Ionization Methods

1. Gas-Phase Ionization Methods
   - A. Electron Impact Ionization
   - B. Chemical Ionization
2. Desorption Ionization Methods
   - A. Field Desorption Ionization
   - B. Fast Atom Bombardment (FAB) Ionization
   - C. Plasma Desorption Ionization
   - D. Laser Desorption Ionization
3. Evaporative Ionization Methods
   - A. Thermospray Mass Spectrometry
   - B. Electrospray Mass Spectrometry

Gas-Phase Ionization Methods

A. Electron Impact Ionization

- EI was available before other ionization methods were developed
- Must have a stable molecule in the vapor phase (no salts, no proteins or peptides, sugars) Only ~20% of organic molecules
- Stability of Molecular ions: Aromatics> conjugated olefins > alicyclics > sulfides > unbranched hydrocarbons > mercaptans >ketones > amines > esters > ethers > carboxyllic acids > branched hydrocarbons > alcohols
- Must have a lifetime $>10^{-6}$ sec

Disadvantages of EI

- Must be vaporized to 10^{-6} torr (not applicable to fragile low vapor , pressure species e.g. salts, sugars, peptides, proteins, polymers)
- EI often leads to extensive fragmentation and no molecular ion is observed
- Rearrangements can cause difficulty in explaining structure.

Advantages of EI:

- Relatively simple to build
- Fragments seen enables library of EI mass spectra containing several 100K spectra (Note there are about 60,000 cpds. common in commerce.)
- Compatible with GC
- Fragments can tell something about structure
- Ion profiles can confirm presence or absence without examining entire spectrum.

B. Chemical Ionization

- The most important "soft ionization" technique to avoid extensive fragmentation in EI.
- In CI: Sample molecules are not subjected to bombardment by high energy electrons
- Reagent gas (usually methane, isobutane, ammonia) is introduced into the source and ionized.
- Sample molecules collide with ionized reagent gas molecules (CH_5^+, $C_4H_9^+$, etc) and undergoes secondary ionization by:
  1. Proton transfer producing an $[M+1]^+$ ion
  2. Electrophilic addition producing $[M+15]^+$, $[M+43]^+$ ion
  3. $[M+18]^+$ with NH_4^+
- CI sometimes give prominent $[M-1]^+$ because of hydride abstraction
- Main use: detection of molecular ion and hence molecular weight

1. Electron beam generates proton donor (via EI of reagent gas)

$$CH_4 + e^- \longrightarrow CH_4^{+\bullet} + 2e^-$$

$$CH_4^{+\bullet} + CH_4 \longrightarrow CH_3^{\bullet} + \boxed{CH_5^{+}}$$ proton donor

$$NH_3 + e^- \longrightarrow NH_3^{+\bullet} + 2e^-$$

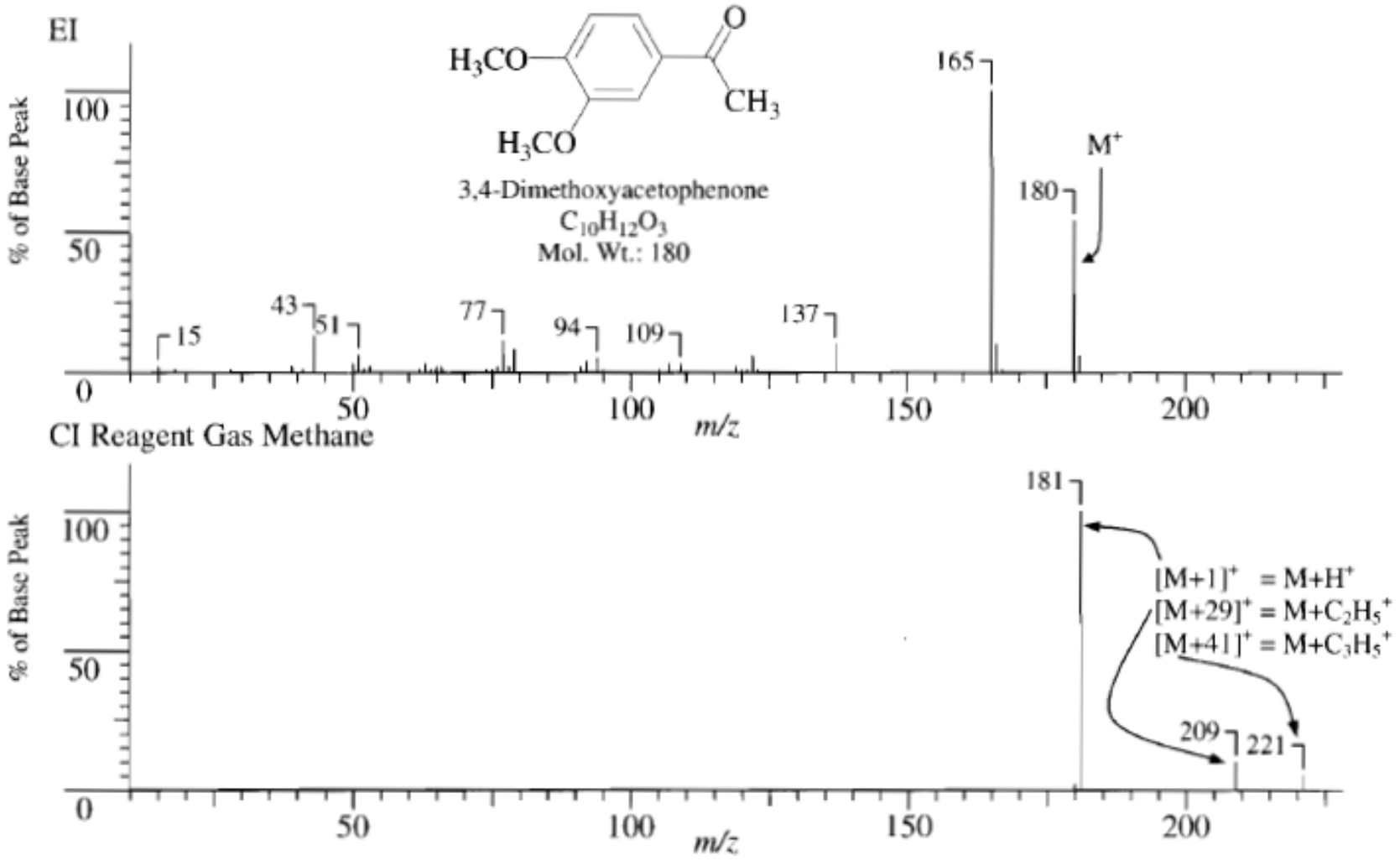
$$NH_3^{+\bullet} + NH_3 \longrightarrow NH_2^{\bullet} + \boxed{NH_4^{+}}$$ proton donor

