Ultra-Sensitive Proteome Quantification on the timsTOF SCP Mass Spectrometer

USHUPO 2022, P17.04

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Introduction

Single cell proteomics is a relatively young niche of proteomics compared to single cell genomics. In recent years, significant progress has been made in sample handling and boosting the sample signal by multiplexing with isobaric labels) is the first commercial mass spectrometer designed to meet the challenge of single cell proteomics. The modified front-end (orthogonal ion-guiding) of the instrument increases the ion transfer up to five times and keeps ultra-high robustness – the default attribute of the timsTOF platform. Here we demonstrate the performance of the instrument for low sample loads in the range of 125 pg to 20 ng in combination with robust low flow rate delivery from the Evosep system.

Methods

Human cervical cancer cell digests (HeLa, Pierce) were loaded on Evotips (EV2001) according to Evosep sample loading instructions for reproducibility assessment of 5 ng on tip and for dilution series at of 0.125, 0.25, 0.5, 0.75 1, 1.5, 2, 5, 10, and 20 ng on tip. Peptides were separated on a 15 cm performance column (15 cm x 75 µm, 1.9 µm) with the Whisper 40 samples per day method on the Evosep One coupled to a timsTOF SCP mass spectrometer via a CaptiveSpray ionization source. Eluting peptides were analyzed with a dia-PASEF method with high sensitivity mode enabled. For dia-PASEF acquisition, a window placement scheme consisting of 8 TIMS ramps with 3 mass ranges per ramp spanning from 400 – 1000 m/z and from a mobility range of 0.64 – 1.40 1/K0 with a cycle time of 0.9 seconds, including one MS1 frame, was utilized (Figure 1). All dia-PASEF data were processed in DIA-NN v1.8 (2) in library-free mode without match between runs using the human reviewed protein sequence database including isoforms (Uniprot, downloaded November 2021).

Results

For a quantitative analysis, a dilution series from 20 ng down to 125 pg peptide loads of a HeLa digest on Evotips was prepared (6 replicates) and processed with DIA-NN v 1.8 in library-free mode. From 125 pg, this yielded 2,870 precursors and 2,850 peptides corresponding to 690 protein groups. When increasing the load to 20 ng, 45,700 precursors and 42,800 peptides were identified corresponding to 5,400 protein groups (Figure 3a – c). The mean CV of the quantified protein groups for all concentrations was less than 10%, where the lowest peptide loads resulted in the highest mean CV, and the highest peptide loads resulted in the lowest mean CV (Figure 3d). For visualization of the quantification accuracy assessment the protein high mobility group box 1 (HMGB1) was used, because it was quantified in each sample of each concentration throughout the entire concentration range loaded onto the Evotips. The mean protein group area (sum of the area under the curve of all precursors quantified from HMGB1) per concentration was correlated with the concentration range. The Pearson correlation (PC) score was 0.999, demonstrating a linear concentration response (Figure 4a). This was also performed for all protein groups with quantitative values (n=6) in at least three concentrations (3991 protein groups) (Figure 4b). The mean PC score was 0.94 with 3539 protein groups showing a correlation greater than 0.9 demonstrating excellent concentration responses at the whole proteome level.

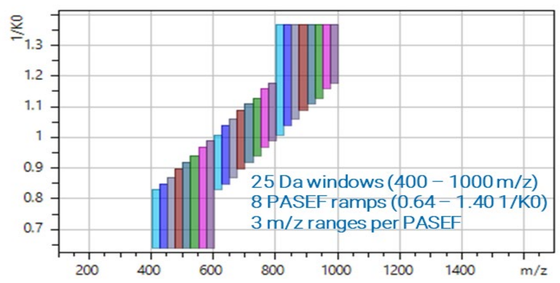

Figure 1: dia-PASEF window placement scheme. The cycle time was 0.9s

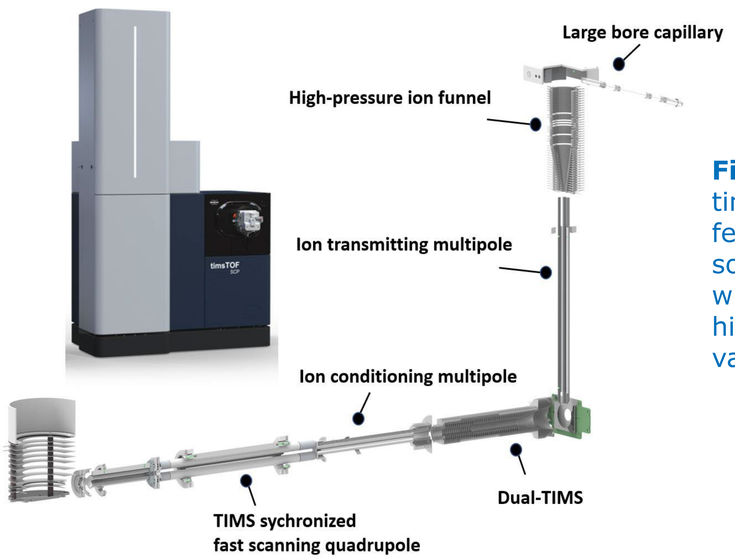

Figure 2: timsTOF SCP features new ion source geometry with additional higher pressure vacuum stage

