Elevating Precision in –Omics Applications: A Breakthrough with Monolithic Silica Columns

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Introduction

Single-cell proteomics is one of several scientific domains in -Omics that aim to understand protein expression within individual cells, contrasting with bulk approaches that average expression across thousands of cells. This presents unique challenges in analysis due to the need for high sensitivity and resolution, making traditional mass spectrometry methods particularly challenging.

Methods

We designed a prototype monolithic silica capillary column tailored for effectively separating low concentration samples, including single cells. This was achieved by harnessing the power of the nanoElute 2 UHPLC system in conjunction with the timsTOF ULTRA platform, and incorporating a 11.5-minute gradient technique we've developed, known as dia-PASEF. Our approach involves a window schema comprising 35 Da windows, spanning a mobility range from 0.7 to 1.3 (Figure 1), and a mass range from 300 to 1200 m/z. Parameters such as ramp and accumulation times were set at 75 milliseconds, resulting in a total cycle time of 0.65 seconds. Tryptic peptides from human lysate digest were used and data analysis was performed on Bruker Proteoscape software.

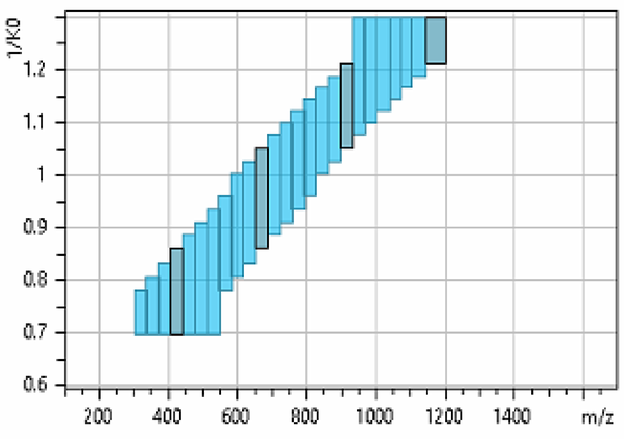
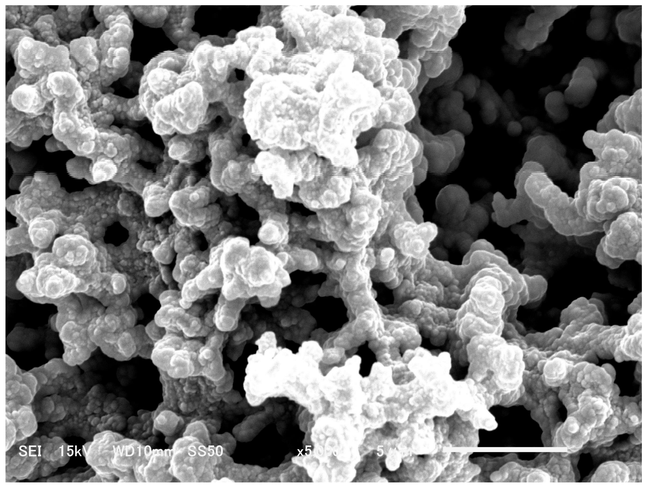


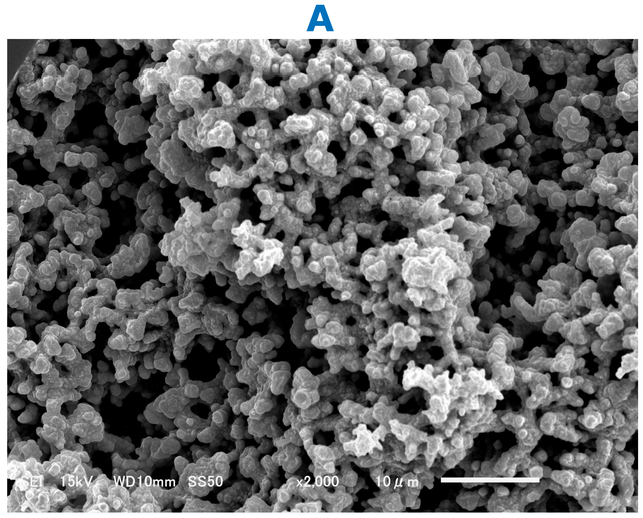
Figure 1: dia-PASEF window placement scheme. The cycle time was 0.65s

Results

We achieved comparable peptides and proteins results (Figure 3) as compared to conventionally packed C18 columns. Notably, we maintained lower pressures, across all injections and near identical chromatographic performance (Figure 4) indicating the broad applicability for various UHPLC systems. In total, our analysis using Proteoscape software enabled the identification of over 5,000 protein groups and 70,000 peptides .



A



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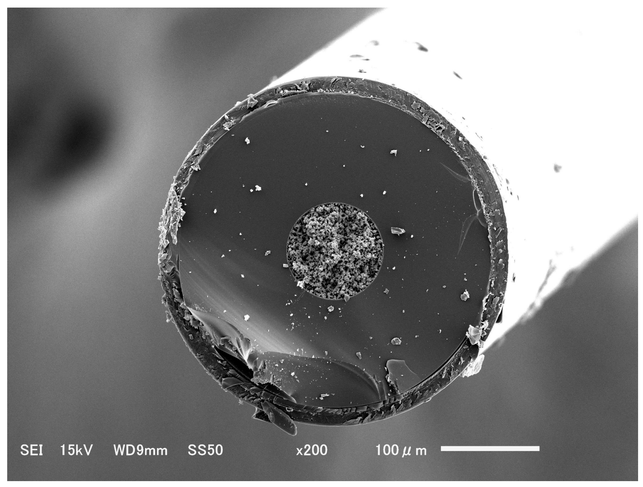

C

Figure 2: Scanning electron microscope images of monolithic column (A) x5000 (B) x2000 (C) x 500

