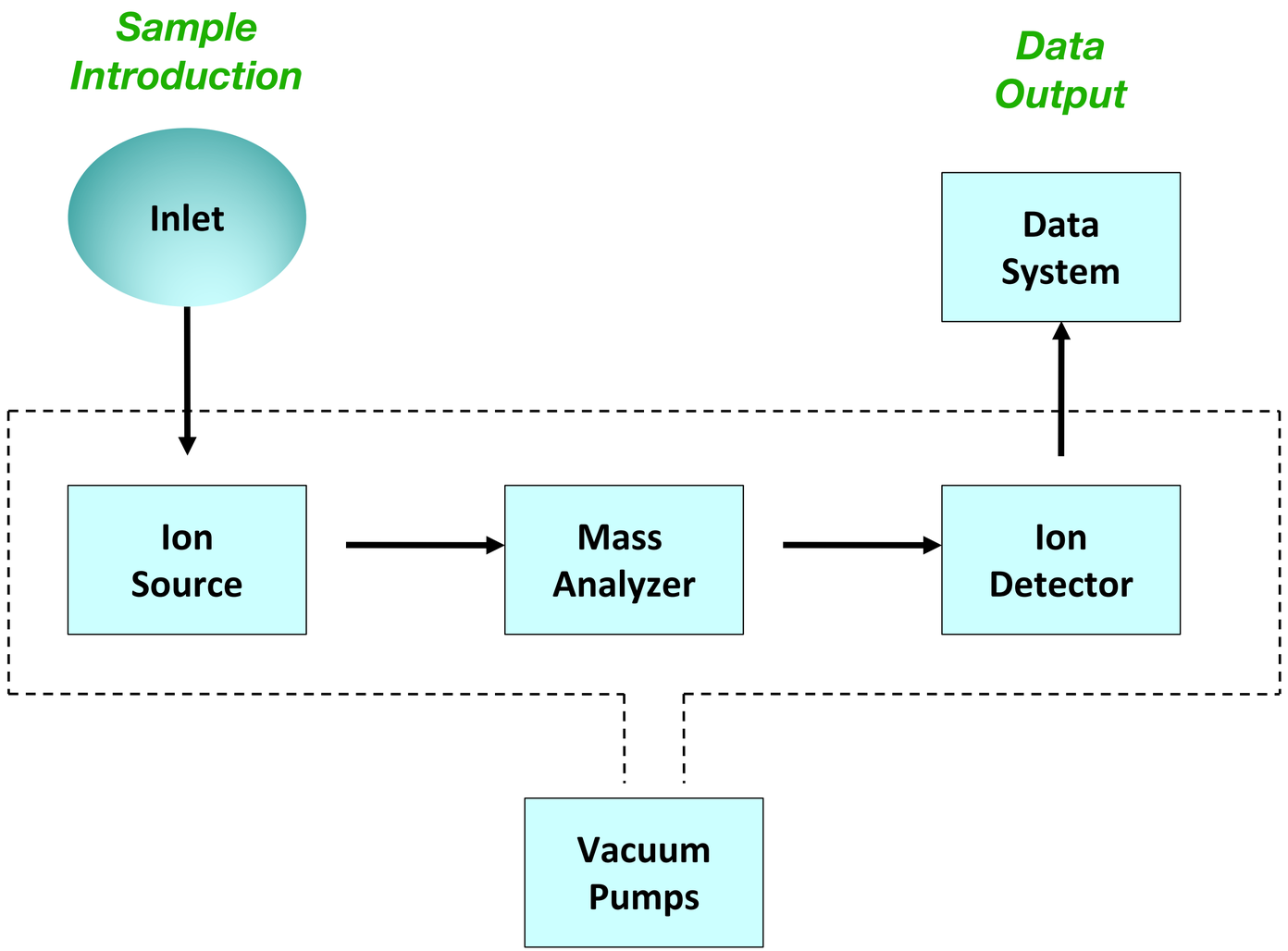
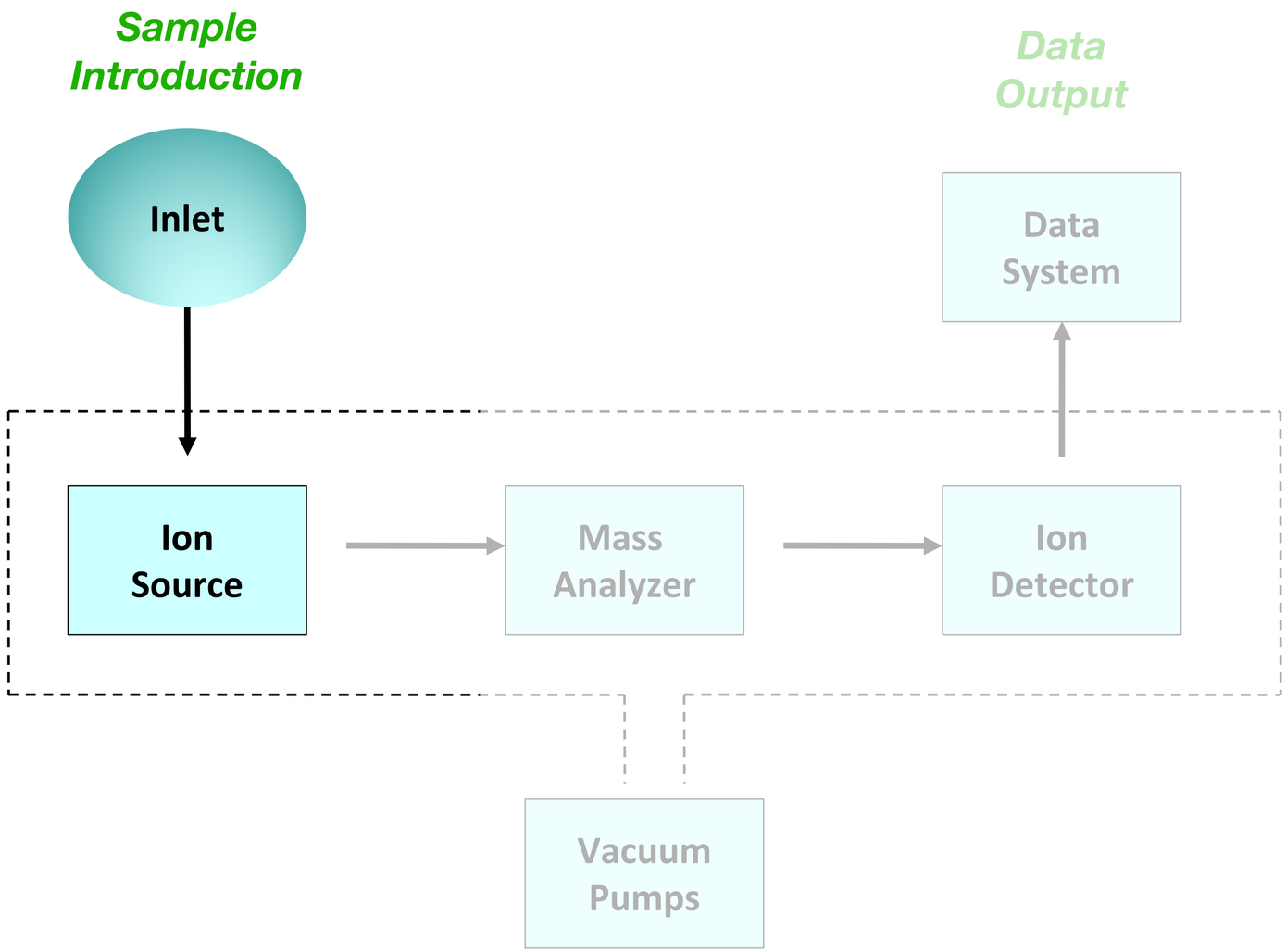
Mass spectrometry instrumentation

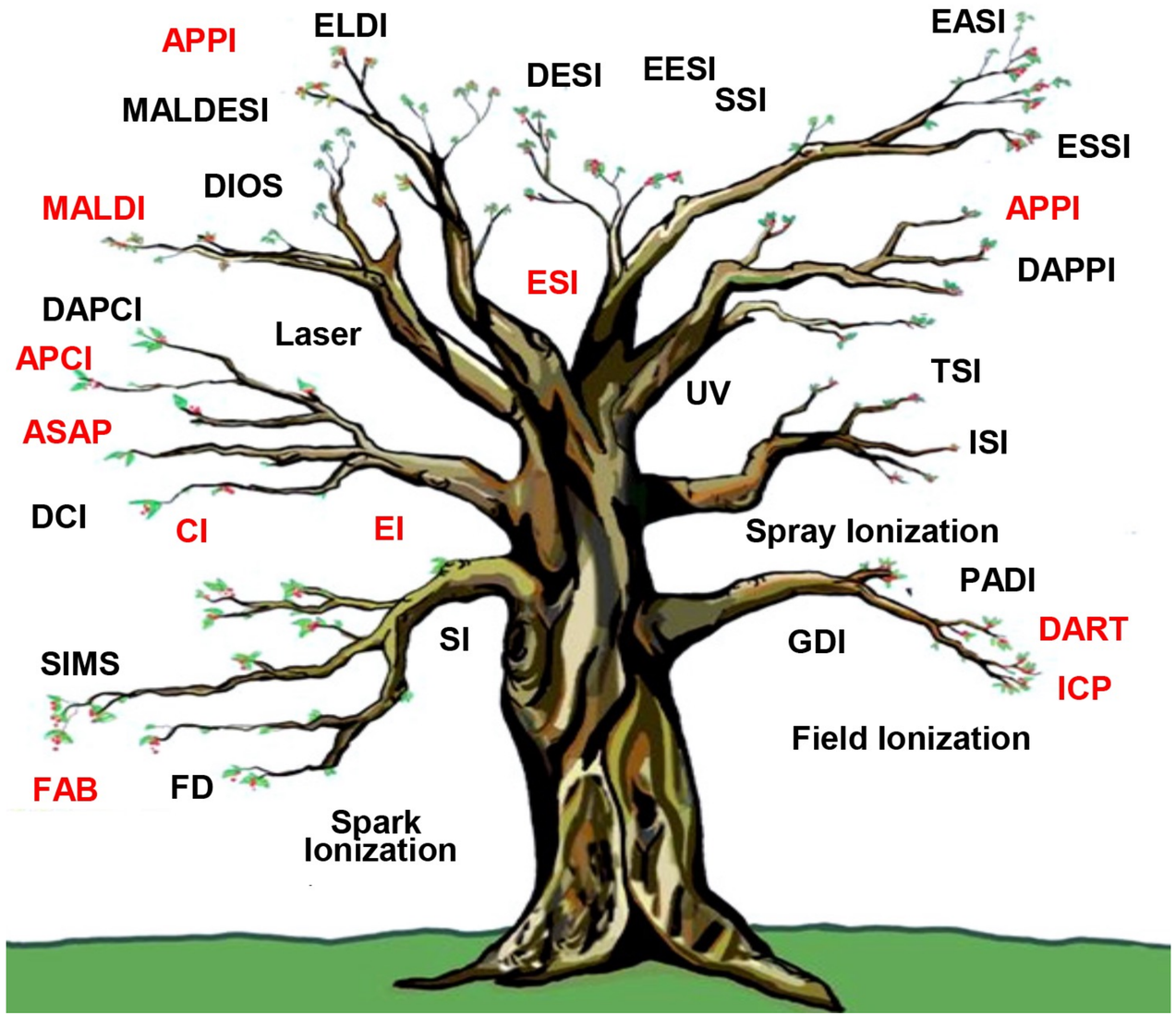
Elements of a mass spectrometer



Ionization methods



Ionization methods



Ionization techniques for molecular analysis

What do we want to achieve?

Desired characteristic	*Characteristics usually achieved*
High ion current	10^{-10} A (10^{9} ions/sec)
High ionization efficiency (neutrals⟶ions)	0.1%
Bipolarity (positive and negative ions)	Except for EI
High molecular weight compounds	Approximately 10^{5} Da
All sample states	All physical states are compatible with MS
Control of fragmentation	By control of energy deposited in the ion

Not every method exhibits all these characteristics.

