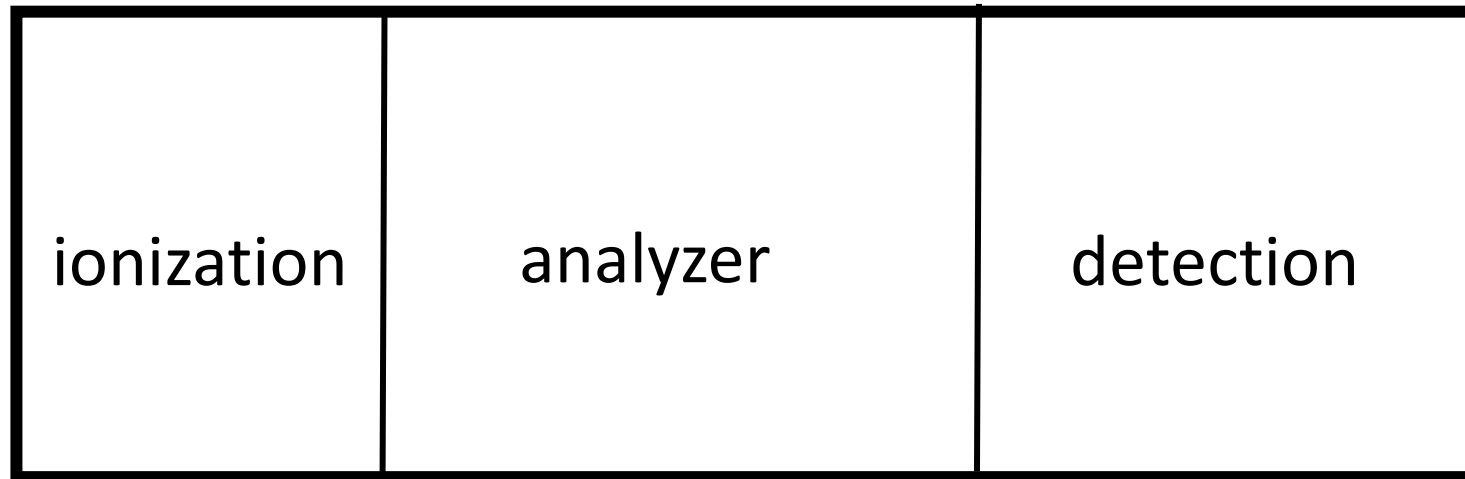


Ion sources

Overview of mass spectrometers



in vacuum
or
not in vacuum

High vacuum

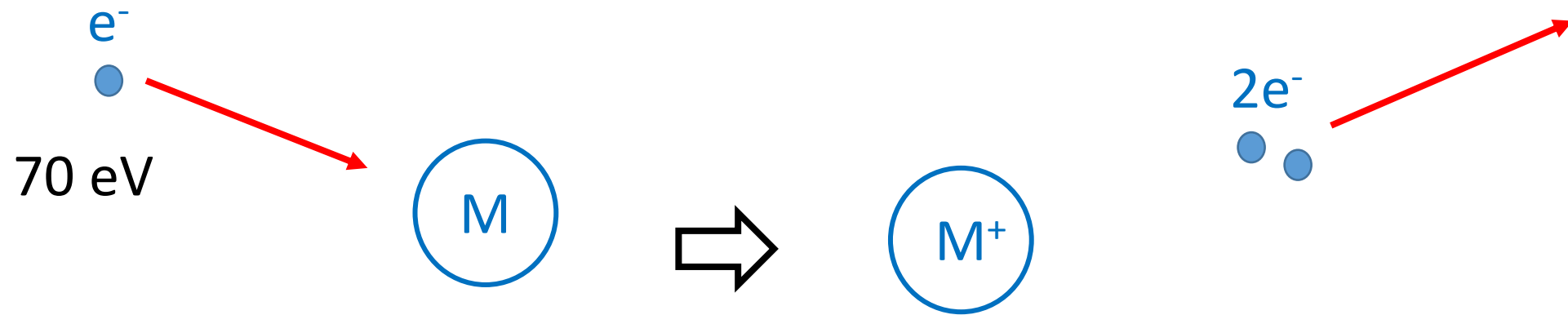
Ionization

1. Electron impact ionization (electron bombardment)
2. VUV ionization
3. Chemical ionization
4. MALDI
5. ESI
6. Multiphoton ionization (MPI)
7. Ambient pressure ionization (DESI, MAI, etc.)

Positive ion {
Loss of electron
Attached cation (proton, Na^+ , etc.)

Negative ion {
Attached electron
Attached anion (Cl^- , etc.)

1. Electron impact ionization (electron bombardment)

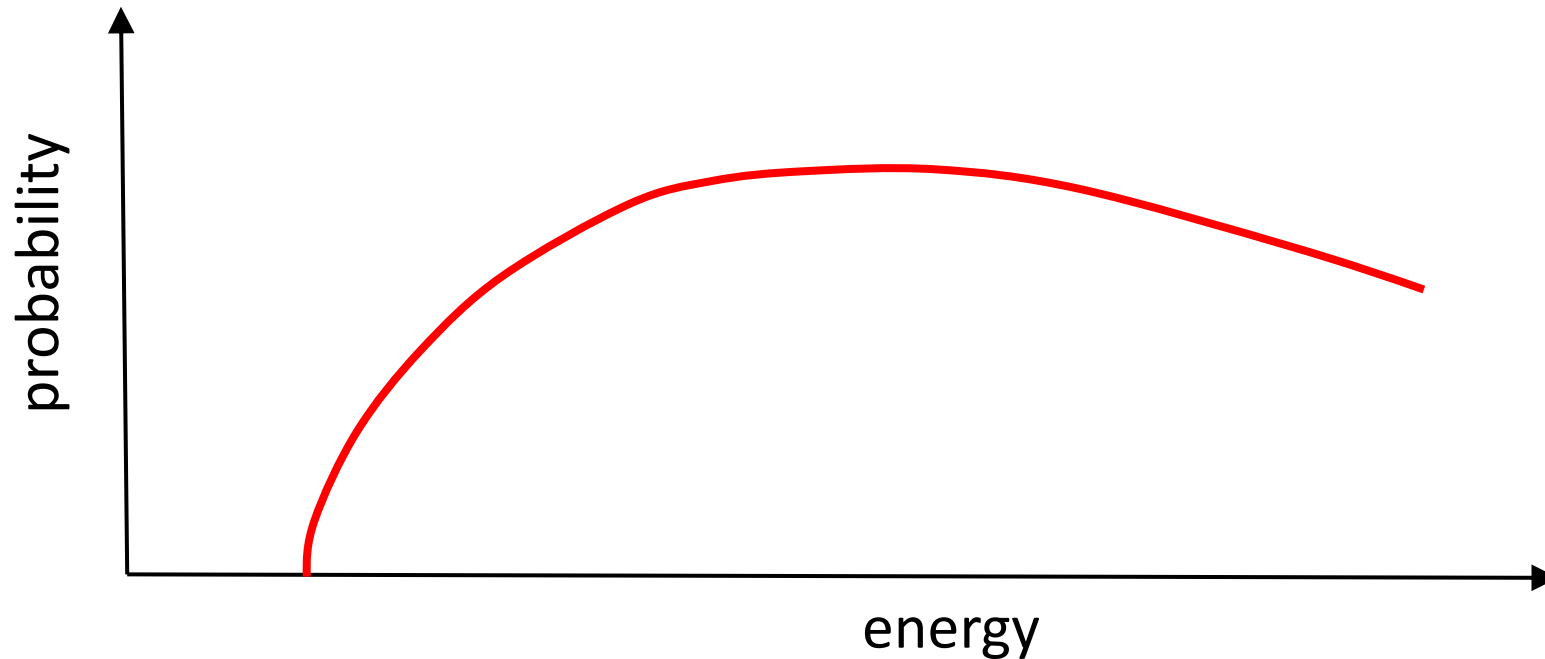


Why 70 eV?

a. Ionization energy (ionization potential):

energy required to remove an electron from molecules

3-15 eV, but 8-11 eV for most molecules



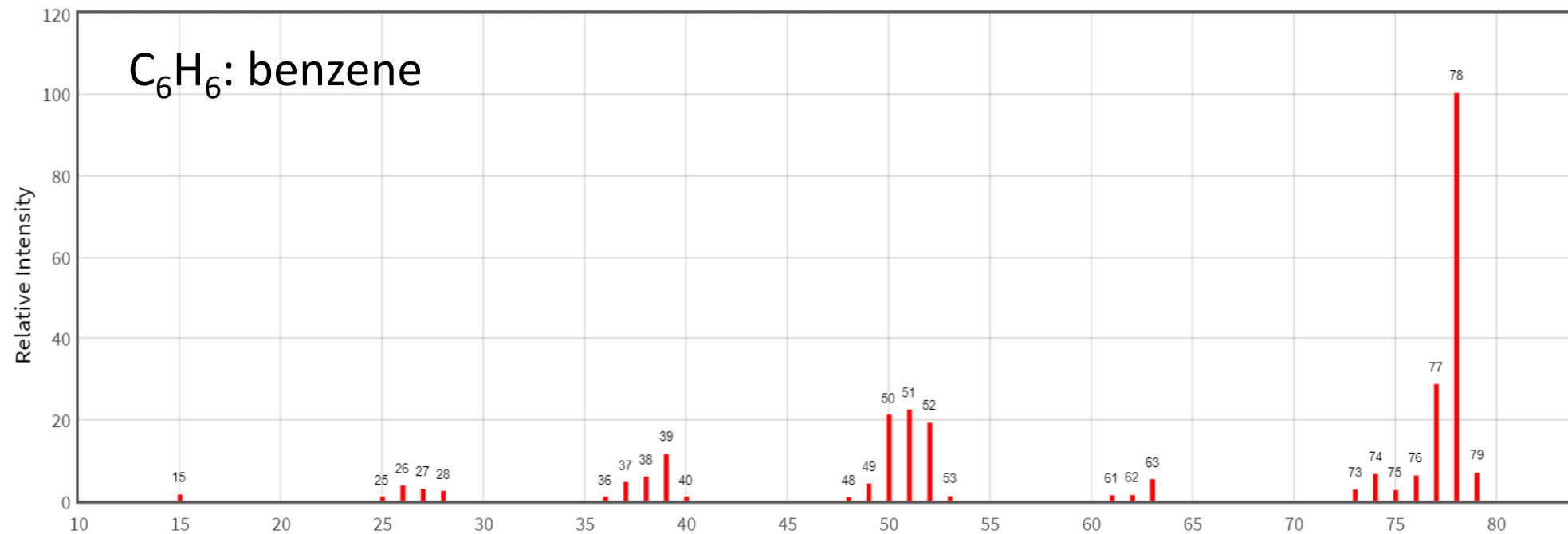
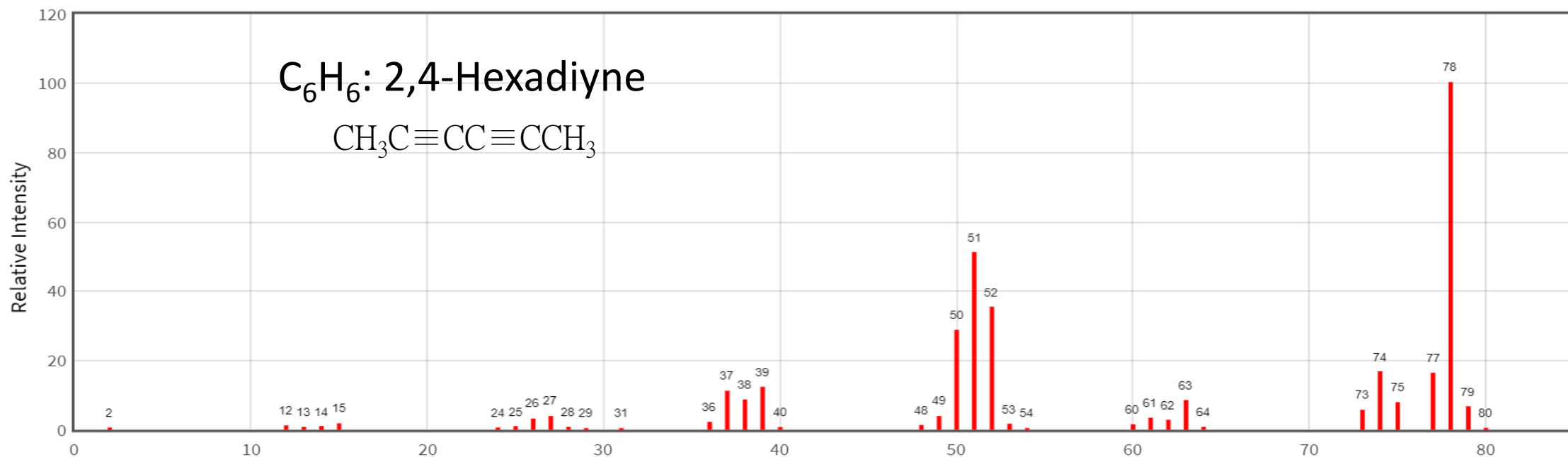
Results of ionization by electron with high kinetic energy:

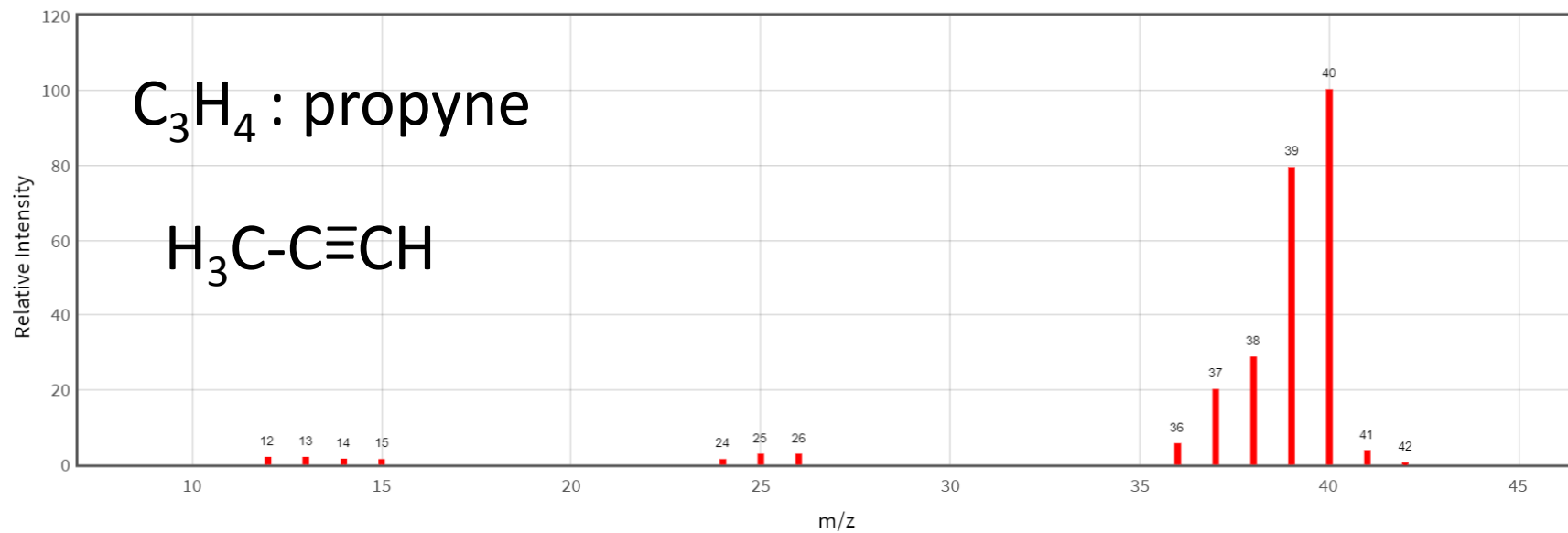
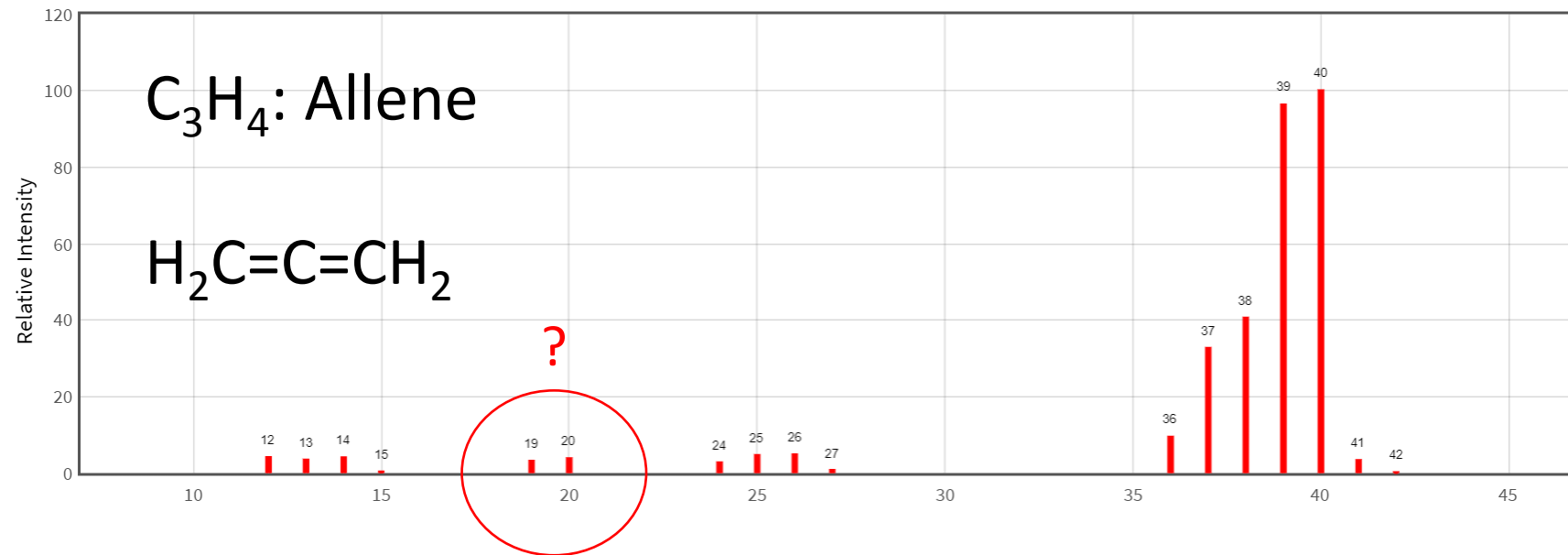
Cracking into small fragments

