Utilising Ion Mobility Spectrometry to Separate Precursors From Background Ions and Species with Different Charges in Automated Tandem MS Experiments

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OVERVIEW

In this work we demonstrate the utility of Ion Mobility Separation in the Automated Tandem MS analysis of peptides.

The separation of candidate precursors from background ions facilitates the use of lower thresholds, giving rise to a highly sensitive technique.

Searched and processed MSMS spectra with <100amol on column.

The IMS characteristics of pseudo cross linked peptides are determined and used to analyse these low level species.

INTRODUCTION

As a technique for characterising proteins, tryptic digestion followed by data dependent LC-MS/MS is well established. However, in complex biological mixtures with a wide dynamic range, the majority of the peptides observed are in the lowest order of magnitude that can be detected. Despite tandem MS experiments, singly charged chemical noise present in the survey spectrum can hinder precursor ion selection and identification.
In addition, cross-linked peptides containing >3 charges are often present at low stoichiometry compared with tryptic peptides, and as such it may be difficult for the mass spectrometer to identify these in the MS survey as candidate precursors.
In this poster, we will show how Ion Mobility Spectrometry is used to separate components by Drift Time and mass-to-charge ratio leading to an increase in the signal-to-noise ratio of low level species as they are separated from the chemical noise. This drift time separation allows the mass spectrometer to more clearly identify and select them as candidates for MSMS and increasing the sensitivity when compared with MS only acquisitions.

METHODS

Mass Spectrometer

A Synapt HDMS (Waters Corporation) was used in these studies, **figure 1**. Ions produced during electrospray ionisation were sampled by a Z-spray source and passed through a quadrupole, which was set to transmit a substantial mass range in the survey mode of operation or to isolate candidate precursors meeting specific criteria in the MSMS mode of operation. When enabled, Ion Mobility Separation was performed in three T-wave devices; Trap, IMS and Transfer. The system pressures during IMS operation were ~ 10^{-2} mbar of Ar in the trap and transfer T-Wave regions and 0.5 mbar of N_2 in the IMS T-Wave. The pressure during MS only operation was ~ 8 x 10^{-3} mbar of Ar in the trap and transfer T-Wave regions.

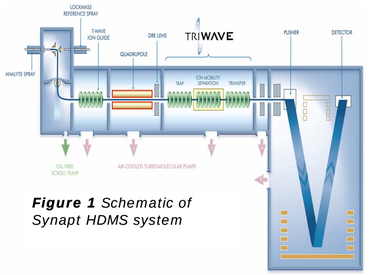

***Figure 1** Schematic of Synapt HDMS system*

In mobility enabled automatic tandem MS experiments, the candidate precursors were identified when they appeared in a region of m/z vs. drift time plot and reached a set intensity. These ions were fragmented on entrance to the Trap T-Wave and/or the Transfer T-Wave. In this mode, the instrument can be operated with a High Duty Cycle (HDC) where the pusher is synchronised to the release of ions from the Transfer T-Wave device with a delay dependent upon the mobility separation of the ions of interest. This ensures that the ion of interest is within the oa-extraction region as the oa-field is applied and leads to a signal increase over the entire mass range for the selected charge state.
In MS only DDA, ions were selected for MSMS based on charge state recognition and intensity. Fragmentation was performed in the Trap region.

Mass scale Calibration: Fragment ions from Glu Fibrinopeptide B (GFP) were used for sensitivity experiments and NaICsI was used in the BSA disulphide intact experiments.

Automated Tandem MS integration times
1 second Survey, 3 seconds MSMS (sensitivity), 10 seconds MSMS (BSA disulphide intact).

Nanoscale LC	Waters NanoAcquity UPLC
Trap Column	180µm ID x 20mm long, Symmetry C18
Analytical Col.	75µm ID x 200mm long, BEH 1.7µm
Solvent A	Aqueous 0.1% formic acid
Solvent B	Acetonitrile + 0.1% formic acid
Injection	Partial Loop mode
Trapping	100% solvent A at 15µL/min for 1 min
Gradient	1—40% B in 30 minutes at 300nL/min

Samples
1) Sensitivity / Dilution Series: Equimolar mixture of four protein tryptic digests; Bovine Serum Albumin, Yeast Enolase, Yeast Alcohol Dehydrogenase and Rabbit Phos B.
2) Cross Linked: Tryptic Digest of Bovine Serum Albumin, prepared without a reduction and alkylation stage to preserve the disulphide bonds.

