Accelerating Discovery: High-Throughput Single-Cell Proteomics with Rapid Analysis and Deep Protein Coverage

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Introduction

Single-cell proteomics reveals protein-level differences masked by bulk analysis, offering critical insights into disease, immune responses, and therapy resistance. This is key for advancing oncology, immunology, and neurodegenerative research. We present a high-sensitivity, high-throughput C18 column optimized for low-input samples, enabling deep, scalable proteome coverage. This workflow supports large-scale single-cell studies, accelerating biomarker discovery and advancing precision medicine.

Methods

Diluted K562 tryptic digests were analyzed using a high-sensitivity LC-MS workflow optimized for low-input proteomic samples, with specific application to single-cell proteomics. Chromatographic separation was performed on a short, high-efficiency C18 column designed for low-concentration sample loading, enabling sharp peak shapes and minimal carryover. The system was integrated with the nanoElute 2 UHPLC and coupled to a timsTOF ULTRA platform, leveraging trapped ion mobility spectrometry (TIMS) and parallel accumulation–serial fragmentation (PASEF) for maximal sensitivity and acquisition speed. A 10-minute dia-PASEF acquisition method employing 26 Da precursor isolation windows was used, providing efficient ion sampling across a mobility range of 0.7–1.3 1/K_0 and a mass range of 300–1200 m/z. With a TIMS ramp and accumulation time of 65 ms, the total cycle time was reduced to 0.85 s, enabling high-throughput data acquisition with minimal duty cycle losses. This configuration enhances proteome coverage, increases quantification precision, and is well-suited for large-scale, single-cell-level studies, offering a scalable solution for ultra-low-input proteomics.

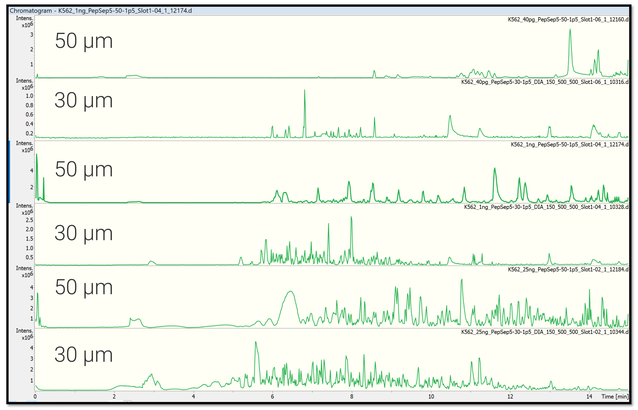

Figure 1. *Analysis of three concentrations of K562 cell lysate (40 pg, 1 ng, and 25 ng) using the nanoElute 2 system coupled to a timsTOF Ultra mass spectrometer. Samples were injected onto two short 5 cm analytical columns with internal diameters of 30 µm and 50 µm, each packed with Dr. Maisch C18 media. The setup was optimized to assess performance across varying sample loads and column dimensions*

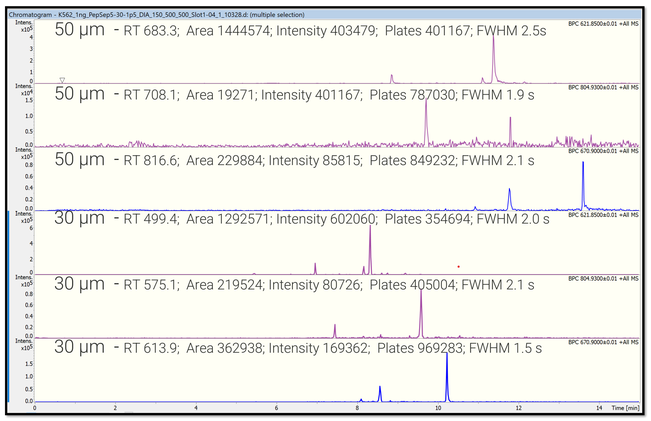

Figure 2. *Comparison of extracted ion chromatograms (XICs) for 1 ng K562 digest using 5 cm columns with 30 µm vs. 50 µm I.D. Separation was performed on a Bruker nanoElute 2 system coupled to a timsTOF Ultra, illustrating performance differences at ultra-low sample loads.*

Results

We evaluated a high-throughput nanoLC-MS workflow using 5 cm C18 columns (30 µm and 50 µm i.d., packed with Dr. Maisch media) coupled to the nanoElute 2 and timsTOF Ultra platform. Despite the shorter column length and faster run time, proteome coverage, peak width and protein/peptides ids remained comparable to traditional, longer-gradient approaches, confirming that significant throughput gains can be achieved without compromising data quality.

