TANDEM ION MOBILITY COUPLED WITH MASS SPECTROMETRY FOR GAS PHASE UNFOLDING STUDIES

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OVERVIEW

- **The cyclic IMS-enabled QTof is a flexible platform for native MS and collision-induced unfolding studies.**
- **Multiple rounds of ion mobility separation can be performed using the cyclic mobility geometry.**
- **IMS^2 and IMS^3 are demonstrated with intervening collisional activation.**
- **Mobility selection combined with activation facilitates detailed protein unfolding studies.**

INTRODUCTION

Over more than a decade ion mobility spectrometry (IMS) has been employed to great effect in the field of native mass spectrometry-based structural biology.

In this field IMS has been used for separating protein oligomeric states, co-populated conformations and for measuring experimental collision cross-sections for use in determining protein complex architecture[1].

In addition, from an early stage the potential of pre-IMS activation in probing gas phase protein unfolding pathways and stabilities was pursued. This experiment, called collision-induced unfolding (CIU) has spawned a whole field of research into probing protein domain architecture[2], protein-ligand stabilization[3], and therapeutic antibody comparisons[4].

Here we describe an IMS-based instrument platform with IMS^n functionality which allows novel protein CIU studies to be performed in which protein sub-populations can be mobility-selected for further rounds of IMS. Furthermore, by adding an activation step in between IMS experiments, sequential rounds of unfolding can be performed on the same ion populations allowing greater insight into protein unfolding pathways.

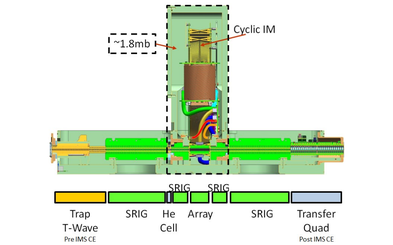
In addition to unfolding studies the ability to mobility-select after quadrupole isolation and activation allows high mobility-resolution interrogation of ligands released from protein-ligand complexes.

METHODS

Human TTR (Sigma Aldrich) was prepared at a concentration of 4 micromolar in 200 mM ammonium acetate. It was subjected to one round of buffer exchange using Biorad MicroBioSpin 6 spin gel filtration columns. Proteins were electrosprayed from PicoTip GlassTip borosilicate glass nanocapillaries (2 um I.D. NewObjective) mounted on a nanolockspray ionisation source.

Native ion mobility experiments were performed on a cyclic ion mobility-enabled Q-Tof (ESI-Q-cIM-Tof) system fitted with an extended time-of-flight mass analyser, a segmented quadrupole transfer ion guide and dual gain ADC.

Data were acquired using a custom version of MassLynx and DriftScope software. The cyclic IMS device and array (Figure 1) provide a 98cm, single pass, mobility path length at 90° to the instrument axis offering IMS resolution of 60-70 Ω/ΔΩ. By controlling the array region ions can be made to do further passes increasing the IMS path length by 1 meter per pass. In this way IMS resolution in excess of 200 is possible. Nitrogen was used as the mobility buffer gas and collision gas in the trap cell, transfer quadrupole and intervening stacked-ring ion guides (SRIG). Helium was employed in the cIM entrance cell.



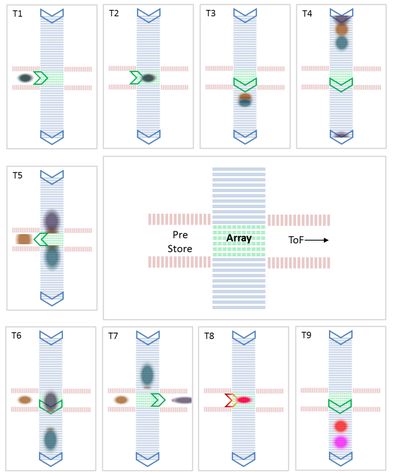


Figure 2. Schematic of ion manipulation in IMS^n mode of operation. T1-9 show events in an IMS^2 sequence. Inset shows a representation of the pre store, multifunctional array, and cIM region.

Figure 2 describes the events in an IMS^2 experiment. In time points 1 and 2 (T1 and T2) ions are injected from store (red) into the array (green). During T3 the direction of T-waves in the array (green arrow) change to match those in cIM region (blue arrows); ions begin to drift around cIM device. After certain number of passes three species appear separated (T4). Point T5 shows IM selection; as brown population of ions drifts through the array region, the array T-wave direction changes and the selected ion population is ejected into the pre store. Ions remaining in the cIM travel for an additional pass around cIM (T6) before being ejected out (T7). At point T8 ions from the pre store are re-injected into the array at high energy and form product ions (either fragments or unfolded species). Finally, product ions originating from brown precursor population are mobility separated after certain number of passes (T9).

RESULTS

Human TTR is a well-studied tetrameric protein complex standard for native MS with a molecular weight of 56 kDa.

The native mass spectrum (Figure 3A.) displays broad charge state signals consistent with a wide range of isoforms making up the tetramer.

The tetramer was subjected to single cIM pass. Each charge state populates a single conformational family (Figure 3B.).

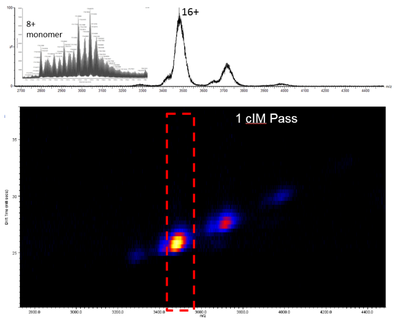


Figure 3. Native cIMS of the TTR tetramer. A) The native mass spectrum of the TTR tetramer. Inset; the dissociated monomer showing sample heterogeneity. B) cIM data after one pass through the device.

After subjecting the 16+ charge state to elevated energy in the trap cell, collision-induced unfolding was induced. At a collision voltage of 40 V the compact 16+ tetramer ion evolves into an arrival time distribution exhibiting approximately five major unfolded conformational families.

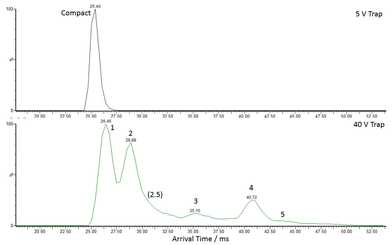


Figure 4. Collision-induced unfolding of the TTR tetramer. A) Single pass cIMS of native TTR tetramer. B) Arrival time distribution of 16+ charge state after activation in the trap collision cell.

Tandem-IMS unfolding

By timing precisely the travelling waves on the multifunctional array unfolded ions were selectively ejected into the pre-array store where they were held briefly before reinjection into the cIM device for an additional pass. Species 1-4 were separately subjected to this sequence (Figure 5.).

Perhaps unsurprisingly no further evolution of the selected arrival time distributions was observed after the second round of IMS.

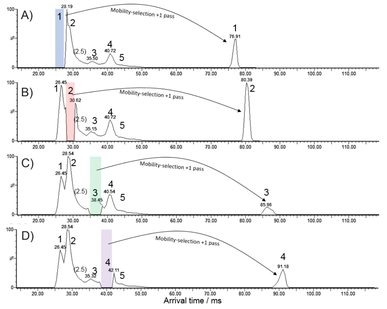


Figure 5. IMS^2 of unfolded states of the TTR tetramer. A) Species 1 selected by ejection to the pre store followed by reinjection for a single cIM pass. B-D) the same for species 2-4 respectively.

During this type of experiment it is possible to apply an accelerating voltage to the mobility-selected ions upon reinjection in the cIM array. This allows a further unfolding step to be performed in between rounds of IMS separation. This was performed on species 1-4 from the unfolding profile of the TTR tetramer (Figure 6.).

Inspection of the arrival time distribution for unfolded conformation **1** after activation showed further unfolding into conformations **2**, **2.5**, **3**, **4** and **5**.

After activation of conformation **2** a similar arrival time distribution was observed showing **2.5**, **3**, **4**, and **5**. Performing the same experiment of conformation **3** showed unfolding to **4** and **5** and conformation **4** formed a small amount of **5** at voltage used. From this data we can observed that only lower mobility species are generated, no collapse is detected.

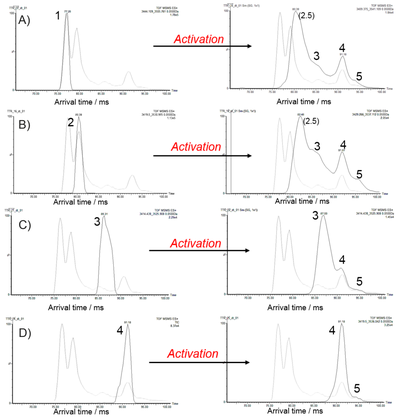


Figure 6. IMS^2 with additional activation. A) The selected species 1 is subjected to activation upon reinjection into the cIM array causing unfolding. B-D) Species 2-4 undergo the same procedure. Generally for TTR, only lower mobility species are generated in this manner, no collapse is observed.

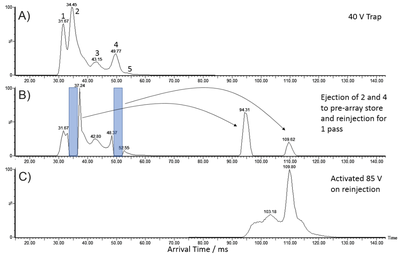


Figure 7. Multiple mobility selections. A) the arrival time distribution of unfolded TTR showing species 1-4. B) An example of multiple selection regions. Species 2 and 4 are mobility-selected by ejection to the pre store and reinjected for an additional cIM pass. No re-equilibration of the arrival time distributions are observed as expected. C) Mobility-selected species 2 and 4 after activation at 85 V upon reinjection. Species 2 readily unfolds further whereas species 4 does not.

The control software on the cyclic IMS-enabled QTof is highly flexible, meaning the user can specify easily the portions of the arrival time range to be selected. Furthermore, multiple mobility-selections can be made per round of IMS, removing intermediate species.

IMS^3 and beyond for following unfolding pathways

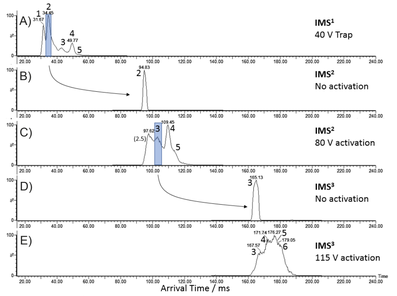


Figure 8. IMS^n on the cyclic IMS-enabled QTof. A) Selection of species 2 from the unfolding profile of TTR. B) The selected species after reinjection and 1 cIM pass. C) The selected species after activation at 80 V upon reinjection. Species 3 is generated directly from species 2. D) Species 3 after selection from the unfolding profile of species 2. E) Species 3 after activation at 115 V upon reinjection.

The cIM geometry allows IMS^3 (Figure 8.) and higher. Selection and activation of species **2** generates **2.5**, **3**, **4** and **5**. Subsequent selection and activation of species **3** generates **4**, **5** and **6**. This multi-stage approach offers the opportunity of delineating gas phase unfolding pathways.

CONCLUSION

- **Human TTR was investigated as a model system for collision-induced unfolding**
- **The geometry of the cyclic IMS-enabled instrument is highly flexible allowing multi-stage gas phase separations**
- **IMS^2 experiments with activation showed a reproduction of the stepwise TTR unfolding pathway**
- **Multiple mobility selections can be made per round of IMS**
- **IMS^3 experiments provide greater detail into the TTR unfolding pathway**

References

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