Low pg Level Protein Detection in Cell Lysates with the timsTOF SCP

Thomas Kosinski[1], Amalia Apalategui[1], Oliver Raether, Markus Lubeck[1], Gary Kruppa[2], Matthew Willetts[3] and Nagarjuna Nagaraj[2]

[1]Bruker Daltonik, GmBH & Co, KG, Bremen, Germany; [2]Bruker S.R.O, Czech Rep; [3]Bruker Daltonics, Billerica, MA

Introduction

Recent reports have shown multiple strategies to tackle the problem of detecting low abundant peptides, but no approach has succeeded in increasing the raw sensitivity of the mass spectrometer in a robust manner. Parallel Accumulation and Serial Fragmentation (PASEF) [1] on the timsTOF Pro platform makes efficient usage of the ion beam and, with intelligent precursor placement within a TIMS cycle, achieves rapid sequencing speeds. 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. Here we present ultra-high sensitivity measurements of peptides from as little as 250 pg of protein digest

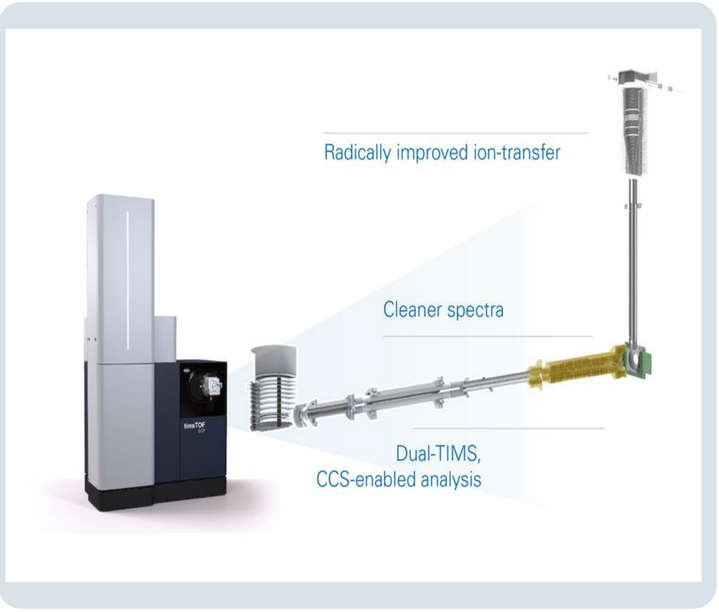

Figure 1: timsTOF SCP features a new ion source geometry with additional higher pressure vacuum stage resulting in a 4-5 x increase in ion current while maintaining the industry leading robustness of the timsTOF Pro.

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 Whisper 40SPD method that offers a constant flow of 100 nL/min. Evotips were loaded with K562 (Promega) peptides according to the vendor instructions. The column was connected to a CaptiveSpray nanoLC ion source using a 10 µm CaptiveSpray emitter. Data were acquired in a dia-PASEF mode with window placements as shown (Figure-2C). All data were processed using Spectronaut software version 14 with default settings applying a hybrid library.

Results

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.

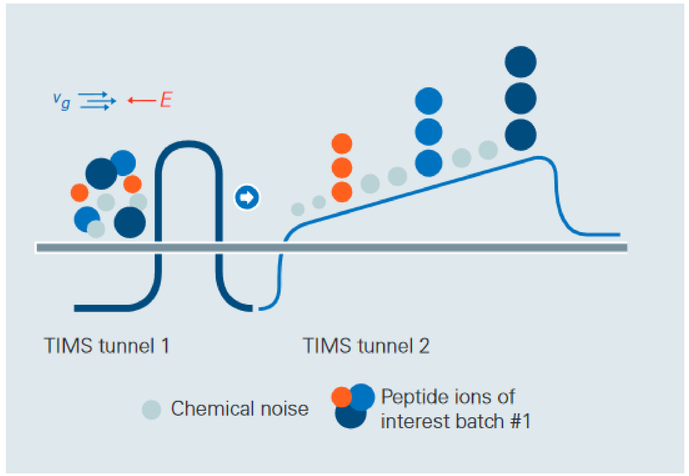

Figure 2: TIMS (Trapped Ion Mobility Spectrometry) accumulates and concentrates ions of a given mass and mobility, while removing chemical noise, enabling an increase in sensitivity and speed.

