timsTOF Pro and PASEF

Multiplying Sequencing Speed and Sensitivity in Mass Spectrometry-based Proteomics

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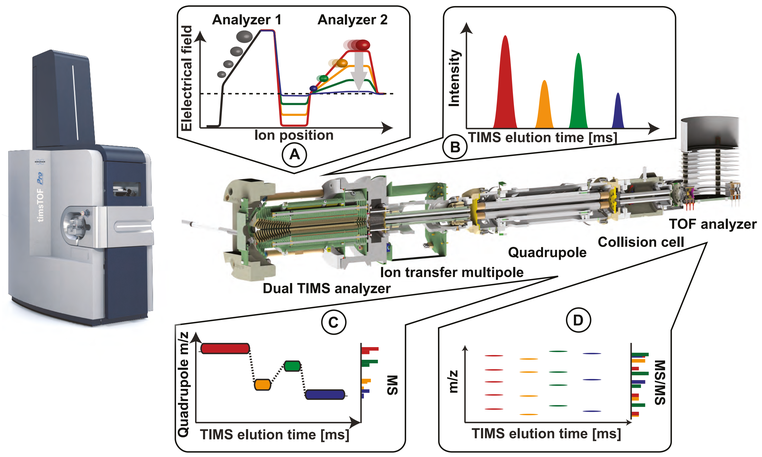
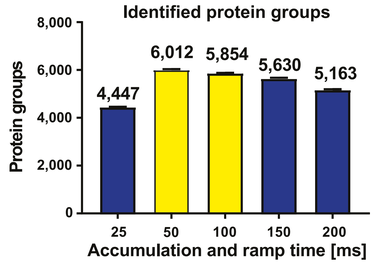
1 Background

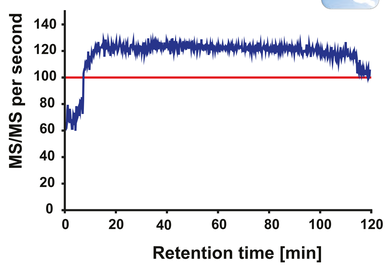
Trapped Ion-Mobility Mass-Spectrometry (TIMS) fits seemlessly between the chromatographic and the quadrupole time-of-flight analyzer time-scales. The combination of TIMS with the Parallel-Accumulation followed by SErial-Fragmentation (PASEF) scan-mode promises to overcome long-standing limitations in speed, sensitivity, and robustness.

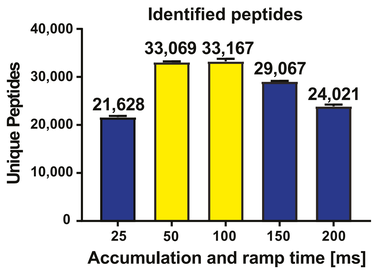
In PASEF, incoming ions are accumulated in parallel to the separation and focussing of dense ion packages in two independent analyzers (A). These ion packages are subsequentially 'eluted' from the TIMS-dimension according to their collisional cross sections (B). The quadrupole mass position is synchronized with the mobility elution profile of the trapped ions in the TIMS dimension (C), which multiplies sequencing-speed and -sensitivity, and results in the fragmentation of the stored precursor ions during PASEF MS/MS scans, as well as an up to 100 % duty cycle (D) on the timsTOF Pro.



2 Single-Shot performance - 200 ng HeLa digest in 120 min

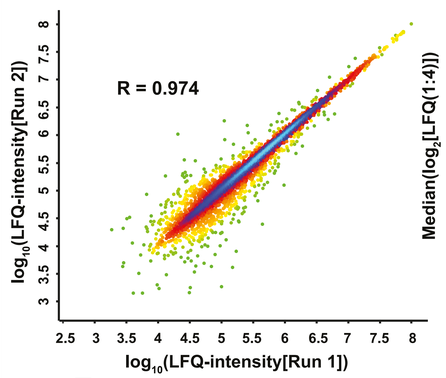


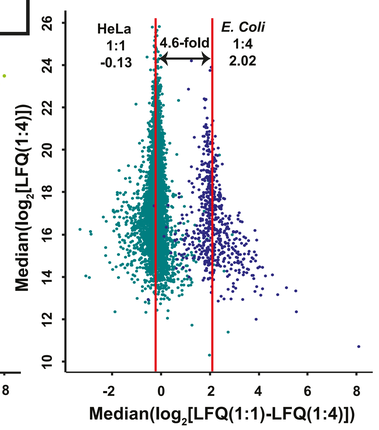


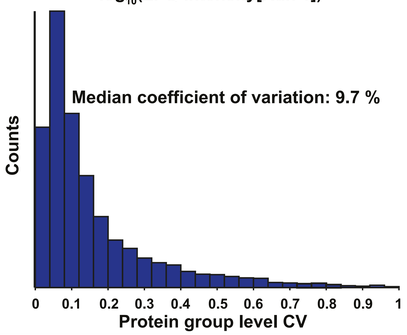


In quadruplicate single-shot experiments with 200 ng of HeLa digest and a 120 min LC gradient we identified ***more than 6,000 protein groups*** and ***33,000 unique peptides***. The ***sequencing speed of >120 MS/MS per second*** and the PASEF scan-mode nearly completely alleviated the missing value problem and allowed the ***label-free quantification of more than 5,900 protein groups in MaxQuant without matching between runs.*** The ***median identified peptide length is 15 amino acids.***

