2014 Taiwan International Graduate Program
Academia Sinica

FUNDAMENTAL OF MASS SPECTROMETRY

Yi-Sheng Wang

Academia Sinica
Genomics Research Center

OUTLINE

WHAT

- *Basic concept of mass spectrometry*
- *Configuration and history*
- *Definition of mass spectrometric terms*

HOW

- *Charges in MS*
- *Ionization of samples*
- *Mass analyzers*
- *Fragmentation/tandem mass spectrometry*

WHY

- *Advantages over other analytical tools*
- *Limitations/bottlenecks*

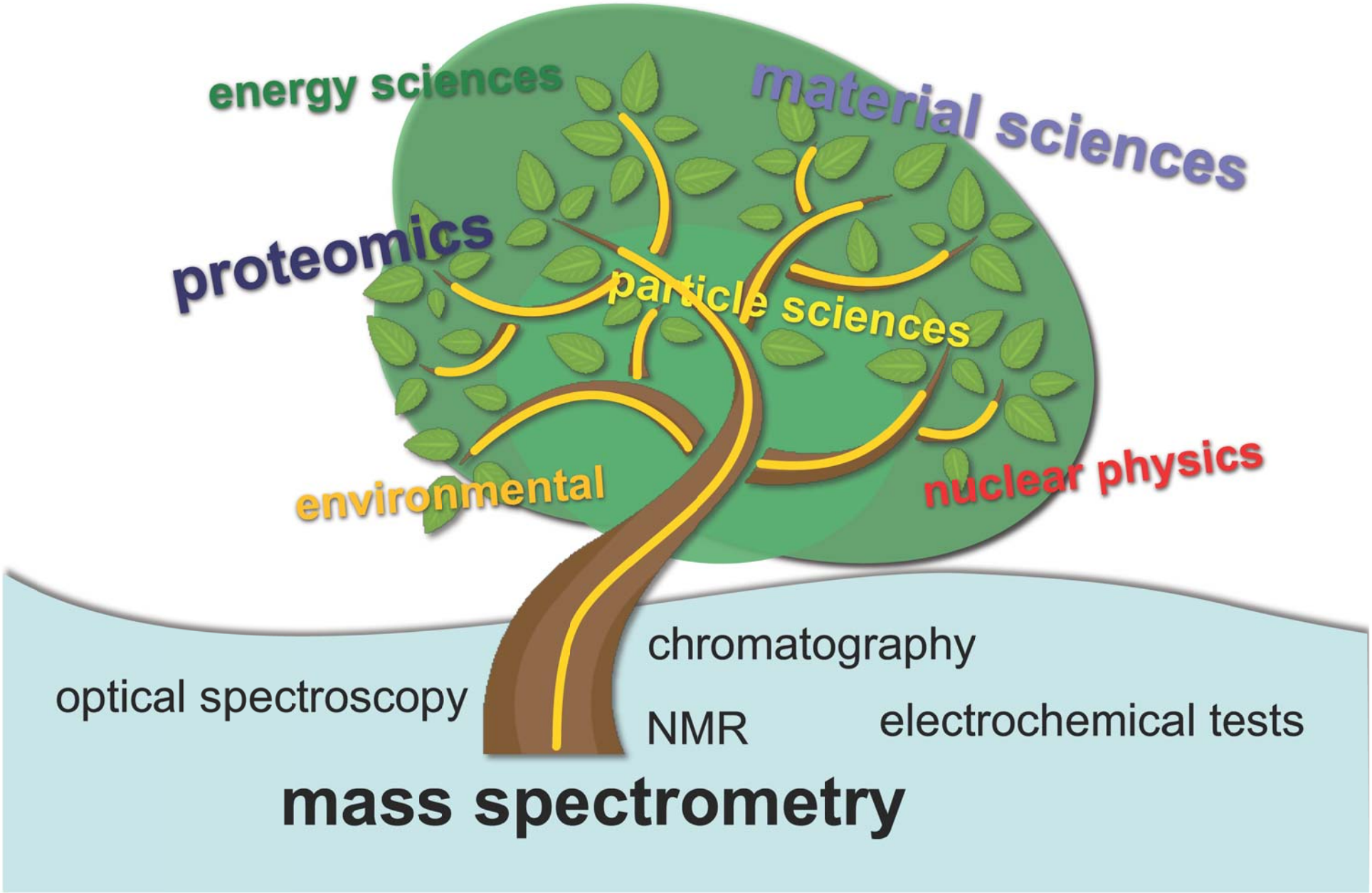
WHEN

- *Identification of unknown molecules*
- *Imaging mass spectrometry*
- *Discussions and perspectives*

WHAT

- *Basic concept of mass spectrometry*
- *Configuration and history*
- *Definition of mass spectrometric terms*

Important Methods for Sample Analysis



Basic Concept of Mass Spectrometry

Mass spectrometry is a powerful analytical technique that is used to ***identify unknown compounds***, to ***quantify known compounds***, and to ***elucidate the structure and chemical properties of molecules.*** Detection of compounds can be accomplished with very minute quantities (as little as 10^{-12}g, 10^{-15} moles for a compound of mass 1000 Daltons). This means that compounds can be identified at very low concentrations (one part in 10^{12}) in chemically complex mixtures. Mass spectrometry provides valuable information to a wide range of professionals: physicians, astonomers, and biologists, to name a few.

ASMS - American Society for Mass Spectrometry

The Primary Function of MS

To identify the analytes according to the mass (molecular weight).

MS is different from conventional balances in the applicable mass range.*

Conventional Balances

100 ±1 kg

5 ±0.1 kg

1 ±0.0001 g

object weight

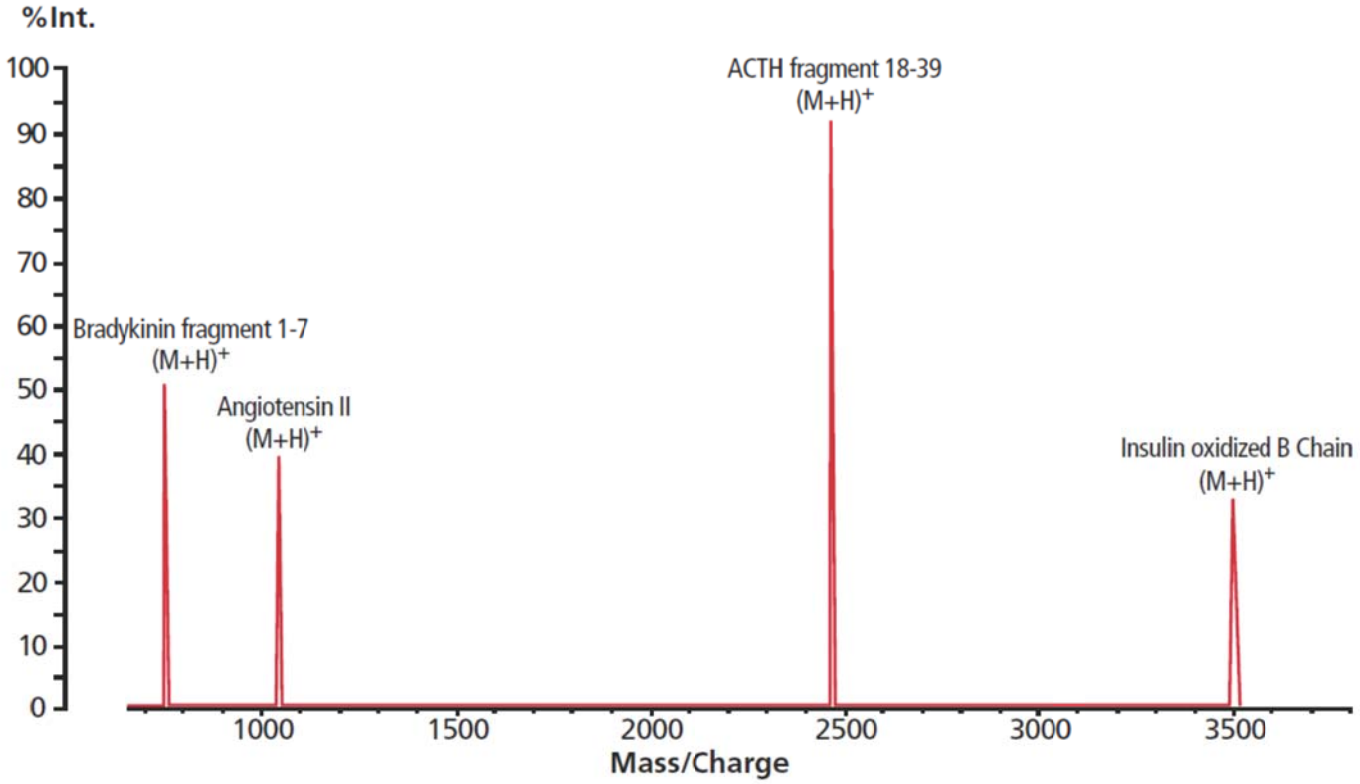
< 10^{-18} g

Mass spectrometer

The Second Function of MS:

To provide quantity information of the analytes.

Relative quantitation is feasible, but not absolute quantitation.*



SIGMA

ProteoMass™ Peptide MALDI-MS Calibration Kit

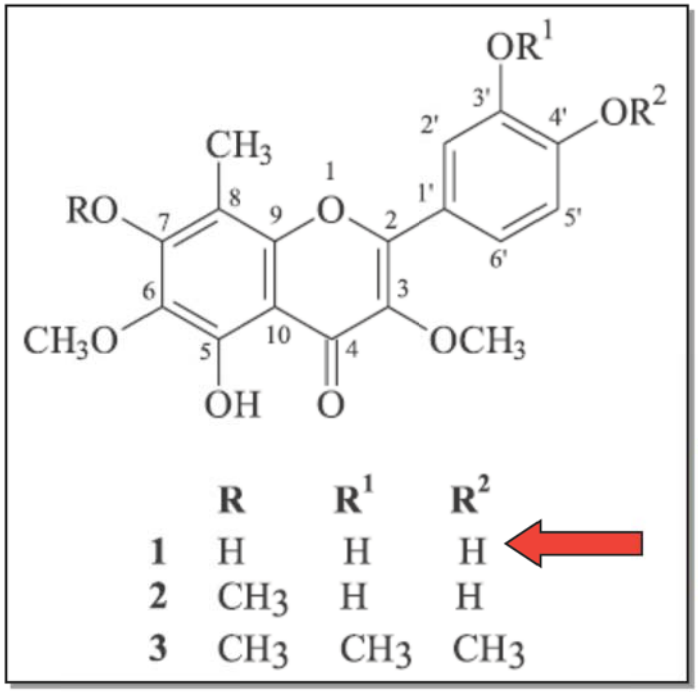
For the calibration, tuning and sensitivity testing of MALDI-MS instruments

Mass range of standards: 757 Da to 3,494 Da

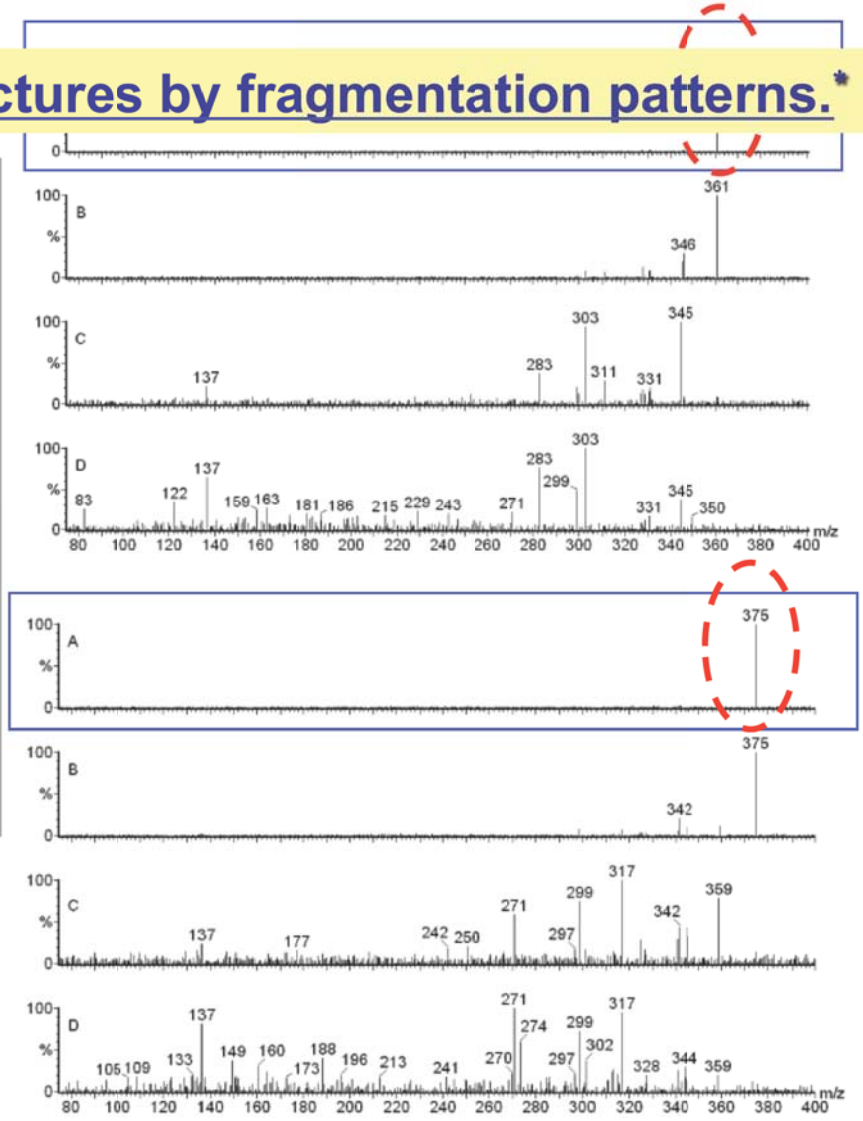
The Third Function of MS:

To provide the structural (fragment) information of the analytes.

MS identifies molecular structures by fragmentation patterns.*



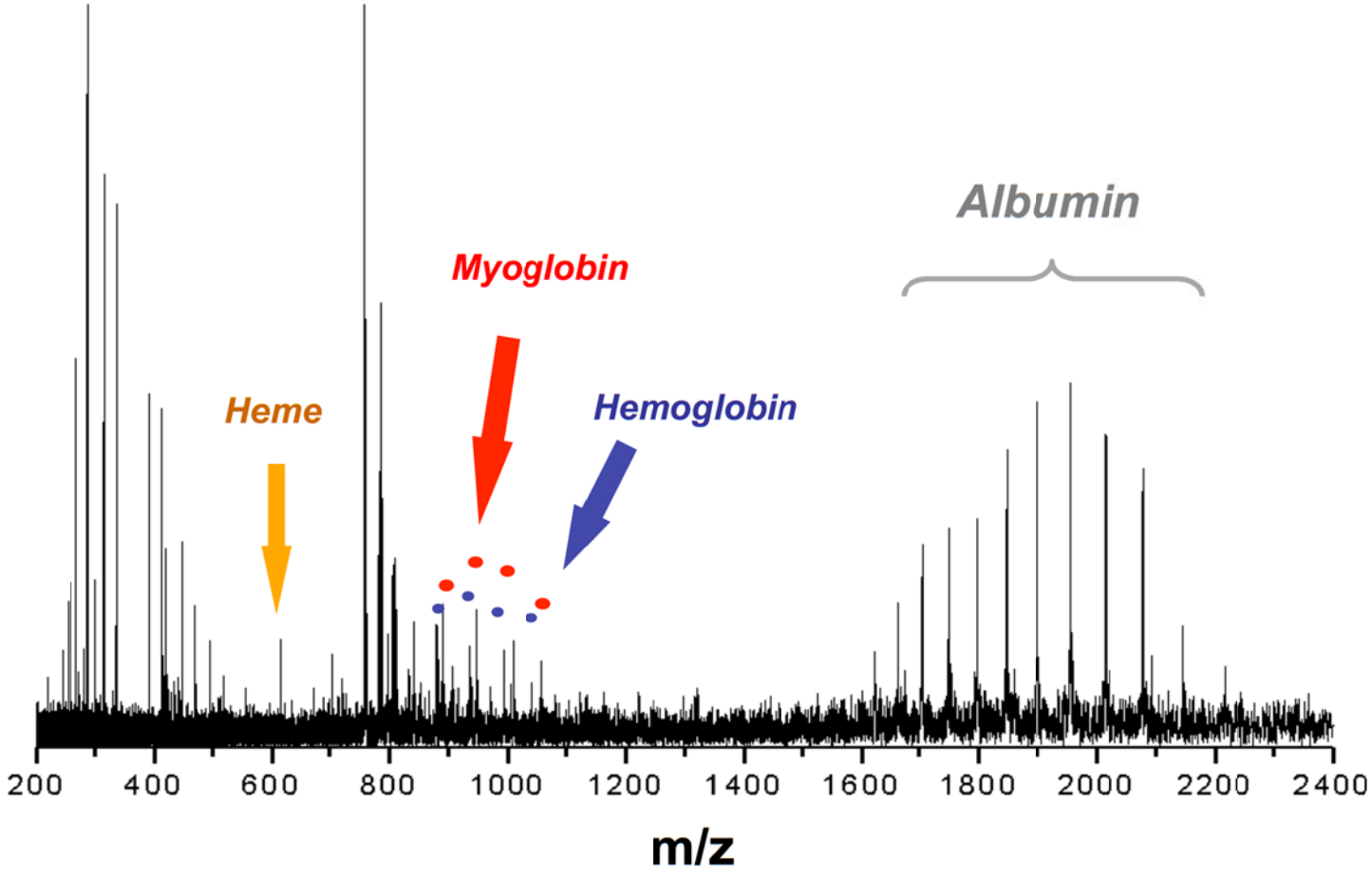
	R	R^1	R^2
1	H	H	H
2	CH_3	H	H
3	CH_3	CH_3	CH_3



BRANCO, Alexsandro, PINTO, Ângelo C., IFA, Demian R. *et al. J. Braz. Chem. Soc.*, June 2002, vol.13, no.3, p.318-323. ISSN 0103-5053.

