INSTITUT DES SCIENCES ANALYTIQUES

cnrs

Wednesday 8th of March, 2023

Application of omics and MS imaging in Ecotoxicology

Molecular characterization of lipid metabolism in *G. fossarum*

Sophie AYCIRIEX

Multidimensional approaches for complex samples (MDMC)
ANABIO-MS team
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Commission européenne

Co-funded by the Horizon 2020 Framework Programme of the European Union under the grant N° *952306*

UNTOIN

G. fossarum : a sentinel species in freshwater risk assessment

© Lab. Ecotoxicologie INRAe

https://biomae.fr

Crustacean amphipod

High environmental relevance

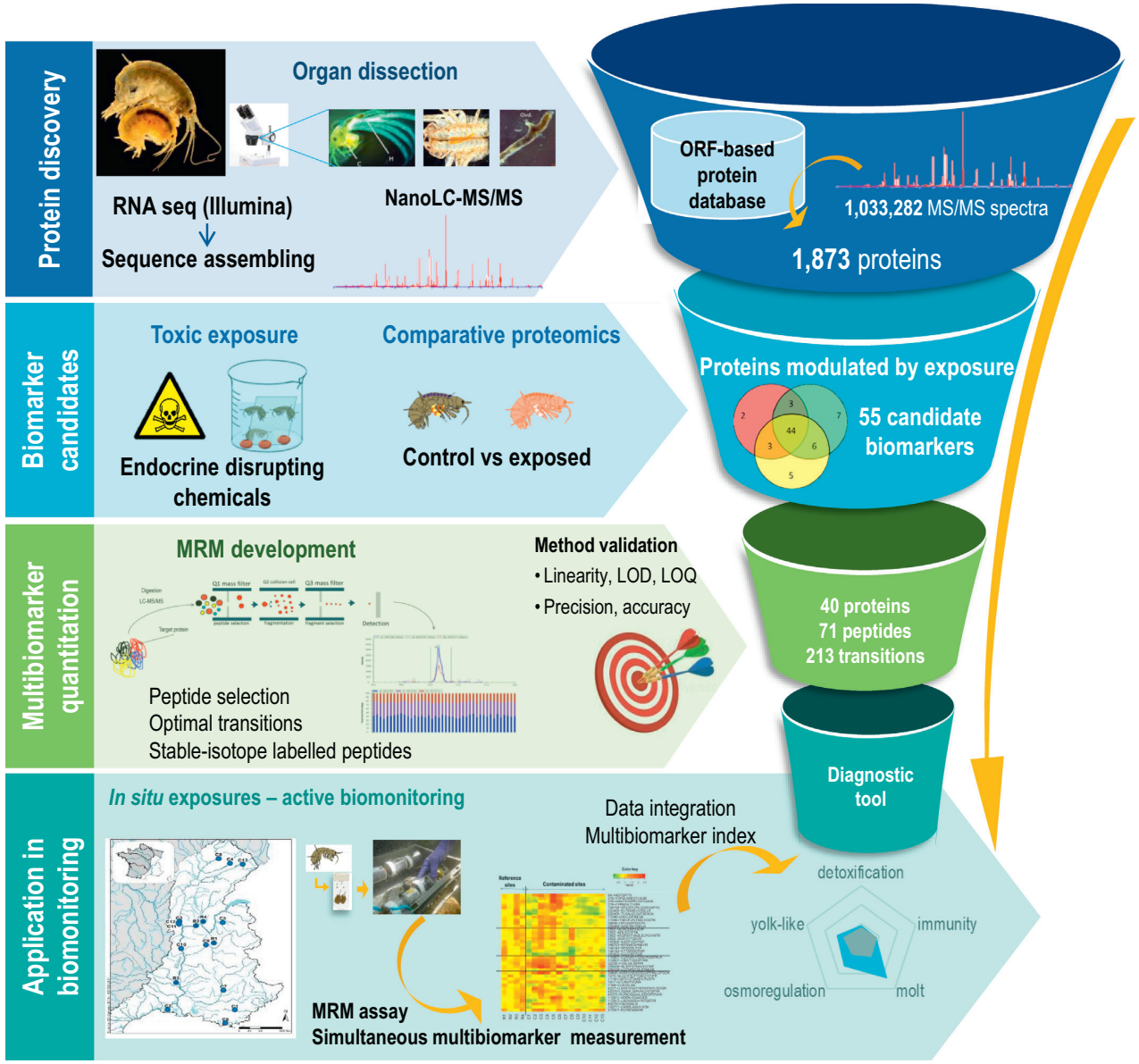
Easy to manipulate (calibration, stabulation)

Sensitive to contaminants

(EDC, metals, pharmaceutics..)

INRAE-RIVERLY
Ecotox. team headed by Dr. A. Chaumot

Proteomics as a performant molecular tools for biomonitoring



J. Armengaud (CEA Marcoule)
A. Chaumot, D. Degli-Esposti,
O. Geffard (INRAe)
A. Salvador, S. Ayciriex (ISA)
(ANR ProteoGam, APPROve)

- To propose Proteomics as a tool for biomarker discovery
- Biomonitoring of water quality in aquatic environment
- What about small molecules ?

Gouveia, D., *et al.* (2019) *J. Proteomics* 198:66-77

INRAE

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Proteomics and Lipidomics Approaches for understanding molecular mechanisms of environmental chemical toxicity in conjunction with lipid metabolism in *Gammarus fossarum* during reproduction cycle (PLAN-TOX)

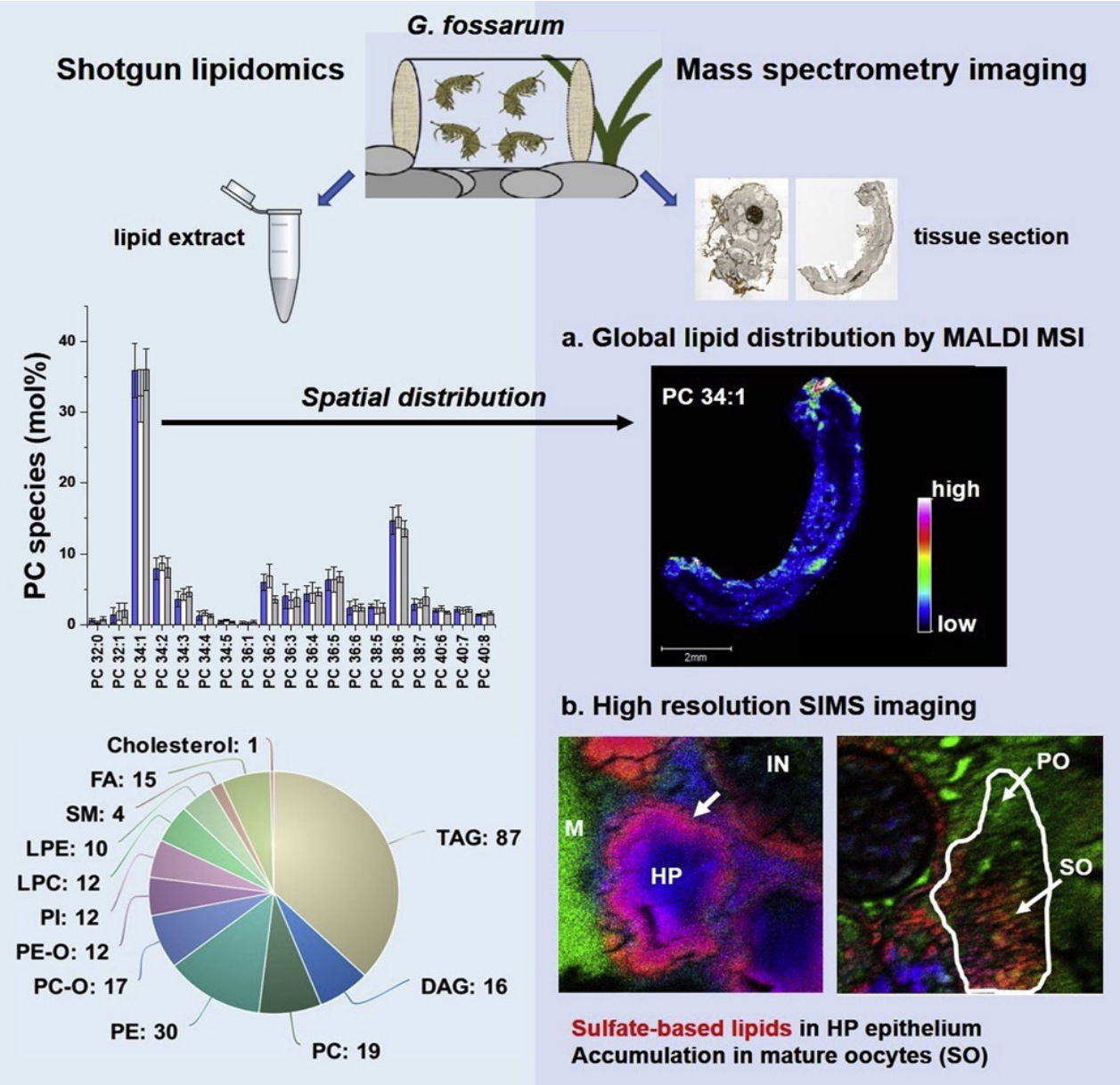
Multi-omics approaches for the characterization of metabolic perturbations in *G. fossarum* after exposure to drug residues

anr

ANR JCJC PLAN-TOX (ANR-18-CE34-0008)

