SEPARATION AND DETECTION OF PROTEIN POST-TRANSLATIONAL MODIFICATIONS BY LIQUID CHROMATOGRAPHY COUPLED WITH A NOVEL ION MOBILITY MASS SPECTROMETER

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OVERVIEW

- **A new class of mass spectrometer, Synapt™ High Definition Mass Spectrometry (HDMS™) system (Waters Corp.) has been developed which combines high efficiency Ion Mobility Separations (IMS) and oa-Time-of-Flight mass spectrometry . This combination allows the separation of biomolecules in the gas phase by size, shape, and charge prior to mass spectrometric detection.**
- **This poster presents the design and theory behind the Synapt HDMS system, and shows the different modes of analysis available for the analysis of post translational modifications**
- **The instrument described here is equipped with both MALDI and atmospheric pressure ionisation sources.**
- **Separation of differentially phosphorylated peptides, based upon ion mobility, has been demonstrated with fragmentation in both the Trap and Transfer T-Wave devices providing additional flexibility**

INTRODUCTION

Post-translational modification (PTM) of proteins plays a fundamental role in cellular processes and their determination is one of the main goals of modern proteomics research. Among more than 200 known PTM's, phosphorylation, glycosylation and acetylation are the best characterized. However, the variety, diversity and heterogeneity of these modifications requires novel analytical tools for qualitative and quantitative assessment of their structural and functional roles.

We have investigated the potential of a novel travelling wave ion mobility spectrometer for the separation, detection and mass determination of post-translationally modified proteins.

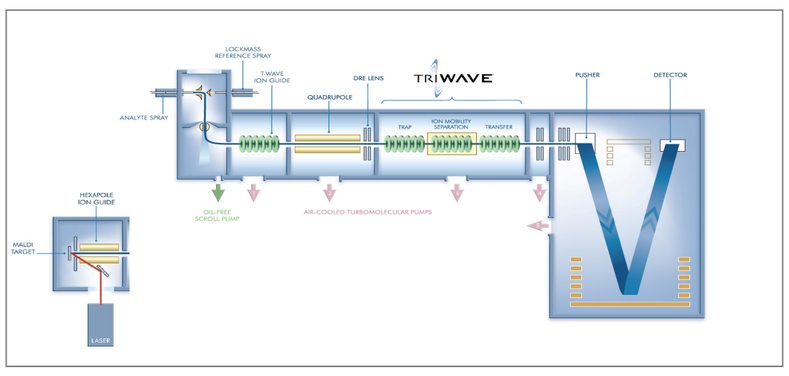


Figure 1. Schematic representation of the Synapt HDMS system used for ion mobility separation (IMS) experiments with ESI source and inset MALDI source

