Application Note

# Heat stabilization prior to successful tissue sectioning and MALDI Imaging analysis

## Introduction

MALDI imaging is gaining increasing interest in pharmaceutical research, as it is a valuable tool when mapping molecules in situ on tissue sections. When working with tissue samples, one must be aware that post mortem molecular changes occur rapidly, causing variations to the molecular content. To maintain the sample, preservation as early on as possible after tissue harvesting is fundamental. The Stabilizor™ system uses rapid heating to preserve tissue samples as close as possible to their in vivo state. Heat stabilization permanently prevents post-mortem changes from the moment of sampling and thereby increases the accuracy and quality of analytical results.
Heat stabilization prior to MSI analysis is therefore a useful method to measure the distribution and abundance of molecules in organs. All results presented in this application note are extracted from the work by Dr. R. Goodwin and can be found in Journal of Proteomics, 2012.

## Results

In the featured study, rat brain was embedded in CMC and cryosections were cut and attached to conductive carbon tape on glass slides.This methodology allows for preservation of the tissue morphology during sectioning, and does not affect ionization or quantitation of the sample (figure 1). Mapping of PEP-19 in snap-frozen, as well as in heat-stabilized brain tissue, confirm that peptidomic MSI analysis is not altered after tape mounting, (figure 2).


Fig 1. Morphology is well preserved in heat stabilized tissue samples during sectioning

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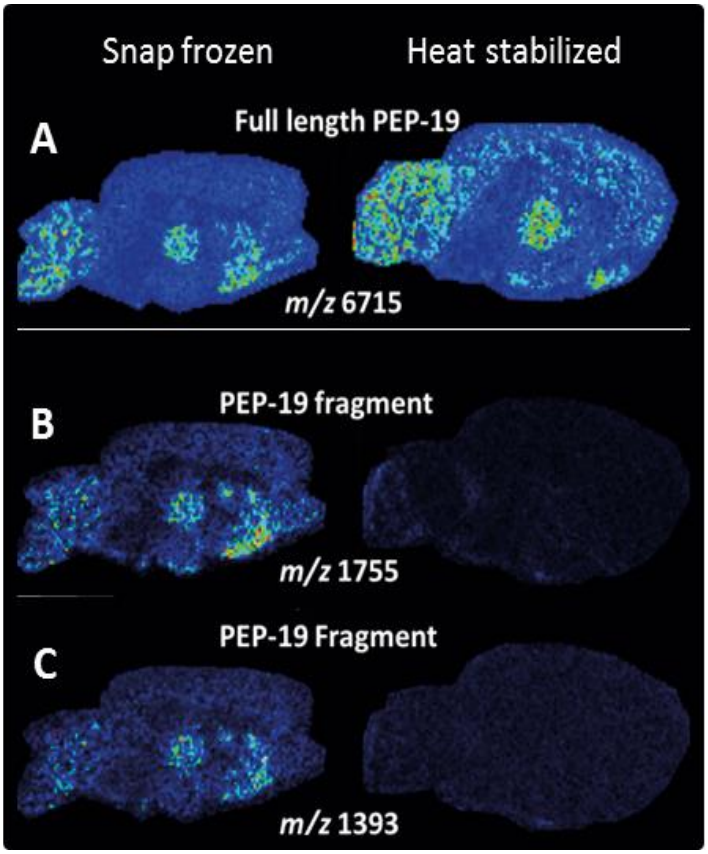


Figure 2. MALDI Imaging of full length PEP-19 and metabolites in snap-frozen vs. heat stabilized brain tissue samples

The neuropeptide PEP-19 (m/z 6715) is detected at higher levels in the heat-stabilized brain than in snap-frozen brain (figure 2A) indicating preservation. Further proof of prevention of port-mortem degradation is the two well-known fragments of PEP-19 (m/z 1755 and m/z 1393) that are only found in snap-frozen tissue (figure 2B and C). This suggests extensive degradation of the peptide during sample preparation and emphasizes the need for heat stabilization of the tissue in order to avoid such changes. The ability to effectively analyze heat-stabilized tissue with MALDI Imaging will benefit both pharmaceutical and proteomic MSI researchers.

## Methods

Rat brains were removed, bisected and one half snap frozen in liquid nitrogen while the other hemisphere was heat stabilized prior to freezing. Both hemispheres were embedded in CMC.
Double-sided adhesive carbon tape (NEM) was placed onto the sample surface and sagittal sections (20µm) were cut at -20°C using a computerized cryostat macrotome. The sections on tape were placed on the MALDI targets. Samples for proteomic analysis were washed with ethanol and dried with oxygen-free nitrogen.
CHCA or DHB were used as matrix and MALDI-TOF in positive ion linear mode and reflectron modes was performed. Typically 300 laser shots per raster point with ~50 µm spot diameter were used. Data was analysed using FlexImaging 2.0-3.0. For further details on methodology, please view the original publication

## References

Sample preparation for mass spectrometry imaging: Small mistakes can lead to big consequences, Goodwin et al., Journal of Proteomics, 2012

Stopping the clock on proteomic degradation by heat-treatment at the point of tissue excision, Goodwin et al., Proteomics, 2010

Conductive carbon tape used for support and mounting of both whole animal and fragile heat-treated tissue sections for MALDI MS imaging and quantification, Goodwin et al., Journal of Proteomics, 2012