- Advantage: a method to differentiate isomers (?)
- Disadvantage: sometime loss of precursor ion

Precursor ion, parent ion

Fragment ion, daughter ion

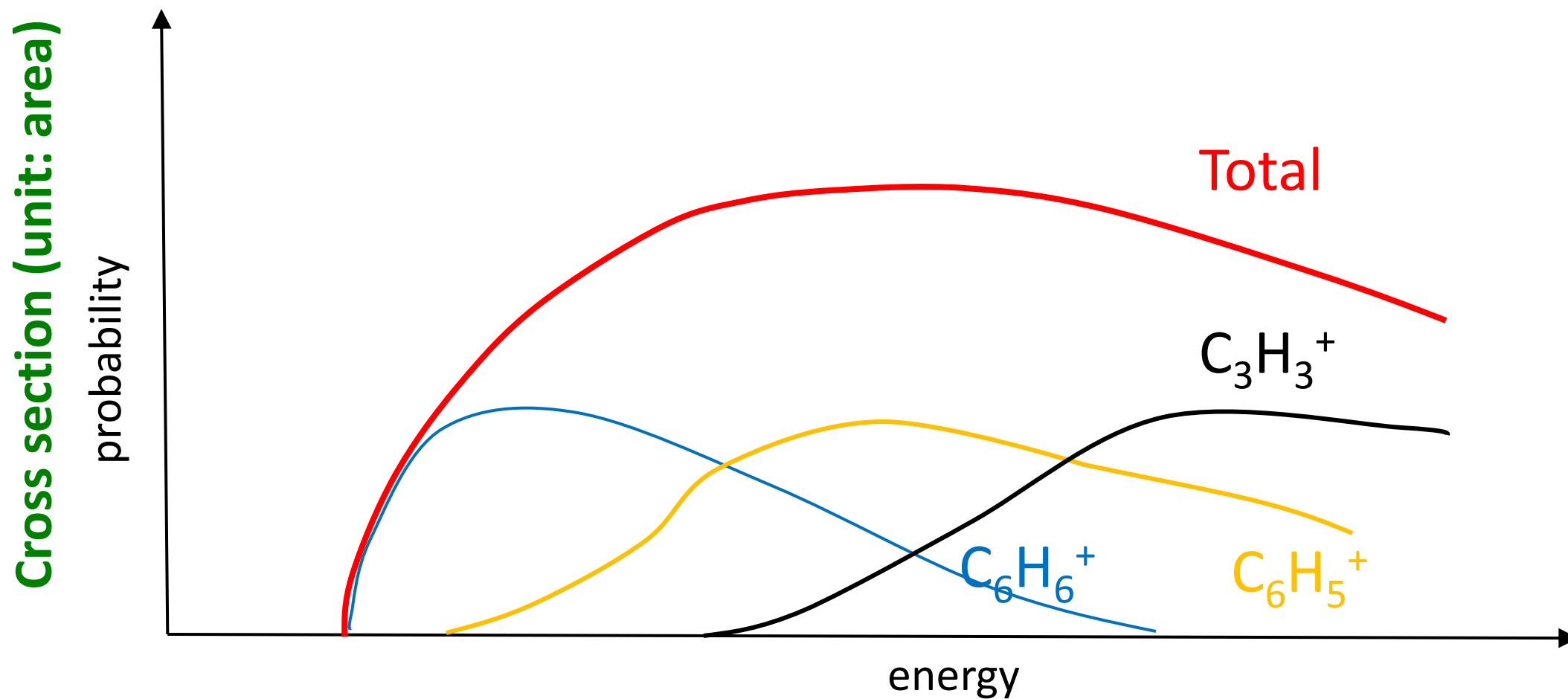




Ionization efficiency:

1. Ionization cross section
2. Density of electrons in ionization region
3. How long is the interaction time between neutral and electrons?

Total ionization cross section vs partial ionization cross section



Ionization cross section:

1. Typical values from experimental measurement:

The ionization cross-section by electron ionization at 70 eV is in the range from 4×10^{-17} cm, for a small atom like He, to 1.5×10^{-15} cm² for a large molecule like benzene or 1.7×10^{-15} cm² for hexan-3-one.

Ionization cross section:

2. Estimated values from additivity rule:

- The ionization cross-sections of atoms are measured.
- Ionization cross-sections of molecules are obtained from atoms by applying the additivity rule postulated by Otvos and Stevenson in 1956.
- The above additivity rule gives a value which differs from the experimental one by not more than a factor of 2 (total cross section only).

Ionization efficiency:

1. Ionization cross section

2. Density of electrons in ionization region

3. How long is the interaction time between
neutral and electrons?

Depends on the
design of ionizer

- Density of electron is limited by the space-charge effect
- Interaction time is limited by velocity of neutral molecules
typically $< 2 \times 10^{-4}$

Soft ionization: little fragmentation in ionization

1. Electron with low energy (~ 10 eV)
2. Vacuum ultraviolet (VUV) ionization
3. Chemical ionization
4. MALDI
5. ESI

Electron with low energy (~10 eV)

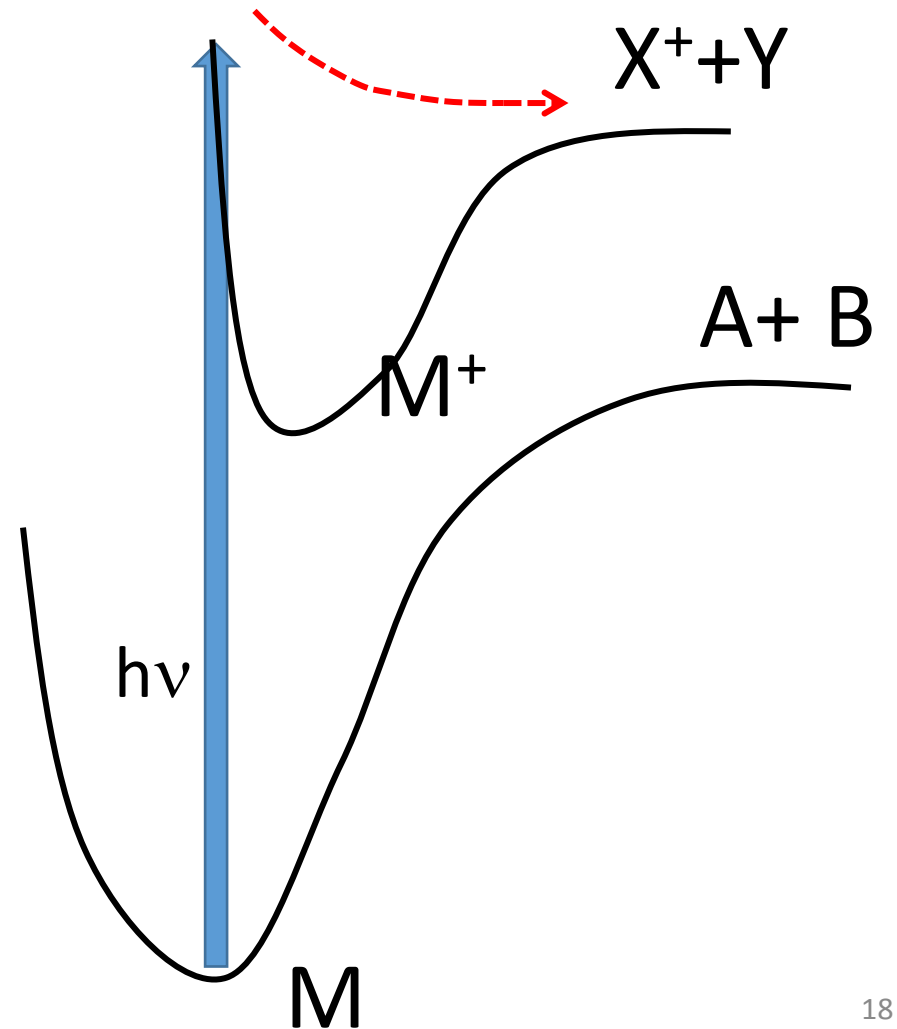
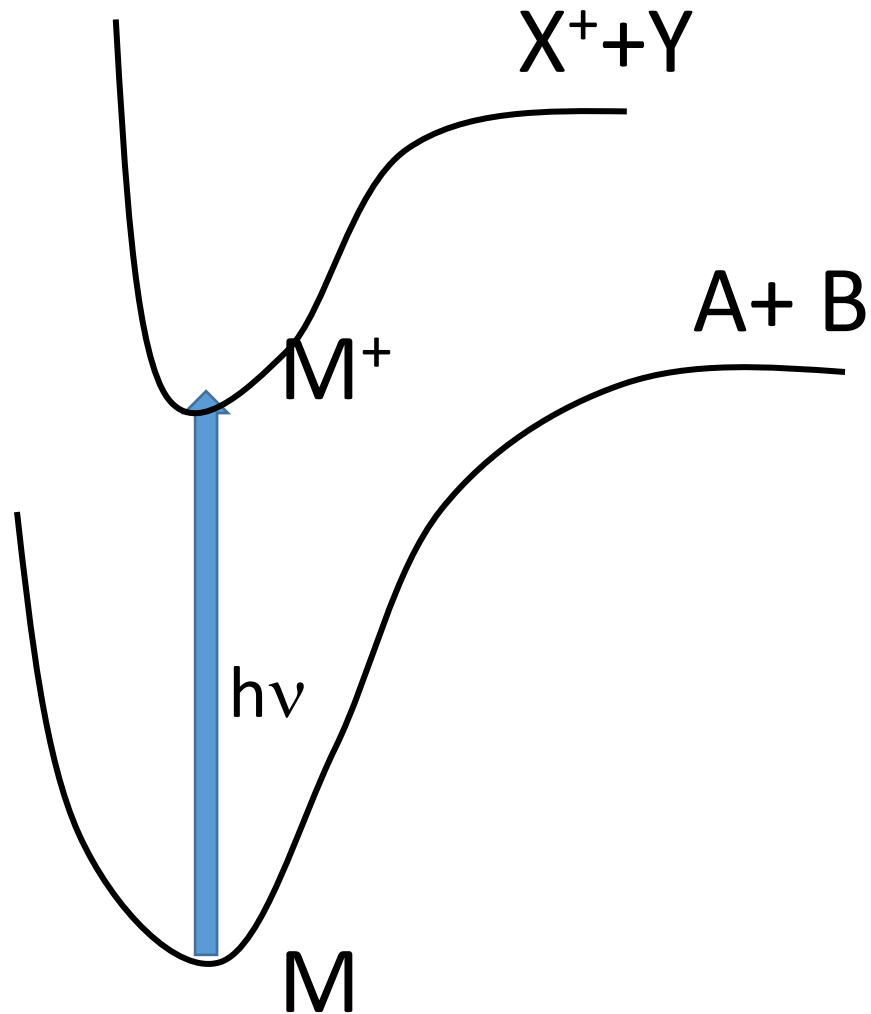
- Ionization energy 8-11 eV
- Energy left in molecules is small, not able to crack into smaller fragments
- Low efficiency: small ionization cross section and low density of electrons

2. VUV ionization

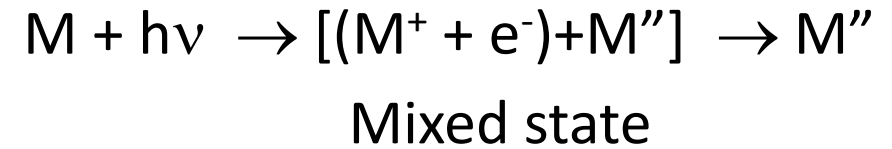
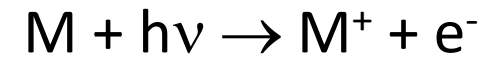
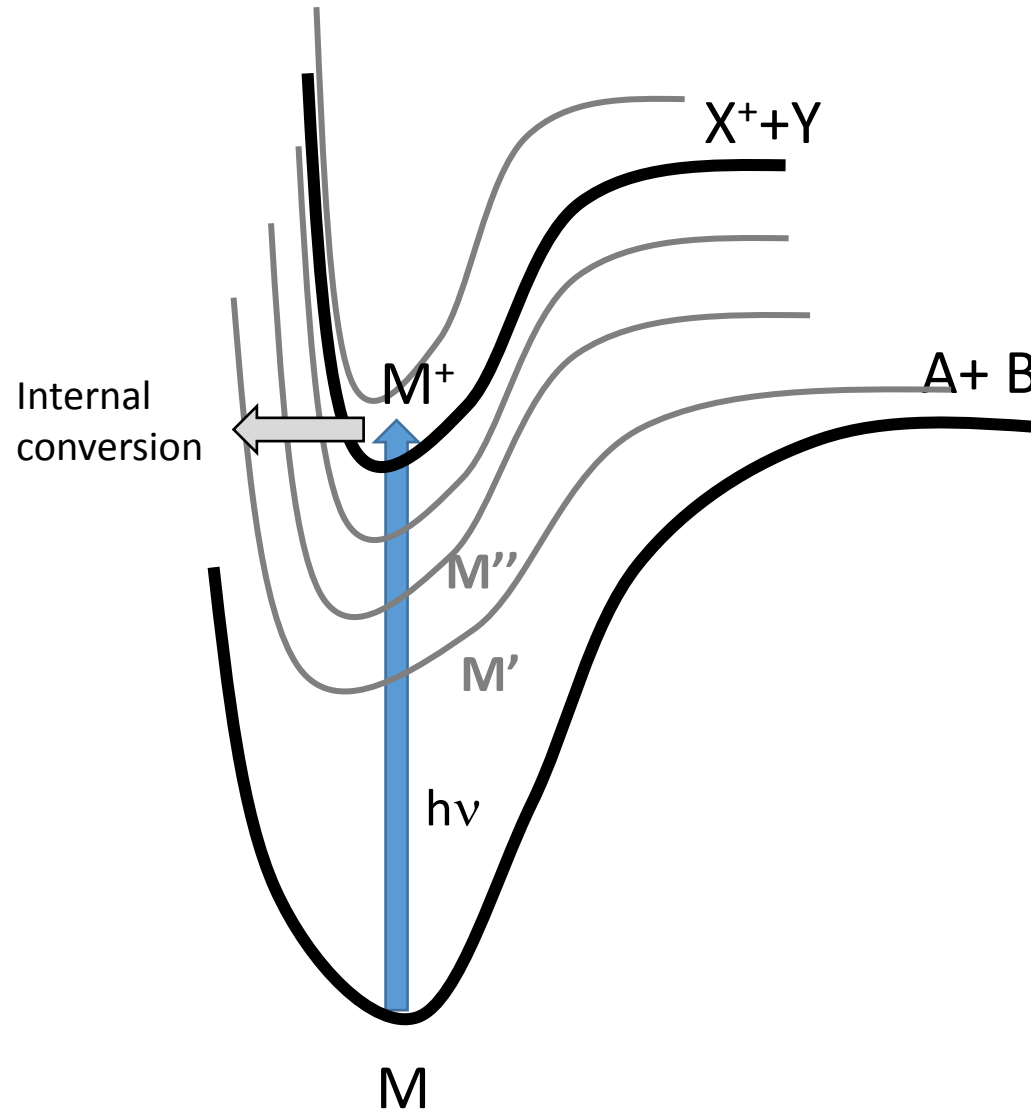
- Vacuum ultraviolet (VUV): $\lambda < 200$ nm, absorbed by air
- Photon energy: tunable from 6.2 eV-20 eV
- Ionization energy of molecules: 3-15 eV (typically, 8-11 eV)
- Tune the photon energy slightly above ionization energy, so energy left in molecules is small, not able to crack into smaller fragments

