Poster Reprint

ASMS 2024
Poster number MP 473

Enabling Protein and Oligonucleotide Ion Mobility Data Analysis in BioConfirm with PNNL PreProcessor Data Conversions

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Introduction

Ion mobility spectrometry has long played a key role in biomolecular research with many early applications focusing on protein and oligonucleotide structure. Proteomics workflows now include IM as more efficient protein coverage is made possible by the additional mobility separation and corresponding fragmentation. Oligonucleotide workflows benefit from the ease of fragmenting multiple charge states within the same experiment. Despite the adoption of IM into these workflows to perform efficient experiments, downstream software does not typically support the additional IM dimension. To avoid this common barrier to data analysis, a newly developed conversion in the PNNL Preprocessor takes advantage of linking precursor and fragment ions in the IM dimension and generates a traditional DDA LC-MS data file that many software can process.

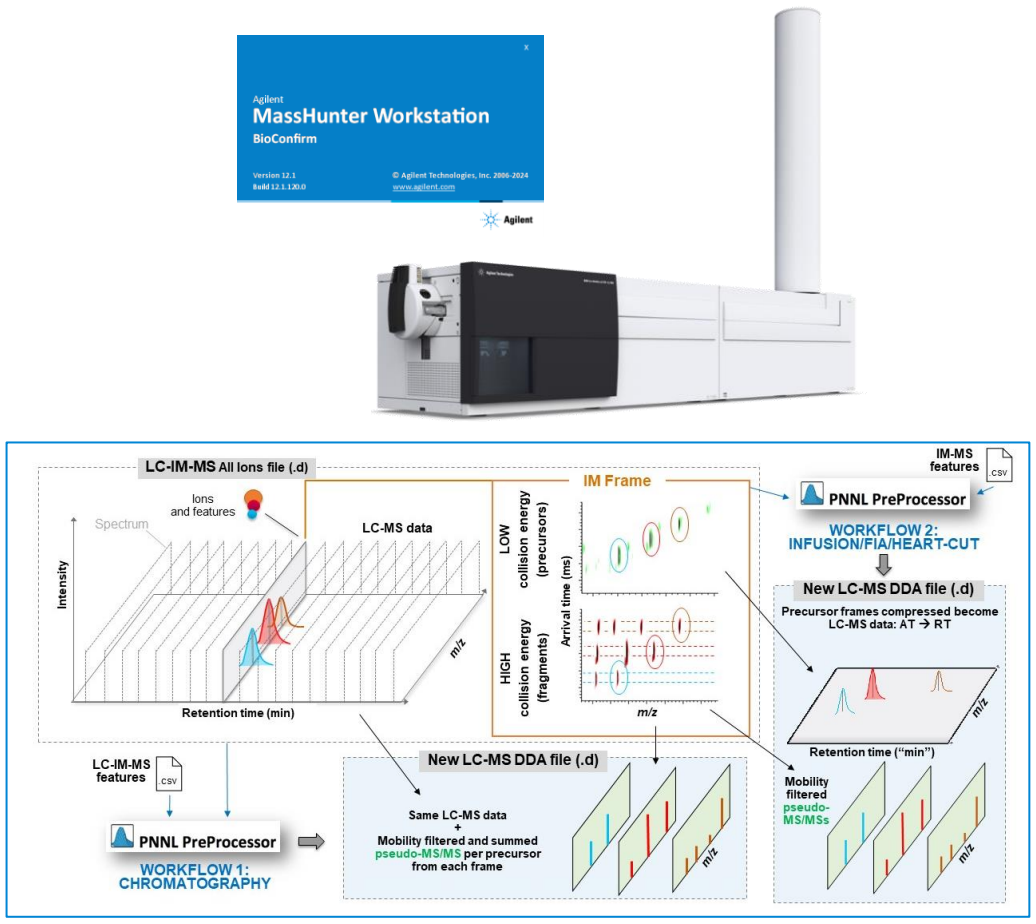


Figure 1. Diagram for IM-to-DDA conversion

