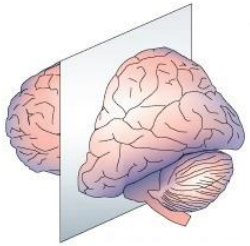
ÚOCHB AV ČR
IOCB PRAGUE

Tissue imaging with MALDI-MS

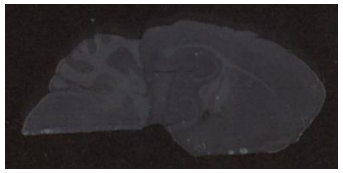
Ing. Štěpán Strnad Ph.D. | MS Group Days 2022

Mass spectrometry imaging

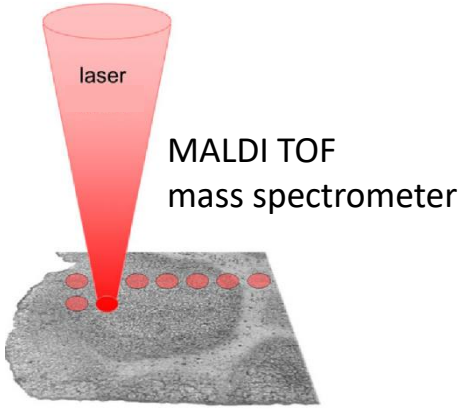


Tissue sectioning
(12 µm)

Tissue mounting

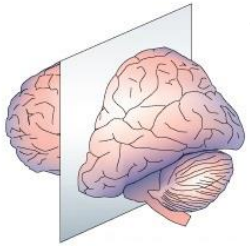


Matrix deposition



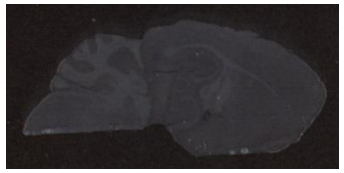
Tissue covered with matrix

- **visualization of the distribution**
- **lipids**, drugs, metabolites, proteins, peptides
- **label free technique**

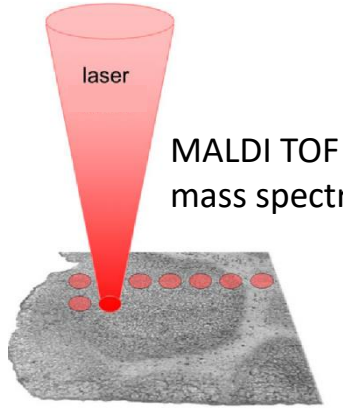


Tissue sectioning
(12 µm)

Tissue mounting



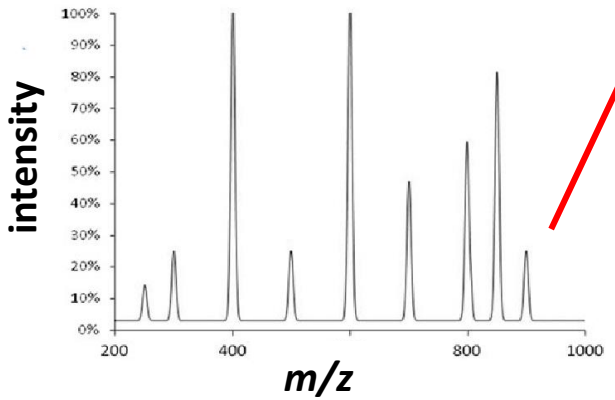
Matrix deposition



MALDI TOF
mass spectrometer

Tissue covered with matrix

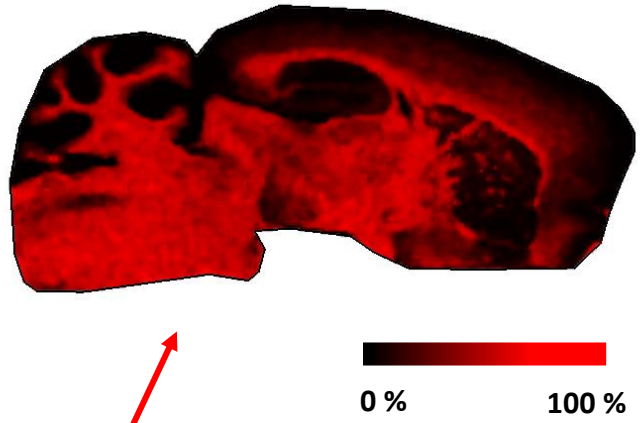
Data processing



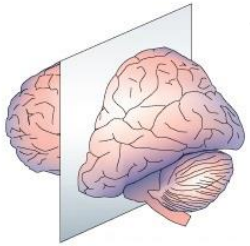
(mass/charge)

Image reconstruction

sulfatide
ST 24:0 $[M - H]^-$

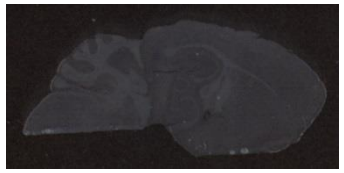


0 % **100 %**

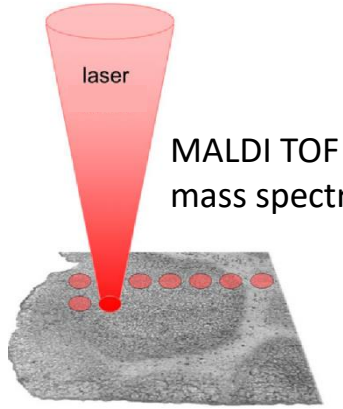


Tissue sectioning
(12 µm)

Tissue mounting



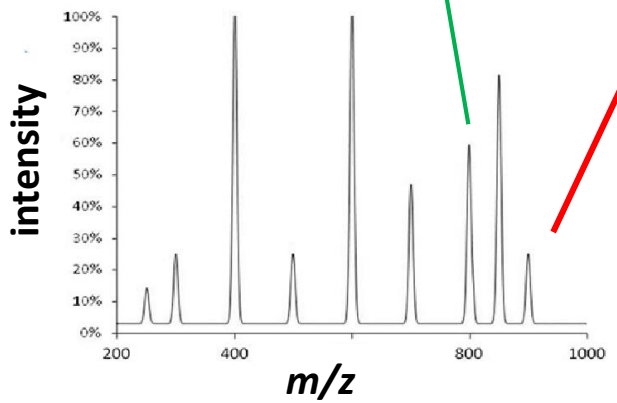
Matrix deposition



MALDI TOF
mass spectrometer

Tissue covered with matrix

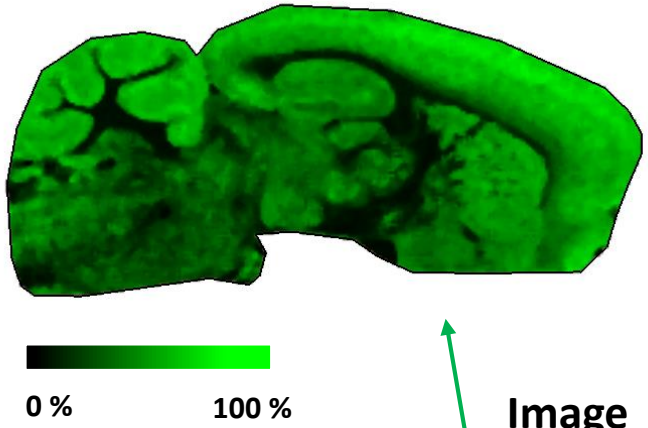
Data processing



(mass/charge)

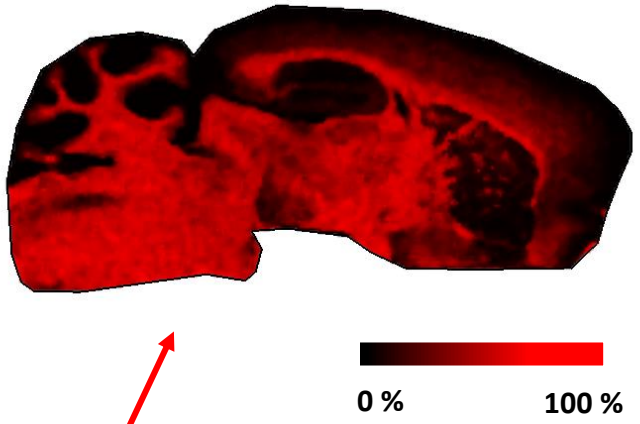
Image reconstruction

phosphatidylethanolamin
PE 40:6 $[M - H]^-$



0 % 100 %

sulfatide
ST 24:0 $[M - H]^-$



0 % 100 %

MSI workflow - optimized methods

Study of lipids, small molecules

matrix: **10 mg/ml DAN 70% ACN**

deposition: **iMatrixSpray**

matrix: **DHB**

deposition: **sublimation**

Cholesterol

analyte: cholesterol, unsaturated FA

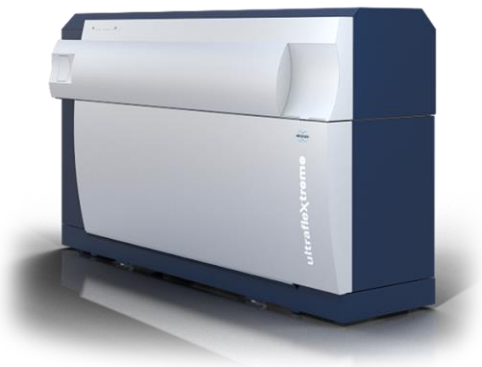
matrix: **silver**

deposition: **thermal evaporation**

- reproducible, high spatial resolution capabilities (10 - 20 µm)

MSI workflow - measurement

Bruker ultrafleXtreme (MALDI-TOF/TOF)



data **acquisition**
(flexControl, flexImaging)

import

data **processing, analysis, visualization, statistics**

SCiLS Lab

June 15, 2022 (Wednesday)

GUIDED TOURS & DEMONSTRATION OF THE INSTRUMENTS (MS Group Labs)

13:00 - 15:00

Orbitrap LTQ XL – High resolution and tandem MS *(A.1.83; Josef Cvačka)*
QTRAP – Quantification of small molecules *(A.2.84; Karel Čížek)*
Orbitrap Lumos – Proteomics *(A.1.80; Martin Hubálek)*
MALDI – Large and small molecules *(A.1.80; Vladimír Vrkoslav)*

MSI workflow - identification

m/z

- databases (**SwissLipids**, lipidmaps), literature

Name	m/z ([M-H]-)
Triacylglycerol (55:9)	885.69775
Phosphatidylinositol (38:4)	885.54987
Phosphatidate (O-51:7)	885.67426
Phosphatidylglycerol (44:2)	885.659
Phosphatidate (50:7)	885.63788

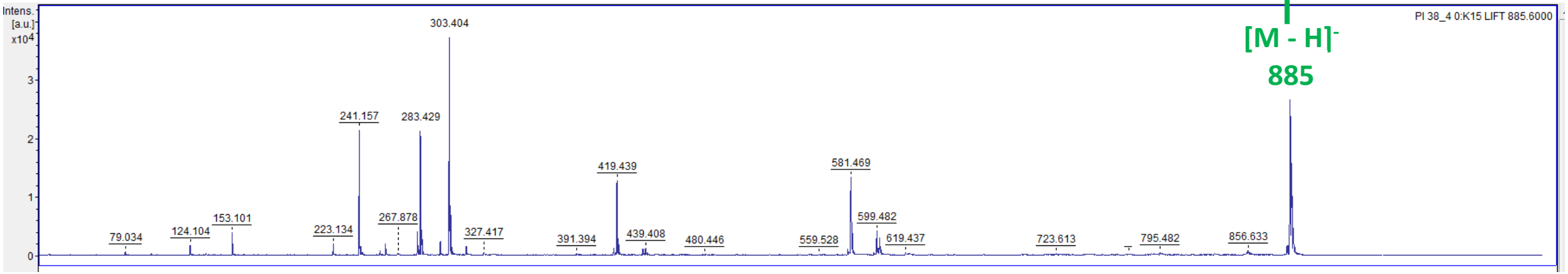
confirmation:

- standard measurement - comparison
- MS^2 (LIFT) characteristic fragments

??? *m/z* 885.6

***m/z* 885,6 ± 0,1**

[M - H]⁻
885



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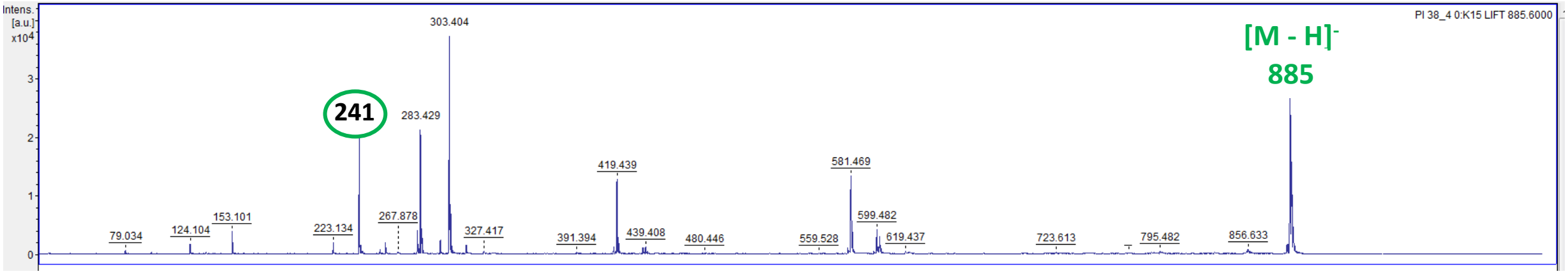
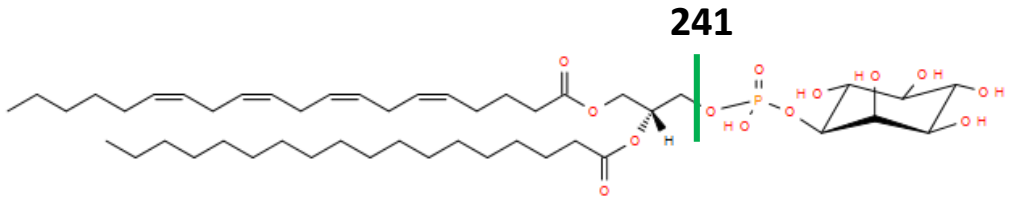


MSI workflow - identification



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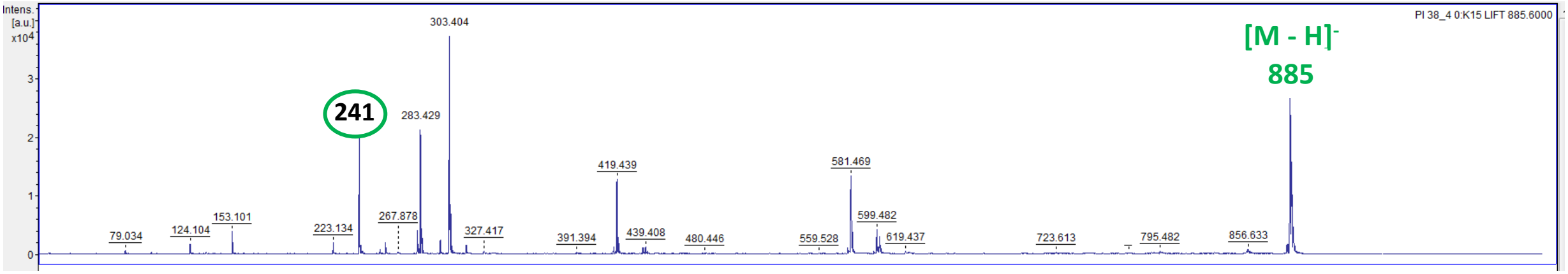
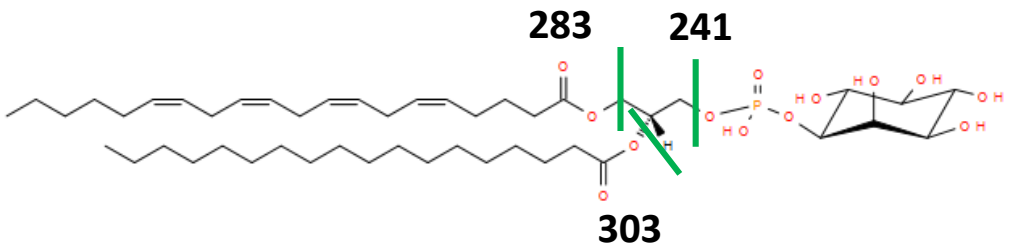


MSI workflow - identification



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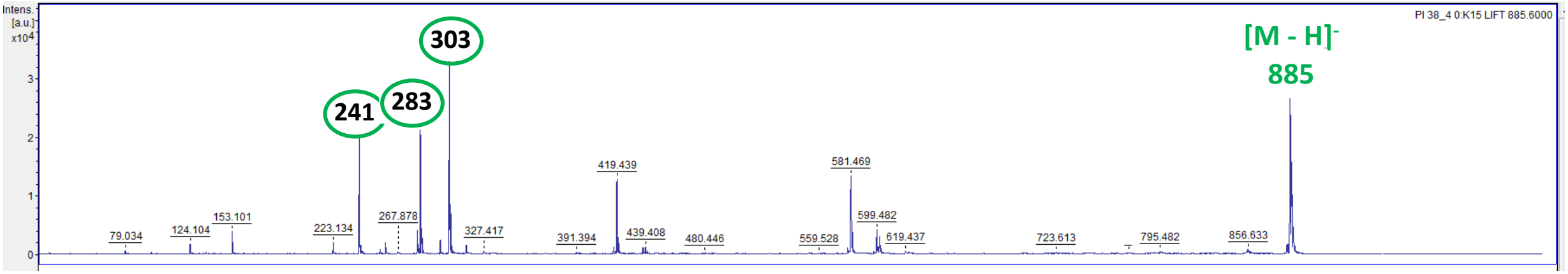
PI 38:4 *m/z* 885,6

