PASEF for sensitive shotgun proteomics: toward single cell analysis

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Introduction

Acquiring proteomics data with single cell resolution is highly desirable to investigate fundamental biological processes in more detail without the variation resulting from individual cell types in a tissue or different stages during cell cycle.

The PASEF[1)] approach on the timsTOF Pro should be particularly beneficial for low sample amounts as the mobility based pre-focussing of ions prior to quadrupole isolation allows to fragment around 10-20 times the number of precursors without any sensitivity loss.

Methods

To evaluate the performance of the MS instrument independent from the challenging sample preparation of single cell digests, a commercially available tryptic digest from the myelogenous leukemia cell line K562 (Promega, Madison, WI) was diluted accordingly.

Samples were loaded onto a 250 mm, 75µm ID column with pulled emitter (IonOpticks, Melbourne, Australia) and separated using a linear gradient from 4-30% buffer B (80% Acetonitrile, 0.1% formic acid) at a flow rate of 250 nl/min over 60 min.

LC-TIMS-MS/MS data were acquired using 166 ms TIMS accumulation time, 166 ms TIMS ramp of the mobility ($1/K_0$) range from 0.7 to 1.3 Vs/cm²; 1 MS1 followed by 10 PASEF MSMS frames per cycle, 1.8 s total cycle time.

Data analysis was performed using PEAKS X+ (Bioinformatics Solutions Inc., Waterloo, ON). Peptide spectrum matches as well as protein numbers were normalized to less than 1.0% false discovery rate.

Results

For sample amounts of 0.15/0.31 ng PASEF allowed to reliably identify 3300/5100 unique sequences leading to 680/980 protein groups (Fig. 1). These values would be around the 0.22 ng protein content reported for single HeLa cells[2)].

Given the stochastic nature of data dependent acquisition especially close to the detection limit, peptide identification increases significantly when results from technical replicates are combined (Fig. 2).

Although signal intensities are low in this concentration range, quantitative information can be reproducibly extracted even without further optimization of the feature extraction algorithm. The mobility separation dimension seems to help here separating peptide signals from chemical background (Fig. 3).

