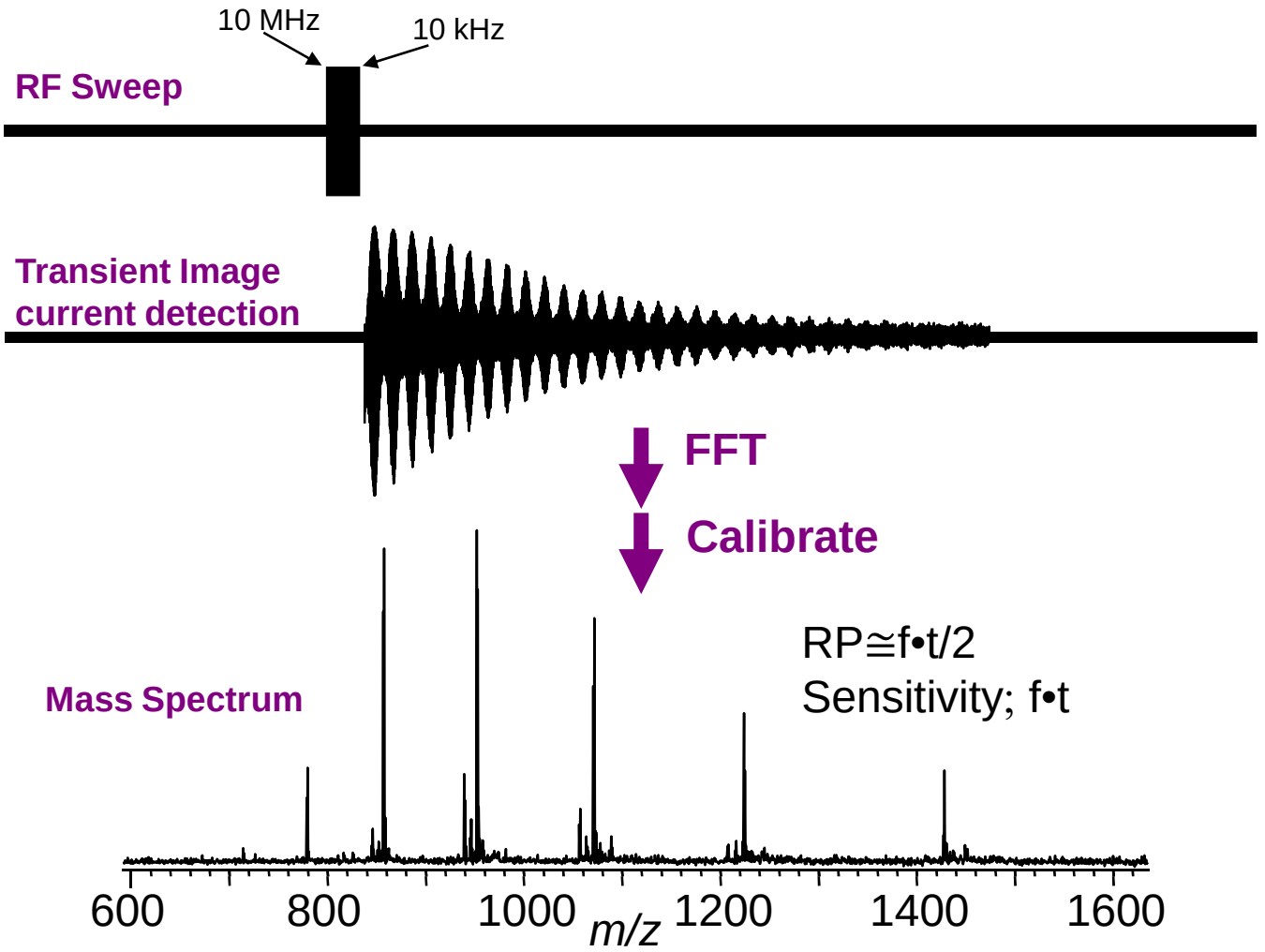


Excitation sweeps resonant ions into a large, coherent cyclotron orbit



Preamplifier and digitizer pick up the induced potentials on the cell.

How Does FTMS Work?



High Resolution (~50,000 FWHM)

High mass accuracy (~1 ppm)

High sensitivity (femtomoles)

Protein Digest of TV60

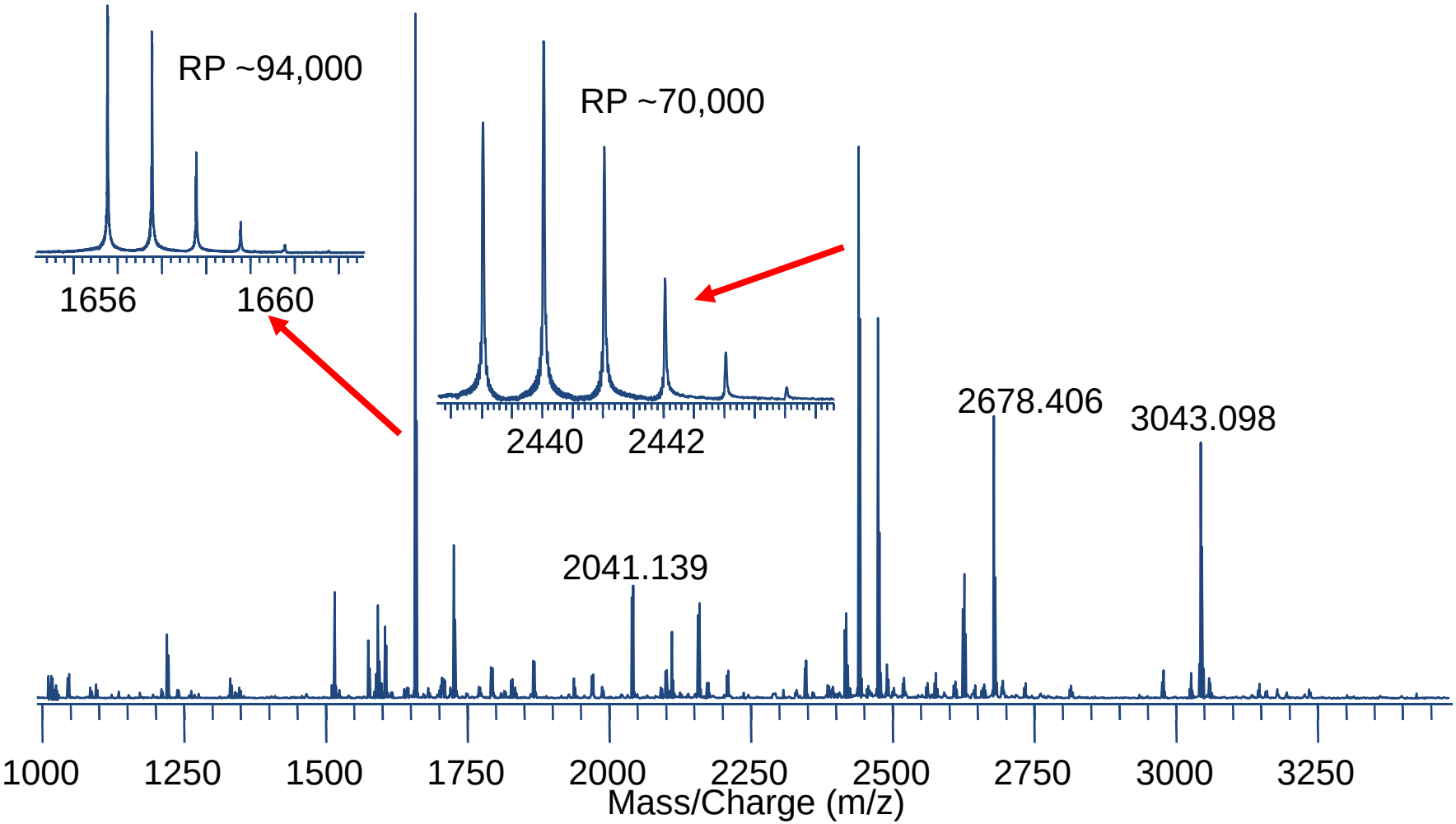
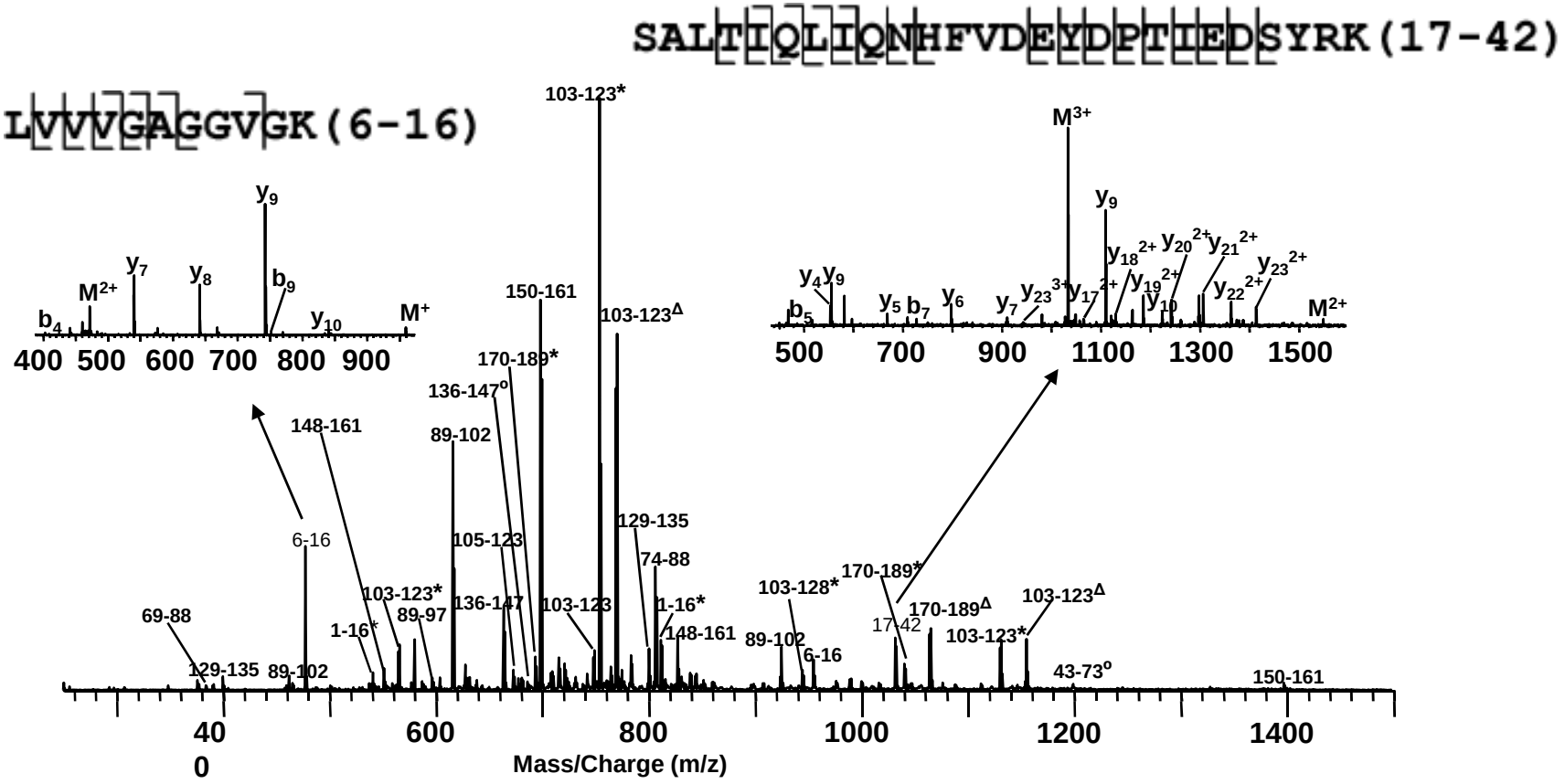


Figure 3. A tryptic digest MS spectrum of oxidized human p21Ras. The two insets show MS/MS spectra, confirming the identity of two peptides which were then used for internal calibration, allowing ~1 ppm mass accuracy on all peaks.



A new instrument – the orbitrap

Anal. Chem. **2000,** *72,* 1156−1162

Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

Alexander Makarov*

HD Technologies Ltd., Atlas House, Simonsway, Manchester, M22 5PP, U.K.

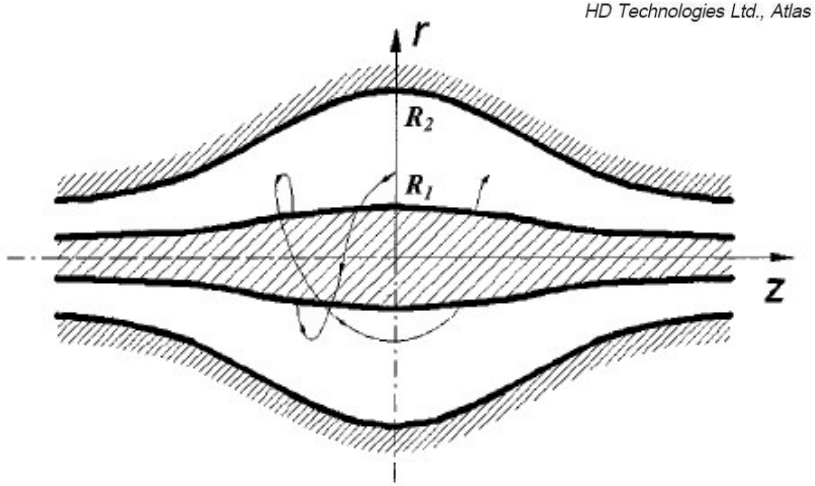


Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

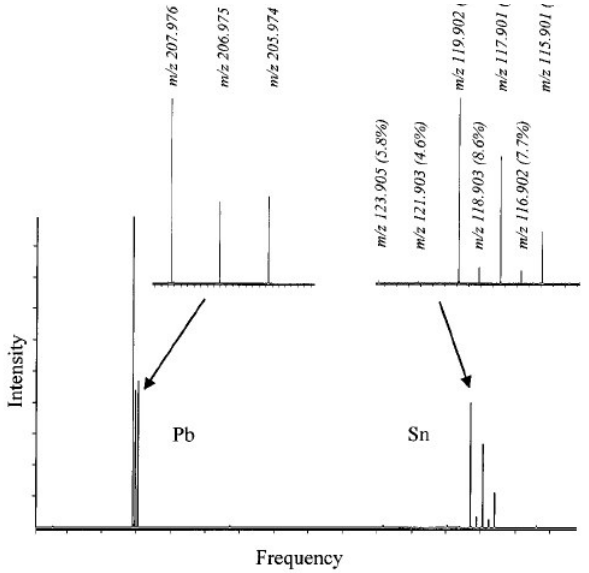


Figure 8. Panoramic mass spectrum of laser-ablated solder alloy (40%:60% lead/tin) in the frequency domain. Accurate masses of most abundant isotopes are shown along with their natural abundance.

Break Time (10 Minutes)

Back to the Fundamentals!

What information is in a mass spectrum?

What information is in Mass Spectra?

1. Masses
   Useful for testing your theory of your chemical structure
2. Elemental compositions
   Or at least estimates thereof...
   Nitrogen Rule
3. Mixture Compositions
4. Abundances (quantitation)
5. Charge states
6. Mass differences (MS/MS)
   - Sequences (Proteins, peptides, polymers, DNA, etc)
   - **Linkages (carbohydrates, lipids, hydrogen bonding, etc.**
7. Fragment stabilities
   Breakdown curves yield relative (Net) transition state energies
8. Higher order structure
   - Hydrogen/Deuterium Exchange (HDX) experiments
   - Electron Capture/Transfer Dissociation
   - **Rings plus Double Bonds**

What is Molecular Mass?

Mass: $M = \Sigma m_e \cdot n_e$,

m_e – mass of an element

n_e – number of atoms of this element in the molecule

Isotope	*Mass*	*Abundance*	*Chemical mass*	*Deviation from the whole number*
^{1}H	**1.00782510**	**99.9852%**	**1.00794**	**+0.0079**
^{2}H (D)	**2.01410222**	**0.0148%**		
^{12}C	**12.0(0)**	**98.892%**	**12.011**	**+0.011**
^{13}C	**13.0033544**	**1.108%**		
^{14}N	**14.00307439**	**99.635%**	**14.00674**	**+0.007**
^{15}N	**15.0001077**	**0.365%**		
^{16}O	**15.99491502**	**99.759%**	**15.9994**	**-0.0006**
^{17}O	**16.9991329**	**0.037%**		
^{18}O	**17.99916002**	**0.204%**		
^{31}P	**30.9737647**	**100%**	**30.9737647**	**-0.0262**
^{32}S	**31.9720737**	**95.0%**	**32.066**	**+0.066**
^{33}S	**32.9714619**	**0.76%**		
^{34}S	**33.9678646**	**4.22%**		
^{36}S	**35.967090**	**0.014%**		

Some Typical EI spectra of lipids

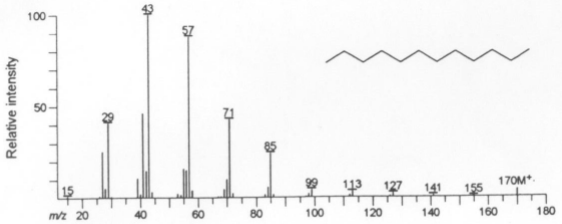


Figure 3.2. Mass spectrum of dodecane.

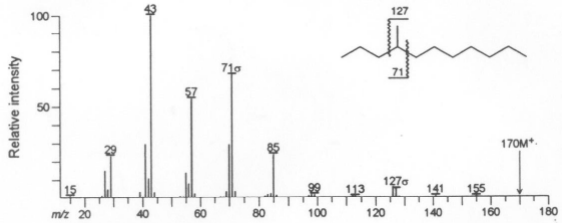


Figure 3.3. Mass spectrum of 4-methylundecane.

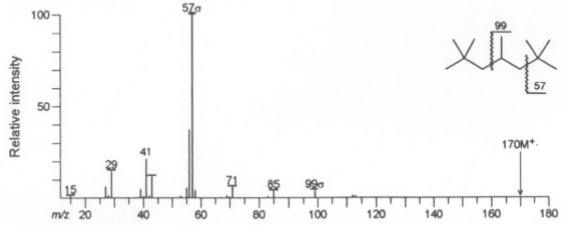


Figure 3.4. Mass spectrum of 2,2,4,6,6-pentamethylheptane.

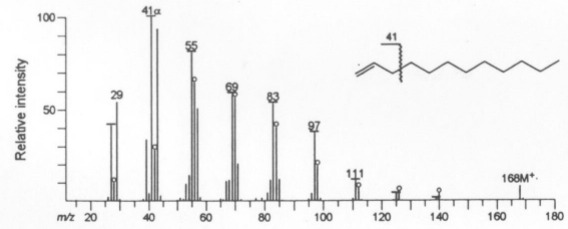


Figure 3.5. Mass spectrum of 1-dodecene.

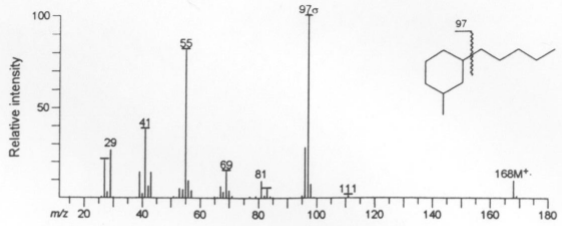


Figure 3.6. Mass spectrum of 1-methyl-3-pentylcyclohexane.

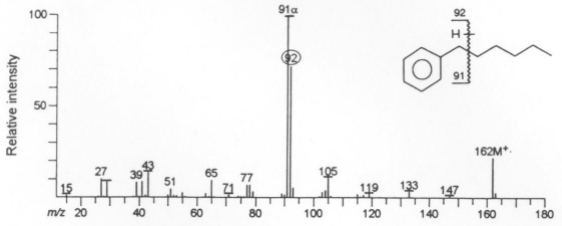


Figure 3.7. Mass spectrum of 1-phenylhexane.

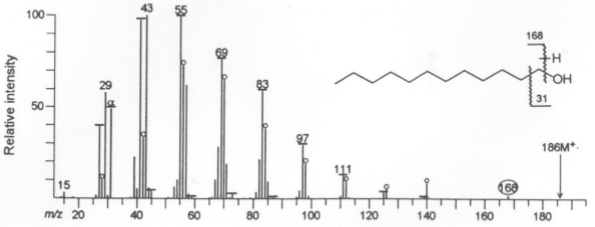


Figure 3.8. Mass spectrum of 1-dodecanol.

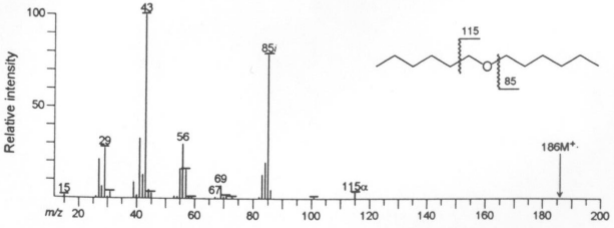


Figure 3.9. Mass spectrum of hexyl ether.

Rings plus Double Bonds

What elemental compositions are realistic chemically?

Because of basic valence orbital arrangments, a simple equation can be used to calculate the number of double bonds (or rings) in a molecule.

$$X - Y/2 + Z/2 + 1 = R+DB$$

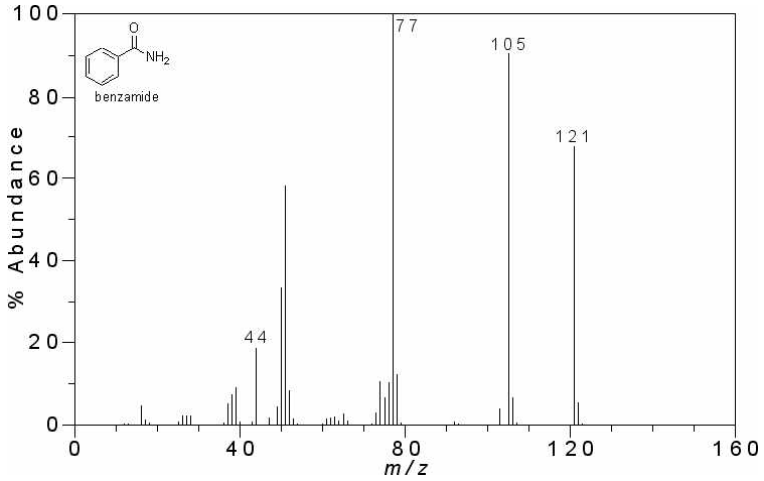
X = carbon, silicon
Y = hydrogen, chlorine, fluorine, etc.
Z = nitrogen, phosphorus

Values ending in ½ correspond to even electron ions.
Values lower than –½ are not possible chemically.

The nitrogen rule

Odd electron ions: a molecule containing the elements C, H, O, N, S or halogen has an odd nominal mass if it contains an odd number of nitrogen atoms.

Even electron ions: a molecule containing the elements C, H, O, N, S or halogen has an odd nominal mass if it contains an Even number of nitrogen atoms.



http://www.chemistry.ccsu.edu/glagovich/teaching/316/ms/nrule.html

Caveats:

1. no metals please!
2. mass “defects” eventually accumulate to > 1 Da, inverting the rule

Switching gears entirely….

Tandem Mass Spectrometry

“Tandem in Time” – FTMS, QITMS

“Tandem in Space” – Triple quad, TOF/TOF, sector

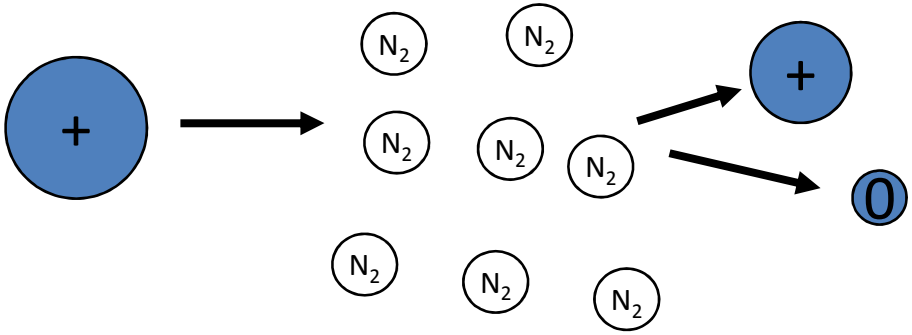
Fragmentation Methods

Breaking up a molecule requires putting energy into it's vibrational modes or causing a reaction that breaks a bond.

- Collisional Activation
- Photodissociation
- Surface Induced Dissociation
- Electron capture dissociation and Electron transfer dissociation

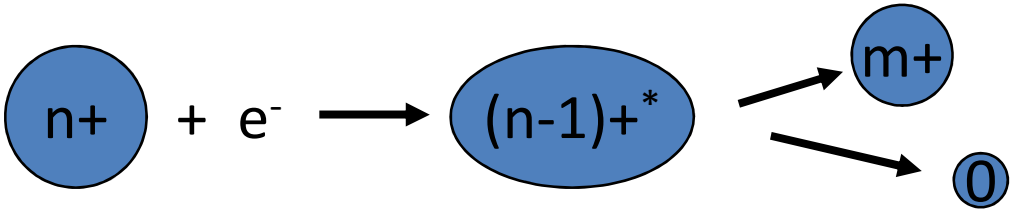
Collisionally Activated Dissociation

also called Collision Induced Dissociation (CID)



- Ion's smack into neutral gas molecules and break up
- Energy of the collision is controlled by changing the kinetic energy of the ion.
- Fragments scatter radially
- By far the most common MS/MS technique
- slow fragmentation method, deposits vibrational energy throughout the molecule prior to fragmentation.
- SORI-CAD, $ITMS^n$, Triple quad, TOF/TOF, etcetera

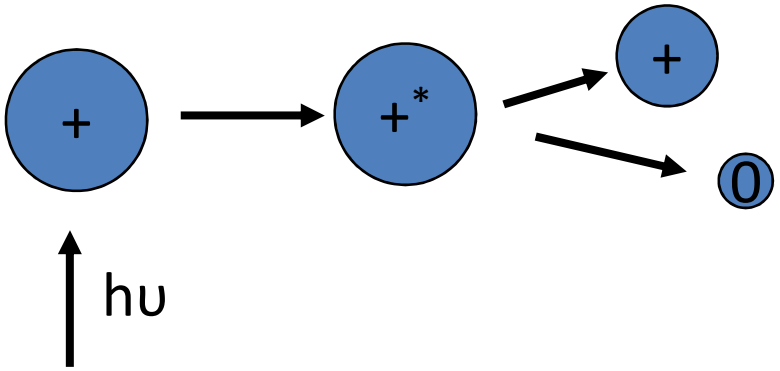
Electron Capture Dissociation



- Multiply charged ions capture a slow electron
- Energy of the fragmentation is determined by coulombic recombination.
- no scattering, but if both fragments are charged, coulombic repulsion will occur

- Fast fragmentation method involving a radical rearrangement in the region of the backbone carbonyl (for proteins)
- Generates very predicable and very even sequence ladder
- Nobody knows how it works on things other than proteins

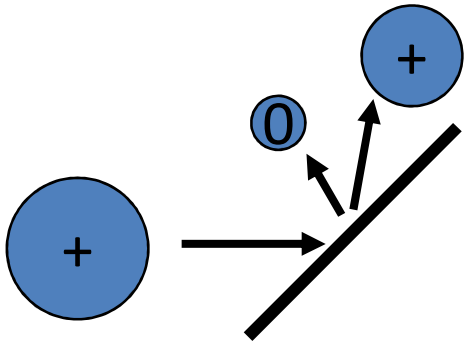
Photo-Dissociation



- Ion absorbs photon(s) and break
- Energy of the fragmentation is controlled by changing the photon's wavelength.
- No scattering, except for multiply charged ions

- slow fragmentation method, deposits vibrational energy throughout the molecule prior to fragmentation (depends on wavelength).
- IRMPD, UVPD, BIRD

Surface induced fragmentation



- Ion smack into a surface, break, and rebound
- Energy of the fragmentation is controlled by changing the ion kinetic energy.
- Fragments scatter radially

- slow fragmentation method, deposits vibrational energy throughout the molecule prior to fragmentation.
- Ions are lost by neutralization at the surface (much better with perfluorinated surfaces)

What information is in Mass Spectra?

