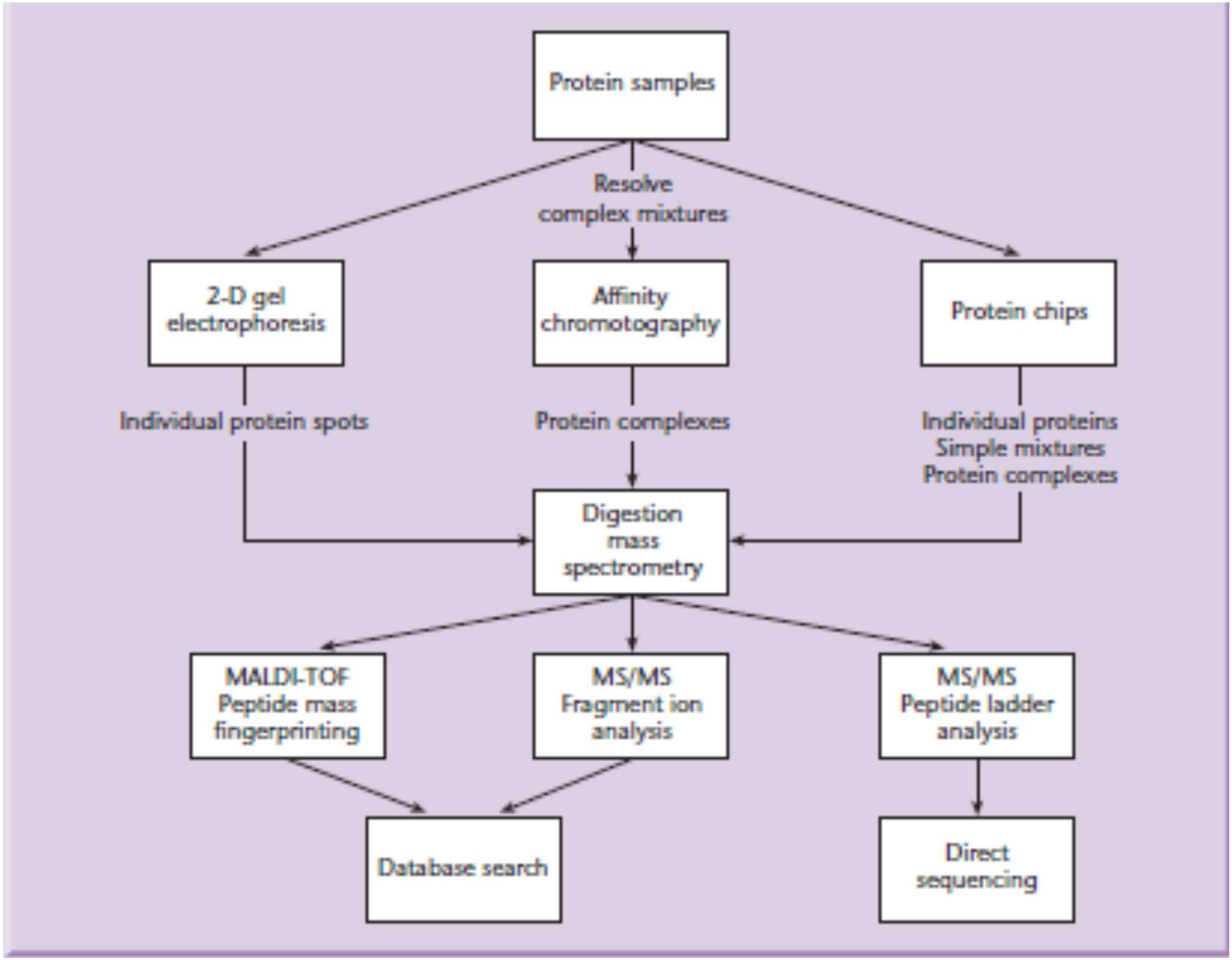
Mass spectrometry involves the ionization of target molecules in a vacuum, and accurate measurement of the mass of the resulting ions.