Janv. 2019 – Dec. 2022

Characterization of the lipidome by shotgun lipidomics and MS imaging



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Post-doctorante
Tingting Fu

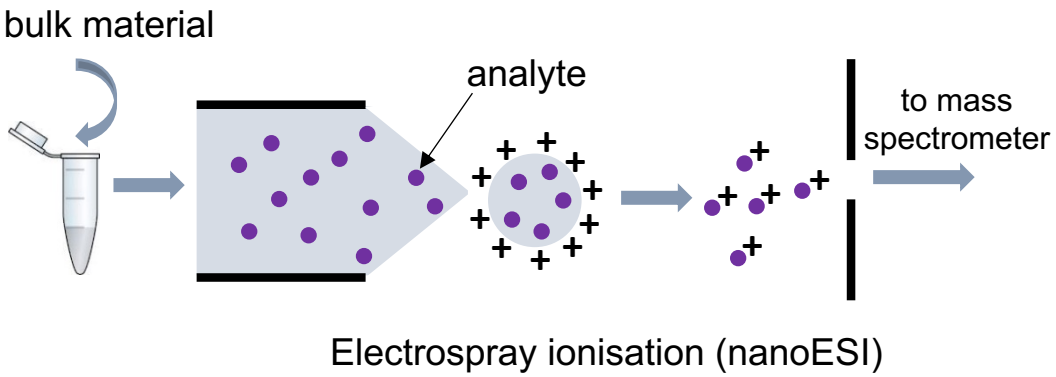
- First exhaustive characterization of the lipidome (12 classes of lipids)
- Lipidome variation according to the development stage
- Spatial distribution of lipids by MSI (MALDI, ToF-SIMS, MALDI IMS)
- Discovery of novel lipids

Fu, T., *et al.*, Ayciriex S. (2020) J. Mass Spec. 55(9):e4513
Fu, T., *et al.*, Ayciriex S. (2021) *iScience*. 24(2)

ANR JCJC PLAN-TOX (ANR-18-CE34-0008)

Methodology: untargeted, spatially resolved lipidome profiling

Shotgun lipidomics by high resolution mass spectrometry (Orbitrap)

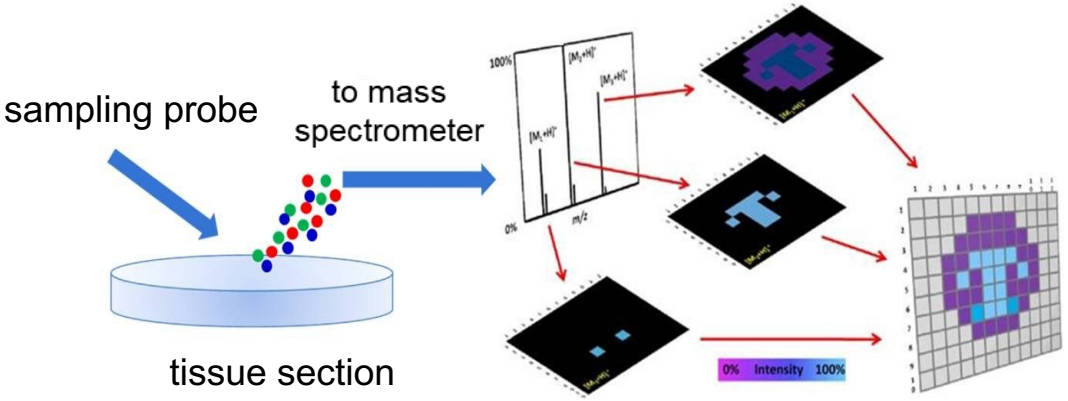


Gammarus fossarum

- **comprehensive lipidome profile**
- **quantitative analysis**

+

Mass spectrometry imaging (MSI: MALDI and SIMS)



- **spatial distribution**
- **semi-quantitative comparison**
- **cellular localization**

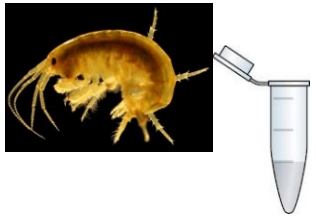
MALDI: matrix-assisted laser desorption/ionization **SIMS: secondary ion mass spectrometry**

Shotgun lipidomics workflow

LIPIDOMICS Informatics for Life Sciences

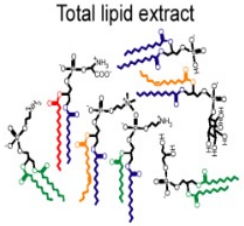
de NBI GERMAN NETWORK FOR BIOINFORMATICS INFRASTRUCTURE

Sample → Lipid extraction → Direct Infusion → Mass Spectrometer → Data Analysis



Adult gammarids (male, female)

10 biological replicates



MTBE/MeOH + ISTD (1 lipid per class)

TriVersa Nanomate HD system (Advion) - nanoESI

Q Exactive – polarity switch + fragmentation experiments

LipidXplorer

PeakStrainer (SIM stitching)

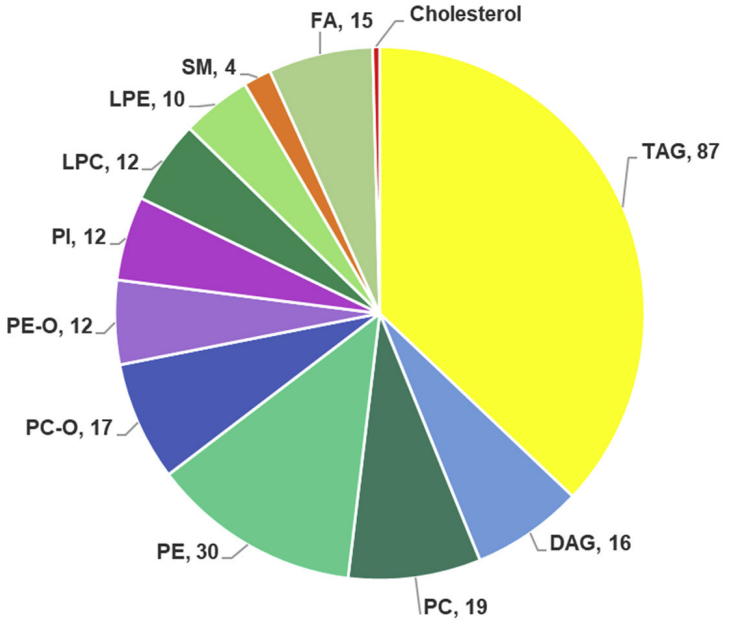
LipidXplorer 1.2.7

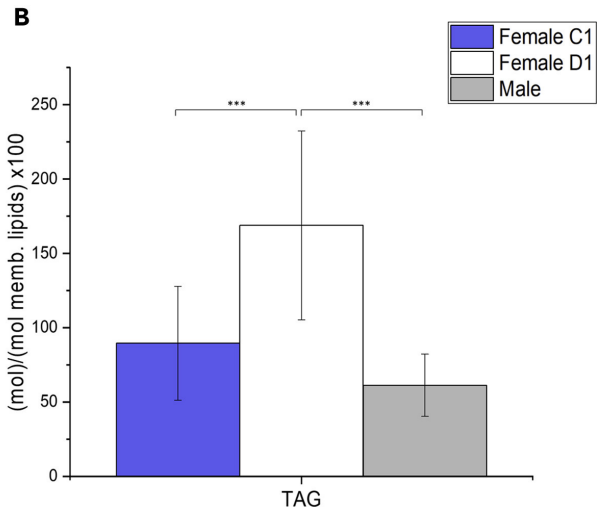
https://lifs-tools.org/tools/68-lipidxplorer-1-2-8-release.html

Herzog R, *et al.*, PLoS ONE 2012;7(1): e29851

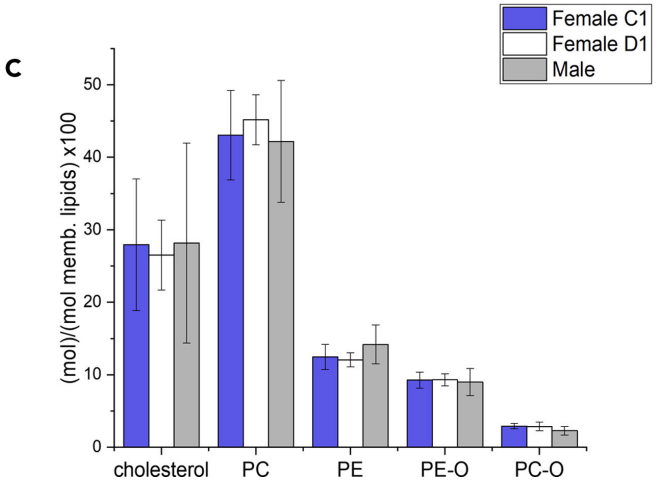
Herzog R, *et al.*, Genome Biol. 2011;12(1):R8

