

ION MOBILITY-MASS SPECTROMETRY AS A TOOL FOR STRUCTURAL INVESTIGATION OF HIGH M/Z SPECIES

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OVERVIEW

- Investigating ion mobility separation (IMS) capabilities for high m/z species
- Evaluation on a hybrid quadrupole/IMS/oa-ToF instrument (Synapt HDMS)
- Ion mobility separation of CsI clusters, intact and activated GroEL (800kDa) and human haemoglobin (64kDa)

INTRODUCTION

Over the past 10 years interest in high mass non-covalent protein analysis has increased due to the ability of the current mass spectrometers and electrospray sources to preserve the non-covalent protein/protein subunit interactions, allowing one to analyse proteins in their native conformation and stoichiometry. The transfer of non-covalently associated protein-protein complexes from solution to the gas phase using electrospray ionisation generally results in the formation of ions possessing relatively few charges. As a result the m/z values can be quite large, making mass analysis using time-of-flight instrumentation amongst the most efficient.

The utility of ion mobility separation (IMS) in probing the structures of relatively large complexes has been highlighted previously [1]. Here we report further evaluation of a hybrid ion mobility / time-of-flight instrument for analysis of high m/z species, including the 800kDa GroEL complex, under non-activation and collisional activation conditions.

METHODS

Instrumentation

The instrument used in these studies was a Synapt HDMS System (Waters Corporation), shown in **Figure 1**, which has a hybrid quadrupole/IMS/oa-ToF geometry [2]. Briefly, samples were introduced by a borosilicate glass nano electrospray-spray tip and sampled into the vacuum system. The ions pass through a quadrupole mass filter to the IMS section of the instrument. This section comprises three travelling wave (T-Wave) ion guides. The trap T-Wave accumulates ions whilst the previous mobility separation is occurring, then these ions are released in a packet into the IMS T-Wave in which the mobility separation is performed. The transfer T-Wave is used to deliver the mobility separated ions into the oa-ToF analyser. Each ion mobility run was 51ms long and the ions were released from the trap in 500µs wide packets. The gas pressure in the trap and transfer T-Wave regions was 0.07 mbar (Argon) and the pressure in the IMS T-Wave was 0.5 mbar (Nitrogen). The travelling wave used in the IMS T-Wave for mobility separation was operated at a velocity of 250m/sec. The wave amplitude was ramped from 0 to 30V over the period of the mobility separation for optimum performance over the large m/z range used (m/z 1000 to 32,000).

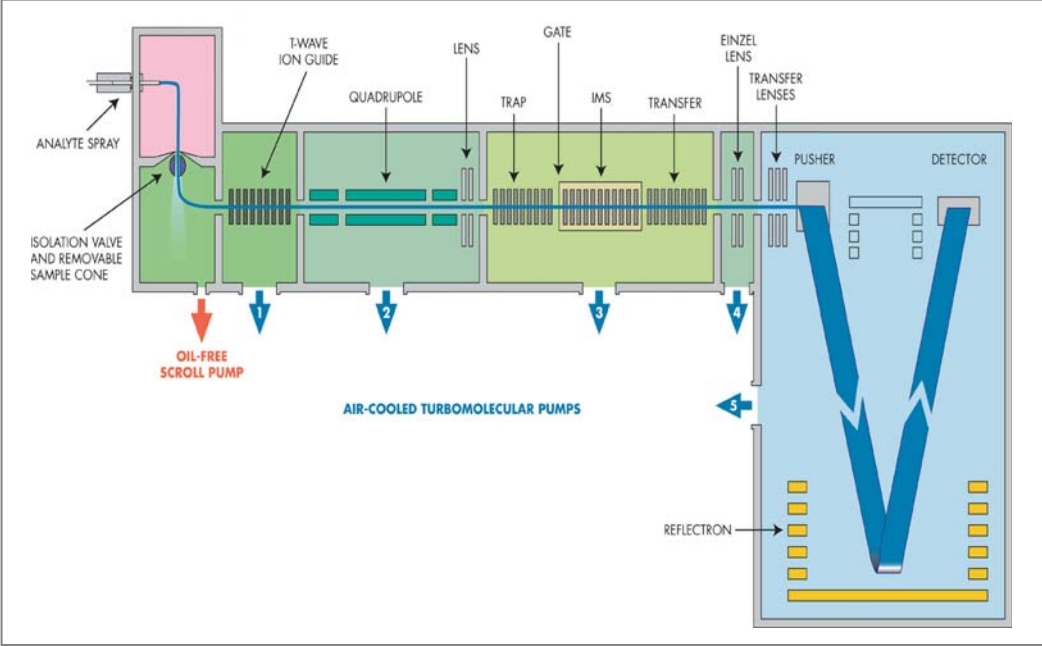


Figure 1. Diagram of the Synapt HDMS System instrument.

