Unveiling High-Throughput and Sensitive Single-Cell Proteomics: with Rapid LC-MS Workflows

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Michael Krawitzky[1], Andy Phan[1]; Jose Luis Marin Rubio[2]; Petra Martinovic[3]

[1]Bruker Scientific, Billerica, USA
[2]Memorial Sloan Kettering Cancer Center, New York, USA
[3]Bruker Daltonics, Bremen, Germany

Introduction

Single-cell proteomics allows for precise protein expression analysis at the individual cell level, surpassing the limitations of bulk averaging and enabling deeper biological insights. This study introduces a high-sensitivity, high-throughput C18 column specifically designed for single-cell proteomics, addressing the challenges posed by low-concentration samples while delivering enhanced sensitivity and resolution.

Methods

A commercial K562 dilution series was prepared ranging from 625 ng to 8pg. Our approach utilizes a short, high-efficiency C18 column (PepSep 5 cm x 30µm x 1.5µm) optimized for low-concentration separation, such as single-cell samples, integrated with the nanoElute 2 UHPLC and timsTOF ULTRA platforms. By employing a 10-minute dia-PASEF gradient (15 min total) Figure 2 with 26 Da isolation windows, (Figure 1) we effectively cover a mobility range of 0.64–1.45 and a mass range of 400–1000 m/z. The optimized method, with a 100 ms ramp and accumulation time, achieved a cycle time of just 0.95 seconds, significantly enhancing throughput without compromising data for dia-PASEF acquisition. Tryptic peptides from a human lysate digest were analyzed, and data processing was conducted using Bruker Proteoscape (tims DIA-NN). The FASTA database used was from uniport.org (human). The Spectral library was the Bruker human spectral library.

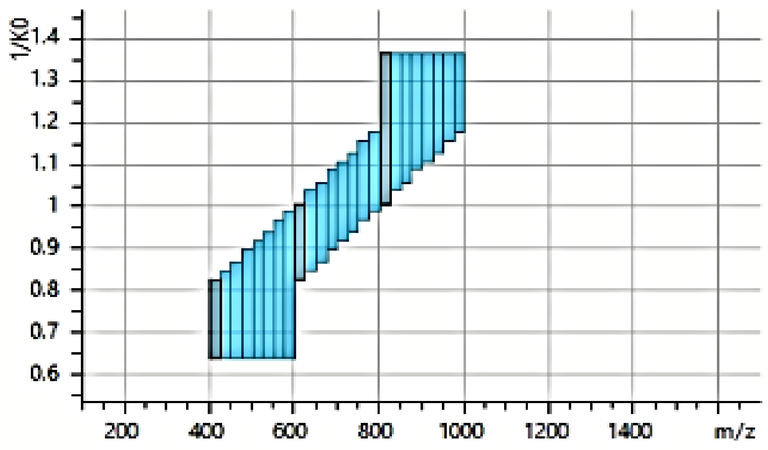


Figure 1: 26 Da window (24 total windows) dia-PASEF window placement covering a mobility range of 0.64–1.45 and a mass range of 400–1000 m/z. The cycle time was 0.95s.

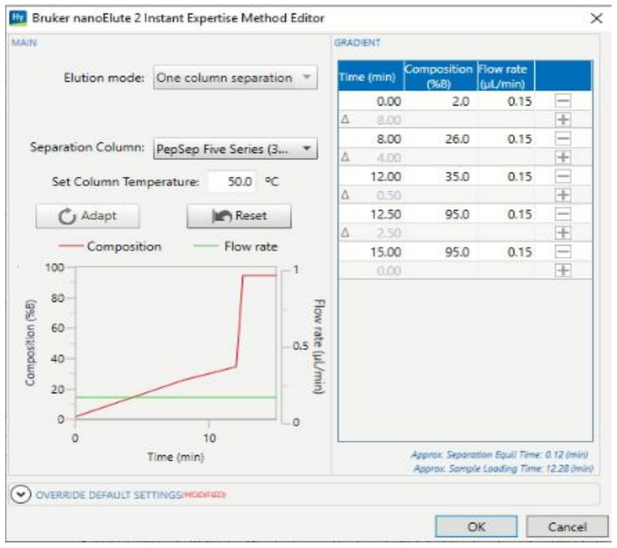
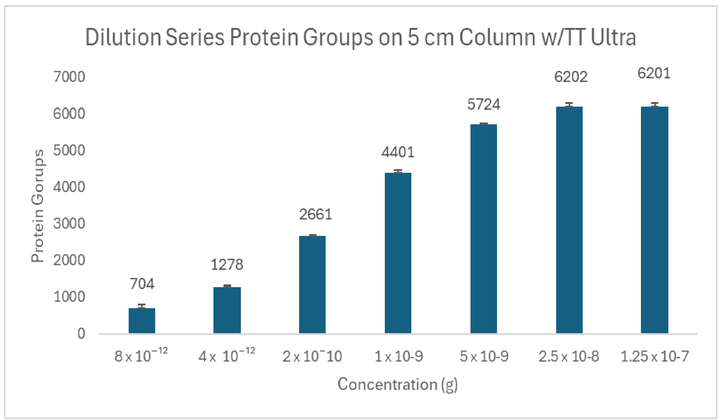
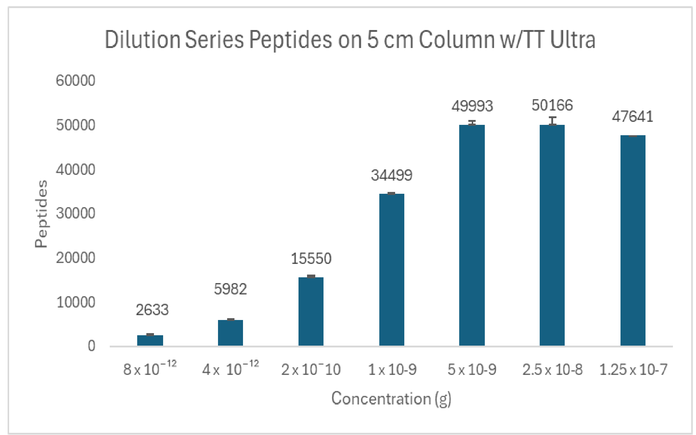


