

Mass Spectrometry of Imaging

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City Center



Sart Tilman



Chemistry Building



Campus Gembloux



Campus Arlon

The golden standards of the analytical chemist

- Identify the analytes (targeted or non targeted)
- Locate the analytes in the sample
- Quantify the analytes
- All quickly, on a minute amount of sample...

What is the contribution of Mass Spectrometry and particularly MRMS

Outlook

1. Basis for identification using Mass Spectrometry
2. Families of compounds with a common property: increasing mass with a constant chemical composition group. Good candidates for Kendrick Mass Defect (lipids, lipopeptides)
3. Separation methods: classical methods, LC MS/MS, CE MS, TLC
4. Mass Spectrometry Imaging using MALDI/SALDI
5. Application to lipids
6. Application to lipopeptides
7. Applications to biopolymers

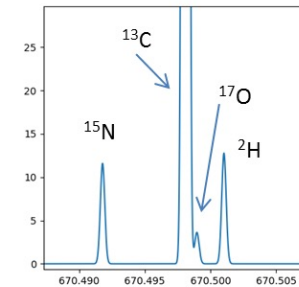
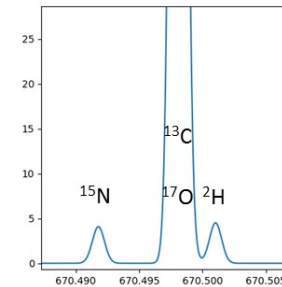
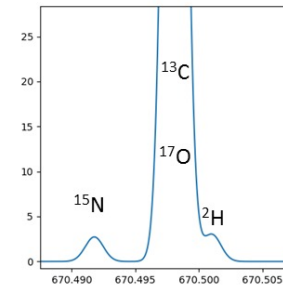
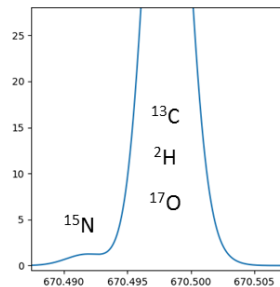
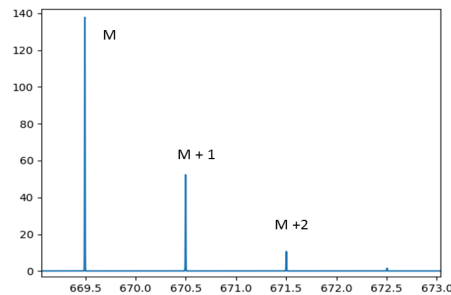
1. Basis for identification using Mass Spectrometry

- Mass
- MS/MS: analysis of fragments
- Energetics: Breakdown curves, when possible after IMS separation to identify isomers
- Shape ion mobility
- Properties from separation methods (if any)

https://www.wada-ama.org/sites/default/files/resources/files/WADA_TD2010IDCRv1.0_Identification%20Criteria%20for%20Qualitative%20Assays_May%2008%202010_EN.doc.pdf
https://www.nist.gov/system/files/documents/2019/04/22/chsac_tox_identification_in_forensic_toxicology_for_asb_and_website_1.pdf

1.1 Mass Resolution and accuracy

- resolving power and the mass accuracy reached routinely record values
 - 10^6 RP: separates 1000 from 1000,001
 - Sub-ppm accuracy depends on calibration and instrumental mass shift
- isotopic signature: qualitative or quantitative (relative intensities)
- The result is a molecular formula, not yet a fully characterized compound

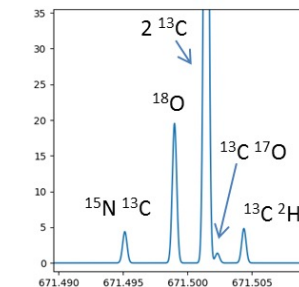
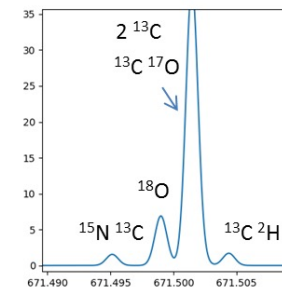
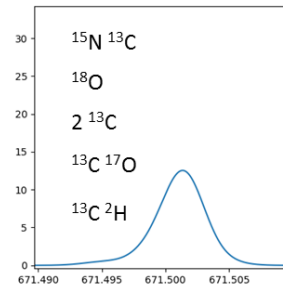
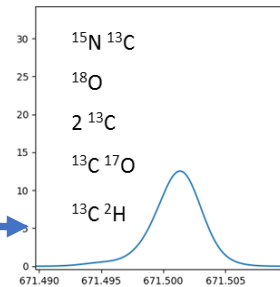


M+1

Calculated model of isotopic peak separation

Sphingomyelin (d18:1/12:0): $C_{35}H_{71}N_2O_6P$

TOF limit
Orbitrap limit



M+2

180K

400K

600K

1,7 M

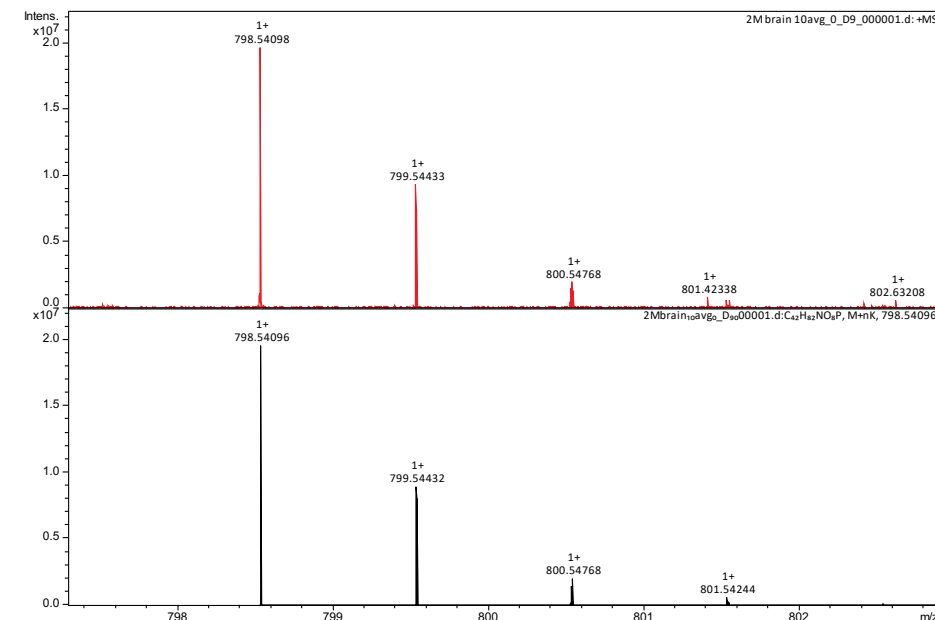
1.1 Real case

Delta max: 0,001

Input Mass	Matched Mass	Delta	Name	Formula	Ion	LMSD Examples
798.54098	798.5408	.0002	PC O-37:7	C45H78NO7PNa	[M+Na] ⁺	
798.54098	798.5408	.0002	PE O-40:7	C45H78NO7PNa	[M+Na] ⁺	https://lipidmaps.org/data/chemdb_lm_text_ontology.php?ABBREV=PE O-40:7
798.54098	798.5410	.0000	CerP 42:2;O4	C42H82NO8PK	[M+K] ⁺	
798.54098	798.5410	.0000	LPC 34:2;O	C42H82NO8PK	[M+K] ⁺	
798.54098	798.5410	.0000	PC 34:1	C42H82NO8PK	[M+K] ⁺	https://lipidmaps.org/data/chemdb_lm_text_ontology.php?ABBREV=PC 34:1
798.54098	798.5410	.0000	PC O-34:2;O	C42H82NO8PK	[M+K] ⁺	
798.54098	798.5410	.0000	PE 37:1	C42H82NO8PK	[M+K] ⁺	https://lipidmaps.org/data/chemdb_lm_text_ontology.php?ABBREV=PE 37:1
798.54098	798.5410	.0000	PE O-37:2;O	C42H82NO8PK	[M+K] ⁺	

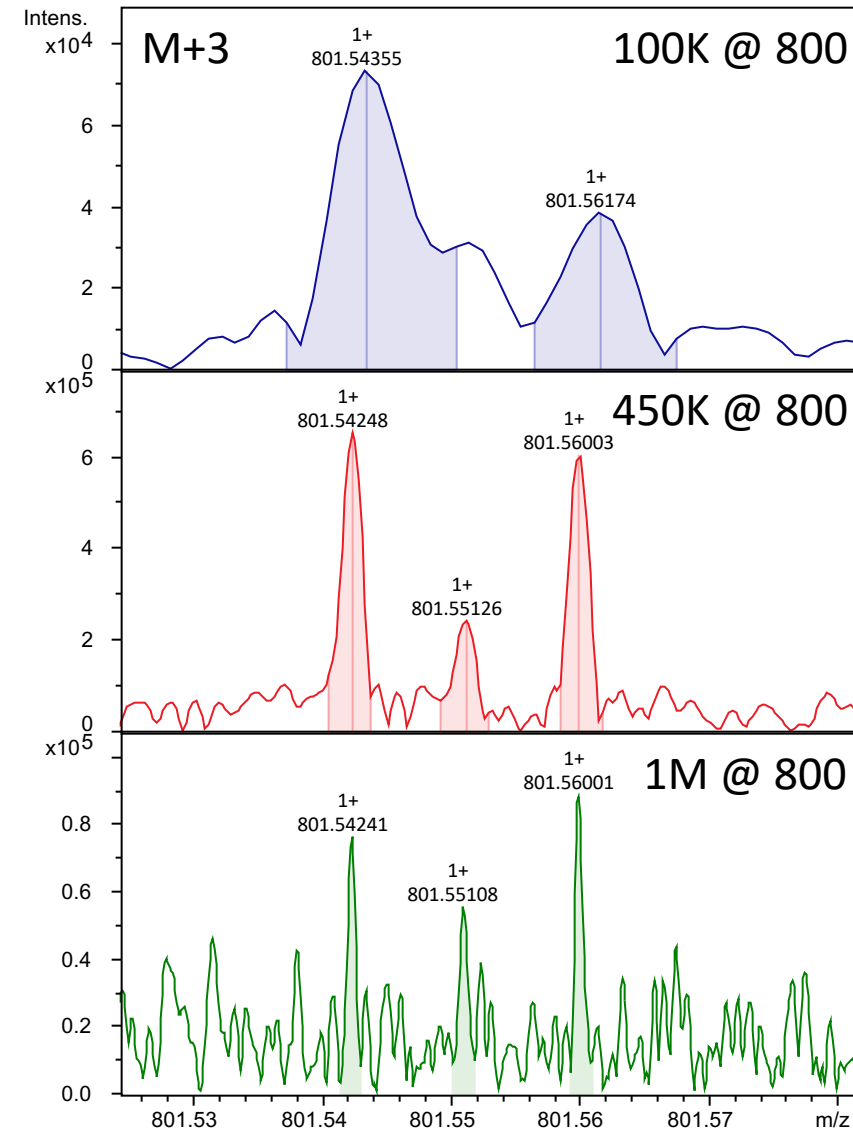
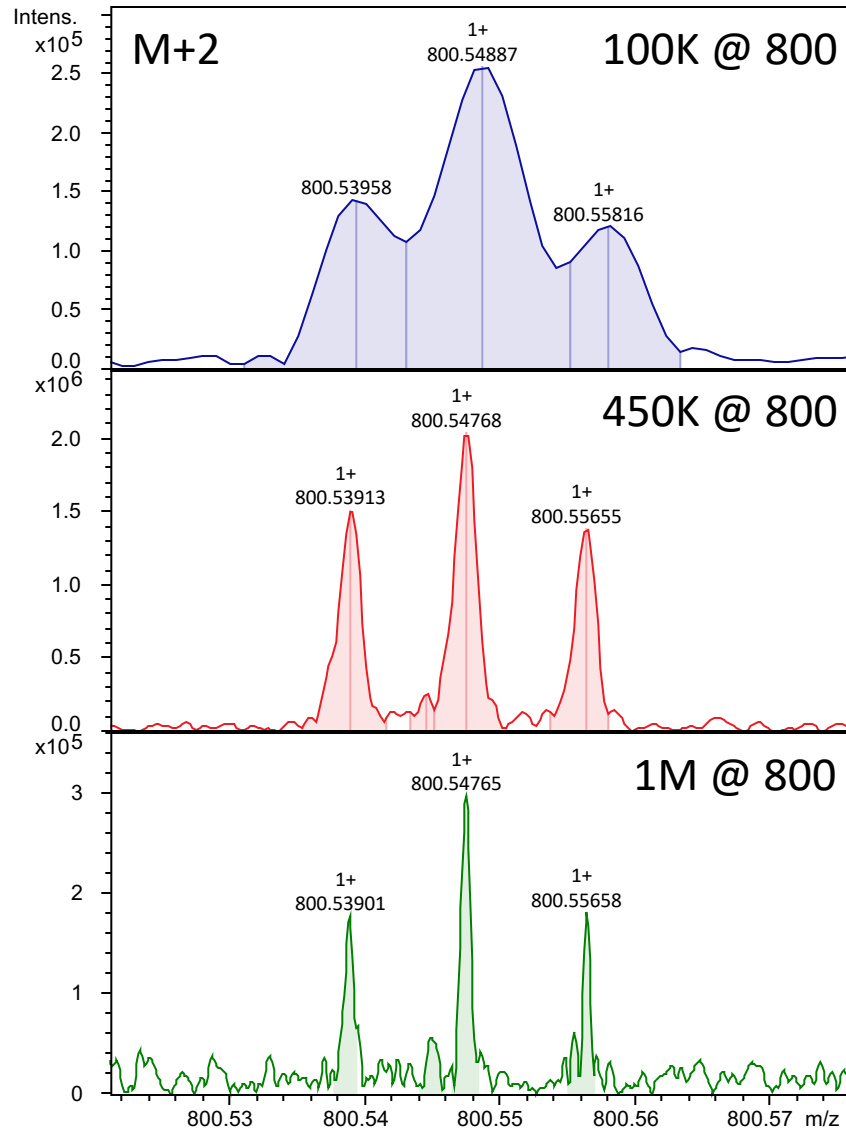
Delta max: 0,0005

Input Mass	Matched Mass	Delta	Name	Formula	Ion	LMSD Examples
798.54098	798.5408	.0002	PC O-37:7	C45H78NO7PNa	[M+Na] ⁺	
798.54098	798.5408	.0002	PE O-40:7	C45H78NO7PNa	[M+Na] ⁺	https://lipidmaps.org/data/chemdb_lm_text_ontology.php?ABBREV=PE O-40:7
798.54098	798.5410	.0000	CerP 42:2;O4	C42H82NO8PK	[M+K] ⁺	
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798.54098	798.5410	.0000	PC 34:1	C42H82NO8PK	[M+K] ⁺	https://lipidmaps.org/data/chemdb_lm_text_ontology.php?ABBREV=PC 34:1
798.54098	798.5410	.0000	PC O-34:2;O	C42H82NO8PK	[M+K] ⁺	
798.54098	798.5410	.0000	PE 37:1	C42H82NO8PK	[M+K] ⁺	https://lipidmaps.org/data/chemdb_lm_text_ontology.php?ABBREV=PE 37:1
798.54098	798.5410	.0000	PE O-37:2;O	C42H82NO8PK	[M+K] ⁺	



The best accuracy is mandatory
Other informations will be required:
Retention time against a standard
MS/MS
High resolution ion mobility

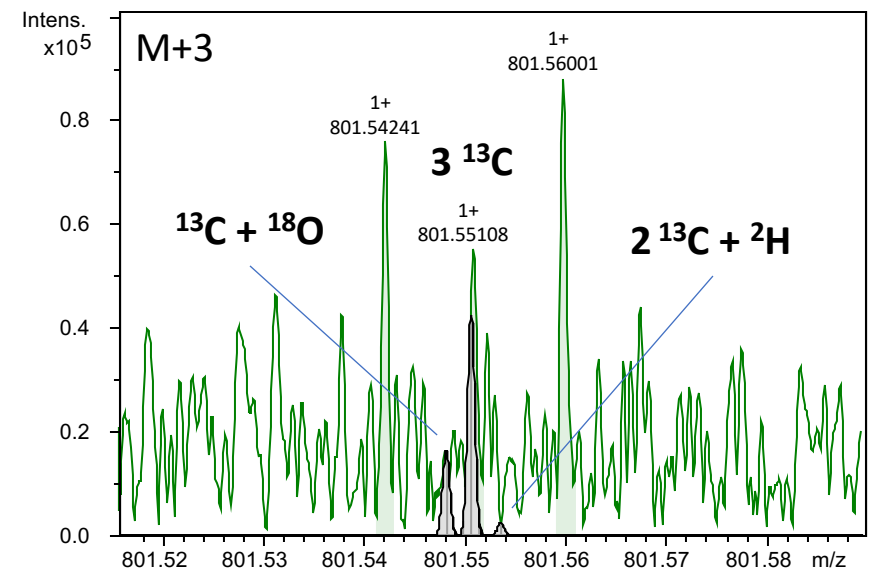
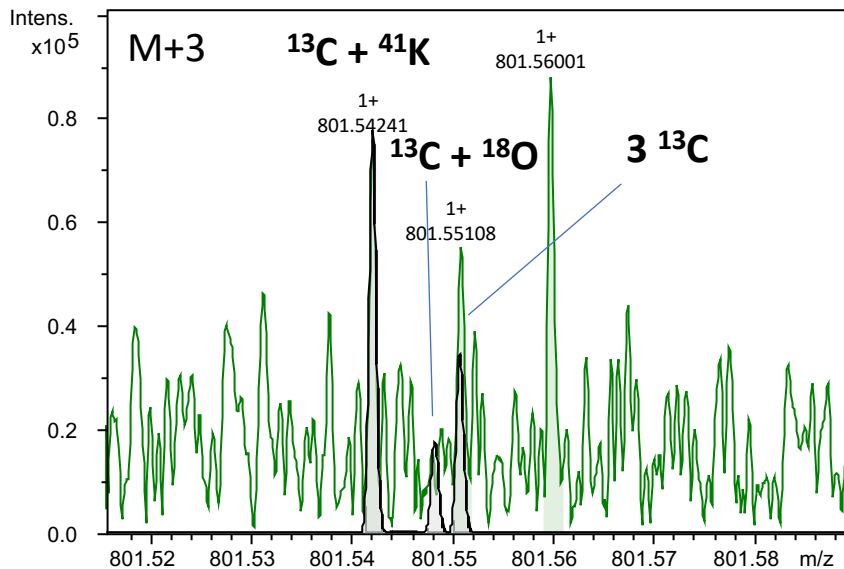
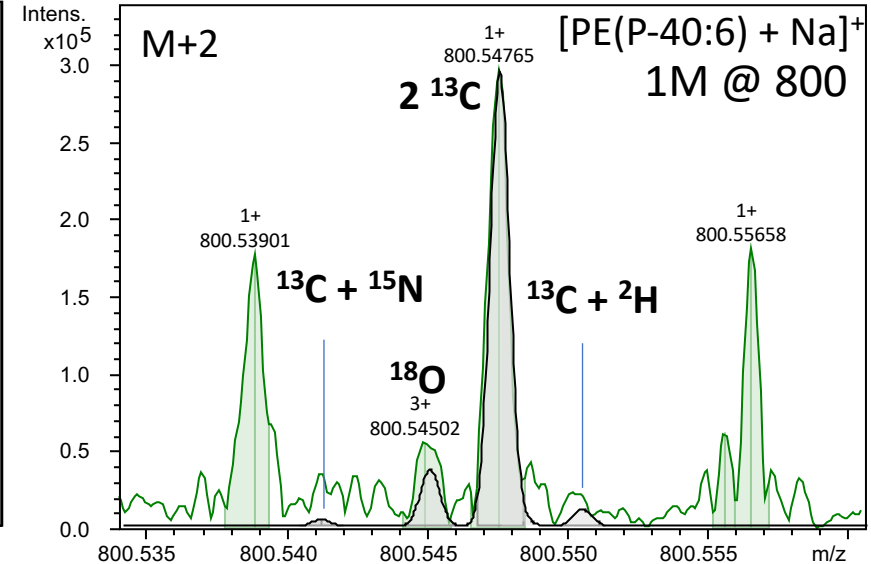
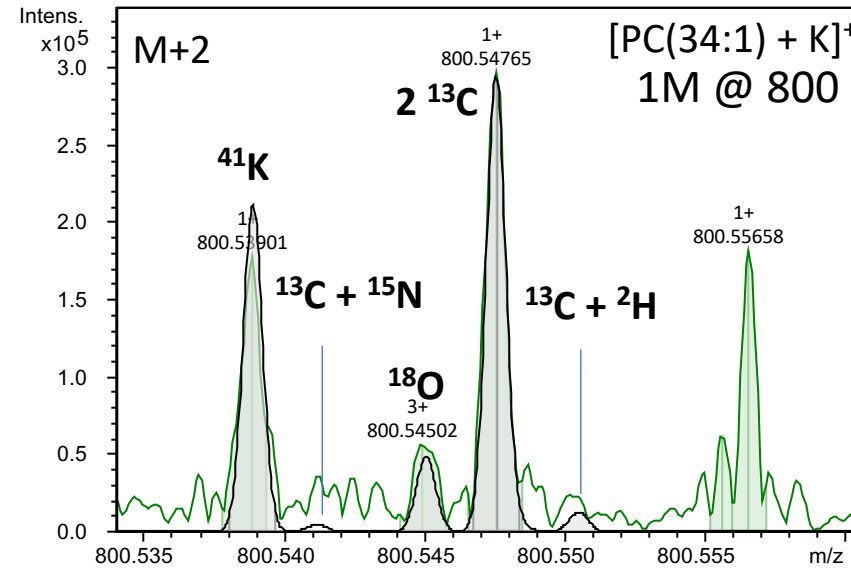
1.1 Real case Isotope fine structure vs R.P.



1.1 Isotopic pattern simulation for identification

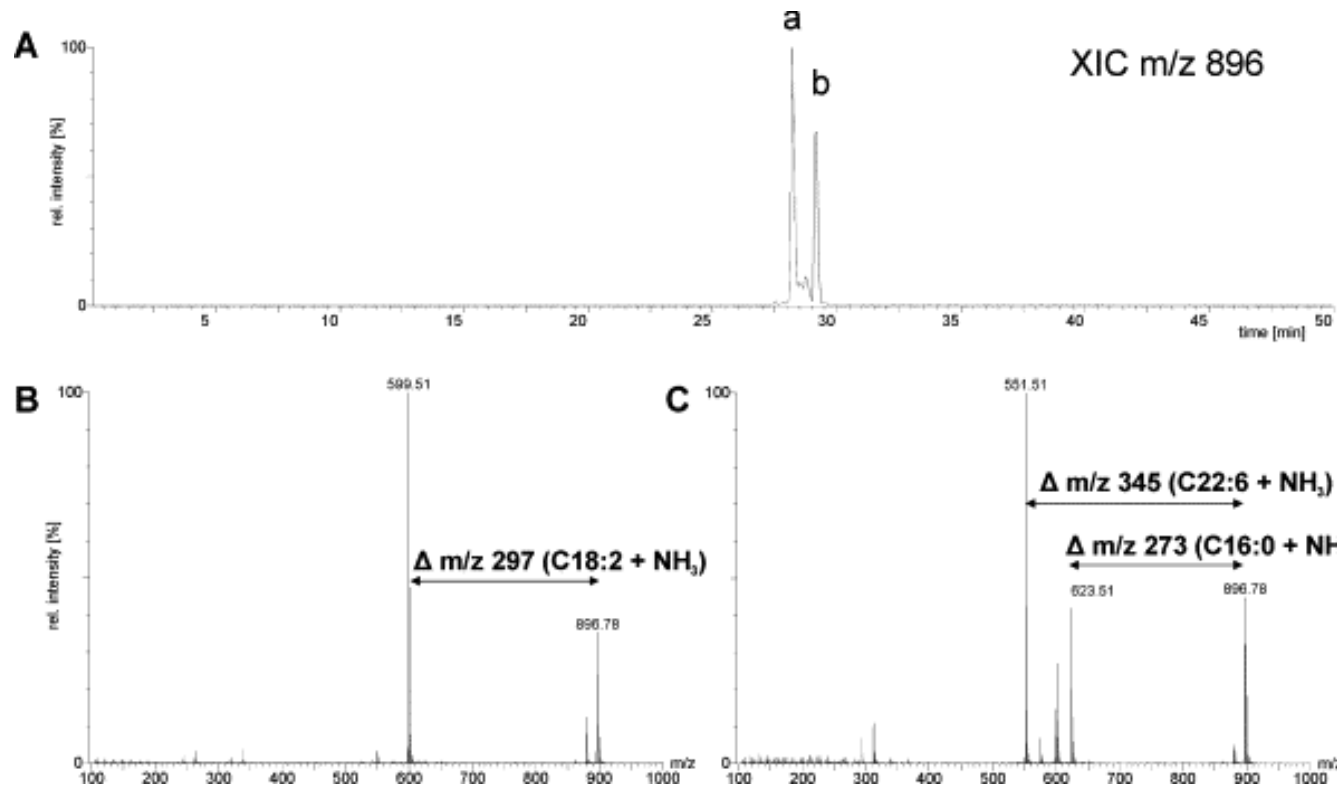
The isotopic pattern allow
To improve the selection of
candidates

For isomers, additionnal
criteria should be used:
MS/MS, IMS...



1.2 MS/MS

Activation performed usually using collision activation (CID)



TG 54:6: 54 carbon atoms, 6 double bonds
Cn:x: number of carbons, number of DB

65 isomers possible

But who is who ?

Separation of two isomers of TG 54:6. 54

A. XIC of m/z 896 ([TG 54:6 + NH₄]⁺) shows two separated peaks.
B. MS² spectrum of m/z 896 at peak a (28.2 min).

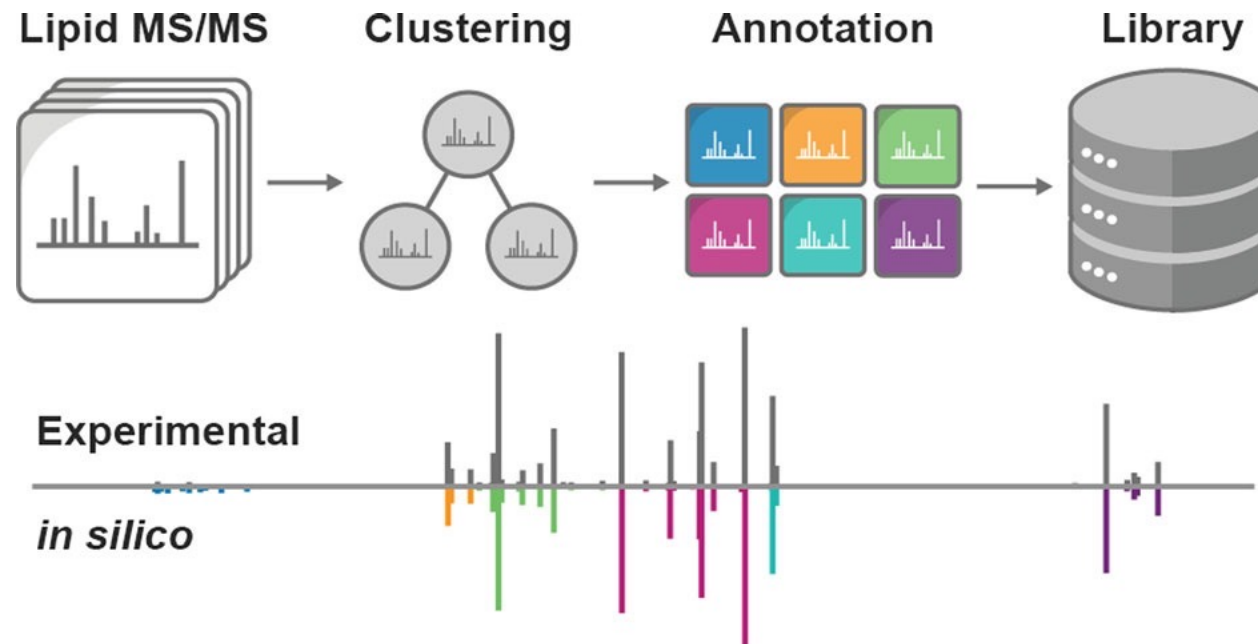
The major fragment is caused by the loss of C18:2 fatty acid.

