Development of a Robust Protocol for Profiling Peptides by MALDI-MSI

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Introduction

MALDI-MSI has emerged as a powerful tool for the spatial mapping of a wide range of molecules in a variety of tissue types, including fresh frozen tissues, FFPE tissue, and tissue microarrays to aid in the identification of potential biomarkers. Here we describe the development of a robust protocol for profiling the spatial distribution of peptides within fresh frozen and FFPE tissues. Specifically, varying trypsin digestion time and relative humidity during digestion were optimized. Relative humidity was modified during trypsin digestion through the addition of different salts. The selection of optimized conditions involved assessing the number of identified peptide IDs in METASPACE and evaluating analyte delocalization by examining off-tissue signals within SCiLS.

Methods

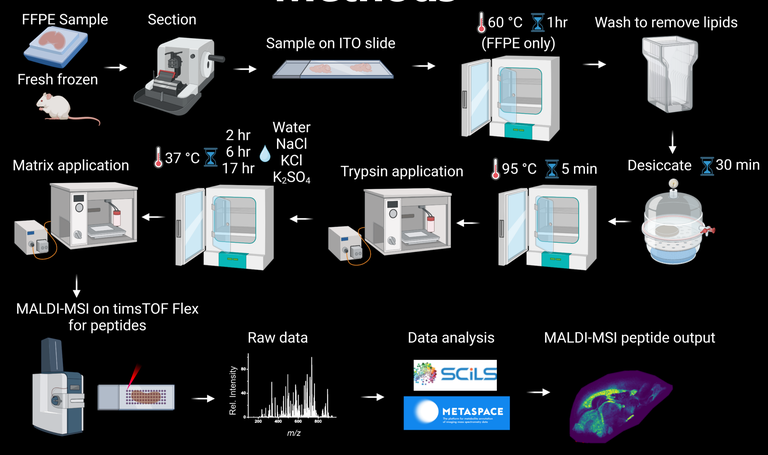

Figure 1: Experimental workflow and optimization parameters

FFPE and fresh frozen tissue were sectioned at 10 µm thickness. FFPE slides were heated, dewaxed, and rehydrated for heat-induced antigen retrieval. Fresh frozen tissue only underwent ethanol washes. Trypsin was applied to the slides using a TM-Sprayer. Trypsin incubation times tested were 2, 6, and 16 hours. In addition, NaCl, KCl, and K_2SO_4 were introduced to humidity chambers to saturation to change the relative humidity at 37 °C. CHCA matrix was sprayed onto the slides. Bruker timsTOF Flex MALDI2 was operated in positive ion mode. Imaging data was imported into the SCiLS lab software, exported to imzML, and the resulting files were submitted to METASPACE for data processing using a custom database generated by LC-MS/MS of bulk tissues.

Building Custom Search Database

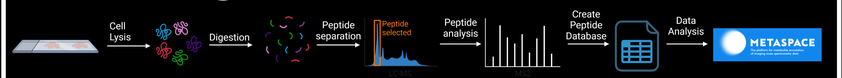

Figure 2: Experimental workflow of database creation.

Custom peptide lists was obtained through a standard LC-MS/MS proteomics with extracted peptide chemical formula through in-house software. We successfully identified peptides with confidence within the dataset through importing the custom peptide database to METASPACE for efficient data processing and visualization.

Fresh Frozen Tissue Optimization

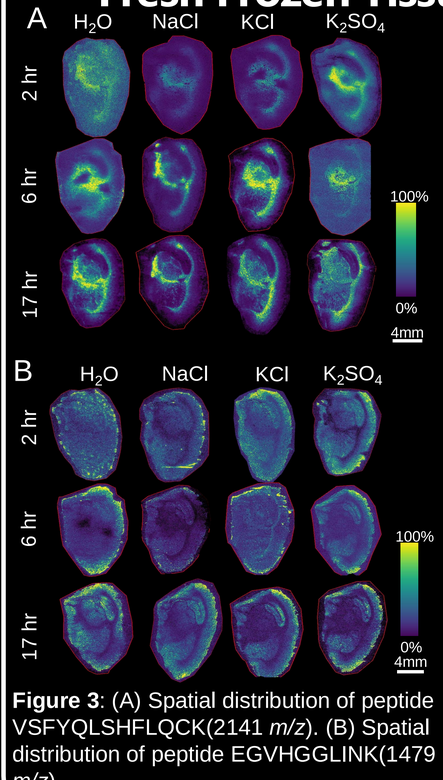

Figure 3: (A) Spatial distribution of peptide VSFYQLSHFLQCK(2141 *m/z*). (B) Spatial distribution of peptide EGVHGGLINK(1479 *m/z*).

Sample	Number of Annotations in METASPACE
2 hr Water	55
2 hr NaCl	58
2 hr KCl	69
2 hr K_2SO_4	72
6 hr Water	69
6 hr NaCl	71
6 hr KCl	89
6 hr K_2SO_4	108
17 hr Water	74
17 hr NaCl	95
17 hr KCl	102
17 hr K_2SO_4	128

Table 1: Number of peptides in fresh frozen rat brain that were annotated within METASPACE through the creation of a custom database.

Increased incubation time and humidity allowed for more peptides to be annotated in fresh frozen rat brain.