1. Masses
   Useful for testing your theory of your chemical structure
2. Elemental compositions
   Or at least estimates thereof...
   Nitrogen Rule
3. Mixture Compositions
4. Abundances (quantitation)
5. Charge states
6. Mass differences (MS/MS)
   - **Sequences (Proteins, peptides, polymers, DNA, etc)**
   - Linkages (carbohydrates, lipids, hydrogen bonding, etc.
7. Fragment stabilities
   Breakdown curves yield relative (Net) transition state energies
8. Higher order structure
   - Hydrogen/Deuterium Exchange (HDX) experiments
   - Electron Capture/Transfer Dissociation
   - Rings plus Double Bonds

Part 1: “Top-Down” vs. “Bottom-Up” Protein Characterization

“Bottom-up” is also called peptide mass fingerprinting or peptide digest mapping of proteins.

Typical “Proteomics” experiment using a MALDI-TOF

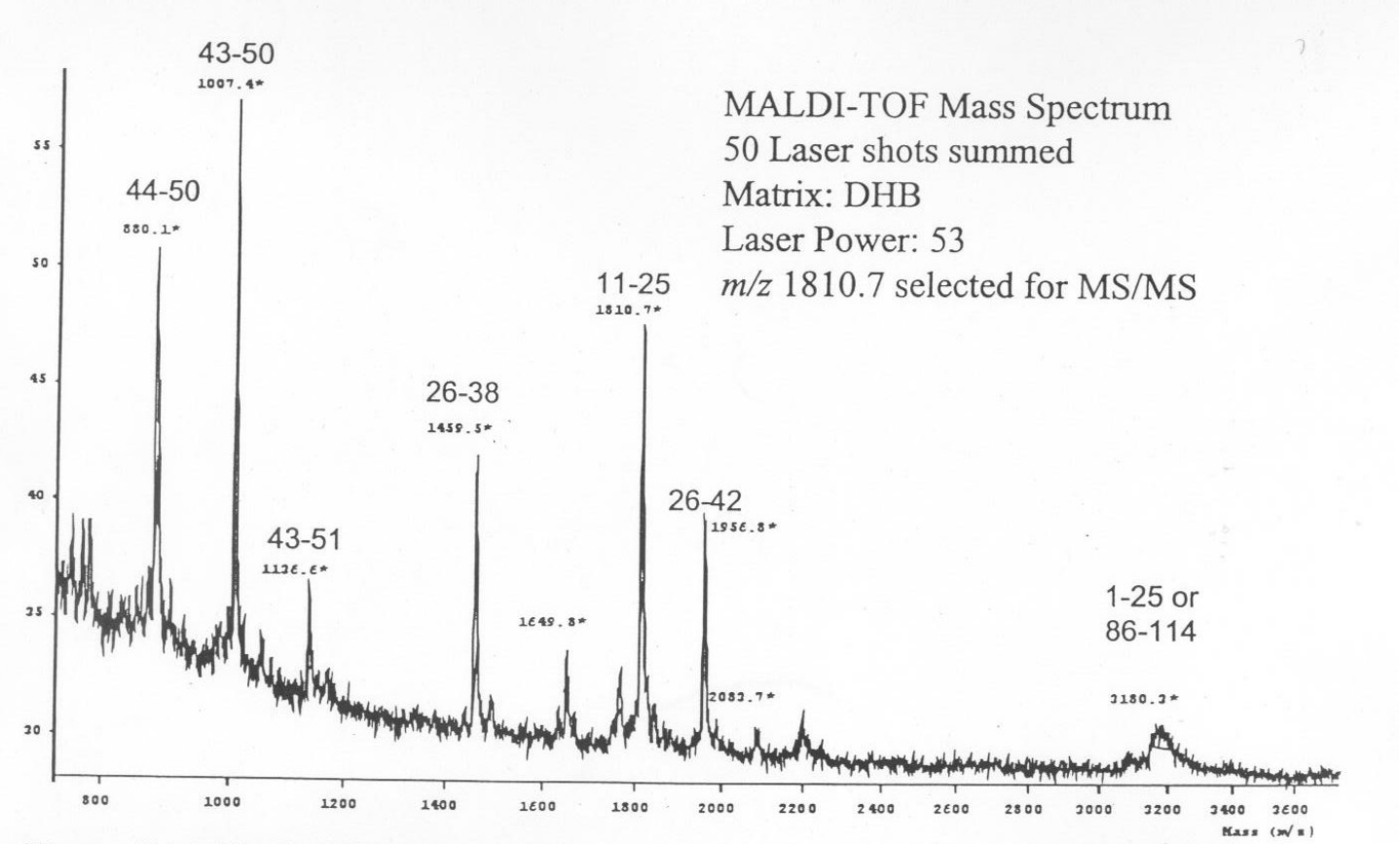
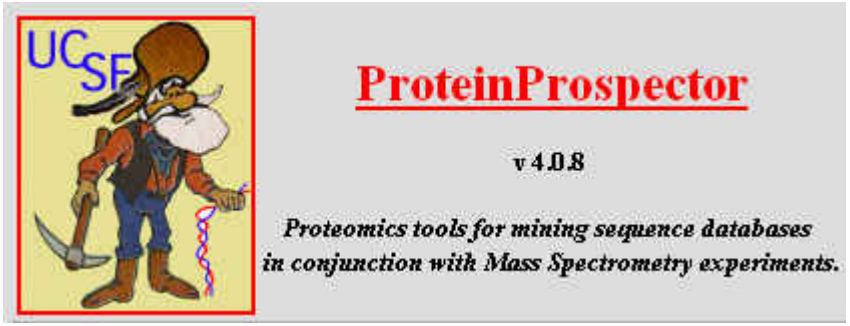


Figure 6A MALDI-TOF MS spectrum of tryptic peptides from Spot 4, pellicle 5-8

2) What would the MALDI-TOF mass spectrum of the tryptic digest of human cytochrome c look like (don't forget the heme)? What about a mutation?



http://prospector.ucsf.edu/

A mutation just shifts the mass, for example, G30A = +14.015 Da on the fourth peptide, so that 1168.62 becomes 1182.63

MS-Digest Search Results

Parameters

Database: **User Protein**
Considered modifications: | **Peptide N-terminal Gln to pyroGlu** | **Oxidation of M** | **Protein N-terminus Acetylated** |
Digest Used: **Trypsin**
Max. # Missed Cleavages: **0**
User AA Formula 1: **C2 H3 N1 O1**
Cysteine Modification: **acrylamide**
Instrument Name: **MALDI-TOF**
Minimum Digest Fragment Mass: **800**
Maximum Digest Fragment Mass: **4000**
Minimum Digest Fragment Length: **5**

pI of Protein: **9.6**
Protein MW: **11749**
Amino Acid Composition: **A6 C2 D3 E8 F3 G13 H3 I8 K18 L6 M4 N5 P4 Q2 R2 S2 T7 V3 W1 Y5**

1 MGDVEKGKKI FIMKCSQCHT VEKGGKHKTG PNLHGLFGRK TGQAPGYSYT AANKNKGIIW GEDTLMEYLE NPKKYIPGTK
81 MIFVGIKKKE ERADLIAYLK KATNE

Number	m/z (mi)	m/z (av)	Modifications	Start	End	Missed Cleavages	Sequence
1	807.4797	808.0802		81	87	0	(K)MIFVGIK (K)
1	823.4746	824.0796	1Met-ox	81	87	0	(K)MIFVGIK(K)
1	906.5295	907.1043		93	100	0	(R)ADLIAYLK (K)
1	1168.6222	1169.3366		29	39	0	(K)TGPNLHGLFGR (K)
1	1176.5136	1177.3515		15	23	0	(K)CSQCHTVEK (G)
1	1428.6754	1429.5383		41	54	0	(K)TGQAPGYSYTAANK (N)
1	2007.9732	2009.2995		57	73	0	(K)GIIWGEDTLMEYLENPK (K)
1	2023.9681	2025.2989	1Met-ox	57	73	0	(K)GIIWGEDTLMEYLENPK(K)

MS-Digest in ProteinProspector 4.0.8

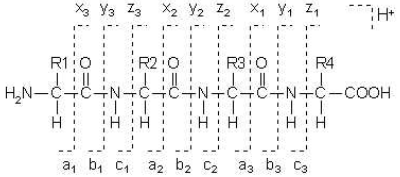
Note: I did neglect the heme, but you need to look up it's structure, and add that mass to the peptide that binds it at positions 15 and 18.

http://www.matrixscience.com/help/fragmentation_help.html

Peptide Fragmentation

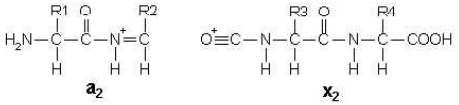
Sequence Ions

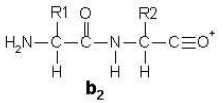
The types of fragment ions observed in an MS/MS spectrum depend on many factors including primary sequence, the amount of internal energy, how the energy was introduced, charge state, etc. The accepted nomenclature for fragment ions was first proposed by Roepstorff and Fohlman [Roepstorff, 1984], and subsequently modified by Johnson *et. al.* [Johnson, 1987].

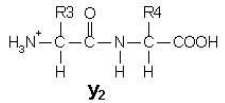


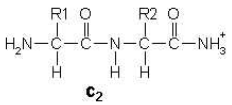
Fragments will only be detected if they carry at least one charge. If this charge is retained on the N terminal fragment, the ion is classed as either *a*, *b* or *c*. If the charge is retained on the C terminal, the ion type is either *x*, *y* or *z*. A subscript indicates the number of residues in the fragment.

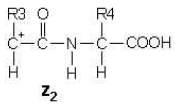
In addition to the proton(s) carrying the charge, *c* ions and *y* ions abstract an additional proton from the precursor peptide. Thus, the structures of the six singly charged sequence ion are:







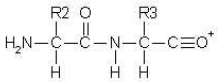




Note that these structures include a single charge carrying proton. In electrospray ionisation, tryptic peptides generally carry two or more charges, so that fragment ions may carry more than one proton.

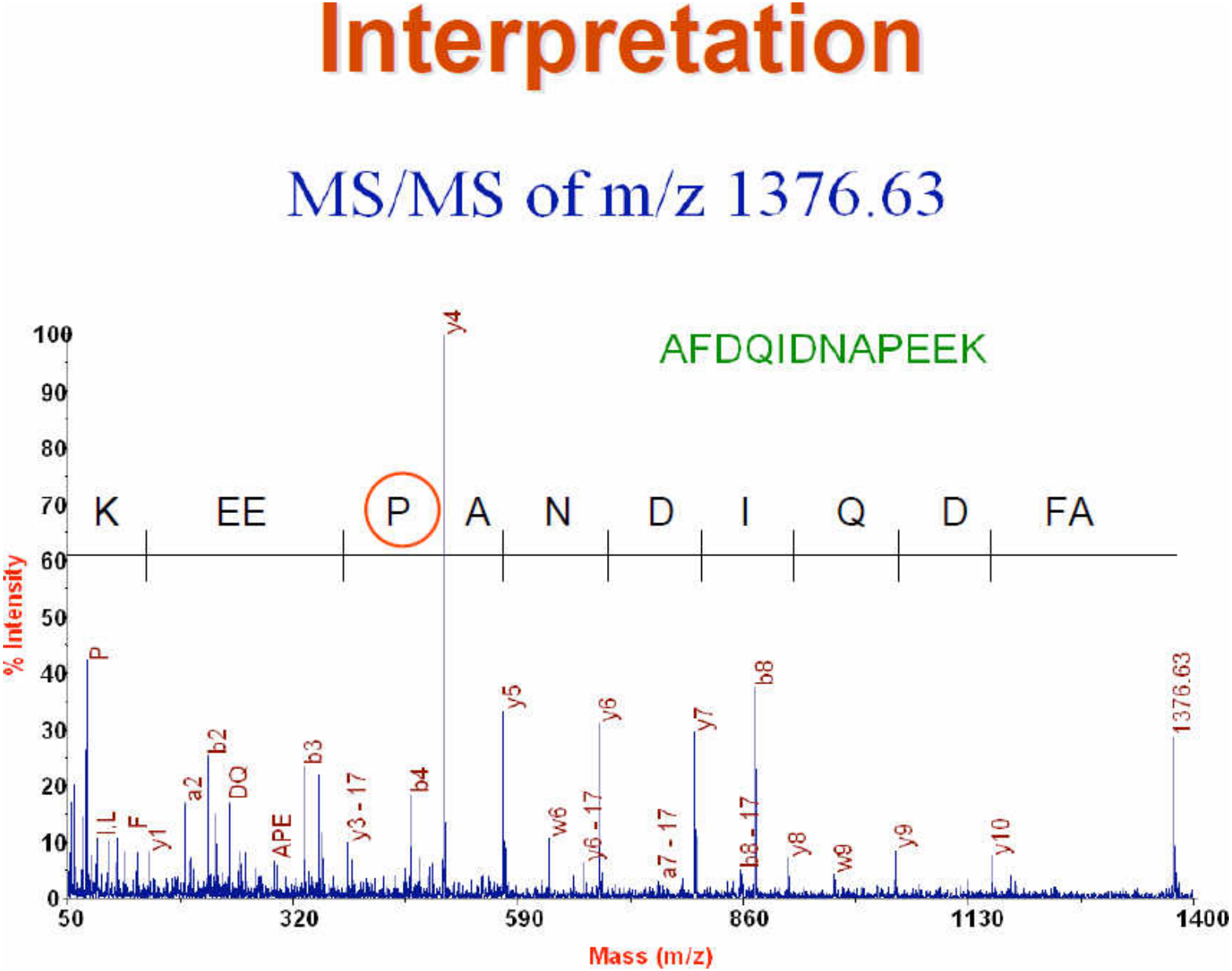
Internal Cleavage Ions

Double backbone cleavage gives rise to internal fragments. Usually, these are formed by a combination of *b* type and *y* type cleavage to produce the illustrated structure, an amino-acylium ion. Sometimes, internal cleavage ions can be formed by a combination of *a* type and *y* type cleavage, an amino-immonium ion. Internal fragments are labelled with their 1 letter amino acid code.



Immonium Ions

Interpretation

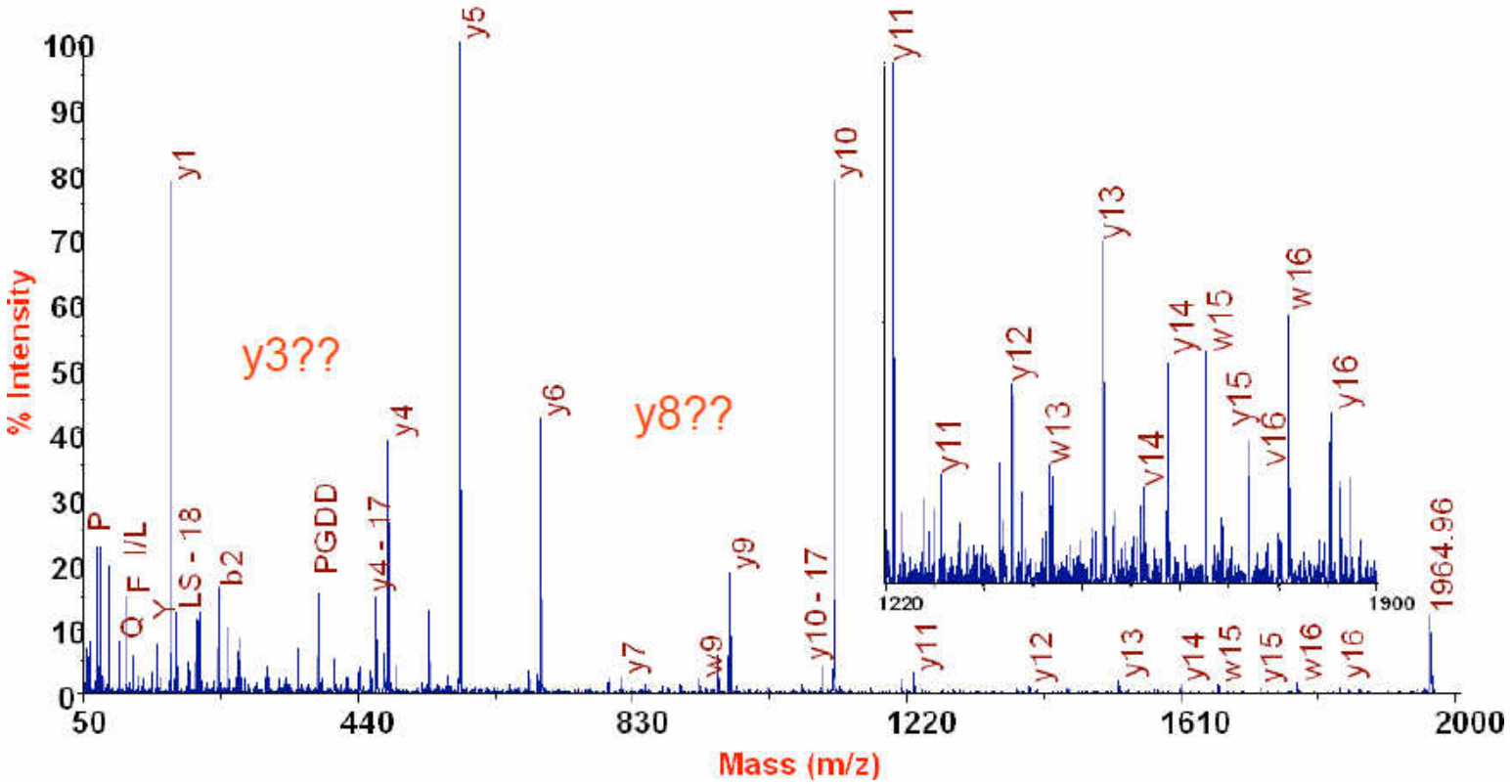


http://www.enghild-lab.dk/downloads/proteomics_course2004/CAF.pdf

Interpretation

MS/MS of m/z 1964.96

ELLSQYDFPGDDTPIVR

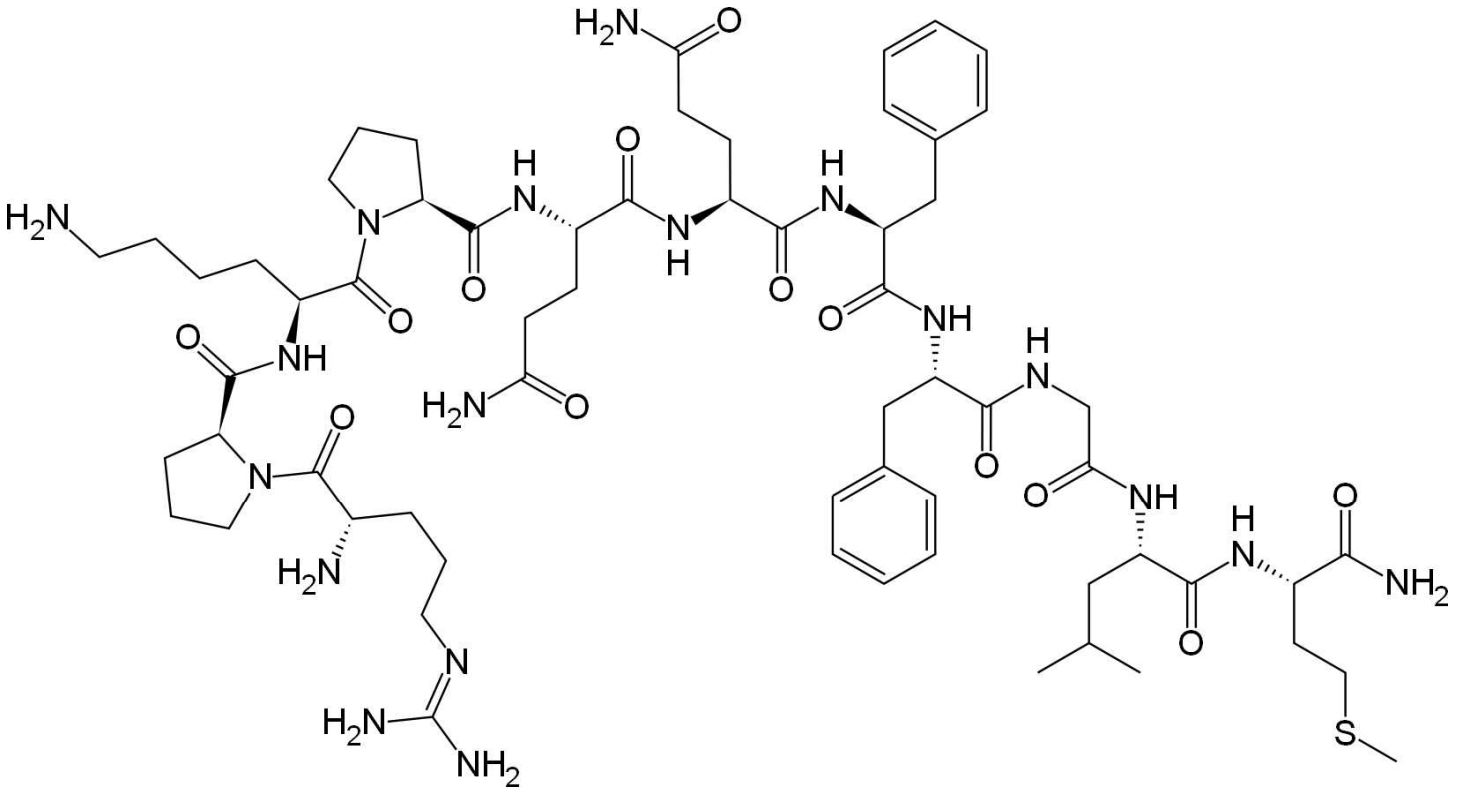


http://www.enghild-lab.dk/downloads/proteomics_course2004/CAF.pdf

Example #2

Calculate the b, c, y, and z ion series for Substance P.

Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met = RPKPQQFFGLM



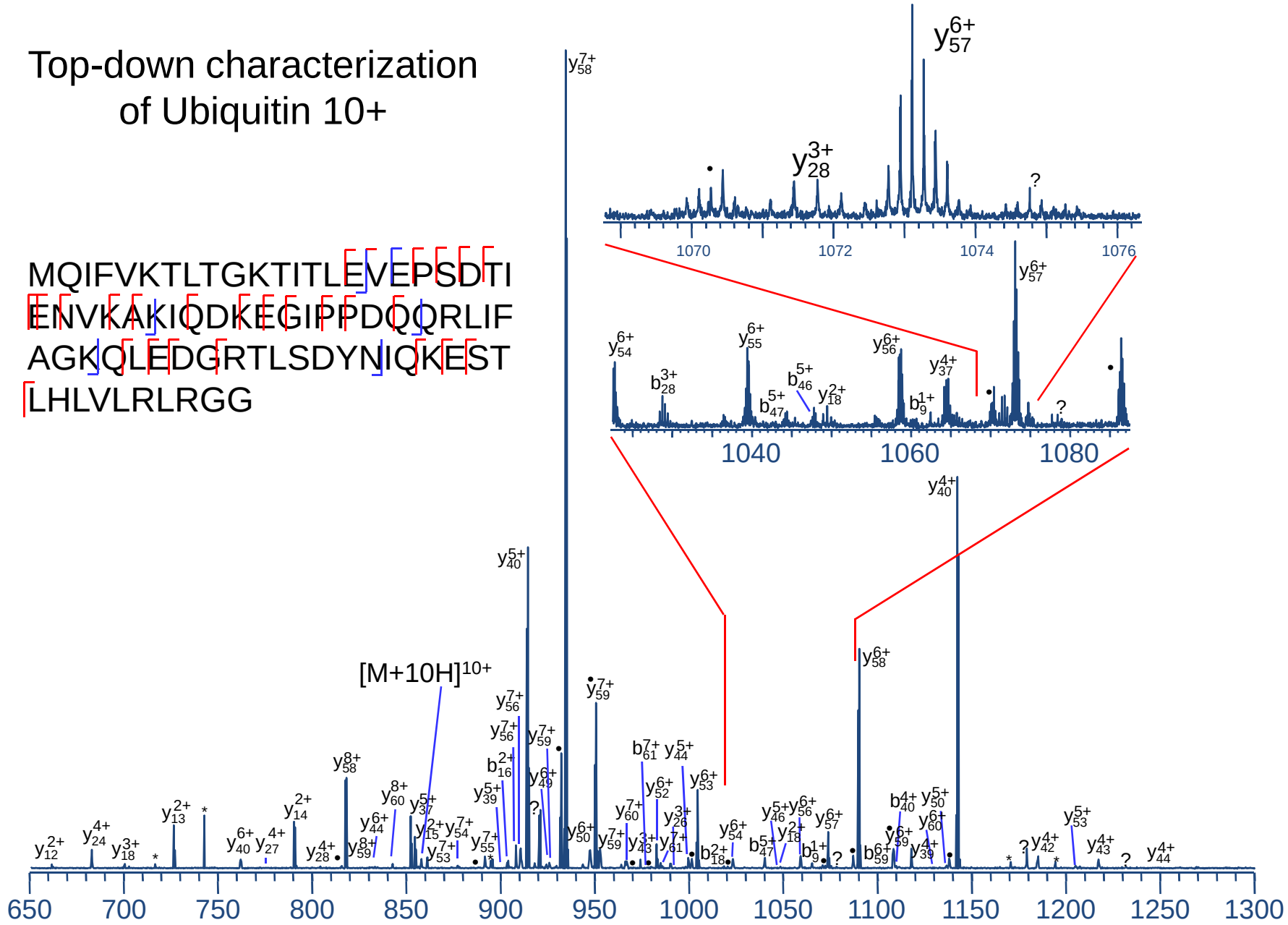
“Top-Down” MS of Proteins

Determine molecular weight of a whole protein with >1 Da accuracy.

Fragment a whole protein in MS^n experiments to localize modifications

Top-down characterization of Ubiquitin 10+

MQIFVKTLTGKTITLEVEPSDTI
ENVKAKIQDKEGIPPDQQRLIF
AGKQLEDGRTLSDYNIQKEST
LHLVLRLRGG



Jebanathirajah, J. A.; Pittman, J. L.; Thomson, B. A.; Budnik, B. A.; Kaur, P.; Rape, M.; Kirschner, M.; Costello, C. E.; O'Connor, P. B. Characterization of a new qQq-FTICR mass spectrometer for post-translational modification analysis and top-down tandem mass Spectrometry of whole proteins *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1985-1999.

*: electronic noise •: Water Loss

What's the advantage of Top-Down?

- Mutants are immediately obvious as a mass error in the protein molecular ion.
- Post-Translational modifications are obvious because of a mass shift in the molecular ion
- 100% sequence coverage (although not necessarily all inter-residue bonds are cleaved)

What’s the advantage of Bottom-Up?

- It’s easy
- You don’t need 100% sequence coverage to id a protein (typically 3-5 peaks with <5 ppm mass accuracy will do)
- You work in a mass region where the mass spectrometers work best
- Don’t need as much sample cleanup

What’s needed for Top-Down?

