Part 1 : Mass spectrometry

Chapter 5 : Tandem mass spectrometry

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Tandem mass spectrometry

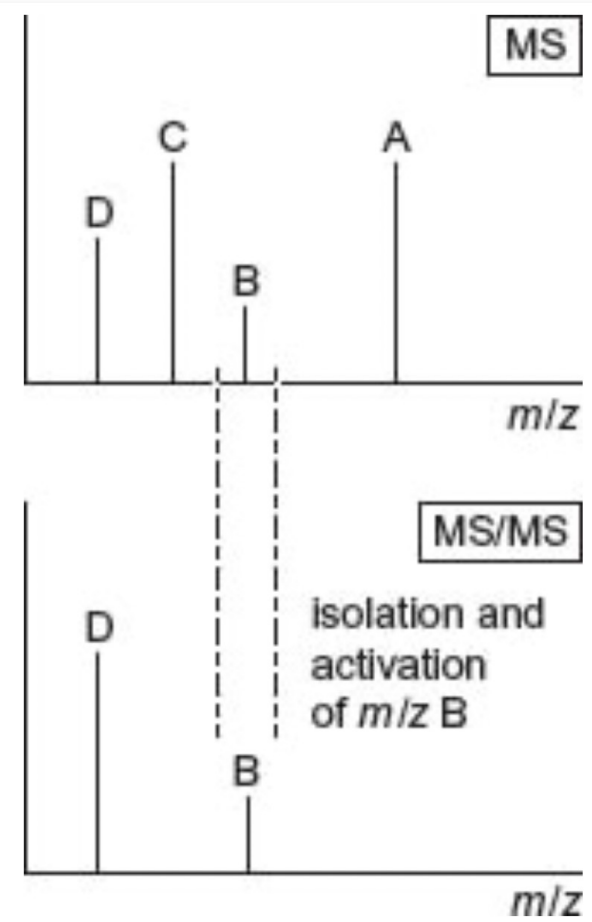
Introduction

- As well as being able to make accurate *m/z* measurements from which relative molecular masses can be determined, mass spectrometry is also capable of providing structural information through the generation of fragment ions.
- Some methods of ionization, such as **electron ionization (EI),** impart sufficient internal energy into the newly formed molecular ion to promote a significant amount of fragmentation. **(hard ionization)**
- Other types of ionization, such as electrospray ionization and field ionization, produce ions with very low residual energies and induce little or no fragmentation. **(soft ionization)**
- In all of these cases, ions can be **isolated inside tandem mass spectrometers**, according to their m/z, and dissociated to enable examination of the resulting products.

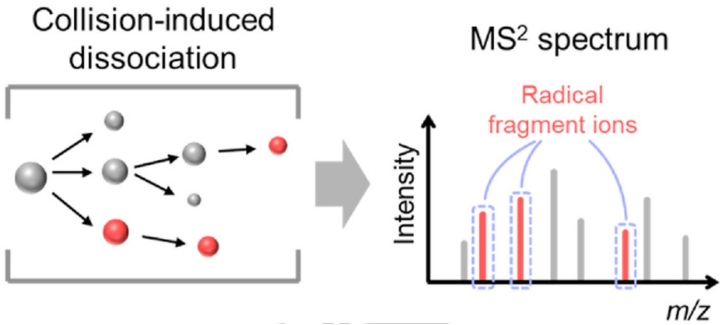
Tandem mass spectrometry

Introduction

- Tandem mass spectrometry, or mass spectrometry/mass spectrometry (MS/MS), is the acquisition and study of the spectra of ions following m/z selection.
- It is usually combined with a method for inducing ion dissociation by energy transfer (activation).
- Figure on the right shows how an MS/MS spectrum can be generated by isolation and activation of an ion from the corresponding MS spectrum.



Ion dissociation

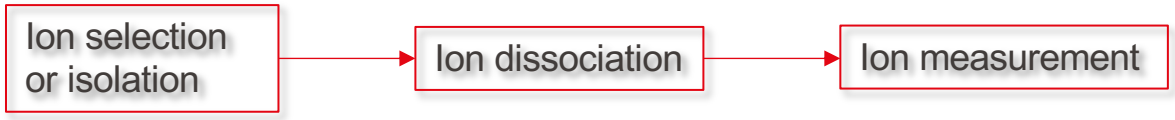


Shipei Xing, Tao Huan, Radical fragment ions in collision-induced dissociation-based tandem mass spectrometry, Analytica Chimica Acta, Volume 1200, 2022, doi.org/10.1016/j.aca.2022.339613.

Tandem mass spectrometry

Ion dissociation

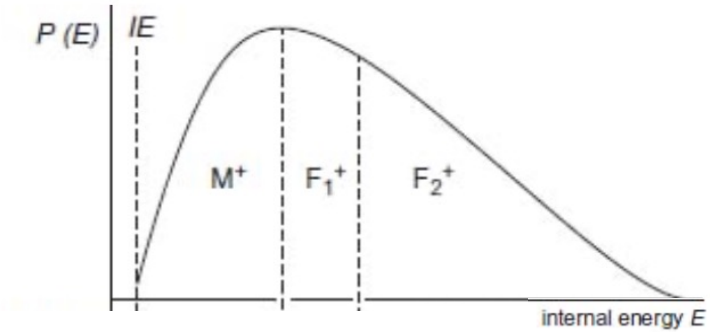
- Precursor ion => Product ions
- or ‘Parent ion’ => ‘Daughter ion’ but in principle outdated



- In order for ions to dissociate they need to possess internal energies E > activation energy E_0, such that chemical bonds can be broken.
- Ions generated by EI, for example, often possess energies that exceed E_0, and so some fragmentation takes place as a consequence of the ionization process.
- Peaks corresponding to fragment ions appear in the MS spectrum.
- Softer ionization methods (CI, APCI, MALDI) usually require an extra energy transfer step which provides protonated molecules with energies greater than E_0.

