TOP DOWN SEQUENCING USING AN ELECTROSPRAY CHIP COUPLED TO A TRAVELLING WAVE ION MOBILITY / TIME OF FLIGHT MASS SPECTROMETER

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OVERVIEW

- In this work we have used reversed phase liquid chromatography to separate intact protein mixtures and collected fractions for subsequent Top Down sequence analysis by MS/MS followed by IMS.
- Ion Mobility separation of the different fragment ions, predominantly by their charge, leads to the generation of highly specific tandem mass spectra.
- This approach provides significant sequence coverage increases compared with traditional tandem MS/MS data.

INTRODUCTION

Top down proteomics allows the identification and characterization of intact proteins from tandem mass spectrometry experiments, enabling the identification of Post Translational Modifications (PTMs). The Top Down approach provides direct measurement of the intact mass of the protein, as well as fragment ion information relating to the amino acid sequence. This is not possible in a bottom up proteomics strategy and as such crucial information may be lost, for example, if a tryptic peptide on which a PTM resides does not ionise well. However, even with a small to medium sized protein the large number of ions and the range of charge states produced during the fragmentation of a single charge state leads to a spectrum that can be extremely complex and challenging to interpret.

In this poster, we have separated protein mixtures by LC and collected fractions for infusion by ESI MS. For each fraction, we have fragmented different intact protein charge states and used ion mobility separation prior to Time of Flight mass analysis to enable the mobility separation of the different charged state fragments. By post processing of data, we have produced simplified top down fragment spectra containing ions of mainly one charge state. These ion mobility separations and region selections reveal low abundance species that are masked by larger species in a spectrum where no mobility separation is used.

METHODS

Mass Spectrometer

A Synapt HDMS (Waters Corporation) was used in these studies, figure 1. Ions produced during electrospray ionisation are sampled by a Z-spray source and first pass through a quadrupole, which may be set to transmit a substantial mass range or to isolate specific ions. Mobility Separation is then performed in three T-wave devices [1]; Trap, IMS and Transfer. The pressure in the accumulation 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. Ions may be fragmented on entrance to the Trap T-Wave and/or the Transfer T-Wave.

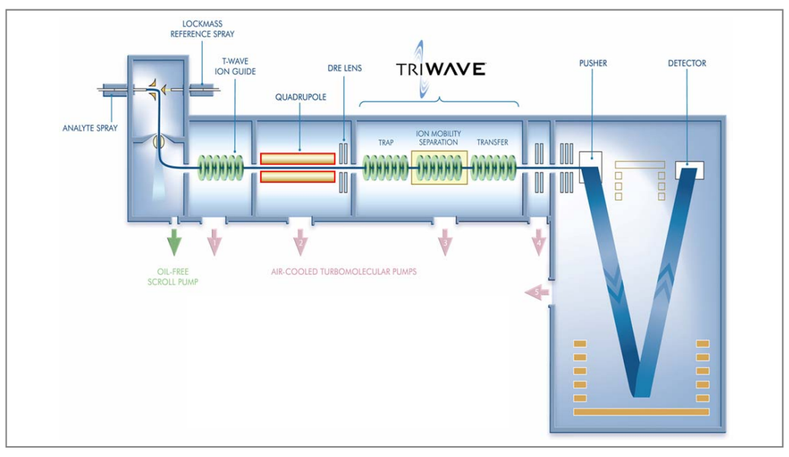

Figure 1. Schematic diagram of the Waters Synapt HDMS instrument. In Top Down sequence experiments, fragment ions are produced in the Trap region before the Ion Mobility Device, where they are separated based on charge state, size and shape. The ions are subsequently passed to the oa-ToF analyser for mass analysis. Typical collision energies used in this study were 25-55V.

Mass Scale Calibration was performed from 50 to 3520amu using Caesium Iodide solution.

