

### THE APPLICATION OF SYNAPT HIGH DEFINITON MASS SPECTROMETRY FOR THE CONFORMATIONAL STUDIES OF PROTEIN COMPLEXES

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### INTRODUCTION

Electrospray (ESI) is a gentle form of ionization that enables the transfer of large multi-protein structures with little or no fragmentation into the gas phase. The coupling of ESI to mass spectrometry (MS) allows the detection and accurate mass measurement of non-covalently assembled macromolecular protein complexes. This 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. As such, the m/z values are often above 10,000 and, in some cases MS/MS activation of such complexes can produce ions with m/z values in excess of 20,000.

The Waters<sup>®</sup> Synapt<sup>™</sup> High Definition Mass Spectrometry<sup>™</sup> (HDMS<sup>™</sup>) System combines high-efficiency ion mobility-based (IMS) measurements and separations with a hybrid quadrupole orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer. This combination enables both accurate mass measurements of intact biomolecular complexes and the potential to measure their collisional cross sections, including differences in cross section, produced upon activation. The IMS separation allows the detection of subtle conformation differences that are not evident from spectral data alone.<sup>1</sup>

In this application note, we describe the analysis of GroEL (Figure 1), an 800 kDa non-covalently associated protein-protein complex using the Synapt HDMS System.



Figure 1. Space-filling model of the protein complex, GroEL, illustrating the overall ring shaped topology from both arial(left) and side views (right), respectively.

## EXPERIMENTAL

#### Sample

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



Figure 2. Schematic of the Synapt HDMS System, which incorporates Triwave technology.

#### Instrumentation

Samples were introduced using nano-electrospray ionization. Ions produced were sampled by the ZSpray<sup>TM</sup> source of the Synapt HDMS System (Figure 2).

The ions pass through a quadrupole and are either set to transmit a substantial mass range or to select a particular m/z before entering the Triwave™ device. Triwave is comprised of three T-Wave™ devices.<sup>2</sup> The first device, the Trap T-Wave, accumulates ions. These stored ions are gated (500 µsec) into the second device, the IMS T-Wave, where they are separated according to their mobilities. The final Transfer T-Wave is used to transport the separated ions into the oa-TOF for MS analysis. The pressure in the Trap and Transfer T-Wave regions was 7e-2 mbar of Argon and the pressure in the IMS T-Wave was 0.5 mbar of Nitrogen.

TOF-MS analysis was carried out to determine the accurate mass of the intact GroEL tetradecamer complex from the m/z of the different charge-states present. The instrument was calibrated over the m/z range 1000 to 32,000 using a solution of caesium iodide.

In a second analysis, the precursor ions from the intact tetradecamer were selected using the quadrupole, which in this instrument enables selection of ions up to an m/z value of 32,000 Da. The selected ions were then fragmented in the gas-filled Trap T-Wave. HDMS mode analysis was also conducted whereby mobility separations in the IMS T-Wave enabled the separation of species by their mobilities and drift time data could be derived.

### RESULTS AND DISCUSSION

The mass spectrum obtained for the intact GroEL tetradecamer protein is shown in Figure 3 (left), where multiply charged ions distributed around m/z 12,000 can be seen. These represent charge states centered around +68, which demonstrate that under these native conditions it is possible to preserve the interactions of the 14, 57 kDa subunits. As a result, the intact mass observed is that of the intact tetradecamer (14 mer, 800 kDa) with a mass of 800 kDa.

Activation and subsequent fragmentation of the GroEL complex, Figure 3 (right), occurs in the Trap T-Wave with injection voltages of up to 150 V, which disrupts the large macromolecular assemblies. Operating the Trap T-Wave at elevated pressures allows for efficient fragmentation of the GroEL complex. These high pressures also



Figure 3. Schematic representation of the activation of the GroEL complex and the MS and MS/MS spectra obtained.

provide efficient collisional cooling and focusing of the intact 14 mer and subsequent 13 mer generated from the activation process.

The acquired HDMS data was analyzed in Driftscope<sup>™</sup> Software, where the drift time of each ion is plotted vs. the m/z of the ion. The intensity of an ion is indicated using a color scale from least (blue/red) to most (yellow/white) intense. The multiply-charged series of peaks for the intact GroEL, Figure 4 (left), range in m/z from 11,000 to 12,000 with drift times ranging from 13 msec for the higher charge states to 19 msec for the lower charge states.

Activation of the intact GroEL and subsequent ion mobility separation, Figure 4 (right), shows that there are clear differences in drift time of the monomer, the precursor ion, and the remaining 13 mer (744 kDa) complex. This 13 mer carries relatively few charges (between +33 and +50). An expanded Driftcope plot of the 13 mer region is shown in Figure 5, where the individual charge states have well defined drift time differences.



Figure 5. Expanded drift time vs. m/z region of Figure 4, showing the conformational heterogeneity of the GroEL 13 mer.



Figure 4. Left - HDMS analysis (drift-time vs. m/z) of the intact GroEL tetradecamer. Right - HDMS analysis (Drift-time vs. m/z) of GroEL (+68 charge state), activated with 150 V in the TRAP T-Wave. Driftscope Software where each pixel represents one ion with color representing intensity from low (blue) to high (yellow).

It is evident that there are two distinct drift time populations for the 13 mer (744 kDa), region A and region B. When the mass spectra are extracted from these regions, Figure 6, there are two different charge state envelopes, both of which deconvolute to the mass of the 13 mer (744 kDa). Region A is far more intense than region B. The two different populations are postulated to be a result of the monomer being ejected from a different position within the GroEL 14 mer, with one mechanism of ejection being favoured over another, and thus represent different conformations of the GroEL 13 mer.



Figure 6. Extracted spectra from region A and region B in Figure 5, showing the different charge-state distributions for each potential confirmation of the GroEL 13 mer.

### CONCLUSION

- The Synapt HDMS System was used to separate and mass analyze different conformations of a large intact protein species in the gas phase.
- Ions with high m/z ratios can be isolated for fragmentation (MS/MS) using a Synapt HDMS System equipped with a 32 kDa quadrupole.
- HDMS analysis provided new evidence on the fragmentation mechanism of the GroEL protein complex.
- This additional dimension of specificity obtained in HDMS analysis has provided insight into protein complex conformation (and fragmentation pathways), which would be impossible by MS or MS/MS alone.

#### References

- 1. Ruotolo, Giles, Campuzano, Sandercock, Bateman & Robinson, Science, 9th December 2005, vol 310, 1569-1724
- "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.
- 3. The traveling wave device described here is similar to that described by Kirchner in US Patent 5,206,506; 1993.



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