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Assessing cellular responses to treatment in single cell and low cell count spheroids using the cellenONE with proteoCHIP EVO 96 workflow on the timsTOF Ultra2

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Introduction

For cancer treatment, common strategies are the establishment of cell cultures from cancer tissue biopsies to assess probabilities in treatment success or drug responsiveness. Typically, 2D tissue culture is performed, however, this artificial lab environment is altering inter cell connectivity, communication, and cell to cell microenvironments and hence responses to treatment as well. 3D spheroid-based cell culture simulates structural properties of solid tumors better. Single cell proteomics aims to decipher heterogeneity on cell microenvironment. Therefore, we want to assess treatment responses in small spheroids versus 2D culture derived single cells using the cellenONE proteoCHIP EVO 96 platform for small spheroid and single cell isolation, direct transfer to Evotips and proteome analysis using the timsTOF platform.

Methods

2D cultured cells as well as small spheroids were isolated into the proteoCHIP® EVO 96, directly lysed and digested using the cellenONE platform. Samples were transferred by centrifugation onto Evotips (96 tip box). Prepared boxes were placed on top of an Evosep One and analyzed using either the Whisper Zoom40 SPD method with peptides separation on a 15 cm Aurora Elite C18 column (IonOpticks) or the Whisper Zoom 80 or 120SPD mehods with peptides separation on a 5 cm Aurora Rapid 75 C18 column (IonOpticks). Peptides were eluted into a timsTOF Ultra2 with data acquisition in dia-PASEF® mode with optimized window placement using pyDIAid [1]. Collected data were processed with Spectronaut 19 (Biognosys) using directDIA+.

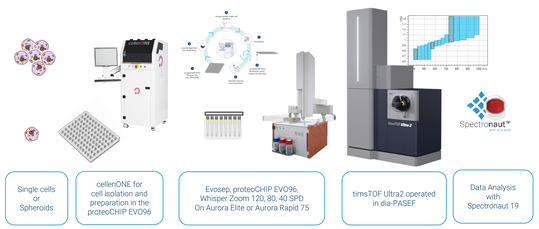


Figure 1:
Single cell and Spheroid proteomics workflow with the Evosep One with Whisper Zoom 120, 80, and 40SPD using the proteoCHIP EVO96 for pipetting-free sample transfer onto Evotips Pure and pyDIAid [1] optimized dia-PASEF data acquisition on **timsTOF Ultra2** with data analysis in Spectronaut 19.

Results

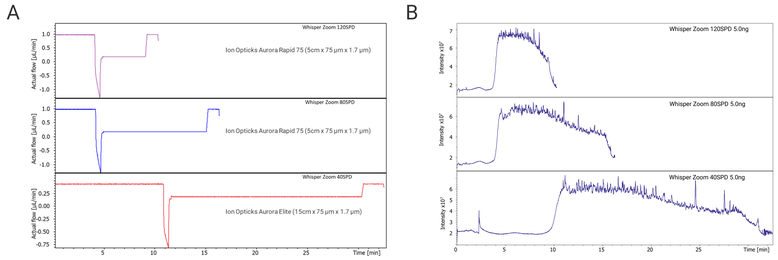


Figure 2:
A) Overview of isocratic high pressure pump flow rates (µL/min) in Whisper Zoom 120, 80 and 40 SPD with 200 nL/min during peptide elution and 1 µL/min (W120 and W80 SPD) or 450 nL/min (W40 SPD) during transfer of sample in pre-formed gradient to the analytical column. B) Typical total ion currents (TIC) MS1 of 5 ng HeLa peptide digest (Pierce) analyzed in Whisper Zoom 120, 80 and 40 SPD.

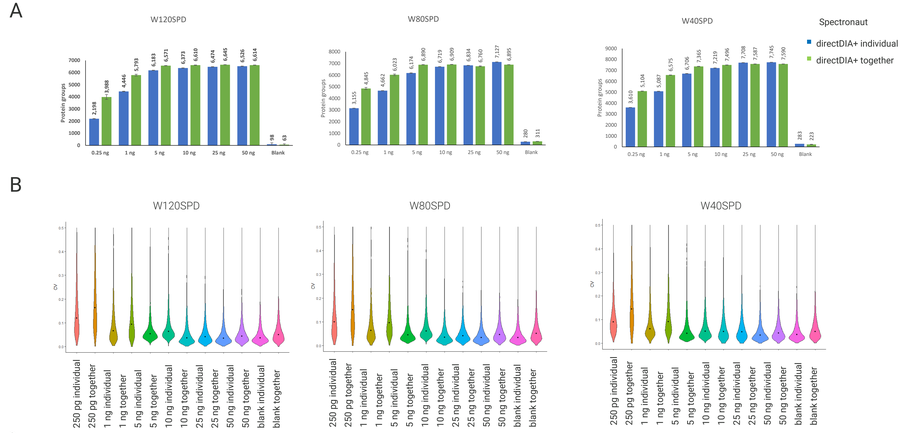
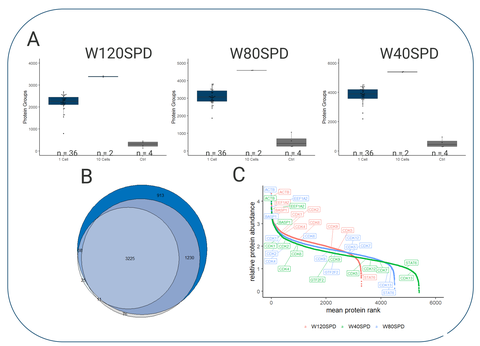


Figure 3:
A) Protein group identifications of HeLa peptide digest (Pierce) dilution series from 50 ng to 250 pg loaded on Tip, acquired in dia-PASEF mode (window placement optimized using pyDIAid [1]) and analyzed in Spectronaut with directDIA+ either processed individually per group or the entire dilution series together. Protein group identification at 250 pg increase from 2.200/4.000 (indv./together) at 120 SPD to 3.600/5.100 (indv./together) at 40SPD. B) Violin plots showing CVs within each concentration group per Whisper zoom method and processing type with median CVs for almost all conditions below 10%.