A mass spectrometer has three component parts: an ionizer, which converts the anylate into gas phase ions; a mass analyzer which separates the ions according to their mass/charge ratio (m/z); and an ion detector.

Large molecules such as proteins and nucleic acids are broken up and degraded by the ionization procedure, recently, sensitive instruments that are capable of *soft ionization*, i.e. the ionization of large molecules without significant degradation, have been developed.

This allows accurate mass measurements of whole proteins and peptide fragments, data that can be used to search protein databases to identify particular proteins.



Route of protein annotation in proteomics

Mass spectrometry is used for protein characterization

Two major strategies used to characterize proteins

***Peptide mass fingerprinting*, which is often carried out using a mass spectrometer with an electrospray ionization (ESI) or a matrix assisted laser desorption ionization (MALDI) source coupled with a time of flight (TOF) analyzer.**

Briefly, protein spots are excised from a 2D gel and digested with a specific endopeptidase, such as trypsin, to generate peptide fragments.

These are then analyzed by mass spectrometry to determine their molecular masses, and these data are used to search protein databases.

Computer algorithms have been developed by a number of groups for correlating MS-determined peptide masses with virtual peptide masses derived from protein databases.

Mass spectrometry

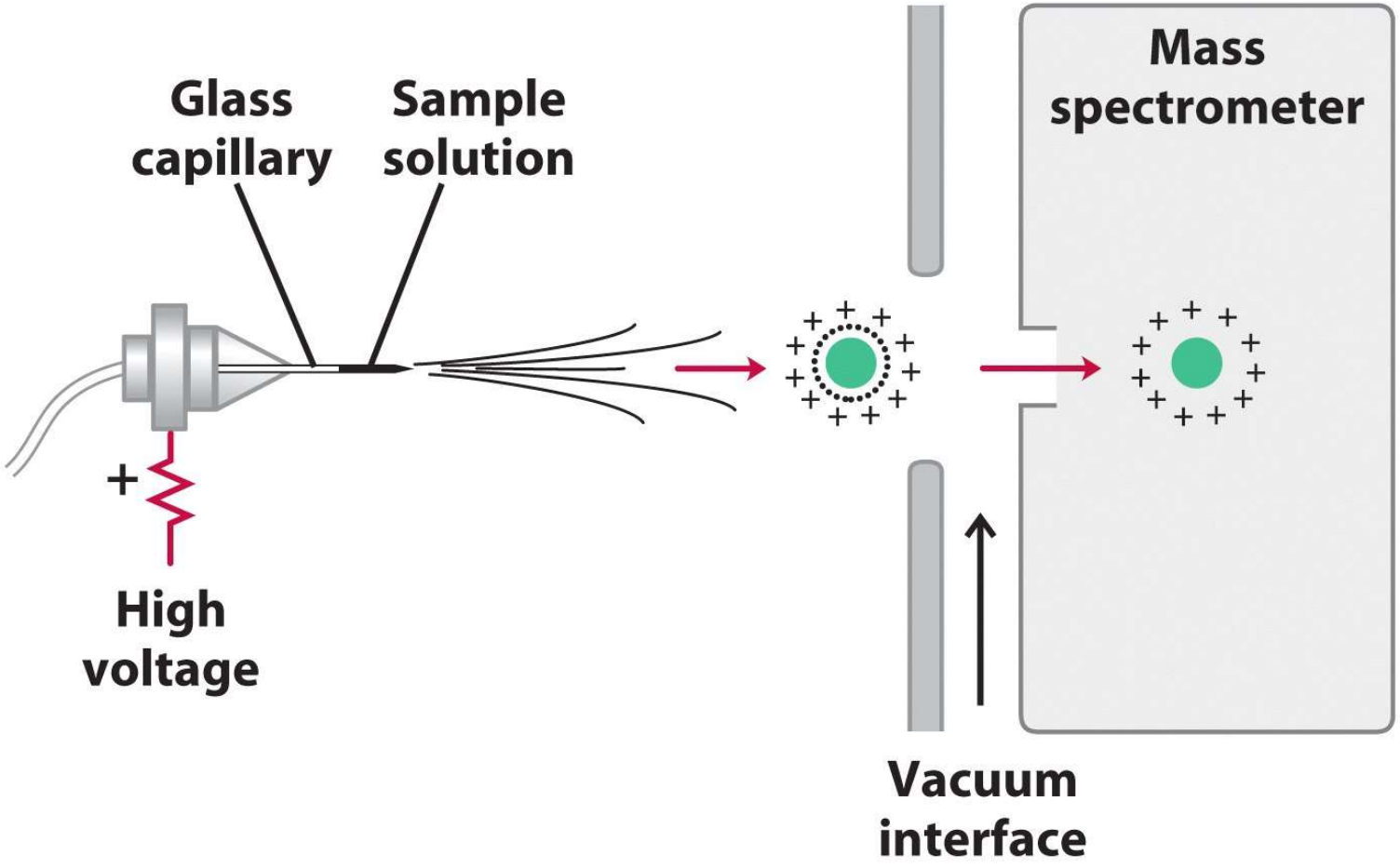
A technique for accurately determining molecular masses by calculating the mass : **charge ratio of ions in a vacuum**. A mass spectrometer is an instrument combining a source of ions, a mass analyser that can separate ions according to their mass : charge ratio, and an ion detector.