2. Proton donor protonates sample

$$M + XH^{+} \longrightarrow MH^{+} + X^{-}$$

Parent mass + 1

The EI and CI mass Spectra of 3,4-dimethoxyacetophenone



Ionization Methods

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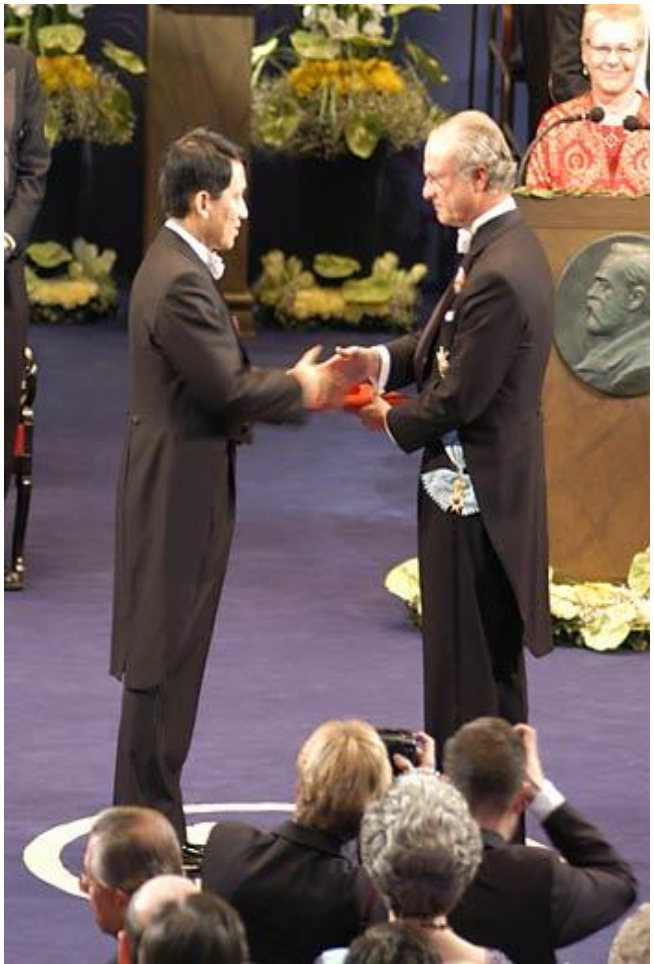
Nobel Prize 2002

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

Koichi Tanaka (Japan)

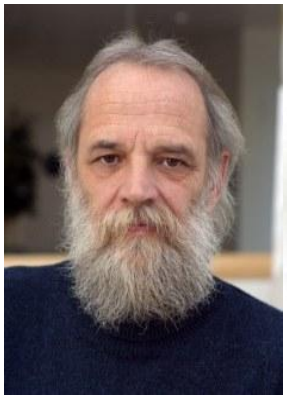


John B. Fenn (USA)

Matrix Assisted Laser Desorption/Ionization (MALDI)

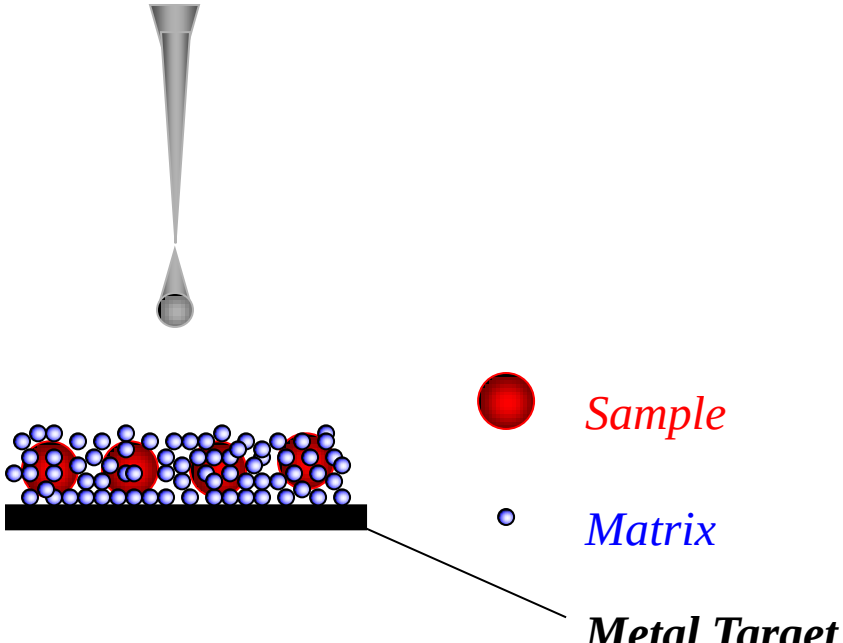
- Limitations of Mass Spectrometry
  - Biomolecules and organic macromolecules are fragile
  - Molecular ions or meaningful fragments were limited to only 5-10 kDa at the time

- In 1987, Michael Karas and Franz Hillenkamp successfully demonstrated the use of a matrix to ionize high molecular weight compounds

