

Purdue-UAB Botanicals Center for Age-Related Disease

Principles of MALDI-TOF mass spectrometry

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Shining the light on samples

- A focused laser beam, either in the UV or infrared 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)



MALDI-TOF ionization



Protein/peptide/nucleotide/saccharide deposited on crystal surface

How a TOF instrument works



Protein analysis 2002





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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



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 μ l spotted

LC-MS analysis of mouse bile



Mouse bile does not contain ANY glycine conjugates of bile acids, only taurine conjugates. Several isomers are present.



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?