Biological Mass spectrometry

-basics and protein identification

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MASS SPECTROMETRY

is an analytical technique that identifies the chemical composition of a compound or sample based on the mass-to-charge ratio of charged particles

ION SOURCE

MASS ANALYZER

DETECTOR

Mass spectrometry

ION SOURCE: molecules of interest are ionized

↓

MASS ANALYZER:
ions are separated according to their m/z-ratios

↓

DETECTOR: separated ions are detected

MS of small molecules

-In 1918, Arthur Jeffrey Dempster developed the first modern mass spectrometer, and established the basic theory and design of mass spectrometers that is still used to this day

-1919 Francis Aston constructs the first velocity focusing mass spectrograph with mass resolving power of 130 (1922 Nobel Prize in chemistry)

-The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred McLafferty

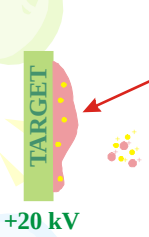
-Ionization modes: chemical ionization (CI) and electron ionization (EI), not suitable for labile biomolecules

Biological mass spectrometry

-two modes of ionization: MALDI (matrix assisted laser desorption ionization) and ESI (electrospray ionization)

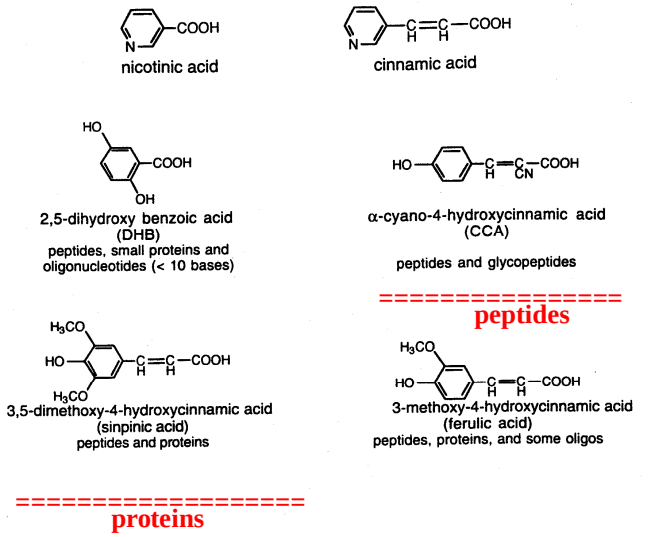
developed in 1980's

Nobel-price in Chemistry 2002

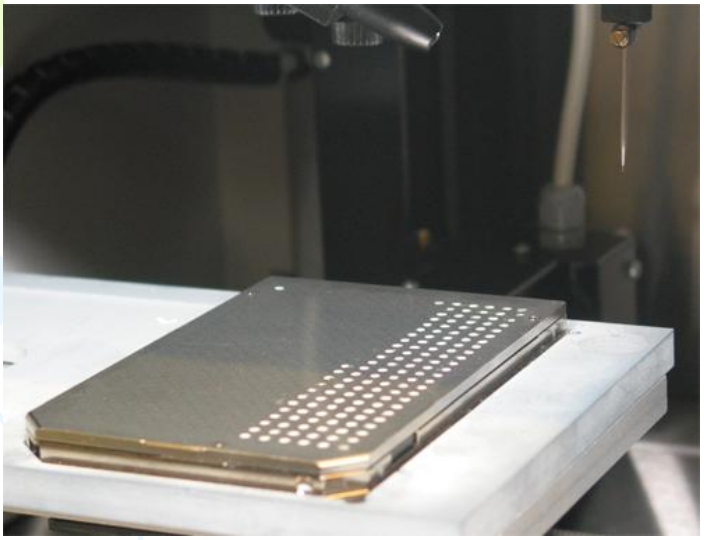


MALDI = matrix assisted laser desorption ionization

An ionization process suitable for mass spectrometric analysis of large molecules, like proteins and peptides. The analyte substance is embedded in a crystallized matrix, which is irradiated by a laser. The power of the laser beam is usually adjusted in a way that it has enough energy to ionize the biomolecules and matrix molecules but does not split the large analyte molecule. Thus MALDI belongs to the "soft" ionization techniques.