Tandem mass spectrometry

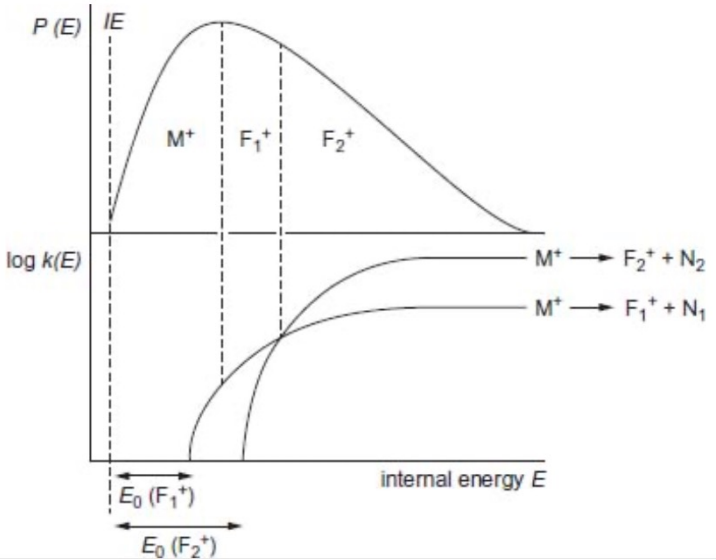
Ion dissociation



- Left figure **shows the probability P(E) of a population of ions possessing some value of internal energy E.**
- At the lower end (left) of the energy distribution, we see ions which have **insufficient internal energy to dissociate**, and are therefore detected as intact molecular ions **M^+** (EI) or protonated/deprotonated molecules (ESI, MALDI, APCI, etc.).
- At the higher-energy end (center, right), we see ions that **possess internal energies > E_0**, and that can therefore dissociate to the **fragment ions F_1^+ and F_2+.**
- In the case of EI, this fragmentation takes **place in the source.**
- In soft ionization methods, such as ESI, it may require additional activation, e.g. **in a collision cell**

Tandem mass spectrometry

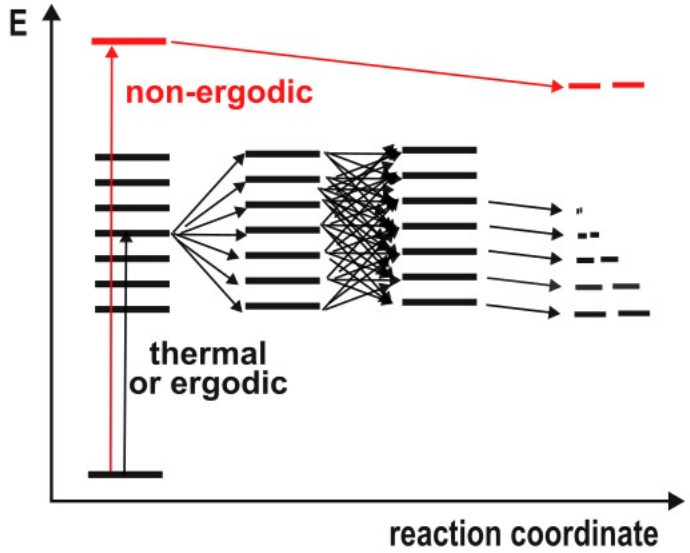
Ion dissociation



- **Wahrhaftig diagram** includes, below the probability distribution, a **complementary rate plot of *logk(E)* in function of the internal energy for dissociation** of the molecular ion or protonated/deprotonated molecules to F_1^+ and F_2^+.
- Although **dissociation to F_1^+ occurs at a lower value of E_0 than for F_2^+, the latter is the dominant process due to its enhanced rate at higher values of internal energy, E.**
- The result of this is that F_2^+ will be the major fragment ion in the mass spectrum.
- **Wahrhaftig diagrams** can be used to **rationalize fragment ion abundance in a mass spectrum.**

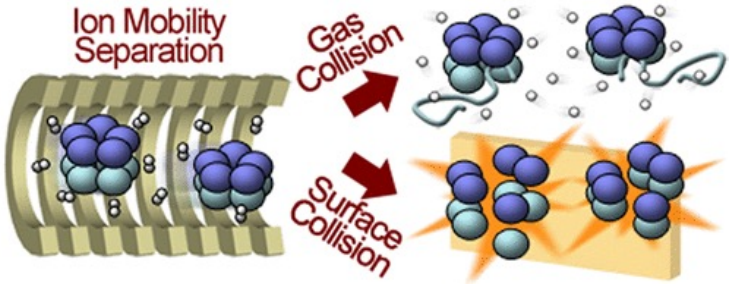
Tandem mass spectrometry

Ion dissociation



- For most methods of ion activation, **the rate of dissociation is much slower than the rate of energy dissipation throughout the ion**.
- => energy **is statistically distributed prior** to bond cleavage, **the process is said to be thermal, or ergodic**.
- There are a **few methods of ion activation where dissociation is extremely fast** such that **energy is not spread** throughout the ion before bond fission (i.e. the process is non-ergodic).

Methods of ion activation and dissociation for MS/MS



Anal. Chem. 2012, 84, 14, 6016–6023
doi.org/10.1021/ac300810u

Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

Collision-induced dissociation CID

- **Collision-induced dissociation (CID),** sometimes known as **collisionally activated dissociation (CAD),** is the **most commonly used method** for ion dissociation in MS/MS.
- Ions are **accelerated to high kinetic energy by an electric field**, and **collided with neutral gas molecules/atoms** such as argon, nitrogen, or helium.
- A portion of the precursor ion's kinetic energy is converted into internal energy to induce fragmentation.
- The **total amount of energy transferable** is the **centre-of-mass energy** E_{com} , which is a function of the **ion's translational kinetic energy** E_{lab} and **the masses of the colliding particles** (equation on the right).

$$E_{com} = \left(\frac{m_N}{m_p + m_N}\right) E_{lab}$$

Where, m_N is the mass of the neutral gas molecule/atom, and m_p is the mass of the precursor ion.

Tandem mass spectrometry

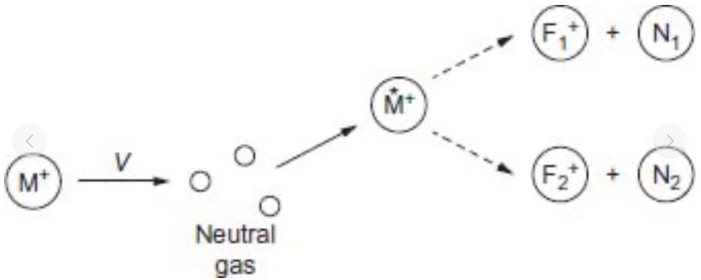
Methods of ion activation and dissociation for MS/MS

Collision-induced dissociation CID

- E_{lab}, being a kinetic energy, can be calculated from the product of ionic charge z and accelerating voltage V:

$$E_{lab} = zeV$$

- **General mechanism of CID :** The illustration below shows a generic positively charged ion. In reality this could be a radical cation, radical anion, or positively or negatively charged molecule



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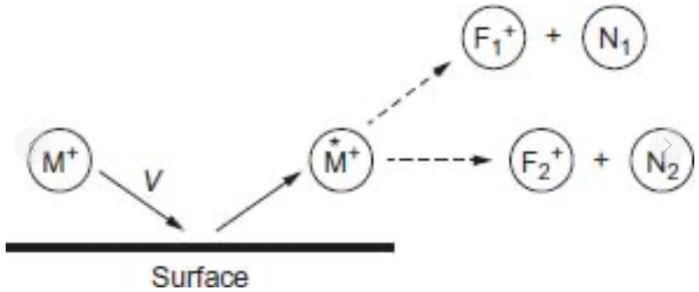
Methods of ion activation and dissociation for MS/MS

Surface-induced dissociation SID

- This method of ion dissociation resembles CID in that **ions are accelerated to high kinetic energy**, but **an inert solid surface is used as the target instead of neutral gas molecules**.



