Label-free single cell analysis workflow on the timsTOF SCP using the cellenONE platform

International Mass Spectrometry Conference 2022 – Maastricht, Netherlands

Christoph Krisp[1]; Anjali Seth[2]; David Hartlmayr[2]; Jonathan Krieger[3]; Tharan Srikumar[3]; Pierre-Olivier Schmit[4]; Guilhem Tourniaire[2]; Markus Lubeck[1]; Gary Kruppa[5]

1 Bruker Daltonics GmbH & Co. KG, Bremen, Germany; 2 Cellenion, Lyon, France; 3 Bruker Ltd., Milton, ON; 4 Bruker Daltonique S.A, Wissembourg, France; 5 Bruker s.r.o., Brno, Czech Republic

Introduction

Single cell proteomics is a rapidly developing field with the potential to make important contributions to the understanding of cellular heterogeneity. Enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry established in the timsTOF SCP [1] 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.

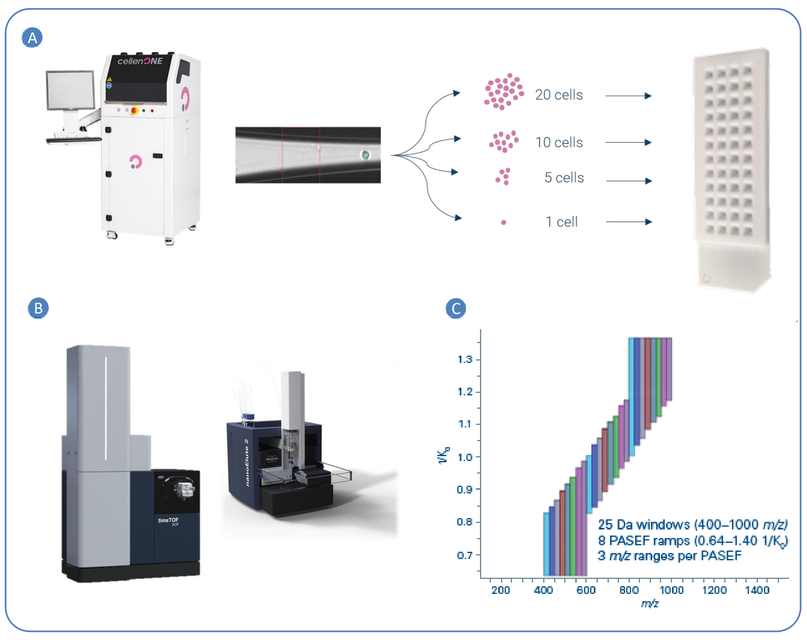

Figure 1:
Schematic of cell sorting on the cellenONE ®, an instrument for cell isolation and picolitre dispensing, using the label-free proteoCHIP ® for sample preparation; B) LC-MS/MS setup using a timsTOF SCP mass spectrometer coupled to nanoElute 2 UPLC; C) dia-PASEF window placement scheme.

Methods

- Cell number and Type: 20, 10, 5 and 1 HEK 293 and HeLa cells
- Sorting, lysis, and protein digested using the cellenONE® platform (Cellenion)
- Tryptic peptide injection from low protein binding autosampler vials and the label-free proteoCHIP
- Injections onto a 25 cm x 75 µm Aurora C18 column (IonOpticks) using a nanoElute 2.
- 30 min ACN gradient and eluted into a timsTOF SCP (Bruker).
- Data acquisition in dia-PASEF mode (data independent acquisition in parallel accumulation serial fragmentation)
- Data analysis with TIMS-DIA-NN
  - Spectral library generated by DDA from a deeply fractioned human cell line containing 573,610 precursors from 13,679 proteins
  - No match-between-runs