The diagram shows the two workflows for mapping All Ions IM/MS data to LC-MS DDA format. For data files where the LC separation is to be maintained (Workflow 1) in the resulting data file the MS/MS spectra are mobility filtered and summed per precursor for each individual *m/z* vs. drift time frame. Individual MS/MS spectra are written for isomers, but most downstream data analysis tools will ultimately combine them during analysis. For infusion/FIA/heartcut data where all data is summed to one *m/z* vs drift time frame (Workflow 2), the IM separation is mapped to the LC axis in the resulting LC-MS DDA format where IM isomer separation is maintained.

Experimental

Experiments were performed on a 6560 IM-QTOF (Agilent Technologies, Santa Clara, CA) using an All Ions fragmentation mode. A commercial LC (1290 Infinity II series, Agilent Technologies, Santa Clara, CA) was used for LC separations prior to analysis with IM-MS. A trypsin digested BSA MS Standard (New England BioLabs Inc., Ipswich, MA) and a single stranded oligonucleotide standard (21mer DNA) (Integrated DNA Technologies, Coralville, IA) were analyzed to evaluate their respective workflows.

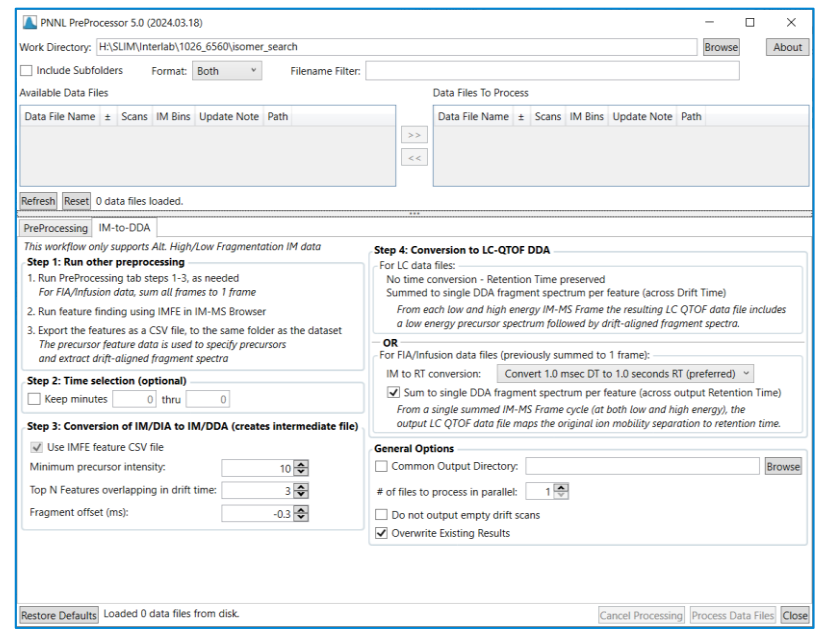
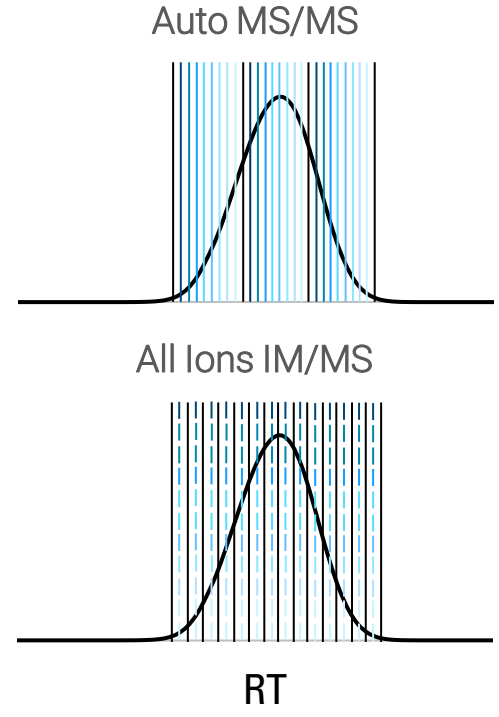


