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GoToS3
ALPO

State-of-the-art mass spectrometry methods for the analysis of secondary metabolites

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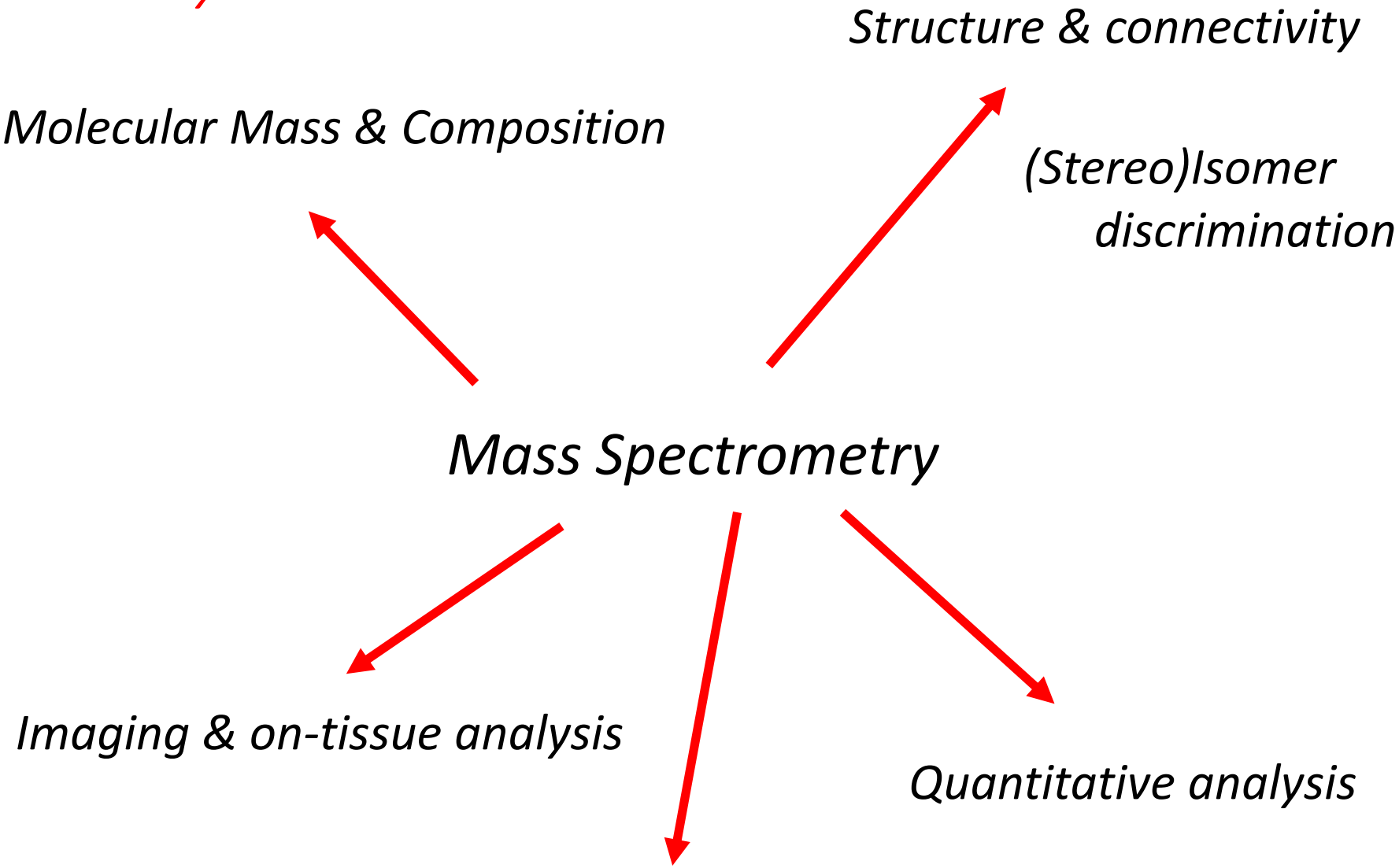
University of Mons, Belgium

Faculté
des Sciences

S²MOs

What do you need ?



Mass spectrometry : an indirect analytical method !

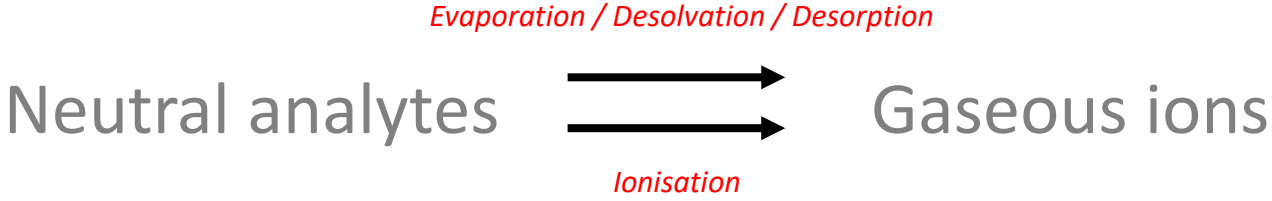
Mass spectrometry is

- an analytical technique based on the measurement of the m / z ratio of ions in the gas phase
- an analytical technique that allows the determination of the composition of ions in the gas phase
- an analytical technique that affords information on the structure of the gas phase ions
- an analytical technique that allows to determine the quantity of gaseous ions associated with a given m / z

in order to

- determine the molecular mass of a molecule (neutral) in the condensed phase (solid / liquid / solution)
- determine the elemental composition of a molecule (neutral)
- determine the structure of the molecule (neutral)
- determine the concentration of a molecule within a complex mixture / matrix

But ...



so many ….

benchtop …

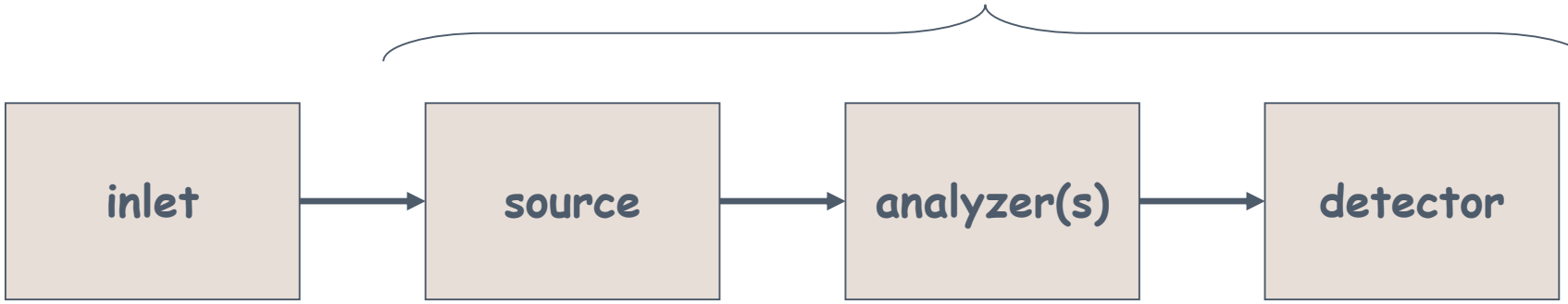
Really expensive …

portable …

There are all the same…

Mass spectrometer

… in principle



- direct introduction **DI**
  - solid
  - liquid
  - gas
  - solution
- hyphenated methods
  - **GC-MS**
  - **LC-MS**

- Electron ionization **EI**
- Chemical ionization **CI**
- ElectroSpray ionization **ESI**
- Matrix-assisted laser desorption/ionization **MALDI**

- Sector instruments **B / E**
- Quadrupole **Q**
- Ion trap **IT / LIT**
- Time-of-Flight instruments **ToF**
- Ion Cyclotron Resonance **ICR / FT-MS**
- orbitrap

The selection of the instrument is analyte dependent and is motivated by the objectives of the analysis !!!

Source & Analyzer

Nature of the analytes ?

Mass range limit ?

20 MDa

nano-objects

- Virus capsids

macromolecular assemblies

- ds-DNA
- Protein assemblies
- Protein/DNA

macromolecules

- Polymers
- DNA
- Proteins

molecules

source selection

- Organic molecules
- Organometallic complexes
- Non covalent complexes

atoms

1 Da

? volatile or non volatile molecules ?

Angewandte Communications

Mass Spectrometry of Proteins

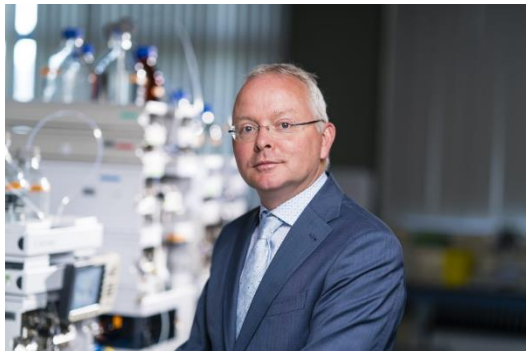
DOI: 10.1002/anie.201210197

Studying 18 MDa Virus Assemblies with Native Mass Spectrometry**

*Joost Snijder, Rebecca J. Rose, David Veesler, John E. Johnson, and Albert J. R. Heck**

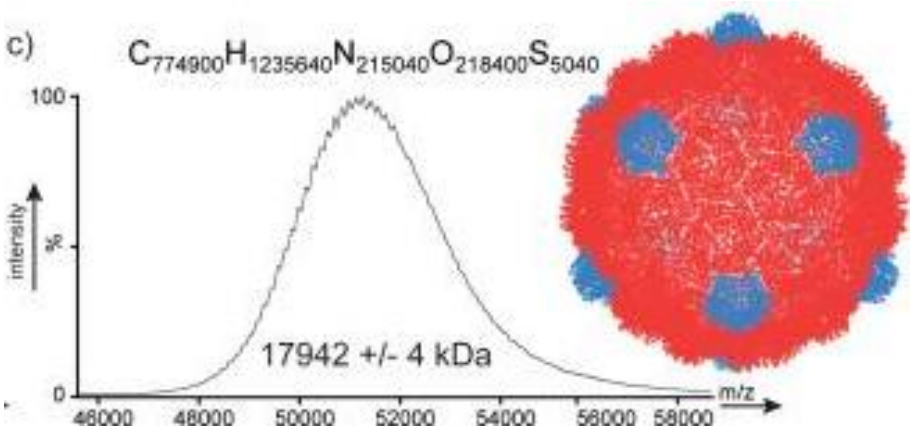
Following John Fenn's Nobel Laureate lecture ("Electrospray Wings for Molecular Elephants"),[1] native mass spectrometry (MS) has been developed as a powerful analytical technique to study large noncovalent protein complexes.[2] Using soft nano-electrospray ionization (nESI), these protein complexes can be transferred intact into the gas phase without significant loss of quaternary or tertiary structure. Minor modifications of commercially available MS instruments[3] can greatly improve the transmission of large ions, therefore yielding accurate and precise mass analysis of intact protein complexes. Although there is no theoretical upper mass limit for a ToF analyzer, achieving good-quality spectra of assemblies several MDa in size has proven not to be trivial.

We used a modified QToF instrument for the analysis of intact 18 MDa capsids from the bacteriophage HK97. HK97 is an important model system for studying bacteriophage assembly and maturation.[8] In vitro, the $T=7$ capsid assembles from a mixture of pentameric and hexameric capsomers (made of the capsid protein gp5) and the viral protease gp4 to form a first icosahedral intermediate termed Prohead-1.