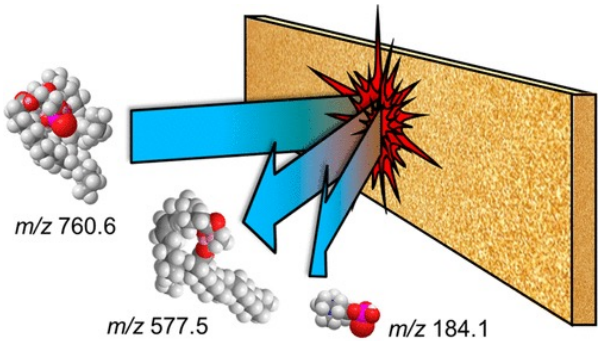
- An **difference between SID and CID** is the **mass of the neutral collision partner**. With SID, E_{com} **is no longer limited by the mass of the gas molecules, and** $E_{com} \approx E_{lab}$

Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

Surface-induced dissociation SID

- SID is characterized by a single collision producing excited-state **precursor ions with a relatively narrow distribution of internal energies**.
- **SID offers greater control over energy transfer than CID**, since all ions strike the surface at essentially the same angle.
- As with CID, **SID can be used for MS/MS experiments on both small molecules and biomolecules.**
- Currently, SID is finding application in the **study of native multiprotein complexes**, where its properties of single collision at well-defined energy provide charge-partition and dissociation modes complementary to those seen by low-energy CID.

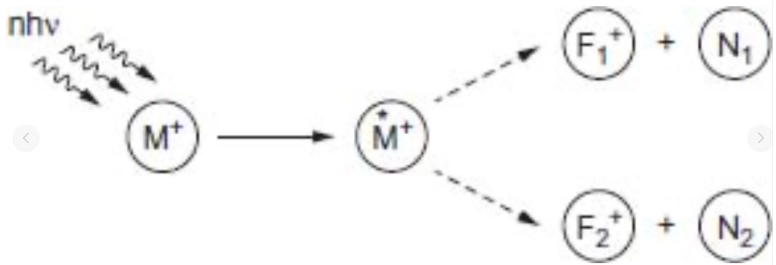


Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

Infrared multiphoton dissociation IRMPD

- Infrared multiphoton dissociation (IRMPD) is the **most common photodissociation method** used for ion activation and dissociation.
- Precursor ions are **trapped and exposed to IR radiation**, most commonly from a **CO_2 laser operating at 10.6 µm.**
- The **stepwise absorption of multiple photons results in activation of vibrational modes** within the ion. **Rapid redistribution of energy occurs prior to bond cleavage (ergodic)** and, as a consequence, **thermal-type fragmentation is seen**.



Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

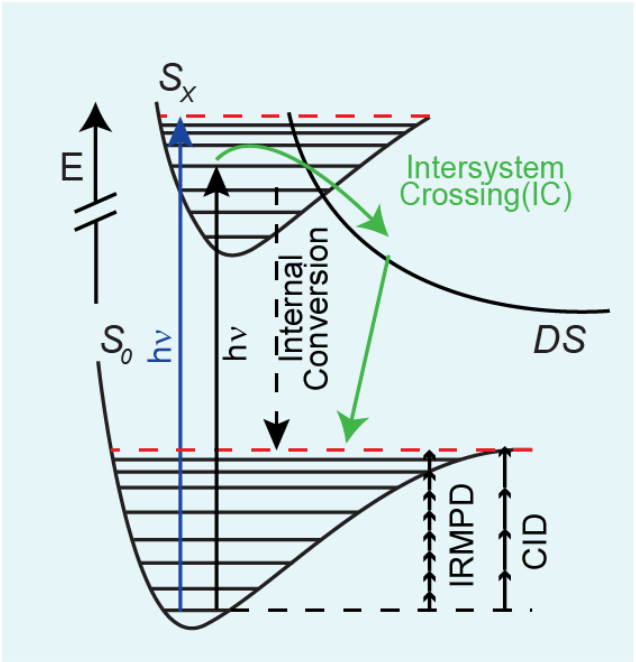
Infrared multiphoton dissociation IRMPD

- Efficient IRMPD requires **precursor ion trapping**, and for this reason it is usually performed in **FTICR or ion-trap** instruments.
- In these cases, irradiation takes place directly within the mass analyser, via an optical window.
- Depending on the size of the ion cloud, IRMPD typically requires irradiation times of 10–100 ms.
- The advantages of IRMPD are the **delivery of well-defined energy (0.117 eV per photon at 10.6 μm) and efficient fragmentatio**n.
- Disadvantages include the **need for a vibrational mode capable of absorbing the IR radiation (not normally a problem at 10.6 μm),** and the presence of a trapping device in the instrument.
- IRMPD can be used to induce MS/MS fragmentation for **small and large molecules** in a manner similar to low-energy CID.

Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS
UV photodissociation UVPD

- By using the UV range of the EM spectrum, electronic states of the precursor ion are excited in the first instance.
- This may lead to **direct dissociation of the electronically excited state**, or indirect dissociation via dissipation into vibrational modes within the ion.
- A commonly used **wavelength is 193 nm**, generated by an ArF excimer laser.
- **Photon delivers 6.4 eV of energy, meaning that single photon absorption is sufficient to induce bond fission in even large protein ions.**



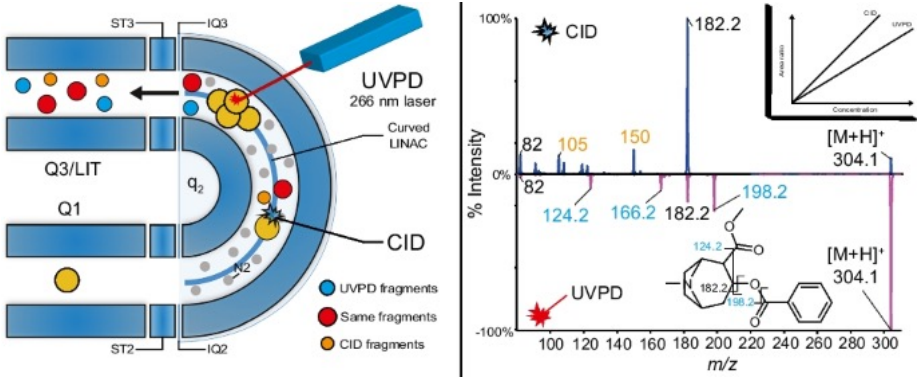
https://www.unigiessen.de/en/faculties/f08/departments/iaac/spengler/data_heiles/uvpd-msMSI

Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

UV photodissociation UVPD

- Like IRMPD, UVPD usually requires some form of **ion trapping for efficient operation**, but the **need for only single-photon absorption reduces irradiation times**.
- Like the other MS/MS ion activated methods discussed so far, UVPD **can be applied to both small molecules and large biomolecules**.
- The presence of an **aromatic chromophore often enhances fragmentation efficiency**, but is not essential, as most organic molecules exhibit some absorption at 193 nm.
- UVPD **is particularly attractive in the analysis of peptides and proteins**, where its fast, high-energy activation produces a rich array of fragments and makes it suitable for the analysis of protein modifications



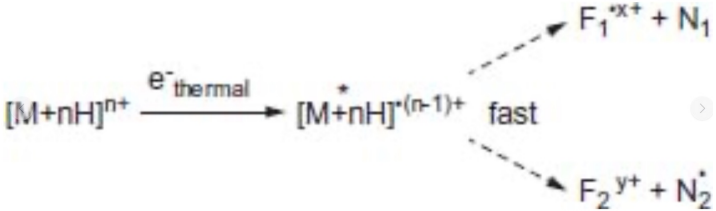
Giraud, R., Le Blanc, Y.J.C., Guna, M. *et al.* Ultraviolet photodissociation and collision-induced dissociation for qualitative/quantitative analysis of low molecular weight compounds by liquid chromatography-mass spectrometry. *Anal Bioanal Chem* **415**, 7117–7126 (2023). https://doi.org/10.1007/s00216-023-04977-0

Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

Electron-capture dissociation ECD

- In electron-capture dissociation (ECD), multiply-charged cations interact with low-energy electrons.
- The ions capture these electrons, resulting in charge reduction and an increase in energy.
- Removal of an unpaired electron from a multiply-charged odd-electron cation $[M+nH]^{\bullet n+}$ requires 5–7 eV, hence, if an electron is captured by an even-electron species $[M+nH]^{n+}$ to give $[M+nH]^{\bullet(n-1)+}$, 5–7 eV of energy is deposited into the ion, which can induce dissociation



Example : Activation and dissociation of an ion $[M+nH]^{n+}$ by ECD (thermal electrons) generate fragment ions $F_1^{\bullet x+}$ and F_2^{y+}, and associated neutrals (N_1 and N_2).

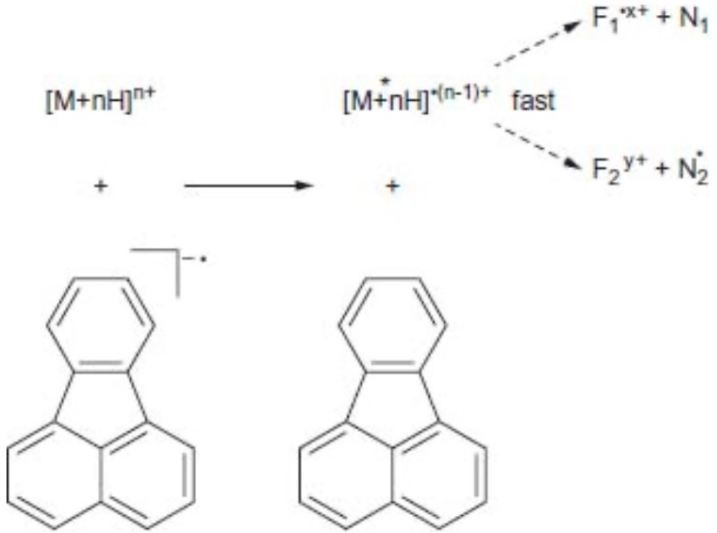
Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

Electron-transfer dissociation ETD

- This method of ion activation and dissociation is similar to ECD in that it delivers electrons to multiply-charged cations to induce fragmentation.
- The difference between ETD and ECD is that, in ETD, an electron-transfer reagent is used, rather than electrons themselves.

Example : ETD process with fluoranthene as the transfer reagent.

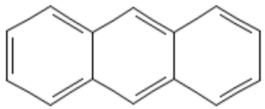


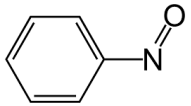
Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

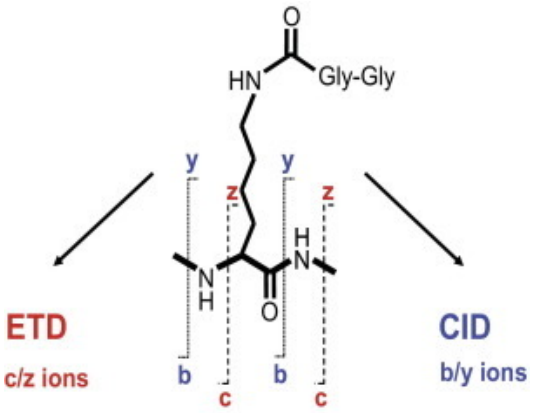
Electron-transfer dissociation ETD

- **Multiply-charged cations** (usually peptides produced by ESI) are allowed to **react with fluoranthene radical anions**, which are produced by a **dedicated ETD chemical ionization (CI) source**.
- Upon transfer of an electron, the charge-reduced, activated radical cation fragments by mechanisms similar to those in ECD.
- **ETD can be performed in beam instruments**, **as well as ion traps**, providing there is a region where ions can be stored for approximately 100 ms to enable the transfer reaction.
- Other reagents used as electron donors in ETD include anthracene and nitrosobenzene.





- The advantage is that it can be performed in ion traps and other trapping devices that are not suitable for ECD.



Frank Sobott, Stephen J. Watt, Julia Smith, Mariola J. Edelmann, Holger B. Kramer, Benedikt M. Kessler, Comparison of CID Versus ETD Based MS/MS Fragmentation for the Analysis of Protein Ubiquitination, Journal of the American Society for Mass Spectrometry, Volume 20, Issue 9, 2009, Pages 1652-1659, doi.org/10.1016/j.jasms.2009.04.023.