Soft ionization

The ionization of large molecules, proteins and nucleic acids, without causing significant amounts of fragmentation.

Electrospray ionization (ESI)

A soft ionization method used for fragment ion searching. The analyte is dissolved in an appropriate solvent and pushed through a narrow capillary. A potential difference is applied across the capillary such that charged droplets emerge and form a fine spray. Under a stream of heated inert gas, each droplet rapidly evaporates so that the solvent is removed as the analyte enters the mass analyser and the ions are accelerated towards the detector.



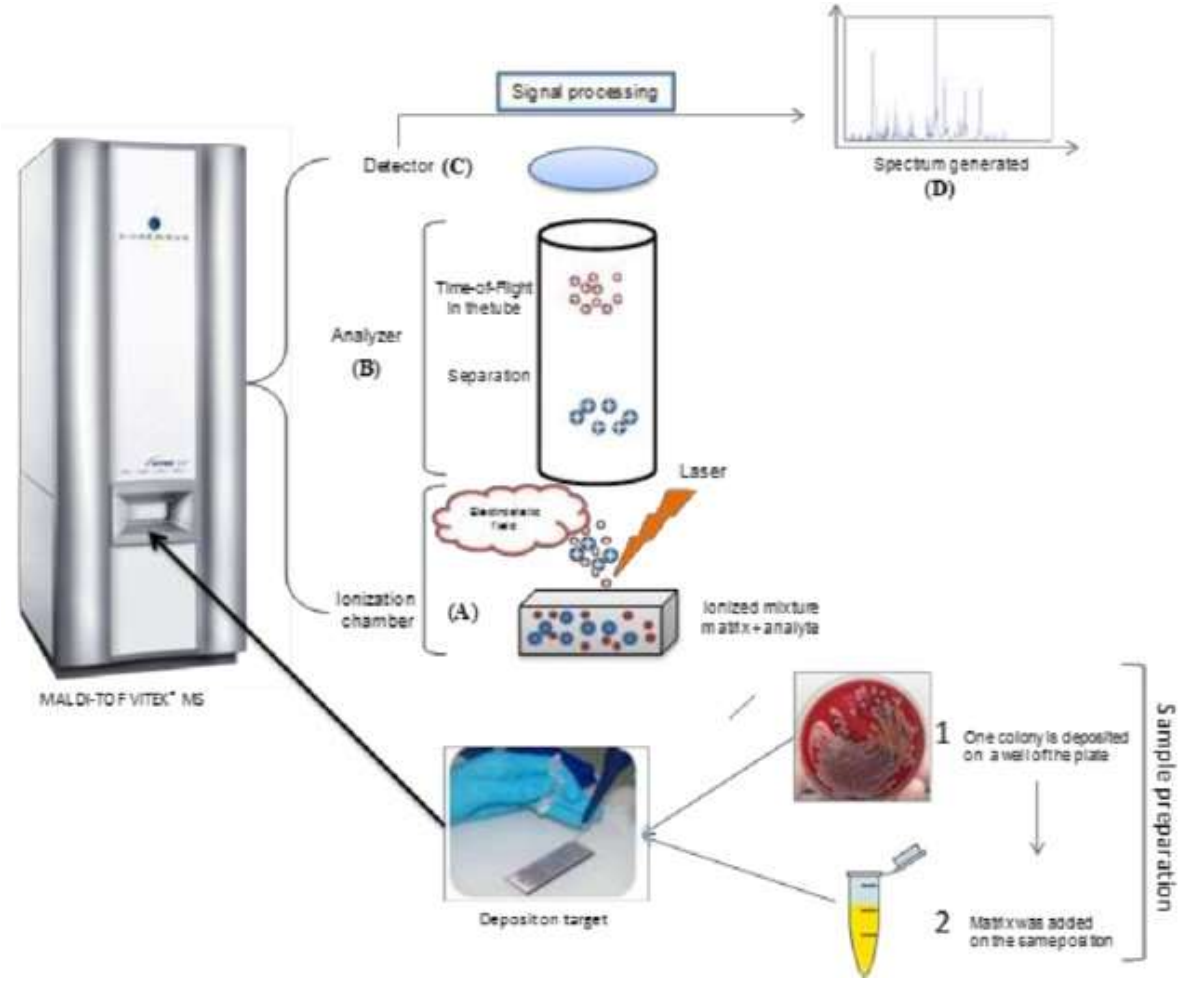
Matrix-assisted laser desorption ionization (MALDI)