C. MS² spectrum of m/z 896 at peak b (29.1 min).

The major fragments are caused by the loss of C22:6 and C16:0 fatty acid.

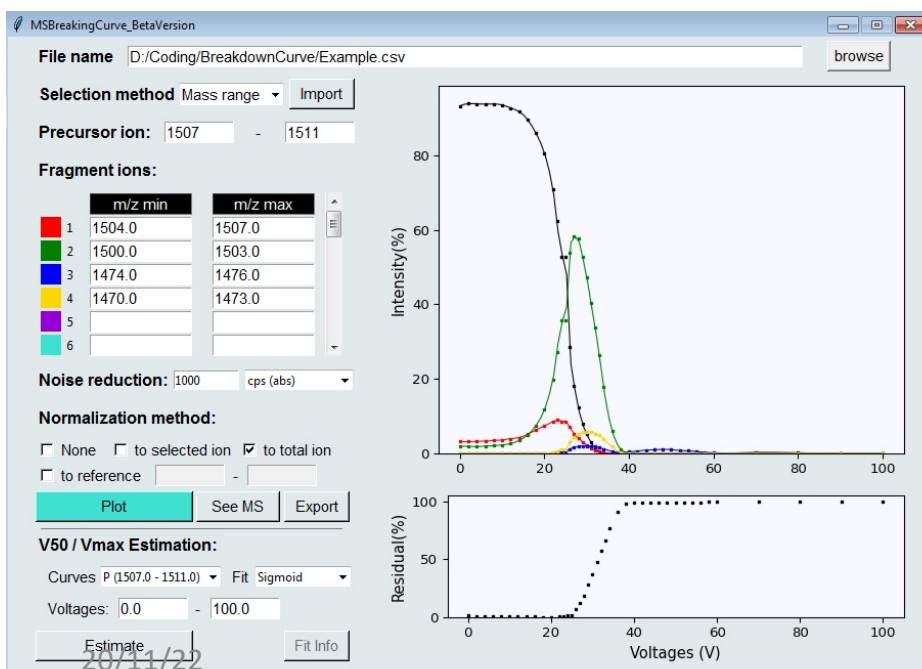
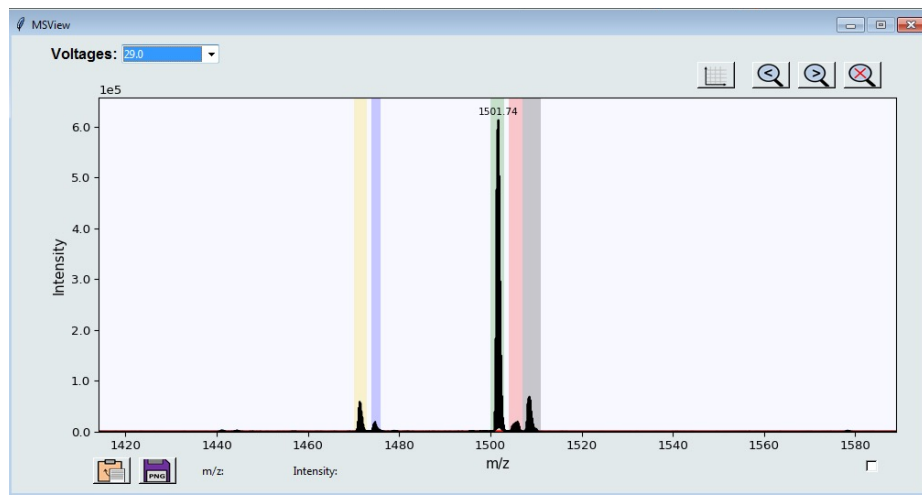
1.2 Fragmentation (MS/MS)

A large dataset exist for MS/MS spectra of lipids allowing automated identification

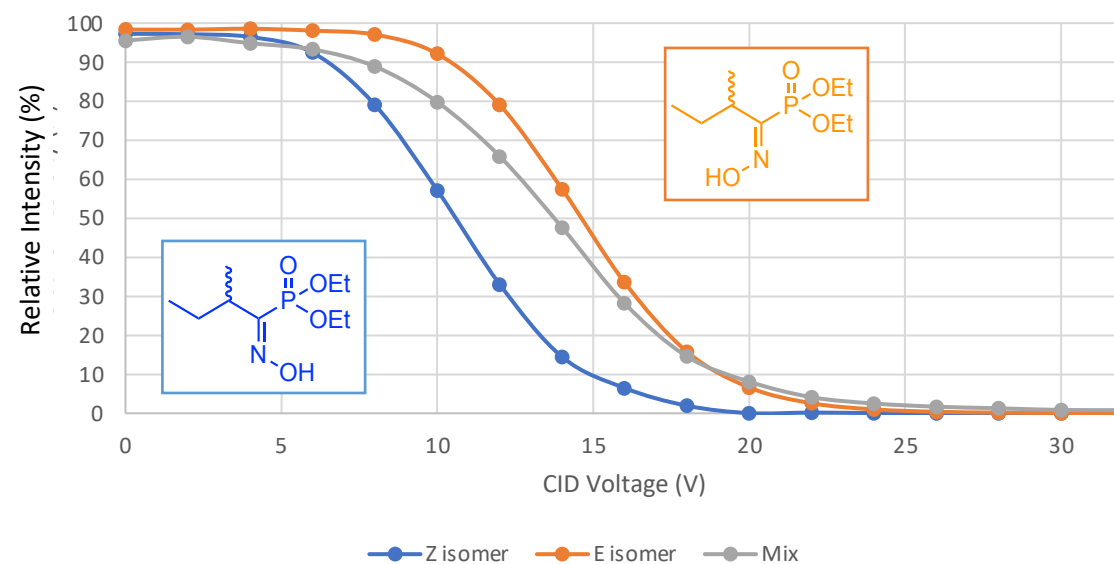


Paul D. Hutchins, Jason D. Russell, Joshua J. Coon
J. Am. Soc. Mass Spectrom. (2019) 30:659Y668 [DOI: 10.1007/s13361-018-02125-y](https://doi.org/10.1007/s13361-018-02125-y)

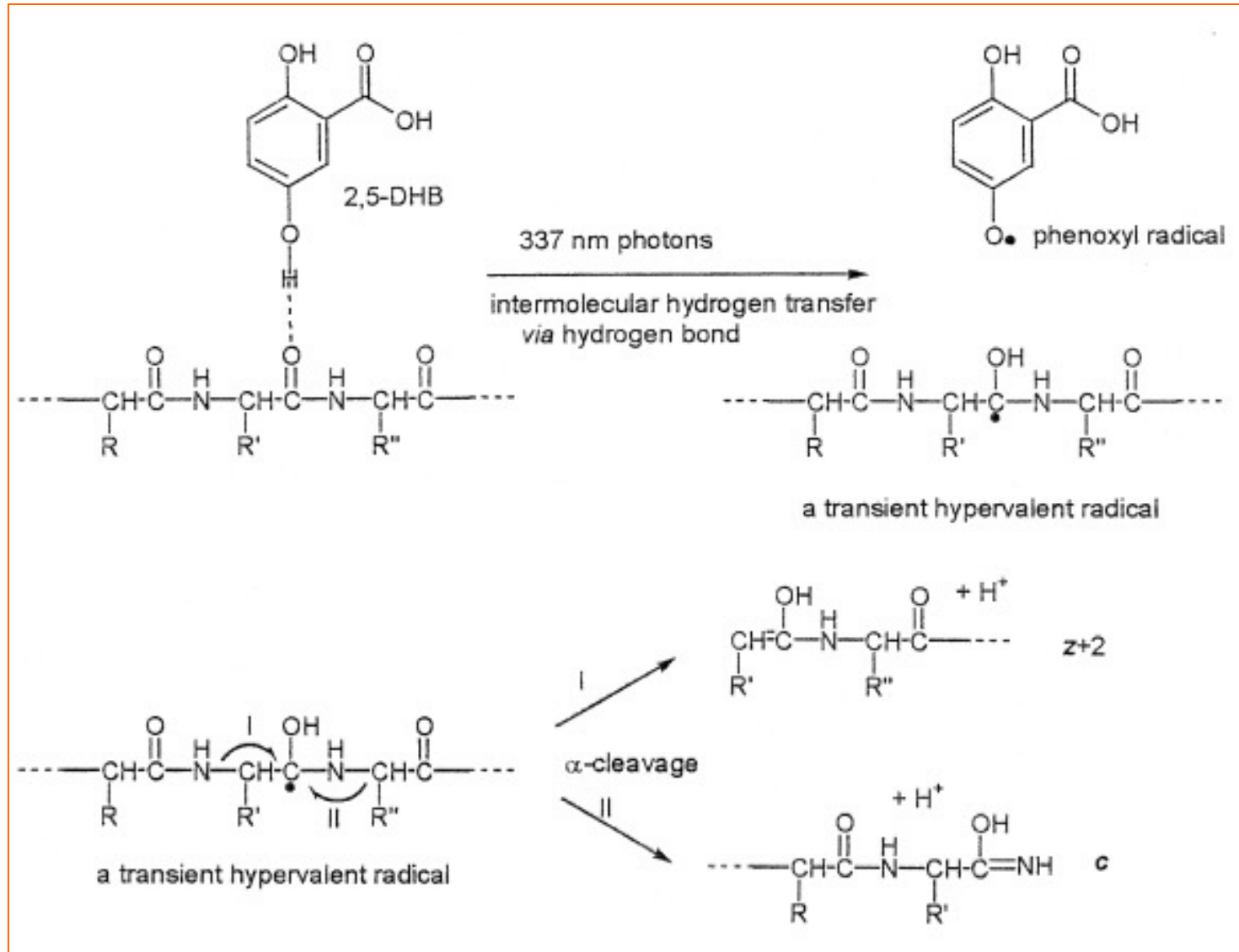
1.3 CID breaking down curves are extracted from MS Data using an in-house Python Software

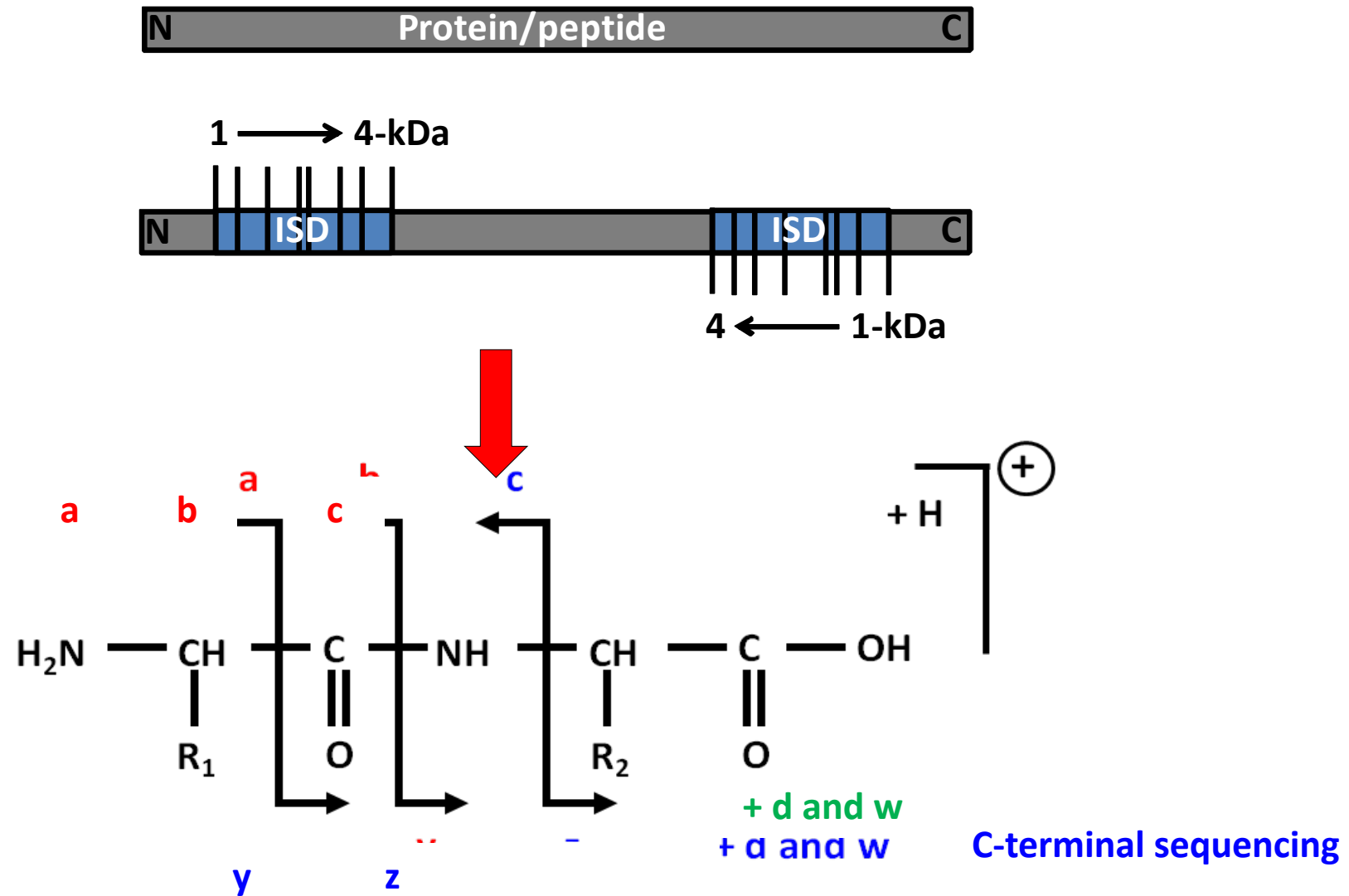


Hydroxylamine isomer ions

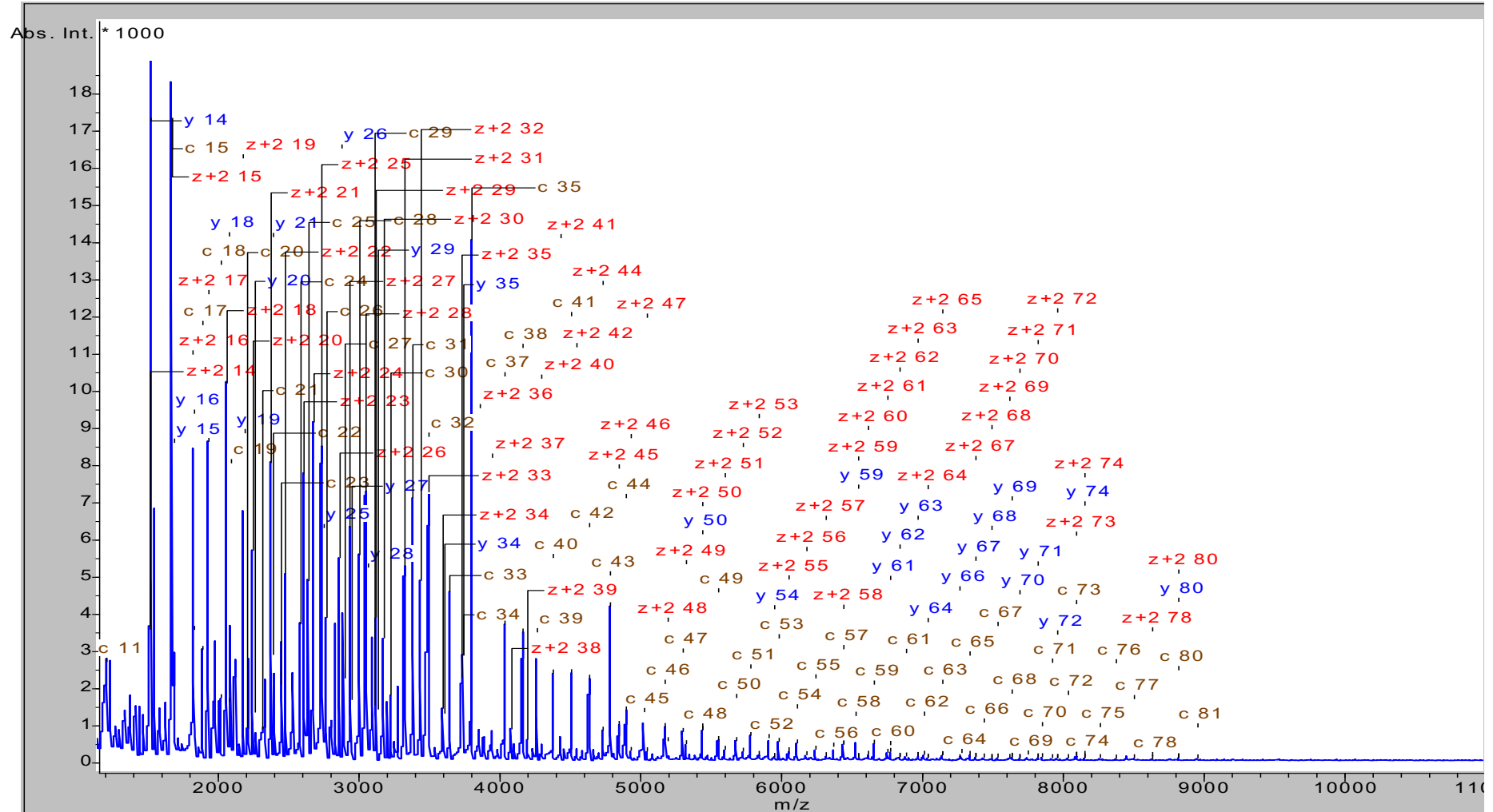


Toupy T. and al., Org. Chem. Front., 2022, 9, 173-182





ISD top down sequencing using c- and z- ions



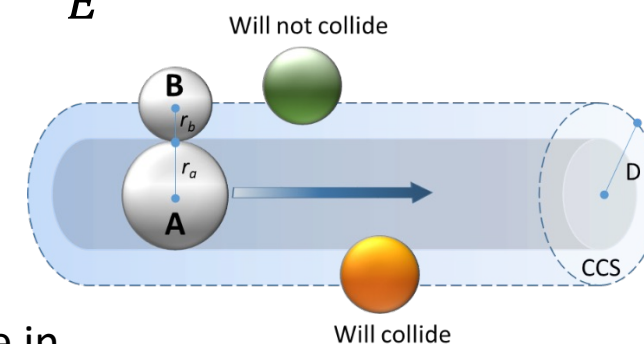
1.4 Shape (ion mobility)

Gas-phase separation

Motion of ions in an electric field undergoing collisions (friction) with a buffer gas

Intrinsic ion mobility value K ($\text{m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$)

$$K = \frac{v_d}{E}$$



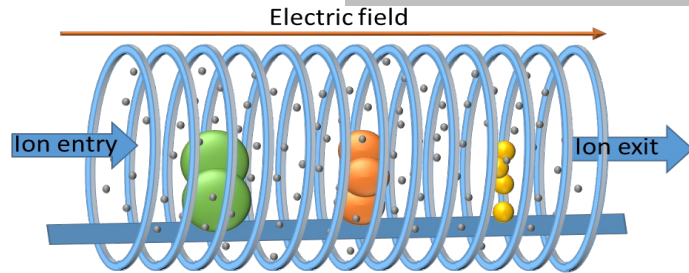
From the mobility K , it is possible to extract the **collision cross section (CCS or Ω)** value in \AA^2 . This is only valid for DT. For other methods, calibration is required. **CCS** value reflects the conformation and shapes adopted by ions in the gas phase under defined experimental conditions and can be calculated according to Mason-Schamp equation:

$$CCS = \frac{3}{16} \times \sqrt{\frac{2 \times \pi}{\mu \times k \times T}} \times \frac{z}{N \times K}$$

Applicable for low-field limit
Low (E/N) ratio

The CCS of an ion may be carefully be used as **a structure descriptor**

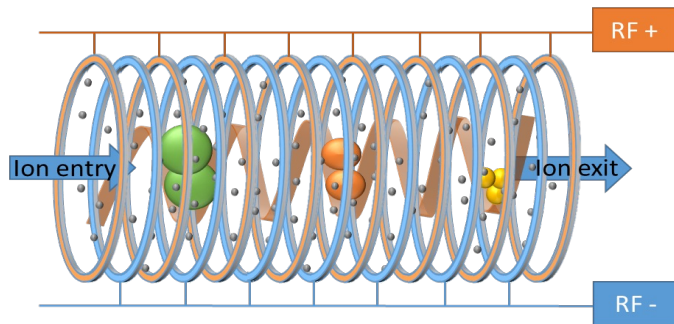
1.4 Different types of IMS



Drift tube IMS (DTIMS) constant velocity

$$K = \frac{v_d}{E} \longrightarrow K = \frac{L}{t_a \times E} \longrightarrow \text{DTCCS value}$$

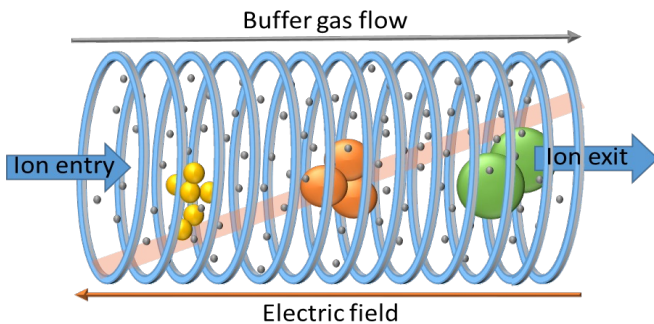
Smaller first



Travelling wave IMS (TWIMS) constant average velocity

$$t_a \longrightarrow \text{TWIMS CCS} = f(t_a)$$

Smaller first

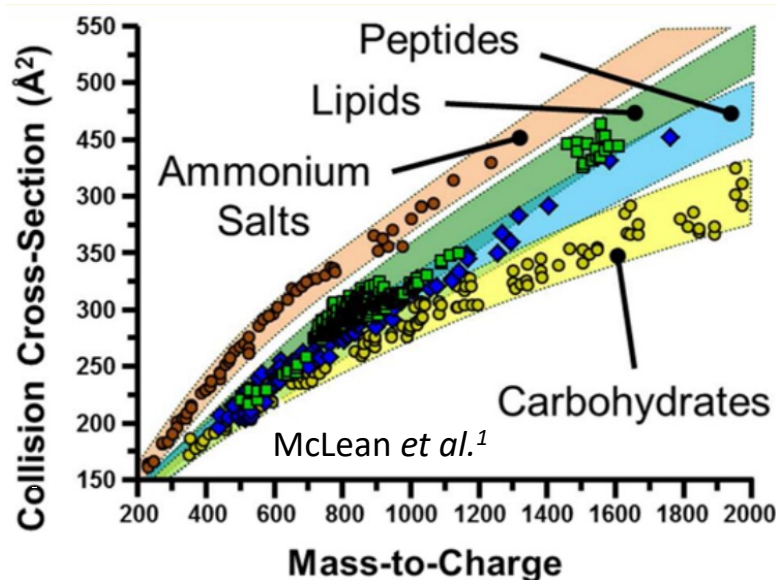


Trapped Ion Mobility (TIMS) ZERO velocity

$$E_e \longrightarrow \text{TIMS CCS} = f(E_e)$$

Larger first

1.4 Mass/CCS trends

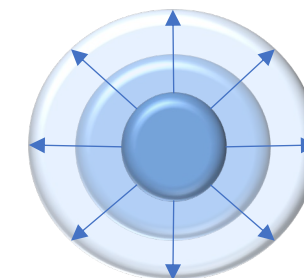


Class-specific CCS/mass trends

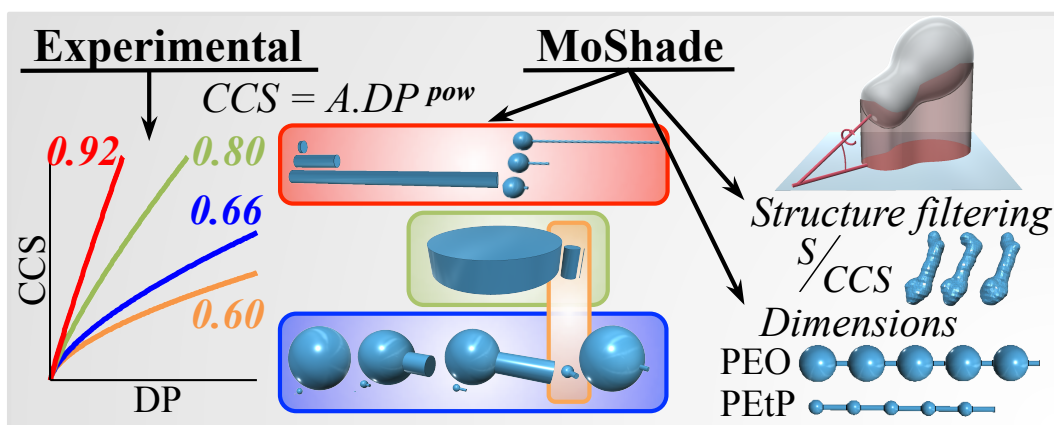
$$CCS = A \times m^{POW}$$

Haler et al.²

$A \rightarrow$ Apparent density
 $POW \rightarrow$ Ion shape



Considering that systems **grow with a constant density** and a **constant global spherical shape**, POW parameter would be equal to 0.66.
If we consider a **spherical**, **cylindrical** or **ball-cylindrical** shape evolution, POW parameter range is **defined between 0.5 and 0.9**.



Family-specific CCS/mass trends ?

