In situ isobaric/isomeric lipid mapping by MALDI-Ion Mobility Separation-MSI

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Introduction

The highly diverse chemical structures of lipids make their analysis directly from biological tissue sections extremely challenging. Here we report the *in-situ* mapping and identification of lipids in a freshwater crustacean *Gammarus fossarum* using MALDI mass spectrometry imaging (MSI) in combination with an additional separation dimension using ion mobility spectrometry (IMS) [1]. The high-resolution trapped ion mobility spectrometry (TIMS) allowed efficient separation of isobaric/isomeric lipids showing distinct spatial distributions. The structures of the lipids were further characterized by MS/MS analysis. It is demonstrated that MALDI MSI with mobility separation is a powerful tool for distinguishing and localizing isobaric/isomeric lipids.

Methods

The workflow for MALDI-MSI sample preparation and data analysis is outlined in Figure 1. Fresh frozen female gammarid was sectioned at 12 µm and thaw mounted onto an ITO slide (Sigma-Aldrich). After drying, sections were sprayed with 10 mg/ml DHB in ACN/H_2O/TFA (70:30:0.1, *v/v/v*) using a TM sprayer (HTX Technologies, Chapel Hill, NC, USA). Tissues were imaged using the following parameters if not indicated otherwise: *m/z* range: 100-1000, 400 shots, 10 kHz laser frequency, pitch: 20 µm. For ion mobility separation, ions were separated and eluted in the second part of the dual TIMS device using a ramp time of 300 ms and a $1/K_0$ range of 0.6-1.8. Imaging data processing was performed with SCiLS Lab version 2020a. Ion mobility data was visualized with TIMS data viewer. Assignment of the ions were achieved by interrogating open source databases including METLIN [2], Lipid Maps [3] and CCS Compendium[4].

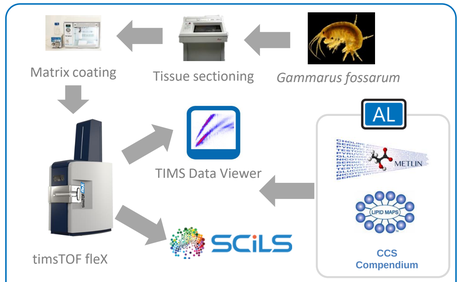


Fig. 1: MALDI-MSI workflow

Results

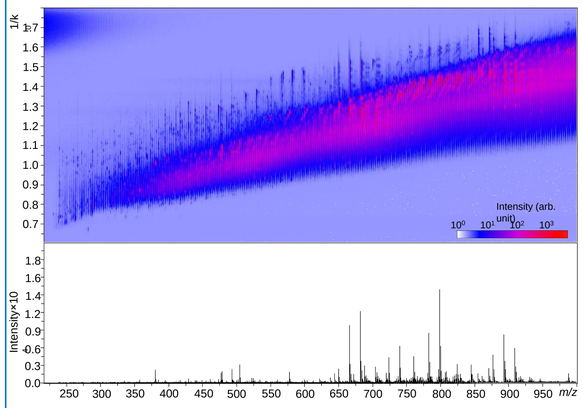


Fig. 2: Ion mobility heat map (top) and average mass spectrum (bottom) generated from the tissue section of female *G. fossarum*.

- Good sensitivity at high lateral resolution.
- Various ion species with different $1/K_0$ values detected.
- Richer molecular information compared to non-TIMS Imaging