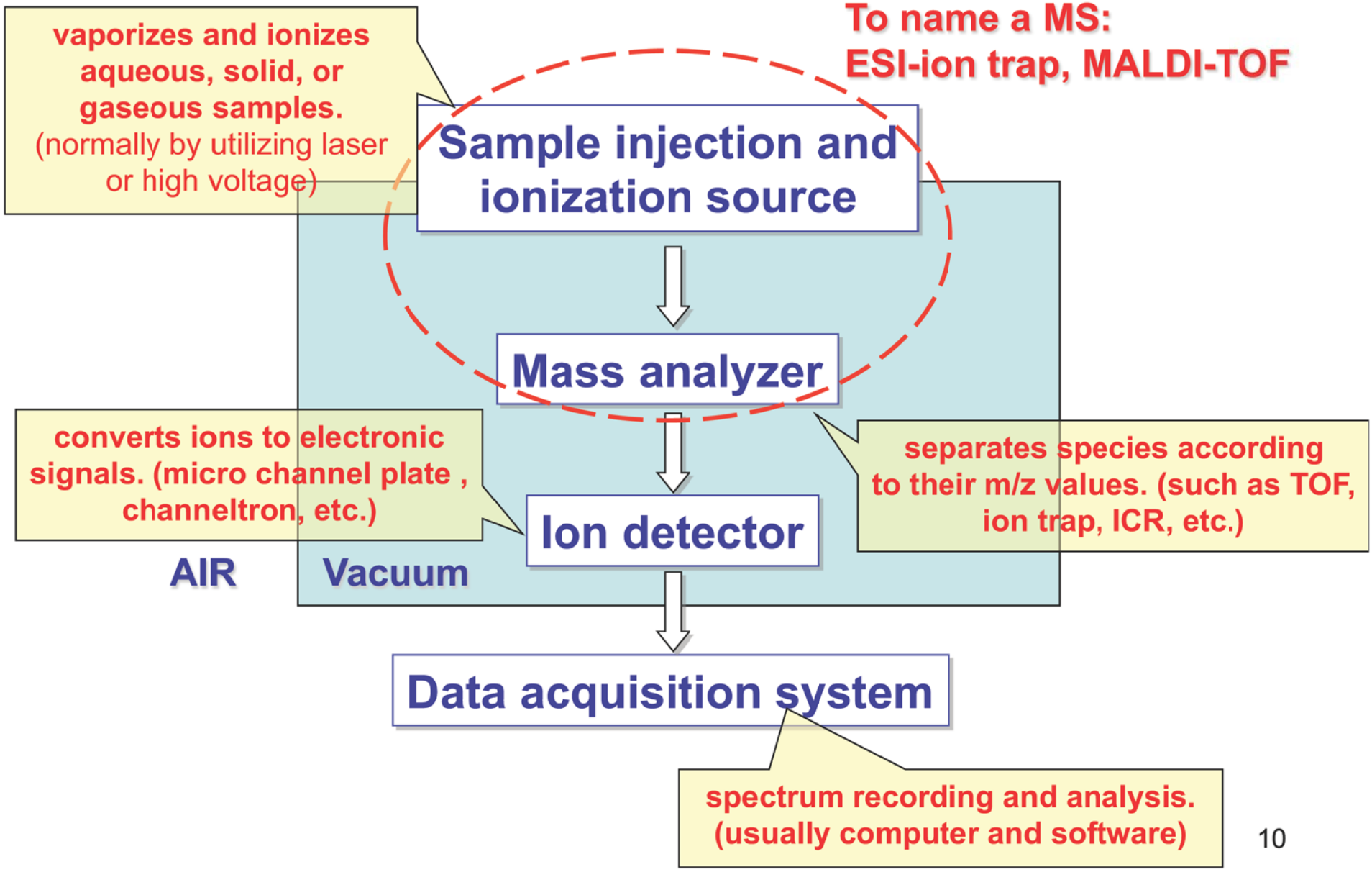
● Another Important Feature of MS

To separate/sort ions according to mass-to-charge ratio



A mass spectrum of human serum

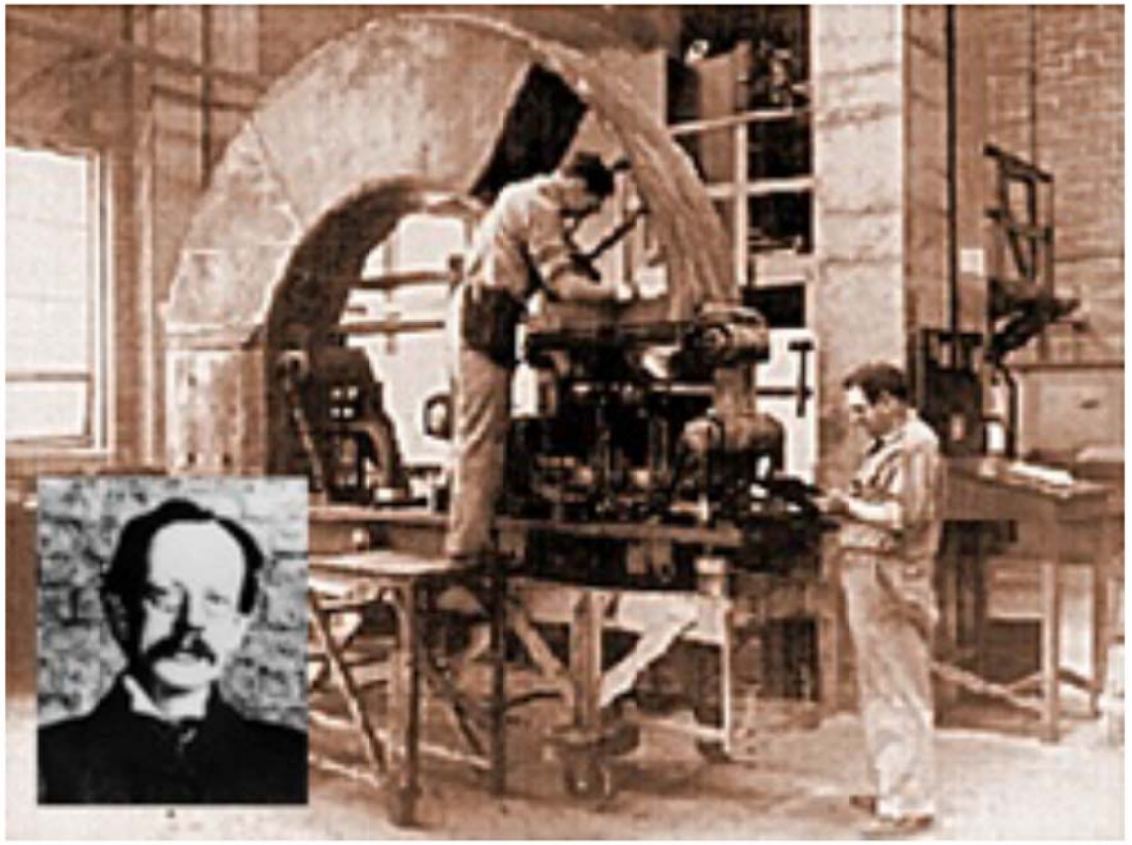
General Configuration of Mass Spectrometer



Back to the history.....

The first mass spectrometer - parabola spectrograph

1912
J. J. Thomson



History of Mass Spectrometry

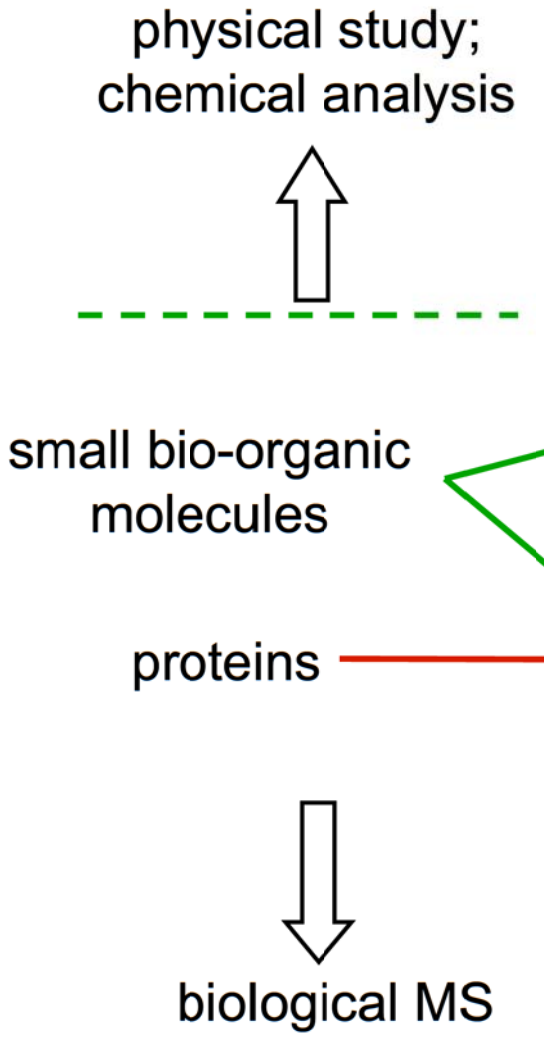


Table 1. Historical Developments in MS

Investigator(s)	Year	Contribution
Thomson	1899–1911	First mass spectrometer
Dempster	1918	Electron ionization and magnetic focusing
Aston	1919	Atomic weights using MS
Stephens	1946	Time-of-flight mass analysis
Hipple, Sommer, and Thomas	1949	Ion cyclotron resonance
Johnson and Nier	1953	Double-focusing instruments
Paul and Steinwedel	1953	Quadrupole analyzers
Beynon	1956	High-resolution MS
Biemann, Cone, Webster, and Arsenault	1966	Peptide sequencing
Munson and Field	1966	Chemical ionization
Dole	1968	Electrospray ionization
Beckey	1969	Field desorption MS of organic molecules
MacFarlane and Torgerson	1974	Plasma desorption MS
Comisarow and Marshall	1974	FT-ICR MS
Yost and Enke	1978	Triple quadrupole MS
Barber	1981	Fast atom bombardment (FAB)
Tanaka, Karas, and Hillenkamp	1983	Matrix-assisted laser desorption/ionization
Fenn	1984	ESI on biomolecules
Chowdhury, Katta, and Chait	1990	Protein conformational changes with ESI MS
Mann and Wilm	1991	MicroESI
Ganem, Li, and Henion Chait and Katta	1991	Noncovalent complexes with ESI MS
Pieles, Zurcher, Schär, and Moser	1993	Oligonucleotide ladder sequencing
Henzel, Billeci, Stults, Wong, Grimley, and Watanabe	1993	Protein mass mapping
Siuzdak, Bothner, Fuerstenau, and Benner	1996–2001	Intact viral analysis

Millstones of Mass Spectrometry

Nobel Laureates

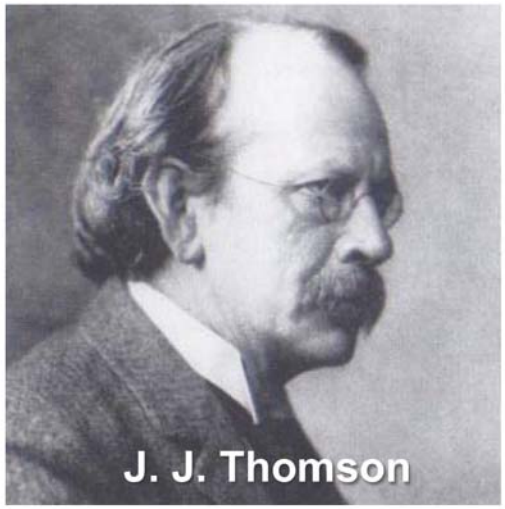
1906 Thomson --- Electricity

1922 Aston --- Isotopes

1939 Lawrence --- Cyclotron

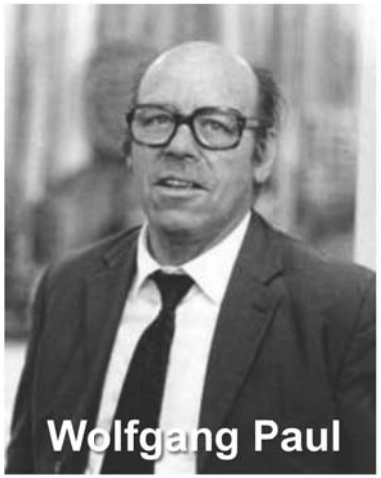
1989 Paul --- Quadrupole

2002 Fenn & Tanaka --- ESI/SLDI

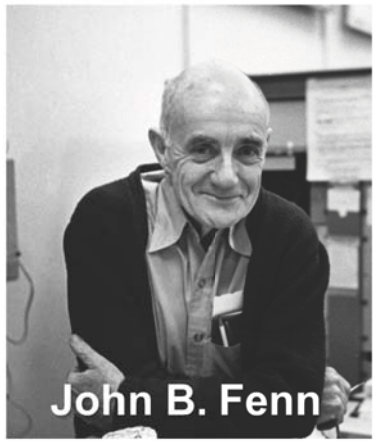

J. J. Thomson


Aston


Lawrence

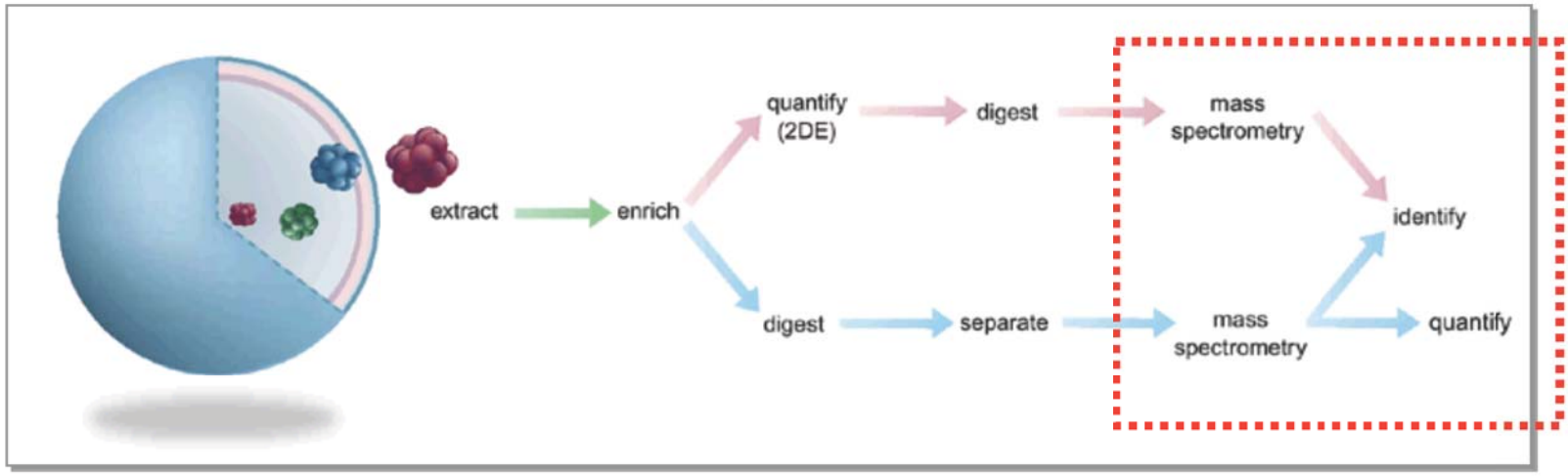

Wolfgang Paul


田中 耕一
Koichi Tanaka


John B. Fenn

Modern Biological Mass Spectrometry

protein ID* & quantification*



Quantitative protein analysis from the cell to the identified protein.
The two most common processes for quantitative proteome analysis are shown. In the first (top), 2DE is used to separate and to quantify proteins, and selected proteins are then isolated and identified by mass spectrometry. In the second (bottom), LC-MS/MS is used to analyze enzyme digests of unseparated protein mixtures, and accurate quantification is achieved by labeling the peptides with stable isotope. Both processes are compatible with protein fractionation or separation methods, such as subcellular fractionation, protein complex isolation and electrophoresis and chromatography, thereby providing additional biological context to the protein samples being analyzed.

Scott D. Patterson & Ruedi H. Aebersold, Nature Genetics ***33****, 311 - 323 (2003)*

Soft Ionization of DNA Using MALDI

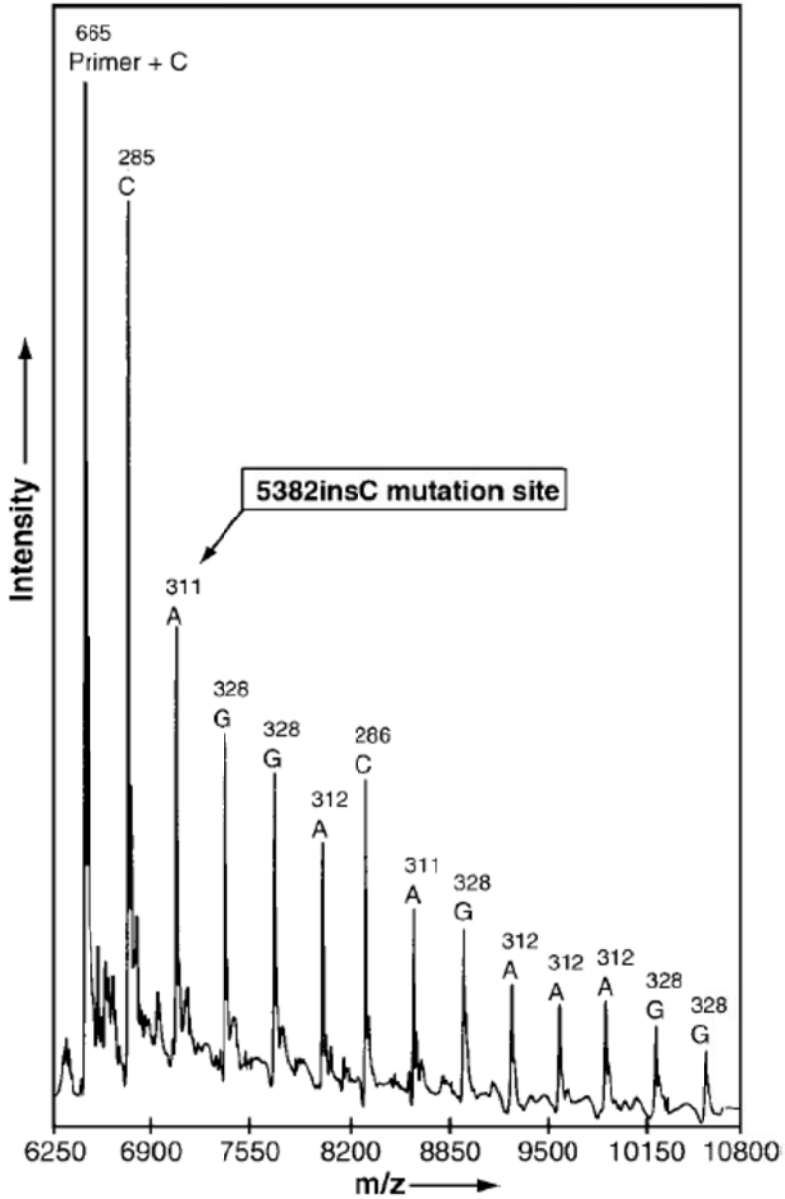


Fig. 5.7 MALDI-TOF mass spectrum of a Sanger sequencing ladder generated by primer extension from PCR products using a mixture of normal elongators (dNTPs) and biotinylated terminator nucleotides (ddNTPs). The termination products are purified on a streptavidin-coated solid support. The sequence of the target region is derived by calculating the mass difference between the termination products. Each mass signal in the spectrum is marked with the mass difference to the preceding mass signal and the corresponding nucleotide that this mass difference represents (C = 289.2 Da; A = 313.2 Da; G = 329.2 Da; T = 304.2 Da).