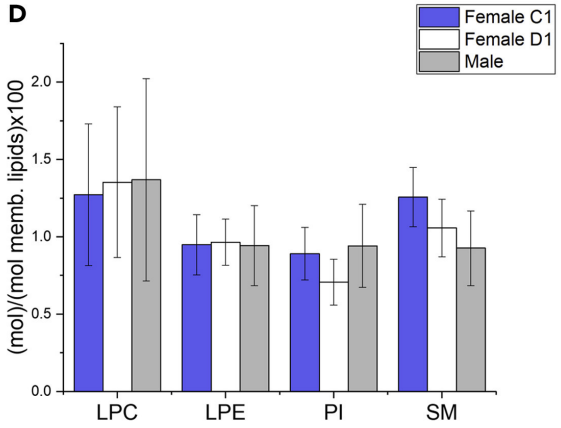
Diversity in lipid composition





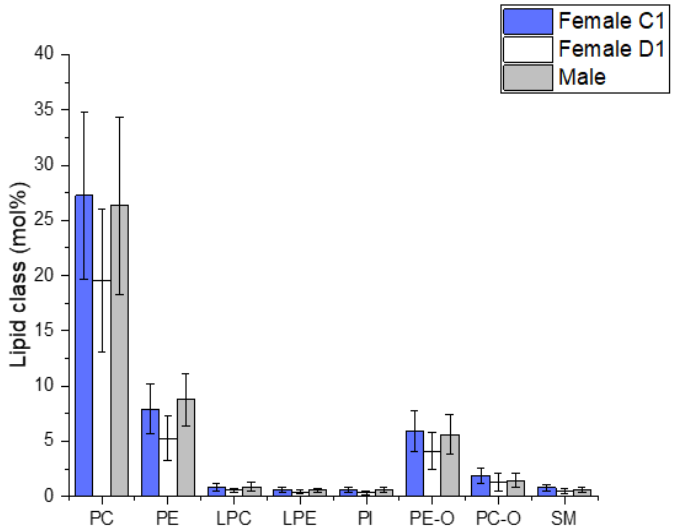
Gammarus fossarum

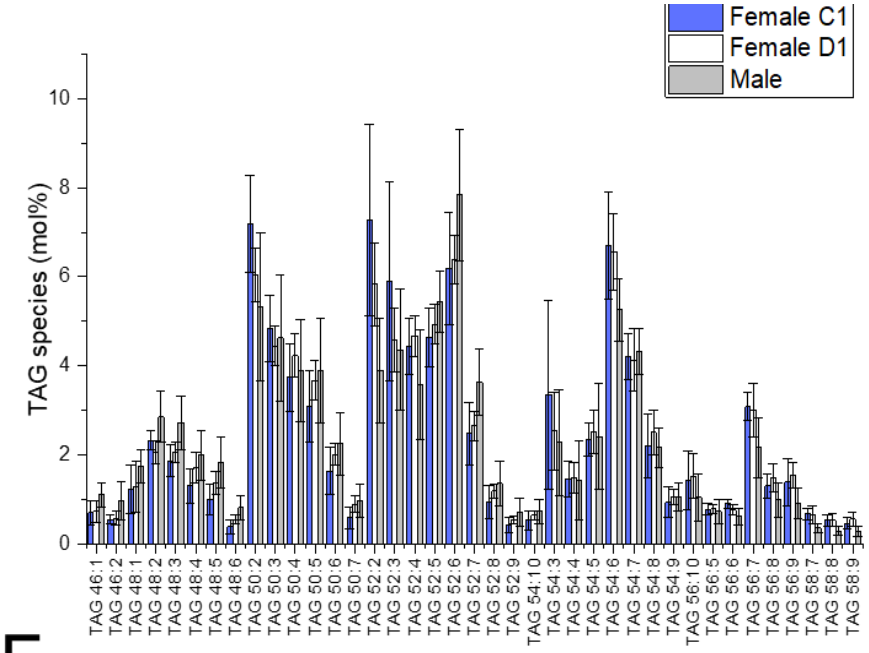




- 12 lipid classes depicted
- > 200 molecular lipid species
- Prominence of TAG species
- Correlation with vitellogenin

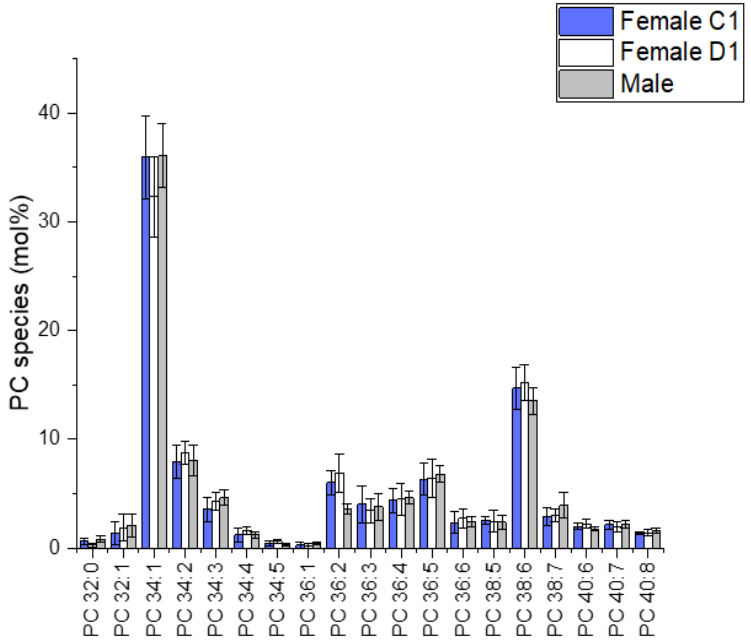
Fu T, *et al.*, iScience (2021) 24(2):102115

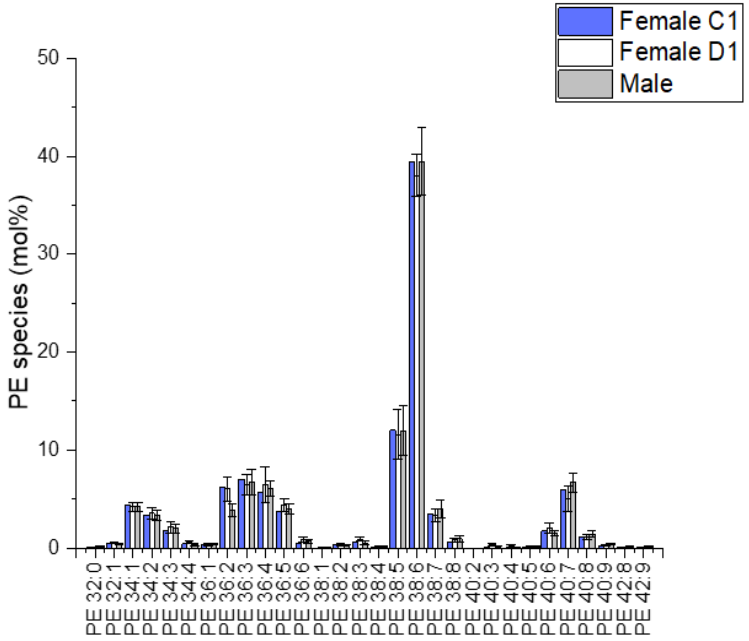




E.

F.





Fu T, *et al.*, iScience (2021) 24(2):102115

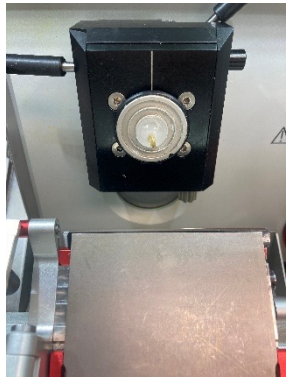
MS imaging workflow

Samples

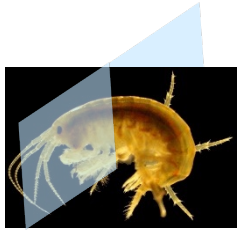
G. fossarum

- Collected from a referenced site and acclimatized to laboratory conditions
- Plunge frozen in liquid N_2 for storage and cryosectioning

Cryo-sectioning



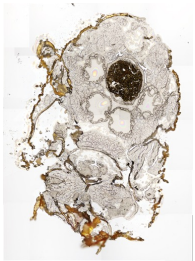
longitudinal



transverse

- 12 µm thickness
- - 23 °C

Mass spectrometry imaging



MALDI imaging:

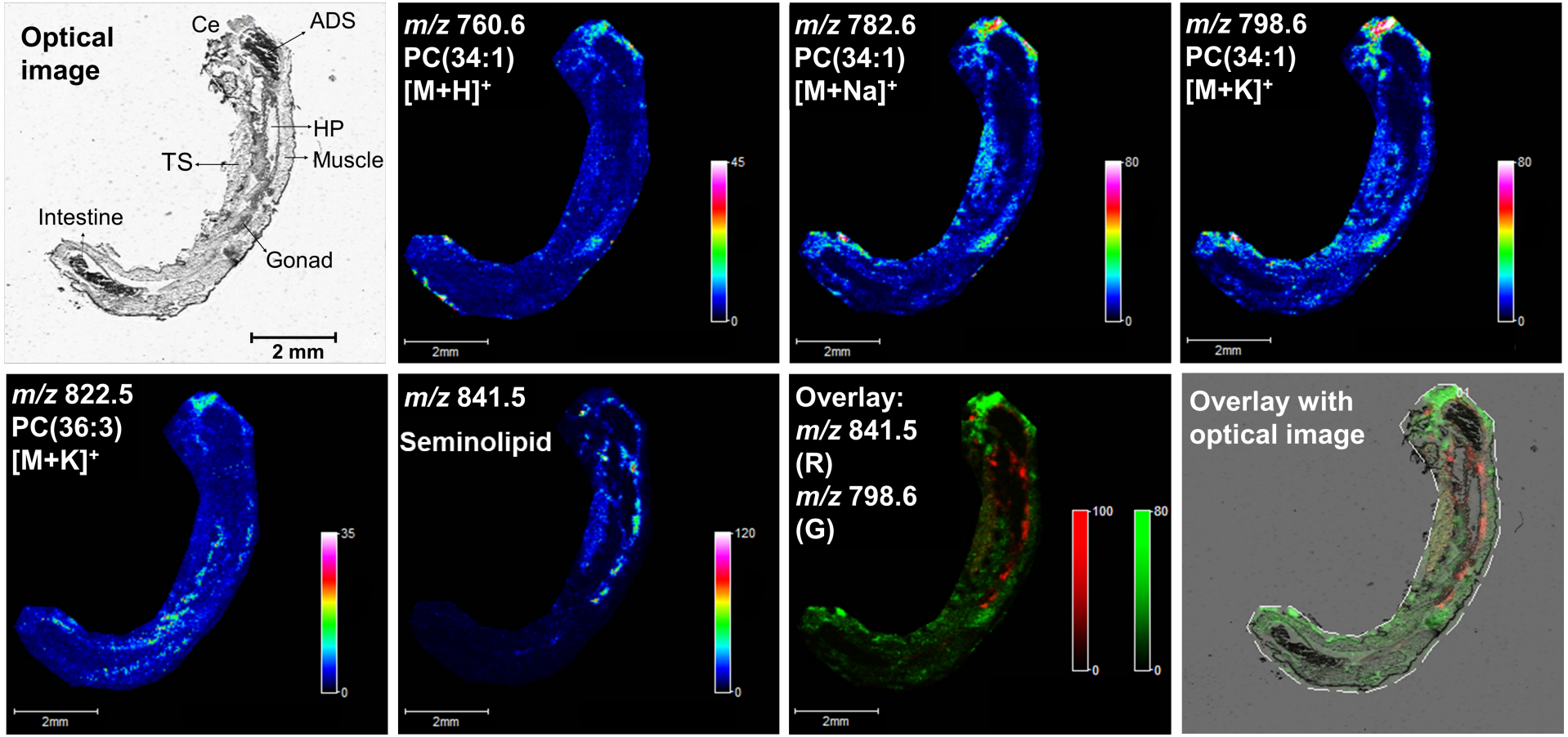
- CHCA for positive ion mode, 10 mg/mL (ACN/H_2O/TFA, 70:30:0.1, *v*/*v*/*v*)
- 9-AA for negative ion mode, 10 mg/mL (EtOH/H_2O, 70:30, *v*/*v*)
- 40 µm/pixel

SIMS imaging:

- 25 keV Bi_3^+
- 2 µm/pixel

Lipid distribution in whole-body gammarid by MALDI-MSI

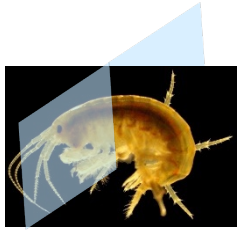
@ 40 µm/pixel



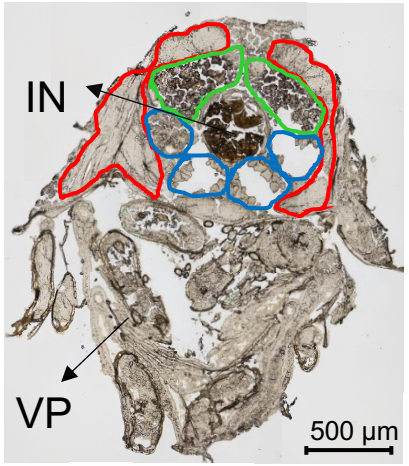
Ce: Cephalon; ADS: anterior digestive system; HP: hepatopancreas; TS: thorax segments

Fu T, *et al.*, iScience (2021) 24(2):102115

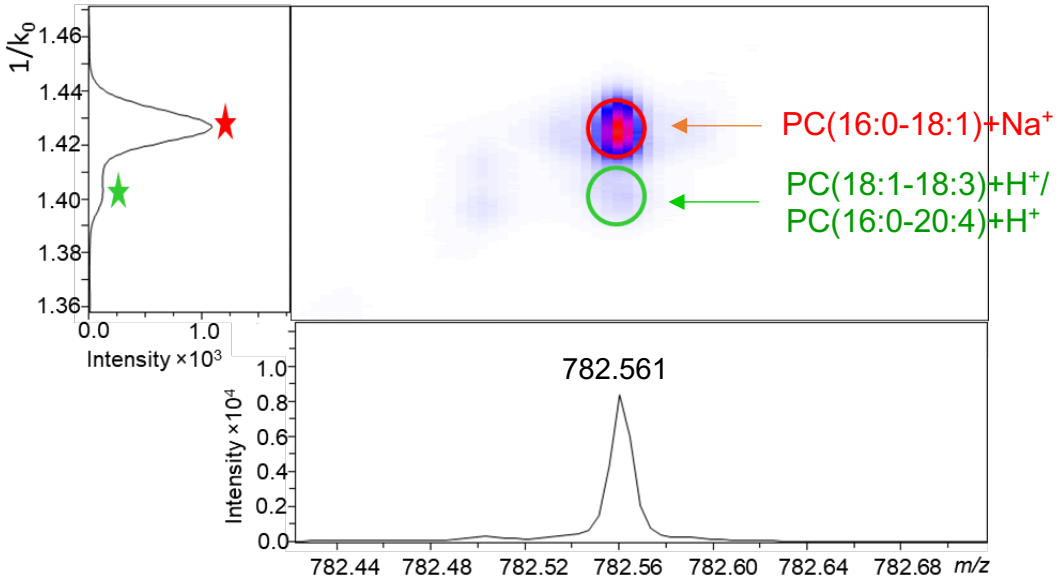
Isobaric lipid separation by MALDI-ion mobility separation MSI



transversal

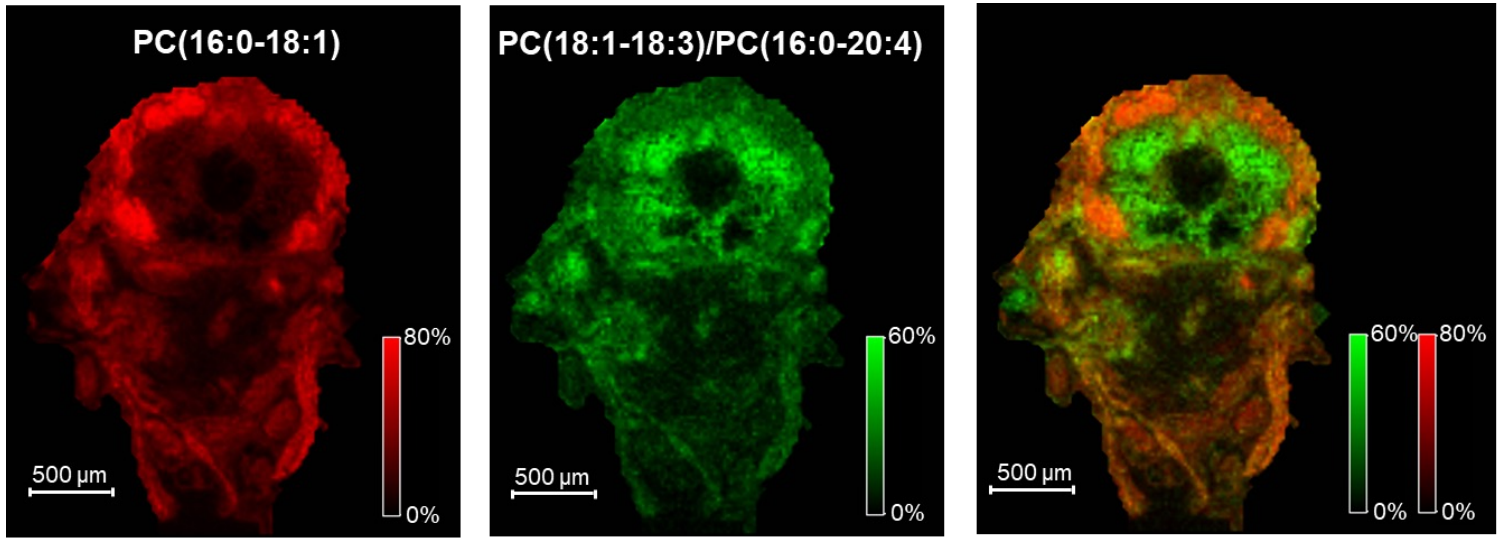


Muscle Oocytes HP



timsTOF flex, Bruker (collaboration)

