Ionization Methods – EI, FAB, MALDI, ESI, ICP

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Learning Goals

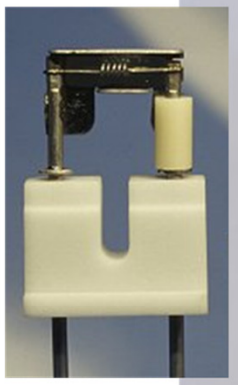
based on an understanding of the ionization mechanism, you should be able to

- judge the scope and limitations of the different methods discussed
- decide which of the ionization methods matches best your sample and chemical question to be answered
- prepare the sample for mass analysis
- roughly estimate the amount of internal energy deposited in the ions during ionization and how it changes the appearance of the spectra

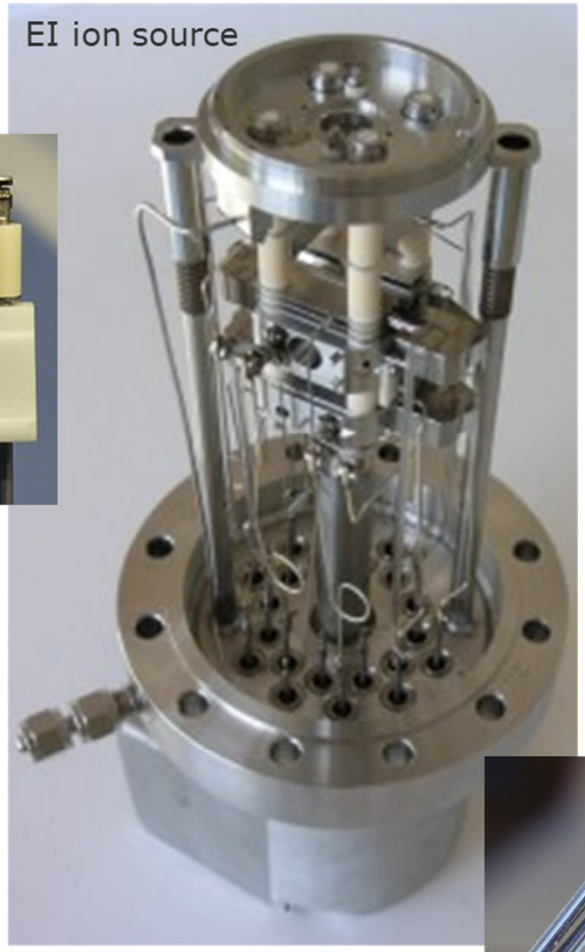
Electron Ionization (EI)

Electron Ionization: A Harsh Ionization Method

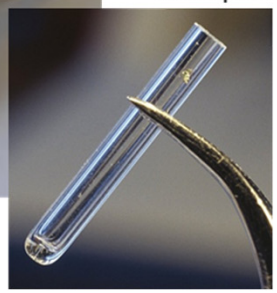
filament

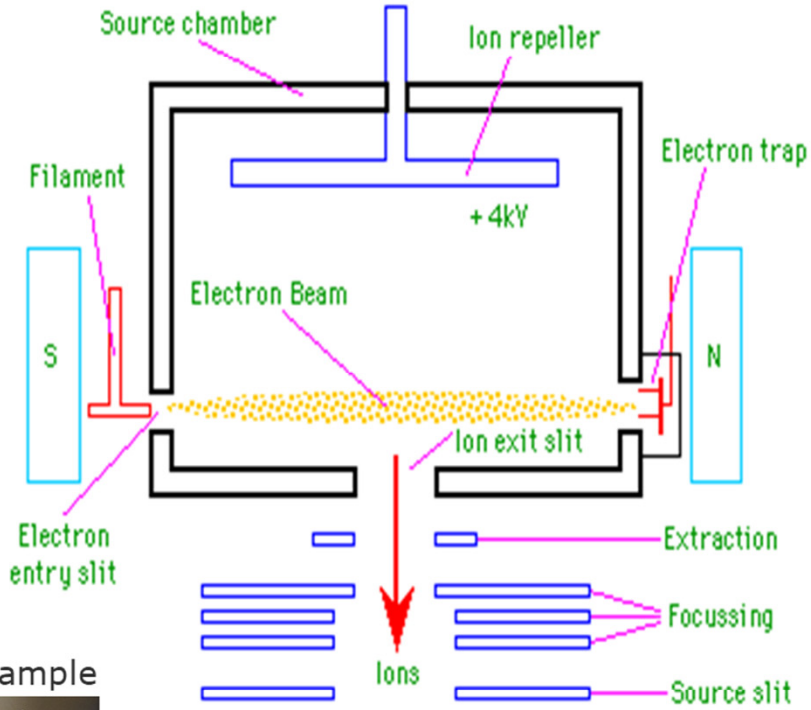


EI ion source



sample





Electron Ionization: Ionization Processes

Ionization by high-energy collisions with fast electrons:

1.	$AB + e^-$	$\rightarrow$	$[AB^{+\bullet *}] + 2e^-$	*electron impact*
	$[AB^{+\bullet *}]$	$\rightarrow$	$AB^{+\bullet}$	*collisional cooling*
2.	$AB + e^-$	$\rightarrow$	$A^+ + B^- + e^-$	*collisional dissociation*
3.	$AB + e^-$	$\rightarrow$	$A^+ + B^\bullet + 2e^-$	*collisional dissociation*
4.	$AH + e^-$	$\rightarrow$	$AH^* + e^-$	*collisional excitation*
	$AH^* + AH$	$\rightarrow$	$[AH+H]^+ + A^-$	*self-chemical ionization*

Fragmentation:

$[AB^{+\bullet *}]$	$\rightarrow$	$A^+ + B^\bullet$	*70 eV ≈ 7000 kJ/mol*

Electron Ionization: Scope and Limitations

- extremely well reproducible spectra due to exactly reproducible internal energies; spectral databases available; identification of unknowns possible through database searches

- sample needs to be vaporized; EI limited to m/z < 1000 for polar and to m/z < 2500 for unpolar substances

- electron energy (70 eV) is rather high; many parent ions destroyed by fragmentation

  advantage: structure information encoded in the fragments
  disadvantage: parent ion not always easy to identify

- open-shell radical cations $[M]^{+\bullet}$ produced with higher reactivity than closed-shell $[M+H]^{+}$

Fast Atom Bombardment (FAB)

Secondary Ion Mass Spectrometry (SIMS)

Fast Atom Bombardement: FAB and SIMS

Two related ionization methods

SIMS (secondary ion mass spectrometry; 1950 by R. E. Honig):
analyte in liquid matrix
bombarded with fast primary ions
e.g. Cs^+ with 25 keV kinetic energy

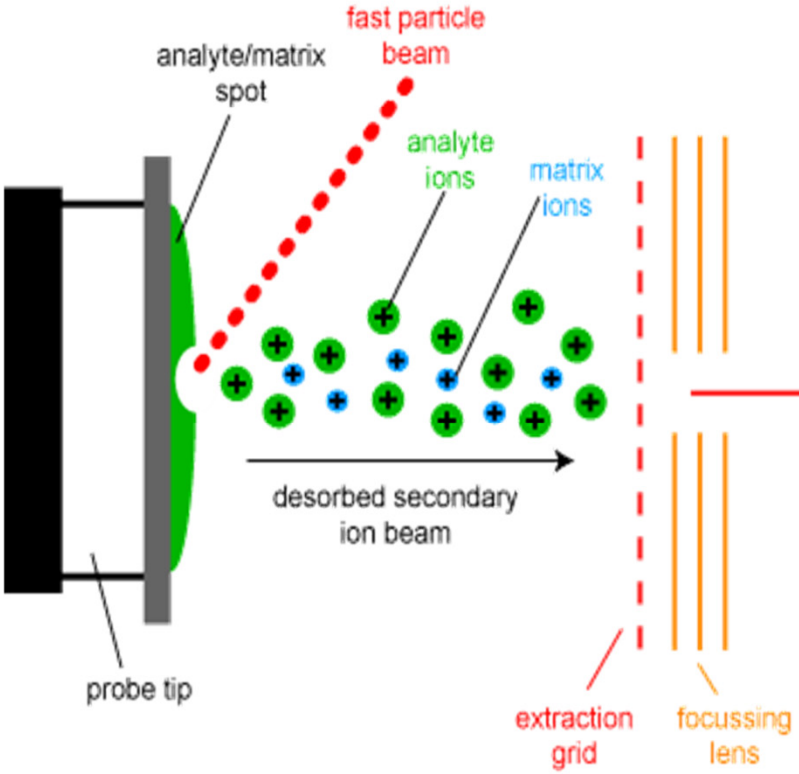
FAB (fast atom bombardment; 1981 by Barber *et al.*):
analyte in liquid matrix
bombarded with fast atoms
e.g. Xe with typically 8 – 10 keV kinetic energy

Result

large, polar and even ionic molecules (e.g. larger peptides) can be ionized;
fragmentation is moderate

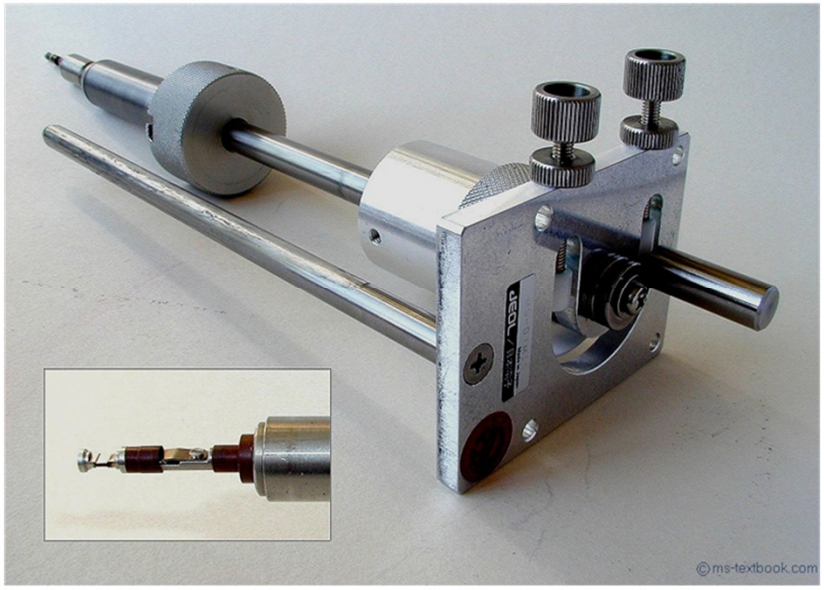
M. Barber, R. S. Bordoli, G. J. Elliott, D. Sedgwick, A. N. Tyler, *Anal. Chem.* **1982**, *54*, 645A
J. Sunner, *Org. Mass Spectrom.* **1993**, *28*, 805