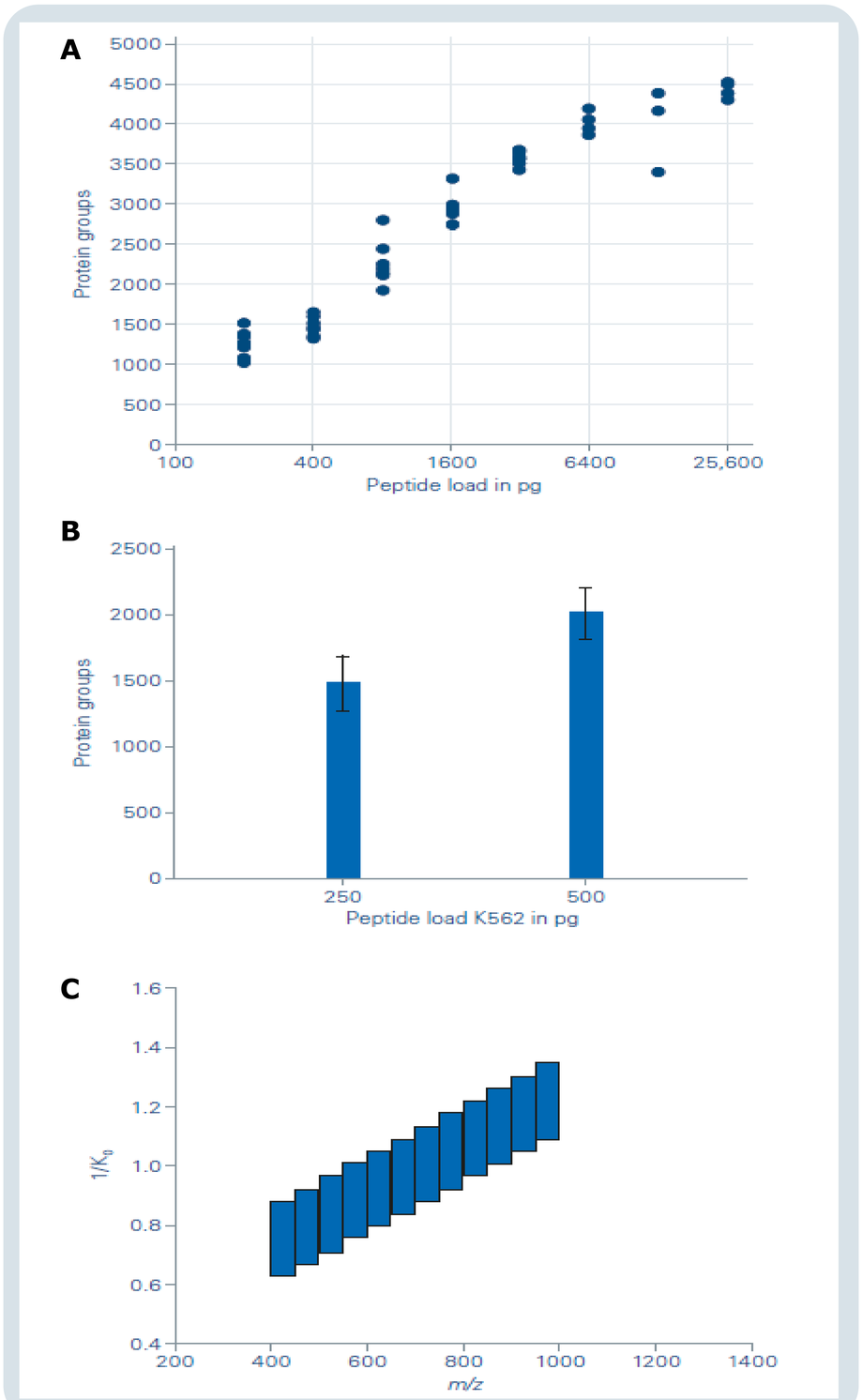

Figure 3: A Dilution series of peptides with Whisper 40SPD. **B** Multiple injections of 250 and 500 pg runs using Whisper 40SPD. **C** dia-PASEF window scheme used for low sample amount.

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 One provide a robust and sensitive platform to perform ultra low abundance proteomics.

1)Meier F, et al. (2018), Molecular & Cellular Proteomics.

timsTOF Pro