Application to the glycosphingolipid family

$$CCS = A \times DP^{POW}$$

The relation is optimal when the mass increment is used as unit

1.4 Prediction of CCS for small molecules

SMILES

```
CH(O)
=NaCC
CH[OH]CH
BrCCC@
(H2)CN1C=
NC2=C1CH
```



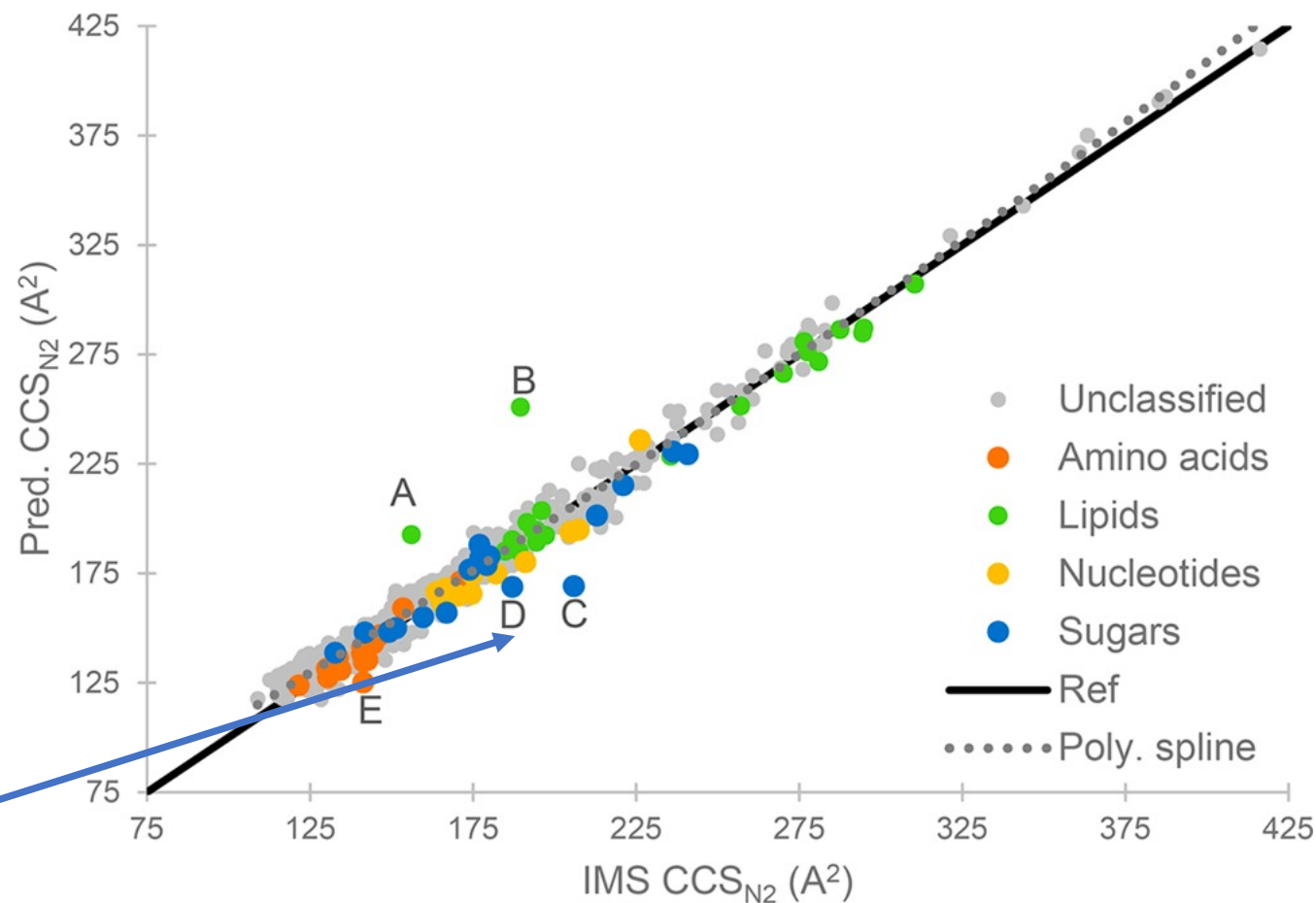
DeepCCS

CCS

```
192.34;
181.92;
314.7;126.5;
281.34;473;
192.0;174.2;
411.6;138.9;
```

Deep Learning CCS prediction

Outlayers when intramolecular interactions
restric the shape



Pier-Luc Plante and al. [Anal. Chem. 2019, 91, 5191–5199](#)

2.1 Re-scaling the IUPAC mass scale to the Kendrick mass scale

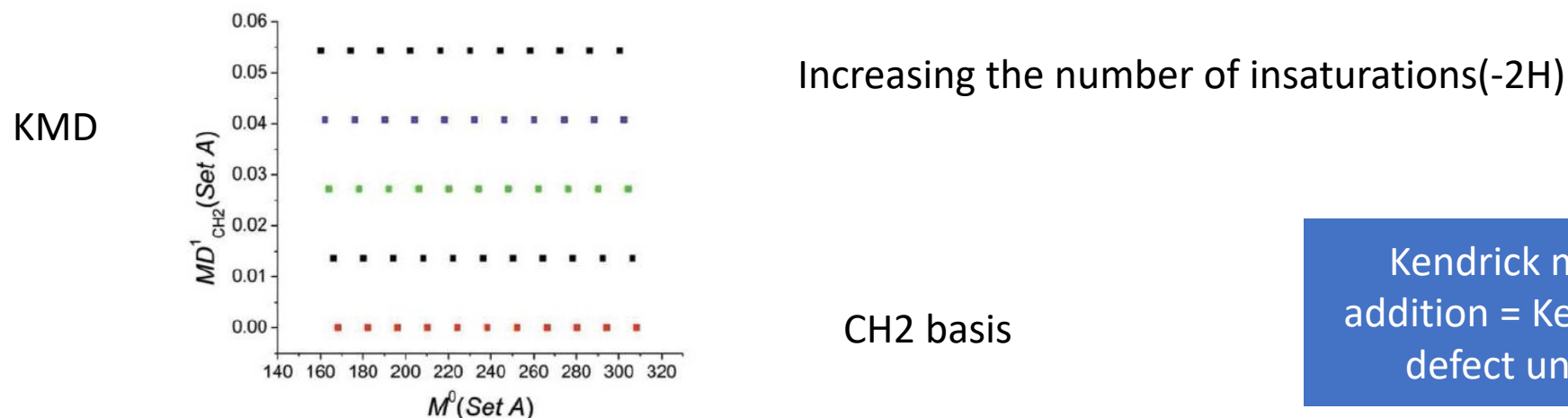
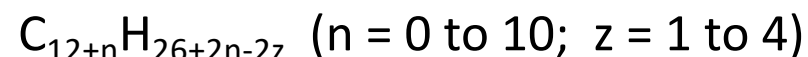
$$-\text{CH}_2- = 14.01565$$

$$-\text{CH}_2- = 14.00000$$

$$\text{Kendrick mass} = \text{IUPAC mass} \times \frac{14.00000}{14.01565}$$

$$\text{Kendrick mass defect } KMD = \text{round}(KM) - KM$$

In this mode, all the component having the same constitution of heteroatoms but different numbers of $-\text{CH}_2-$ units will have identical Kendrick mass defect (KMD)



Kendrick mass basis
addition = Kendrick mass
defect unchanged

2.2 Questions

- Calculate the KMD of C₁₄H₃₀ C₁₅H₃₂ C₁₅H₃₀ C₁₅H₂₈

Formula	UIPAC	KM	KMD				
C ₁₄ H ₃₀	198,23473	198,013379	-0,01337933				
C ₁₅ H ₃₂	212,25038	212,013379	-0,01337933				
C ₁₅ H ₃₀	210,23473	209,99998	1,99777E-05				
C ₁₅ H ₂₈	208,21908	207,986581	0,013419285				
	Conversion factor		14	14,01565		0,99888339	

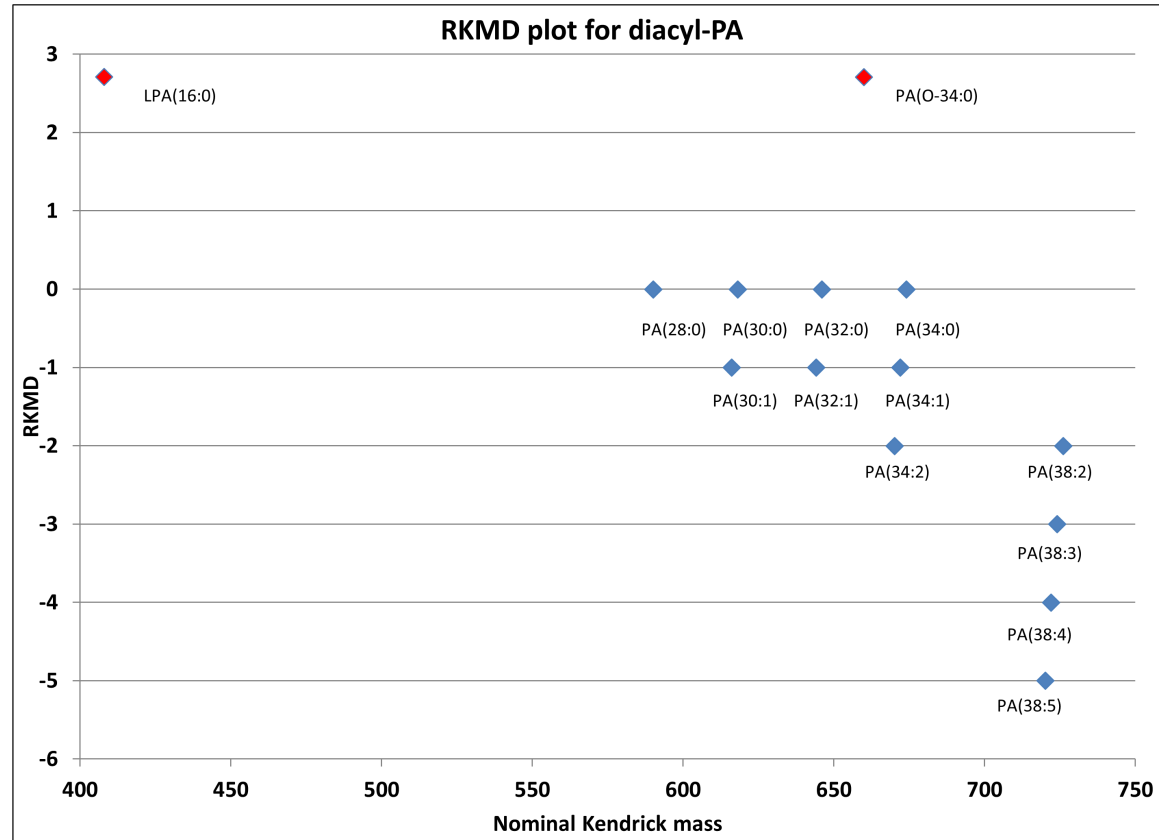
0 as all CH₂

What are your observations?

2.3 Example from the Lipid maps site

Other methods have been proposed for the rounding and the representation of the aliphatic chain

https://www.lipidmaps.org/tools/ms/kendrick_form.php

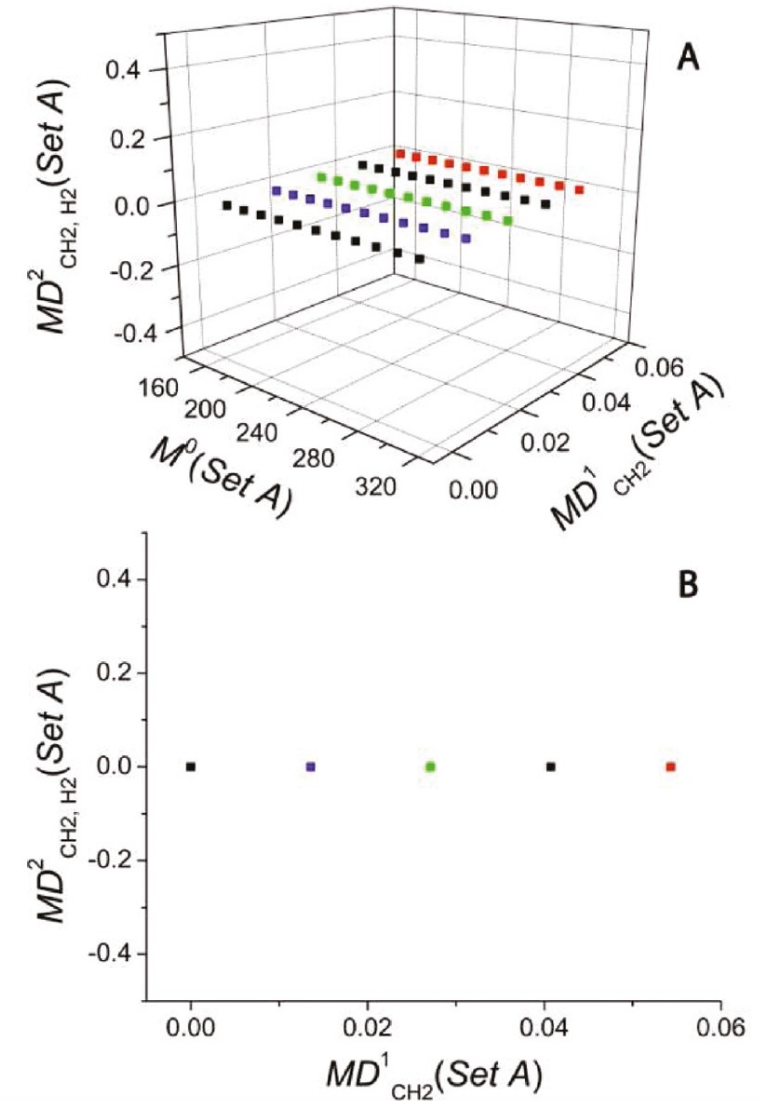


2.4 Second order Kendrick mass defect

Use of a second base (here H₂) to renormalize mass defects, reducing the number of points in the representation

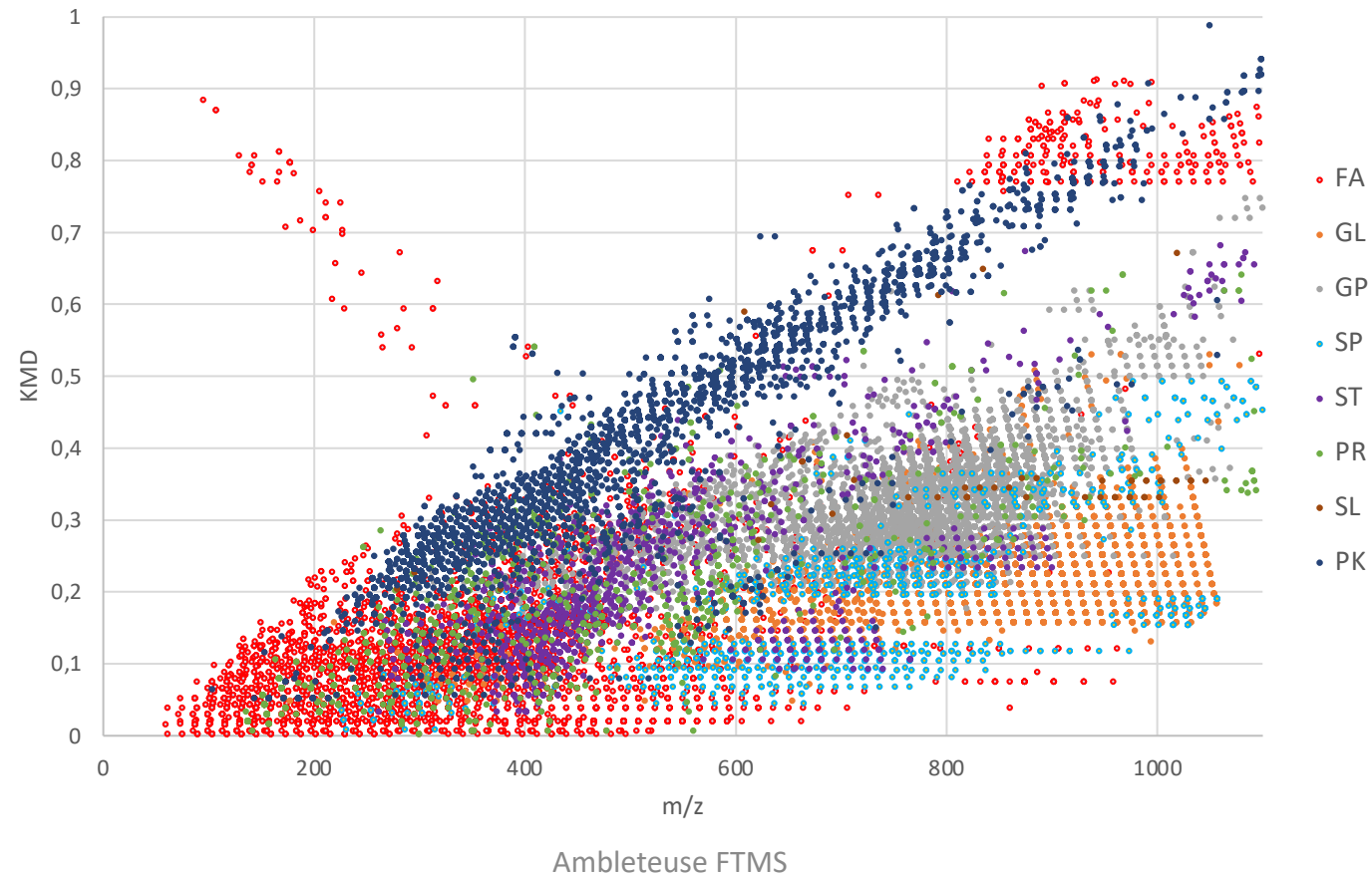
$$M_{B1, B2}^2(\text{peak}) = \frac{MD_{B1}^1(\text{peak})}{MD_{B1}^1(B2)}$$

$$MD_{B1, B2}^2 = MD_{B1, B2}^2(\text{peak}) - \text{ceiling}(M_{B1, B2}^2(\text{peak}), 1)$$



2.5 KMD reference plot

- Theoretical KMD plot for lipids



3. Separation methods

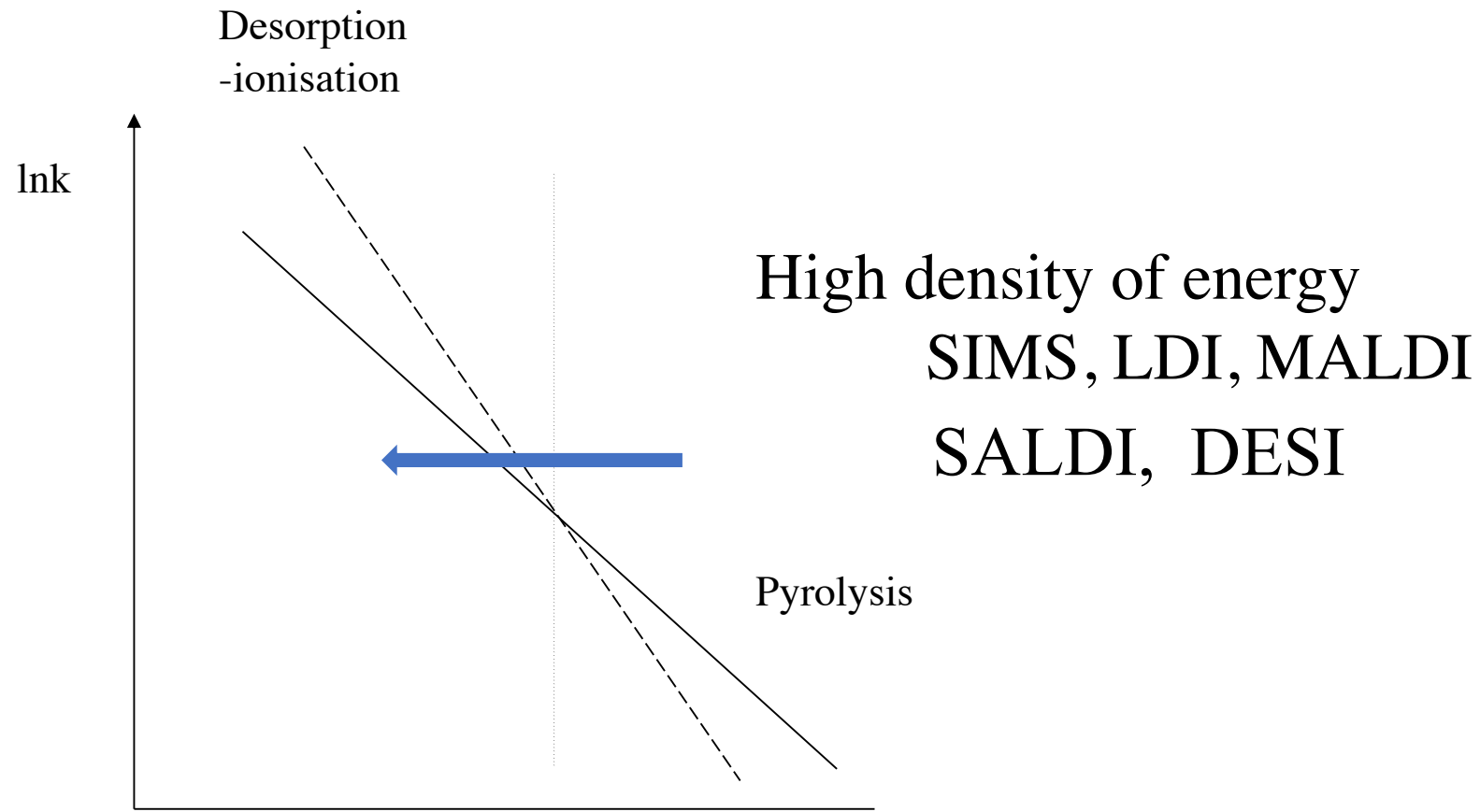
- HPLC RP HPLC for target analysis, HILIC for family based analysis for small polar compounds
- Capillary electrophoresis « ion mobility in solution »: special cases
- « Old » re-emerging method: Thin Layer Chromatography

Mass spectrometry imaging

Reassembling the whole set of properties of MS AND locate the analytes

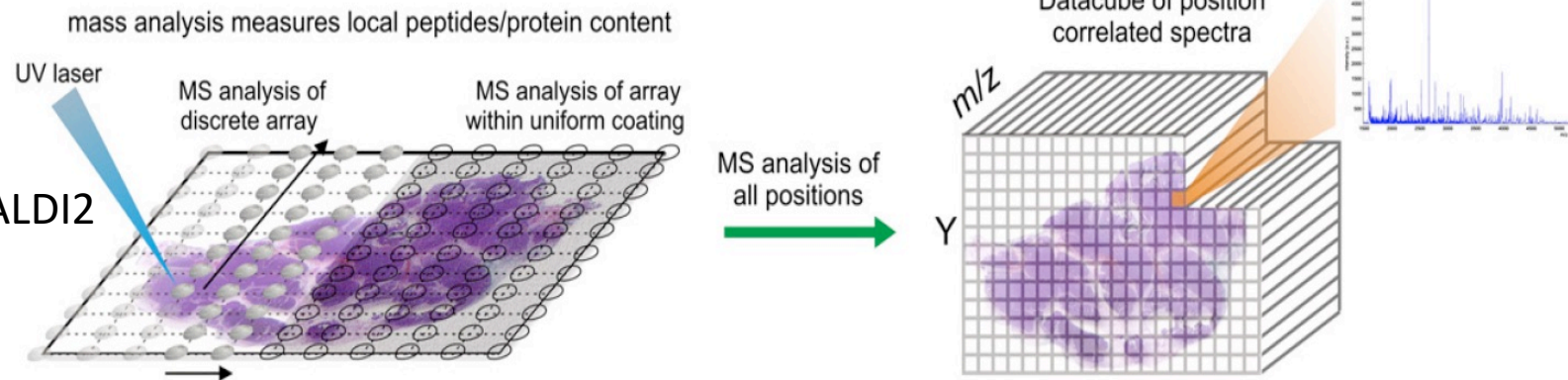
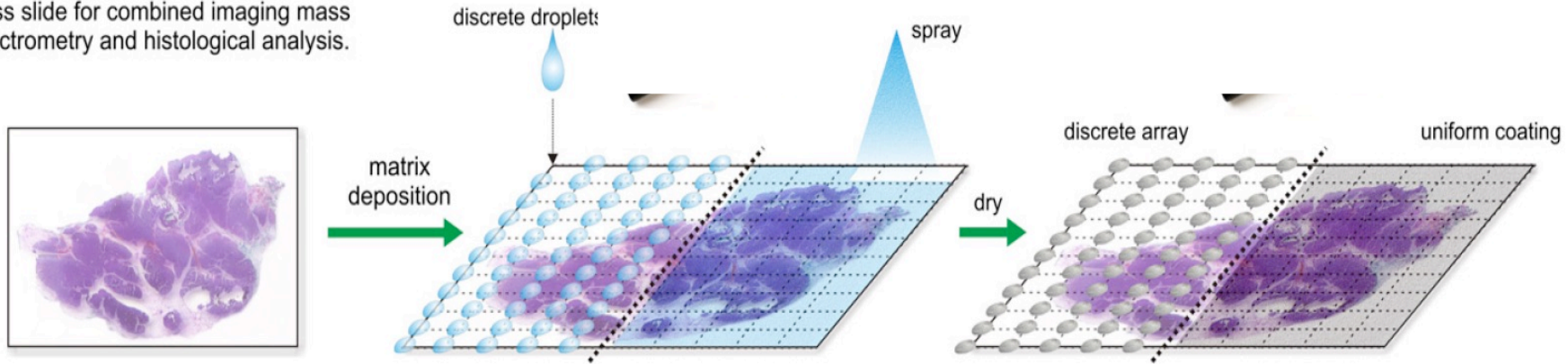
This not only applies to a single spectrum but to a whole set of spectra

Desorption methods from solids



MS Imaging

Tissue section placed onto a conductive glass slide for combined imaging mass spectrometry and histological analysis.

