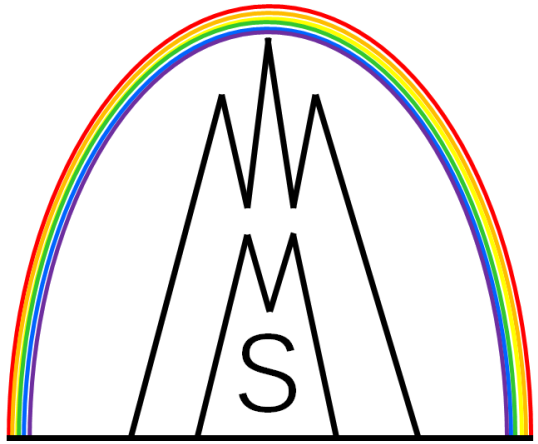
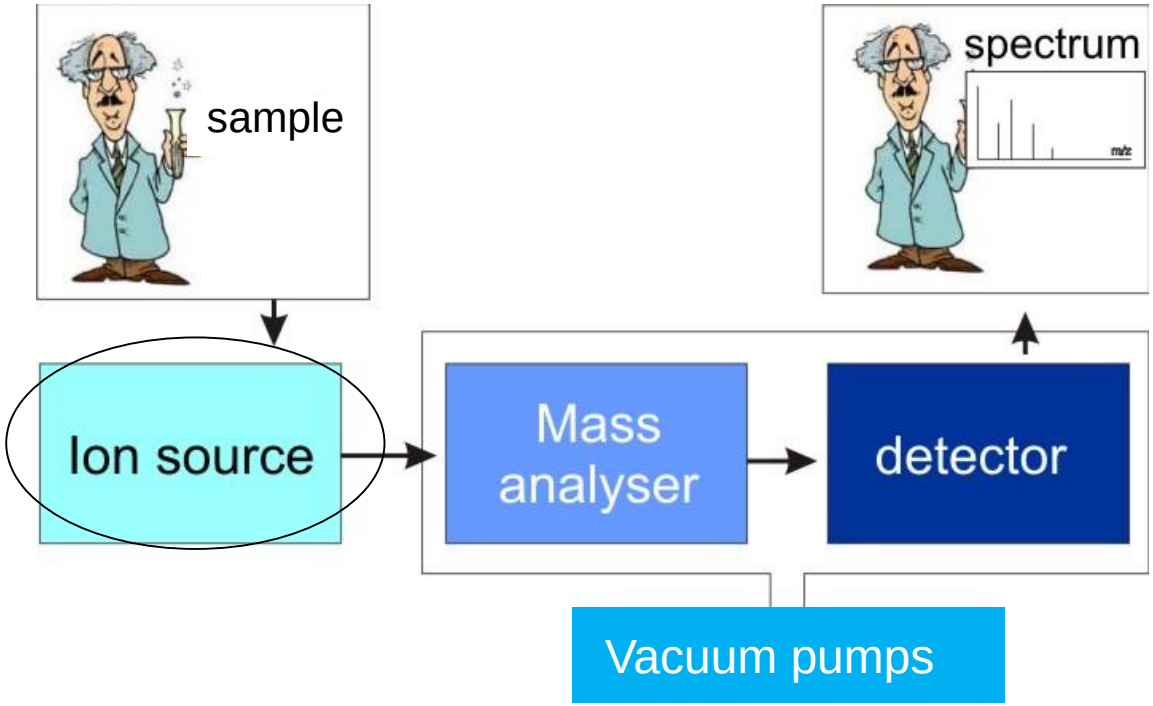


Ionization and ion sources, detector, vacuum systems

Mass spectrometer



- Ion source - devices which produce positive or negative electrically charged molecules in gas phase
- Mass analysers - separate the ions according to their mass-to-charge ratio (m/z)
- Detectors - record the charge induced or the current produced, when an ion passes by or hits a surface

Ion source

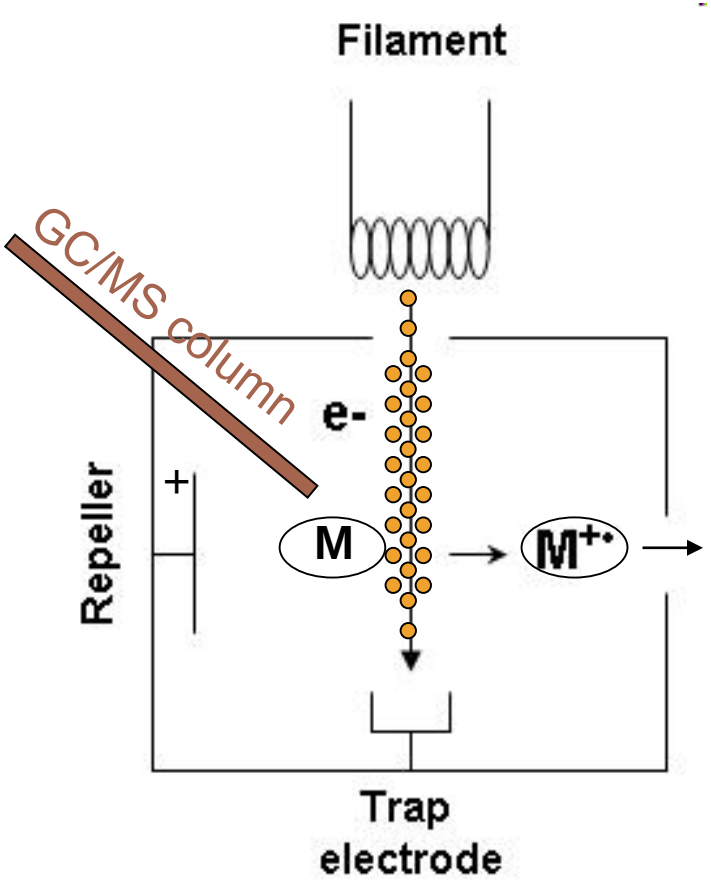
Produce **positive** or negative electrically charged molecules in **gas phase**