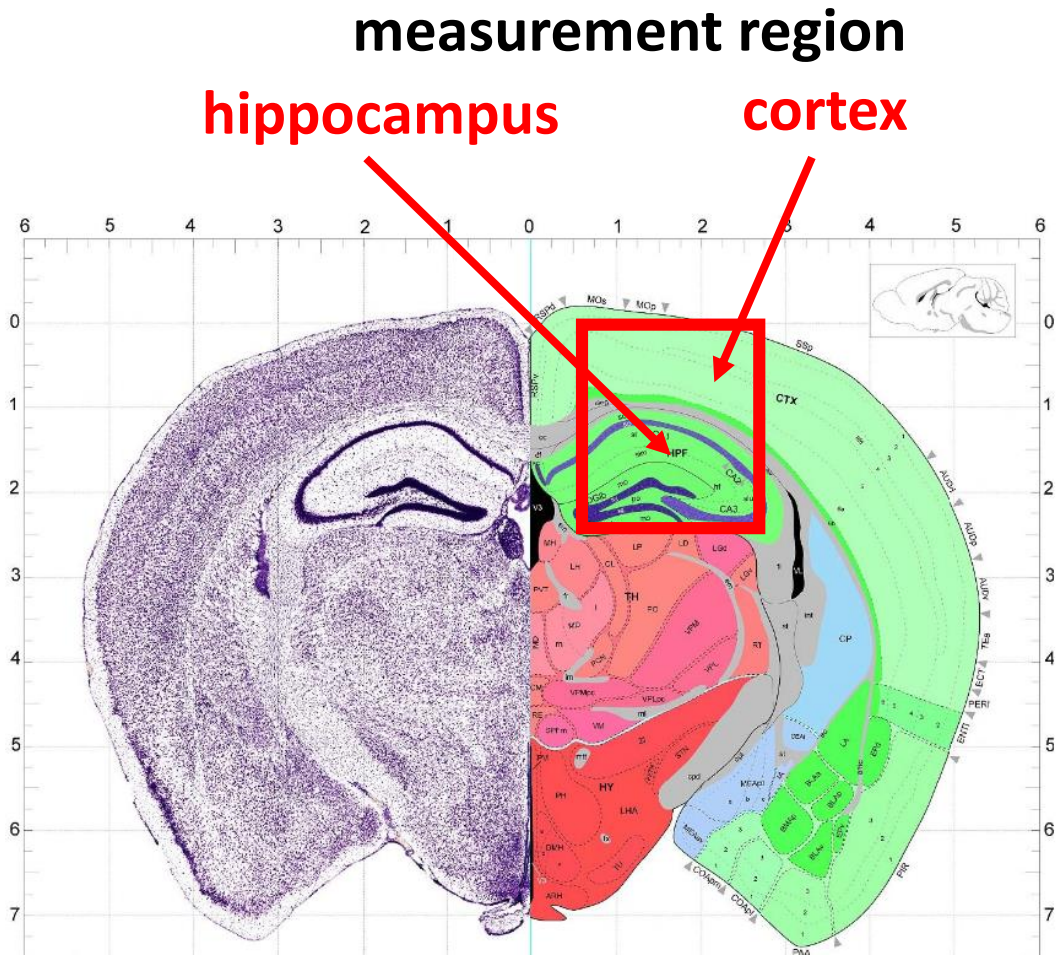


Study of lipid changes – neurodegeneration

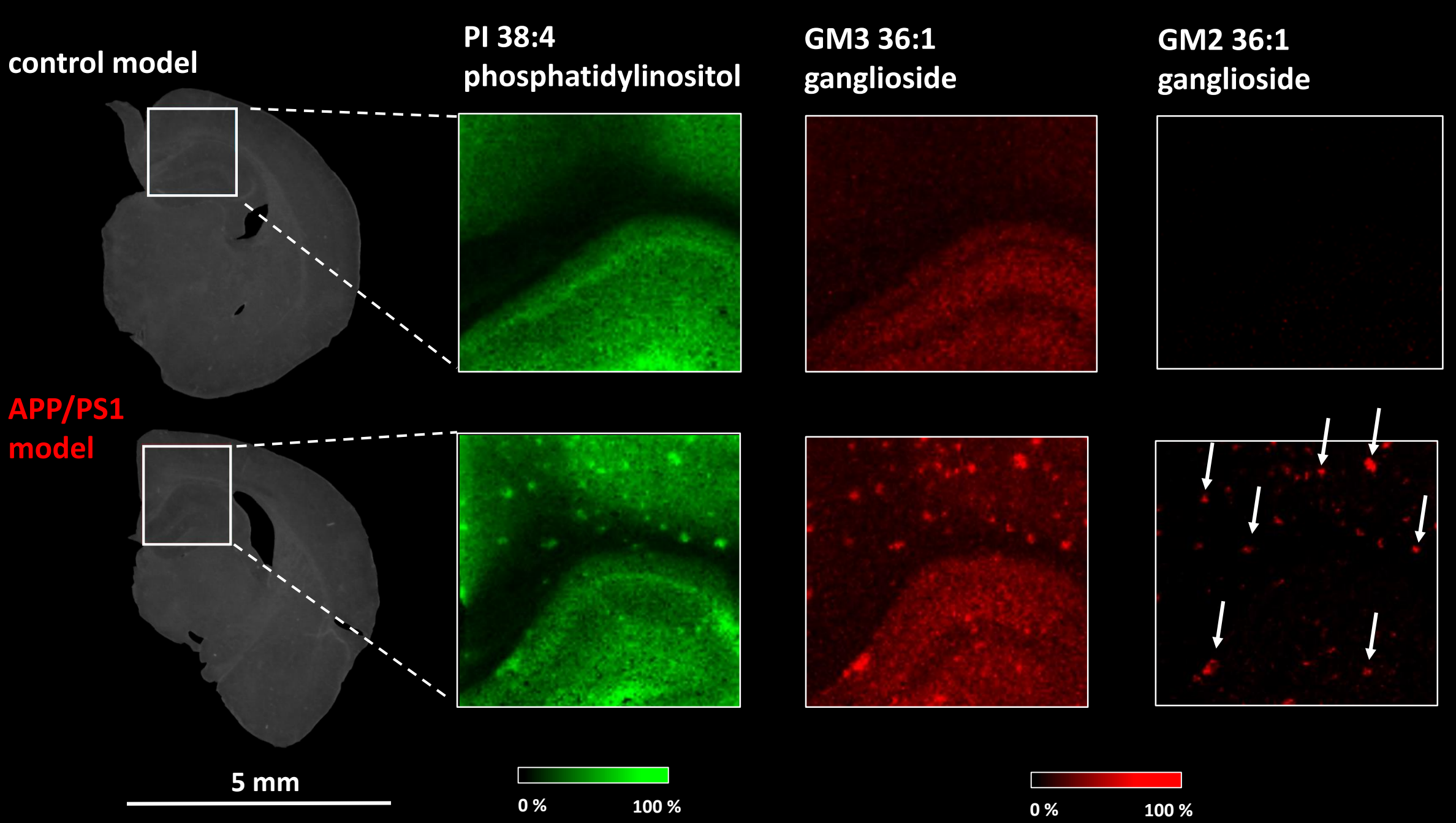
APP/PS1 mouse neurodegeneration model

- exhibits senile plaques
- neurodegeneration starts in:

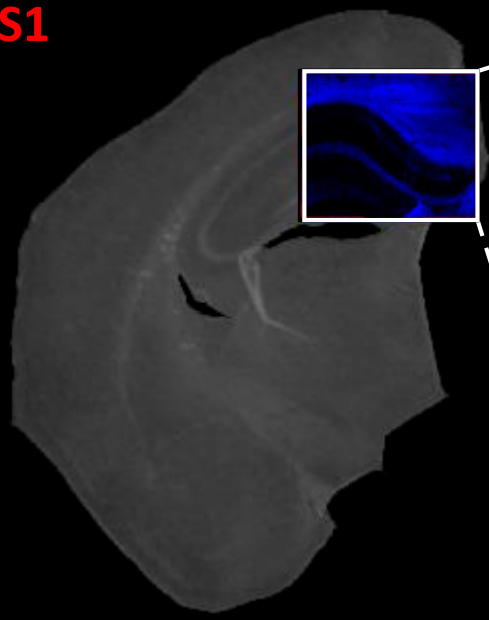
  hippocampus, cortex
- APP/PS1 vs age-matched control model

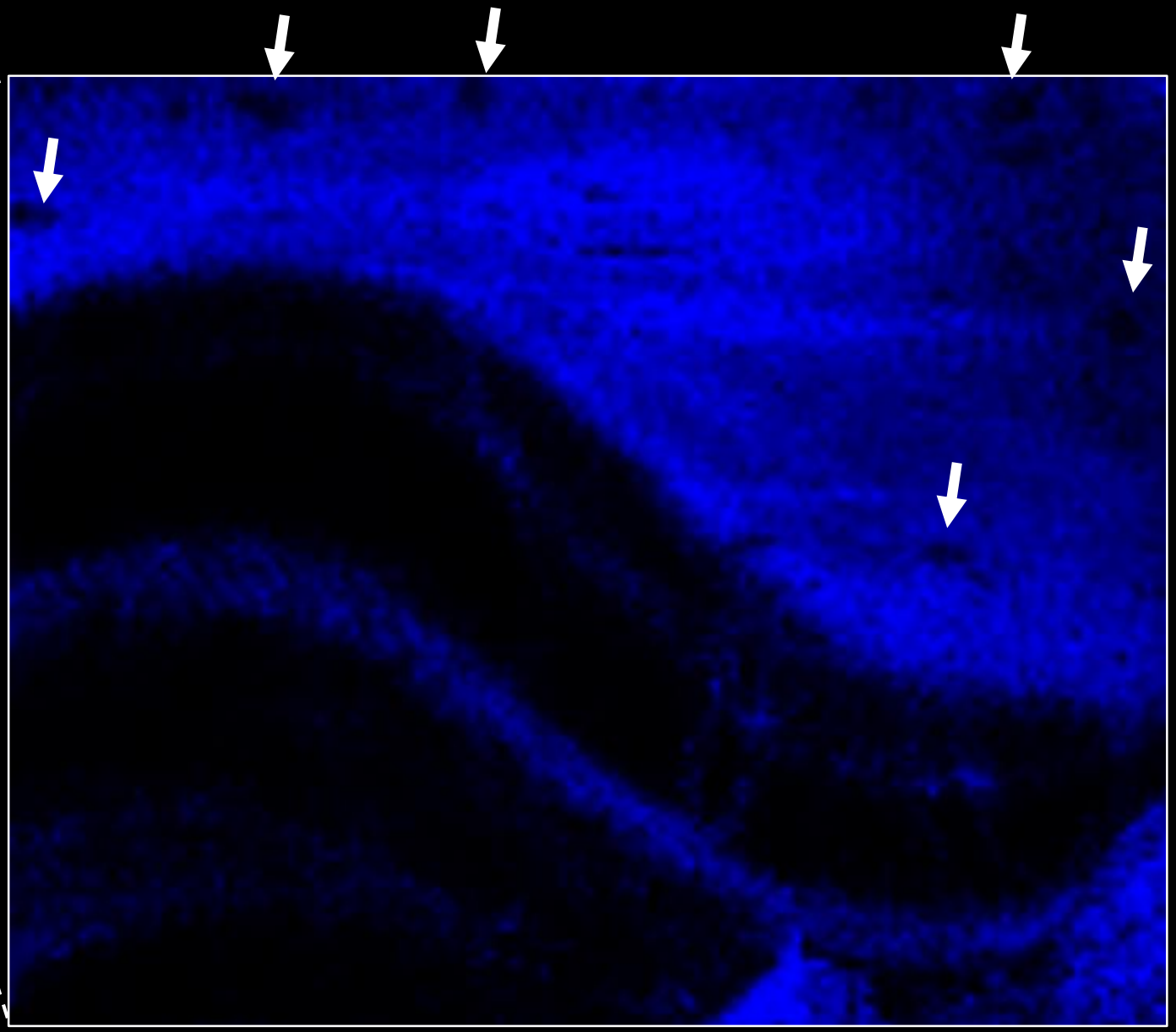


www.mouse.brain-map.org/


control model
PI 38:4
phosphatidylinositol
GM3 36:1
ganglioside
GM2 36:1
ganglioside
APP/PS1
model
5 mm
0 %
100 %
0 %
100 %

