PC 235: Mass Spectrometry and Proteomics

Lecture 1

May 11th, 2009

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Course Outline

Lectures: 10hrs:

10am-12 in Genentech Hall Room S271, Mission Bay Campus

	Date	Lecturer	Topic
Lecture1	Monday, May 11	S. Guan	Mass Spectrometry Fundamentals: Instrumentation; ion optics, resolution and mass accuracy; why these are important at protein vs peptide vs PTM level.
Lecture2	Wednesday, May 13	R. Chalkley	Sample preparation: Gels and Chromatography; IP/Tags. What shouldn't be in the sample. Contaminants. Digestion. Basics of peptide fragmentation.
Lecture3	Monday, May 18	R. Chalkley	Protein Identification: Database searching. How to measure the reliability of assignments.
Lecture4	Wednesday, May 20	K. Medzihradszky	PTMs: Protein vs peptide analysis. PTM enrichment, modification specific scans/ions.
Lecture5	Friday, May 22	R. Chalkley	Quantitation strategies. Cross-linking for identifying complexes/binding partners and interfaces.

Course Outline – cont.

Laboratory: Approximately 30hrs total

Will include a half-day tutorial on instrumentation and how data is acquired. There will also be training on data interpretation and bioinformatic analysis.

11 May Lecture 1	12May	13 May Lecture 2	14 May Digest Samples	15 May Extract Peptides Acquire Data
18 May Lecture 3	19 May Data Analysis Training	20 May Lecture 4	21 May	22 May Lecture 5
25 May Analyze Results and Prepare Final Presentation	26 May	27 May	28 May Present Results	29 May

What is Mass Spectrometry?

IUPAC Definition: The branch of science dealing with all aspects of mass spectrometers and the results obtained with these instruments.

My Definition: An analytical instrument that measures the mass-to-charge ratio of charged particles.

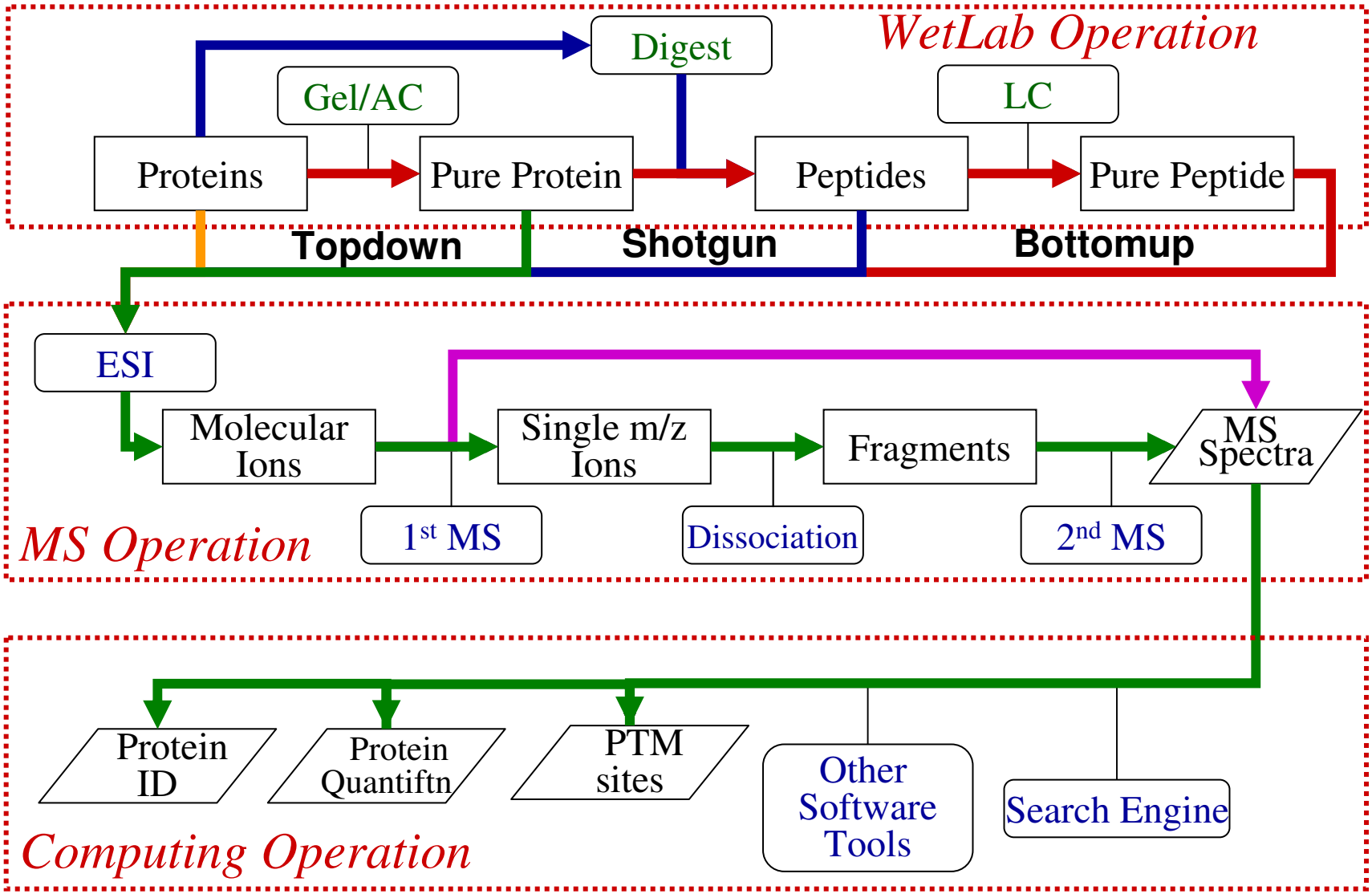
Applications:

1. identification
2. Quantification
3. Molecular structure
4. higher-order structure (H/D exchange, cross-link)
5. gas-phase ion chemistry
6. tissue imaging

What do we use Mass Spectrometry for in this course?

1. Protein identification, either by direct protein analysis, or by digesting the protein into smaller pieces (peptides), then identifying the peptides.
    - Complex mixture; e.g. cell organelle
    - Immunoprecipitation of protein of interest
        - ID binding partners
2. Identification of post-translational modifications:e.g. phosphorylation, acetylation.
3. Quantifying relative differences in protein/peptide levels between related samples.
4. Quantifying changes in post-translational modifications.

Proteomics Approaches



Outline: Lecture 1

- Mass Measurement
  - Mass definitions
  - Isotopes
  - Characteristics of a mass spectrum

- Instrumentation
  - Ion sources
  - Fragmentation methods
  - Mass analyzers
  - Ion detection methods

Isotopes and Mass Measurement

Mass Definitions

Molecular masses are measured in Daltons (Da) or mass units (u).

One Dalton = 1/12 of the mass of a ^{12}C atom.

Monoisotopic mass = sum of the exact masses of the most abundant isotope of each element present, i.e., ^{1}H=1.007825, ^{12}C=12.000000, ^{16}O=15.994915, etc.

This is the most accurately defined molecular mass and is preferred if a measurement of it can be determined.

Average mass = sum of the abundant averaged masses ("atomic weights") of the constituent atoms of a given molecule.

The result is a weighted average over all of the naturally occurring isotopes present in the compound. This is the common chemical molecular weight that is used for stoichiometric calculations (H=1.0080, C=12.011, O=15.994, etc.). The average mass cannot be determined as accurately as the monoisotopic mass because of variations in natural isotopic abundances.

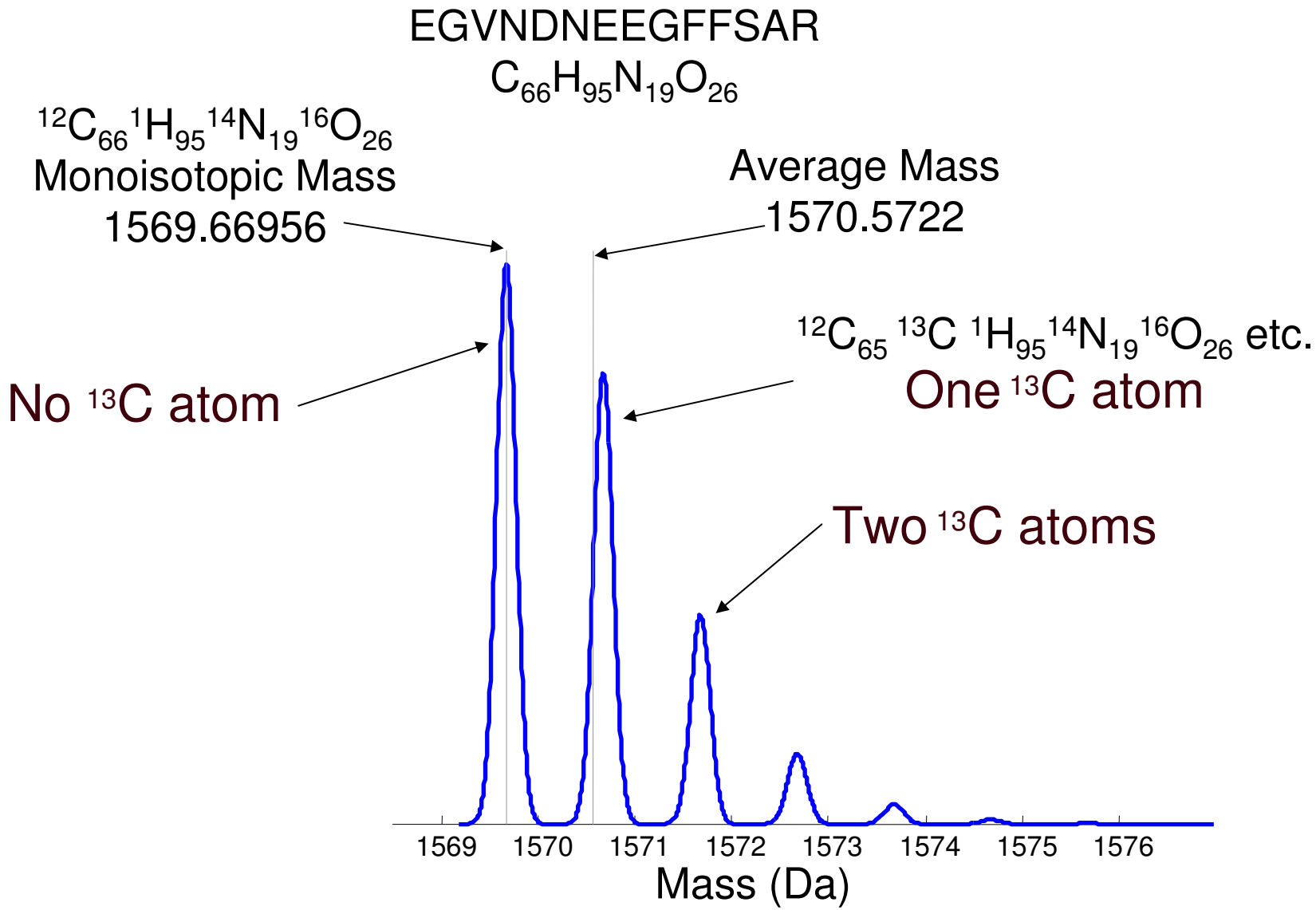
The mass to charge ratio (m/z). A quantity formed by dividing the mass (in u) of an ion by its charge number; unit: Thomson or Th.

Isotopic Abundances of Common Elements

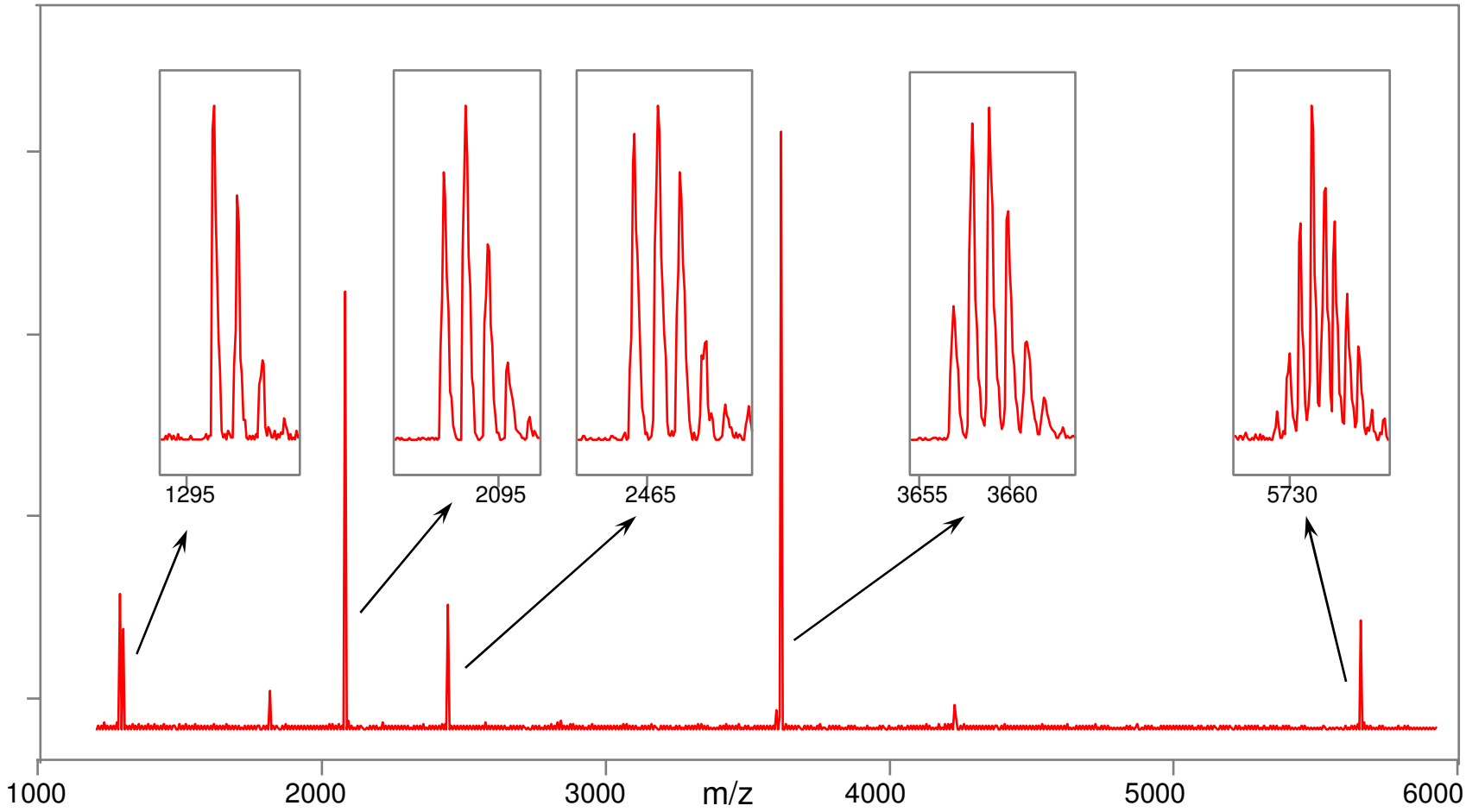
Element	Mass	Natural Abundance
H	1.0078 2.0141	99.985% 0.015
C	12.0000 13.0034	98.89 1.11
N	14.0031 15.0001	99.64 0.36
O	15.9949 16.9991 17.9992	99.76 0.04 0.20
P	30.9737	100
S	31.9721 32.9715 33.9679 35.9671	95.00 0.76 4.22 0.02

By coincidence, the most abundant isotope of common elements has the lowest mass.