FFPE Tissue Optimization

Sample	Number of Annotations in METASPACE
2 hr Water	63
2 hr NaCl	71
2 hr KCl	78
2 hr K_2SO_4	84
6 hr Water	77
6 hr NaCl	80
6 hr KCl	93
6 hr K_2SO_4	113
17 hr Water	88
17 hr NaCl	105
17 hr KCl	114
17 hr K_2SO_4	132

Table 2: Number of peptides in FFPE rat brain that were annotated in METASPACE.

Similar trends were seen in FFPE rat brain when compared to fresh frozen. As optimization continues 6hr K_2SO_4 incubation time could be considered as annotation number is just slightly lagging.

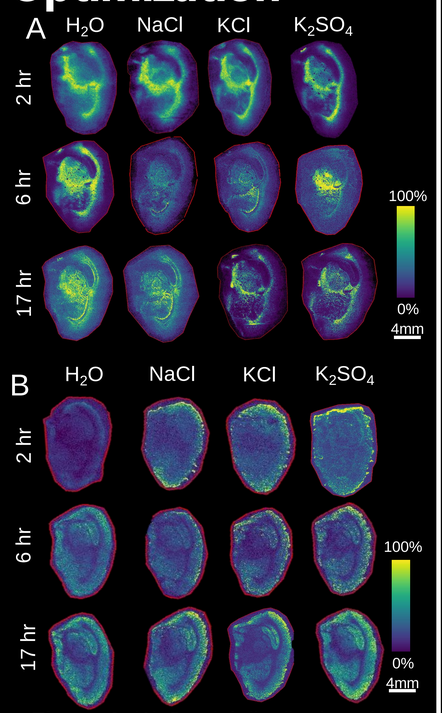

Figure 4: (A) Spatial distribution of peptide VSFYQLSHFLQCK (2141 *m/z*). (B) Spatial distribution of peptide EGVHGGLINKK(1479 *m/z*).

Analyte Delocalization Considerations

It is important to look at how the different conditions are affecting the analytes within the tissue. Pearson correlation coefficients are often generated using an on and off tissue signal to help determine potential analyte delocalization.

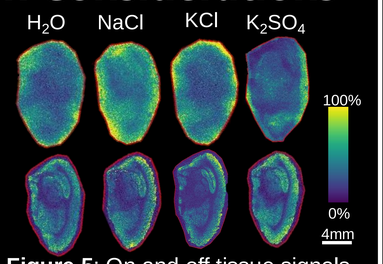

Figure 5: On and off tissue signals used for the Pearson correlation coefficient determination in 17hr incubation of fresh frozen tissue.

Table 3: Pearson correlation coefficients of all conditions tested. Values show less analyte delocalization occurred as incubation time and humidity level increased.

Fresh Frozen Sample	Pearson correlation coefficient	FFPE Sample	Pearson correlation coefficient
2 hr Water	0.297	2 hr Water	0.303
2 hr NaCl	0.161	2 hr NaCl	0.182
2 hr KCl	0.125	2 hr KCl	0.136
2 hr K_2SO_4	0.112	2 hr K_2SO_4	0.117
6 hr Water	0.137	6 hr Water	0.141
6 hr NaCl	0.135	6 hr NaCl	0.132
6 hr KCl	0.114	6 hr KCl	0.126
6 hr K_2SO_4	0.105	6 hr K_2SO_4	0.101
17 hr Water	0.029	17 hr Water	0.036
17 hr NaCl	0.018	17 hr NaCl	0.021
17 hr KCl	0.026	17 hr KCl	0.029
17 hr K_2SO_4	0.016	17 hr K_2SO_4	0.018

Antigen Retrieval for FFPE Tissue

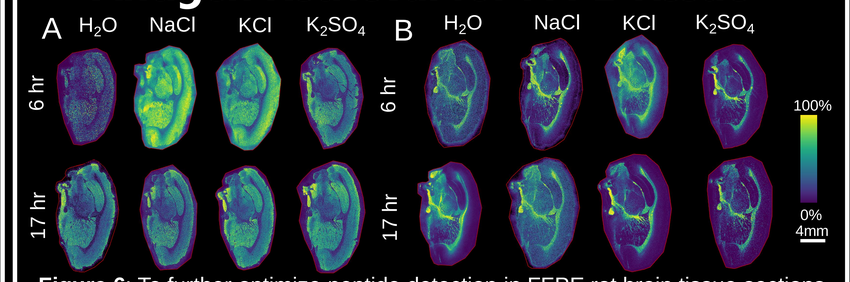

Figure 6: To further optimize peptide detection in FFPE rat brain tissue sections were heated in citrate buffer at 99 °C for 20 mins. Buffer exchanges with water were performed before drying the samples in the desiccator for 30 mins prior to trypsin application. Antigen retrieval overall helped with the detection of peptides after a 6 hr and 17 hr trypsin digestion.

Conclusions and Future Directions

Here we examined both FFPE and fresh frozen tissue to optimize the sample preparation protocol to maximize the number of peptide annotations found as well as reduce the analyte delocalization. Trypsin incubation times tested were 2, 6, and 17 hours. In addition, NaCl, KCl, or K_2SO_4 were added into the humidity chambers to maintain a relative humidity of 75%, 82%, and 96% respectively at 37 °C. Further optimization steps include changing enzyme concentration and incubation temperature. Additional sample types will also be tested.

Mass spectrometry analyses were performed by the Mass Spectrometry Technology Access Center at the McDonnell Genome Institute (MTAC@MGI).