Figure 2. New PNNL PreProcessor tab

The new PNNL PreProcessor conversion (shown above) uses a precursor feature list from untargeted feature finding (IMFE) in IM-MS Browser or manual creation and then extracts the mobility aligned fragment ions and stores them as fragmentation spectra for each precursor. The LC-MS DDA data files are then used for sequence confirmation in BioConfirm 12.1. The ability to collect more fragment spectra across a chromatographic peak is highlighted below. With All Ions IM/MS the IM separation allows for fragmentation spectra to be acquired for all precursors every other frame.

Figure 3. When acquiring data with Auto MS/MS the instrument must cycle through the precursor ions collecting only a few spectra across a given chromatographic peak. With All Ions IM/MS fragmentation spectra are acquired every other frame for all the precursors in the chromatographic peak.



Oligo 21mer – Ion mobility analysis

DNA 21mer, sequence 'CAG TCG ATT GTA CTG TAC TTA' elutes in LC as a single large peak. To examine the benefits that IM may provide, we applied Workflow 2, which involves collapsing the LC dimension, while maintaining the IM drift time dimension. All Ions IM/MS was applied on alternating frames, and data first processed through PNNL PreProcessor resulting in a 'FCSum_DDA_3D' Agilent datafile. Qual 12.1 software was used to examine the drift-time EICs of the various 21mer precursor charge states (Fig 6).

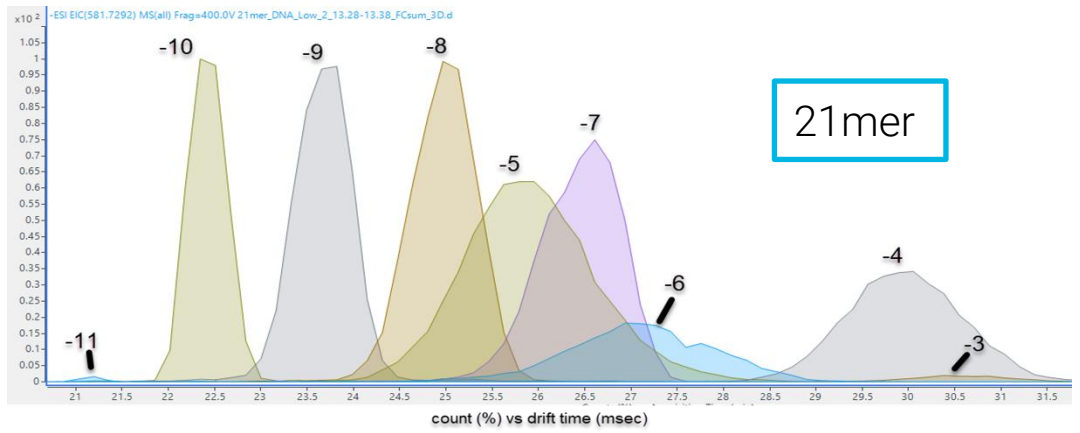


Figure 6. Drift-time extracted ion chromatograms for the 21mer charge states -3 through -11.

Figure 4: BioConfirm 12.1 results for 21mer MS/MS fragments for selected charge states following sequence confirmation analysis

