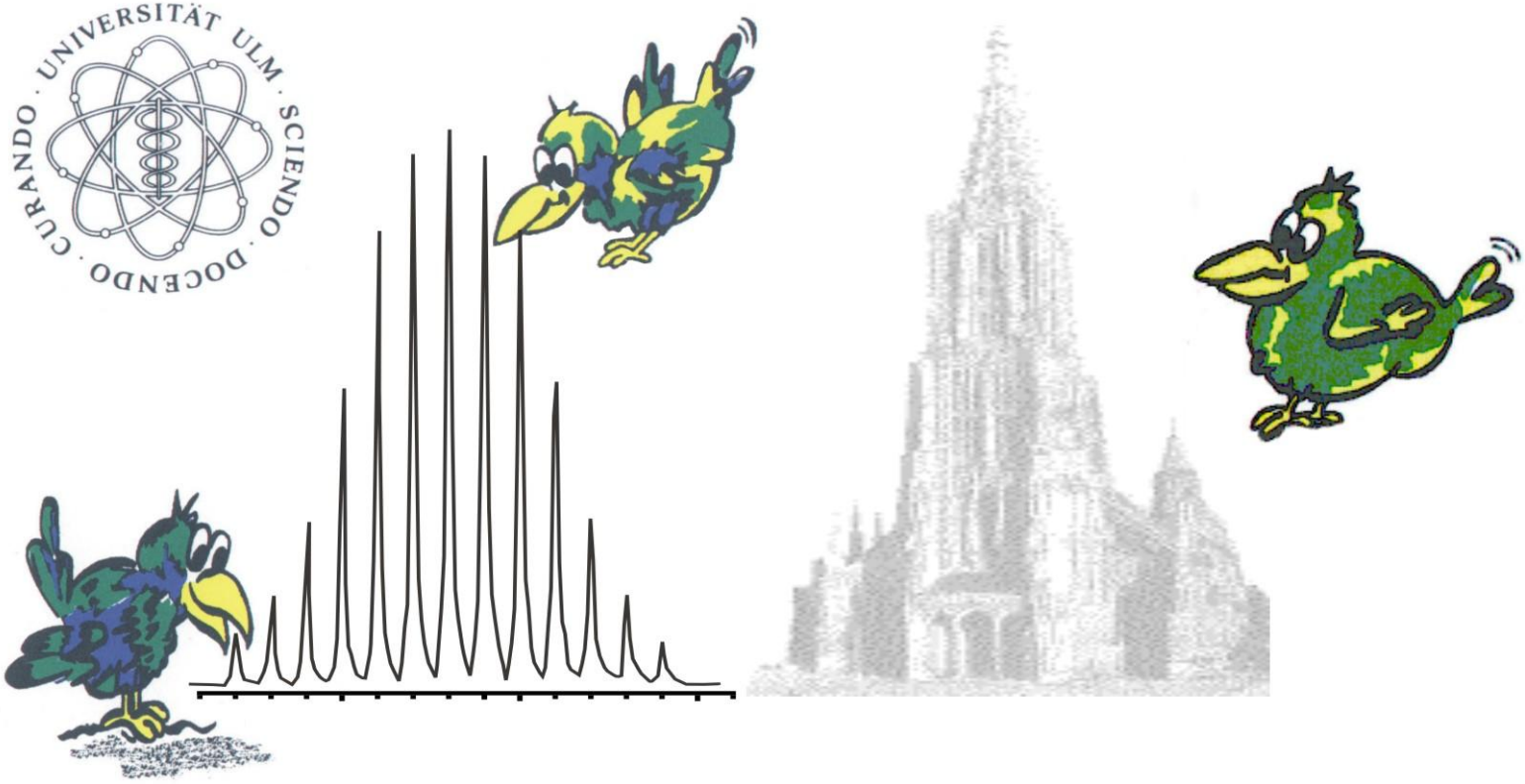


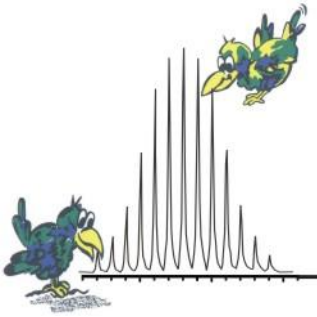
Mass Spectrometry
Frequently Asked Questions

Dr. Markus Wunderlin, Seminar

07.07.2004

Overview

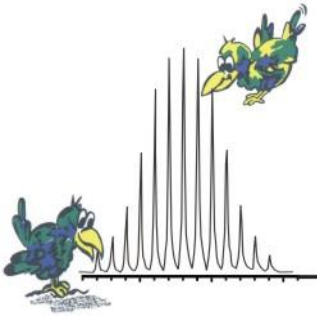
- Mass Spectrometry in a Nutshell - Facts and Basics
- Mass Resolution and Mass Accuracy
- Fragmentation – Dissozation – Adduct Formation
- Impurities - Contamination - Artefacts
- FTICR-MS: The „Ferrari Age" Of MS



Facts and Basics

Mass Spectrometry

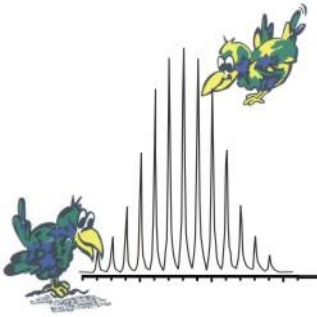
A technique for measuring and analyzing molecules, that involves introducing enough energy into a (neutral) target molecule to cause its ionization and disintegration. The resulting primary ions and their fragments are then analyzed, based on their mass/ charge ratios, to produce a "molecular fingerprint."



Facts and Basics

Difference Between Spectrometric Methods:

Ionization implies a chemical process induced by physical methods. The sample is consumed during the measurement. Their is no defined stimulation of molecular energy levels through interaction with electromagentic radiation, where you can get the sample back without modification.



Structural Information by MS

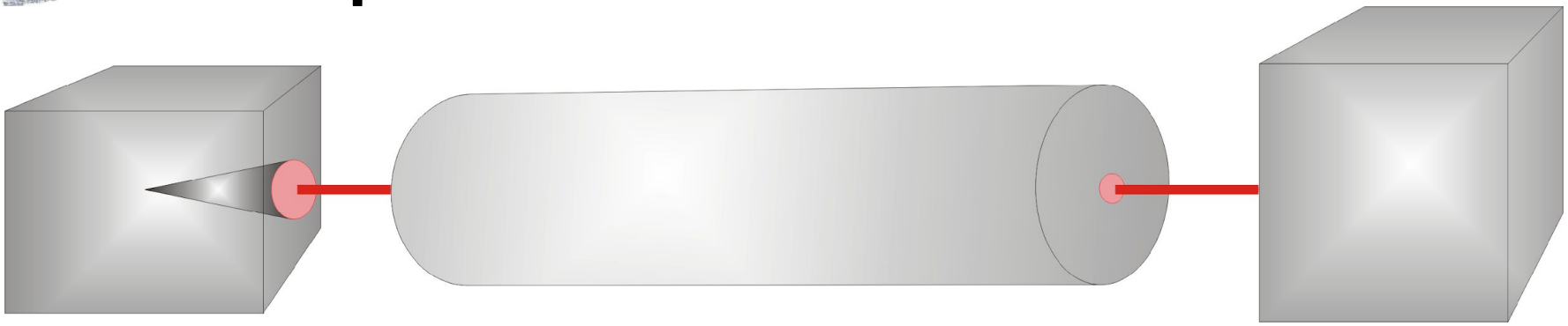
MW determination

- nominal
- accurate (elemental composition)
  - Isotope pattern
  - High resolution

Fragmentation

- Fragmentation rules
- Libraries („fitting“)
- MS/MS (or MS^n)

Components Of A Mass Spectrometer



Ionisation

Ion Source

- Electron Ionisation (EI)
- Chemical Ionisation (CI)
- Fast Atom Bombardment (FAB)
- Electrospray Ionisation (ESI)
- Matrix-Assisted Laserdesorption/ Ionisation (MALDI)

Ion Separation

Mass Analyser

- Quadrupole
- Magnetic Sector Field
- Electric Sector Field
- Time-Of-Flight (TOF)
- Ion Trap

Ion Detection

Detector

- Electron Multiplier
- Multichannel plate
- Faraday Cup

Sektion MS: Mass Spectrometers

	EI	CI	ESI	APCI	MALDI	FAB	MS/MS	Inlet	Status
Bruker Reflex III					+		PSD		
Finnigan SSQ7000	+	+					+	GC, SP, DEP	
Finnigan TSQ700	(+)	(+)	(+)	+		+		GC, SP, DEP	
Finnigan TSQ7000			+					Nano-ESI	

Sektion MS: Info & Data

- Homepage „Sektion Massenspektrometrie“

  http://www.uni-ulm.de/uni/fak/natwis/oc2/massenspektrometrie/index.htm

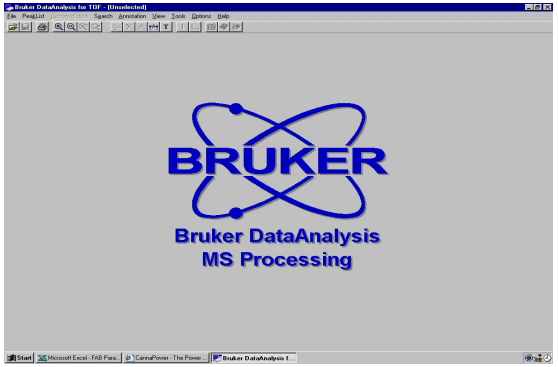
- FTP-Server

  for data collection (MALDI, EI, CI, FAB) like the NMR-service

  Server: 134.60.63.96
  Username:OC2
  PW:Maldi

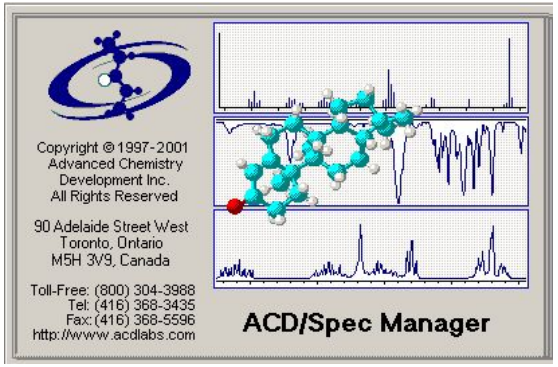
MS Software

- Software for MALDI data analysis

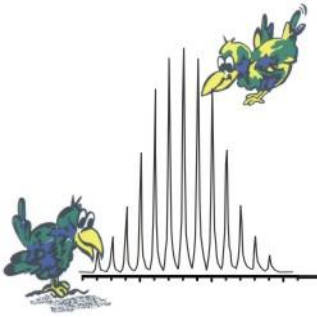


Bruker Data Analysis 1.6d

- Software for EI, CI and FAB data analysis



Labs MS Processor



What type of analysis is needed ?

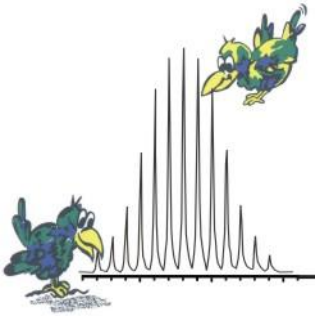
Ionization methods: MALDI, EI, CI, (FAB), (ESI)

– I will select the ionization method unless

- you have previous success with a method
- duplicating literature methods

- Analyses are low resolution

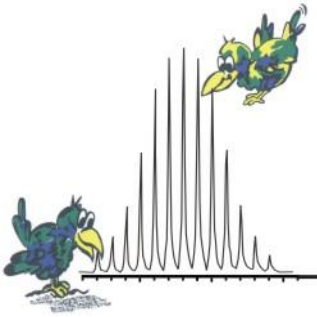
- confirms presence of analyte
- for high mass compounds (m/w >10000) I try to obtain the best resolution possible
- for high mass accuracy internal calibration (standard: external calibration)



What type of analysis is needed ?

Which MS method is best for the compound I want to analyze ?

- Molecular weigth?
- Solvent & solubility?
- Purity?
- Reactivity?
- Would it distill or sublime under HiVac ?
- One compound or mixture?
- Acidic? Basic?
- Ionic?



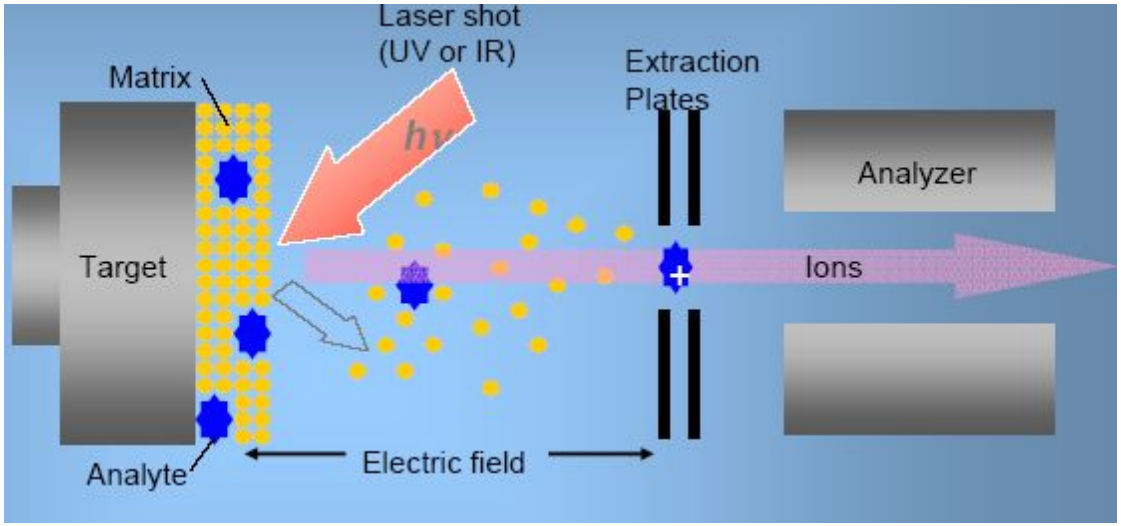
Ionization Methods

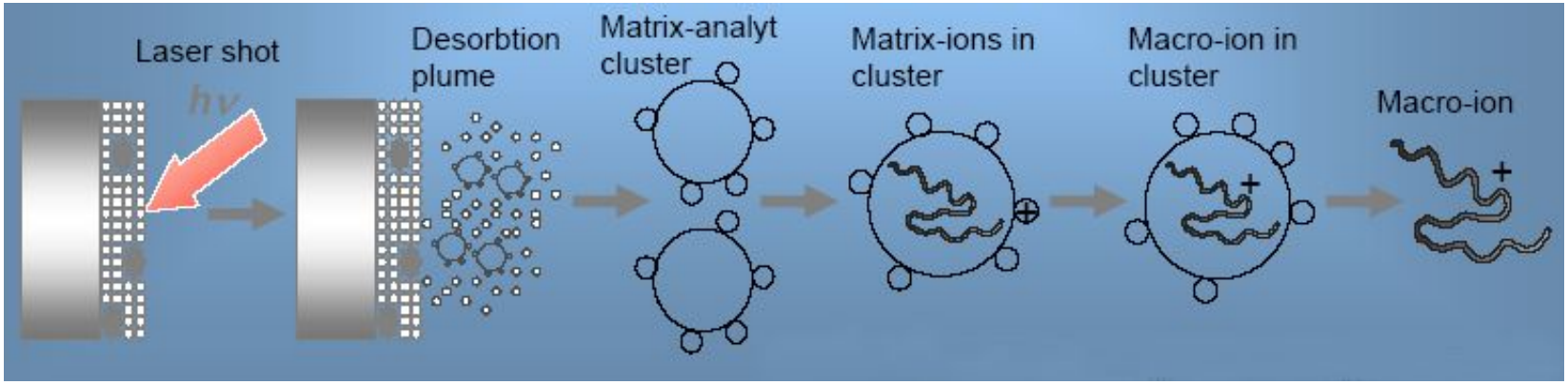
Neutral species Charged species

- Removal/addition of electron(s)
  - $M + e^- \rightarrow (M^{+\cdot})^* + 2e^-$
  - electron ionization

