Rapid identification of chemically-related compounds produced by bacteria by Kendrick mass defect filtering applied to high resolution imaging mass spectrometry

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Introduction

Over the last years, lots of progress have been done in the development of mass spectrometry imaging, making the technique more and more accessible for various applications, such as biomarkers discovery or bioactive compounds identification. However, the progresses made in terms of spatial and instrumental resolution has for consequences the dramatic increase of dataset size, shifting the burden from data production to data analysis.

We propose here to use a semi-targeted method based on Kendrick mass defect (KMD) analysis to immediately identify the chemistry-related compounds in mass spectrometry imaging applied to microbiology samples. In that aim, we developed an in-house software to simplify the analysis of high resolution MS spectra that we then applied to mass spectrometry imaging.
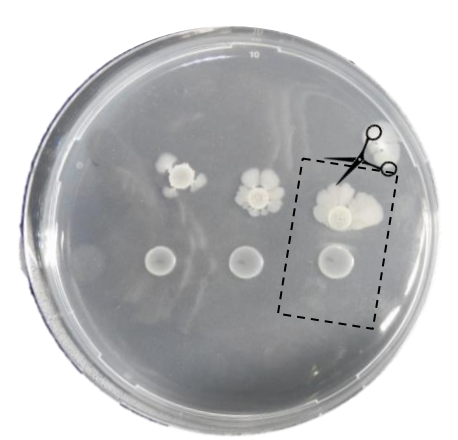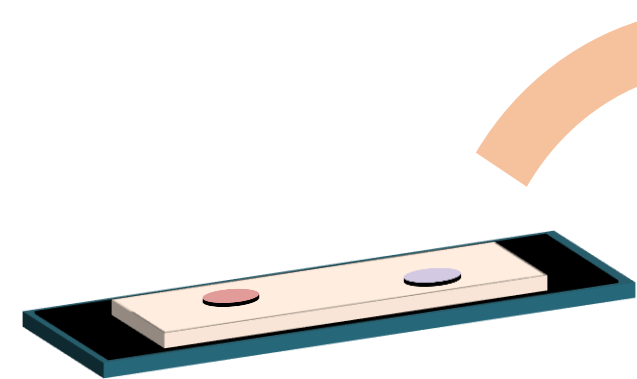
Methods

1) In vivo assay

Strains of two different bacteria were inoculated on a semi-solid agar-based PDA medium (Potato Dextrose Agar) at different distances from each-other (0.5cm and 2cm) and incubated overnight at 30°C
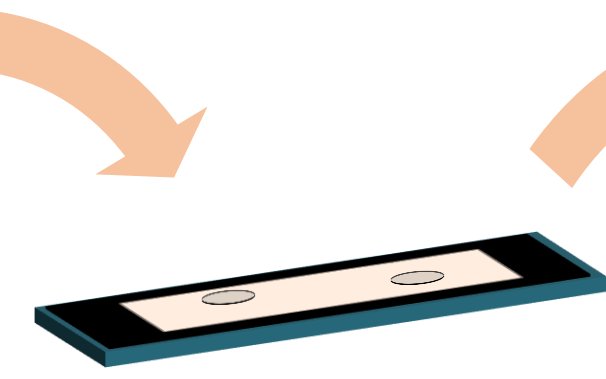
0.5cm