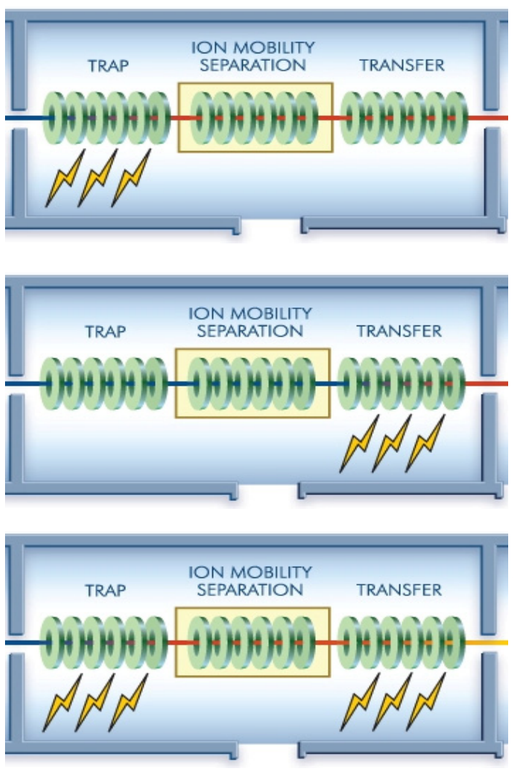
METHODS

HDMS analysis mode

The Waters Synapt HDMS system schematic representation is shown in Figure 1. Ions produced by Electrospray ionization or MALDI using a 200Hz Nd:YAG laser pass through a quadrupole. The quadrupole may be set to transmit a particular m/z or pass a substantial mass range. Ions then enter a novel three stage Triwave device [1]. The Triwave device comprises three traveling wave (T-Wave) ion guides (see Figure 2). The first the Trap T-Wave, accumulates ions and releases them in a short pulse (100µs) every 20 ms into the next device the IMS T-Wave in which the mobility separation is performed. The final device , the Transfer T-Wave is used to transport the separated ions into the oa-ToF for subsequent analysis. The pressure in the Trap and Transfer T-Wave regions was ~ 10^{-2} mbar of Ar and the pressure in the IMS T-Wave was 0.5 mbar of N_2. The T-Wave pulse velocity and voltage were optimised to provide adequate ion mobility separation.

Fragmentation modes

Three different types of ion mobility fragmentation experiments can be performed using the Tri-Wave device. These are illustrated in Figure 2.



Trap fragmentation
Fragment ions are ion mobility separated.

Transfer fragmentation
Precursor ions are ion mobility separated. Fragment ions align with precursor ion drift time.
e.g. useful for isobaric precursor ions

Time aligned parallel (TAP) fragmentation
Ion mobility separation of first generation fragments followed by second fragmentation.
e.g. greatly enhanced structural information.

Figure 2. Options for performing fragmentation experiments using the Triwave device.

Samples

Peptides and phosphopeptides originating from proteolytic digestion of the protein mixture were purified using TiO2 columns as previously described [2]. For Electrospray, samples were solubilised and then 25mM EDTA, 50mM Amm Phosphate added prior to separation using Waters nanoACQUITY UPLC in trapping mode described previously [3].Phosphopeptide standards (Waters Milford) were used for infusion experiments.

MALDI IMS experiments were performed on released glycans from IgG. The released glycans were initially analysed using IMS MS then further analysis was performed on the individual glycans using TAP fragmentation. The glycans were released from the glycoprotein through treatment with PNGase F, the released glycans were then immobilized on a HILIC µElution Plate (Waters, Milford, MA), washed and finally eluted. Glycans were spotted at an approximate level of 1pmol on target. The matrix used was DHB.

RESULTS

The 2 phosphopeptides ions at m/z 706.3 corresponding to the di-phosphorylated peptide EQLSTSEENSK and the ion at m/z 666.3 corresponding to the mono-phosphopeptide species colelute at 11.5 minutes. However they have different drift time profiles when ion mobility is employed as an orthogonal separation technique shown in Figure 3 below.

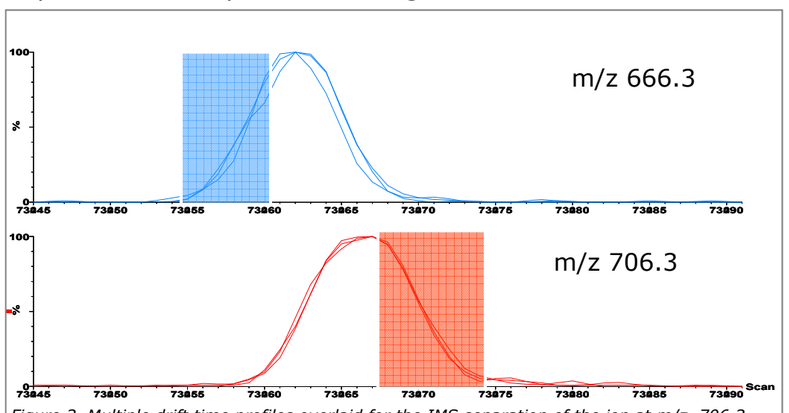


Figure 3. Multiple drift time profiles overlaid for the IMS separation of the ion at m/z 706.3 corresponding to the di-phosphorylated peptide EQLSTSEENSK and the ion at m/z 666.3 corresponding to the mono-phosphopeptide species.