3 Label-Free Quantification

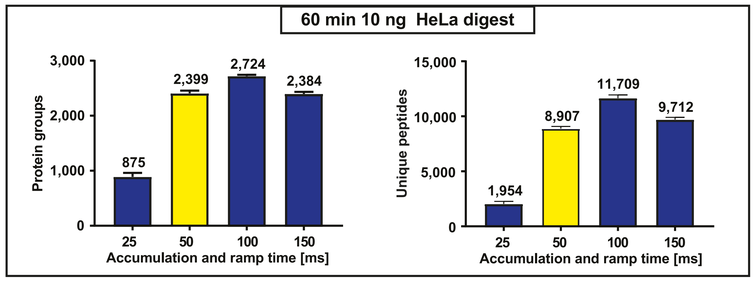


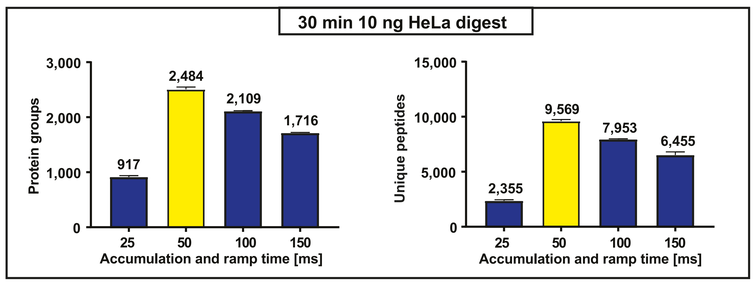




The label-free quantitative reproducibility of single-shot experiments with ***200 ng HeLa digest*** and ***120 min LC gradients*** analyzed with MaxQuant was ***R > 0.97***. Also, the median ***coefficient of variation on protein group level is routinely below 10 %.*** In a ***HeLa and E. coli mixture experiment at a fixed ratio of 1 to 4,*** measured in quadruplicates and a 120 min LC gradient, ***we quantified more than 5,100 HeLa and 1,200 E. coli protein groups*** and obsered a ***clear separation of both species at the defined ratio.***

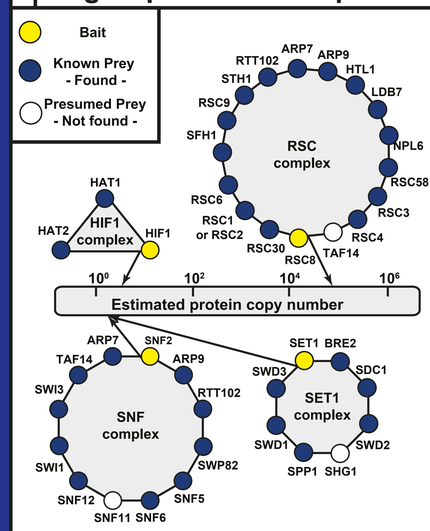
4 Speed and Sensitivity - 10 ng HeLa digest in 30 min



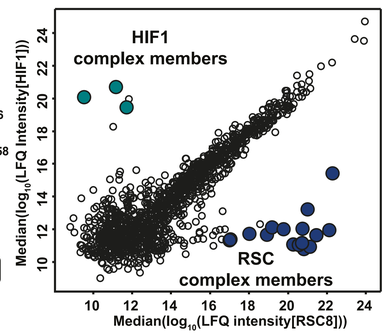


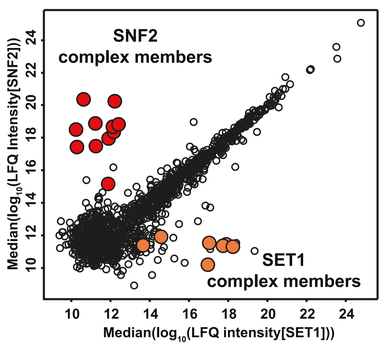
In quadruplicate single-shot experiments with only ***10 ng of HeLa digest, a 30 min LC gradient, and 50 ms accumulation/ramp time***, we consistently identified ***more than 2,400 protein groups*** and ***9,000 unique peptides***. Decreasing the measurement time from 60 min to 30 min does not decrease the number of identified protein groups and peptides much. Higher accumulation/ramp times only increase the number of peptides by less than 10 % on the protein group level and less than 20 % on the peptide level.

5 High-Speed and complete Yeast interactomes in 23 min



We performed triplicate single-shot measurements with only ***100 ng of Yeast digest*** and a ***23 min LC gradient*** of pulldowns with protein copy numbers ranging from <150 to >4,500. ***All validated protein complex members such as the RSC-, SNF-, HIF-1, and SET-1 complex were found and quantified.***





6 Conclusion

The timsTOF Pro in combination with the PASEF scan-mode promises to overcome ***long-standing limitations in the field of proteomics*** including ***sequencing-speed, sensitivity, and robustness***.

Our setup enables a ***routine sequencing-speed of >120 MS/MS per second*** in the PASEF data-dependent acquisition scan-mode, while still keeping the ***cycle time at 1.1 sec*** and the ***duty cycle at 100 %***. This enabled the identification and quantification of ***more than 6,000 protein groups in 120 min in the MaxQuant software*** environment with a reproducibility of ***R > 0.97*** and a ***median coefficient of variation of <10 % on protein group level***. The identification of ***more than 2,400 protein groups*** from only ***10 ng of HeLa digest in 30 min*** highlights the ***ultra-high sensitivity*** and sequencing speed of the timsTOF pro in combination with the PASEF scan-mode. These instrument characteristics enabled the measurement of ***yeast pulldowns across the whole protein copy number range from ~140 to ~4,400 protein copies per cell in 23 min and 100 ng - without any compromise in sensitivity***.

The instrument operation was very robust, which argues well for clinical applications.

Parallel Accumulation-Serial Fragmentation (PASEF); Meier,..., Mann et al., JPR, 2015

Trapped ion mobility spectrometry: A short review; Ridgeway,..., Park et al., Int. J. MS, 2018

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