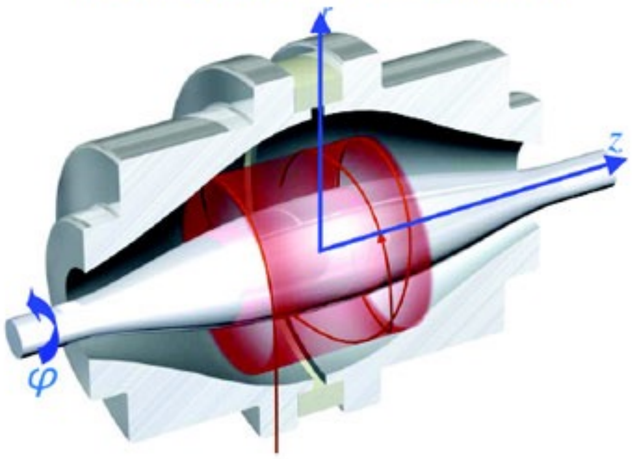
Orbitrap mass spectrometer

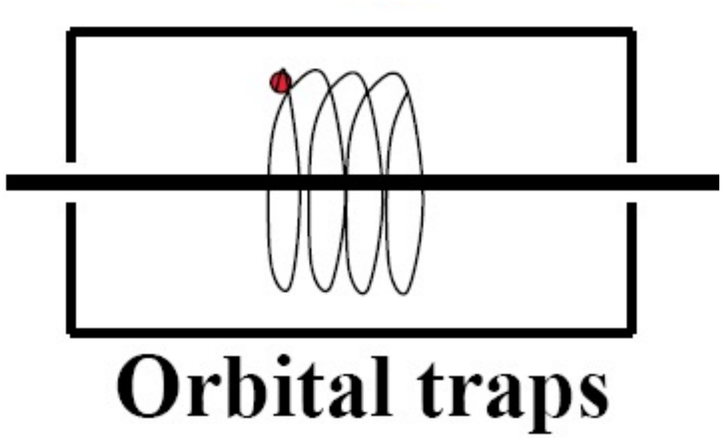
The only new mass spectrometer concept to be developed in the last 30 years

The only commercial instrument that can come close to the performance of an FTICR



Orbitrap: Principle of ion trapping

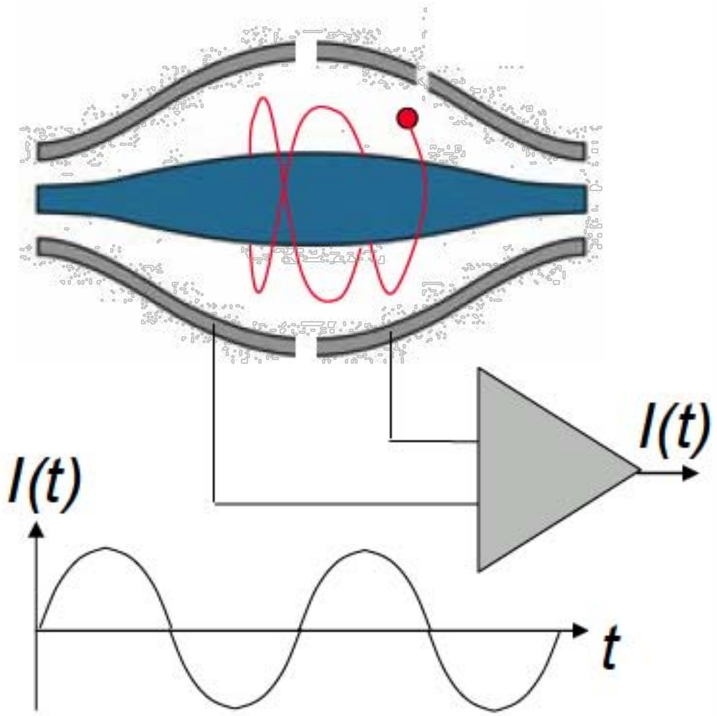
A satellite is trapped in orbit as the gravitational force compensates the centrifugal force



An ion can be trapped in orbit around a wire as the electrostatic potential compensates the centrifugal force

What Could We Do With Ions In The orbitrap?

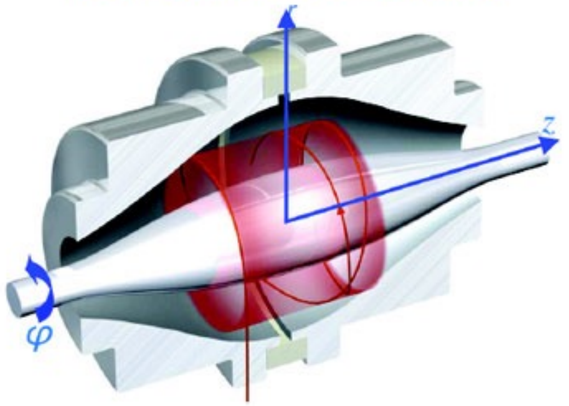
Image current detection



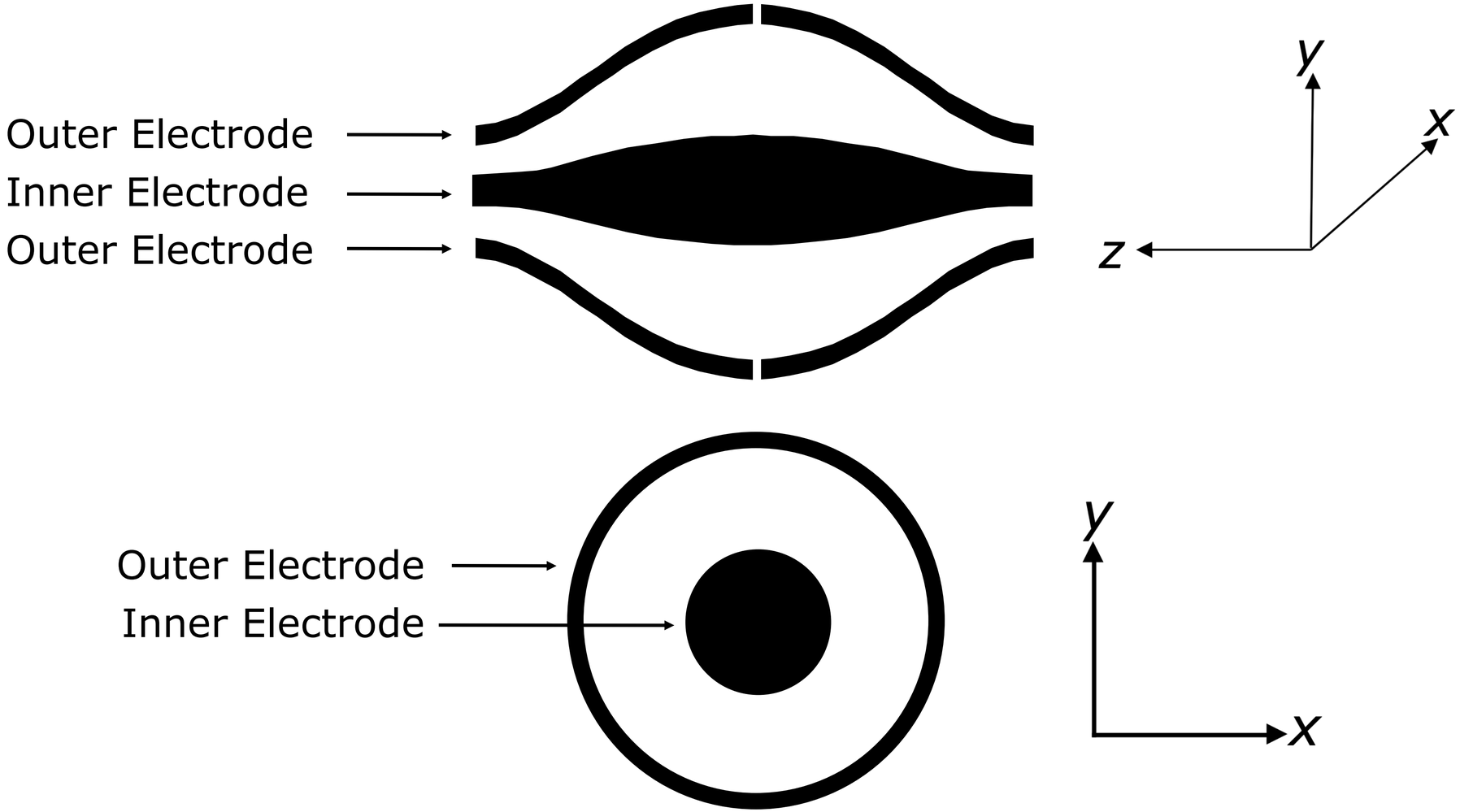
Notes:
-All-mass detection
-Noise equiv. to 20 ions (1 sec)

When the size does matter !

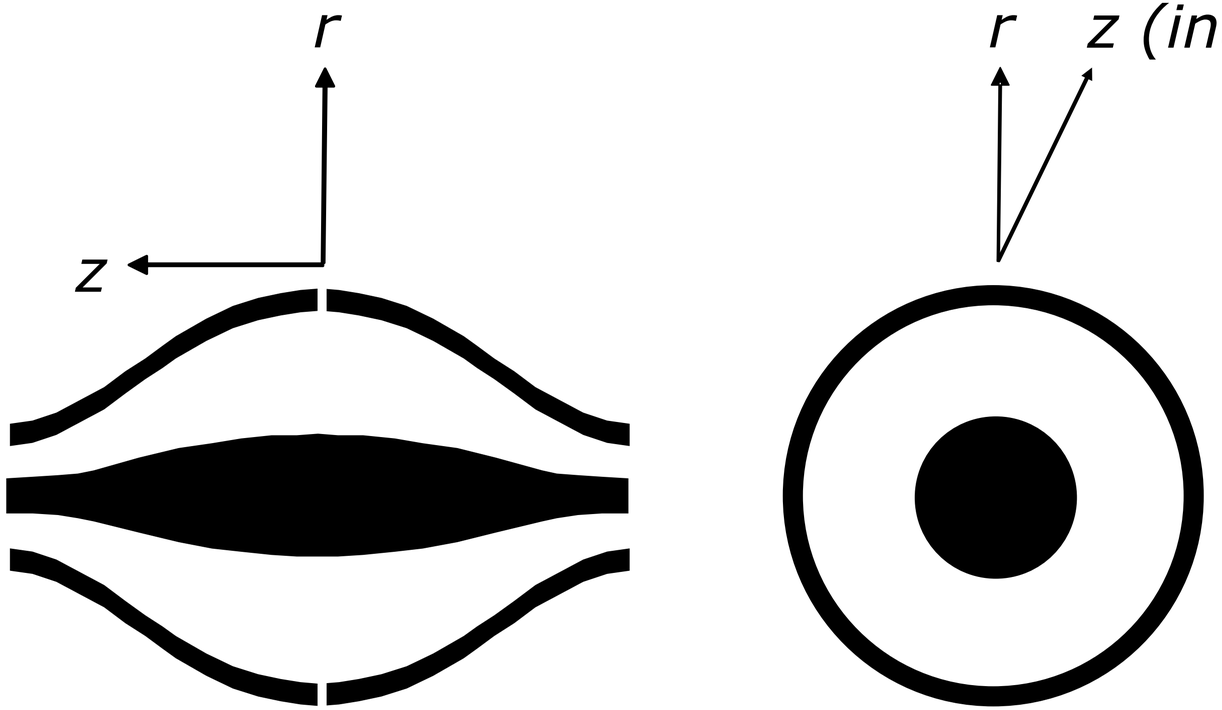
First published theory: 1981;
First instrument: 2006.

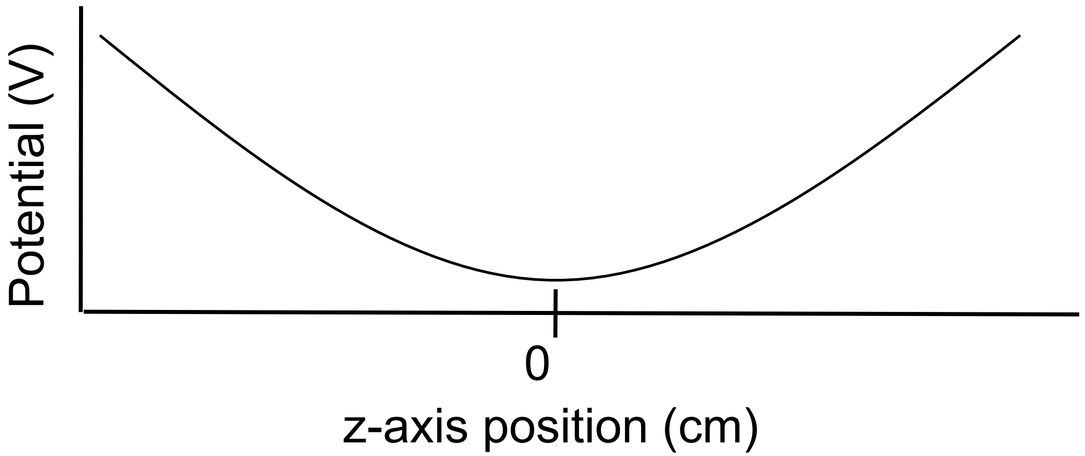


Orbitrap


Outer Electrode
Inner Electrode
Outer Electrode
y
x
z
Outer Electrode
Inner Electrode
y
x

Electrostatic field in the orbitrap

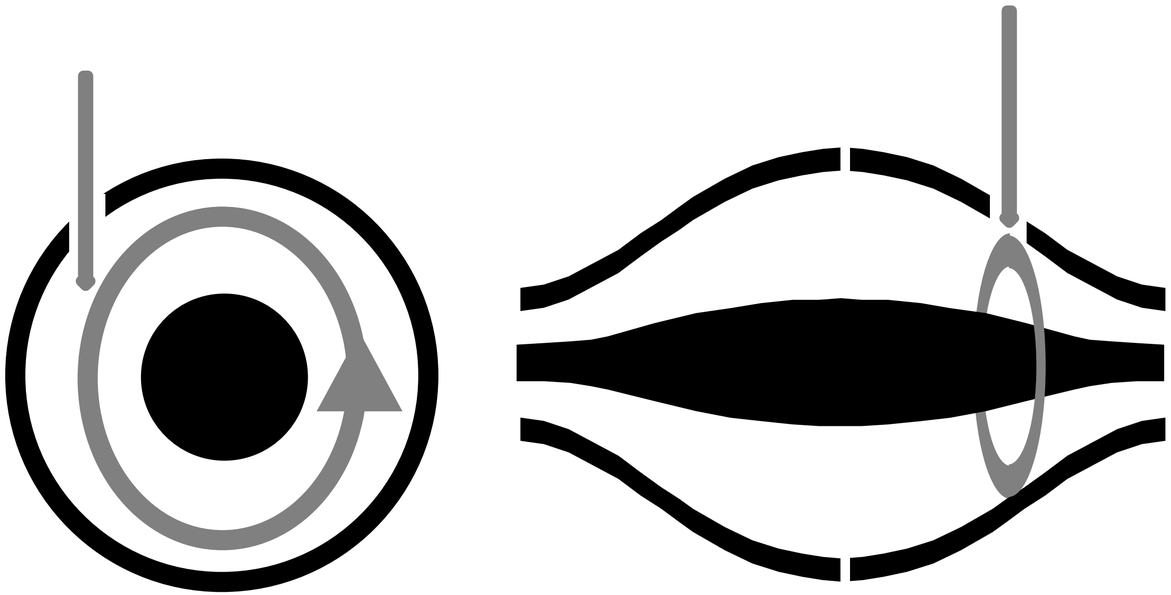


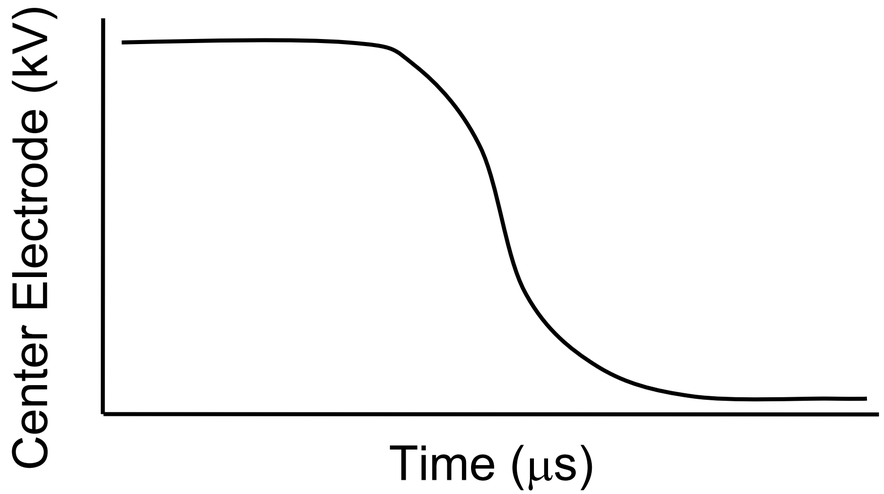


$$E(r,z) = \frac{k_0}{2}\left[z^2 - \frac{r^2}{2} + R_m^2 \ln\left(\frac{r}{R_m}\right)\right] + C$$

- E, electrostatic potential distribution
- r, z, cylindrical coordinates
- k_o, field curvature constant;
- R_m, characteristic radius (trapped ions must have $r < R_m$)
- C, constant potential offset

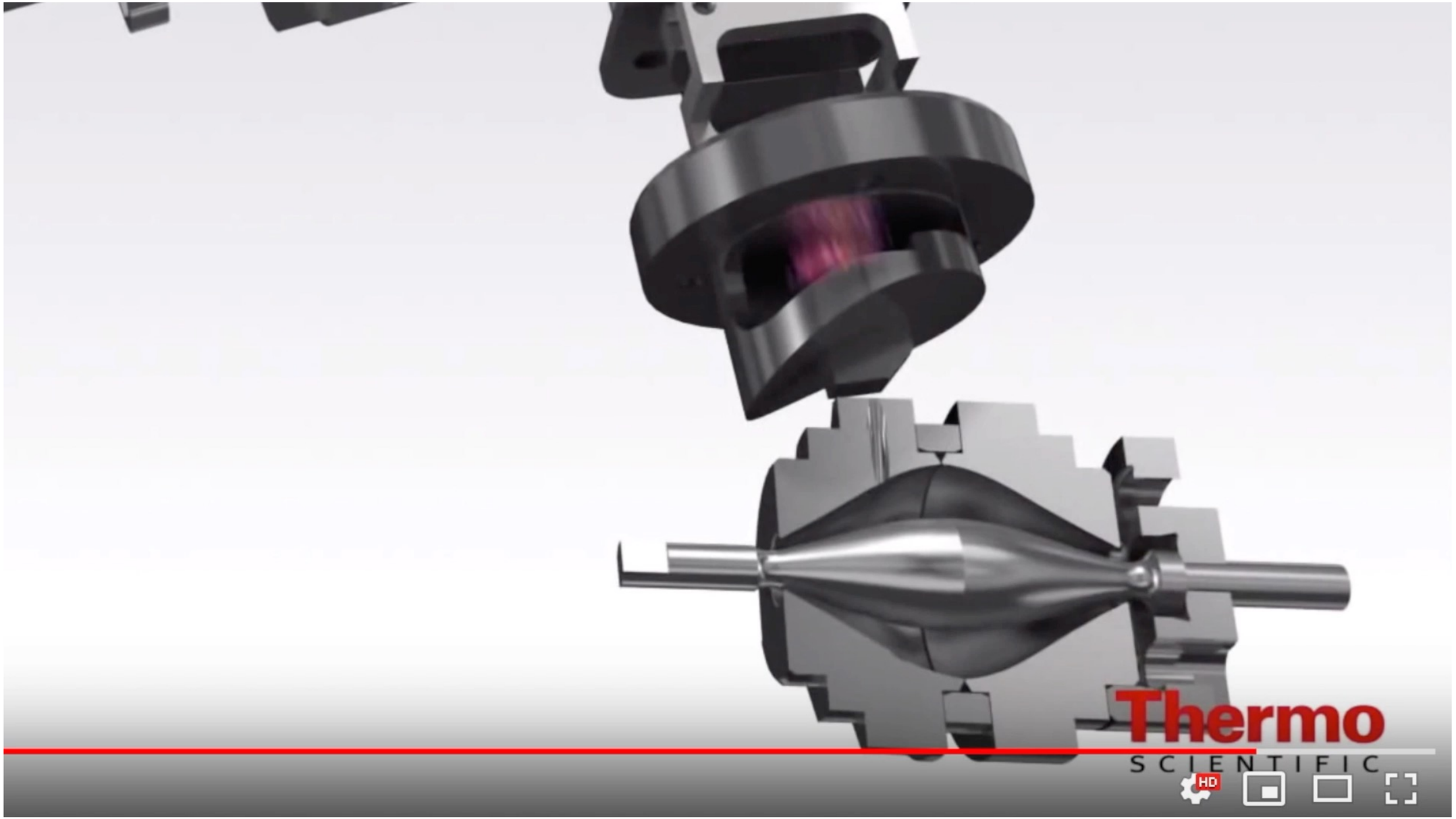
Establishing rotational and axial oscillation



