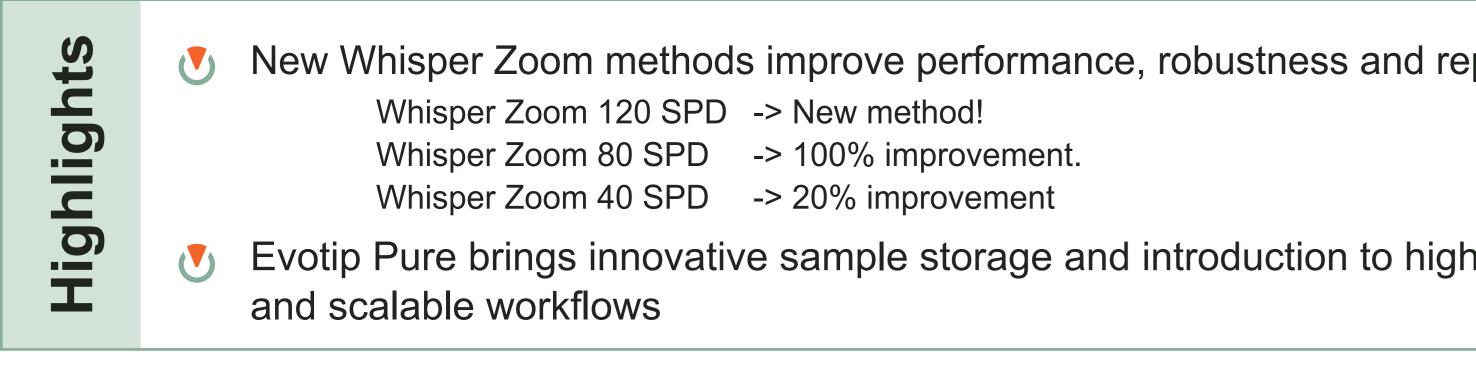
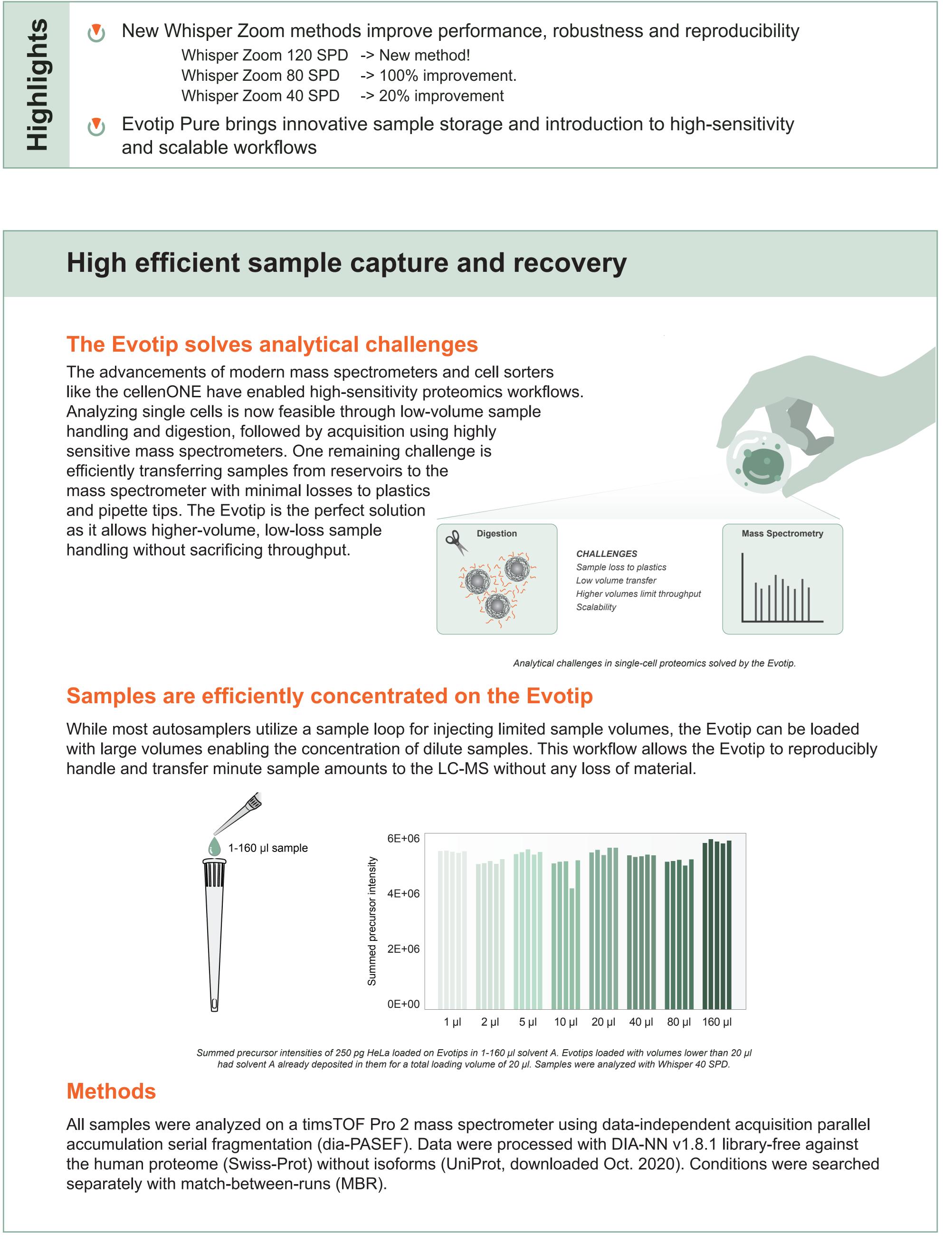
Enabling scalable single-cell proteomics by utilizing the unique analytical properties of the Evotip Pure with new Whisper Zoom methods

