Principles of MALDI-TOF mass spectrometry

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Mass Spectrometry Methods in Botanicals Research
Shining the light on samples

- A focused laser beam, either in the UV or infra-red ranges, can “evaporate” compounds from the solid phase

- The resulting ions are injected into a tube (1 - 2 m in length), accelerated and allowed to drift towards a detector. Their *time-of-flight* is proportional to their $(MW)^{1/2}$
Matrix-Assisted Laser Desorption Ionization (MALDI)

- Short laser pulse
- Flight tube and drift region to measure the time-of-flight (TOF)
- Accelerating pulse
- Detector
MALDI-TOF ionization

N₂ laser pulse (337 nm)

\[ [M+H]^+ \]

\[ [M-H]^- \]

matrix ions

Protein/peptide/nucleotide/saccharide deposited on crystal surface
How a TOF instrument works

Source → Linear flight tube (1.0 - 1.5 m) → Oscilloscope to time ion arrival (1 µsec)

Accelerating pulse

Effective flight tube (3.0 m) → Reflectron

Oscilloscope to time ion arrival
Protein analysis 2002

- destain
- Eppendorf tube
- Speed-Vac
- Incubate overnight
- trypsin 1:20
- MALDI plate
- Desalting Ziptip
- Water Bath 37°C

Counts

Mass (m/z)
Applications of time-of-flight

- Whole proteins - modified antibodies
- Peptide mixtures following trypsinolysis of spots or bands from 2D-gels
- Polyphenols
- Bile acids and many other biologically interesting molecules
Cytochrome C Modified by HNE
MALDI-TOF Mass Spectrum

MALDIMALDI--TOF Mass Spectrum

Mass (m/z)

1000 8800 16600 24400 32200 40000

12365.22 12521.80 12680.12 12837.86 6260.97 25207.30

[M+H]  [M+H]^{2+} [2M+H]^{+}

CONTROL PROTEIN + ONE 4HNE PROTEIN + TWO 4HNE PROTEIN +THREE 4HNE

% Intensity
Trypsin Digest of porin-P1; Voltage-dependent anion-selective channel
MALDI-TOF Spectrum for Daidzein
MALDI-TOF of mouse bile

Matrix: α-cyano-4-hydroxycinnamic acid
N₂ laser 337 nm, 1:100 fold dilution of bile, 1 µl spotted
Mouse bile does not contain ANY glycine conjugates of bile acids, only taurine conjugates. Several isomers are present.
Connecting CE and LC to MALDI analysis

- CE analysis
- nanoLC analysis

Creates 20 mm wide tracks that can be scanned by MALDI laser for MS analysis

Parallel capture of effluents of 8 nanoLC separations on Mylar - can be scanned simultaneously by fast laser
Pros/Cons of laying down LC or EC separations on matrix plate

- Allows off-line analysis both in real time and then in a retrospective mode
- MALDI-TOF analysis is very fast
- Can do TOF-TOF MS-MS analysis
- BUT what happens chemically on the acidic environment on the surface of the plate during storage
- Also, can the laser beam cause chemical changes?