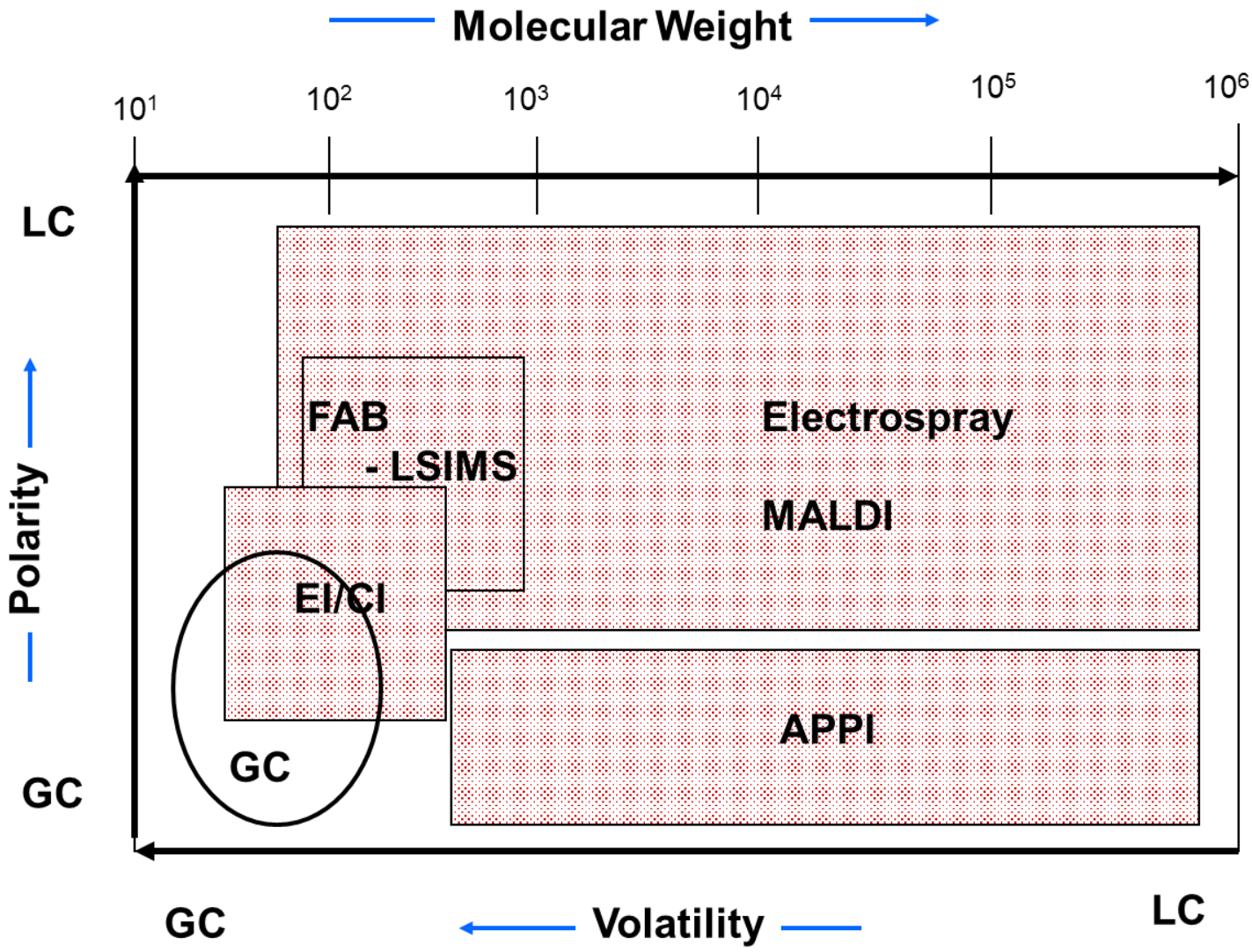


Prof Albert Heck – Utrecht

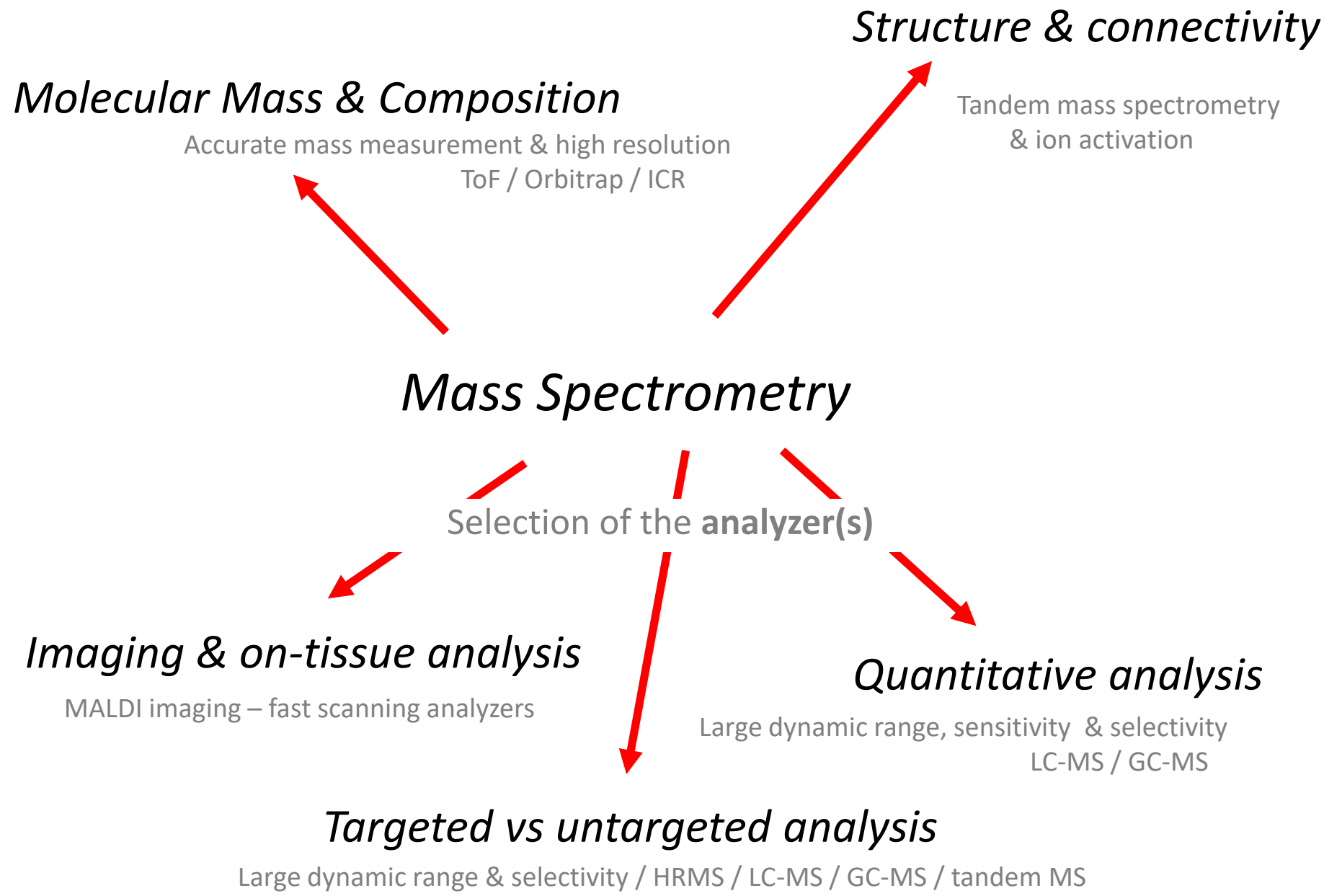
655 papers / h-index 86



How to select the ionization method ?

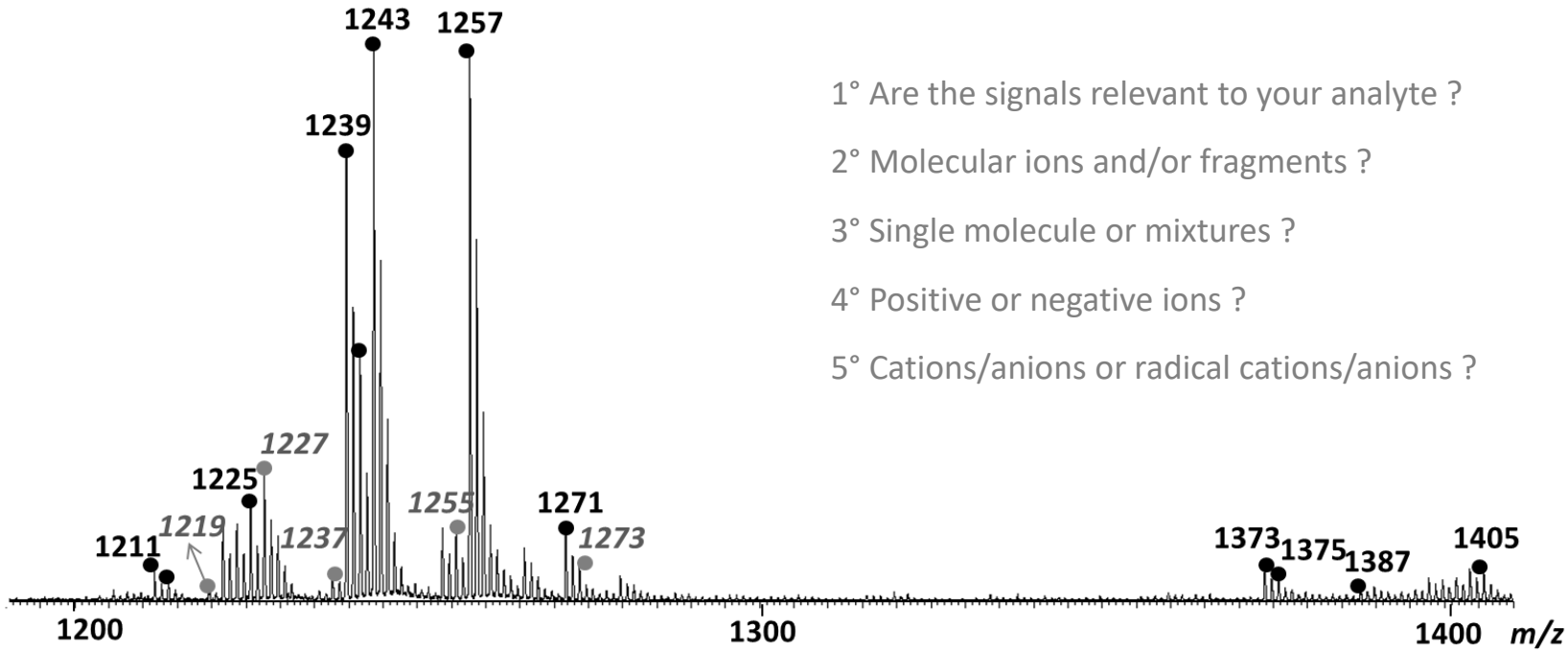


What do you want to know about your samples ?



Then you get a mass spectrum ….

and a huge set of questions…



1° Are the signals relevant to your analyte ?

2° Molecular ions and/or fragments ?

3° Single molecule or mixtures ?

4° Positive or negative ions ?

5° Cations/anions or radical cations/anions ?

Highly dependent on the source conditions, including the pressure conditions

Neutral analytes ⟶ Gaseous ions * (to be evacuated)

Energy

What about MS and research ?

Research with Mass Spectrometry

Mass Spectrometry

- *Natural products*
- *Synthetic Macromolecules*
- *Non covalent interactions*

Research in Mass Spectrometry

Research & Development of the MS-based- methods

Ionization processes / analyzer development / ion activation and ion decomposition / energetics and kinetics / gaseous ion structure vs solution phase structure ….

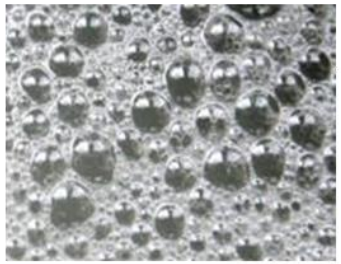
State-of-the-art mass spectrometry methods for the analysis of secondary metabolites

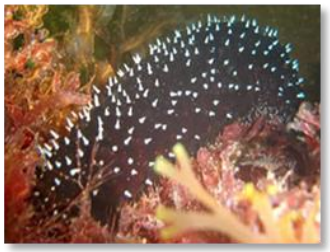
- the saponin case -

What is a saponin ?

