High-mass Q-ToF upgrade for MS/MS studies of intact non-covalent complexes

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Introduction

Standard quadrupole time-of-flight (Q-Tof) mass spectrometers have multiple fundamental limitations in their performance in the analysis of high-mass species. This poster discusses an MS Vision instrument upgrade, available for Q-Tof 1, 2 and Ultima, which results in the unique capability to isolate ions up to m/z 30,000 in the quadrupole and perform ToF MS/MS studies of these. Using some example non-covalent protein complexes, we demonstrate the powerful mass spectrometry performance the upgrade enables for high-mass native species studies.

Instrument Limitations and Solutions

The following instrument limitations for high mass analysis were identified:

SOURCE:

Although high mass species are readily ionised using electrospray ionisation (ESI), the resultant ions are too energetic to be efficiently transported into the transfer region of the instrument.

→ The source pressure was increased by a factor of 10 by adding a speedivalve and hexapole sleeve. This resulted in sufficient collisional cooling of the ions to enable their successful transit into the hexapole region.

QUADRUPOLE:

The quadrupole mass range of standard instruments is 4,000 m/z and simply too low for useful native mass spectrometry studies.

→ The RF frequency was dropped which resulted in a m/z range increase to 31,000 m/z. Although this reduces the quadrupole resolution it is still more than sufficient to allow selection of an individual charge state from a charge state envelope.

COLLISION CELL:

The collision cell pressures and maximum collisional energy are too low for efficient CID. At high collisional energy, ions are not sufficiently cooled before exiting the cell.

→ The collision cell pressure was increased by a factor of 10 by modification of the gas plumbing system. In addition, the maximum collision voltage was doubled to 400V by modifying the electronics in order to give the required energy to break large complexes.

ToF:

The maximum range of the ToF of 22 kDa (Q-Tof1) or 33 kDa (Q-Tof2 and Ultima) is insufficient for high mass studies.

→ A 'pusher' timing modification extended the ToF range by a factor of 100.

CONTROL:

Standard software settings and electronic pressure trip settings negate the required working range required.

→ Pressure trip and MassLynx software edits were made to allow the instrument to run with the modifications

Figure 1 The MS Vision High-mass Q-Tof.

Testing the performance of the modified instrument

To demonstrate the new capabilities, a GroEL sample was used. GroEL is an *E.coli* heat shock protein that facilitates protein folding; it is a 14-mer consisting of two stacked rings (1). A solution of 5-10 µM was introduced using offline electrospray nanoflow needles. The speedivalve was set to give a pressure of 4.0E-5 mBar in the analyzer chamber. Argon was then introduced to give an analyzer pressure at 3.4E-4 mBar. Cone voltage was set to 150V.

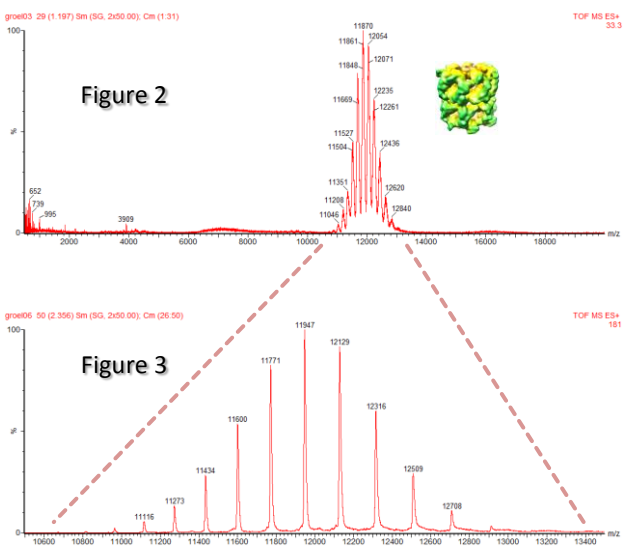
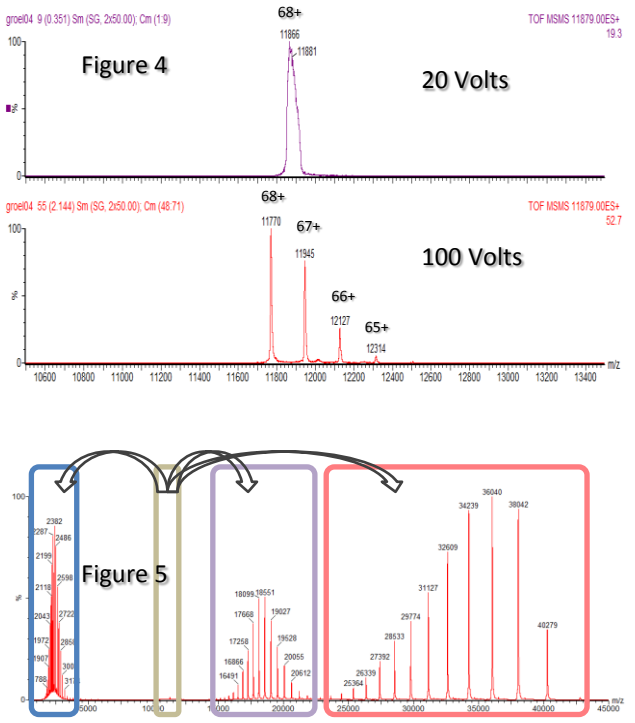


Figure 2 shows the intact GroEL complex appearing around 11.8 kDa m/z. The peaks in the distribution are approximately 90 Da wide, this is due to solvent and other adducts trapped within the structure. The low resolution makes it impossible to determine the charge state. In order to improve the resolution the collision energy was raised to 100V . The high energy in the collision cell causes a lot of the trapped solvent to be removed. The resolution shown in **figure 3** is >1,000. We are now able to perform deconvolution of the charge envelope. We calculate 801 kDa for the total mass of the complex, equating to 57.2 kDa for each subunit.