MALDI MS: A Practical Guide to Instrumen
-tation, Methods and Applications. Wily-VCH

Basic Terms of Mass Spectrometry

Angiotensin I — nominal = 1296

$[C_{62}H_{89}N_{17}O_{14} + H]^+$ — (C=12, H=1, N=14, O=16, …)

Precise masses of various common elements (Audi and Wapstra, 1995)

^{1}H = 1.00782503214 u ± 0.00035 μu (one standard deviation)
^{2}H = 2.01410177799 u ± 0.00036 μu
^{3}H = 3.0160492675 u ± 0.0011 μu
^{3}He = 3.01602930970 u ± 0.00086 μu
^{4}He = 4.0026032497 u ± 0.0010 μu
^{13}C = 13.0033548378 u ± 0.0010 μu
^{14}C = 14.0032419884 u ± 0.0040 μu
^{14}N = 14.00307400524 u ± 0.00086 μu
^{15}N = 15.00010889844 u ± 0.00092 μu
^{16}O = 15.9949146221 u ± 0.0015 μu
^{17}O = 16.999131501 u ± 0.22 μu
^{18}O = 17.999160419 u ± 0.9 μu

Basic Terms of Mass Spectrometry

Angiotensin I

$[C_{62}H_{89}N_{17}O_{14} + H]^+$

nominal = 1296

monoisotopic mass

exact mass → 1296.68518

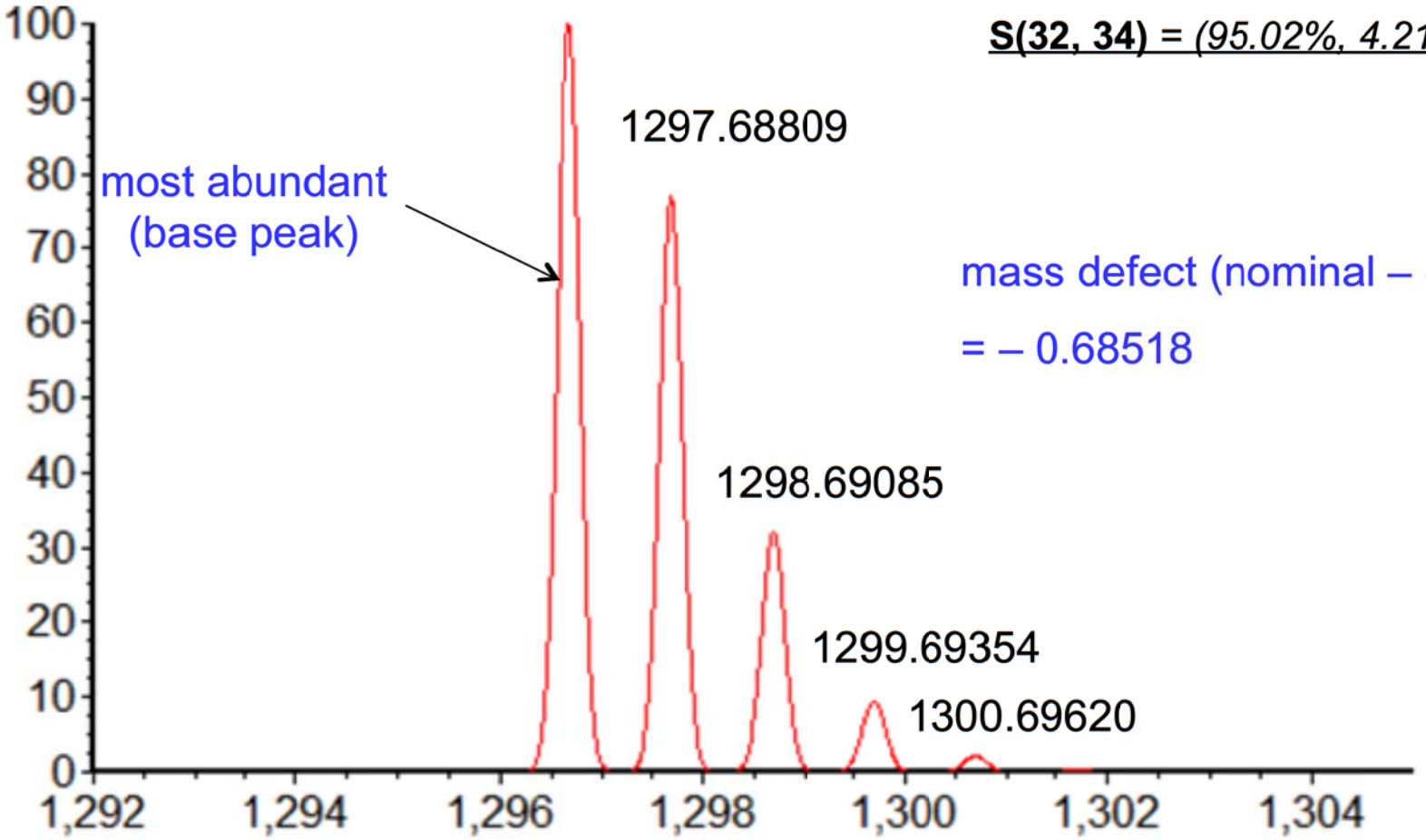
Isotope Abundance:

C(12,13) = *(98.90%,1.10%);*

N(14,15) = *(99.63%, 0.37%);*

O(16,18) = *(99.76%, 0.20%);*

S(32, 34) = *(95.02%, 4.21%)*



mass defect (nominal – exact)

= – 0.68518

Basic Terms of Mass Spectrometry

Defining the masses

Mass peak width ($\Delta m_{50\%}$)
Full width of mass spectral peak at half-maximum peak height

Mass resolving power ($m/\Delta m_{50\%}$)
A well-isolated single mass spectral peak

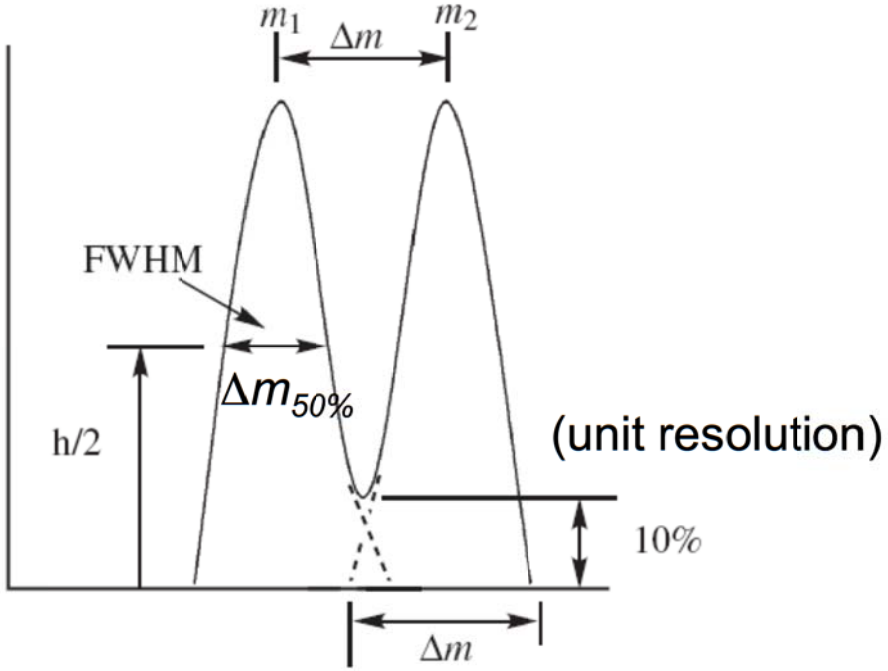
Mass resolution ($m_2 - m_1$ in Da, or $(m_2 - m_1)/m_1$ in ppm)
The smallest mass difference between equal magnitude peaks such that the valley between them is a specified fraction of either peak height

Mass precision
Root-mean-square deviation in a large number of repeated measurements

Mass accuracy
Difference between measured and actual mass

Mass defect
Difference between exact and nominal mass

Marshall et al., Anal. Chem. 74 (9), 252 A-259 A (2002).

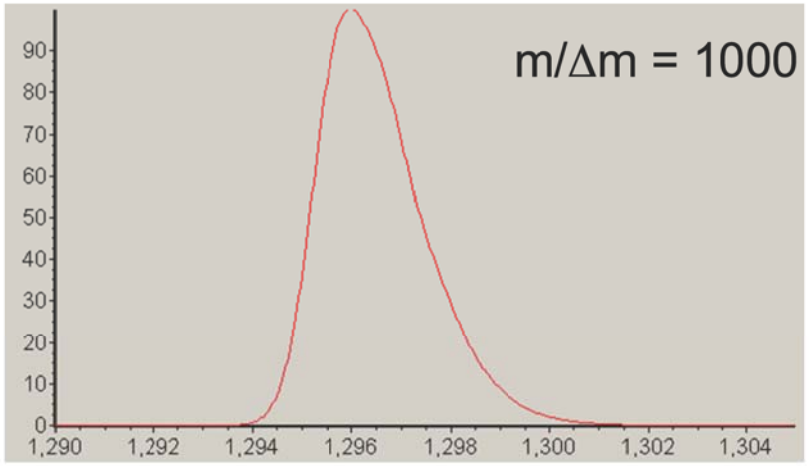


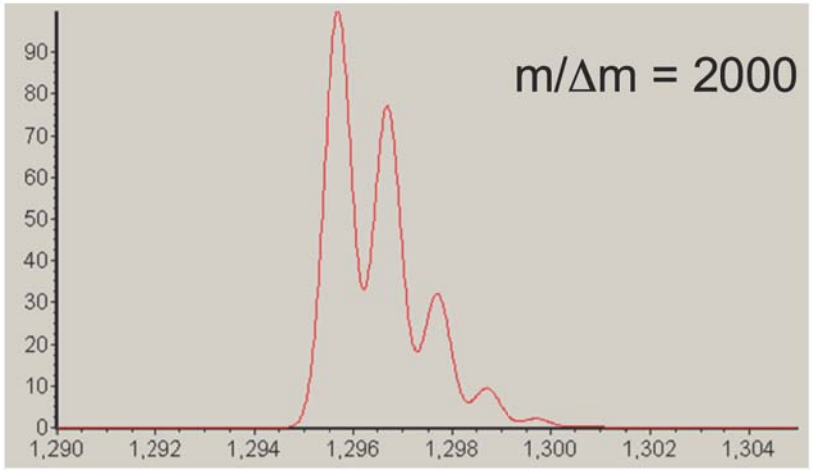
C. Dass, Fundamentals of Contemporary Mass Spectrometry, John Wiley& Sons, 2007.

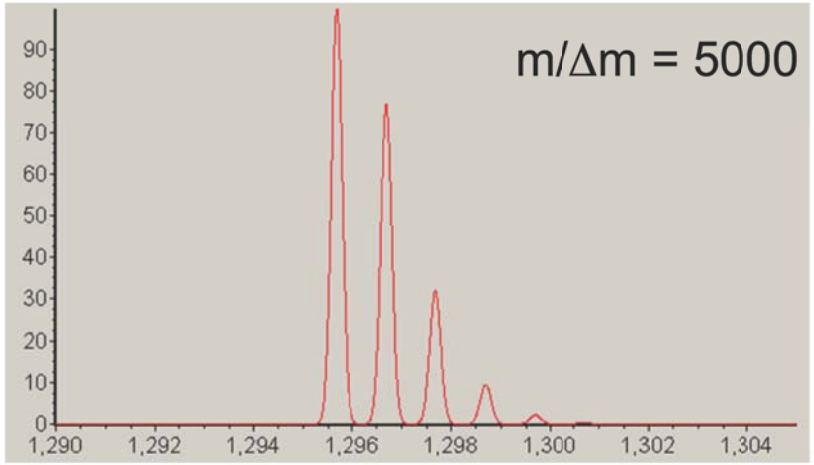
Importance of Mass Resolving Power

Angiotensin I
$[C_{62}H_{89}N_{17}O_{14} + H]^+$

1296.68518	45%
1297.68809	35%
1298.69085	14%
1299.69354	4%
1300.69620	1%







Peak Separation vs Charge State

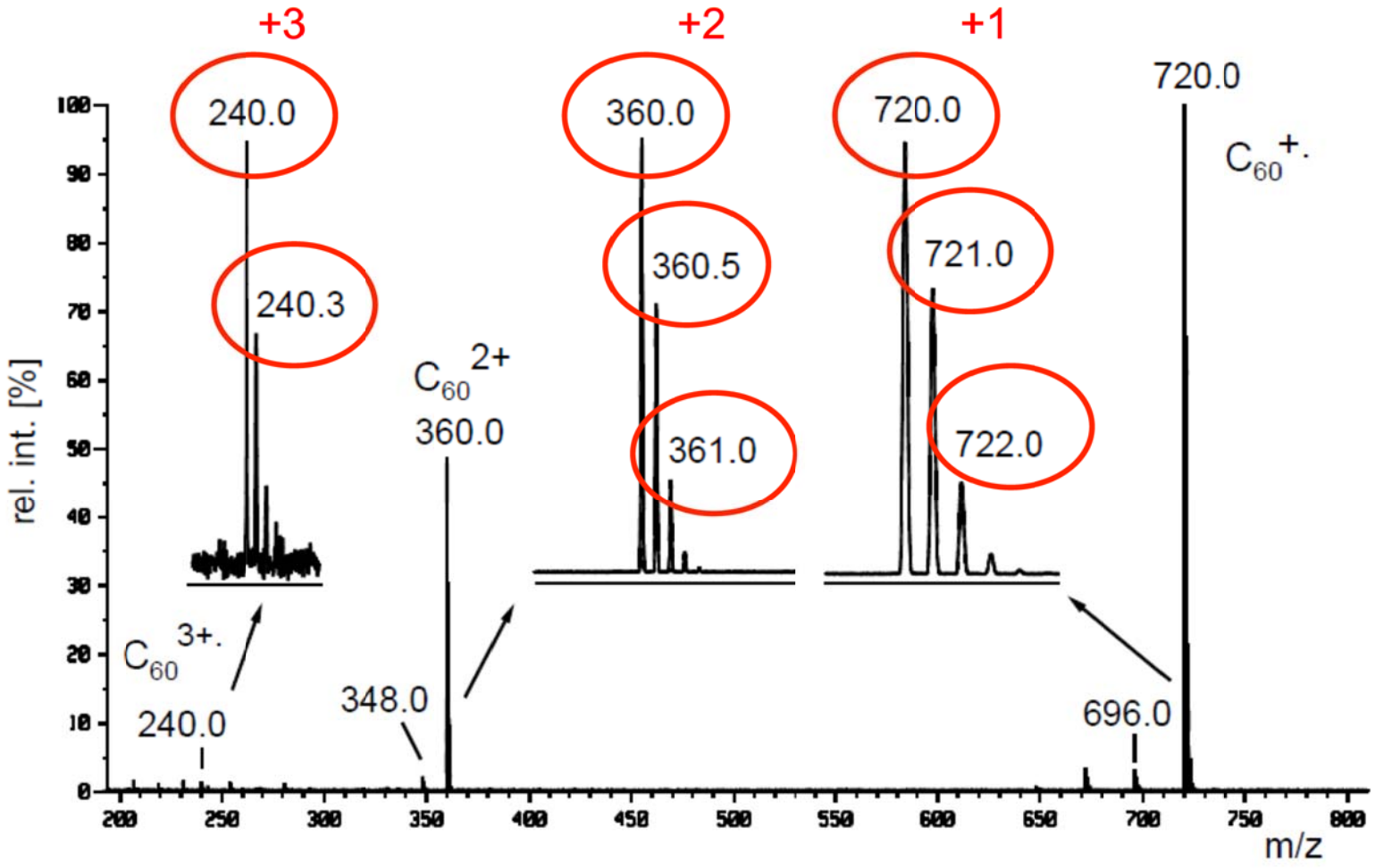


Fig. 3.34. [60]Fullerene – EI mass spectrum.. The insets show the expanded signals of $M^{+\bullet}$, M^{2+}, and $M^{3+\bullet}$ ions. The signals of the patterns are $\Delta m/z = 1$, 0.5, and 0.33, respectively. The intensity scale has been normalized in the insets to allow for easier comparison of the isotopic patterns. By courtesy of W. Krätschmer, Max Planck Institute for Nuclear Physics, Heidelberg.

WHY

- *Advantages over other analytical tools*
- *Limitations/bottlenecks*

Advantage of MS Over Other Methods

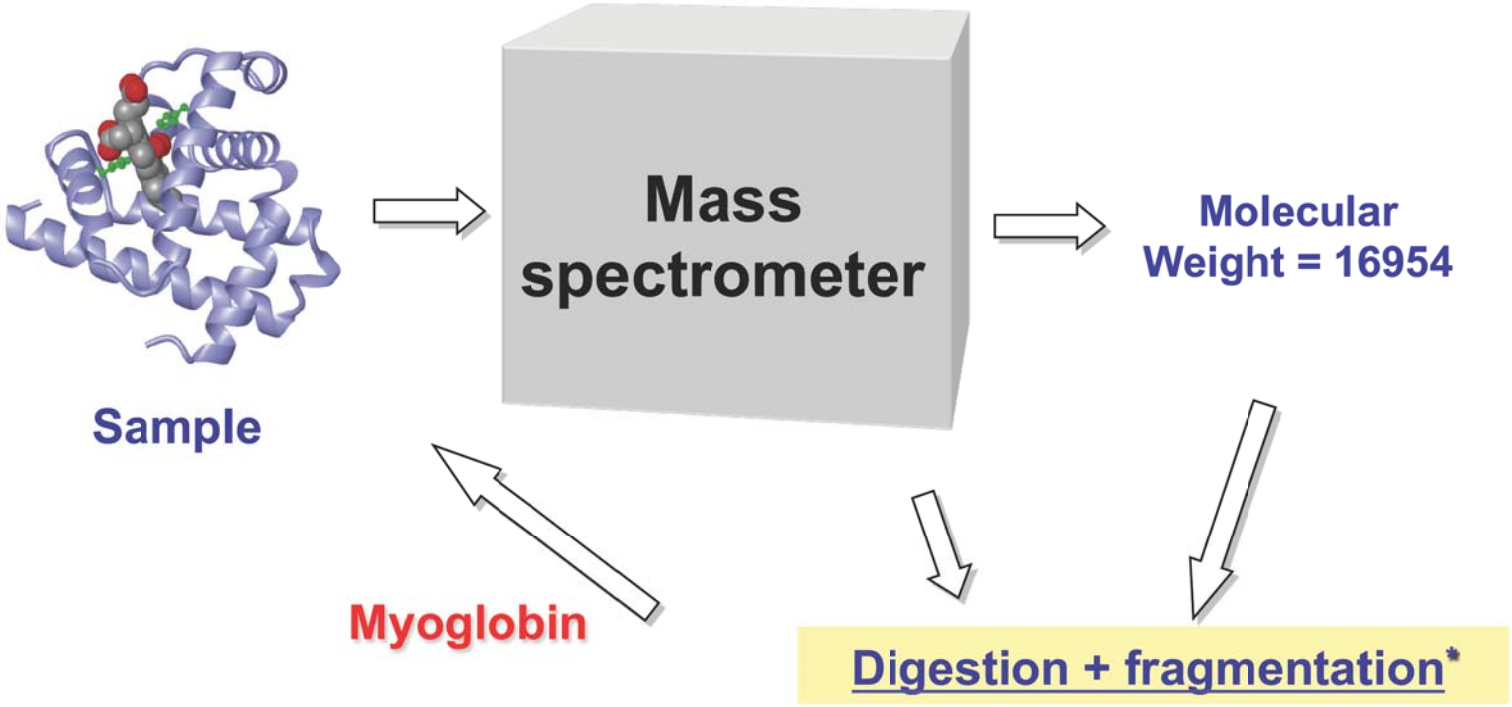
a. **Sensitive** *(< pmole of sample, HPLC requires ~nmole)*

b. **Rapid** *(<1 minute acquisition, HPLC requires >30 min)*

c. **Accurate** *(± 1 ppm)*

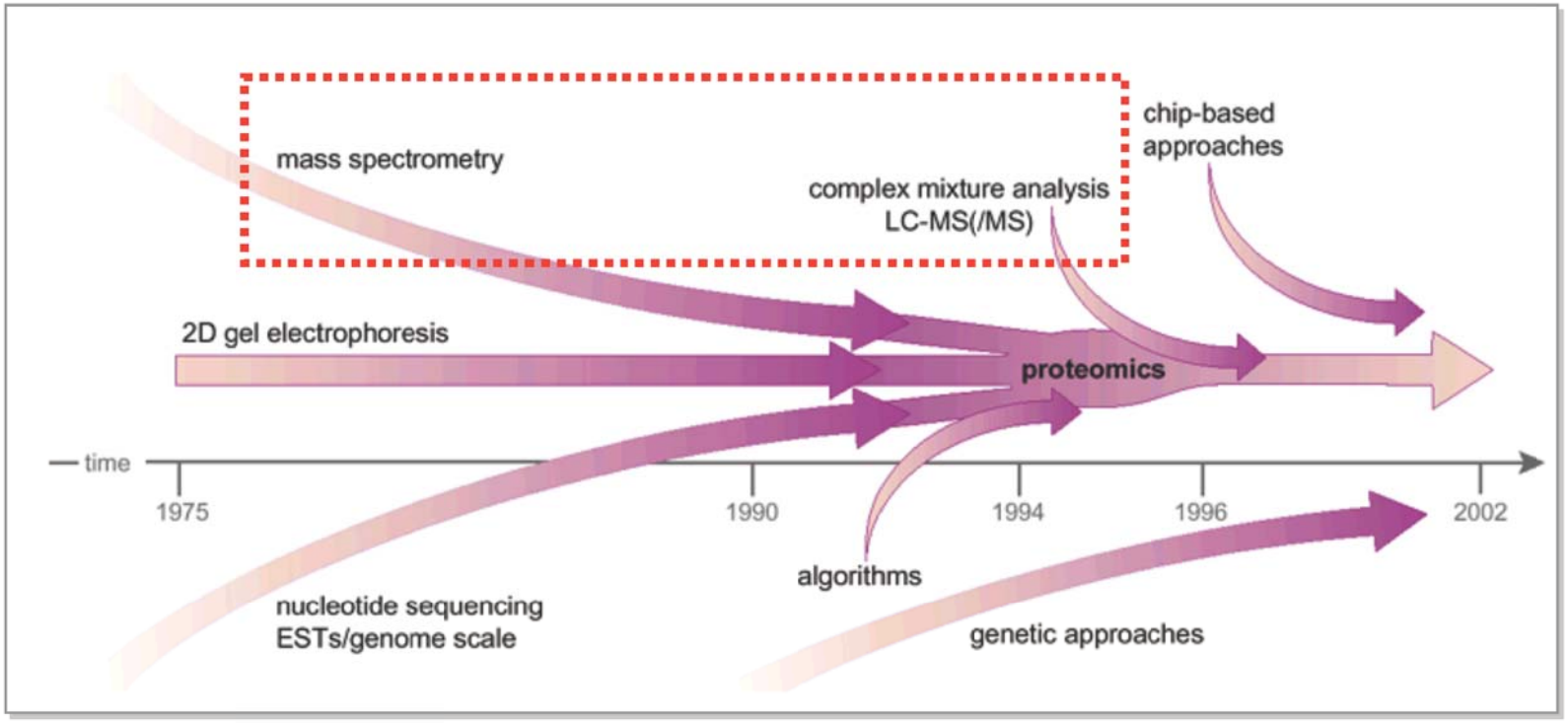
d. **Precise** *(0.005 amu)*

e. **Multifunctional** *(mass detection, structural analysis, quantity information, molecular interaction, spatial distribution)*



Sequence

```
1                 11                21                31                41
GLSDGEWQQV LNVWGKVEAD IAGHGQEVLI RLFTGHPETL EKFDKFKHLK
51                61                71                81                91
TEAEMKASED LKKHGTVVLT ALGGILKKKG HHEAELKPLA QSHATKHKIP
101               111               121               131               141               151
 IKYNEFISDA IIHVLHSKHP GDFGADAQGA MTKALELFRN DIAAKYKELG FQG
```



Time line indicating the convergence of different technologies and resources into a proteomic process. Advances in mass spectrometry and the generation of large quantities of nucleotide sequence information, combined with computational algorithms that could correlate the two, led to the emergence of proteomics as a field.

