Development and Application of SLIM-based Mobility-Aligned Fragmentation for Protein Analysis

Lauren C Royer; Joshua K McBee, PhD; Kelly L Wormwood Moser, PhD; Daniel DeBord, PhD; Jacob McCabe, PhD | MOBILion Systems, Inc. Chadds Ford, PA

Abstract

The combination of high-resolution ion mobility (HRIM) with high resolution mass spectrometry (MS) represents an incredibly powerful analytical approach for proteomic analysis. However, for unambiguous identification of peptide digests, fragmentation analysis is required. Many approaches have been developed to achieve fragmentation-enabled ion mobility mass spectrometry measurements, including SWATH-MS [1], HDMS^E [2], PASEF [3], and CV-stepping [4]. Here, we introduce an alternative approach termed mobility-aligned fragmentation (MAF) which exploits the HRIM domain of a 13m SLIM device to generate arrival-time aligned precursor and fragment ions. Rather than isolating with the quadrupole (e.g., typical DDA or DIA methods), the MS/MS spectra are filtered based on the ATD of the MS1 peptides. A standard Bovine Serum Albumin (BSA) digest was used to prototype the LC-IM-MS/MS data analysis workflow and demonstrate successful fragmentation analysis.

Methods

Data Acquisition - Waters MassPREP BSA digestion standard was prepared and analyzed on a MOBIE™ HRIM module (MOBILion Systems) coupled to a 6546 Q-TOF (Agilent Technologies). An Agilent 1290 Infinity II LC was used for sample introduction. The standard was analyzed by 30 and 90-minute reverse phase LC gradients and by direct infusion. Duplicate runs with collision energies (CE) of 0V and 30V were used to generate precursor and product ion spectra.

LC-IM-MS/MS Data Analysis - HRIM Data Processor (HRIM-DP) and PNNL Preprocessor Version 3.0 (2021.04.21) were used to prepare the MAF data files for downstream analysis in Skyline. A Proteomics search was initiated by importing the BSA FASTA sequence into Skyline to build a library of common tryptic BSA peptides for DIA MS/MS. A custom target library was created based on the 90-minute gradient data file. Retention time, *m/z*, and arrival time peaks were confirmed by manual review. Assignments of precursor and product ions were confirmed based on mobility peak alignment in the IM-MS heatmap (IM-MS Browser, Agilent) and exported arrival time values from Skyline generated reports.

IM-MS/MS Data Analysis - Direct infusion data were imported into Skyline as separate high and low energy files with the mobility separation (Collision Cross Section calibrated) substituted for LC retention time. CCS values from the mobility library were used as retention time windows in the Skyline feature list.

MAF Data Processing Workflow

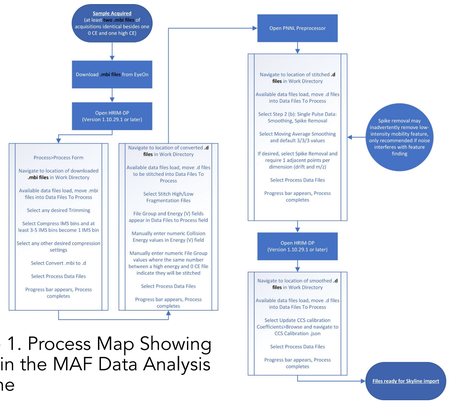


Figure 1. Process Map Showing Steps in the MAF Data Analysis Pipeline

MAF Data File Creation (Stitching)

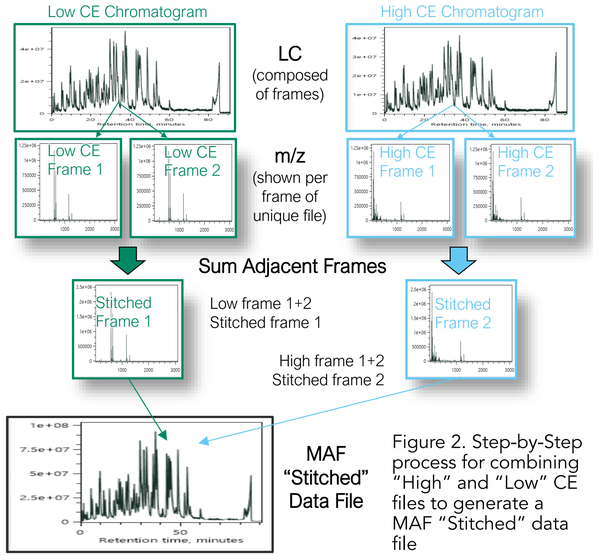


Figure 2. Step-by-Step process for combining "High" and "Low" CE files to generate a MAF "Stitched" data file