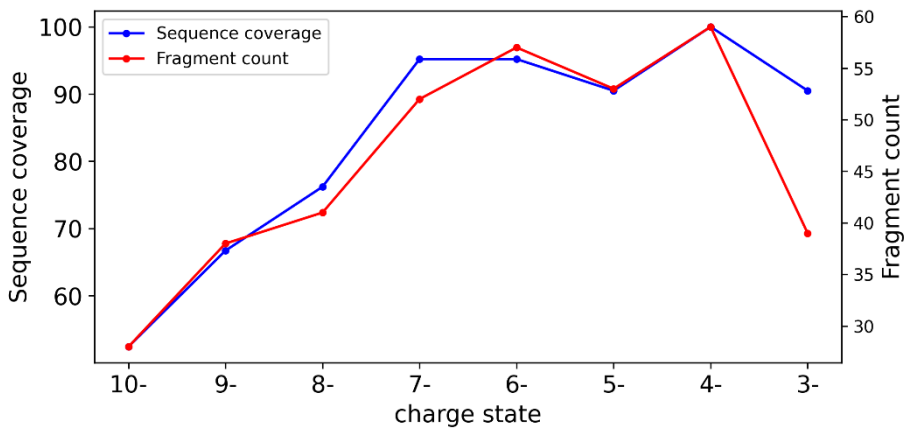


Figure 5: Sequence coverage and fragment number at various charge states

BioConfirm Oligo Sequence Confirmation Workflow

The 'Sequence Confirmation' workflow in BioConfirm 12.1 aims to provide detailed MS/MS fragmentation details of a single oligo. High sequence coverage is highly desirable, often requiring multiple injections at various CE's and targeting specific charge states with each injection. Ion-mobility allows concurrent analysis of many charge states, many of which are separated from one another, shown above. The conversion of the IM-MS Agilent datafile to the 'FCSum_DDA_3D' format now allows processing within standard BioConfirm workflows, taking advantage of the IM dimension.

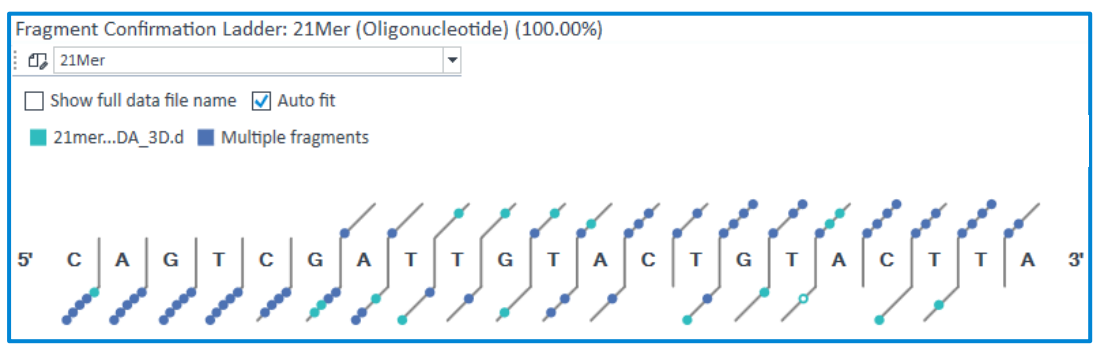


Figure 7: Fragment confirmation ladder for the 21mer following BioConfirm sequence confirmation analysis

Summary – oligo analysis

- First time IM-MS/MS oligo analysis using BioConfirm.
- Clear delineation of most precursor charge-states in the IM dimension.
- Excellent sampling of many MS/MS fragments for many charge-states
- Some charge states are better than others for sequence coverage, even if precursors are lower in abundance

