BRAIN GLYCEROPHOSPHOLIPIDS AND CHOLESTEROL IMAGING BY MASS SPECTROMETRY

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BRAIN GLYCEROPHOSPHOLIDS AND CHOLESTEROL IMAGING BY MASS SPECTROMETRY

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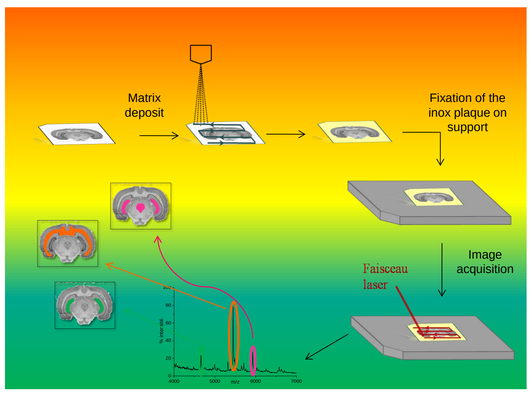
Lipids are the most abundant biomolecules found in the brain, following water, representing up to 50% of its dry weight. These compounds are structural components of cell membranes, and have various functions of precursors, biomessengers, and signal transduction. Marked alterations in their composition have been reported to occur during neurological disorders.

Until now, many studies have been interested in brain lipid content. However, the methods used to identify lipids always involve extraction prior to analysis, which destroys any information relevant to tissue localization. Lipid staining in tissues is possible with fluorescent or classic histological dyes but is not specific. Rare are the specific molecular probes such as immunohistochemical tools able to detect lipids in tissue. Therefore, brain tissue lipidomic imaging is crucial to precise anatomic localization of lipid species in cerebral structures.

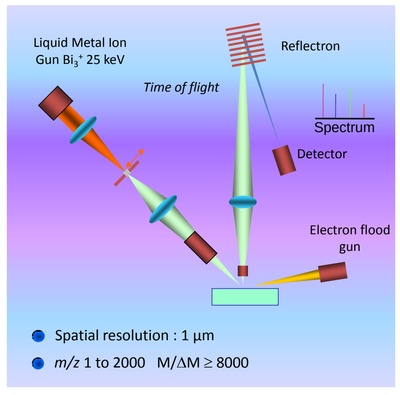
In this study, we used two powerful mass spectrometry imaging methods, MALDI-TOF/TOF[1] (matrix-assisted laser desorption /ionization) and TOF-SIMS[2] (time-of-flight secondary ion mass spectrometry), in order to detect and localize lipid species in rat brain and human temporal cortex, both at regional and cellular levels. The first method can perform *in situ* structural identifications by MS/MS, while the second is able to localize species with a spatial resolution of less than 1 µm [3].

We focused on cholesterol and on the most quantitatively relevant glycerophospholipids (PC, PE, PS, PI) described in literature, their composition greatly altering neural membrane stability, fluidity and permeability. The results showed differential repartition of these lipids in cell bodies or dendrites of grey matter, and in myelinated axons.

