HIGH-EFFICIENCY ION MOBILITY COUPLED WITH DIRECT MALDI MS IMAGING FOR VISUALIZATION AND IN-SITU IDENTIFICATION OF PEPTIDES

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Emmanuelle Claude[1]; Jonathan Stauber[2]; Marten F. Snel[1]; Ron M.A. Heeren[2]; Therese McKenna[1]; James Langridge[1]
[1]Waters Corporation, Manchester, UNITED KINGDOM; [2]FOM-Institute for Atomic and Molecular Physics, Amsterdam, NETHERLANDS

INTRODUCTION

Imaging Mass spectrometric imaging (MSI) is an emerging tool in proteomics, lipidomics and metabolomics. Biomolecules (i.e. proteins, lipids) and drugs are analysed directly from a tissue section, providing spatial information.

Proof of concept has been published[1, 2] whereby diagnostic biomarkers have been localized on either formalin fixed paraffin embedded (FFPE) and frozen tissues using IMS and changes occurring in disease have been visualised at the molecular level . Further development of this technique is the identification of the diagnostic biomarkers in situ using on-tissue tryptic digestion of proteins followed by MS/MS analysis of the tryptic peptides.

One limitation of MALDI imaging MS of tissue sections is the complexity of the data collected due to the presence of different classes of molecules, i.e. lipids and peptides which can hinder the localization and/or identification. In this case a further dimension of separation is required which is orthogonal to the mass spectrometer. The incorporation of a high efficiency ion mobility separation (IMS) based on travelling wave (T-wave) technology coupled with time-of-flight mass spectrometry offers such a separation and is a means of increasing the separating power of a MALDI imaging experiment.

TriWave consists of three T-wave devices (see Figure 1). The first T-wave (Trap) is used to trap ions during the period when an IMS separation is being performed in the second T-Wave, thus greatly enhancing the efficiency of the IMS process. The final T-wave (Transfer) transports the separated ions to the TOF analyser. In addition fragmentation experiments can be performed in either or both the Trap and Transfer T-Wave regions *e.g.* providing Transfer fragmentation of two isobaric species that have been separated by their ion mobilities.

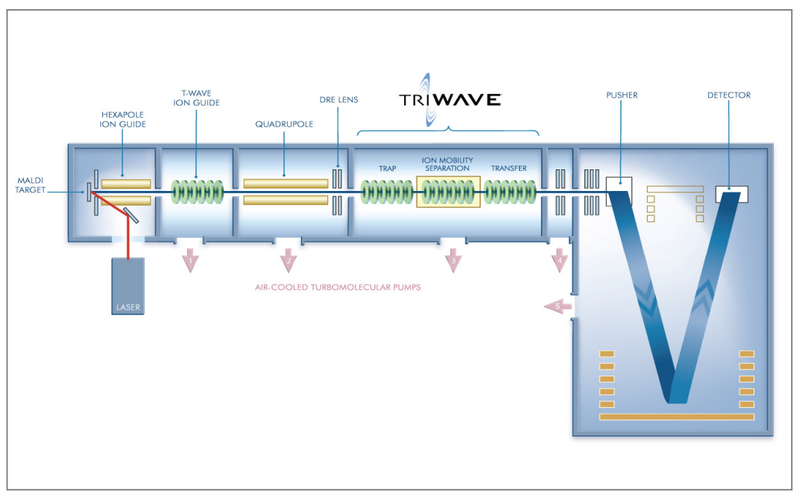

Figure 1. Schematic of the MALDI Synapt HDMS system, showing the TriWave Ion Mobility Separation device.