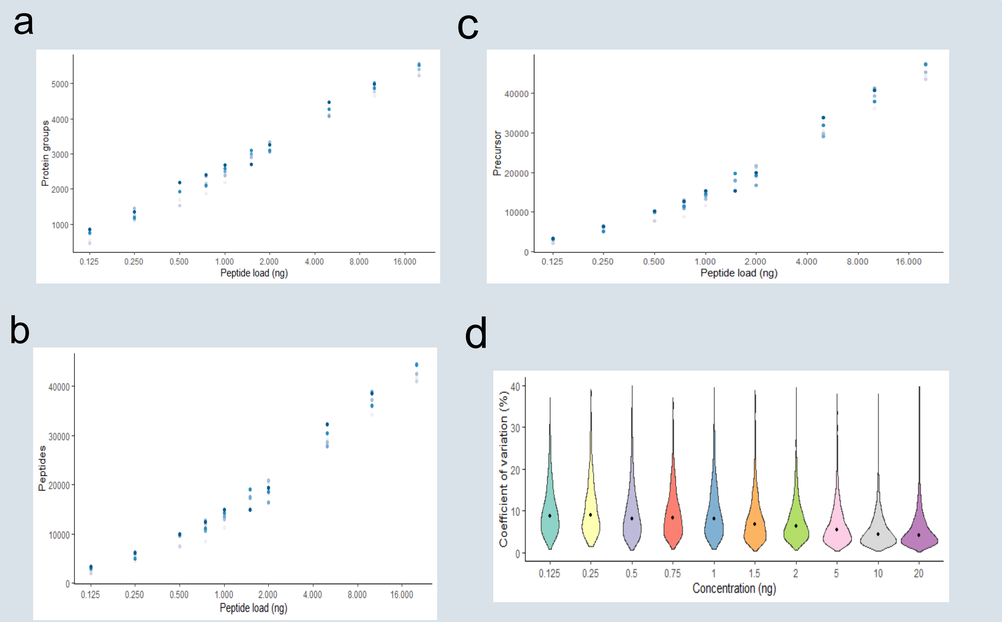

Figure 3: Quantitative assessment of HeLa digest dilution series a) reported protein groups, b) stripped peptide sequences and d) precursors of a HeLa digest dilution series with peptide loads of 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2, 5, 10 and 20 ng processed with DIA-NN v1.8 in library-free mode, d) CV of quantification within individual concentration ranges (n=6).

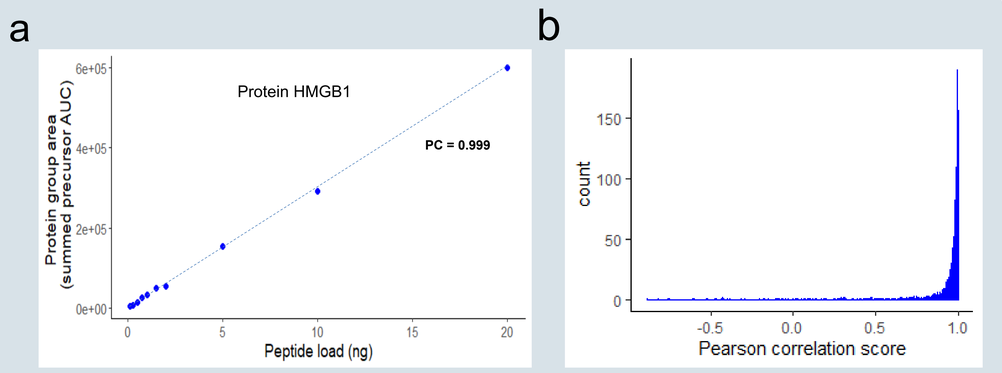

Figure 4: a) Correlation of peptide load on Evotip with protein group area (summed precursor area under the curve), b) Histogram of Pearson correlation scores of protein groups with quantitative values (quantified in all replicates) in at least 3 concentrations with the peptide amount loaded onto an Evotip.

Conclusions

- Ultra sensitive identification of 5600 peptides from 1250 protein from 250 pg of HeLa
- Linear concentration response in quantification from 125 pg up to 20 ng
- Low flow Evosep Whisper methods provide with good chromatographic reproducibility and robustness with short gradients and low overhead time between gradients

timsTOF SCP

1) Budnik, B et al, Genome Biology 19, 161 (2018)
2) Demichev, V et al., Nat Methods, 2020