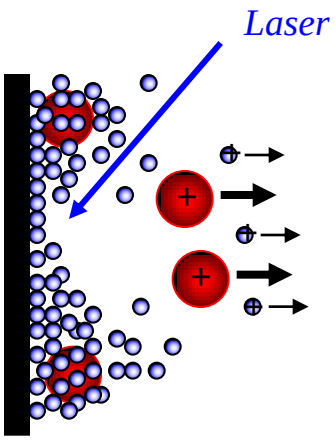


Michael Karas

Franz Hillenkamp



MALDI Sample Prep

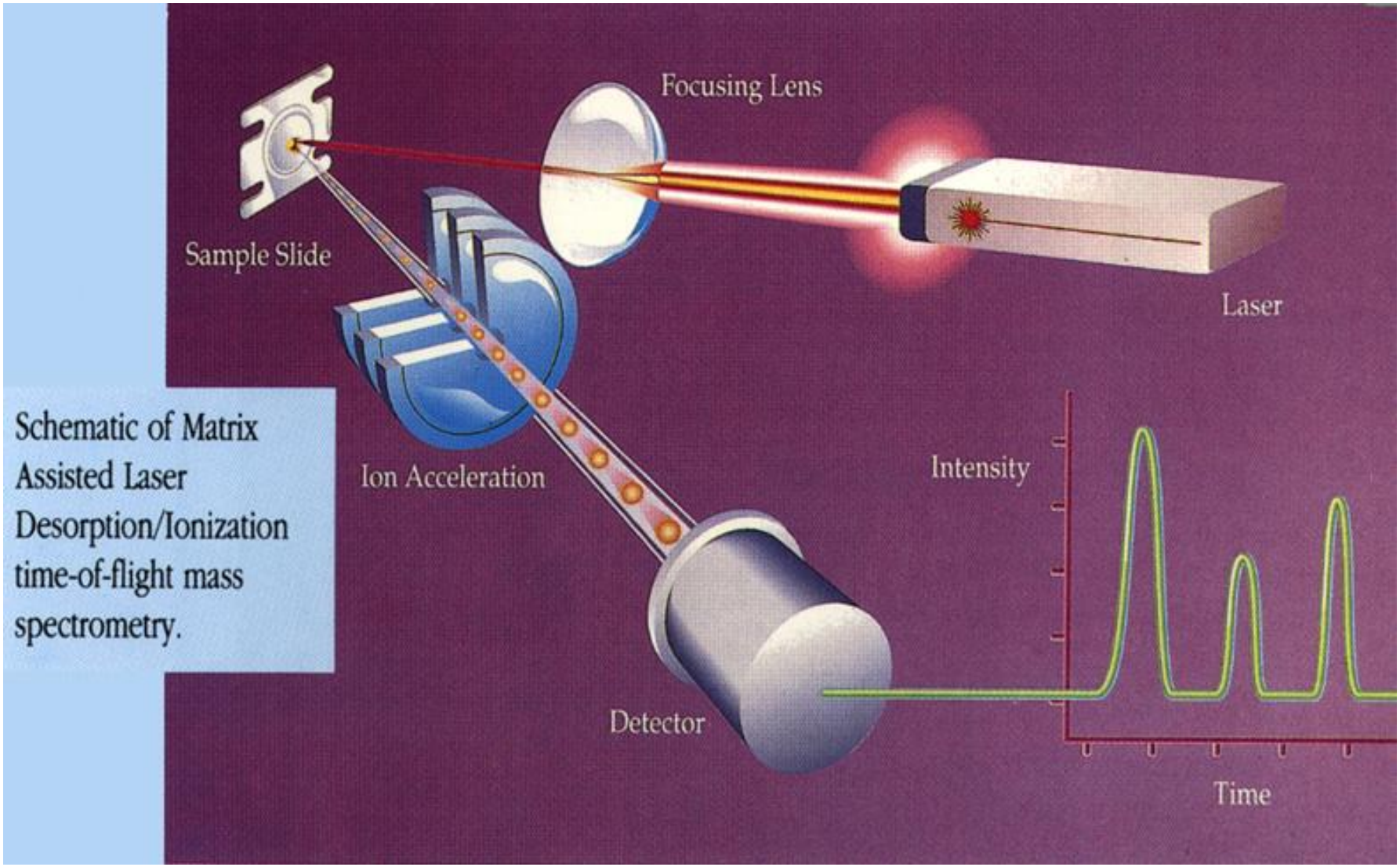


1. Sample (A) is mixed with excess matrix (M) and dried on a MALDI plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules are ionized by proton transfer from matrix:

$MH^+ + A \rightarrow M + AH^+$.

Koichi Tanaka

Nobel Prize 2002

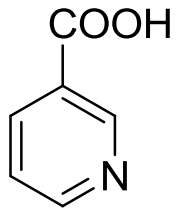


Schematic of Matrix Assisted Laser Desorption/Ionization time-of-flight mass spectrometry.

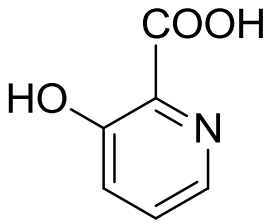
MALDI

- Method where a laser is used to generate ions of high molecular weight samples, such as proteins and polymers.
- Analyte is embedded in to crystal matrix.
- The presence of an aromatic matrix causes the large molecules to ionize instead of decomposing.
- MALDI involves incorporation of the analyte into a matrix, absorption/desorption of laser radiation, and then ionization of the analyte.
- **"MALDI 'theory' or 'models' are still crude and are far away from having predictive power."---** Michael Karas, Chem Rev. 2003, 103, 427-439
- **Challenges for MALDI Theories:**
  - It is a complex chemical event
  - Happens on a time scale of nanoseconds
- The MALDI technique combined with a MS detector (MALDI-MS) became an indispensable tool in analysis of biomolecules and organic macromolecules.

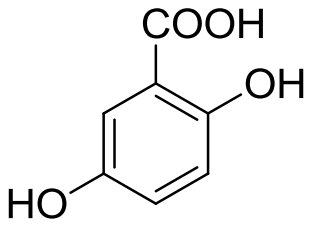
MALDI Matrix Substances



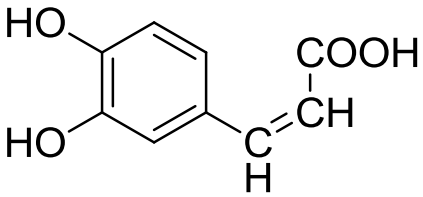
Nicotinic Acid (NA)



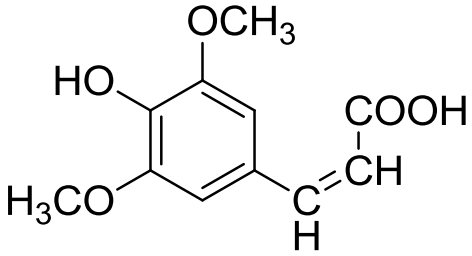
3-Hydroxypicolinic Acid (HPA)



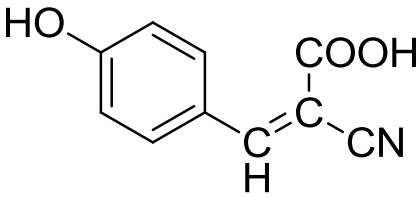
2,5-Dihydroxybenzoic Acis (DHBA)



Caffeic Acid (CA)



Sinapinic Acid (SA)



α-Cyano-4-Hydroxycinnamic Acid (CHCA)

MALDI Advantages:

- Gentle Ionization technique
- High molecular weight analyte can be ionized
- Molecule need not to be volatile
- Sub-picomole sensitivity easy to obtain
- Wide array of matrices

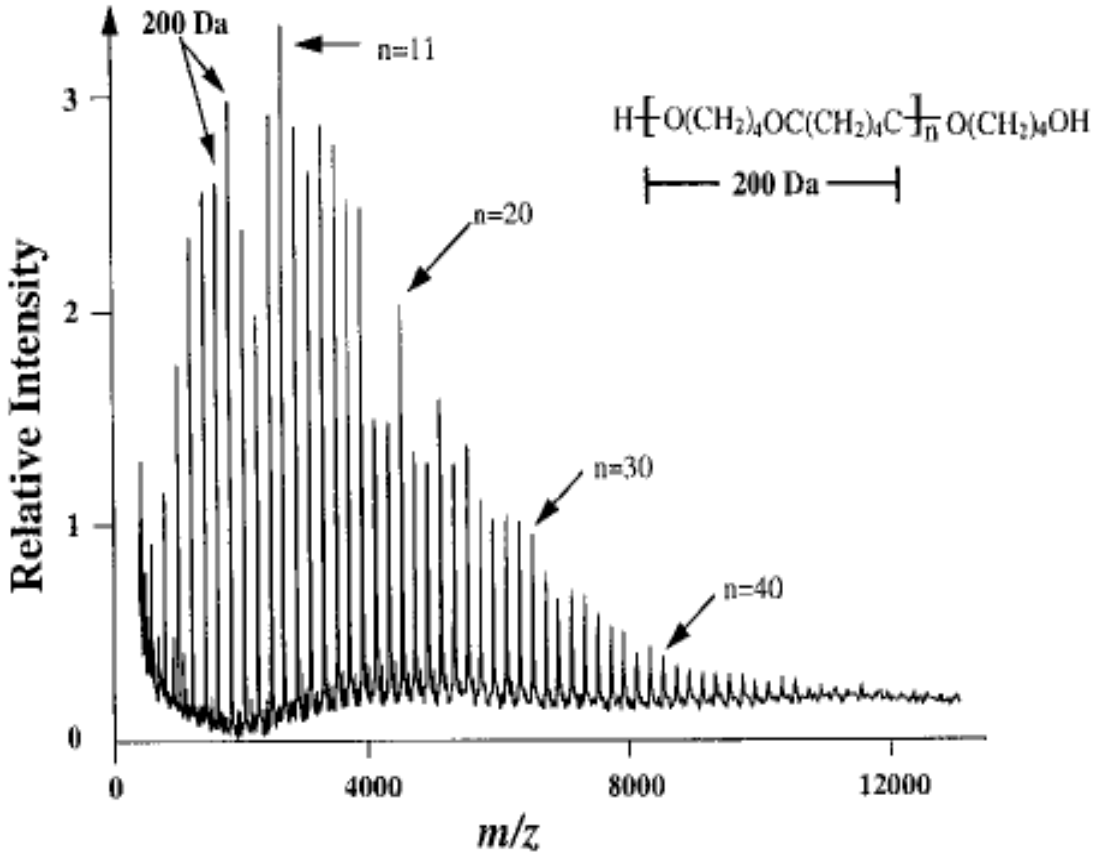
MALDI Disadvantages:

- MALDI matrix cluster ions obscure low m/z species (<600)
- Analyte must have very low vapor pressure
- Pulsed nature of source limits compatibility with many mass analyzers
- Coupling MALDI with chromatography can be difficult
- Analytes that absorb the laser can be problematic
  - Fluorescein-labeled peptides

Applications of MALDI

- Peptides and proteins
- Synthetic polymers
- Oligonucleotides
- Oligosaccharides
- Lipids
- Inorganics
- Bacterial identification
- Proteomics

Polymer Analysis



Bacterial Identification

- MALDI-TOF-MS is used for bacterial identification.
- MALDI-TOF-MS can determine mass of proteins of 1-40 kDa.
- Used to diagnose diseases and monitor contamination.
- Identified by:

  -Biomarkers

  -Cellular protein content

Mass spectral analysis of protein extracts

Biomarkers for Bacteria Protein Extracts

Organism	Genus Biomarkers (m/z)	Species Biomarkers (m/z)	Strain Biomarkers (m/z)
Bacillus anthracis, vollum	6680, 6837	2385, 3991, 4313	4505
Bacillus anthracis, sterne	6680, 6837	2385, 3991, 4313	2789
Bacillus anthracis, zimbabwe	6680, 6837	2385, 3991, 4313	2850
Bacillus thuringiensis, 4A1	6680, 6837	3932	5916
Bacillus thuringiensis, 4A2	6680, 6837	3932	4871, 7845
Bacillus thuringiensis, 4L2	6680, 6837	3932	2864, 4074, 4548, 5781
Bacillus cereus, 6E1	6680, 6837	5269, 5537, 7365, 9533	N/A
Bacillus subtilis, 3A1	6680[a]	3757, 3871, 6304, 9146	N/A
Brucella melitensis, melitensis wild	7379, 8031, 9060	N/A	N/A
Brucella melitensis, REV-1 vaccine	7354, 8030, 9071	N/A	N/A
Brucella melitensis, Suis wild	7378, 8020, 9080	N/A	5844
Brucella melitensis, abortus wild	7379, 8020, 9088	N/A	N/A
Yersinia pestis, India	2460, 3223, 3297, 4361, 4437	N/A	7335
Yersinia pestis, La Paz	3223, 3297, 4361, 4437	N/A	N/A
Yersinia pestis, Nairobi	2453, 3223, 3297, 4361, 4437	N/A	5196, 5638, 7599, 9637
Francisella tularensis, type B	2622, 2770, 3205, 3274, 4120, 4747	N/A	N/A