2cm
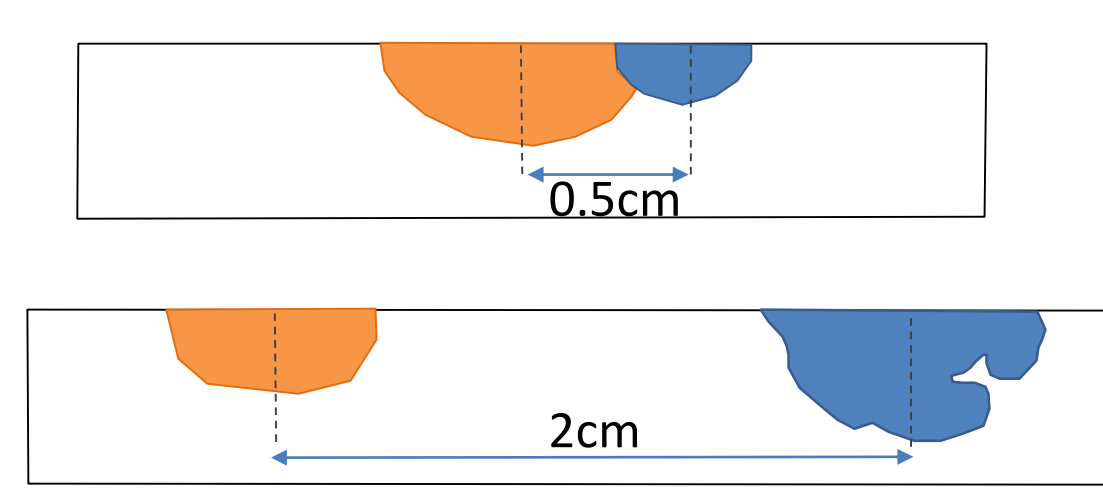
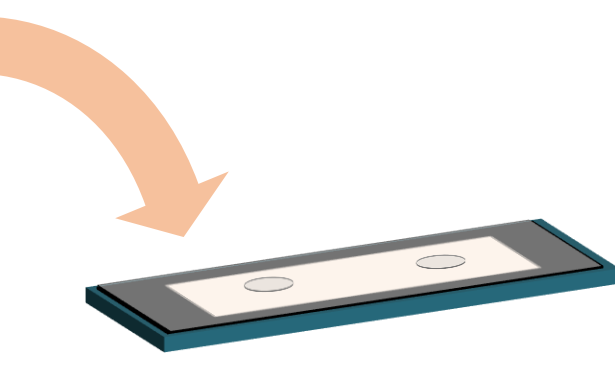### 2) Sample preparation for MALDI-MS imaging[1]

Region of interest is directly cut from the agar-plate.

Agar is transferred to and ITO-coated glass slide, previously covered with double sided conductive carbon tape.

The assembly is put in a vacuum desiccator until dryness (Overnight)

HCCA 5mg/mL 70% ACN 0.2% TFA matrix solution is spread onto the sample using the Sunchrom spraying system.

3) Data acquisition

- High resolution FT-ICR MS Solarix 9.4T (Bruker Daltonics, Bremen, Germany)
- Calibration from 200m/z to 2000m/z with red Phosphorous (err. >0.5 Ppm)
- Stable TIC was obtained for MS Imaging with the following conditions:
  Laser power 50% - Laser shots per pixel : 10 - Frequency : 200 Hz
- Pixel step set to 80 µm
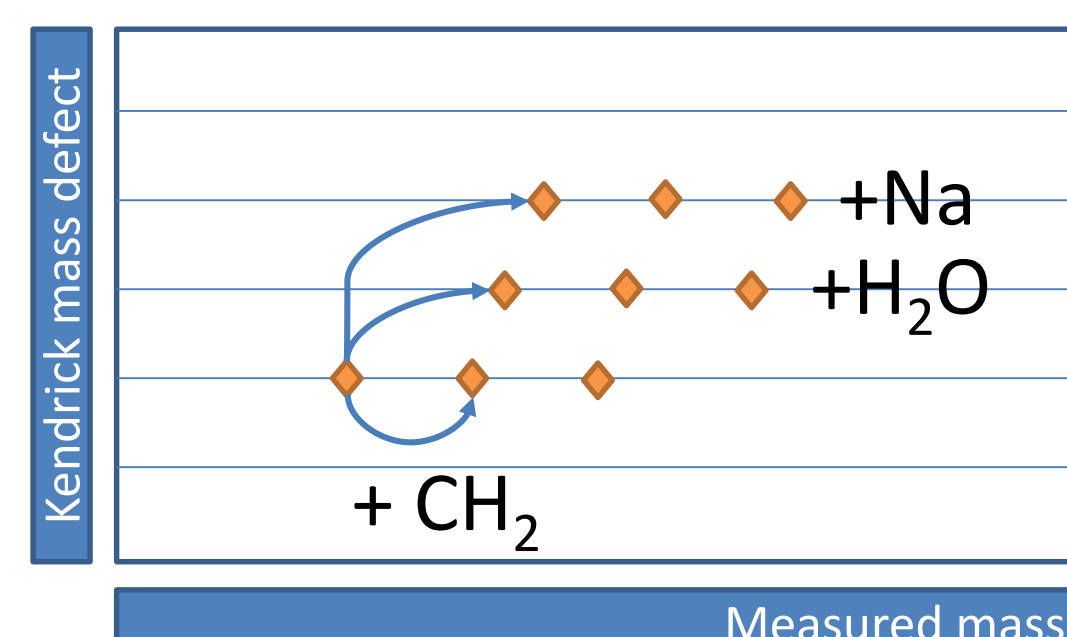
4) Kendrick principle[2]

