

# Ultra-high sensitive Single Cell Proteomics on the timsTOF Ultra

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## Introduction

For single cell proteome analysis, ultra-high sensitivity mass spectrometry is a key to reach a proteome coverage necessary for understanding the cellular heterogeneity on a cell-by-cell level. The latest enhancements in ion transfer with a larger transfer capillary, an additional higher-pressure segment for more effective ion collection and two orthogonal deflections, to maintain robustness, pushes the limits of detection to the single cell level. Combined with automated single cell isolation and sample preparation using the cellenONE® platform for protein-loss reduced preparation and transfer with the proteoCHIP format leads to deep proteome coverage and high reproducibility.

## Single cell proteomics Ecosystem

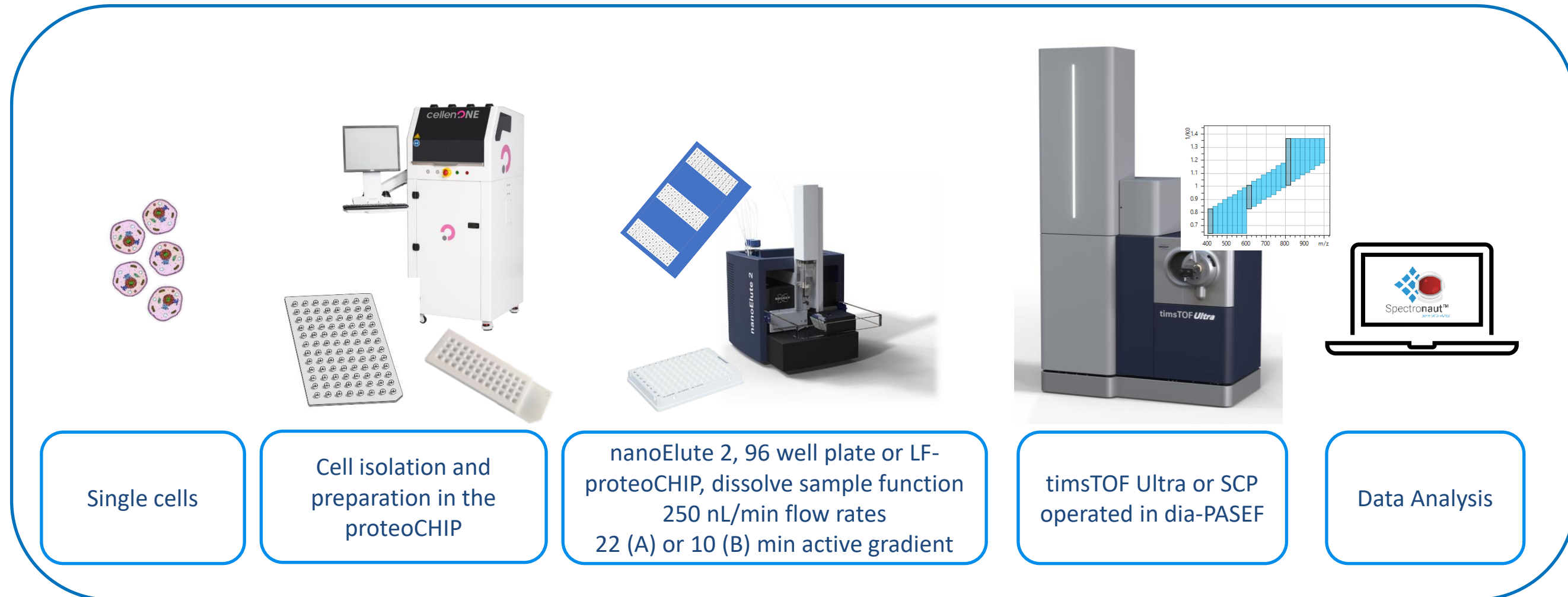


Figure 1: Single cell proteomics workflow with the nanoElute 2 dissolve sample function for pickup of lyophilized samples from the label-free proteoCHIP or a 96-well plate on **timsTOF Ultra**.

