A modified Orbitrap Tribrid MS enables simultaneous manipulation of three ion populations to improve sample throughput, coverage, and sensitivity

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Abstract

Here, we evaluated a modified Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometer (MS) that enables simultaneous manipulation of three ion populations to improve sample throughput, coverage, and sensitivity. Specifically, we analyzed new hardware including, dual ion routing multipoles (IRMs), that increase proteome coverage by interrogating more precursors in less time, and a modified ion funnel designed to capture ions under gentle conditions to improve the transfer of labile compounds including phosphopeptides. We analyzed 1µg of a human cell digest using 30min gradients and a wide-window acquisition strategy. We found that using the same gradient we could produced a 34% increase in unique peptides identified, or we could shorten our gradient and obtain similar coverage. To test the softer source, we analyzed 0.5 µg of phophopeptides enriched from a human cell line using a 90 min gradient, and we observed a 18% improvement in the numbers of unique phosphopeptides identified, and a corresponding 25% improvement in the modification site localization. In summary, we observed that the modified Orbitrap Tribrid MS, recently introduced as the Thermo Scientific™ Orbitrap Ascend™ Tribrid MS, enables improved proteome coverage in less time than previous instruments.

Introduction

To better understand the function of proteins in complex biological systems, it is necessary to measure changes in protein abundance across temporal and biological conditions. Unlike affinity-based approaches that are dependent on the specificity of consumable reagents, mass spectrometry-based proteomics can measure the proteoform diversity of sequence variations, splice isoforms, and post-translational modifications (PTMs) with high sensitivity and selectivity. Recent advances in mass spectrometry have allowed for faster and more sensitive acquisition of proteomics data. This is especially true using the Orbitrap Tribrid architecture, where there are a plethora of analyses available including multiplexing[1] and real time search[2] for protein or metabolomics research. Herein we will discuss recent architectural changes to the Orbitrap Tribrid MS line which allow for, in addition to all previous experimental types, the most robust and highest quality data of any system yet. This includes allowing for more labile analytes to be transferred more gently into the MS. Phosphorylation is arguably one of the most important and studied PTMs in proteomics, but it is notoriously labile. Losing the phosphorylation site means losing vital cellular information. Proof of concept data utilized to demonstrate the versatility of the new system includes the benchmark HeLa standard, a standard phosphopeptide enrichment, and native GroEL, a 14mer of approximately 800,000 Da size. These are commonly implemented benchmarks for instrument capabilities and limitations.

Materials and methods

0.5 µg of enriched phosphopeptides were analyzed using a 90min gradient on using a 50cm Thermo Scientific ™ EASY-spray column OT/OT scans of high resolution Orbitrap MS1 scan of 120,000 FWHM resolution @ 200 m/z, and Orbitrap MS2 scan of 7.5K FWHM @ 200 m/z with 27 ms max ion injection.

1 µg of Thermo Scientific ™ Pierce™ HeLa protein digest standard was analyzed using a 50cm EASY-spray column with 4 amu isolation window for MS2 scan. Data was processed in Thermo Scientific™ Proteome Discoverer software 3.0 include CHIMERYS™ by MSAID ™ @1% false discovery rate (FDR).

GroEL sample was diluted to 1.25 µM in 100 mM ammonium acetate and infused via static nanospray. Tune parameters were optimized to in-source CID of 225 V and source compensation of 0.15, and a MS resolution of 7,500.

ALELFR was purchased and diluted in 50/50 Methanol/Water with 0.1% Formic Acid to a concentration of approximately 10 pmol/µl. Sample was infused into the MS with a mass range or 150-2000 Th, source RF set to 60% and Capillary temperature set to 300ºC.

Figure 1. The Orbitrap Tribrid Ascend MS on the right coupled to a Thermo Scientific™ FAIMS Duo Pro interface and Thermo Scientific™ EASY-nano ion source, and a Thermo Scientific™ Vanquish Neo ™ UHPLC system on the left. The new architecture has not resulted in a significant physical benchtop change in the instrumentation, and existing dedicated lab-space can be utilized for any upgraded systems.