LC, Fraction Collection and Infusion

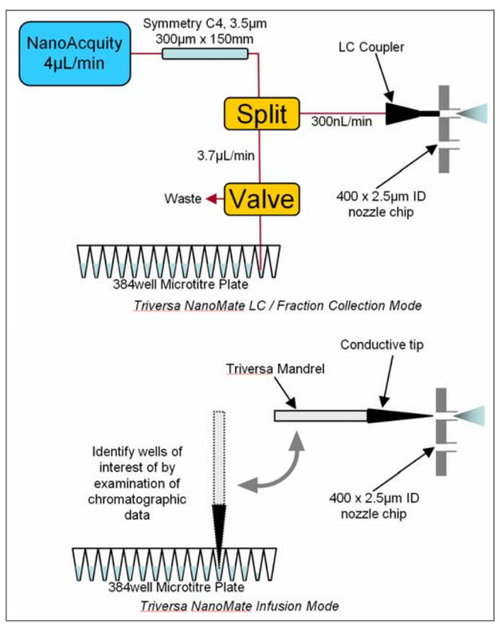

Figure 2. Intact protein mixtures were separated using reversed phase liquid chromatography. Column eluent was split inside the Triversa Nanomate (Advion BioSciences) between the LC coupler and fraction collection into a 384 well plate. 5µL acetonitrile was added to each protein containing well and a sample volume of 1µL was aspirated. The infused flow rate was 20–60nL/min.

Samples

Mixture of proteins containing 3pmol/µL each of Ribonuclease, Cytochrome C, Ubiquitin and Beta Lactoglobulin.
4 –5µg Human Histones in 0.04M H_2SO_4

Data Processing

Ion Mobility separated data were viewed in m/z vs drift time plots, from which regions were selected and exported to produce new datafiles containing simplified spectra of mainly one charge state. For z>1, these spectra were deconvoluted using Maximum Entropy algorithms. Fragment ion stretches were then identified from the amino acid sequence of the protein. The identified sequence information was compared to data that would have been obtained with no mobility separation.

RESULTS

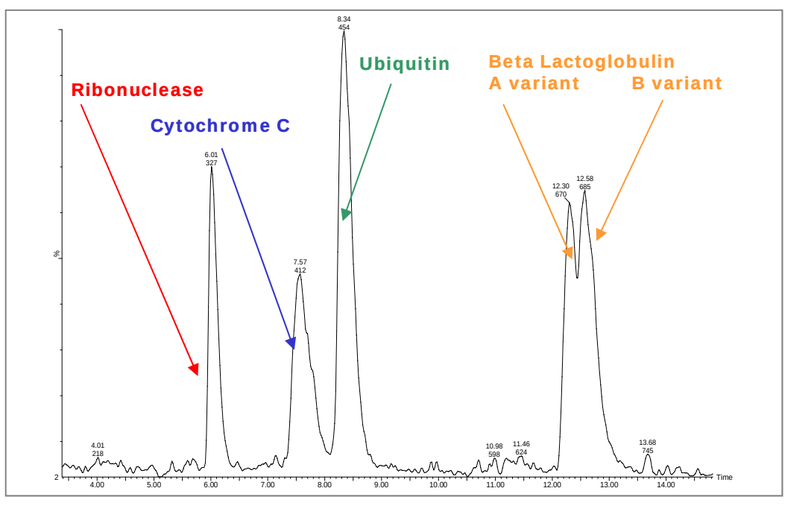

Figure 3. Base Peak Ion Chromatogram from the separation of the standard intact protein mixture, 1µL injected. Gradient used was 20% to 40% 0.1% formic acid in acetonitrile (solvent B) in ten minutes, then ramped to 95% at twelve minutes. Solvent A was aqueous 0.1% formic acid. Fractions collected were 1-minute wide.

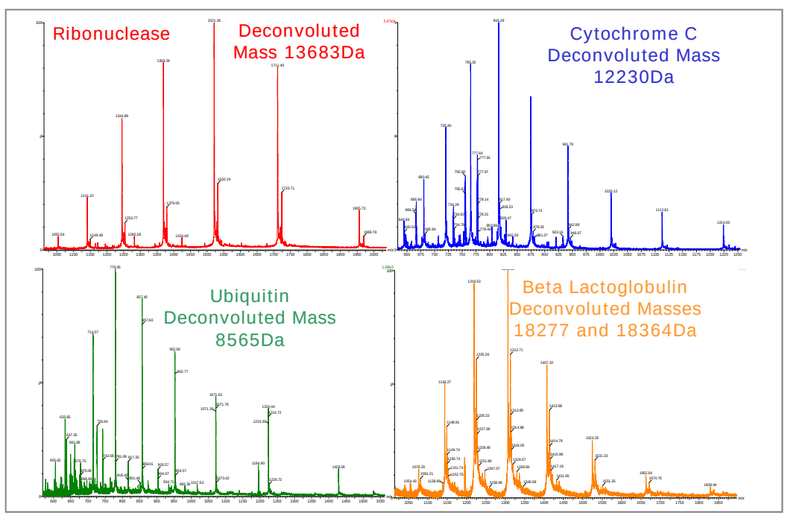

Figure 4. ToF MS spectra of intact protein species. Acquisition parameters were 50 to 3500amu in 2secs.