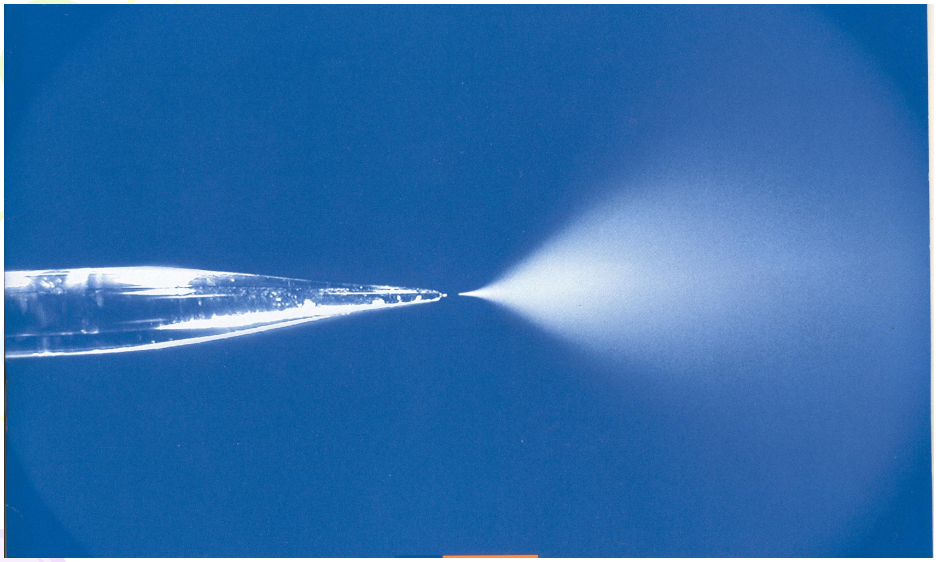


Commonly used matrixes for MALDI-TOF mass spectrometry



A 384 position MALDI-TOF sample target plate.

Electrospray ionization (ESI):
Creation of ions by spraying a solution into an electrical field. This process, which belongs to the "soft" ionization techniques, enables the analysis of intact biomolecules, such as e.g. proteins and peptides by mass spectrometry.



Electrospray of peptides in 0.1% FA /ACN from a 15 µm I.D. fused silica glass needle. The liquid flow is 200 nl/min and the needle has a potential of 2000V as compared to the cone inlet of the mass spectrometer.

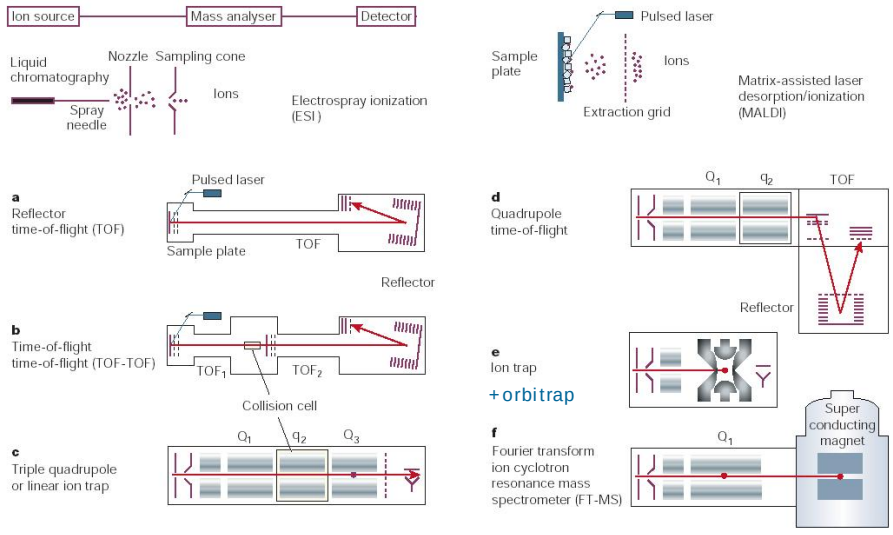
MALDI

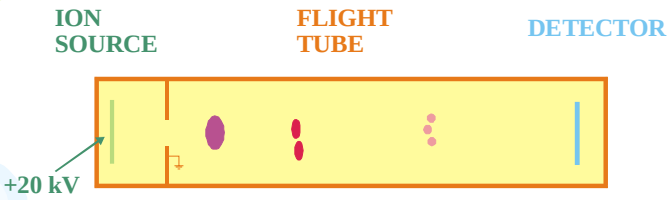
- sample is crystallized
- produces mainly singly charged ions
- simple, easy-to-use
- more tolerant to salts+other contaminants in the sample than ESI

ESI

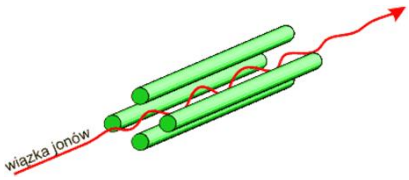
- liquid sample
- produces multiply charged ions
- easy to couple with HPLC

Mass analyser types





Time-of-flight mass spectrometry (TOFMS) is a method of mass spectrometry in which ions are accelerated by an electric field of known strength. This acceleration results in an ion having the same kinetic energy as any other ion that has the same charge. The velocity of the ion depends on the mass-to-charge ratio. The time that it subsequently takes for the particle to reach a detector at a known distance is measured.



Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize ions passing through a radio frequency (RF) quadruple field. The quadrupole consists of four parallel metal rods. Each opposing rod pair is connected together electrically and a radio frequency voltage is applied between one pair of rods, and the other. A direct current voltage is then superimposed on the R.F. voltage. Ions travel down the quadrupole in between the rods. Only ions of a certain m/z will reach the detector for a given ratio of voltages: other ions have unstable trajectories and will collide with the rods. This allows selection of a particular ion, or scanning by varying the voltages

MS in protein chemistry/ proteomics/structural biology

-protein identification

-protein MW determination

-characterisation of post-translational modifications

-relative quantification of proteins between samples

-analysis of protein complexes

-MS imaging