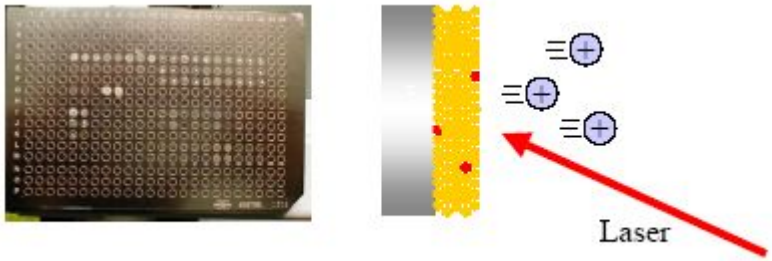
- Removal/addition of proton(s)
  - $M + (Matrix)\text{-}H \rightarrow MH^+ + (Matrix)^-$
  - chemical ionization (CI)
  - atmospheric pressure CI (APCI)
  - fast atom bombardment (FAB)
  - electrospray ionization (ESI)
  - matrix assisted laser desorption/ionization (MALDI)

Matrix Assisted Laser Desorption





Matrix Assisted Laser Desorption



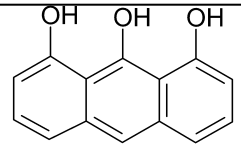
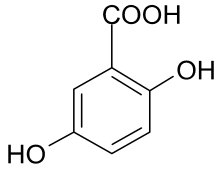
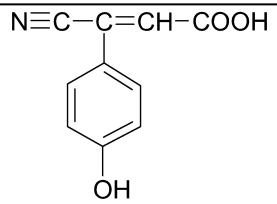
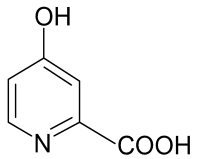
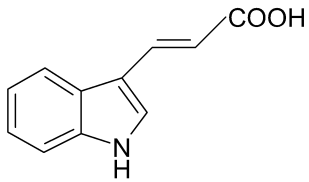
TOF Parameters

Simple, cheap (in theory), robust, sensitive.

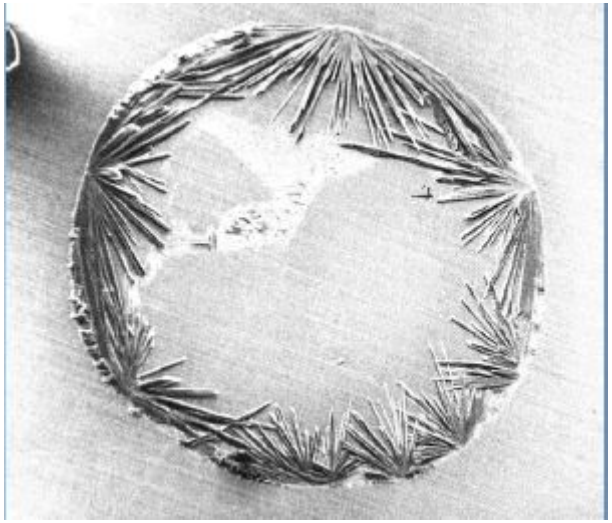
A good modern TOF should give:

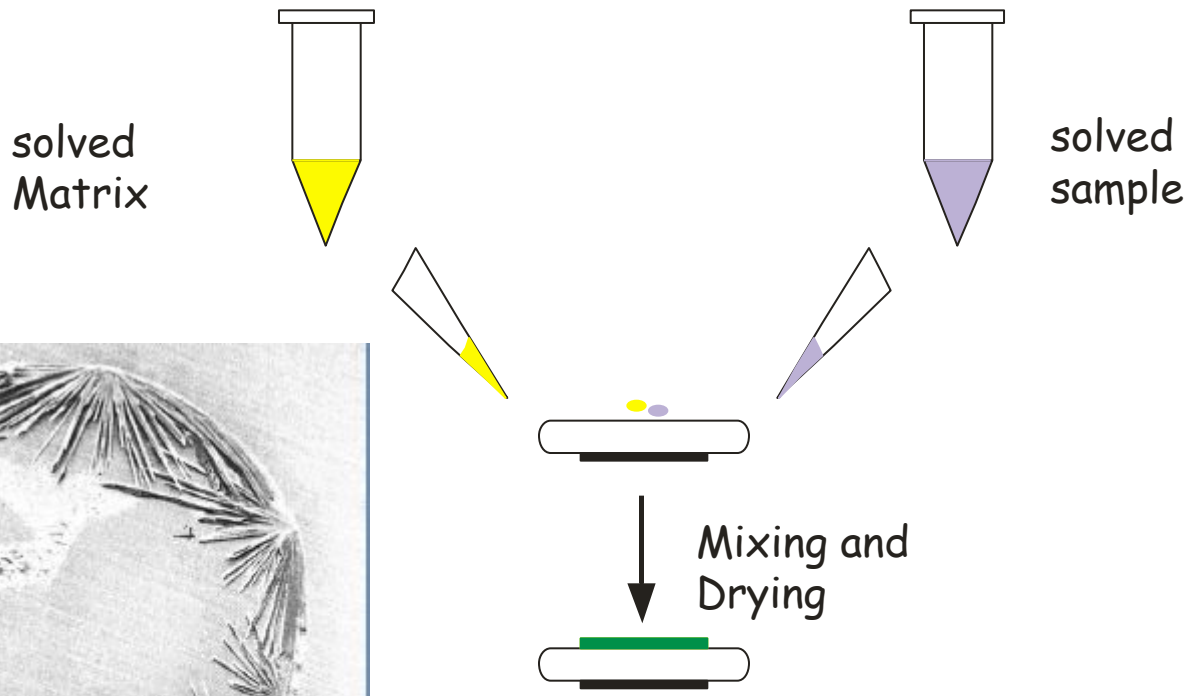
- >10k Resolving power
- ~1-10 fmol sensitivity (single scan)
- ~10 ppm mass accuracy internally calibrated (5 ppm if the peak is particularly large or clean).
- >1000 scans/second
- Unlimited mass range

Matrices

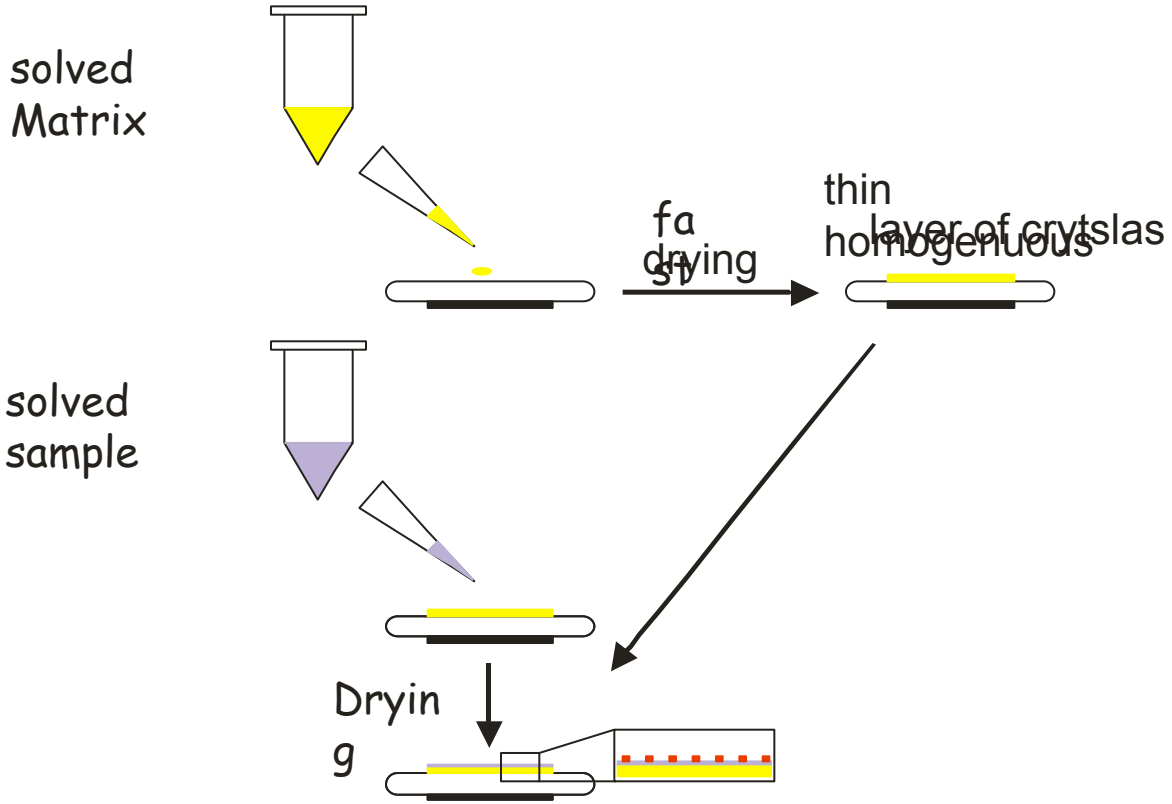
Matrix		
1,8,9-Trihydroxyanthracen (Dithranol)		polymers
2,5-Dihydroxybenzoic acid (DHB)		proteins, peptides, polymers
α-Cyano-4-hydroxycinnamic acid		peptides, (polymers)
4-Hydroxypicolinic acid		oligonucleotides
Trans-Indol-3-acrylacid (IAA)		polymers