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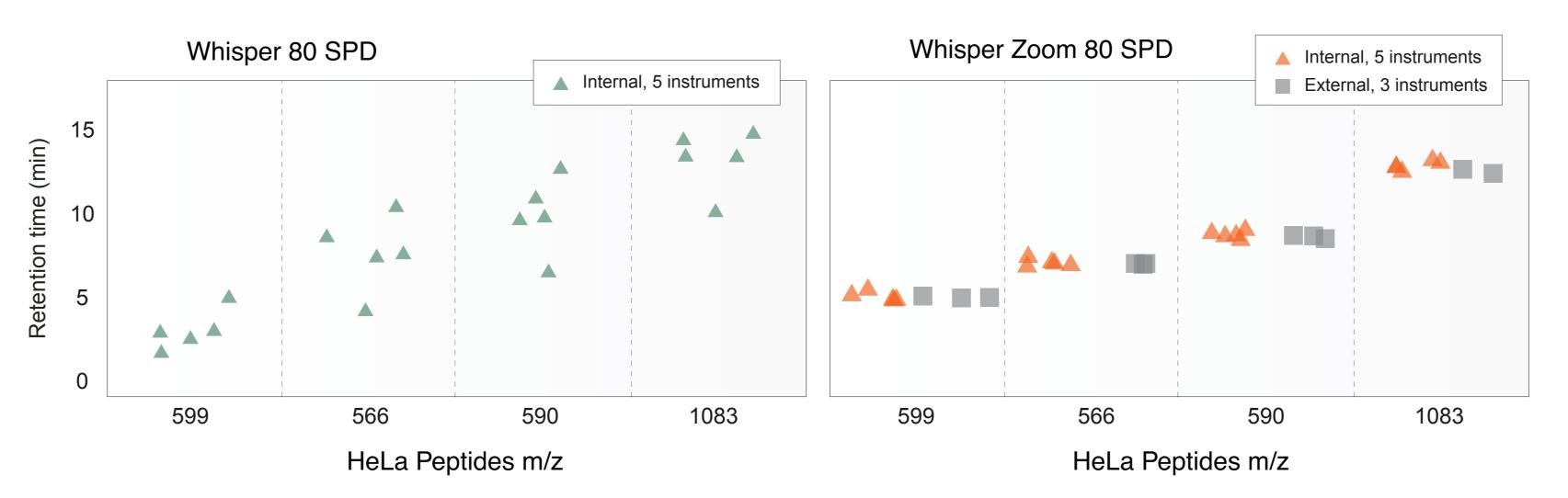


