

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

BINDESH SHRESTHA<sup>1</sup>; Peter Nemes <sup>1</sup>; Javad Nazarian<sup>2</sup>; Eric P. Hoffman<sup>2</sup>; Akos Vertes <sup>1</sup>

<sup>1</sup>George Washington University, Washington, DC; <sup>2</sup>Children's National Medical Center, Washington, DC

**Keywords:** Ionization, Matrix-Assisted Laser Desorption (MALDI); Laser Ablation; Lipid; Mass Spectrometry/Infrared; Metabolic Profiling;

**Novel Aspect:** Two novel ambient ionization techniques were demonstrated for the direct analysis of lipids and other metabolites in mouse brain tissue.

## Introduction

Lipids such as glycerophospholipids, composing over half of the human and mouse brain, play a vital role in brain function at both molecular and physiological levels. The direct and rapid ambient analysis of brain tissue is required to study cell specific molecular profiles. Sample processing of biopsies and cellular disintegration of postmortem autopsied samples often cause the loss of molecular information related to specific cells such as malignant tumor cells. In this contribution we demonstrate the utility of two novel ambient ionization techniques, atmospheric pressure infrared matrix-assisted laser desorption/ionization (AP IR-MALDI) and laser ablation electrospray ionization (LAESI) 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 biological tissue due to their OH vibrations at mid-infrared wavelengths. The tissue samples were ablated at 2.94  $\mu\text{m}$  wavelength by a Nd:YAG laser-driven optical parametric oscillator. In AP IR-MALDI the generated ions were directly detected by an orthogonal acceleration time-of-flight mass spectrometer, Q-TOF, with a custom made interface. In the LAESI experiments, the neutrals and particulates were postionized by an electrospray. Transverse cross-sections (~200-400  $\mu\text{m}$  thick) of mouse brain obtained from normal and healthy C57Bl/10 strain of mice (age 12-14 months) were kept frozen during the analysis using a Peltier cooling stage to prevent rapid biochemical changes present at higher temperatures.

## Preliminary results

The ambient direct analysis of mouse brain by AP IR-MALDI and LAESI produced mass spectra dominated primarily by phospholipids such as glycerophosphocholines(PC), and glycerophosphoethanolamines(PE), such as PC[34:1], PC[32:1], PE[38:4], etc. Certain ions were detected by both techniques, whereas in other cases the methods provided complementary information. For example, non-polar lipids such as cholesterol, one of the primary components in the brain, was only detected by AP IR-MALDI at  $m/z$  369.353, 409.3401 and 425.3224 as  $[M-H_2O+H]^+$ ,  $[M+Na]^+$  and  $[M+K]^+$ , respectively. Many small metabolites in the brain such as creatine, arginine, gamma-aminobutyric acid etc., were also detected alongside the lipids. The mouse brain tissue was also analyzed with traditional vacuum UV-MALDI after application of DHB matrix and as extracted samples using electrospray ionization. For the lipids and small metabolites, the traditional methods did not provide significant advantage over direct AP IR-MALDI and LAESI analysis in either selectivity or sensitivity. The tentative assignment of the lipid peaks was obtained by accurate mass match, and isotope distribution analysis. For peaks of interest, the structure was further confirmed by tandem mass spectrometry using collision activated dissociation (CAD). In LAESI, the analyte molecules desorbed from the tissue sample formed adducts with the ions (e.g.,  $Li^+$ ) present in the electrosprayed solution. The CAD of lithiated glycerophosphocholines helped the identification of individual fatty acyl chains. For example, CAD of a lithiated glycerophosphocholine at  $m/z$  766.5938 produces  $m/z$  510.3614 and 484.3454 showing the loss of palmitic acid [16:0] and oleic acid [18:1], respectively, which identifies the molecule as PC[16:0/18:1]. Our results on brain tissue samples indicate that the direct analysis of lipids at atmospheric pressure by AP IR-MALDI and LAESI could be useful in broader lipidomics and metabolomics applications such as profiling of brain tumors.