

Mass Spectrometry and Proteomics

Lecture 1

30 March, 2010

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Lectures: 10hrs:

12noon-2pm

Course Outline

	Date	Topic
Lecture1	Tuesday, March 30	Mass Spectrometry Fundamentals: Instrumentation; ion optics, resolution and mass accuracy
Lecture2	Wednesday, March 31	MS based methodology in system biology proteomics - sample preparation gel-based/LC methods - topdown/bottom up Microfluidics and MS
Lecture3	Tuesday, April 6	Posttranslational modifications phosphorylation glycosylation/O-GlcNAc ubiquitination methylation/acetylation - histones cross-linking/other chemical biology methods.
Lecture4	Friday, April 9	Infomatics for MS preprocessing - deisotoping simulated spectra/spectral libraries database search engines denovo sequencing algorithms
Lecture5	Tuesday, April 13	Quantification chemical/metabolic labeling label free H/D exchange/protein turnover

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.

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., $^1\text{H}=1.007825$, $^{12}\text{C}=12.000000$, $^{16}\text{O}=15.994915$, 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 averaged masses (“isotope abundant weighted”) of the constituent elements 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 ($\text{H}=1.0080$, $\text{C}=12.011$, $\text{O}=15.994$, 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	Isotope	Mass	Natural Abundance
H	^1H	1.0078	99.99%
	^2H	2.0141	0.015
C	^{12}C	12	98.89
	^{13}C	13.0034	1.11
N	^{14}N	14.0031	99.64
	^{15}N	15.0001	0.36
O	^{16}O	15.9949	99.76
	^{17}O	16.9991	0.04
	^{18}O	17.9992	0.2
P	^{31}P	30.9737	100
S	^{32}S	31.9721	95
	^{33}S	32.9715	0.76
	^{34}S	33.9679	4.22
	^{36}S	35.9671	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)

EGVNDNEEGFFSAR



$^{12}\text{C}_{66}\text{H}_{95}\text{N}_{19}\text{O}_{26}$
Monoisotopic Mass
1569.66956

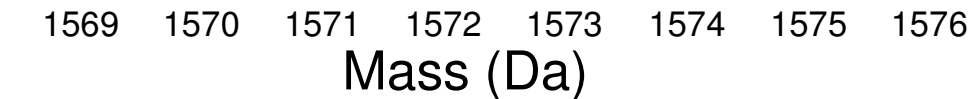
Average Mass
1570.5722

No ^{13}C atom

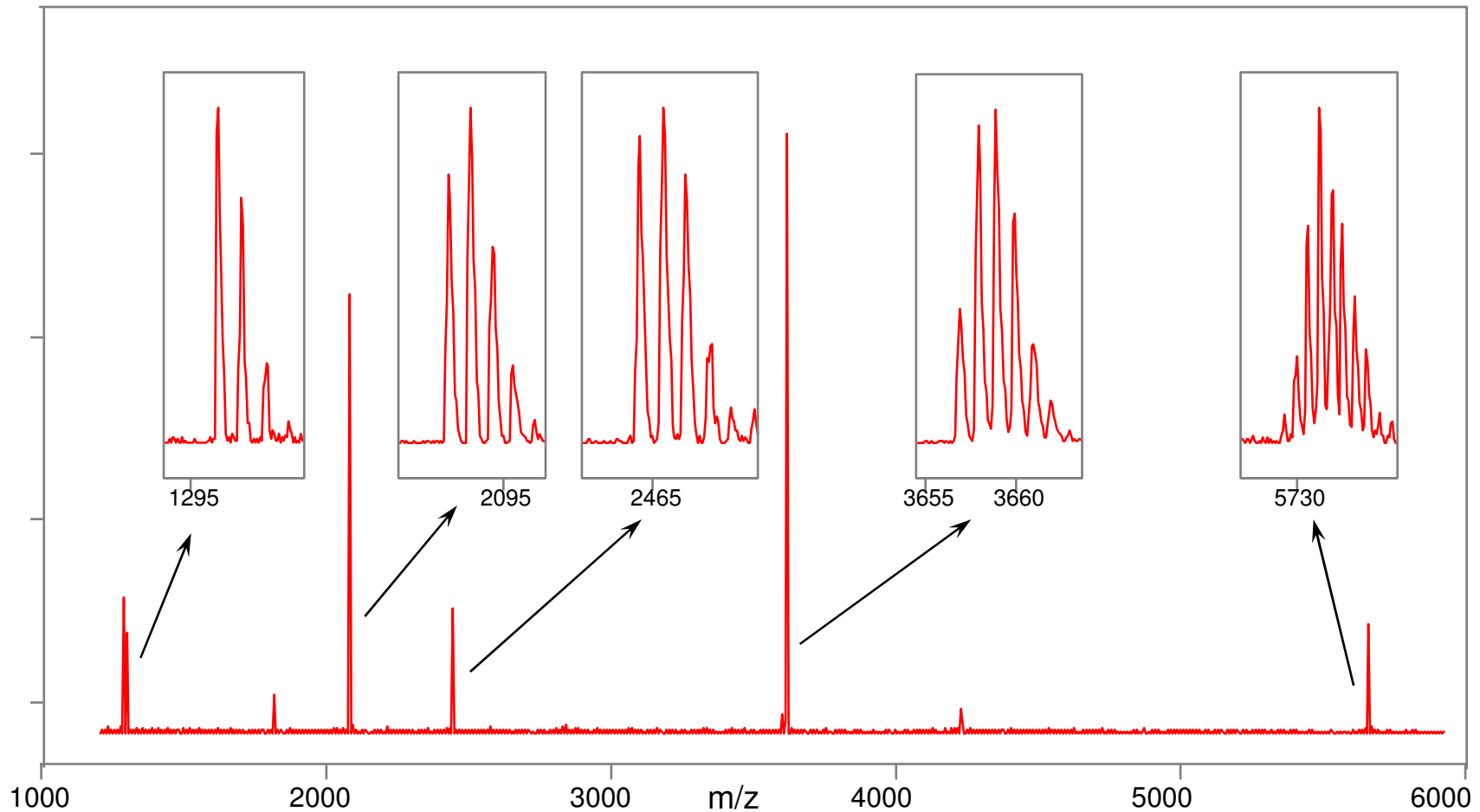
$^{12}\text{C}_{65}\text{ }^{13}\text{C}\text{H}_{95}\text{N}_{19}\text{O}_{26}$ etc.
One ^{13}C atom

Two ^{13}C atoms

$\Delta m \approx 1$



Isotope Pattern Changes with Mass

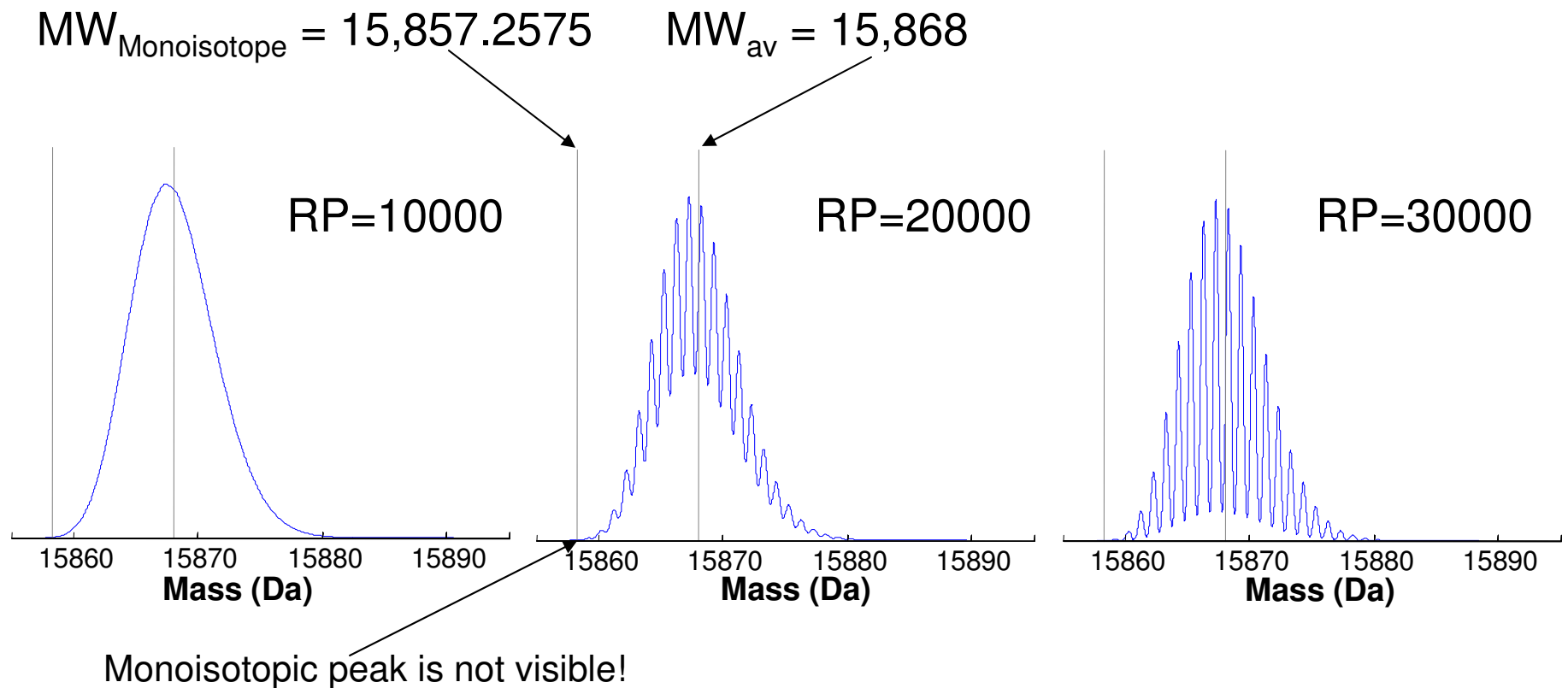
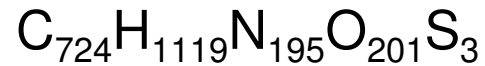


As the number of C-atoms in the molecule increases, the peaks due to higher mass isotopes increase in relative abundance. Data are for a series of peptides.

Protein Mass Measurement

- Protein masses are normally reported as average masses

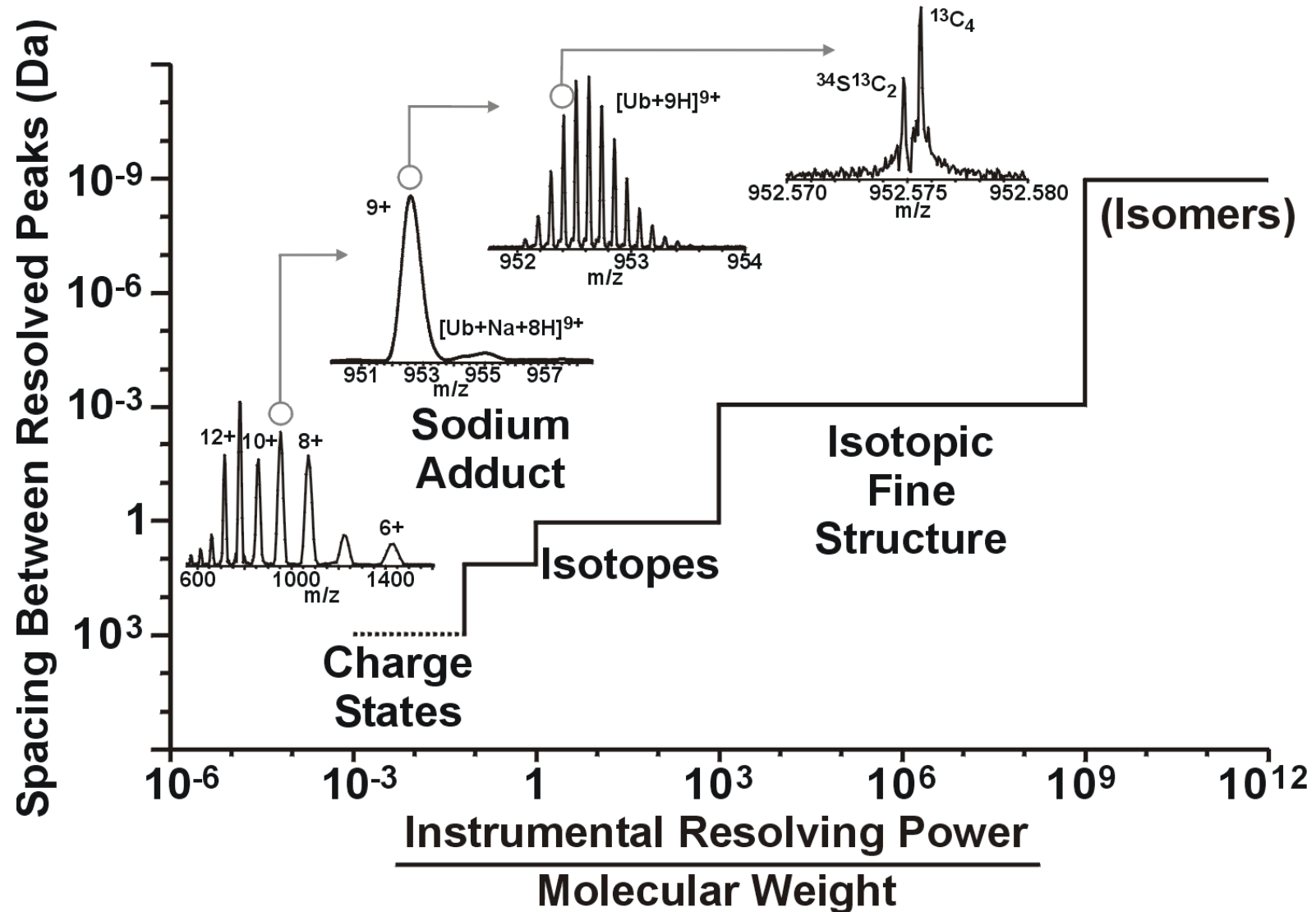
Effect of different resolving power on Hemoglobin beta chain peak,



Information from MS Resolution

Bovine Ubiquitin

MW_{monoisotopic} = 8559.6158 Da



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

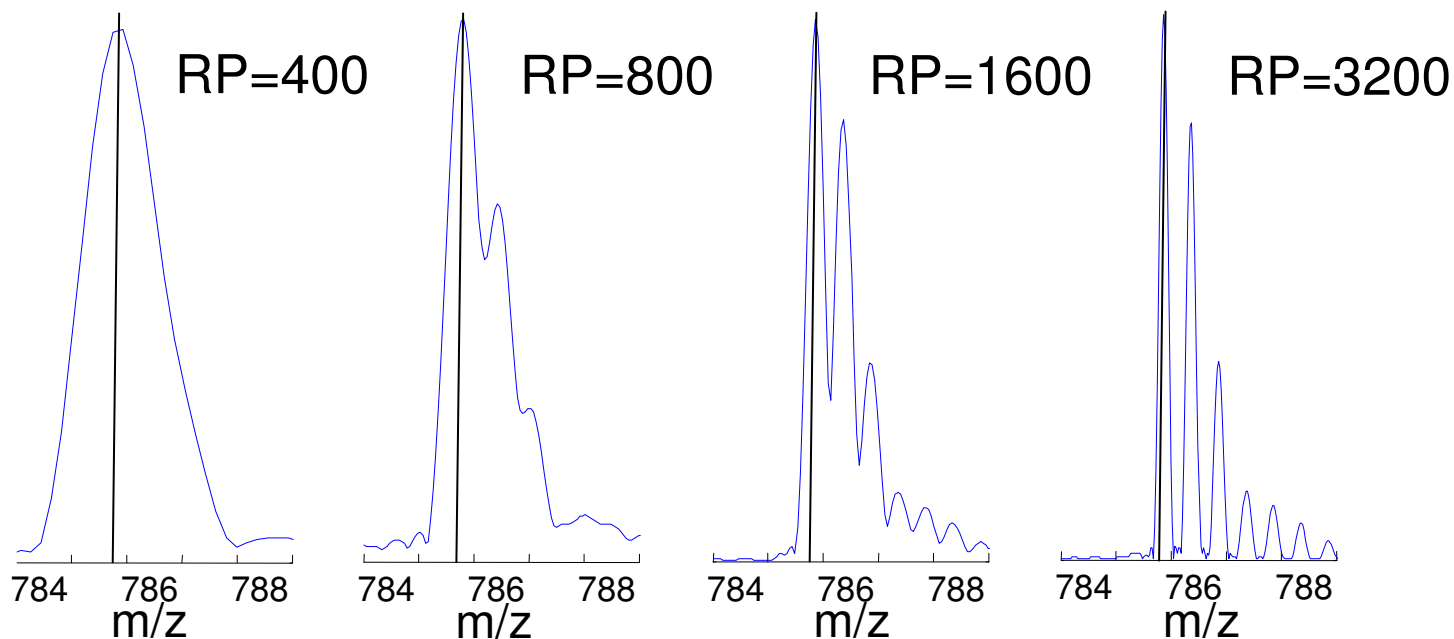
- Determine charge state. Resolve mixtures. High resolving can also improve mass accuracy.