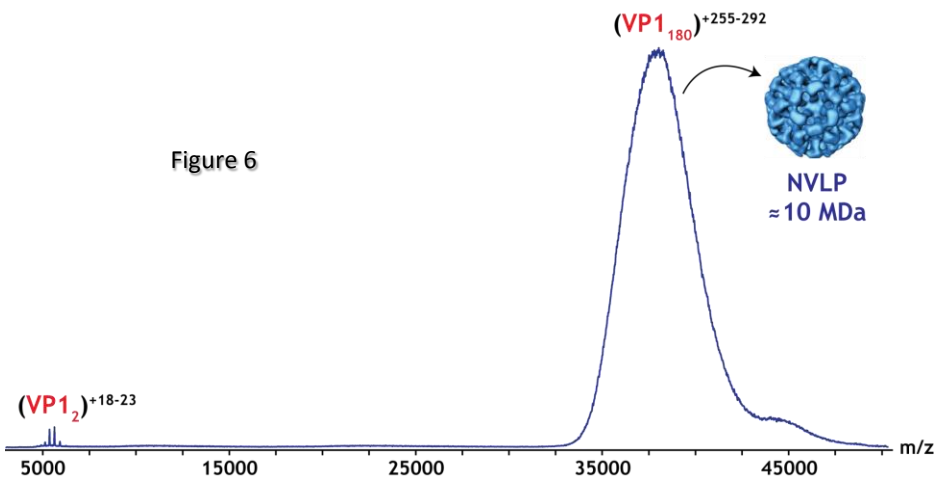


When setting the parent mass for MS/MS precursor selection, it is important to park the quad at the mass before full desolvation, as this takes place after the ions exit the quadrupole. **Figure 4** shows the 68+ charge state of GroEL at 20V and 100V collision energies. At 100V, trapped solvent and adducts, amounting to a weight of ~7,400 Da on average are removed. Also some charge stripping can be seen. In order to break the non-covalent bonds between the GroEL sub-units, more energy is needed.

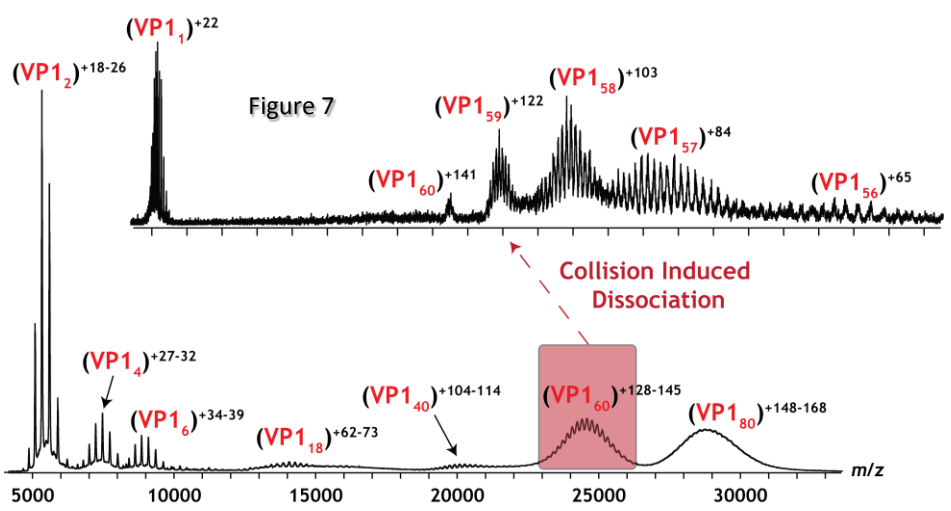
Figure 5 shows MS/MS at 200V collisional energy using Xenon gas at higher pressures for better fragmentation and cooling. The non-covalently bound 14-mer (green) falls apart into monomer (blue), 13-mer (purple) and 12-mer (red). The apparent high mass cutoff is caused by charge depletion.

State of the Art: some recent results on intact virus capsids

Over the past years many different proteins and protein complexes have been analyzed on our modified instruments. Recently, intact virus capsids weighing millions of Daltons have been measured. **Figure 6** is a ToF mass spectrum of a Norovirus capsid of 10 MDa under Native conditions (pH = 7).



This capsid, comprising 180 subunits, is too large to be fragmented in the collision cell. At pH = 9, disassembly takes place and smaller complexes form. One such complex ($VP1_{60}$ of 3.3 MDa) has been subjected to MS/MS at 375V collisional energy, see **figure 7**.



Acknowledgments

Data courtesy of the group of Albert Heck at Utrecht University, The Netherlands

1) Yifrach, O. and Horovitz, A. (2000) PNAS USA 97: 1521-1524