Results and Discussion

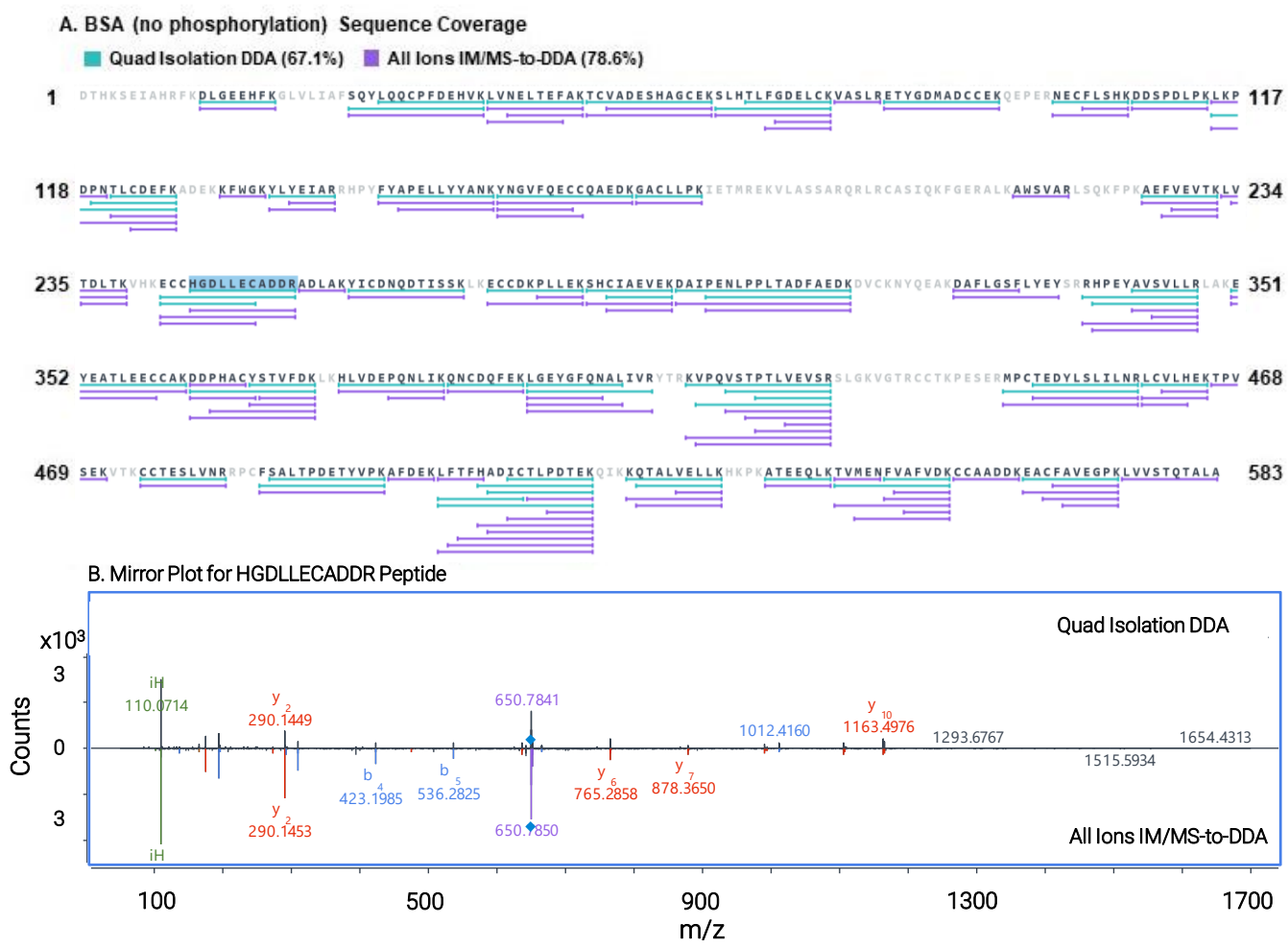


Figure 8. BSA sequence coverage map (A) and mirror plot for selected peptide (B).

BioConfirm Protein Digest Workflow

A BSA tryptic digest sample was evaluated with two different fragmentation methods in BioConfirm. Data was acquired with both a Quad Isolation DDA experiment (teal) and an All Ions IM/MS experiment. The All Ions IM/MS data was then converted to DDA format using the PNNL PreProcessor (purple). The sequence coverage (A.) for the All Ions IM/MS-to-DDA data file was higher (78.6% vs. 67.1%) than the Quad Isolation DDA due to fragmentation data for singly charged ions which are not included in Quad Isolation DDA experiments in order to spend more time on higher charge state ions. For the 66 sequence matches that were common to both data files the average score for the Quad Isolation DDA file was 10.7 ± 4.1 and for the All Ions IM/MS-to-DDA file was 12.0 ± 5.8. The mirror plot for a peptide found in both data files shows very similar spectra with only slightly more noise in the All Ions IM/MS-to-DDA data file from closely eluting peptide ions.

