Fast Top-Down Analysis via Orbitrap and Astral Analyzers

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Abstract

Purpose: Demonstration of intact protein measurement and analysis with the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer.

Methods: Optimization of instrument parameters and measurement of several infused protein samples, along with DDA measurement of Pierce™ Intact Protein Standard Mix.

Results: Measurement of intact proteins and their MS/MS analysis is shown to be feasible at Astral acquisition rates. Modification to acquisition software is required for compatibility with antibody full-MS, but appears fundamentally feasi

Introduction

Intact protein analysis imposes considerable challenge to mass spectrometer hardware. High charge loads from multiply charged ions, often at high m/z, combine with innately complex spectra, made worse by collisional scatter and minor fragmentation processes. Top-down analysis in Orbitrap™ -based instruments is consequently relatively slow, typically generating MS/MS spectra at 4 Hz.

A high mass mode of operation has been developed for the Orbitrap™ Astral™ mass spectrometer[1], to allow parallelized operation of Astral™ and Orbitrap analyzers for top-down methods. The Astral analyzer and its ion processor, shown in Figure 1, have been optimized and its high-speed, high-resolution acquisition assessed for measurement of a range of intact proteins. A hybrid Top-down DDA method could then be applied, far faster than Orbitrap analyzer-only experiments.

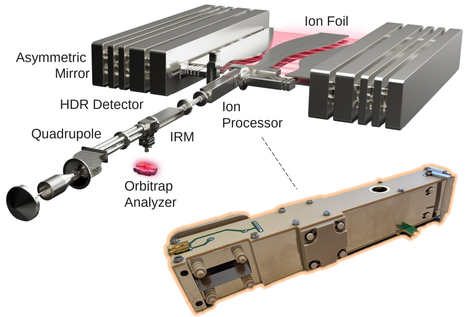

Figure 1. a) Ion optical layout of the Orbitrap Astral mass spectrometer. b) Photograph of the ion processor.

Method

An 'Intact protein' application mode was implemented combining optimization of key instrument parameters such as pressure with advanced peak deconvolution and charge state detection algorithms. Here the pressure in the ion processor, a dual pressure region extraction ion trap[2], was lowered along with that of the IRM, primarily to reduce the number of ions lost to collisional scatter.

The analyzer was assessed with the aid of infused protein standards such as myoglobin, carbonic anhydrase and the antibodies Humira and NISTmAb standard, and measured spectra compared to those produced by the conjoined Orbitrap analyzer.

Top-down LC/MS experiments were also performed with Pierce™ Intact Protein Standard Mix, injected into a Thermo Scientific™ Vanquish Neo™ LC system and separated on a Thermo Scientific™ MAbPac™ Capillary Reversed Phase HPLC Column (0.15 mm x 150 mm) then passed into the Orbitrap Astral mass spectrometer running a 22-minute Top 10 DDA method with Orbitrap Full-MS and Astral MS/MS analysis.

Results

Infused Intact Proteins.

Figures 2 and 3 show profile spectra of infused myoglobin and carbonic anhydrase respectively. Each spectrum is composed of 100 averaged shots, and at least several averages were required to build up sufficient ion statistics to show reasonable profiles. Compared to equivalent Orbitrap spectra it is notable how heavily populated the spectra are, with a large spread of single ions peaks around the known m/z values. There must be significant numbers of background peaks, including fragmented or scattered ions forming a chemical noise bed that is then averaged down. Reduction of the ion processor pressure was very helpful here. The level of averaging required to discern signal is also clearly mass dependent, as carbonic anhydrase full-MS proved more challenging to generate attractive isotopic envelopes. ~30 kDa is closing on the limit of what may be isotopically resolved.

Orbitrap mass analyzers destructive interference and possibly space charge effects serve to eliminate weaker signals producing relatively clean spectra. This is in many ways advantageous, though overall sensitivity is also attenuated; thus, the push towards single ion measurement methods.

Charge states were detectable via the Advanced Peak Detection algorithm. Deconvolution of overlapping peaks was generally successful, though for busy spectra occasional errors were observed producing apparently intense peaks.

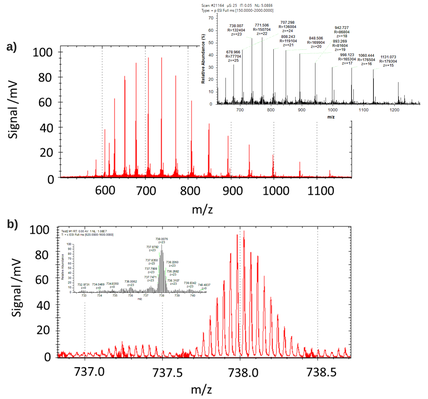

Figure 2. Astral mass spectra of intact 17 kDa myoglobin showing a) the charge state distribution and b) the isotopic envelope of at m/z 738.

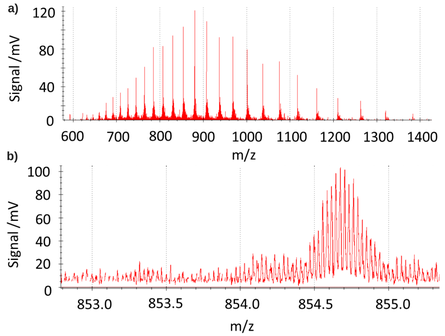

Figure 3. Astral mass spectra of intact 29 kDa carbonic anhydrase showing a) the charge state distribution and b) the isotopic envelope around m/z 855

Astral MS/MS

Carbonic Anhydrase was infused and m/z 854.7 isolated and fragmented. RAW files were recorded at CE=16, which produced good looking Orbitrap fragmentation, and similar to NCE-35 at this charge state. MS2 spectra are shown in Figure 4. The Astral fragmentation pattern looks similar to Orbitrap MS/MS, although the ratios of high m/z fragments appeared slightly lower, as no collision cell calibration had been made.

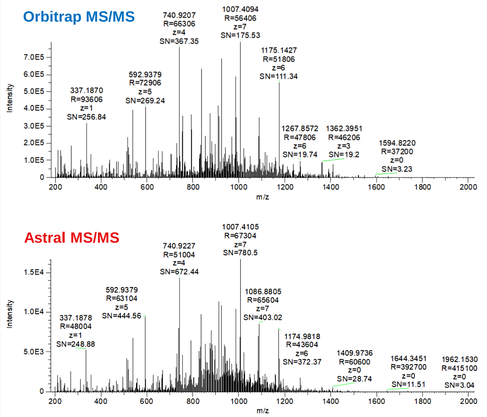

Figure 4. Comparison of carbonic anhydrase m/z 855 MS/MS measured in Orbitrap and Astral analyzers.

2.5 s of data were taken from the Orbitrap and Astral acquisitions and searched via ProSight™ Lite, with results shown in Figure 5. The Orbitrap search found 65 matching fragments, with 25% Residue Cleavages. The Astral data found 79 matching fragments and 28% Residue Cleavages. Only a small proportion of peaks were explained in the Astral, likely due to its sensitivity to large numbers of background and scattered single ions.

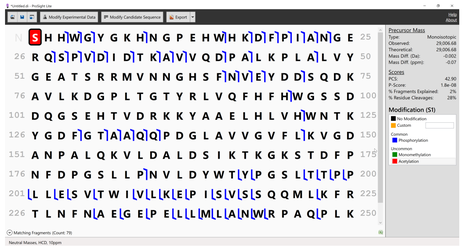

Figure 5. ProSight Lite analysis of carbonic anhydrase Astral MS/MS.

6-Protein DDA

22-minute DDA acquisition of the standard protein mixture demonstrated a good Orbitrap full MS chromatogram and many 10x averaged Astral data-dependent MS/MS. Many scans, such as shown in Figure 6, look very suitable for analysis, with good fragmentation and assignment of charge state. The results were analyzed with a modified Thermo Scientific™ Proteome Discoverer™ 3.1 (utilizing the ProSightPD™ node). All searches were performed against custom databases. All proteins were identified with a minimum score of >45 after filtering using an E-value cutoff of 1x10^{-5} and search engine rank 1.

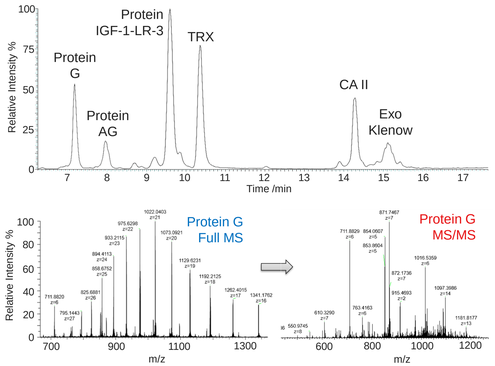

Figure 6. MS chromatogram and example 10 µscan MS/MS spectrum from a Top10 DDA measurement of Pierce 6-protein mixture.

Antibodies

Profile spectra generated from infusions of Humira and NISTmAB are shown in Figure 7. From a distance these look reasonable, with glycoforms resolved, and implies that the Astral analyzer is not fundamentally incompatible with such measurements. Currently however the peaks cannot be discerned by the instrument software, and the vast number of datapoints overburdens the existing peak picking algorithm. It may be that a suitable way forward is to simply bin together otherwise slow the acquisition rate of datapoints at the digitizer to better reflect the lower resolving power of antibody acquisition.

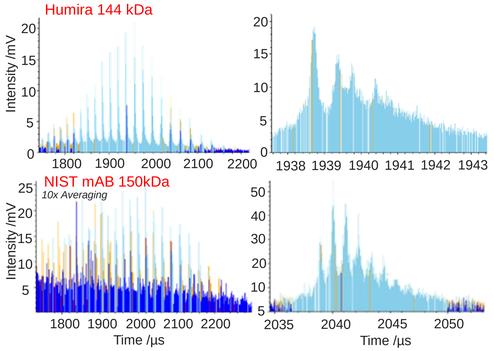

Figure 7. Profile spectra of the large antibodies Humira and NISTmAB, showing the resolved glycoforms.

Conclusions

The results so far demonstrate that the Astral analyzer, in low pressure protein mode, can produce high quality intact and top-down mass spectra.

- Intact measurement requires some averaging, more for higher m/z proteins, eventually hitting limits of resolving power and current instrument control software.
- Astral MS/MS of carbonic anhydrase is very comparable to Orbitrap measurement.
- Top10 DDA is functional, generating good spectra and promising for future development.

References

1. Stewart et al (2023). *Analytical Chemistry*, *95*(42), 15656-15664.
2. Stewart et al (2023). *Journal of the American Society for Mass Spectrometry*, *35*(1), 74-81.
3. Viner et al (2017). *Proceedings of the 65th ASMS Conference*, Indianapolis, Indiana.

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