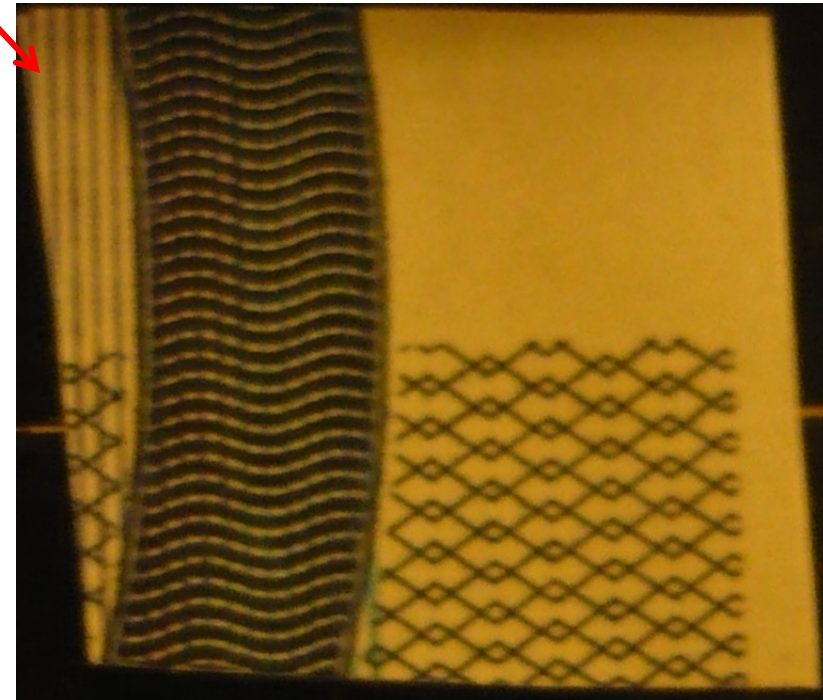


X Peptide and protein imaging mass spectrometry in cancer research
McDonnell L. et al., J Proteomics. 2010,73(10):1921-1944

Each spectrum is a pixel, the distribution of each peak gives an image

Can also be DESI
Improvements with MALDI2

Small molecules imaging

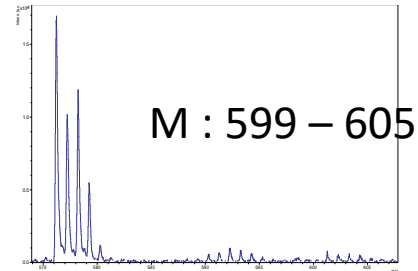


2. LDI, no matrix

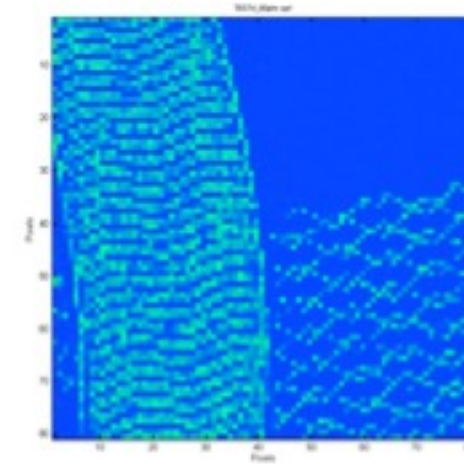
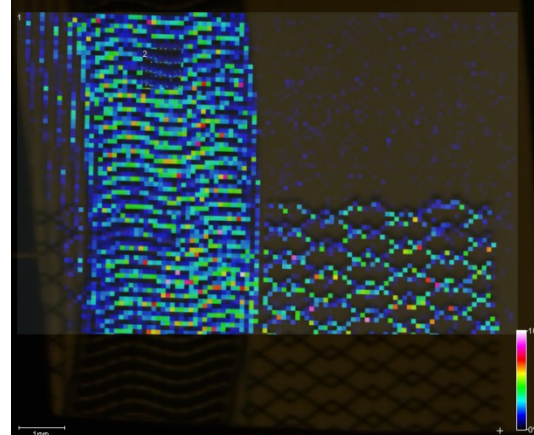
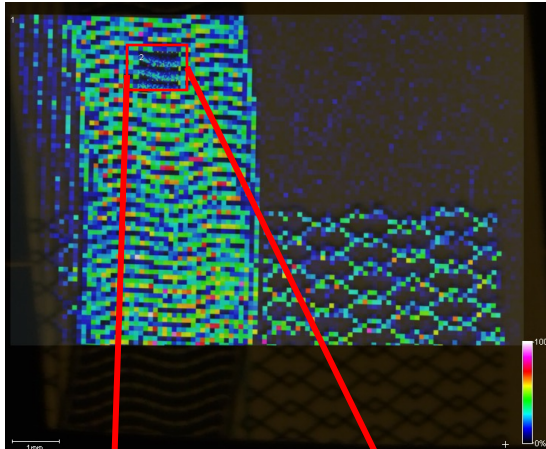
With matrix: no signal

REGION 1 :

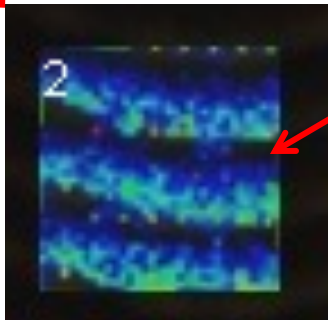
M : 574,5 – 580,5



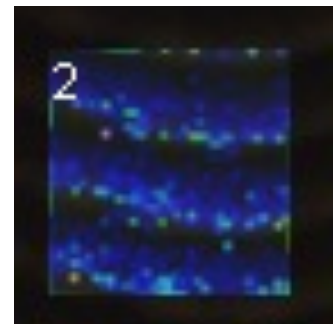
RAMAN, exc 633nm



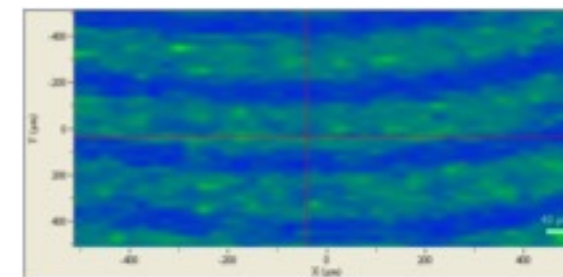
REGION 2 :



gap: ~ 50 μm

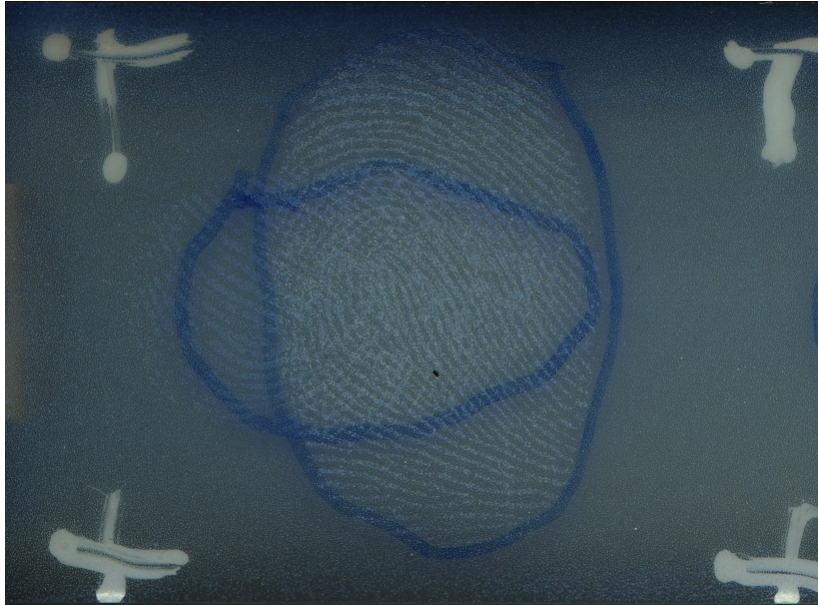


Ambleteuse FTMS

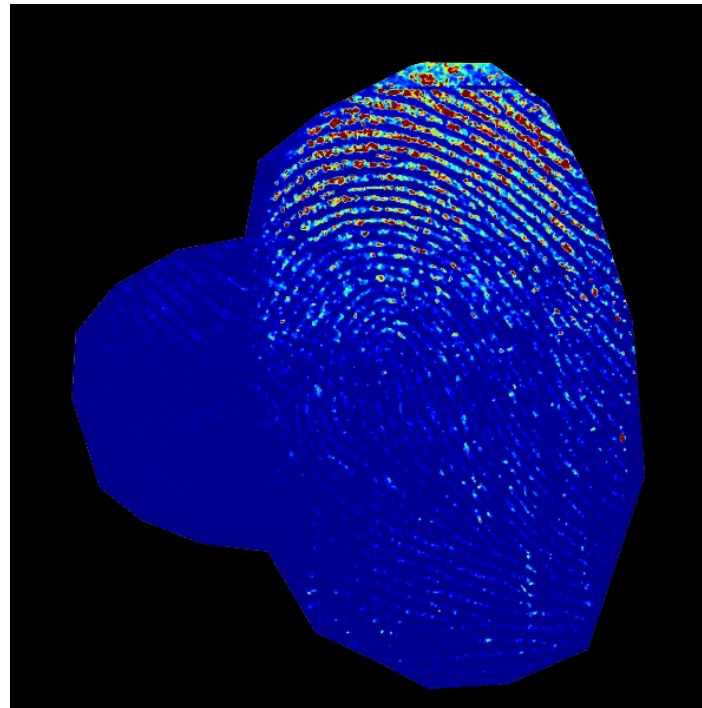
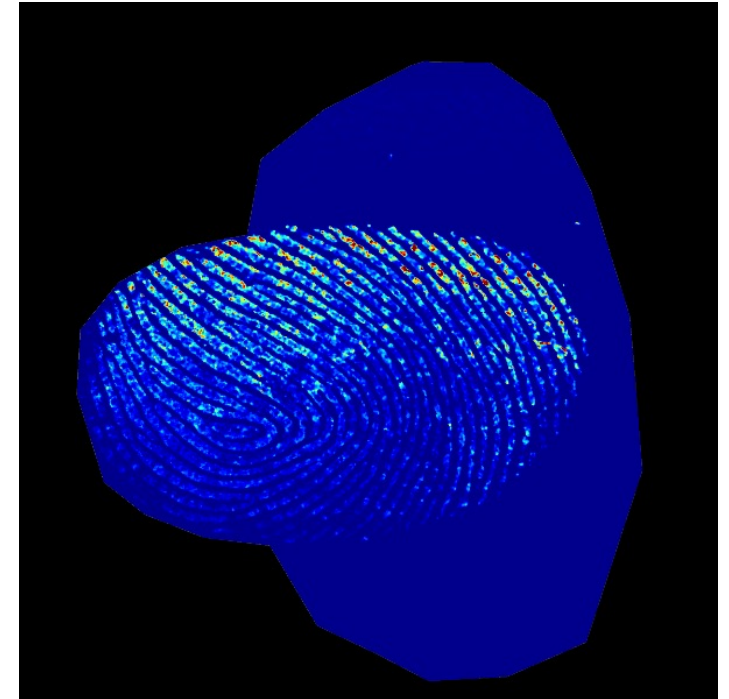


4.2 Fingerprints by MALDI

Empreinte inférieure



Empreinte supérieure

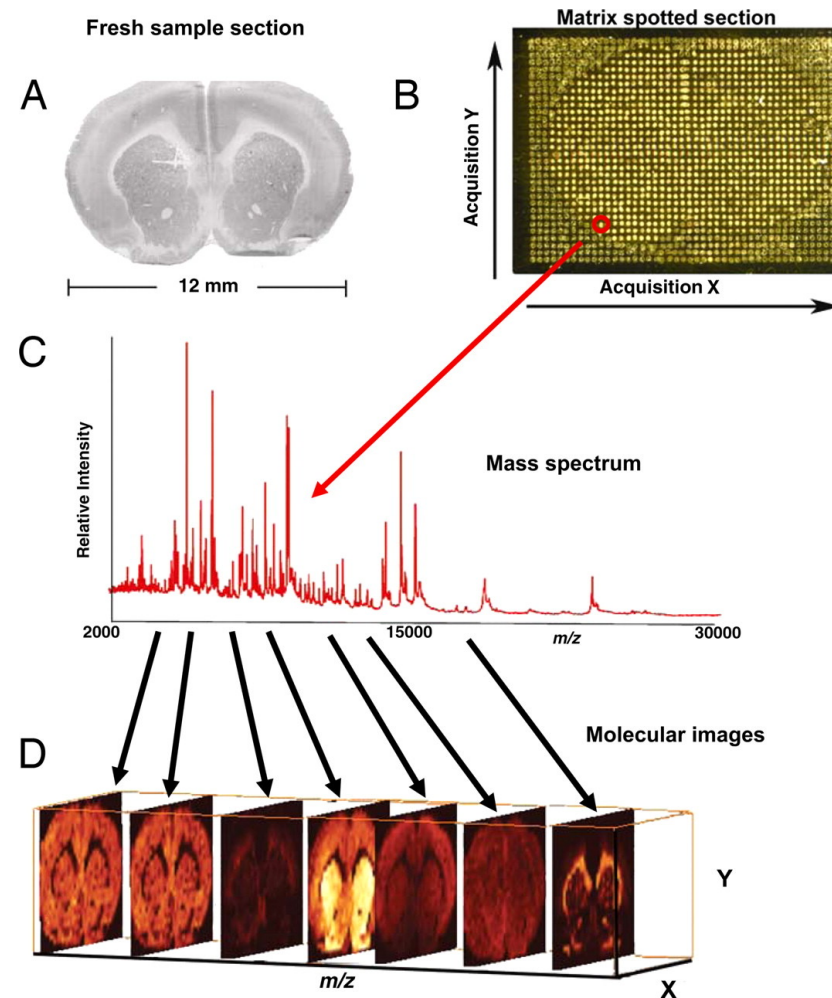


$494,578 \pm 0,06 \text{ Da}$

$283,314 \pm 0,04 \text{ Da}$

Ambiteuse FTMS

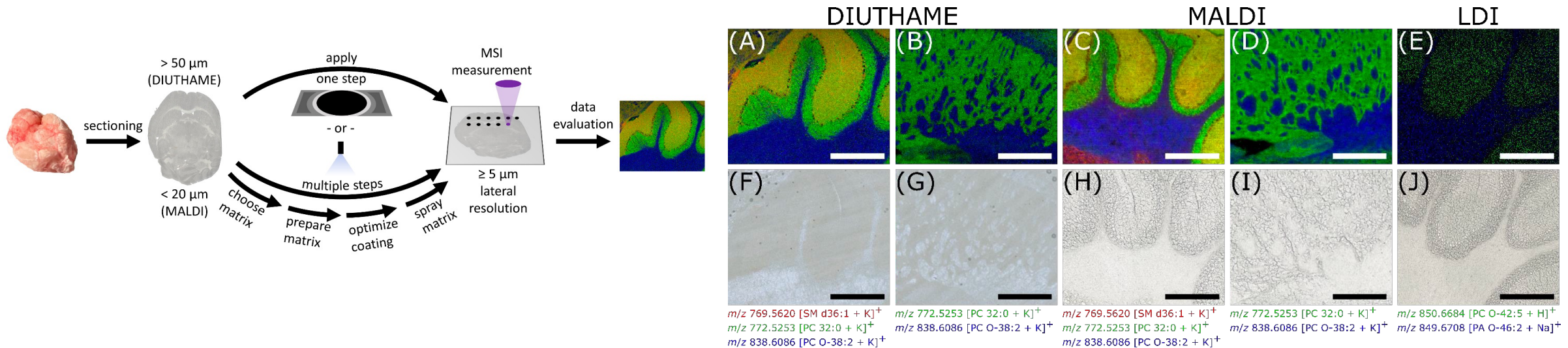
Mass spectrometry imaging, the concept



Seeley E H , Caprioli R M PNAS 2008;105:18126-18131

PNAS

5.5 SALDI imaging



Comparison of DIUTHAME, MALDI and LDI MSI from adjacent mouse brain tissue sections, acquired with a pixel size of 5 µm, an image size of 300 Å ~ 250 pixels, a laser focal diameter of 5 µm, in a mass range of m/z 600–1000. (A), (F), DIUTHAME MS image of mouse brain cerebellum with corresponding microscopic image. (B,G), DIUTHAME MS image of mouse brain striatum ventral region with corresponding microscopic image. (C,H), MALDI MS image of mouse brain cerebellum with corresponding microscopic image. (D,I), MALDI MS image of mouse brain striatum ventral region with corresponding microscopic image. (E,J), LDI MS image of mouse brain cerebellum with corresponding microscopic image. Thickness of tissue sections (A,B), 50 µm, (C–E), 20 µm. Scale bars: 500 µm.

Max A. Müller, Dhaka R. Bhandari and Bernhard Spengler *Metabolites* 2021, 11, 624.
<https://doi.org/10.3390/metabo11090624>

3. ISD: classical fragments (thermal like) or chemically induced (radical ion precursor)

Matrices with high hydrogen radical donor abilities, such as the 1,5-DAN favor the radical-induced pathway (*c*-, *z*-, *w*- and *d*-type fragments)

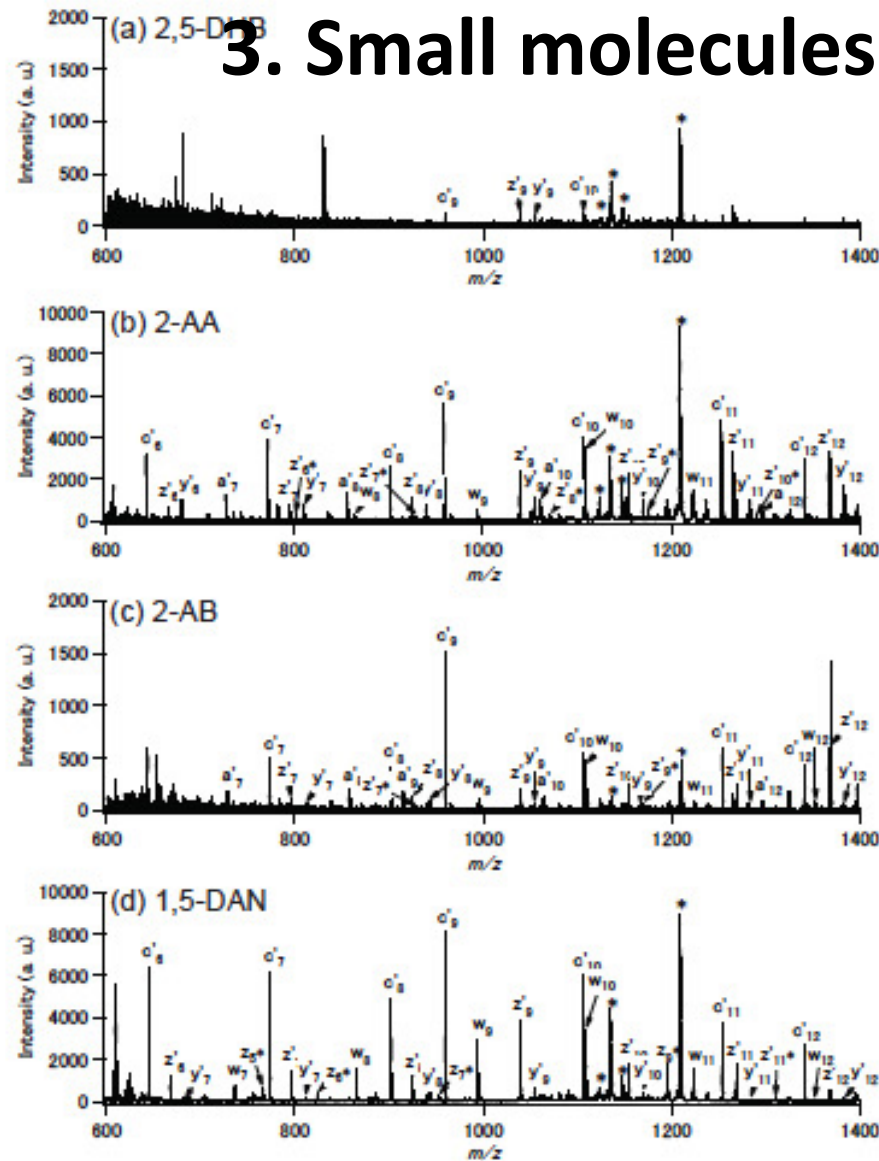
Protons play a major role in thermal like fragmentations

Matrices with a low proton affinity, as the 2,5-DHB, favor the thermal pathway (*a*-, *b*- and *y*-type fragments) and the formation of the *d*-type fragments.

Matrices may present an intermediate behavior such 5-Aminosalicylic acid (5-ASA)

CID is more sensitive to laser fluence than ISD

3. Small molecules: Peptides



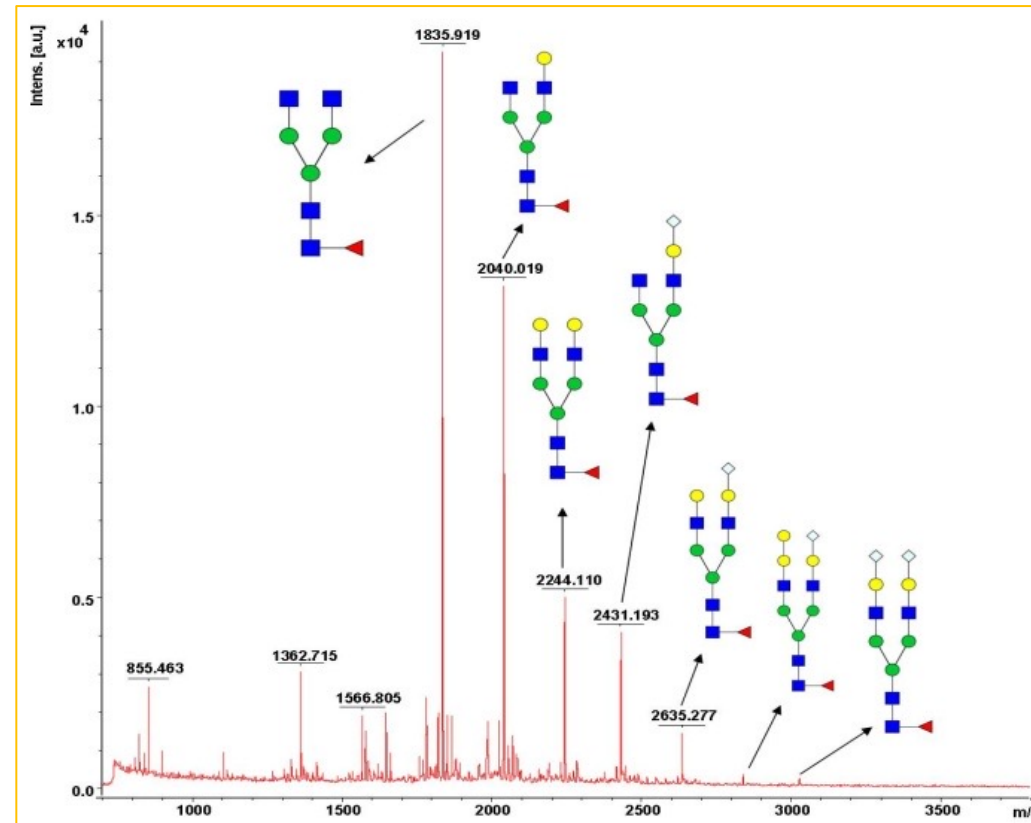
Initial velocity is an important parameter in the balance between fragmentation pathways

Figure 3. Negative-ion MALDI-MS/MS spectra of (Glu1)-fibrinopeptide B obtained with (a) 2,5-DHB, (b) 2-AA, (c) 2-AB and (d) 1,5-DAN. The asterisk for z ions denotes the matrix adduct of the z ions. Asterisk indicates metastable peaks.

3. Glycan analysis - Glycosylations of proteins

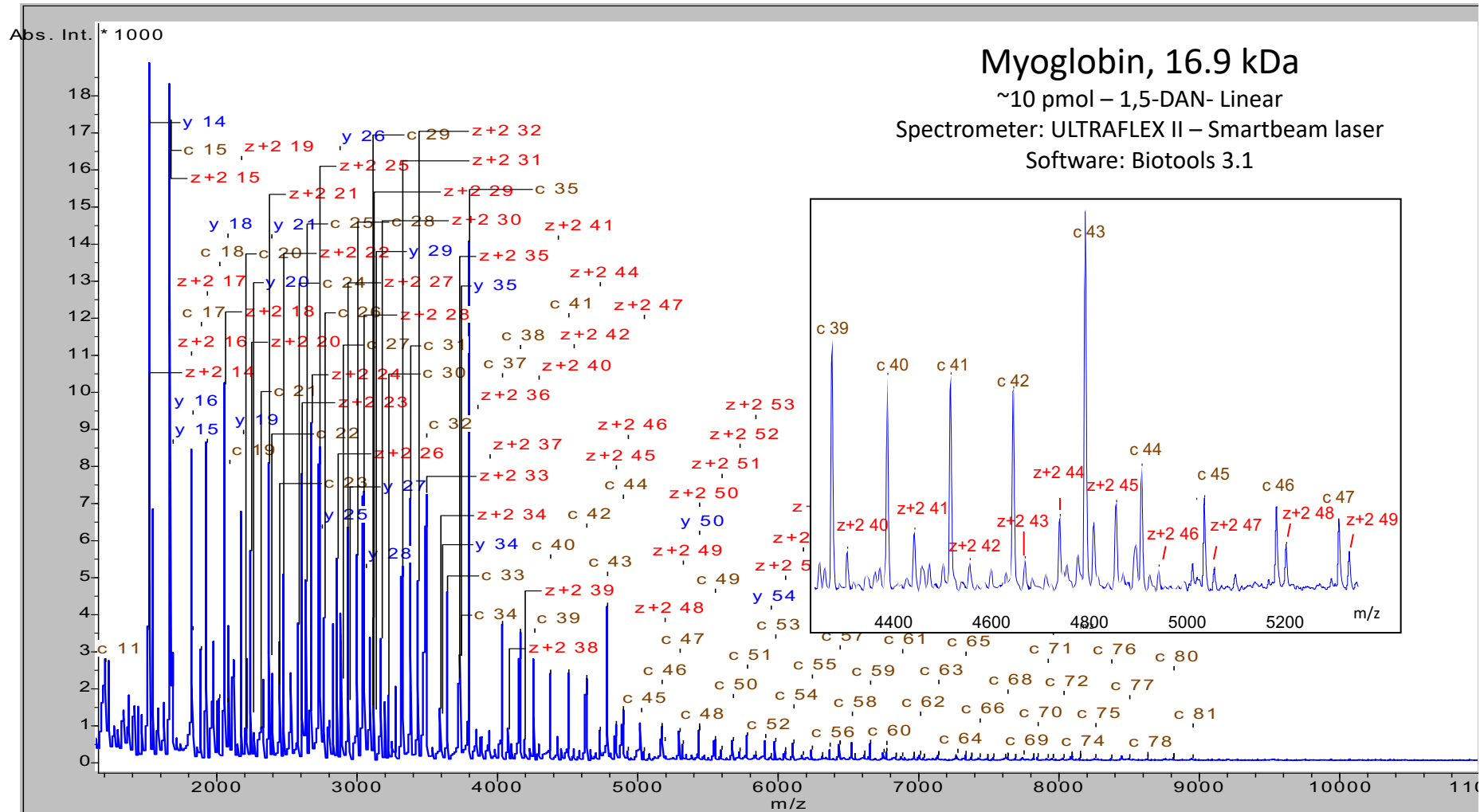
- *N*- and *O*-Glycans repertoire determination (on cell extract or pure proteins)
- Glycopeptides enrichment methods
- Identification of Glycosylation sites on proteins

