A method for the structural analysis and time resolved imaging of bio-macromolecular assemblies in mass spectrometry using Timepix detector

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Introduction

The retrieval of 3D structural features of bio-macromolecular assemblies (MMAs) with mass spectrometry (MS) is of great interest since the structure of these complexes strongly defines their functions and interactions with other molecular species. Currently, MS based techniques (native MS, Ion mobility, etc.) only provide 2D structural information. Complementary methods such as X-ray diffraction, electron microscopy and nuclear magnetic resonance require large amount of purified samples or rely on extensive crystallization trials. We investigate an Orbitrap™-Time of flight (ToF) instrument that utilizes MS combined with position-and time-sensitive Timepix (TPX) detectors and a UV laser to obtain both 3D structural and molecular information at the same time on single molecules of MMAs..

Method

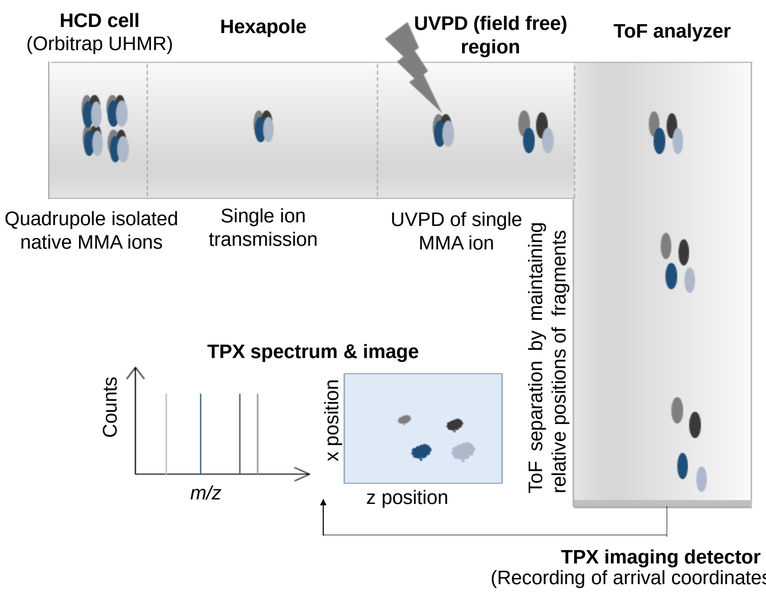


Figure 1 The interaction of single gas phase native MMA ion with 193 nm UV laser leads to the rapid fragmentation. The ion fragments are then accelerated to the TPX through a ToF analyzer by maintaining relative positions for the time resolved imaging.

Ion optics simulation results

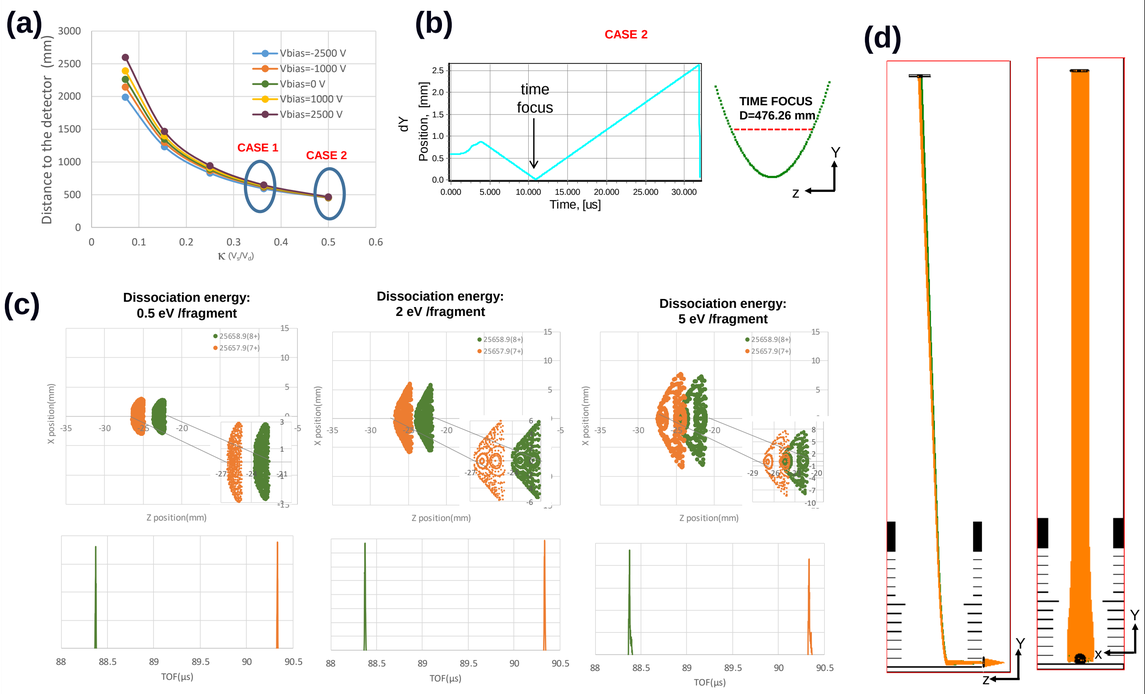


Figure 2 ToF analyzer consists of two-stage acceleration (s and d) field and a bias electrode. (a) shows the effect of bias electrode voltage on the position of the time focus. If the value of κ is adjusted to ~0.5 (case 2), the position of the time focus is least sensitive to the variations of the bias voltage. Adjustments of the bias voltage can then be applied to control beam divergence (x direction) and accommodate different isotropic velocity distributions while maintaining the fragments in their relative positions (c & d) and without displacing the detector (b). Simulation conditions (c)- Precursor: mass= 51317.14 Da , axial energy (z)= 450eV, Fragment 1: mass= 25658.9 Da , axial energy= 225.004 eV, Fragment 2: mass= 25657.9 Da , axial energy= 224.996 eV, fragmentation energy=0.5, 2, 5eV, V_{pusher}= 15 kV.

Overview-MMA imaging device

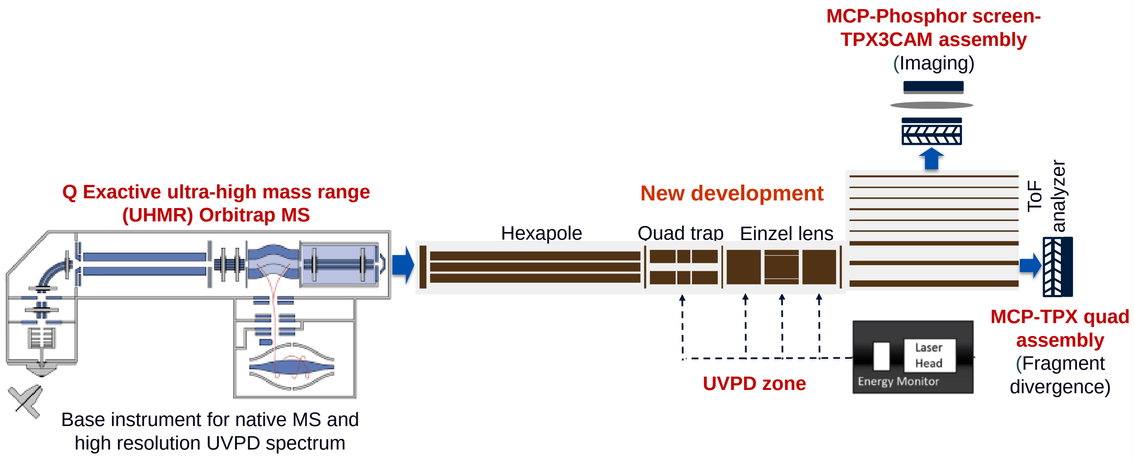


Figure 3 MMA imaging device can be operated in the following modes:

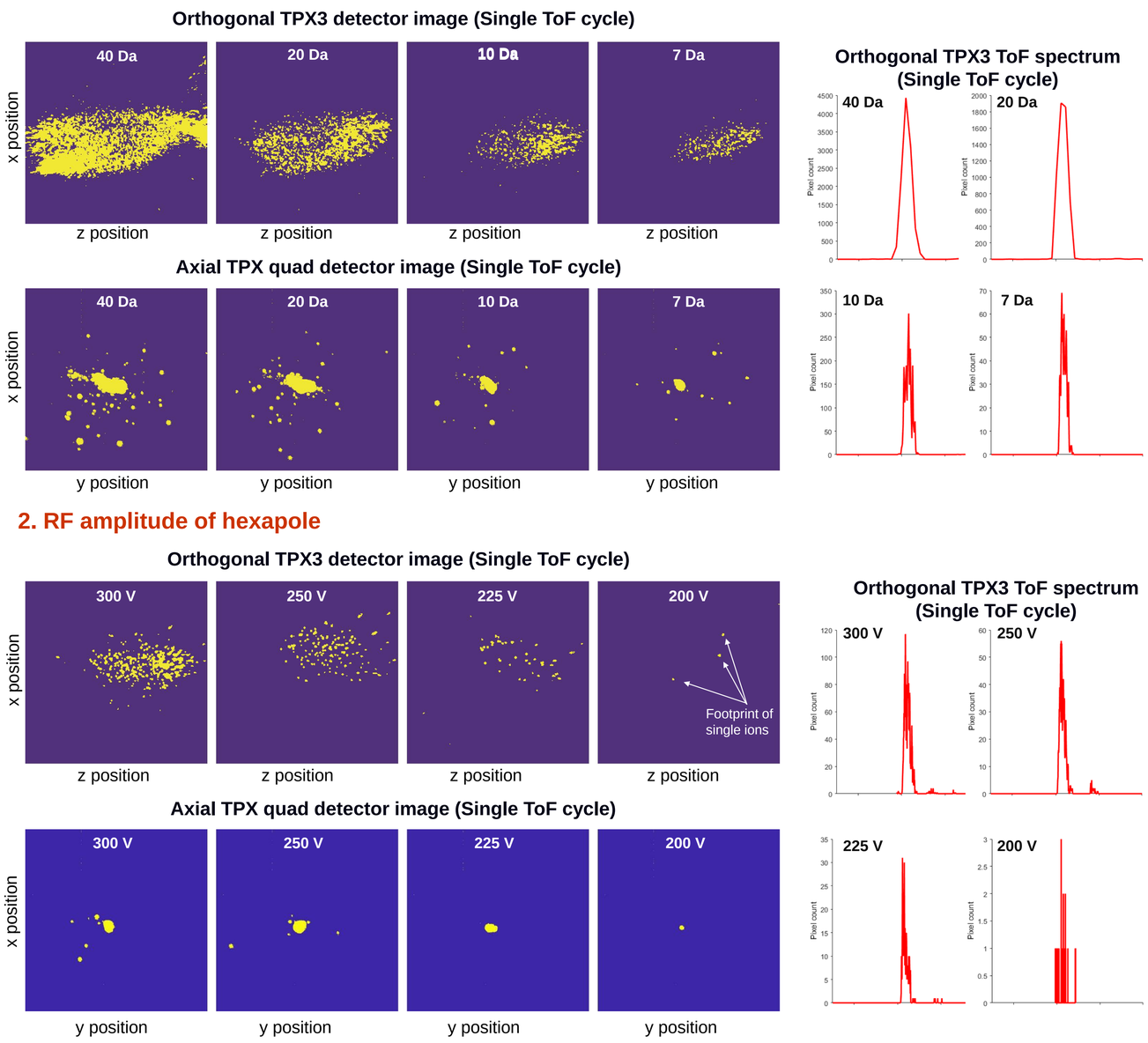
- Standard Orbitrap mode, in which MMA ions not send to the newly installed assembly.
- Quad trapping mode, in which ions are transported from HCD cell of the Orbitrap to the quad trap of the newly mounted part, trapped and sent back to the Orbitrap analyzer for the high resolution molecular analysis (will be used for obtaining the high resolution UVPD spectrum in the future).
- Axial mode, in which ions from the HCD cell are transported to the quad trap, trapped and sent to the axial TPX quad detector.
- Orthogonal TOF mode, in which ions from the HCD cell are transported to the quad trap, trapped and sent to the orthogonal detector.

Experimental results

Figure 4 Orbitrap spectrum recorded in (a) standard Orbitrap mode (b) Quad trapping mode, ToF spectrum acquired in orthogonal ToF mode using ETP detector with a time delay (time difference between trigger signal from the Orbitrap MS and pusher pulse) of (c) 150 µs and (d) 300 µs, by spraying Cesium Iodide mix (m/z range: 392 to 11304 Da).

Single ion transmission and detection

1. m/z isolation window



2. RF amplitude of hexapole

Figure 5 Single ion sensitivity has been achieved by the isolation of the ion of interest (Concanavalin A tetramer, mass= 102 kDa, m/z= 5147) with the quadrupole mass filter of the Orbitrap MS, and limiting the ion transmission by controlling RF voltage of the hexapole (placed between Orbitrap MS and TOF analyzer).

Conclusion

We discussed the design and development of a new Orbitrap-ToF MS based instrument combined with TPX imaging detectors and a UV laser, which is targeted to obtain structural and molecular details of MMAs at the same time. The important aspects of the ion optics design and achievement of single ion transmission and detection of MMAs were presented. This innovative method based on photofragment ion imaging is expected to have a significant impact in structural /molecular biology.

Acknowledgments

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