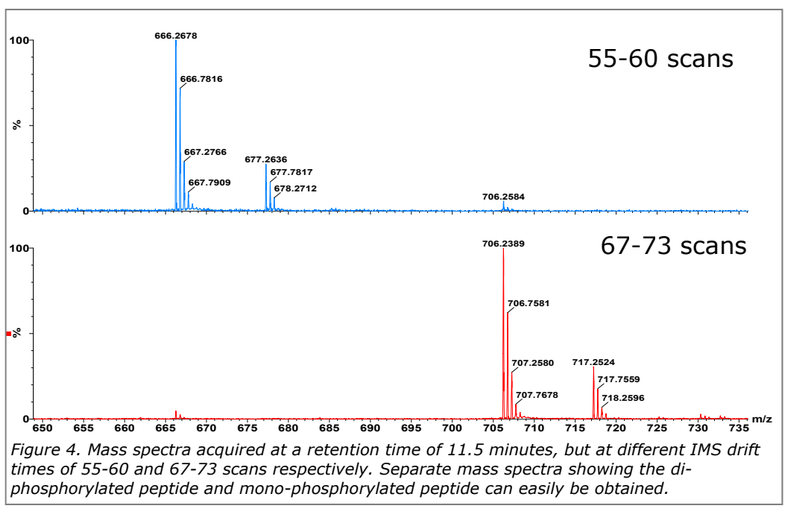


Figure 4. Mass spectra acquired at a retention time of 11.5 minutes, but at different IMS drift times of 55-60 and 67-73 scans respectively. Separate mass spectra showing the di-phosphorylated peptide and mono-phosphorylated peptide can easily be obtained.

Trap T-wave fragmentation

The di-phosphopeptide VNQIGTLSESIK ion at m/z 724.8 (2+) was isolated with the quadrupole, with limited fragmentation induced in the Trap T-wave prior to HDMS analysis. The sequential neutral loss of H3PO4 can be clearly observed, separated from the intact peptide both by m/z and drift time.

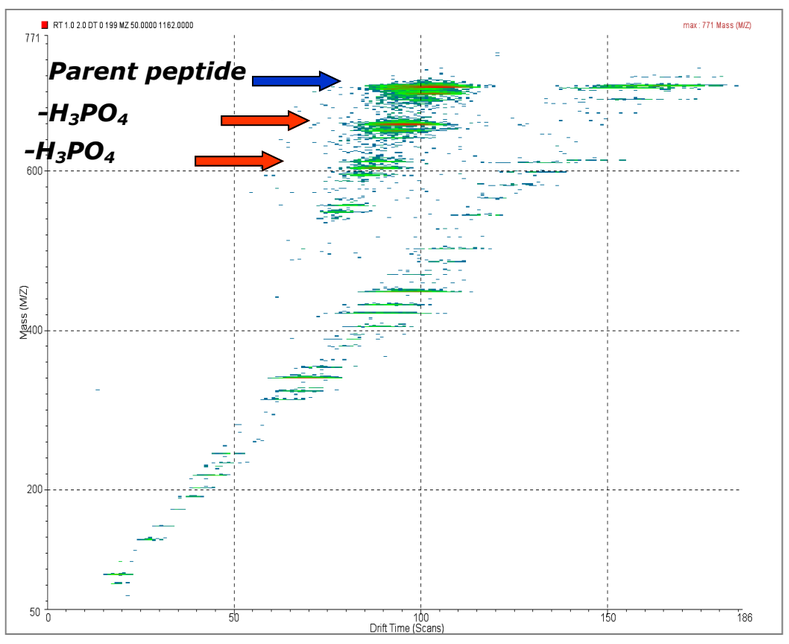


Figure 5. Drift time vs m/z plot for the MS-IMS-MS analysis of the phosphopeptide VNQIGTLSESIK; m/z 724.8 (2+).

