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Mass spectrometry and metabolic imaging

Pierre Pétriacq

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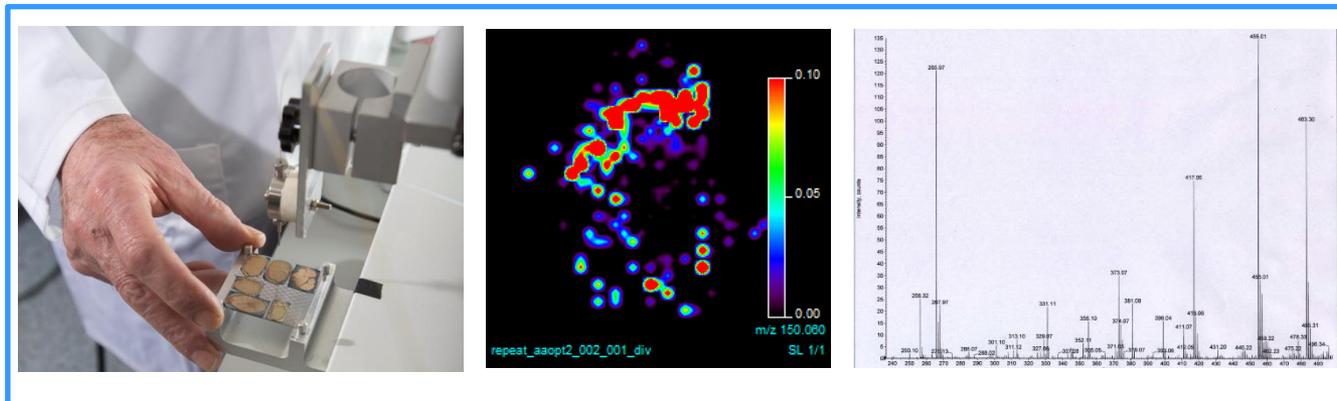
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Mass Spectrometry and metabolic imaging

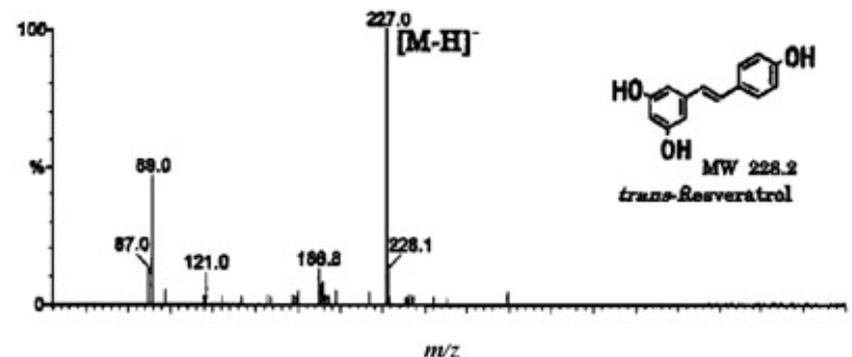
Master 2 IOB

Pierre Pétriacq, PhD, FHEA



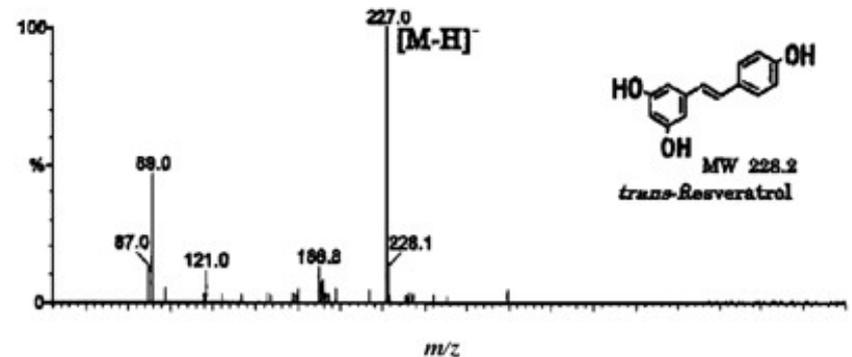
Outlines

- 1. Introduction
- 2. Mass spectrometer
 - Ion source
 - Mass analyser
 - Tandem MS (MS/MS)
- 3. Mass spectrum
- 4. MS analysis
- 5. MS imaging
- 6. Conclusion



Outlines

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Mass spectrometry

1. Introduction

✓ Principle

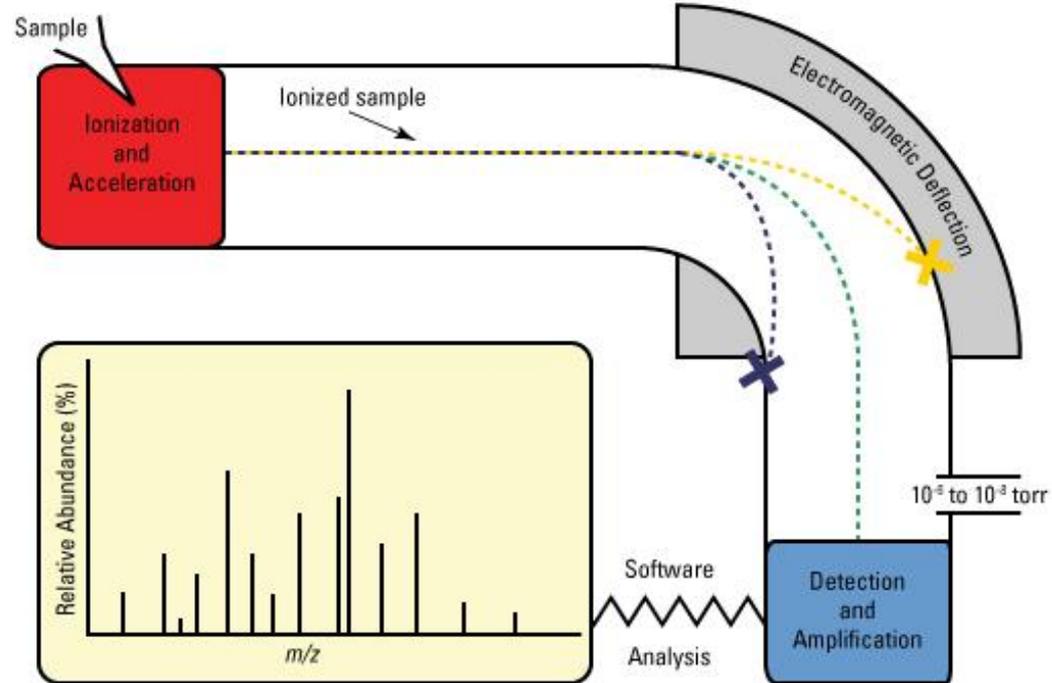


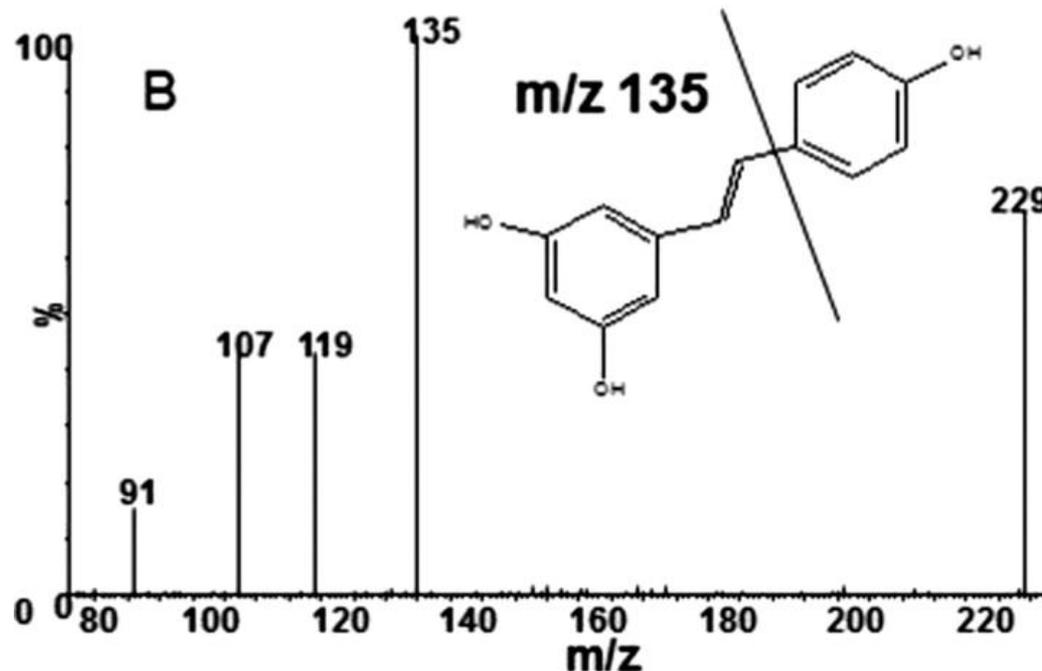
Diagram of a sector mass spectrometer

1. A sample is injected into the mass spectrometer, and **the molecules are ionised** and **accelerated**.
2. The ions are **separated** by mass and charge by the **mass analyser** via electromagnetic deflection.
3. The ions that are properly aligned are **detected**
3. The data that is generated report on the **relative abundance** of each ion based on its **mass-to-charge (m/z) ratio**.

1. Introduction

✓ Objectives

- Mass spectrometry (MS) measures the m/z ratio of ions to identify, and quantify molecules in simple and complex mixtures



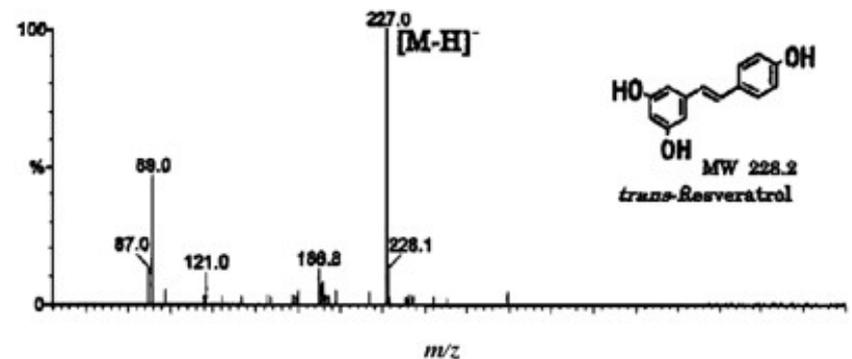


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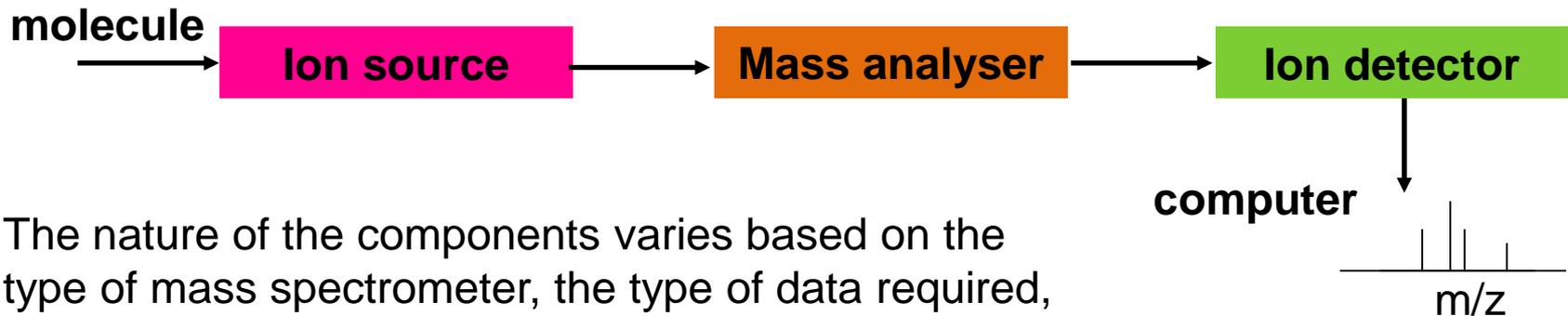
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2. Mass spectrometer

- ✓ All mass spectrometers have 3 main components
 - an ion source
 - a mass analyser
 - an ion detector



The nature of the components varies based on the type of mass spectrometer, the type of data required, and the physical properties of the sample.

Mass spectrometry

2. The ion source

- ✓ **ionisation:** production of gas phase ions suitable for resolution in the mass analyser
- ✓ **several ion sources available** with advantages and disadvantages for particular applications

Hard ionisation techniques:
large degrees of fragmentation

electron ionisation (EI)

Soft ionisation techniques:
limited fragmentation.

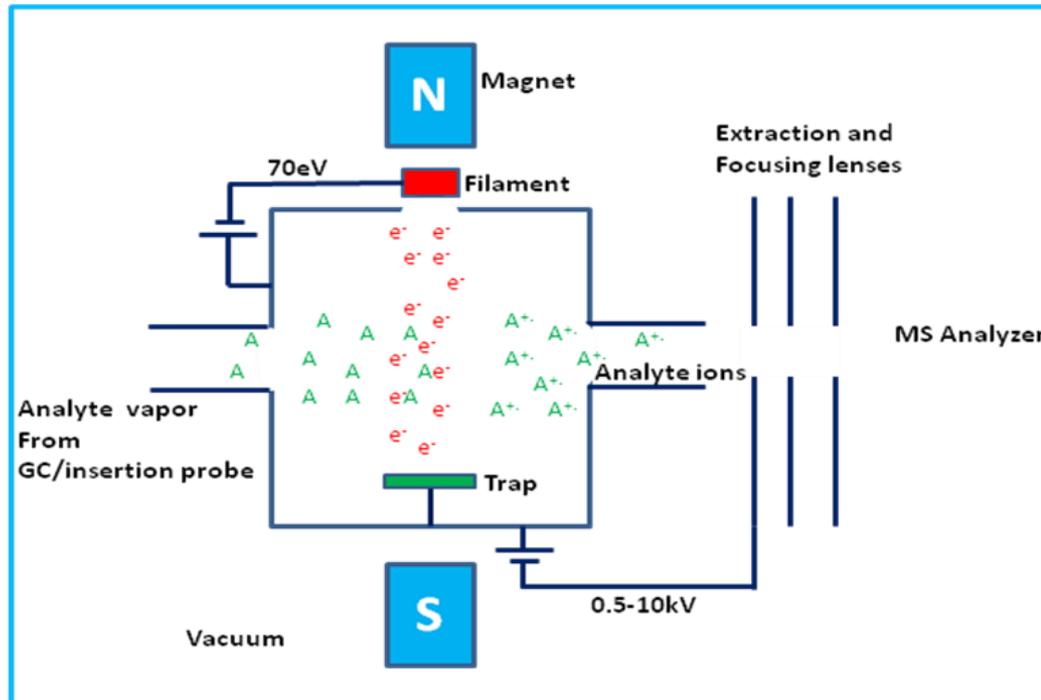
fast atom bombardment (FAB),
chemical ionisation (CI)
atmospheric-pressure chemical
ionisation (APCI)
electrospray ionisation (ESI)
**matrix-assisted laser
desorption/ionisation (MALDI)**

Mass spectrometry

2. The ion source

✓ electron ionisation (EI)

- electrons interact with solid or gas phase atoms or molecules to produce ions

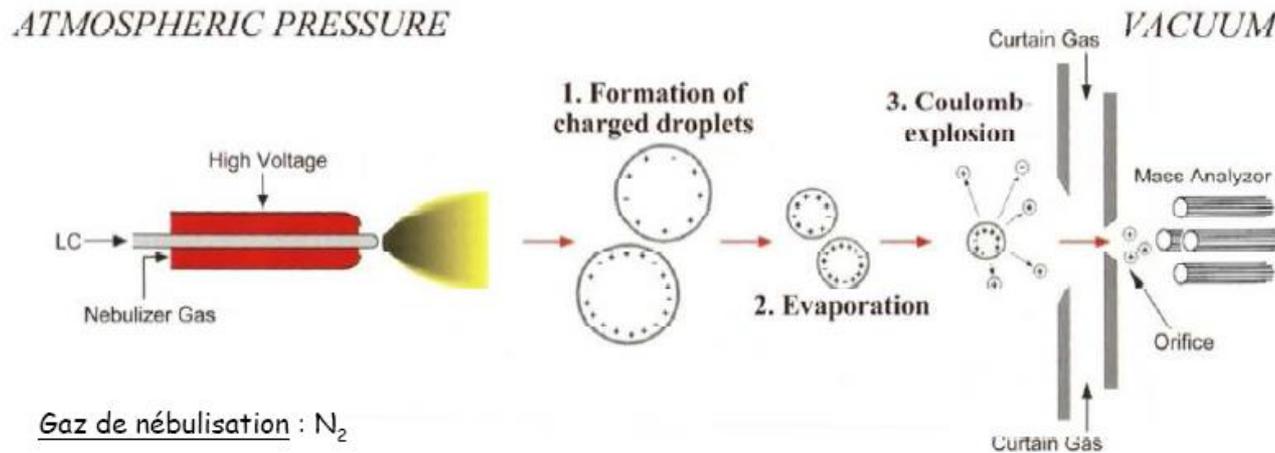


- First and most common ionisation technique
- **Extensive fragmentation:** structural information for interpreting unknown spectra
- **Not suitable for coupling to HPLC (LC-MS)** – burn out of the filament at atmospheric pressure

Mass spectrometry

2. The ion source

- ✓ **electrospray ionisation (ESI)**
 - high voltage is applied to a liquid to create an aerosol



- Especially useful in producing ions from **macromolecules** (avoids macromolecule fragmentation)
- **Source of choice for LC-MS**

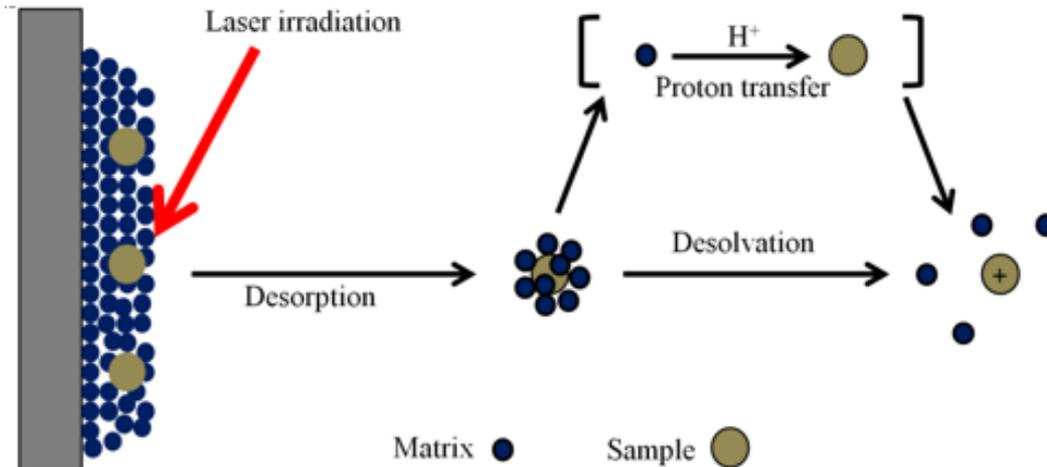
Mass spectrometry

2. The ion source

✓ Matrix-assisted laser desorption/ionisation (MALDI)

