

Direct analysis of lipids and other metabolites in mouse brain tissue with infrared laser ablation and mass spectrometry

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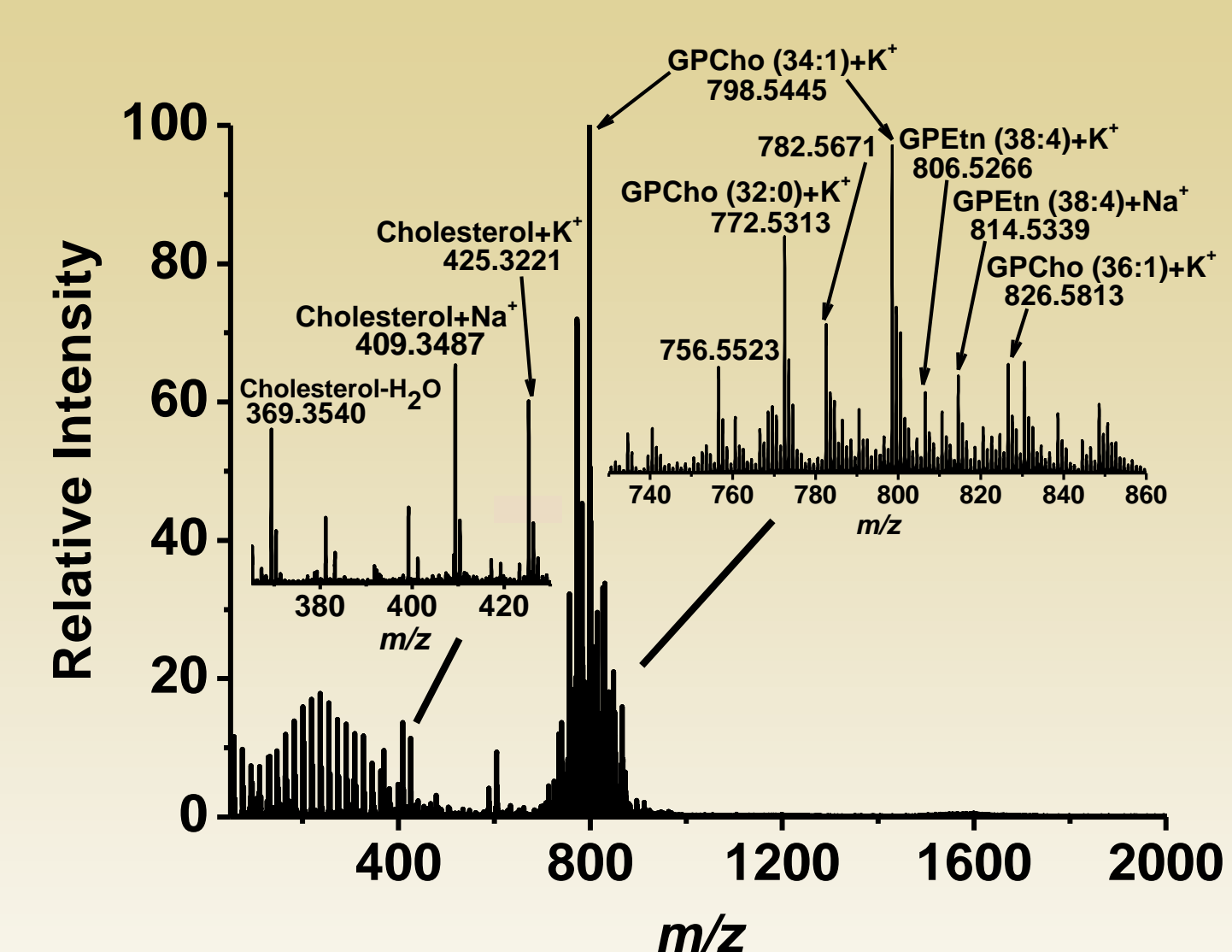
Introduction

- Significant portion of the brain is composed of lipids, predominantly glycerophospholipids, which have vital roles in the physiological functioning of the brain. These have been studied by various soft ionization methods.¹⁻³
- Sample preparation and processing during analysis might lead to biochemical changes.
- A direct and rapid ambient analysis of the brain tissue is required to avoid any postmortem changes in its biochemical composition.
- Two ambient ionization techniques, atmospheric pressure (AP) infrared (IR) matrix-assisted laser desorption/ionization (MALDI)⁴ and laser ablation electrospray ionization (LAESI)^{5,6} were utilized in this study for the direct analysis of lipids and other metabolites in the mouse brain.