Fragmentation by VUV ionization

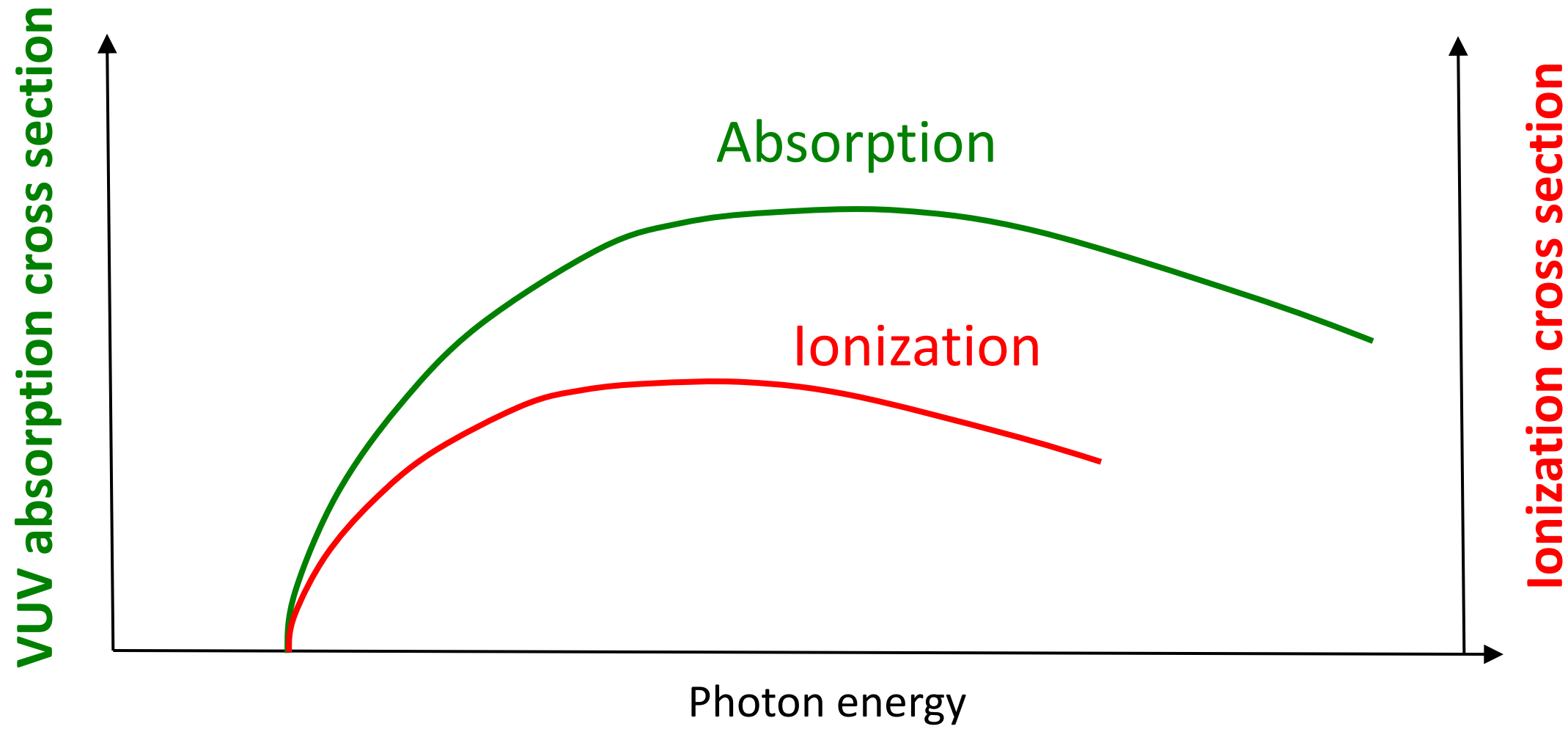
(adiabatic ionization vs vertical ionization energy)



Photoabsorption and photoionization



How large the molecules can be ionized?

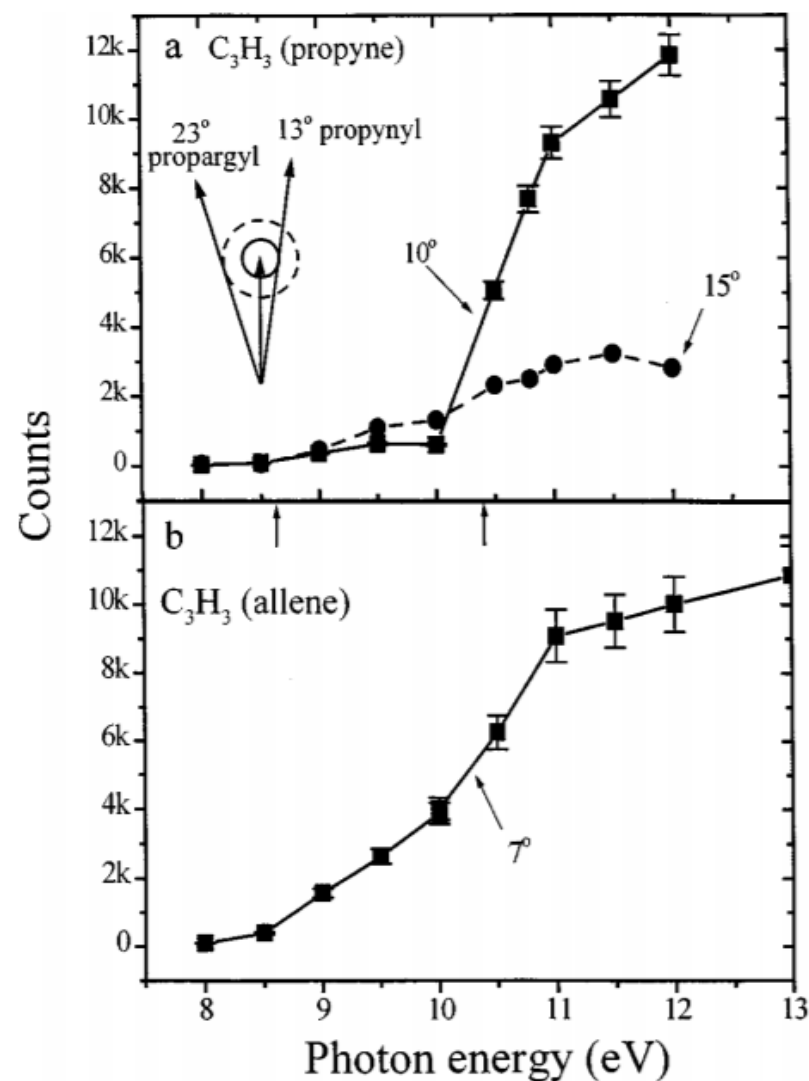


Differentiation of isomers

Propyne: 10.36 eV

Allene: 9.6 eV

J. Chem. Phys., Vol. 110, No. 9, 1 March 1999

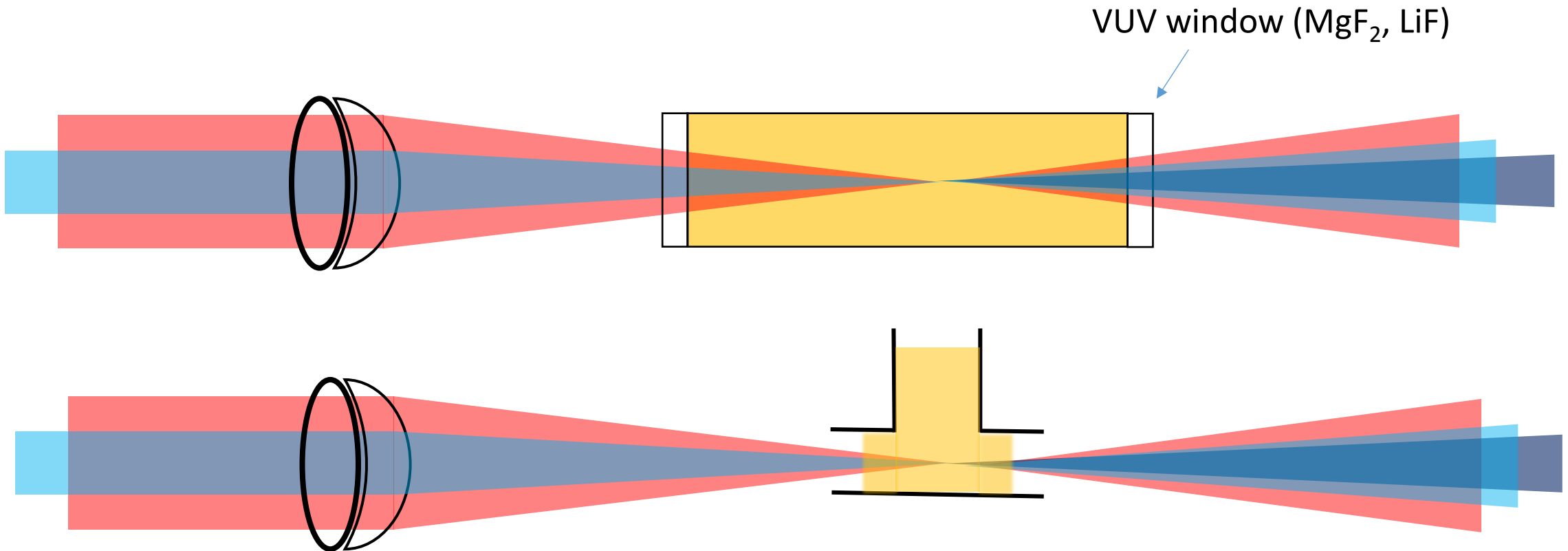




National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan

VUV laser

1. Commercial laser: 193 nm, 157 nm excimer laser
2. Four wave mixing from UV-VIS laser (efficiency $<0.1\%$, typically $10^{-5} \sim 10^{-9}$)



VUV photon ionization efficiency

- Ionization cross section
- Photon density
- Interaction time

Unit: 1Mb = 10^{-18} cm²

Ionization cross section:

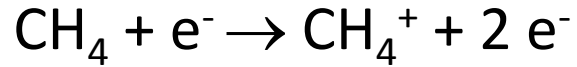
0 Mb below ionization potential ~ 50 Mb above IP

Intensive VUV photon: $10^{12} \sim 10^{15}$ photons

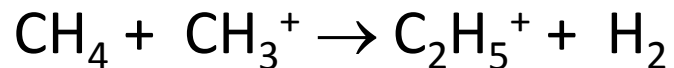
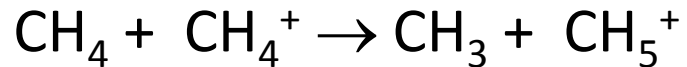
Ionization efficiency: 10^{-2} - 10^{-4} for molecules with large ionization cross section

3. Chemical ionization (CI): soft ionization

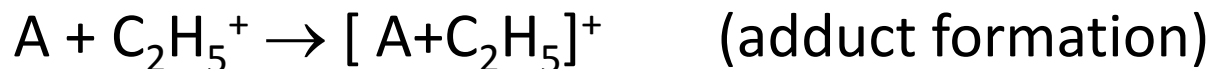
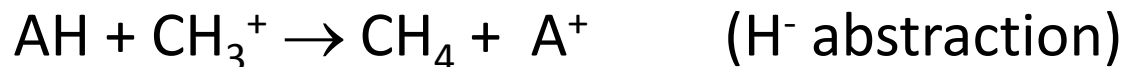
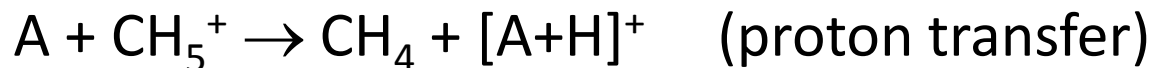
Primary ion formation (methane, ammonia, isobutene)