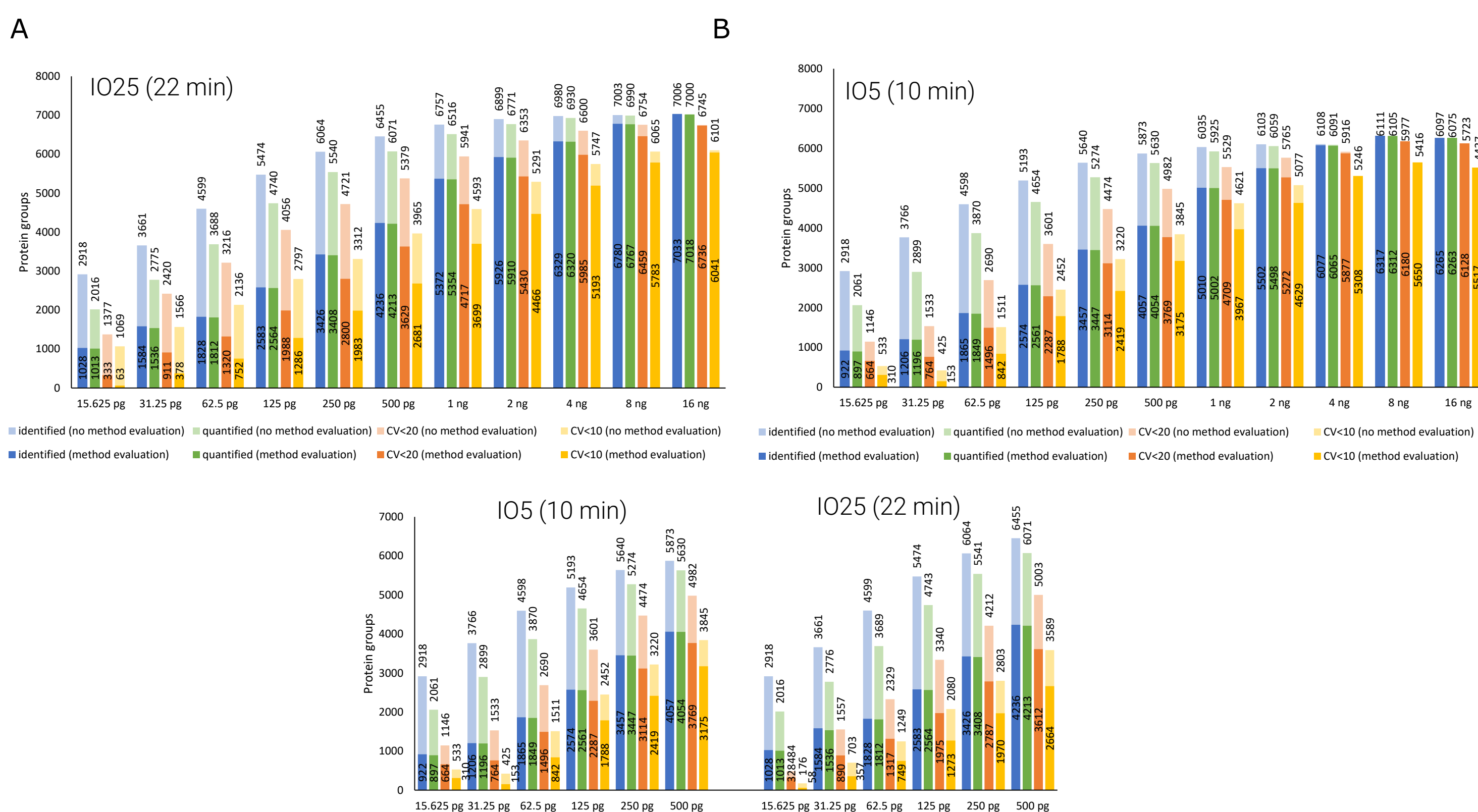


Figure 2: A) Protein group identification rates of a K562 peptide dilution series from 16 ng to 15.625 pg loaded on column, acquired in dia-PASEF mode and analyzed in Spectronaut 18 with directDIA+, grouped by IDs total, quantified, CV < 20 and CV < 10 either with method evaluation enabled for each concentration group or without method evaluation (no method evaluation) using a Human protein sequence data base (20,598 entries). B) Same coverage using a 10 minute (80 SPD) method with 5 cm Aurora Rapid 75 column (IonOpticks).