- Charge state determination
  - Sufficiently High resolving power to determine charge state of the fragments
- lot’s of time (or good computer programs) for going through spectra
- relatively homogeneous samples

Pitfalls for top-down

- Sample must be clean-clean-clean
- Protein must be pure
- Excessive heterogeneity in modifications will distribute the signal over many peaks
- Generally need picomoles of sample at least

Pitfalls for Bottom-Up

- If the protein isn’t clean, you can’t determine which peptide comes from which protein
- You usually lose many of the peptides (30-60% sequence coverage is typical in good cases) so if there’s a modification, you might miss it.
- You are making a simple spectrum (1 protein) into a complicated one (many peptides), which can make assignment of the peaks difficult.
- You often cannot assign many of the peaks, thus wasting information.

The Thiaminase Story

- 42 kDa protein
- n-terminal heterogeneity
- c-terminal fragments were all wrong because of a frame shift in the DNA

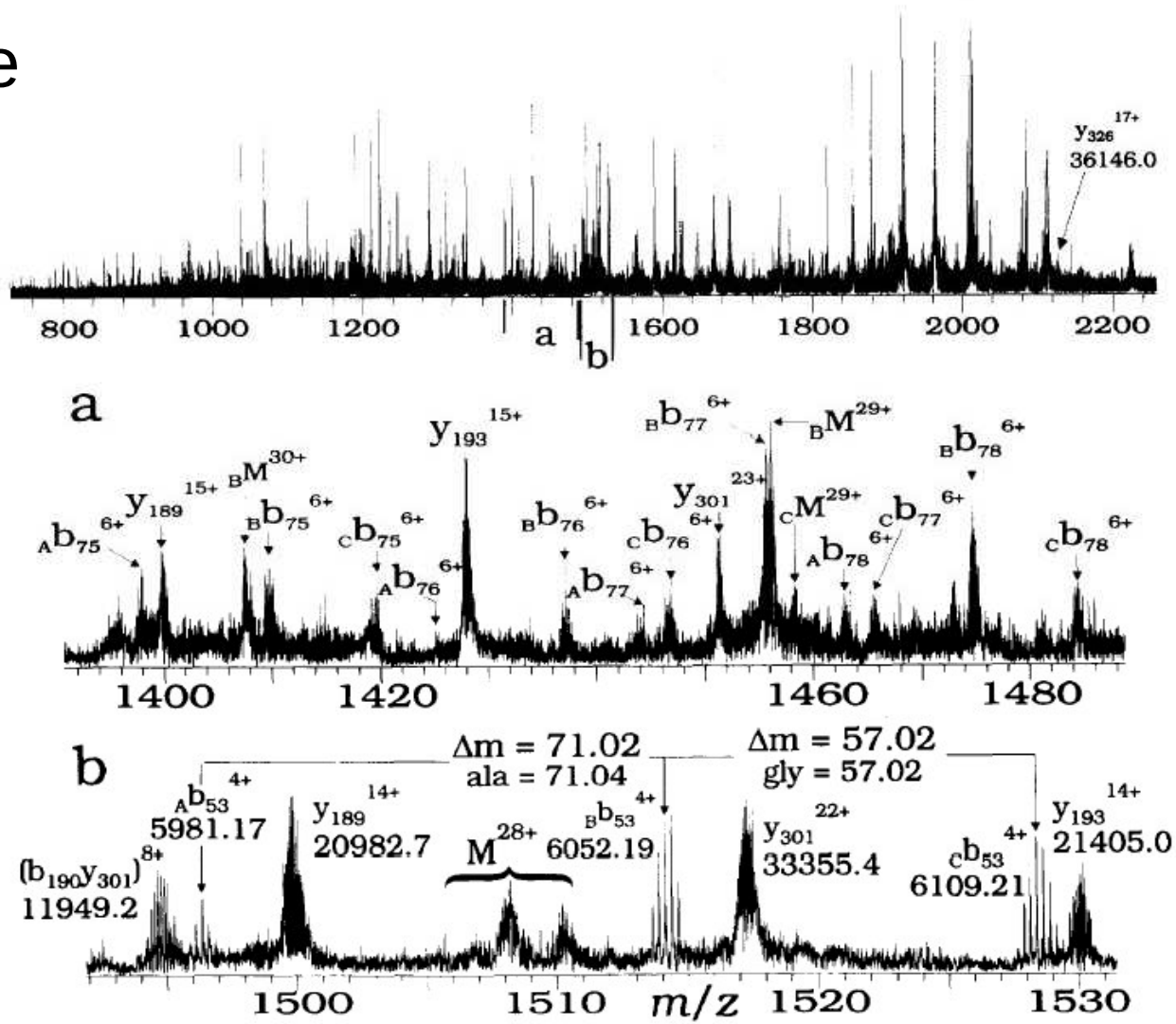


Figure 2. Nozzle–skimmer (130 V) dissociation spectrum of thiaminase I, 30 scans; preceding subscripts A, B, or C denote origin from the smallest, middle, or largest molecule species, respectively.

Kelleher, N. L.; Costello, C. A.; Begley, T. P.; McLafferty, F. W. Thiaminase I (42 Kda) Heterogeneity, Sequence Refinement, and Active Site Location From High-Resolution Tandem Mass Spectrometry *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 981-984.

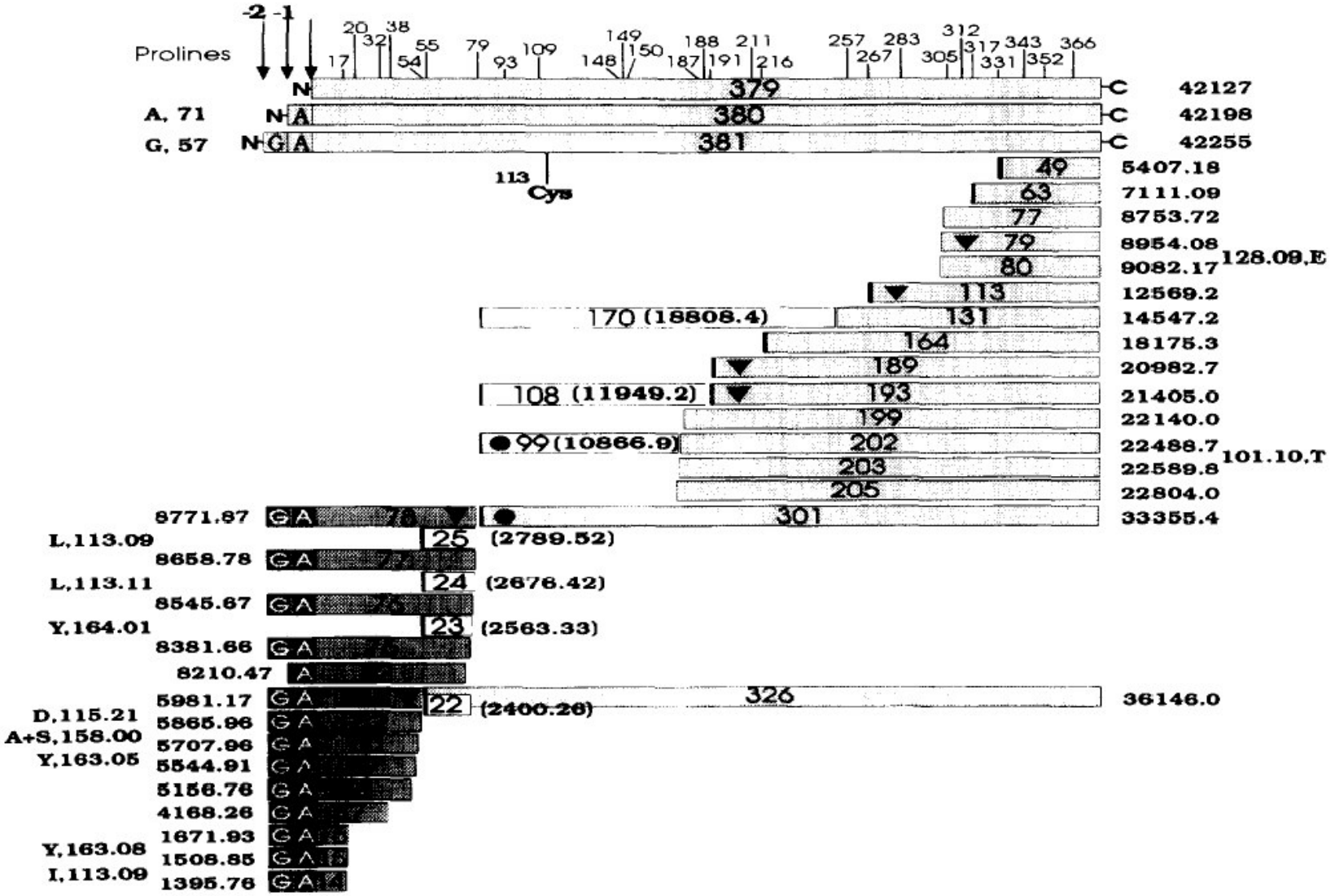


Figure 3. Thiaminase I fragmentation data; y ions on right; b ions on left. Upper left arrows, post-translational cleavage sites. ●, fragment shifts + 108 Da upon inactivation; ▼ fragment does not shift.

Kelleher, N. L.; Costello, C. A.; Begley, T. P.; McLafferty, F. W. Thiaminase I (42 Kda) Heterogeneity, Sequence Refinement, and Active Site Location From High-Resolution Tandem Mass Spectrometry *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 981-984.

“Golden” Complementary Pairs

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

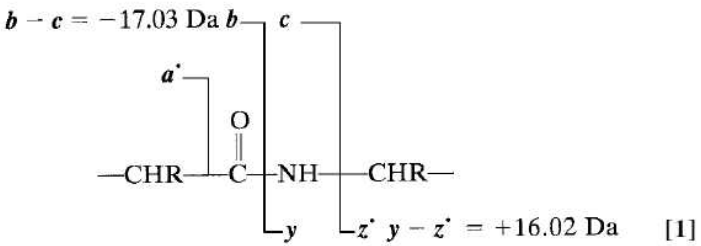
A *de novo* sequencing program for proteins is described that uses tandem MS data from electron capture dissociation and collisionally activated dissociation of electrosprayed protein ions. Computer automation is used to convert the fragment ion mass values derived from these spectra into the most probable protein sequence, without distinguishing Leu/Ile. Minimum human input is necessary for the data reduction and interpretation. No extra chemistry is necessary to distinguish N- and C-terminal fragments in the mass spectra, as this is determined from the electron capture dissociation data. With parts-per-million mass accuracy (now available by using higher field Fourier transform MS instruments), the complete sequences of ubiquitin (8.6 kDa) and melittin (2.8 kDa) were predicted correctly by the program. The data available also provided 91% of the cytochrome *c* (12.4 kDa) sequence (essentially complete except for the tandem MS-resistant region K^{13}–V^{20} that contains the cyclic heme). Uncorrected mass values from a 6-T instrument still gave 86% of the sequence for ubiquitin, except for distinguishing Gln/Lys. Extensive sequencing of larger proteins should be possible by applying the algorithm to pieces of ≈10-kDa size, such as products of limited proteolysis.

Fourier transform MS | electrospray ionization | electron capture dissociation

Mass spectrometry (MS) has proven to be a valuable method for characterizing linear biomolecules, especially peptides and proteins (1–4). “Soft” ionization tech-

(27), or MS “ladder sequencing” using mixtures from N-terminal Edman (15) and C-terminal carboxypeptidase (28) cleavages. However, chemical or enzymatic treatment of the sample greatly increases sample requirements; without this, MS sequence information has been obtained from $\approx 10^{-17}$ moles of peptides (29) and proteins (30).

A new MS/MS method, electron capture dissociation (ECD) (31–35), induces far more general backbone cleavage through nonergodic dissociation, deriving extensive sequence information from proteins as large as 42 kDa (36). In contrast to fragment ions from CAD, ECD fragments always contain either the N or C terminus, and these can be distinguished if dissociations between the same residue pair yield both a *y* and a *c* or $z^{\cdot}$ ion (Eq. **1**). For ubiquitin



[1]

http://www.pnas.org/content/97/19/10313.full.pdf

What information is in Mass Spectra?

1. Masses
   Useful for testing your theory of your chemical structure
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   Or at least estimates thereof...
   Nitrogen Rule
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4. Abundances (quantitation)
5. Charge states
6. Mass differences (MS/MS)
   - Sequences (Proteins, peptides, polymers, DNA, etc)
   - **Modifications to the sequence.....**
   - Linkages (carbohydrates, lipids, hydrogen bonding, etc.
7. Fragment stabilities
   Breakdown curves yield relative (Net) transition state energies
8. Higher order structure
   - Hydrogen/Deuterium Exchange (HDX) experiments
   - Electron Capture/Transfer Dissociation
   - Rings plus Double Bonds

Histone modifications

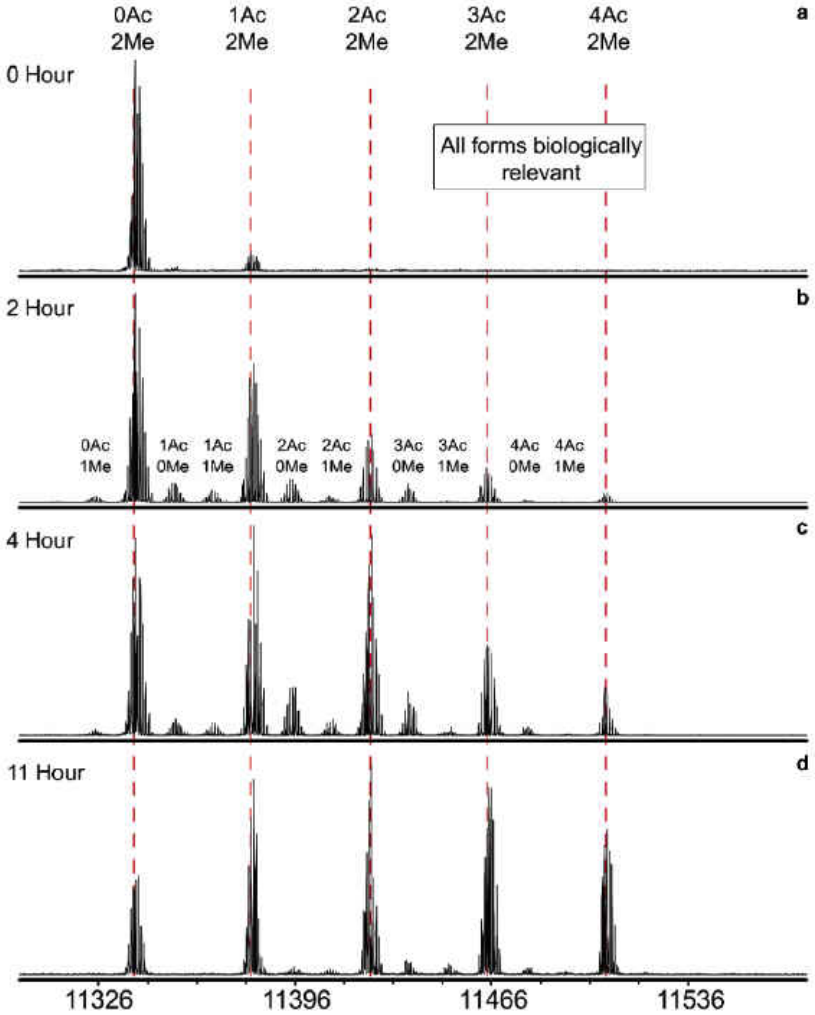


Figure 6. (a–d) HeLa H4 FTMS profiles after treatment with sodium butyrate. (a) The initial profile of H4 shows that a majority of molecules are unacetylated with dimethylated K20. (b–d) After 2 h, all possible acetylation states are observed: mono-, di-, tri-, and tetraacetylation. As butyrate treatment continues, H4 becomes progressively acetylated resulting in the higher abundance of tri- and tetraacetylated forms.

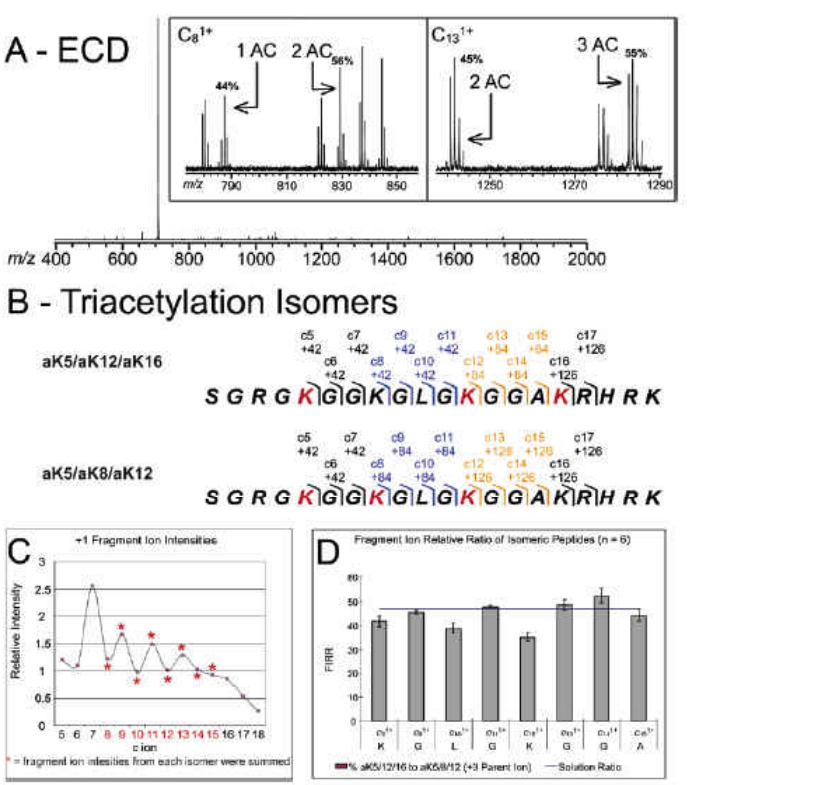


Figure 2. (a) Resolution of an isomeric H4 peptide mixture by ECD FIRRs. The inset shows the FIRRs of a monoacetylated c_8 vs diacetylated c_8 and the FIRRs of a diacetylated c_{13} vs a triacetylated c_{13}. (b) A fragmentation map of the two triacetylated peptides in (a). Isomer-specific fragment ions are colored in blue and orange. (c) Relative intensity plot for each observed c ion. The relative intensity of c_8–c_{15} and corresponding acetylated c_8–c_{15} were added together (*). (d) The average FIRRs for c_8–c_{15} from six independent experiments vs corresponding c ion. The true solution ratio of aK5/aK12/aK16 to aK5/aK8/aK12 H4 peptide (47%) is shown by a straight line.

Pesavento, J. J.; Mizzen, C. A.; Kelleher, N. L. Quantitative Analysis of Modified Proteins and their Positional Isomers by Tandem Mass Spectrometry: Human Histone H4 *Anal. Chem.* **2006**, *78*, 4271-4280.

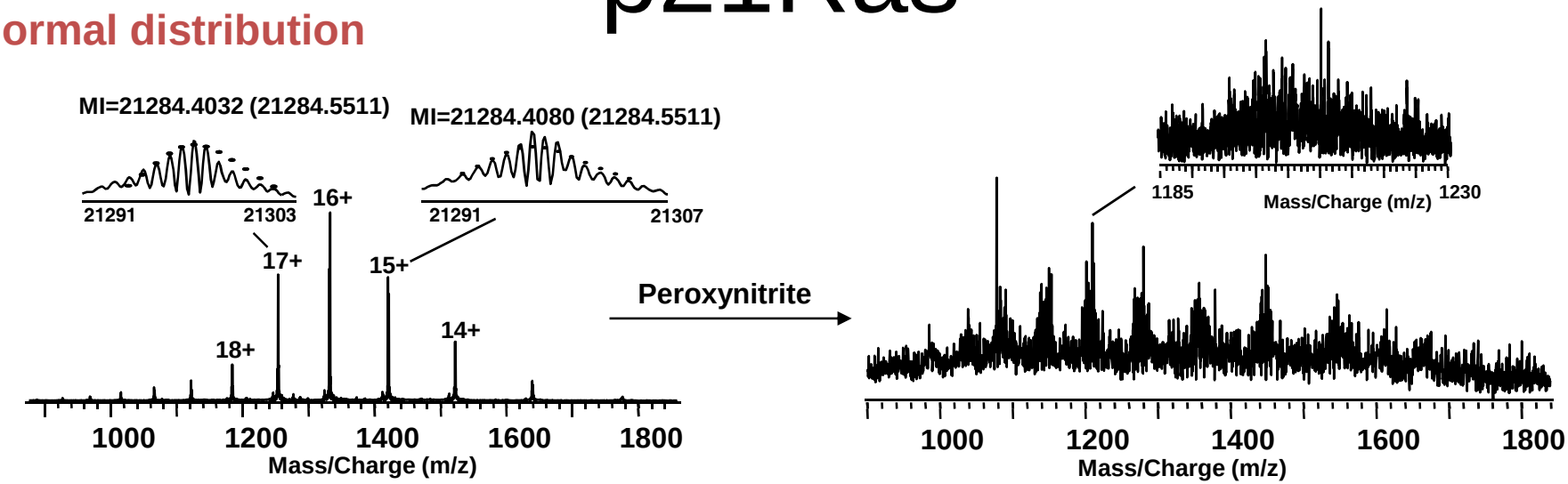
Oxidative stress modifications of p21Ras

- p21Ras = product of oncogene

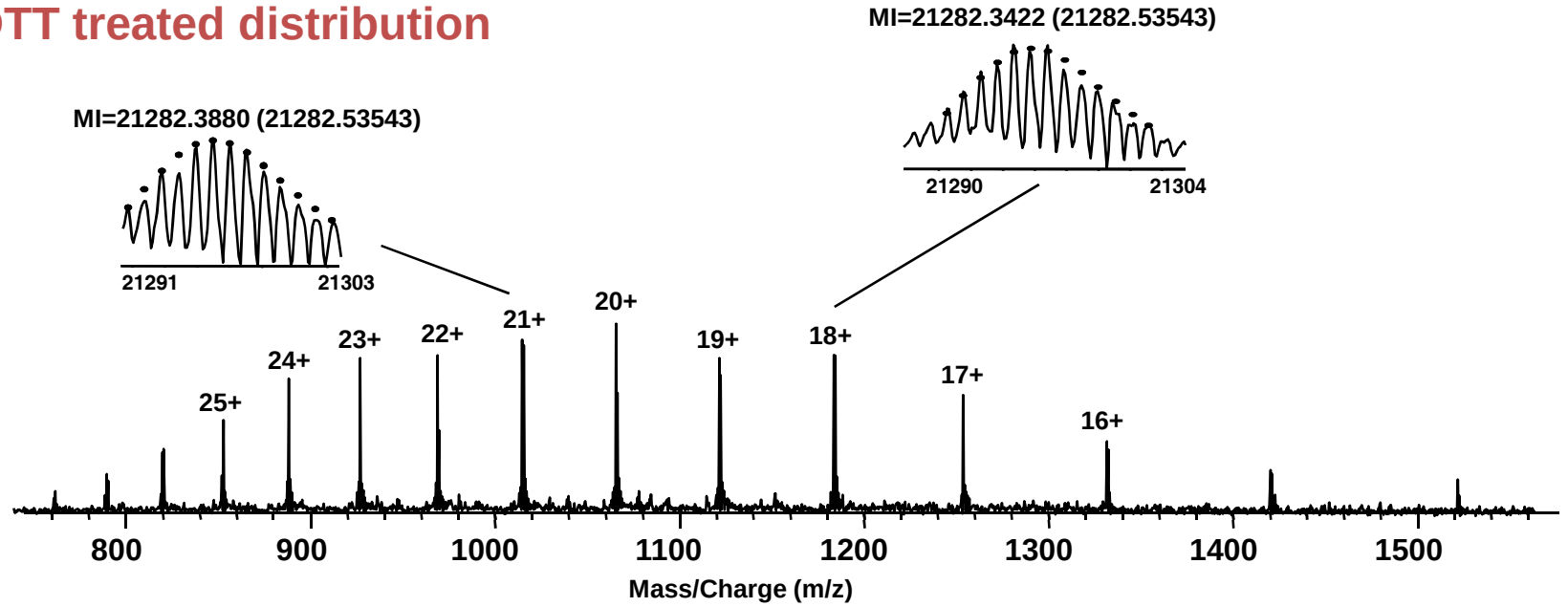
Zhao, C.; Sethuraman, M.; Clavreul, N.; Kaur, P.; Cohen, R. A.; O'Connor, P. B. A Detailed Map of Oxidative Post-translational Modifications of Human p21ras using Fourier Transform Mass Spectrometry *Anal. Chem.* **2006**, *78*, 5134-5142.