- Three-step process :

1. The sample is mixed with a **suitable matrix material**
2. Pulsed laser irradiates the sample → **ablation and desorption**
3. **Ionisation** of the analyte in the hot plume of ablated gases



- Similar in character to ESI but fewer multiply charged ions

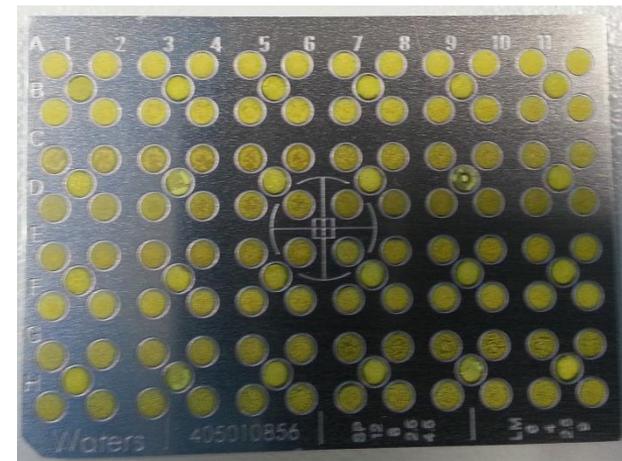
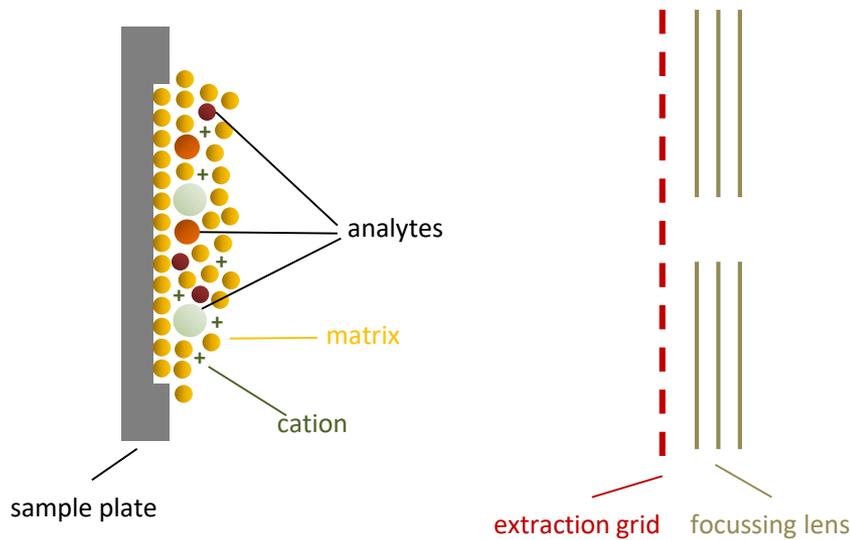
2. The ion source

REMINDER: Matrix Assisted Laser Desorption/Ionization (MALDI)

Method where a laser is used to generate ions in a relatively gentle manner

Commonly used in proteomics for high molecular weight species

Analytes are embedded into a crystal matrix that causes the molecules to ionise instead of decomposing



Mass spectrometry

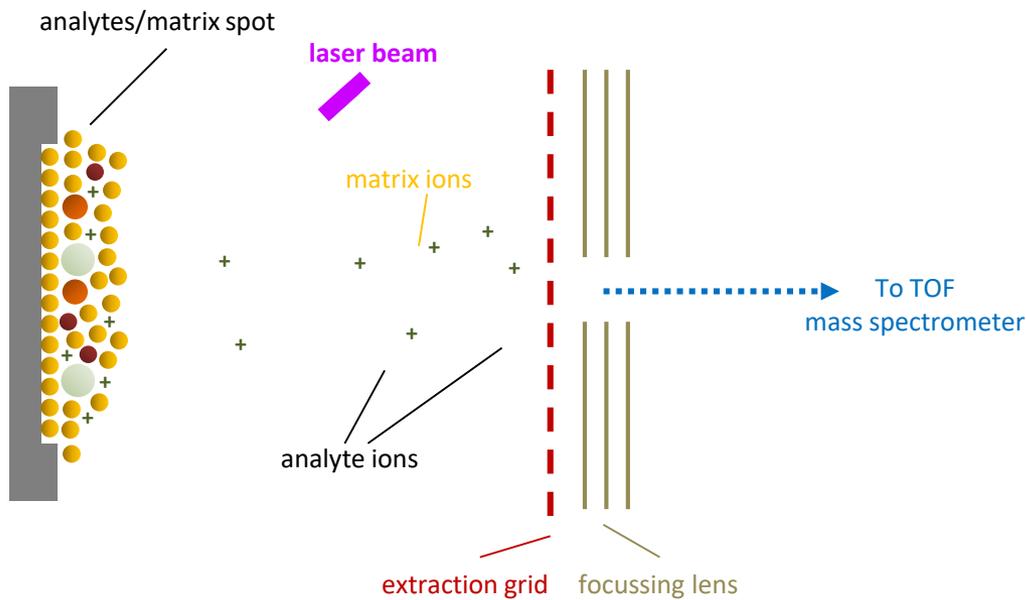
2. The ion source

REMINDER: Matrix Assisted Laser Desorption/Ionization (MALDI)

Uncertain mechanism (absorption of light by the matrix)

Transfer of this energy to the analyte which then ionises into the gas phase as a result of high energy absorbed

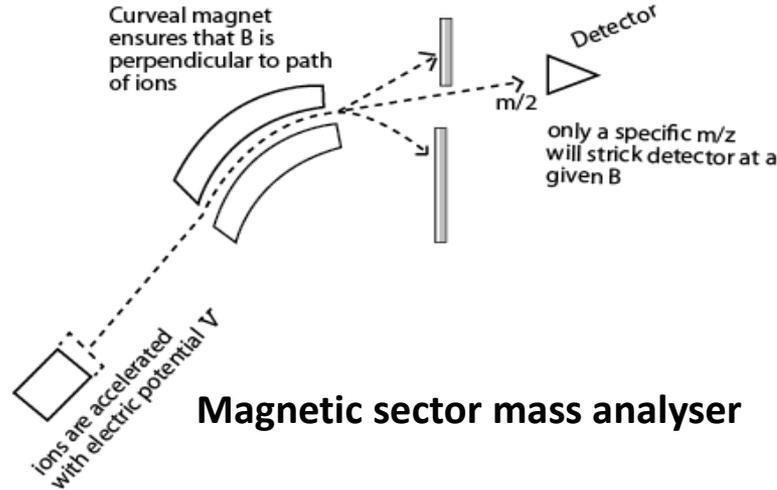
Resulting ions are subjected to a high electrical field to accelerate them into a flight-tube in the MS



Mass spectrometry

2. The mass analyser

- **Mass selection:** separates the ions according to the mass-to-charge ratio (m/z)



Electric field: acceleration

$$v = \sqrt{\frac{2qV}{m}}$$

Magnetic field: circular motion

$$R_B = \frac{v}{B} \times \frac{m}{q}$$

✓ Several mass analyser:

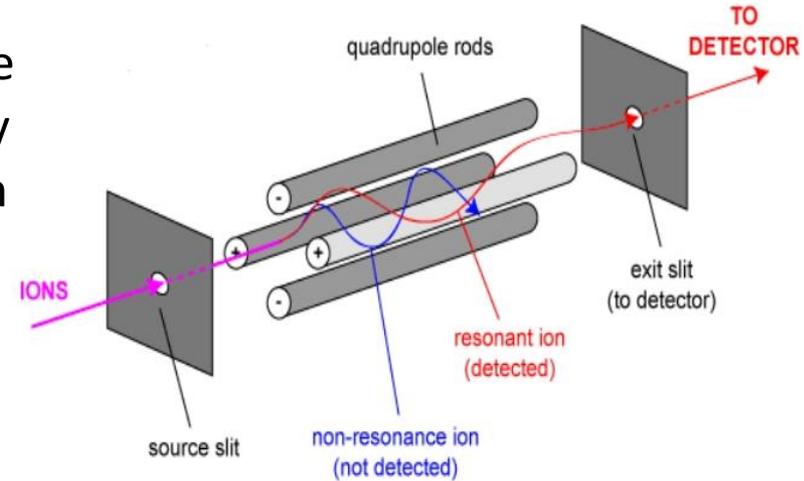
- Sector instruments, **quadrupoles**, **ion trap**, **Time-of-flight (TOF)**, **Orbitrap**

Mass spectrometry

2. The mass analyser

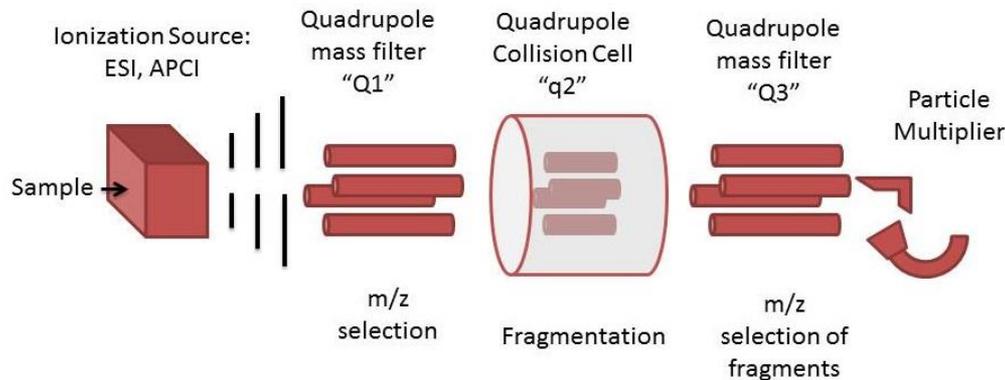
✓ Quadrupole

- Oscillating electrical fields to select the ions passing through a radio frequency (RF) quadrupole field created between 4 parallel rods



triple quadrupole (triple quad)

three consecutive quadrupole stages

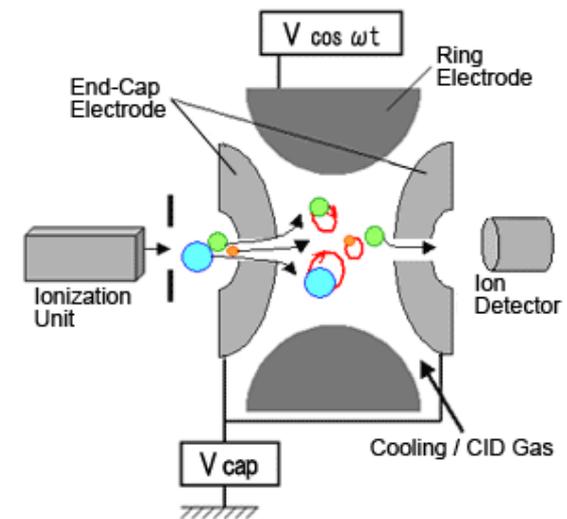
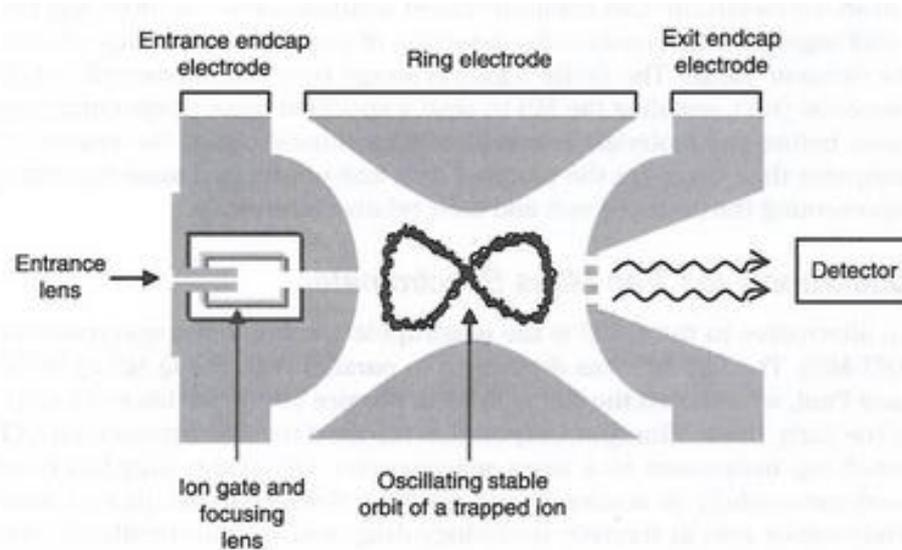


- Tandem mass spectrometry
- Quantification

2. The mass analyser

✓ Ion trap

- same physical principles as the quadrupole mass analyser, but the ions are trapped and sequentially ejected

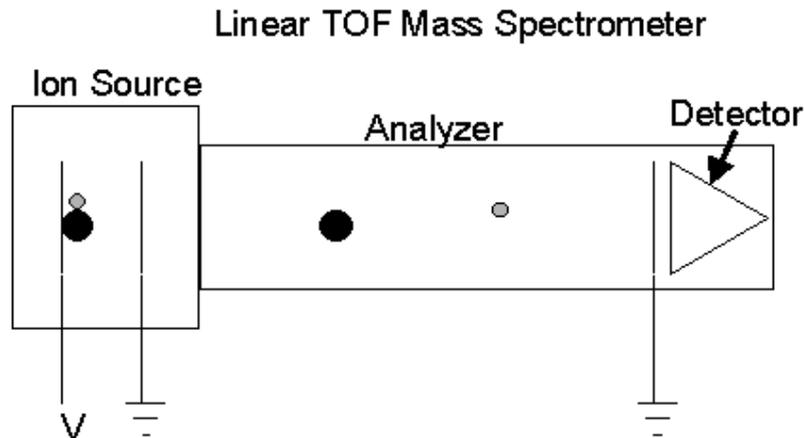


Mass spectrometry

2. The mass analyser

✓ Time-of-flight (TOF)

- Electric field to accelerate the ions through the same potential
- Measure of the time to reach the detector



the ions of lesser m/z will have greater velocity than ions of greater m/z . Therefore, as ions traverse the analyser, they separate in space.

$$\frac{1}{2} m v^2 = q U$$

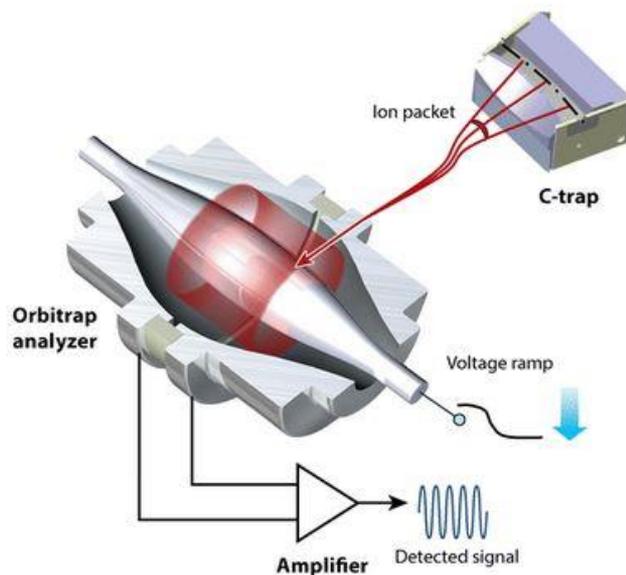
$$\frac{1}{2} m (L/t)^2 = q U$$

$$\frac{m}{q} = \frac{2 U}{L^2} t^2$$

2. The mass analyser

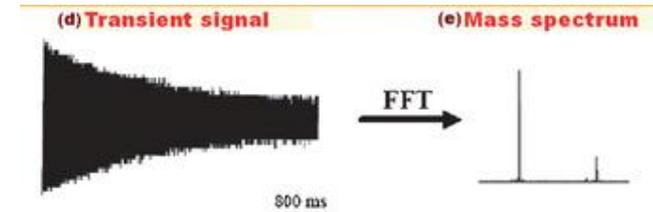
✓ Orbitrap

- Ion trapped in an orbit around a central electrode
- Orbit and oscillation induce an image current
- **Fourier transformation** → mass spectrum



Axial frequency :

$$\omega = \sqrt{\frac{k}{m/z}}$$



2. The mass analyser

General Comparison of Mass Analyzers

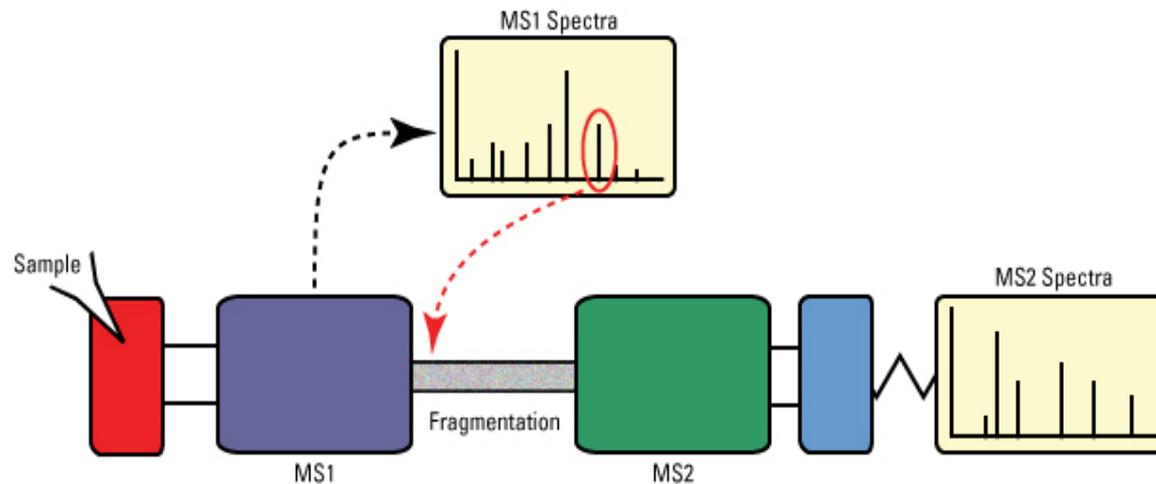
Mass analyzer	Typical mass range and resolution	Advantages	Disadvantages
Quadrupole	Range m/z 3000 Resolution 2000	Tolerant of high pressures Well-suited for electrospray Ease of switching between positive/negative ions Small size Relatively low cost	Mass range limited to about 3000 m/z Poor adaptability to MALDI
Ion trap	Range m/z 2000 Resolution 1500	Small size Medium resolution Simple design, low cost Well-suited for tandem mass spectrometry ($MS^n, n \leq 4$) Easy for positive/negative ions	Limited mass range of current commercial versions, however, progress is being made in their development
Magnetic sector	Range m/z 20,000 Resolution 10,000	Capable of high resolution Capable of exact mass Medium mass range Can be very reliable, manufacturer dependent	Not tolerant of high pressures Expensive Instrumentation is massive Relatively slow scanning
Time-of-flight (TOF)	Range m/z ∞ Resolution 350	Highest mass range Very fast scan speed Simple design, low cost Ease of adaptation to MALDI	Low resolution Difficulty of adaptation to electrospray
Time-of-flight reflectron	Range m/z ∞ Resolution 1500	Good resolution Very fast scan speed Simple design, low cost	Good resolving power has limited m/z range Lower sensitivity than TOF
Fourier transform-mass spectrometry (FT-MS)	Range m/z 10,000 Resolution 30,000	High resolution Well-suited for tandem mass spectrometry ($MS^n, n \leq 4$)	High vacuum ($<10^{-7}$ Torr) required Superconducting magnet required, expensive Instrumentation massive