Methods

- Both AP IR-MALDI and LAESI take advantage of the strong absorption of native water molecules in the brain due to their OH vibrations at mid-infrared wavelengths (2.94 μm).
- Ablation was carried out by a Nd:YAG laser-driven optical parametric oscillator.
- Ions were detected by an orthogonal acceleration time-of-flight mass spectrometer, Q-TOF with a custom made interface.
- Transverse cross-sections (~200-400 μm thick) of mouse brain were obtained from normal and healthy C57Bl/10 strain of mice (age 12-14 months).
- To prevent rapid biochemical changes at higher temperatures, samples were kept around -5°C during analysis by a Peltier cooling stage.

Analysis by AP IR-MALDI



- The mass spectra are dominated by cationized species, customary for MALDI at elevated pressures.
- Most ions, detected within the m/z 700-900 mass range are phospholipids.
- Cholesterol is observed as cationized peaks as well as a $-H_2O$ peak.
- List of few tentative metabolite and lipid assignments is presented below.

Metabolites

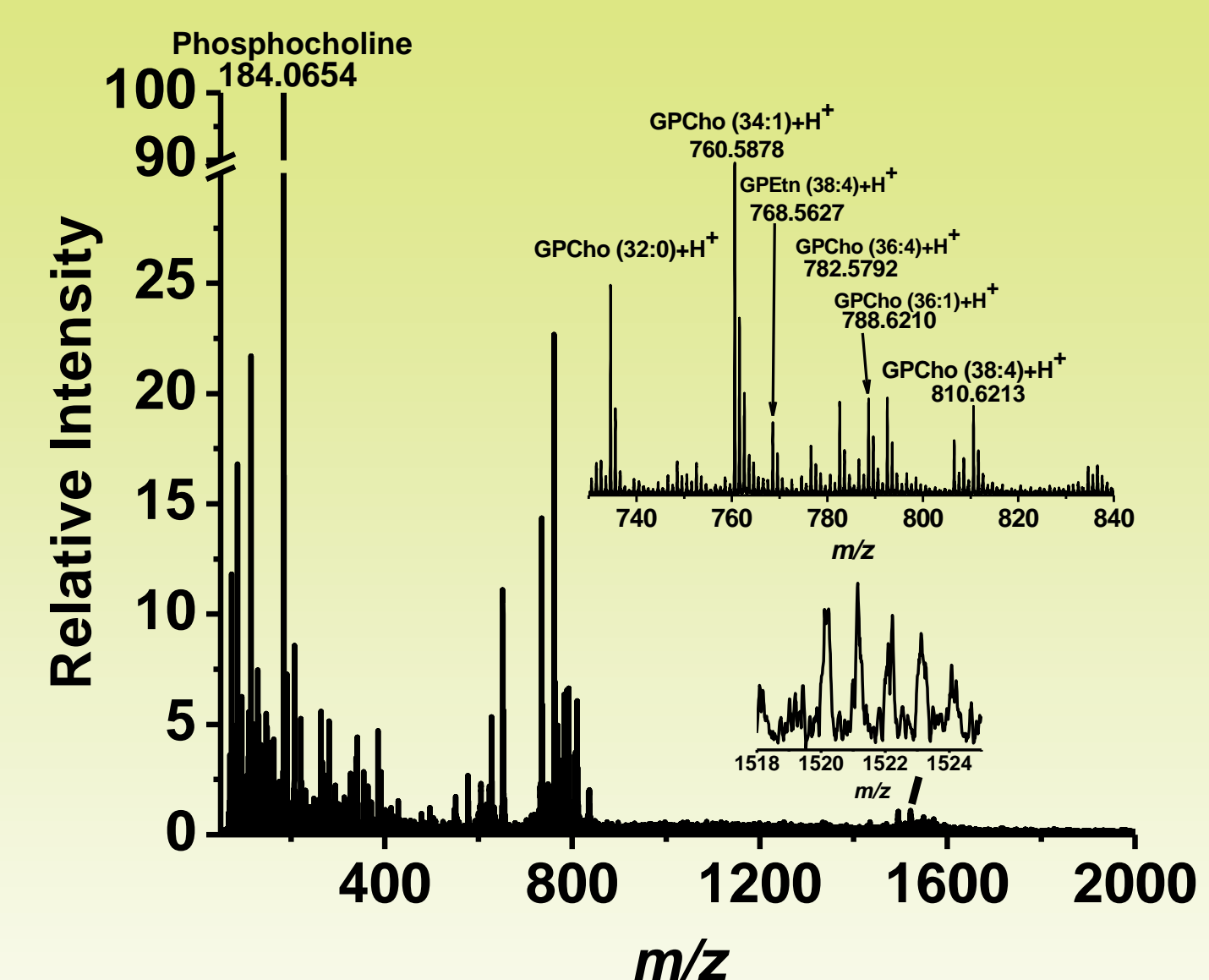
Alanine
Aminobutyric acid
Choline
Creatine
Homocysteine
Hypoxanthine
Spermidine
Dopamine
Arginine
Carnosine

