Combining the Accurate Mass and Time Tag and Ion Mobility for Label-free and Missing Peptide Analysis

HUPO 2021

Robin Park[1], Titus Jung [3], Sven Brehmer[2], Patrick Garrett[3], Vijayaraja Gnanasambandan[1], Marc-Antoine Beauvais[1], Casimir Bamberger[3], Hyunsoo Kim[3], Christopher Adams[1], Dennis Trede[2], John R. Yates, III[3], Rohan Thakur[1]

[1]Bruker Daltonics Inc., USA
[2]Bruker Daltonik GmbH, Germany
[3]Scripps Research, San Diego, USA

Introduction

An accurate mass and time (AMT) tag approach for proteomic analyses has been developed to validate peptide identifications using mass measurement accuracy and retention time. Because of the complexity of the proteome, ambiguities in peptide assignments can occur when two peptides are assigned to the same mass value or when PTM peptides are identified with low localization scores. In this study, we applied high throughput 4-D proteomics strategy to the AMT tag by adding the ion mobility dimension (CCS-centric AMT tag), which plays a critical role in clarifying such ambiguities. The timsTOF Pro and timsTOF fleX instruments provide four-dimensional data space called 4D-Proteomics with CCS values and parallel accumulation serial fragmentation (PASEF) to improve ion utilization efficiency and data acquisition speed. In this study, we utilized CCS-centric AMT for finding missing peptides when we compared multiple experiments. We acquired the spectra on a timsTOF PRO from 500ng of digested Hela samples. The search space included all tryptic peptide candidates that fell within the precursor mass tolerance window with three miscleavage constraints. Parallel Database Search Engine in Real-time (PaSER) performed the database search while the instrument was acquiring spectra on the UniProt human database. Census reconstructed chromatograms for each identified peptide. We built a CCS-centric AMT library storing accurate precursor mass, retention time, charge states, and CCS values. When peptides are not identified in all the relevant replicates, Census went through spectra, searching them using information from the library to detect missing peptides. To increase accuracy for finding peptide precursors, we compared CCS values from library and target spectra. For peptide abundance, we applied smoothing and calculated Pearson product-moment correlation coefficient comparing theoretical and experimental isotope distributions.

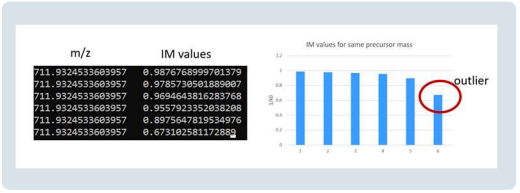

Figure 1: Precursor Ion Mobility Values Challenge of the quantitative analysis is to select true precursor peaks. Co-eluting peaks having similar or same masses make it difficult to calculate the abundance of peptide accurately. Ion mobility values provide another dimension to differentiate such co-eluting or noisy peaks.

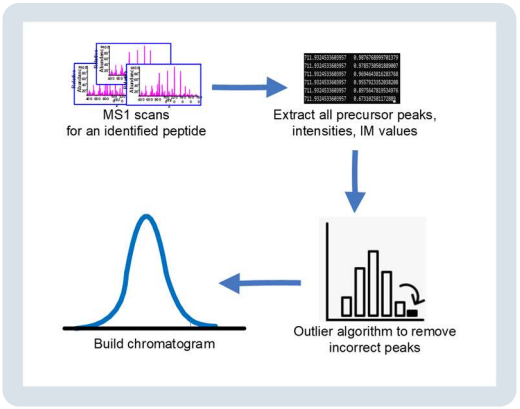

Figure 2: CCS-centric Quantitative Analysis Workflow We collect all precursor peaks from within mass tolerance from identified spectrum. Peak finding algorithms expand to neighboring ms1 frame spectra to further collect peaks. Then, we apply outlier algorithm to check ion mobility values and remove outlier peaks. Next, we reconstruct XIC to generate abundance of peptides.

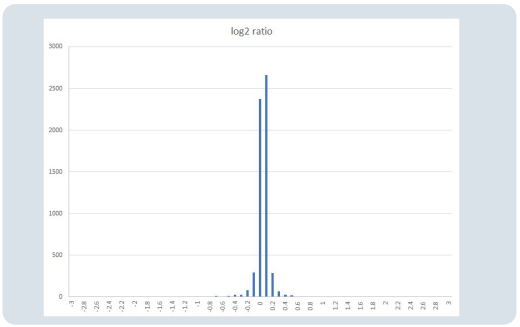

Figure 3: Ratio Distribution We compared two HeLa sample data from timsTOF Pro, Bruker Daltonics with expected ratio 1:1. We applied CCS-centric quantitative algorithm to calculate ratio. We converted the ratio in log scale to generate histogram.

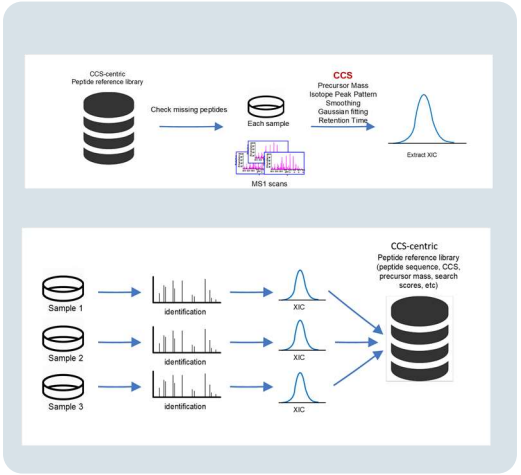

Figure 4: Accurate Mass, Time Tag and Ion Mobility library We collect all precursor peaks from within mass tolerance from identified spectrum. Peak finding algorithms expand to neighboring ms1 frame spectra to further collect peaks. Then, we apply outlier algorithm to check ion mobility values and remove outlier peaks. Next, we reconstruct XIC to generate abundance of peptides.

sequences	charge state	ratio with CCS	ratio without CCS
TERPVNSAALSPNYDHVVLGGGQEAMDVTTTS TR	3	-0.2	2.0
EKLCYVALDFEQEMATAASSSSLEK	3	-0.1	3.2
DLAAATAESAPNAAILVISNPVNSTVPIVAQVLK	3	0.4	3.3

Table 1: Application of CCS filter on Outlier Peptides in MBR When retrieving missing peptides, there are peptides having outlier ratios. These peptides ratios can be improved by applying CCS filter

Conclusions

- Ion mobility is critical metric to find correct precursor peaks from co-eluting or noisy peaks.
- Accurate Mass, Time Tag and ion mobility library is useful for analyzing large cohort experiments efficiently

timsTOF Pro