Figure 2: LC separation gradient run on nanoElute 2. Gradient length was set for 12 min with a total time of 15 min including wash. The flow rate was 150 nl/min



A



B

Figure 3: Dilution series of commercial K562 digest (A) Protein Groups, (B) Peptides analyzed using a PepSep Custom (5 cm x 30 µm x1.5 µm) column, coupled to the nanoElute2® and timsTOF ULTRA. The concentration injected was 8pg to 125 ng utilizing a dia-PASEF method with 24 total windows, using 3 steps was used. 60 Samples per day throughput was achieved with a 15-minute gradient. Ion mobility (IM) was set at 0.64 (1/k0 start) to 1.45 (1/k0 end). Data analysis was conducted using Bruker Proteoscape.

Results

Despite the reduced run time, our results closely matched those of longer columns, running longer gradients, with consistently lower pressures across injections and higher sensitivity. From 8 pg K562 sample, we identified over 700 protein groups and 2,600 peptides in 15 minutes. 5ng resulted in more than 5700 protein groups and nearly 50,000 peptides, showcasing the depth of proteomic coverage achievable with rapid analysis

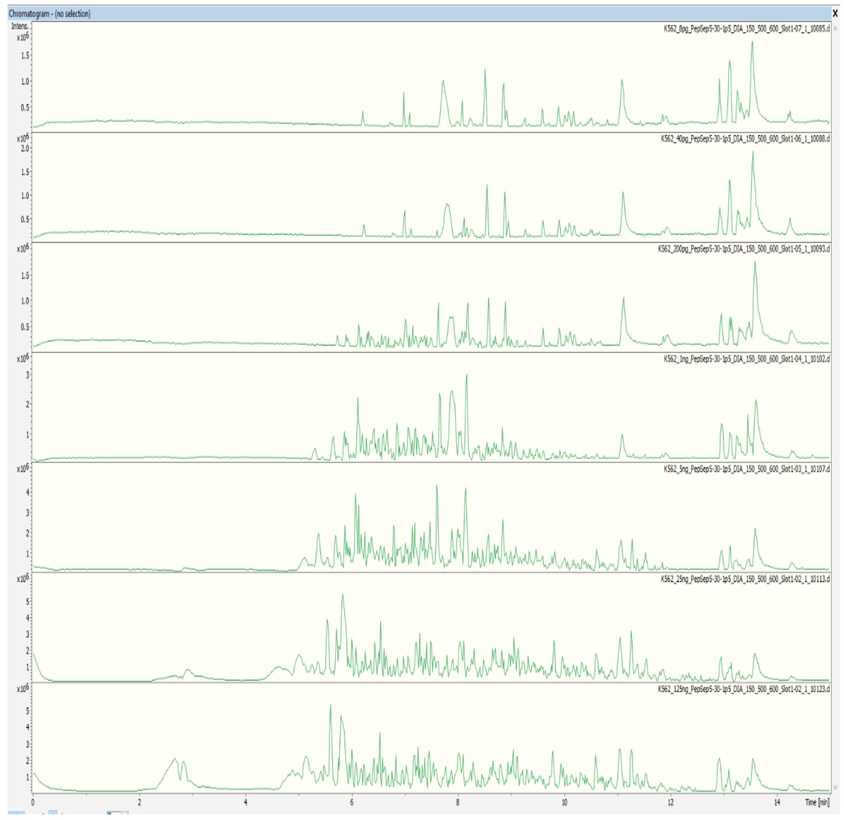


Figure 4: Base peak chromatogram comparison between a dilution series of K562. K562 peptides were separated on a 15 min gradient run on a PepSep custom column coupled to nanoElute2 and timsTOF Ultra mass spectrometer

Conclusions

This study highlights the advantages of high-throughput single-cell analysis, demonstrating how shorter, faster runs can maintain high proteomic depth while improving efficiency. These advancements accelerate single-cell proteomics and broader LC-MS applications, providing richer insights into protein expression at the individual cell level.

Highlights

High Sensitivity: Short runs maintained comparable results to longer gradients, with improved sensitivity.

Deep Proteomic Coverage in Minutes: 8 pg K562 sample yielded 700+ protein groups and 2,600 peptides in 15 minutes, while 5 ng identified 5,700+ protein groups and ~50,000 peptides.

Advancing Single-Cell Proteomics: Faster, high-throughput LC-MS runs enhance efficiency while preserving proteomic depth, accelerating insights into protein expression at the single-cell level.

LC/MS Columns