** MALDI-MS and MSMS on permethylated glycans*



Ambleuse FTMS

4. ISD top down sequencing using c- and z- ions

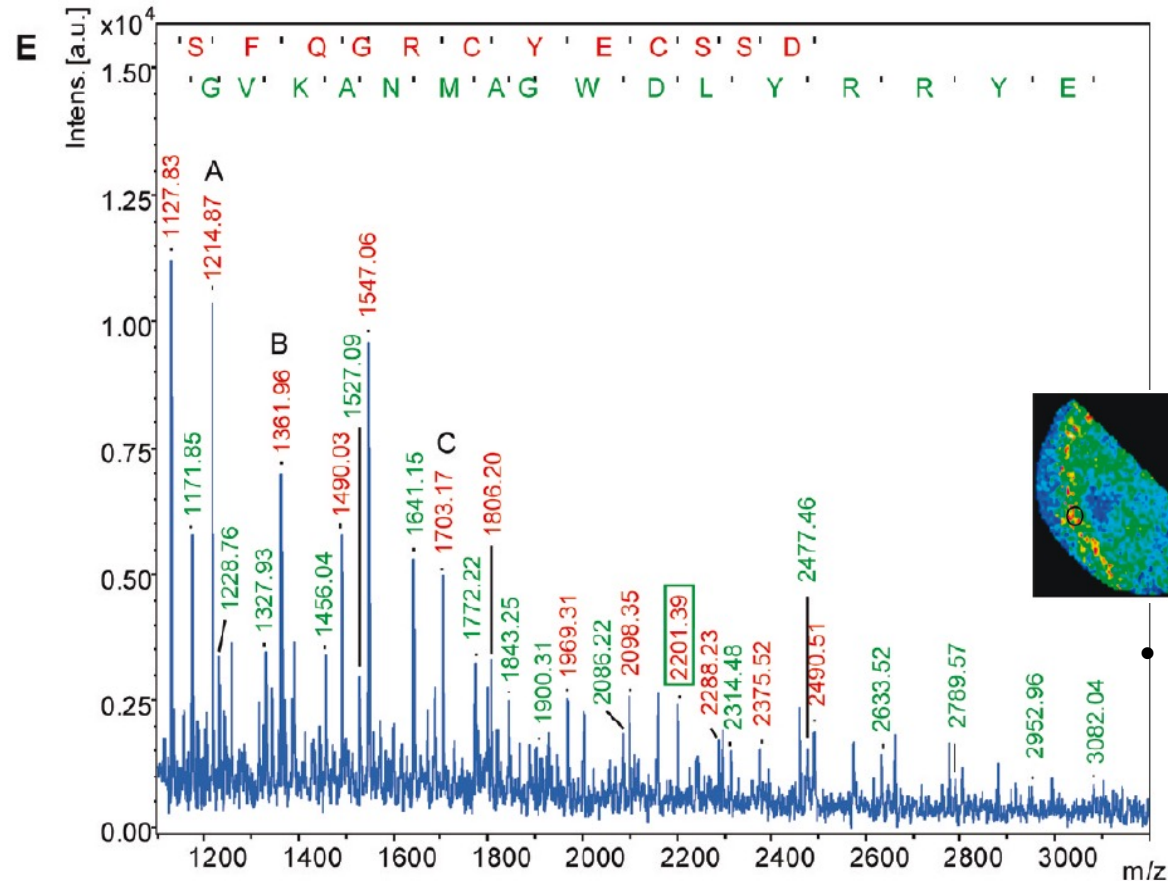


Large series of c- and z-ions are effectively generated allowing TOP-DOWN sequencing

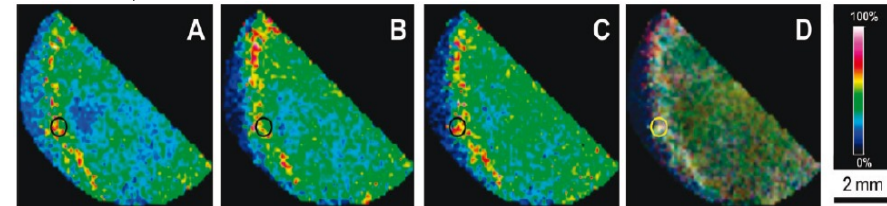
Proteins identification : *in situ* top-down ISD

MALDI – In Source Decay Applied to Mass Spectrometry Imaging : A new toll for protein identification

Debois D. et al.. Anal. Chem..2010.82(10):4036-4045



- Direct β -crystallin B2 identification by MALDI-ISD

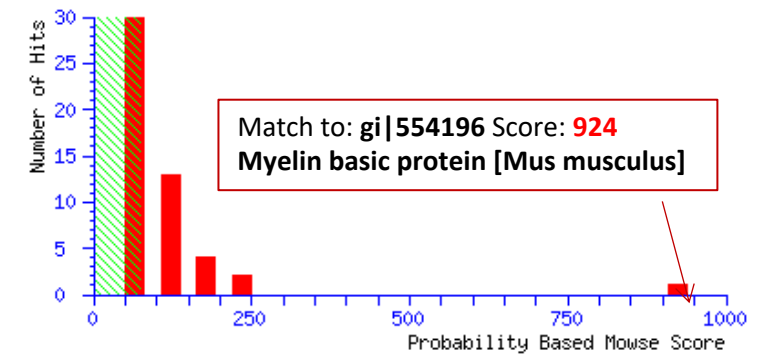
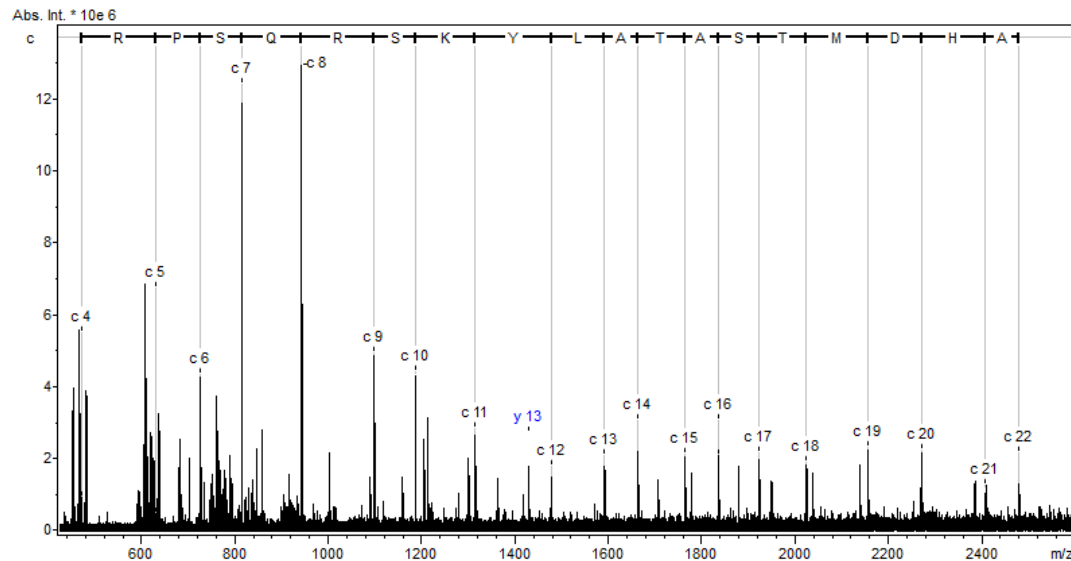
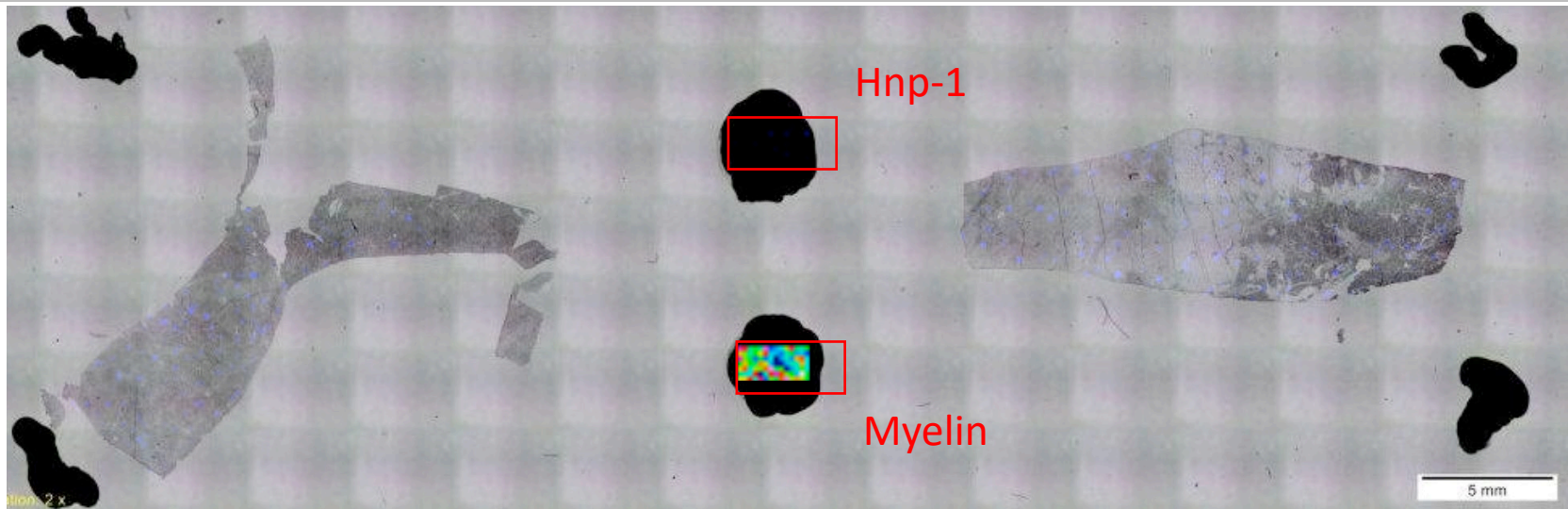


- β -crystallin B2 localization in eye lens

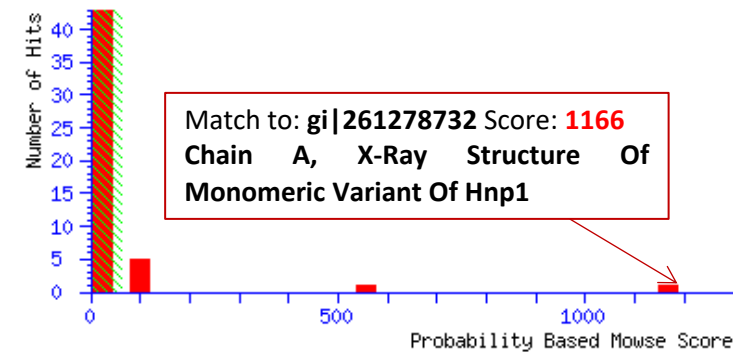
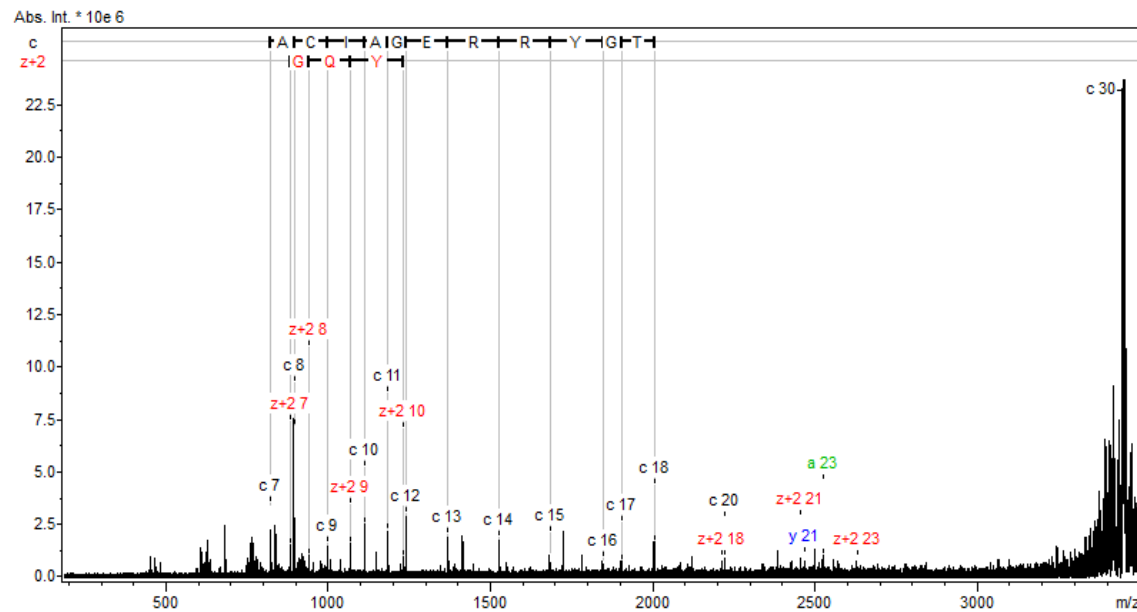
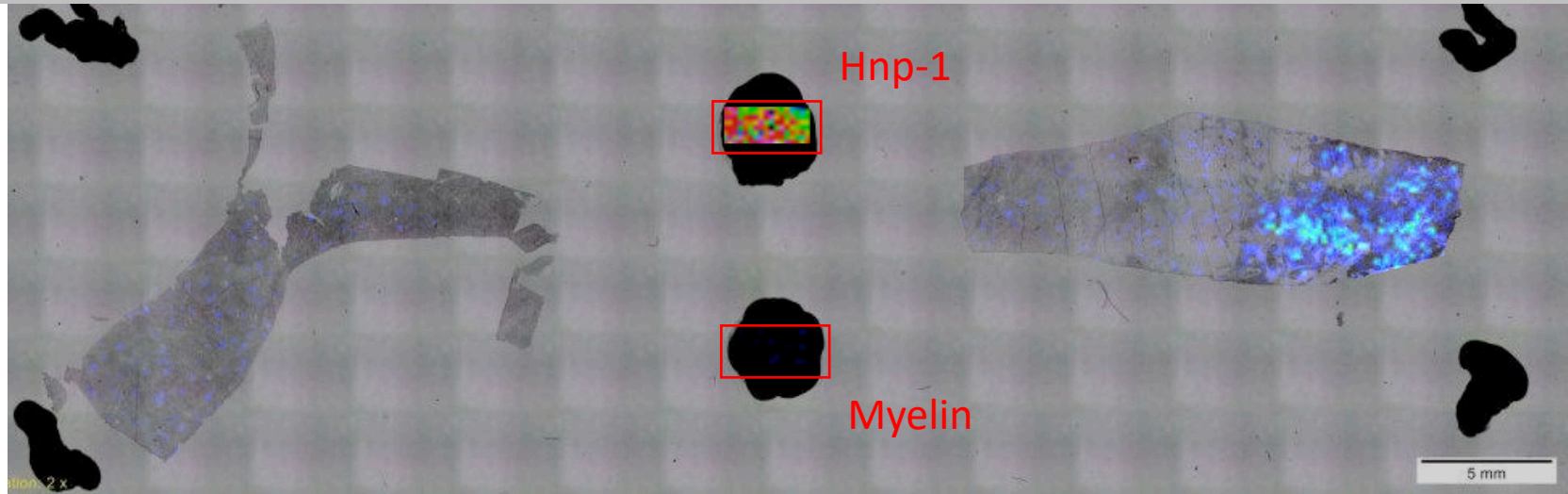
Possibility to identify proteins by ISD

without tissue slice treatment (use of matrix such as 1,5 DAN) and selection of precursor ion

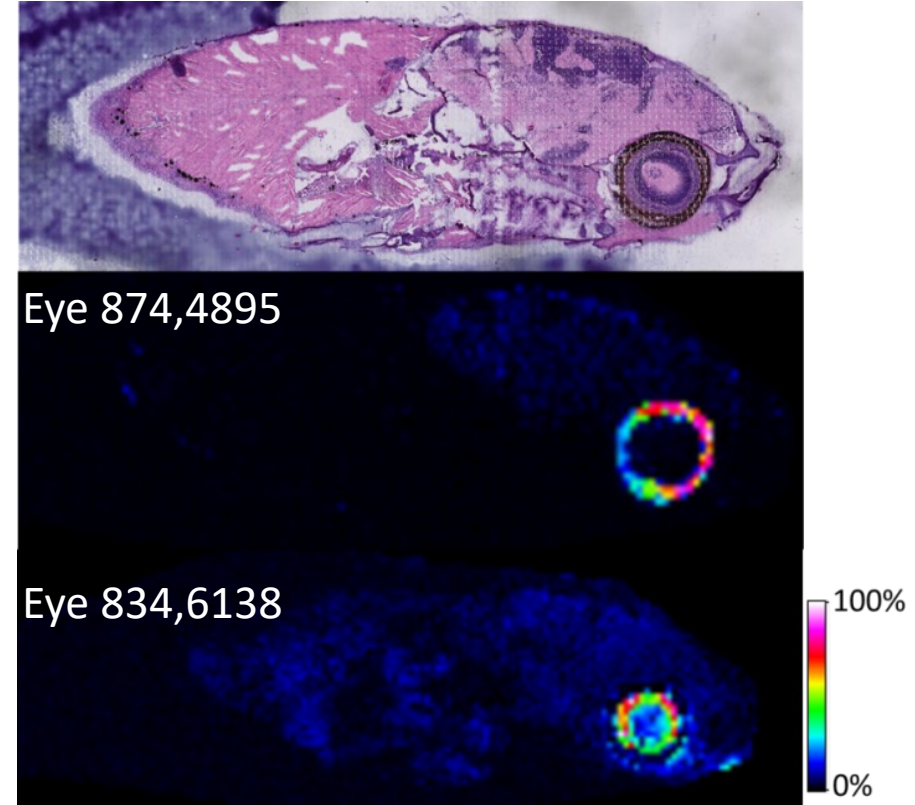
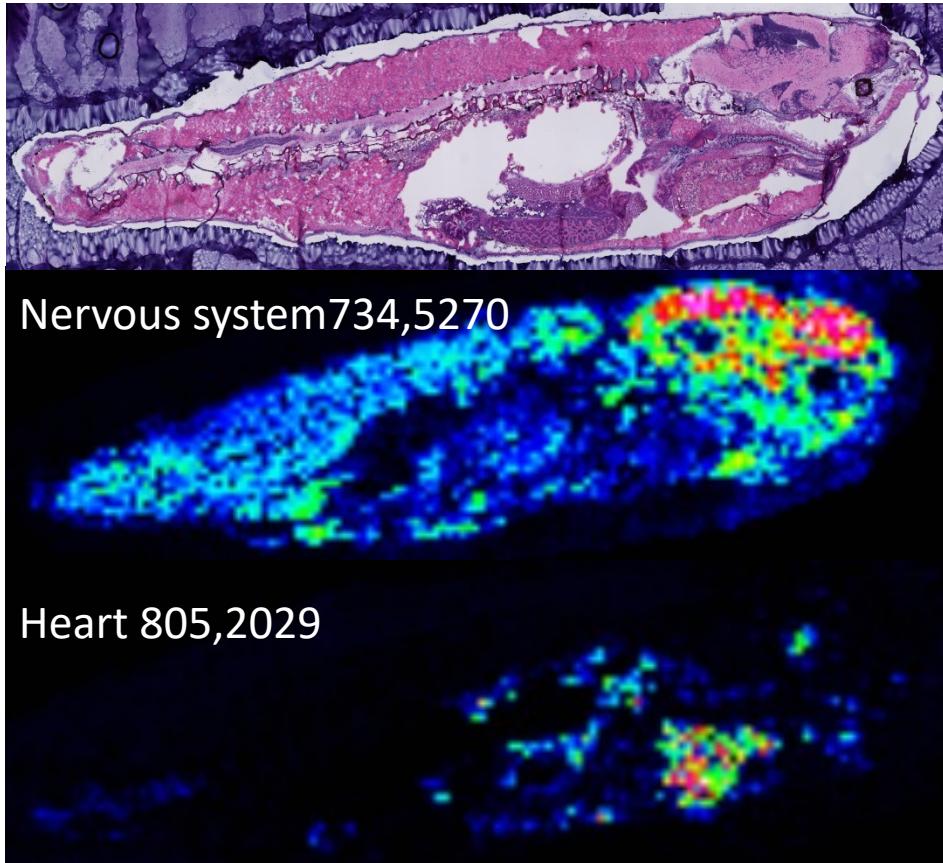
4.4 Proteins identification by MALDI-MSI in source decay



Proteins identification by localization and MALDI-MS/MS profile matching

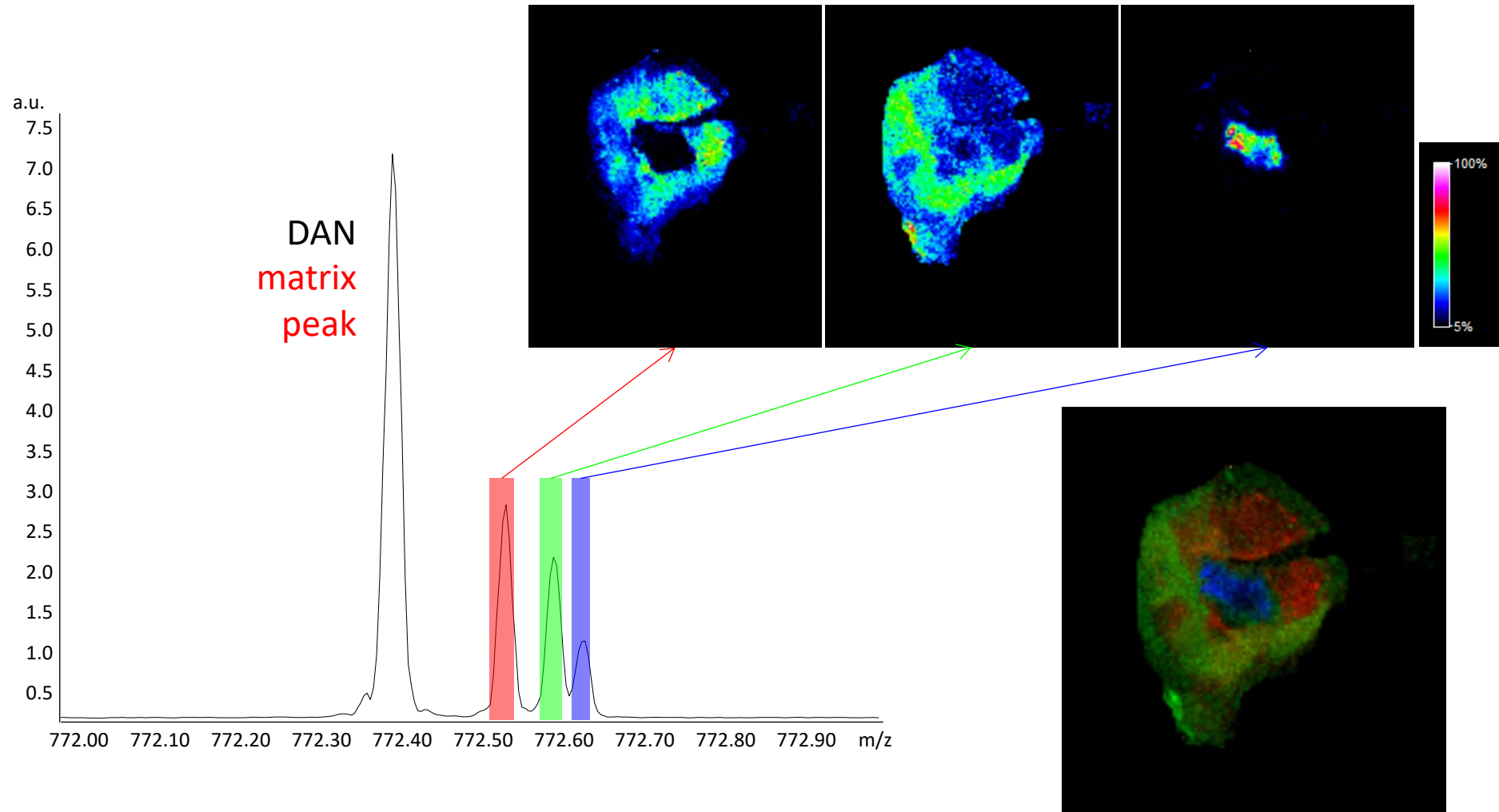


4.3 Molecular Histology

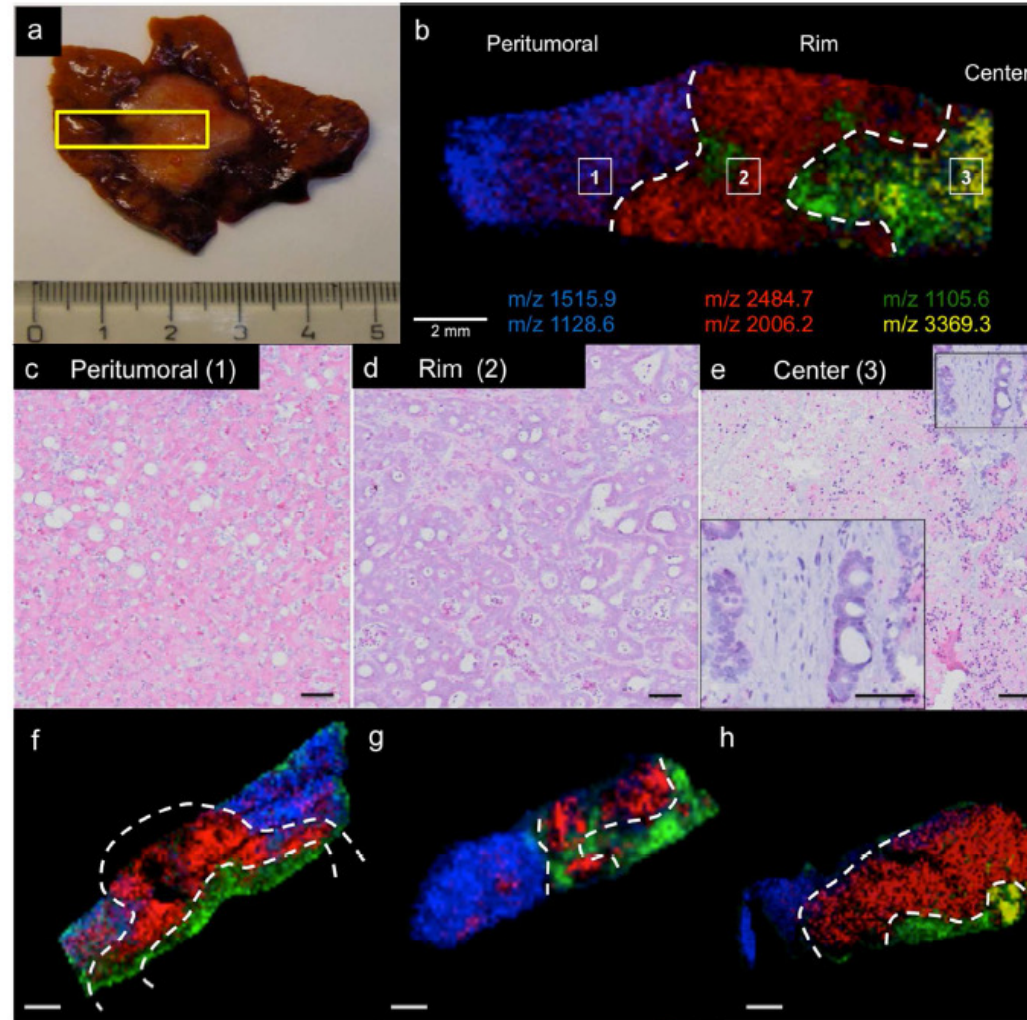


Careful with suppression effects

4.5 High mass resolution for better lateral resolution



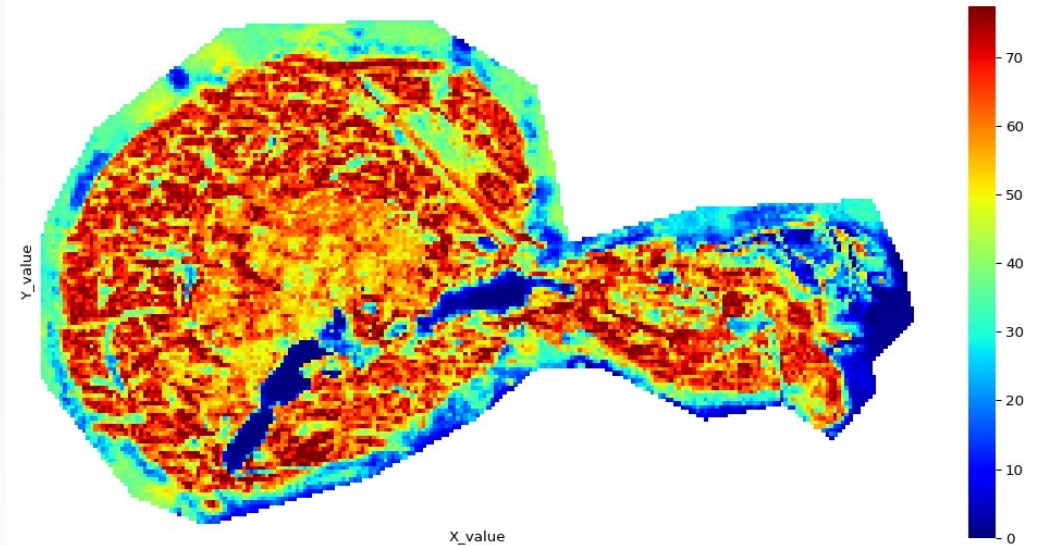
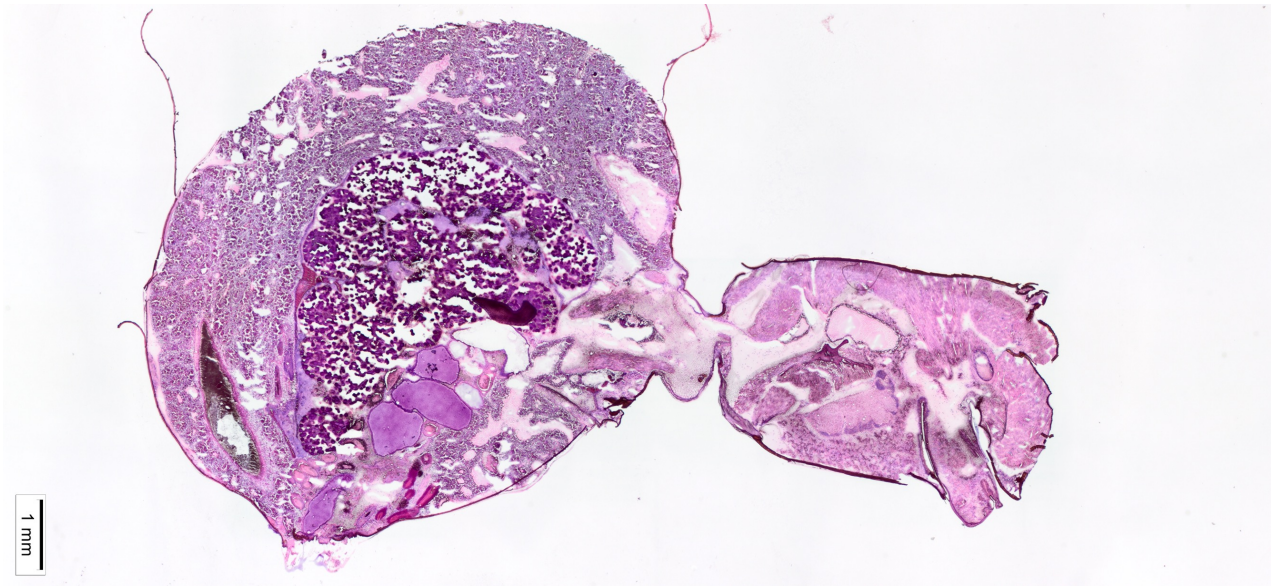
4.6 Scaling down proteomics thanks to Laser Microdissection guided by MALDI