Ionization methods

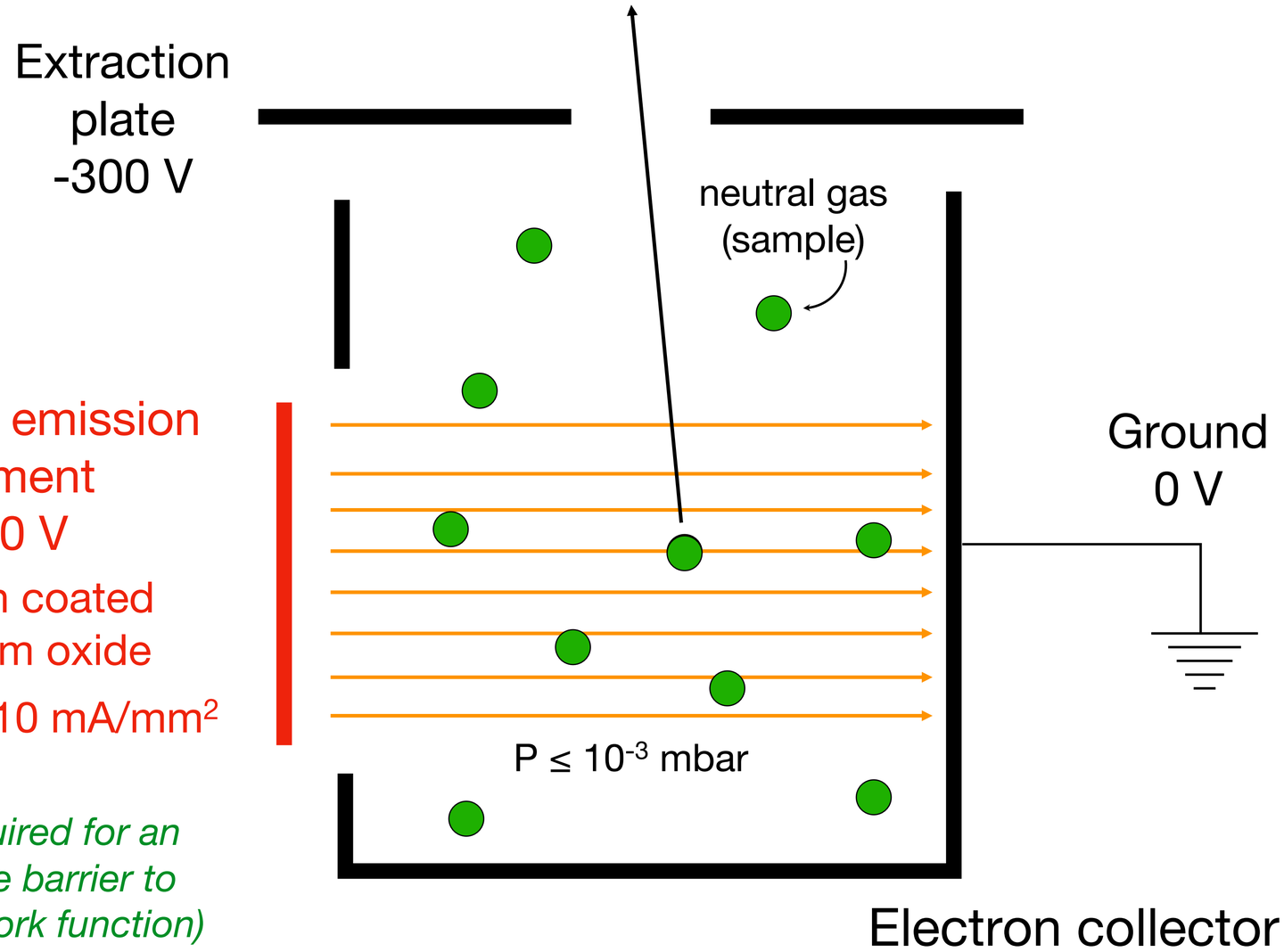
Molecular Analysis

- Electron impact ionizaton (EI)
- Chemical ionization (CI)
- Photoionization (PI)
- Electrospray ionization (ESI)
- Matrix-assisted laser desorption/ionization (MALDI)

Elemental Analysis

- Thermal desorption
- Spark ionization
- Inductively coupled plasma
- Glow discharge
- Secondary ion mass spectrometry (SIMS)

Electron impact ionization (EI)



High temperature is required for an electron to overcome the barrier to get to the gas phase (work function)

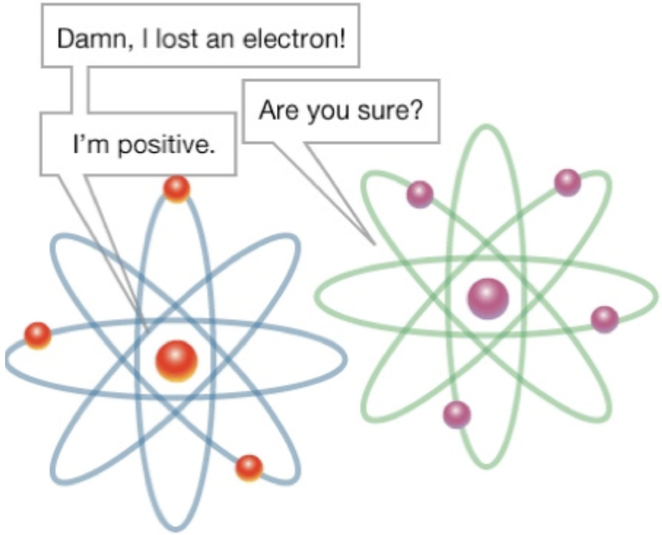
Electron impact ionization (EI)

Ionization step: $M + e^- \rightarrow M^{+\bullet} + 2e^-$

Subsequent fragmentation:

Open shell ion $M^{+\bullet}$

- EE^+ (even ion) + $R^\bullet$ (radical)
- $OE^{+\bullet}$ (odd ion) + N (molecule)



Must determine the molecular ion:

- The MI must have the highest mass in the spectrum (except isotopes)
- The MI must be an odd electron ion, $M^{+\bullet}$
- The MI may fragment generating odd-electron fragments.

Electron impact ionization (EI)

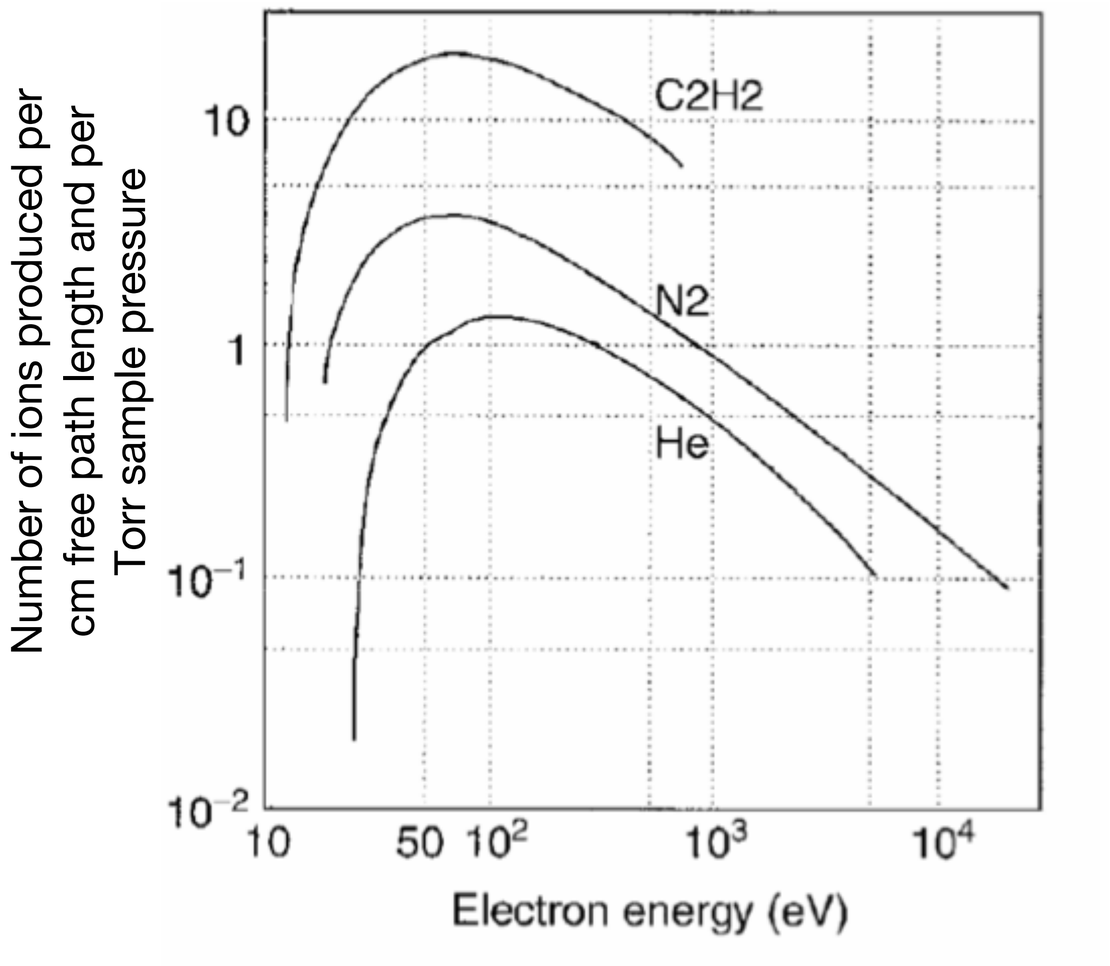
- The ionization potential is the electron energy that will produce a molecular ion. The appearance potential for a given fragment ion is the electron energy that will produce that fragment ion.
- Most mass spectrometers use electrons with an energy of 70 electron volts (eV) for EI.
- Decreasing the electron energy can reduce fragmentation, but it also reduces the number of ions formed.

Electron energy dependence

Number of ions produced as a function of the electron energy

$I=\alpha \cdot i \cdot p \cdot V$

i – filament current
p – pressure
V - volume



Note the wide maximum at around 70 eV

Quiz

Why does the ionization cross section of molecules increase from 10 eV to 70 eV?

A. Valence electrons are typically bound by approximately 70 eV.

B. An electron of 70 eV is resonant with a transition to a particular excited electronic state.

C. The electron size better matches the size of the covalent bond.

D. None of the above.

Ionization efficiency

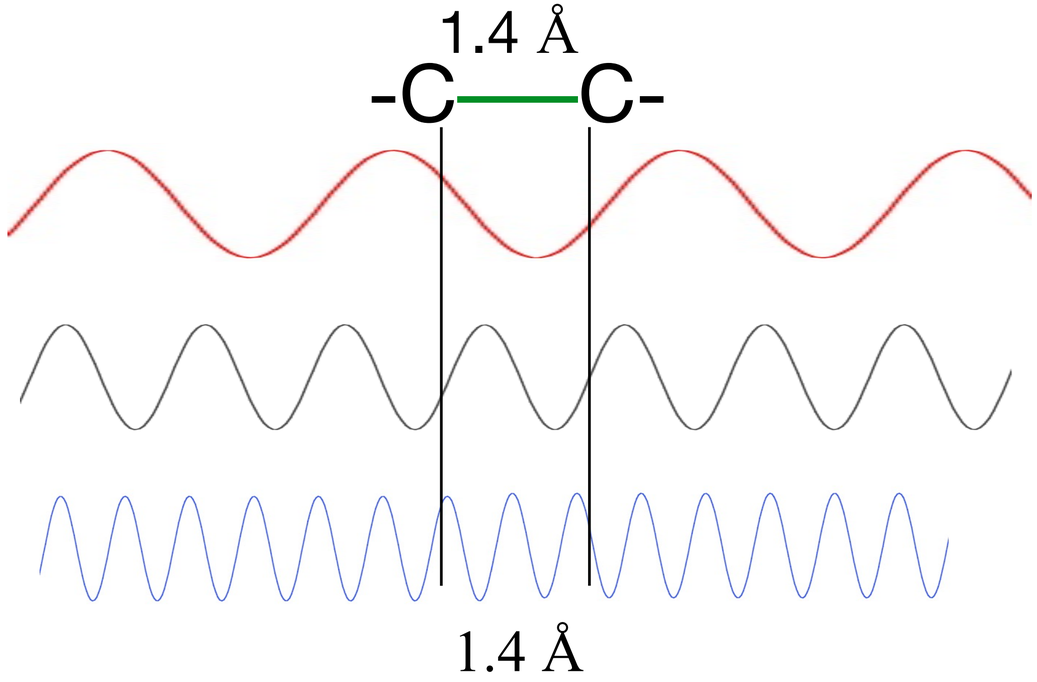
Best condition for energy transfer is when the size of an external electron (width of probability) is close to the bond length (“size” of the valent electron).