- Natural products in plants and marine animals
- Secondary metabolites
  (defense, reproduction, symbiosis)
- **Amphiphilic molecules**
- Natural surfactants

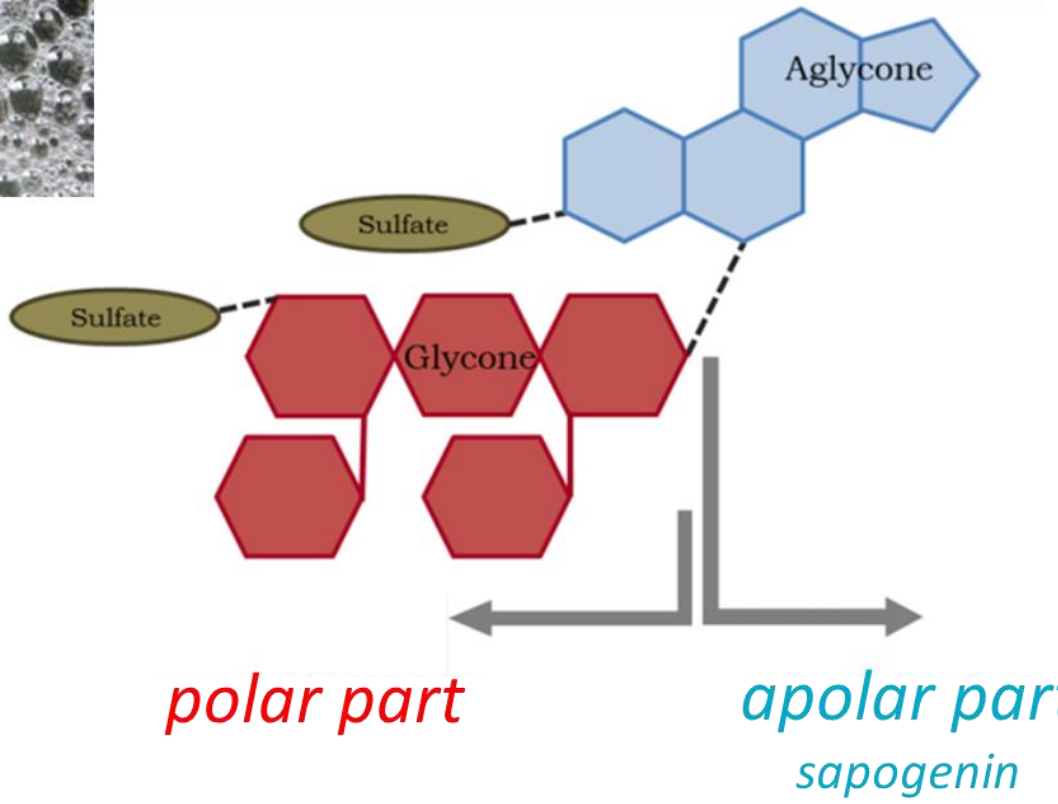
Quillaja saponaria (Chili) Sapindus mukorossi (Inde)





Sea cucumbers
Holothuries

Sea stars - Starfishes



How complex is the saponin structure ?

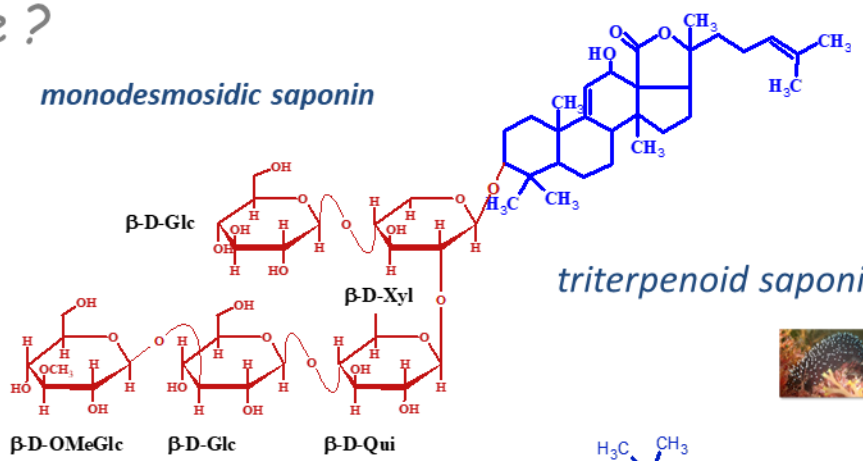
Asteroside C

Sulfate

Steroid (Aglycone)

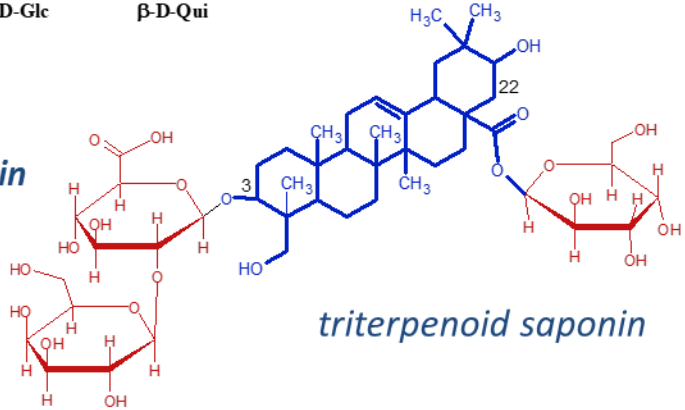
Oligosaccharide (Glycone)

monodesmosidic saponin



triterpenoid saponin

bidesmosidic saponin

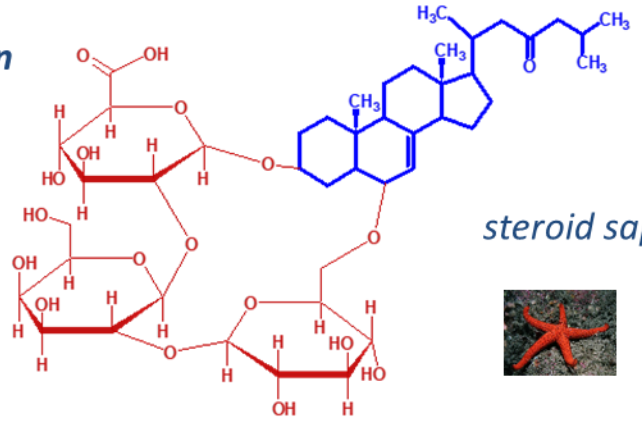


triterpenoid saponin

Huge structural diversity

- sapogenin : *steroid / triterpenoid*
- oligosaccharide chain :
  n = 1 – 10 / linear or branched
- topology :
  mondesmosidic / bidesmosidic / macrocyclic

macrocyclic saponin

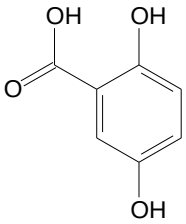


steroid saponin

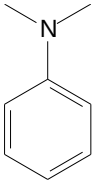
STEP 1 - Screening of the saponin compositions by MALDI-MS