Tandem mass spectrometry

Methods of ion activation and dissociation for MS/MS

Fragmentation approach	Acronym	Energy	Common analyser combinations	Applications	Usage
Collision-induced dissociation	CID/CAD	High & Low	Q-TOF, Q-Orbitrap, Q-FTICR, QqQ, IT, Q-IT	Proteomics, peptide, and small molecule analysis	High
Surface-induced dissociation	SID	High & Low	Q-TOF, Q-FTICR, QqQ, IT, Q-IT	Native multiprotein complexes and small molecules	Low
Infrared multiphoton dissociation	IRMPD	Low	FTICR, IT	Small and large molecules	Medium
UV photodissociation	UVPD	Low	FTICR, IT	Peptides and proteins	Low
Electron-capture dissociation	ECD	Low	FTICR	Peptides, proteins and synthetic polymers	Medium
Electron-transfer dissociation	ETD	Low	IT, IT-Orbitrap, Q-TOF	Proteins and oligomers, phosphoproteomics	Medium

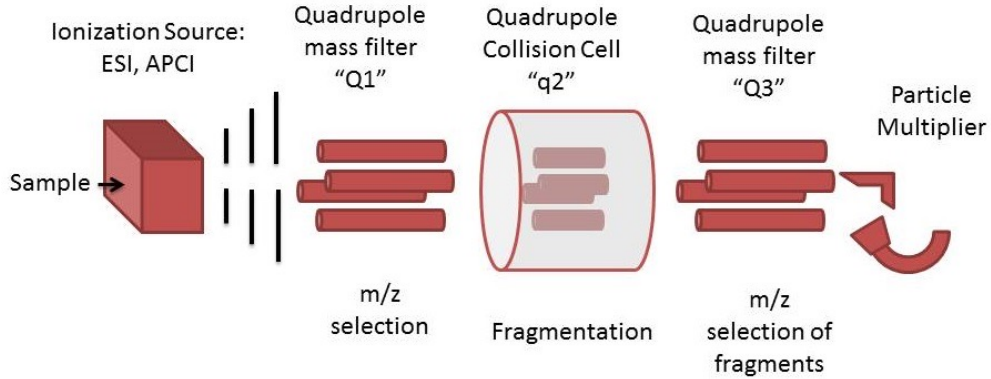
MS/MS instruments

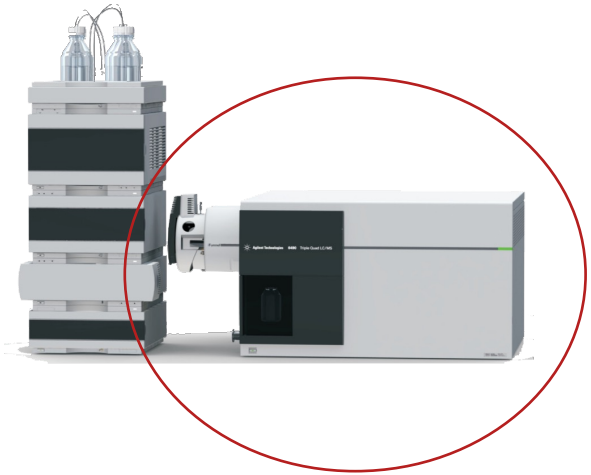
Tandem mass spectrometry

MS/MS instruments

Tandem quadrupole analysers

- Quadrupoles can be combined in series to produce a tandem quadrupole instrument.
- The most well-known arrangement is the triple-quadrupole (TQ), where Q1 and Q3 are used as mass analysers, and q2 (it is convention to designate a non-mass separating quadrupole with lower-case q) is operated in a collision cell in RF-only mode.





https://en.wikipedia.org/wiki/Triple_quadrupole_mass_spectrometer

Tandem mass spectrometry

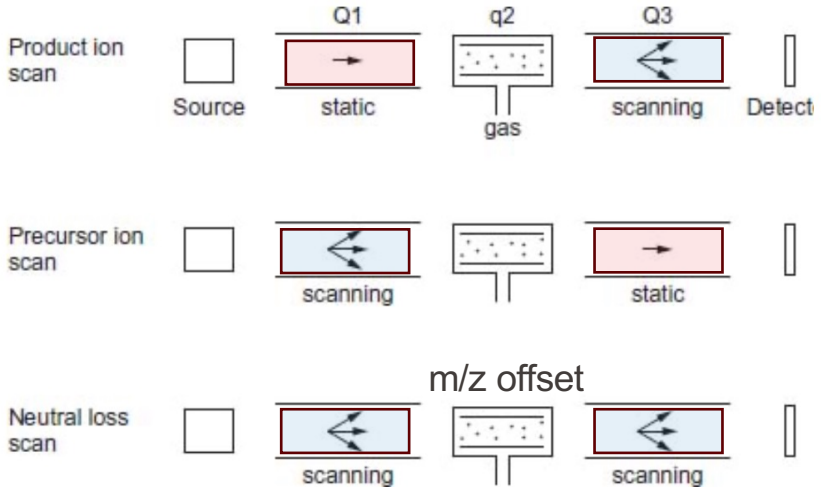
MS/MS instruments

Tandem quadrupole analysers

- In **product ion scan mode**, Q1 is set to transmit a particular precursor ion m/z (sim), and Q3 is scanned for the product ions resulting from CID of that precursor in the collision cell (scan). This way the fragment ions originating for the chosen precursor can be studied.

- **In precursor ion scan mode**, Q3 is set to transmit a particular product ion m/z (sim) and Q1 is scanned for precursors that give rise to the product during CID in the collision cell (scan). Precursor ion scanning allows all the precursors of a particular product to be identified.

- **In neutral ion scanning**, both Q1 and Q3 are scanned with an m/z offset corresponding to a particular neutral loss (e.g. 44 for CO_2). This experiment allows all ions which fragment with that neutral loss to be identified.



Tandem mass spectrometry

MS/MS instruments

Tandem quadrupole analysers

- **Tandem quadrupole mass spectrometers are powerful and flexible instruments, providing high selectivity and sensitivity.**
- **They can be coupled to a full range of ion sources**, although their limited mass range (**approximately m/z 4000**) makes their use with MALDI sources impractical.
- **ESI sources are by far the most commonly utilized**.
- Tandem quadrupoles are often employed **in combination with HPLC** for quantifying trace impurities and metabolites

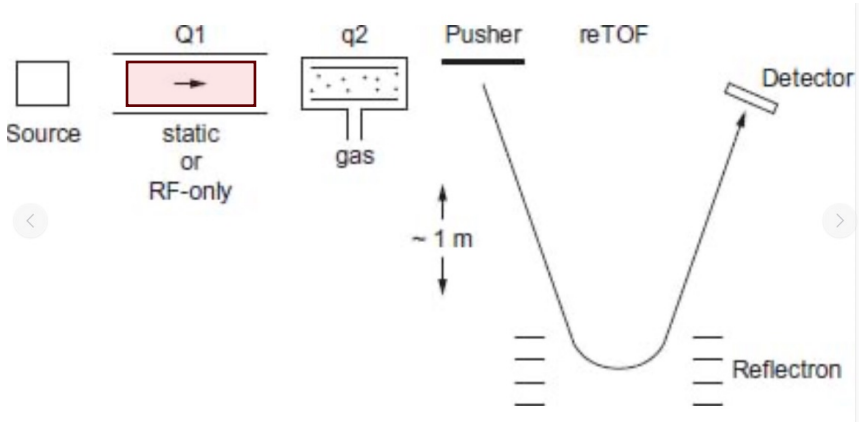
Agilent - 8900 Triple Quadrupole ICP-MS

Tandem mass spectrometry

MS/MS instruments

Quadrupole-time-of-flight analysers

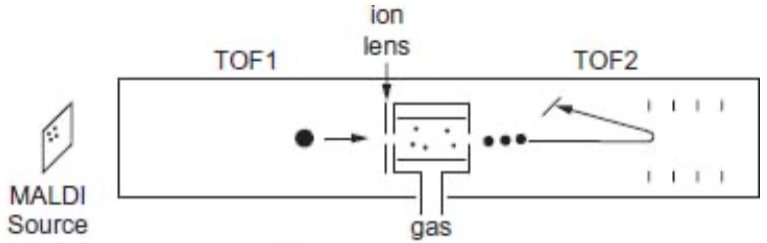
- **Q-TOFs** can be thought of as variants of tandem quadrupoles where **Q3 is replaced by a time-of-flight (TOF)** analyser (**almost always a reflectron TOF).**
- TOF analysers have a number of advantages over quadrupoles, **especially in m/z range and resolving power.**
- As with tandem quadrupoles, **the main form of MS/MS activation is low-energy CID**, although commercial instruments are available with ETD capability in the collision cell.