For organic molecules the average bond length is around ***l***~ 1.4 Å . DeBroglie wavelength: $\lambda = h/mv$

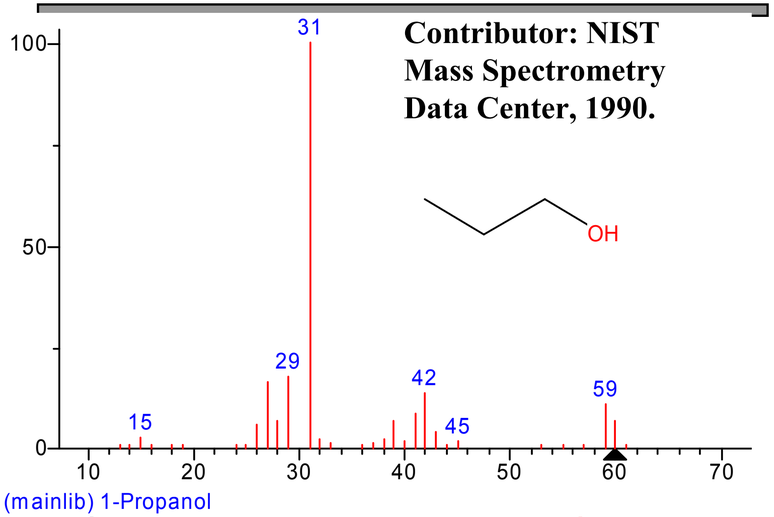
E=15 eV: $\lambda \sim 3$ Å

E=70 eV: $\lambda \sim 1.4$ Å

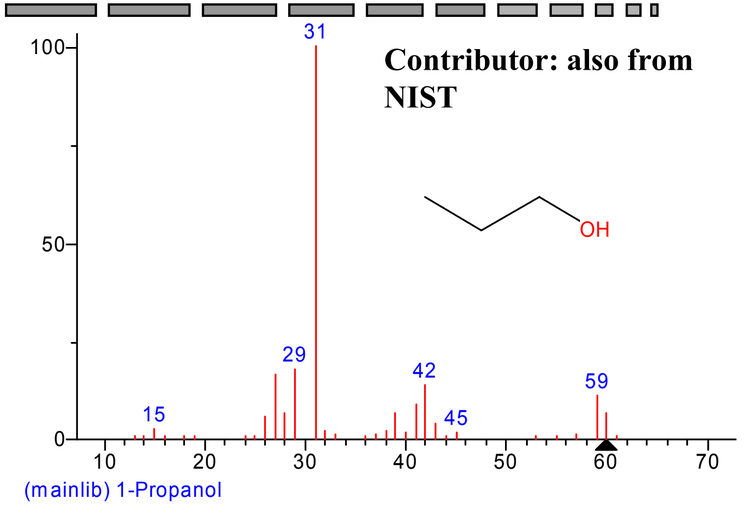
E=140 eV $l \sim 0.7$ Å



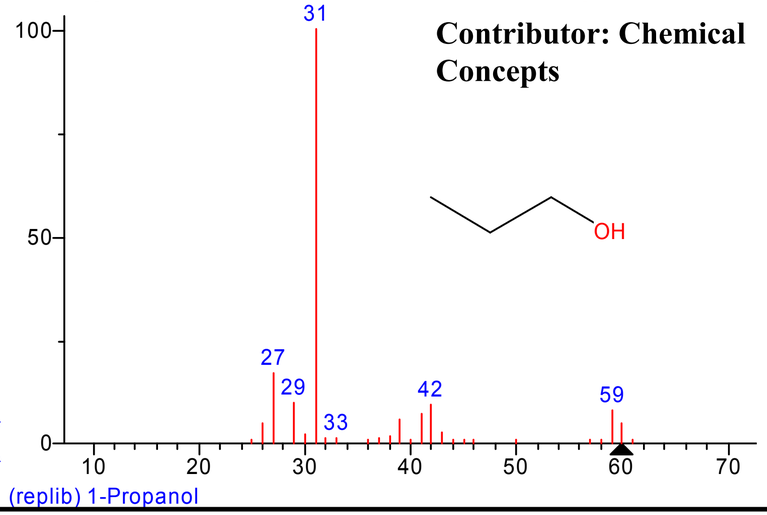
Reproducibility: 4 Spectra of 1-Propanol in NIST



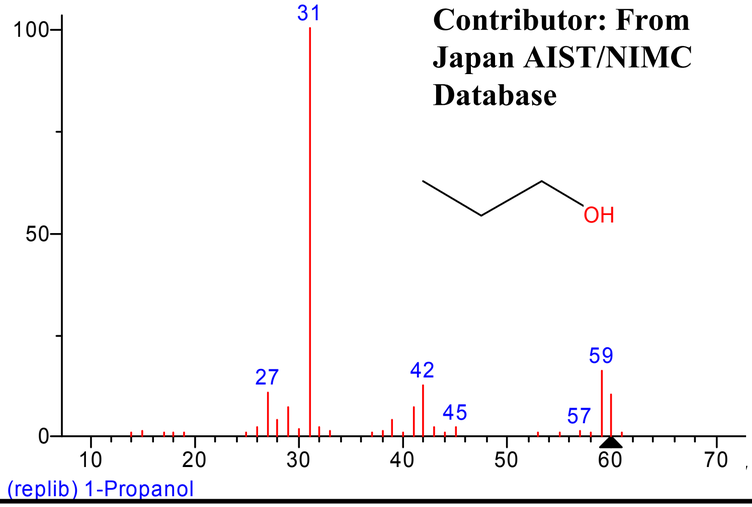
Contributor: NIST Mass Spectrometry Data Center, 1990.



Contributor: also from NIST



Contributor: Chemical Concepts



Contributor: From Japan AIST/NIMC Database

Electron impact ionization

Advantages

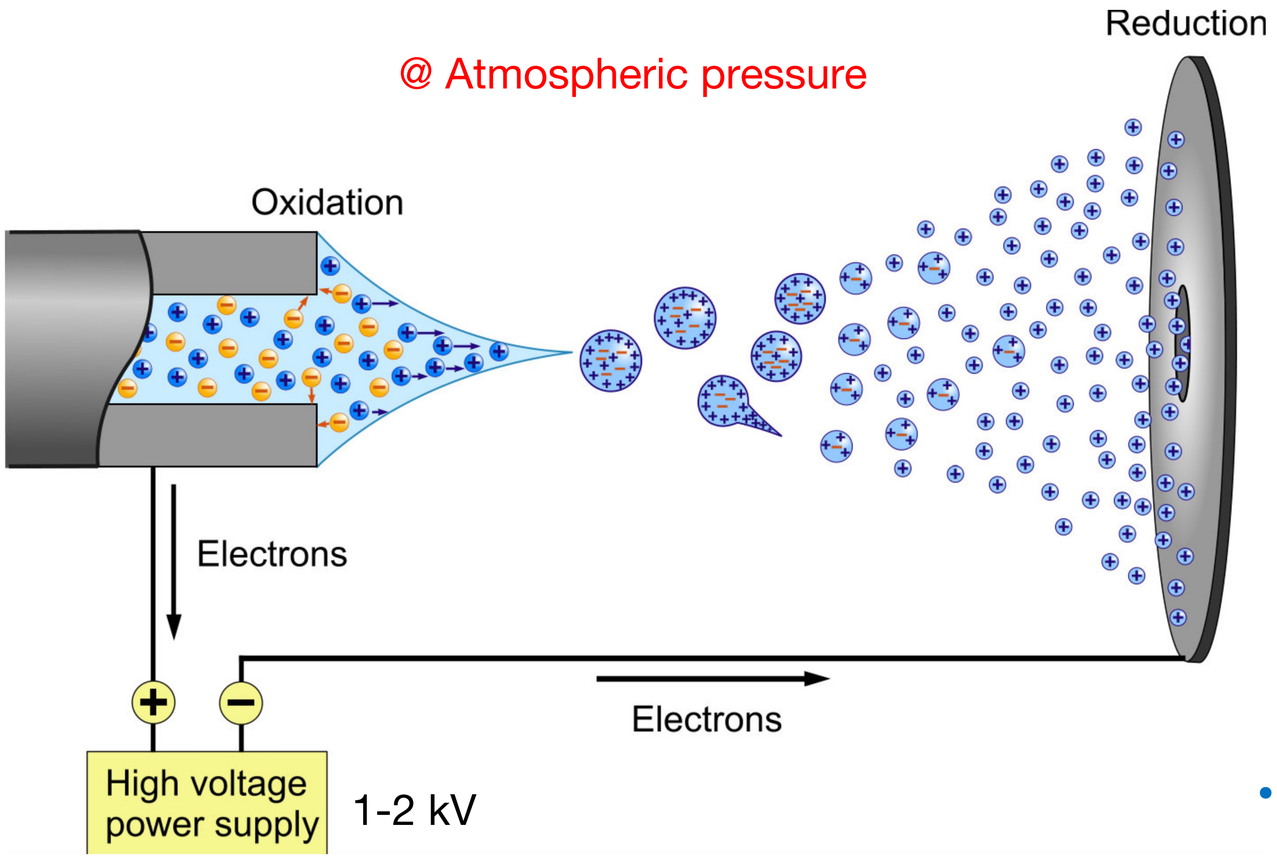
- Reproducible and well understood
- Extensive fragmentation occurs
- High ionization efficiency
- Universal to all vaporized molecules
- Libraries of EI spectra allow for compound identification and quantification
- Very sensitive: 1 in 1000 molecules ionized

Disadvantages

- Only positive ions
- Extensive fragmentation occurs
- Radical cations formed
- Sample must be volatile and hence low MW
- Ionization is non-selective
- All vaporized molecules contribute

