Bringing proteomics sensitivity to single cell level with Evosep whisper on the timsTOF SCP

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Introduction

Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry, established in the timsTOF SCP [1], paired with fast and robust liquid chromatography, enabled by the Evosep One, open new possibilities for proteome analyses at the single cell level. Paired with automated single cell sorting and sample preparation realized with the cellenONE platform, allows for sensitive proteome analyses at the single cell level. Coupled to developments in processing of data independent acquisition (DIA) mode data files using deep learning with neuronal networks (e.g., DIA-NN [2]) further improves detectability and quantifiability of proteins from minimal input samples such as single cells. Single cell proteomics has the potential to make important contributions to the understanding of cellular heterogeneity.

Results

C18 Evotips with 15 cm Performance column and 10 µm Emitter in Whisper 40SPD (old 28 min method)

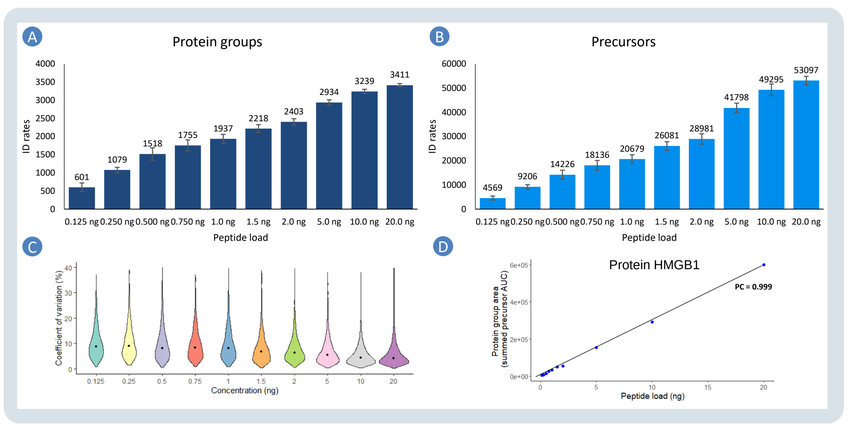


Figure 1:
A) Protein group and B) precursor identification rates in a dilution series of a commercial HeLa cell digest with peptide loads from 20 ng down to 125 pg (n = 8 per concentration), C) assessment of the cariation in protein group areas reported for the different peptide loads on Evotips, D) correlation of the protein group area of the Protein HMGB1 with the peptide amount loaded on Evotips.

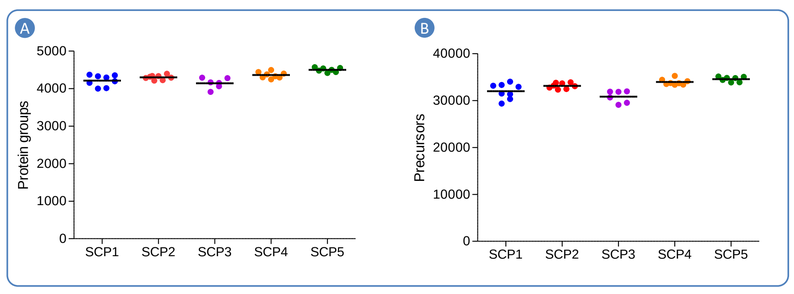
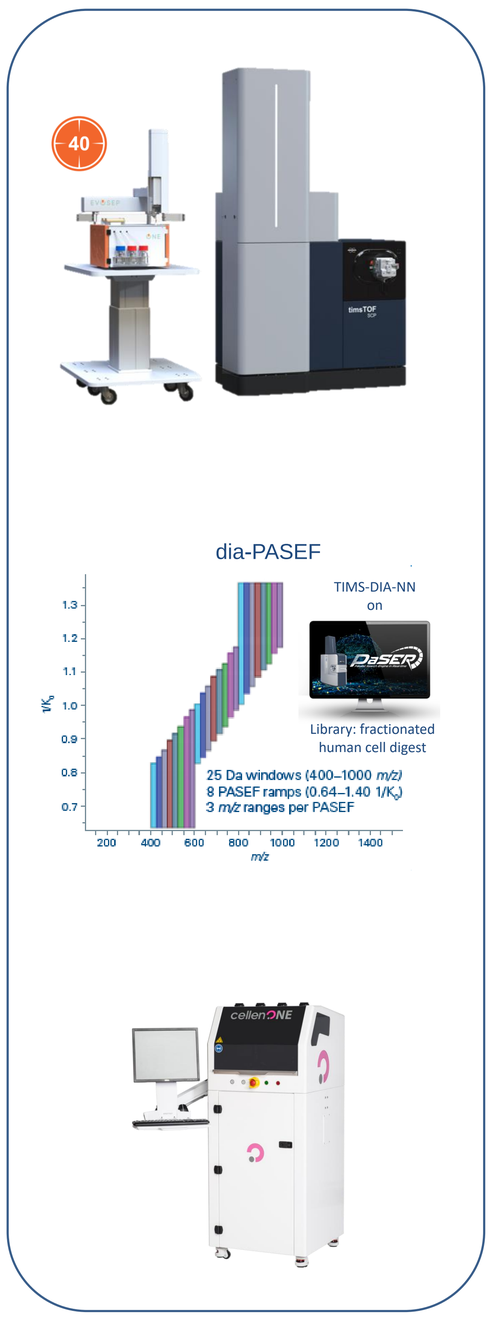