TAP fragmentation

In Figure 6 the ion at 724.8 (2+) was isolated and fragmentation induced in both the Trap and Transfer T-Waves i.e. TAP fragmentation. Here spectra can be acquired for the parent phosphopeptide and neutral loss species in parallel.

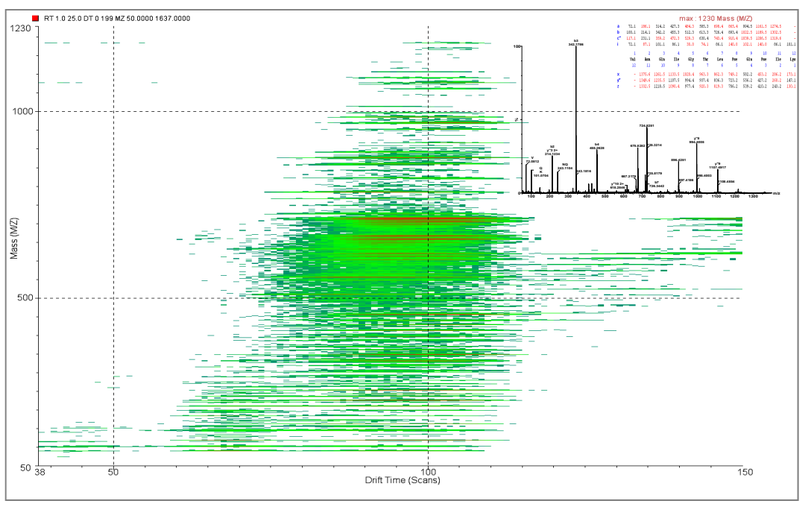


Figure 6. Driftscope plot from the MS-IMS-MS analysis of the phosphopeptide m/z 724 (2+) with inset TAP fragmentation spectrum.

Glycan analysis using MALDI HDMS

Using the Driftscope software sodiated glycan ions from a MALDI HDMS experiment can be separated from background ions by filtering out a large part of the chemical background, based on its mobility difference from the ions of interest. This is particularly useful for low intensity ions. The Driftscope plot showing mobility separation is shown in Figure 7 with the spectrum obtained with ToF MS and HDMS mode in Figure 8. TAP fragmentation was performed on each of these glycans simultaneously. The results from the ion with m/z 1485.5 are shown here Figure 9. TAP fragmentation produces more analytical information than fragmentation conducted in either the Trap T-Wave or Transfer T-wave alone as seen in Figure 10. Individual second generation fragment ion spectra can be extracted to obtain further structural information, as illustrated in Figure 11.

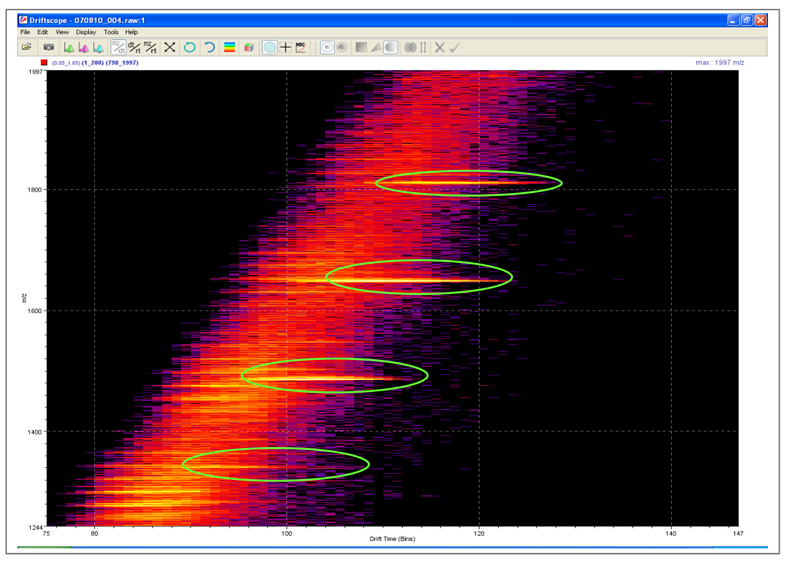


Figure 7. Driftscope plot of released glycans. Sodiated glycan ions are circled. The glycans