⇒ Harsh, universal, highly reproducible method for volatile molecules

Electrospray ionization



John Fenn
Nobel Prize
2002

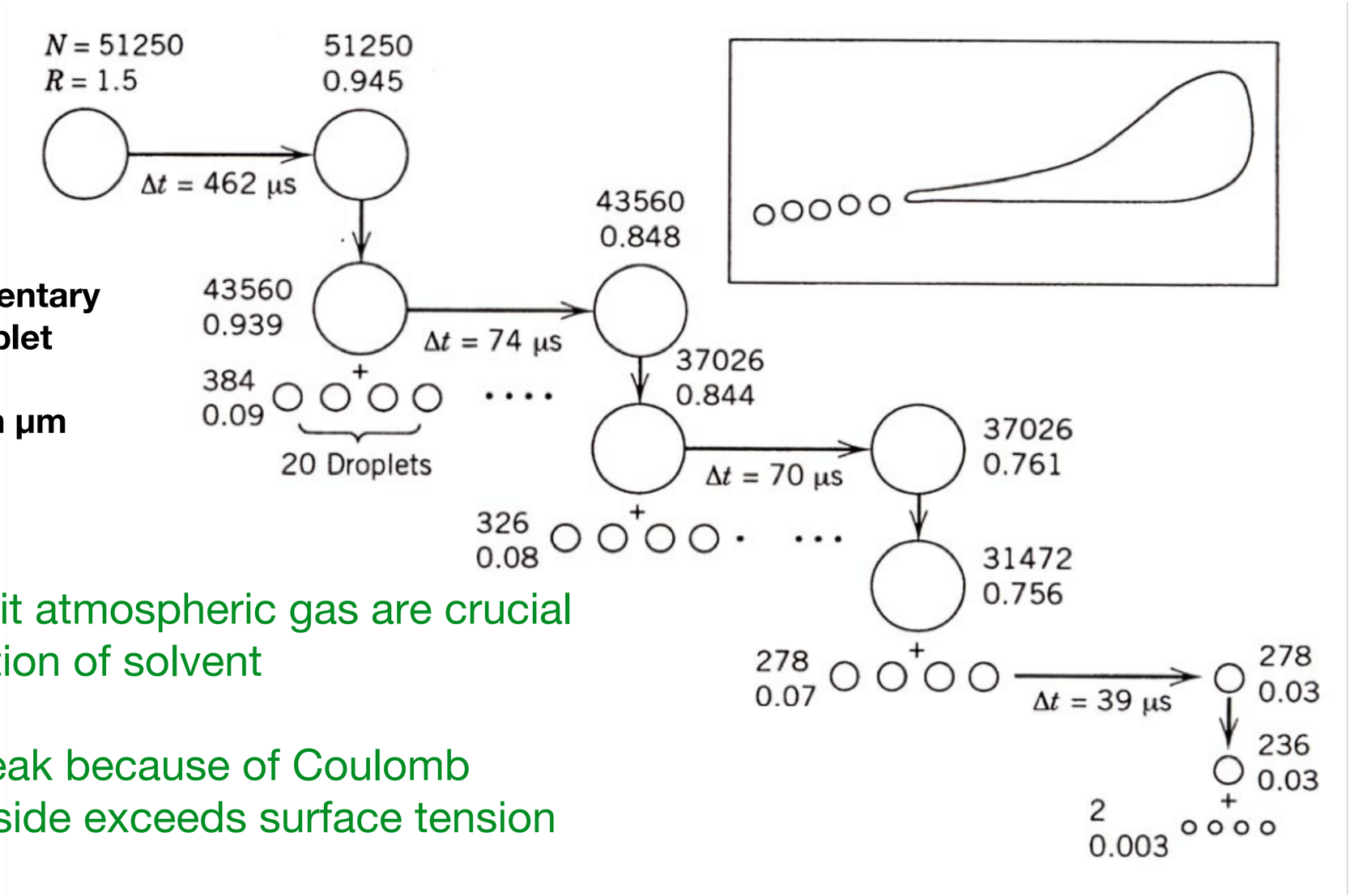
- Produces multiply-charged ions
- Soft: can retain non-covalent complexes, including solvent-ion and protein-protein.

- Sample is introduced directly from the liquid
- A spray of charged droplets are formed
- Desolvation and breakup of droplets occurs
- Ions emerge from charged droplets
- Ions are sampled from the atmosphere

Desolvation/Ionization mechanism

N - number of elementary charges on the droplet

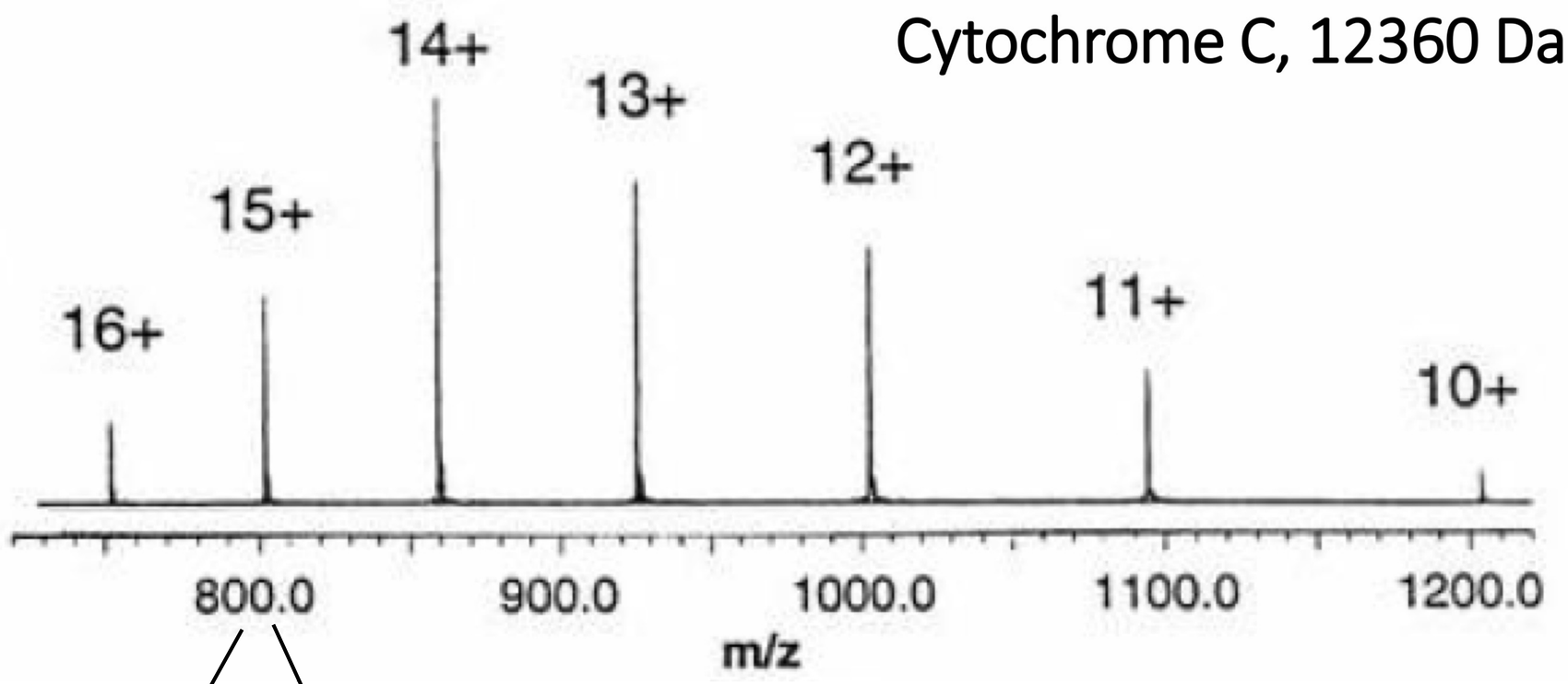
R - droplet radius in μm



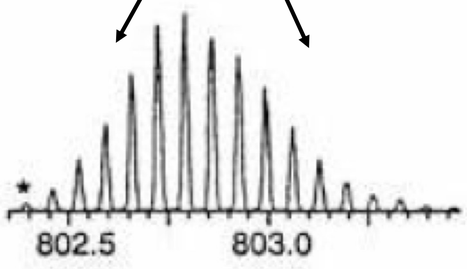
- Collisions wit atmospheric gas are crucial for evaporation of solvent
- Droplets break because of Coulomb repulsion inside exceeds surface tension

Multiple Charging in Electrospray Ionization

When spraying large, biological molecules, ESI tends to produced multiply-charged ions.



isotope peaks

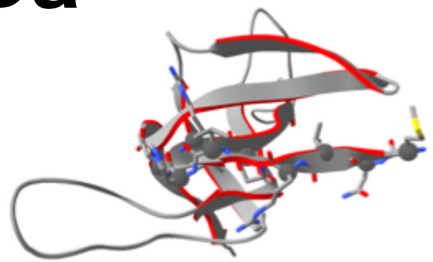


Multiple charge states in ESI allows for MS of heavy molecules in instruments with limited MAX m/z.

Analysis of multiply charged ions from ESI

Protein GroES

10.3 kDa



Spacing of isotopic peaks give you the charge state.

Analysis of multiply protonated ions from ESI

No isotopic distribution !

How to find *Z* and *M* from charge distribution MS ?

1. Numerate peaks in charge distribution; take any as the first and go to higher m/z.
2. For any two peaks ***i*** and ***j*** one can write:

$$z_i \cdot m_i = M + z_i \cdot m_p; \quad \text{and} \quad z_j \cdot m_j = M + z_j \cdot m_p .$$

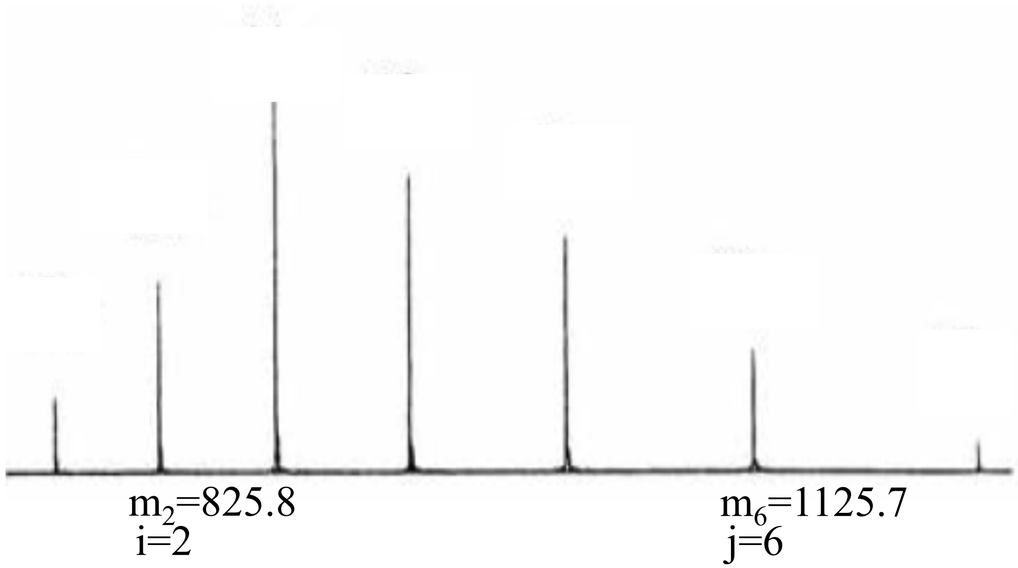
M –mass of the molecule;
m and ***z*** – mass and charge of an ion

3. Combining and solving for z_i and M:

$$z_i = \frac{(j-i)\cdot(m_j - m_p)}{(m_j - m_i)} = \frac{4\cdot 1124.7}{299.9} = 15.001$$

$$M = z_i(m_i - m_p) = \frac{(j-i)\cdot(m_j - m_p)\cdot(m_i - m_p)}{(m_j - m_i)}$$

$$M = \frac{4\cdot(1125.7-1)\cdot(825.8-1)}{(1125.7-825.8)} = 12372.8$$



Low-resolution mass spectrum of a protonated molecule produced with ESI

Analysis of multiply protonated ions from ESI

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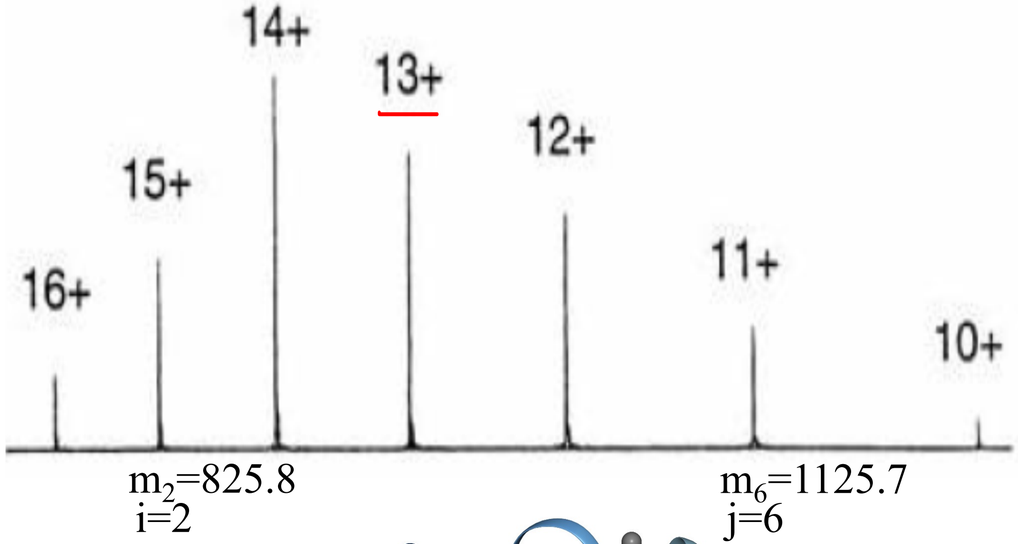
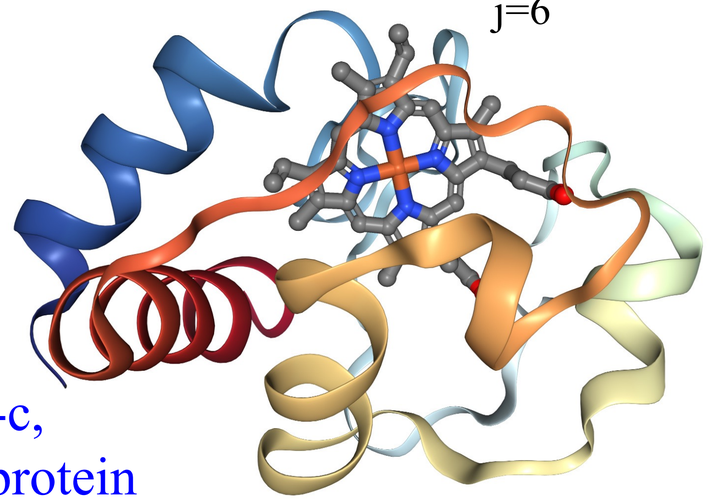




