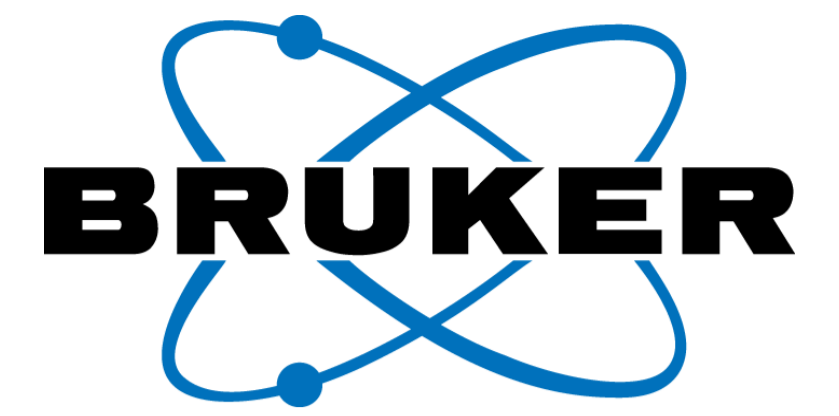


Ultra-sensitive proteome quantification on the timsTOF SCP mass spectrometer



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Introduction

The timsTOF SCP is the first commercially introduced mass spectrometer dedicated for single cell proteomics. The modified front-end (orthogonal ion-guiding, fig. 1) and the brighter ion beam (wider glass capillary) increase the ion transfer up to five times with keeping ultra-high robustness. 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.