FAB: The Ionization Mechanism

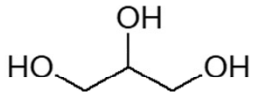


momentum transfer from fast atom into matrix

generation of quasi-molecular ions, e.g. $[M+H]^+$, $[M+Na]^+$

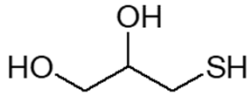


FAB: Liquid, Non-Volatile Matrices



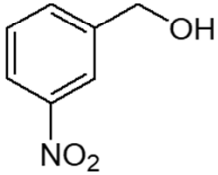
glycerol

for polar compounds



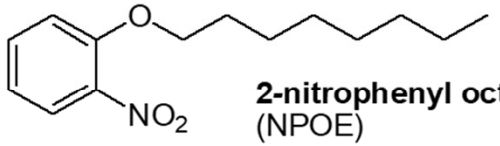
1-thioglycerol

for polar compounds
often increased $[M+H]^+$



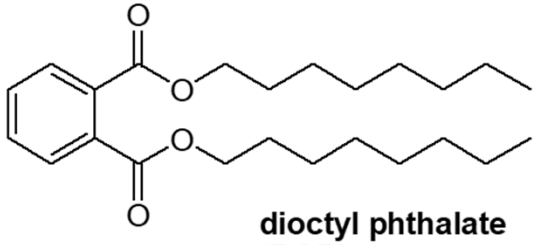
***m*-nitrobenzyl alcohol**
(mNBA)

good for less polar molecules

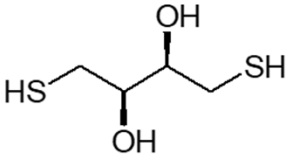


2-nitrophenyl octyl ether
(NPOE)

aprotic FAB matrices, thus excellently suited for organometallics and other reactive compounds

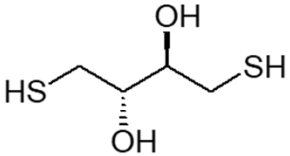


dioctyl phthalate
(DOP)



dithiothreitol

5 : 1

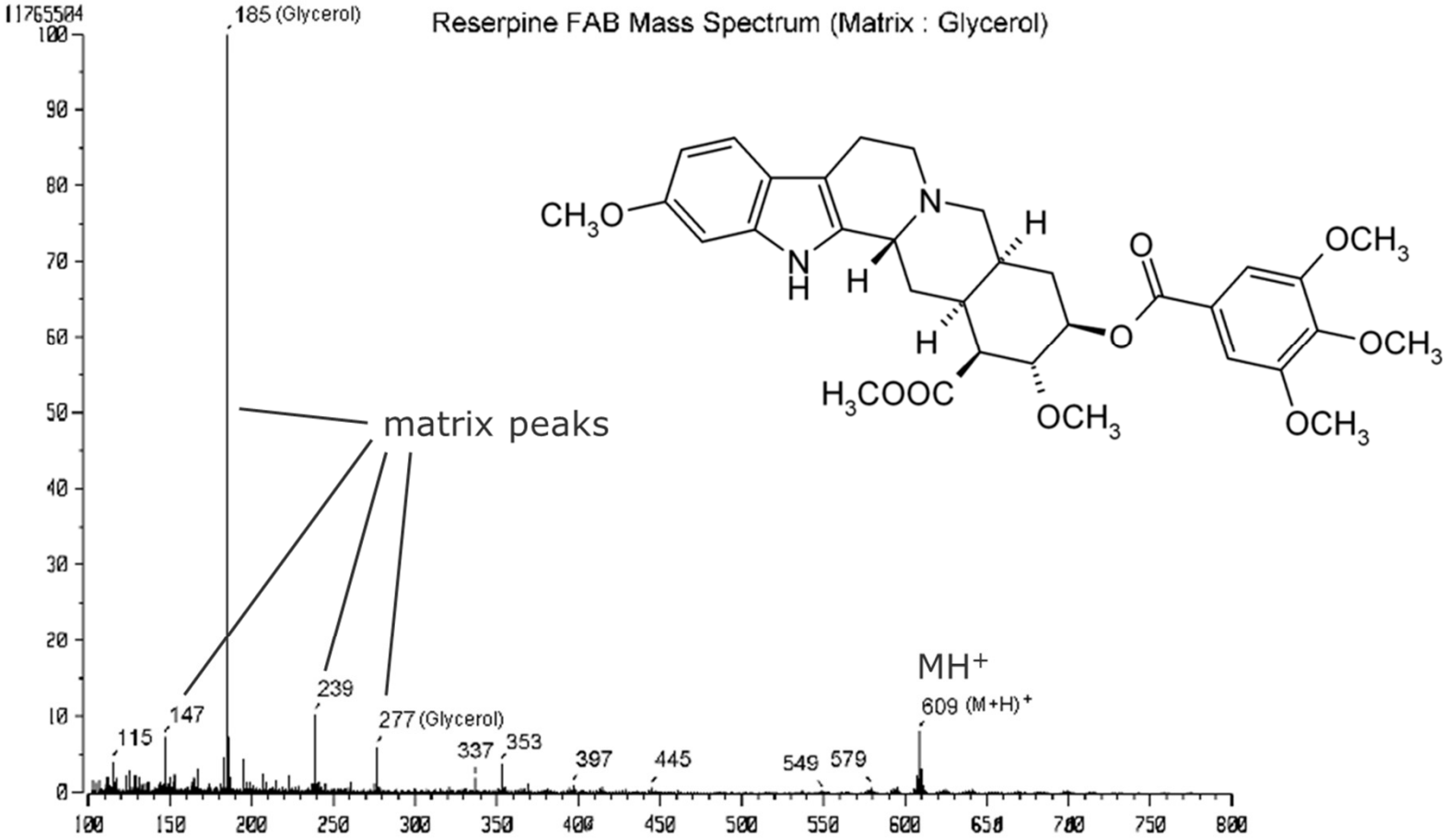


dithioerythritol

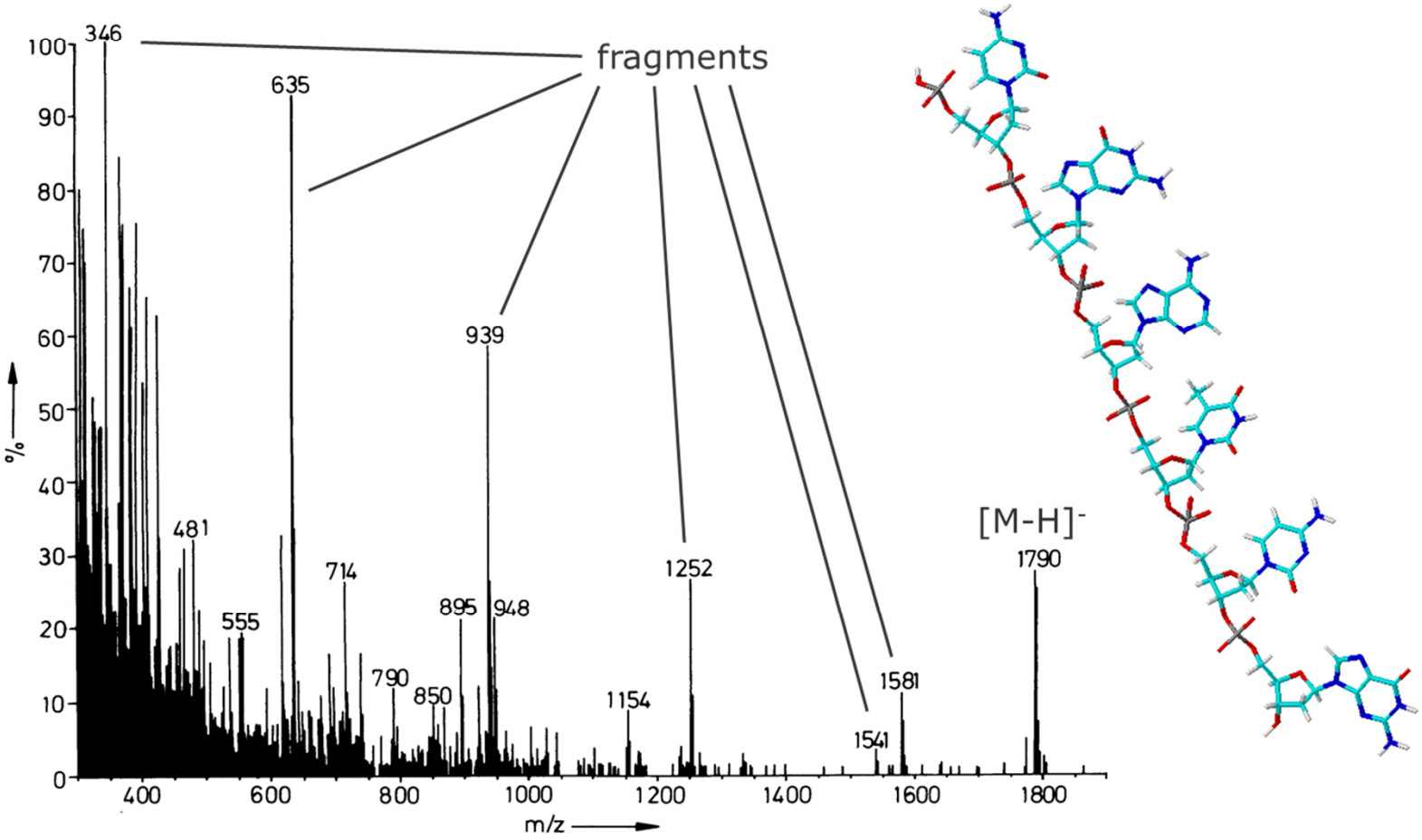
"magic bullet"

E. De Pauw, *Mass Spectrom. Rev.* **1986**, *5*, 191
E. De Pauw, A. Agnello, F. Derwa, *Mass Spectrom. Rev.* **1991**, *10*, 283