Kendrick mass defect analysis is a powerful tool for compounds identification in complex spectra, by plotting the data according to the contribution of a repeated mass unit (here; CH_2).
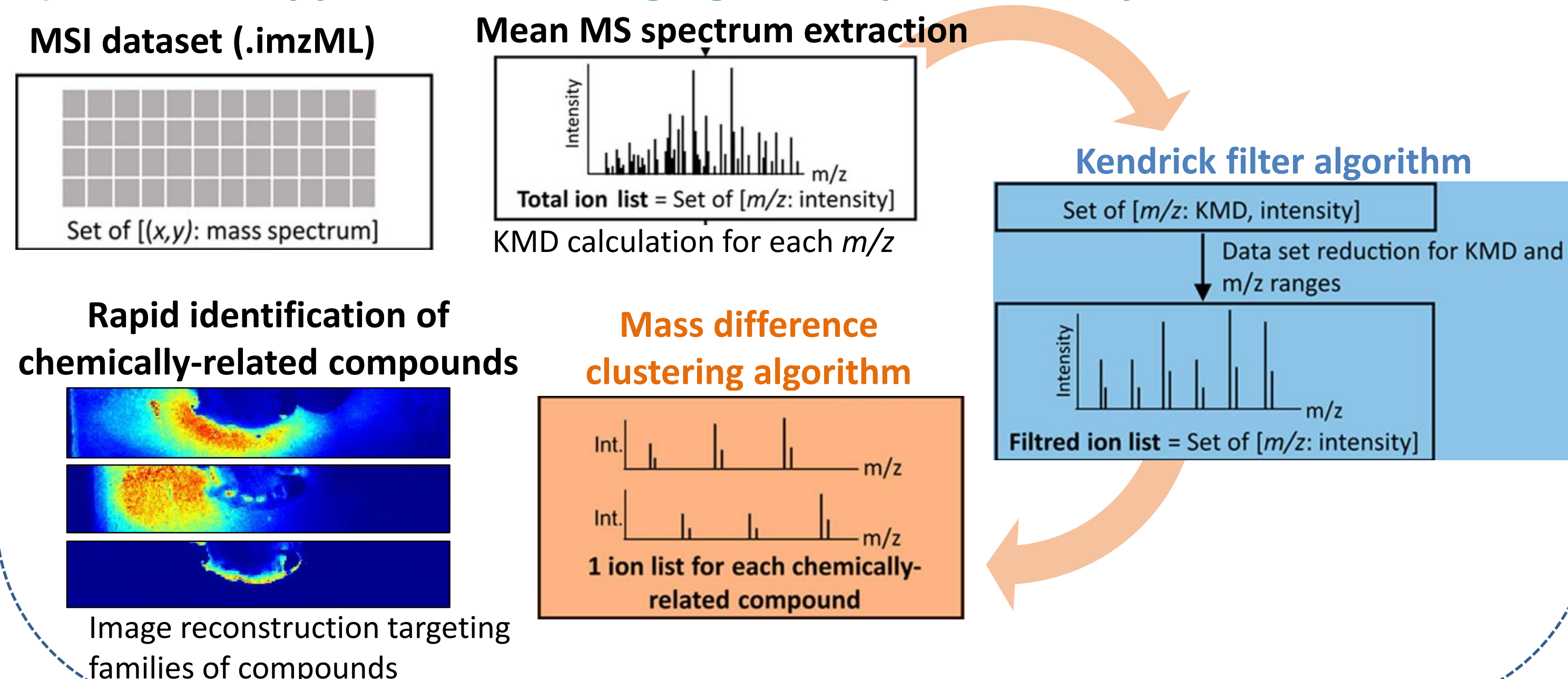To do so, the measured masses are first converted into Kendrick mass :