Mass spectrum of peptide with 66 C-atoms (14 amino acid residues)



Isotope Pattern Changes with Mass

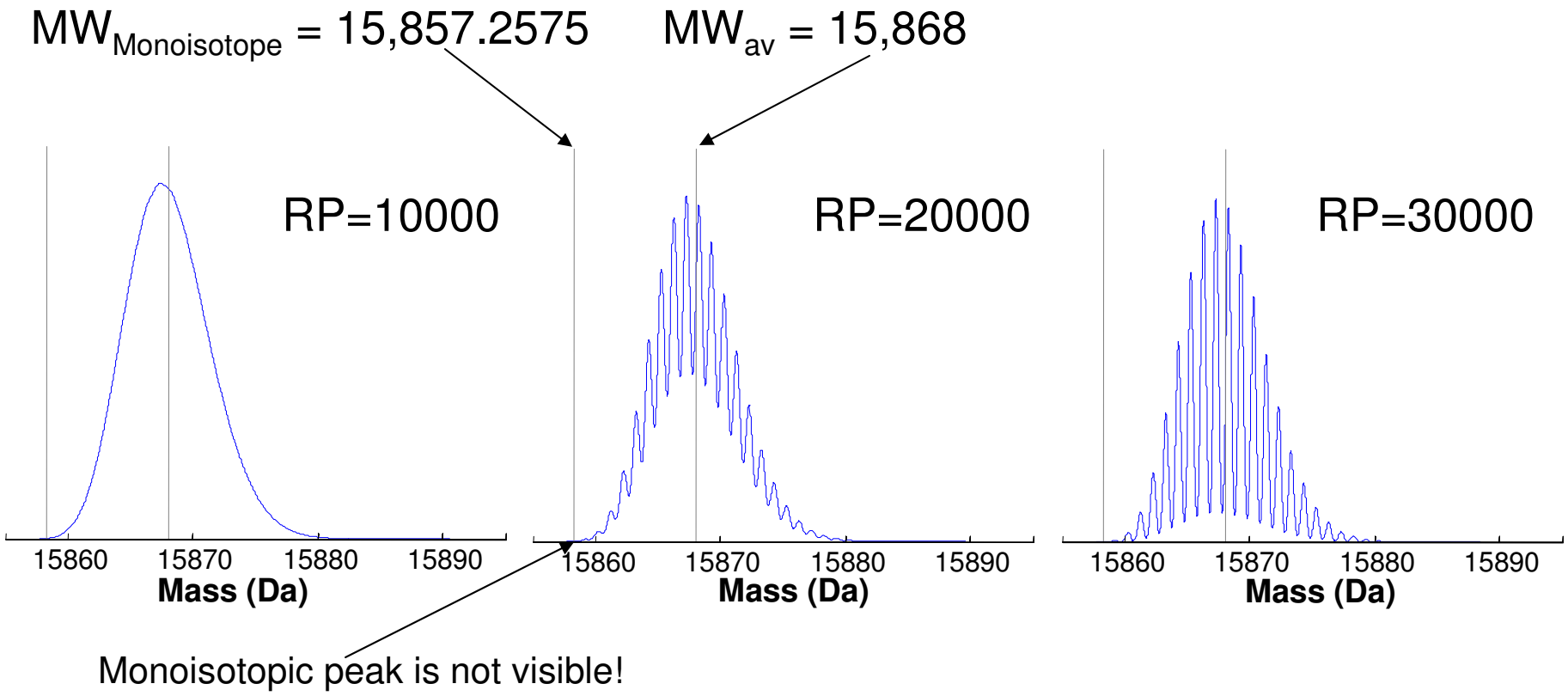


As the number of C-atoms in the molecule increases, the peaks due to higher mass isotopes increase in relative abundance. Data are for a series of peptides.

Protein Mass Measurement

•Protein masses are normally reported as average masses

Effect of different resolving power on Hemoglobin beta chain peak, $C_{724}H_{1119}N_{195}O_{201}S_3$



Three Important Properties to Assess Performance of a Mass Spectrometer

1. Sensitivity

•Minimum quantity of sample needed (always estimate how much sample you have, in femtomoles!)

2. Mass Accuracy

•Needed for identifying samples by database searching or to determine elemental composition

3. Resolving Power

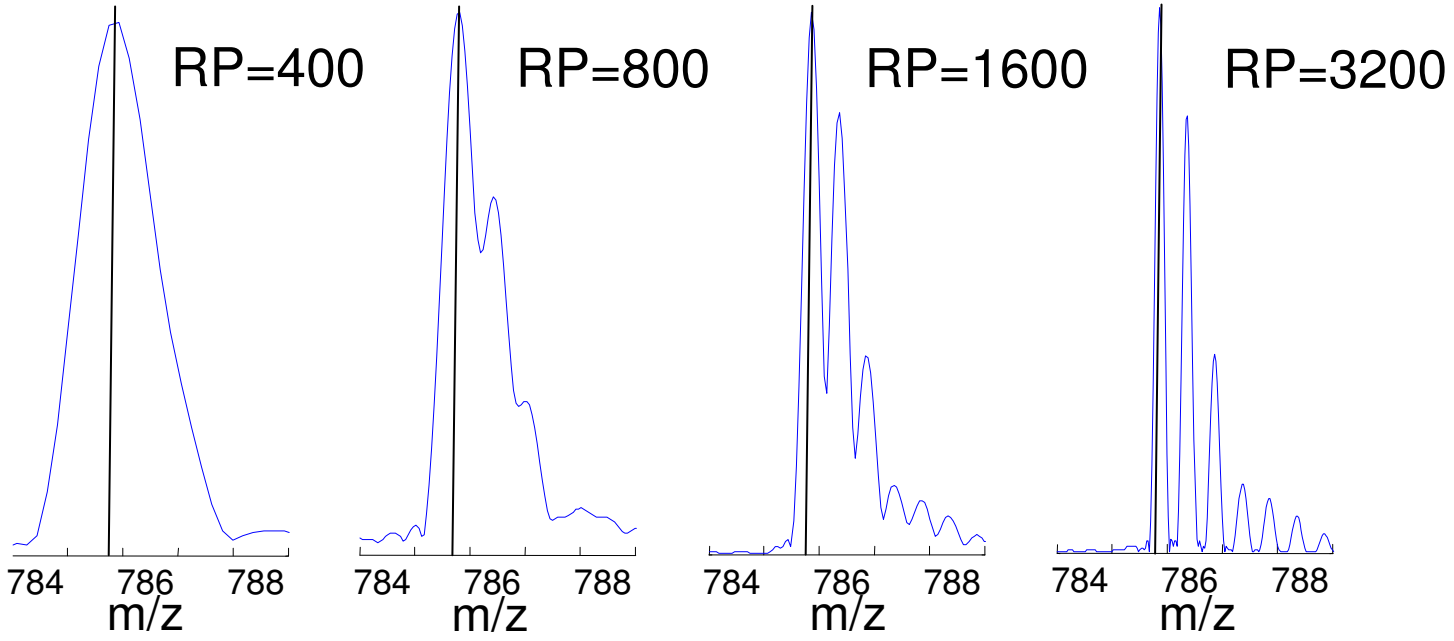
•Determine charge state. Resolve mixtures. High resolving can also improve mass accuracy.

Peptide Mass Measurement

Monoisotopic (neutral) mass, *M* of peptide can be calculated from measured *monoisotopic* mass-to-charge ratio *(m/z)* and charge state (*z*) of protonated ion

$$M_{monoisotopic} = (m/z)_{monoisotopic} \times z - M_{proton} \times z\ , \quad M_{proton}=1.007276$$

m/z:	785.838	785.782	785.853	785.853
M:	1569.661	1569.549	1569.720	1569.720



Mass (Measurement) Accuracy

Mass Accuracy or Mass Measurement Error is the difference between the experimental mass (M_{exp}) and the theoretical value (M_{theo}), calculated from elemental composition.

In absolute term, $MA = M_{\exp} - M_{theo}$, in Da or milli-Da

In relative term, $MA = \frac{M_{\exp} - M_{theo}}{M_{theo}}$, unit-less (ppm for high resolution MS)

Example:

M_{exp} = 1569.684
M_{theo}= 1569.66956

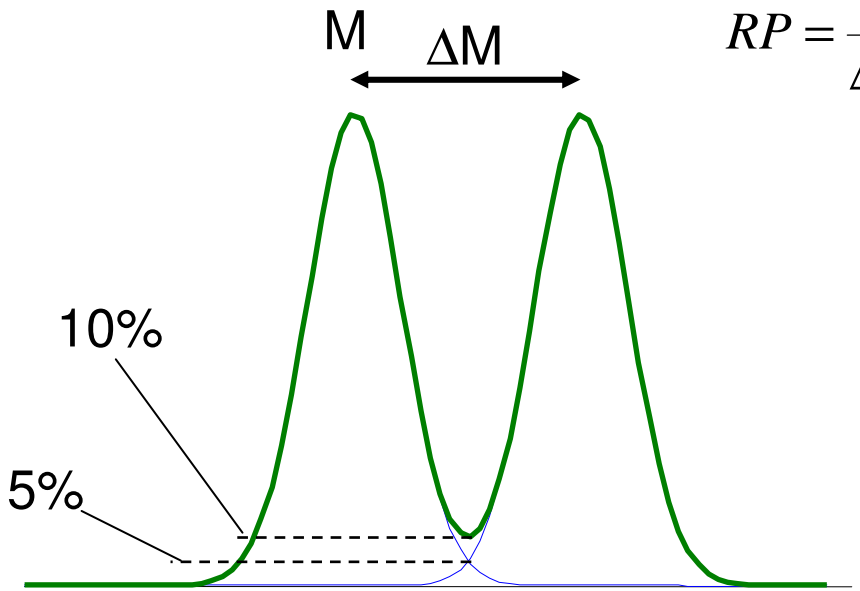
Mass Measurement Error = 0.014Da or 9.2ppm

http://physics.nist.gov/PhysRefData/Elements/per_noframes.html

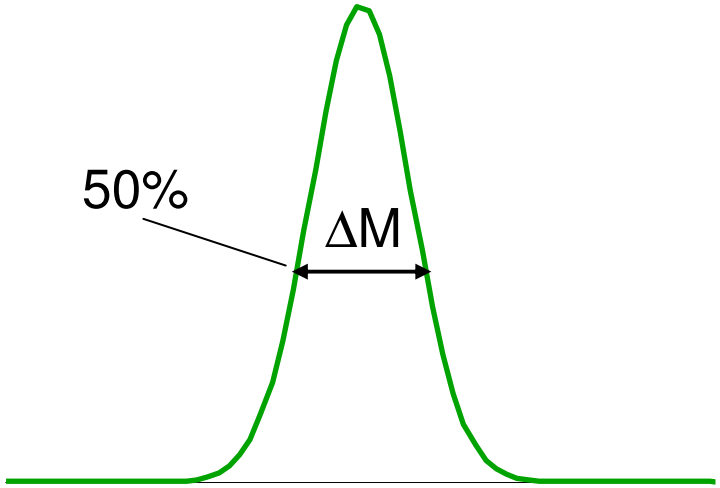
Resolving Power

- Measure of the ability to differentiate between components of similar mass.
- Two definitions:
  - Valley Definition: Neighboring peaks overlap at 10% peak apex height.
  - Full Width Half Maximum (FWHM): Width of a single peak measured at 50% peak apex. This is the most commonly used definition nowadays (because it is simpler).

$$RP = \frac{M}{\Delta M}$$



10% Valley Definition



FWHM Definition

Resolution vs Resolving Power

Resolution (Mass) – The smallest mass difference (ΔM) between two equal magnitude peaks such that the valley between them is a specified fraction of the peak height.

-IUPAC definition

For most people in the field, mass resolution and mass resolving power are used interchangeably.

