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Imaging Capabilities of a Low-Cost Benchtop MALDI-TOF Mass Spectrometer

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Introduction

MALDI mass spectrometry imaging (MSI) is a powerful technique that utilizes the capabilities of the spectrometer to collect thousands of individual spectra at various positions into a data cube. Using dedicated software, the resulting ions can be spatially represented with a pseudo-color image to visualize key molecules relative to their function in biological tissues. The MALDI-8020 time of flight (TOF) mass spectrometer can generate top quality MS images, such as lipid MSI of rat brains (Figure 1). We have previously demonstrated MALDI imaging for a variety of target analytes on the MALDI-TOF benchtop system with fingerprints, soybeans, and PET films [1] . Here, we demonstrate the capability of our imaging platform for intact protein and on-tissue digestion MS imaging of rat brain tissue in linear mode.

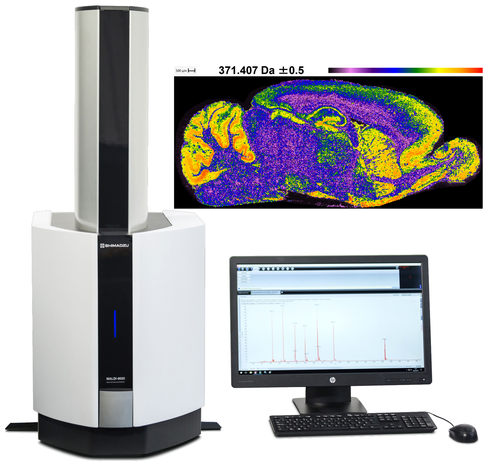

Figure 1: MALDI-8020 Benchtop linear mass spectrometer with rat brain lipid mass spectrometry imaging example highlighted.

Methods

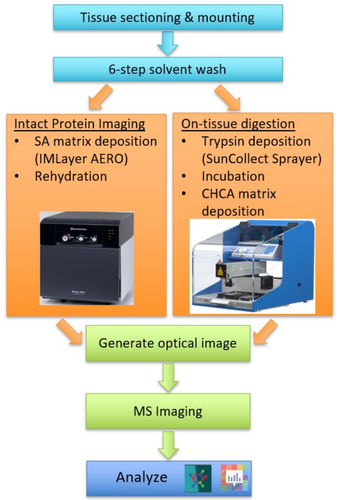

Figure 2: Workflow for protein and peptide MS imaging with Sinapinic acid (SA) and α-cyano-4-hydroxycinnamic acid (CHCA) matrices respectively.

Sagittal rat brain tissue samples sections were purchased from AMSBIO (Oxford, UK) and mounted on custom ITO slides. Two serial sections were washed using a 6-step solvent wash to remove lipids and salts [2] – one section was prepared for on-tissue digestion while the other was prepared for intact protein imaging.

For on-tissue digestion, porcine trypsin (67 ng/µL, 90:10 of 20 mM Am. Bic.: ACN) and the CHCA (10 mg/mL, 50:50 ACN: 0.1 % TFA) were applied using the SunCollect Sprayer (SunChrom, Germany). For intact protein analysis, sinapinic acid (10 mg/mL, 70:30 ACN: 0.1 % TFA) was sprayed onto the tissue using the new IMLayer AERO spraying device (Shimadzu, Japan). (Figure 2).

Samples were analysed at 50 µm spatial resolution in linear mode on a benchtop MALDI-TOF MS modified for MALDI imaging (MALDI-8020, Shimadzu, Japan). Data was analyzed in IonView software (Shimadzu, Japan).

Results

On-tissue digestion and protein imaging protocols were successful on a MALDI-TOF-TOF (MALDI-7090 (Shimadzu, Japan)) instrument, and we have transferred these methods to a benchtop linear MALDI-TOF MS (MALDI-8020) instrument.

In the absence of MS/MS it was still possible to tentatively assign peptides based on mass, to commonly observed rat brain proteins, e.g., myelin basic protein (MBP) produces characteristic peptides that are localized in the white matter while some are specific to grey matter or other cell types (Figure 3) [3].