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

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

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, $MA = M_{\text{exp}} - M_{\text{theo}}$, in Da or milli-Da

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

Example:

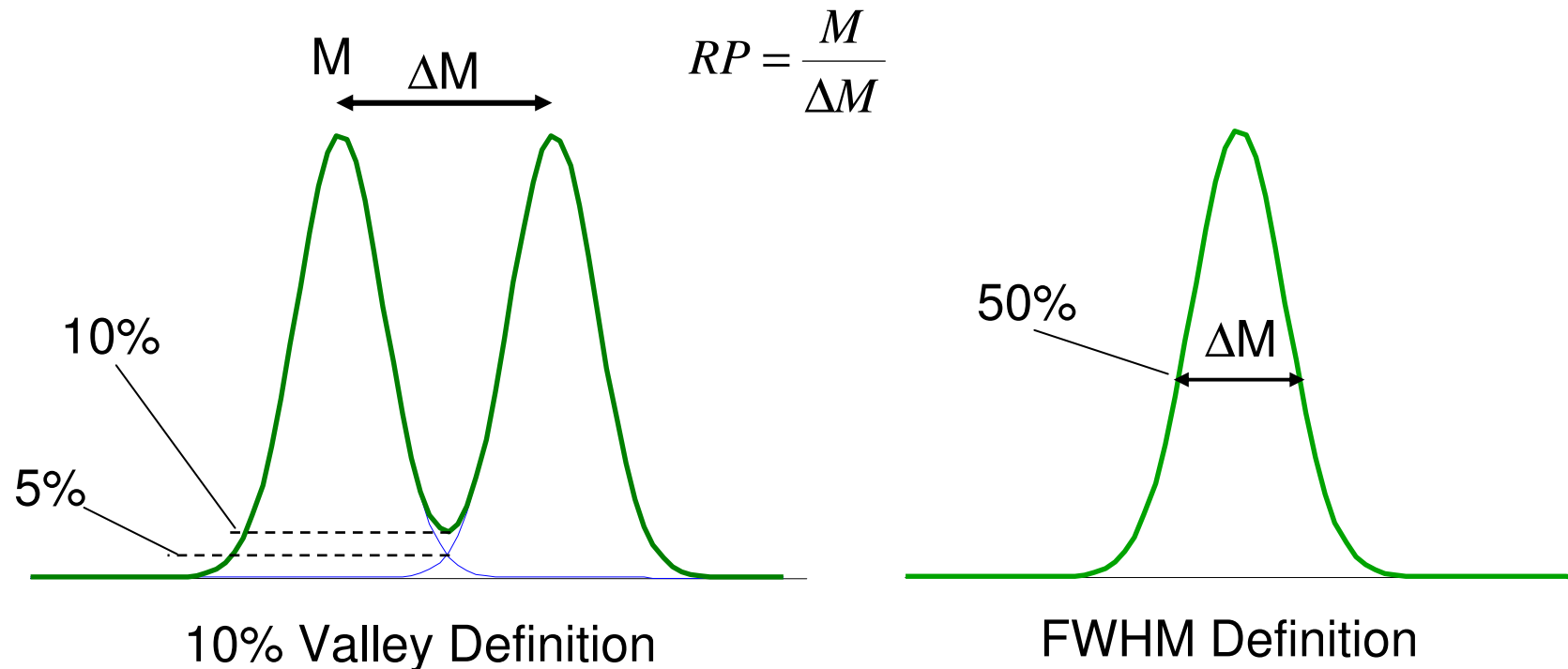
$$M_{\text{exp}} = 1569.684$$

$$M_{\text{theo}} = 1569.66956$$

Mass Measurement Error = 0.014Da or 9.2ppm

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).



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 $\Delta(m/z)$,

$z=1/\Delta(m/z)$ or more accurately $z=1.00235/\Delta(m/z)$

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]

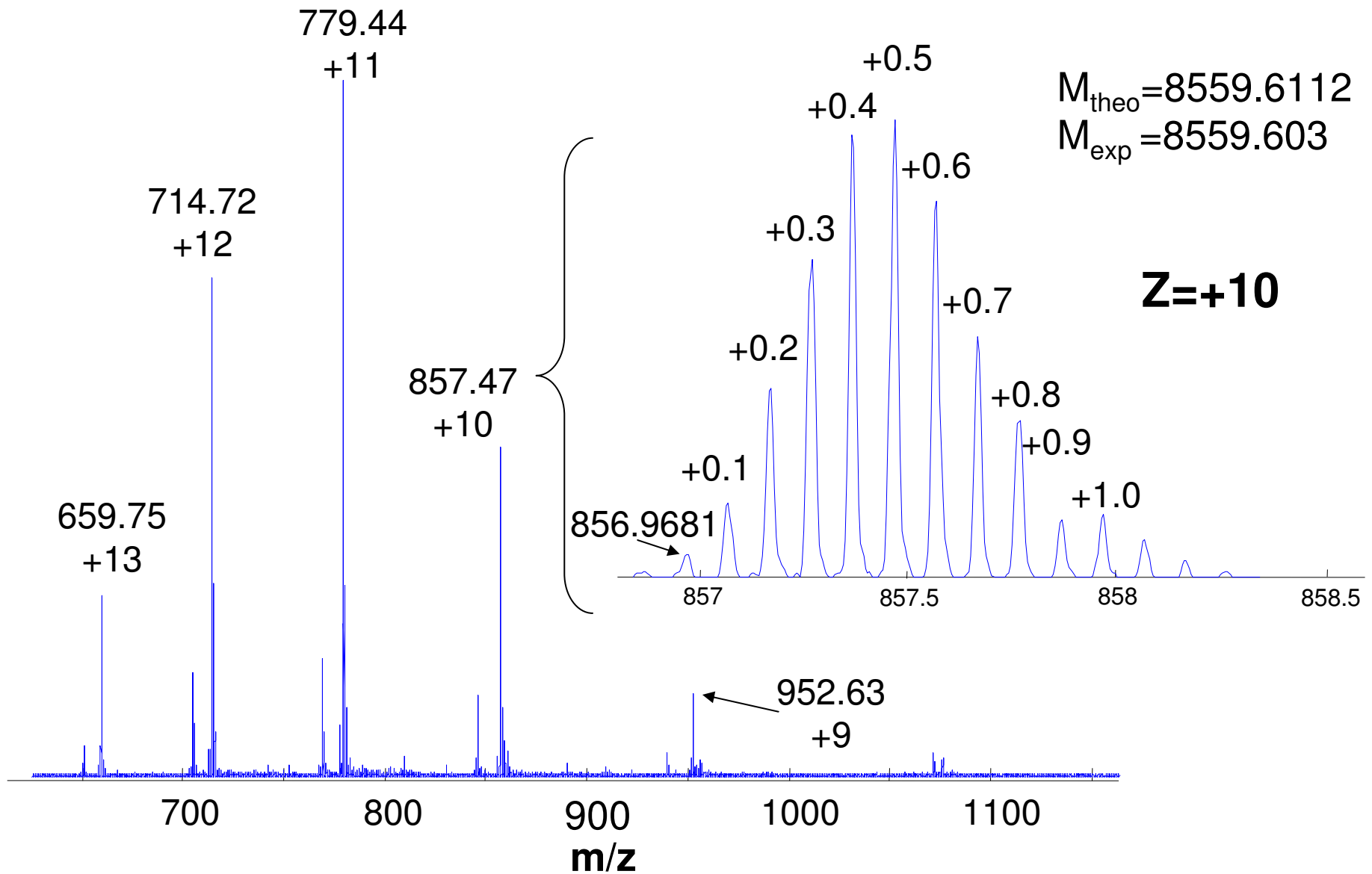
Solve the following linear equations

for z (for $(m/z)_1$) and M (neutral mass)

$$(m/z)_1 X z - z = M$$

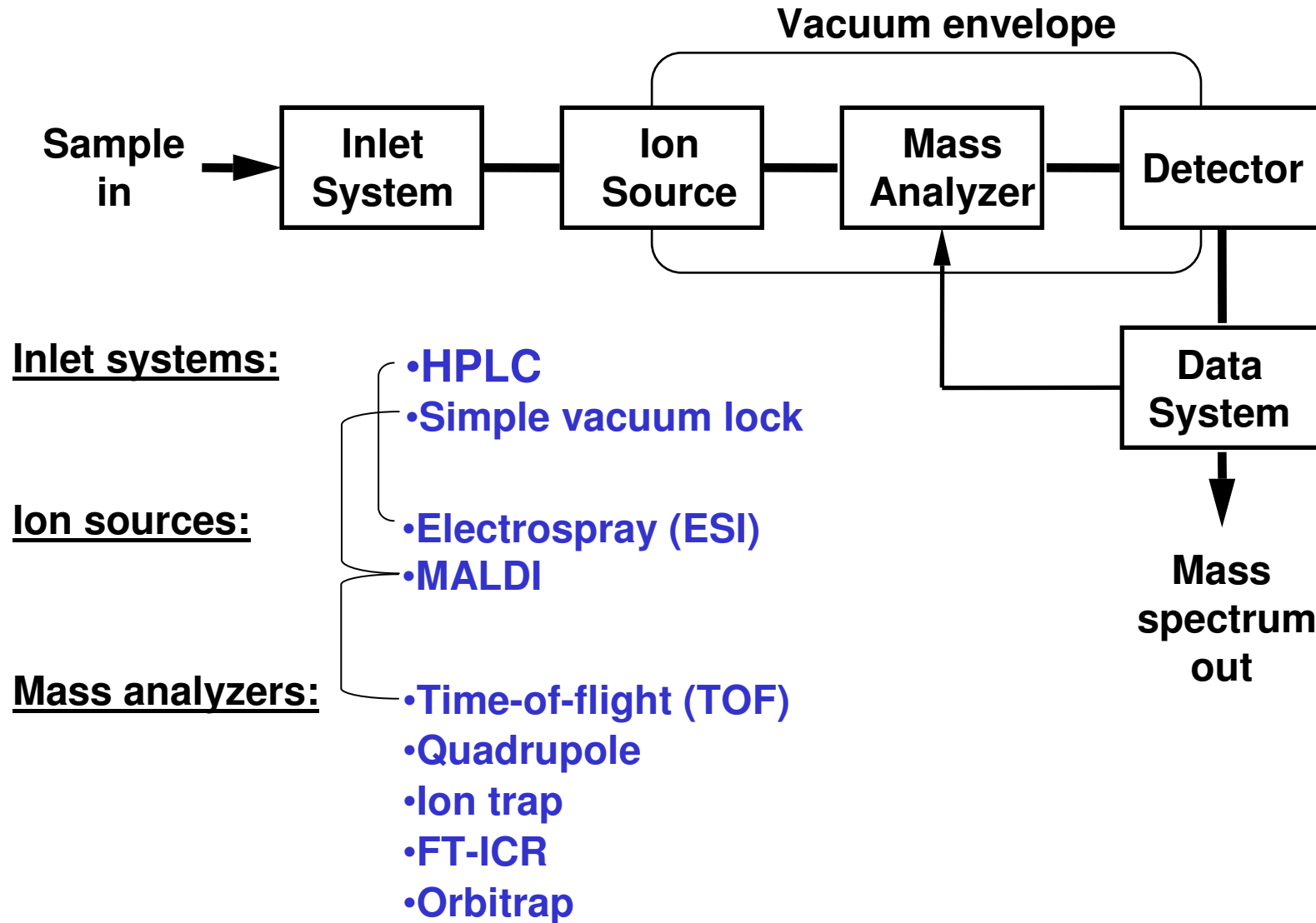
$$(m/z)_2 X (z-1) - (z-1) = M$$

Electrospray Mass Spectrum of Bovine Ubiquitin



Instrumentation

Mass Spectrometer Schematic



Ion sources

MALDI & ESI

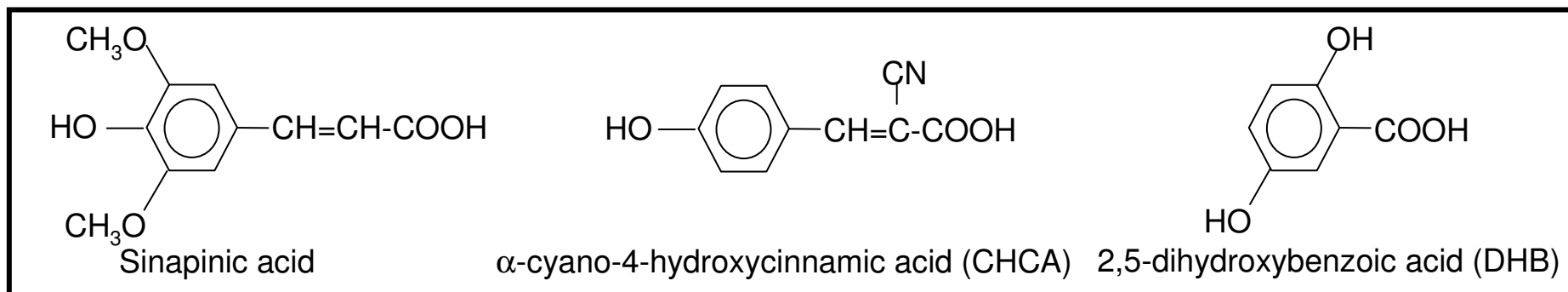
Matrix-Assisted Laser Desorption/Ionization (MALDI)