G. Siuzdak. "Mass Spectrometry for Biotechnology." Academic Press, 1996. QP519.9.M3 S58 1996 at CU Libraries.

Mass spectrometry

2. Tandem mass spectrometry (MS/MS)

- ✓ Additional information about specific ions
- ✓ Selected ions are fragmented by a number of methods of dissociation
- ✓ Fragments are separated based on their individual m/z ratios in a second round of MS



Take home message

- **Mass spectrometry measures m/z ratios**
- **It allows for quantification and identification of compounds**
- **Fragmentation is orchestrated**
- **The technique measures the abundance of the fragments**
- **Analysis may be refined to a single fragment**

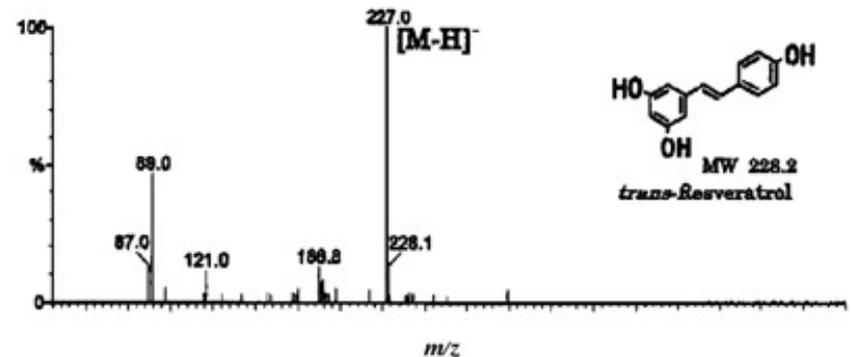


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Outlines

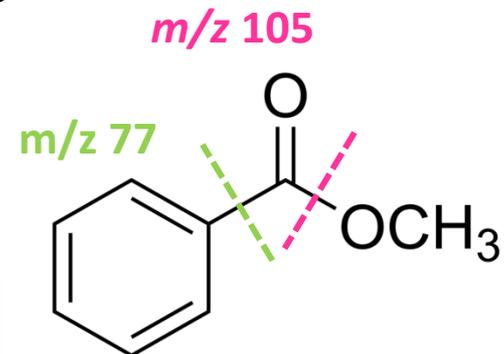
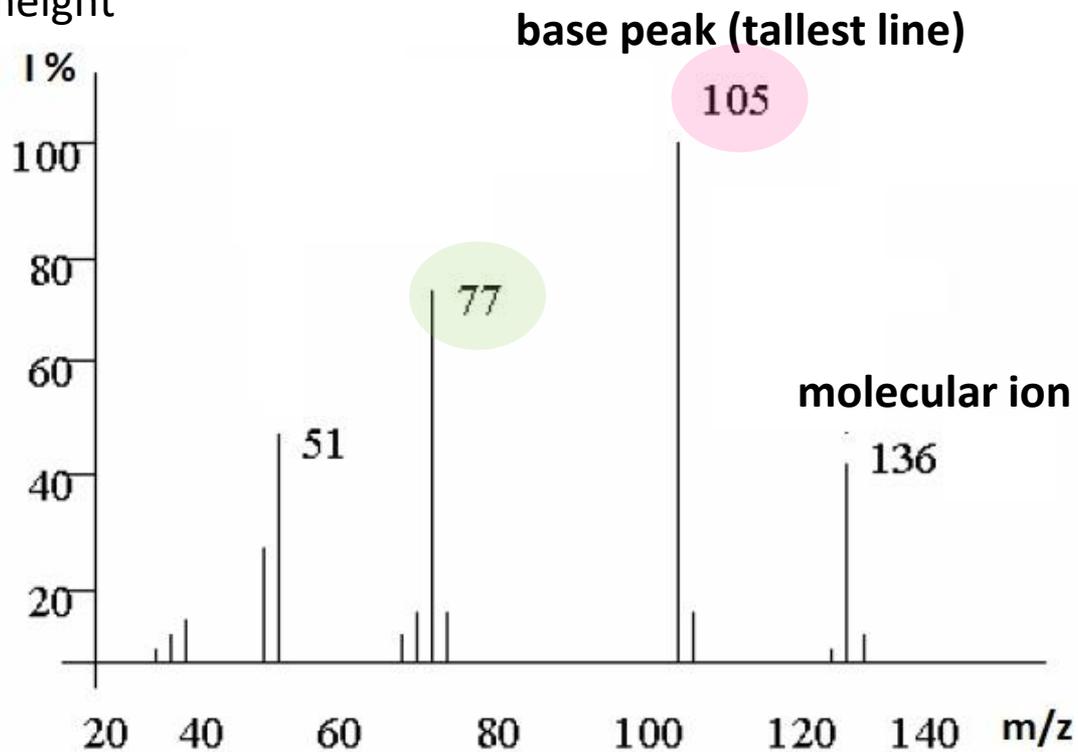
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3. Mass spectrum

✓ fragmentation patterns of organic molecules

arbitrary height
of 100%

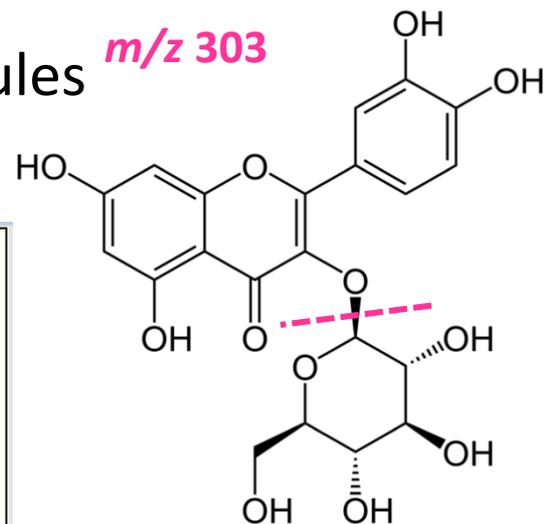
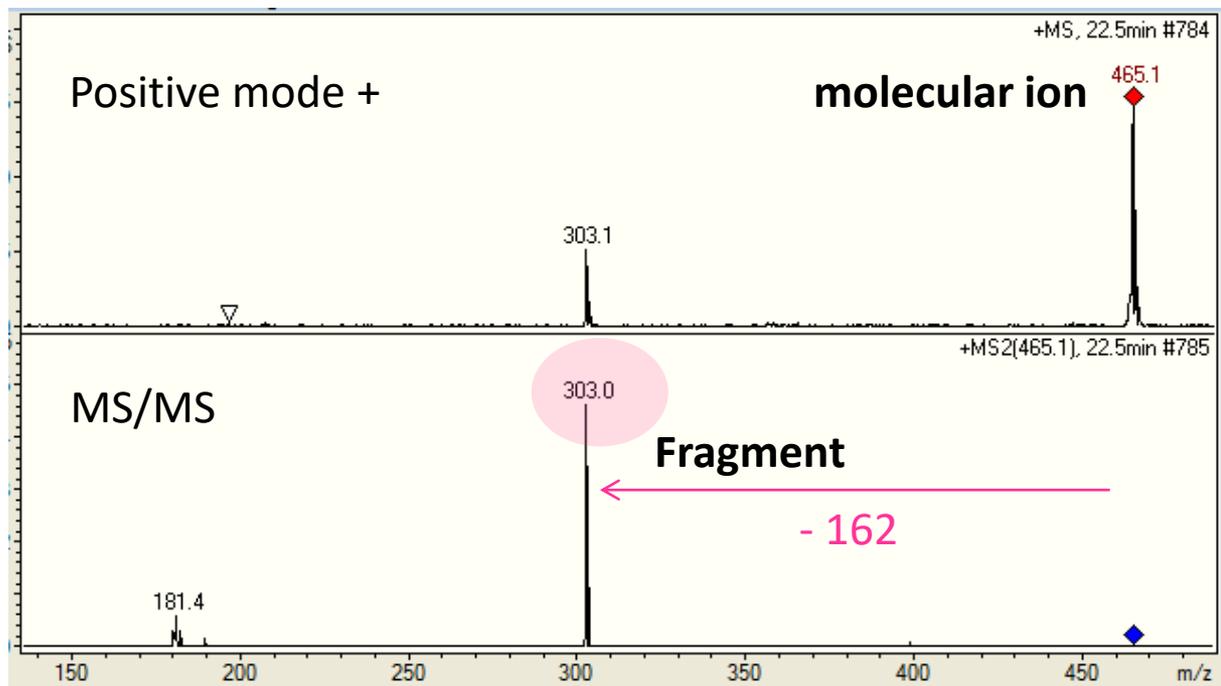


Methyl benzoate
MW = 136

Fragmentation
 $M^+ \rightarrow X^+ + Y$

3. Mass spectrum

✓ fragmentation patterns of organic molecules



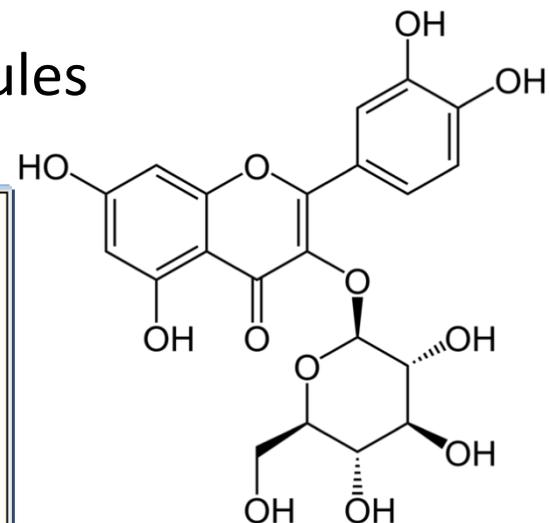
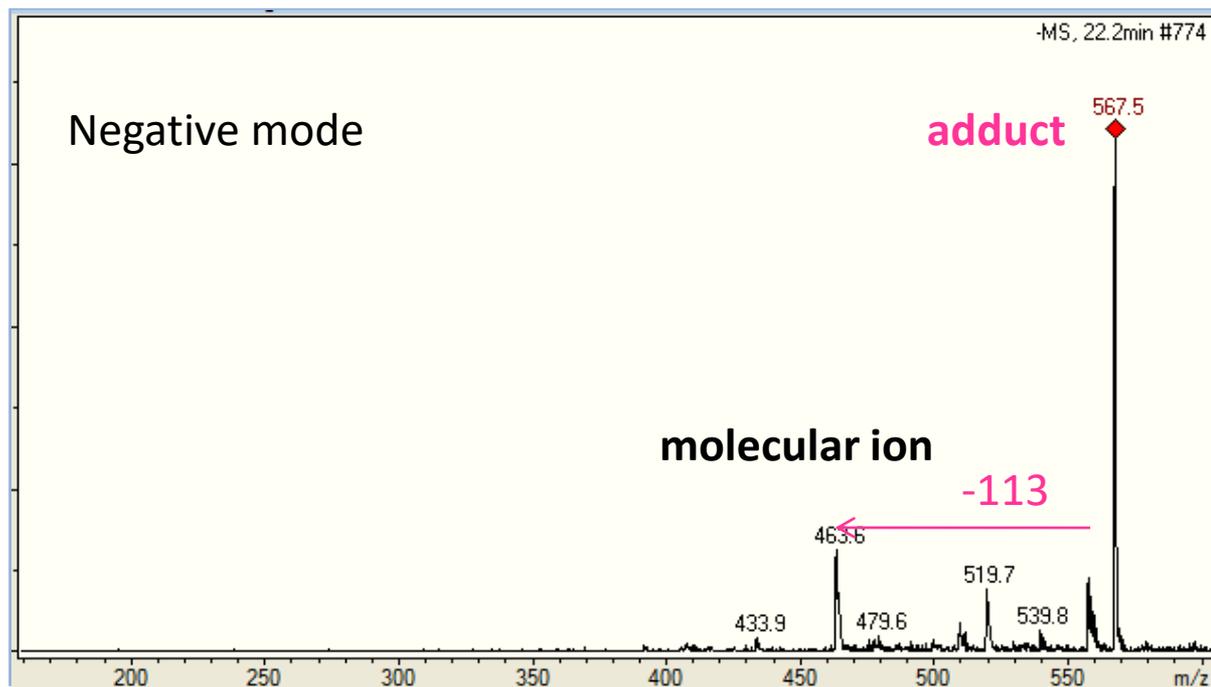
- Formula: $C_{21}H_{20}O_{12}$
- Molecular Weight 464

Quercetin hexoside

Position and 3D structure unknown !!!!

3. Mass spectrum

✓ fragmentation patterns of organic molecules



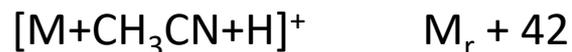
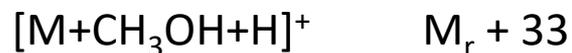
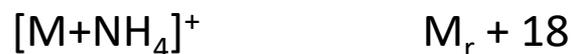
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Quercetin hexoside

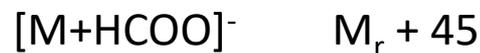
3. Mass spectrum

✓ Adduct Ions

Cationized Adducts



Anionized Adducts



3. Using a mass spectrum to find a molecular formula

✓ High resolution mass spectrometry

- Accurate isotopic masses (**4 decimal places**)

^1H	1.0078
^{12}C	12.0000
^{14}N	14.0031
^{16}O	15.9949

✓ Example:

m/z of propane (C_3H_8) and ethanal (CH_3CHO) = 44

C_3H_8	44.0624
CH_3CHO	44.0261

high resolution mass easily distinguishes propane and ethanal

Mass spectrometry

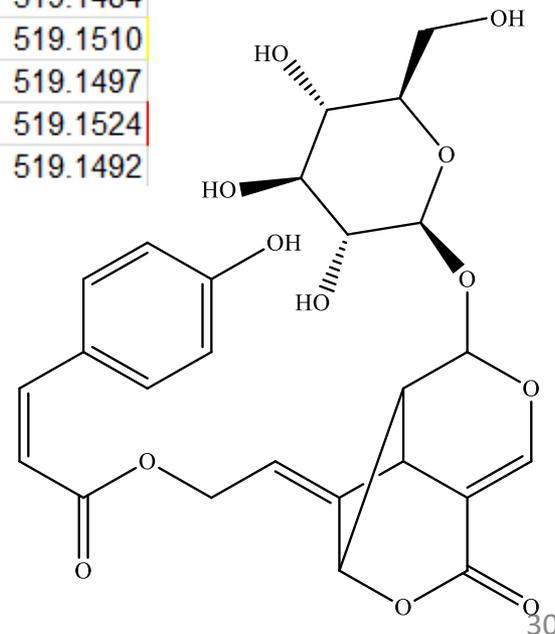
3. Using a mass spectrum to find a molecular formula

✓ High resolution mass spectrometry

- Accurate isotopic masses (4 decimal places)

	Meas. m/z	#	Formula	Score	m/z
[M+H] ⁺	519.1503	1	C ₂₅ H ₂₇ O ₁₂	100	519.1497
[M+H] ⁺	519.1503	2	C ₂₂ H ₁₉ N ₁₀ O ₆	41.71	519.1484
[M+H] ⁺	519.1503	3	C ₂₆ H ₂₃ N ₄ O ₈	73.43	519.1510
[M+H] ⁺	519.1503	4	C ₂₃ H ₁₅ N ₁₄ O ₂	69.33	519.1497
[M+H] ⁺	519.1503	5	C ₂₇ H ₁₉ N ₈ O ₄	22.5	519.1524
[M+H] ⁺	519.1503	6	C ₃₈ H ₁₉ N ₂ O	8.38	519.1492

Formula: C₂₅H₂₆O₁₂
 Exact Mass: 518.1424



Take home message

- **Knowing the spectrum of a compound can help determining its formula**
- **3D structures remain debatable**
- **Adducts can be formed**
- **Accurate mass determination can help finding the right formula**