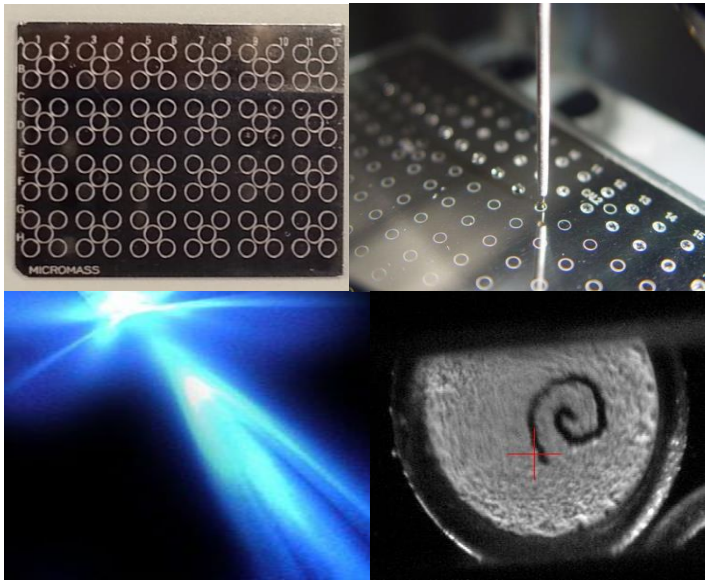
Matrix based on ionic liquid

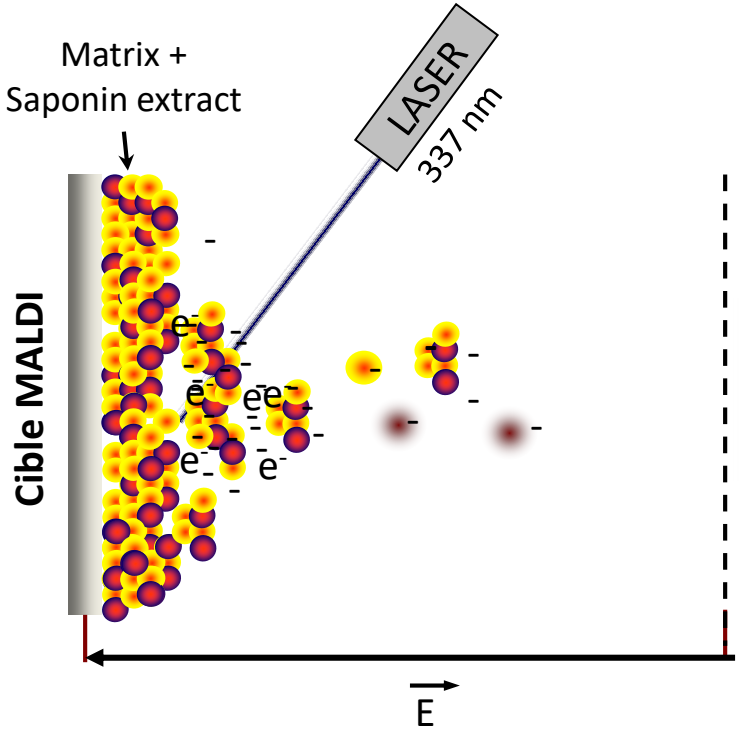
DHB



DMA







MS

Waters Q-ToF Premier

Our favorite mass spectrometers are the Q-ToF instruments

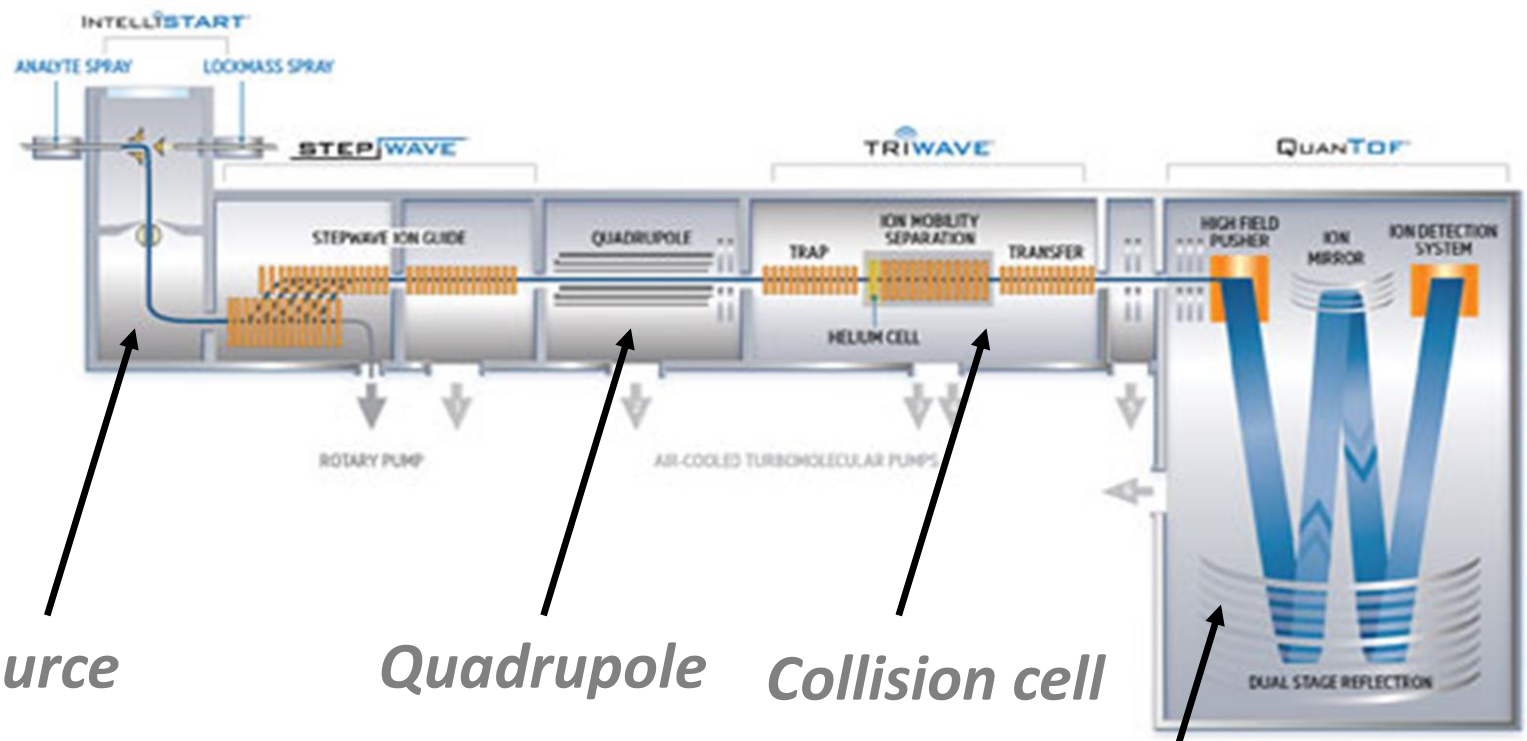
MALDI Q-ToF Premier

ESI Q-ToF API-US

ESI Synapt G2-Si

Q-ToF mass spectrometers

introduction → source → analyzer(s) → detector



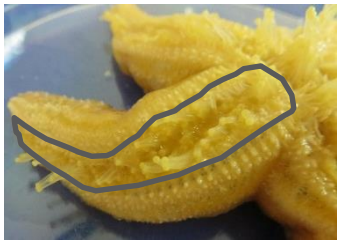
source *Quadrupole* *Collision cell*

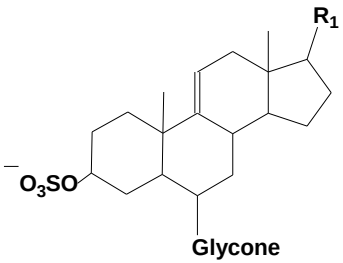
Q-ToF mass spectrometer

=> HRMS => composition
=> MSMS => structure
=> ion mobility => structure

Time-of-Flight analyzer

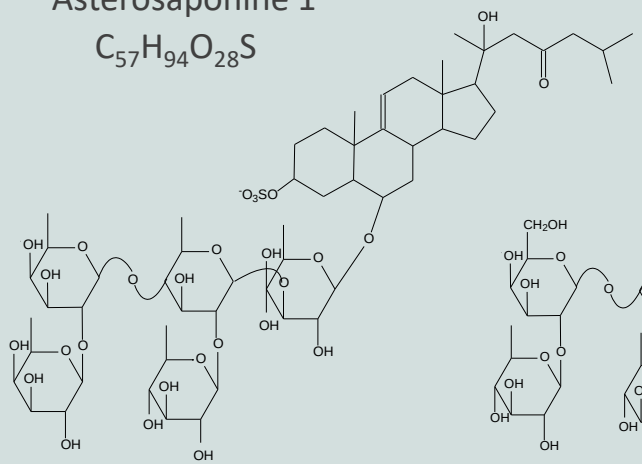
MALDI(-)-MS podia extract from Asterias ruben



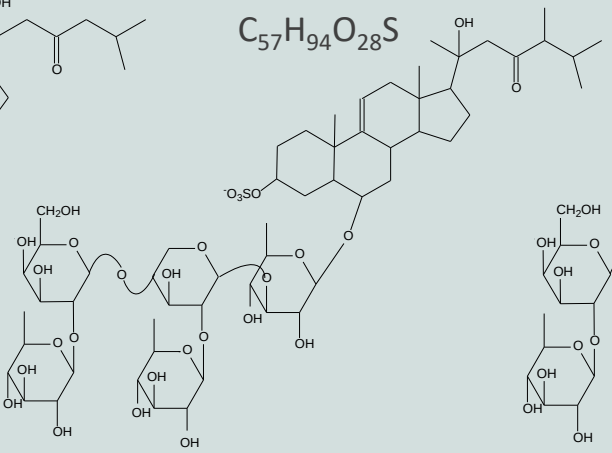


- 16 detected compositions
- 12 known saponin compositions
- 4 new saponin compositions

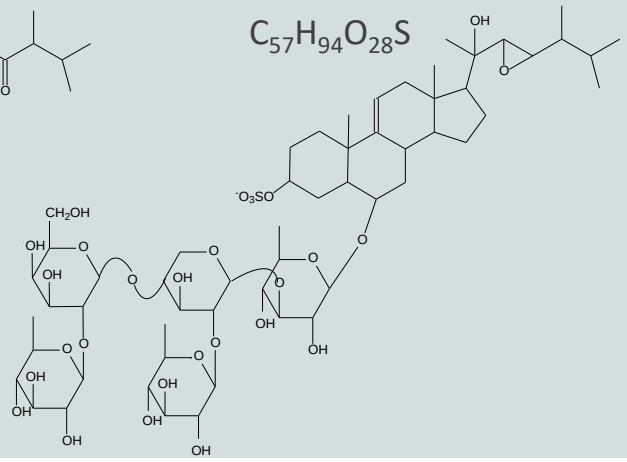
Astérosaponine 1
$C_{57}H_{94}O_{28}S$



Astéroside C
$C_{57}H_{94}O_{28}S$



Rubéroside F
$C_{57}H_{94}O_{28}S$

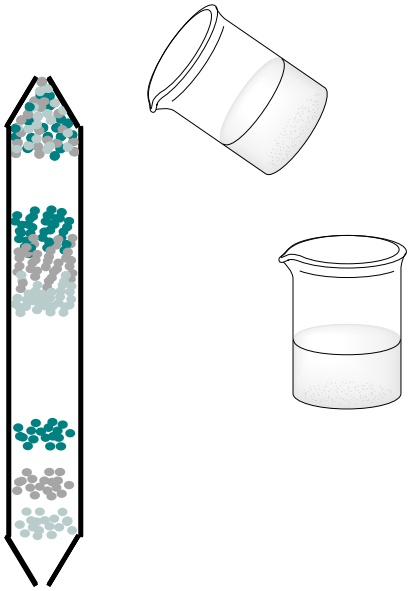


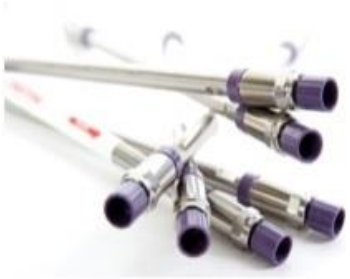
⇒ ***3 isomers are proposed in the literature ?***

⇒ ***LC-ESI(-)-MSMS***

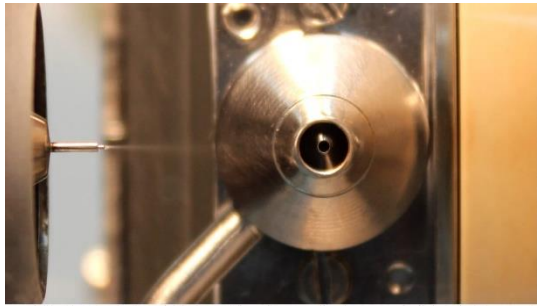
*Sandvoss M. *et al.* J. Chrom. A 2001; 917:75-86.

STEP 3 –LC-MS analysis of the podia extract

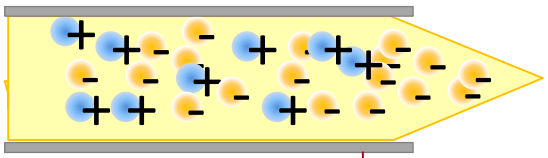




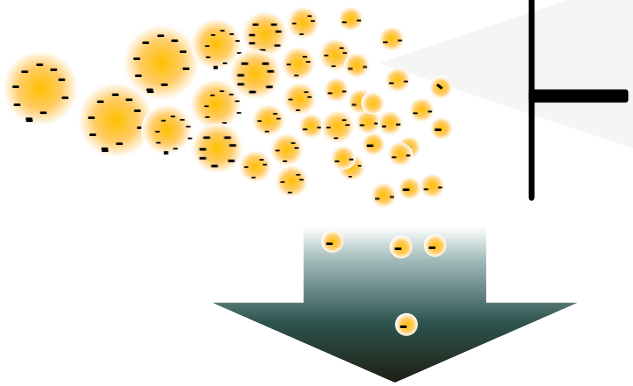
HPLC or UPLC



Capillaire



-3,1 kV

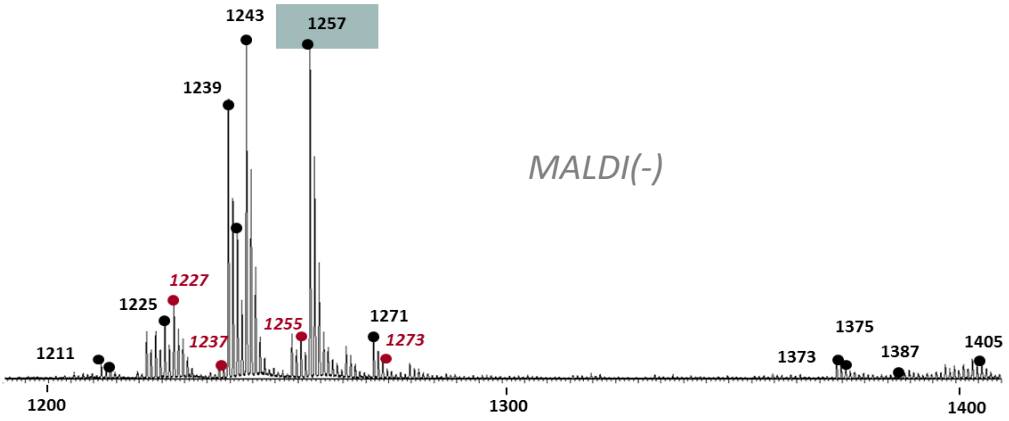


Waters Synapt G2-Si

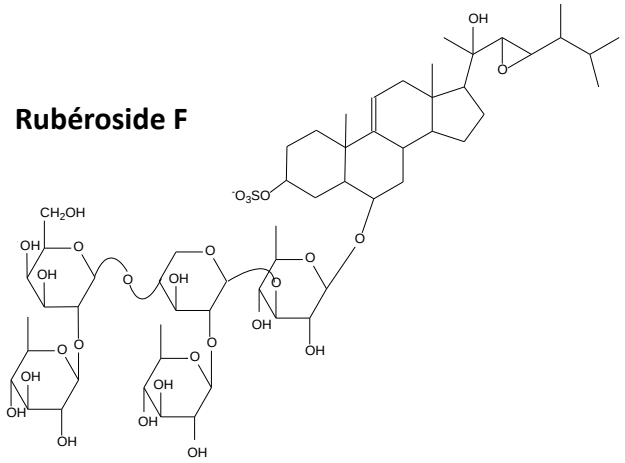
ESI (ElectroSpray Ionization)