METHODS

Rat brain (formalin-fixed-paraffin embedded, FFPE tissue) and human cerebellum (frozen tissue) are sectioned in 10 µm thick sections with a cryo-microtome at –20°C.
Paraffin is removed from FFPE tissue by two baths of 5 minutes in toluene and lightly rehydrated with graded ethanol (100°, 96°, 70° and 30° baths before drying at room temperature.
All tissues are digested with trypsin (0.05 µg/µL in water) using a micro-spotting system (CHIP, Shimadzu) at 34°C during approximately 1 hour of matrix deposition. The whole tissue section was micro-spotted in a 150 µm spacing raster scheme.
The alpha-cyano-4-hydroxycinnamic acid matrix (10 mg/mL in MeCN/TFA 0.1%, 6:40, v/v) is deposited by a vibrational spraying system (ImagePrep, Bruker Daltonics) to cover the brain tissue.
All data were acquired on a MALDI hybrid orthogonal acceleration time-of-flight mass spectrometer, MALDI Synapt (Waters Corporation, Manchester, UK) operated in HDMS mode over the m/z range of 600 to 3000.
The area to be imaged was selected using MALDI Imaging Pattern Creator (Waters Corporation, Manchester, UK). After acquisition, HDMS data evaluation was perfomed using DriftScope (Waters Corporation, Manchester, UK). Regions of drift time vs m/z were exported into MassLynx. Data were converted into Analyze file format and subsequently visualised using BioMap (Novartis, CH).
ProteinLynx Global SERVER 2.3 (PLGS 2.3) (Waters, Manchester, UK) was used for database searching, using MASCOT algorithm (MatrixScience, London, UK).

RESULTS

Ion mobility separates class compounds directly on frozen tissue.

Lipids belong to a class of compounds which are very abundant on tissue because they are in every cell membranes acting as structural component. Certain families of lipids ionize very well using the MALDI ionization technique. Analyzing directly from tissue by MALDI imaging can be challenging for species with m/z around 600-900. Figure 2 shows the DriftScope display of MS data acquired directly from tissue after on tissue tryptic digestion where peptides, lipids and matrix species are present. A large number of peaks are observed in the mass range of 600-1000 m/z, with mass difference of 1 or 2 Da, resulting in an elevated baseline level and peaks without any isotopic profile. This large number of peptides can be challenging when MS/MS experiments are carried out using MALDI instruments with ion-gate type precursor selections.

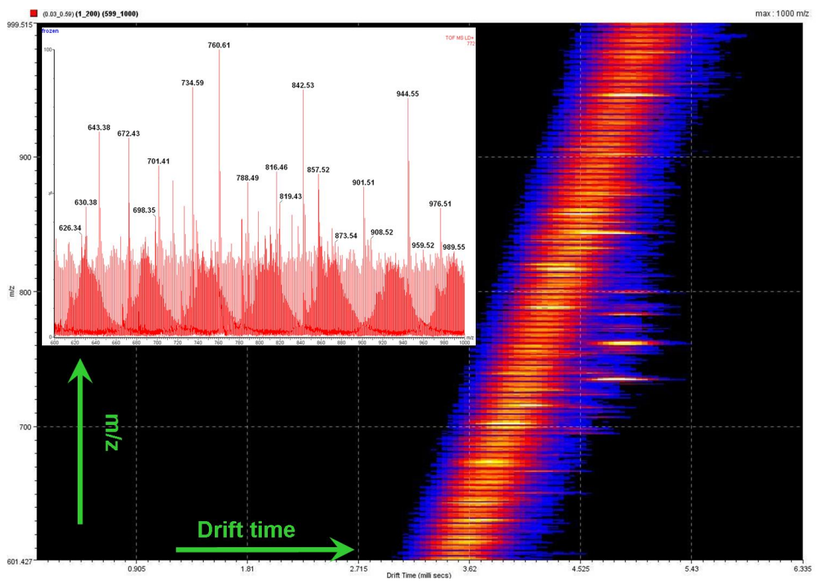

Figure 2. DriftScope display of data acquired directly from frozen tissue. Insert shows the MS spectrum equivalent.

The ion mobility cell allows the separation of isobaric compounds which have different ion conformation in the gas phase, resulting in different cross-sections.
Using DriftScope software, it is possible to separate the different class of compounds and extract the MS spectra of the lipids only and peptides+matrix (as seen in Figure 3 A and B respectively).