- Choice depends on compound
  - Universal ionization technique does not exist
- Differentiation
  - By energy
    - Hard (EI)
      - Cation radical with high energy - fragmentation in ion source – many fragments in the spectra
    - Soft (CI, ESI, APCI, MALDI,...)
      - Molecular adduct with low energy – no or a few fragments in the spectra
  - By pressure
    - Vacuum (EI, CI, MALDI,...)
    - Atmospheric pressure (ESI, APCI, APPI, AP MALDI,...)
      - Ambient ionization technique
- Ions
  - $M + e^- \rightarrow M^{+\bullet} + 2\ e^-$ Cation radicals
  - $M + HA \rightarrow [M+H]^+ + A^-$ Molecular adducts
  - $M + B^- \rightarrow [M-H]^- + HB$ Deprotonatet molecules

Different ionization technique

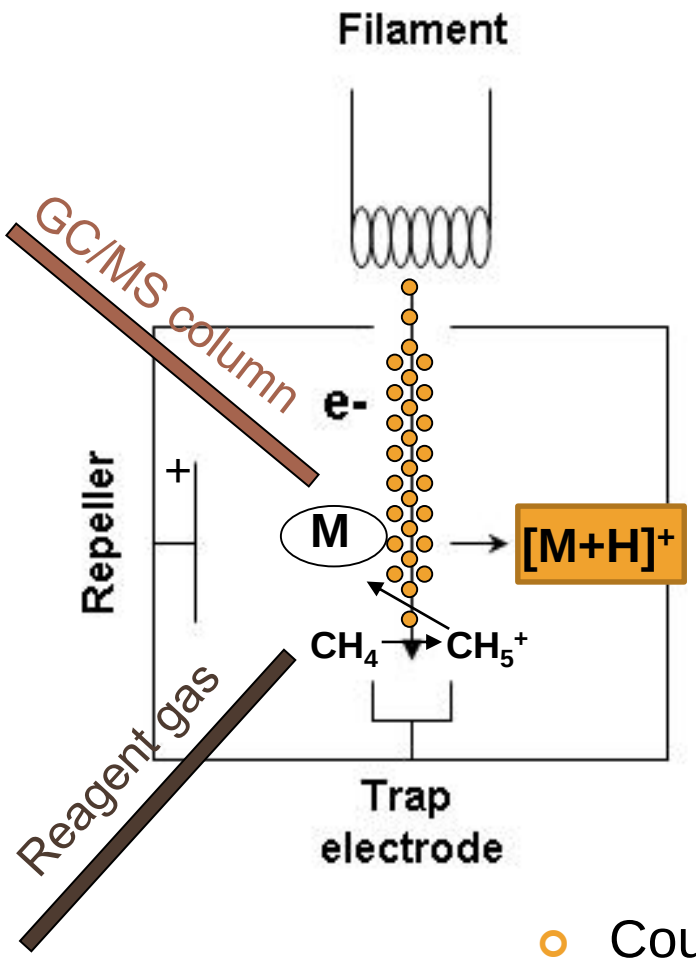
- **Molecular Analysis**
  - **Electron Ionization** (EI)
  - **Chemical Ionization** (CI)
  - **Electrospray** (ESI)
    - **Nanoelektrospray** (nanoESI)
  - **Atmospheric Pressure Chemical Ionization** (APCI)
  - Atmospheric Pressure Photoionization (APPI)
  - **Matrix-Assisted Laser Desorption/Ionization** (MALDI)
  - **Laser Desorption Ionization** (LDI)
  - Secondary Ion Mass Spectrometry (SIMS)
  - Fast Atom Bombardment (FAB)
  - Termospray (TSI)
    - Ambient ionization technique
      - Desorption Electrospray Ionization (DESI)
      - Desorption Atmospheric Pressure Photoionization (DAPPI)
      - Direct Analysis in Real Time (DART)
  - .....
  - .....

Electron ionization (EI)



- An ionization method in which energetic electrons interact with gas phase molecules to produce ions.
  - Electron emission by heating a tungsten wire filament
  - Good reproducibility – spectral library – easy interpretation
    - (energy of the electrons 70eV)
- $M + e^- \rightarrow M^{+\bullet} + 2\ e^-$
  - M is the analyte molecule being ionized
  - e^- is the electron and
  - $M^{+\bullet}$ is the resulting ion
- Widely used for volatile organic molecules
- Often coupled with GC = GC/EI-MS

Chemical Ionization (CI)



- Analyzed ions are produced through the collision of the analyte with ions of a reagent gas, that are present in the ion source
  - **Methane**, ammonia, isobutane, acetonitrile,.....
- Soft ionization technique
  - $[M+H]^+$, $[M+\text{reagent gas}]^+$, fragments (depend on condition)
- Example
  - CH_4 as a reagent gas