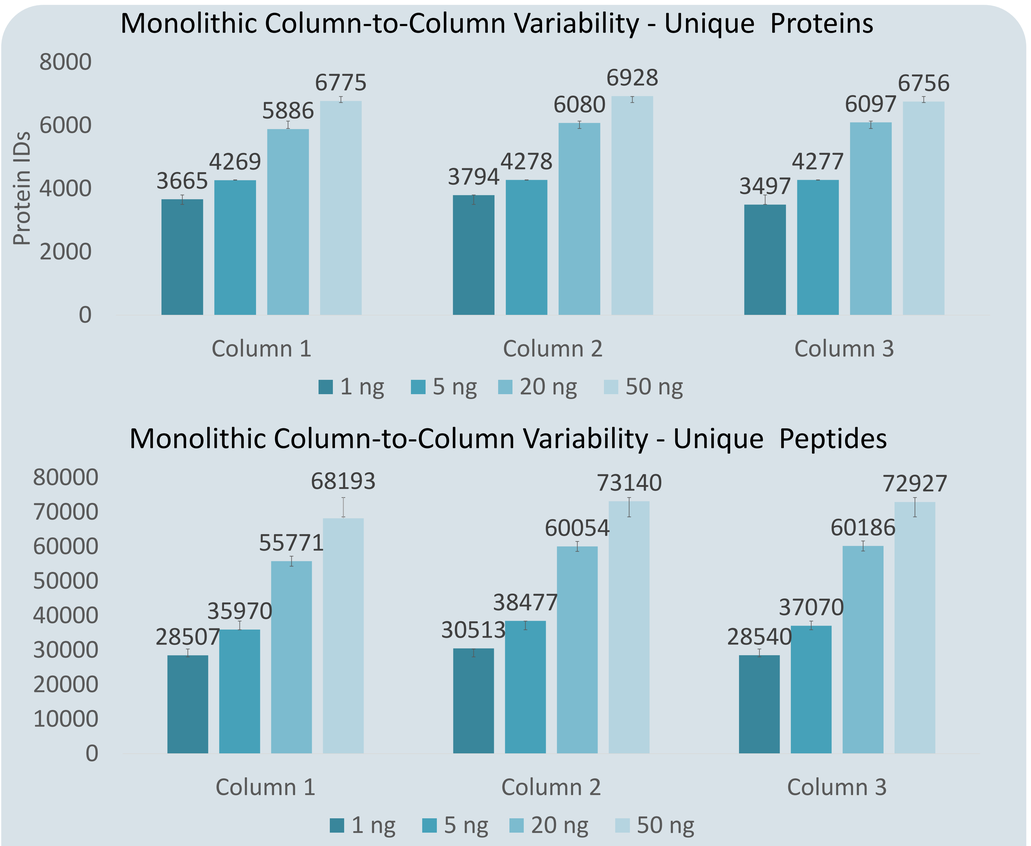

Figure 3: Analysis of commercial K562 digest using Monolithic columns (15 cm x 75 µm), coupled to the nanoElute2® and timsTOF ULTRA. A dia-PASEF method with 25 variable (width and mobility) windows, using 3 steps was used. One-hundred samples-per-day throughput was achieved with an 11.5-minute gradient. Ion mobility (IM) was set at 0.7 (1/k0 start) to 1.3 (1/k0 end). Data analysis was conducted using Bruker Proteoscape (no MBR), and triplicate injections were performed per concentration per column.

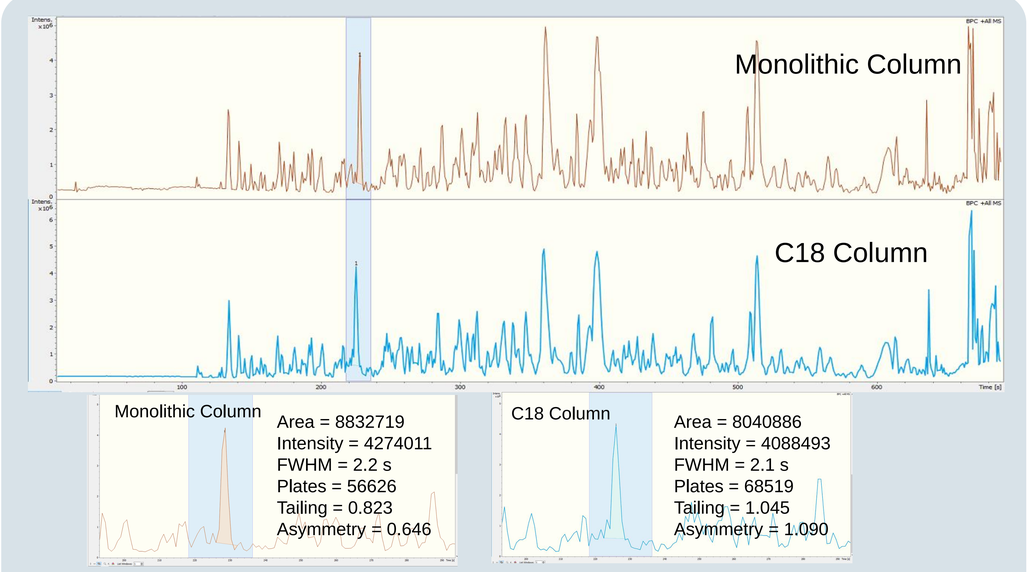

Figure 4: Base peak chromatogram comparison between conventional packed c18 column vs Monolithic Column running 50 ng K562 on 11.5 min gradients

Conclusions

- Improved sensitivity with Monolithic column vs Conventional C18 column
- Optimal for single-cell and low sample amount applications
- Versatile for wider application in LC-MS and multiomics applications

LC/MS Columns