EVUSEP

Whisper Zoom drives scalable workflows

Unmatched robustness and reproducibility

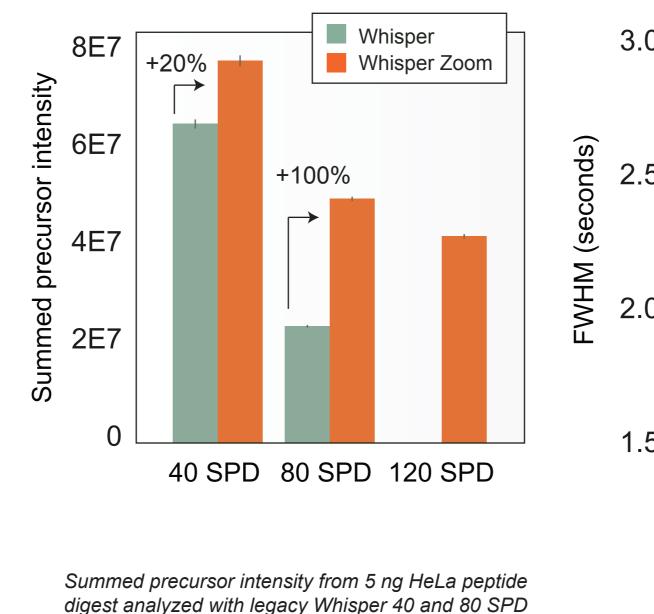
Whisper Zoom consist of newly developed methods that offer enhanced robustness and reproducibility with a throughput of up to 120 samples per day (SPD). All Whisper Zoom methods (20/40/80/120 SPD) operate with a gradient flow of 200 nl/min. The Aurora Rapid 75 column (IonOpticks) is recommended for the Whisper Zoom 80 and 120 SPD methods. To evaluate the robustness and reproducibility of Whisper Zoom, we analyzed 5 ng HeLa samples using the legacy Whisper 80 SPD method on 5 different Evosep instruments and compared it to the Whisper Zoom 80 SPD on the same 5 Evosep instruments. In addition, the Whisper Zoom methods were also compared across instruments from three external labs. Collectively, the Whisper Zoom method significantly enhances retention time stability and inter-instrument reproducibility, while delivering sharper peaks and more reproducible peak widths across different Evosep instruments.



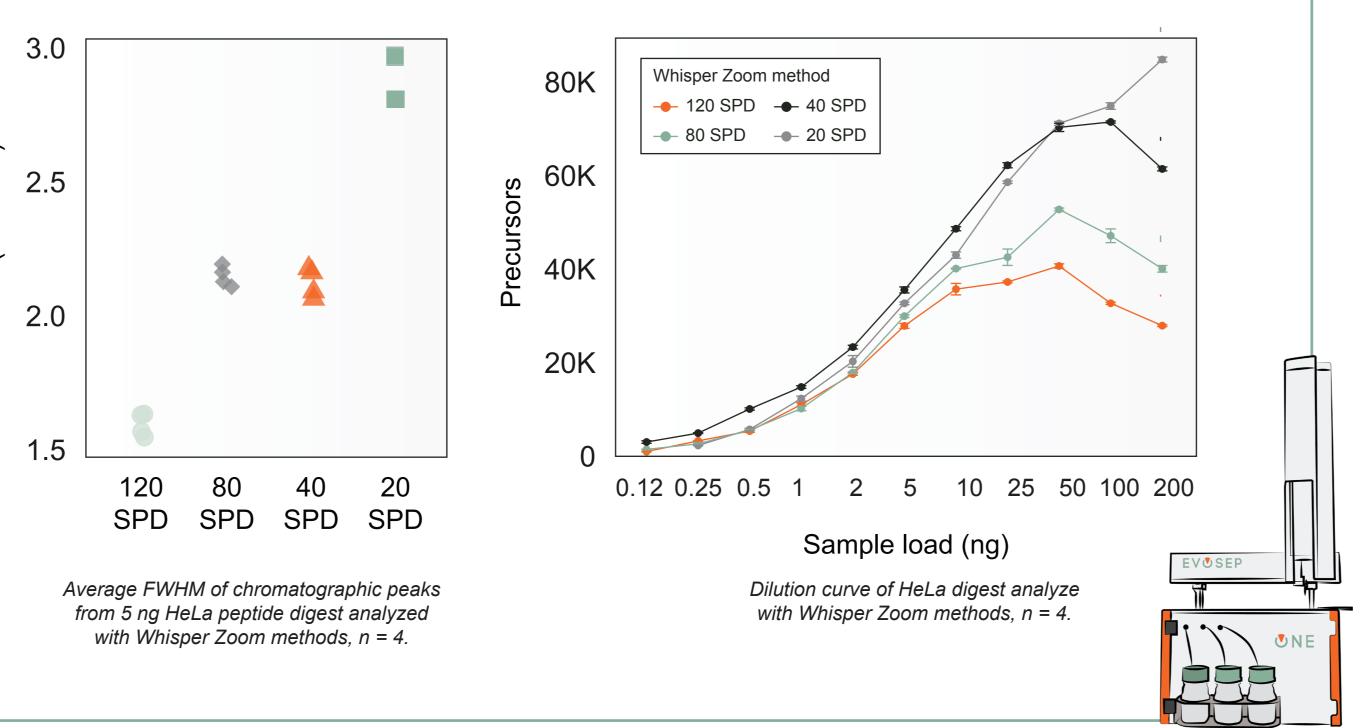
Significantly improved performance gain with Whisper Zoom