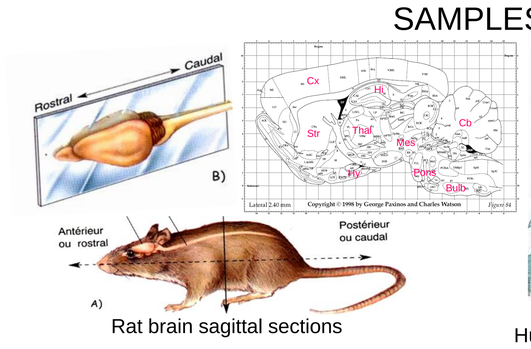
MALDI-TOF/TOF



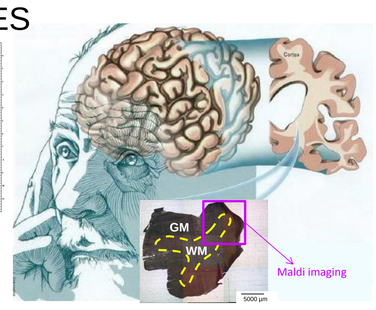
TOF-SIMS



SAMPLES



Rat brain sagittal sections



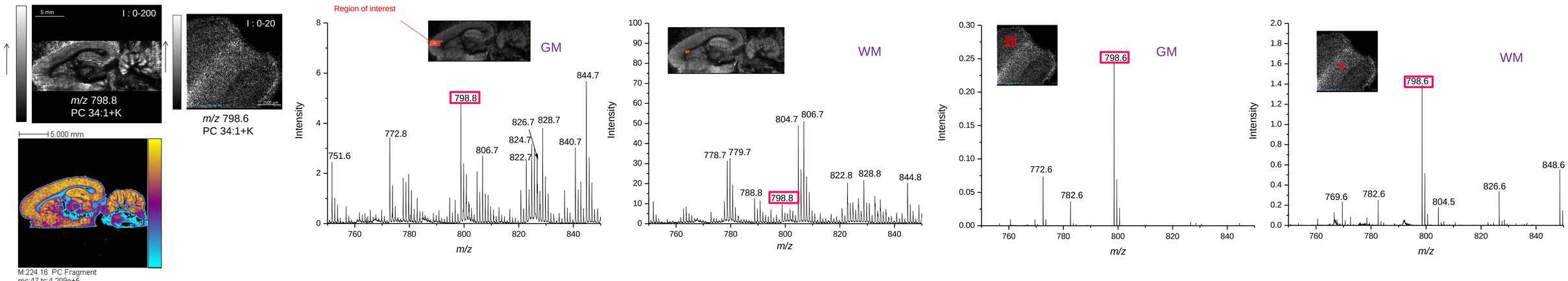
Human brain sections :Temporal cortex ★

Species	Localization and abundance	Grey Matter (GM)	White Matter (WM)	Structures
Cholesterol *$[M+H-H_2O]^+$ m/z 369.35* *$[M-H]^-$ m/z 385.35*	38±2% of total lipids in rat brain cortex [6] 14-16 mg/g brain ww [11]		✓	
PC PC 34 :1 16 :0/18 :1 *$[M+K]^+$ m/z 798.54*	20-23 mg/g brain ww [7,8,9,10] 20±1% of total lipids in brain cortex [6] 30-40% of PC in cortex (PlsPC 34:0 :1-2% of PC) [5,6]	✓		
PE PE 40 :6 (18 :0/22 :6) *$[M-H]^-$ m/z 790.54* PE O-40:6 (p18 :0/22 :6) *$[M-H]^-$ m/z 774.54* PE O-36 :2 (p18 :1/18 :1) *$[M-H]^-$ m/z 726.54*	15-20 mg/g brain ww [7,8,9,10] 22±2% of total lipids in brain cortex[6] 20-35% of PE in cortex[6] 10-20% of PE in cortex [4,5,6] 8-15% of PE in cortex[4,5,6]	✓ ✓	✓	
PS PS 40:6 (18:0/22:6) *$[M-H]^-$ m/z 834.53*	4-8 mg/g brain ww[7,8,9,10] 35-50% of PS in cortex[5,6]	✓		
PI PI 38:4 (18:0/20:4) *$[M-H]^-$ m/z 885.55*	1-2 mg/g brain ww[7,8,910] 50-70% of PI in cortex[5,6]	✓		

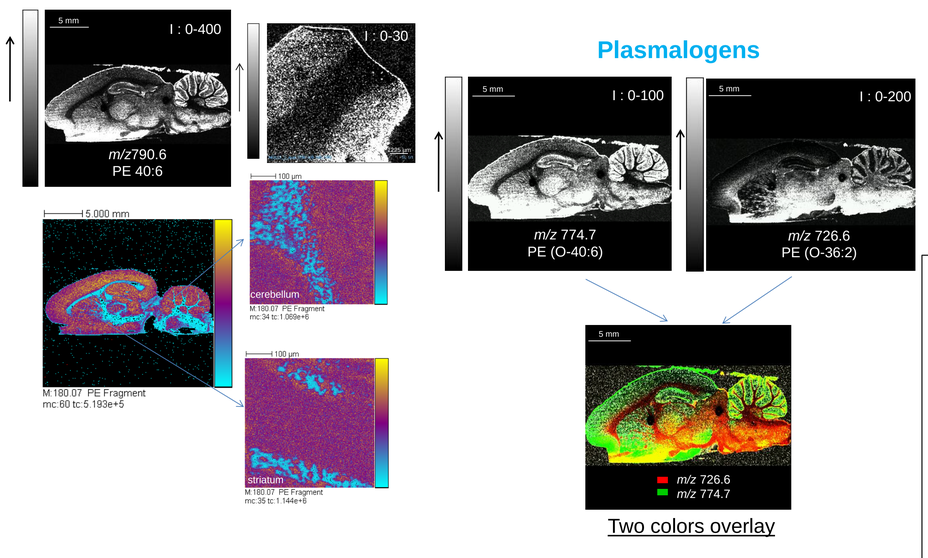
Cholesterol represents 40% of total lipids in brain, Glycerophospholipids : 36%, Sphingolipids : 15%, Plasmalogens : 9.5% [6]
Total amount of PL in rat brain = 42±1mg/g brain, wet weight [9]
PC and PE represent 80% of total glycerophospholipids