Figure 4:
A) Protein groups identified from single HeLa cells, 10 Hela cells and no-cell dispense controls analyzed in 3 Whisper Zoom methods 120, 80, 40 SPD using a spectral library generated from the respective 10 cell runs in directDIA+. Protein groups show a steady increase in identifications from 120 SPD (mean 2.300 protein groups) to 80 SPD (mean 3.000 protein groups) to 40 SPD (mean 4.000 protein groups). B) Venn-diagram showing excellent overlap of protens identified at Whisper Zoom 120, 80 and 40SPD. C) Mean protein group rank plots of proteins identified in single HeLa cells showing same dynamic range across the 3 Whisper Zoom methods with increasing proteome coverage correlating with the gradient lengths. Protein abundance rank remains comparable between the methods, as shown for selected structural proteins, transcription factors and cell cycle regulating proteins. Proteins gained with the longer gradient methods are from the lower abundance range.



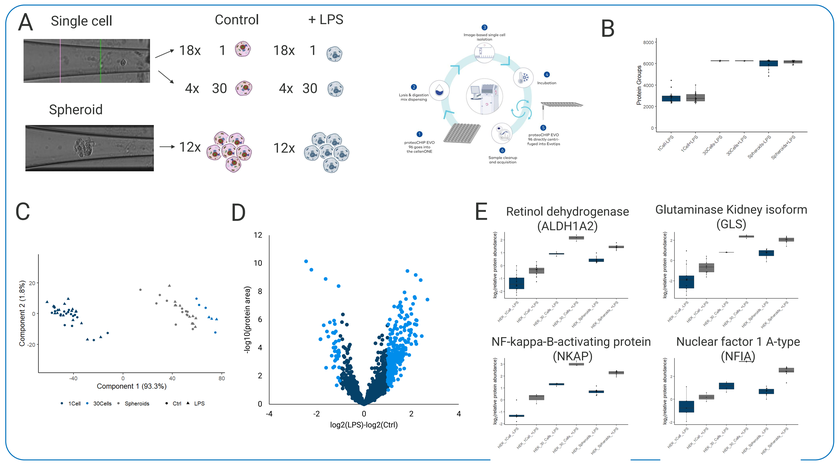
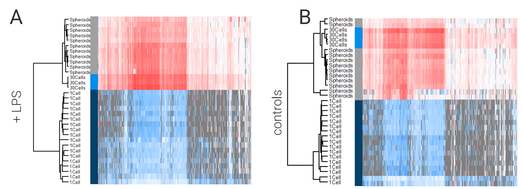


Figure 5:
A) Isolation scheme of 2D cultured HEK cells and small HEK cell spheroids into the proteoCHIP EVO96 followed by transfer by centrifugation of digested proteins onto Evotips and analysis with Whisper Zoom 40 SPD. B) Boxplot showing protein group identification rates across the 3 conditions with mean 2.800 protein groups for single HEK cells, mean 6.200 for spheroids and 6.300 protein groups for the 30 cell bulk. C) Differentiation on protein abundance level based in cell numbers per sample in a PCA projection plot, spheroids cluster closely with 30 cell bulk. D) Volcano plot of LPS treatment vs controls after condition-based data normalization showing significant changes in protein abundance in response to treatment. E) Example protein abundance profiles of proteins with abundance increase in response to treatment in single cells, 30 cell bulks and spheroids. Largest variation seen on single cell level.

Figure 6:
Heatmap representation of abundance profiles of proteins identified in at least 50% of all samples per condition A) cells + LPS and B) controls. Heatmaps demonstrate high degree of similarity in protein abundance distribution in the spheroids and 30 cell bulk samples. Proteins identified in the single cells manly represent proteins at higher abundance level in the 30 cell bulk and spheroid LPS treated and control samples



Conclusion

- Highly reproducible and robust low sample amount analysis on the timsTOF Ultra2 using Evosep Whisper Zoom methods in 120 SPD, 80 SPD and 40 SPD.
- Scalable single cell analysis with high sensitivity and reproducibility on identification and quantification demonstrated on HeLa cells with protein depth of more than 4,000 protein groups identified per single HeLa cell.
- Excellent proteome depths for small single spheroids isolated on the cellenONE exceeding 6.000 proteins.
- Response to LPS treatment compared to untreated cells conserved from single cell to spheroids to 30 cell bulk with alterations in protein abundance in stress response and inflammation response processes.

timsTOF Ultra2

References

[1] P. Skowronek, Matthias Mann et al. Mol Cell Proteomics, 2022, 21, 9, 100279

Further reading

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

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

Application Note, Bruker Daltonics, LCMS-206, 1815135, 2023

Application Note, Bruker Daltonics, LCMS-213, 1901456, 2023

Application Note, Bruker Daltonics, LCMS-222, 1911577, 2024

Conflict of Interest
CK, VT, ML are employees at Bruker Daltonics GmbH & Co. KG; AS, DH, GT are employees at Cellenion; DBJ, NB are employees at Evosep.