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

Kendrick mass defect

+Na
+H$_2$O
+ CH$_2$
Measured mass

Then, the Kendrick mass defect is calculated based on the difference between the Kendrick nominal mass (the integer) and the Kendrick mass :

$$Kendrick\ mass\ defect = nominal\ Kendrick\ mass - Kendrick\ mass$$

5) Kendrick application to imaging mass spectrometry[3]

MSI dataset (.imzML)
Set of [(x,y): mass spectrum]

Mean MS spectrum extraction
Intensity
m/z
Total ion list = Set of [m/z: intensity]
KMD calculation for each m/z

Kendrick filter algorithm
Set of [m/z: KMD, intensity]
Data set reduction for KMD and m/z ranges
Intensity
m/z
Filtred ion list = Set of [m/z: intensity]

Rapid identification of chemically-related compounds
Image reconstruction targeting families of compounds

Mass difference clustering algorithm
Int.
m/z
Int.
m/z
1 ion list for each chemically-related compound

Results

Rapid data filtration by Kendrick mass defect plot

a.i.
9x10^6
6x10^6
3x10^6
0.0
300 600 900 1200 1500 1800 2100 m/z

Kendrick Mass Defect
0.9 0.6 0.3 0.0
300 600 900 1200 1500 1800 2100 m/z

KMD plot for MSI data enables to
- Filter the data to keep only the signal containing information
- Rapidly detect the compounds of interest (lipids, lipopeptides)

Dextrose (agar media)
Lipopeptides
Lipids

Lipopeptides detection based on KMD plot

Kendrick Mass Defect
0.6991 — Iturins [M+K]$^+$
0.6875 — Unknown 1
0.6567 — Iturins [M+Na]$^+$
0.6397 — Surfactins [M-H+2K]$^+$
0.6335 — Unknwon 2
0.5993 — Surfactins [M-H+Na+K]$^+$
0.5939 — Unknown 3
0.5557 — Surfactins [M+K]$^+$
0.5512 — Surfactins [M-H+2Na]$^+$
0.5103 — Surfactins [M+Na]$^+$

+Na$^+$
-Na$^+$, +K$^+$
+K$^+$

Rapid screening of different possible adducts
Immediate visualization of chemically-related compounds

Surfactin identification based on exact mass

	Exact mass	Measured mass	Delta (Da)
[M+H]$^+$	1022.67476	N/A	
[M+Na]$^+$	1044.65671	1044.65664	0.00007
[M+K]$^+$	1060.63064	1060.62706	0.00358
[M-H+2Na]$^+$	1066.63865	1066.63370	0.00495
[M-H+Na+K]$^+$	1082.61259	1082.61009	0.00250
[M-H+2K]$^+$	1098.58652	1098.58532	0.00120

Surfactin structure

Rapid visualization of lipopeptides distribution using Kendrick

0.5cm

2cm

Iturins [M+K]$^+$ KMD= 0.6991
Unknown #1 KMD= 0.6875
Iturins [M+Na]$^+$ KMD = 0.6567
Unknown #2 KMD = 0.6335
Unknwon #3 KMD = 0.5939
Surfactin [M+K]$^+$ KMD = 0.5557
Surfactin [M+Na]$^+$ KMD = 0.5103

0% 100%
0% 100%

Conclusions and prospects

- Kendrick mass defect filtering is particularly adapted for mass spectrometry imaging enabling :
  - Rapid compound screening and identification of chemically related comounds based on their repetitive unit
  - Immediate visualization of the different adducts

- Our in-house software enables to reconstruct the images according to a specific gorup of molecules selected on their KMD values. This software can be used with any type of data (HPLC, IM-MS, IMS).