- 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:

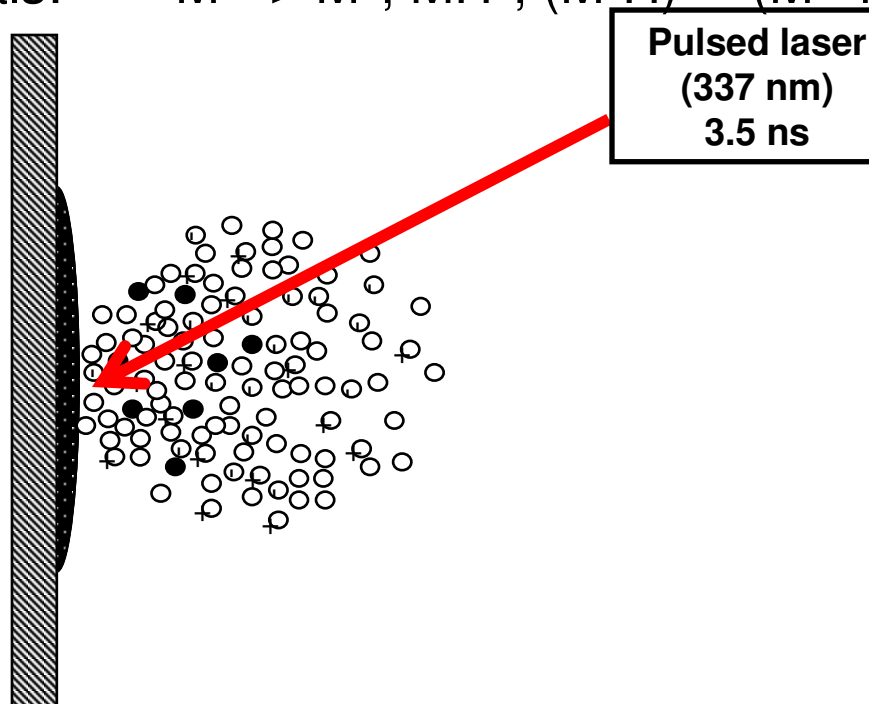
- It must co-crystallize with typical analyte molecules
- It must absorb radiation at the wavelength of the laser (usually 337 nm)
- To transfer protons to the analyte it should be acidic

Typical successful matrices for UV MALDI are aromatic carboxylic acids.

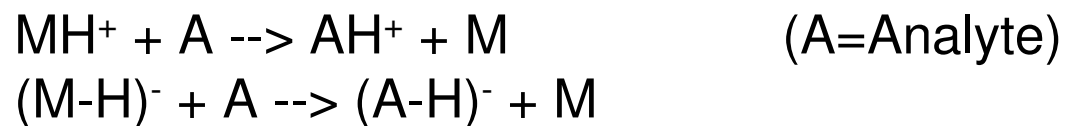


MALDI Ionization Mechanism

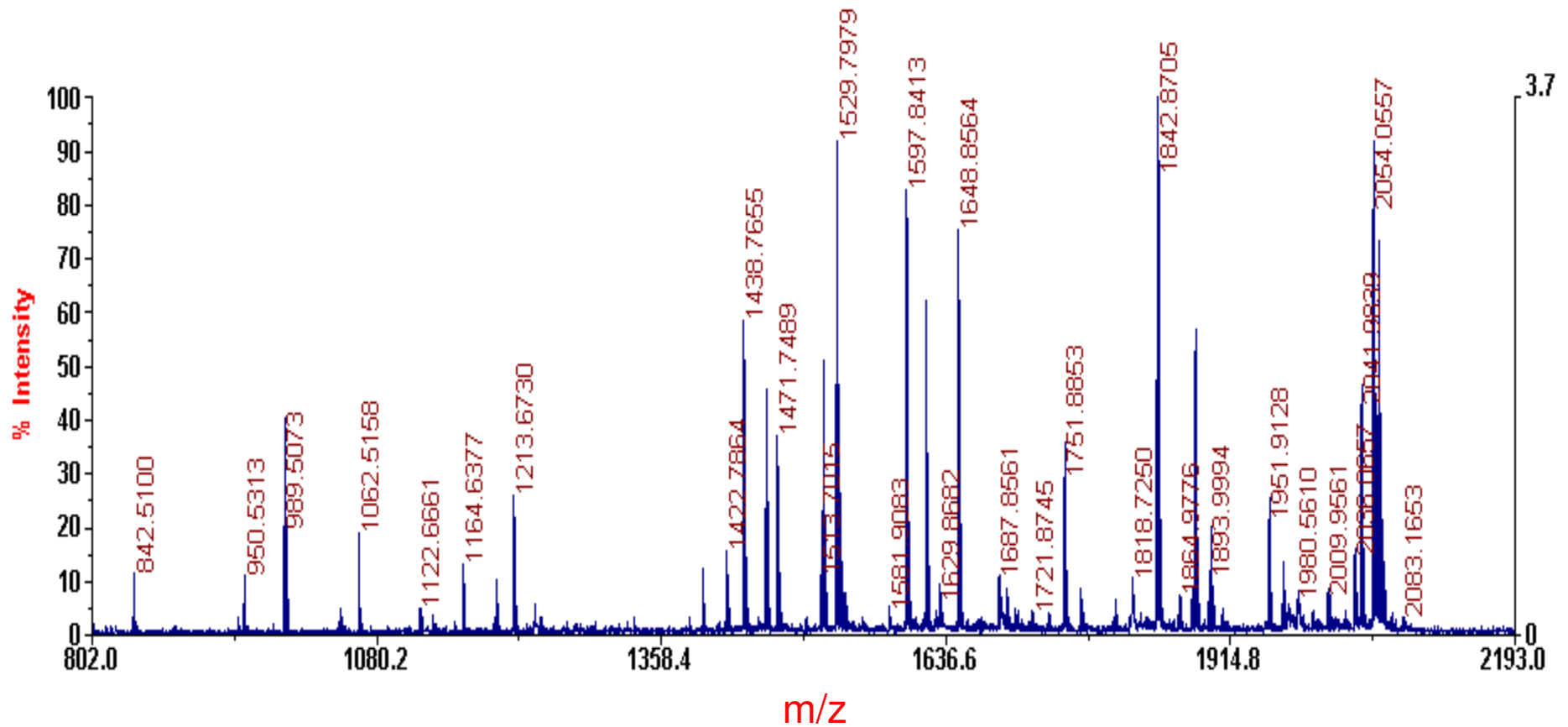
1. Laser pulse produces matrix neutrals, + and - ions, and sample neutrals:
sample neutrals: $M \rightarrow M^*, MH^+, (M-H)^-$ (M= Matrix)



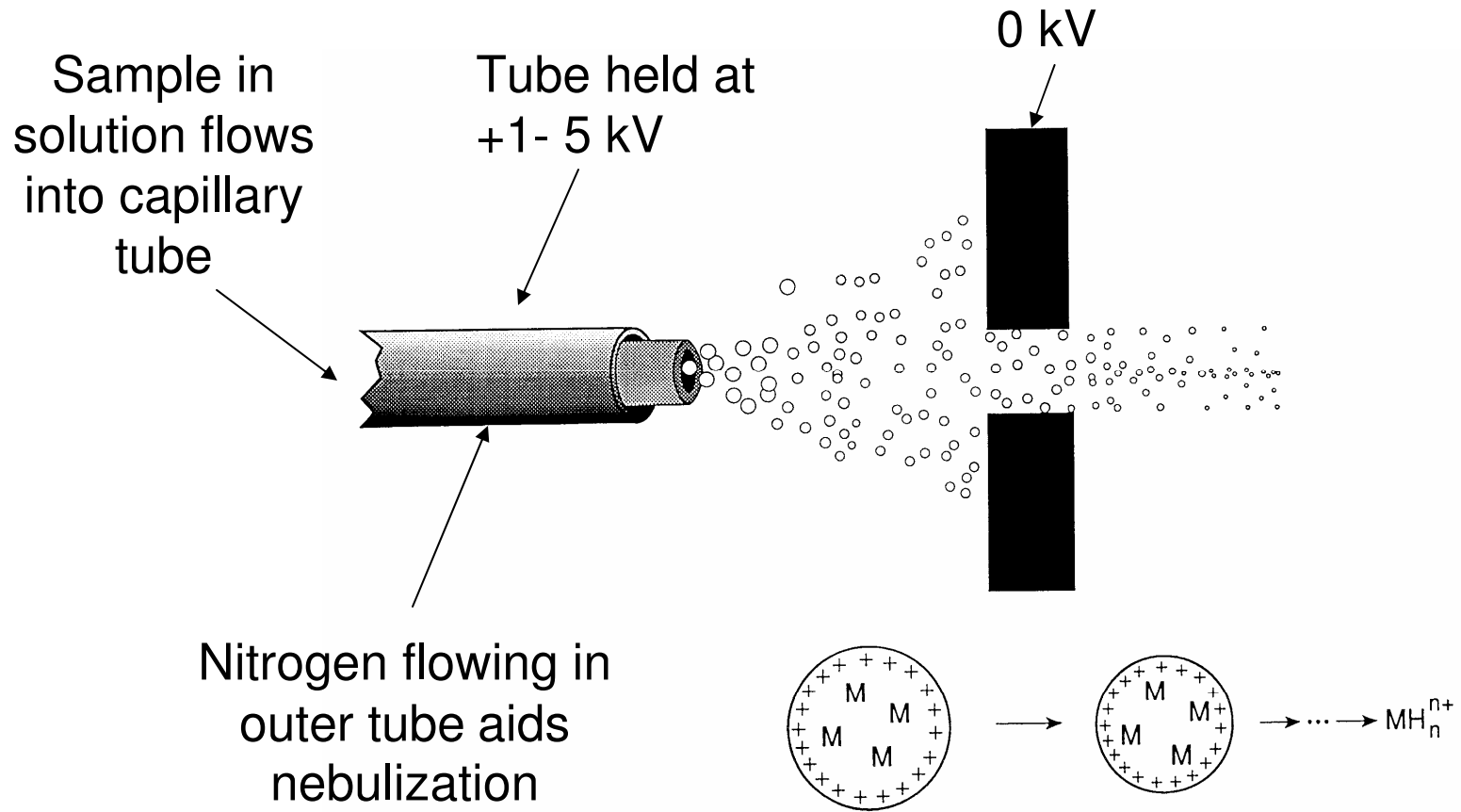
2. Some analyte molecules are ionized by gas-phase proton transfer:



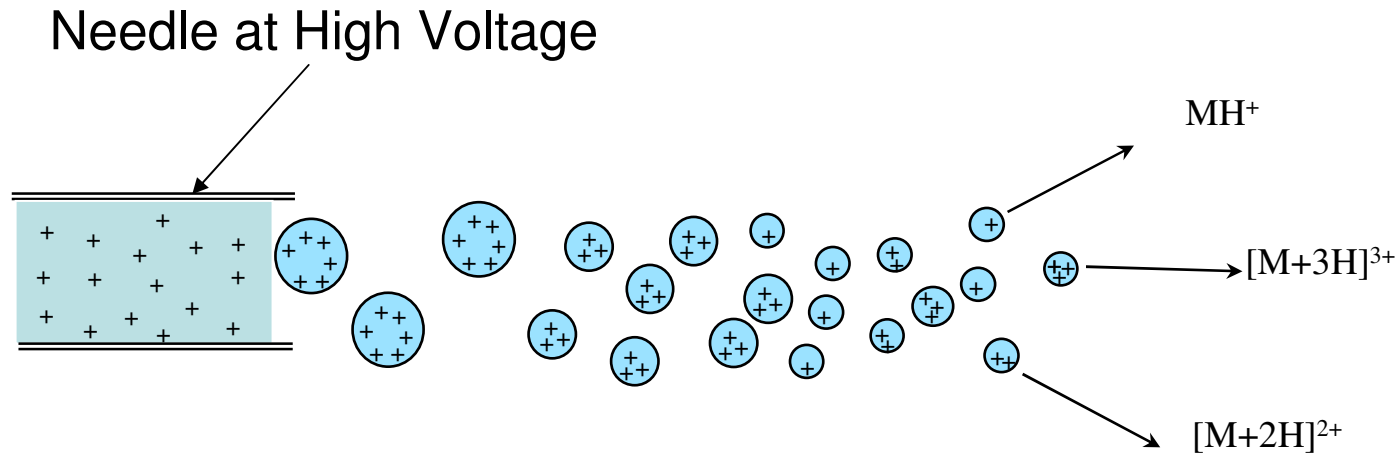
MALDI Mass Spectrum of Protein Tryptic Digest



Electrospray Ionization



Electrospray Ion Formation



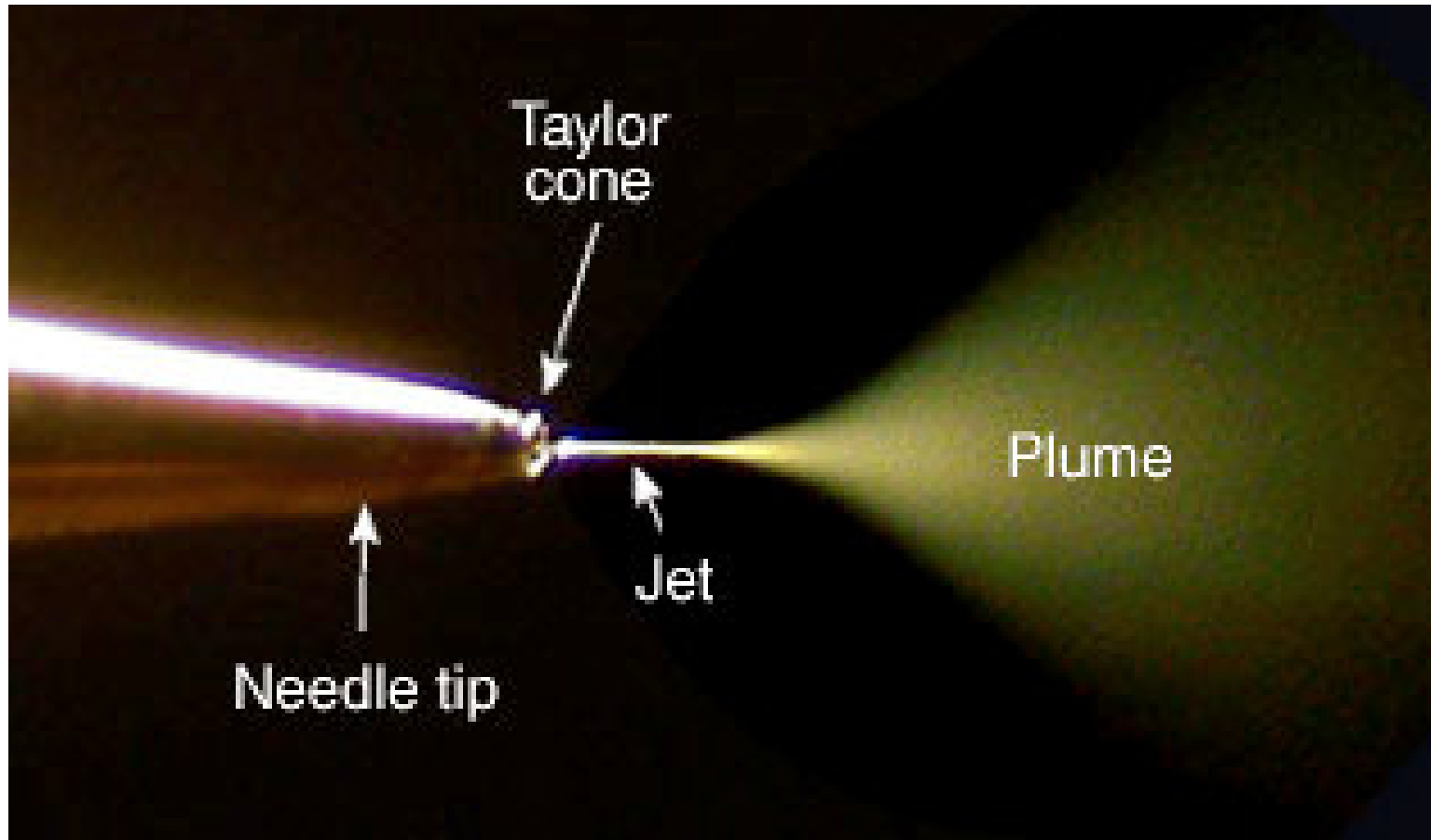
Droplets formed in electric field have excess positive ions.