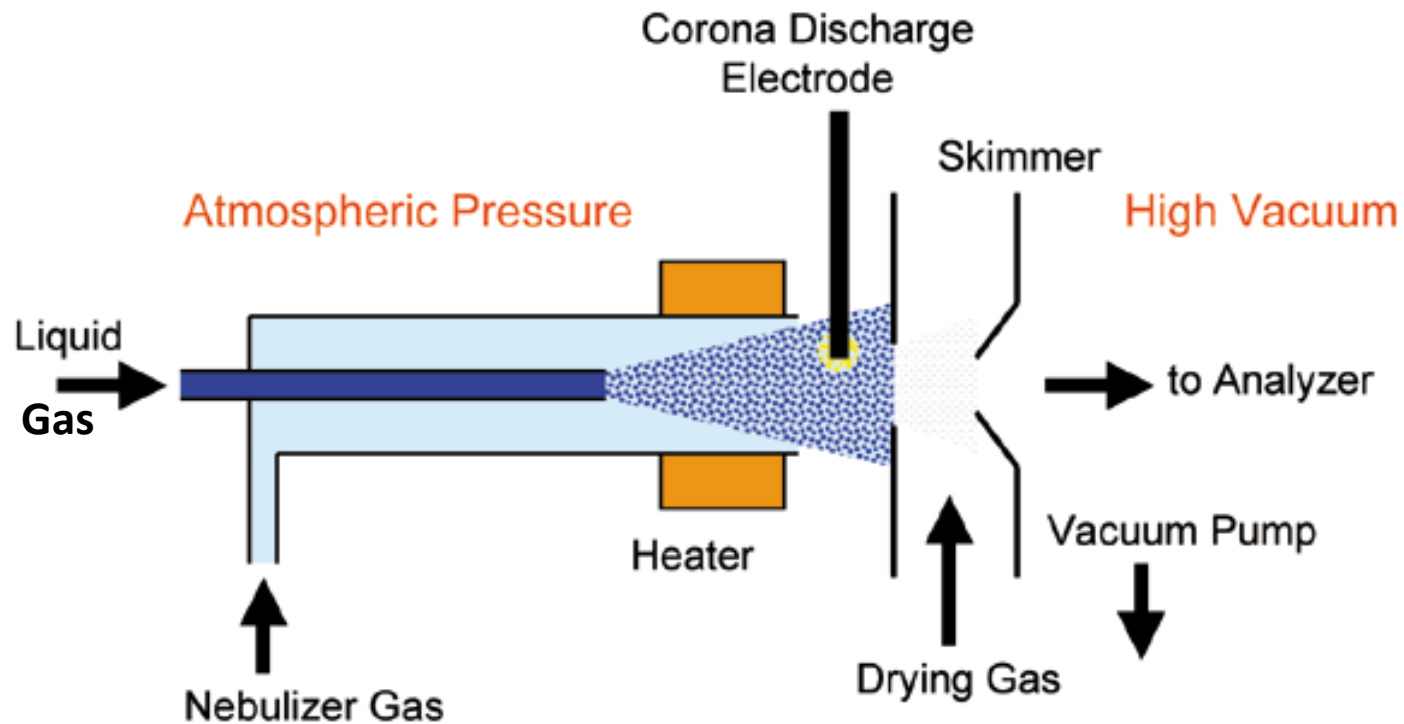
Secondary reagent ions



Product ion formation

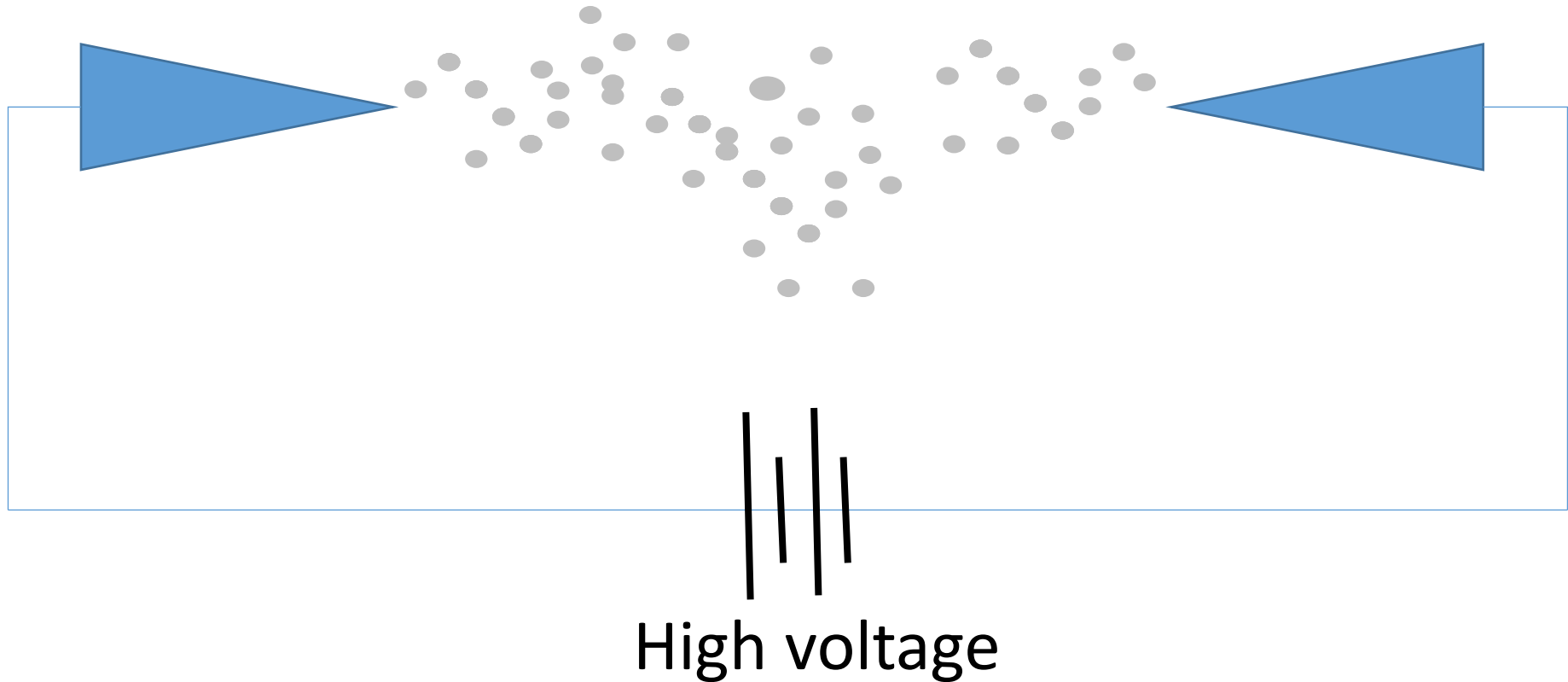


Combination of GC or LC mass spectrometer



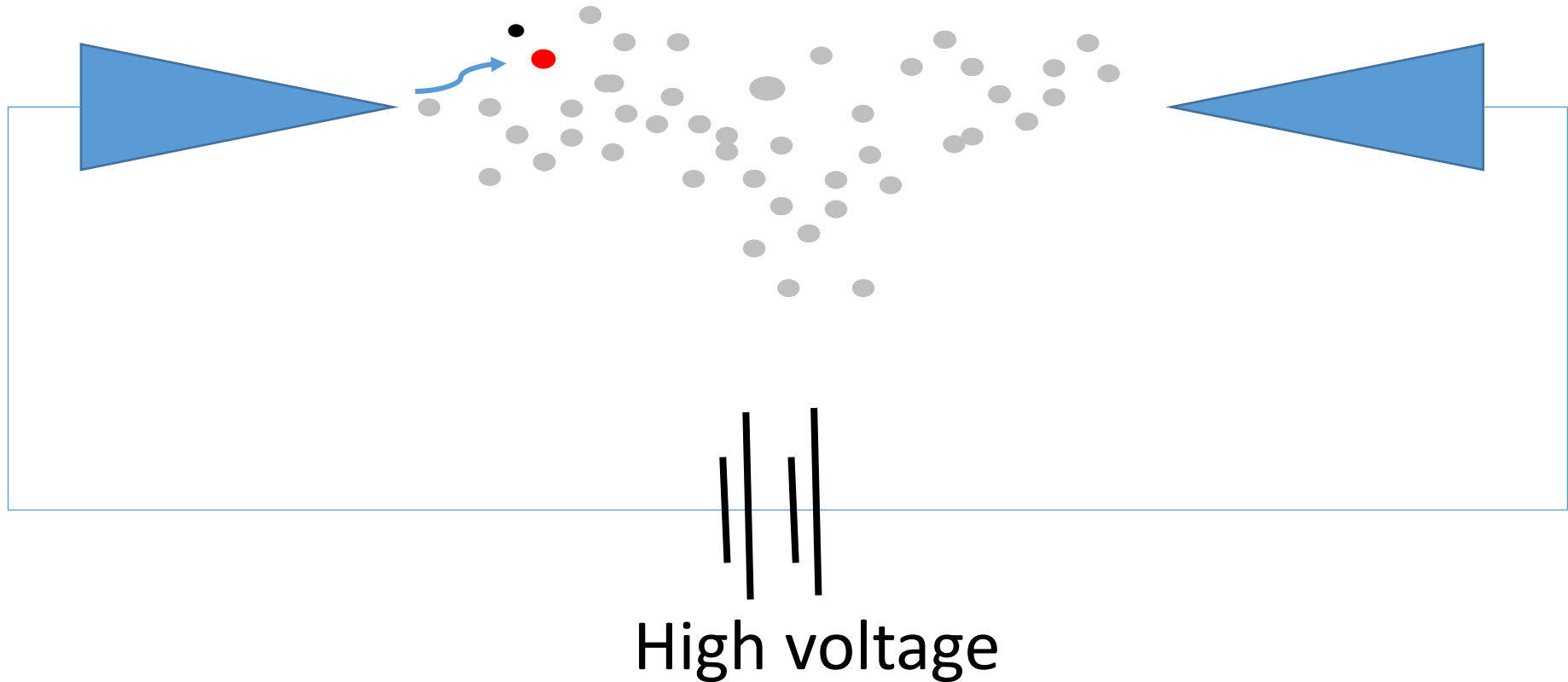
Corona discharge

Neutrals flow through high voltage electrode



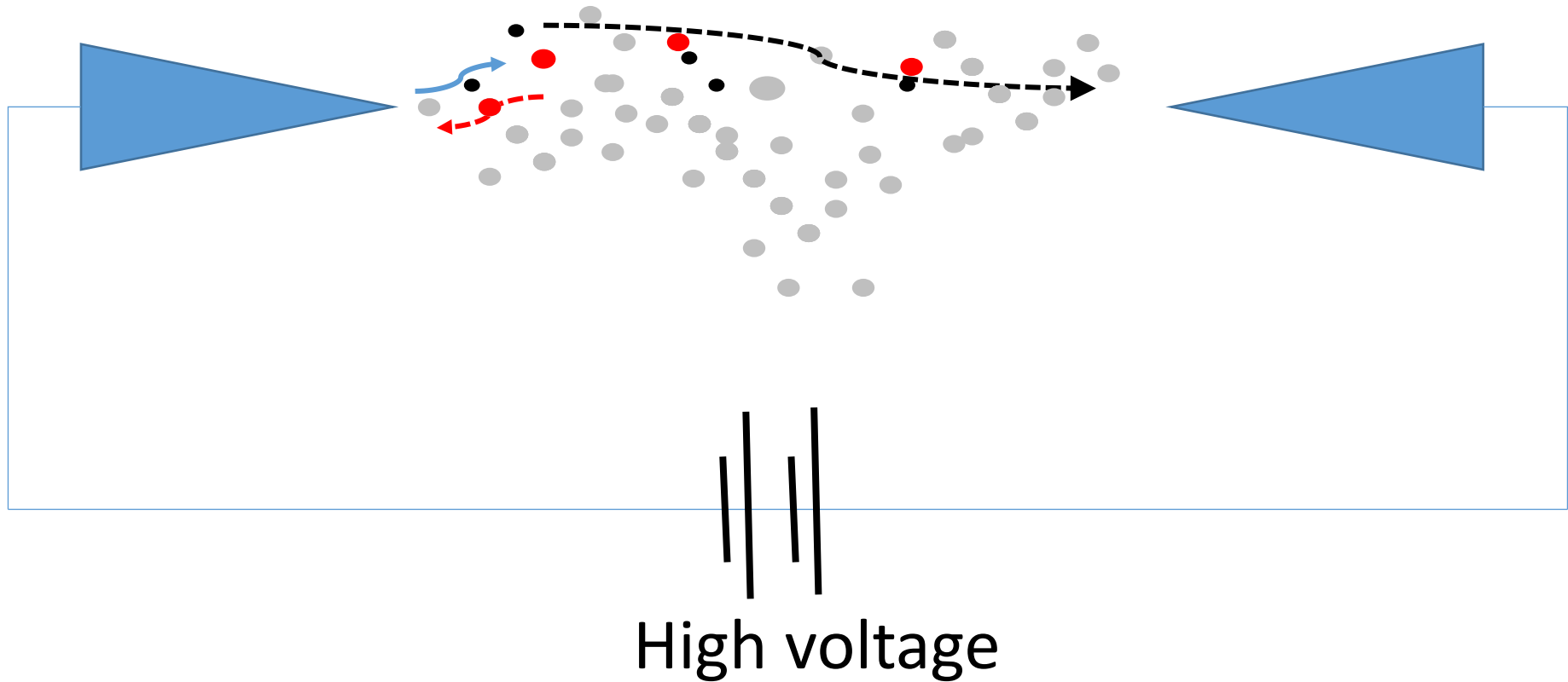
Corona discharge

Initial ionization by high energy particle (electron, VUV, ...)



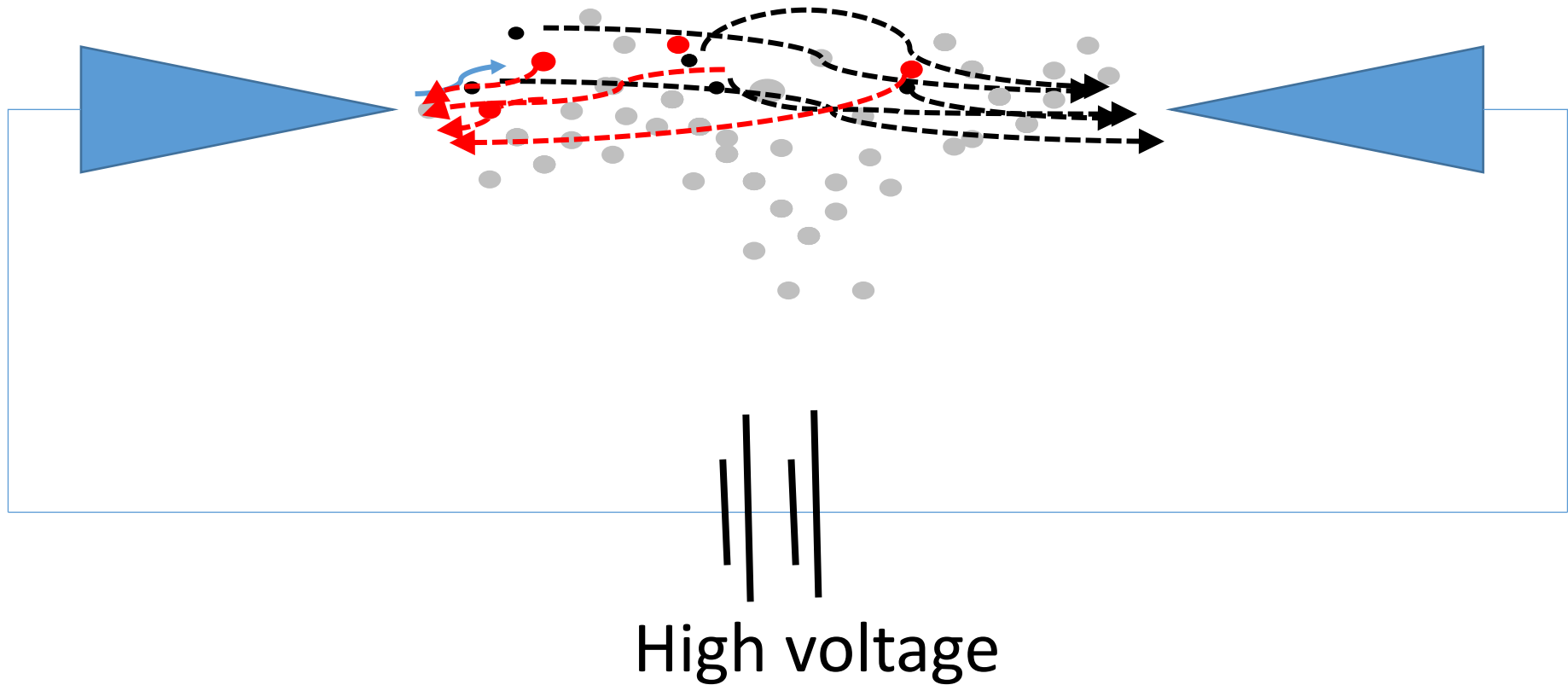
Corona discharge

Electrical breakdown



Corona discharge

Electrical breakdown



4. MALDI: Matrix-Assisted Laser Desorption Ionization



Koichi Tanaka's soft laser desorption method inspired the development of matrix assisted laser desorption ionization.

He shared the Nobel Prize in Chemistry in 2002 with John Fenn and Kurt Wuthrich.

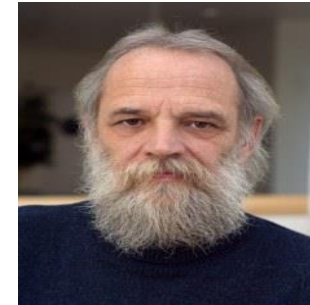
History of MALDI

- 1985

*Matrix-assisted desorption ionization (MALDI)
at Universitat Munster, Germany*

*Influence of the Wavelength in High-Irradiance
Ultraviolet Laser Desorption Mass Spectrometry of
Organic Molecules*

*M. Karas, et al and F. Hillenkamp;
Anal. Chem. 1985, 57, p2935*



- 1988

*Application of MALDI mass spectrometric analysis
to biological macromolecules*

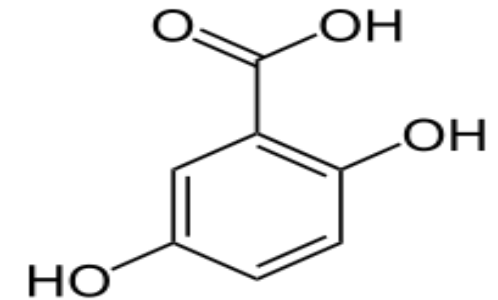
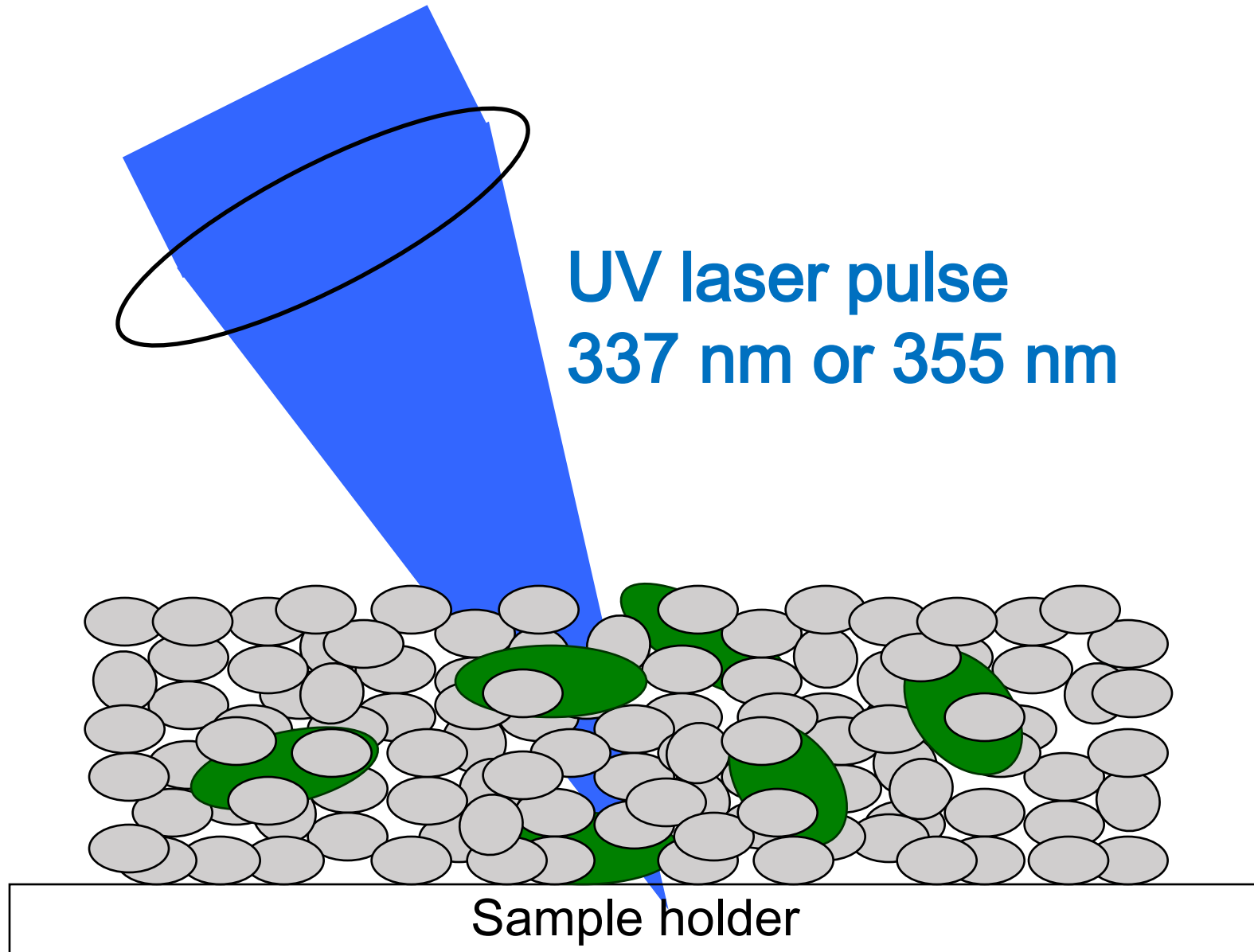
*Protein and polymer analyses up to m/z 100000 by
laser ionization time-of-flight mass spectrometry*