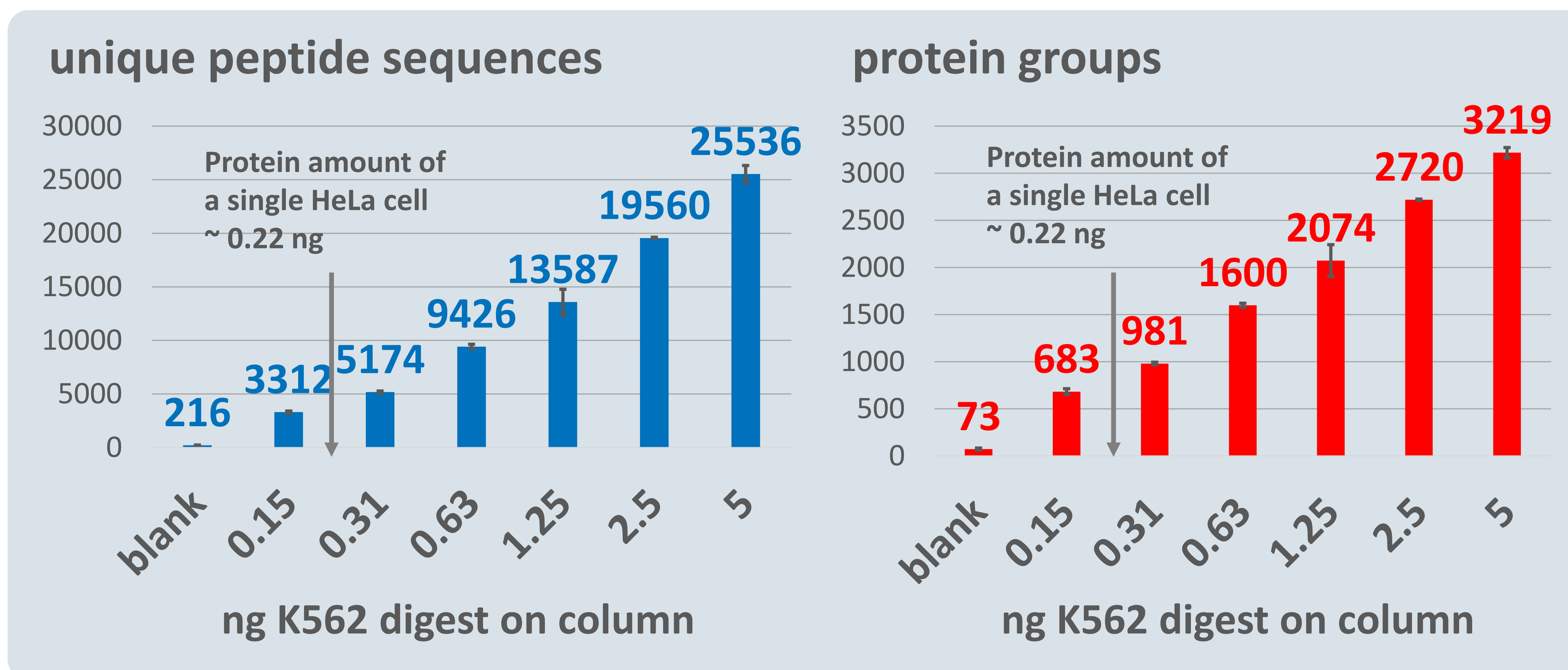

Fig. 1: Identification results of dilution series of K562 cell digest separated using 60 min gradients at 250 nl/min (average of triplicate measurements, one sigma error bars).

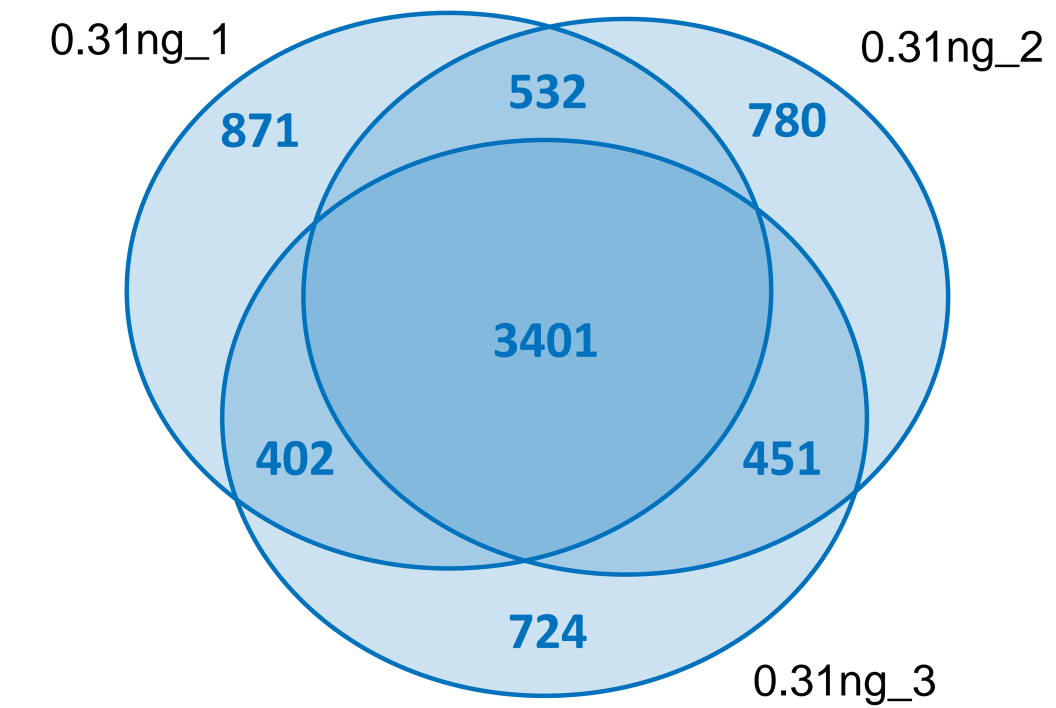

Fig. 2: Unique peptide sequences identified in three replicates of 0.31 ng K562 digest. The average number of unique sequences per individual run is ~5170, combining all three results increases IDs by 38% to ~7160.

