High-Plex, Multiomic and Multimodal Imaging of PANTHOS Neuronal Degeneration in Model Alzheimer's Transgenic Mouse Brain using MALDI-IHC

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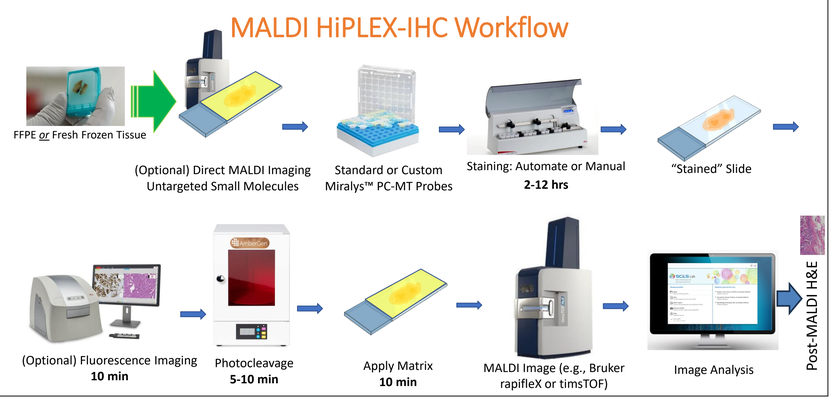
Introduction

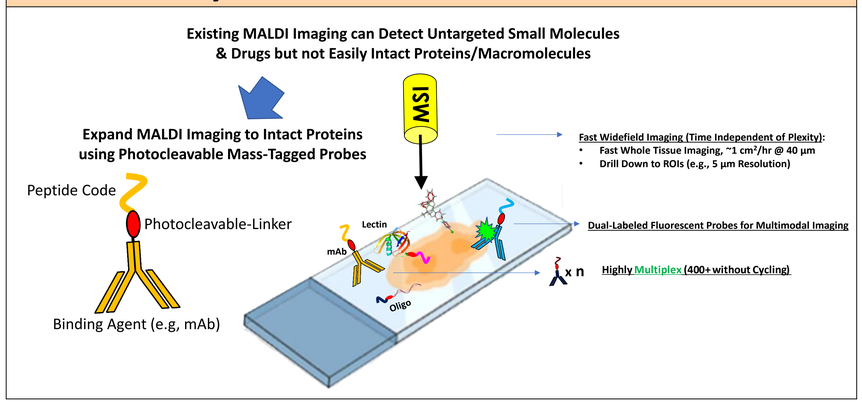
Alzheimer's Disease (AD) kills more people in the U.S. every year than breast and prostate cancers combined. Recent progress has been made in understanding AD pathology using transgenic AD mouse models, including 5XFAD-TRGL transgenic mice, where a fusion of the LC3 autophagosome marker with mRFP-eGFP is expressed selectively in neurons using a Thy1 promoter, enabling fluorescence imaging of relative pH in autophagy–lysosomal neuronal processes. This reveals that autophagy dysfunction arises from exceptionally early failure of autolysomal/lysosomal acidification, driving downstream AD pathogenesis. This process involves the formation in compromised, yet still intact neurons, of large autophagic waste-filled plasma membrane blebs which have a flower-like perikaryal rosette structure termed PANTHOS - **P**oisonous flower (**ANTHOS**) (Lee, J-H et al. Nat Neurosci **25**, 688 (2022)).

Methods

To further explore the molecular composition of PANTHOS structures and proximal cells, we imaged brain tissue from 5XFAD-TRGL and other AD transgenic mouse models using a new, highly multiplex, multiomic and multimodal method termed MALDI-IHC. This method combines the relative strengths of mass spectrometry imaging (MSI) and immunohistochemistry by using novel photocleavable mass-tagged antibodies to probe the spatial location of a panel of intact proteins as well as to serve as markers of cell lineage. MSI was performed on a Bruker rapifleX Tissuetyper or timsTOF flex equipped with a microGRID accessory which enables 5 µm spatial resolution. Multiomic imaging combines untargeted label-free MSI with MALDI-IHC, and multimodal imaging combines fluorescence and/or brightfield microscopy with MALDI-IHC, all performed on the same tissue section.



Key Features of MALDI HiPLEX-IHC



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Elucidating the Mechanisms of Alzheimer's Disease with Multimodal MALDI-IHC