[a]Doubly charged ion, corrected mass/charge ratio
N/A - Data not available

Electrospray Ionization Mass Spectrometry

John Fenn
Nobel Prize 2002

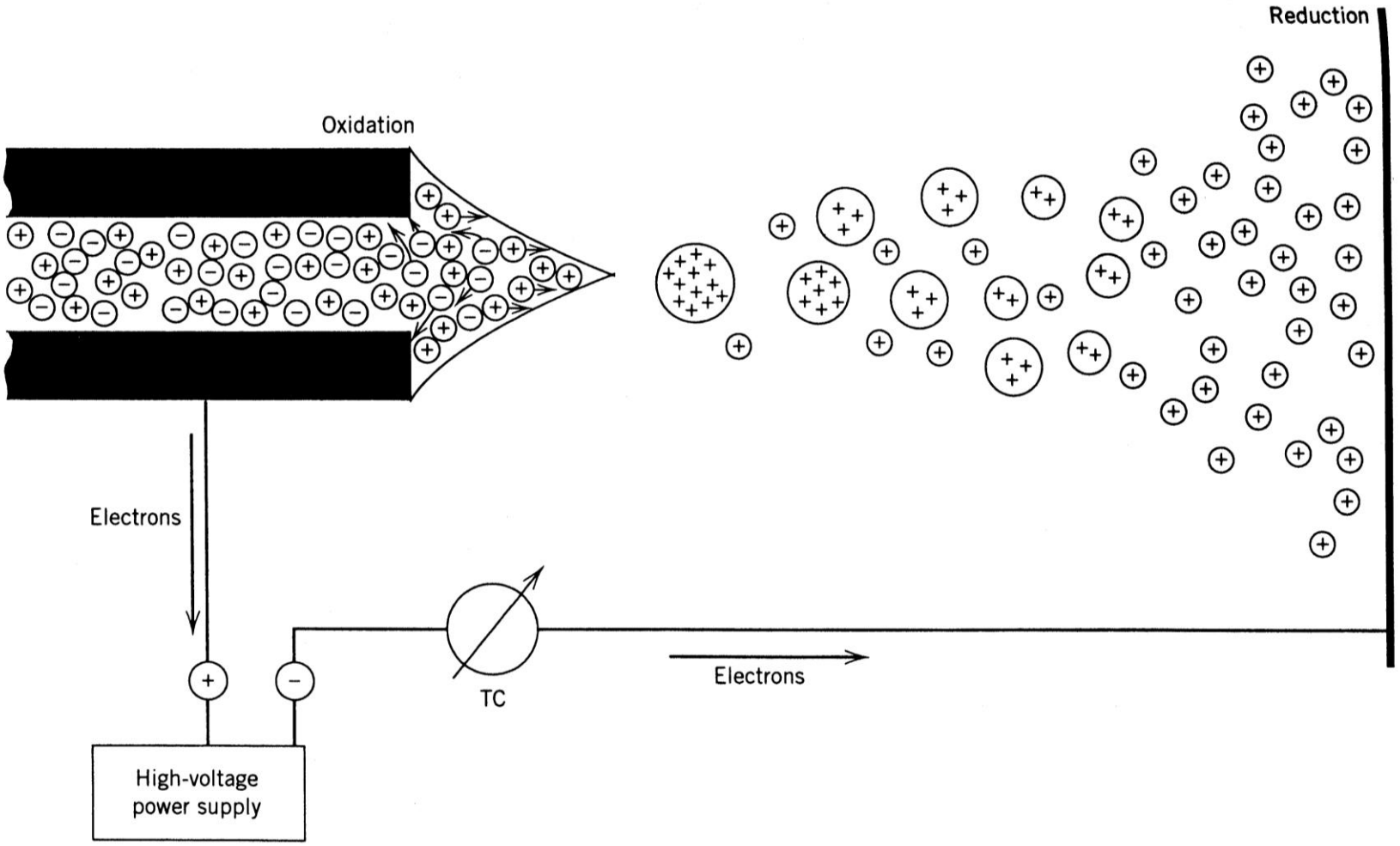
ESI changed the field of mass spectrometry and opened the world of biology to mass spectrometry and enabled:

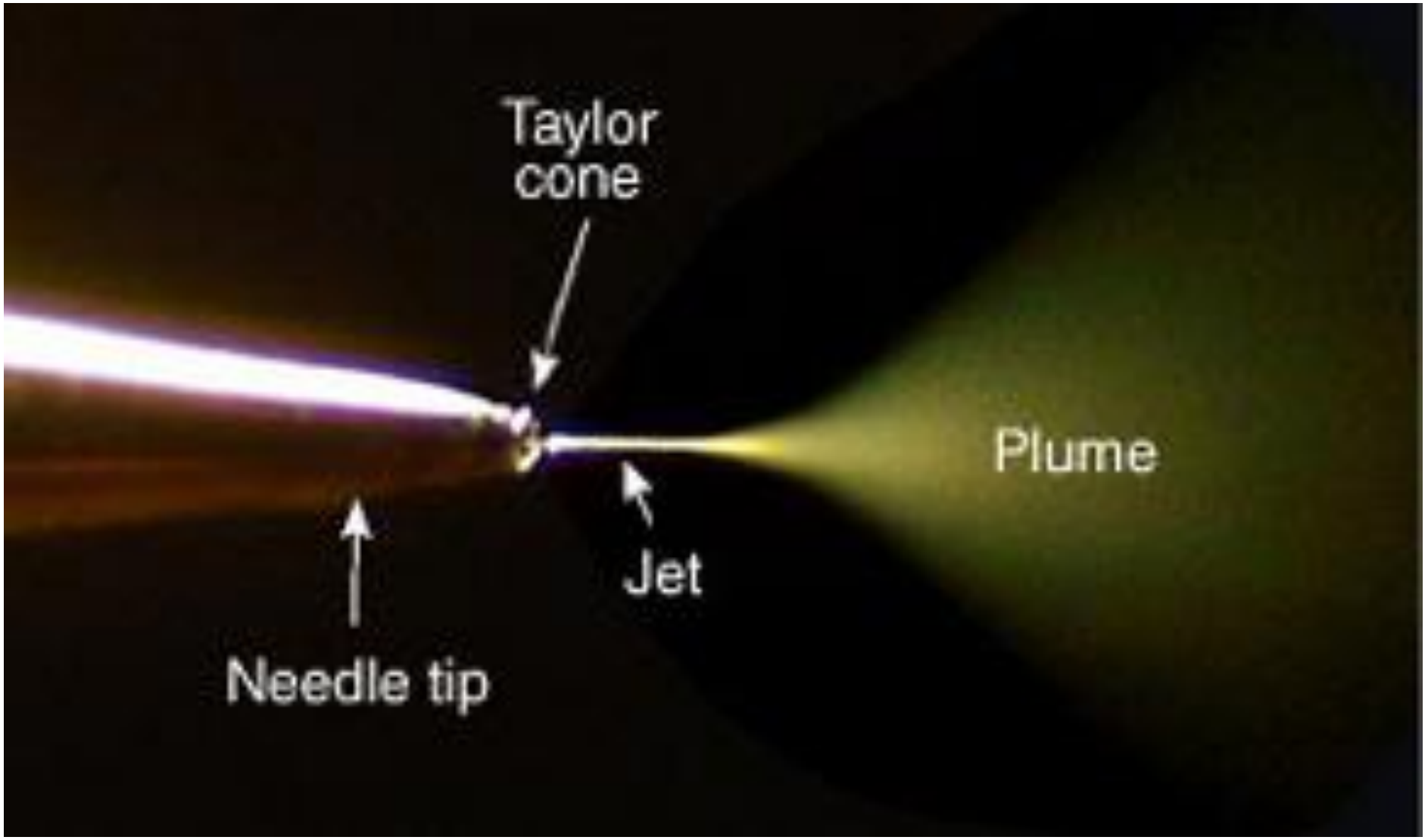
- Proteomics
- Metabolomics
- Systems biology