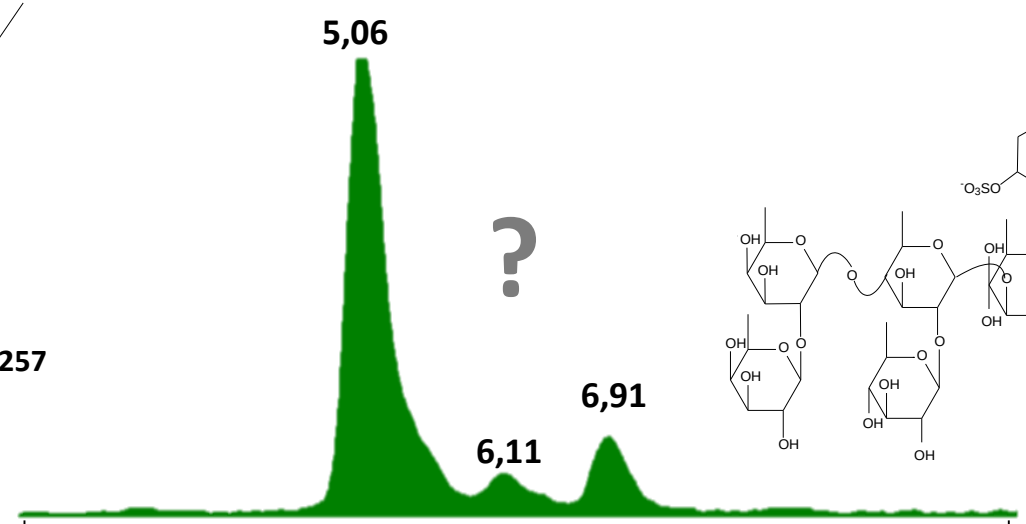
MS

LC-ESI(-)-MS podia extract

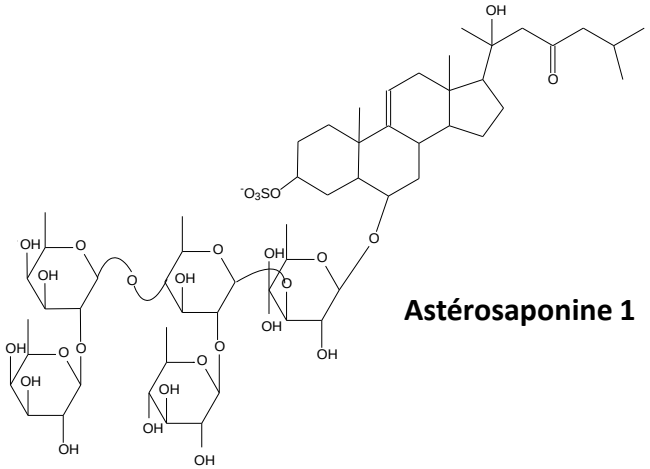


Rubéroside F



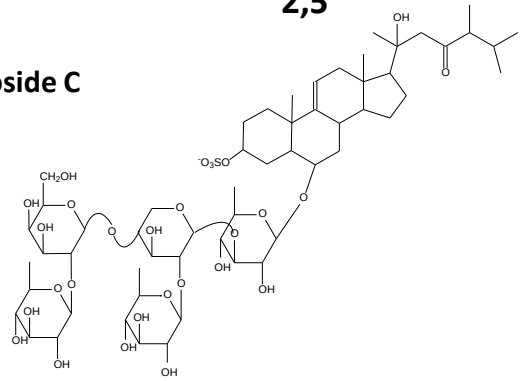


Astérosaponine 1



Extracted Ion Current for ***m/z* 1257**

Astéroside C



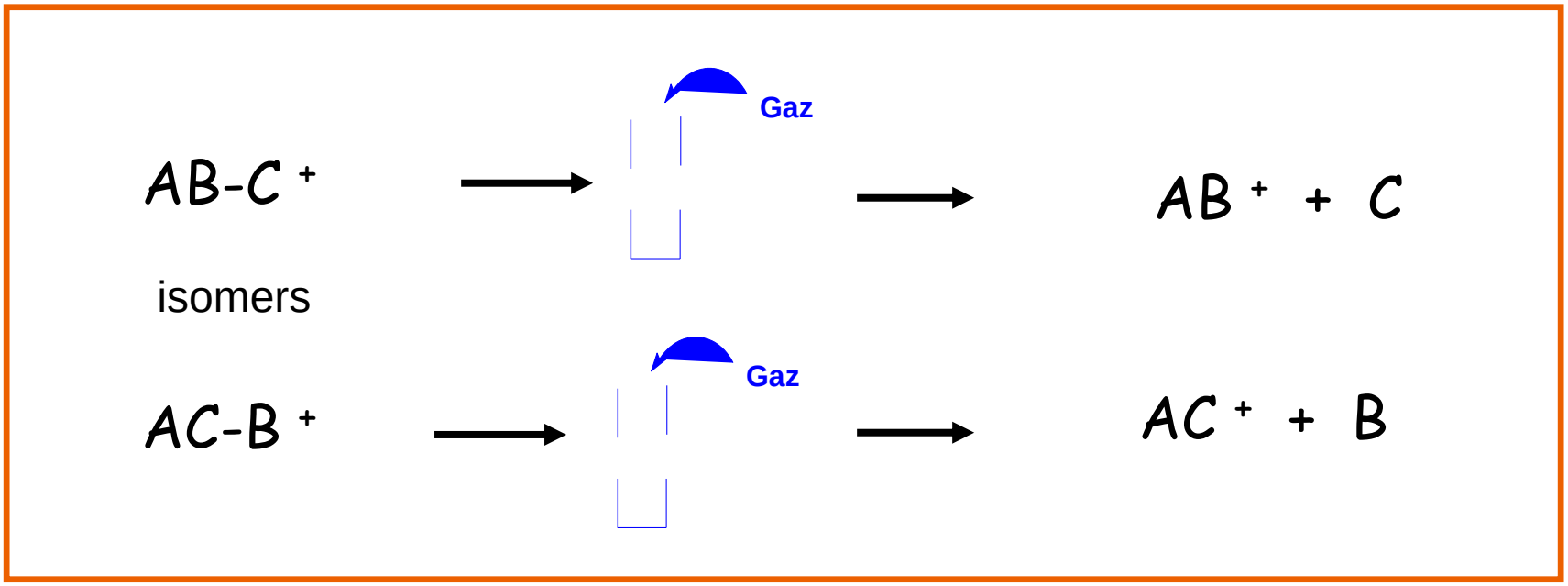
⇒ ***LC-ESI(-)-MSMS***

⇒ ***Tandem Mass spectrometry***

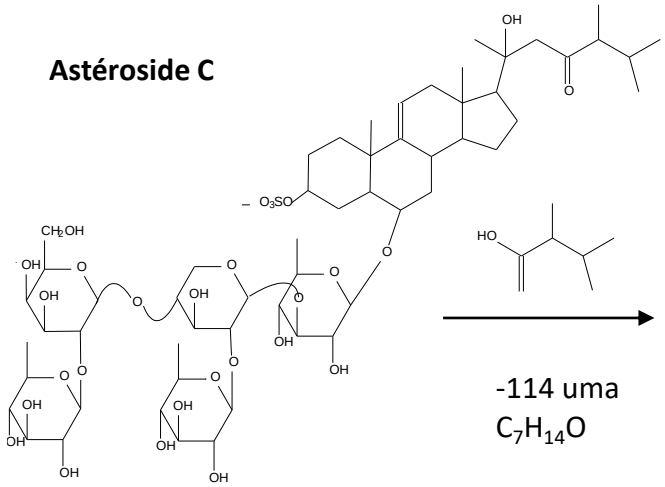
⇒ ***Collision-induced dissociation (CID)***

Tandem Mass Spectrometry (MSMS)

Ion activation / Collision-induced Dissociation (CID)

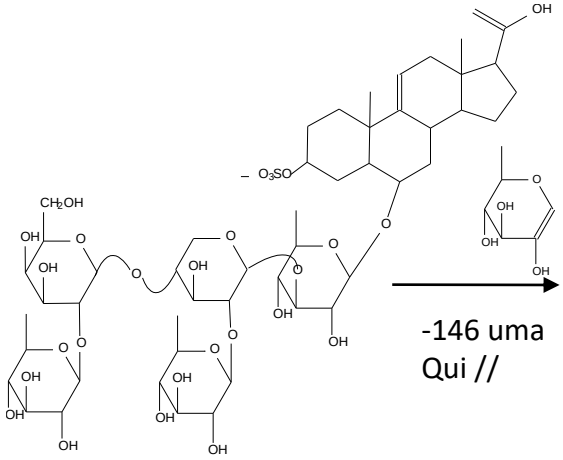


Astéroside C



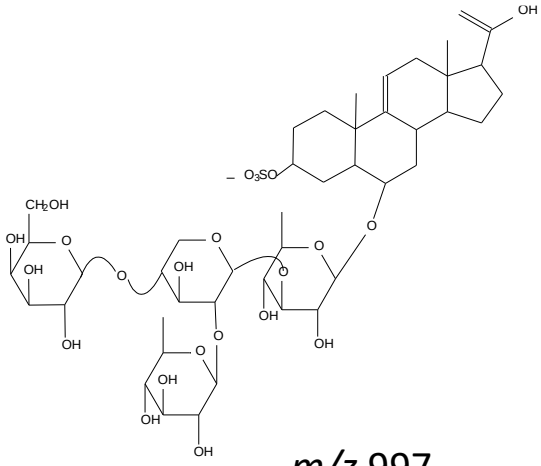
m/z 1257

-114 uma
$C_7H_{14}O$



m/z 1143

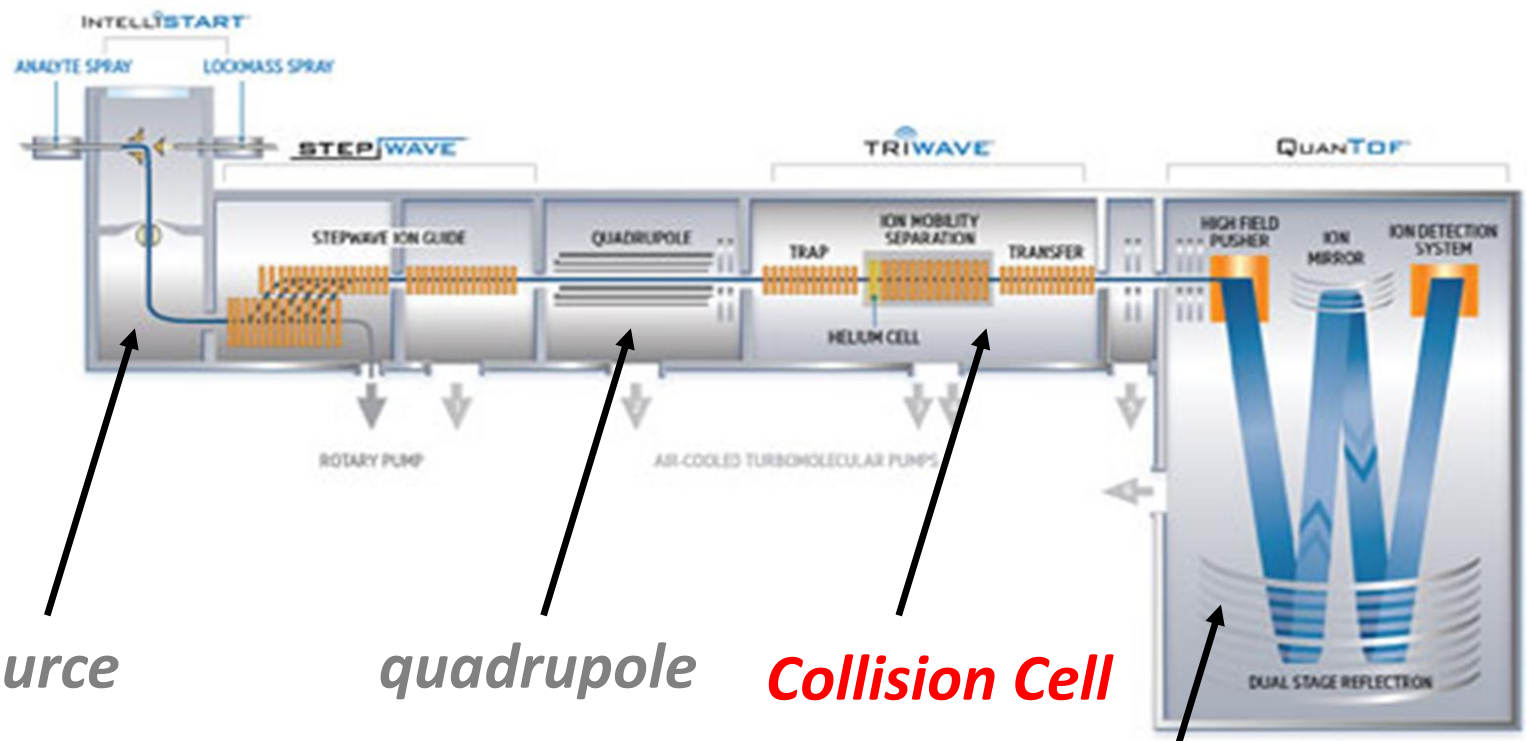
-146 uma
Qui //



m/z 997

Q-ToF mass spectrometers

introduction → source → analyzer(s) → detector

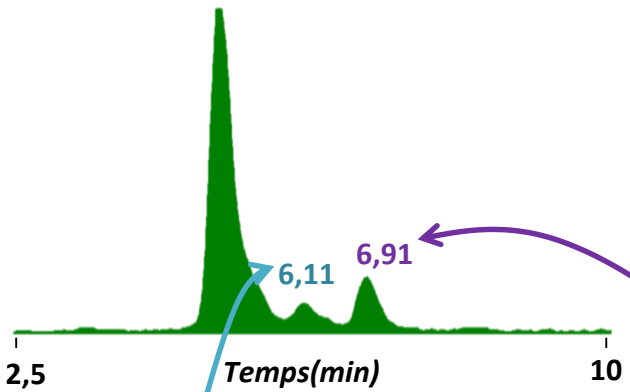


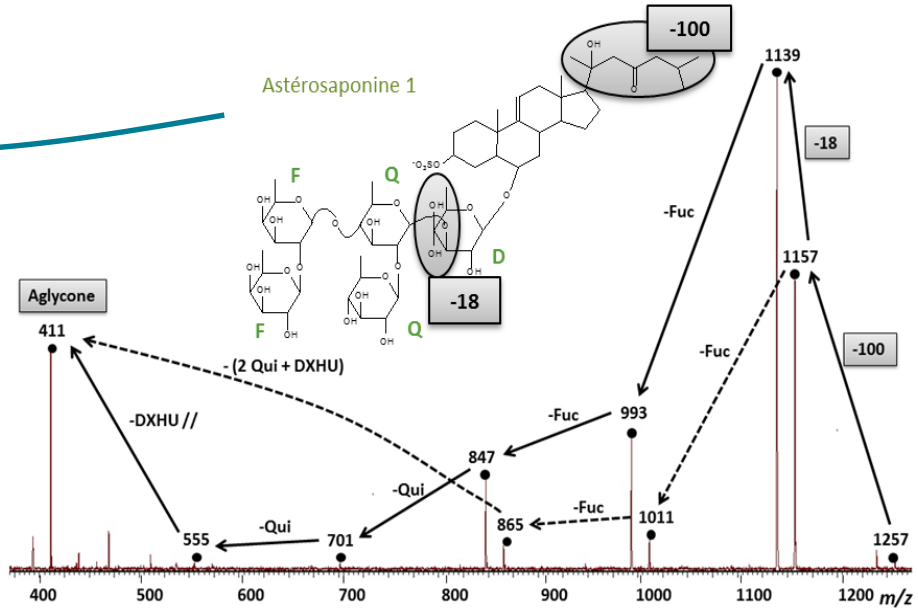
source *quadrupole* ***Collision Cell*** *ToF analyzer*