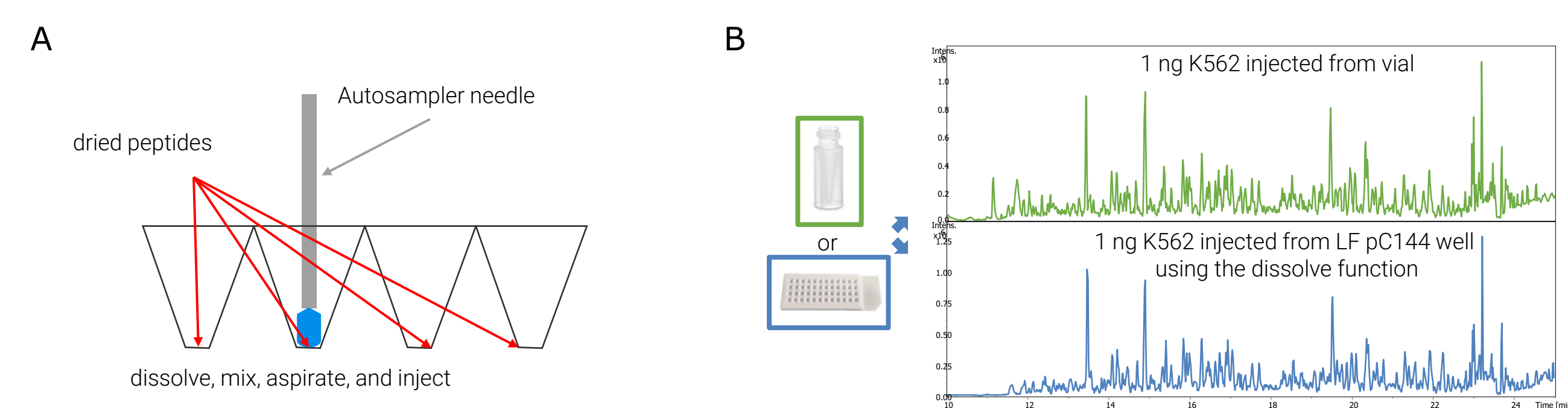


Figure 3: A) schematic of the dissolve sample function using the nanoElute 2 CTC autosampler arm. B) BPC of 1 ng K562 cell digest either injected from vial or resuspended with the dissolve sample function from the LF proteoCHIP.