Sample Preparation: Dried Droplet

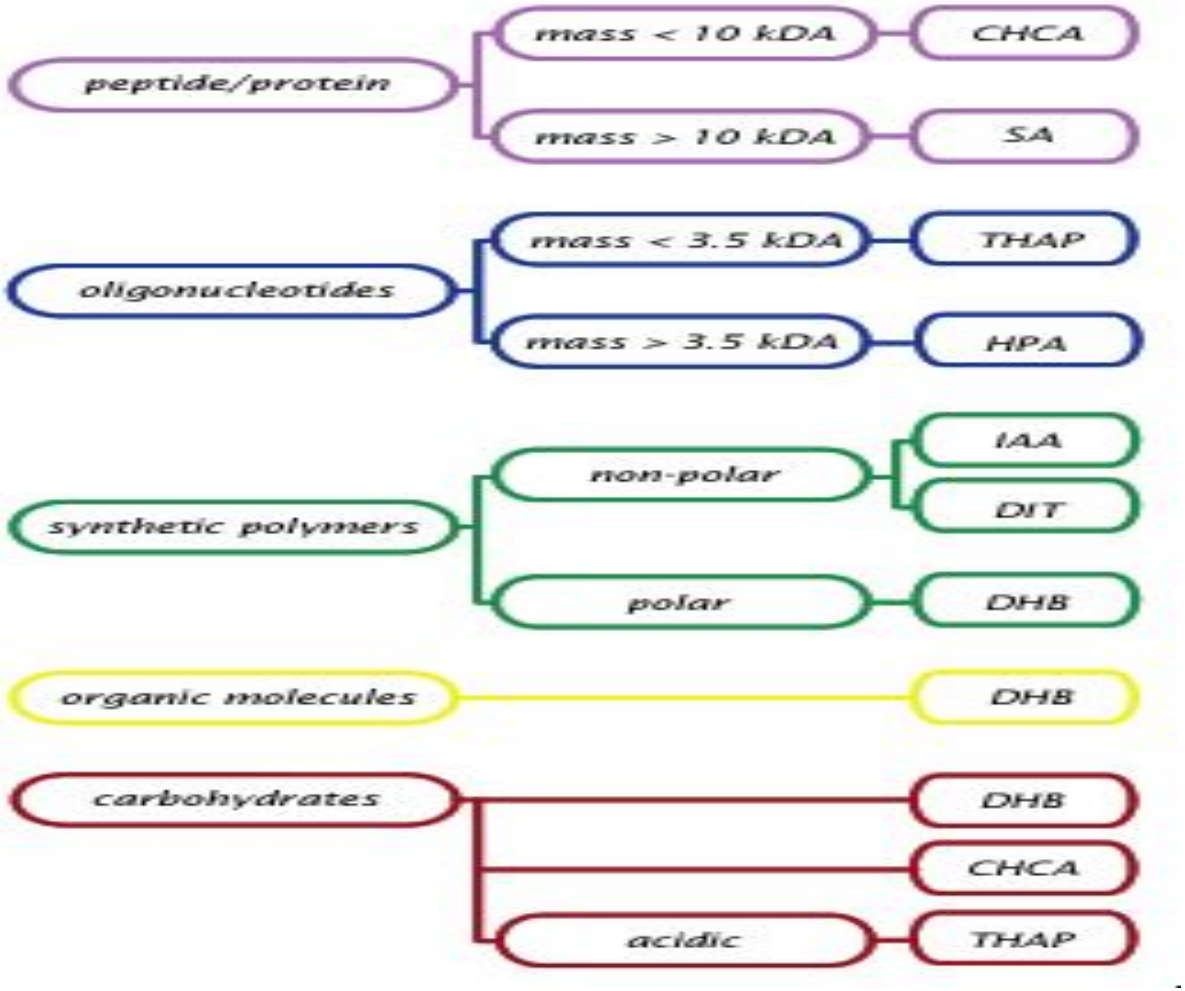


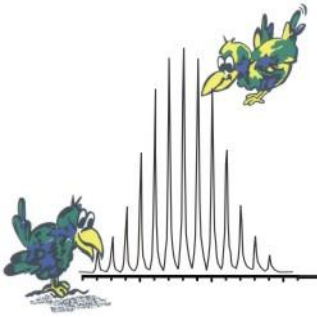


Sample Preparation: Thin Layer



Guide to Sample Preparation





Reflector

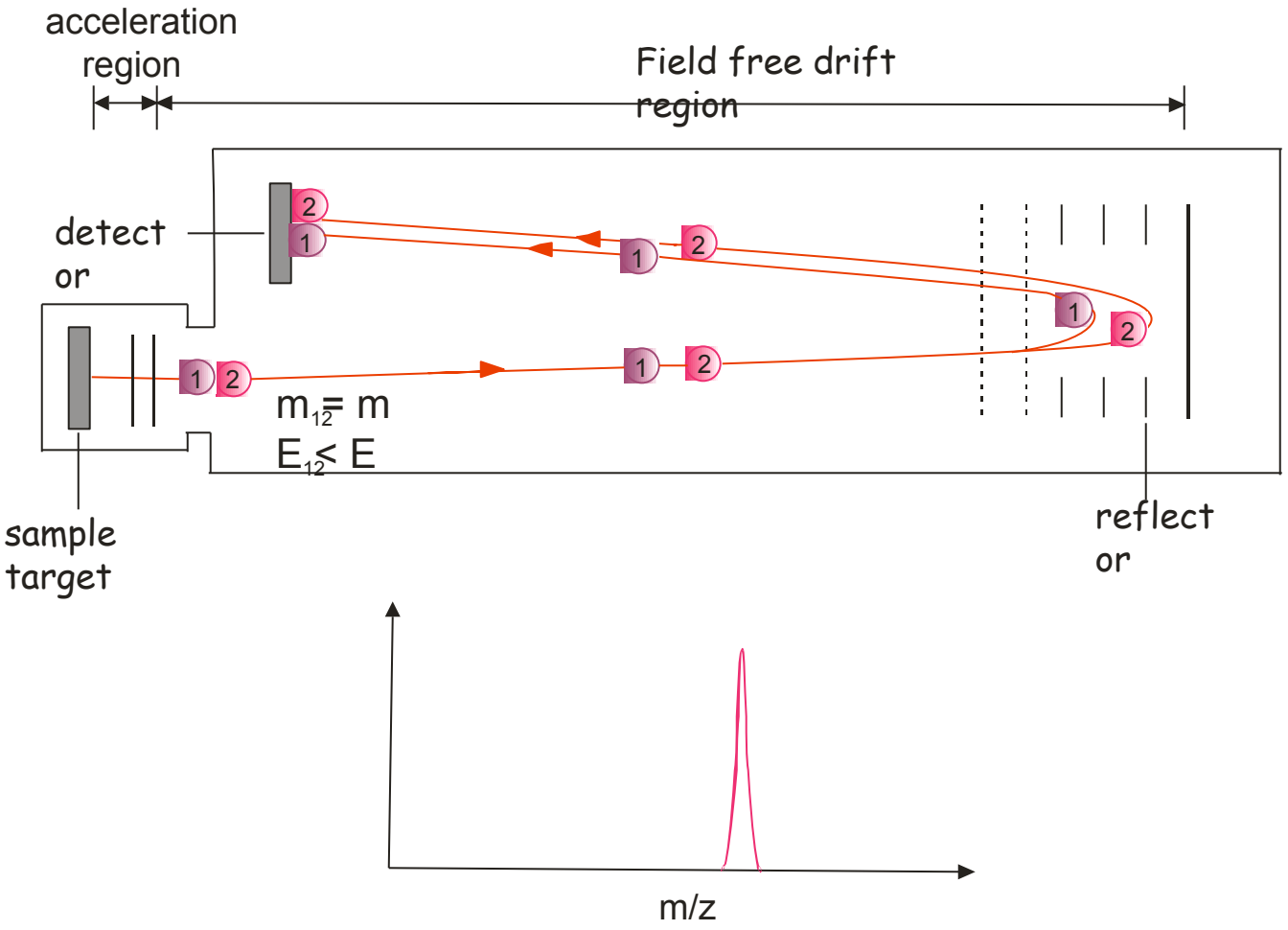
- Through ionisation there is an activation energy distribution (energy-, position- and time uncertainty, electronic repulsion energy, shielding effects)

- Electric field after the field free drift region that reverses the direction of travel of the ion (reflects)

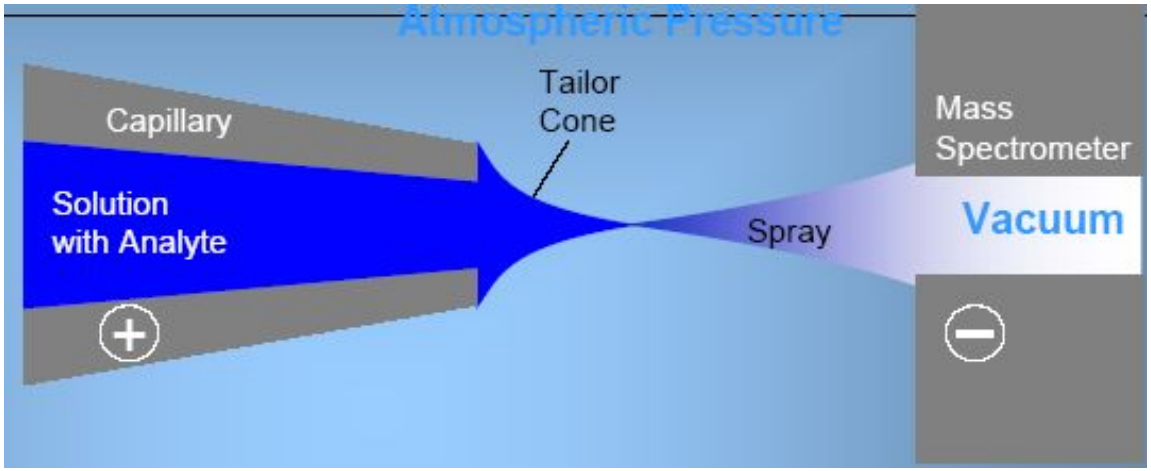
- Ions with same m/z ratio but higher kinetic energy penetrate deeper into the reflector, delaying their time of arrival at the reflector relative to the slower low-energy ions

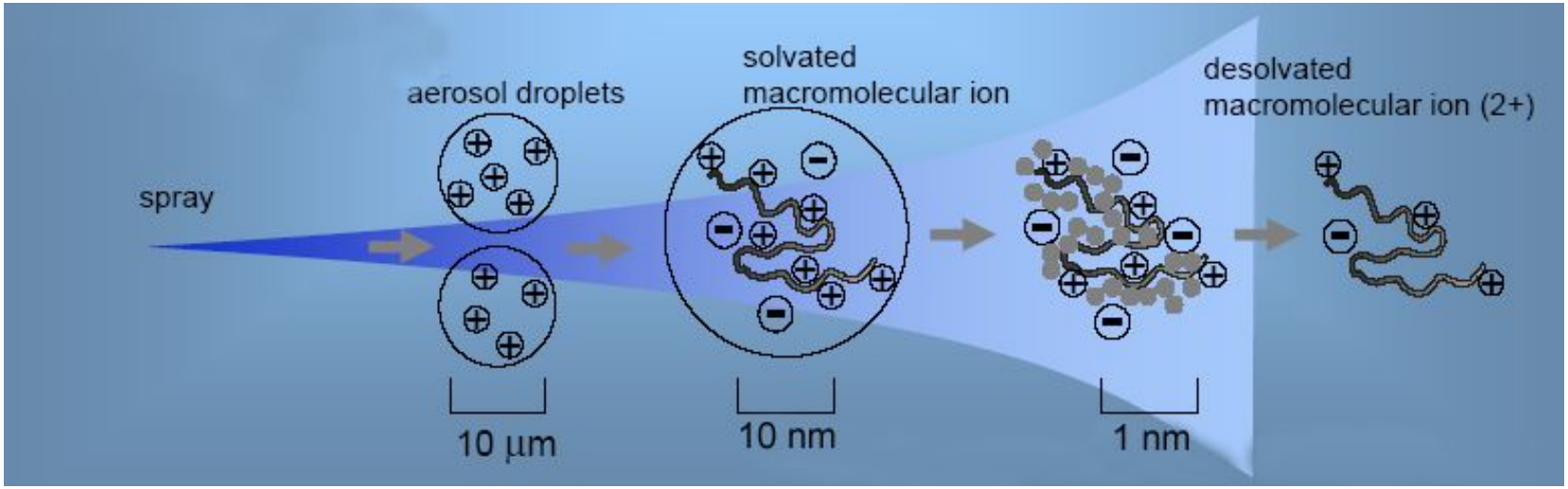
☞ Improved resolution, increase in mass accuracy

Principle Of Reflector-TOF



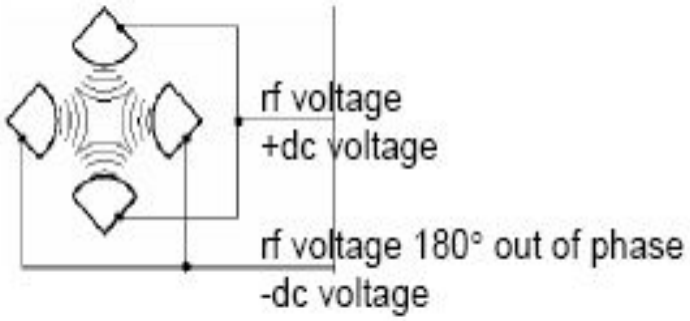
Electrospray (ESI)





Mass Analyzer: Quadrupole (Q)

Four parallel rods or poles through which the ions being separated are passed.
Poles have a fixed DC and alternating RF voltages applied to them.

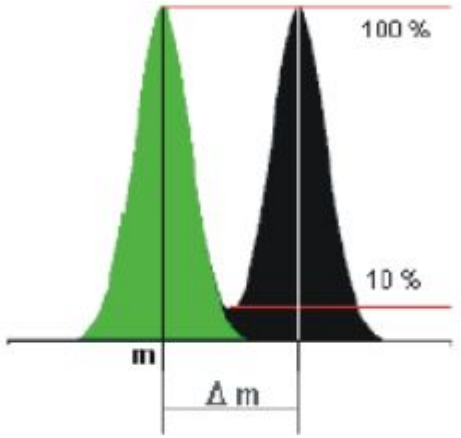


- Depending on the produced electric field, only ions of a particular m/z will be focused on the detector, all the other ions will be deflected into the rods.
- Scanning by varying the amplitude of the voltages (AC/DC constant)

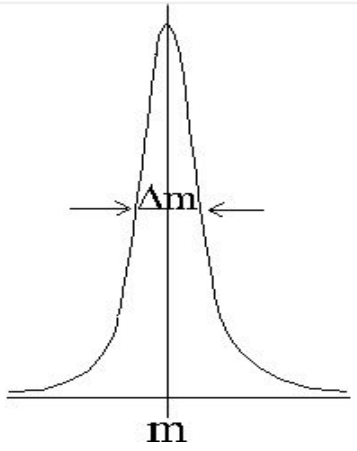
Resolution

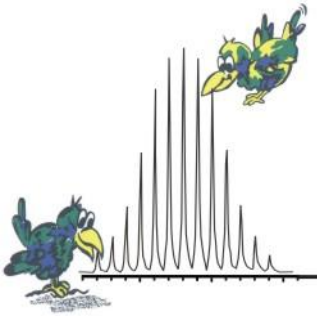
Ability of a mass spectrometer to distinguish between ions of different m/z ratios.

$$R = m/\Delta m$$



- Δm is the mass difference between two adjacent peaks that are just resolved
- m is the mass of the first peak (or the mean mass of two peaks)
- although this definition is for two peaks, it is acceptable to measure the resolution from a single peak (MALDI-TOF). In that case
- **Δm is the width of the peak at half maxima (FWHM) of the peak corresponding to m.**