We assessed the sensitivity of the four Whisper Zoom methods with 5 ng of tryptic HeLa peptides. Whisper Zoom methods vastly improve the signal intensity of identified precursors compared to legacy Whisper methods and thus improve analysis sensitivity. Whisper Zoom methods utilize the shortest possible flowpath in the Evosep. This enables sharp symmetric peaks as exemplified with an average FWHM of ~1.5 seconds with the Whisper Zoom 120 SPD method and \sim 2.2 seconds using the Whisper Zoom 40 SPD method.

We further evaluated the sensitivity of the Whisper Zoom 20, 40, 80, and 120 SPD methods with a dilution series of 0.125 pg to 200 ng tryptic HeLa peptides using the timsTOF Pro 2. The Whisper Zoom 120 SPD shows saturating levels of precursor identifications at 10 ng load where additional peptide input does not improve analysis depth. For the Whisper Zoom 40 and 80 SPD methods, saturation occurs at 50 ng, while the Whisper Zoom 20 SPD method is saturated at or above 200 ng input.



and Whisper Zoom 40, 80, and 120 SPD methods, n = 4.

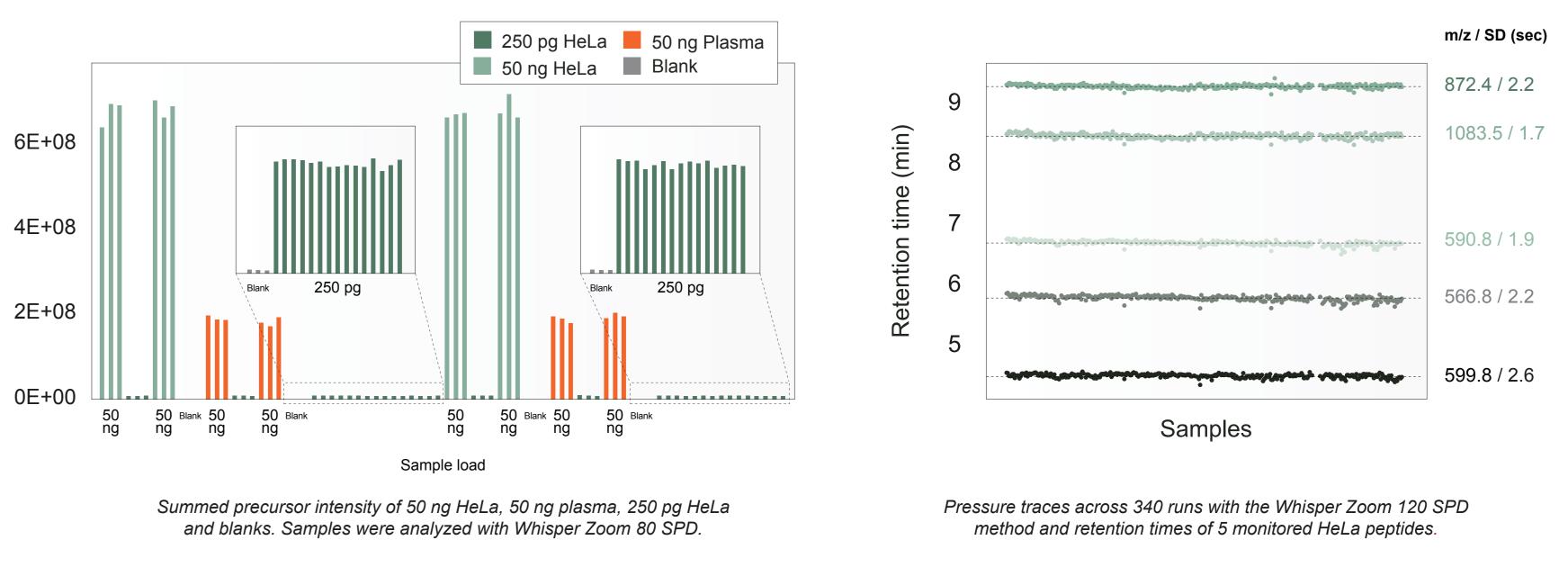


provement in inter-instrument retention time (RT) reproducibility of Whisper Zoom 80 SPD internally (n=5) and externally (n=3). RT from four peptides were monitored from 5 ng injections (n=4).

