WP 376

# Multimodal Omics Imaging of Human Brain Using MALDI HiPLEX-IHC


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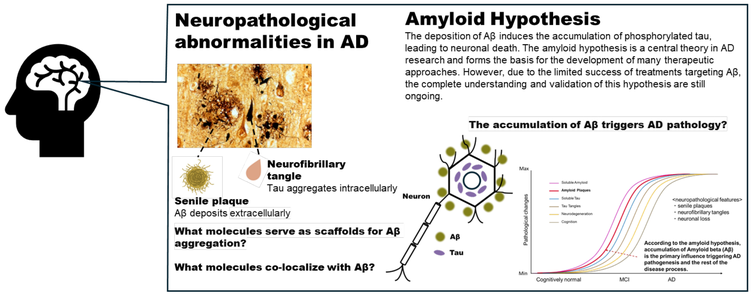
## Introduction

MALDI HiPLEX-IHC enables layered imaging of unlabeled small molecules and target proteins. In this study, multimodal omics imaging was conducted on human brains from Alzheimer's disease (AD) patients and control subjects. The aim is to elucidate the localization of AD-related proteins using photo-cleavable probes and enhance the pathological understanding of AD brains by overlaying lipid imaging on these findings. AD is pathologically characterized by the aggregation of the Amyloid β (Aβ) species. However, detailed information about the co-localization of specific small molecules with these protein aggregates remains unclear. Understanding these neurological and pathological changes is crucial for comprehending the progression of AD and is a significant focus of research aimed at developing new treatments.




## Methods

### Subjects

Human cortical specimens for MALDI-MSI and MALDI-IHC were obtained from those brains that were removed, processed and placed in -80˚C within 8h postmortem at the Brain Bank at Tokyo Metropolitan Institute of Gerontology. For all brains registered, we obtained written informed consents for their use for medical research from patients or patient's family. Each brain specimen was taken from occipital cortex of 3 AD patients and 3 controls (Table1). Fresh frozen sections were used at a thickness of 10 um.

### MALDI-MSI for lipids

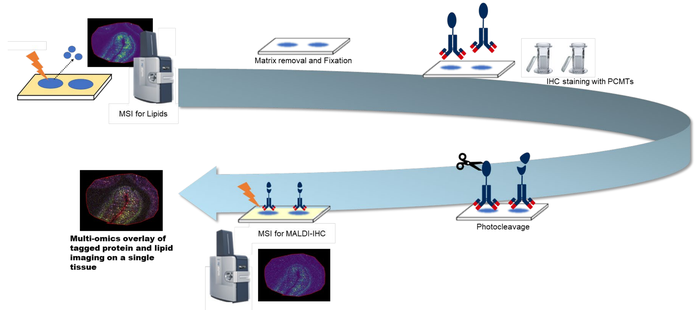
Matrix: DHA, TM-Sprayer
Spatial resolution : 40 um
Mass range : *m/z* 200-2000
Mode : Negative
Instrument : tims-TOF-fleX

| case | gender | age at death | Braak Stage | CAA |
|---|---|---|---|---|
| 1 | M | 88 | V | 1 |
| 2 | M | 78 | VI | 1 |
| 3 | M | 83 | V | 1 |
| 4 | M | 84 | I | 0 |
| 5 | M | 78 | I | 0 |
| 6 | M | 81 | I | 0 |

Table1 Clinical and pathological data of AD / CAA cases and control. The Braak stage and CAA score represent the degree of progression.

### MALDI-IHC with photocleavable mass-tags (PCMTs)

PCMTs: Aβ42, pTau(pS404, Thr205), α-synuclein,
Amyloid precursor protein(APP), Nicastrin, NLF, MBP,GFAP
Matrix: CHCA, TM-Sprayer
Spatial resolution: 40 um
Mass range: *m/z* 500-2000
Mode: Positive
Instrument: tims-TOF-fleX




## Results and Discussions

### ➢MALDI-IHC for AD-related proteins

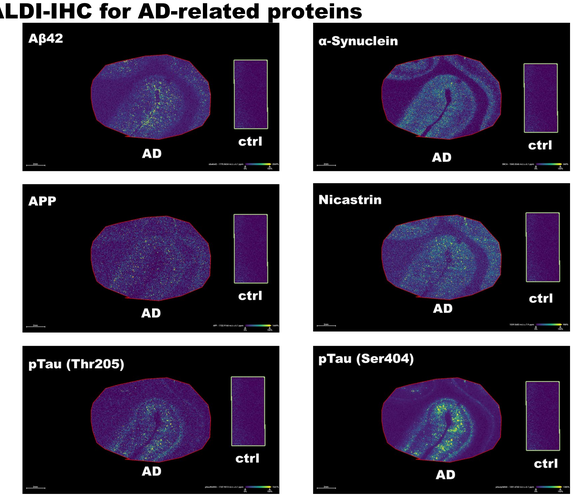



Figure. 1 Single peak imaging of tagged proteins
Aβ42 *m/z* 1770.88, α-synuclein *m/z* 1045.57, APP *m/z* 1722.92, Nicastrin *m/z* 1039.56, pTau (Thr205) *m/z* 1747.92, pTau(Ser404) *m/z* 1201.69, scale bars = 2 mm