p21Ras

Normal distribution

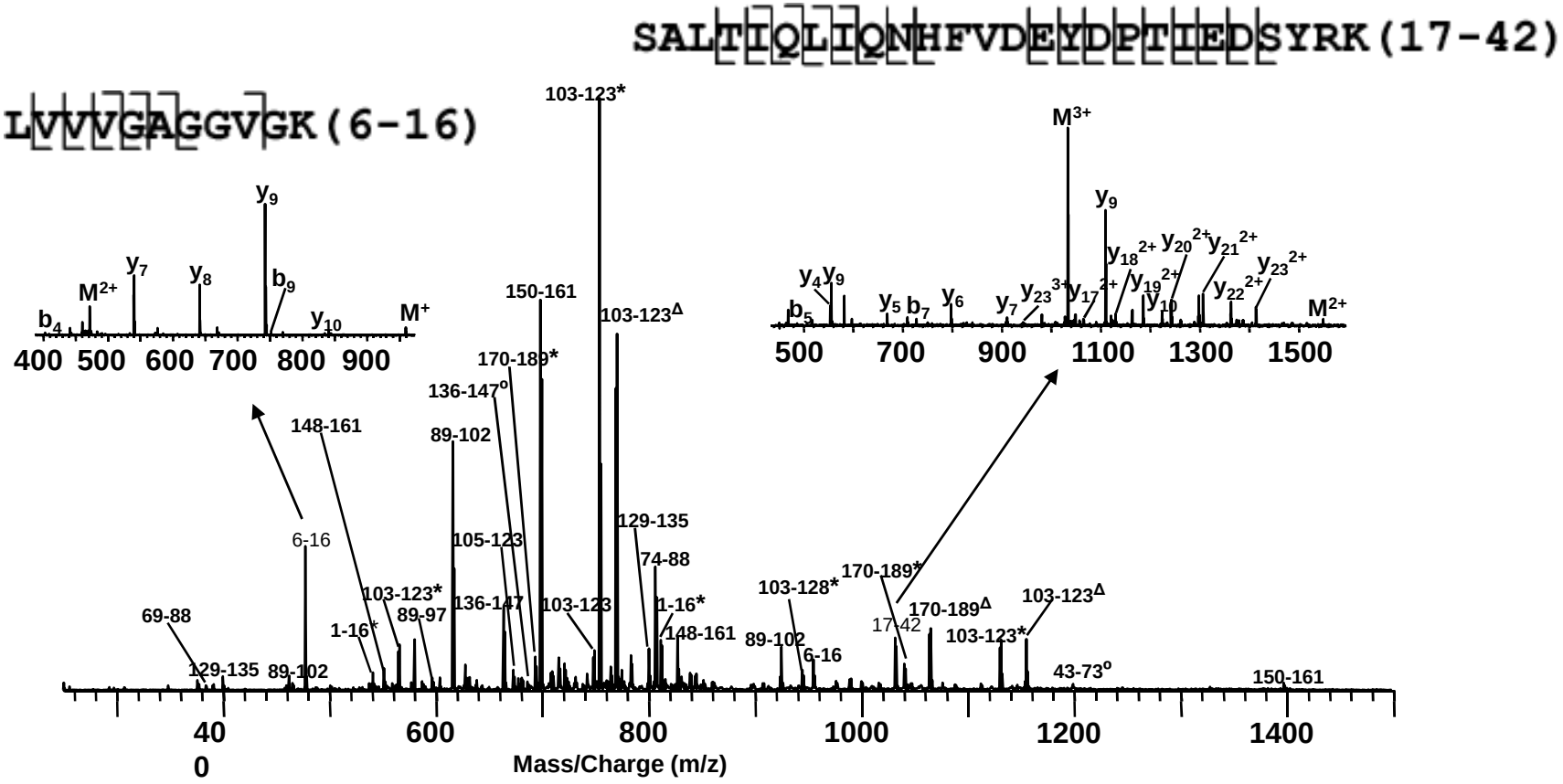


DTT treated distribution



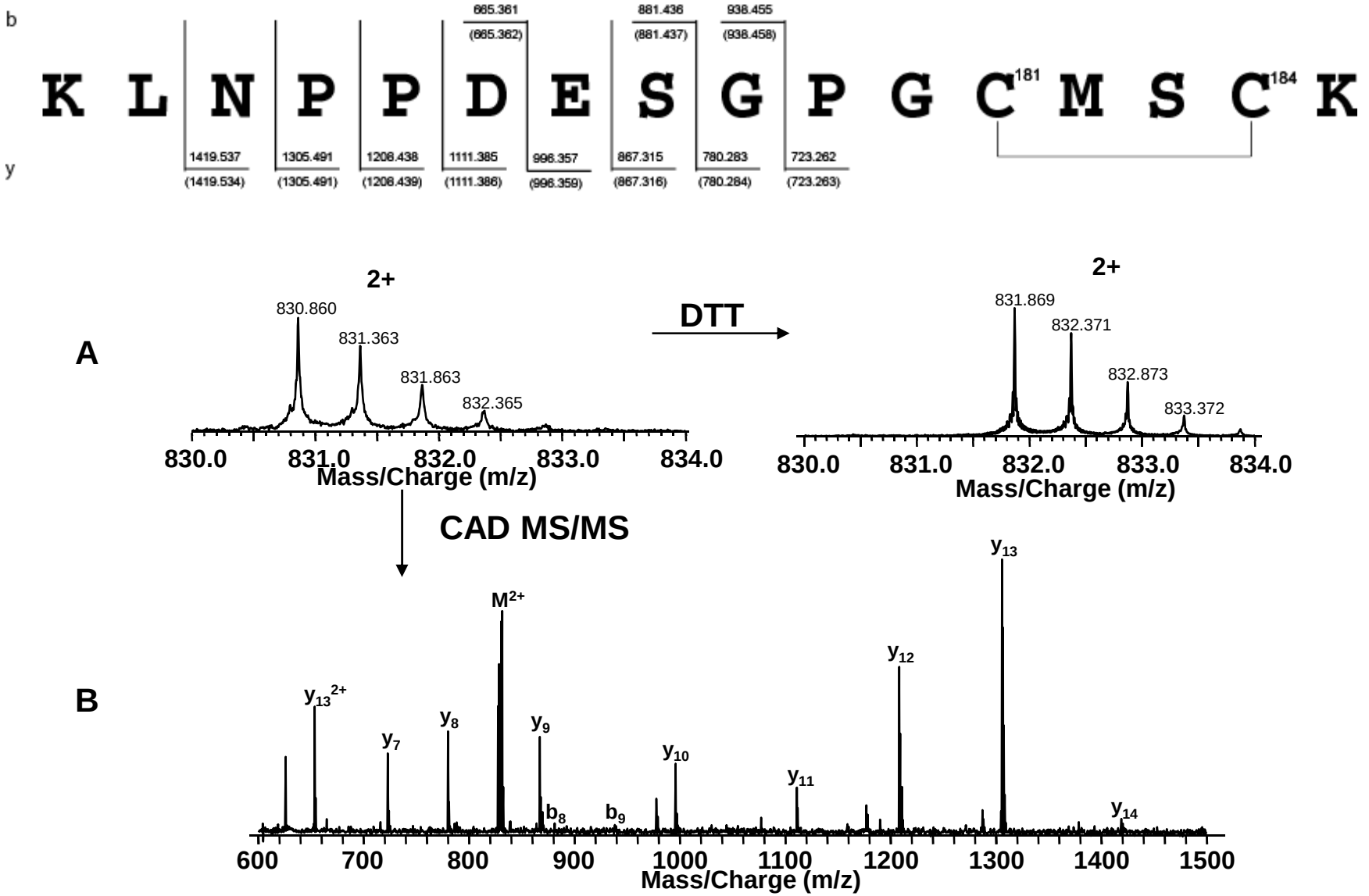
Zhao, C.; Sethuraman, M.; Clavreul, N.; Kaur, P.; Cohen, R. A.; O'Connor, P. B. A Detailed Map of Oxidative Post-translational Modifications of Human p21ras using Fourier Transform Mass Spectrometry *Anal. Chem.* **2006**, *78*, 5134-5142.

Full tryptic digest of p21Ras

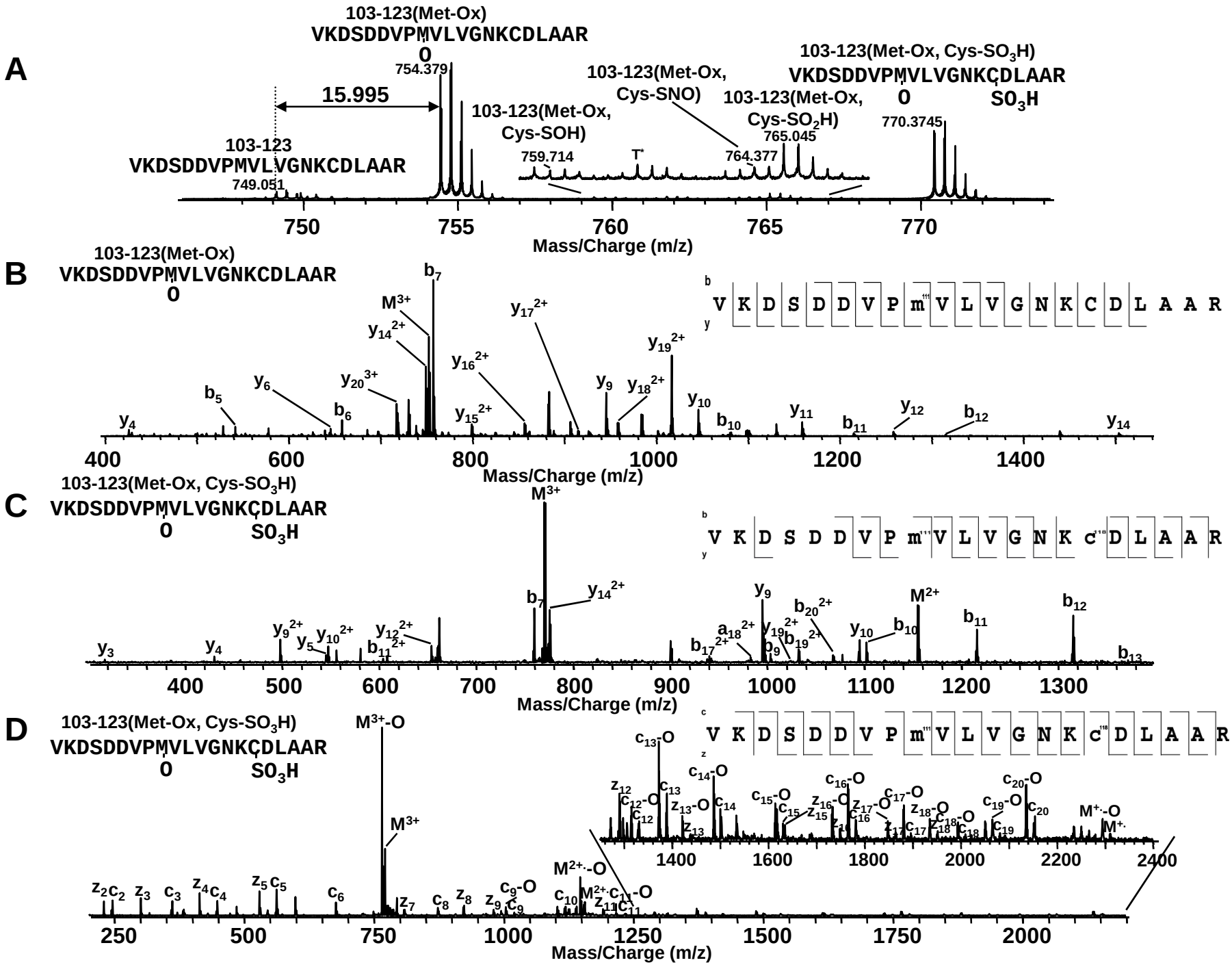


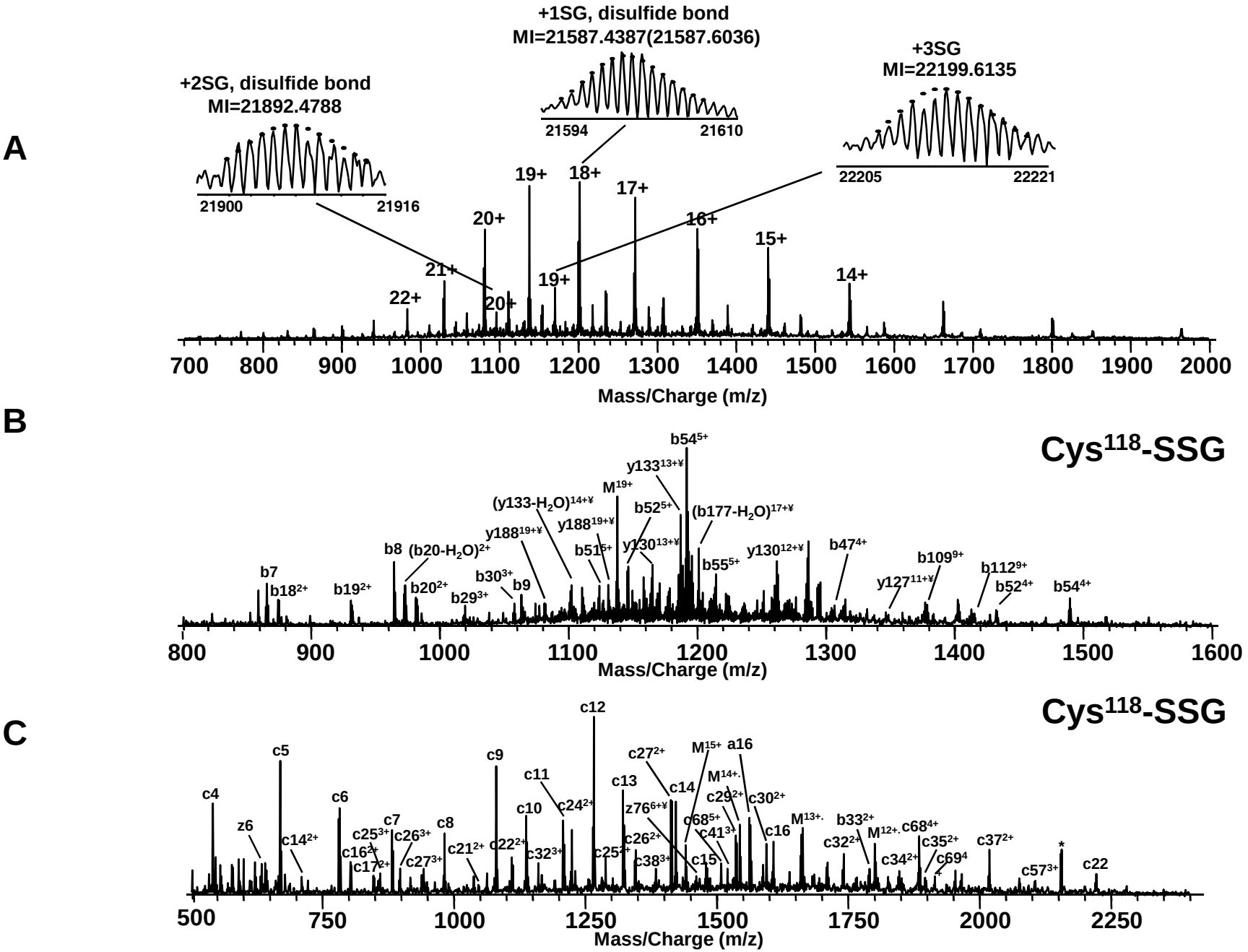
Zhao, C.; Sethuraman, M.; Clavreul, N.; Kaur, P.; Cohen, R. A.; O'Connor, P. B. A Detailed Map of Oxidative Post-translational Modifications of Human p21ras using Fourier Transform Mass Spectrometry *Anal. Chem.* **2006**, *78*, 5134-5142.

c-terminal peptide of p21Ras

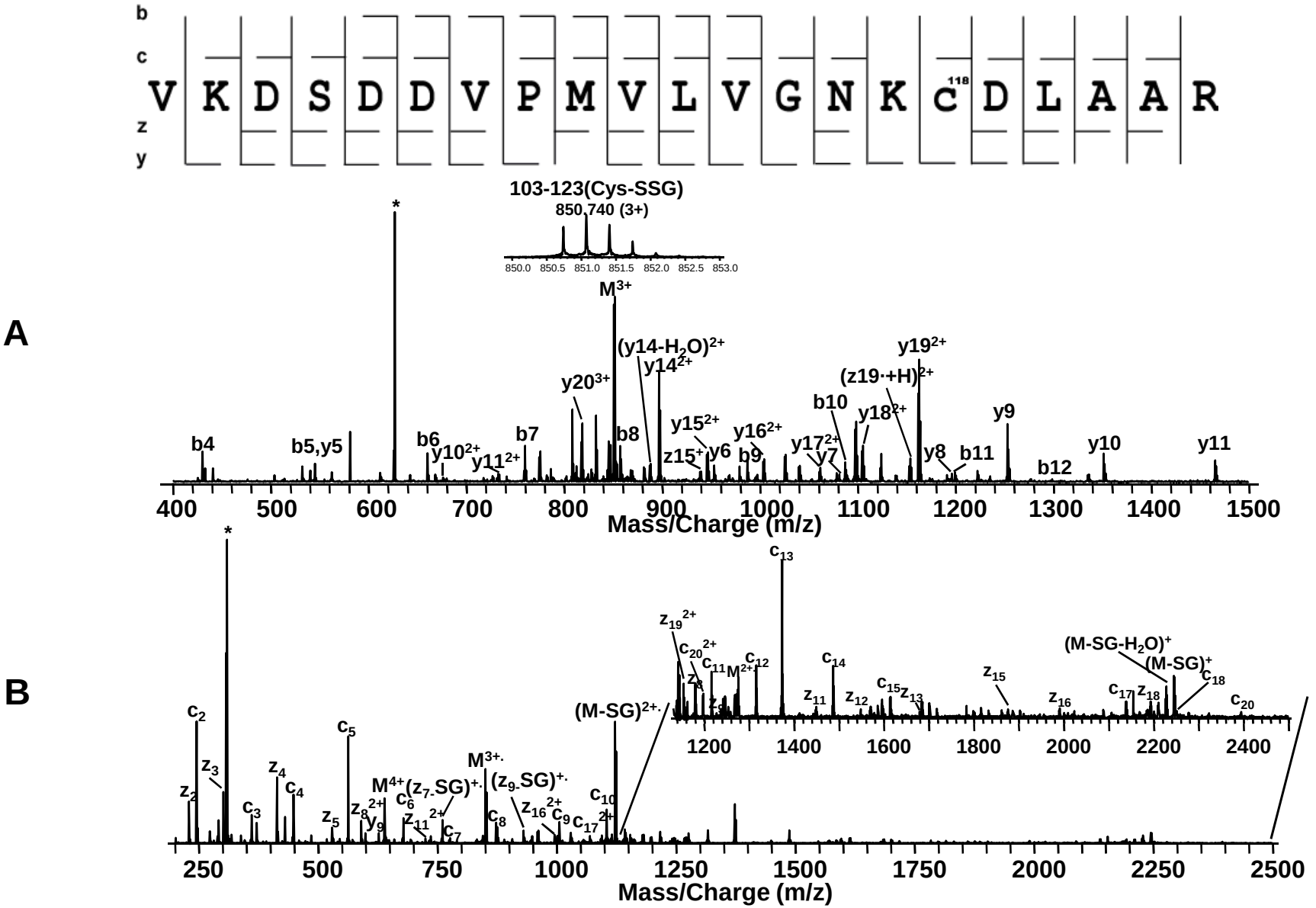


Zhao, C.; Sethuraman, M.; Clavreul, N.; Kaur, P.; Cohen, R. A.; O'Connor, P. B. A Detailed Map of Oxidative Post-translational Modifications of Human p21ras using Fourier Transform Mass Spectrometry *Anal. Chem.* **2006**, *78*, 5134-5142.


A
103-123(Met-Ox)
VKDSDDVPMVLVGNKCDLAAR
0
754.379
15.995
103-123
VKDSDDVPMVLVGNKCDLAAR
749.051
103-123(Met-Ox,
Cys-SOH)
759.714
T*
103-123(Met-Ox,
Cys-SNO)
764.377
103-123(Met-Ox,
Cys-SO2H)
765.045
103-123(Met-Ox, Cys-SO3H)
VKDSDDVPMVLVGNKCDLAAR
0
SO3H
770.3745
750
755
760
765
770
Mass/Charge (m/z)
B
103-123(Met-Ox)
VKDSDDVPMVLVGNKCDLAAR
0
VKDSDDVPm'''VLVGNKCDLAAR
400
600
800
1000
1200
1400
Mass/Charge (m/z)
C
103-123(Met-Ox, Cys-SO3H)
VKDSDDVPMVLVGNKCDLAAR
0
SO3H
VKDSDDVPm'''VLVGNKc'''DLAAR
400
500
600
700
800
900
1000
1100
1200
1300
Mass/Charge (m/z)
D
103-123(Met-Ox, Cys-SO3H)
VKDSDDVPMVLVGNKCDLAAR
0
SO3H
VKDSDDVPm'''VLVGNKc'''DLAAR
1400
1600
1800
2000
2200
2400
250
500
750
1000
1250
1500
1750
2000
Mass/Charge (m/z)



Zhao, C.; Sethuraman, M.; Clavreul, N.; Kaur, P.; Cohen, R. A.; O'Connor, P. B. A Detailed Map of Oxidative Post-translational Modifications of Human p21ras using Fourier Transform Mass Spectrometry *Anal. Chem.* **2006**, *78*, 5134-5142.



Zhao, C.; Sethuraman, M.; Clavreul, N.; Kaur, P.; Cohen, R. A.; O'Connor, P. B. A Detailed Map of Oxidative Post-translational Modifications of Human p21ras using Fourier Transform Mass Spectrometry *Anal. Chem.* **2006**, *78*, 5134-5142.

Table 1

Position	Exp. Peak (In.)	Charge State	M+H(In.)	Theo. Mass	Error(ppm)
1-16(1Met-Ox)	541.9650	3	1623.8794	1623.8780	-0.83
1-16(1Met-Ox)	812.4440	2	1623.8802	1623.8780	-1.34
1-16(1Met-Ox,1Nit)	834.9350	2	1668.8622	1668.8631	0.55
6-16	478.3010	2	955.5942	955.5940	-0.18
6-16	955.5930	1	955.5930	955.5940	1.05
17-41	990.1520	3	2968.4404	2968.4406	0.07
17-42	774.6400	4	3095.5365	3095.5326	-1.27
17-42	1032.5170	3	3095.5354	3095.5326	-0.89
17-42(1Nit)	1047.5090	3	3140.5114	3140.5176	1.99
43-73(2Met-Ox)	899.4150	4	3594.6365	3594.6402	1.02
74-88	538.6060	3	1613.8024	1613.7998	-1.58
74-88	807.4040	2	1613.8002	1613.7998	-0.23
74-102	1148.5759	3	3443.7121	3443.7170	1.44
89-97	597.7820	2	1194.5562	1194.5544	-1.49
89-101	564.9510	3	1692.8374	1692.8346	-1.62
89-102	462.9900	4	1848.9365	1848.9357	-0.45
89-102	616.9830	3	1848.9334	1848.9357	1.27
89-102	924.9720	2	1848.9362	1848.9357	-0.26
89-102(1Nit)	631.9800	3	1893.9244	1893.9207	-1.93
102-123(1Met-Ox)	806.4150	3	2417.2294	2417.2281	-0.52
102-123(1Met-Ox,1CysO3)	822.4110	3	2465.2174	2465.2128	-1.85
103-117(1Met-Ox)	544.6170	3	1631.8354	1631.8314	-2.42
103-123	749.0510	3	2245.1374	2245.1321	-2.34
103-123	1123.0700	2	2245.1322	2245.1321	-0.03
103-123(1Met-Ox)	566.0380	4	2261.1285	2261.1270	-0.67
103-123(1Met-Ox)	754.3790	3	2261.1214	2261.1270	2.50
103-123(1Met-Ox)	1131.0670	2	2261.1262	2261.1270	0.36
103-123(1Met-Ox,1CysO)	759.7140	3	2277.1264	2277.1219	-1.95
103-123(1Met-Ox, 1Cys(NO))	764.3770	3	2291.1154	2291.1200	2.02
103-123(1Met-Ox,1CysO2)	765.0450	3	2293.1194	2293.1168	-1.11
103-123(1Met-Ox,1CysO3)	578.0340	4	2309.1125	2309.1117	-0.36
103-123(1Met-Ox,1CysO3)	770.3745	3	2309.1079	2309.1117	1.67
103-123(1Met-Ox,1CysO3)	1155.0620	2	2309.1162	2309.1117	-1.94
103-117(1Met-Ox)	816.4200	2	1631.8322	1631.8314	-0.47
103-128(1Met-Ox)	709.1110	4	2833.4205	2833.4188	-0.61
103-128(1Met-Ox)	945.1450	3	2833.4194	2833.4188	-0.19
105-123	673.3270	3	2017.9654	2017.9687	1.66
105-123(1Met-Ox)	678.6610	3	2033.9674	2033.9636	-1.84
105-123(1Met-Ox)	1017.4840	2	2033.9602	2033.9636	1.68
129-135	401.2150	2	801.4222	801.4219	-0.34
129-135	801.4230	1	801.4230	801.4219	-1.37
136-147	664.8410	2	1328.6742	1328.6738	-0.28
136-147(1Nit)	687.3350	2	1373.6622	1373.6589	-2.38
148-161	552.2905	3	1654.8559	1654.8553	-0.33
148-161	827.9320	2	1654.8562	1654.8553	-0.53
148-161(1Nit)	850.4240	2	1699.8402	1699.8404	0.13
150-161	466.5740	3	1397.7064	1397.7065	0.11
150-161	699.3560	2	1397.7042	1397.7065	1.66
150-161	1397.7030	1	1397.7030	1397.7065	2.50
150-161(1Nit)	721.8510	2	1442.6942	1442.6916	-1.78
162-169	540.3260	2	1079.6442	1079.6438	-0.35
170-185(1Met-Ox)	560.2475	3	1678.7269	1678.7239	-1.76
170-185(1Met-Ox)	839.8670	2	1678.7262	1678.7239	-1.36
170-189	688.9800	3	2064.9244	2064.9227	-0.80
170-189(1Met-Ox)	694.3110	3	2080.9174	2080.9176	0.12
170-189(1Met-Ox)	1040.9630	2	2080.9182	2080.9176	-0.28
170-189(1Met-Ox,1CysO3)	710.3070	3	2128.9054	2128.9023	-1.43
170-189(1Met-Ox,1CysO3)	1064.9570	2	2128.9062	2128.9023	-1.82
170-189(1Met-Ox,2CysO3)	1088.9490	2	2176.8902	2176.8870	-1.46
170-189(1Met-Ox,3CysO3)	1112.9400	2	2224.8722	2224.8717	-0.21
171-185(1Met-Ox)	775.8190	2	1550.6302	1550.6289	-0.82
171-189(1Met-Ox)	976.9160	2	1952.8242	1952.8226	-0.81
171-189(1Met-Ox,1CysO3)	1000.9070	2	2000.8062	2000.8073	0.56

Error
Average =1.09
σ = 0.74

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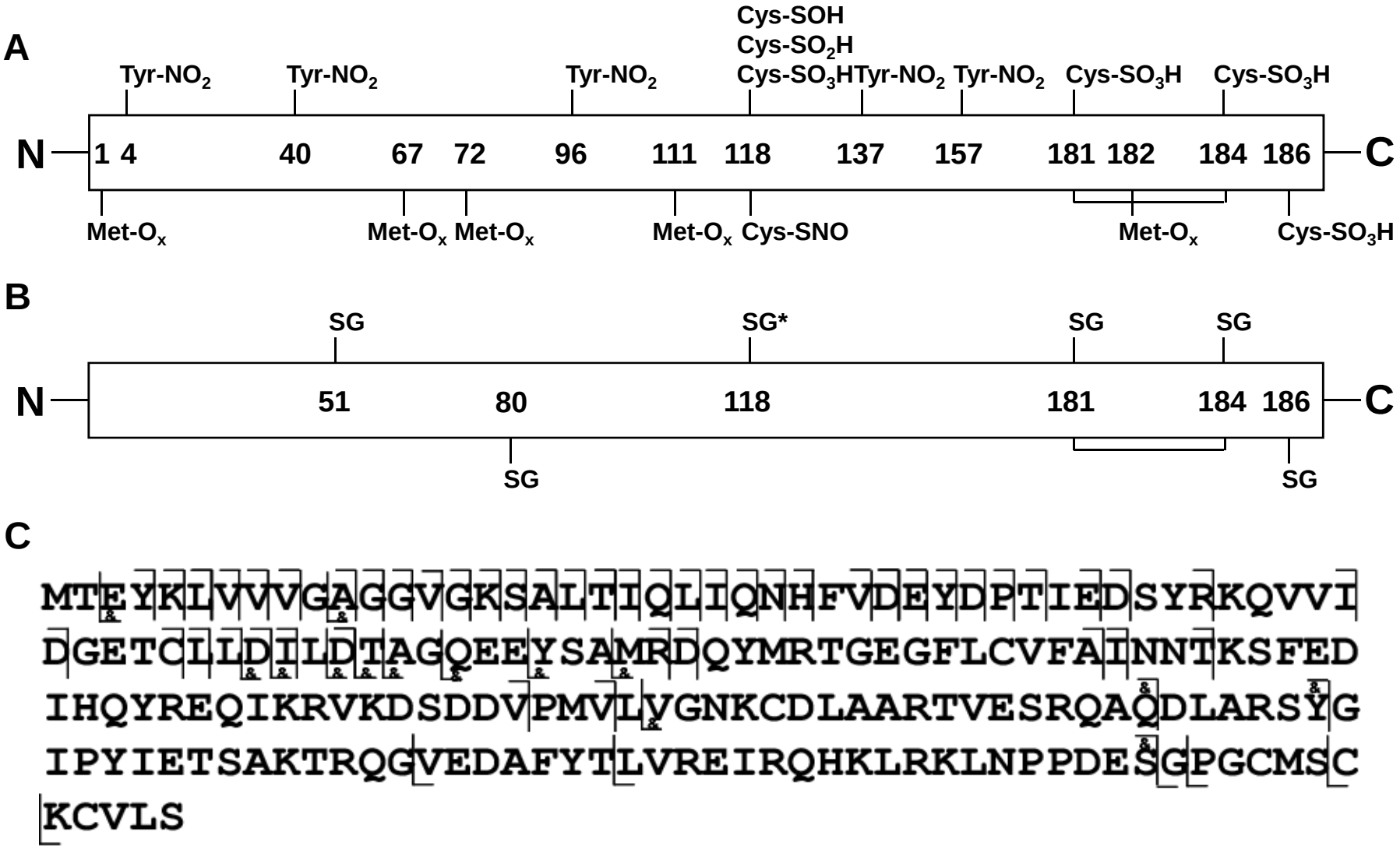
Summary of oxididative PTM's of p21Ras



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What information is in Mass Spectra?