Disposable trap column increases system robustness

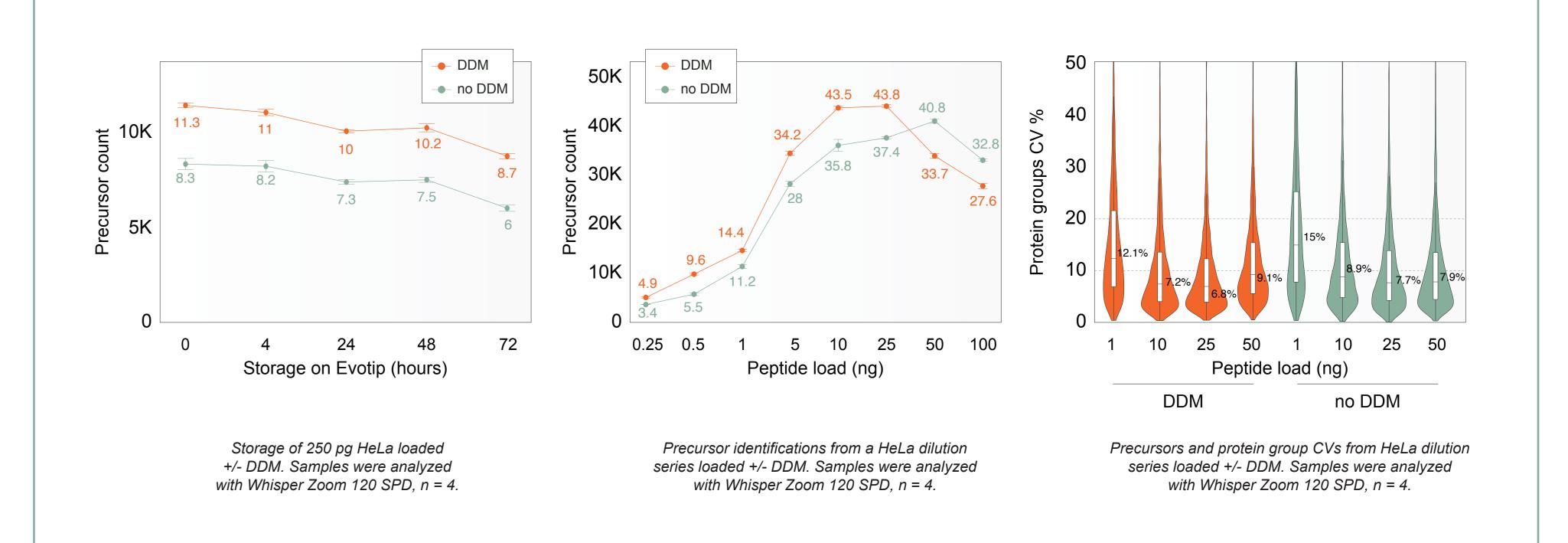
Low carryover enhances versatility and scalability

The high-sensitivity Evotip Pure workflow was evaluated for reproducibility and quantitative precision by injecting single-cell equivalents of HeLa (250 pg) consecutively with high-load plasma (50 ng) and HeLa digest (50 ng). The analyzed samples were mixed with blank Evotips to monitor carryover as outlined below using the Whisper Zoom 80 SPD method. This experiment was repeated 8 times. The carryover in single-cell equivalent (250 pg HeLa) samples was 0.6% and 0.02% following 50 ng Plasma and HeLa, respectively. In blank injections, carryover was 0.2% after both 50 ng plasma and HeLa samples. Additionally, single-cell levels of HeLa (250 pg) were analyzed with Whisper Zoom 120 SPD, demonstrating high retention time stability across 340 samples with retention time standard deviations of less than 2.6 seconds for all monitored peptides. These data shows that Evosep One can be used for versatile samples without compromising the performance.



Sensitive sample storage on the Evotip

Sample storage is essential for robust and scalable single-cell proteomics, as it allows for the preparation and execution of experiments over extended periods. Storing samples on Evotips for up to 72 hours showed that over 90% of precursor identifications are preserved after 48 hours, with nearly 75% preserved after 72 hours. We also evaluated the impact of DDM on sample loading using a dilution series of HeLa (Pierce). The results reveal that DDM positively affects low sample loads until saturation occurs at higher loads. Overall, DDM enhances precursor identifications and quantitative precision for low loads without affecting storage capability, demonstrating its beneficial impact on sample preparation workflows upstream of the Evotip.



The Evosep One instrument is for Research Use Only