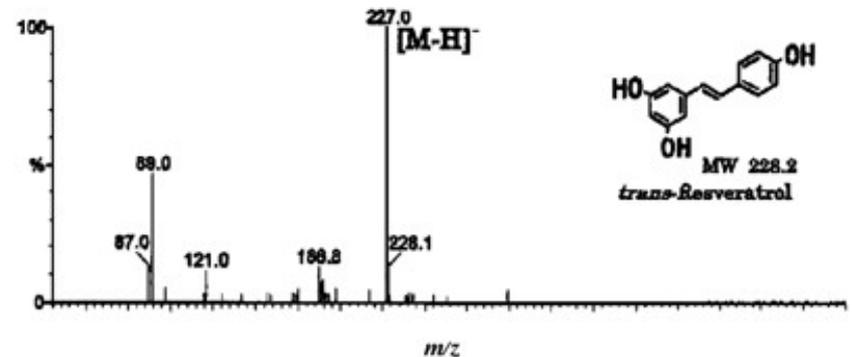


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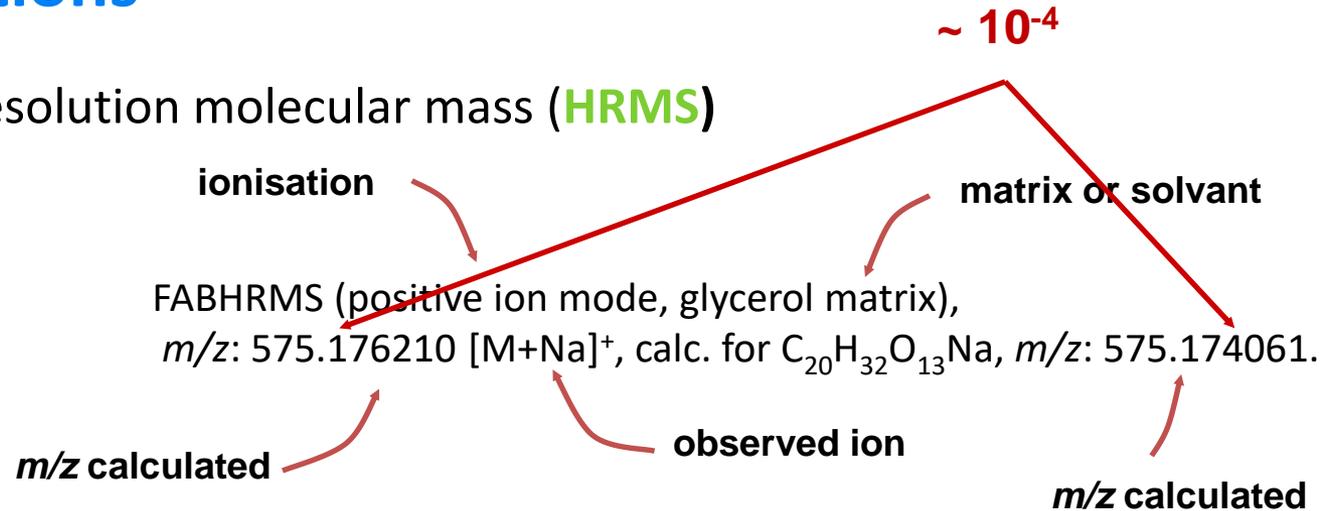
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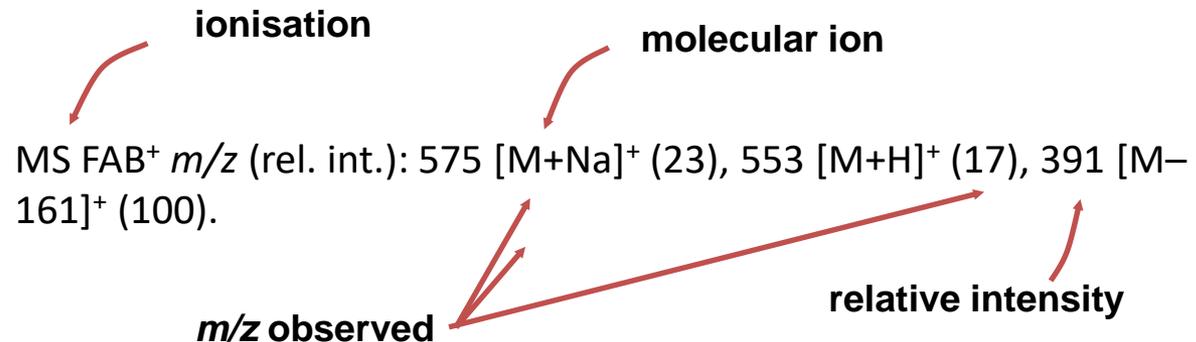
Mass spectrometry

4. MS publications

- High Resolution molecular mass (**HRMS**)



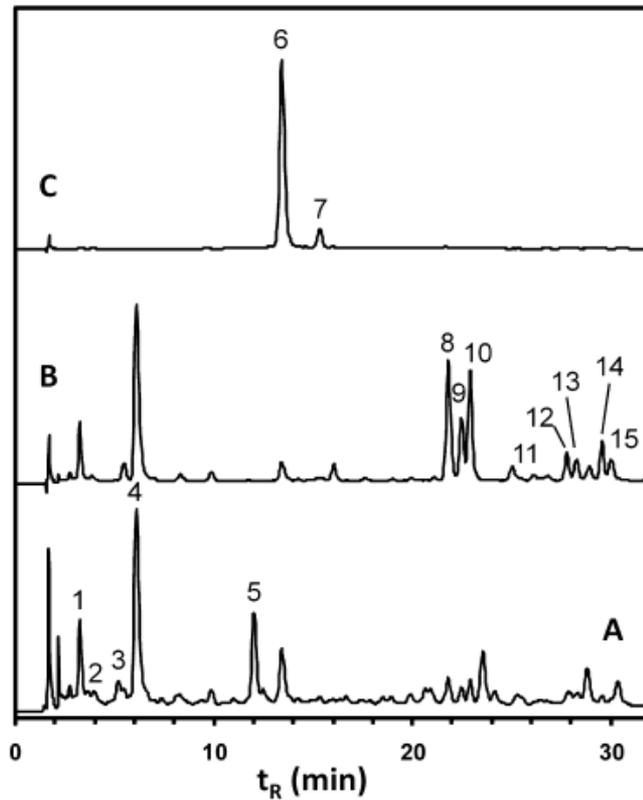
- Low resolution molecular mass



Mass spectrometry

4. LC-MS analysis

✓ HPLC analysis of mixtures



Anthocyanins λ_{max} 520 nm

Flavonols λ_{max} 360 nm

Other phenols λ_{max} 280 nm

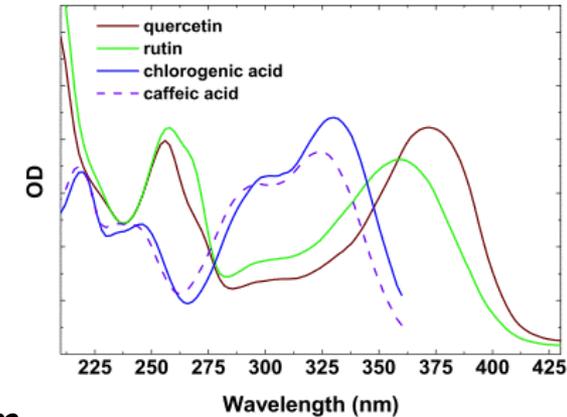
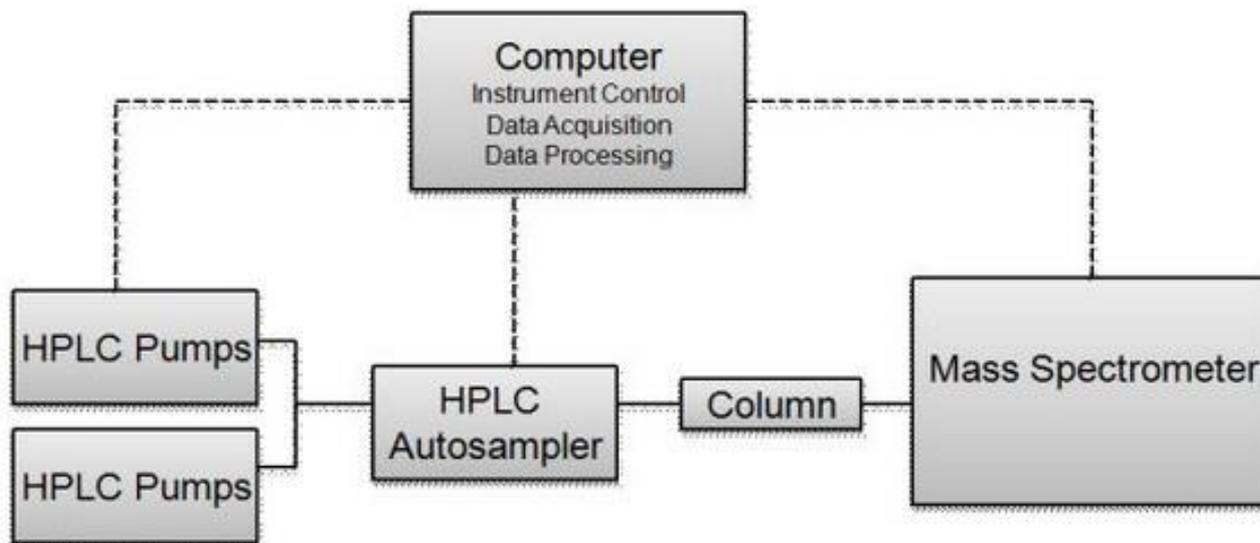


Fig. 1. HPLC chromatograms of Spring Belle extract recorded at 280 nm (A), 360 nm (B) and 520 nm (C).

Mass spectrometry

4. LC-MS analysis

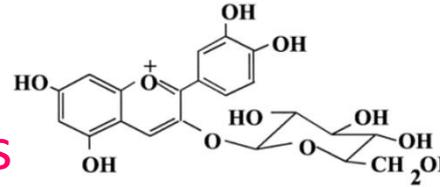
- ✓ Schematic diagram of a liquid chromatography-mass spectrometry system



Mass spectrometry

4. LC-MS analysis

✓ LC-MS analysis of mixtures



delphinidin	302
cyanidin	286
petunidin	316
peonidin	300
malvidin	330

Cyanidin 3-O-β-D-glucoside

Anthocyanins λ_{max} 520 nm

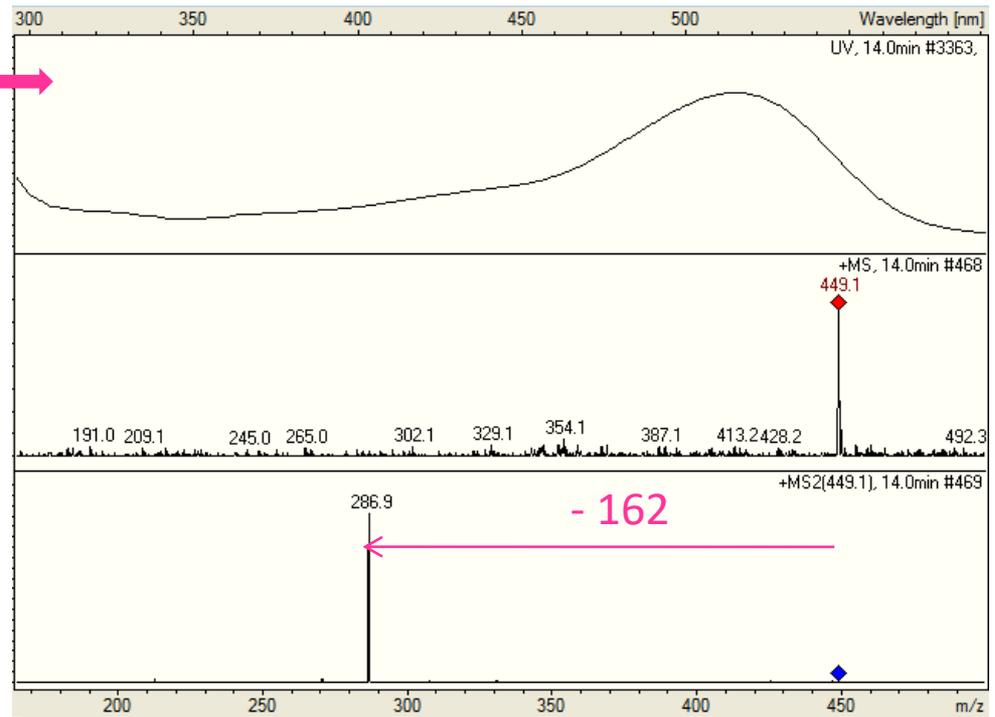
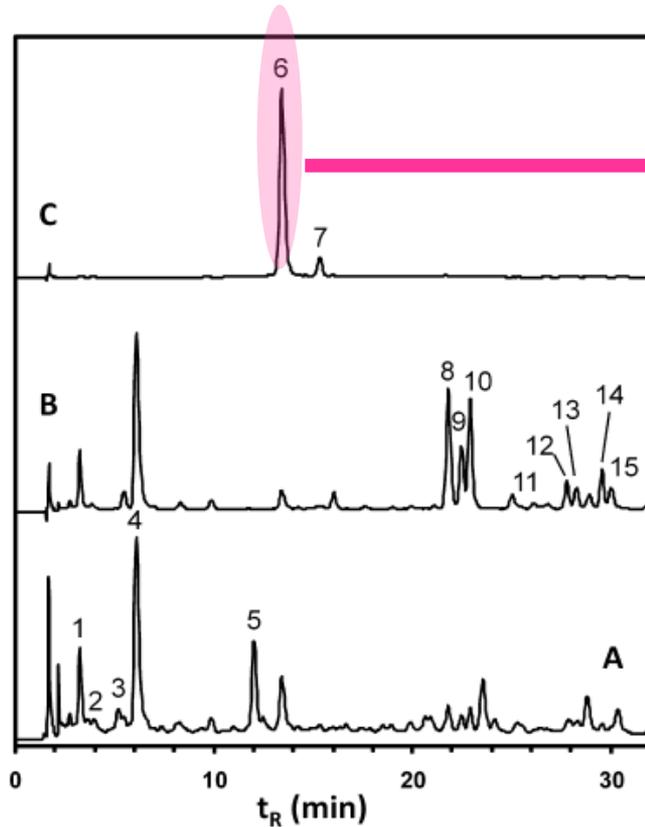


Fig. 1. HPLC chromatograms of Spring Belle extract recorded at 280 nm (A), 360 nm (B) and 520 nm (C).

Take home message

- **Spectrum analysis allows the identification of new molecules**
- **The more accurate is the mass determination, the more accurate is the identification**
- **The 3D structure cannot be solved by MS!**

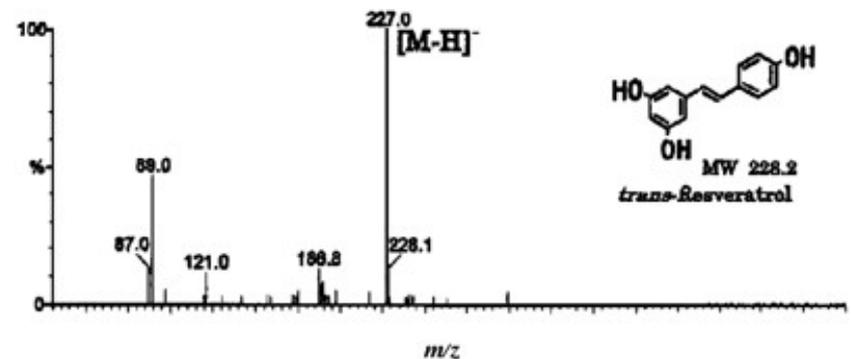


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5. MS imaging

- ✓ What is MS Imaging and why might we use it?
- ✓ Instrumentation
- ✓ Sample Preparation
- ✓ Data Analysis
- ✓ Applications

5. MS imaging

✓ Definition

Mass spectrometry imaging (MSI) is a technique used to visualize the spatial distribution of chemical compositions
e.g. compounds, biomarkers, metabolites, peptides or proteins by their molecular masses.

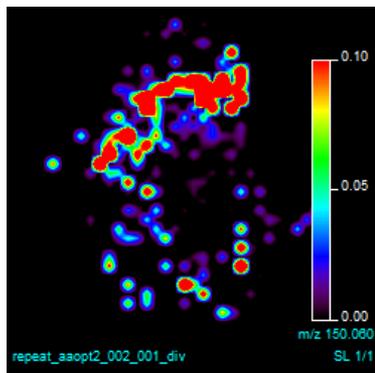
It is an emerging field in science allowing true label-free molecular imaging of flat samples, *e.g.* biological tissue sections.

<https://ms-imaging.org/wp/>

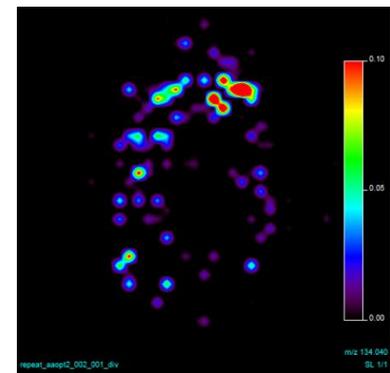
5. MS imaging

✓ Why imaging?

- To study metabolites *in situ*
- Negates the need for complex sample preparation
- Sample preparation could alter metabolism
- Biological samples are not always homogenous
- Tissues are not made up of identical cells



Methionine

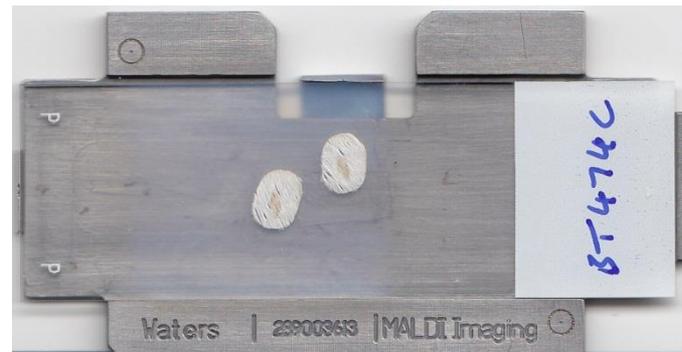


Aspartate

5. MS imaging

✓ Why biology?