Scott D. Patterson & Ruedi H. Aebersold, Nature Genetics ***33****, 311 - 323 (2003)*

insight overview

From genomics to proteomics

Mike Tyers* & Matthias Mann†

**Samuel Lunenfeld Research Institute, Mount Sinai Hospital, and Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Canada M5G 1X5 (e-mail: tyers@mshri.on.ca)*

†Center for Experimental BioInformatics, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark (e-mail: mann@bmb.sdu.dk)

Proteomics is the study of the function of all expressed proteins. Tremendous progress has been made in the past few years in generating large-scale data sets for protein–protein interactions, organelle composition, protein activity patterns and protein profiles in cancer patients. But further technological improvements, organization of international proteomics projects and open access to results are needed for proteomics to fulfil its potential.

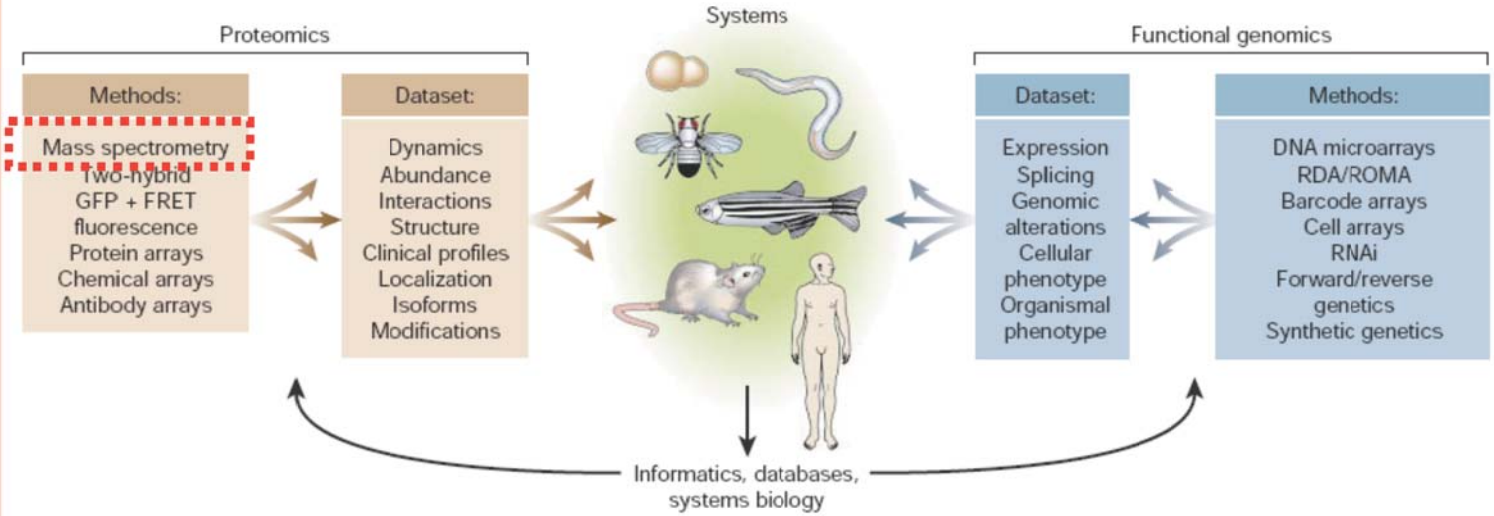


Figure 1 Platforms for proteomics and functional genomics. Methodology is shown in the outer columns, resultant data sets in the middle columns, and model systems in the centre.

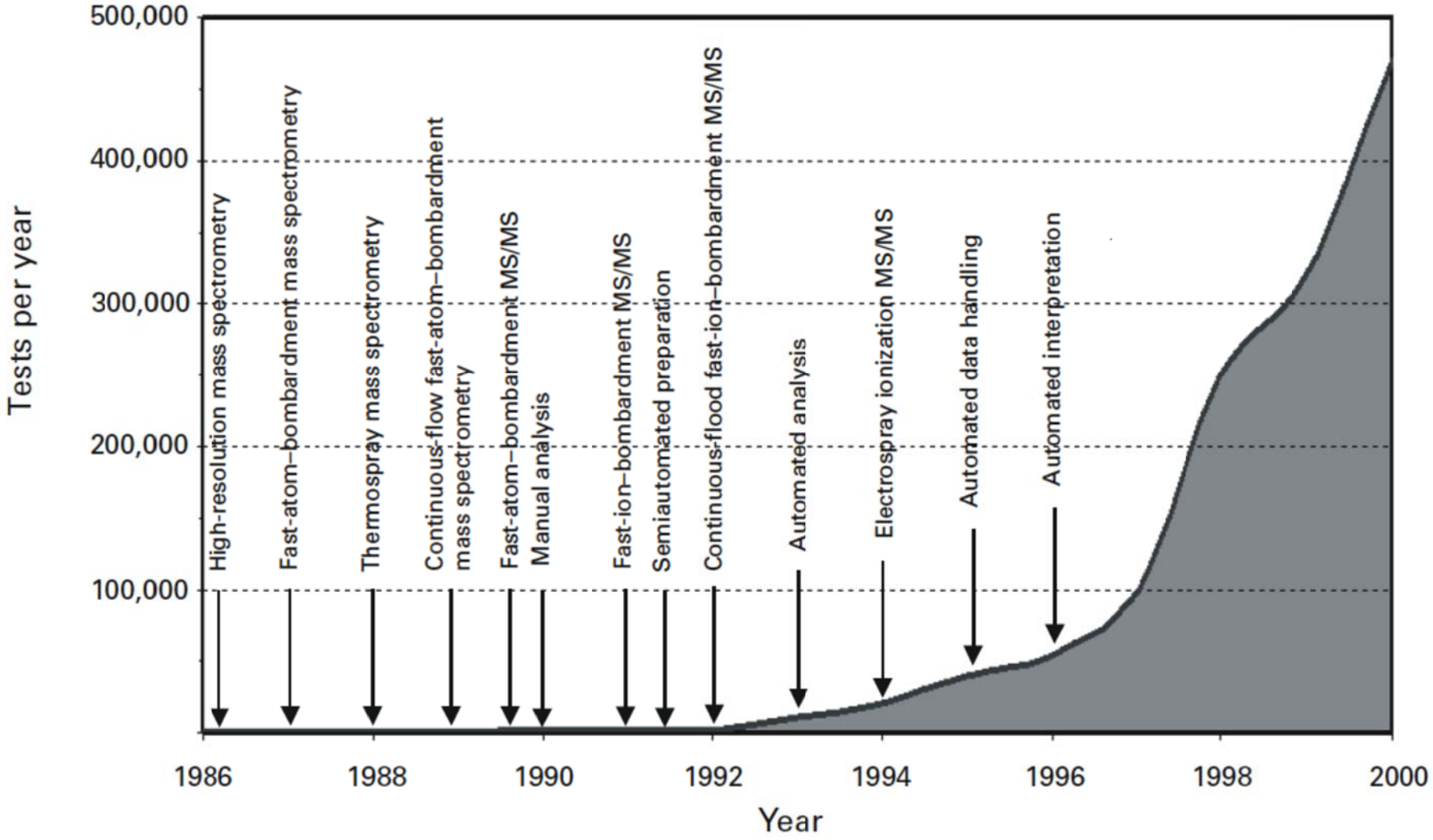
*Mike Tyers & Matthias Mann, Nature **422**, 193 (2003)*

Application of tandem mass spectrometry to newborn screening

TABLE 1. Metabolic disorders detectable in newborns aged 1–5 days by using tandem mass spectrometry

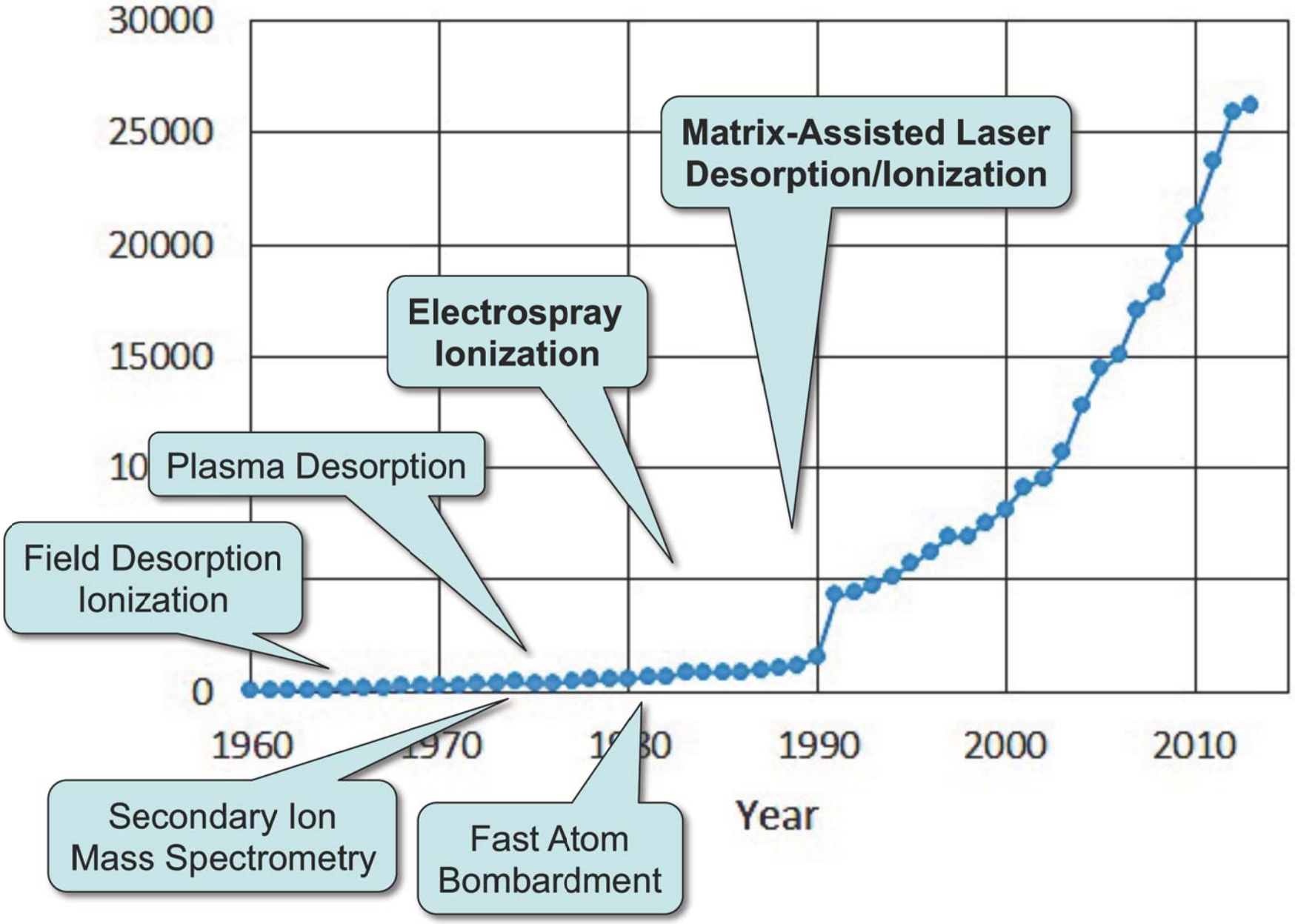
Disorder	Primary metabolic indicator
Amino Acids	
Phenylketonuria	Phe
Maple syrup urine disease	Leu/Ile, Val
Homocystinuria (cystathione synthase deficiency)	Met
Hypermethioninemia	Met
Citrullinemia	Cit
Argininosuccinic aciduria	Cit
Tyrosinemia, type I	Tyr
Fatty Acids	
Medium-chain acyl-CoA dehydrogenase deficiency	C8, C10, C10:1, C6
Very-long–chain acyl-CoA dehydrogenase deficiency	C14:1, C14, C16
Short-chain acyl-CoA dehydrogenase deficiency	C4
Multiple acyl-CoA dehydrogenase deficiency	C4, C5, C8:1, C8, C12, C14, C16, C5DC
Carnitine palmitoyl transferase deficiency	C16, C18:1, C18
Carnitine/acylcarnitine translocase defect	C16, C18:1, C18
Long-chain hydroxy acyl-CoA dehydrogenase deficiency	C16OH, C18:1OH, C18OH
Trifunctional protein deficiency	C16OH, C18:1OH, C18OH
Organic Acids	
Glutaric acidemia, type I	C5DC
Propionic acidemia	C3
Methylmalonic acidemia	C3
Isovaleric acidemia	C5
3-hydroxy-3-methylglutaryl CoA lyase deficiency	C5OH
3-methylcrotonyl CoA carboxylase deficiency	C5OH

Progress leading to the introduction of tandem mass spectrometry (MS/MS) in newborn screening



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention (CDC)

Number of Literature Found with the Key Word: "Mass Spectrometry"



Limitations of MS

- Performance of MS on biological samples:
  Proteins > DNAs > RNAs > Carbohydrates

- Sample preparation is crucial to the experimental result. (contamination, salt, detergent, concentration, etc…)

- MS is unsuitable for absolute quantitation because of unpredictable ionization and detection efficiencies.

- Data reproducibility relies critically on the preparation methods, sample conditions, instrument settings, etc.

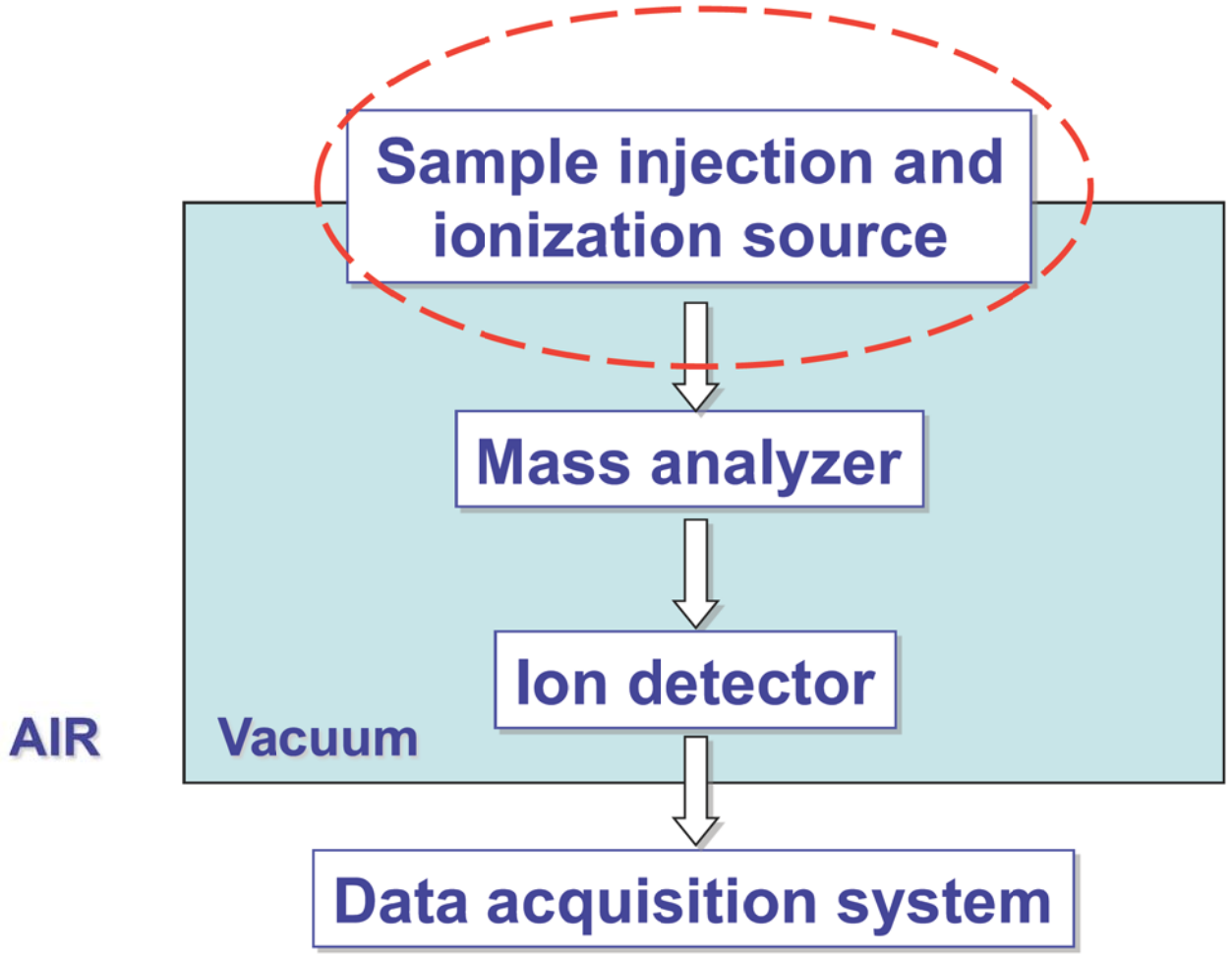
- MS/MS capability is limited by the fragmentation method, chemical property of samples, and ion loss during analysis.

- High accuracy and resolution is NOT always available. Mass calibration and instrument tuning should be performed regularly.

