Ultra-high sensitivity proteomics on the timsTOF SCP

The sensitivity of the mass spectrometer plays a crucial role in analyzing low input samples and single cell proteomics.

Abstract

The timsTOF Pro platform, introduced in 2017, already featured a sensitivity boost from the Parallel Accumulation SErial Fragmentation (PASEF) technology, which provides time and space focusing of the ions in the Trapped Ion Mobility Spectrometry (TIMS) tunnel. The timsTOF SCP platform further enhances the sensitivity with robustly modified ion optic design. This boost in sensitivity enables measurement of low nanogram and sub-nanogram peptide loads resulting in quantification of few thousand protein groups per injection. Our data from as low as 200 pg of peptide loads demonstrates the applicability to unbiased true single cell proteomics in a routine fashion.

Keywords: 4D-Proteomics, PASEF, dia-PASEF, data completeness, single cell protoemics

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Introduction

Single cell omics in recent years have highlighted the microheterogeneity in clonal population. Unlike other omics technologies, single cell proteomics is hindered by lack of amplification techniques for protein molecules. Recently single cell proteomics is beginning to get focus and one common strategy is to multiplex labeled single cells together with carrier samples to boost the sensitivity in detection. Further improvements in sophisticated sample preparation techniques have resulted in increased peptide yield that is delivered to the LC-MS instrumentation [1]. While these improvements upstream of sample measurement have improved the analysis depth, the raw sensitivity requires improvement as well without compromising robustness. Parallel Accumulation and Serial Fragmention (PASEF) [2] on the timsTOF Pro platform makes efficient usage of the ion beam and with intelligent precursor placement within a TIMS cycle achieves rapid sequencing speed. In addition, the ions get focused in space and time within the TIMS cell, resulting in a significant boost in the sensitivity. This enables the analysis of low sample amounts, in the range of low ng peptide loads. The newly designed timsTOF SCP's ion optics allows a 4-5x improvement in ion current by increasing the ion brightness, while maintaining the robustness of the timsTOF Pro. As the yield of the electrospray ionization increases with lower flow rates, we further enhanced the experiment's overall sensitivity by coupling the timsTOF SCP to an Evosep One (Evosep Biosystems) operated with the new low-flow Whisper methods. We have characterized the resulting system's performances by injecting and measuring peptide loads mimicking the amount resulting from a single cell preparation.