APP/PS1 model

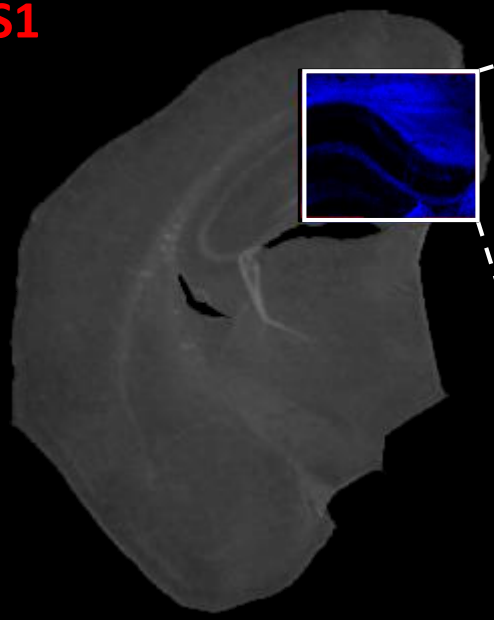


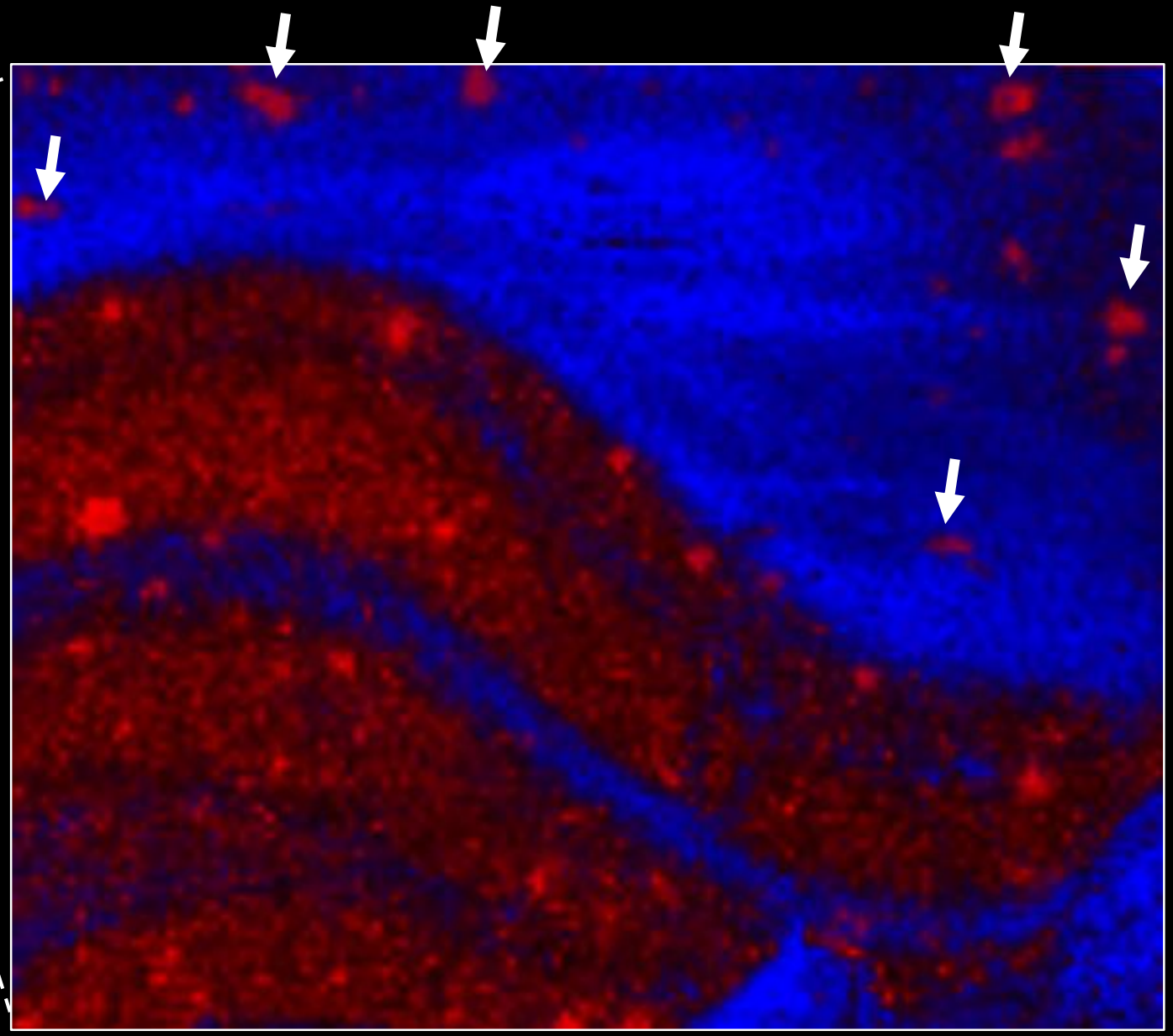


ST-0H 22:0 ↓

lower concentration of sulfatides

APP/PS1 model





ST-0H 22:0 ↓

lower concentration of sulfatides

GM3 36:1 ↑

higher concentration of sulfatides

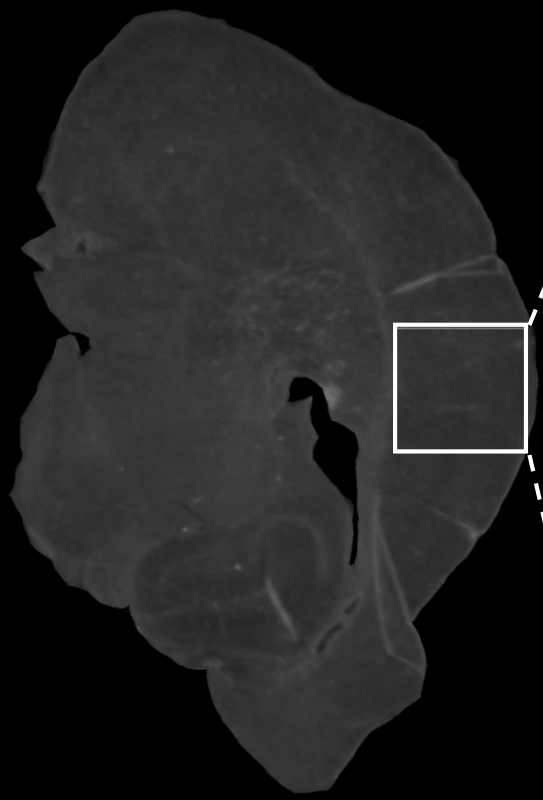
APP/PS1 colocalization with senile plaques

Colocalization experiment design:

- same sample

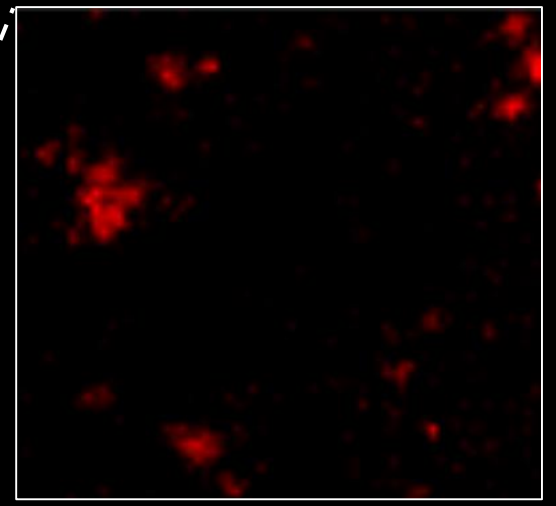
1. step: MALDI MSI
   - lipid analysis

2. step: imunohistochemistry (IHC)
   - Aβ (senile plaques)
   - GFAP (inflamation marker)

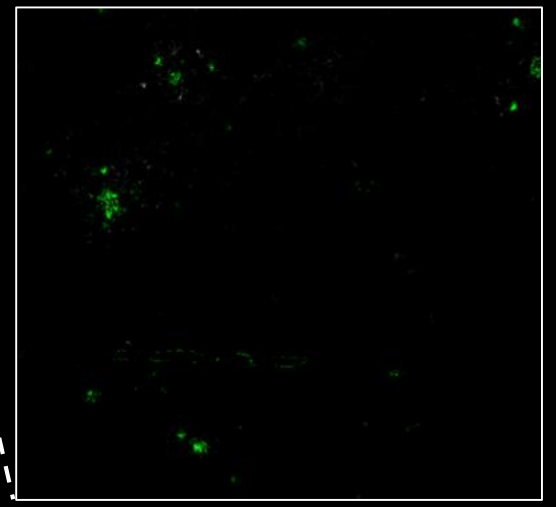


5 mm

ganglioside GM2 36:1 (MSI)

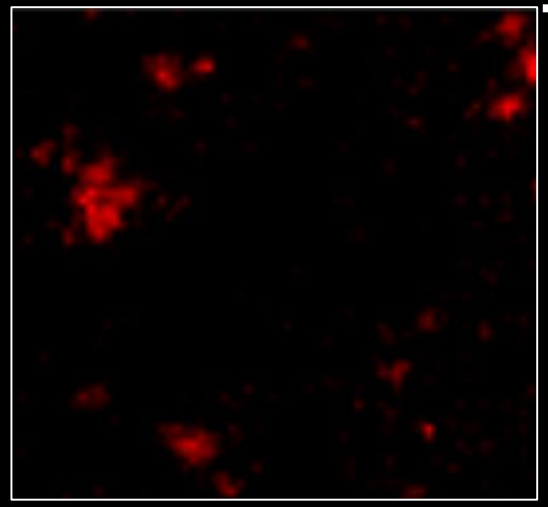


senile plaques (IHC)

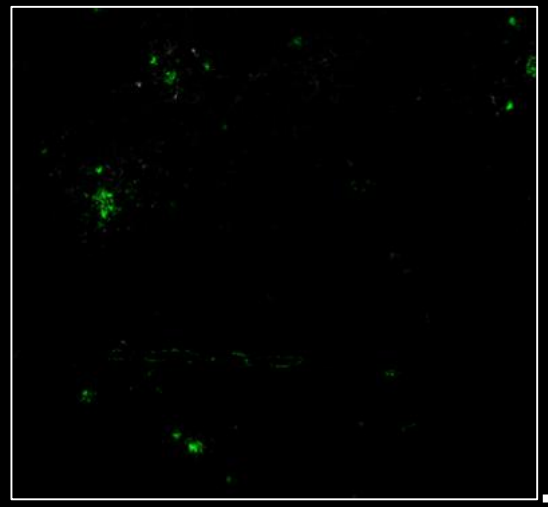


APP/PS1 colocalization

ganglioside GM2 36:1 (MSI)

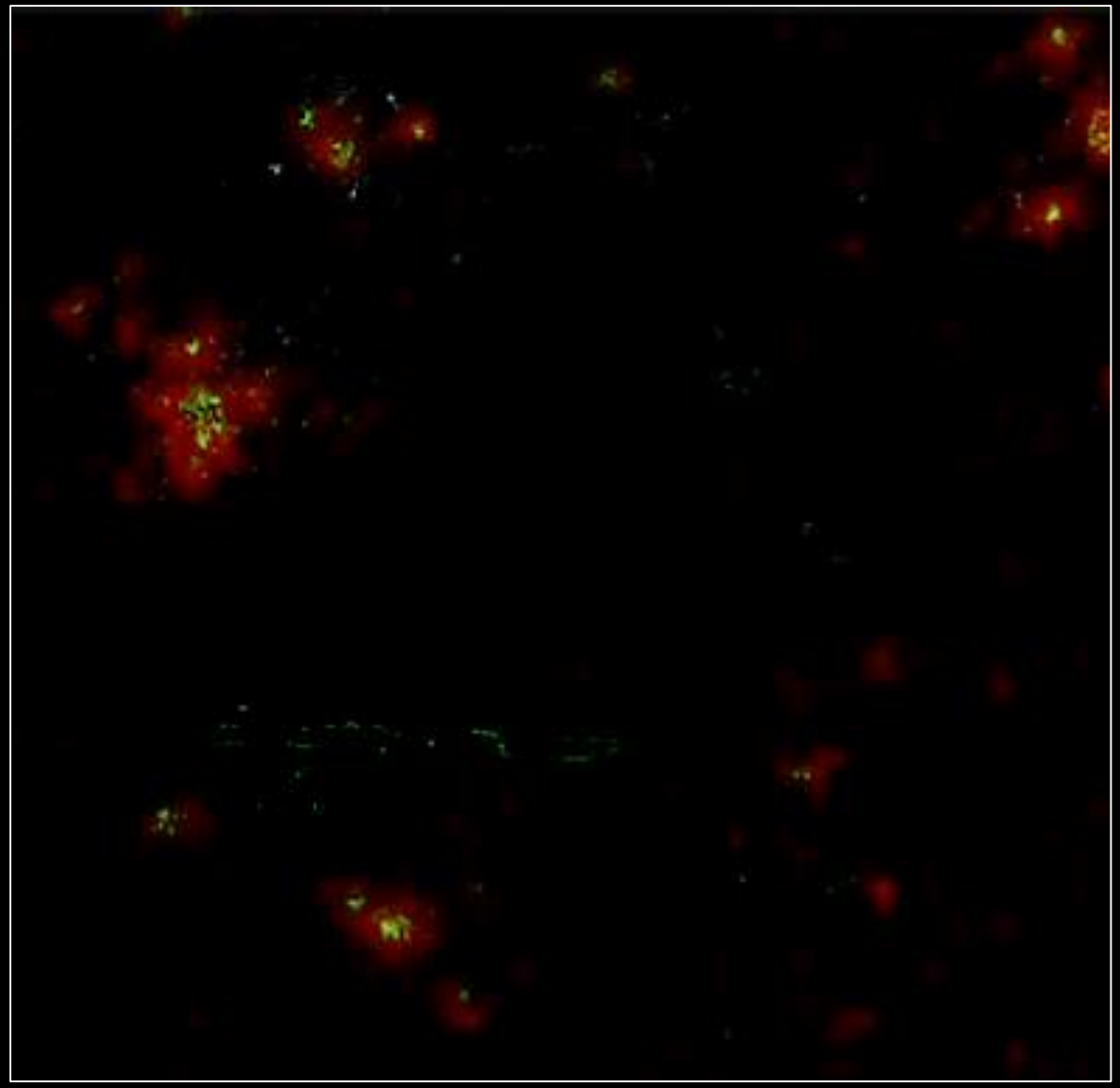


senile plaques (IHC)