Tandem mass spectrometry

MS/MS instruments
TOF/TOF analysers

- This type of tandem instrument is used **almost exclusively with MALDI sources**, and there **are two common types**.
- **The first** is essentially two TOF analysers in sequence with a collision cell placed between them.
- **In MS/MS mode the first TOF is used to select precursor ions of particular m/z** based on their flight time taken to reach the collision cell.

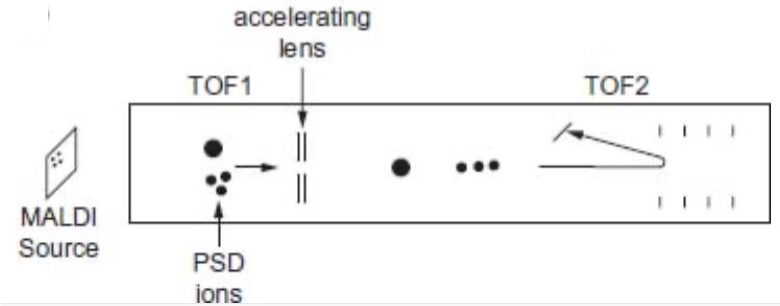


- **An ion lens is placed just before the collision cell, and is opened for a narrow time window to allow the selected m/z to pass through**.
- Following **high-energy CID**, the product ions are analysed using the **second TOF**, usually operated in reflectron mode.

Tandem mass spectrometry

MS/MS instruments

TOF/TOF analysers

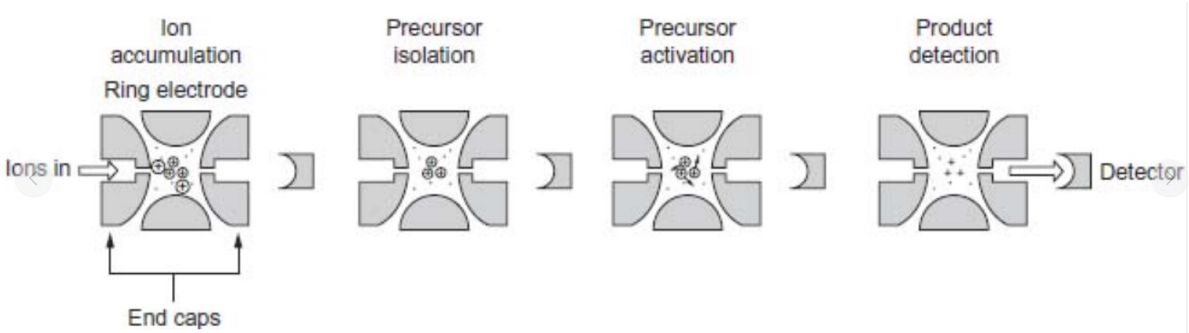


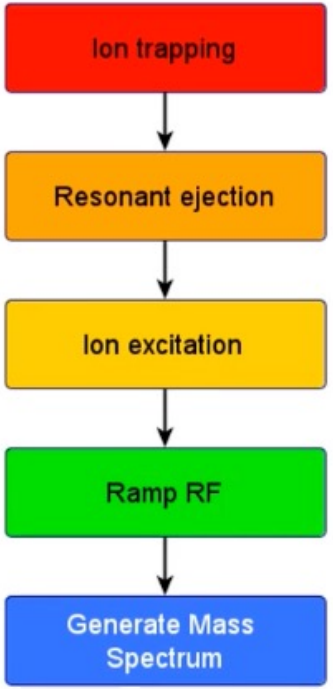
- This type of tandem instrument is used **almost exclusively with MALDI sources**, and there **are two common types**.
- **The second TOF/TOF** design utilizes post-source decay (PSD) of precursor ions to produce an MS/MS spectrum.
- Ions that are accelerated out of the MALDI source intact, but fragment before they reach the detector, have the same velocity as the precursor and hence the same flight time.
- **If, however, they are subjected to a second round of acceleration, after fragmentation, they will possess different velocities, as they have different masses from the original precursor.**
- This means that the **fragment ions will be detected at a time of flight that corresponds to their own m/z**, rather than that of their precursor.
- Thus, rather unusually in this type of TOF/TOF**, ion dissociation occurs before the selection step**.

Tandem mass spectrometry

MS/MS instruments

Ion-trap analysers

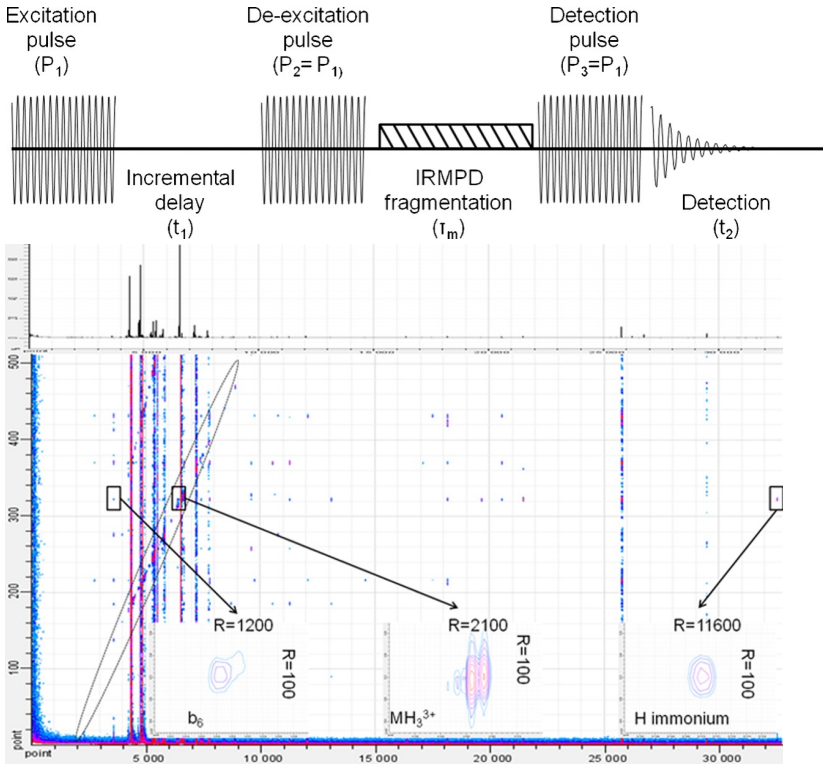




- Once isolated, the precursor ion can be activated (excited) by a range of methods, including CID, ETD, IRMPD, and UVPD.

