WP397

Selective Glycopeptide Signal Enhancement Using the Athena Ion Processor in the Collision Cell on a timsUltra AIP system

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Introduction

Glycopeptide analysis by mass spectrometry remains challenging due to low ionization efficiency, reduced precursor signal intensity, and the labile nature of glycan modifications. These limitations compromise MS/MS spectral quality and fragment ion abundance, particularly for Y ions critical to glycan structure assignment. Conventional approaches to improving sensitivity, such as increased injection amounts or extended gradients, often introduce trade-offs in throughput and sample consumption. Enhancing ion transmission at the hardware level offers a more direct solution. In this study, we evaluate the Athena Ion Processor (AIP), a novel RF-based ion manipulation device integrated into the collision cell of the timsTOF Ultra, for its ability to selectively enhance glycopeptide precursor and fragment ion transmission across defined m/z and ion mobility ranges.

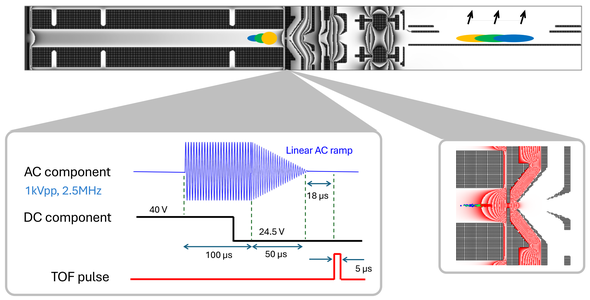

Fig. 1 Schematic illustrating the Athena Ion Processor (AIP) mechanism within the collision cell.

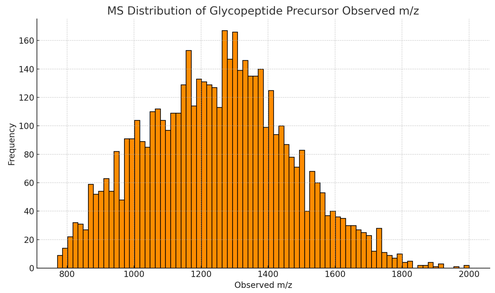

Fig. 2 Distribution of glycopeptide precursor ions across the m/z range, showing their spread relative to charge state.

Methods

Alpha-1-acid glycoprotein digest (10 ng/µL) was used as a standard to optimize AIP ion transmission parameters via direct infusion on a timsTOF Ultra system. AIP RF amplitude, slope, and storage base time were systematically tuned to selectively enhance ion transmission within targeted m/z windows. Performance was assessed by monitoring precursor signal intensity, diagnostic oxonium ions, Y ions, and overall MS/MS spectral quality across multiple AIP configurations. Optimized AIP parameters were then applied to biological samples: HILIC-enriched N-glycopeptides from HeLa cell digests were analyzed on the timsTOF Ultra AIP platform coupled to a nanoElute LC system. For each sample, 100 ng was injected and separated over a 30-minute gradient.

Results

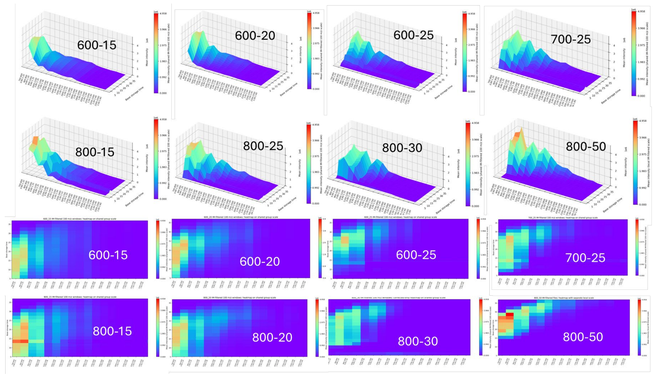

Fig. 3 Targeted m/z range scans (filtered for z>1) acquired under different AIP profiles varying RF, slope, and base storage time. Larger RF and steep slope result in a more focused heatmap for higher m/z

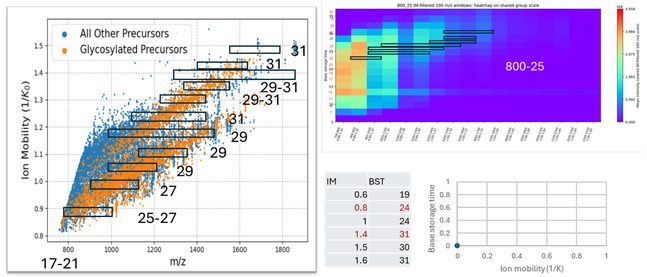

Fig. 4 Longer base storage times preferentially retain larger ions in m/z. Use a mobility-mass heat map to identify the optimal base storage time, then apply it as a guide for ramping base storage time as a function of IM in MS1.