Charge State Determination

High Resolution

– isotope peaks resolved

(1) counting isotope peaks in ONE m/z unit
(2) if the measured spacing of neighboring isotopes is Δ(m/z),
z=1/ Δ(m/z) or more accurately z=1.00235/Δ(m/z)
1.00235 is the average isotope spacing

Low Resolution

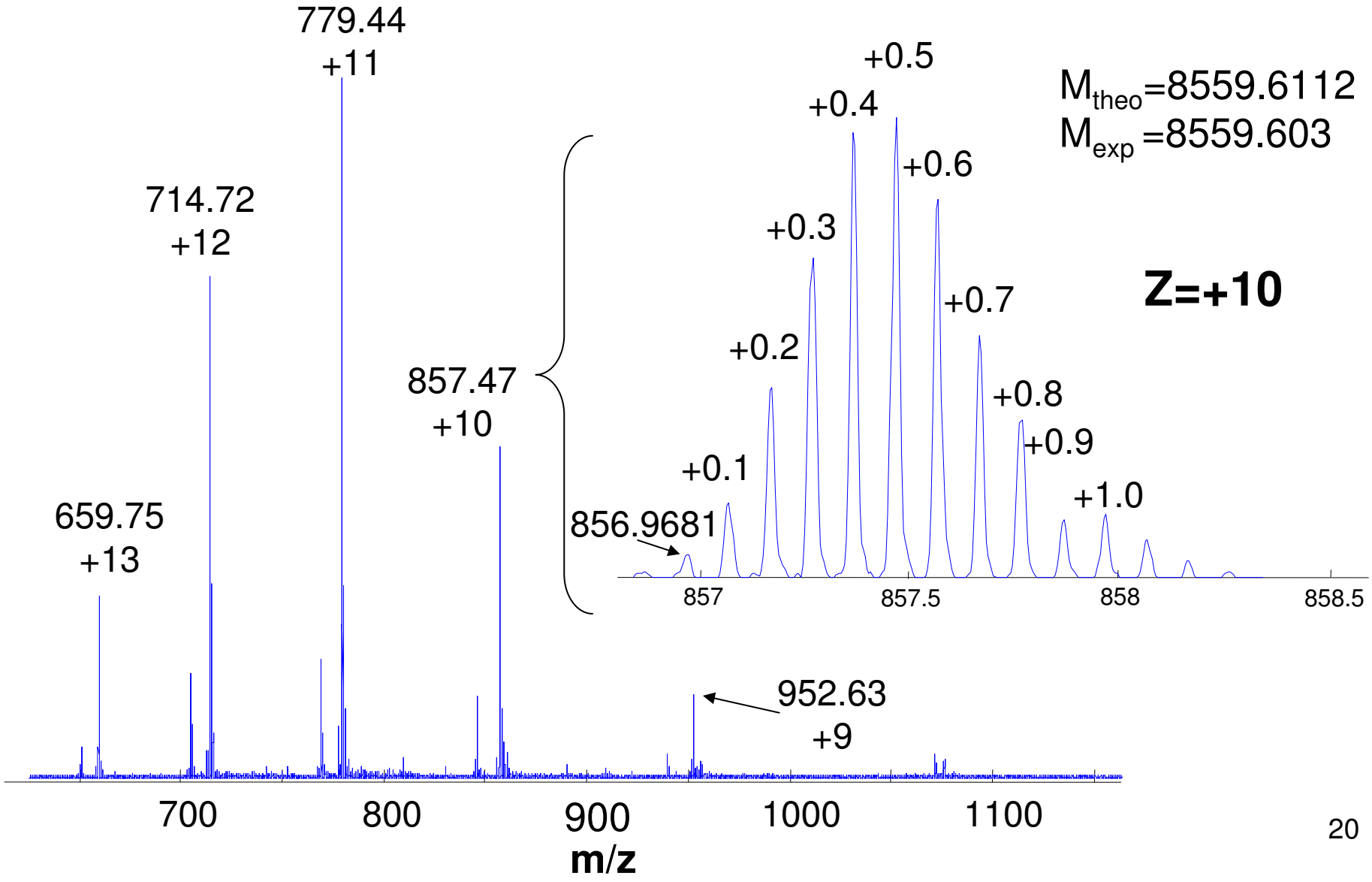
- isotope peaks are not resolved

Use neighboring charge states $(m/z)_1$ [higher charge]
and $(m/z)_2$ [lower charge, higher m/z]

Solve the following linear equations
for z (for $(m/z)_1$) and M (neutral mass)

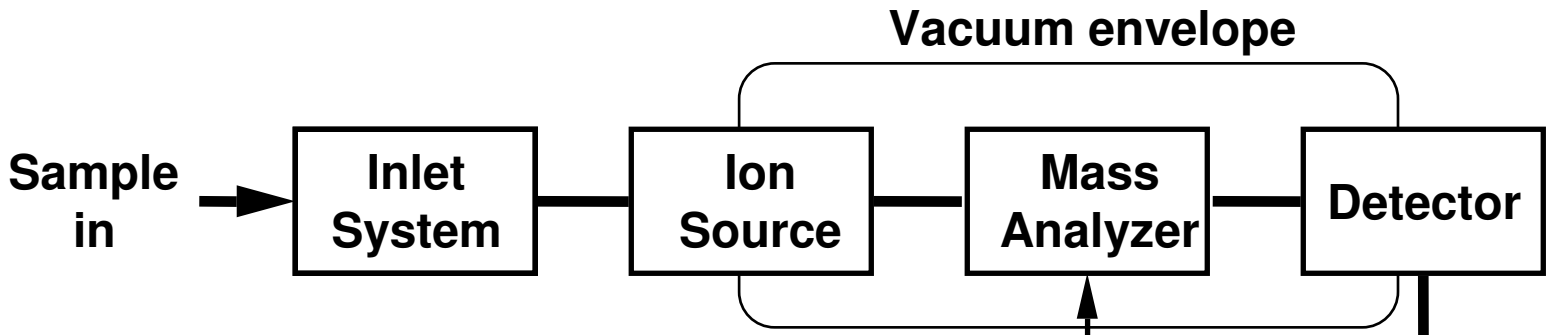
$$(m/z)_1 Xz - z = M$$
$$(m/z)_2 X(z-1) - (z-1) = M$$

Electrospray Mass Spectrum of Bovine Ubiquitin



Instrumentation

Mass Spectrometer Schematic



Inlet systems:

- HPLC
- Simple vacuum lock

Ion sources:

- Electrospray (ESI)
- MALDI

Mass analyzers:

- Time-of-flight (TOF)
- Quadrupole
- Ion trap
- FT-ICR
- Orbitrap

Ion sources

MALDI & ESI

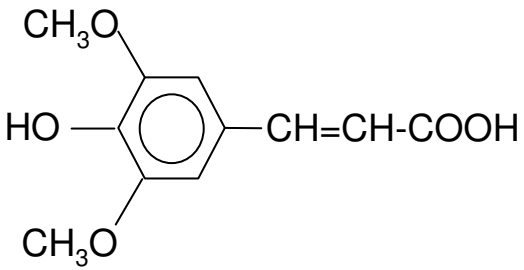
Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Analyte is dissolved in solution with excess matrix (>10^4).
- Sample/matrix mixture is dried on a target and placed in the MS vacuum.

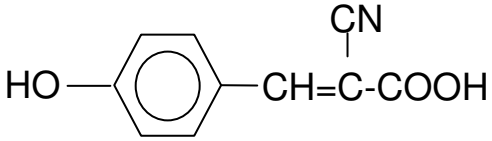
Requirements for a satisfactory matrix:

- It must co-crystallize with typical analyte molecules
- It must absorb radiation at the wavelength of the laser (usually 337 nm)
- To transfer protons to the analyte it should be acidic

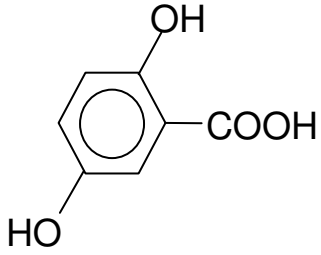
Typical successful matrices for UV MALDI are aromatic carboxylic acids.



Sinapinic acid

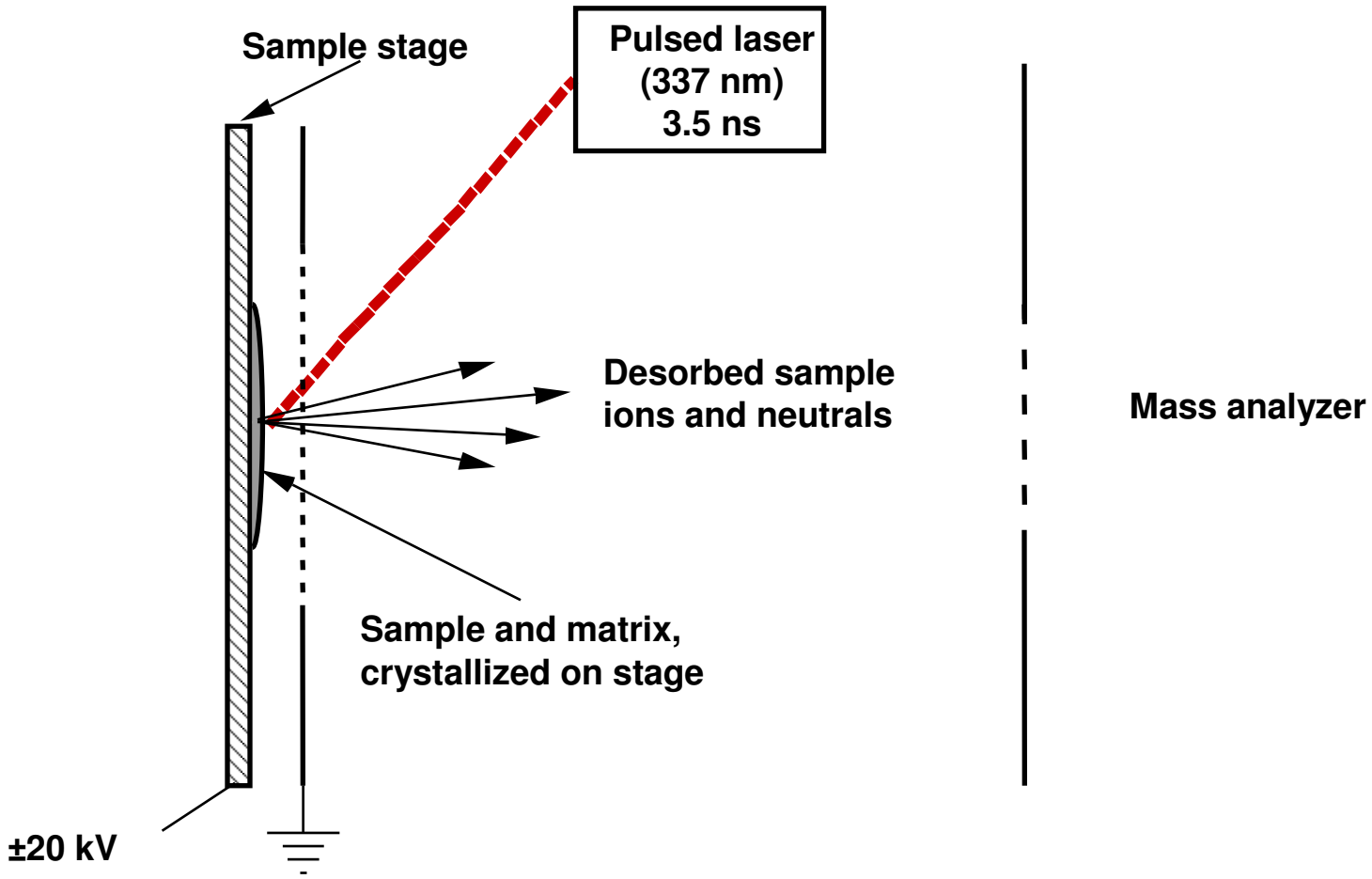


α-cyano-4-hydroxycinnamic acid (CHCA)



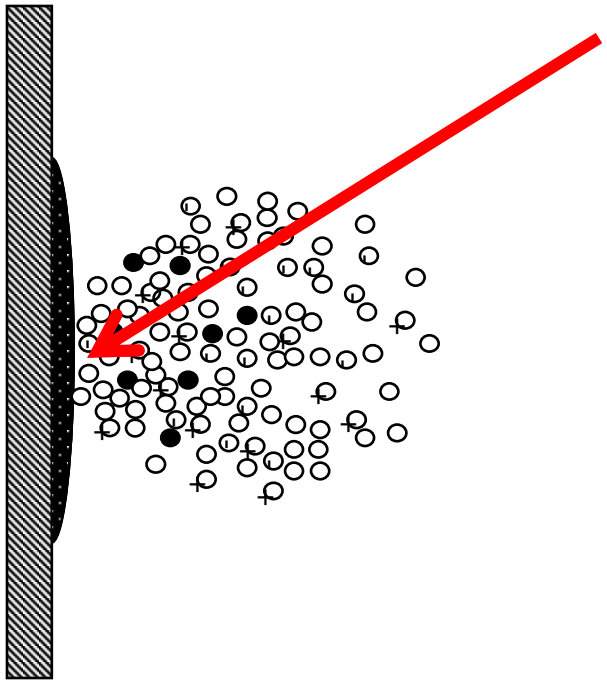
2,5-dihydroxybenzoic acid (DHB)

Matrix-assisted laser desorption ionization (MALDI)