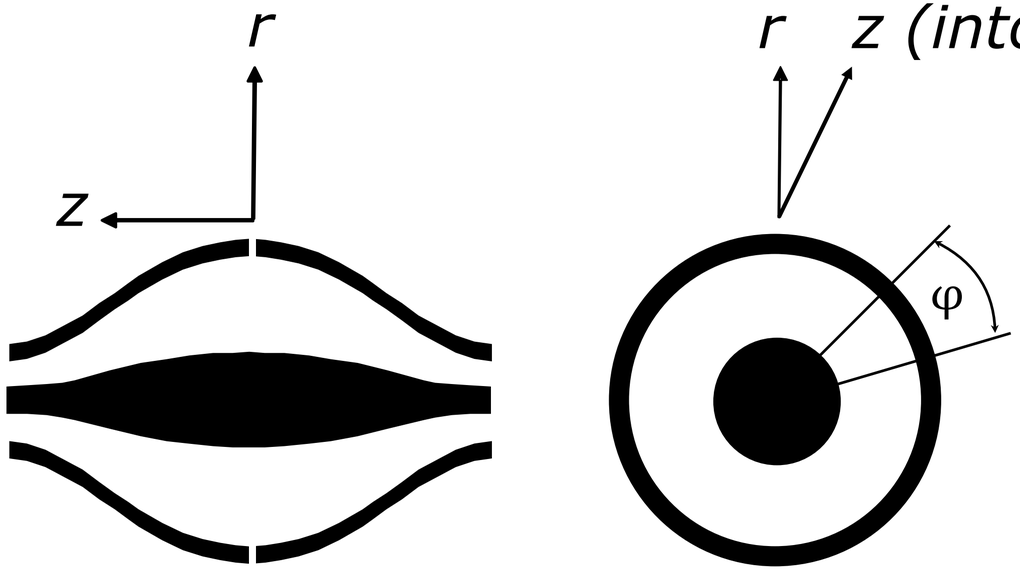


- Ions are rapidly injected as a spatially confined packet at one end of the z axis.
- The center electrode potential is lowered in order to trap the ions in a stable orbit.
- The ions of a given m/z thus begin their z-axis oscillation with the same phase.

Orbitrap motion video



Different oscillation frequencies



$$R = \frac{m}{\Delta m}; \qquad \textbf{Resolution:}$$

$$m \propto \frac{1}{\omega_z^2}; \quad dm \propto \left(\frac{1}{\omega_z^2}\right)' d\omega = \frac{-2}{\omega_z^3} d\omega;$$

$$\frac{m}{\Delta m} \propto \frac{1}{\omega_z^2}\frac{\omega_z^3}{\Delta\omega} \propto \omega_z \cdot t \propto \frac{t}{\sqrt{m}}.$$

– Frequency of radial oscillations: $\omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$

– Frequency of rotation: $\omega_\varphi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1}$

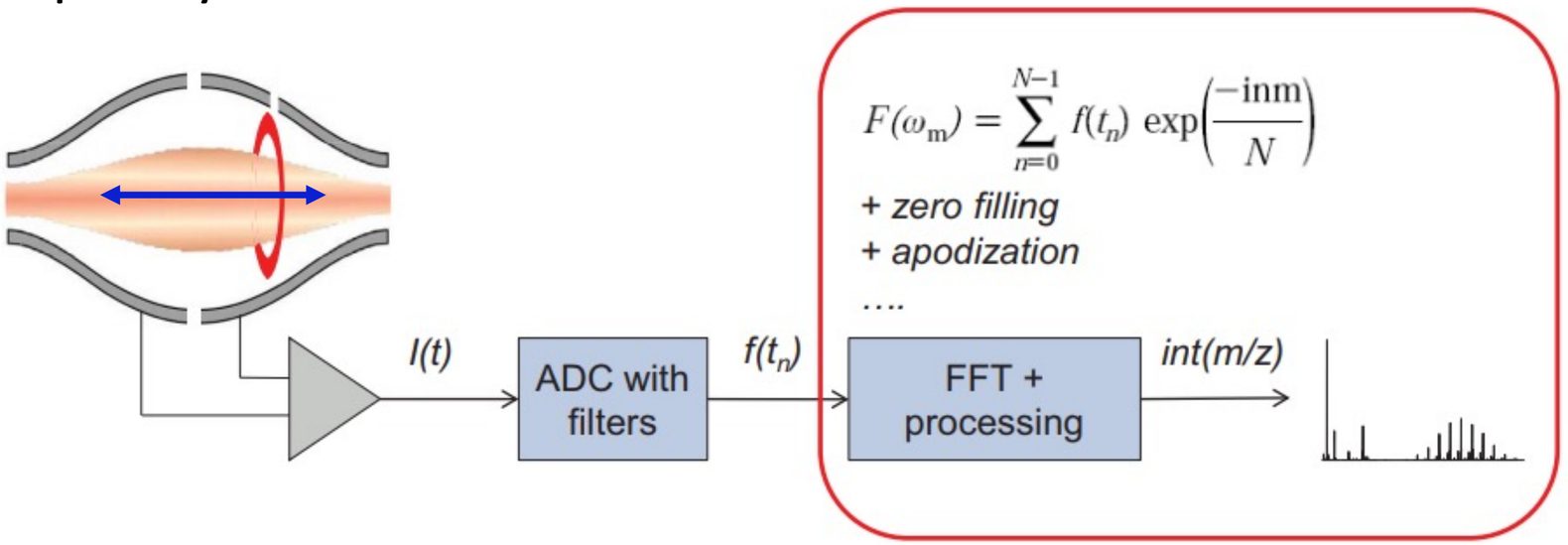
– Frequency of axial motion: $\omega_z = \sqrt{\frac{k_0 z e}{m}}$ → $\boxed{\frac{m}{z} = \frac{k_0 e}{4\pi^2 f_z^2}}$

- ✓ Resolution decreases with m as $\mathbf{m}^{-1/2}$
- ✓ Resolution increases with time of measurements

Only this motion depends on m/z !

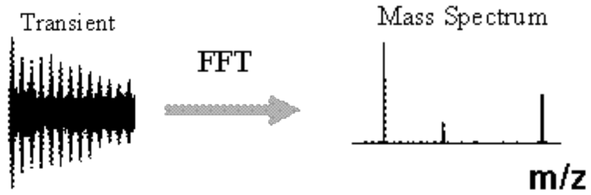
Orbitrap ion current detection

- Similar to FT ICR;
- Frequency of axial oscillations



The image current on each half is differentially amplified and undergoes Analog-to-Digital Conversion before FT algorithm

- Fourier Transform is used to convert signal to *m/z* data



Details of FT will be given in NMR section

3m19s

Orbitrap: high to ultrahigh resolution

Low-Medium	High	Ultra-high
< 100k @ *m/z* 200	**100k-500k @ *m/z* 200**	**> 500k @ *m/z* 200**
	Transients	
≤ 256 ms (120k) ≤ 512 ms (140k)	≤ 1 sec	longer transients (1 to 10 sec)

Orbitrap summary

+ High performance instrument competitive with FT-ICR mass analyzers

+ Mass resolving power up to 240’000 at 400 *m/z*

+ Resolution depends on the transient length: 512 ms for 240K on a Q-Exactive HF.

+ High mass accuracy (1 ppm).

+ Compare with ICR: compact: no LHe is required; low cost; low operation cost.

− Requires ultra-high vacuum to realize sufficiently long transients

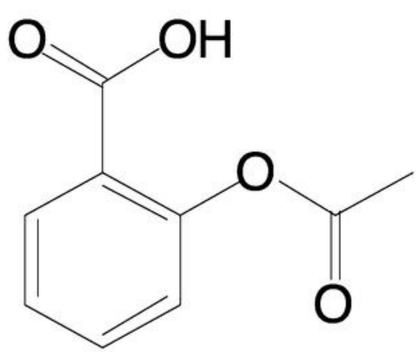
− Resolving power is directly linked to scan speed (lower scan speed yields better RP)

− RP decreases with increasing *m/z*

Fragmentation methods and tandem mass spectrometry

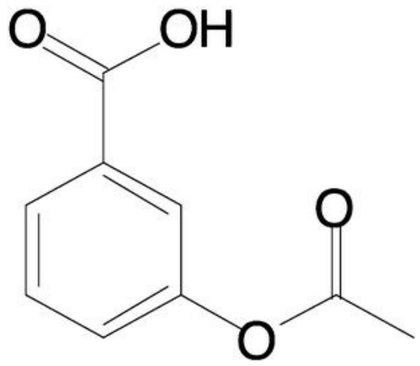
Tandem mass spectrometry

Problem: How do you distinguish two different molecules of exactly the same mass?



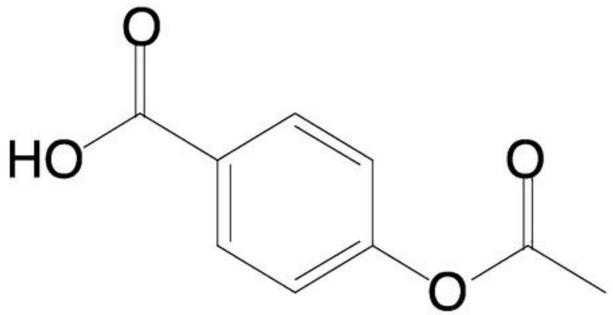
o-Aspirin

(2-acetoxybenzoic)



m-Aspirin

(3-acetoxybenzoic)



p-Aspirin

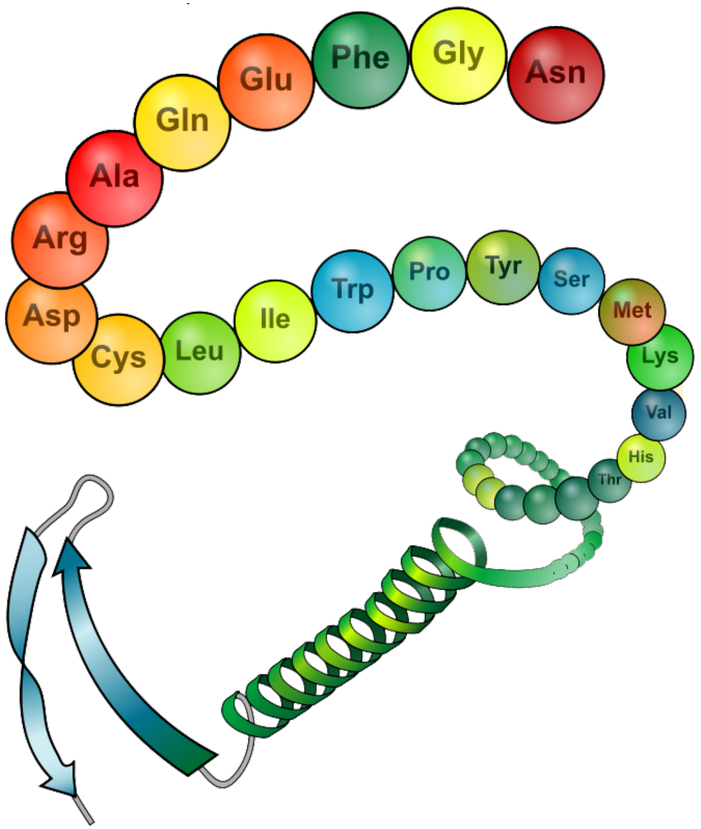
(4-acetoxybenzoic)

same m/z:	180.4169	180.4169	180.4169

Tandem mass spectrometry

Problem: How do you distinguish two different molecules of exactly the same mass?

Another example, proteins are made up of a linear sequence of amino acids.

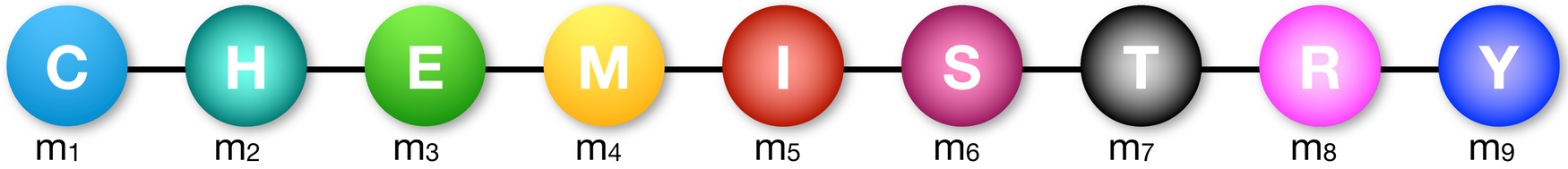


How do you distinguish two proteins if they have the same amino acids but in different order?

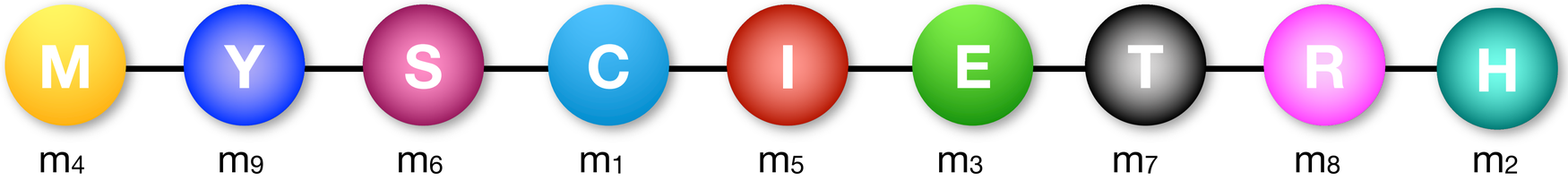
Tandem mass spectrometry

Problem: How do you distinguish two different molecules of exactly the same mass?

Consider the following:



How do we distinguish it from:

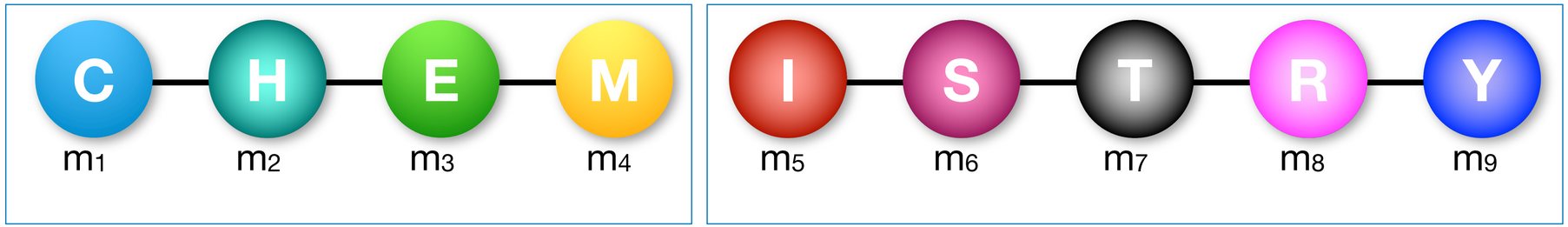


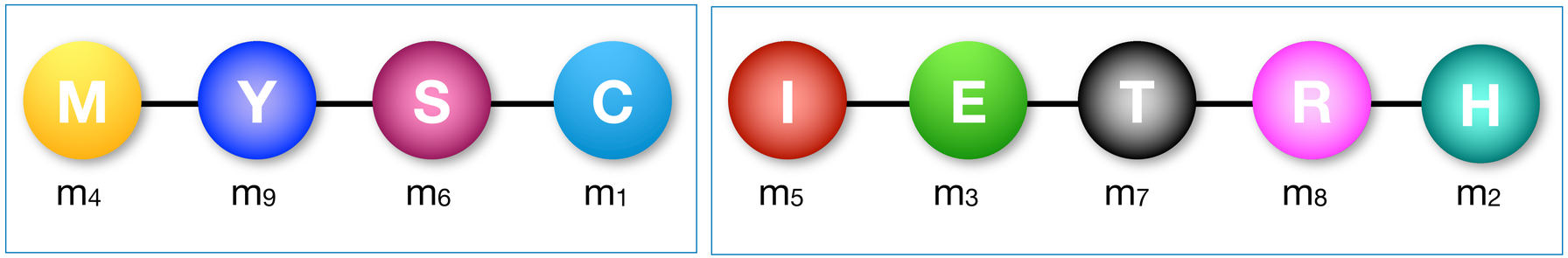
They have exactly the same mass.

Tandem mass spectrometry

Problem: How do you distinguish two different molecules of exactly the same mass?