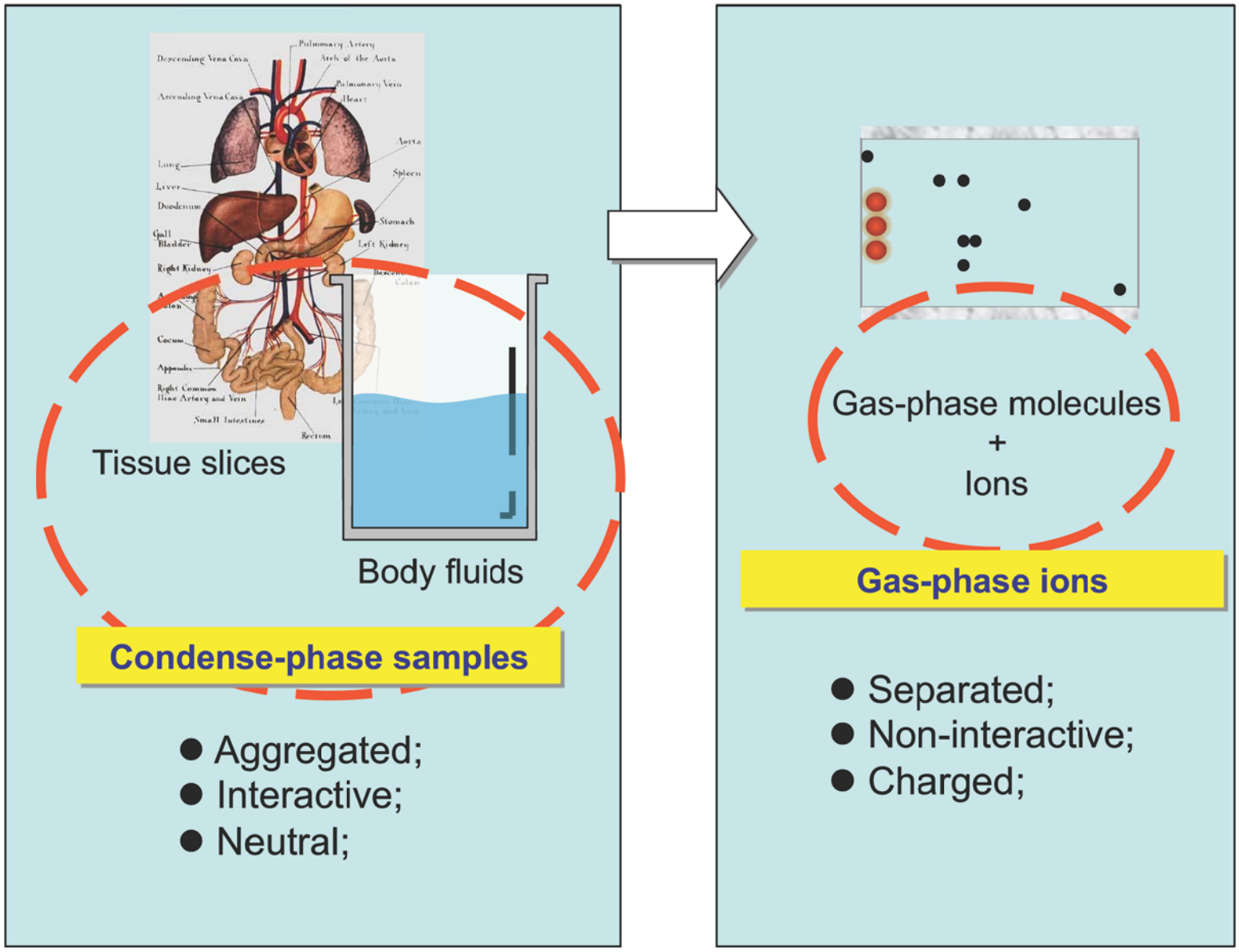
HOW

- *Charges in MS*
- *Ionization of samples*
- *Mass analyzers*
- *Fragmentation/tandem mass spectrometry*

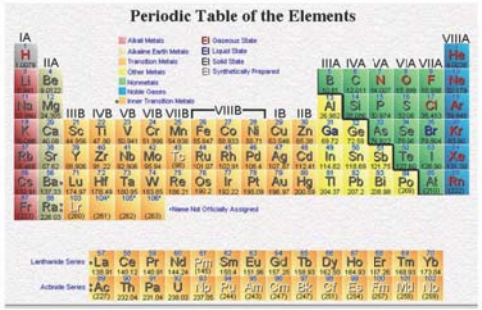
General Configuration of Mass Spectrometer



The Function of Ion Sources



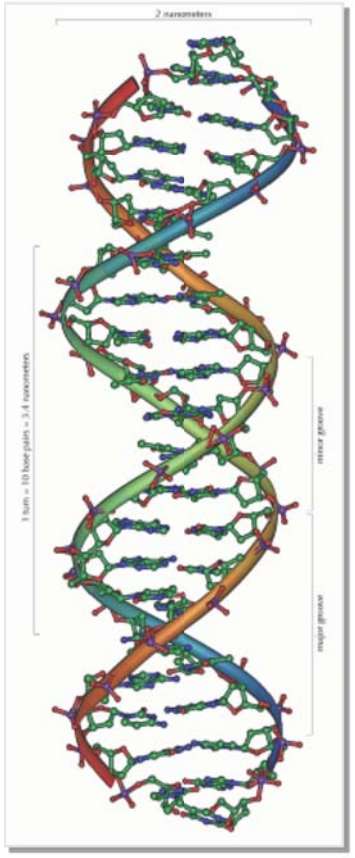
How electric charges contribute to the real world?



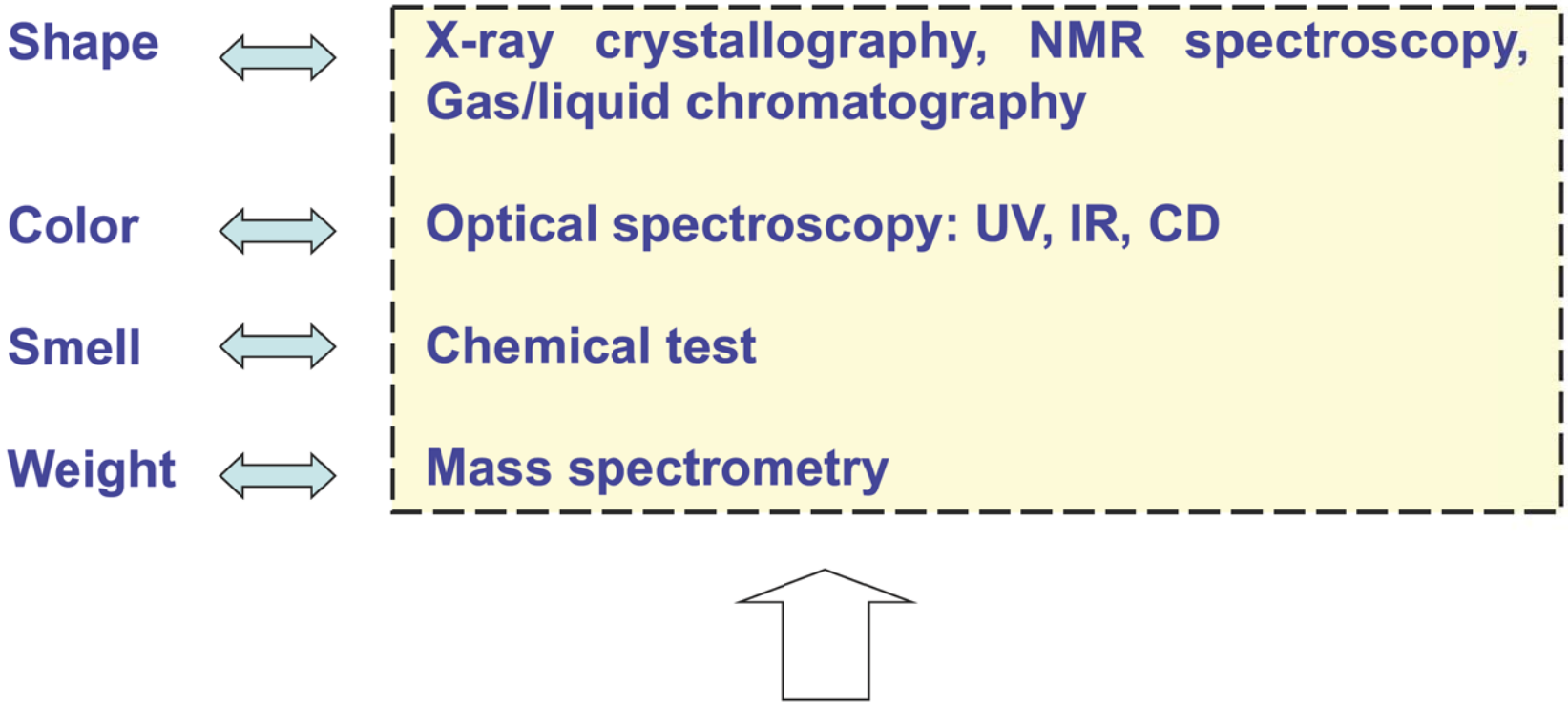
I. Making every atom chemically unique. *(Every atom would be chemically identical if charges do not exist.)*

II. Constructing molecules. *(It determines the physical condition, the color, the chemical reactivity, and the toxicity of molecules.)*

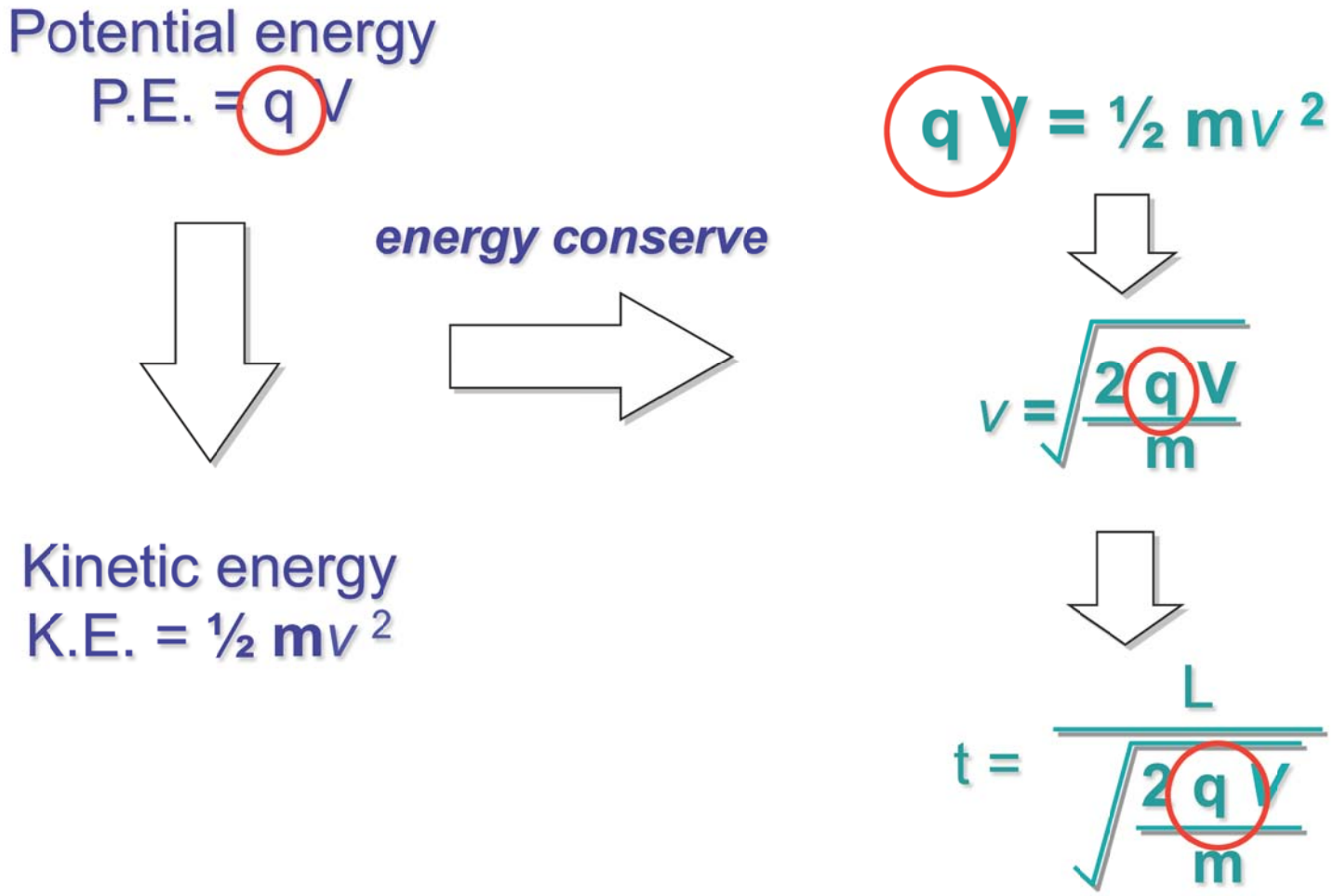
III. Inducing chemical reactions. *(All reactions are attributed to the interaction of electric charges between objects.)*



How humans and scientists identify species?



Principle of Mass Separation
(time-of-flight mass analyzer)



Principle of Other Mass Spectrometers

Time-of-flight MS

$$t = \frac{L}{\sqrt{\frac{2V}{m/q}}}$$

Quadrupole MS

$$a_u = \frac{8ZeU}{mw^2r_0^2} \qquad q_u = \frac{4ZeV}{mw^2r_0^2}$$

FT-ICR MS

$$w_c = (q/m)\,B$$

Charge and Charge Interaction

charge, property of matter that gives rise to all electrical phenomena. The basic unit of charge, usually denoted by *e,* is that on the proton or the electron; that on the proton is designated as positive (+*e*) and that on the electron is designated as negative (-*e*). All other charged elementary particles have charges equal to +*e,* -*e,* or some whole number times one of these

Particle	Mass	Charge
Electron	9.11×10^{-31} kg	-1.6×10^{-19} C
Proton	1.672×10^{-27} kg	$+1.6 \times 10^{-19}$ C
Neutron	1.674×10^{-27} kg	0

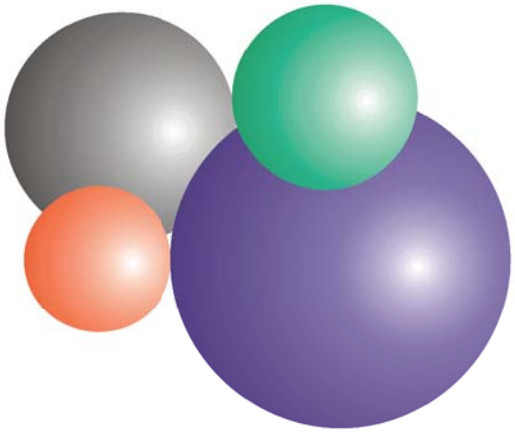
Coulomb's law, in physics, law stating that the electrostatic force between two charged bodies is proportional to the product of the amount of charge on the bodies divided by the square of the distance between them.

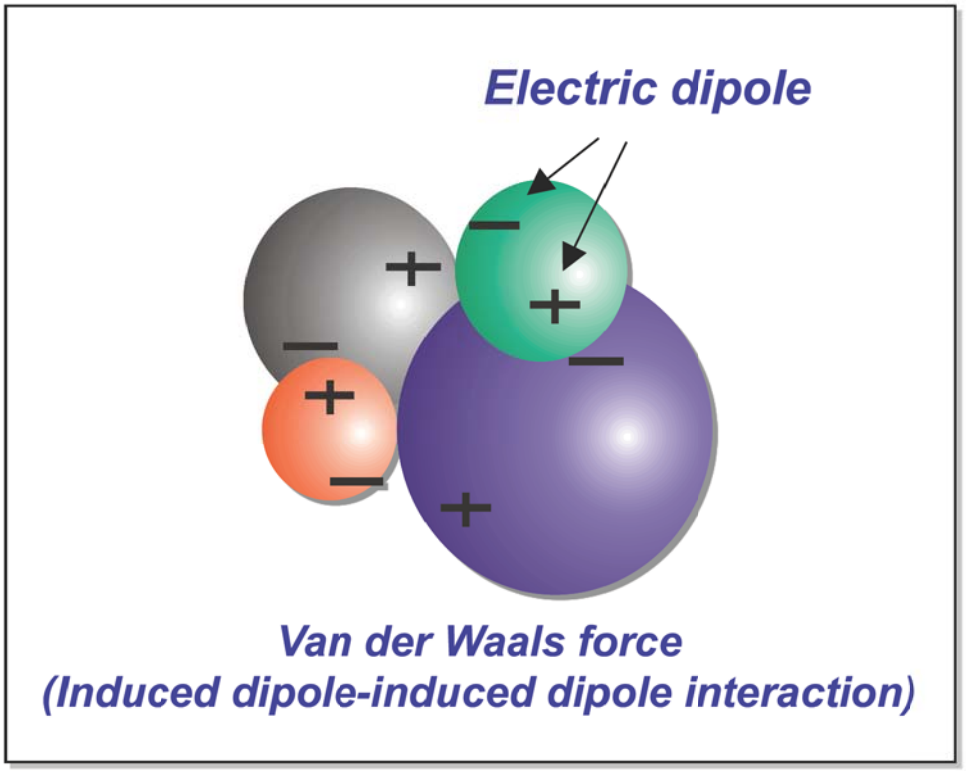
$$F = k\frac{q_a q_b}{r^2}$$

The Columbia Electronic Encyclopedia, 6th ed.

Charge and Charge Interaction

Neutral molecules

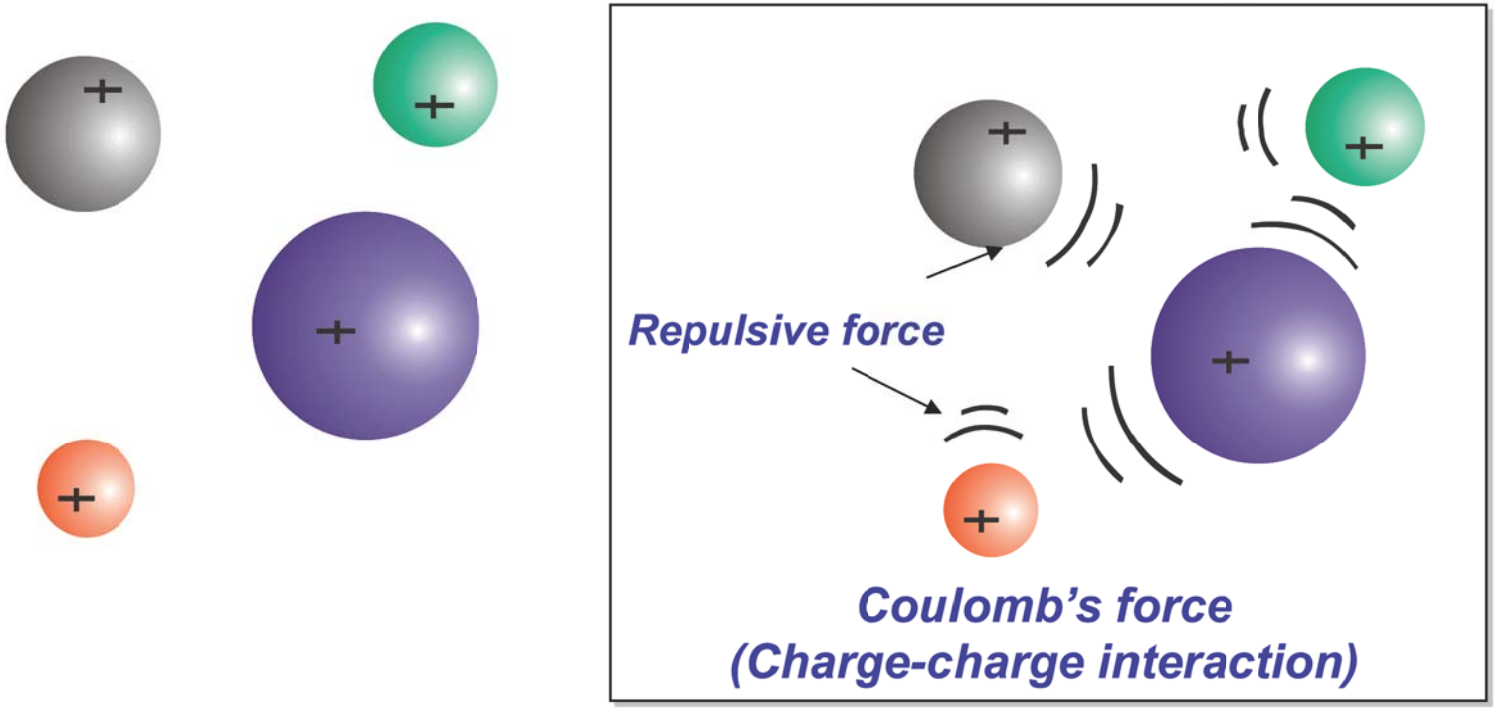




Neutral molecules tend to aggregate because of van der Waals attractive force.