MALDI Ionization Mechanism

1. Laser pulse produces matrix neutrals, + and - ions, and sample neutrals: M --> M*, MH^+, $(M-H)^-$ (M= Matrix)

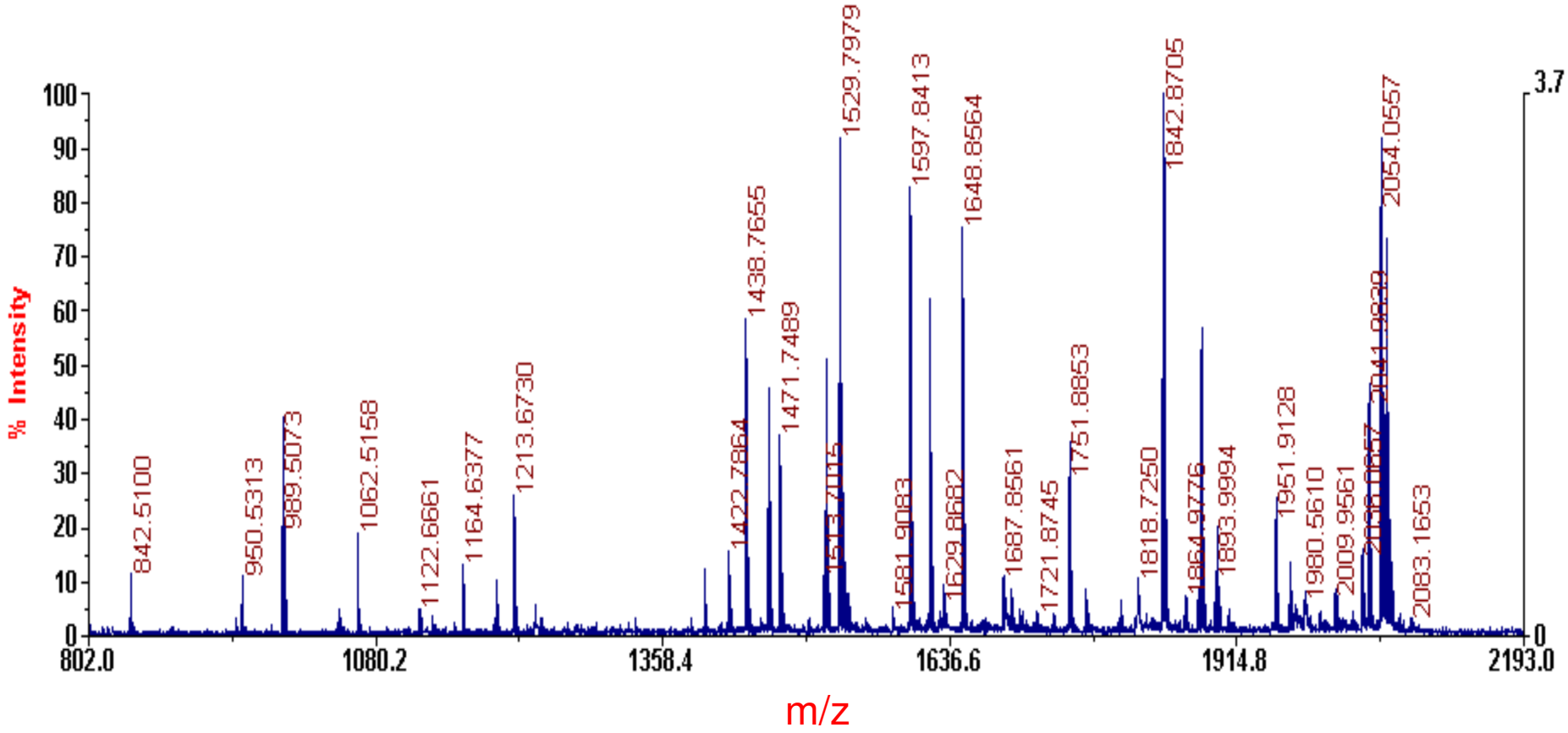


2. Sample molecules are ionized by gas-phase proton transfer:

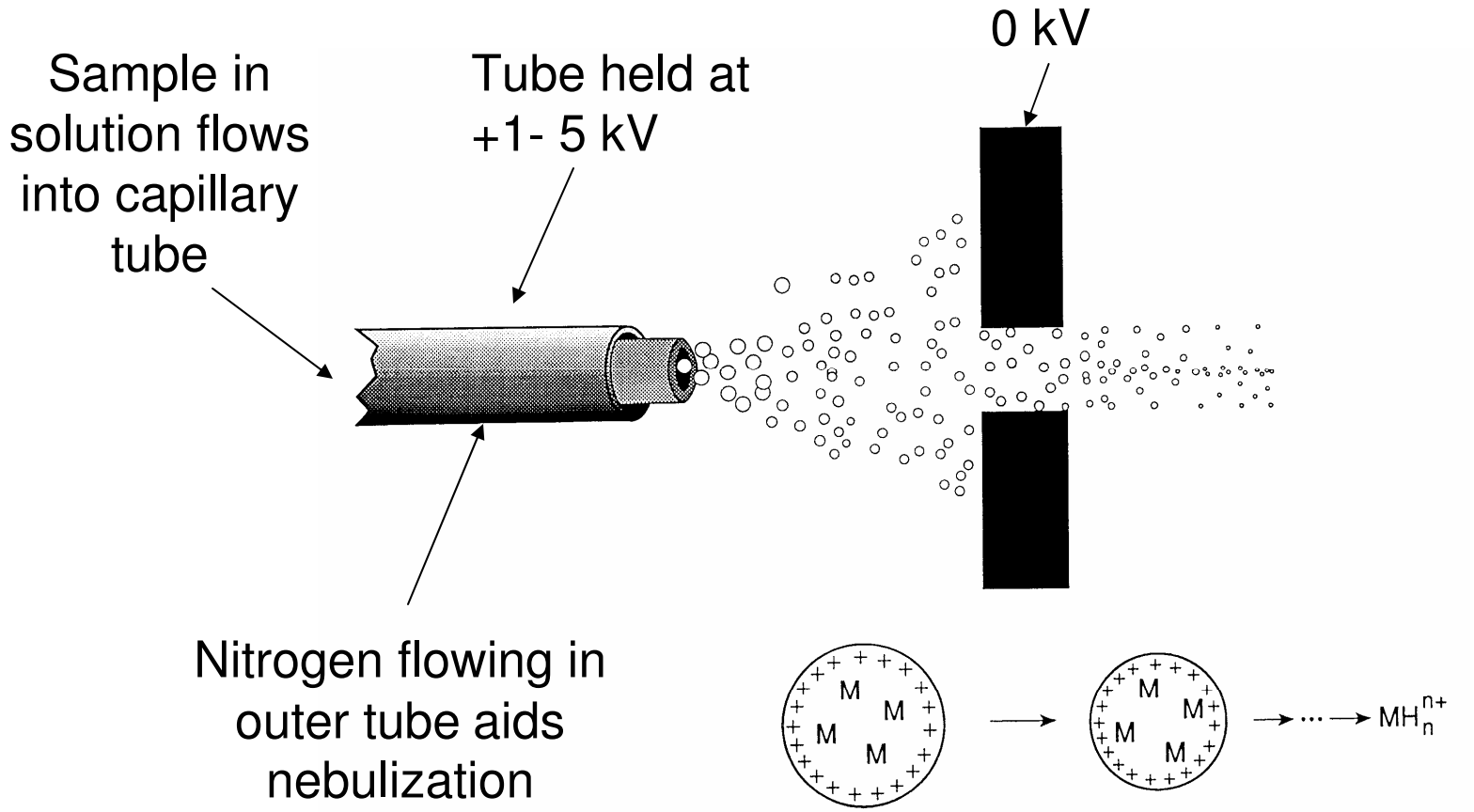
$MH^+ + A \rightarrow AH^+ + M$ (A=Analyte)

$(M-H)^- + A \rightarrow (A-H)^- + M$

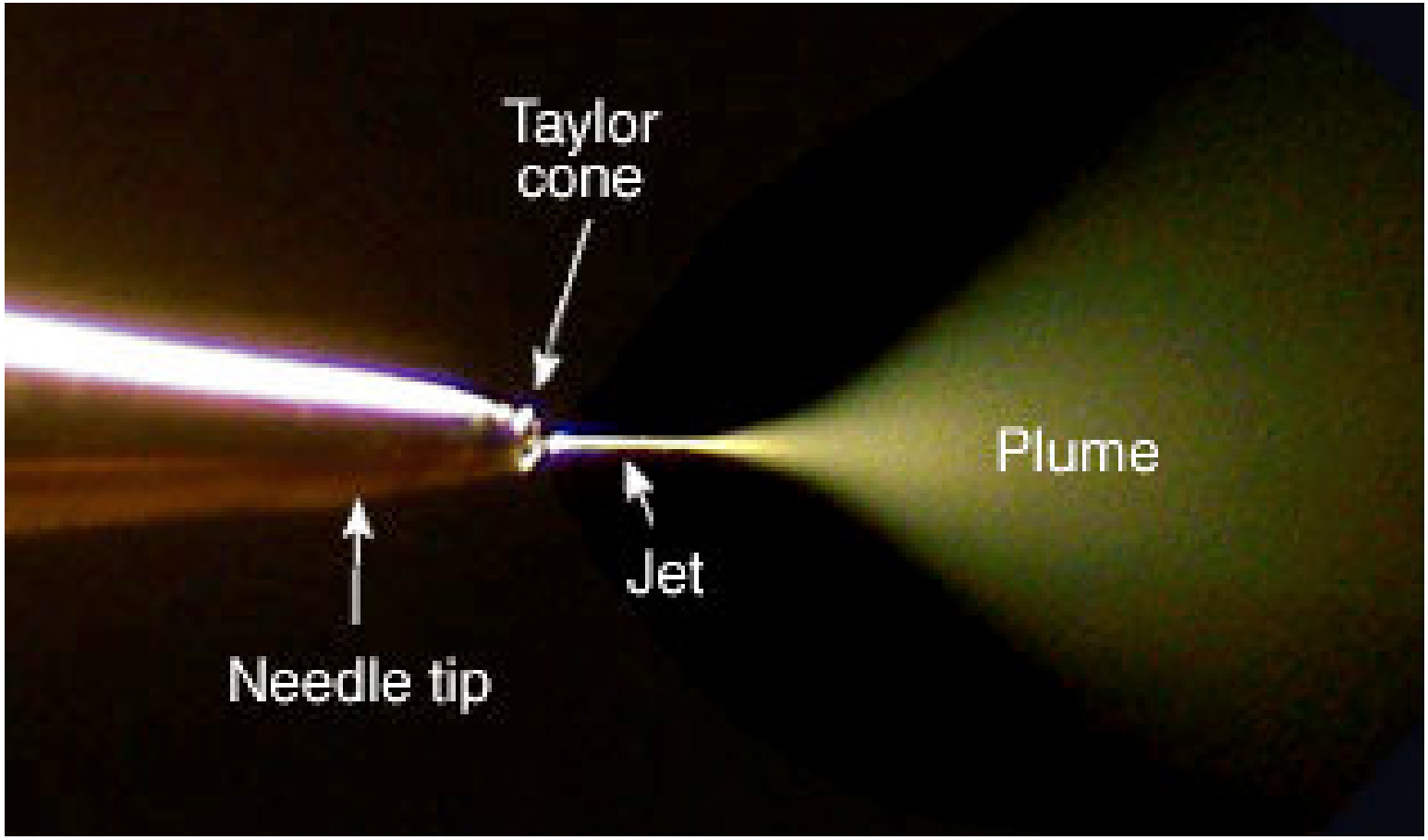
MALDI Mass Spectrum of Protein Tryptic Digest



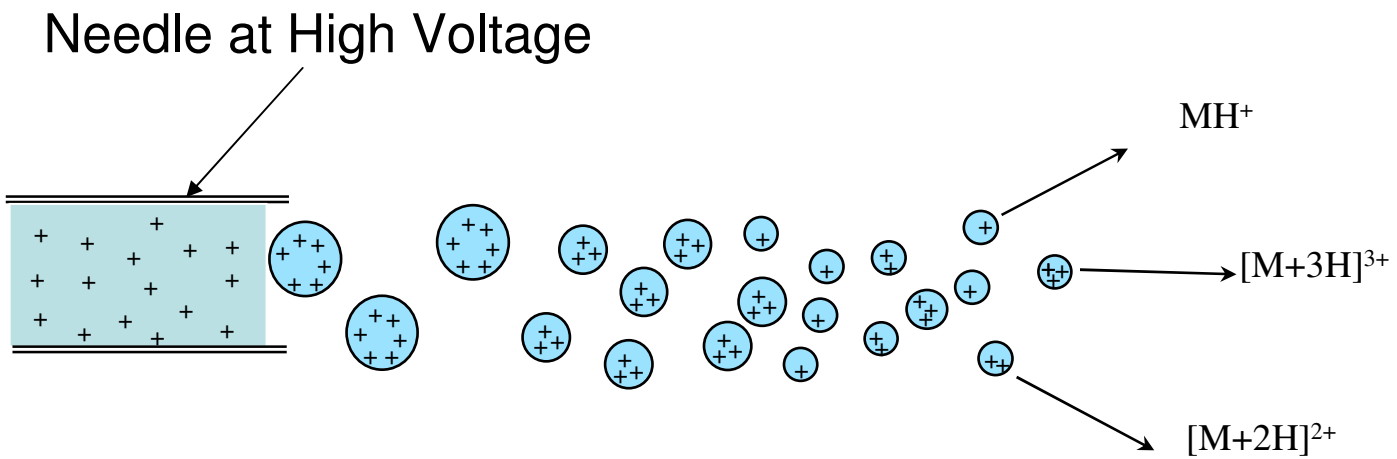
Electrospray Ionization



Electrospray ionization



Electrospray Ion Formation



Droplets formed in electric field have excess positive ions.

Evaporation of neutrals concentrates charge.

Droplets break into smaller droplets.

Eventually one molecule + *n* protons is left.