Cytochrome-c,
“recycling” protein

Electrospray ionization

Advantages

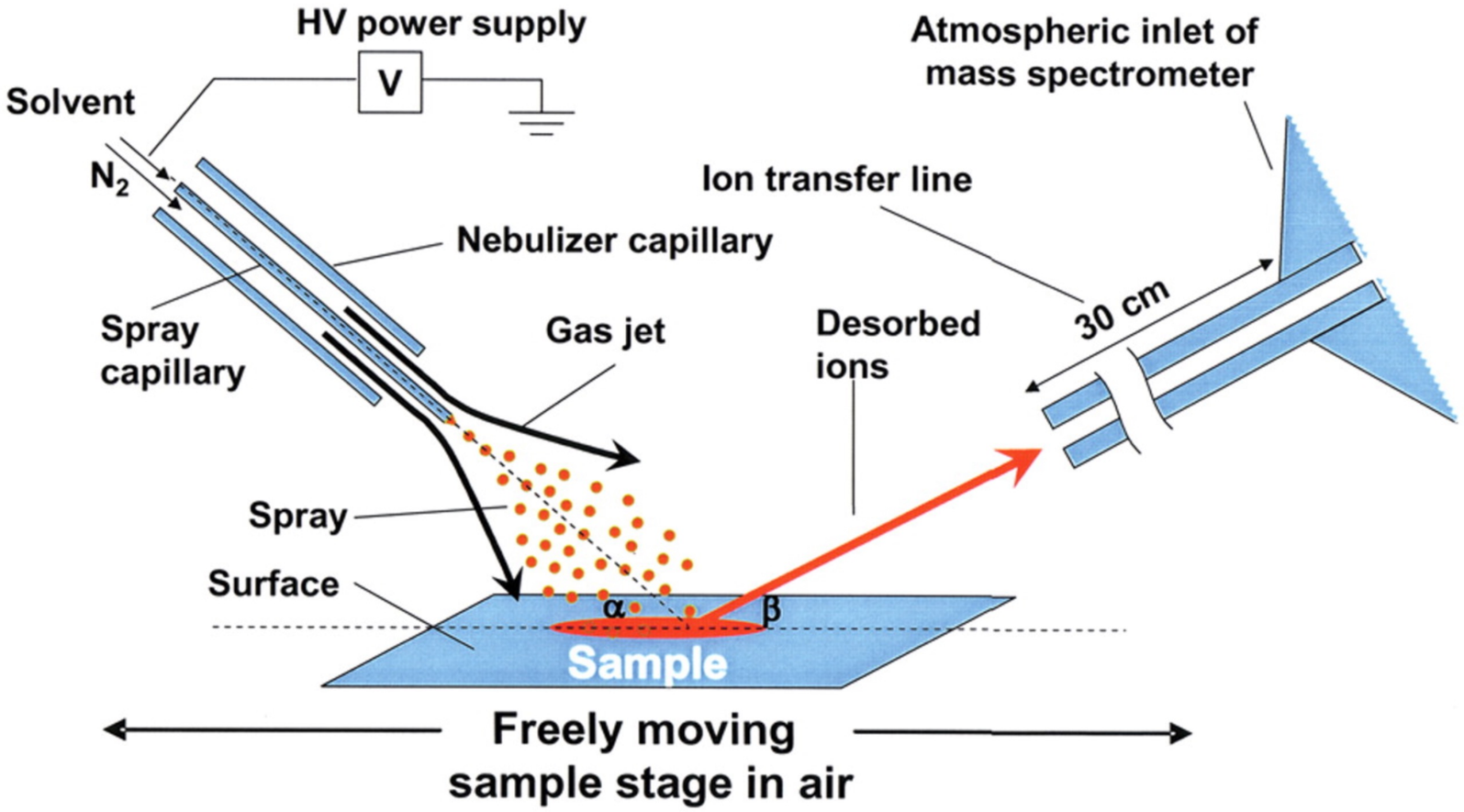
- Soft ionization
- Directly from solution
- Coupling with online separation (i.e., HPLC)
- High accuracy for large molecules
- Large mass range
- Fast
- Preserves non-covalent interactions

Disadvantages

- Complicated spectra
- Less tolerant to salts
- Relatively low ionization efficiency

Variants of Electrospray

Desorption Electrospray Ionization (DESI) was first reported in 2004 by Graham Cooks



DESI Mechanism

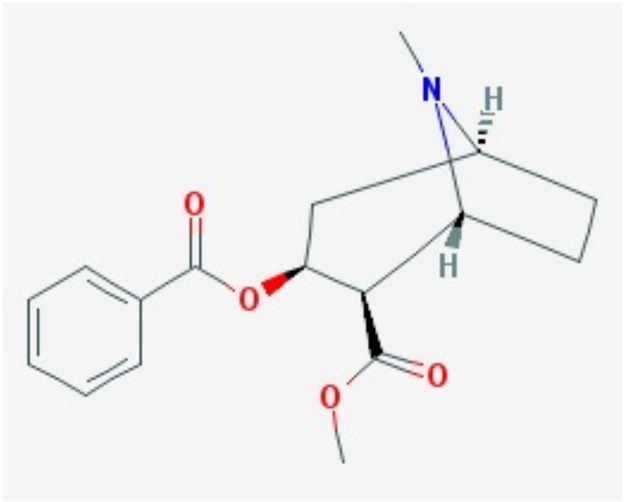
- DESI occurs when analyte particles are solvated by an ionized solvent flow.
  - The solvated analyte is ejected from the sample and swept toward the mass analyzer.
  - The mechanism and spectra are very similar to ESI.

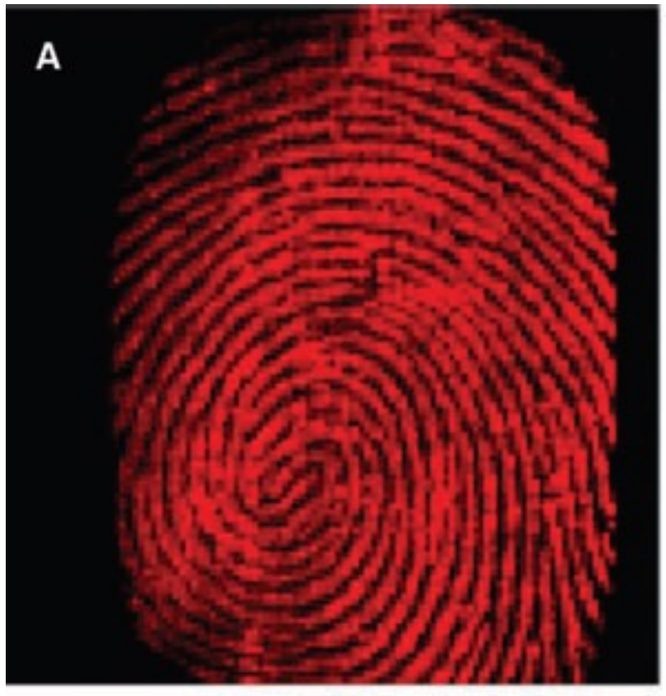
DESI Applications

DESI can be used in a wide variety of applications

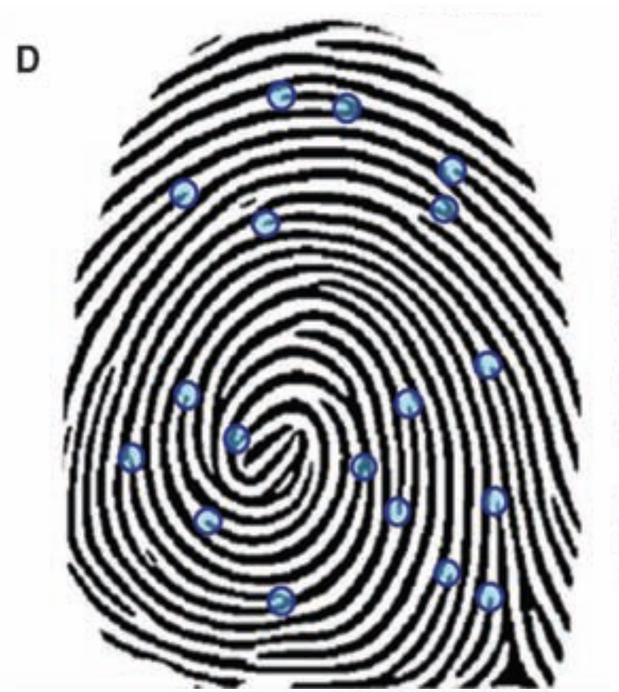
1. Pharmaceutical testing
   - Quality control/assurance
   - Counterfeit identification
2. Chemical weapons
3. Explosive residues
4. Latent fingerprints

Cocaine Fingerprints





DESI of cocaine distribution in a FP on glass.