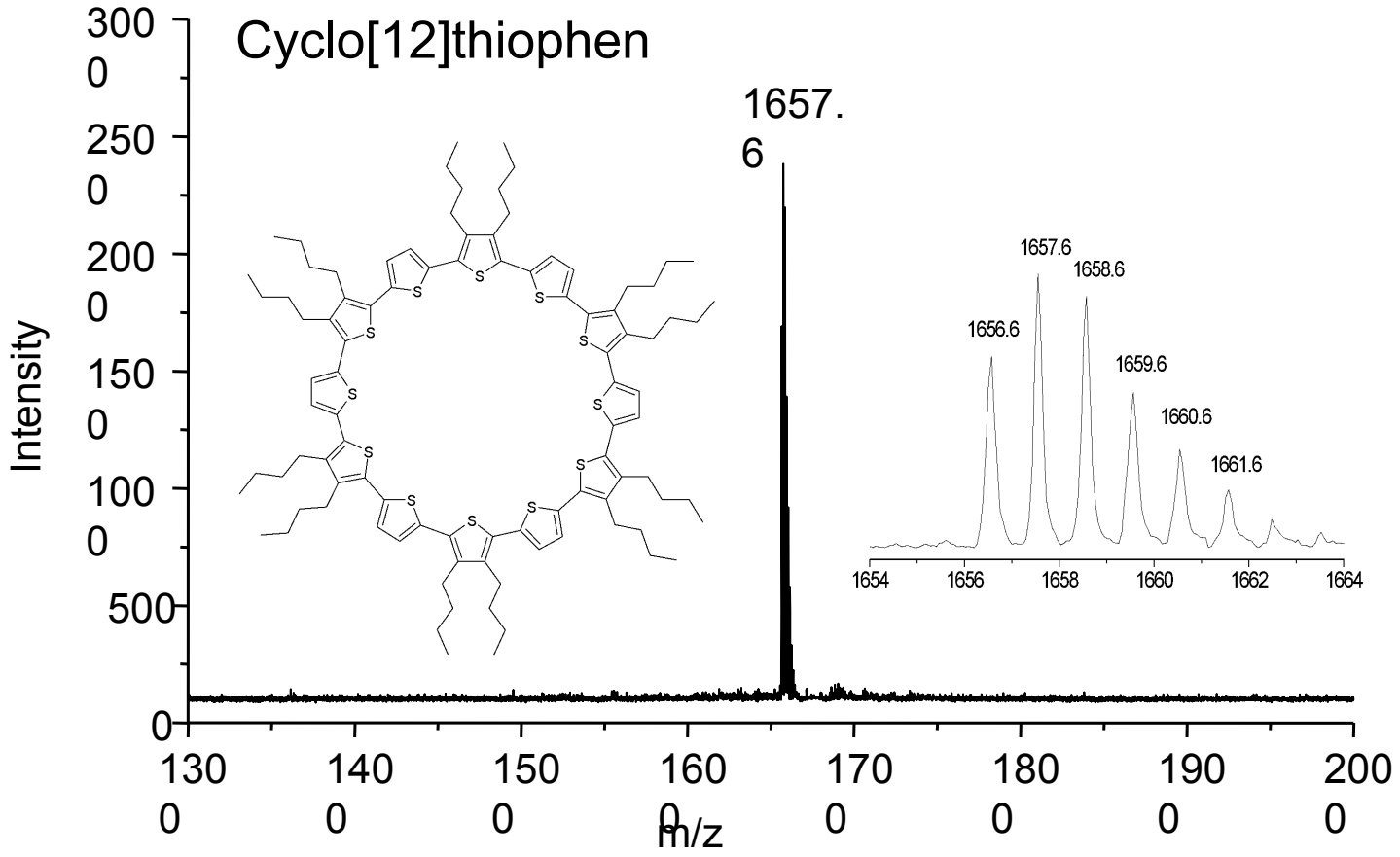




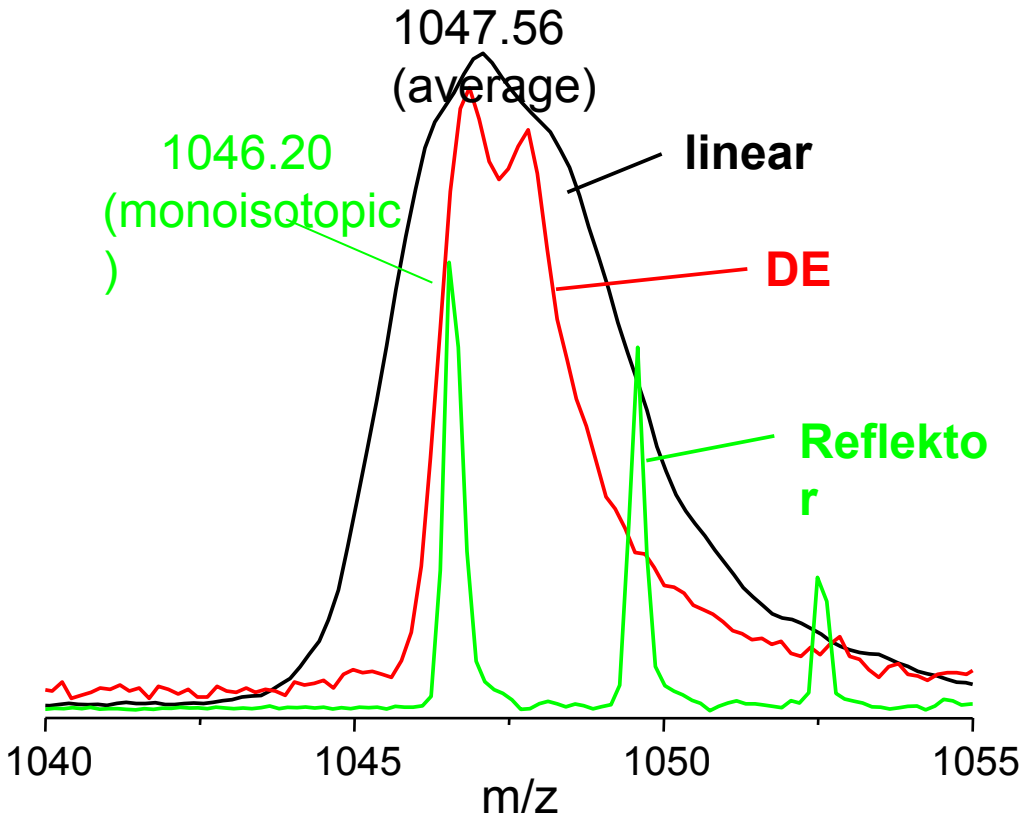
Resolution

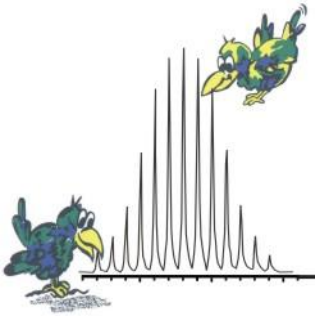
If we have 5000 resolution on a mass spectrometer, we can separate m/z 50.000 from m/z 50.010, or separate m/z 100.000 from m/z 100.020, or separate m/z 1000.000 from m/z 1000.200 (all down to a 10% valley between the two peaks).

Mass Spectra of Cyclothiophen



Mass Spectra of Angiotensin





„Masses“

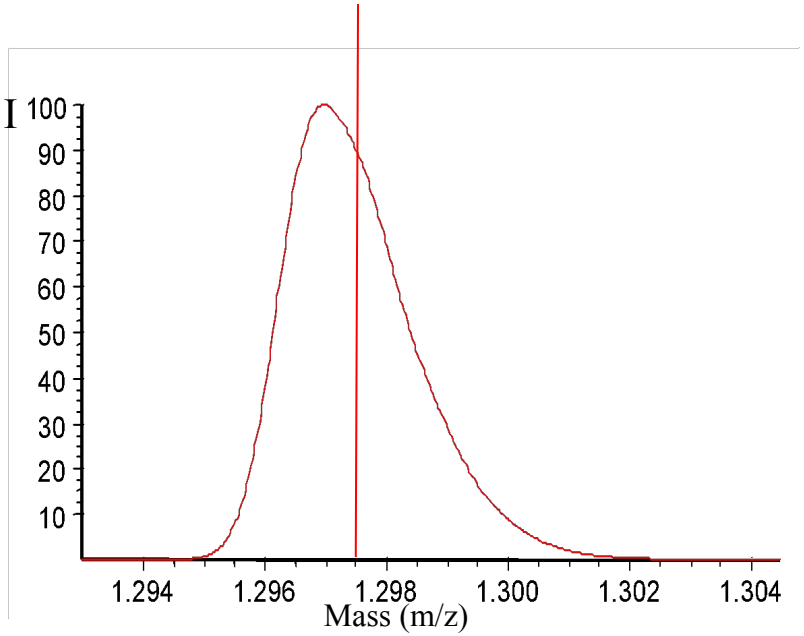
- **Average Mass**
  The sum of the average of the isotopic masses of the atoms in a molecule, e.g. C = 12.01115, H = 1.00797, O = 15.9994.

- **Monoisotopic Mass**
  The sum of the exact or accurate masses of the lightest stable isotope of the atoms in a molecule, e.g. C = 12.000000, H = 1.007825, O = 15.994915.

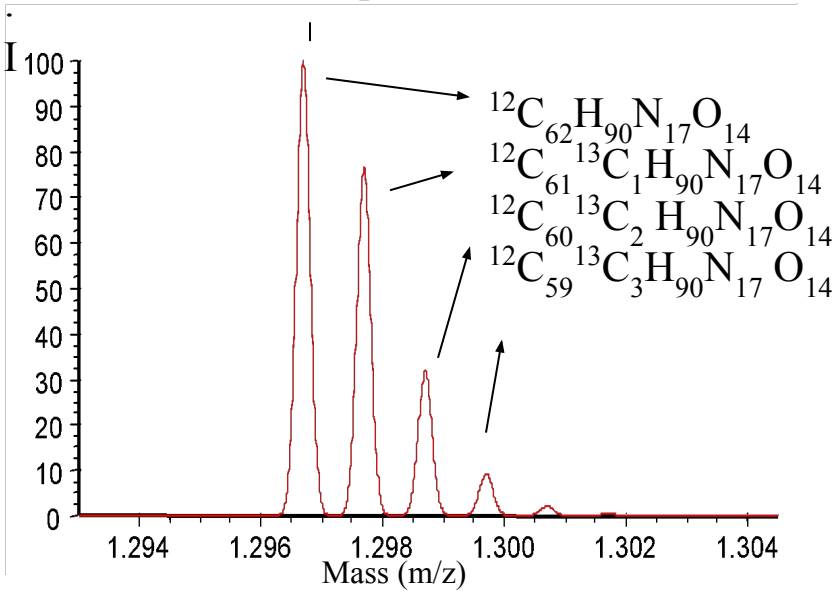
- **Nominal Mass:**
  The integral sum of the nucleons in an atom (also called the atomic mass number), e.g. C = 12, H = 1, O = 16.

Mass spectra of Angiotensin I

average mass 1297.50248



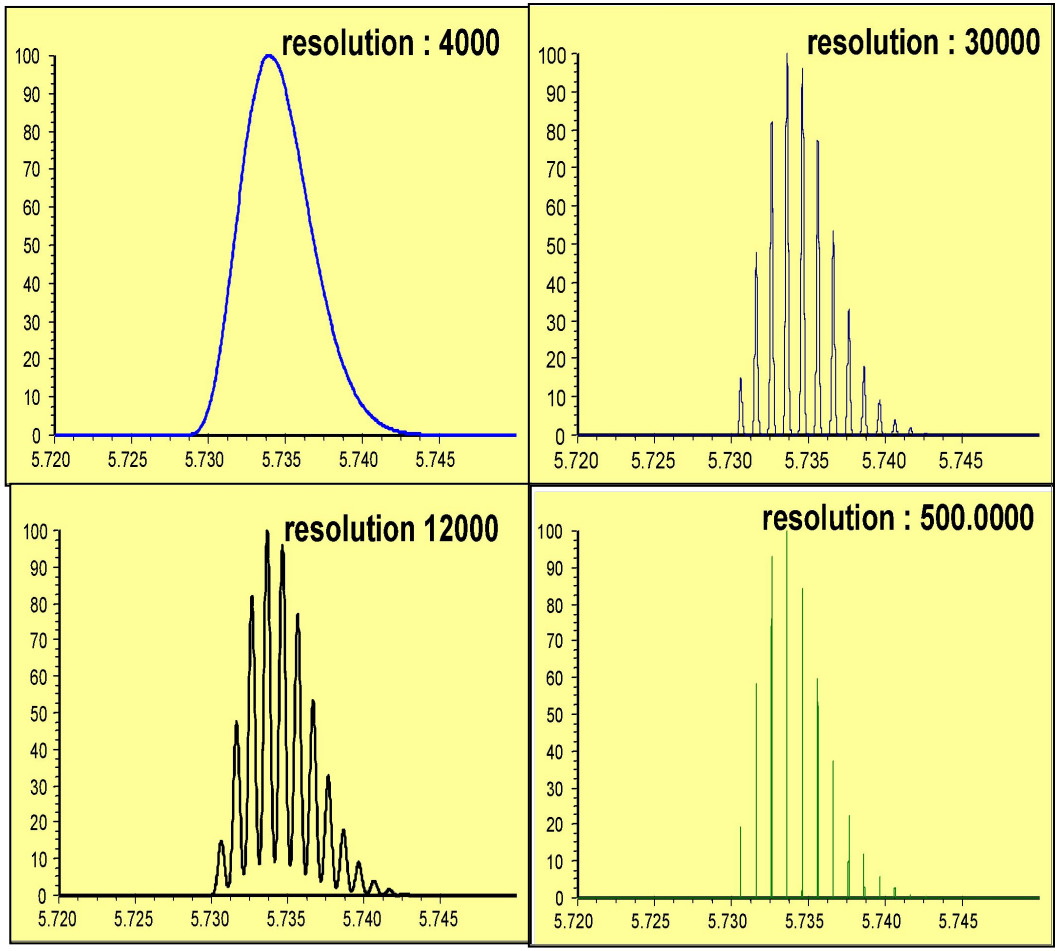
monoisotopic mass 1296.68518

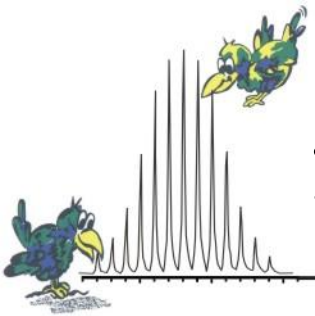


R = 1000

R = 5000

Simulated Spectra of Bovine Insulin

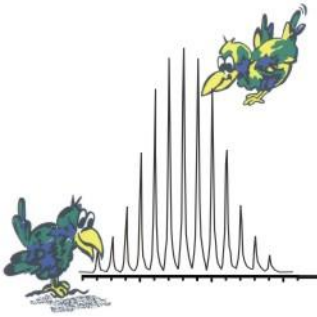




Instrument Resolution and Mass Accuracy

Instrument	Mass Range m/z	Resolution (at m/z 1000)	Accuracy (Error) (at m/z 1000)
GC/MS (Quadrupole)	To 2000	Low Resolution	
Sector	To 4000	50000-100000	0.0005% (5 ppm)
MALDI/TOF	To 400000	15000 (Reflectron)	0.006% (60 ppm) ext. Cal. 0.003% (30 ppm) int. Cal.
FTICR	To 4000	To 3000000	0.0001% (1 ppm)

$$ppm = \frac{(\text{Theoretical MW} - \text{Measured MW})}{\text{Theoretical MW}} \times 10$$

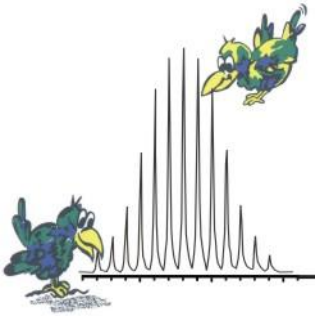


Calibration

- Instrument calibration performed well before sample analysis:

  - EI/CI, GC-MS
  - FAB
  - ESI

- Performed immediately before sample analysis:
  - MALDI-TOF



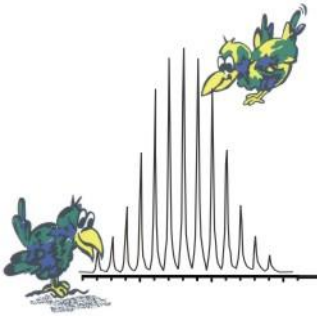
Calibration

Compounds used for calibration include:
– PEG, PBM, peptides, proteins, PFTBA, CsI

External Calibration:
m/z scale is calibrated with a mixture of molecules with different molecular weights; after that the analyte is measured.

.

Internal Calibration:
Analyte and a mixture of molecules with different molecular weigths are mixed and measured together. Then the spectrum is calibrated by assigning the right masses to the well known calibration standards (perfect: mass of analyte is between the mass of two standards).