Nanospray

Online analysis

~ 20 µm tip ID

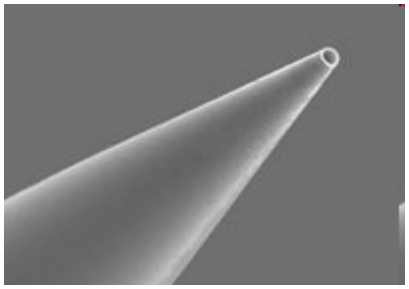
Interface with nanoLC

Flow rate: ~300nL/min

Offline analysis (static infusion)

~ 2 µm tip ID

Flow rate: ~40nL/min



New Objective, Inc.

Requires pure sample free from salt

Ionization Methods for Biomolecule Analysis

Electrospray	MALDI
•Online LC/MS possible	•Very long sample lifetime; repeated measurements possible
•Poor for mixtures without LC	•Good for mixtures
•Quantitation possible	•Matrix peaks can interfere at MW <600
•Good for MW <600	•Salt tolerant
•Generate highly charged ions	•Low maintenance
	•Generate ions with few charges

Mass analyzers

TOF

Quadruploe

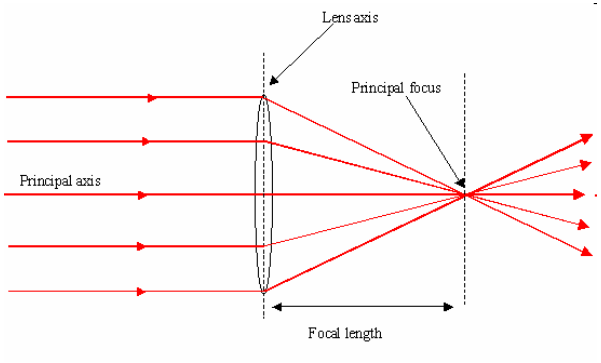
Ion Trap

FTICR

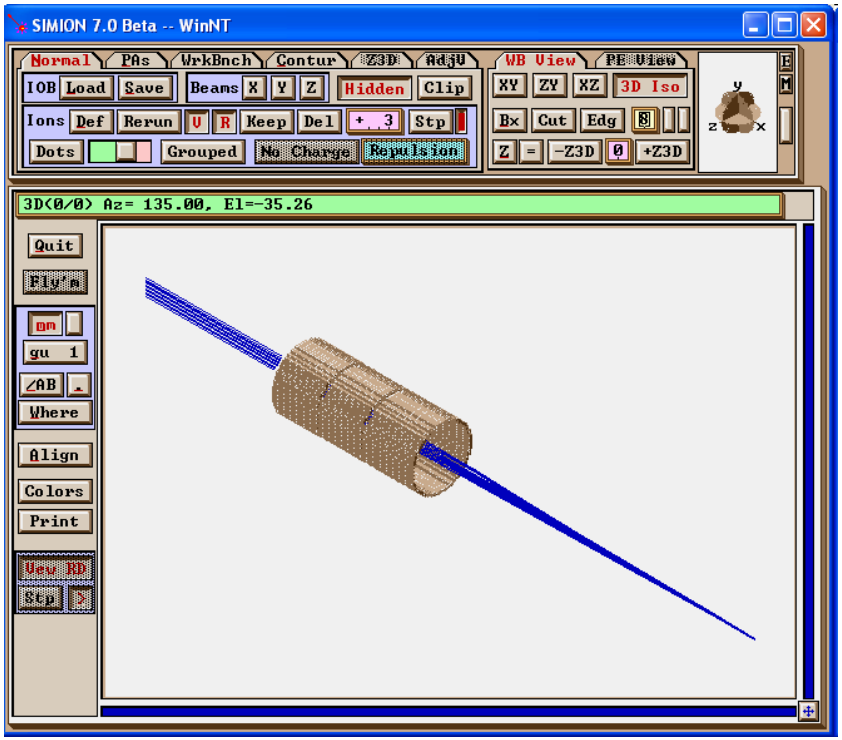
Orbitrap

Ion Optics

A device for manipulating ion beams.
A mass spectrometer consists of many *ion optical* components

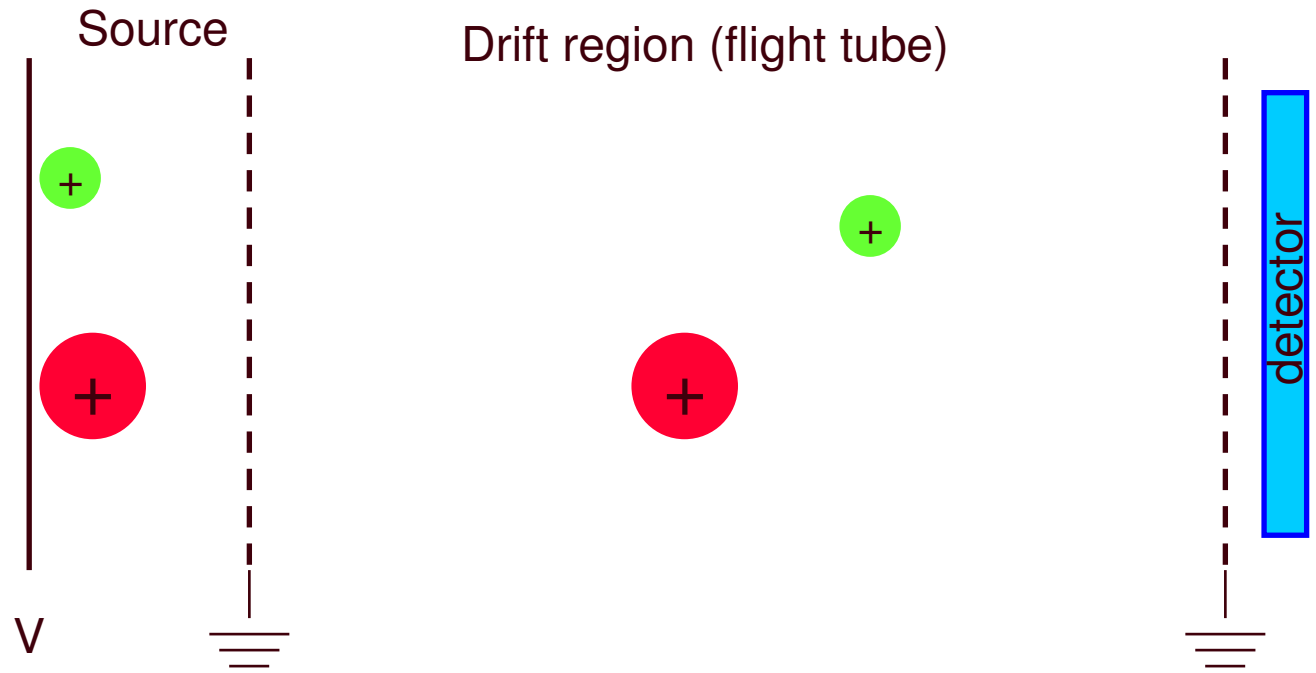


Einzel lens modeled with SIMION ion optics simulation program (computing electric and magnetic fields and ion trajectories)



http://simion.com/

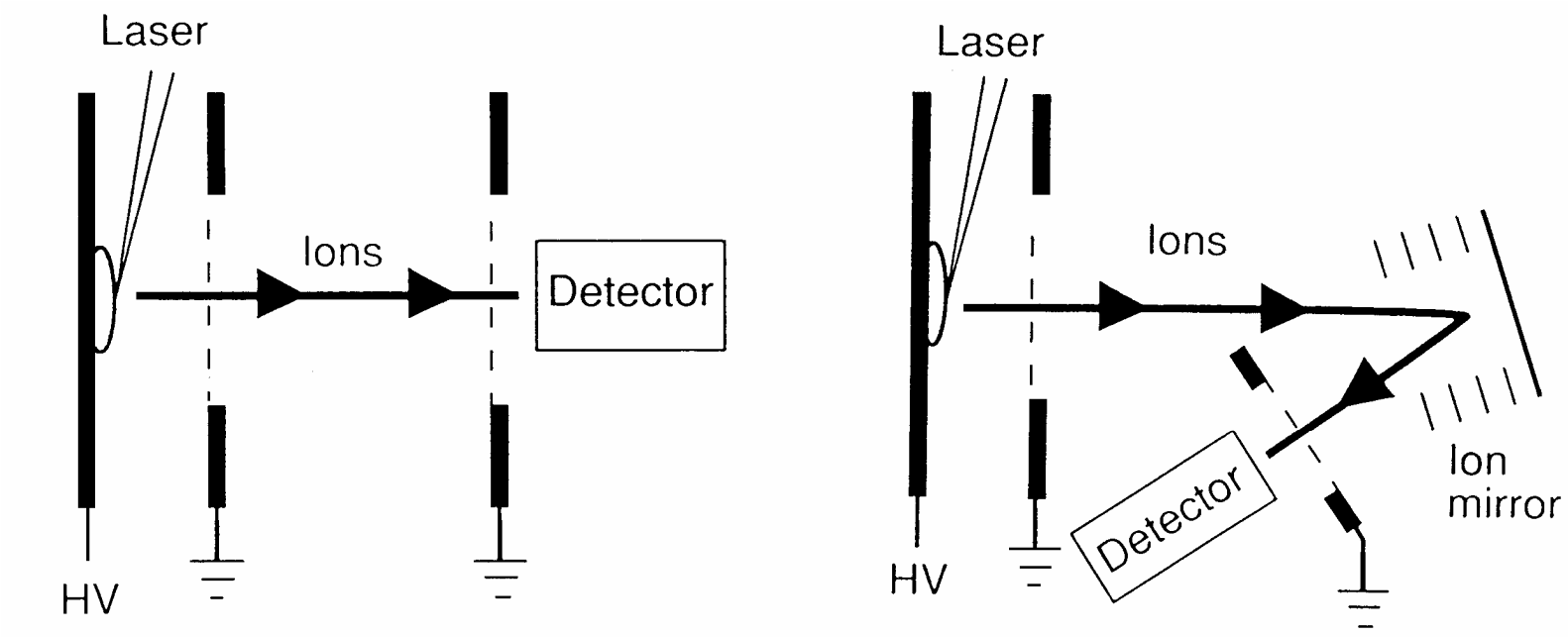
Time-of-Flight (TOF) Mass Analyzer



- Ions formed in pulses.
- Measures time for ions to reach the detector.

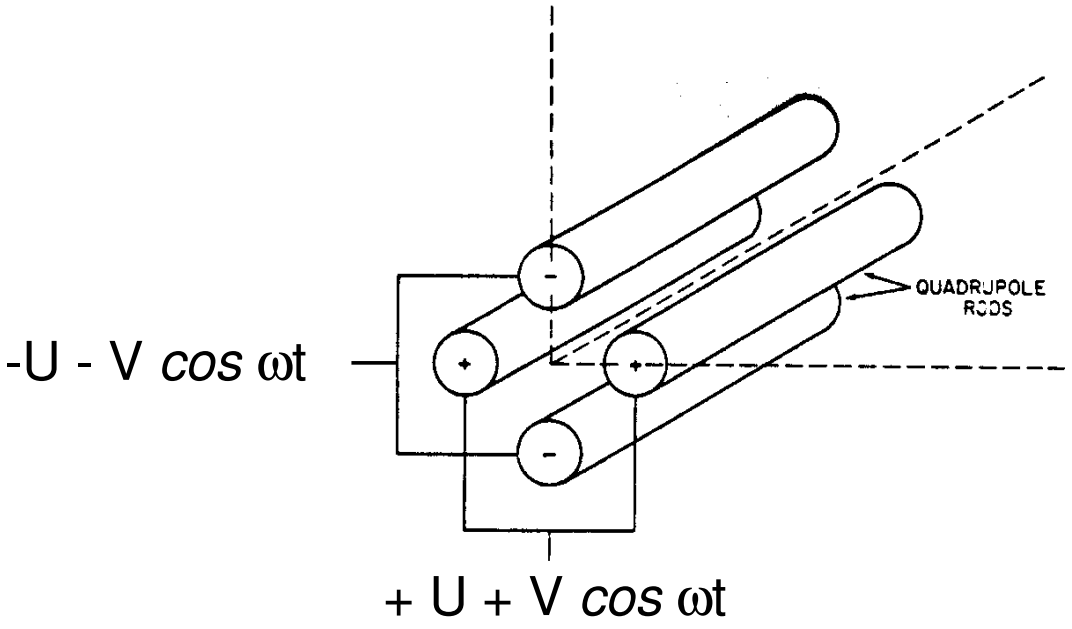
$$m/z = \frac{2t^2V}{L^2} \qquad \text{or} \qquad t \propto \sqrt{m/z}$$

Linear and Reflector TOF Analyzers



Reflector compensates for initial variation in kinetic energy, improving resolving power and mass accuracy.

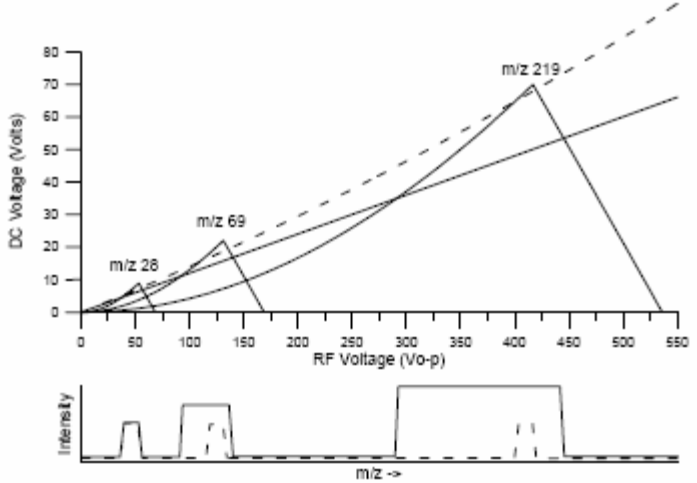
Quadrupole Mass Analyzer/Filter



Uses a combination of RF and DC voltages to operate as a mass filter.