Charge and Charge Interaction

Ions with same charge polarity

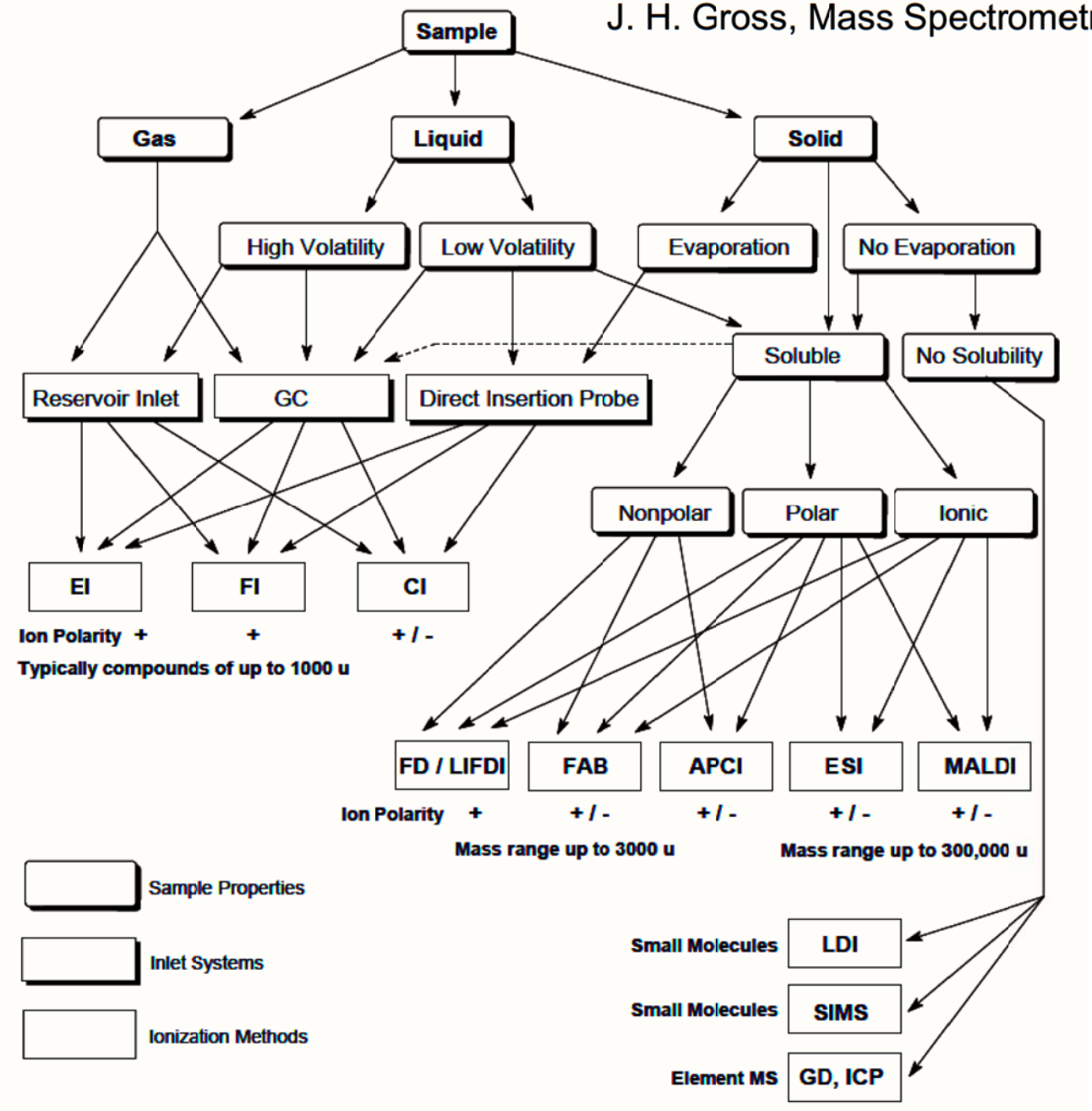


The function of electric charges in MS:*

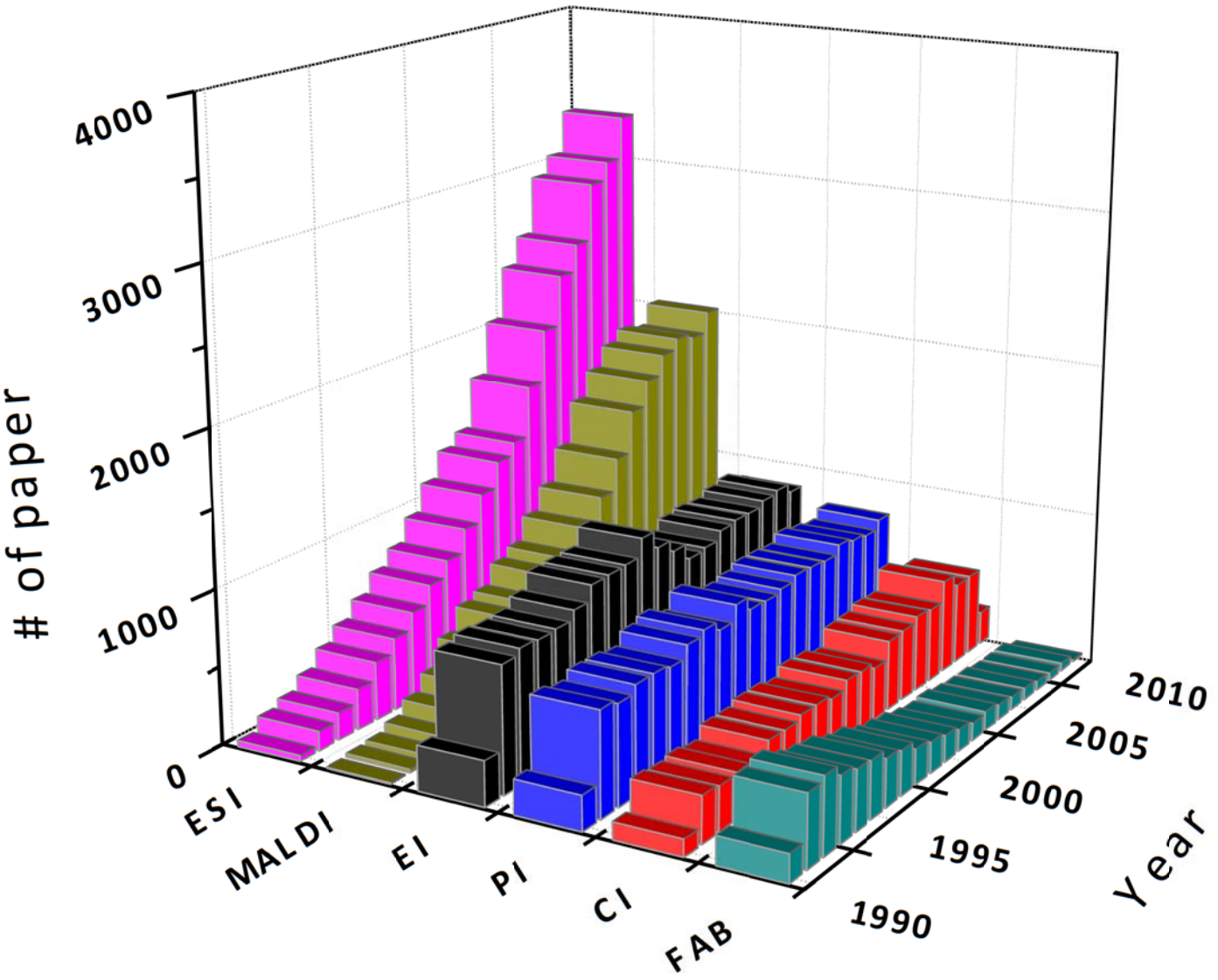
- Particle separation (Coulombic repulsion)
- Trajectory modulation (Coulombic interaction)
- Particle detection (electric current measurement)

Mass Spectrometric Method Selection

J. H. Gross, Mass Spectrometry A Textbook



Number of Literature Associated With Every Ionization Method



Electron Ionization (EI)

- Volatile samples
- Molecular ions (M+)
- Extensive fragmentation

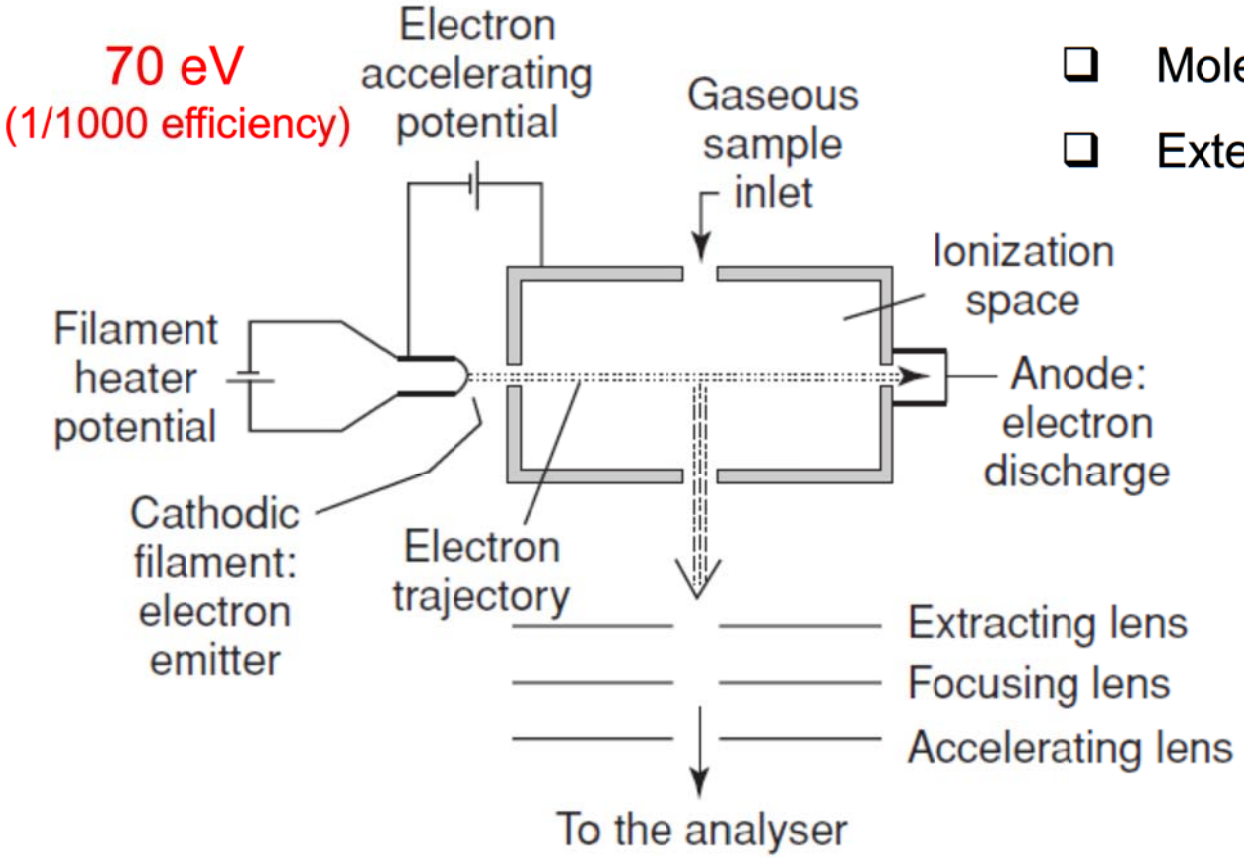


Figure 1.1
Diagram of an electron ionization source.

Hoffmann and Stroobant, Mass Spectrometry: Principles and Applications

Chemical Ionization (CI)

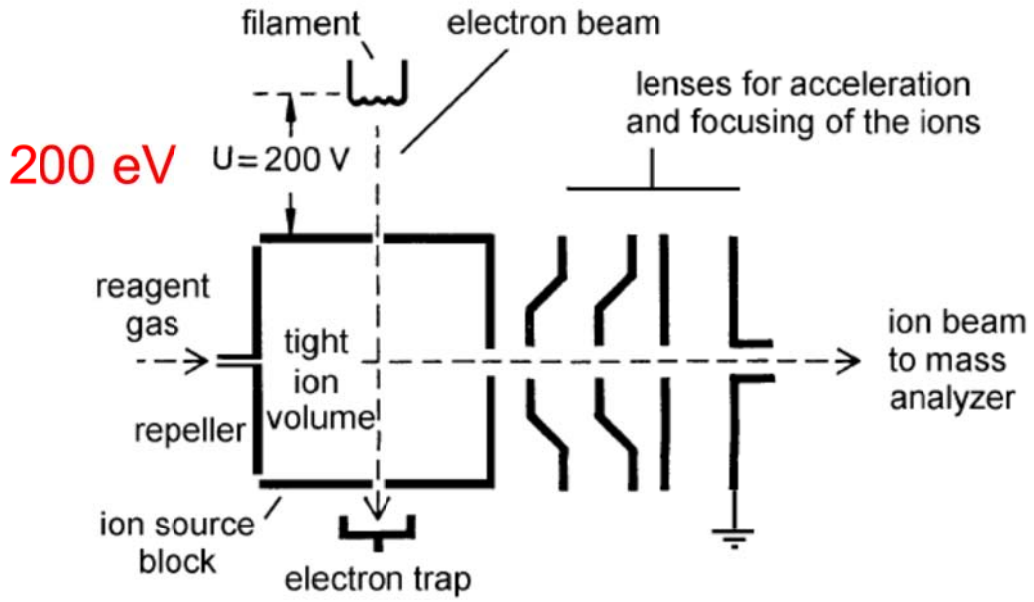
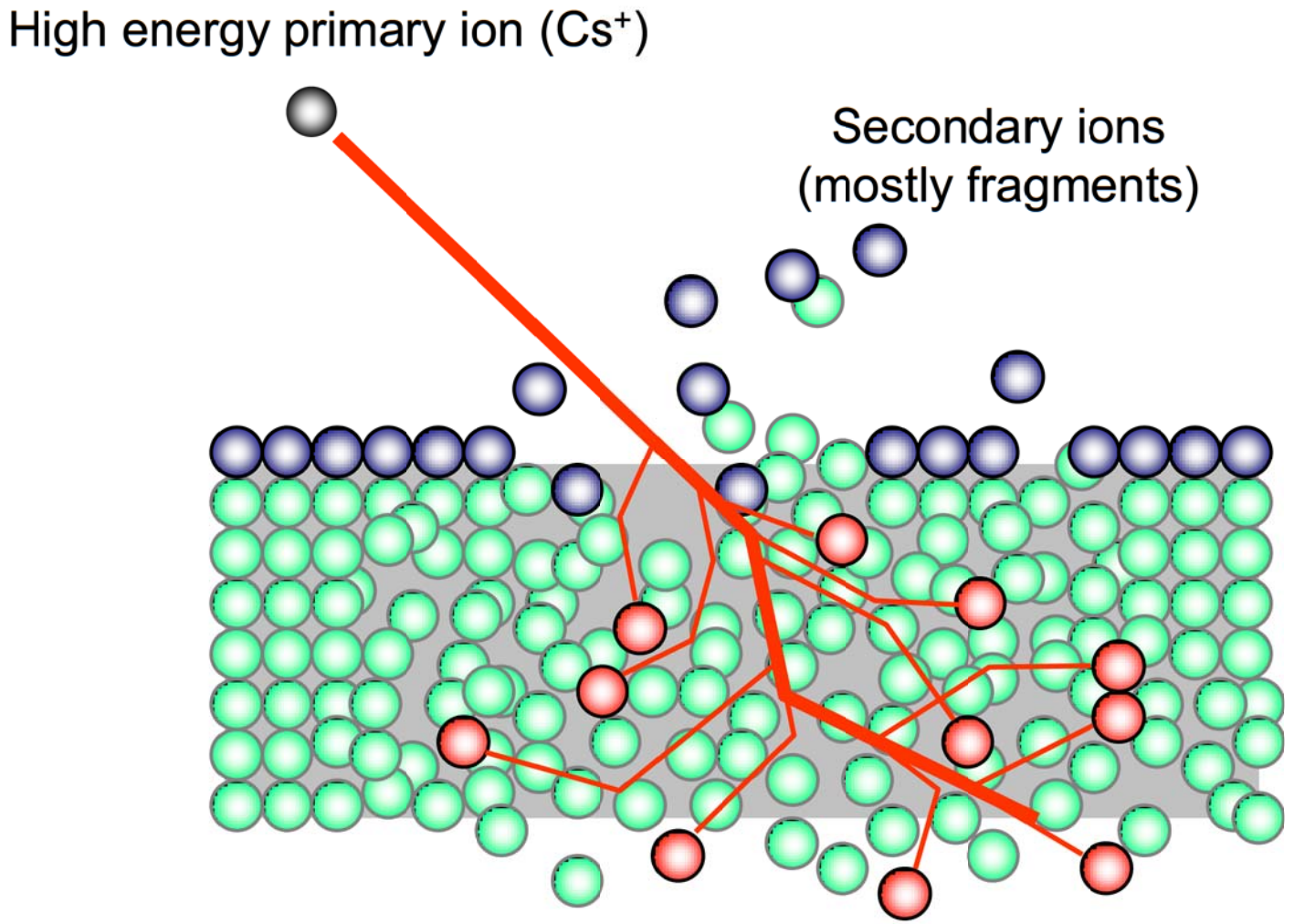


Fig. 7.1. Layout of a chemical ionization ion source. Adapted from Ref. [14] by permission.

$M + [BH]^+ \rightarrow [M+H]^+ + B$	*proton transfer*	(7.1)
$M + X^+ \rightarrow [M+X]^+$	*electrophilic addition*	(7.2)
$M + X^+ \rightarrow [M-A]^+ + AX$	*anion abstraction*	(7.3)
$M + X^{+\bullet} \rightarrow M^{+\bullet} + X$	*charge exchange*	(7.4)

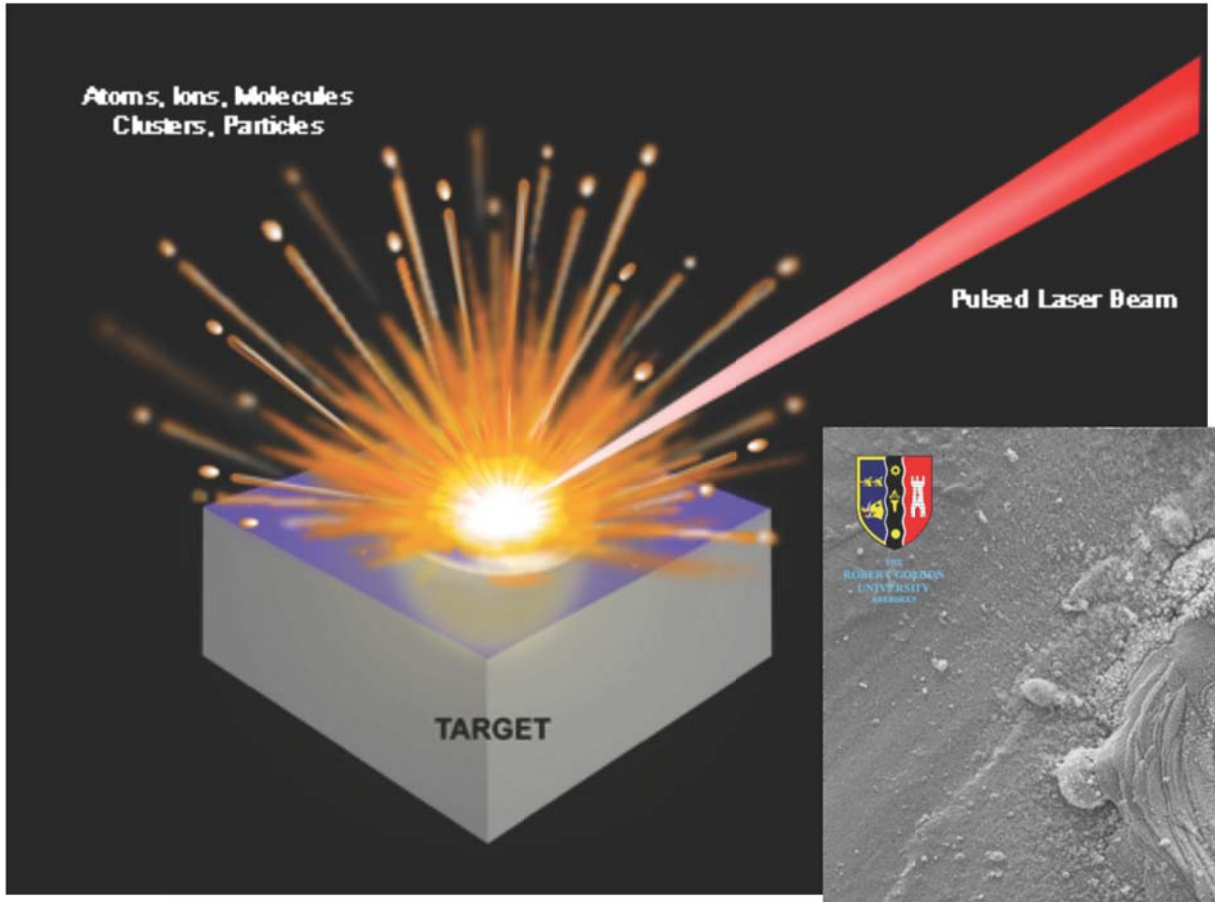
J. H. Gross, Mass Spectrometry A Textbook

Secondary Ion Mass Spectrometry (SIMS)



Schematic of the collision cascade

Laser Ablation



Applied Spectra, Inc.

