Advanced Methods for Differentiating Lipid Isomers in Tissue using Trapped Ion Mobility Imaging Mass Spectrometry

Katerina V. Djambazova[1,2], Lukasz Migas[3], Elizabeth K. Neumann[2,4], Dustin R. Klein[2,4], Emilio S. Rivera[2,4], Martin Dufresne[2,4], Raf Van de Plas[3], Richard M. Caprioli[1,2,4] , Jeffrey M. Spraggins[1,2,4]

[1]Department of Chemistry
[2]Mass Spectrometry Research Center
[3]Delft Center for Systems and Control
[4]Department of Biochemistry
[1,2,4]Vanderbilt University, Nashville, TN
[3]Delft University of Technology, Delft, Netherlands

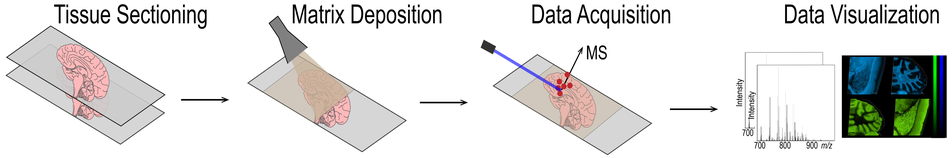
INTRODUCTION

GOAL: Develop matrix-assisted laser desorption/ionization (MALDI) trapped ion mobility mass spectrometry (TIMS)[1] methods for separating lipid isomers from standards and *in situ*.

RESULTS: MALDI-generated lipid isomer standards[2], including those that differ in sn-position, double bond position and geometry, and acyl chain composition, were successfully separated in both positive and negative mode. *In situ* separation of lipid isomers was also demonstrated.

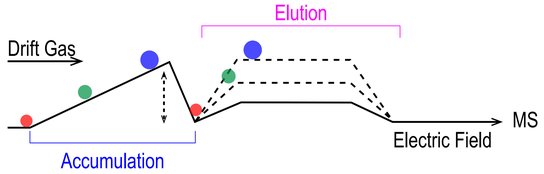
SIGNIFICANCE: Proof of concept, *in situ* TIMS separation and imaging of lipid isomers with distinct spatial distributions was demonstrated.

MATRIX ASSISTED LASER DESORPTION/IONIZATION (MALDI)



TRAPPED ION MOBILITY MASS SPECTROMETRY

- Augmented ion funnel: entrance funnel, ion tunnel, and exit funnel
- Electric field gradient (EFG) applied to the tunnel opposing incoming ions and carrier gas traps ion in order of ascending mobilities. EFG gradually reduced to allow for sequential elution of trapped ions.



METHODS

- Standards: Lipids were mixed with 2',5'-dihydroxyacetophenone (DHA) and spotted onto an Anchorchip plate.

Imaging: Tissues were cryosectioned, thaw mounted onto Indium-tin-oxide (ITO) coated glass slides, and 2',5'-dihydroxybenzoic acid (DHB) was applied using a robotic sprayer.

- All experiments were performed using a prototype timsTOF fleX mass spectrometer (Bruker Daltonics).[3] Mass spectra and ion mobilograms were assessed using DataAnalysis (Bruker Daltonics) and imaging data were visualized using SCiLS and custom in-house software.

CONCLUSIONS

- Gas-phase isobar and isomer separations confirm TIMS as a valuable tool that should be utilized during IMS experiments.
- MS/MS methods, (Paternò-Büchi derivatization, ozone-induced dissociation, and ultraviolet photodissociation) should be integrated in MALDI TIMS workflow to enable a higher degree of lipid structural characterization.