Primary ion formation

$$CH_4 + e^- \rightarrow CH_4^+ + 2e^-$$

Secondary reagent ions

$$CH_4 + CH_4^+ \rightarrow CH_5^+ + CH_3$$

$$CH_4 + CH_3^+ \rightarrow C_2H_5^+ + H_2$$

Product ion formation

$$M + CH_5^+ \rightarrow CH_4 + [M+H]^+$$

$$AH + CH_3^+ \rightarrow CH_4 + A^+$$

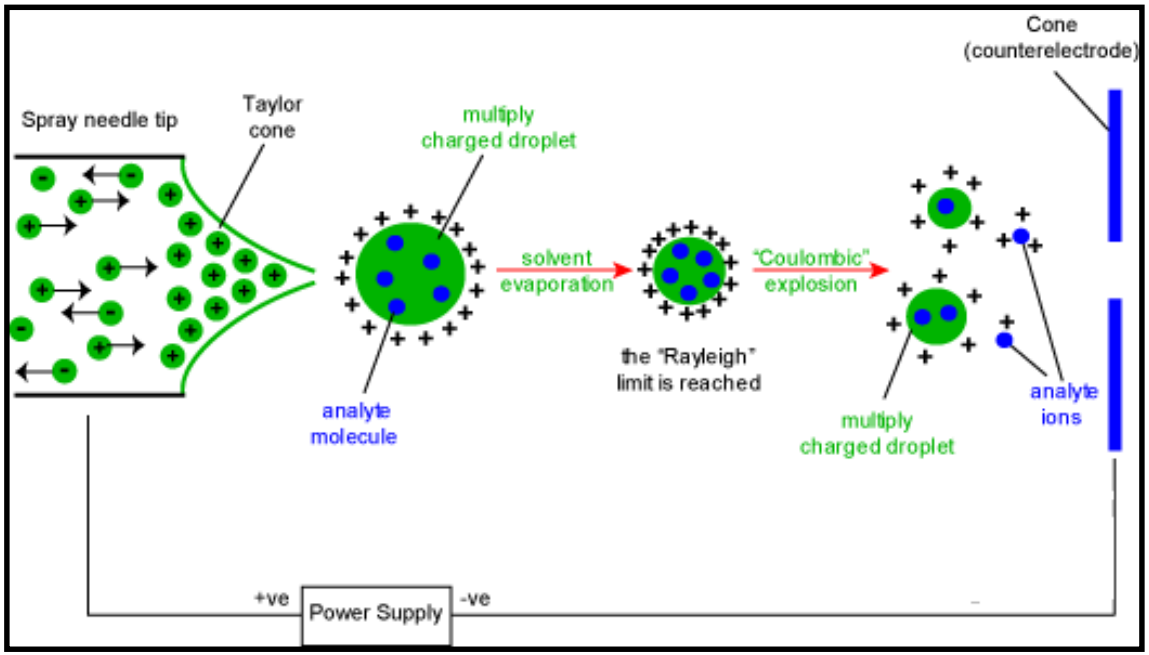
$$M + CH_5^+ \rightarrow [M+CH_5]^+$$

$$A + CH_4^+ \rightarrow CH_4 + A^+$$

- Coupled with GC = GC/CI-MS
- Used for volatile organic molecules

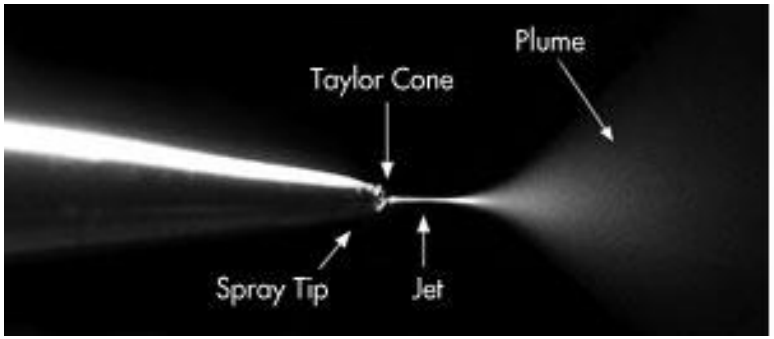
ELECTROSPRAY (ESI)

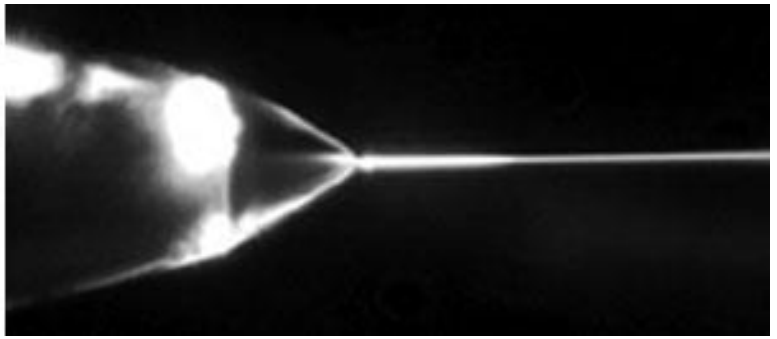
- The liquid containing the analyte(s) is dispersed by electrospray into an aerosol
  - Charged droplets
  - Solvent evaporation
  - Coulombic explosion
- Soft ionization technique
  - **[M+H]** $^{+}$, [M+ Na]$^{+}$, [M+ K]$^{+}$, ... molecular adducts
  - Multiply charged ions, dimers



http://www.chm.bris.ac.uk/ms/theory/esi-ionisation.html

NANOELECTROSPRAY (NANOESI)