FP generated from ink blotted on paper

Imaging drug distribution in tissue

R. Zare, Stanford University

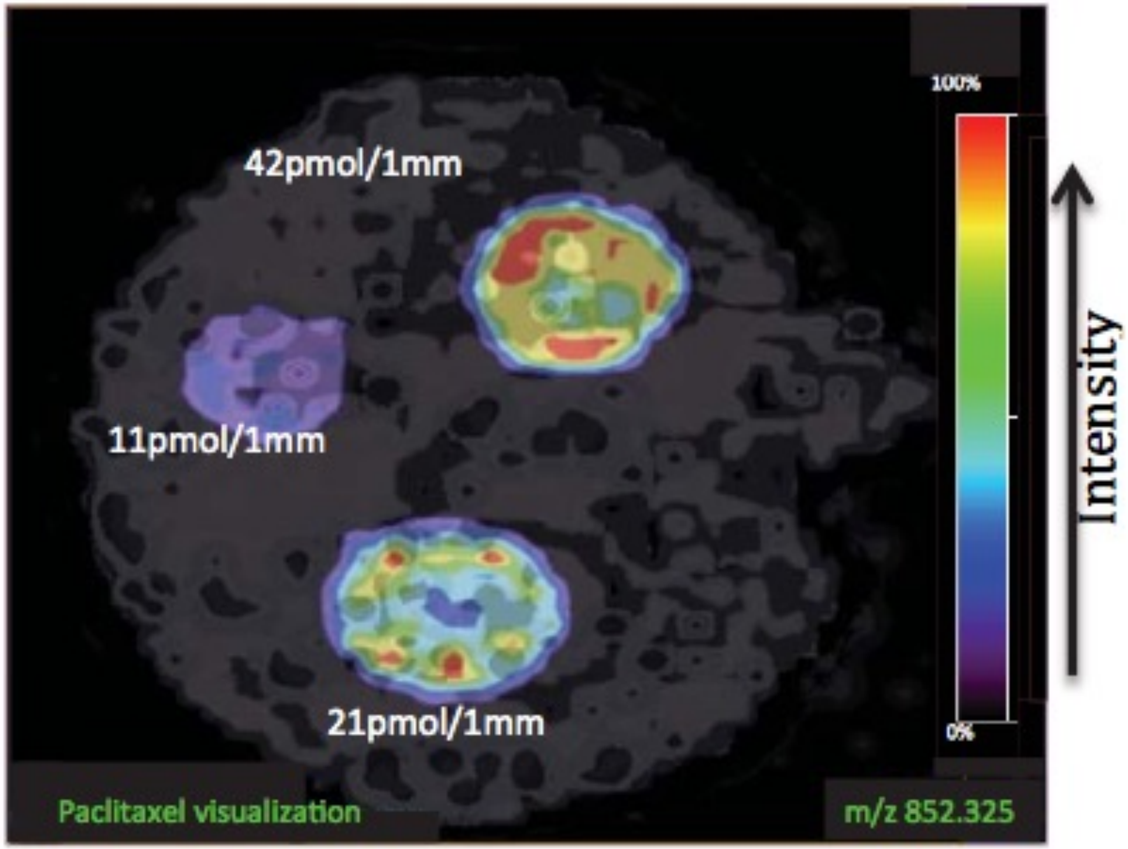
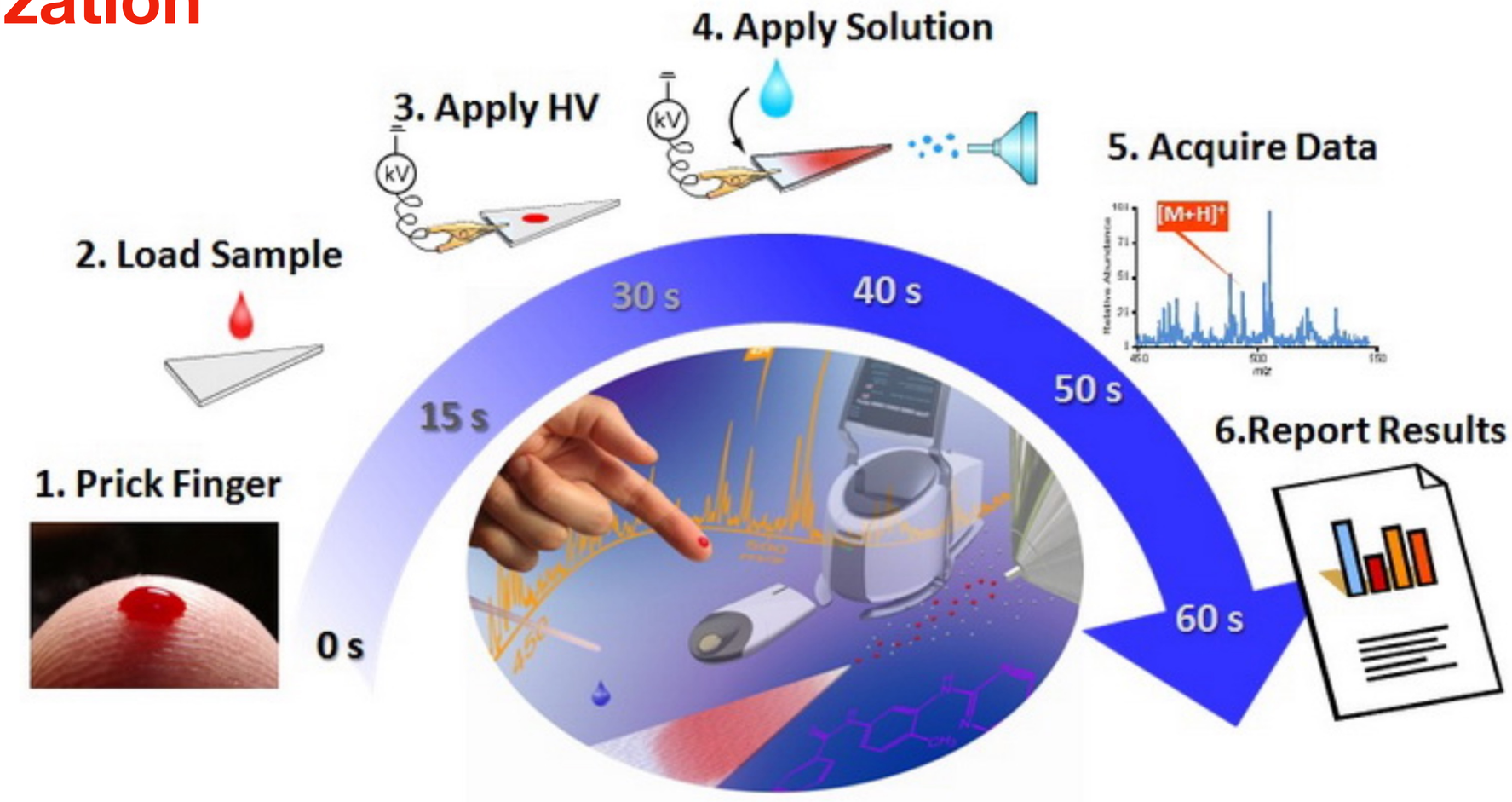


Fig.2 DESI-MSI generated image of anti-tumor drug, paclitaxel, applied in different concentrations on liver tissue (superimposed with the image of the tissue).

Variants of Electrospray

Paper spray ionization

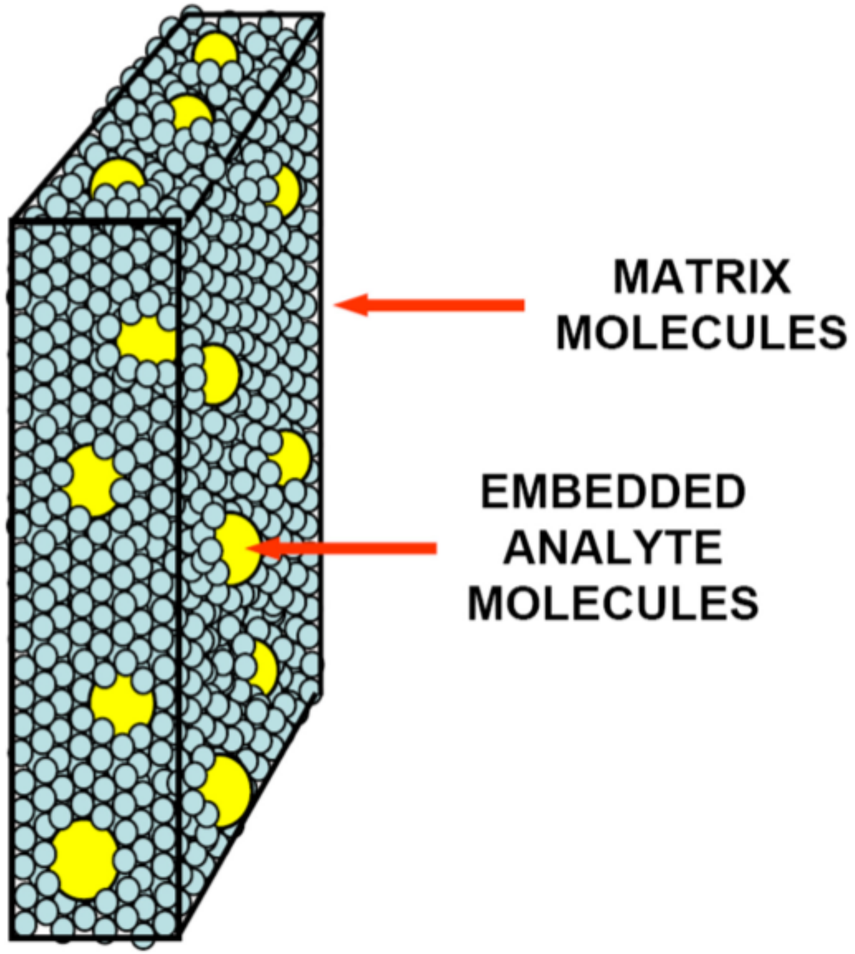
Can be used for fingerprints, blood samples, etc.



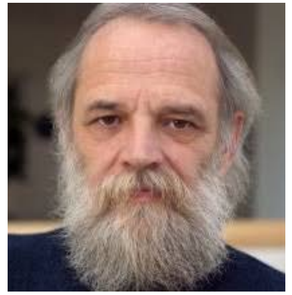
Wang, Liu, Cooks, and Ouyang *Angew. Chemie Int. Ed.,* **2010**, *49*, 877-880

Liu, Jiangjiang; Wang, He; Manicke, Nicholas E.; Lin, Jin-Ming; Cooks, R. Graham; Ouyang, Zheng. *Analytical Chemistry*. **82** (6): 2463–2471.

Matrix-assisted Laser Desorption Ionization (MALDI)



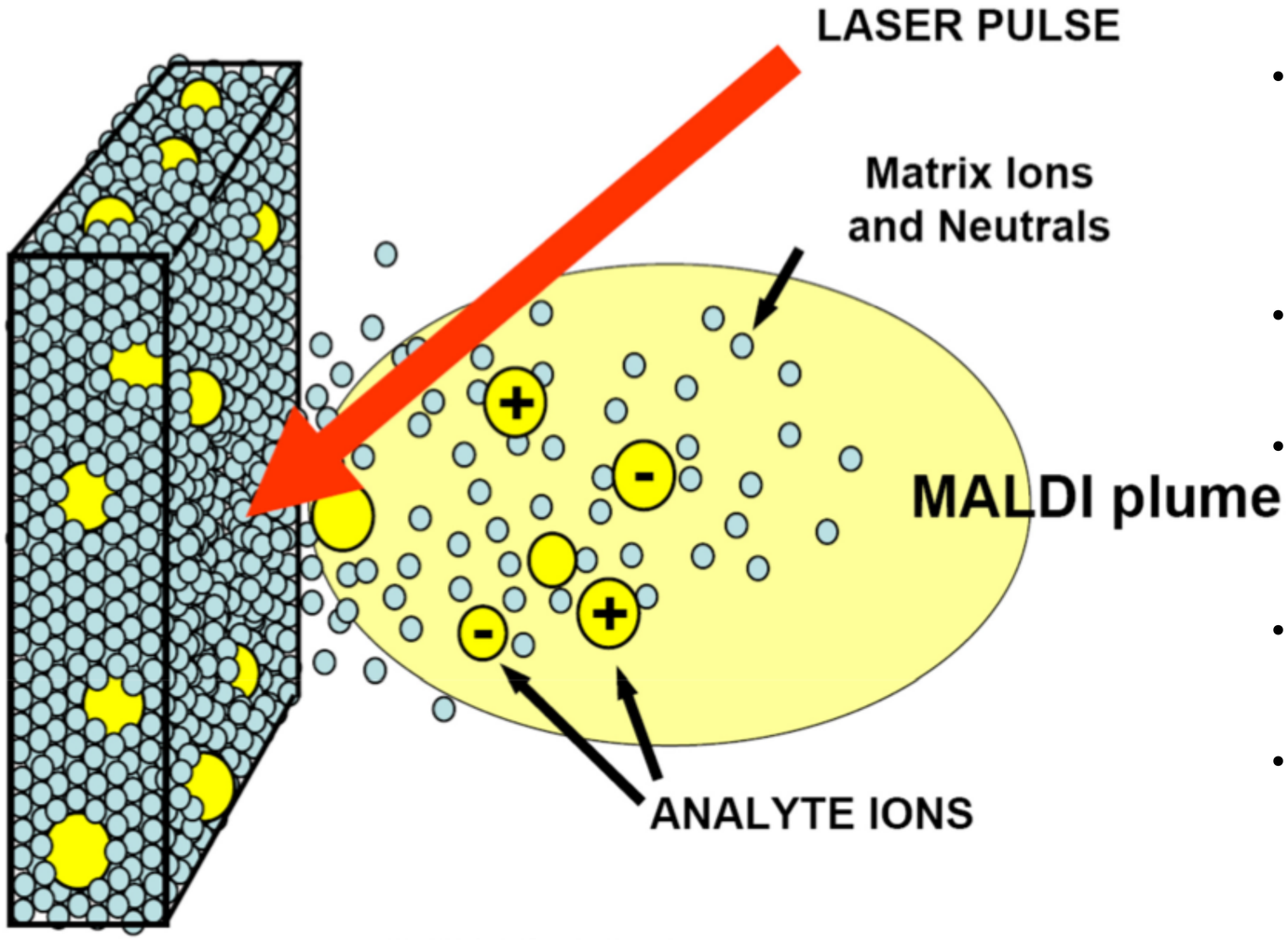
- Analyte molecules are embedded in a matrix after solvent evaporation.
- The dried mixture is struck with a short, intense laser pulse having a wavelength that is strongly absorbed by the matrix (often UV).