IM Isomer Treatment in IM-to-DDA Workflow 1 and 2

The peptide TLPDTEK has two IM isomers indicated below in the IM Browser heat map with the two yellow circles. The LC peak where this peptide elutes is summed and processed via Workflow 2, resulting in two fragmentation spectra in BioConfirm. When the full LC separation is processed via Workflow 1, only one fragmentation spectra results in BioConfirm and is a combination of the two spectra in Workflow 2. In this example all fragmentation spectra are representative of the sample peptide, but only Workflow 2 will ensure that IM isomers are kept separate in downstream data analysis where user parameters to specify otherwise are not always available.

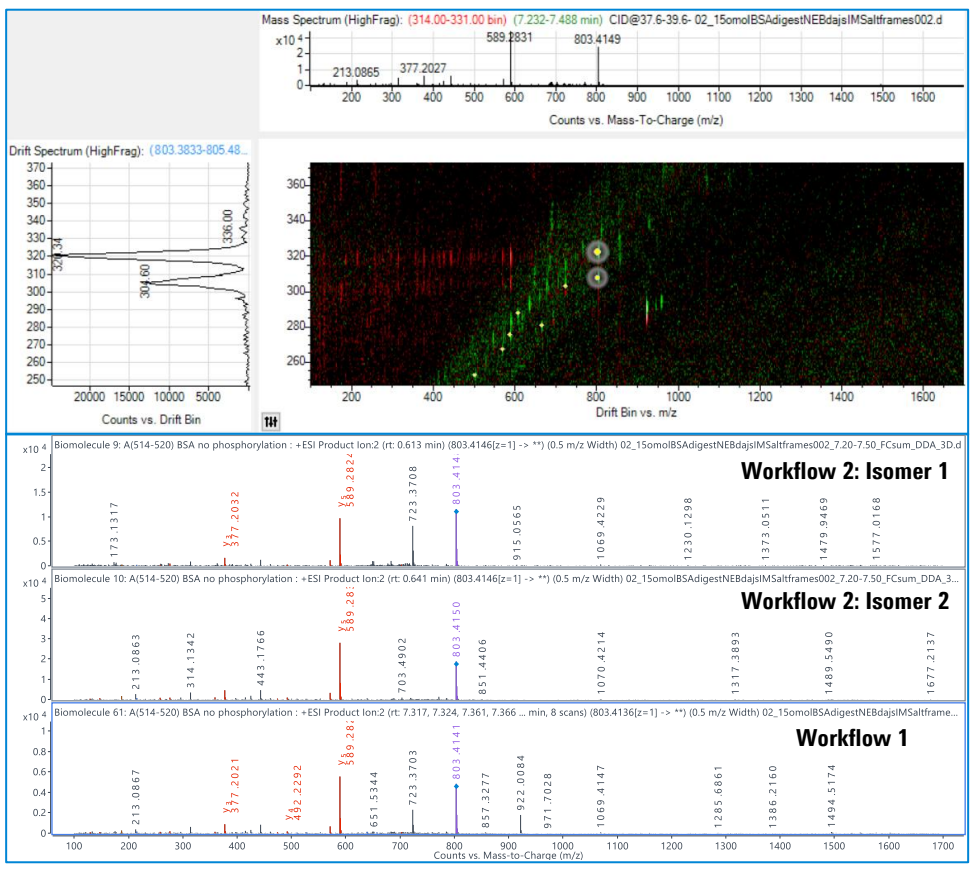


Figure 9. Comparing MS/MS spectra from Workflow 1 and 2 for IM isomers.

Conclusions

- Improved coverage was observed for BSA digest with converted All Ions IM/MS-to-DDA due to inclusion of +1 charge state
- Improved overall scoring for identified peptides from the All Ions IM/MS-to-DDA workflow.
- Workflow 2 ensures that IM isomers are handled separately throughout entire data analysis workflow

References

[1] Bilbao, A. et. al. Journal of Proteome Research 2022, 21 (3), 798-807.

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Published in USA, May 31,2024

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