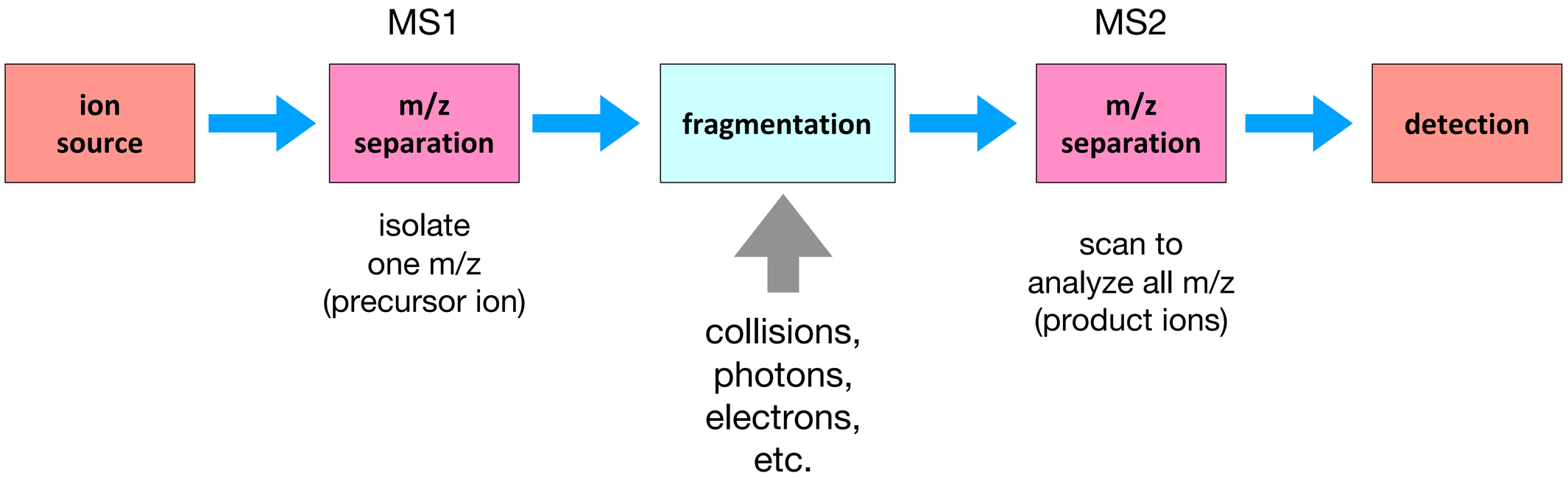
Break them into pieces and analyze the mass of the pieces





In general, the pieces will have different mass.

Tandem mass spectrometry



Fragmentation methods in tandem MS

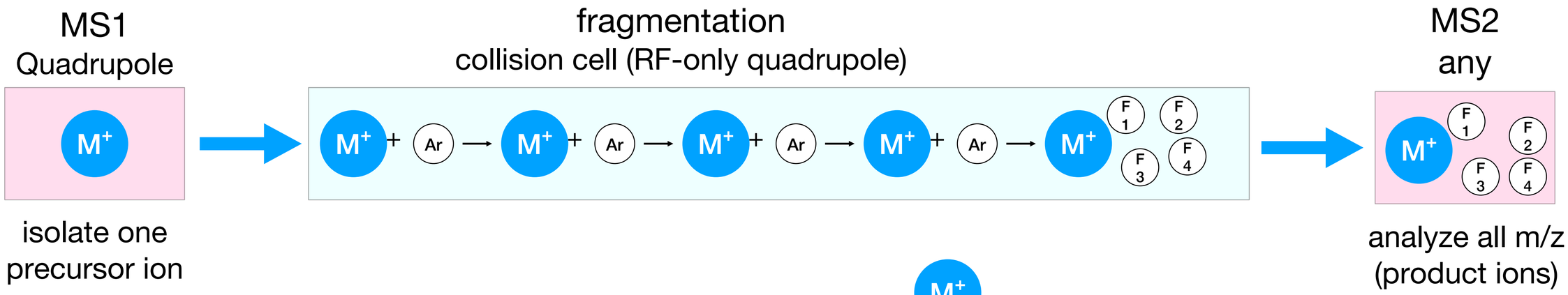
- Collision Induced Dissociation (CID)
  - Low-energy CID
  - High-energy CID (HCD)
- Surface-induced Dissociation (SID)
- Photodissociation
  - Infrared Multi-photon Dissociation (IRMPD)
  - UV Photodissociation (UVPD)
- Electron Capture Dissociation (ECD)
- Electron Transfer Dissociation (ETD)

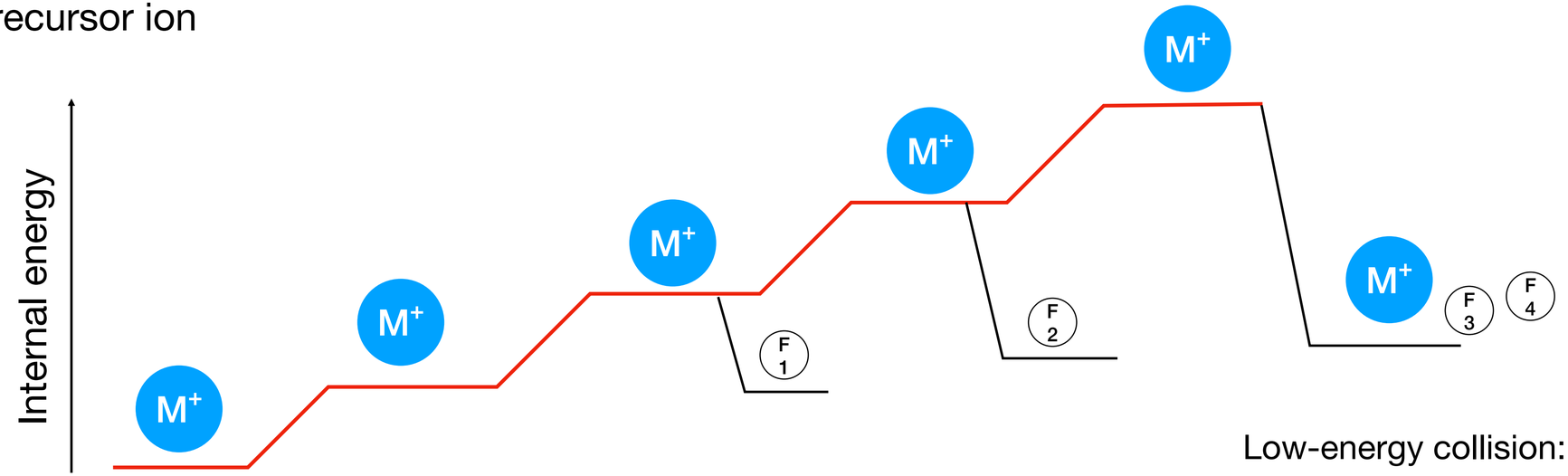
Different fragmentation techniques produce different types of fragments, which is useful for structural analysis.

Different MS techniques are best suited to specific fragmentation methods.

Collision-induced dissociation (CID)

Low energy: Energy deposited into a molecule slowly through multiple, low-energy collisions.





Low-energy collision:
- 1-100 eV
- Excitation is mostly vibrational
- Rearrangements can occur

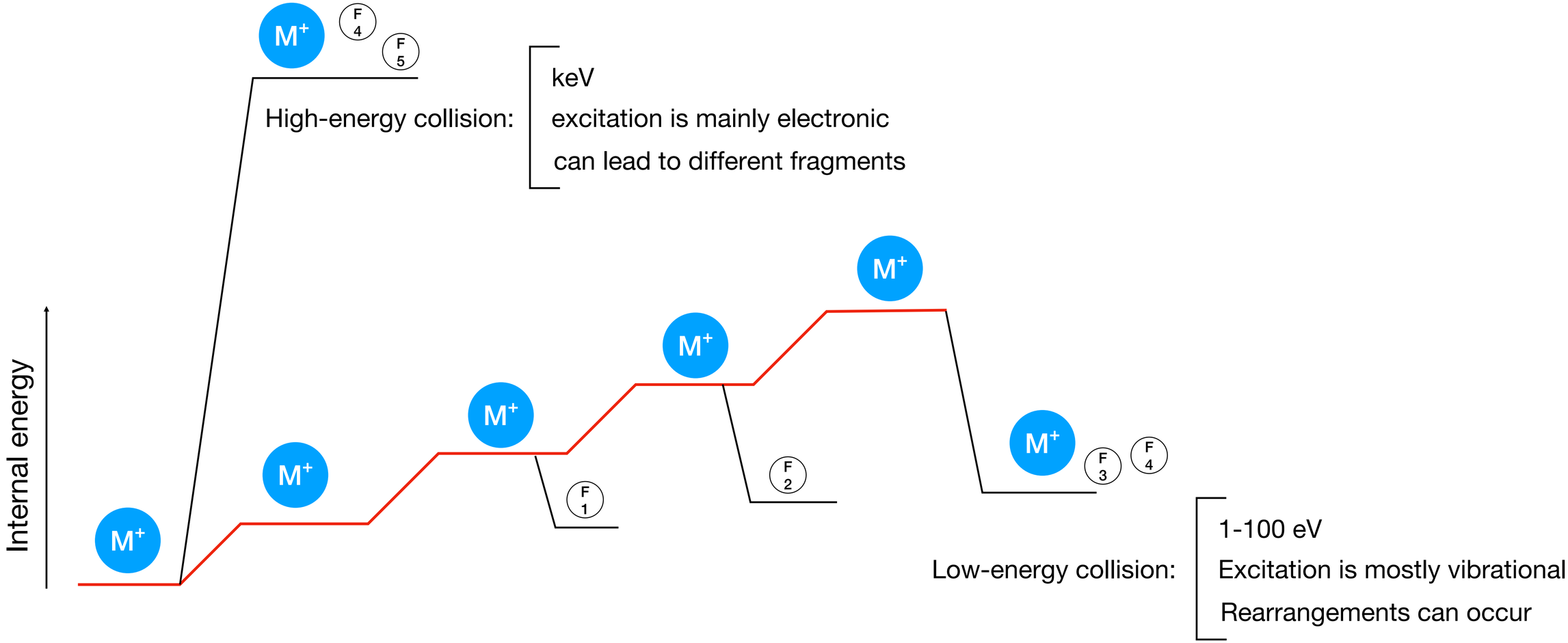
Two step process:

(i) Energy deposited into a molecule slowly through multiple, low-energy collisions.

(ii) Unimolecular dissociation of the activated ion

Collision-induced dissociation (CID)

High energy: Energy deposited into a molecule through a single, high-energy collision.



CID concepts

- Collisions are accompanied by an increase in internal energy
- This energy is redistributed among all the ion vibrational modes, inducing decomposition
- The dissociation products that are observed result from a series of competitive and consecutive reactions
- The total energy available for conversion from kinetic energy to internal energy must be considered in the center-of-mass reference frame.

Ion kinetic energy in the lab

Mass of the neutral

Available energy in the CM

$$E_{CM} = E_{lab} \frac{M_N}{M_i + M_N}$$

Mass of the ion

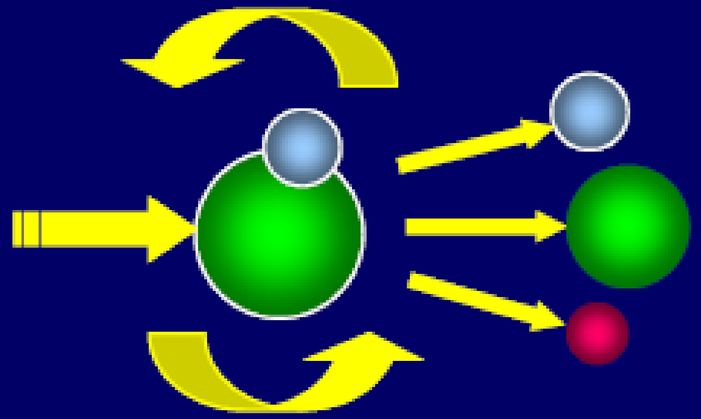
Consider the collision of a ping-pong ball with a bowling ball compared with that between two bowling balls.

Low (LE) and High (HE) Energy CID

Low energy CID

(E_{Lab} 1 - 200 V)

E_{Cm} = 0.2 eV



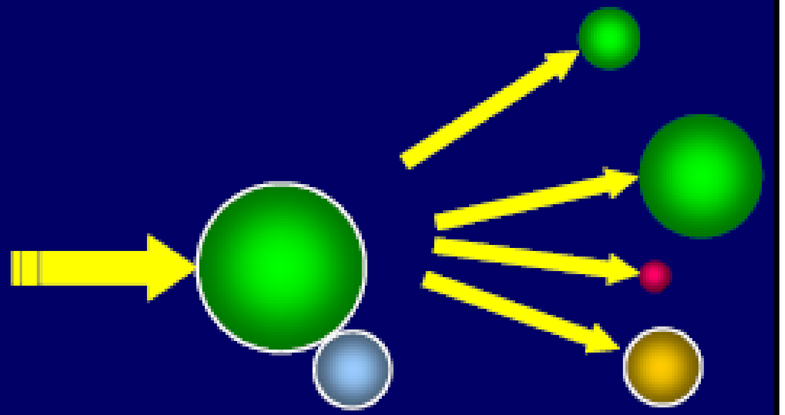
Vibrational excitation

t_{IA} 10^{-14} s

High energy CID

(E_{Lab} ≥ 800 V)

E_{Cm} = 4 eV (long tail!)



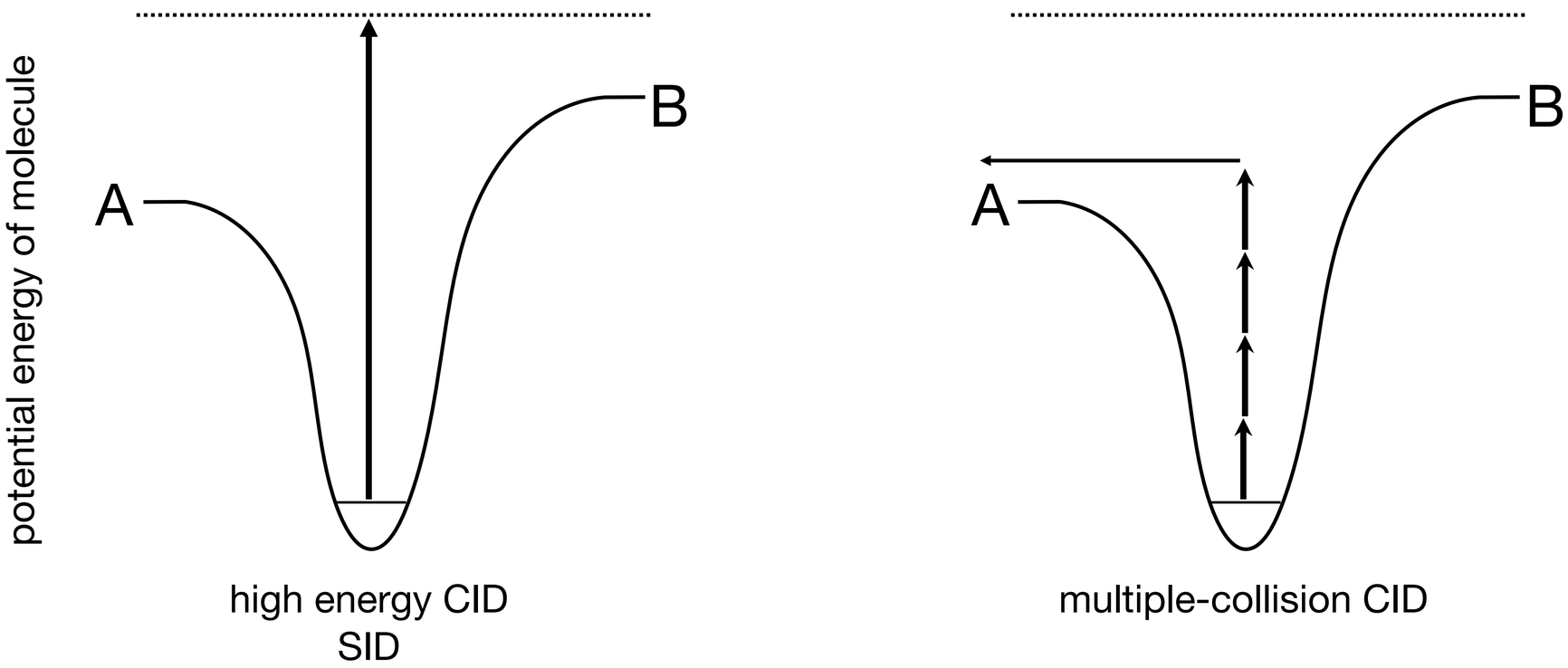
Electronic excitation

t_{IA} 10^{-15} s

Surface-induced dissociation (SID)

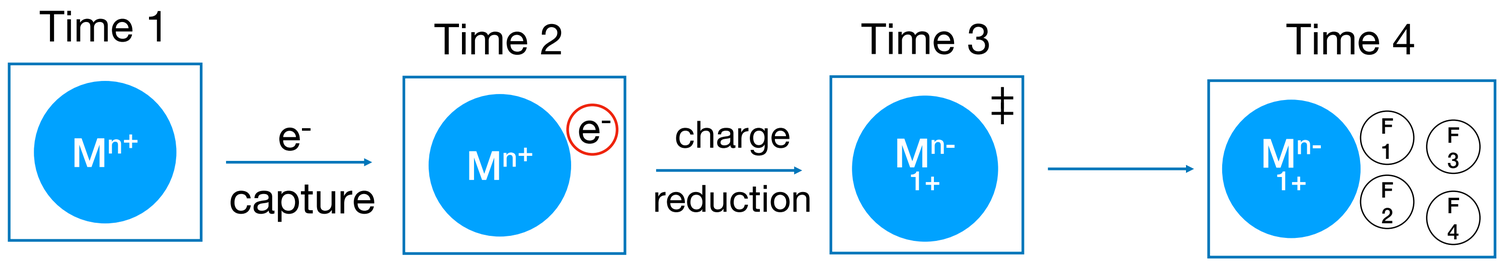
- Using a surface as the neutral collision partner, the mass becomes “infinite”, allowing maximal energy transfer.