A soft ionization method used for peptide mass fingerprinting.

The analyte, a mixture of peptide fragments resulting from tryptic digestion of a particular protein, is first mixed with a light-absorbing “matrix compound” such as dihydroxybenzoic acid, in an organic solvent.

The solvent is then evaporated to form crystals and these are transferred to a vacuum. The dry crystals are targeted with a laser beam.

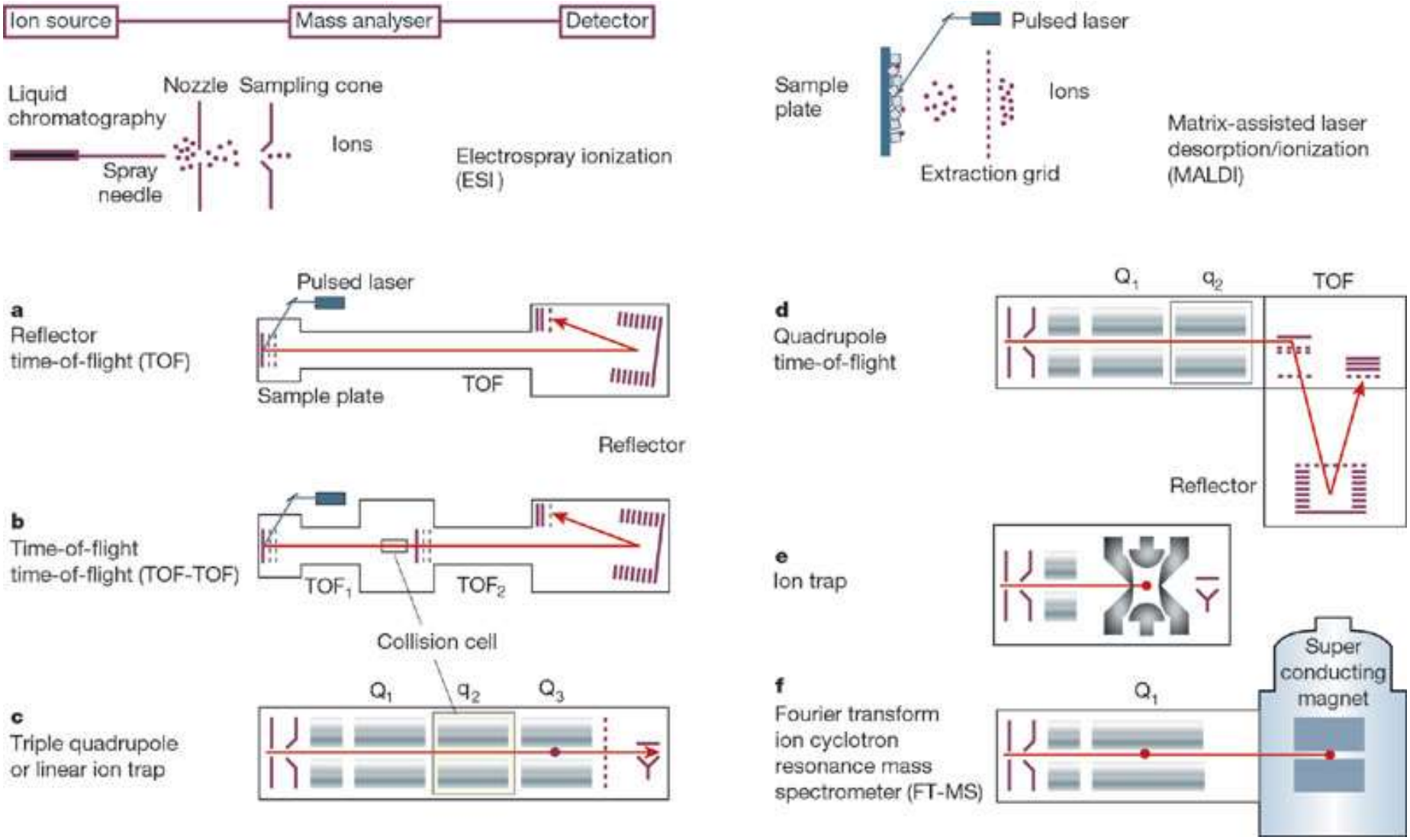
The laser energy is absorbed and then emitted (desorbed) as heat, resulting in expansion of the matrix and anylate into the gas phase. A high voltage is applied across the sample to ionize it, and the ions are accelerated towards the detector.