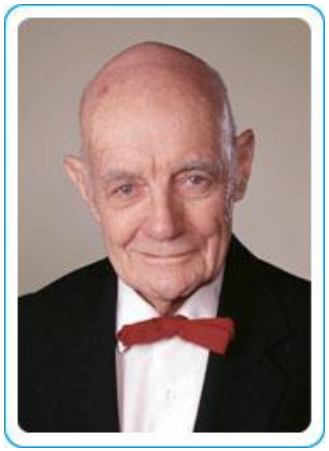


- Flow of mobile phase usually hundreds nl/min
  - Higher sensitivity
  - $[M+H]^+$, ~~$[M+Na]^+$, $[M+K]^+$, ...~~
  - Easer interpretation of the spectra

- Important parameters
  - Mobile Phase Flow
  - Tip Inner Diameters (IDs)
  - Mobile Phase Composition
  - Applied Voltage

Electrospray technique

- Polar analytes in broad mass range
- Obtaining multiply charged
  - Analysis of molecules with Mr behind the range of analyzer
- Coupled with HPLC or UHPLC
  - Polar solvent (mobile phase) as a donor of H^+

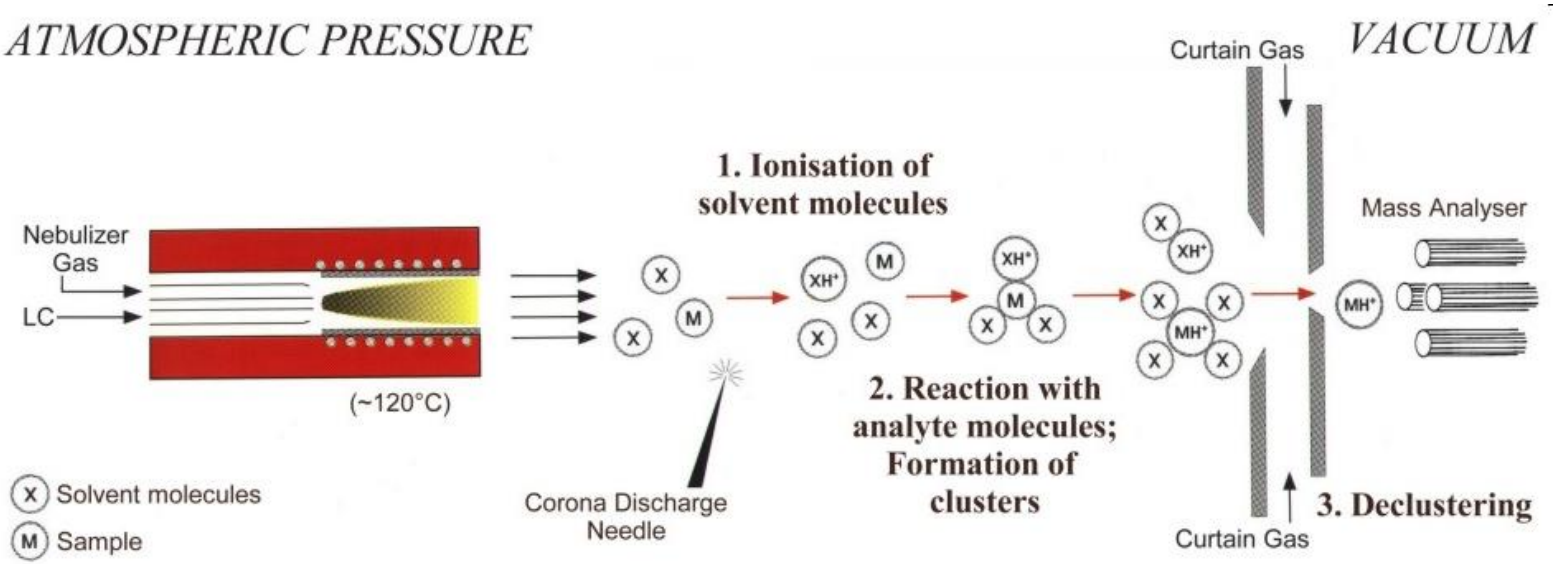


Electrospray Wings for Molecular Elephants (Nobel Lecture)

John B. Fenn
Nobel prize in Chemistry
2002

Atmospheric Pressure Chemical ionization (APCI)

- The mobile phase containing eluting analyte is heated to high temperature (above 400°C), sprayed with high flow rates of nitrogen
- Molecules of solvent and gas are ionized by corona discharge
- Analyte are ionized by ionized gas molecules and solvent



Atmospheric Pressure Chemical ionization (APCI)

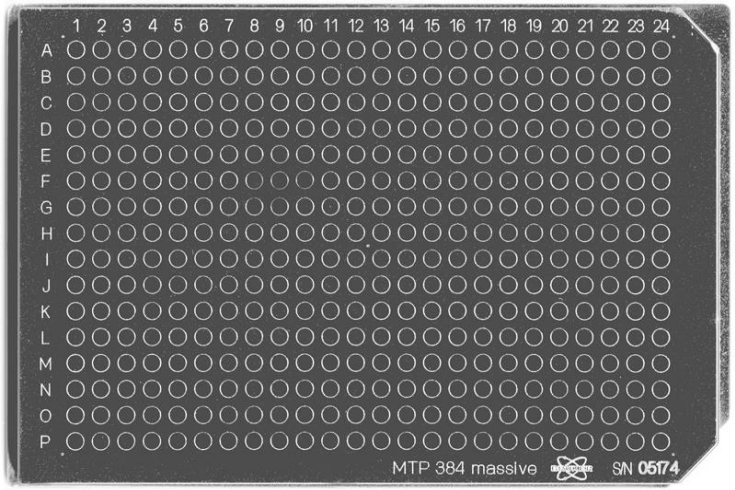
- APCI can be performed in a modified ESI source
  - Device similar to ESI source
  - However, mechanism of ionization similar to CI
- The ionization occurs in the gas phase