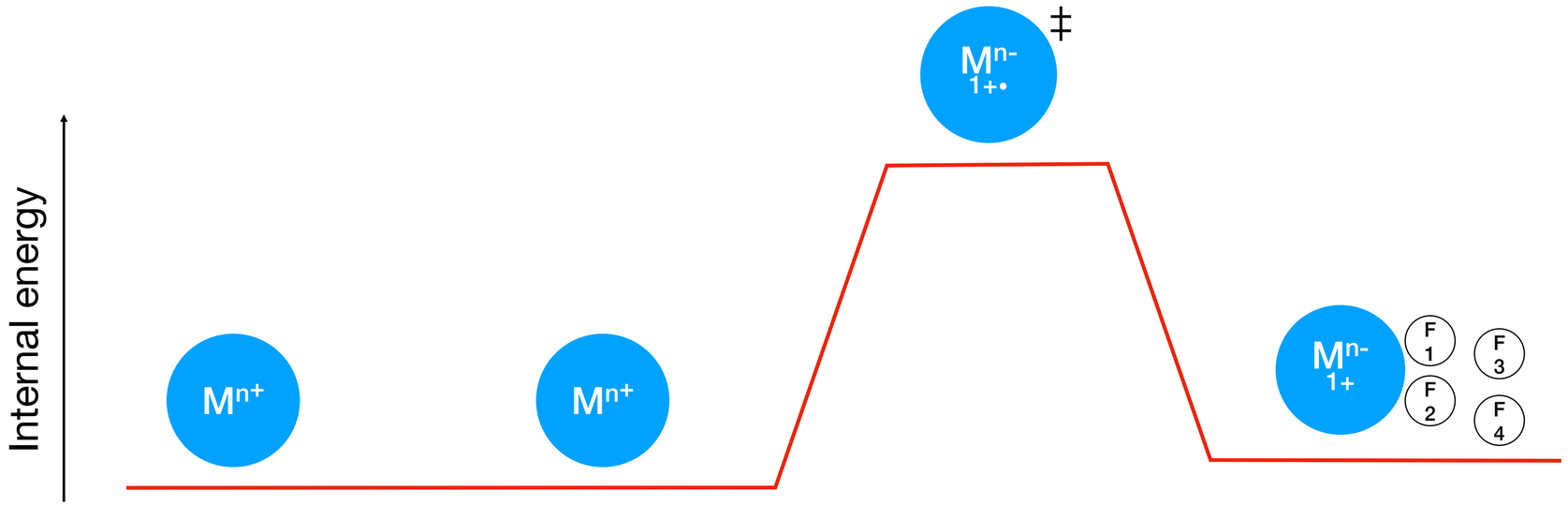
$$E_{CM} = E_{lab} \frac{M_N}{M_i + M_N} \approx E_{lab}$$



Electron capture dissociation (ECD)

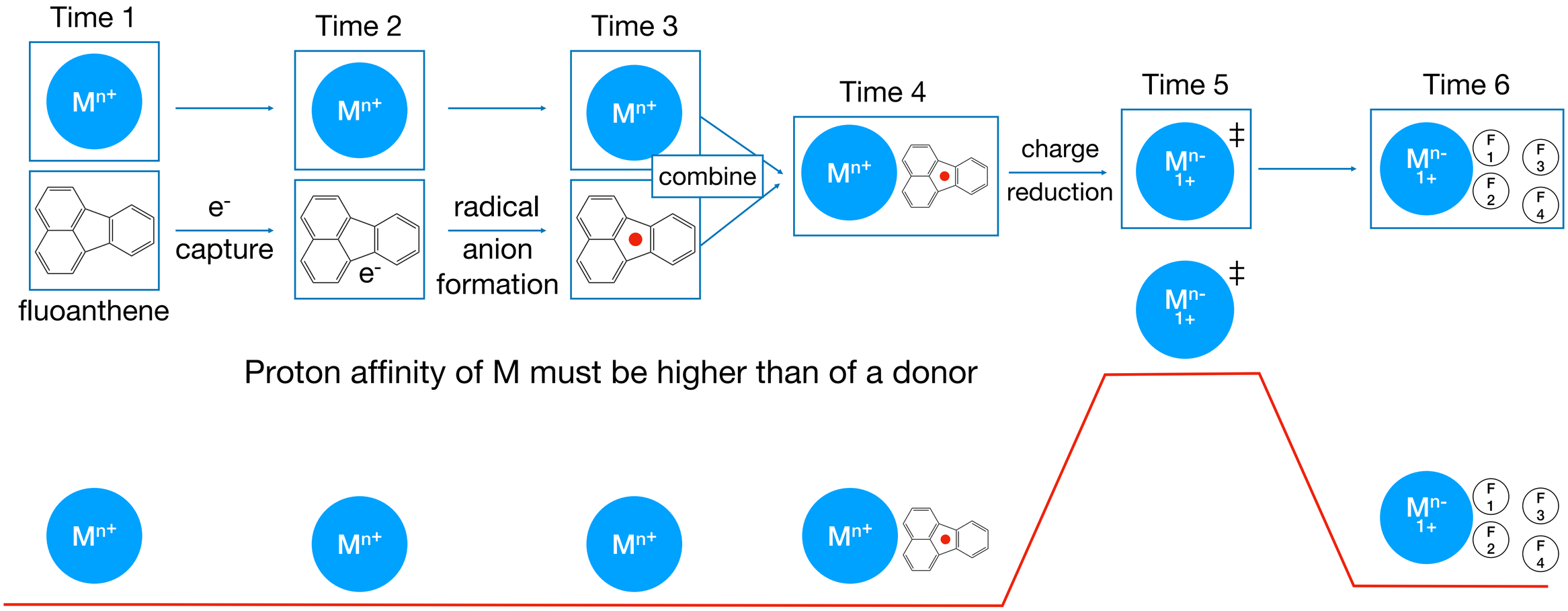
- ECD involves the capture of low-energy electrons by multiply charged ions, with charge-state reduction and subsequent fragmentation
- Works in FTICR but it is difficult to perform in quadrupole traps; not in orbitraps, . . .





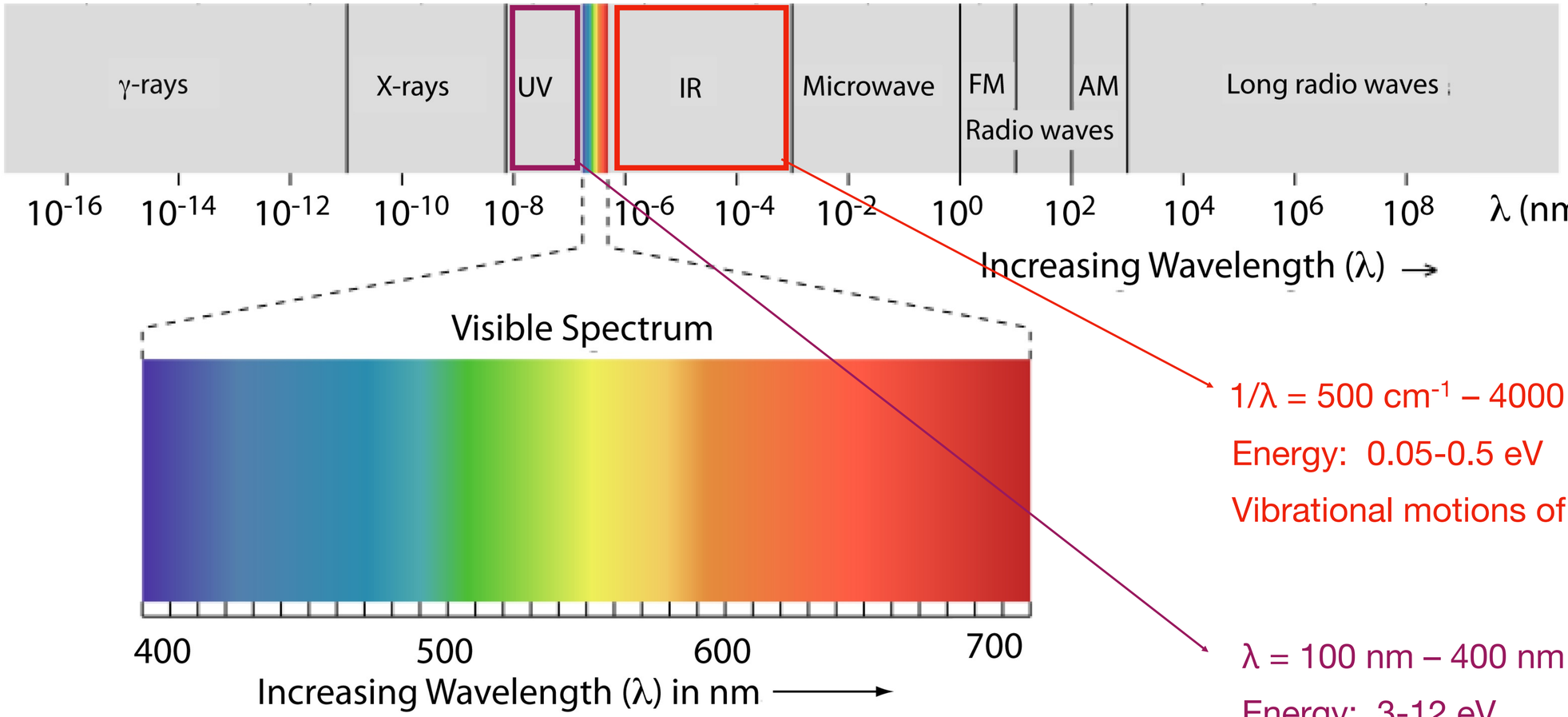
Electron transfer dissociation (ETD)

- ETD is similar to ECD



- Fluoanthene has about a 40% efficiency for electron transfer

Photon-induced dissociation



$1/\lambda = 500\ cm^{-1} – 4000\ cm^{-1}$

Energy: 0.05-0.5 eV

Vibrational motions of the molecule

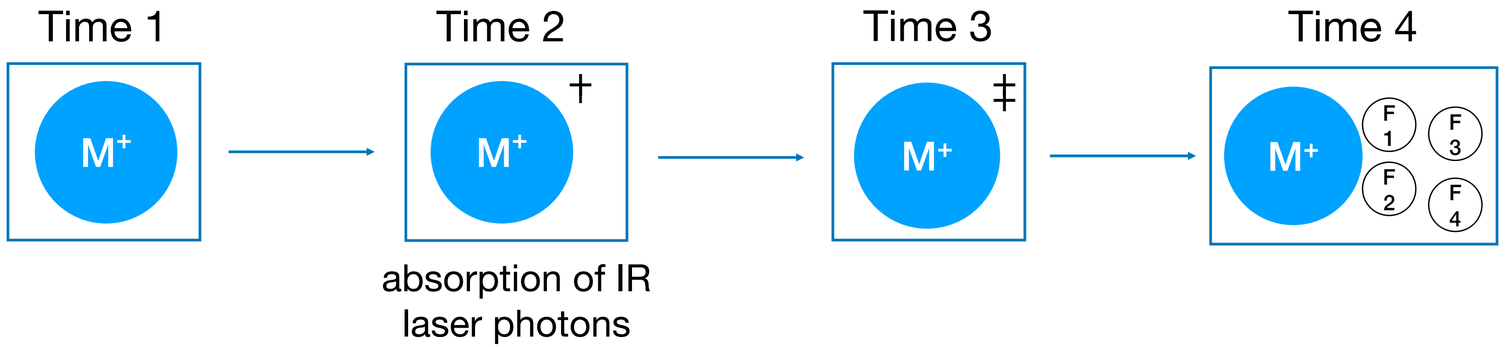
$\lambda = 100\ nm – 400\ nm$

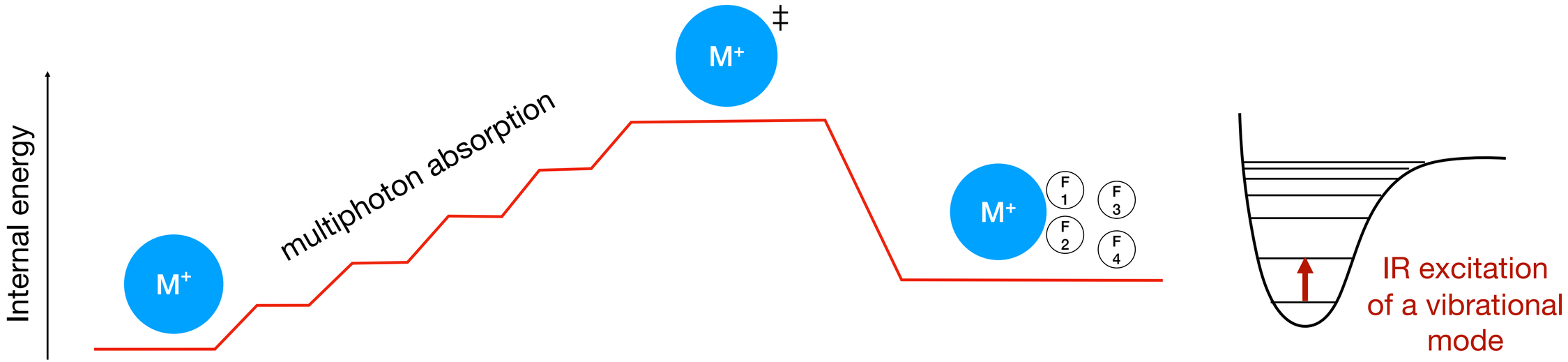
Energy: 3-12 eV

Electronic excitations of the molecule

Infrared multiphoton dissociation (IRMPD)

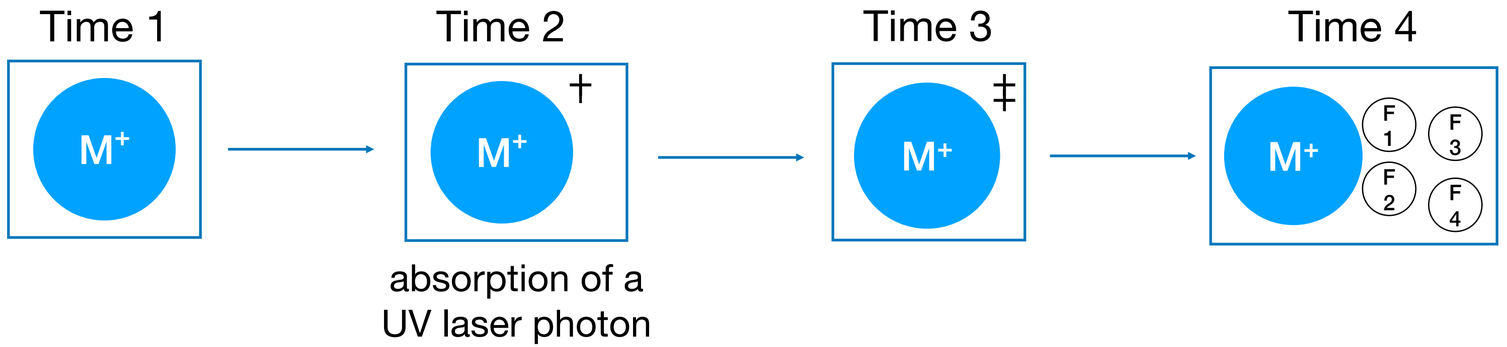
- Involves excitation of multiple IR photons by IR active modes
- After photon absorption, rapid redistribution of energy over all vibrational degrees of freedom occurs.
- Statistical internal energy distribution (like in CID)

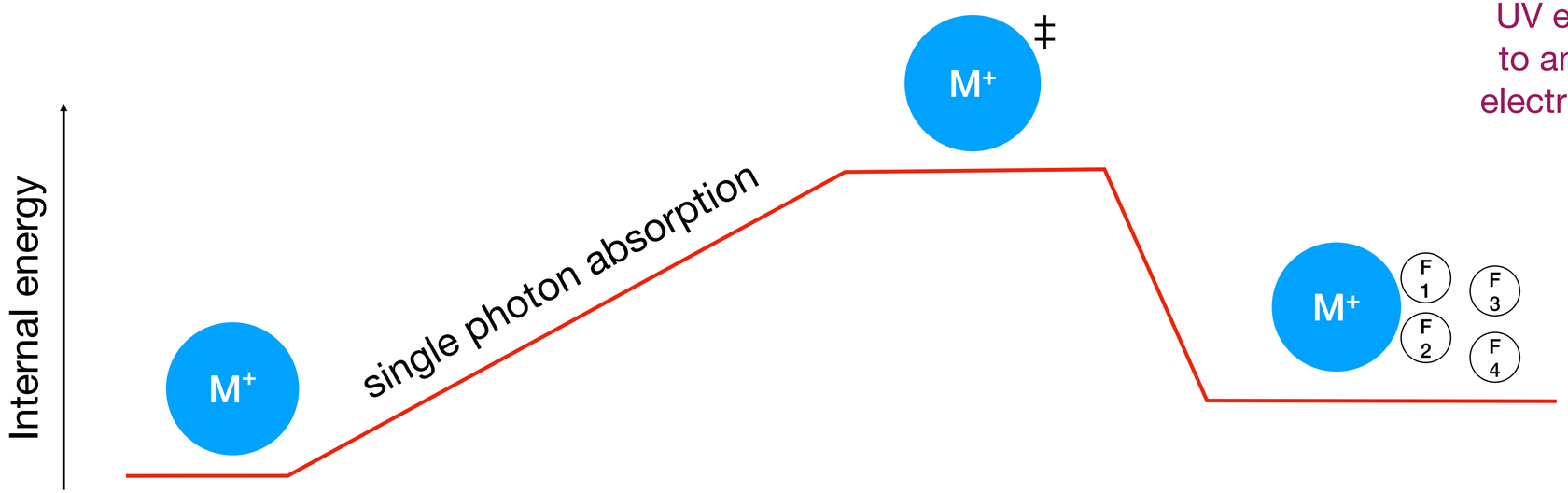


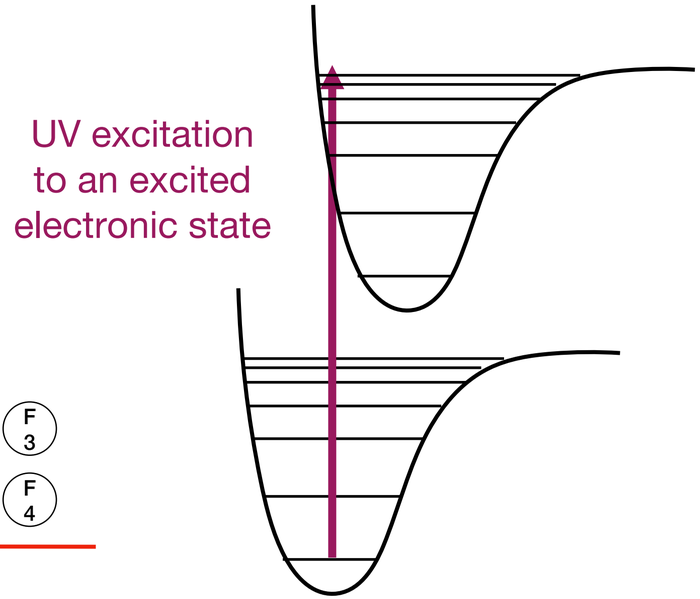


UV photodissociation (UVPD)

- Activation occurs through the absorption of a single UV photon
- The photon causes a vibronic transition, which is a simultaneous change both in electronic and vibrational state