1. Masses
   Useful for testing your theory of your chemical structure
2. Elemental compositions
   Or at least estimates thereof...
   Nitrogen Rule
3. Mixture Compositions
4. Abundances (quantitation)
5. Charge states
6. Mass differences (MS/MS)
   - Sequences (Proteins, peptides, polymers, DNA, etc)
   - Modifications to the sequence.....
   - Linkages (carbohydrates, lipids, hydrogen bonding, etc.
7. Fragment stabilities
   Breakdown curves yield relative (Net) transition state energies
8. **Higher order structure**
   - **Hydrogen/Deuterium Exchange (HDX) experiments**
   - Electron Capture/Transfer Dissociation
   - Rings plus Double Bonds

H/D exchange

- Solution phase
  - measurement of unfolding rates (D. Smith)
  - AA positional measurement of unfolding rates using NS-CAD-FTMS (I. Kaltashov)
- Gas phase
  - Cyt. C has different conformations in the gas phase (F. Mclafferty)

H/D Exchange of CRABP

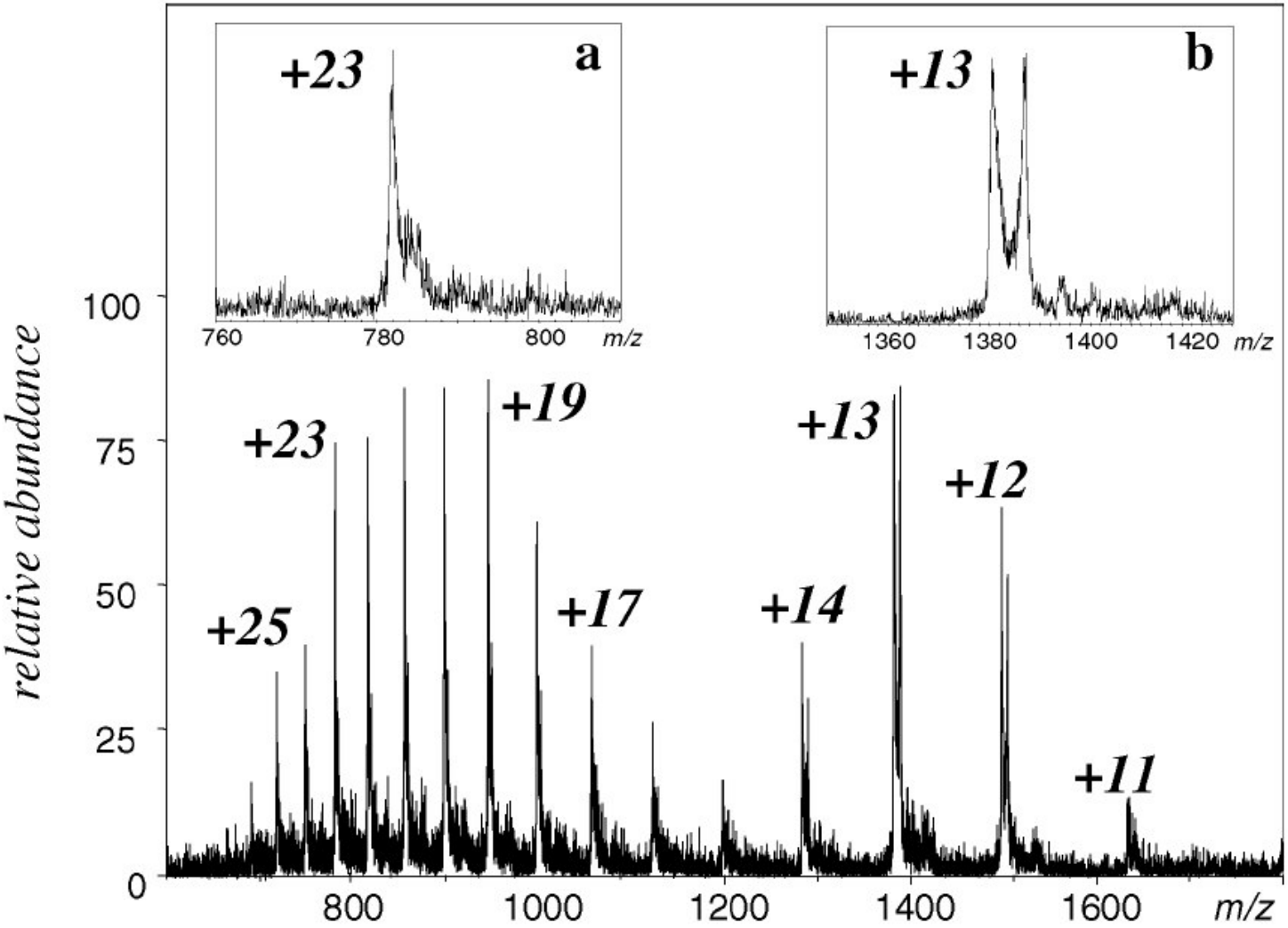


Figure 2. ESI time-of-flight mass spectrum of CRABP I-*d* acquired after 10 min of H–D isotope exchange in water–ethanol (9:1, v/v) solution. Insets: peak shapes of (a) high charge-state ions which have undergone complete exchange by this time point and (b) low charge density ions which exhibit a bimodal exchange pattern.

1.Eyles, S. J.; Dresch, T.; Gierasch, L. M.; Kaltashov, I. A. Unfolding dynamics of a beta-sheet protein studied by mass spectrometry *J. Mass Spectrom.* **1999**, *34*, 1289-1295.

MS/MS of CRABP during H/D back-exchange

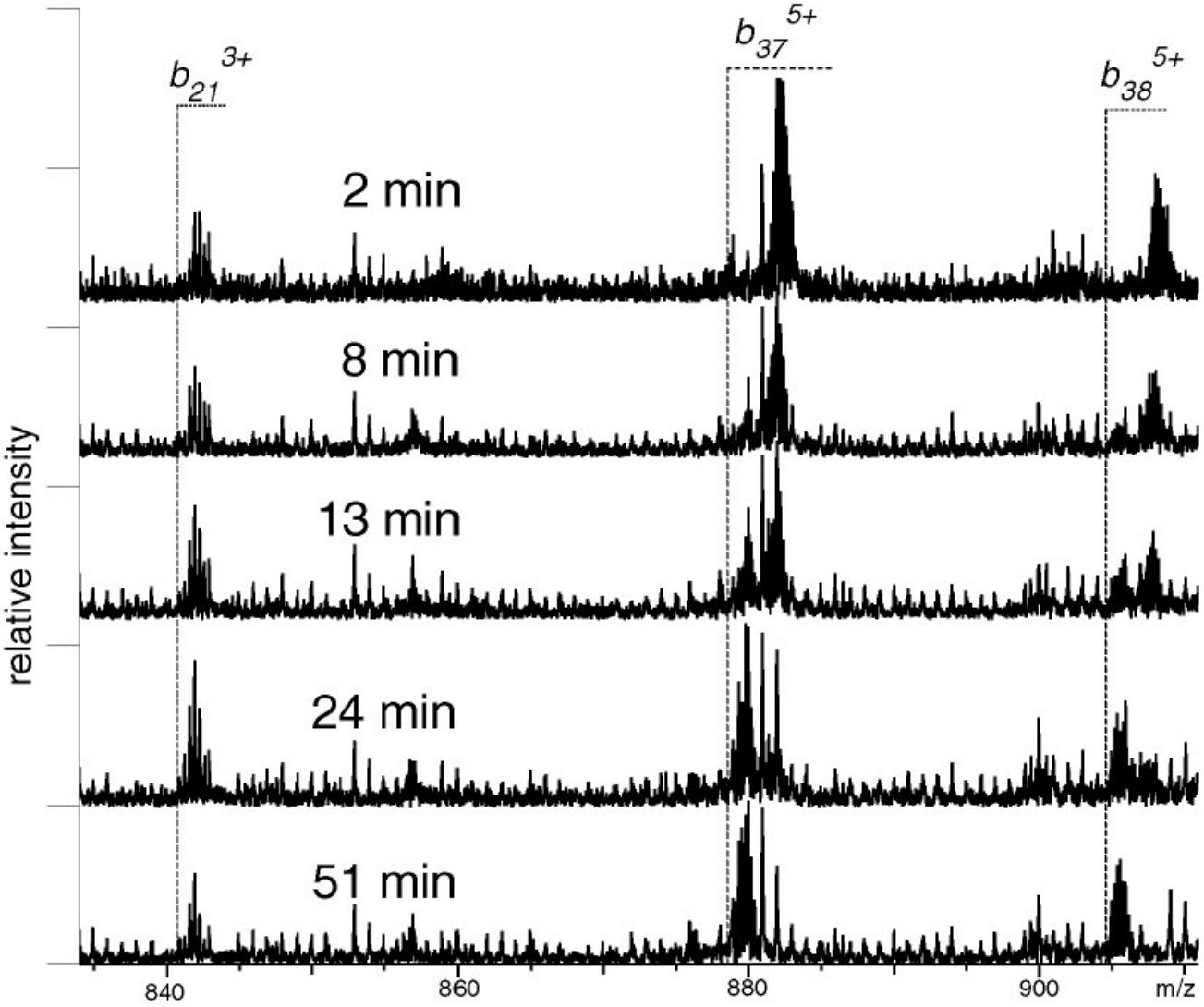


Figure 5. Time evolution of fragment ions peak from a CAD spectrum of multiply charged ions of CRABP I (in-source collisional activation). Charge state envelope of intact protein ions ranges from +11 to +14. The dashed line on the left of each isotopic cluster indicates the position of the fully exchanged fragment ion peak (monoisotopic mass).

1.Eyles, S. J.; Dresch, T.; Gierasch, L. M.; Kaltashov, I. A. Unfolding dynamics of a beta-sheet protein studied by mass spectrometry *J. Mass Spectrom.* **1999**, *34*, 1289-1295.

Melting Curves

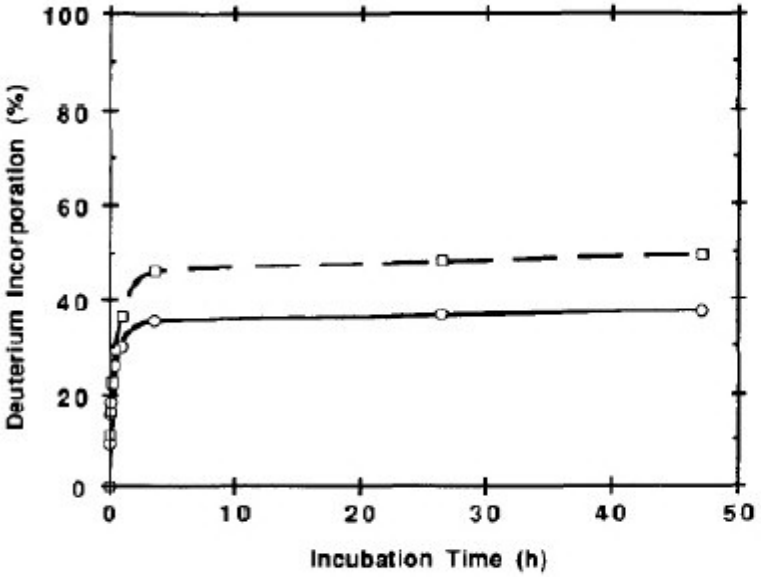


Fig. 4. Deuterium exchange into the 95–104 segment of cytochrome *c* (expressed as % of peptide amide positions deuterated) as a function of incubation time (pD 6.8; 25 °C, time = 1, 3, 8, 24, min/1, 3.6, 26.5, 47.1 h) before (○) and after (□) adjustment for deuterium gain/loss during digestion and analysis.

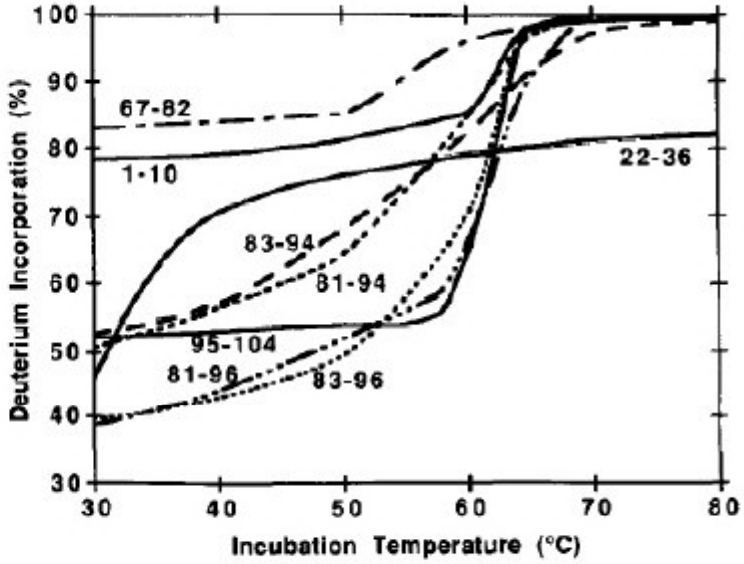


Fig. 5. Plot of deuterium incorporation into specific segments of cytochrome *c* as a function of the incubation temperature. The incubation time was adjusted to minimize the effect of temperature on the intrinsic rate of hydrogen exchange.

(1) Zhang, Z. Q.; Smith, D. L. Determination of amide hydrogen-exchange by mass-spectrometry - a new tool for protein-structure elucidation *Protein Science* **1993**, *2*, 522-531.

Detecting structural changes in viral capsids by hydrogen exchange and mass spectrometry

LINTAO WANG,[1] LESLIE C. LANE,[2] AND DAVID L. SMITH[1]

[1]Department of Chemistry, University of Nebraska–Lincoln, Lincoln, Nebraska 68588, USA
[2]Department of Plant Pathology, University of Nebraska–Lincoln, Lincoln, Nebraska 68588, USA

(RECEIVED January 2, 2001; FINAL REVISION March 14, 2001; ACCEPTED March 28, 2001)

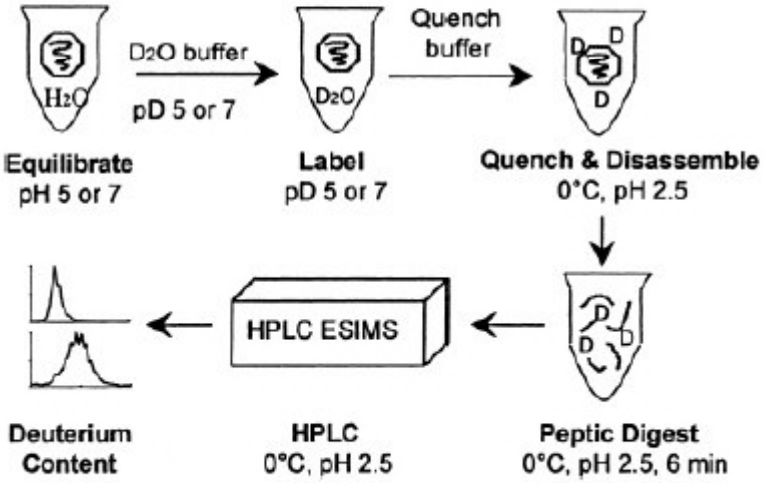


Fig. 2. General procedure used to label the BMV capsid protein in the intact viral particles and to determine deuterium levels at peptide amide linkages by HPLC ESI MS.

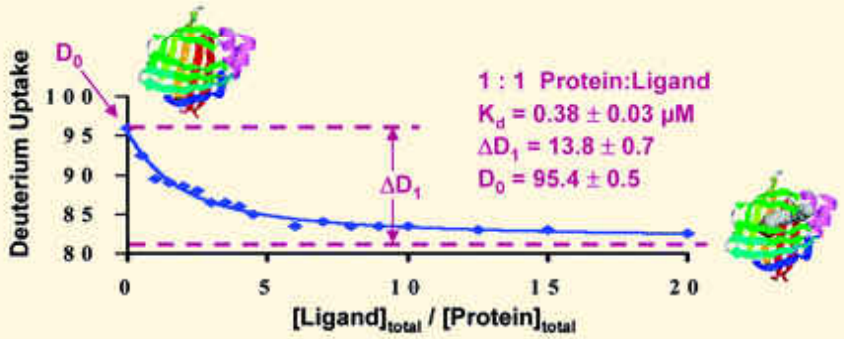
(1) Wang, L. T.; Lane, L. C.; Smith, D. L. Detecting structural changes in viral capsids by hydrogen exchange and mass spectrometry *Protein Science* **2001**, *10*, 1234-1243.

PLIMSTEX: Simple titration using deuterium incorporation as the readout

Quantification of Protein-Ligand Interactions by Mass Spectrometry, Titration, and H/D Exchange: PLIMSTEX

Mei M. Zhu, Don L. Rempel, Zhaohui Du, and Michael L. Gross

pp 5252 - 5253; **(Communication)** DOI: 10.1021/ja029460d



Gas phase HD exchange

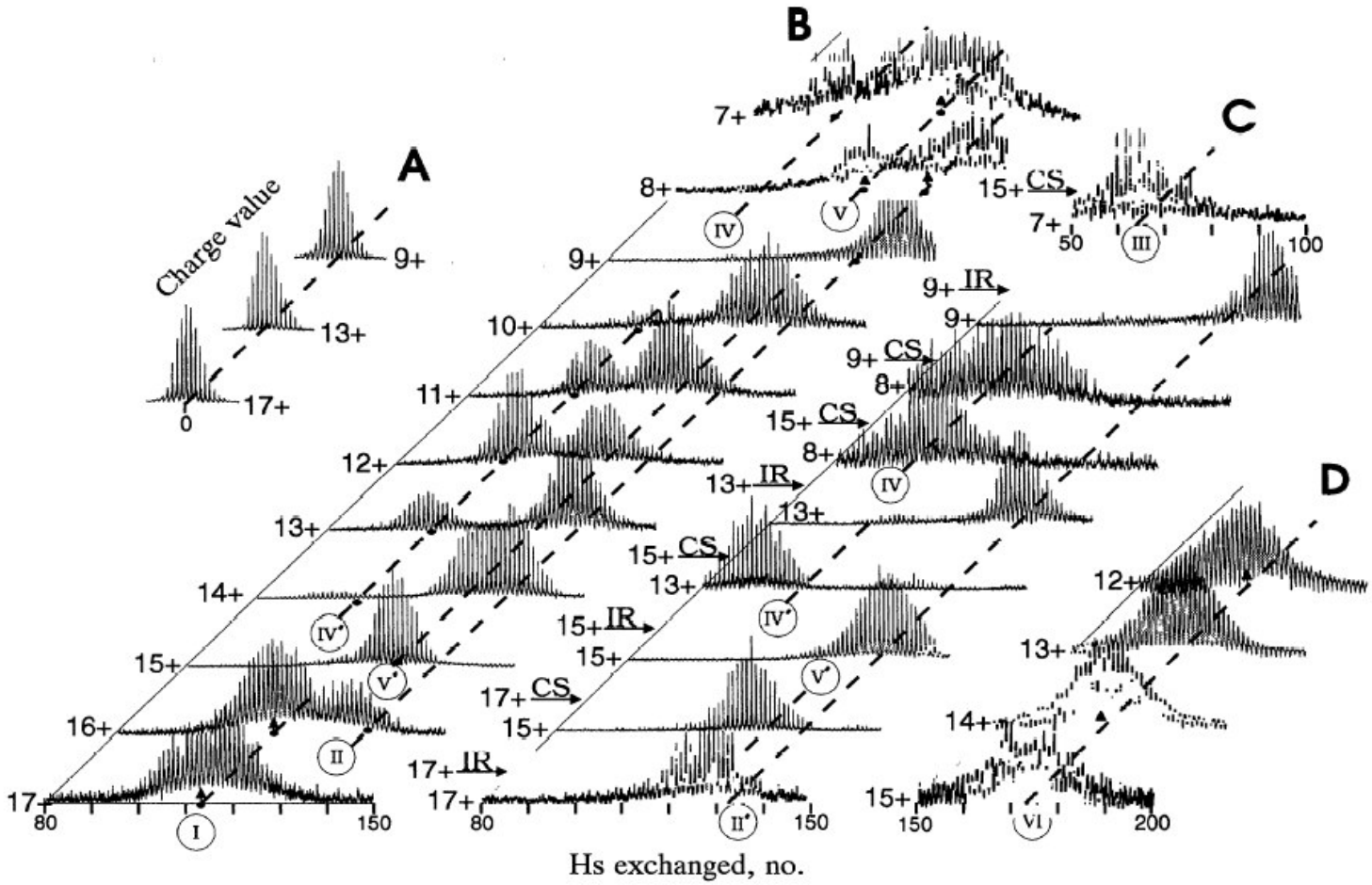


FIG. 1. Isotopic peak clusters of electrosprayed equine cytochrome *c*. (*A*) Typical precursor $(M + nH)^{n+}$ ions (most abundant isotopic peak contains seven ^{13}C atoms and ^{56}Fe). (*B*) After gaseous D_2O exchange. (*C*) After IR irradiation or charge-stripping (CS). (*D*) After quadrupolar axialization collisions.

1.Wood, T. D.; Chorush, R. A.; Wampler, F. M. I.; Little, D. P.; O'Connor, P. B.; McLafferty, F. W. Gas Phase Folding and Unfolding of Cytochrome c Cations *Proc. Nat. Acad. Sci. USA* **1995**, *92*, 2451.

Gas phase HD exchange

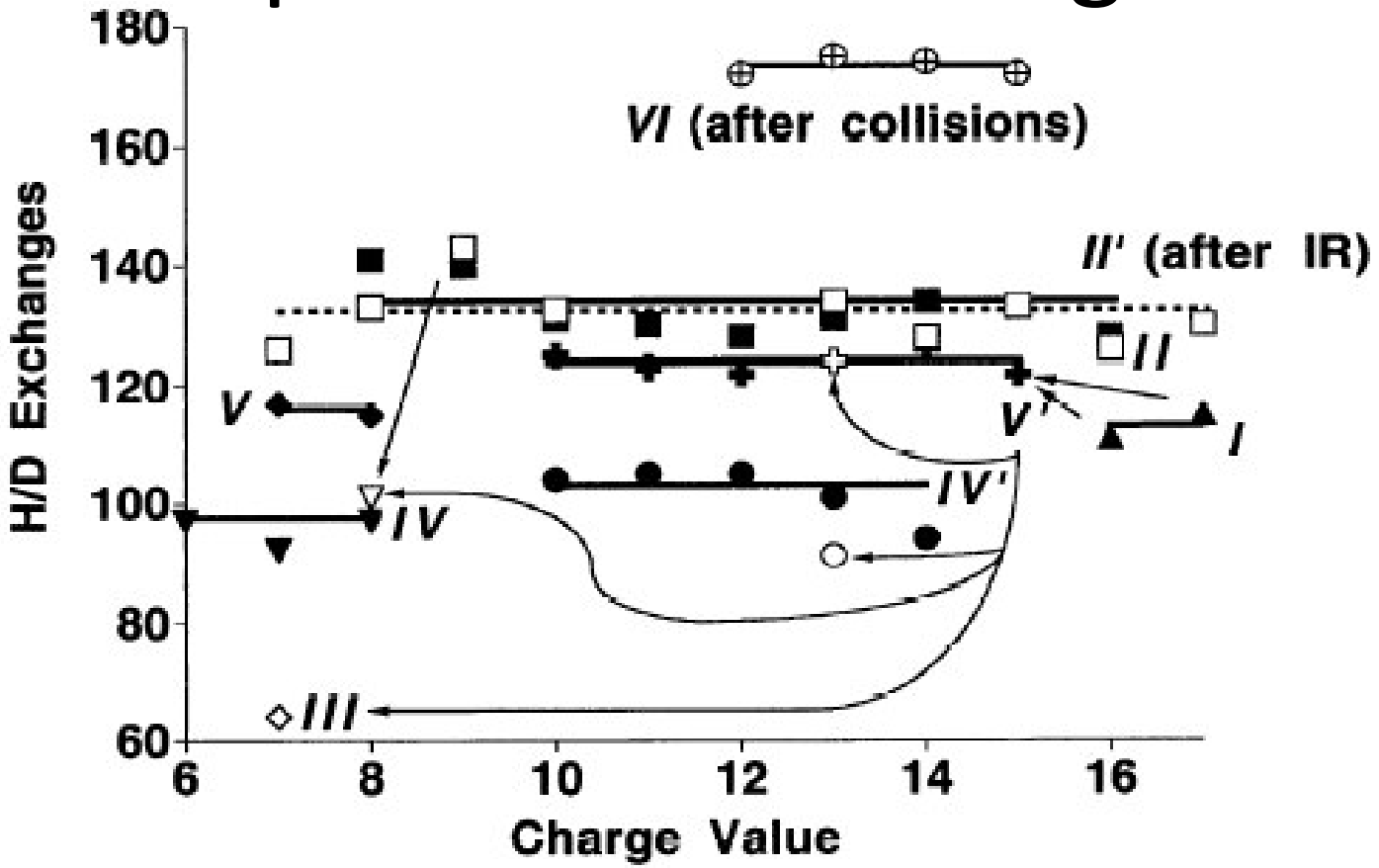


FIG. 2. H/D exchange levels (no. of H/D exchanges) vs. charge value. Solid symbols, states from electrosprayed ions; open symbols, states altered by irradiation, charge-stripping, or collisions.

1.Wood, T. D.; Chorush, R. A.; Wampler, F. M. I.; Little, D. P.; O'Connor, P. B.; McLafferty, F. W. Gas Phase Folding and Unfolding of Cytochrome c Cations *Proc. Nat. Acad. Sci. USA* **1995**, *92*, 2451.

Pitfalls to H/D exchange experiments

- Nothing is more hygroscopic than water (the backexchange problem)
- How much is water involved in protein folding?
- When performing MS/MS experiments on H/D exchanged proteins, is there proton scrambling?

