A DEMONSTRATION OF THE APPLICATION OF ION MOBILITY MASS SPECTROMETRY FOR THE ANALYSIS OF ACTIVATED BIOMOLECULES

Iain Campuzano, Kevin Giles, Thérèse McKenna, Keith Compson, Christopher J Hughes and James I Langridge
Waters Corporation MS Technologies Centre, Wythenshawe, Manchester, UK

- **Ion mobility separation of intact and activated GroEL (800kDa) tetradecamer complex.**
- **MS/MS precursor ion selection using a 32kamu quadrupole and subsequent ion mobility analysis.**
- **The Synapt HDMS is an intermediate structural characterisation technique fitting between already existing low and high resolution characterisation techniques.**

INTRODUCTION

Electrospray ionization is a very gentle form of ionization, which enables the intact transfer into the gas phase and detection of large multi-protein structures with little or no fragmentation and when coupled to mass spectrometry, non-covalently assembled macromolecular protein complexes can be detected and accurately mass measured.

The transfer of non-covalently associated protein-protein complexes from solution to the gas phase generally results in the formation of ions possessing relatively few charges and as such, the m/z values are often above 10,000. In some of the data presented in this poster, MS/MS activation of such biomolecular complexes can produce ions with m/z values in excess of 20,000.

By coupling an ion mobility separator (IMS) with a time-of-flight (ToF) mass spectrometer, one can not only accurately mass measure intact biomolecular complexes, but also one can also measure their collisional cross sections and differences in cross section produced upon activation and detect subtle conformation differences, which are not evident from spectral data alone [1].

Here we show the analysis of several different, non-covalently associated protein-protein complexes, which differ vastly in mass and collisional cross section, by IMS-ToF-MS and IMS-ToF-MS/MS.

METHODS

Instrumentation

The Mass Spectrometer used in these studies was a Synapt HDMS (Waters Corporation), **Figure 1**. Briefly, samples were introduced by a borosilcate sampled into the Z-spray source. They pass through a quadrupole which may be set to transmit a substantial mass range or to select a particular m/z. The Tri-Wave comprises three T-Wave devices [2]. The first device (trap T-Wave) accumulates ions for periods up to 51msec in the experiments described in this poster. The stored ions were then gated (500usec) into the IMS device (IMS T-Wave) in which the mobility separation is performed.

The final device (transfer T-Wave) is used to transport the separated ions into the oa-ToF for subsequent analysis. Ions may be fragmented on entrance to the accumulation T-Wave and/or in the transfer T-Wave. The pressure in the accumulation and transfer T-Wave regions was $7e^{-2}$ mbar of Argon 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 ion mobility separation.

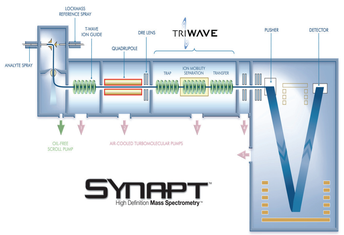

Figure 1. Schematic diagram of the Waters Synapt HDMS instrument.

Sample

The GroEL was buffer exchanged into an aqueous solution of 100mM ammonium acetate, to a final working protein concentration of 3uM.

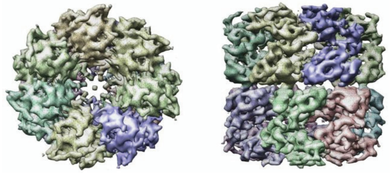

Figure 2. *3D Crystal of the GroEL tetradecamer complex*

Experiment

Intact protein ToF-MS and MS/MS were carried out to determine the accurate mass of the intact GroEL tetradecamer complex and it's activated fragments. Within the same acquisition ion mobility mass spectra were acquired in order to determine the mass of the protein and the m/z of the different charge states. The quadrupole was then set to transmit a single charge state and the trap collision energy raised to produce a fragment ion spectrum. The instrument was calibrated over the m/z range 1000-32,000 using a solution of caesium iodide.

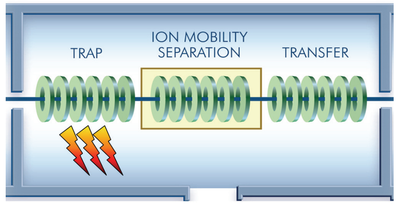

Figure 3. Synapt Trap T-Wave Fragmentation. Fragment ions produced before the Ion Mobility device are separated based on charge state, size and shape. They are subsequently passed to the oa-ToF analyser by the Tranfer T-Wave ion guide for mass analysis.

Precursor ion selection occurs in the quadrupole region of the instrument. The quadrupole in this instrument has an extended m/z range, allowing mass selection of an ion up to m/z 32,000. Activation and subsequent fragmentation of the GroEL complex (800kDa, **Figure 2**) occurs in the Trap T-Wave, with acceleration voltages of up to 150eV to induce disruption of the large macromolecular assemblies (**Figure 3**). Operating the Trap T-Wave at elevated pressures, ($7e^{-2}$mbar) 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.

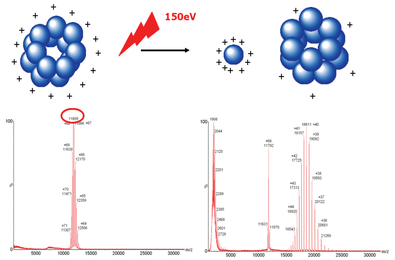

Figure 4. Schematic representation of the activation of the GroEL tetradecameric complex and the corresponding MS and MS/MS spectra.