FAB: Example 1 - Reserpine



FAB: Example 2 – An Oligonucleotide



FAB: Scope and Limitations

Limitations of FAB/SIMS:

- matrix required: higher noise in particular at low m/z, matrix cluster ions
- analyte must dissolve in liquid matrix
- limited measuring time due to volatility of matrix
- limited mass range (m/z < 6.000 – 8.000)
- intensities often not very constant in a series of scans
- intensities often too low for efficient MS/MS experiments

FAB/SIMS today almost completely replaced by ESI and MALDI

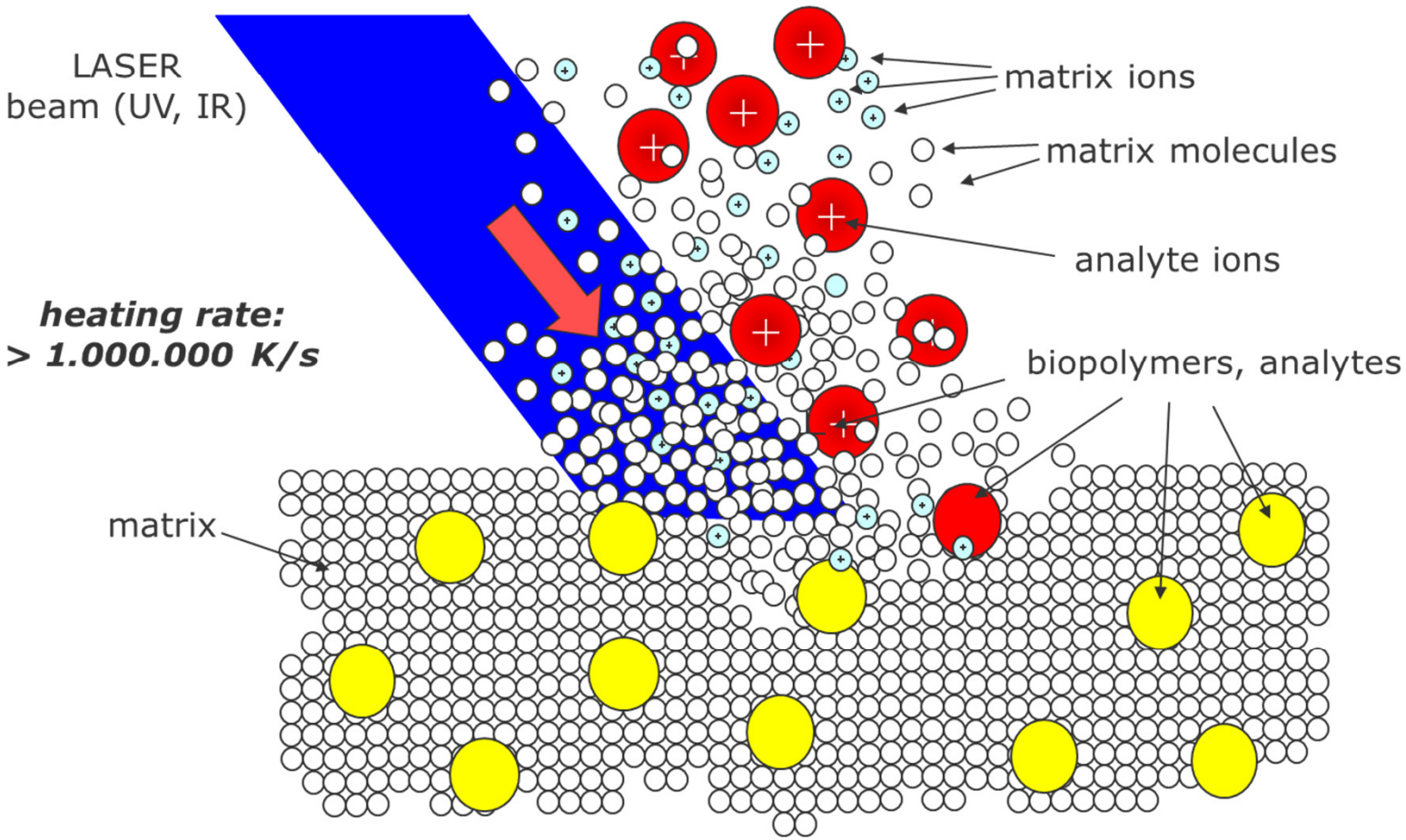
Main field of application:

- photo- (MALDI-laser)/electrochemically (ESI-HV) labile compounds
- compounds labile to oxidation or hydrolysis
- metal complexes

Matrix-Assisted Laser Desorption/Ionization

(MALDI)

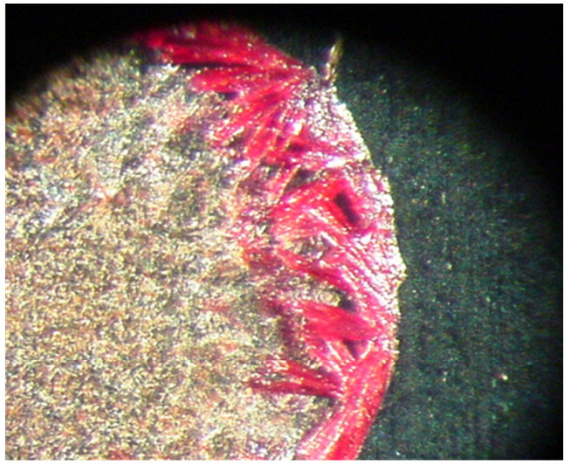
MALDI: Matrix-Assisted Laser Desorption/Ionization



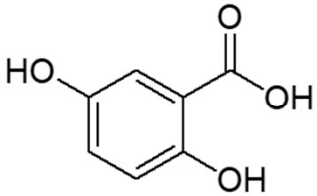
M. Karas, U. Bahr, U. Gießmann, *Mass Spectrom. Rev.* **1991**, *10*, 335

MALDI: The Role of the Matrix

- usually solid matrices, thus cocrystallization with analyte required (still under debate)
- matrix absorbs laser light (typically N_2 laser at 337 nm), must contain a suitable chromophore
- matrix protonates analyte and thus provides the charge for ion generation
- collisional cooling of ions in the plume; protection against fragmentation

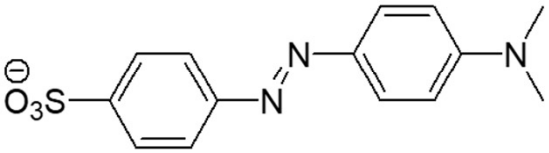


Problem: Matrix may change analyte or even react with it



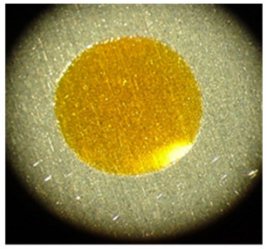
matrix: 2,5-dihydroxy benzoic acid (DHB)

$pK_a = 2.0$

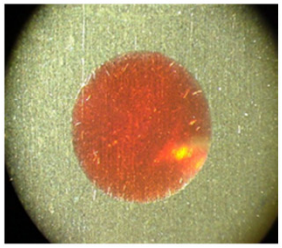


analyte: methyl orange

$pK_a = 4.2$



without DHB



with DHB

MALDI: Matrices – A Small Selection

There are thousands of matrices …

1.	α-cyanohydroxy cinnamic acid (CHCA)	proteins < 10 kDa, carbohydrates
2.	sinapinic acid (SA)	proteins > 10 kDa, fullerenes
3.	succinic acid	
4.	2,6-dihydroxy acetophenon	
5.	ferula acid	
6.	caffeic acid	
7.	4-nitroaniline (liquid, IR-MALDI)	
8.	glycerol (liquid, IR-MALDI)	
9.	2,4,6-trihydroxy acetophenone (THAP)	oligonucleotides < 3.500 Da, carbohydrates
10.	3-hydroxy picolinic acid (HPA)	oligonucleotides > 3.500 Da
11.	anthranilic acid	
12.	nicotinic acid	
13.	*trans*-3-indolacrylic acid (IAA)	nonpolar synthetic polymers
14.	dithranol (DIT)	oligonucleotides, dendrimers, lipids
15.	dihydroxy benzoic acid (DHB)	polar synthetic polymers
16.	1-isochinoline	oligosaccharides
17.	…	

M. A. R. Meier, N. Adams, U. S. Schubert, *Anal. Chem.* **2007**, *79*, 863
K. Strupat, et al., *Int. J. Mass Spectrom. Ion Processes* **1997**, *169/170*, 31 and 43

MALDI: Sample Preparation Alchemy

… and dozens of sample preparation procedures

1. dried droplet
2. vacuum drying
3. crushed crystal
4. fast evaporation
5. overlayer
6. sandwich
7. spin-coating
8. slow crystallization
9. electrospray
10. quick and dirty
11. matrix precoated target
12. …

Most common preparation procedure:

- **solution A:** dissolve sample in a volatile solvent (some acid can be helpful)
  concentration: ca. 10^{-6} - 10^{-8} mol/L

- **solution B:** dissolve matrix in the same or a similar solvent
  concentration: ca. 5 - 10 mg/mL

- mix solutions **A** and **B** in a 1:1 ratio

- spot ca. 2 µl of the mixture on a MALDI sample plate, the MALDI target

- slow evaporation of the solvent leads to crystallization; sample is ready

MALDI: Ion Formation Mechanisms

- direct photo ionization

  ⇒ only possible for UV-absorbing substances

- gas-phase proton transfer through matrix photo ionization

  ⇒ appears to be important

- gas-phasecationization

  ⇒ important only for small neutral molecules (e.g. small sugars)

- electron transfer

  ⇒ not clear, but does not seem to be of great importance
  (mainly secondary reaction/charge reduction)