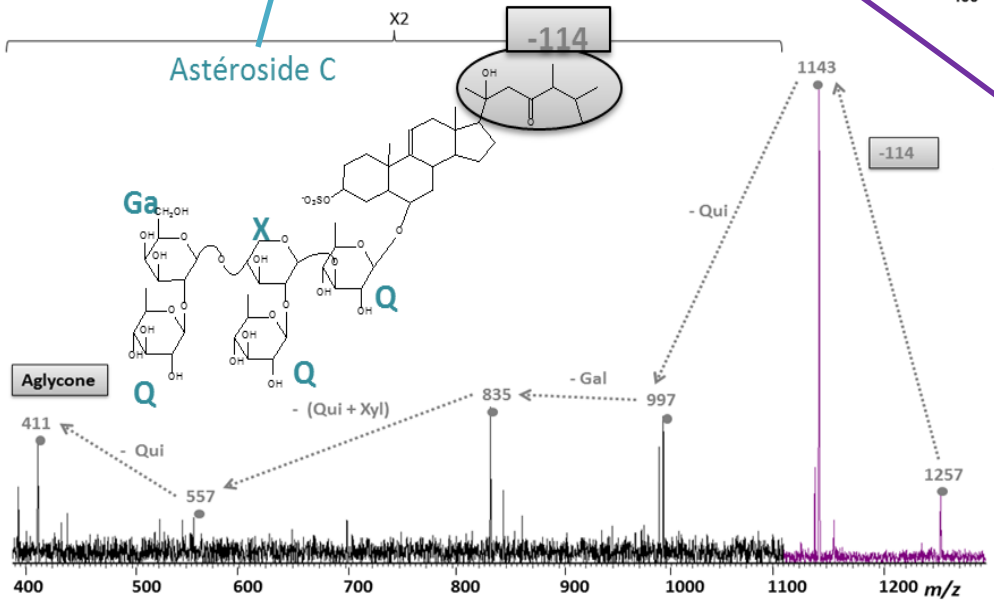
Q-ToF mass spectrometer

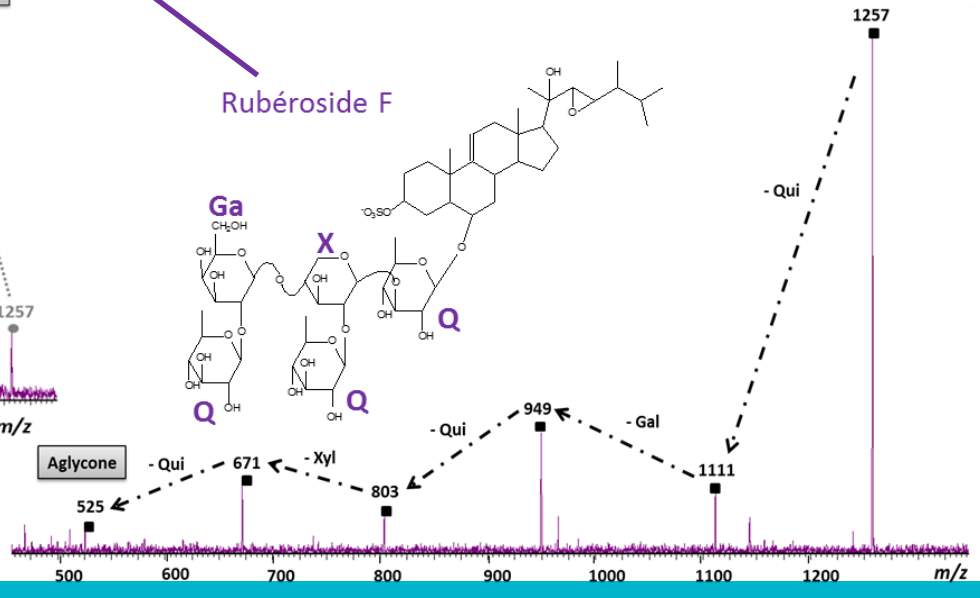
=> HRMS => composition
=> MSMS => structure
=> ion mobility => structure

LC(-)-MSMS analysis of the m/z 1257 ions



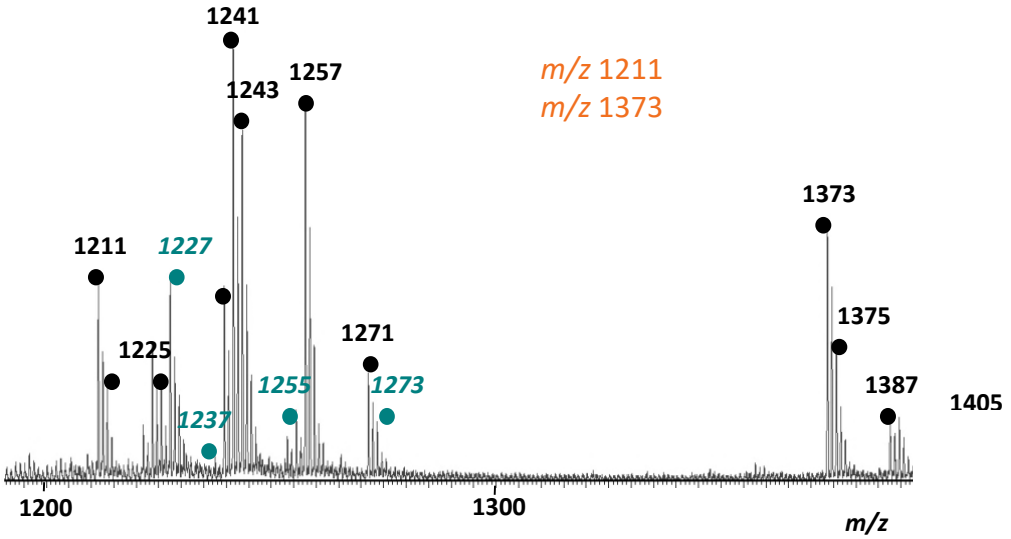






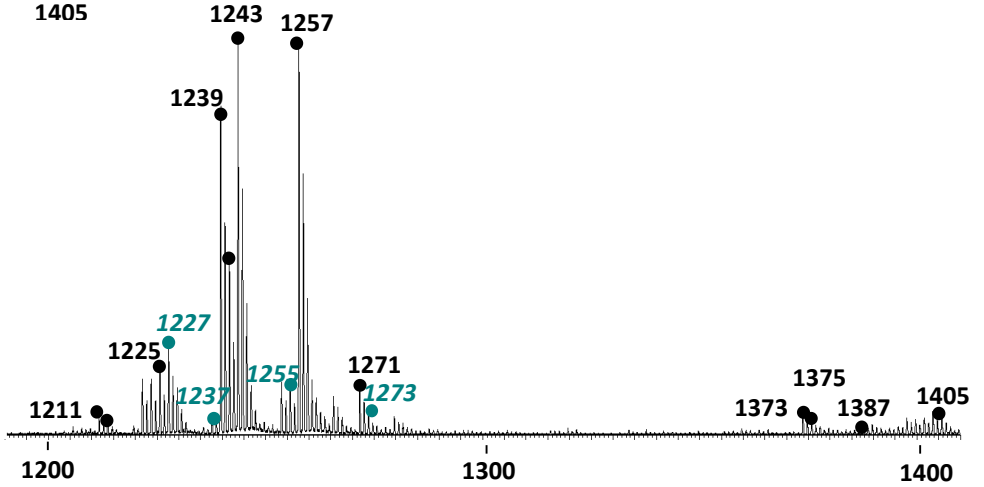
STEP 4 – Inter-organ distribution of saponins

MALDI(-)-MS of ***tegument extract***

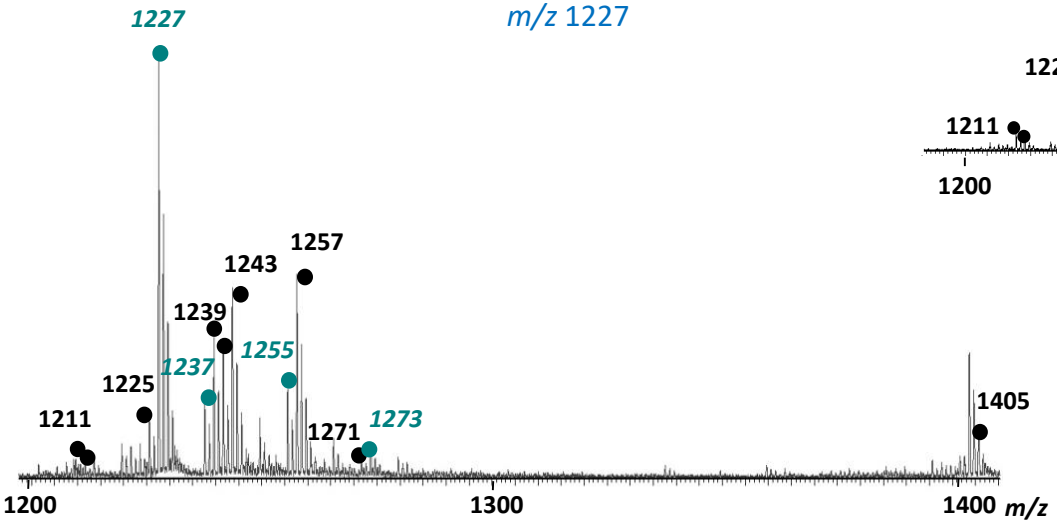


m/z 1211
m/z 1373

MALDI(-)-MS of ***podia extract***



MALDI(-)-MS ***gonad extract***



m/z 1227

Specific saponin content in function of

- → **the organ**
- → **the season**
- → **female / male**
- → **animal development**

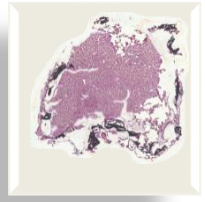
Comparative Biochemistry & Physiology, Part.B, 168 (**2014**) 1-11

STEP 5 - intra-organ distribution with MALDI imaging

Prof Fournier (ULille)

PRISM
Protéomique • Réponse Inflammatoire • Spectrométrie de Masse
Laboratoire de Spectrométrie de Masse Biologique Fondamentale et Appliqué
EA 4550, université Lille 1