Tandem mass spectrometry

MS/MS instruments
FTICR analysers – 2D FTICR



- MS/MS ion activation methods that are still commonly performed in the ICR cell include ECD, IRMPD, and UVPD

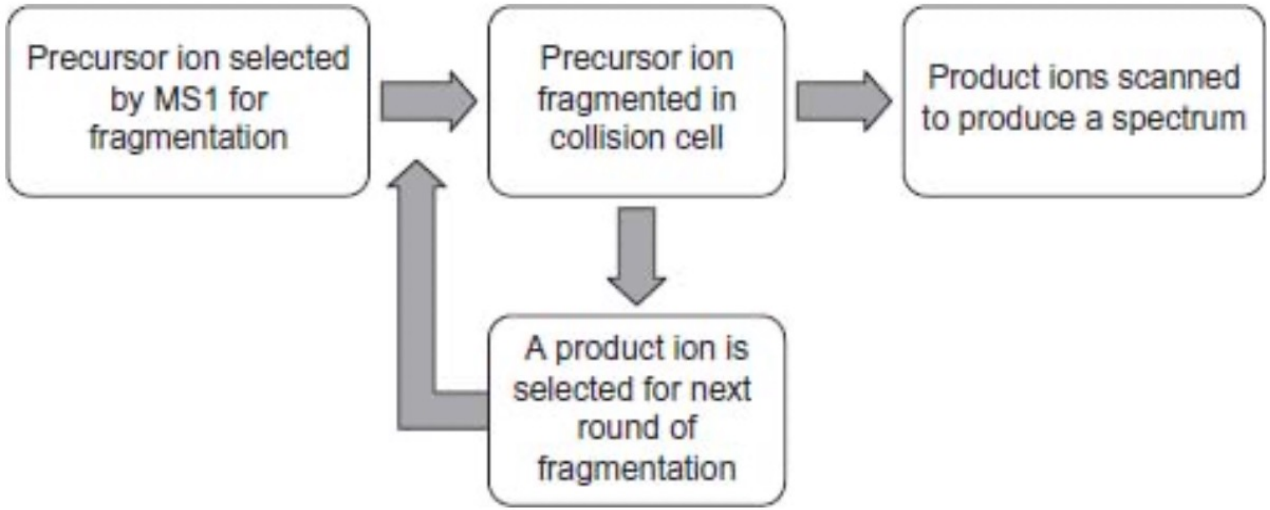
Maria A. van Agthoven, Marc-André Delsuc, Christian Rolando, Two-dimensional FT-ICR/MS with IRMPD as fragmentation mode, International Journal of Mass Spectrometry, Volume 306, Issues 2–3, 2011, doi.org/10.1016/j.ijms.2010.10.034.

MS/MS experiments

Tandem mass spectrometry

MS/MS experiments

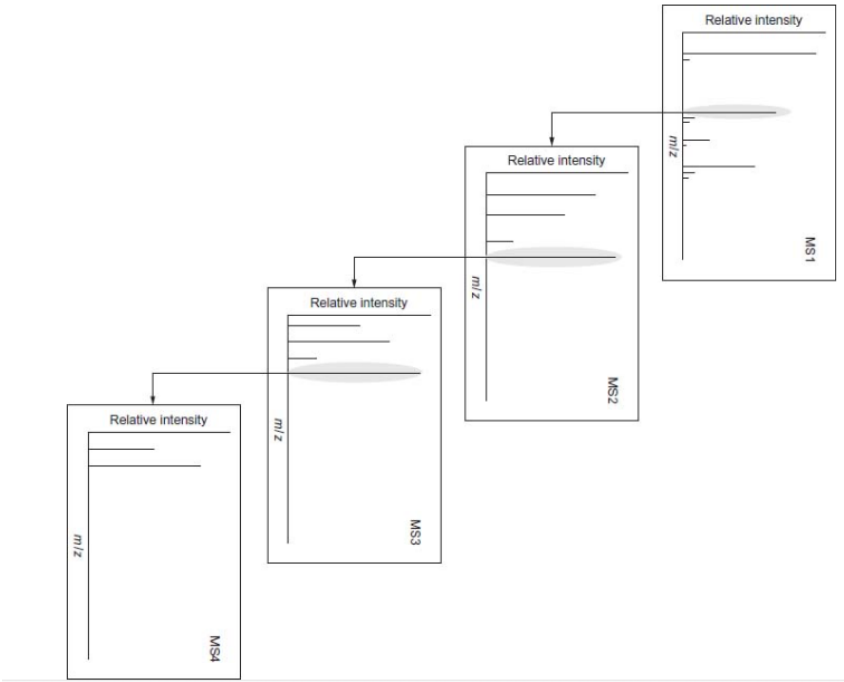
MS^n

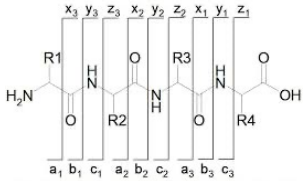


Tandem mass spectrometry

MS/MS experiments

MS^n





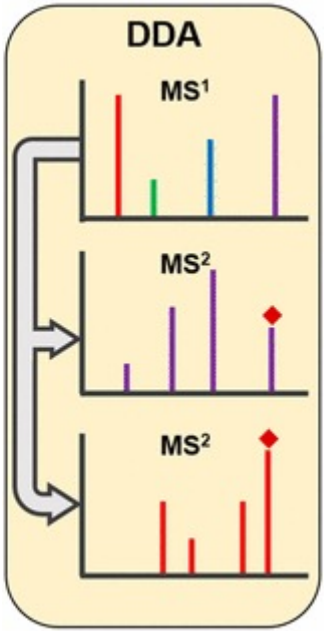
Structure elucidation (fragmentation pattern)

Compound identification (database comparison)

Tandem mass spectrometry

MS/MS experiments

Data-dependent experiments (DDA) and data-independent experiments (DIA)



Overview:
DDA involves acquiring a full MS¹ scan and then selecting the most intense precursor ions for fragmentation and MS² analysis. Selection is based on signal intensity, hence “data-dependent.”

How it works:
1. A full-scan MS¹ is recorded.
2. The instrument **automatically selects the top N most intense ions**.
3. Those ions are fragmented, producing **MS² spectra** for structural elucidation.