Since the tissue used was embedded in a media to improve the sectioning and storage of the rat brain specimen, it was difficult to detect protein species greater than 20 kDa [4]. Peaks corresponding to common proteins in the rat brain (e.g., MBP) were detected and the results were localized to their structural function correlating to that of the peptides (Figure 4) [5, 6].

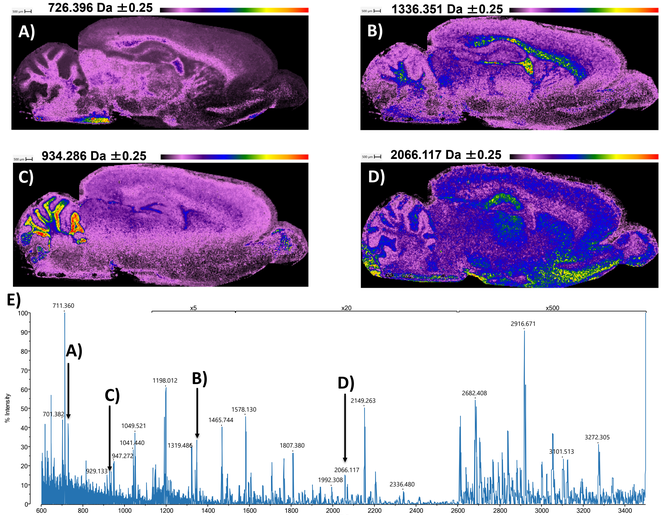

Figure 3: MS Images of digested peptides: A) MBP peptide: HGFLPR, T160-165 (*m/z* 726.394), B) MBP peptide: YLATASTMDHAR, T148-159 (*m/z* 1336.633), C) & D) unknown peptides localized in the cerebellum and grey matter respectively, E) TIC spectrum of rat brain peptides

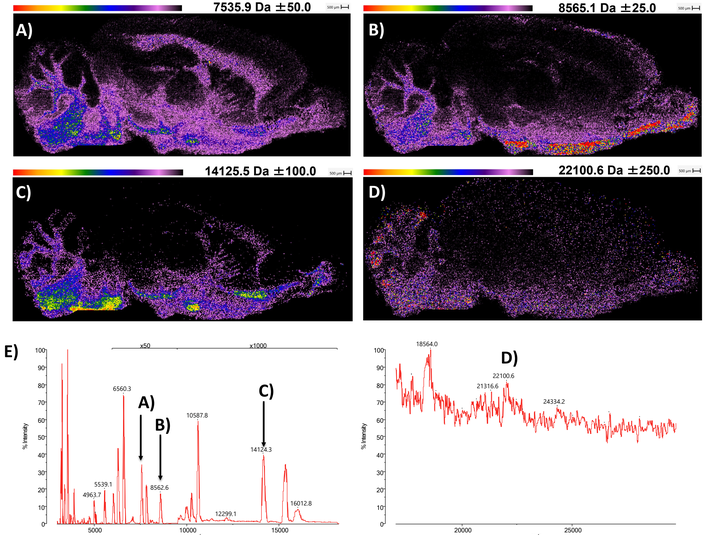

Figure 4: MS Images of intact proteins: A) Neurogranin (*m/z* 7537), B) Ubiquitin (m/z 8565), C) Myelin Basic Protein (*m/z* 14124), D) Brain Acid Soluble Protein I (*m/z* 22104), E) TIC spectrum of rat brain proteins with a zoomed view of the high mass, low resolution peaks

Conclusions

We have demonstrated the capability of a low-cost benchtop MALDI-TOF instrument for the analysis of *in situ* peptides and proteins. Proteins up to 22 kDa were detected and corresponding peptides from the on-tissue digestion were consistent with theoretical values from an *in-silico* digestion and localized observations. This compact and robust instrument would be ideal for those new to MALDI imaging and would represent a great resource for universities and teaching laboratories.

References

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