M. Karas

F. Hillenkamp

Matrix-assisted Laser Desorption Ionization (MALDI)



- Upon pulsed laser irradiation, rapid heating of the matrix causes sublimation and expansion into the gas phase in a fast cooling plasma called a MALDI plume.
- The most widely accepted ionization mechanism is gas-phase proton transfer.
- Efficient, “soft” (i.e., little fragmentation), and relatively universal (the wavelength is independent of the analyte).
- Allows analysis of large (100’s of kDa) intact biopolymers.
- Not quantitative due to heterogeneity.

K. Tanaka
Nobel Prize
2002

Properties of MALDI matrices

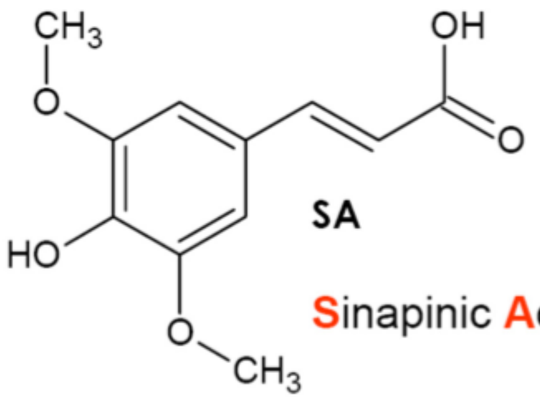
The ideal matrix should have the following properties:

- Strong absorption at the laser wavelength (chromophore)
- Solvent compatible with sample
- Homogeneous solid-state mixing with the analyte (co-crystallization)
- **Proton donor M⟶$[M+H]^+$**
  - Ability to undergo photochemical reaction leading to proton transfer to the analyte
- **Proton acceptor M⟶$[M-H]^-$**
  - Ability to undergo photochemical reaction leading to proton transfer from the analyte

Different from ESI: MALDI produces only low-charged ions!

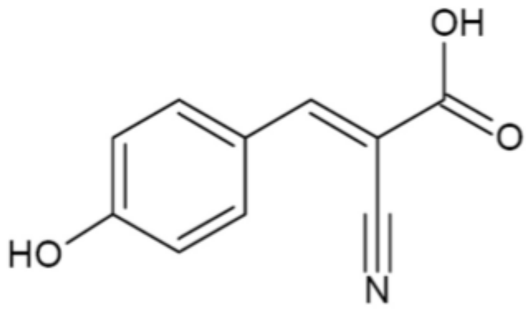
MALDI matrices

Structure of the most versatile matrices



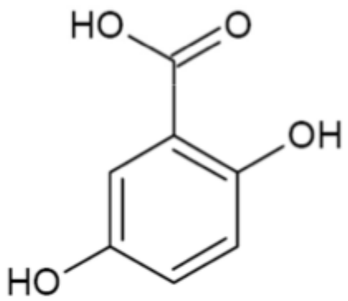
SA

Sinapinic **A**cid



HCCA (α-CN)

α-**C**yano-4-**H**ydroxy**C**innamic **A**cid

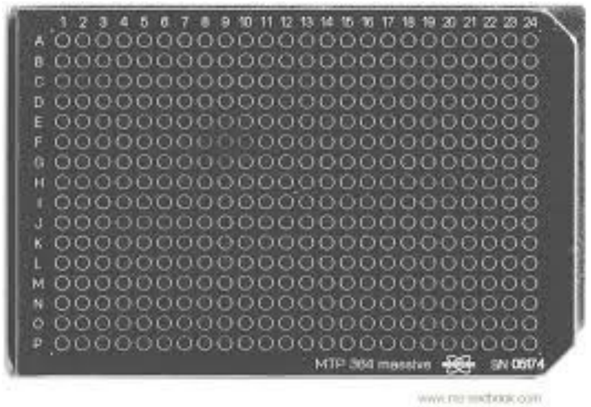


DHB

2,5-**Di**H**B**enzoic Acid

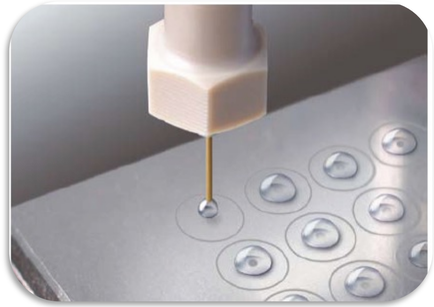
- Absorbance @ 337 and 355 nm
- Easy crystallization
- Soluble in aqueous organic solvents

MALDI targets



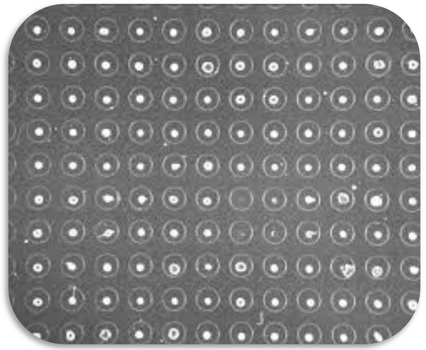
Manual deposition

MALDI spotter



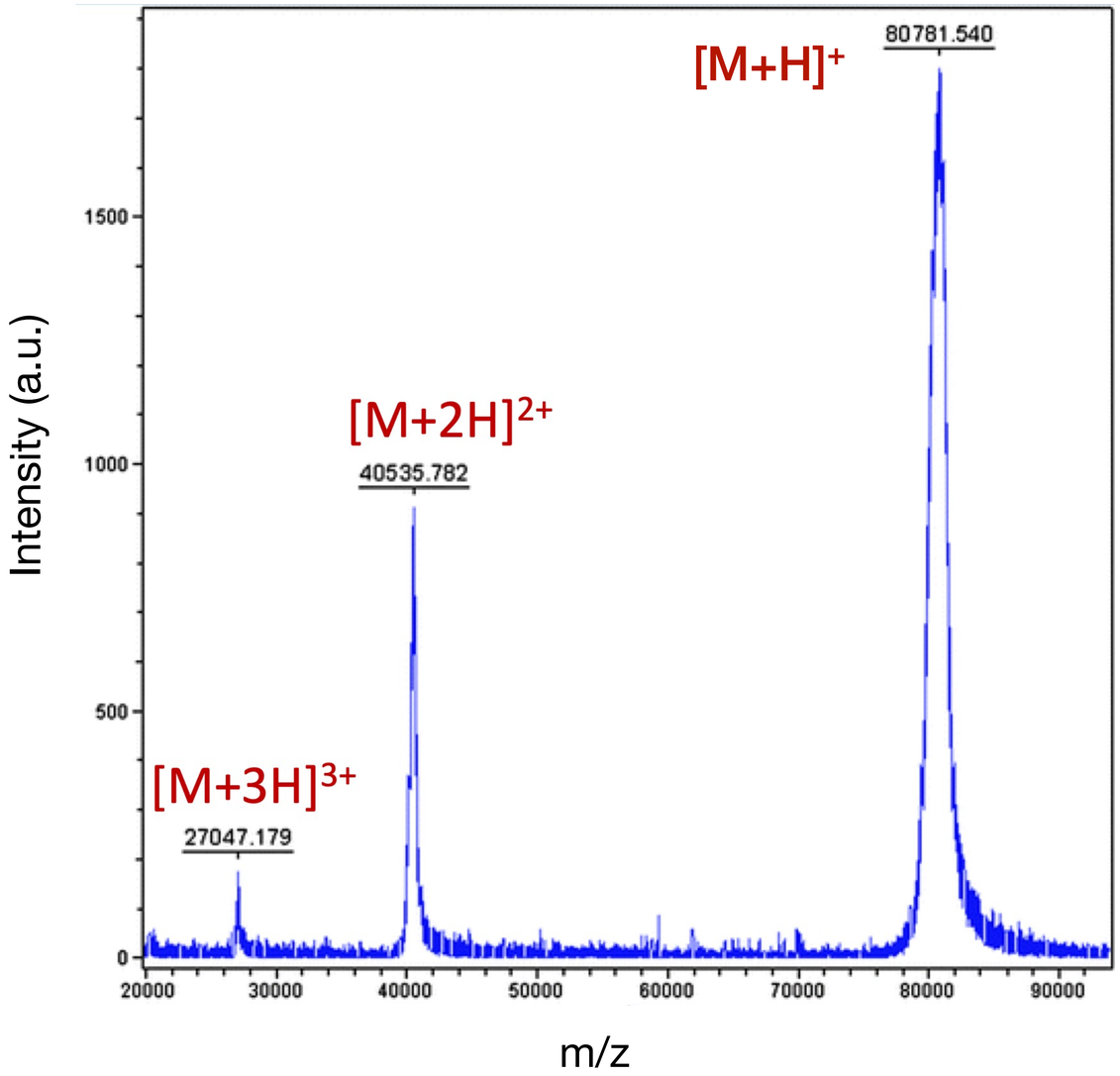
Crystallization

Anchorchip (Bruker)

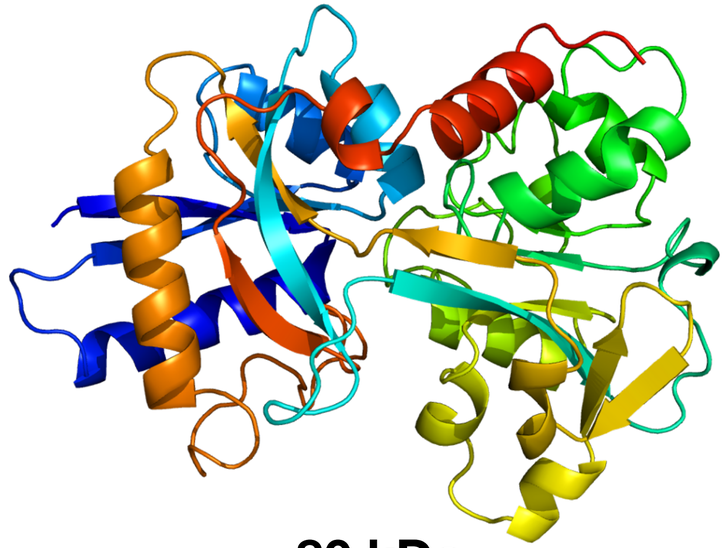


- Sample is mixed with matrix and 1 µL is deposited on the target
- Many different protocols: Dried-droplets (DD), Sandwich…

MALDI of Biomolecules



Transferrin



~80 kDa

Protein is co-crystalized with SA matrix

Proteins up to 200 kDa can be measured

Matrix-assisted Laser Desorption Ionization (MALDI)

Advantages

- Preferable for large molecules
- Very fast
- Sensitive to small amounts of sample
- Simple spectra
- Accurate
- Not affected by salts
- Soft ionization