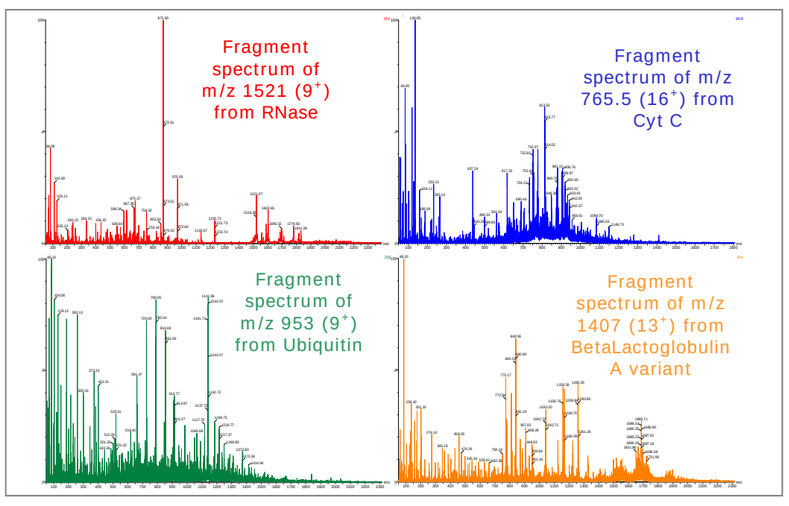

Figure 5. Tof MSMS data (non-mobility) produced by the quadrupole isolation and fragmentation of ions identified in Tof MS data. The fragment spectra contain species of many different charge states and can be challenging to interpret. Three precursor ions per protein were fragmented in the experiments. Acquisition parameters were 50 to 3500amu in 2secs.

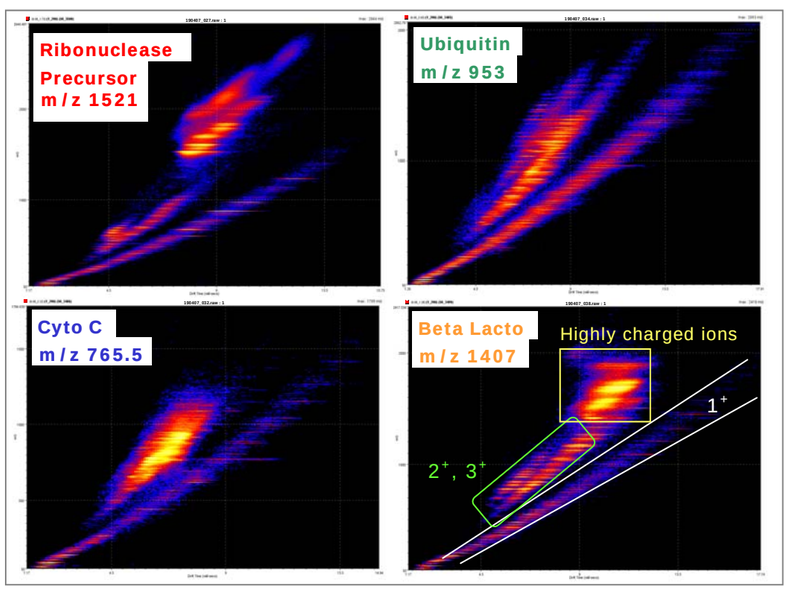

Figure 6. m/z vs. drift time plots for the trap fragmentation of one of the precursor ions per protein. Each of the m/z vs drift time plots show distinct regions and bands. The charge state bands are indicated in the Beta Lactoglobulin plot at the bottom.

These regions and bands are typical in every case of trap fragmentation data. The bands can be exported from the mobility viewing software to create a new raw file and the different bands are found to correspond to different charge states or classes of ions. Selection and exporting the entire plot leads to data equivalent to that acquired without mobility.

Comparing the spectra produced by specific selection with non mobility data reveals information that is not observed or hidden by more intense species in the non mobility acquisition.

