

Ultra-sensitive proteome quantification on the timsTOF SCP mass spectrometer

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Introduction

The timsTOF SCP is the first commercially introduced mass spectrometer dedicated for single cell proteomics (fig. 1). The modified front-end (orthogonal ion-guiding, fig. 2) and the brighter ion beam (wider glass capillary) increase the ion transfer up to five times with keeping ultra-high robustness. Previous work showed the timsTOF SCP combined with robust low flow Evosep One and efficient sample preparation yielded exciting results on the changes upon perturbation in the single cell proteome [1]. Here, we demonstrate the performance of the instrument for low sample loads in the range of 125 pg to 25.6 ng in combination with robust low flow rate delivery from the Evosep system.



Fig. 1 Evosep One – timsTOF SCP bundle for single cell proteomics



Fig. 2 Modified ion source geometry with a 1 mm capillary

Methods

Commercially available K562 tryptic peptide digests (Promega) were loaded on the Evtotips according to the recommended protocol provided by the vendor. Peptide amounts from 125 pg to 25.6 ng were measured with the Evosep one (fig. 3, right) coupled to a captive spray ionization source using a 10-micron ID zero-dead volume emitter. Samples were analyzed using a dia-PASEF method tuned for high sensitivity measurements (fig. 3, left). Raw data were processed in Spectronaut 15 with a spectral library consisting of 5,200 protein groups and about 54,000 peptides and a second library of 9,300 protein groups and about 116,000 peptides. Additionally, DIA-NN 1.8 was used for a library-free processing approach[2].

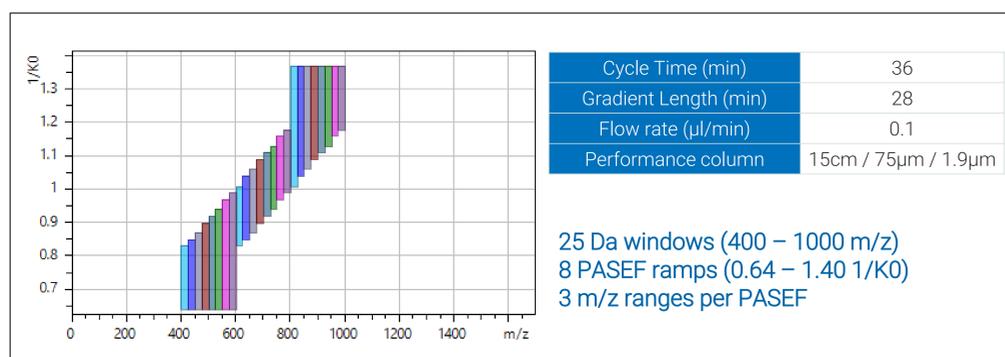


Fig. 3 Low sample input dia-PASEF method and Evosep one Whisper 40 method

References

- [1] <https://doi.org/10.1101/2020.12.22.423933>
- [2] Demichev *et al.*; Nat Methods 2020, 17: 41–44

Results

- Significant boost in sensitivity due to hardware improvements and additional improvement of electrospray ionization with the new low-flow Whisper methods of the Evosep One (fig. 3, right)
- Optimization of dia-PASEF method with respect to window size and speed (fig. 3, left)
- Two dilution series of peptide loads starting from 125 pg to 25.6 ng in replicates were processed with two different sized spectral libraries in Spectronaut (fig. 4)
- Using the bigger library improved the number of quantified protein groups by an average factor of 16%
- About 1,700 protein groups were quantified from the 200 pg peptide loads; the number increased to an excess of 5,200 protein groups for 25.6 ng loads (fig. 4)

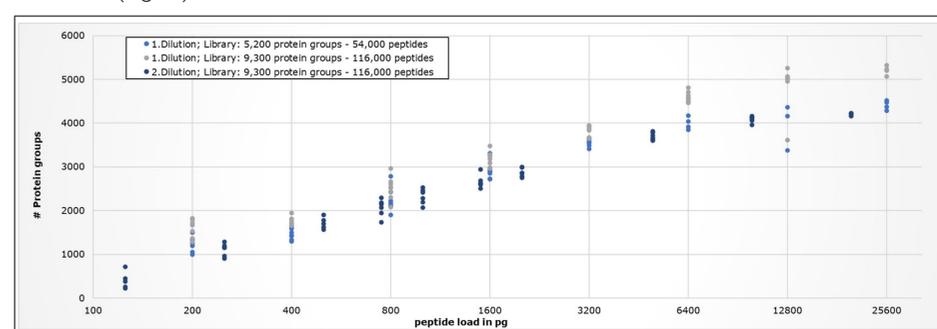


Fig. 4 Dilution series of peptides with Whisper 40 SPD and dia-PASEF

- 48 replicates of 250 and 500 pg loads (mimicking the number of peptides resulting from the digestion of one or two isolated cell) were used to test the accessible proteome depth
- From 250 and 500 pg loads on average 1,433 and 1,916 protein groups were quantified using Spectronaut, respectively (fig 5)
- Using the library-free approach from DIA-NN [2], quantification results could be further enhanced up to 2,512 and 3,130 protein groups (fig 5)

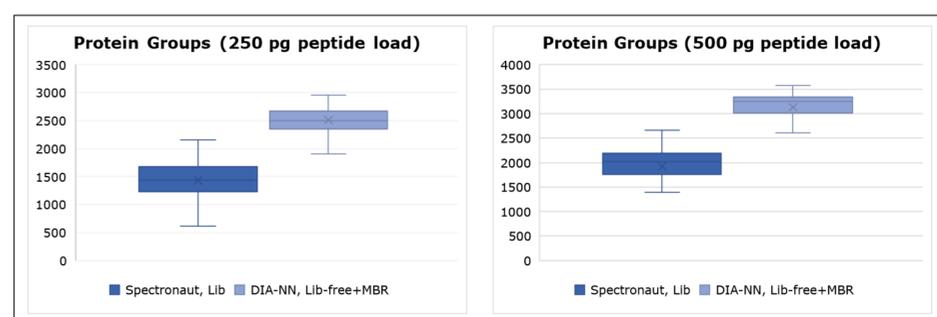


Fig. 5 Multiple injections of 250 and 500 pg runs processed with Spectronaut 15 and DIA-NN 1.8

Conclusion

- Combination of timsTOF SCP with Whisper methods on the Evosep provide a robust and sensitive platform to perform single cell proteomics
- timsTOF SCP provides robust proteome coverage with peptide loads in the range of 250 pg
- Dilution series showed excellent results with respect to sensitivity
- DIA-NN's library-free approach further improved protein group quantitation by more than 60%

Technology