Evaporation of neutrals concentrates charge.

Droplets break into smaller droplets.

Eventually one molecule + n protons is left.

Electrospray ionization



Nanospray

ESI: 1-100 μ L/min flow

Online analysis

~ 20 μ m tip ID

Interface with nanoLC

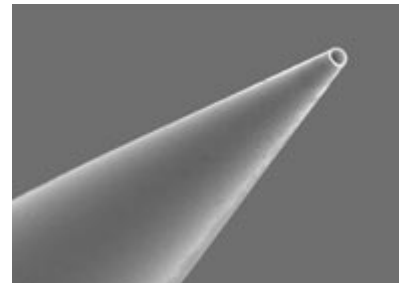
Flow rate: ~300nL/min

Offline analysis (static infusion)

~ 2 μ m tip ID

Flow rate: ~40nL/min

Requires pure sample free from salt



New Objective, Inc.

Ionization Methods for Biomolecule Analysis

Electrospray	MALDI
<ul style="list-style-type: none">• Online LC/MS possible• Poor for mixtures without LC• Quantitation possible• Good for MW <600• Generate highly charged ions	<ul style="list-style-type: none">• Very long sample lifetime; repeated measurements possible• Good for mixtures• Salt tolerant• Matrix peaks can interfere at MW <600 <p>Generate ions with few charges</p>

Mass analyzers

TOF

Quadrupole

Ion Trap

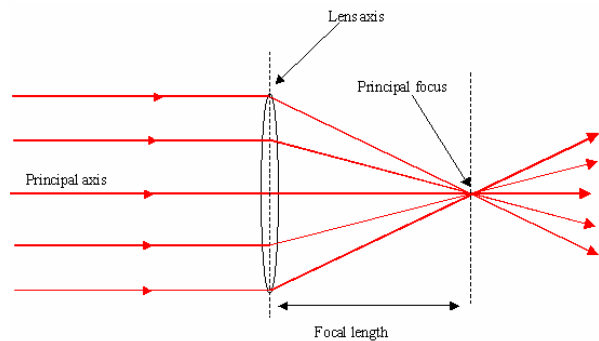
FTICR

Orbitrap

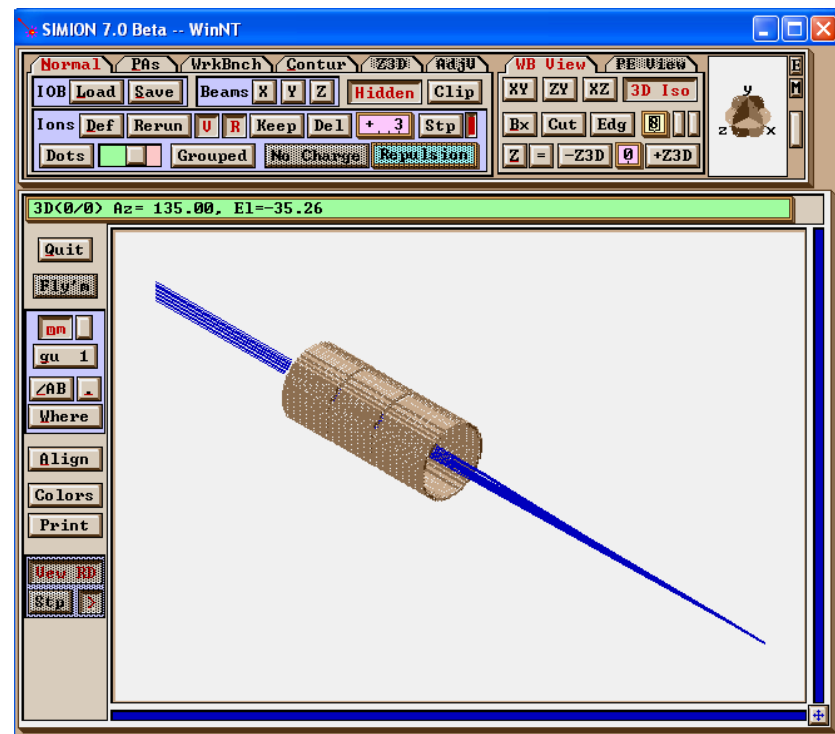
Ion Optics

A device for manipulating ion beams.

A mass spectrometer consists of many *ion optical* components

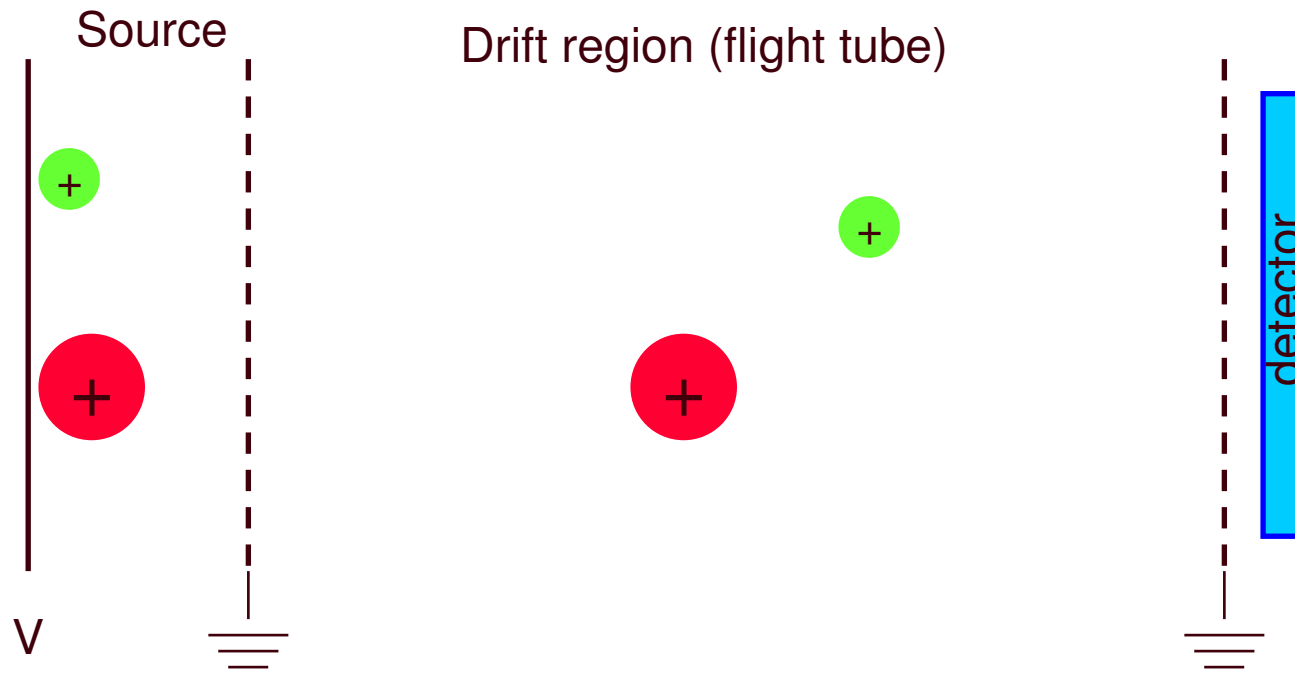


Einzel lens modeled with SIMION ion optics simulation program (computing electric and magnetic fields and ion trajectories)



<http://simion.com/>

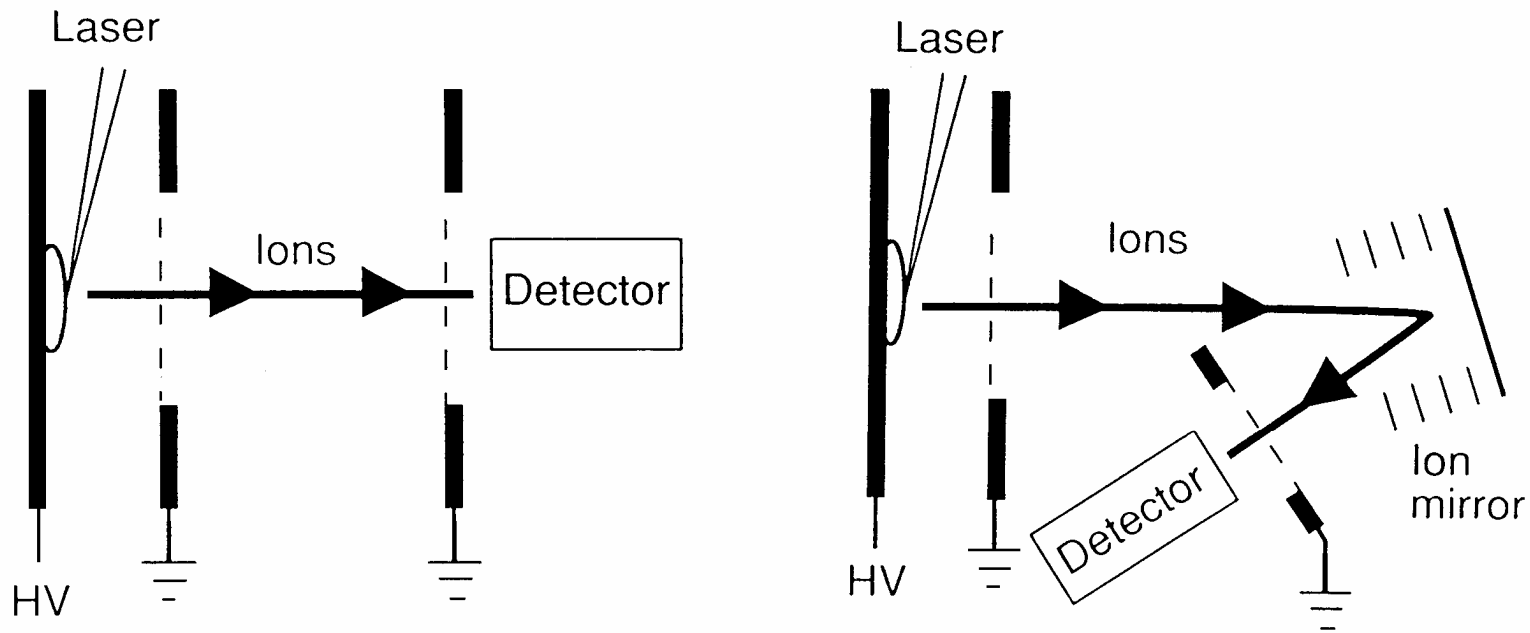
Time-of-Flight (TOF) Mass Analyzer



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

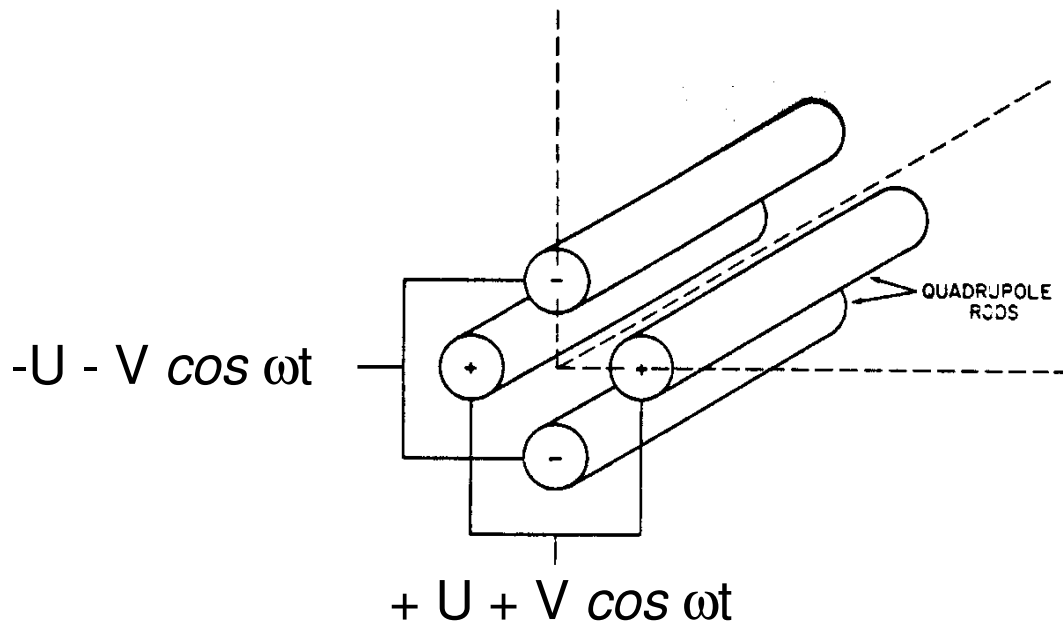
$$m/z = \frac{2t^2V}{L^2} \quad \text{or} \quad t \propto \sqrt{m/z}$$