#	m/z	IM (1/K0)	z	Peptide	Glycan
1	887.697	0.91-1.05	3	QNGTLSK	2N(1913)
2	893.029	0.9-1.15	3	QNGTLSK	2N(1929)
3	990.061	0.95-1.0	3	QNGTLSK	2N(2220)
4	1002.928	1.0-1.15	2	QNGTLSK	2N(1257)
5	1111.771	1.0-1.1	3	QNGTLSK	2N(2585)
6	1156.473	1.15-1.2	2	QNGTLSK	2N(1564)
7	1339.039	1.25-1.3	2	QNGTLSK	2N(1929)
8	1438.102	1.35-1.4	2	TFMLAASWNGTK	9N(1548)
9	1476.59	1.3-1.37	2	QNGTLSK	2N(2204)
10	1774.213	1.45-1.51	2	TFMLAASWNGTK	9N(2220)

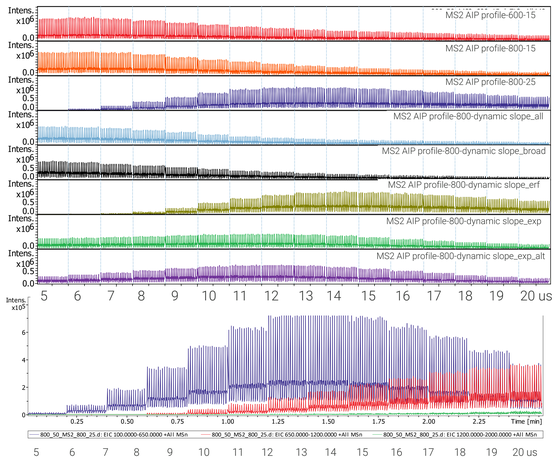

Fig. 5 MS2 AIP settings require broader transmission spanning lower-mass oxonium ions, medium-mass b,y ions, and larger-mass Y ions. MRM-based experiments were used to evaluate MS/MS spectral quality of target glycopeptides.

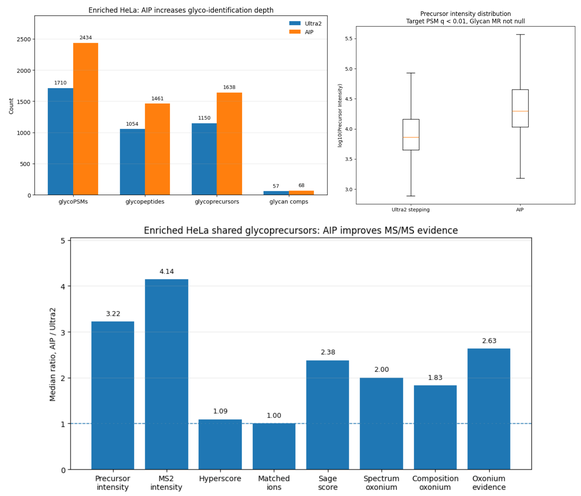

Fig. 6 In a 30-min run of HILIC-enriched HeLa, AIP increased glycoPSMs by 42.3%, unique glycopeptides by 38.6%, and unique glycoprecursors by 42.4% relative to Ultra2, with improved precursor intensity and MS/MS spectral quality.

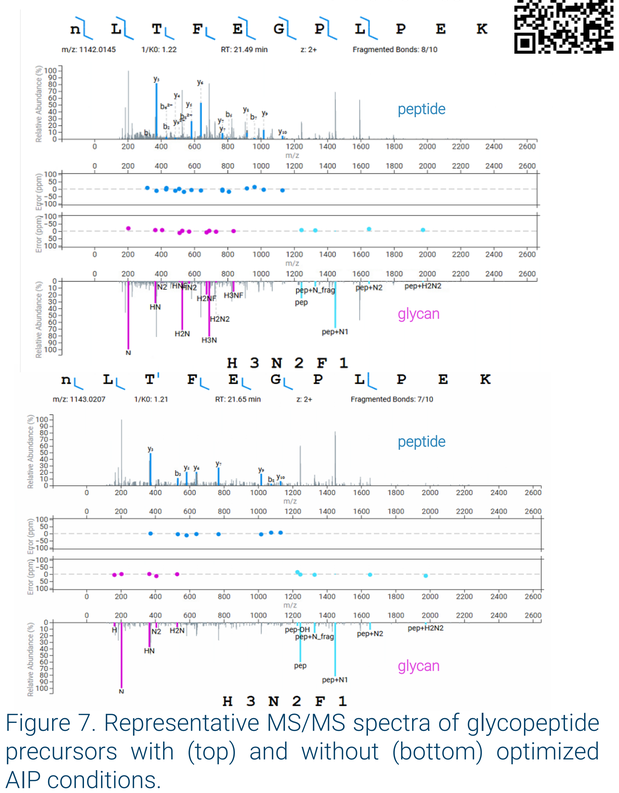

Figure 7. Representative MS/MS spectra of glycopeptide precursors with (top) and without (bottom) optimized AIP conditions.

Summary

Systematic optimization of the Athena Ion Processor (AIP) on the timsTOF Ultra significantly improved glycopeptide analysis. Tuning RF amplitude, slope, and base storage time enabled selective enhancement of precursor ion transmission across defined m/z and ion mobility ranges. Optimized AIP settings increased precursor abundance and improved MS/MS spectral quality, with enhanced Y ion intensities critical for confident glycan structure assignment.

Conclusion

- AIP RF tuning selectively enhanced glycopeptide precursor transmission and improved MS/MS spectral quality.
- Ion mobility–guided AIP ramping enabled m/z-specific enhancement, increasing Y-ion intensity.
- The optimized AIP workflow improved glycopeptide identification in HILIC-enriched HeLa samples.

Technology

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