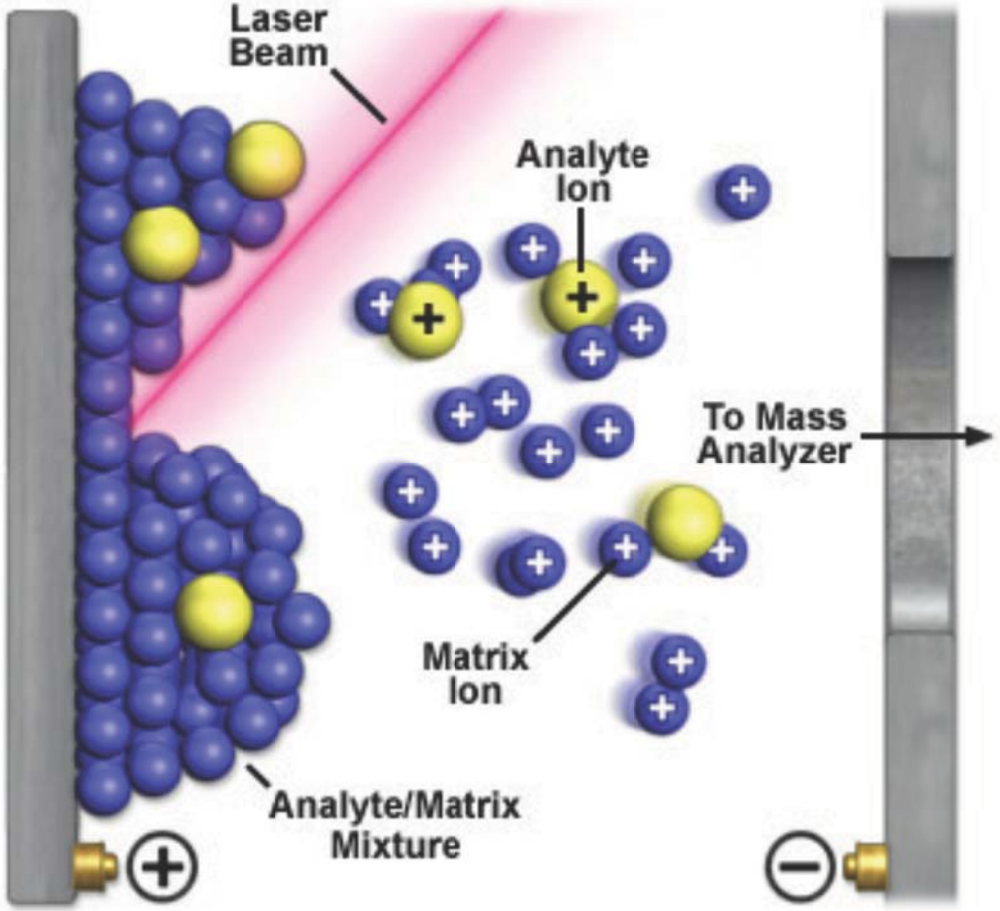
Fluence ≈100 J/cm^2

Power ≈10^{12} W/cm^2

steel surface

Robert Gordon University

Matrix-Assisted Laser Desorption/Ionization (MALDI)



Fluence ≈0.01 J/cm^2

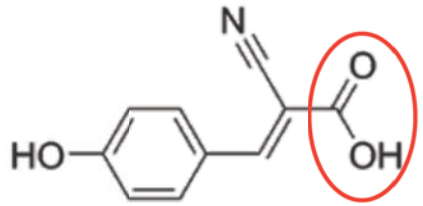
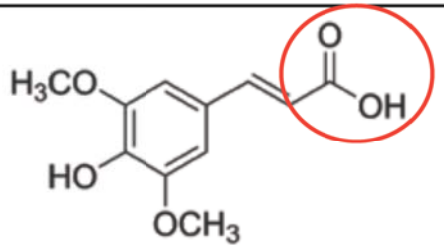
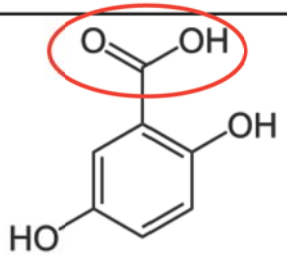
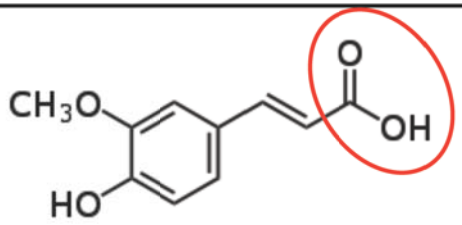
Power ≈10^6 W/cm^2

Sample solution + matrix solution (1:5000)*

Matrix is a photon absorber and a proton provider.*

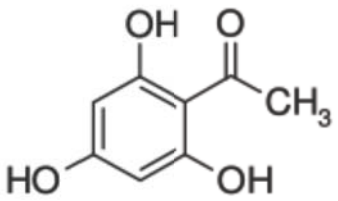
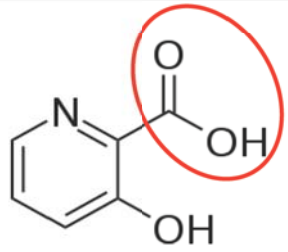
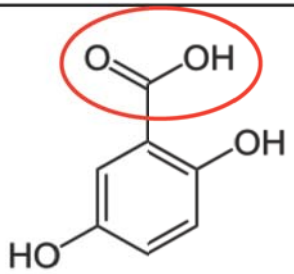
Correct choice of matrix

– Proteomics application

Recommended Matrix		Application
α-cyano-4-hydroxycinnamic acid (CHCA)		Peptides and proteins (<10kDa)
Sinapinic acid (3,5-dimethoxy-4-hydroxy cinnamic acid)		Peptides and proteins (>10kDa)
2,5-dihydroxybenzoic acid (DHB)		Peptides and proteins (<10kDa) Carbohydrates
4-hydroxy-3-methoxycinnamic acid (Ferulic acid)		For proteins

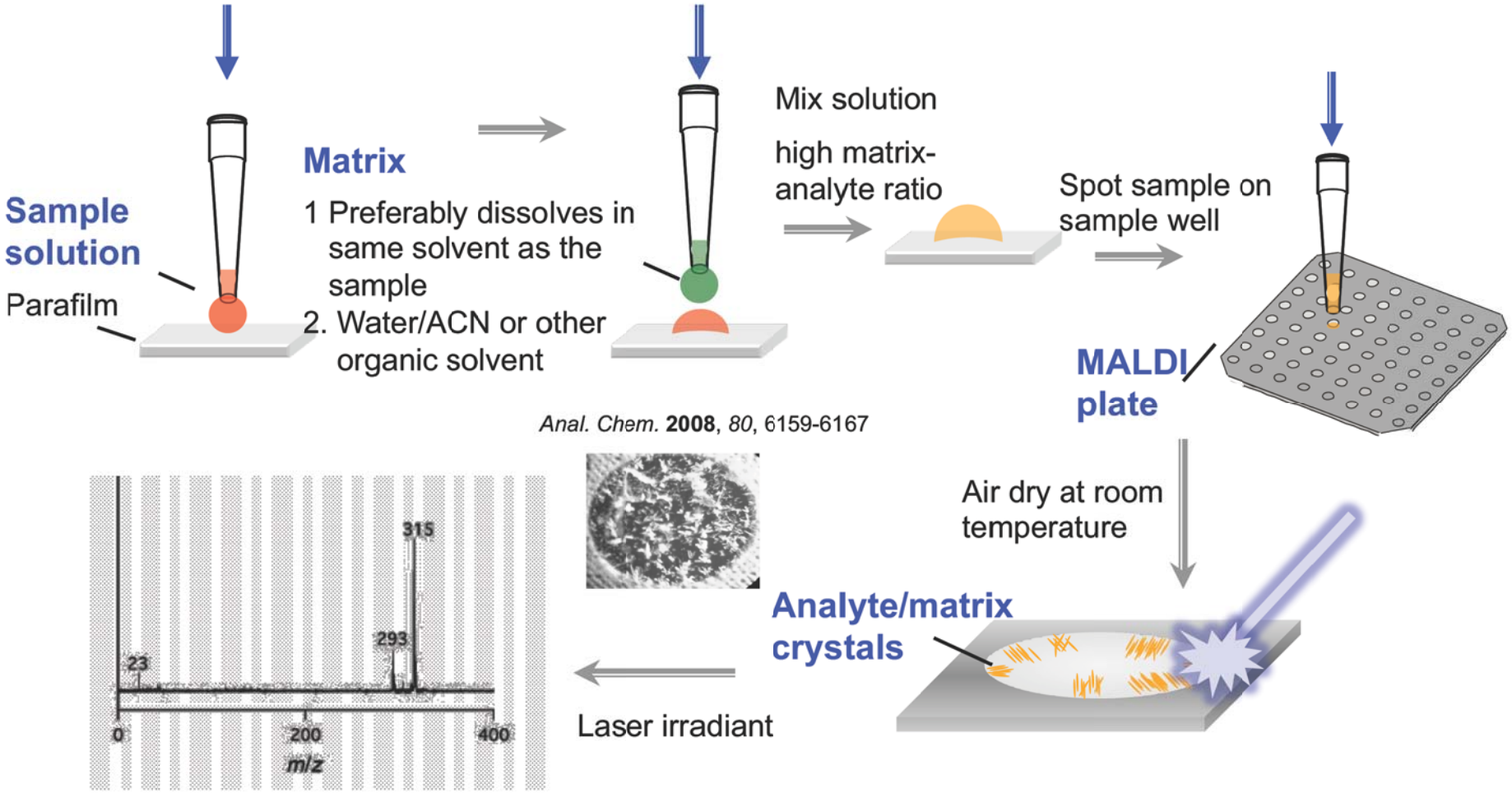
Correct choice of matrix

– Proteomics application

Recommended Matrix		Application
2,4,6-Trihydroxyacetophenone (THAP)		• Small oligonucleotides <3500 Da • Acidic carbohydrates • Acidic glycopeptides • Acidic-sensitive compounds
3-hydroxypicolinic acid (3-HPA)		Large oligonucleotides >3500 Da
2,5-dihydroxybenzoic acid (DHB)		• Neutral or basic carbohydrates • Glycolipids • Synthetic polymers • Small molecules

Sample preparation in MALDI MS

– Dried-droplet crystallization



Anal. Chem. **2008**, *80*, 6159-6167

Advantages: Simple

Disadvantages: The analyte/matrix crystals are inhomogeneous and irregularly distributed, which is the reason MALDI users often end up searching for "sweet spots" on their sample surfaces.

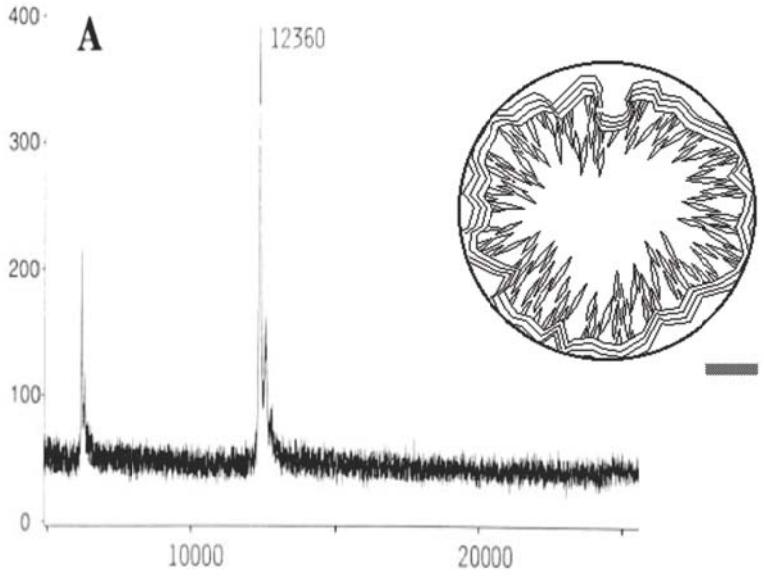
Sample spotting techniques

- **Dried droplet**

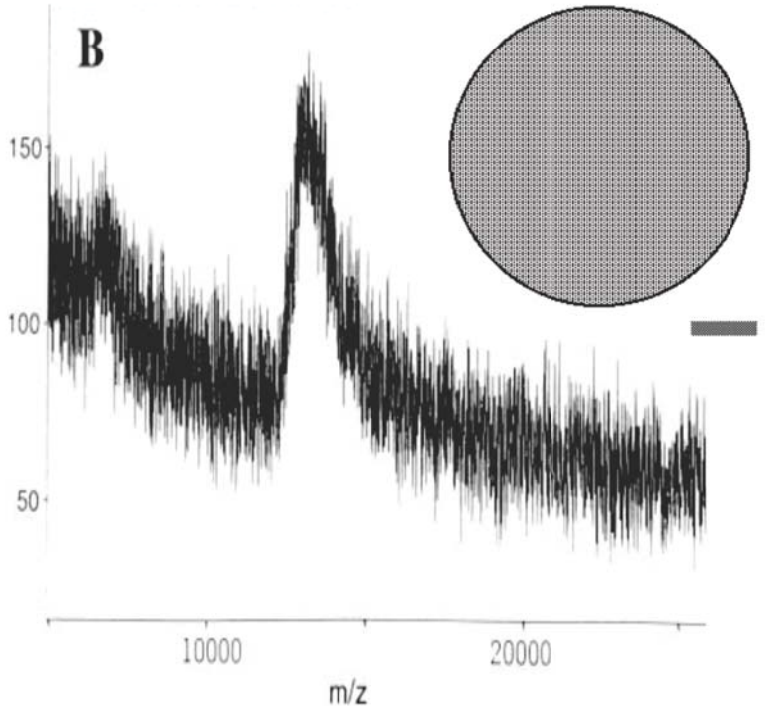
"Sweet Spot" -
Inhomogeneous sample crystallization

Homogeneous MALDI spot

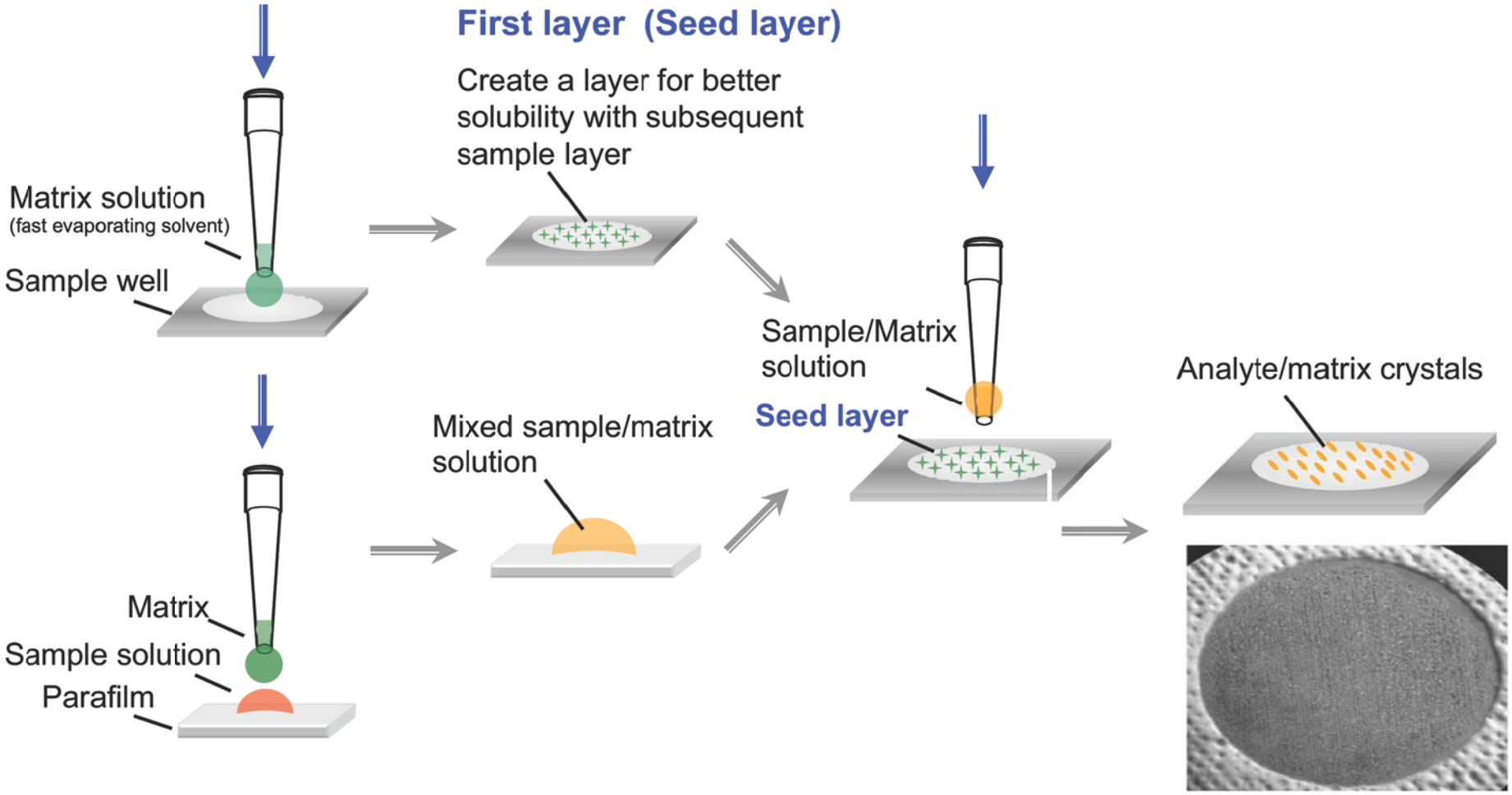
- **Fast evaporation**
- **Crushed crystal**
- **Thin layer (acetone)**
- **Sandwich**



Fast Evaporation



Thin-layer method (two layer)



Anal. Chem. **2008**, *80*, 6159-6167

Advantages: (good for quantification)

