# Exploring the Aβ Plaque microenvironment in Alzheimer's disease model mice by multimodal Lipid-Protein-Histology Imaging on a benchtop mass spectrometer

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## Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the leading cause of dementia in the elderly population. Key pathological features include extracellular lipid-containing amyloid-β plaques, and a complex cellular and molecular microenvironment, indicating that it is also involved in the pathogenesis.

MALDI Imaging is an advanced technique to visualize the spatial distribution of different biomolecular classes and cell types in tissue sections. Here, we demonstrate the feasibility of multiomic and multimodal imaging of lipids, protein markers, and histology, all on the same tissue section. The MALDI HiPLEX-IHC technology was used to visualize amyloid-β plaques and known cell/protein markers.

## Methods

Fresh-frozen APP/PS1 or wild-type mouse brain sections were prepared for lipid imaging on a neofleX™ (Bruker) using reflector negative mode at 20 µm pixel size. Relevant lipids in the amyloid-β plaque region were fragmented using MS/MS. The same slide was processed by MALDI HiPLEX-immunohistochemistry (IHC) using Miralys™ antibodies (AmberGen). MALDI Imaging was conducted in reflector-positive mode at 20 µm pixel size. H&E staining was performed on the same slide. All matrices were applied by pneumatic sprayers (HTX Technologies). Analysis and visualization of the data were done in SCiLS™ Lab, SCiLS Scope, and M²aia software.

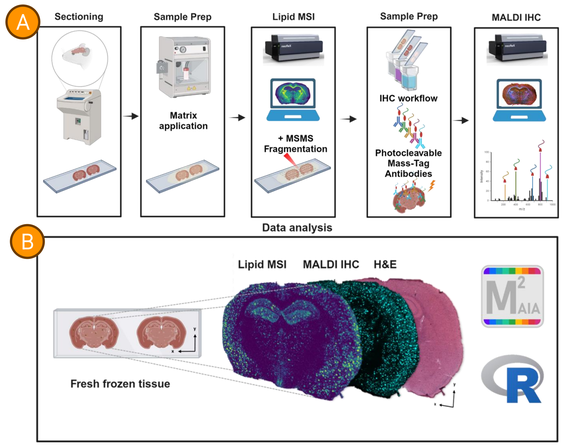
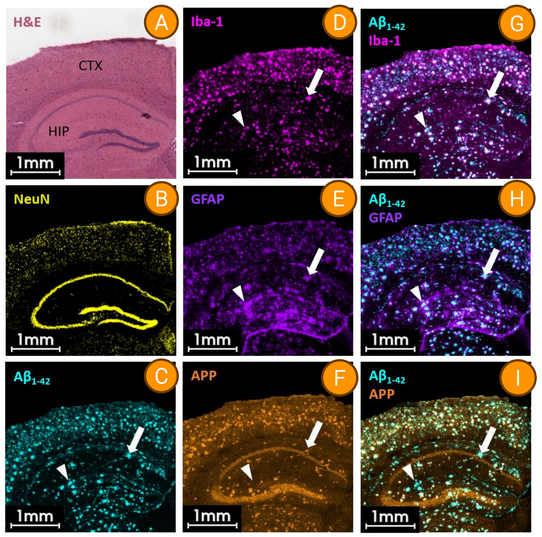



**Figure 1**: **(A)** Workflow for sample preparation and multimodal MALDI Imaging on a neofleX. Lipid imaging was followed by MALDI HiPLEX-immunohistochemistry and then H&E staining of the Aβ plaque microenvironment in AD model mice.

**(B)** For data analysis, the order was reversed from acquisition, i.e., HiPLEX then lipids were analyzed to focus on spatial analysis of protein/cell markers before correlation with lipids.

## Results and Discussion

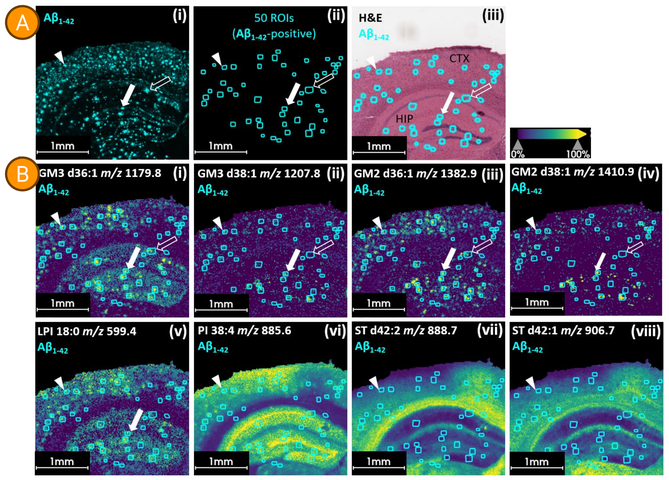



The focus of AD research has expanded beyond Aβ plaques and hyper-phosphorylated tau proteins to now include additional molecular characteristics in close association with Aβ deposits. A targeted protein panel was analyzed to determine plaque regions.

