A novel Ion Processor Device for High-Throughput Analysis in a High-Resolution Mass Analyzer

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Introduction

Ion accumulation devices are routinely used to shape ion packets prior to pulsed extraction into an analyzer. A prominent example is the C-Trap coupled to a Thermo Scientific™ Orbitrap™ analyzer. Compared to conventional orthogonal extractors found in Q-ToF instruments, these benefit from vastly higher duty cycle but require a compromise between buffer gas pressure to capture and thermalize ions and minimized fragmentation and scatter during extraction.

A novel ion accumulation and extraction device formed of two pressure regions for parallel accumulation/fragmentation and extraction is introduced. It has a unified, phase-locked RF pseudopotential channel for seamless transfer of pre-cooled analyte ions to overcome common limitations of ion transfer energy, cooling time, device size and buffer gas pressure. Axial accumulation and movement are performed via DC electrodes, allowing fast movement/processing at reduced pressures.

Figure 1 shows a schematic of the ion processor. In a), the general scheme including ion movement inside the ion processor is presented. The ion processor is divided into two regions, a High Pressure Region and a Low Pressure Region. The High Pressure Region is optimized to accumulate/fragment ions while the Low Pressure Region is preparing ions for injection into the Thermo Scientific™ Orbitrap™ Astral™ analyzer. Both regions are operated in parallel, i.e. accumulation is done in parallel to preparation/extraction into the Astral analyzer. In b), crucial components if the ion processor are highlighted. At the entrance of the ion processor, a steel aperture acts as entrance lens. DC potentials are changed as a function of time in order to transfer ions between the regions and prior to extraction into the analyzer.

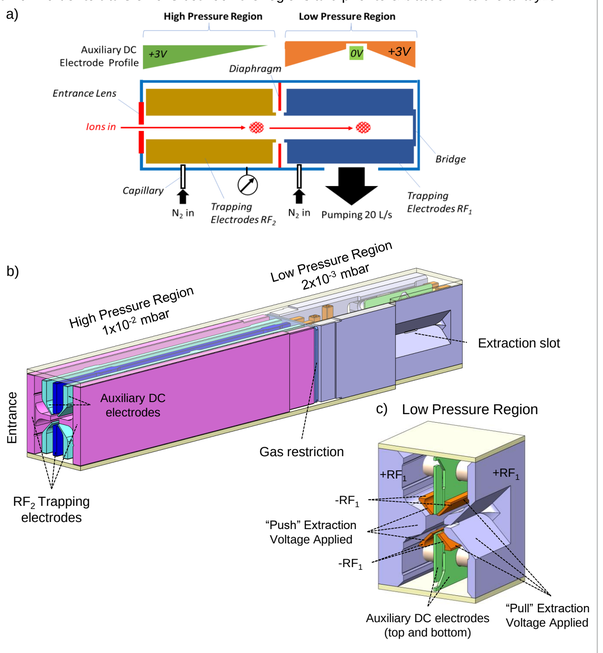

Figure 1. a) General schematic of the ion processor including ion movement inside the device. b) Isometric view of the ion processor. c) View of the extraction region.

Experimental

A prototype instrument was constructed based on a Thermo Scientific™ Orbitrap Exploris™ platform mass spectrometer coupled to the novel Thermo Scientific™ Orbitrap™ Astral™ analyzer. The position of the ion processor is highlighted in Figure 2. The instrument is geared for high quality full-MS scans in the Orbitrap at slow speed and parallel high-speed handling of of many MS/MS acquisitions in the Astral analyzer.

The key performance characteristics of the ion processor were measured with electrosprayed ions of infused Thermo Scientific™ Pierce™ FlexMix™ calibration solution and intact proteins. Ions could be isolated with quadrupole mass filter before sending them to the ion processor.

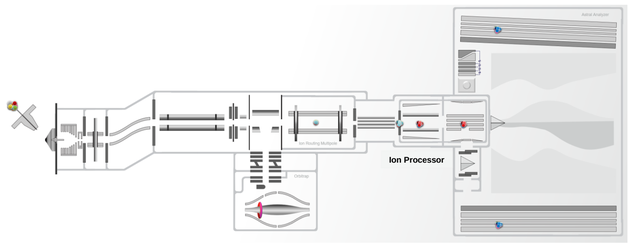

Figure 2. Experimental instrument incorporating Orbitrap analyzer and Ion processor combined with Astral analyzer.

Results

FlexMix spectrum: Figure 3 shows a typical 10,000 ion FlexMix full-MS spectrum, acquired at a high repetition rate (single acquisition).

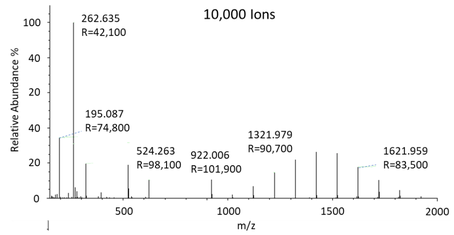

Figure 3. 10,000 ion FlexMix mass spectrum.

Fragmentation: Ion fragmentation is performed by applying a DC offset potential to the high-pressure region of the ion processor. Ions are accelerated into the high-pressure where they undergo high-energy collisions and consequently dissociate. After dissociation, ions accumulate in the backend of the first region before being transferred to the low-pressure region. Ion transfer is initiated by changing the DC potential of the first region. After transfer, ions are trapped by DC gradients in the low-pressure region. After successful trapping, ions are elevated to 4kV before the extraction sequence starts. The high-pressure region is immediately switched back into ion receiving/fragmentation mode so that the next ion package can arrive. In Figure 5, fragmentation spectra for MRFA m/z 524 and Ultramark m/z 1522 are presented. For comparison, an Ultramark MS/MS acquired in the Orbitrap analyzer is shown in c).

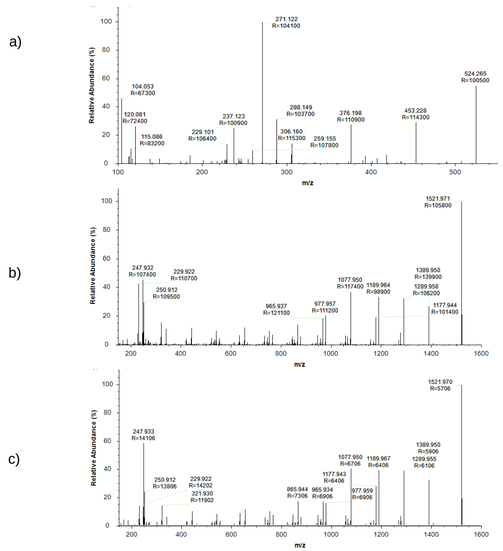

Figure 5. a) MS/MS spectrum of MRFA m/z 524 for low number of ions recorded at 200Hz. b) Comparison of Ultramark m/z 1522 MS/MS spectrum between Astral analyzer and c) Orbitrap analyzer.

Ion Capacity: Trapping capacity and linear response with injection time is crucial for many applications. This was probed by scanning ion accumulation time under varying conditions like for example varying isolation windows. Intensities of individual ion species and overall ion current have been evaluated. A typical example can be found in Figure 6.

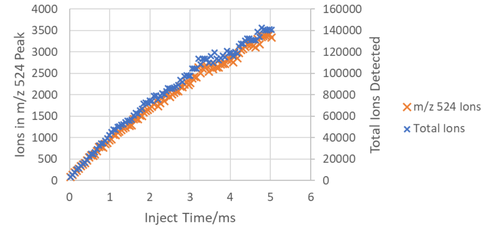

Figure 6. Comparison of number of ions for MRFA, m/z 524 (left axis) and total number of ions (right axis) as function of inject time. Isolation range of 190-3000 and RF amplitude of 1475Vpp.

Intact Proteins: The ion processor is also suitable for handling intact proteins. Figure 7 shows 100x averaged profile spectra of infused myoglobin (17KDa) over both, a wide mass range and zoomed into a single charge envelope. The device still allows the acquisition of individual spectra at 200Hz. In order to get good quality spectra however, at least 10x averaging is required. Unwanted fragmentation and scatter during extraction is well controlled.

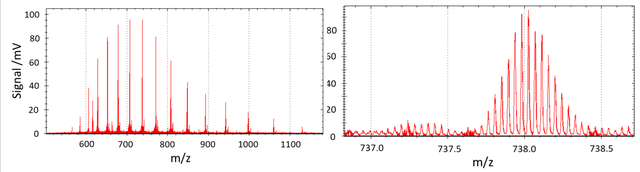

Figure 7. Myoglobin full-MS profile spectrum and zoom in of m/z 738 envelope

Applications: The combination of the speed of the ion processor, high transmission and sensitivity of the Astral analyzer allow for unprecedented performance. This is especially true for demanding high-throughput applications. Figure 8a) shows the results for 8-minute HeLa DIA-experiments (5.5min gradient) with unprecedented depth of analysis at high throughput (180 SPD). Figure 8b) presents a comparison between Orbitrap Astral and Orbitrap Exploris for TMT applications. For TMT11-plex TKO standard, >2.3x protein groups could be quantified for identical experimental and processing conditions (500ng sample, 50min gradient).

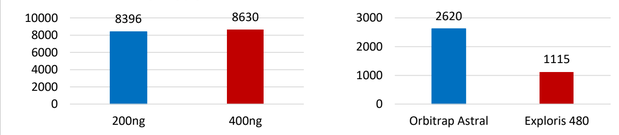

Figure 8. a) Identified protein groups for 8-minute HeLa DIA experiments. b) Comparison of Orbitrap Astra MS and Orbitrap Exploris MS for TMT applications.

CONCLUSION

A powerful new ion processor suitable for high-paced extraction (200 Hz) into a novel analyzer has been developed. It comprises of two pressure regions that have an apertureless interface. The device is capable of ion fragmentation, fast ion transfer and can handle ion capacities >>10^5 charges. Combined with the novel Astral analyzer it is ideally suited for a wide range of scientific applications.

REFERENCES

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TRADEMARKS/LICENSING

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