Lipids^a

Cholesterol
GPCho (32:0)
GPCho (34:1)
GPCho (36:1)
GPCho (36:2)
GPCho (38:4)
GPCho (40:6)
GPEtn (38:4)
GPEtn (40:6)
SM (18:0)

^aGlycerophosphocholines (GPCho), phosphatidylethanolamine (GPEtn), are denoted with total length and number of double bonds for both acyl chains. Sphingolipids (SM) are denoted by the length and number of double bonds of acyl chains attached to the sphingosine base (18:1).

Analysis by LAESI



- The mass spectra are dominated by protonated ions of phospholipids between m/z 700-900.
- The Most intense peak at m/z 184.0654 is the phosphocholine head group of glycerophosphocholines.
- Cluster ions, such as the dimer of GPCho (34:1) are detected at nominal m/z 1519.
- List of few tentative metabolite and lipid assignments is presented below.

Metabolites

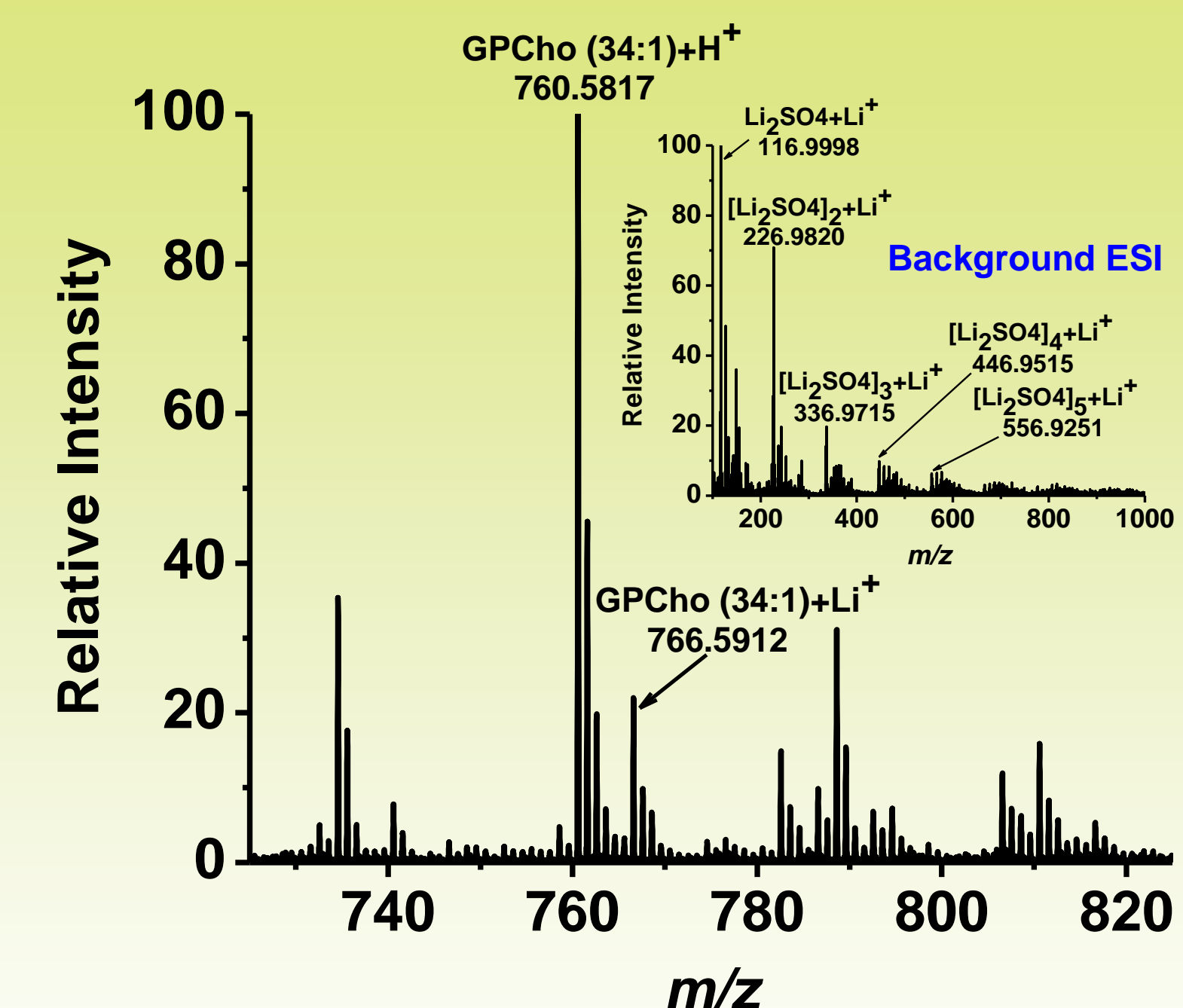
Pyrimidine
Phosphocholine
Aminobutyric acid
Choline
Threonine
Creatine
Phosphocholine
Epinephrine
Acetylcholine
Cetoleic acid