- Mass analyzer.
- Mass selection device
- Ion transport device (RF-only collision cell).

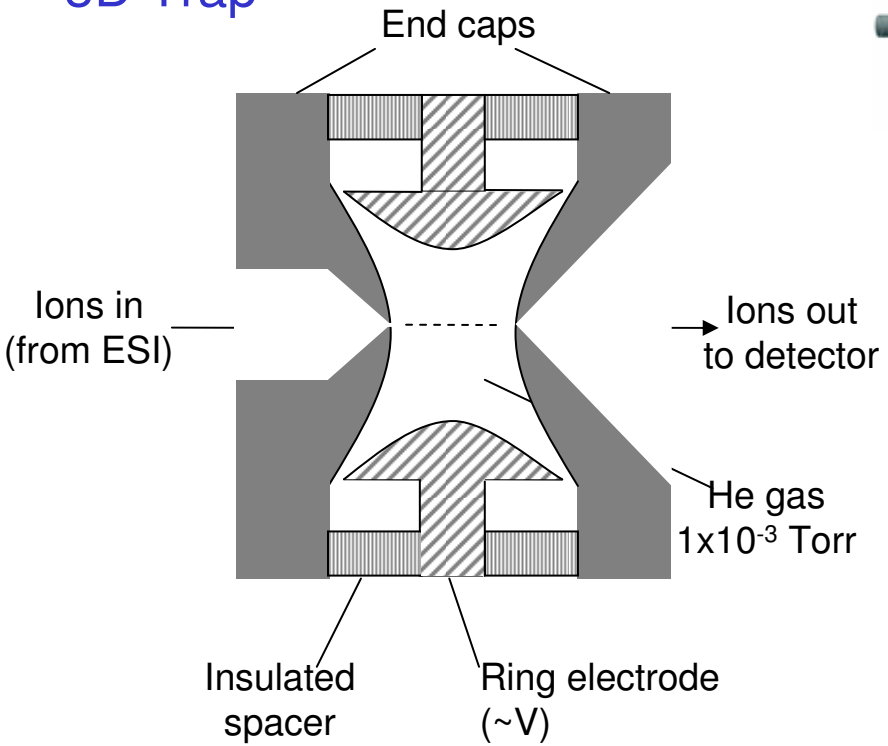
Mass scan and stability diagram

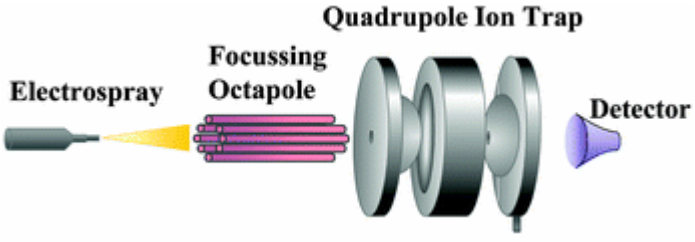


Randall E. Pedder
Extrel Application Note

Quadrupole Ion Trap

3D Trap





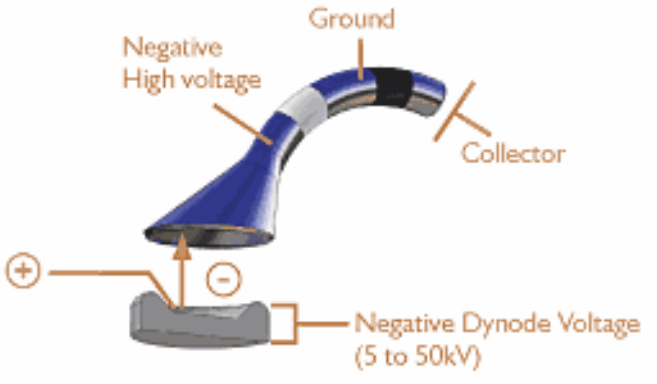
- Uses a combination of DC and RF fields to trap ions
- Ions are sequentially ejected by scanning the RF voltage

Linear Trap

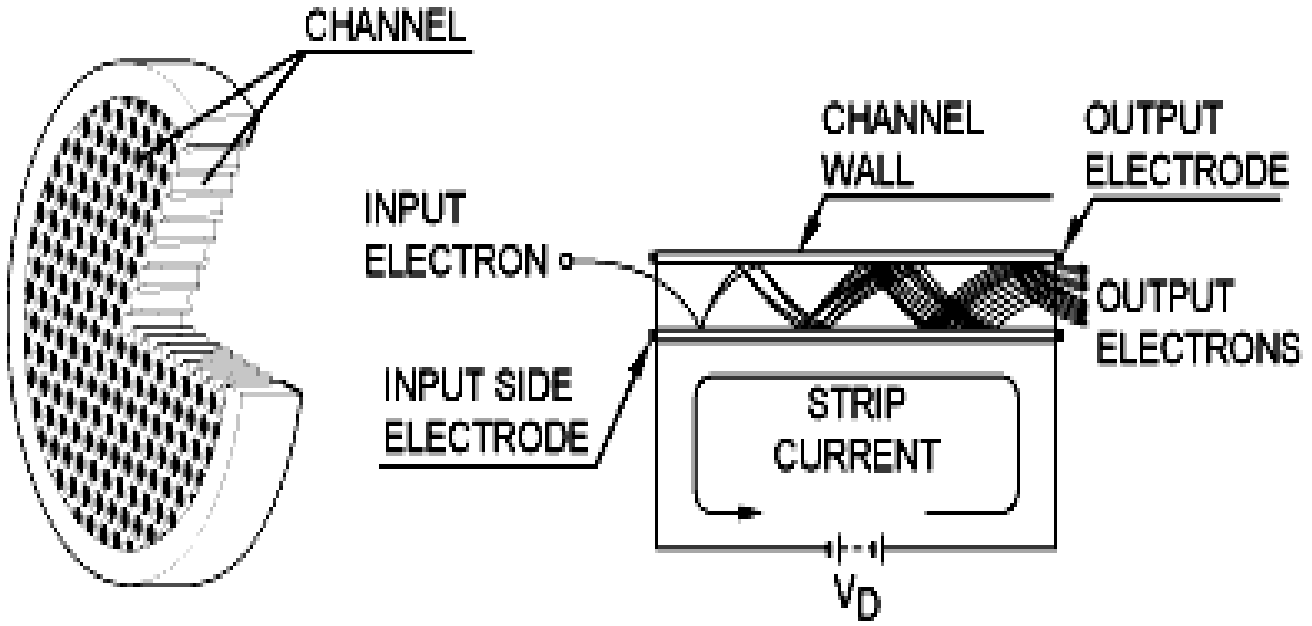
- Essentially a quadrupole with end-caps
- Advantage: Larger ion storage capacity, leading to better dynamic range

Raymond E. March, JOURNAL OF MASS SPECTROMETRY, VOL. 32, 351È369 (1997)

Electron Multiplier



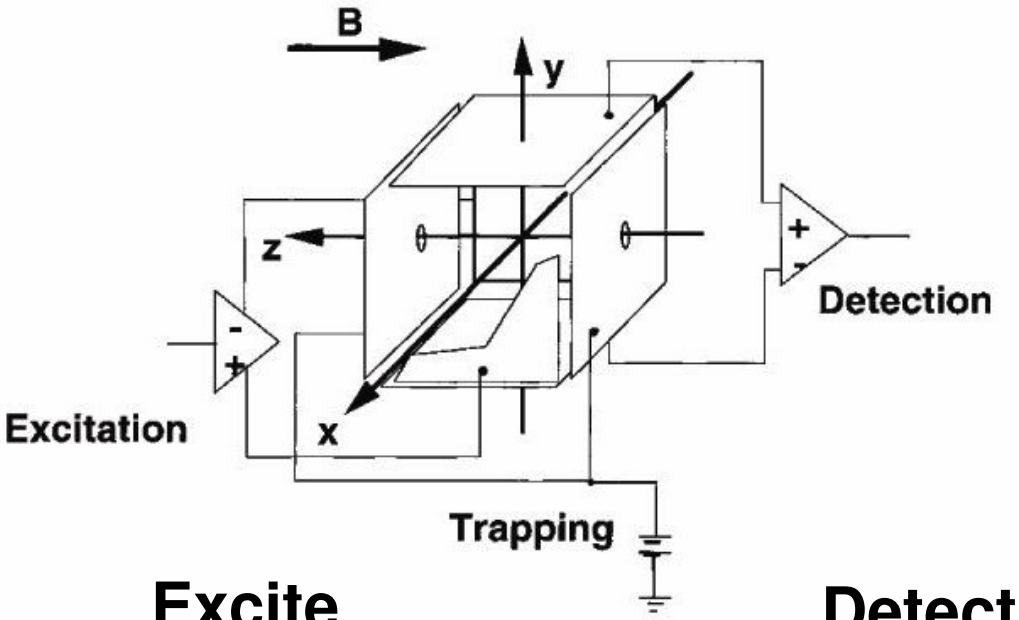
Multi-Channel Plate (MCP)



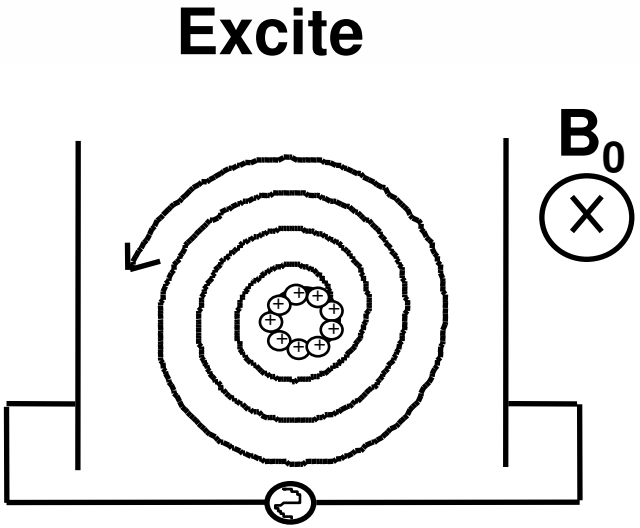
From Detector Technolgy: http://www.detechinc.com/

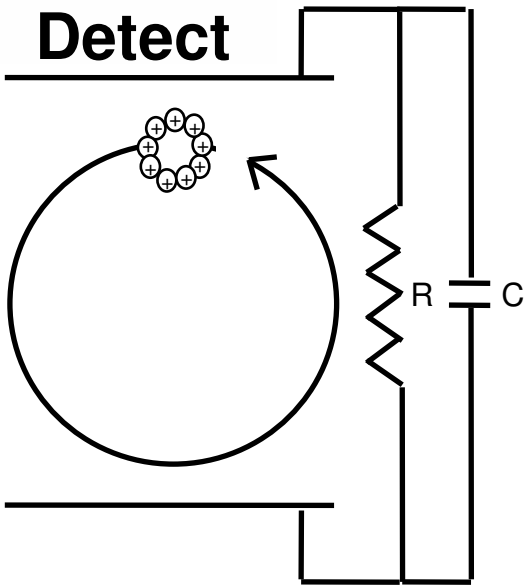
B. Brehm et al., Meas. Sci. Technol. 6 (1995) 953-958.

Fourier Transform Ion Cyclotron Resonance (FT-ICR)



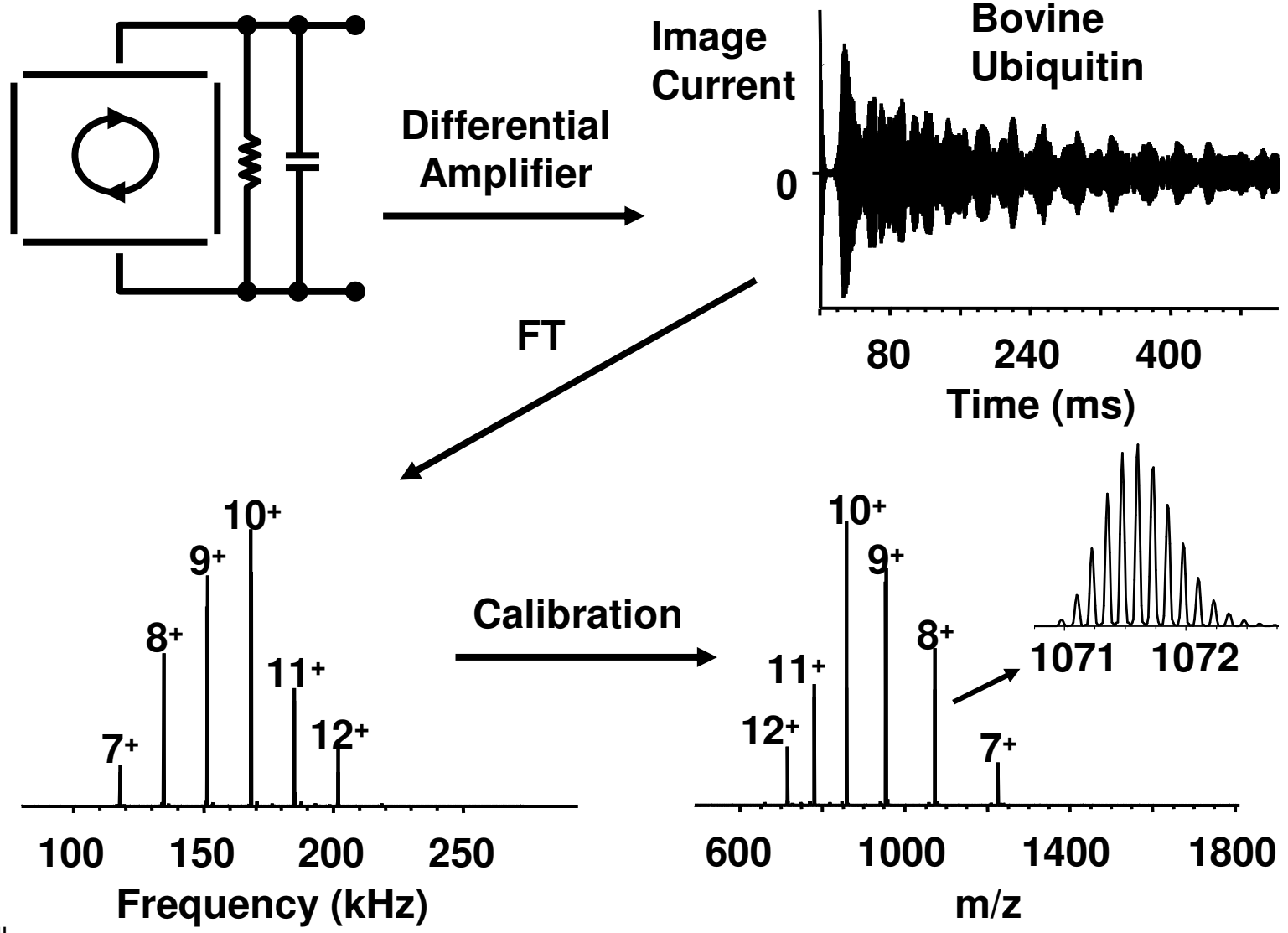
- Ions trapped and measured in ultrahigh vacuum inside a superconducting magnet.