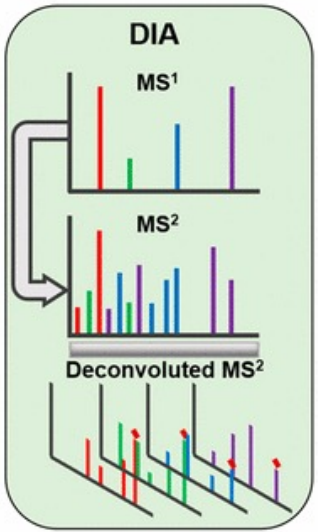
Pros:
- High-quality MS² spectra for the most abundant ions.
- Well-established workflows for **peptide/protein identification** in proteomics.
- Efficient database searching due to cleaner MS² spectra.

Cons:
- **Biased toward high-abundance ions** — low-abundance species may be missed.
- Poor reproducibility in complex samples (different ions may be selected in each run).
- Limited sampling depth.

Tandem mass spectrometry

MS/MS experiments

Data-dependent experiments (DDA) and data-independent experiments (DIA)



Overview:
DIA overcomes DDA's bias by fragmenting **all ions within predefined m/z windows**, rather than selecting based on intensity. It provides a comprehensive MS² dataset for all detectable species.

How it works:
1.A full MS¹ scan is acquired.
2.The m/z range is divided into **sequential isolation windows** (e.g., 25 Da each).
3.All ions in each window are fragmented simultaneously, and MS² spectra are recorded for all.

Pros:
- Simple, fast, and unbiased.
- Provides a comprehensive view of all ions present.
- Ideal for **quantitative analysis** of known targets.

Cons:
- MS² spectra are complex (contain fragments from many precursors).
- Data analysis is computationally intensive and requires spectral libraries or advanced algorithms.
- Slightly less sensitive for individual ions compared to DDA.

Tandem mass spectrometry

MS/MS experiments
Selective reaction monitoring (SRM)

Use of MS/MS to improve selectivity, reduce detection limits, and enhance quantitative analysis.

- **Definition:** A targeted MS/MS technique where a specific precursor ion (from a known analyte) and one specific fragment ion (product ion) are selected and monitored.

- **How it works:**
  - The first quadrupole (Q1) isolates a single precursor ion (SIM)
  - The second quadrupole (q2, collision cell) fragments it.
  - The third quadrupole (Q3) monitors one specific product ion (SIM)

- **Purpose:** Highly selective and sensitive quantification of a known compound.

- **Use case:** Ideal for quantifying a small number of known analytes in complex samples (e.g., biomarkers, targeted proteomics).

Tandem mass spectrometry

MS/MS experiments
Multiple Reaction Monitoring (MRM)

Use of MS/MS to improve selectivity, reduce detection limits, and enhance quantitative analysis.

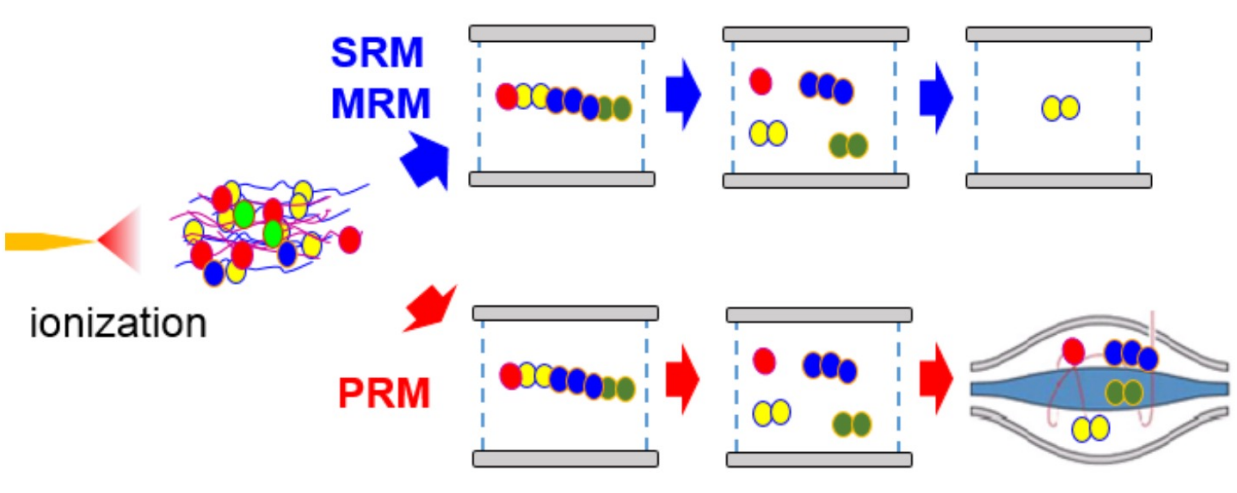
- **Definition:** An extension of SRM where **multiple** precursors product ion pairs (transitions) are monitored in the same run.
- **How it works:**
  - Similar to SRM, but Q1 and Q3 cycle through several predefined ion pairs rapidly. (Sequenced SIM)
- **Purpose:** Increases analytical throughput by enabling simultaneous quantification of several analytes.
- **Use case:** Widely used in targeted metabolomics and proteomics for quantifying multiple peptides or compounds with high specificity and sensitivity.

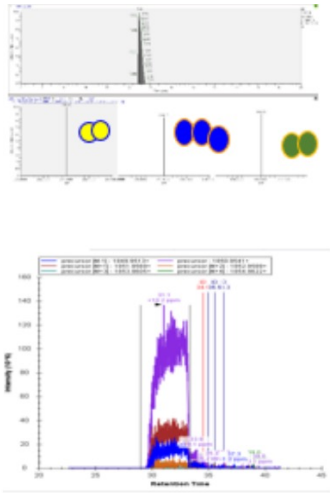
Pros SRM/MRM

- Minimal fractionation only (second separation in the MS)
- Better sensitivity
- Better linear range (4-5 orders of magnitude)
- Less interfering ions than in simple SIM mode

Tandem mass spectrometry

MS/MS experiments
Parallel Reaction Monitoring (PRM)





Tandem mass spectrometry

MS/MS experiments
Parallel Reaction Monitoring (PRM)

- **Definition:** A targeted MS/MS technique where a specific precursor ion is isolated, but **all** of its fragment ions are detected in parallel with a high-resolution mass analyzer (e.g., Orbitrap or TOF).

- **How it works:**
  - Q1 isolates the target precursor ion.
  - Q2 fragments it.
  - Instead of selecting one or a few product ions (as in SRM/MRM), the high-resolution analyzer records the **full MS/MS spectrum** of all product ions simultaneously.

- **Purpose:** Provides high specificity and confidence in analyte identification and quantification by analyzing the complete fragmentation pattern.

- **Use case:** Targeted proteomics and validation studies where accuracy and specificity are more important than absolute throughput.