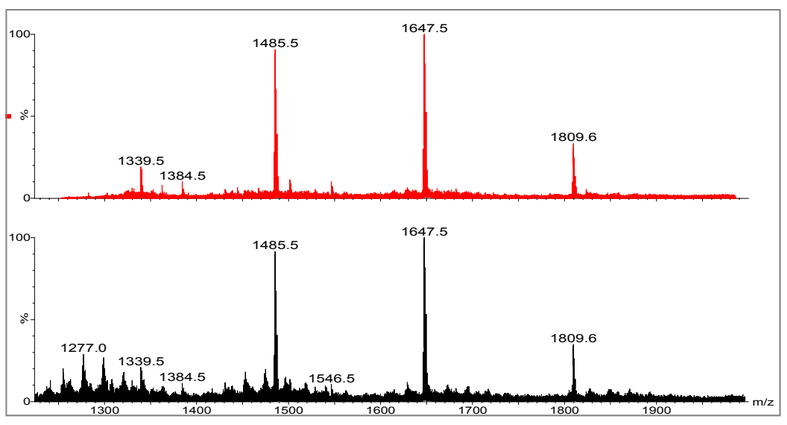


Figure 8. Top spectrum, background ions filtered out based on ion mobility, bottom spectrum conventional MALDI spectrum.