*K. Tanaka, et al;
Rapid Commun. Mass Spectrom. 1988, 2, p151*



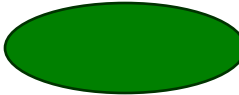
2002 Nobel Prize for Chemistry

Introduction of MALDI

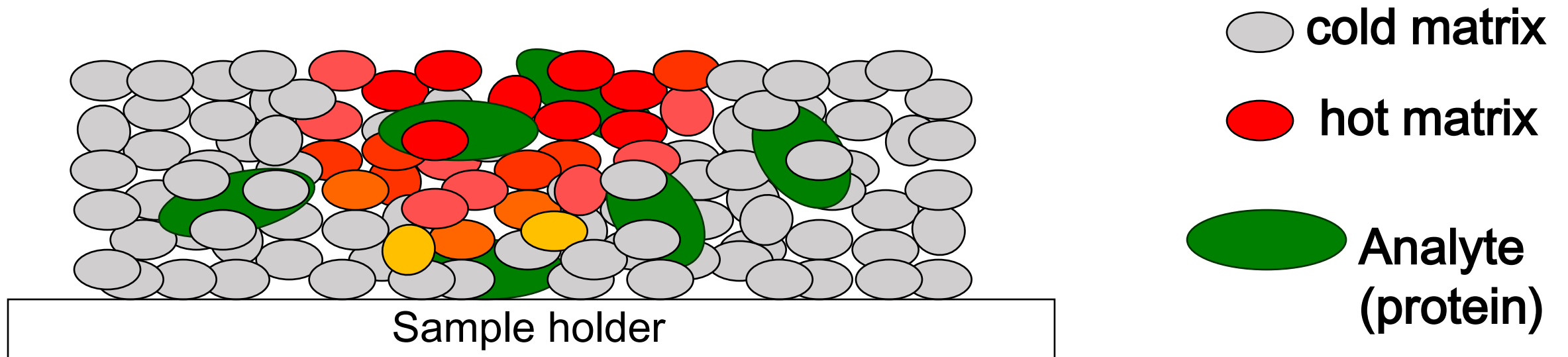


25DHB

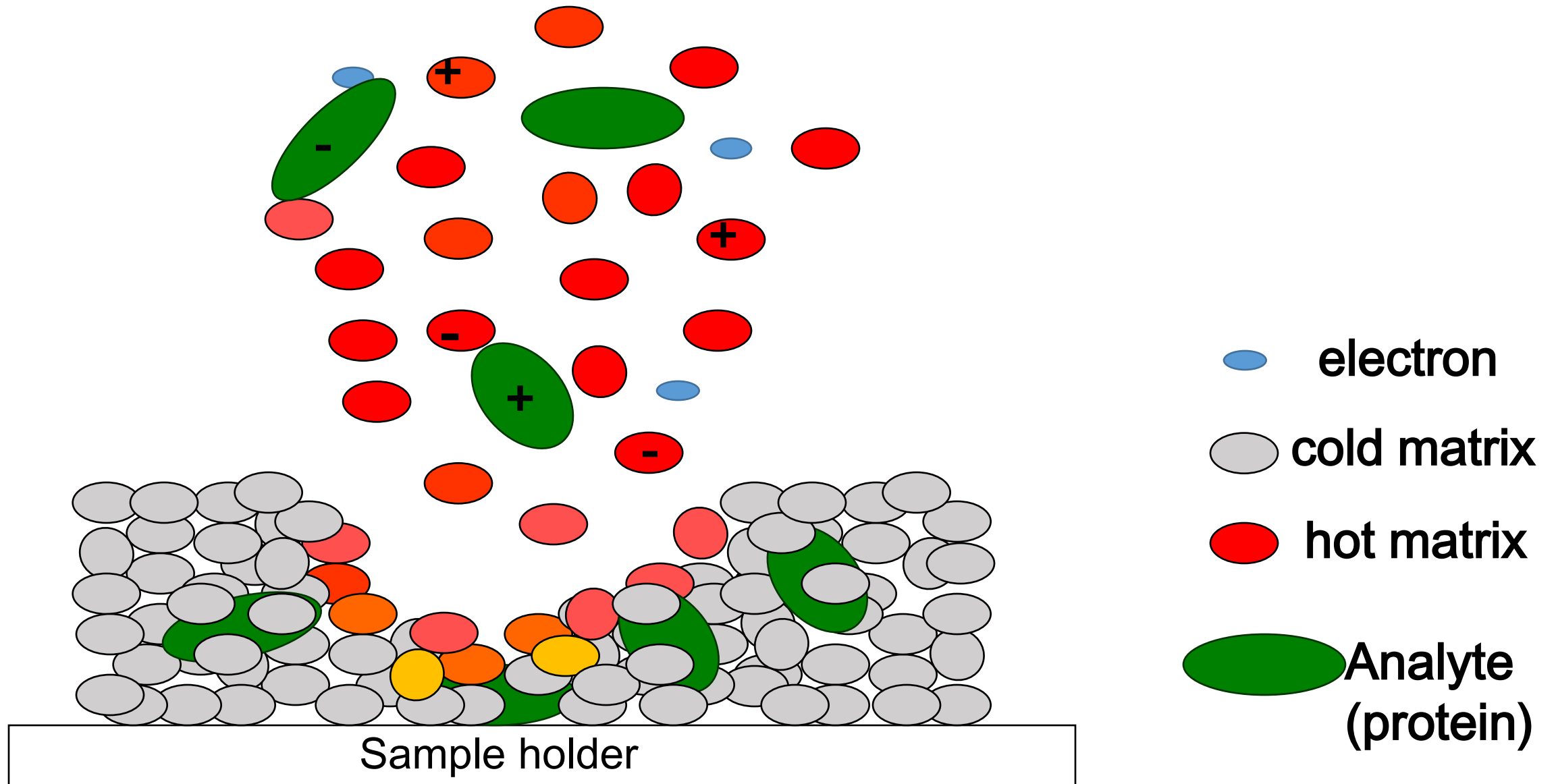
 matrix

 Analyte
(protein)

Introduction of MALDI



Introduction of MALDI



Advantages MALDI

1. High sensitivity
2. Easy to use

Disadvantages MALDI

1. Large signal fluctuation
2. Not suitable for quantitative analysis
3. Choice of matrix is trial and error
4. Low sensitivity for carbohydrates

Ionization mechanism of MALDI

	Matrix	Analyte
Molar ratio	100~10000	1

$$I/N \text{ (matrix)} = \frac{\text{Desorbed Matrix Ions}}{\text{Desorbed Matrix Neutrals}} = 10^{-5} \sim 10^{-9}$$

$$I/N \text{ (analyte)} = \frac{\text{Desorbed Analyte Ions}}{\text{Desorbed Analyte Neutrals}} = 10^{-2} \sim 10^{-9}$$

Ion-to-neutral ratios measured in previous studies

Ref	laser	Ion-to-neutral ratio
1	308 nm	2.5×10^{-4} for analyte near threshold (300 J/m ²)
2	248 nm	$10^{-4} \sim 10^{-5}$ for analyte near threshold, Ratio for matrix is smaller than analyte (matrix: analyte = 9:1 or 1:1)
3	355 nm	10^{-7} (100 J/m ²) for matrix, 5×10^{-6} (400 J/m ²) for matrix
4	193 nm	1.4×10^{-4} (~230 J/m ²) for matrix (1cm x 2cm spot size)
5	337 nm	No report

1. W. Ens, Y. Mao, F. Mayer, K. G. Standing. *Rapid Commun. Mass Spectrom.* 1991, 5, 117.
2. C. D. Mowry, M. V. Johnston. *Rapid Commun. Mass Spectrom.* 1993, 7, 569.
3. P. Quist, T. Huth-Fehre, B. U. R. Sundqvist. *Rapid Commun. Mass Spectrom.* 1994, 8, 149.
4. A. A. Puretzky, D. B. Geohegan. *Chem. Phys. Lett.* 1998, 286, 425.
5. K. Dreisewerd, M. Schürenberg, M. Karas, F. Hillenkamp. *Int. J. Mass Spectrom. Ion Proc.* 1995, 141, 127.

Mechanisms proposed for the primary ion generation

1. Direct multi-photon ionization (Hillenkam)
2. Excited state proton transfer
3. Polar fluid model (Chait and Beavis)
4. Pneumatic assistance
5. Thermal ionization of photoexcited matrix (Allwood, Dyer, Dreyfus)
6. Energy pooling (Sundqvist, and Knochenmuss)
7. Preformed ions (Karas)
8. Solid state photoexcited model (Lia, Lin, Lee, and Wang)

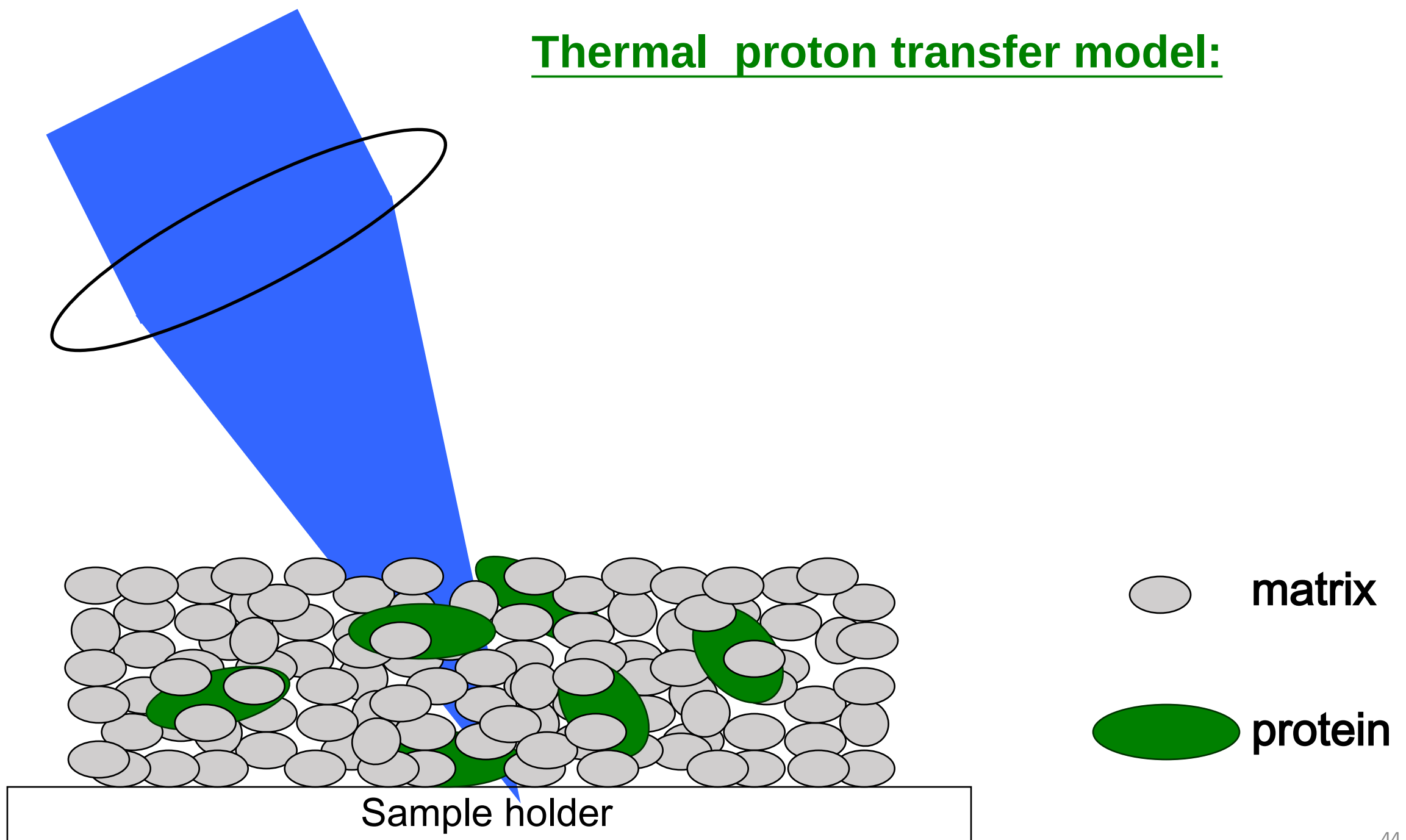
High ion-to-neutral ratio of analyte (which is 10^{-2} - 10^{-4}) was mistakenly used as ion-to-neutral ratio of matrix (which should be 10^{-5} - 10^{-9}) in all the aforementioned theoretical models in the past 25 years.

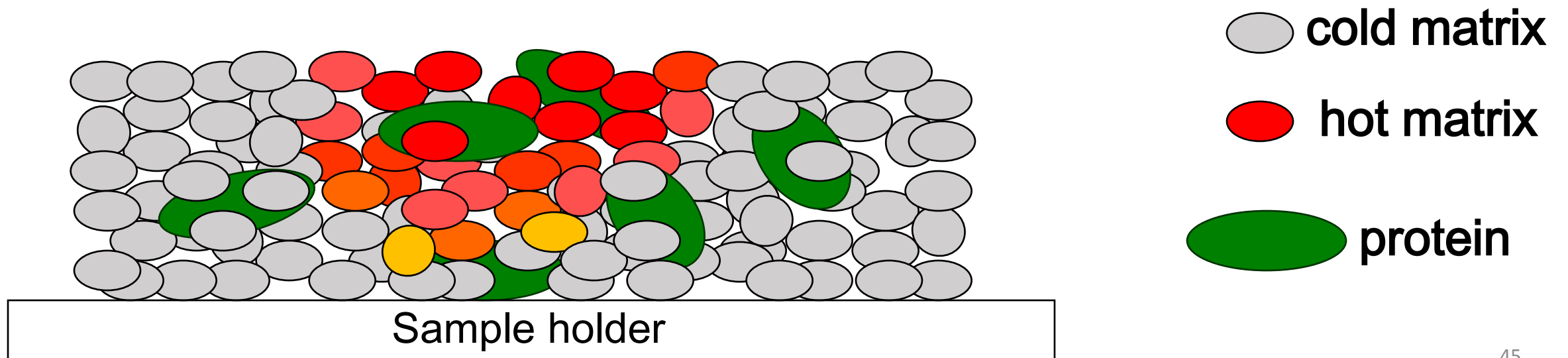
Mechanisms proposed for the primary ion generation

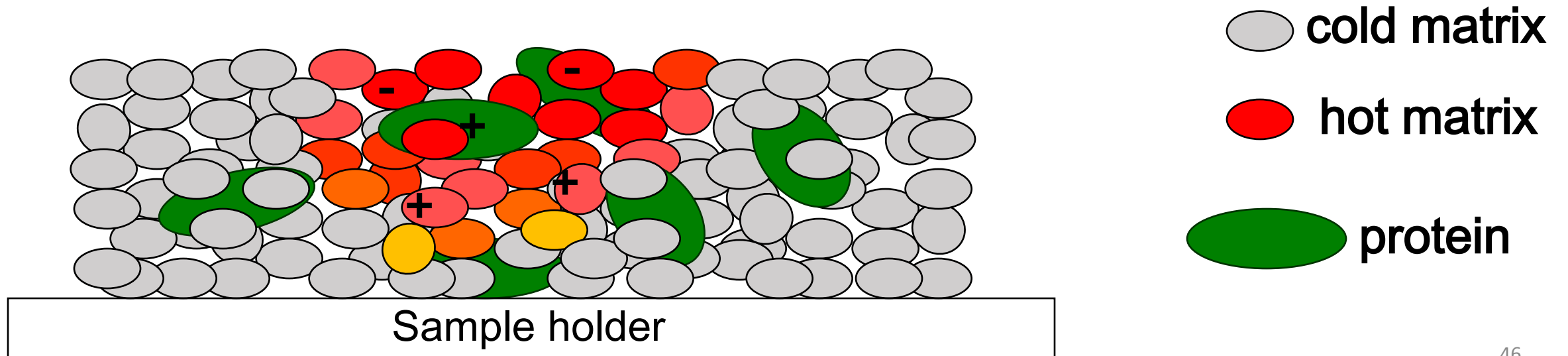
1. Direct multi-photon ionization (Hillenkam)
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3. Polar fluid model (Chait and Beavis)
4. Pneumatic assistance
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6. Energy pooling (Sundqvist, and Knochenmuss)
7. Preformed ions (Karas)
8. Solid state photoexcited model (Lia, Lin, Lee, and Wang)
9. Thermal proton transfer model (Lee and Ni/ 2014)