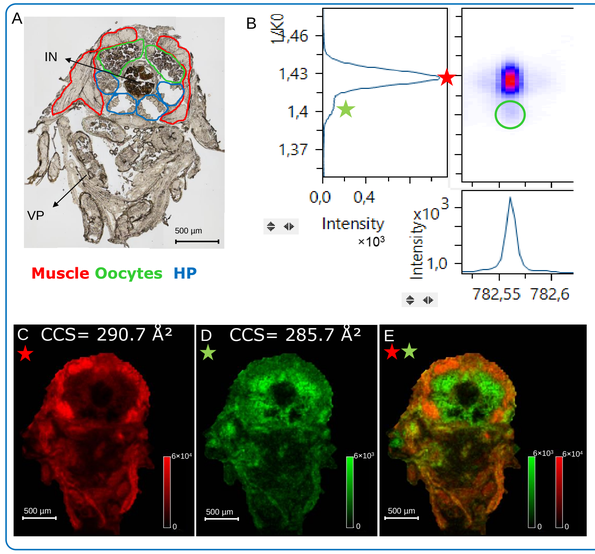


Fig. 3: MALDI-IMS-MSI of female *G. fossarum* tissue section with 20 µm pixel size.

A. Optical image of the tissue after MALDI Imaging analysis. HP: hepatopancreas; In: Intestine. VP: ventral pouch.
B. Ion mobility separation of two isobaric lipid species.
C. Ion image of the ion at *m/z* 782.561 with $1/K_0$ = 1.426 ± 0.01(CCS = 290.7 Å²).
D. Ion image of ion at *m/z* 782.561 with $1/K_0$ = 1.402 ± 0.01 (CCS = 285.7 Å²).
E. Overlay of C and D.

Table 1: Most intense ions colocalizing to different organs. (Assignments by interrogating METLIN [2]).

Organ	*m/z*	Tentative assignment
Muscle	713.452	PC(32:0)+K^+-N(CH_3)$_3$
	772.525	PC(32:0)+K^+
	834.679	GlcCer(d18:1/24:0)+Na^+
	883.678	TAG(53:8)+Na^+
Oocyte	766.574	PC(O-16:0/20:5)+H^+/PC(P-16:0/20:4)+H^+
	792.590	PC(O-16:0/22:6)+H^+/PC(P-16:0/22:5)+H^+
	794.606	PC(O-16:0/22:5)+H^+/PC(P-16:0/22:4)+H^+
	806.569	PC(38:6)+H^+
Hepatopancreas	332.783	
	348.758	Not assigned
	375.065	

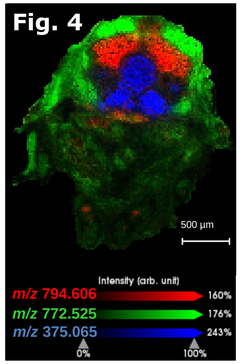
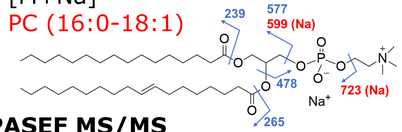


Fig. 4: High resolution MALDI imaging resolves fine tissue structures in a very small sample. The three-color overlay of ions at *m/z* 794.606 (red), *m/z* 772.525 (green) and *m/z* 375.065 (blue) illustrates the specific distributions of different chemical species in the organs of female *G. fossarum.*

Fig. 5: Identification of isobaric lipid species at *m/z* 782.56.

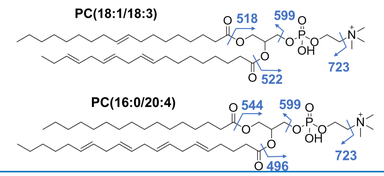
***In-situ* MALDI MS/MS**

CCS= 290.7 Å²
$[M+Na]^+$
PC (16:0-18:1)



ESI PASEF MS/MS

CCS= 285.7 Å²
$[M+H]^+$
PC (36:4)



Conclusions

- MALDI TIMS Imaging leads to richer molecular information content.
- Isobaric and isomeric lipids were mapped to specific locations in a tiny crustacean sample.
- The combination of *in-situ* MALDI MS/MS and ESI-PASEF is a powerful tool to retrieve lipid identifications with different distributions.

MS-Imaging

References 1. Fu *et al.*, doi: 10.1002/jms.4531 2. https://metlin.scripps.edu 3. http://lipidmaps.org/ 4. Picache *et al.*, Chem. Sci., 2019, 10, 983

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