Samples

The GroEL and human haemoglobin were buffer exchanged into an aqueous solution of 100mM ammonium acetate, to a final working protein concentration of 1.5µM. Cesium iodide was used at a concentration of 50µg/µL in 50% acetonitrile.

Experiment

The instrument was calibrated over the m/z range 1000-32,000 using a solution of caesium iodide. Intact protein ToF-MS were carried out to determine the accurate mass of the intact GroEL tetradecamer and the human haemoglobin complex. In the same acquisition ion mobility spectra were acquired in order to determine the drift times of the species observed. Fragment ion mass and mobility spectra were obtained for individual charge state species using the quadrupole in resolving mode and high injection energies into the gas-filled trap T-Wave.

RESULTS

Caesium Iodide Clusters

Upon MS acquisition of a concentrated caesium iodide solution, intense ion clusters can be observed up to m/z 20,000, with each cluster's composition based on the formula Cs_(n+1)I_n. From the mass spectrum generated it is evident that a number of overlapping series, differing in charge state and intensity profile, are present over the m/z scale (**Figure 2**). However, when analysed in IMS mode, the different charge states and distributions become very clear as can be seen in **Figure 3**.

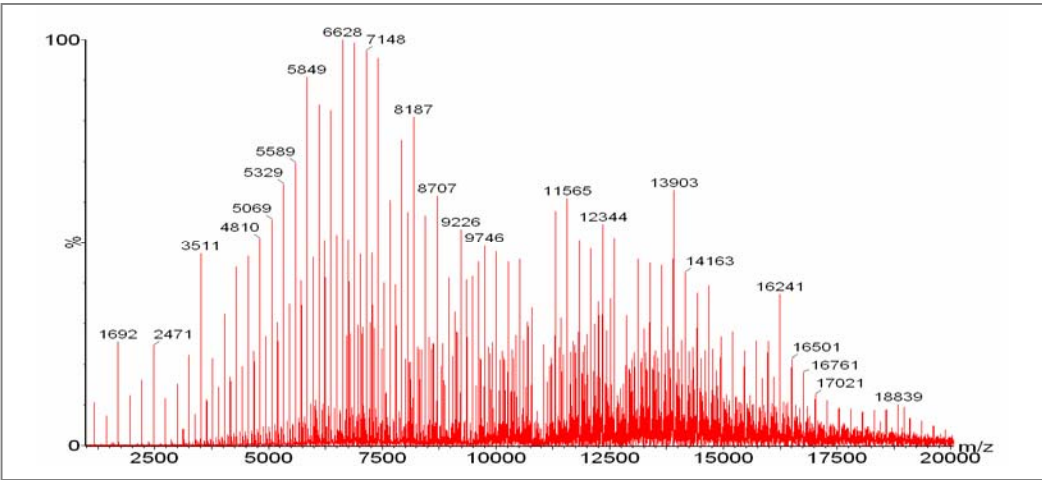


Figure 2. Mass spectrum of CsI (m/z 1000-20,000)