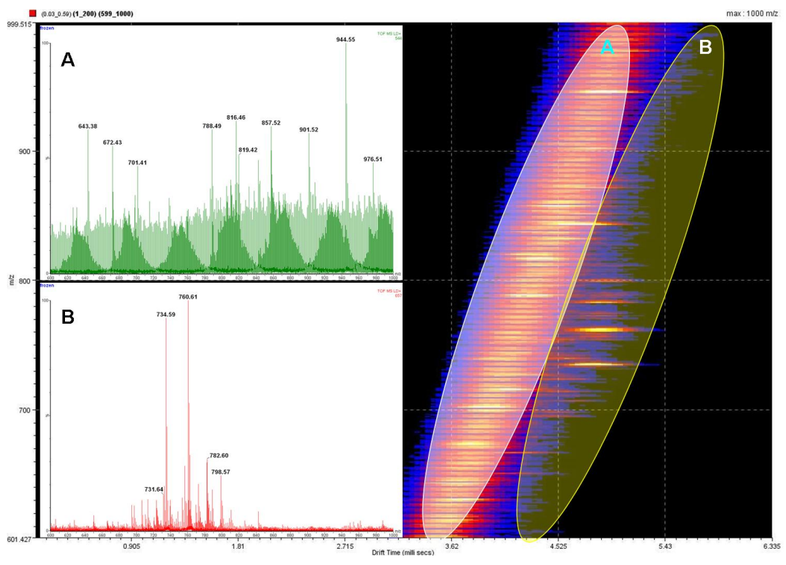

Figure 3. DriftScope display where MS spectrum A is the selection of peptides+matrix region and MS spectrum B is the selection of the lipid region.

Figure 4 shows an example of MS/MS experiment carried out directly from tissue for the precursor mass 944.5 Da where there are two isobaric species.
The precursors are not fragmented in the TRAP collision cell. They are separated in the drift cell, followed by fragmentation in the TRANSFER collision cell, before mass analysis in the oa-TOF. As a consequence, the drift time separation is conserved after dissociation. The precursor and its fragments have the same drift time.
Species A has a higher mobility than species B.
Species B produces fragments at m/z 184 and 86 which are characteristic fragments of a lipid phosphatidylcholine.

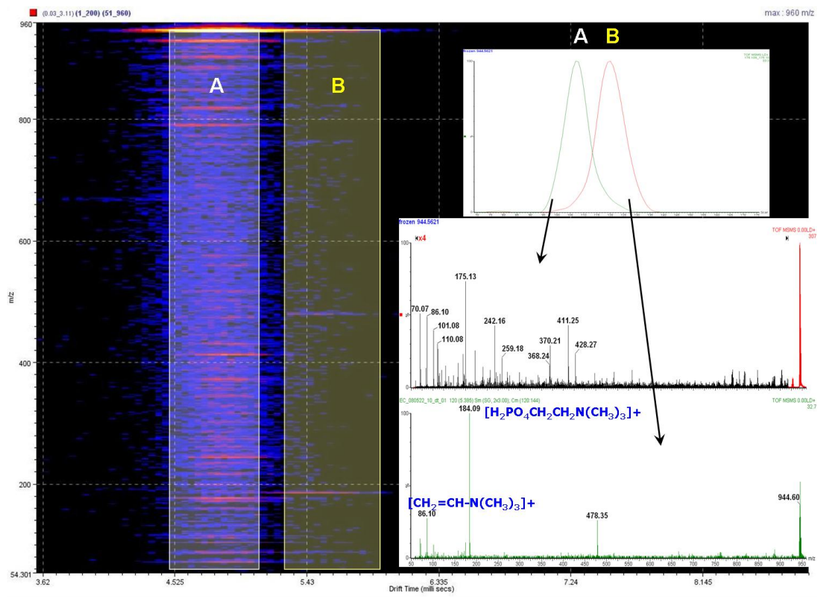

Figure 4. DriftScope display where TRANSFER MS/MS spectrum is a peptide and TRANSFER MS/MS spectrum B is PC(47:0).

The fragment ion at m/z 175 observed in spectrum A indicates that it is a peptide, with an arginine C-Terminal. A database search was carried out using ProteinLynx Global Server with the MASCOT algorithm and an Histone H2A type 1 was identified with a confident MOWSE score of 40 (Individual ions scores > 25 indicate identity with p<0.05) (Figure 5).

