Data-dependent auto-MSMS 3D-precursor selection for bottom-up proteomics with Parallel-Accumulation SErial-Fragmentation (PASEF) on a Trapped-Ion-Mobility quadrupole-Time-Of-Flight mass spectrometer (TIMS-QTOF)

ASMS 2008, MP-119

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Introduction

The recently introduced PASEF acquisition mode on a TIMS-QTOF[1)] separates the incoming ion-beam mobility-dependent in time and elutes spatially condensed ion-packages from the TIMS device. In PASEF, precursors are detected in the m/z- and mobility-dimensions. The quadrupole isolates distinct precursor species during the few milliseconds they actually elute from the TIMS device and immediately switches to the next precursor resulting in improved speed and sensitivity compared to traditional MSMS scan modes. Here, different approaches for the precursor selection algorithm are evaluated, which also match the time constrains dictated by the chromatographic retention length.

Methods

Tryptic digests of a human cancer cell line (HeLa) were separated by nanoLC with 90min gradients and analyzed on a timsTOF pro instrument with modified acquisition software. The quality of acquired MSMS spectra was evaluated using Mascot and PEAKS search engines; peptide spectrum matches were normalized to 1% FDR.

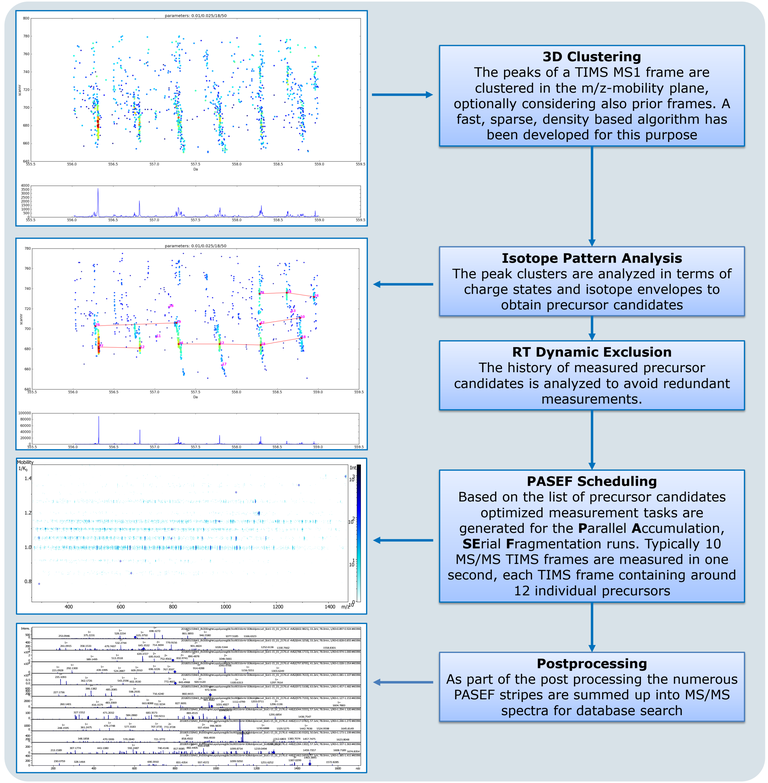

Fig. 1: Overall workflow of the new 3D-clustering based precursor selection for PASEF measurements. Goal: optimize the number and quality of MS/MS spectra which can be obtained in a LC-TIMS-QTOF bottom-up experiment.

Results

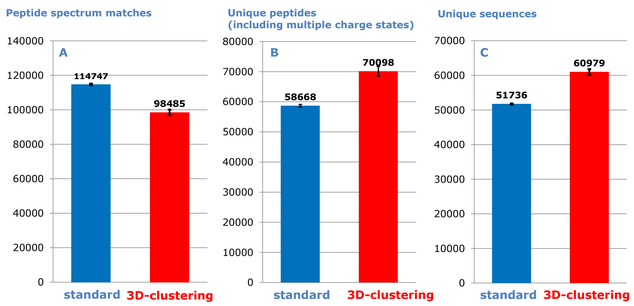

Fig. 2: Peptide identification results (Mascot, average of triplicate measurements) from 200 ng HeLa digest separated by a 90min nanoLC gradient using the current standard PASEF precursor selection algorithm (peak picking on overlapping mobilogram slices) or the new 3D clustering approach. The number of unique peptides (different charge states of the same sequence counted as peptide) and also the number of unique sequences could be increased by ~15%. Due to the more accurate precursor detection and dynamic exclusion, unwanted replicate measurements could be significantly reduced, thus the number of peptide spectrum matched decreased.

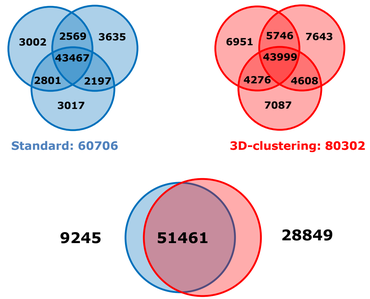

Fig. 3: Venn diagrams of unique sequences obtained during triplicate measurements of the standard and new 3D-clustering approach (top), and comparison between both algorithms (bottom).

Summary

A new 3D-clustering based precursor selection has been compared with a mobilogram peak picking approach using overlapping slices. An improved recognition and separation of nearby precursors has been observed.

The algorithm is fast enough to be used with a standard PC without additional accelerators. In complex samples precursor determination takes around 300ms, so that even more sophisticated approaches can be added.

References

[1)] Meier et al., *J. Proteome Res.*, **2015**, 14 (12), pp 5378–5387

Conclusions

- The new algorithm has shown to give an improved yield of unique peptide sequences.
- Further improvement can be expected from an online 4D-clustering under development. Using the individual mobility width of the clusters for the scheduling is also an option for further improvement.

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