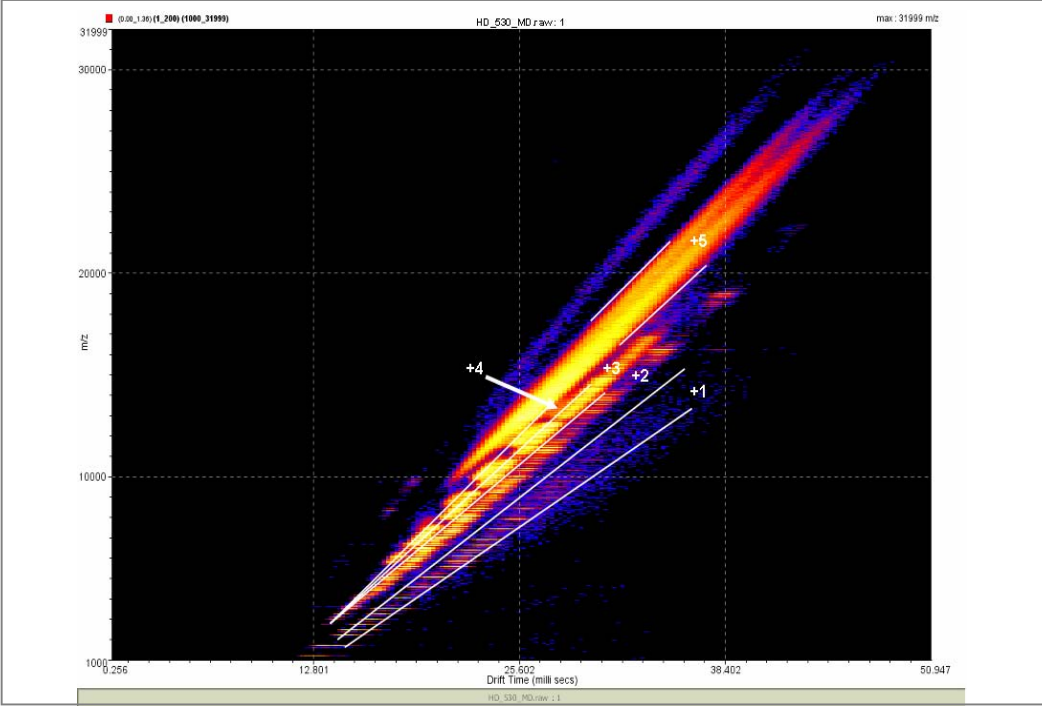


Figure 3. Drift-time vs m/z plot of CsI (m/z 1000-32,000)

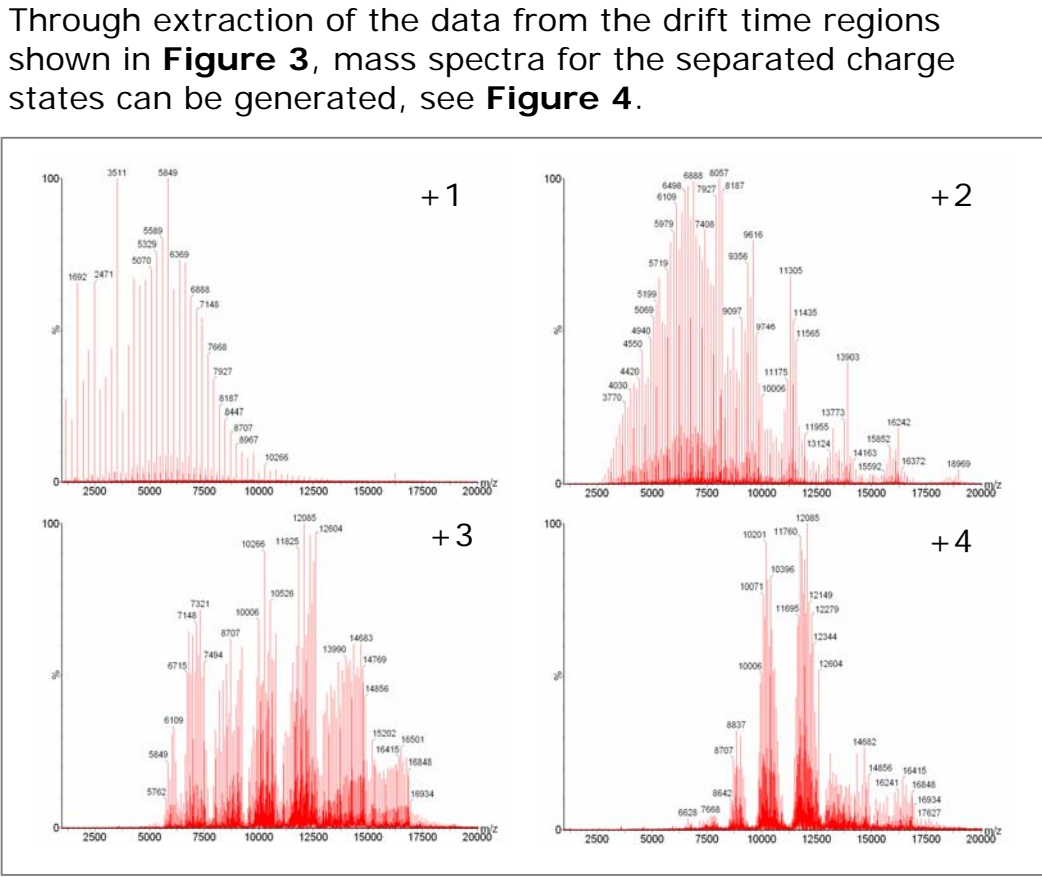


Figure 4. Charge state distributions of CsI.