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   Useful for testing your theory of your chemical structure
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   Or at least estimates thereof...
   Nitrogen Rule
3. Mixture Compositions
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   Breakdown curves yield relative (Net) transition state energies
8. Higher order structure
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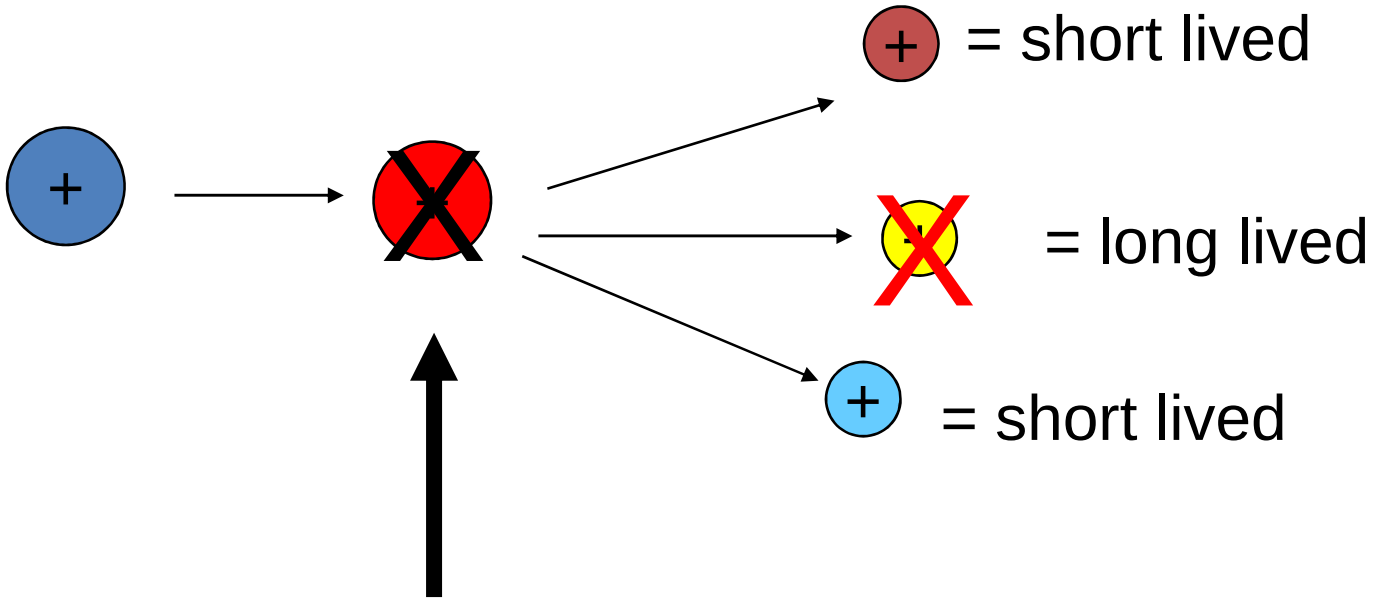
   - **Electron Capture/Transfer Dissociation**
   - Rings plus Double Bonds

Double Resonance

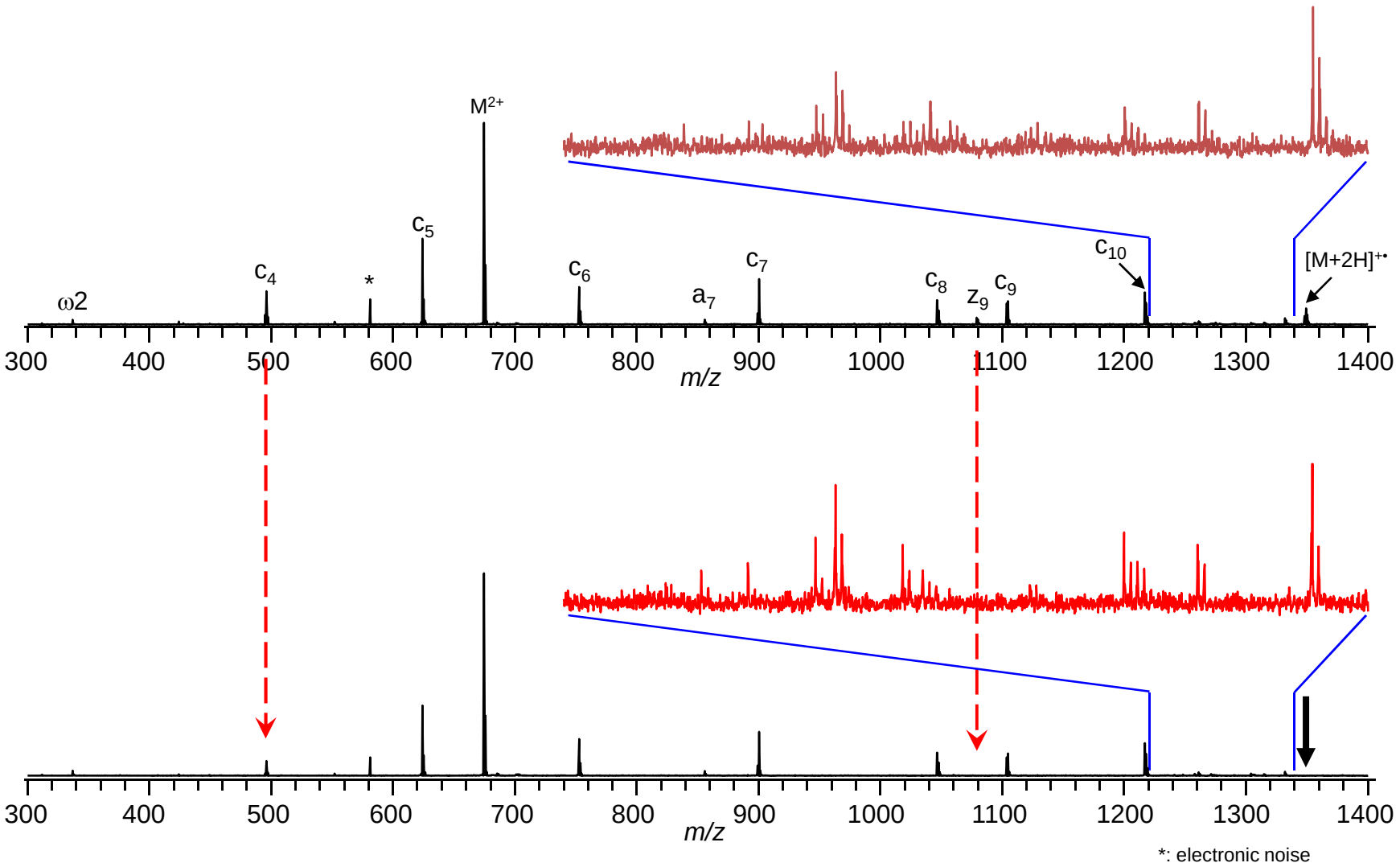


Resonantly Eject

Timeframe = 0.01 – 1 msec

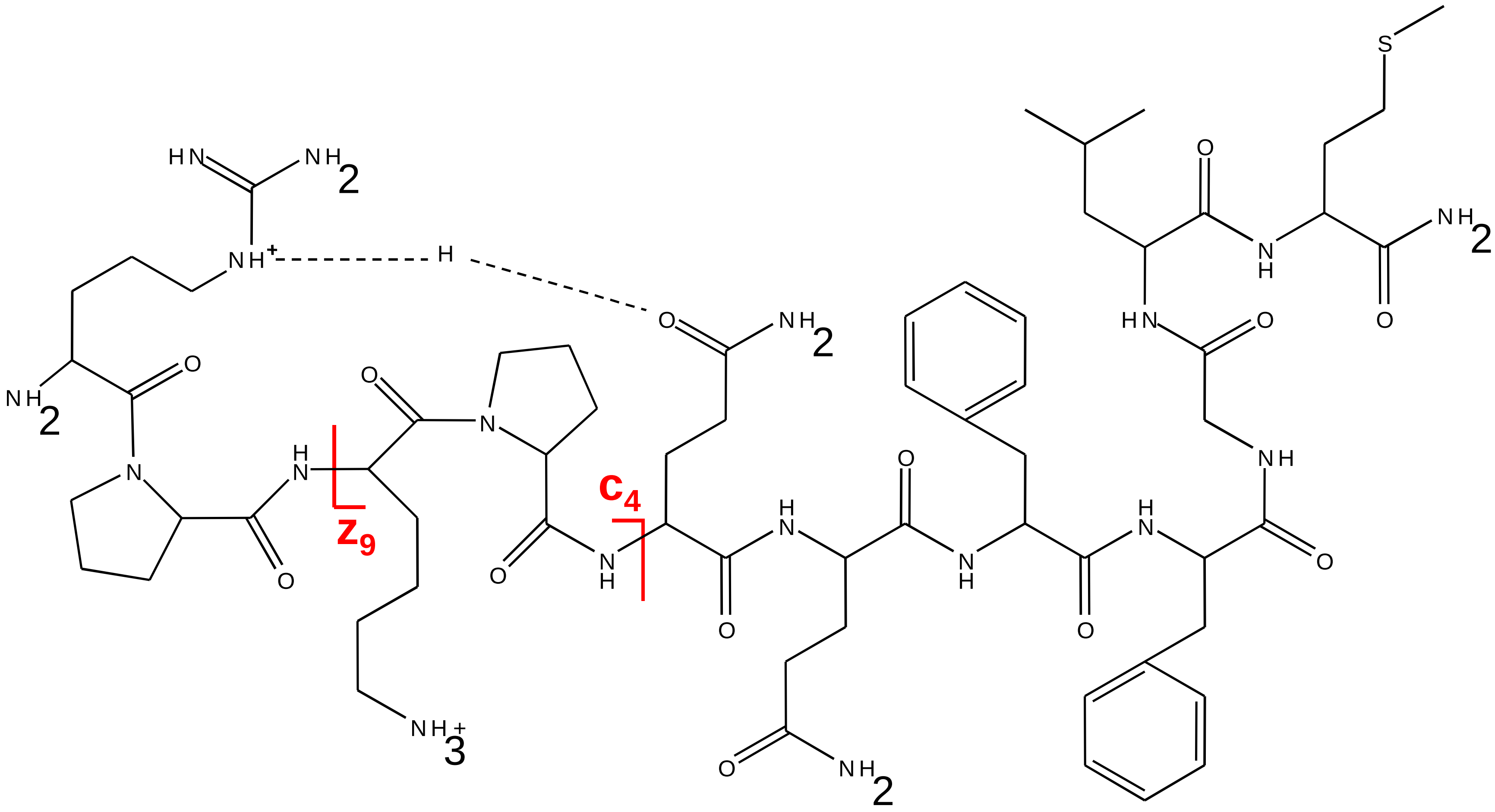
Substance P ECD

RPKPQQFFGLM-NH_2



*: electronic noise

Substance P



HN
NH2
NH+
H
O
NH2
O
NH2
N
H N
O
O
N
N H
O
c4
z9
NH3+
H N
O
N H
O
H N
O
O
NH2
S
O
N H
NH2
HN
O
O
NH

Glycans

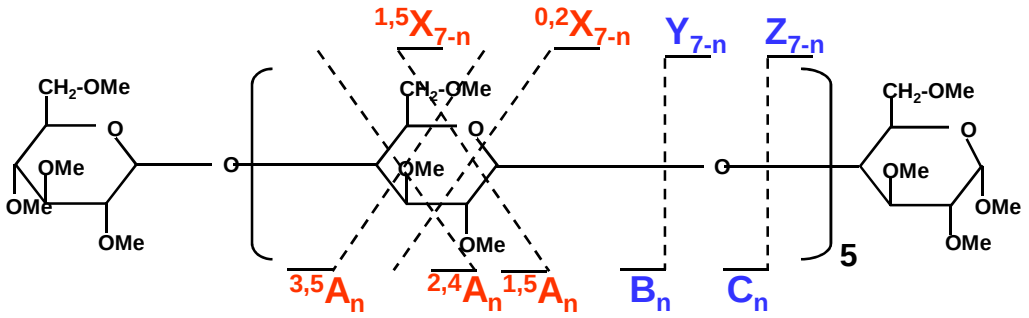
Supplemental: ESI FT-MS/MS (CAD) of a Permethylated Maltoheptaose

m/z of B_n=*m/z* of Z_n

m/z of C_n=*m/z* of Y_n

m/z of $^{0,2}X_n$=*m/z* of $^{2,4}A_{n+1}$

❖ **Symmetrical Structure**



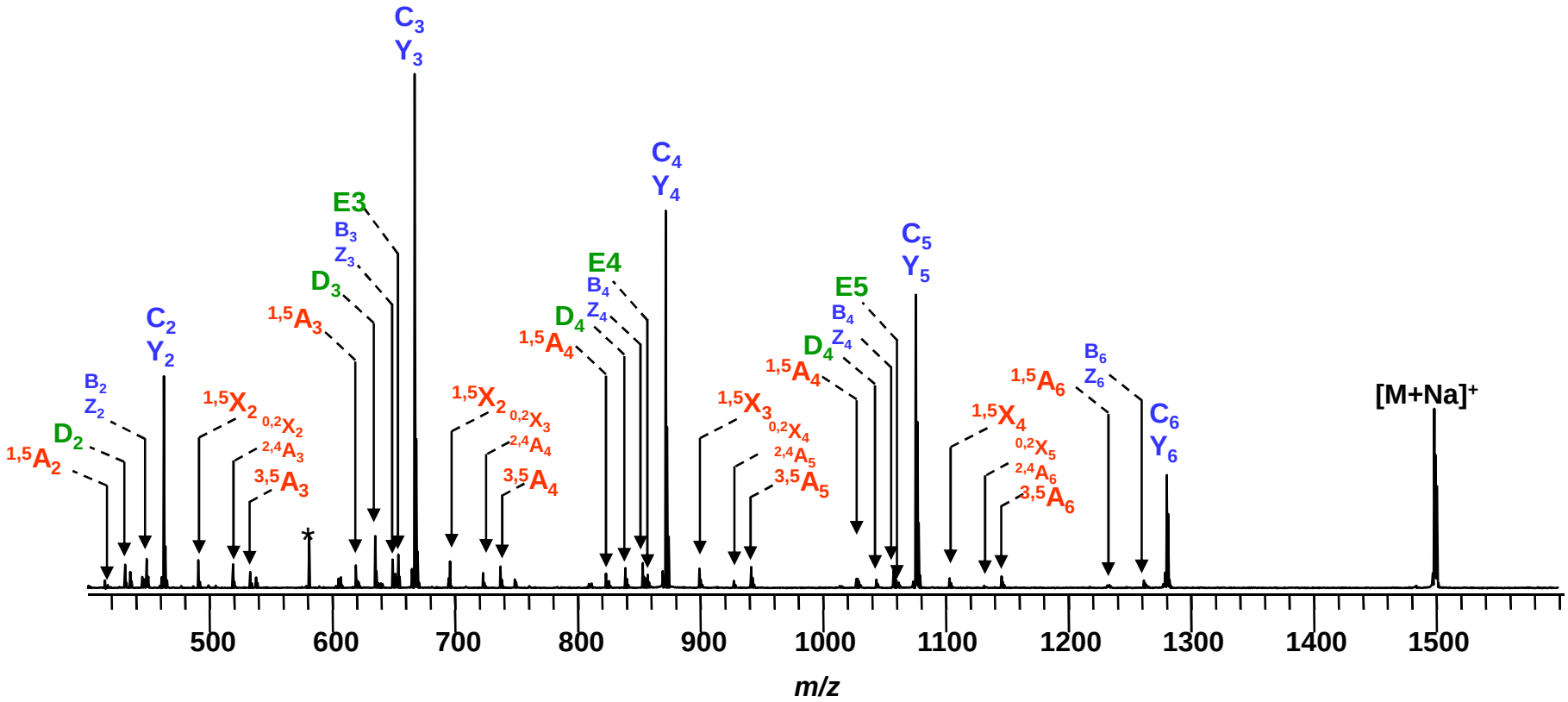


Fig.1 ESI FT-MS/MS of Reduced and Permethylated Maltoheptaose

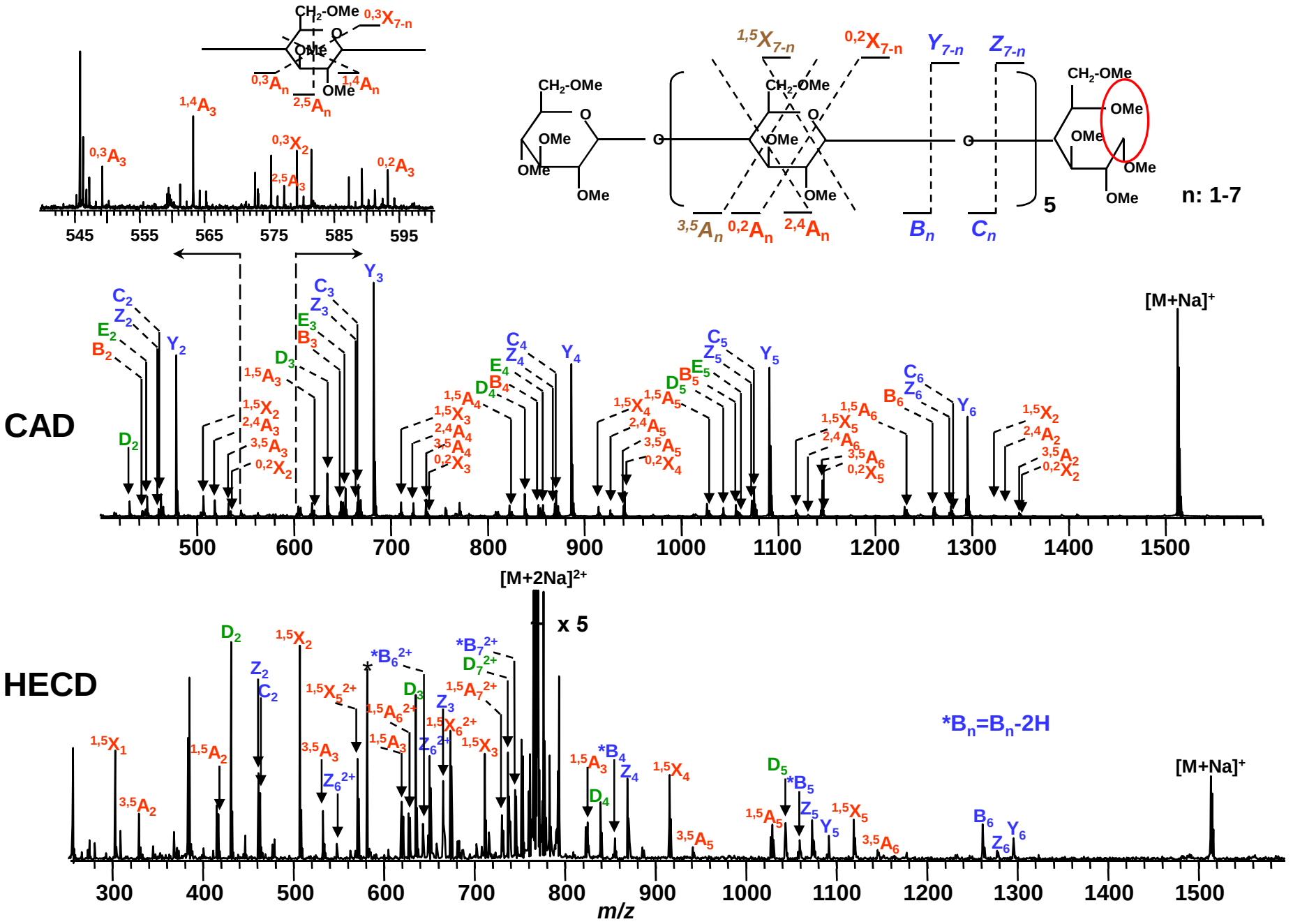
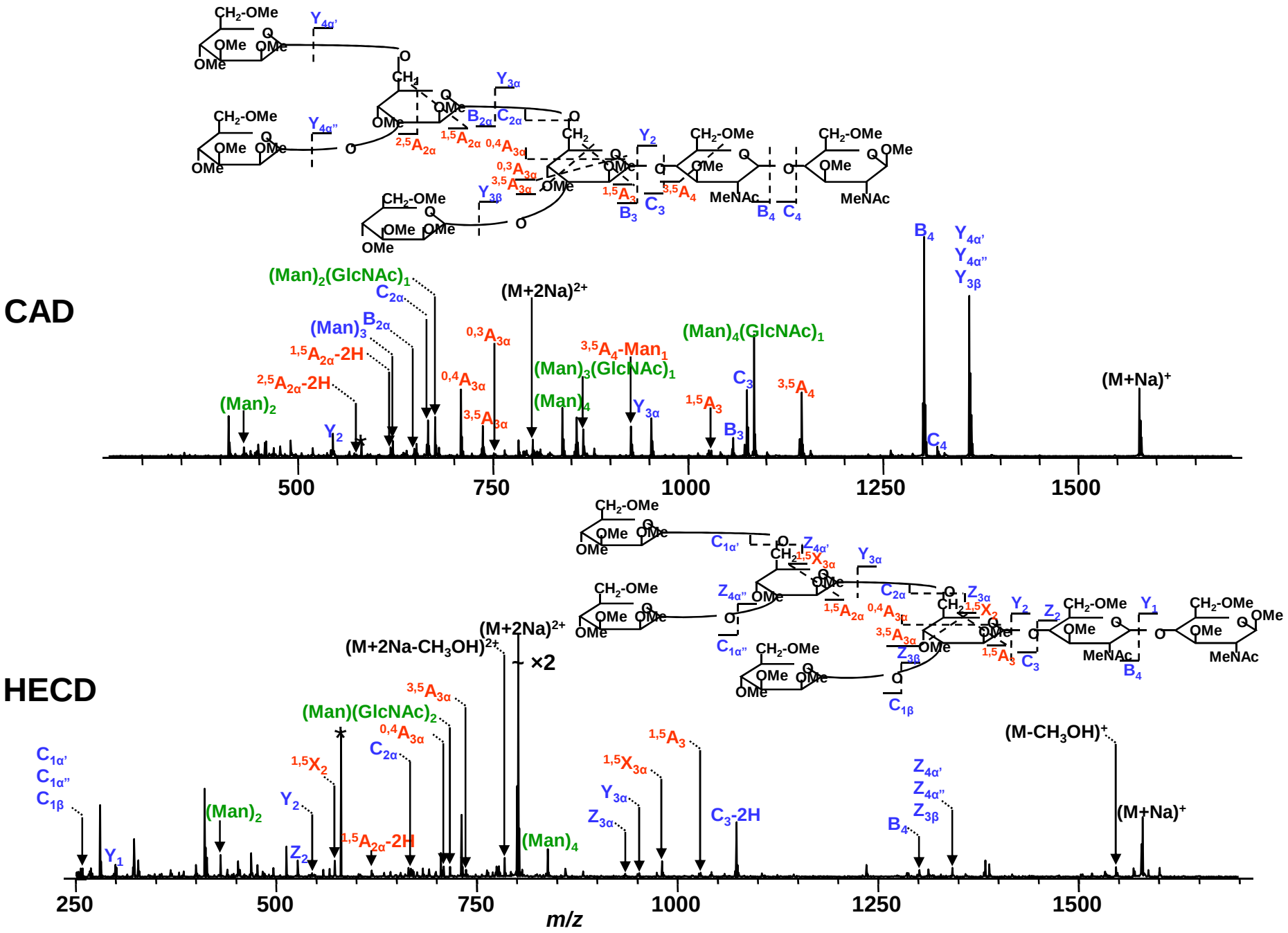


Fig. 2 ESI FT-MS/MS of Permethylated Man5 Glycan



Summary

Mass spectrometry is far more sensitive than other major analytical instruments (such as NMR, IR, etc).

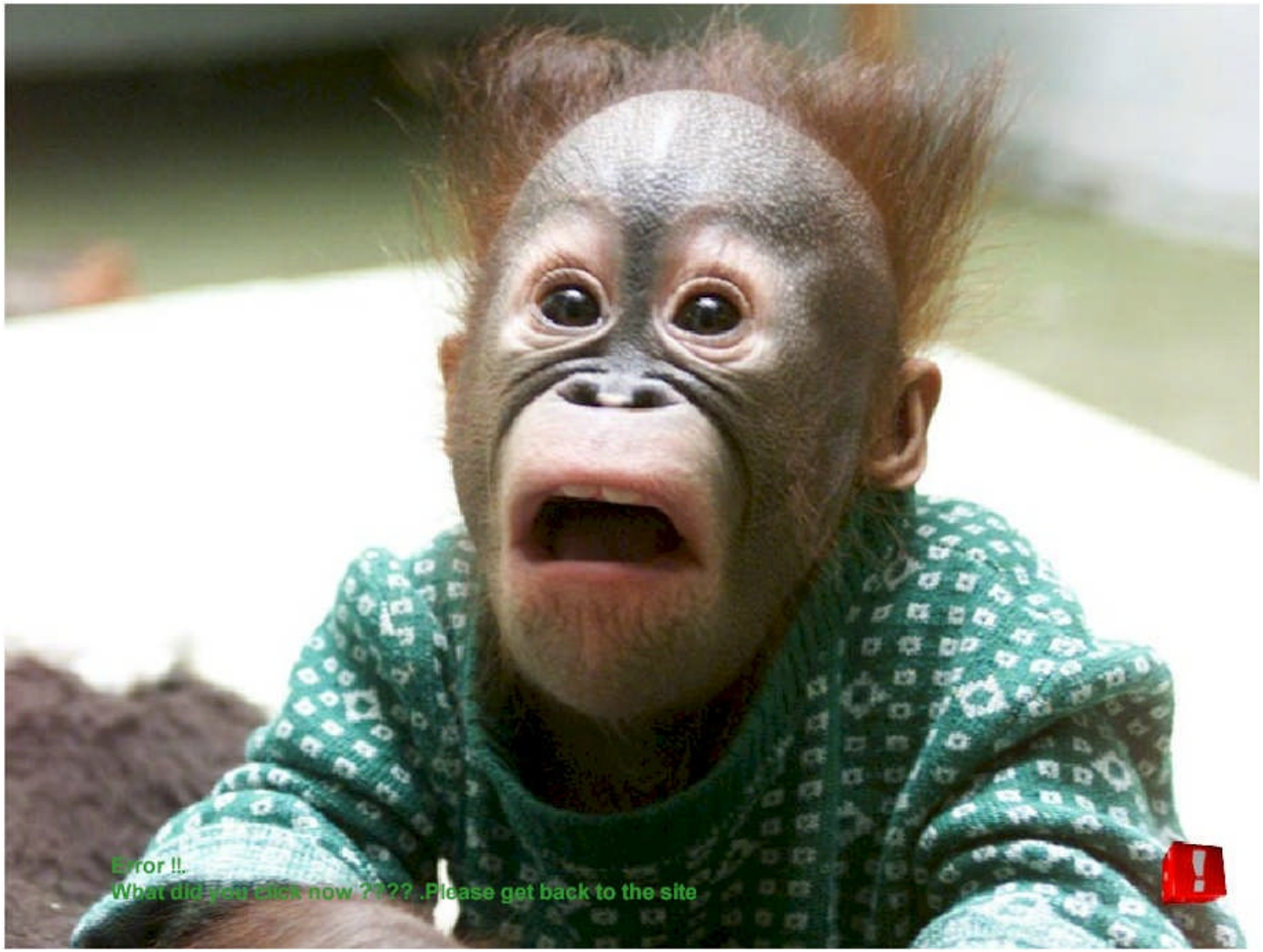
- so don't ever run spectra more concentrated than 10 μM !!!
- If you do, your spectra will be no better, and you'll just make the source dirty for everyone else.
- Almost always, if you can't see it, it is because the source is dirty, or the sample is heterogeneous or contaminated. MS detects what's there, not what you want to see.

Mass Spectrometry can determine sequence, branching linkages, post-translational modifications and sometimes higher-order structure of biomolecules (with femtomole sensitivity)

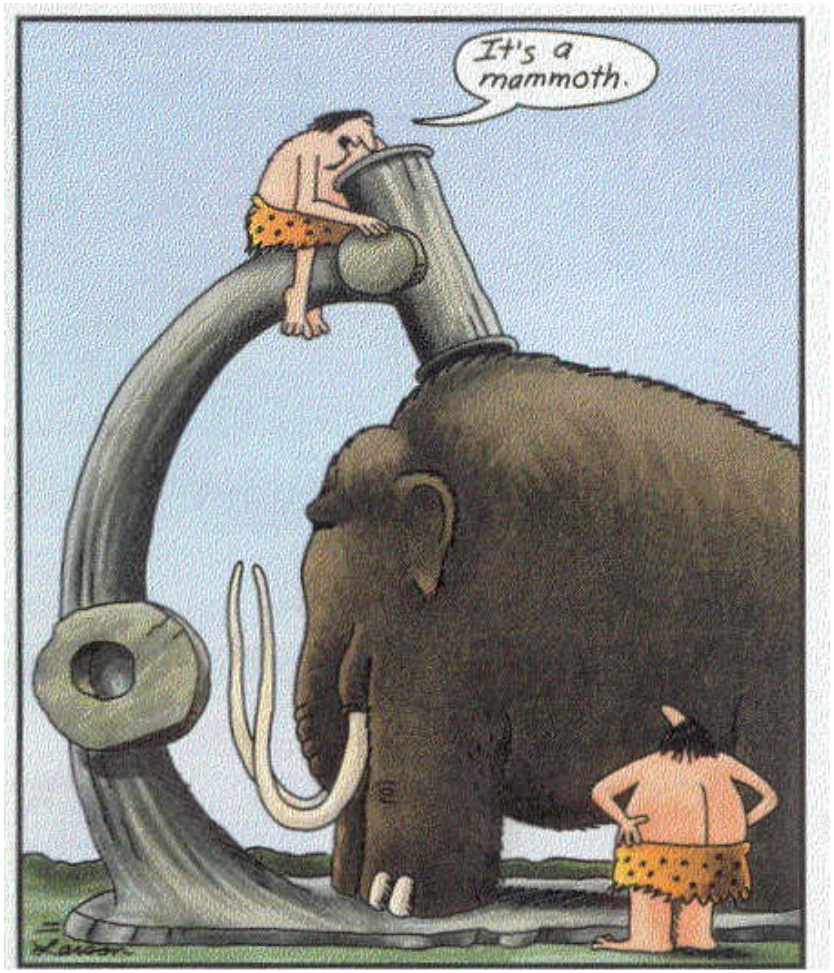
With enough resolution/accuracy, MS can determine the exact elemental composition.