Fragmentation – Dissozation – Adduct Formation
Comparison of Ionization Methods

	EI	CI	ESI	MALDI	FAB
Additional mass due to Positive Ionisation	No	Yes	H, Na, K etc. (+1, +23, +39 etc.)	H, Na, K etc. (+1, +23, +39 etc.)	H, Na, K etc. (+1, +23, +39 etc.)
Loss of mass due to negative ionisation	-	No	Loss of H(-1)	Loss of H(-1)	Loss of H(-1)
Number of charges added	1	1	1-many (dependent upon mass)	1-2	1-2
Matrix peaks?	No	No	Yes	Yes	No

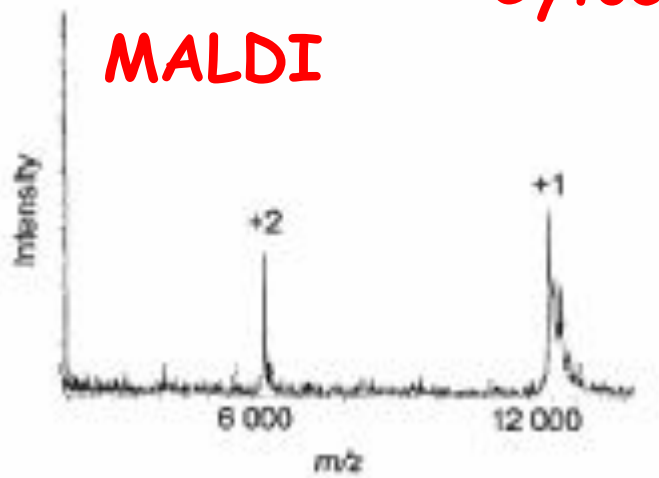
Fragmentation – Dissozation – Adduct Formation

Singly-, doubly-, triply-, etc. charged ion

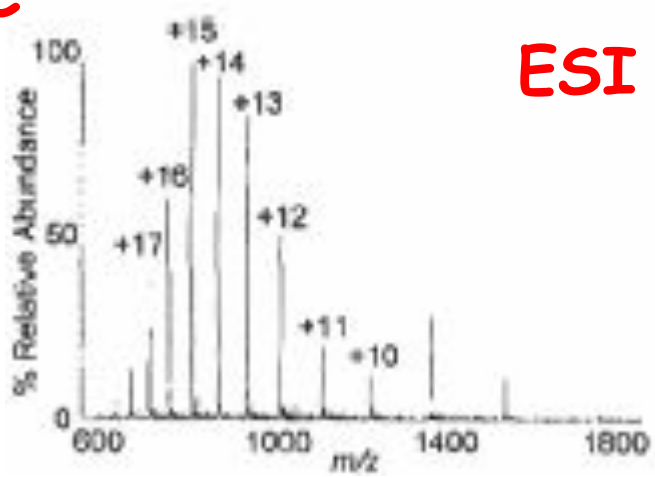
Molecule or molecular moiety which has gained or lost respectively one, two, three or more electrons/protons.

Cytochrom C

MALDI



ESI



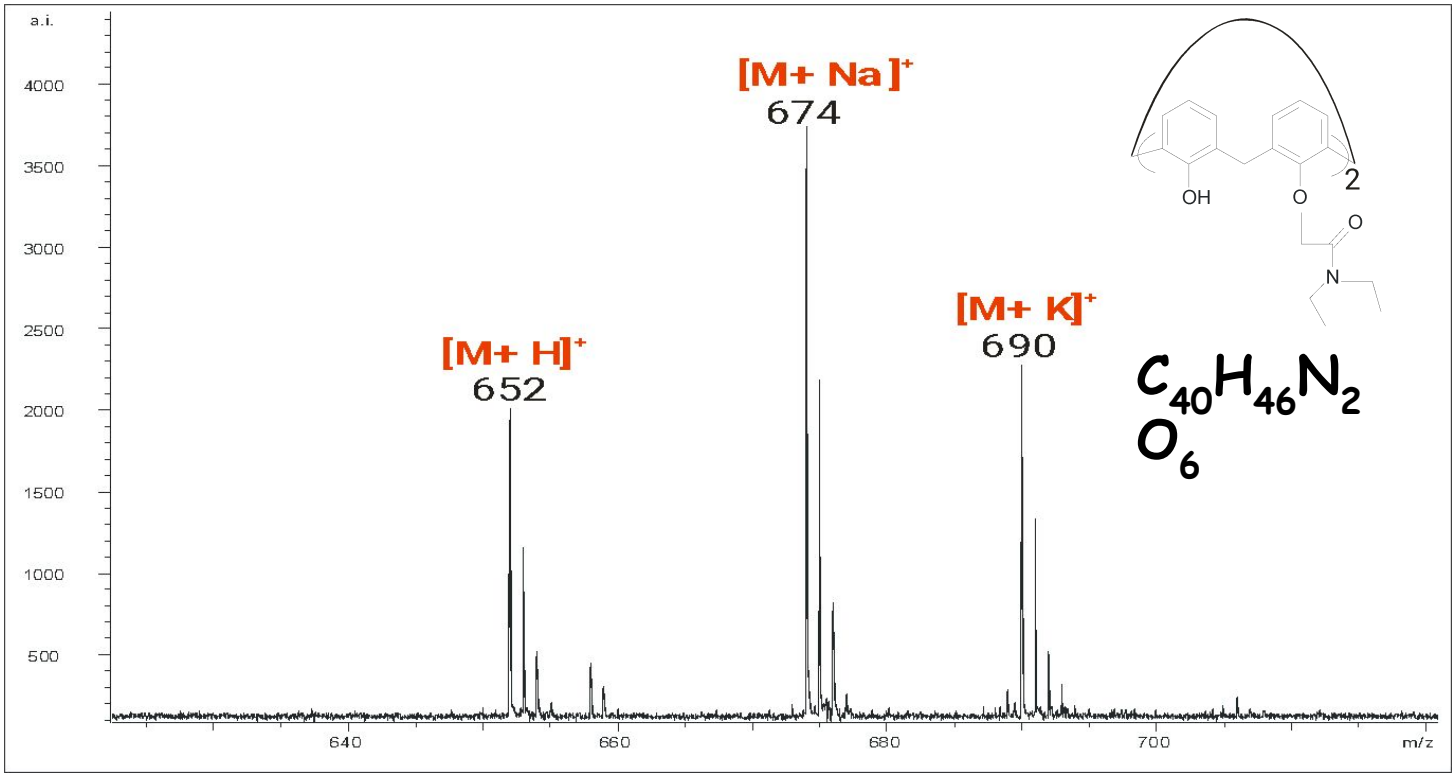
Dimeric ion

Ion formed when a chemical species exists in the vapour as a dimer and can be detected as such, or when a molecular ion can attach to a neutral molecule within the ion source e.g. $[2M+H]^+$

Fragmentation – Dissozation – Adduct Formation

Adduct ions

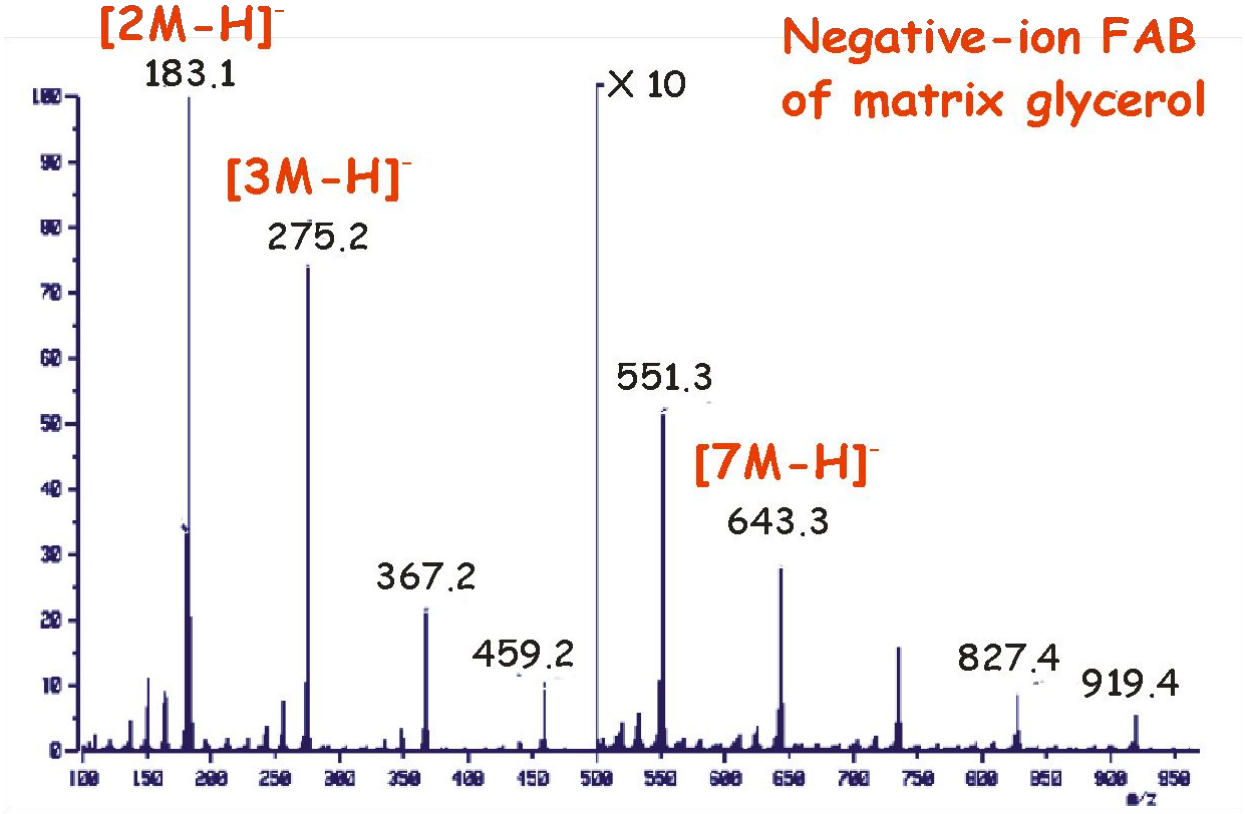
An ion formed by interaction of two species, usually an ion and a molecule, and often within an ion source, to form an ion containing all the constituent atoms of one species as well as an additional atom.



Fragmentation – Dissozation – Adduct Formation

Cluster ion

An ion formed by the combination of two or more atoms, ions or molecules of a chemical species, often in association with a second species.

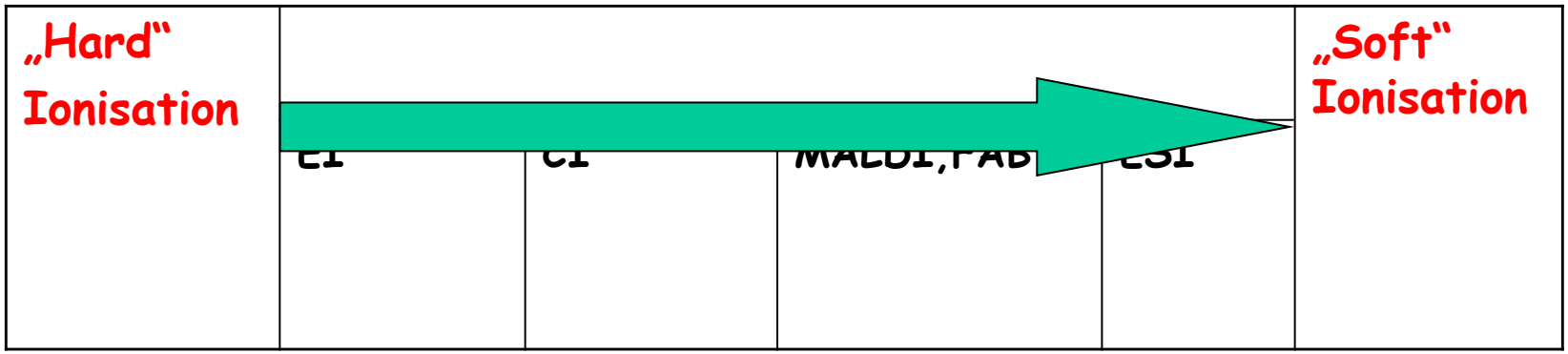


Fragmentation – Dissozation – Adduct Formation

Fragment ion

An electrically charged dissociation product of an ionic fragmentation. Such an ion may fragmentate further to produce other electrically charged molecular or atomic moieties of successively lower formula weight.

Fragmentation Break Of Covalent Bond
Dissociation Break of Non-covalent complex



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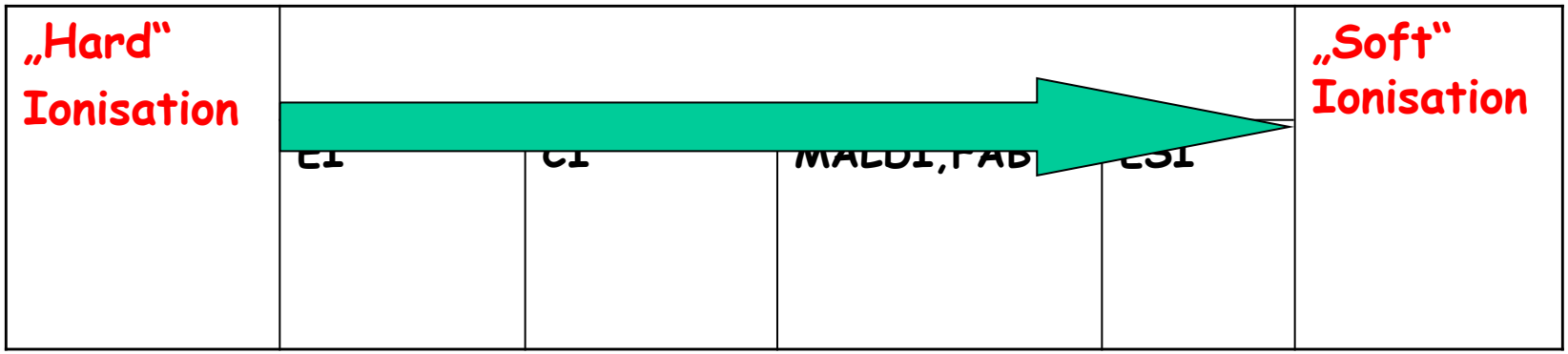