- APCI is a less "soft" ionization technique than ESI
  - Generates more fragment ions

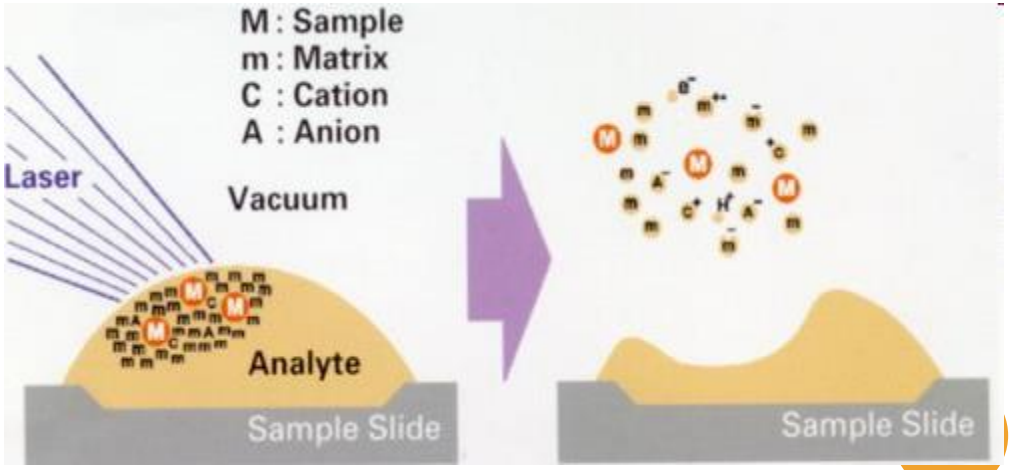
- Coupled with HPLC or UHPLC
  - Advantage of APCI - it is possible to use a nonpolar solvent (mobile phase)

Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Laser-based soft ionization method
  - Matrix and analyt are mixed on the target plate
  - The laser (**UV**, IR) shoots the mixture
  - The energy is transferred to the matrix, which is vaporized, carrying analyte into the vapour phase and charging it
- The mechanism of MALDI is still debated



www.ms-textbook.com



http://www.fasmatech.net/content-61-2.html

Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Analysis of
  - Biomolecules (DNA, proteins, peptides and sugars)
  - Large organic molecules (polymers, dendrimeres,...)
    - Which tend to fragment, when are ionized by more conventional ionization methods.
  - Singly charged molecular adduct
    - Molecular adducts (**$[M+H]^+$**, $[2M+H]^+$, $[M+2H]^{2+}$) or loss of proton $[M-H]^-$
    - Other molecular adducts $[M+metal]^+$ with salts in sample (**Na**, K, ...,)

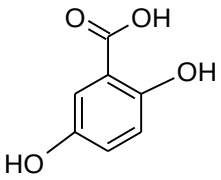
Franz Hillenkamp

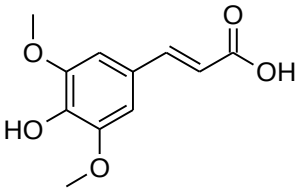
Koichi Tanaka

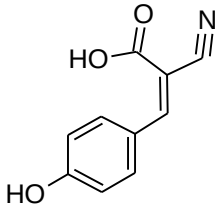
Nobel prize in Chemistry 2002

MALDI matrices: Properties and Requirements

- Small molecules, usually small organic acids
  - 2,5-dihydroxybenzoic acid, sinapic acid, α-cyano-4-hydroxycinnamic acid,.....

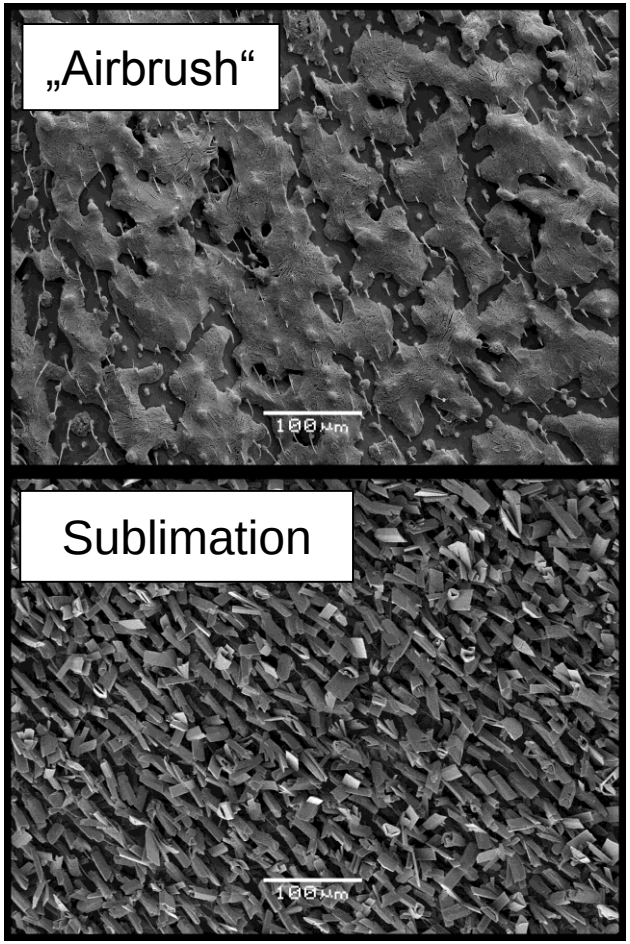






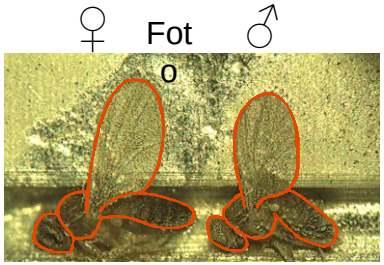
- The MALDI matrix of requirements
  - Be able to embed analytes (by co-crystallization)
  - Be soluble in solvents compatible with analyte
  - Be vacuum stable
  - Absorb the laser wavelength
  - Cause co-desorption of the analyte upon laser irradiation
  - Promote analyte ionization