- Imaging has a wide scope of application among most types of biological molecules, including metabolites, lipids, low-MW carbohydrates, peptides, and proteins, as well as drugs and other xenobiotics
- High spatial resolution of ($\sim 30 \mu\text{m}$)
- Sample preparation is relatively straightforward
- Modern instruments are exceedingly fast
- No target specific reagents are needed, such as antibodies.
- Reagent costs for the analysis are exceedingly low



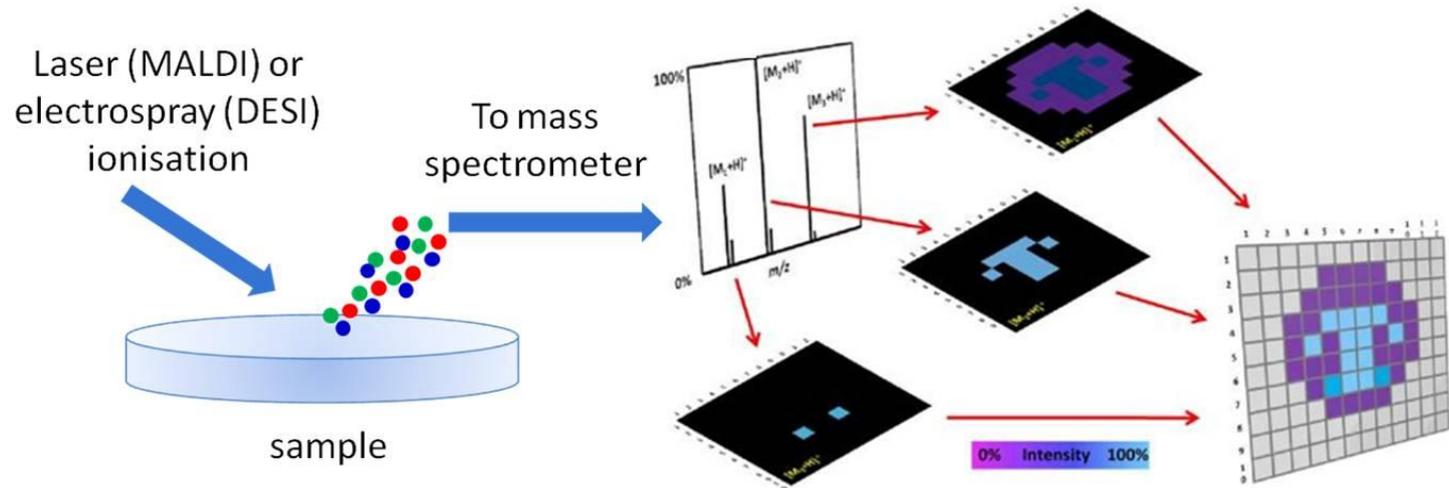
5. MS imaging

✓ Instrumentation

- MALDI MSI
 - Matrix-assisted laser desorption/ionisation mass spectrometric imaging
- DESI
 - Desorption electrospray ionisation mass spectrometric imaging
- SIMS
 - Secondary ion mass spectrometric imaging
- LA ICP MSI
 - Laser ablation inductively coupled plasma mass spectrometric imaging

5. MS imaging

✓ Workflow

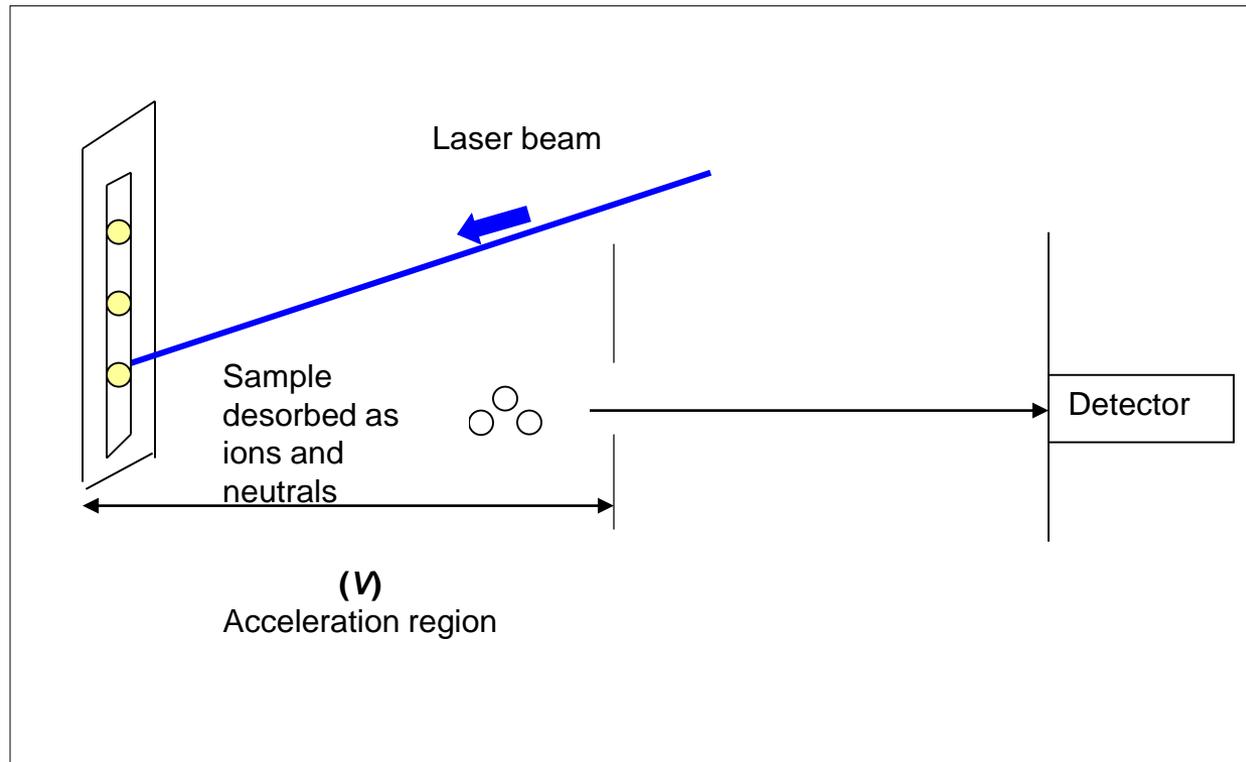


Schematic courtesy of Waters UK LTD

<http://blog.waters.com/do-you-see-what-we-see-mass-spectrometry-imaging-is-revealing-insights-in-biomedical-research>

5. MS imaging

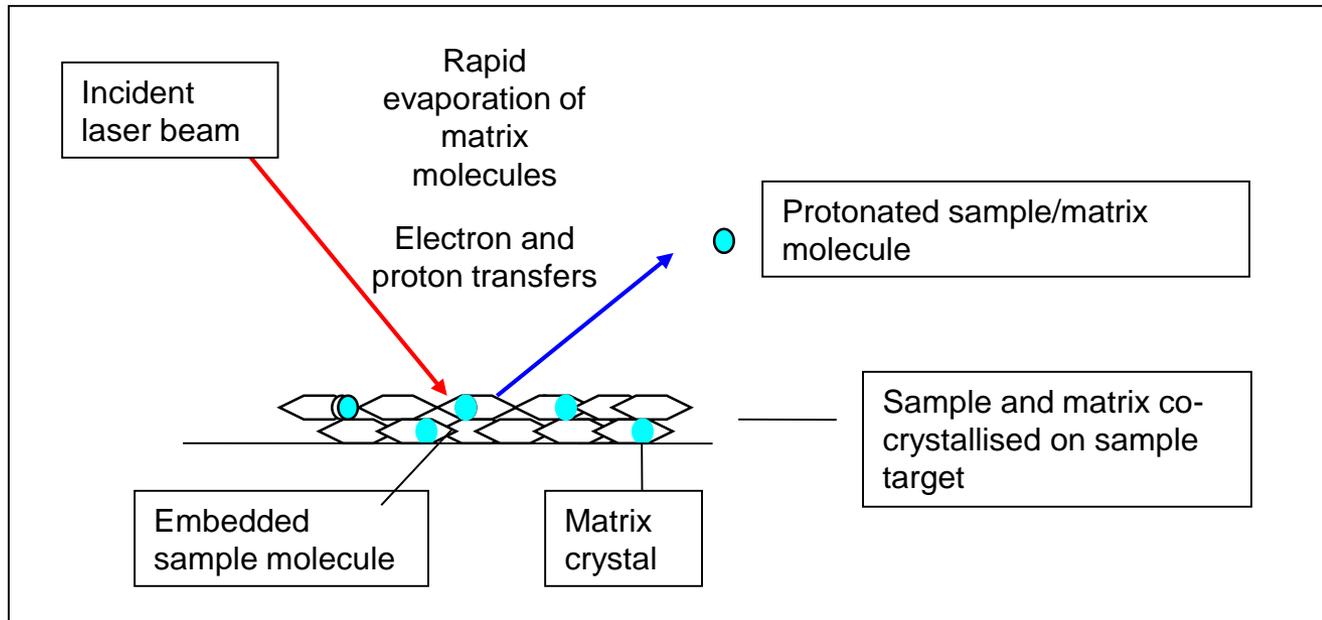
✓ Schematic Overview of MALDI-TOF-MS



5. MS imaging

✓ MALDI

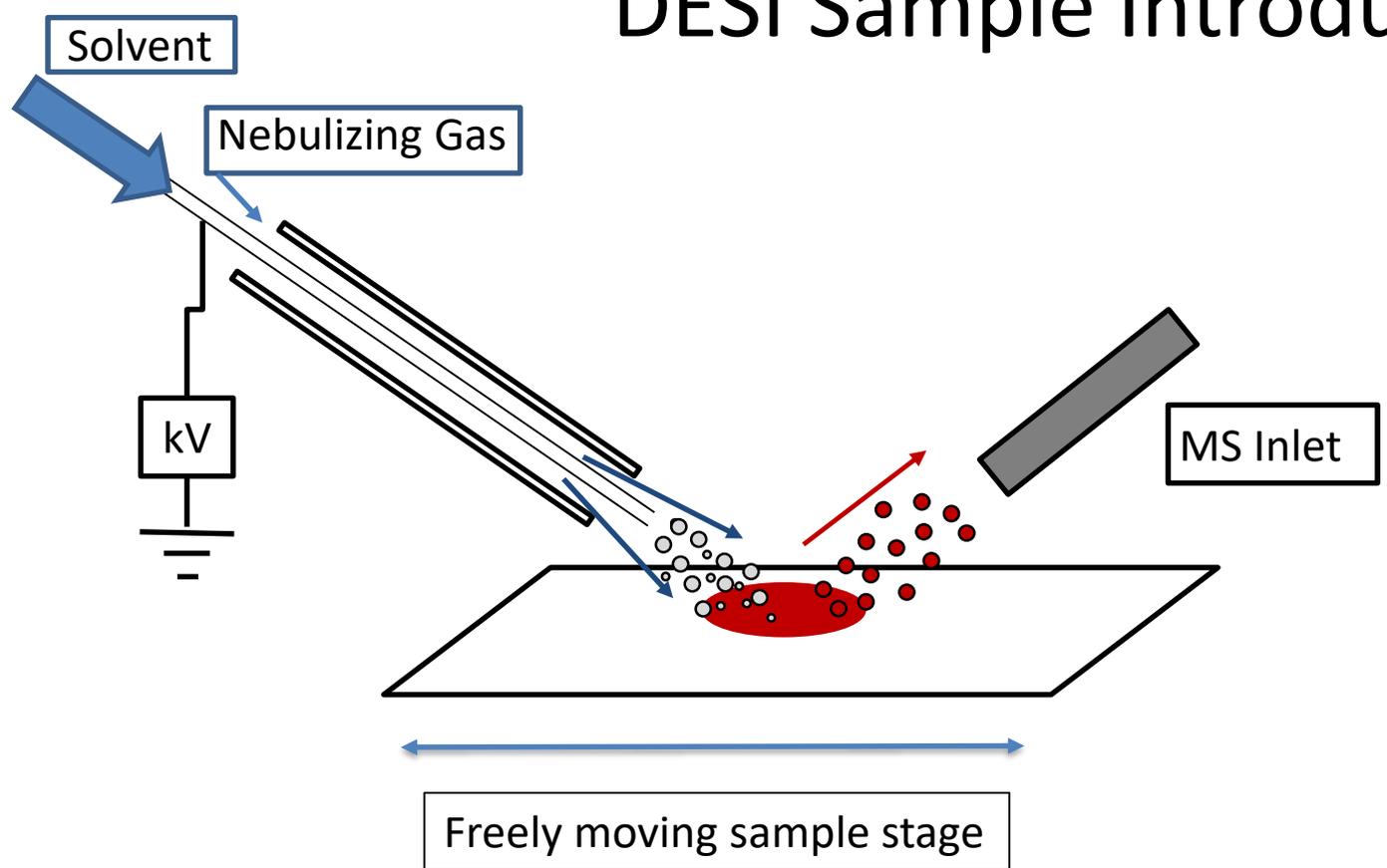
Energy absorption, ionisation and formation of molecular ions.



5. MS imaging

✓ DESI

DESI Sample Introduction



5. MS imaging

✓ Optimisation

MALDI

- Matrix concentration
- Laser Power
- Voltage on sample plate/ MS inlet

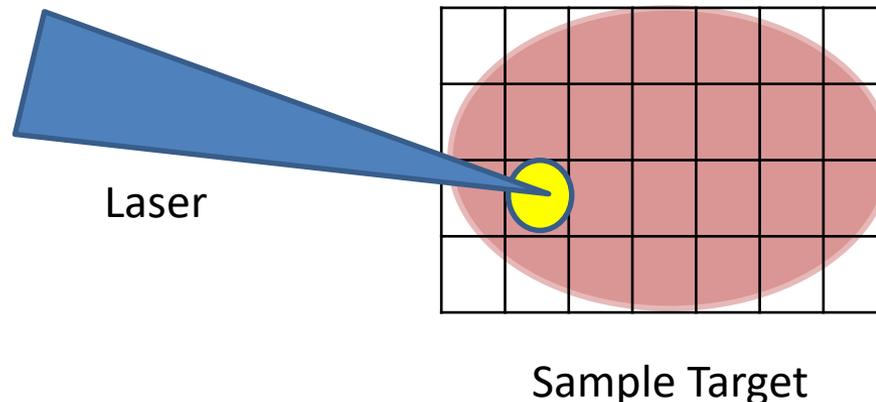
DESI

- Voltage on capillary tube
- Solvent composition and flow
- Angle of inlet tube/solvent spray

5. MS imaging

✓ Resolution of Imaging

- Resolution refers to the size of the “pixel” used to determine spatial distribution.
- Resolution is reliant but not wholly dependant upon spot size of laser (MALDI) or desorbing liquid (DESI)
- Lowest spatial resolution achieved by commercial instruments is around the 30–50 μm range.



5. MS imaging

✓ MALDI/DESI advantages

MALDI

- Characterised technique
- Use of matrix allows matrix peaks to be used as markers to easily line up section/normalise data

DESI

- Sample not destroyed by technique
- No sample preparation needed
- Allows desorption using different solvents on same sample therefore able to sequentially extract ions
- Atmospheric pressure technique

5. MS imaging

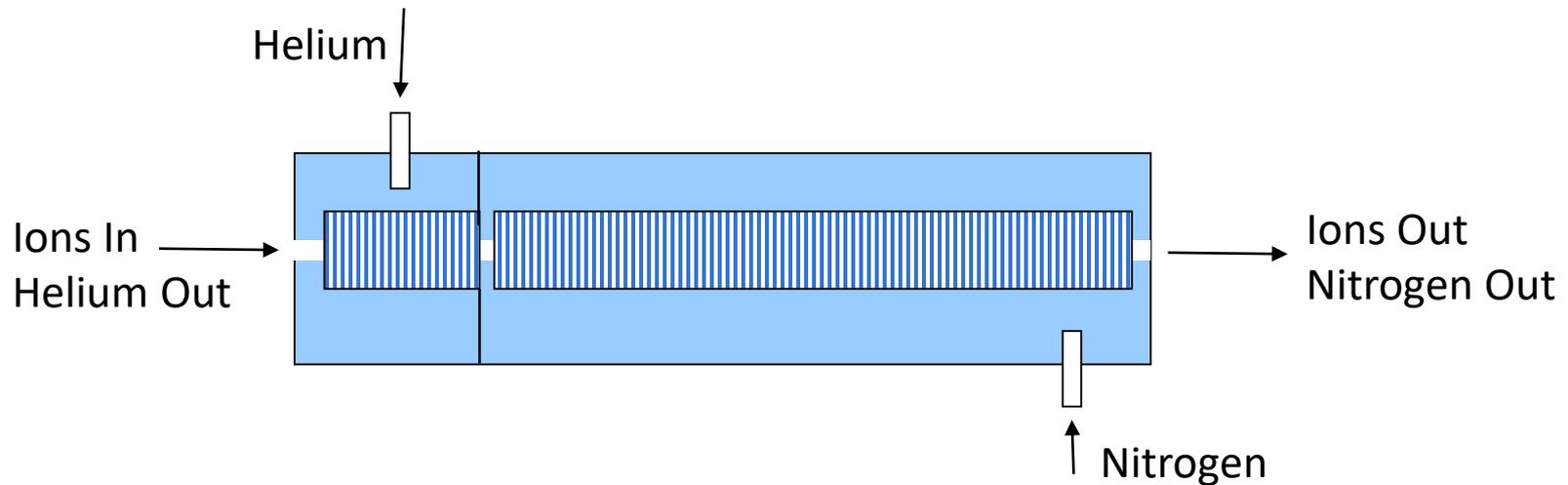
✓ What is ion mobility?

- Separation of ionic species as they drift through a gas under the influence of an electric field
- Rate of drift is dependant on ion mobility through the gas
- Mobility is dependant upon
 - Mass
 - Charge
 - Shape (cross-sectional)
- Ability to separate isobaric species if they have different interaction cross sections

5. MS imaging

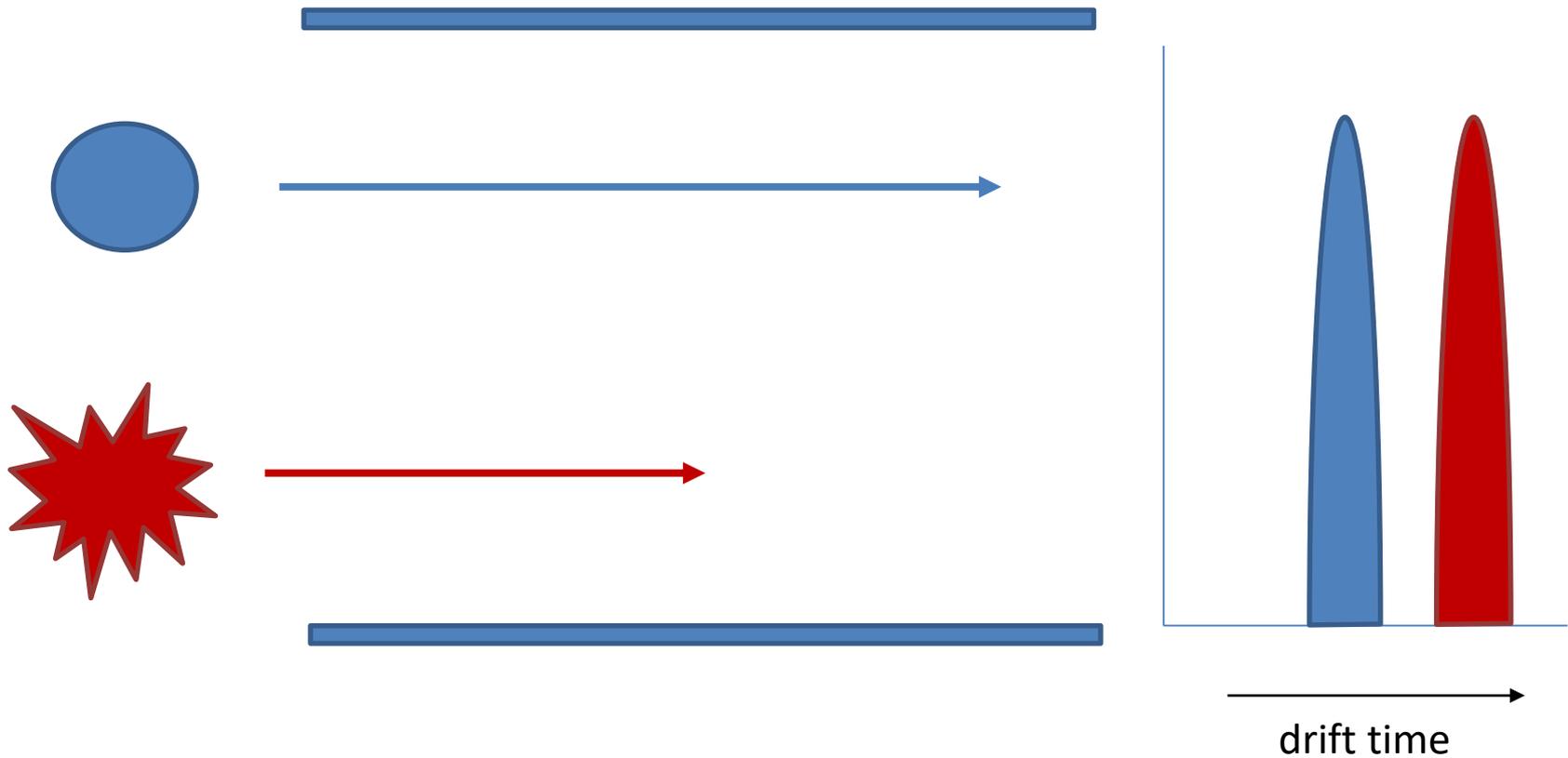
✓ What is ion mobility?