The drift times of the cluster ions seem to increase monotonically with increasing m/z value for the different charge state species, although with increasing charge state series up to +4, distinct m/z stability regions become clear.

GroEL

The mass spectrum obtained for the intact GroEL tetradecamer protein is shown in **Figure 5** where an m/z distribution around 12,000 can be seen and represents charge states around +68. Activation and subsequent fragmentation of the GroEL complex (**Figure 5**) occurs in the Trap T-Wave, with injection voltages of up to 150V to induce disruption of the large macromolecular assemblies. Operating the Trap T-Wave at elevated pressures allows for efficient fragmentation of the GroEL complex, but also it provides efficient collisional cooling and focussing of the intact 14mer and subsequent 13mer generated from the activation process.

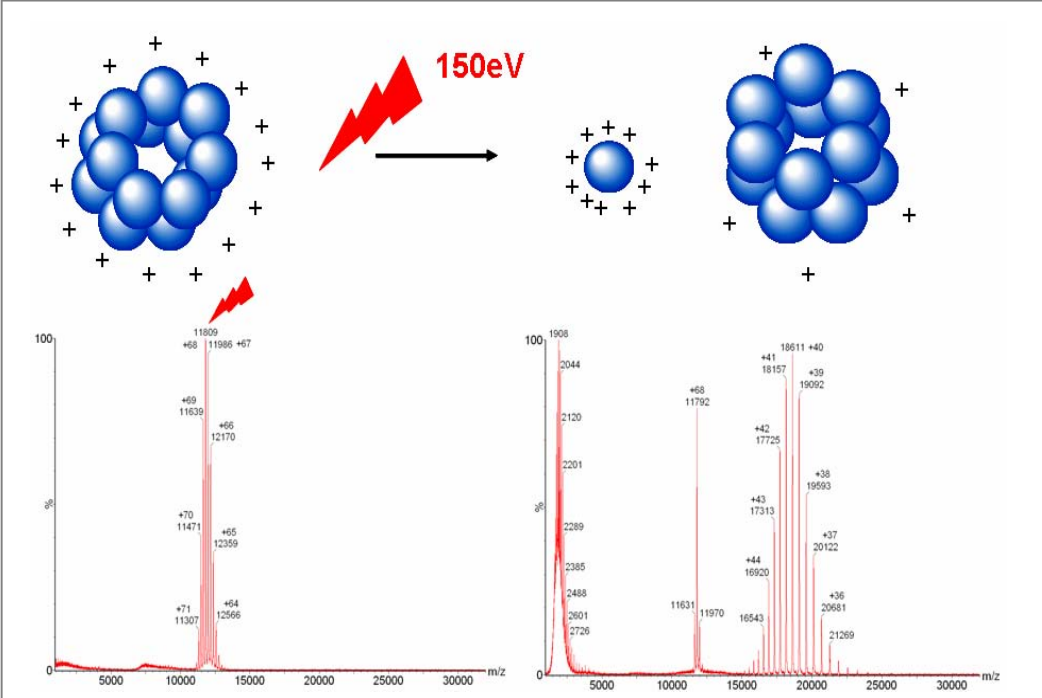


Figure 5. Schematic representation of the activation of the GroEL complex and the MS and MS/MS spectra.

The fragmentation of large non-covalently assembled complexes follow an asymmetric fragmentation pattern (**Figure 5**). For example, in the case of GroEL (800kDa), when a precursor ion m/z 11,809 (+68) from the intact complex is selected in the quadrupole and activated with 150V, a highly charged single monomer (57kDa) is ejected, m/z 1000-3000. As a result the remaining intact non-covalently assembled 13mer (744kDa) possesses relatively few charges and appears very high in the m/z scale, between m/z 15,000 and 25,000. The mobility separation achieved of both the intact and activated GroEL are shown below in **Figure 6**.