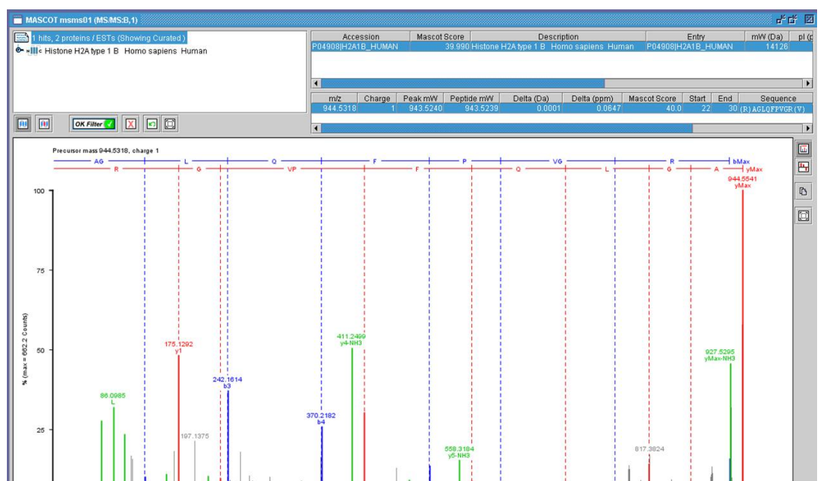

Figure 5. PLGS 2.3 display results for database search of 944.5. Histone H2A type 1 was identified.

FFPE tissue: Separation and identification of two isobaric peptides. .

In FFPE tissue, lipids are less abundant than in frozen tissue. Figure 6 shows the MS spectrum acquired during an imaging experiment. Ion reconstituted images are displayed for several peptides from 800-3000 Da, where the Ion-mobility dimension is not taken into account.

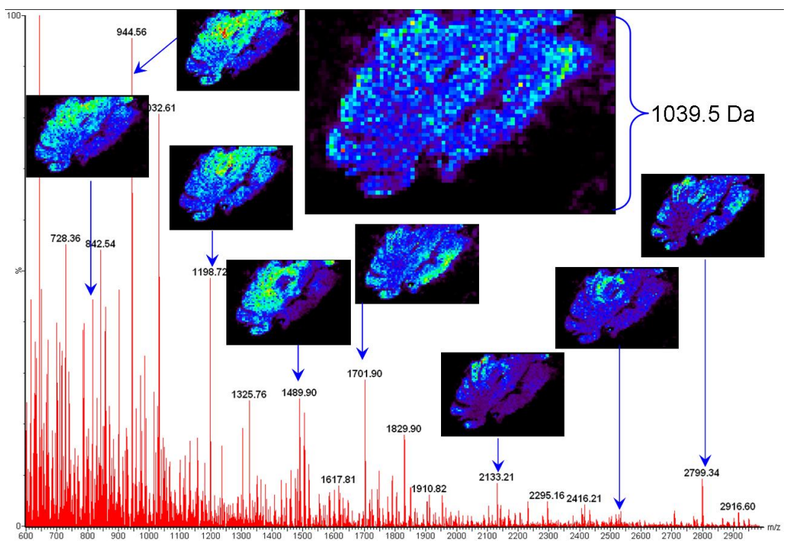

Figure 6. MS spectrum combining all pixels from an imaging experiment on FFPE tissue. Displaying ion reconstituted of peptides

MS/MS analysis of several peptides was performed. The precursor ion at m/z 1039.5 Da was identified as a peptide from Tubulin with a MOWSE score of 15 i.e. a score below the 95% confidence score of 20.
Figure 7 shows that at m/z 1039.5 Da, two species are present and can be separated in the drift cell. By performing a Transfer MS/MS experiment the peptide with the high mobility conformation was identified as Tubulin with a much higher confidence score of 59 as displayed in Figure 8.
The reconstituted ion image with the IMS dimension is similar to the one without the IMS dimension. However the peptide with the low mobility conformation was identified as Ubiquitin with a confident score of 27. The reconstituted ion-image for this ion shows localization of the peptide, on the outskirts of the brain.