- Chamber on the front of the IMS cell filled with Helium
 - Same pressure as Nitrogen drift gas
 - Reduces fragmentation (lower centre of mass)
 - Maintains transmission at higher pressures



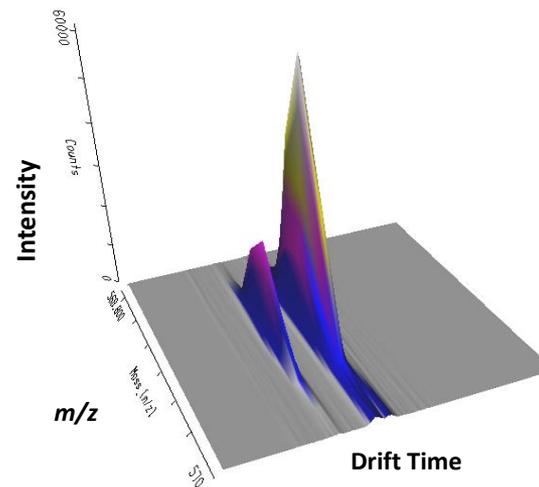
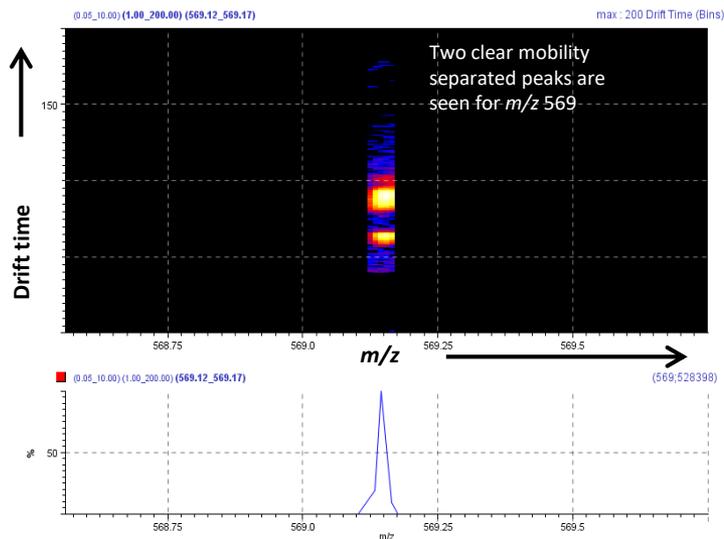
5. MS imaging

✓ Ion mobility separation



5. MS imaging

✓ Ion mobility separation



5. MS imaging

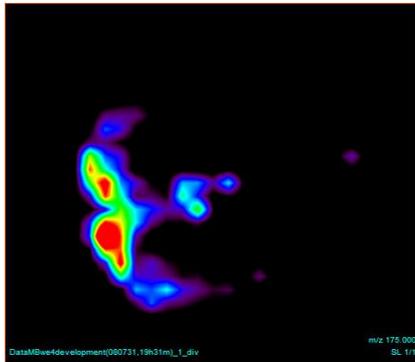
✓ Applications: MALDI-MSI Wheat Grain

- Type of protein and its content in wheat grain is an important quality trait and dictates the end use of the crop
- The phloem in wheat leaves contains all of the amino acids required for protein synthesis
- Amino acids are used not only for protein synthesis but in the developing wheat grain protein is the sink for most of the nitrogen

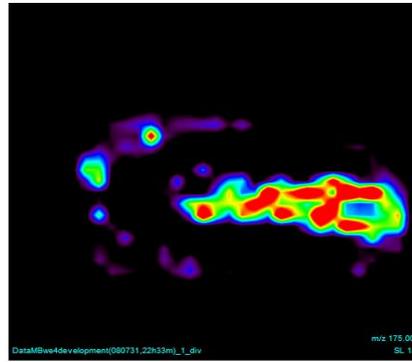
5. MS imaging

✓ Applications: MALDI-MSI Wheat Grain

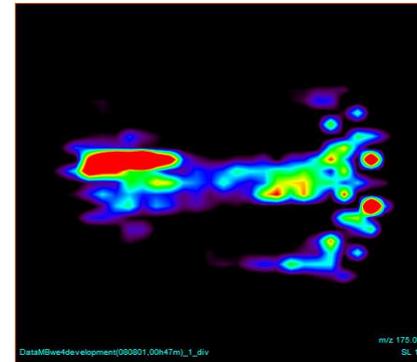
Example –Wheat Seed Distribution of Arginine



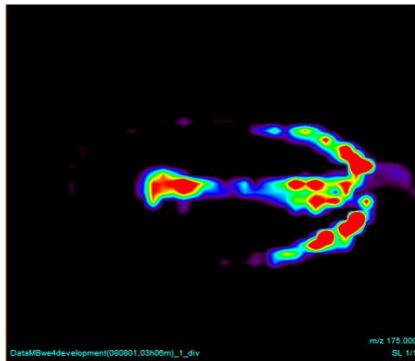
7dpa



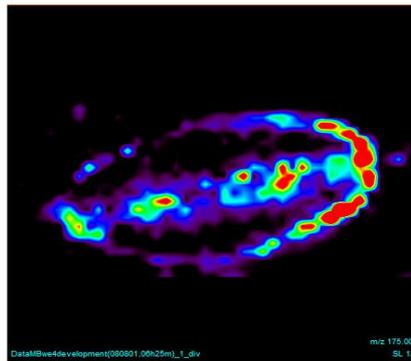
9dpa



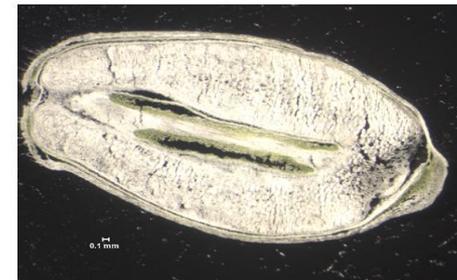
11dpa



13dpa



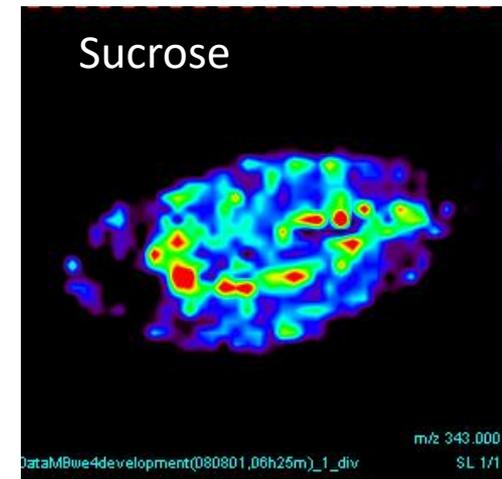
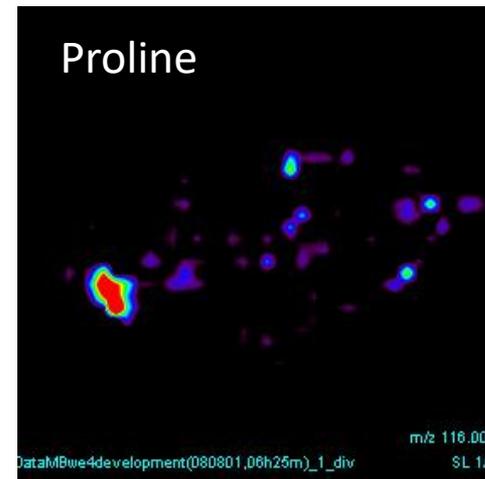
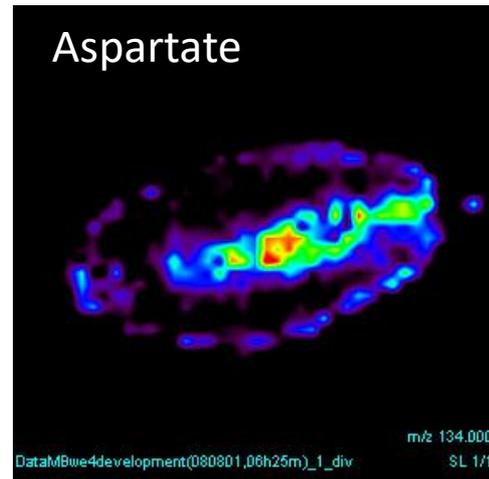
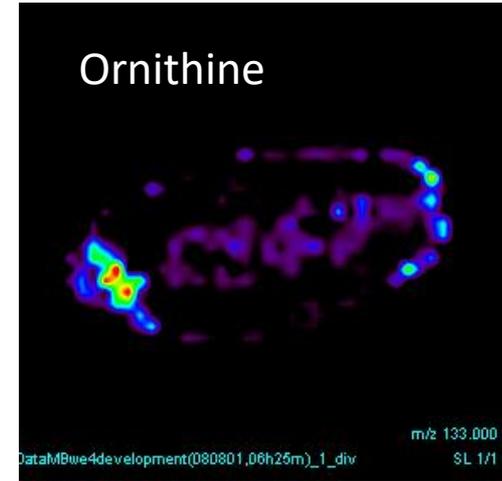
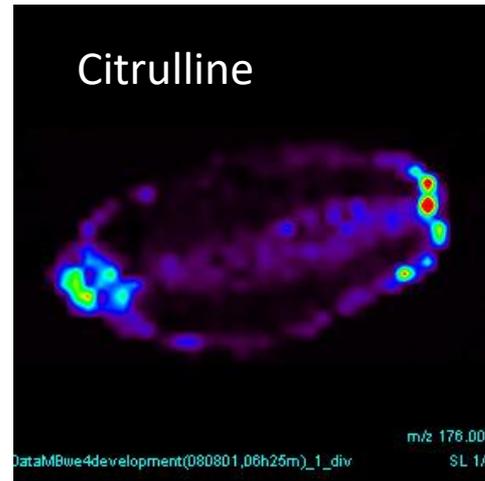
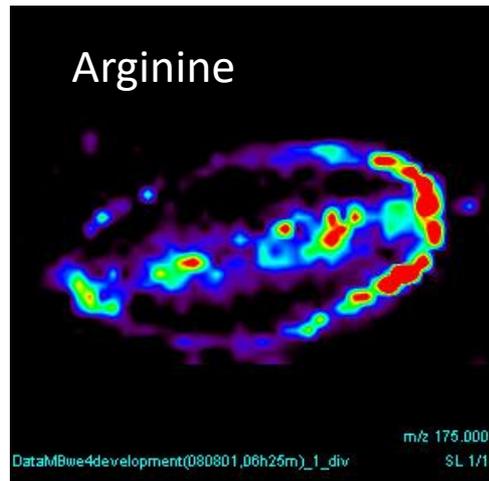
15dpa



Section at 15dpa

5. MS imaging

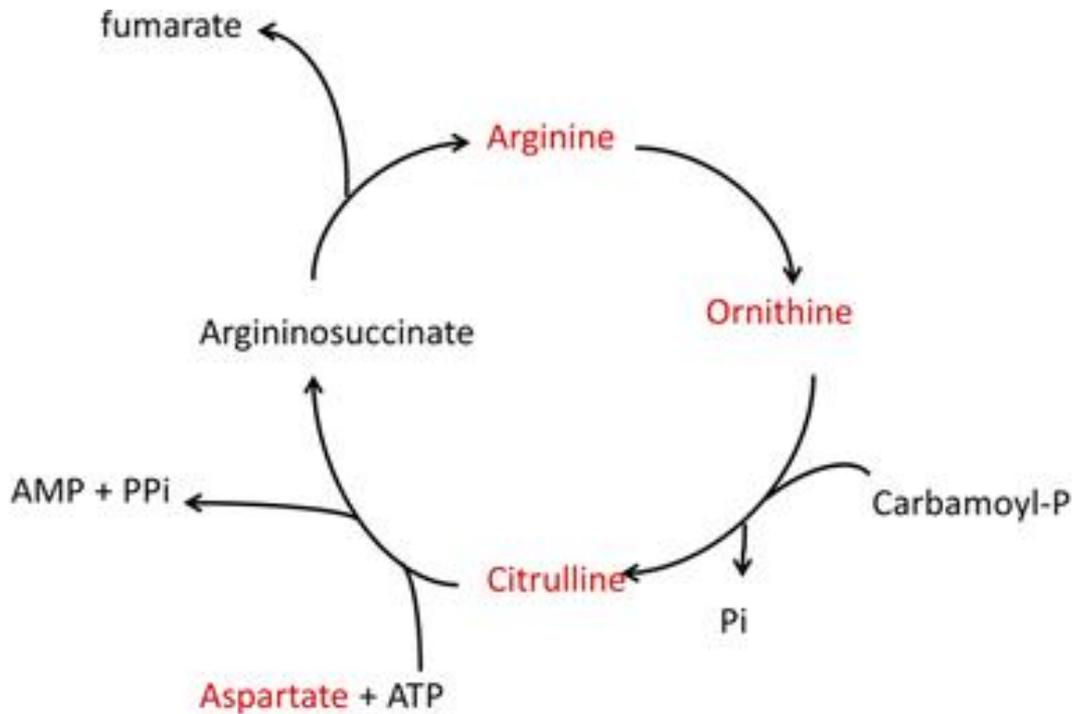
✓ Applications: MALDI-MSI Wheat Grain



Mass spectrometry

5. MS imaging

✓ Applications: MALDI-MSI Wheat Grain



5. MS imaging

✓ Applications: Fingerprint Analysis by MALDI-MSI

Identification of suspects by fingerprint analysis is one of the mainstays of forensic science.

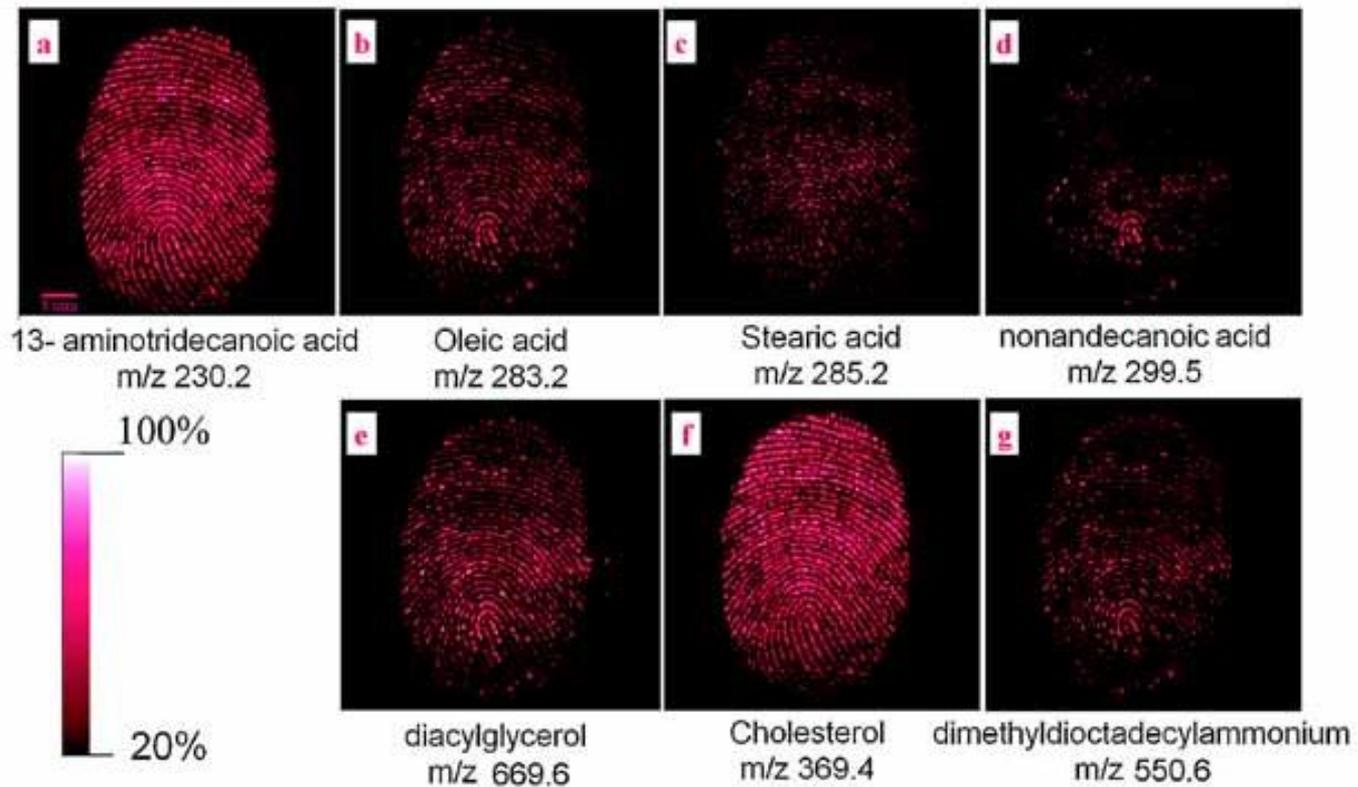
The success in matching fingerprints, strongly relies on the components present in the fingerprints and the method adopted for fingerprint recovery.

MALDI-MSI has been used to image endogenous lipids from fresh and aged, groomed and un-groomed samples.