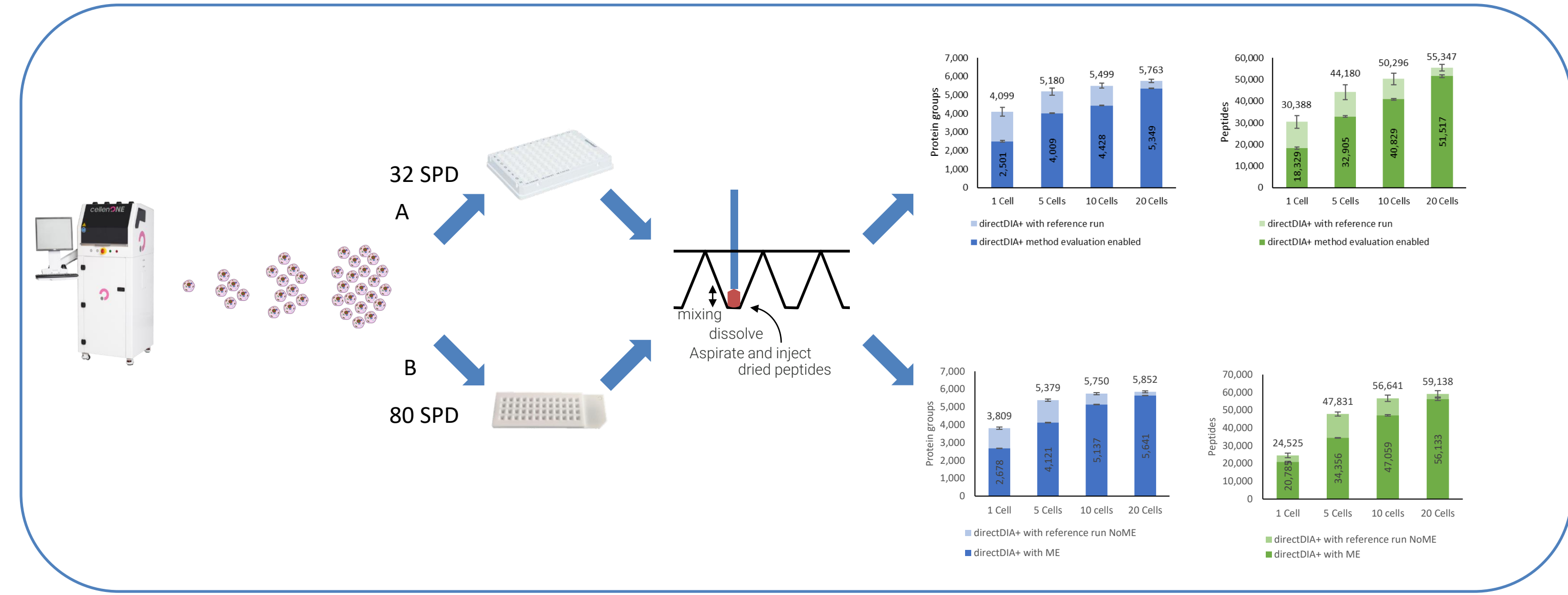


Figure 4: Protein group and peptide identification rates of HeLa cells isolated at counts of 1, 5, 10, and 20 cells per well (n = 3). Samples were prepared in the proteoCHIP LF 48, A) transferred into a 96-well plate, dried, dissolved immediately prior to injection and analyzed at 32 SPD or B) kept in the proteoCHIP LF 48 dried, dissolved immediately prior to injection and analyzed at 80 SPD. The entire sample was loaded onto column, acquired in dia-PASEF mode. and the resulting data were analyzed in directDIA+ using Spectronaut 18 in method evaluation mode for each cell count and method evaluation disabled with 8 ng K562 peptide loads in 32 or 80 SPD as reference runs.