ITO plate



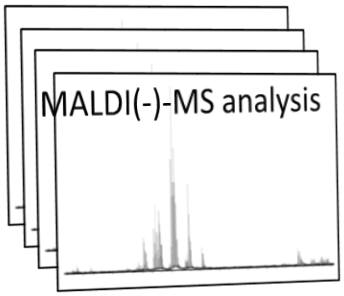
1 cryo cross section

2 Matrix

4 histologic coloration

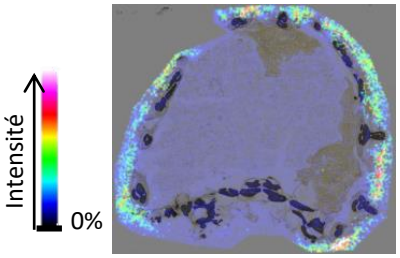
3 MALDI imaging

~ 25 000 mass spectra



Software analysis

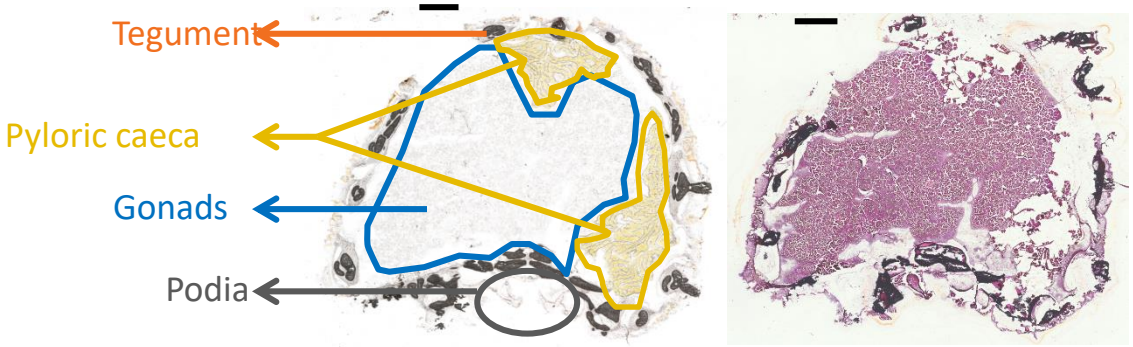
Ions *m/z* 1211

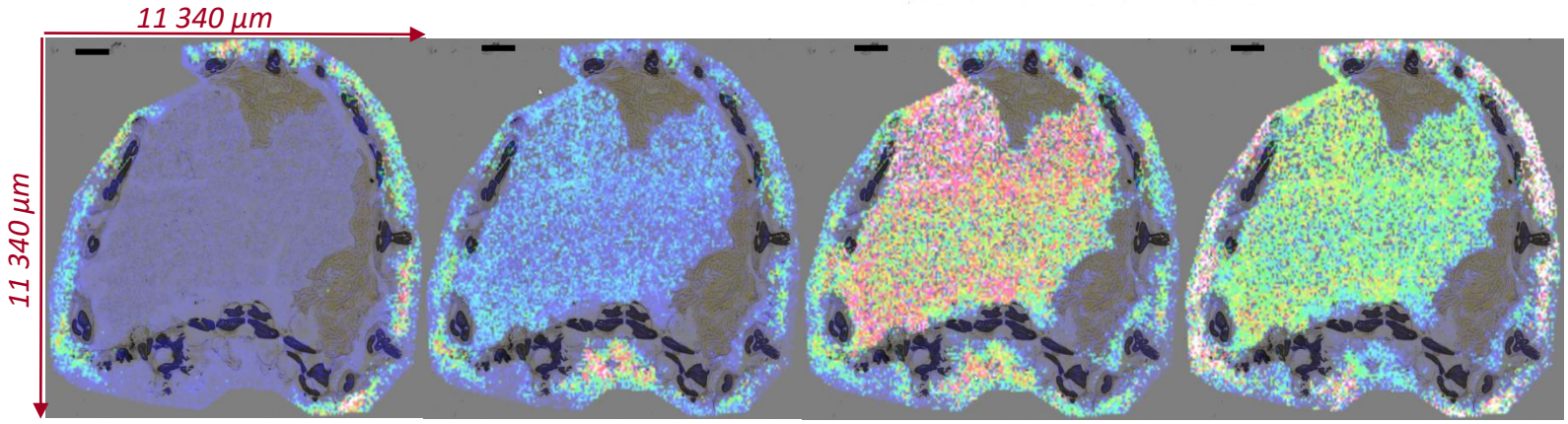


Spatial localization of the *m/z* 1211

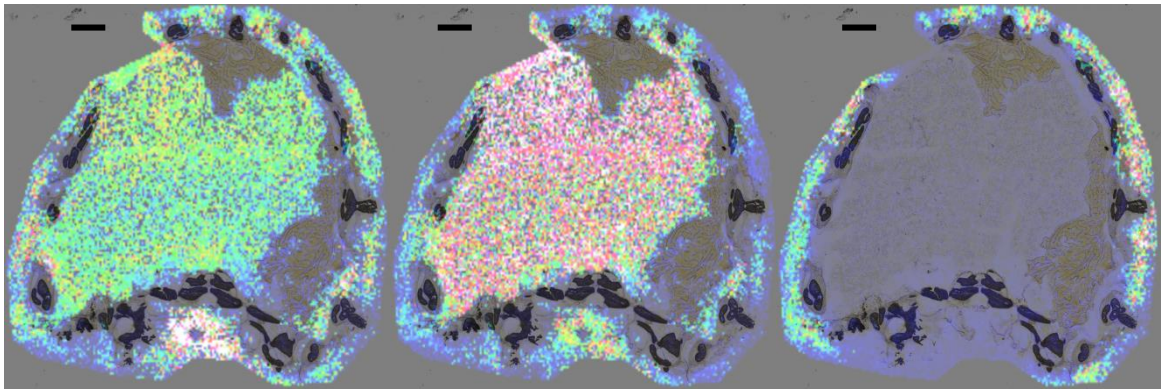
MALDI-imaging of A ruben

Spatial resolution of **120x120 µm²**





m/z 1211 *m/z* 1225 *m/z* 1227 *m/z* 1241

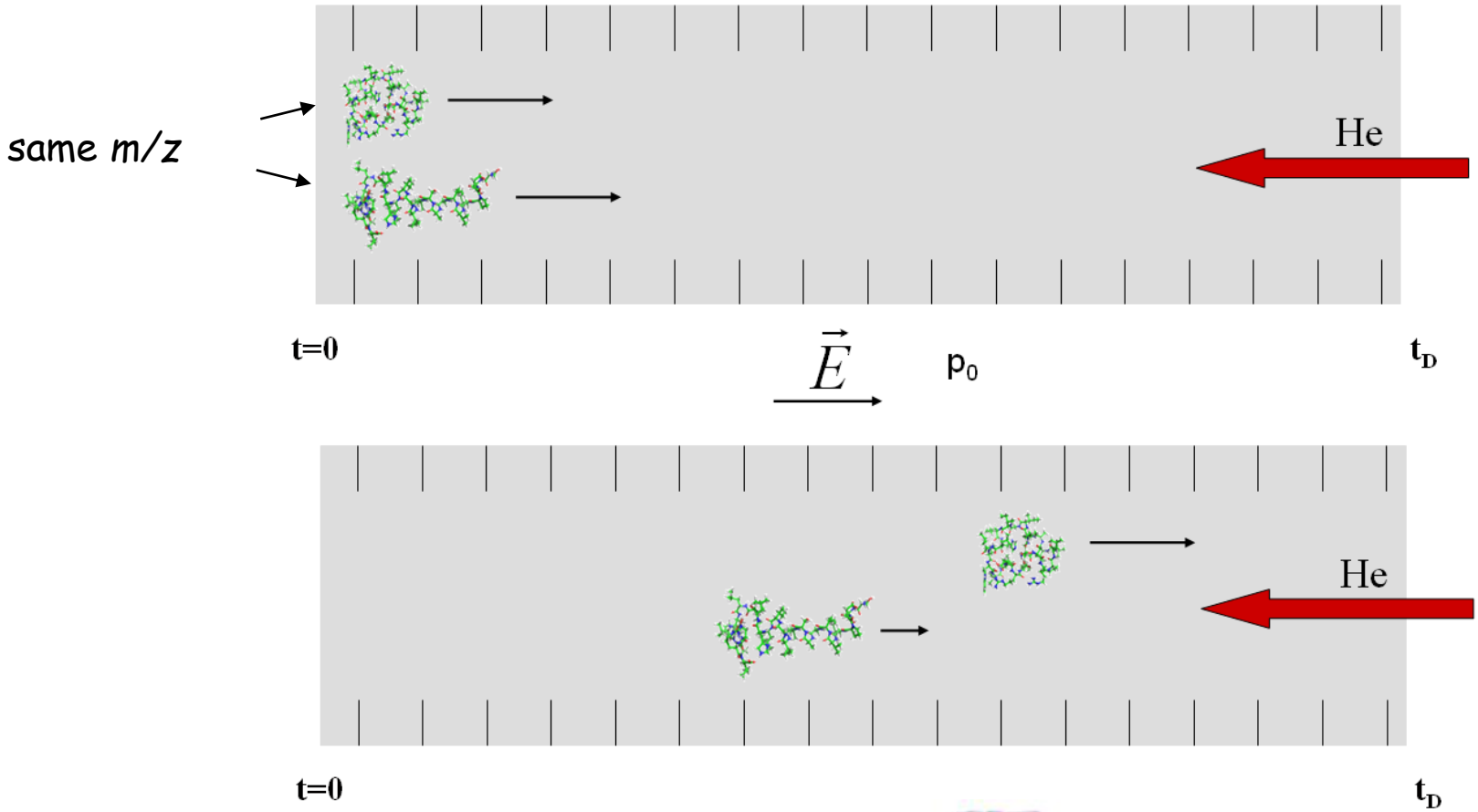


m/z 1243 *m/z* 1257 *m/z* 1373

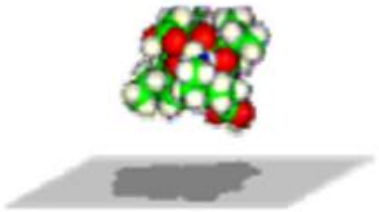
1 000 µm

Anal.Bioanal.Chem. 407 (**2015**) 8813–8824

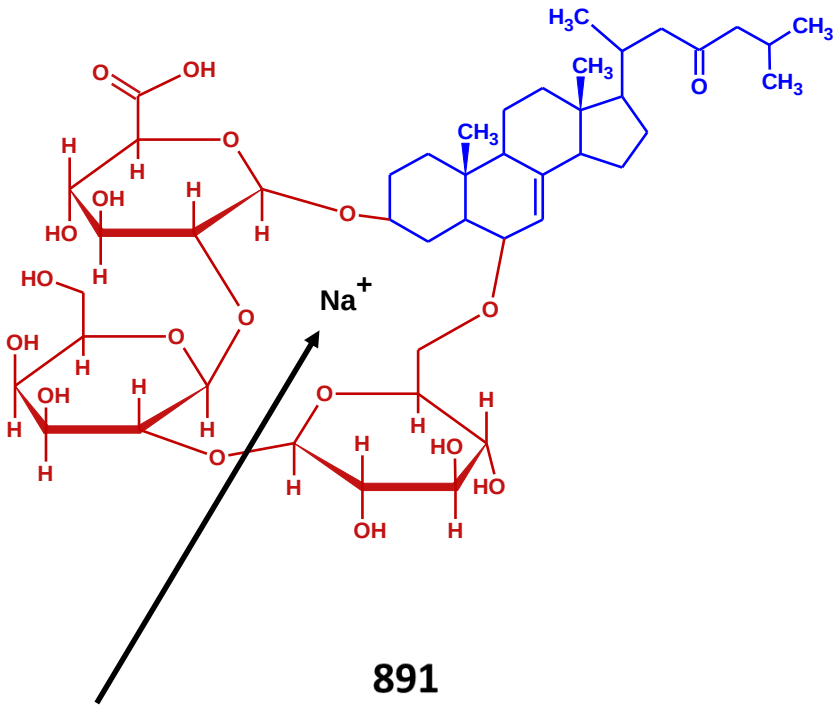
3D structure : ion mobility spectrometry – mass spectrometry