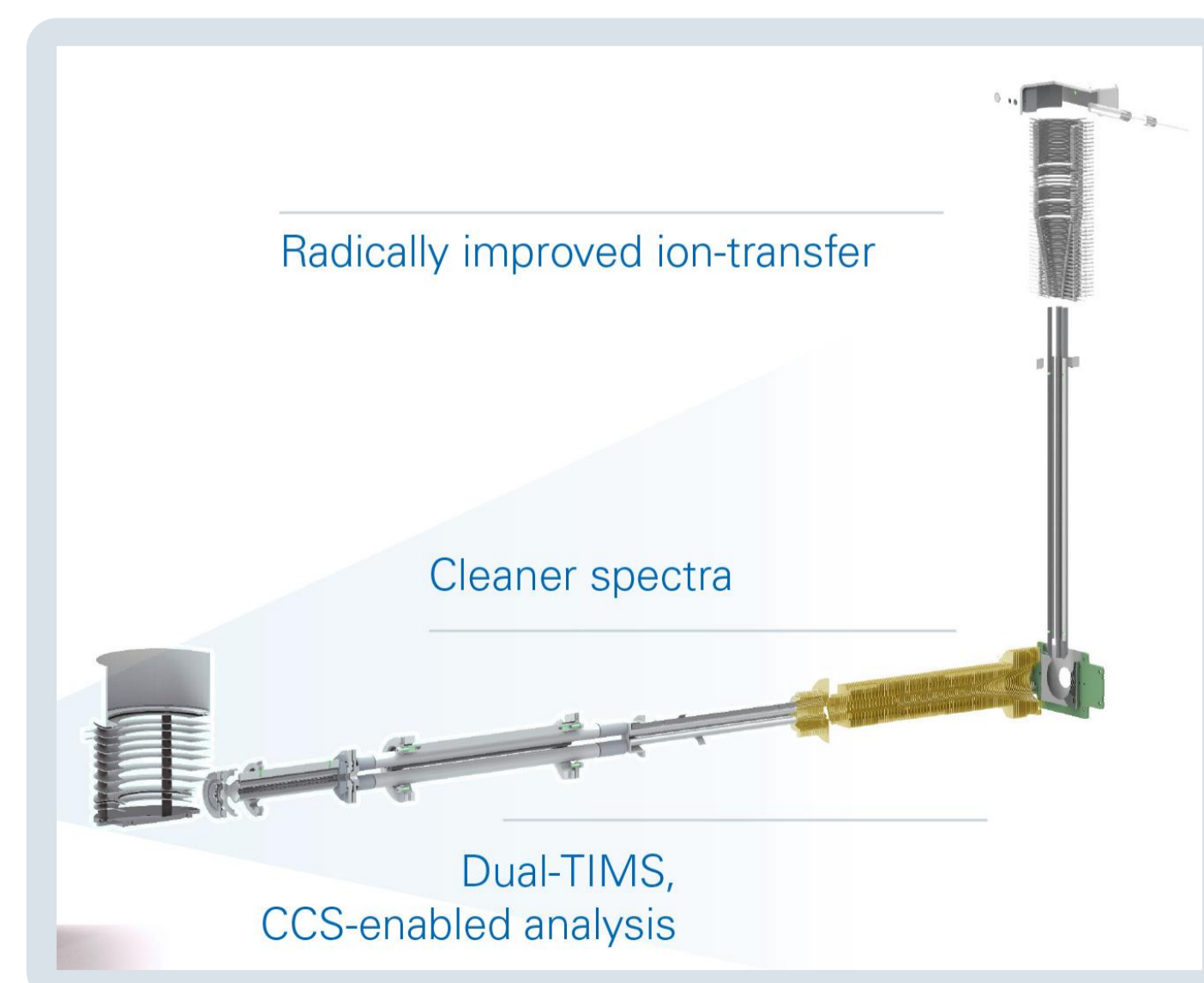


Fig. 1 Modified ion source geometry with a 1 mm capillary.

Cycle Time (min)	36
Gradient Length (min)	28
Flow rate (µl/min)	0.1
Performance column	15cm / 75µm / 1.9µm

Fig. 2 Evosep one Whisper 40 method.