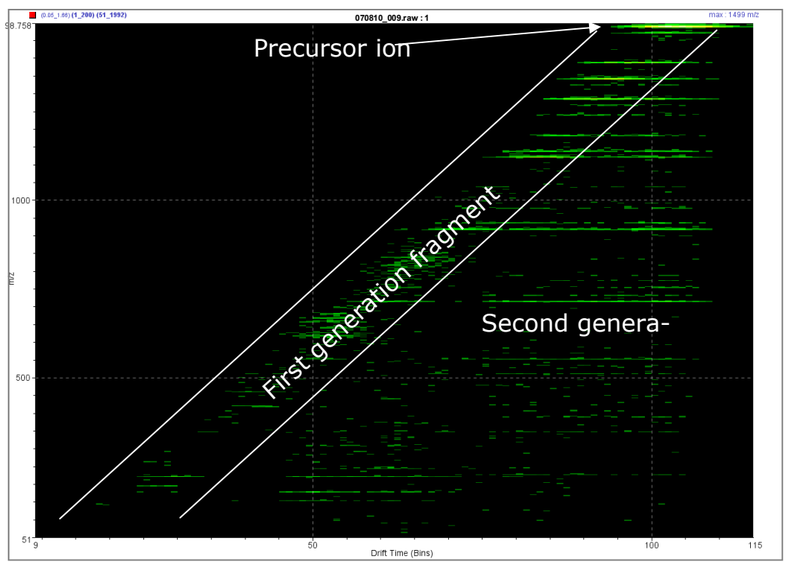


Figure 9. Driftscope ion mobility plot of TAP fragmented glycan with m/z 1485.5

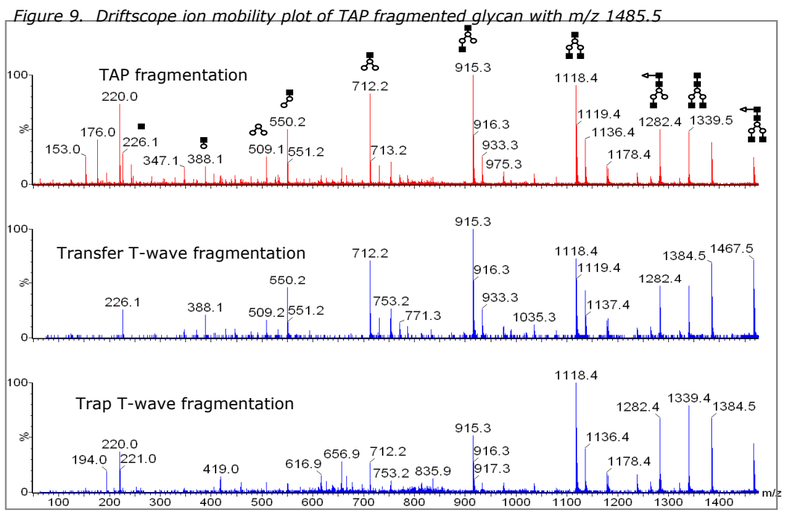


Figure 10. Comparison of TAP fragmentation with Trap and Transfer fragmentation.

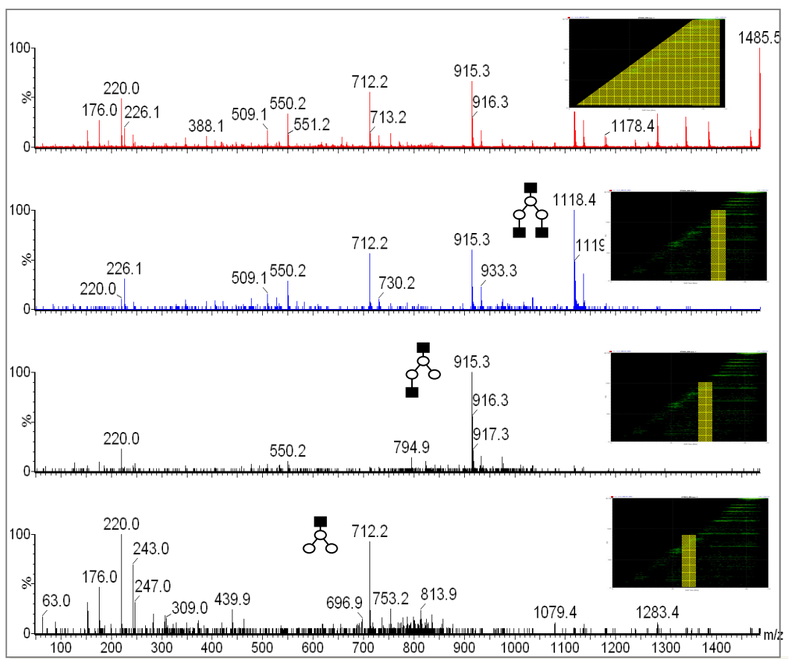


Figure 11 Selected second generation MS spectra showing different fragmentation patterns.

CONCLUSION

- **The use of ion mobility combined with oa-TOF mass spectrometry for the analysis of phophopeptides has been presented.**
- **Separation of mono and di-phosphorylated peptides using IMS has been shown.**
- **Sodiated glycan ions were successfully separated from background ions using their lower ion mobility as the differentiating factor.**
- **The use of IMS-MS-IMS allows fragmentation patterns from both the intact phosphopeptide and peptide neutral loss in parallel.**
- **TAP fragmentation was used to generate secondary fragment ion information not available using conventional MALDI MS/MS.**

Acknowledgements

Many thanks go to John Gebler and Ying Qing Yu, Waters Corporation, Milford MA, for providing the glycan samples and Martin Larsen for the phosphopeptides

References

1. K. Giles, S. Pringle, K. Worthington, D. Little, J. Wildgoose, R. Bateman, *Rapid Commun. Mass Spectrom.*, 2004; 18: 2401-2414
2. Larsen MR et al *Mol Cell Proteomics* 2005, 4(7), 873-86

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