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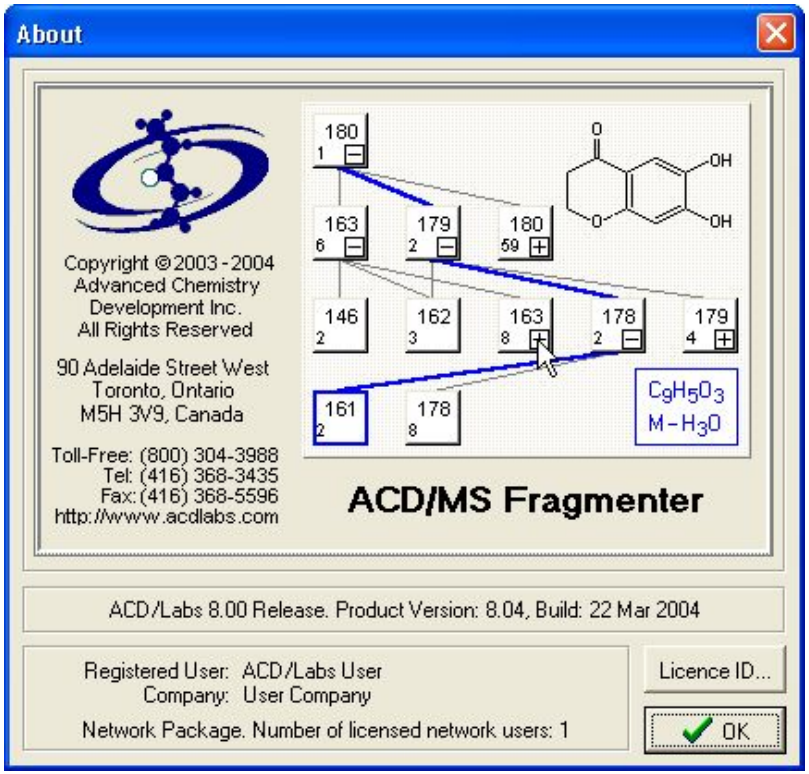
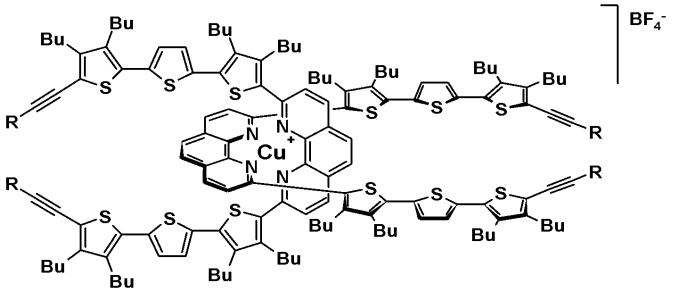
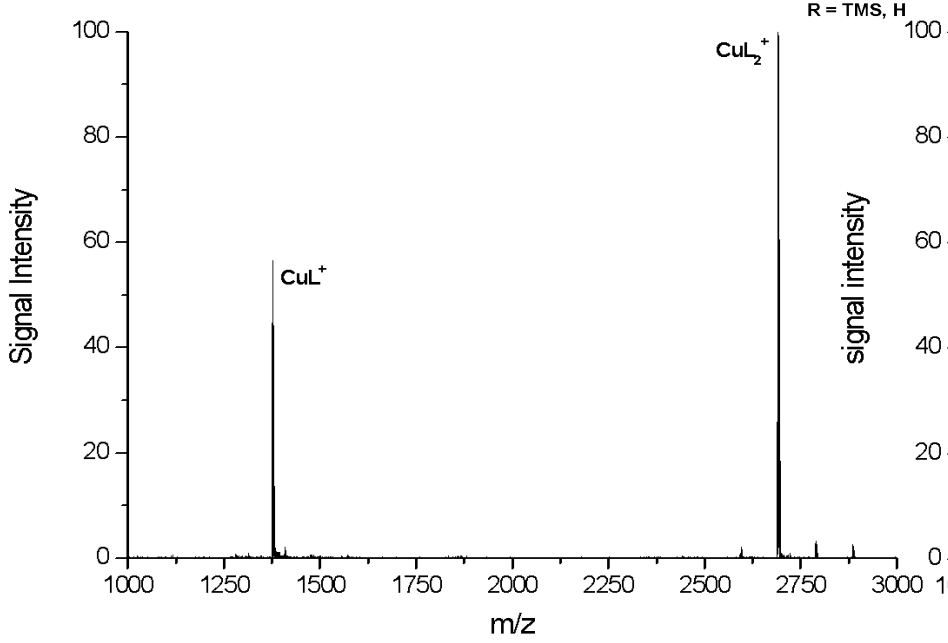


New Software:
ACD/MS Fragmenter

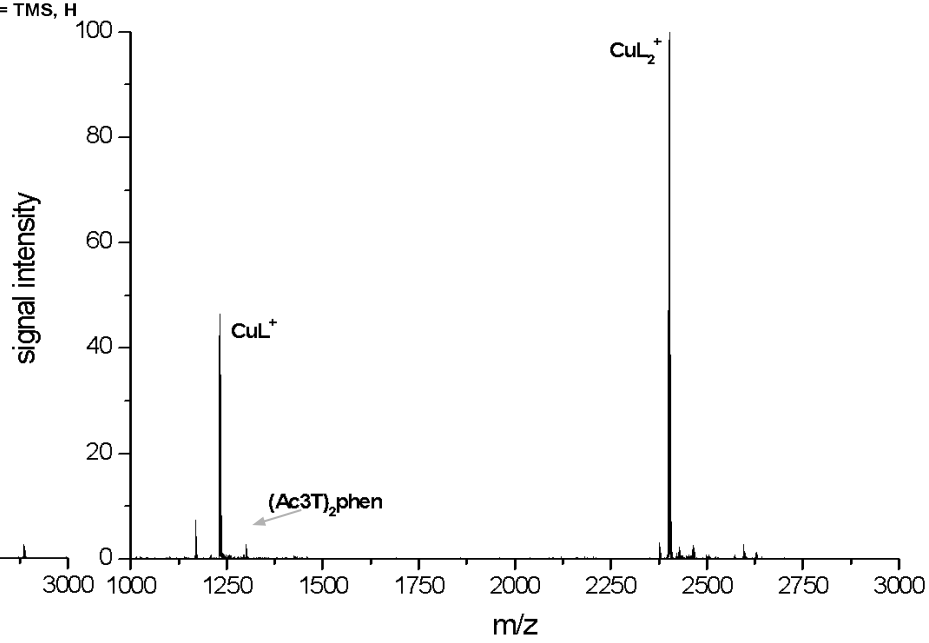
- predicting of possible schemes of mass spectral fragmentation for chemical structures
- Selection fragmentation-rule parameters to mimic different ionization techniques that range from EI to low energy protonation techniques such as ESI or APCI
- Recognition of fragments within an aquired mass spectra

Fragmentation – Dissozation – Adduct Formation



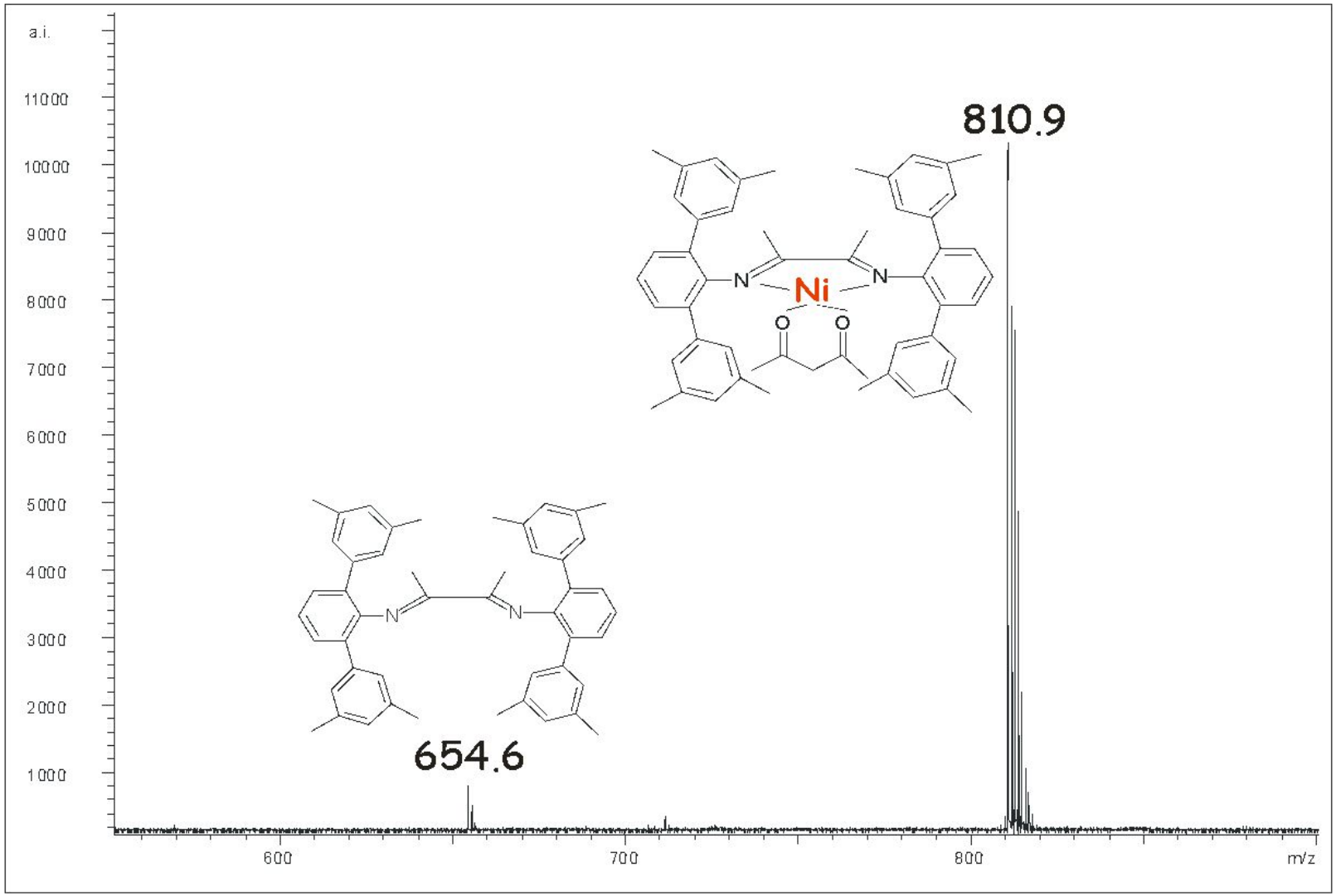


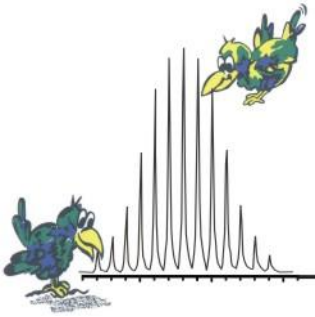
TMS-protected complex



deprotected complex

Fragmentation – Dissozation – Adduct Formation





Impurities - Contamination - Artefacts

Impurity

e.g. antioxidantia in organic solvents, side products not separated after synthesis, additional components after insufficient isolation from biological material

Contamination

Compound which was putinto the sample subsequently, e.g. through chromatographic column

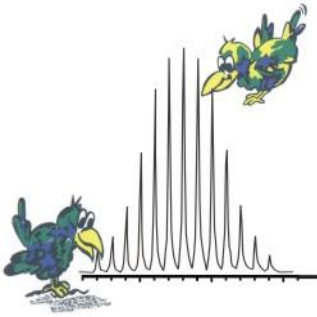
Artefact

MS-specific „key ions", e.g. CI with CH4 as ionisation gas:

$CH_4 + e^- \rightarrow CH_4^{+\cdot}$ (formation of primary ion)

$CH_4^{+\cdot} \rightarrow CH_3^+ + H^\cdot$

$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$ formation of adducts with m/z +28



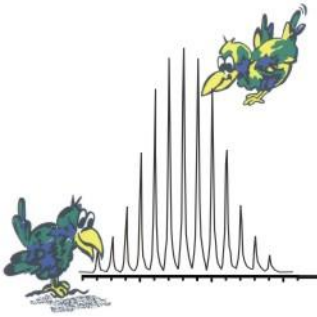
Impurities - Contamination - Artefacts

Contamination	Source	Detection		
		EI, CI	FAB	MALDI, ESI
Alkali salts	Solvents, glas etc.	-	++	++/++
Heavy metal salts	Sample vessels, HPLC pumps	-	++	+/+
Alkyl(benzol)sulfonate	Columns, IE, detergents	-	++	-/+
Alkylammounium salts	Columns, IE, detergents	-	++	++/++
HC	Grease	+	+	+/+
Polyphenylether	Grease, pump oil	+	+	+/+
Longchain carbonic acids	Chromatographic columns	++	+	(+)/(+)
Siloxane	Silicon grease, DC plate, plastic	++	+	-/(+)

General Sample Handling

Mass spectrometry is a sensitive technique
(for impurities and contamination, too!)

- **Sample Storage**
  - Glass vials can leach salts (Na/K) into sample
  - Ideal storage vial is siliconized polypropylene tubes
- Use Freshly prepared, high purity reagents and water
- Omit high concentrations of
  buffer salts (NaCl, KH_2PO_4!!!), Detergents (Tween, Triton, SDS)
  Urea, guanidine salts
  - Cleaning of the sample: dialysis, RP-HPLC, Zip-Tips, ion exchange
  - Use of removable buffer salts (z.B. NH4Ac)
  - Use of removable solvents like water, acetonitrile, methanol



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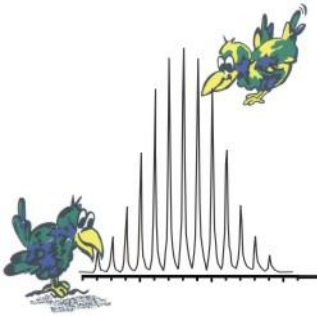


Mass Spectra of Synthetic Polymers

Information:

- monomer unit
- end group
- average masses
  Mn = ∑(NiMi) / Mi
  Mw = ∑(NiMi2) / (NiMi)
- polydispersity D = Mw/Mn

Problems:

Synthetic polymers are polydisperse

☞ bad signal-noise-ratio

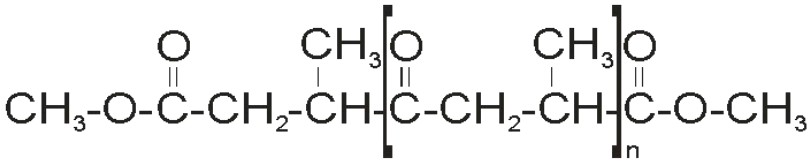
☞ „mass discrimination"/detector sättigung at D > 1.1