Linear and Reflector TOF Analyzers



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

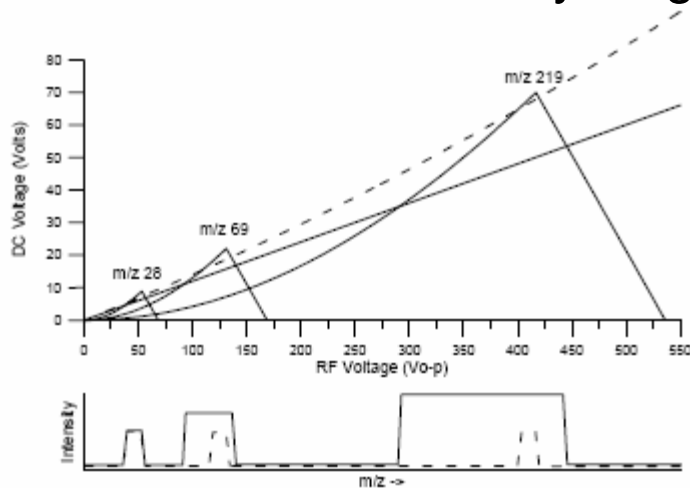
Quadrupole Mass Analyzer/Filter



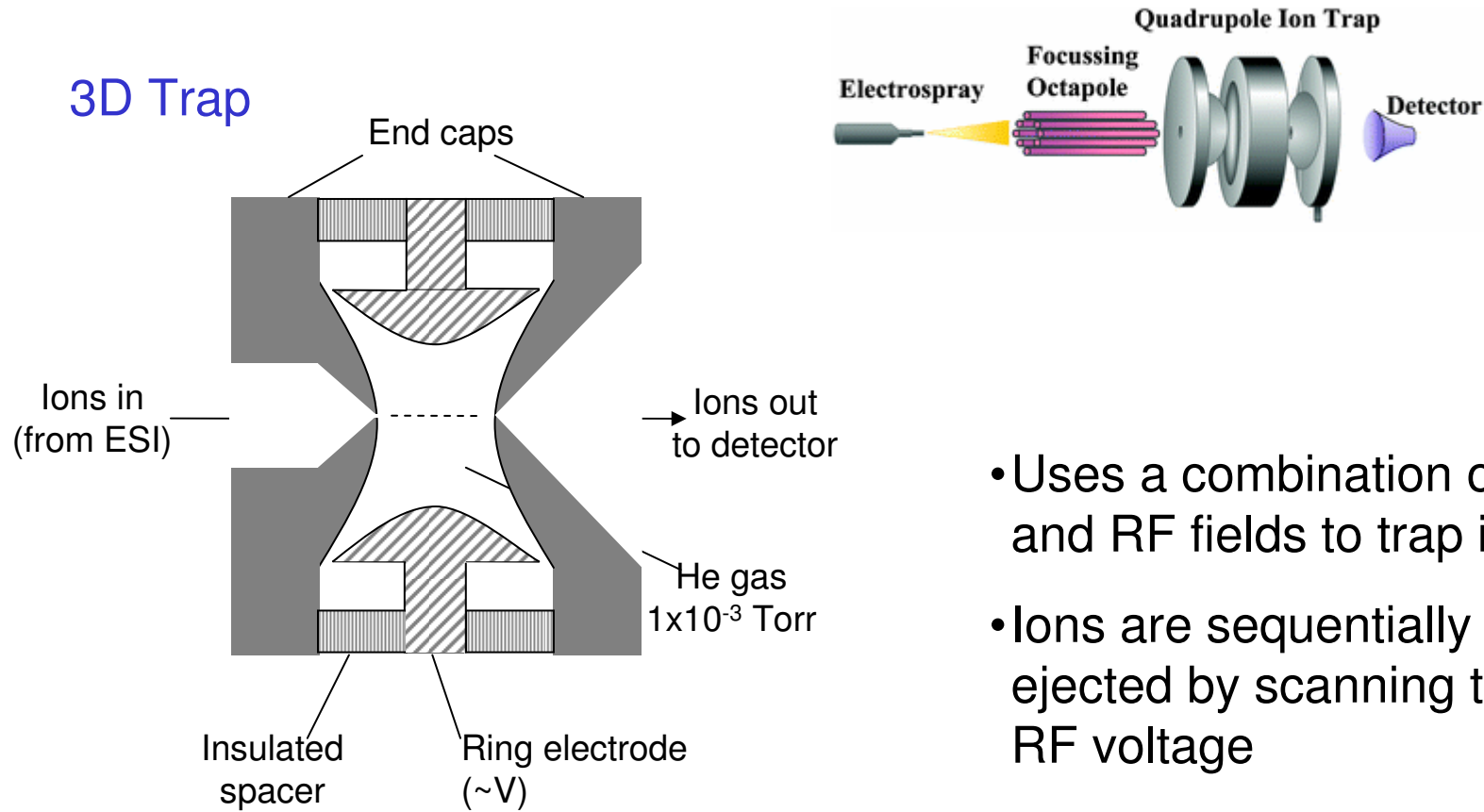
Uses a combination of RF and DC voltages to operate as a mass filter.

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

Mass scan and stability diagram



Quadrupole Ion Trap

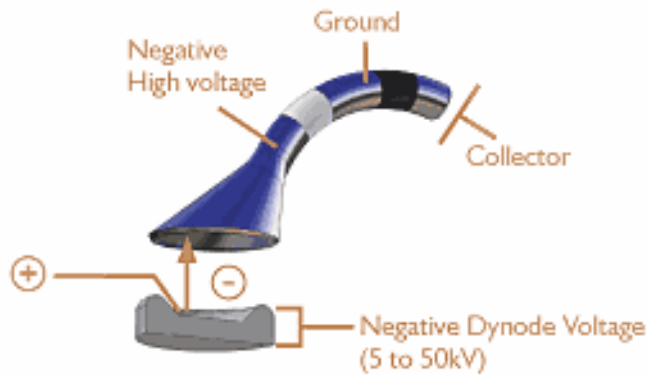


- Uses a combination of DC and RF fields to trap ions
- Ions are sequentially ejected by scanning the RF voltage

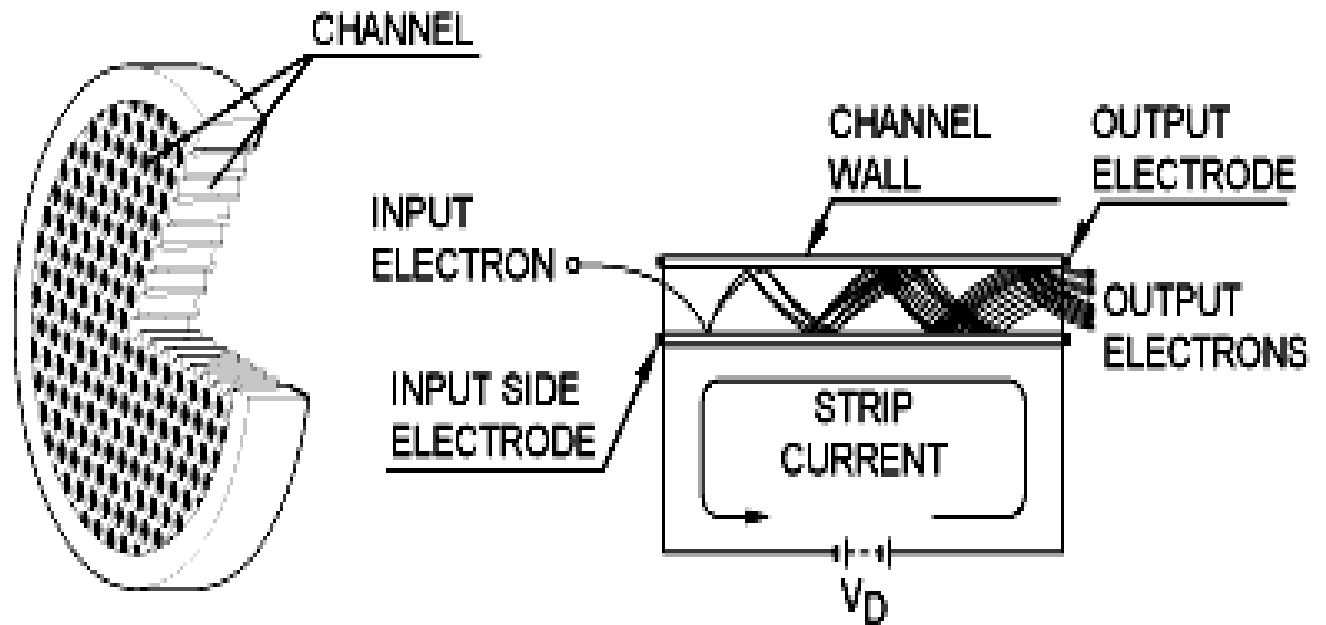
Linear Trap

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

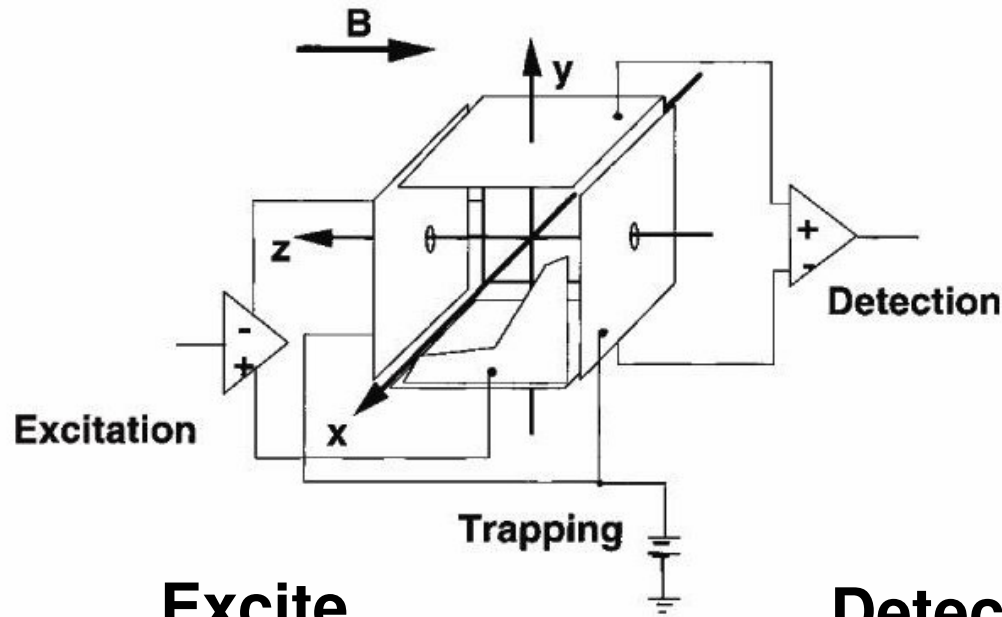
Electron Multiplier



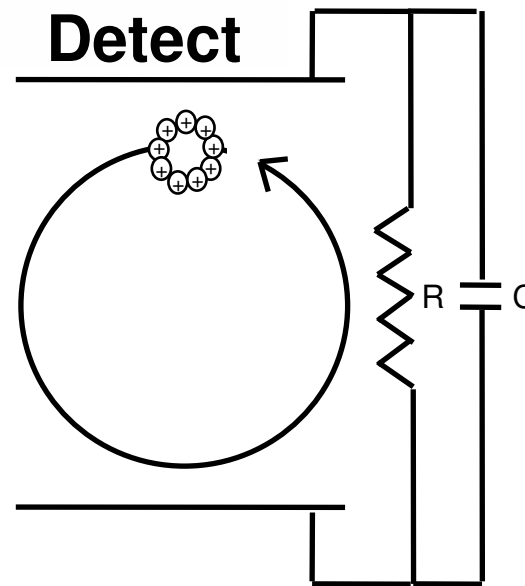
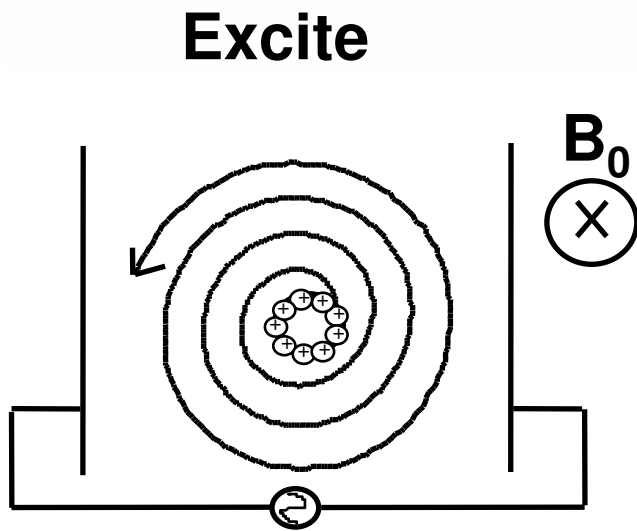
Multi-Channel Plate (MCP)



Fourier Transform Ion Cyclotron Resonance (FT-ICR)

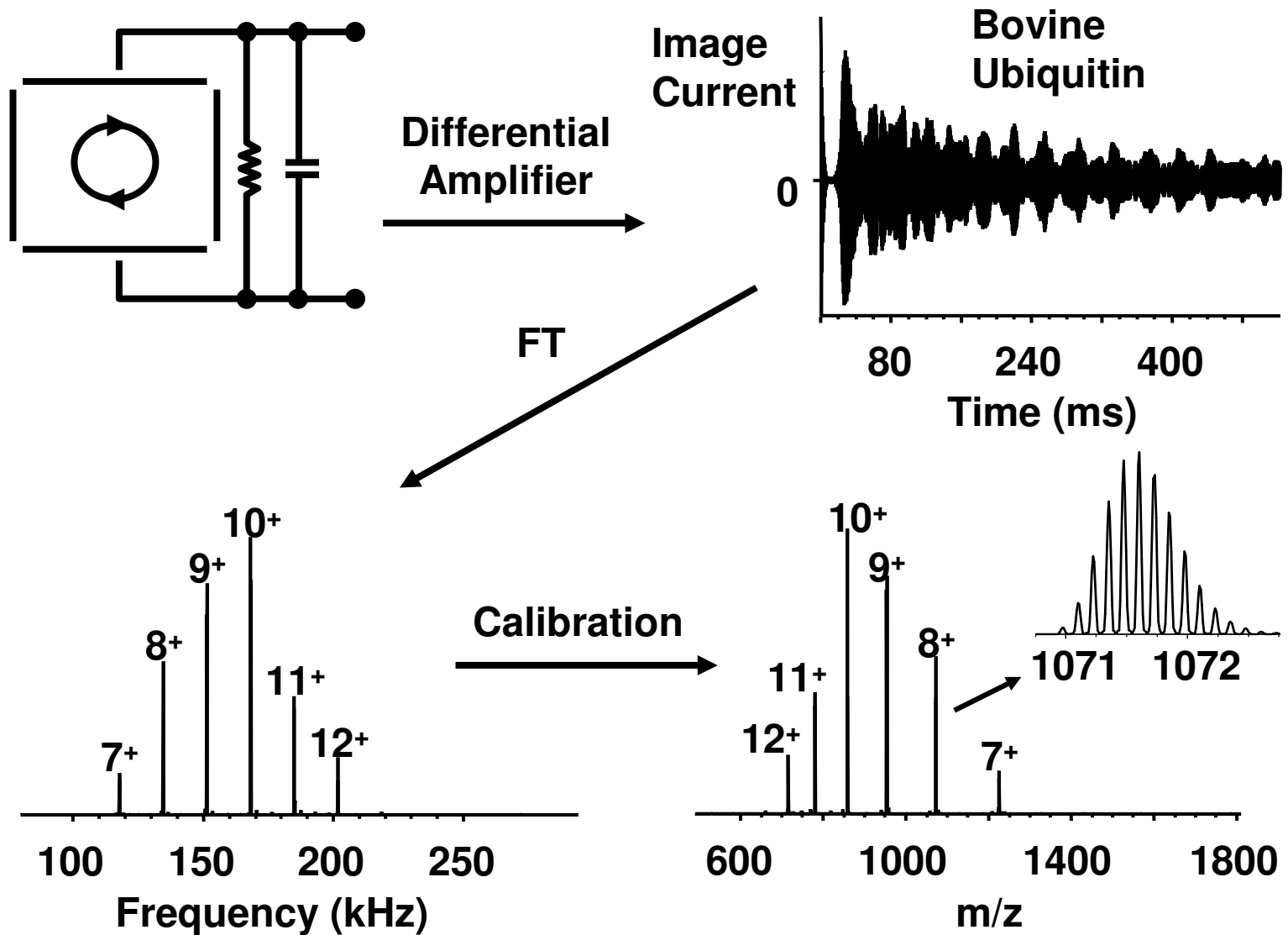


- Ions trapped and measured in ultrahigh vacuum inside a superconducting magnet.