Quantitation in MS is very difficult due to signal suppression effects, but can be done in a pinch.

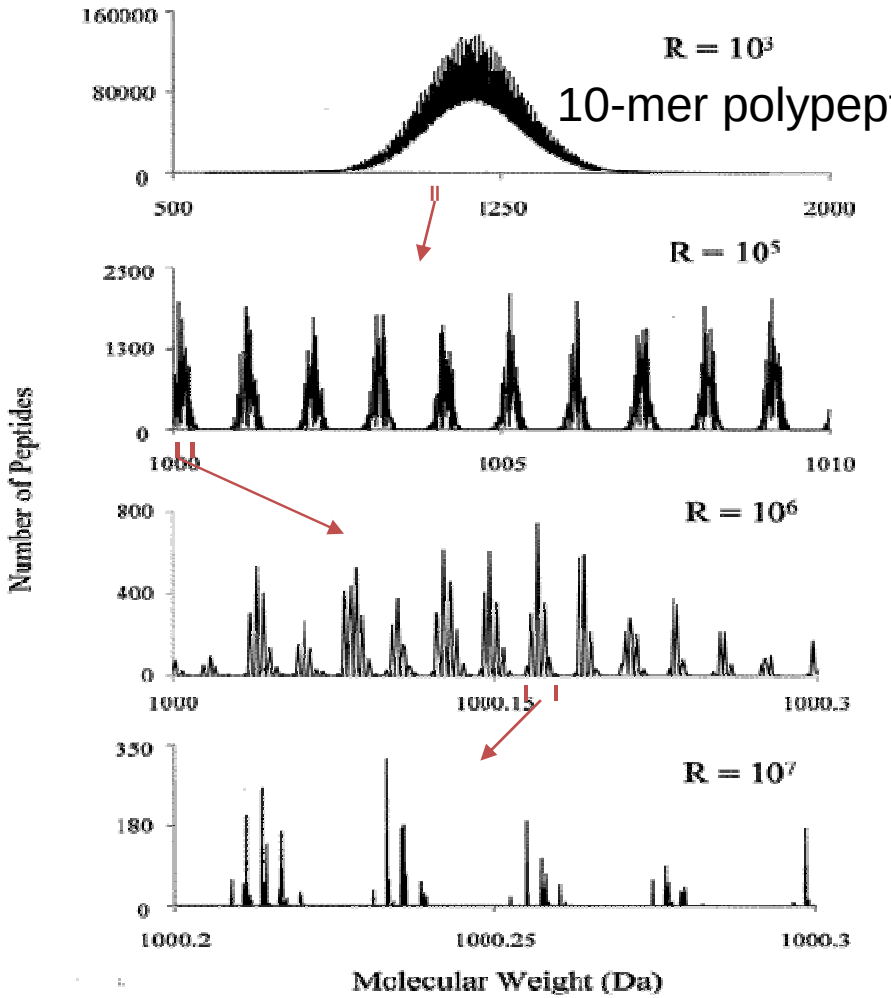
Fini... for now...



What is Molecular Mass?



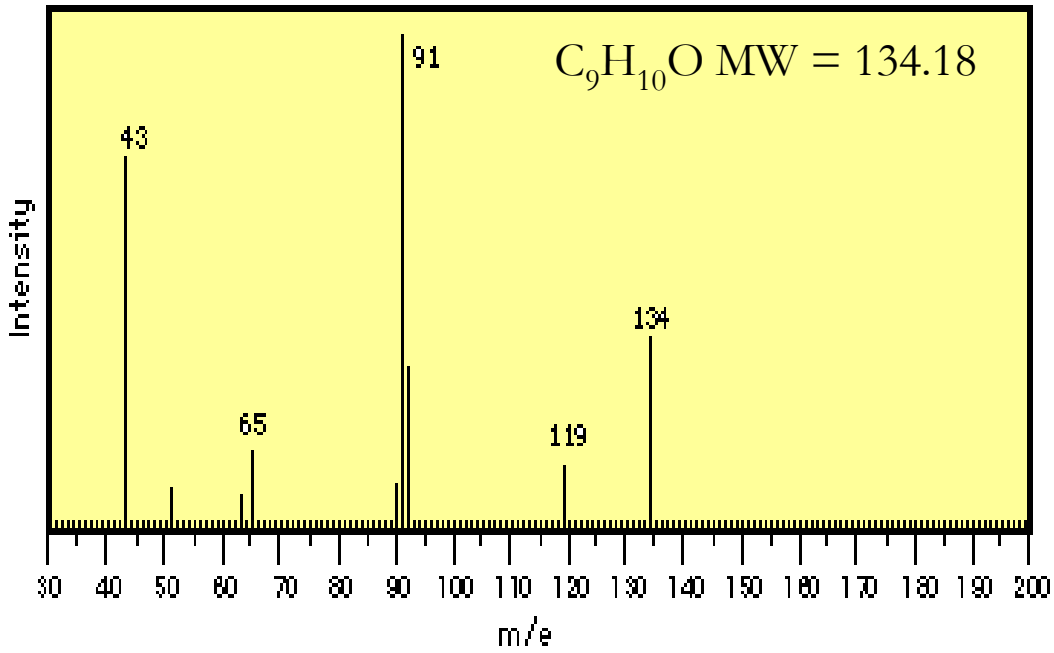
Conrads TP, Anderson GA, Veenstra TD, Pasa-Tolic L, Smith RD, Anal. Chem. 2000, *72,* 3349-3354.

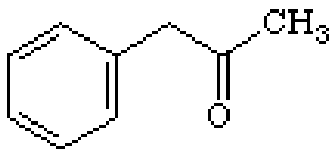


Practice Example 3

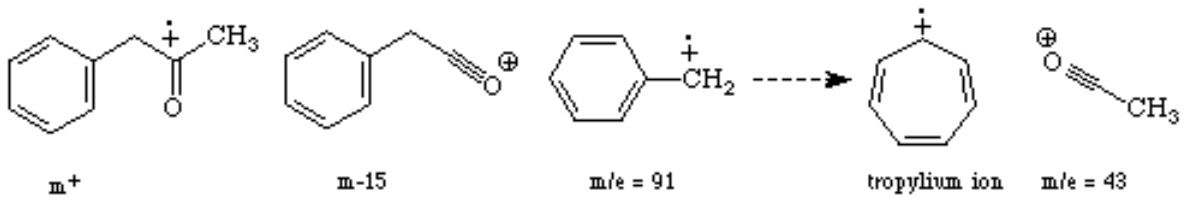
Peaks:

- m/z = 134 (molecular ion)
- m/z = 119 (m-15): labile CH_3
- m/z = 91 (m-43): base peak
  - indicative of benzyl cation
  - suggests loss of $CH_2CH_2CH_3$
- m/z = 65; loss of neutral acetylene from tropylium ion
- m/z = 43; intense
  - Suggests methyl ketone, which fragments to form acylium ion.





3-pentanone
(diethyl ketone)



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Calculating isotopic distributions using Microsoft Excel

1. For each heavy isotope calculate:
2. Number_s = current isotope index
3. Trials = total number of atoms of that element
4. P = natural abundance of that heavy isotope
5. Add the distributions of each element.

BINOMDIST

See Also

Returns the individual term binomial distribution probability. Use BINOMDIST in problems with a fixed number of tests or trials, when the outcomes of any trial are only success or failure, when trials are independent, and when the probability of success is constant throughout the experiment. For example, BINOMDIST can calculate the probability that two of the next three babies born are male.

Syntax

BINOMDIST(number_s,trials,probability_s,cumulative)

Number_s is the number of successes in trials.

Trials is the number of independent trials.

Probability_s is the probability of success on each trial.

Cumulative is a logical value that determines the form of the function. If cumulative is TRUE, then BINOMDIST returns the cumulative distribution function, which is the probability that there are at most number_s successes; if FALSE, it returns the probability mass function, which is the probability that there are number_s successes.

Remarks

- Number_s and trials are truncated to integers.
- If number_s, trials, or probability_s is nonnumeric, BINOMDIST returns the #VALUE! error value.
- If number_s < 0 or number_s > trials, BINOMDIST returns the #NUM! error value.
- If probability_s < 0 or probability_s > 1, BINOMDIST returns the #NUM! error value.
- The binomial probability mass function is:

$$b(x;n,p) = \binom{n}{x} p^x (1-p)^{n-x}$$

where:

$$\binom{n}{x}$$

is COMBIN(n,x).

The cumulative binomial distribution is:

$$B(x;n,p) = \sum_{y=0}^{x} b(y;n,p)$$

Example

The example may be easier to understand if you copy it to a blank worksheet.

How?

	A	B
1	**Data**	**Description**
2	6	Number of successes in trials
3	10	Number of independent trials
4	0.5	Probability of success on each trial
	Formula	**Description (Result)**
	=BINOMDIST(A2,A3,A4,FALSE)	Probability of exactly 6 of 10 trials being successful (0.205078)

COMBIN

See Also

Returns the number of combinations for a given number of items. Use COMBIN to determine the total possible number of groups for a given number of items.

Syntax

COMBIN(number,number_chosen)

Number is the number of items.

Number chosen is the number of items in each combination.

Remarks

- Numeric arguments are truncated to integers.
- If either argument is nonnumeric, COMBIN returns the #VALUE! error value.
- If number < 0, number_chosen < 0, or number < number_chosen, COMBIN returns the #NUM! error value.
- A combination is any set or subset of items, regardless of their internal order. Combinations are distinct from permutations, for which the internal order is significant.
- The number of combinations is as follows, where number = n and number_chosen = k:

$$\binom{n}{k} = \frac{P_{k,n}}{k!} = \frac{n!}{k!(n-k)!}$$

where:

$$P_{k,n} = \frac{n!}{(n-k)!}$$

Example

The example may be easier to understand if you copy it to a blank worksheet.

▶ How?

	A	B
1	**Formula**	**Description (Result)**
2	=COMBIN(8,2)	Possible two-person teams that can be formed from 8 candidates (28)

Odd vs. Even Electron Fragmentation

- Even electron = proton rearrangements
- Odd electron = radical rearrangements

- Non-ergodic fragmentation = FAST!!

J. Am. Chem. Soc. **1998**, *120*, 3265−3266

Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process

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

Received October 6, 1997

Neutralization-reionization mass spectrometry (MS)[1] is of unique value for preparing and characterizing highly reactive and unstable neutral species, such as the intermediate in the dissociative−recombination reaction $H_3O^+ + e^- \rightarrow H_2O + H + 6.4$ eV.[2] Following an earlier suggestion,[3] using neutralization accompanying surface-induced dissociation (SID)[4] to form an unstable site did not yield new cleavage reactions[5] in multiply charged protein

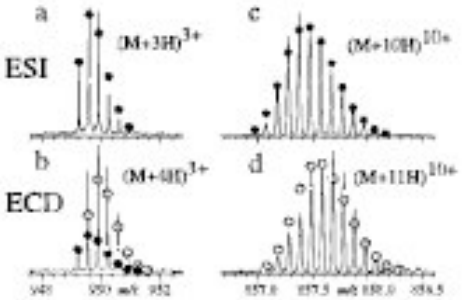


Figure 1. Isotopic distributions of (a, b) melittin 3+ and (c, d) ubiquitin 10+ ions obtained by (a, c) ESI and (b, d) ECD. Closed and open circles, theoretically predicted isotopic abundance distributions for $(M + nH)^{n+}$ and $(M + nH)^{n-1+}$, respectively;[6] those of 1b should sum to the measured abundances.

these are mainly $(M + 11H)^{10+}$ ions (Figure 1d), 1 Da heavier

Some Typical EI spectra of lipids

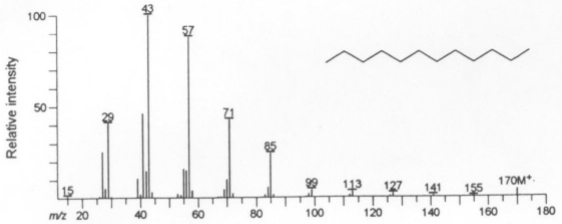


Figure 3.2. Mass spectrum of dodecane.

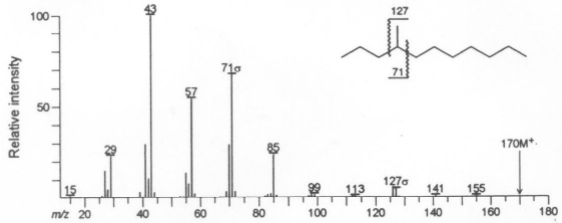


Figure 3.3. Mass spectrum of 4-methylundecane.

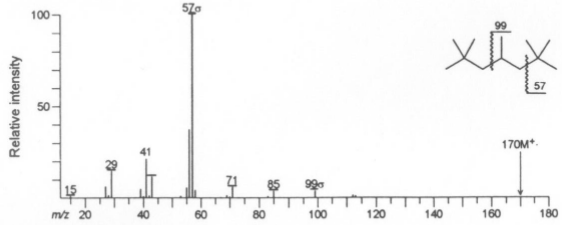


Figure 3.4. Mass spectrum of 2,2,4,6,6-pentamethylheptane.

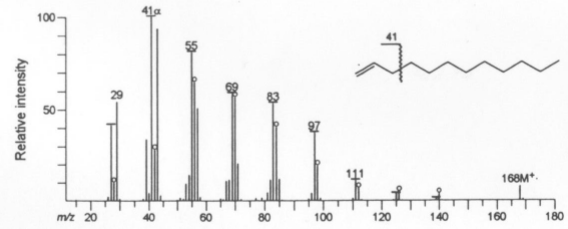


Figure 3.5. Mass spectrum of 1-dodecene.

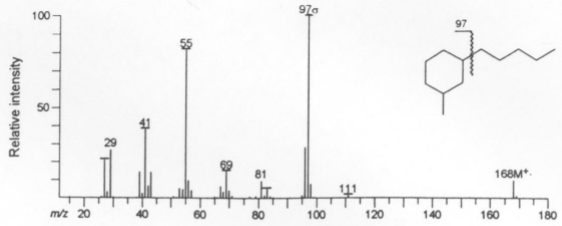


Figure 3.6. Mass spectrum of 1-methyl-3-pentylcyclohexane.

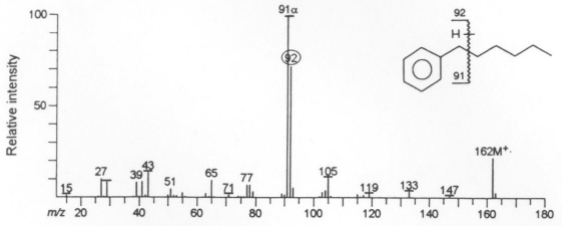


Figure 3.7. Mass spectrum of 1-phenylhexane.

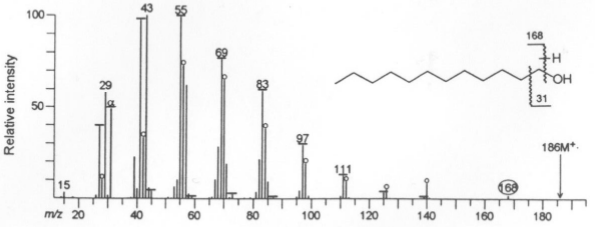


Figure 3.8. Mass spectrum of 1-dodecanol.

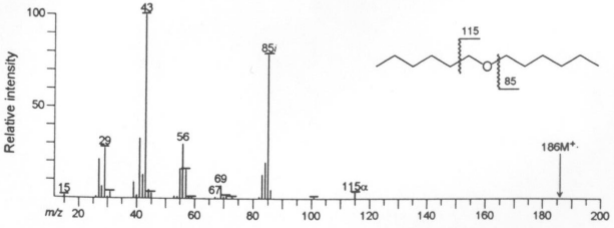
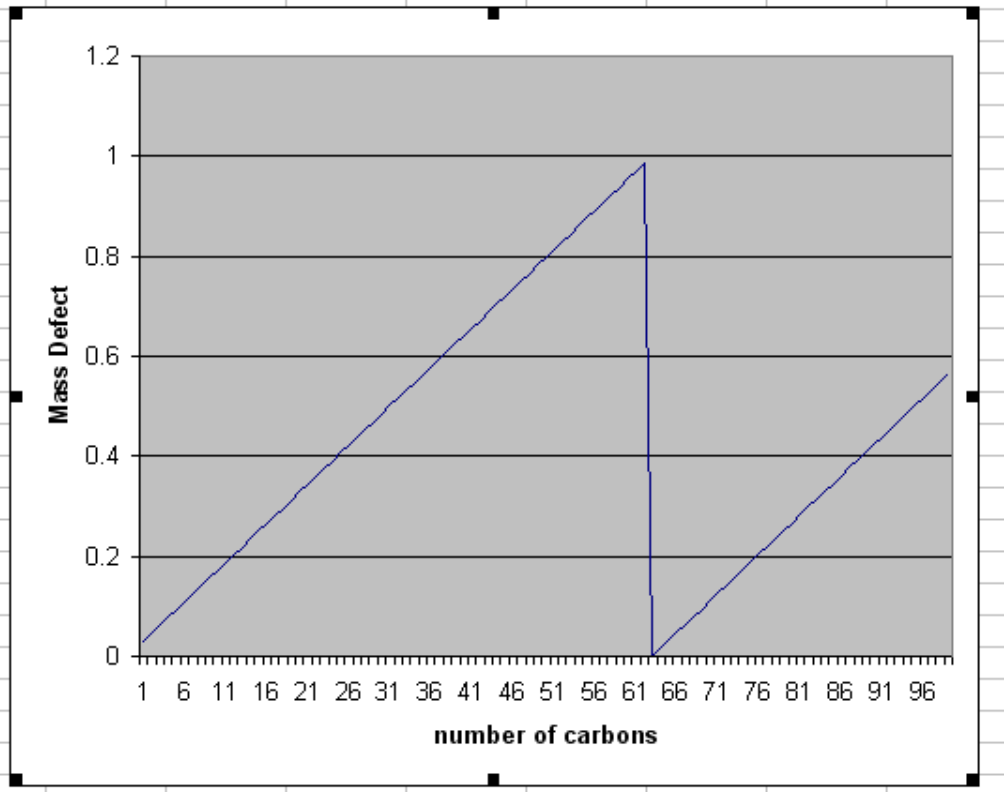


Figure 3.9. Mass spectrum of hexyl ether.

Mass Defect plot for Hydrocarbons

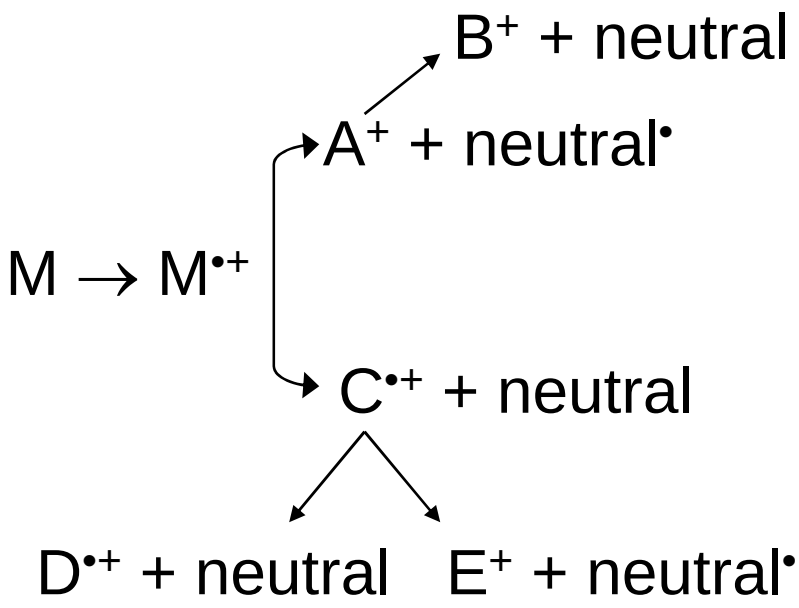
carbon	12		
hydrogen	1.007825		
hydrocarbons			
c	h	mass	mass defect
1	4	16.0313	0.0313
2	6	30.04695	0.04695
3	8	44.0626	0.0626
4	10	58.07825	0.07825
5	12	72.0939	0.0939
6	14	86.10955	0.10955
7	16	100.1252	0.1252
8	18	114.1409	0.14085
9	20	128.1565	0.1565
10	22	142.1722	0.17215
11	24	156.1878	0.1878
12	26	170.2035	0.20345
13	28	184.2191	0.2191
14	30	198.2348	0.23475
15	32	212.2504	0.2504
16	34	226.2661	0.26605
17	36	240.2817	0.2817
18	38	254.2974	0.29735
19	40	268.313	0.313
20	42	282.3287	0.32865
21	44	296.3443	0.3443
22	46	310.36	0.35995
23	48	324.3756	0.3756
24	50	338.3913	0.39125
25	52	352.4069	0.4069
26	54	366.4226	0.42255
27	56	380.4382	0.4382



Structural information from EI

- EI can cause fragmentation
- Fragmentation follows regular chemical rules

- Molecular ion has unpaired *e*
  - radical cation, $^{\bullet}$
  - can lose radical (A^+)
  - can form another rad. cation ($C^{\bullet+}$)
  - don't see neutral fragments



EI fragmentation rules

1. Many molecules are too fragile under EI for a molecular ion to be observed.

2. The radical is formed on the heteroatom (if it exists) and undergoes radical chemistry from there.

3. The intense peaks are usually due to the radical being stuck at a particularly stable site

   For example: for branched alkanes, 1° < 2° < 3°

Commonly seen masses

Commonly Lost Fragments

m-15 $\cdot CH_3$

m-17 $\cdot OH$

m-26 $\cdot CN$

m-28 $H_2C{=}CH_2$

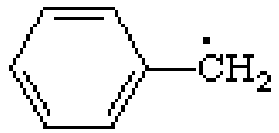
m-29 $\cdot CH_2CH_3$ $\cdot CHO$

m-31 $\cdot OCH_3$

m-35 $\cdot Cl$

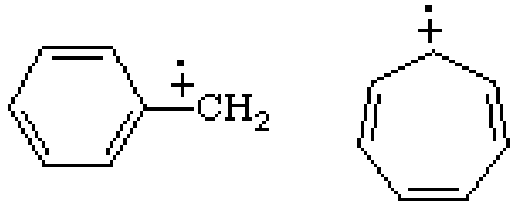
m-43 $CH_3\dot{C}{=}O$

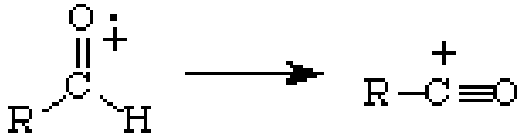
m-45 $\cdot OCH_2CH_3$

m-91 

Common Stable Ions

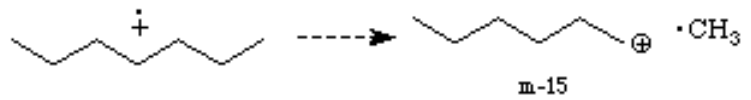
m/e = 43 $CH_3{-}C{\equiv}O^+$

m/e = 91 

m/e = m-1 

Common fragment patterns

- **Alkanes**: tend to undergo fragmentation by initial loss of a methyl group to form a (m-15) species.
  - Weakening of C-C bonds by loss of electrons (C-H doesn't fragment)
  - Stepwise cleavage of carbocation down alkyl chain, expelling neutral two-carbon units (ethylene).
  - Branched hydrocarbons form more stable secondary and tertiary carbocations
    - May not see molecular ion at all



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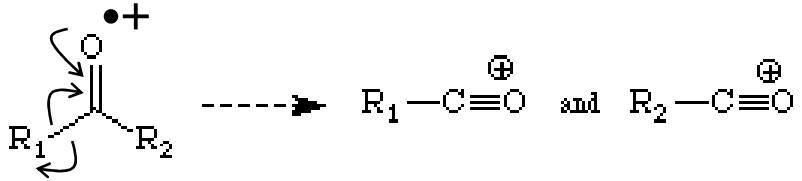
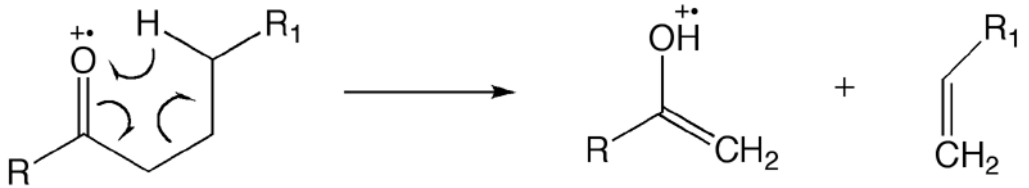
Common fragment patterns

- **Heteroatom cleavage:** (O, S, N, X) have lone pair; radical cation localized on heteroatom, get cleavage of b bonds
- **Aldehydes/ketones (carbonyls)**: predominate cleavage is loss of one of the side-chains to generate the substituted oxonium ion.



  - Very favorable cleavage (ion is often base peak)
  - methyl derivative (CH_3CO^+) is called "acylium ion" (m/z = 43).

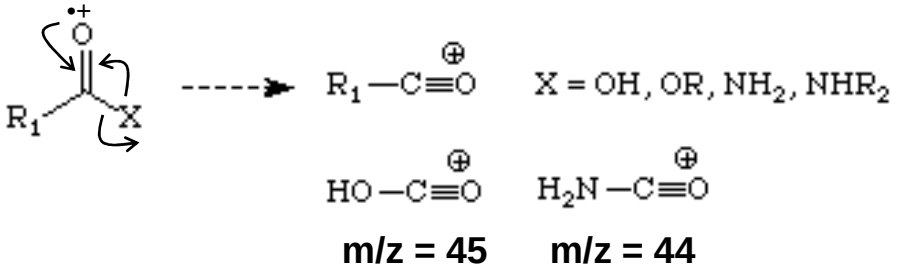
- *McLafferty rearrangement:* expulsion of neutral alkene



e.g. Identify substituents on two halves of ketone

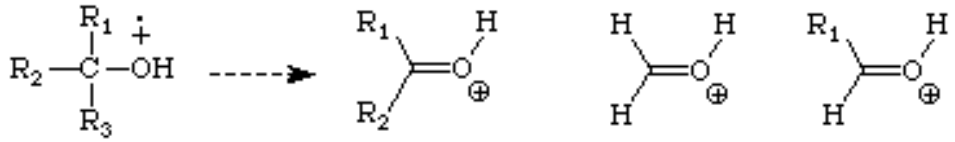
Common fragment patterns

- **Esters, Acids and Amides**: As with ketones etc, major cleavage involves expulsion of the "X" group to form substituted oxonium ion.