See serie of papers of Remi Longuespée

5.4 Imaging lipids on special samples

- MALDI imaging of a female spider



Examples

5. Lipids

6. Lipopeptides

5. Lipids

Category or family	Structures in Database
Fatty acyls (FA)	10,078
Glycerolipids (GL)	7,680
Glycerophospholipids (GP)	9,993
Sphingolipids (SP)	4,906
Sterol Lipids (ST)	3,184
Prenol Lipids (PR)	1,596
Saccharolipids (SL)	1,335
Polyketides (PK)	7,026

Golden standards methods are based on HPLC MS/MS

Reliable, quantitative, require internal standards

Compound targeted method

Slow and expensive method

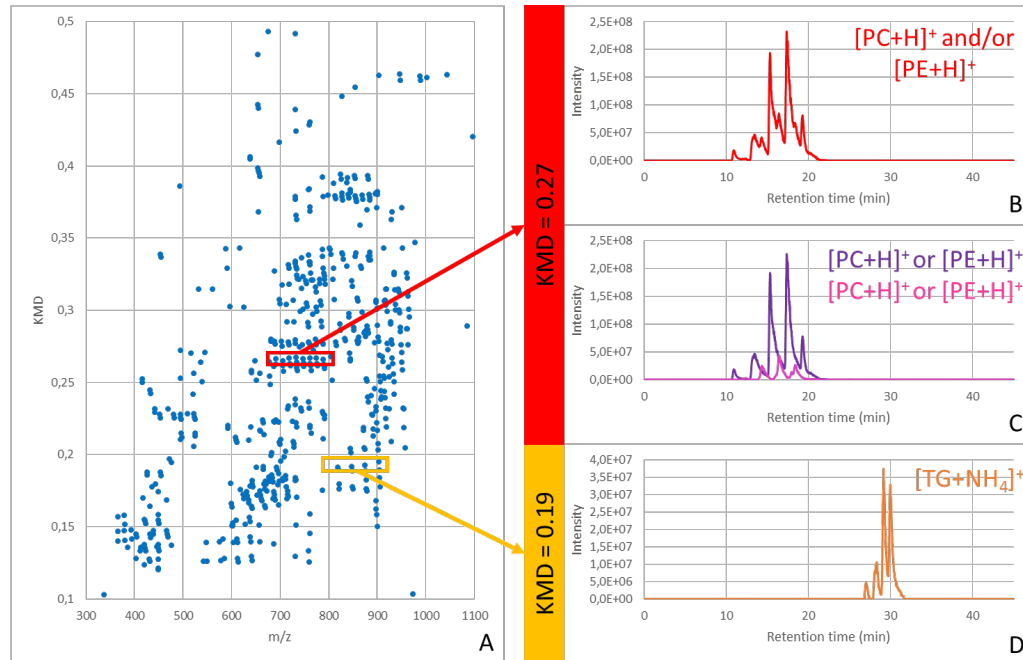
Fast screening method: infusion

Table 1 : Lipid categories of the comprehensive classification system and the number of structure in the LIPID MAPS database.

The LIPID MAPS structure database (LMSD, Nature Lipidomics Gateway, <http://www.lipidmaps.org>), currently contains 43,798 unique lipid structures (as of 03/02/2022)¹².

5. 1 Lipids HPLC MS/infusion

No need to check all peaks, automatically detected



Chromatogram extraction from the Kendrick plot.

(A) Kendrick mass defect plot of the spectra obtained by LC-MS.

by selecting a specific horizontal line in the Kendrick plot, it is possible to filter the Kendrick plot and provide the extracted ions chromatogram of this selection box.

(B) Chromatogram from a specific line of protonated phosphatidylcholines (PC) and/or phosphatidylethanolamines (PE) which are isomers.

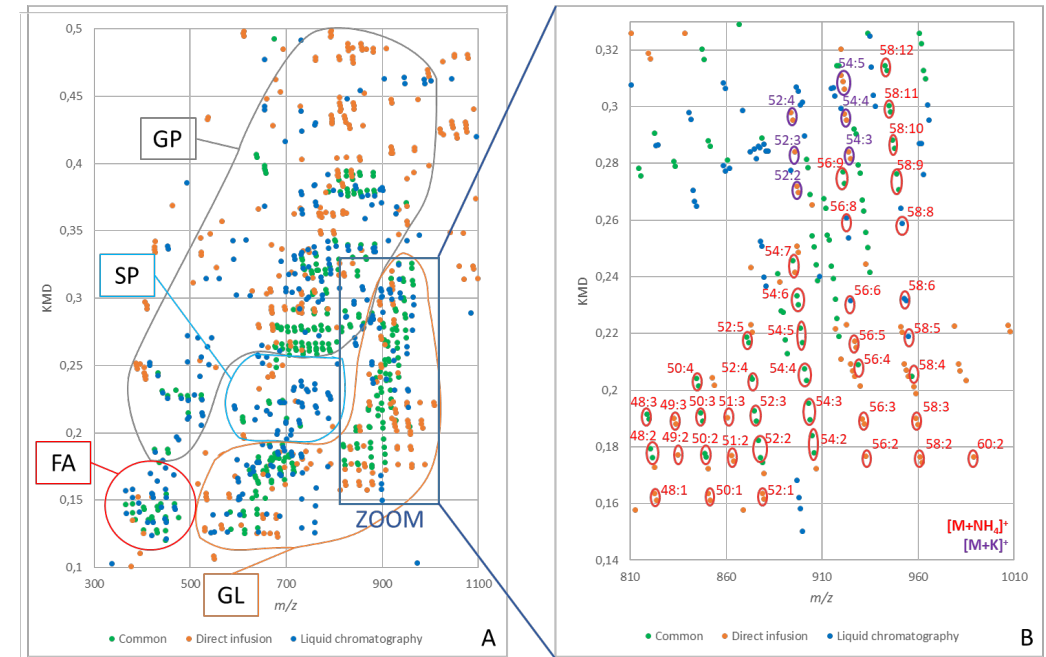
(C) Extraction of chromatogram B allow us to separate two families of PC and/or PE from CH_2 spacing.

(D) Chromatogram from a specific line of triglycerides (TG detected as ammonium adducts).

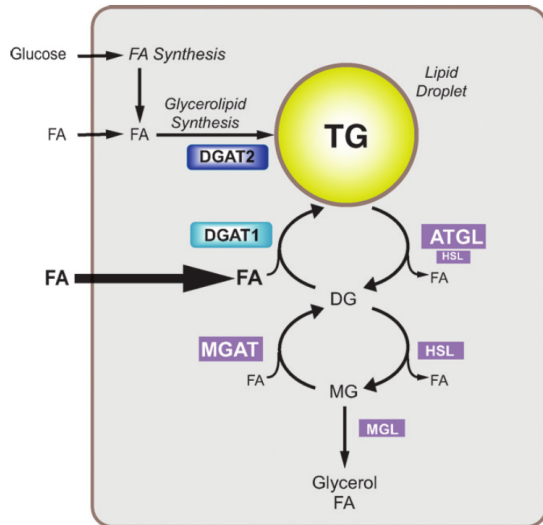
The retention time is different from the PC and PE one due to the size of the lipids

Comparison HPLC/infusion

(A) Lipids families comparison by the Kendrick plots of the yeast sample analysed by nESI-FT-ICR and RP-UPLC-ESI-FT-ICR. The area containing the fatty acyls (FA) is circled in red, the glycerolipids (GL) in orange, the sphingolipids (SP) in blue and the glycerophospholipids (GP) in grey.
(B) Deep triglycerides comparison

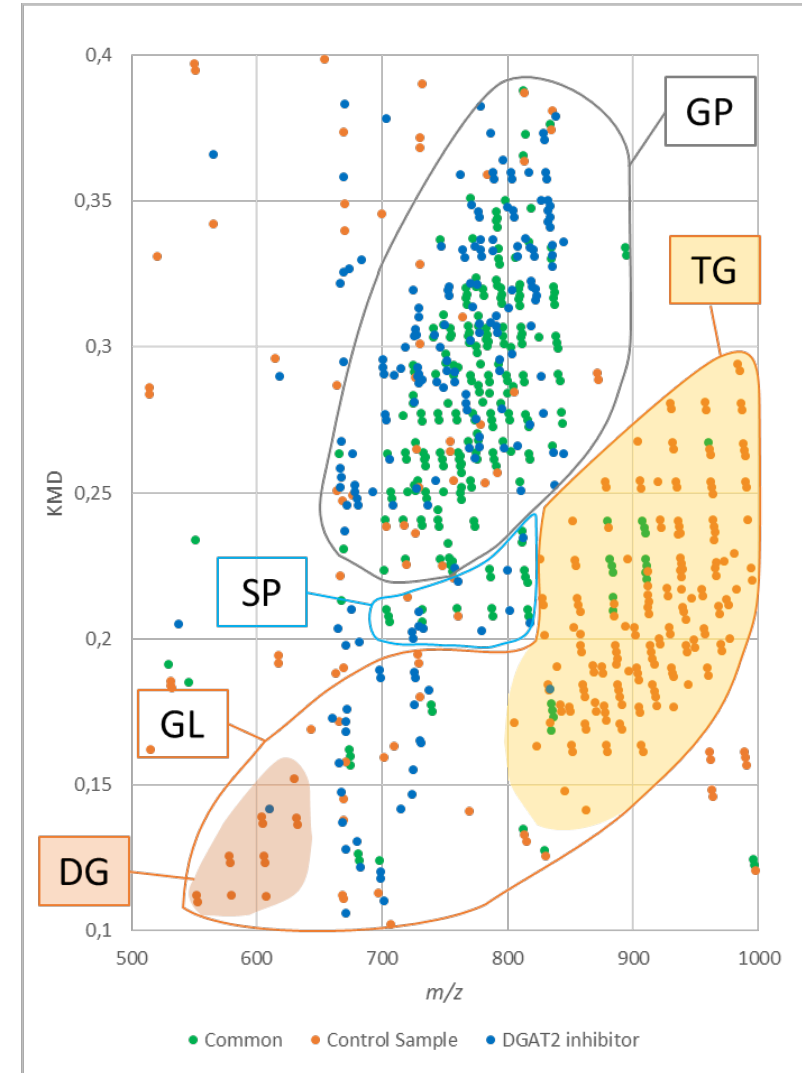


5.2 Quick evaluation of the lipids composition of a normal and modified cell line



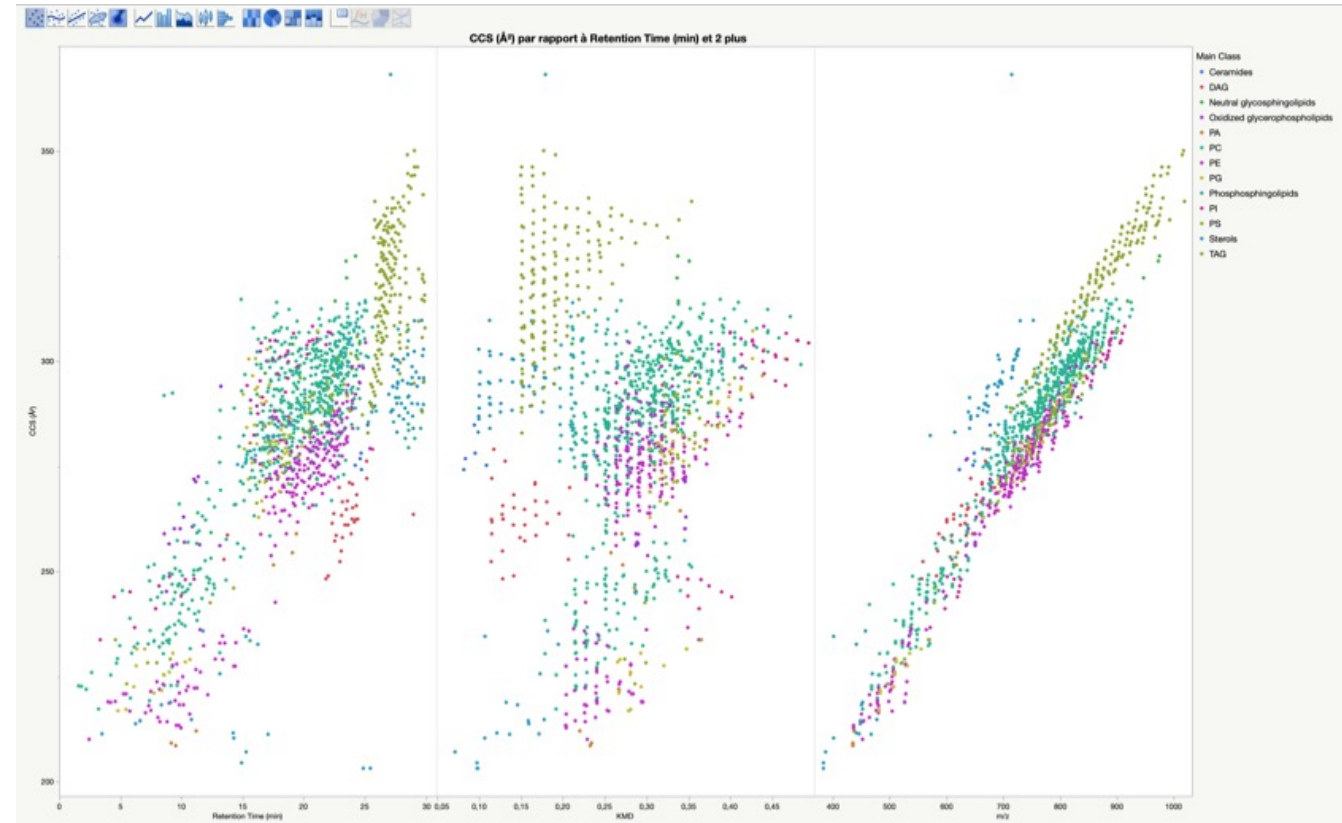
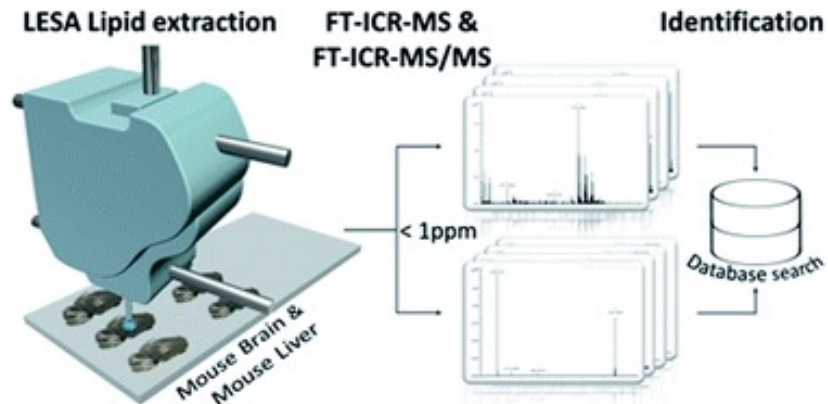
Infusion
KMD map

Intracellular role of DGAT2. DGAT2 is an enzyme involved in the triglycerides synthesis and which are themselves transformed in diglycerides with the action of the adipose-tissue triglyceride lipase



5.3 Additionnal informations

- Retention time
- CE elution time
- TLC RF
- LESA extraction
- LASER microdissection

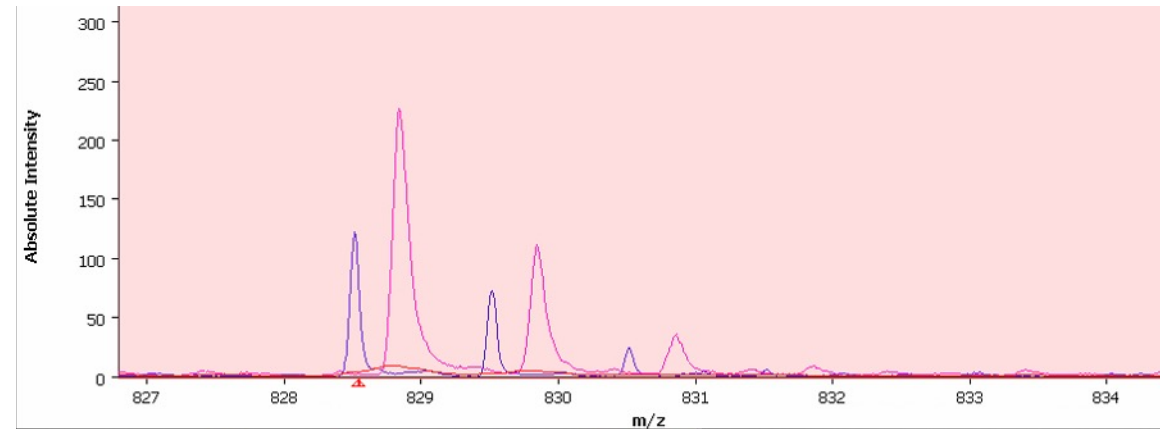
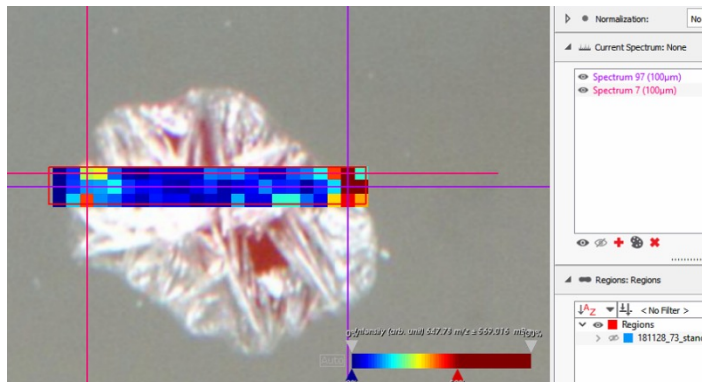


Data from <https://www.nature.com/articles/s41467-019-14044-x>

From Haler and al. <https://pubs.rsc.org/en/content/getauthorversionpdf/c8ay02739k>

5.6 Imaging lipids: the problem of mass shift

- Accuracy can be good on a pixel but summing spectra to obtain the mean spectrum of the image can reduce resolution and accuracy
- Mass shift



What are the origins of the problem?

For TOF: planeity of the sample
For FTICR: number of ions in the cell

5.7 Considerations for a robust MS calibration for MSI

- Mass shift prevention due to heterogeneous sample topology: Important for TOF instruments, control of the matrix deposition
- Mass shift prevention due to the range of the TIC: Important for FTICR instruments
- Mass shift reduction by data post processing: Requires the identification of some known compounds for pixel by pixel recalibration

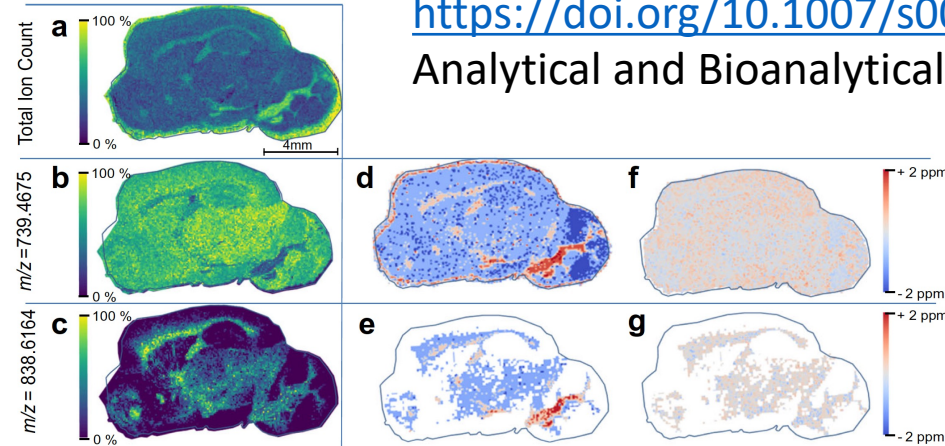


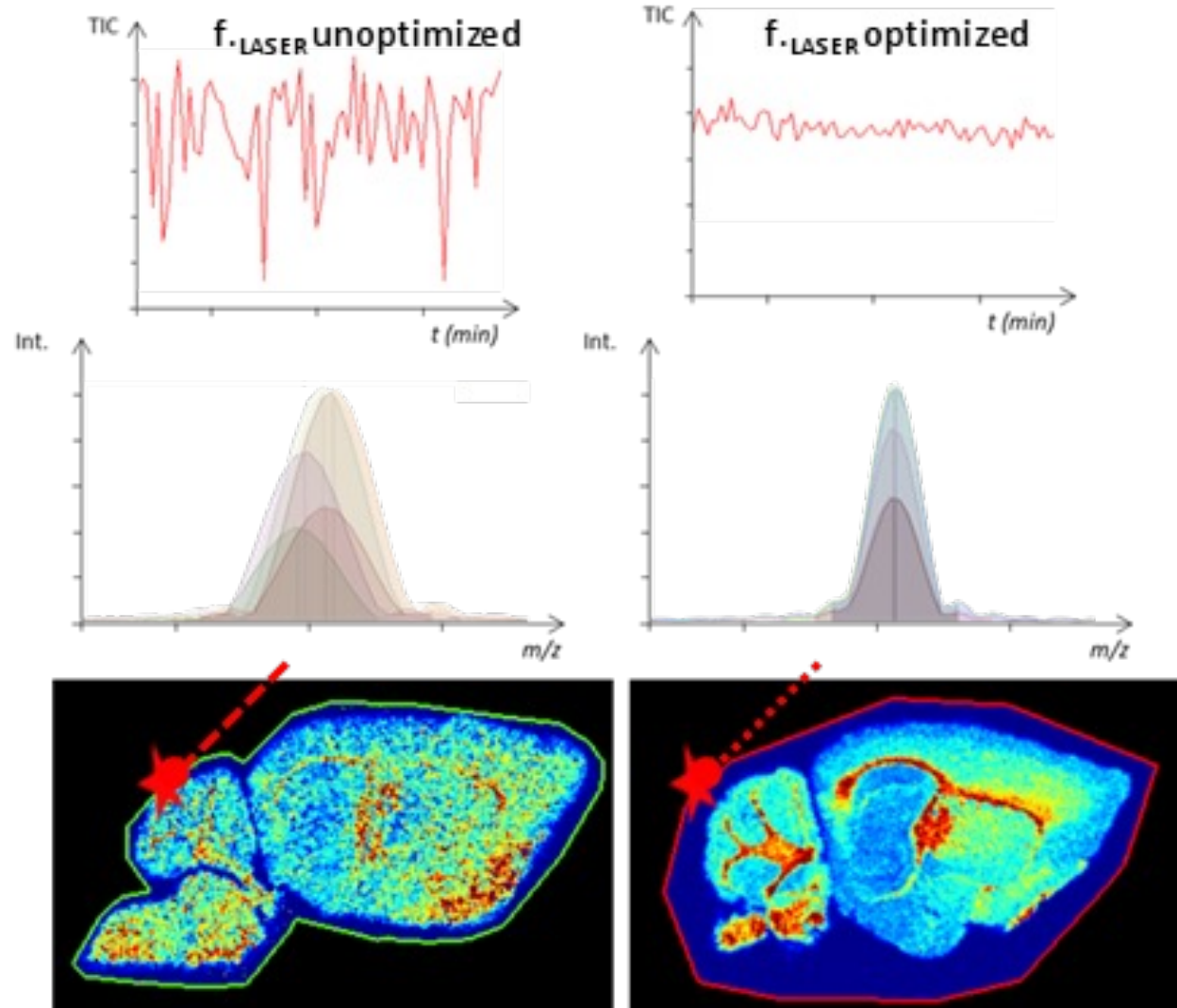
Fig. 6 Mass spectrometry imaging of mouse tissue section acquired on a solarix XR 9.4T MALDI FT-ICR using our recommended optimized workflow (i.e., flat tissue section, homogeneous HCCA matrix deposition, 20 laser shots per pixel for TIC (total ion current) stabilization) at nominal resolving power FWHM higher than 220,000 at m/z 800. **a** is the image of the TIC. **b** and **c** depict mass spectrometry images of [PA 36:2+