What Is ESI?

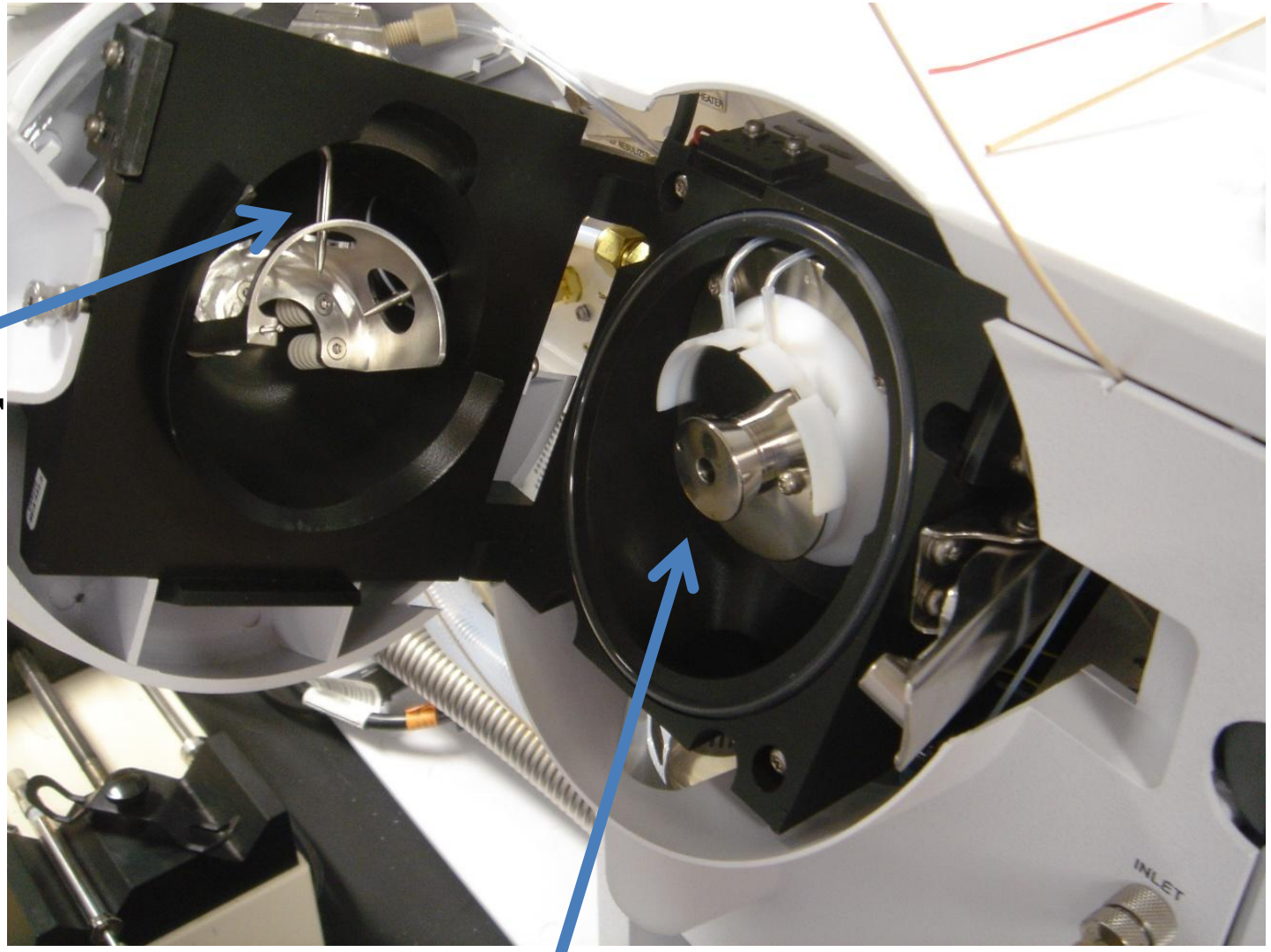
- The transfer and ionization of molecules from solution to gas phase by electrospray (An electrical nebulization of liquid that results in the formation of charged micro droplets)
- Vaporization increases the charge density on the surface of the droplets (Electrostatic repulsion increases).
- When the electrostatic repulsion exceeds the surface tension, the droplet undergoes coulombic fission (Formation of daughter droplets)
- The formation of charged ions in the gas phase

Electrospray Ionization (ESI)