MALDI-TOF MS's operating principle and the sample preparation step for identification. The principle of this measurement is based on the ability of an electric and/or magnetic field to deflect a flow of ions, each with a mass and a charge proportional to their trajectories. Overall, mass spectrometry can be divided into three steps: the ionization chamber that produces ions in the gas phase (A), the analyzer which selects ions by mass-to-charge ratio (m/z) (B), and the detector that converts the ionic current into electric current (C).

Bombing with a laser beam generates ions in the ionization chamber. These ions are accelerated into an electric field which directs them to the analyzer that separates them according to their time-of-free flight (TOF: Time-Of-Flight). The smaller molecules grasp the detector first, followed by the biggest, according to the m/z ratio. Those which have the same m/z ratio are then separated by an electrostatic mirror. The detector converts the received ions into electrical current which is amplified and digitized (D).

Mass spectrometers



Quadrupole

A mass analyzer that determines the mass : charge ratio of an ion by varying the potential difference applied across the ion stream, allowing ions of different mass : charge ratios to be directed towards the detector.

A quadrupole comprises four metal rods, pairs of which are electrically connected and carry opposing voltages that can be controlled by the operator.

More than one quadrupole may be connected in series, as in triple quadrupole mass spectrometry. Varying the voltage steadily over time allows a mass spectrum to be obtained.

Time of flight (TOF)

A mass analyzer that determines the mass : charge ratio of an ion by measuring the time taken by ions to travel down a flight tube to the detector.

Collision-induced dissociation (CID)

The use of a collision cell between mass analyzers to excite ions and make them dissociate into fragments.

Mass Analyzers (MS)

Quadrapole

- High Sensitivity, acceptable mass accuracy and resolution
- Easily coupled to chromatography

Time of Flight

- High Sensitivity, high mass accuracy, high resolution
- Limited to small m/z ratios
- Not easily coupled to chromatography
- Easily coupled to MALDI

Ion Trap

- High Sensitivity
- Low mass accuracy and resolution

Fourier Transform ion cyclotron

- High sensitivity, mass accuracy, resolution, dynamic range
- Expensive, difficult to operate, low fragmentation efficiency