Lipids^a

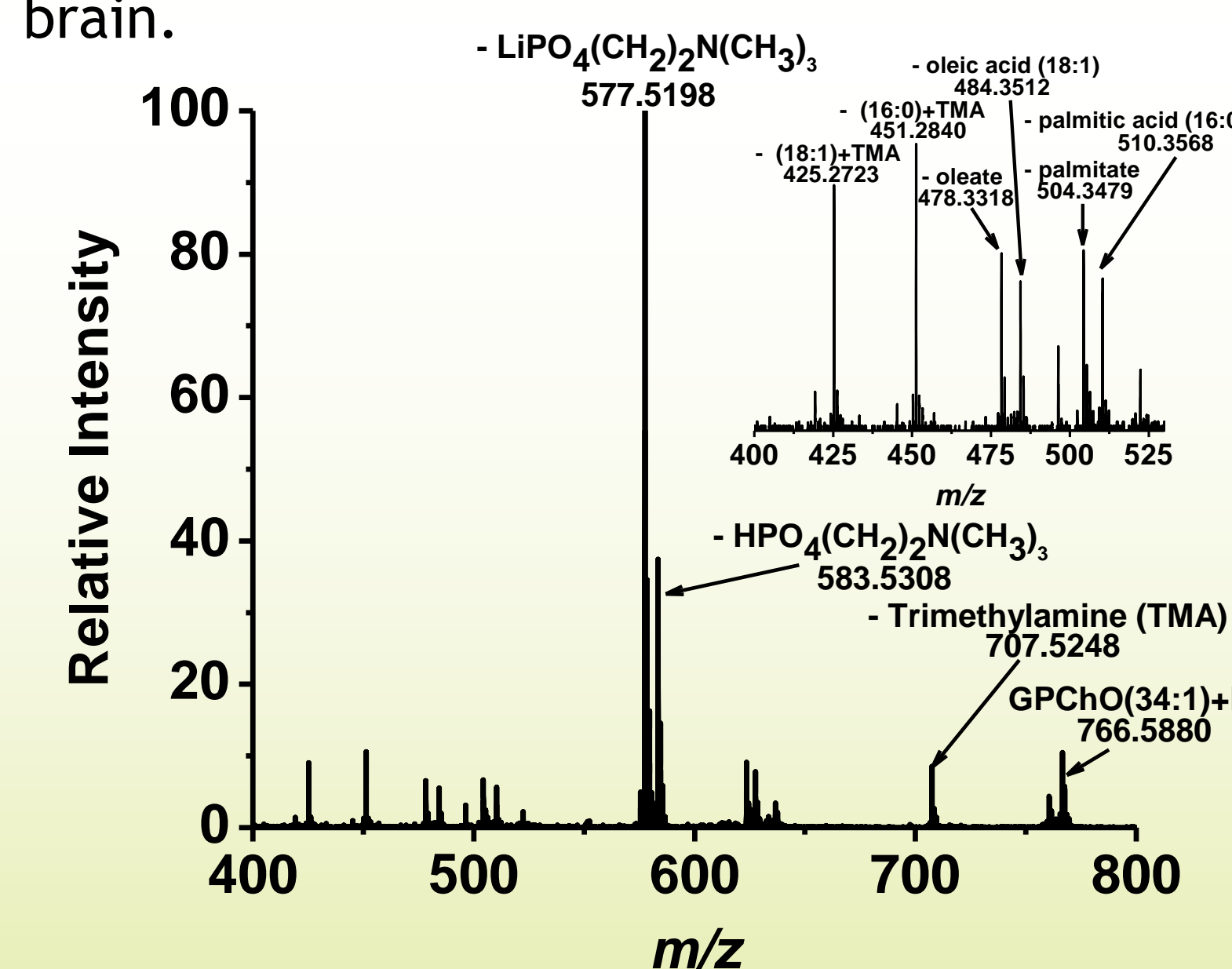
GPCho (36:1)
GPCho (36:2)
GPCho (38:4)
GPCho (38:5)
GPCho (38:6)
GPCho (40:6)
GPEtn (38:4)
GPEtn (40:6)
GPSer (40:6)
SM (18:0)