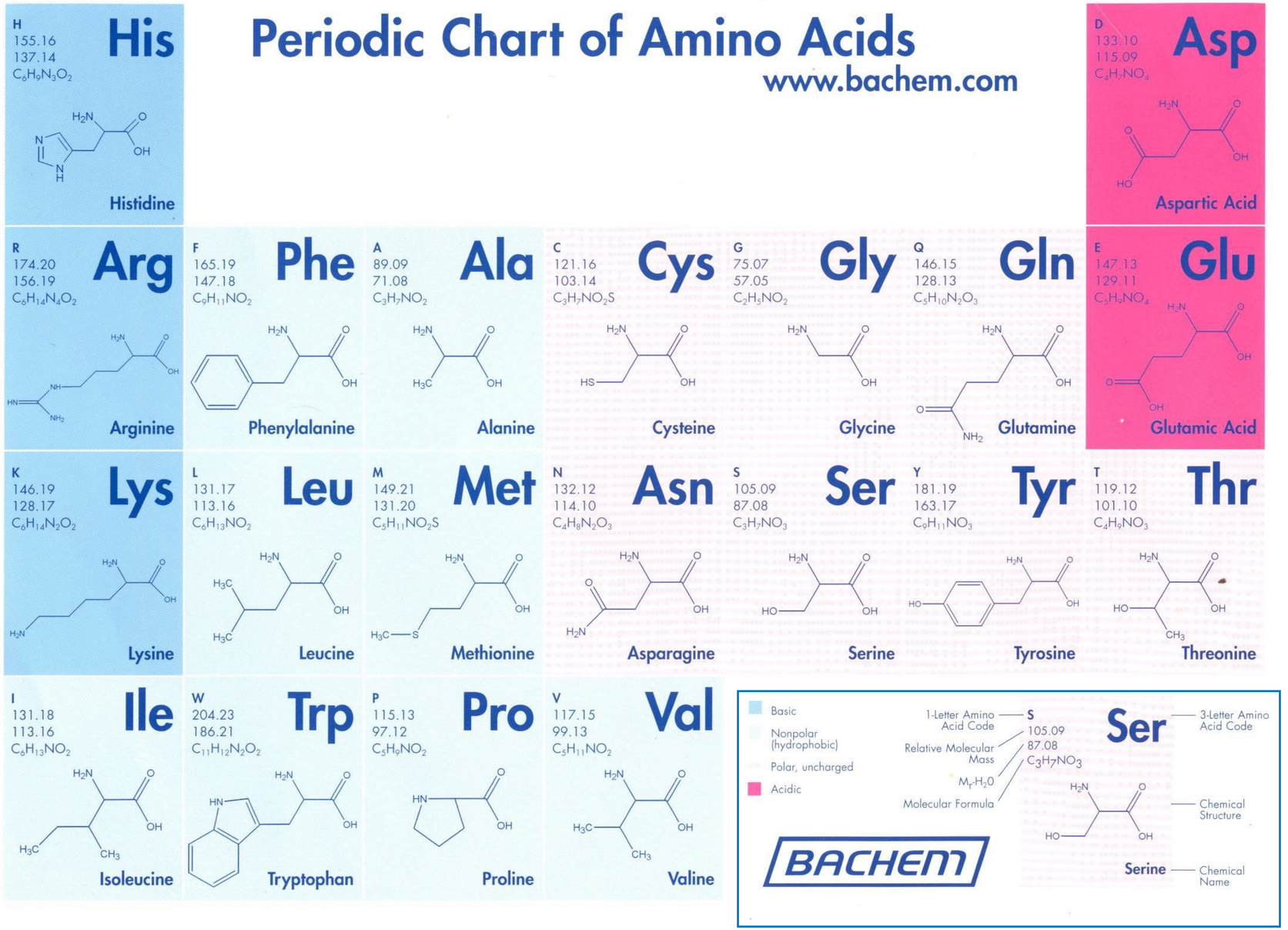




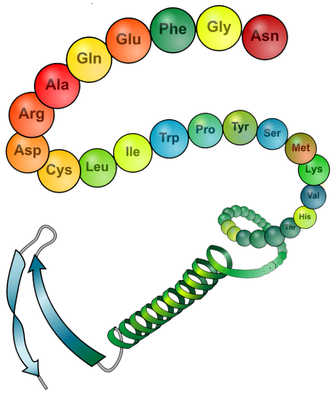
Comparison of fragmentation methods

+		-
Simple Large diversity of fragments Applicable to any charge state	**CID**	Unpredictable fragments Loss of PTMs No info on 3D structure
Similar to CID; Retains some PTMs;	**HCD**	Unpredictable fragmentation Loss of high fraction of PTMs No info on 3D structure
Similar to CID, but doesn't require collisions; Can be wavelength selective;	**IRMPD**	The same as for CID; Technical complexity
Non-statistical, gives specific fragments; Retains PTMs, reflects 3D structure + Highly specific to ions/isomers	**UVPD** **UVPD-MS**	Technical complexity Moderate dissociation yield Additional technical complexity
Cleaves very wide types of bonds; High fragment abundance; Retains some PTMs;	**VUVPD**	Technical complexity; No info on 3D structure
Non-statistical, highly predictable bond cleavage (c, z fragments); Retains most of PTMs	**ETD/ECD**	Low efficiency; Not applicabale to singly-charged and negative ions; Very limited info on 3D structure

Tandem MS of peptides and proteins

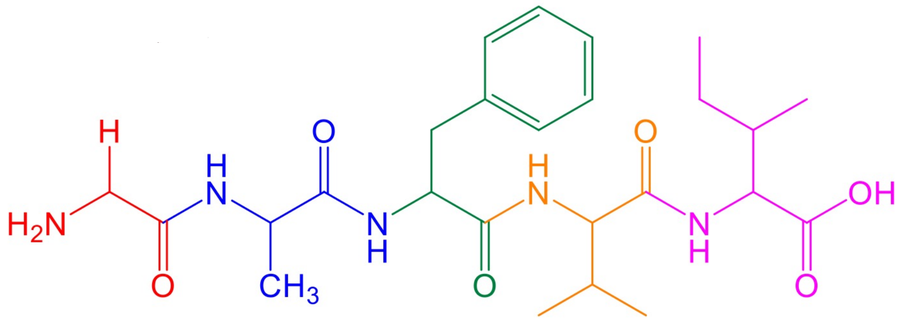


Tandem MS of peptides and proteins



Primary sequence of a peptide or protein: the order of its amino acids

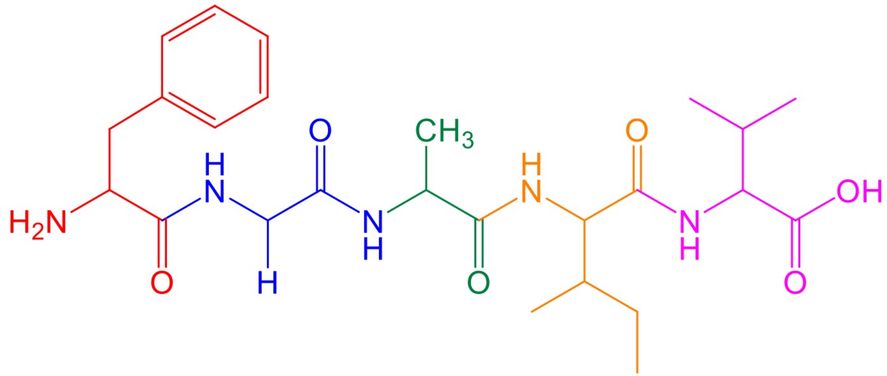
Strategy: break it up into smaller pieces (peptides), determine sequence of peptides, and then piece back together



Three-letter code: Gly-Ala-Phe-Val-Ile

One-letter code: GAFVI

MW 505.61

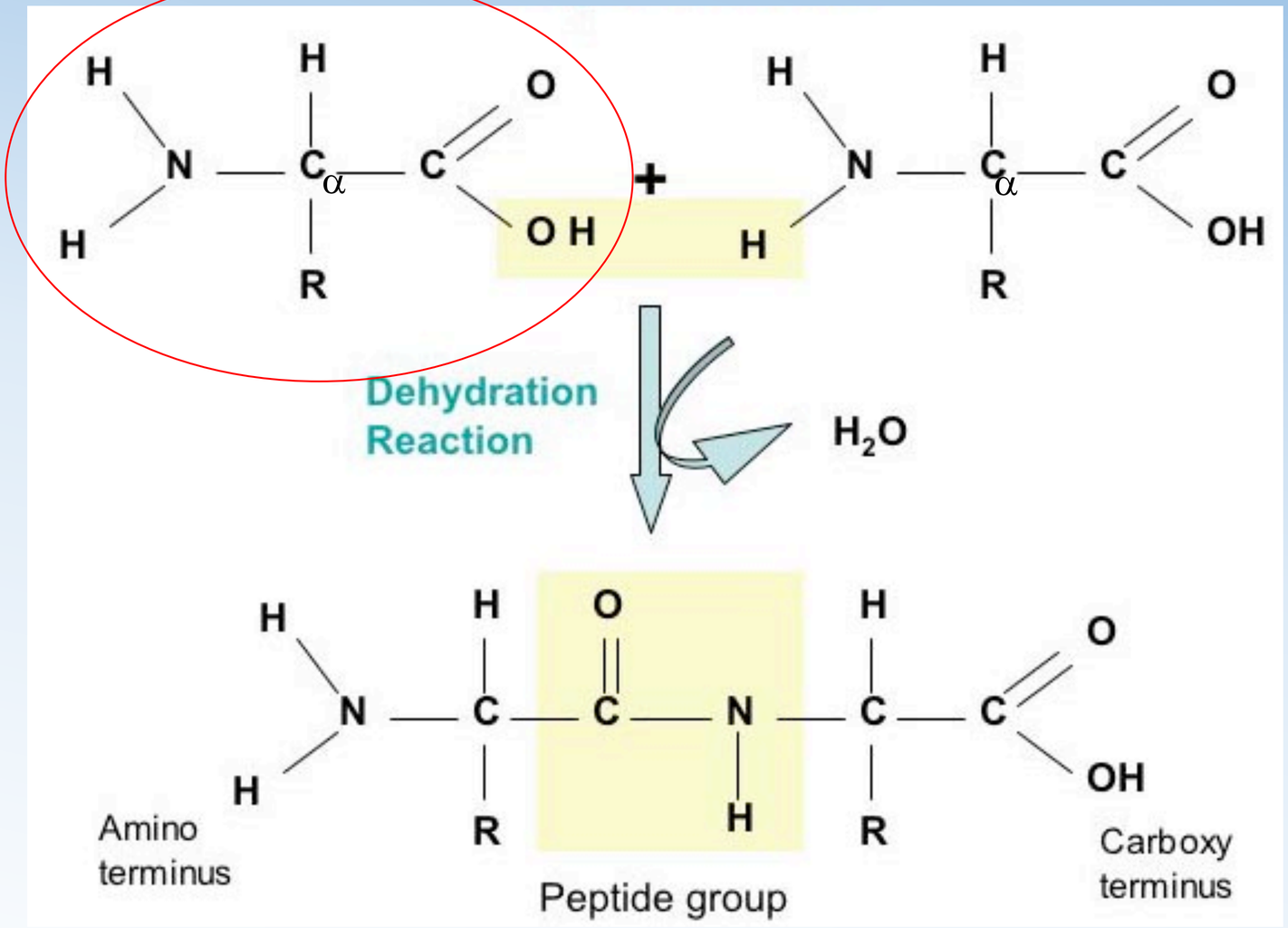


Three-letter code: Phe-Gly-Ala-Ile-Val

One-letter code: FGAIV

MW 505.61

Peptide structure

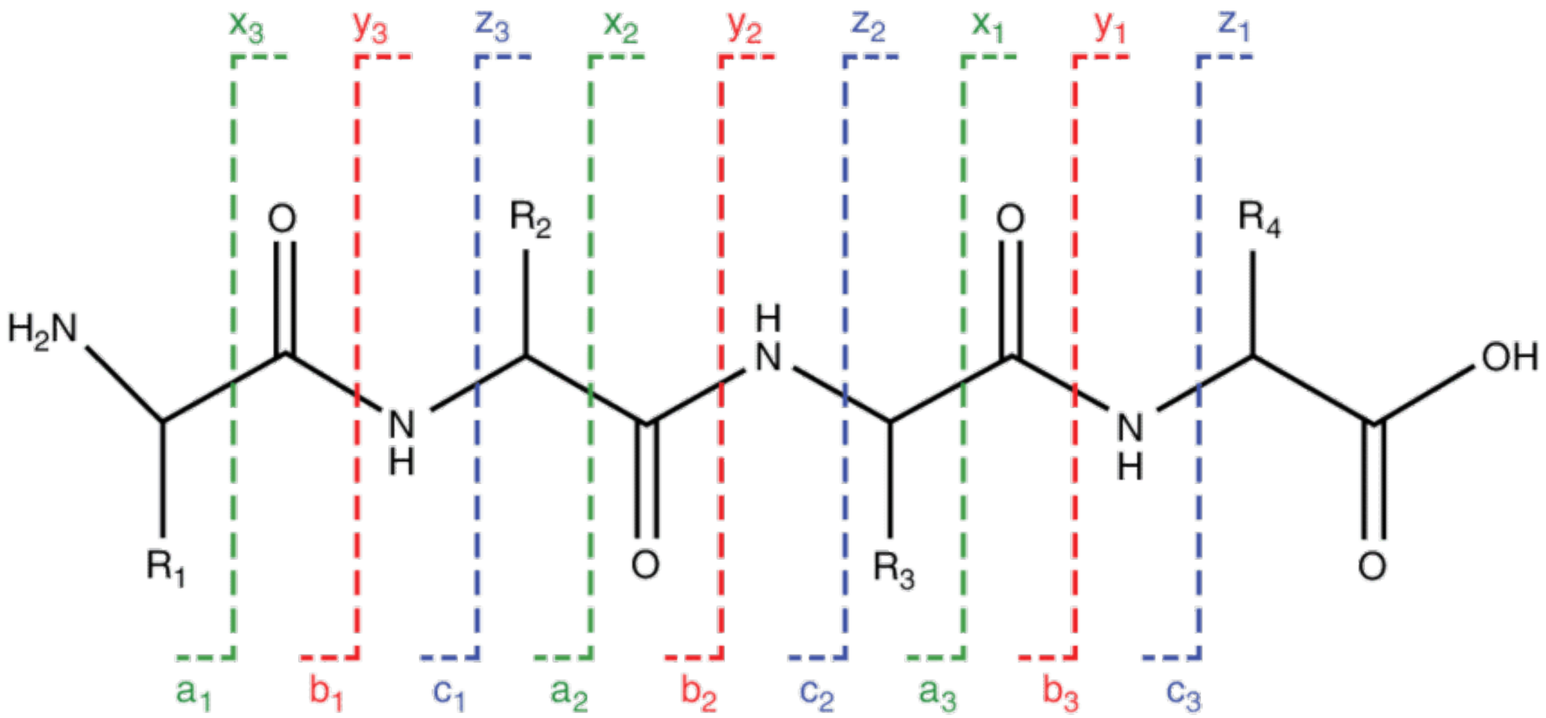


Tandem MS of peptides and proteins

Nomenclature for peptide fragments (example with 4 residues):

For x, y, and z fragments, the charge remains on the fragment to the right.

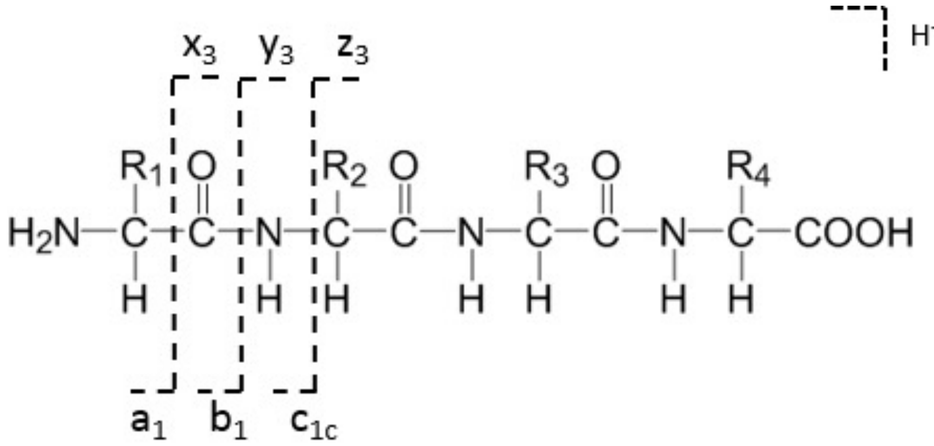
N-terminus

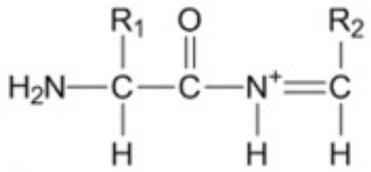


C-terminus

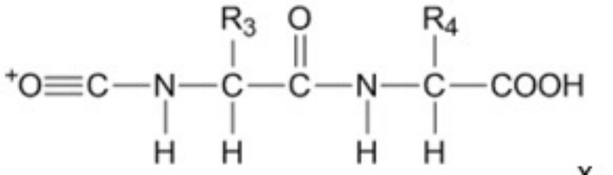
For a, b, and c fragments, the charge remains on the fragment to the left

Peptide fragmentation

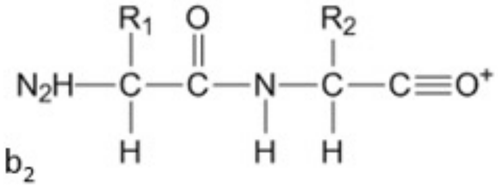




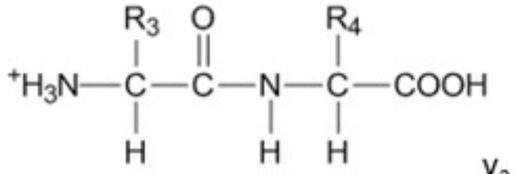
a2



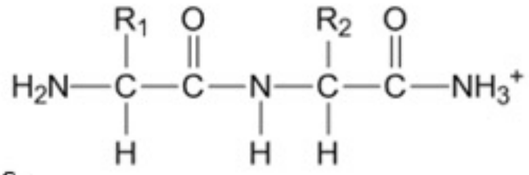
x2



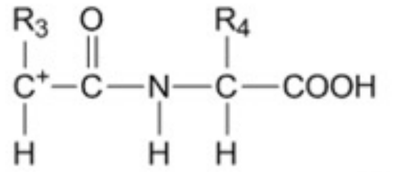
b2



y2

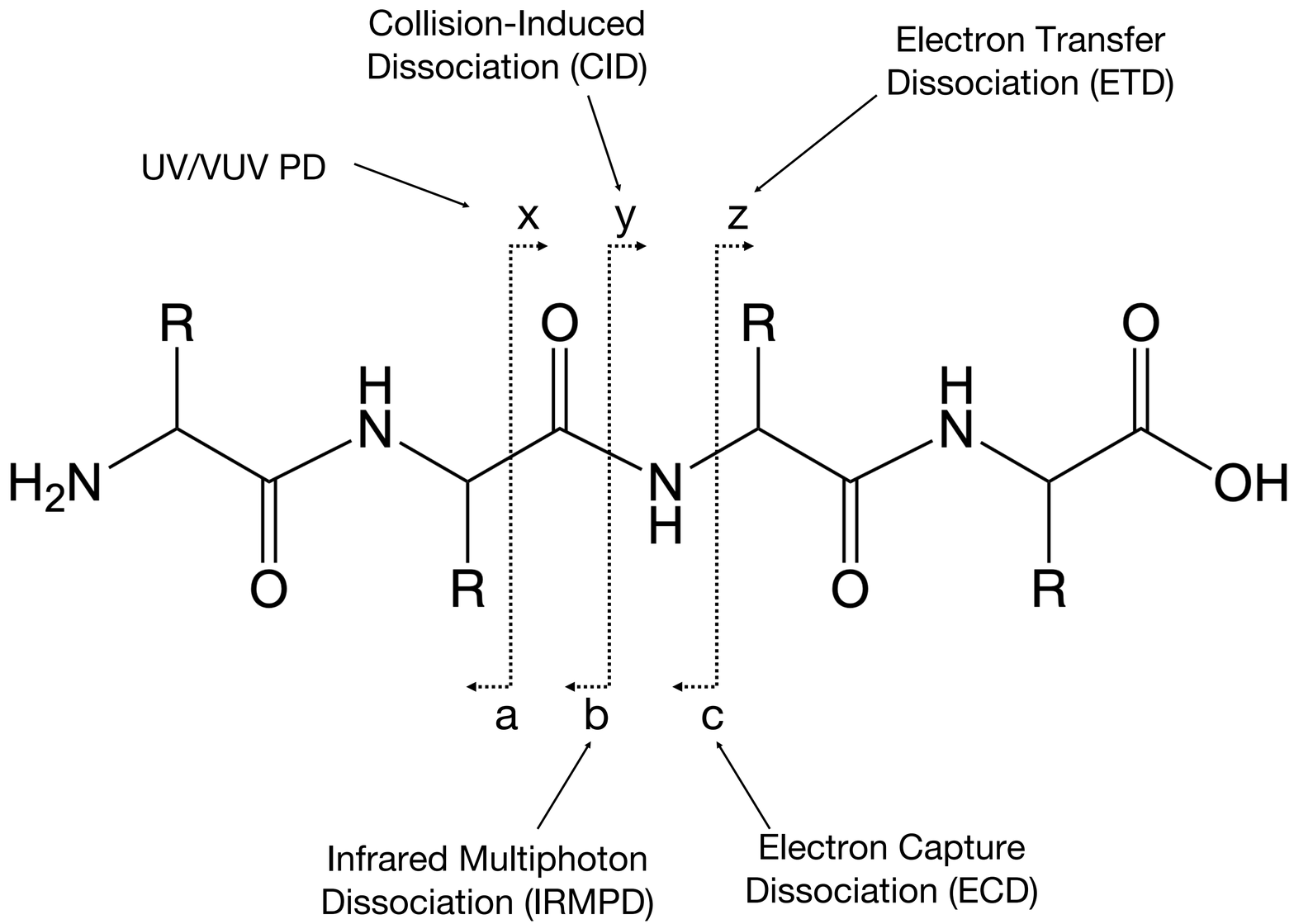


c2



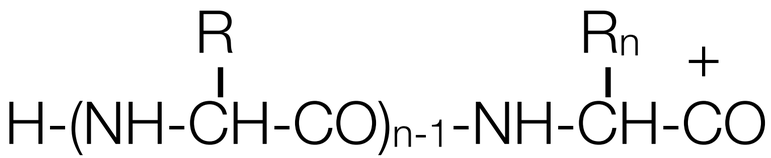
z2

Peptide fragmentation



How to calculate b/y fragments

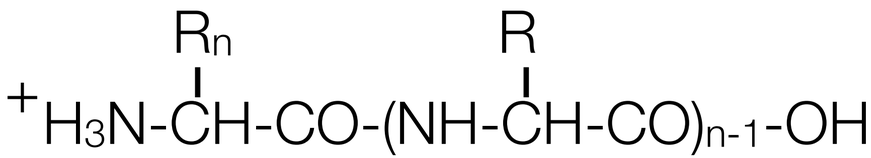
b_n



$$m(b_n) = \sum_{i=1}^{n-1} m(R_i + C_2H_2NO) + m(R_n) + m(C_2H_3NO^+)$$

Enumeration starts from N-terminus

y_n



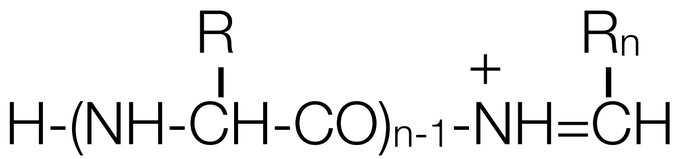
$$m(y_n) = \sum_{i=1}^{n-1} m(R_i + C_2H_2NO) + m(R_n) + m(C_2H_5NO_2^+)$$