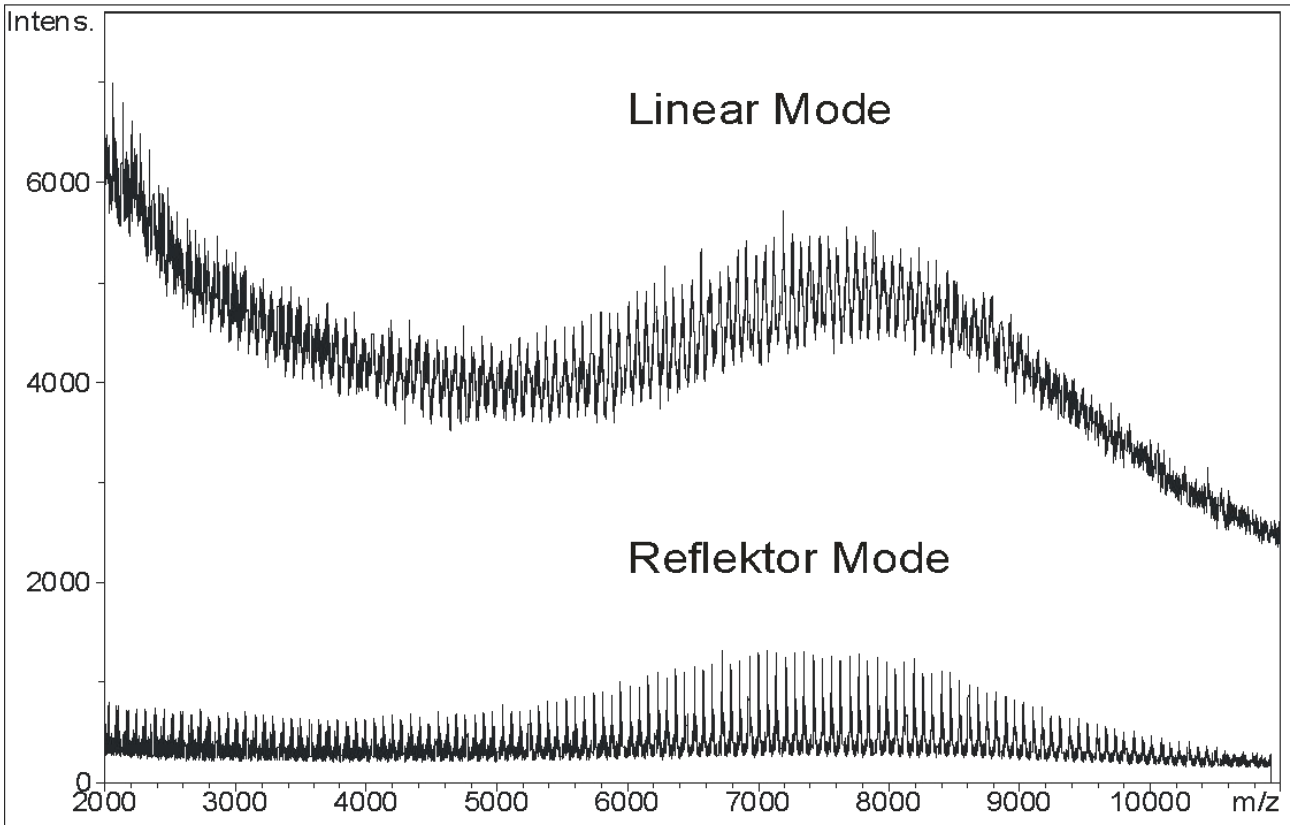
Polymers without ionisationable functional groups

☞ metal ion add-on

z.B. Polystyrol ↔ Ag+; PEG ↔ Na+, K+ etc.

Mass Spectra of Synthetic Polymers





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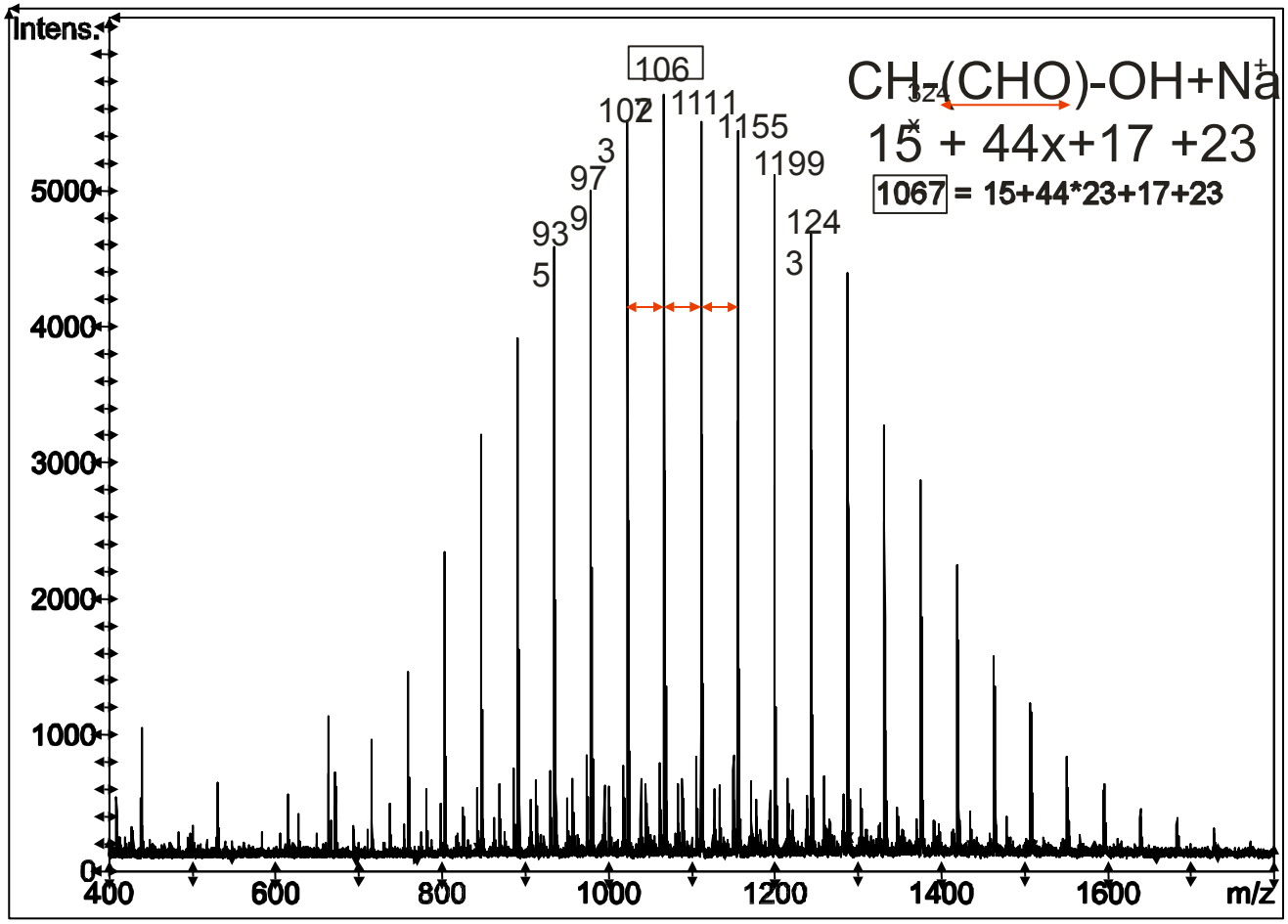
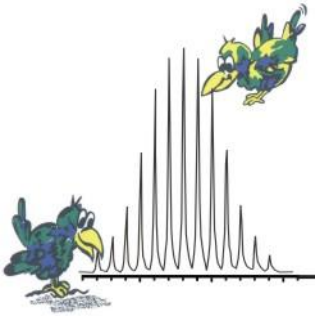


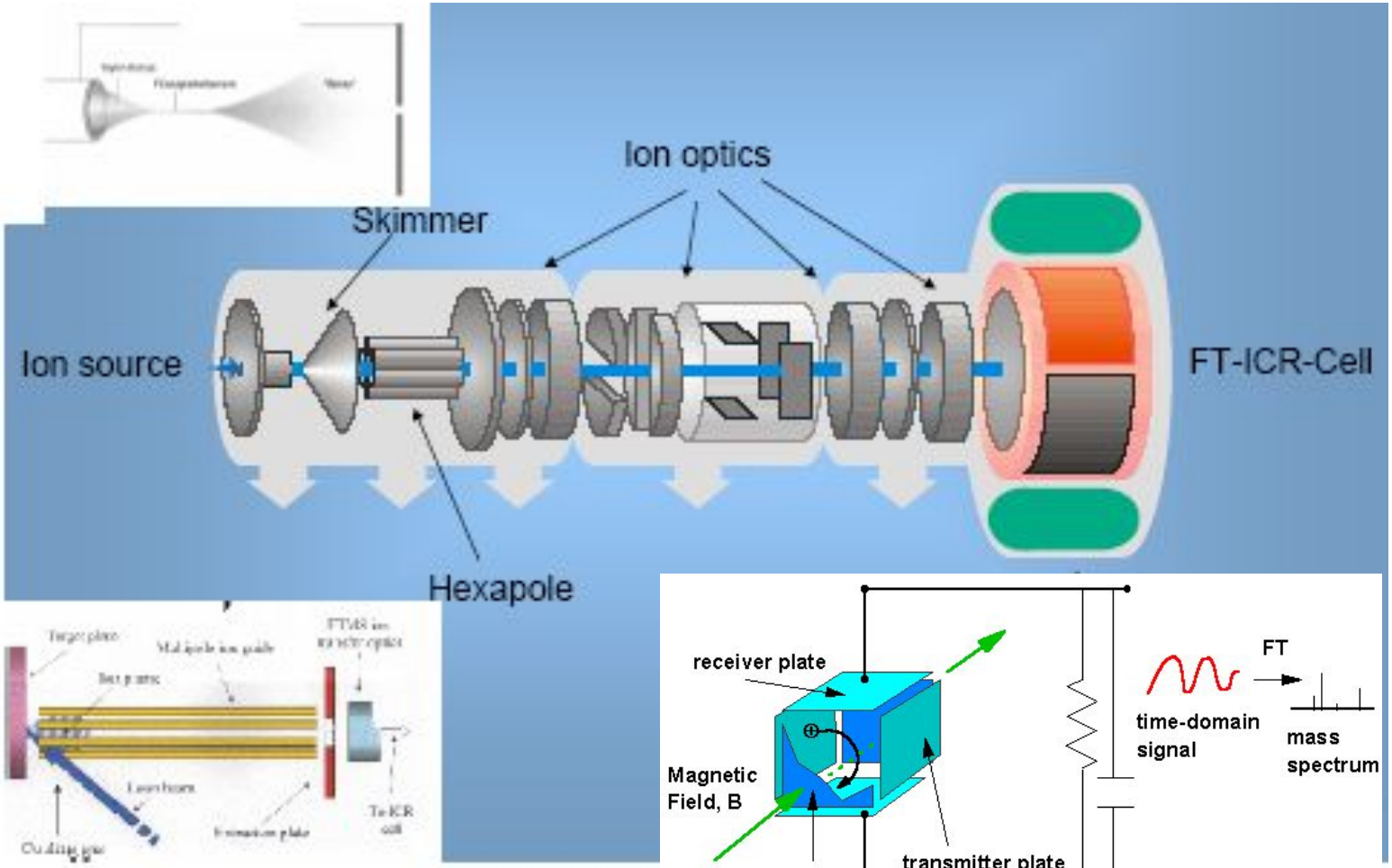


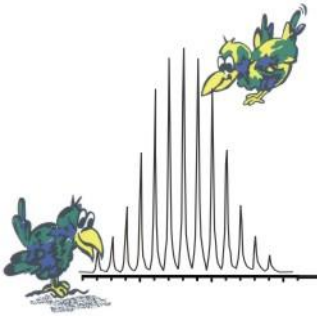
New aspects in mass spectrometry: Hybrid Mass Spectrometers

Perhaps hundreds of hybrids have been explored.
Some of the more successful:
Triple quadrupole
IT-TOF
Q-TOF
Quadrupole-FTMS
TOF/TOF

New aspects in mass spectrometry: FT-ICR-MS

FT-ICR-MS instrument general scheme





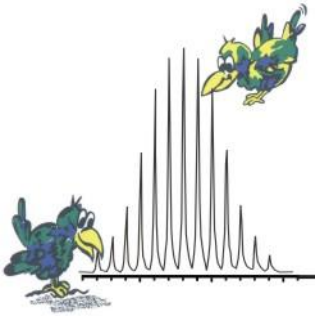
Fouriertransform-ICR: New Dimensions of High Performance Mass Spectrometry

- A high-frequency mass spectrometer in which the cyclotron motion of ions, having different m/z ratios, in a constant magnetic field, is excited essentially simultaneously and coherently by a pulse of a radio-frequency electric field applied perpendicularly to the magnetic field.

- The excited cyclotron motion of the ions is subsequently detected on receiver plates as a time domain signal that contains all the cyclotron frequencies excited.

- Fourier transformation of the time domain signal results in the frequency domain FT-ICR signal which, on the basis of the inverse proportionality between frequency and m/z ratio, can be converted to a mass spectrum.

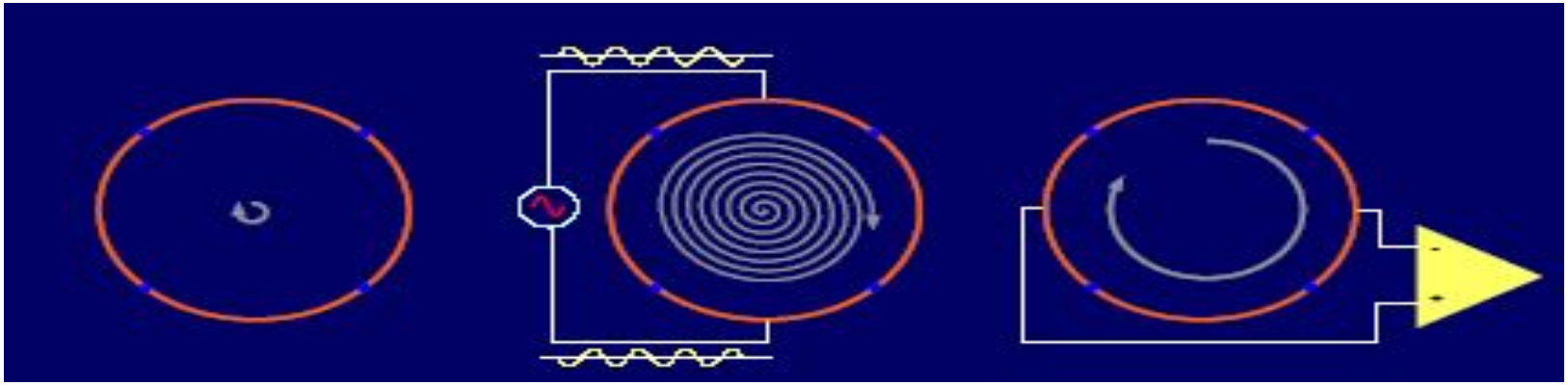
- The ions are to be detected, with a selected m/z ratio, absorb maximum energy through the effect of a high-frequency field and a constant magnetic field perpendicular to it. Maximum energy is gained by ions that satisfy the cyclotron resonance condition and as a result these are separated from ions of different mass/charge.