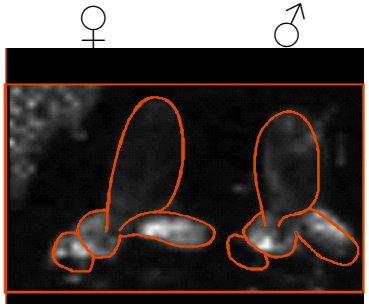
MALDI IMAGING



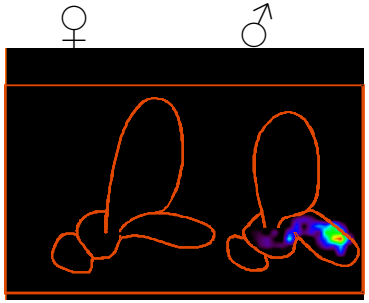
V. Vrkoslav, A. Muck, J. Cvačka, A. Svatoš, *J. Am. Soc. Mass Spectrom*. **21** (2010) 220–231

D. melanogaster



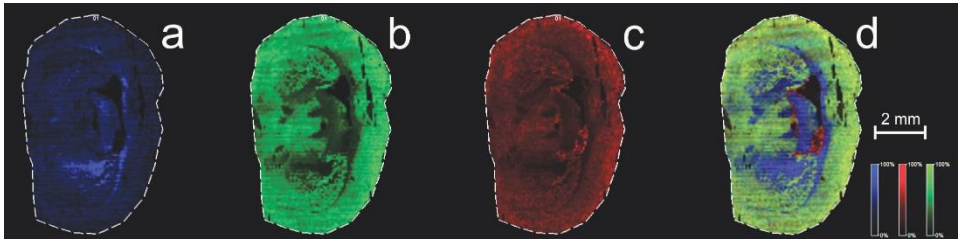


TIC

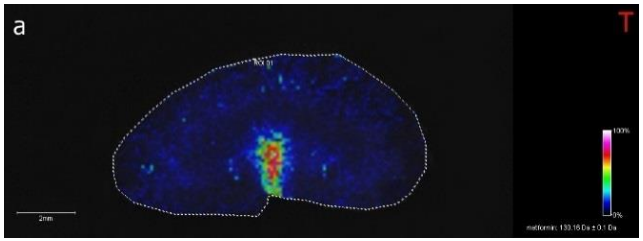


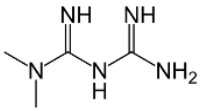
cis-vacenyl acetate

Cholesterol PC SM d=a+b+c.