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

Fourier Transform Ion Detection



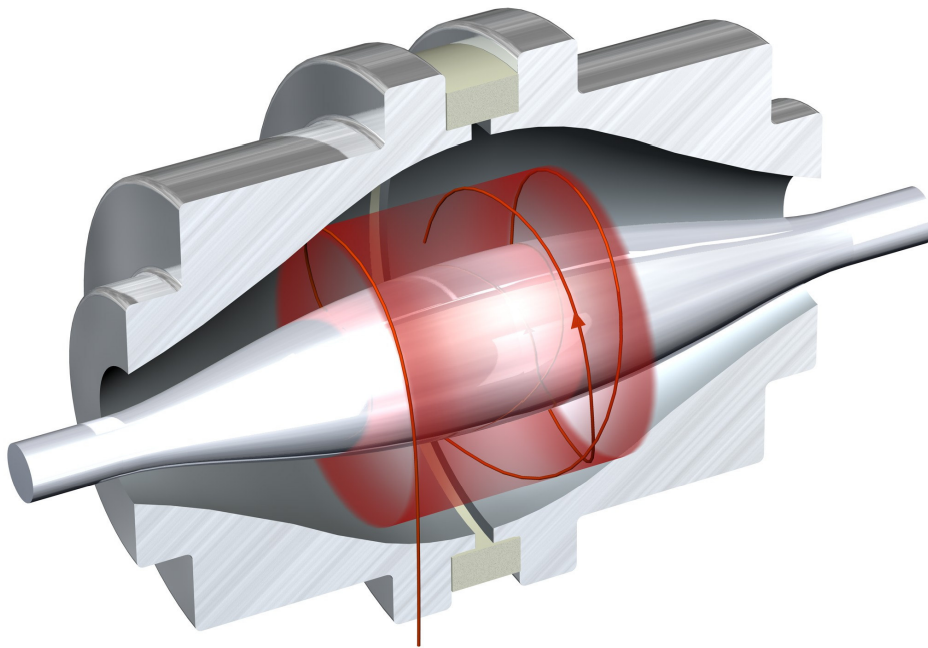
Orbitrap

TOF

- Simultaneous excitation

FTICR

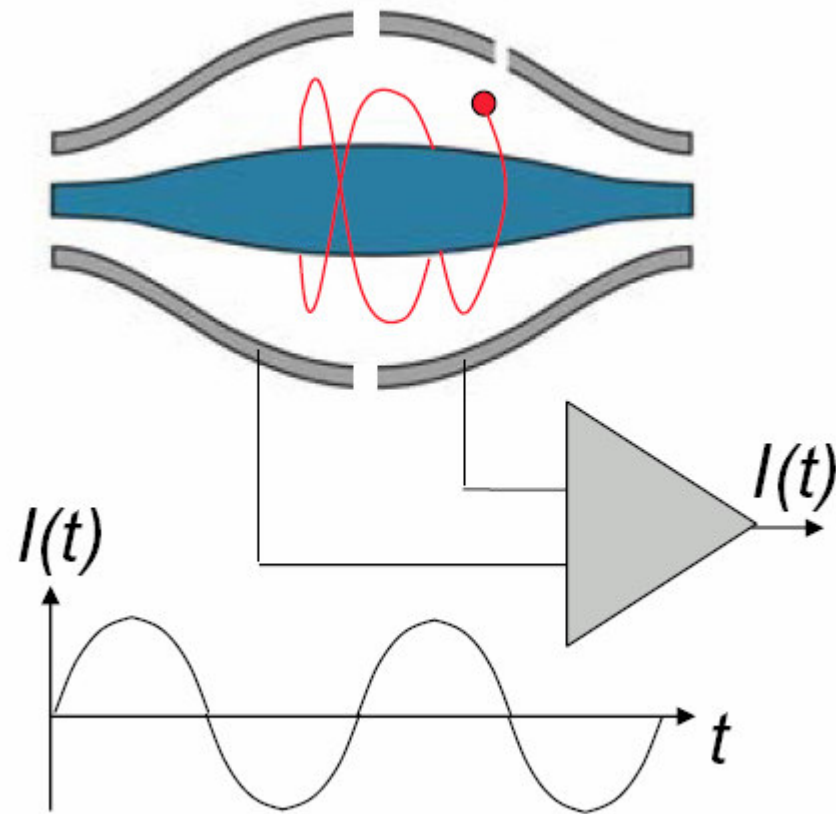
- Confined ion trajectory
- Image current detection
- Fourier transform data conversion



Unique to Orbitrap

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

Image Current Detection in Orbitrap



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 4)

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)

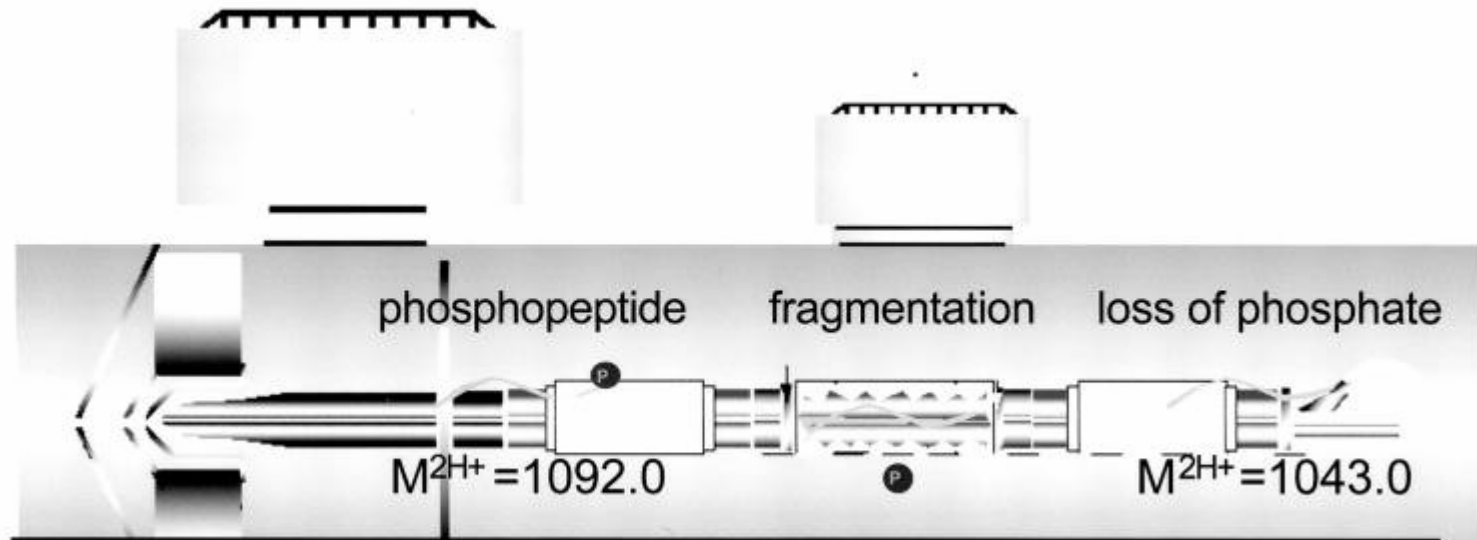
ECD: FTICR, reagent: electron

ETD: ion traps, reagent: free radical anion

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

Multi-Reaction Monitoring (MRM)

A



B

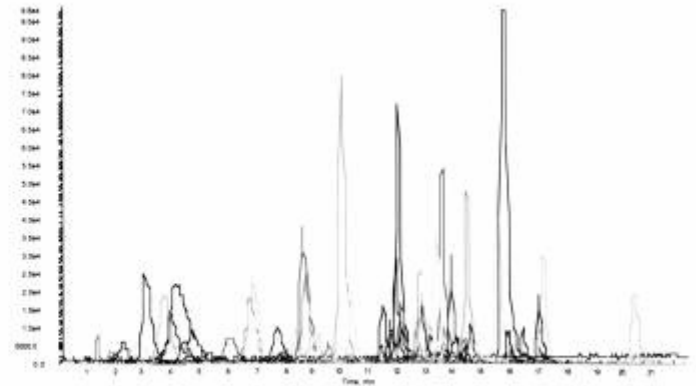
MGRKKIQITRIMDERNRQVTFKRFGLMKKAYELS
 VLCDCEIALIIFNSSNKLQYASTDMDKVLLKYTEYN
 EPHEsrTNSDIVEALNKKehRGCDSPDPDTSYVLT
 PHTEEKYKKINEEFDNMMRNHkiAPGLPPQNFsMS
 VTVPVtSPNALSYtNPGSSLVSPSLAASSTLTDSSM
 LSPpQTTLHRNVSPGAPQRPPSTGNAGGMLSTTD
 LTVpNGAGSSPVGNFVNSRASPNLIGATGANSLG
 KVMPTKSPPPGGNlGMNSRkPDLRVVIPPSSK
 GMMpPLeEEEELELNTQRiSSSQATQPLATPVVSV
 TTPSLPPQGLVYSAMPTAYNTDYSLTsADLSALQG
 FNSPGMLSLGQVSAWQqHHLGQAALSSLVAGGQL
 SQGSNLSINTNqNISIKSEPiSPPRDRMTPSGFQQQ
 QQQQQQPPPPPPQPQPQPQPRQEMGRSP
 VDSLSSSSSYDGSdREDPRGDFHSP
 IVLGRPPNTEDRESpSVKRRMDAWVT

sequence



Q1	Q3	Sequence
537.3	488.3	NRQVTFK
545.2	496.2	MRMDAWVT
571.8	522.8	NFIAVSAANR
617.3	568.3	SEPiSPPRDR
635.8	586.8	KNFIAVSAANR
642.3	593.3	TNSDIVEALNK
651.8	602.8	ISSGALDDDDK
677.8	628.8	IQITRIMDER
699.8	650.8	LFQYASTMDK
706.3	657.3	TNSDIVEALNKK
709.4	660.4	NFIAVSAANRFK

MRM transitions



mass spec detection

Multi-Reaction Monitoring (MRM)