Enumeration starts from C-terminus

Hydrogen jumps to y-side

How to calculate a/x fragments

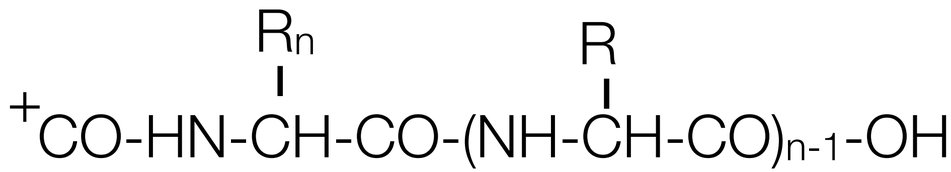
a_n



$$m(a_n) = \sum_{i=1}^{n-1} m(R_i + C_2H_2NO) + m(R_n) + m(CH_3N^+)$$

Enumeration starts from N-terminus

x_n



$$m(x_n) = \sum_{i=1}^{n-1} m(R_i + C_2H_2NO) + m(R_n) + m(C_3H_3NO_3^+)$$

Enumeration starts from C-terminus

Ion spectroscopy

Ion spectroscopy

A third way to distinguish two molecules of the same mass:
by their optical spectra

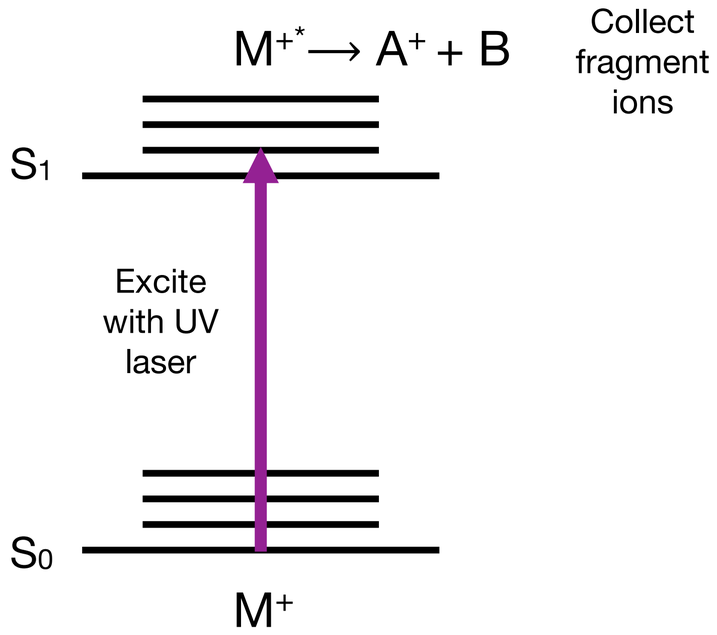
- Spectral transitions are fundamental to ions
- Spectra are extremely sensitive to structural differences of molecules.
- Spectra can distinguish between isomers.

Problem:

There are too few ions in a mass spectrometer to do absorption spectroscopy.

Action spectroscopy of ions

UV photofragmentation



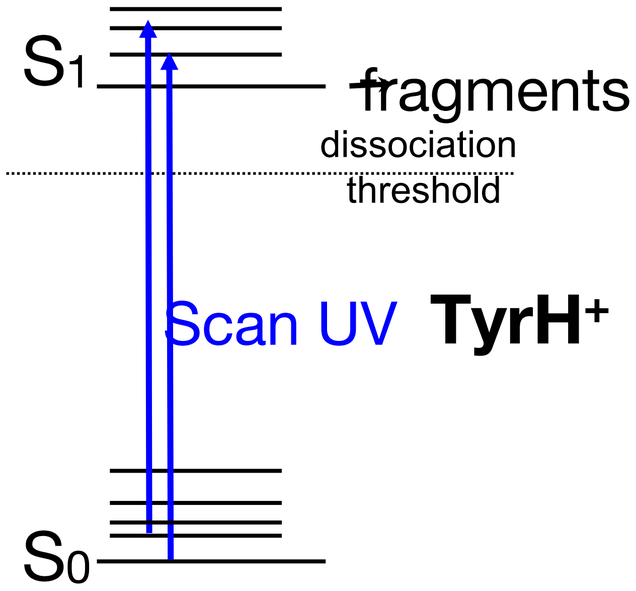
Advantages:

- Can probe different conformations of molecule
- Provide structural info in MS^3 UVPD measurements
- One can efficiently collect fragment ions (100%)

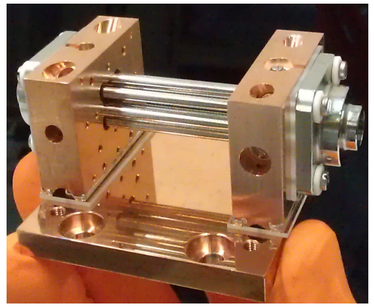
Disadvantages:

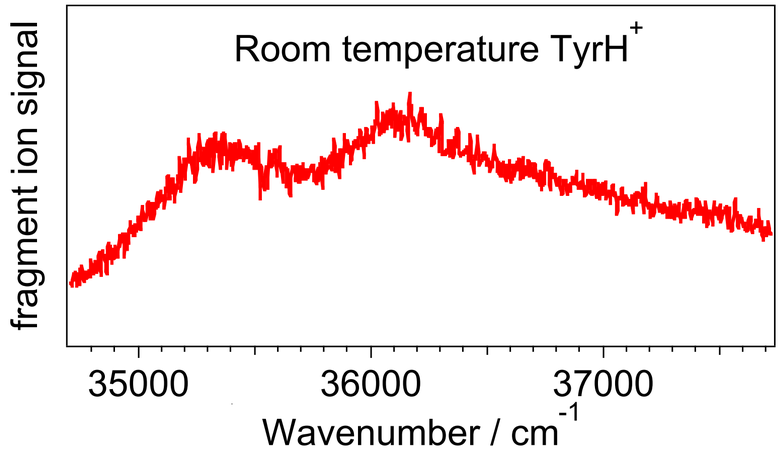
- Need UV chromophore
- Need long enough lifetime for lines to be sharp
- UV spectra are difficult to calculate

UV photodissociation spectroscopy

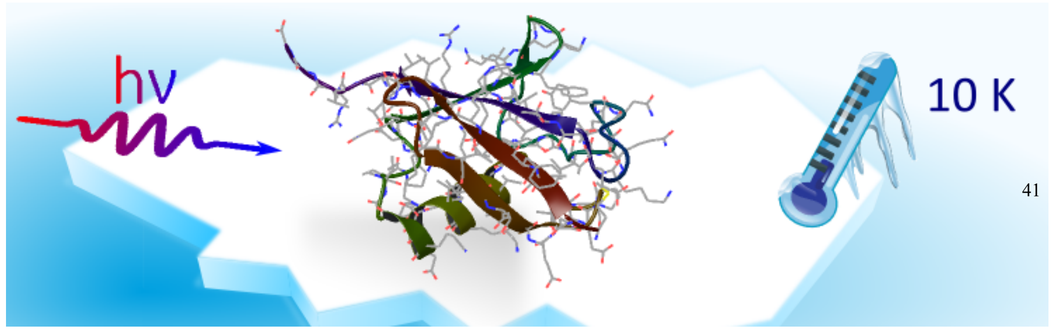


T = 6 K



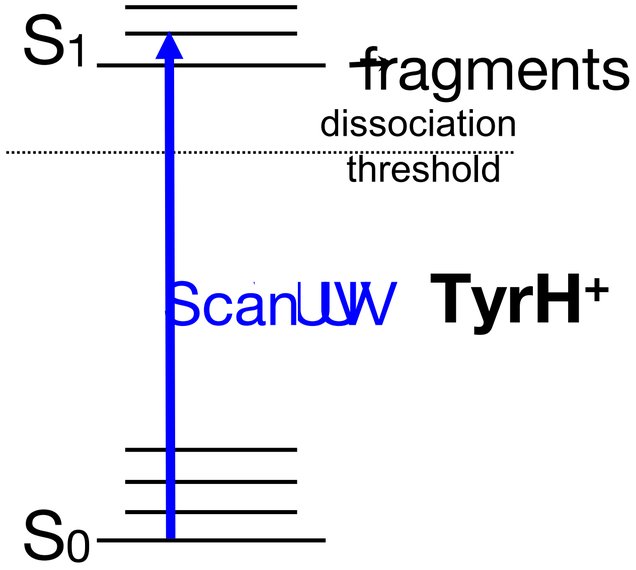


Gas-phase but solution-like

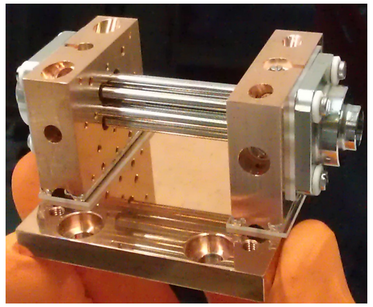


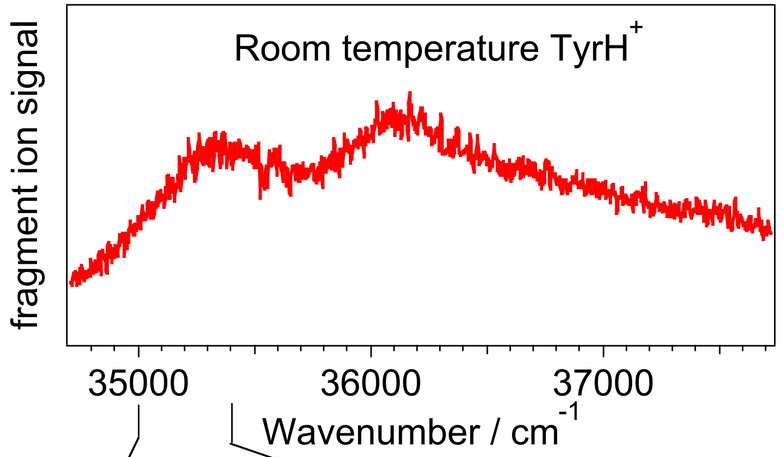
- UVPD produces charged fragments

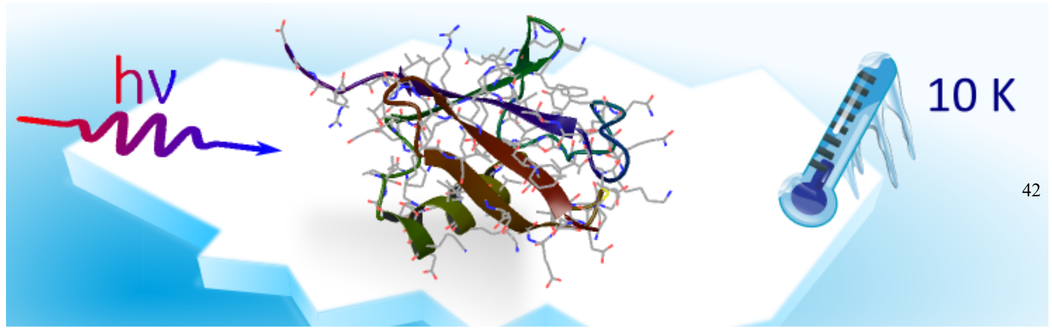
UV photodissociation spectroscopy of cold ions