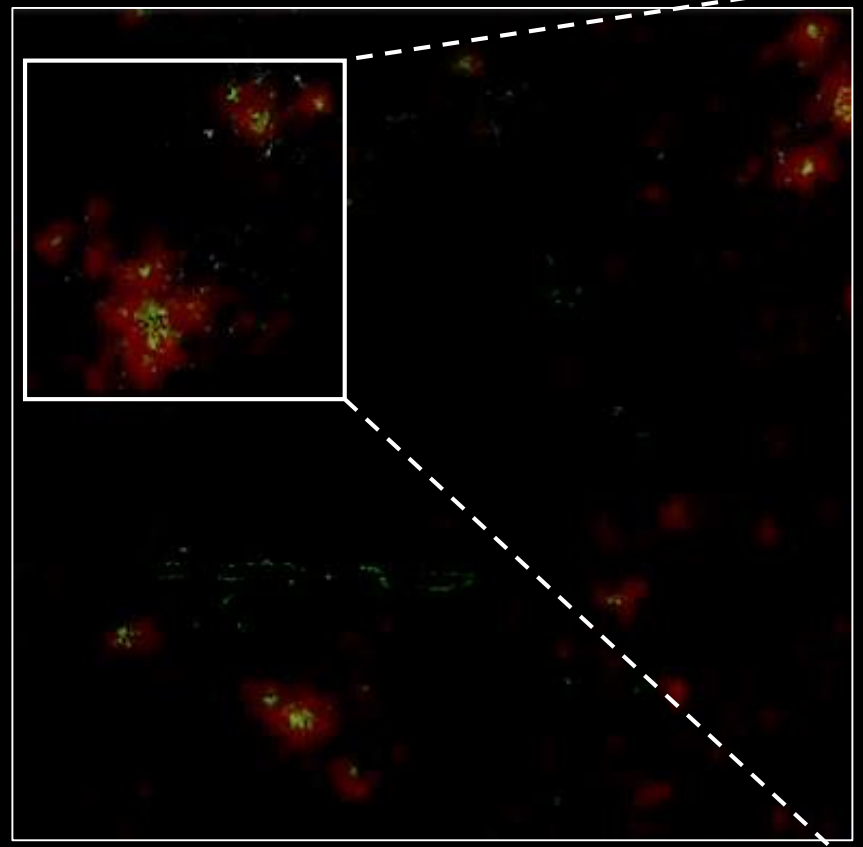
Merge MSI + IHC

GM2 36:1 + senile plaques

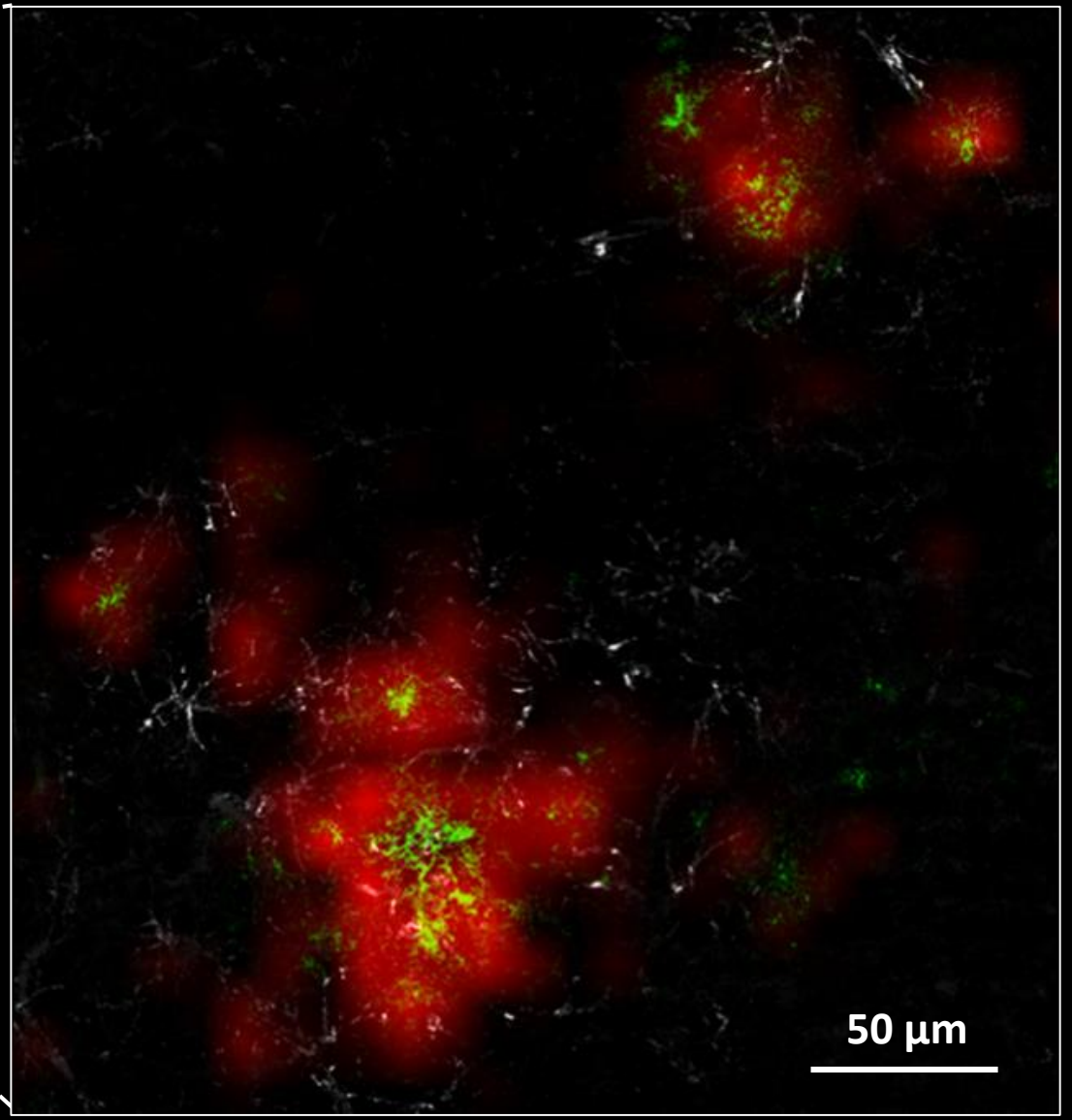


APP/PS1 colocalization

Merge MSI + IHC



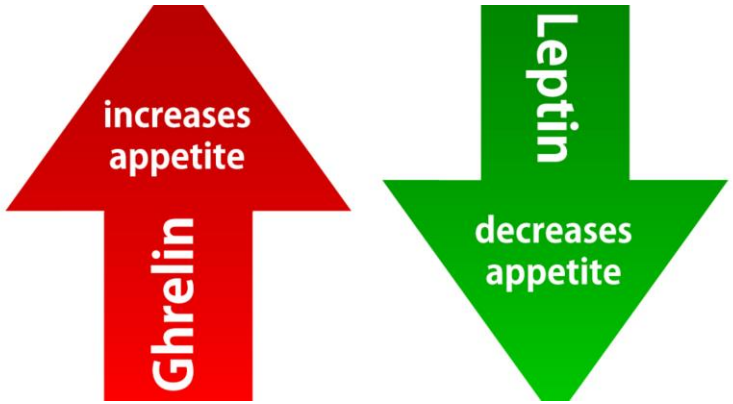
GM2 36:1 + senile plaques + inflamation

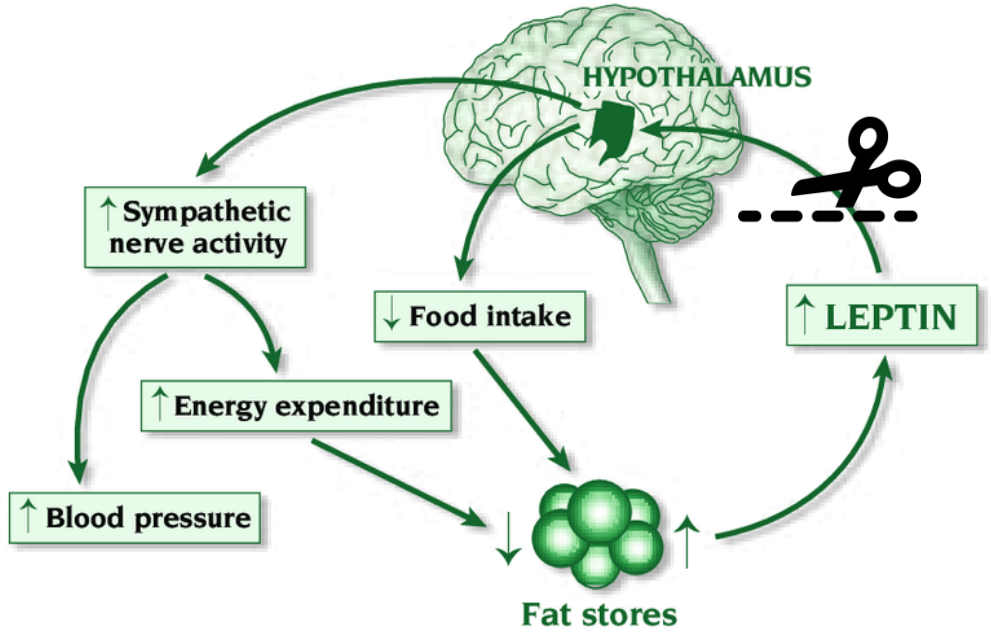


colocalization of lipid changes with senile plaques and inflammation

Study of lipid changes – obesity

- **Zucker (fa/fa) rats** widely used model of genetic obesity
- insulin, **leptin** resistence

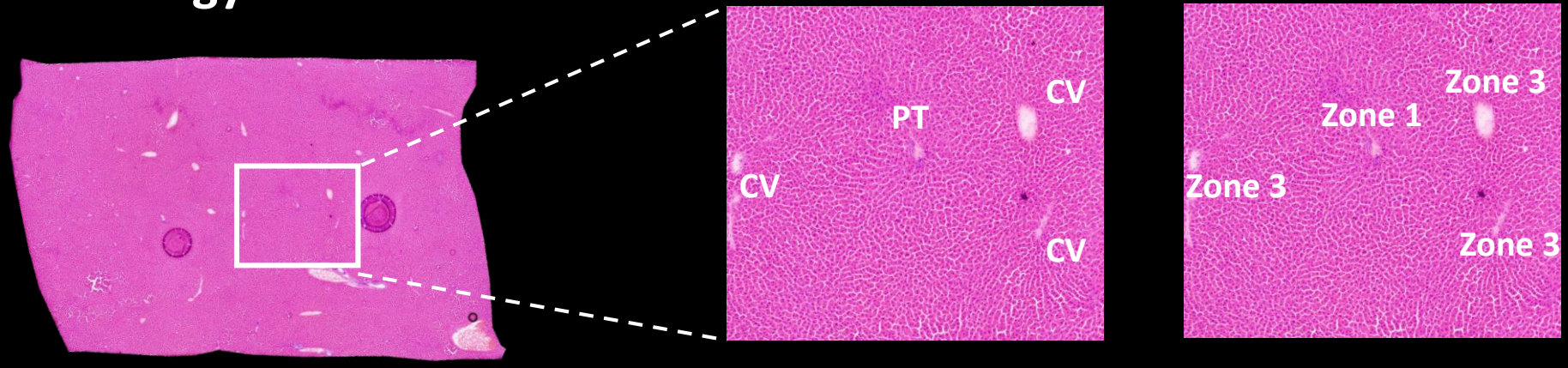




- fa/fa model vs lean control model

Liver zonation

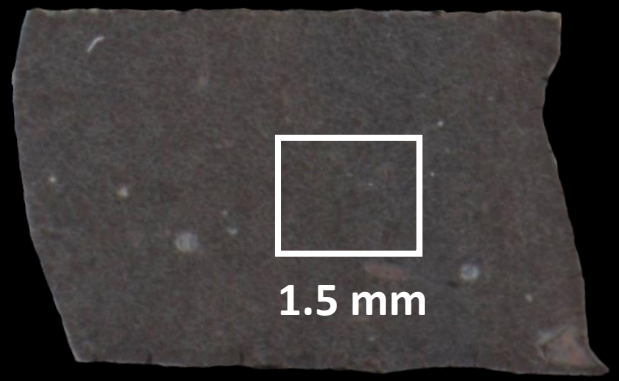
Histology



CV…central vein
PT…portal triad

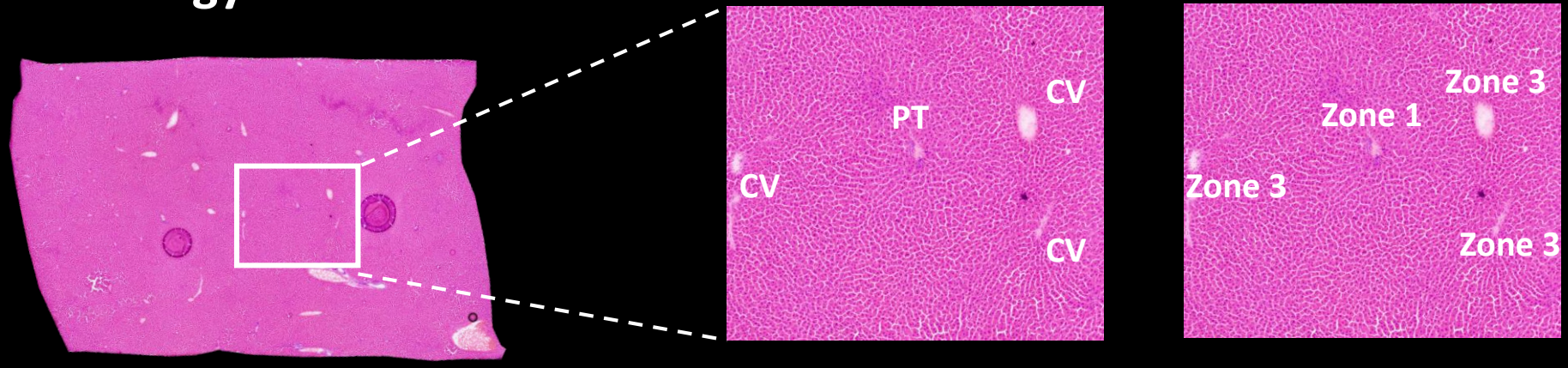
Zone 1…Z1
Zone 3…Z3

MSI



Liver zonation

Histology



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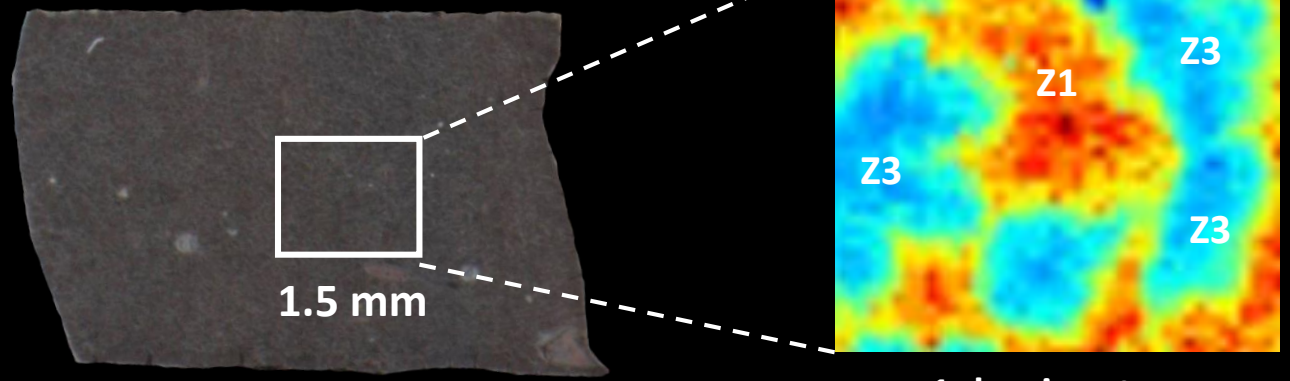
MSI

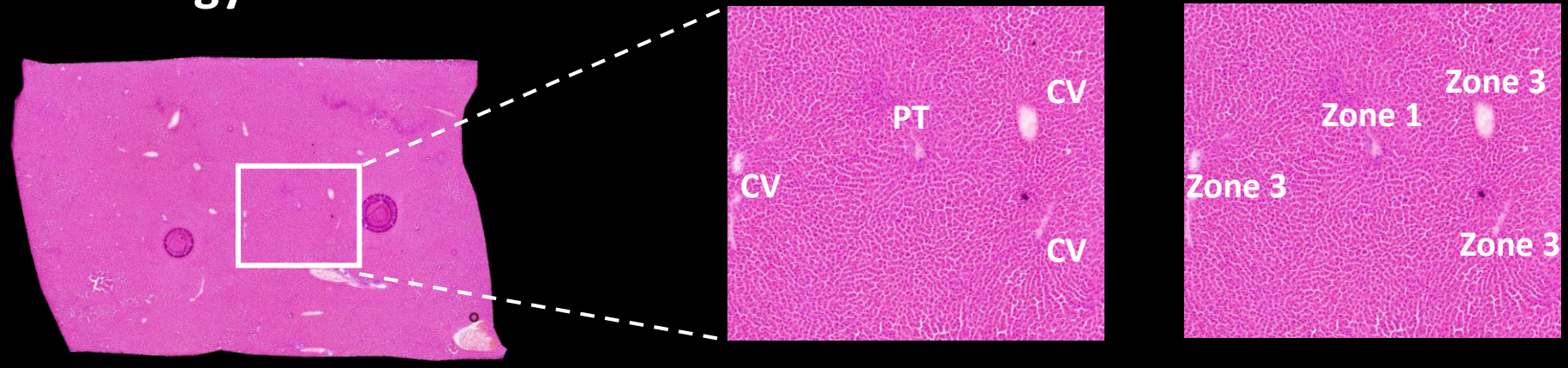


PC 34:2

zone 1 dominant distribution

Liver zonation

Histology



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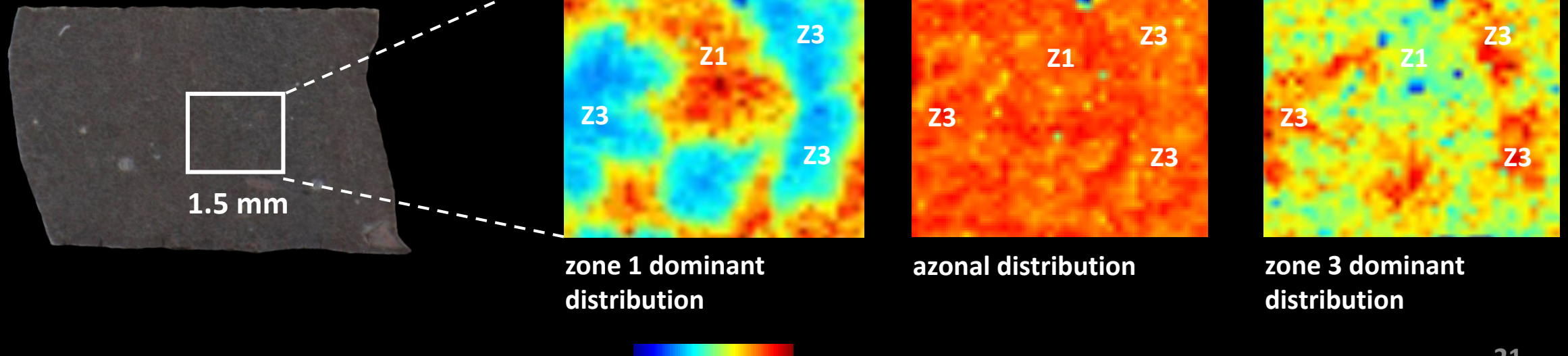


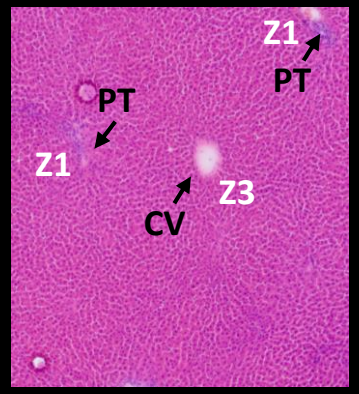
zone 1 dominant distribution

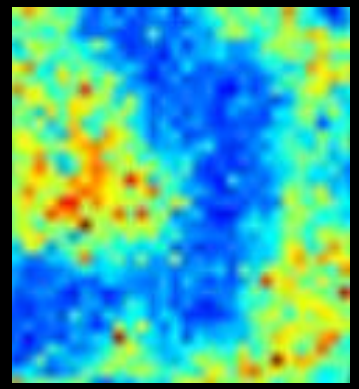
azonal distribution

zone 3 dominant distribution

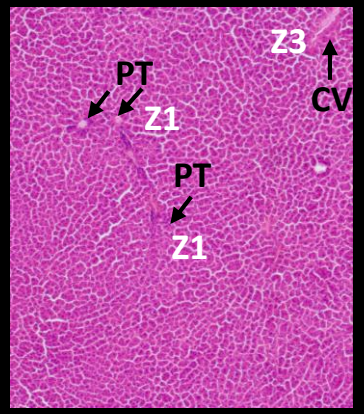
control model

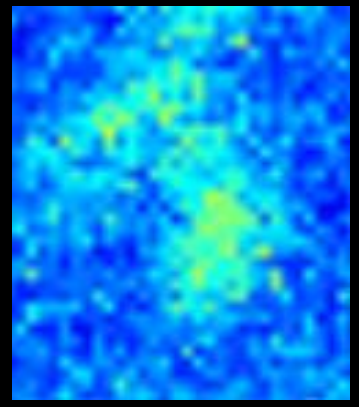
PE 34:2





fa/fa model

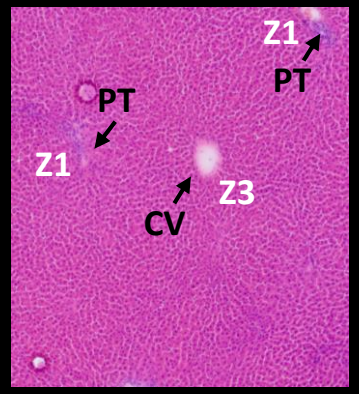


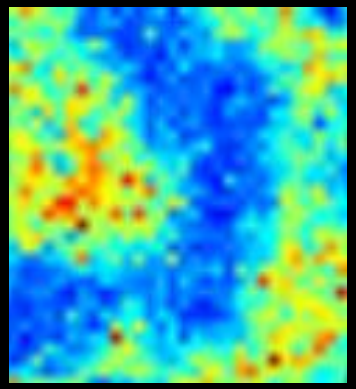


1 mm

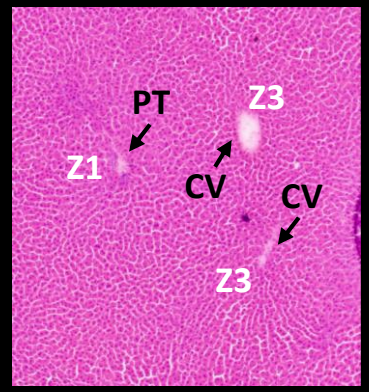
control model

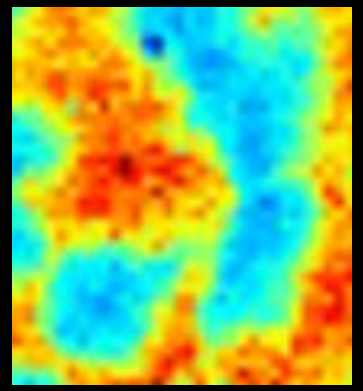
PE 34:2



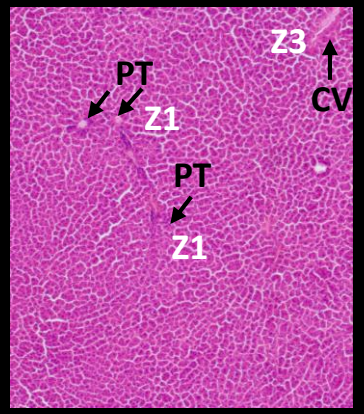


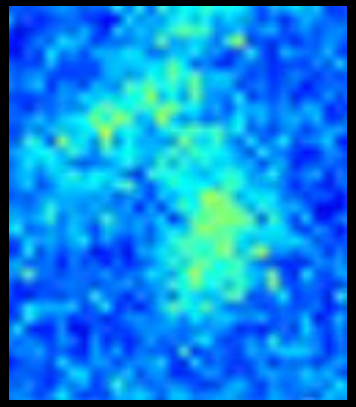
PC 34:2

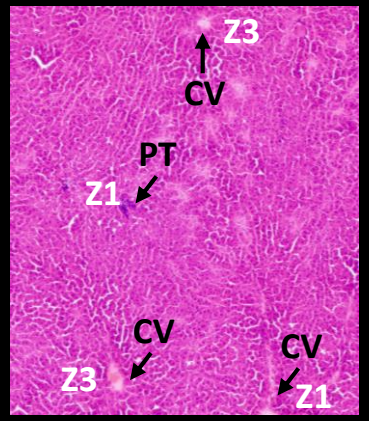


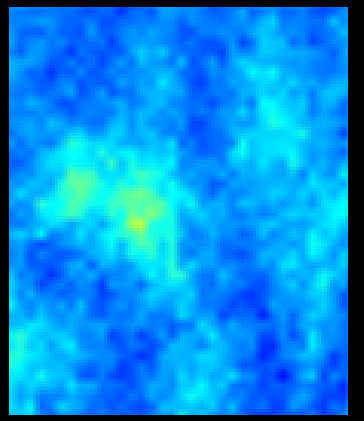


fa/fa model





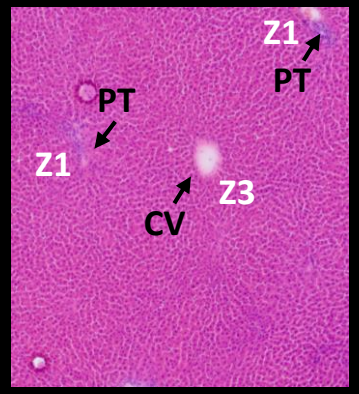


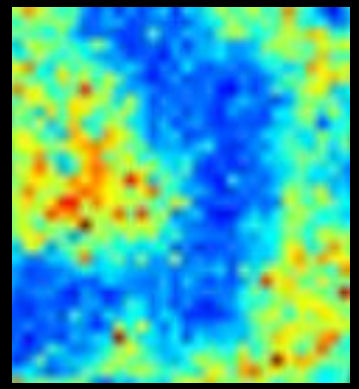


1 mm

control model

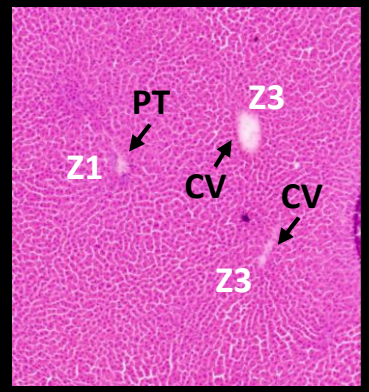
PE 34:2

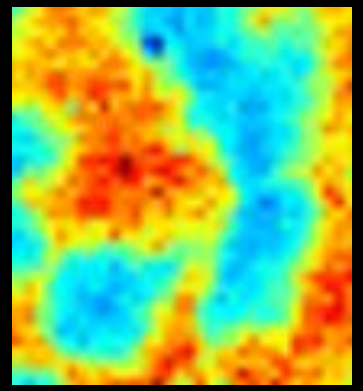


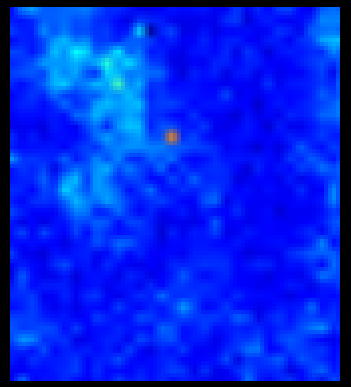


PC 34:2

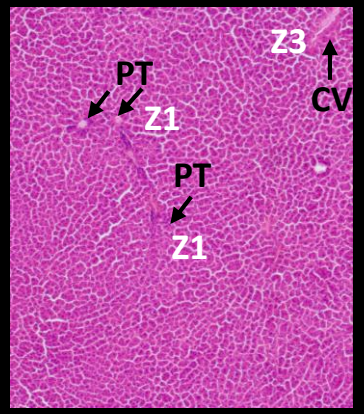
TG 52:3

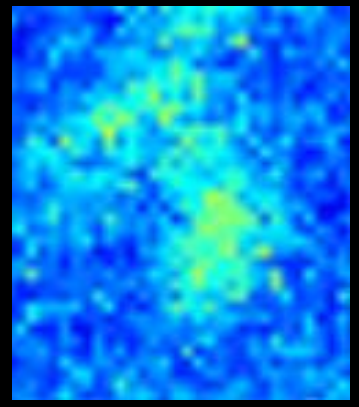


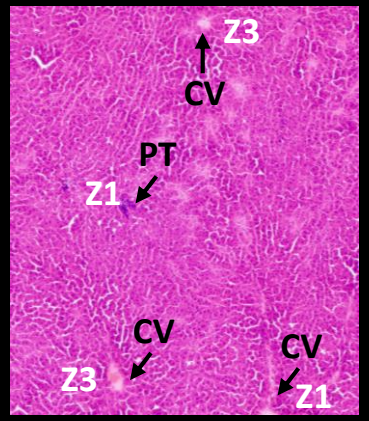


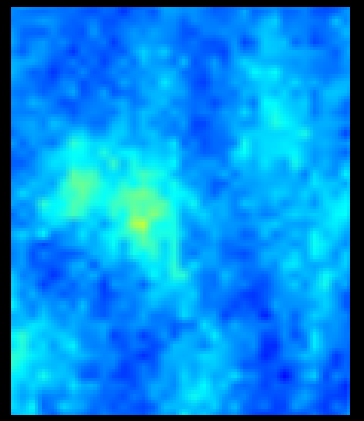


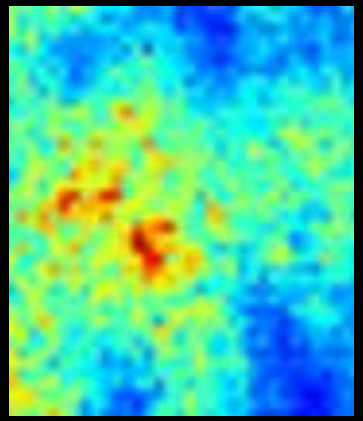
fa/fa model











1 mm

Study of lipid changes – obesity

- **Zucker (fa/fa) rats** vs. controls
- disruption in phospholipid metabolism

lower concentration:

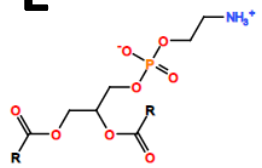
PC (34:2, 36:2, 38:5)

PE (34:2, 36:2)

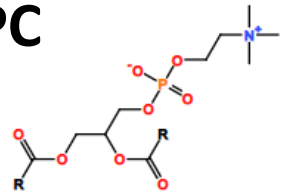
higher concentration:

TAGs (50:2, 52:1, 52:2, 52:3, 54:2)

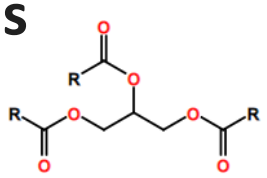
PE



PC



TAGs



30 % PC from PE

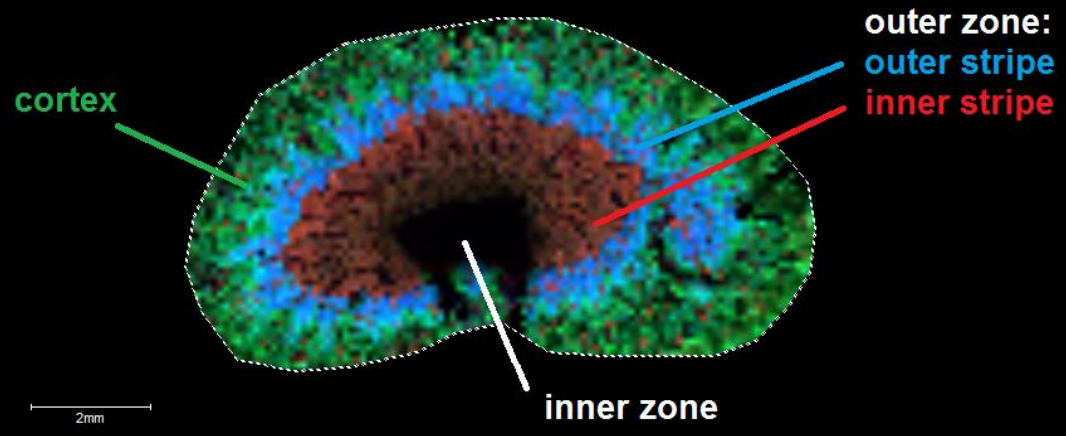
65 % TAGs from PC

- **TGs accumulation mainly in zone 1 - periportal**

Pharmacokinetic study - metformin

- mice - metformin p.o. 100 mg/kg

Lipid distribution: PC 38:6 cortex
PC 40:6 outer stripe
PC 38:4 inner stripe

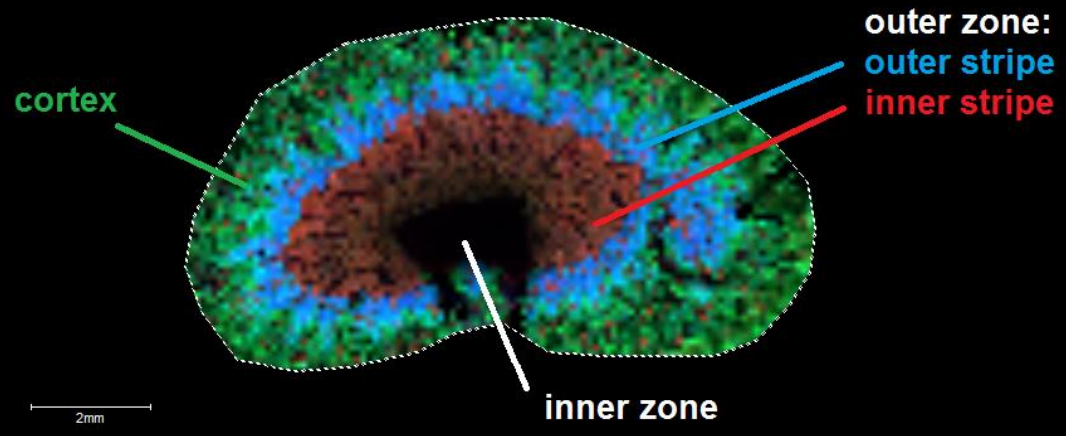


- distribution of phospholipids in the mouse **kidney** corresponds very well with histological features