AB SCIEX QTRAP® 5500 System

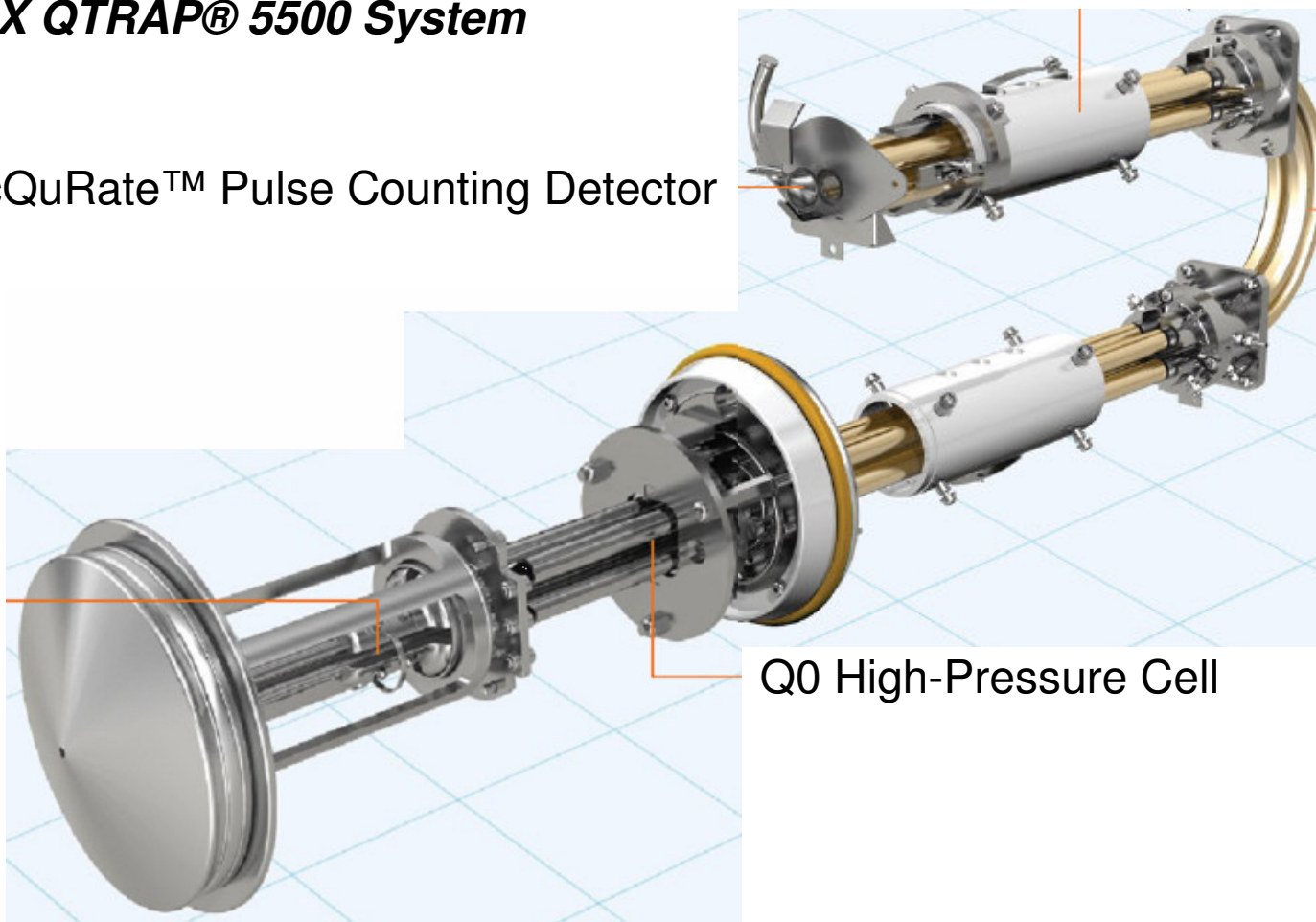
AcQuRate™ Pulse Counting Detector

Linear Accelerator™ Trap

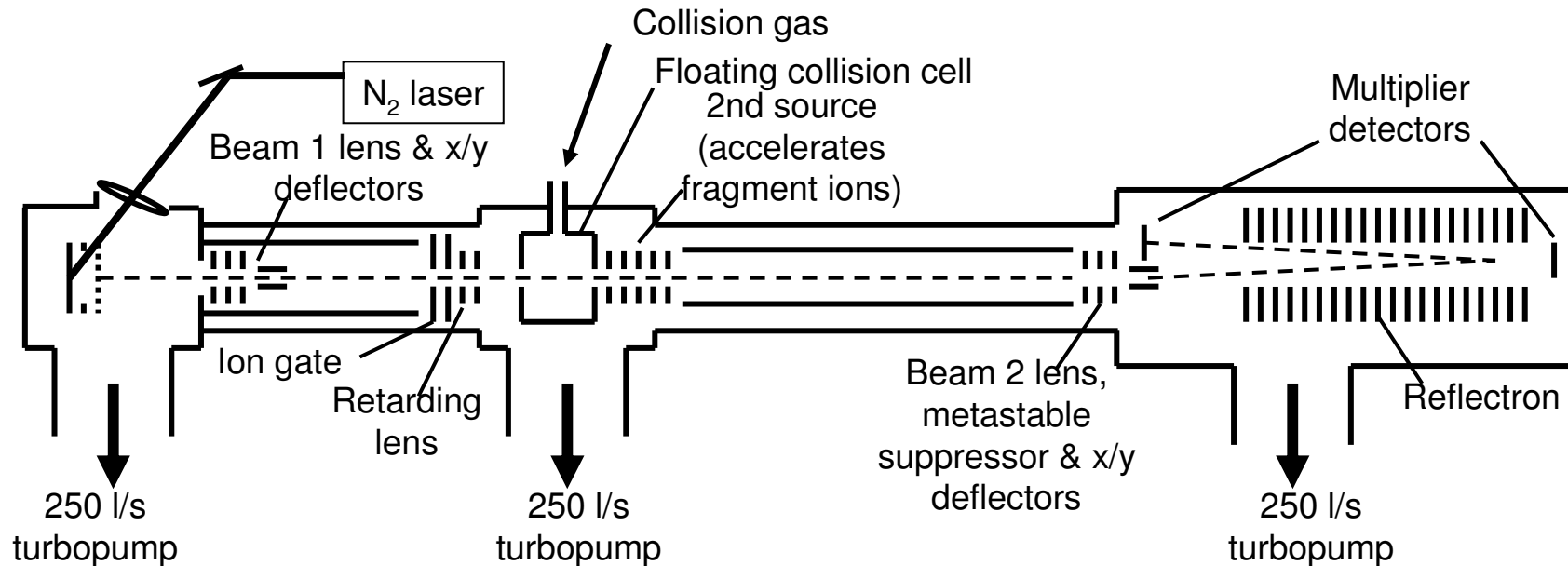
QJet® 2 Ion Guide

Q0 High-Pressure Cell

Curved LINAC® Collision Cell

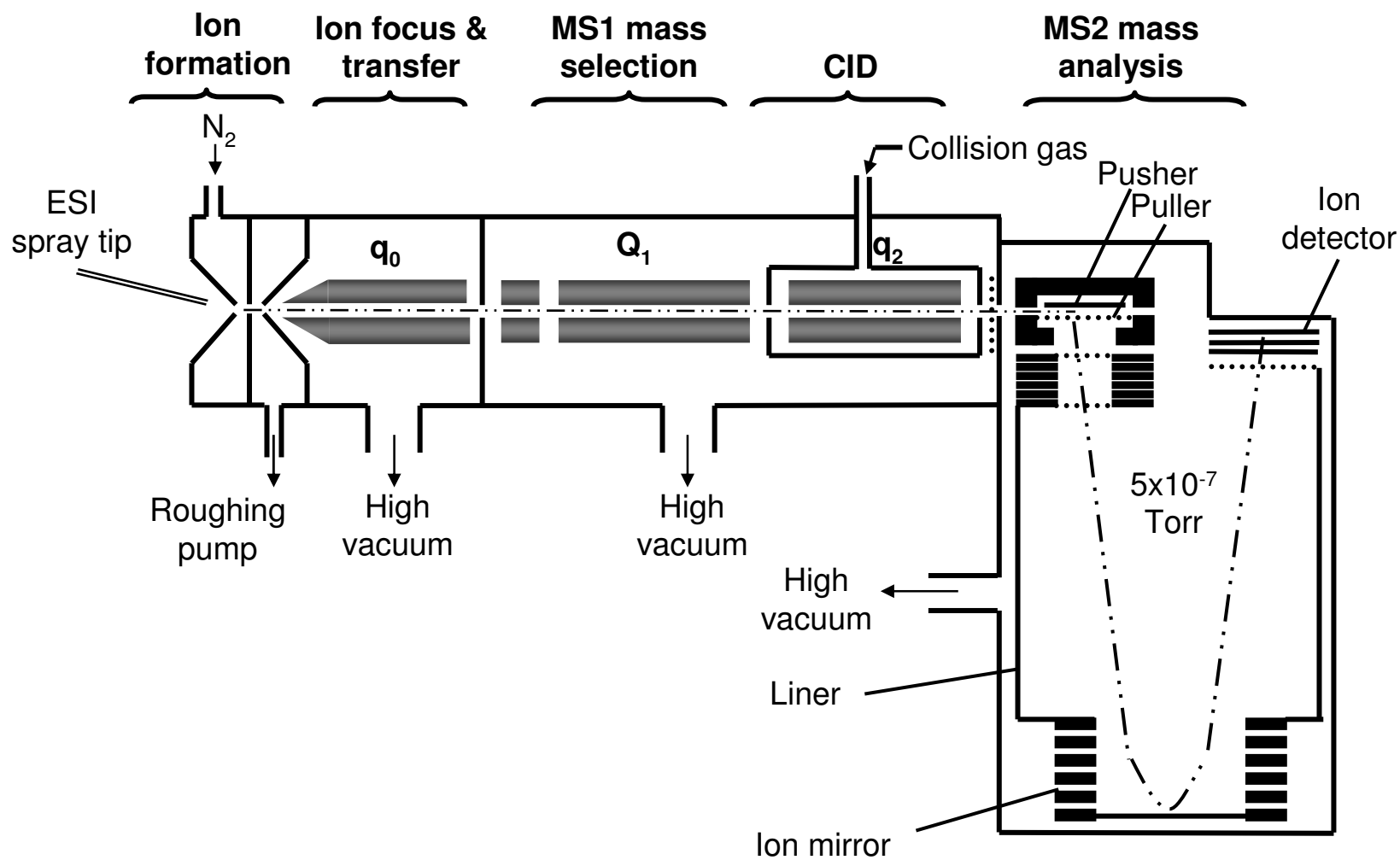


MALDI-TOF/TOF (4700 Proteomics Analyzer)

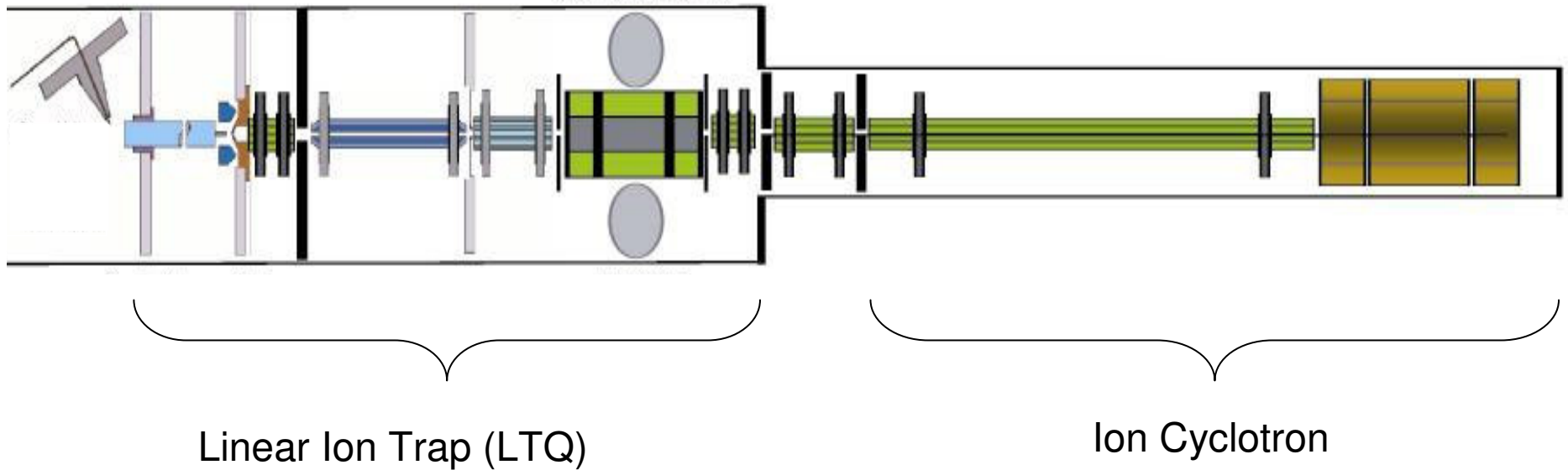


- High performance TOF analysis for MS1 and MS2 give high resolving power and good mass accuracy.
- High accelerating voltage allows high energy CID, giving a wider range of fragment ions and facilitating side-chain cleavages that distinguish isomeric amino acids Ile and Leu.

Hybrid Instrument: QqTOF Mass Spectrometer (QSTAR)



Linear Ion Trap – FT-ICR (LTQ-FT)



Data Dependent Acquisition

- *Data Dependent Scans*

MSMS based on intensity ranking of precursor ions

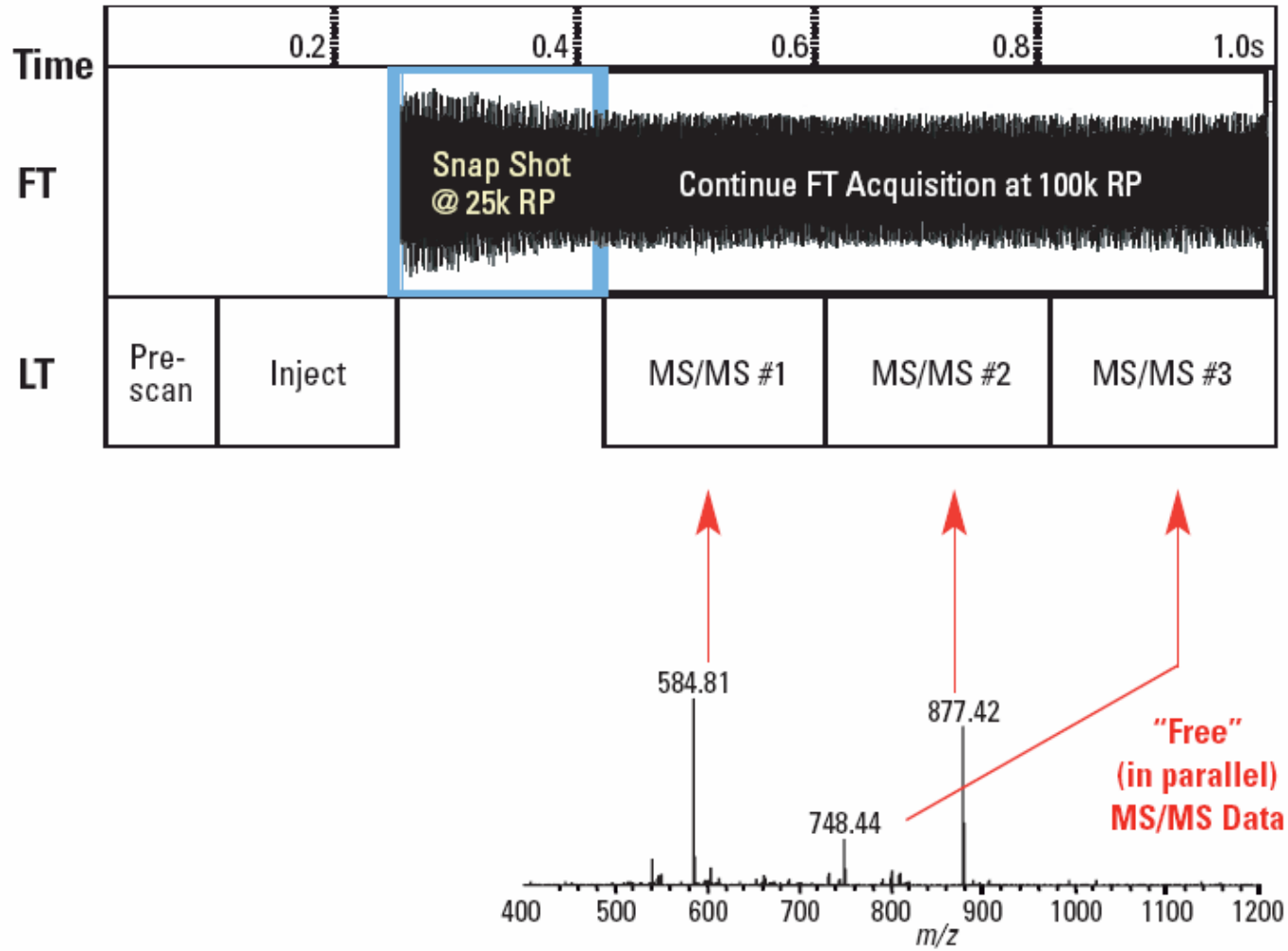
- *Dynamic Exclusion*

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

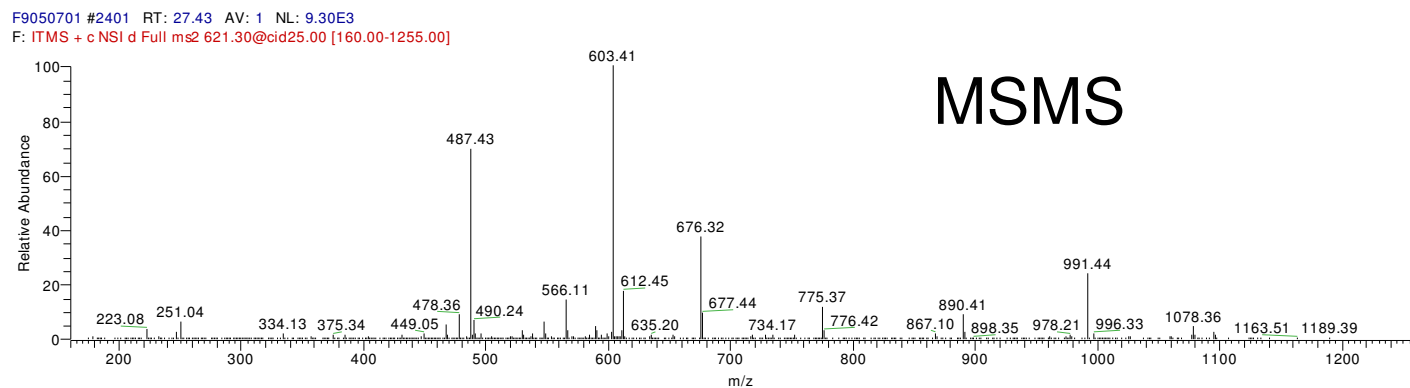
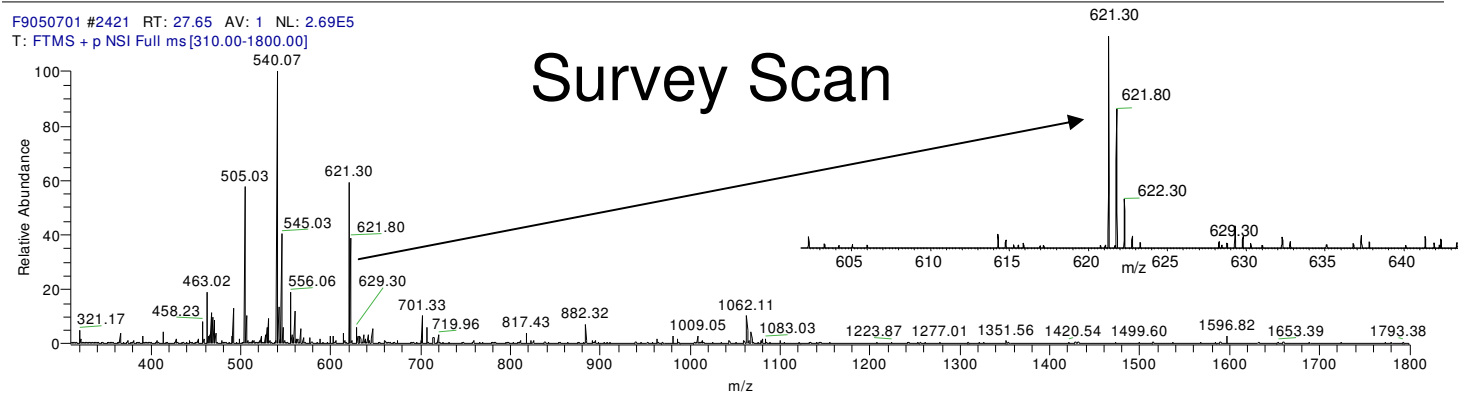
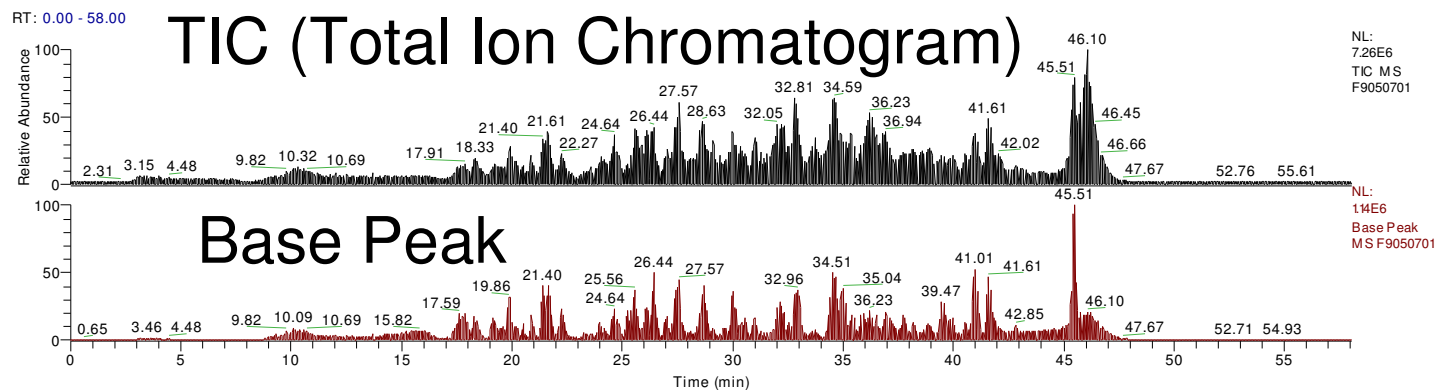
Scan Sequence of LTQFT