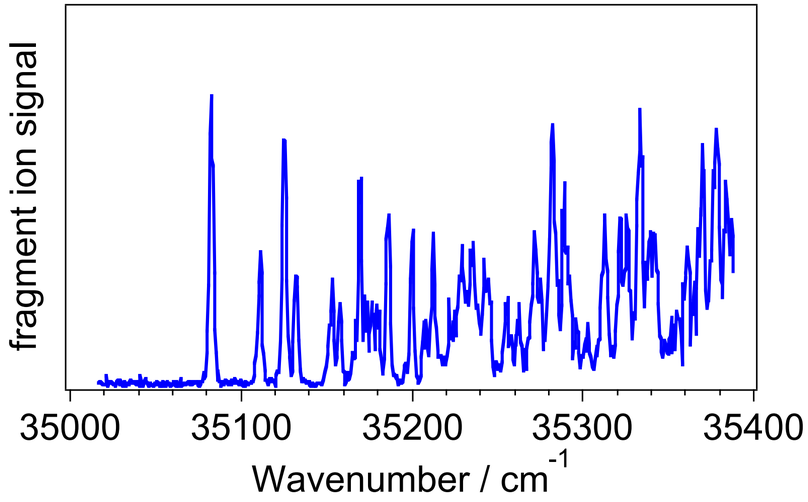


T = 6 K



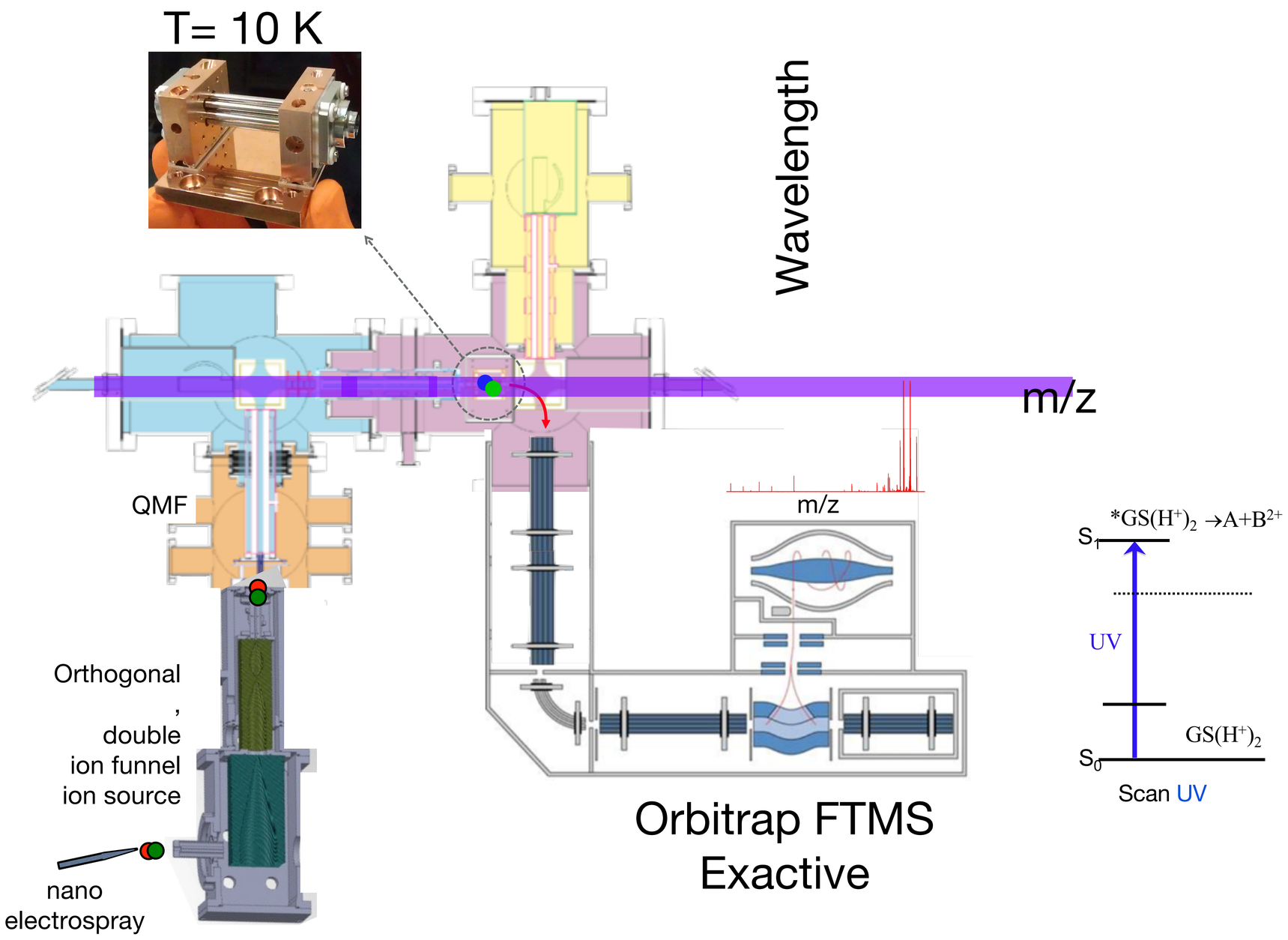






- Cooling enables vibrational resolution
- UVPD produces charged fragments

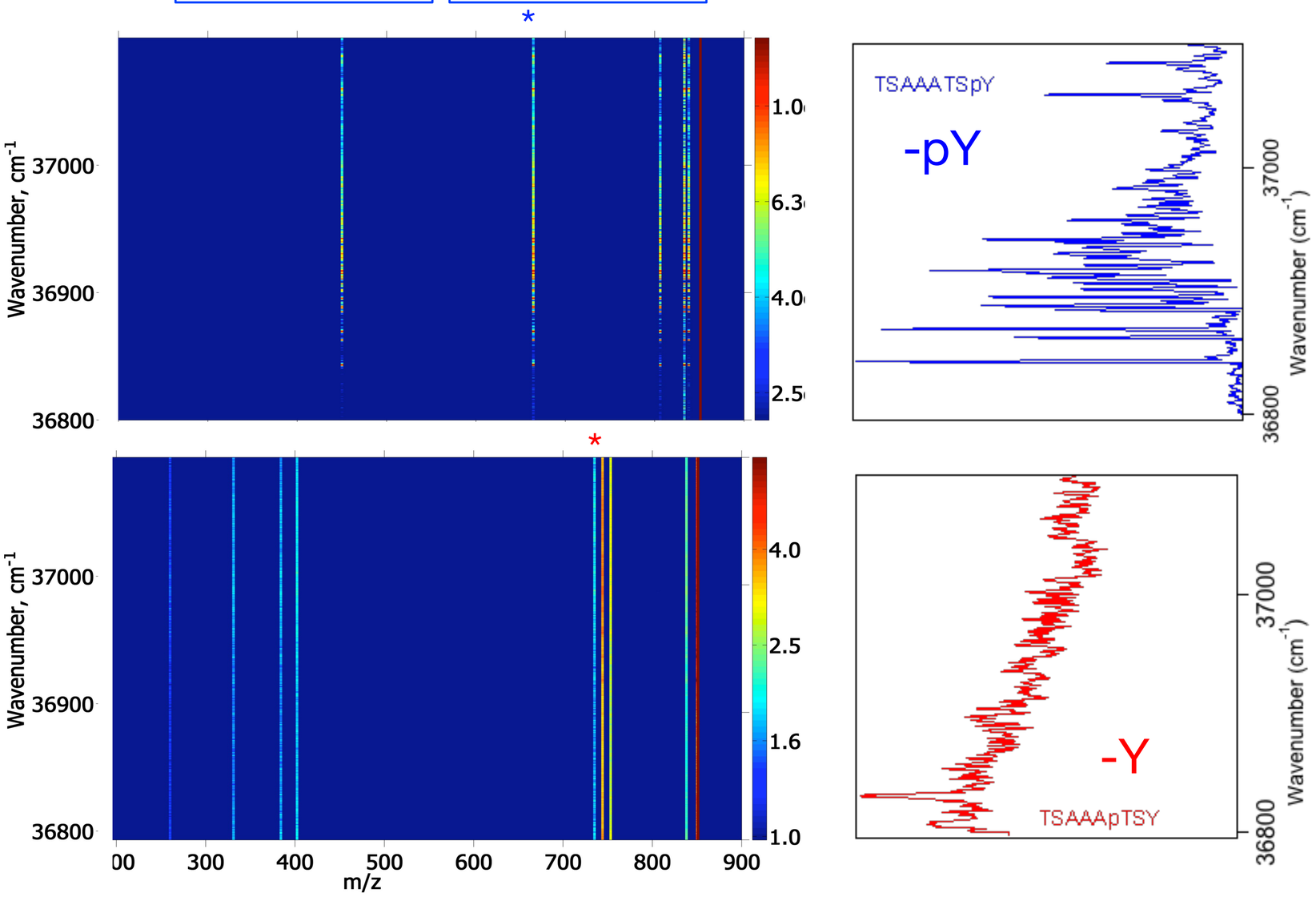
Cold ion spectrometer-Orbitrap MS



Identification of components in a mixture of isobaric peptides

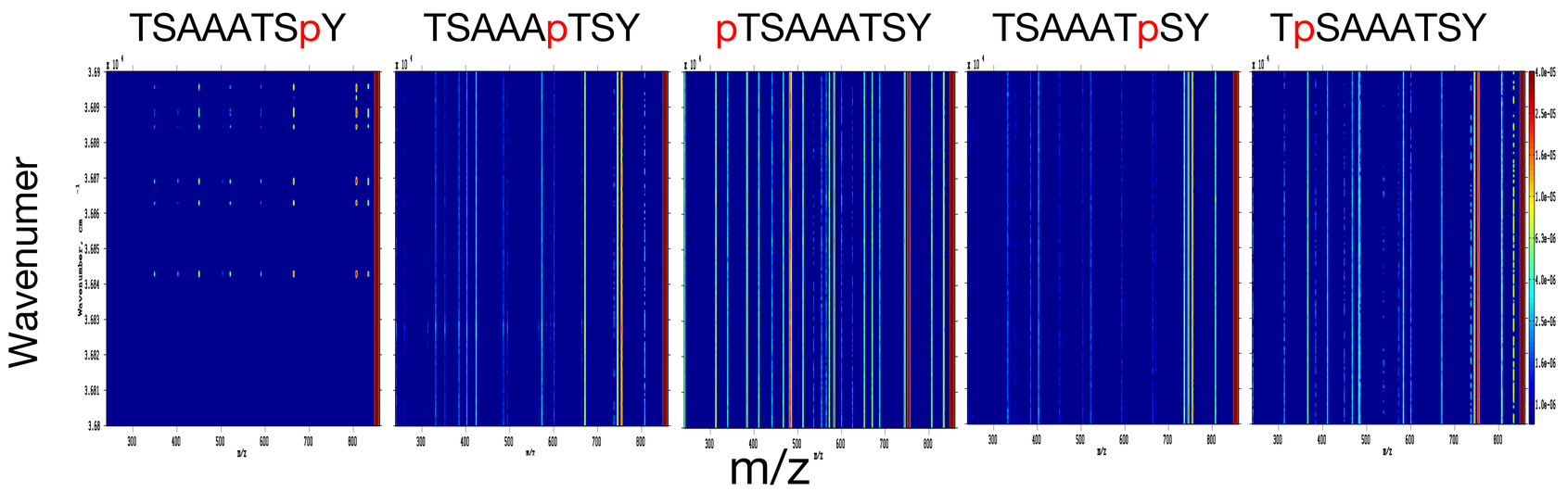
Isomeric phosphopeptides:

TSAAATSpY | TSAAApTSY

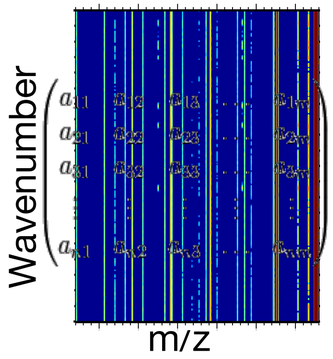


Identification of components in mixtures of isomeric peptides

2D UV-MS Library of ions with m=771.3525 Da

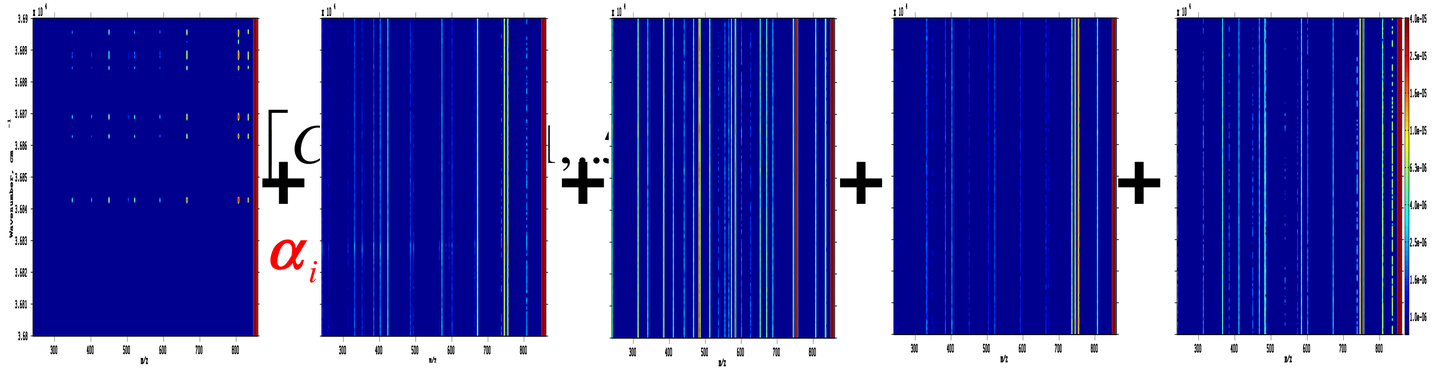


Unknown Solution mixture Calculated:



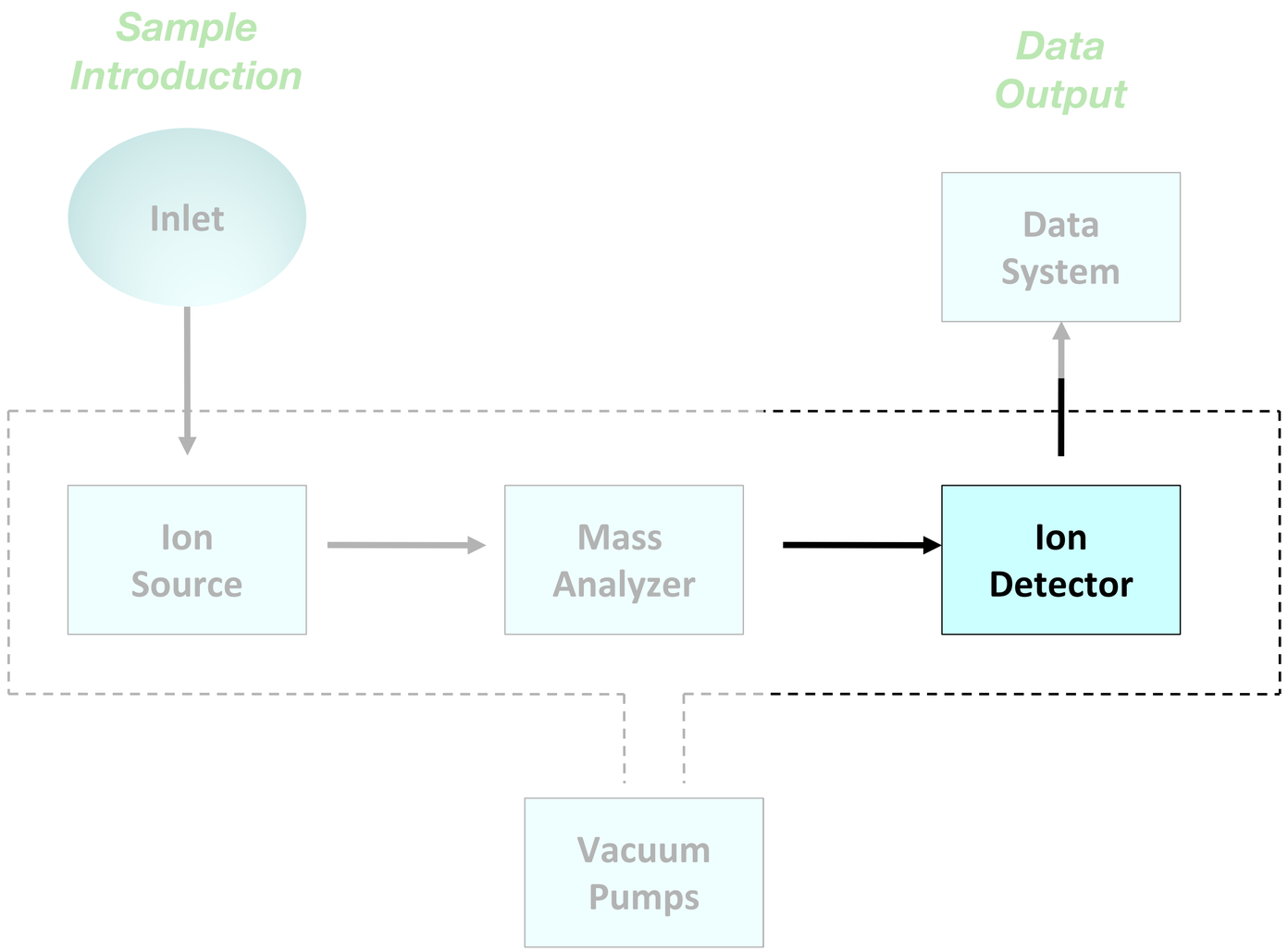
Potentially mixed isomers:

TSAAATSpY	TSAAApTSY	pTSAAATSY	TSAAATpSY	TpSAAATSY
49%	23%	1.5%	24%	2.6%
50%	25%	0%	25%	0%



Solution conc.:

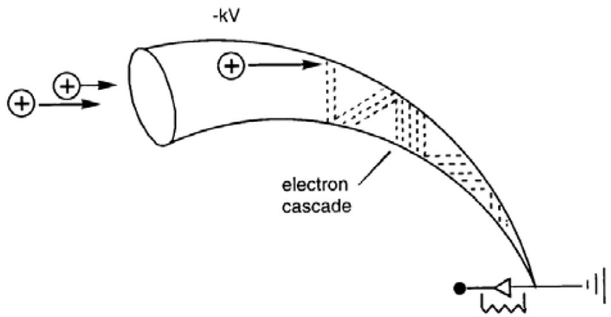
Ion detectors



Ion detectors for different mass analyzers

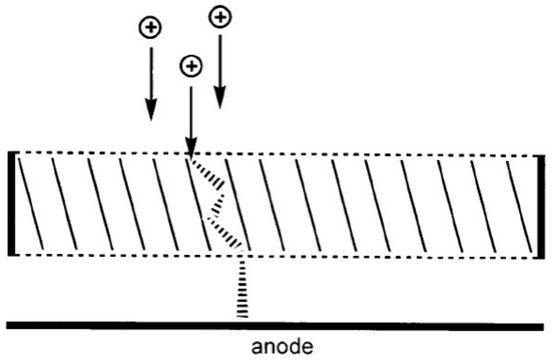
Electron multiplier

- Quadrupole
- Ion Trap



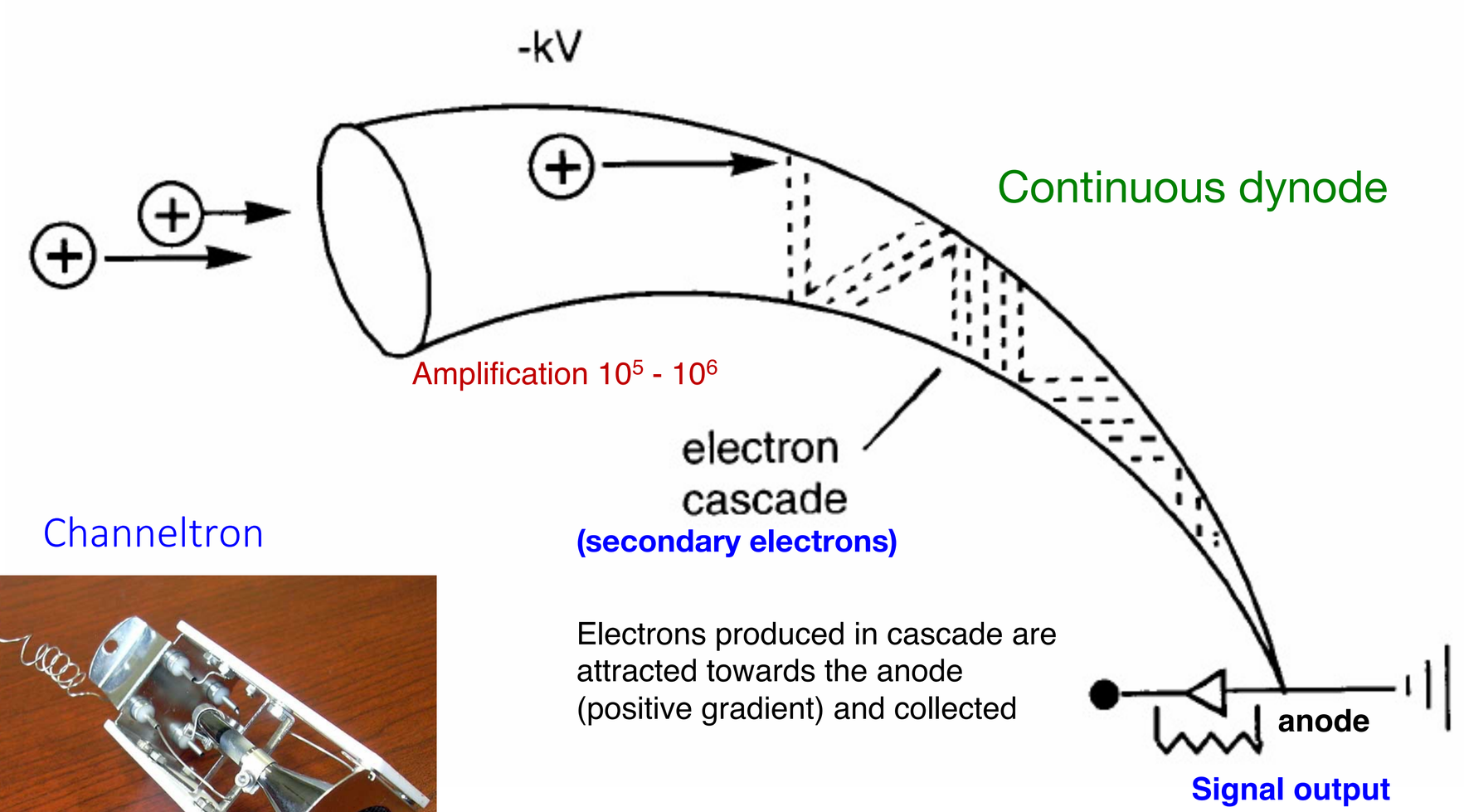
Multichannel plate

- Linear TOF
- Reflectron TOF



Continuous dynode electron multiplier

Vacuum-tube structure that multiplies incident charges by secondary emission of electrons