Results

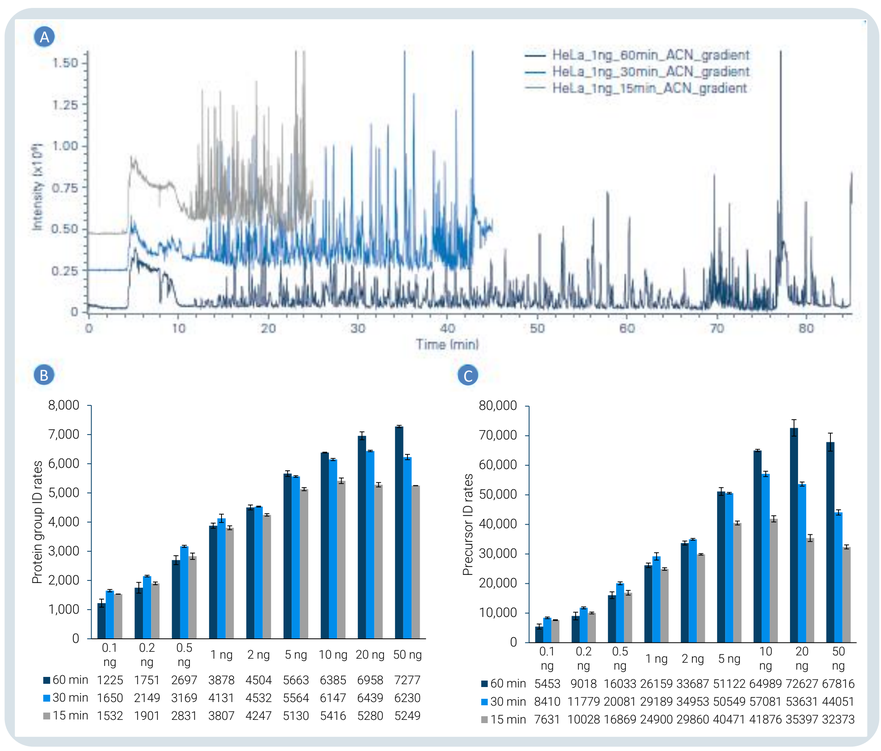

Figure 2:
Gradient length versus HeLa peptide load optimization using HeLa protein digests (Pierce, Cat. 88328) to prepare dilution series with concentrations of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, and 50 ng/µL.
A) representative base peak chromatograms of 1 ng HeLa peptides on column separated with a 15 (grey), 30 (blue), and 60 min (dark blue) ACN gradient; B) protein group and C) precursor identification rates of HeLa peptide dilution series of the three different gradient length. the 60 min ACN gradient showed best performance for peptide loads greater 5 ng, the 30 min ACN gradient performed best for concentration ranges ≤ 5 ng and was used for the analysis of cellenONE prepared single to few cell numbers.

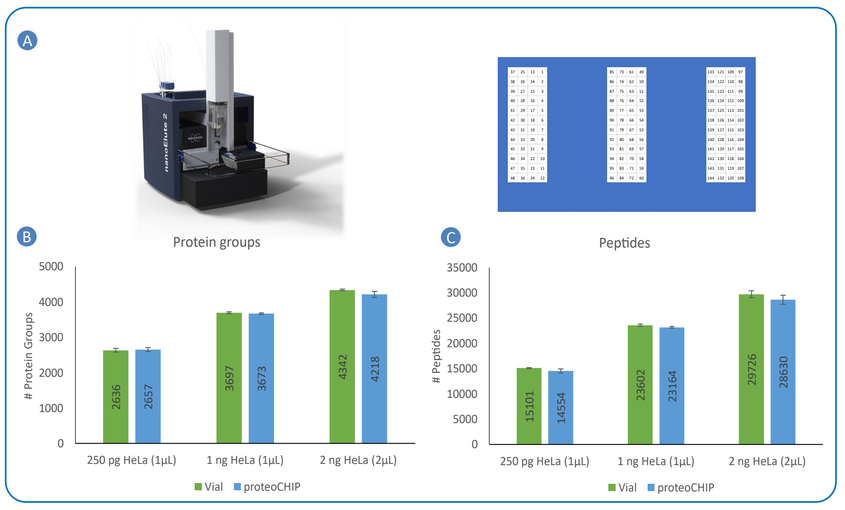

Figure 3:
A) schematic of the label-free proteoCHIP holder designed for direct injection of cellenONE prepared (single) cells using the nanoElute 2. Repetitive injections of 250 pg, 1 ng and 2 ng of peptides from a HeLa cell lysate digest either injected from an autosampler vial (green) or from the label-free proteoCHIP (blue) with indicated volumes pipetted into each well show excellent reproducibility on protein group (B) and peptide (C) level.