Results

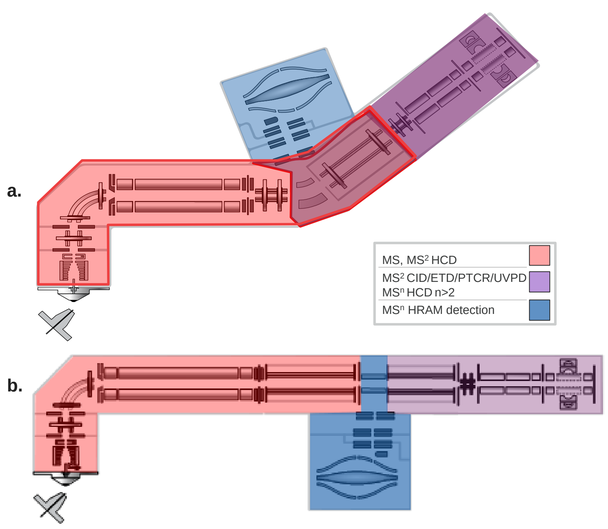
The modifications in the new Orbitrap Ascend Tribrid MS resulted in major improvements to identification of multiple different test cases. Our first test utilized a complex HeLa protein digest standard, which resulted in a 33% increase in MS2 scans possible in the same time period compared to the Thermo Scientific™ Orbitrap Eclipse Tribrid MS. This increase in the number of MS2 is a result of utilizing a front and back IRM for simultaneous ion fill and ion fragmentation and injection into the c-trap/orbitrap. This duality was not possible in the previous design, though in precious iterations, ions could still be parallelized, just not to this extent. The faster scan speed –due to the increase in ion parallelization efficiency- led to a 34% increase in the number of unique peptides identified. Interestingly, if we look at the number of unique peptides or the number of protein groups identified, we have nearly identical number of IDs from a 45min gradient on the Orbitrap Eclipse Tribrid MS, and the 30 min gradient on the Orbitrap Ascend Tribrid MS. Concluding that we can decrease the gradient by up to 50% and still achieve the same number of IDs using the new architecture.

In the second test case, we ran a phosphopeptide enriched sample to not only test that there would be an increase in the number of identifications, but to prove that the softer ion source will produce more intact peptides, allowing for more confident identification of the phosphorylation site. This was shown in a 25% increase in modification site ID and a general density distribution in the Xcorrs being shifted to the right on the Orbitrap Ascend Tribrid MS compared to the Orbitrap Eclipse Tribrid MS. We attribute this to the softer ion source retaining more of the PTM, allowing for more accurate, correct fragmentation patterns through Thermo Scientific™ Proteome Discoverer™ software analysis.

Our last test case was to see how the extended mass range (HMRn+) mode would work using GroEL, a 14mer complex in native mode. We were able to see the complex centered

Alterations in the Obritrap Tribrid Architecture Improve Ion Management

Figure 2. The original architecture of the Orbitrap Tribrid series is shown in **figure a** with the curved c-trap and single ion routing multipole (IRM). This revolutionary structure allowed for ions to be packeted into three regions of the system over the course of the run. Ions are packeted into the IRM (for cooling or HCD fragmentation) in parallel with m/z anaylsis. Ions can also be sent to the ion traps for secondary detection, fragmentation, or isolation, separate from storage in the IRM or orbitrap. However, there has always been a limiting factor in that ions cannot be collected in the IRM while another packet is being cooled or fragmented. This can be seen in the mixture of the peach and blue color overlapping the IRM and the whole region is highlighted in red. In the new architecture seen in **figure b**, there is a vital addition which allows for ion accumulation and HCD fragmentation in the front IRM and cooling and injection into the orbitrap in the back IRM. This change allows for ions to fully utilize the parallelization of ion accumulation.

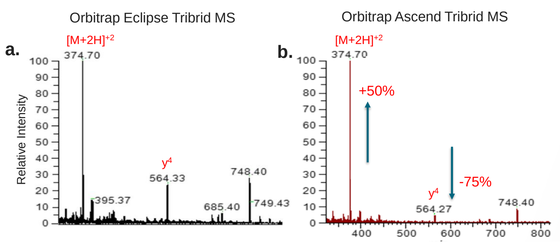


Faster Scan Speed Leads to More Identifications

Figure 3. Comparative data from HeLa protein digest standard run on the Obritrap Eclipse Tribrid MS (Blue) and the Orbitrap Ascend Tribrid MS (Red). The amount of MS/MS spectra acquired within a 45 min run **(a.)** gives nearly a 33% increase in MS2 scans aquired. In terms of actual identifications, we see a large increase in the number of unique peptides **(b.),** with a 30 min run giving a 34% increase in identifications. When we look at protein groups **(c.),** we can see that the 30 min run on the Ascend results in a 15% increase. Interestingly, whether by peptide or protein IDs, we see that the 30min Orbitrap Ascend Tribrid MS data, and the 45 min Orbitrap Eclipse Tribrid MS data have nearly identical number of IDs, thus enabling increased sample throughput on Orbitrap Ascend Tribrid MS without sacrificing coverage.