Taylor cone
Plume
Jet
Needle tip

ESI Nebulizer



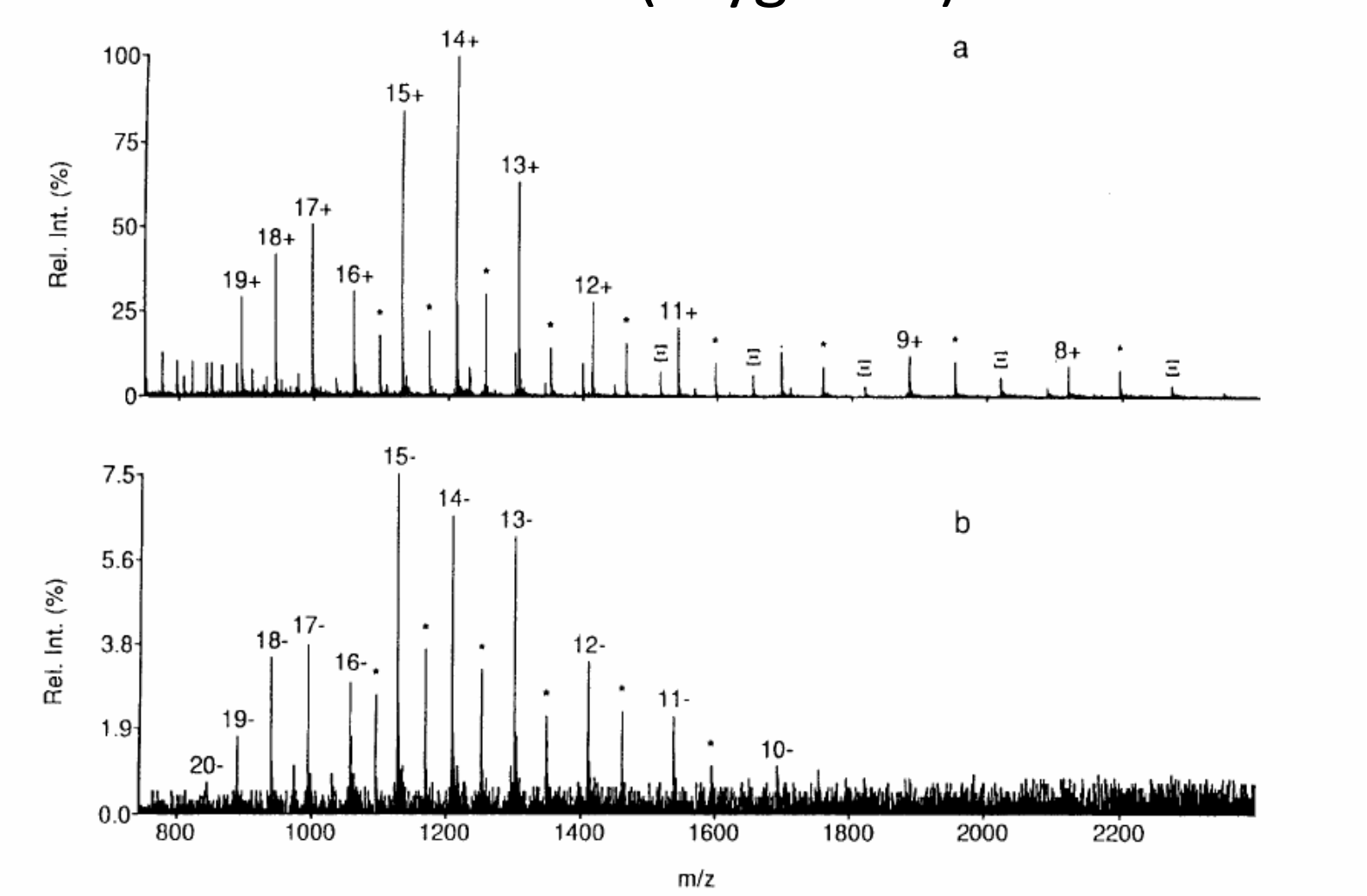
Heated Inlet to MS

Requirements for Electrospray Ionization:

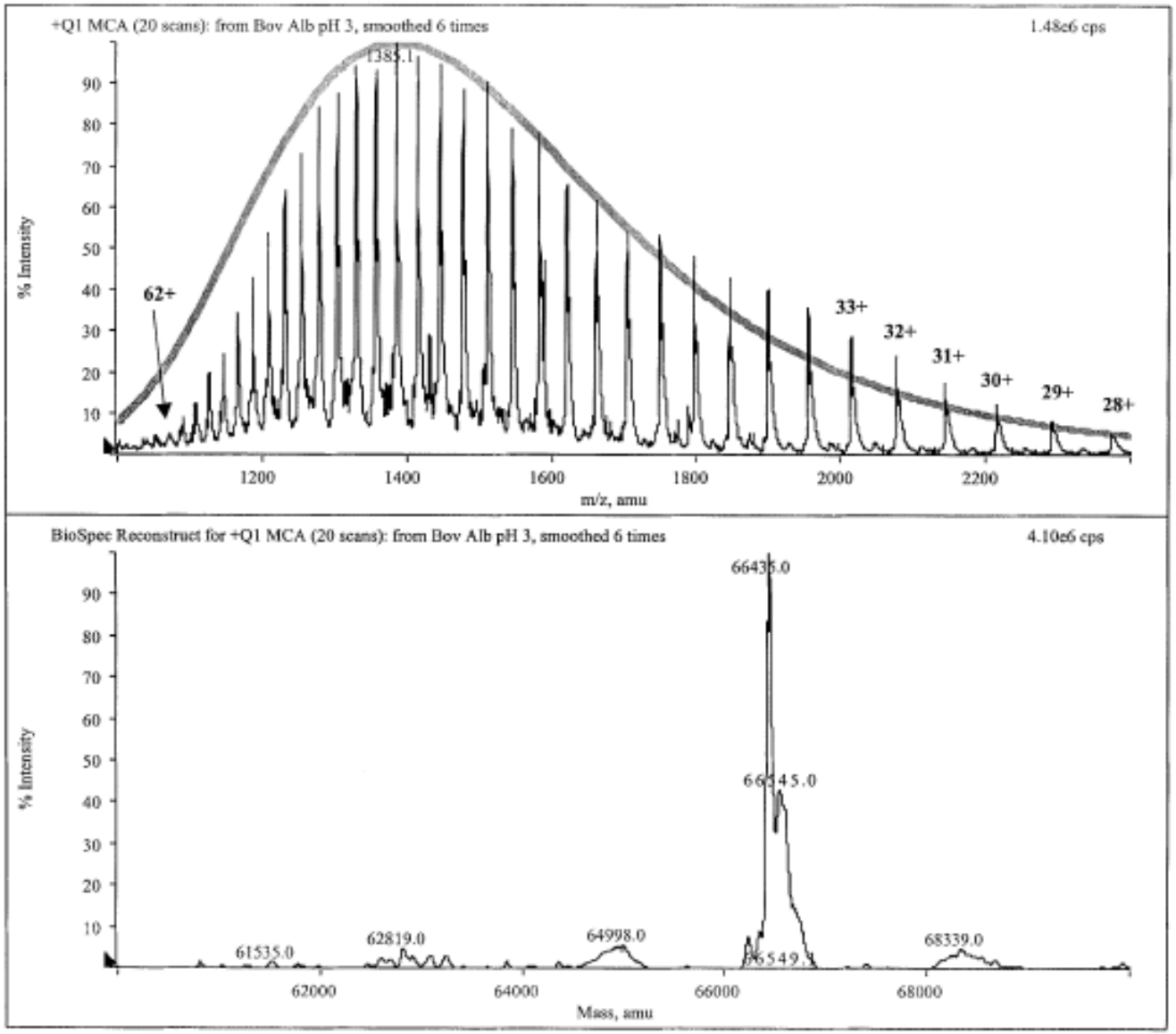
- Soluble in solvent (water, methanol, acetonitrile)
- Avoid DMSO and DMF
- Pseudomolecular ion is formed **MH^+ ,$[M+Na]^+$, etc.**
- Very Gentle
- MW to megadalton range
- Preformed ions – Salts, peptides at low pH
- Electrochemical reactions can take place
- Often need a basic site; otherwise add a cation (Na+)