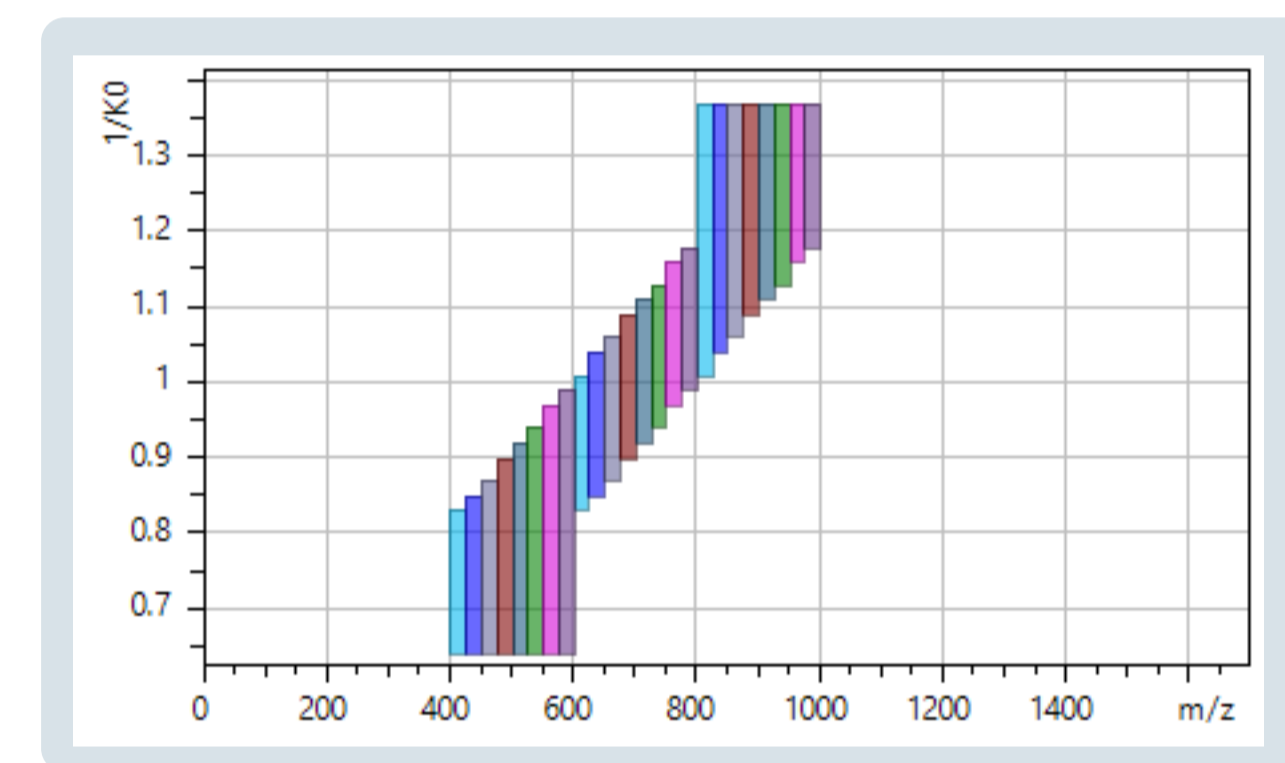


Fig. 3 diaPASEF method.

Methods

Commercially available K562 tryptic peptide digests (Promega) were loaded on the Evtips according to the recommended protocol provided by the vendor. Peptide amounts from of 125 pg to 25.6 ng were measured with the Evosep one (fig. 2) 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). 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.

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.
- Optimization of diaPASEF method with respect to window size and speed (fig. 3).
- 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 # 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).