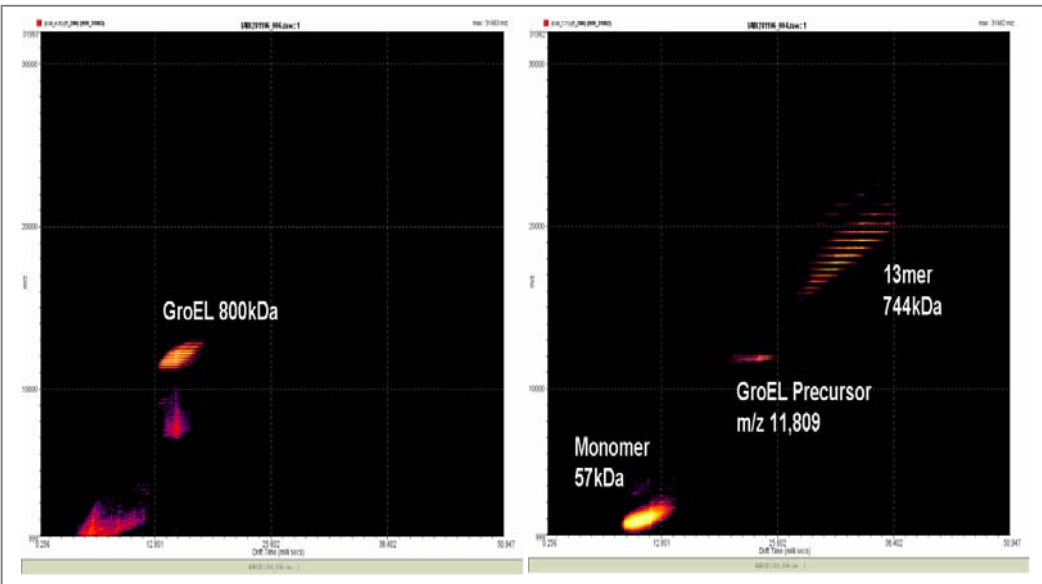


Figure 6. The drift-time vs m/z of the intact GroEL tetradecamer (left). Drift-time vs m/z at 150V trap activation of the +68 charge state of GroEL (right).

The multiply charged series of peaks for the intact GroEL range in m/z from 11,000 to 12,000 with drift times ranging from 13msec for the higher charge states to 19msec for the lower charge states. Upon activation of the intact GroEL and subsequent ion mobility separation, there are clear differences in drift time of the monomer, activated precursor ion and 13mer (744kDa) complex, which carries relatively few charges (ranging from +33 to +50), as seen in **Figure 6**. An expanded drift time versus m/z plot of the 13mer region can be seen in **Figure 7**. The individual charge states have well defined drift time differences.