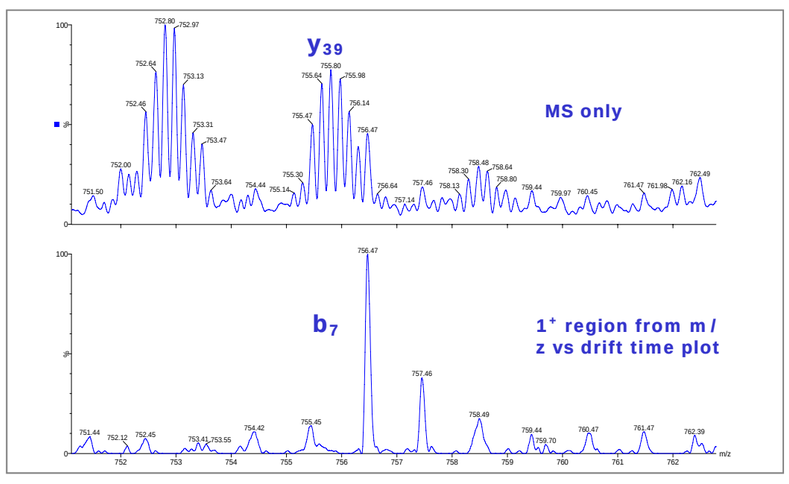

Figure 7. Selection of the 1^+ region from the fragmentation of the m/z 765.5 from Cytochrome C reveals the b7 ion which is masked by the more intense $y39^{6+}$ ion in the non-mobility data.

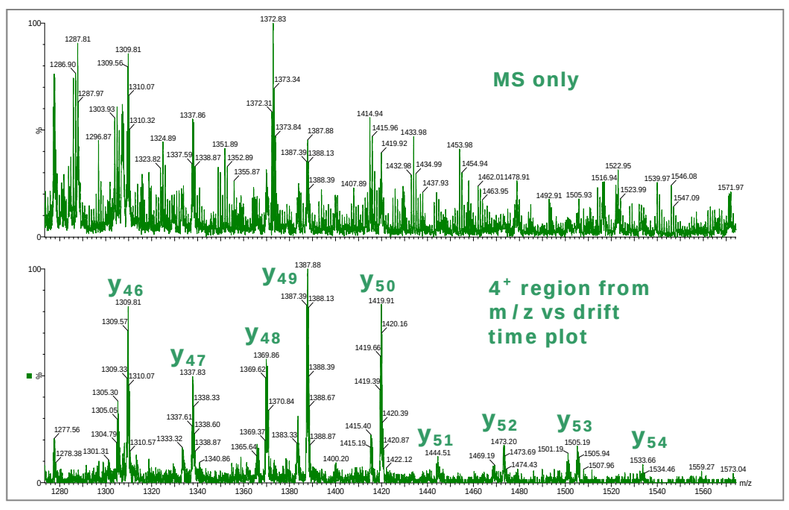

Figure 9. Selection of the 4^+ region from the fragmentation of the m/z 953 from Ubiquitin leads to an increase in signal to noise compared to the non-mobility data and hence clearer assignment of fragment ions.

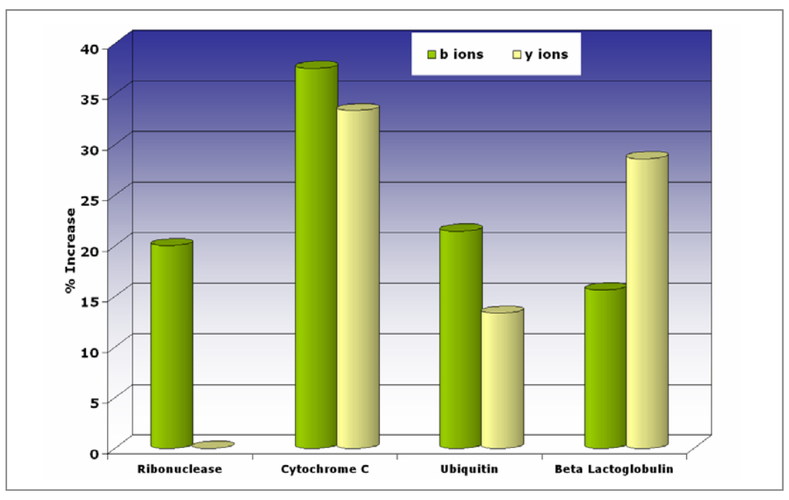

Figure 10. The percentage increase in sequence information (b and y ions) when comparing mobility separated data with non-mobility data. Data from all three precursors per protein are combined. Increases in the number of sequence ions are observed in the majority of the proteins.