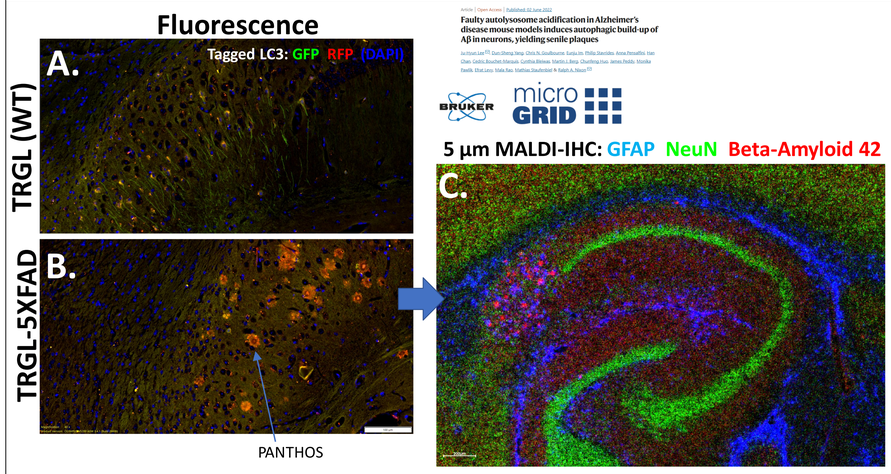

Figure 1. Multimodal Mouse Brain Imaging with MALDI-IHC in a Transgenic Alzheimer's Model in Collaboration Prof. Randy Nixon (Nathan S. Kline Institute and NYU). (A-B.) Fluorescence microscopy of a recombinantly expressed marker of autophagosomes (LC3) containing a tandem fluorescent protein tag (GFP and RFP), the so-called TRGL transgene. (A.) WT mouse with TRGL transgene. (B.) 5XFAD Alzheimer's mouse with TRGL transgene. The Nixon group has shown that neuronal endosomal/lysosomal dysfunction is one of the earliest processes leading to amyloid deposits and the pathogenesis of Alzheimer's Disease. The so-called PANTHOS structures observed are compromised, dying neurons exhibiting this endosomal/lysosomal dysfunction. (C.) 10-plex MALDI-IHC (3 antibodies shown) on TRGL-5XFAD tissue (5 µm spatial resolution using Bruker's timsTOF fleX with microGRID).

Cyclic MALDI-IHC (cMIHC): Whole-Tissue High-Plex Overview Scan in <2hr Followed by Detailed ROI Scan

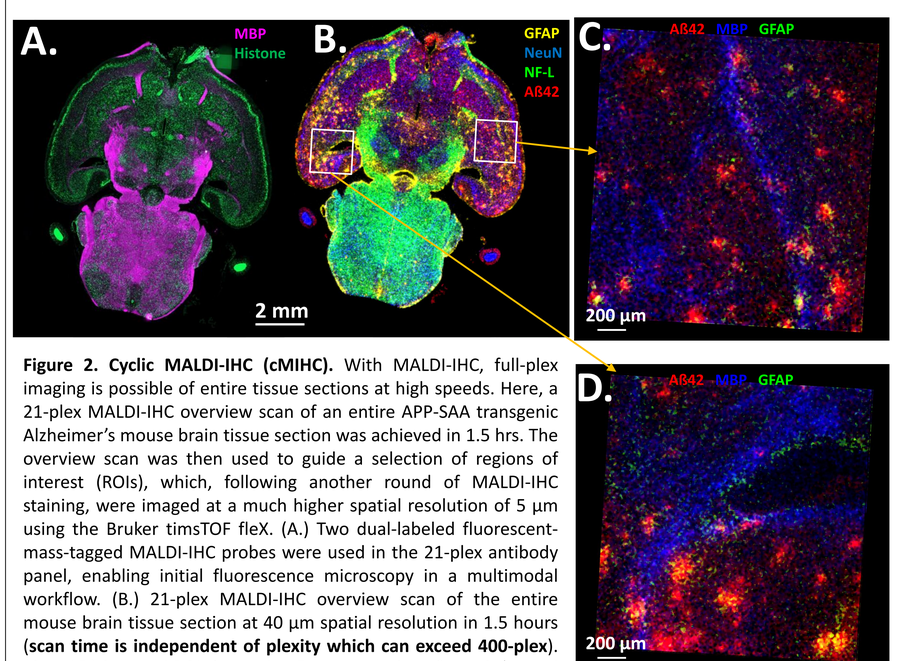

Figure 2. Cyclic MALDI-IHC (cMIHC). With MALDI-IHC, full-plex imaging is possible of entire tissue sections at high speeds. Here, a 21-plex MALDI-IHC overview scan of an entire APP-SAA transgenic Alzheimer's mouse brain tissue section was achieved in 1.5 hrs. The overview scan was then used to guide a selection of regions of interest (ROIs), which, following another round of MALDI-IHC staining, were imaged at a much higher spatial resolution of 5 µm using the Bruker timsTOF fleX. (A.) Two dual-labeled fluorescent-mass-tagged MALDI-IHC probes were used in the 21-plex antibody panel, enabling initial fluorescence microscopy in a multimodal workflow. (B.) 21-plex MALDI-IHC overview scan of the entire mouse brain tissue section at 40 µm spatial resolution in 1.5 hours (**scan time is independent of plexity which can exceed 400-plex**). (C and D) Selected ROIs imaged at 5 µm resolution after a 2nd round of MALDI-IHC staining.

Trimodal Imaging on the Same Whole-Brain Section from an APP-SAA Transgenic Alzheimer's Mouse

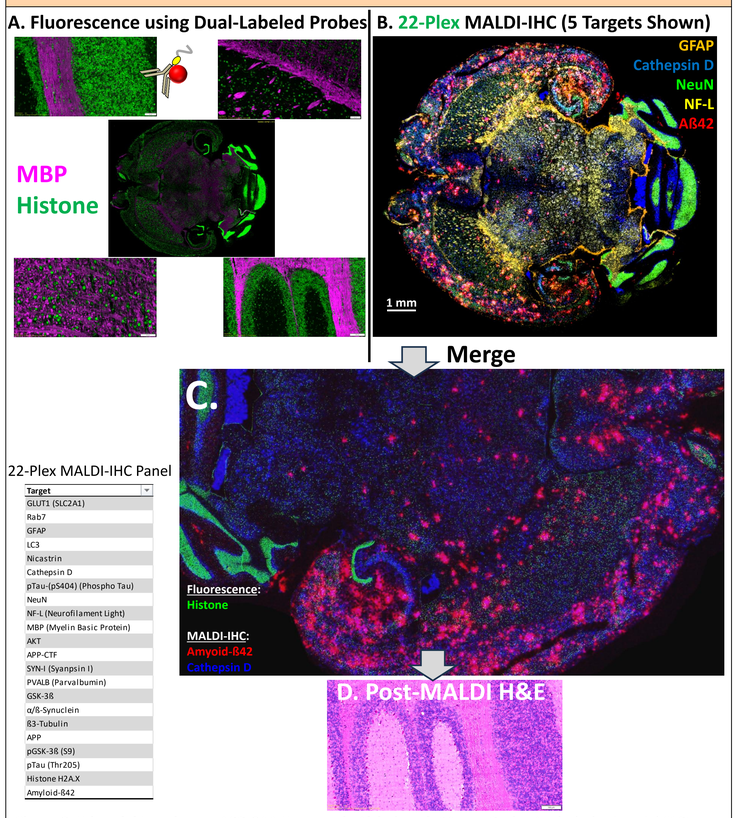

Figure 3. Trimodal Imaging on APP-SAA Transgenic Alzheimer's Mouse Brain. A whole-brain axial FFPE tissue section from an APP-SAA transgenic Alzheimer's mouse was subjected to 3 modes of imaging (all on the same tissue section). Tissues were stained with a 22-plex MALDI-IHC antibody panel (see Table), however, 2 antibodies (MBP and Histone) were dual-labeled and therefore additionally contained fluorophores. (A.) Fluorescence microscopy of magnified regions and the whole mouse brain (40x objective). (B.) 22-Plex MALDI-IHC (20 µm spatial resolution), with 5 antibodies shown for example (see Table for list of the 22 antibodies). (C.) Since the fluorescence and MALDI-IHC images are from the same tissue section, the images are readily co-registered. Here, the fluorescence for the histone antibody is displayed as green and co-registered with MALDI-IHC (Amyloid-ß42 and Cathepsin D, red and blue, respectively). (D.) Following MALDI-IHC imaging, the CHCA MALDI matrix is removed with methanol and the tissue subjected to standard H&E staining followed by brightfield microscopy.

Results and Conclusions

Results demonstrate the capability of combining fluorescence and brightfield microscopy modalities with multiplexed MALDI-IHC-based targeted protein imaging on the same tissue specimen. This enables high-information-content spatial biology analyses on entire tissue specimens quickly and at ultra-high plex (ultimately 400+ plexity without cycling) in order to elucidate the mechanisms of Alzheimer's pathology. The lower plex but higher spatial resolution fluorescence and brightfield microscopy can complement MALDI-IHC in several ways. For example, i) visualization of intrinsic transgenic expressed fluorescent proteins, ii) cell segmentation, and iii) effective enhancement of the spatial resolution of the MALDI-IHC through image registration and the use of predictive algorithms. Ultimately, a multiomic approach can be employed by leveraging the power of mass spectrometry for untargeted, label-free, small molecule imaging such as of drugs, lipids and metabolites on the same tissue section as MALDI-IHC (not shown here but see references 1-2 listed at the bottom of this poster). Moreover, cyclic MALDI-IHC (cMIHC) allows high-plex overview scans of entire tissue sections in <2 hrs (**scan time independent of plexity**), region of interest selection, and subsequent equally high-plex imaging of the selected regions at much higher spatial resolution. The net result is seeing the "big picture" from high-plex, multiomic and multimodal imaging of entire tissue sections, rather than only small sub-regions of interest, and at high speeds.

References: 1. Yagnik G, Liu Z, Rothschild KJ, Lim MJ. Highly Multiplexed Immunohistochemical MALDI-MS Imaging of Biomarkers in Tissues. J Am Soc Mass Spectrom. 2021;32(4):977-88. doi: 10.1021/jasms.0c00473. 2. Lim MJ, Yagnik G, Henkel C, Frost SF, Bien T, Rothschild KJ. MALDI HiPLEX-IHC: multiomic and multimodal imaging of targeted intact proteins in tissues. Front Chem. 2023;11:1182404. doi: 10.3389/fchem.2023.1182404.