Mass spectrometry

5. MS imaging

✓ Applications: Fingerprint Analysis by MALDI-MSI



Take home message

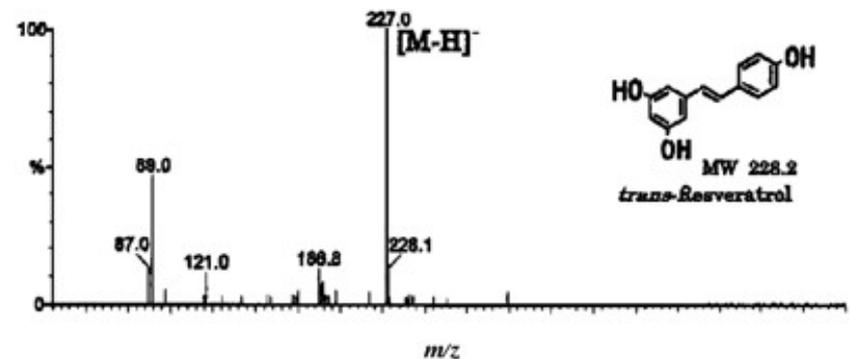
- **MS imaging is a challenging technique which is rapidly progressing within the field of medical sciences and biology in general**
- **Use of ion mobility technique is very promising**
- **The race for lower resolution (pixel) but higher resolution (mass accuracy) is key to develop this methodology even further... and engage with more life scientists**



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Outlines

- 1. Introduction
- 2. Mass spectrometer
 - Ion source
 - Mass analyser
 - Tandem MS (MS/MS)
- 3. Mass spectrum
- 4. MS analysis
- 5. MS imaging
- 6. Conclusion



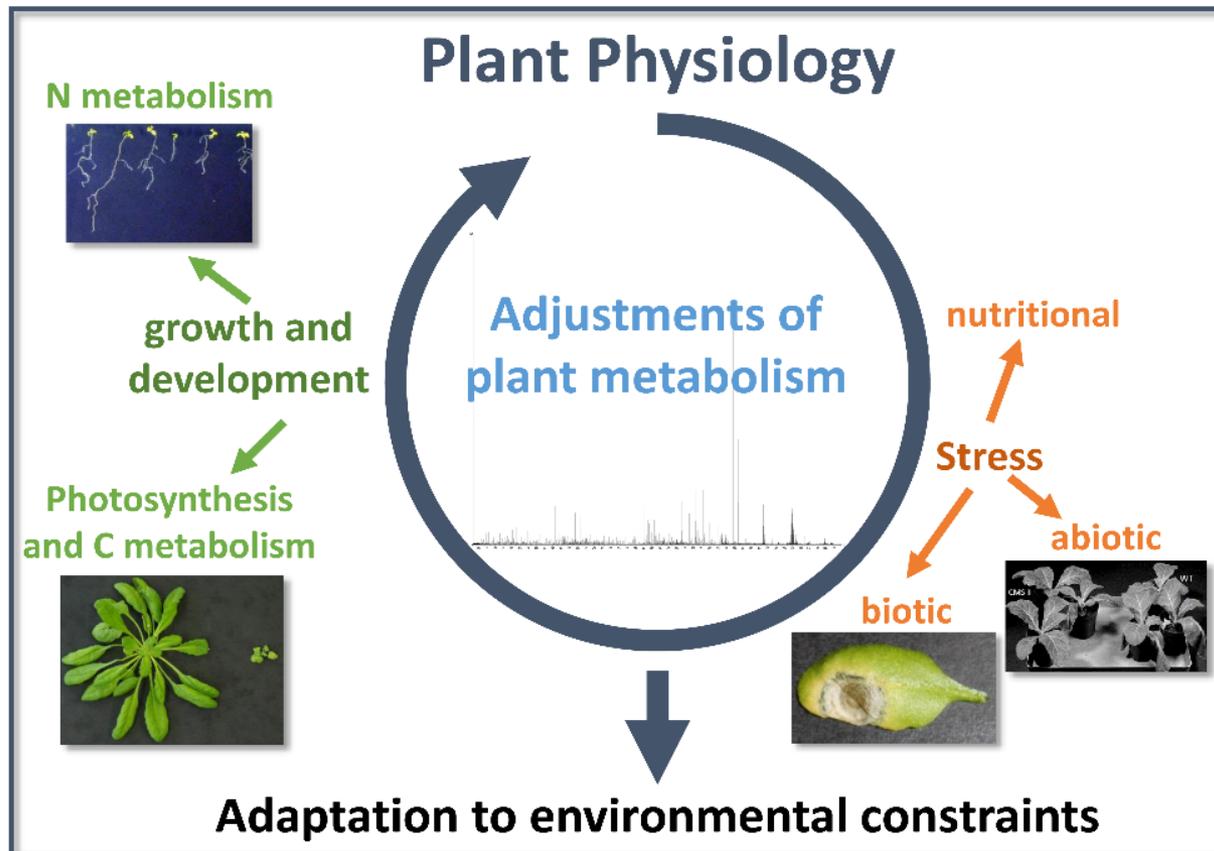
Conclusion

- **Mass spectrometry is now considered as an indispensable tool for the study of life sciences**
- **Accessibility, versatility and sensitivity of MS are major advantages**
- **The technology is still evolving in areas including higher spatial resolution, increased sensitivity, robotics for easier sample preparation, easier molecular identifications, along with other areas of technical development**
- **IMS brings the unique advantage of molecular specificity ensuring it a prominent role in both fundamental science, and clinical research and diagnosis**



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Case studies in molecular phytopathology



Spore Density Determines Infection Strategy by the Plant Pathogenic Fungus *Plectosphaerella cucumerina*^{1[OPEN]}

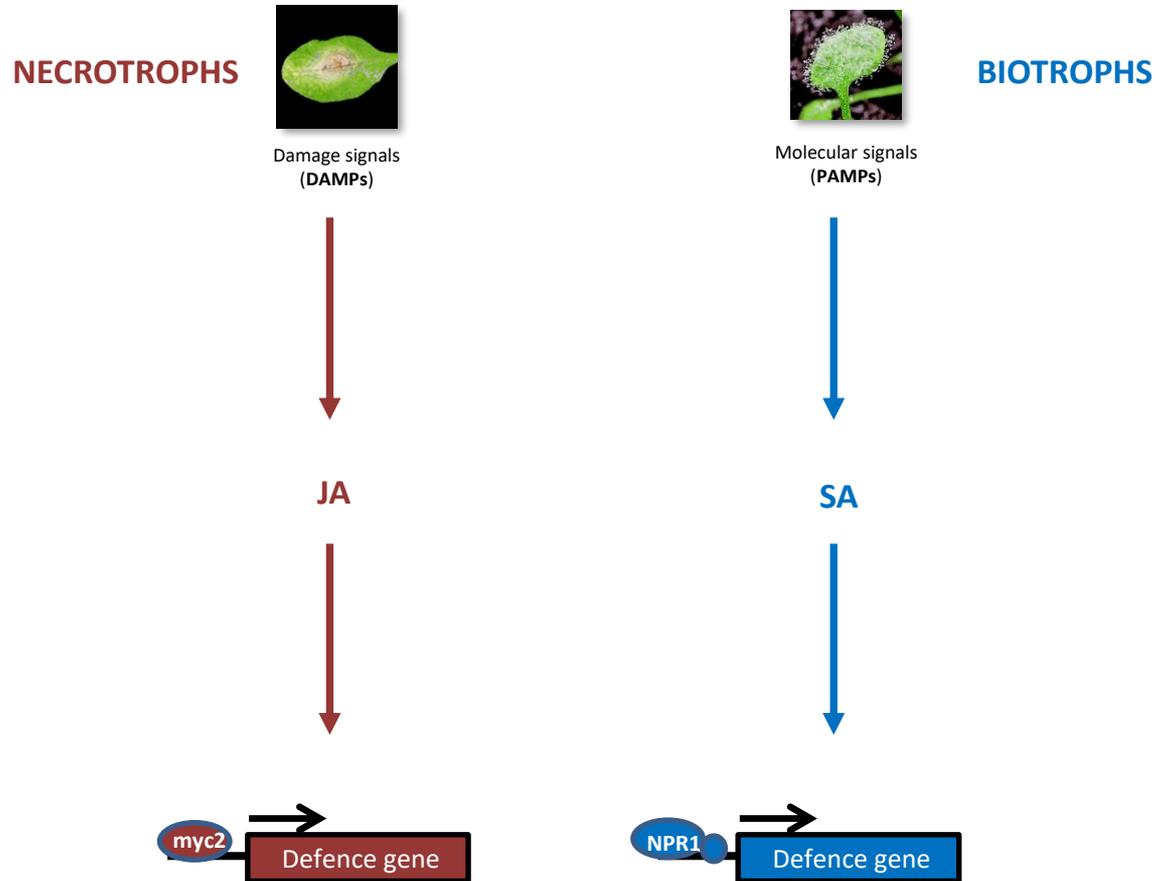
Pierre Pétriacq, Joost H.M. Stassen, and Jurriaan Ton*

Plant Environmental Signaling Group (P.P., J.H.M.S., J.T.) and biOMICS Facility (P.P.), Department of Animal and Plant Sciences, University of Sheffield, S10 2TN Sheffield, United Kingdom

ORCID IDs: 0000-0001-8151-7420 (P.P.); 0000-0001-5483-325X (J.H.M.S.); 0000-0002-8512-2802 (J.T.).

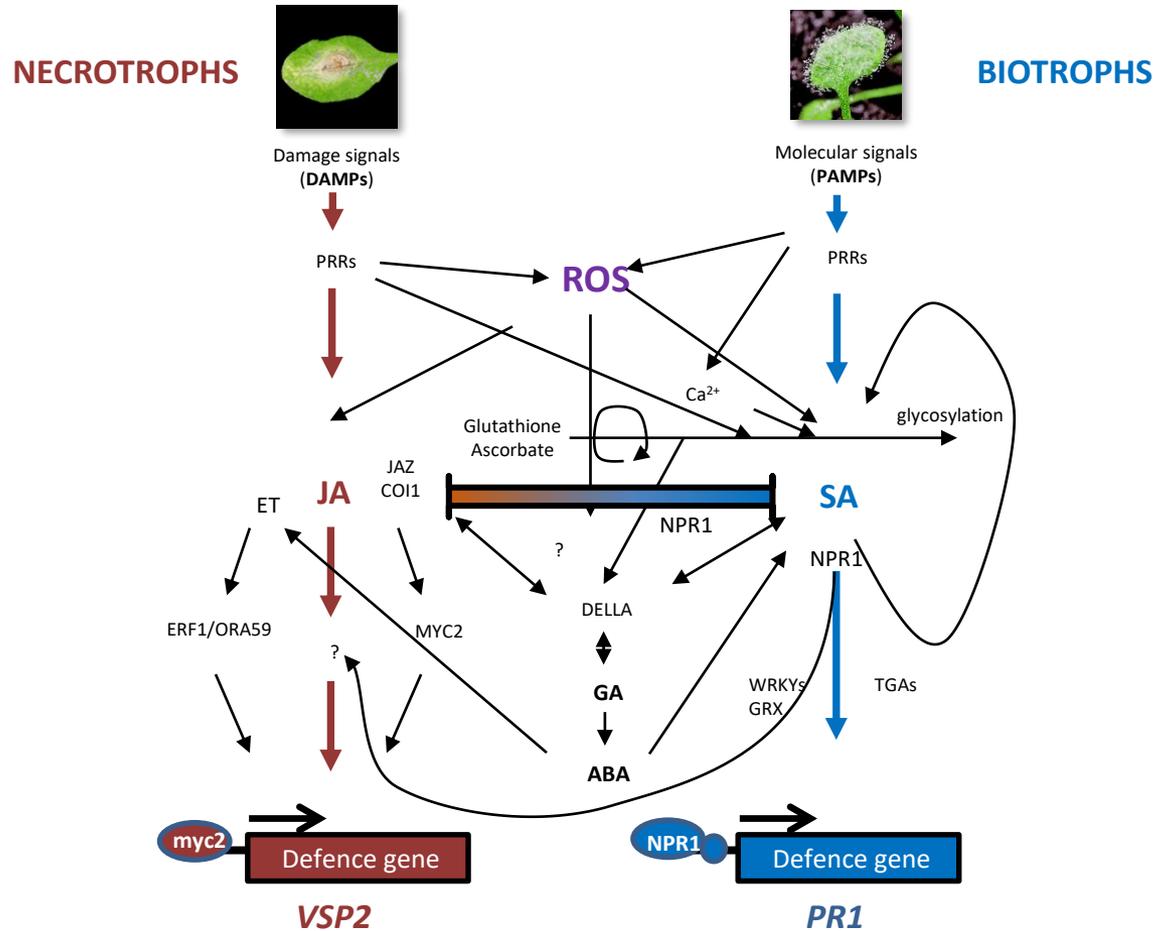
Necrotrophic and biotrophic pathogens are resisted by different plant defenses. While necrotrophic pathogens are sensitive to jasmonic acid (JA)-dependent resistance, biotrophic pathogens are resisted by salicylic acid (SA)- and reactive oxygen species (ROS)-dependent resistance. Although many pathogens switch from biotrophy to necrotrophy during infection, little is known about the signals triggering this transition. This study is based on the observation that the early colonization pattern and symptom development by the ascomycete pathogen *Plectosphaerella cucumerina* (*P. cucumerina*) vary between inoculation methods. Using the Arabidopsis (*Arabidopsis thaliana*) defense response as a proxy for infection strategy, we examined whether *P. cucumerina* alternates between hemibiotrophic and necrotrophic lifestyles, depending on initial spore density and distribution on the leaf surface. Untargeted metabolome analysis revealed profound differences in metabolic defense signatures upon different inoculation methods. Quantification of JA and SA, marker gene expression, and cell death confirmed that infection from high spore densities activates JA-dependent defenses with excessive cell death, while infection from low spore densities induces SA-dependent defenses with lower levels of cell death. Phenotyping of Arabidopsis mutants in JA, SA, and ROS signaling confirmed that *P. cucumerina* is differentially resisted by JA- and SA/ROS-dependent defenses, depending on initial spore density and distribution on the leaf. Furthermore, in situ staining for early callose deposition at the infection sites revealed that necrotrophy by *P. cucumerina* is associated with elevated host defense. We conclude that *P. cucumerina* adapts to early-acting plant defenses by switching from a hemibiotrophic to a necrotrophic infection program, thereby gaining an advantage of immunity-related cell death in the host.

Immune hormonal network



Metabolomics for phytopathology

Immune hormonal network



Glazebrook, 2005
Nishimura and Dangl, 2010
Pel and Pieterse, 2013

Metabolomics for phytopathology

Not that binary though...



Genus *Botrytis*, an archetypal necrotroph, but with a biotrophic endophyte lifestyle (van Kan *et al.*, 2014)

Rice blast fungus *Magnaporthe oryzae*, classified as a hemi-biotrophic leaf pathogen, can adopt a biotrophic infection in root tissues (Marcel *et al.*, 2010)

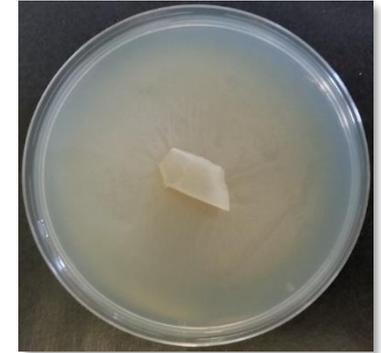
→ Little is known about the signal triggering the trophic transition !

Plectosphaerella cucumerina (*P. cucumerina*)

Filamentous **ascomycete**

commonly present in soil and decomposing vegetal tissues

Responsible for sudden death and blight
in **crops and weeds** (*Arabidopsis*)



Harrington *et al.*, 2000;
Chen *et al.*, 1999; Palm *et al.*, 1995

A wide range of inducible defence strategies in *Arabidopsis*

Phytohormones (JA, SA, ET, ABA and IAA)

Glucosinolates (including indole and aliphatic)

Callose deposition

Reactive Oxygen Species (ROS)

Gamir *et al.*, 2012; 2014; Pastor *et al.*, 2013; Sánchez-Vallet *et al.*, 2010; 2012;
Ton & Mauch-Mani, 2004; Berrocal-Lobo *et al.*, 2002; etc.

→ Resisted through a wide spectrum of both **anti-biotroph** and **anti-necrotroph** defences

Metabolomics for phytopathology

From an odd observation...

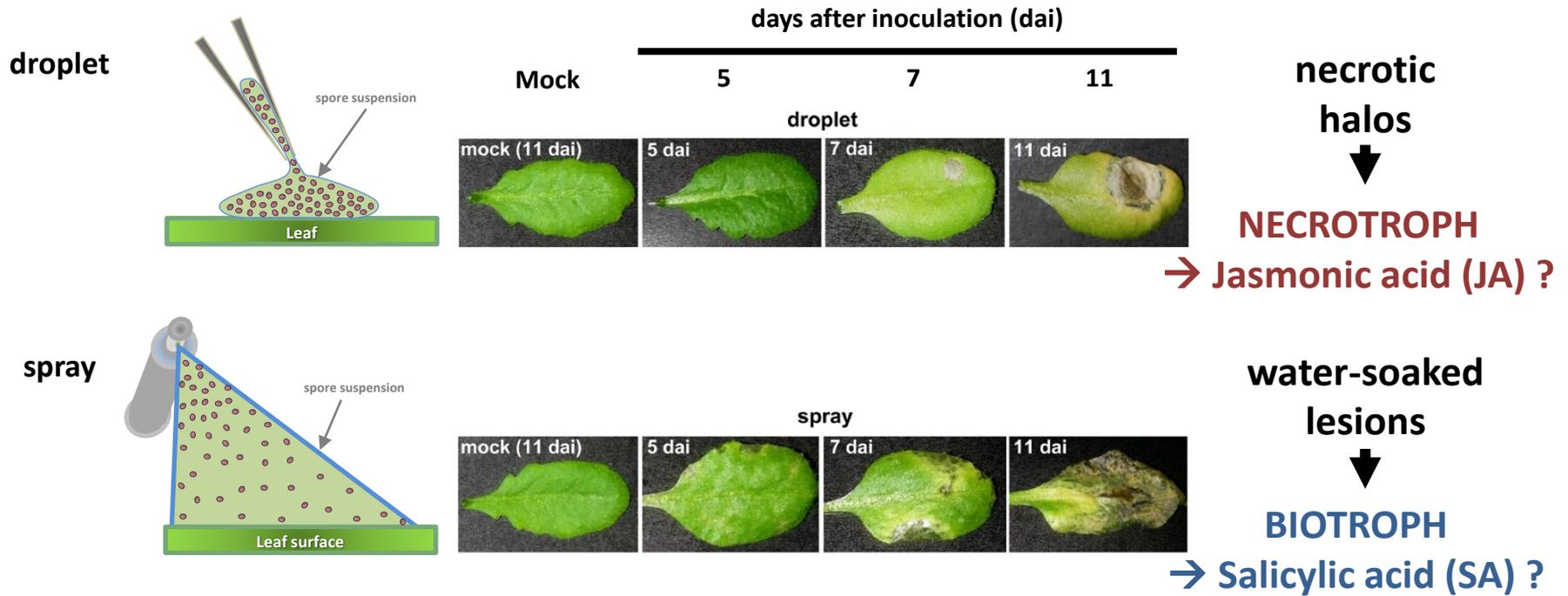
droplet



spray

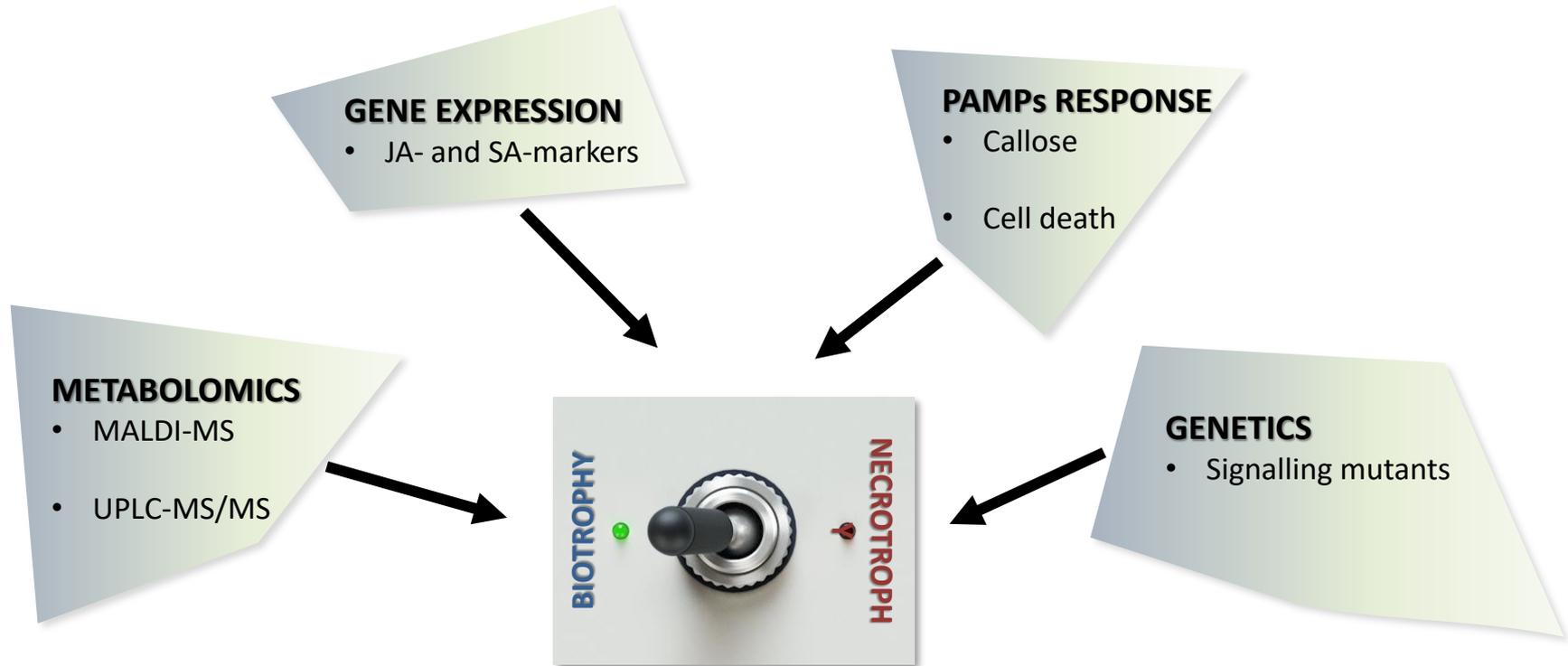


Necrotrophic ascomycete
Plectosphaerella cucumerina



→ Droplet- and spray-inoculations cause differences
in aggressiveness and symptoms severity