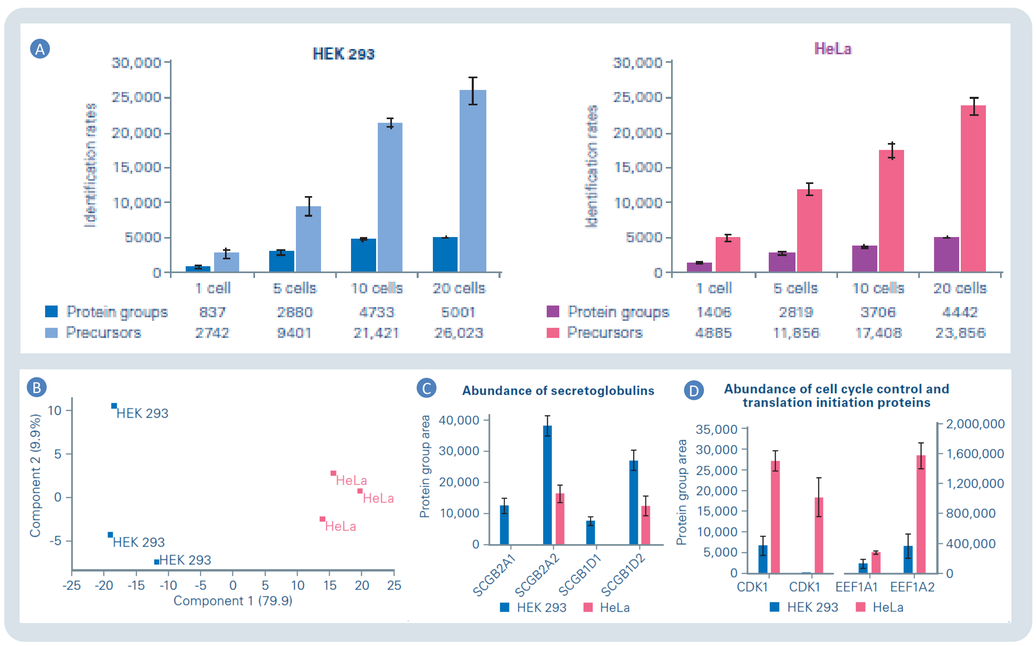

Figure 4:
A) Protein group and precursor identification rates for 1, 5, 10 and 20 HEK 293 cells and HeLa cells acquired in dia-PASEF and analyzed using TIMS DIA-NN. for single HEK cells in average 837 protein groups, with a steady increase towards 20 cells with, in average, 5001 protein groups identified. For HeLa cells, in average, 1400 protein groups for single HeLa cells and for 20 cells 4400 protein groups were identified. B) Component 1 versus component 2 loadings of principal component analysis of proteins quantifiable in single HEK 293 and HeLa cells; Protein group area distribution of C) secretoglobulins and of D) cell cycle control proteins CDK1 and CDK2, and eukaryotic translation initiation proteins EEF1A1 and A2 in single HEK 293 and single HeLa cells.

References

[1] Brunner et al. Mol Systems Biol, 2022, 18: e10798

[2] Demichev *et al*.; Nat Methods, 2020, 17: 41–44

Further reading

Application Note, Bruker Daltonics, LCMS-193, 1894933, 2022

Application Note, Bruker Daltonics, LCMS-194, 1895627, 2022

Conclusions

- Excellent protein group and precursor identification rates at concentration ranges at single to few cell levels simulated with commercial HeLa protein digest dilutions.
- Comparable and reproducible protein group and peptide identification rates for injections from autosampler vial and the label-free proteoCHIP using the nanoElute 2
- Good proteome coverage for single cell samples sorted with a cellenONE ® system with more than 1400 protein groups identified per single cell.
- High quantification accuracy at single cell level with high single cell to single cell reproducibility on protein group level.