$K]^+$ and [HexCer 40:1;O3+K] $^+$ respectively (black pixel in (c) corresponds to pixel where [HexCer 40:1;O3+K] $^+$ is not detected). **d** and **f** depict mass shifts heatmaps of [PA 36:2+K] $^+$ before and after post-processing recalibration respectively. **e** and **g** are the mass shifts heatmaps of [HexCer 40:1;O3+K] $^+$ before and after post-processing recalibration respectively

<https://doi.org/10.1007/s00216-021-03174-1>

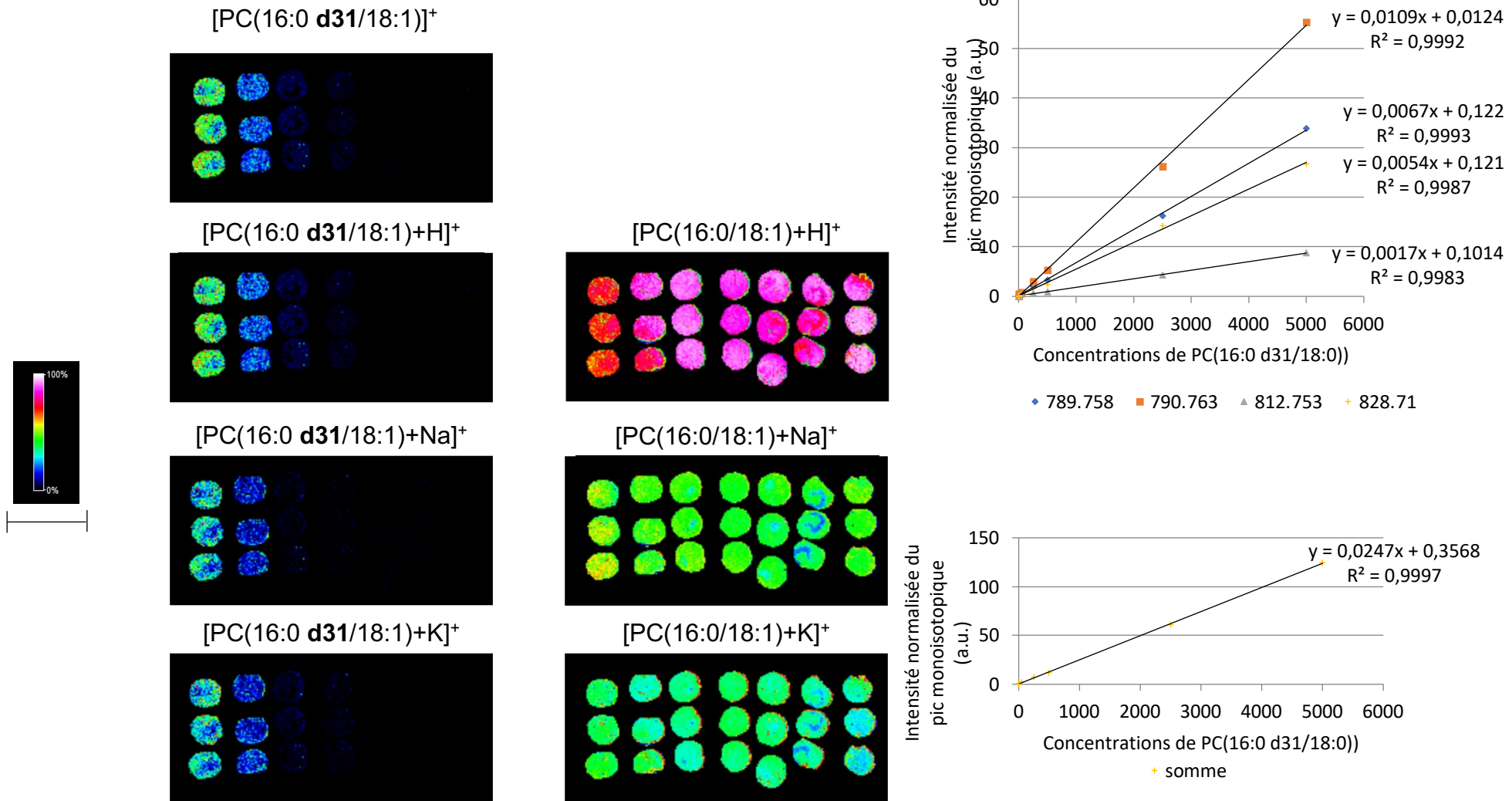
Analytical and Bioanalytical Chemistry (2021) 413:2831–2844

5.8 Image improvements



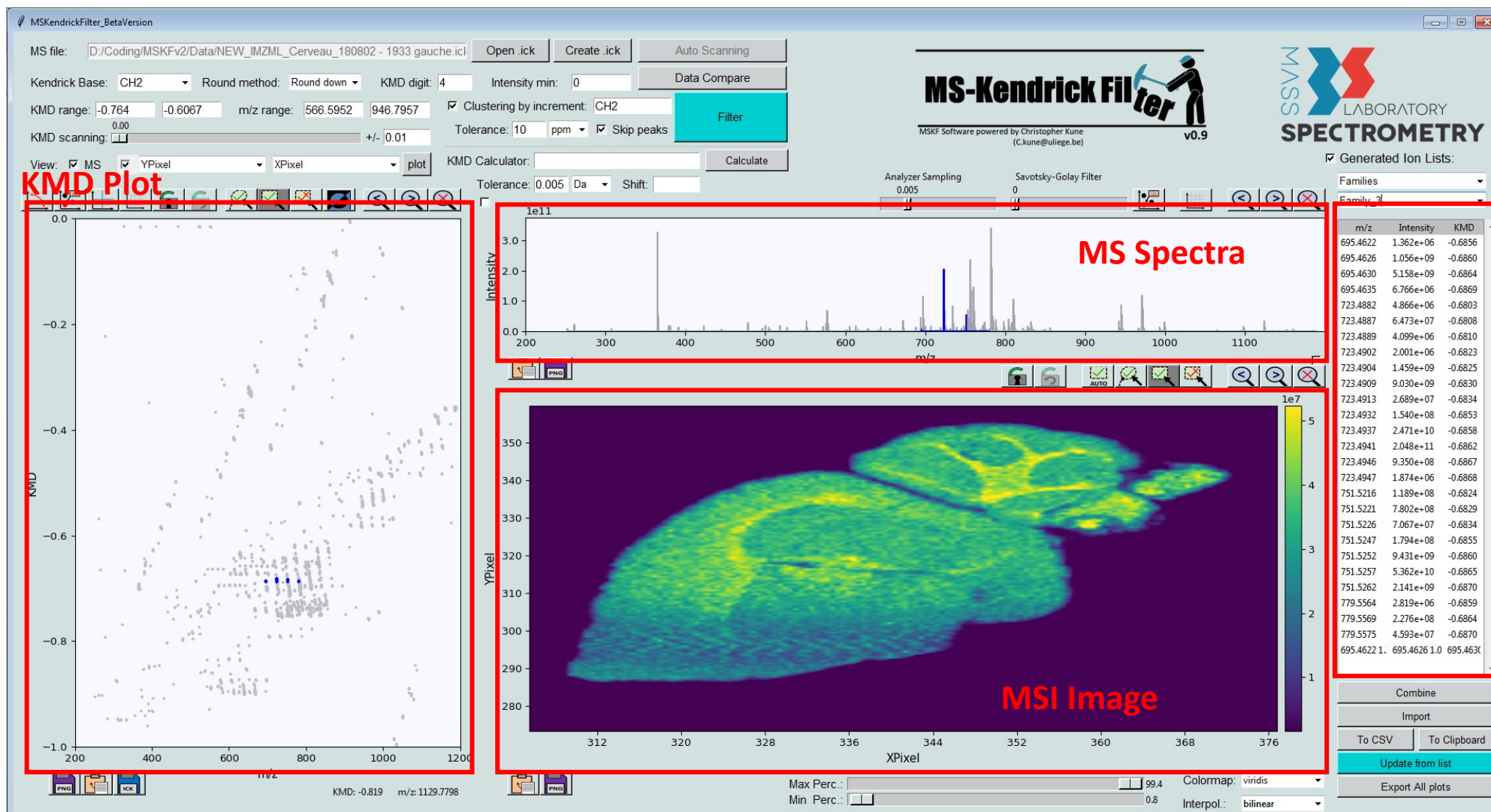
Tiquet and al
Analytical Chemistry

5.9 Lipids quantification



brain homogenate doped with (5000, 2500, 500, 250, 50, 10 µg/g) of deuterated standard PC(16:0 d31/18:1)

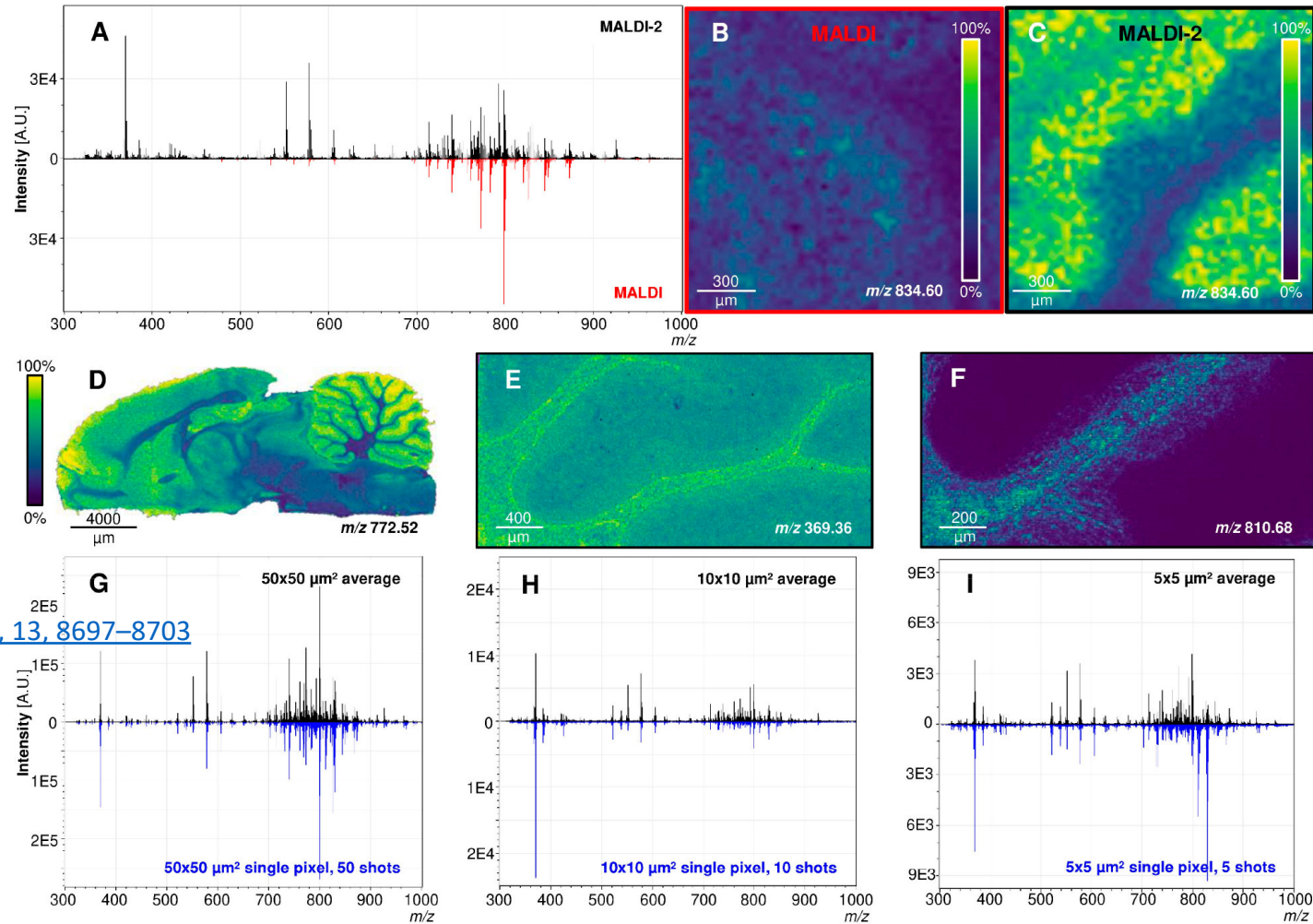
5.10 MS-Kendrick Filter Software on MSI data (in-house GUI-Based Python software)



This software allows data filtering based on KMD on:

- mzml files generated from MS data, 1D MS data (e.g. LC-MS, GC-MS), 2D MS data (e.g. LCxIM-MS, GCxGC-MS)
- Imzml files generated from MSI data

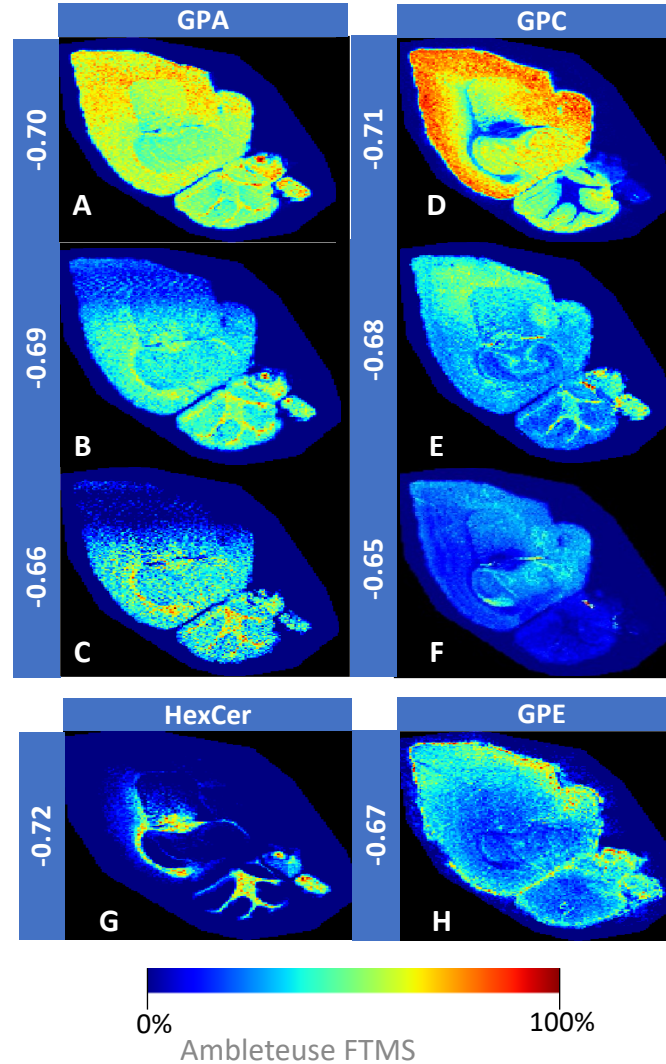
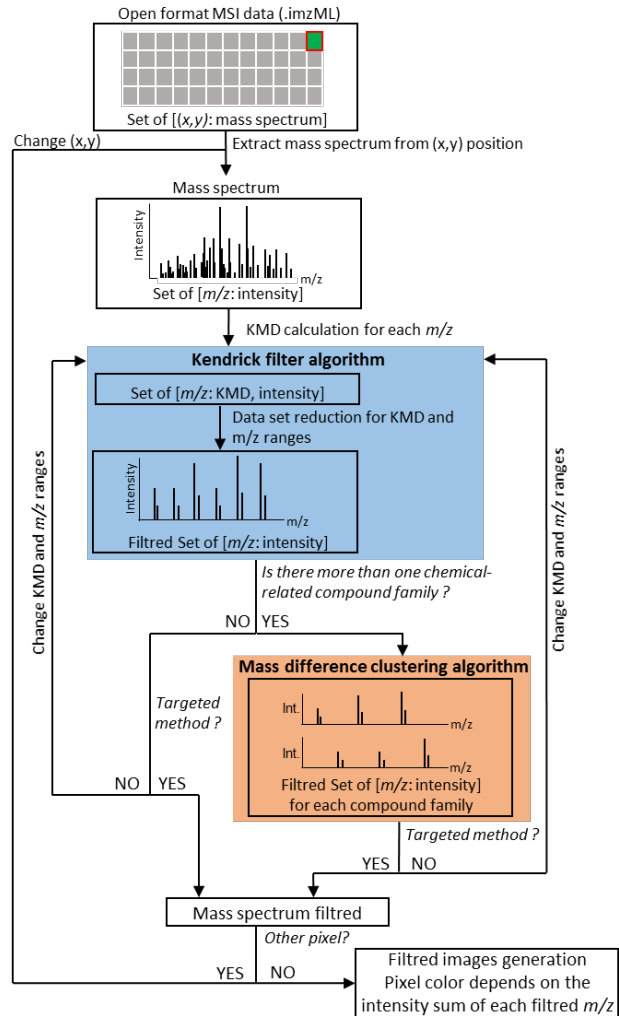
5.13 MALDI 2



Two consecutive laser shots

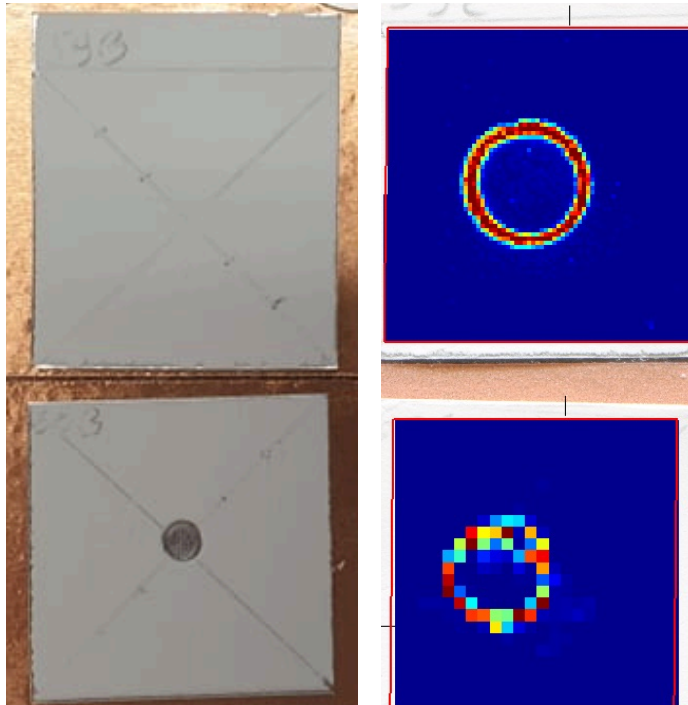
Jens Soltwisch and al. [Anal. Chem. 2020, 92, 13, 8697–8703](#)

5.11 KMD analysis of images

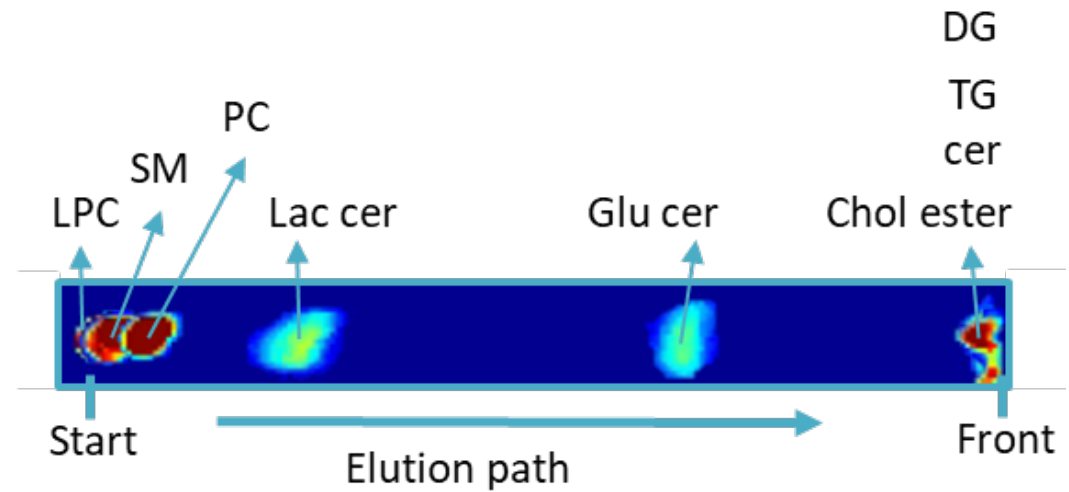


5.12 MALDI imaging of lipids TLC separations

Radial separation



Linear separation



6. Visualisation of Chemical Communication using MALDI MSI of Lipopeptides

Cyclic lipopeptides (CLPs) are composed of

- hydrophobic fatty acid tail
- amphipathic oligopeptide
- cyclization bond

Cyclic lipopeptides producing organisms

- Bacteria and yeasts
- *Bacillus* and *Pseudomonas*

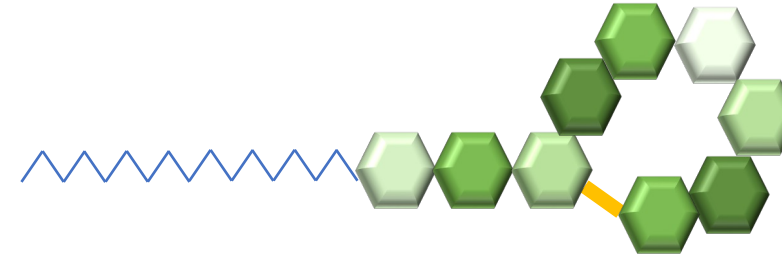
Large structural diversity

- length and branching mode of the fatty acyl chain
- length and sequence of amino acid residue
- nature of the peptide cyclisation

Modular organization of its synthesis

Large biosynthetic gene clusters encode for
Multi-modular non ribosomal peptide synthetases

Impressive functional diversity



Quorum sensing

Biofilm formation
and colonisation

Virulence

Anti oomycetes

Soil remediation

Anti protozoa

Cell motility

Anti bacterial

Anti fungal

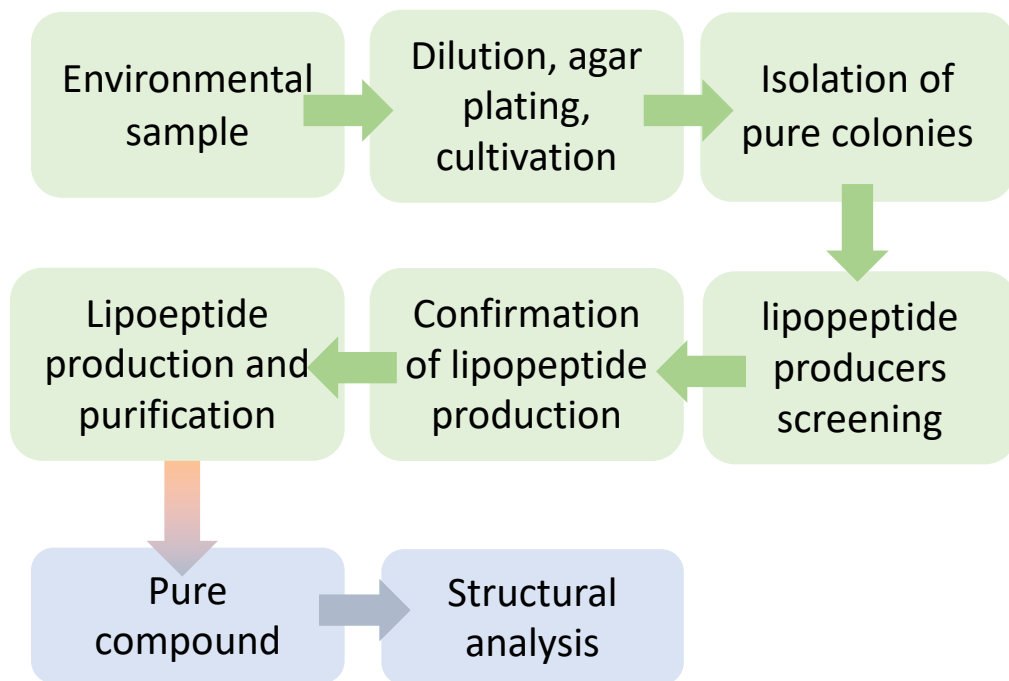
Anti cancer



Anti viral

6.1 Chemical communication: General workflow

In vivo method



Limitations

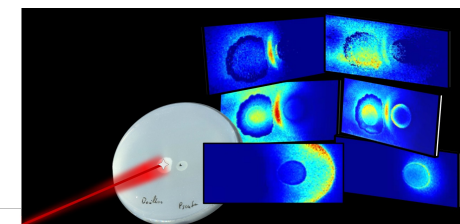
Low proportion of positive hits (Few percent range)

Limited to microbes able to grow and produce CLP on a synthetic medium

MS based tools for lipopeptide screening

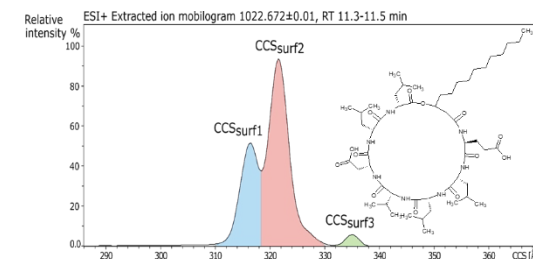
Mass spectrometry imaging (MSI)

Label-free 2D mapping and identification of the lipopeptide production directly on the agar.