$$\omega \propto \frac{1}{m/z}$$

Fourier Transform Ion Detection

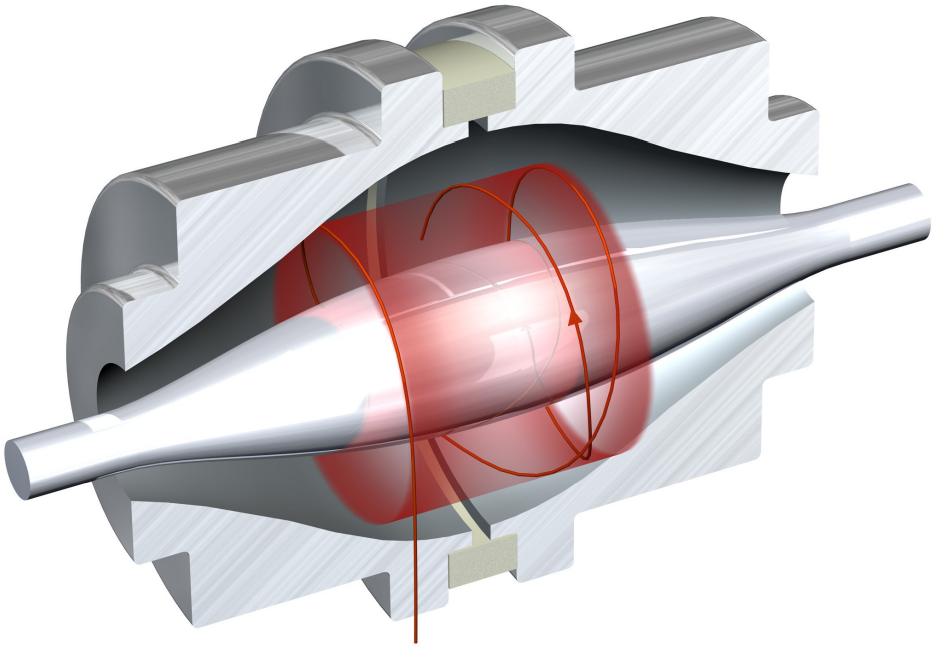


A.G. Marshall

Orbitrap

TOF
- Simultaneous excitation

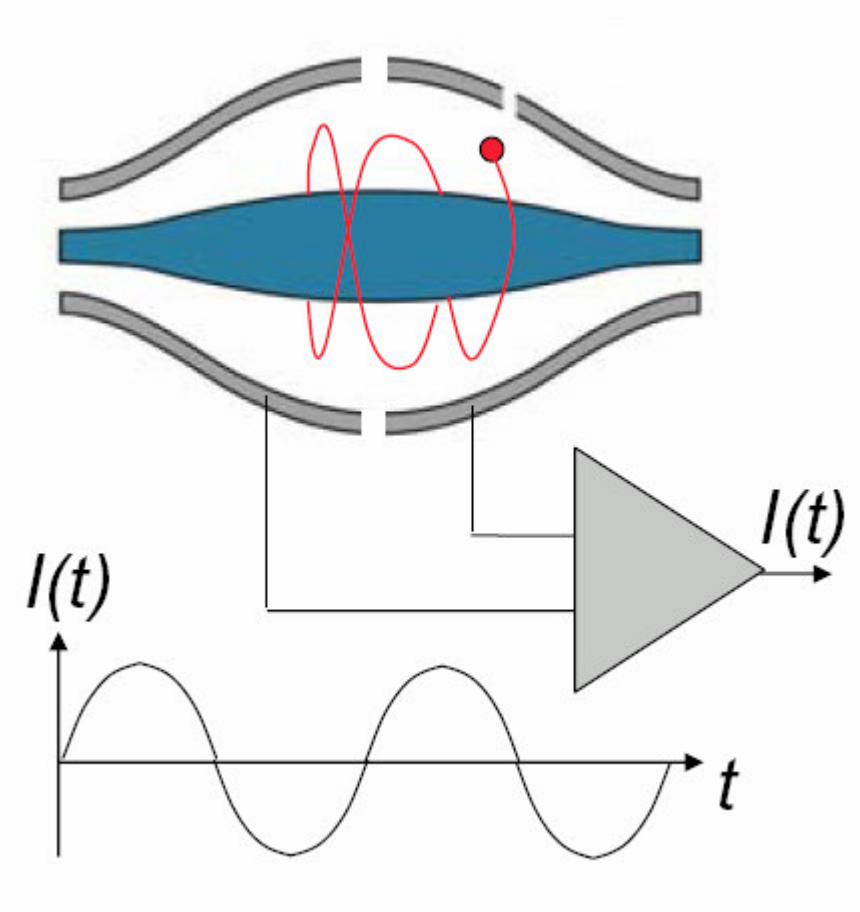
FTICR
- Confined ion trajectory
- Image current detection
- Fourier transform data conversion



Unique to Orbitrap
- 3D electric field trapping
- No need for magnet
- Easy access
- Final detection device

Alexander Makarov, Anal. Chem. 2000, 72, 1156-1162

Image Current Detection in Orbitrap



From Alexander Makarov's 2008 ASMS Award Address

Comparison of Analyzer Types

	Ion Trap/ Quadrupole	TOF	OrbiTrap	FT-ICR
Sensitivity	+++	++* to +++	++*	+*
Mass Accuracy	+**	++	+++	+++**
Resolving Power	+**	++	+++	++++**
Dynamic Range	+ to +++**	++	+++	++**
Upper m/z	+	++++	+++	++

*Sensitivity lowered due to losing ions on way to analyzer, rather than inherent sensitivity.
**Can be improved by scanning narrower mass range or slower.

Hybrid/Tandem Instruments

- Combine (1) ion selection, (2) ion dissociation, and (3) mass analyzer devices
  - Quadrupoles and ion traps good for selective isolation of precursor ions and for fragmentation (required for MSMS - Topic of Lecture 2)
  - Reflectron TOF, FT-ICR, and OrbiTrap have higher mass accuracy and resolving power (high mass accuracy is good for identification – Lecture 3)

Ion Isolation

- Quadupole
  - Continuous ion beam

- Quadrupole ion trap
  - Pulsed-mode operation; space charge issue

- SWIFT in FTICR
  - Ultrahigh selectivity; only works well in ICR traps

- TOF
  - Only implemented on TOF/TOF

Ion Dissociation

•Collision Induced Dissociation (CID or Collision Activated Dissociation (CAD)

ion traps: off-resonance excitation

rf-only multi-poles: higher kinetic energy (HCD) and cascaded CID

TOF/TOF: single collision

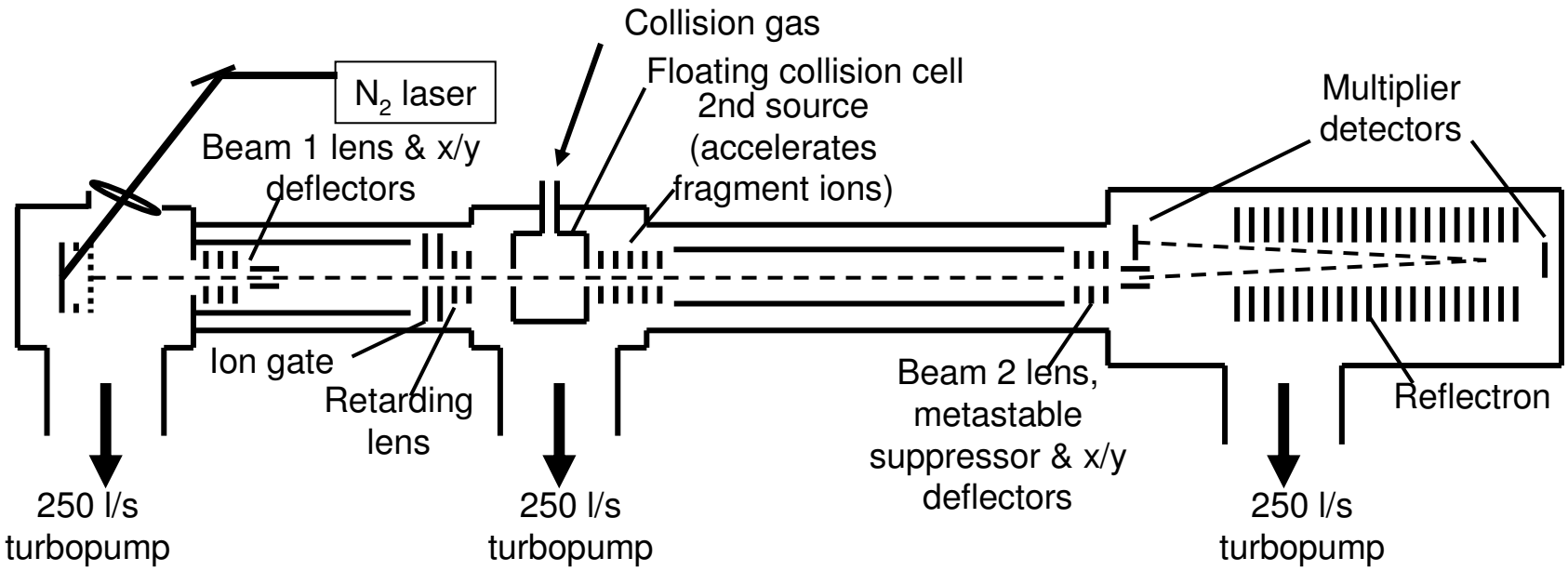
•Electron capture dissociation (ECD) and Electron transfer dissociation (ETD)

ECD: FTICR, reagent: electron

ETD: ion traps, reagent: free radical anion

Other important factors to consider: how product ions are collected and detected

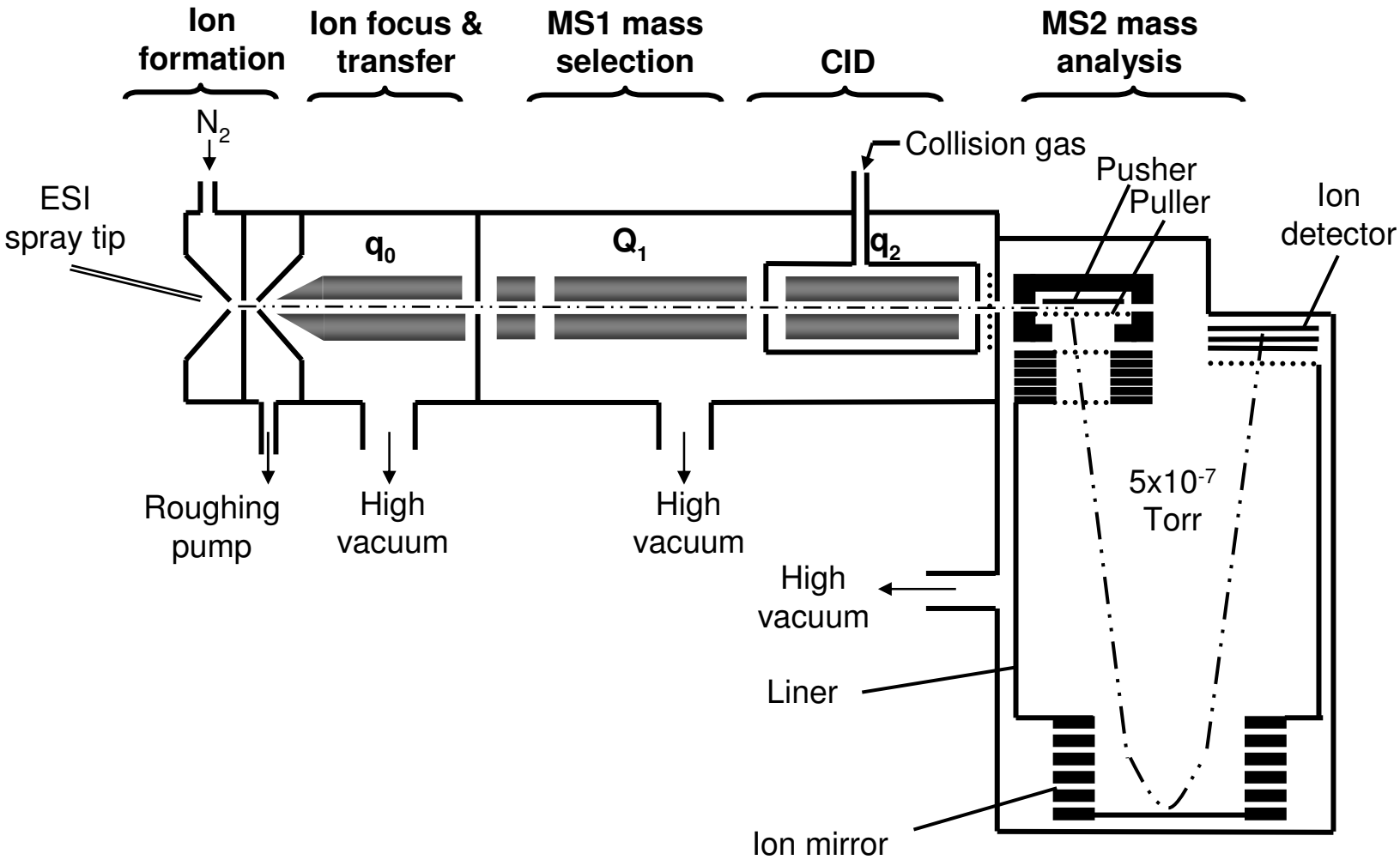
MALDI-TOF/TOF (4700 Proteomics Analyzer)



- High performance TOF analysis for MS1 and MS2 give high resolving power and good mass accuracy.
- High accelerating voltage allows high energy CID, giving a wider range of fragment ions and facilitating side-chain cleavages that distinguish isomeric amino acids Ile and Leu.