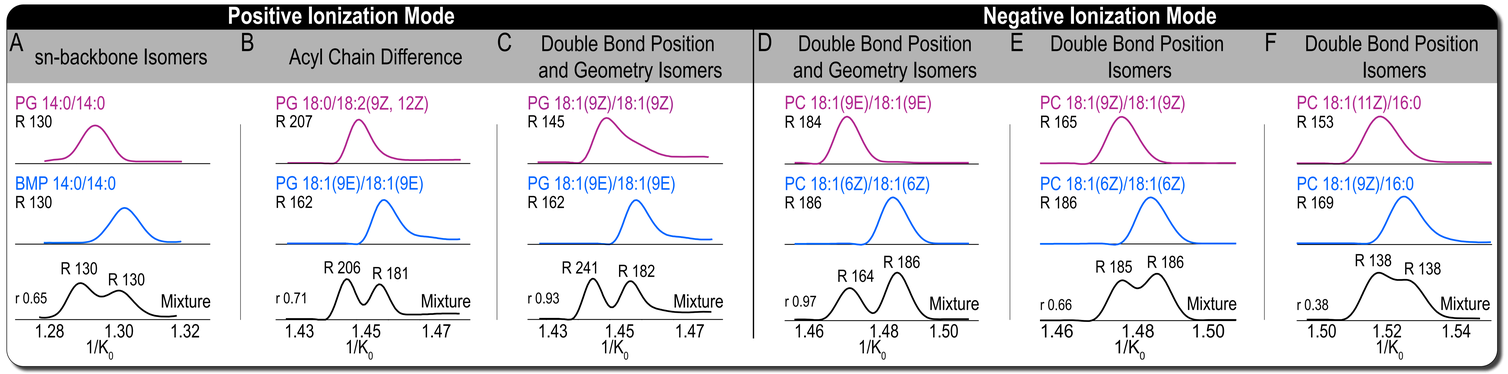
RESULTS

LIPID ISOMER STANDARD SEPARATIONS

Mobilograms of MALDI-generated ions of isomeric lipid standards are shown for both negative (A-C) and positive ionization mode (D-F).

Negative mode Analysis ($[M-H]^-$):
(A) PG (14:0/14:0) and BMP (14:0/14:0), *m/z* 665.49
(B) PG 18:0/18:2 (9Z, 12Z) and PG 18:1/18:1 (9E), *m/z* 773.53
(C) PG (18:1(9Z)/18:1 (9Z)) and PG ((18:1(9E)/18:1(9E)), *m/z* 773.53

Positive mode analysis ($M+Na]^+$):
(D) PC (18:1(9E)/18:1(9E)) and PC (18:1/18:1(6Z)), *m/z* 808.57
(E) PC(18:1(9Z)/18:1(9Z)) and PC(18:1(6Z)/18:1(6Z)), *m/z* 808.57
(F) PC (18:1(11Z)/16:0) and PC18:1(9Z)/16:0) ([M+Na]+, *m/z* 782.56

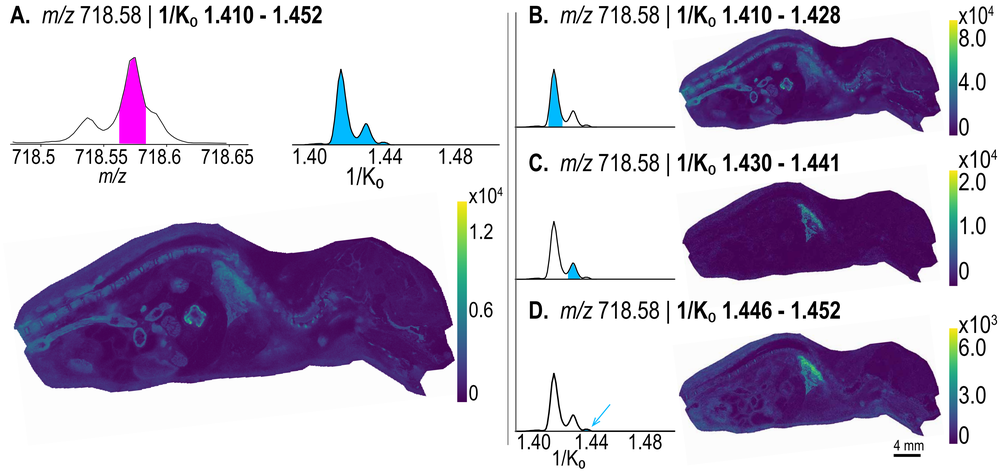


IN SITU LIPID ISOMER SEPARATION

TIMS enables the separation of lipid isomers in whole-body mouse pup tissues - $[CerP(t40:1) + H]^+$, $[PC(O-32:1) + H]^+$ and $[PC(P-32:0) +H]^+$ (0.14 ppm error)

(A) Composite image of all three peaks in the extracted ion mobilogram ($1/K_0$ 1.410 – 1.452) of *m/z* 718.58
(B) $1/K_0$ 1.410 - 1.428
(C) $1/K_0$ 1.430 - 1.441
(D) $1/K_0$ 1.446 - 1.452

- Composite image is dominated by the spatial distributions of the highest intensity ion (B), and spatial distributions of lower intensity ions (B) and (C) are lost.



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