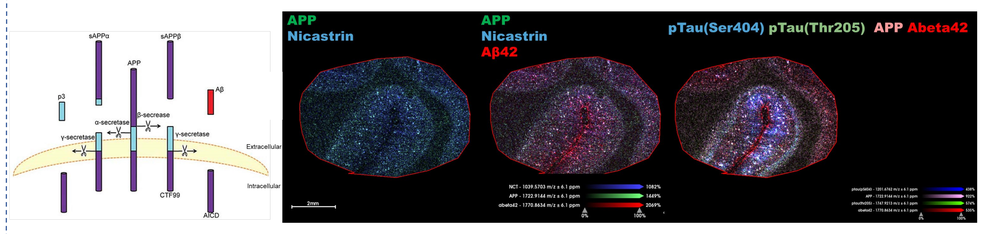



Figure.2 Multiplex staining imaging of tagged proteins
APP is sequentially cleaved by β-secretase and γ-secretase, resulting in the production of Aβ. Nicastrin is a component of the γ-secretase and serves as its active site. In the AD brain, APP and nicastrin exhibited the same localization pattern. On the other hand, Aβ42 exhibited a specific deposition pattern along meningeal blood vessels and the brain surface. Different phosphorylation sites of tau molecules were overlaid with APP and Aβ42. Tau phosphorylated at the 205 tyrosine and 404 serine residues exhibited a distribution along the structure of the cerebral cortex, demonstrating a similar localization pattern.

### ➢The combination of targeted protein imaging and non-targeted lipid imaging

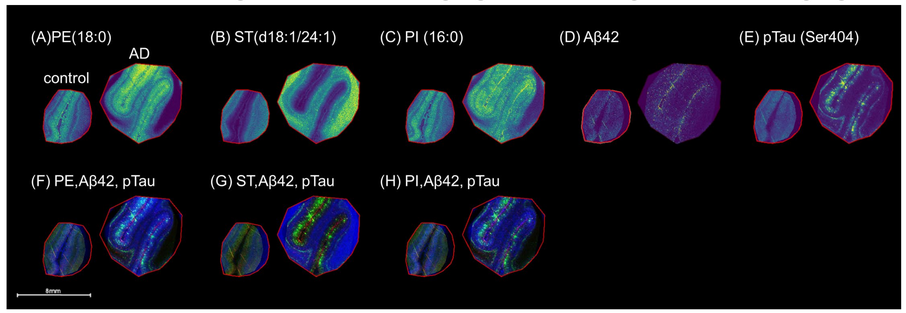



Figure.3 Overlay tagged proteins imaging and lipids imaging
Upper: Single peak imaging of lipids and tagged proteins, Lower: Merged imaging (Red: Aβ42, Green: ptau, Blue: Lipid), scale bar = 8 mm
(A)PE(18:0) *m/z* 480.3101, (B)ST (d18:1/24:1) *m/z* 888.6257, (C) PI(16:0) *m/z* 571.2898, (D) Aβ42 *m/z* 1770.88, (E) pTau (Ser404) *m/z* 1201.69
(F) Blue: PE(18:0), Red: Aβ42, Green: ptau (G) Blue: ST(d18:1/24:1), Red: Aβ42, Green: ptau (H) Blue: PI(16:0),Red: Aβ42, Green: ptau
PE and PI demonstrated distribution patterns in the gray matter, complemented by ST which showed distribution in the white matter.
PE, PI, and ST species have been reported to fluctuate in the brains of AD patients and are known to be involved in the Aβ production pathway.

## Summary

Performing multi-omics imaging with MALDI-IHC has the potential to reveal pathologically specific interactions and correlations among different molecular classes. This study successfully visualized the localization of AD-related proteins in the brain, which were difficult to detect with previous protein-targeted MALDI-MSI and classic IHC staining.

Aβ species exhibit differences in aggregation and toxicity due to variations in structure and sequence. Microdomains located in specific regions of the cell membrane are deeply associated with AD pathology. As a next step, visualizing the co-localization of different Aβ molecular species in the AD brain in addition to current strategy.


No potential conflicts of interest were disclosed.