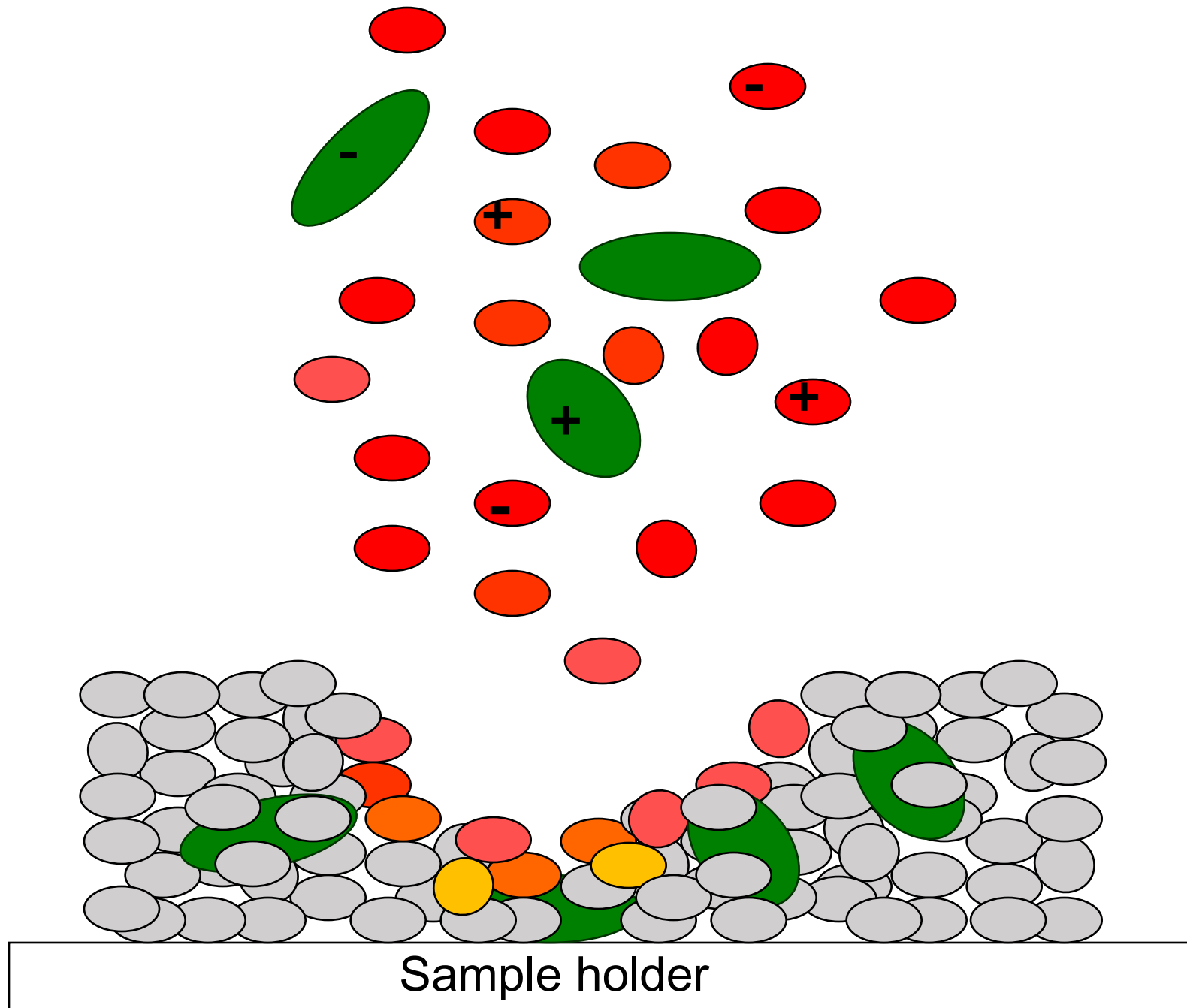
Our proposed model:
Thermal proton transfer model

Thermal proton transfer model:









**MALDI
Laser**

$M+Na^+$

$M+Cl^-$

$M+Cl^-$

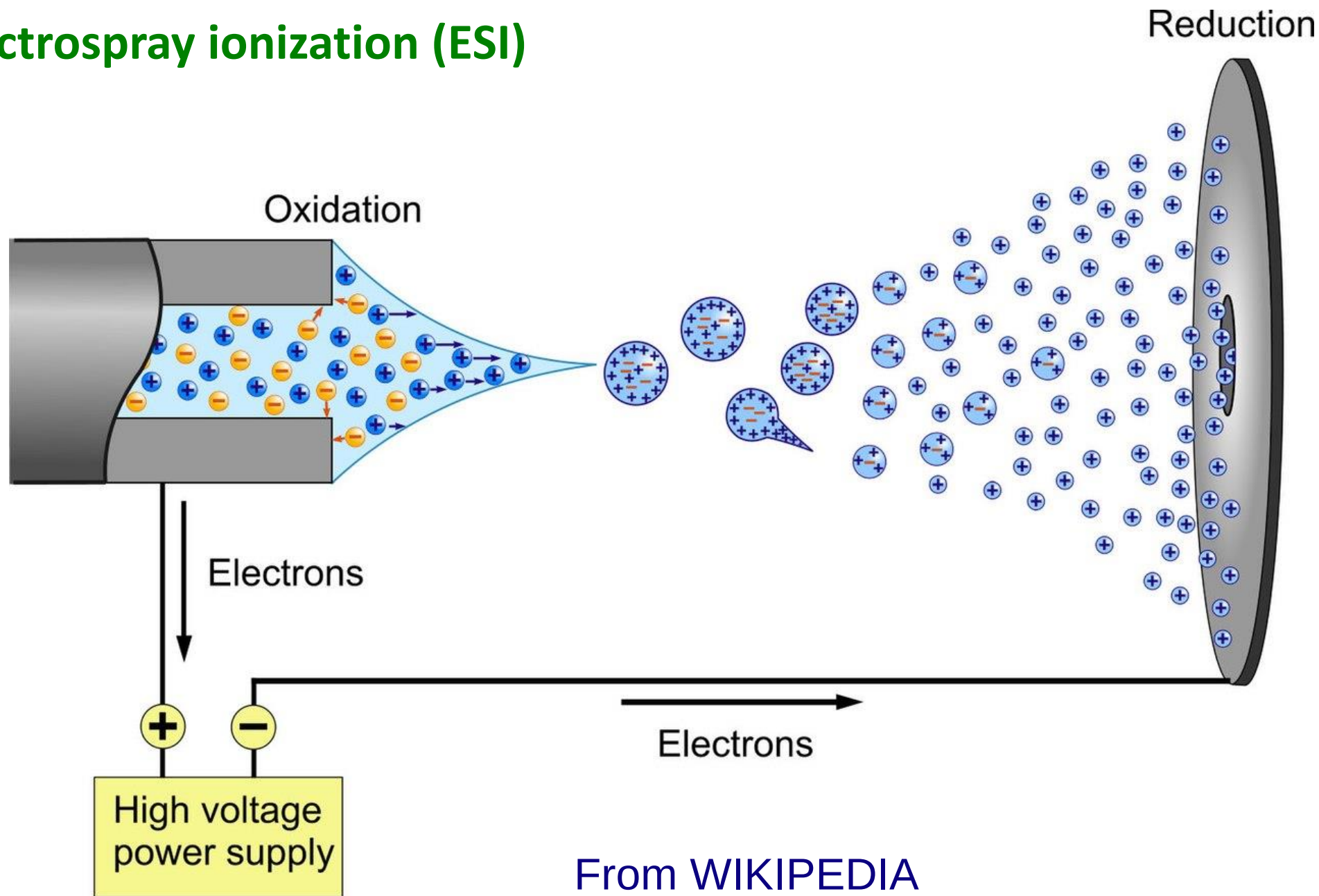
$M+Na^+$

Na^+ Cl^-

NaCl

Matrix

5. Electrospray ionization (ESI)

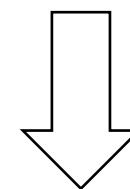
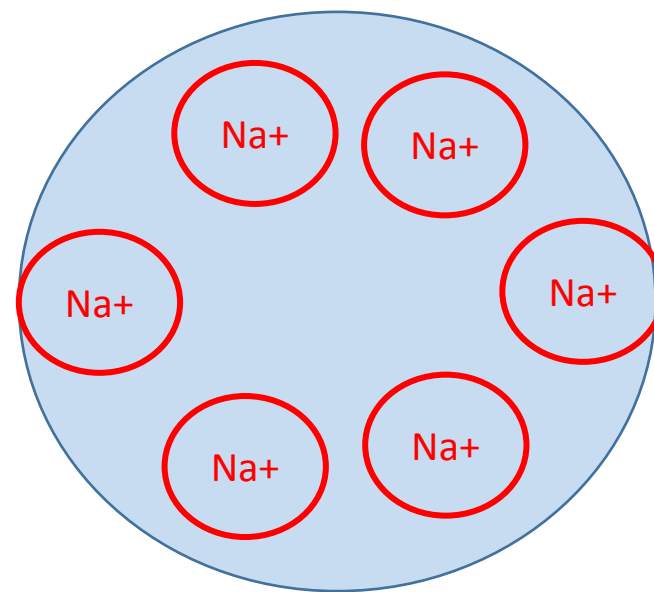
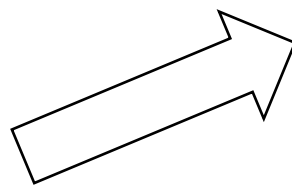
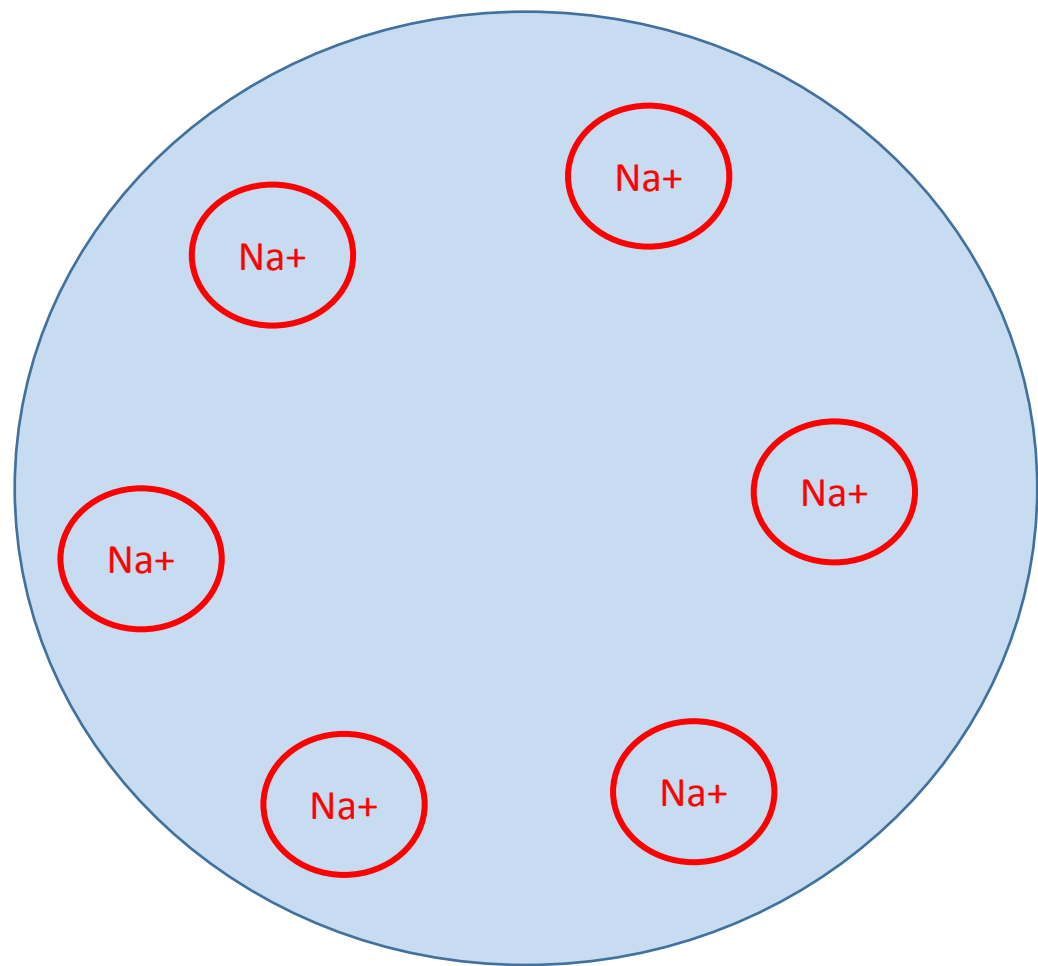


From WIKIPEDIA

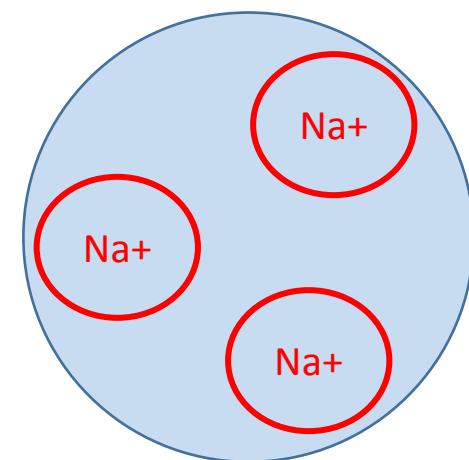
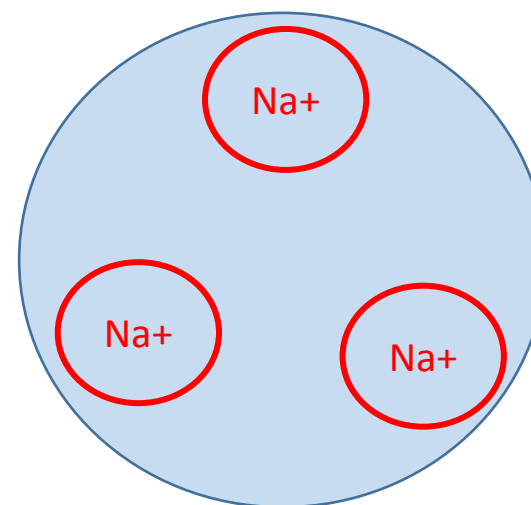
ESI mechanisms

From WIKIPEDIA

- Ion evaporation model (IEM): droplet reaches a certain radius the field strength at the surface of the droplet becomes large enough to assist the [field desorption](#) of solvated ions
- Charge residue model (CRM): electrospray droplets undergo evaporation and fission cycles, eventually leading progeny droplets that contain on average one analyte ion or less.
- A third model invoking combined charged residue-field emission has been proposed.



Coulomb explosion



ESI properties:

1. **Very little fragmentation**
2. **Diameter of emitter:** shift to higher charge when delivered by nanospray versus standard electrospray
3. **Spectrum:** H^+ , Na^+ , K^+
4. **Solvent:**
5. **Supercharging agents:**

The term “supercharging” describe the increased charging observed in spectra obtained from solvents supplemented with m-nitrobenzyl alcohol (m-NBA, 0–20%), glycerol (0–50%), dimethyl sulfoxide (DMSO, 0–50%), m-chlorophenol (0–40%), formamide (0–10%), and 2-methoxyethanol (0–25%).