The system consistently operated at lower backpressures across all injections, resulting in improved run-to-run stability, peak width (Figure 3), and greater robustness.

The performance characteristics (Figures 4 and 5) make the workflow highly compatible with diverse UHPLC systems and scalable to large cohort studies.

We assessed proteome coverage across a range of K562 digest inputs, including ultra-low sample amounts relevant to single-cell proteomics:

8 pg: ~1000 peptides (30um), ~2,400 peptides (50 um)

40 pg: > ~5200 peptides (30 um), ~7,650 peptides (50 um)

1 ng: > ~32,750 peptides (30 um), >30,000 peptides (50 um)

5 ng: > ~61,000 peptides (30 um), >55,000 peptides (50 uM)

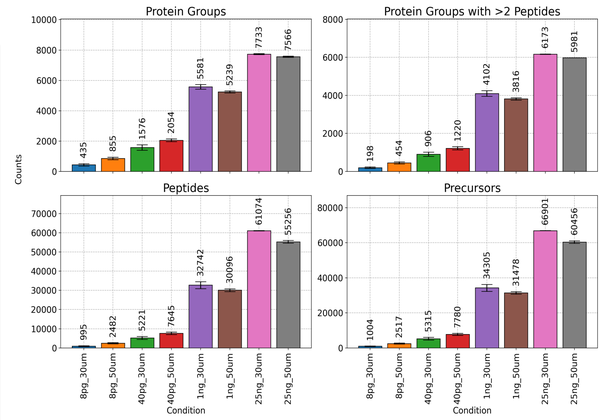

Figure 3. Quantitative comparison of average protein groups, peptides, and precursors identified using the DIA-PASEF workflow across triplicate injections of three sample concentrations. Experiments were conducted on 5 cm analytical columns with 30 µm and 50 µm I.D., separated on a Bruker nanoElute 2 system and analyzed on a timsTOF Ultra, highlighting the impact of column format on proteomic depth and reproducibility."

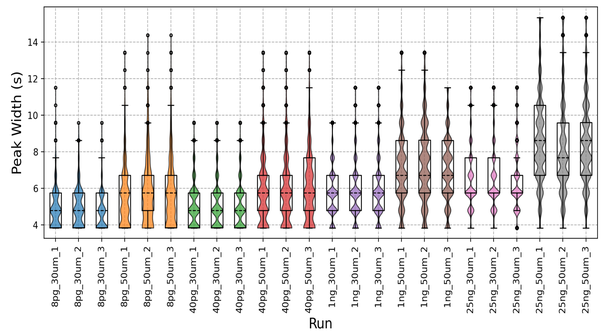

Figure 4. Assessment of average peak width for K562 digest at 8 pg, 40 pg, 1 ng, and 25 ng concentrations using 30 µm and 50 µm I.D. 5 cm columns. Triplicate DIA-PASEF injections were performed on a Bruker nanoElute 2 system coupled to a timsTOF Ultra, highlighting the influence of column format and sample load on chromatographic resolution."

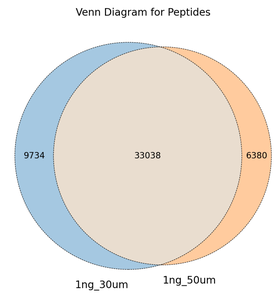

Figure 5. Comparison of unique peptide identifications from 1 ng K562 digest using 5 cm columns with 30 µm and 50 µm I.D. The Venn diagram highlights complementary peptide coverage achieved with each format. Data were acquired using DIA-PASEF on a Bruker nanoElute 2 coupled to the timsTOF Ultra.

Summary

These results underscore the method's exceptional sensitivity and depth, even at picogram levels. The optimized chromatographic and MS parameters allowed for efficient data acquisition, high reproducibility, and precise quantitation—all essential for high-resolution single-cell proteomics.

This workflow enables comprehensive profiling of thousands of single cells with minimal sample consumption, accelerating biomarker discovery, rare cell population identification, and dynamic protein expression analysis. Moreover, its broad applicability extends to clinical, translational, and population-scale proteomics where time, sensitivity, and reproducibility are critical.

Conclusion

- **Short, fast gradients** deliver **comparable proteome depth** to traditional long-column setups.
- **High sensitivity** enables deep proteomic coverage from as little as **8 pg of input**.
- **Over 5,500 protein groups and 32,000 peptides** identified from 1 ng of K562 digest.
- Ideal for **high-throughput studies** in precision medicine and translational research.

Single Cell II