Data Processing / Searching
ProteinLynx Global Server 2.3 / Mascot / Swissprot DB

RESULTS

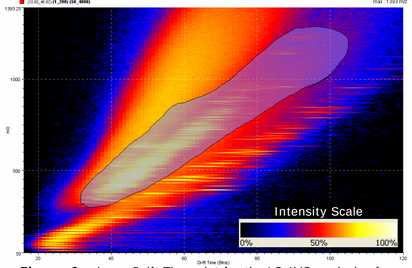

***Figure 2** m/z vs. Drift Time plot for the LC-IMS analysis of a protein tryptic digest. A 'Rule File' for use in automated Tandem MS experiments is generated by the selection of, in this case, the region encompassing species of charge states 2^+ and 3^+ and excluding the predominantly singly charged background. The rule file effectively sets the mass range for the Mobility DDA experiment and 'charge strips' the background detected.*

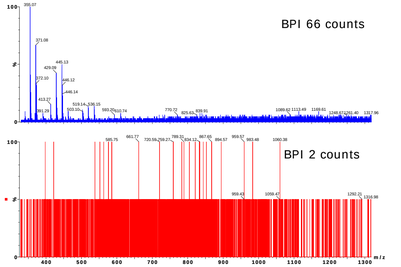

***Figure 3** Comparison of background spectra prior to species eluting from the column. The top spectrum is the MS only signal whereas the bottom shows the lower intensity background spectrum in mobility mode when a rule file, or charge stripping, is applied. These data show how lower thresholds can be set in mobility mode with the rule file applied. A threshold of 3 counts/sec was used in the Mobility DDA experiments.*

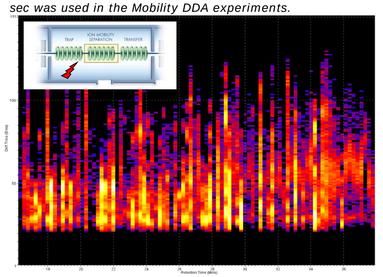

***Figure 4** Trap Fragmentation (inset), 1fmol each protein injected. The drift time vs. RT plot for MSMS shows the range of fragment ion drift times present in each MSMS spectrum.*

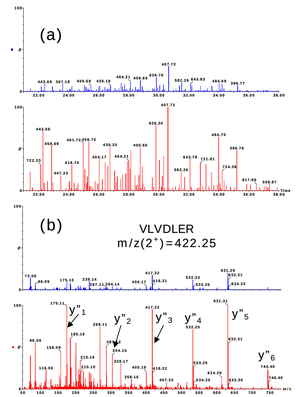

***Figure 5** MSMS Chromatograms (a) and an example of fragment ion spectra (b) showing a comparison of HDC on (bottom) vs. HDC off (unsynchronised). Data is normalised to the HDC 'ON' signal and the injected amount is 1fmol each. Each vertical line in the chromatograms represents an automatic switch into MSMS.
HDC is calibrated using the drift times from Trap T-Wave fragments of GFP.*

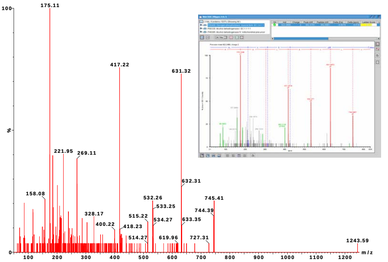

***Figure 6** Sensitivity Example: The MSMS spectrum of m/z 422.25 from the injection of 50amol of each protein using Trap fragmentation and HDC mode. The fragment spectrum shows good coverage of y" ions and the PLGS Search (inset) using the Swissprot database returned a positive hit from this spectrum.*

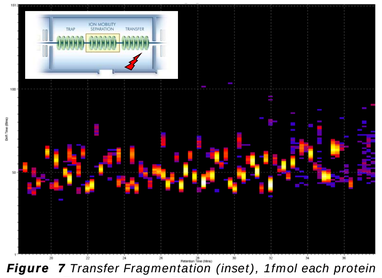

***Figure 7** Transfer Fragmentation (inset), 1fmol each protein injected. The drift time vs. RT plot for MSMS indicates that all fragments exhibit one drift time, which is the same drift time as the precursor ion.*