PHOSPHATIDYLCHOLINES

MALDI-TOF Positive ion mode



PHOSPHATIDYLETHANOLAMINES



Plasmalogens

Two colors overlay

Color scale for TOF-SIMS imaging

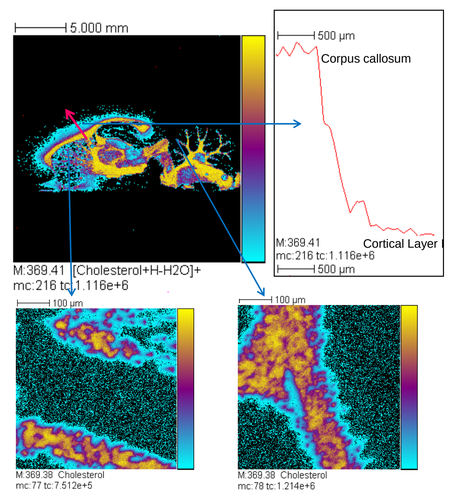
mc = maximal number of counts in a pixel

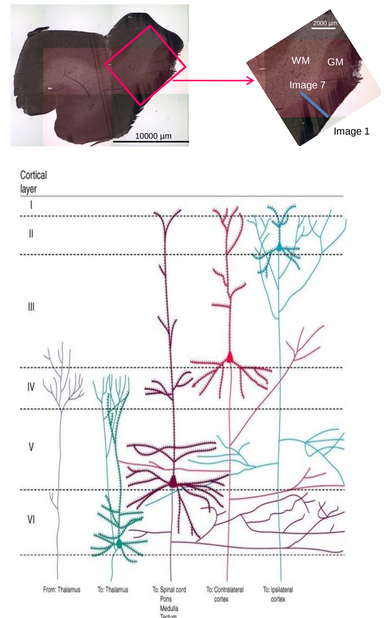
0

tc = total number of counts for the whole image

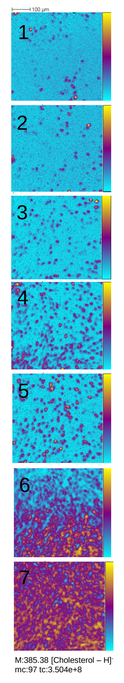
CHOLESTEROL

TOF-SIMS
Positive ion mode

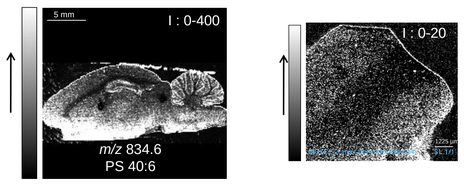




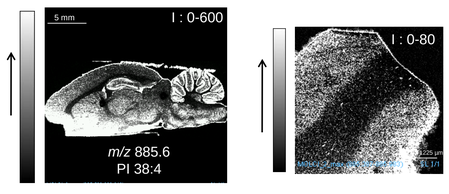
TOF-SIMS
Negative ion mode



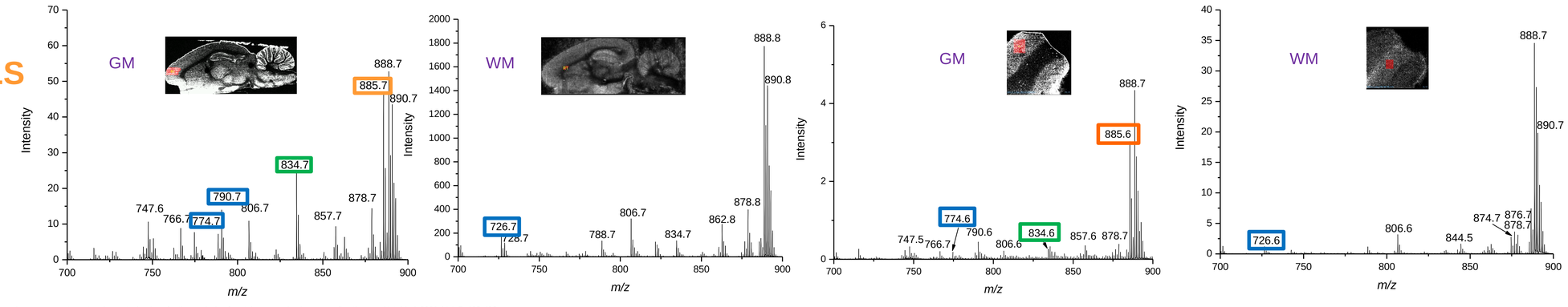
PHOSPHATIDYLSERINES



PHOSPHATIDYLINOSITOLS



MALDI-TOF Negative ion mode



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