- This method can be applied on many different types of compounds with a repeated unit : lipids, sugards, polymers, lipopeptides.
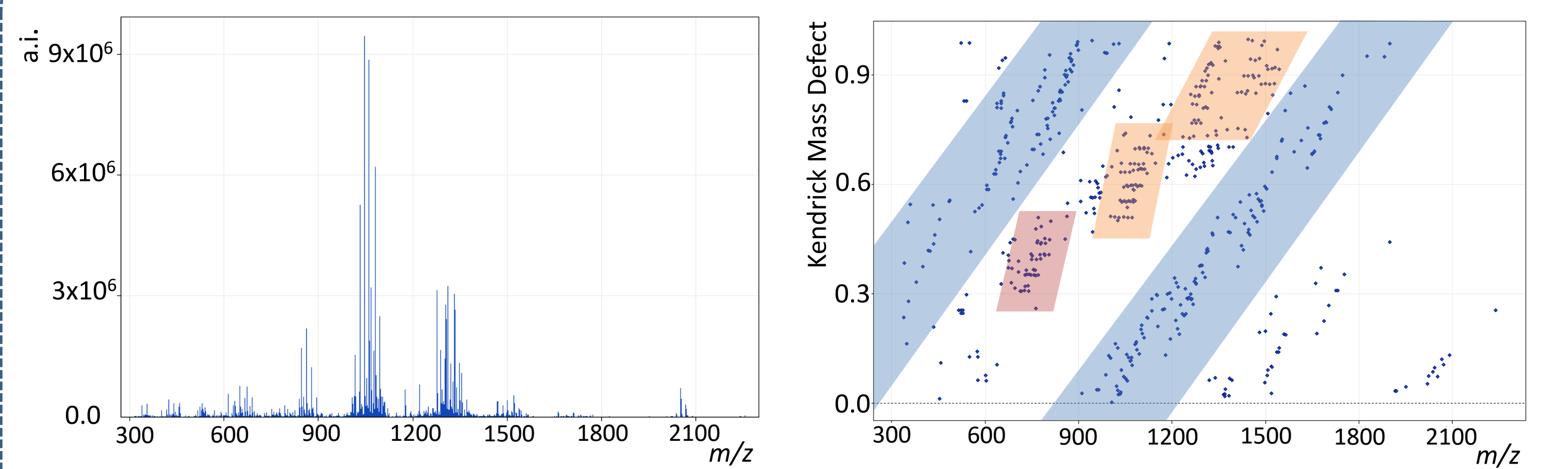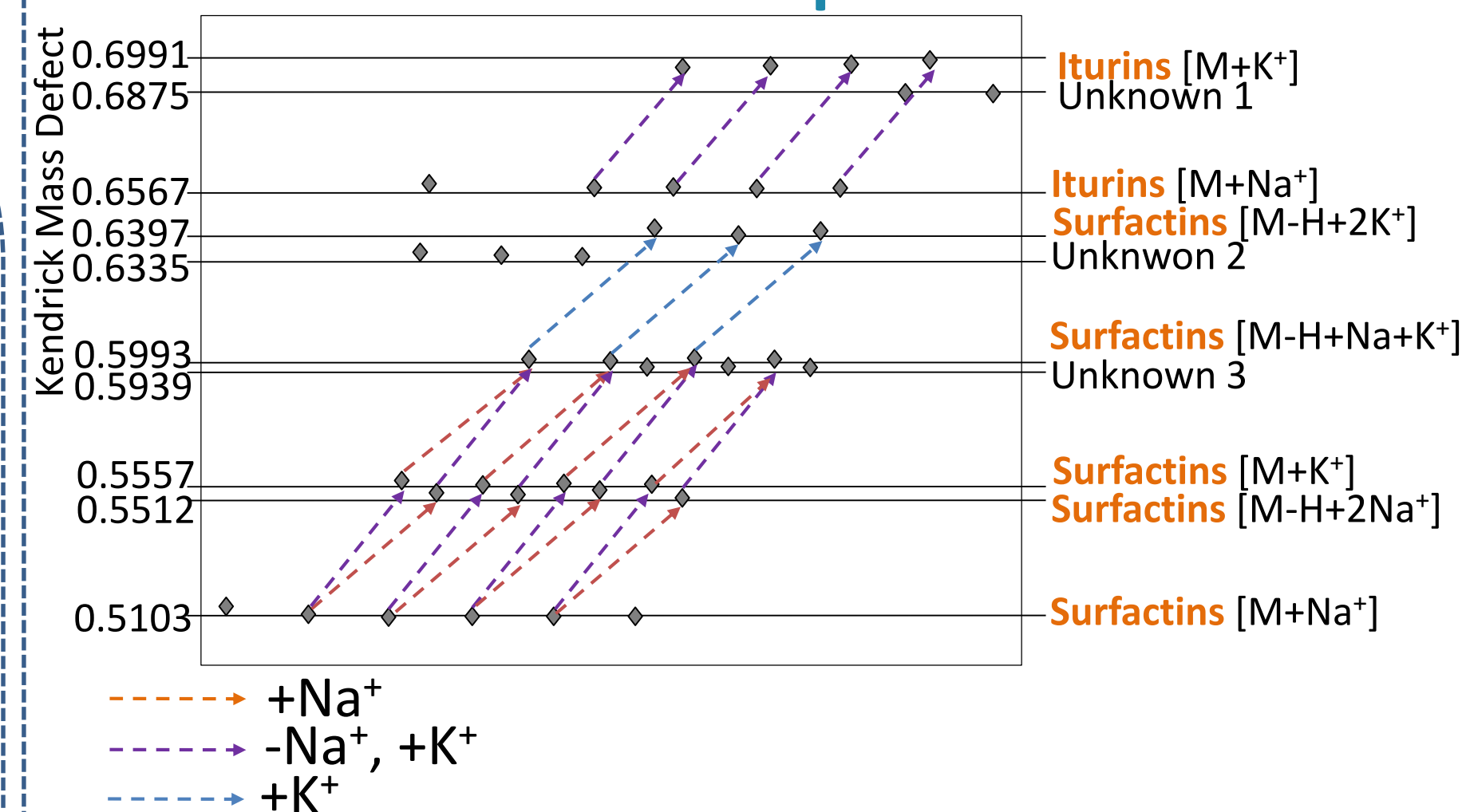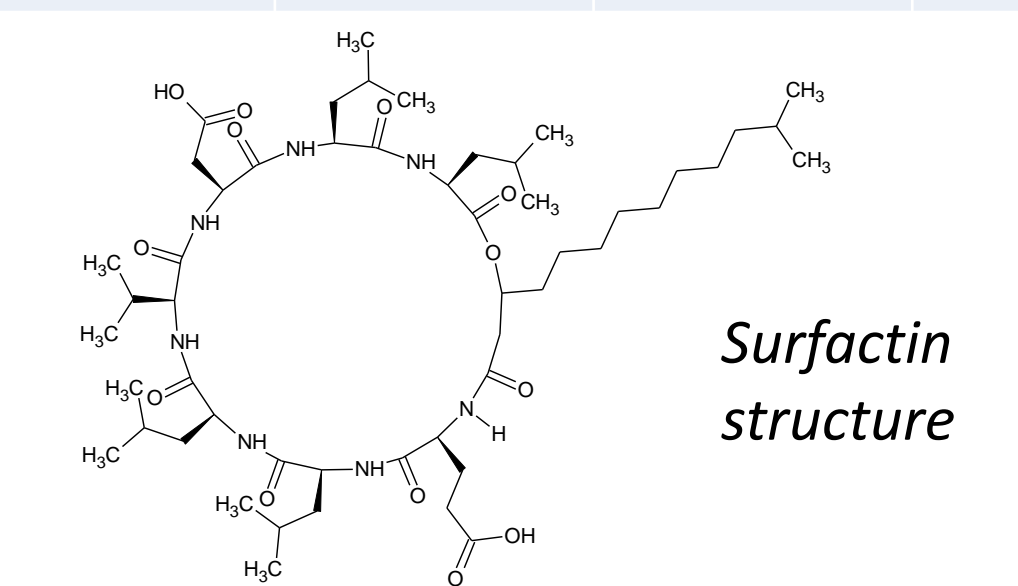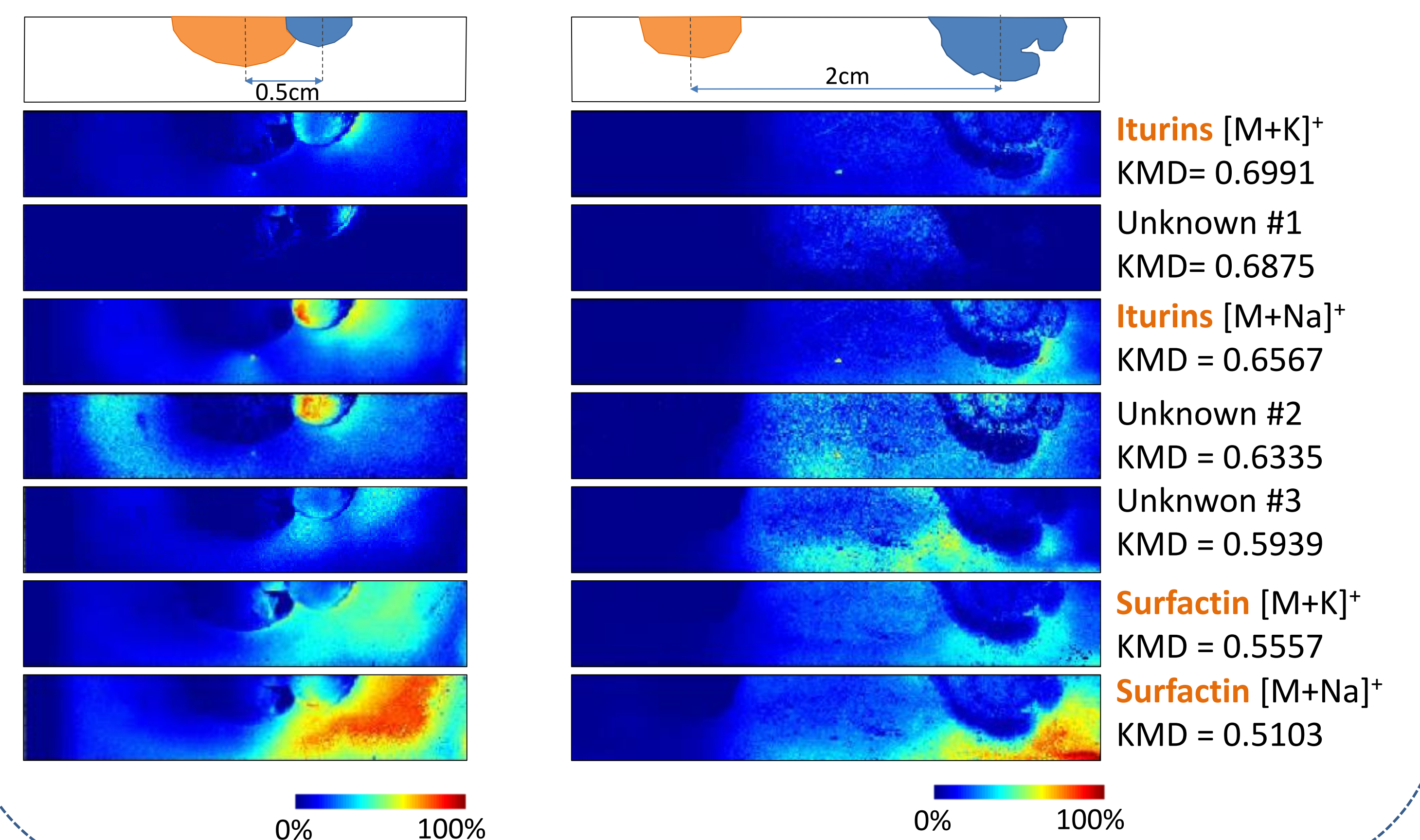
Literature

[1]Debois, D., et al. (2013). "MALDI-FTICR MS imaging as a powerful tool to identify Paenibacillus antibiotics involved in the inhibition of plant pathogens." J Am Soc Mass Spectrom 24(8): 1202-1213
[2]Hughey, C. A., et al. (2001). "Kendrick Mass Defect Spectrum: A Compact Visual Analysis for Ultrahigh-Resolution Broadband Mass Spectra." Analytical Chemistry 73(19): 4676-4681.
[3]Kune C, McCann A, La Rocca R, et al. (2019) "Rapid visualization of chemically related compounds using Kendrick mass defect as a filter in mass spectrometry imaging". Analytical Chemistry 91(20): 13112-13118.

Acknowledgments