FTICR: New Dimensions of High Performance Mass Spectrometry

High mass resolution	> 3 000 000
Accuracy of mass determination	< 0.1 ppm
Sensitivity (ESI, Octapeptide)	ca. 50 attomol
Structure-specific fragmentation	MS/MS , MS^n

FTICR: New Dimensions of High Performance Mass Spectrometry

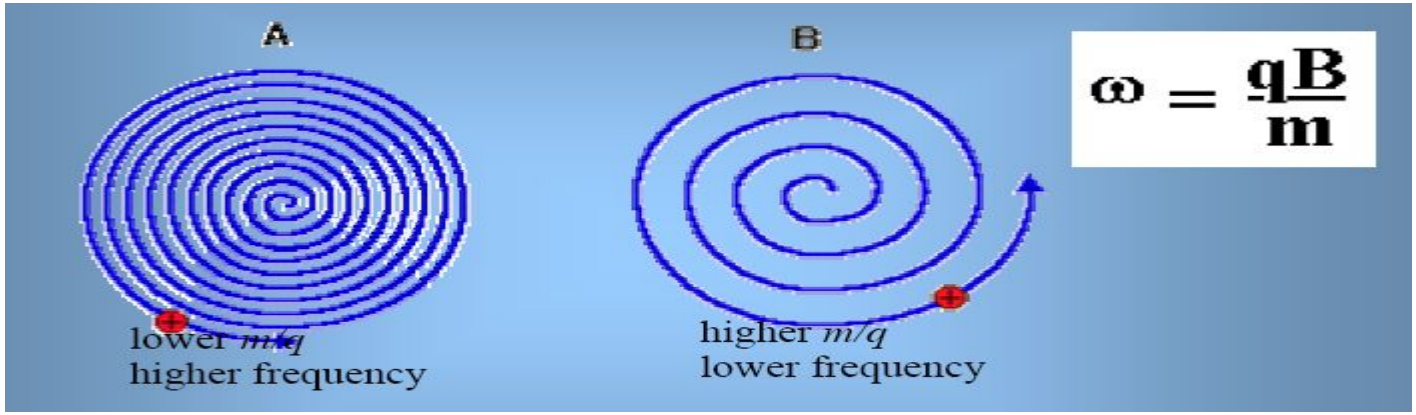


Ions are trapped and oscillate with low, incoherent, thermal amplitude

Excitation sweeps resonant ions into a large, coherent cyclotron orbit

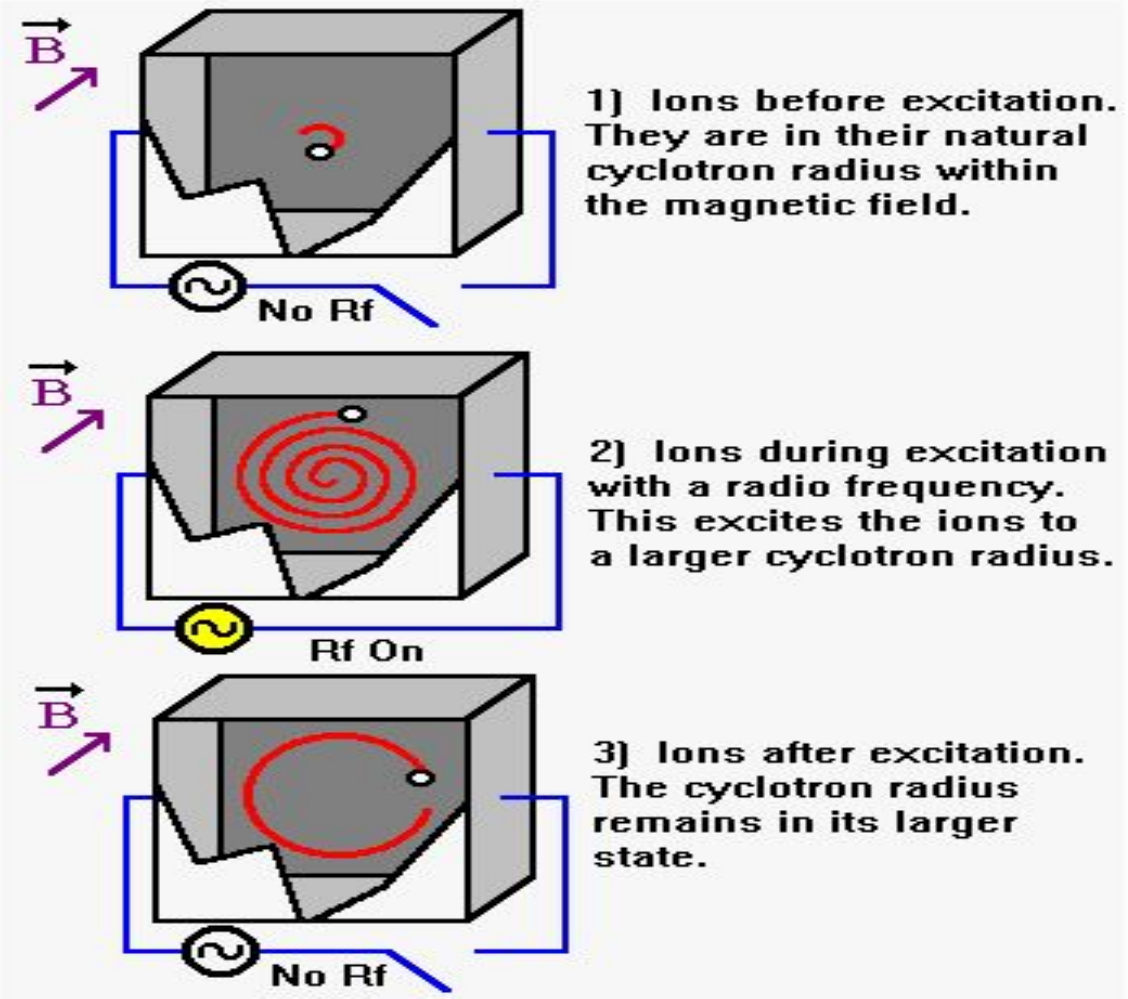
Preamplifier and digitizer pick up the induced potentials on the cell.

FTICR: New Dimensions of High Performance Mass Spectrometry



The frequency of the cyclotron gyration of an ion is inversely proportional to its mass-to-charge ratio (*m/q*) and directly proportional to the strength of the applied magnetic field B.

FTICR: New Dimensions of High Performance Mass Spectrometry



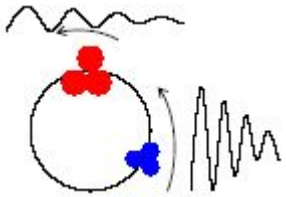
FTICR: New Dimensions of High Performance Mass Spectrometry

In the presence of a magnetic field, sample ions orbit according to cyclotron frequency, fc

- Cyclotron frequency related to charge of ion (z), magnetic field strength (B) and mass of ion (m).

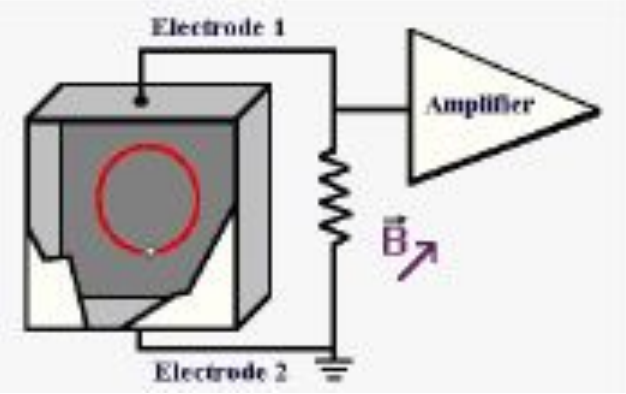
$$\frac{m}{z} = \frac{B}{2\pi f_c}$$

All ions of same m/z will have same cyclotron frequency at a fixed B and will move in a coherent ion packet.



FTICR: New Dimensions of High Performance Mass Spectrometry

Ion packets produce a detectable image current on the detector cell plates.

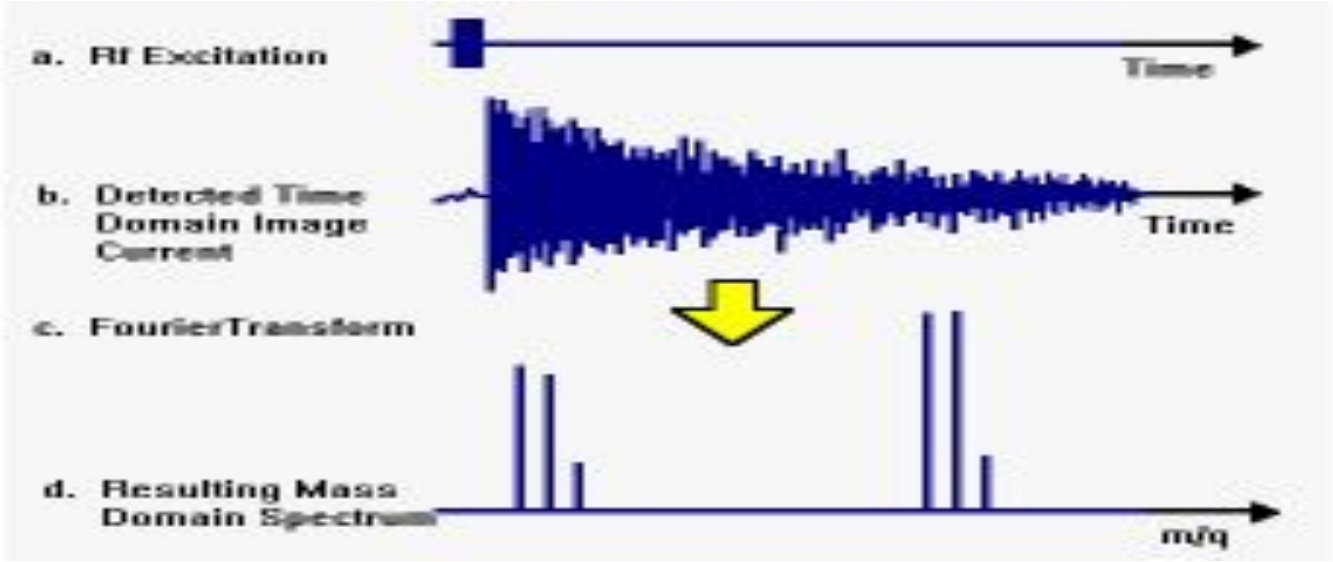


As the ion(s) in a circular orbit approach the top plate, electrons are attracted to this plate from ground. Then as the ion(s) circulate towards the bottom plate, the electrons travel back down to the bottom plate. This motion of electrons moving back and forth between the two plates produces a detectable current.

FTICR: New Dimensions of High Performance Mass Spectrometry

Image is Fourier transformed to obtain the component frequencies and amplitudes (intensity) of the various ions.

Cyclotron frequency value is converted into a m/z value to produce mass spectrum with the appropriate intensities.



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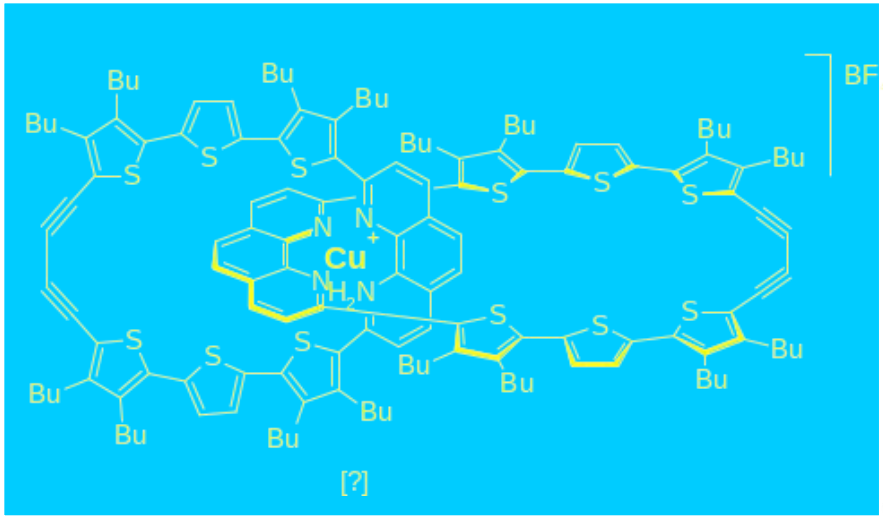

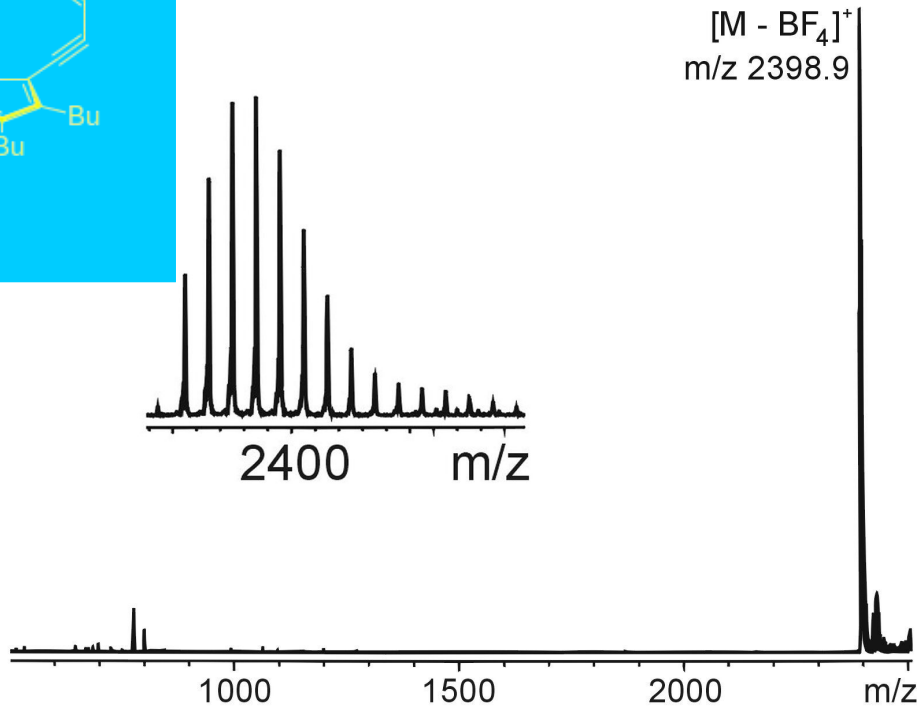

The End