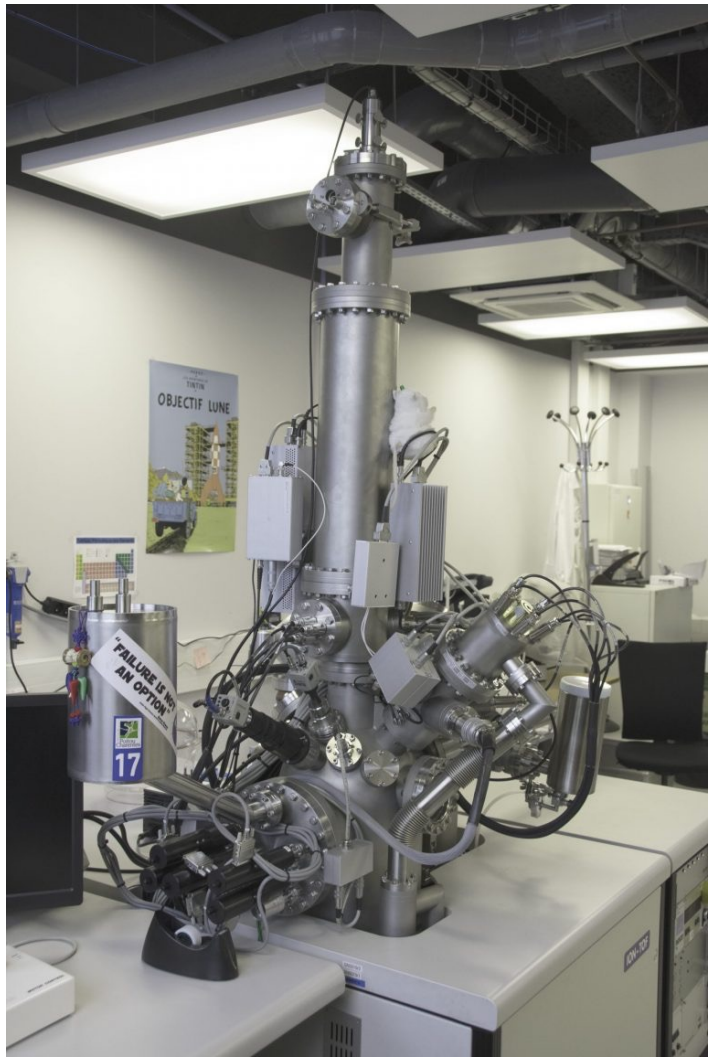
Resolution: 20 µm



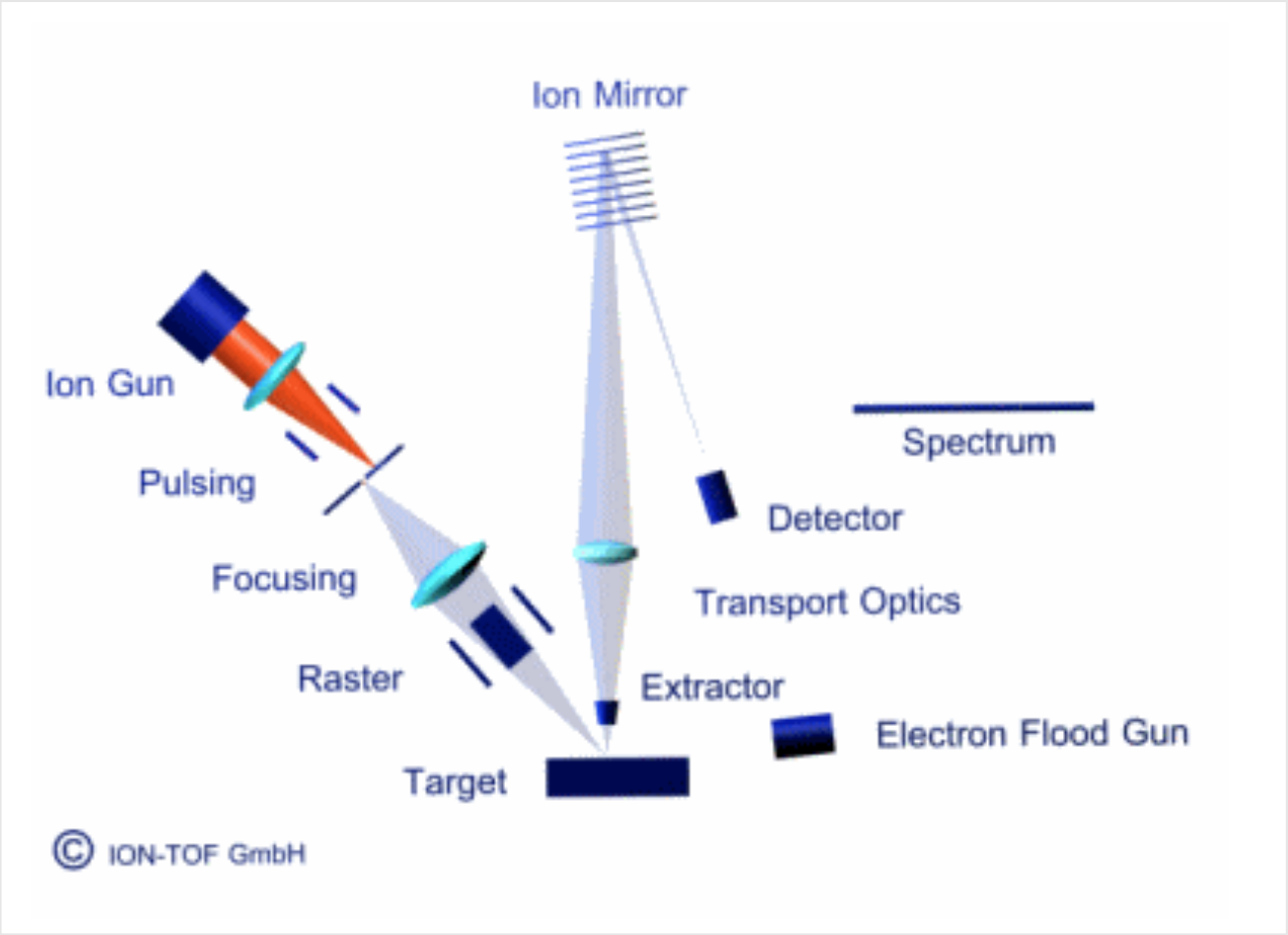
- PC 16:0/18:1, Na+ → muscle
- PC 18:1-18:3 / PC 16:0-20:4 → oocytes

Fu, T. *et al*. *J Mass Spectrom* (2020), 55:e4531

TOF-SIMS principle



http://www.umr-lams.fr/tof-sims/

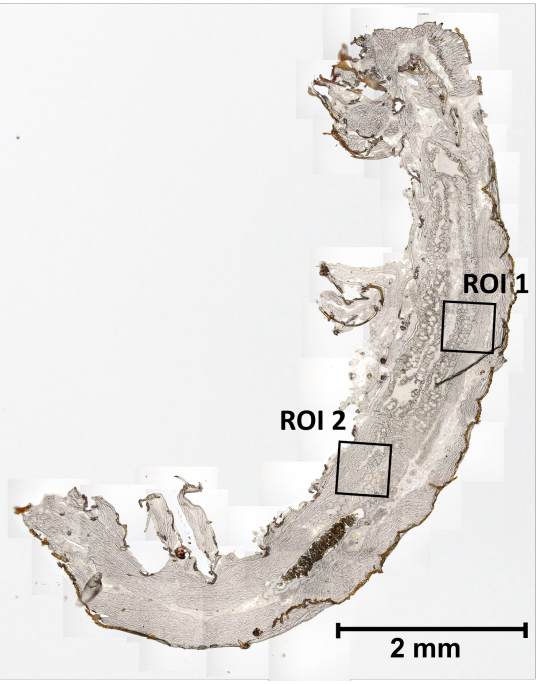


- Extreme surface (50-100 Å) elemental and molecular analysis technique
- A focused beam of ions (primary ions) irradiates the surface of a sample from which secondary ions characteristic of the surface are emitted

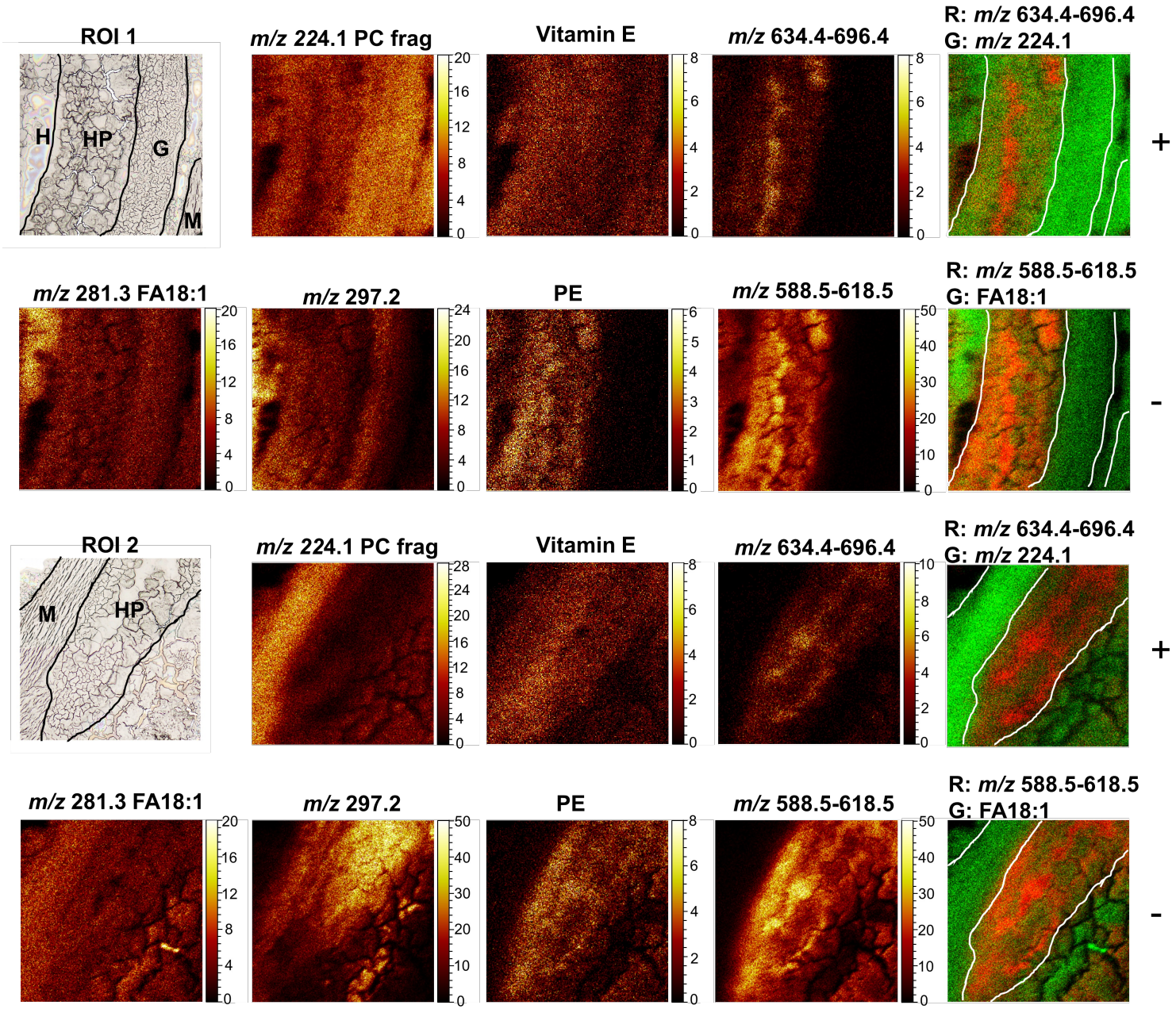
High resolution SIMS imaging of targeted organs

@ 2 µm/pixel

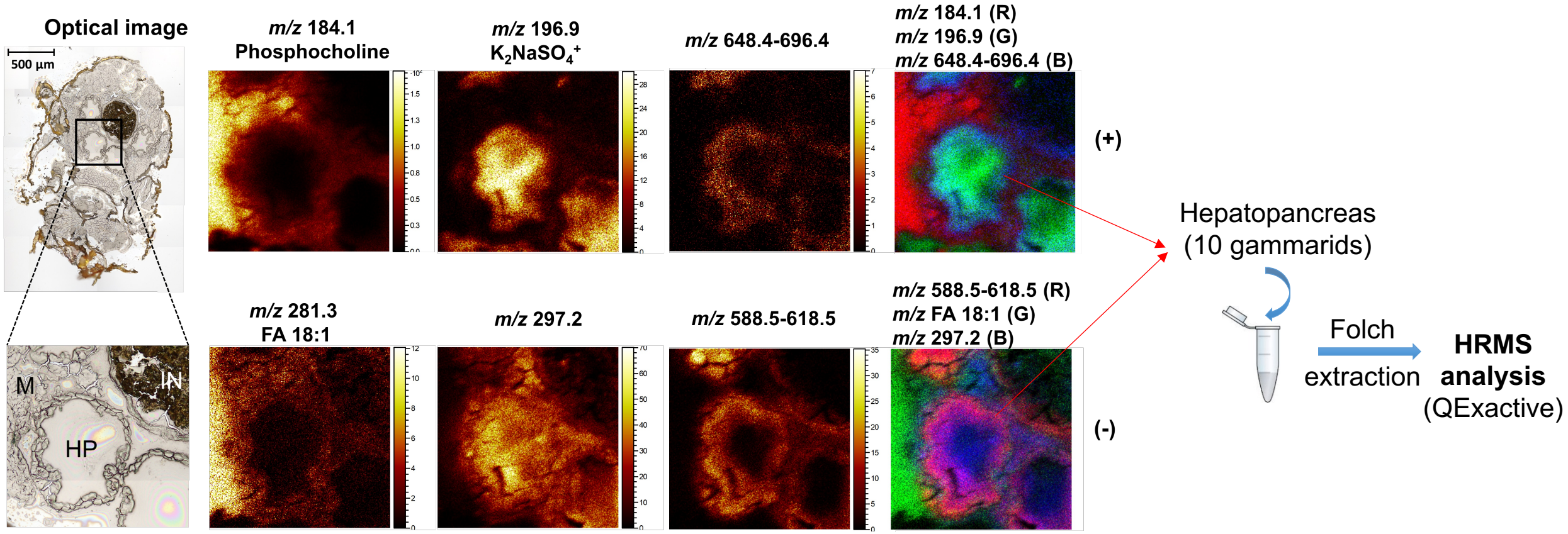
Optical image



H: haemocoel
HP: hepatopancreas
M: Muscle
G: Gonad



Lipid distribution and identification in hepatopancreas



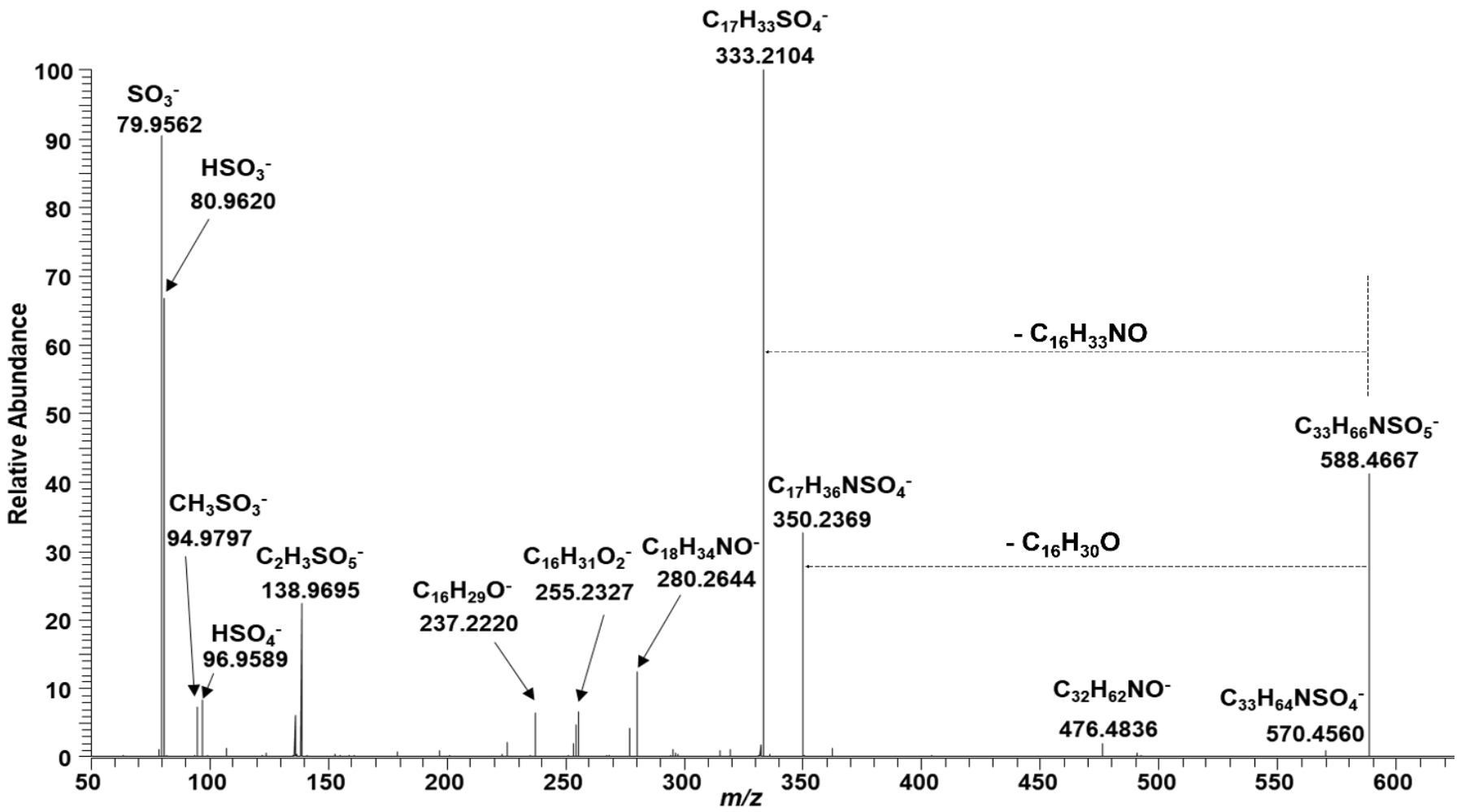
HP: hepatopancreas
IN: Intestine
M: Muscle

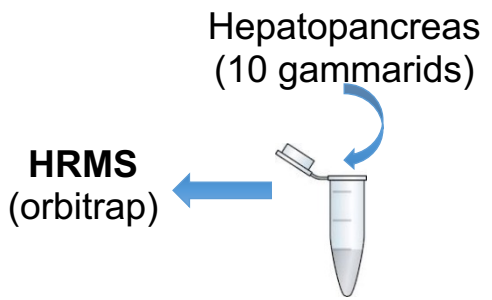
***m/z* 588.5-618.5**: *m/z* 588.5, 602.5, 604.5, 616.5, 618.5

Fu T, *et al.*, iScience (2021) 24(2):102115

Sulfate-based lipid identification in hepatopancreas

MS/MS spectra of *m/z* 588.5





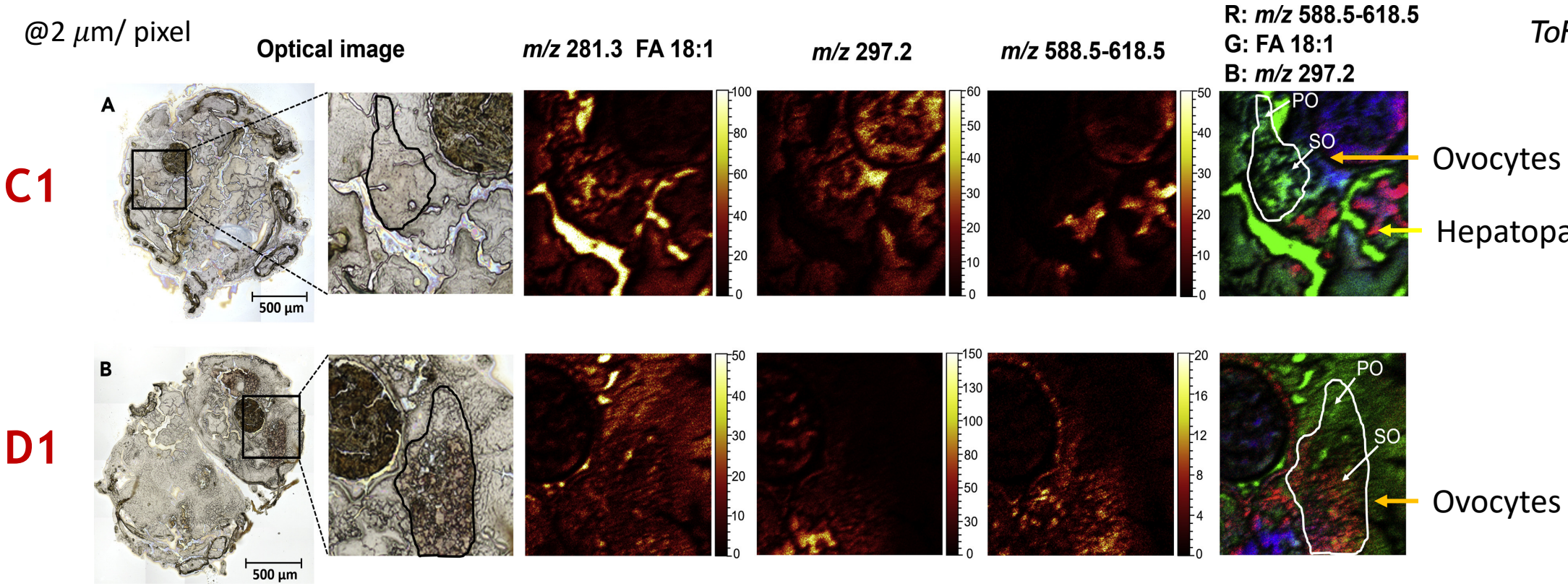
m/z	Assignment	(ppm)
588.4667	$C_{33}H_{66}NSO_5^-$	0.8
570.4560	$C_{33}H_{64}NSO_4^-$	0.7
476.4836	$C_{32}H_{62}NO^-$	1.0
350.2369	$C_{17}H_{36}NSO_4^-$	1.1
333.2104	$C_{17}H_{33}SO_4^-$	1.2
280.2644	$C_{18}H_{34}NO^-$	1.4
255.2327	$C_{16}H_{31}O_2^-$	1.2
237.2220	$C_{16}H_{29}O^-$	0.8
138.9695	$C_2H_3SO_5^-$	-4.3
96.9589	HSO4$^-$	-6.7
94.9797	$CH_3SO_3^-$	-6.3

Fu T, *et al.*, iScience (2021) 24(2):102115

Changes in the lipid composition of oocytes during development

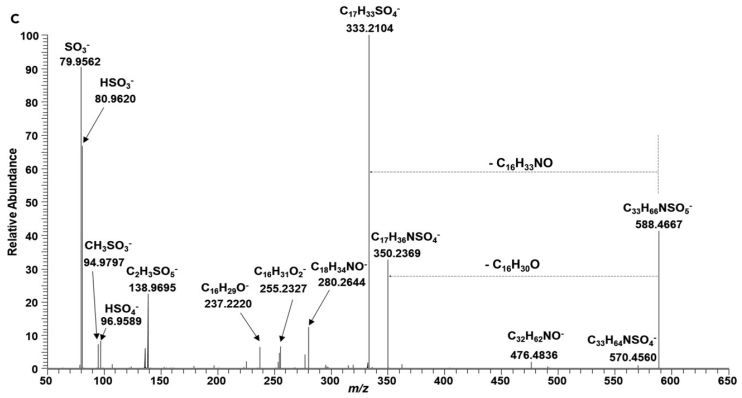
@2 μm/ pixel

ToF-SIMS imaging



MS^2 spectra

m/z 588.4667



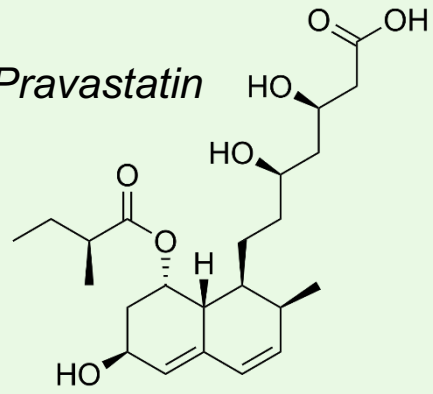
- **Discovery of sulfated lipids in HP**
- **Migration of sulfated lipids from C1 (HP) to D1 ovocytes**

Fu, T., *et al.*, Ayciriex S. (2021) *iScience*. 24(2)

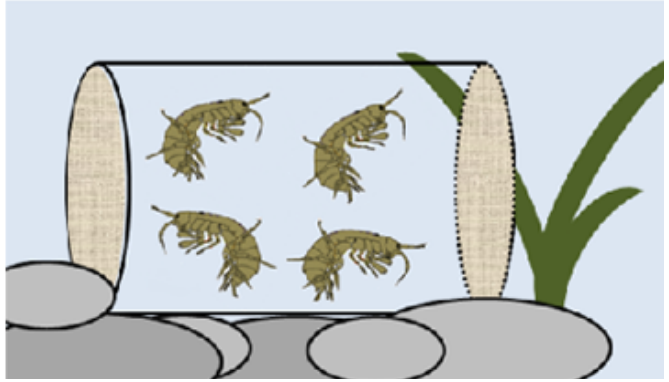
Evaluation of reprotoxicity and lipid dysregulation after pravastatin exposure

Effluent

Pravastatin

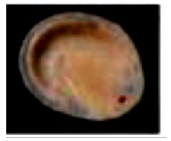


Exposure experiments

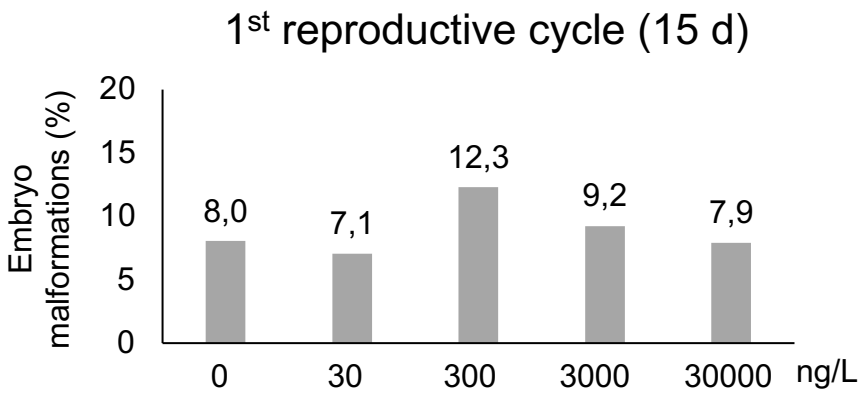


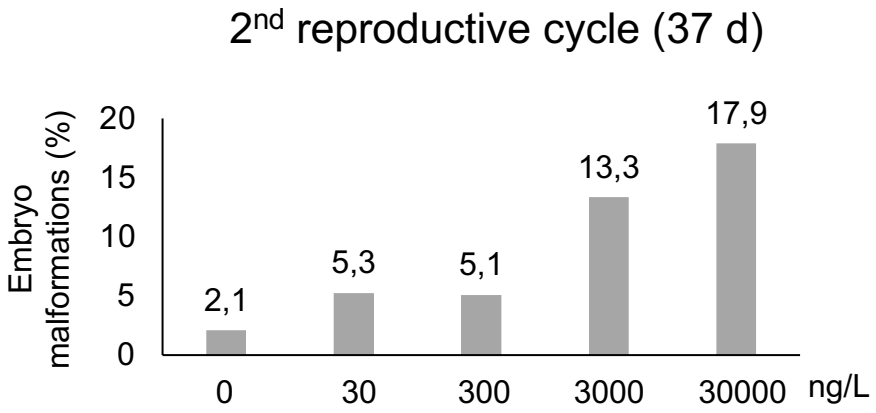
Pravastatin: 0 ng/L-30,000 ng/L

Brood pouch



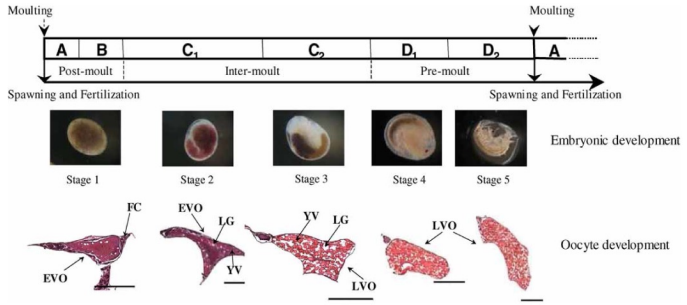
Examination of malformed embryos

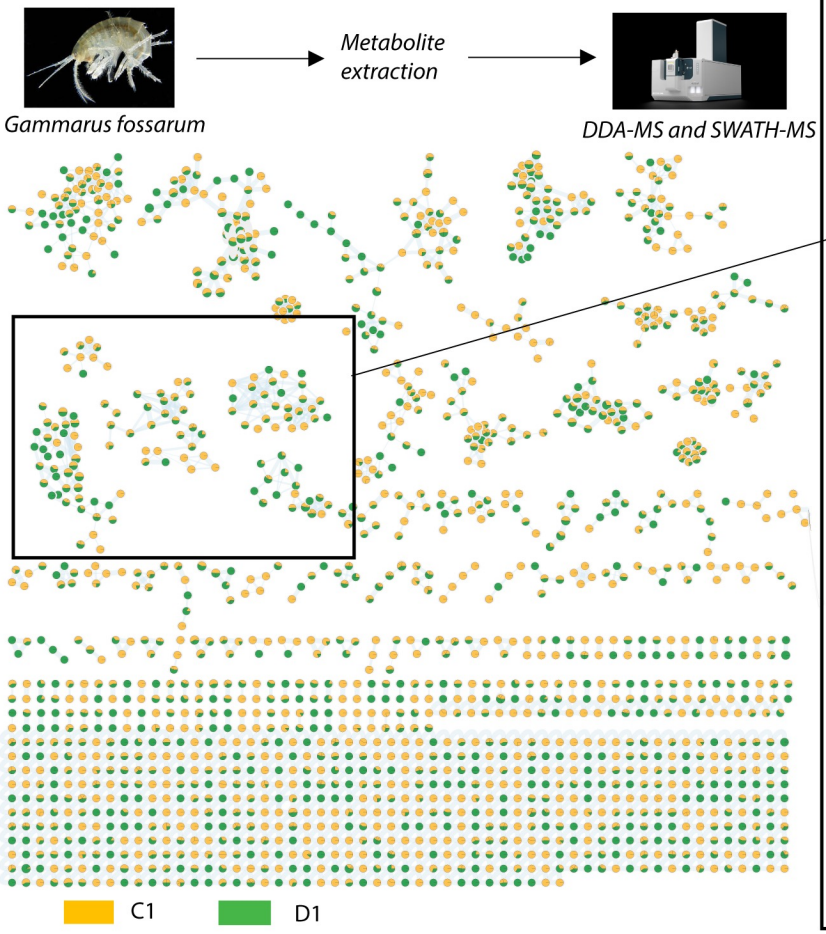


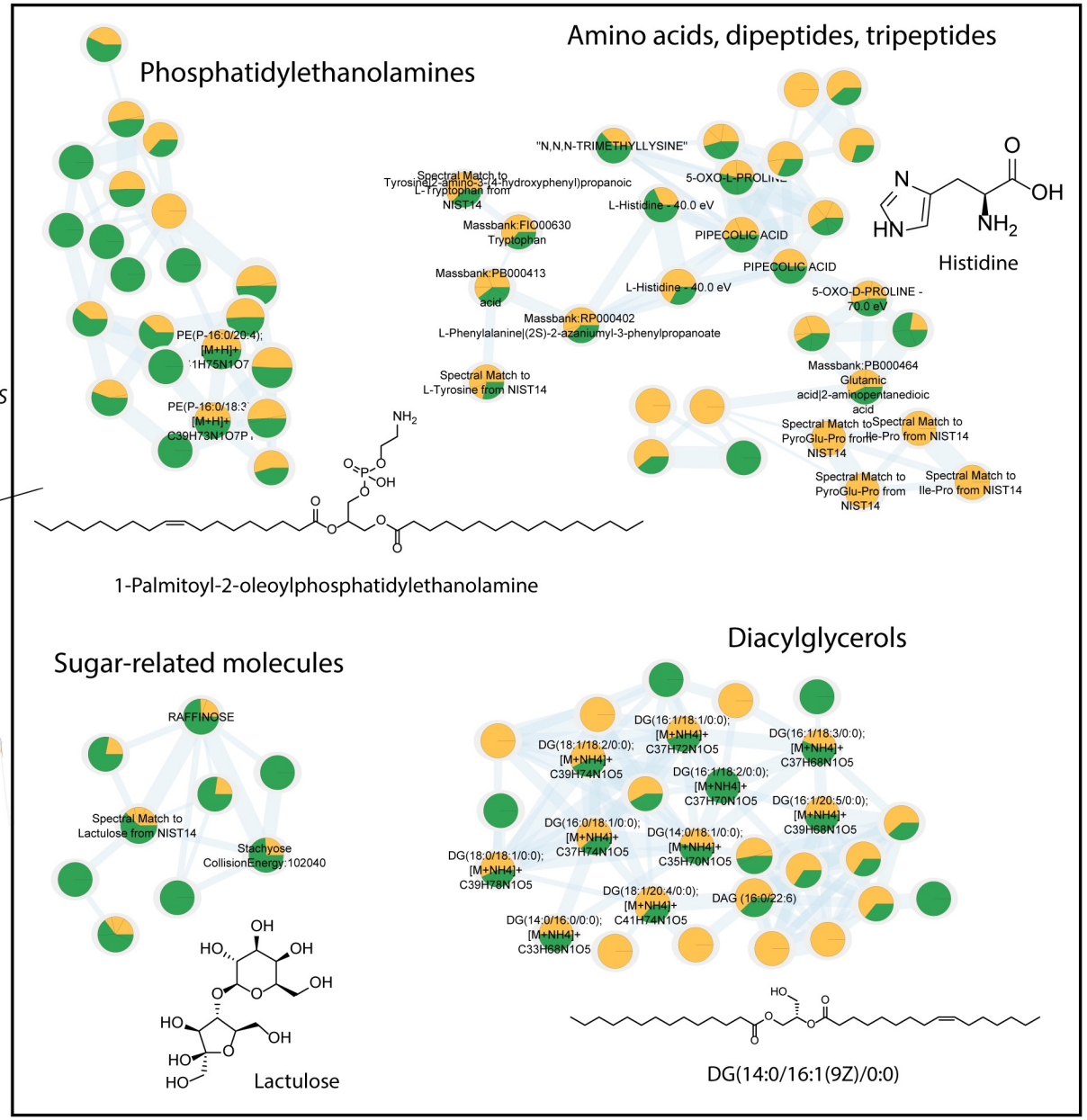


Fu, T., *et al.*, Ayciriex S. (en preparation pour Aquatic Toxicology)

In-depth structural characterization of metabolites : use of molecular networks







Post-doctorante
Valentina Calabrese

INSTITUT DES SCIENCES ANALYTIQUES

cnrs

Wednesday 8th of March, 2023

MERCI POUR VOTRE ATTENTION !

Sophie AYCIRIEX

Multidimensional approaches for complex samples (MDMC)
ANABIO-MS team
sophie.ayciriex@univ-lyon1.fr

Commission européenne

Co-funded by the Horizon 2020 Framework Programme of the European Union under the grant N° *952306*

UNTOIN