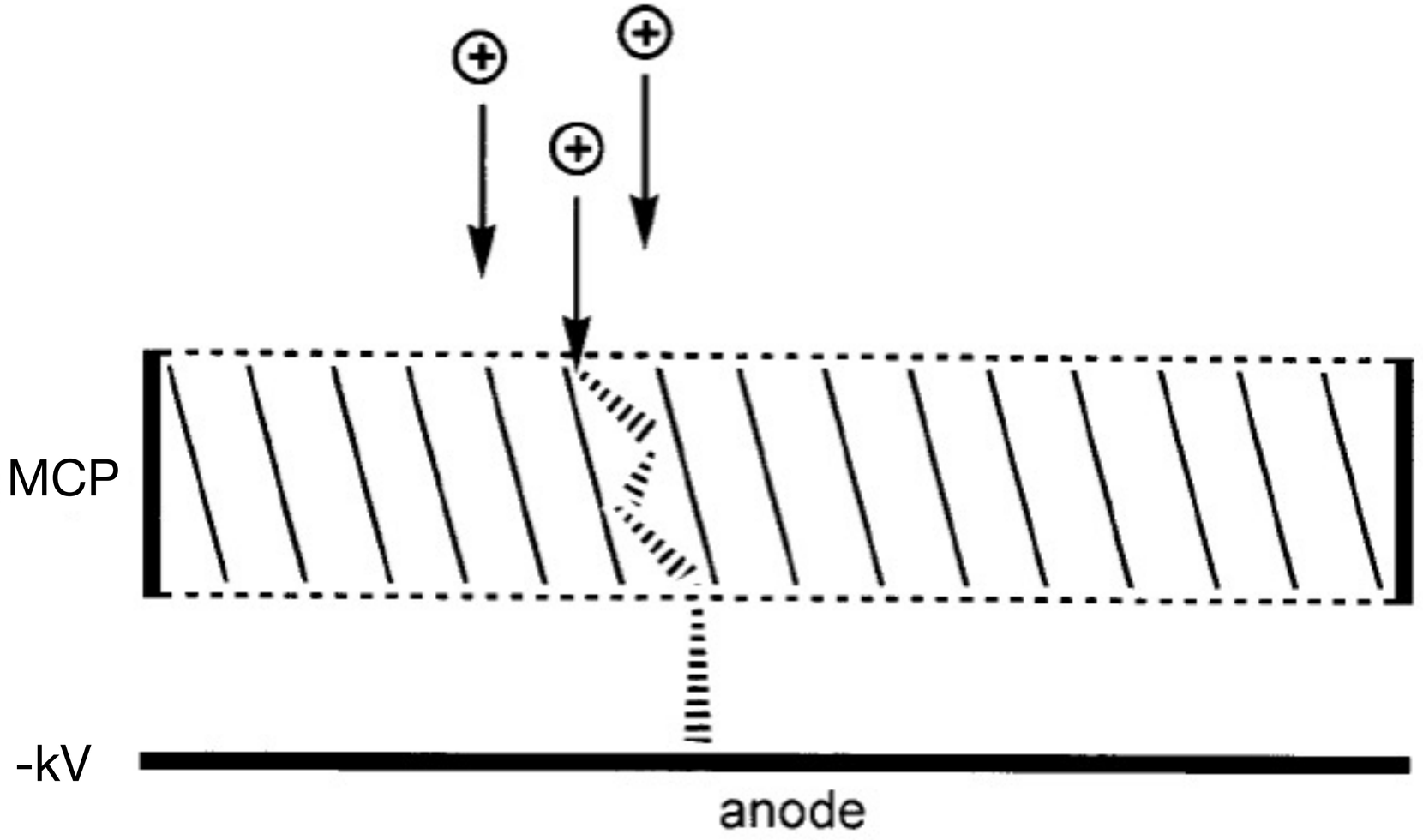


Channeltron

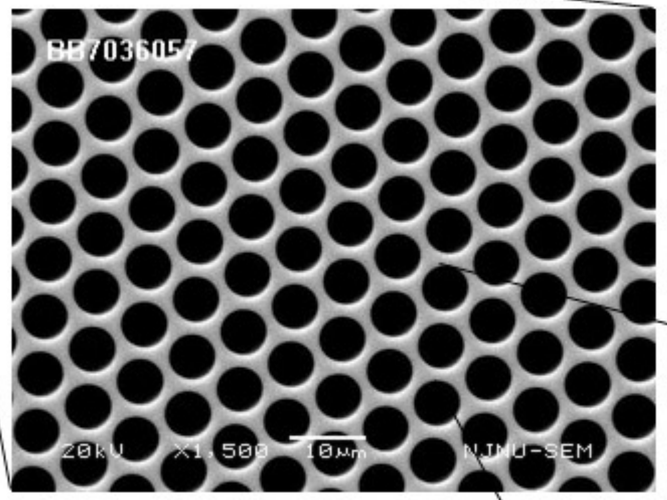
The final result is a measurable current proportional to the number of particles that hit the cathode

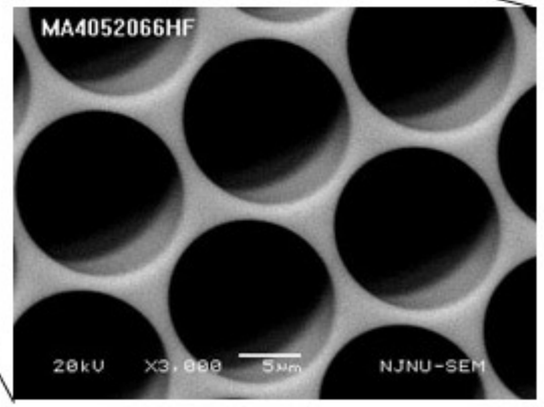
Microchannel plate detectors (MCP)

Just another geometry of a continuous dynode electron multiplier

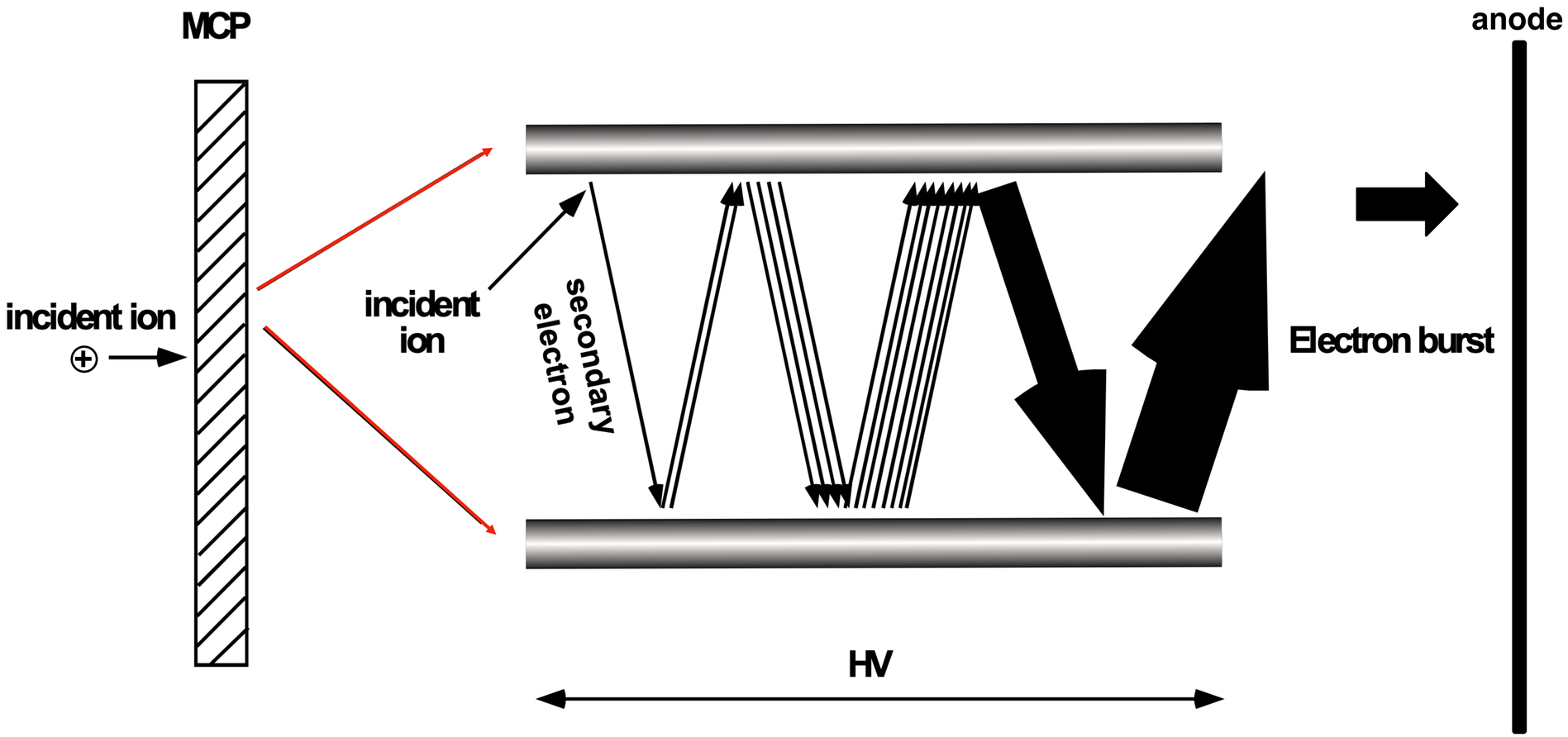


Microchannel plate detectors (MCP)





Microchannel plate detectors (MCP)



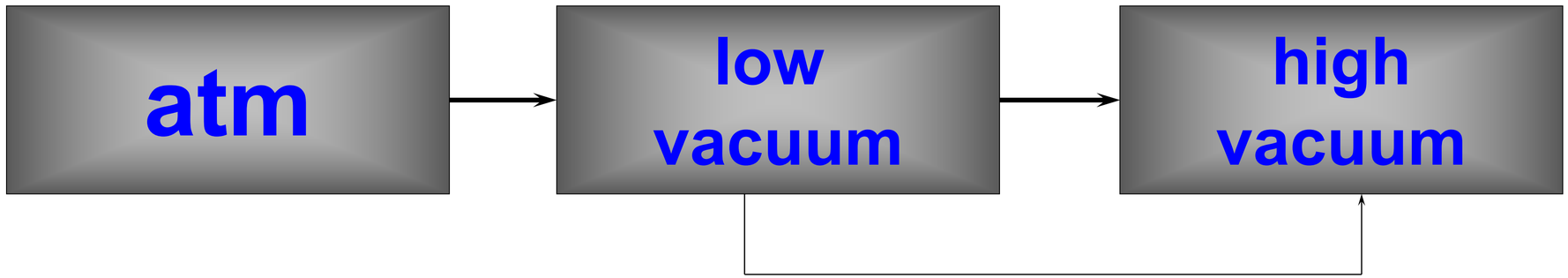
- Each channel is covered by a semiconductor substance
- Electron path very short: very fast response detector
- Well suited for TOFs which need precise arrival times and narrow pulse widths

Why Do We Need a Vaccum?

Reduce ion collisions with residual molecules (longer flight path, less pronounced non-desirable fragmentation and reactions, higher ion coherence, better ion focusing, analytical characteristics)

Prevent condensation from building up inside the instruments (coating lenses and contacts, creating electrical discharges)

Preventing detector from moisture and oxidation



Ion free path (vacuum requirements)

Boltzmann constant **temperature, K**

$$L = \frac{kT}{\sqrt{2}\,p\sigma} \qquad L \approx \frac{0.7}{n \cdot \sigma}$$

mean free path, m

pressure, Pa

cross-section, m²

$$\sigma = \pi \cdot d^2$$

Pressure units:

1 pascal (Pa) = 1 newton (N) m^{-2}
1 bar = 10^6 dyn cm^{-2} = 10^5 Pa
1 millibar (mbar) = 10^{-3} bar = 10^2 Pa
1 atmosphere (atm) = 1.013 bar = 101 308 Pa
1 Torr = 1 mmHg = 1.333 mbar = 133.3 Pa
1 psi = 1 pound per square inch = 0.07 atm

d- is the summ of the radii of the stationary molecule and the colliding ion

MS entrance → **MS detector**

atm → **10^{-10} Torr**

Mean free path (example)

Air molecular diameter ~ 0.375 nm

At 1 mtorr = 1.33×10^{-1} Pa (between medium and high vacuum)

At 300 K (near room temperature)

$$\lambda = \frac{kT}{\sqrt{2}\pi d_0^2 P} = \frac{\left(1.38\times10^{-23}\,\mathrm{J/K}\right)\left(300\mathrm{K}\right)}{\sqrt{2}\pi\left(1.33\times10^{-1}\mathrm{Pa}\right)\left(3.75\times10^{-10}\,\mathrm{m}\right)^2}$$

$$\lambda = 4.95\times10^{-2}\,m = 4.95\,cm$$

$P \cdot \lambda = 5$ (mtorr·cm)

Significance of mean free path

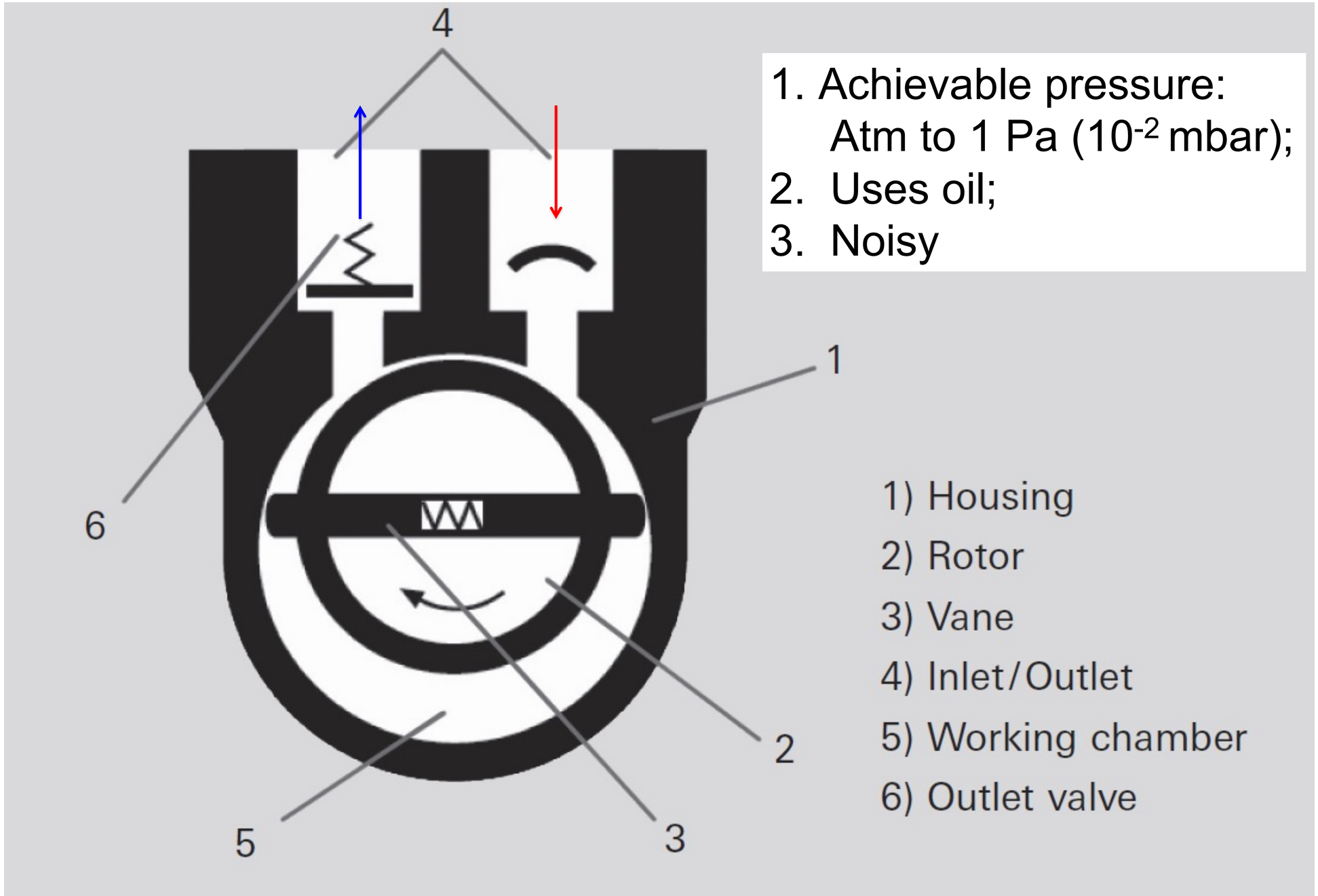
- $\lambda << L$ (chamber size)
  - Viscous flow; most collisions with other atoms
  - 10^{-4} torr to 10 torr
  - Mechanical pumps
- $\lambda >> L$
  - Molecular flow; most collisions with walls
  - 10^{-8} torr to 10^{-4} torr
  - Diffusion, Turbomolecular, and Cryogenic pumps.

Vacuum Quality Classification

Atmospheric pressure	760 Torr	101 kPa
Low vacuum	760 to 25 Torr	100 to 3 kPa
Medium vacuum	25 to 1×10^{-3} Torr	3 kPa to 100 mPa
High vacuum	1×10^{-3} to 1×10^{-9} Torr	100 mPa to 100 nPa
Ultra high vacuum	1×10^{-9} to 1×10^{-12} Torr	100 nPa to 100 pPa
Extremely high vacuum	$<1 \times 10^{-12}$ Torr	<100 pPa
Outer Space	1×10^{-6} to $<3 \times 10^{-17}$ Torr	100 µPa to <3fPa
Perfect vacuum	0 Torr	0 Pa

40 km @ 10^{-7} Pa

Rotary or Roughing Pumps



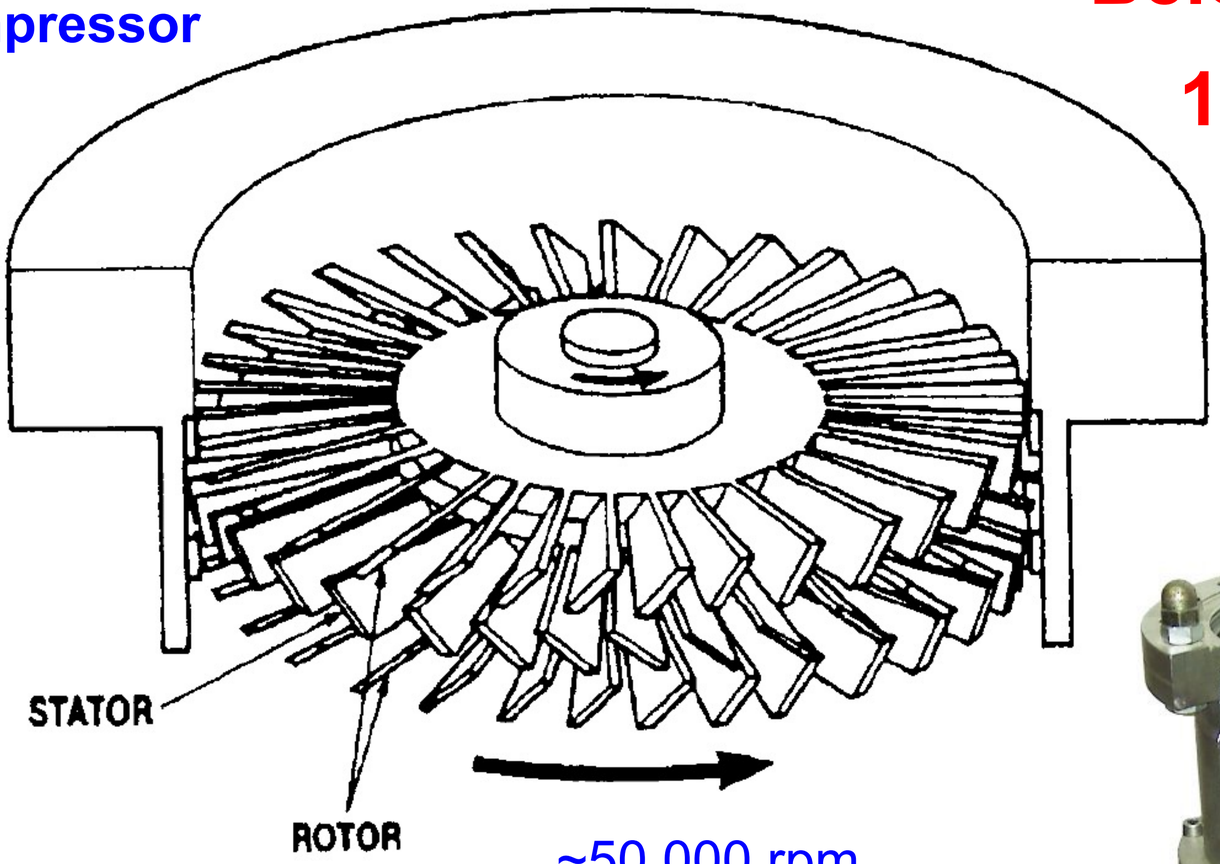
1. Achievable pressure:
   Atm to 1 Pa (10^{-2} mbar);
2. Uses oil;
3. Noisy

Turbomolecular Pumps

Below 10^{-6} Pa

10-100 L/s

Turbine compressor



~50 000 rpm

No oil exposed to the vacuum
Go to speed quickly than the diffusion pumps
Requires backing pump
Expensive

Vacuum System of FT-ICR MS