^aGlycerophosphocholines (GPCho), phosphatidylethanolamine (GPEtn), are denoted with the total length and number of double bonds for both acyl chains. Sphingolipids (SM) are denoted by the length and number of double bonds of acyl chains attached to the sphingosine base (18:1).

Identification of Lipids - LAESI MS/MS



- In the LAESI approach, the electrospray solution with Li_2SO_4 produced lithiated phosphocholine ions without direct application of Li_2SO_4 to the mouse brain.



- Collision-activated dissociation MS/MS of lithiated phosphatidycholine at m/z 766.5880 produced fragment ions m/z 510.3568 and 484.3512 showing the loss of palmitic acid (16:0) and oleic acid (18:1) respectively identifying the GPCho (34:1) molecule as GPCho (16:0/18:1).

Conclusions

Ambient Analysis of Brain Section. AP IR-MALDI and LAESI were used for *in situ* detection of metabolites and lipids in a brain section without sample preparation for lipidomics and metabolomics applications.

Structural Elucidation of Lipids by LAESI-MS/MS. Fragmentation of lithiated lipid molecules, formed in LAESI by adding Li_2SO_4 to the electrospray solution, helped in their structural elucidation.

Future Directions. Future studies include spatial mapping of metabolites and lipids in the mouse brain tissue to study the related biological processes or pathological changes.

References

- [1] Uphoff, A.; Hermansson, M.; Haimi, P.; Somerharju, P., Analysis of complex lipidomes. In *Medical Applications of Mass Spectrometry*, Vekey, K.; Telekes, A.; Vertes, A., Eds. Elsevier Science: St. Louis, MO, 2007; pp 217-243.
- [2] Pulfer, M.; Murphy, R. C., Electrospray mass spectrometry of phospholipids. *Mass Spectrometry Reviews* 2003, 22, (5), 332-364.
- [3] Schiller, J.; Su, R.; Arnold, J.; Fuchs, B.; Leig, J.; Muller, M.; Petkovic, M.; Spalteholz, H.; Zschornig, O.; Arnold, K., Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research. *Progress in Lipid Research* 2004, 43, (5), 449-488.
- [4] Li, Y.; Shrestha, B.; Vertes, A., Atmospheric Pressure Infrared MALDI Imaging Mass Spectrometry for Plant Metabolomics. *Analytical Chemistry* 2008, 80, 407-420.
- [5] Nemes, P.; Vertes, A., Laser Ablation Electrospray Ionization for Atmospheric Pressure, *In Vivo*, and Imaging Mass Spectrometry. *Analytical Chemistry* 2007, 79, (21), 8098-8106.
- [6] Nemes, P.; Li, Y.; Barton, A. A.; Vertes, A., Molecular imaging of live tissues by infrared laser ablation electrospray ionization mass spectrometry. *Analytical Chemistry* 2008, Web Released.

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