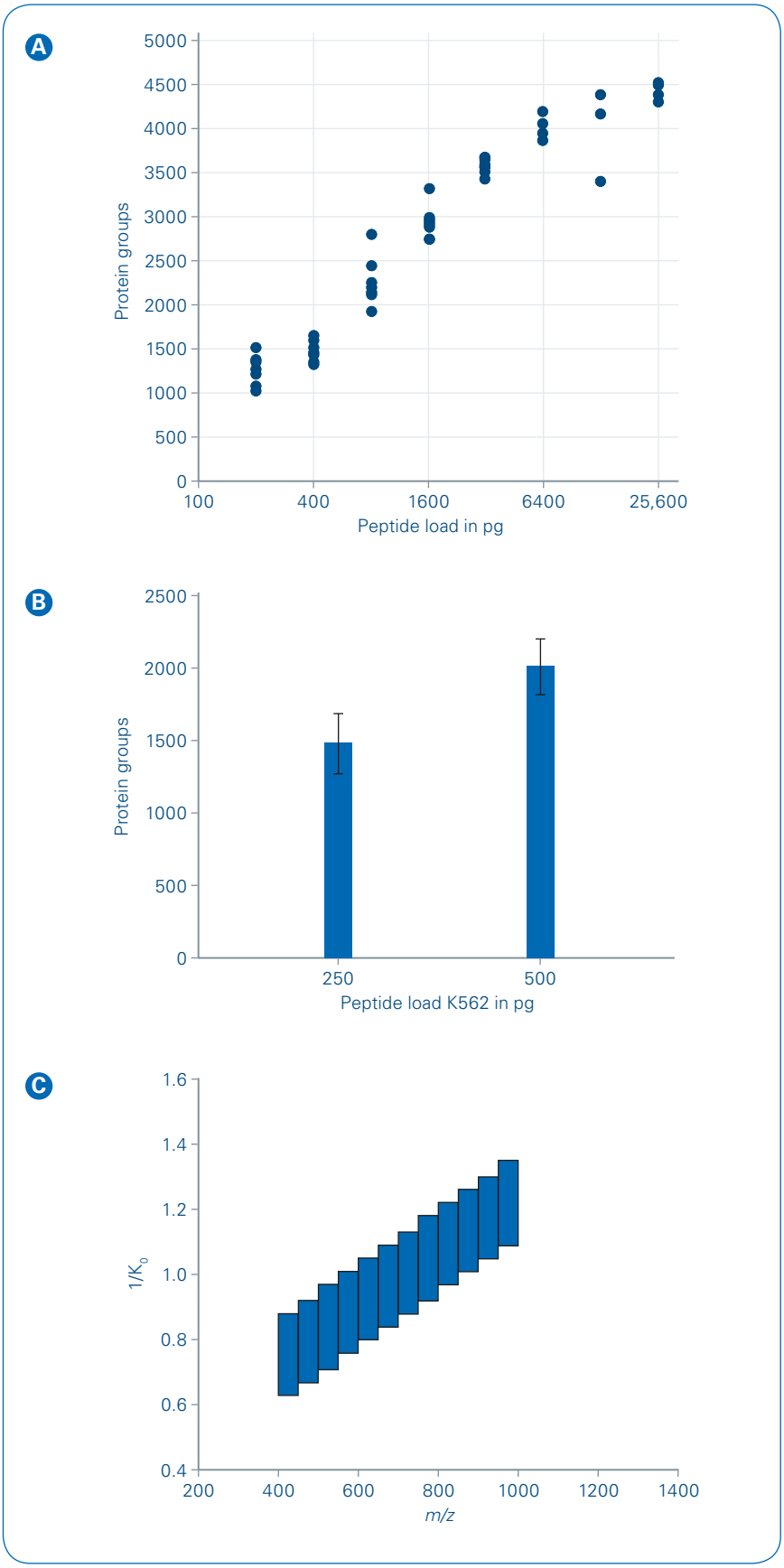

Figure 1. (A) Dilution series of peptides with Whisper 40SPD. (B) Multiple injections of 250 and 500 pg runs using Whisper 40SPD. (C) dia-PASEF window scheme used for low sample amount.

Methods

The new ion optic design of the timsTOF SCP system includes switching the orientation of the ion optics with inclusion of an additional ion funnel and additional orthogonal turns of the ion beam to preserve the robustness of the instrument. The source contained a wider glass capillary orifice that draws more ions into an additional funnel housed in a multi-stage differentially pumped region. Our initial experiments demonstrated that in addition to the brighter ion beam these dedicated modifications were crucial in order to gain a factor five boost in ion current. For ultra-high sensitivity measurements from 200 pg to few ng peptide amount we coupled an Evosep One system (Evosep Biosciences) to the timsTOF SCP instrument and used a ~28 minute gradient Whiper 40SPD method that offers a constant flow of 100 nL/min. Evotips were loaded with K562 (Promega) peptides according to the vendor instructions. Data were acquired in a DIA mode with window placements as shown (Figure-1B). All data were processed using Spectronaut software version 14 with default settings applying a hybrid library.

Results and Discussion

A dilution series of peptide load was performed starting from 200 pg to 25.6 ng in replicates using the ultralow flow method – Whisper 40SPD - from Evosep Biosystems. This method delivers gradient at a flow rate of 100 nL/min further boosting the sensitivity of the platform. About 1200 protein groups could be quantified from the 200 pg loads, and that number increased to an excess of 4000 protein groups for 6,4 ng loads. 250 and 500 pg loads, mimicking the amount of peptides resulting from the digestion of one or two isolated cells, were used to test the accessible proteome depth. These samples were analyzed using the Whisper 40 samples per day (SPD) method applying dia-PASEF methods with a 0.7 cycle time method that covers between 400 and 1000 *m/z*. The data were processed with a library consisting of 5200 protein groups and about 54,000 peptides. From 250 and 500 pg loads on average 1542 and 2146 protein groups were quantified, respectively.

Work done in the labs of Prof.Matthias Mann with the timsTOF SCP combined with robust low flow Evosep One when applied with efficient sample preparation yields exciting results on the biology of the cell cycle [3,4].

Conclusions

- timsTOF SCP provides robust proteome coverage with peptide loads in the range of 250 pg.
- Combination of timsTOF SCP with Whisper methods on the Evosep provide a robust and sensitive platform to perform single cell proteomics.

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