Figure 2:
Assessment of A) protein group and B) precursor identification rate reproducibility of 5 ng peptide load of HeLa cell digests onto Evotips (n = 8 per instrument) across 5 timsTOF SCP instruments.



Evotips Pure with 15 cm IonOpticks Aurora Elite column in Whisper 40 SPD (new 31 min method)

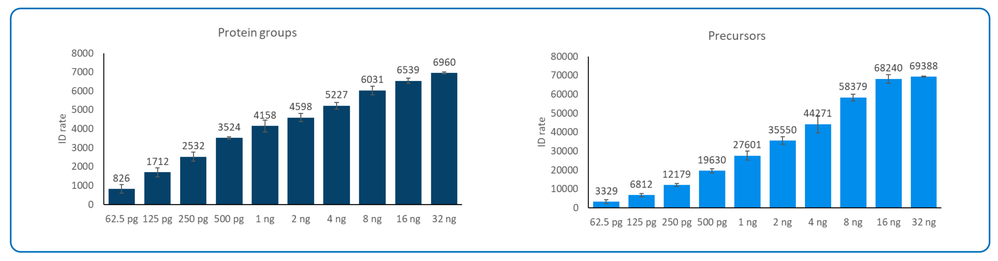


Figure 3:
Protein group and precursor identification rates in a dilution series of HeLa cell digests with peptide loads from 32 ng down to 62.5 pg (n = 6 per concentration).