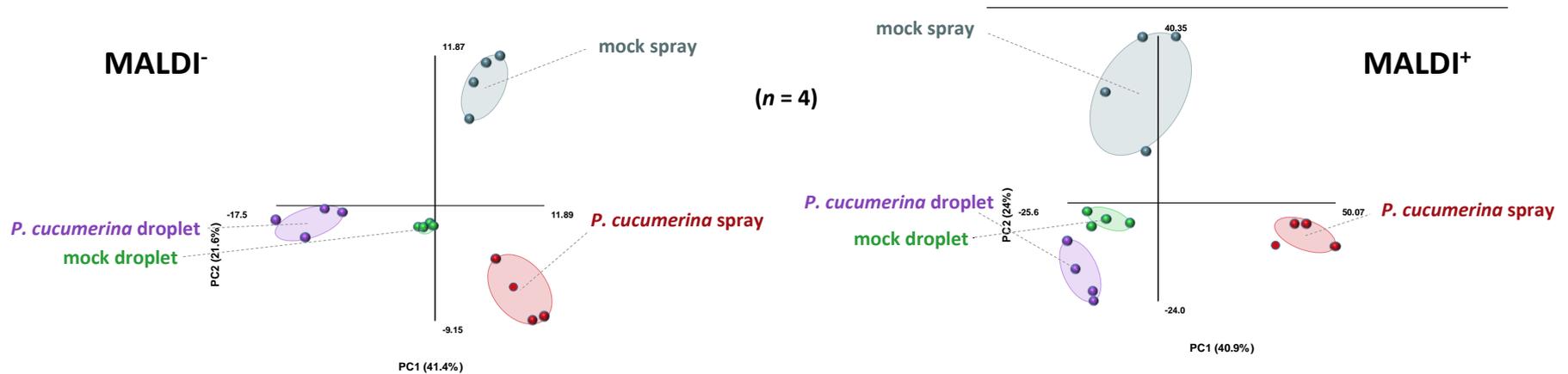
Biotrophy-to-necrotrophy switch ?



Metabolomics for phytopathology

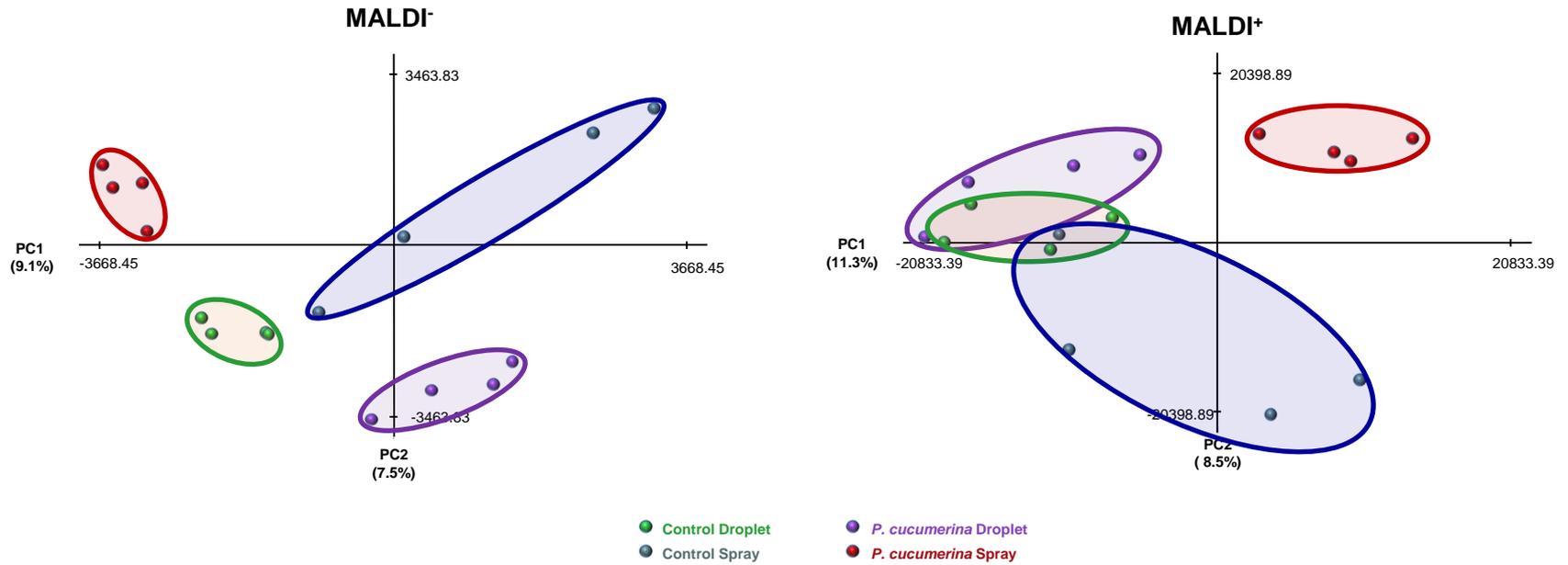
Untargeted MALDI-qTOF-MS profiling 2 dai

Global impacts on the metabolome (ANOVA + PCA)



→ Droplet and spray inoculations elicit distinct plant metabolic profiles

Unbiased PCA



Metabolomics for phytopathology

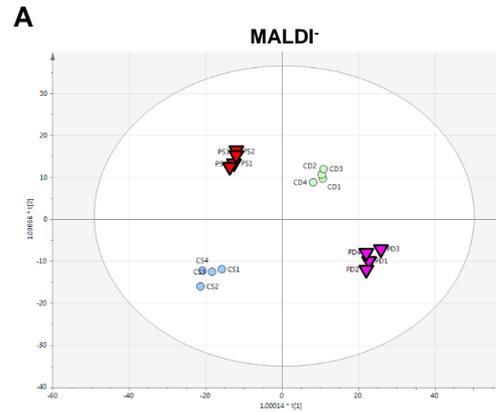
OPLS-DA

Figure S2: Bidirectional orthogonal partial least square discriminant analysis (O2PLS-DA) of unfiltered m/z values from MALDI-Q-TOF analysis in negative (A) and positive (B) mode.

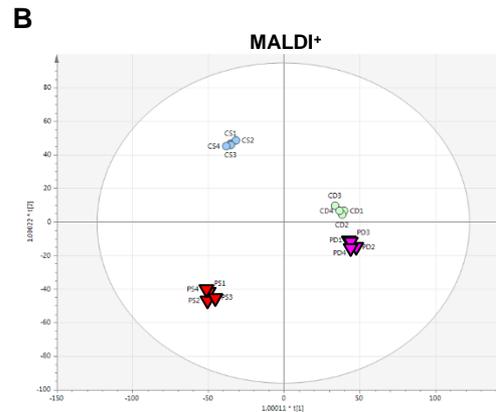
Shown are score plots of replicate samples (A, B) and loadings plots of m/z values (C, D).

The O2PLS discrimination was validated in SIMCA for MALDI⁻ ($R^2X=0.65$; $Q^2=0.94$; CV-ANOVA $p=7.02 \cdot 10^{-6}$) and MALDI⁺ ($R^2X=0.68$; $Q^2=0.92$; CV-ANOVA $p=1.81 \cdot 10^{-3}$).

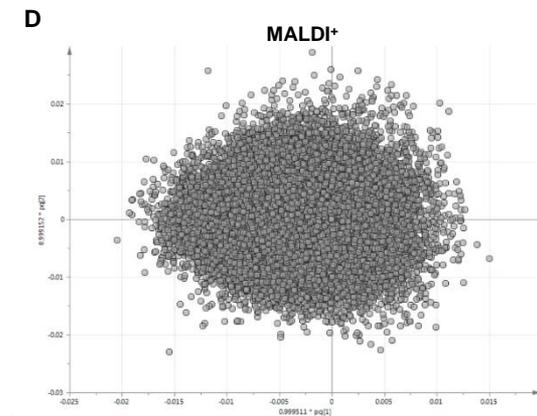
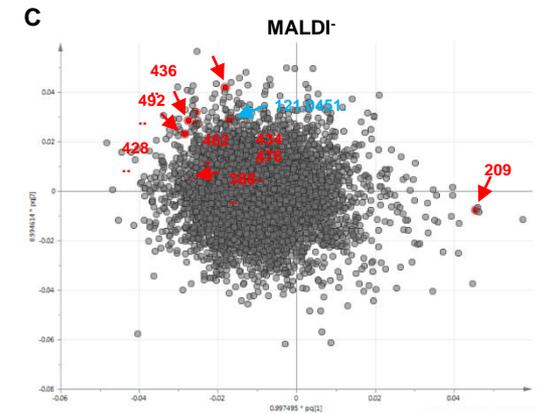
Despite the Log transformation of the data, important variables detected in MALDI⁻ are visible on the loadings plots in red (C).



○ Control Droplet ▽ *P. cucumerina* Droplet
○ Control Spray ▽ *P. cucumerina* Spray



○ Control Droplet ▽ *P. cucumerina* Droplet
○ Control Spray ▽ *P. cucumerina* Spray



Pathways analysis: inoculation method x *P. cucumerina*

SIGNIFICANT MARKERS (two-ways ANOVA, $P < 0.01$)

Accurate <i>m/z</i> value	MALDI mode	Adduct	Predicted mass	Putative compound (MarVis)	Pathway	Fold change Droplet	Fold change Spray	O2PLS-DA VIP Ranking
450.0536	-	<i>m-H</i>	451.0641	5-methylsulfinylpentyl glucosinolate	Glucosinolates	0.10	7.89	36
462.0879	-	<i>m-H</i>	463.0952	7-methylthioheptyl glucosinolate	Glucosinolates	NI	25.61	61
428.1081	-	<i>m-H</i>	427.1087	7-octenyl glucosinolate	Glucosinolates	0.10	6.27	135
476.1009	-	<i>m-H</i>	477.1161	8-methylthiooctyl glucosinolate	Glucosinolates	NI	489.39	16
434.0212	-	<i>m-H</i>	435.0285	Glucoberberoin	Glucosinolates	0.08	400.96	136
420.0445	-	<i>m-H</i>	421.0518	Glucoerucin	Glucosinolates	0.07	491.81	9
492.0947	-	<i>m-H</i>	493.1020	Glucohirsutin	Glucosinolates	NI	14.87	10
436.0375	-	<i>m-H</i>	437.0448	Glucoraphanin	Glucosinolates	0.11	7.19	2
388.0424	-	<i>m-H</i>	389.0497	Progoitrin	Glucosinolates	0.02	447.61	1028
157.1203	+	<i>m+H</i>	156.1130	3-indoleacetonitrile	Glucosinolates	NI	11.66	905
293.1700	+	<i>m+H</i>	292.1627	12-OPDA	JA metabolism	19640.00	NI	788
209.9645	-	<i>m-H</i>	210.9718	JA	JA metabolism	4.23	NI	15
324.2425	+	<i>m+H</i>	323.2352	JA-Ile	JA signaling	55.09	NI	303
125.0156	+	<i>m+H</i>	124.0083	Salicyl alcohol/catechol monomethyl ether	SA degradation	0.32	2933.40	4962
121.0451	-	<i>m-H</i>	122.0524	Benzoic acid/salicyl aldehyde	SA metabolism	0.04	435.41	244

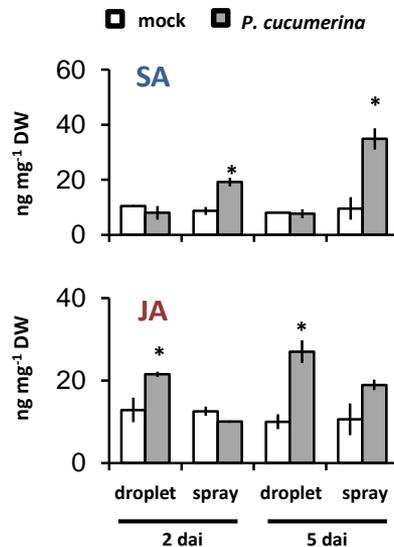
→ Does JA/SA antagonism activate the trophic switch ?

Metabolomics for phytopathology

Differences in JA- and SA-induction / responses

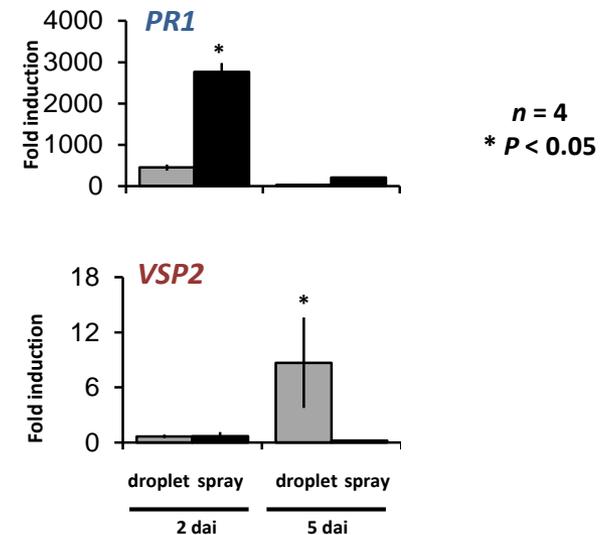
Targeted UPLC-MS/MS confirmed

- Elevated **JA** levels in droplet-inoculated leaves
- Increased **SA** levels in sprayed-infected leaves



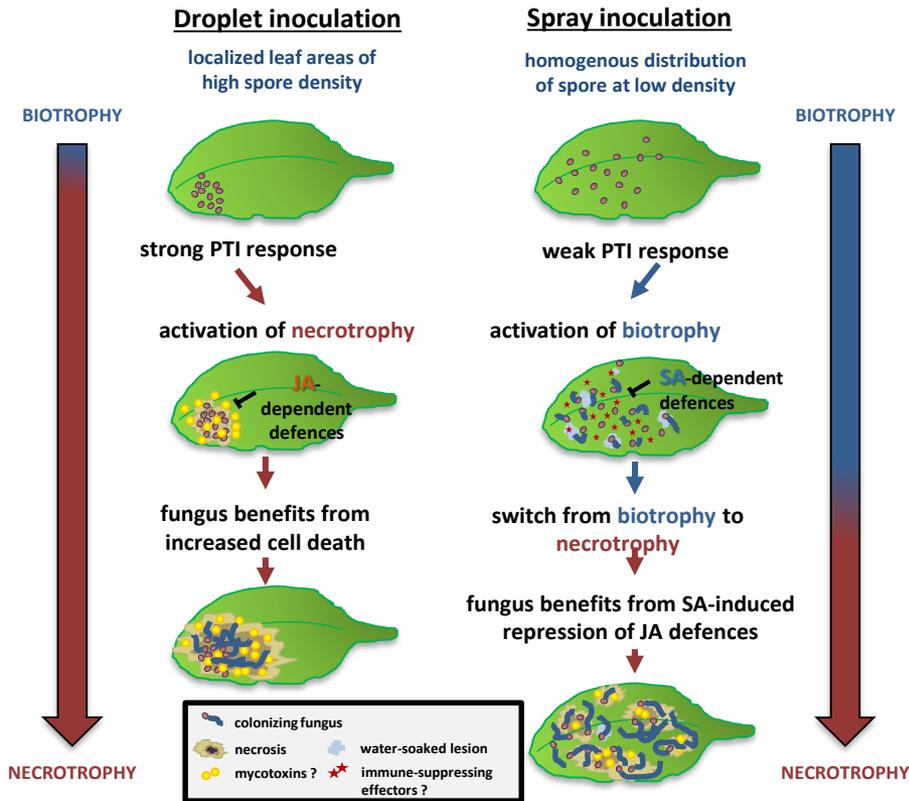
RT-qPCR revealed

- Induced **VSP2** expression after droplet
- Higher induction of **PR1** after spraying, then repression



→ Droplet- and spray-inoculations differentially trigger JA- and SA- pathways

Biotrophy-to-necrotrophy switch : summary



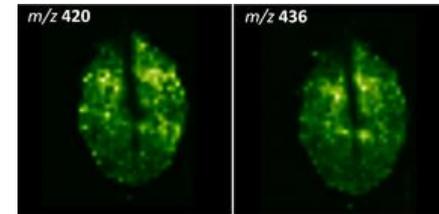
→ Extra caution when using *P. cucumerina* as a typical necrotrophic pathogen !

Metabolomics for phytopathology

To be continued...

Which molecular signals trigger the trophic switch ?

- Identify *P. cucumerina* mycotoxin(s), if possible
- Investigate **glucosinolates** metabolism and its relation with SA/ROS-related defences
- Benefit from **MALDI-MS imaging** to picture the **dynamic distribution of defence compounds** upon droplet- and spray-inoculation



Glucosinolates Shroff *et al.*, 2015



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