Metformin - $[M+H]^+$; m/z 130,16



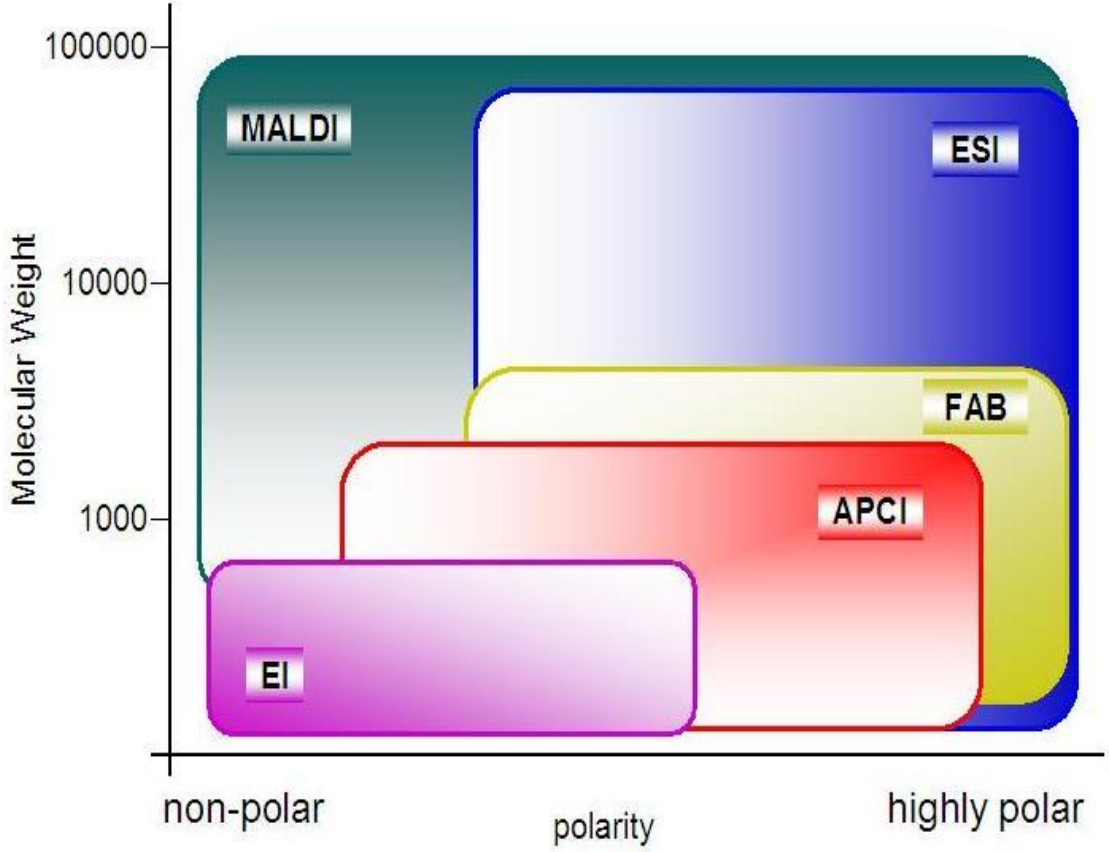


LASER DESORPTOIN/IONIZATION (LDI)

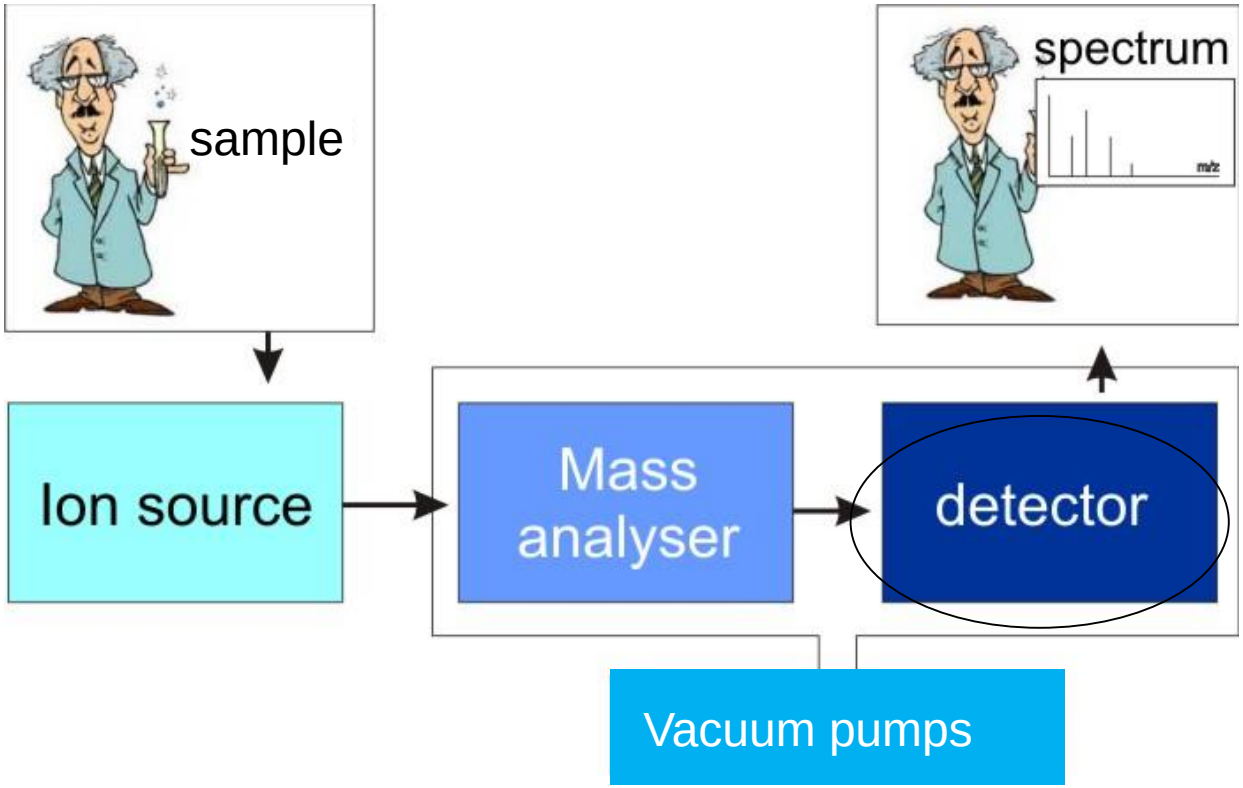
- **LDI** (laser desorption/ionization)
  - Energy of laser is directly absorbed by analyte
  - Without matrix
  - For small molecules only
    - Spectra without matrix ions
    - Better reproducibility then MALDI
    - Harder ionization technique then MALDI – fragments in spectra
    - **Sensitivity depends on analyte**
  - **Useful for imaging**
  - **Instrumentation is same as for MALDI**

Choice of ionization technique

- Almost all compounds can by ionized by more then one technique
  - Depends on **molecular mass, polarity, ionization energy,** solubility, ...



Detector



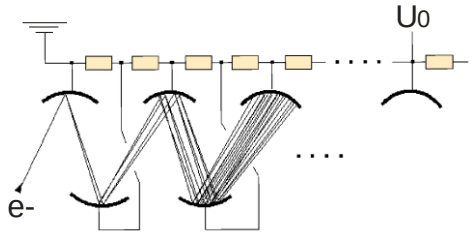
- Detectors - record the charge induced or the current produced, when an ion passes by or hits a surface

Detectors

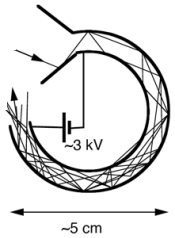
- Records the current produced, when an ion hits a surface of detector
- In commercial instrument detectors with conversion dynode
  - Ions strike a conversion dynode to produce electrons – electron multiplied by
    - Electron multiplier
    - Ion-to-photon detector
- Record the charge induced, when an ion passes by
  - FT-ICR-MS and Orbitrap
    - The detector is part of analyser
    - Ions only pass near the electrodes