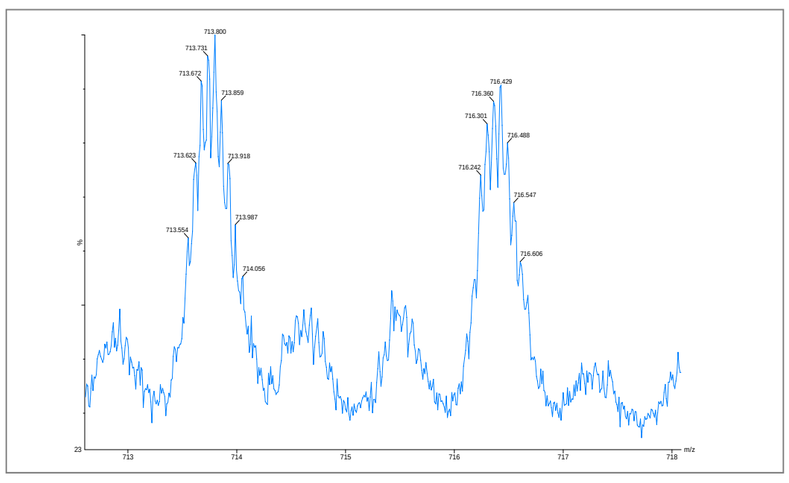

Figure 11. Expanded view of the 16^+ region of intact Histone H4. A reversed phase gradient of 1 –40% organic over 30minutes was used to separate the human histones. Two - minute wide fractions were collected and infused.

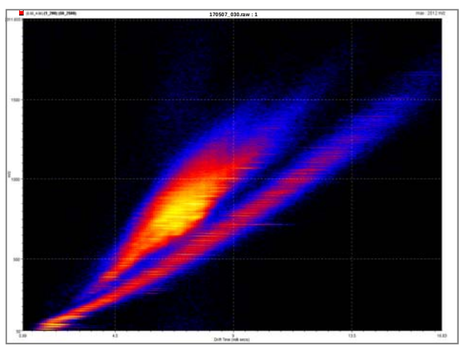

Figure 12. m/z vs. drift time plot for the Trap fragmentation of m/z 713.8 (16+, H4) after the LC separation and subsequent infusion of human histones.

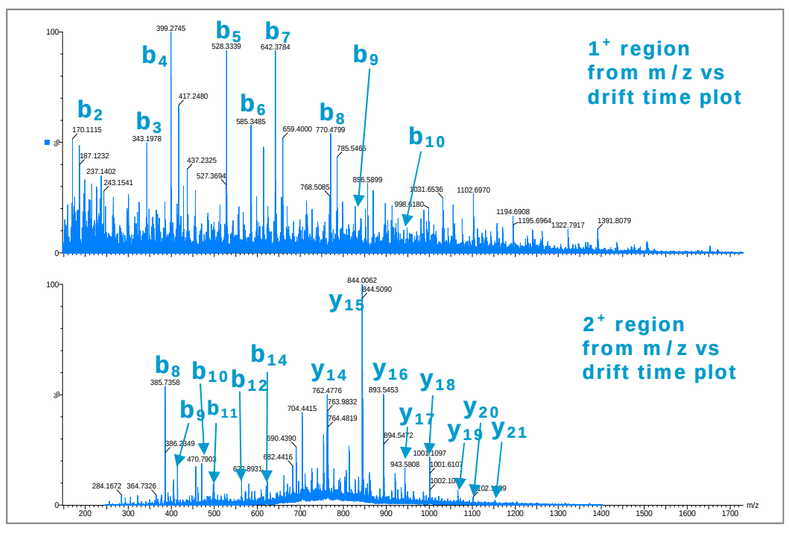

Figure 13. Sequence information for Histone H4 obtained from the selection of the 1+ (top) and 2+ bottom regions of the m/z vs. drift time plot in figure 12. The fragment ions b_9 and b_{10} are greatly enhanced in the 1+ spectrum compared with MS only and the fragment ions y_{20} and y_{21} are greatly enhanced in the 2+ spectrum compared with MS only. The particular charge state chosen for fragmentation is from N-terminal (serine) acetylated Histone H4.

CONCLUSION

- **Reversed phase LC separation of protein mixtures and fraction collection allows more time to be spent for fragmentation of numerous charge states for intact protein species.**
- **The ion mobility separation of fragment ions produced in Top Down Proteomics can generate simplified spectra and allow identification of low intensity species that are hidden in analysis without IMS.**
- **For the small to medium sized proteins analysed, sequence coverage increases are observed when comparing IMS and non-IMS data.**
- **Future work will involve further characterisation of the different Histones.**

References

1. "Travelling Wave Ion Propulsion in Collision Cells" K. Giles, S.Pringle, K. Worthington and R. Bateman— Presented at the 51st ASMS Conference, Montreal, Canada 2003. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

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