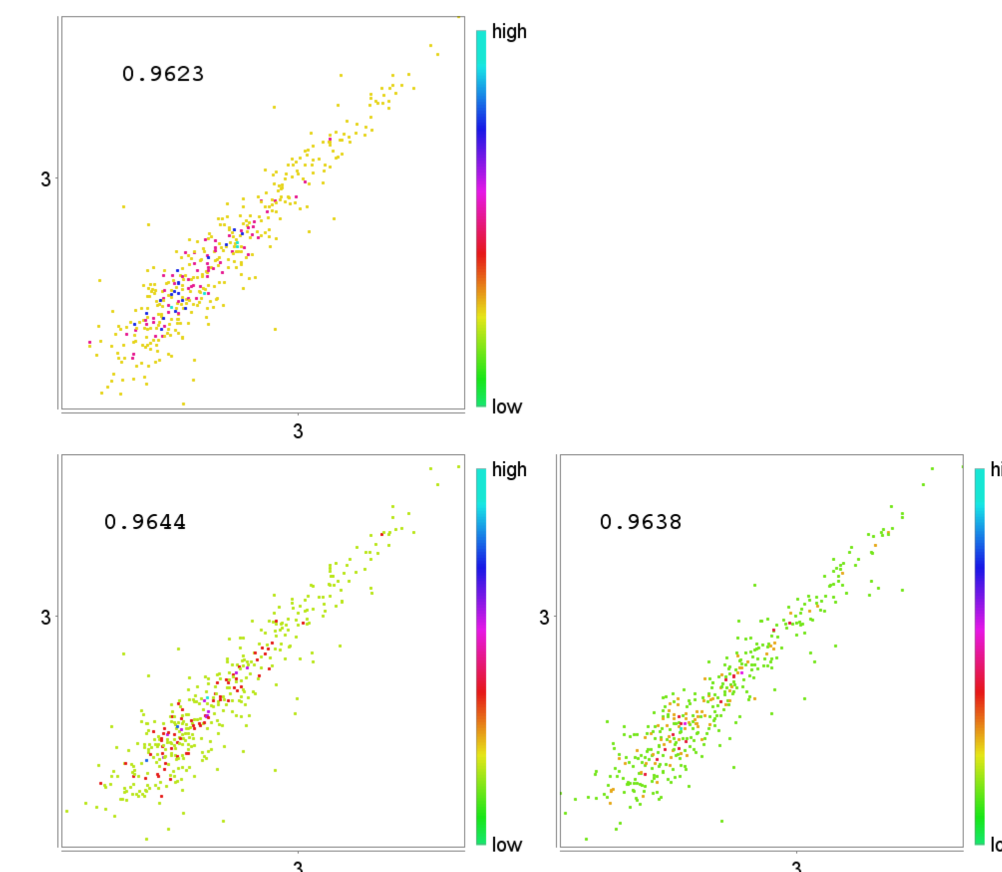

Fig. 3: Correlation of peptide feature intensities among three replicates of 0.31ng K562 digest.

Even at the high PASEF sensitivity and acquisition speed, the variation in IDs among technical replicates (Fig 2.) indicates that time limits the ID rate and more information could be obtained by matching MS1 features between individual runs. With the increased specificity due to the additional ion mobility property of a peptide feature this matching could be extended even further. For results on real single cell applications see talk "ThOF am 09:50".

References

1) https://doi.org/10.1074/mcp.TIR118.000900

2) https://doi.org/10.1021/acs.analchem.8b03692

Conclusions

- PASEF enables direct identification of thousands of peptides and hundreds of proteins from sample amounts that correspond to a single cell.
- no isobaric labelling or booster channel with higher sample amount required.

timsTOF PASEF

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