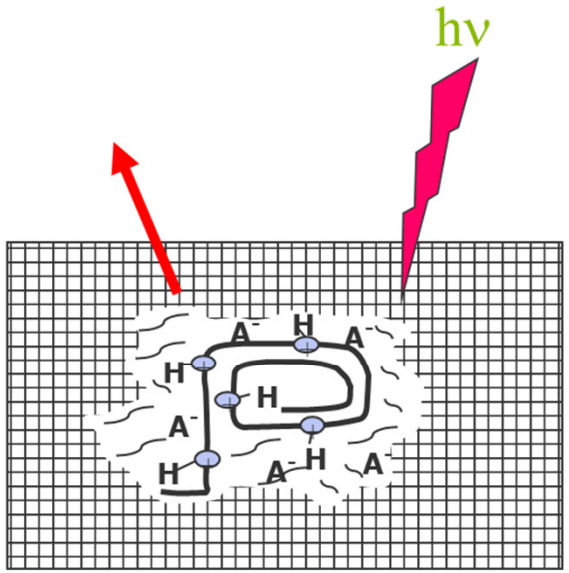
H. Ehring, M. Karas, F. Hillenkamp, *Org. Mass Spectrom.* **1992**, *27*, 427

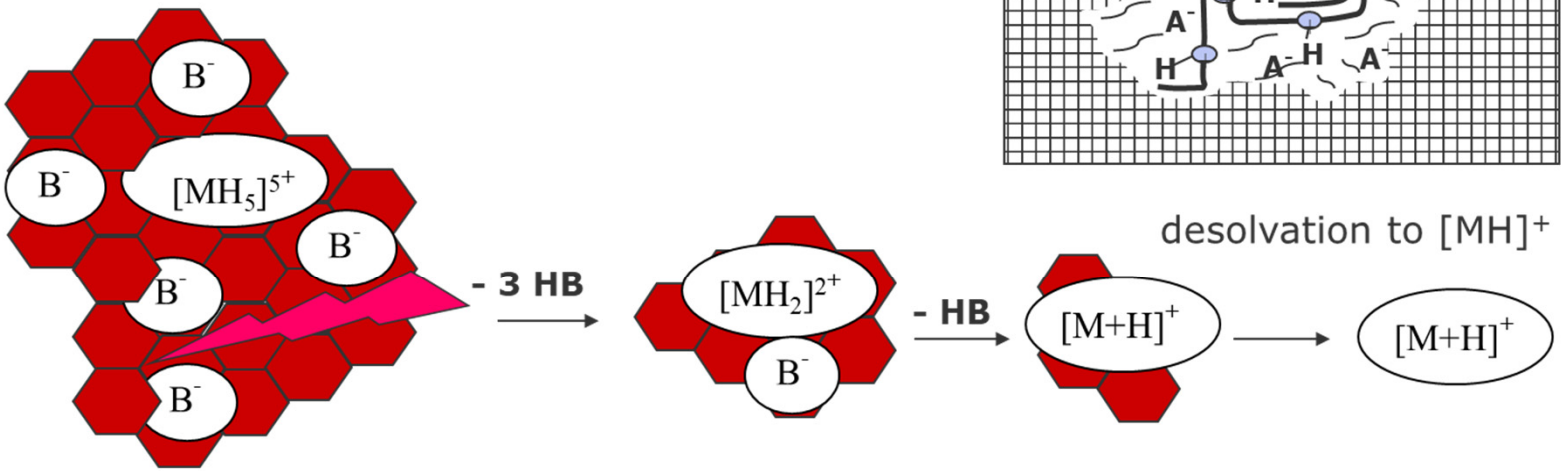
MALDI: Ion Formation Mechanisms

predominant primary ion formation pathway

formation of charged clusters that are desolvated to thermodynamically stable ions

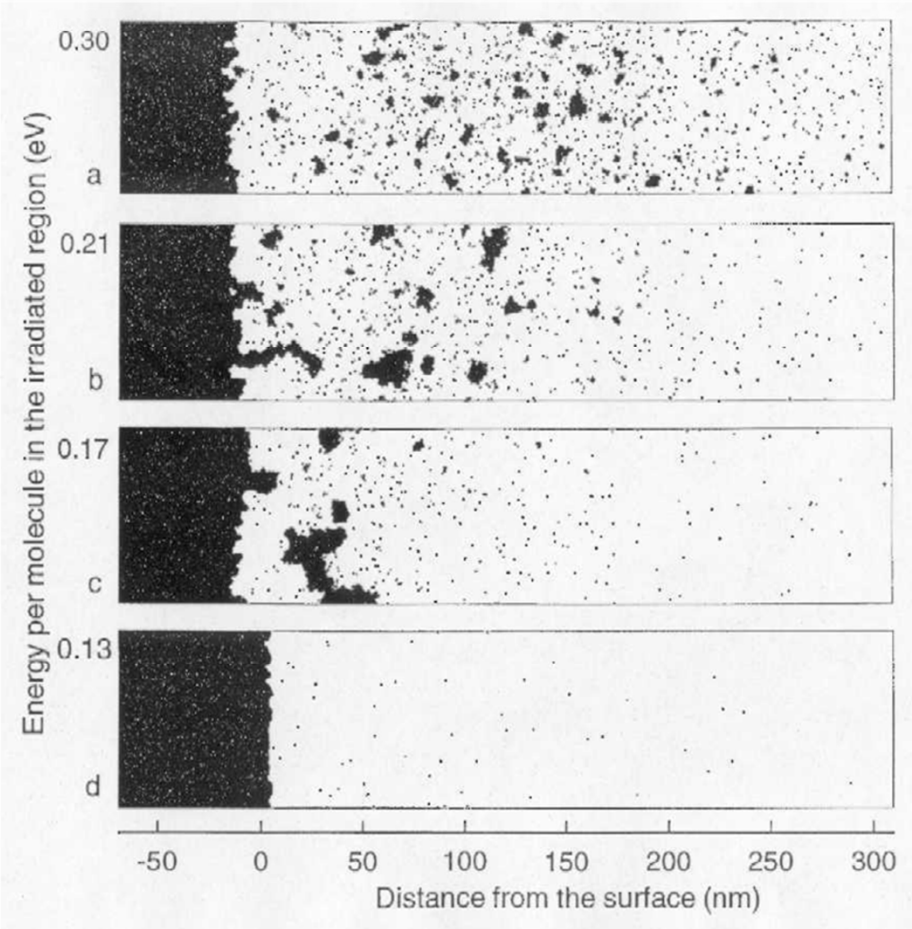
additionally, proton- (and electron-) transfer reactions occur





M. Karas, R. Kruger, *Chem. Rev.* **2003**, *103*, 427

MALDI: The Role of Laser Power



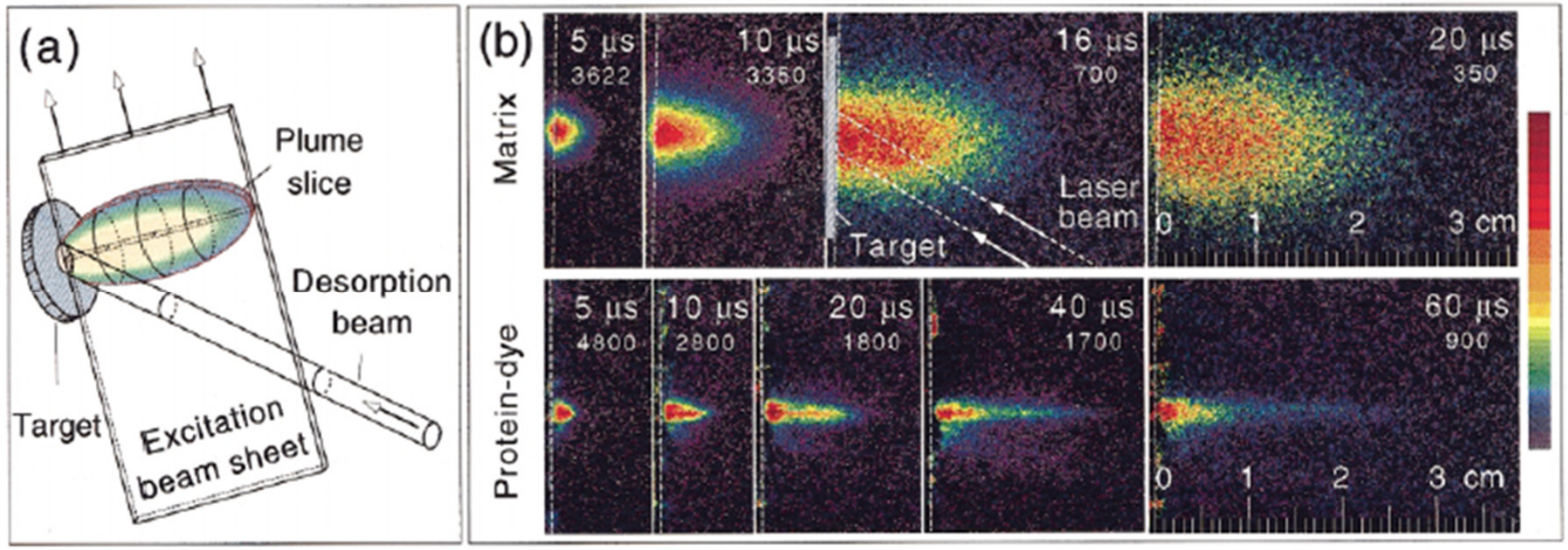
above threshold:

collective ejection or ablation of clusters

below threshold: evaporation of single molecules

MALDI: The Plume

Imaging the MALDI plume



processes in the plume:
desolvation of clusters, proton transfer, electron transfer (rare), collisional cooling of ions, …

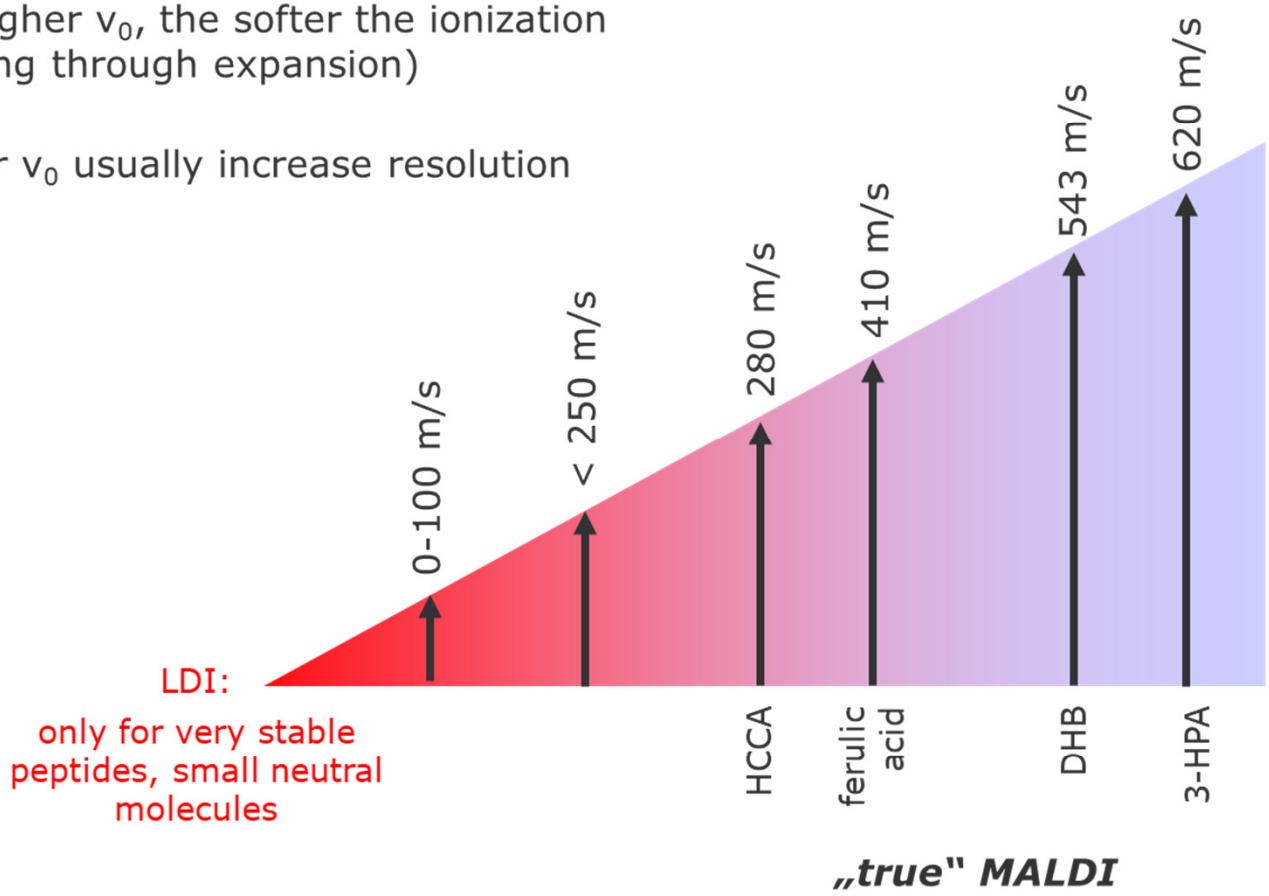
Initial velocities of clusters away from the surface differ from matrix to matrix

R. Knochenmuss, R. Zenobi, *Chem. Rev.* **2003**, *103*, 441

MALDI: Why Initial Velocities are Important

the higher v_0, the softer the ionization (cooling through expansion)

higher v_0 usually increase resolution



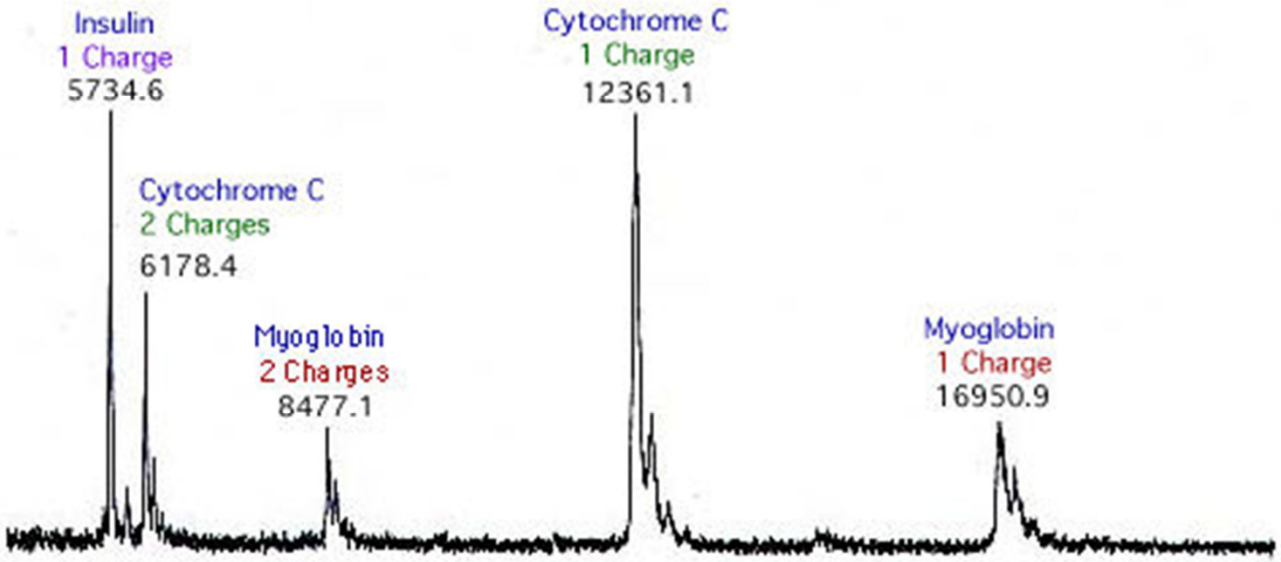
S. Berkenkamp, C. Menzel, F. Hillenkamp, K. Dreisewerd, *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 209

MALDI: Scope and Limitations

first advantage of MALDI:

ions with narrow charge distribution (normally z = 1 > 2 >> 3)

easy spectrum interpretation even from analyte mixtures

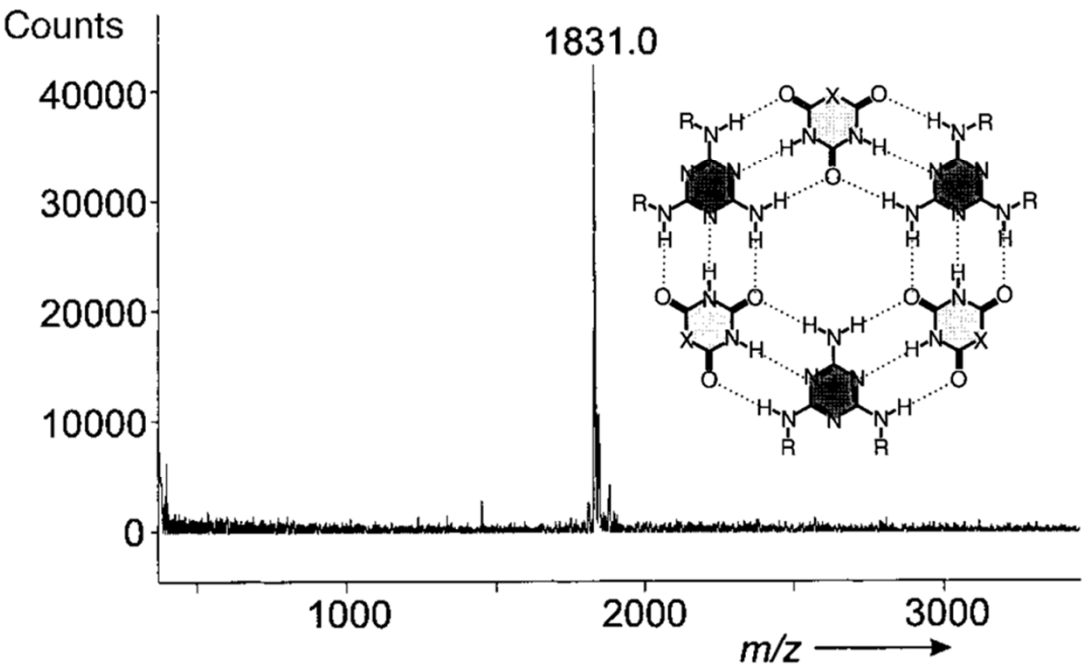


MALDI Spectrum of Insulin, Horse Cytochrome C and Myoglobin

MALDI: Scope and Limitations

MALDI useful for large molecules including (bio-)polymers, dendrimers and some non-covalent biomolecule complexes

few examples for intact ionization of synthetic non-covalent complexes



MALDI still quite harsh

matrices often competitive, for example with hydrogen bonded systems

K.A. Jolliffe, C.M. Calama, R. Fokkens, N.M.M. Nibbering, P. Timmerman, D.N. Reinhoudt, *Angew. Chem. Int. Ed.* **1998**, *37*, 1247

MALDI: Scope and Limitations

second advantage of MALDI

in situ recrystallisation during sample preparation

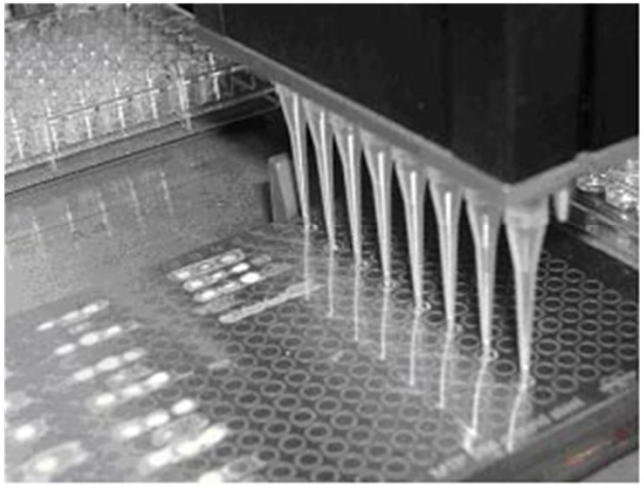
relatively high tolerance against buffers, surfactants etc.

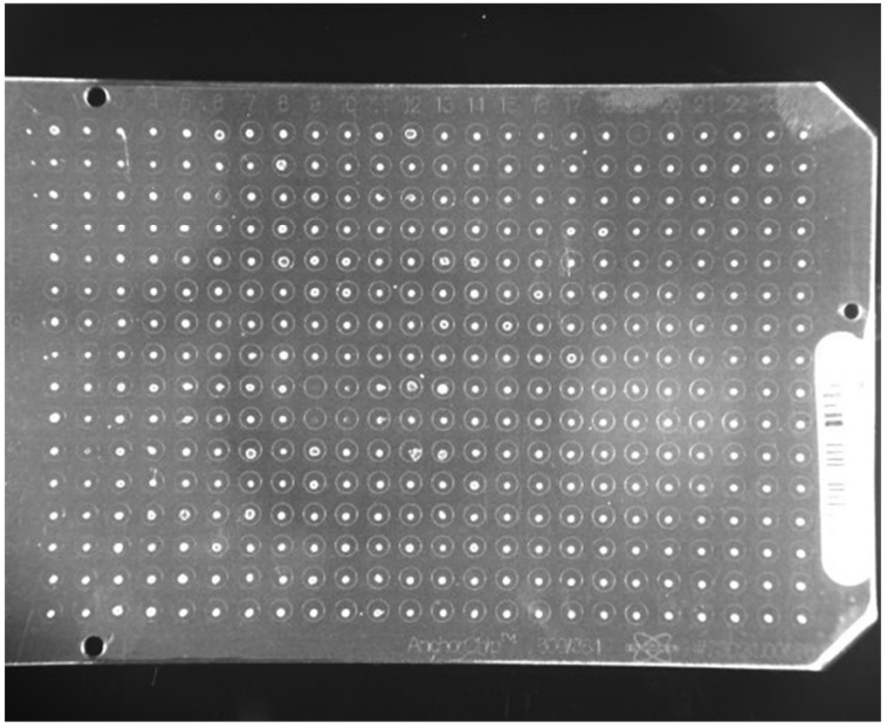
	Highest tolerable contamination (ca.)
urea	0.3 M
guanidinium-HCl	0.5 M
dithiothreitol	0.5 M
glycerol	1% (matrix for IR-MALDI)
alkaline salts	< 0.3M
tris buffer	0.05 M
NH_4HCO_3	0.05 M
phosphate buffer	0.01 M
detergents (not SDS)	0.1%
SDS	0.01%

MALDI: Scope and Limitations

third advantage of MALDI

automating sample preparation and MALDI experiments save cost- and time





robots for pipetting, automatic
spectrum recording,
automatic data evaluation

Electrospray Ionization

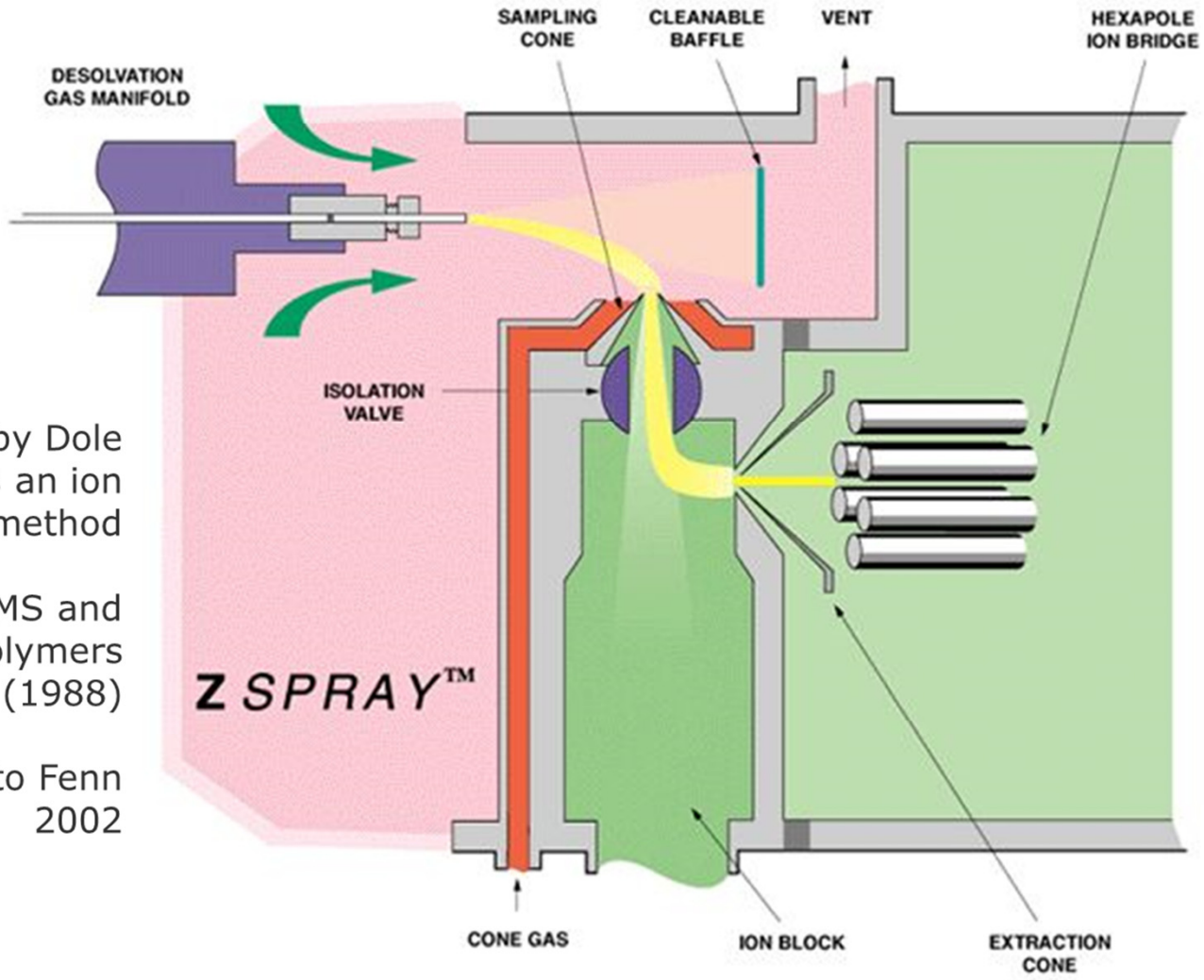
(ESI)

ESI: Electrospray Ionization

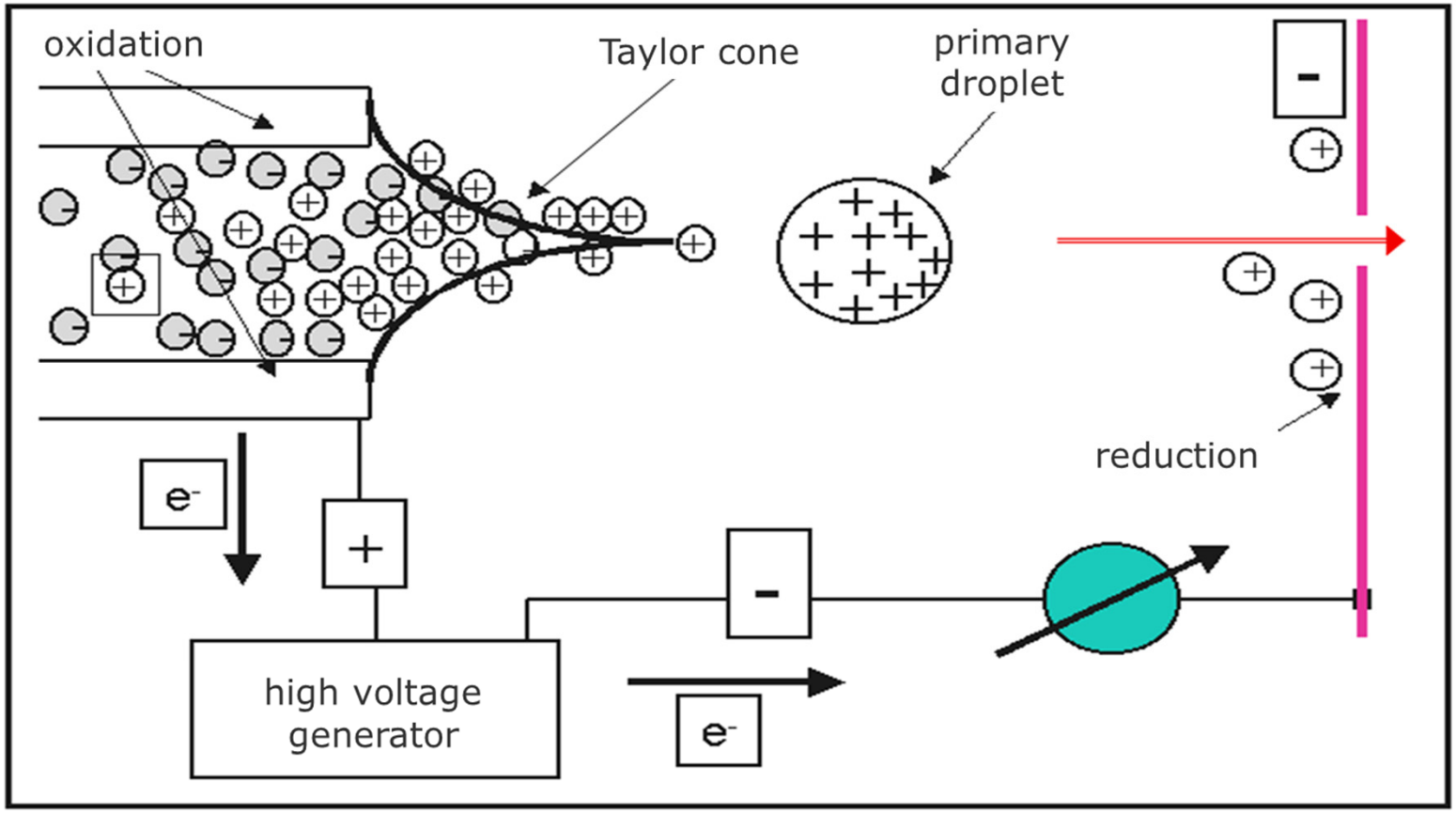
invented by Dole (1968) as an ion generating method

coupled to MS and applied to biopolymers by Fenn (1988)

Nobel prize to Fenn 2002

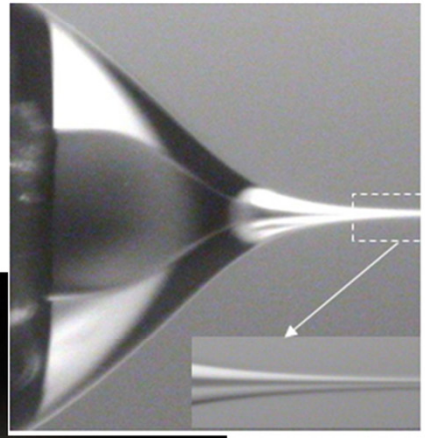


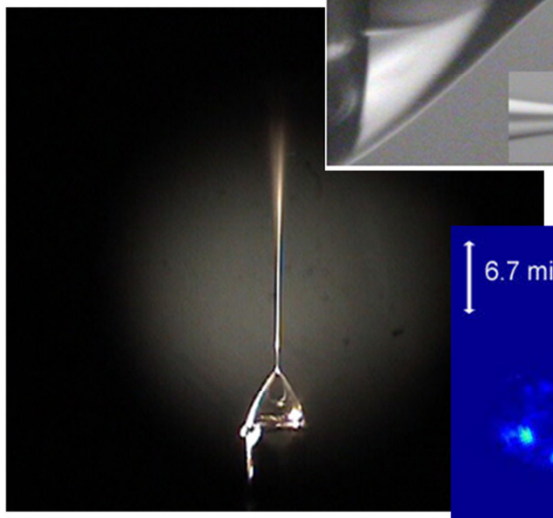
ESI: Ion Formation – The Taylor Cone



J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Mass Spectrom. Rev.* **1990**, *9*, 37
N. B. Cech, C. G. Enke, *Mass Spectrom. Rev.* **2001**, *20*, 362

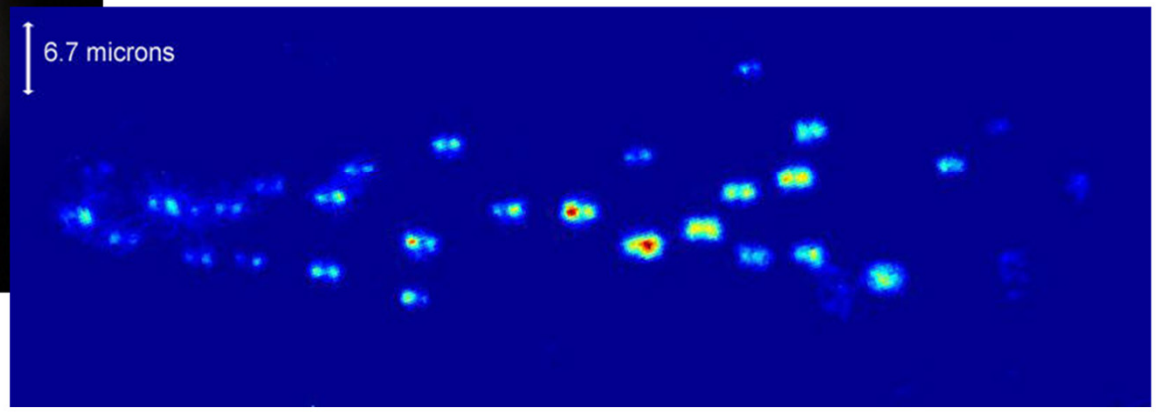
ESI: Ion Formation – The Taylor Cone





diameter of the droplets depends on:
- solvent viscosity, conductivity, volatility
- capillary diameter
- flow rate
- high voltage

ESI flow rate: 2 to 500 µL/min:
diameter of droplets <10 µm



ESI: Ion Formation – The Rayleigh Limit

droplet size limited by the number of charges and the surface tension of the solvent in the droplet

$$q = \sqrt{8\pi^2 \cdot \varepsilon_0 \cdot \gamma \cdot d^3}$$

q: number of charges in droplet
ε_0: vacuum permittivity for electrostatic interactions
γ: surface tension of the droplet solvent
d: droplet diameter

if the number of charges in the droplet is higher, the droplet undergoes Coulomb fission

ESI: Ion Formation – Two Mechanisms

CRM (Charge Residue Model)

after several Coulomb explosions droplets so small (ca. 1 nm), that they contain only ONE charged ion

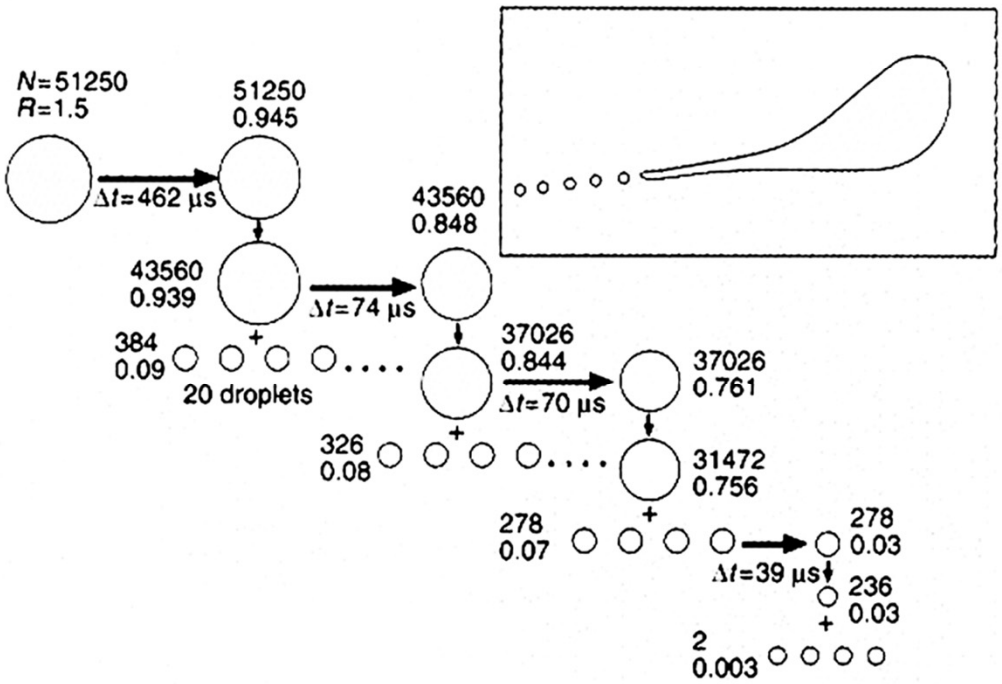
remaining solvent evaporates and desolvated ion is formed

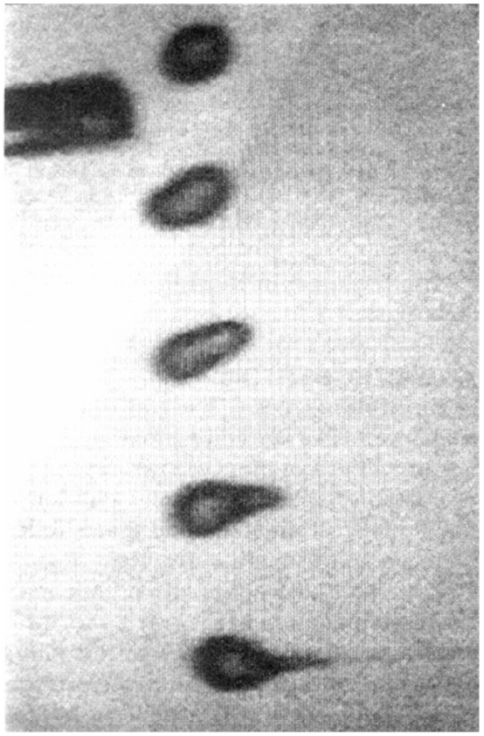
IEM (Ion Evaporation Model)

large droplets may emit small droplets as well as single unsolvated ions, when beyond the Rayleigh limit

G. Wang, R. B. Cole, *Anal. Chim. Acta* **2000**, *406*, 53
E. A. C. Spencer, T. Ly, R. R. Julian, *Int. J. Mass Spectrom.* **2008**, *270*, 166

ESI: Ion Formation – Charge Residue Model





jet formation on droplets:

Coulomb fission does not necessarily lead to smaller droplets with uniform size distribution

P. Kebarle, L. Tang, *Anal. Chem.* **1993**, *65*, A972

ESI: What is Seen in the Spectra

sign of the voltage applied on the sprayer defines the kind of ions:

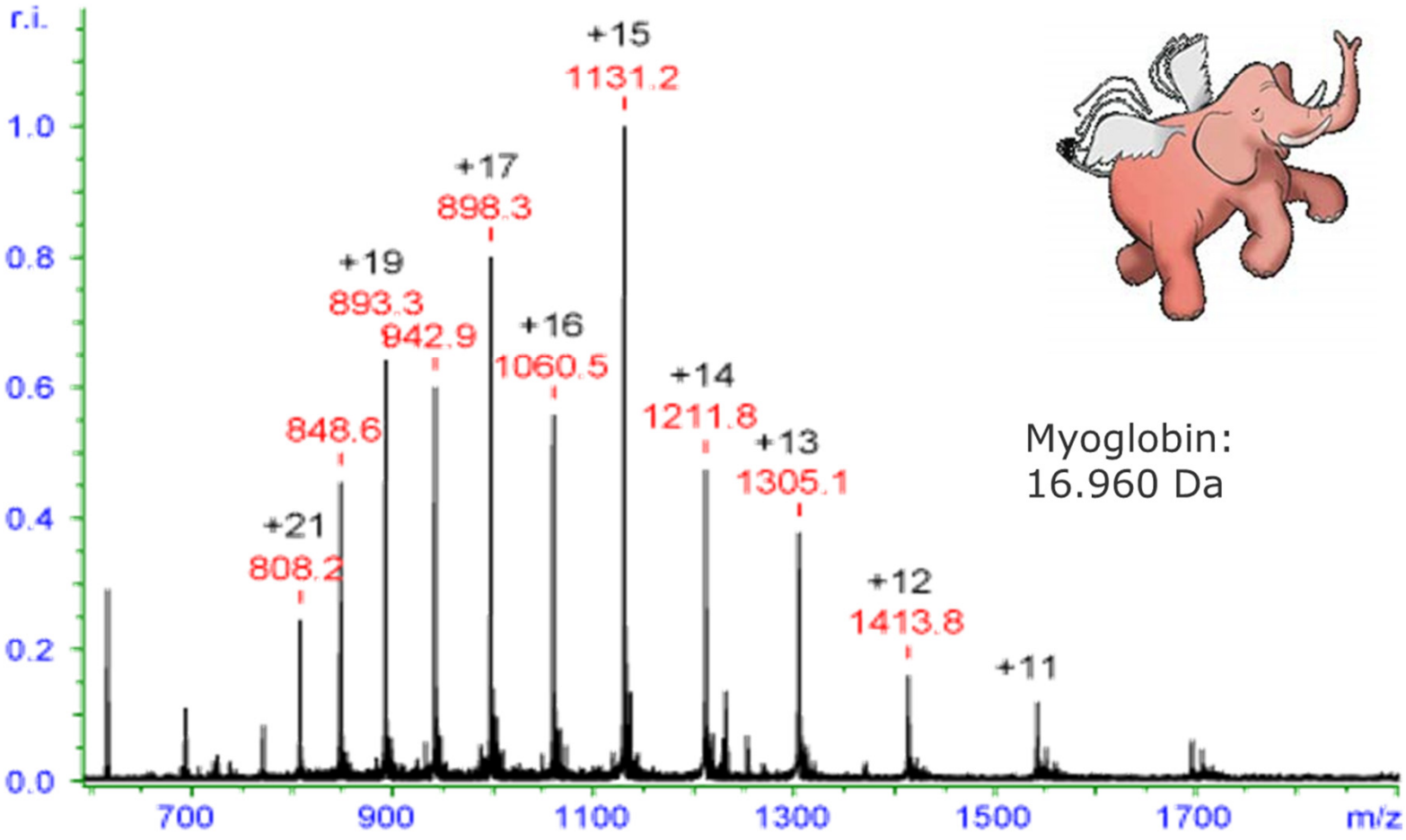
positive voltage leads to the formation of positively charged ions,
negative voltage to negative ions