**Figure 2: (A)** H&E staining highlighted distinct brain structures e.g., the hippocampus (HIP) and cortex (CTX) which are distinct pathology that is observed in many of the images. MALDI HiPLEX-IHC images of five targeted proteins including **(B)** the brain structural marker neuronal nuclei (NeuN). NeuN is a marker that indicates regions with a high density of neuronal cell bodies like the pyramidal neuron layer.

Single ion images show the distribution of: **(C)** the amyloid-$\beta_{1\text{-}42}$ peptide, **(D)** the microglia marker Iba-1, **(E)** glial fibrillary acidic protein (GFAP), and **(F)** the amyloid precursor protein (APP). **(G)** Overlay of $A\beta_{1\text{-}42}$ with Iba-1 shows almost 100% co-localization of both signals in the CTX and HIP. **(H)** Overlay of $A\beta_{1\text{-}42}$ and GFAP indicated high loads of GFAP clusters in HIP, but with less co-localization in CTX. **(I)** $A\beta_{1\text{-}42}$ and APP overlay showed some degree of co-localization in HIP and CTX.

The filled white arrow shows co-localization of $A\beta_{1\text{-}42}$ with all other biomarkers. The filled white arrowhead shows $A\beta_{1\text{-}42}$ co-localization with Iba-1 and GFAP, but not for APP. Co-localization of $A\beta_{1\text{-}42}$ plaques with microglia, activated astrocytes, and the amyloid precursor protein APP in APP/PS1 mouse brain.




After determination of regions of interest (ROIs) from the $A\beta_{1\text{-}42}$ marker (**Figure 3Ai-ii**), lipid composition in the plaque regions were analyzed for a multiomic comparison with the goal of identifying potential lipid biomarkers. The ROIs were also visualized with histology (**Figure 3Aiii**).

Lipid accumulation and depletion has been previously studied in mouse models and human AD samples. Here, single ion images of key lipids in the ROIs of interest provide a multiomic contextualization from the benchtop neofleX.

Plaque-associated gangliosides (GM2 and GM3, represented in **Figure 3Bi-iv**) were elevated in the AD mouse model. GMs are major components in lipid rafts neuronal membranes, making them essential for cell signaling and processing. Lysophosphatidyl inositol (LPI 18:0) and phosphatidylinositol (PI 38:4) also showed accumulation at plaque-positive ROIs (**Figure 3Bv-vi**). LPI 18:0 was primarily visualized in the vicinity of plaque areas, PI 38:4 was also present in the pyramidal layer of the HIP and some of the CTX.

Depletion of two sulfatides (STs) are shown in **Figure 3Bvii-viii.** STs had a high intensity in the white matter as well. STs are major components of the myelin sheath, a key component of healthy nerve cell signal transduction.

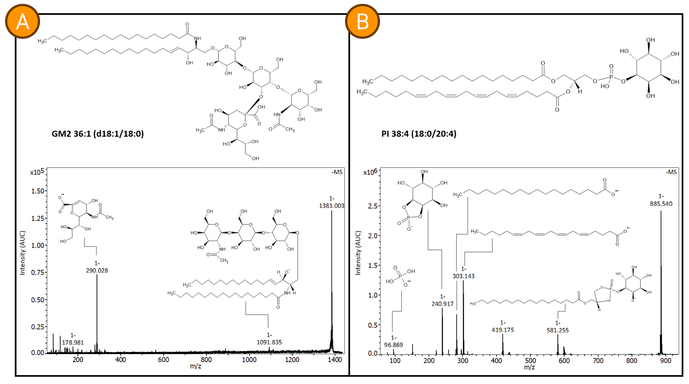



**Figure 4**: Identification of candidate lipid biomarkers by on-tissue TOF/TOF fragmentation analysis using a bench top mass spectrometer.

On-tissue MALDI TOF/TOF fragmentation of ten lipid features was performed with successful annotation. After MALDI Imaging lipid data was collected, precursors were manually selected for fragmentation. **Figure 4A** shows the fragmentation and structural annotation of putative GM2 d36:1 (18:1/18:0). Loss of sialic acid (*m/z* 1091.8) and the counterpart (*m/z* 290.0) confirm the ganglioside, but exact arrangement and chain length were not defined. Fragmentation of putative PI 38:4 (**Figure 4B**) resulted in *m/z* 581.3, the triglyceride backbone connected to the inositol phosphate headgroup. Additional fragments that are indicative of fatty acid chains and head group components were observed.

- Multiomic and multimodal data revealed extensive co-localization of Aβ plaques with the peptides' precursor protein, with a defined subset of lipids, in particular gangliosides, and with reactive glia cells on a single brain section of APP/PS1 mice.
- A multi-purpose benchtop mass spectrometer that is capable of MALDI Imaging and TOF/TOF fragmentation was demonstrated to be applicable to disease-based research.

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**COI Disclosure**. D.N., K.S., and M.E. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.