Alterations in Source for Gentle Conditions

Figure 4. MS1 spectra of the fragile peptide ALELFR where 374.70 Th is the +2 charge state of the precursor, 748.40 Th is the +1 charge state of the precursor, and 564.33 Th is the most abundant fragment (y^4) of the peptide. MS1 spectrum from the Orbitrap Eclipse Tribrid MS **(a.)** compared to MS1 spectrum from the Obritrap Ascend Tribrid MS with a modified source **(b.)** shows a gentler transmission. We see a 50% increase in the intact precursor and 75% decrease in the fragment on the Orbitrap Ascend Tribrid.



Phosphopeptide identification/characterization comparison between old and new architecture

Figure 5. Phosphorylation is a biologically relevant and important post translational modification. Its importance is undercut by its fragility in common MS analysis. Below, we see the difference between the Orbitrap Eclipse Tribrid MS (Blue) and the Orbitrap Ascend Tribrid MS (Red) in the identification of phosopeptides (left) and the exact determination of the phosphorylations' modification site (right). We see a 17% increase in phosphopeptide analysis in the Orbitrap Ascend Tribrid MS compared to the Orbitrap Eclipse Tribrid MS. That increase can be due to a combination of the increased speed of the new architecture, and the implementation of the softer ion funnel source. There is a 25% increase in the identification of the modification site when comparing the Orbitrap Eclipse Tribrid MS and the Orbitrap Ascend Tribrid MS. This increase is a direct result of less in-source dissociation, allowing for the labial modification site to be more retained in the new system when compared to the previous iteration.

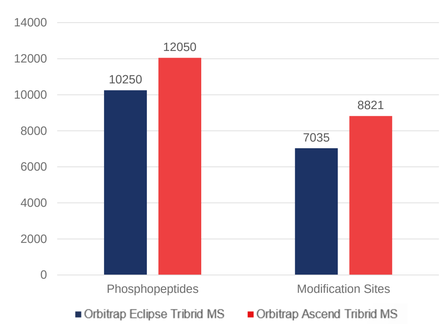
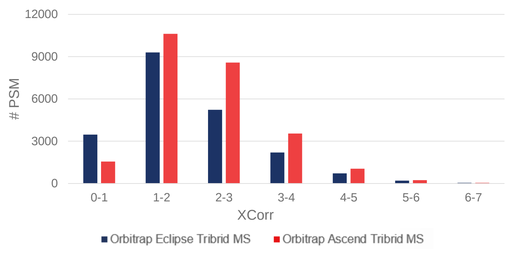
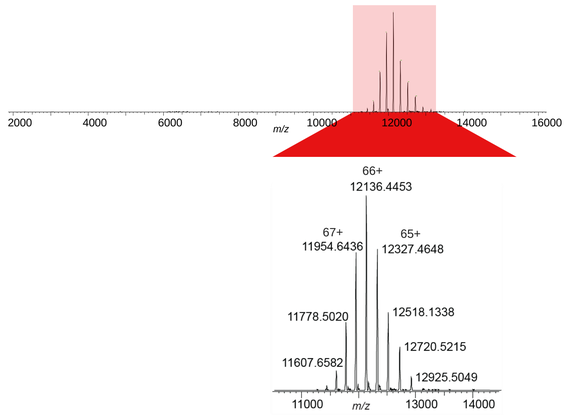


Figure 6. The Xcorr (cross correlation) measures the goodness of the fit between a peptide's theoretical fragmentation spectrum and the experimental fragmentation MS/MS. The Xcorr is used in conjunction with the SEQUEST® algorithm to assess the quality of the match. The larger the corss correlation, the more the experimental spectrum matches the theoretical. In the figure below, we show that in the analysis of phosphopeptides, the gentler source on the Orbitrap Ascend Tribrid MS leads to better matching of the fragmentation pattern due to better retention of the labile modification.



Extended m/z Range Allows Analysis of Large Complexes

Figure 7. The Orbitrap Ascend Tribrid MS has double the extended m/z range of the Orbitrap Eclipse Tribrid MS. This allows for the analysis of larger complexes, including GroEL, shown below. GroEL has a theoretical native intact mass as a 14mer of approximately 801 kDa.



Conclusions

- Dual IRMs allow for 33% faster gradient acquisition for same number of IDs as old architecture and 34% increase in the number of IDs on the same gradient length.
- Softer modified ion source shows 50% increase in the intact precursor for ALEFFR peptide and 75% decrease in the y4 fragment ion compared to usual ion funnel.
- The combination of changes detailed above allow for a 25% increase in modification site ID. The increase can be explained by higher quality MS2 matches by Xcorr.
- Extended m/z range will allow the first in the Orbitrap Tribrid MS series to scan up to 16,000 m/z, seeing the 14mer complex GroEL in native state with clear, distinct charge state distribution and signal to noise.

References

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