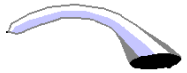
Detectors

- Elektromultiplier with discrete dynodes
  - Amplification 10^6

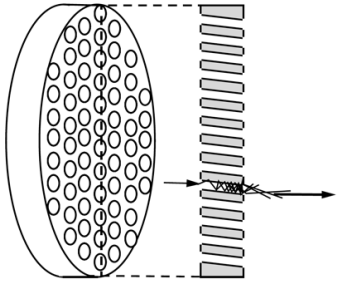


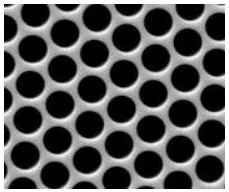
- Chaneltron
  - PbO - sensitive surface
  - Amplification 10^6



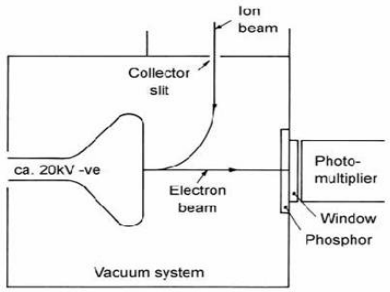


- Microchannel Plate Detectors (MCP)
  - PbO - sensitive service
  - Amplification 10^3
    - Two detectors – 10^6
  - For TOF analyser

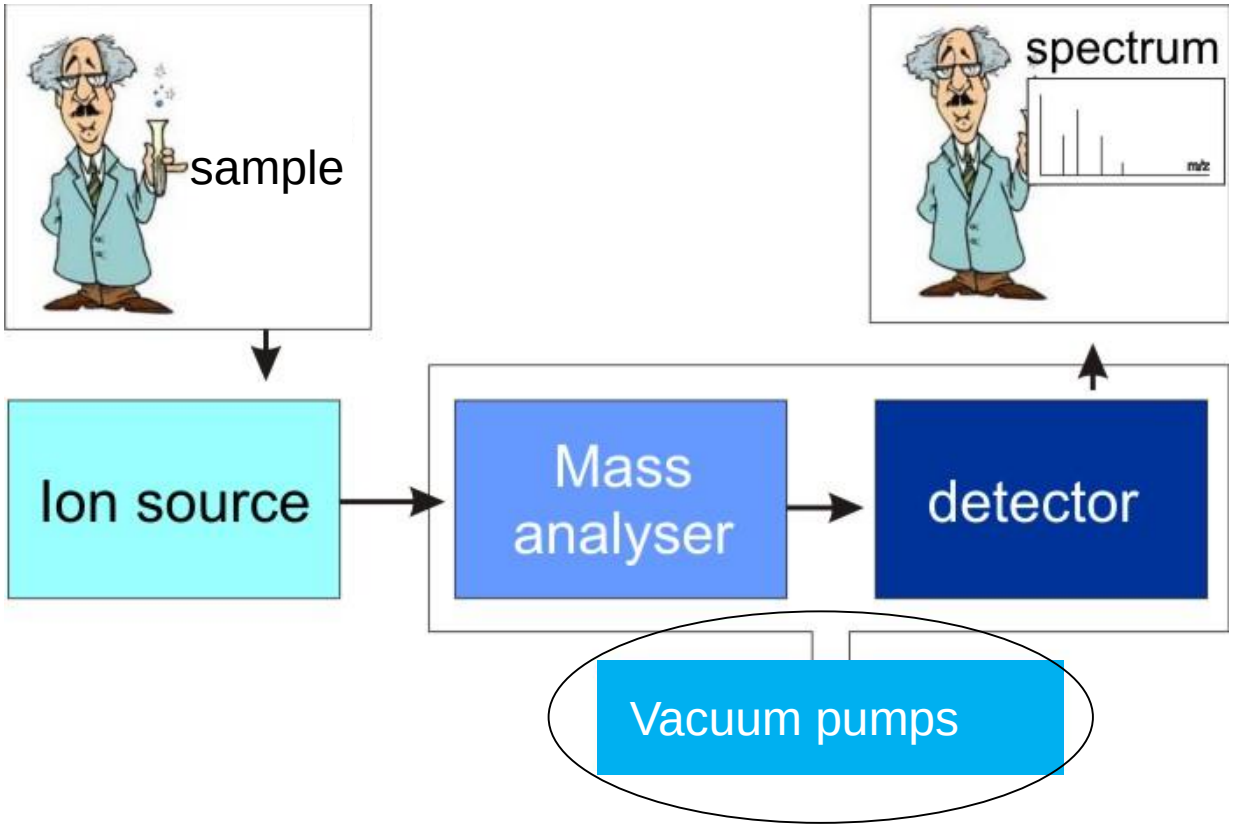




- Ion-to-photon detector
  - Electron strike a phosphor and the resulting photons are detected by a photomultiplier



VACUUM SYSTEM



Vacuum systems

- Usually two steps
  - Rough vacuum (roughing pump - membrane pump, scroll pump, oil-sealed roughing pump)
    - 100 – 0.1 Pa
    - all type of instruments
  - High vacuum (**turbomolecular pump**, diffusion pump)
    - $0.1\text{-}10^{-6}$ Pa,
    - TOF, Q, IT
  - Ultra-high vacuum (turbomolecular pump)
    - (10^{-10}-10^{-12} Pa)
    - Orbitrap, ICR