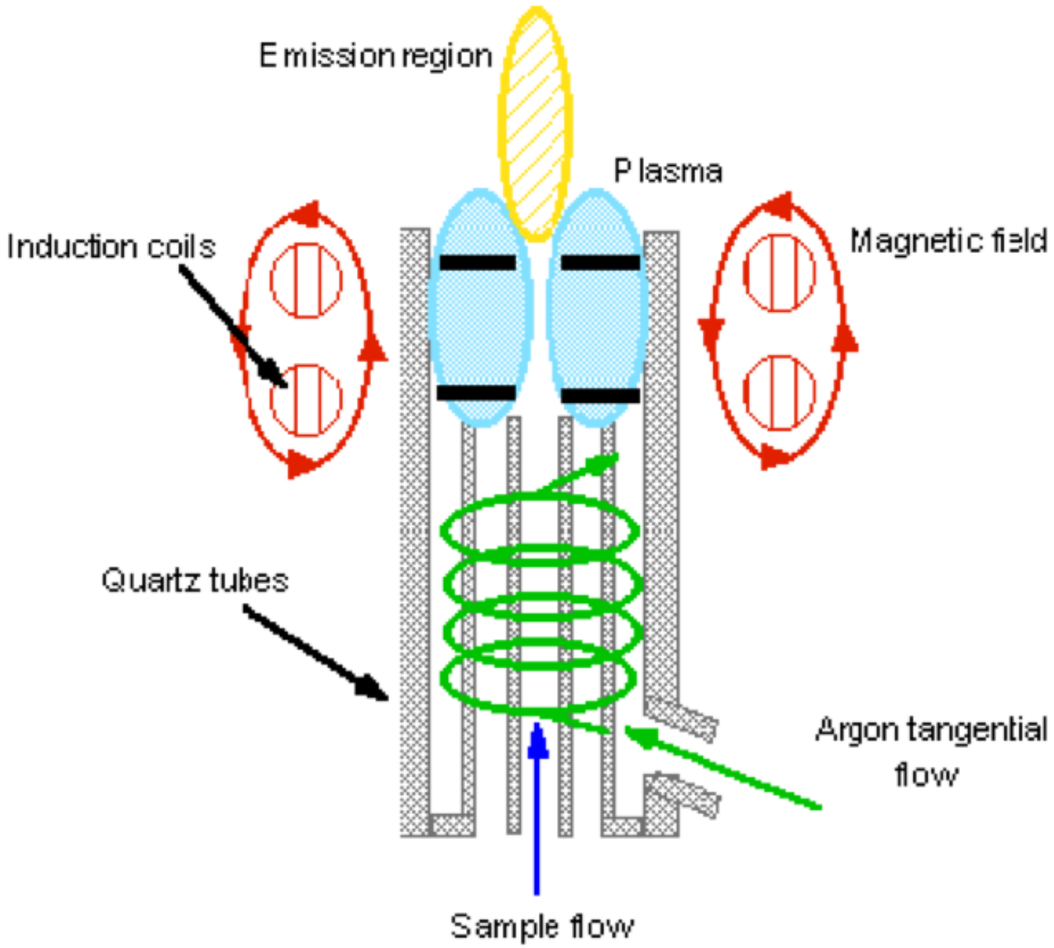
Disadvantages

- Needs preparation of crystals, adjusting intensity of laser, finding crystals on plate with sample
- Low shot to shot reproducibility
- Short sample life
- Singly charged ions

The Inductively Coupled Plasma

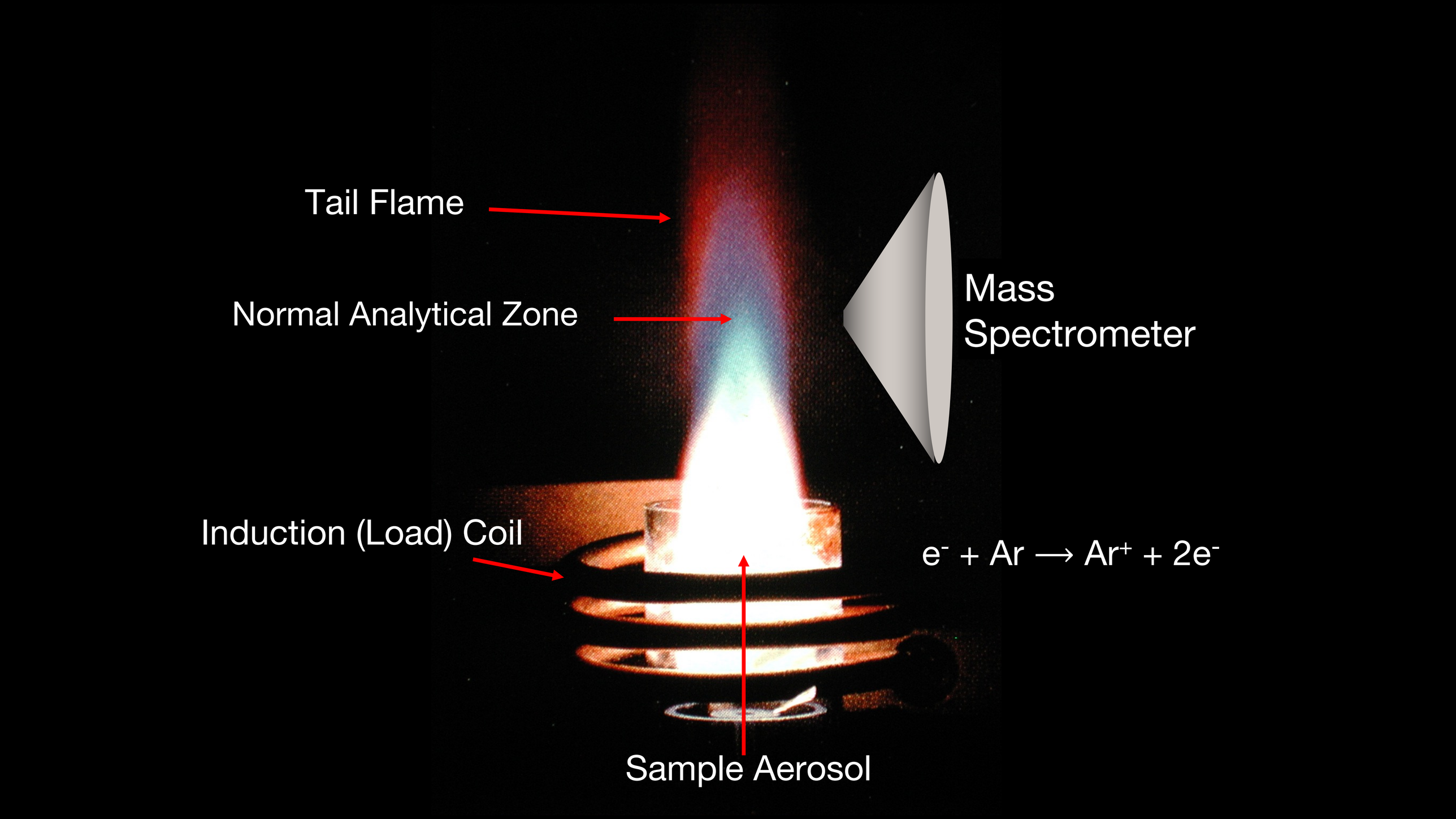
- A plasma is a hot, partially ionized gas.
- The ICP is an argon-based, radio frequency plasma.
- The input rf frequency is either 27 or 40 MHz at powers from 1 to 2 kW.
- The plasma is formed and contained in a three tube quartz torch.
- The temperature in the central analyte channel ranges from about 6000 to 8000° K.
- At these temperatures most elements are largely atomized and ionized

Inductively Coupled Plasma (ICP)

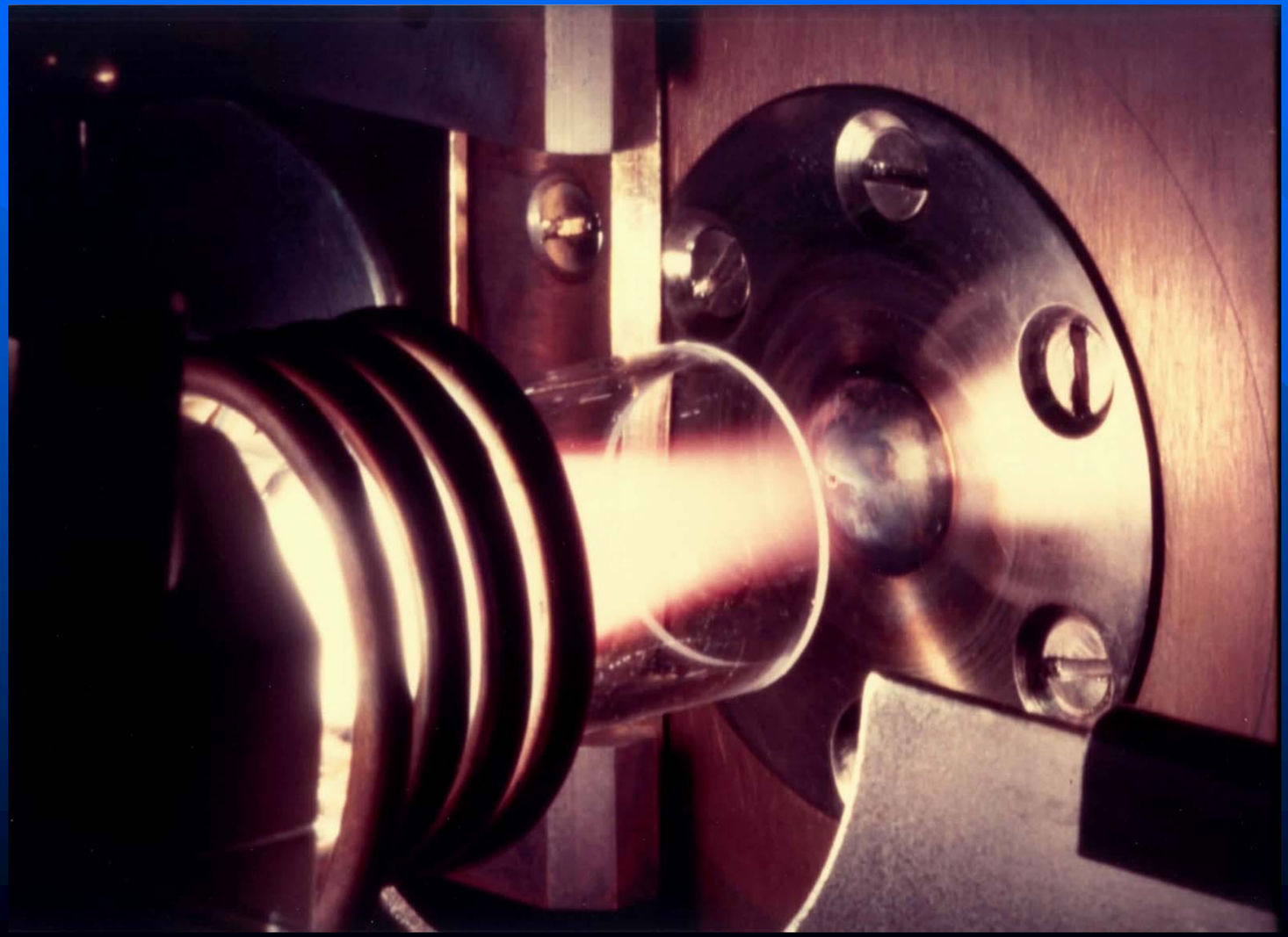


1. Ionization of Ar in a radio frequency plasma.
2. Atomization of sample due to high temperature and ionization by the plasma.

The temperature in the central analyte channel ranges from about 6000 to 8000° K


Tail Flame
Normal Analytical Zone
Mass Spectrometer
Induction (Load) Coil
$e^- + Ar \longrightarrow Ar^+ + 2e^-$
Sample Aerosol

Inductively Coupled Plasma - Mass Spectrometry



Inductively Coupled Plasma MS

Advantages

- Multi-element analysis
- High sensitivity
- Nearly complete isotope coverage
- Liquid, solid or gas samples
- Short analysis times
- Less “art”

Disadvantages

- Isobaric interferences
- Relatively noisy
- Wide ion energy spread
- Inefficient
- Spectral complexity