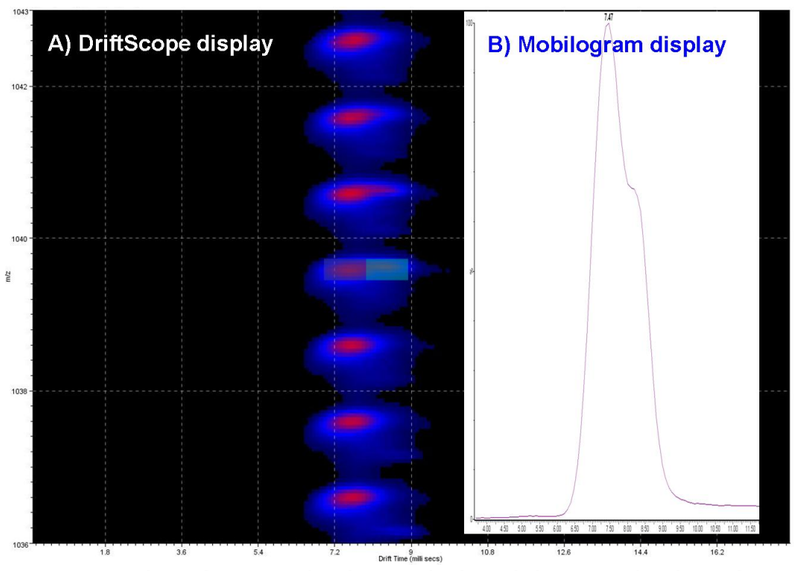

Figure 7. A) DriftScope display and B) Mobilogram display of 1039.5 Da where two species are present with different drift time.

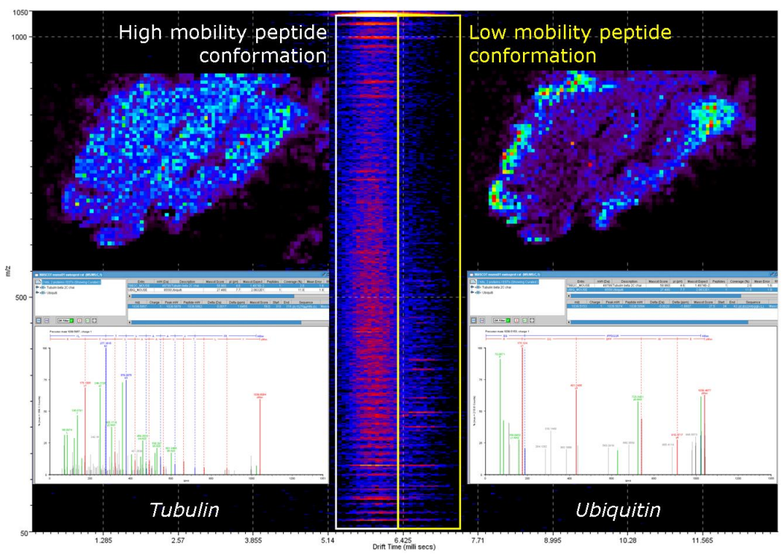

Figure 8. Transfer MS/MS DriftScope display of 1039.5 Da.

CONCLUSION

- **The combination of high efficiency ion mobility separation with MALDI provides a unique separation dimension to further enhance mass spectrometric imaging.**
- **Using MALDI orthogonal acceleration TOF mass spectrometry on a MALDI Synapt HDMS system, nominally isobaric species are separated and their identification is enhanced using TRANSFER MS/MS experiment directly from tissue.**
- **IMS can be used to produce images without the interference of ions of similar mass. This can remove ambiguity from imaging experiments and lead to more precise localisation of the compound of interest.**

References

[1]:Reyzer ML, Caprioli RM. J Proteome Res 4 (4): 1138-42, 2005.
[2]: Liam A. McDonnell, Ron M.A. Heeren. Mass Spectrometry Reviews 26 (2007) 606-643

50 YEAR LEGACY
VISION ■ CONTRIBUTION ■ ADVANCEMENT

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS