Improvements for high resolution analysis on a modified Tribrid mass spectrometer

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Overview

Purpose: This work shows improvements in Orbitrap analysis, especially with respect to peak coalescence and resolution.

Methods: A Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer was modified by (1) improvements to the vacuum system aimed at reducing the amount of gas in the ion injection region and (2) careful matching of Orbitrap electrodes.

Results: Improvements in coalescence performance, resolution, and in examples from applications in top-down proteomics, lipidomics, and metabolomics are observed.

Introduction

The utility of FTMS analysis is limited by the nature of the trajectory of ions' orbits, namely the rate of decay of oscillations and by second-order effects such as additional fields due to machining imperfections and other ions. These factors govern available resolution, peak shape, space charge tolerance, and other quantities. In the case of Orbitrap instruments, Orbitrap injection is of critical importance, as the incoming energy defines the shape of the orbital path. In particular, for large molecules, collisions with neutral gas molecules are possible upon ejection (at ~2kV) from the C-trap and within the Orbitrap itself, leading to fragmentation and loss of packet coherence, causing ion loss and premature transient decay [1]. In this work we focus efforts on reducing the amount of gas that is allowed to pass into the Orbitrap chamber, leading to improvements in signal-to-noise (S/N) for intact proteins and improved results for top-down experiments.

Another recurring problem in FTMS is that of peak coalescence or self-bunching [2-3], which results when two ion clouds closely spaced in frequency couple together, producing a single measured frequency in the transformed data. In the case of Orbitrap instruments, this behavior can be minimized by carefully choosing electrode sets that closely match the ideal profile. Both of the above changes can provide benefits for analysis at resolutions up to 1M (at m/z 200).

Methods

Samples, infusion, and liquid chromatography

The standard calibration solution (Figs. 5, 6, 8): a mix of n-butylamine, caffeine, the peptide MRFA, and Ultramark dissolved in 50:49.9:0.1 acetonitrile, water, and formic acid. ***Intact proteins (Figs. 3, 4)***: Horse heart myoglobin and carbonic anhydrase II from bovine erythrocytes (Sigma-Aldrich, St. Louis) were dissolved in 50:49.9:0.1 water:methanol:formic acid to a concentration of 1 µM. ***Egg PC (Fig. 7)***: 25 mg of L-α-phosphatidylcholine (chicken egg) was purchased as a solution in 100% chloroform (Avanti Polar Lipids, Alabaster, Alabama), and diluted 10:1 in 1:1 isopropyl alcohol and methanol. ***Infusion***: All infusions were done at a flow rate of 3 µL/min. ***Metabolomics (Fig. 9)***: a urine sample was taken after a single oral dose of lansoprazole (30 mg) with collection pre-dose and from 0-8 hours after dose. The urine was prepared by solid phase extraction on a Thermo Scientific™ Hypersep™ C18 column. 10mL of urine was loaded onto the column, washed, and eluted with 1 volume each of methanol and ACN. The eluate was evaporated under nitrogen gas and reconstituted in 500µL of 90:10 water:ACN, and analyzed as described. ***LC/MS***: sample was eluted at 400 µL/min over a 20-min water/ACN gradient, 5-30% ACN (min 1-9), and 30-95% ACN (min 9-16), using a Thermo Scientific™ Dionex™ UltiMate™ 3000 system. All mass spectrometry was performed on a modified Orbitrap Fusion mass spectrometer (Fig. 1). Note, all quoted resolutions are with respect to *m/z* 200.

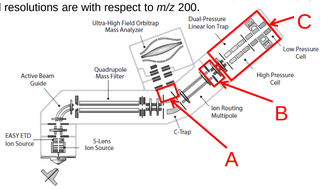

FIGURE 1. Modified Orbitrap Fusion mass spectrometer, with areas of modification: A, material removed to allow better gas conductance; B, nitrogen gas allowed to pass into dual linear trap chamber; and C, increased pumping capacity from 80 l/s to 290 l/s to handle the increased gas load from (B).

Results

Metric for assessing gas flow to analyzer chamber

We evaluated the effectiveness of the modifications in Figure 1 by performing internal leak rate tests, wherein the ion routing multipole (IRM) collision gas is varied, the cold-cathode gauge (Orbitrap analyzer chamber) monitored, and the slope recorded. This leak rate is limited by conductance limits in the system, and can be inflated in cases of malfunctioning hardware. We generally observed a decrease of about 1.5-2.0x in this slope on instruments with these modifications compared to instruments without these modifications, as indicated in Figure 2.

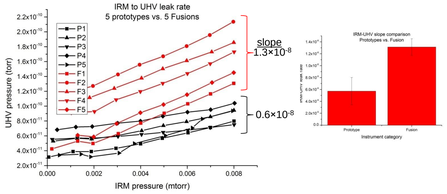

FIGURE 2. IRM to UHV leak rates, prototypes with vacuum modifications compared with Orbitrap Fusion systems. Higher base pressure in Orbitrap Fusion systems is due to initial system pumpdown.

Signal-to-noise ratio improvements

Reducing the amount of nitrogen at the exit of the C-trap reduces the probability of ion-neutral collisions which can result in signal loss in the Orbitrap, as described in the Introduction. We observed a 30-50% improvement in decay rate of the +32 charge state of carbonic anhydrase on the same instrument, over data collected prior to the hardware changes. The improvement is pressure dependent, and is larger at higher pressures, as shown in Figure 3 (top).

Trapping and transfer efficiency play a large role for S/N as well, and at the low pressures needed for protein mode, efficient ion capture prior to Orbitrap analysis is easier with the described changes. We observed similar S/N at lowest pressures but improved S/N at higher IRM pressures, comparing a prototype instrument with the changes to an unmodified Orbitrap Fusion MS, as shown in Figure 3 (bottom).

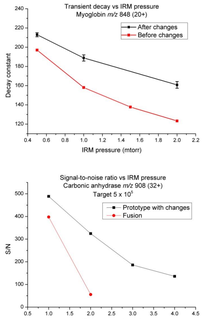

FIGURE 3. (top) Transient decay versus IRM pressure, on the same instrument before and after the changes. Decay is faster (smaller time constant) at higher pressures prior to the changes. Myoglobin 20+ is used because isolating this charge state results in a transient comprised of 8 beats. Therefore at higher pressures where fewer beats are visible, decay can still be accurately measured.

(bottom) Signal-to-noise ratio for carbonic anhydrase for two different instruments, a prototype with the changes and an unmodified Orbitrap Fusion MS. S/N is comparable at lowest pressures (< 1 mtorr), while improvements are observed at higher pressures (≥ 1 mtorr).

Improvement for top-down proteomics

Reduced IRM pressure is typically necessary for obtaining isotopic resolution of medium-sized proteins (< 40 kDa), and typically lower pressures are required for larger proteins. Diminishing returns result, however, as pressure is lowered beyond the point where ions can be trapped, possibly fragmented, and transferred efficiently. Below we show improved protein coverage for carbonic anhydrase for HCD and CID fragmentation.

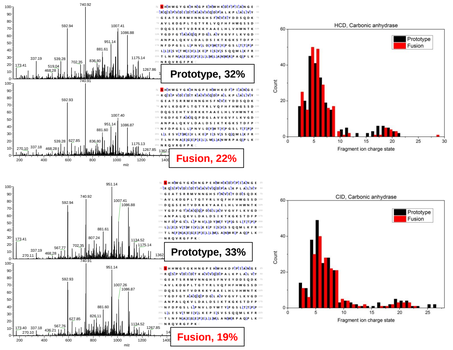

FIGURE 4. HCD (top) and CID (bottom) fragmentation of carbonic anhydrase II, showing improvement in sequence coverage, likely due to better transmission of higher charge state fragments. Note that coverage maps show more large fragments in the prototype data. Spectra shown are taken at 50 µscans, 240k resolving power, and 1 mtorr IRM pressure, except for Orbitrap Fusion MS HCD data, taken at 3 mtorr IRM pressure.

Optimal electrode matching: improvement for coalescence

Peak coalescence results from ion-ion interactions that couple two ion clouds of different nominal m/z, such that they oscillate together at a single resultant frequency [2-3]. A method for measuring this effect and comparing system-to-system is to measure the signal-to-noise ratio (S/N) at which two peaks coalesce.

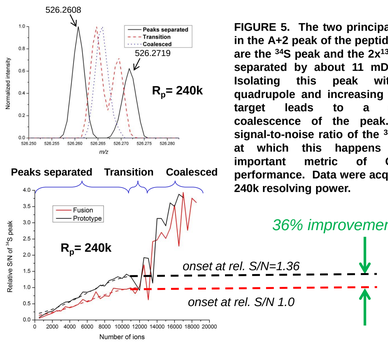

FIGURE 5. The two principal peaks in the A+2 peak of the peptide MRFA are the ^{34}S peak and the $2x^{13}C$ peak, separated by about 11 mDa (left). Isolating this peak with the quadrupole and increasing the ion target leads to a gradual coalescence of the peak. The signal-to-noise ratio of the ^{34}S peak at which this happens is an important metric of Orbitrap performance. Data were acquired at 240k resolving power.

Optimal electrode matching: improvement for coalescence, continued

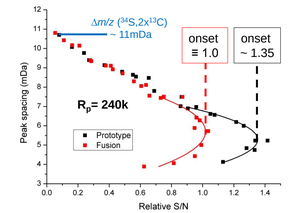

Figure 6. Replotting of data in Figure 5, to account for difference in transmission between the two systems. Peak spacing decreases from the correct initial spacing of ~11mDa down to ~5 mDa at onset. Results show a modest but significant gain in coalescence behavior for the prototype. Data collected at 240k resolving power.

Application: separating closely spaced peaks at 500,000 resolving power

One challenge of general concern is the ability to separate a low-abundance ion from a much more abundant ion of very similar *m/z*. In the example below, we show separation of the monounsaturated form of chicken egg phosphatidylcholine (PC 16:0,18:1), from the $2x^{13}C/^{18}O$ isotope of the doubly-unsaturated form, at 500k resolving power.

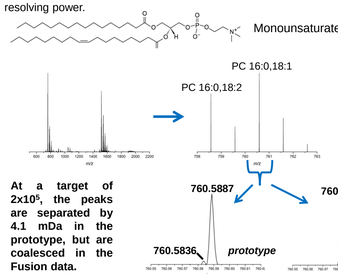

FIGURE 7. The monounsaturated form at m/z 760.588 is nominally about 9 mDa separated from the $2x^{13}C/^{18}O$ isotope of the doubly unsaturated form.

At a target of $2x10^5$, the peaks are separated by 4.1 mDa in the prototype, but are coalesced in the Fusion data.

Optimizing performance for high resolution applications

For resolving power ≥ 500,000 (at *m/z* 200), performance demands are elevated and acceptable performance is available across a narrower range of device parameters. In particular, it becomes difficult to find systems which can adhere to isotope ratio specifications while simultaneously delivering better-than-average performance in terms of peak coupling and coalescence (as measured by the test described in Figures 5 and 6). However, by carefully matching electrodes and with careful tuning, such a balance between "hard" and "soft" traps can be found [3].

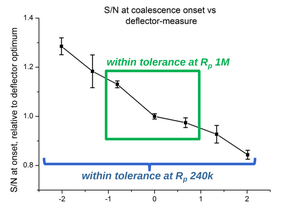

FIGURE 8. Subset of acceptable tuning range. All displayed points pass production specifications for isotope ratio fidelity and coalescence, but only the points in the green box pass isotope ratio specifications at 1M resolving power. Without careful electrode matching, no parameters can be found yielding acceptable performance at higher resolutions.

Application: high resolution metabolomics

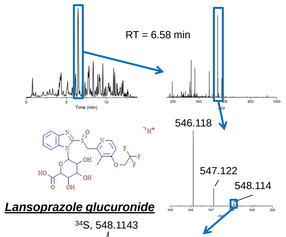

FIGURE 9. Drug metabolism is an important subject in biology, medicine, and the pharmaceutical industry. Here we show an example from the metabolism of the drug lansoprazole. At bottom left we show the glucuronide metabolite of lansoprazole, showing higher oxygen content compared with bare lansoprazole (bottom right) from the same sample. The separation between the 18O isotope and the 2x13C isotope is 2.1 mDa.

Conclusions

High resolution analysis is improved with modified vacuum configurations and carefully matched Orbitrap analyzer electrodes as follows:

- Improvements in transient decay and signal-to-noise ratio.
- Improvements in results from top-down proteomics experiments where both isotope resolution and trapping and transfer characteristics are important
- Improvements in coalescence characteristics as well as in performance metrics for resolving powers ≥ 500,000
- Improved results from examples in lipidomics and metabolomics

References

1. A. Makarov and E. Denisov, *J. Am. Soc. Mass Spec.* (2009), **20**, 1486-1495.
2. D.W. Mitchell and R.D. Smith, *Phys. Rev. A*, (1995) **52** (4), 4366; A. Kharchenko *et al., J. Am. Soc. Mass Spec.* (2012), **23**, 977-987.
3. A. Makarov, et al., "Crowd Control of ions in Orbitrap mass spectrometry," ASMS 2012.

Acknowledgements

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Please see the following ASMS 2015 presentations for more information:

L. Fornelli et al., "Improved Top-Down and Middle-Down Characterization of Complex Biopharmaceuticals on a Modified Tribrid Mass Spectrometer," Oral, WOH, 2:30-4:30pm Wednesday 6/3

S. Sharma, et al., "Optimizing Top Down Analysis of Proteins on an Orbitrap Fusion Mass Spectrometer," Poster 393, Tuesday 6/2

R. Viner, et al., "Optimization of LC/MS intact/top-down protein analysis on an Orbitrap Fusion mass spectrometer," Poster 395, Tuesday 6/2