$$\Omega_{avg} = f(t_d)$$



Collisional Cross Section



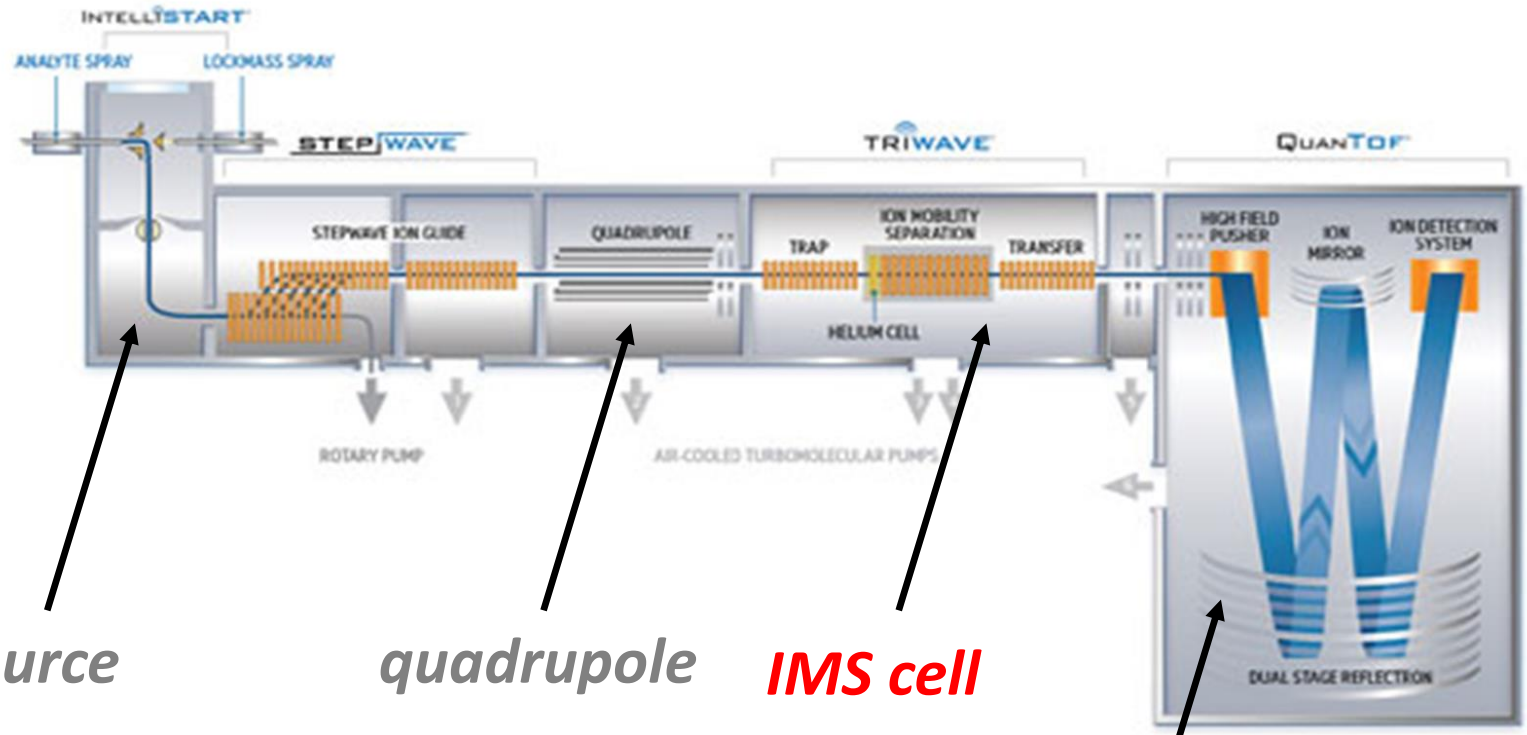
Echinaster sepositus

MALDI(+)

Rapid Commun.Mass Spectrom., 2018, in press.

Q-ToF mass spectrometers

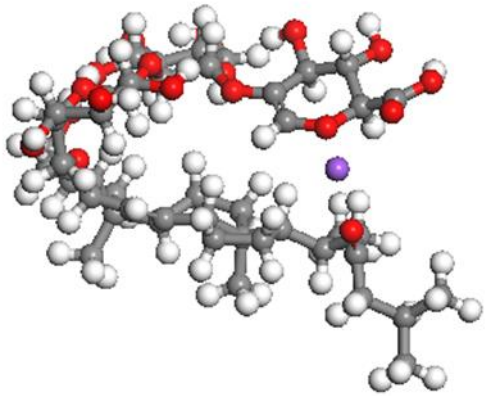
introduction → source → analyzer(s) → detector

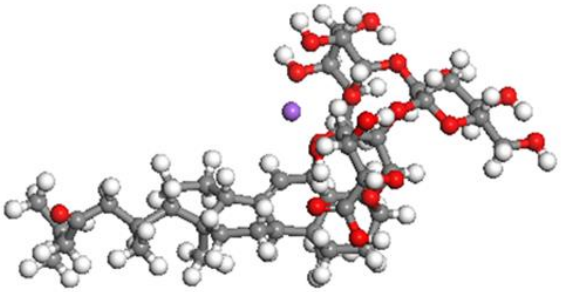


source *quadrupole* ***IMS cell*** *ToF analyzer*

Q-ToF mass spectrometer

=> HRMS => composition
=> MSMS => structure
=> ion mobility => structure





$\Omega_{th} = 225\ Å^2$

$\Omega_{th} = 242\ Å^2$

7,69

m/z 921

236 Å²

216 Å²

6,86

Time

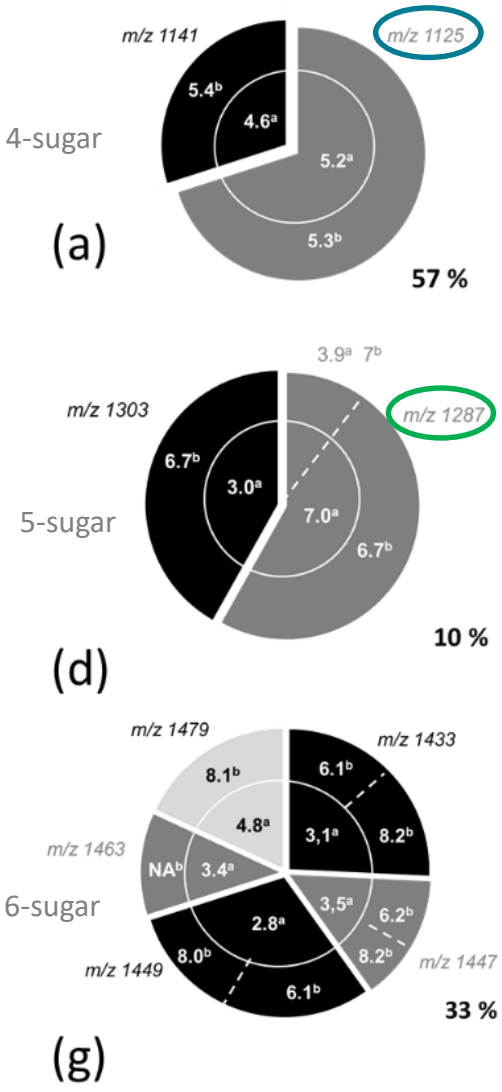
5,00

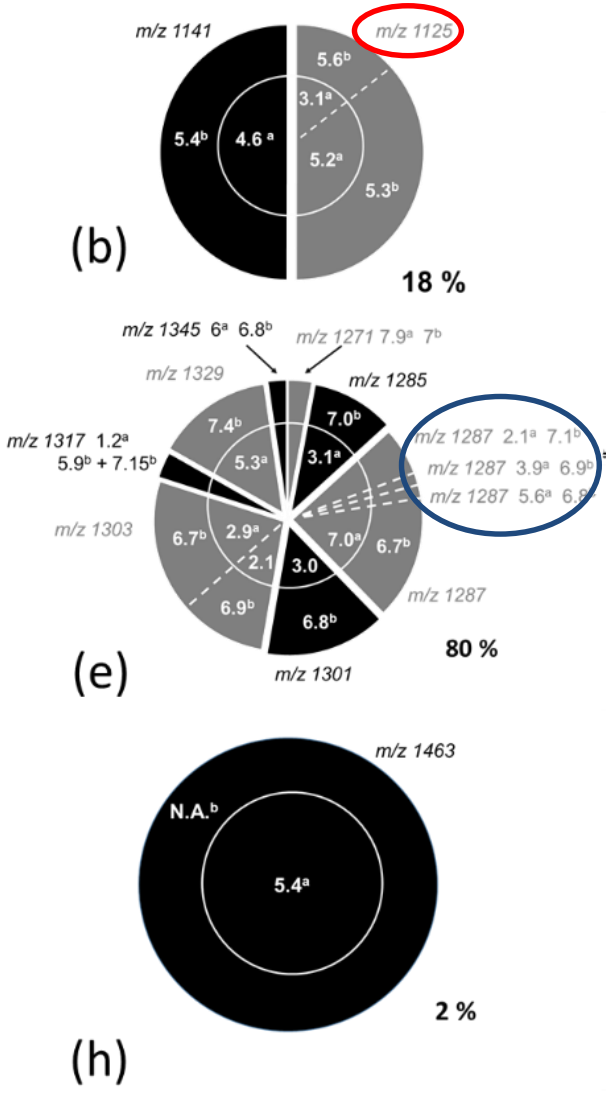
10,00

(msec)

Molecular Dynamics simulation (Prof Cornil – CMN)

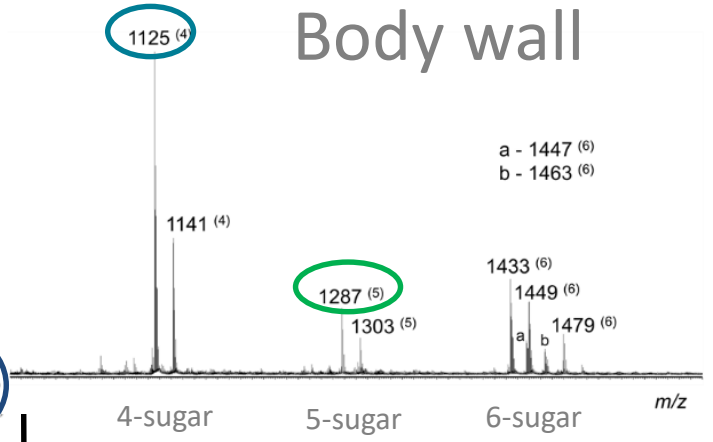
STEP 7 – Data compilation : MALDI + LC + CID + IMS

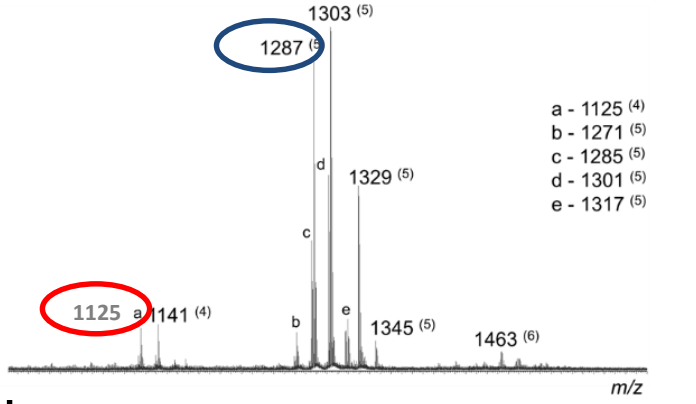




Body wall

Gonads

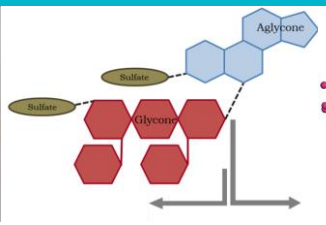


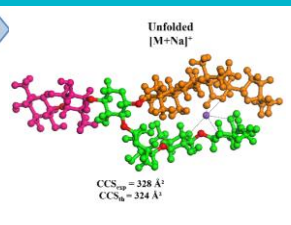


Anal.Bioanal.Chem., 409, (2017), 3115-3126









- **Saponins are secondary metabolites in plants and marine animals**

- **Saponins are amphiphilic molecules and the saponin family is characterized by a huge structure diversity**

- **Saponins are membranolytic molecules**

Elucidation of the structure/activity relationship is a multidisciplinary research project

- **To build the structure/activity relationship, structural characterization is also mandatory**

- **We are developing MS-based methods for saponin characterization**

  by associating MALDI-MS, LC-MS, CID and ion mobility / MD

- **We evaluate the biological activities of saponin extracts & pure saponins**

  hemolytic activity / bactericidal activity / fungicide activity / antioxydant activity…

Many thanks to …

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Thank you for your attention