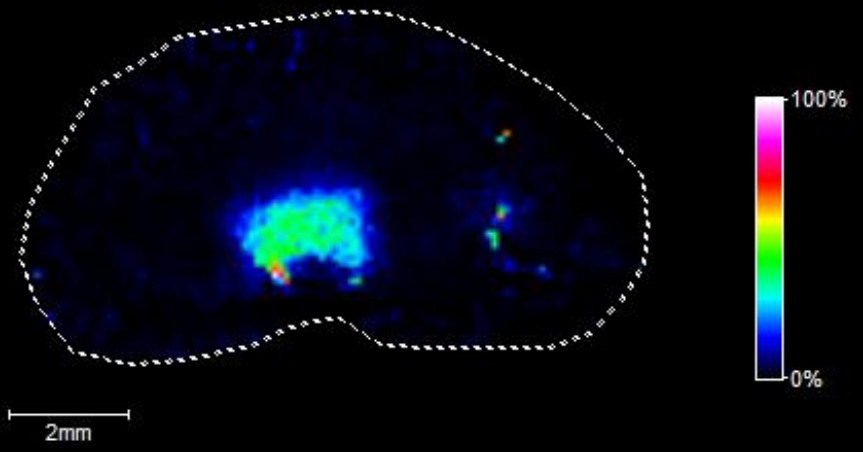
Pharmacokinetic study - metformin

- mice - metformin p.o. 100 mg/kg

Lipid distribution: PC 38:6 cortex
PC 40:6 outer stripe
PC 38:4 inner stripe



Metformin distribution:

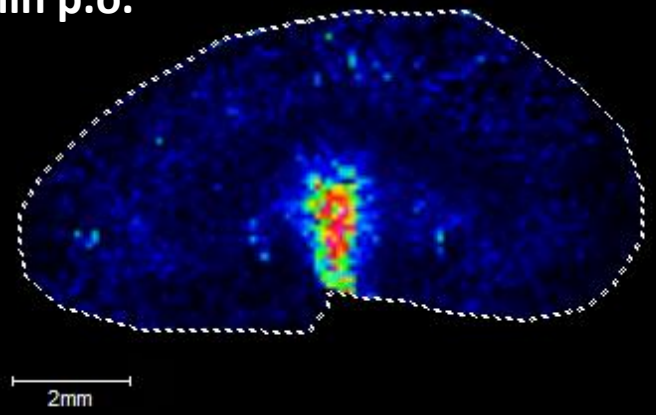


- distribution of phospholipids in the mouse **kidney** corresponds very well with histological features

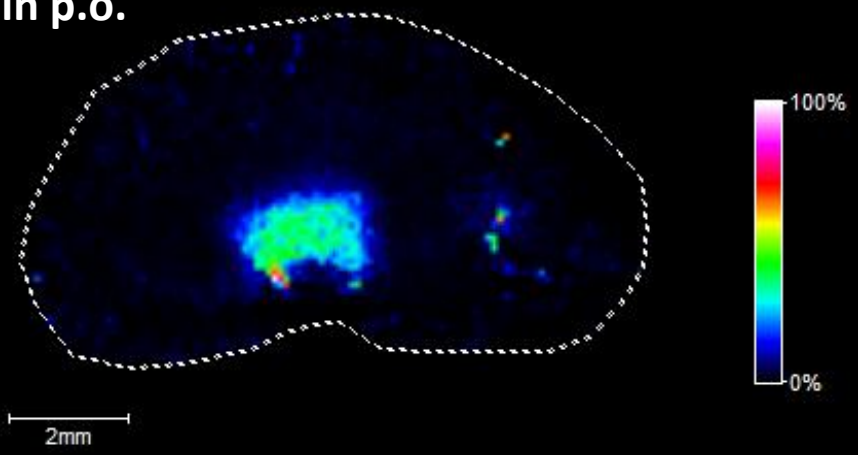
Pharmacokinetic study - metformin

Kidney:

30 min p.o.



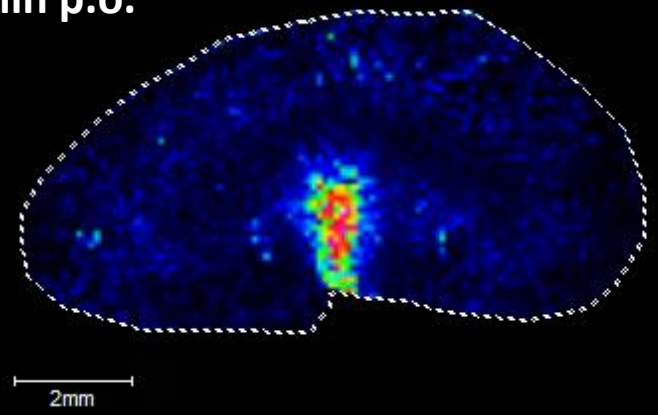
60 min p.o.



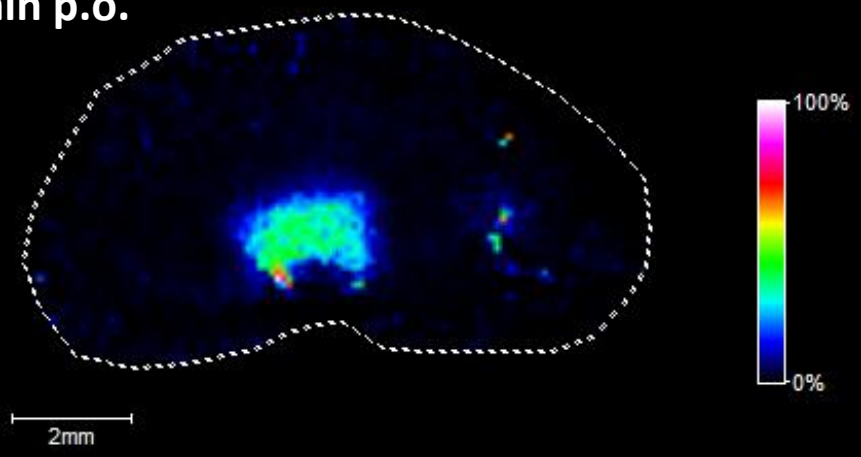
Pharmacokinetic study - metformin

Kidney:

30 min p.o.

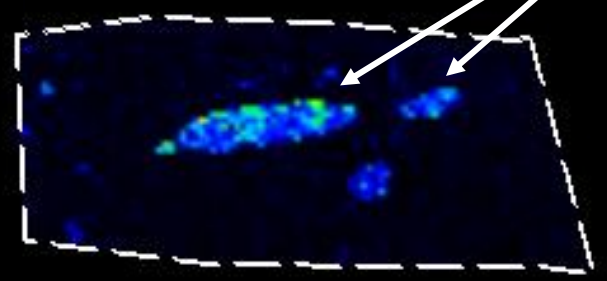


60 min p.o.

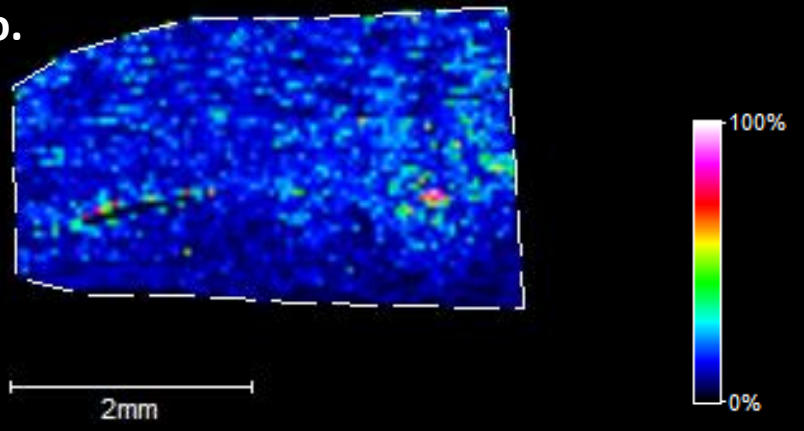


Liver:

15 min p.o.



30 min p.o.



New REQUEST

My requests

CREATE NEW REQUEST

Small molecules analysis

CREATE

The analysis covers (i) measurement of full-scan nominal-resolution mass spectra of compounds using ESI, EI/CI, APCI, or MALDI; (ii) measurement of full-scan high-resolution spectra using the same ionization methods as above to confirm expected elemental composition or suggest elemental compositions for unknows (mass accuracy 5 ppm or less).

Quantitative analysis of small molecules

CREATE

The aim of the analysis is the targeted detection and quantification of small molecules (approximately up to 2000 Da). Sensitive detection of the analytes is achieved by the measurement of compound-specific fragment ions (MRM transitions). The amount (concentration) of the analytes is determined using either a calibration curve with an internal standard or a standard addition method.

Proteomics analysis

CREATE

The main focus of the analysis is the identification of proteins, their post-translational modifications, and/or quantification of proteins either via label-free or labeling strategy. The workflows are predominantly based on proteolytic digestion and analysis of resulting peptides.

Lipidomics analysis

CREATE

Untargeted lipidomics analysis begins with liquid-liquid extraction of raw biological material (biofluids, cells, tissues, etc.) and is based on liquid chromatography coupled to high-resolution mass spectrometry. The experiments are designed to acquire data on many individual lipid species and compare their relative abundances between experimental conditions (wild type vs. knockout etc.).

Mass spectrometry imaging

CREATE

Mass spectrometry imaging (MSI) is used to evaluate the spatial distribution of compounds in tissue sections.

Biomacromolecules

CREATE

The aim of the analysis is to acquire mass spectra of intact biopolymers like peptides, proteins, nucleic acid, polysaccharides, etc., using MALDI or ESI.

REQUEST - mass spectrometry imaging

Provided services for collaboration:

1) **MALDI MSI lipid analysis**
2) **customized method development** of MALDI imaging for other analytes (time-consuming)

- MSI sample preparation (cryosectioning of frozen samples, matrix application)
- data analysis

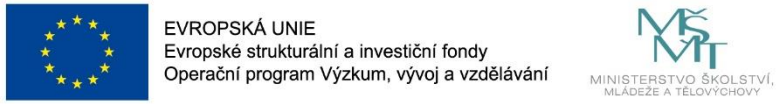
- MSI analysis (**suitable for abundant components**)

Consultation:

Štěpán **Strnad** and Vladimír **Vrkoslav** (phone: 347, room: A.1.85).

Acknowledgment

The work was supported from European Regional Development Fund; OP RDE; Project: “Chemical biology for drugging undruggable targets (ChemBioDrug)” (No. CZ.02.1.01/0.0/0.0/16_019/0000729)



Thank you for your attention

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