From J. Am. Soc. Mass Spectrom. (2014) 25, 1675

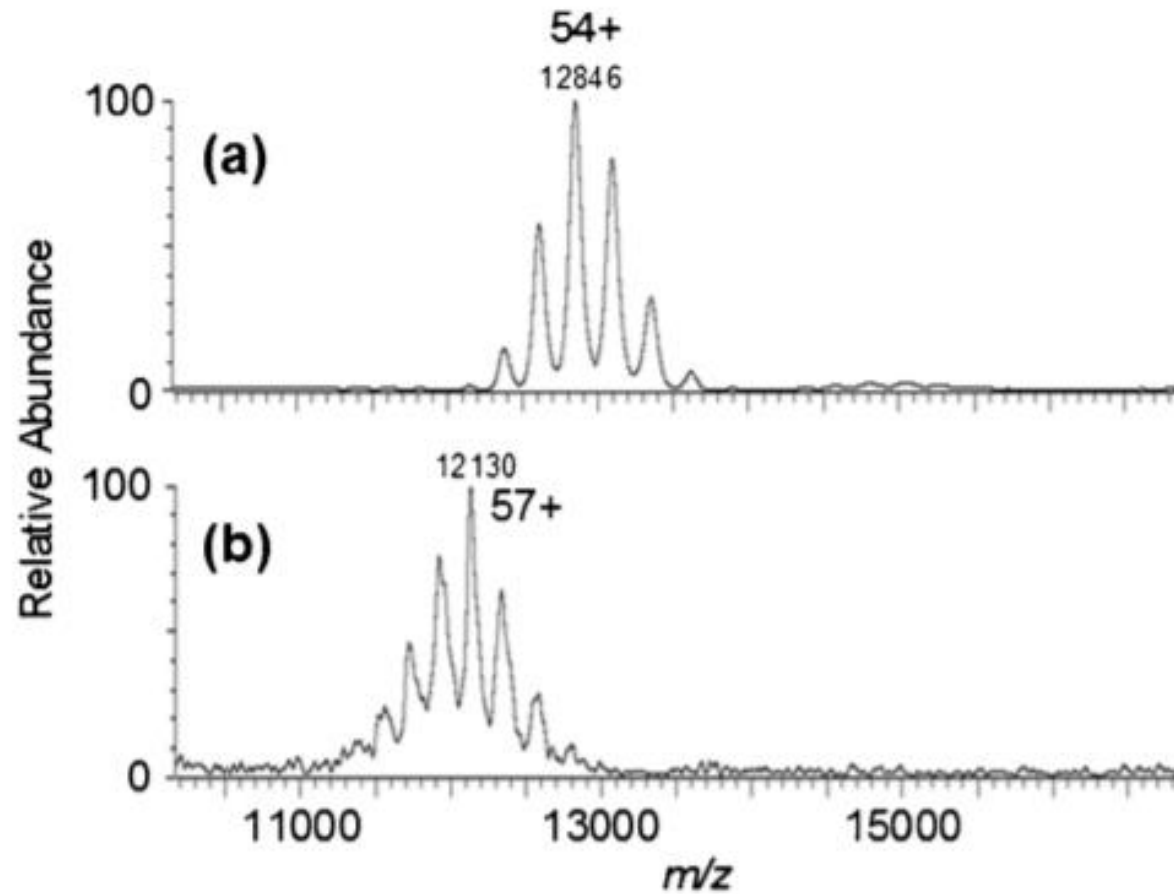
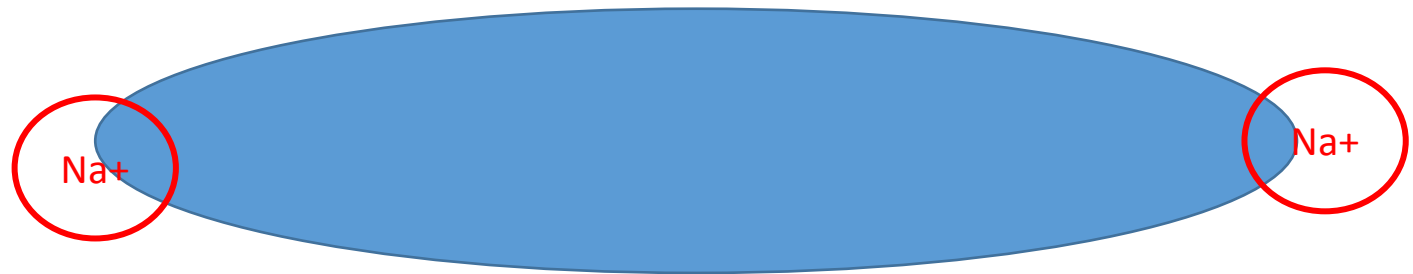
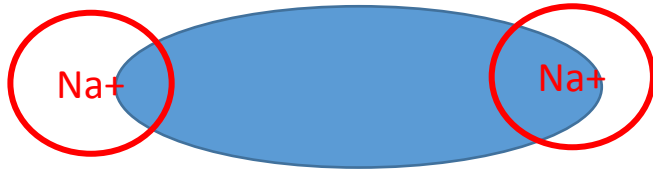


Figure 3. Subunit interactions are maintained, despite the increased charge borne by a 28-mer complex. ESI-MS of *Methanosarcina thermophila* 20S proteasome (690 kDa) (a) without, and (b) with 0.25% *m*-NBA. Reprinted Figure S1, with permission from Lomeli et al. [53]

How to generate multiple charge ion?



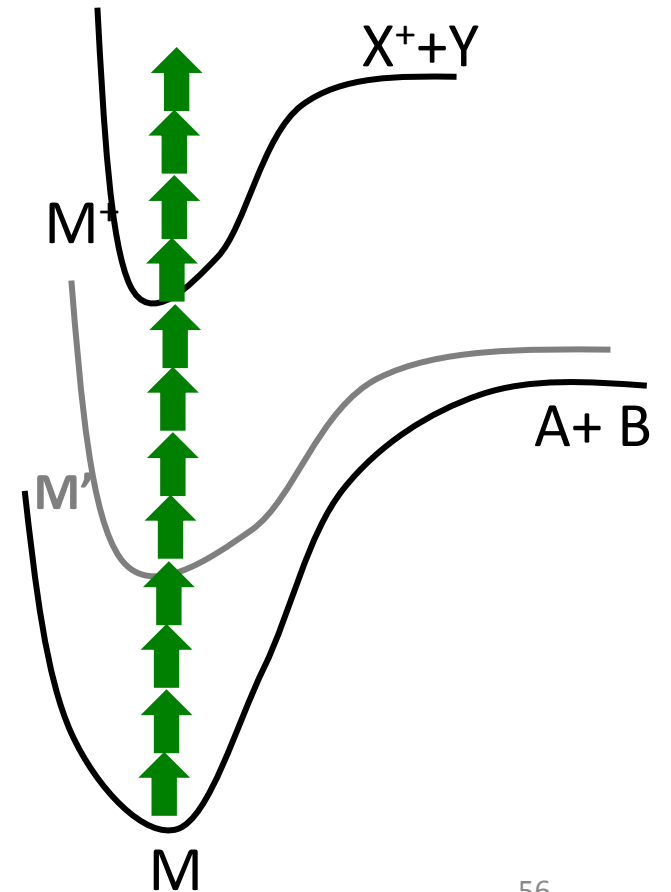
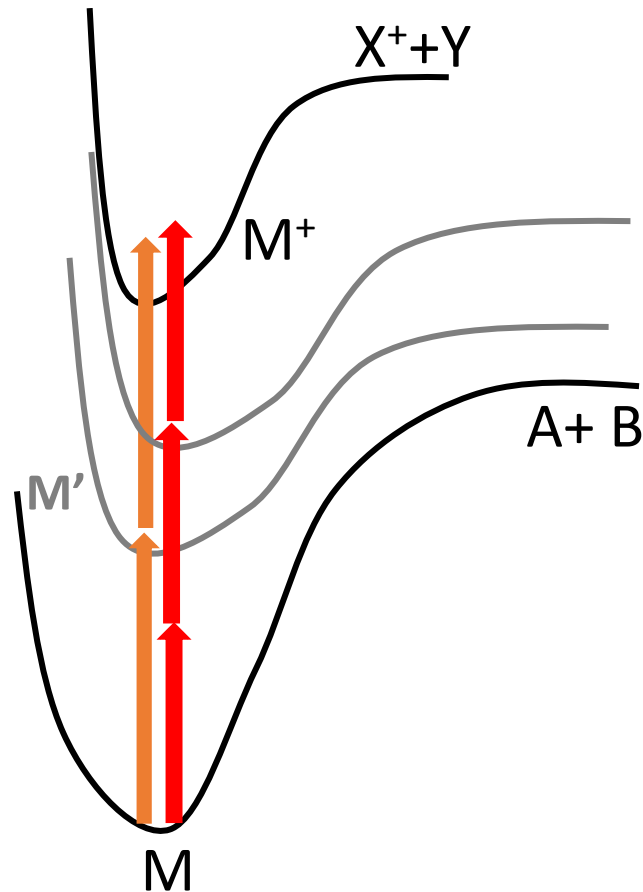
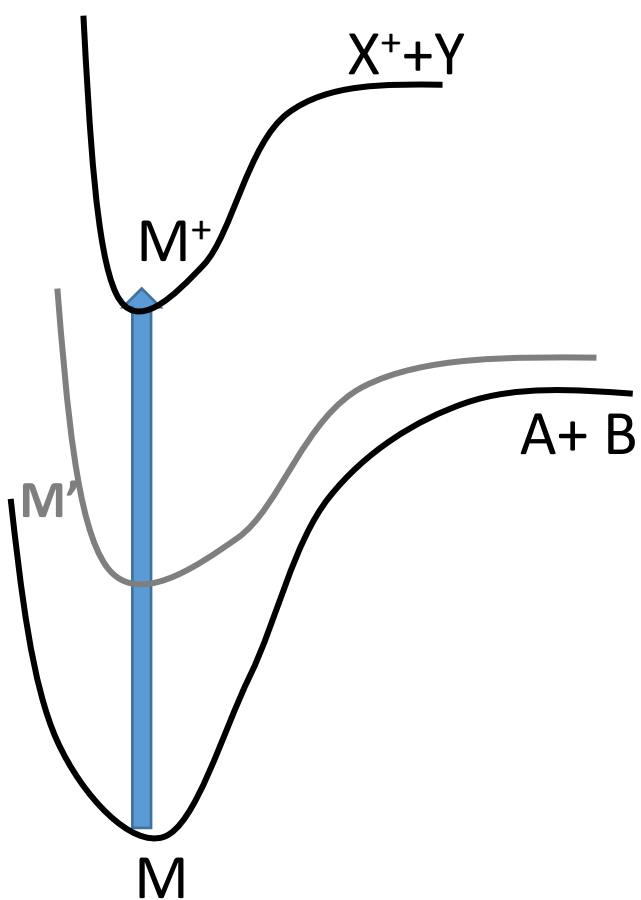
How to reduce fragmentation in ESI?

Reduced corona discharge

6. Multiphoton ionization (MPI)

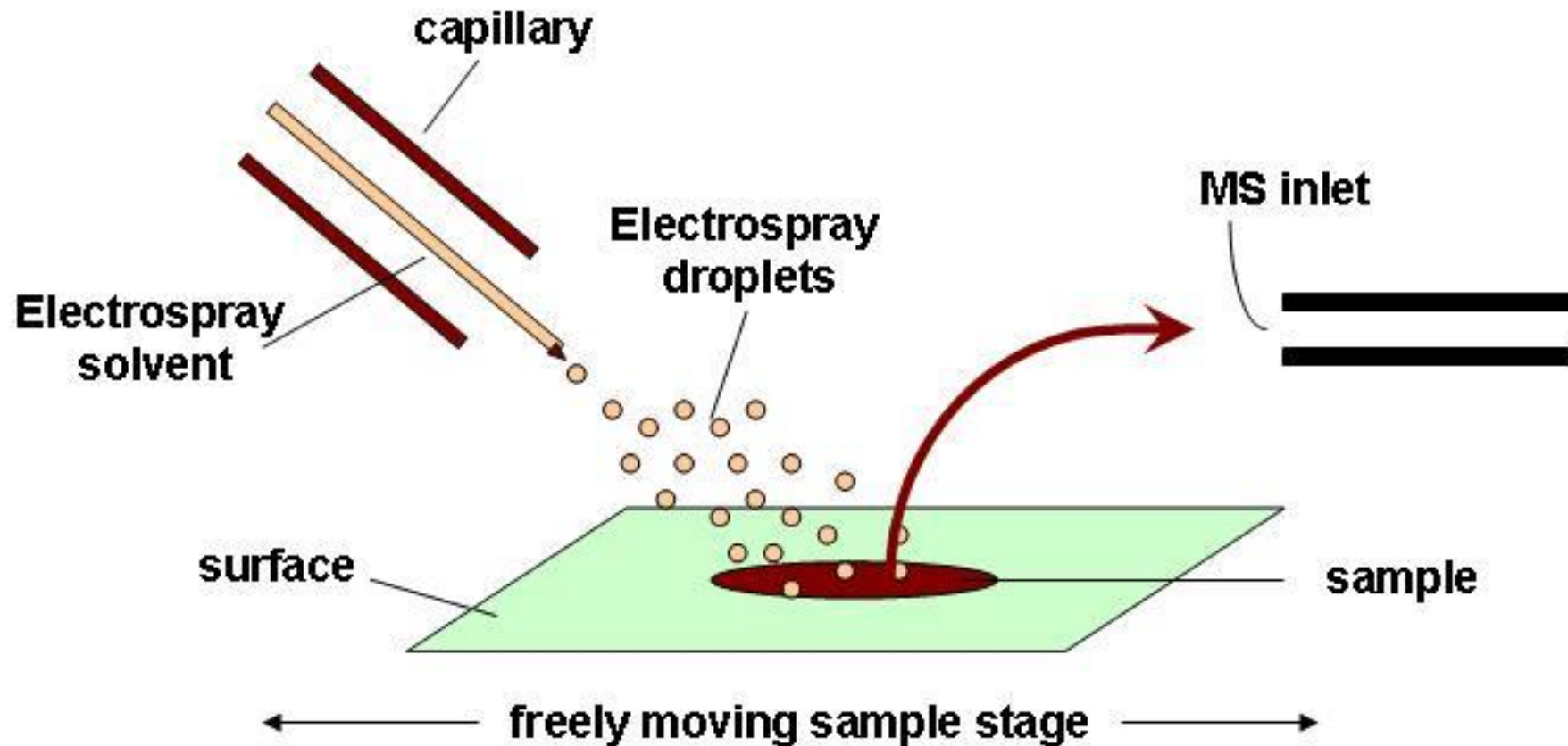
Resonance multiphoton ionization (REMPI)

Non-resonance multiphoton ionization



7. Ambient ionization

Desorption electrospray ionization (DESI) is one of the original ambient ionization sources.



Sensitivity:

Ionization efficiency and
collection efficiency (efficiency of ions into analyzer)

Mass Analyzers

1. Sector mass spectrometer
2. Time-of-flight mass spectrometer
3. Quadrupole mass spectrometer
4. Quadrupole ion trap mass spectrometer
5. Fourier-transform ion cyclotron resonance mass spectrometer
6. Orbit trap mass spectrometer

Charge particle motion: changed by electric and magnetic fields:

$$\mathbf{F} = q(\mathbf{\hat{E}} + \mathbf{V} \times \mathbf{B})$$

where

$\mathbf{F}$: force;

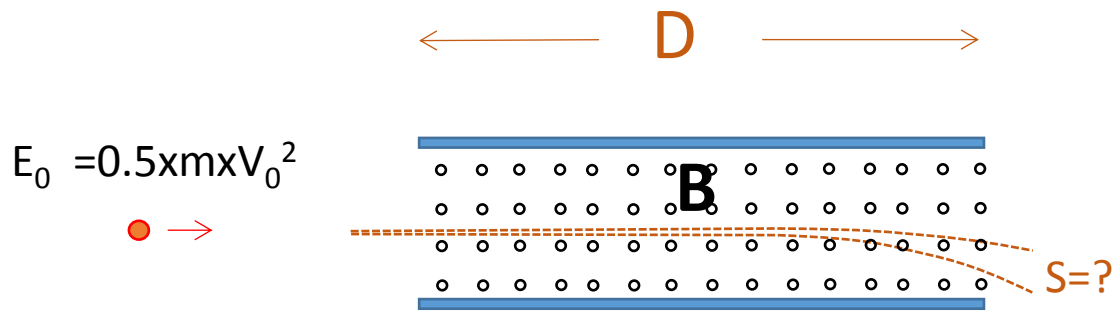
q : charge of particle;

$\mathbf{E}$: electric field;

$\mathbf{B}$: magnetic field;

$\mathbf{V}$: velocity of charge particle

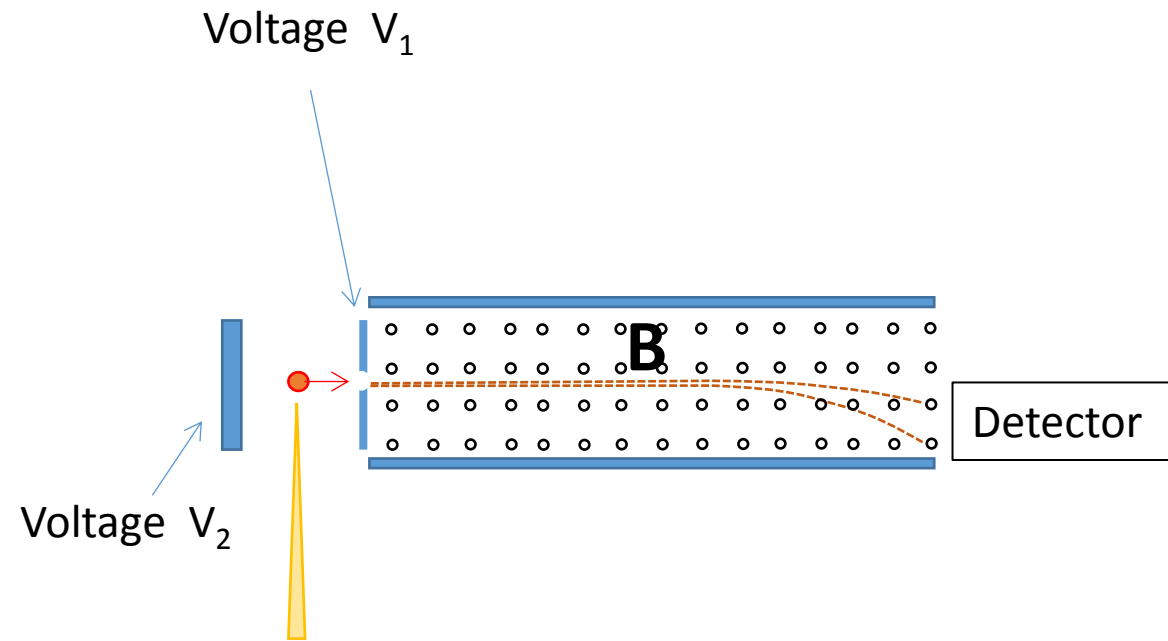
Magnetic and electric sector mass spectrometer