Application of ESI

Proteins (Myglobin)



Bovine Serum Albumin



Mass spectrometry and viral analysis

Gary Siuzdak[1]*, Brian Bothner[1], Mark Yeager[2], Christophe Brugidou[3], Claude M Fauquet[3], Kenway Hoey[4] and Cheng-Ming Chang[2]

Departments of [1]Chemistry and [2]Cell Biology and [3]The International Laboratory for Tropical Agriculture Biotechnology, The Scripps Research Institute,10666 North Torrey Pines Road, La Jolla, CA 92037, USA and [4]Peptide Synthesis Laboratory, R.W. Johnson Pharmaceutical Research Institute, San Diego, CA 92121, USA

Background: Electrospray ionization (ESI) mass spectrometry is a powerful new approach for analyzing biomolecules and biomolecular complexes. Previous studies have provided evidence that non-covalent biomolecular complexes can be observed by ESI mass spectrometry; it is not clear, however, whether the native conformation of the biomolecules is maintained throughout the ionization and analysis process. We set out to address this question using live viruses.

Results: Viral ions have been generated in the gas phase using electrospray ionization mass spectrometry. These ions have been collected, following ion filtering through the mass analyzer, and then analyzed by transmission electron microscopy. Transmission electron microscopy revealed that rice yellow mottle virus and tobacco mosaic virus retained their respective spherical and rod-like ultrastructure. The viability of the isolated tobacco mosaic virus was confirmed by inoculation and infection of tobacco plants.

Conclusions: These results demonstrate the utility of electrospray for supramolecular complexes with molecular weights of over 40 million Da and offer conclusive evidence that native biomolecular structures can be conserved through the electrospray process.

Chemistry & Biology January 1996, **3**:45–48

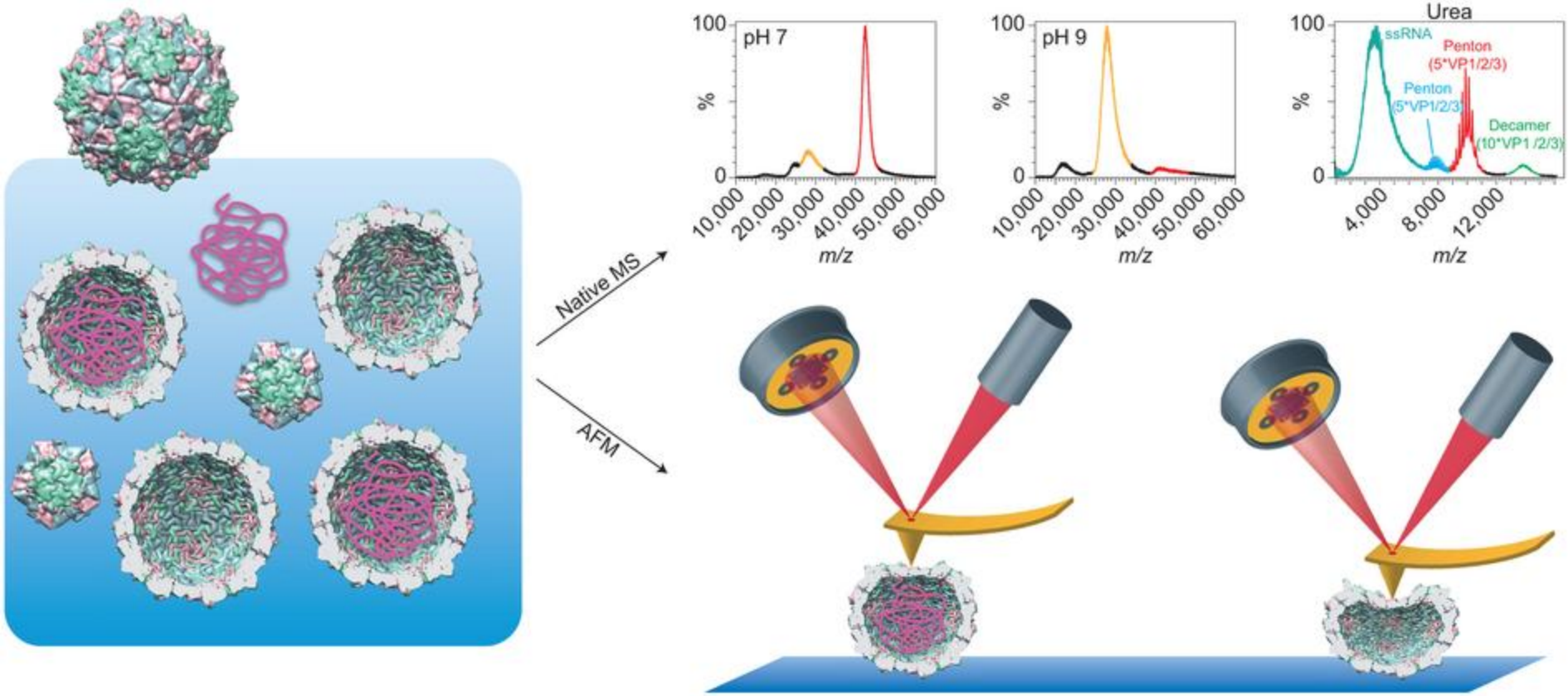
Key words: electron microscopy, electrospray, mass spectrometry, non-covalent, virus

BIOPHYSICAL CHEMISTRY

Unravelling capsid transformations

The interactions between a virus capsid and its cargo are essential for viral infection as well as in the design of synthetic virus-like particles. Now a combination of analytical techniques has unravelled key steps in the transformation of a model virus and the release of its RNA cargo.

Masaki Uchida and Trevor Douglas



Nature Chemistry 2013, p. 44

THANK YOU