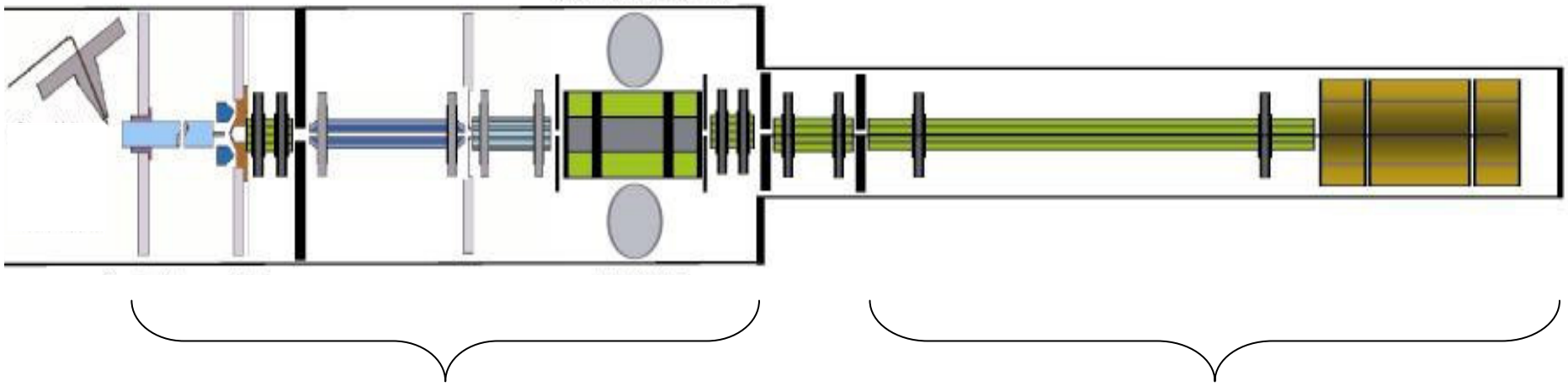
Marvin L. Vestal and Jennifer M. Campbell, Methods Enzymol. 2005 ;402 :79-108

Hybrid Instrument: QqTOF Mass Spectrometer (QSTAR)



Chernushevich, Loboda and Thomson, J. Mass Spectrom. 2001; 36: 849–865

Linear Ion Trap – FT-ICR (LTQ-FT)



Linear Ion Trap (LTQ)

Ion Cyclotron

John E. P. Syka, et al, Journal of Proteome Research 2004, 3, 621-62

Data Dependent Acquisition

- *Data Dependent Scans*

  MSMS based on intensity ranking of precursor ions

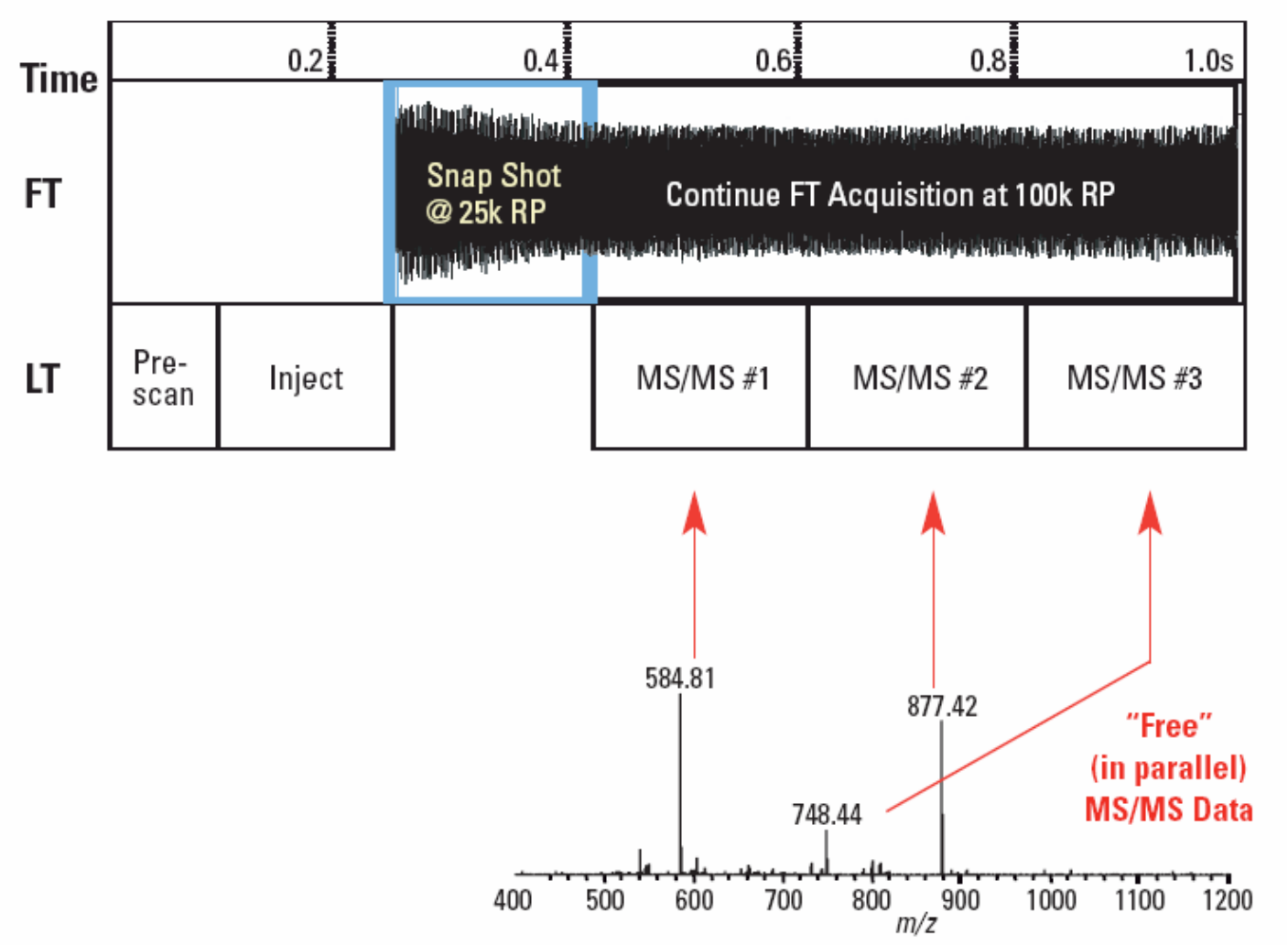
- *Dynamic Exclusion*

  Precursor m/z of previous MSMS are memorized and no MSMS done on them during a defined time period

- *Automatic Gain Control* (AGC, unique to ion trap)

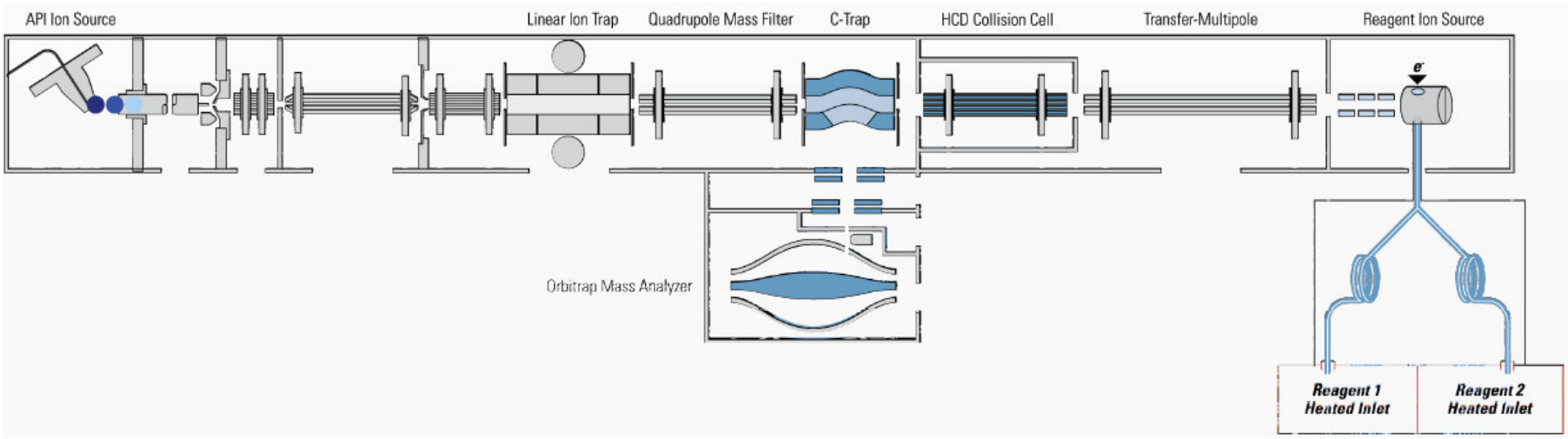
  Control how many ions are scanned – to achieve signal/noise ratio and to minimize space charge effect

Scan Sequence of LTQFT



Thermo Application Note: 30046

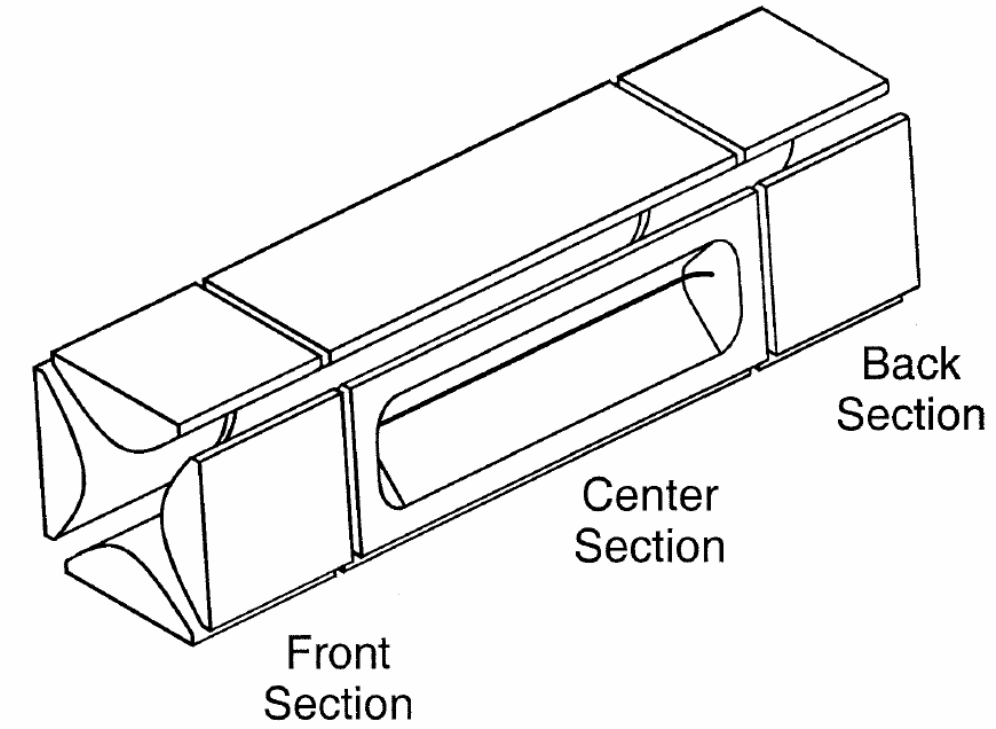
Linear Ion Trap - Orbitrap - ETD



Alexander Makarov, Stevan Horning, et al, Anal. Chem. 2006, 78, 2113-2120

G. C. McAlister, et al, J Proteome Res. 2008, 7, 3127-36

Linear quadrupole ion trap (LTQ) video clip



File_146.exe

J. Schwartz, et al, JASMS2002v13p659

Have you filled out the attendance sheet?

I will e-mail a pdf covering the topics of this introduction to all addresses on this list.

Mass Spectrometry Online Resources

NIH NCRR Mass Spectrometry Facility, UCSF

http://ms-facility.ucsf.edu/

American Society for Mass Spectrometry (ASMS)

http://www.asms.org

Molecular & Cellular Proteomics

http://www.mcponline.org

Ninth International Symposium on Mass Spectrometry in the Health and Life Sciences: Molecular and Cellular Proteomics

http://ms-facility.ucsf.edu/symposium/

August 23-27, 2009

Hotel Nikko, San Francisco

Abstract submission deadline is June 12, 2009

Early registration now open until June 12, 2009