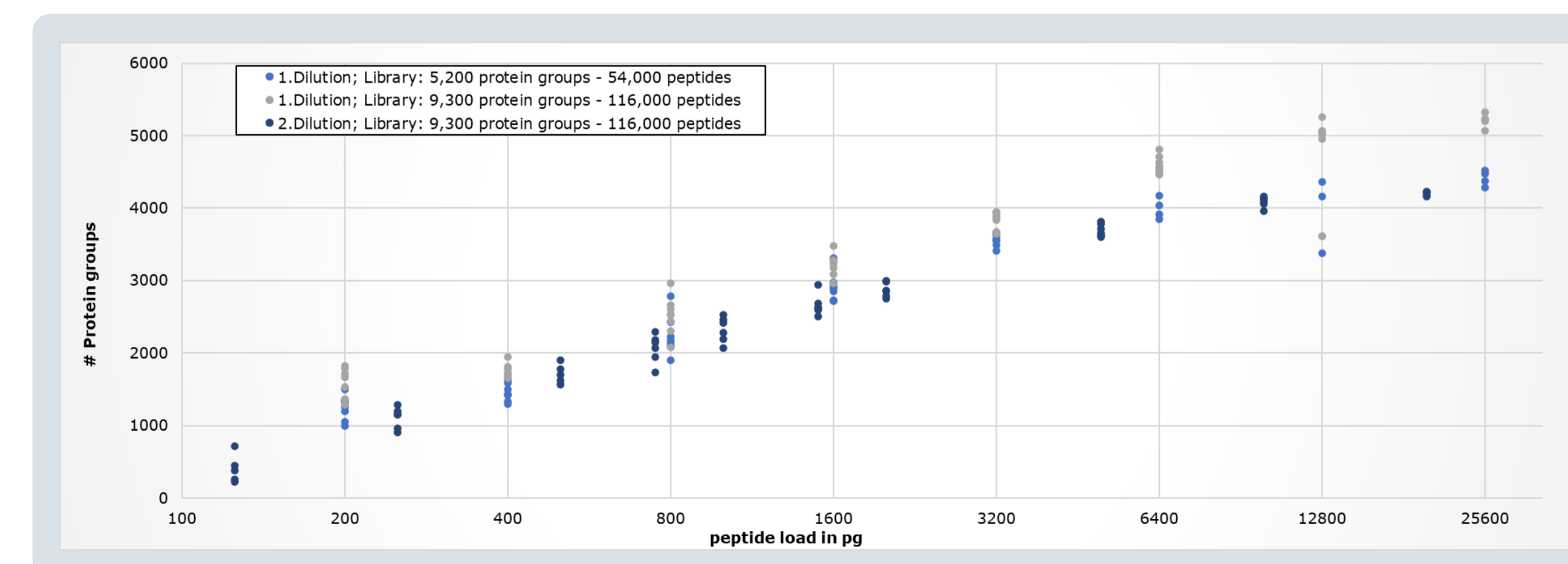


Fig. 4 Dilution series of peptides with Whisper 40 SPD and diaPASEF

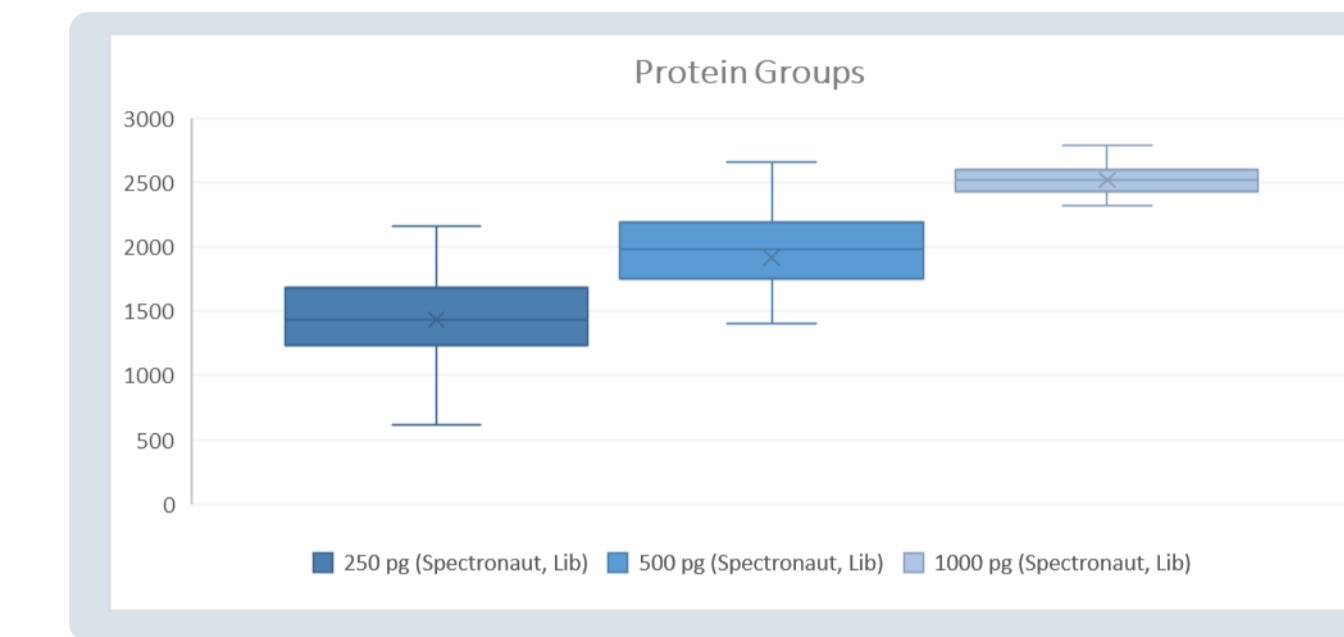


Fig. 5 Multiple injections of 250, 500 and 1,000 pg runs.

- 48 replicates of 250, 500 (mimicking the number of peptides resulting from the digestion of one or two isolated cell) and 1000 pg loads were used to test the accessible proteome depth.
- From 250, 500 and 1,000 pg loads on average 1,433, 1,916 and 2,519 protein groups were quantified, respectively (fig 5).

Summary

Work done in the labs of Prof. Matthias Mann with 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] and with respect to deep visual proteomics [2].

References

- (1) <https://doi.org/10.1101/2020.12.22.423933>
- (2) <https://doi.org/10.1101/2021.01.25.427969>
- (3) Demichev *et al.*; Nat Methods 2020, 17: 41–44

Conclusions

- Combination of timsTOF SCP with Whisper methods on the Evosep provide a robust and sensitive platform to perform single cell proteomics.
- timsFOF SCP provides robust proteome coverage with peptide loads in the low nano to pico gram range.
- Dilution series showed excellent results with respect to sensitivity down to 125 pg.

**Ion Mobility:
Applications**