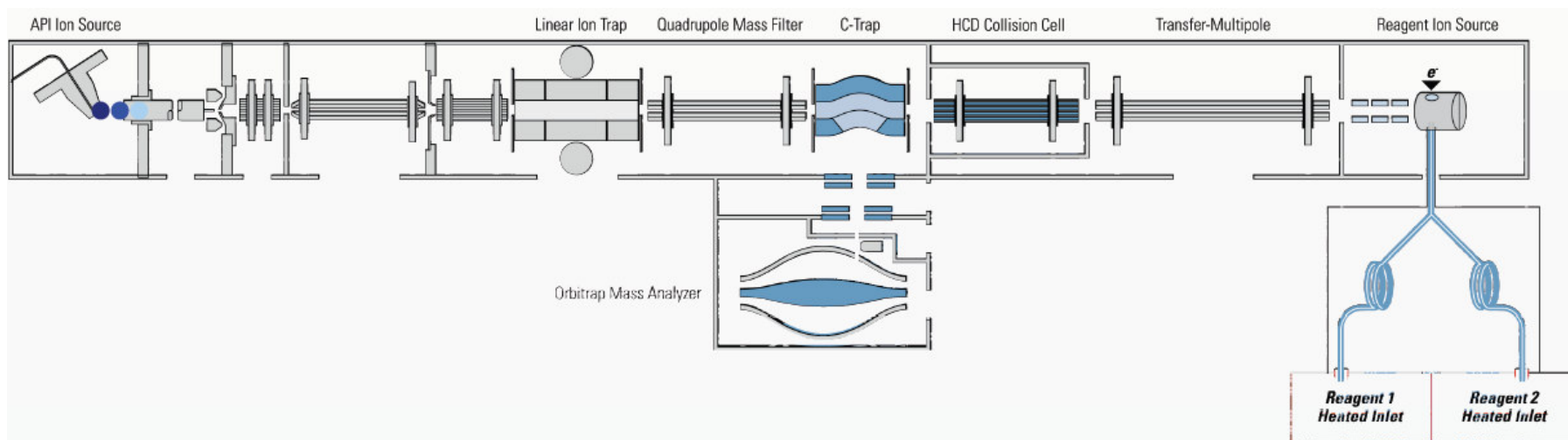
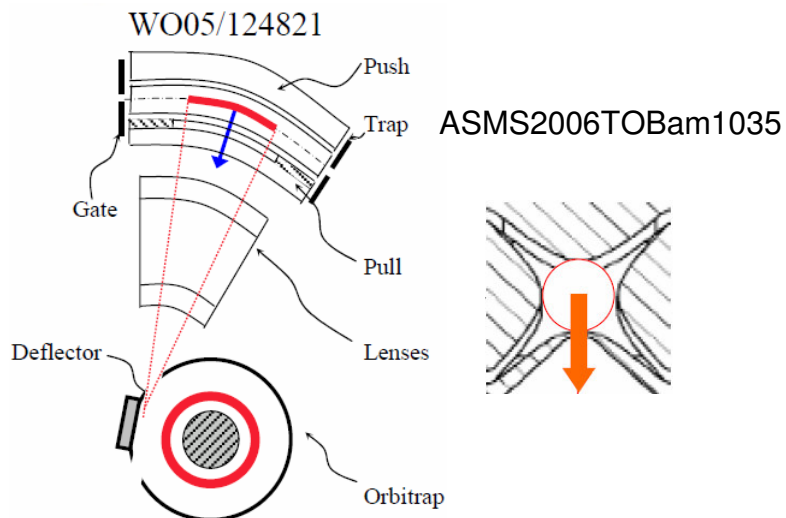
LCMSMS on LTQFT

C:\Documents and Settings\...\F9050701

5/7/2009 8:02:10 AM



Linear Ion Trap - Orbitrap - ETD



AnalChem2006v78p2113

JProteomeRes2008v7p3127

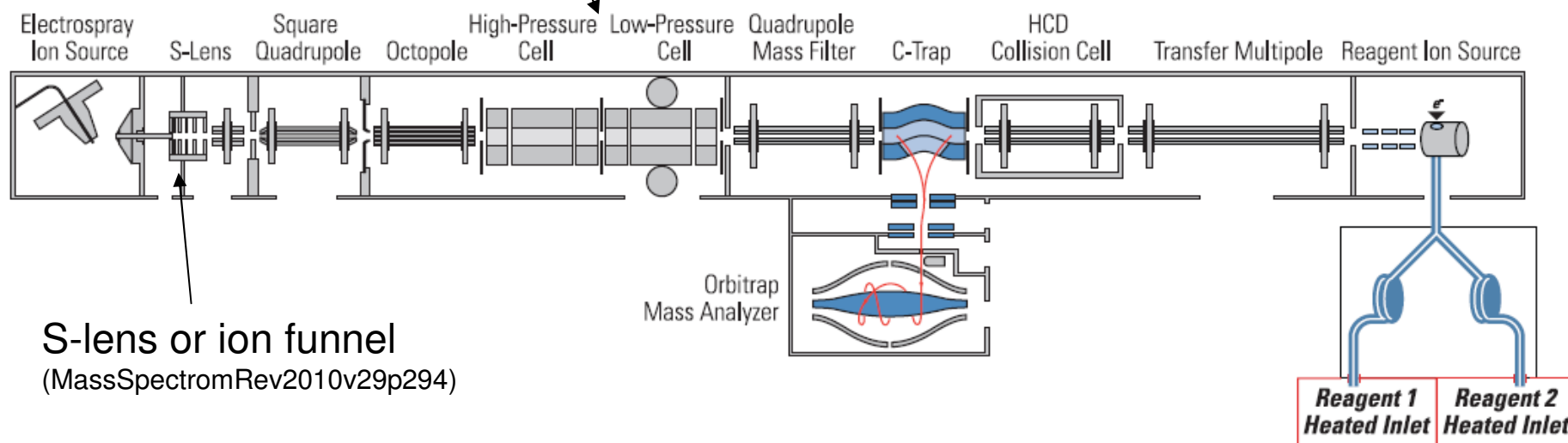
Newest Velos - Orbitrap - ETD

Dual Cell Linear Ion Trap

HP cell: ion accumulation and dissociation

LP cell: fast detection

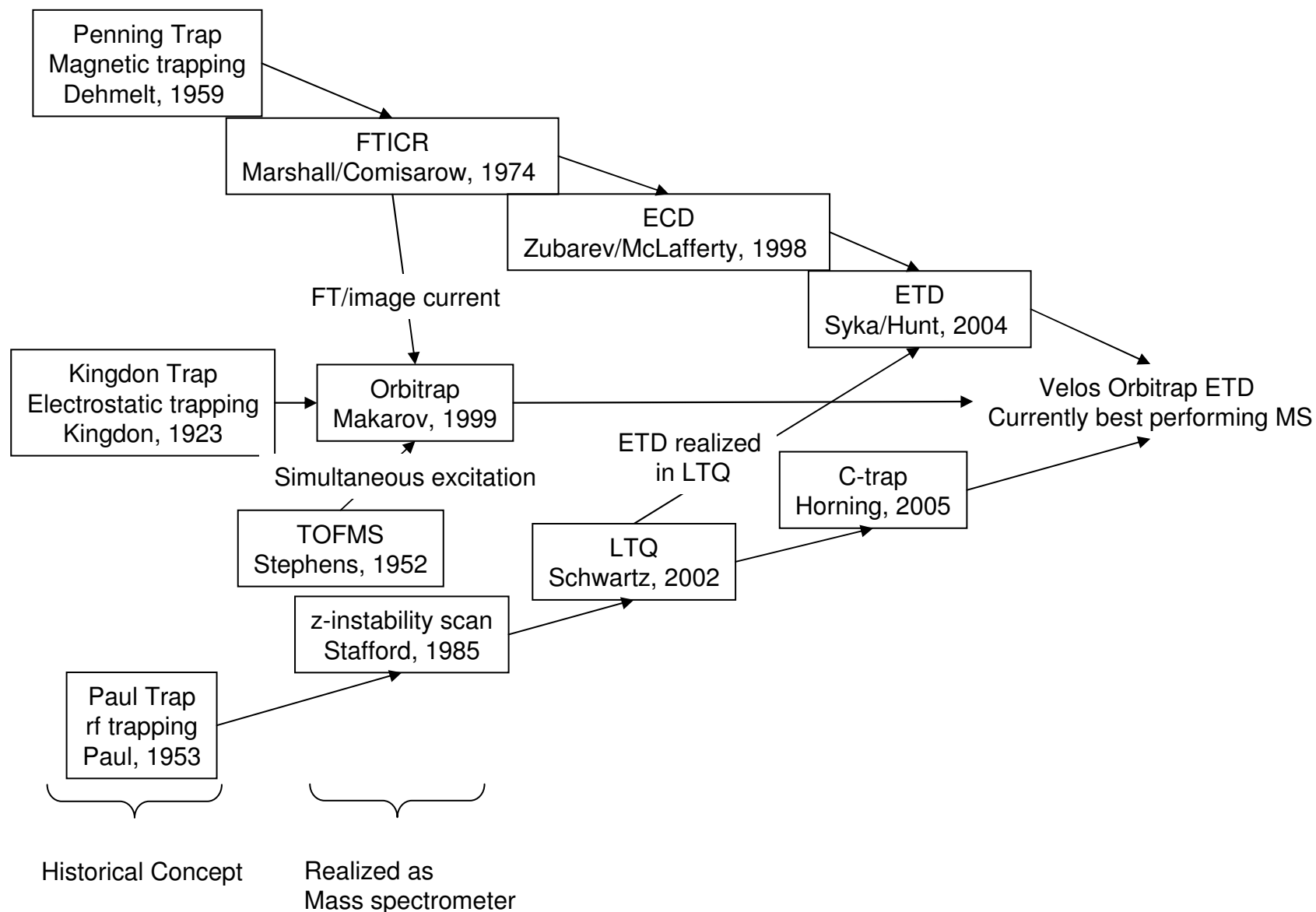
(ASMS2008WPAA039)



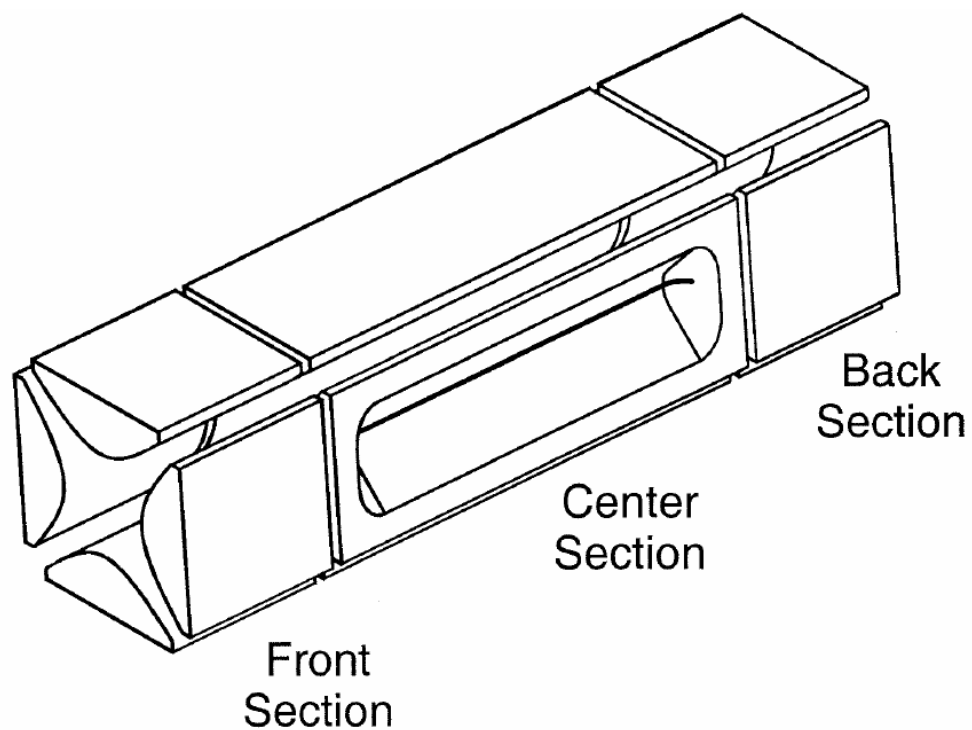
S-lens or ion funnel

(MassSpectromRev2010v29p294)

Key Milestones Leading to LTQ Orbitrap ETD



Linear quadrupole ion trap (LTQ) video clip



File_146.exe

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>