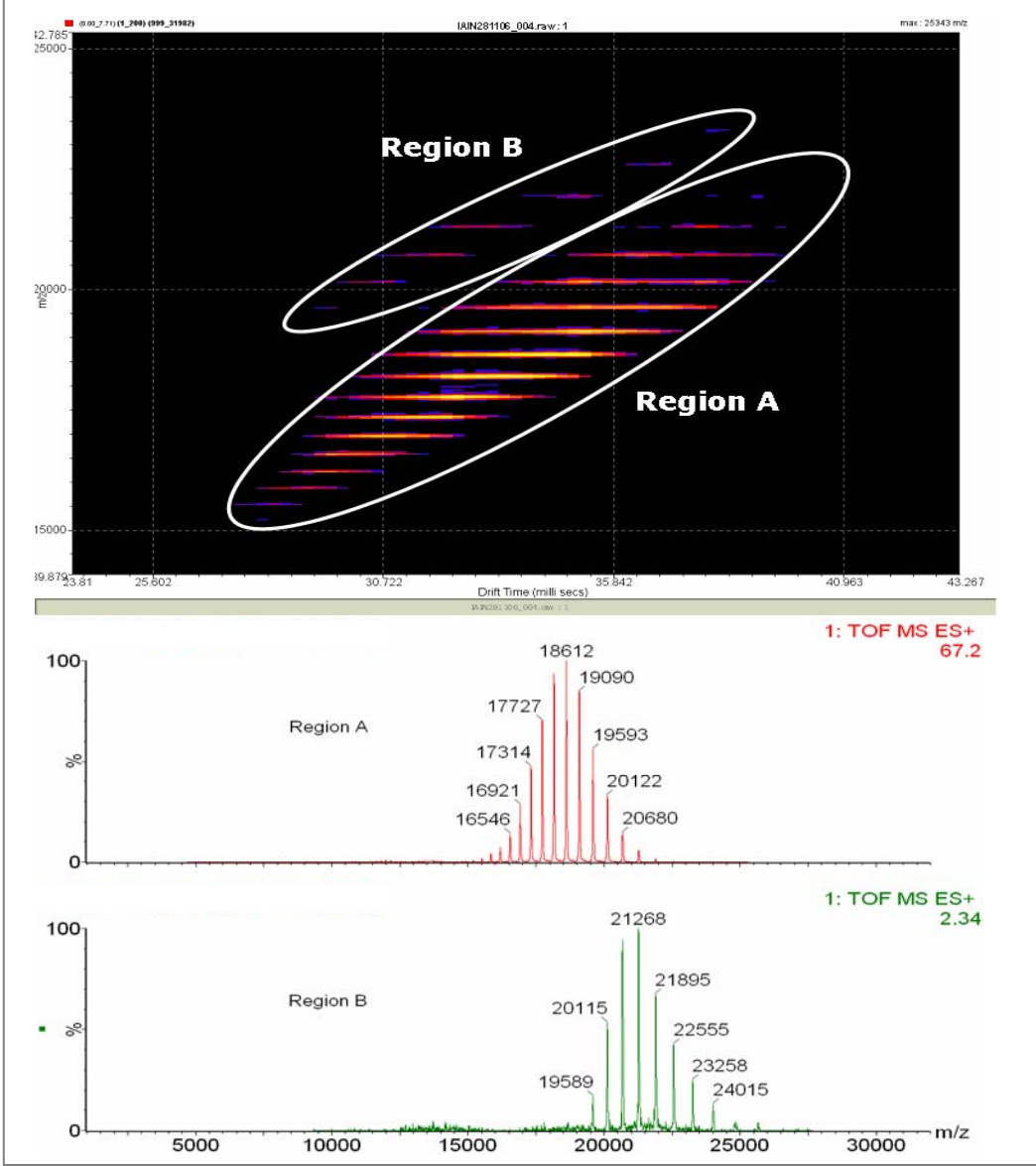


Figure 7. Expanded drift time vs. m/z region of Figure 6, and extracted spectra from Region A and Region B.

What can also be observed is that there are 2 distinct drift time populations for the 13mer (744kDa), **Region A** and **Region B**. When the mass spectra are extracted from these regions, **Figure 7**, there are two different charge state envelopes, which both deconvolute to the mass of the 13mer (744kDa). The two different populations could be a result of the monomer being ejected from a different position within the GroEL 14mer, with one mechanism of ejection being favoured over another.

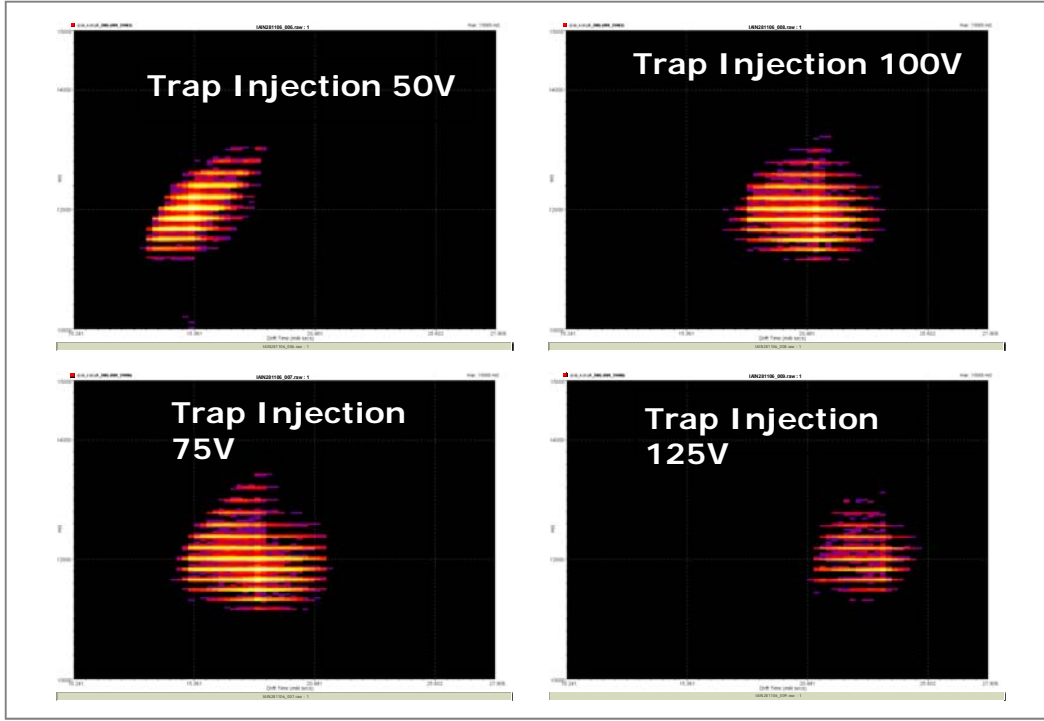


Figure 8. Investigating the effect of raising the trap injection voltage on the intact GroEL.