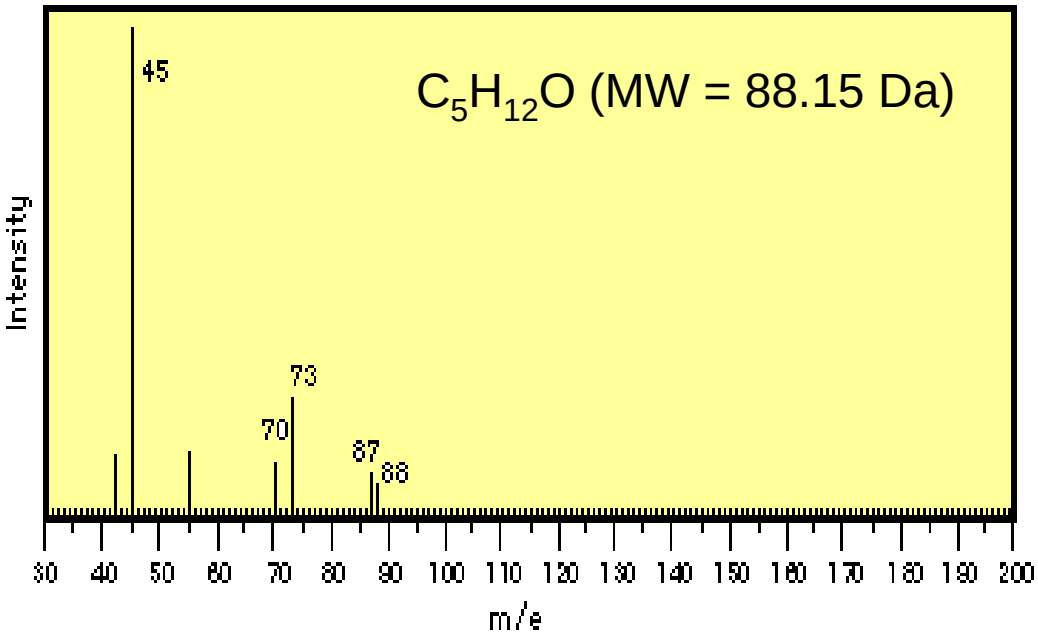
  - For carboxylic acids and unsubstituted amides, peaks at m/z = 45 and 44 are also often observed.

- **Alcohols**: Usually lose a proton (m-1) and hydroxy radical (m-17), H_2O (m-18) & an alkyl group to form oxonium ions (base peak). 3° alcohols almost never show molec. ion



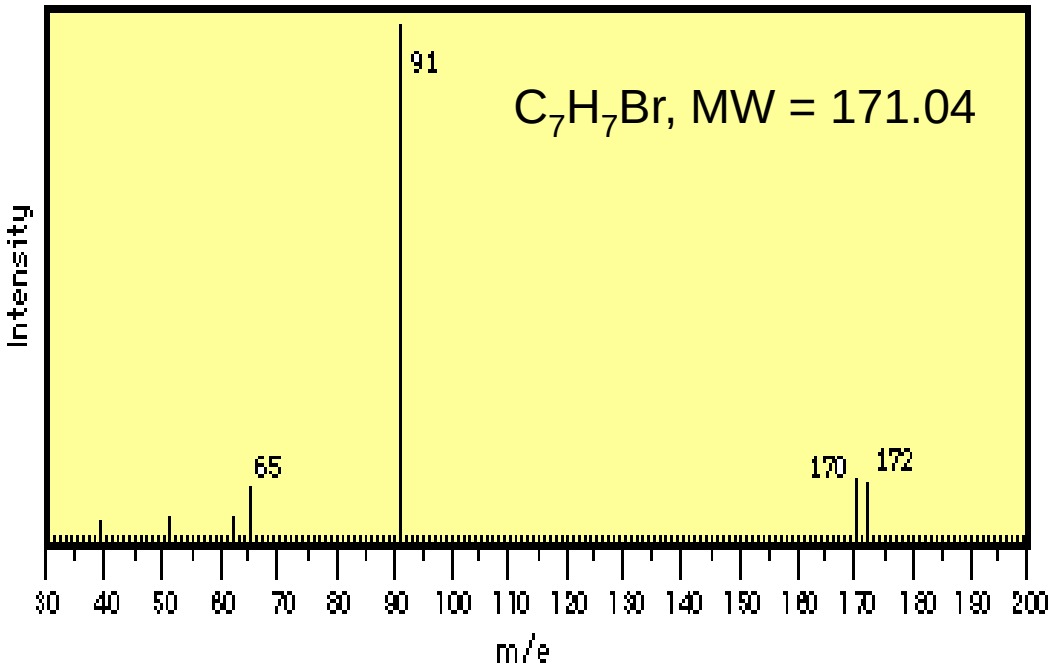
Practice Example 1

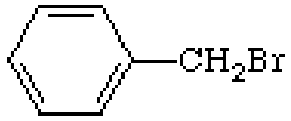
- If we are given the molecular formula, first step is to calculate the degree unsaturation (i.e. double bond equivalents)
  - General form: for formula $C_aH_bN_cO_d$; **DBE = [(2a+2)-(b-c)]/2**
- In example: DBE = [(12)-(12)]/2 = 0
    - No aldehyde, carbonyl, aromatic
- Peaks:
  - m/z = 88 (molecular ion)
  - m/z = 87 (m-1): loss of H
    - Small; suggests alcohol
  - m/z = 73 (m-15): loss of CH_3
  - m/z = 70 (m-18): loss of H_2O
  - m/z = 45 (m-43): base peak, oxonium ion (secondary alcohol, R=CH_3)



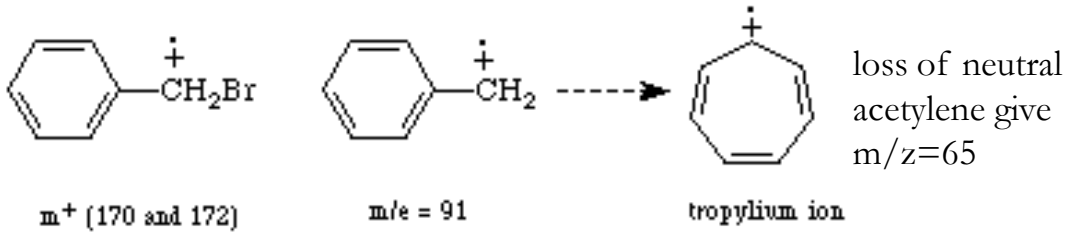
Practice Example 2

- DBE = [(16)-(7)]/2 = 4.5
  - Contains double bonds, aromatic rings
- Peaks:
  - m/z = 170 and 172 (peaks of equal intensity, near M^+)
    - contains bromine (^{79}Br and ^{81}Br).
  - m/z = 91 (m-79): base peak, loss of Br
    - peak at m/z = 91 also indicative of tropylium.
  - m/z = 65; loss of neutral acetylene from tropylium ion





bromomethyl benzene
(benzyl bromide)



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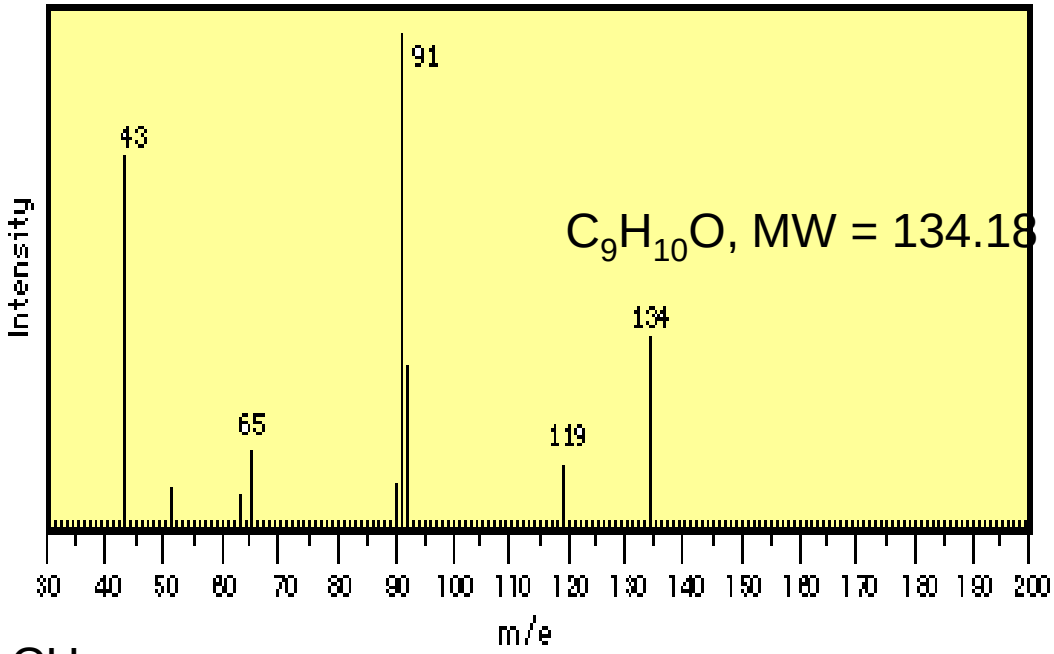
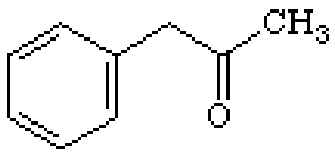
Practice Example 3

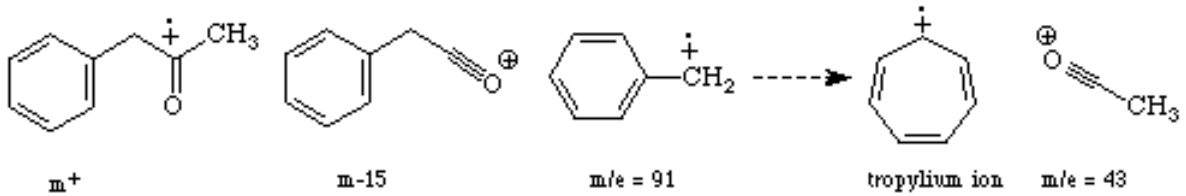


$C_9H_{10}O$, MW = 134.18

- DBE = [(20)-(10)]/2 = 5
  - Contains double bonds, aromatic rings
- Peaks:
  - m/z = 134 (molecular ion)
  - m/z = 119 (m-15): labile CH_3
  - m/z = 91 (m-43): tropylium
    - suggests loss of $CH_2CH_2CH_3$
  - m/z = 65; loss of neutral acetylene from tropylium ion
  - m/z = 43; intense
    - Suggests methyl ketone, which fragments to form acylium ion.



3-pentanone
(diethyl ketone)



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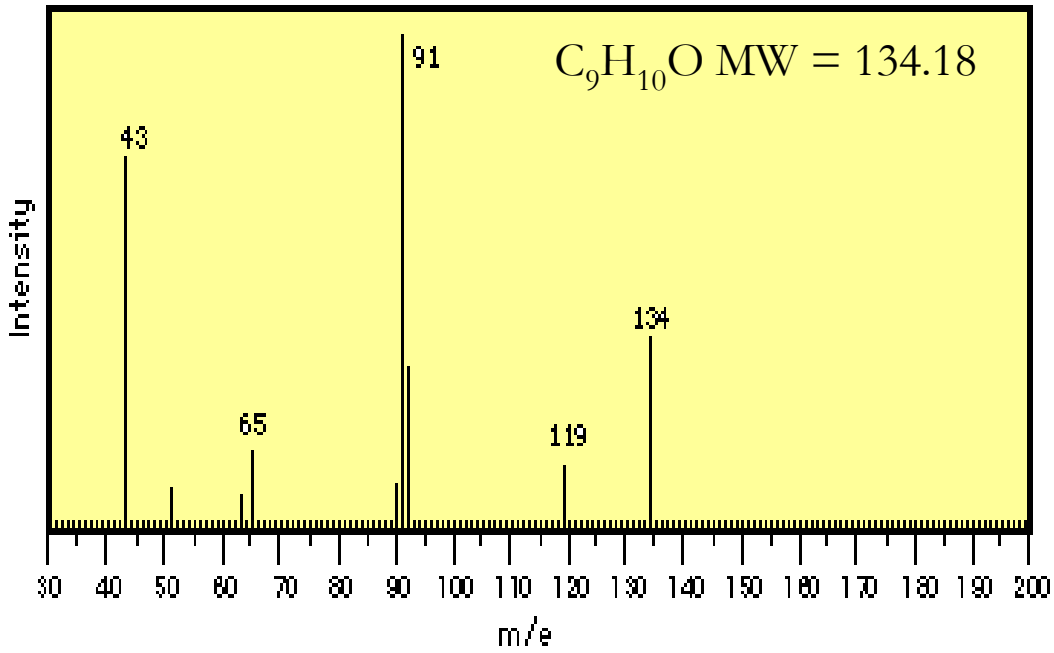
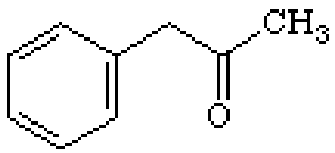
Practice Example 3

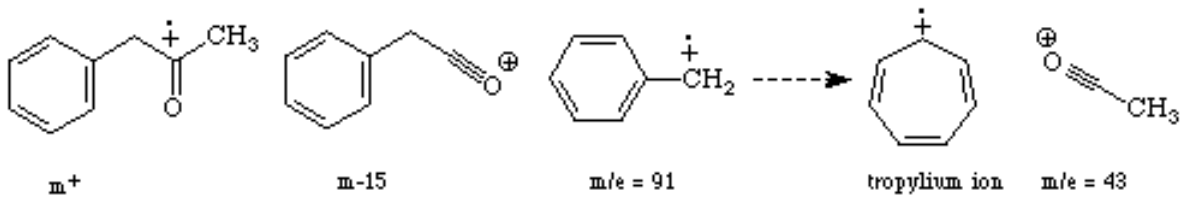
Peaks:

- m/z = 134 (molecular ion)
- m/z = 119 (m-15): labile CH_3
- m/z = 91 (m-43): base peak
  - indicative of benzyl cation
  - suggests loss of $CH_2CH_2CH_3$
- m/z = 65; loss of neutral acetylene from tropylium ion
- m/z = 43; intense
  - Suggests methyl ketone, which fragments to form acylium ion.





3-pentanone
(diethyl ketone)



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Common Neutral Losses

Table A.5. *Common neutral fragments.*

Δ[a]	Mass	Formula	Example[b,c]
−4	79	Br	R–Br
	121	$C_7H_5O_2$	Benzoates
	51 65	C_3HN, C_4H_3N	Some nitrogen heterocyclic compounds
−3	38	H_6O_2	Some polycarboxylic acids
−2	39 53 67	C_nH_{2n-3}	Allyl esters and some cyclic carbonates—specific rearrangement loss of (C_nH_{2n-1} − 2H); some propargyl and allenic derivatives
−1	26 40 54	C_nH_{2n-2}	Aromatics; alkenyl aryl ethers
	54 68 82	C_nH_{2n-2}	4-Y-cycloalkenyls; $M^{+\cdot} - 69 - (68)_n$ in polyisoprenes[c]
	54	C_3H_2O	Cyclic—CO—CH=CH—
	26 40	$C_nH_{2n}CN$	R–CN, R–CH_2CN (stable R^+ only)
0	27 41 55 69, etc.	C_nH_{2n-1}	RCOOR′-specific rearrangement loss of (R − 2H) or (R′ − 2H) + (R − H), also from carbonates, amides, larger ketones, etc.; loss of activated C_nH_{2n-1} groups
	27	HCN	Nitrogen heterocyclic compounds, cyanides, aryl-NH_2, enamines, imines
+1	(±14: Homologous impurity)		
	28 42 56	C_nH_{2n}	RCH_2COCH_2R-specific rearrangement loss of (R − H) or $(R - H)_2$, also from many unsaturated functional groups; retro-Diels-Alder[c]
	14	N	Aryl—NO
	28	N_2	Aryl—N=N—Aryl, >C=N_2, cyclic—N=N—
	28	CO	Aromatic oxygen compounds (carbonyls, phenols), cyclic ketones, R–C=O^{+c}
	42 56 70	$C_nH_{2n}CO$	Unsaturated acetamides, alkanoates; di-, cyclic, and complex ketones; specific H rearrangement loss of —CR_2—CO—
+2	29	CH_3N	Some unsaturated-, aryl—$N(CH_3)_2$
	43 57 71	HNCO, $C_nH_{2n-1}NO$	Loss of—NR—CO—from carbamates, cyclic amides, uracils
	1	H	Labile H; aryl—CH_2—H, RC≡CH, alkyl cyanides, lower fluorides and aldehydes (stable RCO^+), cyclopropyl compounds
	15 29 43 57 71, etc.	C_nH_{2n+1}	Alkyl loss: α-cleavage or branched site favored (loss of largest R); elimination from cycloalkyl group with H rearrangement[b]
	29 43 57	$C_nH_{2n+1}CO$	$C_nH_{2n+1}CO$–R (stable R^+ only)

Common Neutral Losses

+2							127	I	R⟨–I
+3	2	16	30	44	58	72,	etc.	C_nH_{2n+2}	Loss of RH from alkane branched site; loss of H_2 or CH_4 mainly from EE^+ ion
		16						NH_2	Aromatic and other amides and amines; often not abundant
			30					NO	Nitroaromatics, nitroesters, *N*- or metal nitroso compounds
				44				$CONH_2$	R⟨–$CONH_2$ (stable R^+ only)
		16						O	N-oxides, some sulfoxides; smaller for epoxides, nitro compounds, quinones, alicyclic ketoximes, diketoenamines
			30					CH_2O	$ROCH_2OR$, cyclic ethers, methoxy aromatics[c]
				44	58	72,	etc.	$C_nH_{2n+2}CO$	$RCOCH_2R'$-specific hydrogen rearrangement ⟶ $(R' - H)^{+\cdot}$ (stable ion only)[c]
				44				CO_2	Carbonates, cyclic anhydrides,[c] lactones, CH_3—N— and aryl—*N*—phthalimides
				44				CS	Thiophenols, aryl—S—aryl
	2,3,4							H_n	Boranes, silanes, phosphines.
+4		17						NH_3	Amines: uncommon unless other group to stabilize charge
		17						OH	Acids, oximes; rearrangement (e.g., *o*-$NO_2C_6H_4CH_3$)
			31	45	59			$C_nH_{2n+1}O$	R⟨–OR′; RCO⟨–OR′
					59	73		$C_nH_{2n+1}CO_2$	R⟨–OCOR′, R⟨–COOR′ (stable R^+, small R′ only)[c]
				45				CHS	Thiophenes, thiophenois
						73		C_3H_9Si	$(CH_3)_3Si$—
+5		18						H_2O	Alcohols (primary favored); higher mol. wt. aldehydes, ketones, ethers[c]
			32	46	60	74		$C_nH_{2n+1}OH$ ($n = 0, 1, 2$), $C_nH_{2n+1}OH + C_nH_{2n}$	Loss of ROH from $R'CH_2OR$; from R′COOR with labile hydrogen; these with further loss of C_nH_{2n} ($n > 1$)
				46	60			$CO + C_nH_{2n+1}OH$	Loss of CO + HOR from R′COOR with labile H; cyclic—OCHRO—

Common Neutral Losses

Table A.5. *Common neutral fragments (continued).*

Δ[a]	Mass						Formula	Example[b,c]
+5			46				NO_2	Nitroaromatics, R–NO_2, R–ONO
				60	74		$C_nH_{2n+1}COOH$	R′COO—R—H (stable $R^{+\cdot}$, small R′)
				60			COS	Thiocarbonates
		32					S	Sulfides, polysulfides,[c] aryl thiols
			46				CH_2S	Methyl aryl sulfides, some cyclothioalkanes
+6		33					$CH_3 + H_2O$	Some alcohols
	19	33	47				$C_nH_{2n}F$	Fluoroalkanes
		33	47	61			$C_nH_{2n+1}S$	R–SR′, R^+ more stable than R'^+; $(M-33)^+$ in aryl thiols, isothiocyanates
+7	20						HF	R–HF (primary favored)
		34	48	62	76		H_2S, $H_2S + C_nH_{2n}$	Thiols (primary favored); methyl sulfides; these with further loss of C_nH_{2n} ($n > 1$)
			48	62	76	90	$C_nH_{2n+3}SiOH$	Silyl ethers with a labile H, e.g., mass 90 from $(CH_3)_3SiOR$
+8					77	91	C_nH_{2n-7}	R′—C_6H_4—R (stable R^+)
		35	49				$HF + C_nH_{2n+1}$	C_nH_{2n+1}–R–HF
		35	49	63			$C_nH_{2n}Cl$	Chlorides (labile bond cleaved)
+9		36	50	64			$(C_nH_{2n+1}OH)_2$	Dialcohols, methoxyalcohols, etc.
		36					HCl	R–HCl (distinctive ^{37}Cl, primary favored)
			50				CF_2	Aryl—CF_3
				64			SO_2	RSO_2R, Aryl—SO_2OR[c]

[a] Δ = mass − 14n + 1 (Dromey 1976).

[b] Specific cleavages giving a major peak are usually indicative of a particular structural moiety (Chapter 4). Lower mass even-electron ions which are formed through secondary decompositions involving randomizing rearrangements ("ion series") are often of significant abundance, so that such ions are generally useful to indicate compound types, not specific structural moieties.

[c] Also see elimination rearrangement in Table 8.4.

Components of a MS

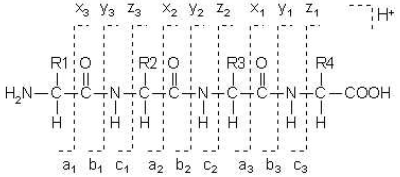
- Vacuum pump
  - Ions may have to travel over a metre (or many kilometers) to detector
  - Without vacuum, ions would collide with gas molecules in instrument (lose ions)
- Inlet
  - Sample introduced in various ways depending on the nature of the sample.
  - Solid or liquid samples: introduced via a direct insertion probe (DIP); sample then heated to vaporise
    - GC-MS/LC-MS: samples introduced directly from a gas or liquid chromatograph
  - Gas sample: introduced by a "gaseous leak"

http://www.matrixscience.com/help/fragmentation_help.html

Peptide Fragmentation

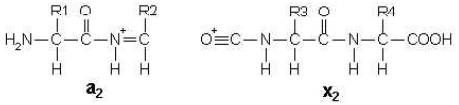
Sequence Ions

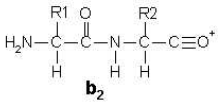
The types of fragment ions observed in an MS/MS spectrum depend on many factors including primary sequence, the amount of internal energy, how the energy was introduced, charge state, etc. The accepted nomenclature for fragment ions was first proposed by Roepstorff and Fohlman [Roepstorff, 1984], and subsequently modified by Johnson *et. al.* [Johnson, 1987].

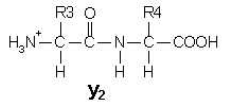


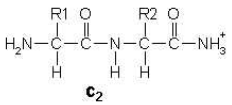
Fragments will only be detected if they carry at least one charge. If this charge is retained on the N terminal fragment, the ion is classed as either *a*, *b* or *c*. If the charge is retained on the C terminal, the ion type is either *x*, *y* or *z*. A subscript indicates the number of residues in the fragment.

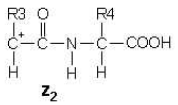
In addition to the proton(s) carrying the charge, *c* ions and *y* ions abstract an additional proton from the precursor peptide. Thus, the structures of the six singly charged sequence ion are:







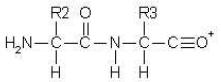




Note that these structures include a single charge carrying proton. In electrospray ionisation, tryptic peptides generally carry two or more charges, so that fragment ions may carry more than one proton.

Internal Cleavage Ions

Double backbone cleavage gives rise to internal fragments. Usually, these are formed by a combination of *b* type and *y* type cleavage to produce the illustrated structure, an amino-acylium ion. Sometimes, internal cleavage ions can be formed by a combination of *a* type and *y* type cleavage, an amino-immonium ion. Internal fragments are labelled with their 1 letter amino acid code.



Immonium Ions

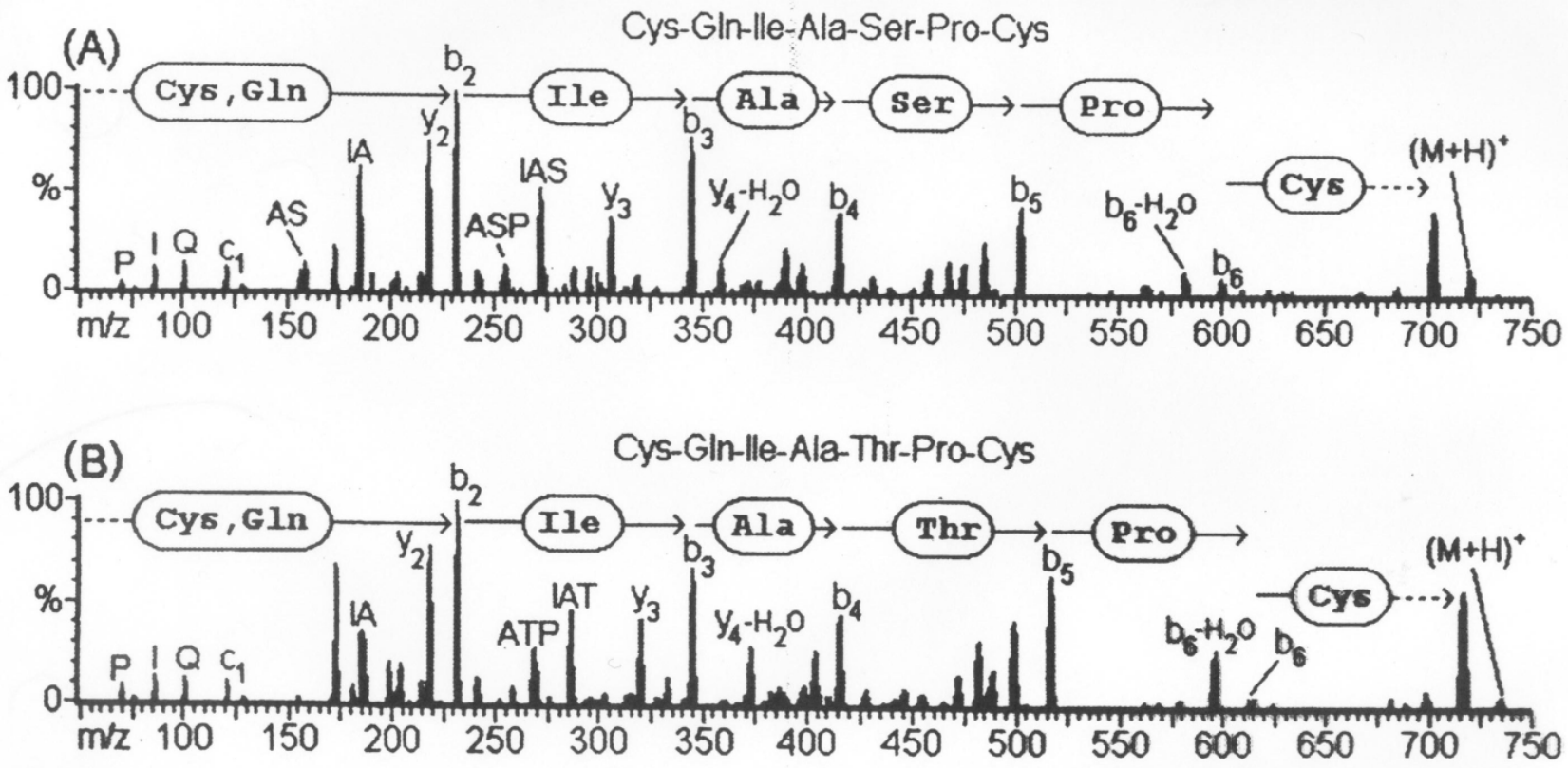


FIGURE 3. Low-energy tandem CID mass spectra of two related peptides differing by one amino acid. Both precursor ions, generated by electrospray ionization, were singly protonated. The spectra look very similar, but ions indicative of the amino-acid substitution have shifted by 14 u between (**A**) and (**B**).