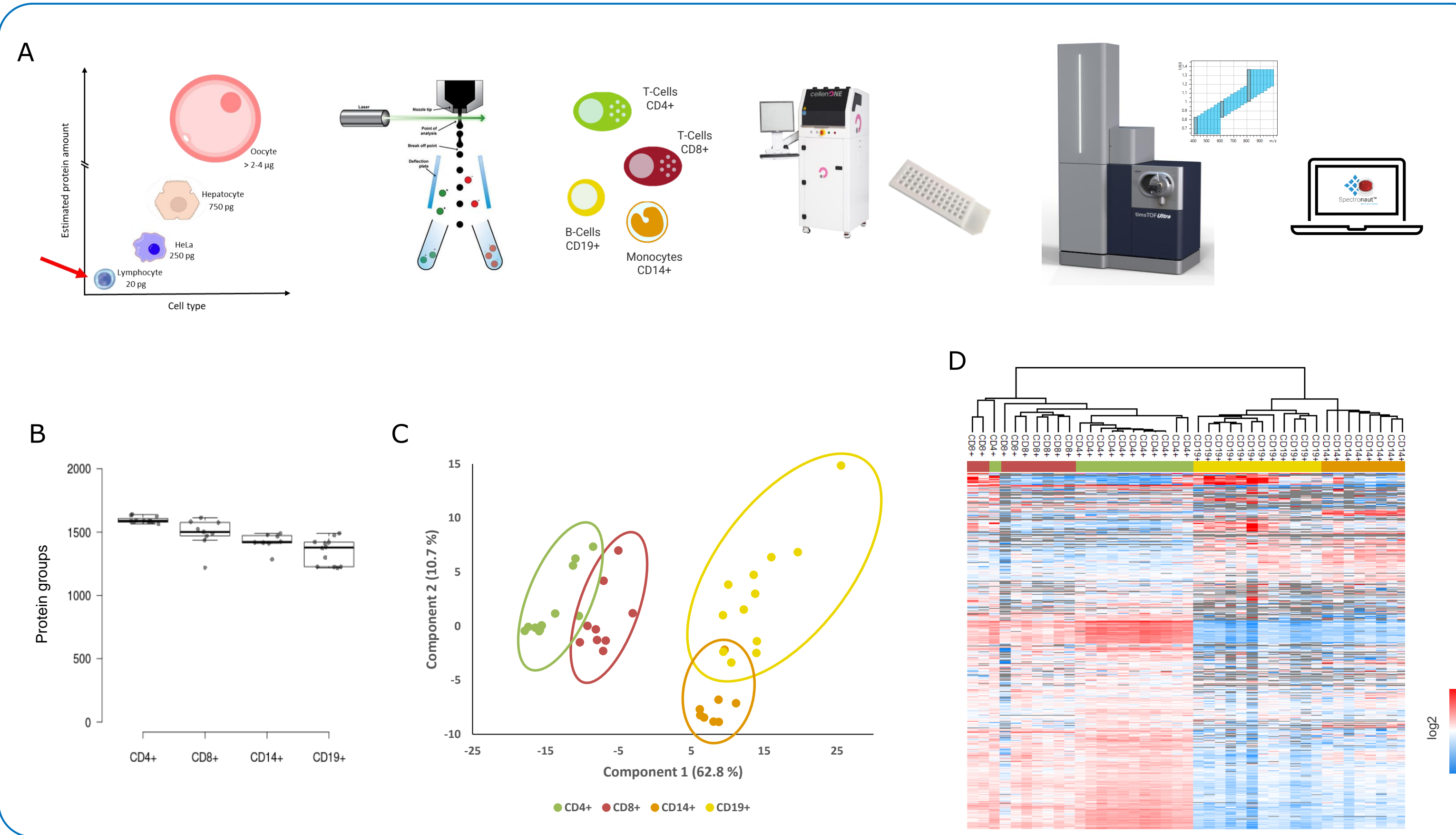


Figure 5: A) PBMC analysis Workflow from FACS sorting of T-Cells (CD4+, CD8+) B-Cells (CD19+) and monocytes (CD14+), single cell isolation with the cellenONE into LF proteoCHIP, mass spectrometric analysis on the **timsTOF Ultra** using the 10 min gradient on I05 and data analysis in Spectronaut in directDIA+ identifying in total 1,713 protein groups. B) Box plot of protein group identification rates across the 4 different cell types demonstrating good protein identification rate reproducibility for each cell type group. C) Differentiation on protein abundance of the 4 cell types in a PCA projection plot. D) Heatmap of protein group abundance pattern shows distinct clustering by cell type with good reproducibility with a cell type group.

## Conclusions

- Hands-free and pipetting-free workflow using the dissolve sample function of the nanoElute 2 for resuspension of lyophilized peptide pickup from the LF proteoCHIP
- High sensitivity with good chromatographic reproducibility and robustness with 10 min (80SPD) or 30 min (32 SPD) run time
- Good quantification accuracy at single cell level with good single cell to single cell reproducibility on protein level with protein depth of more than 4,000 protein groups per single HeLa cell on the timsTOF Ultra
- Sorted PBMC analysis workflow shows good proteome coverage with distinct protein abundance profiles for different the 4 cell types

## Further reading

Application Note, Bruker Daltonics, LCMS-193, 1894933, 2022; Application Note, Bruker Daltonics, LCMS-194, 1895626, 2022; Application Note, Bruker Daltonics, LCMS-206, 1815135, 2023; Application Note, Bruker Daltonics, LCMS-213, 1901456, 2023