Figure 8 shows the effect of increasing the injection energy of the intact GroEL ions into the Trap T-Wave. As the energy is increased, the drift-time for the intact GroEL increases. This effect is most likely as a result of an increase in the size of the GroEL complex due to activation which would be expected to occur prior to fragmentation.

Haemoglobin

Under native conditions haemoglobin is in the form of a tetramer made up of 2 alpha chains (Mr 15,126) two beta chains (Mr 15,867) and four haem groups (Mr 616). On selection and fragmentation of the +18 charge state of the haemoglobin tetramer, m/z 3582, monomeric alpha and beta chains (with and without haem non-covalently bound) are observed in the m/z range 1200-2500. Trimeric (mixed alpha 2/beta, beta 2/alpha, beta 3) species are also observed in the m/z range 5000-7000. The haem group is also observed at m/z 616. All the species produced upon MS/MS fragmentation have well defined drift times as observed in **Figure 9**.

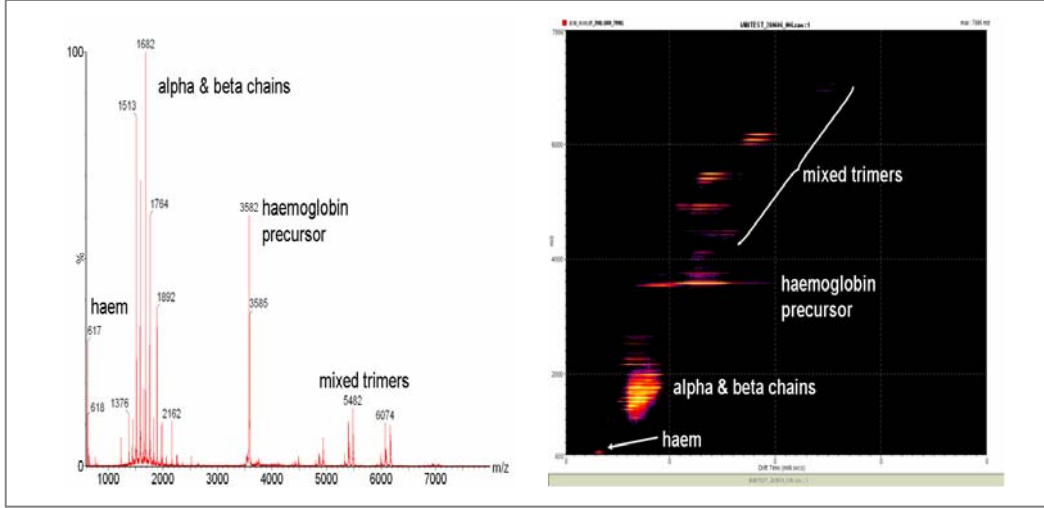


Figure 9. MS/MS spectrum of the +18 charge state of human haemoglobin and corresponding drift-time vs m/z plot.

CONCLUSION

- A novel quadrupole/ion mobility/oa-ToF mass spectrometer was used to mobility separate and analyse large m/z species
- Mobility separation of up to 5 charge states series of CsI clusters up to m/z 20,000 has been shown
- Ion mobility separation of the MS and MS/MS ions of large biomolecular species has been demonstrated
- Mobility separation of the GroEL 13mer has potentially provided new information on the fragmentation mechanism.
- This additional dimension allows us to gain insights into cross sectional areas and fragmentation pathways which would otherwise be impossible by MS or MS/MS alone.

References

- Ruotolo, Giles, Campuzano, Sandercock, Bateman & Robinson, *Science*, 310 (2005) 1658
- Pringle, Giles, Wildgoose, Williams, Slade, Thalassinou, Bateman, Bowers, Scrivens, Int. *J. Mass Spectrom.*, 261 (2007) 1
The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).