ESI can be tuned to **very soft ionization conditions**
non-covalent aggregates often ionized with limited fragmentation

quasi molecular ions are usually detected
e.g. $[M+H]^+$ or $[M+Na]^+$ or $[M-H]^-$)

multiply charged ions $[M+nH]^{n+}$ are often formed from larger molecules
(biopolymers etc.)

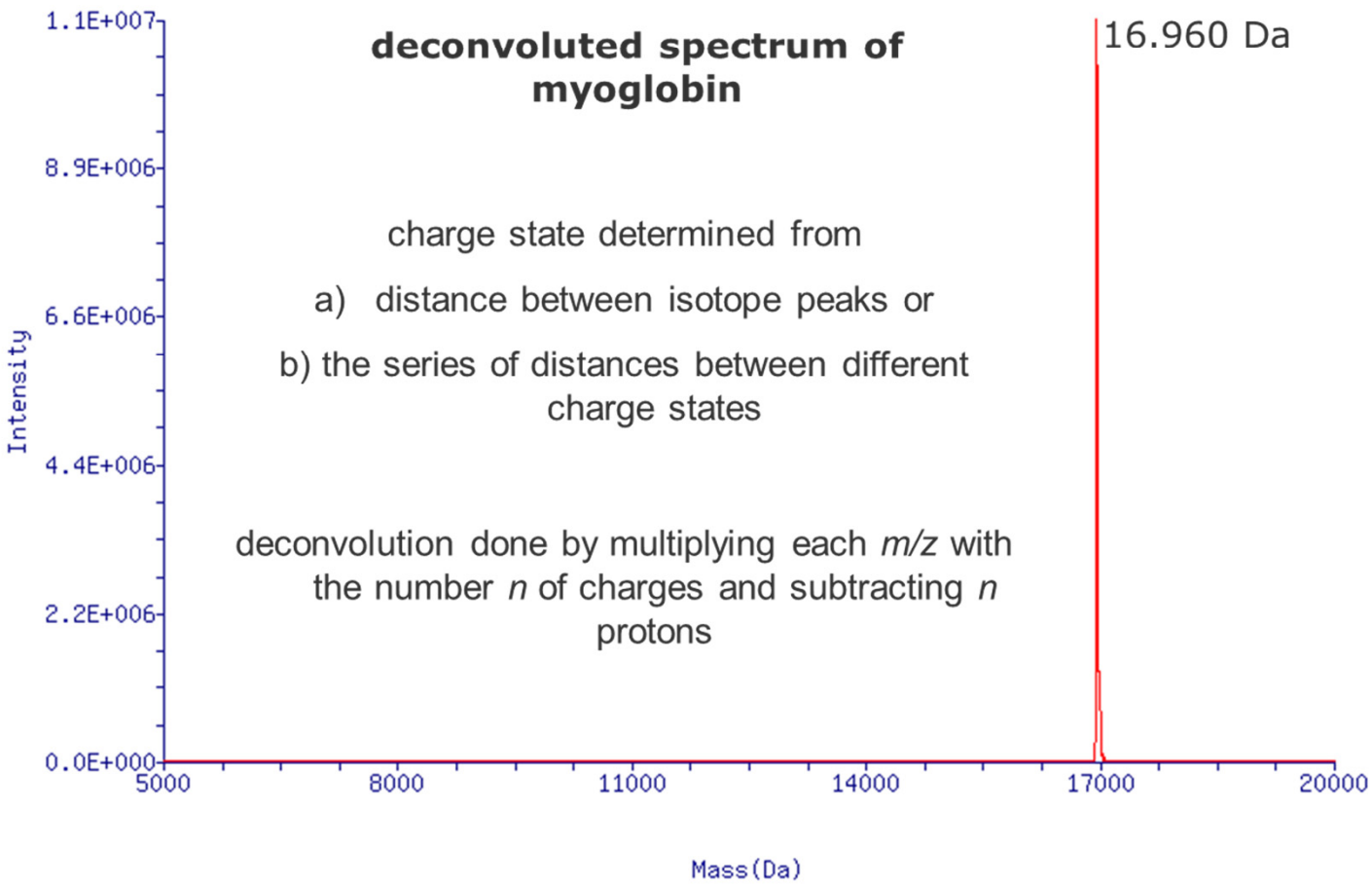
ESI: Myoglobin as an Example



Myoglobin:
16.960 Da

J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Science* **1989**, *246*, 64

ESI: Deconvolution of Charge States



ESI: Suitable Analytes

- analyte must be **completely soluble**
  (blocking of the capillary)

- **suppression effect** in complex mixtures
  plasticizer, (nonvolatile) salts, other impurities (e.g. polyethylene glycol) interfere!

- analyte must be **stable against electrochemical oxidation**/ **reduction**
  (e.g. some metal complexes decompose when ionized)

- analyte should be **stable against water and oxygen**

- analyte should have **ionizable functional groups**
  (polarity must be comparably high)

ESI: Suitable Spray Solvents

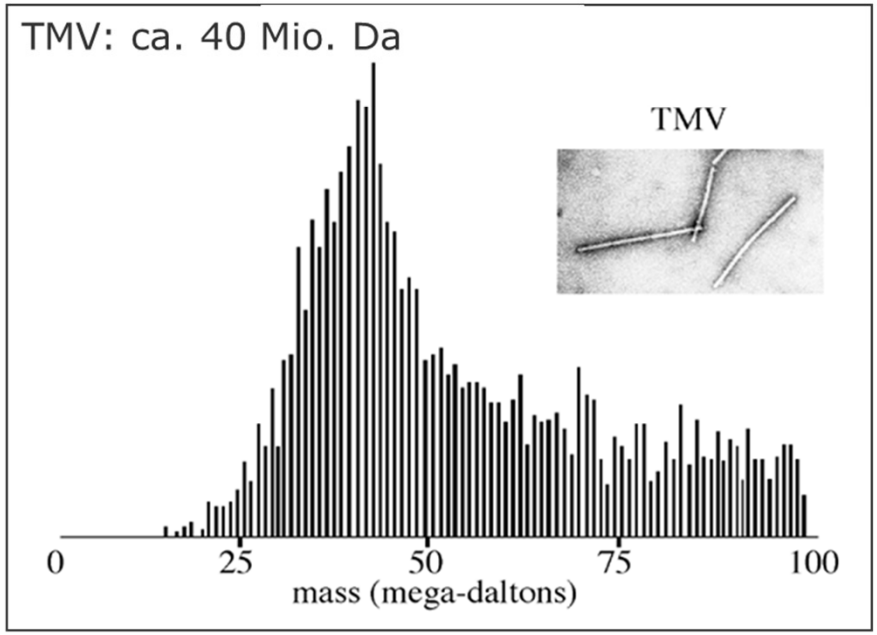
- solvents should be quite **volatile**, provide **conductivity**
  rule of thumb: miscible with water = good solvent
  (DMSO and DMF are non-ideal solvents, they form stable ions)

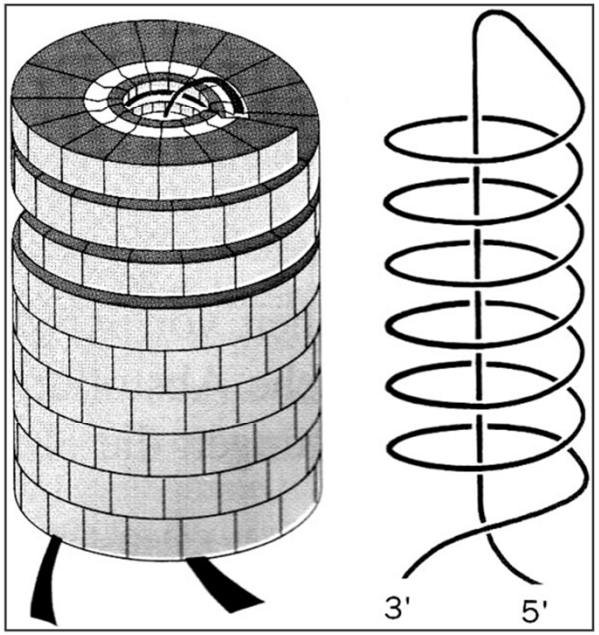
- **typical examples**
  water with ≥ 20% organic solvent, ACN, MeOH, acetone, 2-propanol

- to improve protonation, **volatile acids/bases** can be added
  e.g. formic acid, ammonium acetate, triethyl amine, …

- if **aprotic solvents** are to be used, a charge must be provided by any suitable means (protonation impossible)

ESI: Non-Covalent Complexes - A Highlight





Tobacco Mosaic Virus: 2131 non-covalently bound building blocks

structural integrity after ionization can be proven by collecting viruses from the gas phase. They are still infectious

S.D. Fuerstenau, W.H. Benner, J.J. Thomas, C. Brugidou, B. Bothner, G. Siuzdak, *Angew. Chem. Int. Ed.* **2001**, *40*, 541; G. Siuzdak, B. Bothner, M. Yeager, C. Brugidou, C.M. Fauquet, K. Hoey, C M. Chang, *Chem. Biol.* **1996**, *3*, 45

Ionization Methods: A Comparison