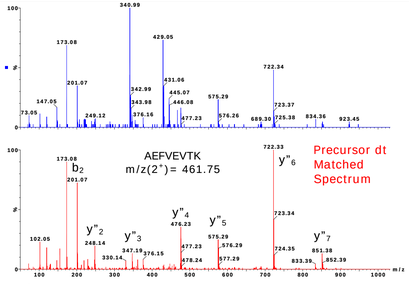

***Figure 8** PostIMS Precursor Drift Time Matching: Fragment ions produced by background species close in m/z to precursor ions but with different drift times are automatically removed from the spectrum. In this example, m/z 340, 429 and 445 in the top spectrum are fragments from a singly charged ion at m/z 462.1. This is within the resolving quadrupole window of the detected precursor at m/z 461.75 . These background fragment ions are removed in the precursor matched spectrum.*

Injected amount (amol)	PreIMS HDC on			PostIMS Precursor Matched			MS Only		
	Proteins	Peptides	Switches	Proteins	Peptides	Switches	Proteins	Peptides	Switches
50	2	5	21*	1	4	19*	0	0	311*
100	4	9	33*	3	10	35*	1	2	30#
250	4	25	48*	4	20	53*	3	6	48#
500	4	38	66*	4	33	77*	4	14	63#
1000	4	45	104*	4	45	85*	4	31	51*
2500	4	73	162*	4	51	143*	4	52	82*
5000	4	70	174*	4	57	162*	4	57	98*

***Figure 9** Summary of search results for the system operating in Mobility and MS only mode. All four proteins were identified at the 100amol level (and above) in HDC 'on' mobility mode, at 250amol in Precursor matched mobility mode and at the 500amol level (and above) in MS only mode. The high number of switches in MS only mode at 50amol is due to an initially low threshold (3 counts/sec, signified by * above) where the system switched on background ions. The threshold was subsequently increased in MS only to 6# and then 10* counts/sec.*

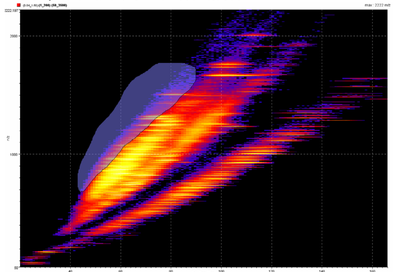

***Figure 10** Analysis of a disulphide bond intact BSA digest, infused at 1µL/min at a concentration of 1µM. Highlighted in the m/z vs. drift time plot is the region chosen for the rule file where species with >3 charges are present.*

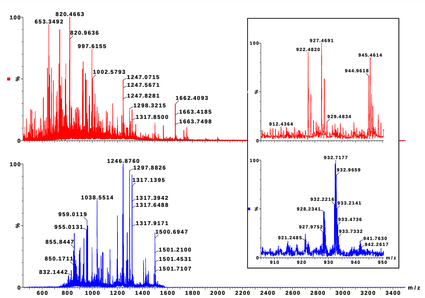

***Figure 11** MS Only (top) and charge stripped (mobility) spectrum. Inset is the expanded m/z region from 900 to 950 and the charge stripped spectrum here shows the emergence of a 4^+ species at m/z 932.7. This species was amongst those automatically selected for MSMS interrogation by the mass spectrometer.*

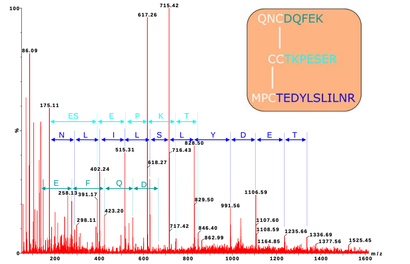

***Figure 12** PostIMS (Transfer CE = 52V) precursor drift time matched fragmentation spectrum for the 4^+ species at m/z 932.7. The spectrum shows coverage for the regions highlighted.*

CONCLUSION

- **By using IMS to separate potential precursors from background, we have shown the sensitivity limits for automated tandem MS experiments in the analysis of tryptic digest mixtures can be extended compared with MS only experiments.**
- **Future work will investigate the sensitivity limits of this new technique with more complex mixtures with a wider dynamic range.**
- **We have also shown how IMS can separate highly charged, low level species from more abundant species of lower charge states, thus enabling the detector to identify these species for automated MSMS analysis.**

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