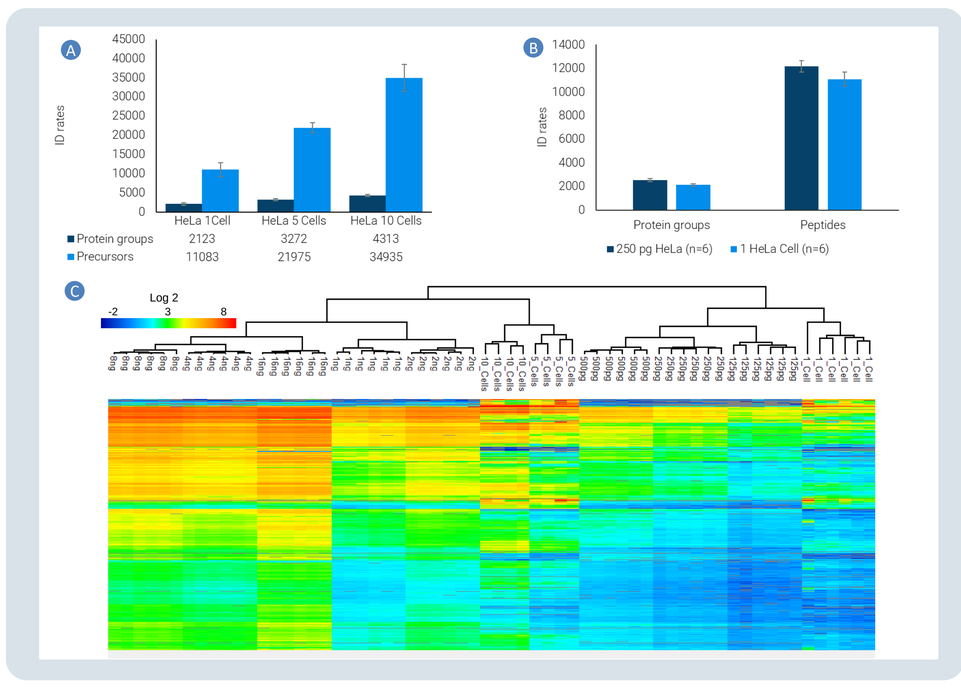


Figure 4:
A) Protein group and precursor identification rates of cellenONE sorted and prepared single, 5 and 10 cells, B) comparison of protein group and precursor identification rates in single HeLa cells and 250 pg peptide loads of commercial HeLa cell digest, C) hierarchical clustering of protein group areas of the commercial HeLa cell digest dilution series and the cellenONE sorted and prepared cells.

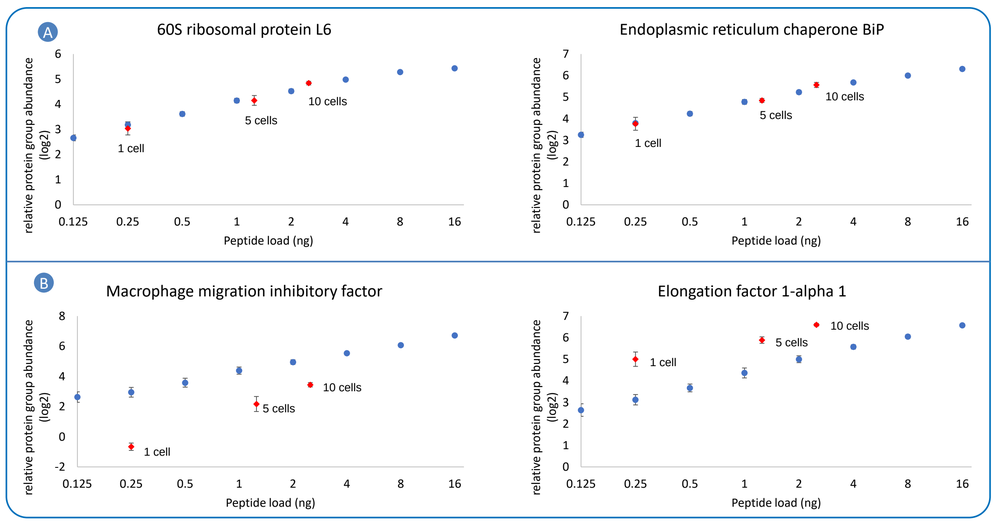


Figure 5:
Correlation of the protein group area with peptide load on Evotips (blue) from the commercial HeLa cell digest dilution series and the protein group area of the cellenONE sorted and prepared cells (red) at the expected protein amount (1 cell ~ 250 pg, 5 cells ~ 1.25 ng, 10 cells ~ 2.5 ng) of proteins with A) matching protein group area and B) where the protein group area was either higher or lower in the cells.

Further reading

Application Note, Bruker Daltonics, LCMS-193, 1894933, 2022;
Application Note, Bruker Daltonics, LCMS-194, 1895627, 2022;
Application Note, Evosep, AN-018B 22/06

References

[1] Brunner et al. Mol Systems Biol, 2022, 18: e10798
[2] Demichev *et al*.; Nat Methods, 2020, 17: 41–44

Conclusions

- The low flow Evosep Whisper methods (100 nL/min in the core part of the gradient) enables high sensitivity with good chromatographic reproducibility and robustness with short gradients and low overhead time between gradients
- High timsTOF SCP instrument to instrument identification rate reproducibility demonstrated with 5 ng peptides of a HeLa digest loaded onto Evotips
- High identification rates with the standard whisper 40SPD setup and the new 15 cm Aurora Elite column from IonOpticks
- High quantification accuracy at a single cell level with high single cell to single cell reproducibility on protein level with good proteome coverage for single cell samples sorted with a cellenONE system with more than 2000 protein groups identified per single cell