Infusion Data Reduction via Arrival Time to Retention Time Conversion

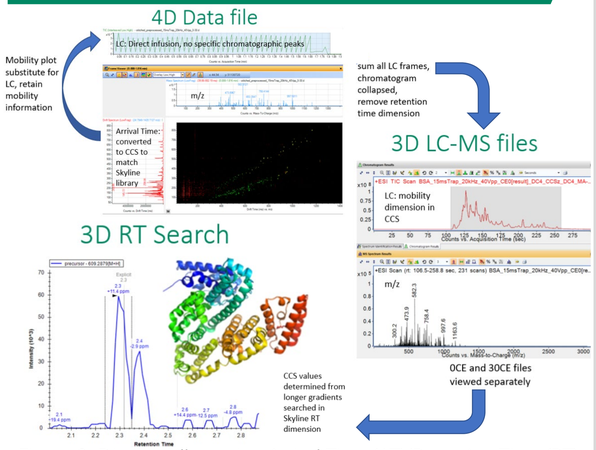


Figure 3. Diagram illustrating Arrival Time (AT)/Retention Time (RT) substitution of direct infusion sample and adjusted feature list for Skyline search

High/Low MAF Data Visualization

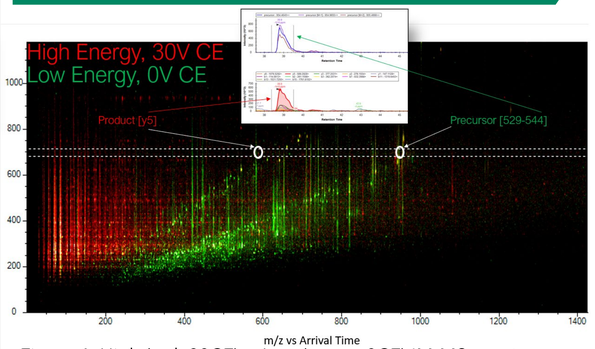
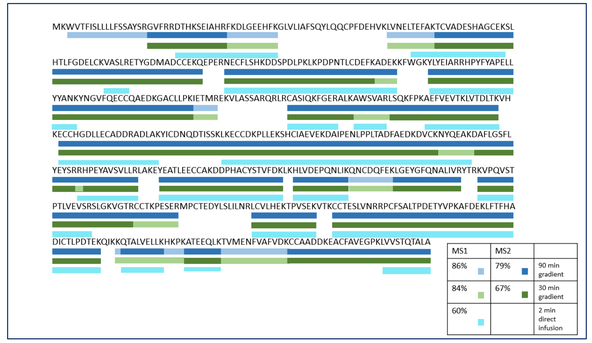


Figure 4. High (red, 30CE) + Low (green, 0CE) IM-MS spectra overlay illustrating AT alignment of precursor & product ions from 90 min BSA digest analysis. The +2 precursor ion of BSA [529-544] is aligned by arrival time with the [y5] product ion in the IM-MS Browser heatmap. These ions also elute in the same LC peak as highlighted in the Skyline chromatogram plot.

BSA MAF MS/MS Sequence Coverage



MS1	MS2	
86%	79%	90 min gradient
84%	67%	30 min gradient
60%		2 min direct infusion

Figure 5. MS1 & MS2 sequence coverage across 90 min LC, 30 min LC, and 2 min direct infusion BSA digest analyses.

Conclusions

- LC-HRIM-MS acquisitions of unique collision energies produce files with data recognized as precursor and product ions (MS/MS) in Skyline
- Skyline data file compatibility enables semi-automated processing equivalent with traditional MS/MS analysis
- Mobility Aligned Fragmentation analysis provides a data independent acquisition methodology capable of rapid MS2 spectra generation using HRIM precursor isolation
- Initial experiments demonstrate proof of concept for a DIA protein fragmentation workflow via direct infusion analysis

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1. Scientific Data, 2020, 7(1), 389. 2. Nature Methods, 2014, 11(2), 167-170.3. Journal of Proteome Research, 2015, 14(12), 5378-5387. 4. Analytical Chemistry, 2018, 90(15): 9529-9537.