The fragmentation of large non-covalently assembled complexes follow an asymmetric fragmentation pattern (**Figure 4**). 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 150eV, a highly charged single monomer (57kDa) is ejected, *m/z* 1000-3000. As a results 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.

RESULTS

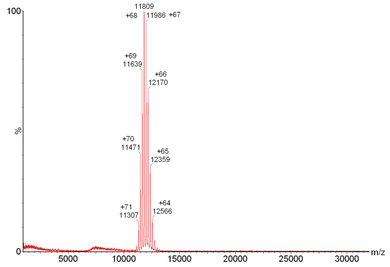

Figure 5. TOF-MS spectra obtained on the intact GroEL tetradecamer complex. The multiply charged series between m/z 11,000 and 12,500 represent the intact mass of 800kDa.

When GroEL is infused into the Synapt under native conditions it is possible to preserve the native interactions of the 14 57kDa subunits. As a result, the intact mass observed is the intact tetradecamer (14mer, 800kDa) with a mass of 800kDa. The intact complex possess relatively few charges (in the order of 64 to 71) for such a large molecule, therefore, appearing very high in the m/z scale, between m/z 11,000 and 12,500 (**Figure 5**).

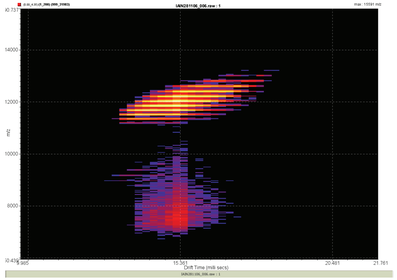

Figure 6. The ion mobility separation of the intact GroEL tetradecamer.

Since the intact GroEL 14 can be preserved within the Synapt, it is also therefore, possible to perform ion mobility measurements. **Figure 6** shows the multiply charged series of peaks with m/z values of 11,000 to 12,000 having a mobility drift time distribution from 13msec for the higher charge states, to 19msec for the lower charge states.

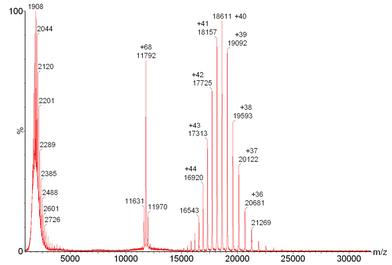

Figure 7. MS/MS quadrupole selection of the GroEL precursor ion m/z 11,809 (Figure 5) followed by Trap activation with a collision voltage of 150V.

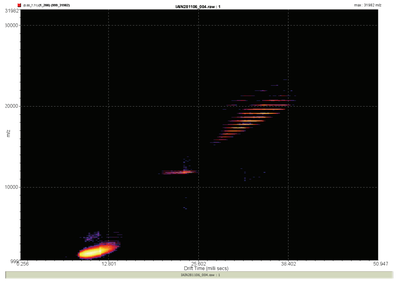

Figure 8. The ion mobility separation of the multiply charged species produced in Figure 7.

Upon activation of the Intact GroEL and subsequent ion mobility separation, there is a clear difference in drift times of the monomer, activated precursor ion and remaining 13mer (744kDa) complex, possessing relatively few charges, ranging from +33 to +50, as shown in **Figure 8**. The measured drift time of the 13mer which is produced upon activation can be seen in **Figure 9**, which has a very late drift time, in the order of 23 to 43msec. The individual charge states have very well defined drift time differences. 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 region A and region B, **Figure 10**, there are two different charge state envelopes, both deconvoluting to the mass of the 13mer (744kDa). Region A being far more intense than Region B. The two different populations could be a result of the highly charged monomer being ejected from a different position from the GroEL 14mer, or one mechanism of ejection being favoured over another.

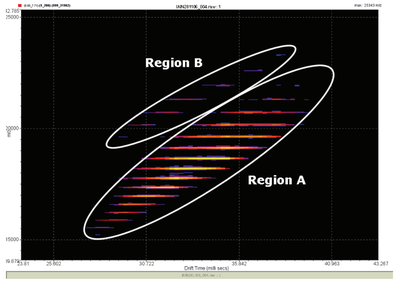

Figure 9. Expanded region (drift time 23 to 43msec) of Figure 8.

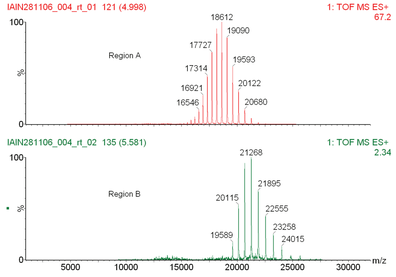

Figure 10. Extracted spectra from Region A and Region B.

CONCLUSION

- A novel quadrupole/Tri-Wave oa-Tof mass spectrometer (Synapt HDMS) was used to mobility separate and analyse intact protein species.
- We have demonstrated that we can acquire high mass non-covalent data on the Synapt HDMS system.
- Macromolecules can be selected using the 32k amu quadrupole option
- We can obtain IMS-TOF-MS and MS/MS data on large biomolecular species.
- 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

1. Ruotolo, Giles, Campuzano, Sandercock, Bateman & Robinson, Science, 9th December 2005, vol 310, 1569-1724

2. "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).

720002150EN

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