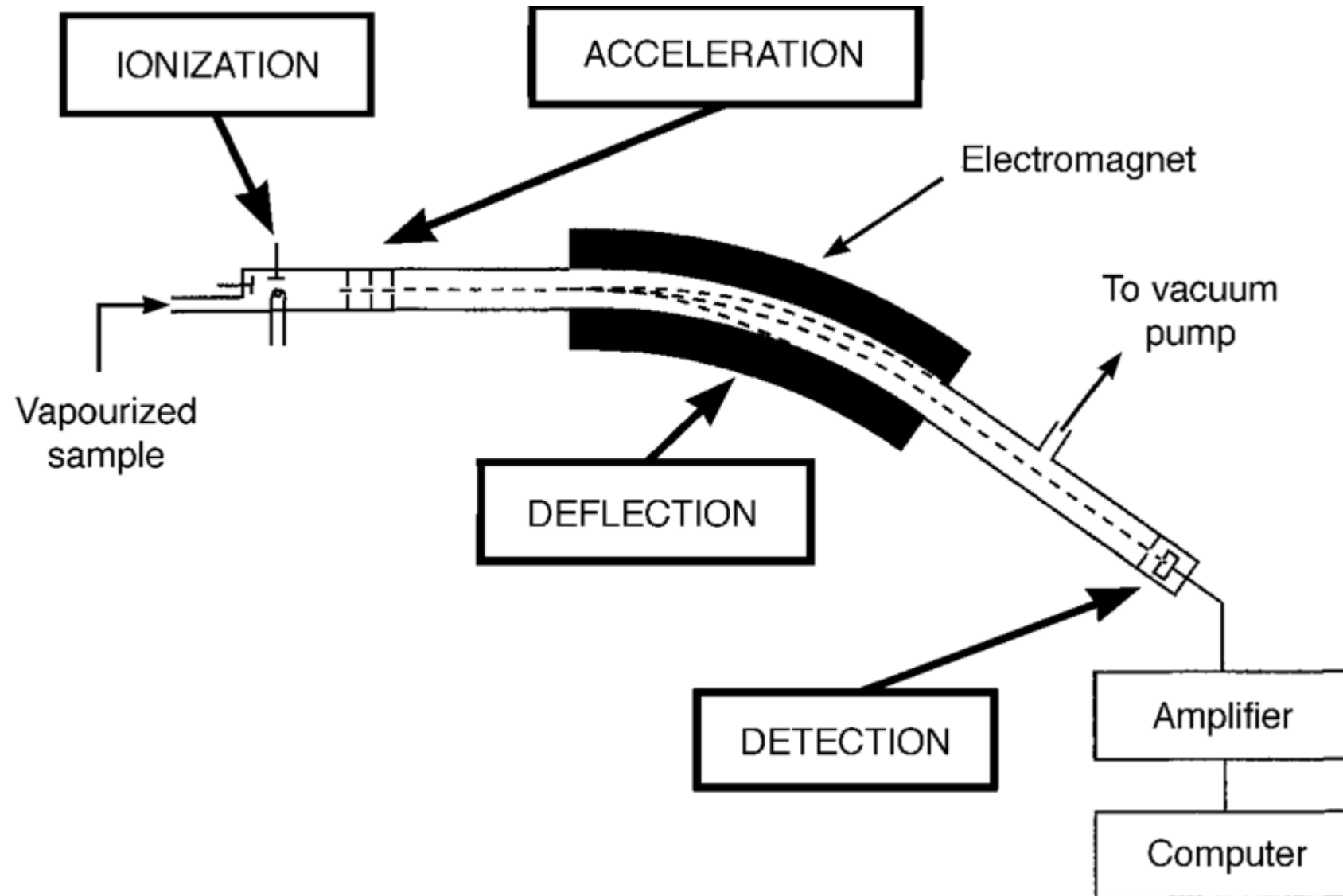
Distance deviated by magnetic field

$$\begin{aligned}
 S &= V_0 t + 0.5 a t^2 \\
 &= V_0 (D/V_0) + 0.5 (F/m) (D/V_0)^2 \\
 &= D + 0.5 (q V_0 B / m) (D/V_0)^2 \\
 &= D + 0.5 q B D^2 / (m V_0) \\
 &= D + 0.5 q \hat{E} D^2 / \sqrt{2 m E_0}
 \end{aligned}$$

If initial kinetic energy is the same, the distance deviated by magnetic field **depends** on mass of charge particle. For large mass, the distance is small.



$$E_0 = 0.5qx(V_2 - V_1), \text{ does not depend on mass}$$



Example:

A typical magnetic sector instrument operated with an accelerating voltage of 2500 V, a magnetic field of 0.4 Tesla

For an singly charged ion, $q = 1.6 \times 10^{-19} \text{ C}$ and $m = 100 \text{ Da}$

Assume $m = 100 \times 10^{-3} / (6 \times 10^{23}) \text{ kg}$, kinetic energy = 2500 eV

$$2500 \text{ eV} \times 1.6 \times 10^{-19} \text{ J/eV} = 0.5 \times 100 \times 10^{-3} \text{ kg} / (6.02 \times 10^{23}) \times V^2$$
$$V = 70000 \text{ m/s}$$

$$F = 1.6 \times 10^{-19} \text{ C} \times 70000 \text{ m/s} \times 0.4 \text{ tesla} = 4.5 \times 10^{-15} \text{ N}$$

$$\text{Flight distance } 1 \text{ m, time } t = 1/70000 = 1.4 \times 10^{-5} \text{ s}$$

$$S = V_0 \times t + 0.5 \times a \times t^2$$
$$= 0.5 \times 4.5 \times 10^{-15} \text{ N} / [100 \times 10^{-3} / (6 \times 10^{23})] \times (1.4 \times 10^{-5})^2$$
$$= 2.75 \text{ m}$$

Effect by earth magnetic field ($20\text{-}60 \times 10^{-6} \text{ Tesla}$)

$$\Delta S = 0.027 \text{ cm for } 40 \times 10^{-6} \text{ Tesla}$$

Example:

A typical magnetic sector instrument operated with an accelerating voltage of V, a magnetic field of 0.4 Tesla

For an singly charged ion, $q = 1.6 \times 10^{-19} \text{ C}$

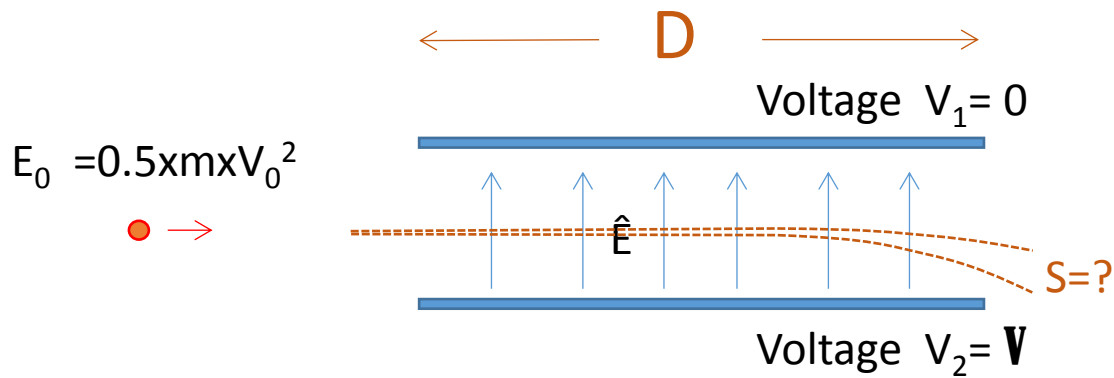
$$S = q \times B \times D^2 / [2 \times \sqrt{2 \times m \times E}]$$

where q: charge, B magnetic field, m: mass, E: kinetic energy

$$\Delta S (m+1, m) = 27.5 \times [1/\sqrt{m} - 1/\sqrt{m+1}] \text{ (m in unit of Da)}$$

For $m_1 = 100$, $m_2 = 100.1$, $\Delta S = 0.13 \text{ cm}$

Separation can be increased by: reducing E, increase B and D



Distance deviated by electric field

$$\begin{aligned}
 S &= V_0 t + 0.5 a t^2 \\
 &= V_0 (D/V_0) + 0.5 (F/m) (D/V_0)^2 \\
 &= D + 0.5 (q \hat{E} / m) (D/V_0)^2 \\
 &= D + 0.5 q \hat{E} D^2 / (m V_0^2) \\
 &= D + 0.5 q \hat{E} D^2 / (2 E_0)
 \end{aligned}$$

If initial kinetic energy is the same, the distance deviated by electric field is the same. The deviated distance **does not** depend on mass of charge particle, only depends on initial kinetic energy.

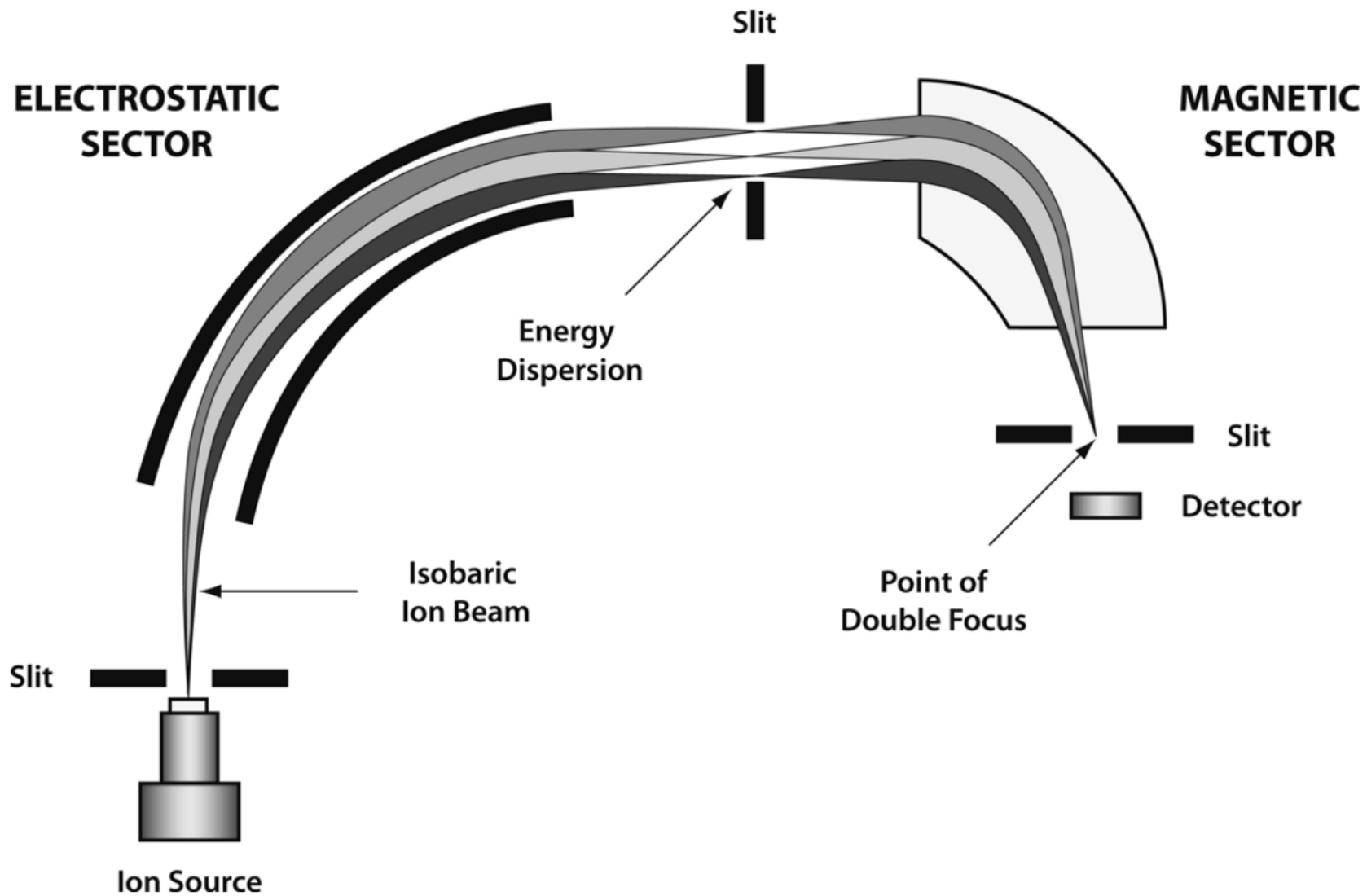


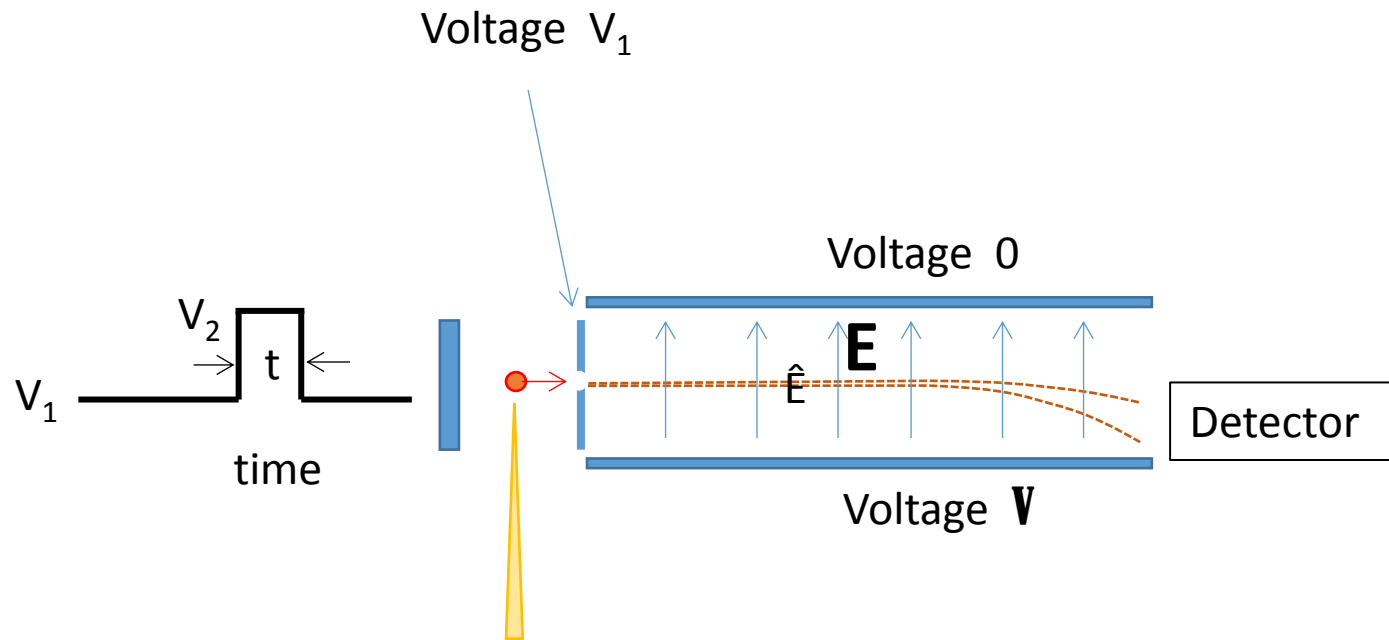
Figure 1.7 The operating principles of a double focusing sector mass analyzer.

Charge particle with the same kinetic energy:

For different masses, the trajectories are the same in electric field,
but not the same in magnetic field

Charge particle with the same momentum:

For different masses, the trajectories are different in electric field,
but are the same in magnetic field



$$mxV_0 = Fxt = qxEx t = \text{const}$$

$$E_0 = 0.5x(\text{const})^2/m$$

Distance deviated by electric field

$$S = V_0 t + 0.5 a t^2$$

$$= V_0 (D/V_0) + 0.5 x (F/m) x (D/V_0)^2$$

$$= D + 0.5 x [qx\hat{E}/m] x (D/V_0)^2$$

$$= D + 0.5 x qx\hat{E} x D^2 / (mxV_0)^2$$

$$= D + 0.5 qx\hat{E} x D^2 / (2E_0)$$

$$= D + 0.5 qx\hat{E} x D^2 x m / (\text{const})^2$$

Applying a constant momentum to charge particles,
distance deviated by electric field depends on mass

Constant momentum mass spectrometer in IAMS, Academia Sinica