Uniform crystalline deposits

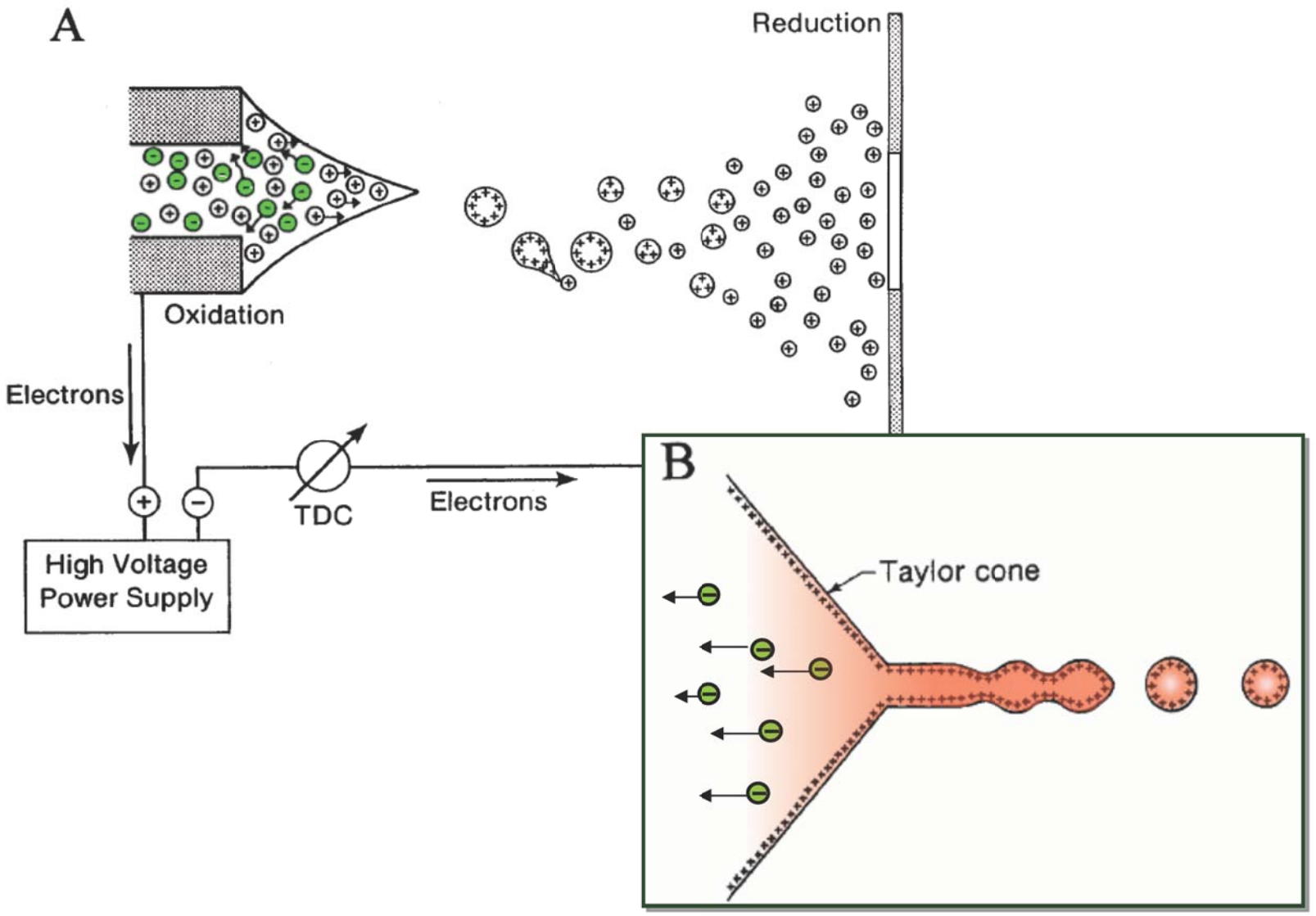
Provides enhanced sensitivity and excellent spot-to-spot reproducibility

Ionization Energetics of Biomolecules

Amino acid	Proton affinity (kcal/mol)	Na^+ affinity (kcal/mol)	Ionization potential (eV)
R	251	55	-
K	238	-	9.1
H	236	51	8.2
W	227	47	7.9
Q	224	50	-
N	222	49	-
Y	221	45	8.3
G	212	36	9.2
2,5 DHB	204	38	8
Glucose	188	43	-
Water	168	23	13

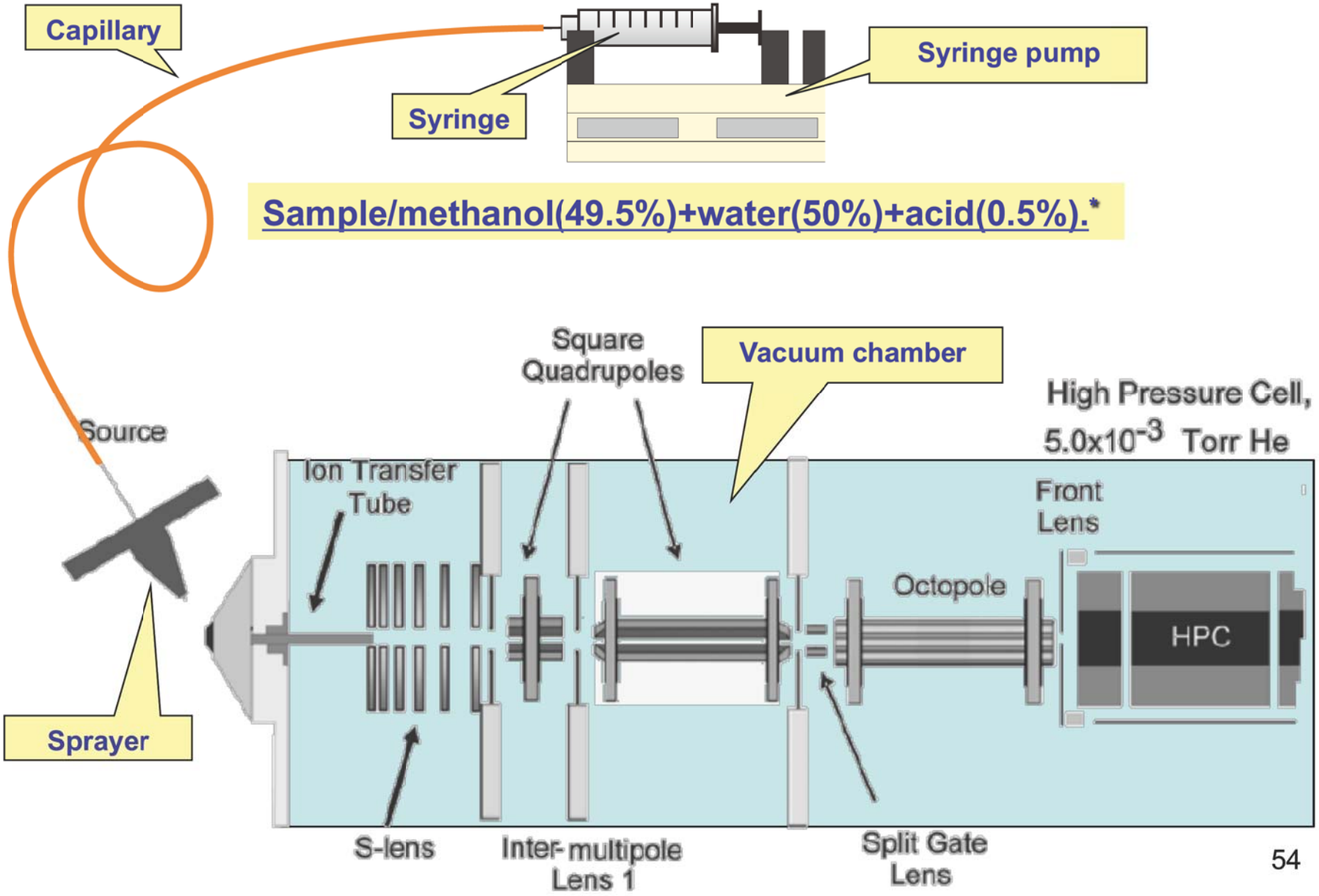
The higher charge affinity, the better signal quality*

Electrospray Ionization (ESI)



Kebarle, *J. Mass Spectrom.*, 2000, *35*, 804-817.

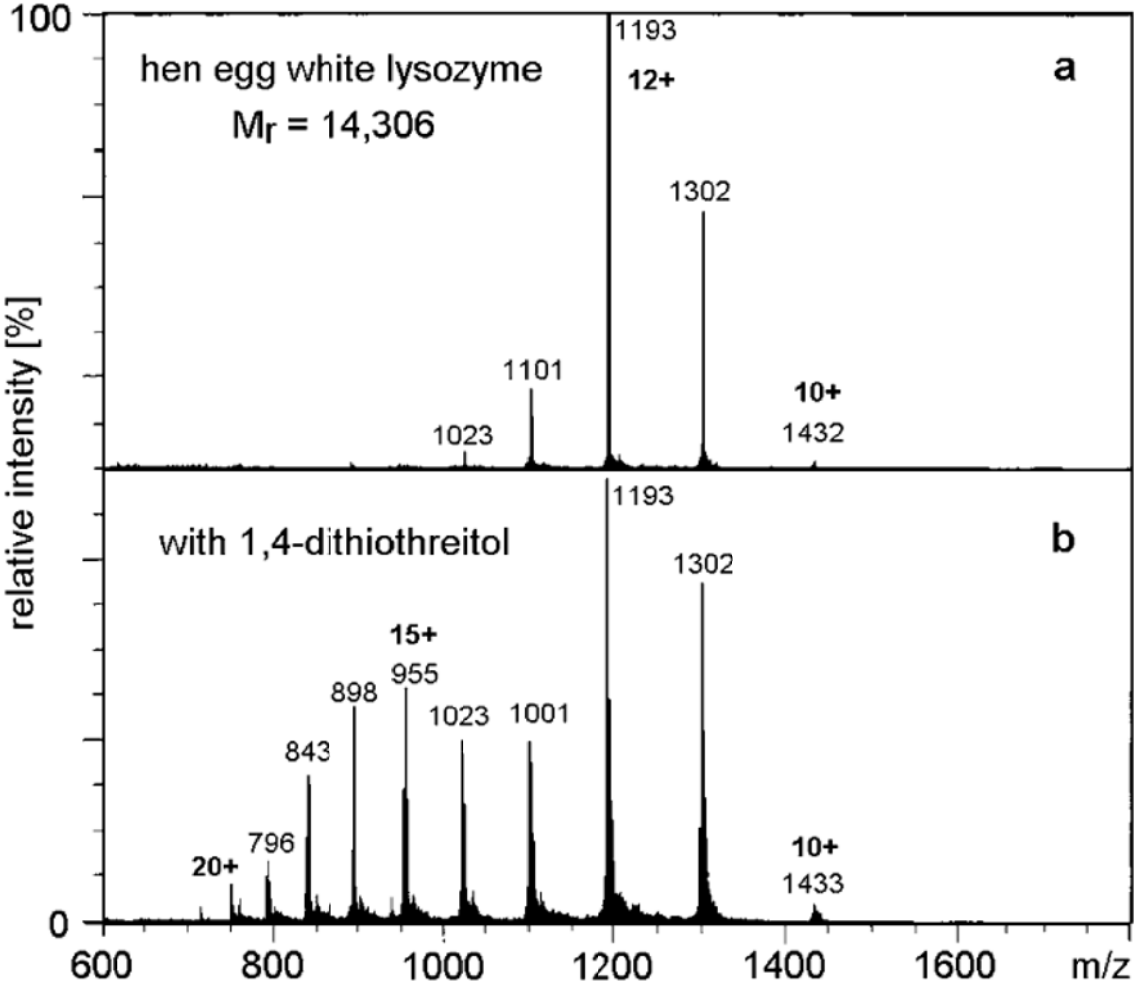
Electrospray Ionization (ESI)



Electrospray Ionization (ESI)

M → $[M+H]^{+}$, $[M+2H]^{2+}$, $[M+3H]^{3+}$, $[M+4H]^{4+}$, ...

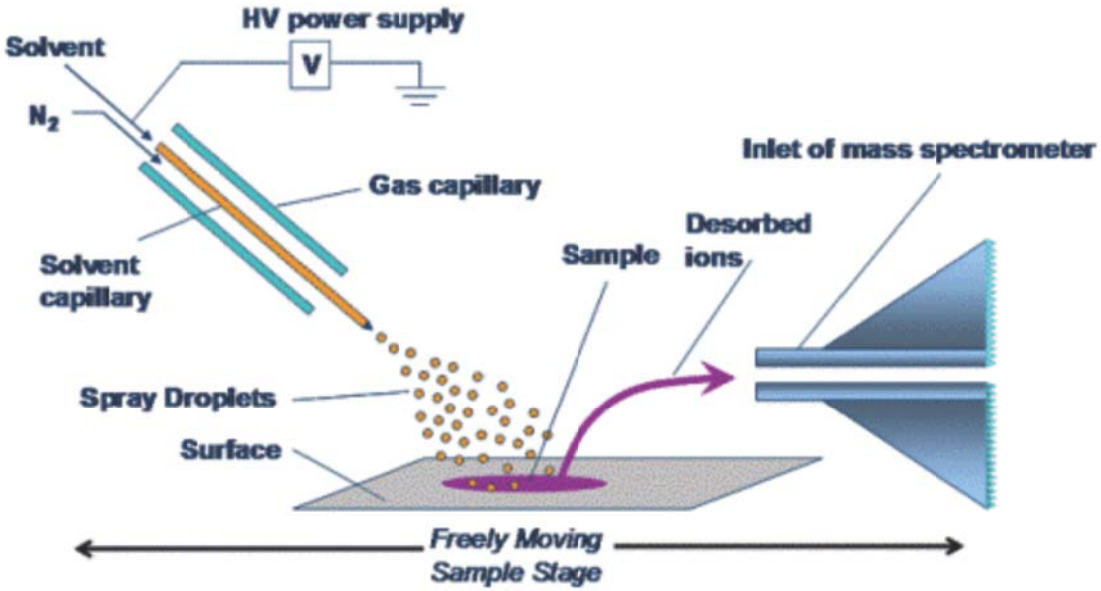
→ $[M-H]^{-}$, $[M-2H]^{2-}$, $[M-3H]^{3-}$, $[M-4H]^{4-}$, ...



Jurgen H. Gross, *Mass Spectrometry: A Textbook, 2nd Ed., 2011, Springer.*

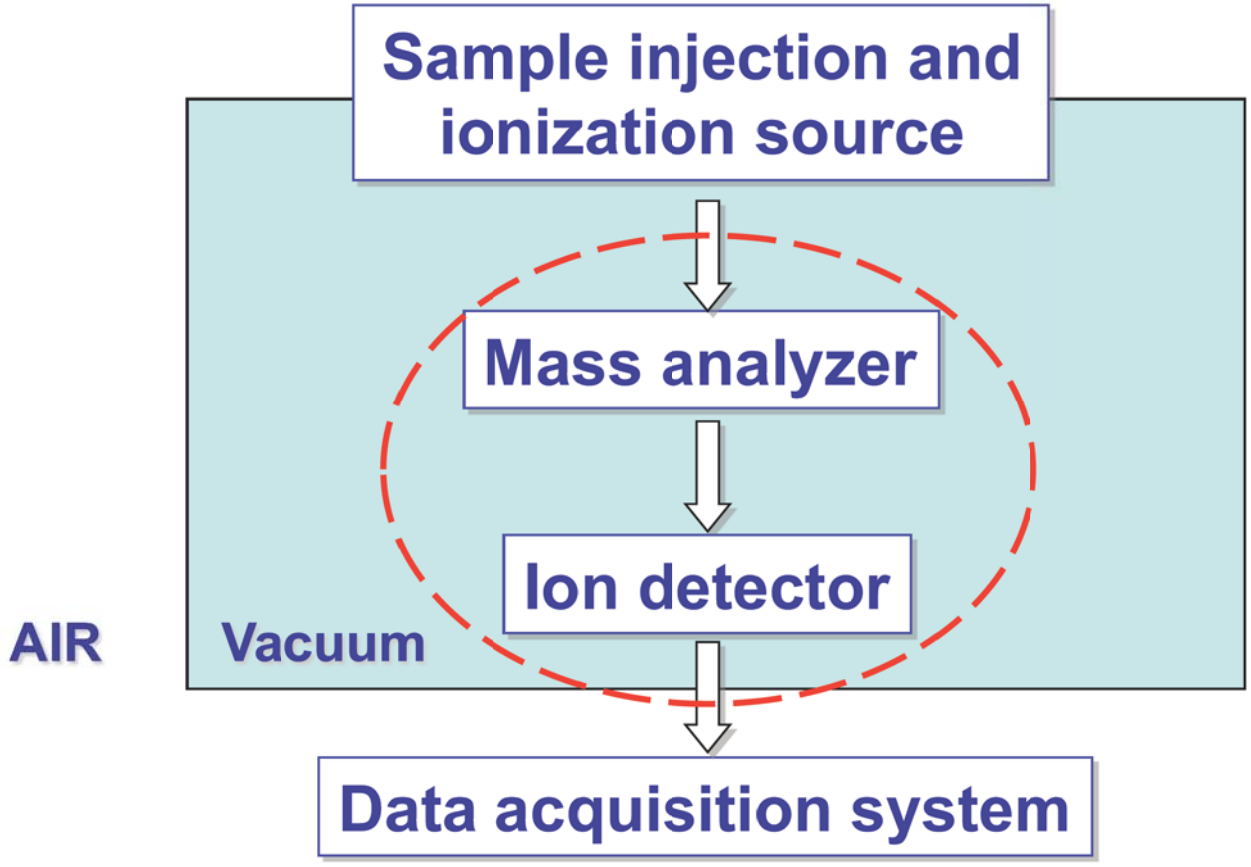
Atmospheric Pressure Ionization Source

Desorption electrospray ionization source

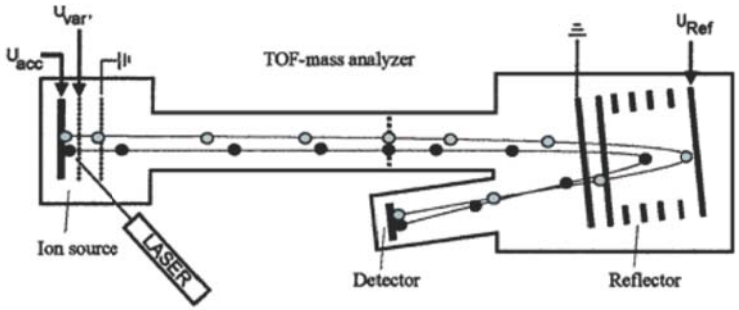


Prosolia Inc.

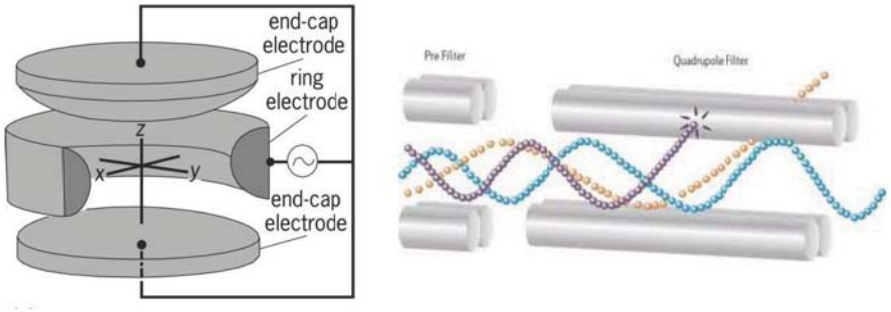
General Configuration of Mass Spectrometer



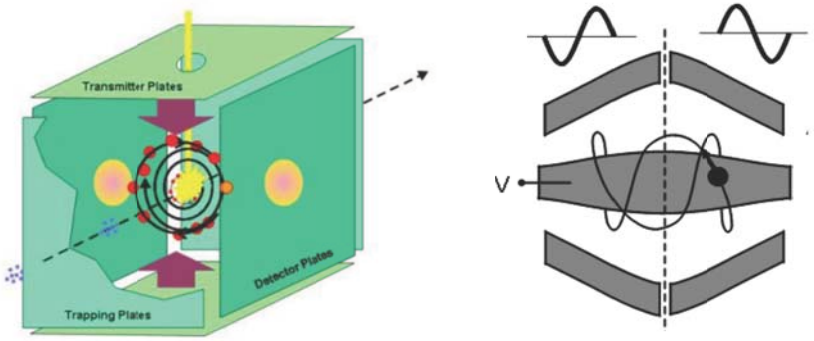
Important Mass Analyzers



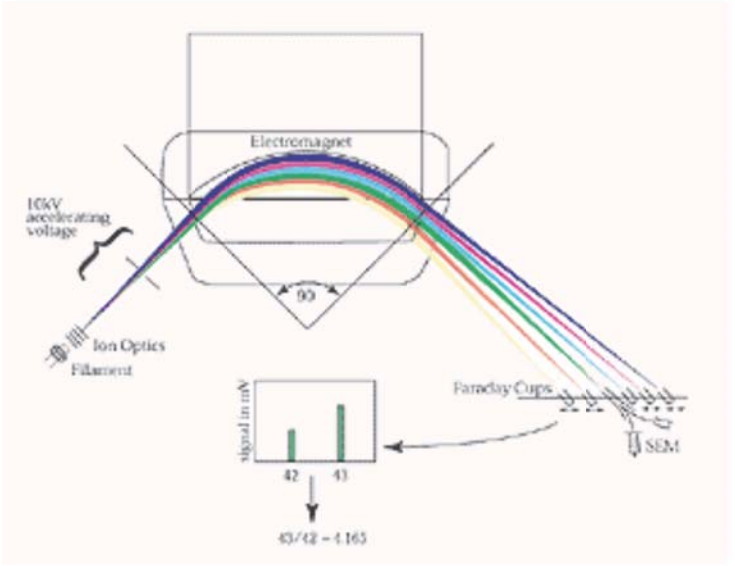
Time-of-Flight (TOF)



Ion Trap/Quadrupole



Fourier-Transform Mass Analyzer



Magnet Sector