Ion mobility mass spectrometry

identification of lipopeptide based on their ion mobility measurement



6.2 Experimental conditions



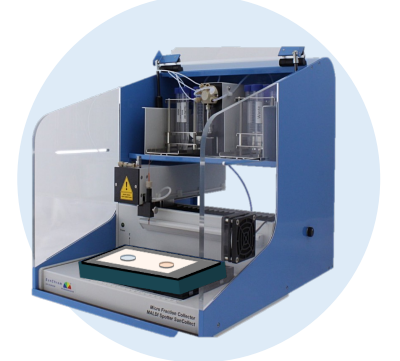
Extraction of the zone of interest



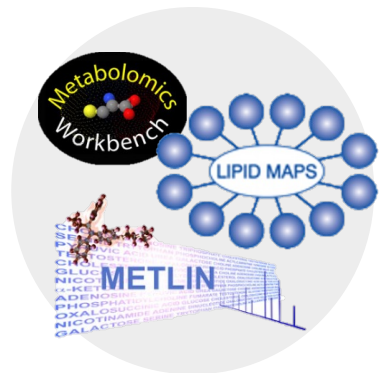
Sample deposition onto an ITO-glass slide



Overnight vacuum dry



Automated HCCA matrix deposition (Sunchrom)



20/11/22

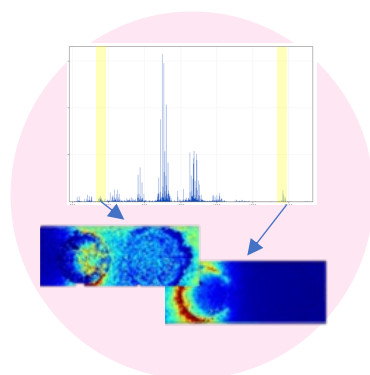
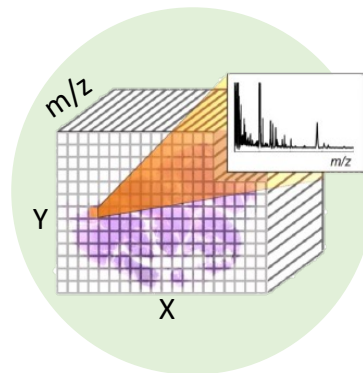
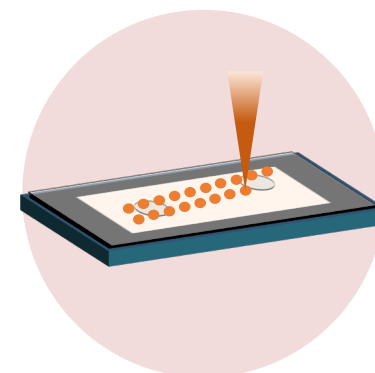


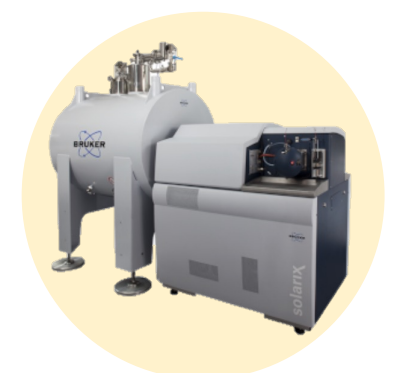
Image analysis – mean spectra generation



Datacube of position correlated spectra
Ambleteuse FTMS

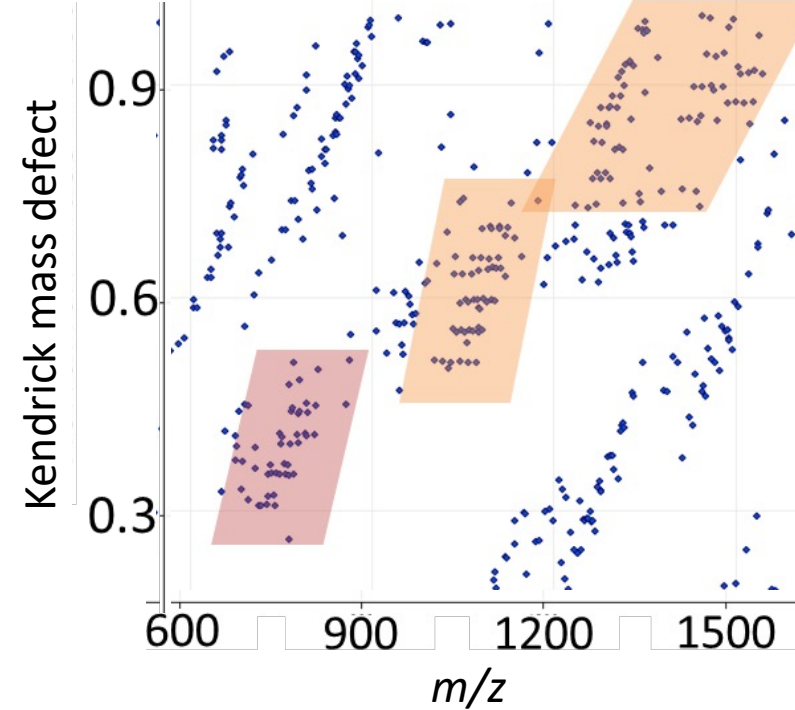
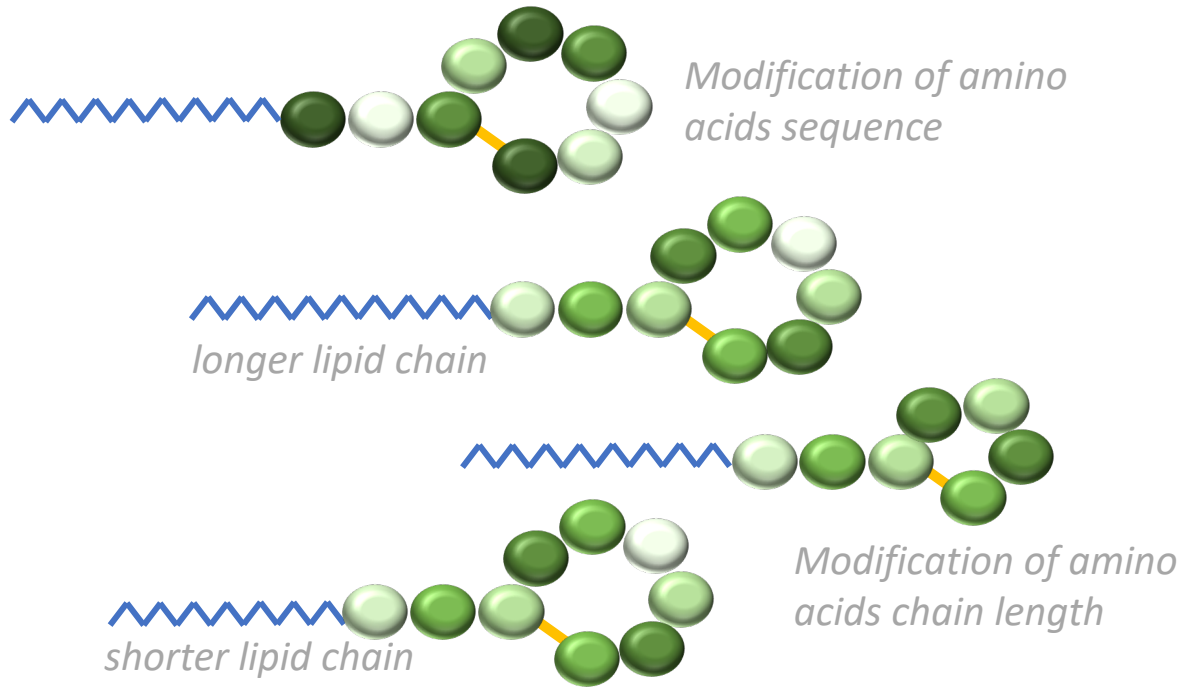


MALDI spectra pixel by pixel (X_i, Y_j)



Ready for MS imaging

6.4 Lipopeptides



The solution



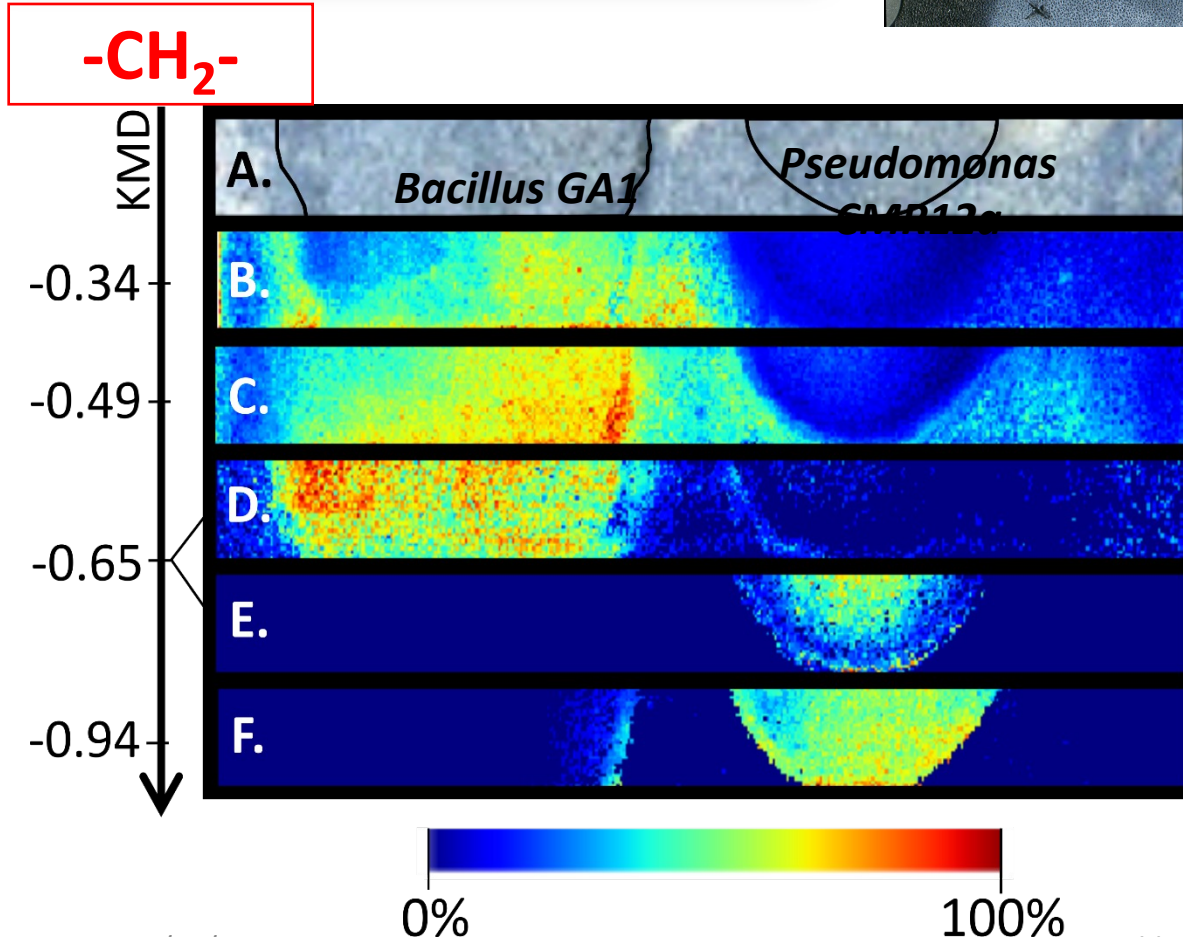
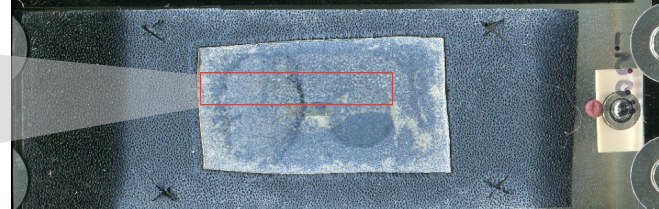
Kendrick mass defect filter

→ Filters the compounds presenting a repetitive mass unit

→ Suitable for lipopeptide !

6.3 Imaging lipopeptides in co-cultures

Bacillus GA1 *Pseudomonas*
CMR12a



A. Optical image

B. Iturins (*Lipopeptides*)

C. Surfactins (*Lipopeptides*)

D. Phosphatidyletanolamines PE (*Lipids*)

E. Phosphatidylcholines¹ (PC) (*Lipids*)

F. Sessilins² (*Lipopeptides*)

1) Fauland, A. et al, A comprehensive method for lipid profiling by liquid chromatography-ion cyclotron resonance mass spectrometry. *Journal of Lipid Research* **52**(12): 2314-2322.

2) Sofija Andric, et al. Chelator sensing and lipopeptide interplay mediates molecular interspecies interactions between soil bacilli and pseudomonads. *bioRxiv* 2021.02.22.432387

6.5 Kendrick analysis

First step : Kendrick mass defect filtration:

Filtrated range: 500 – 1900 m/z

Kendrick mass unit: CH₂

- Noise removal
- 40 detected families of lipids and lipopeptides

Second step: CCS-mass trend filtration

Filtrated range : 40 retained families by KMD

POW parameter range: 0.5 – 0.9

$R^2 > 0.9$

- 24 detected families of lipids and lipopeptides

Third step: Database identification

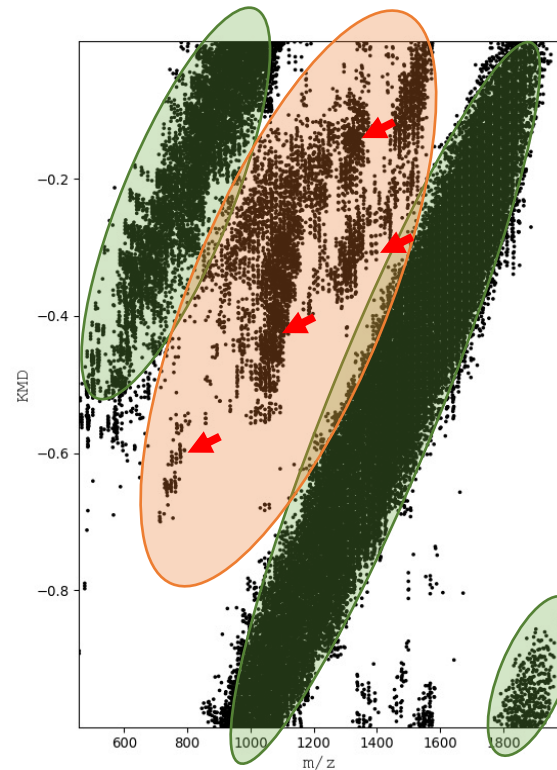
MS database search mass tolerance: 20ppm

IM database search CCS tolerance: 2%

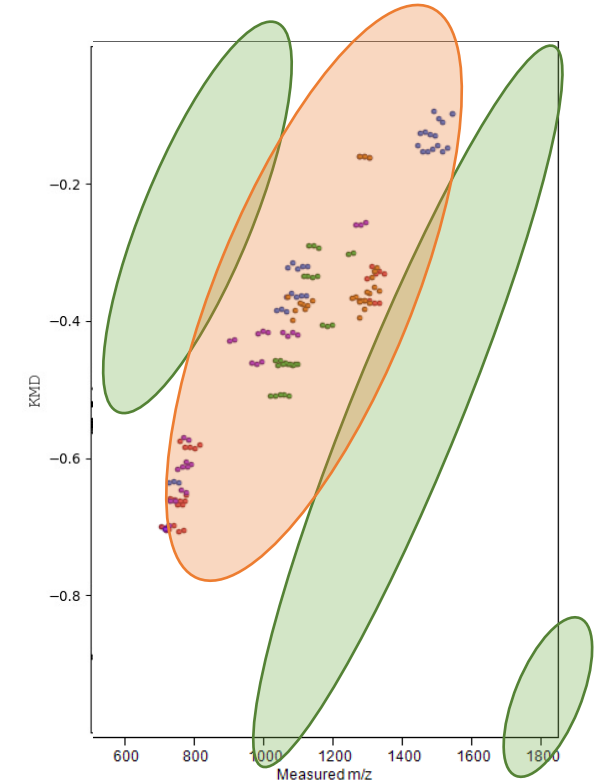
Databases : Lipidmaps / Metlin / Metabolomics Workbench / PamDB

- Only 6 families of compounds were “unknown” after the database search.

Kendrick mass defect plot



Kendrick mass defect plot after KMD filtration



McCann, A., *et al* "Rapid visualization of lipopeptides and potential bioactive groups of compounds by combining ion mobility and MALDI imaging mass spectrometry." Drug Discovery Today: Technologies.

6.6 Mass trends for lipopeptide families identification

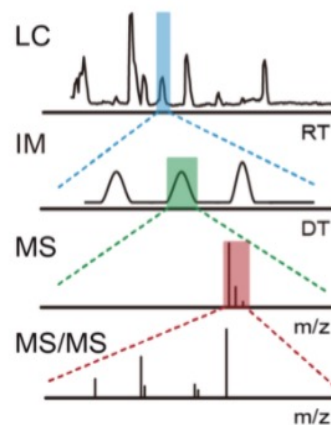
We have seen that **CCS-mass trends** can be used to identify a **family of lipopeptide**.

Can we consider a CCS as an additional molecular descriptor of lipopeptide ?

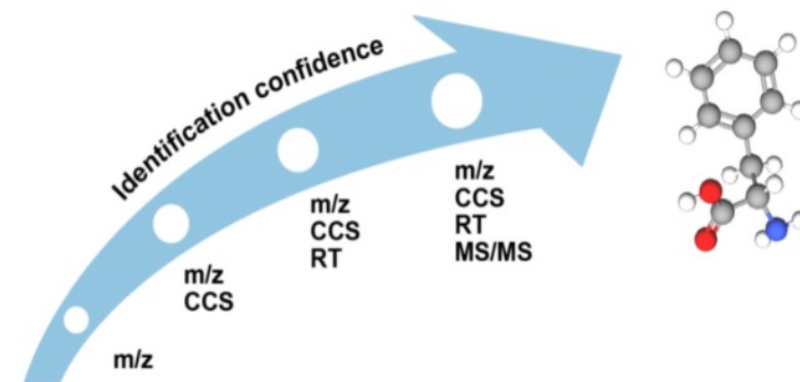
CCS = molecular descriptor for lipids and metabolites

CCS is now used as an additional molecular descriptor (McLean group). If this can be done for lipids and metabolites, it might not be the case for all biomolecules, as they may behave differently depending on the ionization and separation conditions.

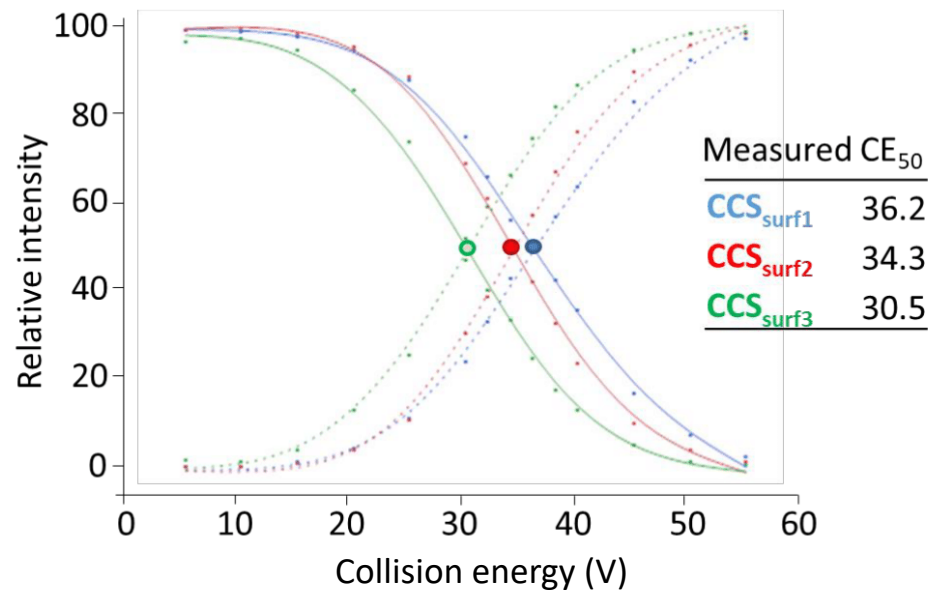
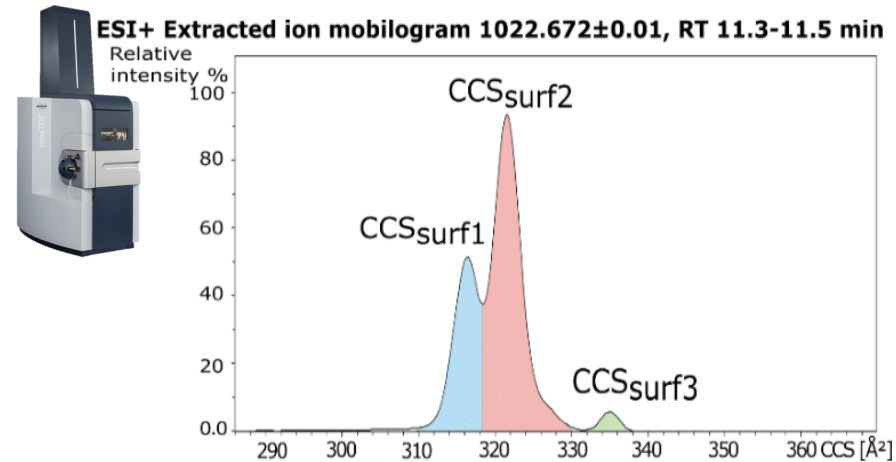
Four-dimensional data



Metabolite/Lipid identification



Can the CCS always help for identification



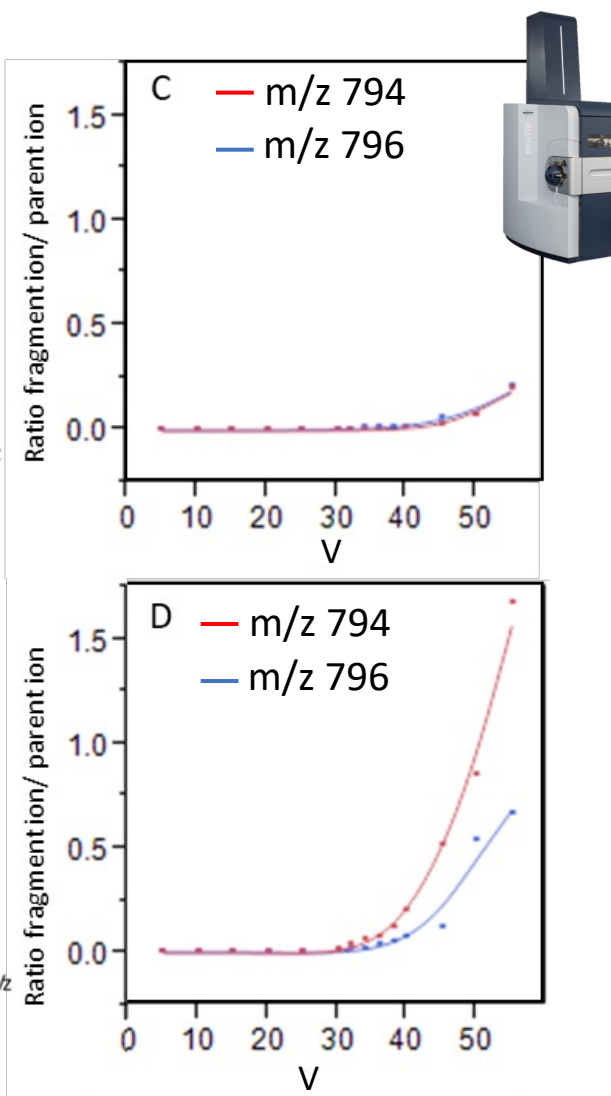
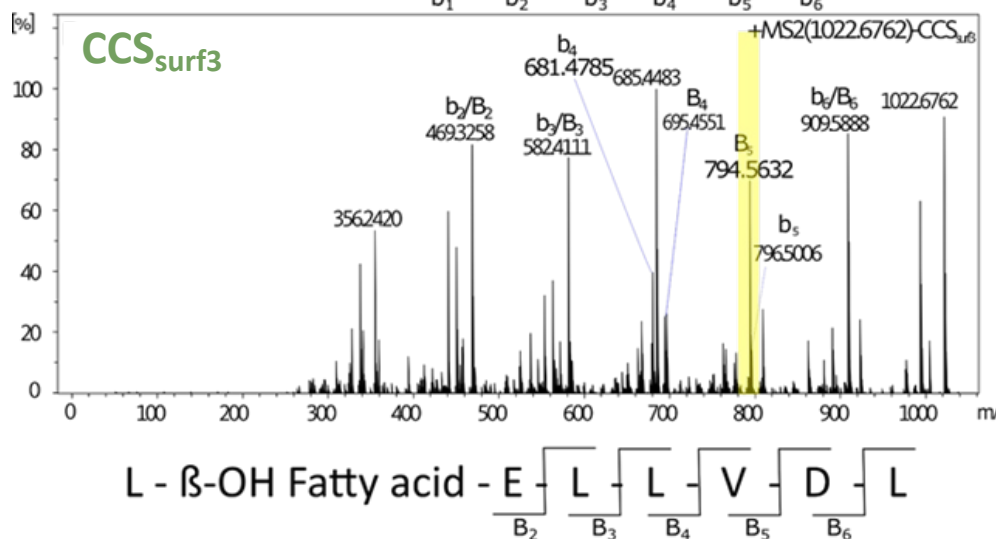
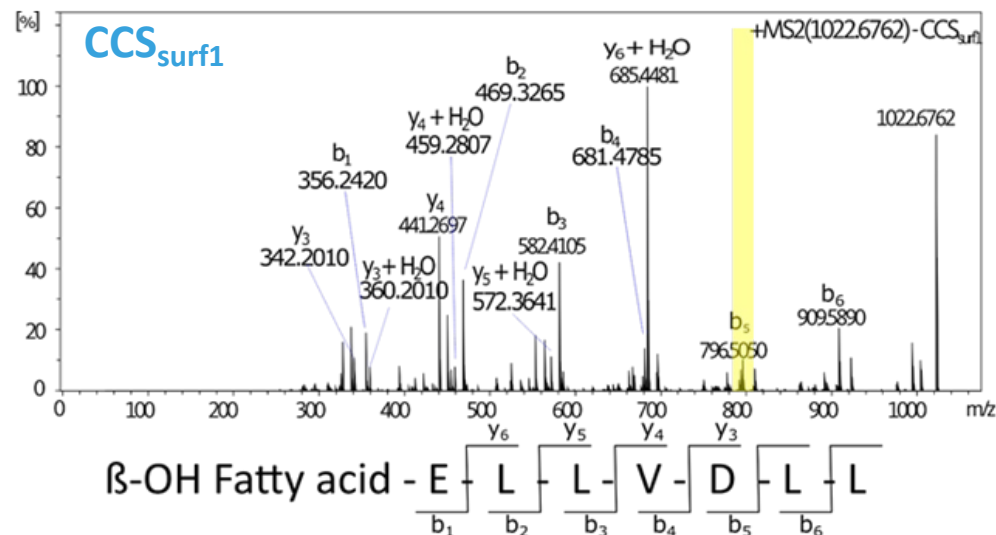
MS/MS may be required

Identical ion structure = same fragmentation pattern

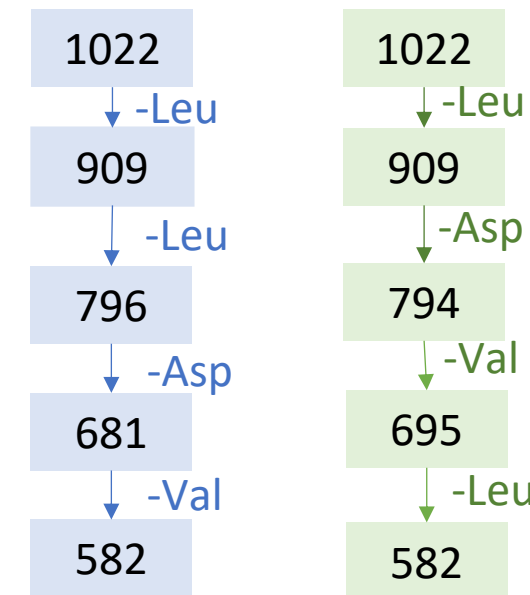
Fragmentation of CCS_{surf1}, CCS_{surf2} and CCS_{surf3} at different collision energies (ranging from 5V to 55V)

Survival yield of the three CCS_{surf} shows three different CE₅₀ → in agreement with the protomer hypothesis

Fragmentation routes of protomers



m/z Ester bond opening m/z Peptide bond opening



Two different cycle openings observed

Confirmed with the presence of two distinct fragments :

m/z 796
 m/z 794

Take home message

- Resolution and calibration are a must, in particular in imaging where they should be optimal for the whole image
- FTICR is slow but produces a huge amount of data when dealing with complex sample. Automated data handling is mandatory
- FTICR may not be sufficient: separation methods, MS/MS and ion mobility are excellent adds on
- IMS is not yet «routinely available » on FTICR but working off line with another instrument can help
- Imaging MS is still tricky but a good control of the sample preparation and acquisition helps
- Microextraction methods (Laser microdissection, LESA...) are interesting options to validate identifications and quantify

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You for your attention

